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Triterpenoids with Anticancer Properties and Their Mechanism of Action

Ph.D. Thesis

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Declaration of originality

I hereby confirm that I am the sole author of this Ph.D. thesis and it is entirely my original work. I have used no other literature sources except as referred by citations. I also declare that neither this thesis nor its substantial part have been used for awarding of any other academic degree.

In Olomouc ..... 2018 .....

Lucie Borková

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Abstract

Pentacyclic triterpenes are natural compounds produced by the majority of living organisms and may be found especially in fungi, algae, marine invertebrates, and most commonly in plants. Being secondary metabolites, they are not a part of the main metabolic pathways and it seems that their role is to protect their producers from various diseases. This may be confirmed by the fact that many triterpenes are anticancer, antiviral, antibacterial, antifungal, antiparasitic, and anti-inflammatory, etc. Despite their low toxicity and good availability from the natural resources, their clinical use is still limited by their higher values of IC_{50} and worse pharmacological properties than in the currently used therapeutics. Several approaches have been used to increase the activity and to improve the bioavailability of natural triterpenes. Among the most important is to prepare their semi-synthetic derivatives with higher selectivity and better pharmacological properties. As a part of this research, my thesis was focused on the preparation of new semi-synthetic triterpenoids more suitable for the potential clinical use. This work may be divided into three major topics: (I) Synthesis and anticancer properties of triterpenoid thiazoles; (II) Cytotoxic triterpenoids substituted in the position 2; (III) Preparation of new betulinic acid derivatives by Suzuki-Miyaura cross-coupling.

Abstrakt

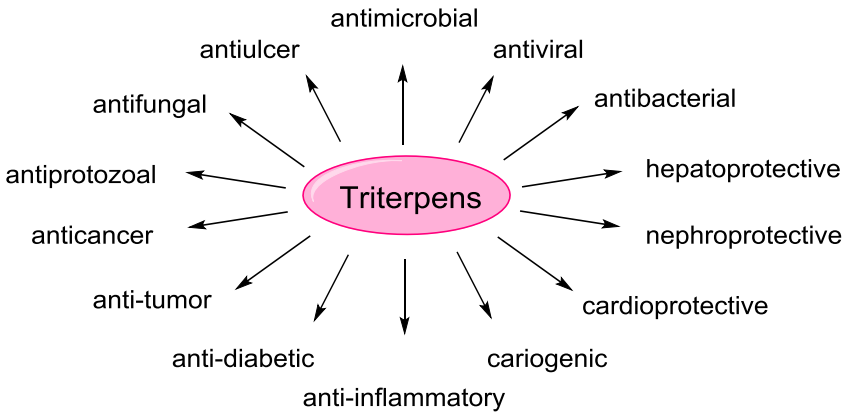
Pentacyklické triterpeny jsou přírodní sloučeniny produkované velkým množstvím živých organismů, například houbami, řasami, mořskými bezobratlými živočichy a nejšastěji rostlinami. Jakožto sekundární metabolity nejsou součástí hlavních metabolických drah a jejich rolí je zřejmě ochrana svých producentů před různými chorobami a škůdci, což může být potvrzeno faktem, že mají velké množství biologických účinků, jsou např. protirakovinné, antivirální, antibakteriální, antifunglální a protizánětlivé. Navzdory jejich nízké toxicitě a jednoduché dostupnosti z přírodních zdrojů je klinické použití triterpenů výrazně limitováno jejich vyššími hodnotami IC_{50} a horšími farmakologickými vlastnostmi, než mají v současnosti používaná léčiva. Existuje řada přístupů, jak zvýšit aktivitu a zlepšit biodostupnost přírodních triterpenů; jedním z nejúčinnějších je příprava jejich semi-syntetických derivátů s vyšší selektivitou a vhodnějšími farmakologickými vlastnostmi. Cílem mojí práce, která je součástí tohoto výzkumu, bylo připravit semi-syntetické triterpenoidy vhodnější pro potenciální klinické použití. Práci je možno rozdělit na tři hlavní témata, všechny se týkají modifikace triterpenoidů na A-kruhu: (I) Syntéza a protirakovinné účinky triterpenoidních thiazolů; (II) Cytotoxické triterpenoidy substituované v poloze 2 a (III) Příprava nových derivátů kyseliny betulinové pomocí Suzuki-Miyaura cross-couplingu.

Contents

1 Introduction

Pentacyclic triterpenes are natural compounds usually occurring in plants,[1–3] fungi,[4–6] algae,[7,8] bacteria[9] and marine invertebrates.[10–12] Hundreds of new triterpenes are being isolated from natural resources every year.[13,14] As secondary metabolites, they do not play a key role in the growth, development, or reproduction of the organisms. Instead, they usually protect and defend their producers against various diseases and pests,[15] although the role of many pentacyclic triterpenes still remains elusive. It is known that pentacyclic triterpenes have a wide range of biological activities (Figure 1).[16] They are not only antimicrobial,[17] antifungal,[18] antiulcer,[19] and antiprotozoal[20,21] (including antimalarial activity),[22] but also antibacterial,[23,24] and antiviral[25–27] (including anti-HIV activity).[28–30] They often reduce inflammation and oxidative demage[31–34] and have hepatoprotective,[35] nephroprotective,[36] cardioprotective,[37] and anti-diabetic effects.[38,39] A large number of triterpenes are cytotoxic against various cancer cell lines[40–42] and their anti-tumor activity was also observed in preclinical animal models.[43] Contemporary research reveals, that triterpenes are biologically active *via* a variety of mechanisms of action.[44–48] This makes their research including structure-activity relationship (SAR) studies complicated.

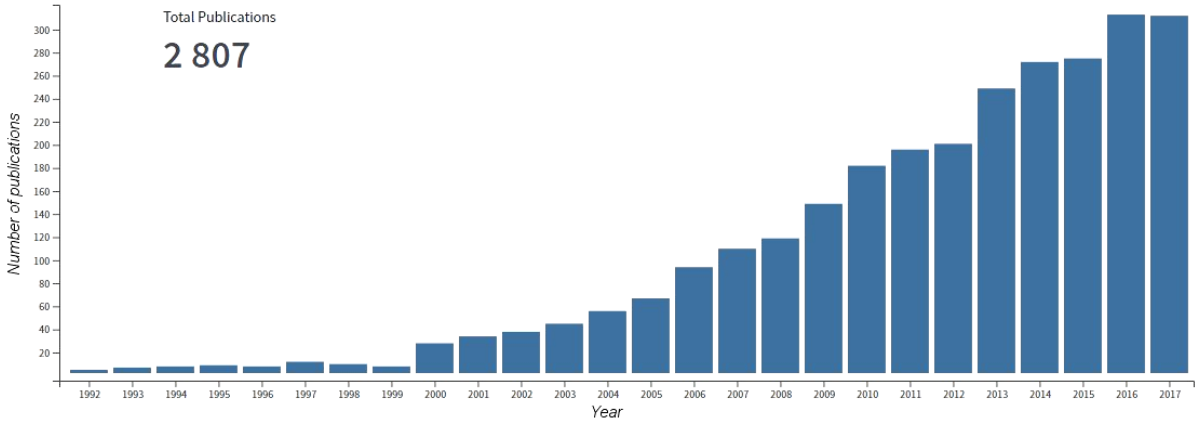
Figure 1. Biological activities of triterpenes.



Triterpenes have a vast number of biological activities, they are easily available from natural resources, and they are usually nontoxic.[49] Despite that, they are still not commonly used in the therapies since the majority of the parent compounds have some major drawbacks. First, in many cases the effective concentrations of triterpenes are not low enough for their therapeutic use. The lowest values of IC_{50} are usually in low micromolar and rarely in

submicromolar range which usually cannot compete with the activity of already available drugs. In addition, low selectivity may exclude some of the potential candidates with otherwise high activity. Therefore, significant improvement of the activity with the respect to the selectivity is one of the main goals of many research groups dealing with triterpenoids.[50–57] A growing interest in triterpenes and triterpenoids and their cancer research can be documented by the significant increase in number of scientific publication in this field during past 25 years (Figure 2).

Figure 2. Number of scientific publications containing research on anti-cancer triterpenes or triterpenoids in past 25 years. Data was obtained from the Web of Science database by searching the keywords (triterpene OR triterpenoid) AND (cancer OR tumor).



Inappropriate pharmacological properties of triterpenes are the second disadvantage. Their low solubility in water is one of the main reasons why compounds with high *in vitro* activities and high selectivity against cancer cells often fail during the advanced *in vivo* screening. Their bioavailability from gastro-intestinal tract is usually limited because they are often very lipophilic and do not adsorb well. These properties make triterpenes difficult to pass through preclinical and clinical tests, although there are few examples of compounds in phase I-III of clinical trials.[34,58–61] Recently, several patents emerged on betulinic acid derivatives dealing with low solubility in water while retaining selectivity against cancel cell lines.[62] All in all, these examples show that such problems can be solved by derivatization, prodrug approach, or by appropriate formulations.[63]

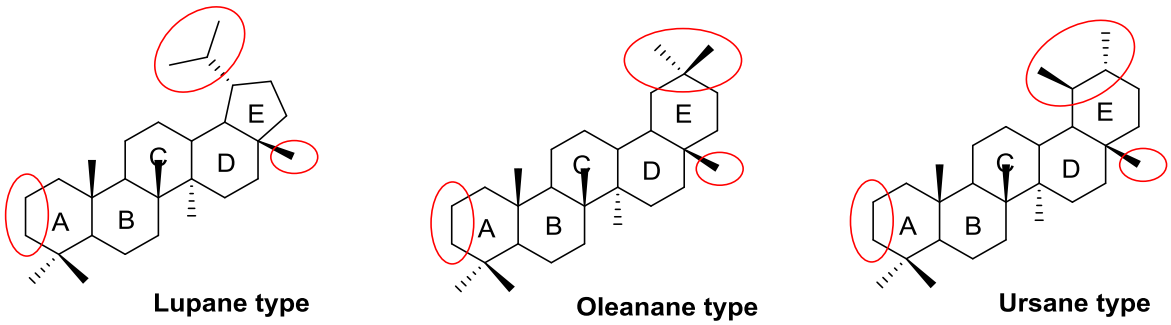
To find appropriate *in silico* models for evaluation of structure-activity relationships (SARs) and designing new compounds with better pharmacological properties is also difficult

because triterpenes usually interact with multiple proteins in the organism and the final biological activity is the result of combined mechanisms. Triterpenes are typical examples of multi-target compounds. On the other hand, some of the mechanisms of action of triterpenes are quite unique and theoretically, they may be used to overcome resistances or to cure diseases with currently no available drugs. Their great potential to become new drugs may be documented by a number of patents (summarized in the lit.[62,64]) that protect thousands of promising compounds.

Often, the biological activities (such as anti-oxidant) and low toxicity of triterpenes make them important components of natural medicine, cosmetics and food supplements which do not need to be approved by the strict and expensive process for the approval of new therapeutics.[65,66] In natural extracts, triterpenes are often bound to sacharides as glycosides – saponins[17,67,68] which have better bioavailability in comparison to the free triterpenes. Saponins are often the active components of raw extracts, ointments or tinctures made from medicinal plants. As a result, new plant species are being explored to find new molecules with potential biological activities. In the same time, semisynthetic compounds with higher activity, improved pharmacological properties, and more favorable therapeutic index are being prepared.[40]

In the research group of assoc. prof. Urban, we work with several structural types of triterpenes. Our attention is directed mainly towards lupane type, oleanane type, and ursane type derivatives, which are the most abundant in the nature. (Figure 3) The chemical reactivity of such robust molecules is often limited due to the steric hindrance by a number of skeletal methyl groups. The reactive sites are almost exclusively limited to the A-ring and to the E-ring of all structure types, and the isopropyl moiety at the lupane type structure.

Figure 3. Three most abundant structural types of triterpenes and their reactive sides.

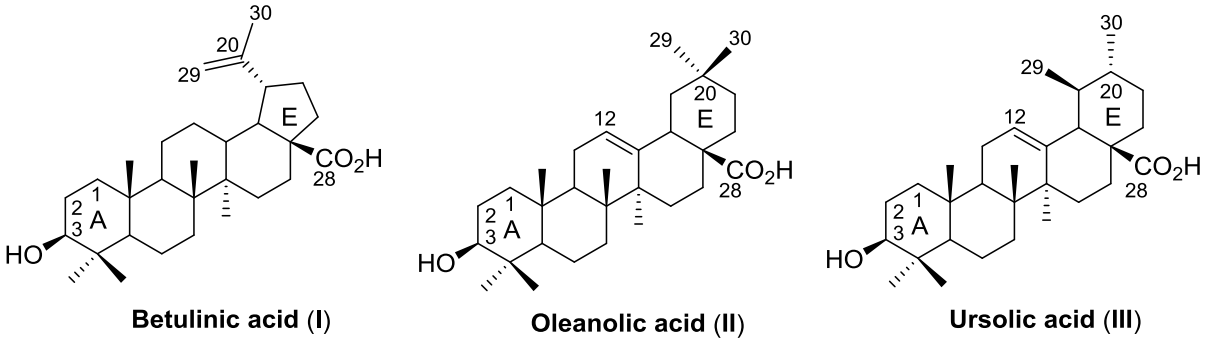


2 State of the art

2.1 Chemical modifications of the triterpenoid structure

Triterpenoids may be modified at their skeleton by expansion or contraction of the A-ring or the E-ring,[69,70] by cleavage of the A-ring or the E-ring to form seco- and nor-derivatives[71,72] or highly oxidized des-E derivatives[73] or by expanding of the molecule e.g. by their fusion with heterocycles.[74] Another way how to modify triterpenoids is at their hydroxyl, carboxyl, or other functional groups to form prodrugs or prodrug-like structures.[75–77] Derivatives with modified position C-3,[78–80] C-20,[81] or C-28,[82] and heterocyclic derivatives[74] are among the most common (Figure 4).

Figure 4. Selected triterpenoids and their positions suitable for modifications.



The main interest in our group is to modify the structure of triterpenoids to improve their solubility in water and to increase their cytotoxic activity and selectivity against cancer cells. Among a large number of new compounds, significant anticancer effects were found at many derivatives of lupane, ursane, and oleanane.[75,83–86] In addition, tools for the identification of molecular targets are being developed.[87] Based on this knowledge, simplified SAR assumptions were made and some trends how the chemical structure may affect the anti-cancer activity were discovered. In general, increasing hydrophobicity leads to less active compounds. On the other hand, derivatives with modified A-ring[88] are among the most promising. By introducing an electronegative substituent into the position 2 of the skeleton the cytotoxicity increases significantly, which works particularly well for lupane

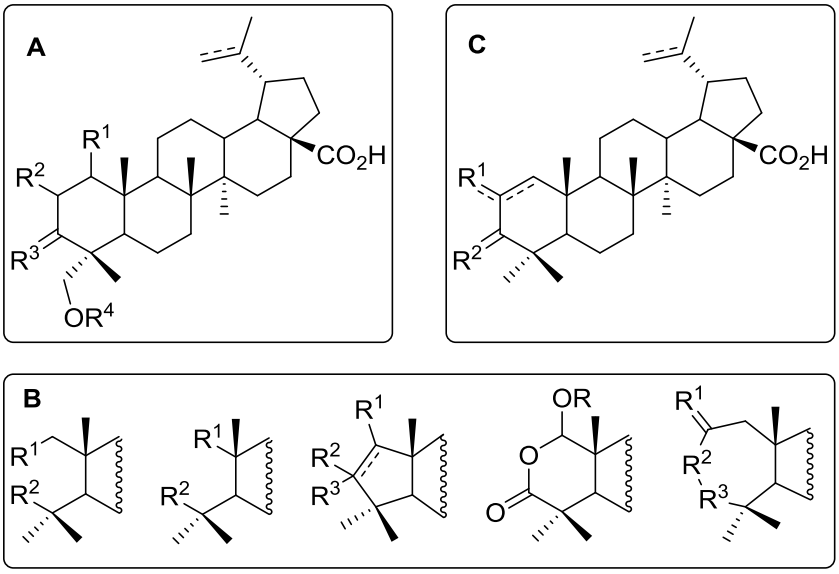
derivatives.[86,88–90] More specifically, derivatives of betulinic acid modified at the A-ring were probably the most successful in anti-cancer research and are the closest to the drug development. Moreover, triterpenoid derivatives with fused heterocycle to the A-ring belong to the large group of compounds with potential cytotoxic activities which are currently being explored by number of research groups all over the world.[74]

2.2 Betulinic acid derivatives with modified A-ring and their development as potential drug candidates

During the work on my thesis, I have been continuously doing literature research focused on derivatives of betulinic acid with modified A-ring. By the beginning of 2018, I have collected enough data to summarize this literature research in a review article: Borkova, L.; Hodon, J.; Urban, M. Modifications at the A-ring of Betulinic Acid and Betulonic Acid. *Asian J. Org. Chem.* **2018**, *7*, 1542-1560.[91]

The review deals with betulinic acid derivatives substituted at the positions 1, 2, and or 3, seco-derivatives, nor-derivatives, compounds with contracted or expanded A-ring, and compounds containing Michael acceptor and similar derivatives (Figure 5).

Figure 5. Types of betulinic acid derivatives described in the review.[91] A: derivatives modified at the positions 1, 2, and or 3. B: seco-derivatives, nor-derivatives, 5-membered ring derivatives, and 7-membered ring derivatives. C: Michael acceptor and similar derivatives.



The review summarizes all biological activities of betulinic acid derivatives found to date in the literature including anticancer, anti-angiogenic, anti-inflammatory, and antiviral. Some of the betulinic acid derivatives described in the review were isolated from medicinal plants; however, most of them were prepared by researchers from all over the world including us. Detailed synthetic procedures were included which may be useful for other researchers in their attempts to modify the A-ring of betulinic acid (**I**) and or other triterpenes and triterpenoids in the future. Heterocyclic derivatives of betulinic acid were not reviewed since another review has been published on this topic in 2015 covering heterocyclic derivatives of many triterpenoids modified both at the A-ring and the E-ring and their biological activities.[74] In the past four years, two reviews concerning triterpenoid prodrugs including betulinic acid esters at the 3-OH were published, such compounds were therefore also excluded.[75,76]

2.3 Introduction to the subprojects

2.3.1 Triterpenoid Thiazoles

A comprehensive review summarizing research in triterpenoids modified with heterocycles and their biological activities was published by our research group in 2015 covering publications from 1962 to 2014.[74] Many cytotoxic derivatives were found among triterpenes that contain a heterocycle fused to the A-ring of the triterpenoid skeleton. The most active compounds were indoles,[92,93] pyrazoles,[84,92,94–98] isoxazoles,[99,100] triazoles,[101] quinoxalines,[92] pyrimidines,[99] pyrazines,[85,92,96,102,103] and triazines.[74,104]

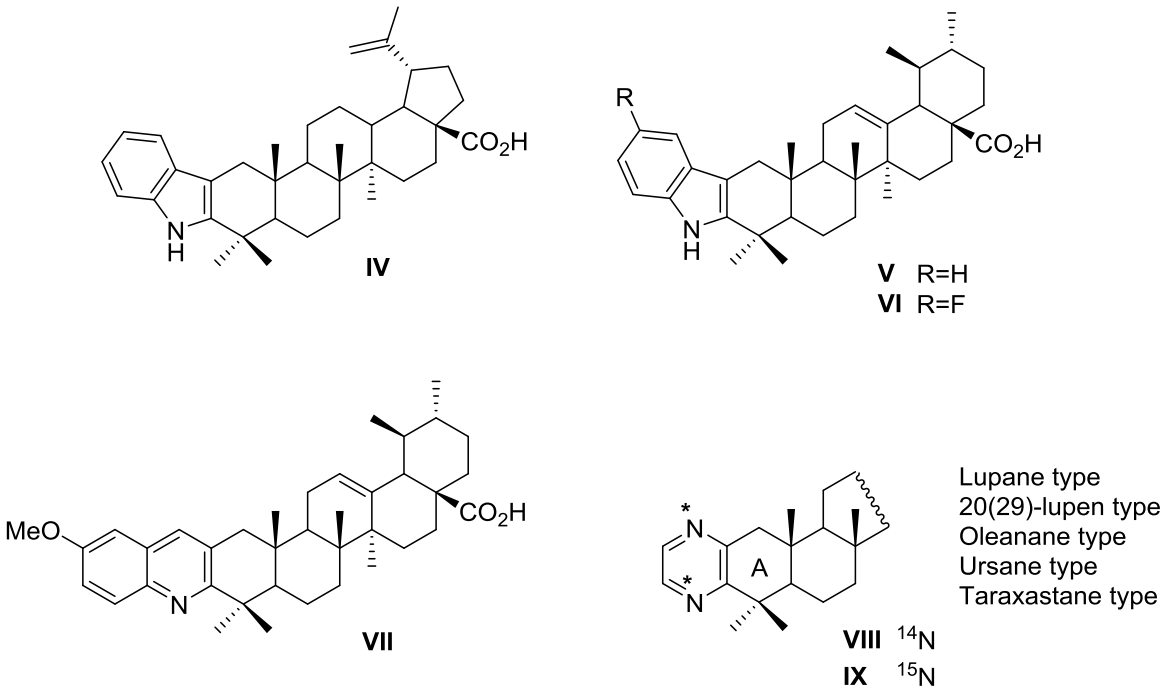
Several new publications concerning heterocyclic triterpenoids fused to the A-ring were published since the beginning of 2015. A-ring fused indoles and pyrazines of lupane, ursane, oleanane, and dammarane triterpenoids were synthesized and their anti-diabetic properties were tested in the lit.[105] The most promising results were obtained for unsubstituted indole derivative of betulinic acid **IV** which inhibits activity of α-glucosidase with IC_{50} value of 1.8 μM (Figure 6).

Antimicrobial and cytotoxic activities of substituted indole derivatives of ursolic acid were examined in the lit.[56] Derivatives **V** and **VI** (Figure 6) belong to the most active compounds with significant antibacterial activity (MIC values 15.6 μg/mL against *B. subtilis* and *E. coli*); however, their cytotoxicity is just moderate (IC_{50} 15-40 μM against SMMC-7721 and HepG2).

Quinoline derivatives of ursolic acid and their anticancer activity were studied in the lit.[57] 6-methoxyquinoline derivative **VII** (Figure 6) exhibited the most potent anticancer activity against all tested cancer cells (MDA-MB-231, HeLa, and SMMC-7721 with IC_{50} 0.61 μM, 0.36 μM, and 12.5 μM, resp.) and was non-toxic for normal hepatocytes (IC_{50} >40 μM against QSG-7701). Moreover, compound **VII** induces apoptosis of MDA-MB-231 cells and arrests the cell cycle at G0/G1 phase in dose-dependent manner.

Vlk et al prepared a set of ^{15}N labelled triterpenoid pyrazines **IX** derived from seven skeletal types along with their non-labeled analogues **VIII** (Figure 6).[106] All ^{15}N labelled compounds were prepared with abundance higher than 98 % confirmed by HRMS; their biological activity was not reported.

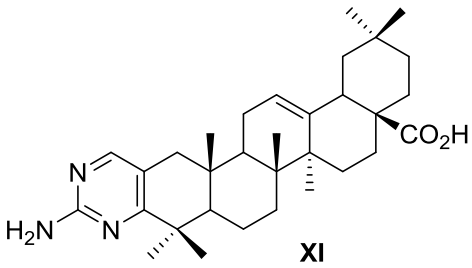
Figure 6. Biologically active indole (**IV-VI**) and quinoline (**VII**) derivatives of triterpenoids and ^{15}N labelled (**IX**) and non-labeled (**VIII**) triterpenoid pyrazines.



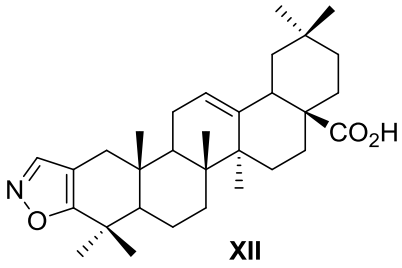
Pyrimidine and isoxazole derivatives of oleanolic acid were prepared by Pan et al.[107] and their molecular mechanism of action was studied. Both compounds **XI** and **XII** (Figure 7) induce apoptosis in human leukemia K562 cells by strong inhibitory effect on their viability in a dose-dependent manner and significantly increase chromatin condensation and formation of apoptotic bodies. Results from the flow cytometry suggest that the compounds induce inhibition of proliferation of K562 cells in the G1 phase. Further biological assays show that compounds **XI** and **XII** may be involved in inactivation of Akt1 signaling pathway.

Isoxazole derivative **XII**, its analogues with various amides at C-28, as well as pyrazole derivatives of oleanolic acid were prepared to study their effect on osteoporosis.[108] Most of the compounds had better inhibitory activity on RANKL-induced osteoclast formation generated from RAW264.7 cells than oleanolic acid (**II**). When various phenyl sulfonyl groups were introduced to the pyrazole ring and carboxylic acid was modified to form various alkylamides, some derivatives displayed even better inhibitory activity. The best results were obtained when testing the compound **XIII** (IC_{50} value of 24 nM); pyrazole **XIII** is non-toxic and obviously prevents ovariectomy-induced bone loss *in vivo* (Figure 7).

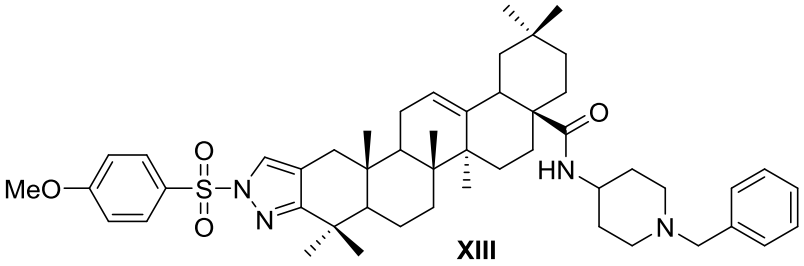
Figure 7. Pyrimidine (**XI**), isoxazole (**XII**) and pyrazole (**XIII**) derivatives of oleanolic acid with biological activities.



XI



XII

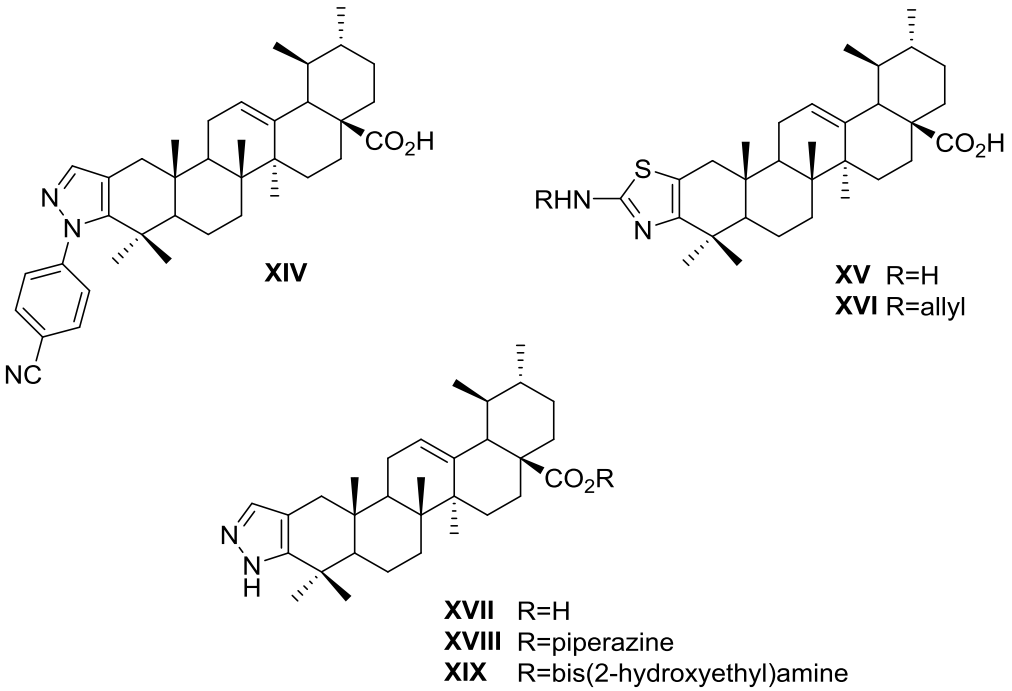


XIII

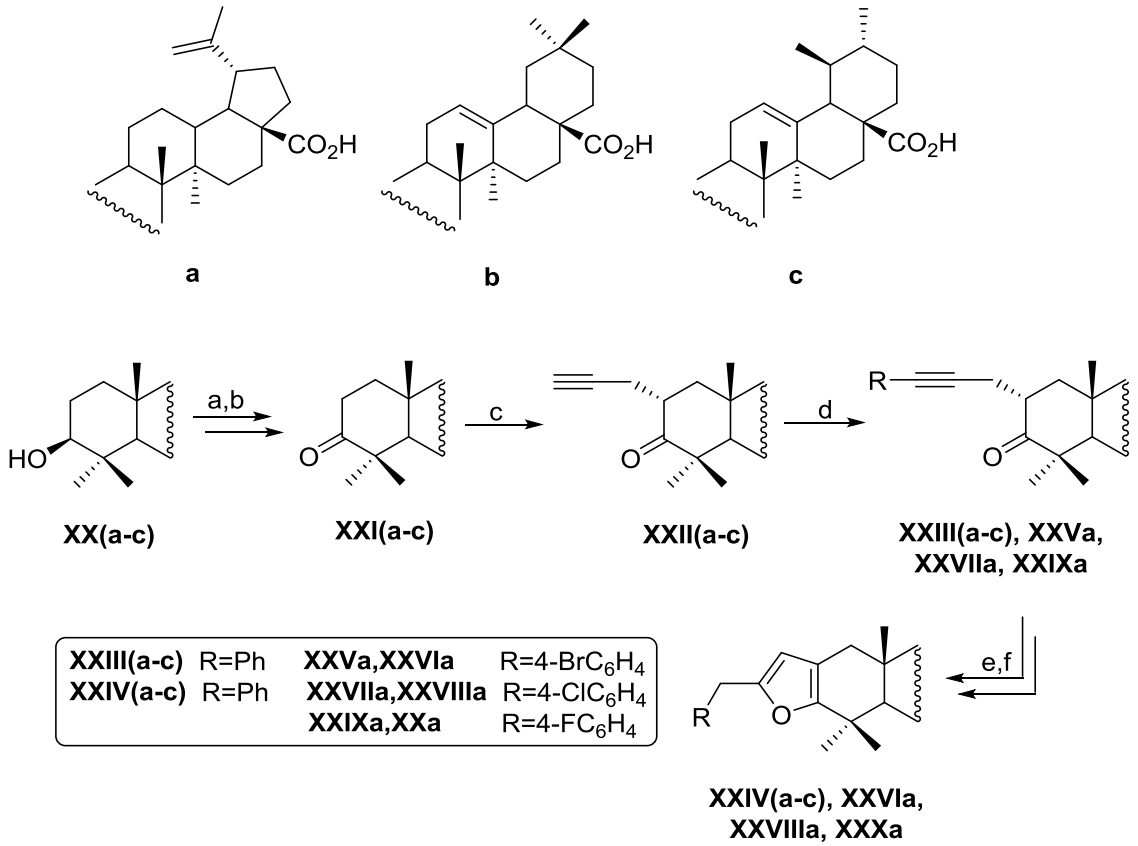
A set of various substituted pyrazoles of ursolic acid was prepared by Sun et al.[109] Among them, derivative **XIV** was found to induce cancer cell death through hyperstimulation of macropinocytosis leading to methuosis, nonapoptic cell death (Figure 8).

Unsubstituted pyrazole derivatives and aminothiazole derivatives fused too the A-ring of ursolic acid were synthesized and pharmacologically evaluated.[55] Both aminothiazoles **XV** and **XVI** had just moderate cytotoxic activity (IC_{50} ~ 40 μM) against human promyelocytic leukemia (HL-60) cell line. On the contrary, pyrazoles **XVII-XIX** had cytotoxicity in low micromolar range (<10 μM) with the best result for amphiphilic pyrazole amid **XVIII** with IC_{50} value of 0.91 μM. Both pyrazoles **XVII** and **XVIII** modulate the apoptotic signaling pathway as well as PI3K/Akt pathway. Moreover, compound **XVIII** can self-assemble into stable and well-defined nanoparticles in an aqueous solution (Figure 8).

Figure 8. Pyrazole (**XIV**, **XVII-XIX**) and aminothiazole (**XV** and **XVI**) derivatives of ursolic acid with biological activities.



An atom-economical synthetic procedure for the preparation of furan-fused pentacyclic triterpenoids was published.[110] Triterpenoids with a terminal or internal triple bond undergo gold-catalyzed cycloisomerization under very mild conditions (Scheme 1). The scope of the method was demonstrated by efficient synthesis of furan-fused triterpenoids containing various aromatic and heteroaromatic functional groups. Only derivatives with free C-28 carboxyl group are presented in the Scheme 1; other derivatives were prepared as methyl esters. Biological activities were not tested.

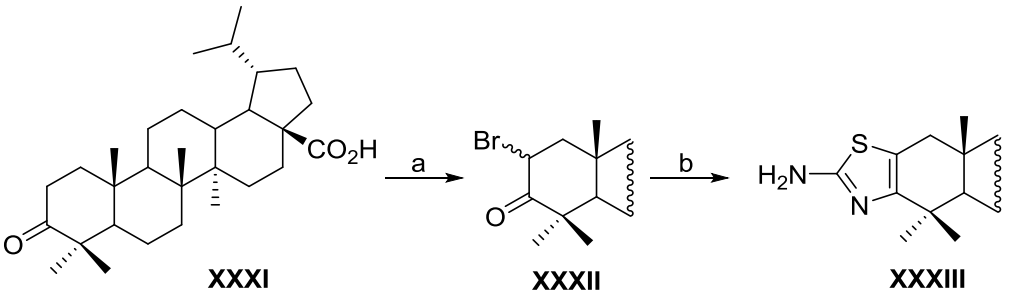


Scheme 1. Novel synthetic procedure for the preparation of furane-fused triterpenoids. Reagents and conditions: (a) CrO_3, H_2SO_4, acetone, or PCC, DCM; (b) CH_2N_2, Et_2O; (c) $KN(SiMe_3)_2$-Et_3B, propargyl bromide, DME; (d) ArI, $PdCl_2(PPh_3)_2$, CuI, TEA, DMF, Ar, 20 °C; (e) $AuCl(PPh_3)_2$/AgOTf, toluene, 20 °C or KHMDS, DME, Ar, 20 °C; (f) LiI, DMF, refl. Ar.

A set of triterpenes modified with a 5-membered ring was synthesized in our research group and among them aminothiazole **XXXIII** (Scheme 2) was the most active on multiple cancer cell lines,[84] which sparked our interest in such compounds.

To further explore structure-activity relationships among triterpenoid aminothiazoles, in this thesis, I chose to add substituents of various size and shape to the NH_2 group. Based on the preliminary results from ongoing pull-down assays it was assumed that many of the potential targets of triterpenoids are enzymes sensitive to lipophilic substrates or membrane proteins that contain large lipophilic surface areas. To improve the ligand-target binding, rather lipophilic substituents were chosen. In order to get more data about the influence of the triterpenic part of the molecule on the cytotoxic activity, a variety of basic triterpenes was chosen as well (lupane, lup-20(29)-ene, oleanane, 18α-oleanane, and ursane). Thus, in the first

part of this thesis, triterpenoids modified with aminothiazoles were prepared and their cytotoxicity was tested. The classical Hantzsch synthesis of aminothiazoles does not give good yields in the case of triterpenoids and steroids because of harsh reaction conditions,[111,112] also the reaction does not allow to simply obtain compounds substituted at the amino group. Therefore, an alternative approach was used involving the cyclization reaction of 3-oxo-2-thiocyanato triterpenoids with various ammonium acetates.[113] Using this method, I was able to synthesize a large variety of substituted aminothiazoles. Moreover, this was the first time that triterpenoid aminothiazoles were prepared using this synthetic procedure.

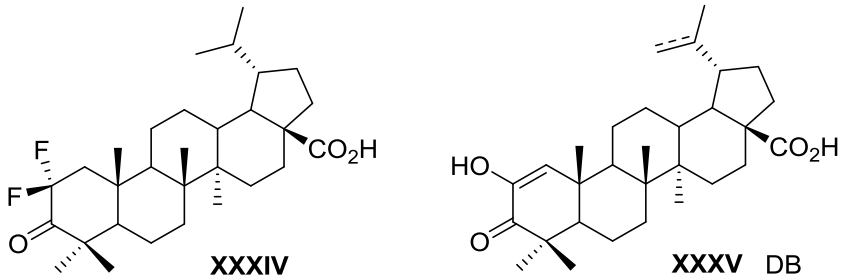


Scheme 2. Synthesis of aminothiazole of dihydrobetulinic acid **XXXIII** - the most active compound with the 5-membered heterocyclic ring prepared in the lit.[84] Reagents and conditions: (a) Br_2, AcOH, NaOAc; (b) thiourea, morpholine, refl.

2.3.2 Lupane derivatives substituted in the position 2 with electron withdrawing groups

Earlier in the research, we have seen a significant trend that introducing an electronegative substituent to the position 2 of lupane skeleton increases cytotoxicity significantly and this works especially well for betulinic acid derivatives. The examples are 2,2-difluoro derivatives of dihydrobetulinic acid, such as **XXXI**,[86] and diosphenols, such as **XXXV** and **XXXVI**,[88,89,114] (Figure 9). Among the derivatives with an electronegative substituent at the position 2, as expected, 2,2-difluoro derivatives were the most cytotoxic. However, their cytotoxicity was not limited to cancer cells only and therefore their further development as drugs was compromised. Their toxicity did not let the compounds to pass into further biological evaluation.[86]

Figure 9. The most active compounds with EWG(s) at C-2 position – difluoro derivative **XXXIV** and diosphenols **XXXV** and **XXXVI**.



XXXIV **XXXV** DB
XXXVI SB

Therefore, one of the subprojects of this work was to further explore triterpenes with other substituent types at the position 2. As the triterpene, dihydrobetulonic acid was chosen because this molecule is significantly cytotoxic and the influence of the substituent would be easily notable. New compounds were prepared *via* nucleophilic substitution of 2-bromo derivatives or by selective nitration of the 2-position in 3-oxocompounds. New derivatives were designed to contain heteroatoms such as nitrogen, sulfur, and oxygen.

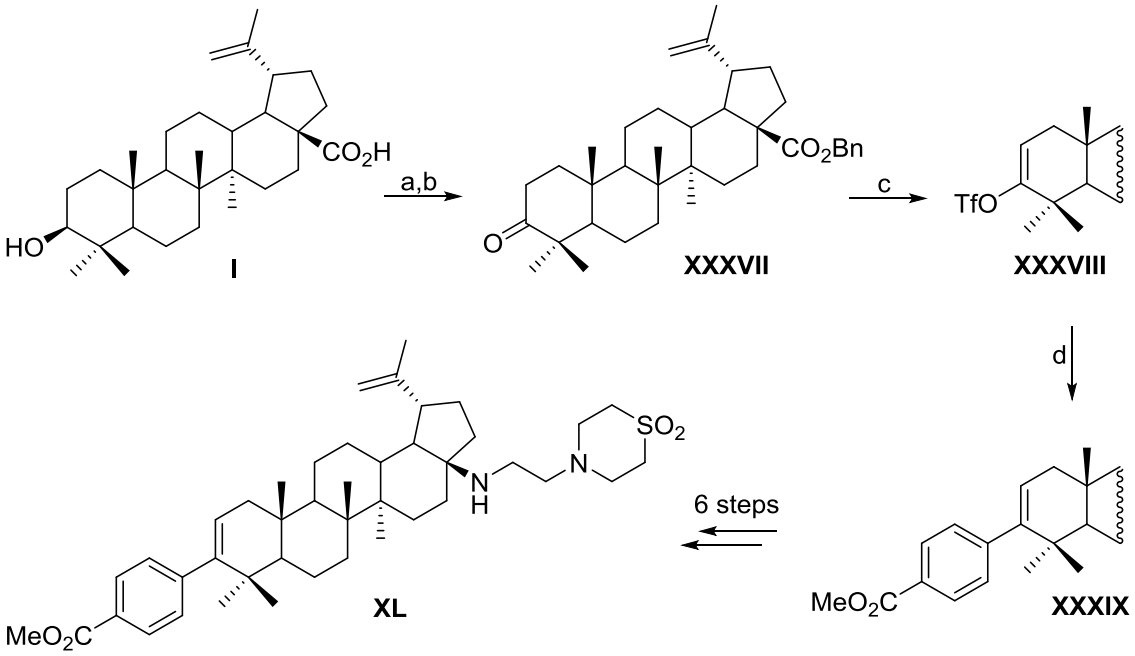
2.3.3 Suzuki-Miyaura cross-coupling on triterpenoids

Suzuki-Miyaura cross-coupling is a modern and well-known method in organic chemistry synthesis generating compounds by forming a new C-C bond.[115–120] Despite that, in the chemistry of triterpenoids there is only one published precedent of the use of this procedure.[121] Since it may open an access to almost unlimited amount of new compounds, a part of this work was focused on the study of the utilization of Suzuki-Miyaura cross-coupling for the preparation of various betulinic acid derivatives substituted at the C-2 or C-3 positions, and on derivatization of allobetulon at C-2 position.

In the lit.,[121] Suzuki-Miyaura cross-coupling reaction was used for the preparation of the second generation of betulinic acid derivatives - HIV inhibitors. Synthesis starts from betulinic acid (**I**) that is in the first step protected as benzyl ester, then oxidized with pyridinium chlorochromate in dichloromethane to benzyl betulonate (**XXXVII**). Ester **XXXVII** reacted with *N*-phenyl-bis(trifluoromethanesulfonimide) and potassium bis(trimethylsilyl)amide in tetrahydrofuran at -78 °C to yield triflate of benzyl betulonate **XXXVIII**. Triflate **XXXVIII** was then used to Suzuki-Miyaura cross-coupling reaction with (4-(methoxycarbonyl)phenyl)boronic acid in presence of sodium carbonate and $Pd(PPh_3)_4$ in

the mixture of solvents 1,4-dioxane, 2-propanol and water to yield methyl ester **XXXIX** in 68 %. Derivative **XL** was than prepared in a sequence of next six reaction steps (Scheme 3).

The main goal of this subproject was to find the appropriate reaction conditions, limitations and scope of Suzuki-Miyaura cross-coupling in the chemistry of triterpenoids and to prepare a small library of new compounds, although at the beginning of such a new project no one is usually able to say if those new derivatives will or will not have any interesting biological activities.



Scheme 3. Preparation of anti-HIV derivative BMS-955176 (**XL**) using Suzuki-Miyaura cross-coupling. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, 60 °C, 3.5 h; (b) PCC, DCM, 6 h; (c) Tf_2NPh, KHMDS, THF, -78°C, 4 h; (d) (4-(methoxycarbonyl)phenyl)boronic acid, Na_2CO_3, $Pd(PPh_3)_4$, 1,4-dioxane, IPO, H_2O, refl., 14.5 h.

3 Aims of the work

The topic of my PhD work was targeted to the modification of the A-ring of several triterpenoids in order to increase their biological activities, primarily anticancer properties. My project may be divided into three main subprojects:

- The first part was focused on the preparation of new heterocycle-containing derivatives of triterpenoids and study of their impact on cancer cells. The goal was to synthesize a library of substituted aminothiazoles fused to the positions C-2 and C-3 of seven selected triterpenoids to get more data about the structure-activity relationships (SARs) within this set of compounds.
- The aim of the second subproject was to confirm or rebut the theory: *The higher electronegativity of the substituent at the C-2 of lupane triterpenoids, the higher cytotoxic activity*. For this purpose, dihydrobetulonic acid – a lupane type derivative - was substituted at the position C-2 with various electronegative substituents and cytotoxic activity of prepared molecules was tested.
- The ambition of the third project was to develop and optimize the conditions for the preparation of new lupane type derivatives with aryl substituents at the positions C-2 or C-3 by Suzuki-Miyaura cross-coupling and test the cytotoxicity of these derivatives.

3.1 Summary of the presented aims

Figure 10. Synthesis of a series of substituted aminothiazoles derived from seven selected triterpenoids.

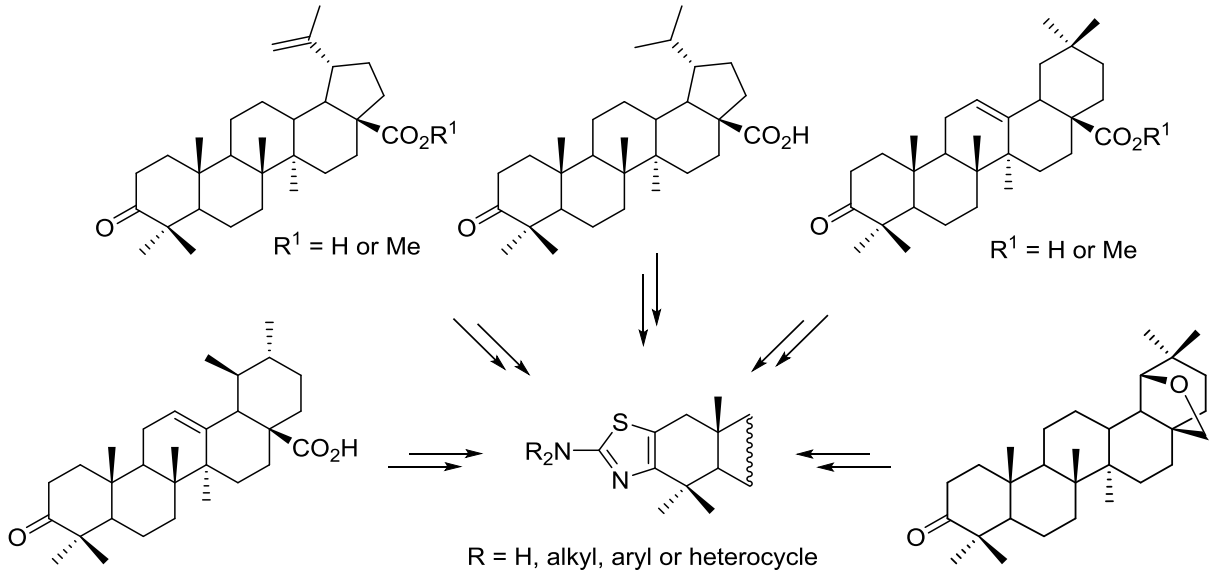


Figure 11. Synthesis of dihydrobetulonic acid derivatives with different electron withdrawing groups (EWGs) at the position C-2.

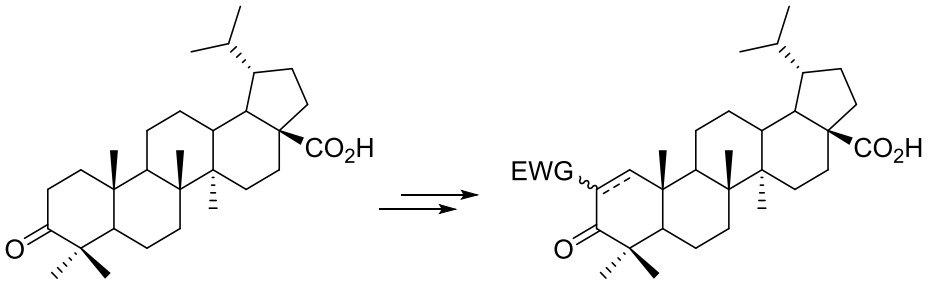
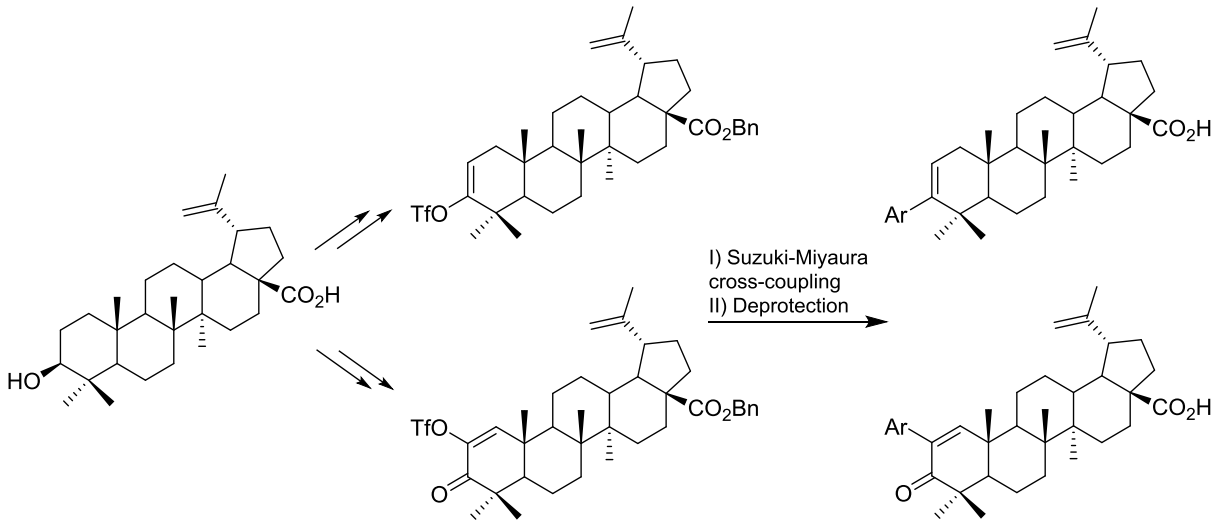


Figure 12. Development of synthesis of aryl-substituted lupane type derivatives applying Suzuki-Miyaura cross-coupling reaction.



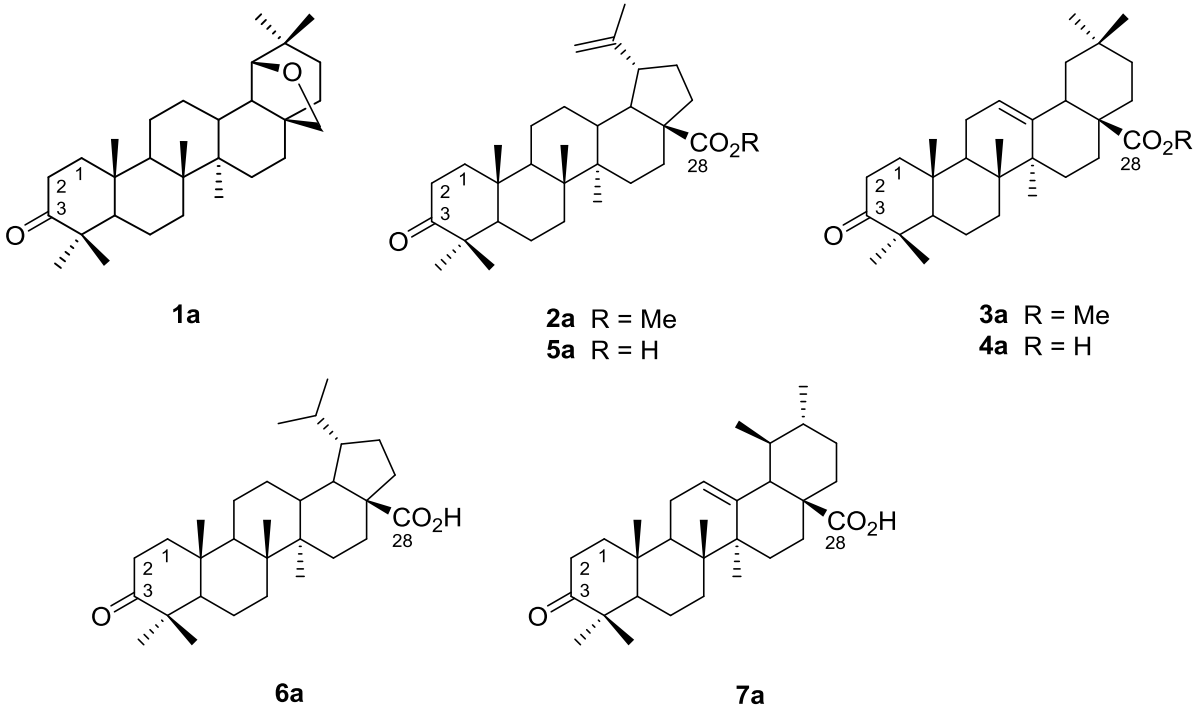
4 Results and Discussion

4.1 Synthesis and anticancer properties of triterpenoid thiazoles

The background of this subproject was described in the chapter *3.3.1 Triterpenoid Thiazoles* (starting page 15).

Four series of *N*-substituted aminothiazoles derived from allobetulon (**1a**), methyl betulonate (**2a**), methyl oleanonate (**3a**), and oleanonic acid (**4a**) were prepared in the first set. After the evaluation of their cytotoxic activity, SAR assumptions were made and another three series of *N*-substituted aminothiazoles derived from betulonic acid (**5a**), dihydrobetulonic acid (**6a**), and ursonic acid (**7a**) were synthesized as the second set. (Figure 13) Altogether, more than 80 compounds including the reaction intermediates were prepared and characterized and their cytotoxic activities were tested.

Figure 13. Structures of the starting 3-oxotriterpenoids **1a**-**7a**.



4.1.1 Synthesis and cytotoxic activity of triterpenoid thiazoles derived from allobetulin, methyl betulonate, methyl oleanonate, and oleanonic acid

This chapter was published in: Borkova, L.; Adamek, R.; Kalina, P.; Drasar, P.; Dzubak, P.; Gurska, S.; Rehulka, J.; Hajduch, M.; Urban, M.; Sarek, J. *ChemMedChem* **2017**, *2017*, 12, 390-398.[122]

In the lit.[84] the aminothiazole derivative **XXXIII** (Scheme 2) was obtained in rather low yield (57 %). The compound contains a free amino and carboxylic group which may form ions and lower its solubility in organic solvents and this causes troubles during the purification step. Here we started with allobetulon (**1a**), methyl betulonate (**2a**), and methyl oleanonate (**3a**) because they do not contain the free carboxylic group and simplier purification and higher yields were expected. Although lower yields during the synthesis were expected, we added free oleanonic acid (**4a**) to this set in order to see if methyl esters (made from **3a**) are less active than analogous free acids (made from **4a**). This is unfortunatelly a common trend in triterpenoids.[11,29,81] Starting compounds **1a**, **2a**, **3a**, and **4a** are derivatives of betulinic acid, oleanolic acid, and 18α-oleanane, these triterpenes are commonly studied for their high cytotoxic activities.[123–126]

4.1.1.1 Synthesis

The starting 3-oxotriterpenoids **2a**, **3a**, and **4a** were brominated by modified procedure with $CuBr_2$ in a mixture of EtOAc, and MeOH at r.t.[127,128] or by modified procedure with Br_2 in $CHCl_3$ at r.t. (for **1a**).[84,129,130] The crude 2-bromo-3-oxoderivatives **1b**, **2b**, **3b**, and **4b** were purified by column chromatography and used for the synthesis of 3-oxo-2-thiocyanato derivatives **1c**, **2c**, **3c**, and **4c** by the nucleophilic substitution of bromine by ammonium thiocyanate in NMP at 50 °C or by potassium thiocyanate in DMSO at 90 °C. 2-thiocyanato-3-oxoderivatives **1c**, **2c**, **3c**, and **4c** were obtained as mixtures of 2α/2β epimers, which were not separated because the final cyclization and aromatization of each epimer leads to the same flat, aromatic system. The structures of compounds **1c** – **4c** was proven by spectral data. In the ^{1}H NMR spectra, 2-bromo-3-oxoderivatives had a characteristic multiplet signal around 5.17 – 5.01 ppm assigned to the H-2 whereas 2-thiocyanato-3-oxoderivatives had a similar characteristic multiplet signal around 4.76 – 4.67 ppm assigned to the H-2. Moreover, a band around 2158 – 2152 cm^{-1} was found in the IR spectra of 2-thiocyanoderivatives assigned to the C≡N bond vibration.

Novel thiazole derivatives **1d** – **1l**, **2d** – **2l**, **3d** – **3l** and **4d** – **4l** were prepared by the procedure similar to the lit.[99] Each thiocyanate was stirred with five equivalents of the corresponding freshly prepared alkyl ammonium acetate at r.t. for 1 to 7 days as needed (Schemes 4-7). The yields of the cyclizations were usually moderate to high (**1d-1l** 76-96 %; **2d-2l** 35-61 %; **3d-3l** 40-90 %; **4d-4l** 9-79 %) depending on the solubility of the starting triterpene and the product in organic solvents used for the reaction, which influenced the work-up procedures and the purification. Lower yields were generally observed at compounds with longer aliphatic substituents (dibutyl- or dioctyl-derivatives) or by cyclohexyl moiety which caused difficulties during the purification. It is worth mentioning that as expected, the yields of free oleanonic acid derivatives (**4d-4l**) were much lower in comparison to methyl oleanonate derivatives (**3d-3l**); compounds with the free carboxylic function were difficult to separate by column chromatography.

The structure of all prepared substituted aminothiazoles was confirmed by spectral data:

^{1}H NMR spectra contained the following signals of the aminothiazoles' substituents:

- multiplet around 3.48 – 3.31 ppm with integral 4 was assigned to the **piperidine moiety**, the other signals of piperidine were in the same area as the signals of skeletal triterpenic hydrogens and were not identified,
- multiplet around 3.64 – 3.22 ppm with integral 4 and multiplet around 2.00 – 1.94 ppm with integral 4 were assigned to the **pyrrolidine moiety**,
- multiplet around 3.51 – 3.32 ppm with integral 4 and multiplet or triplet around 1.26 – 1.16 ppm with integral 6 were assigned to the **diethyl moiety**,
- three multiplets around 3.48 – 3.18 ppm, 1.63 – 1.57 ppm, and 1.36 – 1.31 ppm, all with integral 4, and multiplet or triplet around 0.96 – 0.92 ppm with integral 6 were assigned to the **dibutyl moiety**,
- three multiplets around 3.46 – 3.17 ppm, 1.65 – 1.57 ppm, and 1.31 – 1.26 ppm with integrals 4, 4, 20, and multiplet or triplet around 0.91 – 0.85 ppm with integral 6 were assigned to the **dioctyl moiety**,
- broad singlet around 7.72 ppm with integral 1, multiplet around 7.38 – 7.27 ppm with integral 5 and multiplet around 4.40 – 4.30 ppm with integral 2 were assigned to the **benzylamine moiety**,

- two multiplets around 3.85 – 3.75 ppm and 3.45 – 3.30 ppm, both with integral 4 were assigned to the **morpholine moiety**,

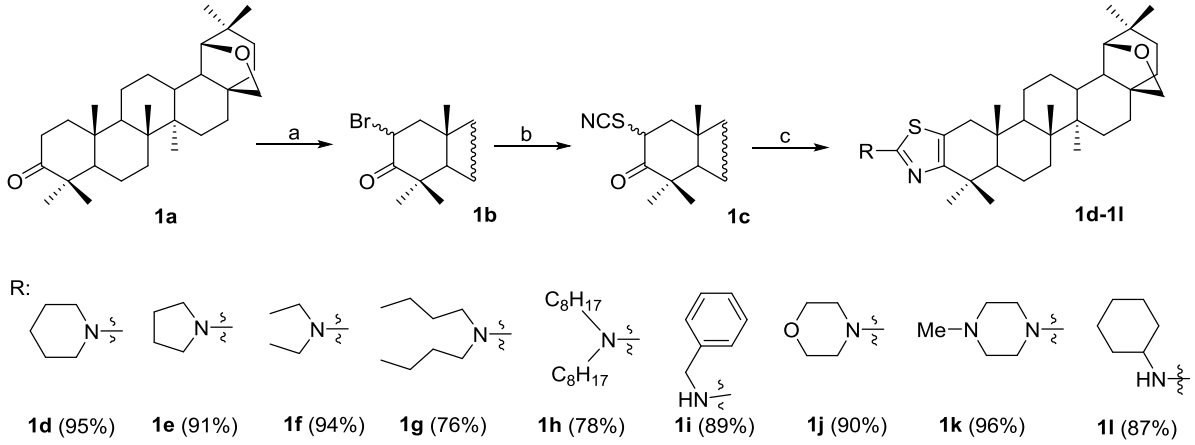
- two multiplets around 3.50 – 3.35 ppm and 2.65 – 2.50 ppm, both with integral 4, and singlet around 2.36 – 2.33 ppm with integral 3 were assigned to the ***N*-methylpiperazine moiety**,

- multiplet around 7.21 – 7.06 ppm with integral 1 and multiplet around 3.06 – 2.97 ppm with integral 1 were assigned to the **cyclohexylamine moiety**, the other signals of cyclohexyl moiety were in the same area as the signals of skeletal triterpenic hydrogens and were not identified.

IR spectra contained the following bands of the aminothiazoles' substituents:

- NH bond vibration around 3520 – 3500 cm^{-1} (benzylamine and cyclohexylamine substituents),

- C-O-C bond vibration around 1101 cm^{-1} (morpholine substituent).

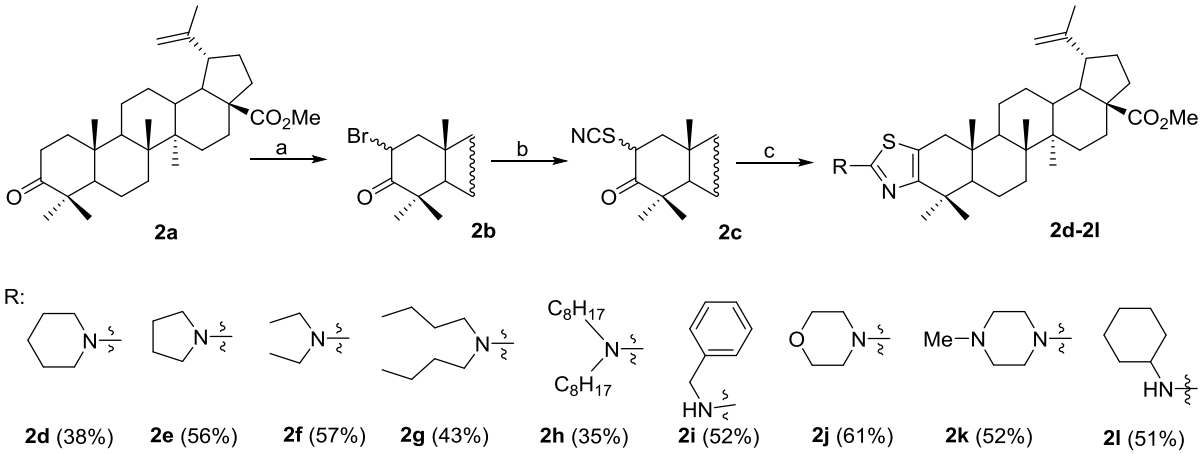


Scheme 4. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t., 30 min; (b) KSCN, DMSO, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 24 hours.

Table 1. Cytotoxic activities of allobetulon derivatives **1a** – **1l** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

	IC_{50} (πM/L)[a]										
Comp.	CCRF-CEM	CEM-DNR	HCT116	HCT116 $p53^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
1a[c]	>50	>50	>50	>50	>50	>50	>50	>50	>50	-	-
1b	**5.2**	>50	21.5	22.8	11.7	**7.5**	>50	47.5	>50	>50	>9.6
1c	43.0	>50	35.6	>50	41.1	>50	>50	>50	>50	>50	>1.2
1d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1f	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
1l	42.8	>50	>50	>50	>50	>50	>50	>50	>50	>50	>1.2

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts. Compounds with IC_{50} > 50 μM are considered inactive. [c]Parent compound used as a standard.

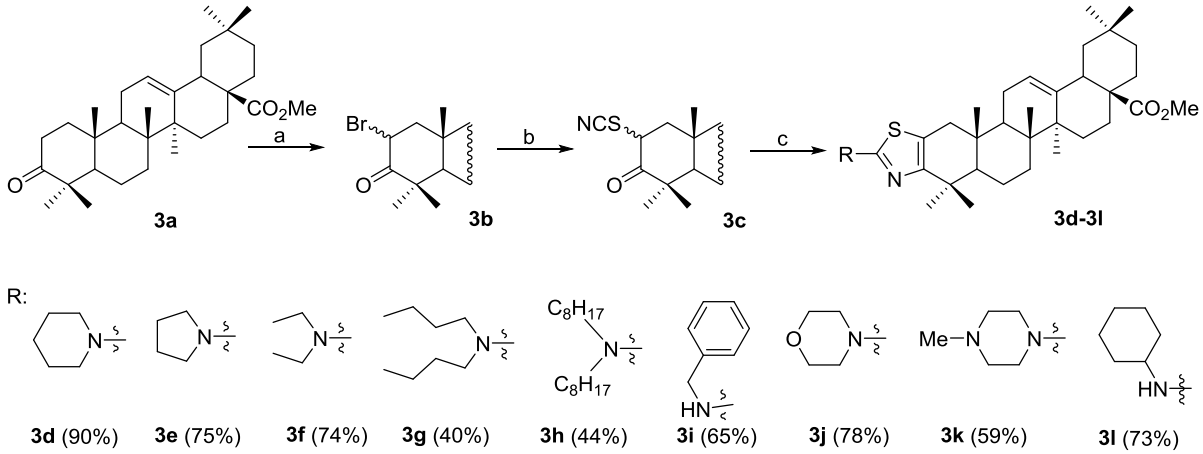


Scheme 5. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) NH_4SCN, NMP, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36 hours.

Table 2. Cytotoxic activities of methyl betulonate derivatives **2a** – **2l** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

	IC_{50} (μM/L)[a]										
Comp.	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
2a[c]	>50	>50	>50	>50	>50	>50	>50	>50	>50	-	-
2b	**2.9**	15.0	14.9	12.4	**9.0**	11.0	>50	13.5	22.9	26.3	8.5
2c	10.8	>50	34.6	29.9	15.5	>50	46.2	>50	32.3	36.1	3.2
2d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2f	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
2l	42.8	>50	>50	>50	>50	>50	>50	>50	>50	>50	-

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts. Compounds with IC_{50} > 50 μM are considered inactive. [c]Parent compound used as a standard.

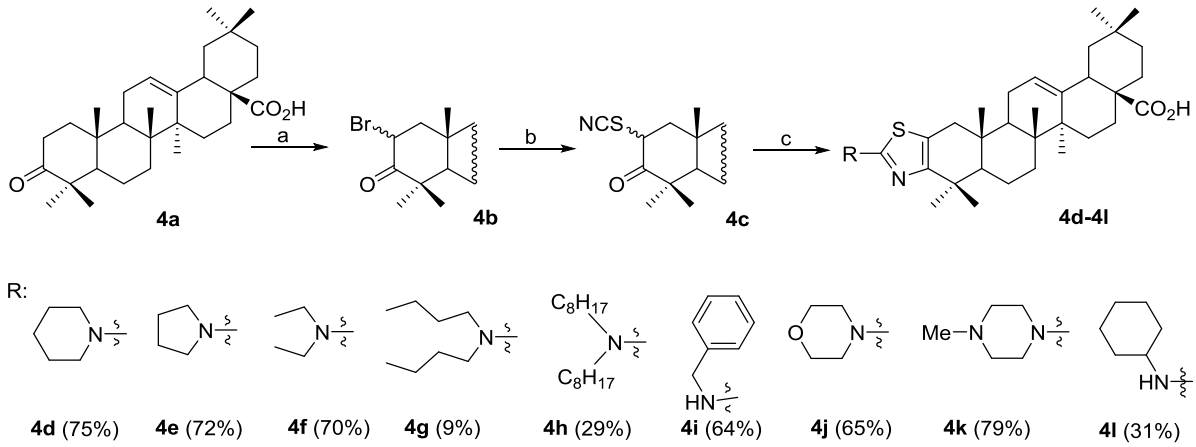


Scheme 6. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 12 h; (b) NH_4SCN, NMP, 50 °C, 6 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36-48 h.

Table 3. Cytotoxic activities of methyl oleanonate derivatives **3a** – **3l** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

	IC_{50} (μM/L)[a]										
Comp.	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
3a[c]	>50	>50	>50	>50	>50	>50	>50	>50	>50	-	-
3b	14.0	22.2	>50	>50	19.9	**7.2**	>50	>50	>50	48.2	>3.5
3c	29.0	48.5	>50	38.5	46.9	>50	>50	47.8	>50	45.7	>1.7
3d	>50	>50	>50	>50	>50	>50	>50	>50	46.5	>50	-
3e	>50	49.6	>50	>50	>50	>50	>50	>50	>50	>50	-
3f	41.8	42.6	44.9	43.6	42.6	42.3	44.2	43.2	>50	42.4	>1.1
3g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
3h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
3i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
3j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
3k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
3l	42.8	>50	>50	>50	>50	32.4	>50	>50	>50	>50	-

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts. [c]Parent compound used as a standard.



Scheme 7. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 12 h; (b) NH_4SCN, NMP, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36-48 h.

Table 4. Cytotoxic activities of oleanonic acid derivatives **4a – 4l** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

	IC_{50} (μM/L)[a]										
Comp.	CCRF-CEM	CEM-DNR	HCT116	HCT116 $p53^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
4a[c]	15.1	17.4	45.1	>50.0	>50.0	21.5	49.5	48.5	>50.0	>50.0	5.3
4b	**4.5**	14.8	**4.1**	**8.2**	**2.1**	13.5	15.8	29.3	27.7	20.0	5.0
4c	**6.4**	31.1	23.6	16.3	28.6	>50	21.7	46.1	>50	48.0	>7.7
4d	>50	>50	>50	>50	>50	>50	>50	>50	46.5	>50	-
4e	>50	49.6	>50	>50	>50	>50	>50	>50	>50	>50	-
4f	**9.7**	>50	>50	48.7	>50	>50	>50	>50	>50	>50	>5.2
4g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
4h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
4i	23.5	>50	>50	>50	>50	>50	>50	>50	>50	>50	>2.1
4j	25.6	48.5	>50	21.1	46.1	>50	>50	48.7	>50	>50	>2.0
4k	11.4	31.1	>50	>50	>50	14.4	>50	>50	>50	>50	>4.4
4l	42.8	>50	>50	>50	>50	14.3	>50	>50	>50	>50	-

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts. [c]Parent compound used as a standard.

4.1.1.2 Biological assays – cytotoxicity

The cytotoxic activity of all synthesized compounds was tested *in vitro* against eight human cancer cell lines of different histogenetic origin and two non-tumor fibroblasts by using the standard MTS test (Tables 1-4).[131] The testing was performed by the Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc (LEM). The cancer cell lines were derived from T-lymphoblastic leukemia (CCRF-CEM), leukemia (K562) and their multi-drug resistant counterparts (CEM-DNR, K562-TAX), solid tumors including lung (A549) and colon (HCT116, HCT116p53-/-) carcinomas, osteosarcoma cell line (U2OS), and for comparison, on two human non-cancer fibroblast cell lines (BJ, MRC-5).[131] In general, the CCRF-CEM line was the most sensitive cancer cell line to the prepared compounds with only a few exceptions. Therefore, SAR assumptions were mostly based on the activities on CCRF-CEM cells.

Among the starting material and intermediates, 2-bromo-3-oxoderivatives **1b**, **2b**, and **4b** were cytotoxic against the CCRF-CEM line in a low micromolar range of 3-5 μM. This is surprising because compound **1b** is a derivative of allobetulon (**1a**), analogues of which are often inactive.[85,87] In addition, the active compound **2b** is a methyl ester and triterpenic methyl esters are also usually not active.[85,87] Even bromo derivative **3b**, also a methyl ester, had a moderate IC_{50} of 14 μM. 2-thiocyanato derivatives **2c** and **4c** had IC_{50} values of 6-10 μM. In contrast, compounds **1c** and **3c** had IC_{50} values in higher micromolar ranges (Tables 1 and 3). The cytotoxicity of most of the substituted aminothiazoles was below the detection limit with two exceptions, **4f** and **4k** with cytotoxicity of 9.7 μM and 11.3 μM (CCRF-CEM), respectively, which were considered enough active to be interesting for further studies towards the mechanism of action. The activity of derivatives **1l**, **3f**, **4i**, **4j**, and **4l** was better than the detection limit; however, not sufficient for more tests. The activity of almost all derivatives with $IC_{50} < 50$ μM on the resistant cell lines CEM-DNR and K562-TAX is worse in comparison to parental cell lines CCRF-CEM and K562 which indicates the possible mechanism of resistance by MDR transporter proteins. To sum up, within this part of the study, 8 compounds (**1b**, **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **4f**, and **4k**) had higher cytotoxic activity than their parent compounds **1a**, **2a**, **3a**, and **4a**.

4.1.1.3 *Biological assays – analysis of apoptosis, cell cycle, and DNA/RNA synthesis*

All promising compounds with an IC_{50} below or around 10 μM in CCRF-CEM cell line (**1b**, **2b**, **2c**, **4b**, **4c**, and **4f**) were further investigated for their mechanisms of action at LEM. The analysis of apoptosis, cell cycle, and DNA and RNA synthesis in CCRF-CEM cells at 1 × or 5 × IC_{50} were measured (Table 5).[132,133]

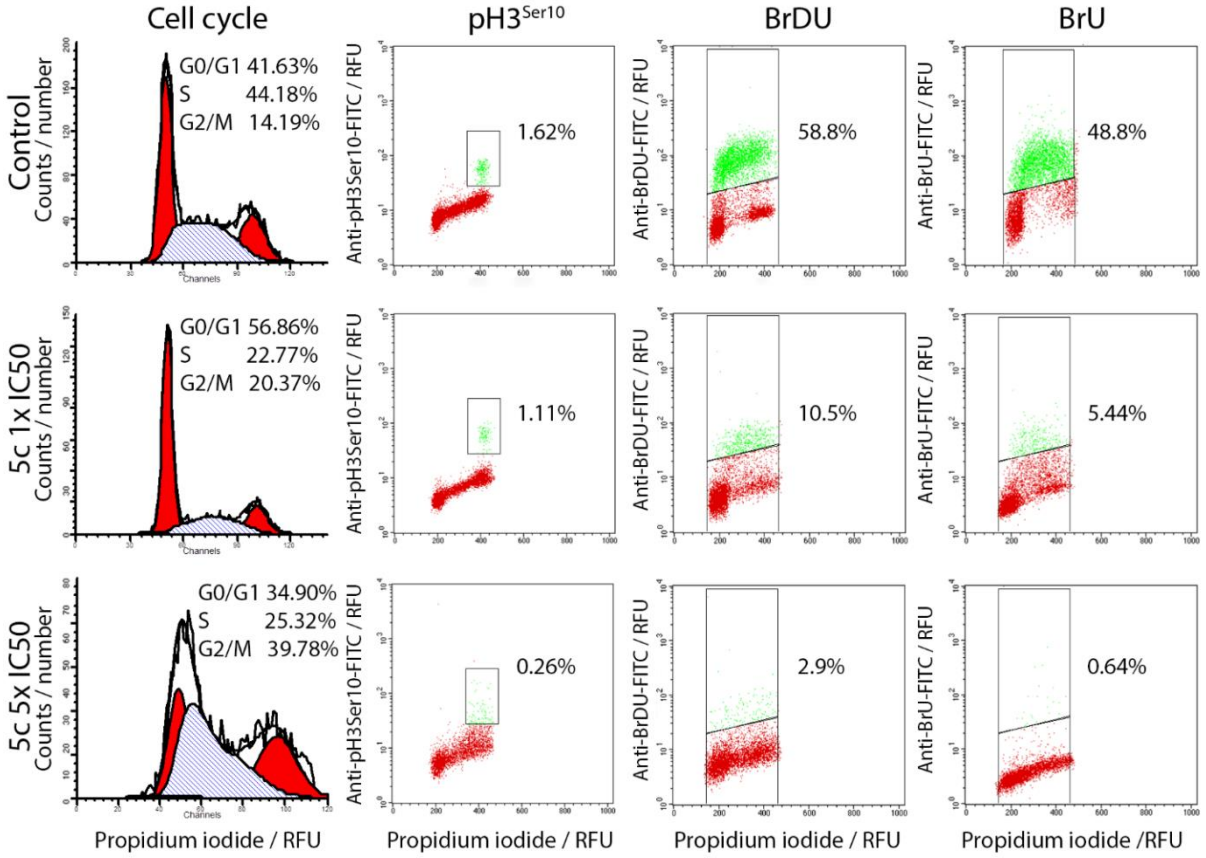
Highly active compound **4c** led to the accumulation of cells in G2 phase. Nucleic acid synthesis was almost completely inhibited by **4c** at both tested concentrations (Figure 14), pointing to a possible mechanism of action somewhere within the regulatory mechanisms of cell cycle proliferation. Since **4c** had no toxicity on fibroblasts, is the most promising candidate for further cell biology studies and drug development. Similar phenomenon (inhibition of DNA/RNA synthesis) but at higher concentrations (5 × IC_{50}) was detected for compounds **2b** and **2c**; however, the mechanical proposal is more difficult in this case. Other tested derivatives had no significant effect on the cell cycle and DNA/RNA synthesis.

Table 5. Influence of **1b**, **2b**, **2c**, **4b**, **4c**, and **4f** on cell cycle and DNA and RNA synthesis inhibition at 1 × and 5 × IC_{50}.

	Used conc. (μM)	Sub G1 (%)	G0/G1 (%)	S (%)	G2/M (%)	$pH3^{Ser10}$ (%)	DNA synthesis	RNA synthesis
Control		3.38	41.63	44.18	14.19	1.62	58.76	48.80
1b	5.2[a]	5.84	44.78	40.95	14.27	0.99	52.18	41.37
1b	21.2[b]	43.57	44.30	37.92	17.77	1.74	37.24	31.77
2b	2.9[a]	7.76	40.23	38.24	21.53	2.79	25.98	49.31
2b	14.5[b]	32.49	45.58	31.06	23.35	2.44	**13.34**	**1.37**
2c	10.8[a]	3.42	36.14	47.92	15.93	2.09	41.05	47.46
2c	54.0[b]	58.50	30.90	41.29	27.81	1.02	**13.82**	**0.97**
3b	4.5[a]	5.96	42.73	42.51	14.77	1.11	61.70	53.54
3b	22.5[b]	57.69	45.40	39.88	14.71	1.55	36.20	24.60
3c	6.4[a]	41.55	56.86	22.77	**20.37**	1.11	**10.52**	**5.44**
3c	32.0[b]	83.11	34.90	25.32	**39.78**	0.26	**2.86**	**0.64**
3f	9.7[a]	4.27	36.59	48.14	15.27	1.18	40.16	67.11
3f	48.5[b]	7.79	40.35	49.43	10.22	1.87	32.95	58.95

[a]The values were obtained at 1 × IC_{50}. [b]The values were obtained at 5 × IC_{50}. Control are cells treated with vehicle.

Figure 14. Graphs and dot plots of flow cytometry analysis are showing the cell cycle inhibition in G2/M phase and almost complete DNA, RNA synthesis inhibition by the best compound **4c**, monitored by incorporation of BrDU/BrU into the DNA.



In this sub-chapter, 44 triterpenoid derivatives were synthesized, and their cytotoxicity was evaluated followed by more advanced biological experiments in the most promising compounds. 42 of the compounds were not published before. From the 3-oxocompounds **1a**, **2a**, **3a**, and **4a**, 2-bromo-3-oxoderivatives, 2-thiocyanato-3-oxoderivatives, and substituted aminothiazoles were prepared. Nine different types of substituents at the amino group were used in order to evaluate their influence on the cytotoxicity. Substituents were chosen rather lipophilic, because we expected to improve interactions of the terpenes with lipophilic areas on the potential protein targets. The most active compounds were the intermediate 2-bromo-3-oxoderivatives **1b**, **2b**, **4b**, and thiocyanates **2c**, and **4c**. Although the majority of the final aminothiazoles were inactive, there were two exceptions, **4f** (diethylamin substituent) and **4k** (*N*-methylpiperazine substituent), with cytotoxicity around 10 μM (CCRF-CEM). Both compounds are derivatives of oleanonic acid with the free 28-carboxyl groups which led us to the future direction of this research on aminothiazoles. New compounds should be more

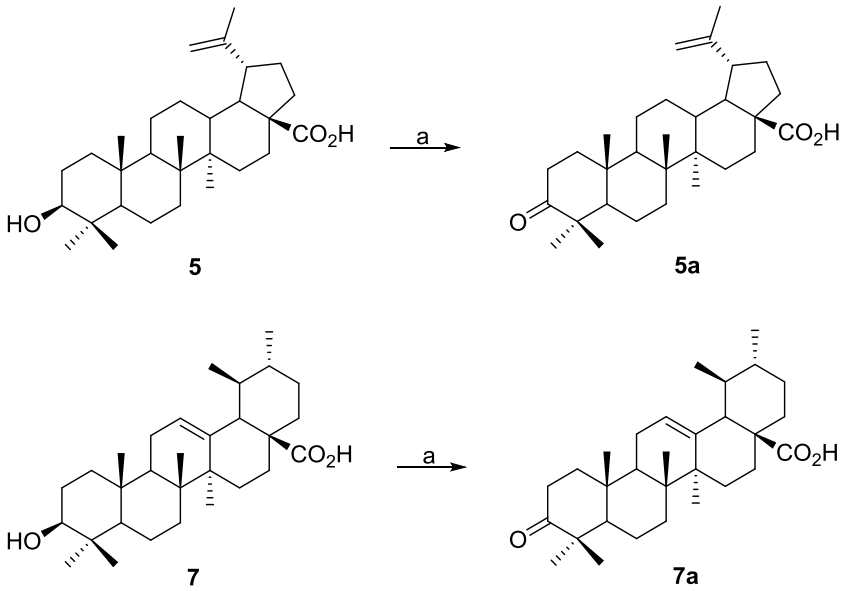
oriented towards the free triterpenic acids despite the lower yields from the synthetic procedures.

4.1.2 Synthesis and cytotoxic activity of triterpenoid thiazoles derived from free betulinic acid, dihydrobetulinic acid, and ursonic acid

The results presented in the previous chapter showed that compounds with the free carboxyl group (C-28) had higher cytotoxic activity compared to the corresponding methyl ester derivatives. Therefore, a second set of triterpenoid thiazoles derived from three free triterpenoid acids: betulinic acid (**5**), dihydrobetulonic acid (**6a**), and ursolic acid (**7**) was synthesized. The starting compounds and their derivatives are more and more often studied for their high cytotoxic activities.[63,134–140] The experimental set up from the first project was preserved in order to be able to combine the results from the both sets and select several most promising compounds for the advanced biological tests. The same reaction procedures as well as the same substituents on the amino group of the aminothiazole part were used. To finalize both sets, I additionally prepared three unsubstituted aminothiazoles, analogous to the compound **XXXIII** made from the other basic triterpenes.

4.1.2.1 Synthesis

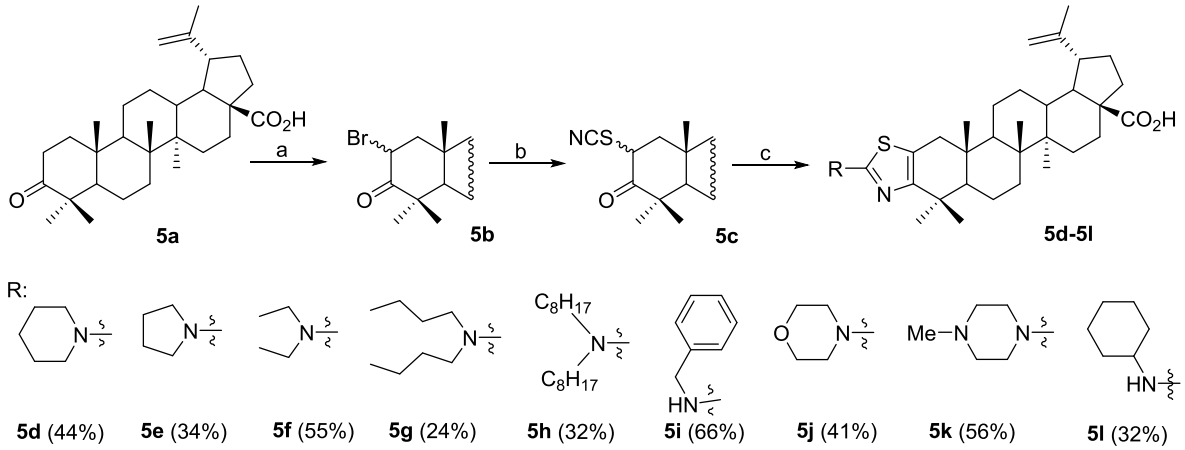
First, betulinic acid (**5**) and ursolic acid (**7**) were converted into 3-oxoderivatives **5a** and **7a** using sodium dichromate, sodium acetate and a mixture of solvents 1,3-dioxane, acetic acid and acetic anhydride at r.t. (Scheme 8).[84] Then, betulonic acid (**5a**) and ursonic acid (**7a**) were brominated by copper(II) bromide in a mixture of ethyl acetate and methanol at r.t., similar introduction of bromine into the position C-2 was used in the lit.[55,96], while dihydrobetulonic acid (**6a**) was brominated by bromine solution in chloroform at r.t. under modified conditions.[84] Bromoketones **5b**, **6b**, and **7b** were purified by column chromatography and used as mixtures of α- and β-epimers to the next reaction step. A nucleophilic substitution of bromoketones **5b**, **6b**, and **7b** by potassium thiocyanate in DMSO at 90 °C led to the thiocyanato derivatives **5c**, **6c**, and **7c** which were after purification used to the cyclization reaction which was similar to the reaction used in the lit.[99] Analogous signals in the NMR spectra and bands in the IR spectra to those described in the previous chapter confirmed the structures of 2-bromo-3-oxoderivatives and 2-thiocyanato-3-oxoderivatives.



Scheme 8. Preparation of the starting compounds from commercially available triterpenic acids. Reagents and conditions: (a) $Na_2Cr_2O_7.2H_2O$, AcONa, 1,3-dioxane, AcOH, Ac_2O, r.t., 18 h.

Compounds **5c**, **6c**, and **7c** reacted with freshly prepared alkyl ammonium acetates in chloroform at r.t. for one to seven days to give substituted aminothiazoles **5d-5l**, **6d-6l**, and **7d-7l** which were purified by column chromatography (Schemes 9-11). Aminothiazoles derived from betulinic acid **5d-5l** were prepared in 24-66 % yield. Aminothiazoles derived from dihydrobetulinic acid **6d-6l** were obtained in 8-94 % yield. Aminothiazoles prepared from ursolic acid **7d-7l** were synthesized in 15-64 % yield. The lower yields were most often caused by the difficult purification during which some parts of the products kept remaining on silica gel and were just slowly washed out. These troubles were probably caused by the combination of the free carboxylic group and the amino group, as was predicted. In addition, some difficulties with respect to the substituents were also observed. Longer aliphatic chains, such as dibutyl or dioctyl, and cyclohexylamine moiety were partly responsible for problems during purification. Since the compounds for biological tests must be of the highest purity, sometimes multiple column chromatography was needed which often was done on account to low yields. Neither HPLC purification nor crystallization led to sufficiently pure compounds in a single step and had to be repeated.

The spectral characterization of the prepared substituted aminothiazoles was analogous to the characterization described in the previous chapter. Analogous ^{1}H NMR signals and IR bands of appropriate substituents were observed.

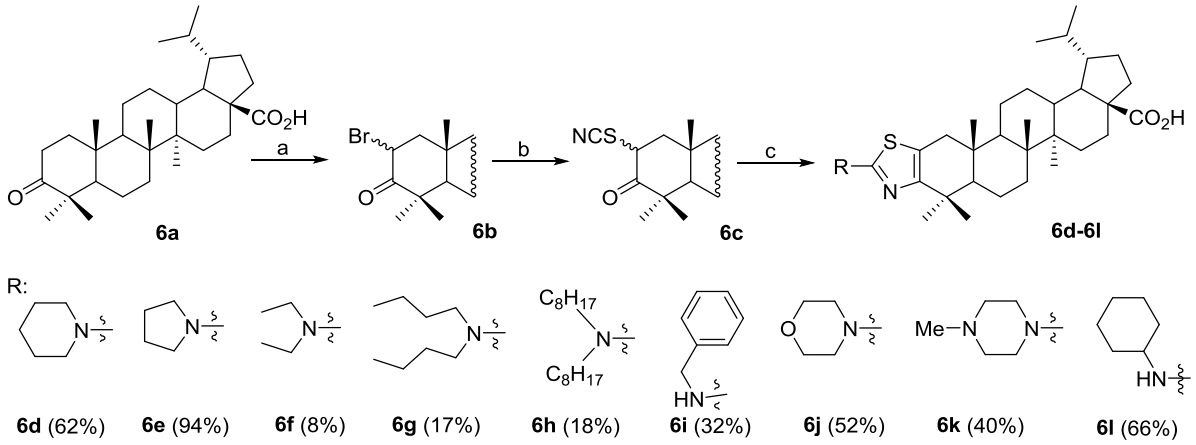


Scheme 9. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 3-7 days.

Table 6. Cytotoxic activities of betulinic acid derivatives **5a-5l** on eight tumor (including resistant) cell lines and two normal fibroblast cell lines.

Comp.	IC_{50} (μM)[a]										
	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
5a[c]	**8.4**	11.2	38.1	49.4	13.6	14.9	40.0	38.0	39.5	29.5	4.1
5b	**3.0**	17.9	31.1	32.7	35.5	23.5	24.0	23.4	33.5	20.4	9.0
5c	**2.4**	**6.8**	**3.3**	11.9	28.5	16.6	21.2	20.2	19.8	18.7	8.0
5d	**9.3**	>50	>50	>50	35.9	>50	>50	>50	>50	50	>5.4
5e	**7.0**	>50	>50	>50	>50	22.9	>50	>50	>50	>50	>7.1
5f	**8.5**	>50	>50	31.6	29.5	46.8	>50	>50	>50	23.31	>4.3
5g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
5h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
5i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
5j	**7.2**	>50	>50	>50	11.2	>50	>50	>50	>50	>50	>6.9
5k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
5l	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts (BJ and MRC-5). [c]Parent compound used as a standard.

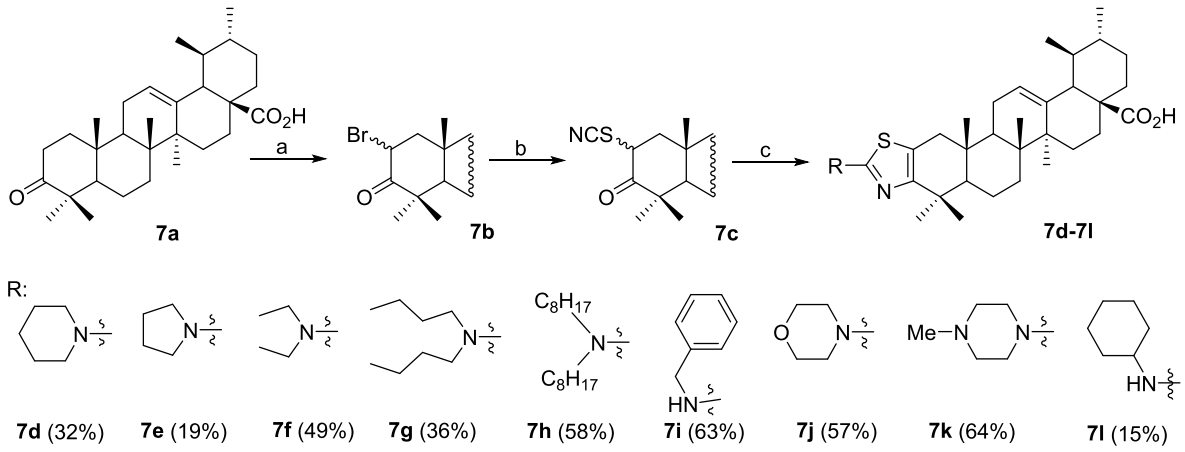


Scheme 10. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t. 30 min; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 1-7 days.

Table 7. Cytotoxic activities of dihydrobetulinic acid derivatives **6a-6l** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

Comp.	IC_{50} (μM)[a]										
	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
6a[c]	**6.2**	48.3	33.5	36.4	11.6	11.1	22.2	27.5	>50	>50	>8.1
6b	**3.1**	15.6	**1.0**	**5.2**	**0.7**	**5.1**	15.2	10.0	21.8	**8.0**	4.8
6c	**0.9**	14.5	**3.5**	**3.4**	**3.4**	**5.4**	12.5	15.1	**5.8**	**4.6**	5.8
6d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6e	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6f	19.7	>50	>50	>50	16.3	>50	>50	45.78	>50	>50	>2.5
6g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
6l	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts (BJ and MRC-5). [c]Parent compound used as a standard.



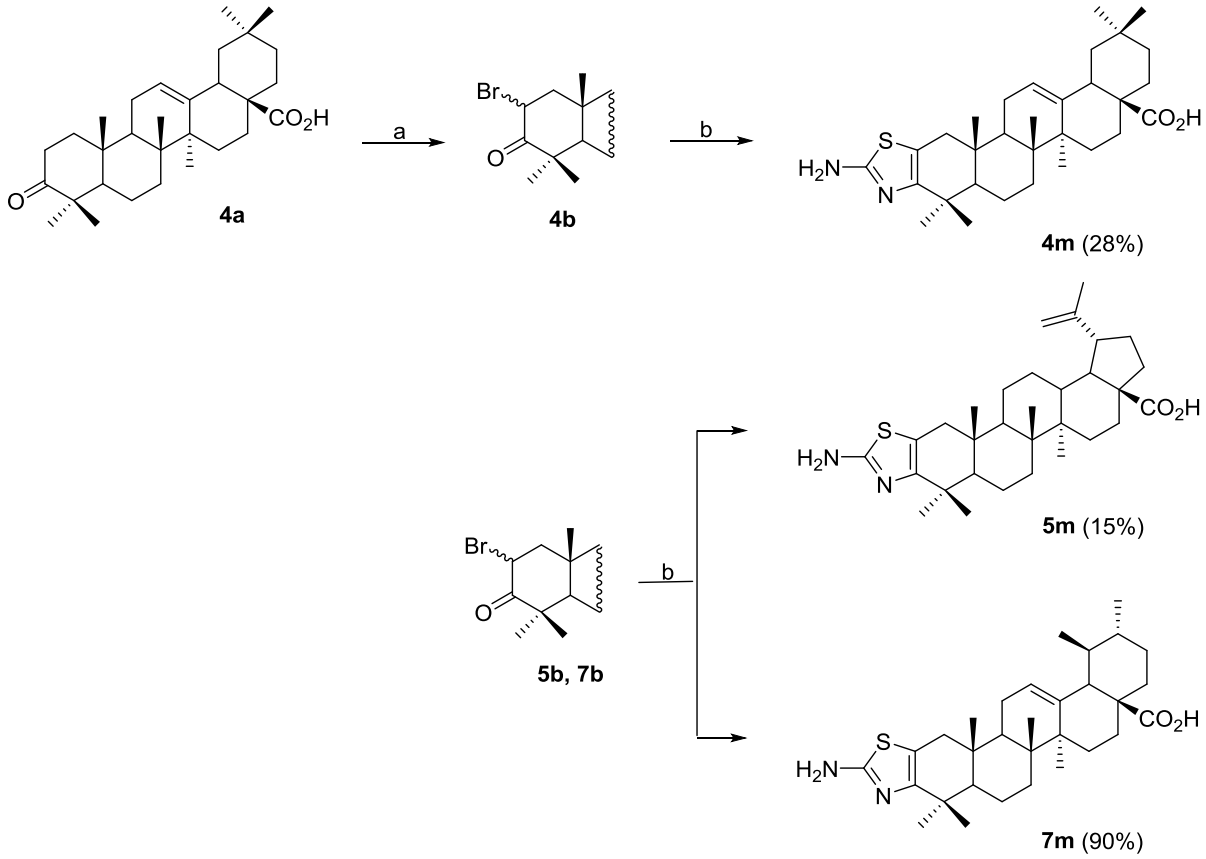
Scheme 11. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 4-7 days.

Table 8. Cytotoxic activities of ursolic acid derivatives **7a-7l** on eight tumor (including resistant) cell lines and two normal fibroblast cell lines.

Comp.	IC_{50} (μM)[a]										
	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
7a[c]	<50[d]	34.0	49.7	>50	>50	35.5	>50	>50	>50	>50	/
7b	**4.7**	28.7	31.5	32.3	33.3	25.0	32.5	29.7	>50	32.1	>8.7
7c	**4.7**	28.2	32.1	32.3	34.7	29.0	42.5	33.1	>50	39.8	>9.6
7d	<50 [d]	>50	>50	>50	>50	>50	>50	>50	>50	>50	/
7e	<50 [d]	39.0	39.0	>50	>50	37.5	>50	>50	>50	>50	/
7f	<50 [d]	>50	41.3	>50	>50	>50	>50	>50	>50	>50	/
7g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
7h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
7i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	-
7j	**8.5**	23.8	23.6	37.9	<50 [d]	29.7	28.8	29.1	>50	>50	>5.9
7k	47.6	>50	>50	>50	>50	>50	>50	>50	>50	>50	1.1
7l	<50 [d]	>50	>50	>50	>50	>50	>50	>50	>50	>50	/

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts (BJ and MRC-5). [c]Parent compound used as a standard. [d]Specific results are not available.

A set of three unsubstituted aminothiazoles, analogous to the compound **XXXIII**, were synthesized to compare the influence of the triterpenic part of the molecule on the cytotoxicity. Starting from oleanonic acid (**4a**), 2-bromoketone (**4b**) was prepared by the reaction with copper(II) bromide in a mixture of solvents ethyl acetate and methanol at r.t. Intermediate **4b** along with other bromoketones **5b** and **7b** were used for cyclization with thiourea in ethanol at r.t. The aminothiazoles **4m**, **5m**, and **7m** were formed after two to seven days according to the reactivity of the substrate (Scheme 12). NMR experiments of **4m** and **5m** were measured in DMSO-d_6 which allowed us to see in the ^{1}H NMR spectrum the characteristic broad singlet around 12.07 ppm assigned to the COOH group and the singlet around 6.61 with integral 2 assigned to the NH_2 group.



Scheme 12. Aminothiazoles with the free NH_2 group derived from oleanonic acid (**4a**), betulonic acid (**5a**), and ursonic acid (**7a**). Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 12 h; (b) thiourea, EtOH, r.t., 2-7 days.

Table 9. Cytotoxic activities of free prepared aminothiazoles **4m**, **5m**, and **7m** and known aminothiazole **XXXIII** on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

Comp.	IC_{50} (μM)[a]										
	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
XXXIII	**3.5**	11.2	**5.1**	**4.3**	**4.8**	**6.9**	**7.0**	/	24.9	15.7	5.8
4m	**4.6**	14.9	26.2	>50	>50	20.5	>50	>50	>50	>50	>11
5m	**7.9**	13.3	36.9	49.7	/	15.4	28.8	50	50	50	6.3
7m	19.0	29.5	43.0	>50	>50	33.1	>50	49.6	>50	>50	>2.6

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts (BJ and MRC-5).

4.1.2.2 Biological assays – cytotoxicity

All compounds prepared in this study were tested *in vitro* for their cytotoxic activity against eight human cancer cell lines and two non-cancer fibroblasts at LEM. The compounds were tested in triplicates by standard MTS assay. CCRF-CEM cell line is the most chemosensitive cell line on the panel and therefore it was used for the SARs assumptions (Tables 6-9).

Similar trends were observed as for the first set.[122] Bromoketones **5b**, **6b**, and **7b** together with thiocyanato ketones **5c**, **6c**, and **7c** were the most active compounds with cytotoxicity in the range 0.9 μM (thiocynato derivative **6c**) to 4.7 μM (derivatives **7b** and **7c**) on CCRF-CEM cell line. Their therapeutic index ranged from 4.8 to >9.6 (thiocyanato derivative of ursonic acid **7c**). On the other hand, a vast number of final products with the free carboxylic group were moderately cytotoxic. The highest number of active aminothiazoles was derived from ursolic acid (**7**); six derivatives **7d**, **7e**, **7f**, **7j**, **7k**, and **7l** had cytotoxic activity below 50 μM on CCRF-CEM with the best result of IC_{50} 8.5 μM for the derivative **7j** (morpholine substituent). Four substituted aminothiazoles derived from betulinic acid **5d**, **5e**, **5f**, and **5j** had IC_{50} in range 7.0 to 9.3 μM (CCRF-CEM); other compounds were inactive. Only one derivative of dihydrobetulonic acid **6f** had cytotoxicity below the detection limit (19.7 μM on CCRF-CEM). Free aminothiazoles **4m** and **5m** (derivatives of oleanonic acid

and betulonic acid) were active in the same range as dihydrobetulonic acid derivative **XXXIII** (4.6 μM and 7.9 μM, resp. compare to 3.5 μM on CCRF-CEM); however, they were less toxic on healthy cells and therefore their therapeutic index showed higher selectivity compared to **XXXIII** (>11 and 6.3, resp.; compare to 5.8). Aminothiazole **7m** (derivative of ursolic acid) had only moderate cytotoxicity (19.0 μM on CCRF-CEM) and low selectivity (>2.6).

4.1.2.3 Biological assays – pharmacological parameters

The most active compounds from both series (IC_{50} < 5 μM) were also subjected to the tests of pharmacological parameters at LEM. The intermediates **5c**, **6c**, **7b**, and **7c** as well as the prepared unsubstituted aminothiazoles (**4m** and **5m**) together with compound **XXXIII** were tested for their stability, plasma protein binding, and membrane permeability *in vitro* (Table 10).

Isothiocyanates **5c**, **6c**, and **7c** would be interesting because of their low IC_{50} values, compound **6c** is the most cytotoxic from all of them with the IC_{50} on CCRF-CEM cells in submicromolar range; however, they were found unstable in stock solution, in plasma, and in microsome; therefore, they are not suitable for further drug development and had to be abandoned. In addition, some of them were toxic on non-cancer cells.

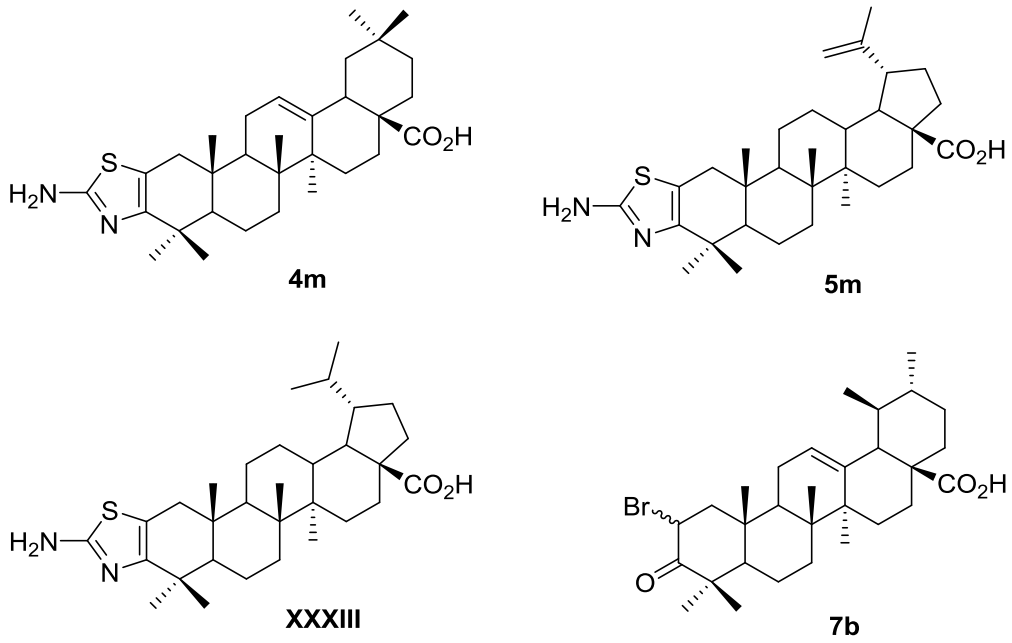
Compound **4m**, aminothiazole of oleanolic acid is stable but it seems that it does not pass through the cellular membranes well (in PAMPA models). Despite that, the compound deserves more evaluation, because there are many other mechanisms how cells transport the molecules into them. Compounds **XXXIII**, **5m** and **7b** are the best compounds of the series, they are active enough, they are stable enough, and they penetrate through the cellular membranes well. All of them bind to plasma proteins which is similar to steroids and, at this point, it is not a hurdle.

Compounds **4m**, **XXXIII**, **5m** and **7b** (Figure 15) are cytotoxic, selective and have appropriate pharmacological parameters (*in vitro*); therefore, they were selected for the future *in vivo* ADME-Tox evaluatiuon on mice. If they pass this part of tests, they will be subjeceted to *in vivo* anti-tumor testing.

Table 10. Pharmacological parameters of the unsubstituted aminothiazoles **XXXIII**, **4m**, and **5m** and intermediates **5c**, **6c**, **7b**, and **7c**. Data were provided by Dr. Lišková from LEM.

Compound	Metabolism							Plasma protein binding	Permeability	
	Chemical stability		Plasma stability		Microsomal stability			Rapid equilibrium dialysis	PAMPA	
	Time (min)	Comp. remaining (%)	Time (min)	Comp. remaining (%)	Time (min)	Comp. remaining (%)	Intrinsic clearence	Fraction bound (%)	log Pe	Category
XXXIII	0	100	0	100	0	100	Low	98,5	-5,340	Medium
	15	99	15	99	15	98				
	30	96	30	100	30	93				
	60	80	60	99	60	91				
	120	76	120	100						
4m	0	100	0	100	0	100	Low	98,9	-7,162	Low
	15	91	15	99	15	97				
	30	77	30	99	30	90				
	60	75	60	99	60	87				
	120	75	120	99						
5c	0	100	0	100	0	100	Medium	11,8	-5,245	Medium
	15	100	15	99	15	90				
	30	67	30	76	30	86				
	60	54	60	36	60	57				
	120	50	120	8						
5m	0	100	0	100	0	100	Medium	95,8	-5,338	Medium
	15	100	15	99	15	31				
	30	91	30	99	30	26				
	60	91	60	98	60	26				
	120	87	120	98						
6c	0	100	0	100	0	100	High	7,0	-6,295	Low
	15	100	15	99	15	63				
	30	99	30	95	30	35				
	60	91	60	57	60	2				
	120	70	120	14						
7b	0	100	0	100	0	100	Medium	82,4	-4,934	Medium
	15	100	15	98	15	93				
	30	99	30	98	30	81				
	60	99	60	95	60	66				
	120	89	120	86						
7c	0	100	0	100	0	100	High	28,5	-6,519	Low
	15	90	15	99	15	61				
	30	49	30	100	30	55				
	60	37	60	77	60	15				
	120	15	120	37						

Figure 15. The most promising derivatives from this study are aminothiazoles **4m**, **5m**, and **XXXIII** and 2-bromo-3-oxoderivative **7b**.



To sum up, 82 compounds were prepared in the thiazole subproject. In general, the most active compounds were the intermediate bromoketones and thiocyanato ketones, unsubstituted aminothiazoles with the free amino group and the free C-28 carboxylic group, and some *N*-substituted aminothiazoles with the free C-28 carboxylic group. The highest therapeutic index was calculated for aminothiazole **4m** (TI >11). The most active intermediates (**5c**, **6c**, **7b**, and **7c**) as well as the prepared unsubstituted aminothiazoles (**4m** and **5m**) together with compound **XXXIII** were chosen for the further biological screening. Their stability and membrane permeability are being tested *in vitro*. A manuscript summarizing this project is in preparation.

The main results of this part of the research are:

A) Important trends useful for SAR assumptions were found:

(1) The free C-28 carboxylic group of triterpenoids is esential for the activity.

(2) The intermediates with -SCN and -Br substituent at the C-2 were the most active but some of them were toxic on non-cancer cells, especially those with –SCN at C-2.

(3) The aminothiazole derivatives with polar substituents (such as morpholine) and especially the derivatives with the free amino group were more active than the aminothiazoles

with non-polar substituents. This disproved our original theory that the lipophilic substituents would increase the activity.

(4) The highest number of active compounds was among derivatives of betulinic acid (**5**) and ursolic acid (**7**), in this order, compare to the derivatives of other triterpenoids.

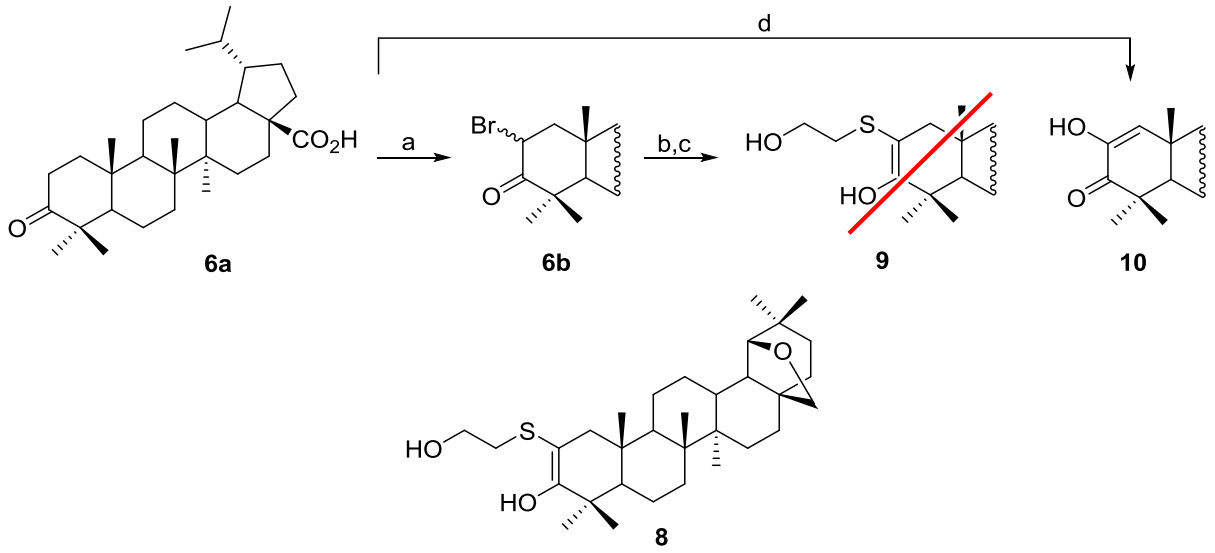
B) Most importantly, four compounds of this series (**4m**, **5m**, **XXXIII**, and **7b**; Figure 15) had high and selective cytotoxicity against cancer cell lines and favorable pharmacological properties. Therefore, they were selected for the future *in vivo* evaluations of the ADME-TOX and then for the evaluations of the anti-cancer properties. These compounds have the highest potential to become new anti-cancer drugs from all compounds synthesized here.

4.2 Lupane derivatives substituted in the position 2, their cytotoxicity and influence on cancer cells

The background of this subproject was described in the chapter *3.3.2 Lupane derivatives substituted in the position 2 with electron withdrawing groups* (starting page 20).

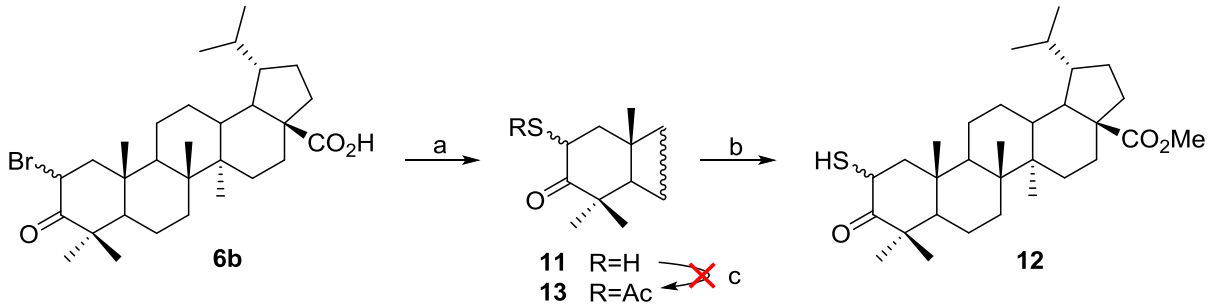
4.2.1 Synthesis of sulfur containing derivatives

Earlier, compound **8** was prepared[141] in our research group. It is, however allobetulon **1a** derivative and the cytotoxic activity was below the detection limit which is common for allobetulon **1a** derivatives. Therefore, one of the task in this work was to prepare its analogue from dihydrobetulonic acid (**6a**).[84] Derivatives of **6a** are usually more cytotoxic than derivatives of **1a**. Dihydrobetulonic acid (**6a**) was brominated with one equivalent of Br_2 in chloroform to yield epimeric mixture of 2α/β-bromo-dihydrobetulonic acid (**6b**). Nucleophilic substitution of the bromoketone **6b** with mercaptoethanol under various reaction conditions was investigated including the conditions from.[141] The most promising procedure with 1 eq. of NaOH, and 10 eq. of mercaptoethanol in anhydrous ethanol gave a mixture of three compounds among which I expected the desired 2-hydroxyethylsulfanylderivatibe **9** to be present (Scheme 13). However, the only isolable product was known diosphenol **10**.[114] The other two compounds were repeatedly lost during the column chromatography or HPLC, probably due to decomposition. Formation of **10** is similar as for many attempts to substitute bromine atom in 2-bromo-3-oxoterpenes described in the lit.[84] Diosphenol **20b** is usually being prepared directly by the oxidation of dihydrobetuolonic acid (**2b**) with air, in a solution of potassium *tert*-butanolate in *tert*-butanol at 40 °C (Scheme 13).[89,142]



Scheme 13. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t.; (b) mercaptoethanol (1 eq.), NaOH, EtOH (anh.), 0 °C to r.t.; (c) mercaptoethanol (used as cosolvent), NaOH, EtOH (anh.), 0 °C to r.t; (d) air, *t*-BuOK, *t*-BuOH, 40 °C.

The reaction of bromoketone **6b** with sodium sulfide in *N*-methylpyrrolidone at r.t. gave sulfanylderivative **11** which was lost while chromatographed (the compound probably stayed on silica gel) but when **11** was methylated by diazomethane before the HPLC chromatography, it was possible to obtain and characterize it as methyl ester **12** (Scheme 14). The structure of **12** was confirmed by LC-HRMS and other spectral data. During the following attempts for the synthesis of larger amounts it was found that both derivatives **11** and **12** succumb to slow decomposition while being purified on silica gel column or on HPLC. An attempt to prepare more stable acetyl sulfide of dihydrobetulonic acid **13** led to decomposition of the starting compound **11** (Scheme 14). This part of the project was then abandoned.

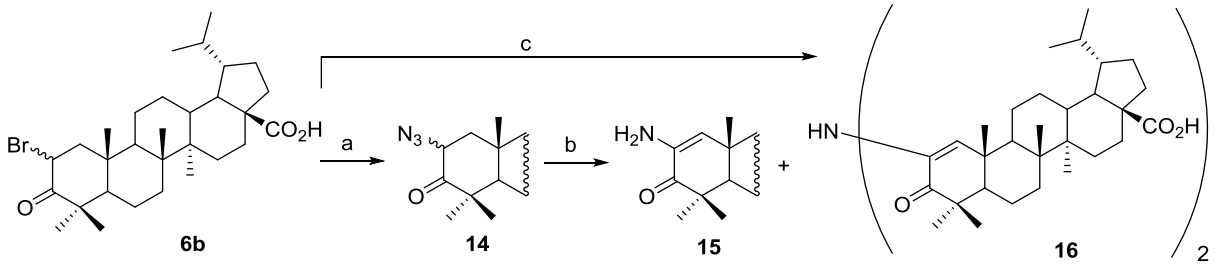


Scheme 14. Reagents and conditions: (a) Na_2S, N-methylpyrolidone, r.t.; (b) CH_2N_2, Et_2O, $CHCl_3$, r.t.; (c) Ac_2O, AcOH, r.t.

4.2.2 Synthesis of nitrogen containing derivatives

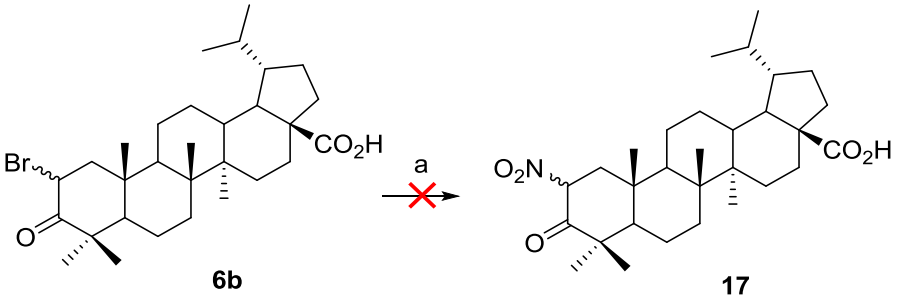
The reaction of bromoketone **6b** with sodium azide was performed in *N*-methylpyrrolidone catalyzed by acetic acid according to a precedent from steroid chemistry[143] (Scheme 15). An attempt to purify the crude azido acid **14** on a silica gel column failed due to fast decomposition which yielded yellow enaminoketone **15** that spontaneously dimerizes to imine **16** the same way as it was described for analogous betulinic acid derivative in the lit.[144] (Scheme 15). The decomposition could be easily fastened by adding silica gel, triphenylphosphine or by heating. Finally, the sufficiently pure 2α/β-azidodihydrobetulonic acid (**14**) was obtained when the reaction mixture was poured to a tenfold volume of water; the precipitate was filtered on a frit (S4), washed with a small portion of water, dried on a vacuum line and recrystalized from chloroform/methanol under nitrogen flow. The structure was confirmed by spectral data, most importantly, in the ^{1}H NMR spectrum of **14**, there is a doublet of doublets of H-2α from 2β isomer at 4.29 ppm and doublet of doublets of H-2β from 2α isomer at 4.25 ppm (according to them, the stereomers are in ratio around 60:40); most importantly, in the IR spectrum of **14** the band at 2100 cm^{-1} proves the presence of the -N_3 moiety.

Unlike azide **14**, both enaminoketone **15** and dimer **16** were stable enough and I was able to isolate and characterize them as a mixture without any problems. In the ^{1}H NMR spectrum of **15** and **16** a broad singlet at 11.42 ppm with integral 2 was assigned to the NH_2 group, singlet at 6.55 ppm with integral 3 was assigned to the H-1, H-1', and NH of the dimer, singlet at 6.14 ppm with integral 1 was assigned to the H-1of the monomer. In the IR spectrum of **15** and **16** bands at 3337 cm^{-1} and 1675 cm^{-1} were found and assigned to the NH bond, resp. C=C bond vibration. Moreover, compounds **15** and **16** were prepared directly and faster when a reaction of bromoketone **6b** with sodium azide was stirred in a solution of DMSO containing a drop of H_2SO_4 at 70 °C (Scheme 15).



Scheme 15. Reagents and conditions: (a) NaN_3, NMP, AcOH, r.t.; (b) spontaneous decomposition at r.t., increased by silica gel, Ph_3P, heating etc.; (c) NaN_3, DMSO, H_2SO_4, 70 °C.

A series of optimization reactions with sodium nitrite was tried to find the best reaction conditions for preparation of 2-nitrodihydrobetulonic acid (**17**) by nucleophilic substitution. Bromoketone **6b** reacted with sodium nitrite (5eq.) in DMSO without any additive or with floroglucinole or with tetrabutylammonium chloride at r.t. or at 50 °C. The starting material was consumed in all cases; however, all reactions led to complicated mixtures with usually four to six spots on the TLC plate, which were difficult to separate and purify (Scheme 16). Therefore, I decided to do the direct nitration of dihydrobetulonic acid (**6a**) with nitric acid according to the method that was described by Shernyukov et al.[28] who used it to nitrate allobetulon (**1a**).

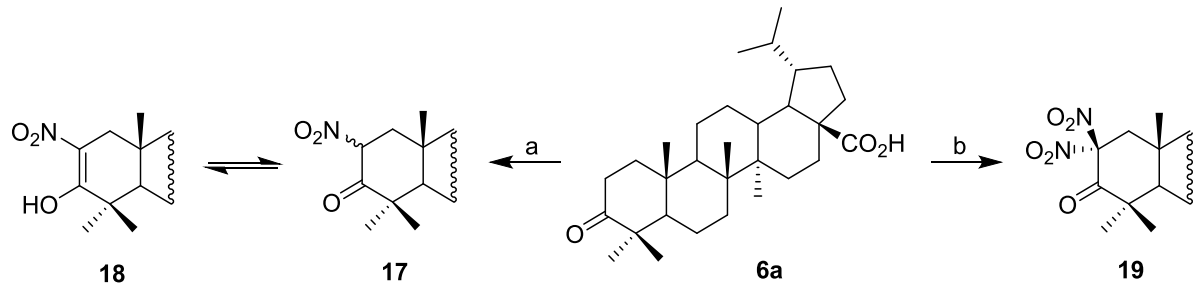


Scheme 16. (a) $NaNO_2$, no additive or floroglucinole or TBAC, DMSO, r.t. or 50 °C, 3 - 24 h.

When the reaction procedure from the lit.[28] was followed (compound **6a** was dissolved by heating in AcOH and treated with 57% nitric acid at 25 ºC for 24 h), the starting material stayed unreacted. Next time, I have used 67% nitric acid while other reaction conditions were not changed (Scheme 17). Mononitroderivative **17** was identified as the only spot on the TLC after 25 hours with some impurities at the start; however, after work-up, it was obtained in rather low yield (23 %). Part of the starting material **6a** probably decomposed during the

reactin. The compound **17** was characterized as the enol **18**. In the ^{1}H NMR spectrum of **18** the doublet at 2.84 ppm with integral 1 and coupling constants 15.5 Hz was assigned to the H-1a, the doublet at 1.98 ppm with integral 1 and coupling constant 15.5 Hz was assigned to the H-1b. In the IR spectrum of **18** the band at 1607 cm^{-1} was found and assigned to the C=C bond vibration. This was in agreement with the spectrum of analogous compound (2-nitroallobetulon) published in the lit.[28] It is worth to mention, that in general, 3-oxotriterpenes with an electronegative substituent at C-2 often occur in their enol-forms.

2,2-dinitrodihydrobetulonic acid (**19**) was the main product when the same reaction mixture was heated to 35 ºC for 6 h (Scheme 17). The structure of compound **19** was confirmed by spectral data, especially in the ^{1}H NMR spectrum of **19** the doublets of H-1a at 3.10 ppm and H-1b at 2.93 ppm with integral 1 and the coupling constant 16.0 Hz prove that there is no hydrogen in the position 2 and instead, electronegative substituents were introduced, two nitro groups in this case. Final proof of the structure was obtained from LC-HRMS.



Scheme 17. Reagents and conditions: (a) 67% HNO_3, AcOH, 25 °C, 25 h; (b) 67% HNO_3, AcOH, 35 °C, 6 h.

The reaction of bromoketone **6b** with sodium cyanide in DMSO gave 2-cyanodihydrobetulonic acid (**20**) which was characterized in its enol-form **21**, as it was described in the lit.[141] (Scheme 18). Reduction of compound **20** was then performed by lithium aluminum hydride.

When cyanoketone **20** in THF was stirred and heated under reflux with $LiAlH_4$ which was added as powder, the starting material was consumed in 30 min to give the crude 2α/β-(aminomethylene) dihydrobetulinic acid (**22**, Scheme 18). The work-up procedure and isolation of the product, however, caused many troubles because of the presence of insoluble aluminum salts. Moreover, the purification of **22** was very difficult because the product kept remaining on the silica gel and was just slowly washed out by methanol. These troubles were

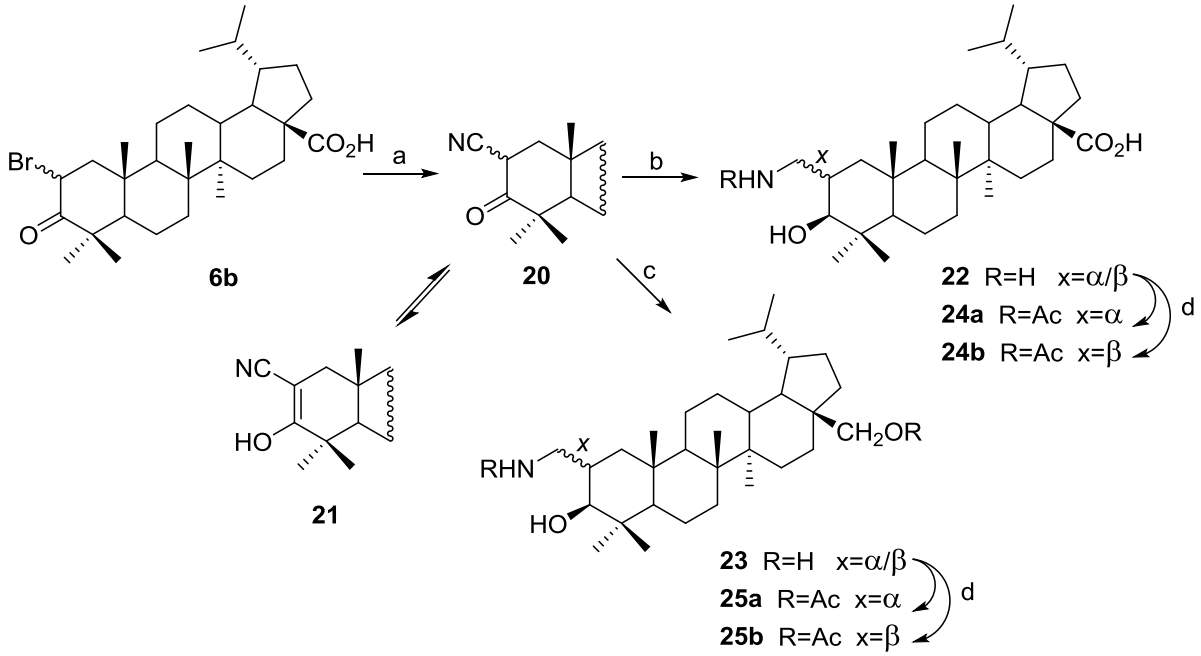
very likely caused by the combination of the free amino group along with the free carboxylic group in one molecule as mentioned earlier in this thesis. Even though the compound was poorly soluble in organic solvents, small amount of the epimeric mixture **22** was anyways collected. Acetylation of the crude amino alcohol **22** gave the epimeric mixture of 2-acetamidomethylenederivatives **24a** and **24b** which were then separated and characterized as α-epimer **24a** and β-epimer **24b** (Scheme 18).

The structure of the α-epimer **24a** was confirmed by the characterictic signals in the ^{1}H NMR spectrum measured in DMSO-d_6; the broad singlet at 11.97 ppm was assigned to the COOH, the doublet of doublets at 7.65 ppm was assigned to the NH, the singlet at 4.28 ppm was assigned to the OH, the doublet of doublets at 3.60 ppm with the coupling constant 13.8 Hz and 7.6 Hz was assigned to the H-3, the doublet of doublets at 2.90 ppm with the coupling constant 13.6 Hz and 3.0 Hz was assigned to the H-2, multiplet at 2.12 – 2.19 ppm with integral 2 was assigned to the H-31, and singlet at 1.83 with integral 3 was assigned to the methyl of CH_3CON- group. The presence of NH group was also confirmed by IR spectrum as the associated NH stretch with bands at 3441 cm^{-1} and 3334 cm^{-1}. The remaining COOH group was confirmed by the associated envelope of the carboxylic OH stretch at 3500 – 2500 cm^{-1} and the band of carbonyl stretch at 1707 cm^{-1}.

The structure of β-epimer **24b** was confirmed by the ^{1}H NMR spectrum in $CDCl_3$; the doublet of doublets at 5.72 ppm was assigned to the NH, the doublet of doublets at 3.86 ppm with the coupling constant 2.8 Hz was assigned to the H-3, multiplet at 3.18 – 3.14 ppm was assigned to the H-2, multiplet at 2.27 – 2.18 ppm was assigned to the H-31, and the singlet at 2.03 with integral 3 was assigned to the acetate. The presence of NH and COOH groups was confirmed also in this case by IR spectrum with bands at 3411, 3280, 3500 – 2800, and 1702 cm^{-1}. In this reaction set-up, the carboxylic group at C-28 remained unreduced. The purity and the structure were also proved by LC-HRMS.

Much better results were obtained, when cyanoketone **20** reacted with 1M-solution of $LiAlH_4$ in THF (Scheme 18). An epimeric mixture of dihydroxyamine **23** formed after heating the reaction mixture under reflux for 2 h which was clear from the TLC. Product **23** was unfortunately almost insoluble in organic solvents and all attempts for its purification (crystallization, chromatography on silica gel) failed. Therefore, the reaction was repeated and the crude 2α/β-(aminomethylene)-3β,28β-dihydroxylupane **23** was acetylated to form the

separable mixture of the α- and β-epimers **25a** and **25b**, respectively (Scheme 18). Both acetamides were fully characterized.



Scheme 18. Reagents and conditions: (a) NaCN, DMSO, r.t. 24 h; (b) $LiAlH_4$, THF, refl. 30 min, (c) $LiAlH_4$.THF, THF, refl., 2 h; (d) Ac_2O, pyridine, r.t., 4 h (for **24a**, **24b**) or 6.5 h (for **25a**, **25b**).

The structure of acetyl 2α-(acetamidomethylen)-3β,28-dihydroxylupane (**25a**) was confirmed by these characterictic signals in ^{1}H NMR spectrum: mutiplet at 5.96 – 5.90 ppm was assigned to the NH, doublet at 4.23 ppm with coupling constant 10.8 Hz was assigned to the H-28a, multiplet at 3.87 – 3.77 ppm with integral two was assigned to the H-31a and H-28b, doublet of doublets at 3.16 ppm with coupling constant 13.9 Hz and 2.5 Hz was assigned to the H-31b, broad singlet at 2.79 ppm was assigned to the OH, two singlets at 2.05 ppm and 2.02 ppm, both with integral 3, were assigned to the CH_3COO- and CH_3CON- group, respectively.

The structure of the 2β-(acetamidomethylen)-3β,28-dihydroxylupane (**25b**) was confirmed by these characterictic signals in ^{1}H NMR spectrum: doublet of doublets at 5.59 ppm with the coupling constant 8.3 Hz and 4.4 Hz was assigned to the NH, doublet at 4.24 ppm with coupling constant 11.1 Hz was assigned to the H-28a, broad singlet at 4.17 ppm was assigned to the OH, multiplet at 3.97 – 3.86 ppm with was assigned to the H-31a, multiplet at 3.86 – 3.77 ppm with with was assigned to the H-28b, doublet of triplets at 3.15 ppm with coupling constant 13.8 Hz and 3.5 Hz was assigned to the H-31b, two singlets at

2.05 ppm and 2.02 ppm, both with integral 3, were assigned to the CH_3COO- and CH_3CON-group, respectively. Unlike previously, in this case, carboxylic group at C-28 was reduced to alcohol as expected.

4.2.3 Biological assays – cytotoxicity

Cytotoxic activity of all synthesized compounds and stable intermediates was investigated *in vitro* against CCRF-CEM cell line using the standard MTS test (Table 11). The testing was performed at LEM. All derivatives with IC_{50} below 50 μM were further examined on seven cancer cell lines and two human non-cancer fibroblasts (Table 12). From this set of derivatives, containing a substituent at the position C-2 of triterpenic skeleton, most compounds showed moderate to low cytotoxicity with the best IC_{50} value of 10.1 μM on myelogenous leukemia cell line for the 2-nitroderivative **17**. Only the compounds **6b**, **10** whose biological properties are already known from the lit.[84,114] as well as the starting dihydrobetulonic acid (**6a**) had cytotoxicity in low micromolar range on T-lymphoblastic leukemia cell line.

Table 11. Cytotoxic activity of prepared compounds against the acute T-lymphoblastic leukemia CCRF-CEM cell line.

Comp.	IC_{50} (μmol/L) [a] CCRF-CEM	Comp.	IC_{50} (μmol/L) [a] CCRF-CEM	Comp.	IC_{50} (μmol/L) [a] CCRF-CEM	Comp.	IC_{50} (μmol/L) [a] CCRF-CEM
6a[b]	6	**14**	18	**19**	29	**25a**	17
6b	3	**15**[c]	27	**20**	10	**25b**	25
10	6	**16**[c]	27	**24a**	18		
12	45	**17**	32	**24b**	9		

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Parent compound used as a standard. [c]Compounds were tested as a mixture.

Table 12. Cytotoxic activities of prepared compounds on eight tumor cell lines (including resistant) and two normal fibroblast cell lines.

Comp.	IC_{50} (μM/L)[a]										
	CCRF-CEM	CEM-DNR	K562	K562-TAX	A549	HCT116	HCT116p53$^{-/-}$	U2OS	BJ	MRC-5	TI[b]
6b	**3.1**	15.6	**1.0**	**5.2**	**0.7**	**5.1**	15.2	10.0	21.8	**8.0**	4.8
10	5.8	<50[d]	<50[d]	<50[d]	<50[d]	<50[d]	<50[d]	<50[d]	>50	>50	>8.6
12	45.3	>50	>50	>50	>50	>50	>50	>50	>50	>50	>1.1
14	17.5	16.0	14.2	10.6	25.7	>50	>50	48.9	>50	>50	>2.9
15[c]	26.6	33.9	>50	20.1	>50	>50	>50	>50	>50	>50	>1.9
16[c]	26.6	33.9	>50	20.1	>50	>50	>50	>50	>50	>50	>1.9
17	31.8	36.1	10.1	20.6	31.3	>50	>50	>50	>50	>50	>1.6
19	28.6	46.6	>50	29.9	>50	>50	>50	>50	>50	>50	>1.7
20	10.1	33.7	**5.8**	15.1	14.4	**10.0**	10.6	31.2	15.5	**2.6**	0.9
24a	18.3	40.4	43.3	17.6	>50	>50	>50	>50	>50	>50	>2.7
24b	**8.5**	17.2	12.5	**7.8**	>50	>50	>50	50.0	>50	>50	>6.0
25a	17.3	27.0	19.2	50.0	26.7	27.3	26.2	24.5	32.4	29.6	1.8
25b	24.8	45.1	23.8	14.0	27.9	26.8	27.3	22.9	30.1	30.5	1.2

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts [c]Compounds were tested as a mixture. [d]Specific results are not available.

Some of the prepared triterpenoid derivatives from this subproject are highly cytotoxic. Unfortunately, selectivity towards cancer cell lines was lower than needed, compounds were too toxic against non-cancer cells and therefore they did not pass further into the *in vivo* anticancer tests.

To sum up, I have prepared a set of 12 new triterpenoid compounds to supplement a larger series of compounds prepared earlier in our research group. Within this larger set we could clearly see that the higher electronegativity of the substituents present at the C-2 position resulted in compounds with higher IC_{50} which is in agreement with our original hypothesis. However, in most of the new derivatives, the cytotoxicity was not limited to cancer cell lines exclusively, they are toxic against non-cancer fibroblasts and therefore their use as therapeutics is unlikely. A lot of difficulties had to be overcome during the synthesis. For example, some reactions produced mixtures of epimers, which were impossible to separate and therefore the biological testing had to be done with epimeric mixtures. In many cases, compounds were almost insoluble in both water and organic solvents, they were often

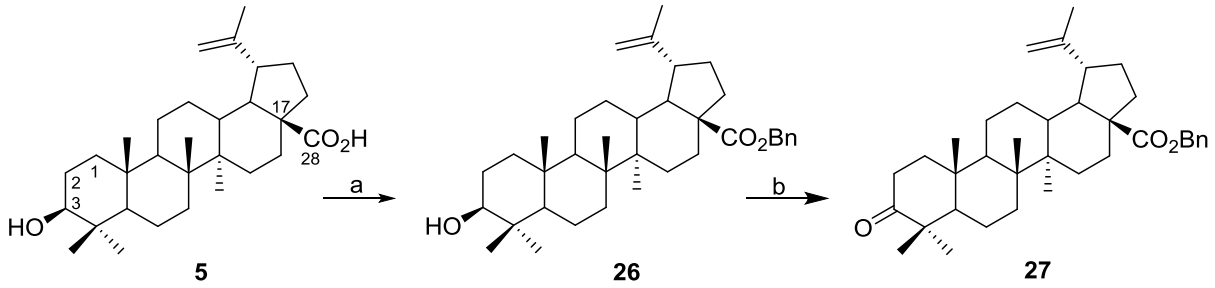
lost or partly lost during the extraction and chromatography procedures and this was the reason for low yields. Despite all difficulties with the synthesis and a small chance to find a new therapeutic agent among the presented set of triterpenes, these compounds gave us important data points for our structure-activity relationship evaluations. Based on those data, structures of better anti-cancer inhibitors may be proposed in the future research and both the difficulties with the low solubility and selectivity may be overcome by prodrug approach or by the synthesis of conjugates such as.[63] This subproject became a significant part of the publication: Borkova, L.; Gurska, S.; Dzubak, P.; Burianova, R.; Hajduch, M.; Sarek, J.; Popa, I.; Urban, M. *Eur. J. Med. Chem.* **2016**, *121*, 120-131.[132]

4.3 Preparation of new betulinic acid derivatives by Suzuki-Miyaura cross-coupling: scope and limitations of the method

The background of this subproject was described in the chapter *3.3.4 Suzuki-Miyaura cross-coupling on triterpenoids* (starting page 21).

4.3.1 Reactions on betulinic acid

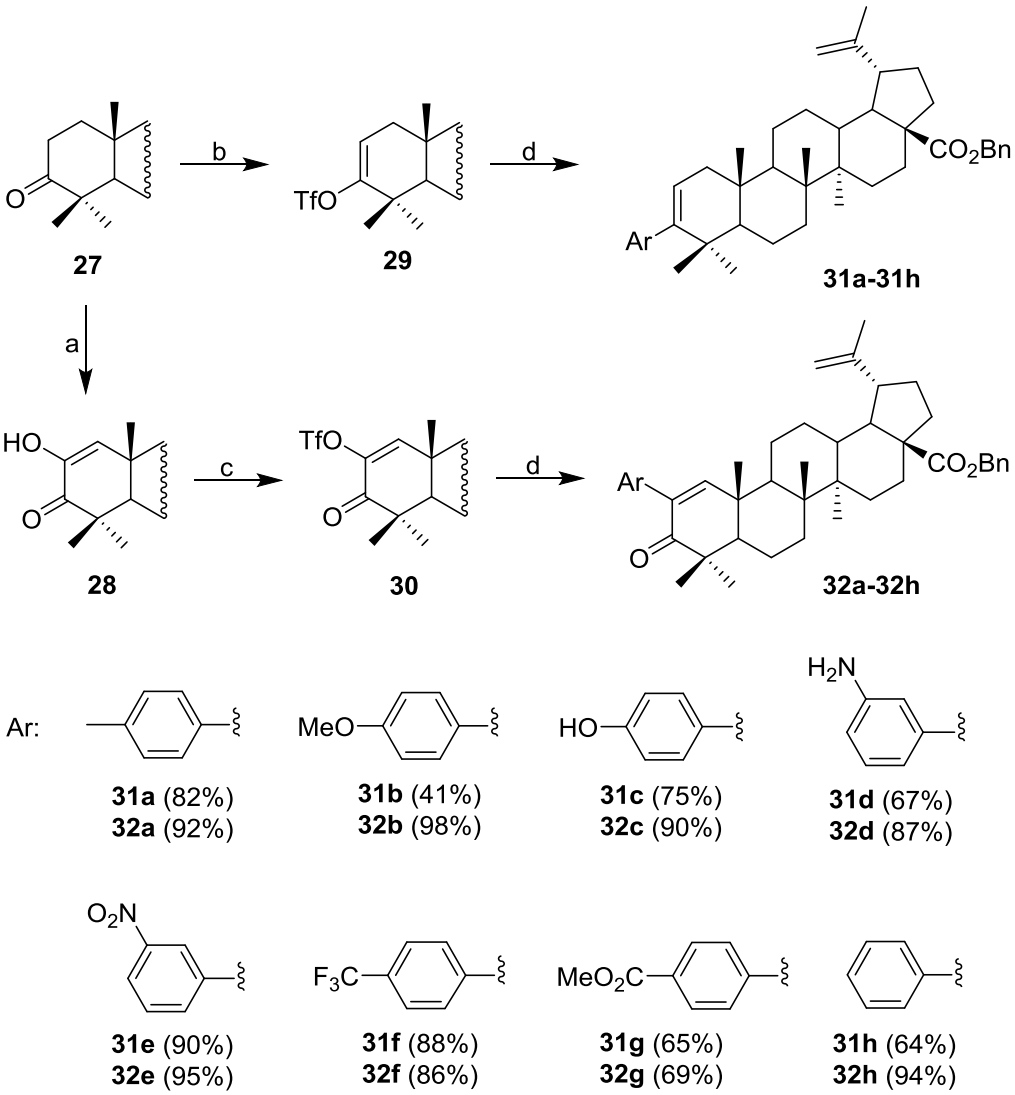
Starting from betulinic acid (**5**), triterpenic triflates **29** and **30** were prepared in three, resp. four steps in overall yield 75 % and 57 %. First, betulinic acid (**5**) was protected with benzyl ester by the reaction with benzyl bromide and potassium carbonate in dimethylformamide and acetonitrile at 60 °C. Benzyl betulinate (**26**) was then oxidized with sodium dichromate and sodium acetate in 1,3-dioxane and acetic acid at r.t. to give benzyl betulonate (**27**, Scheme 19); both reactions are known.[86] Triflate **29** was prepared directly from ester **27** by its reaction with Tf_2NPh and KHMDS in the mixture of tetrahydrofuran and toluene at -78 °C;[121] this procedure is also known from the steroid chemistry.[145] Triflate **30** was prepared in two-step reaction from benzyl betulonate (**27**). First, ester **27** was oxidized with air and potassium *tert*-butoxide in *tert*-butanole at 40 °C to form diosphenol **28**.[89] The subsequent introduction of triflate was first tried with diosphenol **37** – an allobetulon derivative (we often use allobetulon derivatives to try/find/optimize new reaction conditions since allobetulon derivatives are the most stable triterpenes from our repertoir that usually do not undergo any side-reactions, on the other hand, their cytotoxicity is usually low), see the chapter *4.3.2 Reactions on allobetulon*. Then reaction conditions were applied to diosphenol **28** which reacted with Tf_2NPh, dimethylaminopyridine, and trimethylamine in dichloromethane at r.t. to give triflate **30** which was purified on column chromatography (Schemes 20).[146] The structure of compound **30** was confirmed by these signals in ^1H NMR spectrum: multiplet at 7.42 – 7.29 ppm with integral 5 and two doublets at 5.16 and 5.10 ppm, both with integral one, were assigned to the benzyl group, singlet at 7.03 was assigned to the H-1. It is worth to mention, that triflate **30** was stable during the work-up procedures, chromatography, crystallization etc. and it was not necessary to pay any special attention for its storage. It only succumbs to slow decomposition when heated to 50 °C or more. The same is true for the triflate **29**.



Scheme 19. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, MeCN, 60 °C, 48 h; (b) $Na_2Cr_2O_7$, AcONa, 1,3-dioxane, AcOH, r.t., 16 h.

Suzuki-Miyaura cross-coupling procedure from the lit.[121] was used but the reaction conditions had to be optimized. The original procedure[121] uses 2 eq. of boronic acid, 4 eq. of Na_2CO_3, $Pd(PPh_3)_4$ (5 mol %) as the catalyst, and the mixture of solvents 1,4-dioxane/2-propanol/water 2:2:1. The reaction of triflate **29** with *p*-tolylboronic acid was chosen for the reaction conditions optimization (Scheme 20). First, the catalyst $Pd(PPh_3)_4$ from the lit.[121] was replaced with the more air-stable and cheaper catalyst $PdCl_2(PPh_3)_2$. Then its amount was gradually decreased from 8 mol % to 4 mol % and finally to 2 mol %; all amounts allowed for the full consumption of the triflate **29** and formation of **31a** as a single product. This not only saved the catalyst but also resulted in easier reaction work-up and higher reaction yields (from 73 % to 82 %). Next, the amount of the base was lowered from 4 equivalents of sodium carbonate to 2 equivalents. The amount of boronic acid and the ratio of solvents was not changed because the attempt to change the mixture of solvents to 1,4-dioxane and water only resulted in lower yield. To conclude, in my work, the best results were obtained with 2 eq. of boronic acid, 2 eq. of Na_2CO_3, and 2 mol % of $PdCl_2(PPh_3)_2$ when heating the reaction mixture 18 hours under reflux in a mixture of solvents 1,4-dioxane/2-propanol/water 2:2:1.

Once the best conditions for the cross-coupling reaction were found, two series of aryl-substituted betulinic acid derivatives were prepared. Triflates **29** and **30** reacted with a set of eight aromatic boronic acids with various electron-donating (**a**-**d**) and electron-withdrawing (**e**-**g**) substituents or without any substituent (**h**). A small library of 16 new compounds was synthesized in medium to high yields with respect to the substituents (**31a-31h** 41-90 % yields; **32a-32h** 69-98 % yields; Scheme 20).



Scheme 20. Reagents and conditions: (a) air, *t*-BuOK, *t*-BuOH, 40 °C, 2 h; (b) Tf_2NPh, KHMDS, THF, toluene, -78 °C, 4 h; (c) Tf_2NPh, DMAP, TEA, DCM, r.t., 90 min; (d) $ArB(OH)_2$, Na_2CO_3, $PdCl_2(PPh_3)_2$ (2mol %), dioxane, IPO, H_2O, refl., 18 h.

The presence of the appropriate substituent was confirmed by their characteristic signals in ^1H NMR spectra:

- two multiplets within the range 7.19 – 7.01 ppm, both with integral 2, and singlet around 2.34 ppm with integral 3 were assigned to the **4-methylphenyl moiety**,
- two multiplets around 7.24 – 7.01 ppm and 6.87 – 6.77 ppm, both with integral 2, and singlet at 3.80 ppm with integral 3 were assigned to the **4-methoxyphenyl moiety**,
- two multiplets around 7.16 – 6.96 ppm and 6.77 – 6.70 ppm, both with integral 2, were assigned to the **4-hydroxyphenyl moiety**,

- multiplets around 7.13 – 7.02 ppm and 6.70 – 6.48 ppm, together with integral 4, and broad singlet aroung 4.89 ppm with integral one were assigned to the **3-aminophenyl moiety**,

- multiplets around 8.16 – 7.96 ppm, 7.66 – 7.61 ppm, and 7.50 – 7.46 ppm, together with integral 4, were assigned to the **3-nitrophenyl moiety**,

- two multiplets or dublets around 7.56 – 7.50 ppm and 7.35 – 7.23 ppm, both with integral 2, were assigned to the **4-trifluoromethylphenyl moiety**,

- two multiplets around 8.00 – 7.88 ppm and 7.33 – 7.15 ppm, both with integral 2, and singlet at 3.91 ppm with integral 3 were assigned to the **4-(methoxycarbonyl)phenyl moiety**,

- multiplets within the range 7.40 – 7.10 ppm, together with integral 5, were assigned to the unsubstituted **phenyl moiety**,

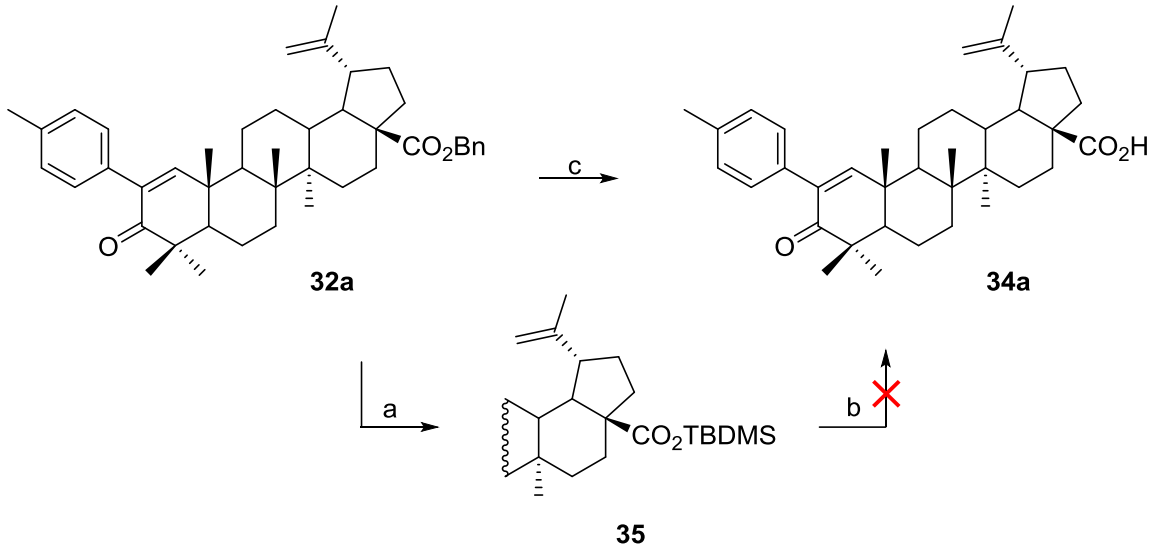
- multiplet around 7.44 – 7.27 ppm with integral 5 and two doublets around 5.16 and 5.10 ppm, both with integral one, were assigned to the **benzyl group**,

- beside the standard signals of the triterpenic part, doublet of doublets around 5.27 ppm was assigned to the H-2 and doublet of doublets around 2.10 ppm was assigned to the H-1a in the spectra of derivatives **31a-31h** and **33a-33h**, signal of H-1b is (as usually) hidden by signals of other triterpenic hydrogens,

- beside the standard signals of the terpenic part, singlet or multiplet in range 7.24 – 7.07 ppm with integral one was assigned to the H-1 in the spectra of derivatives **32a-32h** and **34a-34h**.

As mentioned earlier, the free carboxylic function at C-28 is usually important for the cytotoxic activity of triterpenoids. For this reason, benzyl esters **31a-31h** and **32a-32h** were deprotected *via* reductive debenzylation. First, two-step selective deprotection of benzyl ester described in the lit.[121] was used, however, the desired compound **34a** did not form (Scheme 21). Betulinic acid derivatives **33a-33c**, **33e-33h**, **34a-34c**, and **34e-34h** were then prepared by catalytical hydrogenation of appropriate benzyl esters with the use of cyclohexa-1,3-dien as the hydrogen source in absolute ethanol at 50 °C.[147] Yields ranged between 25-95 % (Scheme 22). Generally, to avoid the formation of dihydrobetulonic acid derivatives (hydrogenation of the double bond between C-20 and C-29) it was necessary to add maximum 15 mol % of Pd/C and no more than 14 eq. of cyclohexa-1,3-dien. Within this set of

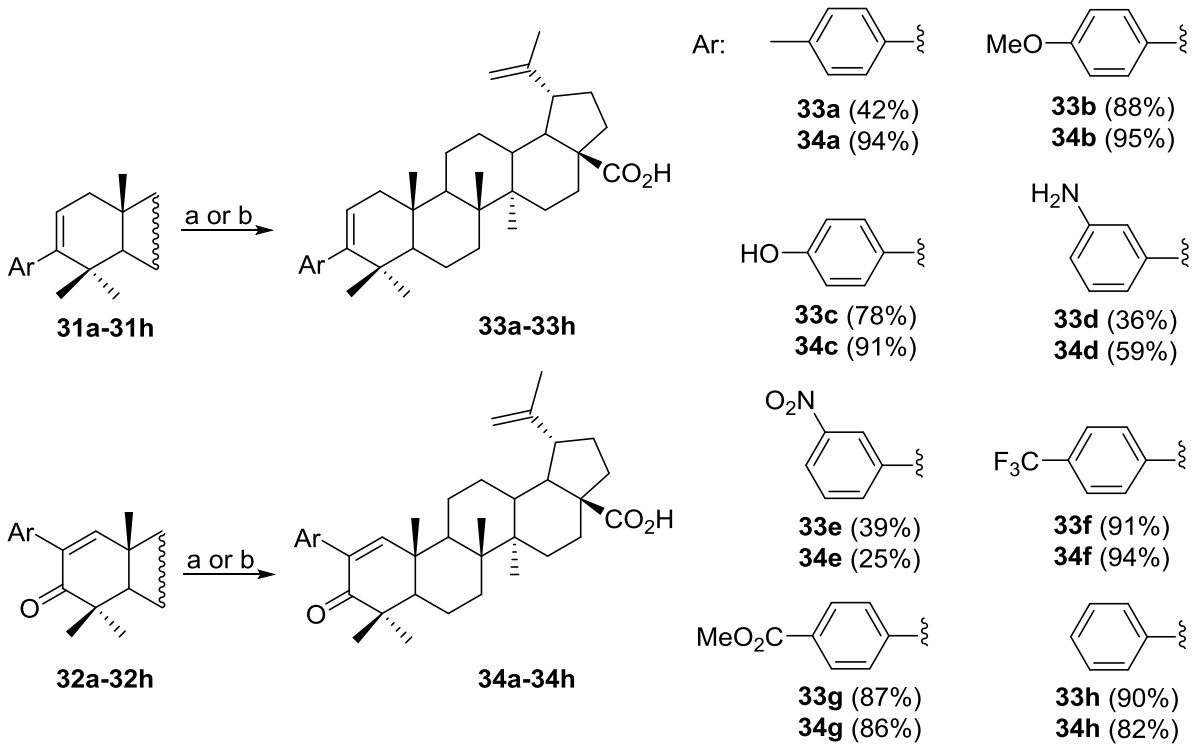
compounds, anilin derivative **34d** formation was observed when more than 7 eq. of cyclohexa-1,3-dien were added to the reaction mixture with nitrophenyl derivative **32e**. The ratio was: aniline derivative **34d** 59 % and nitrophenyl derivative **34e** 25 %. Since it was possible to separate both compounds by chromatography, the reaction was not further optimized to yield either pure **34d** or **34e**. Medium to low yields of some products (**33e** 39 %; **33a** 42 %) were caused by difficulties with the purification and/or by residues of the unreacted starting compound in the reaction mixture (unreacted compound was separated and recycled). Often, the balance between debenzylation and hydrogenation of the double bonds in the molecule had to be achieved.



Scheme 21. Reagents and conditions: (a) TBDMSH, DCM, TEA, $Pd(OAc)_2$, 60 °C, 24 h; (b) TBAF, 1,4-dioxane, THF, r.t., 4 h; (c) cyclohexa-1,3-dien, Pd/C, EtOH, 50 °C, 24 h.

Aniline derivatives **31d** and **32d** did not react with cyclohexa-1,3-dien; therefore, another procedure had to be tried. Reductive debenzylation with H_2 on Pd/C was applied. A series of optimization reactions was done to find the best conditions for the deprotection. It was observed that the choice of the solvent was the major determinant for the side-reactions, the hydrogenation of the 20(29)-double bond or the 1(2)-double bond. Methanol, ethanol, 2-propanol, and a mixture of DCM with methanol 2:1 were used as solvents. The best results were obtained with the mixture DCM/MeOH; nevertheless, the reaction still could not be left to react till the 100% consumption of the starting material and had to be stopped earlier to avoid the formation of the side-products with reduced double-bond(s). Thus, derivatives **33d** and **34d** were prepared by reaction with H_2 and Pd/C in DCM/MeOH 2:1 at r.t. (Scheme 22). Yields were 36 % and 34 %, respectively, the starting material was the rest and it was

separated and deprotected again, here I present the yield of a single deprotection step. The characteristic signals of the final products in ^{1}H NMR spectra remained analogous to the signals described earlier in this chapter with respect to the absence of signals belonging to the benzyl ester protective group.

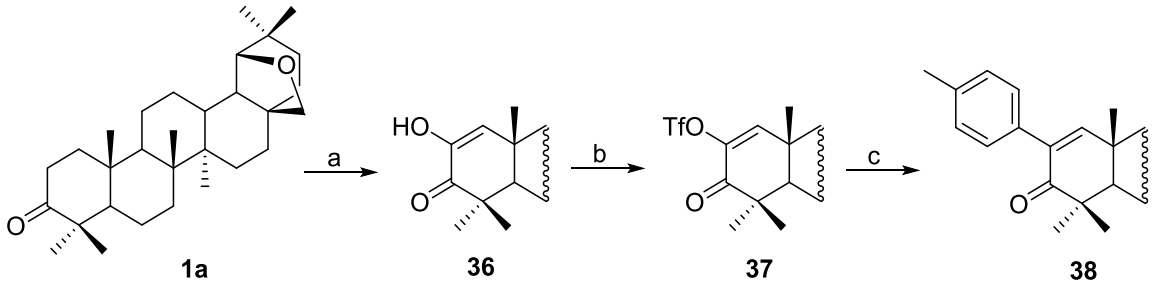


Scheme 22. Reagents and conditions: (a) cyclohexa-1,3-dien, Pd/C, EtOH, 50 °C, 5-48 h; (b) H_2, Pd/C, IPO, r.t., 2-3 h (for **33d** and **34d** only).

4.3.2 Reactions on allobetulon

In order to find out whether the Suzuki-Miyaura cross-coupling works on other triterpenoid skeletons, allobetulon **1a** was chosen for further investigation. First, compound **1a** was oxidized with an air and potassium *tert*-butoxide in *tert*-butanol at 40 °C. The reaction was completed after 5 h of vigorous stirring yielding diosphenol **36** in 93 % yield.[129] Triflate **37** was prepared by the reaction of **36** with Tf_2NPh, dimethylaminopyridine, and trimethylamine in dichloromethane at r.t.[146] and as mentioned earlier, this was the first time this protocol was used in triterpenoid chemistry and was optimized and then applied on benzyl betulonate **28**. After 90 minutes, the reaction was completed. After the standard work up procedure, the crude allobetulon triflate **37** was dried on the vacuum line and used to the next reaction step without further purification. Triflate **37** then reacted with tolylboronic acid under optimized Suzuki-Miyaura cross-coupling conditions. After complete consumption of

the starting triflate **37** the reaction mixture was processed, and the crude product was purified on the column chromatography. Tolyl derivative **38** was obtained in 54 % yield and its structure was confirmed by spectral data (Scheme 23). In the ^{1}H NMR spectrum of **38** multiplet at 7.21 – 7.18 ppm with integral 2 and multiplet at 7.16 – 7.12 ppm with integral 3 were found and assigned to the phenyl moiety and H-1, singlet at 2.34 was assigned to the methyl group.



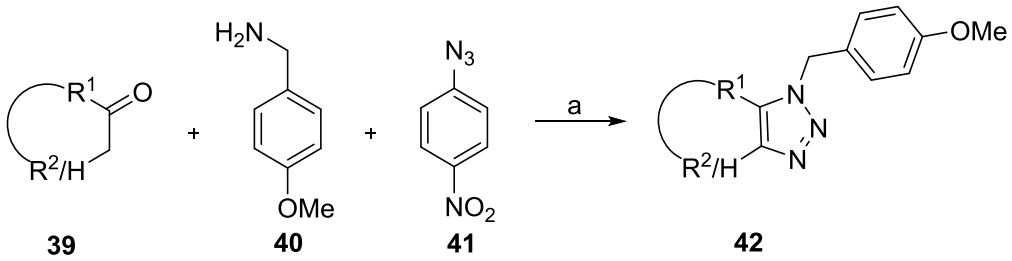
Scheme 23. Reagents and conditions: (a) air, *t*-BuOK, *t*-BuOH, 40 °C, 5 h; (b) Tf_2NPh, DMAP, TEA, DCM, r.t., 90 min; (c) $ArB(OH)_2$, Na_2CO_3. $PdCl_2(PPh_3)_2$ (2 mol %), dioxane, IPO, H_2O, refl., 18 h.

In conclusion, Suzuki-Miyaura cross-coupling reaction was applied to triterpenoids in order to find its scope and limitations and to synthesize a series of new potentially biologically active compounds. Betulinic acid (**5**) and allobetulon (**1a**), compounds derived from lupane and 18α-oleanane skeletal types, were chosen for the derivatization. Introduction of substituents into position C-2 on allobetulon (**1a**) and positions C-2 and C-3 on betulinic acid (**5**) were studied. Reaction conditions for preparation of triflates and cross-coupling products were found and optimized. An example of Suzuki-Miyaura cross-coupling into the position C-2 on allobetulon (**1a**) was demonstrated. Two sets of benzyl betulonate derivatives modified at positions C-2 and C-3 by aryl-substituents were prepared. The method for reductive debenzylation of benzyl betulonate derivatives was developed and the final products **33a-33h** and **34a-34h** with the free C-28 carboxylate were prepared. Cytotoxic properties of all compounds are currently being examined on eight cancer cell lines and two non-cancer fibroblasts. Preliminary results show that compounds protected by benzyl ester are inactive which is common. To summarize it, Suzuki-Miyaura cross-coupling reaction is a robust method for derivatization of the A-ring of triterpenoids and it will be tried on other triterpenoid rings in the future. A manuscript based on this project is in preparation.

4.4 Triazolization reaction and triterpenoid chemistry

Recently, triazolylation reactions were introduced to the triterpenoid chemistry and it has been used in our research group to connect triterpenes with another small molecules of the interest.[148] Meanwhile, a large number of other scientists have begun to explore this area and published quite interesting results; therefore, my colleagues and I summarized this research area and published a review: Pokorny, J.; Borkova, L.; Urban, M. Click Reactions in Chemistry of Triterpenes - Advances Towards Development of Potential Therapeutics. *Current Med. Chem.* **2018**, *25* (5), 636–658, which describes all results of this new approach to improve both the activity and ADME-Tox properties of triterpenes by connecting them to another modifying molecules using click reactions.[149] As the second author, I wrote a significant part of this article.

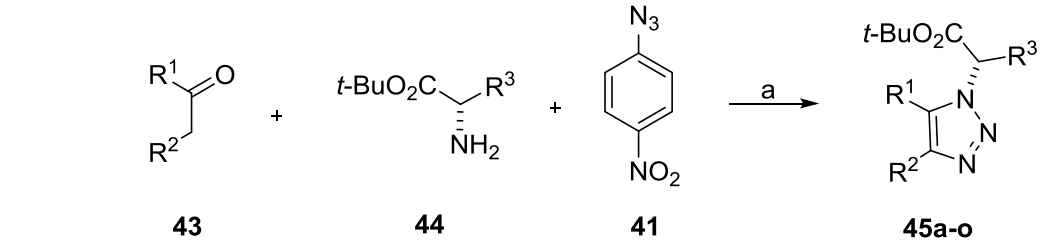
In the research group of prof. Wim Dehaen, they invented a completely new method of preparation of 1,2,3-triazoles from three building blocks, simply called the *Triazolization reaction*.[150] The method relies on using abundantly available enolizable ketones, primary amines and *p*-nitrophenylazide as a dinitrogen source. This general multi-component strategy can be applied to the synthesis of 1,4,5-trisubstituted, 1,5-disubstituted, and 4,5-fused 1,2,3-triazoles, such as **42** (Scheme 24).[151]



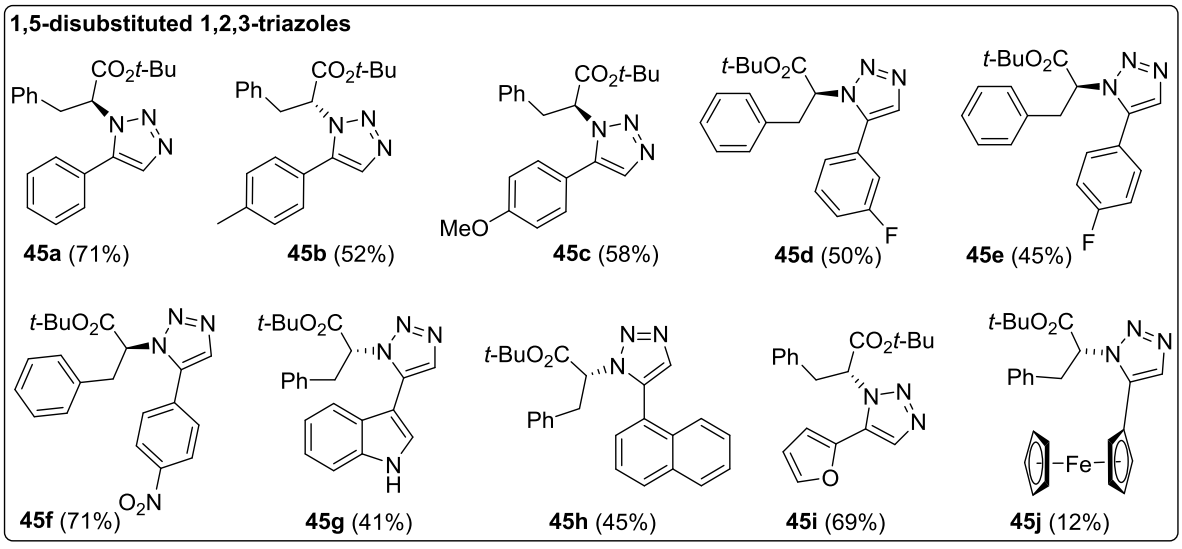
Scheme 24. A general reaction scheme of the Triazolization reaction with *p*-methoxy-benzylamine as a model of primary amine. Reagents and conditions: (a) **39** (1.0 eq.), **40** (1.3eq.), **41** (1.0 eq.), AcOH (30 mol %), 4Å molecular sieves, toluene, 100 °C, 12 h.[151]

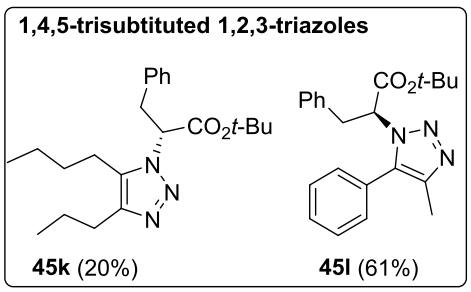
From this reason, I have decided to go to their research group for three months stay during which I have learned this method in order to introduce it to the triterpenoid chemistry. As a result, I am a co-author of the publication: Silveira-Dorta, G.; Jana, S.; Borkova, L.; Thomas, J.; Dehaen, W. Straightforward Synthesis of Enantiomerically Pure 1,2,3-Triazoles

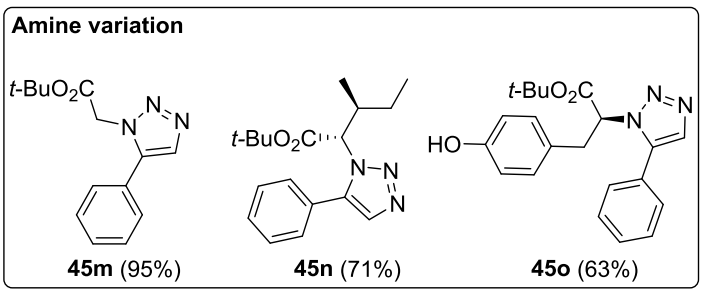
Derived from Amino Esters. *Org. Biomol. Chem.* **2018**, *16* (17), 3168–3176. I have optimized the reaction conditions for the synthesis of enantiomerically pure 1,5-disubstituted and 1,4,5-trisubstituted 1,2,3-triazoles derived from diversely substituted, commercially available ketones and amino esters. After optimization, the reaction was carried out in toluene with 1.0 eq. of appropriate ketone (**43**), 1.2 eq. of *p*-nitrophenylazide (**41**) and 1.4 eq. of appropriate amino acid ester (**44**) under nitrogen atmosphere at 100 °C overnight. The obtained results showed that the reaction is independent either of the electron donating ability (**45b**, **45c**) or the electron withdrawing ability (**45d-45f**) of the substituents, diverse heterocyclic ketones (**45g-45j**), and ketones providing trisubstituted triazoles (**45k**, **45l**). The amino acid diversity scope was also demonstrated (**45m-45o**, Scheme 25).[152]



43 **44** **41** **45a-o**

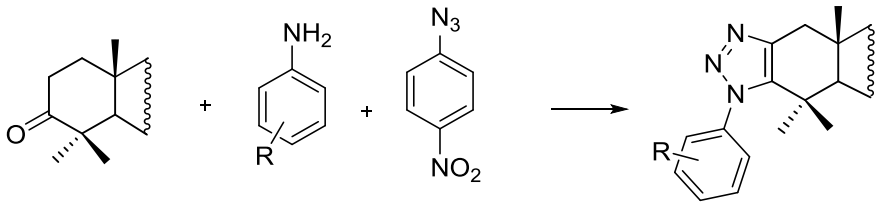






Scheme 25. Chemically diverse library of 1,5-disubstituted and 1,4,5-trisubstituted 1,2,3-triazoles. Reagents and conditions: (a) **43** (1.0 eq.), **44** (1.4 eq.), **41** (1.2 eq.), toluene, 100 °C, 18 h.

This reaction will be very useful to prepare new heterocyclic triterpenes with a 5-membered heterocyclic ring (substituted triazole) condensed to the triterpenic A-ring (Scheme 26).



Scheme 26. The planned synthesis of fused triterpenoid 1,2,3-triazoles from 3-oxotriterpenoids, substituted anilines, and *p*-nitrophenylazide.

5 Conclusions and future directions

Efficient and universal three-step synthestic pathway towards *N*-substituted aminothiazoles fused to the A-ring of triterpenoids (3-oxoderivatives) was developed and optimized. Seven series of variously substituted triterpenoid thiazoles, their unsubstituted analogues and intermediates was synthesized. Together, 82 compounds, 75 of them new, was prepared and fully characterized and their cytotoxicity was measured on eight cancer cell lines and two non-cancer fibroblasts. The most active compounds against T-lymphoblastic leukemia cell line were aminothiazoles **4m** and **5m** with the free NH_2 group, 2-bromo-3-oxoderivative **7b**, and 2-thiocyano-3-oxoderivatives **5c**, **6c**, and **7c**. Pharmacological parameters of the most active derivativatives were measured and these trends in SAR were found: An active compound must have the free C-28 COOH group; derivatives with the free amine on the ainothiazole ring are active; sometimes derivatives with morpholine substituent are active; derivatives with the substituents of low polarity are inactive; intermediates (2-bromo and 2- thiocyanoderivatives) are the most active but their selectivity is usually insufficient. Four compounds of this sub-project **4m**, **5m**, **XXXIII**, and **7b** had high and selective cytotoxicity against cancer cells as well as favorable pharmacological properties. Since these compounds have the highest potential to become new anti-cancer drugs, they will be further tested *in vivo* for ADME-Tox parameters and anti-cancer properties.

Substitution of the C-2 position of dihydrobetulonic acid (**6a**) with electronegative substituents was performed and our hypothesis about the relationship between electronegativity of the substituents and cytotoxicity of the molecule was confirmed: The higher electronegativity of a substituent, the more active compound. 15 compounds, 12 of them new, were prepared and evaluated for their cytotoxicity. Although we found some interesting trends, no further tests are planed since most of the compounds were only moderately active and the rest was not selective towards cancer cells. Also, some of the reactions had low yields and produced unseparable mixtures of epimers which also is not favorable in medicinal chemistry.

Suzuki-Miyaura cross-coupling, on the other hand, was found to be a great tool for the preparation of libraries of new terpenoids. Conditions for the preparation of triflate intermediates and conditions for Suzuki-Miyaura cross-coupling were found and optimized. The reaction is a robust methodology that allows for almost unlimited numbers of the

derivatives of triterpenes, in this work, compounds were modified at the positions 2 and 3 but there are strong expectations that this method may be expanded to derivatize other triterpenic rings. The aryl-substituted derivatives of allobetulon in the position C-2 (**38**) and benzyl betulonate in positions C-2 (**31a-31h**) and C-3 (**32a-32h**) were prepared. A procedure for deprotection of benzyl betulonate derivatives to final products was developed and two sets of aryl-substituted betulinic acid derivatives (**33a-33h** and **34a-34h**) were prepared. Together, 40 compounds, 33 of them new, were synthesized in this subproject and their cytotoxicity is being tested. Preliminary results show that benzyl esters are, as usually, inactive, while compounds with the free 28-COOH have $IC_{50} < 50$ μM.

To sum up, 137 compounds, 120 of them new, with potential biological activities derived from seven triterpenoid skeletons were prepared and characterized by common chemical and analytical methods. Both final products and intermediates were or are being tested on their cytotoxic activity against eight cancer cell lines of a different histogenetic origin, including multi-drug resistant cell lines, and two normal fibroblasts. Influence on the cell cycle and on the inhibition of DNA and RNA synthesis were measured for the most potent compounds as well as metabolic stability and membrane permeability was tested for some derivatives. All biological assays were performed at the department of MUDr. Hajdúch, Ph.D. in the Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc (LEM). The summary of the results of cytotoxicity screening and conclusions about SARs were made by me under supervision of the LEM members. Conclusions from the advanced tests such as cell cycle observations were made by the LEM members with my participation.

Specification of the work of Lucie Borková and other co-authors of the articles

The first subproject was started in collaboration with the research group of RNDr. Jan Šarek, Ph.D., whose students Ing. Petr Kalina and Bc. Filip Korda prepared several allobetulon aminothiazoles and a few oleanonic acid derivatives. I have finished all series, optimized the syntheses, collected all data from the synthesis, characterization and biological assays, and I wrote the majority of the published article. Several dihydrobetulonic aminothiazoles were prepared by Mgr. Richard Adámek under my supervision and ursolic acid derivatives were prepared by Bc. Nikola Jakubcová under my supervision. Most importantly, I have optimized the synthesis of 2-bromo-3-oxoderivatives, the synthesis of 2-

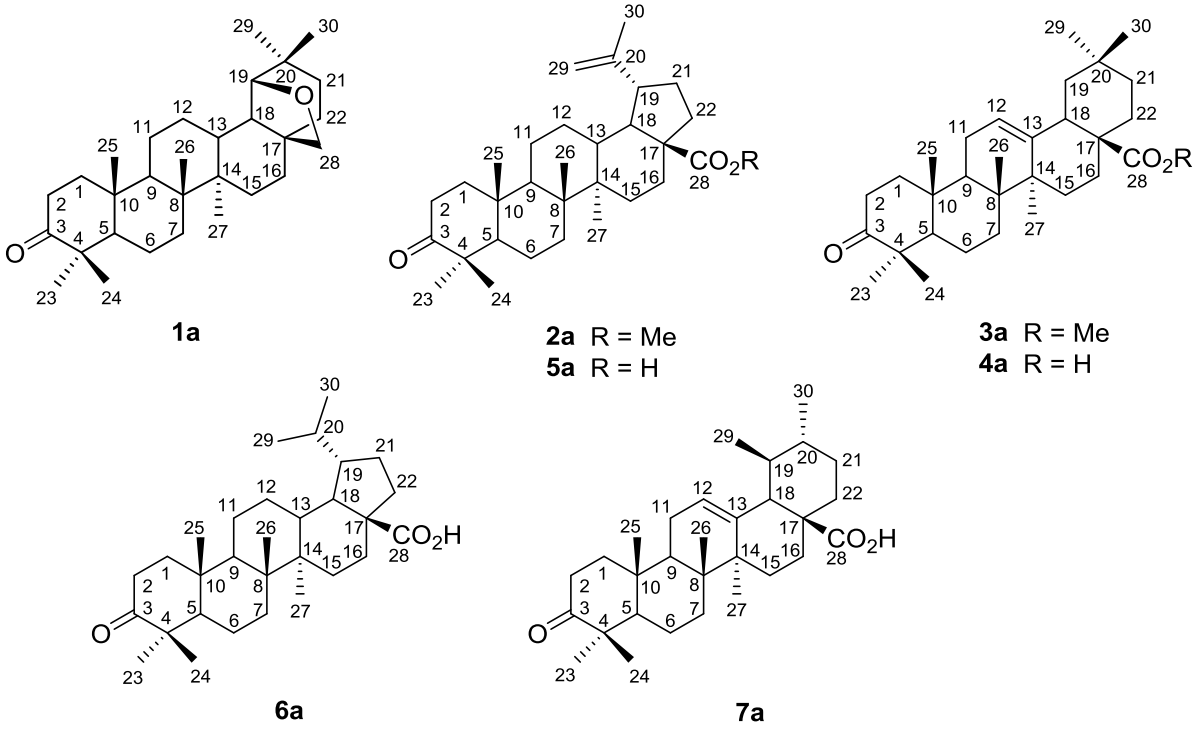
thiocyano-3-oxo derivatives, and the synthesis of aminothiazoles with the free NH_2 group. I have prepared 43 compounds out of 82.

Other subprojects (Substitutions on the C-2 and Suzuki-Miyaura cross-coupling) were solely mine. In this regard, several betulinic acid derivatives were prepared by Bc. Barbora Vránová under my supervision.

6 Experimental part

6.1 Numbering of triterpenic skeletones and their characterictic spectral features

Figure 16. Numering of skeletones of allobetulon (**1a**), methyl betulonate (**2a**), methyl oleanonate (**3a**), oleanonic acid (**4a**), betulonic acid (**5a**), dihydrobetulonic acid (**6a**), and ursonic acid (**7a**).



Characteristic signals of allobetulone (**1a**) derivatives in ^{1}H NMR spectra: doublet around 3.80 ppm with the coupling constant around 7.6 Hz belongs to the H-28a, singlet around 3.55 ppm belongs to the H-19, and doublet around 3.46 with the coupling constant around 7.6 Hz belongs to the H-28b, all of the signals are with integral one, seven singlets in range 1.25 – 0.80 ppm, all with integral 3, belong to the seven skeletal methyl groups (H-23, H-24, H-25, H-26, H-27, H-29, and H-30). Characteristic bands of **1a** derivatives in IR spectra: vibration of C=O bond around 1700 cm^{-1} and vibration of C-O-C bond around 1035 cm^{-1}.

Characteristic signals of oleanonic acid (**4a**) derivatives in ^{1}H NMR spectra: multiplet around 5.30 ppm with integral one belongs to the H-12, doublet of doublets or multiplet around 2.85 ppm belongs to the H-18, seven singlets in range 1.28 – 0.78 ppm, all with integral 3, belong to the seven skeletal methyl groups (H-23, H-24, H-25, H-26, H-27, H-29, and H-30). ^{1}H NMR spectra of derivatives of methyl oleanonate (**3a**) contains an additional singlet around 3.64 ppm with integral three which is refered to the methyl ester.

Characteristic signals of betulonic acid (**5a**) derivatives in ^{1}H NMR spectra: two multiplets with integral one around 4.75 ppm and 4.60 ppm belong to the H-29a and H-29b, doublet of triplets or multiplet around 3.02 ppm belongs to the H-19β, singlet around 1.70 ppm belongs to the methyl group H-30, five singlets in range 1.25 – 0.80 ppm, all with integral 3, belong to the five skeletal methyl groups (H-23, H-24, H-25, H-26, and H-27). ^{1}H NMR spectra of derivatives of methyl betulonate (**2a**) contains an additional singlet around 3.68 ppm with integral three which is refered to the methyl ester.

Characteristic signals of dihydrobetulonic acid (**6a**) derivatives in ^{1}H NMR spectra: multiplet around 2.30 ppm belongs to the H-18, seven singlets and/or doublets in range 1.24 – 0.76 ppm, all with integral 3, belong to the seven skeletal methyl groups (H-23, H-24, H-25, H-26, H-27, H-29, and H-30).

Characteristic signals of ursonic acid (**7a**) derivatives in ^{1}H NMR spectra: multiplet around 5.27 ppm with integral one belongs to the H-12, doublet of doublets or multiplet around 2.22 ppm belongs to the H-18, seven singlets, dublets and/or multiplets in range 1.29 – 0.78 ppm, all with integral 3, belong to the seven skeletal methyl groups (H-23, H-24, H-25, H-26, H-27, H-29, and H-30).

6.2 Materials and instruments

Melting points were determined using either the Büchi B-545 apparatus or the STUART SMP30 apparatus and are uncorrected.

Optical rotations were measured on an Autopol III (Rudolph Research, Flanders, USA) polarimeter in MeOH at 25 °C and are in [10^{-1} deg cm^{2} g^{-1}].

Infrared spectra were recorded on a Nicolet Avatar 370 FTIR and processed in the OMNIC 9.8.372. DRIFT stands for Diffuse Reflectance Infrared Fourier Transform.

^{1}H, ^{13}C and 2D NMR experiments were performed on Jeol ECX-500SS (500 MHz for ^{1}H), Varian^{UNITY} Inova 400 (400 MHz for ^{1}H) or Varian^{UNITY} Inova 300 (300 MHz for ^{1}H) instruments, using $CDCl_3$, DMSO-d_6, CD_3OD or THF-d_8 as solvents (25 °C). Chemical shifts (δ) were referenced to the residual signal of the solvent ($CDCl_3$, DMSO-d_6, CD_3OD or THF-d_8) and are reported in parts per million (ppm). Coupling constants (*J*) are reported in Hertz (Hz). NMR spectra were processed in the ACD/NMR Processor Academic Edition 12.01, MestReNova 6.0.2-5475 or JEOL Delta v5.0.5.1.

EI-MS spectra were recorded on an INCOS 50 (Finnigan MAT) spectrometer at 70 eV and an ion source temperature of 150 °C. The samples were introduced from a direct exposure probe at a heating rate of 10 mA/s. Relative abundances stated are related to the most abundant ion in the region of $m/z > 180$.

HRMS analysis was performed using an LC-MS Orbitrap Elite high-resolution mass spectrometer with electrospray ionization (Dionex Ultimate 3000, Thermo Exactive plus, MA, USA). Spectra were taken at positive and negative mode in the range of 400 – 700 *m/z*. The samples were dissolved in MeOH and injected to the mass spectrometer over autosampler after HPLC separation: precolumn Phenomenex Gemini (C18, 50 × 2 mm, 2.6 μm), mobile phase isokrat MeOH/water/HCOOH 95:5:0.1.

The course of the reactions was monitored by TLC on Kieselgel 60 F_{254} plates (Merck) detected first by UV light (254 nm) and then by spraying with 10% aqueous H_2SO_4 and heating to 150 °C – 200 °C. Mobile phase is listed as a volume ratio of solvents at each experiment.

Purification was performed using column chromatography on Silica gel 60 (Merck 7734). Mobile phase is listed as a volume ratio of solvents at each experiment.

Allobetulon (**1a**), methyl betulonate (**2a**), methyl oleanonate (**3a**), oleanonic acid (**4a**), betulinic acid (**5**), dihydrobetulonic acid (**6a**), and ursolic acid (**7**) were purchased from company Betulinines (www.betulinines.com), which manufactures them from betulin, betulinic acid and oleanolic acid in bulk scale. All other chemicals and solvents were obtained from Sigma-Aldrich, Lachner or Across Chemicals.

MTS assay, Cell Cycle and Apoptosis Analysis, BrDU Incorporation Analysis (DNA synthesis), and BrU Incorporation Analysis (RNA synthesis) were performed at the department of MUDr. Hajdúch, Ph.D. in the Laboratory of Experimental Medicine, Institute

of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc.

6.3 Usual procedures for processing of reaction mixtures

Procedure A

The reaction mixture was several times washed with water, the chloroform layer was dried over magnesium sulfate, filtered, and chloroform was evaporated under reduced pressure.

Procedure B

The reaction mixture was poured into water and the product was extracted into ethyl acetate or chloroform. The collected organic layers were washed successively with water. Then the organic phase was dried over magnesium sulfate, filtered, and the solvents were evaporated under reduced pressure.

Procedure C

The reaction mixture was poured into water and the product was extracted into organic solvent. The collected organic layers were washed successively in this order with saturated aqueous sodium hydrogen carbonate, brine, and water. The organic phase was then dried over magnesium sulfate, filtered, and the solvents were evaporated under reduced pressure.

Procedure D

The reaction mixture was poured into diluted aqueous HCl (3.5%) and the product was extracted into organic solvent. The collected organic layers were washed successively in this order with water, saturated aqueous sodium hydrogen carbonate, and again water. The organic phase was then dried over magnesium sulfate, filtered, and the solvents were evaporated under reduced pressure.

Procedure E

The reaction mixture was poured into saturated aqueous solution of ammonium chloride and the product was extracted into organic solvent. The collected organic layers were washed successively with brine and water. The organic phase was then dried over magnesium sulfate, filtered, and the solvents were evaporated under reduced pressure.

6.4 General synthetic procedures

General procedure for the synthesis of 2-bromo-3-oxotriterpenoids with Br_2 (I)

The starting 3-oxoderivative was dissolved in chloroform. Solution of bromine in chloroform (3%) was added dropwise to the reaction mixture. After 30 minutes of vigorous stirring at r.t. the reaction was completed, monitored by TLC (mobile phase at each experiment). The reaction mixture was processed by the procedure A. The crude product was purified by column chromatography on silica gel (mobile phase at each experiment). Collected fractions were evaporated to give 2-bromoderivative as a mixture of 2α/2β epimers.

General procedure for the synthesis of 2-bromo-3-oxotriterpenoids with $CuBr_2$ (II)

The starting 3-oxoderivative was dissolved in a mixture of solvents chloroform, ethyl acetate, and methanol, ratio is at each experiment. Anhydrous $CuBr_2$ was added, and the reaction mixture was stirred for 3-12 hours at r.t. until the starting material was fully consumed, (mobile phase at each experiment). Then, the precipitate of copper(I) bromide was filtered off, organic solvents were washed with water, dried over magnesium sulfate, and the crude product was purified by chromatography on silica gel (mobile phase at each experiment) unless otherwise stated. Evaporation of solvents gave 2-bromoderivative as a mixture of 2α/2β epimers.

General procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids with KSCN (I)

Starting 2-bromoderivative was dissolved in dimethyl sulfoxide. Potassium thiocyanate was added, and the reaction mixture was stirred for 2-24 h at 90 °C until the reaction was completed (controlled by TLC, mobile phase at each experiment). Then, the reaction mixture was processed by the procedure B. The crude product was purified by column chromatography on silica gel (mobile phase at each experiment), which gave 2-thiocyanato derivative as a mixture of 2α/2β epimers.

General procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids with $(NH_4)SCN$ (II)

Starting 2-bromoderivative was dissolved in *N*-methylpyrrolidone. Ammonium thiocyanate was added, and the reaction mixture was stirred for 4-6 hours at 50 °C until the reaction was completed (controlled by TLC, mobile phase at each experiment). Then, water was added, resulting solid precipitate was filtered off and organic phase was washed with

water. The crude product was purified by column chromatography on silica gel (mobile phase at each experiment), which provided 2-thiocyanato derivative as a mixture of 2α/2β epimers.

General procedure for the synthesis of substituted aminothiazole derivatives

Alkyl ammonium acetate of each corresponding amine was added to the solution of 2-thiocyanato derivative in chloroform. The reaction mixture was left stirring for 1 to 7 days at r.t. monitored by TLC (mobile phase at each experiment). The reaction mixture was then processed by the procedure A. The crude product was purified by column chromatography on silica gel (mobile phase at each experiment). Collected fractions were evaporated to give substituted aminothiazole derivative.

General procedure for the synthesis of unsubstituted aminothiazole derivatives

To the suspension of bromoketone in anhydrous ethanol thiourea (10 mol eq.) was added. A white reaction mixture was stirred at r.t. under inert atmosphere for 2-7 days monitored by TLC (mobile phase at each experiment). The reaction mixture was processed by the procedure B. The crude product was purified by column chromatography (mobile phase at each experiment). Collected fractions were evaporated to give an appropriate aminothiazole derivative with a free NH_2 group.

General procedure for the synthesis of diosphenols

Potassium tert-butoxide was added to the solution of 3-oxo triterpenoid in *tert*-butanole. The reaction mixture was bubbled through a needle with air supply at 40 °C, monitored by TLC (mobile phase at each experiment). After 2-5 hours the reaction mixture was processed by the procedure D. The crude product was purified by column chromatography (mobile phase at each experiment). Collected fractions were evaporated to give an appropriate diosphenol.

General procedure for the synthesis of compounds by Suzuki-Miyaura cross-coupling reaction

A round-bottom flask equipped with a stirrer was annealed and then cooled with a stream of nitrogen and closed with a septum. Solutions of starting compounds were injected to the prepared flask in this order: a solution of triflate in 1,4-dioxane, a solution of boronic acid (2 eq.) in 2-propanol and a solution of anhydrous sodium carbonate (2 eq.) in water. Finally, $PdCl_2(PPh_3)_2$ (2 mol %) was added to the reaction mixture and the flask was equipped with a reflux condenser, flushed with nitrogen. The reaction mixture was heated to reflux and stirred overnight, monitored by TLC (mobile phase at each experiment), and quenched by

adding a double volume of saturated aqueous solution of ammonium chloride. The reaction mixture was processed according to the procedure E and a crude product was purified by column chromatography on silica gel (mobile phase at each experiment) and recrystalized from hexane unless otherwise stated.

General procedure for reductive debenzylation of benzyl betulonate derivatives with cyclohexa-1,3-dien (I)

To a freshly degassed solution of benzyl betulonate in anhydrous ethanol in round-bottom flask equipped with a stirrer cyclohexa-1,3-dien (7 eq.) and Pd/C (10%) were added. The reaction mixture was stirred at 50 °C and a course of the reaction was monitored by TLC (mobile phase at each experiment). After specified time, the reaction was quenched by evaporation of the solvent or another portion of reagents was added to the reaction mixture and the whole proces was repeated till at least 80 % of starting compound was consumed. After solvent evaporation, the reaction mixture was suspended in a mobile phase (stated at each experiment) and chromatographed on silica gel with a celite pad on the top. Solvents from collected fractions were evaporated to give an appropriate betulinic acid derivative.

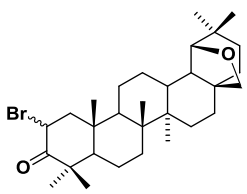
General procedure for reductive debenzylation of benzyl betulonate derivatives with H_2 (II)

To a freshly degassed solution of benzyl betulonate in anhydrous DCM and methanol in round-bottom flask equipped with a stirrer Pd/C (10%) was added. The reaction mixture was flushed with hydrogen gas from a ballon for 30 min and then it was stirred for another 30 min at r.t., monitored by TLC (mobile phase at each experiment). The whole process was repeated several times unless the starting material was consumed till at least 80 %. The reaction was quenched by evaporation of solvents. The reaction mixture was suspended in a mobile phase (stated at each experiment) and chromatographed on silica gel with a celite pad on the top. Solvents from collected fractions were evaporated to give an appropriate aminophenyl derivative of betulinic acid.

6.5 Experimental procedures

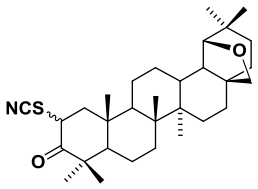
6.5.1 Triterpenoid aminothiazoles derived from allobetulin, methyl betulonate, methyl oleanonate, and oleanonic acid

2α/2β-bromo allobetulon (1b)



Epimeric mixture of **1b** was prepared according to the general procedure for the synthesis of 2-bromo-3-oxotriterpenoids (I) from allobetulon **1a** (2 g; 4.5 mmol) dissolved in chloroform (30 mL) and bromine solution (7 mL), monitored by TLC (hexane/ethyl acetate 5:1). After the standard work up and purification (toluene/Et_2O 20:1) white crystals of compound **1b** (1.98 g; 84 %) were obtained: R_f = 0.50 (silica gel, toluen/Et_2O 20:1); MP: 222-224 °C (hexane/MeOH); $[\alpha]_D^{20}$ = +68° (c = 0.55 in $CHCl_3$); (lit.[129,132] MP: 216-225 °C; $[\alpha]_D^{20}$ = +74°).

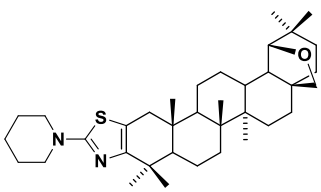
2α/2β-thiocyanato allobetulon (1c)



Epimeric mixture of **1c** was prepared according to the general procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids (I) from **1b** (500 mg; 4.5 mmol) and potassium thiocyanate (390 mg; 4.8 mmol) in dimethyl sulfoxide (25 mL) after 2 h, monitored by TLC (hexane/ethyl acetate 5:1). After the standard work up and purification (gradient elution: toluene → toluene/diethylether 5:1) compound **1c** was obtained as a mixture of epimers in the ratio 0.3:0.7 determined by the intensity of signals in NMR, white crystals (426 mg; 89 %): R_f = 0.20 (silica gel, toluene/diethylether 5:1); MP: 150-156 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +5° (c = 1.5 in $CHCl_3$); IR (DRIFT): 2930, 2860, 2152 (CN), 1700 (C=O), 1457, 1033 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.81, 0.82, 0.91, 0.95, 0.96, 0.97, 1.05, 1.12, 1.13, 1.15, 1.17, 1.20 (all s, 42H, 7 × CH_3 from both epimers), 2.02 (dd, J_1 = 13.5 Hz, J_2 = 8.3 Hz, 1H, H-1a from one epimer), 2.52 (t, J = 12.9 Hz, 1H, H-1b from one epimer), 2.75 (dd, J_1 = 12.9 Hz, J_2 = 6.0 Hz, 1H, H-1b from the other epimer), 3.46 (d, J = 7.7 Hz, 2H, H-28a from both epimers), 3.54 (s, 2H, H-19 from both epimers), 3.78 (d, J = 7.5 Hz, 2H, H-28b from both epimers), 4.71 – 4.76 (m, 2H, H-2 from both epimers); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.39, 15.02, 15.85, 16.43, 18.81, 18.98, 19.56, 19.85, 21.22, 21.31, 22.07, 24.50, 24.52, 24.76, 26.08, 26.14, 26.17, 26.26, 26.36, 26.38, 28.74, 29.14, 32.24, 32.66, 33.46, 34.02, 34.36, 36.24, 36.68, 38.53, 39.16, 40.45, 40.79, 40.82, 40.92, 41.40, 41.43, 46.67, 46.72,

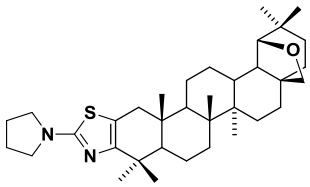
47.34, 49.51, 49.53, 50.24, 50.68, 51.05, 52.23, 52.35, 53.98, 57.41, 71.18, 71.20, 87.87, 87.88, 112.21, 112.46, 209.35, 211.62; MS (ESI) *m/z* (%): 498.4 (100) $[M+H]^+$; Anal. calcd for $C_{31}H_{47}NO_2S$: C 74.80, H 9.52, S 6.44, N 2.81; found C 74.80, H 9.64, S 6.02, N 2.57.

Thiazole derivative 1d



Compound **1d** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and piperidinium acetate (732 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1d** (536 mg; 95 %); R_f = 0.35 (silica gel, toluene/diethylether 5:1); MP: 152-155 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +50° (c = 0.79 in $CHCl_3$); IR (DRIFT): 2935, 2858, 1704 (C=O), 1553, 1524, 1415, 1035 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.91 (s, 3H), 0.94 (s, 6H), 1.03 (s, 3H), 1.13 (s, 3H), 1.23 (s, 3H, 7 × CH_3), 1.58 – 1.73 (m, 6H, H-piperidine), 2.15 (d, *J* = 15.2 Hz, 1H, H-1a), 2.61 (d, *J* = 14.9 Hz, 1H, H-1b), 3.30 – 3.43 (m, 4H, H-piperidine), 3.46 (d, *J* = 7.9 Hz, 1H, H-28a), 3.55 (s, 1H, H-19), 3.80 (d, *J* = 7.6 Hz, 1H, H-28b); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.49, 15.41, 16.51, 19.51, 21.45, 21.91, 24.25, 24.55, 25.15, 26.22, 26.45, 28.79, 29.68, 30.24, 32.68, 33.03, 34.25, 36.25, 36.70, 37.13, 38.56, 39.28, 40.59, 40.70, 41.48, 46.75, 49.41, 49.66, 52.92, 71.25, 87.88, 112.25, 143.65, 153.22, 166.55; MS (ESI) *m/z* (%): 565.4 (100) $[M+H]^+$; Anal. calcd for $C_{36}H_{56}N_2OS$: C 76.54, H 9.99, N 4.96, S 5.68; found C 76.70, H 9.81, N 4.42, S 5.44.

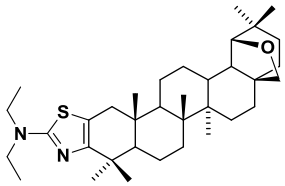
Thiazole derivative 1e



Compound **1e** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and pyrrolidinium acetate (655 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1e** (501 mg; 91 %): R_f = 0.20 (silica gel, toluene/diethylether 5:1); MP: 151-155 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +58° (c = 1.26 in $CHCl_3$); IR (DRIFT): 2944, 2865, 1702 (C=O), 1546, 1453, 1034 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.82 (s, 3H), 0.92 (s, 3H), 0.95 (s, 6H), 1.03 (s, 3H), 1.17 (s, 3H), 1.27 (s, 3H, 7 × CH_3), 1.96 – 2.04 (m, 4H, H-pyrrolidine), 2.16 (d, *J* = 15.2 Hz, 1H, H-1a), 2.61 (d, *J* = 15.3 Hz, 1H, H-1b), 3.36 – 3.51 (m, 5H, H-28a, H-pyrrolidine), 3.56 (s, 1H, H-19), 3.81 (d, *J* = 7.9 Hz, H-28b); ^{13}C NMR (75

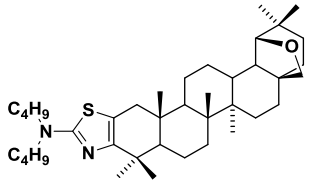
MHz, $CDCl_3$): δ ppm 13.50, 15.42, 16.49, 19.52, 21.45, 21.84, 24.55, 25.65, 26.22, 26.46, 28.80, 30.22, 32.69, 33.03, 34.26, 36.26, 36.71, 37.22, 38.57, 39.41, 40.59, 40.70, 41.48, 46.75, 49.66, 53.00, 71.25, 87. 89, 113.01, 143.31, 153.14, 164.67; MS (ESI): *m/z* (%) = 551.4 (100) $[M+H]^+$; Anal. calcd for $C_{35}H_{54}N_2OS$: C 76.31, H 9.88, N 5.09, S 5.82; found C 76.53, H 9.91, N 4.12, S 5.64.

Thiazole derivative 1f



Compound **1f** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and diethyl ammonium acetate (665 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1f** (520 mg; 94 %): R_f = 0.35 (silica gel, toluene/diethylether 5:1); MP: 152-156 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +57° (c = 0.43 in $CHCl_3$); IR (DRIFT): 2925, 2865, 1705 (C=O), 1535, 1468, 1035 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.93 (s, 3H), 0.94 (s, 6H), 1.03 (s, 3H), 1.12 (s, 3H), 1.23 (s, 3H, 7 × CH_3), 1.20 (t, *J* = 7.0 Hz, 6H, $(CH_3CH_2)_2N$), 2.14 (d, *J* = 15.3 Hz, 1H, H-1a), 2.59 (d, *J* = 15.0 Hz, 1H, H-1b), 3.35 – 3.47 (m, 5H, H-28a, $(CH_3CH_2)_2N$), 3.56 (s, 1H, H-19), 3.81 (d, *J* = 7.6 Hz, 1H, H-28b); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 12.72, 13.51, 15.44, 16.56, 19.55, 21.44, 21.92, 24.55, 26.23, 26.47, 28.80, 30.25, 32.70, 33.96, 34.26, 36.26, 36.72, 37.14, 38.61, 39.31, 40.60, 40.70, 41.48, 44.98, 46.77, 49.69, 52.93, 71.27, 87.90, 112.48, 143.17, 153.11, 166.19; MS (ESI) *m/z* (%): 553.4 (100) $[M+H]^+$; Anal. calcd for $C_{35}H_{56}N_2OS$: C 76.03, H 10.21, N 5.07, S 5.80; found C 75.54, H 10.36, N 4.64, S 5.57.

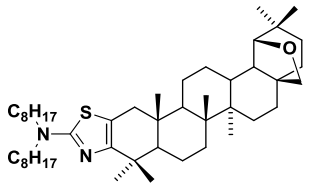
Thiazole derivative 1g



Compound **1g** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and dibutyl ammonium acetate (947 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1g** (463 mg; 76 %): R_f = 0.40 (silica gel, toluene/diethylether 5:1); MP: 148-152 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +55° (c = 0.51 in $CHCl_3$); IR (DRIFT): 2925, 2864, 1700 (C=O), 1538, 1465, 1035 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.92 – 0.97 (m, 15 H), 1.03 (s, 3H), 1.11 (s, 3H), 1.21 (s, 3H, 7 × CH_3, $(CH_3CH_2CH_2CH_2)_2N$), 1.28

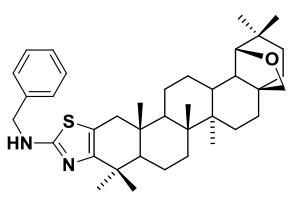
– 1.40 (m, 8H, $(CH_3CH_2CH_2CH_2)_2N$), 2.13 (d, J = 15.2 Hz, 1H, H-1a), 2.58 (d, J = 15.0 Hz, 1H, H-1b), 3.24 – 3.43 (m, 4H, $(CH_3CH_2CH_2CH_2)_2N$), 3.46 (d, J = 7.6 Hz, 1H, H-28a), 3.55 (s, 1H, H-19), 3.80 (d, J = 7.6 Hz, 1H, H-28b); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.51, 13.92, 15.44, 16.58, 19.55, 20.16, 21.42, 21.92, 24.55, 26.23, 26.48, 27.41, 28.80, 29.62, 30.23, 32.70, 33.07, 34.26, 36.26, 36.72, 37.14, 38.61, 39.31, 40.60, 40.70, 41.48, 46.77, 49.69, 50.81, 52.89, 71.28, 87.90, 112.27, 143.78, 153.01, 166.74; MS (ESI) *m/z* (%): 609.4 (100) $[M+H]^+$; Anal. calcd for $C_{39}H_{64}N_2OS$: C 76.92, H 10.59, N 4.60, S 5.27; found C 76.92, H 10.61, N 4.33, S 5.24.

Thiazole derivative 1h



Compound **1h** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and dioctyl ammonium acetate (1.50 g; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1h** (562 mg; 78 %): R_f = 0.45 (silica gel, toluene/diethylether 5:1); MP: 146-152 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +51° (c = 1.18 in $CHCl_3$); IR (DRIFT): 2925, 2856, 1706 (C=O), 1539, 1453, 1036 (C-O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.86 – 0.91 (m, 6H, $CH_3(CH_2)_7N$), 0.93 (s, 3H), 0.95 (s, 6H), 1.03 (s, 3H), 1.11 (s, 3H), 1.22 (s, 3H, 7 × CH_3), 1.25 – 1.33 (m, 20H, $(CH_3(CH_2)_6CH_2)_2N$), 2.14 (d, J = 15.2 Hz, 1H, H-1a), 2.59 (d, J = 14.9 Hz, 1H, H-1b), 3.23 – 3.41 (m, 4H, $(CH_3(CH_2)_6CH_2)_2N$), 3.46 (d, J = 7.9 Hz, 1H, H-28a), 3.56 (s, 1H, H-19), 3.81 (d, J = 7.9 Hz, 1H, H-28b); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.52, 14.11, 15.44, 16.58, 19.57, 21.43, 21.93, 22.65, 24.55, 26.24, 26.49, 26.98, 27.42, 27.46, 28.80, 29.24, 29.34, 30.25, 31.82, 32.70, 33.08, 34.27, 36.27, 36.73, 37.15, 38.62, 39.33, 40.61, 40.71, 41.49, 46.78, 49.70, 51.19, 52.90, 71.28, 87.91, 112.27, 143.09, 153.02, 166.72; MS (ESI) *m/z* (%): 721.6 (100) $[M+H]^+$; Anal. calcd for $C_{47}H_{80}N_2OS$: C 78.27, H 11.18, N 3.88, S 4.45; found C 77.65, H 11.09, N 3.53, S 4.43.

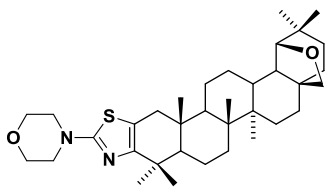
Thiazole derivative 1i



Compound **1i** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and benzyl ammonium acetate (836 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished

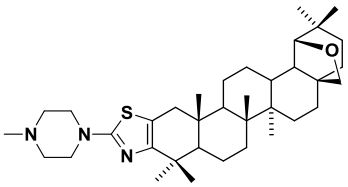
1i (522 mg; 89 %): R_f = 0.10 (silica gel, toluene/diethylether 5:1); MP: 148-154 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +56° (c = 0.61 in $CHCl_3$); IR (DRIFT): 3520 (NH), 2934, 2865, 1702 (C=O), 1542, 1463, 1035 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.92 (s, 3H), 0.95 (s, 6H), 1.03 (s, 3H), 1.14 (s, 3H), 1.24 (s, 3H, 7 × CH_3), 2.15 (d, *J* = 15.5 Hz, 1H, H-1a), 2.59 (d, *J* = 15.2 Hz, 1h, H-1b), 3.46 (d, *J* = 7.9 Hz, 1H, H-28a), 3.55 (s, 1H, H-19), 3.80 (d, *J* = 7.9 Hz, 1H, H-28b), 4.39 (s, 2H, PhCH_2), 5.59 (bs, 1H, NH), 7.29 – 7.44 (m, 5H, Ph); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.49, 15.43, 16.51, 19.45, 21.45, 21.99, 24.55, 26.22, 26.43, 26.47, 28.80, 30.23, 32.69, 33.00, 34.25, 36.26, 36.71, 37.00, 38.66, 39.28, 40.60, 40.71, 41.48, 46.75, 49.68, 50.13, 52.80, 71.25, 87.89, 114.31, 127.63, 127.72, 128.62, 137.71, 142.82, 151.94, 166.77; MS (ESI) *m/z* (%): 587.4 (100) $[M+H]^+$; Anal. calcd for $C_{38}H_{54}N_2OS$: C 77.76, H 9.27, N 4.77, S 5.46; found C 77.69, H 9.28, N 4.55, S 4.93.

Thiazole derivative 1j



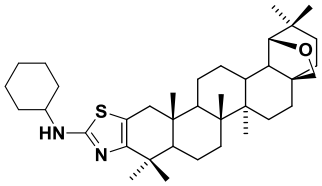
Compound **1j** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and morpholinium acetate (736 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1j** (510 mg; 90 %): R_f = 0.20 (silica gel, toluene/diethylether 5:1); MP: 151-155 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +65° (c = 0.95 in $CHCl_3$); IR (DRIFT): 2924, 2856, 1701 (C=O), 1529, 1452, 1036 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.91 (s, 3H), 0.95 (s, 6H), 1.03 (s, 3H), 1.12 (s, 3H), 1.22 (s, 3H, 7 × CH_3), 2.17 (d, *J* = 15.2 Hz, 1H, H-1a), 2.63 (d, *J* = 15.2 Hz, 1H, H-1b), 3.39 (t, *J* = 4.9 Hz, 4H, O(CH_2CH_2)$_2$N), 3.46 (d, *J* = 8.1 Hz, 1H, H-28a), 3.55 (s, 1H, H-19), 3.79 – 3.81 (m, 5H, H-28b, O(CH_2CH_2)$_2$N); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.49, 15.43, 16.49, 19.52, 21.47, 21.98, 24.55, 26.23, 26.45, 26.47, 28.79, 30.29, 32.71, 33.04, 34.27, 36.27, 36.73, 37.22, 38.63, 39.30, 40.62, 40.72, 41.49, 46.78, 48.57, 49.69, 52.89, 66.28, 71.27, 87.90, 115.21, 153.34, 168.39; MS (ESI) *m/z* (%): 567.4 (100) $[M+H]^+$; Anal. calcd for $C_{35}H_{54}N_2O_2S$: C 74.16, H 9.60, N 4.94, S 5.66; found C 73.43, H 9.63, N 4.59, S 6.03.

Thiazole derivative 1k



Compound **1k** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and *N*-methylpiperazinium acetate (802 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1k** (557 mg; 96 %): R_f = 0.15 (silica gel, toluene/diethylether 5:1); MP: 147-154 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +61° (c = 0.99, $CHCl_3$); IR (DRIFT): 2940, 2865, 1710 (C=O), 1529, 1453, 1035 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.80 (s, 3H), 0.90 (s, 3H), 0.94 (s, 6H), 1.02 (s, 3H), 1.11 (s, 3H), 1.21 (s, 3H, 7 × CH_3), 2.15 (d, *J* = 15.2 Hz, 1H, H-1a), 2.35 (s, 3H, *N*-CH_3), 2.52 – 2.55 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 2.61 (d, *J* = 15.2 Hz, 1H, H-1b), 3.40 – 3.48 (m, 5H, H-28a, $CH_3N(CH_2CH_2)_2N$), 3.54 (s, 1H, H-19), 3.79 (d, *J* = 7.6 Hz, 1H, H-28b); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.50, 15.42, 16.51, 19.51, 21.45, 21.98, 24.55, 26.22, 26.44, 28.79, 30.28, 32.69, 33.03, 34.25, 36.26, 36.71, 37.19, 38.59, 39.28, 40.60, 40.71, 41.48, 46.12, 46.75, 48.07, 49.66, 52.87, 54.27, 71.25, 87.88, 114.97, 147.97, 153.28, 168.03; MS (ESI) *m/z* (%): 580.4 (100) $[M+H]^+$; Anal. calcd for $C_{36}H_{57}N_3OS$: C 74.56, H 9.91, N 7.25, S 5.53; found C 73.13, H 9.44, N 6.58, S 5.23.

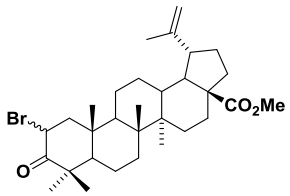
Thiazole derivative 1l



Compound **1l** was prepared according to the general procedure from 2α/2β-thiocyanato allobetulon (**1c**) (500 mg; 1.0 mmol) and cyclohexyl ammonium acetate (797 mg; 5.0 mmol) in chloroform (20 mL) after 24 hours controlled by TLC (toluene/diethylether 5:1). Dissolved crude product was filtered through a short pad of silica gel. Evaporation of solvent furnished **1l** (504 mg; 87 %): R_f = 0.10 (silica gel, toluene/diethylether 5:1); MP: 149-151 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +60° (c = 0.92 in $CHCl_3$); IR (DRIFT): 3500 (NH), 2927, 2856, 1705 (C=O), 1538, 1452, 1032 (C-O) cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.92 (s, 3H), 0.94 (s, 6H), 1.03 (s, 3H), 1.12 (s, 3H), 1.22 (s, 3H, 7 × CH_3), 2.14 (d, *J* = 15.2 Hz, 1H, H-1a), 2.58 (d, *J* = 15.3 Hz, 1H, H-1b), 3.08 – 3.20 (m, 1H, H-cyclohexyl), 3.46 (d, *J* = 7.9 Hz, 1H, H-28a), 3.55 (s, 1H, H-19), 3.80 (d, *J* = 7.6 Hz, 1H, H-28b), 5.30 (bs, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 13.49, 15.42, 16.50, 19.44, 21.45, 21.94, 24.54, 24.64, 25.52, 26.21, 26.44, 26.46, 28.80, 30.20, 32.68, 33.00, 33.04, 33.08, 34.24, 36.26, 36.70, 36.89, 38.64, 39.30, 40.59, 40.71, 41.48, 46.75, 49.68, 52.81,

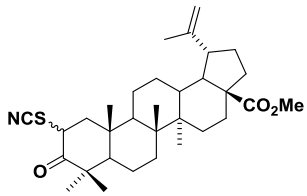
55.25, 71.25, 87.89, 113.27, 145.26, 151.93, 166.25; MS (ESI) *m/z* (%): 578.4 (100) $[M+H]^+$; Anal. calcd for $C_{37}H_{58}N_2OS$: C 76.76, H 10.10, N 4.84, S 5.54; found C 76.23, H 10.06, N 4.87, S 5.40.

Methyl 2α/2β-bromo betulonate (2b)



Epimeric mixture of **2b** was prepared according to the general procedure from methyl betulonate (**2a**) (1.0 g; 2.1 mmol) and $CuBr_2$ (1.0 g; 4.5 mmol) in mixture of ethyl acetate (20 mL) and methanol (10 mL) after 3 hours, monitores by TLC (toluene/Et_2O 5:1). After the standard work up and purification (hexane/EtOAc 7:1) compound **2b** (853 mg; 73 %) was obtained; MP: 104-107 °C (toluene/diethylether); R_f = 0.47 and 0.53 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2945, 2869, 1724 (C=O), 1642 (C=C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.80, 0.91, 0.95, 0.97, 1.00, 1.09, 1.12, 1.12, 1.13, 1.19 (all s, 30H, 10 × CH_3 from both epimers), 1.68 (s, 3H, H-30 from one epimer), 1.69 (s, 3H, H-30 from the other epimer), 2.04 (dd, J_1 = 13.5 Hz, J_2 = 9.4 Hz, 1H, H-1a from one epimer), 2.20 – 2.28 (m, 4H), 2.46 (dd, J_1 = 13.4 Hz, J_2 = 11.4 Hz, 1H, H-1b from one epimer), 2.64 (dd, J_1 = 12.9 Hz, J_2 = 6.3 Hz, 1H, H-1b from the other epimer), 2.97 – 3.02 (m, 2H, H-19 from both epimers), 3.67 (s, 3H, CO_2CH_3 from one epimer), 3.68 (s, 3H, CO_2CH_3 from the other epimer), 4.60 – 4.61 (m, 2H, H-29a from both epimers), 4.74 – 4.74 (m, 2H, H-29b from both epimers), 5.04 – 5.10 (m, 2H, H-2 from both epimers); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.67, 15.39, 16.00, 16.14, 18.93, 19.33, 19.37, 19.46, 20.02, 20.11, 21.26, 21.84, 22.12, 25.36, 25.63, 26.34, 29.39, 29.68, 29.78, 30.60, 30.62, 32.13, 32.19, 32.93, 33.94, 37.01, 38.23, 38.57, 39.44, 40.11, 40.72, 40.91, 42.56, 42.64, 46.97, 47.04, 47.53, 49.35, 49.40, 49.46, 49.99, 50.05, 51.38, 51.40, 51.51, 52.29, 52.62, 52.88, 54.23, 56.53, 56.58, 56.73, 56.74, 109.84, 109.88, 150.41, 150.41, 176.65, 176.67, 207.11, 209.22; HRMS (ESI): *m/z* calcd for $C_{31}H_{47}BrO_3$ $[M+H]^+$ 547.2781, found 547.2784.

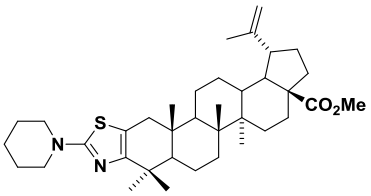
Methyl 2α/2β-thiocyanato betulonate (2c)



Epimeric mixture of **2c** was prepared according to the general procedure from compound **2b** (5.0 g; 9 mmol) and ammonium thiocyanate (3.6 g; 45 mmol) in *N*-methylpyrrolidone (50 mL) after 4 hours at 50 °C, controlled by TLC (toluene/diethylether 5:1). After standard work up and purification (gradient elution: toluene → toluene/diethylether 5:1) compound **2c** (3.5 g; 74 %) was obtained; MP: 116-118 °C (toluene/diethylether); R_f = 0.39

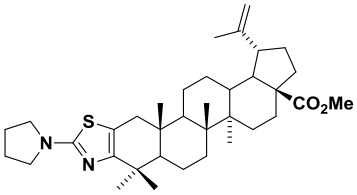
(silica gel, toluene/diethylether 40:1); IR (DRIFT): 2949, 2867, 2155 (CN), 1723 (C=O), 1642 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): mixture of epimers in the ratio 0.3:0.7: 0.79, 0.92, 0.96, 0.99, 1.01, 1.11, 1.12, 1.14, 1.15, 1.17 (all s, 30H, 10 × CH_3 from both epimers), 1.68, 1.70 (both s, 6H, H-30 from both epimers), 1.96 (dd, 1H, J_1 = 13.7 Hz, J_2 = 8.6 Hz, H-1a from one epimer), 2.22 – 2.29 (m, 4H), 2.49 (dd, 1H, J_1 = 13.2 Hz, J_2 = 12.0 Hz, H-1b from one epimer), 2.72 (dd, 1H, J_1 = 12.6 Hz, J_2 = 6.1 Hz, H-1b from other epimer), 2.97 – 3.02 (m, 2H, H-19 from both epimers), 3.67, 3.68 (both s, 6H, CO_2CH_3 from both epimers), 4.61 – 4.63 (m, 2H, H-29a from both epimers), 4.69 – 4.73 (m, 2H, H-2 from both epimers), 4.74 – 4.75 (m, 2H, H-29b from both epimers); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.09, 14.56, 14.58, 15.22, 16.07, 16.10, 18.57, 19.02, 19.24, 19.26, 19.52, 19.87, 21.18, 21.23, 22.02, 24.76, 25.18, 25.43, 29.12, 29.56, 30.46, 31.56, 32.02, 32.09, 32.67, 33.88, 36.91, 38.08, 38.42, 38.44, 39.08, 40.55, 40.87, 42.48, 42.58, 46.91, 46.94, 47.37, 49.26, 49.35, 49.40, 49.48, 49.81, 50.21, 50.91, 51.30, 52.20, 52.26, 53.95, 56.38, 56.43, 57.29, 109.87, 109.90, 112.17, 112.38, 150.17, 150.20, 176.55, 176.55, 209.41, 211.67; HRMS (ESI): *m/z* calcd for $C_{32}H_{47}NO_3S$ $[M+H-CH_3COOH]^+$ 526.3349, found 526.3352.

Thiazole derivative 2d



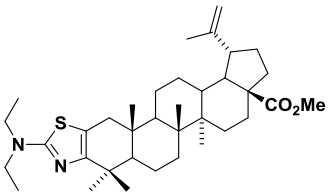
Compound **2d** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and piperidinium acetate (415 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (gradient elution: toluene → toluene/diethylether 10:1) compound **2d** (130 mg; 38 %) was obtained; MP: 166-168 °C (toluene/diethylether); R_f = 0.52 (silica gel, toluene/diethylether, 40:1); IR (DRIFT): 2936, 2866, 1720 (C=O), 1670 (C=C), 1523, 1452 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.88, 0.97, 1.00, 1.11, 1.21 (all s, 15H, 5 × CH_3), 1.70 (s, 3H, H-30), 2.11 (d, J = 15.5 Hz, 1H, H-1a), 2.24 – 2.29 (m, 2H), 2.56 (d, J = 15.5 Hz, 1H, H-1b), 3.00 – 3.05 (m, 1H, H-19), 3.37 (t, J = 5.7 Hz, 4H, piperidine), 3.68 (s, 3H, CO_2CH_3), 4.61 (s, 1H, H-29a), 4.75 (s, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.10, 14.69, 15.62, 16.14, 19.36, 19.59, 21.36, 21.98, 22.63, 24.30, 25.16, 25.57, 29.73, 30.26, 30.58, 31.57, 32.11, 33.49, 36.94, 37.12, 38.37, 38.46, 39.09, 40.70, 42.40, 46.92, 49.20, 49.41, 51.26, 52.78, 56.57, 109.58, 114.13, 150.54, 153.01, 168.67, 176.66; HRMS (ESI): *m/z* calcd for $C_{37}H_{56}N_2O_2S$ $[M+H]^+$ 593.4135, found 593.4136.

Thiazole derivative 2e



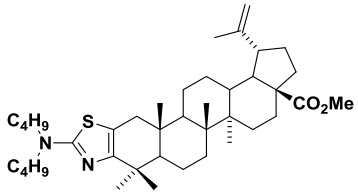
Compound **2e** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and pyrrolidinium acetate (380 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (gradient elution: toluene → toluene/diethylether 20:1) compound **2e** (184 mg; 56 %) was obtained; MP: 218-220 °C (toluene/diethylether); R_f = 0.15 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2945, 2869, 1725 (C=O), 1670 (C=C), 1541, 1448 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.89, 0.97, 1.00, 1.14, 1.24 (all s, 15H, 5 × CH_3), 1.70 (s, 3H, H-30), 1.96 – 2.09 (m, 4H, pyrrolidine), 2.11 (d, *J* = 15.6 Hz, 1H, H-1a), 2.22 – 2.29 (m, 2H), 2.56 (d, *J* = 15.1 Hz, 1H, H-1b), 2.99 – 3.05 (m, 1H, H-19), 3.41 (t, *J* = 6.3 Hz, 4H, pyrrolidine), 3.68 (s, 3H, CO_2CH_3), 4.62 (s, 1H, H-29a), 4.76 (s, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.69, 15.60, 16.13, 19.35, 19.60, 21.34, 21.92, 25.56, 25.63, 29.73, 30.25, 30.57, 32.10, 33.48, 36.92, 37.20, 38.36, 38.45, 39.21, 40.67, 42.39, 46.92, 49.19, 49.25, 49.38, 51.27, 52.85, 56.56, 109.58, 113.12, 150.54, 153.27, 164.68, 176.66; HRMS (ESI): *m/z* calcd for $C_{36}H_{54}N_2O_2S$ $[M+H]^+$ 579.3979, found 579.3978.

Thiazole derivative 2f



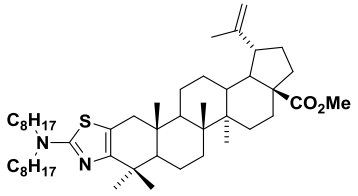
Compound **2f** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and diethyl ammonium acetate (380 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene) compound **2f** (189 mg; 57 %) was obtained; MP: 179-182 °C (toluene/diethylether); R_f = 0.48 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2933, 2868, 1725 (C=O), 1665 (C=C), 1541, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.90 (s, 3H), 0.98 (s, 3H), 1.00 (s, 3H), 1.12 (s, 3H), 1.19 (t, *J* = 6.8 Hz, 6H, $(CH_3CH_2)_2N$), 1.22 (s, 3H, 5 × CH_3), 1.70 (s, 3H, H-30), 2.10 (d, *J* = 14.9 Hz, 1H, H-1a), 2.23 – 2.29 (m, 2H), 2.55 (d, *J* = 15.5 Hz, 1H, H-1b), 3.00 – 3.05 (m, 1H, H-19), 3.35 – 3.48 (m, 4H, $(CH_3CH_2)_2N$), 3.69 (s, 3H, CO_2CH_3), 4.62 (s, 1H, H-29a), 4.76 (d, *J* = 2.3 Hz, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 12.72, 14.70, 15.63, 16.20, 19.37, 19.62, 21.36, 21.94, 25.59, 29.68, 29.74, 30.23, 30.59, 32.12, 33.51, 36.94, 37.12, 38.39, 38.52, 39.11, 40.70, 42.40, 45.00, 46.93, 49.21, 49.41, 51.25, 51.26, 52.80, 56.58, 109.57, 112.57, 150.56, 153.05, 166.19, 176.67; HRMS (ESI): *m/z* calcd for $C_{36}H_{56}N_2O_2S$ $[M+H]^+$ 581.4135, found 581.4136.

Thiazole derivative 2g



Compound **2g** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and dibutyl ammonium acetate (540 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene) compound **2g** (155 mg; 43 %) was obtained; MP: 136-137 °C (toluene/diethylether); R_f = 0.75 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2933, 2867, 1727 (C=O), 1675 (C=C), 1538, 1452 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.90 (s, 3H), 0.95 (t, J = 7.4 Hz, 6H, ($CH_3CH_2CH_2CH_2$)$_2$N), 0.98 (s, 3H), 1.00 (s, 3H), 1.11 (s, 3H), 1.21 (s, 3H, 5 × CH_3), 1.30 – 1.38 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.57 – 1.63 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.70 (s, 3H, H-30), 2.10 (d, J = 15.5 Hz, 1H, H-1a), 2.23 – 2.29 (m, 2H), 2.54 (d, J = 14.9 Hz, 1H, H-1b), 3.00 – 3.05 (m, 1H, H-19), 3.26 – 3.41 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 3.69 (s, 3H, CO_2CH_3), 4.62 (s, 1H, H-29a), 4.76 (s, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 13.91, 14.10, 14.71, 15.64, 16.23, 19.39, 19.63, 20.17, 21.36, 21.95, 22.64, 25.60, 29.65, 29.76, 30.22, 30.60, 31.58, 32.13, 33.52, 36.96, 37.14, 38.39, 38.54, 39.11, 40.72, 42.40, 46.95, 49.22, 49.43, 50.84, 51.26, 52.76, 56.60, 109.58, 112.37, 150.57, 152.97, 166.75, 176.67; HRMS (ESI): *m/z* calcd for $C_{40}H_{64}N_2O_2S$ $[M+H]^+$ 637.4761, found 637.4761.

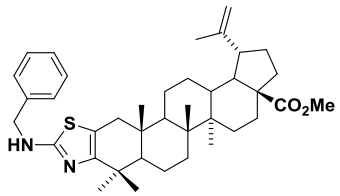
Thiazole derivative 2h



Compound **2h** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and dioctyl ammonium acetate (865 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene) compound **2h** (148 mg; 35 %) was obtained; MP: 136-138 °C (toluene/diethylether); R_f = 0.74 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2925, 2855, 1728 (C=O), 1669 (C=C), 1539, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.87 – 0.89 (m, 9H), 0.97 (s, 3H), 0.99 (s, 3H), 1.10 (s, 3H), 1.20 (s, 3H, (CH_3(CH_2)$_7$)$_2$N, 5 × CH_3), 1.28 – 1.30 (m, 20H, (CH_3(CH_2)$_6$$CH_2$)$_2$N), 1.40 – 1.45 (m, 8H), 1.60 – 1.63 (m, 6H), 1.70 (s, 3H, H-30), 2.09 (d, J = 15.0 Hz, 1H, H-1a), 2.24 – 2.29 (m, 2H), 2.53 (d, J = 15.01 Hz, 1H, H-1b), 3.00 – 3.05 (m, 1H, H-19), 3.23 – 3.39 (m, 4H, (CH_3(CH_2)$_6$$CH_2$)$_2$N), 3.68 (s, 3H, CO_2CH_3), 4.62 (s, 1H, H-29a), 4.76 (s, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.10, 14.70, 15.62, 16.22, 19.37, 19.62, 21.34, 21.95, 22.64, 25.58, 26.97, 27.41, 29.22, 29.34, 29.74, 30.22, 30.58, 31.81, 32.11, 33.50, 36.94,

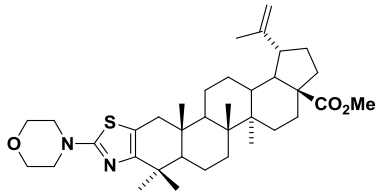
37.12, 38.36, 38.53, 39.10, 40.69, 42.39, 46.93, 49.20, 49.40, 51.19, 51.27, 52.74, 56.58, 109.58, 112.36, 150.57, 152.95, 166.70, 176.67; HRMS (ESI): *m/z* calcd for $C_{48}H_{80}N_2O_2S$ $[M+H]^+$ 749.6013, found 749.6006.

Thiazole derivative 2i



Compound **2i** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and benzyl ammonium acetate (480 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 10:1) compound **2i** (184 mg; 52 %) was obtained; MP: 167-168 °C (toluene/diethylether); R_f = 0.11 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 3377 (NH), 2943, 2867, 1724 (C=O), 1670 (C=C), 1541, 1460, 698 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.89, 0.97, 0.99, 1.12, 1.22 (all s, 15H, 5 × CH_3), 1.70 (s, 3H, H-30), 1.89 – 1.95 (m, 2H), 2.10 (d, *J* = 15.5 Hz, 1H, H-1a), 2.24 – 2.29 (m, 2H), 2.54 (d, *J* = 15.1 Hz, 1H, H-1b), 3.00 – 3.05 (m, 1H, H-19), 3.68 (s, 3H, CO_2CH_3), 4.38 (s, 2H, Ph*CH*$_2$), 4.62 (s, 1H, H-29a), 4.75 (s, 1H, H-29b), 5.37 (bs, 1H, NH), 7.29 – 7.39 (m, 5H, Ph); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.67, 15.61, 16.15, 19.40, 19.53, 21.35, 22.02, 25.54, 29.72, 30.24, 30.57, 32.09, 33.45, 36.92, 37.02, 38.34, 38.54, 39.13, 40.67, 42.39, 46.96, 49.19, 49.38, 50.09, 51.25, 52.69, 56.56, 109.57, 114.46, 127.55, 127.65, 127.71, 128.56, 128.61, 137.96, 150.53, 152.45, 166.62, 176.64; HRMS (ESI): *m/z* calcd for $C_{39}H_{54}N_2O_2S$ $[M+H]^+$ 615.3979, found 615.3976.

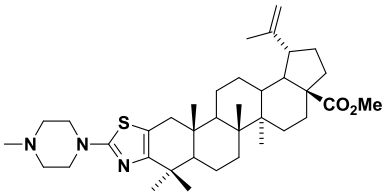
Thiazole derivative 2j



Compound **2j** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and morpholinium acetate (420 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 10:1) compound **2j** (208 mg; 61 %) was obtained; MP: 193 °C (toluene/diethylether); R_f = 0.21 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2945, 2863, 1725 (C=O), 1671 (C=C), 1529, 1458, 1119 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.88, 0.97, 1.00, 1.11, 1.21 (all s, 15H, 5 × CH_3), 1.70 (s, 3H, H-30), 2.13 (d, *J* = 15.4 Hz, 1H, H-1a), 2.24 – 2.29 (m, 2H), 2.58 (d, *J* = 15.2 Hz, 1H, H-1b), 2.99 – 3.04 (m, 1H, H-19), 3.38 (t, *J* = 4.5 Hz, 4H, O(CH_2*CH*$_2$)$_2$N), 3.68 (s, 3H, CO_2CH_3), 3.79 (t, *J* = 4.9 Hz, 4H, O(*CH*$_2$$CH_2$)$_2$N), 4.61 (s, 1H, H-29a), 4.75 (1H, H-29b); ^{13}C NMR

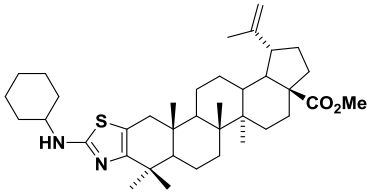
(126 MHz, $CDCl_3$): δ ppm 14.67, 14.68, 15.60, 16.13, 19.36, 19.56, 21.36, 21.98, 25.54, 29.72, 30.24, 30.57, 32.08, 33.46, 36.92, 37.18, 38.34, 38.51, 39.07, 40.69, 42.39, 46.91, 48.56, 49.19, 49.39, 51.26, 52.71, 56.55, 66.26, 109.58, 115.26, 150.51, 153.23, 168.35, 176.62; HRMS (ESI): *m/z* calcd for $C_{36}H_{54}N_2O_3S$ $[M+H]^+$ 595.3928, found 595.3929.

Thiazole derivative 2k



Compound **2k** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (460 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (methanol/chloroform 3:1) compound **2k** (179 mg; 52 %) was obtained; MP: 169 °C (toluene/diethylether); R_f = 0.52 (silica gel, methanol/chloroform 5:1); IR (DRIFT): 2939, 2868, 1726 (C=O), 1669 (C=C), 1522, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.88, 0.97, 0.99, 1.11, 1.21 (all s, 15H, 5 × CH_3), 1.69 (s, 3H, H-30), 2.11 (d, *J* = 15.0 Hz, 1H, H-1a), 2.23 – 2.29 (m, 2H), 2.33 (s, 3H, CH_3N), 2.50 (t, *J* = 4.7 Hz, 4H, $CH_3N(CH_2CH_2)_2N$), 2.57 (d, *J* = 15.1 Hz, 1H, H-1b), 2.99 – 3.05 (m, 1H, H-19), 3.42 (t, *J* = 5.2 Hz, 4H, $CH_3N(CH_2CH_2)_2N$), 3.68 (s, 3H, CO_2CH_3), 4.61 (s, 1H, H-29a), 4.75 (s, 1H, H-29b); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.68, 15.60, 16.14, 19.32, 19.38, 19.56, 21.35, 21.99, 25.53, 29.72, 30.25, 30.56, 32.09, 33.46, 36.90, 37.14, 38.34, 38.48, 39.05, 40.67, 42.39, 46.23, 46.87, 46.95, 48.20, 49.19, 49.38, 51.25, 52.72, 54.35, 56.55, 109.62, 114.93, 150.53, 153.19, 168.11, 176.65; HRMS (ESI): *m/z* calcd for $C_{37}H_{57}N_3O_2S$ $[M+H]^+$ 608.4244, found 608.4245.

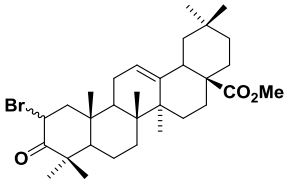
Thiazole derivative 2l



Compound **2l** was prepared according to the general procedure from compound **2c** (300 mg; 0.6 mmol) and cyclohexyl ammonium acetate (455 mg; 2.9 mmol) in chloroform (12 mL) after 36 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 10:1) compound **2l** (175 mg; 51 %) was obtained; MP: 187-188 °C (toluene/diethylether); R_f = 0.10 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 3345 (NH), 2933, 2865, 1724 (C=O), 1670 (C=C), 1538, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.88, 0.97, 0.99, 1.11, 1.20 (all s, 15H, 5 × CH_3), 1.70 (s, 3H, H-30), 2.09 (d, *J* = 15.6 Hz, 1H, H-1a), 2.23 – 2.29 (m, 2H), 2.52 (d, *J* = 15.0 Hz, 1H, H-1b), 2.98 – 3.05 (m, 1H, H-19), 3.06 – 3.18 (m, 1H, cyclohexyl), 3.68 (s,

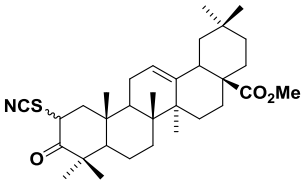
3H, CO_2CH_3), 4.61 (s, 1H, H-29a), 4.75 (s, 1H, H-29b), 5.15 (bs, 1H, NH); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.65, 15.60, 16.13, 19.31, 19.38, 19.46, 21.35, 21.90, 24.68, 25.51, 29.71, 30.13, 30.54, 32.07, 33.03, 33.43, 36.84, 36.88, 36.92, 38.32, 38.54, 39.06, 40.66, 42.38, 46.84, 46.95, 49.18, 49.37, 51.25, 51.28, 52.66, 56.55, 109.57, 119.89, 150.54, 166.25, 176.64; HRMS (ESI): *m/z* calcd for $C_{38}H_{58}N_2O_2S$ $[M+H]^+$ 607.4292, found 607.4292.

Methyl 2α/2β-bromo oleanonate (3b)



Epimeric mixture of **3b** was prepared according to the general procedure from methyl oleanonate (**3a**) (8.0 g; 17 mmol) and $CuBr_2$ (8.0 g; 35 mmol) in mixture of ethyl acetate (80 mL) and methanol (3 mL) after 12 hours, monitores by TLC (toluene/Et_2O 5:1). After standard work up and purification (gradient elution toluene → toluene/EtOAc 30:1) compound **3b** (6.7 g; 84 %) was obtained; MP: 97-100 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +75.9° (c = 1.6, $CHCl_3$); R_f = 0.41 (silica gel, toluene/diethylether 30:1); IR (DRIFT): 2945, 2864, 1721 (C=O), 1696 (C=C), 1456 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.78 (s, 3H), 0.91 (s, 3H), 0.93 (s, 3H), 1.11 (s, 3H), 1.16 (d, J = 2.6 Hz, 3H), 1.21 (s, 3H), 1.22 (s, 3H, 7 × CH_3), 2.57 (dd, J_1 = 12.9 Hz, Hz, J_2 = 6.2 Hz, 1H, H-1b), 2.87 (dd, J_1 = 14.0 Hz, J_2 = 2.6 Hz, 1H, H-18), 3.64 (s, 3H, CO_2CH_3), 5.05 – 5.13 (m, 1H, H-2), 5.28 – 5.33 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.25, 16.96, 19.27, 19.98, 21.97, 22.96, 23.46, 23.49, 23.60, 25.86, 26.30, 27.64, 30.69, 32.18, 32.29, 33.08, 33.80, 39.36, 39.84, 41.24, 41.73, 45.75, 47.01, 49.35, 51.58, 56.63, 118.32, 121.58, 144.08, 174.04, 183.94; HRMS (ESI): *m/z* calcd for $C_{31}H_{47}BrO_3$ 547.61, found $[M-H+Na]^+$ 569.26; Anal. calcd for $C_{31}H_{47}BrO_3$: C 66.99, Br 14.59, H 8.65; found C 67.81, Br 15.84, H 8.79.

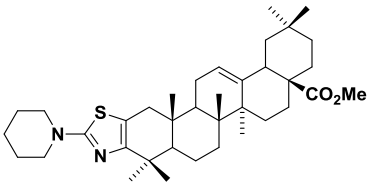
Methyl 2α/2β-thiocyanato oleanonate (3c)



Epimeric mixture of **3c** was prepared according to the general procedure from **3b** (6.0 g; 11 mmol) and ammonium thiocyanate (8.0 g; 104 mmol) in *N*-methylpyrrolidone (60 mL) after 6 hours at 60 °C, controlled by TLC (toluene/diethylether 40:1). After standard work up and purification (gradient elution: toluene → toluene/diethylether 40:1) compound **3c** (4.2 g; 70 %) was obtained; MP: 124-125 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +16.3° (c = 1.5, $CHCl_3$); R_f = 0.31 (silica gel, toluene/diethylether, 40:1); IR (DRIFT): 2948, 2870, 2155 (CN), 1722 (C=O), 1640 (C=C), 1458 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.79, 0.91, 0.94, 1.12, 1.14, 1.16, 1.26 (all s, 21H, 7 × CH_3), 2.64 (dd, J_1 = 12.9 Hz, J_2 = 6.2 Hz, 1H, H-

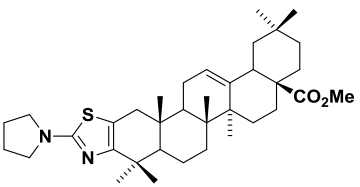
1b), 2.89 (dd, J_1 = 13.5 Hz, J_2 = 2.9 Hz, 1H, H-19), 3.64 (s, 3H, CO_2CH_3), 4.70 – 4.76 (m, 1H, H-2), 5.29 – 5.34 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.42, 16.99, 19.08, 21.44, 22.92, 23.56, 23.59, 24.88, 25.88, 27.62, 30.68, 31.21, 32.26, 33.07, 33.79, 38.90, 39.39, 41.15, 41.71, 45.72, 46.61, 47.30, 48.96, 49.51, 51.59, 53.88, 57.18, 112.50, 121.44, 144.04, 178.19, 209.36; HRMS (ESI): *m/z* calcd for $C_{32}H_{47}NO_3S$ 525.79, found $[M+Na]^+$ 548.32, $[M+H]^+$ 526.33; Anal. calcd for $C_{32}H_{47}NO_3S$: C 73.10, H 9.01, S 6.10, N 2.66; found C 72.08, H 9.13, S 5.63, N 2.30.

Thiazole derivative 3d



Compound **3d** was prepared according to the general procedure from compound **3c** (700 mg; 1.3 mmol) and piperidinium acetate (730 mg; 5.0 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 40:1). After standard work up, purification (gradient elution: toluene → toluene/diethylether 40:1) and lyophilization compound **3d** (560 mg; 80 %) was obtained; MP: 133-135 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +91.0° (c = 1.5, $CHCl_3$); R_f = 0.50 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2945, 2864, 1725 (C=O), 1695 (C=C), 1522, 1450 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.80, 0.91, 0.94, 0.96, 1.14, 1.16, 1.23 (all s, 21H, 7 × CH_3), 1.61 – 1.67 (m, 6H, piperidine), 2.17 (d, J = 15.2 Hz, 1H, H-1a), 2.51 (d, J = 15.3 Hz, 1H, H-1b), 2.90 (dd, J_1 = 13.7 Hz, J_2 = 3.8 Hz, 1H, H-19), 3.34 – 3.42 (m, 4H, piperidine), 3.64 (s, 3H, CO_2CH_3), 5.32 – 5.36 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.36, 16.56, 19.66, 22.14, 23.11, 23.30, 23.60, 24.30, 25.16, 25.76, 26.92, 27.72, 30.43, 30.69, 32.09, 32.37, 33.10, 33.88, 36.99, 37.20, 38.27, 38.60, 39.34, 41.41, 41.79, 45.86, 46.16, 46.77, 49.44, 51.53, 52.67, 113.84, 122.33, 143.70, 153.01, 168.65, 178.29; HRMS (ESI): *m/z* calcd for $C_{37}H_{56}N_2O_2S$ 592.92, found $[M+H]^+$ 593.41; Anal. calcd for $C_{37}H_{56}N_2O_2S$: C 74.95, H 9.52, S 5.41, N 4.72; found C 74.25, H 9.46, S 5.27, N 4.42.

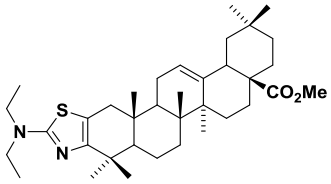
Thiazole derivative 3e



Compound **3e** was prepared according to the general procedure from compound **3c** (700 mg; 1.3 mmol) and pyrrolidinium acetate (700 mg; 5.3 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 40:1). After standard work up, purification (gradient elution: toluene → toluene/diethylether 40:1) and lyophilization compound **3e** (525 mg; 75 %) was obtained; MP: 143-145 °C

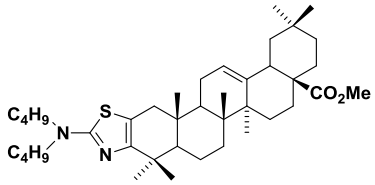
(toluene/diethylether); $[\alpha]_D^{20}$ = +101.9° (c = 1.6, $CHCl_3$); R_f = 0.23 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2945, 2864, 1725 (C=O), 1695 (C=C), 1542, 1460 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.79 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.97 (s, 3H), 1.16 (s, 6H), 1.25 (s, 3H, 7 × CH_3), 1.96 – 2.01 (m, 4H, pyrrolidine), 2.18 (d, J = 15.2 Hz, 1H, H-1a), 2.51 (d, J = 15.3 Hz, 1H, H-1b), 2.87 (dd, J_1 = 13.1 Hz, J_2 = 4.1 Hz, 1H, H-19), 3.37 – 3.46 (m, 4H, pyrrolidine), 3.64 (s, 3H, CO_2CH_3), 5.32 – 5.37 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.34, 16.56, 19.67, 22.06, 23.10, 23.29, 23.59, 25.64, 25.76, 27.71, 30.41, 30.69, 32.08, 32.36, 33.10, 33.87, 36.95, 37.08, 38.26, 38.71, 39.32, 41.40, 41.79, 45.84, 46.14, 46.77, 49.30, 51.54, 52.75, 112.80, 122.32, 143.69, 149.48, 164.66, 178.30; HRMS (ESI): *m/z* calcd for $C_{36}H_{54}N_2O_2S$ 578.89, found $[M+H]^+$ 579.40; Anal. calcd for $C_{36}H_{54}N_2O_2S$: C 74.69, H 9.40, S 5.54, N 4.84; found C 74.01, H 9.26, S 5.02, N 4.28.

Thiazole derivative 3f



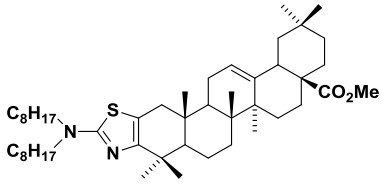
Compound **3f** was prepared according to the general procedure from compound **3c** (700 mg; 1.3 mmol) and diethylamonium acetate (700 mg; 5.3 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 40:1). After standard work up, purification (gradient elution: toluene → toluene/diethylether 40:1) and lyophilization compound **3f** (518 mg; 74 %) was obtained; MP: 99-101 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +100.0° (c = 1.5, $CHCl_3$); R_f = 0.35 (silica gel, toluene/diethylether, 40:1); IR (DRIFT): 2943, 2870, 1725 (C=O), 1695 (C=C), 1538, 1462 cm^{-1} ; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.80 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.98 (s, 3H), 1.16 (s, 3H), 1.16 (t, J = 3.9 Hz, 6H, $(CH_3CH_2)_2N$), 1.20 (s, 3H), 1.23 (d, J = 2.1 Hz, 3H, 7 × CH_3), 2.16 (d, J = 15.2 Hz, 1H, H-1a), 2.49 (d, J = 15.2 Hz, 1H, H-1b), 2.90 (dd, J_1 = 13.4 Hz, J_2 = 4.1 Hz, 1H, H-19), 3.32 – 3.53 (m, 4H, $(CH_3CH_2)_2N$), 3.64 (s, 3H, CO_2CH_3), 5.32 – 5.37 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 12.72, 15.43, 16.59, 19.68, 22.10, 23.12, 23.31, 23.61, 25.75, 27.06, 27.73, 30.38, 30.70, 32.11, 32.37, 33.11, 33.88, 37.00, 38.31, 38.61, 39.35, 41.42, 41.80, 45.02, 45.23, 45.87, 46.17, 46.79, 51.55, 52.69, 112.26, 122.36, 143.70, 151.68, 166.17, 178.31; HRMS (ESI): *m/z* calcd for $C_{36}H_{56}N_2O_2S$ 580.91, found $[M+H]^+$ 581.41; Anal. calcd for $C_{36}H_{56}N_2O_2S$: C 74.43, H 9.72, S 5.52, N 4.82; found C 74.15, H 9.78, S 5.39, N 4.57.

Thiazole derivative 3g



Compound **3g** was prepared according to the general procedure from compound **3c** (300 mg; 0.6 mmol) and dibutyl ammonium acetate (290 mg; 1.5 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 20:1) compound **3g** (146 mg; 40 %) was obtained; MP: 148-151 °C (toluene/diethylether); R_f = 0.75 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2948, 2862, 1727 (C=O), 1695 (C=C), 1538, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.80, 0.91, 0.94 (all s, 9H, 3 × CH_3), 0.95 (t, J = 7.3 Hz, 6H, ($CH_3CH_2CH_2CH_2)_2N$), 0.98, 1.13, 1.16, 1.22 (all, s, 12H, 4 × CH_3), 1.29 – 1.39 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 1.59 – 1.67 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 2.16 (d, J = 15.5 Hz, 1H, H-1a), 2.48 (d, J = 15.6 Hz, 1H, H-1b), 2.90 (dd, J_1 = 13.5 Hz, J_2 = 4.2 Hz, 1H, H-19), 3.26 – 3.41 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 3.64 (s, 3H, CO_2CH_3), 5.34 (t, J = 3.8 Hz, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 13.91, 15.44, 16.58, 19.69, 20.16, 22.10, 23.12, 23.29, 23.60, 25.75, 27.72, 29.63, 30.37, 30.69, 32.11, 32.37, 33.10, 33.87, 36.99, 38.31, 38.60, 39.35, 41.42, 41.79, 45.87, 46.16, 46.78, 50.82, 51.53, 52.64, 112.05, 122.39, 143.70, 152.95, 166.73, 178.31; HRMS (ESI): *m/z* calcd for $C_{40}H_{64}N_2O_2S$ $[M+H]^+$ 637.4761, found 637.4761.

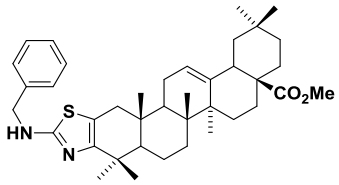
Thiazole derivative 3h



Compound **3h** was prepared according to the general procedure from compound **3c** (300 mg; 0.6 mmol) and dioctyl ammonium acetate (310 mg; 1.0 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 20:1) compound **3h** (189 mg; 44 %) was obtained; MP: 139-140 °C (toluene/diethylether); R_f = 0.84 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2924, 2854, 1727 (C=O), 1694 (C=C), 1538, 1450 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.80, 0.89 (t, J = 7.2 Hz, 6H, ($CH_3(CH_2)_7)_2N$), 0.91, 0.94, 0.98, 1.13, 1.16, 1.22 (all s, 21H, 7 × CH_3), 1.28 – 1.31 (m, 20H, ($CH_3(CH_2)_6CH_2)_2N$), 2.16 (d, J = 15.5 Hz, 1H, H-1a), 2.48 (d, J = 15.1 Hz, 1H, H-1b), 2.90 (dd, J_1 = 14.0 Hz, J_2 = 4.2 Hz, 1H, H-19), 3.24 – 3.39 (m, 4H, ($CH_3(CH_2)_6CH_2)_2N$), 3.64 (s, 3H, CO_2CH_3), 5.32 – 5.36 (m, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.10, 15.43, 16.58, 19.69, 22.10, 22.64, 23.12, 23.29, 23.60, 25.75, 26.97, 27.42, 27.72, 29.23, 29.34, 30.38, 30.69, 31.81, 32.11, 32.37, 33.09, 33.87, 36.99, 38.31, 38.61, 39.35, 41.42, 41.79,

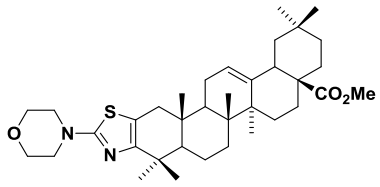
45.87, 46.17, 46.78, 51.20, 51.52, 52.65, 112.04, 122.39, 143.69, 152.95, 166.69, 178.30; HRMS (ESI): *m/z* calcd for $C_{48}H_{80}N_2O_2S$ $[M+H]^+$ 749.6013, found 749.6005.

Thiazole derivative 3i



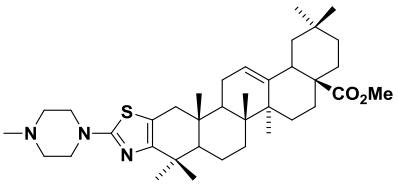
Compound **3i** was prepared according to the general procedure from compound **3c** (300 mg; 0.6 mmol) and benzyl ammonium acetate (310 mg; 1.9 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (toluene/diethylether 7:1) compound **3i** (229 mg; 65 %) was obtained; MP: 146-148 °C (toluene/diethylether); R_f = 0.14 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 3369 (NH), 2945, 2862, 1724 (C=O), 1698 (C=C), 1541, 1458 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.80, 0.91, 0.94, 0.97, 1.14, 1.16, 1.23 (all s, 21H, 7 × CH_3), 2.17 (d, J = 14.9 Hz, 1H, H-1a), 2.50 (d, J = 15.4 Hz, 1H, H-1b), 2.90 (dd, J_1 = 13.2 Hz, J_2 = 3.5 Hz, 1H, H-19), 3.64 (s, 3H, CO_2CH_3), 4.38 (s, 2H, Ph*CH*$_2$), 5.30 (bs, 1H, NH), 5.34 (t, J = 3.5 Hz, 1H, H-12), 7.28 – 7.39 (m, 5H, Ph); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 15.37, 16.56, 19.62, 22.19, 23.10, 23.28, 23.59, 25.73, 27.70, 30.41, 30.68, 32.06, 32.35, 33.08, 33.87, 36.91, 38.32, 38.63, 39.33, 41.40, 41.79, 45.86, 46.16, 46.76, 50.09, 51.53, 52.61, 114.21, 122.26, 127.56, 127.72, 127.72, 128.59, 128.59, 137.99, 143.74, 152.58, 166.59, 178.27; HRMS (ESI): *m/z* calcd for $C_{39}H_{54}N_2O_2S$ $[M+H]^+$ 615.3979, found 615.3979.

Thiazole derivative 3j



Compound **3j** was prepared according to the general procedure from compound **3c** (700 mg; 1.3 mmol) and morpholinium acetate (700 mg; 4.8 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 40:1). After standard work up, purification (gradient elution: toluene → toluene/diethylether 40:1) and lyophilization compound **3j** (427 mg; 61 %) was obtained; MP: 132-133 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +83.3° (c = 1.6, $CHCl_3$); R_f = 0.17 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 2946, 2858, 1724 (C=O), 1695 (C=C), 1524, 1451 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.79, 0.91, 0.94, 0.96, 1.13, 1.16, 1.22 (all s, 21H, 7 × CH_3), 2.19 (d, J = 15.5 Hz, 1H, H-1a), 2.53 (d, J = 15.5 Hz, 1H, H-1b), 2.90 (dd, J_1 = 13.7 Hz, J_2 = 4.1 Hz, 1H, H-19), 3.35 – 3.43 (m, 4H, O(CH_2*CH*$_2$)$_2$N), 3.64 (s, 3H, CO_2CH_3), 3.76 – 3.84 (m, 4H, O(*CH*$_2$$CH_2$)$_2$N), 5.31 – 5.38 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.36, 16.56, 19.64, 21.45, 22.15, 23.09, 23.29, 23.59, 25.75, 27.70, 30.42, 30.69, 32.04,

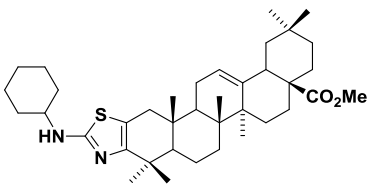
32.34, 33.10, 33.86, 37.05, 38.30, 38.57, 39.33, 41.39, 41.78, 45.84, 46.14, 46.76, 48.56, 51.55, 52.59, 66.27, 114.98, 122.24, 143.74, 153.23, 168.37, 178.30; HRMS (ESI): *m/z* calcd for $C_{36}H_{54}N_2O_3S$ 594.89, found $[M+H]^+$ 595.39; Anal. calcd for $C_{36}H_{54}N_2O_3S$: C 72.68, H 9.15, S 5.39, N 4.71; found C 71.89, H 9.21, S 5.05, N 4.29.



Thiazole derivative 3k

Compound **3k** was prepared according to the general procedure from compound **3c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (310 mg; 1.9 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 10:1). After standard work up and purification (methanol/chloroform 5:1) compound **3k** (270 mg; 78 %) was obtained; MP: 188-192 °C (toluene/diethylether); R_f = 0.53 (silica gel, methanol/chloroform 5:1); IR (DRIFT): 2939, 2843, 1726 (C=O), 1692 (C=C), 1523, 1456 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.79, 0.91, 0.94, 0.96, 1.13, 1.16, 1.22 (all s, 21H, 7 × CH_3), 2.18 (d, J = 15.4 Hz, 1H, H-1a), 2.34 (s, 3H, CH_3N), 2.50 – 2.53 (m, 5H, H-1b, $CH_3N(CH_2CH_2)_2N$), 2.89 (dd, J_1 = 13.7 Hz, J_2 = 4.0 Hz, 1H, H-19), 3.43 (m, 4H, H $CH_3N(CH_2CH_2)_2N$), 3.64 (s, 3H, CO_2CH_3), 5.34 (t, J = 3.4 Hz, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 15.36, 16.56, 19.63, 22.14, 23.10, 23.29, 23.59, 25.75, 27.70, 30.42, 30.68, 32.06, 32.35, 33.09, 33.87, 37.02, 38.28, 38.59, 39.33, 41.40, 41.78, 45.85, 46.15, 46.21, 46.76, 48.21, 51.52, 52.64, 54.34, 114.64, 122.28, 143.72, 153.21, 168.09, 178.27; HRMS (ESI): *m/z* calcd for $C_{37}H_{57}N_3O_2S$ $[M+H]^+$ 608.4244, found 608.4244.

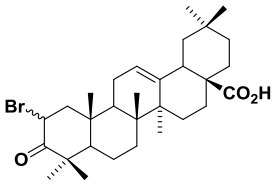
Thiazole derivative 3l



Compound **3l** was prepared according to the general procedure from compound **3c** (300 mg; 0.6 mmol) and cyclohexyl ammonium acetate (310 mg; 1.9 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 7:1). After standard work up and purification (toluene/diethylether 20:1) compound **3l** (204 mg; 59 %) was obtained; MP: 197-199 °C (toluene/diethylether); R_f = 0.07 (silica gel, toluene/diethylether 40:1); IR (DRIFT): 3349 (NH), 2928, 2854, 1724 (C=O), 1698 (C=C), 1538, 1456 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.79, 0.91, 0.94, 0.97, 1.12, 1.16, 1.21 (21H, 7 × CH_3), 2.16 (d, J = 15.0 Hz, 1H, H-1a), 2.48 (d, J = 15.1 Hz, 1H, H-1b), 2.89 (dd, J_1 = 13.5 Hz, J_2 = 3.6 Hz, 1H, H-19), 3.08 – 3.19 (m, 1H, H-cyclohexyl), 3.64 (s, 3H, CO_2CH_3),

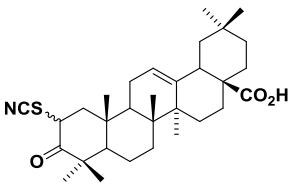
5.13 (bs, 1H, NH), 5.34 (t, J = 3.6 Hz, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 15.37, 16.56, 19.60, 22.13, 23.09, 23.29, 23.60, 24.68, 25.54, 25.73, 27.70, 30.35, 30.68, 32.07, 32.35, 33.09, 33.14, 33.86, 36.78, 38.31, 38.62, 39.33, 41.41, 41.80, 45.85, 46.16, 46.76, 51.53, 52.60, 55.19, 113.12, 122.25, 143.74, 152.04, 166.17, 178.28; HRMS (ESI): *m/z* calcd for $C_{38}H_{58}N_2O_2S$ $[M+H]^+$ 607.4292, found 607.4291.

2α/2β-bromo oleanonic acid (4b)



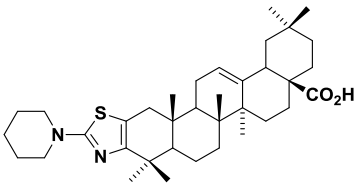
Epimeric mixture of **4b** was prepared according to the general procedure from oleanonic acid (**4a**) (5.0 g; 11 mmol) and $CuBr_2$ (5.0 g; 22 mmol) in mixture of ethyl acetate (50 mL) and methanol (2 mL) after 12 hours, monitores by TLC (toluene/Et_2O 5:1). After standard work up and purification (gradient elution toluene → toluene/diethylether 5:1) compound **4b** (853 mg; 73 %) was obtained; MP: 172-175 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +72.5° (c = 1.5, $CHCl_3$); R_f = 0.55 (silica gel, toluene/diethylether 5:1); IR (KBr): 3560 (OH), 2944, 2867, 1722 (C=O), 1692 (C=C), 1460 cm^{-1} ; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.80, 0.91, 0.93, 1.10, 1.12, 1.20, 1.21 (all s, 21H, 7 × CH_3), 2.57 (dd, J_1 = 12.9 Hz, J_2 = 6.2 Hz, 1H, H-1b), 2.83 (dd, J_1 = 14.6 Hz, J_2 = 3.8 Hz, 1H, H-18), 5.04 – 5.13 (m, 1H, H-2), 5.27 – 5.29 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.24, 16.34, 17.09, 18.13, 19.18, 19.91, 20.20, 20.76, 21.90, 22.76, 22.82, 23.43, 23.45, 23.48, 23.51, 24.18, 25.70, 25.84, 26.27, 27.56, 27.99, 29.43, 30.64, 31.46, 32.11, 32.31, 33.01, 33.71, 33.74, 36.84, 39.09, 39.31, 39.33, 39.81, 40.85, 41.09, 41.66, 41.80, 45.64, 45.68, 46.47, 46.59, 46.66, 46.95, 47.48, 49.31, 51.06, 52.21, 52.28, 52.35, 53.22, 56.54, 121.81, 122.15, 143.42, 143.77, 177.09, 184.12, 206.77, 209.14; HRMS (ESI): *m/z* calcd for $C_{30}H_{45}BrO_3$ $[M+H]^+$ 533.58, found $[M-H+Na]^+$ 555.24; Anal. calcd for $C_{30}H_{45}BrO_3$: C 67.53, Br 14.98, H 8.50; found C 67.32, Br 15.19, H 8.63.

2α/2β-thiocyanato oleanonic acid (4c)



Epimeric mixture of **4c** was prepared according to the general procedure from **4b** (3.5 g; 6 mmol) and ammonium thiocyanate (4.0 g; 52 mmol) in *N*-methylpyrrolidone (35 mL) after 4 hours at 40 °C, controlled by TLC (toluene/ diethylether 10:1). After standard work up and purification (gradient elution: toluene → toluene/diethylether 10:1) compound **4c** (2.7 g; 77 %) was obtained; MP: 173-176 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +10.3° (c = 1.4, $CHCl_3$); R_f = 0.38 (silica gel, toluene/diethylether 10:1); IR (KBr): 3560 (OH), 2949, 2873,

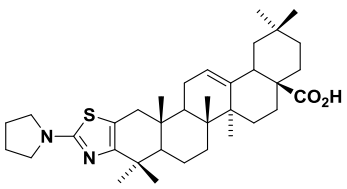
2158 (CN), 1722 (C=O), 1640 (C=C), 1458 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.82 (s, 3H), 0.92 (s, 3H), 0.94 (s, 3H), 1.13 (s, 6H), 1.16 (s, 3H), 1.26 (s, 3H, 7 × CH_3), 2.64 (dd, J_1 = 12.6 Hz, J_2 = 5.9 Hz, 1H, H-1b), 2.85 (dd, J_1 = 13.8 Hz, J_2 = 3.2 Hz, 1H, H-18), 4.70 – 4.76 (m, 1H, H-2), 5.29 – 5.32 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.42, 17.12, 21.39, 23.47, 23.51, 24.87, 25.87, 27.56, 30.65, 32.12, 32.31, 33.01, 33.71, 38.22, 38.90, 39.25, 39.38, 40.82, 41.67, 45.65, 46.44, 47.27, 47.40, 49.47, 53.83, 57.09, 112.50, 121.70, 143.75, 184.06, 209.22; HRMS (ESI): *m/z* calcd for $C_{31}H_{45}NO_3S$ 511.76, found $[M+Na]^+$ 534.30, $[M+K]^+$ 550.28, $[M+H]^+$ 512.32; Anal. calcd for $C_{31}H_{45}NO_3S$: C 72.76, H 8.86, S 6.27, N 2.74; found C 71.91, H 8.92, S 5.88, N 2.29.



Thiazole derivative 4d

Compound **4d** was prepared according to the general procedure from compound **4c** (500 mg; 1.0 mmol) and piperidinium acetate (500 mg; 3.4 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 4:1). After standard work up, purification (toluene/diethylether 4:1) and lyophilization compound **4d** (375 mg; 75 %) was obtained; MP: 210-213 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +100.0° (c = 1.5, $CHCl_3$); R_f = 0.57 (silica gel, toluene/diethylether 4:1); IR (KBr): 3600 (OH), 2935, 2858, 1754 (C=O), 1696 (C=C), 1523, 1451 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.81 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.15 (s, 6H), 1.23 (s, 3H, 7 × CH_3), 2.16 (d, J = 15.5 Hz, 1H, H-1a), 2.50 (d, J = 15.6 Hz, 1H, H-1b), 2.79 – 2.90 (m, 1H, H-18), 3.30 – 3.52 (m, 4H, piperidine), 5.28 – 5.37 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.37, 16.78, 19.54, 22.06, 22.90, 23.26, 23.53, 24.22, 25.15, 25.75, 27.65, 30.31, 30.66, 31.17, 32.00, 32.37, 33.05, 33.79, 36.79, 36.93, 38.25, 38.53, 39.31, 40.98, 41.73, 45.81, 46.15, 46.58, 49.52, 52.65, 108.81, 122.48, 143.52, 153.10, 162.44, 184.18; HRMS (ESI): *m/z* calcd for $C_{36}H_{54}N_2O_2S$ 578.89, found $[M+H]^+$ 579.40; Anal. calcd for $C_{36}H_{54}N_2O_2S$: C 74.69, H 9.40, S 5.54, N 4.84; found C 73.62, H 9.59, S 5.28, N 4.11.

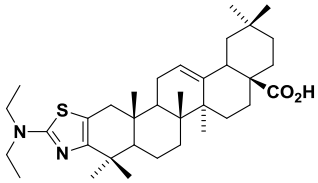
Thiazole derivative 4e



Compound **4e** was prepared according to the general procedure from compound **4c** (500 mg; 1.0 mmol) and pyrrolidinium acetate (500 mg; 3.8 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 4:1). After

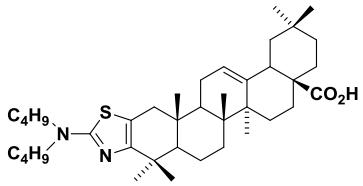
standard work up, purification (toluene/diethylether 4:1) and lyophilization compound **4e** (360 mg; 72 %) was obtained; MP: 196-199 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +93.6° (c = 1.6, $CHCl_3$); R_f = 0.29 (silica gel, toluene/diethylether 4:1); IR (KBr): 3700 (OH), 2945, 2860, 1734 (C=O), 1695 (C=C), 1543, 1460 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.82 (s, 3H), 0.91 (s, 3H), 0.93 (s, 3H), 0.96 (s, 3H) 1.16 – 1.28 (9H, 7 × CH_3), 1.96 – 2.03 (m, 4H, pyrrolidine), 2.16 (d, J = 15.5 Hz, 1H, H-1a), 2.49 (d, J = 15.3 Hz, 1H, H-1b), 2.85 (dd, J_1 = 12.9 Hz, J_2 = 2.7 Hz, 1H, H-18), 3.31 – 3.61 (m, 4H, pyrrolidine), 5.30 – 5.37 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.35, 16.80, 19.53, 21.84, 22.93, 23.25, 23.53, 25.73, 27.65, 29.68, 30.09, 30.66, 31.17, 32.03, 32.37, 33.05, 33.81, 36.98, 38.24, 38.64, 39.30, 41.01, 41.76, 45.87, 46.14, 46.55, 52.79, 97.46, 112.58, 122.37, 143.59, 152.25, 164.84, 183.50; HRMS (ESI): *m/z* calcd for $C_{35}H_{52}N_2O_2S$ 564.86, found $[M+H]^+$ 565.38; Anal. calcd for $C_{35}H_{52}N_2O_2S$: C 74.42, H 9.28, S 5.68, N 4.96; found C 72.32, H 9.35, S 4.92, N 4.01.

Thiazole derivative 4f



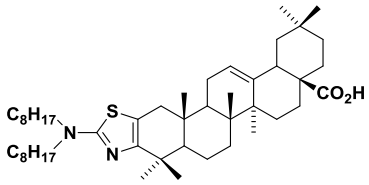
Compound **4f** was prepared according to the general procedure from compound **4c** (500 mg; 1.0 mmol) and diethyl ammonium acetate (500 mg; 3.8 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 4:1). After standard work up, purification (toluene/diethylether 4:1) and lyophilization compound **4f** (350 mg; 70 %) was obtained; MP: 193-194 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +100.0° (c = 1.6, $CHCl_3$); R_f = 0.46 (silica gel, toluene/diethylether, 4:1); IR (KBr): 3650 (OH), 2942, 2870, 1742 (C=O), 1695 (C=C), 1539, 1462 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ ppm 0.82 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.98 (s, 3H), 1.16 (s, 3H), 1.16 (t, J = 6.4 Hz, 6H, $(CH_3CH_2)_2N$), 1.20 (s, 3H), 1.23 (s, 3H, 7 × CH_3), 2.16 (d, J = 15.5 Hz, 1H, H-1a), 2.49 (d, J = 15.3 Hz, 1H, H-1b), 2.85 (dd, J_1 = 13.5 Hz, J_2 = 2.9 Hz, 1H, H-18), 3.31 – 3.54 (m, 4H, $(CH_3CH_2)_2N$), 5.29 – 5.36 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 12.69, 15.42, 16.79, 19.55, 22.06, 22.93, 23.26, 23.53, 25.75, 27.67, 30.25, 30.65, 31.17, 32.02, 32.37, 33.05, 33.80, 36.93, 38.29, 38.54, 39.31, 40.98, 41.73, 45.07, 45.83, 46.17, 46.58, 52.67, 112.11, 122.51, 143.50, 152.97, 166.21, 184.16; HRMS (ESI): *m/z* calcd for $C_{35}H_{54}N_2O_2S$ 566.88, found $[M+H]^+$ 567.40; Anal. calcd for $C_{35}H_{54}N_2O_2S$: C 74.16, H 9.60, S 5.66, N 4.94; found C 72.99, H 9.63, S 5.50, N 4.35.

Thiazole derivative 4g



Compound **4g** was prepared according to the general procedure from compound **4c** (300 mg; 0.6 mmol) and dibutyl ammonium acetate (300 mg; 1.6 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 4:1). After standard work up and purification (toluene/diethylether 5:1) compound **4g** (31 mg; 8 %) was obtained; MP: 149-152 °C (toluene/diethylether); R_f = 0.63 (silica gel, toluene/diethylether 4:1); IR (DRIFT): 3750 (OH), 3100 – 2400, 2929, 2862, 1730 (C=O), 1694 (C=C), 1538, 1452 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.83, 0.91, 0.94 (all s, 9H, 3 × CH_3), 0.95 (t, *J* = 7.3 Hz, 6H, ($CH_3CH_2CH_2CH_2)_2N$), 0.98, 1.11, 1.16, 1.21 (all s, 12H, 4 × CH_3), 1.29 – 1.39 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 1.57 – 1.65 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 2.16 (d, *J* = 15.6 Hz, 1H, H-1a), 2.48 (d, *J* = 15.1 Hz, 1H, H-1b), 2.85 (dd, J_1 = 13.5 Hz, J_2 = 3.1 Hz, 1H, H-18), 3.26 – 3.42 (m, 4H, ($CH_3CH_2CH_2CH_2)_2N$), 5.31 – 5.36 (m, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.03, 15.55, 16.92, 19.71, 20.26, 22.19, 23.06, 23.37, 23.64, 25.85, 27.79, 29.72, 29.79, 30.44, 30.76, 32.16, 32.50, 33.16, 33.92, 37.07, 38.42, 38.71, 39.44, 41.15, 41.85, 45.96, 46.29, 46.71, 50.94, 52.73, 112.11, 122.69, 143.61, 166.86, 183.98; HRMS (ESI): *m/z* calcd for $C_{39}H_{62}N_2O_2S$ $[M+H]^+$ 623.4605, found 623.4604.

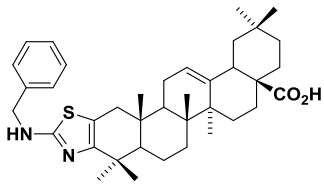
Thiazole derivative 4h



Compound **4h** was prepared according to the general procedure from compound **4c** (300 mg; 0.6 mmol) and dioctyl ammonium acetate (300 mg; 1.0 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 4:1). After standard work up and purification (toluene/diethylether 5:1) compound **4h** (102 mg; 24 %) was obtained; MP: 118 °C (toluene/diethylether); R_f = 0.72 (silica gel, toluene/diethylether 4:1); IR (DRIFT): 3720 (OH), 3000 – 2500, 2924, 2854, 1725 (C=O), 1694 (C=C), 1539, 1457 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.83 (s, 3H, CH_3), 0.89 (t, *J* = 7.0 Hz, 6H, ($CH_3(CH_2)_7)_2N$), 0.91, 0.94, 0.98, 1.11, 1.16, 1.21 (all s, 18H, 6 × CH_3), 1.28 – 1.31 (m, 20H, ($CH_3(CH_2)_6CH_2)_2N$), 2.16 (d, *J* = 15.3 Hz, 1H, H-1a), 2.49 (d, *J* = 15.3 Hz, 1H, H-1b), 2.86 (dd, J_1 = 13.5 Hz, J_2 = 3.4 Hz, 1H, H-18), 3.26 – 3.40 (m, 4H, ($CH_3(CH_2)_6CH_2)_2N$), 5.31 –

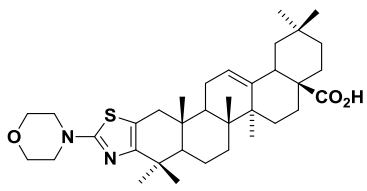
5.36 (m, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 14.11, 15.44, 16.82, 19.61, 22.09, 22.65, 22.98, 23.28, 23.55, 25.74, 26.97, 27.41, 27.71, 29.23, 29.28, 29.34, 30.35, 30.66, 31.78, 31.81, 32.08, 32.41, 33.05, 33.84, 36.97, 38.32, 38.60, 39.35, 41.03, 41.75, 45.89, 46.20, 46.62, 51.20, 52.64, 112.02, 122.59, 143.54, 152.96, 166.71, 184.05; HRMS (ESI): *m/z* calcd for $C_{47}H_{78}N_2O_2S$ $[M+H]^+$ 735.5857, found 735.5851.

Thiazole derivative 4i



Compound **4i** was prepared according to the general procedure from compound **4c** (300 mg; 0.6 mmol) and benzyl ammonium acetate (300 mg; 1.8 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 4:1). After standard work up and purification (toluene/diethylether 3:1) compound **4i** (227 mg; 64 %) was obtained; MP: 238-240 °C (toluene/diethylether); R_f = 0.15 (silica gel, toluene/diethylether 4:1); IR (DRIFT): 3710 (OH), 3231 (NH), 3100 – 2400, 2941, 2866, 1730 (C=O), 1694 (C=C), 1548, 1454 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.81, 0.91, 0.92, 0.95, 1.14, 1.15, 1.21 (all s, 21H, 7 × CH_3), 2.11 (d, J = 15.6 Hz, 1H, H-1a), 2.42 (d, J = 15.3 Hz, 1H, H-1b), 2.91 (dd, J_1 = 12.9 Hz, J_2 = 2.3 Hz, 1H, H-18), 4.34 (s, 2H, PhCH_2), 5.27 – 5.34 (m, 1H, H-12), 7.27 – 7.37 (m, 5H, Ph); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 15.38, 16.83, 19.50, 21.67, 23.03, 23.24, 23.63, 25.70, 27.78, 29.67, 29.77, 30.74, 32.05, 32.60, 33.15, 33.97, 36.60, 38.41, 39.33, 41.38, 41.84, 46.00, 46.13, 46.57, 50.17, 52.64, 112.66, 121.91, 126.96, 127.46, 127.62, 128.52, 128.98, 129.74, 137.43, 144.15, 169.30, 183.31; HRMS (ESI): *m/z* calcd for $C_{38}H_{52}N_2O_2S$ $[M+H]^+$ 601.3822, found 601.3822.

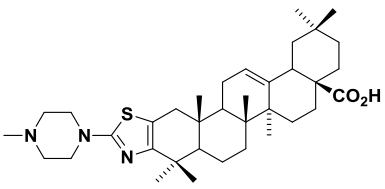
Thiazole derivative 4j



Compound **4j** was prepared according to the general procedure from compound **4c** (500 mg; 1.0 mmol) and morpholinium acetate (500 mg; 3.4 mmol) in chloroform (20 mL) after 36 hours controlled by TLC (toluene/diethylether 4:1). After standard work up, purification (toluene/diethylether 4:1) and lyophilization compound **4j** (325 mg; 65 %) was obtained; MP: 272 °C (toluene/diethylether); $[\alpha]_D^{20}$ = +96.2° (c = 1.5, $CHCl_3$); R_f = 0.33 (silica gel, toluene/diethylether, 4:1); IR (KBr): 3710 (OH), 2944, 2864, 1728 (C=O), 1696 (C=C), 1524, 1451 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 0.82 (s, 3H), 0.91 (s, 3H), 0.94 (s, 3H), 0.96 (s, 3H), 1.12 – 1.16 (m, 6H), 1.28 (s, 3H, 7 × CH_3), 2.18 (d, J = 15.6 Hz, 1H, H-1a), 2.52 (d, J = 15.5 Hz, 1H, H-1b), 2.82 – 2.88 (m, 1H, H-18), 3.27 – 3.51

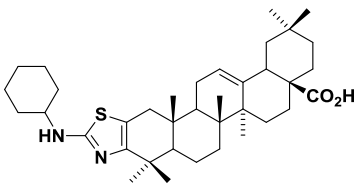
(m, 4H, O(CH_2CH_2)$_2$N), 3.75 – 3.88 (m, 4H, O(CH_2CH_2)$_2$N), 5.27 – 5.36 (m, 1H, H-12); ^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 15.37, 16.77, 19.54, 22.91, 23.26, 23.53, 25.76, 27.65, 28.16, 30.43, 30.67, 31.19, 31.97, 32.38, 33.05, 33.78, 37.00, 38.32, 38.51, 39.31, 41.00, 41.74, 42.91, 45.81, 46.15, 46.57, 48.59, 52.59, 66.24, 115.54, 122.40, 143.57, 152.93, 168.40, 183.91; HRMS (ESI): *m/z* calcd for $C_{35}H_{52}N_2O_3S$ 580.86, found $[M+H]^+$ 581.38; Anal. calcd for $C_{35}H_{52}N_2O_3S$: C 72.37, H 9.02, S 5.52, N 4.82; found C 71.37, H 9.25, S 5.11, N 4.35.

Thiazole derivative 4k



Compound **4k** was prepared according to the general procedure from compound **4c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (300 mg; 1.9 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 4:1). After standard work up and purification (methanol/chloroform 3:1) compound **4k** (276 mg; 79 %) was obtained; MP: 227-231 °C (toluene/diethylether); R_f = 0.58 (silica gel, methanol/chloroform 5:1); IR (DRIFT): 3705 (OH), 3100 – 2400, 2942, 2866, 1729 (C=O), 1698 (C=C), 1522, 1450 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.78 (s, 3H), 0.85 (s, 3H), 0.88 (d, J = 4.9 Hz, 3H), 1.06 (s, 3H), 1.10 (s, 3H), 1.15 (s, 3H), 1.20 (s, 3H, 7 × CH_3), 2.12 (d, J = 15.5 Hz, 1H, H-1a) 2.29 (s, 3H, CH_3N), 2.45 (d, J = 15.6 Hz, 1H, H-1b), 2.48 – 2.52 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 2.80 (dd, J_1 = 13.0 Hz, J_2 = 2.6 Hz, 1H, H-18), 3.36 (br s, 4H, $CH_3N(CH_2CH_2)_2N$), 5.22 – 5.29 (m, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$):15.22, 16.46, 19.50, 21.87, 22.95, 23.15, 23.37, 25.53, 27.57, 29.50, 29.53, 30.14, 30.55, 31.99, 32.42, 32.92, 33.78, 36.88, 38.12, 38.43, 39.18, 41.23, 41.77, 45.63, 45.86, 46.01, 46.35, 47.80, 52.52, 53.93, 114.96, 121.98, 143.81, 153.05, 168.25, 181.03; HRMS (ESI): *m/z* calcd for $C_{36}H_{55}N_3O_2S$ $[M+H]^+$ 594.4088, found 594.4089.

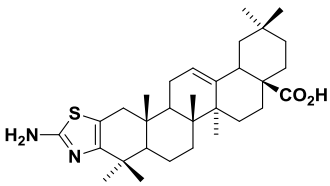
Thiazole derivative 4l



Compound **4l** was prepared according to the general procedure from compound **4c** (300 mg; 0.59 mmol) and cyclohexyl ammonium acetate (300 mg; 1.9 mmol) in chloroform (12 mL) after 48 hours controlled by TLC (toluene/diethylether 4:1). After standard work up and purification (toluene/diethylether 5:1) compound **4l** (108 mg; 31 %) was obtained; MP: 238-241 °C (toluene/diethylether); R_f = 0.07 (silica gel, toluene/diethylether, 4:1); IR (DRIFT): 3232 (NH), 3100 – 2400, 2928, 2854, 1729 (C=O), 1694 (C=C), 1539, 1450 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.85 (s, 3H), 0.91 (s, 3H),

0.95 (s, 6H), 1.12 (s, 3H), 1.16 (s, 3H), 1.20 (s, 3H, 7 × CH_3), 2.14 (d, J = 15.5 Hz, 1H, H-1a), 2.44 (d, J = 15.1 Hz, 1H, H-1b), 2.92 (dd, J_1 = 13.7 Hz, J_2 = 3.7 Hz, 1H, H-18), 2.98 – 3.05 (m, 1H, cyclohexyl), 5.29 – 5.34 (m, 1H, H-12); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 15.36, 17.04, 19.54, 21.57, 23.06, 23.33, 23.63, 24.45, 24.50, 25.49, 25.68, 27.79, 29.75, 30.77, 32.16, 32.49, 32.55, 32.61, 33.17, 34.03, 36.55, 38.41, 38.54, 39.40, 41.51, 41.90, 46.05, 46.58, 52.71, 55.71, 111.60, 121.85, 144.23, 149.66, 168.19, 183.11; HRMS (ESI): *m/z* calcd for $C_{37}H_{56}N_2O_2S$ $[M+H]^+$ 593.4135, found 593.4136.

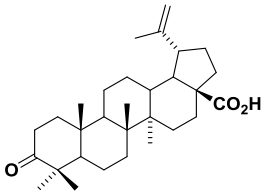
Thiazole derivative 4m



Compound **4m** was prepared according to the general procedure from 2α/2β-bromo oleanonic acid **4b** (205 mg; 0.4 mmol) and thioure (286 mg; 3.8 mmol) in ethanol (5 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 1:1 + AcOH 0,1 %) white crystals of compound **4m** (56 mg; 28 %) were obtained; MP: 241-242 °C (hexane); IR (DRIFT): 3295 (NH), 3168 (OH), 2973, 2917, 2849, 1704 (C=O), 1692 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 12.07 (bs, 1H, COOH), 6.61 (s, 2H, NH_2), 5.26 – 5.15 (m, 1H, H-12), 2.82 – 2.71 (m, 1H, H-18), 2.38 (d, J = 15.4 Hz, 1H, H-1a), 2.09 (d, J = 15.4 Hz, 1H, H-1b), 2.01 – 1.76 (m, 3H), 1.12 (s, 6H), 1.03 (s, 3H), 0.92 – 0.85 (m, 9H), 0.78 (s, 3H, 7 × CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ ppm 178.63, 165.14, 151.34, 143.69, 121.56, 112.16, 52.07, 45.71, 45.54, 41.50, 40.93, 37.93, 36.47, 33.36, 32.84, 32.08, 31.77, 30.44, 27.28, 25.41, 23.36, 22.74, 22.68, 22.17, 19.27, 16.61, 15.26; HRMS (ESI): *m/z* calcd for $C_{31}H_{47}N_2O_2S$ $[M+H]^+$ 511.3353, found 511.3352.

6.5.2 Triterpenoid aminothiazoles derived from betulinic acid, dihydrobetulinic acid, and ursonic acid

Betulonic acid (5a)

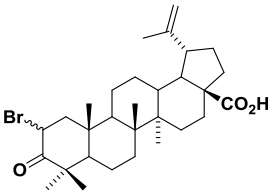


Acid **5a** was prepared according to the procedure.[84] Betulinic acid (**5**) (90%, 11.0 g; 21.9 mmol) was dissolved in the mixture of solvents 1,4-dioxane (250 mL), acetic acid (85 mL), and acetic anhydride (35 mL). Sodium acetate trihydrate (2.7 g; 33.1 mmol) and sodium dichromane dihydrate (11.6 g; 38.8 mmol) were added to the solution. The reaction mixture was stirred 8 hours at r.t. monitored by TLC (hexane/EtOAc 5:1). Then, the reaction mixture was poured into an ice-bath (1.5 L); the resulting soild precipitate was filtered off through a pad of celite

and a frit S3, washed with water and dried at 55 °C for two days. The crude product **5a** was purified on column chromatography (hexane/EtOAc/$CHCl_3$ 10:2:1) to give white crystals of **5a** (9.3 g; 93 %); R_f = 0.40 (silica gel, hexane/EtOAc 5:1); MP: 250-254 °C (methanol).[89] ^{1}H NMR spectrum was consistent with the literature.[153]

2α/2β-bromo betulonic acid (5b)

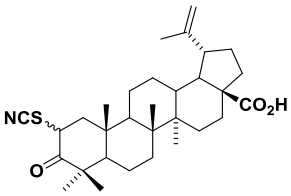
The derivative **5b** was known from the literature;[96] however, it was only used as an crude intermediate and its spectral data were not reported. For this reason, characterisation of bromketone **5b** by spectral data is included.



Epimeric mixture of **5b** was prepared according to the general procedure for the synthesis of 2-bromo-3-oxotriterpenoids (II) from **5a** (4.2 g; 9.2 mmol) and CuBr2 (4.2 g; 18.8 mmol) in mixture of ethyl acetate (85 mL) and methanol (40 mL) after 3 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). Then, the precipitate of copper(I) bromide was filtered off the reaction mixture was poured to water (300 mL) and sturated aqueous solution of sodium thiosulphate (100 mL) and the product was extracted into ethyl acetate. The collected organic layers were washed with water, dried over magnesium sulfate, filtered over a pad of silica gel and the solvents were evaporated under reduced pressure to give light yellow crystals of compound **5b** (4.7 g; 95 %) which were used to the next reaction. A small part was purified on column chromatography (hexane/EtOAc 3:1 + AcOH 0.05 %) to yield white crystals of epimeric mixture of bromoketone **5b**; R_f = 0.36 (silica gel, hexane/EtOAc 5:1 with a drop of AcOH); MP: 248-250 °C (hexane); IR (DRIFT): 3366 (OH), 2928, 2868, 1716 (C=O), 1667 (C=C), 1152 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) mixture of epimers in the ratio 0.2:0.8: δ ppm 5.17 – 5.02 (m, 2H, H-2 from both epimers), 4.77 – 4.71 (m, 2H, H-29a from both epimers), 4.63 – 4.58 (m, 2H, H-29b from both epimers), 3.08 – 2.95 (m, J = 10.6, 5.1 Hz, 2H, H-18 from both epimers), 2.63 (dd, J = 13.0, 6.3 Hz, 1H, H-1a from one epimer), 2.54 – 2.44 (m, 2H, H-1b from both epimers), 2.40 (ddd, J = 15.7, 7.6, 4.4 Hz, 1H, H-1a from one epimer), 2.36 – 2.14 (m, 3H, from both epimers), 1.69, 1.68, 1.12, 1.08, 1.07, 1.01, 1.00, 0.99, 0.98, 0.97, 0.96, 0.92 (all s, 30H, 10 × CH_3 from both epimers); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 218.33, 218.26, 209.24, 207.14, 182.34, 182.24, 150.44, 150.26, 110.09, 109.93, 56.76, 56.54, 55.07, 54.25, 52.60, 52.30, 50.03, 49.98, 49.40, 49.33, 47.58, 47.47, 47.04, 46.98, 42.74, 42.63, 40.97, 40.77, 40.15, 39.74, 39.49, 38.80, 38.66, 38.47, 37.17, 37.06, 34.26, 33.74, 32.24, 32.16, 30.69, 30.62, 29.82, 29.78, 29.44, 26.78, 26.40,

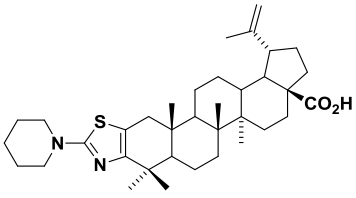
25.63, 21.88, 21.51, 21.14, 20.13, 19.76, 19.51, 16.10, 16.06, 15.99, 15.96, 14.77, 14.73; HRMS (ESI): *m/z* calcd for $C_{30}H_{44}BrO_3$ $[M-H]^-$ 531.2468, found 531.2468, isotope 533.2448.

2α/2β-thiocyanato betulonic acid (5c)



Epimeric mixture of **5c** was prepared according to the general procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids (I) from **5b** (4.7 g; 8.9 mmol) and potassium thiocyanate (5.1 g; 62.3 mmol) in dimethyl sulfoxide (180 mL) after 24 h, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and and purification (toluene/diethylether 7:1 + AcOH 0,1 %) compound **5c** was obtained as a mixture of epimers white crystals (2.97 g; 66 %); R_f = 0.50 and 0.38 (silica gel, toluene/diethylether 7:1 with a drop of AcOH); MP: 236-238 °C (hexane); IR (DRIFT): 3068 (OH), 2980, 2933, 2847, 2154 (C≡N), 1715 (C=O), 1688 (C=C), 1573, 1538, 881 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 4.76 – 4.73 (m, 2H, H-29a from both epimers), 4.73 – 4.67 (m, 2H, H-2 from both epimers), 4.65 – 4.59 (m, 2H, H-29b from both epimers), 3.00 (td, *J* = 10.7, 4.8 Hz, 2H, H-18 from both epimers), 2.71 (dd, *J* = 12.8, 6.1 Hz, 1H, H-1a from one epimer), 2.54 – 2.45 (m, 1H, H-1a from one epimer), 2.33 – 2.26 (m, 2H), 2.25 – 2.18 (m, 2H), 2.07 – 1.91 (m, 6H), 1.70, 1.69, 1.17, 1.15, 1.13, 1.10, 1.02, 1.00, 0.97, 0.93, 0.79 (all s, 30H, 10 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 211.76, 209.50, 182.41, 150.14, 150.12, 112.57, 112.30, 110.23, 110.19, 57.41, 56.47, 56.41, 54.09, 52.37, 52.31, 51.04, 50.31, 49.90, 49.63, 49.54, 49.30, 49.21, 47.53, 47.05, 47.02, 42.80, 42.68, 41.04, 40.73, 39.24, 38.76, 38.60, 38.44, 37.19, 34.04, 32.82, 32.22, 32.14, 30.59, 29.76, 29.74, 29.27, 25.56, 25.32, 24.92, 22.13, 21.37, 21.30, 20.02, 19.66, 19.40, 19.39, 19.17, 18.72, 16.27, 16.23, 15.43, 14.74; HRMS (ESI): *m/z* calcd for $C_{31}H_{46}NO_3S$ $[M+H]^+$ 512.3193, found 512.3198.

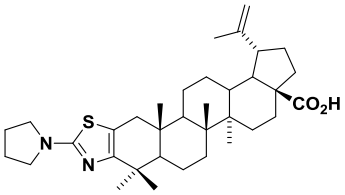
Thiazole derivative 5d



Compound **5d** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and piperidinium acetate (427 mg; 3.0 mmol) in chloroform (12 mL) after 3 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) white crystals of compound **5d** (150 mg; 44 %) were obtained; MP: decomposition at 280 °C (hexane); IR (DRIFT): 3100 – 2500 (OH), 2934, 2867, 1713 (C=O), 1689 (C=C), 1536, 882 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 4.78 – 4.73 (m, 1H, H-29a),

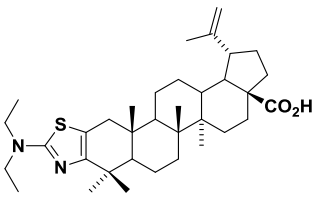
4.65 – 4.59 (m, 1H, H-29b), 3.44 – 3.31 (m, 4H, piperidine), 3.04 (td, *J* = 10.6, 4.8 Hz, 1H, H-18), 2.55 (d, *J* = 15.3 Hz, 1H, H-1a), 2.37 – 2.21 (m, 2H), 2.10 (d, *J* = 15.3 Hz, 1H, H-1b), 2.06 – 1.91 (m, 2H), 1.70 (s, 3H, H-30), 1.21, 1.10, 1.00, 0.99, 0.88 (all s, 15H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.19, 169.04, 153.07, 150.58, 114.28, 109.84, 56.59, 53.00, 49.66, 49.36, 49.33, 47.03, 42.60, 40.87, 39.28, 38.66, 38.62, 37.29, 37.19, 33.65, 32.28, 30.73, 30.35, 29.93, 25.72, 25.34, 24.44, 22.07, 21.50, 19.73, 19.54, 16.33, 15.89, 14.84; HRMS (ESI): *m/z* calcd for $C_{36}H_{55}N_2O_2S$ $[M+H]^+$ 579.3979, found 579.3979.

Thiazole derivative 5e



Compound **5e** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and pyrrolidinium acetate (380 mg; 3.0 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) white crystals of compound **5e** (112 mg; 34 %) were obtained; MP: 188-192 °C (hexane); IR (DRIFT): 3000 – 2500 (OH), 2917, 2844, 1710 (C=O), 1691 (C=C), 1540, 1266 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 4.76 – 4.72 (m, 1H, H-29a), 4.64 – 4.58 (m, 1H, H-29b), 3.48 – 3.35 (m, 4H, pyrrolidine), 3.07 (td, J = 11.0, 4.6 Hz, 1H, H-18), 2.52 (d, J = 15.3 Hz, 1H, H-1a), 2.43 (td, J = 12.8, 3.3 Hz, 1H), 2.36 – 2.27 (m, 1H), 2.09 (d, J = 15.3 Hz, 1H, H-1b), 2.00 – 1.95 (m, 4H, pyrrolidine), 1.69 (s, 3H, H-30), 1.24, 1.16, 0.99, 0.97, 0.88 (all s, 15H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 179.63, 165.84, 152.60, 151.02, 113.14, 109.63, 56.47, 53.32, 49.52, 49.39, 49.23, 46.89, 42.61, 40.90, 39.44, 38.59, 38.19, 37.33, 37.18, 33.70, 32.32, 30.76, 29.95, 29.89, 25.89, 25.75, 21.91, 21.66, 19.84, 19.52, 16.37, 15.99, 14.75; HRMS (ESI): *m/z* calcd for $C_{35}H_{53}N_2O_2S$ $[M+H]^+$ 565.3822, found 565.3822.

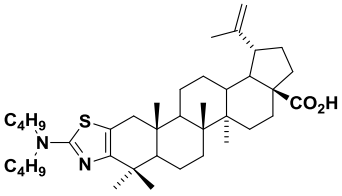
Thiazole derivative 5f



Compound **5f** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (215 mg; 0.4 mmol) and diethyl ammonium acetate (277 mg; 2.1 mmol) in chloroform (10 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,04 %) white crystals of compound **5f** (132 mg; 55 %) were obtained; MP: 224-228 °C (hexane); IR (DRIFT): 3100 – 2500 (OH), 2966, 2869, 1709 (C=O), 1685 (C=C) cm^{-1}; ^1H

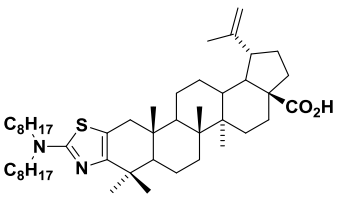
NMR (500 MHz, $CDCl_3$): δ ppm 4.78 – 4.73 (m, 1H, H-29a), 4.64 – 4.59 (m, 1H, H-29b), 3.52 – 3.31 (m, 4H, $(CH_3\mathit{CH_2})_2N$), 3.03 (td, *J* = 10.7, 4.8 Hz, 1H, H-18), 2.53 (d, *J* = 15.2 Hz, 1H, H-1a), 2.33 – 2.21 (m, 2H), 2.09 (d, *J* = 15.2 Hz, 1H, H-1b), 2.04 – 1.93 (m, 2H), 1.70 (s, 3H, H-30), 1.22 – 1.16 (m, 9H, $(\mathit{CH_3}CH_2)_2N$, 1 × CH_3), 1.10, 1.00, 0.90, 0.89 (all s, 12H, 4 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.06, 166.47, 153.17, 150.58, 112.70, 109.85, 56.58, 53.00, 49.37, 49.35, 47.04, 45.19, 42.61, 40.88, 39.29, 38.68, 37.29, 37.18, 33.67, 32.28, 30.72, 30.35, 29.94, 25.73, 22.05, 21.49, 19.75, 19.54, 16.38, 15.89, 14.85, 12.88; HRMS (ESI): *m/z* calcd for $C_{35}H_{55}N_2O_2S$ $[M+H]^+$ 567.3979, found 567.3979.

Thiazole derivative 5g



Compound **5g** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and dibutyl ammonium acetate (549 mg; 2.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) white crystals of compound **5g** (89 mg; 24 %) were obtained; MP: 164-168 °C (hexane); IR (DRIFT): 3000 – 2600 (OH), 2930, 2867, 1712 (C=O), 1686 (C=C), 1539 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 4.77 – 4.73 (m, 1H, H-29a), 4.64 – 4.60 (m, 1H, H-29b), 3.41 – 3.23 (m, 4H, $(CH_3CH_2CH_2\mathit{CH_2})_2N$), 3.03 (td, *J* = 10.8, 4.9 Hz, 1H, H-18), 2.53 (d, *J* = 15.2 Hz, 1H, H-1a), 2.34 – 2.20 (m, 2H), 2.09 (d, *J* = 15.3 Hz, 1H, H-1b), 2.06 – 1.94 (m, 2H), 1.70 (s, 3H, H-30), 1.63 – 1.56 (m, 4H, $(CH_3CH_2\mathit{CH_2}CH_2)_2N$, 1.37 – 1.29 (m, 4H, $(CH_3\mathit{CH_2}CH_2CH_2)_2N$), 1.20, 1.09, 1.00, 0.99 (all s, 12H, 4 × CH_3), 0.94 (t, *J* = 7.4 Hz, 6H, $(\mathit{CH_3}CH_2CH_2CH_2)_2N$), 0.89 (s, 3H, 1 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.17, 166.97, 153.11, 150.58, 112.50, 109.85, 56.60, 52.92, 51.01, 49.39, 49.33, 47.05, 42.60, 40.88, 39.27, 38.70, 37.30, 37.19, 33.66, 32.29, 30.73, 30.37, 29.95, 29.81, 25.73, 22.07, 21.48, 20.33, 19.76, 19.55, 16.40, 15.90, 14.85, 14.07; HRMS (ESI): *m/z* calcd for $C_{39}H_{63}N_2O_2S$ $[M+H]^+$ 623.4605, found 623.4603.

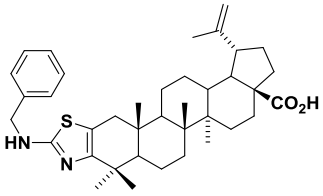
Thiazole derivative 5h



Compound **5h** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and dioctyl ammonium acetate (874 mg; 2.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether

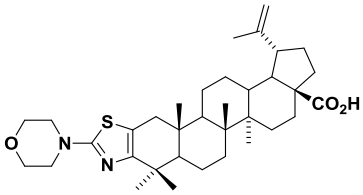
10:1 + AcOH 0,1 %) white crystals of compound **5h** (138 mg; 32 %) were obtained; MP: 124-126 °C (hexane); IR (DRIFT): 3000 – 2500 (OH), 2922, 2854, 1714 (C=O), 1693 (C=C), 1535 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.77 – 4.74 (m, 1H, H-29a), 4.63 – 4.60 (m, 1H, H-29b), 3.40 – 3.22 (m, 4H, $(CH_3(CH_2)_6CH_2)_2N$), 3.03 (td, *J* = 10.7, 4.8 Hz, 1H, H-18), 2.53 (d, *J* = 15.2 Hz, 1H, H-1a), 2.33 – 2.21 (m, 2H), 2.09 (d, *J* = 15.3 Hz, 1H, H-1b), 2.06 – 1.95 (m, 2H), 1.70 (s, 3H, H-30), 1.32 – 1.25 (m, 20H, $(CH_3(CH_2)_6CH_2)_2N$), 1.20, 1.09, 1.00, 0.99 (all s, 12H, 4 × CH_3), 0.88 (t, *J* = 7.0 Hz, 9H, $(CH_3(CH_2)_7)_2N$, 1 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.04, 166.93, 153.11, 150.61, 112.51, 109.83, 56.61, 52.94, 51.38, 49.39, 49.34, 47.06, 42.61, 40.89, 39.29, 38.70, 38.68, 37.30, 37.21, 33.67, 32.31, 31.99, 30.75, 30.38, 29.95, 29.50, 29.39, 27.59, 27.14, 25.74, 22.81, 22.07, 21.49, 19.76, 19.55, 16.40, 15.91, 14.85, 14.25; HRMS (ESI): *m/z* calcd for $C_{47}H_{79}N_2O_2S$ $[M+H]^+$ 735.5857, found 735.5857.

Thiazole derivative 5i



Compound **5i** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and benzyl ammonium acetate (874 mg; 2.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) white crystals of compound **5i** (138 mg; 32 %) were obtained; MP: 202-204 °C (hexane); IR (DRIFT): 3400 – 2900 (OH), 3245 (NH), 2916, 2846, 1713 (C=O), 1682 (C=C), 1272 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 11.17 (s, 1H, COOH), 7.71 (t, *J* = 5.6 Hz, 1H, NH), 7.37 – 7.28, 7.26 – 7.19 (both m, 5H, Ph), 4.70 (s, 1H, H-29a), 4.57 (s, 1H, H-29b), 4.40 – 4.25 (m, 2H, PhCH_2), 3.02 – 2.92 (m, 1H, H-18), 2.44 (d, *J* = 15.5 Hz, 1H, H-1a), 2.29 (bs, 1H), 2.17 – 2.08 (m, 1H), 2.04 (d, *J* = 15.3 Hz, 1H, H-1b), 1.88 – 1.76 (m, 2H), 1.65 (s, 3H, H-30), 1.13 (s, 3H), 1.02 (s, 3H), 0.97 – 0.89 (m, 6H), 0.81 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 165.40, 162.09, 151.51, 139.33, 127.94, 127.49, 126.61, 120.64, 112.08, 109.21, 55.30, 52.11, 48.48, 48.36, 47.82, 46.43, 41.95, 38.27, 37.98, 37.55, 36.50, 35.55, 32.93, 30.62, 30.05, 29.14, 25.04, 21.76, 20.83, 18.99, 18.87, 15.83, 15.35, 14.20; HRMS (ESI): *m/z* calcd for $C_{38}H_{53}N_2O_2S$ $[M+H]^+$ 601.3822, found 601.3821.

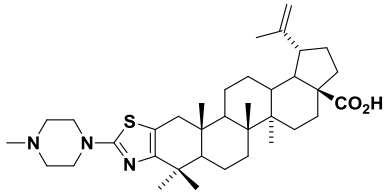
Thiazole derivative 5j



Compound **5j** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol)

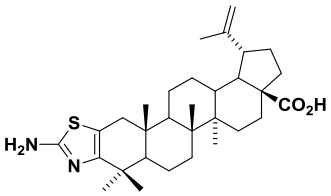
and morpholinium acetate (427 mg; 2.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **5j** (139 mg; 41 %) were obtained; MP: >290 °C (hexane); IR (DRIFT): 3000 – 2500 (OH), 2940, 2868, 1718 (C=O), 1684 (C=C), 1275 (C-O), 1102 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 4.77 – 4.73 (m, 1H, H-29a), 4.64 – 4.60 (m, 1H, H-29b), 3.85 – 3.72 (m, 4H, O(CH_2CH_2)$_2$N), 3.43 – 3.33 (m, 4H, O(CH_2CH_2)$_2$N), 3.03 (td, *J* = 10.7, 4.8 Hz, 1H, H-18), 2.57 (d, *J* = 15.4 Hz, 1H, H-1a), 2.33 – 2.21 (m, 2H), 2.12 (d, *J* = 15.4 Hz, 1H, H-1b), 2.07 – 1.93 (m, 2H), 1.70 (s, 3H, H-30), 1.20 (s, 3H), 1.10 (s, 3H), 1.00 (s, 3H), 0.99 (s, 3H), 0.88 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.14, 168.63, 153.38, 150.53, 115.42, 109.86, 66.44, 56.58, 52.89, 49.36, 49.31, 48.76, 47.04, 42.61, 40.87, 39.25, 38.68, 37.35, 37.18, 33.62, 32.27, 30.72, 30.39, 29.93, 25.69, 22.11, 21.50, 19.70, 19.54, 16.32, 15.89, 14.84; HRMS (ESI): *m/z* calcd for $C_{35}H_{53}N_2O_3S$ $[M+H]^+$ 581.3771, found 581.3771.

Thiazole derivative 5k



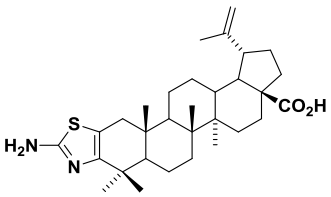
Compound **5k** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (315 mg; 2.9 mmol) in chloroform (12 mL) after 5 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (gradient elution: toluene/diethylether 1:1 → diethylether → chloroform → methanol) white crystals of compound **5k** (196 mg; 56 %) were obtained; IR (DRIFT): 3000 – 2500 (OH), 2940, 2866, 1739 (C=O), 1685 (C=C), 1523 cm^{-1}; ^{1}H NMR (500 MHz, THF-d_8): δ ppm 4.73 – 4.70 (m, 1H, H-29a), 4.59 – 4.55 (m, 1H, H-29b), 3.38 – 3.29 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 3.08 (td, *J* = 10.6, 4.6 Hz, 1H, H-18), 2.57 (d, *J* = 15.3 Hz, 1H, H-1a), 2.46 – 2.37 (m, 5H, $CH_3N(CH_2CH_2)_2N$), 2.24 (s, 3H, CH_3N), 2.12 (d, *J* = 15.4 Hz, 1H, H-1b), 1.98 – 1.86 (m, 2H), 1.69 (s, 3H, H-30), 1.18 (s, 3H), 1.09 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.89 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, THF-d_8): δ ppm 168.75, 153.67, 151.52, 115.20, 109.68, 56.74, 55.06, 53.63, 50.03, 49.98, 48.91, 47.85, 46.22, 43.15, 41.49, 39.51, 39.24, 39.01, 37.77, 37.56, 34.30, 32.88, 31.31, 30.54, 30.51, 26.45, 25.62, 22.22, 22.11, 20.36, 19.43, 16.46, 16.05, 14.83; HRMS (ESI): *m/z* calcd for $C_{36}H_{56}N_3O_2S$ $[M+H]^+$ 594.4088, found 594.4086.

Thiazole derivative 5l



Compound **5l** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **5c** (300 mg; 0.6 mmol) and cyclohexyl ammonium acetate (469 mg; 2.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 4:1 + AcOH 0,05 %) white crystals of compound **5l** (112 mg; 32 %) were obtained; MP: >290 °C (hexane); IR (DRIFT): 3216 (NH), 3200 – 2700 (OH), 2931, 2856, 1703 (C=O), 1686 (C=C), 1575 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 12.08 (s, 1H, COOH), 7.21 – 7.06 (m, 1H, NH), 4.70 (s, 1H, H-29a), 4.57 (s, 1H, H-29b), 3.26 – 3.15 (m, 1H, cyclohexyl), 3.01 – 2.90 (m, 1H, H-18), 2.44 (d, *J* = 15.4 Hz, 1H, H-1a), 2.32 – 2.23 (m, 1H), 2.17 – 2.08 (m, 1H), 2.04 (d, *J* = 15.3 Hz, 1H, H-1b), 1.96 – 1.87 (m, 2H), 1.86 – 1.75 (m, 2H), 1.65 (s, 3H, H-30), 1.12 (s, 3H), 1.01 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.81 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 177.23, 164.81, 151.55, 150.29, 111.21, 109.61, 55.44, 53.49, 52.17, 48.46, 48.43, 46.57, 42.03, 40.23, 38.36, 38.07, 37.69, 36.60, 36.28, 33.00, 32.37, 31.63, 30.23, 30.09, 29.28, 25.36, 25.09, 24.45, 21.95, 20.90, 19.15, 18.96, 16.05, 15.40, 14.37; HRMS (ESI): *m/z* calcd for $C_{37}H_{57}N_2O_2S$ $[M+H]^+$ 593.4135, found 593.4135.

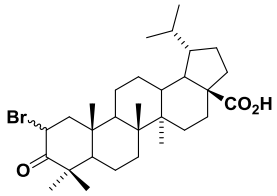
Thiazole derivative 5m



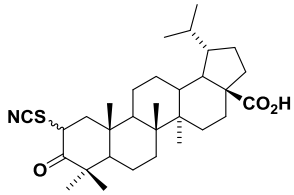
Epimeric mixture of **5m** was prepared according to the general procedure from 2α/2β-bromo betulonic acid (**5b**) (300 mg; 0.6 mmol) and thioure (427 mg; 5.6 mmol) in ethanol (6 mL) after 7 days controlled by TLC (toluene/diethylether 1:1). After the standard work up and purification (toluene/diethylether 1:1) white crystals of compound **5m** (42 mg; 15 %) were obtained; MP: decomposition at 230 °C (hexane); IR (DRIFT): 3292 (NH), 3000 – 2500 (OH), 2934, 2866, 1742 (C=O), 1688 (C=C), 1022 (C-O), 798 (NH) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 12.09 (s, 1H, COOH), 6.58 (s, 2H, NH_2), 4.74 – 4.66 (m, 1H, H-29a), 4.57 (bs, 1H, H-29b), 2.96 (td, *J* = 10.6, 5.0 Hz, 1H, H-18), 2.49 – 2.37 (m, 2H), 2.28 – 2.22 (m, 1H), 2.19 – 1.98 (m, 3H), 1.89 – 1.74 (m, 3H), 1.70 (d, *J* = 10.3 Hz, 1H), 1.66 (s, 3H, H-30), 1.11 (s, 3H), 1.01 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.82 (s, 3H, 5 × CH_3); ^{13}C NMR (101 MHz, DMSO-d_6): δ ppm 177.28, 165.14, 150.32, 128.91, 128.21, 112.45,

55.47, 52.11, 48.45, 48.41, 46.59, 42.05, 38.34, 38.10, 37.70, 36.59, 36.30, 33.00, 31.63, 30.26, 30.11, 29.31, 25.10, 22.00, 20.92, 19.15, 18.99, 16.05, 15.43, 14.38; HRMS (ESI): *m/z* calcd for $C_{31}H_{47}N_2O_2S$ $[M+H]^+$ 511.3353, found 511.3353.

2α/2β-bromo dihydrobetulonic acid (6b)

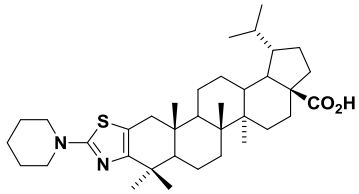


Epimeric mixture of **6b** was prepared according to the general procedure for the synthesis of 2-bromo-3-oxotriterpenoids (I) from **6a** (2 g; 4.4 mmol) dissolved in chloroform (32 mL) and bromine solution (8 mL), monitored by TLC (toluene/diethylether 10:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,05 %) white crystals of compound **6b** (2.3 g; 98 %) were obtained; R_f = 0.37 (silica gel, toluene/diethylether 10:1 with a drop of AcOH). ^{1}H NMR spectrum was consistent with the literature.[84,141]



2α/2β-thiocyanato dihydrobetulonic acid (6c)

Epimeric mixture of **6c** was prepared according to the general procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids (I) from **6b** (2 g; 3.7 mmol) and potassium thiocyanatee (2.5 g; 26.1 mmol) in dimethyl sulfoxide (70 mL) after 24 h, monitored by TLC (toluene/diethylether 10:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) compound **6c** was obtained as a white crystals (1.7 g; 89 %); R_f = 0.40 and 0.31 (silica gel, toluene/diethylether 10:1 with a drop of AcOH). ^{1}H NMR spectrum was consistent with the literature.[141]

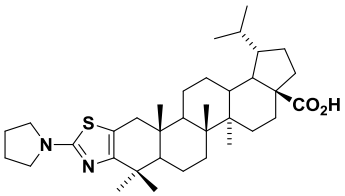


Thiazole derivative 6d

Compound **6d** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and piperidinium acetate (427 mg; 2.9 mmol) in chloroform (15 mL) after 24 hours controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 15:1 + AcOH 0,1 %) white crystals of compound **6d** (207 mg; 62 %) were obtained; MP: 305-307 °C (hexane); IR (DRIFT): 3100 – 2400 (OH), 2933, 2865, 1741 (C=O), 1693 (C=C) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 3.48 – 3.32 (m, 4H, piperidine), 2.57 (d, *J* = 15.3 Hz, 1H, H-1a), 2.32 – 2.21 (m, 3H), 2.11 (d,

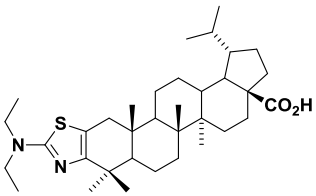
J = 15.3 Hz, 1H, H-1b), 1.89 (dd, *J* = 12.3, 7.4 Hz, 1H), 1.85 – 1.76 (m, 1H), 1.21 (s, 3H), 1.10 (s, 3H), 0.98 (s, 6H), 0.88 (s, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.76 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 182.52, 168.99, 153.09, 114.23, 57.03, 52.91, 49.64, 49.06, 48.83, 44.28, 42.73, 40.86, 39.23, 38.59, 38.48, 37.53, 37.28, 33.69, 32.16, 30.36, 29.90, 27.08, 25.33, 24.43, 23.15, 22.89, 22.08, 21.50, 19.71, 16.29, 15.94, 14.83, 14.74; HRMS (ESI): *m/z* calcd for $C_{36}H_{57}N_2O_2S$ $[M+H]^+$ 581.4135, found 581.4135.

Thiazole derivative 6e



Compound **6e** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and pyrrolidinium acetate (380 mg; 2.9 mmol) in chloroform (15 mL) after 24 hours controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **6e** (310 mg; 94 %) were obtained; MP: 276-278 °C (hexane); IR (DRIFT): 3300 – 3000 (OH), 2950, 2868, 1742 (C=O), 1693 (C=C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 3.45 – 3.37 (m, 4H, pyrrolidine), 2.55 (d, *J* = 15.2 Hz, 1H, H-1a), 2.37 (td, *J* = 12.5, 3.4 Hz, 1H, H-18), 2.32 – 2.18 (m, 3H), 2.11 (d, *J* = 18.2 Hz, 1H, H-1b), 2.00 – 1.94 (m, 4H, pyrrolidine), 1.89 (dd, *J* = 12.2, 7.2 Hz, 1H), 1.84 – 1.76 (m, 1H), 1.72 – 1.65 (m, 1H), 1.24 (s, 3H), 1.15 (s, 3H), 0.97 (s, 6H), 0.89 (s, 3H), 0.86 (d, *J* = 6.8 Hz, 3H), 0.76 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 180.91, 165.50, 152.98, 113.13, 56.94, 53.20, 49.51, 49.14, 48.77, 44.17, 42.77, 40.91, 39.42, 38.58, 38.22, 37.55, 37.36, 33.77, 32.22, 30.09, 29.94, 27.16, 25.85, 23.17, 22.91, 21.95, 21.62, 19.81, 16.31, 15.99, 14.85, 14.69; HRMS (ESI): *m/z* calcd for $C_{35}H_{55}N_2O_2S$ $[M+H]^+$ 567.3979, found 567.3979.

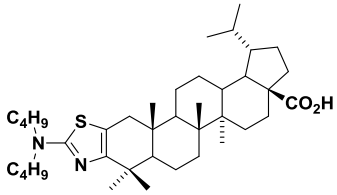
Thiazole derivative 6f



Compound **6f** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and diethyl ammonium acetate (386 mg; 2.9 mmol) in chloroform (15 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 15:1 + AcOH 0,1 %) white crystals of compound **6f** (23 mg; 7 %) were obtained; MP: 168-170 °C (hexane); IR (DRIFT): 3400 – 2300 (OH), 2956, 2867, 1731 (C=O), 1693 (C=C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 3.49 – 3.33 (m, 4H, $(CH_3CH_2)_2N$), 2.55 (d, *J* = 15.1 Hz, 1H, H-1a), 2.32

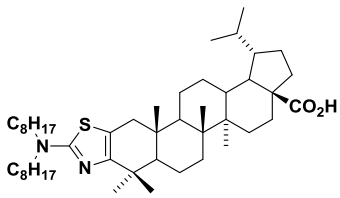
– 2.20 (m, 4H), 2.11 (d, J = 15.2 Hz, 1H, H-1b), 1.89 (dd, J = 12.4, 7.3 Hz, 1H), 1.85 – 1.77 (m, 1H), 1.73 – 1.67 (m, 1H), 1.21 (s, 3H), 1.19 (t, J = 7.1 Hz, 6H, (CH_3CH_2)$_2$N), 1.10 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.90 (s, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.76 (d, J = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 166.39, 153.26, 112.68, 56.96, 52.95, 49.12, 48.87, 45.18, 44.28, 42.77, 40.90, 39.28, 38.68, 38.51, 37.52, 37.31, 33.76, 32.18, 30.40, 29.94, 29.90, 29.86, 27.12, 23.14, 22.88, 22.10, 21.53, 19.77, 16.35, 15.95, 14.84, 14.77, 12.90; HRMS (ESI): *m/z* calcd for $C_{36}H_{57}N_2O_2S$ $[M+H]^+$ 581.4135, found 581.4135.

Thiazole derivative 6g



Compound **6g** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and dibutyl ammonium acetate (549 mg; 2.9 mmol) in chloroform (15 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 15:1 + AcOH 0,1 %) white crystals of compound **6g** (58 mg; 17 %) were obtained; MP: 192-194 °C (hexane); IR (DRIFT): 3200 – 2350 (OH), 2955, 2866, 1730 (C=O), 1693 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.40 – 3.24 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 2.54 (d, J = 15.2 Hz, 1H, H-1a), 2.31 – 2.20 (m, 4H), 2.10 (d, J = 15.1 Hz, 1H, H-1b), 1.89 (dd, J = 12.3, 7.3 Hz, 1H), 1.85 – 1.77 (m, 1H), 1.73 – 1.66 (m, 1H), 1.63 – 1.57 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.36 – 1.31 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.20 (s, 3H), 1.09 (s, 3H), 0.98 (s, 3H), 0.98 (s, 3H), 0.94 (t, J = 7.4 Hz, 6H, ($CH_3CH_2CH_2CH_2$)$_2$N), 0.90 (s, 3H), 0.87 (d, J = 6.9 Hz, 3H), 0.76 (d, J = 6.8 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 166.94, 153.17, 112.47, 56.96, 52.89, 51.01, 49.11, 48.87, 44.28, 42.76, 40.90, 39.27, 38.69, 38.51, 37.53, 37.31, 33.75, 32.18, 30.39, 29.95, 29.89, 29.82, 27.13, 23.14, 22.88, 22.10, 21.51, 20.34, 19.77, 16.37, 15.95, 14.84, 14.77, 14.07; HRMS (ESI): *m/z* calcd for $C_{39}H_{65}N_2O_2S$ $[M+H]^+$ 625.4761, found 625.4761.

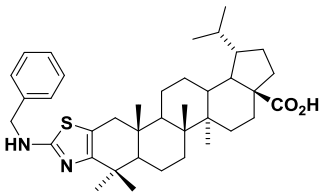
Thiazole derivative 6h



Compound **6h** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and dioctyl ammonium acetate (874 mg; 2.9 mmol) in chloroform (15 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 15:1 + AcOH 0,1 %) white crystals of compound **6h** (78 mg; 18 %) were obtained; MP: 207-209 °C

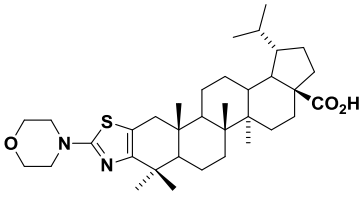
(hexane); IR (DRIFT): 3300 – 2400 (OH), 2924, 2855, 1733 (C=O), 1693 (C=C) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 3.40 – 3.21 (m, 4H, ($CH_3(CH_2)_6CH_2)_2N$)), 2.55 (d, *J* = 15.2 Hz, 1H, H-1a), 2.31 – 2.22 (m, 3H), 2.10 (d, *J* = 15.2 Hz, 1H, H-1b), 1.89 (dd, *J* = 12.3, 7.5 Hz, 1H), 1.85 – 1.78 (m, 1H), 1.73 – 1.66 (m, 1H), 1.64 – 1.59 (m, 4H, ($CH_3(CH_2)_6CH_2)_2N$)), 1.31 – 1.26 (m, 20H, ($CH_3(CH_2)_6CH_2)_2N$)), 1.20 (s, 3H), 1.09 (s, 3H), 0.99 (s, 3H), 0.98 (s, 3H), 0.90 – 0.86 (m, 12H, ($CH_3(CH_2)_7)_2N$), 0.77 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.42, 166.92, 153.16, 112.47, 57.04, 52.90, 51.39, 49.10, 48.87, 44.30, 42.75, 40.89, 39.27, 38.68, 38.52, 37.55, 37.30, 33.74, 32.19, 31.99, 30.38, 29.95, 29.90, 29.51, 29.39, 27.60, 27.15, 23.15, 22.90, 22.81, 22.09, 21.51, 19.76, 16.36, 15.95, 14.84, 14.76, 14.26; HRMS (ESI): *m/z* calcd for $C_{47}H_{81}N_2O_2S$ $[M+H]^+$ 737.6013, found 737.6008.

Thiazole derivative 6i



Compound **6i** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and benzyl ammonium acetate (485 mg; 2.9 mmol) in chloroform (15 mL) after 7 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **6i** (112 mg; 18 %) were obtained; MP: 200-204 °C (hexane); IR (DRIFT): 3085 (NH), 3100 – 2500 (OH), 2942, 2870, 1719 (C=O), 1683 (C=C), 1273, 1102 (C-O) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ ppm 11.96 (bs, 1H, COOH), 7.72 (bs, 1H, NH), 7.37 – 7.21 (m, 5H, Ph), 4.33 (qd, *J* = 15.2, 5.2 Hz, 2H, PhCH_2), 2.46 (d, *J* = 15.5 Hz, 1H, H-1a), 2.33 – 2.26 (m, 1H), 2.25 – 2.08 (m, 3H), 2.06 (d, *J* = 15.5 Hz, 1H, H-1b), 1.74 (dd, *J* = 11.6, 6.6 Hz, 2H), 1.13 (s, 3H), 1.03 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.84 – 0.81 (m, 6H), 0.74 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, DMSO-d_6): δ ppm 177.29, 165.55, 128.43, 128.13, 127.67, 127.31, 126.82, 112.10, 55.87, 52.09, 48.15, 48.02, 47.91, 43.68, 42.16, 38.34, 38.05, 37.47, 36.70, 36.61, 33.06, 31.59, 30.18, 29.35, 29.27, 26.54, 26.31, 22.96, 22.44, 21.93, 20.91, 19.12, 16.01, 15.43, 14.52, 14.26; HRMS (ESI): *m/z* calcd for $C_{38}H_{55}N_2O_2S$ $[M+H]^+$ 603.3979, found 603.3979.

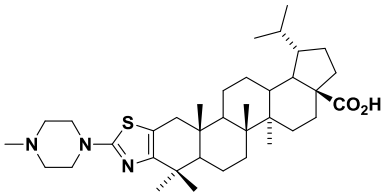
Thiazole derivative 6j



Compound **6j** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and morpholinium acetate (427 mg; 2.9 mmol) in chloroform

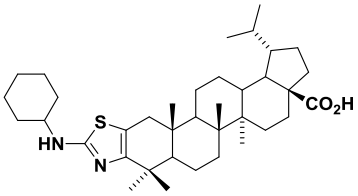
(15 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **6j** (175 mg; 52 %) were obtained; MP: 336-338 °C (hexane); IR (DRIFT): 3400 – 3000 (OH), 2933, 2865, 1741 (C=O), 1697 (C=C), 1101 (C-O-C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.85 – 3.74 (m, 4H, O(CH_2CH_2)$_2$N), 3.42 – 3.34 (m, 4H, O(CH_2CH_2)$_2$N), 2.59 (d, *J* = 15.3 Hz, 1H, H-1a), 2.33 – 2.19 (m, 4H), 2.13 (d, *J* = 15.4 Hz, 1H, H-1b), 1.89 (dd, *J* = 12.4, 7.5 Hz, 1H), 1.85 – 1.77 (m, 1H), 1.74 – 1.67 (m, 1H), 1.21 (s, 3H), 1.10 (s, 3H), 0.98 (s, 6H), 0.89 (s, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.77 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 181.75, 168.59, 153.45, 115.39, 66.45, 57.00, 52.86, 49.08, 48.86, 48.76, 44.30, 42.77, 40.89, 39.24, 38.68, 38.51, 37.53, 37.37, 33.69, 32.17, 30.42, 29.93, 29.91, 29.85, 27.07, 23.14, 22.89, 22.14, 21.53, 19.71, 16.29, 15.94, 14.84, 14.75; HRMS (ESI): *m/z* calcd for $C_{35}H_{55}N_2O_3S$ $[M+H]^+$ 583.3928, found 583.3928.

Thiazole derivative 6k



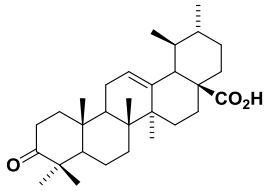
Compound **6k** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (465 mg; 2.9 mmol) in chloroform (15 mL) after 2 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (chloroform/methanol 10:1) white crystals of compound **6k** (136 mg; 40 %) were obtained; MP: 319-321 °C (hexane); IR (DRIFT): 3400 – 3100 (OH), 2932, 2865, 1741 (C=O), 1698 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 3.51 – 3.39 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 2.64 – 2.51 (m, 5H, $CH_3N(CH_2CH_2)_2N$, H-1a), 2.36 (s, 3H, CH_3N), 2.33 – 2.20 (m, 4H), 2.12 (d, *J* = 15.4 Hz, 1H, H-1b), 1.87 (dd, *J* = 12.3, 7.6 Hz, 1H), 1.85 – 1.77 (m, 1H), 1.73 – 1.67 (m, 1H), 1.20 (s, 3H), 1.10 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.88 – 0.85 (m, 6H), 0.76 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 180.40, 168.18, 153.46, 56.93, 54.12, 52.87, 49.08, 48.87, 48.03, 45.86, 44.32, 42.78, 40.91, 39.23, 38.68, 38.47, 37.55, 37.35, 33.71, 32.29, 30.43, 29.98, 29.93, 29.85, 27.10, 23.15, 22.94, 22.19, 21.56, 19.74, 16.36, 15.95, 14.85, 14.75; HRMS (ESI): *m/z* calcd for $C_{36}H_{58}N_3O_2S$ $[M+H]^+$ 596.4244, found 596.4244.

Thiazole derivative 6l



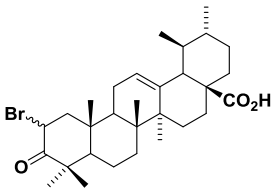
Compound **6l** was prepared according to the general procedure from 2α/2β-thiocyanato betulonic acid **6c** (300 mg; 0.6 mmol) and cyclohexyl ammonium acetate (462 mg; 2.9 mmol) in chloroform (15 mL) after 7 days controlled by TLC (hexane/EtOAc 3:1 with a drop of AcOH). After standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 % → chloroform → methanol) white crystals of compound **6l** (136 mg; 40 %) were obtained; MP: decomposition at 267 °C (hexane); IR (DRIFT): 3232 (NH), 3200 – 2700 (OH), 2938, 2902, 1717 (C=O), 1671 (C=C), 1288, 1115 (C-O) cm^{-1}; ^1H NMR (500 MHz, CD_3OD + $CDCl_3$): δ ppm 3.05 – 2.98 (m, 1H, cyclohexyl), 2.50 (d, *J* = 15.3 Hz, 1H, H-1a), 2.38 – 2.28 (m, 1H), 2.28 – 2.13 (m, 3H), 2.07 (d, *J* = 15.4 Hz, 1H, H-1b), 1.14 (s, 3H), 1.05 (s, 3H), 0.96 (s, 3H), 0.94 (s, 3H), 0.85 (s, 3H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.72 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, CD_3OD + $CDCl_3$): δ ppm 167.81, 151.15, 113.41, 57.33, 55.91, 52.98, 44.51, 42.93, 41.01, 39.32, 38.81, 38.50, 37.99, 37.03, 33.86, 33.27, 33.21, 32.73, 30.09, 27.35, 25.70, 25.02, 23.57, 23.12, 23.05, 21.90, 21.77, 19.76, 16.31, 15.89, 14.84, 14.72; HRMS (ESI): *m/z* calcd for $C_{37}H_{59}N_2O_2S$ $[M+H]^+$ 595.4292, found 595.4293.

Ursonic acid (7a)



Acid **7a** was prepared according to the procedure.[84] Ursolic acid (**7**) (5,0 g; 11.0 mmol) was dissolved in the mixture of solvents 1,4-dioxane (125 mL), acetic acid (40 mL), and acetic anhydride (17 mL). Sodium acetate trihydrate (2.3 g; 16.5 mmol) and sodium dichromane dihydrate (5.2 g; 19.8 mmol) were added to the solution. The reaction mixture was stirred 24 hours at r.t. monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). Then, the reaction mixture was processed by the procedure C and the resulting light green crystals were purified on column chromatography (toluene/diethylether 15:1 + AcOH 0,1 %) to give white crystals of **7a** (4,6 g; 92 %); R_f = 0.32 (silica gel, toluene/diethylether 5:1 with a drop of AcOH); MP: 232-240 °C (hexane); lit.[135] MP: 255-258 °C (hexane). ^1H NMR spectrum was consistent with the literature.[154]

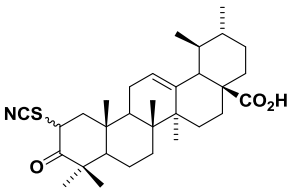
2α/2β-bromo ursonic acid (7b)



The derivative **7b** was known from the literature;[55,135] however, it was only used as a crude intermediate and its spectral data were not reported. For this reason, spectral data of **5b** are included.

Epimeric mixture of **7b** was prepared according to the general procedure for the synthesis of 2-bromo-3-oxotriterpenoids (II) from **7a** (2.6 g; 5.7 mmol) and $CuBr_2$ (2.6 g; 11.6 mmol) in mixture of ethyl acetate (52 mL) and methanol (26 mL) after 3 hours, monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). Then, the precipitate of copper(I) bromide was filtered off the reaction mixture was poured to water (300 mL) and sturated aqueous solution of sodium thiosulphate (100 mL) and the product was extracted into ethyl acetate. The collected organic layers were washed with water, dried over magnesium sulfate and purified on column chromatography (toluene/diethylether 7:1 + AcOH 0.1 %) to give white crystals of epimeric mixture of bromoketone **7b** (2.8 g; 92 %); R_f = 0.53 and 0.38 (silica gel, toluene/diethylether 5:1 with a drop of AcOH); MP: 144-147 °C (hexane); IR (DRIFT): 3200 – 2500 (OH), 2973, 2918, 2853, 1730 (C=O), 1691 (C=C) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) mixture of epimers in the ratio 0.2:0.8: δ ppm 5.30 – 5.22 (m, 2H, H-12 from both epimers), 5.12 – 5.01 (m, 2H, H-2 from both epimers), 2.59 (dd, J = 12.9, 6.2 Hz, 2H, H-1a from both epimers), 2.56 – 2.49 (m, 2H from both epimers), 2.24 – 2.17 (m, 2H from both epimers), 1.21, 1.19, 1.13, 1.11, 1.09, 1.06, 0.95, 0.94, 0.93, 0.88, 0.86, 0.85, 0.83, 0.78 (all s, 42H, 14 × CH_3 from both epimers); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 206.80, 183.17, 138.29, 125.01, 56.56, 52.50, 52.41, 52.09, 49.30, 47.93, 46.87, 42.11, 39.75, 39.61, 38.98, 38.80, 36.63, 32.47, 30.56, 27.96, 26.45, 24.00, 23.54, 23.41, 21.99, 21.13, 19.26, 17.12, 16.96, 15.39; HRMS (ESI): *m/z* calcd for $C_{30}H_{45}BrO_3$ $[M+H]^+$ 533.2625, found 533.2627, isotope 535.2615.

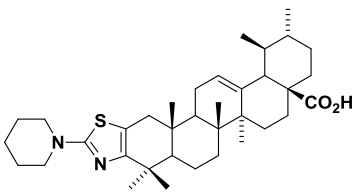
2α/2β-thiocyanato ursonic acid (7c)



Epimeric mixture of **7c** was prepared according to the general procedure for the synthesis of 2-thiocyanato-3-oxotriterpenoids (II) from **7b** (2,1 g; 3.9 mmol) and potassium thiocyanatee (2.7 g; 28.4 mmol) in dimethyl sulfoxide (75 mL) after 24 h, monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 7:1 + AcOH 0,1 %) compound **7c** was obtained as a light yellow crystals (1.27 g; 62 %); R_f = 0.33 and 0.25 (silica gel, toluene/diethylether 5:1 with a

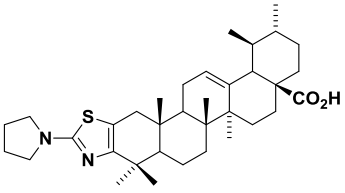
drop of AcOH); MP: 158-164 °C (hexane); IR (DRIFT): 3300 – 2500 (OH), 2924, 2857, 2155 (NC), 1743 (C=O), 1692 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of one epimer: δ ppm 5.29 – 5.25 (m, 1H, H-12), 4.73 (dd, *J* = 13.6, 6.0 Hz, 1H, H-2), 2.67 (dd, *J* = 12.8, 6.0 Hz, 1H, H-1a), 2.22 (d, *J* = 11.2 Hz, 1H), 1.29 (s, 3H), 1.16 (s, 3H), 1.13 (s, 3H), 1.08 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.88 – 0.85 (m, 6H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of one epimer: δ ppm 209.19, 183.08, 138.30, 124.86, 112.48, 57.18, 53.79, 52.51, 49.51, 49.07, 47.90, 47.21, 42.14, 39.70, 38.99, 38.87, 38.79, 36.59, 32.49, 30.55, 27.94, 24.96, 23.95, 23.58, 23.52, 21.48, 21.13, 19.10, 17.13, 16.95, 15.64; HRMS (ESI): *m/z* calcd for $C_{31}H_{48}NO_3S$ $[M+H]^+$ 512.3193, found 512.3187.

Thiazole derivative 7d



Compound **7d** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (270 mg; 0.5 mmol) and piperidinium acetate (334 mg; 2.6 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up, first purification (toluene/diethylether 5:1 + AcOH 0,1 %) and second purification (hexane/EtOAc 5:1 + AcOH 0,1 %) white crystals of compound **7d** (97 mg; 32 %) were obtained; MP: 118-122 °C (hexane); IR (DRIFT): 3300 – 2400 (OH), 2924, 2858, 1740 (C=O), 1692 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.31 – 5.26 (m, 1H, H-12), 3.42 – 3.31 (m, 4H, piperidine), 2.53 (d, *J* = 15.0 Hz, 1H, H-1a), 2.25 – 2.15 (m, 2H), 1.26 (s, 3H), 1.10 (s, 3H), 0.97 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 6H), 0.84 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.12, 168.78, 138.09, 125.95, 113.98, 52.91, 49.62, 48.24, 46.29, 42.33, 39.74, 39.29, 39.04, 39.00, 38.40, 37.15, 36.88, 32.58, 32.08, 30.82, 29.85, 29.52, 28.19, 25.34, 24.45, 24.32, 23.63, 23.45, 22.85, 21.30, 19.68, 17.12, 17.08, 15.75, 14.27; HRMS (ESI): *m/z* calcd for $C_{36}H_{55}N_2O_2S$ $[M+H]^+$ 579.3979, found 579.3976.

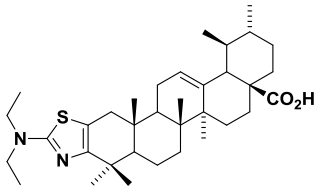
Thiazole derivative 7e



Compound **7e** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (200 mg; 0.4 mmol) and pyrrolidinium acetate (221 mg; 2.0 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **7e** (41 mg; 19 %) were obtained; MP: 168-172 °C

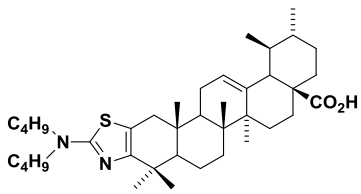
(hexane); IR (DRIFT): 3400 – 3000 (OH), 2867, 1740 (C=O), 1691 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.35 – 5.24 (m, 1H, H-12), 3.64 – 3.22 (m, 4H, pyrrolidine), 2.52 (d, *J* = 15.2 Hz, 1H, H-1a), 2.26 – 2.15 (m, 2H), 1.26 (s, 3H), 1.17 – 1.13 (m, 3H), 1.10 (s, 3H), 0.98 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.4 Hz, 3H), 0.84 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.93, 164.96, 138.12, 125.86, 112.83, 53.05, 52.90, 48.21, 46.28, 42.33, 39.73, 39.30, 39.15, 38.99, 38.39, 37.20, 36.87, 32.59, 30.83, 30.48, 30.36, 29.85, 28.18, 25.87, 24.32, 23.61, 23.44, 22.05, 21.31, 19.70, 17.11, 15.72; HRMS (ESI): *m/z* calcd for $C_{35}H_{53}N_2O_2S$ $[M+H]^+$ 565.3822, found 565.3820.

Thiazole derivative 7f



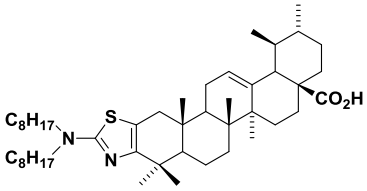
Compound **7f** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (200 mg; 0.4 mmol) and diethyl ammonium acetate (225 mg; 2.0 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **7f** (108 mg; 49 %) were obtained; MP: 160-162 °C (hexane); IR (DRIFT): 3200 – 2600 (OH), 2921, 2854, 1741 (C=O), 1693 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.32 – 5.27 (m, 1H, H-12), 3.51 – 3.32 (m, 4H, $(CH_3CH_2)_2N$), 2.51 (d, *J* = 15.1 Hz, 1H, H-1a), 2.24 – 2.15 (m, 2H), 2.09 – 1.83 (m, 5H), 1.26 – 1.16 (m, 12H, $(CH_3CH_2)_2N$, 2 × CH_3), 1.10 (s, 3H), 0.99 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.84 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.38, 166.43, 138.07, 125.98, 112.35, 52.95, 52.88, 48.24, 46.29, 45.22, 42.30, 39.74, 39.28, 39.05, 39.00, 38.43, 37.14, 36.88, 32.58, 30.81, 30.48, 29.85, 28.19, 24.31, 23.63, 23.44, 22.19, 21.30, 19.71, 17.12, 17.07, 15.79, 12.85; HRMS (ESI): *m/z* calcd for $C_{35}H_{55}N_2O_2S$ $[M+H]^+$ 567.3979, found 567.3983.

Thiazole derivative 7g



Compound **7g** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (196 mg; 0.4 mmol) and dibutyl ammonium acetate (327 mg; 1.9 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 7:1 + AcOH 0,1 %) light yellow crystals of compound **7g** (87 mg; 37 %) were obtained; MP: 128-130 °C (hexane); IR (DRIFT): 3400 – 2500 (OH), 2922, 2858, 1739

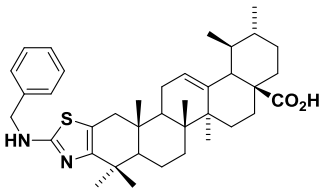
(C=O), 1693 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.31 – 5.27 (m, 1H, H-12), 3.46 – 3.18 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 2.50 (d, *J* = 15.5 Hz, 1H, H-1a), 2.21 (d, *J* = 11.6 Hz, 1H), 2.18 (d, *J* = 15.4 Hz, 1H, H-1b), 2.09 – 1.97 (m, 2H), 1.97 – 1.83 (m, 2H), 1.63 – 1.57 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.36 – 1.31 (m, 4H, ($CH_3CH_2CH_2CH_2$)$_2$N), 1.22 – 1.17 (m, 3H), 1.10 (s, 6H), 0.99 (s, 3H), 0.96 – 0.92 (m, 9H, ($CH_3CH_2CH_2CH_2$)$_2$N), 0.87 (d, *J* = 6.5 Hz, 3H), 0.84 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.12, 166.94, 153.11, 138.06, 126.10, 112.23, 52.90, 51.02, 48.23, 46.27, 42.31, 39.75, 39.29, 39.00, 38.45, 37.15, 36.89, 32.60, 31.98, 30.82, 30.54, 29.82, 29.51, 29.39, 28.20, 24.32, 23.60, 23.44, 22.81, 22.26, 21.31, 20.32, 19.76, 17.14, 17.08, 15.82, 14.25, 14.06; HRMS (ESI): *m/z* calcd for $C_{39}H_{63}N_2O_2S$ $[M+H]^+$ 623.4605, found 623.4603.



Thiazole derivative 7h

Compound **7h** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (200 mg; 0.4 mmol) and dioctyl ammonium acetate (554 mg; 2.0 mmol) in chloroform (12 mL) after 6 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 7:1 + AcOH 0,1 %) light yellow crystals of compound **7h** (167 mg; 58 %) were obtained; MP: 78-80 °C (hexane); IR (DRIFT): 3300 – 2600 (OH), 2921, 2854, 1740 (C=O), 1693 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.31 – 5.27 (m, 1H, H-12), 3.46 – 3.17 (m, 4H, ($CH_3(CH_2)_6CH_2$)$_2$N), 2.50 (d, *J* = 15.2 Hz, 1H, H-1a), 2.21 (d, *J* = 11.6 Hz, 1H), 2.18 (d, *J* = 15.5 Hz, 1H, H-1b), 2.09 – 1.98 (m, 2H), 1.96 – 1.83 (m, 2H), 1.31 – 1.26 (m, 20H, ($CH_3(CH_2)_6CH_2$)), 1.10 (s, 6H), 0.99 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.91 – 0.85 (m, 12H, ($CH_3(CH_2)_6CH_2$)$_2$N), 0.84 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.13, 166.93, 153.11, 138.06, 126.06, 112.23, 52.90, 51.41, 48.23, 46.29, 42.31, 39.75, 39.29, 39.08, 39.00, 38.44, 37.15, 36.88, 32.58, 31.98, 30.83, 30.54, 29.51, 29.38, 28.21, 27.59, 27.14, 24.34, 23.60, 23.45, 22.81, 22.28, 21.31, 19.73, 17.14, 17.08, 15.82, 14.25; HRMS (ESI): *m/z* calcd for $C_{47}H_{79}N_2O_2S$ $[M+H]^+$ 735.5857, found 735.5865.

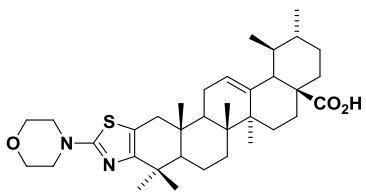
Thiazole derivative 7i



Compound **7i** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (200 mg; 0.4 mmol) and benzyl ammonium acetate (291 mg; 2.0 mmol) in chloroform

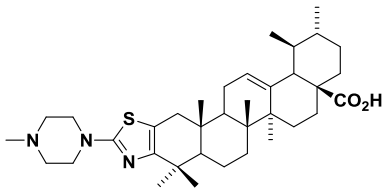
(12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up, purification (toluene/diethylether 5:1 + AcOH 0,1 %) and crystalization from hexane white crystals of compound **7i** (147 mg; 63 %) were obtained; MP: 160-163 °C (hexane); IR (DRIFT): 3500 – 2600 (OH), 3231 (NH) 2916, 2852, 1568, 734 (Ph), 701 (Ph) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.38 – 7.27 (m, 5H, Ph), 5.28 – 5.24 (m, 1H, H-12), 4.40 – 4.30 (m, 2H, PhCH_2), 2.44 (d, *J* = 15.3 Hz, 1H, H-1a), 2.25 (d, *J* = 11.4 Hz, 1H), 2.14 (d, *J* = 15.6 Hz, 1H, H-1b), 2.04 – 1.83 (m, 4H), 1.21 (s, 3H), 1.13 (s, 3H), 1.09 (s, 3H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.93 (s, 3H), 0.86 (d, *J* = 6.4 Hz, 3H), 0.83 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.97, 169.29, 138.54, 137.59, 128.73, 127.68, 127.64, 125.40, 112.89, 53.13, 52.86, 50.39, 48.19, 46.24, 42.36, 39.75, 39.36, 39.04, 38.90, 38.53, 37.01, 36.79, 32.57, 30.95, 30.04, 29.85, 28.27, 24.40, 23.62, 23.39, 21.91, 21.36, 19.65, 17.16, 17.13, 15.75.; HRMS (ESI): *m/z* calcd for $C_{36}H_{53}N_2O_2S$ $[M+H]^+$ 601.3822, found 601.3819.

Thiazole derivative 7j



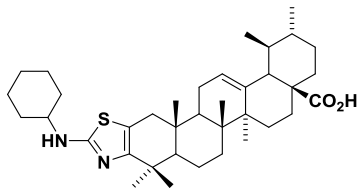
Compound **7j** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (300 mg; 0.6 mmol) and morpholinium acetate (378 mg; 3.0 mmol) in chloroform (15 mL) after 4 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 10:1 + AcOH 0,1 %) light yellow crystals of compound **7j** (192 mg; 57 %) were obtained; MP: 178-180 °C (hexane); IR (DRIFT): 3400 – 2400 (OH), 2921, 2854, 1739 (C=O), 1693 (C=C), 1116 (COC) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.32 – 5.25 (m, 1H, H-12), 3.85 – 3.74 (m, 4H, O($CH_2$$CH_2$)$_2$N), 3.45 – 3.30 (m, 4H, O($CH_2$$CH_2$)$_2$N), 2.54 (d, *J* = 15.1 Hz, 1H, H-1a), 2.25 – 2.18 (m, 2H), 2.09 – 1.98 (m, 2H), 1.97 – 1.84 (m, 2H), 1.23 – 1.18 (m, 3H), 1.18 – 1.11 (m, 3H), 1.10 (s, 3H), 0.97 (s, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.84 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.44, 168.57, 153.39, 138.12, 125.87, 115.10, 66.42, 52.87, 48.79, 48.23, 46.27, 42.30, 39.73, 39.27, 38.99, 38.43, 37.21, 36.87, 32.54, 30.80, 30.53, 28.18, 24.29, 23.63, 23.44, 22.30, 21.30, 19.68, 17.11, 17.07, 15.73.; HRMS (ESI): *m/z* calcd for $C_{35}H_{53}N_2O_3S$ $[M+H]^+$ 581.3771, found 581.3772.

Thiazole derivative 7k



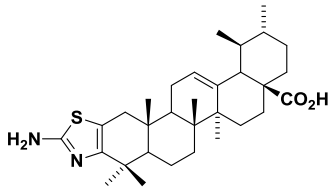
Compound **7k** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (300 mg; 0.6 mmol) and *N*-methylpiperazinium acetate (420 mg; 3.0 mmol) in chloroform (15 mL) after 4 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (chloroform/methanol 25:1) white crystals of compound **7k** (222 mg; 64 %) were obtained; MP: 198-202 °C; IR (DRIFT): 3500 – 2500 (OH), 2921, 2854, 1740 (C=O), 1695 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD + $CDCl_3$): δ ppm 5.29 – 5.20 (m, 1H, H-12), 3.50 – 3.34 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 2.65 – 2.52 (m, 4H, $CH_3N(CH_2CH_2)_2N$), 2.50 (d, *J* = 15.3 Hz, 1H, H-1a), 2.33 (s, 3H, CH_3N), 2.22 – 2.11 (m, 2H), 2.03 – 1.78 (m, 4H), 1.16 (s, 3H), 1.07 (s, 3H), 1.06 (s, 3H), 0.92 (s, 3H), 0.90 (d, *J* = 6.1 Hz, 3H), 0.84 – 0.79 (m, 6H, 7 × CH_3); ^{13}C NMR (126 MHz, CD_3OD + $CDCl_3$): δ ppm 181.15, 169.12, 153.62, 138.83, 125.92, 115.92, 54.54, 53.63, 53.34, 48.43, 46.68, 45.94, 42.77, 40.10, 39.74, 39.52, 39.37, 38.70, 37.52, 37.38, 33.03, 31.22, 30.60, 28.57, 24.77, 23.79, 23.76, 22.29, 21.39, 20.13, 17.35, 17.21, 15.93; HRMS (ESI): *m/z* calcd for $C_{36}H_{56}N_3O_2S$ $[M+H]^+$ 594.4088, found 594.4088.

Thiazole derivative 7l



Compound **7l** was prepared according to the general procedure from 2α/2β-thiocyanato ursonic acid **7c** (200 mg; 0.4 mmol) and cyclohexyl ammonium acetate (248 mg; 2.0 mmol) in chloroform (12 mL) after 7 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (gradient elution: toluene/diethylether 5:1 + AcOH 0,1 % → toluene/diethylether 2:1 + AcOH 0,1 %) light yellow crystals of compound **7l** (35 mg; 15 %) were obtained; MP: 168-170 °C (hexane); IR (DRIFT): 3500 – 2400 (OH), 3320 (NH), 2925, 2855, 1742 (C=O), 1696 (C=C) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.32 – 5.24 (m, 1H, H-12), 3.06 – 2.97 (m, 1H, cyclohexyl), 2.45 (d, *J* = 15.3 Hz, 1H, H-1a), 2.25 (d, *J* = 11.4 Hz, 1H), 2.16 (d, *J* = 15.6 Hz, 1H, H-1b), 2.07 – 1.86 (m, 9H), 1.20 (s, 3H), 1.11 (s, 3H), 1.10 (s, 3H), 0.96 (s, 3H), 0.94 (d, *J* = 6.3 Hz, 3H), 0.87 (d, *J* = 6.5 Hz, 3H), 0.85 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.96, 168.31, 138.54, 125.35, 111.60, 56.22, 53.12, 52.82, 48.18, 46.29, 42.39, 39.77, 39.35, 39.04, 38.94, 38.56, 36.94, 36.82, 36.59, 32.69, 32.59, 30.94, 29.92, 28.25, 25.59, 24.65, 24.38, 23.60, 23.44, 21.70, 21.34, 19.57, 17.16, 15.73; HRMS (ESI): *m/z* calcd for $C_{37}H_{57}N_2O_2S$ $[M+H]^+$ 593.4135, found 593.4137.

Thiazole derivative 7m



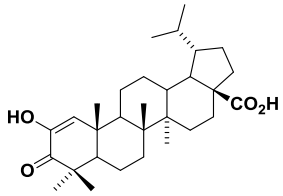
The derivative **7m** was known from the literature;[55] however, the compound was characterized only by ^{1}H NMR spectrum and HRMS spectrum with a deviation higher than 0.0020 *m/z*; other spectral data were not reported. For this reason, spectral data of **5b** including ^{1}H NMR, ^{13}C NMR, IR, and HRMS are included.

Compound **7m** was prepared according to the general procedure from 2α/2β-bromo ursonic acid **7b** (183 mg; 0.4 mmol) and thioure (261 mg; 3.4 mmol) in ethanol (4 mL) after 6 days controlled by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 30:1 + AcOH 0,1 %) white crystals of compound **7m** (157 mg; 90 %) were obtained; MP: 246-250 °C (hexane); IR (DRIFT): 3400 – 2500 (OH), 2921, 2867, 1689, 1656, 776 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.27 (t, *J* = 3.5 Hz, 1H, H-12), 3.79 (s, 2H, NH_2), 2.40 (d, *J* = 16.4 Hz, 1H, H-1a), 2.26 (d, *J* = 16.4 Hz, 1H, H-1b), 2.20 (d, *J* = 11.3 Hz, 1H), 2.05 – 1.95 (m, 2H), 1.94 – 1.81 (m, 2H), 1.57 (dd, *J* = 11.5, 6.2 Hz, 1H), 1.10 (s, 3H), 1.08 (s, 3H), 1.04 – 1.02 (m, 3H), 1.01 – 0.98 (m, 3H), 0.95 (d, *J* = 6.3 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H), 0.79 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 183.61, 158.32, 138.06, 125.75, 106.45, 61.54, 52.84, 52.56, 51.43, 48.22, 46.03, 42.24, 41.99, 39.58, 39.25, 38.98, 38.82, 36.86, 32.31, 30.79, 28.47, 28.14, 24.23, 23.60, 23.38, 21.29, 19.90, 19.61, 17.09, 15.79; HRMS (ESI): *m/z* calcd for $C_{31}H_{47}N_2O_2S$ $[M+H]^+$ 511.3353, found 511.3349.

6.5.3 Lupane derivatives substituted in the position 2

6.5.3.1 Reaction of bromoderivative 6b with mercaptoethanol

2-hydroxy-3-oxolup-1-en-28-oic acid (10)

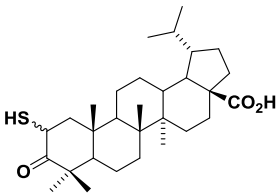


The mixture of sodium hydroxide (19 mg; 0.5 mmol), mercaptoethanol (330 µL; 4.7 mmol) and anhydrous ethanol (15 mL) was stirred at r.t. until the solution was completed. The reaction mixture was colled to 0 – 5 °C (an ice-bath) and then bromoderivative **6b** (250 mg; 0.5 mmol) was slowly added. The ice-bath was removed and the reaction mixture was stirred 3 days at r.t., monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). The reaction mixture was poured to tenfold volume of water and extracted to an organic solvent. The organic phase was collected, washed with water and the solvents were

removed in vacuo. The mixture of products was separated by column chromatography on silica gel (gradient elution: toluene/diethylether 15:1 + AcOH 0,1 % → toluene/diethylether 1:1 + AcOH 0,1 %) to give diosphenol **10** (46 mg; 21 %). The other compounds decomposed during the attempts for their purification.

An alternative way of preparation of diosphenol **10**: Compound **10** was prepared according to the general procedure for synthesis of diosphenols from dihydrobetulonic acid (**6a**) (420 mg; 0.9 mmol) and potassium tert-butoxide (415 mg; 3.7 mmol) in *tert*-butanole (21 mL) after 5 hours monitored by TLC (cyclohexane/EtOAc 2:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 4:1 + AcOH 0,1 %) white crystals of **10** (221 mg; 51 %) were obtained; ^{1}H NMR spectrum was consistent with the literature.[114]

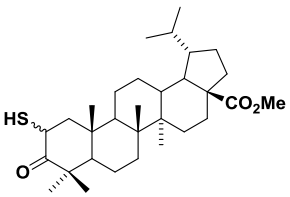
6.5.3.2 Reaction of bromoderivative 6b with sodium sulfide



2α/2β-sulfanyl-3-oxolupane-28-oic acid (11)

Bromoderivative **6b** (1.0 g; 1.9 mmol) was dissolved in NMP (100 mL) and sodium sulfide (800 mg; 10.3 mmol) was added. The reaction was stirred 90 minutes at r.t monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). The reaction was quenched when poured to water and extracted to an organic solvent. The organic phase was collected, washed with water and the solvents were evaporated in vacuo to give crude **11** (854 mg); IR (DRIFT): 3300 – 2500 (OH), 1704 (C=O), 1683 cm^{-1}; MS (ESI^{+}): *m/z* (%) = 528 (15, [M+K]$^{+}$); MS (ESI^{-}): *m/z* (%) = 470 (100, [M-H_2O]). Compound **11** decomposed when being purified on column chromatography.

Methyl 2α/2β-sulfanyl-3-oxolupane-28-oate (12)

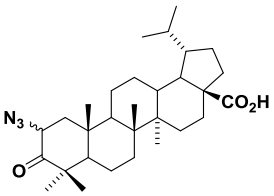


Acid **11** (100 mg; 0.2 mmol) was transformed to methyl ester by the reaction with diazomethane in a mixture of solvents diethylether and chloroform, and purified by HPLC in cyclohexane/EtOAc 13:1 to give methyl ester **12** (8 mg; 8 %); MP: 110-112 °C ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 0.76 (d, 3H, J = 6.9 Hz), 0.87 (d, 3H, J = 6.8 Hz), 0.94 (s, 3H), 0.95 (s, 3H), 0.98 (s, 3H), 1.07 (s, 3H), 1.21 (s, 3H, 7 × CH_3), 2.22 – 2.26 (m, 3H, H-13, H-18, H-19), 2.31 (dd, 1H, J_1 = 13.7 Hz, J_2 = 7.4 Hz, H-2), 3.66 (s, 3H, CH_3), 3.91 (dd, 1H, J_1 = 10.9 Hz, J_2 = 7.5 Hz, SH). ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 14.12, 14.50, 14.66, 15.78,

19.65, 20.08, 22.63, 22.74, 22.97, 26.82, 28.24, 29.60, 29.69, 29.73, 30.92, 31.57, 31.91, 31.99, 37.85, 38.07, 40.60, 42.60, 44.11, 47.72, 48.79, 51.21, 55.22, 56.96, 77.20, 176.85; HRMS (ESI-TOF) *m/z* calcd for $C_{31}H_{50}O_3S$ $[M+H]^+$ 503.3559, found 503.3557.

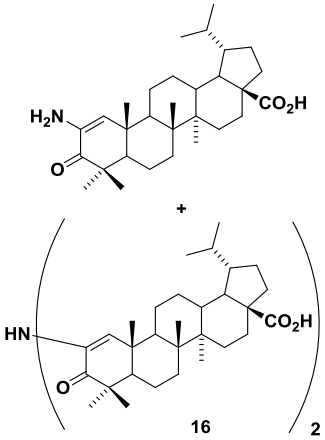
6.5.3.3 Reaction of the acid 6b with sodium azide

2α/2β-azido-3-oxolupane-28-oic acid (14)



Sodium azide (219 mg; 3.4 mmol) was added to the solution of bromoderivative **6b** (300 mg; 0.6 mmol) in NMP (15 mL). After 15 minutes bromderivative **6b** was converted completely to azide **14** according to TLC (toluene/diethylether 5:1 with a drop of AcOH). Azide **14** was precipitated by pouring the reaction mixture to tenfold volume of water, filtered off, dried under vacuum and lyophilized from *t*-butylalcohol to give 183 mg (66 %); MP was not possible to measure due to decomposition of **14** to enaminoketon **15** before melting. All physical and spectral data were obtained from crude mixture of 2α and 2β diastereoisomers (1:1): $[\alpha]_D^{20}$ = +27.8° (c = 0.36); IR (DRIFT): 3500 – 2500 (OH), 2100 (CN), 1719 (C=O), 1656 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 0.75, 0.76, 0.77, 0.86, 0.87, 0.92, 0.94, 0.98, 1.00, 1.08, 1.09, 1.13, 1.14, 1.15 (42H, all s, 14 × CH_3 from both diastereoisomers), 2.18 (t, 1H, H-1α from 2β isomer), 2.22 – 2.28 (m, 6H, H-13, H-18, H-19 all from both isomers), 2.32 (dd, 1H, J_1 = 12.6 Hz, J_2 = 6.0 Hz, H-1β from 2α isomer), 4.25 (dd, 1H, J_1 = 13.3 Hz, J_2 = 6.0 Hz, H-2β from 2α isomer), 4.29 (dd, 1H, J_1 = 11.2 Hz, J_2 = 8.7 Hz, H-2α from 2β izomer). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 14.50, 14.63, 14.66, 15.35, 16.16, 16.31, 17.84, 18.36, 19.07, 19.46, 19.90, 21.14, 21.37, 22.03, 22.72, 22.94, 24.92, 26.60, 26.86, 29.25, 29.57, 29.62, 29.70, 29.73, 30.63, 31.90, 31.98, 32.84, 33.98, 37.02, 37.36, 37.84, 38.12, 38.44, 39.98, 40.56, 40.80, 42.62, 42.74, 44.07, 44.13, 46.56, 46.61, 47.66, 48.59, 48.63, 48.74, 49.48, 49.61, 49.82, 52.03, 56.72, 56.79, 56.95, 59.89, 60.81, 181.80, 181.82, 210.62, 213.43. MS (ESI^+): *m/z* (%) = 498 (100, $[M+H]^+$), 520 (75, $[M+Na]^+$), 540 (40, $[M+K]^+$). MS (ESI^-): *m/z* (%) = 496 (100, $[M-H]^-$). HRMS (ESI-TOF) *m/z* calcd for $C_{30}H_{47}N_3O_3$ $[M+H]^+$ 498.3696, found 498.3692.

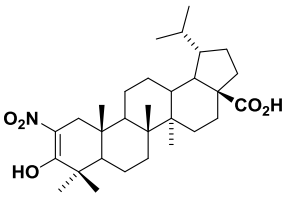
2-amino-3-oxolup-1-en-28-oic acid (15) and its dimer 16



Bromoderivative **6b** (500 mg; 0.9 mmol) was dissolved in DMSO (15 mL) and sodium azide (486 mg; 7.5 mmol) and one drop of sulfuric acid were added. The reaction mixture was stirred at 70 °C, monitored by TLC (toluene/diethylether 5:1). After 4 hours the reaction mixture was poured to double volume of water and extracted to an organic solvent. The organic phase was collected, washed with water and the solvents were removed in vacuo. The crude enaminoketone **15** was purified by column chromatography on a silica gel ($CHCl_3$/EtOAc 10:1) and was spontaneously decomposing. Fractions containing a mixture of compounds 1**5** and **16** were collected and evaporated to give 134 mg (30 %); MP: 242-243 °C ($CHCl_3$/EtOAc); $[\alpha]_D^{20}$ = -41° (c = 0.20); IR (DRIFT): 3337 (NH), 3400 – 2400 (OH), 1697 (C=O), 1675 (C=C), 1613 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 0.78 (d, 9H, *J* = 6.3 Hz, 3 × CH_3), 0.93 (d, 9H, *J* = 6.3 Hz, 3 × CH_3), 0.96 (s, 9H, 3 × CH_3), 1.05 (s, 9H, 3 × CH_3), 1.13 (s, 9H, 3 × CH_3), 1.19 (s, 9H, 3 × CH_3), 1.22 (s, 9H, 3 × CH_3), 2.27 – 2.34 (m, 9H, H-13, H-18, H-19 all from both monomer and dimer), 6.14 (s, 1H, H-1 monomer), 6.55 (s, 3H, H-1, H-1', NH dimer), 11.42 (bs, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ ppm 14.60, 14.72, 16.56, 18.97, 21.12, 21.31, 21.91, 22.77, 23.07, 26.90, 27.72, 29.69, 29.77, 31.96, 34.10, 37.63, 38.17, 38.53, 41.64, 42.97, 44.04, 44.41, 45.74, 48.49, 53.14, 56.96, 132.28, 132.81, 183.45, 200.80. MS (ESI^+): *m/z* (%) = 471 (100, $[M+H]^+$). MS (ESI^-): *m/z* (%) = 469 (100, $[M-H]^-$), 423 (70, $[M-COOH]^-$). HRMS (ESI-TOF) *m/z* calcd for $C_{30}H_{47}NO_3$ $[M+H]^+$ 470.3629, found 470.3628.

6.5.3.4 Reaction of acid 6a with nitric acid

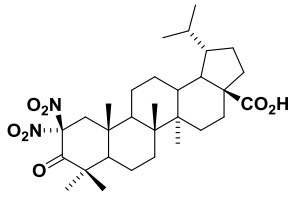
2-nitro-3-hydroxylup-2-en-28-oic acid (18)



Dihydrobetulonic acid (**6a**) (200 mg; 0.4 mmol) was dissolved in acetic acid (4 mL) by heating. The solution was gradually cooled to r.t. and nitric acid (67%, 2 mL) was added dropwise. The reaction mixture was vigorously stirred 25 hours at r.t., monitored by TLC (hexane/EtOAc 5:1). The reaction mixture was poured to tenfold volume of water; the precipitate was filtered off, washed with $KHCO_3$ and water and cryatallized from chloroform and cyclohexane. The crude product was chromatographed on silica gel (DCM:MeOH:AcOH 500:10:1) and purified by column chromatography (cyclohexane/EtOAc 5:1) to give

nitrocompound **18** (51 mg; 23 %); MP: 248-249 °C ($CHCl_3$/cyclohexane); IR (DRIFT): 3200 – 2450 (OH), 1692 (C=O), 1607 (C=C), 1570 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.78 (d, 3H, *J* = 6.9 Hz), 0.87 (s, 3H), 0.88 (d, 3H, *J* = 7.4 Hz), 0.98 (s, 6H), 1.19 (s, 3H), 1.27 (s, 3H, 7 × CH_3), 1.98 (d, 1H, *J* = 15.5 Hz, H-1a), 2.23 – 2.29 (m, 3H, H-13, H-18, H-19), 2.84 (d, 1H, *J* = 15.5 Hz, H-1b). ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 14.52, 14.64, 15.68, 16.07, 19.45, 20.45, 21.40, 22.71, 22.96, 26.68, 28.62, 29.63, 29.71, 31.91, 33.04, 36.31, 37.34, 38.29, 39.99, 40.12, 40.47, 42.62, 44.10, 48.56, 48.61, 51.77, 56.78, 122.88, 178.38, 181.90. MS (ESI^+): *m/z* (%) = 519 (40, $[M+H_2O]^+$). (ESI^-): *m/z* (%) = 500 (100, $[M-H]^-$). HRMS (ESI-TOF) *m/z* calcd for $C_{30}H_{47}NO_5$ $[M+H]^+$ 502.3527, found 502.3527.

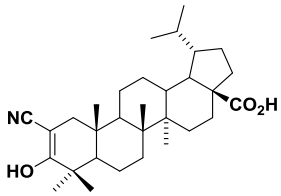
2,2-dinitro-3-oxolupane-28-oic acid (19)



Dihydrobetulonic acid (**6a**) (1.0 g; 2.2 mmol) was dissolved in acetic acid (20 mL) by heating. The solution was gradually cooled to 35 °C and nitric acid (67%, 10 mL) was added dropwise. The reaction mixture was vigorously stirred 6 hours at 35 °C, monitored by TLC (hexane/EtOAc 5:2). The reaction mixture was poured to tenfold volume of water; the precipitate was filtered off, washed with $KHCO_3$ and water and cryatallized from chloroform and cyclohexane. The crude product was purified by column chromatography (DCM:MeOH:AcOH 500:10:1). Fractions containing **19** were collected and evaporated to give shiny white crystals of dinitroderivative **19** (432 mg; 36 %); MP: 226-228 °C ($CHCl_3$/hexane); $[\alpha]_D^{20}$ = +9.6° (c = 0.5, $CHCl_3$); IR (DRIFT): 3300 – 2500 (OH), 1734 (C=O), 1692, 1573 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.78 (d, 3H, *J* = 6.3 Hz), 0.88 (d, 3H, *J* = 6.9 Hz), 0.98 (s, 3H), 1.00 (s, 3H), 1.01 (s, 3H), 1.23 (s, 3H), 1.26 (s, 3H, 7 × CH_3), 2.25 – 2.30 (m , 3H, H-13, H-18, H-19), 2.93 (d, 1H, *J* = 16.0 Hz, H-1a), 3.10 (d, 1H, *J* = 16.0 Hz, H-1b), 10.92 (bs, 1H, COOH). ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 14.50, 14.63, 15.43, 17.32, 19.59, 21.81, 22.71, 22.94, 23.12, 26.57, 29.53, 29.73, 30.34, 31.82, 32.65, 36.73, 37.34, 38.17, 40.56, 42.83, 44.06, 47.49, 48.48, 48.48, 49.32, 52.26, 56.76, 119.13, 182.19, 197.20. MS (ESI^+): *m/z* (%) = 564 (100, $[M+H_2O]^+$). HRMS (ESI-TOF) *m/z* calcd for $C_{30}H_{46}N_2O_7$ $[M+H]^+$ 547.3383, found 547.3385.

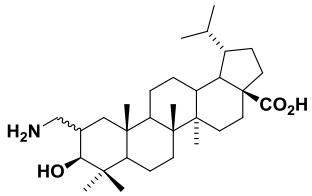
6.5.3.5 Reaction of bromketone 6b with sodium cyanide

2-cyano-3-hydroxylup-2-en-28-oic acid (21)



Sodium cyanide (69 mg; 1.4 mmol) was added to the solution of 2-bromo acid **2c** (150 mg; 0.3 mmol) in DMSO (10 mL). The reaction mixture was stirred for 2 hours at r.t., monitored on TLC (toluene/diethylether 20:1 with a drop of AcOH). The reaction was quenched by adding double volume of 5% acetic acid and then extracted to EtOAc. The organic phase was collected, washed with water and the solvents were removed in vacuo. The crude nitrile **15** (136 mg) was crystallized from cyclohexane and purified by chromatography on silica gel (toluene/diethylether/AcOH 100:10:1). Fractions containing carbonitrile were collected and evaporated to give white acicular crystals of **15** (93 mg; 69 %), MP: 222-224 °C ($CHCl_3$/hexane); $[\alpha]_D^{20}$ = -3.0° (c = 0.50). ^{1}H NMR spectrum was consistent with the literature.[141]

6.5.3.6 Reduction of carbonitrile 20 with $LiAlH_4$ and further transformation of resulting amines

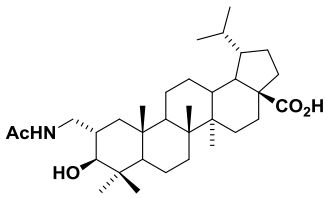


2α/2β-(aminomethyl)-3β-hydroxylupane-28-oic acid (22)

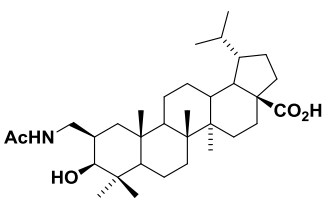
$LiAlH_4$ (207 mg; 6.2 mmol) was slowly added to the vigorously stirred solution of nitrile **20** (260 mg; 0.5 mmol) in dry THF (20 mL) under nitrogen and the mixture was heated under reflux for 30 minutes, while monitored on TLC (hexane/EtOAc 5:1). The reaction was quenched by cooling to r.t. and adding water (3 mL) until hydrogen evolution ceased. The reaction mixture was filtered off, washed with THF, solvents were evaporated under reduced pressure, and the crude product was purified by chromatography on silica gel (gradient elution: hexane/EtOAc 1:1 → hexane/EtOAc 1:20 and then the mobile phase was changed to EtOAc/methanol 1:1). Fractions containing epimeric mixture **22** were collected, evaporated and recrystalized from $CHCl_3$/methanol to give 12 mg (5 %); ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 4.53 (bs, 2H, NH_2), 3.77 (t, J = 7.8 Hz, 1H, H-3), 2.31 – 2.16 (m, 1H), 2.18 – 2.09 (m, 1H), 2.09 – 2.04 (m, 1H), 0.91 (s, 3H), 0.89 (s, 3H), 0.85 (s, 3H), 0.83 (s, 3H), 0.78 (d, J = 6.8 Hz, 3H), 0.69 (d, J = 6.6 Hz, 3H), 0.52 (s, 3H, 5 × CH_3). Compound **22** was prepared again then it was acetylated and characterized as acetamides **24a** and **24b**.

2α-(acetamidomethyl)-3β-hydroxylupane-28-oic acid (24a) and 2β-(acetamidomethyl)-3β-hydroxylupane-28-oic acid (24b)

$LiAlH_4$ (207 mg; 6.2 mmol) was slowly added to the vigorously stirred solution of nitrile **20** (300 mg; 0.6 mmol) in dry THF (20 mL) under nitrogen and the mixture was heated under reflux for 30 minutes, while monitored on TLC (hexane/EtOAc 5:1). The reaction was quenched by cooling to r.t. and adding water (3 mL) until hydrogen evolution ceased. The reaction mixture was filtered off and washed with THF, solvents were evaporated under reduced pressure to give the crude diastereomeric mixture of **22**. Acetanhydride (6 mL; 63.5 mmol) was slowly added to the solution of the crude intermediate **22** in pyridine (10 mL). The reaction mixture was stirred at r.t. for 4 hours monitored by TLC (hexane/EtOAc 5:1). Two reaction mixtures of the same amount of the starting compound were processed together by the procedure D. The crude epimeric mixture of acetate **24** (540 mg) was purified by chromatography on silica gel (hexane/EtOAc 2:3). Fractions containing epimers **24a** and **24b** were collected and evaporated to dryness.



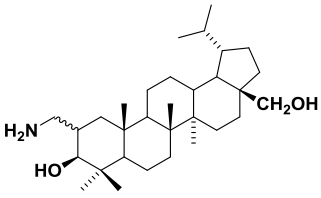
24a: white crystals (190 mg; 58 %); MP: 340-342 °C ($CHCl_3$/hexane); $[\alpha]_D^{20}$ = -18.2° (c = 0.5, $CHCl_3$); IR (DRIFT): 3441 (NH), 3334 (OH), 3500 – 2500 (OH), 1704 (C=O), 1656 cm^{-1}. ^1H NMR (500 MHz, DMSO-d_6): δ ppm 0.73 (d, 3H, J = 6.6 Hz), 0.78 (s, 3H), 0.80 – 0.82 (m, 9H), 0.87 (s, 3H), 0.93 (s, 3H, 7 × CH_3), 1.86 (s, 3H, Ac), 2.12 – 2.19 (m, 2H, H-31), 2.90 (dd, 1H, J_1 = 13.6 Hz, J_2 = 3.0 Hz, H-2), 3.60 (dd, 1H, J_1 = 13.8 Hz, J_2 = 7.9 Hz, H-3), 4.28 (s, 1H, OH), 7.65 (dd, 1H, J_1 = 7.7 Hz, J_2 = 3.5 Hz, NH), 11.97 (bs, 1H, COOH). ^{13}C NMR (125 MHz, DMSO-d_6): δ ppm 14.42, 14.52, 15.80, 16.31, 18.56, 19.87, 20.45, 22.48, 22.55, 23.02, 23.57, 23.91 26.46, 26.53, 29.21, 29.34, 31.70, 34.12, 36.67, 36.79, 37.37, 40.30, 40.57, 42.11, 42.16, 43.73, 48.08, 50.05, 52.64, 55.84, 75.29, 170.39, 177.32. MS (ESI^+): *m/z* (%) = 529 (90, $[M]^+$), 1058 (60, $[2M]^+$). MS (ESI^-): *m/z* (%) = 546 (100, $[M+H_2O-H]^-$). HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{55}NO_4$ $[M+H]^+$ 530.4204, found 530.4202.



24b: white crystals (77 mg; 23 %); MP: 190-192 °C ($CHCl_3$/hexane); $[\alpha]_D^{20}$ = -28.4° (c = 0.4, $CHCl_3$); IR (DRIFT): 3411 (NH), 3280 (OH), 3500 – 2800 (OH), 1702 (C=O), 1621 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ ppm 0.76 (d, 3H, J = 6.9 Hz), 0.86 (d, 3H, J = 6.3 Hz), 0.94 (s, 3H), 0.95 (s, 3H), 0.97 (s, 6H), 1.15 (s, 3H, 7 × CH_3),

2.03 (s, 3H, Ac), 2.18 – 2.27 (m, 5H, H-13, H-18, H-19, H-31), 3.14 – 3.18 (m, 1H, H-2), 3.86 (d, 1H, J = 2.8 Hz, H-3), 5.71 – 5.74 (dd, 1H, J_1 = 8.6 Hz, J_2 = 4.6 Hz, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ ppm 14.53, 14.66, 16.22, 16.45, 18.20, 18.52, 20.80, 22.71, 22.97, 23.13, 26.96, 29.55, 29.69, 30.35, 32.11, 34.41, 35.49, 37.02, 37.41, 37.59, 38.20, 40.72, 42.65, 44.14, 45.19, 48.77, 50.73, 51.56, 56.79, 57.57, 66.04, 172.18, 181.89. MS (ESI$^+$): *m/z* (%) = 529 (100, $[M]^+$), 530 (50, $[M+H]^+$). MS (ESI$^-$): *m/z* (%) = 528 (100, $[M-H]^-$). HRMS (ESI-TOF) *m/z* calcd for $C_{33}H_{55}NO_4$ $[M+H]^+$ 530.4204, found 530.4201.

2α/2β-(aminomethyl)-3β,28-dihydroxylupane (23)

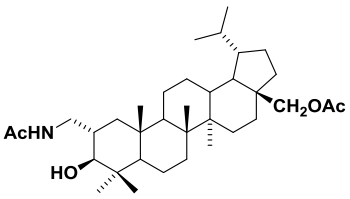


1M $LiAlH_4$.THF (3.9 mL; 3.9 mmol) was slowly added to the vigorously stirred solution of nitrile **20** (188 mg; 0.4 mmol) in dry THF (10 mL) under nitrogen and the mixture was heated under reflux for 4 hours, while monitored on TLC (hexane/EtOAc 1:1). The reaction was quenched by cooling to r.t. and adding the saturated aqueous solution of sodium-potassium tartrate (1.5 mL) until hydrogen evolution ceased. The white precipitate was extracted to ethyl acetate and washed with another portion of sodium-potassium tartrate. Collected organic phases were washed with water, dried over magnesium sulphate and the solvents were evaporated. The crude compound was purified (chloroform with traces of ammonia) to give diastereomeric mixture of **23**; IR (DRIFT): 3600 – 3200 (OH, NH), 2952, 2867, 1557, 1407, 1025 (C-O) cm^{-1}. It was impossible to separate both epimers; therefore, 2-aminoderivative **23** was transformed to acetamide and characterized as individual epimers **25a** and **25b**.

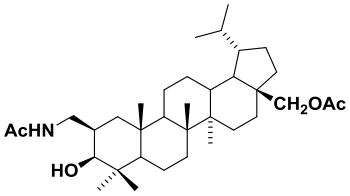
Acetyl 2α-(acetamidomethyl)-3β,28-dihydroxylupane (25a) and acetyl 2β-(acetamidomethyl)-3β,28-dihydroxylupane (25b)

1M $LiAlH_4$.THF (2.0 mL; 2.0 mmol) was slowly added to the vigorously stirred solution of nitrile **20** (100 mg; 0.2 mmol) in dry THF (10 mL) under nitrogen and the mixture was heated under reflux for 2 hours, while monitored on TLC (hexane/EtOAc 1:1). After the reaction was completed, the reaction was quenched by cooling to r.t. and adding the saturated aqueous solution of sodium-potassium tartrate until hydrogen evolution ceased. The white precipitate was extracted to ethyl acetate and washed with another portion of sodium-potassium tartrate. Collected organic phases were washed with water, dried over magnesium sulphate and the solvents were evaporated to give a crude product **23** (69 mg). Acetic anhydride (3 mL) was added to the solution of **23** (60 mg; 0.1 mmol) in pyridine (6 mL). The reaction mixture was

stirred for 6.5 hours at r.t. monitored by TLC (hexane/EtOAc 1:1). Then, the reaction mixture was processed by the procedure D and purified by column chromatography on silica gel (hexane/EtOAc 1:1). Fractions containing epimers **25a** and **25b** were collected, evaporated and dried in the drier at 65 °C.



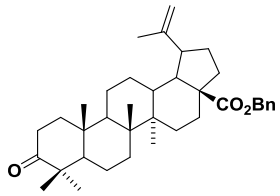
25a: white crystals (23 mg; 33 %); IR (DRIFT): 3600 – 3100 (OH, NH), 2952, 2867, 1737 (C=O), 1653 (C=O), 1234 (C-O), 1030 (C-O), 802 (NH) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.96 – 5.90 (m, 1H, NH), 4.23 (d, *J* = 10.8 Hz, 1H, H-28a), 3.87 – 3.77 (m, 2H, H-31a, H-28b), 3.16 (dd, *J* = 13.9, 2.5 Hz, 1H, H-31b), 2.79 (bs, 1H, OH), 2.05 (s, 3H, AcO), 2.02 (s, 3H, AcN), 1.02 (s, 3H), 0.95 (s, 3H), 0.88 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.75 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 171.76, 171.15, 62.96, 53.21, 50.34, 48.13, 46.52, 44.62, 42.95, 42.33, 41.08, 41.00, 37.37, 37.20, 34.70, 34.41, 29.91, 29.49, 27.60, 26.99, 26.75, 23.98, 23.37, 22.98, 21.68, 21.13, 20.82, 19.54, 18.99, 16.55, 16.13, 14.90; HRMS (ESI): *m/z* calcd for $C_{35}H_{58}NO_3$ $[M+H-H_2O]^+$ 540.4411, found 540.4413.



25b: white crystals (18 mg; 25 %); IR (DRIFT): 3404 (OH) 3277 (NH), 2953, 2867, 1741 (C=O), 1651 (C=O), 1234 (C-O), 1027 (C-O), 801 (NH) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 5.59 (dd, *J* = 8.3, 4.4 Hz, 1H, NH), 4.24 (d, *J* = 11.1 Hz, 1H, H-28a), 4.17 (bs, 1H, OH), 3.97 – 3.86 (m, 1H, H-31a), 3.86 – 3.77 (m, *J* = 10.6 Hz, 2H, H-28b), 3.15 (dt, *J* = 13.8, 3.4 Hz, 1H, H-31b), 2.23 (d, *J* = 13.7 Hz, 1H), 2.05 (s, 3H, AcO), 2.02 (s, 3H, AcN); 1.15 (s, 3H), 1.06 (s, 3H), 0.97 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.84 (d, *J* = 6.7 Hz, 3H), 0.76 (d, *J* = 6.7 Hz, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 172.20, 171.75, 66.15, 63.00, 57.69, 51.79, 50.72, 48.29, 46.66, 45.38, 44.64, 43.12, 41.14, 37.76, 37.25, 37.13, 35.66, 34.83, 34.48, 30.51, 30.06, 29.56, 27.10, 26.98, 23.32, 23.05, 21.73, 21.20, 20.88, 18.73, 18.39, 16.58, 16.31, 15.03, 14.76; HRMS (ESI): *m/z* calcd for $C_{35}H_{58}NO_3$ $[M+H-H_2O]^+$ 540.4411, found 540.4414.

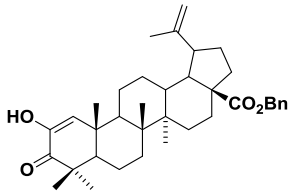
6.5.4 Betulinic acid derivatives prepared by Suzuki-Miyaura cross-coupling

Benzyl betulonate (27)



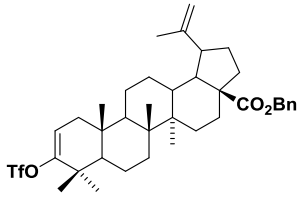
Benzyl bromide (6.0 mL; 50.2 mmol) was added to the solution of betulinic acid (**5**) (20.0 g; 44.1 mmol) and potassium carbonate (12.2 g; 88.1 mmol) in DMF (240 mL) and MeCN (40 mL). The reaction mixture was stirred for 2 days at 60 °C monitored by TLC (hexane/EtOAc 10:1). The reaction mixture was cooled to the r.t., processed by the procedure D and dried in the drier at 50 °C. Then, the crude benzyl betulinate (**26**) was dissolved in the mixture of solvents 1,3-dioxane (400 mL), acetic acid (160 mL), and acetic anhydride (50 mL). Trihydrate of sodium acetate (5.2 g; 63.4 mmol) and dihydrate of sodium dichromate (15.0 g; 50.3 mmol) were added to the reaction mixture. The reaction mixture was stirred for 16 hours at r.t. mointored by TLC (hexane/EtOAc 10:1) and then it was processed by the procedure C. The crude product was divided into two parts which were dried in the drier at 50 °C and purified separately by column chromatograpgy on silica gel (400 mL; hexane/EtOAc 10:1 for both parts) to give benzyl ester **27** (18.6 g; 93 %) as white crystals. ^{1}H NMR spectrum was consistent with the literature.[155]

Benzyl 2-hydroxybetulon-1-enoate (28)



Compound **28** was prepared according to the general procedure for synthesis of diosphenols from benzyl betulonate (**27**) (6.0 g; 11.0 mmol) and potassium tert-butoxide (5.0 g; 44.6 mmol) in *tert*-butanole (250 mL) after 2 hours monitored by TLC (toluene/diethylether 10:1). After the standard work up and without further purification white crystalic foam of **10** (6.1 g; 99 %) was obtained; ^{1}H NMR spectrum was consistent with the literature.[50]

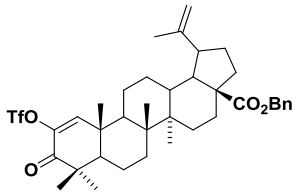
Benzyl 3-{[(trifluoromethyl)sulfonyl]oxy}betulin-2-enoate (29)



The reaction was performed under nitrogen atmosphere. A solution of **27** (1.0 g; 1.8 mmol) in dry THF (10 mL) was cooled to -78 °C and KHMDS (0.5 M in toluen, 7.35 mL; 3.7 mmol) was added dropwise. The yellow solution was being stirred for 1 hour at -78 °C when a solution of Tf_2NPh (720 mg; 2.0 mmol) in THF (2.5 mL) and toluene (2 mL) was added dropwise. The starting compound was fully consumed after 3 hours when the reaction mixture was stirred at -78 °C, monitored by TLC (hexane/EtOAc 10:1). The reaction was

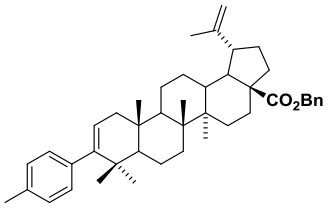
quenched with water (10 mL) and the resulting mixture was processed by the procedure E. The crude product was purified on column chromatography (hexane/toluene 1:1). Evaporation of collected fractions gave shiny white crystals of **29** (1.2 g; 99 %); R_f = 0.45 (silica gel, hexane/EtOAc 20:1); MP: 143-144 °C (hexane); IR (DRIFT): 2937, 2867, 1716 (C=O), 1643 (C=C), 1209 (C-O), 1140 (C-O), 880 cm^{-1}. Triflate **29** slowly decomposes upon heating to 50 °C; however, it can be stored at r.t. in a fume hood for at least three weeks without a trace of decomposition. ^{1}H NMR spectrum was consistent with the literature.[121]

Benzyl 2-{[(trifluoromethyl)sulfonyl]oxy}betulon-1-enoate (30)



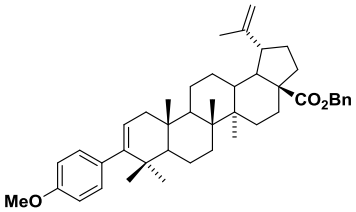
The reaction was performed in a Schlenk tube under nitrogen atmosphere. TEA (1.5 mL; 10.7 mmol) and Tf_2NPh (1.9 g; 5.4 mmol) were added to the solution of diosphenol **28** (2.0 g; 3.6 mmol) and DMAP (44 mg; 0.6 mmol) in dry DCM (20 mL). A light yellow reaction mixture got dark red while stirring at r.t., monitored by TLC (hexane/EtOAc 9:1). After 90 minutes the reaction was quenched by evaporation of DCM. The resulting mixture was dissolved in EtOAc, washed with diluted aqueous HCl (1:10), saturated aq. solultion of $KHCO_3$ and water to neutral pH. The organic phase was dried under magnesium sulphate, filtered, and the solvents were removed under reduced pressure to give a red honey-like mass. The crude product was purified on column chromatography (hexane/EtOAc 18:1 + AcOH 0.05 %). Evaporation of collected fractions gave colorless oil of triflate **30** (2.0 g; 81 %); R_f = 0.41 (silica gel, hexane/EtOAc 9:1); IR (DRIFT): 2972, 2862, 1720 (C=O), 1697 (C=C), 1421, 1205 (C-O), 1140 (C-O), 782 (C-F) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.42 – 7.29 (m, 5H, Ph), 7.03 (s, 1H, H-1), 5.16 (d, J = 12.2 Hz, 1H, $PhCH_2$-a), 5.10 (d, J = 12.2 Hz, 1H, $PhCH_2$-b), 4.79 – 4.70 (m, 1H, H-29a), 4.66 – 4.58 (m, 1H, H-29b), 3.03 (td, J = 11.0, 4.6 Hz, 1H, H-18), 2.35 – 2.22 (m, 2H), 1.97 – 1.85 (m, 2H), 1.85 – 1.77 (m, 1H), 1.69 (s, 3H, H-30), 1.21 (s, 3H), 1.15 (s, 3H), 1.13 (s, 3H), 0.96 (s, 3H), 0.82 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 196.10, 175.83, 150.37, 147.73, 142.92, 136.57, 128.66, 128.47, 128.28, 118.75 (q, $^1J_{C\text{-}F}$ = 320.5 Hz), 110.03, 65.97, 56.58, 53.22, 49.40, 47.02, 46.19, 44.83, 42.89, 41.92, 40.87, 38.34, 37.01, 33.67, 32.14, 30.64, 29.53, 27.55, 25.42, 21.51, 21.43, 19.47, 19.33, 18.89, 16.43, 14.68; HRMS (ESI): *m/z* calcd for $C_{38}H_{50}F_3O_6S$ $[M+H]^+$ 691.3275, found 691.3278.

Benzyl 3-(*p*-methylphenyl)betulin-2-enoate (31a)



Compound **31a** was prepared according to the general procedure from triflate **29** (100 mg; 0.15 mmol) in dioxane (0.6 mL), 4-methylphenylboronic acid (40 mg; 0.3 mmol) in IPO (0.6 mL), sodium carbonate (31 mg; 0.3 mmol) in water (0.3 mL) and $PdCl_2(PPh_3)_2$ (2 mg), controlled by TLC (hexane/EtOAc 25:1). After the standard work up and purification (hexane/EtOAc 25:1) white crystals of compound **31a** (75 mg; 82 %) were obtained; MP: 153-156 °C (hexane); IR (DRIFT): 2941, 2867, 1727 (C=O), 1641 (C=C), 1121 (C-O), 881, 813, 696 (CH) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.41 – 7.28 (m, 5H, Ph), 7.06 (d, *J* = 7.8 Hz, 2H, Me*Ph*), 7.01 (d, *J* = 8.0 Hz, 2H, Me*Ph*), 5.26 (dd, *J* = 6.1, 1.5 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.77 – 4.72 (m, 1H, H-29a), 4.64 – 4.58 (m, 1H, H-29b), 3.05 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.34 (s, 3H, *Me*Ph), 2.32 – 2.21 (m, 2H), 2.07 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.97 – 1.83 (m, 2H), 1.70 (s, 3H, H-30), 0.98 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.91 (s, 3H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 175.99, 150.76, 146.83, 140.85, 136.68, 135.48, 130.05, 128.65, 128.40, 128.21, 127.94, 123.71, 109.72, 65.89, 56.79, 53.11, 49.72, 49.61, 47.10, 42.53, 42.03, 40.68, 38.57, 37.65, 37.11, 36.42, 33.79, 32.24, 30.77, 29.77, 29.58, 25.86, 21.47, 21.21, 21.14, 20.02, 19.56, 16.56, 15.70, 14.81; HRMS (ESI): *m/z* calcd for $C_{44}H_{59}O_2$ $[M+H]^+$ 619.4510, found 619.4510.

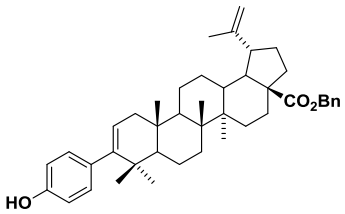
Benzyl 3-(*p*-methoxyphenyl)betulin-2-enoate (31b)



Compound **31b** was prepared according to the general procedure from triflate **29** (100 mg; 0.15 mmol) in dioxane (0.6 mL), 4-methoxyphenylboronic acid (45 mg; 0.3 mmol) in IPO (0.6 mL), sodium carbonate (31 mg; 0.3 mmol) in water (0.3 mL) and $PdCl_2(PPh_3)_2$ (2 mg), controlled by TLC (hexane/EtOAc 20:1). After the standard work up and purification (hexane/EtOAc 20:1) white crystals of compound **31b** (38 mg; 41 %) were obtained; MP: 128-132 °C (hexane); IR (DRIFT): 2939, 2870, 1724 (C=O), 1684 (C=C), 1507 (C-C), 1243, 1120 (C-O), 1037, 822, 698 (CH) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.30 (m, 5H, Ph), 7.06 – 7.01 (m, 2H, MeO*Ph*), 6.82 – 6.77 (m, 2H, MeO*Ph*), 5.26 (dd, *J* = 6.2, 1.6 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.4 Hz, 1H, Ph*CH*$_2$), 4.76 – 4.72 (m, 1H, H-29a), 4.62 – 4.59 (m, 1H, H-29b), 3.80 (s, 3H, *Me*OPh), 3.05 (td, *J* = 10.8, 4.7 Hz, 1H, H-18), 2.33 – 2.20 (m, 2H), 2.06 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.95 – 1.83 (m, 2H), 1.69 (s, 3H, H-30), 0.97 (s, 3H), 0.94 (s, 3H), 0.92 (s,

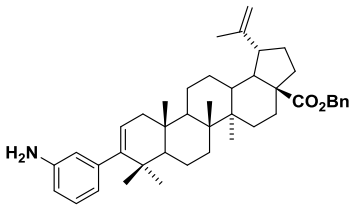
3H), 0.90 (s, 3H), 0.82 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 175.99, 157.95, 150.76, 146.43, 136.64, 136.20, 131.10, 128.64, 128.39, 128.20, 123.97, 112.59, 109.73, 65.87, 56.75, 55.30, 53.03, 49.67, 49.54, 47.06, 42.49, 42.00, 40.62, 38.51, 37.72, 37.09, 36.37, 33.74, 32.20, 30.71, 29.85, 29.73, 29.53, 25.81, 21.43, 21.09, 20.02, 19.53, 16.57, 15.67, 14.79; HRMS (ESI): *m/z* calcd for $C_{44}H_{59}O_3$ $[M+H]^+$ 635.4459, found 635.4454.

Benzyl 3-(*p*-hydroxyphenyl)betulin-2-enoate (31c)



Compound **31c** was prepared according to the general procedure from triflate **29** (200 mg; 0.3 mmol) in dioxane (1.2 mL), 4-hydroxyphenylboronic acid (80 mg; 0.6 mmol) in IPO (1.2 mL), sodium carbonate (62 mg; 0.6 mmol) in water (0.6 mL) and $PdCl_2(PPh_3)_2$ (4 mg), controlled by TLC (toluene/diethylether 30:1). After the standard work up and purification (toluene/diethylether 30:1) white crystals of compound **31c** (141 mg; 75 %) were obtained; MP: 192-194 °C (hexane); IR (DRIFT): 3397 (OH), 2936, 2867, 1729 (C=O), 1683 (C=C), 1163 (C-O), 697 (CH) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.29 (m, 5H, Ph), 7.00 – 6.96 (m, 2H, HO*Ph*), 6.74 – 6.70 (m, 2H, HO*Ph*), 5.26 (dd, *J* = 6.3, 1.8 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, PhCH_2), 5.11 (d, *J* = 12.3 Hz, 1H, PhCH_2), 4.75 – 4.71 (m, 1H, H-29a), 4.62 – 4.58 (m, 1H, H-29b), 3.04 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.32 – 2.21 (m, 2H), 2.06 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.95 – 1.86 (m, 2H), 1.69 (s, 3H, H-30), 0.98 (s, 3H), 0.94 (s, 3H), 0.92 (s, 3H), 0.90 (s, 3H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 176.06, 153.92, 150.75, 146.41, 136.65, 136.38, 131.29, 128.65, 128.39, 128.21, 124.05, 114.09, 109.72, 65.92, 56.80, 53.10, 49.71, 49.61, 47.09, 42.53, 42.04, 40.67, 38.57, 37.72, 37.11, 36.40, 33.78, 32.25, 30.76, 29.76, 29.54, 25.86, 21.46, 21.10, 20.05, 19.55, 16.56, 15.69, 14.81; HRMS (ESI): *m/z* calcd for $C_{43}H_{57}O_3$ $[M+H]^+$ 621.4302, found 621.4302.

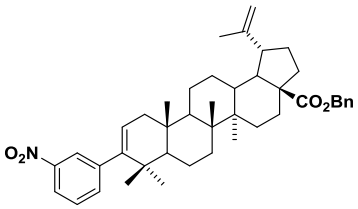
Benzyl 3-(*m*-aminophenyl)betulin-2-enoate (31d)



Compound **31d** was prepared according to the general procedure from triflate **29** (200 mg; 0.3 mmol) in dioxane (1.2 mL), 3-aminophenylboronic acid (102 mg; 0.6 mmol) in IPO (1.2 mL), sodium carbonate (62 mg; 0.6 mmol) in water (0.6 mL) and $PdCl_2(PPh_3)_2$ (4 mg), controlled by TLC (toluene/diethylether 30:1). After the standard work up and purification (toluene/diethylether 30:1) white crystals of compound **31d** (122 mg; 67 %) were obtained; MP: 167-170 °C (hexane); IR (DRIFT): 3383 (NH), 2956,

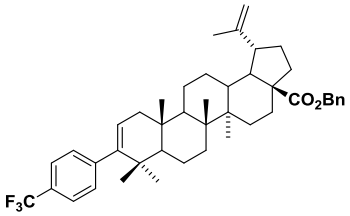
2864, 1709 (C=O), 1143 (C-O), 697 (CH) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.27 (m, 5H, Ph), 7.06 – 7.02 (m, 1H, NH_2Ph), 6.61 – 6.57 (m, 1H, NH_2Ph), 6.57 – 6.54 (m, 1H, NH_2Ph), 6.50 – 6.48 (m, 1H, NH_2Ph), 5.26 (dd, *J* = 6.2, 1.6 Hz, 1H, H-2), 5.16 (d, *J* = 12.3 Hz, 1H, PhCH_2), 5.10 (d, *J* = 12.2 Hz, 1H, PhCH_2), 4.75 – 4.71 (m, 1H, H-1a), 4.62 – 4.58 (m, 1H, H-1b), 3.04 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.32 – 2.20 (m, 2H), 2.06 (dd, *J* = 17.0, 6.3 Hz, 1H, H-1a), 1.69 (s, 3H, H-30), 0.97 (s, 3H), 0.93 (s, 6H), 0.92 (s, 3H), 0.82 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 175.99, 150.75, 147.03, 145.09, 144.97, 136.68, 128.64, 128.39, 128.20, 128.05, 123.38, 121.08, 117.48, 113.13, 109.72, 65.88, 56.79, 53.15, 49.72, 49.61, 47.09, 42.52, 41.98, 40.67, 38.56, 37.60, 37.11, 36.40, 33.78, 32.24, 30.76, 29.76, 29.73, 25.86, 21.47, 21.29, 19.98, 19.55, 16.56, 15.69, 14.81; HRMS (ESI): *m/z* calcd for $C_{43}H_{58}O_2N$ $[M+H]^+$ 620.4462, found 620.4466.

Benzyl 3-(*m*-nitrophenyl)betulin-2-enoate (31e)



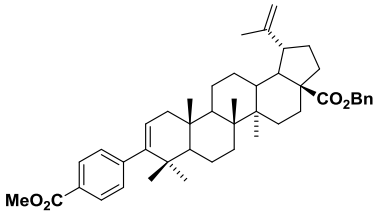
Compound **31e** was prepared according to the general procedure from triflate **29** (100 mg; 0.15 mmol) in dioxane (0.6 mL), 3-nitrophenylboronic acid (49 mg; 0.3 mmol) in IPO (0.6 mL), sodium carbonate (31 mg; 0.3 mmol) in water (0.3 mL) and $PdCl_2(PPh_3)_2$ (2 mg), controlled by TLC (hexane/EtOAc 20:1). After the standard work up and purification (hexane/EtOAc 20:1) white crystals of compound **31e** (86 mg; 90 %) were obtained; MP: 86-89 °C (hexane); IR (DRIFT): 2941, 2868, 1724 (C=O), 1643 (C=C), 1526, (N-O), 1347 (N-O), 1143, 1122 (C-O), 694 (CH) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ ppm 8.12 – 8.07 (m, 1H, NO_2Ph), 8.01 – 7.96 (m, 1H, NO_2Ph), 7.74 – 7.28 (m, 7H, NO_2Ph, Ph), 5.33 (dd, *J* = 6.2, 1.7 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, PhCH_2), 5.10 (d, *J* = 12.3 Hz, 1H, PhCH_2), 4.77 – 4.70 (m, 1H, H-29a), 4.63 – 4.57 (m, 1H, H-29b), 3.04 (td, *J* = 10.9, 4.6 Hz, 1H, H-18), 2.34 – 2.19 (m, 2H), 2.12 (dd, *J* = 17.2, 6.4 Hz, 1H, H-1a), 1.97 – 1.82 (m, 2H), 1.69 (s, 3H, H-30), 0.98 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.93 (s, 3H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 175.96, 150.73, 147.60, 145.31, 145.17, 136.66, 136.38, 128.65, 128.40, 128.20, 125.80, 124.88, 121.38, 109.74, 65.89, 56.76, 52.90, 49.67, 49.56, 47.07, 42.53, 41.87, 40.66, 38.51, 37.61, 37.09, 36.39, 33.68, 32.21, 31.74, 30.73, 29.74, 29.44, 25.80, 22.80, 21.48, 21.10, 19.92, 19.55, 16.62, 15.68, 14.81, 14.26; HRMS (ESI): *m/z* calcd for $C_{43}H_{56}O_4$ $[M+H]^+$ 650.4204, found 650.4209.

Benzyl 3-(*p*-trifluoromethylphenyl)betulin-2-enoate (31f)



Compound **31f** was prepared according to the general procedure from triflate **29** (100 mg; 0.15 mmol) in dioxane (0.6 mL), 4-trifluoromethylphenylboronic acid (56 mg; 0.3 mmol) in IPO (0.6 mL), sodium carbonate (31 mg; 0.3 mmol) in water (0.3 mL) and $PdCl_2(PPh_3)_2$ (2 mg), controlled by TLC (hexane/EtOAc 25:1). After the standard work up and purification (hexane/EtOAc 25:1) colorless oil of compound **31f** (87 mg; 88 %) was obtained; IR (DRIFT): 2945, 2869, 1723 (C=O), 1684 (C=C), 1322, 1121 (C-O), 753 (CF) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ ppm 7.50 (d, *J* = 8.0 Hz, 2H, F_3CPh), 7.41 – 7.28 (m, 5H, Ph), 7.23 (d, *J* = 7.9 Hz, 2H, F_3CPh), 5.32 – 5.24 (m, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.74 (s, 1H, H-29a), 4.60 (s, 1H, H-29b), 3.04 (td, *J* = 10.8, 4.3 Hz, 1H, H-18), 2.35 – 2.19 (m, 2H), 2.10 (dd, *J* = 17.1, 6.3 Hz, 1H, H-1a), 1.99 – 1.80 (m, 2H), 1.69 (s, 3H, H-30 0.98 (s, 3H), 0.95 (s, 3H), 0.92 (s, 6H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ ppm 175.98, 150.75, 147.53, 146.05, 136.67, 130.45, 128.65, 128.40, 128.22, 124.65, 124.23, 124.20, 109.74, 65.90, 56.77, 52.98, 49.69, 49.58, 47.08, 42.53, 41.88, 40.67, 38.53, 37.59, 37.10, 36.40, 33.72, 32.22, 31.75, 30.75, 29.75, 29.51, 25.82, 22.81, 21.47, 21.12, 19.93, 19.56, 16.59, 15.69, 14.80, 14.27; HRMS (ESI): *m/z* calcd for $C_{44}H_{56}F_3O_2$ $[M+H]^+$ 673.4227, found 673.4230.

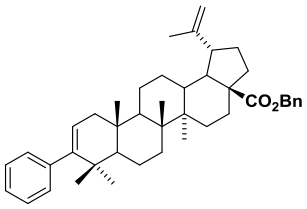
Benzyl 3-(*p*-methoxycarbonylphenyl)betulin-2-enoate (31g)



Compound **31g** was prepared according to the general procedure from triflate **29** (225 mg; 0.33 mmol) in dioxane (1.4 mL), 4-(methoxycarbonyl)phenylboronic acid (120 mg; 0.67 mmol) in IPO (1.4 mL), sodium carbonate (71 mg; 0.67 mmol) in water (0.7 mL) and $PdCl_2(PPh_3)_2$ (4.6 mg), controlled by TLC (hexane/EtOAc 15:1). After the standard work up and purification (hexane/EtOAc 10:1 + AcOH 0.1 %) white crystals of compound **31g** (144 mg; 65 %) were obtained; MP: 182-185 °C (hexane); IR (DRIFT): 2937, 2902, 2870, 1717 (C=O), 1659 (C=C), 1287 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.97 – 7.88 (m, 2H, MeO_2CPh), 7.45 – 7.30 (m, 5H, Ph), 7.22 – 7.15 (m, 2H, MeO_2CPh), 5.28 (dd, *J* = 6.2, 1.8 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.79 – 4.67 (m, 1H, H-29a), 4.65 – 4.56 (m, 1H, H-29b), 3.91 (s, 3H, *Me*O_2CPh), 3.04 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.33 – 2.27 (m, 1H), 2.27 – 2.20 (m, 1H), 2.10 (dd, *J* = 17.2, 6.4 Hz, 1H, H-1a), 1.96 – 1.83 (m, 2H), 1.69 (s, 3H, H-30), 0.98 (s, 3H), 0.96 (s, 3H), 0.92 (s, 3H), 0.92 (s, 3H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ

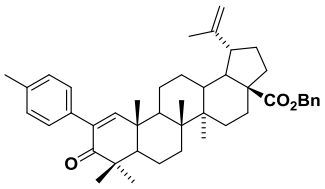
ppm 175.98, 167.40, 150.75, 148.94, 146.39, 136.68, 130.22, 128.65, 128.40, 128.21, 128.06, 124.29, 109.73, 65.90, 56.78, 53.04, 52.12, 49.70, 49.60, 47.09, 42.54, 41.90, 40.68, 38.55, 37.63, 37.10, 36.41, 33.74, 32.23, 30.76, 29.76, 29.59, 25.84, 21.49, 21.19, 19.93, 19.56, 16.58, 15.69, 14.81; HRMS (ESI): *m/z* calcd for $C_{45}H_{57}O_5$ $[M+H]^+$ 677.4201, found 677.4207.

Benzyl 3-phenylbetulin-2-enoate (31h)



Compound **31h** was prepared according to the general procedure from triflate **29** (400 mg; 0.6 mmol) in dioxane (2.4 mL), phenylboronic acid (144 mg; 1.2 mmol) in IPO (2.4 mL), sodium carbonate (124 mg; 1.2 mmol) in water (1.2 mL) and $PdCl_2(PPh_3)_2$ (4 mg), controlled by TLC (hexane/EtOAc 30:1). After the standard work up and purification (hexane/EtOAc 30:1) white crystals of compound **31h** (231 mg; 64 %) were obtained; MP: 117-120 °C (hexane); IR (DRIFT): 2918, 2850, 1726 (C=O), 1693 (C=C), 1121 (C-O), 703 (CH) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.30 (m, 5H, Ph), 7.26 – 7.19 (m, 3H, Ph), 7.14 – 7.10 (m, 2H, Ph), 5.27 (dd, *J* = 6.3, 1.8 Hz, 1H, H-2), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.80 – 4.69 (m, 1H, H-29a), 4.65 – 4.56 (m, 1H, H-29b), 3.04 (td, *J* = 10.9, 4.8 Hz, 1H, H-18, 2.32 – 2.21 (m, 2H), 2.08 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.97 – 1.83 (m, 2H), 1.69 (s, 3H, H-30), 0.98 (s, 3H), 0.96 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.83 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 176.00, 150.77, 146.98, 143.78, 136.69, 130.18, 128.65, 128.41, 128.21, 127.23, 126.05, 123.79, 109.73, 65.89, 56.80, 53.10, 49.73, 49.61, 47.10, 42.54, 42.00, 40.69, 38.57, 37.65, 37.12, 36.43, 33.79, 32.25, 30.77, 29.77, 29.58, 25.87, 21.48, 21.15, 20.01, 19.56, 16.57, 15.71, 14.82; HRMS (ESI): *m/z* calcd for $C_{43}H_{57}O_2$ $[M+H]^+$ 605.4353, found 605.4353.

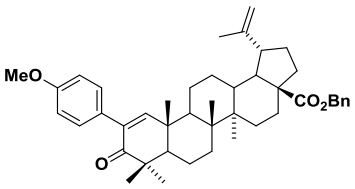
Benzyl 2-(*p*-methylphenyl)betulon-1-enoate (32a)



Compound **32a** was prepared according to the general procedure from triflate **30** (200 mg; 0.3 mmol) in dioxane (1.2 mL), 4-methylphenylboronic acid (80 mg; 0.6 mmol) in IPO (1.2 mL), sodium carbonyte (62 mg; 0.6 mmol) in water (0.6 mL) and $PdCl_2(PPh_3)_2$ (4 mg), controlled by TLC (hexane/EtOAc 15:1). After the standard work up and purification (hexane/EtOAc 15:1) white crystals of compound **32a** (168 mg; 92 %) were obtained; MP: 120-123 °C (hexane); IR (DRIFT): 2921, 2864, 1723 (C=O), 1678 (C=C), 1284 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.43 – 7.30 (m, 5H, Ph), 7.19 – 7.16

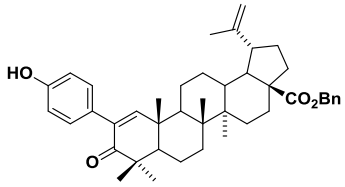
(m, 2H, Me*Ph*), 7.13 – 7.11 (m, 3H, Me*Ph*, H-1), 5.17 (d, *J* = 12.2 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.2 Hz, 1H, Ph*CH*$_2$), 4.77 – 4.70 (m, 1H, H-29a), 4.65 – 4.56 (m, 1H, H-29b), 3.05 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.33 (s, 3H, *Me*Ph), 2.32 – 2.23 (m, 2H), 1.97 – 1.84 (m, 2H), 1.82 – 1.75 (m, 1H), 1.68 (s, 3H, H-30), 1.18 (s, 3H), 1.16 (s, 3H), 1.06 (s, 3H), 0.96 (s, 3H), 0.85 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.86, 175.89, 155.45, 150.52, 137.35, 136.57, 136.18, 134.43, 128.92, 128.64, 128.43, 128.25, 128.10, 109.88, 65.92, 56.62, 52.87, 49.42, 47.00, 45.42, 45.00, 42.78, 41.59, 39.09, 38.59, 37.05, 33.69, 32.18, 30.64, 29.59, 28.78, 25.74, 21.74, 21.46, 21.29, 19.60, 19.48, 19.40, 16.31, 14.66; HRMS (ESI): *m/z* calcd for $C_{44}H_{57}O_3$ $[M+H]^+$ 633.4302, found 633.4303.

Benzyl 2-(*p*-methoxyphenyl)betulon-1-enoate (32b)



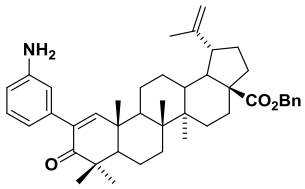
Compound **32b** was prepared according to the general procedure from triflate **30** (200 mg; 0.3 mmol) in dioxane (1.2 mL), 4-methoxyphenylboronic acid (88 mg; 0.6 mmol) in IPO (1.2 mL), sodium carbonyte (62 mg; 0.6 mmol) in water (0.6 mL) and $PdCl_2(PPh_3)_2$ (4 mg), controlled by TLC (hexane/EtOAc 15:1). After the standard work up and purification (hexane/EtOAc 15:1) white crystals of compound **32b** (185 mg; 98 %) were obtained; MP: 78-80 °C (hexane); IR (DRIFT): 2952, 2862, 1719 (C=O), 1669 (C=C), 1511, 1147 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.30 (m, 5H, Ph), 7.24 – 7.19 (m, 2H, MeO*Ph*), 7.09 (s, 1H, H-1), 6.87 – 6.82 (m, 2H, MeO*Ph*), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.76 – 4.71 (m, 1H, H-29a), 4.64 – 4.57 (m, 1H, H-29b), 3.80 (s, 3H, *Me*OPh), 3.04 (td, *J* = 10.9, 4.7 Hz, 1H, H-18), 2.34 – 2.22 (m, 2H), 1.97 – 1.83 (m, 2H), 1.82 – 1.75 (m, 1H), 1.68 (s, 3H, H-30), 1.18 (s, 3H), 1.16 (s, 3H), 1.05 (s, 3H), 0.96 (s, 3H), 0.84 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 205.00, 175.90, 159.22, 154.97, 150.52, 136.57, 135.73, 129.82, 129.39, 128.65, 128.43, 128.25, 113.68, 109.89, 65.93, 56.62, 55.45, 52.84, 49.42, 47.00, 45.42, 45.02, 42.79, 41.59, 39.06, 38.60, 37.05, 33.70, 32.19, 30.64, 29.84, 29.59, 28.81, 25.76, 21.75, 21.47, 19.60, 19.46, 16.31, 14.67; HRMS (ESI): *m/z* calcd for $C_{44}H_{57}O_4$ $[M+H]^+$ 649.4251, found 649.4245.

Benzyl 2-(*p*-hydroxyphenyl)betulon)-1-enoate (32c)



Compound **32c** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), 4-hydroxyphenylboronic acid (60 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 10:1 + AcOH 0.05 %) white crystals of compound **32c** (124 mg; 90 %) were obtained; MP: 86-89 °C (hexane); IR (DRIFT): 3411 (OH), 2925, 2867, 1726 (C=O), 1653 (C=C), 1513, 1123 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.30 (m, 5H, Ph), 7.16 – 7.11 (m, 2H, HO*Ph*), 7.08 (s, 1H, H-1), 6.77 – 6.72 (m, 2H, HO*Ph*), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.75 – 4.72 (m, 1H, H-29a), 4.62 – 4.58 (m, 1H, H-29b), 3.04 (td, *J* = 10.9, 4.8 Hz, 1H, H-18), 2.34 – 2.23 (m, 2H), 1.96 – 1.85 (m, 2H), 1.81 – 1.75 (m, 1H), 1.68 (s, 3H, H-30), 1.18 (s, 3H), 1.16 (s, 3H), 1.05 (s, 3H), 0.96 (s, 3H), 0.85 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 205.36, 175.97, 155.42, 155.20, 150.51, 136.59, 135.84, 129.81, 129.59, 128.66, 128.45, 128.27, 115.26, 109.90, 65.97, 56.68, 52.89, 49.48, 47.03, 45.47, 45.06, 42.83, 41.64, 39.11, 38.65, 37.08, 33.75, 32.23, 30.69, 29.84, 29.63, 28.82, 25.80, 21.77, 21.49, 19.62, 19.50, 19.46, 16.34, 14.69; HRMS (ESI): *m/z* calcd for $C_{43}H_{53}O_4$ $[M-H]^-$ 633.3938, found 633.3933.

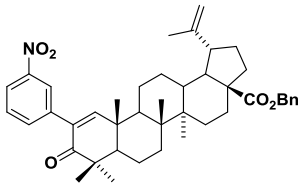
Benzyl 2-(*m*-aminophenyl)betulon-1-enoate (32d)



Compound **32d** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), 3-aminophenylboronic acid (77 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 5:1) white crystals of compound **32d** (120 mg; 87 %) were obtained; MP: 128-132 °C (hexane); IR (DRIFT): 3367 (NH), 2927, 2868, 1718 (C=O), 1667 (C=C), 1152 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.28 (m, 5H, Ph), 7.13 – 7.07 (m, 2H, NH_2*Ph*, H-1), 6.70 – 6.59 (m, 3H, NH_2*Ph*), 5.16 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.10 (d, *J* = 12.2 Hz, 1H, Ph*CH*$_2$), 4.75 – 4.70 (m, 1H, H-29a), 4.62 – 4.58 (m, 1H, H-29b), 3.03 (td, *J* = 11.0, 4.7 Hz, 1H, H-18), 2.34 – 2.22 (m, 2H), 1.97 – 1.83 (m, 2H), 1.81 – 1.74 (m, 1H), 1.67 (s, 3H, H-30), 1.17 (s, 3H), 1.15 (s, 3H), 1.04 (s, 3H), 0.96 (s, 3H), 0.84 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.76, 175.91, 155.90, 150.52, 138.51, 136.62, 136.37, 129.21, 128.66, 128.45, 128.26, 119.08, 115.48, 114.92, 109.90, 65.95, 56.67,

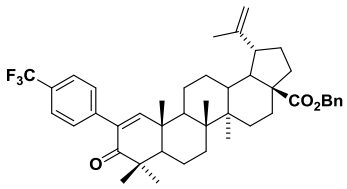
52.90, 49.49, 47.05, 45.50, 45.04, 42.83, 41.65, 39.13, 38.66, 37.08, 33.74, 32.23, 30.69, 29.63, 28.81, 25.79, 21.80, 21.45, 19.64, 19.50, 19.35, 16.34, 14.69; HRMS (ESI): *m/z* calcd for $C_{43}H_{56}NO_3$ $[M+H]^+$ 634.4255, found 634.4258.

Benzyl 2-(*m*-nitrophenyl)betulon-1-enoate (32e)



Compound **32e** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), 3-nitrophenylboronic acid (72 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 15:1) white crystals of compound **32e** (137 mg; 95 %) were obtained; MP: 120-124 °C (hexane); IR (DRIFT): 2924, 2854, 1719 (C=O), 1669 (C=C), 1530 (N-O), 1346 (N-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 8.16 – 8.10 (m, 2H, NO_2Ph), 7.66 – 7.61 (m, 1H, NO_2Ph), 7.48 (t, *J* = 7.9 Hz, 1, NO_2Ph), 7.41 – 7.29 (m, 5H, Ph), 7.24 (s, 1H, H-1), 5.17 (d, *J* = 12.2 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.76 – 4.71 (m, 1H, H-29a), 4.63 – 4.57 (m, 1H, H-29b), 3.04 (td, *J* = 11.0, 4.6 Hz, 1H, H-18), 2.35 – 2.25 (m, 2H), 1.98 – 1.85 (m, 2H), 1.84 – 1.77 (m, 1H), 1.68 (s, 3H, H-30), 1.20 (s, 3H), 1.19 (s, 3H), 1.11 (s, 3H), 0.98 (s, 3H), 0.86 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 203.92, 175.88, 158.18, 150.43, 148.30, 139.09, 136.61, 134.83, 134.58, 128.98, 128.67, 128.47, 128.28, 123.31, 122.47, 109.99, 65.97, 56.64, 53.00, 49.48, 47.04, 45.48, 44.93, 42.88, 41.78, 39.55, 38.59, 37.06, 33.70, 32.22, 30.67, 29.61, 28.76, 25.73, 21.74, 21.57, 19.54, 19.50, 19.40, 16.40, 14.72; HRMS (ESI): *m/z* calcd for $C_{43}H_{54}NO_5$ $[M+H]^+$ 664.3997, found 664.3993.

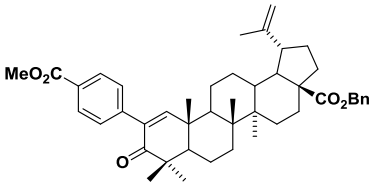
Benzyl 2-(*p*-trifluoromethylphenyl)betulon-1-enoate (32f)



Compound **32f** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), 4-trifluoromethylphenylboronic acid (83 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 15:1) white crystals of compound **32f** (129 mg; 86 %) were obtained; MP: 156-160 °C (hexane); IR (DRIFT): 2921, 2872, 1721 (C=O), 1665 (C=C), 1324, 1123 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.56 (d, *J* = 8.2 Hz, 2H, F_3CPh), 7.41 – 7.30 (m, 7H, F_3CPh, Ph), 7.19 (s, 1H, H-1), 5.17 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.76 – 4.72 (m, 1H, H-29a), 4.62 – 4.58 (m,

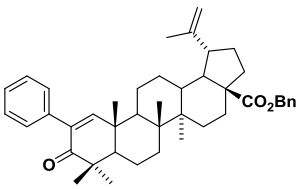
1H, H-29b), 3.04 (td, *J* = 11.0, 4.6 Hz, 1H, H-18), 2.35 – 2.25 (m, 2H), 1.99 – 1.84 (m, 2H), 1.83 – 1.76 (m, 1H), 1.68 (s, 3H, H-30), 1.19 (s, 3H), 1.18 (s, 3H), 1.09 (s, 3H), 0.97 (s, 3H), 0.86 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.08, 175.72, 157.40, 150.33, 136.45, 135.27, 128.56, 128.51, 128.30, 128.12, 124.97, 124.94, 109.76, 65.81, 56.49, 52.81, 49.32, 46.87, 45.33, 44.84, 42.70, 41.58, 39.25, 38.45, 36.90, 33.56, 32.05, 30.52, 29.69, 29.46, 28.59, 25.60, 21.61, 21.35, 19.41, 19.35, 19.19, 16.22, 14.54; HRMS (ESI): *m/z* calcd for $C_{44}H_{54}O_3F_3$ $[M+H]^+$ 687.4020, found 687.4014.

Benzyl 2-(*p*-methoxycarbonylphenyl)betulon-1-enoate (32g)



Compound **32g** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), 4-(methoxycarbonyl)phenylboronic acid (78 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 10:1) colorless oil of compound **32g** (101 mg; 69 %) was obtained; IR (DRIFT): 2927, 2867, 1721 (C=O), 1673 (C=C), 1275 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.00 – 7.95 (m, 2H, MeO_2CPh), 7.42 – 7.30 (m, 7H, MeO_2CPh, Ph), 7.21 (s, 1H, H-1), 5.17 (d, *J* = 12.3 Hz, 1H, PhCH_2), 5.10 (d, *J* = 12.3 Hz, 1H, PhCH_2), 4.79 – 4.69 (m, 1H, H-29a), 4.67 – 4.57 (m, 1H, H-29b), 3.91 (s, 3H, MeO_2CPh), 3.04 (td, *J* = 10.8, 4.5 Hz, 1H, H-18), 2.39 – 2.20 (m, 2H), 1.98 – 1.84 (m, 2H), 1.84 – 1.75 (m, 1H), 1.68 (s, 3H, H-30), 1.19 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H), 0.97 (s, 3H), 0.86 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 175.89, 167.13, 157.31, 150.51, 142.09, 136.61, 135.75, 129.52, 129.19, 128.67, 128.46, 128.34, 128.27, 109.92, 65.96, 56.66, 52.91, 52.20, 49.48, 47.04, 45.54, 44.98, 42.86, 41.72, 39.41, 38.62, 37.07, 33.70, 32.22, 30.69, 29.85, 29.63, 28.77, 25.78, 21.80, 21.49, 19.60, 19.52, 19.34, 16.37, 14.71; HRMS (ESI): *m/z* calcd for $C_{45}H_{57}O_5$ $[M+H]^+$ 677.4201, found 677.4200.

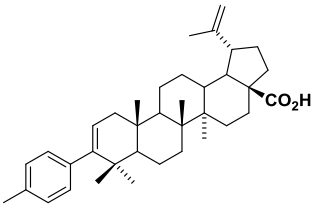
Benzyl 2-phenylbetulon-1-enoate (32h)



Compound **32h** was prepared according to the general procedure from triflate **30** (150 mg; 0.2 mmol) in dioxane (0.9 mL), phenylboronic acid (53 mg; 0.4 mmol) in IPO (0.9 mL), sodium carbonyte (47 mg; 0.4 mmol) in water (0.45 mL) and $PdCl_2(PPh_3)_2$ (3 mg), controlled by TLC (hexane/EtOAc 10:1). After the standard work up and purification (hexane/EtOAc 10:1) white crystals of compound **32h** (126 mg; 94 %) were obtained; MP:

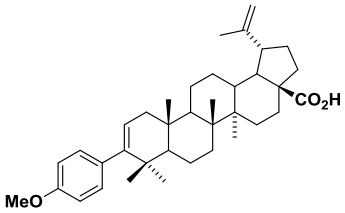
114-117 °C (hexane); IR (DRIFT): 2926, 2856, 1705 (C=O), 1637 (C=C), 1202 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.40 – 7.24 (m, 10H, 2 × Ph), 7.15 (s, 1H, H-1), 5.17 (d, *J* = 12.2 Hz, 1H, Ph*CH*$_2$), 5.11 (d, *J* = 12.3 Hz, 1H, Ph*CH*$_2$), 4.76 – 4.72 (m, 1H, H-29a), 4.63 – 4.59 (m, 1H, H-29b), 3.05 (td, *J* = 11.0, 4.7 Hz, 1H, H-18), 2.35 – 2.25 (m, 2H), 1.97 – 1.85 (m, 2H), 1.83 – 1.76 (m, 1H), 1.68 (s, 3H, H-30), 1.19 (s, 3H), 1.18 (s, 3H), 1.08 (s, 3H), 0.97 (s, 3H), 0.86 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.53, 175.72, 155.93, 150.34, 137.26, 136.45, 136.24, 128.49, 128.28, 128.14, 128.09, 128.06, 127.45, 109.73, 65.78, 56.49, 52.79, 49.32, 46.87, 45.29, 44.89, 42.66, 41.50, 39.02, 38.48, 36.90, 33.58, 32.06, 30.52, 29.68, 29.46, 28.62, 25.63, 21.61, 21.32, 19.46, 19.34, 19.22, 16.19, 14.53; HRMS (ESI): *m/z* calcd for $C_{43}H_{55}O_3$ $[M+H]^+$ 619.4146, found 619.4145.

3-(*p*-methylphenyl)betulin-2-enoic acid (33a)

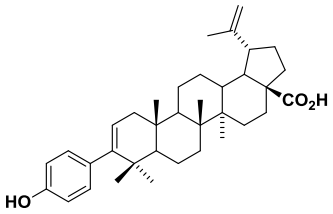


Compound **33a** was prepared according to the general procedure from ester **31a** (100 mg; 0.15 mmol) in EtOH (4 mL), cyclohexa1,3-dien (100 µL; 1.1 mmol) and Pd/C (100 mg; 61 mol %) after 48 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 7:1 + AcOH 0,1 %) colorless oil of compound **33a** (33 mg; 42 %) was obtained; IR (DRIFT): 3000 – 2500 (OH), 2932, 2869, 1702 (C=O), 1690 (C=C), 898, 815 cm^{-1}; ^1H NMR (500 MHz, THF-d_8): δ ppm 10.65 (s, 1H, COOH), 7.02 (d, *J* = 7.9 Hz, 2H, Me*Ph*), 6.96 (d, *J* = 8.0 Hz, 2H, Me*Ph*), 5.23 (dd, *J* = 6.3, 1.7 Hz, 1H, H-2), 4.73 – 4.69 (m, 1H, H-29a), 4.58 – 4.54 (m, 1, H-29b), 3.07 (td, *J* = 10.6, 4.6 Hz, 1H, H-18), 2.54 – 2.36 (m, 2H), 2.28 (s, 3H, *Me*Ph), 2.24 (dt, *J* = 12.7, 3.3 Hz, 1H), 2.09 (dd, *J* = 16.9, 6.4 Hz, 1H, H-1a), 1.98 – 1.87 (m, 2H), 1.69 (s, 3H, H-30), 1.03 (s, 3H), 1.02 (s, 3H), 1.01 (s, 3H), 0.92 (s, 3H), 0.92 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, THF-d_8): δ ppm 177.33, 151.46, 147.65, 141.23, 135.87, 130.45, 128.32, 124.04, 109.73, 56.73, 53.82, 50.40, 50.00, 47.86, 43.08, 42.48, 41.30, 39.07, 38.12, 37.52, 36.99, 34.42, 32.82, 31.29, 30.48, 29.68, 26.51, 22.06, 21.28, 20.92, 20.60, 19.43, 16.71, 16.01, 14.82; HRMS (ESI): *m/z* calcd for $C_{37}H_{51}O_2$ $[M+H]^+$ 572.3884, found 572.3862.

3-(*p*-methoxyphenyl)betulin-2-enoic acid (33b)



Compound **33b** was prepared according to the general procedure from ester **31b** (154 mg; 0.24 mmol) in EtOH (5 mL), cyclohexa-1,3-dien (162 µL; 1.7 mmol) and Pd/C (154 mg; 61 mol %) after 20 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 5:1 + AcOH 0,1 %) white crystals of compound **33b** (120 mg; 88 %) were obtained; MP: 139–142 °C (hexane); IR (DRIFT): 3000 – 2600 (OH), 2932, 2866, 1726 (C=O), 1688 (C=C), 1245 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ ppm 7.02 – 6.97 (m, 2H, MeO*Ph*), 6.78 – 6.74 (m, 2H, MeO*Ph*), 5.28 – 5.18 (m, 1H, H-2), 4.75 – 4.66 (m, 1H, H-29a), 4.62 – 4.52 (m, 1H, H-29b), 3.82 – 3.71 (m, 3H, *Me*OPh), 2.98 (td, *J* = 10.6, 4.5 Hz, 1H, H-18), 2.31 – 2.18 (m, 2H), 2.04 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.99 – 1.86 (m, 2H), 1.66 (s, 3H, H-30), 0.97 (s, 6H), 0.93 (s, 3H), 0.89 (s, 3H), 0.86 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$ + CD_3OD): δ ppm 179.26, 157.88, 150.81, 146.42, 136.24, 131.04, 123.92, 112.58, 109.57, 56.38, 55.26, 53.03, 49.62, 49.30, 47.05, 42.51, 41.97, 40.63, 38.59, 37.68, 37.21, 36.35, 33.73, 32.31, 30.70, 29.80, 29.46, 25.77, 21.41, 21.01, 19.97, 19.43, 16.48, 15.70, 14.72.HRMS (ESI): *m/z* calcd for $C_{37}H_{53}O_3$ $[M+H]^+$ 545.3989, found 545.3989.

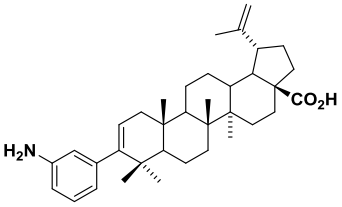


3-(*p*-hydroxyphenyl)betulin-2-enoic acid (33c)

Compound **33c** was prepared according to the general procedure from ester **31c** (105 mg; 0.17 mmol) in EtOH (5 mL), cyclohexa1,3-dien (112 µL; 1.2 mmol) and Pd/C (105 mg; 61 mol %) after 18 hours, monitored by TLC (toluene/diethylether 5:1 with a drop of AcOH). After the standard work up and purification (toluene/diethylether 5:1 + AcOH 0,1 %) white crystals of compound **33c** (71 mg; 78 %) were obtained; MP: >290 °C (hexane); IR (DRIFT): 3391 (OH), 3174 (OH), 2936, 2868, 1704 (C=O), 1690 (C=C), 1210 (C-O), 837 (CH) cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ ppm 6.93 – 6.88 (m, 2H, HO*Ph*), 6.70 – 6.63 (m, 2H, HO*Ph*), 5.21 (dd, *J* = 6.3, 1.8 Hz, 1H, H-2), 4.72 – 4.67 (m, 1H, H-29a), 4.58 – 4.53 (m, 1H, H-29b), 2.97 (td, *J* = 10.7, 4.6 Hz, 1H, H-18), 2.30 – 2.16 (m, 2H), 2.02 (dd, *J* = 17.1, 6.1 Hz, 1H, H-1a), 1.97 – 1.86 (m, 2H), 1.65 (s, 3H, H-30), 0.95 (s, 3H), 0.95 (s, 3H), 0.91 (s, 3H), 0.87 (s, 3H), 0.84 (s, 3H, 5 × CH_3); ^{13}C NMR (101 MHz, $CDCl_3$ + CD_3OD): δ ppm 179.27, 154.75, 150.81, 146.56, 135.38, 131.07, 123.71, 113.94, 109.53, 56.36, 53.02, 49.60, 49.27, 47.04, 42.48, 41.95, 40.59, 38.57, 37.65, 37.19, 36.32, 33.70, 32.29, 30.67, 29.77,

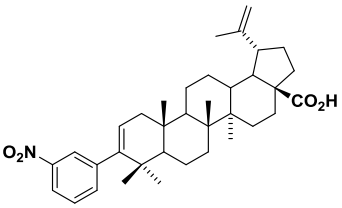
29.42, 25.75, 21.38, 20.96, 19.96, 19.39, 16.43, 15.66, 14.69; HRMS (ESI): *m/z* calcd for $C_{36}H_{51}O_3$ $[M+H]^+$ 531.3833, found 531.3833.

3-(*m*-aminophenyl)betulin-2-enoic acid (33d)



Compound **33d** was prepared according to the general procedure from ester **31d** (104 mg; 0.17 mmol), Pd/C (6 mg; 3.5 mol %) and hydrogen gas in DCM (1.5 mL) and MeOH (0.5 mL), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). The reaction mixture was quenched after 3 hours. After the standard work up and purification (hexane/EtOAc 5:1 + AcOH 0,1 %) colorless oil of compound **33d** (21 mg; 36 %) was obtained; IR (DRIFT): 3317 (NH), 2932, 2868, 1717 (C=O), 1687 (C=C), 1180 (C-O), 784, 708 (NH) cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6): δ ppm 12.03 (s, 1H, COOH), 6.88 (t, *J* = 7.7 Hz, 1H, NH_2*Ph*), 6.44 – 6.38 (m, 1H, NH_2*Ph*), 6.35 – 6.30 (m, 1H, NH_2*Ph*), 6.26 – 6.19 (m, 1H, NH_2*Ph*), 5.20 – 5.13 (m, 1H, H-2), 4.89 (bs, 1H, NH), 4.73 – 4.68 (m, 1H, H-29a), 4.60 – 4.54 (m, 1H, H-29b), 2.96 (td, *J* = 10.6, 5.0 Hz, 1, H-18), 2.34 – 2.23 (m, 1H), 2.18 – 2.08 (m, 1H), 2.08 – 1.97 (m, 1H), 1.91 – 1.74 (m, 2H), 1.66 (s, 3H, H-30), 0.97 (s, 3H), 0.93 (s, 3H), 0.92 (s, 3H), 0.89 (s, 3H), 0.87 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, DMSO-d6): δ ppm 177.25, 150.34, 147.51, 146.98, 143.59, 127.53, 122.12, 117.55, 115.65, 111.79, 109.59, 55.49, 52.45, 48.82, 48.49, 46.58, 41.99, 41.17, 40.42, 37.76, 36.95, 36.31, 35.77, 33.14, 31.64, 30.11, 29.46, 29.22, 25.18, 21.04, 20.92, 19.37, 18.99, 16.18, 15.41, 14.33; HRMS (ESI): *m/z* calcd for $C_{36}H_{52}NO_2$ $[M+H]^+$ 530.3993, found 530.3990.

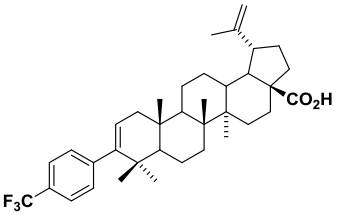
3-(*m*-nitrophenyl)betulin-2-enoic acid (33e)



Compound **33e** was prepared according to the general procedure from ester **31e** (94 mg; 0.14 mmol) in EtOH (2 mL), cyclohexa1,3-dien (96 µL; 1.0 mmol) and Pd/C (20 mg; 13 mol %) after 18 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 8:1 + AcOH 0,1 %) white crystals of compound **33e** (32 mg; 39 %) were obtained; MP: decomposition at 280 °C (hexane); IR (DRIFT): 3000 – 2500 (OH), 2949, 2870, 2833, 1737 (C=O), 1684 (C=C), 1539 (N-O), 1347 (N-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 8.11 – 8.07 (m, 1H, NO_2Ph), 7.99 – 7.96 (m, 1H, NO_2Ph), 7.48 – 7.37 (m, 2H, NO_2Ph), 5.34 (dd, *J* = 6.2, 1.8 Hz, 1H, H-2), 4.80 – 4.73 (m, 1H, H-29a), 4.66 – 4.59 (m, 1H, H-29b), 3.04 (td, *J* = 10.7, 4.8 Hz, 1H, H-18), 2.35 – 2.21 (m, 2H), 2.13 (dd, *J* = 17.2, 6.4 Hz, 1H, H-1a), 2.07 –

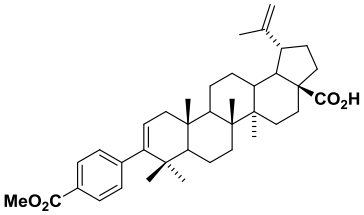
1.96 (m, 2H), 1.71 (s, 3H, H-30), 1.03 (s, 3H), 1.02 (s, 3H), 1.00 (s, 3H), 0.94 (s, 6H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 182.19, 150.55, 147.62, 145.28, 145.22, 136.34, 128.22, 125.78, 124.88, 121.40, 109.88, 56.64, 52.92, 49.60, 49.42, 47.09, 42.58, 41.86, 40.71, 38.77, 37.64, 37.21, 36.44, 33.68, 32.28, 30.75, 29.90, 29.46, 25.75, 21.45, 21.05, 19.92, 19.56, 16.63, 15.92, 14.84; HRMS (ESI): *m/z* calcd for $C_{36}H_{48}NO_4$ $[M-H]^-$ 558.3578, found 558.3571.

3-(*p*-trifluoromethylphenyl)betulin-2-enoic acid (33f)



Compound **33f** was prepared according to the general procedure from ester **31f** (79 mg; 0.12 mmol) in EtOH (2 mL), cyclohexa1,3-dien (77 µL; 0.8 mmol) and Pd/C (20 mg; 15 mol %) after 18 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 8:1 + AcOH 0,1 %) white crystals of compound **33f** (65 mg; 91 %) were obtained; MP: 250-254 °C (hexane); IR (DRIFT): 3000 – 2600 (OH), 2934, 2869, 1691, 1326, 1122 (C-O) cm^{-1}; ^{1}H and ^{13}C NMR spectra were not measured due to low solubility of **33f** in the available deuterated solvents ($CDCl_3$, CD_3OD, DMSO-d_6); HRMS (ESI): *m/z* calcd for $C_{37}H_{48}F_3O_2$ $[M-H]^-$ 581.3601, found 581.3597.

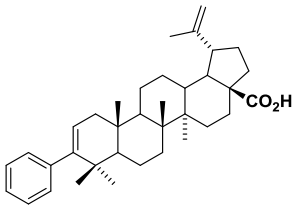
3-(*p*-methoxycarbonylphenyl)betulin-2-enoic acid (33g)



Compound **33g** was prepared according to the general procedure from ester **31g** (105 mg; 0.16 mmol) in EtOH (2 mL), cyclohexa1,3-dien (101 µL; 1.1 mmol) and Pd/C (20 mg; 12 mol %), monitored by TLC (hexane/EtOAc 20:1 with a drop of AcOH). After 24 hours another portion of cyclohexa1,3-dien (101 µL; 1.1 mmol) and Pd/C (20 mg; 12 mol %) was added. After 32 hours third portion of cyclohexa1,3-dien (101 µL; 1.1 mmol) was added. The reaction mixture was quenched after 40 hours. After the standard work up and purification (hexane/EtOAc 40:1 + AcOH 0,1 %) white crystals of compound **33g** (79 mg; 87 %) were obtained; MP: >290 °C (hexane); IR (DRIFT): 3200 – 2500 (OH), 2937, 2832, 1727 (C=O), 1689 (C=C), 1273 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.89 – 7.85 (m, 2H, MeO_2C*Ph*), 7.18 – 7.12 (m, 2H, MeO_2C*Ph*), 5.24 (dd, *J* = 6.2, 1.8 Hz, 1H, H-2), 4.72 – 4.67 (m, 1H, H-29a), 4.58 – 4.53 (m, 1H, H-29b), 3.86 (s, 3H, *Me*O_2CPh), 3.02 – 2.90 (m, 1H), 2.29 – 2.18 (m, 2H), 2.06 (dd, *J* = 17.2, 6.4 Hz, 1H, H-1a), 1.98 – 1.87 (m, 2H), 1.72 – 1.66 (m, 1H), 1.65 (s, 3H, H-30), 0.96 (s,

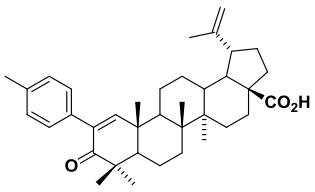
6H), 0.93 (s, 3H), 0.87 (s, 6H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 179.26, 167.61, 150.78, 148.98, 146.28, 130.15, 128.55, 127.82, 124.22, 109.55, 56.35, 52.92, 52.08, 49.58, 49.26, 47.03, 42.49, 41.79, 40.61, 38.54, 37.52, 37.18, 36.32, 33.64, 32.27, 30.67, 29.76, 29.45, 25.73, 21.39, 21.05, 19.82, 19.39, 16.46, 15.65, 14.69; HRMS (ESI): *m/z* calcd for $C_{38}H_{51}O_4$ $[M-H]^-$ 571.3782, found 571.3787.

3-phenylbetulin-2-enoic acid (33h)



Compound **33h** was prepared according to the general procedure from ester **31h** (107 mg; 0.18 mmol) in EtOH (2 mL), cyclohexa1,3-dien (111 µL; 1.2 mmol) and Pd/C (107 mg; 61 mol %) after 24 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (gradient elution: hexane/EtOAc 5:1 + AcOH 0,1 % → hexane/EtOAc 1:1 + AcOH 0,1 %) white crystals of compound **33h** (84 mg; 90 %) were obtained; MP: 278-280 °C (hexane); IR (DRIFT): 3000 – 2500 (OH), 2931, 2855, 1740 (C=O), 1688 (C=C), 895, 765, 702 cm^{-1}; ^{1}H NMR (500 MHz, THF-d_8): δ ppm 10.69 (s, 1H, COOH, 7.24 – 7.12 (m, 3H, Ph), 7.12 – 7.04 (m, 2H, Ph), 5.25 (dd, *J* = 6.3, 1.9 Hz, 1H, H-2), 4.76 – 4.68 (m, 1H, H-29a), 4.62 – 4.54 (m, 1H, H-29b), 3.12 – 3.02 (m, 1H, H-18), 2.49 – 2.45 (m, 2H), 2.44 – 2.39 (m, 1H), 2.24 (dt, *J* = 12.7, 3.3 Hz, 1H), 2.11 (dd, *J* = 17.0, 6.4 Hz, 1H, H-1a), 1.98 – 1.88 (m, 2H), 1.70 – 1.69 (m, 3H, H-30), 1.28 (s, 3H), 1.04 – 1.01 (m, 6H), 0.93 (s, 6H, 5 × CH_3); ^{13}C NMR (126 MHz, THF-d_8): δ ppm 176.36, 150.47, 146.78, 143.19, 129.58, 126.67, 125.58, 123.18, 108.73, 55.73, 52.80, 49.40, 49.00, 46.86, 42.08, 41.45, 40.27, 38.07, 37.09, 36.52, 35.99, 33.41, 31.83, 30.29, 29.44, 28.68, 25.51, 24.62, 21.06, 20.29, 19.58, 18.43, 15.72, 15.01, 13.82; HRMS (ESI): *m/z* calcd for $C_{36}H_{49}O_2$ $[M+H]^+$ 513.3727, found 513.3727.

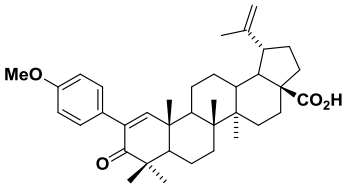
2-(*p*-methylphenyl)betulon-1-enoic acid (34a)



Compound **34a** was prepared according to the general procedure from ester **32a** (110 mg; 0.18 mmol) in EtOH (2 mL), cyclohexa1,3-dien (122 µL; 1.3 mmol) and Pd/C (20 mg; 10 mol %), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After 24 hours another portion of cyclohexa1,3-dien (122 µL; 1.3 mmol) and Pd/C (20 mg; 12 mol %) was added. The reaction mixture was quenched after 40 hours a after the standard work up and purification (hexane/EtOAc 5:1 + AcOH 0,1 %) white crystals of compound **34a** (93 mg; 94 %) were obtained; MP: 166-169 °C (hexane); IR (DRIFT): 3200 –

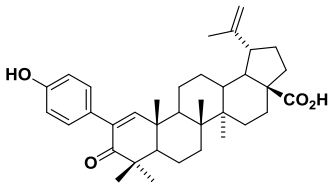
2500 (OH), 2950, 2871, 1730 (C=O), 1692 (C=C), 1510 (C-C), 1245 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.25 – 7.20 (m, 2H, Me*Ph*), 7.10 (s, 1H, H-1), 6.88 – 6.83 (m, 2H, Me*Ph*), 4.80 – 4.73 (m, 1H, H-29a), 4.66 – 4.59 (m, 1H, H-29b), 3.80 (s, 3H, *Me*Ph), 3.03 (td, *J* = 10.8, 4.9 Hz, 1H, H-18), 2.38 – 2.22 (m, 2H), 2.08 – 1.94 (m, 2H), 1.86 – 1.77 (m, 1H), 1.70 (s, 3H, H-30), 1.18 (s, 3H), 1.17 (s, 3H), 1.08 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.92, 182.10, 159.29, 154.79, 150.34, 135.84, 129.84, 129.42, 113.73, 110.04, 56.53, 55.47, 52.91, 49.29, 47.03, 45.45, 45.05, 42.88, 41.69, 39.11, 38.89, 37.20, 33.77, 32.26, 30.67, 29.78, 28.82, 25.75, 21.76, 21.46, 19.62, 19.45, 16.52, 14.74; HRMS (ESI): *m/z* calcd for $C_{37}H_{51}O_3$ $[M+H]^+$ 543.3833, found 543.3831.

2-(*p*-methoxyphenyl)betulon-1-enoic acid (34b)



Compound **34b** was prepared according to the general procedure from ester **32b** (107 mg; 0.17 mmol) in EtOH (2 mL), cyclohexa1,3-dien (118 µL; 1.2 mmol) and Pd/C (20 mg; 11 mol %) after 5 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 4:1 + AcOH 0,1 %) white crystals of compound **34b** (87 mg; 95 %) were obtained; MP: 210-212 °C (hexane); R (DRIFT): 3200 – 2500 (OH), 2921, 2864, 1712 (C=O), 1679 (C=C), 1243 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.21 – 7.16 (m, 2H, MeO*Ph*), 7.15 – 7.10 (m, 3H, MeO*Ph*, H-1), 4.78 – 4.74 (m, 1H, H-29a), 4.65 – 4.61 (m, 1H, H-29b), 3.03 (td, *J* = 10.8, 4.9 Hz, 1H, H-18), 2.34 (s, 3H, *Me*OPh), 2.33 – 2.26 (m, 2H), 2.07 – 1.96 (m, 2H), 1.85 – 1.78 (m, 1H), 1.71 – 1.69 (m, 3H, H-30), 1.18 (s, 3H), 1.17 (s, 3H), 1.09 (s, 3H), 1.05 (s, 3H), 1.01 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 181.86, 155.29, 150.34, 137.39, 136.30, 134.46, 128.94, 128.14, 110.04, 56.53, 52.94, 49.29, 47.02, 45.46, 45.03, 42.88, 41.70, 39.15, 38.88, 37.20, 33.77, 32.26, 30.67, 29.78, 28.79, 25.75, 21.76, 21.46, 21.29, 19.62, 19.50, 19.41, 16.53, 14.73; HRMS (ESI): *m/z* calcd for $C_{37}H_{51}O_4$ $[M+H]^+$ 559.3782, found 559.3782.

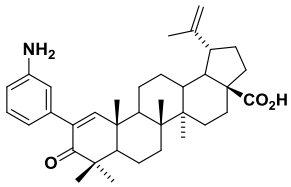
2-(*p*-hydroxyphenyl)betulon-1-enoic acid (34c)



Compound **34c** was prepared according to the general procedure from ester **32c** (124 mg; 0.20 mmol) in EtOH (2 mL), cyclohexa1,3-dien (130 µL; 1.4 mmol) and Pd/C (25 mg; 12 mol %), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After 24 hours another portion of cyclohexa1,3-dien (130 µL; 1.4 mmol) and Pd/C

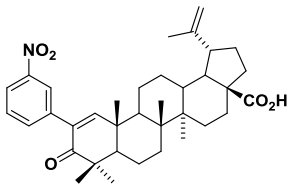
(25 mg; 12 mol %) was added. After 48 hours third portion of cyclohexa1,3-dien (130 µL; 1.4 mmol) and Pd/C (25 mg; 12 mol %) was added. The reaction was quenched after 3 days. After the standard work up and purification (hexane/EtOAc 3:1 + AcOH 0,1 %) white crystals of compound **34c** (96 mg; 91 %) were obtained; MP: 260-262 °C (hexane); IR (DRIFT): 3383 (OH), 3200 – 2500 (OH), 2940, 2867, 1728 (C=O), 1682 (C=C), 1512 (C-C), 835 (C-O), 735 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + CD_3OD): δ ppm 7.14 – 7.09 (m, 2H, HO*Ph*), 7.07 (s, 1H, H-1), 6.77 – 6.72 (m, 2H, HO*Ph*), 4.74 – 4.70 (m, 1H, H-29a), 4.61 – 4.57 (m, 1H, H-29b), 3.00 (td, *J* = 10.9, 4.6 Hz, 1H, H-18), 2.34 – 2.23 (m, 2H), 2.02 – 1.90 (m, 2H), 1.83 – 1.74 (m, 1H), 1.67 (s, 3H, H-30), 1.15 (s, 3H), 1.14 (s, 3H), 1.04 (s, 3H), 1.02 (s, 3H), 0.97 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$ + CD_3OD): δ ppm 205.61, 179.63, 156.15, 155.00, 150.51, 135.92, 129.45, 129.07, 115.17, 109.84, 56.31, 52.81, 49.21, 47.00, 45.44, 45.01, 42.84, 41.63, 39.07, 38.74, 37.20, 33.72, 32.29, 30.64, 29.71, 28.75, 25.73, 21.75, 21.40, 19.58, 19.42, 16.42, 14.65; HRMS (ESI): *m/z* calcd for $C_{36}H_{47}O_4$ $[M-H]^-$ 543.3469, found 543.3472.

3-(*m*-aminophenyl)betulon-2-enoic acid (34d)



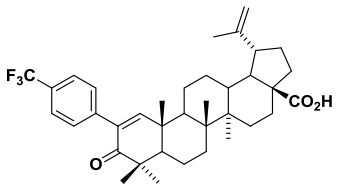
Compound **34d** was prepared according to the general procedure from ester **32d** (66 mg; 0.10 mmol), Pd/C (5.5 mg; 5 mol %) and hydrogen gas in DCM (1 mL) and MeOH (0.3 mL), monitored by TLC (hexane/EtOAc 3:1 with a drop of AcOH). The reaction mixture was quenched after 1 hour. After the standard work up and purification (hexane/EtOAc 3:1 + AcOH 0,1 %) white crystals of compound **34d** (20 mg; 34 %) were obtained; MP: 182-184 °C (hexane); IR (DRIFT): 3358 (NH), 2930, 2865, 1717 (C=O), 1674 (C=C), 1165 (C-O), cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.17 – 7.10 (m, 2H, NH_2*Ph*), 6.79 – 6.65 (m, 3H, NH_2*Ph*, H-1), 4.78 – 4.72 (m, 1H, H-29a), 4.65 – 4.59 (m, 1H, H-29b), 3.02 (td, *J* = 10.8, 4.9 Hz, 1H, H-18), 2.35 – 2.22 (m, 2H), 2.07 – 1.94 (m, 2H), 1.85 – 1.76 (m, 1H), 1.69 (s, 3H, H-30), 1.18 (s, 3H), 1.16 (s, 3H), 1.07 (s, 3H), 1.04 (s, 3H), 1.00 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.73, 181.19, 155.91, 150.31, 138.54, 136.37, 129.25, 119.72, 115.98, 115.43, 110.06, 56.47, 52.90, 49.30, 47.03, 45.51, 44.99, 42.89, 41.71, 39.17, 38.88, 37.19, 33.75, 32.27, 30.67, 29.77, 28.82, 25.74, 21.77, 21.45, 19.62, 19.49, 19.38, 16.53, 14.74; HRMS (ESI): *m/z* calcd for $C_{36}H_{48}NO_3$ $[M-H]^-$ 542.3629, found 542.3631.

2-(*m*-nitrophenyl)betulon-1-enoic acid (34e)



Compound **34e** was prepared according to the general procedure from ester **32e** (127 mg; 0.19 mmol) in EtOH (2 mL), cyclohexa1,3-dien (127 µL; 1.3 mmol) and Pd/C (20 mg; 10 mol %), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After 24 hours another portion of cyclohexa1,3-dien (127 µL; 1.3 mmol) and Pd/C (20 mg; 12 mol %) was added. After 45 hours third portion of cyclohexa1,3-dien (127 µL; 1.3 mmol) and Pd/C (20 mg; 12 mol %) was added. The reaction was quenched after 3 days. After the standard work up and purification (hexane/EtOAc 5:1 + AcOH 0,1 % and DCM 0.05 %) white crystals of compound **34e** (27 mg; 25 %) were obtained as well as white crystals of amino derivative **34d** (61 mg; 59 %) characterized above were obtained; MP: 188-192 °C (hexane); IR (DRIFT): 3100 – 2500 (OH), 2932, 2868, 1729 (C=O), 1692 (C=C), 1524 (N-O), 1347 (N-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.19 – 8.11 (m, 2H, NO_2Ph), 7.67 – 7.63 (m, 1H, NO_2Ph), 7.51 – 7.45 (m, 1H, NO_2Ph), 7.26 (s, 1H, H-1), 4.79 – 4.74 (m, 1H, H-29a), 4.65 – 4.59 (m, 1H, H-29b), 3.03 (td, *J* = 10.9, 4.9 Hz, 1H, H-18), 2.36 – 2.27 (m, 2H), 2.06 – 1.96 (m, 2H), 1.88 – 1.81 (m, 1H), 1.70 (s, 3H, H-30), 1.21 – 1.19 (m, 6H), 1.14 (s, 3H), 1.07 (s, 3H), 1.03 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 203.87, 181.28, 158.04, 150.22, 148.31, 139.05, 134.85, 134.66, 129.00, 123.29, 122.50, 110.16, 52.99, 49.28, 47.01, 45.49, 44.90, 42.94, 41.84, 39.57, 38.82, 37.20, 33.73, 32.24, 30.63, 29.85, 29.76, 28.77, 25.68, 21.73, 21.56, 19.54, 19.48, 19.43, 16.58, 14.77; HRMS (ESI): *m/z* calcd for $C_{36}H_{48}NO_5$ $[M+H]^+$ 574.3527, found 574.3525.

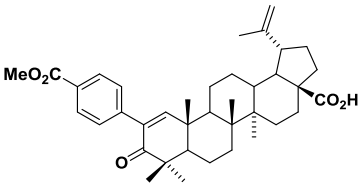
2-(*p*-trifluoromethylphenyl)betulon-1-enoic acid (34f)



Compound **34f** was prepared according to the general procedure from ester **32f** (129 mg; 0.19 mmol) in EtOH (2 mL), cyclohexa1,3-dien (125 µL; 1.3 mmol) and Pd/C (20 mg; 10 mol %), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After 24 hours another portion of cyclohexa1,3-dien (125 µL; 1.3 mmol) and Pd/C (20 mg; 12 mol %) was added. The reaction mixture was quenched after 30 hours and after the standard work up and purification (hexane/EtOAc 15:1 + AcOH 0,1 %) white crystals of compound **34f** (105 mg; 94 %) were obtained; MP: >290 °C (hexane); IR (DRIFT): 3200 – 2500 (OH), 2942, 2870, 1733 (C=O), 1678 (C=C), 1115 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.56 (d, *J* = 8.3 Hz, 2H, F_3CPh), 7.40 (d, *J* = 8.1 Hz, 2H, F_3CPh), 7.21 (s, 1H, H-1), 4.78 – 4.73 (m, 1H, H-29a), 4.65 – 4.60 (m, 1H, H-29b), 3.03 (td, *J* = 10.8, 5.0 Hz, 1H,

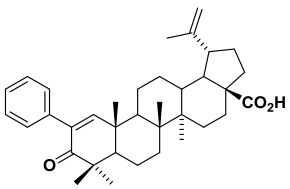
H-18), 2.36 – 2.27 (m, 2H), 2.07 – 1.95 (m, 2H), 1.87 – 1.79 (m, 1H), 1.70 (s, 3H, H-30), 1.20 (s, 3H), 1.19 (s, 3H), 1.12 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.22, 181.69, 157.44, 150.29, 141.01, 135.50, 128.72, 125.14, 110.09, 56.50, 52.95, 49.27, 47.01, 45.50, 44.96, 42.91, 41.79, 39.42, 38.84, 37.20, 33.73, 32.24, 30.65, 29.77, 28.75, 25.71, 21.75, 21.50, 19.56, 19.49, 19.36, 16.56, 14.74; ^{19}F NMR (471 MHz, $CDCl_3$): δ ppm -62.44 (s); HRMS (ESI): *m/z* calcd for $C_{37}H_{48}F_3O_4$ $[M+H]^+$ 597.3550, found 597.3550.

2-(*p*-methoxycarbonylphenyl)betulon-1-enoic acid (34g)



Compound **34g** was prepared according to the general procedure from ester **32g** (101 mg; 0.15 mmol) in EtOH (2 mL), cyclohexa1,3-dien (100 µL; 1.0 mmol) and Pd/C (20 mg; 13 mol %), monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After 24 hours another portion of Pd/C (20 mg; 12 mol %) was added. The reaction mixture was quenched after 30 hours and after the standard work up and purification (hexane/EtOAc 20:1 + AcOH 0,1 %) white crystals of compound **34g** (75 mg; 86 %) were obtained; MP: 250-251 °C (hexane); IR (DRIFT): 3200 – 2500 (OH), 2941, 2869, 1719 (C=O), 1683 (C=C), 1273 (C-O), 1100 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 8.00 – 7.95 (m, 2H, MeO_2C*Ph*), 7.38 – 7.33 (m, 2H, MeO_2C*Ph*), 7.22 (s, 1H, H-1), 4.78 – 4.73 (m, 1H, H-29a), 4.66 – 4.60 (m, 1H, H-29b, 3.91 (s, 3H, *Me*O_2CPh), 3.03 (td, *J* = 10.7, 4.9 Hz, 1H, H-18), 2.35 – 2.27 (m, 2H), 2.06 – 1.96 (m, 2H), 1.88 – 1.78 (m, 1H), 1.70 (s, 3H, H-30), 1.19 (s, 3H), 1.19 (s, 3H), 1.11 (s, 3H), 1.06 (s, 3H), 1.02 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.32, 181.77, 167.14, 157.17, 150.30, 142.05, 135.82, 129.53, 129.21, 128.34, 110.08, 56.51, 52.89, 52.22, 49.28, 47.01, 45.54, 44.94, 42.91, 41.76, 39.42, 38.85, 37.20, 33.72, 32.24, 30.66, 29.77, 28.77, 25.72, 21.77, 21.47, 19.59, 19.49, 19.36, 16.54, 14.74; HRMS (ESI): *m/z* calcd for $C_{38}H_{51}O_5$ $[M+H]^+$ 587.3731, found 587.3728.

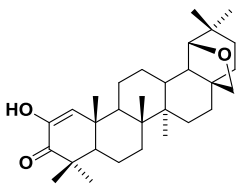
2-phenylbetulon-1-enoic acid (34h)



Compound **34h** was prepared according to the general procedure from ester **32h** (126 mg; 0.20 mmol) in EtOH (2.5 mL), cyclohexa1,3-dien (136 µL; 1.4 mmol) and Pd/C (25 mg; 12 mol %) after 8 hours, monitored by TLC (hexane/EtOAc 5:1 with a drop of AcOH). After the standard work up and purification (hexane/EtOAc 8:1 + AcOH 0,05 %) white crystals of compound **34h** (89 mg; 82 %) were obtained; MP: 111-115 °C (hexane); IR

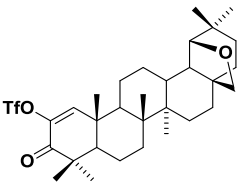
(DRIFT): 3100 – 2500 (OH), 2941, 2868, 1692 (C=O), 1670 (C=C), 752, 731, 695 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.35 – 7.26 (m, 5H, Ph), 7.16 (s, 1H, H-1), 4.79 – 4.74 (m, 1H, H-29a), 4.66 – 4.61 (m, 1H, H-29b), 3.03 (td, *J* = 10.8, 4.9 Hz, 1H, H-18), 2.36 – 2.25 (m, 2H), 2.07 – 1.94 (m, 2H), 1.85 – 1.78 (m, 1H), 1.70 (s, 3H, H-30), 1.19 (s, 3H), 1.18 (s, 3H), 1.10 (s, 3H), 1.06 (s, 3H), 1.01 (s, 3H, 5 × CH_3); ^{13}C NMR (126 MHz, $CDCl_3$): δ ppm 204.69, 181.90, 155.98, 150.32, 137.40, 136.48, 128.31, 128.24, 127.65, 110.05, 56.52, 52.95, 49.29, 47.02, 45.48, 45.02, 42.88, 41.72, 39.21, 38.88, 37.20, 33.77, 32.26, 30.66, 29.78, 28.79, 25.74, 21.76, 21.47, 19.61, 19.49, 19.41, 16.54, 14.74; HRMS (ESI): *m/z* calcd for $C_{36}H_{49}O_3$ $[M-H]^-$ 529.3676, found 529.3679.

2-Hydroxyallobetulon-1-en (36)



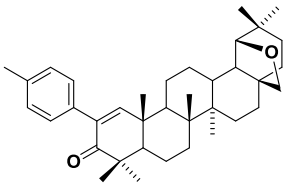
Compound **36** was prepared according to the general procedure from allobetulone (**1a**) (5.0 g; 11.3 mmol) and potassium *tert*-butoxide (5.0 g; 44.6 mmol) in *tert*-butanole (250 mL) after 5 hours, monitored by TLC (hexane/EtOAc 6:1). After the standard work up and purification (hexane/EtOAc 6:1 + $CHCl_3$ 1 %) white crystals of **36** (4.8 g; 93 %) were obtained; R_f = 0.50 (silica gel, hexane/EtOAc 6:1). ^{1}H NMR spectrum was consistent with the literature.[142]

2-{[(trifluoromethyl)sulfonyl]oxy}allobetulon-1-en (37)



The reaction was performed in a Schlenk tube under nitrogen atmosphere. TEA (184 μL; 1.3 mmol) and Tf_2NPh (236 mg; 0.7 mmol) were added to the solution of diosphenol **28** (200 mg; 0.4 mmol) and DMAP (5.4 mg; 10 mmol %) in dry DCM (2 mL). A light yellow reaction mixture got dark red while stirring at r.t., monitored by TLC (hexane/EtOAc 5:1). After 2 hours the reaction was quenched by evaporation of DCM. The resulting mixture was dissolved in EtOAc, washed with diluted aqueous HCl (1:10), saturated aq. solultion of $KHCO_3$ and water to neutral pH. The organic phase was dried under magnesium sulphate, filtered, and the solvents were removed under reduced pressure to give light red crystallic foam. The crude product was used to the next reaction without further purification. A small amount of the triflate **37** was purified on column chromatography (hexane/EtOAc 6:1) to give colorless oil; IR (DRIFT): 2926, 2855, 1704 (C=O), 1692 (C=C), 1204 (C-O), 1140 (C-O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ ppm 7.08 (s, 1H, H-1), 3.79 (d, *J* = 7.9 Hz, 1H, H-28a), 3.59 (s, 1H, H-19), 3.49 (d, *J* = 7.9 Hz, 1H, H-28b), 1.79 – 1.73 (m, 1H), 1.73 – 1.65 (m, 2H), 1.23 (s, 3H), 1.21 (s, 3H), 1.15 (s, 3H), 1.05 (s, 3H), 0.94 (s, 6H), 0.82 (s, 3H, 7 × CH_3).

2-(*p*-methylphenyl)allobetulon-1-en (38)



Compound **38** was prepared according to the general procedure from triflate **37** (250 mg; 0.4 mmol) in dioxane (2 mL), 4-hydroxyphenylboronic acid (138 mg; 1.0 mmol) in IPO (2 mL), sodium carbonate (217 mg; 2.0 mmol) in water (1 mL) and $PdCl_2(PPh_3)_2$ (8 mg), controlled by TLC (hexane/EtOAc 5:1). After the standard work up and purification (hexane/EtOAc 7:1) white crystals of compound **38** (121 mg; 54 %) were obtained; MP: 105-108 °C (hexane); IR (DRIFT): 2928, 2860, 1718 (C=O), 1667 (C=C), 1098 (C-O) cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ ppm 7.21 – 7.18 (m, 2H, Me*Ph*), 7.16 – 7.12 (m, 3H, Me*Ph*, H-1), 3.79 (dd, *J* = 7.8, 1.3 Hz, 1H, H-28a), 3.56 (s, 1H, H-19), 3.47 (d, *J* = 7.8 Hz, 1H, H-28b), 2.34 (s, 3H, *Me*Ph), 1.80 – 1.72 (m, 2H), 1.69 (dd, *J* = 12.6, 2.5 Hz, 1H), 1.20 (s, 3H), 1.18 (s, 3H), 1.11 (s, 3H), 1.08 (s, 3H), 0.95 (s, 3H), 0.94 (s, 3H), 0.81 (s, 3H, 7 × CH_3); ^{13}C NMR (126 MHz, CDCl): δ ppm 204.87, 155.52, 137.43, 136.34, 134.56, 128.98, 128.20, 88.05, 71.41, 53.14, 46.88, 45.52, 45.46, 41.64, 41.58, 41.14, 39.24, 36.86, 36.42, 34.62, 33.35, 32.84, 28.95, 28.81, 26.54, 26.39, 24.70, 21.85, 21.48, 21.29, 19.79, 19.60, 16.29, 13.48. HRMS (ESI): *m/z* calcd for $C_{37}H_{53}O_2$ $[M+H]^+$ 529.4040, found 529.4040.

7 List of abbreviations

Ac	acetyl
AcOH	acetic acid
ADME	absorption, distribution, metabolism, and excretion
Akt	serine-threonine protein kinase
Ar	aryl
Bn	benzyl
BrU	5-bromouridine
BrDU	5-bromo-2´deoxyuridine
CDDO	bardoxolone
DAST	diethylaminosulfur trifluoride
DB	double bond
DCM	dichlormethane
DMAP	4-(dimethylamino)pyridine
DME	1,2-dimethoxyetan
DMF	dimethylfomamide
DMSO	dimethylsulfoxide
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
EWG	electron withdrawing group
Het	heterocycle
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry

IC_{50}	half maximal inhibitory concentration
IR	infrared spectroscopy
IPO	2-propanol
KHMDS	potassium bis(trimethylsilyl)amide
LEM	Laboratory of Experimental Medicine
Me	methyl
MeOH	methanol
MIC	minimum inhibitory concentration
MP	melting point
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxylmethoxyphennyl)-2-(4-sulfophenyl)-2H-tetrazolium
NMP	*N*-methylpyrrolidone
NMR	nuclear magnetic resonance
Nrf-2	nuclear factor (erythroid-derived 2)-like 2
PCC	pyridinium chlorochromate
Pd/C	palladium on activated charcoal
Ph	phenyl
PI3K	phosphatidylinositol-3-kinase
refl.	reflux
r.t.	room temperature
RANKL	receptor activator of nuclear factor-κB ligand
SAR	structure-activity relationship
SB	single bond
t-Bu	*tert*-butyl
t-BuOH	*tert*-butanole
t-BuOK	potassium *tert*-butoxide

TBAC	tetrabutylammonium chloride
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilil
TEA	triethylamine
Tf	triflate
Tf_2NPh	*N*-phenyl-bis(trifluoromethanesulfonimide)
THF	tetrahydrofuran
Tox	toxicity

8 List of cell lines

A549	human lung cancer
BJ	human non-cancer fibroblast
CCRF-CEM	human T-lymphoblastic leukemia
CEM-DNR	human T-lymphoblastic leukemia daunorubicin resistant
HCT116	human colon carcinoma
HCT116p53-	human colon carcinoma p53-deficient form
HeLa	human cervical cancer
HepG2	human hepatocellular carcinoma
HL-60	human promyelocytic leukemia
K562	human leukemia
K562-TAX	human leukemia taxole resistant
MDA-MB-231	human breast cancer
MRC-5	human non-cancer fibroblast
QSG-7701	human non-cancer hepatocyte
RAW264.7	murine macrophage
SMMC-7721	human hepatocellular carcinoma
U2OS	human osteosarcoma

9 List of author's publications

Publications related to the thesis

1. Borkova, L.; Hodon, J.; Urban, M. Modifications at the A-ring of Betulinic Acid and Betulonic Acid. *Asian J. Org. Chem.* **2018**, *7*, 1542-1560.
2. Pokorny, J.; Borkova, L.; Urban, M. Click Reactions in Chemistry of Triterpenes – Advances Towards Development of Potential Therapeutics. *Current Med. Chem*. **2018**, *25*, 5, 636-658.
3. Borkova, L.; Adamek, R.; Kalina, P.; Drasar, P.; Dzubak, P.; Gurska, S.; Rehulka, J.; Hajduch, M.; Urban, M.; Sarek, J. Synthesis and Cytotoxic Activity of Triterpenoid Thiazoles Derived from Allobetulin, Methyl Betulonate, Methyl Oleanonate, and Oleanonic Acid. *ChemMedChem* **2017**, *2017*, 12, 390-398.
4. Borkova, L.; Gurska, S.; Dzubak, P.; Burianova, R.; Hajduch, M.; Sarek, J.; Popa, I.; Urban, M. Lupane and 18alpha-oleanane derivatives substituted in the position 2, their cytotoxicity and influence on cancer cells. *Eur. J. Med. Chem.* **2016**, *121*, 120-131.

Other publications

5. Silveira-Dorta, G.; Jana, S.; Borkova, L.; Thomas, J.; Dehaen, W. Straightforward synthesis of enantiomerically pure 1,2,3-triazoles derived from amino esters. *Org.& Biomolecular Chem.* **2018**, *16* (17), 3168–3176.
6. Borkova, L.; Jasikova, L.; Rehulka, J.; Frisonsova, K.; Urban, M.; Frydrych, I.; Popa, I.; Hajduch, M.; Dickinson, N. J.; Vlk, M.; Dzubak, P.; Sarek, J. Synthesis of cytotoxic 2,2-difluoroderivatives of dihydrobetulinic acid and allobetulin and study of their impact on cancer cells. *Eur. J. Med. Chem.* **2015**, *96*, 482-490.

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Publications

Borkova, L.; Hodon, J.; Urban, M. Modifications at the A-ring of Betulinic Acid and Betulonic Acid. *Asian J. Org. Chem.* **2018**, *7*, 1542-1560.

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PALACKÝ UNIVERSITY OLOMOUC

Faculty of Science

Department of Organic Chemistry

Triterpenoids with Anticancer Properties and Their Mechanism of Action

Summary of the Ph.D. Thesis

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This Ph.D. thesis was elaborated within the framework of the Ph.D. study program P1417 Chemistry, field of study Organic Chemistry, guaranteed by the Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc in the years 2014-2018.

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Abstract

Pentacyclic triterpenes are natural compounds produced by the majority of living organisms and may be found especially in fungi, algae, marine invertebrates, and most commonly in plants. Being secondary metabolites, they are not a part of the main metabolic pathways and it seems that their role is to protect their producers from various diseases. This may be confirmed by the fact that many triterpenes are anticancer, antiviral, antibacterial, antifungal, antiparasitic, and anti-inflammatory, etc. Despite their low toxicity and good availability from the natural resources, their clinical use is still limited by their higher values of IC_{50} and worse pharmacological properties than in the currently used therapeutics. Several approaches have been used to increase the activity and to improve the bioavailability of natural triterpenes. Among the most important is to prepare their semi-synthetic derivatives with higher selectivity and better pharmacological properties. As a part of this research, my thesis was focused on the preparation of new semi-synthetic triterpenoids more suitable for the potential clinical use. This work may be divided into three major topics: (I) Synthesis and anticancer properties of triterpenoid thiazoles; (II) Cytotoxic triterpenoids substituted in the position 2; (III) Preparation of new betulinic acid derivatives by Suzuki-Miyaura cross-coupling.

Abstrakt

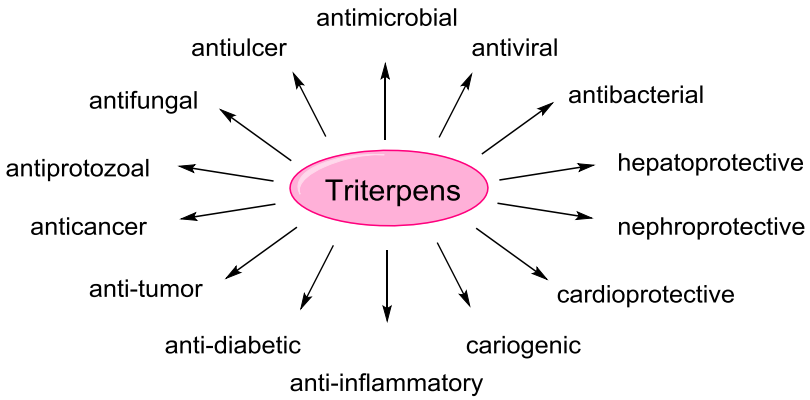
Pentacyklické triterpeny jsou přírodní sloučeniny produkované velkým množstvím živých organismů, například houbami, řasami, mořskými bezobratlými živočichy a nejšastěji rostlinami. Jakožto sekundární metabolity nejsou součástí hlavních metabolických drah a jejich rolí je zřejmě ochrana svých producentů před různými chorobami a škůdci, což může být potvrzeno faktem, že mají velké množství biologických účinků, jsou např. protirakovinné, antivirální, antibakteriální, antifunglální a protizánětlivé. Navzdory jejich nízké toxicitě a jednoduché dostupnosti z přírodních zdrojů je klinické použití triterpenů výrazně limitováno jejich vyššími hodnotami IC_{50} a horšími farmakologickými vlastnostmi, než mají v současnosti používaná léčiva. Existuje řada přístupů, jak zvýšit aktivitu a zlepšit biodostupnost přírodních triterpenů; jedním z nejúčinnějších je příprava jejich semi-syntetických derivátů s vyšší selektivitou a vhodnějšími farmakologickými vlastnostmi. Cílem mojí práce, která je součástí tohoto výzkumu, bylo připravit semi-syntetické triterpenoidy vhodnější pro potenciální klinické použití. Práci je možno rozdělit na tři hlavní témata, všechny se týkají modifikace triterpenoidů na A-kruhu: (I) Syntéza a protirakovinné účinky triterpenoidních thiazolů; (II) Cytotoxické triterpenoidy substituované v poloze 2 a (III) Příprava nových derivátů kyseliny betulinové pomocí Suzuki-Miyaura cross-couplingu.

Contents

11 Introduction

Pentacyclic triterpenes are natural compounds usually occurring in plants,[1–3] fungi,[4–6] algae,[7,8] bacteria[9] and marine invertebrates.[10–12] Hundreds of new triterpenes are being isolated from natural resources every year.[13,14] As secondary metabolites, they do not play a key role in the growth, development, or reproduction of the organisms. Instead, they usually protect and defend their producers against various diseases and pests,[15] although the role of many pentacyclic triterpenes still remains elusive. It is known that pentacyclic triterpenes have a wide range of biological activities (Figure 1).[16] They are not only antimicrobial,[17] antifungal,[18] antiulcer,[19] and antiprotozoal[20,21] (including antimalarial activity),[22] but also antibacterial,[23,24] and antiviral[25–27] (including anti-HIV activity).[28–30] They often reduce inflammation and oxidative demage[31–34] and have hepatoprotective,[35] nephroprotective,[36] cardioprotective,[37] and anti-diabetic effects.[38,39] A large number of triterpenes are cytotoxic against various cancer cell lines[40–42] and their anti-tumor activity was also observed in preclinical animal models.[43] Contemporary research reveals, that triterpenes are biologically active *via* a variety of mechanisms of action.[44–48] This makes their research including structure-activity relationship (SAR) studies complicated.

Figure 17. Biological activities of triterpenes.



Often, the biological activities (such as anti-oxidant) and low toxicity of triterpenes make them important components of natural medicine, cosmetics and food supplements which do not need to be approved by the strict and expensive process for the approval of new therapeutics.[49,50] In natural extracts, triterpenes are often bound to sacharides as glycosides –saponins[17,51,52] which have better bioavailability in comparison to the free triterpenes. Saponins are often the active components of raw extracts, ointments or tinctures made from medicinal plants. As a result, new plant species are being explored to find new molecules with potential biological activities. In the same time, semisynthetic compounds with higher activity, improved pharmacological properties, and more favorable therapeutic index are being prepared.[40]

12 Aims of the work

The topic of my PhD work was targeted to the modification of the A-ring of several triterpenoids in order to increase their biological activities, primarily anticancer properties. My project may be divided into three main subprojects:

- The first part was focused on the preparation of new heterocycle-containing derivatives of triterpenoids and study of their impact on cancer cells. The goal was to synthesize a library of substituted aminothiazoles fused to the positions C-2 and C-3 of seven selected triterpenoids to get more data about the structure-activity relationships (SARs) within this set of compounds.
- The aim of the second subproject was to confirm or rebut the theory: *The higher electronegativity of the substituent at the C-2 of lupane triterpenoids, the higher cytotoxic activity*. For this purpose, dihydrobetulonic acid – a lupane type derivative - was substituted at the position C-2 with various electronegative substituents and cytotoxic activity of prepared molecules was tested.
- The ambition of the third project was to develop and optimize the conditions for the preparation of new lupane type derivatives with aryl substituents at the positions C-2 or C-3 by Suzuki-Miyaura cross-coupling and test the cytotoxicity of these derivatives.

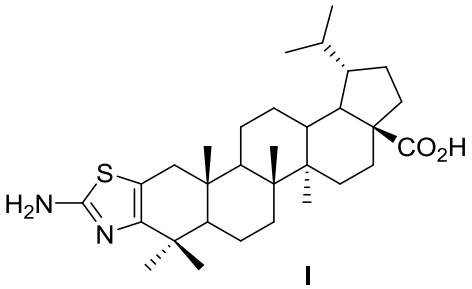
13 Results and Discussion

13.1 Synthesis and anticancer properties of triterpenoid thiazoles

A set of triterpenes modified with a 5-membered ring was synthesized in our research group and among them aminothiazole **I** (Figure 2) was the most active on multiple cancer cell lines,[53] which sparked our interest in such compounds.

To further explore structure-activity relationships among triterpenoid aminothiazoles, in this thesis, I chose to add substituents of various size and shape to the NH_2 group. To improve the ligand-target binding, rather lipophilic substituents were chosen. Substituents were chosen rather lipophilic, because we expected to improve interactions of the terpenes with lipophilic areas on the potential protein targets. In order to get more data about the influence of the triterpenic part of the molecule on the cytotoxic activity, a variety of basic triterpenes was chosen as well (lupane, lup-20(29)-ene, oleanane, 18α-oleanane, and ursane). The classical Hantzsch synthesis of aminothiazoles does not give good yields in the case of triterpenoids and steroids because of harsh reaction conditions,[54,55] also the reaction does not allow to simply obtain compounds substituted at the amino group. Therefore, an alternative approach was used involving the cyclization reaction of 3-oxo-2-thiocyanato triterpenoids with various ammonium acetates.[56] Using this method, I was able to synthesize a large variety of substituted aminothiazoles. Moreover, this was the first time that triterpenoid aminothiazoles were prepared using this synthetic procedure.

Figure 18. Synthesis of aminothiazole of dihydrobetulinic acid **I -** the most active compound with the 5-membered heterocyclic ring prepared in the lit.[53]

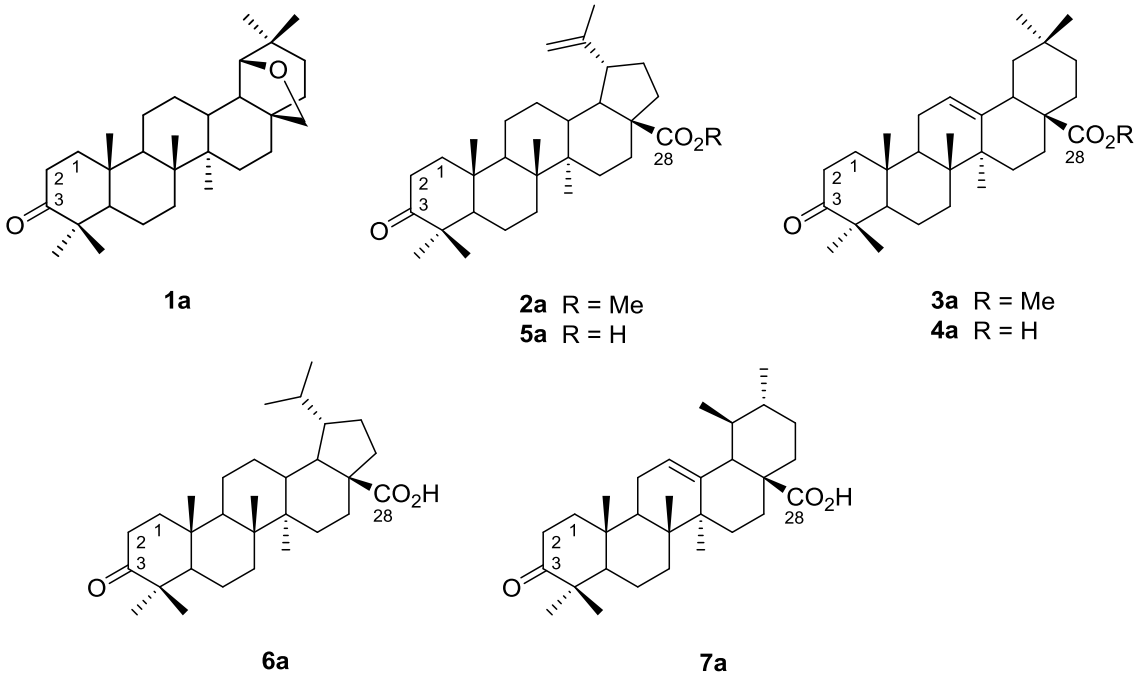


I

Four series of *N*-substituted aminothiazoles derived from allobetulon (**1a**), methyl betulonate (**2a**), methyl oleanonate (**3a**), and oleanonic acid (**4a**) were prepared in the first set. After the evaluation of their cytotoxic activity, SAR assumptions were made and another three series of *N*-substituted aminothiazoles derived from betulonic acid (**5a**), dihydrobetulonic acid (**6a**), and ursonic acid (**7a**) were synthesized as the second set. (Figure 3) Altogether, more than 80 compounds

including the reaction intermediates were prepared and characterized and their cytotoxic activities were tested.

Figure 19. Structures of the starting 3-oxotriterpenoids **1a-7a**.



13.1.1 Synthesis and cytotoxic activity of triterpenoid thiazoles derived from allobetulin, methyl betulonate, methyl oleanonate, and oleanonic acid

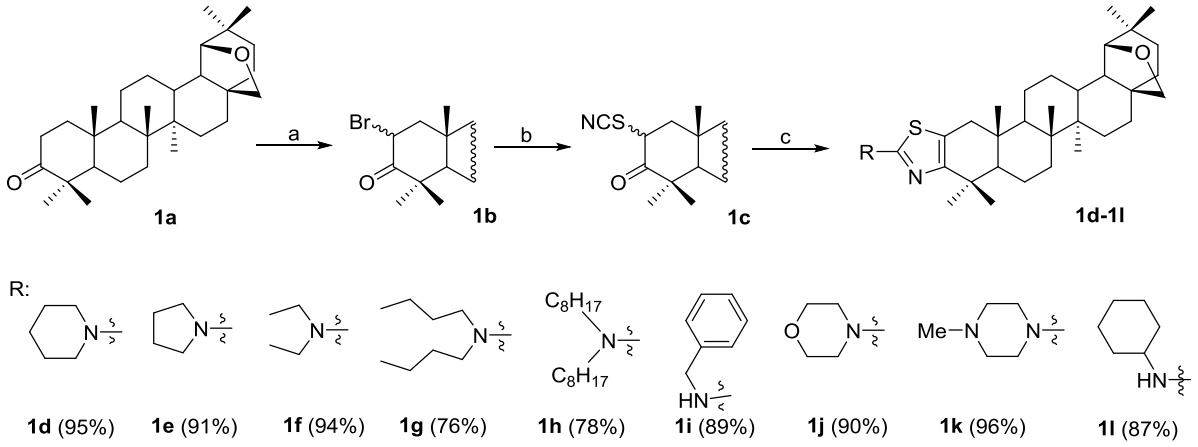
This chapter was published in: Borkova, L.; Adamek, R.; Kalina, P.; Drasar, P.; Dzubak, P.; Gurska, S.; Rehulka, J.; Hajduch, M.; Urban, M.; Sarek, J. *ChemMedChem* **2017**, *2017*, 12, 390-398.[57]

In the lit.[53] the aminothiazole derivative **I** (Figure 2) was obtained in rather low yield (57 %). The compound contains a free amino and carboxylic group which may form ions and lower its solubility in organic solvents and this causes troubles during the purification step. Here we started with allobetulon (**1a**), methyl betulonate (**2a**), and methyl oleanonate (**3a**) because they do not contain the free carboxylic group and simplier purification and higher yields were expected. Although lower yields during the synthesis were expected, we added free oleanonic acid (**4a**) to this set, in order to see if methyl esters (made from **3a**) are less active than analogous free acids (made from **4a**). This is unfortunatelly a common trend in triterpenoids.[11,29,81] Starting compounds **1a**, **2a**, **3a**, and **4a** are derivatives of betulinic acid, oleanolic acid, and 18α-oleanane, these triterpenes are commonly studied for their high cytotoxic activities.[58–61]

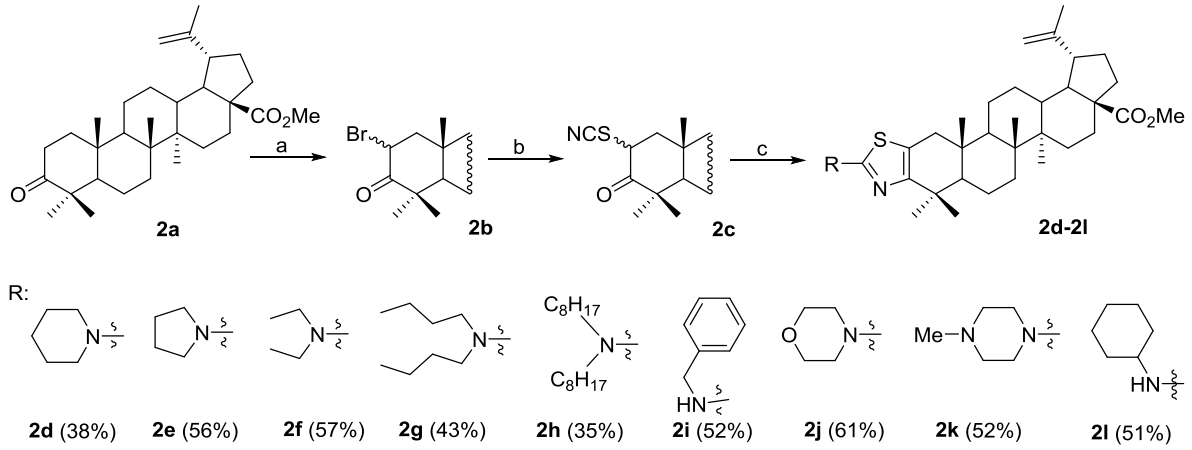
13.1.1.1 Synthesis

The starting 3-oxotriterpenoids **2a**, **3a**, and **4a** were brominated by modified procedure with $CuBr_2$ in a mixture of EtOAc, and MeOH at r.t.[62,63] or by modified procedure with Br_2 in $CHCl_3$ at r.t. (for **1a**).[53,64,65] The crude 2-bromo-3-oxoderivatives **1b**, **2b**, **3b**, and **4b** were purified by column chromatography and used for the synthesis of 3-oxo-2-thiocyanato derivatives **1c**, **2c**, **3c**, and **4c** by the nucleophilic substitution of bromine by ammonium thiocyanate in NMP at 50 °C or by potassium thiocyanate in DMSO at 90 °C (Schemes 1-4). 2-thiocyanato-3-oxoderivatives **1c**, **2c**, **3c**, and **4c** were obtained as mixtures of 2α/2β epimers, which were not separated because the final cyclization and aromatization of each epimer leads to the same flat, aromatic system. The structures of compounds **1c** – **4c** were proven by spectral data.

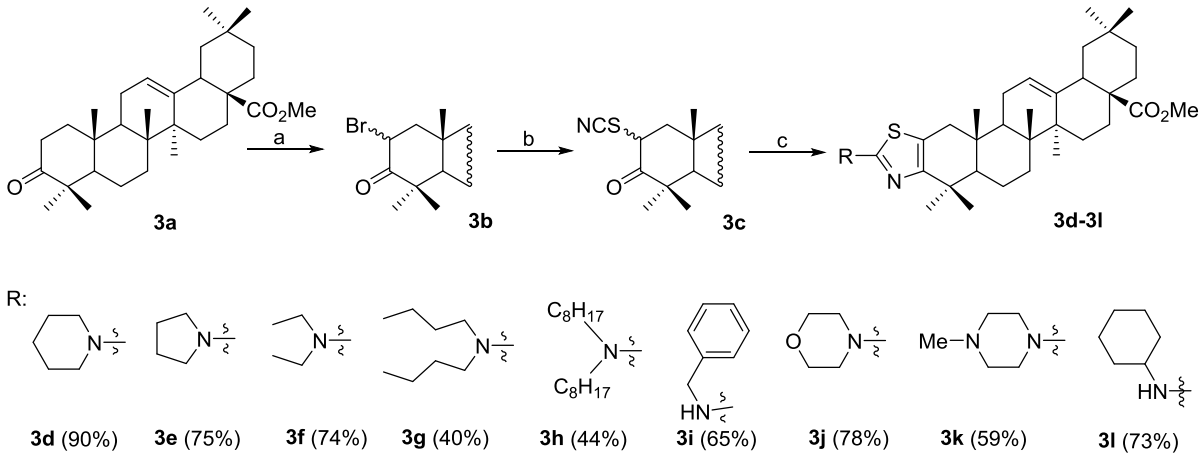
Novel thiazole derivatives **1d** – **1l**, **2d** – **2l**, **3d** – **3l** and **4d** – **4l** were prepared by the procedure similar to the lit.[66] Each thiocyanate was stirred with five equivalents of the corresponding freshly prepared alkyl ammonium acetate at r.t. for 1 to 7 days as needed (Schemes 1-4). The yields of the cyclizations were usually moderate to high (**1d-1l** 76-96 %; **2d-2l** 35-61 %; **3d-3l** 40-90 %; **4d-4l** 9-79 %) depending on the solubility of the starting triterpene and the product in organic solvents used for the reaction, which influenced the work-up procedures and the purification. It is worth mentioning that as expected, the yields of free oleanonic acid derivatives (**4d-4l**) were much lower in comparison to methyl oleanonate derivatives (**3d-3l**); compounds with the free carboxylic function were difficult to separate by column chromatography. The structure of all prepared substituted aminothiazoles was confirmed by spectral data.



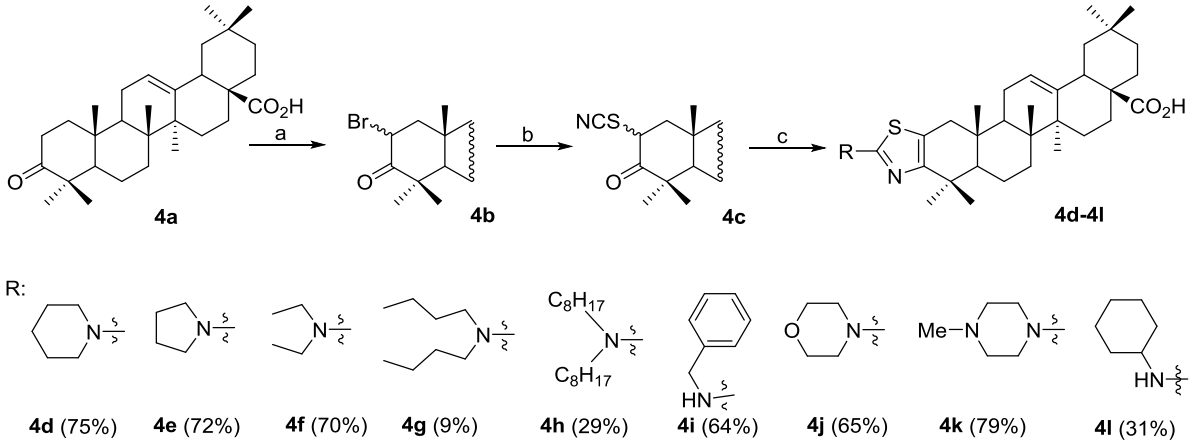
Scheme 27. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t., 30 min; (b) KSCN, DMSO, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 24 hours.



Scheme 28. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) NH_4SCN, NMP, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36 hours.



Scheme 29. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 12 h; (b) NH_4SCN, NMP, 50 °C, 6 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36-48 h.



Scheme 30. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 12 h; (b) NH_4SCN, NMP, 50 °C, 4 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 36-48 h.

13.1.1.2 Biological assays – cytotoxicity

The cytotoxic activity of all synthesized compounds was tested *in vitro* against eight human cancer cell lines of different histogenetic origin and two non-tumor fibroblasts by using the standard MTS test.[67] The testing was performed by the Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc (LEM). The cancer cell lines were derived from T-lymphoblastic leukemia (CCRF-CEM), leukemia (K562) and their multi-drug resistant counterparts (CEM-DNR, K562-TAX), solid tumors including lung (A549) and colon (HCT116, HCT116p53-/-) carcinomas, osteosarcoma cell line (U2OS), and for comparison, on two human non-cancer fibroblast cell lines (BJ, MRC-5).[67] In general, the CCRF-CEM line was the most sensitive cancer cell line to the prepared compounds with only a few exceptions. Therefore, SAR assumptions were mostly based on the activities on CCRF-CEM cells.

Among the starting material and intermediates, 2-bromo-3-oxoderivatives **1b**, **2b**, and **4b** were cytotoxic against the CCRF-CEM line in a low micromolar range of 3-5 μM. This is surprising because compound **1b** is a derivative of allobetulon (**1a**), analogues of which are often inactive.[68,69] In addition, the active compound **2b** is a methyl ester and triterpenic methyl esters are also usually not active.[68,69] Even bromo derivative **3b**, also a methyl ester, had a moderate IC_{50} of 14 μM. 2-thiocyanato derivatives **2c** and **4c** had IC_{50} values of 6-10 μM. In contrast, compounds **1c** and **3c** had IC_{50} values in higher micromolar ranges. The cytotoxicity of most of the substituted aminothiazoles was below the detection limit with two exceptions, **4f** and **4k** with cytotoxicity of 9.7 μM and

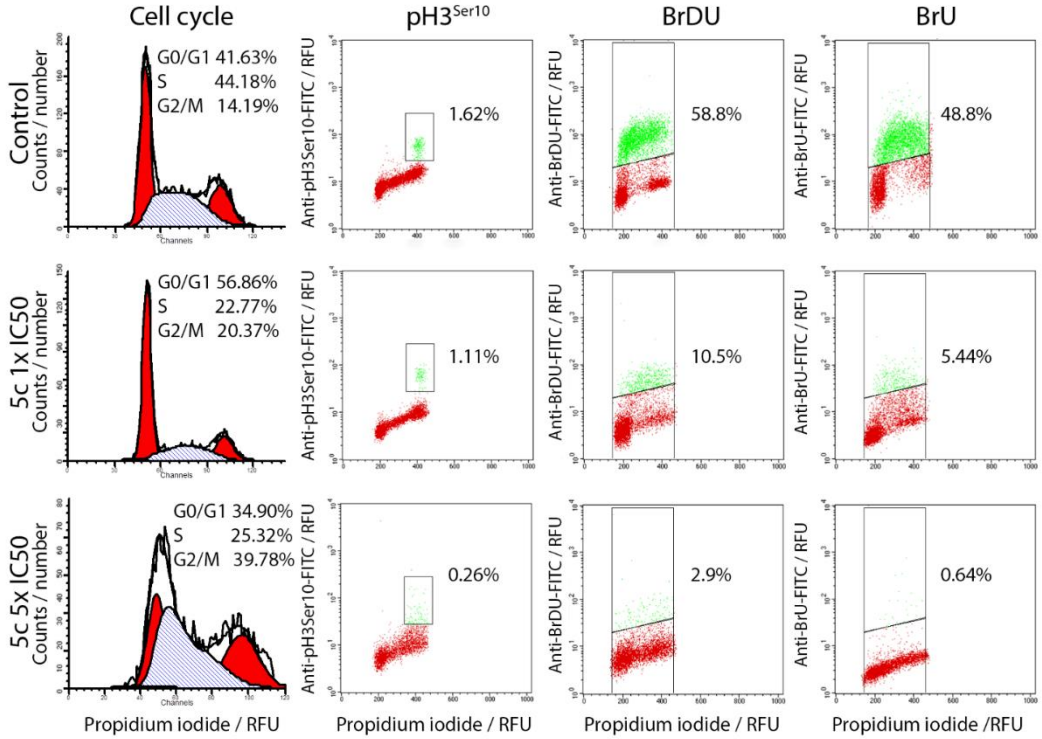
11.3 μM (CCRF-CEM), respectively, which were considered enough active to be interesting for further studies towards the mechanism of action. The activity of derivatives **1l**, **3f**, **4i**, **4j**, and **4l** was better than the detection limit; however, not sufficient for more tests. The activity of almost all derivatives with IC_{50} < 50 μM on the resistant cell lines CEM-DNR and K562-TAX is worse in comparison to parental cell lines CCRF-CEM and K562 which indicates the possible mechanism of resistance by MDR transporter proteins. To sum up, within this part of the study, 9 compounds (**1b**, **2b**, **2c**, **3b**, **3c**, **4b**, **4c**, **4f**, and **4k**) had higher cytotoxic activity than their parent compounds **1a**, **2a**, **3a**, and **4a**.

13.1.1.3 Biological assays – analysis of apoptosis, cell cycle, and DNA/RNA synthesis

All promising compounds with an IC_{50} below or around 10 μM in CCRF-CEM cell line (**1b**, **2b**, **2c**, **4b**, **4c**, and **4f**) were further investigated for their mechanisms of action at LEM. The analysis of apoptosis, cell cycle, and DNA and RNA synthesis in CCRF-CEM cells at 1 × or 5 × IC_{50} were measured.[70,71] Highly active compound **4c** led to the accumulation of cells in G2 phase. Nucleic acid synthesis was almost completely inhibited by **4c** at both tested concentrations (Figure 4), pointing to a possible mechanism of action somewhere within the regulatory mechanisms of cell cycle proliferation. Since **4c** had no toxicity on fibroblasts, is the most promising candidate for further cell biology studies and drug development. Similar phenomenon (inhibition of DNA/RNA synthesis) but at higher concentrations (5 × IC_{50}) was detected for compounds **2b** and **2c**; however, the mechanical proposal is more difficult in this case. Other tested derivatives had no significant effect on the cell cycle and DNA/RNA synthesis.

In this sub-chapter, 44 triterpenoid derivatives were synthesized, and their cytotoxicity was evaluated followed by more advanced biological experiments in the most promising compounds. 42 of the compounds were not published before. From the 3-oxocompounds **1a**, **2a**, **3a**, and **4a**, 2-bromo-3-oxoderivatives, 2-thiocyanato-3-oxoderivatives, and substituted aminothiazoles were prepared. Nine different types of substituents at the amino group were used in order to evaluate their influence on the cytotoxicity. The most active compounds were the intermediate 2-bromo-3-oxoderivatives **1b**, **2b**, **4b**, and thiocyanates **2c**, and **4c**. Although the majority of the final aminothiazoles were inactive, there were two exceptions, **4f** (diethylamin substituent) and **4k** (*N*-methylpiperazine substituent), with cytotoxicity around 10 μM (CCRF-CEM). Both compounds are derivatives of oleanonic acid with the free 28-carboxyl groups which led us to the future direction of this research on aminothiazoles. New compounds should be more oriented towards the free triterpenic acids despite the lower yields from the synthetic procedures.

Figure 20. Graphs and dot plots of flow cytometry analysis are showing the cell cycle inhibition in G2/M phase and almost complete DNA, RNA synthesis inhibition by the best compound **4c**, monitored by incorporation of BrDU/BrU into the DNA.



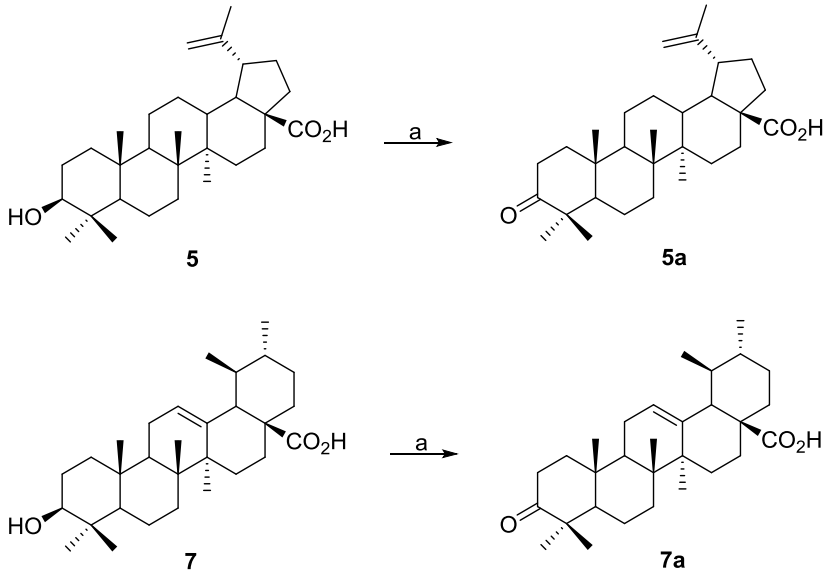
13.1.2 Synthesis and cytotoxic activity of triterpenoid thiazoles derived from free betulinic acid, dihydrobetulinic acid, and ursonic acid

The results presented in the previous chapter showed that compounds with the free carboxyl group (C-28) had higher cytotoxic activity compared to the corresponding methyl ester derivatives. Therefore, a second set of triterpenoid thiazoles derived from three free triterpenoid acids: betulinic acid (**5**), dihydrobetulonic acid (**6a**), and ursolic acid (**7**) was synthesized. The starting compounds and their derivatives are more and more often studied for their high cytotoxic activities.[72–79] The experimental set up from the first project was preserved in order to be able to combine the results from the both sets and select several most promising compounds for the advanced biological tests. The same reaction procedures as well as the same substituents on the amino group of the aminothiazole part were used. To finalize both sets, I additionally prepared three unsubstituted aminothiazoles, analogous to the compound **I** made from the other basic triterpenes.

13.1.2.1 Synthesis

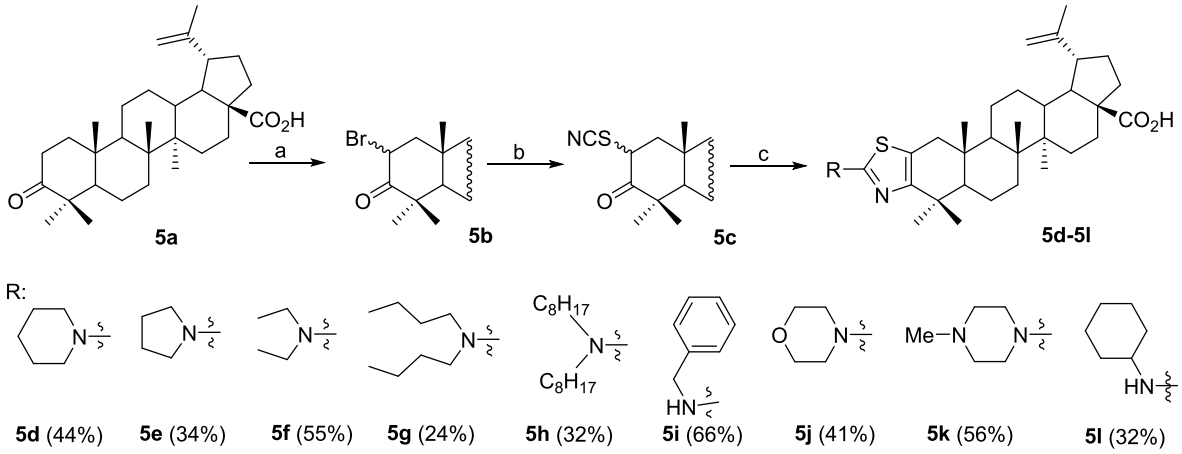
First, betulinic acid (**5**) and ursolic acid (**7**) were converted into 3-oxoderivatives **5a** and **7a** using sodium dichromate, sodium acetate and a mixture of solvents 1,3-dioxane, acetic acid and acetic

anhydride at r.t. (Scheme 5).[53] Then, betulonic acid (**5a**) and ursonic acid (**7a**) were brominated by copper(II) bromide in a mixture of ethyl acetate and methanol at r.t., similar introduction of bromine into the position C-2 was used in the lit.[80,81], while dihydrobetulonic acid (**6a**) was brominated by bromine solution in chloroform at r.t. under modified conditions.[53] Bromoketones **5b**, **6b**, and **7b** were purified by column chromatography and used as mixtures of α- and β-epimers to the next reaction step. A nucleophilic substitution of bromoketones **5b**, **6b**, and **7b** by potassium thiocyanate in DMSO at 90 °C led to the thiocyanato derivatives **5c**, **6c**, and **7c** (Schemes 6-8) which were after purification used to the cyclization reaction which was similar to the reaction used in the lit.[66] The structures of all intermediates were confirmed by spectral data.

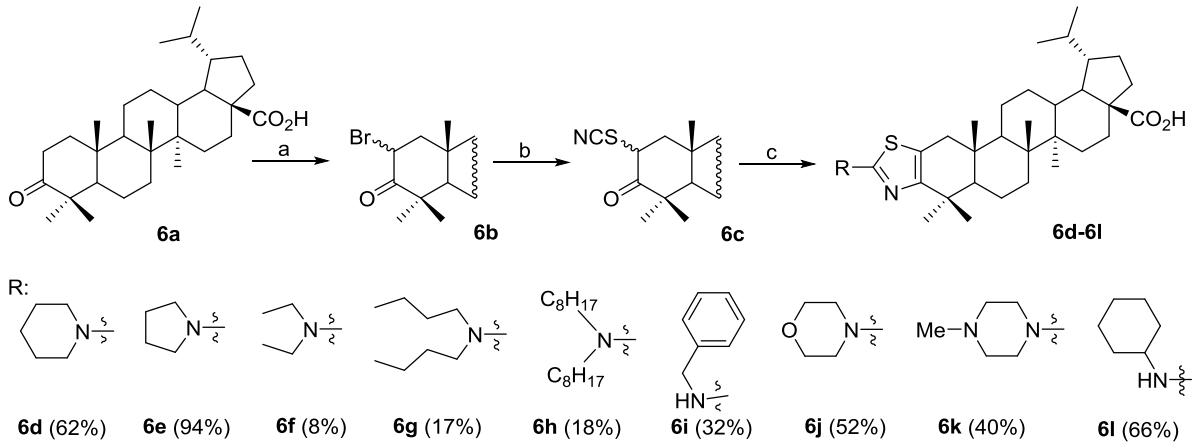


Scheme 31. Preparation of the starting compounds from commercially available triterpenic acids. Reagents and conditions: (a) $Na_2Cr_2O_7.2H_2O$, AcONa, 1,3-dioxane, AcOH, Ac_2O, r.t., 18 h.

Compounds **5c**, **6c**, and **7c** reacted with freshly prepared alkyl ammonium acetates in chloroform at r.t. for 1-7 days to give substituted aminothiazoles **5d-5l** (24-66 % yield), **6d-6l** (8-94 % yield), and **7d-7l** (15-64 % yield) which were purified by column chromatography (Schemes 6-8). The lower yields were most often caused by the difficult purification during which some parts of the products kept remaining on silica gel and were just slowly washed out. These troubles were probably caused by the combination of the free carboxylic group and the amino group, as was predicted. In addition, some difficulties with respect to the substituents were also observed. Longer aliphatic chains, such as dibutyl or dioctyl, and cyclohexylamine moiety were partly responsible for problems during purification. Since the compounds for biological tests must be of the highest purity, sometimes multiple column chromatography was needed which often was done on account to low yields. Neither HPLC purification nor crystallization led to sufficiently pure compounds in a single step and had to be repeated.

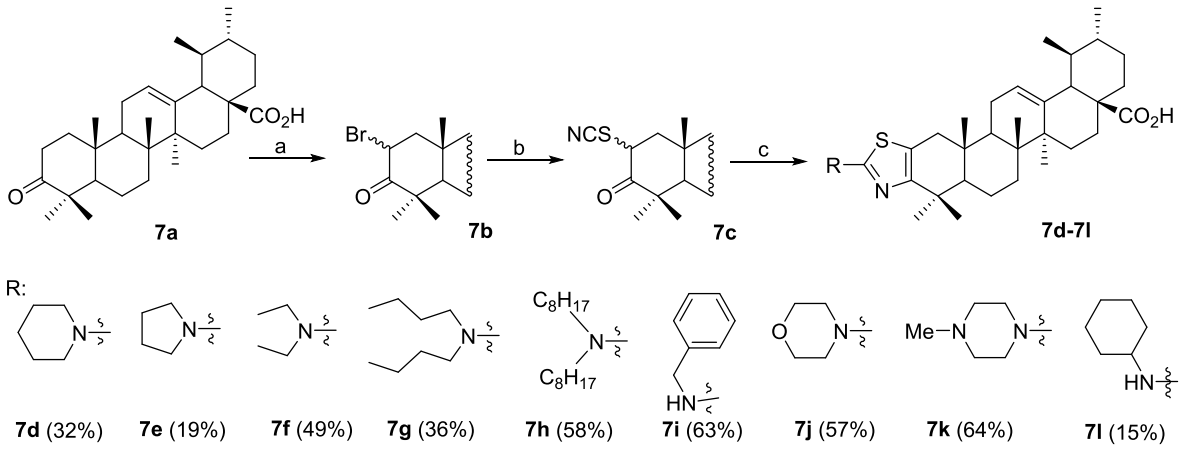


Scheme 32. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 3-7 days.

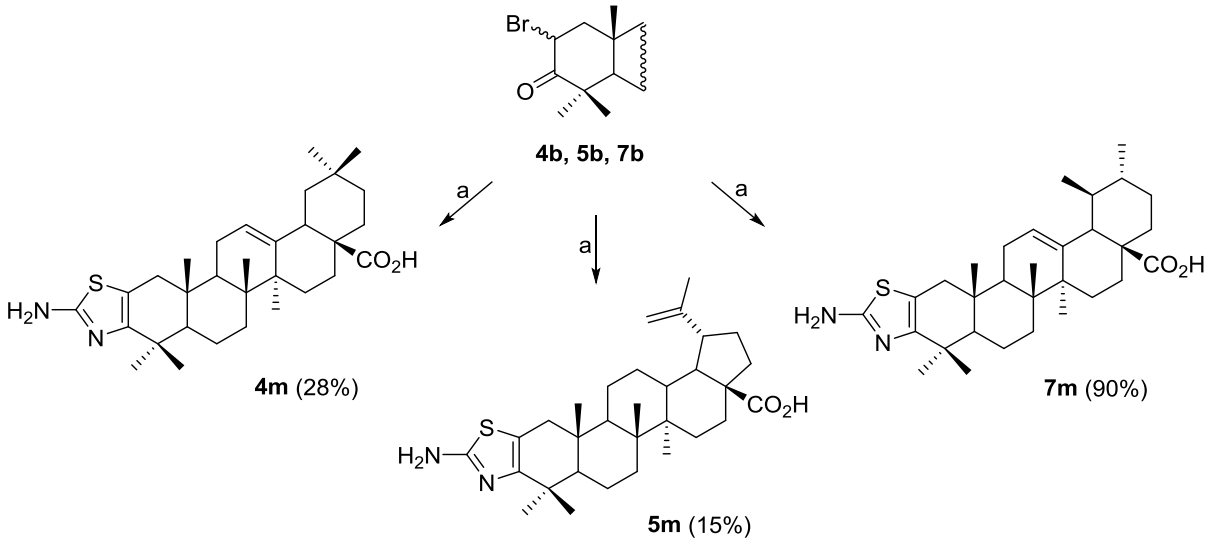


Scheme 33. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t. 30 min; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 1-7 days.

A set of three unsubstituted aminothiazoles, analogous to the compound **I**, were synthesized to compare the influence of the triterpenic part of the molecule on the cytotoxicity. Bromoketones **4b**, **5b**, and **7b** were used for cyclization with thiourea in ethanol at r.t. The aminothiazoles **4m**, **5m**, and **7m** were formed after two to seven days according to the reactivity of the substrate (Scheme 9). The structures of both substituted and unsubstituted aminothiazoles were confirmed by spectral data.



Scheme 34. Reagents and conditions: (a) $CuBr_2$, EtOAc, MeOH, r.t., 3 h; (b) KSCN, DMSO, 90 °C, 24 h; (c) piperidinium acetate (for **d**), pyrrolidinium acetate (for **e**), diethyl ammonium acetate (for **f**), dibutyl ammonium acetate (for **g**), dioctyl ammonium acetate (for **h**), benzyl ammonium acetate (for **i**), morpholinium acetate (for **j**), *N*-methylpiperazinium acetate (for **k**), or cyclohexyl ammonium acetate (for **l**), $CHCl_3$, r.t., 4-7 days.



Scheme 35. Aminothiazoles with the free NH_2 group derived from oleanonic acid (**4a**), betulonic acid (**5a**), and ursonic acid (**7a**). Reagents and conditions: (a) thiourea, EtOH, r.t., 2-7 days.

13.1.2.2 Biological assays – cytotoxicity

All compounds prepared in this study were tested *in vitro* by standard MTS assay for their cytotoxic activity against eight human cancer cell lines and two non-cancer fibroblasts (Table 1) at LEM. Similar trends were observed as for the first set.[57] On the other hand, a vast number of final products with the free carboxylic group were moderately cytotoxic. Moreover, free aminothiazoles **4m** and **5m** (derivatives of oleanonic acid and betulonic acid) were active in the same range as

dihydrobetulonic acid derivative **I** (4.6 μM and 7.9 μM resp., compare to 3.5 μM on CCRF-CEM); however, they were less toxic on healthy cells and therefore their therapeutic index showed higher selectivity compared to **I** (>11 and 6.3 resp., compare to 5.8). Aminothiazole **7m** (derivative of ursolic acid) had only moderate cytotoxicity (19.0 μM on CCRF-CEM) and low selectivity (>2.6).

Table 13. Cytotoxic activities of the free prepared aminothiazoles **4m**, **5m**, and **7m** and known aminothiazole **I** on eight tumor (including resistant) and two normal fibroblast cell lines.

Comp.	IC_{50} (μM)[a]										
	CCRF-CEM	CEM-DNR	HCT116	HCT116 p53$^{-/-}$	K562	K562-TAX	A549	U2OS	BJ	MRC-5	TI[b]
I	**3.5**	11.2	**5.1**	**4.3**	**4.8**	**6.9**	**7.0**	/	24.9	15.7	5.8
4m	**4.6**	14.9	26.2	>50	>50	20.5	>50	>50	>50	>50	>11
5m	**7.9**	13.3	36.9	49.7	/	15.4	28.8	50	50	50	6.3
7m	19.0	29.5	43.0	>50	>50	33.1	>50	49.6	>50	>50	>2.6

[a]The lowest concentration that kills 50 % of cells. The standard deviation in cytotoxicity assays is typically up to 15 % of the average value. Compounds with IC_{50} > 50 μM are considered inactive. [b]Therapeutic index is calculated for IC_{50} of CCRF-CEM line vs average of both fibroblasts (BJ and MRC-5).

13.1.2.3 Biological assays – pharmacological parameters

The most active compounds from both series (IC_{50} < 5 μM) were also subjected to the tests of pharmacological parameters at LEM. The intermediates **5c**, **6c**, **7b**, and **7c** as well as the prepared unsubstituted aminothiazoles (**4m** and **5m**) together with compound **I** were tested for their stability, plasma protein binding, and membrane permeability *in vitro* (Table 2).

Isothiocyanates **5c**, **6c**, and **7c** would be interesting because of their low IC_{50} values, compound **6c** is the most cytotoxic from all of them with the IC_{50} on CCRF-CEM cells in submicromolar range; however, they were found unstable in stock solution, in plasma, and in microsome; therefore, they are not suitable for further drug development and had to be abandoned. In addition, some of them were toxic on non-cancer cells.

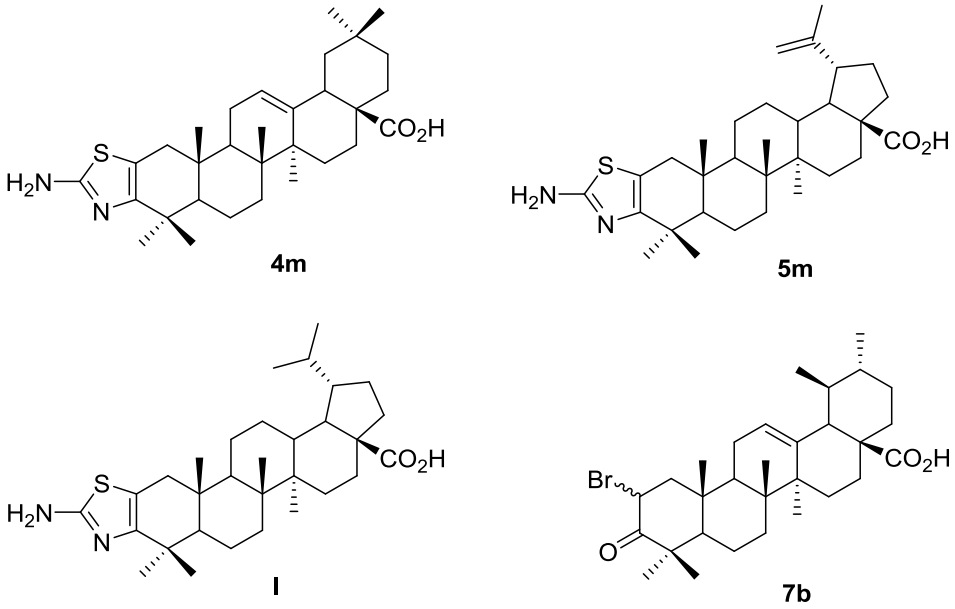
Compound **4m**, aminothiazole of oleanolic acid is stable but it seems that it does not pass through the cellular membranes well (in PAMPA models). Despite that, the compound deserves more evaluation, because there are many other mechanisms how cells transport the molecules into them. Compounds **I**, **5m** and **7b** are the best compounds of the series, they are active enough, they are stable enough, and they penetrate through the cellular membranes well. All of them bind to plasma proteins which is similar to steroids and, at this point, it is not a hurdle.

Table 14. Pharmacological parameters of the unsubstituted aminothiazoles **I**, **4m**, and **5m** and intermediates **5c**, **6c**, **7b**, and **7c**. Data were provided by Dr. Lišková from LEM.

Compound	Metabolism							Plasma protein binding	Permeability	
	Chemical stability		Plasma stability		Microsomal stability			Rapid equilibrium dialysis	PAMPA	
	Time (min)	Comp. remaining (%)	Time (min)	Comp. remaining (%)	Time (min)	Comp. remaining (%)	Intrinsic clearence	Fraction bound (%)	log Pe	Category
I	0	100	0	100	0	100	Low	98,5	-5,340	Medium
	15	99	15	99	15	98				
	30	96	30	100	30	93				
	60	80	60	99	60	91				
	120	76	120	100						
4m	0	100	0	100	0	100	Low	98,9	-7,162	Low
	15	91	15	99	15	97				
	30	77	30	99	30	90				
	60	75	60	99	60	87				
	120	75	120	99						
5c	0	100	0	100	0	100	Medium	11,8	-5,245	Medium
	15	100	15	99	15	90				
	30	67	30	76	30	86				
	60	54	60	36	60	57				
	120	50	120	8						
5m	0	100	0	100	0	100	Medium	95,8	-5,338	Medium
	15	100	15	99	15	31				
	30	91	30	99	30	26				
	60	91	60	98	60	26				
	120	87	120	98						
6c	0	100	0	100	0	100	High	7,0	-6,295	Low
	15	100	15	99	15	63				
	30	99	30	95	30	35				
	60	91	60	57	60	2				
	120	70	120	14						
7b	0	100	0	100	0	100	Medium	82,4	-4,934	Medium
	15	100	15	98	15	93				
	30	99	30	98	30	81				
	60	99	60	95	60	66				
	120	89	120	86						
7c	0	100	0	100	0	100	High	28,5	-6,519	Low
	15	90	15	99	15	61				
	30	49	30	100	30	55				
	60	37	60	77	60	15				
	120	15	120	37						

Compounds **I**, **4m**, **5m** and **7b** (Figure 5) are cytotoxic, selective and have appropriate pharmacological parameters (*in vitro*); therefore, they were selected for the future in vivo ADME-Tox evaluatiuon on mice. If they pass this part of tests, they will be subjeceted to *in vivo* anti-tumor testing.

Figure 21. The most promising derivatives from this study are aminothiazoles **4m**, **5m**, and **I** and 2-bromo-3-oxoderivative **7b**.



To sum up, 82 compounds were prepared in the thiazole subproject. In general, the most active compounds were the intermediate bromoketones and thiocyanato ketones, unsubstituted aminothiazoles with the free amino group and the free C-28 carboxylic group, and some *N*-substituted aminothiazoles with the free C-28 carboxylic group. The highest therapeutic index was calculated for aminothiazole **4m** (TI >11). The most active intermediates (**5c**, **6c**, **7b**, and **7c**) as well as the prepared unsubstituted aminothiazoles (**4m** and **5m**) together with compound **I** were chosen for the further biological screening. Their stability and membrane permeability are being tested *in vitro*. A manuscript summarizing this project is in preparation.

The main results of this part of the research are:

A) Important trends useful for SAR assumptions were found:

(1) The free C-28 carboxylic group of triterpenoids is esential for the activity.

(2) The intermediates with -SCN and -Br substituent at the C-2 were the most active but some of them were toxic on non-cancer cells, especially those with –SCN at C-2.

(3) The aminothiazole derivatives with polar substituents (such as morpholine) and especially the derivatives with the free NH_2 group were more active than the aminothiazoles with non-polar substituents which disproved our original theory that the lipophilic substituents would increase the activity.

(4) The highest number of active compounds was among derivatives of betulinic acid (**5**) and ursolic acid (**7**), in this order, compare to the derivatives of other triterpenoids.

B) Most importantly, four compounds of this series (**4m**, **5m**, **I**, and **7b**; Figure 15) had high and selective cytotoxicity against cancer cell lines and favorable pharmacological properties. Therefore, they were selected for the future *in vivo* evaluations of the ADME-Tox and then for the evaluations of the anticancer properties. These compounds have the highest potential to become new anticancer drugs from all compounds synthesized here.

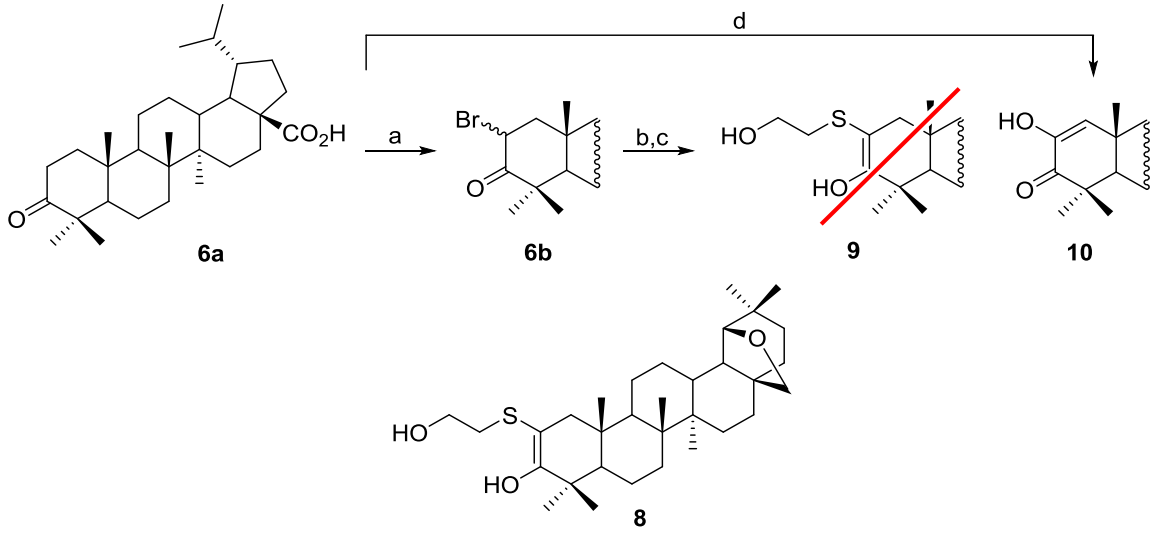
13.2 Lupane derivatives substituted in the position 2, their cytotoxicity and influence on cancer cells

Earlier in the research, we have seen a significant trend that introducing an electronegative substituent to the position 2 of lupane skeleton increases cytotoxicity significantly and this works especially well for betulinic acid derivatives. Among the derivatives with an electronegative substituent at the position C-2, 2,2-difluoro derivatives were the most cytotoxic as expected. However, their cytotoxicity was not limited to cancer cells only and therefore their further development as drugs was compromised.[82] Therefore, one of the subprojects of this work was to further explore triterpenes with other substituent types at the position C-2. As the triterpene, dihydrobetulonic acid (**6a**) was chosen because this molecule is significantly cytotoxic, and the influence of the substituent would be easily notable. New compounds were prepared *via* nucleophilic substitution of 2-bromo derivatives or by selective nitration of the 2-position in 3-oxocompounds. New derivatives were designed to contain heteroatoms such as nitrogen, sulfur, and oxygen.

13.2.1 Synthesis of sulfur containing derivatives

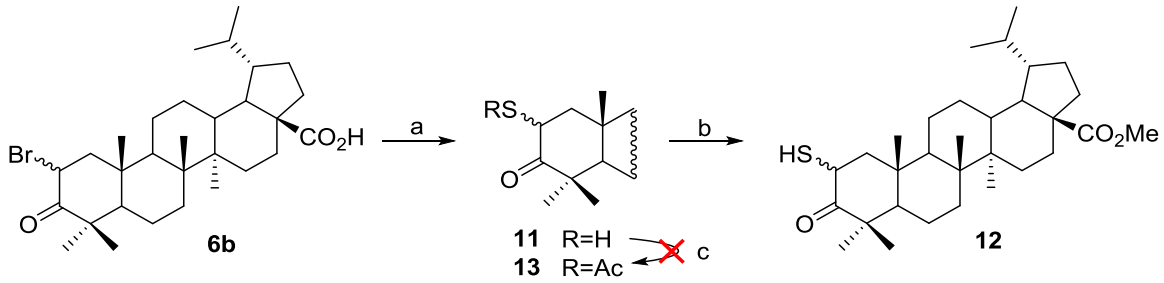
Earlier, compound **8** was prepared in our research group.[83] It is, however, allobetulon **1a** derivative and the cytotoxic activity was below the detection limit which is common for allobetulon **1a** derivatives. Thus, one of the task was to prepare its analogue from dihydrobetulonic acid (**6a**).[53] Derivatives of **6a** are usually more cytotoxic than derivatives of **1a**. Dihydrobetulonic acid (**6a**) was brominated with one equivalent of Br_2 in chloroform to yield epimeric mixture of 2α/β-bromo-dihydrobetulonic acid (**6b**). Nucleophilic substitution of the bromoketone **6b** with mercaptoethanol under various reaction conditions was investigated including the conditions from.[83] The most promising procedure with 1 eq. of NaOH, and 10 eq. of mercaptoethanol in anhydrous ethanol gave a mixture of three compounds among which I expected the desired 2-hydroxyethylsulfanylderivatibe **9** to be present (Scheme 10). However, the only isolable product was known diosphenol **10**.[84] The other two compounds were repeatedly lost during the column chromatography or HPLC, probably due to decomposition. Formation of **10** is similar as for many attempts to substitute bromine atom in 2-bromo-3-oxoterpenes described in the lit.[53] Diosphenol **10** is usually being prepared directly by the

oxidation of dihydrobetuolonic acid (**2b**) with air, in a solution of potassium *tert*-butanolate in *tert*-butanol at 40 °C (Scheme 10).[85,86]



Scheme 36. Reagents and conditions: (a) Br_2, $CHCl_3$, r.t.; (b) mercaptoethanol (1 eq.), NaOH, EtOH (anh.), 0 °C to r.t.; (c) mercaptoethanol (used as cosolvent), NaOH, EtOH (anh.), 0 °C to r.t; (d) air, *t*-BuOK, *t*-BuOH, 40 °C.

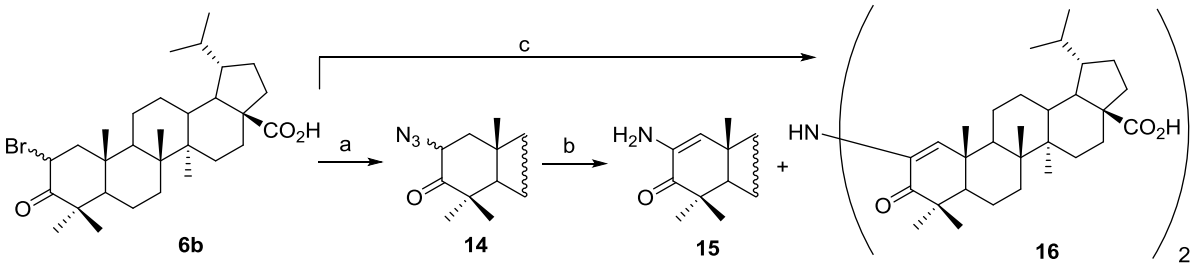
The reaction of bromoketone **6b** with sodium sulfide in *N*-methylpyrrolidone at r.t. gave sulfanylderivative **11** which was lost while chromatographed (the compound probably stayed on silica gel) but when **11** was methylated by diazomethane before the HPLC chromatography, it was possible to obtain and characterize it as methyl ester **12** (Scheme 14). The structure of **12** was confirmed by LC-HRMS and other spectral data. During the following attempts for the synthesis of larger amounts it was found that both derivatives **11** and **12** succumb to slow decomposition while being purified on silica gel column or on HPLC. An attempt to prepare more stable acetyl sulfide of dihydrobetulonic acid **13** led to decomposition of the starting compound **11** (Scheme 11). This part of the project was then abandoned.



Scheme 37. Reagents and conditions: (a) Na_2S, N-methylpyrolidone, r.t.; (b) CH_2N_2, Et_2O, $CHCl_3$, r.t.; (c) Ac_2O, AcOH, r.t.

13.2.2 Synthesis of nitrogen containing derivatives

The reaction of bromoketone **6b** with sodium azide was performed in *N*-methylpyrrolidone catalyzed by acetic acid according to a precedent from steroid chemistry[87] (Scheme 12). An attempt to purify the crude azido acid **14** on a silica gel column failed due to fast decomposition which yielded yellow enaminoketone **15** that spontaneously dimerizes to imine **16** the same way as it was described for analogous betulinic acid derivative in the lit.[88] (Scheme 12). The decomposition could be easily fastened by adding silica gel, triphenylphosphine or by heating. Finally, the sufficiently pure 2α/2β-azidodihydrobetulonic acid (**14**) was obtained when the reaction mixture was poured to a tenfold volume of water; the precipitate was filtered on a frit (S4), washed with a small portion of water, dried on a vacuum line and recrystalized from chloroform/methanol under nitrogen flow. The structure of **14** was confirmed by spectral data. Unlike azide **14**, both enaminoketone **15** and dimer **16** were stable enough and I was able to isolate and characterize them as a mixture without any problems. Moreover, compounds **15** and **16** were prepared directly and faster when a reaction of bromoketone **6b** with sodium azide was stirred in a solution of DMSO containing a drop of H_2SO_4 at 70 °C (Scheme 12).

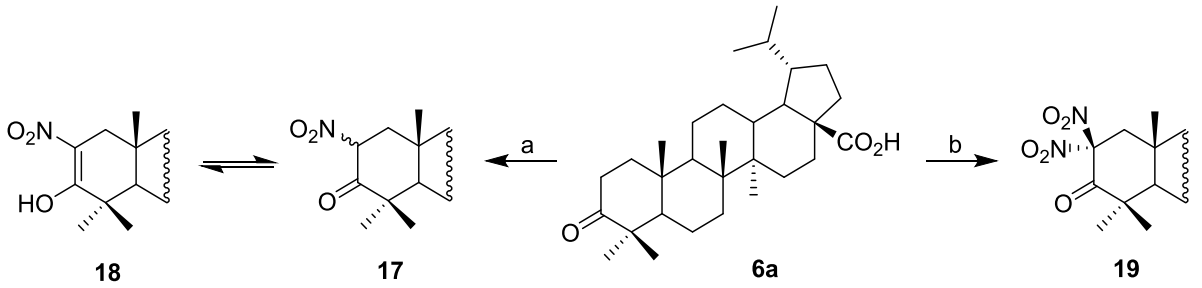


Scheme 38. Reagents and conditions: (a) NaN_3, NMP, AcOH, r.t.; (b) spontaneous decomposition at r.t.; (c) NaN_3, DMSO, H_2SO_4, 70 °C.

A series of optimization reactions with sodium nitrite was tried to find the best reaction conditions for preparation of 2-nitrodihydrobetulonic acid (**17**) by nucleophilic substitution; however, all reactions led to complicated mixtures with usually four to six spots on the TLC plate, which were difficult to separate and purify. Therefore, I decided to do the direct nitration of dihydrobetulonic acid (**6a**) with nitric acid according to the method that was described by Shernyukov et al.[28] who used it to nitrate allobetulon (**1a**).

When the reaction procedure from the lit.[28] was followed (compound **6a** was dissolved by heating in AcOH and treated with 57% nitric acid at 25 ºC for 24 h), the starting material stayed unreacted. Next time, I have used 67% nitric acid while other reaction conditions were not changed (Scheme 13). Mononitroderivative **17** was identified as the only spot on the TLC after 25 hours with some impurities at the start; however, after work-up, it was obtained in rather low yield (23 %). Part

of the starting material **6a** probably decomposed during the reactin. The compound **17** was characterized as the enol **18**. This was in agreement with the spectrum of analogous compound (2-nitroallobetulon) published in the lit.[28] It is worth to mention, that in general, 3-oxotriterpenes with an electronegative substituent at C-2 often occur in their enol-forms. 2,2-dinitrodihydrobetulonic acid (**19**) was the main product when the same reaction mixture was heated to 35 ºC for 6 h (Scheme 13).



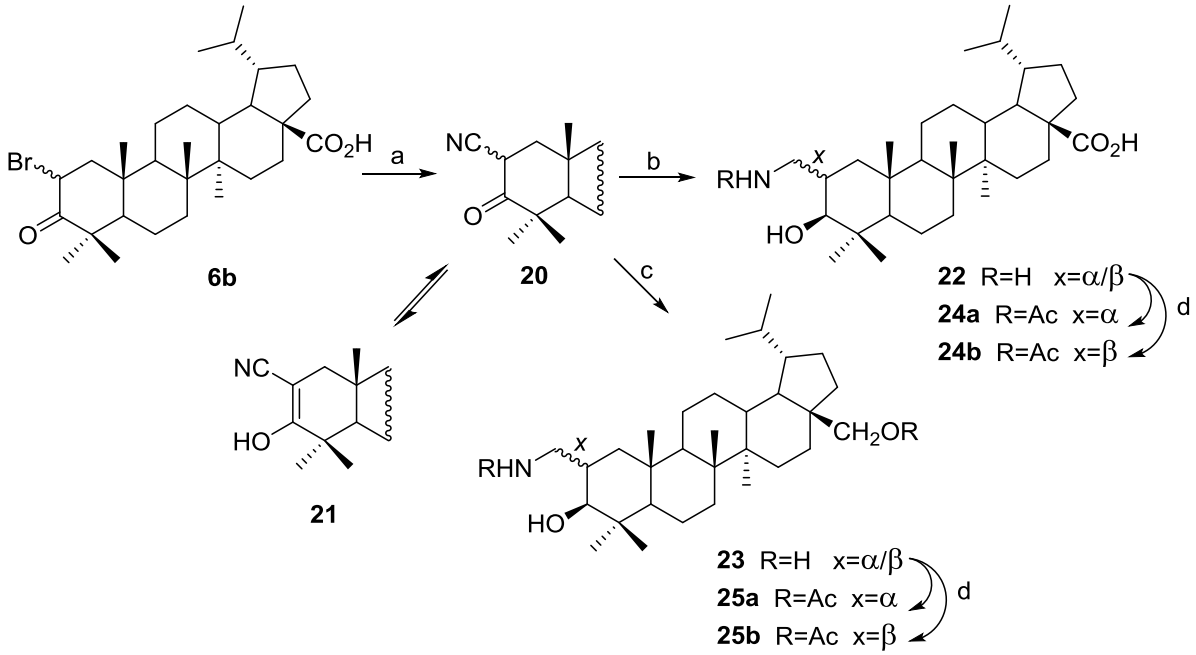
Scheme 39. Reagents and conditions: (a) 67% HNO_3, AcOH, 25 °C, 25 h; (b) 67% HNO_3, AcOH, 35 °C, 6 h.

The reaction of bromoketone **6b** with sodium cyanide in DMSO gave 2-cyanodihydrobetulonic acid (**20**) which was characterized in its enol-form **21**, as it was described in the lit.[83] (Scheme 14). Reduction of compound **20** was then performed by lithium aluminum hydride.

When cyanoketone **20** in THF was stirred and heated under reflux with $LiAlH_4$ which was added as powder, the starting material was consumed in 30 min to give the crude 2α/2β-(aminomethylene) dihydrobetulinic acid (**22**, Scheme 14). The work-up procedure and isolation of the product, however, caused many troubles because of the presence of insoluble aluminum salts. Moreover, the purification of **22** was very difficult because the product kept remaining on the silica gel and was just slowly washed out by methanol. These troubles were very likely caused by the combination of the free amino group along with the free carboxylic group in one molecule as mentioned earlier in this thesis. Even though the compound was poorly soluble in organic solvents, small amount of the epimeric mixture **22** was anyways collected. Acetylation of the crude amino alcohol **22** gave the epimeric mixture of 2-acetamidomethylenederivatives **24a** and **24b** which were then separated and characterized as α-epimer **24a** and β-epimer **24b** (Scheme 14).

Much better results were obtained, when cyanoketone **20** reacted with 1M-solution of $LiAlH_4$ in THF (Scheme 14). An epimeric mixture of dihydroxyamine **23** formed after heating the reaction mixture under reflux for 2 h which was clear from the TLC. Product **23** was unfortunately almost insoluble in organic solvents and all attempts for its purification (crystallization, chromatography on silica gel) failed. Therefore, the reaction was repeated and the crude 2α/2β-(aminomethyl)-3β,28-dihydroxylupane **23** was acetylated to form the separable mixture of the α- and β-epimers **25a** and

25b, respectively (Scheme 14). Both acetamides were fully characterized. Unlike previously, in this case, carboxylic group at C-28 was reduced to alcohol as expected.



Scheme 40. Reagents and conditions: (a) NaCN, DMSO, r.t. 24 h; (b) $LiAlH_4$, THF, refl. 30 min, (c) $LiAlH_4$.THF, THF, refl., 2 h; (d) Ac_2O, pyridine, r.t., 4 h (for **24a**, **24b**) or 6.5 h (for **25a**, **25b**).

13.2.3 Biological assays – cytotoxicity

Cytotoxic activity of all synthesized compounds and stable intermediates was investigated *in vitro* against CCRF-CEM cell line using the standard MTS test. The testing was performed at LEM. All derivatives with IC_{50} below 50 μM were further examined on seven cancer cell lines and two human non-cancer fibroblasts. From this set of derivatives, containing a substituent at the position C-2 of triterpenic skeleton, most compounds showed moderate to low cytotoxicity with the best IC_{50} value of 10.1 μM on myelogenous leukemia cell line for the 2-nitroderivative **17**. Only the compounds **6b**, **10** whose biological properties are already known from the lit.[53,84] as well as the starting dihydrobetulonic acid (**6a**) had cytotoxicity in low micromolar range on T-lymphoblastic leukemia cell line.

To sum up, I have prepared a set of 12 new triterpenoid compounds to supplement a larger series of compounds prepared earlier in our research group. Within this larger set we could clearly see that the higher electronegativity of the substituents present at the C-2 position resulted in compounds with higher IC_{50} which is in agreement with our original hypothesis. However, in most of the new derivatives, the cytotoxicity was not limited to cancer cell lines exclusively, they are toxic against non-cancer fibroblasts and therefore their use as therapeutics is unlikely. A lot of difficulties had to be

overcome during the synthesis. For example, some reactions produced mixtures of epimers, which were impossible to separate and therefore the biological testing had to be done with epimeric mixtures. In many cases, compounds were almost insoluble in both water and organic solvents, they were often lost or partly lost during the extraction and chromatography procedures and this was the reason for low yields. Despite all difficulties with the synthesis and a small chance to find a new therapeutic agent among the presented set of triterpenes, these compounds gave us important data points for our structure-activity relationship evaluations. Based on those data, structures of better anticancer inhibitors may be proposed in the future research and both the difficulties with the low solubility and selectivity may be overcome by prodrug approach or by the synthesis of conjugates such as.[79] This subproject became a significant part of the publication: Borkova, L.; Gurska, S.; Dzubak, P.; Burianova, R.; Hajduch, M.; Sarek, J.; Popa, I.; Urban, M. *Eur. J. Med. Chem.* **2016**, *121*, 120-131.[70]

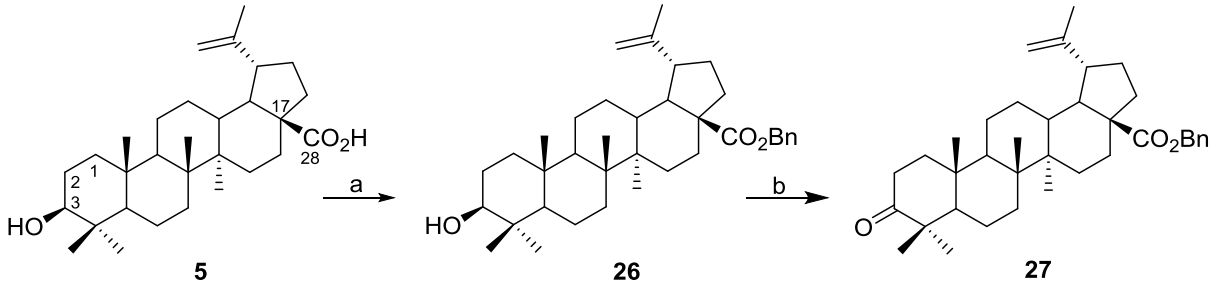
13.3 Preparation of new betulinic acid derivatives by Suzuki-Miyaura cross-coupling: scope and limitations of the method

Suzuki-Miyaura cross-coupling is a modern and well-known method in organic chemistry synthesis generating compounds by forming a new C-C bond.[90–95] Despite that, in the chemistry of triterpenoids there is only one published precedent of the use of this procedure.[96] Since it may open an access to almost unlimited amount of new compounds, a part of this work was focused on the study of the utilization of Suzuki-Miyaura cross-coupling for the preparation of various betulinic acid derivatives substituted at the C-2 or C-3 positions, and on derivatization of allobetulon at C-2 position.

13.3.1 Reactions on betulinic acid

Starting from betulinic acid (**5**), triterpenic triflates **29** and **30** were prepared in three, resp. four steps in overall yield 75 % and 57 %. First, betulinic acid (**5**) was protected with benzyl ester by the reaction with benzyl bromide and potassium carbonate in dimethylformamide and acetonitrile at 60 °C. Benzyl betulinate (**26**) was then oxidized with sodium dichromate and sodium acetate in 1,3-dioxane and acetic acid at r.t. to give benzyl betulonate (**27**, Scheme 15); both reactions are known.[82] Triflate **29** was prepared directly from ester **27** by its reaction with Tf_2NPh and KHMDS in the mixture of tetrahydrofuran and toluene at -78 °C;[96] this procedure is also known from the steroid chemistry.[97] Triflate **30** was prepared in two-step reaction from benzyl betulonate (**27**). First, ester **27** was oxidized with air and potassium *tert*-butoxide in *tert*-butanole at 40 °C to form diosphenol **28**.[85]

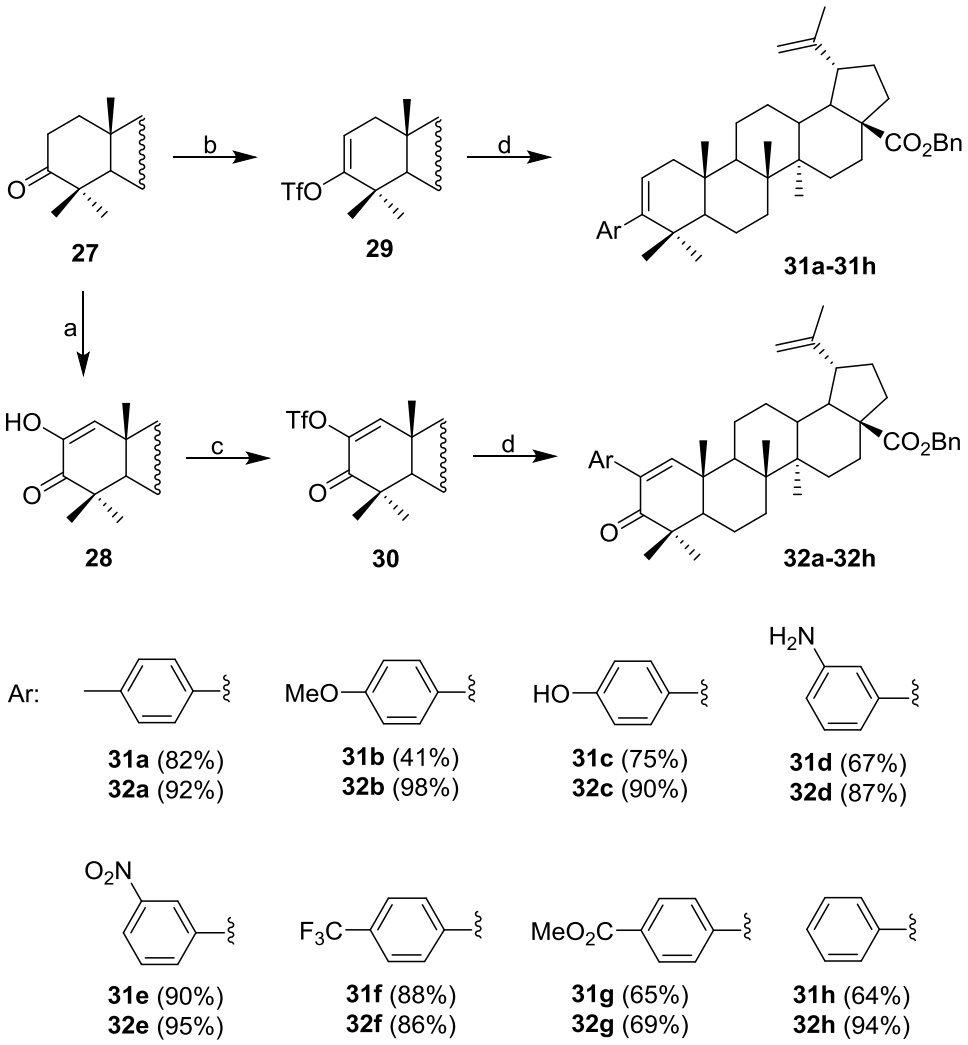
The subsequent introduction of triflate was first tried with diosphenol **37** – an allobetulon derivative (we often use allobetulon derivatives to try/find/optimize new reaction conditions since allobetulon derivatives are the most stable triterpenes from our repertoir that usually do not undergo any side-reactions, on the other hand, their cytotoxicity is usually low), see the chapter *3.3.2 Reactions on allobetulon*. Then the reaction conditions were applied to diosphenol **28** which reacted with Tf_2NPh, dimethylaminopyridine, and trimethylamine in dichloromethane at r.t. to give triflate **30** which was purified on column chromatography (Scheme 16).[98] The structure of compound **30** was confirmed by spectral data. It is worth to mention, that triflate **30** was stable during the work-up procedures, chromatography, crystallization etc. and it was not necessary to pay any special attention for its storage. It only succumbs to slow decomposition when heated to 50 °C or more. The same is true for the triflate **29**.



Scheme 41. Reagents and conditions: (a) BnBr, K_2CO_3, DMF, MeCN, 60 °C, 48 h; (b) $Na_2Cr_2O_7$, AcONa, 1,3-dioxane, AcOH, r.t., 16 h.

Suzuki-Miyaura cross-coupling procedure from the lit.[96] was used but the reaction conditions had to be optimized. The original procedure[96] uses 2 eq. of boronic acid, 4 eq. of Na_2CO_3, $Pd(PPh_3)_4$ (5 mol %) as the catalyst, and the mixture of solvents 1,4-dioxane/2-propanol/water 2:2:1. The reaction of triflate **29** with *p*-tolylboronic acid was chosen for the reaction conditions optimization (Scheme 16). First, the catalyst $Pd(PPh_3)_4$ from the lit.[96] was replaced with the more air-stable and cheaper catalyst $PdCl_2(PPh_3)_2$. Then its amount was gradually decreased from 8 mol % to 4 mol % and finally to 2 mol %; all amounts allowed for the full consumption of the triflate **29** and formation of **31a** as a single product. This not only saved the catalyst but also resulted in easier reaction work-up and higher reaction yields (from 73 % to 82 %). Next, the amount of the base was lowered from 4 equivalents of sodium carbonate to 2 equivalents. The amount of boronic acid and the ratio of solvents was not changed because the attempt to change the mixture of solvents to 1,4-dioxane and water only resulted in lower yield. To conclude, in my work, the best results were obtained with 2 eq. of boronic acid, 2 eq. of Na_2CO_3, and 2 mol % of $PdCl_2(PPh_3)_2$ when heating the reaction mixture 18 hours under reflux in the mixture of solvents 1,4-dioxane/2-propanol/water 2:2:1.

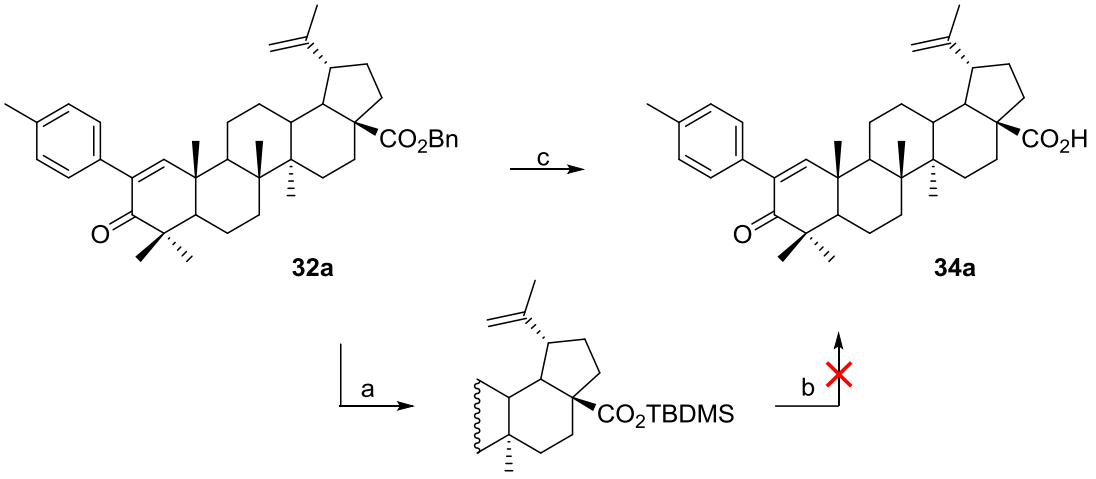
Once the best conditions for the cross-coupling reaction were found, two series of aryl-substituted betulinic acid derivatives were prepared. Triflates **29** and **30** reacted with a set of eight aromatic boronic acids with various electron-donating (**a**-**d**) and electron-withdrawing (**e**-**g**) substituents or without any substituent (**h**). A small library of 16 new compounds was synthesized in medium to high yields with respect to the substituents (**31a-31h** 41-90 % yields; **32a-32h** 69-98 % yields; Scheme 16). The presence of the appropriate substituent was confirmed by their characteristic signals in ^{1}H NMR spectra.



Scheme 42. Reagents and conditions: (a) air, *t*-BuOK, *t*-BuOH, 40 °C, 2 h; (b) Tf_2NPh, KHMDS, THF, toluene, -78 °C, 4 h; (c) Tf_2NPh, DMAP, TEA, DCM, r.t., 90 min; (d) $ArB(OH)_2$, Na_2CO_3, $PdCl_2(PPh_3)_2$ (2mol %), dioxane, IPO, H_2O, refl., 18 h.

As mentioned earlier, the free carboxylic function at C-28 is usually important for the cytotoxic activity of triterpenoids. For this reason, benzyl esters **31a-31h** and **32a-32h** were deprotected *via* reductive debenzylation. First, two-step selective deprotection of benzyl ester described in the lit.[96] was used; however, the desired compound **34a** did not form (Scheme 17). Betulinic acid derivatives

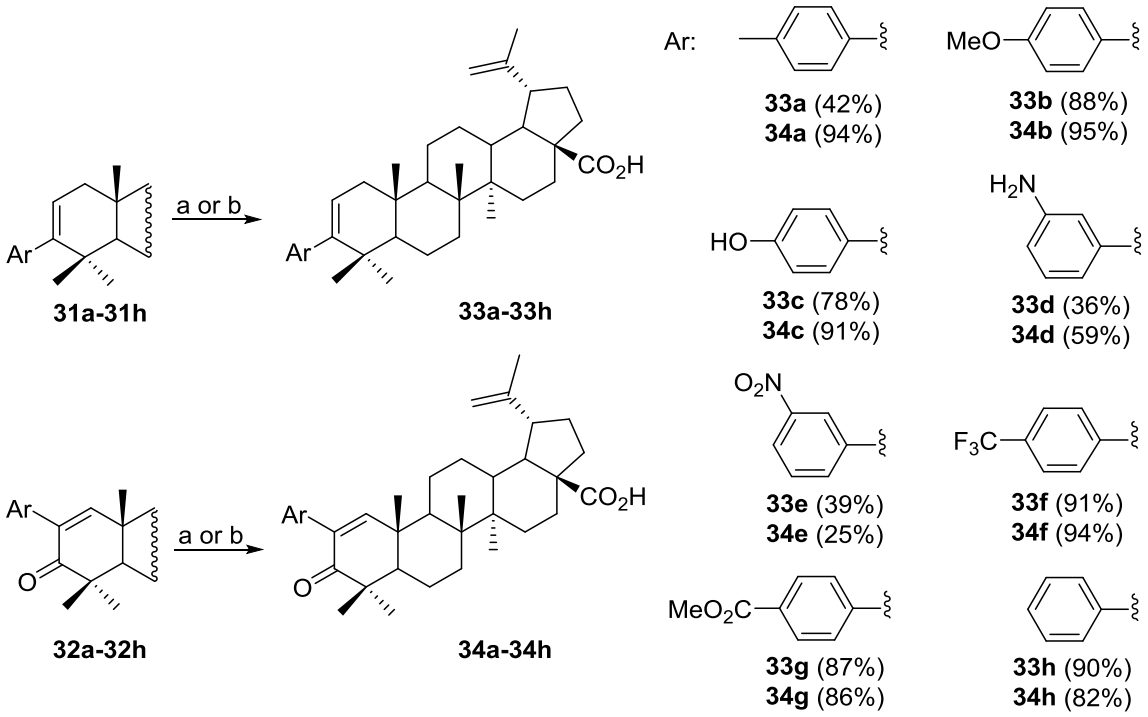
33a-33c, **33e-33h**, **34a-34c**, and **34e-34h** were then prepared by catalytical hydrogenation of appropriate benzyl esters with the use of cyclohexa-1,3-dien as the hydrogen source in absolute ethanol at 50 °C.[99] Yields ranged between 25-95 % (Scheme 18). Generally, to avoid the formation of dihydrobetulonic acid derivatives (hydrogenation of the double bond between C-20 and C-29) it was necessary to add maximum 15 mol % of Pd/C and no more than 14 eq. of cyclohexa-1,3-dien. Within this set of compounds, anilin derivative **34d** formation was observed when more than 7 eq. of cyclohexa-1,3-dien were added to the reaction mixture with nitrophenyl derivative **32e**. The ratio was: aniline derivative **34d** 59 % and nitrophenyl derivative **34e** 25 %. Since it was possible to separate both compounds by chromatography, the reaction was not further optimized to yield either pure **34d** or **34e**. Medium to low yields of some products (**33e** 39 %; **33a** 42 %) were caused by difficulties with the purification and/or by residues of the unreacted starting compound in the reaction mixture (unreacted compound was separated and recycled). Often, the balance between debenzylation and hydrogenation of the double bonds in the molecule had to be achieved.



Scheme 43. Reagents and conditions: (a) TBDMSH, DCM, TEA, $Pd(OAc)_2$, 60 °C, 24 h; (b) TBAF, 1,4-dioxane, THF, r.t., 4 h; (c) cyclohexa-1,3-dien, Pd/C, EtOH, 50 °C, 24 h.

Aniline derivatives **31d** and **32d** did not react with cyclohexa-1,3-dien; therefore, another procedure had to be tried. Reductive debenzylation with H_2 on Pd/C was applied. A series of optimization reactions was done to find the best conditions for the deprotection. It was observed that the choice of the solvent was the major determinant for the side-reactions, the hydrogenation of the 20(29)-double bond or the 1(2)-double bond. Methanol, ethanol, 2-propanol, and a mixture of DCM with methanol 2:1 were used as solvents. The best results were obtained with the mixture DCM/MeOH; nevertheless, the reaction still could not be left to react till the 100% consumption of the starting material and had to be stopped earlier to avoid the formation of the side-products with reduced double-bond(s). Thus, derivatives **33d** and **34d** were prepared by reaction with H_2 and Pd/C

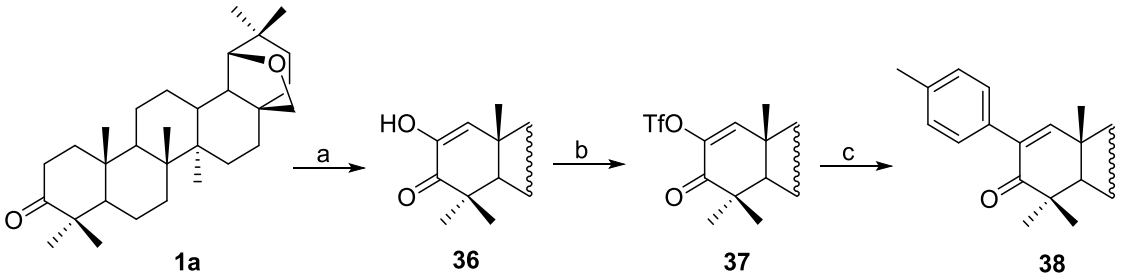
in DCM/MeOH 2:1 at r.t. (Scheme 18). Yields were 36 % and 34 %, respectively, the starting material was the rest and it was separated and deprotected again, here I present the yield of a single deprotection step. The structures of the final products were confirmed by spectral data.



Scheme 44. Reagents and conditions: (a) cyclohexa-1,3-dien, Pd/C, EtOH, 50 °C, 5-48 h; (b) H_2, Pd/C, DCM/MeOH 2:1, r.t., 2-3 h (for **33d** and **34d** only).

13.3.2 Reactions on allobetulon

In order to find out whether the Suzuki-Miyaura cross-coupling works on other triterpenoid skeletons, allobetulon **1a** was chosen for further investigation. First, compound **1a** was oxidized with an air and potassium *tert*-butoxide in *tert*-butanol at 40 °C. The reaction was completed after 5 h of vigorous stirring yielding diosphenol **36** in 93 % yield.[64] Triflate **37** was prepared by the reaction of **36** with Tf_2NPh, dimethylaminopyridine, and trimethylamine in dichloromethane at r.t.[98] and as mentioned earlier, this was the first time this protocol was used in triterpenoid chemistry and was optimized and then applied on benzyl betulonate **28**. After 90 minutes, the reaction was completed. After the standard work up procedure, the crude allobetulon triflate **37** was dried on the vacuum line and used to the next reaction step without further purification. Triflate **37** then reacted with tolylboronic acid under optimized Suzuki-Miyaura cross-coupling conditions. After complete consumption of the starting triflate **37** the reaction mixture was processed, and the crude product was purified on the column chromatography. Tolyl derivative **38** was obtained in 54 % yield and its structure was confirmed by spectral data (Scheme 19).



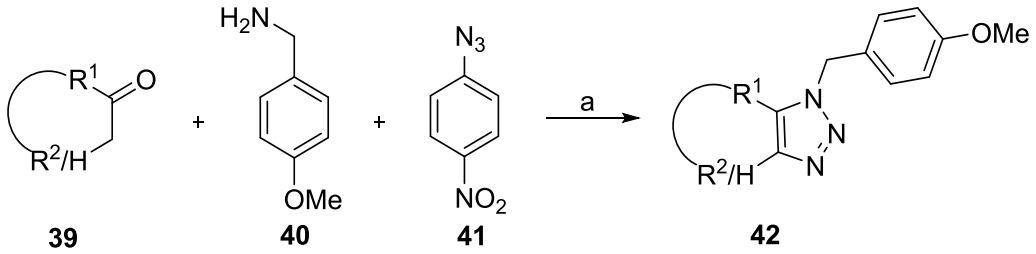
Scheme 45. Reagents and conditions: (a) air, *t*-BuOK, *t*-BuOH, 40 °C, 5 h; (b) Tf_2NPh, DMAP, TEA, DCM, r.t., 90 min; (c) $ArB(OH)_2$, Na_2CO_3. $PdCl_2(PPh_3)_2$ (2 mol %), dioxane, IPO, H_2O, refl., 18 h.

In conclusion, Suzuki-Miyaura cross-coupling reaction was applied to triterpenoids in order to find its scope and limitations and to synthesize a series of new potentially biologically active compounds. Betulinic acid (**5**) and allobetulon (**1a**), compounds derived from lupane and 18α-oleanane skeletal types, were chosen for the derivatization. Introduction of substituents into the position C-2 on allobetulon (**1a**) and the positions C-2 and C-3 on betulinic acid (**5**) were studied. Reaction conditions for preparation of triflates and cross-coupling products were found and optimized. An example of Suzuki-Miyaura cross-coupling into the position C-2 on allobetulon (**1a**) was demonstrated. Two sets of benzyl betulonate derivatives modified at positions C-2 and C-3 by aryl-substituents were prepared. A method for the reductive debenzylation of benzyl betulonate derivatives was developed and the final products **33a-33h** and **34a-34h** with the free C-28 carboxylate were prepared. Cytotoxic properties of all compounds are currently being examined on eight cancer cell lines and two non-cancer fibroblasts. Preliminary results show that compounds protected by benzyl ester are inactive which is common. To summarize it, Suzuki-Miyaura cross-coupling reaction is a robust method for derivatization of the A-ring of triterpenoids and it will be tried on other triterpenoid rings in the future. A manuscript based on this project is in preparation.

13.4 Triazolization reaction and triterpenoid chemistry

Recently, triazolylation reactions were introduced to the triterpenoid chemistry and it has been used in our research group to connect triterpenes with another small molecules of the interest.[100] Meanwhile, a large number of other scientists have begun to explore this area and published quite interesting results; therefore, my colleagues and I summarized this research area and published a review: Pokorny, J.; Borkova, L.; Urban, M. Click Reactions in Chemistry of Triterpenes - Advances Towards Development of Potential Therapeutics. *Current Med. Chem.* **2018**, *25* (5), 636–658, which describes all results of this new approach to improve both the activity and ADME-Tox properties of triterpenes by connecting them to another modifying molecules using click reactions.[101] As the second author, I wrote a significant part of this article.

In the research group of prof. Wim Dehaen, they invented a completely new method of preparation of 1,2,3-triazoles from three building blocks, simply called the *Triazolization reaction*.[102] The method relies on using abundantly available enolizable ketones, primary amines and *p*-nitrophenylazide as a dinitrogen source. This general multi-component strategy can be applied to the synthesis of 1,4,5-trisubstituted, 1,5-disubstituted, and 4,5-fused 1,2,3-triazoles, such as **42** (Scheme 20).[103]

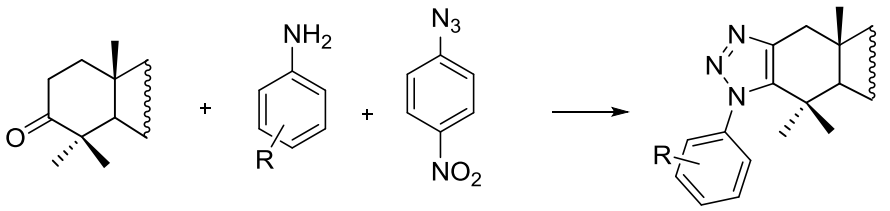


39 **40** **41** **42**

Scheme 46. A general reaction scheme of the Triazolization reaction with *p*-methoxy-benzylamine as a model of primary amine. Reagents and conditions: (a) **39** (1.0 eq.), **40** (1.3 eq.), **41** (1.0 eq.), AcOH (30 mol %), 4Å molecular sieves, toluene, 100 °C, 12 h.[103]

In order to introduce this reaction to the triterpenoid chemistry, I have decided to go to their research group for three months stay during which I have learned this method. As a result, I am a co-author of the publication: Silveira-Dorta, G.; Jana, S.; Borkova, L.; Thomas, J.; Dehaen, W. Straightforward Synthesis of Enantiomerically Pure 1,2,3-Triazoles Derived from Amino Esters. *Org. Biomol. Chem.* **2018**, *16* (17), 3168–3176.

This reaction will be very useful to prepare new heterocyclic triterpenes with a 5-membered heterocyclic ring (substituted triazole) condensed to the triterpenic A-ring (Scheme 21).



Scheme 47. The planned synthesis of fused triterpenoid 1,2,3-triazoles from 3-oxotriterpenoids, substituted anilines, and *p*-nitrophenylazide.

14 Conclusions and future directions

Efficient and universal three-step synthestic pathway towards *N*-substituted aminothiazoles fused to the A-ring of triterpenoids (3-oxoderivatives) was developed and optimized. Seven series of variously substituted triterpenoid thiazoles, their unsubstituted analogues and intermediates were synthesized. Together, 82 compounds, 75 of them new, was prepared and fully characterized and their cytotoxicity was measured on eight cancer cell lines and two non-cancer fibroblasts. The most active compounds against T-lymphoblastic leukemia cell line were aminothiazoles **4m** and **5m** with the free NH_2 group, 2-bromo-3-oxoderivative **7b**, and 2-thiocyano-3-oxoderivatives **5c**, **6c**, and **7c**. Pharmacological parameters of the most active derivativatives were measured and these trends in SAR were found: An active compound must have the free C-28 COOH group; derivatives with the free amine on the ainothiazole ring are active; sometimes derivatives with morpholine substituent are active; derivatives with the substituents of low polarity are inactive; intermediates (2-bromo and 2-thiocyanato derivatives) are the most active but their selectivity is usually insufficient. Four compounds of this subproject **4m**, **5m**, **I**, and **7b** had high and selective cytotoxicity against cancer cells as well as favorable pharmacological properties. Since these compounds have the highest potential to become new anticancer drugs, they will be further tested *in vivo* for ADME-Tox parameters and anticancer properties.

Substitution of the C-2 position of dihydrobetulonic acid (**6a**) with electronegative substituents was performed and our hypothesis about the relationship between electronegativity of the substituents and cytotoxicity of the molecule was confirmed: The higher electronegativity of a substituent, the more active compound. 15 compounds, 12 of them new, were prepared and evaluated for their cytotoxicity. Although we found some interesting trends, no further tests are planed since most of the compounds were only moderately active and the rest was not selective towards cancer cells. Also, some of the reactions had low yields and produced unseparable mixtures of epimers which also is not favorable in medicinal chemistry.

Suzuki-Miyaura cross-coupling, on the other hand, was found to be a great tool for the preparation of libraries of new terpenoids. Conditions for the preparation of triflate intermediates and conditions for Suzuki-Miyaura cross-coupling were found and optimized. The reaction is a robust methodology that allows for almost unlimited numbers of the derivatives of triterpenes, in this work, compounds were modified at the positions 2 and 3 but there are strong expectations that this method may be expanded to derivatize other triterpenic rings. The aryl-substituted derivatives of allobetulon in the position C-2 (**38**) and benzyl betulonate in the positions C-2 (**31a-31h**) and C-3 (**32a-32h**) were prepared. A procedure for deprotection of benzyl betulonate derivatives to final products was

developed and two sets of aryl-substituted betulinic acid derivatives (**33a-33h** and **34a-34h**) were prepared. Together, 40 compounds, 33 of them new, were synthesized in this subproject and their cytotoxicity is being tested. Preliminary results show that benzyl esters are, as usually, inactive, while compounds with the free 28-COOH have $IC_{50} < 50$ μM.

To sum up, 137 compounds, 120 of them new, with potential biological activities derived from seven triterpenoid skeletons were prepared and characterized by common chemical and analytical methods. Both final products and intermediates were or are being tested on their cytotoxic activity against eight cancer cell lines of a different histogenetic origin, including multi-drug resistant cell lines, and two normal fibroblasts. Influence on the cell cycle and on the inhibition of DNA and RNA synthesis were measured for the most potent compounds as well as metabolic stability and membrane permeability was tested for some derivatives. All biological assays were performed at the department of MUDr. Hajdúch, Ph.D. in the Laboratory of Experimental Medicine, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University Olomouc (LEM). The summary of the results of cytotoxicity screening and conclusions about SARs were made by me under supervision of the LEM members. Conclusions from the advanced tests such as cell cycle observations were made by the LEM members with my participation.

Specification of the work of Lucie Borková and other co-authors of the articles

The first subproject was started in collaboration with the research group of RNDr. Jan Šarek, Ph.D., whose students Ing. Petr Kalina and Bc. Filip Korda prepared several allobetulon aminothiazoles and a few oleanonic acid derivatives. I have finished all series, optimized the syntheses, collected all data from the synthesis, characterization and biological assays, and I wrote the majority of the published article. Several dihydrobetulonic aminothiazoles were prepared by Mgr. Richard Adámek under my supervision and ursolic acid derivatives were prepared by Bc. Nikola Jakubcová under my supervision. Most importantly, I have optimized the synthesis of 2-bromo-3-oxoderivatives, the synthesis of 2-thiocyano-3-oxo derivatives, and the synthesis of aminothiazoles with the free NH_2 group. I have prepared 43 compounds out of 82.

Other subprojects (Substitutions on the C-2 and Suzuki-Miyaura cross-coupling) were solely mine. In this regard, several betulinic acid derivatives were prepared by Bc. Barbora Vránová under my supervision.

15 List of abbreviations

Ac	acetyl
AcOH	acetic acid
ADME	absorption, distribution, metabolism, and excretion
Ar	aryl
Bn	benzyl
BrU	5-bromouridine
BrDU	5-bromo-2´deoxyuridine
DB	double bond
DCM	dichlormethane
DMAP	4-(dimethylamino)pyridine
DMF	dimethylfomamide
DMSO	dimethylsulfoxide
Et	ethyl
EtOAc	ethyl acetate
EtOH	ethanol
EWG	electron withdrawing group
HIV	human immunodeficiency virus
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IC_{50}	half maximal inhibitory concentration
IPO	2-propanol
KHMDS	potassium bis(trimethylsilyl)amide
LEM	Laboratory of Experimental Medicine
Me	methyl
MeOH	methanol
MTS	3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxylmethoxyphennyl)-2-(4-sulfophenyl)-2H-tetrazolium
NMP	*N*-methylpyrrolidone

NMR	nuclear magnetic resonance
PCC	pyridinium chlorochromate
Pd/C	palladium on activated charcoal
Ph	phenyl
refl.	reflux
r.t.	room temperature
SAR	structure-activity relationship
SB	single bond
t-Bu	*tert*-butyl
t-BuOH	*tert*-butanole
t-BuOK	potassium *tert*-butoxide
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilil
TEA	triethylamine
Tf	triflate
Tf_2NPh	*N*-phenyl-bis(trifluoromethanesulfonimide)
THF	tetrahydrofuran
Tox	toxicity

16 List of author's publications

Publications related to the thesis

7. Borkova, L.; Hodon, J.; Urban, M. Modifications at the A-ring of Betulinic Acid and Betulonic Acid. *Asian J. Org. Chem.* **2018**, *7*, 1542-1560.
8. Pokorny, J.; Borkova, L.; Urban, M. Click Reactions in Chemistry of Triterpenes – Advances Towards Development of Potential Therapeutics. *Current Med. Chem*. **2018**, *25*, 5, 636-658.
9. Borkova, L.; Adamek, R.; Kalina, P.; Drasar, P.; Dzubak, P.; Gurska, S.; Rehulka, J.; Hajduch, M.; Urban, M.; Sarek, J. Synthesis and Cytotoxic Activity of Triterpenoid Thiazoles Derived from Allobetulin, Methyl Betulonate, Methyl Oleanonate, and Oleanonic Acid. *ChemMedChem* **2017**, *2017*, 12, 390-398.
10. Borkova, L.; Gurska, S.; Dzubak, P.; Burianova, R.; Hajduch, M.; Sarek, J.; Popa, I.; Urban, M. Lupane and 18alpha-oleanane derivatives substituted in the position 2, their cytotoxicity and influence on cancer cells. *Eur. J. Med. Chem.* **2016**, *121*, 120-131.

Other publications

11. Silveira-Dorta, G.; Jana, S.; Borkova, L.; Thomas, J.; Dehaen, W. Straightforward synthesis of enantiomerically pure 1,2,3-triazoles derived from amino esters. *Org.& Biomolecular Chem.* **2018**, *16* (17), 3168–3176.
12. Borkova, L.; Jasikova, L.; Rehulka, J.; Frisonsova, K.; Urban, M.; Frydrych, I.; Popa, I.; Hajduch, M.; Dickinson, N. J.; Vlk, M.; Dzubak, P.; Sarek, J. Synthesis of cytotoxic 2,2-difluoroderivatives of dihydrobetulinic acid and allobetulin and study of their impact on cancer cells. *Eur. J. Med. Chem.* **2015**, *96*, 482-490.

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Curriculum Vitae

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Education

2014 – present	**Ph.D. study in Organic chemistry** Palacký University Olomouc, Faculty of Science (supervision: assoc. prof. Milan Urban)
2012 – 2014	**Master's degree in Bioorganic chemistry (Mgr.)** Palacký University Olomouc, Faculty of Science (supervision: assoc. prof. Milan Urban)
2009 – 2012	**Bachelor's degree in Bioorganic chemistry (Bc.)** Palacký University Olomouc, Faculty of Science (supervision: Jan Šarek, Ph.D.)
2001 – 2009	**Grammar School Uherské Hradiště**

Work Experience and Traineeships

2014 – present	**Palacký University Olomouc, Olomouc, Czech republic** Researcher in organic chemistry at the Department of Organic Chemistry, Faculty of Science
5/2017 – 7/2017	**Katholieke Universiteit Leuven, Leuven, Belgium** Traineeship under supervision of prof. Wim Dehaen at the Molecular Design and Synthesis Division, Chemistry Department
8/2013 (3 weeks)	**Teva Czech Industries s.r.o., Opava, Czech republic** Traineeship under supervision of Tomáš Holas, Ph.D. in the pilot plant, and research and development laboratories
1/2013 – 6/2013	**University of Southern Denmark, Odense, Denmark** Erasmus study stay
2011 – 2012 (4 weeks)	**RNDr. Jan Šarek, Ph.D. - Betulinines, Stříbrná Skalice, Czech rep.** Traineeship under supervision of Jan Šarek, Ph.D. in the pilot plant, and research and development laboratories

Pedagogical Activities

2016 – present	Presentations of popularization-educational lectures in the field of medicinal chemistry at high schools and grammar schools
2015 – present	Supervision of the final work of bachelor's students at the Dept. of Organic Chemistry, Palacký University Olomouc (3 defended)
2014 – 2015	Teaching of Practice in Organic Chemistry at Palacký University Olomouc

Publications

Borkova, L.; Hodon, J.; Urban, M. Modifications at the A-ring of Betulinic Acid and Betulonic Acid. *Asian J. Org. Chem.* **2018**, *7*, 1542-1560.

Silveira-Dorta, G.; Jana, S.; **Borkova, L.**; Thomas, J.; Dehaen, W. Straightforward synthesis of enantiomerically pure 1,2,3-triazoles derived from amino esters. *Org.& Biomolecular Chem.* **2018**, *16* (17), 3168–3176.

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Conferences

Oral presentations

17th Blue Danube Symposium on Heterocyclic Chemistry, Linz, Austria, **2017**

XVI. Interdisciplinary Meeting of Young Biologists, Biochemists and Chemists, Milovy, Czech republic, **2016**

Poster presentations

52nd Advances in Organic, Bioorganic and Pharmaceutical Chemistry „Liblice 2017“, Lázně Bělohrad, Czech republic, **2017**

51st Advances in Organic, Bioorganic and Pharmaceutical Chemistry „Liblice 2016“, Lázně Bělohrad, Czech republic, **2016**

252nd ACS National Meeting & Exposition, Philadelphia, USA, **2016**

50th Advances in Organic, Bioorganic and Pharmaceutical Chemistry „Liblice 2015“, Olomouc, Czech republic, **2015**

16th European Congress on Biotechnology, Edinburgh, Scotland, **2014**

18th European Symposium on Organic Chemistry, Marseille, France, **2013**

47th Advances in Organic, Bioorganic and Pharmaceutical Chemistry „Liblice 2012“, Lázně Bělohrad, Czech republic, **2012**

46th Advances in Organic, Bioorganic and Pharmaceutical Chemistry „Liblice 2011“, Lázně Bělohrad, Czech republic, **2011**

Awards

Jean-Marie Lehn Award for chemistry in 2018 – 2nd place

Dean’s Award in 2018, PhD section, chemistry – 3rd place

Rector’s Award in 2013 for the Bachelor Thesis