24th Conference on Isoprenoids
Białystok, Poland, September 9-12, 2018

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PROGRAM

Program of 24th Conference on Isoprenoids, Białystok, Poland September 9-12, 2018

SUNDAY (Sept. 9)

Ibis Styles Hotel

16:00 - 19:00	**REGISTRATION (Ibis Styles Hotel)**
17:00 - 18:30	Białystok Sightseeing Tour (walk around the city)
19:00 - 23:00	**WELCOME PARTY (Ibis Styles Hotel)**

MONDAY (Sept. 10)

University of Białystok Campus

8:15		**Departure of the conference bus from Ibis Styles Hotel to the Campus**
8:30 - 9:00		**REGISTRATION (University of Białystok Campus)**
9:00 - 9:15		**CONFERENCE OPENING *Jacek W. Morzycki and Jerzy Wicha***
		Morning Session
		Chair: Giovanni Appendino
9:15 - 10:00	**L1**	**Erick M. Carreira** New strategies for the synthesis of isoprostanoids and accompanying studies of their biology
10:00 - 10:30	**L2**	**Alain Tissier** Engineering plant diterpenoid pathways in yeast: increasing yield and expanding product diversity
10:30 - 11:00	**L3**	**Thomas B. Poulsen** Synthesis of the strongylophorines
11:00 - 11:30		**COFFEE BREAK**
		Chair: Pavel Drašar
11:30 - 12:00	**L4**	**Andreas Koeberle** Omega-oxidized terpenoids: from traditional African medicine to novel insights into human vitamin physiology
12:00 - 12:30	**L5**	**Ang Li** Total synthesis of polycyclic natural products
12:30 - 12:45	**O1**	**Yulia A. Volkova** A strategic development toward functionalized extranuclear heterosteroids diversity
12:45 - 13:00	**O2**	**Piotr Przybylski** Basket-like lactam macrolides – modifications and structure – activity relationship studies
13:00 - 13:15		**GROUP PHOTO**
13:15 - 14:15		**LUNCH**
14:15 - 14:45		**A visit to Professor A. Myrcha Natural Science Museum (University of Białystok Campus)**

Afternoon session		
		Chair: Biao Yu
14:45 - 15:15	**L6**	**Giovanni B. Appendino** Cannabinoquinoids: synthesis and biological profile
15:15 - 15:45	**L7**	**Alaksiej L. Hurski** Phytosterols and steroidal human metabolites as targets for testing new synthetic strategies
15:45 - 16:15	**L8**	**Miroslav Strnad** New isoprenoid cytokinins exhibiting interesting biological activities
16:15 - 16:30	**O3**	**Vladimir Zhabinskii** New azole derivatives of [17(20)*E*]-21-norpregnene: synthesis and inhibition of prostate carcinoma cell growth
16:30 - 16:45	**O4**	**Yury V. Kuznetsov** New steroidal estrogen receptor modulators. Design, synthesis, and biological evaluation
16:45 - 17:00		**Veli-Pekka Hyttinen**, SciFinder
17:00 - 17:30		***CAS* COFFEE BREAK**
		Chair: Orazio Taglialatela-Scafati
17:30 - 18:00	**L9**	**Hidayat Hussain** New strategies to identify new chemical diversity for drug development
18:00 - 18:30	**L10**	**Rita Skoda-Földes** Application of ionic liquids in the synthesis of steroid derivatives
18:30 - 18:45	**O5**	**Oldřich Lapčík** Immunoanalysis of anabolic steroids
18:45 - 19:00	**O6**	**Mihalj Poša** Peculiar self association of sodium isoursodeoxycholate in water: H-bonds in the primary micelles
19:10		**Departure of the conference bus from the Campus to Ibis Styles Hotel**
19:30 - 21:30		**DINNER (Ibis Styles Hotel)**

TUESDAY (Sept. 11)

University of Białystok Campus

8:15		**Departure of the conference bus from Ibis Styles Hotel to the Campus**
Morning Session		
		Chair: Ludger A. Wessjohann
8:45 - 9:15	**L11**	**Masayuki Inoue** Radical-based approach for synthesis of complex natural products
9:15 - 9:45	**L12**	**Vladimir Khripach** Recent advances in brassinosteroid research
9:45 - 10:15	**L13**	**Adriaan J. Minnaard** Tuberculosinyl adenosine; an isoprenoid that makes *Mycobacterium tuberculosis* pathogenic
10:15 - 10:45	**L14**	**Pingping Tang** Total synthesis of schilancitrilactones B, C and fluorine chemistry
10:45 - 11:15		**COFFEE BREAK**

		Chair: Douglas F. Covey
11:15 - 11:45	**L15**	**Miguel A. Maestro** Design and synthesis of active and selective vitamin D analogs
11:45 - 12:15	**L16**	**Rafał R. Siciński** Novel 19-norvitamin D compounds with elongated substituents at C-2 or C-26
12:15 - 12:30	**O7**	**Dmitry Dovbnya** Efficient production of hexahydroindanone derivatives by bioconversion of phytosterol with mutants of *Mycobacterium smegmatis*
12:30 - 12:45	**O8**	**Kinga Kuczyńska** Transformation of betulin core
12:45 - 13:45		**LUNCH**
		Afternoon session
		Chair: Agnieszka Wojtkielewicz
13:45 - 14:15	**L17**	**Kenji Mori** Recent results in pheromone synthesis
14:15 - 14:45	**L18**	**Vladimir I. Kalinin** Uncommon aglycones of sea cucumber triterpene glycosides. Structure, biosynthesis, evolution
14:45 - 15:15	**L19**	**Biao Yu** Total synthesis of echinoside A, a representative triterpene glycoside of sea cucumbers
15:15 - 15:30	**O9**	**Timofey V. Malyarenko** Unusual starfish steroidal glycosides: structure and biological activity
15:30 - 15:45	**O10**	**Volodymyr S. Kravets** Brassinosteroids in regulation of plant metabolism
15:45 - 16:45		**POSTER SESSION** **COFFEE BREAK**
17:00 - 23:00		**EXCURSION TO TYKOCIN AND KIERMUSY** **BANQUET IN KIERMUSY**

WEDNESDAY (Sept. 12)

Ibis Styles Hotel

		Morning Session
		Chair: Adriaan J. Minnaard
8:15 - 8:45	**L20**	**Ludger A. Wessjohann** Metabolic profiling based discovery of new isoprenoids, mode of action and (bio-)synthesis of derivatives
8:45 - 9:15	**L21**	**Martin A. Iglesias-Arteaga** Synthesis and some properties of symmetrical, unsymmetrical and hybrid steroid dimers
9:15 - 9:45	**L22**	**Zdzisław Paryzek** Steroidal dimers, macrocycles and conjugates based on bile acid scaffolds
9:45 - 10:00	**O11**	**Barbara Bednarczyk-Cwynar** Current approach in synthesis of triterpenoid dimers

10:00 - 10:15	**O12**	**Sergazy M. Adekenov** Bimolecular compounds based on the natural sesquiterpene lactones
10:15 - 10:45		**COFFEE BREAK**
		Chair: Vladimir Khripach
10:45 - 11:15	**L23**	**Jeroen S. Dickschat** Tracing terpenes with isotopes
11:15 - 11:45	**L24**	**Stanisław Witkowski** Convergent synthesis of vitamin K2 (MK-7)
11:45 - 12:00	**O13**	**Jacek Ścianowski** New benzisoselenazol-3(2*H*)-ones *N*-functionalized with terpene skeletons
12:00 - 12:15	**O14**	**Michał Michalak** NHCCuX-catalyzed Friedländer-type annulation of fluorinated aminophenones with alkynes on water. Unexpected dibenzodiazocine formation
12:15 - 13:15		**LUNCH**
		Afternoon session
		Chair: Rita Skoda-Földes
13:15 - 13:45	**L25**	**Pavel Nagorny** Convergent total synthesis and biological studies of cardiotonic steroid
13:45 - 14:00	**O15**	**Tomáš Zimmermann** Targeting phorbol derivatives toward cancers
14:00 - 14:15	**O16**	**Sergey M. Khomutov** The effect of cyclodextrins on oxidation of DHEA by fungal laccase
14:15 - 14:30	**O17**	**Eva Kudova** S.M.A.R.T. Steroids: Steroidal Molecules As Rapid-acting Therapeutics
14:30 - 14:45	**O18**	**Shingo Nagano** Crystal structures of an indole prenyltransferase, IptA: implications for substrate tolerance and its expansion
14:45 - 15:15		**COFFEE BREAK**
		Chair: Miroslav Strnad
15:15 - 15:30	**O19**	**Izabella Jastrzębska** New selenoorganic reagents in steroid chemistry
15:30 - 16:00	**L26**	**Orazio Taglialatela-Scafati** Plasticity of the isoprenoid moiety in phytocannabinoids
16:00 - 16:45	**L27**	**Douglas F. Covey** Neurosteroid click chemistry for ligand-gated ion channels Luis M. and Mary Fieser Memorial Lecture
16:45 - 17:15		**CONFERENCE CLOSING**
17:25		**Departure of the special bus from Ibis Styles Hotel to the Białystok railway station**

Town Hall on Kościuszko Square, Białystok

PLENARY LECTURES

LIST OF LECTURES

L1 **Erick M. Carreira**, New strategies for the synthesis of isoprostanoids and accompanying studies of their biology

L2 **Alain Tissier**, Engineering plant diterpenoid pathways in yeast: increasing yield and expanding product diversity

L3 **Thomas B. Poulsen**, Synthesis of the strongylophorines

L4 **Andreas Koeberle**, Omega-oxidized terpenoids: from traditional African medicine to novel insights into human vitamin physiology

L5 **Ang Li**, Total synthesis of polycyclic natural products

L6 **Giovanni Appendino**, Cannabinoquinoids: synthesis and biological profile

L7 **Alaksiej L. Hurski**, Phytosterols and steroidal human metabolites as targets for testing new synthetic strategies

L8 **Miroslav Strnad**, New isoprenoid cytokinins exhibiting interesting biological activities

L9 **Hidayat Hussain**, New strategies to identify new chemical diversity for drug development

L10 **Rita Skoda-Földes**, Application of ionic liquids in the synthesis of steroid derivatives

L11 **Masayuki Inoue**, Radical-based approach for synthesis of complex natural products

L12 **Vladimir Khripach**, Recent advances in brassinosteroid research

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L15 **Miguel A. Maestro**, Design and synthesis of active and selective vitamin D analogs

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L17 **Kenji Mori**, Recent results in pheromone synthesis

L18 **Vladimir I. Kalinin**, Uncommon aglycones of sea cucumber triterpene glycosides. Structure, biosynthesis, evolution

L19 **Biao Yu**, Total synthesis of echinoside A, a representative triterpene glycoside of sea cucumbers

L20 **Ludger A. Wessjohann**, Metabolic profiling based discovery of new isoprenoids, mode of action and (bio-)synthesis of derivatives

L21 **Martin A. Iglesias-Arteaga**, Synthesis and some properties of symmetrical, unsymmetrical and hybrid steroid dimers

L22 **Zdzisław Paryzek**, Steroidal dimers, macrocycles and conjugates based on bile acid scaffolds

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L24 **Stanisław Witkowski**, Convergent synthesis of vitamin K2 (MK-7)

L25 **Pavel Nagorny**, Convergent total synthesis and biological studies of cardiotonic steroid

L26 **Orazio Taglialatela-Scafati**, Plasticity of the isoprenoid moiety in phytocannabinoids

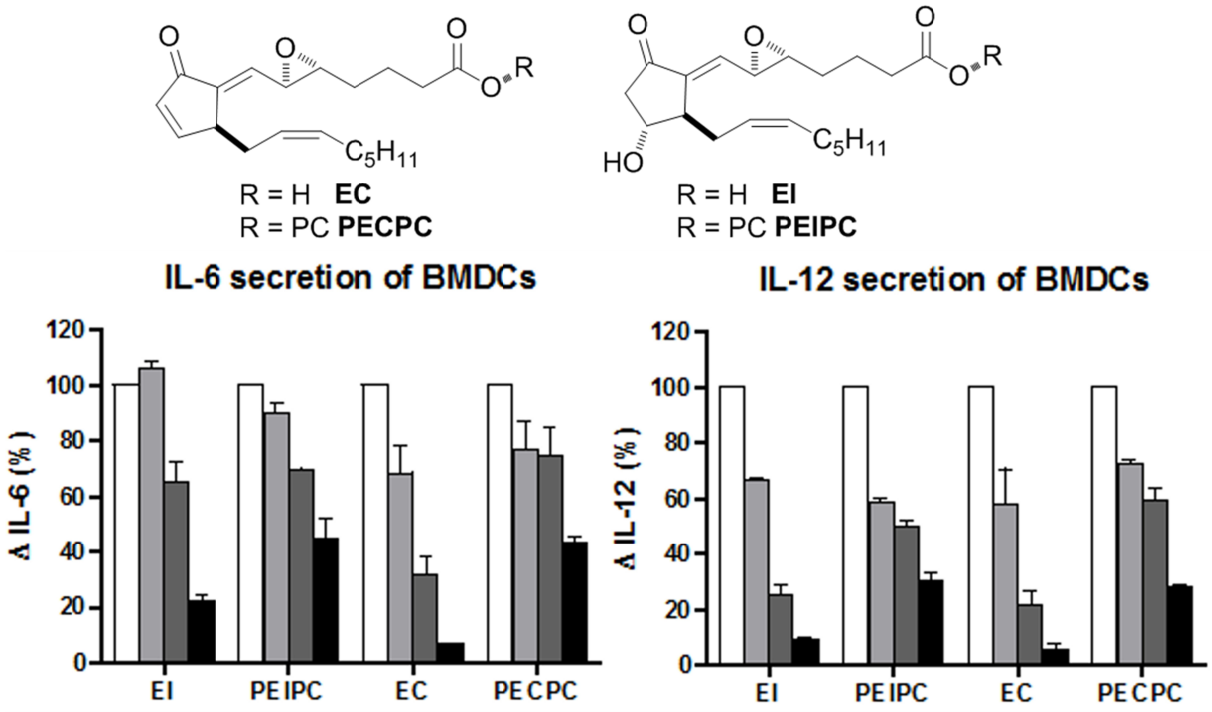
L27 **Douglas F. Covey**, Neurosteroid click chemistry for ligand-gated ion channels
Luis M. and Mary Fieser Memorial Lecture

New strategies for the synthesis of isoprostanoids and accompanying studies of their biology

Erick M. Carreira

ETH-Zürich; http://www.carreira.ethz.ch/

The talk will include discussion and analysis of recent isoprostanoid synthesis from our group and their analogs. The methods involve novel, unexpected reactivity and unusual building blocks that are fully integrated to lead to efficient routes. Studies of natural products present in humans highlight new opportunities for the study of human biology and the discovery of new therapies, which will also be a focal point of the presentation.



Engineering plant diterpenoid pathways in yeast: increasing yield and expanding product diversity

Alain Tissier

Leibniz-Institute of Plant Biochemistry, Department of Cell and Metabolic Biology, Weinberg 3, 06120 Halle (Saale)

We are using the yeast *Saccharomyces cerevisiae* as a platform for engineering the biosynthesis of plant diterpenoids. For this, we developed a set of yeast expression vectors for hierarchical modular cloning based on Golden Gate cloning which we call MoClo-Yeast [1]. A set of parts was established that are compatible with MoClo-Yeast, including yeast native promoters, synthetic promoters (inducible or constitutive), yeast native terminators and synthetic terminators. With MoClo-Yeast up to 6 transcription units can be assembled in just two cloning steps and over 15 in three cloning steps. MoClo-yeast is particularly well suited for combinatorial cloning. We have employed this for example to optimize the production of *cis*-abienol, a tobacco diterpenoid which can be used as a precursor for the synthesis of Ambrox. The highest titer in shake-flasks was over 1 g/l *cis*-abienol, boding well for further optimization in improved culture conditions.

Carnosic acid is a phenolic diterpenoid present in species of the Lamiaceae, including rosemary (*Rosmarinus officinalis*) and several sage species (*Salvia sp*.). Carnosic acid and its derivative carnosol are potent antioxidants which are used in the food and cosmetic industry as natural preservatives. With MoClo-Yeast, we were able to elucidate and reconstitute the biosynthesis of carnosic acid which comprises two diterpene synthases (diTPS) and two cytochrome P450 oxygenases (CYPs) [1]. By combining CYPs with the diTPS we could generate novel products and are now combining related diTPS which produce similar labdanoid diterpene skeletons with a set of CYPs from various plant species and that are known to act on labdane-diterpenes. Progress in the combinatorial engineering of plant labdanoid diterpenes will be presented.

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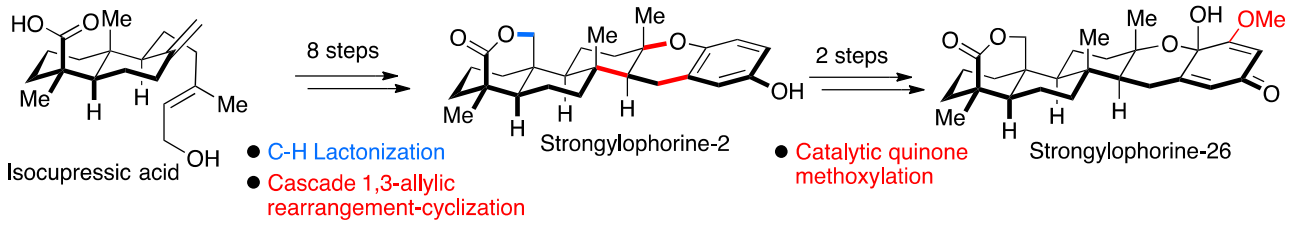
Synthesis of the strongylophorines

Thomas B. Poulsen

Department of Chemistry, Aarhus University, 8000-Aarhus C, Denmark

The strongylophorines is a family of complex marine meroterpenoids that display a series of interesting anti-cancer activities. Notably strongylophorine-26 (STR-26), one of the most structurally complex members of the family, exhibits strong cytotoxicity and ability to depolarize invasive breast cancer cells by a unique effect on the actin cytoskeleton. In my lecture, I will outline our recent efforts that has resulted in efficient syntheses of several STR-family members, including STR-26 [1, 2]. We have developed a semi-synthetic strategy starting from isocupressic acid, a highly abundant diterpene, that can be isolated in gram-scale from the bark and needles of the *Ponderosa* Pine tree. Several powerful synthetic operations, including a C-H lactonization under photoredox conditions and a new catalytic quinone methoxylation method, was developed as part of the synthetic work.



Overall synthetic flowchart from isocupressic acid to strongylophorine-26 with strongylophorine-2 as an intermediate.

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Omega-oxidized terpenoids: from traditional African medicine to novel insights into human vitamin physiology

Helmut Pein[1], Simona Pace[1], Veronika Temml[2,3], Ulrike Garscha[1], Birgit Waltenberger[2], Hermann Stuppner[2], Fiorentina Roviezzo[4], Jean-Jacques Helesbeux[5], Denis Séraphin[5], Alexander Mosig[6], Daniela Schuster[3], Antonietta Rossi[4], Pascal Richomme[5], Oliver Werz[1] and Andreas Koeberle[1*]

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[4] *Department of Pharmacy, School of Medicine, University of Naples Federico II, Naples, Italy*
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Garcinia kola is used in African ethno-medicine to treat inflammatory diseases but its mode of action is incompletely understood. We identified 5-lipoxygenase, a key enzyme in the biosynthesis of chemoattractant and vasoactive leukotrienes [1], as high-affinity target of the ω-carboxylated terpenoide δ-trans-tocotrienolic acid (garcinoic acid) from *Garcinia kola* nut extracts. Garcinoic acid potently inhibited 5-lipoxygenase (IC_{50} = 35 nM) in a reversible and substrate concentration-independent manner and selectively suppressed leukotriene formation by immune cells *in vitro* and *in vivo*. Molecular docking suggests that garcinoic acid allosterically targets an unexploited cavity of 5-lipoxygenase, which was confirmed by pull-down studies and site-directed mutagenesis. The critical pharmacophores of garcinoic acid were unraveled by screening an in-house library that encompasses 20 vitamin E metabolites with potential physiological relevance in humans as well as 55 semi-synthetic analogues [2-4]. One of the hit compounds, 13-((2*R*)-6-hydroxy-2,5,7,8-tetra-methylchroman-2-yl)-2,6,10-trimethyltridecanoic acid (α-T-13'-COOH), was biosynthesized from α-tocopherol in a human liver-on-chip and was detected in human and mouse plasma at concentrations (9-49 nM) that inhibited 5-lipoxygenase in human leukocytes [5]. This physiological metabolite of vitamin E accumulates at sites of inflammation and essentially contributes to the anti-inflammatory properties of vitamin E in mouse models of acute peritonitis and asthma through selective inhibition of 5-lipoxygenase. Further structural optimization of vitamin E metabolites yielded derivatives that inhibit leukotriene formation in human leukocytes with $IC_{50} \geq 19$ nM and show favorable efficacy and selectivity *in vitro* and *in vivo*. To sum up, we provide a rational for the anti-inflammatory activity of *Garcinia kola*, which contains ω-oxidized tocotrienols as potent, allosteric 5-lipoxygenase inhibitors. Moreover, our data suggest that the well-recognized immune functions and protection against inflammation by vitamin E critically depend on its metabolic conversion to the endogenous metabolite α-T-13'-COOH that inhibits 5-lipoxygenase as high-affinity target.

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Total synthesis of polycyclic natural products

Ang Li

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We have developed a number of strategies for the synthesis of polycyclic terpenoids. The 6π electrocyclization/aromatization strategy was exploited to construct the multisubstituted arenes of rubriflordilactones. The Prins cyclization strategy was responsible for the introduction of the tetrasubstituted olefins of epoxyeujindole A and 14-hydroxyaflavinine. The late-stage intermolecular Diels−Alder strategy was used to assemble the sterically congested domains of taiwaniadducts and hybridaphniphylline B.

Cannabinoquinoids: synthesis and biological profile

Orazio Taglialatela-Scafati[1], Eduardo Muñoz[2*], Giovanni Appendino[3*]

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[2] *Departement of Cell Biology, Maimonides Biomedical Research Institute of Córdoba, Reina Sofía University Hospital, Physiology and Immunology, University of Córdoba, Spain*
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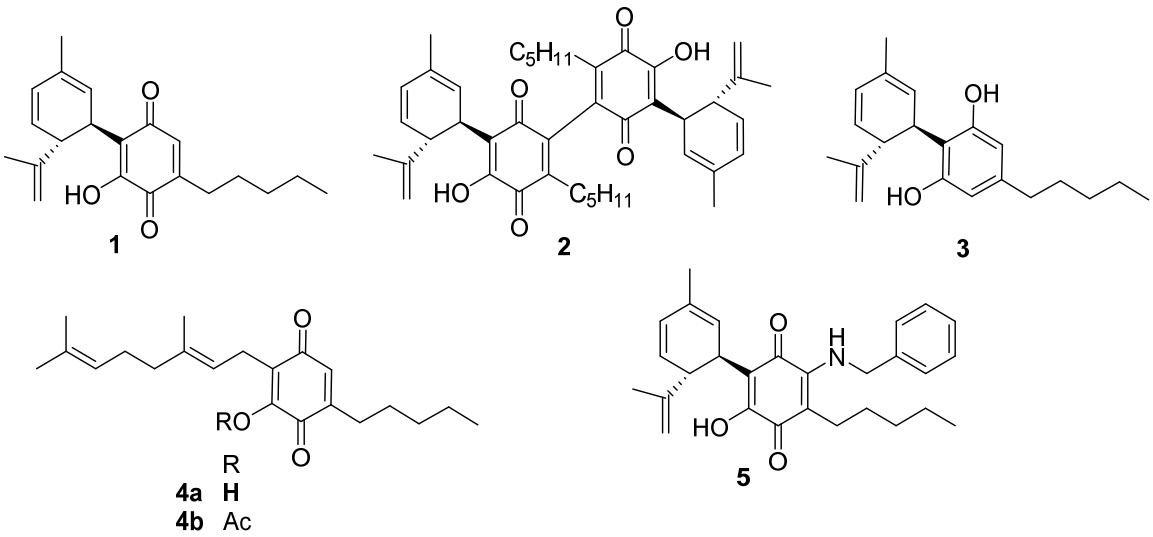
In 1911 it was serendipitously discovered that Cannabis flowers, leaves, and resin give an intense purple color when treated with ethanolic KOH [1]. The reaction has a remarkable specificity for Cannabis, and has been extensively used, as Beam test, in forensic chemistry [2]. The nature of the pigments formed in the reaction was clarified by Mechoulam in the Sixties, characterizing the highly colored quinone **1** and its dimer **2** from the reaction of cannabidiol (CBD, **3**) with KOH [3]. Interest in **1** was rekindled by the discovery of its remarkable selective cytotoxicity [4], while the quinone of cannabigerol (**4a**) and its acetate (**4b**) were reported from a high potency marijuana extract [5]. Despite promising initial results, no further development of **1** (HU-331) was reported, presumably because chemical instability precluded further, more advanced, studies.

Spurred by the discovery of a promising neuroprotective activity for cannabinoquinones, we have systematically investigated the oxidation of cannabinoids to their quinone forms, the mechanism of their degradation, and the effects of substituents on their stability. From these studies, the chemically stable aminoquinone **5** (VCE-004.8) emerged as a powerful immunomodulatory agent [6], currently endowed with orphan drug status in USA and EU for the management of sclerodemia.



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Phytosterols and steroidal human metabolites as targets for testing new synthetic strategies

Alaksiej L. Hurski*, Vladimir N. Zhabinskii, Vladimir A. Khripach

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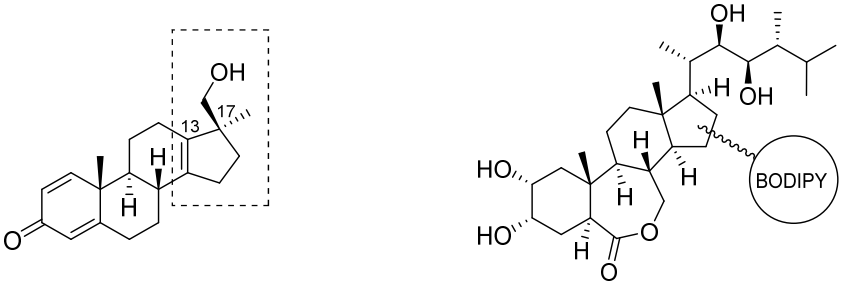
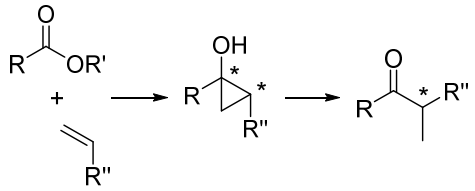
Steroids are isoprenoids, which play a vital role in regulating biological processes in living organisms. Research interests in our laboratory cover synthesis of natural steroids and development of new synthetic methods suitable for construction of their fragments. This talk will be focused on application of C-H activation and hydroxycyclopropanation strategies in synthesis of steroids. Using these methods, we have successfully prepared 17β-hydroxymethyl-17α-methyl-13-androstenes which are on demand in anti-doping analysis, conjugates of the plant growth hormone 24-epibrassinolide with dyes and other steroids. During the work on synthesis of the target metabolites and phytosterols, we have also developed new synthetic methods. We have found a new directing group that promotes palladium-catalyzed C-H functionalization with an unusual regioselectivity and proposed a new way to chiral α-methylketones. The latter developed approach was found to be useful for attachment of side chains to steroidal cores.

C-H activation strategy in synthesis of steroids:



Stereoselective synthesis and reactions of cyclopropanols:



-diastereoselective synthesis of cyclopropanols from alkenes and esters;
-ring-opening reactions of cyclopropanols;
-application of the cyclopropanation strategy to the synthesis of steroids

We thank Belarusian Republican Foundation for Fundamental Research (project x17-073) for the financial support.

New isoprenoid cytokinins exhibiting interesting biological activities

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Cytokinins are phytohormones identified originally as substances that promote plant cell division in the presence of another phytohormone, auxin [1, 2]. Regarding their chemical structure, cytokinins are adenine derivatives substituted at the N^6-position with either an isoprenoid or aromatic side chain. Isoprenoid cytokinins include *cis*- and *trans*-zeatin and their analogues with a saturated side chain (dihydrozeatin) or without a hydroxyl group (N^6-isopentenyladenine, iP). Cytokinins are phytohormones that regulate plant growth, development and senescence. Experiments both *in vitro* and *in vivo* demonstrated that they can also have diverse effects on animal cells and tissues. Particularly interesting is their ability to protect cells against various forms of stress and prevent some detrimental effects of cell aging. For example, human skin fibroblasts cultured in the presence of kinetin or *trans*-zeatin retain some characteristics of cells of lower passage. Kinetin is even able to increase the lifespan of invertebrates various components of the animal purinome. [3] The anti-proliferative activity of cytokinin ribosides (through induction of cell cycle block or/and cell death) and bases (through induction of cell differentiation) has prompted studies into their potential utility for the therapy of proliferative diseases like leukemias, cancers and psoriasis. Recent interest in cytotoxic anti-cancer ribosides has been stimulated by molecular studies of their mechanism of action, e.g., by microarray analyses and discovery of the high anticancer activity of BARs hydroxylated on the phenyl ring. [1, 2] Further, inhibitors of cyclin-dependent kinases olomoucine, bohemine, roscovitine, developed in our laboratory, were inspired by cytokinins (for a review see [4]). In this presentation, the protective effects of cytokinins in animals at molecular, cellular, tissue and organismal levels will be discussed. We will also discuss potential application of cytokinins for the treatment of age-related diseases, including neurodegenerations, inflammatory diseases and disorders caused by aberrant cell proliferation.

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New strategies to identify new chemical diversity for drug development

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It has been reported that natural products (NPs) have an excellent track record in the discovery of lead compounds to treat various human diseases [1]. A characteristic feature of natural products is their chemical diversity, which is created by a sequence of enzymatic reactions in the producing organisms. The phytochemical investigations of plants and fungi provided a continuous supply of the natural products of interest but with limited number of their derivatives [1]. Moreover synthetic chemists are applying rational approaches to the generation of lead-like libraries to achieve increased potency, broader biological activity and fewer side effects and thus, a number of clinically important NP-derived analogues have reached the market [1, 2].

We have isolated a number of natural products (viz., triterpenes, polyketides, and phenazines etc.) from fungi and plants which have interesting chemical diversity. Following the natural product inspired diversity oriented synthesis strategy [3], various derivatives of these bioactive natural products have been prepared with the objective to obtain compounds with greater anticancer and antimalarial activities. In addition to simple chemical modification, various monomers, homodimers and heterodimers of bioactive natural products were prepared in order to enhance the biological effects as well as to create a basis for Structure Activity Relationships (SARs). A detailed discussion about natural and synthetic chemical diversity and their role in drug discovery will be presented.

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Application of ionic liquids in the synthesis of steroid derivatives

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Ionic liquids (ILs) have attracted increasing interest in the last decades with a diversified range of applications. In organic synthesis ILs can be used not only as just alternative reaction media. They can act as solvents, as multifunctional compounds like solvents and ligands, solvents and catalysts, or stabilising agents for the catalysts or intermediates. However, in most cases the use of these IL assisted methods is restricted to the conversion of simple model compounds. In this lecture, some examples for the application of ionic liquids in the synthesis of steroid derivatives with potential pharmacological activity are presented. In all cases, a careful optimisation of the structure of the ILs was carried out to achieve optimal results. Beyond ensuring catalyst recycling, the application of ILs usually resulted in other advantages, such as the possibility to use milder reaction conditions or to achieve higher selectivity and sometimes even led to a surprising change in the outcome of the reaction.

Some topics to be included in the presentation are as follows.

Neutral or slightly acidic ILs were found to be efficient and recyclable catalysts for ring-opening of steroidal epoxides in the presence of amines and thiols. By this methodology, the need for the application of a high excess of the nucleophile as well as long reaction times could be eliminated during the synthesis of close analogues of neuromuscular blocking agents.

Acidic ILs were shown to catalyse an unusual Wagner-Meerwein rearrangement of 16α, 17α-androstanes and estranes resulting in the selective formation of an unnatural steroid core with a cis junction at rings C and D. Some derivatives bearing this steroid skeleton were found to decrease the activation of TRPV1 receptor, an important regulator of nociceptive and inflammatory processing.

16-Dehydropregnenolone derivatives with $C_{17,20}$-lyase inhibitory activity were obtained by aza-Michael reactions catalysed by basic ILs. Besides ensuring efficient recycling, the use of ILs again made it possible to decrease the amount of the reagent necessary for the success of the reaction.

In a base catalysed Claisen-Schmidt condensation of steroidal ketones, catalyst recycling was effected by converting the catalyst into an ionic liquid with the help of CO_2 and an alcohol. After extraction of the product, the IL was converted back to the molecular form, *i.e.* the base catalyst that could be recycled. The products were analogues of steroidal 17β-HSD1 and 17β-HSD2 inhibitors that can be used for the treatment of hormone dependent tumours.

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Radical-based approach for synthesis of complex natural products

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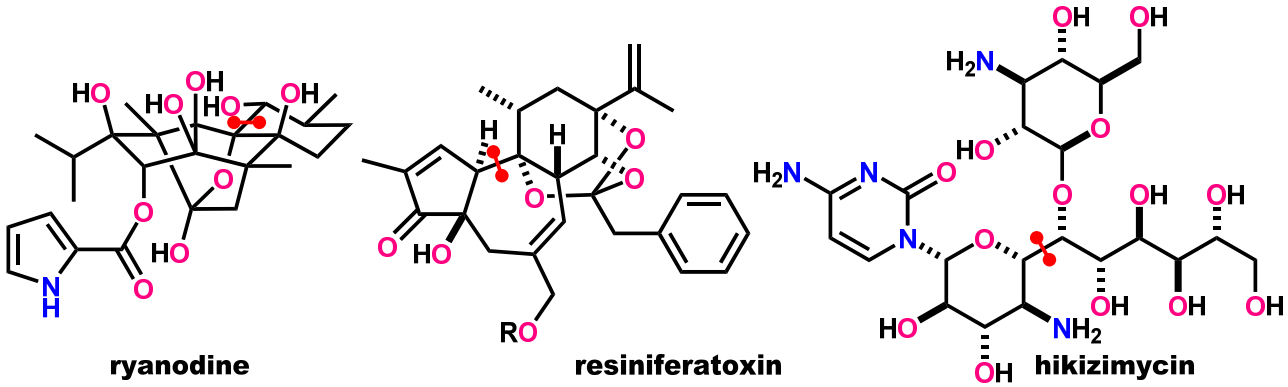
Natural products have been tremendously important in biology and human medicine because of their power to modulate signal transductions of biological system. Our research group focuses on efficient, practical and flexible syntheses of biologically important natural molecules with architecturally complex structures. At the core of this research program is the development of new strategies for assembling the complex natural products in a concise fashion. These synthetic developments would enable unified synthesis of new artificial analogs by modification of natural products templates.

Ryanodine (**1**), resiniferatoxin (**2**) and hikizimycin (**3**) are densely oxygenated natural products. [1-3] The synthetically challenging structures of **1-3** present an ideal platform to devise efficient strategies for building carboskeletons with multiple oxygenated carbons. We developed an α-alkoxy bridgehead radical methodology for construction of the hindered C(sp^3)-C(sp^3) bonds between tertiary alkoxy carbons and tertiary/quaternary carbons. [4] Furthermore, we reported efficient radical-radical homo- and heterocoupling reactions of α-alkoxy radicals, derived from the common pentose and hexoses. [3] In this presentation, we describe investigation of these transformations and the synthetic studies of 1-3 by applying the radical-based strategies.



Representative Target Molecules in Our Laboratory.

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Recent advances in brassinosteroid research

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Brassinosteroids (BS) actually recognized as an important class of plant hormones attract attention of investigators and specialists of different areas because of their multifunctional behavior not only as plant-growth regulating, stress-protecting and crop-increasing agents, but also as multipurpose tools for application in human and veterinary medicine, functional foods and bio-organic agro-preparations [1, 2]. All these became clearly seen in a relatively short time (next year we will celebrate the fortieth anniversary of the discovery of the first hormone of this group - brassinolide) because of the extremely rapid progress in brassinosteroid research. No one from the other phytohormones got such quick development from discovery to practice.

Although being quite well investigated in many respects, BS at the same time remain scarcely available and expensive natural compounds. The same is true for their modified derivatives, which are necessary very often for physiological and mechanistic studies, analytical needs, etc. Quite serious and mostly unsolved problems relate to a complex character of BS-bioactivity dependence from different conditions and very thin tuning the result by a combination of factors, changes in which can lead to opposite effects even for the same species. The third set of problems closely related to practical aspects of BS has their unusually low effective doses as a major reason. In fact, there are no other active ingredients neither in agriculture, nor among medications that act in such small doses as BS do. For some cases, this means that existing approaches cannot be applied for necessary BS-studies like it takes place, for example, in studies of their pharmacokinetics in humans. All mentioned above indicates the necessity of further development of synthetic, biological and analytical branches in brassinosteroid research to get them closer to real understanding the physiological machinery in plants and humans and to wide practical use.

Studies on synthetic approaches to BS were a field of activity with which our laboratory of steroid chemistry has been occupied for a number of years. They gave us a reliable access to all major representatives of natural BS, their derivatives, synthetic analogs and economically reasonable technologies of large-scale production for some of them that formed a basis of BS-use in agriculture. At the same time, namely well-developed synthetic direction in our research underlies extensive cooperation with physiological, bio-medical and agricultural scientists and specialists that makes possible getting new results in different areas via joining the efforts.

New data covering some synthetic, analytical, bio-medical and applied aspects of BS will be presented.

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Tuberculosinyl adenosine; an isoprenoid that makes *Mycobacterium tuberculosis* pathogenic

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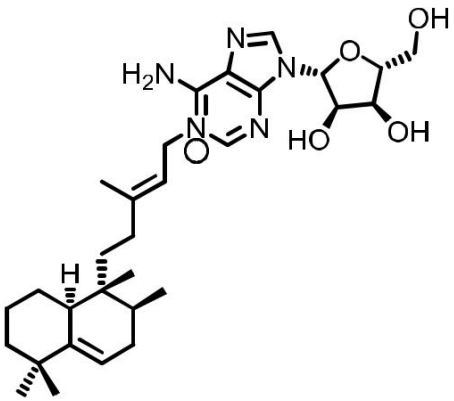
Tuberculosis bacteria are able to survive in late endosomes, a property that explains there "stealth behaviour" and is one of the major causes of their persistence [1]. Five years ago, a compound was isolated that is key to this survival [2]. The journey of the isolation, structure identification and total synthesis of this compound, tuberculosinol adenosine, will be presented. The compound turned out to be a chimaera of a diterpene with the rare halimane skeleton and adenosine. [3] As a strategy for the enantioselective synthesis of halimanes was lacking, it was developed making use of a demanding chiral auxiliary assisted Diels-Alder reaction. The compound is now readily available from synthesis and studies by groups in immunology world-wide. [4]



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This lecture is dedicated to the memory of my promoter Prof. Dr. Ae. De Groot.

Total synthesis of schilancitrilactones B, C and fluorine chemistry

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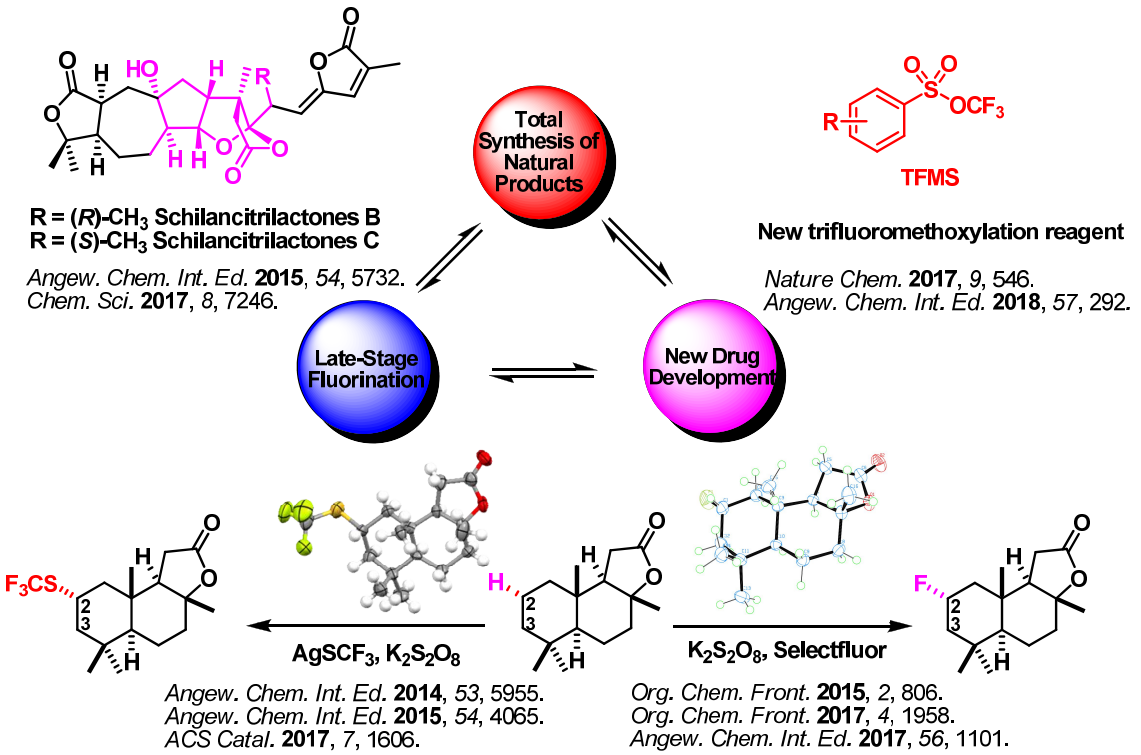
Schilancitrilactones B and C were isolated from the stems of Schisandra Lancifolia in 2012 by Sun and co-workers. Preliminary biological assays indicated schilancitrilactones C showed biological activities for inhibiting HIV-1 while schilancitrilactones B was not bioactive. We present the first total synthesis of schilancitrilactones B, C by employing an intramolecular radical cyclization, late-stage iodination and intermolecular radical addition as key steps in 17 steps (longest linear sequence) from commercial available materials. [1]

The growing importance of fluorinated organic compounds in pharmaceuticals, agrochemicals and materials has driven the development of new methods for the introduction of fluorine into small molecules. We developed the unactivated aliphatic C-H functionalization including fluorination and trifluoromethylthiolation through the radical mechanism, which can be applied to late-stage fluorination of complex small molecules. In addition, we reported a new trifluoromethoxylation reagent (TFMS), which was used to develop the new trifluoromethoxylation methods. [2-5]



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Design and synthesis of active and selective vitamin D analogs

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Increasing synthetic efforts have been directed toward the development of noncalcemic analogs of the natural hormone 1α,25-dihydroxyvitamin D_3 for treatment of specific disorders, but only a few of them have found clinical applications. [1, 2] Various classic synthetic 1,25D analogs have been shown to have anti-tumor activities. [2] Highly active 1,25D-analogs that exert less calcemic activity than the natural hormone by a non rapid metabolism and unknown mechanism have also been developed. The most notable structural features of these compounds include lack of the 19-methylene group, unsaturation at the side chain, unsaturation at **D**-ring, 14-epi-configuration, 3-epi-configuration, short nonhydroxylated side chains, and carboranic side chains.

Recently we described a flexible and efficient convergent synthetic approach for the preparation of vitamin D analogs. [3] This synthesis has been used to access the natural hormone 1,25D and a variety of vitamin D analogs modified at the side-chain, triene system, **C**-ring, and **A**-ring, that were required to establish structure-function relationships for the development of active ligands of the VDR with potential therapeutic value.

In a search for vitamin D analogs that are highly active in the circulation, but with low or negligible calcemic effects, we will describe a modification of the reported synthetic approach for rapid and economic access to active and selective novel vitamin D analogs and present their biological activities.

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Novel 19-norvitamin D compounds with elongated substituents at C-2 or C-26

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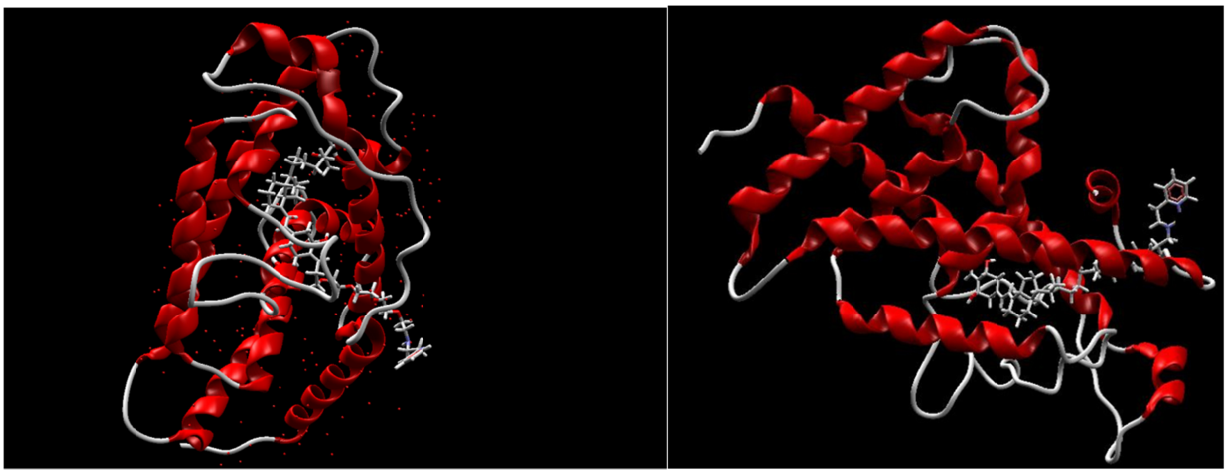
As a continuation of our structure-activity studies of vitamin D compounds, the synthesis of new analogs of 19-nor-1α,25-$(OH)_2D_3$ (19-norcalcitriol) is presented. Calcitriol analogs lacking the exomethylene moiety at C-10 are well recognized as potential drug candidates due to their lower calcemic activity and increased stability [1]. The target 19-norvitamins, characterized by a presence of a long nitrogen-containing substituents attached to C-2 or C-26, have been designed on the basis of molecular modeling and docking experiments. The terminal moieties of these substituents are expected to protrude from the vitamin D receptor (VDR) binding pocket [2] and thus could form complexes with metal cations.



View of the three-dimensional structure of ligand binding cavity of the human VDR with the docked 19-norvitamin D analogs.

The convergent synthesis of 19-norcalcitriol compounds, described in this presentation, consisted of preparation and subsequent combining of appropriate building blocks. In the case of 2-substituted analogs, the crucial part of the synthesis involved preparation of the desired cyclohexanone A-ring fragment with an elongated alkylidene substituents. For 26-substituted compounds, the synthesis of the appropriate Grundmann ketones with the required side chain modifications was the major challenge. Then, the obtained synthons were coupled with the corresponding C/D-ring sulfone [3] or A-ring phosphine oxide [4], respectively. Next synthetic steps consisted of further elaboration of the substituents, and the final hydroxyl deprotection. Binding affinity of the synthesized 19-norcalcitriol analogs to the VDR was also assessed.

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Recent results in pheromone synthesis

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(A) Synthesis of the racemate and the enantiomers of (*anti*-1,2-dimethyl-3-methylenecyclopentyl)acetaldehyde, the female sex pheromone of the pineapple mealybug, *Dysmicoccus brevipes* [1, 2]. Chirality was introduced by means of lipase-catalyzed asymmetric acetylation of (±)-2,3-dimethyl-2-cyclopenten-1-ol. The absolute configuration of a synthetic intermediate was established by X-ray analysis. The natural pheromone was identified with the (1*S*,2*S*)-aldehyde by comparing the specific rotation, enantioselective GC retention time and pheromone activity.

(B) Synthesis of the racemate and the enantiomers of (*E*)-*cis*-6,7-epoxy-2-nonenal, the male pheromone of the red-necked longhorn beetle, *Aromia bungii* [3]. The pheromone epoxides were synthesized by olefin cross metathesis between crotonaldehyde and (±)-, (+)- and (-)-*cis*-3,4-epoxy-7-octene, which was prepared by the Grignard coupling between allylmagnesium bromide and (±)-, (+)- and (-)-*cis*-2,3-epoxypentyl triflate. The epoxide enantiomers were synthesized by the Sharpless asymmetric epoxidation of (*Z*)-2-penten-1-ol.

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Uncommon aglycones of sea cucumber triterpene glycosides. Structure, biosynthesis, evolution

Vladimir I. Kalinin*, Aleksandra S. Silchenko, Sergey A. Avilov and Valentin A. Stonik

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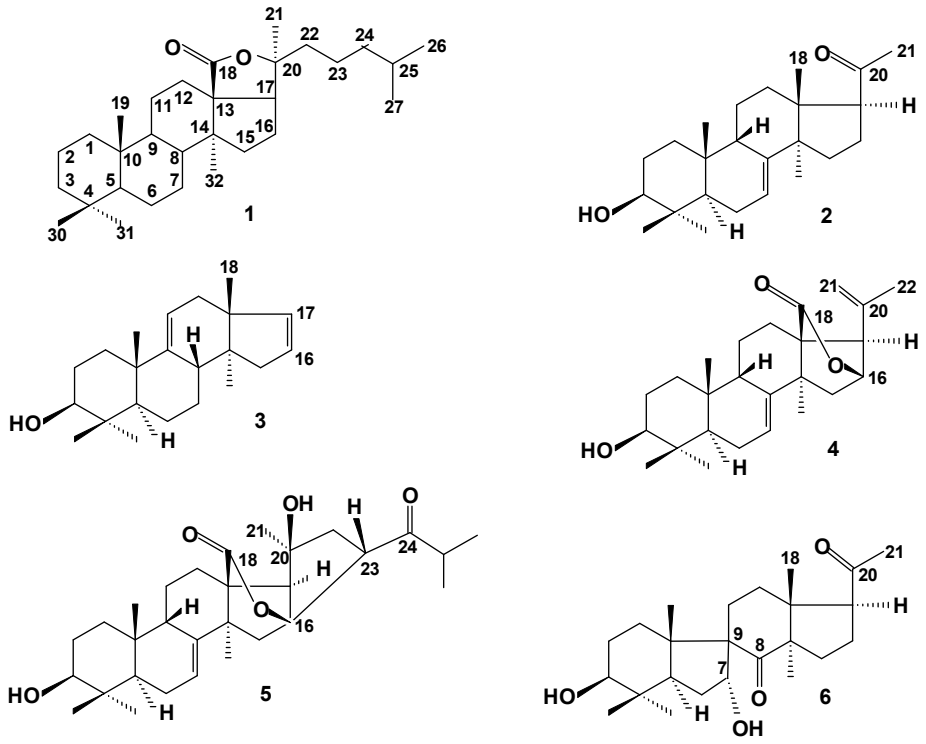
Triterpene glycosides are characteristic metabolites for sea cucumbers (Holothurioidea, Echinodermata). All of them possess lanostane derivatives as aglycones and the most of them has 18(20)-lactone i.e. also belongs to so called holostane series. Nevertheless there are so called non-holostane aglycones i.e. lanostane derivatives without lactone or having 18(16)-lactone (with normal or shortened side chain).



1 – holostane – a model lanostane derivative having 18(20)-lactone basal for the most of sea cucumber triterpene glycosides; **2** – example of 22,23,24,25,26,27-hexanorlanostane aglycone without a lactone; **3** – example of 20,21,22,23,24,25,26,27-octanorlanostane aglycone without a lactone; **4** – example of 23,24,25,26,27-pentanorlanostane aglycone with 18(16)-lactone; **5**, **6** – examples of aglycones with lanostane rearranged carbocyclic system.

There are also very rare examples of non-holostane aglycones having rearranged lanostane skeletons as products of intramolecular aldol condensation of 1,6-diketo (16,24-diketo) precursors or Meinwald rearangement of 1,2-epoxy precursor or pinacol-pinacolone rearrangement of fully substituted 1,2-diol (7,8-epoxy or diol) precursor. The structure, biosynthesis and evolution of non-holostane aglycones of sea cucumber triterpene glycosides and biological activities of the glycosides having such aglycones are discussed.

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Total synthesis of echinoside A, a representative triterpene glycoside of sea cucumbers

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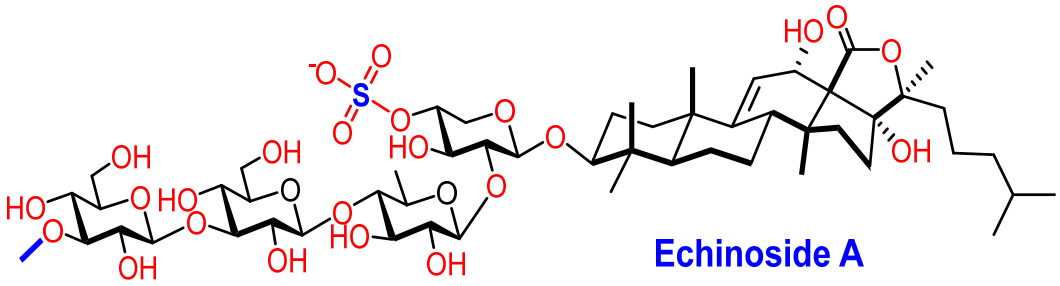
Echinoside A represents a typical triterpene glycoside of sea cucumbers. In fact, hundreds of its congeners have been characterized, and the majority bear such a lanostane-18(20)-lactone (namely holostane) as aglycone and over half embed with the $\Delta^{9,11}$ double bond and 12α hydroxy group. The glycans of these molecules are attached exclusively at the lanostane-3-OH, which start mostly with D-xylose (Xyl) and is elongated via its 2-OH with D-quinovose (Qui) and D-glucose (Glc); the sulphonyl residue at 4-OH of the Xyl unit and the methyl substituent at 3-OH of the terminal Glc unit are also common features. These characteristic metabolites of the slow-moving sea cucumbers are produced to defense against their predators and parasites, therefore are not surprised to show a variety of pharmacological activities.

Herein, we account the synthesis of echinoside A, that represents the first synthesis of a triterpene glycoside of sea cucumbers. The synthesis starts with readily available materials (i.e., lanostanol, glucose, and xylose) with a longest 35 linear operations (in a total of 63 steps). This synthetic approach is easily adaptable to the synthesis of congeners and analogs, thus shall facilitate in-depth studies on the promising biological effects of the sea cucumber saponins.



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Metabolic profiling based discovery of new isoprenoids, mode of action and (bio-)synthesis of derivatives

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Most plants contain functionally interesting isoprenoids. We will present some discoveries from our latest findings, e.g. on sweet tasting or neuroactive compounds. [1]

But how can the biologically or chemically interesting isoprenoids be identified and separated better from the rest of mostly known or irrelevant metabolites without either separating all compounds, or following lengthy bioassay-guided isolation procedures? We will show, how metabolomics and reverse metabolomics will allow the selection of interesting species and the discovery of new (mero-)terpenoids with separation of complex mixtures like plant extracts. [2] As example the prenylated compounds from *Hypericum spec.* will be used.

Following the discovery phase, synthetic efforts to the natural products and especially their derivatives are of our interest. For this, medicinal chemistry methods are used. Starting with ligand and receptor modelling and interaction studies, we continue with the synthesis of derivatives. Depending on the problem, this may involve classical chemical synthesis or biosynthetic steps based on biosynthetic pathway knowledge.

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Synthesis and some properties of symmetrical, unsymmetrical and hybrid steroid dimers

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Steroids are a family of lipophilic compounds that are widespread in nature. Their intrinsic biological activity has kept these compounds in the focus of attention for more than a century.

The subfamily of steroid dimers added the first members when a few compounds were isolated from nature or occurred as by-products in various reactions. The interesting chemical, biological and physical properties of the increasing number of steroid dimers isolated from living organisms or obtained by chemical synthesis has prompted intensive activity in this developing field [1].

We have recently initiated a project directed to prepare steroid dimers and to explore properties like chemical reactivity, crystal structure, cytotoxic activity and fluorescence. This presentation will summarize the methodologies that we have followed or developed for the synthesis of symmetrical, unsymmetrical and hybrid steroid dimers.

SUMMARY

Acid catalyzed dimerizations

- Aldol condensations (Lewis catalysis)
- Double ketalizations (Brønsted–Lowry catalysis)

Dimerization via esterification

- Terephthalates
- Isophthalates

Pd-catalyzed dimerizations

- Alkyne homocoupling
- Sonogashira coupling
- Suzuki-Miyaura coupling
- Pd-catalyzed spiroketalization
- Multicomponent reactions

Gold-catalyzed di-spiroketalization

The relevant details of NMR and X-ray characterization and some of the properties of the obtained dimers will be briefly discussed.

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Steroidal dimers, macrocycles and conjugates based on bile acid scaffolds

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Dedicated to the memory of Professor Marian Kocór

Steroids have been used as building blocks for designing and construction of molecular receptors which serve for recognition of guest molecules of diverse chemical nature [1]. Among them, the dimeric steroids – mainly derivatives of bile acids – have received special attention [2]. The cleft type receptors which are head-to-tail, head-to-head and tail-to-tail dimers of bile acids have been reported. The dimers show interesting properties [3] and are potential substrates for the preparation of macrocyclic cholaphanes [4].

Organic carbamates are compounds of various important biological and medicinal properties and have been used, among others, as pharmaceuticals, intermediates in organic synthesis and as linkers in supramolecular and combinatorial chemistry [5].

All three hydroxy groups of bile acid esters may be transformed to the stable, albeit very reactive, 3-, 7- and 12-chlorocarbonyl derivatives [6]. Thus, the steroidal 3-*O*-chlorocarbonyl derivatives reacted with *N,N'*-dinucleophiles (e.g. ethylene- or phenylenediamine) to give bis-carbamate bridged dimeric bile acid esters of the molecular cleft-type [4]. Similarly, the reaction with isomeric dihydroxybenzenes afforded the respective bis-carbonates. Depending on the structure of the dinucleophile, 3.3'-dimers of variable length, polarity and predicted geometry of the spacer group were synthesized. The macrocyclization of the selected dimers has also been studied. In our approach to cholaphanes, the cyclization step utilized bis-amide bond formation or ring-closing metathesis (RCM) of the dimers comprising two terminal *N*-allyl amide groups. The RCM reaction (Grubbs I catalyst) afforded a series of cholaphanes, predominantly, as *E*-isomers.

In another investigation, a series of a new type amphiphilic dimers derived from deoxycholic and cholic acid esters having the 12,12'-bis-carbamoyloxa-bridge have been synthesized from the 12-*O*-chlorocarbonyl derivatives. Furthermore, some novel bile acid – α-aminoacid conjugates and molecular tweezers with the carbamate linkage have been prepared in the reaction of chlorocarbonyl derivatives of bile acid esters and α-aminoacid methyl esters.

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Tracing terpenes with isotopes

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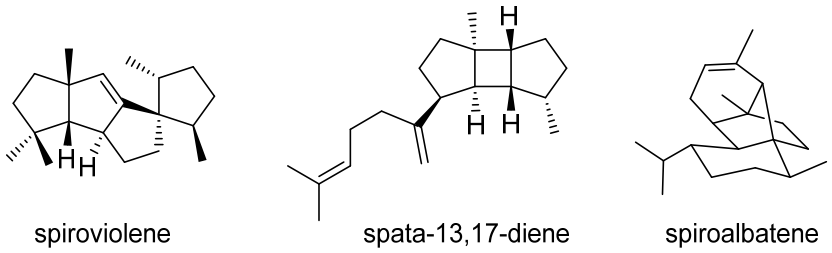
The recent advances in genome sequencing have revealed a large number of terpene cyclases in bacteria [1], fungi [2], and eukaryotic microorgansism such as social amoebae [3]. Several of the encoded enzymes have been characterised during the past two decades by genome mining approaches. The terpene cyclase catalysed reactions frequently yield only one specific product with a high degree of stereocontrol. The complex mechanisms of terpene cyclisations can be addressed by quantum chemical calculations [4], or experimentally by the use of isotopically labelled probes. During the past few years my group has synthesised various isotopically labelled oligoprenyl diphosphates that can be used to efficiently unravel the cyclisation mechanisms of terpene cyclases [5]. Their application in mechanistic investigations on the most interesting newly discovered terpene cyclases, making the products shown below, will be discussed.



Recently identified products of bacterial diterpene cyclases.

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Convergent synthesis of vitamin K2 (MK-7)

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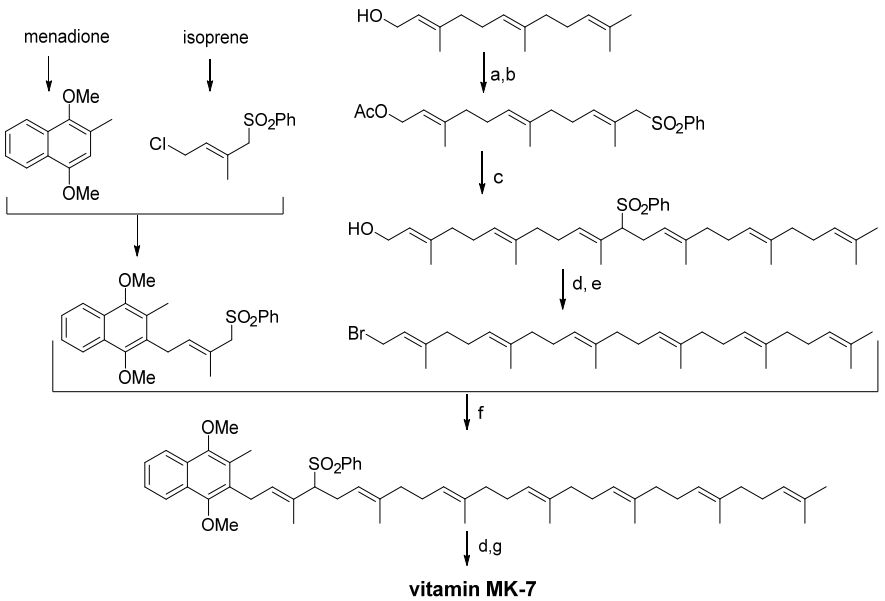
Vitamin K is the collective term for compounds: phylloquinone or vitamin K1 (2-methyl-3 phytyl-1,4-naphthoquinone) and menaquinones (MK-n) or vitamin K2 (polyisoprenylquinones, several species).

Vitamin K plays an essential role in many biological processes including blood coagulation, maintenance of bone health, inhibition of arterial calcification, and cell growth regulation [1].

Vitamin K, particularly MK-7, is currently enjoying a genuine boom in the health products branch. Nevertheless, the concentration of MK-7 in food products is too low for therapeutic applications. The digestion and utilization of MK-7 from these sources in human bodies becomes less efficient as aging occurs [2]. MK-7 shows a rapid increase of serum levels after administration, and the estimated half-life time is three days. All of these are showing the need of industrial production of supplementary MK-7.

An efficient convergent synthesis of menaquinone-7 was designed according to the following scheme:



Reagents and conditions: a) 1. SeO_2, *t*-BuOOH, salicylic acid; 2. $NaBH_4$ b) 1. PBr_3; 2. $PhSO_2Na$; c) 1. *t*-BuOK; 2. farnesyl bromide; d) $LiEt_3BH$, $Pd(dppe)Cl_2$; e) PBr_3; f) *t*-BuOK; g) CAN

Stereoselective synthesis of MK-7 was achieved through "1 + 6" convergent strategy by condensation of menadione monoprenylated derivative (fragment "1") with hexaprenyl bromide (fragment "6"). The high-purity (99,9%) product was obtained, which can be used as a dietary supplement as well as an active pharmaceutical ingredient.

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Convergent total synthesis and biological studies of cardiotonic steroid

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Cardiotonic steroids have many centuries-long history of serving as effective drugs for the treatment of various heart conditions. Many recent studies suggest that these compounds hold great potential as the therapeutic agents for the treatment of cancer, inflammation, immune and metabolic diseases, and in various important health-related areas such as contraception. The development of flexible synthetic routes enabling the preparation of various members of cardenolide family is highly desirable as it may greatly enhance the medicinal exploration of these compounds. This presentation will describe the application of stereoselective Michael/Aldol cascade reactions to the convergent total synthesis of oxygenated cardenolides including ouabagenin, sarmentiologenin, 19-hydroxysarmentogenin and cannogenol. This presentation will also describe new strategies for the site-selective glycosylation of polyhydroxylated cardenolides and biological evaluation of glycosylated and non-glycosylated steroid derivatives.

Plasticity of the isoprenoid moiety in phytocannabinoids

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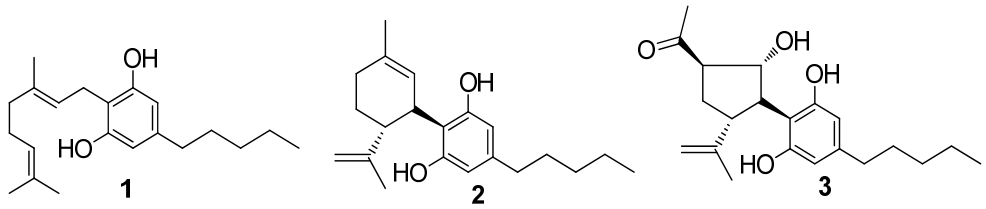
Phytocannabinoids are a diverse group of isoprenylated resorcinyl polyketides produced mainly, but not exclusively, by *Cannabis sativa* L (Cannabaceae). The amazing diversity of cannabis phytocannabinoids (about 140 members have been recently listed [1]) can be largely ascribed to the plasticity of their isoprenoid moiety. Indeed, while this moiety is almost invariably monoterpenoidic (the single C_{15} derivative is sesqui-CBG [2]), the further enzymatic and non-enzymatic transformations of a set of major constituents (CBG (**1**), CBD (**2**), THC and CBC, the so called "big four") generate the greatest diversity. For example cannabimovone (CBM, **3**), one of the latest additions to this family [3, 4], is formally the result of the oxidative fragmentation of the endocyclic bond of CBD followed by intramolecular aldolization.



Chemical structures of CBG (**1**), CBD (**2**) and CBM (**3**).

The rich chemical diversity of natural phytocannabinoids can be further expanded with chemical manoeuvres, that has the potential to discover new reaction pathways and identify biogenetically overlooked relationships [5]. Inspired by the growing evidence that iodine, either alone or in combination with oxidants or phosphines, can trigger interesting reactions of polyolefins, we have discovered that simple treatment with iodine can remarkably expand the structural diversity of phytocannabinoids, with a different outcome on acyclic (e.g. CBG), *p*-menthane- (e.g. CBD), and chromene-type (e.g. CBC) phytocannabinoids. These transformations will be discussed in the light of their general mechanistic relevance and of the biological potential of their reaction products.

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Neurosteroid click chemistry for ligand-gated ion channels

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The endogeneous neurosteroids allopregnanolone and pregnenolone sulfate are positive allosteric modulators (PAMs) of γ-aminobutyric acid type-A (GABA-A) and NMDA type glutamate receptors, respectively. Analogues of both of these compounds are of therapeutic interest for the treatment of clinical depression and other medical conditions. Identification of amino acids at putative binding sites for these PAMs on their respective receptors has been achieved using molecular biology methods. However, much remains to be learned about the molecular details of neurosteroid interactions with the sites thus far identified. Such information is useful for the design of new drugs that act at these sites. In this regard, photoaffinity labeling of the sites by neurosteroid analogues provides information about the location, orientation and number of binding sites for neurosteroids on these receptors. Photoaffinity labeling when combined with click chemistry (click photolabels) provides an enhanced ability to obtain this information using mass spectrometry and molecular modeling. Click photolabels also can be useful for identifying binding sites that may not yet have been detected by site-directed mutagenesis studies. Additionally, click photolabels can be used to study the distribution of neurosteroids in cells and to identify other proteins that bind neurosteroids thereby affecting their pharmacological actions. This presentation will discuss results obtained with neurosteroid click photolabels for GABA-A and NMDA receptors.

Louis and Mary Fieser were distinguished chemists and chemical educators. Their books *"Natural Products Related to Phenanthrene"* and *"Steroids"* were the foremost reference books for information on steroid chemistry during the 1950s and 1960s when the pharmaceutical industry developed many steroid drugs. Before the advent of online chemistry databases, their book series "*Reagents for Organic Synthesis*" was widely used and acknowledged to be the most convenient source of information on reagents used for organic synthesis.

This work was supported by NIH grants GM108799, MH110550 and The Taylor Institute for Innovative Psychiatric Research.

Branicki Palace, also known as the "Polish Versailles", Białystok

View of Białystok with Branicki Palace

ORAL COMMUNICATIONS

LIST OF ORAL COMMUNICATIONS

O1 **Yulia A. Volkova**, A strategic development toward functionalized extranuclear heterosteroids diversity

O2 **Piotr Przybylski**, Basket-like lactam macrolides – modifications and structure – activity relationship studies

O3 **Vladimir Zhabinskii**, New azole derivatives of [17(20)*E*]-21-norpregnene: synthesis and inhibition of prostate carcinoma cell growth

O4 **Yury V. Kuznetsov**, New steroidal estrogen receptor modulators. Design, synthesis, and biological evaluation

O5 **Oldřich Lapčík**, Immunoanalysis of anabolic steroids

O6 **Mihalj Poša**, Peculiar self association of sodium isoursodeoxycholate in water: H-bonds in the primary micelles

O7 **Dmitry Dovbnya**, Efficient production of hexahydroindanone derivatives by bioconversion of phytosterol with mutants of *Mycobacterium smegmatis*

O8 **Kinga Kuczyńska**, Transformation of betulin core

O9 **Timofey V. Malyarenko**, Unusual starfish steroidal glycosides: structure and biological activity

O10 **Volodymyr S. Kravets**, Brassinosteroids in regulation of plant metabolism

O11 **Barbara Bednarczyk-Cwynar**, Current approach in synthesis of triterpenoid dimers

O12 **Sergazy M. Adekenov**, Bimolecular compounds based on the natural sesquiterpene lactones

O13 **Jacek Ścianowski**, New benzisoselenazol-3(2*H*)-ones *N*-functionalized with terpene skeletons

O14 **Michał Michalak**, NHCCuX-catalyzed Friedländer-type annulation of fluorinated aminophenones with alkynes on water. Unexpected dibenzodiazocine formation

O15 **Tomáš Zimmermann**, Targeting phorbol derivatives toward cancers

O16 **Sergey M. Khomutov**, The effect of cyclodextrins on oxidation of DHEA by fungal laccase

O17 **Eva Kudova**, S.M.A.R.T. Steroids: Steroidal Molecules As Rapid-acting Therapeutics

O18 **Shingo Nagano**, Crystal structures of an indole prenyltransferase, IptA: implications for substrate tolerance and its expansion

O19 **Izabella Jastrzębska**, New selenoorganic reagents in steroid chemistry

A strategic development toward functionalized extranuclear heterosteroids diversity

Yulia A. Volkova[1*], Alexander M. Scherbakov[2], Elena I. Chernoburova[1], Igor V. Zavarzin[1]

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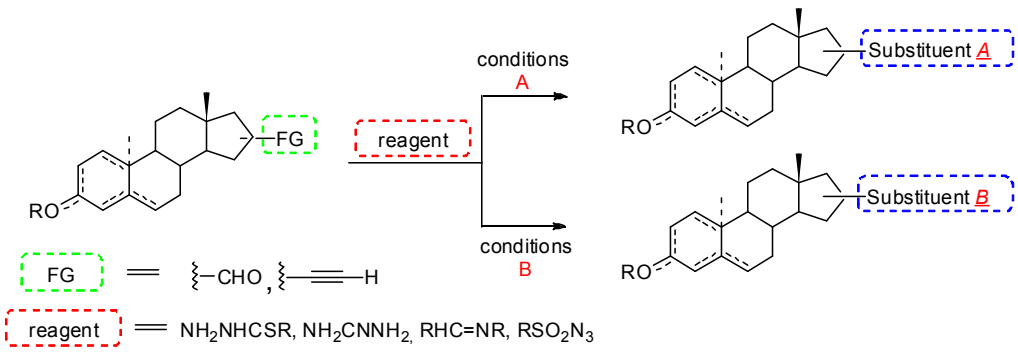
Extranuclear heterosteroids, over the years, have been considered to be privileged scaffold for drug discovery due to their outstanding biological activity, which is especially true for A- and D-ring modified steroids [1]. Thus, synthetic azasteroids encompass a wide range of compounds with various biological activities, e.g., reductase inhibitors such as finasteride, the high-affinity agonist ligands for the glucocorticoid receptor e.g., cortivazol, GnRH agonists such as danazol, aromatase inhibitors such as formestane and exemestane and neuromuscular junction blocking agents such as pipecuronium.

This study was focused on search of facile flexible strategies, in which a common intermediates could be used in a conjunctive fashion to form an array of structurally diverse *N*-heterocycles attached or fused to a steroid core [2]. In this regard we studied thiohydrazides as simple "versatile agents" for the modification of steroids bearing a carbonyl group. Namely, a flexible approach to unknown pyrazole, 1,3,4-thiadiazole, thiadiazine, and pyridazine derivatives of steroids with selective control of heterocyclization patterns was disclosed [2]. Alternatively syntheses of steroidal propargylamines, imidazo[1,2-a]pyridines, 1,2,3-triazoles, *N*-sulfonyl imidates were achieved from ethynyl steroids using copper catalysis. The synthesized compounds were screened for inhibition activities in different cancer cells and were found to be highly promising for development of anticancer agents, in particular, against breast and prostate cancers.



Flexible strategies for the synthesis of heterosteroids.

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Basket-like lactam macrolides – modifications and structure – activity relationship studies

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Ansamycin macrolides as e.g. geldanamycin and rifamycins (3-formylrifamycin SV) are characterized by unique basket-like structure which enable them to overcome the natural barriers like lipid bilayers and to dock at different target sites of action in cells (**Fig. 1**). Rifamycins, such as rifampicin, are typical antibacterial ansamycins due to allosteric inhibition of RNA polymerase activity from different bacteria strains [1], whereas geldanamycin is an example of anticancer ansamycin blocking the action of chaperones (Hsp60, Hsp70, Hsp90 etc.) by the formation of complexes, stabilized via hydrogen bonds at ADP/ATP-binding pocket [2]. In our studies we have performed chemical modifications ansamycins using nucleophilic substitution, reductive amination and Huisgen dipolar cycloaddition (CuAAC) and structural analysis to obtain semisynthetic antibiotics of well–balanced water solubility and lipophilicity, improved mode of binding to the target site of action and better biological potency [3-6].

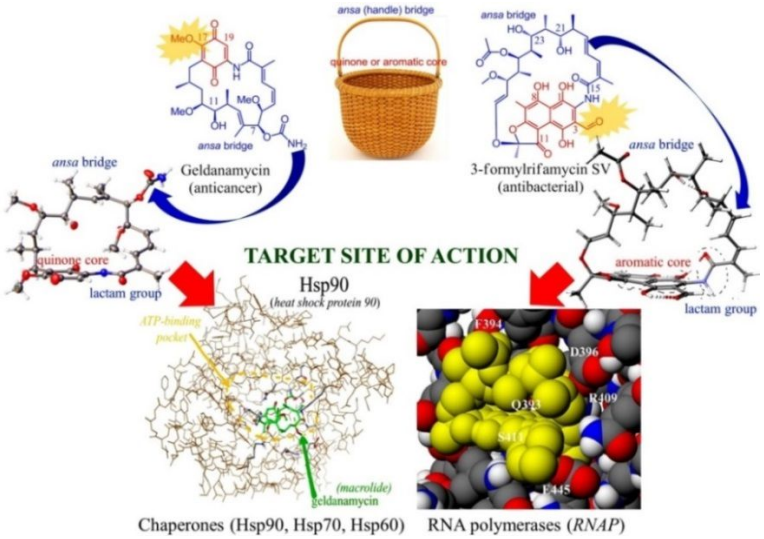

Fig. 1 Structure, biological targets and modification regions (marked by orange) of the two types ansamycin antibiotics.

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New azole derivatives of [17(20)*E*]-21-norpregnene: synthesis and inhibition of prostate carcinoma cell growth

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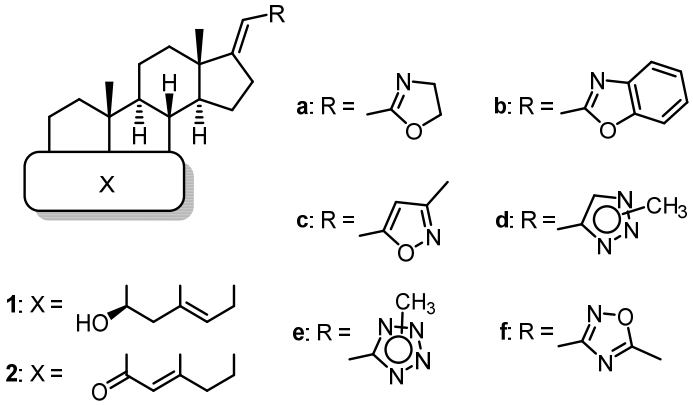
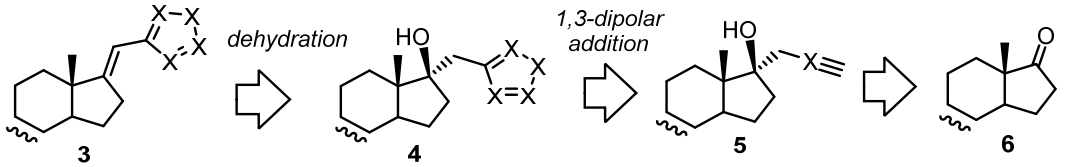
The common structural feature of steroid-based drugs for prostate cancer is the presence of an azole moiety (including pyridine, imidazole, pyrazole, isoxazole, triazole and tetrazole heterocycles) directly linked to C17 of the tetracyclic skeleton. Less studied are homologous [17(20)*E*]-21-norpregnene steroids. Recently, it was shown that oxazolinyl derivatives **1a** and **2a** were able to suppress catalytic activity of CYP17A1 and to inhibit growth of prostate carcinoma LNCaP and PC-3 cells [1-3]. Speculating that biological activity of [17(20)*E*]-21-norpregnene derivatives modified with other nitrogen containing heterocycles also may be of interest and importance, in this work we have synthesized new steroids **1c-f** and **2c-f,** comprising isoxazole, 1,2,3-triazole, tetrazole, and 1,2,4-oxadiazole substituents, and evaluated their potency to inhibit CYP17A1 and to suppress prostate carcinoma LNCaP and PC-3 cell growth.



The key steps for the synthesis of isoxazoles, 1,2,3-triazoles, and tetrazoles were (i) 1,3-dipolar cycloaddition of nitrile oxides or azides to acetylenes or nitriles **5** and (ii) dehydration of 17β-hydroxy-17α-methylene-azoles **4** to [17(20)*E*]-21-norpregnene derivatives **3**. 1,2,4-Oxadiazoles were prepared through the formation of acetimidamides.



Among the new azole derivatives, four compounds were found possessing high anti-proliferative activity.

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New steroidal estrogen receptor modulators. Design, synthesis, and biological evaluation

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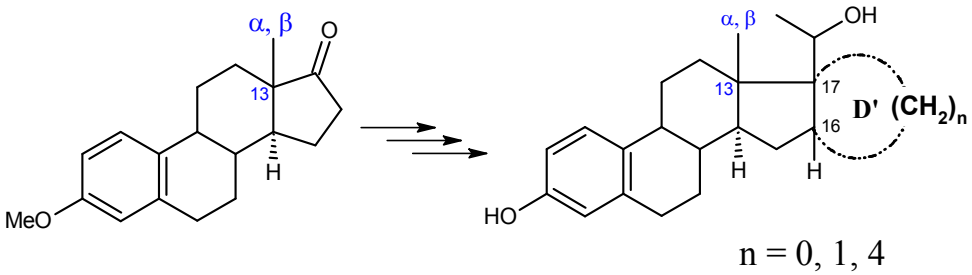
Researches in the field of estrogen receptor modulators for the treatment of hormone-dependent breast cancer remain the actual strategy, in terms of which the development of compounds inhibiting improper effects of estrogens but devoid of intrinsic estrogenic activity is very promising.

Further to our work on pentacyclic steroids [1] we synthesized previously unknown steroid compounds that combine in its structure an aromatic ring A and additional three- and six-membered ring D' at the 16α,17α-positions of natural [2] and distorted 13-episteroid core, and examined *in vitro* their cytotoxic and ERα transcriptional activities.



All target compounds exhibited significant cytotoxic effect against estrogen-dependent breast cancer cells and had low toxicity in the MCF-10A normal epithelial cells. The ability of the 13-episteroid compounds to inhibit the growth of NCI/ADR-RES doxorubicin-resistant cells was also demonstrated. All target compounds suppressed the estradiol-induced ERα transcriptional activity in MCF-7 cells with the exception of highly cytotoxic 16α,17α-cyclopropane derivative of 13-epi-series which proved to be an ERα activator.

Docking studies using AutoDock Vina were performed to evaluate possible differences of receptor binding modes causing different transcriptional activity. The combination of flexible and rigid docking models was found to be appropriate way for the primary assess of the potential ligand-receptor interactions in the case of previously unknown structures.

This report provides the examples of syntheses, distinctive properties of the intermediate 13α/β-compounds, biological effects (cytotoxicity and gene reporter assay data), and molecular modeling of the resulted modulators of estrogen receptor α.

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Immunoanalysis of anabolic steroids

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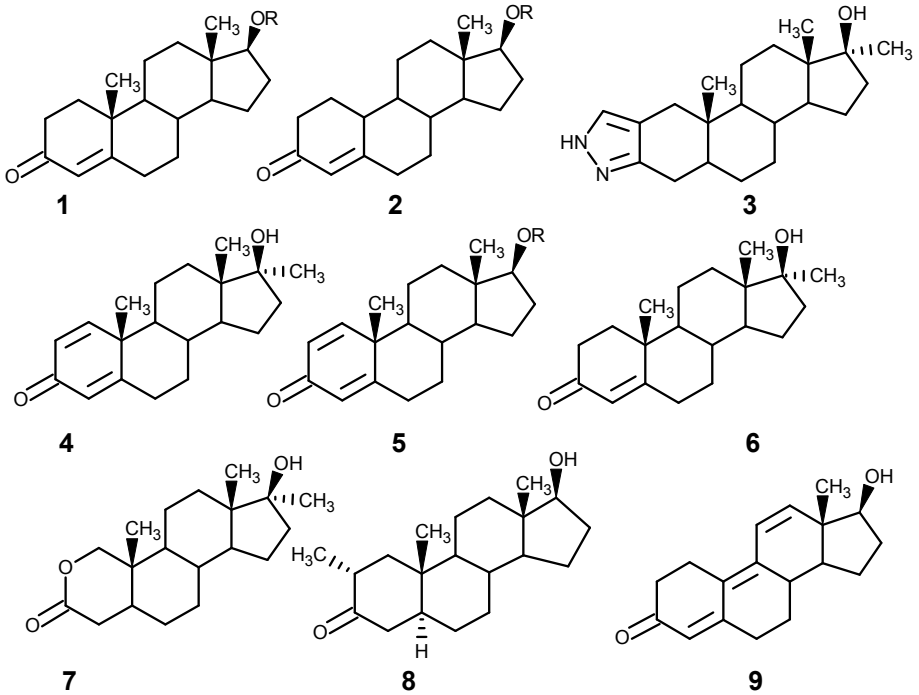
According to Anti-Doping Committee statistics, the most commonly abused anabolic androgen steroids (AAS) in the Czech Republic are: testosterone **1** and 19-nortestosterone **2** together with their esters, stanozolol **3**, methanedienone **4**, boldenone **5** esters, methyltestosterone **6**, oxandrolone **7**, drostanolone **8** and trenbolone **9** esters. These compounds are often distributed by eshop websites, the orally active 17α-methyl steroids **3**, **4**, **6**, **7** occasionally even as undeclared components of vitamin and protein food supplements for athletes and body builders. Anti-doping control and detection of steroid substances in food supplements is currently performed on LC/MS or GC/MS in specialized laboratories. Immunoassay represent a complementary tool, suitable namely for screening purposes, enabling to analyze large number of samples. User-friendly formats of immunoassay may be used without instrumentation in field conditions. We have developed a set of immunoassays for the above-mentioned AAS **1-9** in indirect competitive ELISA and LFIA (Lateral Flow Immunoassay) formats. The synthesis of immunogens and the parameters and applications of developed systems will be presented.



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Peculiar self association of sodium isoursodeoxycholate in water: H-bonds in the primary micelles

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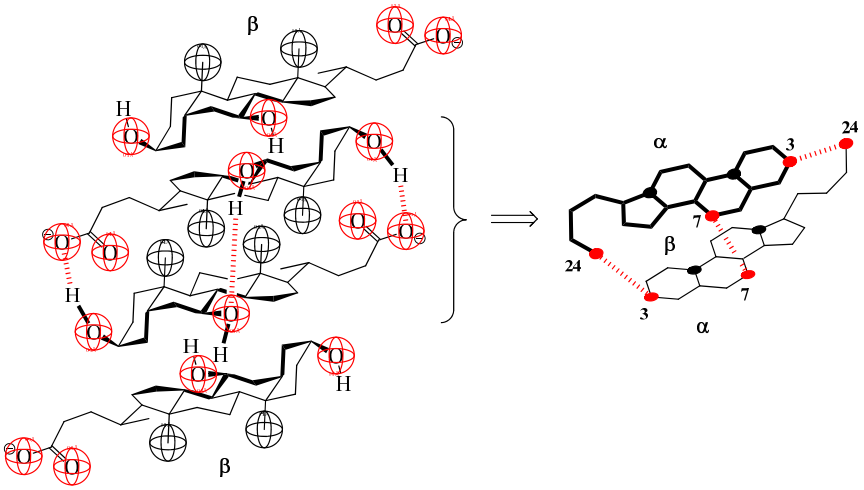
Bile acids are well known in the physiology of digestion i.e. as receptor modulators. Also, they are used in construction of carriers for certain drugs. Although their form relatively small micelles, the small value of their haemolytic potential justifies their biopharmaceutical application [1]. Due to the rigid conformation of the steroid skeleton their self-association differs from self-association of surfactants of type polar head, hydrophobic tail. Above critical micelle concentration (CMC) they form hydrophobic Smalls' primary micelles, while on concentrations above 3CMC they form secondary micelles (association of primary micelles over H-bonds) [2]. For the anion of 6-epimer of hyodeoxycholic acid as well as for the anion of α-muricholic acid it is found that H-bonds between building units are possible in the primary micelles as well [3, 4]. In this paper self-association of the anion of isochenodeoxycholic acid (3-epimer of chenodeoxycholic acid, ICD) and the anion of isoursodeoxycholic acid (3-epimer of ursodeoxycholic acid, IUD) are examined. Using method of the isothermal titration calorimetry (ITC), the heat capacities of demicellisation are determined (ΔC_p) for ICD (185 $Jmol^{-1}K^{-1}$) and IUC (367 $Jmol^{-1}K^{-1}$). These values of the ΔC_p suggest that in the aggregate of IUC the hydrophobic surfaces of building units are largely sheltered from the hydratation than in the micelle of ICD, which is probably the consequence of the H-bond formation between equatorial OH groups and carboxylate group of the side chain of IUC anion. H-Bonds mutually fix building units of the IUC micelle (i.e. prevent mutual slipping between hydrophobic surfaces of the steroid skeleton) thereby preventing hydrophobic hydration [3].



H-bonds in the micelle of IUC: in the steroid skeleton of IUC hydrophobicity inverses, α-side of the steroid skeleton becomes more hydrophobic than the β side.

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Efficient production of hexahydroindanone derivatives by bioconversion of phytosterol with mutants of *Mycobacterium smegmatis*

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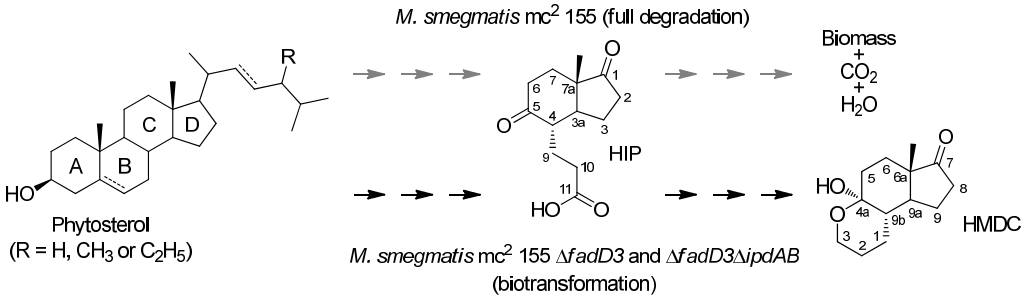
Modifications of sterol catabolic pathways in actinobacteria provide a basis for industrial bio-production of a variety of steroid intermediates and precursors of pharmaceutically valuable steroids [1]. During the last decade the data on genetic regulation of the late steps of steroid core degradation were intensively revealed and classified [2, 3]. Recently the functions of the key genes belonging to the actinobacterial KstR2-regulon and involved in the oxidation of steroid rings C and D were confirmed [4]. These findings allow rational engineering of strains capable for accumulation of useful bi- and tricyclic intermediates.

Herein we report the construction and high biotechnological capabilities of *Mycobacterium smegmatis* mc^2 155 knockout mutants able to selectively produce two hexahydroindanone derivatives from natural sterols.

The appropriate recombinant plasmids were constructed on the basis of non-replicable in mycobacteria vector pBlueScript II KS; knockout of *fadD3* encoding HIP-CoA synthetase and the same gene in combination with *ipdAB* encoding α- and β-subunits of putative acyl-CoA (COHEA-CoA) hydrolase were carried out. The obtained mutants converted cholesterol and phytosterol alternatively to **3aα-H-4α(3'-propanoate)-7aβ-methylhexahydro-1,5-indanedione (HIP)** or **4a-hydroxy-6a-methyldecahydro-cyclopenta[f]chromen-7(*8H*)-one (HMDC)** as confirmed with HRMS and 2D NMR-spectroscopy (see figure). Depending on the application of different clones, single or double mutants and cultivation conditions the biotransformation activity and the route substantially varied. Under the optimized conditions in a laboratory fermenter the mutants selectively produced more than 80% (mol/mol) of HIP or more than 90% of HMDC from 10 – 40 g/l of phytosterol.



Products of phytosterol conversion by wild type and mutants of *M. smegmatis.*

The hexahydroindanone-based isoprenoid derivatives functionalized as cyclic hemiketal (HMDC) or carboxylic acid (HIP) are suggested to be inexpensive precursors for module syntheses of estrogens and similar steroid compounds alternatively to sitolactone.

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Transformation of betulin core

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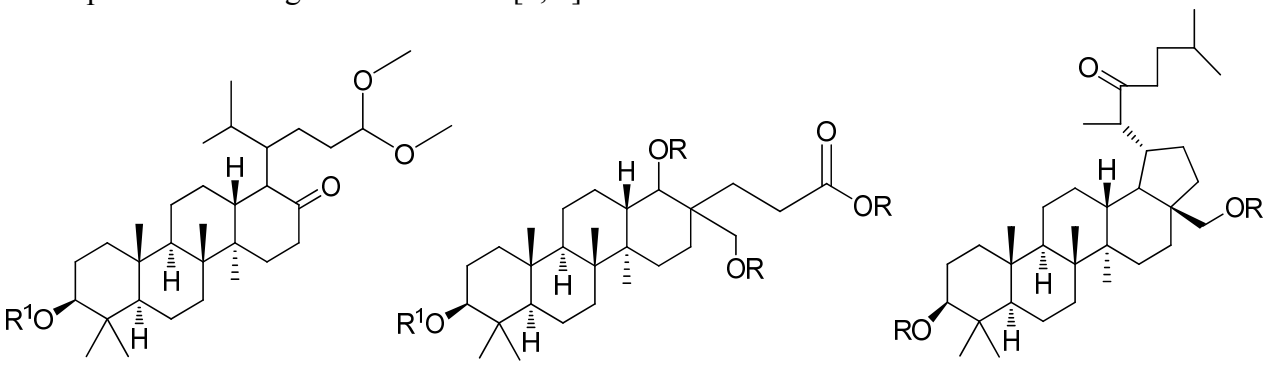
Betulin, widely widespread in nature, belongs to the lupane triterpenes. The richest source of betulin is the outer layer of a birch bark. Betulin and its derivatives show high biological activity, eg. cytotoxicity, anti-inflammatory and antiviral effects. The broad spectrum of biological activity, low toxicity and high availability of betulin attract attention of the pharmaceutical and cosmetic industries.

In our laboratory we have developed synthesis of new modified lupane triterpenoids and lupane saponins. In vitro studies have shown the correlation of cytotoxic activity with the presence of heteroatom in the side chain linked to E ring. Creating the large library of derivatives made it possible to pre-determine the structure of the active sites of lupanes. This encouraged us to undertake broader research into the influence of betulin E-ring modification on the biological activity. Modifications include the opening of the E ring and preparation of derivatives containing a modified side chain at C-19 position. As we expect, it could enhance anticancer effect. Details will be presented during the conference. [1, 2]



R= H, Ac
R^1= H, Sugar

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Unusual starfish steroidal glycosides: structure and biological activity

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Starfish (*Echinodermata*, *Asteroidea*) are characterized by a diversity of polar steroids, including polyhydroxylated steroids, structurally related mono-, bi- and triosides, and steroid oligoglycosides (asterosaponins) with carbohydrate chains comprising five or six sugars. Another rare structural group of polar steroids are cyclic glycosides with a trisaccharide chain, which form a macrocycle between C-3 and C-6 of the Δ^7-3β,6β-dihydroxysteroidal aglycon moiety, and a glucuronic acid residue in the carbohydrate moiety. Steroidal metabolites from starfish, especially glycosides, have been reported to show a wide spectrum of biological activities, including cytotoxic, antiviral, antibacterial, anticancer, neuritogenic and antifungal effects. The recent studies of polar steroidal compounds from starfish led to the isolation of new substances with unusual structures.

Two new polyhydroxysteroidal glycosides, anthenosides A_1 and A_2 were isolated from extract of the tropical starfish *Anthenea aspera*. Anthenosides A_1 and A_2 contain a 2-acetamido-2-deoxy-4-*O*-methyl-β-D-glucopyranosyl residue, found in the starfish steroidal glycosides for the first time. Ten new polyhydroxysteroidal glycosides, anthenosides L−U, with rare positions of carbohydrate fragment attachments, were isolated from the same species of starfish. The unoxidized Δ^{22}-24-nor-cholestane, (24*S*)-Δ^{22}-24-methylcholestane, and Δ^{22}-cholestane side chains of the steroidal aglycons, 3-*O*-methyl-β-D-galactofuranosyl residue and 5α-cholest-8(14)-ene-3α,7β,16α-trihydroxysteroidal nucleus have not been found previously in starfish polar steroidal compounds. Anthenoside O exhibited a potential immunomodulatory action, decreasing the intracellular reactive oxygen species (ROS) content in RAW 264.7 murine macrophages, induced by pro-inflammatory endotoxic lipopolysaccharide (LPS) from *E. coli*.

A new unique steroidal glycoside, granulatoside C, was isolated from the ethanol extract of the starfish *Choriaster granulatus*. This glycoside is unusual one, because combines some characteristic structural peculiarities of steroids from several different phyla of marine invertebrates, it has a range of structural features that were not previously found in starfish polyhydroxysteroidal glycosides, such as Δ^5-3β,7α,16α-trihydroxysteroidal nucleus, $\Delta^{24(28)}$-21-hydroxy-24-methyl-cholestane side chain and 16,21-diglycosylation by D-fucopyranosyl residues, but also characteristic of sponges, ophiuroids and soft corals, respectively.

Six new related polyhydroxysteroidal glycosides, anthenosides S1–S6, along with a mixture of two previously known glycosides, anthenosides J and K, were isolated from the methanolic extract of the starfish *Anthenea sibogae*. The 4-*O*-methyl-β-D-glucopyranose residue and Δ^{24}-cholestane side chain have not been found earlier in the starfish steroidal glycosides. The mixture of anthenosides J and K slightly inhibited the proliferation of human breast cancer T-47D cells and decreased the colony size in the colony formation assay.

Five new cyclic steroid glycosides, luzonicosides B−E, and a related open carbohydrate chain steroid glycoside, luzonicoside F, were isolated from the starfish *Echinaster luzonicus* along with the previously known cyclic steroid glycoside luzonicoside A. Luzonicoside A was shown to be potent in lysosomal activity stimulation, intracellular ROS level elevation, and NO synthesis up-regulation in RAW 264.7 murine macrophages.

Seven new asterosaponins, pentaregulosides A−G, including two furostane-type steroid oligoglycosides were isolated from the ethanolic extract of the starfish *Pentaceraster regulus*. The aglycons of pentaregulosides A and C have not previously been observed in starfish steroid oligoglycosides. Pentaregulosides C, D, and E showed a potential immunomodulatory action, reducing ROS formation in the RAW 264.7 cells, co-stimulated with LPS from *E. coli*.

The study was supported by Grants No. 17-14-01065 from the RSF (Russian Science Foundation).

Brassinosteroids in regulation of plant metabolism

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Brassinosteroids (BS) involved in regulation of key stages of plant growth and development as well as induction of plant resistance to different stressors [1-3].

With the use of molecular biology methods based on model systems, a number of transgenic Arabidopsis plants with mutations in the genes for reception and synthesis of BS, as well as in the genes of proteins of phospholipid metabolism, were studied to expand understanding of the complex mechanism of steroid hormone realization in plant systems. The effect of exogenous natural and chemically modified brassinosteroids on the growth and development of plants was analyzed.

Using novel BS – 24-epicastasterone (24-EP-NBD) conjuncted with fluorescent NBD fragment - and confocal microscopy we tested 24-EP-NBD intracellular transport and accumulation. Treatment of *Arabidopsis thaliana* roots with 24-EP-NBD led to strong accumulation of fluorescent BS in lateral roots. 24-EP-NBD accumulated mainly in plasma membrane and membranes of intracellular compartments like endoplasmic reticulum.

In order to deeper understand the possible connections between brassinosteroid, calcium signaling and antioxidant enzymes, *in silico* gene co-expression analysis and prediction of protein-protein interactions in *Arabidopsis thaliana* were performed in public databases. It was found that genes that are involved in glutathione turnover are co-expressed with some calcium-regulated genes and components of brassinosteroid signaling.

On the basis of the obtained results, the idea of the participation of phospholipid signaling in the hormonal regulation of plant metabolism was formed. The ways of formation of phosphatidic acid and its participation in signaling networks of cells of different level of organization under the action of BS are proposed.

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Current approach in synthesis of triterpenoid dimers

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The dimer structure is ubiquitous in natural products. There are plenty of publications concerning both, isolation and obtaining of dimers.

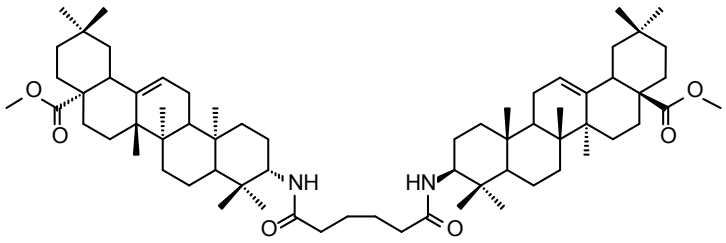
Triterpenoid dimers can be obtained through the covalent linkage of different active functional groups or with the application of active carbon atoms that are in the neighborhood of these groups. Triterpenes form dimer derivatives mainly through the following pathways:

- involving the C-3 atom;
- involving the C-3 hydroxyl group (modified or not);
- involving the C-2 atom;
- involving the C-17 carboxyl group (anhydrides, amides, esters);
- involving other atoms or functional groups at other positions.

The application of the above mentioned functional groups or active atoms of carbon usually allows to obtain linear/quasi-linear dimers, but there are also known some methods that incorporate two functional group or two active atoms of carbon that leads to obtaining of cyclic dimers.

Two monomer units of triterpene usually can be joined into a form of dimer with the application of different atoms or molecules that play a role of a linker (bridge), such as:

- single atom, such as sulphur, selenium, nitrogen, oxygen;
- directly with the application of an additional α,ω-bifunctional molecules, such as: dicarboxylic acids or their simple derivatives (malic, succinic, glutaric, adipic acids or their chlorides), α,ω-diazidohydrocarbons, α,ω-dihalogenohydrocarbons, α,ω-diaminohydrocarbons;
- after further transformation of hemisuccinate derivatives, also with the application of α,ω-bifunctional molecules;
- with the application of triazole system and α,ω-bifunctional molecules;
- with the application of other methods.



The main methods of synthesis of triterpenoid derivatives of dimer character, starting from natural triterpenes or their simple derivatives and the results of biological tests performed for some of these dimers are summarized in review paper by B. Bednarczyk-Cwynar and A. Günther [1].

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Bimolecular compounds based on the natural sesquiterpene lactones

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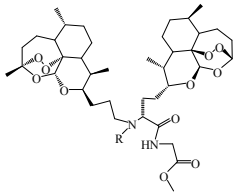
Synthetic modifications of plant metabolites considerably expand the assortment of prospective biologically active compounds. Synthesis of new practically valuable derivatives, including hybrid and dimer molecules, is one of the modern trends in Chemistry of sesquiterpene lactones. In this regard, regio- and stereoselective methods of chemical modification of natural molecules are used.

In the synthetic plan, the following reactions are worth mentioning: the *Ugi* four-component reaction (U-4CR) allowing us to receive a dimer **1** based on artemisinin through a linker binding; the *Sonogashira* cross-coupling reaction between an aryl halide and a terminal alkyne derivative of artemisinin resulting in a bimolecule **2**, as well as a reaction of pseudo-guaianolide helenalin with malonyl dichloride which leads to a dimer **3** through diester linker binding.



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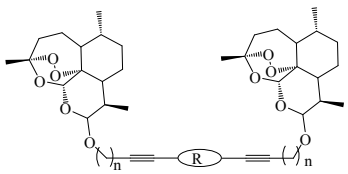

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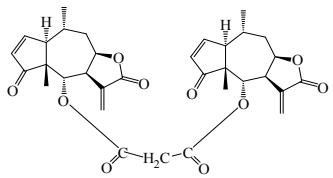

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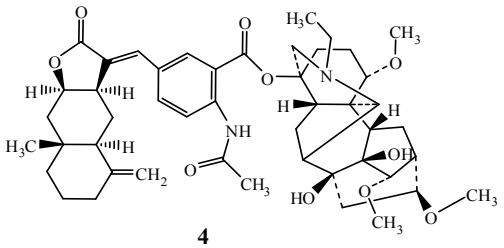
One of the effective methods to obtain hybrid derivatives of sesquiterpene α-methylene-γ-lactones is the *Heck* reaction which proceeds with a preservation of the C11-C13 double bond of a lactone cycle. Arylation of isoalantolactone by the halides lappaconitine and desoxyvasicinone mainly proceeds with the formation of **5** *(E)*-configuration of the C11-C13 double bond as in a hybrid molecule **4**. Moreover, in the course of a reaction with the second halide three other compounds are formed, i.e. diaryl isoalantolactone **6**, a compound **7** with *(Z)*-configuration, and a compound **8** with the C11-C13 double bond shift to position at C7-C11.



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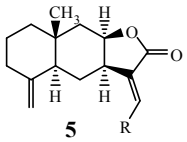

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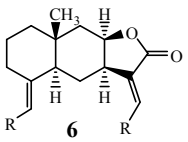

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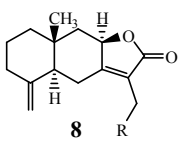

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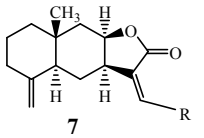

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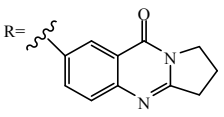
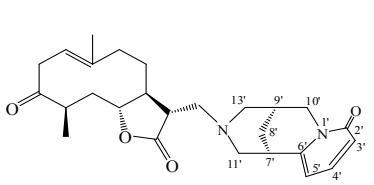


Chemical modification of molecules of practically accessible natural sesquiterpenoids by the *Michael*-type amination, when alkaloids cytisine and anabasine had been used as nucleophilic reagents, allowed us to receive nine new hybrid derivatives of a terpenoid-alkaloid type, including bimolecular compounds **9** and **10** which structural data were later deposited in the Cambridge Structural Database (CSD).



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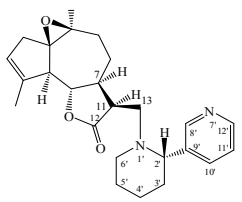

10

New benzisoselenazol-3(2*H*)-ones *N*-functionalized with terpene skeletons

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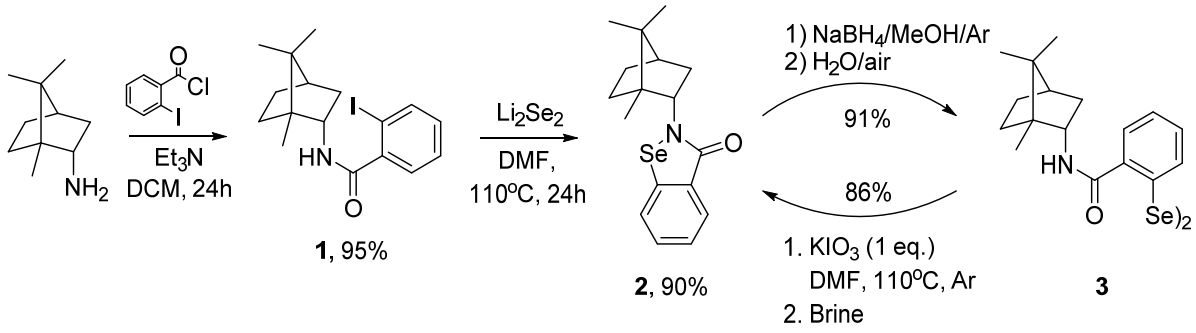
Application of terpenes in the synthesis of chiral reagents and catalysts is highly useful due to their broad structural diversity and commercial availability in enantiomerically pure form. Isoprenoides have been also efficiently applied to obtain several organoselenium derivatives [1]. However, it has not been evaluated if the presence of a terpenyl scaffold can enhance the proven ability of organoselenium compounds to eliminate reactive oxygen species and prevent from diseases induced by oxidative stress. Herein, we present first examples of benzisoselenazolones and corresponding diselenides functionalized on the nitrogen atom with terpene moieties from camphane, *p*-menthane and pinane group. Compounds have been obtained by an efficient, newly developed procedure [2]. The synthesis of *N*-bornyl benzisoselenazolone **2**, by the reaction of *N*-substituted *o*-iodobenzamide **1** with lithium diselenide and corresponding diselenide **3** has been presented below (Scheme 1).



Scheme 1. Synthesis of *N*-bornylbenzisoselenazolone **2** and corresponding diselenide **3**.

Selected compounds were tested as antioxidants by an NMR assay [3]. Compound **2** was additionally evaluated as a cytotoxic agent on various cancer cell lines.

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Work was supported from NCN grant nr UMO-2015/17/B/NZ7/03058.

NHCCuX-catalyzed Friedländer-type annulation of fluorinated aminophenones with alkynes on water. Unexpected dibenzodiazocine formation

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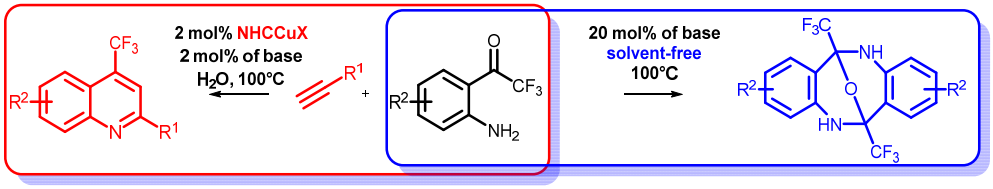
The synthesis of fluorine-containing compounds has attracted much attention due to their unique physical and biological properties. More than 30% of compounds present on agrochemical and pharmaceutical worldwide market contain fluorine or fluorinated groups. The incorporation of fluorine into organic molecules strongly affects on their biological properties. It is well established that the presence of fluorine improves bioavailability and increases lipophilicity and metabolic stability. [1-3]

Among a plethora of fluorinated heterocycles, quinolines and quinolones has retained long-standing interest of the synthetic community. Many of them exhibit remarkable biological properties, such as antimalarial or antibacterial. [4, 5] Among many classical method leading to fluorinated quinolines, including Skraup reaction, Doebner von-Miller reaction, the Gould Jacobs reaction, the Knorr synthesis and the Niementowski synthesis, the Friedländer reaction constitute an obvious choice due to its simplicity. Generally, the classical protocol involves an acid- or base-catalyzed reaction between *o*-aminophenones with α-methylene ketone.



Scheme 1. General reactivity of aminophenones leading to quinolines and dibenzodiazocines.

Herein, we present the first application of *N*-heterocyclic carbene copper(I) complexes (NHCCuX) as efficient catalysts for the synthesis of fluorinated quinolines and naphthydrines via direct catalytic alkynylation/dehydrative cyclization (Friedländer-type reaction) sequence on water (Scheme 1). [4] A series of Friedländer-type reaction were performed with as little as 2 mol% of *N*-heterocyclic carbene copper(I) complex, providing a broad range of fluorinated quinolines and naphthydrines with excellent yield and perfect scalability. The influence of the electronic and steric nature of NHCCuX complex and the scope of method will be discussed in details. In addition, a unique method of synthesis of dibenzodiazocine will be also presented.

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Targeting phorbol derivatives toward cancers

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Phorbol esters are natural products structurally belonging to tigliane diterpenes and present day knowledge about phorbol esters describe their biological activity [1] as proinflammatory, proapoptotic, tumor promoting and causing increase in cytosolic Ca^{2+}. Phorbol esters activity takes place via activation of phosphokinase C (PKC).

PKC [2] plays important role in several cell signal transduction pathways and by direct protein phosphorylation it can participate in diverse biological processes such as cell cycle regulation, p53, p21, cell adhesion, DNA synthesis and transcription, apoptosis, drug resistance, cell growth and differentiation. There are several PKC isozymes and it seems very likely that the corresponding biological effect is also strongly affected by which isozyme is activated [3].

One of the widely used phorbol esters phorbol myristate acetate (PMA), was able to trigger apoptosis of LNCaP cells *via* activation of isozyme PKC-δ [4]. Also a preliminary study in humans of PMA, or PMA combined therapy on amyelocytic leukemia patients resulted in multiple remissions or substantially decreased cancer cell number in bone marrow [5].

These findings led us to propose a strategy for development of phorbol ester prodrugs so that we can maximize the proapoptotic effect and minimize side effects. We used peptides that are specifically cleaved in cancer tissue which were previously utilized with success after being coupled to natural cytotoxin thapsigargin [6]. The plan was to evaluate binding of molecules without peptide to PKC and then to do cytotoxicity assays on LNCaP cells. After positive results from displacement and cytotoxicity assays we prepared final prodrugs of phorbol. These phorbol peptide conjugates have been tested for cytotoxicity with more or less positive results.

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The effect of cyclodextrins on oxidation of DHEA by fungal laccase

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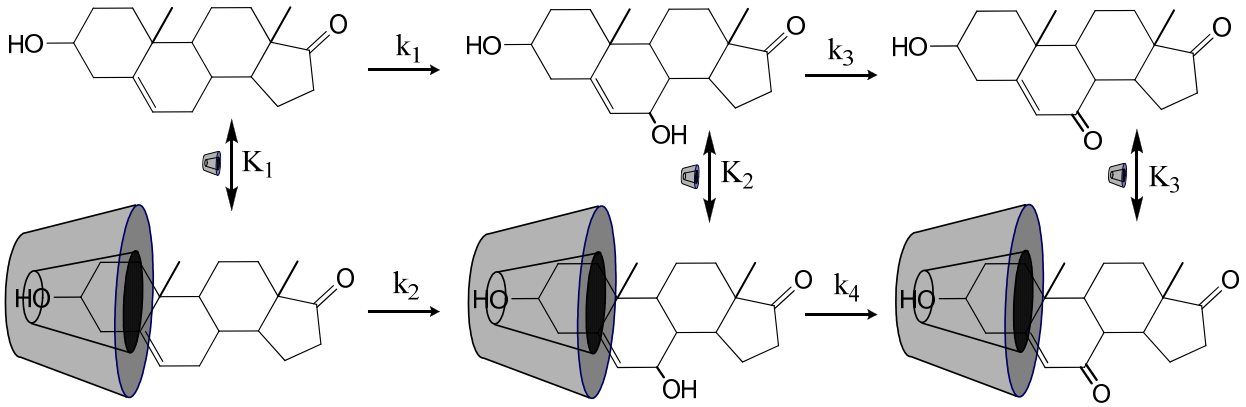
G. K. Skryabin Institute of Biochemistry and Physiology of Microorganisms, RAS, Pushchino, Russia

The introduction of biotechnological methods is a modern trend in the development of ecologically friendly steroid technologies for the pharmaceutical industry. Literature data on the oxidation of steroids with laccase mediator systems (LMS) are restricted by the C–C and C–O dimerization of steroids with phenolic structure of A-ring [1]. Recently we described regiospecific LMS oxyfunctionalization of 3β-hydroxy-Δ^5-steroids in aqueous solutions [2]. However, hydrophobicity of substrates is an obstacle for the development of preparative enzymatic syntheses. We focused on the evaluation of the potential of CDs as solubilization complex agents in green synthesis of 7-keto-derivatives DHEA.



Laccase oxidation of DHEA in CD aqueous solutions.

Enzymatic oxidation of DHEA were carried out in aqueous solutions pH 5.0 at 40 °C by fungal laccase (*Trametes versicolor*) with the use of radical mediator. Parameters of CD-complexation were determined by independent nonlinear spectrometric method [3]. The complex stability constant of CD-DHEA (K_1) at 40 °C were 5544 M^{-1}. The kinetic studies were carried out with HPLC monitoring of LMS conversion. It was established that LMS oxidation of DHEA in homogenic conditions led to formation of 7-keto-DHEA as a main transformation product. The calculation of kinetic constants was made for free (k_1) and CD-complex (k_2) forms of DHEA. It is demonstrated that the effect of CD-complexation was expressed in deactivation of allylic hydrogen (C-7) and impediment of its breakaway as limiting stage in LMS oxidation via radical mechanism ($k_1 >> k_2$). In the same time LMS oxidation of CD-complexes of 7α OH DHEA (k_3) into 7-keto-DHEA and transformation of free steroid forms of 7α-OH-DHEA (k_4) have same kinetic parameters. Summarizing, CDs as solubilizing agents are promising in preparative LMS syntheses of 7-keto-DHEA from the respective alcohols.

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S.M.A.R.T. Steroids: Steroidal Molecules As Rapid-acting Therapeutics

Eva Kudova[1*], Ladislav Vyklicky[2], Jiri Palecek[2], Karel Vales[2], Pavel Mares[2], Michaela Nekardova[3], Marika Matousova[1], Marketa Smidkova[1], Martin Balastik[2], Svetlana Zufanova[2], Radko Soucek[1]

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We have designed and synthesized S.M.A.R.T. Steroids – **S**teroidal **M**olecules **A**s **R**apid-acting **T**herapeutics. S.M.A.R.T. Steroids are neuroactive molecules, targeting primarily the NMDA receptors. Moreover, they show neuroprotective properties and minimal side effects in animal models. *N*-Methyl-D-aspartate receptors (NMDARs) play an important role in development, synaptic plasticity, learning, and memory, however, abnormal activation of NMDA receptors has been shown to mediate neuronal degeneration/cell death. In contrast, positive allosteric modulators that increase the activity of NMDARs may provide a therapeutic aid for patients suffering from neuropsychiatric disorders where NMDARs hypofunction is thought to be involved, such as intellectual disability, autism spectrum disorder or schizophrenia. Our screening pipeline1-9 covers physicochemical and biological properties like: *(i)* lipophilicity (logP, logD, ΔG_{solv}); *(ii)* patch-clamp recordings from HEK293 cells assessing NMDAR modulation; *(iii)* Caco-2 assay, *(iv)* mitotoxicity, hepatotoxicity and ROS induction in HepG2 cells, *(v)* stability in plasma, *(vi)* treatment of glutamate and NMDA-induced neurotoxicity (survival rate, caspase-3, intracellular calcium levels, ROS); *(vii) in vitro* growth of postnatal neurons after neurosteroid administration, *(viii)* models of animal behavior (open field, elevated plus maze, forced swim test, etc.); *(ix)* PTZ-induced seizures; *(x)* paclitaxel-induced peripheral neuropathy; *(xi)* pharmacokinetic properties. Our results indicate that these compounds may be beneficial in treatment of many neurological diseases.

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Crystal structures of an indole prenyltransferase, IptA: implications for substrate tolerance and its expansion

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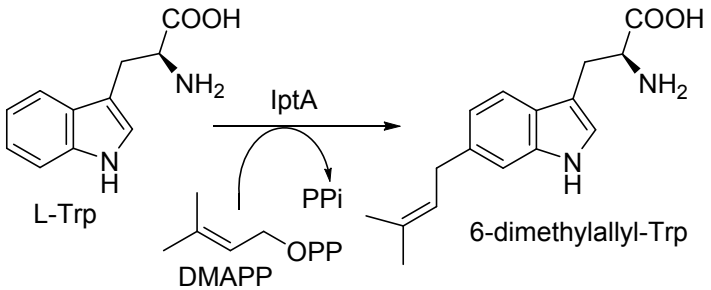
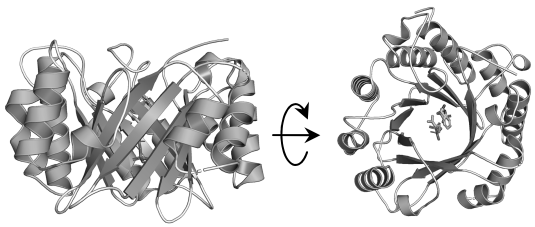
Aromatic prenyltransferases transfer prenyl moiety onto aromatic acceptor molecules, producing diverse primary and secondary metabolites in plants, fungi, and bacteria [1]. IptA, which is a family member of dimethylallyltryptophan synthase (DMATS), catalyzes C6-prenylation of tryptophan using dimethylallyl diphosphate (DMAPP), and exhibits relatively higher substrate tolerance than that of other DMATS family members [2]. Although several crystal structures of DMATS were determined, the structure of IptA and the structural basis of its high substrate tolerance is yet unclear.

In this study, we determined the crystal structures of substrate-free IptA and ternary tryptophan-DMSPP (dimethylallyl *S*-thiolodiphosphate; stable analogue of DMAPP)-enzyme complex of IptA. The overall structure of IptA exhibited typical ABBA-fold, which is commonly found in DMATS family members, and the active site pocket was located inside the ABBA-barrel. The crystal structure of the ternary Trp-DMSPP-enzyme complex suggests that dimethylallyl cation electrophilically attacked the C6 atom of tryptophan, as already suggested for C4 prenylation of tryptophan by FgaPT2 [3]. Although IptA can transfer prenyl group to tryptophan derivatives, such as 5-methyltryptophan, tryptophan snugly fitted into the active site pocket and unoccupied space around the tryptophan was very limited. We also determined the crystal structure of ternary 5-methyltryptophane-DMSPP-enzyme complex and found that 5-methyltryptophan and Leu275 moved further away from each other to accommodate relatively larger prenyl acceptor.

IptA cannot utilize geranyldipohsphate (GPP) as a prenyl donor. To enlarge the substrate biniding pocket, W154A mutation was introduced into the enzyme. As we expected, W154A mutant IptA can produce geranylated tryptophan from tryptophan and GPP. IptA can also transfer prenyl group to several peptides having tryptophan at the C terminus, which can be used for prenylation of bioactive peptide to improve activity.



Prenylation at C6 of Trp by IptA



Overall structure of IptA

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New selenoorganic reagents in steroid chemistry

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An organoselenium moiety can be selectively introduced to a substrate structure in mild reaction conditions by using nucleophilic selenium reagents. [1] Among the latter, PhSeZnCl – the Santi reagent [2] – has recently emerged as the first bench-stable selenium nucleophilic agent having a broad range of application.

The results of reaction with PhSeZnCl (Santi's reagent) and steroid derivatives: epoxides, α,β-unsaturated ketones, and lactone will be presented. The epoxide ring-opening reaction with Santi's reagent appeared to be regio- and stereoselective. This approach led to novel phenylselenium-substituted steroids.

An effective, one-pot method for the oxidation of steroid olefins will be also presented. The method consists of the generation of a reactive species, presumably the benzeneseleninyl cation, by the treatment of BSA with TMSOTf. In the case of BSA/TMSOTf reactions with steroid mono- or disubstituted olefins, the initial step is usually an electrophilic attack of selenium at the less substituted carbon atom. However, the BSA/TMSOTf reactions with tri- and tetrasubstituted steroid olefins proceeded smoothly affording dihydroxylated products in high yields. The products are formed, presumably via the four-membered cyclic intermediate that is formed by a concerted [2+2] cycloaddition of a benzeneseleninyl cation to the olefin. [3]

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View of Białystok with Church of St. Roch

Lipowa Street and Church of St. Roch, Białystok

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Epoxy ring opening in 16α,17α-epoxypregnenolone and its new oxygenated metabolites obtained by biotransformations

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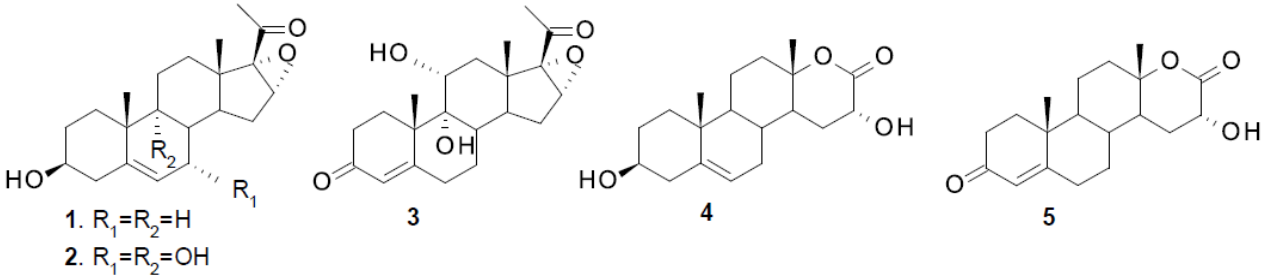
Steroid modifications by selected wild-type and engineered strains of microorganisms have become an effective tool for the production of high-valued steroidal drugs and their precursors for the pharmaceutical industry [1]. 16α,17α-Epoxyprogesterone is an important intermediate for many anti-inflammatory drugs such as hydrocortisone, cortisone, and beclomethasone dipropionate. Although the biotransformation of this compound has already been successfully applied for the production of 11α- [2], 11β- [3], 7β-hydroxy- and 7β,11α-dihydroxy- [4] derivatives, the screening of microbial strains with novel catalytic activities still plays an important role in developing more efficient production processes as well as producing novel steroid compounds.

The established biological activity of 3β-hydroxy-5-ene steroids had encouraged us in the presented work to investigate microbial transformations of 16α,17α-epoxypregnenolone (**1**) in the cultures of *Mortierella isabellina* AM 212 and *Penicillium lanosocoeruleum* KCH 3012 fungi. Both strains were previously used in transformations of steroids, catalyzing 7α- and 7β-hydroxylation [5] and lactonization of DHEA [6], respectively. From reaction with *M. isabellina*, we obtained and identified by chromatographic, spectroscopic and crystallographic methods dihydroxy derivatives of substrate (**2** and **3**) as the main products. When 16α,17α-epoxypregnenolone (**1**) was incubated with *P. lanosocoeruleum*, a mixture of hydroxylactones was formed. The substrate underwent a rare epoxide opening resulting in retention of *alpha* stereochemistry to give 16α-hydroxy metabolites **4** and **5**. A possible metabolic pathway of **1** and tentative mechanism of epoxide opening by *P. lanosocoeruleum* has been proposed.



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Application of α- and β-naphthoflavones as monooxygenase inhibitors in the transformation of 17α-methyltestosterone

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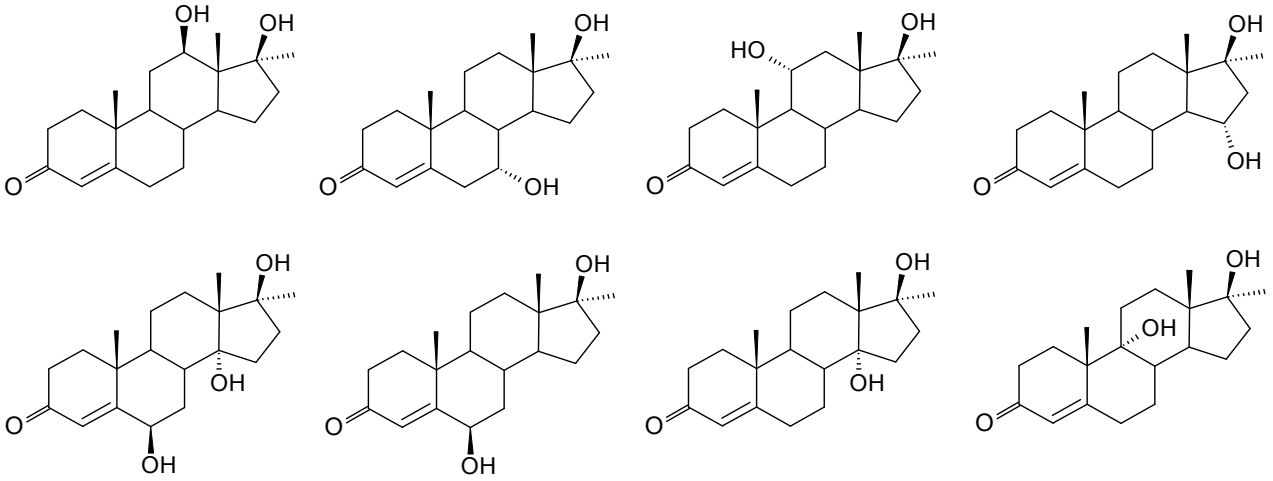
Our study aimed to test methyltestosterone's microbial transformation and then check whether any of the synthesised naphthoflavones would be able to selectively modulate the activity of any of the monooxygenases. 17α-Methyltestosterone was effectively transformed in *Absidia coerulea* KCh 93, *Syncephalastrum racemosum* KCh 105, and *Chaetomium sp*. KCh 6651 cultures. *A. coerulea* KCh 93 cells monooxygenases catalysed methyltestosterone's hydroxylation into 6β-hydroxy- and 12β-hydroxy-17α-methyltestosterone with 44% and 29% yield, respectively, and into four other minor products. In *S. racemosum* KCh 105 cultures, three hydroxylation products at the 7α, 15α, and 11α positions were obtained. In the *Chaetomium sp*. KCh 6651 culture, 15α-, 11α-, 7α- and 6β-hydroxyderivatives were isolated.

Alpha- and beta-naphthoflavone (ANF and BNF, respectively) can be used as either monooxygenase inhibitors or inducers [1-3]. ANF was shown to inhibit 6β-hydroxylation of progesterone and testosterone via cytochrome CYP3A6, in addition to human CYP3A4, in RIF-microsomes [4]. Eight α- and β-naphthoflavones were synthesised and tested for their inhibitory capabilities. A significant reduction in substrate conversion, but no change in the percentage composition, of the obtained products was observed.



Products of 17α-methyltestosterone transformation by tested fungal strains.

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Oleanolic acid dimers with short linkers – synthesis and anticancer activity

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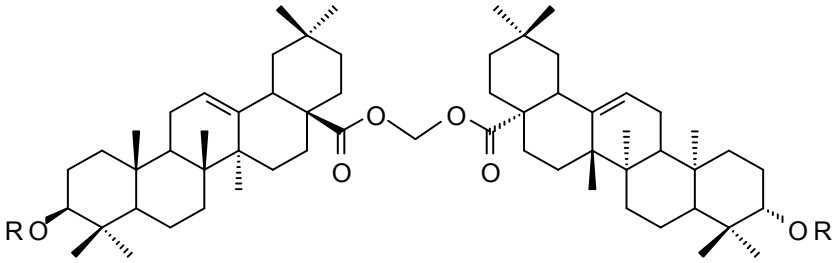
The dimerization of natural and synthetic compounds is one of the most promising approaches for the design of new and potent drugs in the field of medicinal chemistry. Nowadays, there are plenty of papers describing both isolation and synthesis of numerous dimer terpenes. Within this vast group of compounds triterpene dimers comprise quite a unique class of species. The main methods of synthesis of triterpenoid derivatives of dimer character, starting from natural triterpenes or their simple derivatives and the results of biological tests performed for some of these dimers are summarized in review paper [1].

A simple, convenient and efficient method of synthesis of novel oleanolic acid derivatives was developed and a series of compounds was synthetized with the application of this pathway. All of the obtained triterpenes had dimer structure, that was obtained by joining of two molecules of oleanolic acid *via* the C-17 carboxylic function and with the application of methylene iodide. The C-3 hydroxyl group of the received dimers was acylated with carboxylic acid anhydrides (*e.g.* acetic, butyric, benzoic, succinic, glutaric, *o*-phthalic anhydrides) or oxidizied with the Jones reagent and next transformed into oxime. Some dimers of oleanolic acid were subjected to further transformations.

Spectral characterizations (IR, ^{1}H NMR, ^{13}C NMR, DEPT, ESI-MS) of the obtained compounds was performed. Dimeric structure and the presence of another additional functionalities was proved for each compound.



R = e.g. CH_3CO-, $HOOC-CH_2-CH_2-CO$-, C_6H_5-

These triterpenes were tested for cytotoxic activity on human tumor cell lines toward A375 (epithelial melanoma), A2780 (ovarian carcinoma), HT29 (colorectal adenocarcinoma), MCF7 (breast adenocarcinoma), SW1736 (thyroidea carcinoma) as well as non-malignant NIH 3T3 (mouse fibroblasts) applying sulforhodamine B assays. Several of the compounds displayed EC_{50} values < 30 μM.

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Different aspects of chemical activity of oleanolic acid involved in synthesis of its dimers

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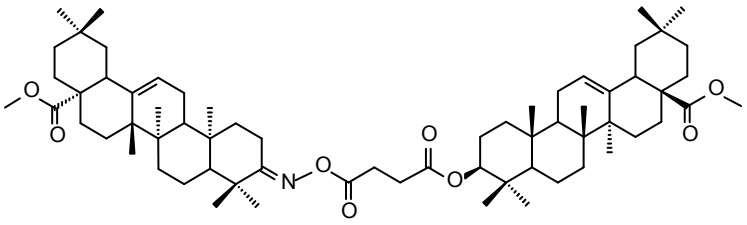
Monomeric units combined into a form of dimers are usually linked through chemical bonds. This idea is inspired by nature and many of the known natural compounds are built of two the same or similar units.

As molecules of various triterpenes are endowed with different functional groups and active carbon atoms, there are diverse possibilities of forming their dimers. The main methods of synthesis of triterpenoid dimers, starting from natural triterpenes or their simple derivatives and the results of biological tests performed for some of these dimers are summarized in review paper [1].

A simple, convenient and efficient methods of synthesis of novel oleanolic acid derivatives were developed. All of the obtained triterpenes had dimer structure, that was obtained by joining of two molecules of oleanolic acid or its simple derivatives with the application of different methods:

- Method A: joining of two molecules of oleanolic acid or some of its derivatives involving the C-17 carboxyl group and with the application of α,ω-dihalogenohydrocarbons;
- Method B: forming of anhydrides of oleanolic acid derivatives with the transformed C-3 hydroxyl group;
- Method C: joining of two molecules of hemisuccinates of oleanolic acid or its derivatives at the C-17 position and with the application of α,ω-dihalogenohydrocarbons;
- Method D: acylating of oleanolic acid oximes with oleanolic acid hemisuccinates owing the transformed C-17 carboxyl function.



Spectral characterizations (IR, ^{1}H NMR, ^{13}C NMR, DEPT, ESI-MS) of the obtained compounds was performed. Dimeric structure and the presence of another additional functionalities was proved for each compound.

Dimerization is a perspective method both from chemical and biological point of view. It is highly likely that the resulted new compounds will exhibit interesting physico-chemical and biological properties which allow to involve them as a pharmaceutical agents as well as new materials for modern application.

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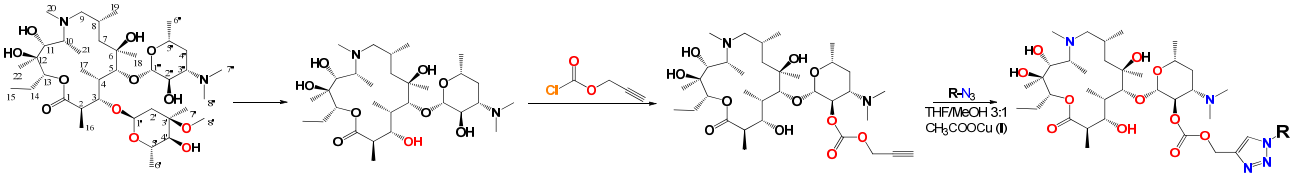
Synthesis and structure-activity relationship of a new derivatives of 14-, 15- and 16-membered macrolide antibiotics containing rebuilt saccharide arms

Anna Janas, Krystian Pyta and Piotr Przybylski

Adam Mickiewicz University in Poznań, Poland

Macrolide antibiotics are large group of natural products produced by various *Streptomyces* strains. They are used against various infectious diseases. Macrolides can be classified by a lot of different criteria. One of them is type and size of the macrolide ring [1] and type of saccharide moieties attached to the aglycone ring as e.g. mycaminose, mycarose, cladinose, forosamine, desosamine [2]. These classifications includes generally lactone macrolides antibiotics, such as 14-membered erythromycins, 15-membered azithromycin and 16-membered leucomycins and spiramycins. The macrolide lactone antibiotics' mechanism of action is based on the inhibition of bacterial protein biosynthesis by reversible binding to the bacterial 50s subunit at the ribosome [3].

In our laboratory we obtaining a novel macrolide antibiotics with improved binding model to biological target and of increased antibacterial and anticancer potency. Our modifications comprising the cascade sequences incorporations of the 1,3-dipolar Huisgen cycloaddition reactions were used to better matching antibiotic with enzyme/protein target. Previously, in our research group we modified aglycone ring of 16-membered antibiotic via regio- and diastereoselective cascade sequences of intramolecular esterifications followed by tandem E1cB eliminations and subsequent 1,2-addition to carbonyl followed by 1,6-conjugate addition α, β, γ, δ –unsaturated aglycone and also via Huisgen reactions. In the result, we obtained entirely new series of macrolide antibiotics of antibacterial and anticancer potency [4, 5]. Currently, with the support of Polish National Science Centre (decision number UMO-2015/19/B/ST5/00231), we are applying this approach to modification of another group of natural macrolide antibiotics - 15-membered azalides, by rebuilt saccharide arms using Huisgen reactions, to obtain efficient alternatives to the currently used antibiotics (azithromycin).



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Influence of C-17 substituents at quinone ring on conformational flexibility of geldanamycins

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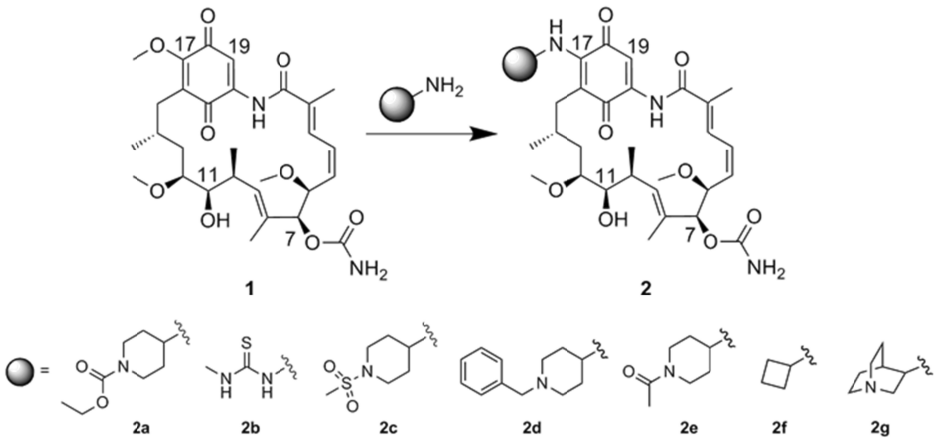
Geldanamycin (**1**), belongs to the natural anticancer antibiotics group, being a part of the larger family of rifamycins, having characteristic macrocyclic structure /basket-like structure/. [1] The anticancer potency of geldanamycin is related to the formation of the protein complex *via* inter-alia hydrogen bonds at ADP/ATP-binding pocket of *N*-binding terminal domain of the highly conserved heat shock proteins as e.g. Hsp90. [2] Our main task is to synthesize less toxic geldanamycins (**2a**-**2g**) with introduced substituents at C7, C17 and C19 of improved binding profile to chaperone proteins. [3] On the other hand, detailed structure determination of new geldanamycin derivatives is the crucial part of the studies to explain structure-bioactivity relationships. It is well known, that ansamycins are able to have the ansa-bridge in different conformations and even in some cases, like for streptovarycins, two atropisomers are observed. Taking into account the above, we took attempts to check out the role of substituent at C17 position on the ansa-bridge conformation and bioactivity.



Geldanamycin and its derivatives structures.

Acquired knowledge contribute to project new geldanamycin derivatives which can better inhibit chaperone proteins and it should be reflected in a higher anticancer potency of these ansa-macrolides.

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Evidence for the presence of brassinosteroids in duckweed *Wolffia arrhiza*

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The Lemnaceae (duckweeds) comprise an aquatic monocotyledon family including only 37 species which are arranged within five genera. They are the smallest angiosperms, some of which may attain a width of only 0.3 mm at maturity [1], however in *Wolffia arrhiza* its body size can be up to 1 mm width. These plants have a crucial role in protection of environment. Duckweeds have also significant applications in aquaculture, i.e. they are food for waterfowl and fish. *Wolffia arrhiza* has neither leaves nor a stem and even lacks roots, but has set flowers and seeds. The simplified morphology of the plant enables it to be a good model for laboratory studies [2].

Brassinosteroids (BRs) belong to polyhydroxylated steroid hormones which are widespread in the plant world. BRs have been isolated from different plant organs such as pollen, anthers, seeds, leaves, stems, roots, flowers, and grain as well as in insect and crown galls. The endogenous level of BRs varies from plant's organ and the age of the plant [3].

BRs are polyhydroxylated compounds that have a four-ringed skeleton, usually composed of either 27, 28 or 29 carbon atoms, with 5α-cholestane or 26-nor-5α-campestanes structures for the C_{27} types, 5α-campestane, 5α-ergostane or 24-methylene-5α-cholestane skeletons for the C_{28} types, and 5α-sitostane, 24(*E*)-ethylidene-5α-cholestane, 25-methyl-5α-campestane and 24-methylene-25-methyl-5α-cholestane structures for the C_{29} types. A greater structural variation is observed in the A-ring, with 15 different structures reported, ranging from $\Delta^{2,3}$-unsaturated to trioxygenated and conjugated BRs. As regards the B-ring oxidation, BRs are divided into 6-oxo-7-oxalactone, 6-oxo, 6-deoxo and 6α-hydroxy types [3].

The present study aimed to determine of BRs in *Wolffia arrhiza* (Lemnaceae). After homogenization (using liquid nitrogen and ball mill) plant material was first extracted with methanol overnight. Then, the exctract was purified using Waters Oasis MAX cartridge (6 ml, 500 mg, 60 μm particle size). After purification, the extract was dried in the vacuum and reconstituted in 100% methanol. The screening process was performed on MS equipped with an electronspray ionization source coupled with LC (Waters XBridge C_{18} column: 250×4.6 mm, 3.5 μm) [4]. Seven compounds have been identified in *Wolffia arrhiza*. The results revealed the presence of brassinolide (**1**, 0.123 ng/g biomass), 24-epibrassinolide (**2**, 0.022 ng/g biomass), 28-homobrassinolide (**3**, 0.176 ng/g biomass), castasterone (**4**, 0.015 ng/g biomass), typhasterol (**5**, 0.193 ng/g biomass), 6-deoxotyphasterol (**6**, 3.129 ng/g biomass) and 6-deoxocastasterone (**7**, 0.214 ng/g biomass). The most commonly detected are C_{28} (**1**, **2**, **4**, **5**, **6**, **7**) BRs, less common – C_{29} (**3**). BRs can reach in wide concentration from 0.015 to 3.129 ng/g biomass. 6-deoxotyphasterol was detected in the highest level. To best of our knowledge, the presence of BRs is the first report in Lemnaceae family.

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Synthesis and cytotoxicity of some new bile acid tetrazoles

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In this work we present a synthesis and cytotoxic properties of some bile acid B-ring fused tetrazoles derived from cholic and chenodeoxycholic acid (Figure 1.). They were obtained trough several synthetic steps, starting from appropriate bile acid, where the Schmidt-reaction of the suitably protected steroidal ketones served as the key transformation for introduction the tetrazole moiety [1].

Starting from cholic acid (CA) methyl 3α,12α-diacetoxy-7-oxo-5β-cholanoate was synthesized trough selective oxidation of CA in position 7 followed by methylation of carboxylic group and acetylation of alcoholic hydroxyl groups. Similarly, another oxo intermediate, the ethyl 3α-acetoxy-7-oxo-5β-cholanoate was prepared from chenodeoxycholic acid (CDCA). These oxo-compounds were submitted to Schmidt-reaction yielding B-ring fused tetrazoles **3** and **4.** In order to find optimal conditions for the synthesis of tetrazoles, reaction was conducted in different solvents exploring two azide sources (hydrazoic acid and trimethylsilyl azide), and different Lewis acids (e.g. $BF_3 \cdot OEt_2$,TMSOTf). The protected tetrazole compounds were obtained in yield of 65 - 87%. Finally, removal of all protecting groups by KOH in ethanol gave the final products **1** and **2**. Structures of all synthesized compounds were determined by NMR and other spectroscopic techniques.

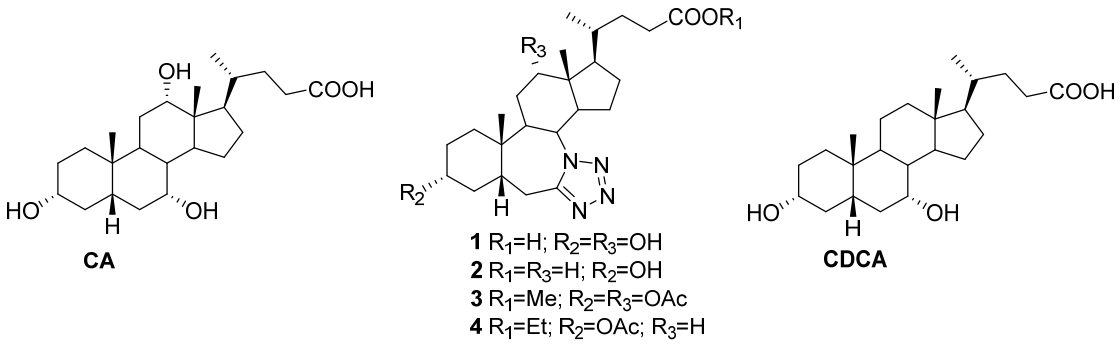

Figure 1. Structures of newly synthesized tetrazoles and the starting bile acids.

Newly synthesized compounds presented in this work are combining the surfactants and drug transport properties of bile acids with tetrazole structural motif known for providing unique physico-chemical and pharmacological properties [2]. Having this in mind, we have investigated the cytotoxic activity towards some human cancer cell lines and one healthy cell line.

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Synthesis and antitumor potential of 3β-acetoxy-5α-halo-6β,19-epoxy-17-oxa-17a-homoandrost-16-ones

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Chemically modified steroids have found significant application in cancer treatment. With this in mind, many researchers have synthesized a number of new steroidal derivatives. Their antitumor activity was also tested, and among the active ones, halogenated derivatives stand out. Taking into account this fact, we have synthesized three new 6β,19-epoxide derivatives **1-3** with halogen atom (F, Cl or Br) on C-5α (Fig. 1). These compounds also contain D-homo lactone ring, and were synthesized starting from dehydroepiandrosterone in several synthetic steps [1, 2]. Key step is transformation of 5α-halo-6β-hydroxy derivatives into 5α-halo-6β,19-epoxides **1-3** with lead tetraacetate.

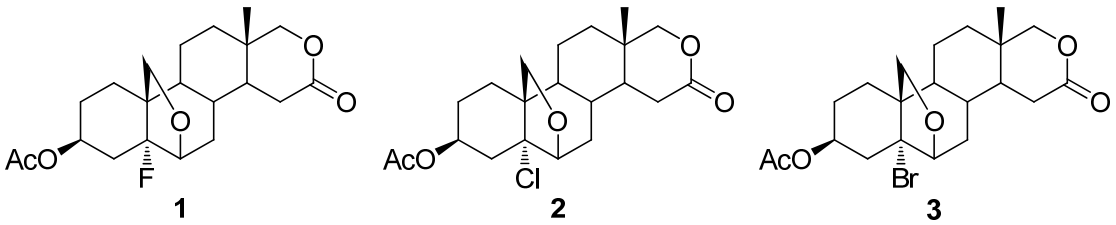

Figure 1.

Newly synthesized compounds were tested for their compliance with the Lipinski rule of five in order to investigate possible drug-like behavior. Last but not least, their cytotoxic activity against selected human cancer cells was measured *in vitro*.

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Novel odiferous organosulfur compounds as high impact materials

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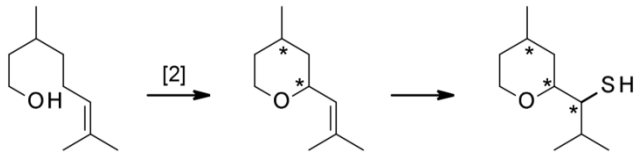
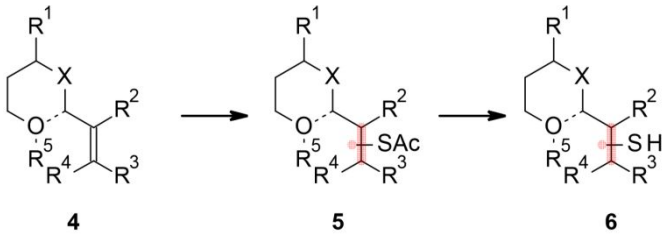
Modern perfumery constantly requires new, creativity-inspiring and powerful materials, which can generate a desired or unique effect in fragrance compositions. Fragrant ingredients with unusually high odor intensity at very low concentration, so-called high impact materials (HIM), help to broaden the creative palette of the perfumer as well as they help to consolidate the understanding of human odor perception and our sensory system. [1] Moreover, they fulfill sustainable criteria as they tremendously reduce the quantities of the used materials.

Herein, we present novel terpene based on organosulfur materials, which are related to the known floral fragrant ingredient Rose oxide **2**. The commercial Rose oxide **2** itself is terpene based and usually industrially made from Citronellol **1**, by e.g. photosensitized oxidation with oxygen (Scheme 1). [2] Starting with different isomeric mixtures of Rose oxide **2** or other starting materials **4**, the desired thiols **6** could be synthesized with high regio selectivity *via* a radical thiol-ene addition and subsequent saponification of the corresponding thioesters **5** in good yield and chemical purity (Scheme 2). [3] The sensory purity was confirmed by GC-sniff methods.

The odiferous compounds are reminiscent to citrus fruits, green, grapefruit and exotic fruits. Chemical and sensory stability in different media have been evaluated and show good performance in shampoo, EDT, detergent liquid and fabric softener.



Scheme 1: Conversion of Citronellol **1** into the desired thiol **3**.



Scheme 2: Synthesis and substrate scope of the fragrant intermediates **5** and fragrant thiols **6** (X = -CH_2-, -O-).

Based on the observed sensory results, we draw the conclusion, that especially β–oxygenated thiols perform very strong and pleasant. Moreover, for sensory impact it is advantageous in final products, to establish secondary or tertiary thiols.

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Approaches to the synthesis of curcumin analogues with tropane ring

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Curcumin (**1**) and its analogues are frequent research objects due to its anti-inflammatory, anti-antioxidant and anti-tumor activities. [1, 2] Despite desired biological activity and negligible side effects, low water solubility and fast degradation limit their potential application. Due to low bioavailability and stability of curcumin we decided to synthesize a range of its analogues containing tropane skeleton (**2**) possessing biological activity of this amine. [3]

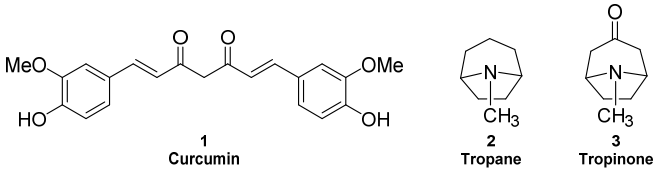
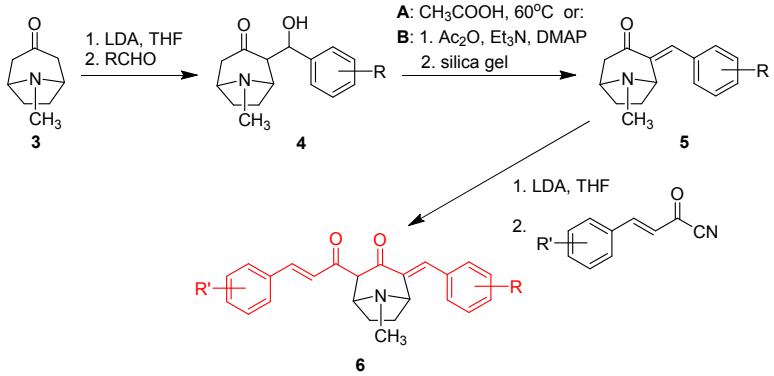


Figure 1. Curcumin, tropane and tropinone.

The first step of syntheses was aldol condensation of tropinone (**3**). Unfortunately this reaction was not trivial because of formation *α,α'*-bisaldol as the major product, even when the excess of ketone was used. [4, 5] The best results were obtained when LDA was used as a base and resulting aldol eliminated in acetic acid. Even in those conditions some retro-aldol product was observed. For this reason in case of acid sensitive and containing nitro group substrates, the aldol was acetylated and resulting ester eliminated on silica gel. The change of the leaving group significantly increased the yield of product **5** (Scheme 1).



Scheme 1. Syntheses of curcumin analogues containing tropane structure.

In the final step enones **5** were *C*-acetylated using cinnamoyl cyanide or another aromatic cyanide giving appropriate curcumin analogues **6** with good yields.

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New latent metathesis catalyst

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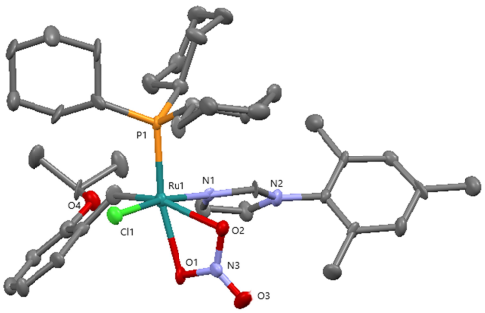
Olefin metathesis is a versatile and powerful tool in modern organic chemistry. Inferring from the Greek words *meta* (change) and *thesis* (position), metathesis means the double exchange of parts of two substances [1]. This reaction is used for the formation of carbon-carbon double bonds by mutual exchange of alkylidene groups between olefins. In 2005 Chauvin, Grubbs and Schrock, shared the Nobel Prize in Chemistry for the development of a metathesis method in organic synthesis [2]. Their discoveries caused great effects on academic research in the development of new polymeric materials and industrial synthesis as well as syntheses of drugs and other biologically active compounds including isoprenoids [3, 4].

In the presented research a new olefin metathesis catalyst was synthesized. The complex has been fully characterized, including X-ray analysis. In addition, the catalyst application profile has been determined.



New olefin metathesis catalyst.

After activation with chemical agents (*e.g.* HCl or TMSCl) the catalyst promote ring-closing metathesis (RCM), enyne and cross-metathesis (CM) reaction. The addition of HCl converts the complex into its active form, that allows to perform RCM of diethyl diallylmalonate very efficiently. An important advantage of new catalyst is its durability in storage and transport, as well as its stability towards air and moisture. It should be noticed that activation by HCl is simple and inexpensive.

The described catalyst can potentially be used in the synthesis of steroidal macrocycles or other isoprenoids.

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Synthesis and antimicrobial properties of steroid based imidazolium salts

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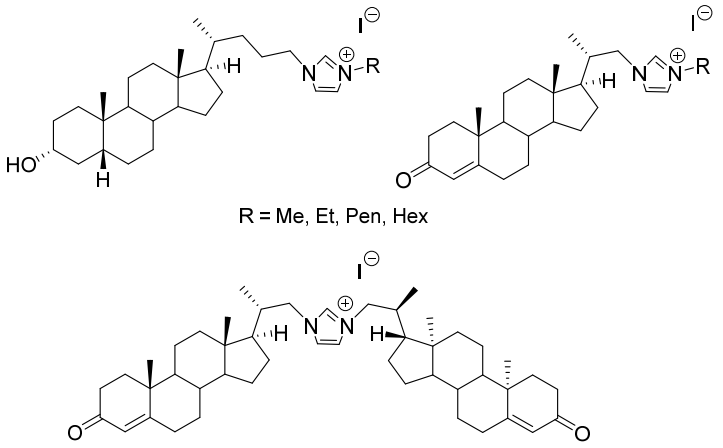
Imidazolium salts, which consist of discrete cation and anion pair, are very important derivatives of imidazole. Their biological activity is connected with ionic character, the presence of azole core and various substituents attached to nitrogen atoms [1]. It should be noted that binding two bioactive molecules as a way to improve biological properties of starting compounds is an emergent practice in medicinal chemistry [2]. In this context it was expected that a compound formed by combining an imidazole moiety and biologically active steroid may enhance biological properties of both fragments. We designed and synthesized series of imidazolium salts of lithocholic acid (LCA) and compound similar to one of LCA metabolites [3] with oxidized 3-hydroxy group and shorter side chain – 3-oxo-23,24-dinorchol-4-en-22-al.



Steroid based imidazolium salts.

New imidazolium salts were biologically tested to evaluate their antibacterial and antifungal properties. For this purpose, four microbial strains (*Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 14579 and *Candida albicans* ATCC 10231) were subjected to these studies. The antimicrobial efficiency was measured by bacterial and fungal growth inhibition expressed as minimal inhibitory concentration (MIC) values. The activities of new salts, especially in relation to Gram-positive bacterial and fungal strains are comparable to the activities of known antibiotics.

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Stereoselective synthesis of α-methylketones from esters and alkenes *via* cyclopropanol intermediates

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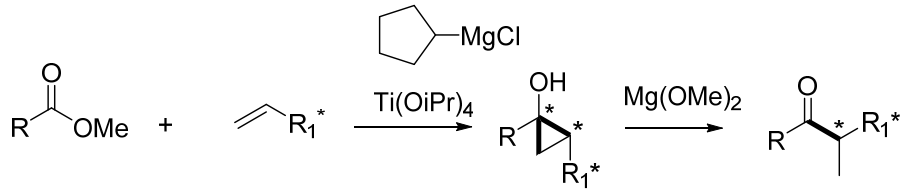
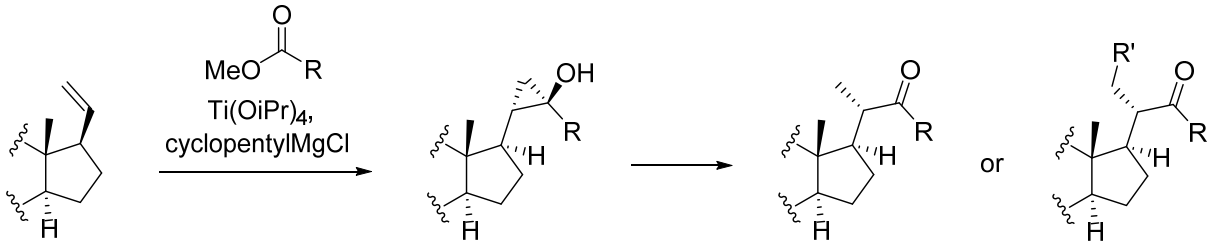
Numerous natural products including those possessing important biological activities bear chiral α-methyl substituted ketone units. In this poster, our results on stereoselective synthesis of α-methyl ketones *via* cyclopropanol intermediates will be presented. We have found a new group of alkenes that undergo Kulinkovich hydroxycyclopropanation with high diastereoselectivity, and developed a new mild protocol for isomerization of the obtained cyclopropanols to the target fragment. The developed approach was successfully applied in synthesis of steroids.

Stereoselective synthesis of α-methyl ketones:



Application of the developed approach in synthesis of steroids:



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Synthetic studies toward Swinhoeisterol A

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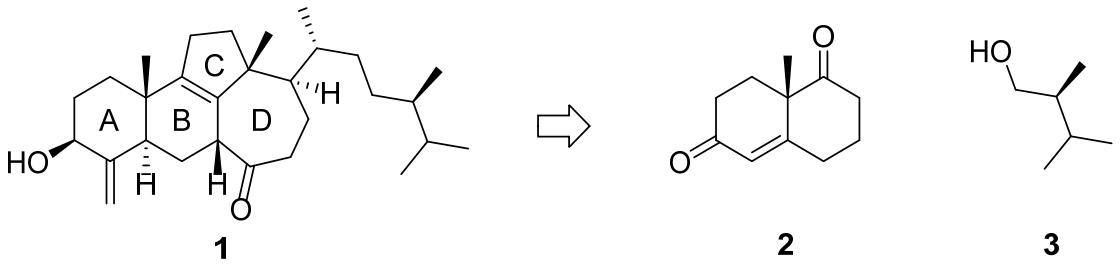
Swinhoeisterols are natural compounds produced by the sponge *Theonella swinhoei.* [1] The structure of these sterols is unique due to presence of 6/6/5/7 tetracyclic ring system. Swinhoeisterol A (**1**) is a cytotoxic compound which also inhibits (h)p300, a histone acetyltransferase associated with the manifestation of cancer. In this poster, synthetic studies toward construction of the unprecedented core of **1** starting from Wieland-Miescher ketone (**2**) will be presented. Enantioselective organocatalytic synthesis of alcohol **3**, which is necessary for construction of the side chain, will be also described.



1 **2** **3**

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Synthesis of brassinosteroid/ecdysteroid hybrids

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Brassinosteroids [1] and ecdysteroids [2] are two classes of steroid hormones that exert their action on plants and insects, respectively. The common structural features of these molecules are hydroxy groups at position C-2, C-3, and C-22, as well as a carbonyl group at C-6 (Fig. 1).

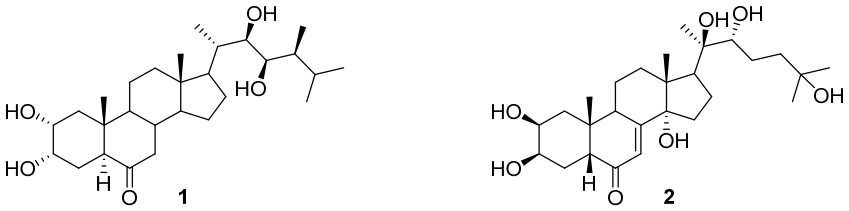

Fig. 1. Structures of castasterone (**1**) and ecdysterone (**2**), belonging to brassinosteroids and ecdysteroids, respectively.

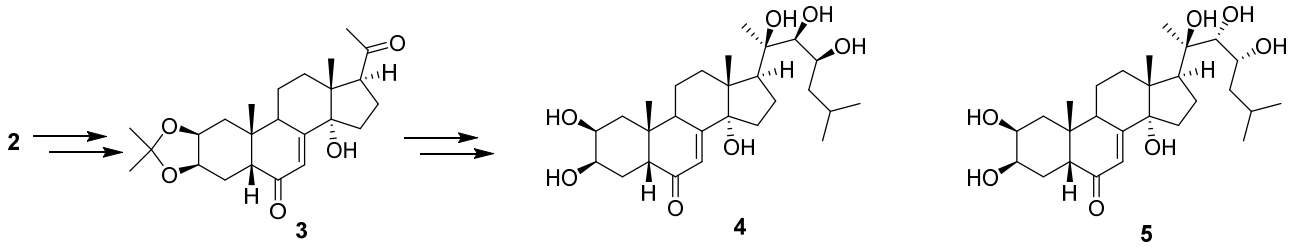

Fig. 2. Synthesis of brassinosteroid/ecdysteroid hybrids **4** and **5**.

The structural similarity of these steroids poses a question about the ability of plants and insects to distinguish them. A great interest in this respect is the study of hybrid structures, combining features of brassinosteroids and ecdysteroids, to determine if they possess brassinosteroid-like, ecdysteroid-like, or both activities. [3, 4]
Synthesis of hybrids **4** and **5** (Fig. 2) from the esily available from ecdisterone (**2**) via ketone intermediate **3** will be presented in this poster.

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Synthesis and bio-activity of brassinosteroids modified with physiologically important carboxylic acids

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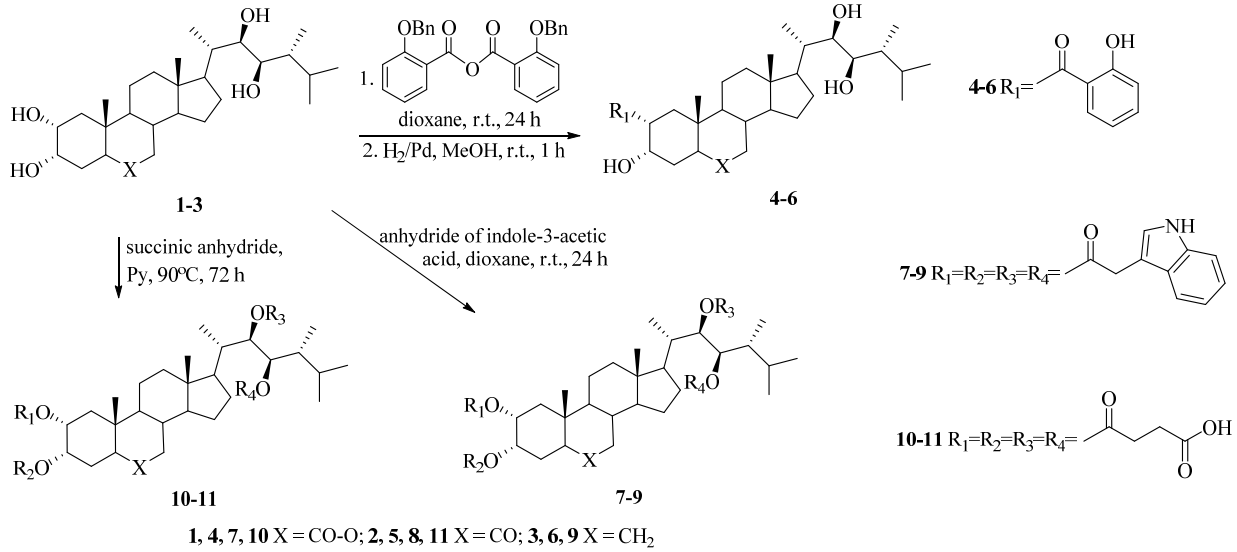
In continuation of our work on the synthesis and study of the activity of brassinosteroid esters with various acids [1-3], a number of physiologically important carboxylic acids esters with brassinosteroids of the 24*R*-methyl group was synthesized.

24-epibrassinolide **1** (EBl), 24-epicastasterone **2** (EBk) and 6-deoxo-24-epicastasterone **3** were used as starting materials. 2-Monosalicylates **4-6**, 2,3,22,23-tetra(indol-3-yl-acetoxy) derivatives **7-9** and 2,3,22,23-tetrasuccinates **10**, **11** were obtained *via* the reaction of BS with anhydrides of the corresponding carboxylic acids.

2-*O*-Benzylsalicylic acid was prepared from salicylic acid according to [4]. 2-*O*-Benzylsalicylic anhydride obtained *in situ* from the acid was used as a reagent. The reaction proceeded for 24 h at room temperature in dioxane with a slight excess of anhydride. After that, the benzyl protection was removed by hydrogenolysis in MeOH over a Pd catalyst. Tetrasubstituted derivatives **7-9** were synthesized by the interaction of BS with indolyl-3-acetic anhydride prepared *in situ* from the acid. Tetrasuccinates **10**, **11** were obtained by the action of large excess of succinic anhydride on EBl **1** and EBk **2** in pyridine at 90 °C for 72 h.



Bio-activity of synthesized BS conjugates will be discussed.

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Synthesis of fluorescent labelled betulinic acid derivatives

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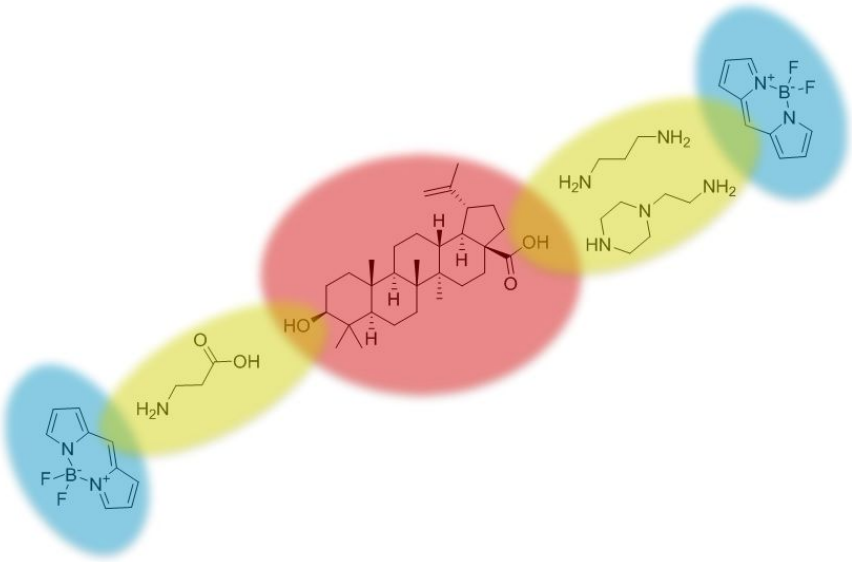
Betulinic acid (BA) is a natural pentacyclic lupane-type triterpene that exhibits a large scale of interesting biological activities. Selective cytotoxicity against a number of cancer cell lines and anti-HIV-1 activity make BA interesting and well accessible building block for the development of new drug. On the other hand, the therapeutic use is limited due to its low water solubility.

In this work we report preparation and study of series of new BA derivatives with improved solubility and potentiated activities. BA and its 3-*O*-(3,3-dimethylsuccinyl) derivative (bevirimat, a well known HIV-1 maturation inhibitor) were conjugated via three different linkers namely 1,3-diamino-propane, 1-(2-aminoethyl)piperazine and β-alanine with a fluorescent BODIPY dye. Used 8-amino-BODIPY is blue emitting fluorescent label with desired properties such as environment insensitivity and intense absorption and emission peaks. Prepared target compounds might serve as an efficient tool for fluorescent microscopy studies of both biological effect mechanism and localization. Newly synthesized compounds, including all intermediates are further investigated *in vitro* to evaluate their cytotoxic potency against different cell lines and anti-HIV activity.



This work was supported by the project MŠMT LO1304.

The development of novel LC-MS/MS method for the simultaneous determination of selected corticosteroids from human plasma

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Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex. They play crucial role in regulation of various physiological processes such as maintenance of whole-body homeostasis, stress response, immune response and regulation of carbohydrate metabolism. The disorders associated with their synthesis may lead to fatal consequences.

While analytical determination of estrogens (by derivatization of phenyl group) and androgens (by derivatization of carbonyl group) is commonly used, determination of some corticosteroids (11-deoxycortisol and 11-deoxycorticosterone) is still quite challenging as the methods of their analysis are financially and time-consuming and often burdened with inaccuracies. However, accurate determination of these cortisol and corticosterone precursors may help in the diagnosis of adrenal corticosteroid production disorders.

A sensitive and specific LC-MS/MS method for determination of selected corticosteroids has been developed and validated. The method uses reactivity of primary hydroxyl functional group and allows the simultaneous determination of selected corticosteroids.

Different extraction agents, derivatizing agents and reaction conditions were tried out. Also the chromatographic and the mass spectrometer conditions were optimized.

1 mL of human plasma was extracted with diethylether and derivatized with fusaric acid. The reaction mixture (fusaric acid, 4-dimethylaminopyridine, 2-methyl-6-nitrobenzoic anhydride, triethylamine, tetrahydrofuran) was incubated for 1 hour at 60 °C. The sample was dissolved in methanol with addition of 5 mM ammonium formate in water, separated by liquid chromatography and subsequently analysed using mass spectrometry. Deuterated internal standards were employed for the correction of losses during sample preparation.

The method will be helpful in the diagnostics of various pathophysiological conditions, especially in diagnosis of adrenal diseases.

This work was supported by the projects MH CZ - DRO (Institute of Endocrinology - EÚ, 00023761), MH CR 17-30528 A from the Czech Health Research Council and MŠMT LO1304.

Determination of neurosteroids in human serum and cerebrospinal fluid by immunoaffinity chromatography combined with UHPLC-MS/MS

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Neurosteroids are defined as steroids, which are synthesized in the CNS or are biologically active in the nervous system [1]. They play an important role in a number of physiological (anxiety, learning and memory processes) and pathophysiological (stress, neurodegenerative diseases) functions [2]. Knowledge of neurosteroid formation and their correct detection can be used to prevent and treat of some neurodegenerative (e.g. multiple sclerosis) and psychiatric diseases.

The aim of our study was to develop highly sensitive analytical method for identification and determination of neurosteroids in various body fluids, based on combination of immunoaffinity chromatography with UHPLC-MS/MS. Immunoaffinity columns were prepared by binding anti-neurosteroid polyclonal antibody to Affi-Gel® 10. Then we determined the optimal conditions for extraction and purification of neurosteroid from body fluids and for UHPLC-MS/MS analysis. Finally we analyzed the neurosteroids in blood serum and cerebrospinal fluid samples of patients with multiple sclerosis and presumed healthy donors. DHEA and testosterone levels differed only physiologically, but in female patients with multiple sclerosis, progesterone levels in serum have increased.

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The novel brassinosteroid analog BR4848 inhibits angiogenesis of human endothelial cells *in vitro*

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Searching for new agents with antitumor or antiangiogenic activity in humans has become an important approach nowadays within plant-derived compounds with effect at the cellular or molecular level. The phytohormones, brassinosteroids (BRs), play an important role in hormone signalling and physiological response in the plant organism. Recently, cytotoxic effects of natural brassinosteroids and their synthetic analogs in human cancer cells derived from tumours and in primary endothelial cells *in vitro* were determined by our group.

New synthetic brassinosteroid analog 2α,3α-dihydroxy-6-oxo-5α-androstan-17β-yl *N*-(tert-butoxycarbonyl)-D,L-valinate (BR4848) inhibited migration of human umbilical vein endothelial cells (HUVECs) after 20 h of treatment. Furthermore, the developed analog exhibited *in vitro* antiangiogenic activity in HUVECs. BR4848 reduced cell adhesion, migration, proliferation and tube formation of endothelial cells by inhibition of FAK, Erk 1/2, CDK5, VEGFR2, TNFα-stimulated production of inducer of endothelial cell migration and proliferation IL-6, and subsequently of angiopoietin-2 and Jagged1.

This work was supported by projects of the Ministry of Education, Youth and Sports CR NPUI LO1204 and 14-27669P of the Grant Agency of the Czech Republic.

Study on the reaction of diosgenin acetate with trimethylsilylazide catalyzed by Lewis acids

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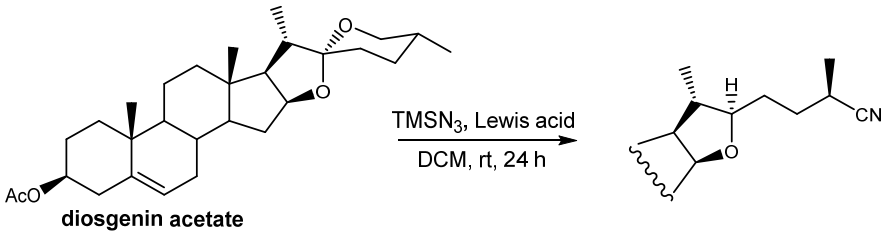
Steroidal sapogenins are the well-known class of natural compounds, widely distributed in plants, especially as glycosides. They exhibit various biological activities as well as they are important starting materials for the syntheses of drugs and different steroid derivatives. [1] The cleavage or the modification of the rings EF spiroketal moiety of sapogenins is a pivotal transformation for the synthesis of different bioactive steroids. Until now a lot of methods of the F-ring opening in steroid sapogenins have been elaborated leading to the open chain derivatives of pseudospirostane or furostane type. Some of these investigations aimed at elaboration of a convenient strategy for the simultaneous F-ring cleavage and the nitrogen atom introduction in the C26 position. [2] Such transformation would provide a short and straightforward route to the synthesis of solasodine from diosgenin. As the spiroketal ring opening reactions are acid catalyzed, the group used as a nitrogen source should exhibit relatively high nucleophilicity and low basicity. So far various amides and sulfamides have been tested in the direct amination reaction of diosgenin spiroketal moiety. The other nitrogen reagent which shows good nucleophilicity under acidic conditions is azide. The introduction of an azide group at C26 instead of amide seemed to be a method of choice since 26-azidopseudodiosgenin can be transformed to solasodine in one step with TMSI. [3] Having this in mind we investigated a ring opening reaction of diosgenin acetate in presence of Lewis acids and trimethylsilyl azide as a facile protocol for synthesis of an open chain derivative with a nitrogen-containing substituent at C26. Furostane 26-nitrile was obtained stereoselectively as the main product (Scheme 1). According to the best of our knowledge, it is unprecedented synthesis of a nitrile derivative directly from sapogenin.



Scheme 1. Ring opening reaction of the steroidal spiroketal with $TMSN_3$ in presence of Lewis acid.

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Enantioselective retro-aza-Michael reaction in continuous flow

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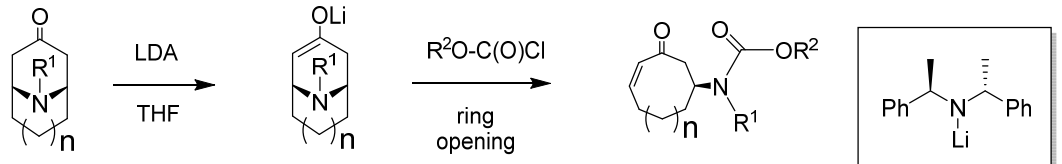
The stereoselective reactions are very important for preparation of optically pure product. This is particularly desirable in the synthesis of biologically active compounds. In principle, a variety of compounds such as 8-membered deoxycarbosugars or homotropan alkaloids could be obtained from functionalized 8-membered ring intermediates (shown in the scheme) if they were readily available.

The retro-aza-Michael reaction of tropinone and granatanone was used for the preparation of substituted 7- and 8-membered rings (scheme). It has been observed that the reaction of tropinone gives the products in good results [1], but the reaction of granatanone (tropinon homologue) gives very low yields or no product [2]. This significant differences in the reactivity of granatanone and tropinone could result from various stereochemical preferences (axial or equatorial position) of substituent at the amine nitrogen in these aminoketones [3].



Tropinone: R^1 = Me, n = 0
N-Benzylnortropinone: R^1 = Bn, n = 0
Granantanone: R^1 = Me, n = 1

retro-aza-Michael product

It turned out that the problem of low conversion can be solved using a reaction in the flow conditions. Due to many advantages, the flow synthesis becomes more and more popular [4]. However there are few examples of using this technique in enantioselective reactions at low temperatures [5]. The performance of the retro-aza-Michael reaction is much better in the flow reactor compared to the results obtained in solution [2]. The results of enantioselective version of the reaction will be presented. Use of the chiral lithium amide in the reaction with granatanone shown in the scheme resulted in low to very good enantioselectivities (19-98% ee for the product of reaction with benzyl and propyl chloroformate).

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26-Thiodiosgenin derivatives oxidated and substituted in the α position

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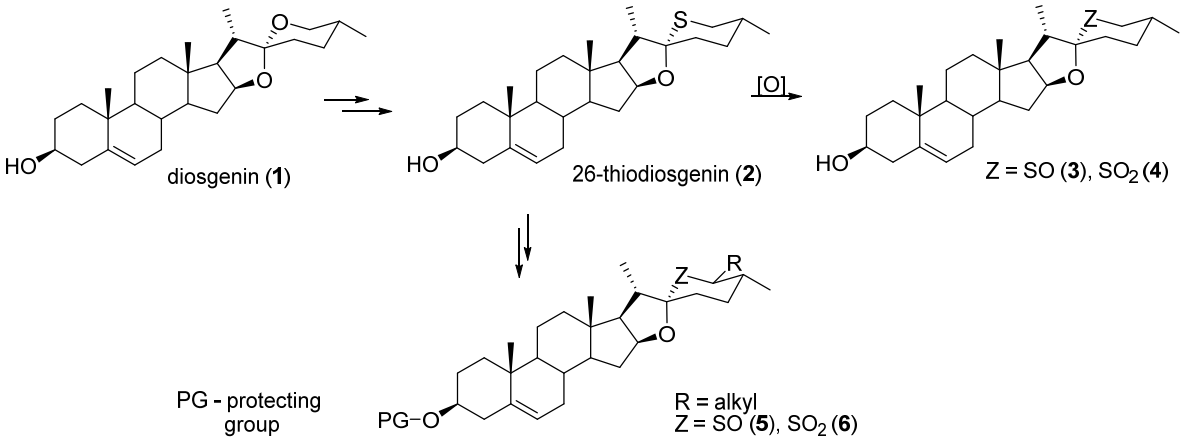
Diosgenin (**1**) and its glycosyl derivatives (saponins) have many interesting biological properties: hemolytic, anti-inflammatory, antibacterial, antifungal and cytotoxic. [1] Scientist are searching for compounds with more favorable therapeutic properties compared to naturally occurring substances. For this purpose, they modify both the sugar and steroid parts of saponins. An example of such a modification was the replacement of an oxygen atom with a sulfur atom in the F ring of diosgenin. [2] The obtained compounds showed similar or slightly higher activity than their oxygen analogs. Since literature reveals much higher cytotoxicity of sulfoxides and sulfones compared to sulfides [3], it can be expected that the oxidation of the sulfur atom in 26-thiodiosgenin (**2**) will also have a positive effect on its biological properties. Due to the presence of acidic protons in the α position, the obtained oxidized forms **3** and **4** can be easily alkylated at this position. A series of sulfoxide and sulfone derivatives have been obtained this way.



Preparation of oxidized and alkylated at C26 derivatives of 26-thiodiosgenin.

All obtained products (**3**, **4**, **5** and **6**) will be subjected to biological tests.

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Synthesis of solasodine analogs *via* Staudinger reaction

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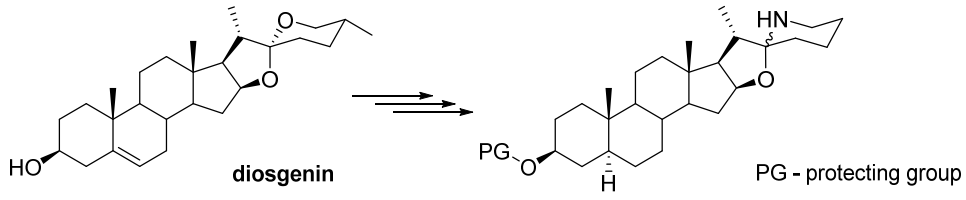
Over 250 million people living mainly in Africa (85% of all cases) suffer from schistosomiasis, a parasitic disease caused by trematode flukes of the genus *Schistosoma*, which is next to malaria the most serious parasitological health problem in the world [1]. One of the methods of combating this, despite many limiting factors, is the elimination of the host snails using synthetic molluscicides [2].

The recent phytochemical investigations indicate that the aqueous extracts of the berries *Solanum aculeastrum* (Solanaceae) are potent to snails [3], because they contain active saponins such as β-solamarine and solamargine. Further studies have shown that these glycosides can also treat gonorrhea [4]. In addition, methanolic extracts of the root bark of *S. aculeastrum* show the presence of a less active steroid saponin – solaculine A, whose weakened effect was due to the structure of polysaccharide rather than the type of aglycone (tomatidenol instead of solasodine) [5].



This presentation reveals the synthetic route to solasodine and tomatidine analogs that may exhibit similar cytotoxic properties as their parent saponins.

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Synthesis of disteroidal macrocyclic molecular rotors with androstane frameworks

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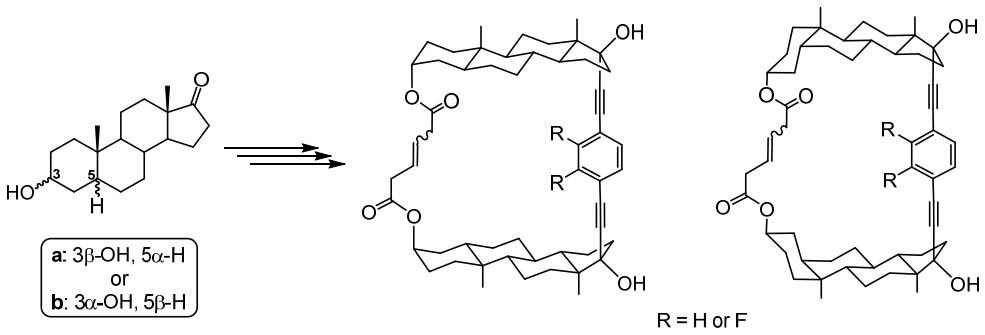
The design of applicable artificial molecular rotors is performed on the basis of structural and dynamic analogies between macroscopic objects and small molecules, trying to integrate them into organized assemblies that can carry out significant work on their immediate environment. The latter was inspired by desirable features presented by biomolecular machines, such as ATP synthase or skeletal muscle [1].

Within this field, the synthesis of compounds having a wheel-and-axis molecular architecture, ensuring controlled rotary motion, was considered [2]. Relatively small group, termed *rotator*, linked to some voluminous groups (called *stators*) with larger moment of inertia would be the most suitable. In this purpose, we have synthesized artificial molecular rotors based on steroidal molecules capable of performing dynamic processes in the crystalline state [3]. Each of the synthesized structures resembles the construction of a child's spinning top toy.



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Oxyfunctionalization of androstenedione by oxidoreductases

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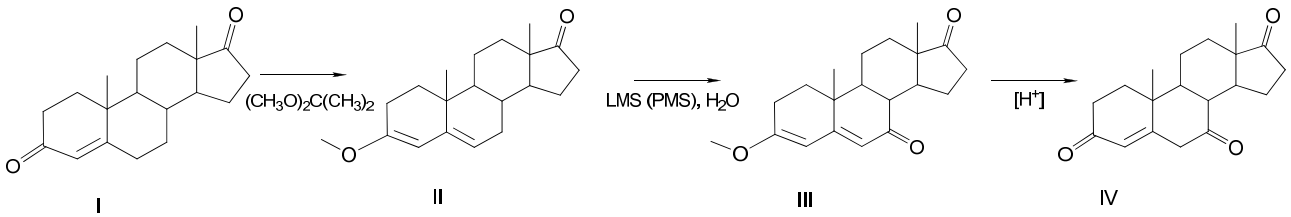
Laccases (E.C.1.10.3.2) are copper-containing oxidoreductases that specifically catalyze the oxidation of a wide range of organic substrates to the corresponding radicals, using molecular oxygen as the final electron acceptor [1, 2]. The reactive radical species rapidly react further to various oxidation products forming water as an only by-product. Recently, we demonstrated efficiency of laccase-mediated systems (LMS) in oxyfunctionalization of 3β-ol-5-ene steroids, such as dehydroepiandrosterone and pregnenolone [3].

In this study, oxyfunctionalization of 3-keto-4-ene steroids by selected oxidoreductases, i.e. laccases and peroxidase (E.C.1.11.1.7) was investigated with special attention to position 7 of the steroid skeleton.

We found that 3-hydroxy-Δ^5-steroids with active allylic hydrogen at C-7 were oxidized by enzyme-mediator systems based on the horseradish peroxidase (PMS) with regiospecific introduction of hydroxylic group at C-7 and further oxidation of the latter to keto-group. However, no LMS and PMS oxidation products were formed from 3-keto-4-en-steroids of androstane and pregnane series (androstenedione (I), testosterone, 9-hydroxy-androstenedione, progesterone) thus indicating-importance of mobile allylic hydrogen in steroid molecule for the reaction. We managed to activate position 7 in 3-oxo-4-en-steroids by preparing of the enol ethers of the steroidal Δ^4-3-ketones with displacement to $\Delta^{5,6}$-double bond (Scheme, I → II). The ethers were stable under bioconversion conditions.



Laccase and peroxidase mediated oxidation of androst-4-en-3,17-dione with 7 activation.

Enzymatic oxidation of methyl ether of the enol forms of AD (Scheme, II → III) were carried out in aqueous solutions pH = 5.0 at 40 °C by fungal laccase (*Trametes versicolor*) and pH = 7.0 at 40 °C by horseradish peroxidase with the use of radical mediator – hydroxybenzotriazole. Both LMS and PMS oxidation of II led to 3-methyl enol ether of 4-androstene-3,7,17-trione (III) as a major product. 7(α/β)-Hydroxy-3-methyl enol ether of 4-androstene-3,17-dione was observed as intermediate products of LMS (PMS) oxidation.

Further studies will be focused on the development of new route for green preparative synthesis of 7-keto-derivatives of 3-keto-4-en-steroids of androstane and pregnane series.

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Synthesis and search for disteryl ethers after high temperature treatment of model rich-sterol samples

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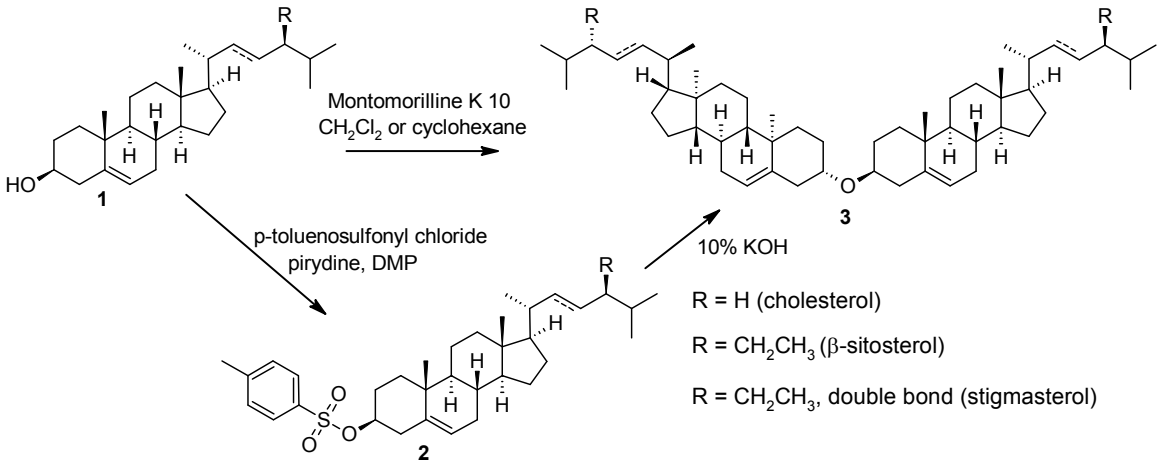
It has been proven that sterols during high temperature treatment can undergo various reactions. Main reaction occurring in sterols, both vegetable and animal origin, is oxidation by radical or reactive forms of oxygen. However, it has also been proven that various sterols subjected to high temperature treatment, can be concatenated, which results in new polymeric structures [1, 2]. Such reaction was studied only on heating sterols themselves. It seems appropriate to conduct study, which will reveal if there is a possibility to form such compounds in biological samples. The aim of the study was to prepare suitable chemical synthesis to obtain such dimeric products in order to search those compounds in biological samples. Synthesized molecules also will allow in the future to evaluate their biological properties and can be used as a starting molecule to synthesize oxidized disteryl ethers.

The appropriate synthesis and purification method were conducted, which resulted in obtaining three dimeric products – 3β,3β'-dicholesteryl ether, 3β,3β'-disitosteryl ether and 3β,3β'-distigmasteryl ether. Molecular structures were confirmed by NMR (^{1}H and ^{13}C). Using prepared compounds sample preparation and instrumental method (GC-MS) was developed and validated in order to evaluate formation of those compounds in model samples.



Scheme 1. Scheme for synthesis of 3β,3β'-disteryl ethers.

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The application of multidimensional NMR analysis to *cis*/*trans* isomers study of menaquinone-7 (vitamine K_2MK-7), identification of the *(E,Z3,E2,ω)*-menaquinone-7 isomer in dietary supplements

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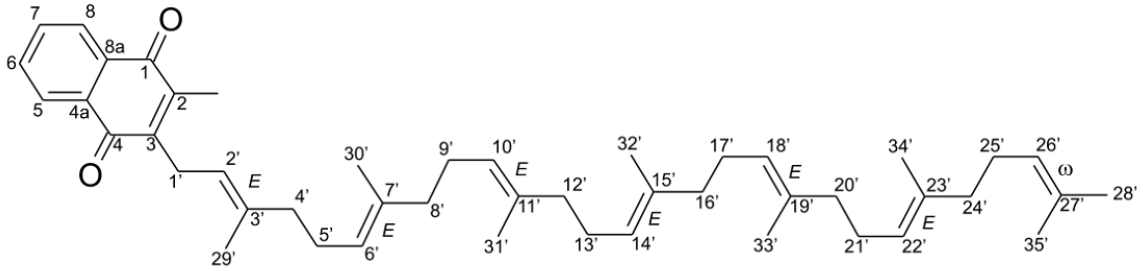
Vitamin K plays an important role in many physiological processes in animals, plants, and bacteria organism. Naturally it exists in two forms: K_1 (phylloquinone or phytomenadione) and K_2 (menaquinone). The menaquinone, usually denoted as MK-n where n is the number of isoprenoid units (from 1 to 14), is a group of compounds with poly-isoprenoid chains [1]. The MK-4 and MK-7 are currently the most important forms of vitamin K_2 used in food supplementation (with constantly growing interest over MK-7). Natural menaquinones have all *E* configurations of double bonds [2], but some commercial preparations are mixtures of compounds with *E* (*trans*) or *Z* (*cis*) configurations of individual double bonds in isoprenoid units [3].

The aim of this work was to determine the chemical structure of *cis/trans* isomers of vitamin K_2MK-7 found in dietary supplements. Vitamin K_2MK-7 was extracted, along with contamination in the form of *cis/trans* isomers of menaquinone-7, from dietary supplements through tetrahydrofuran and then subjected to fractionation using the semi-preparative chromatography column with cholesteryl group phase. The obtained fractions of *cis/trans* isomers of menaquinone-7 underwent analysis with the use of multidimensional NMR methods optimized for maximum signal separation. NMR analysis made it possible to identify the first geometric isomer of (*E,Z3,E2,ω*)-menaquinone-7, which underwent NMR comparative analysis with a known "full *trans*" isomer. It was also possible to confirm the presence of other *cis/trans* isomers in menaquinone-7 in the collected fractions.



Structure of K_2MK-7 all *trans* isomer (*E6,ω*)-menaquinone-7.

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Synthesis of 18-nor-17β-hydroxymethyl-17α-methylandrost-13-enes using visible-light promoted photoredox decarboxylation as a key step

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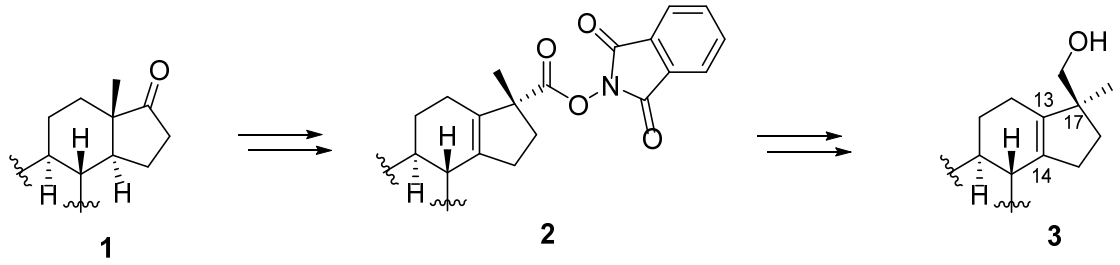
18-Nor-17β-hydroxymethyl-17α-methylandrost-13-ene unit **3** is presented in long-term metabolites of the prohibited in sports androgenic anabolic steroids. [1] Such metabolites are on demand in antidoping analysis as reference compounds. Chemical synthesis of **3** is a challenging task because of simultaneous presence of C13-C14 tetrasubstituted double bond and the quaternary stereocenter at C17. In this paper, a new approach to such steroids is presented. The key step in our synthesis is a visible-light promoted decarboxylative alkylation of a phtalimide ester **2**, which proceeds both regio- and stereoselectively.



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Study of the effects of brassinosteroids in combination with cisplatin on cancer cell growth

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Absolute majority of known antitumor drugs have pronounced side effects, which limits their use. They are: immunosuppression and myelosuppression, organotoxicity, tumor lysis syndrome, peripheral neuropathy and many others [1]. This causes the need of searching for new compounds with minimal destructive side effects or compositions that can reduce them.

During the last two decades evidences appeared that plant hormones brassinosteroids (BS) can inhibit cancer cell growth and tumor angiogenesis, have low cytotoxicity to normal cells and other beneficial effects in mammals, such as neuroprotective, antiviral, anabolic and adaptogenic, immunostimulating, anti-inflammatory, woundhealing, etc [2]. All that refer that BS can not only be perspective antitumor agents, but also can minimize side effects and abate toxicity of existing chemotherapeutics.

In this work, we evaluate in what way some natural BS (24-epibrassinolide and 28-homocastasterone) and their synthetic analogs ((22*S*,23*S*)-24-epibrassinolide and (22*S*,23*S*)-28-homocastasterone) (Fig.1) influence on the cytotoxicity of classical antitumor drug cisplatin.

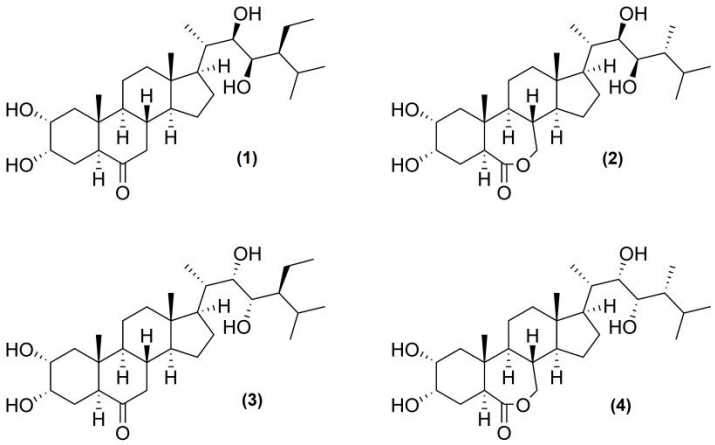

Figure 1. Studied compounds: (**1**) - 28-homocastasterone, (**2**) - 24-epibrassinolide, (**3**) - (22*S*,23*S*) - 28-homocastasterone, (**4**) - (22*S*,23*S*)-24-epibrassinolide.

It was found in two cancer cell models A549 (lung carcinoma) and HepG2 (hepatocellular carcinoma) that all studied BS (except 28-homocastasterone) in combination with cisplatin inhibit cell growth more effectively than cisplatin alone. 10^{-6} M of synthetic BS reduce cisplatin's IC_{50} by almost 2 times. Flow cytometry data on distribution of cell cycle phases showed that combination of synthetic BS with cisplatin reduces the amount of cells in S-phase causing the cell cycle arrest.

The obtained results suggest that biological activity of brassinosteroids strongly depends on the dose used and confirm that cisplatin combined with brassinosteroids can be more effective in cancer therapy. This combination as well can be useful in overcoming negative consequences of chemotherapy by reducing the effective doses of drugs.

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Towards the synthesis of long-term metabolite of Oral-Turinabol for revelation of doping abuse at Beijing and London Olympics

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Recently re-analyzed doping control samples collected at Beijing (2008) and London Olympics (2012) revealed adverse analytical findings (AAF) that remained undetected before. Due to instrumental developments that provided higher sensitivity as well as recent research in long-term metabolism about 10% of these samples now traced back an administration of a prohibited doping substance. One of the compounds most often uncovered herein was dehydrochloro-methyltestosterone (DHCMT, Fig. 1). It was initially developed as doping substance (active pharmaceutical ingredient (API) of Oral-Turinabol®) in the GDR and obviously experienced a revival in recent years [1]. Due to research activities over the last ten years new metabolic pathways have been proposed for 17-methylated androgens that lead to further metabolites with previously unforeseen structure modifications [2-5]. Even if excreted in extremely low concentrations in the urine, these metabolites revealed to be detectable for a largely extended time window after administration. Thus, tracing back a prohibited administration was possible in the stored doping control samples.

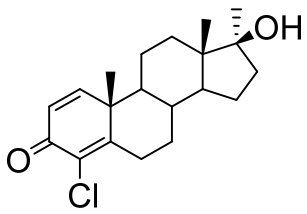

Fig. 1. DHCMT (API in Oral-Turinabol®).

Synthesis of reference material of DHCMT metabolite structures is planned in a combined chemical and biotechnological approach. Different isomeric 4-chloro-17,17-dimethyl-18-nor-13-ene steroids were synthesized as future substrates for 20β-hydroxylation.

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LC-MS determination of osladin in Polypody rhizome

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Polypodium vulgare L. (Polypodiaceae) is a fern used in the traditional Polish medicine as a diuretic and as an expectorant to treat cough, pertussis as well as renal diseases [1]. The osladin (Figure 1) a plant sweetening agent that is 500 times sweeter than sucrose was isolated for the first time from the rhizome of the Polypody in 1971 [2]. Its stereochemistry was fully assigned by Nishizawa and co-workers through a single-crystal X-ray diffraction study of a natural sample and a chemical synthesis [3-4].

Due to the fact that osladin molecule is poorly monitored by UV detector we have elaborated a LC method with MS detector for its evaluation in plant material. All analyses were performed on the Thermo Scientific UHPLC Ultimate 3000 apparatus coupled with the ESI-qTOF mass detector. Identity of osladin was determined by HRMS: $[M-H]^-$ 885.4898 m/z (found) vs. 885.4853 m/z (calcd); equivalent to $C_{45}H_{74}O_{17}$ for neutral molecule. As osladin possesses in its structure the sugar moieties that have influence on water solubility, both the water and the hydroalcoholic extracts were investigated. We have found out that the lowest osladin concentration at polypody rhizome was 0.075 mg/g whereas the highest concentration reached up to 1.8 mg/g.

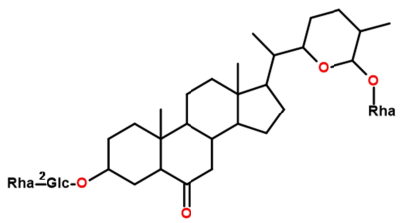

Figure 1. Osladin structure.

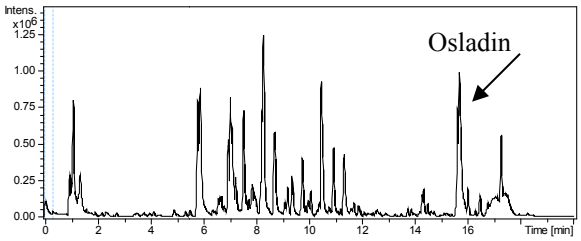

Figure 2. LC-MS chromatogram of Polypody rhizome water extract.

There were only small differences in osladin content between the water and hydroalcoholic extracts. As a result of our study, osladin can be used as an analytical marker for polypody rhizome standardization.

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In search of plant rich in desired compound: primrose case

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In order to improve the quality and value of medicinally important crops both well-known strategies (environment modification) and modern genetic manipulations are applied. The up-to-date trends are focused on soil microorganisms and mycorrhizal fungi. The second strategy to increment the production of key secondary metabolites is a selection of better-yielding relative species. The last approach may still work successfully due to the fact that XXI century knowledge on the medicinal plants is still rudimentary.

The objective of this study was to screen the *Primula* L. genus for species producing the main active compound, primulasaponin 1 (Fig.1), in significant quantities. This triterpenoid glycoside is responsible for this herbal drug activity resulting in secretolytic and expectorant properties. Parts of only two plants belonging to this genus are presently accepted as official herbal medicines [1-3].

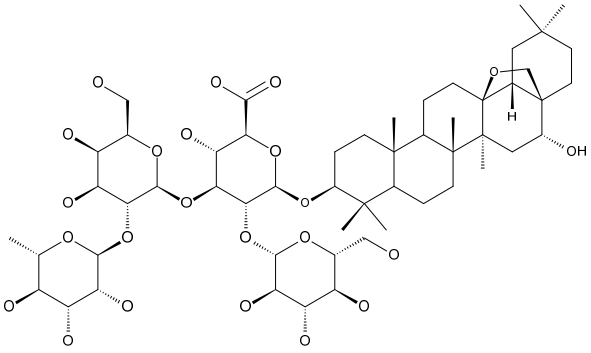

Fig.1. Structure of primulasaponin 1.

The standard of primulasaponin 1 was isolated from pharmacopoeial drug by LC and FC. Its identity was confirmed by HRMS, MS/MS and NMR while its purity was determined by q-HNMR. The roots of the authenticated plants were sequentially dried, milled, extracted with 70% MeOH, filtered and diluted to obtain samples applicable for the assay. MS detector was calibrated prior to each UHPLC analysis to guarantee the accuracy.

The appropriate UHPLC-MS method was developed with the best linearity in the range of 0.05-5.0 µg/mL. Analysis of about 120 species revealed that 8 species of primroses accumulate over 5% of dry weight of primulasaponin 1 in their roots. In one example, the concentration of this compound was significantly higher (exceeding 20% of dry weight). Moreover, the highest yielding species grows easily and produce almost this single metabolite. This study shows that the plant screening may lead to discovery of new species utilisation.

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Synthesis, characterization and antiproliferative properties of 2,3-seco-analogues of brassinosteroids

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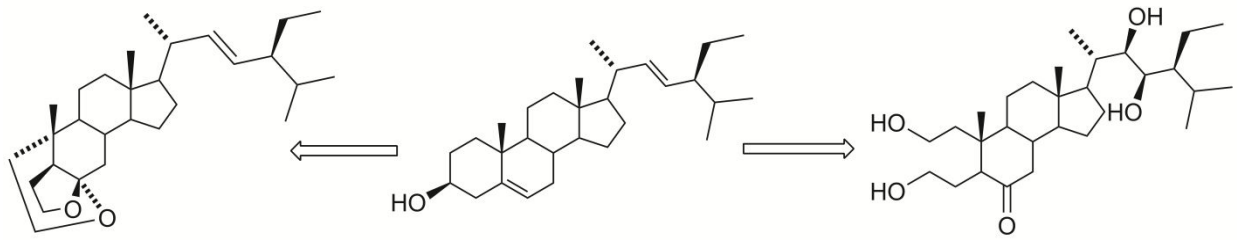
Brassinosteroids (BRs) represent a large group of plant steroids which include more than 70 structurally and functionally related compounds. [1, 2] The most important BRs are castasterone and brassinolide and their 24-epimers and 28-homoanalogues. BRs have been found in a wide range of plant species, including higher and lower plants. They demonstrate various kinds of regulatory action on the growth and development of plants. [3] BRs have structures similar to those of animal steroid hormones. Despite their mostly studied activity on plants, they also act as antiproliferative and antiangiogenic agents in human cancer and/or endothelial cells with the influence on steroid receptors. [4]

Synthesis and structure-activity relationship analysis of a two groups of 2,3-seco analogues of BRs were performed to examine their antiproliferative activities. Two steroid skeletons were chosen for the preparation of seco analogues – cholestane and stigmastane (see scheme). The synthetic strategy consists of multistep reactions and detailed analysis of compounds prepared. We have discovered unprecedented behaviour of 2,3-seco-2,3-dihydroxy-6-ketone leading to instability of seco-analogues of natural BRs.



General scheme of 2,3-secoanalogues preparation.

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Steroid hormones of plants in mineral deposits

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In recent years we have developed a number of enzyme-linked immunosorbent systems for the quantitative determination of brassinosteroids in plant samples and physiological fluids. Among them, systems for the determination of compounds of the series 24-epibrassinolide, brassinolide, 28-homobrassinolide, B-lactones, 6-ketones, and 6-deoxo-24-epibrassinosteroids [1-3]. In this report we present research data obtained using these systems for the determination of BS in 1 sample of peat and in 6 coal samples from various deposits in Belarus. It was noted that all studied samples contain brassinosteroids of the main groups – 24-epibrassinolide, brassinolide and 28-homobrassinolide (see Table). The results of the enzyme immunoassay were confirmed by tandem mass spectrometry (HPLC-MS/MS) on the example of two samples of brown coal with lignin of the Zhitkovichi deposit (**5** and **6**) differing the greatest content of all group of brassinosteroids.

Table – Brassinosteroids content in coal and peat samples

№	Sample, deposit	Depth of occurrence, m	Age, million years	Brassinosteroid content (ng/g)		
				24-epibrass-inolide group	brassinolide group	28-homobras-sinolide group
1	Coal brown soft Lelchitsy field, Belarus.	108	342	6,74 ± 0,04	4,01 ± 0,10	3,41 ± 0,29
2	Coal brown dense Lelchitsy field, Belarus.	162	342	9,90 ± 1,02	not detected	5,45 ±0,96
3	Coal brown dense Bukcha, Belarus	192	164-167	17,20 ± 0,59	5,51 ± 0,54	4,60 ± 0,42
4	Coal brown lignitic earthy Zhitkovichi field, Belarus	40-50	21-27	15,22 ± 2,36	7,42 ± 0,47	18,84 ± 0,50
5	Coal brown lignitic Zhitkovichi field, Belarus	40-50	21-27	17,58 ± 2,99	10,21 ± 0,68	60,84 ±7,46
6	Coal brown lignitic dense Zhitkovichi field, Belarus	40-50	21-27	11,57 ± 1,78	29,96 ± 4,06	56,06 ±11,10
7	Peat The Turshevka-Chertovo deposit of the Krupsky district, Belarus	0,2-0,4	0,004-0,006	37,2±4,97	6,57±0,438	10,5±0,599

Thus, based on the study of coal and peat samples, it can be concluded that these minerals, which are of vegetable origin, contain brassinosteroids in appreciable amounts. The brassinosteroid composition varies depending on the deposit, depth of bedding, and other factors. However, the quantitative content of brassinosteroids is comparable to that in plant objects and medicinal herbs [2]. Obviously, this can be explained by the high stability of the studied objects to transformations under the influence of environmental factors.

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The effect of seed treatment with brassinosteroid-glyphosate mixtures on root growth in fiber flax and spring barley seedlings

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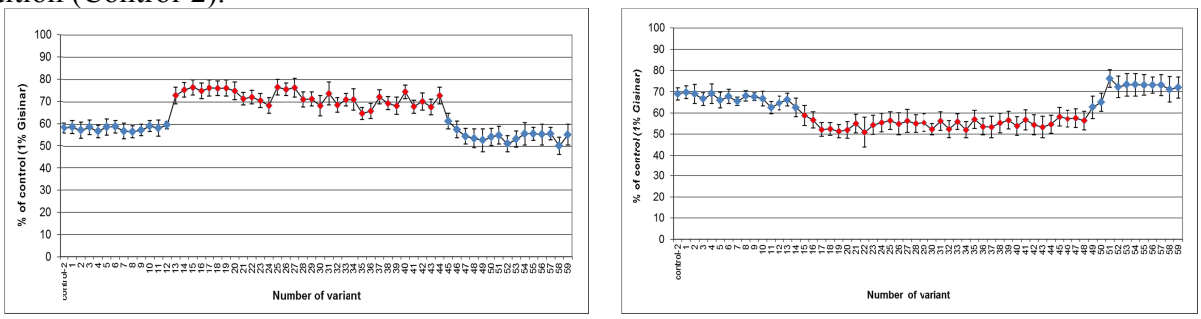
Brassinosteroids (BS) are the phytohormones known as stress adaptogens possessing pronounced growth-stimulating activity [1]. Interest in glyphosate is due to the relevance of studying the effect of hormesis and synergism in glyphosate compositions with physiologically active substances [2-3].

The aim of the study was to determine the «dose-effect» dependence in the action of mixtures of BS (castasterone and epicastasterone) and glyphosate on the seedlings' growth.

The objects of the study are spring barley (vr.Radzimich) and fibre flax (vr.Laska) seedlings. The seeds were incrusted with mixtures of the investigated agents. The inhibitory (40-60%) dose of glyphosate was $5{,}5 * 10^{-2}$ mol for spring barley and $3{,}3 * 10^{-2}$ mol for fibre flax. The range of concentrations of epicastasterone in the experiment was from 10^{-5} to 10^{-11} mol, castasterone – from 10^{-4} to 10^{-8} mol.

Working solutions for seed treatment were prepared by dilution of basic alcohol BS-solution by 1% water solution of film-forming material Gisinar (copolymer of acrylamide and sodium salt of acrylic acid) with a single-step of 1,25 times. Controls are: the variant with treatment with 1% solution of Gisinar (Control-1) and glyphosate in doses of $5{,}5 * 10^{-2}$ and $3{,}3 * 10^{-2}$ mol without BS addition (Control-2).



Root length of seedlings of fiber flax (1) and spring barley (2) depending on the concentration of epicastasterone in mixture with glyphosate.

Intervals of concentrations in which BS increased the effect of glyphosate in comparison with the control-2 were found. For barley seedlings they are from $5{,}9 * 10^{-7}$ to $4{,}1 * 10^{-8}$ mol (18 % below the control) for castasterone and from $8{,}6 * 10^{-7}$ to $5{,}4 * 1\ 0^{-10}$ mol (17% below the control) for epicastasterone. Opposite to barley, fibre flax showed diminishing the growth-inhibiting effect of the herbicide in concentration range of epicastasterone from $4{,}4 * 10^{-7}$ to $3{,}4 * 10^{-10}$ mol (18 % above the control). The differences in the indicators are statistically reliable. Interval of interaction between castasterone and glyphosate on fiber flax seedlings was not found in the experiment.

It was also noted that castasterone in a concentration of $2{,}8 * 10^{-6}$ mol and higher inhibited the root growth of seedlings for both cultures.

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Conformational solvatomorphism of 23*E*-(3'-hydroxy-benzylidene)-tigogenin diacetate

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The modification of the lateral chain of sapogenins has received considerable attention since the middle of the last century, because those transformations have been employed to obtain a great number of bioactive molecules. In this field, our work group recently described the synthesis of 23*E*-benzylidenspirostanes, a new family of compounds that shows new modes of reaction and biological activity. [1]

Following our work to obtain new aromatic substituted benzylidene spirostanes, we have synthesized the novel 23*E*-(3'-hydroxy-benzylidene)-tigogenin diacetate, that was fully characterized by one and two-dimensional NMR experiments. More importantly, solid state characterization of this compound showed its great tendency to generate up to five inclusion complexes depending on the solvent used (dichloromethane/methanol, ethyl ether/hexane, benzene, 1-4-dioxane/water or DMSO) plus another crystal form without solvent when using either DMF/water or pyridine. This unexpected ability to crystallize with different solvent molecules, prompted us to evaluate the conformation and the thermal stability of the crystalline structures for each form. We have found that after desolvation promoted by heating, most of the crystal arrays undergo a phase transition towards the solvent-free form (Figure 1). Herein we describe the synthesis and characterization of this new compound 23*E*-(3'-hydroxy-benzylidene)-tigogenin diacetate and our studies of the crystal structures of each solvate.

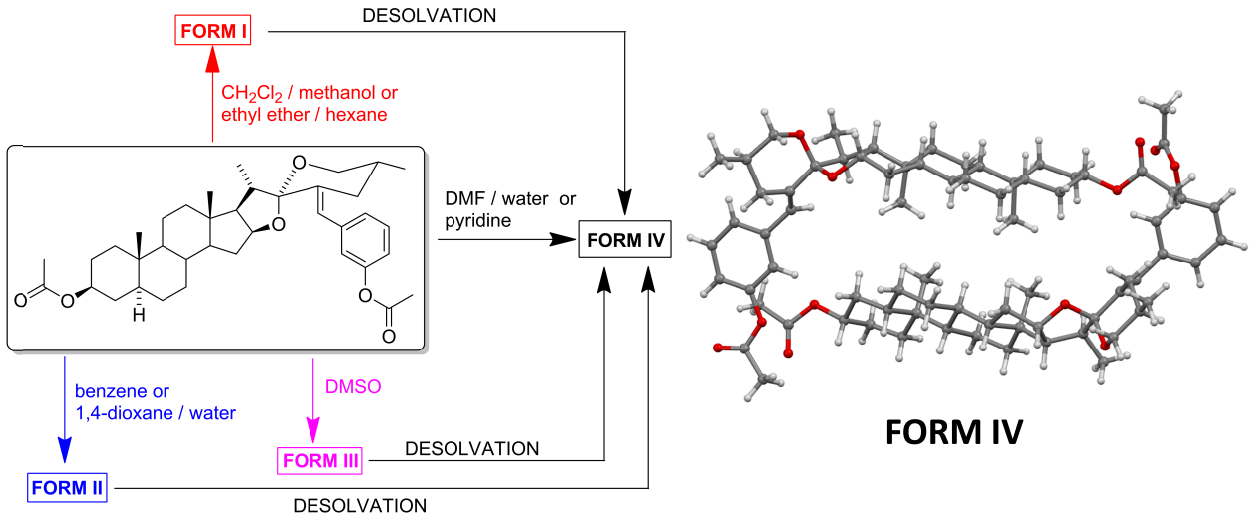

Figure 1. Crystal forms of 23*E*-(3'-hydroxy-benzylidene)-tigogenin.

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Palladium assisted synthesis of cytotoxic benzannulated steroid spiroketals

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Spiroketals are compounds that can be isolated from marine and terrestrial organisms. The growing pharmacological importance of compounds containing spiroketal assemblies has triggered an interest in their synthesis and chemical reactivity. [1] Benzannulated spiroketals constitute a more reduced subfamily that includes from simple compounds to structurally complex substances that have a variety of biological activities. [2]

Herein we describe the synthesis of steroids bearing benzannulated spiroketal moieties in the A-ring derived from cholesterol (**A**), diosgenin (**B**) and testosterone (**C**) and their cytotoxicity against human glioblastoma cell line.

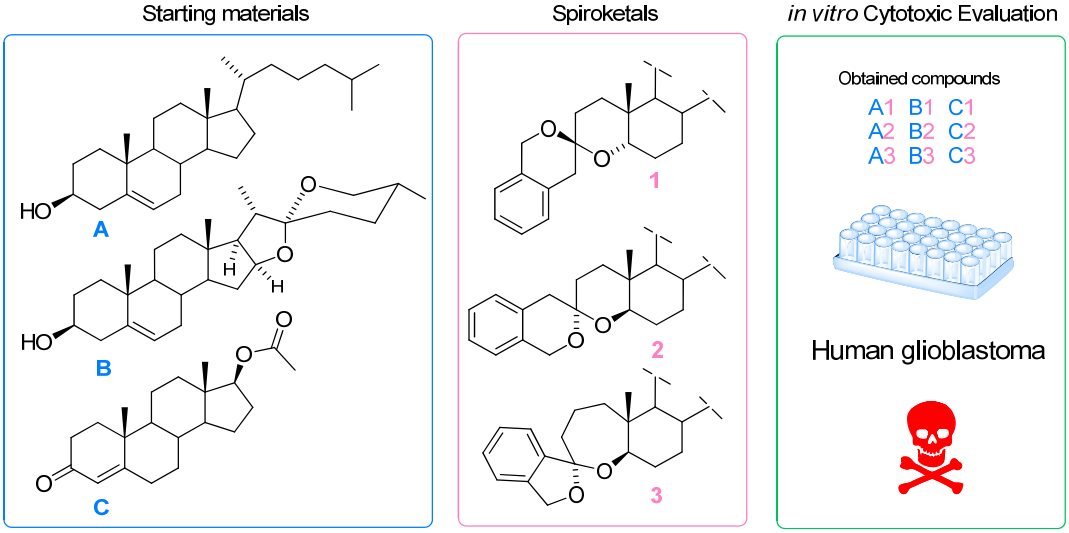

Figure 1. Synthesis of benzannulated steroid spiroketals and the cytotoxic evaluation.

The spiroketals were prepared following our recently described procedure [3] and were characterized by their NMR spectra. The obtained structures were confirmed by X-ray diffraction studies.

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Group-selective immunoanalytical system for the detection of sutherlandiosides

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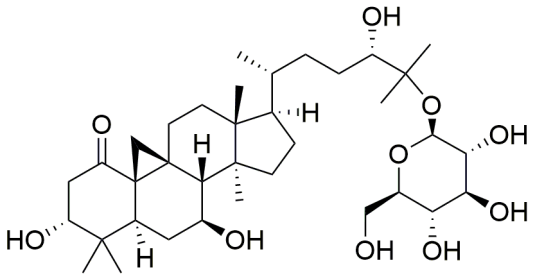
Sutherlandia frutescens (L.) R.Br. (Leguminosae) is an important South African medicinal plant whose major terpenoid-derived constituents and chemotaxonomic marker is a group of cycloartane glycosides, sutherlandiosides A-D. This group of substances is expected to contribute to the observed therapeutic effects of *S. frutescens* [1]. During clinical trials of this plant a need for verifying the patients' compliance arose. Hence, we developed an indirect competitive enzyme-linked immunosorbent assay. This method is based on a group-selective polyclonal antiserum which detects urinary metabolites of sutherlandiosides. Direct assay of urine samples was complicated by matrix effect which lead to insufficient reproducibility of the results. For this reason, we developed time- and cost-effective sample preparation. The procedure consists of urine dilution in 20 mM phosphate buffer (1:4) followed by heating at 95 °C for 5 minutes. Even though such treatment is accompanied by reduction of detected immunoreactivity, the reproducibility of the method was improved. Present results suggest that this method can be used to verify patients' adherence during *S. frutescens* clinical trial.



Major terpenoid-derived constituent of *S. frutescens*, sutherlandioside B.

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Inhibitory effect, cytotoxicity and plasma stability of steroidal inhibitors of *N*-methyl-D-aspartate receptors with C-3 amide structural motif

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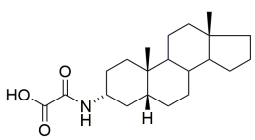
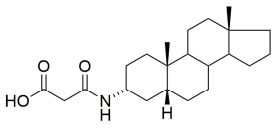
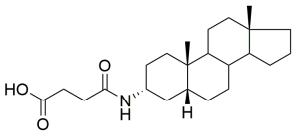
Herein, we report the synthesis, structure-activity relationship study, and biological evaluation of neurosteroid inhibitors of *N*-methyl-D-aspartate receptors that employ an amide structural motif, relative to pregnanolone glutamate (**PAG**) – a compound with neuroprotective properties. Our results demonstrate that all compounds were found to be more potent NMDA inhibitors (IC_{50} values varying from 1.4 to 21.7 μM) than **PAG** (IC_{50} = 51.7 μM). Selected compound **6** was evaluated for its NMDAR subtype selectivity and its ability to inhibit AMPAR/GABAR responses. Compound **6** inhibits the NMDA receptors (8.3 ± 2.1 μM) more strongly than it does the GABA and AMPA receptors (17.0 ± 0.2 μM and 276.4 ± 178.7 μM, respectively). Next, compounds **3**, **5-7**, **9**, and **10** were not associated with mitotoxicity, hepatotoxicity nor ROS induction. Lastly, we were able to show that all compounds have improved rat and human plasma stability over **PAG**.



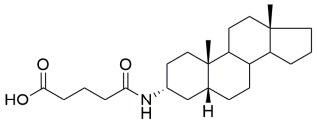
1 (hemi-Oxalylamide-AND)



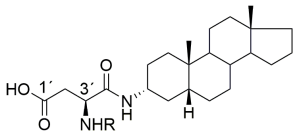
2 (hemi-Malonylamide-AND)



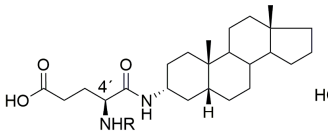
3 (hemi-Succinylamide-AND)



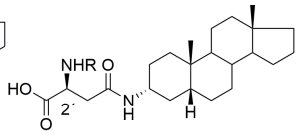
4 (hemi-Glutarylamide-AND)



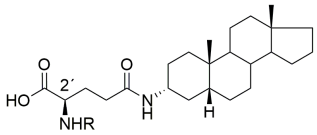
5, R = Boc (3′-N-Boc-L-Asp-AND)
6, R = H (3′-L-Asp-AND)



7, R = Boc (4′-N-Boc-L-Glu-AND)
8, R = H (4′-L-Glu-AND)



9, R = Boc (2′-N-Boc-L-Asp-AND)
10, R = H (2′-L-Asp-AND)



11, R = Boc (2′-N-Boc-L-Glu-AND)
12, R = H (2′-L-Glu-AND)

This work was supported by grant TE01020028 Center for Development of Original Drugs from the Technology Agency of the Czech Republic, ERDF/ESF project "PharmaBrain": CZ.02.1.01/0.0/0.0/16_025/0007444; Czech Academy of Sciences research projects RVO 67985823, RVO 61388963. BIOCEV (CZ.1.05/1.1.00/02.0109) – Biotechnology and Biomedicine Centre of Academy of Sciences and Charles University in Vestec, project supported from European Regional Development Fund. The Czech Science Foundation [P208/12/G016] supported the computational study by MN.

Structural modifications of androstane steroids by micromycetes

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Valuable 11α, 14α- and 7α/β-hydroxylated derivatives of androstane steroids are widely used as anti-inflammatory, immunosuppressive and anabolic agents and also can be applied as key precursors of high-value bile acids from non-animal raws. Filamentous fungi are known as best biocatalysts for steroid hydroxylation, but their hydroxylating activity towards androstanes such as androst-4-ene-3,17-dione (AD) and androsta-1,4-diene-3,17-dione (ADD) is poorly investigated so far.

In this study, selected fungal strains of different phyla were investigated for their hydroxylating activity towards AD and ADD and the methods for effective production of valuable hydroxylated androstanes had been developed.

46 *Ascomycota* and *Zygomycota* fungal strains of 22 different genera were screened for the capability of AD and ADD transformation. Excepting 6 representatives of *Acremonium*, *Sordaria*, *Conidiobolus*, *Fusarium*, *Mucor* and *Rhizomucor* genera all other 40 strains were found to be active biocatalysts of AD and ADD conversions. Two strains of *Aspergillus ochraceus* and *Beauveria bassiana* expressed maximum 11α-hydroxylase activity toward AD. Among the representatives of *Bipolaris*, *Acremonium*, *Fusarium, Gibberella*, *Absidia*, *Cunninghamella* and *Rhizopus* genera 6 strains expressed 14α-hydroxylase activity, 12 strains were found to be the catalysts of 7α- hydroxylation whereas 7β-hydroxylase activity was detected for 7 strains. The yield of target 7β-OH-ADD with the most effective mold exceeded 50% under the optimized conditions.

The capability to express 17β-HSD activity toward androstane substrates was revealed only for the strains of *Bipolaris australiensis*, *Acremonium cereales*, *Doratomyces purpureofuscus* and *Fusarium merismoides*. The last one was able to catalyze the reduction of 17-keto group of ADD molecule only, whereas other three strains were active both towards AD and ADD providing accumulation of more than 15% of testosterone (TS) and 1-dehydrotestosterone (dhTS), correspondingly. The expression of 3-ketosteroid-1(2)-dehydrogenase activity was revealed only for one *Zygomycota* strain of *Cunninghamella echinulata*. Apparently the reaction of 1(2)-dehydrogenation in this strain was induced only in the presence of hydroxyl group at 7β-position. The strains of *Ascomycota* division related to *Bipolaris*, *Acremonium*, *Fusarium* and *Gibberella* genera were able to catalyze 1(2)-reduction of 7α- or 7β-hydroxylated derivatives of ADD with formation of the corresponding reduced 7-hydroxylated derivatives of AD.

The results contribute to the knowledge on biodiversity of steroid transforming micromycetes capable of effective regio- and stereoselective hydroxylating as well as redox reactions of 3-oxo-androstane steroids.

The work was supported by Russian Science Foundation (Grant no.18-14-00361).

Retinoid production from *E. coli* using antibiotic-free expression system

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Industrial production of useful substances in the bio industry mostly uses LMO. In this case, in order to strengthen the expression of the metabolic pathways and the product biosynthesis pathways, the genes are induced by various expression vectors [1]. Vectors have antibiotic resistant genes as selection markers, and during the fermentation process, antibiotics are added to the culture medium for the stable retention of vectors within the host cell. However, when antibiotics are used in the fermentation process, they increase production costs, create risks of environmental pollution and antibiotic resistant mutants, and require additional separation and purification process of residual antibiotics from the final product [2].

In this study, to solve this problem, we have established a new antibiotic marker free system that does not use antibiotics as a selection marker. The system is based on isoprenoid pathway such as MEP pathway and MVA pathway. IPP and DMAPP, which are produced through these two pathways, are essential metabolites to living organisms for growth [3,4]. We applied this system to *Escherichia coli*, and successfully produced retinoids without antibiotics and antibiotic resistant gene.

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This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant#: PJ01326501), Rural Development Administration, Republic of Korea.

Regeneration of NADPH for isoprenoids production in *Escherichia coli*

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Isoprenoids are considered as the compounds with high commercial potential due to their broad applicability as fuels, raw materials for cosmetics, and nutraceuticals, as well as their applicability as pharmaceutically important compounds. *E. coli* has been the organism of choice for the production of isoprenoids by engineering native and heterologous pathways [1]. In the present study, we address some of the main issues associated with *E. coli* as an industrial platform for isoprenoids. In isoprenoid-producing *E. coli*, the cofactor NADPH participates in a variety of anabolic reactions and its availability is considered to play a critical role in biosynthesis processes [2, 3]. So sufficient supply of NADPH is one of the most important factors affecting the productivity of biosynthesis processes. Generally, formate dehydrogenase (FDH) regenerates NADH using NAD^+ as a cofactor. However, *Burkholderia stabilis* FDH produce NADPH instead of NADH [4]. In this study, construction of an efficient NADPH-regenerating system was attempted using NADPH-dependent FDH from *Burkholderia stabilis* for production of α-Bisabolol in *E. coli*. Expression of NADPH-dependent FDH in *E. coli* producing α-Bisabolol resulted in an increase of α-Bisabolol production to 2,416mg/l, an enhancement of 29.5% compared with the control strain.

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Metabolic engineering of methylotrophic bacteria for bio-isoprene production

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Isoprene (2-methyl-1,3-butadiene) is an important organic chemical compound for the production of synthetic rubber and textile manufacturing industries. Currently, the production of isoprene is based on petroleum, which is commercially not feasible because of high refining cost and increasing price of crude oil. However, the microbial production of isoprene (bioisoprene) via metabolic engineering is an alternative and sustainable way to meet a high global demand of isoprene [1, 2]. Bioisoprene is synthesized from dimethylallyl diphosphate (DMAPP), which is derived from mevalonate (MVA) pathway or 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway, by isoprene synthase [3]. Utilization of C1 compounds, which are cheap and abundant, as a fermentation substrate provides a great insight into the development of sustainable and cost-effective production of bioisoprene [4]. In this study, production of isoprene from methanol was explored by using *Methylobacterium organophilum* transformed with plasmid containing isoprene synthase (IspS) gene from *Populus trichocarpa*, which was codon-optimized for the host. To enhance the isoprene production, the endogenous MEP pathway was augmented by overexpression of *dxs*, *dxr*, and *idi* genes. The bottom portion of exogenous MVA pathway was also introduced into the host with supplementation of mevalonate. Gas chromatography (GC-FID) was used to quantify the isoprene production.

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Production of mixed isoprenoid alcohols and their derivatives from metabolically engineered *Escherichia coli*

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The petroleum-based fuels such as gasoline and diesel are complex mixtures of hydrocarbons with different chain lengths. Isoprenoids are hydrocarbon-based compounds with different carbon chain lengths and diverse chemical structures, similar to petroleum based fuels. Thus, isoprenoid-based alcohols such as isopentenol (C_5), geraniol (C_{10}), and farnesol (C_{15}) have been considered to be ideal biofuel candidates. A native phosphatase of *Escherichia coli*, NudB is reported to hydrolyzed isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) into isopentenol. However, no attention has been paid to the substrate specificity of this enzyme toward longer chain length (C_{10}-C_{15}) prenyl diphosphates. In this study, novel substrate specificities of NudB toward geranyl diphosphate (GPP) and farnesyl diphosphate (FPP) were identified and used for the production of isoprenoid-based alcohols mixtures, including isopentenol, geraniol, and farnesol, and their derivatives. *E. coli* was engineered to produce a mixture of C_5 and C_{15} alcohols by overexpressing NudB (dihydroneopterin triphosphate diphosphohydrolase) and IspA (FPP synthase) along with exogenous mevalonate (MVA) pathway, which resulted in a total of up to 1,652 mg/L mixture of C_5 and C_{15} alcohols and their derivatives. The production was further increased to a maximum of 2,027 mg/L by co-overexpression of AphA, in addition to NudB. Furthermore, the DMAPP- and FPP-derived products were significantly increased over IPP-derived products with an increase in the *idi* dose, which encode IPP isomerase (IDI), indicating a potential modulation of the composition of the alcohols mixture. By replacing IspA with its mutant IspA*, generating GPP in the production strain, a total of 1,418 mg/L of the isoprenoid mixture was obtained containing C_{10} alcohols. This is the first successful report on high-titer production of an isoprenoids-based alcohol mixture. The engineering approaches can provide a valuable platform for production of other isoprenoid mixtures via a proportional modulation of prenyl diphosphate precursors, IPP, DMAPP, GPP, and FPP syntheses.

This work was supported by the C1 Gas Refinery Program through the NRF funded by the MSI (NRF-2016M3D3A1A01913246) and a grant (NRF-2016M1A2A2924237) from the National Research Foundation, Korea.

Research of the structure of modified 4-isopropyl-7-methyl-3a,4,7,7a-tetrahydro-4,7-ethanisobenzofuran-1,3-dione

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Turpentine is one of the main products of pine resin processing, which is a mixture of terpenic hydrocarbons. As a result of isomerization of α-pinene, α-terpinene is formed, which is characterized by the presence of conjugated double bonds. This makes it easy to enter into Diels-Alder reaction with maleic anhydride to form of 4-isopropyl-7-methyl-3a,4,7,7a-tetrahydro-4,7-ethanisobenzofuran-1,3-dione.

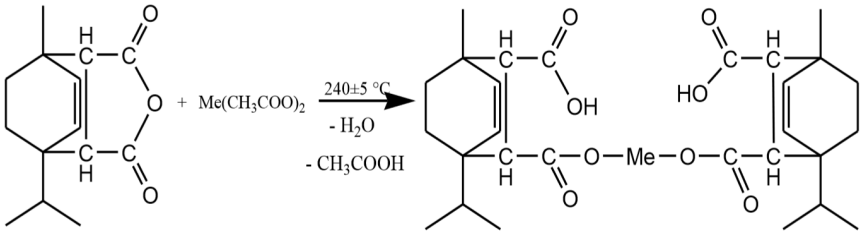
Qualitative analysis of initial adduct was carried out with Agilent Technologies 7890A equipped with a "DB-5MS" column (30m×0.25mm×0.25 μm) and coupled to an Agilent Technologies 5975C mass spectrometer.

The structure of the synthesized substance was proved by NMR spectroscopy.

This product is a promising hardener of epoxy resins, but obtained lacquer coatings have low operational properties.

It is known [1] that the solution of this problem is accomplished by replacing 4-isopropyl-7-methyl-3a,4,7,7a-tetrahydro-4,7-ethanisobenzofuran-1,3-dione on the chemically modified metal acetate.

The synthesis was carried out at a temperature of 240±5 °C for 3 h without access to oxygen according to the following reaction scheme:



The structure of the synthesized modified substance was proved by NMR spectroscopy.

The varnishes obtained with the use of the synthesized product are superior in their performance to varnishes based on epoxy resin [1].

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On reactions of different steroid derivatives with Santi's reagent (PhSeZnCl). The synthesis of selenosteroids

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The last few decades have seen a growing interest in the synthesis of organoselenium compounds because of their promising biological activities. [1-3] Selenium is present in the mammalian organisms primarily in the form of selenoenzymes, having a crucial role as antioxidants that determine protection against the oxidative stress in cells. [4]

The combination of sterols and selenium is quite rare and only a few examples are described in the literature with a very limited selenium moiety. [5] The designed synthesis of new pro-oxidant biomolecules combining oxysterol and selenium-containing compounds has a great potential for the creation of a new library of molecules important for biological applications.

The results of the reactions with PhSeZnCl (Santi's reagent) and steroid derivatives: epoxides, α,β-unsaturated ketones, and lactone will be presented. This reagent has recently emerged as the first bench-stable selenium nucleophilic agent having a broad range of application. [6] The employed procedure involved i*n situ* generation of the organoselenium reagent followed by steroid derivative addition. This approach led to novel phenylselenium-substituted steroids.

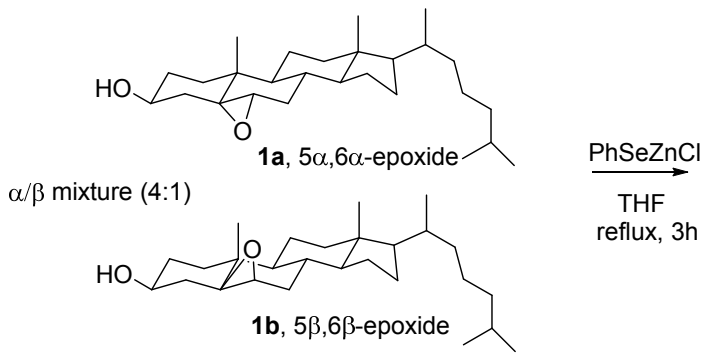

Figure 1. Reaction of 3β-hydroxy-5ζ,6ζ-epoxycholestanes (**1a** and **1b**) with PhSeZnCl.

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DFT and experimental study of vitamin E derivatives

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The XRD measurement and DFT calculations were undertaken for better understanding the structural aspects of biological activity of vitamin E (**1**). The aim of this study concerns the structural features of α-tocopheryl succinate (**3**) - possessing potential anticancer activity [1].

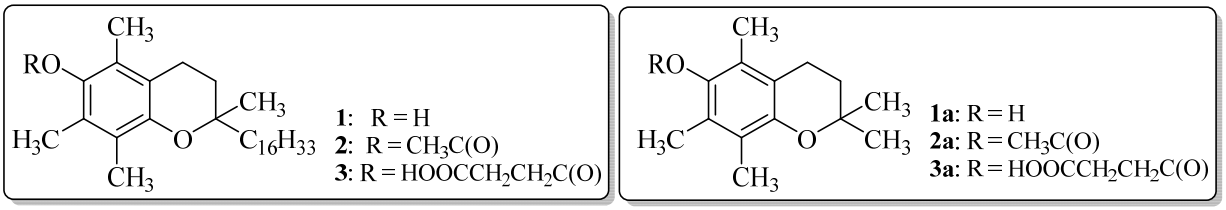

Figure 1. The chemical structure of α-tocopherol (**1**), chroman-6-ol (**1a**) and their acyl derivatives.

The low-energy conformers of: α-tocopherol (**1**), chroman-6-ol (**1a**) and its acetates (**2** and **2a**) and succinates (**3** and **4**) were attributed by population analysis by the natural bond orbital (NBO) at B3LYP/6-31G/(d,p)/CPCM level of theory [2].

Based on NBO charges the electron donor (f^-(r)), electron acceptor (f^+(r)) and dual descriptor (f^2(r)) of the Fukui [3] function in derivatives **1**-**3** and **1a**-**3a** were investigated and discussed. The obtained results were compared with the new X-ray data measured for α-tocopheryl succinate (**3**, CCDC 1560507) and chroman-6-yl succinate (**4**, CCDC 1560510).

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Synthesis of menaquinone-7

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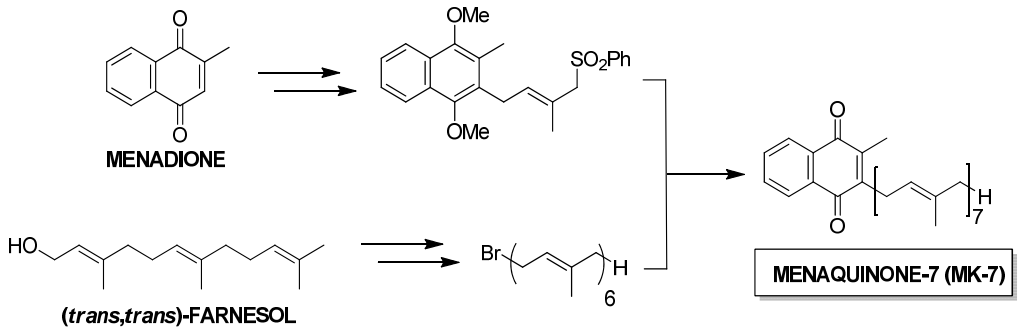
Vitamin K (naphthoquinones) is a group of fat soluble compounds containing 2-methyl-1,4-naphthoquinone system substituted at C3 position with polyisoprenoid lipophilic side chains.

There are two natural forms of vitamin K: K1 and K2. The vitamin K1 (phylloquinone) is main dietary form (80-90%), which occurs mostly in green leafy vegetables. Vitamin K2 (MK-n) is present at lower concentration in animal products (e.g. eggs, meat) and higher in fermented products (e.g. natto, cheese, sauerkraut). Currently, three forms of vitamin K are used in food supplementation: K1, menaquinone-4 (MK-4) and menaquinone-7 (MK-7, from natto). More lipophilic MK-7 shows a better bioavailability, longer half-life and higher activity compared to K1 and MK-4. A growing number of reports conclude that vitamin K2 (MK-4 or MK-7) supplementation have beneficial effects in prevention of osteoporosis, cardiovascular and central nervous system degeneration diseases. The only all-*trans* form of K2 can be used as a dietary supplement as well as an active pharmaceutical ingredient. However, isolation of vitamin MK-7 from natural sources is quite difficult due to low content (up to 1200 μg/100 g) [1-4]. In view of increasing market demand, synthetic works are taken up on efficient methods of production of all-*trans* forms of vitamin K2 (mostly MK-7).



Scheme 1. Convergent synthesis of menaquinone-7 (MK-7).

The aim of our project was to develop an efficient method of vitamin K2 (MK-7) synthesis. Our methodology is based on convergent "1 + 6" coupling of menadione monoprenylated sulphone (fragment "1") with hexaprenyl bromide (fragment "6") (Scheme 1). The high-purity (99,9%) product was obtained [5-6].

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Effect of saponins from *Saponaria officinalis* on model biological membranes

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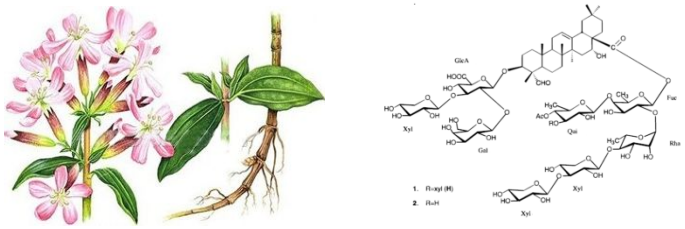
Triterpenoid and steroid surfactants produced by numerous plants, belonging to the group of saponins, are very promising candidates for natural eco-friendly replacements of synthetic surfactants. They possess often comparable ability to lower surface tension and good emulsifying/foaming properties, while offering much lower toxicity and higher biocompatibility. Triterpene saponins found in plant extracts of soapwort (*Saponaria officinalis* L.), washnuts (*Sapindus mukorossi*, Gaertn.), chestnuts (*Aesculus hippocastanum* L.) or Quillaja (*Quillaja saponaria* Molina) already found applications in food, cosmetic and pharmaceutical industries [1].

In the present study, the effect of aqueous extracts of soapwort's rhizomes on two model lipid monolayers mimicking the stratum corneum of the human epidermis is analyzed. The first model consisting of ceramide [AP], cholesterol and stearic acid (1/0.7/1) [2] mimics the intercellular lipids filling the spaces between the corneocytes in stratum corneum. The second model comprises a mixture of dipalmitoylphosphatidylcholine (DPPC) and cholesterol (7/3), which mimics the composition of the cell membrane of corneocytes [3]. The two models are complementary and provide a convenient tool for comparative studies of (bio)surfactants on lipids representing human skin. In the present contribution, the effect of soapwort extracts will be thus compared with that of four typical synthetic surfactants: cocamidopropylbetaine (Cocamidopropyl Betaine), sodium lauryl sulfate (SLS), ethoxylated sodium lauryl sulfate (SLES) and ammonium lauryl sulfate (ALS).



Soapwort plant and a representative saponin identified in its rhizomes (Saponaside A).

The appropriate lipid mixtures were spread on pure water as Langmuir monolayers, compressed to surface pressure of 30 mN/m and their exposure to (bio)surfactants was simulated by a subphase exchange using a dedicated Langmuir trough setup. Surface pressure relaxation combined with surface dilational rheology analysis and fluorescence microscopy observations enabled us to draw conclusions on special character of interaction of the saponin-rich extract with the skin-mimicking lipids. In contrast to the synthetic surfactants, saponins (and possibly other components from the extract) do not solubilize the lipids, but incorporate into the monolayers, significantly enhancing their surface elastic properties.

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Modulators of the *N*-methyl-D-aspartate receptor: search for new neuronal disorders relieving drugs

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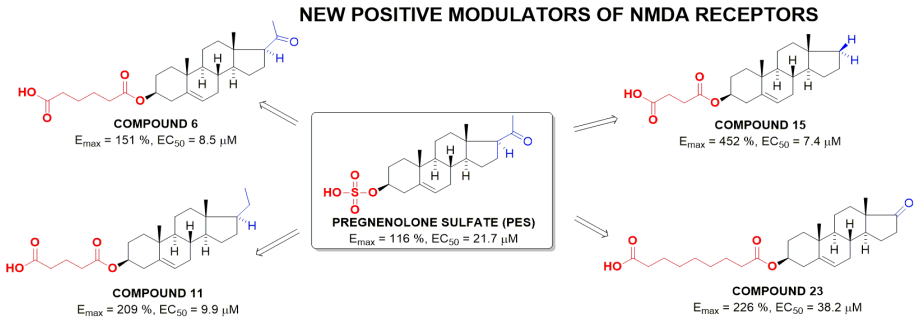
Neurosteroids are endogenous steroidal compounds that can modulate neuronal receptors. *N*-Methyl-D-aspartate receptors (NMDARs) are glutamate-gated, calcium-permeable ion channels that are of particular interest as they participate in synaptic transmission and are implicated in various processes, like learning, memory, or long-term neuronal potentiation. [1] Positive allosteric modulators that increase the activity of NMDARs may provide a therapeutic aid for patients suffering from neuropsychiatric disorders where NMDARs hypofunction is thought to be involved, such as intellectual disability, autism spectrum disorder, or schizophrenia. [2]



Different neurosteroids can exert various effects at NMDARs, apparently mediated by molecular dissimilar mechanisms. A 3β-negatively charged moiety in combination with Δ5-stereochemistry favors potentiation of NMDARs. [3] This class of neurosteroids is represented by endogenous 20-oxo-pregn-5-en-3β-yl sulfate (PES). Hence, a series of PEG-like compounds was synthesized and evaluated for their ability to positively modulate NMDARs. [4] Considering the recommended guidelines for early stage development of new, potent compounds, next, we have introduced mitotoxicity and hepatotoxicity screening on HepG2 cells as a primary tool to rank our compounds. Second, the transport of the tested compounds across the Caco-2 monolayers was studied. Next, the ability of compounds to modulate viability of postnatal neurons was evaluated. Finally, we assessed the stability of PES-like compounds in rat and human plasma to demonstrate their drug-likeness. Our results indicate that these compounds may be beneficial in treatment of many neurological diseases.

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Synthesis of 19-norcalcitriol analogs with modification at C-3

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The chemical structure of vitamin D_3 was established in the 1930s. Broad studies on biological activities and metabolic pathways of this compound resulted in discovery of its biochemical transformations leading to the most active metabolite – calcitriol [1α,25-dihydroxyvitamin D_3; 1α,25-$(OH)_2D_3$]. This active, hormonal form of vitamin D_3 formed in the kidneys, stimulates calcium and phosphorus accumulation in bone.

However, maintaining of the calcium-phosphorus homeostasis is not the only important function of 1α,25-$(OH)_2D_3$. It turned out that a nuclear vitamin D receptor (VDR) has been found in numerous organs of the human body, including, for example, heart, blood vessels, immune system cells, endocrine glands, skin or brain. Interestingly, different cancer cells developing in these tissues and organs also contain vitamin D receptor. The findings that 1α,25-$(OH)_2D_3$ also inhibits proliferation and promotes cells differentiation have prompted chemists to synthesize calcitriol analogs possessing separated calcemic and antiproliferative activities, as the promising anticancer drugs.

Many of the published structural modifications of calcitriol involve different changes in the ring A. Since the modification at C-3, involving an attachment of a short oxygen-containing substituent, seems to be a promising structural change introduced into vitamin D molecule, the goal of our research was to design and obtain new A-ring building fragments useful for construction of such target compounds. We considered commercially available (1*R*,3*R*,4*S*,5*R*)-(-)-quinic acid as a convenient starting material for the planned synthesis.

Synthesis of new 19-norcalcitriol gemini-type analogs

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Calcitriol is the most active metabolite of the vitamin D_3 which represents its hormonal form. Many studies demonstrated that this natural hormone is responsible for calcium and phosphorus homeostasis and plays an important role in cell proliferation and differentiation. However, the usefulness of calcitriol as a cancer chemopreventive agent is significantly limited by its strong calcemic effect that could result in hypercalcemia.

Majority of structural modifications of the calcitriol molecule described in the literature involved its side chain. Such structural changes resulted in many cases in the higher activity of the analogs in comparison to the parent calcitriol. Undoubtedly, an important group of vitamin D_3 derivatives are Gemini compounds. These analogs are characterized by a presence of two side chains which can induce structural rearrangements of the vitamin D receptor's ligand binding domain resulting in the stronger agonistic action of the analogs. Gemini compounds represent a group of vitamin D_3 analogs with limited toxicity, which can be of significant importance when taking into account their anti-tumor properties.

The aim of my research was the synthesis of 19-norcalcitriol derivative, designed on the basis of molecular modeling and docking experiments, and characterized by a presence of steroidal side chain branched at C-20 and an exomethylene moiety at C-2. Such structural modification can potentially decrease the undesired calcemic effect.

Synthesis of 19-norcalcitriol analogs with A-ring modifications

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It has been established that calcitriol (1α,25-dihydroxycholecalciferol) – the active metabolite of vitamin D_3 is crucial for the expression of hundreds of genes and can be effectively used in the regulation of calcium-phosphate homeostasis, cell differentiation and the treatment of immune system diseases. Unfortunately, therapeutic doses of calcitriol are high enough to result in hypercalcemia. Consequently, numerous attempts have been made to develop therapeutic use of this hormone and synthesize new calcitriol analogs that would retain beneficial properties, but also be devoid of calcemic effect.

Biological properties of calcitriol and its analogs are mediated by the nuclear vitamin D receptor (VDR). Therefore, the effective binding to VDR is crucial for biological actions of new analogs. Calcitriol derivatives possessing the A-ring- and side chain-modifications are regarded as the most potent. Thus, based on docking experiments to the vitamin D receptor we selected 19-norcalcitriol derivatives with elongated chains at C-2. Such structural modifications might introduce new important interactions between vitamin D ligands and the ligand binding domain of the VDR and it could influence the activity profiles of the designed molecules.

As a result of multistep synthesis, new 19-norcalcitriol analogs with A-ring modifications have been obtained. The synthesis of the analogs described in this project has been performed using modified Julia and Wittig-Horner approach. The respective A-ring and C/D-ring building blocks have been synthesized starting from the commercially available D-(-)-quinic acid and vitamin D_3, respectively.

Synthesis and biological activity of brassinosteroid analogues with nitrogen-containing groups in the side chain

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Brassinosteroids (BRs) are a class of polyhydroxylated steroidal hormones playing a pivotal role in many aspects of plant growth and development, such as cell elongation, cell division, senescence, vascular differentiation, reproduction, photomorphogenesis, and responses to various stresses. [1]

It is known, that nitrogen is present in many natural or synthetic biologically active compounds. Based on that we have prepared several BRs analogues with amino or amide group in the side chain. Multistep conversion of starting bisnorcholanic acid (**I**) [2, 3] allowed us to receive BRs analogues with different amides (**II**,**III**), amines (**IV**,**V**) and their ammonium salts (Fig.1).

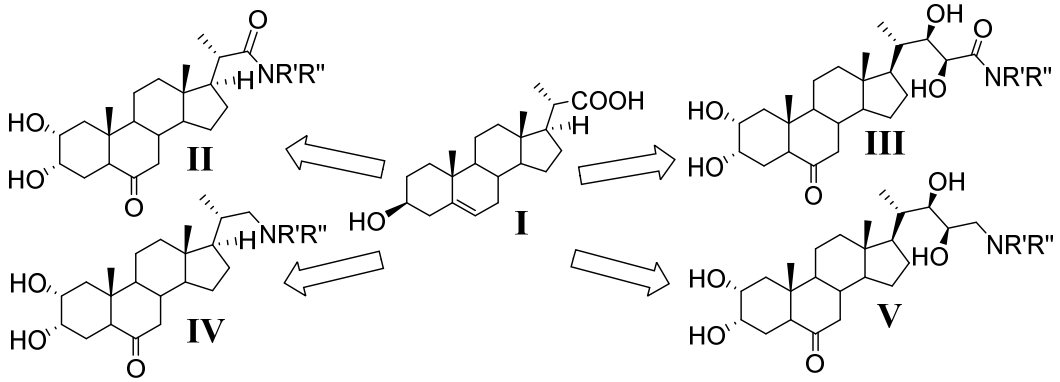

Fig. 1

Biological properties were tested in Arabidopsis BRs root sensitivity and cytotoxic bioassays. As a result of synthesis and biotests, we have also synthesized derivative with primary amine in the side chain as a precursor for other side chain modifications. Investigation of biological activity has shown that some of the newly synthesized compounds are biologically active. This work has shown perspective for the future investigation of this research.

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(1*R*)-(−)-Myrtenal derived chiral thio- and selenoureas

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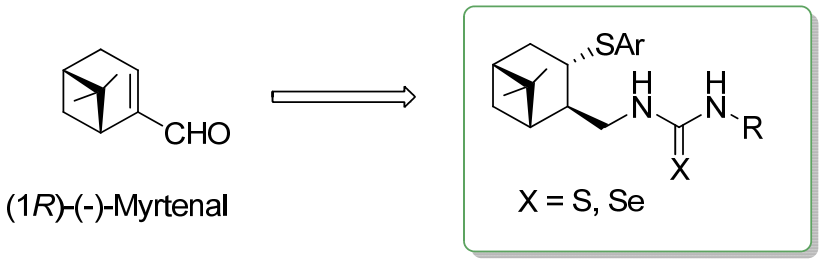
Monoterpene derivatives of the pinane series with oxygen atom are well known and accessible compounds with a high optical purity; they are precursors of biologically active molecules and intermediates in asymmetric syntheses. Recently, a bicyclic skeleton of (1*R*)-(–)-myrtenal was diversely functionalized, resulting in chiral derivatives, effective in various enantioselective transformations [1].

In this contribution, we describe the synthesis of novel chiral thio- and selenourea derivatives containing (1*R*)-(–)-myrtenal chiral backbones, with potential catalytic activity.



The synthetic route consists of a multi-stage sequence involving the preparation of sulfides via sulfa-Michael addition reaction, followed by transformation into alcohols and then amines [2]. Last step includes the coupling of resulted chiral primary amine with selected isothio- and isoselenocyanates giving thio- and selenourea derivatives.

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Synthesis of thermo-responsive polymers with cholesteryl moieties at the end of the polymeric chain

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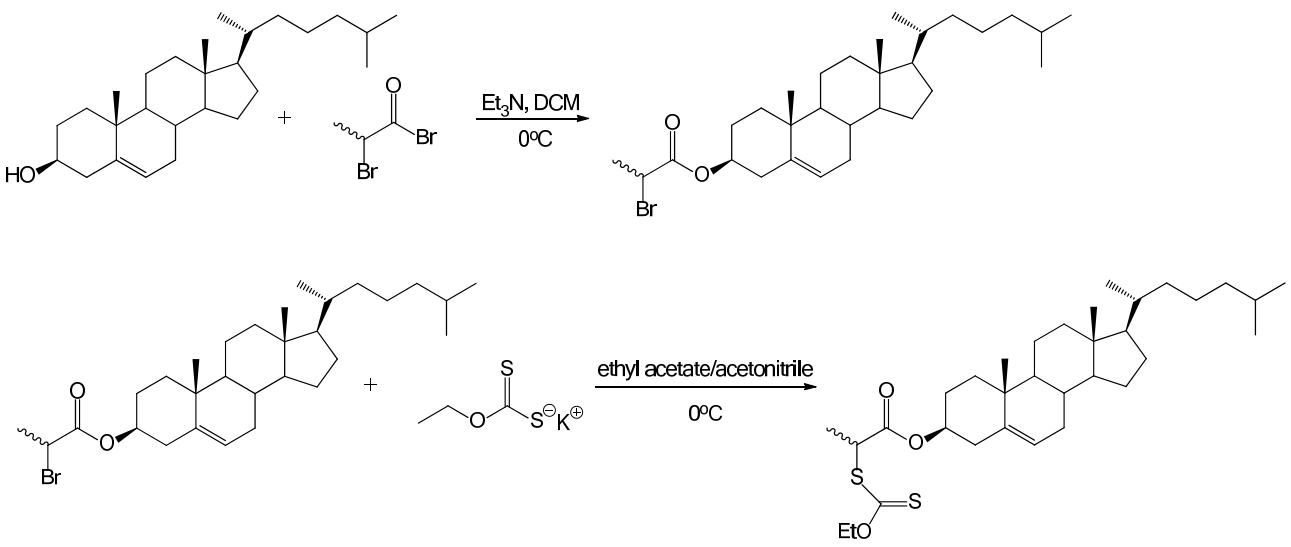
PNIPAm (poly(*N*-isopropylacrylamide)) and pNVCL (poly(*N*-vinylcaprolactam)) and its block and random copolymers were synthesized via Reversible Addition-Fragmentation chain Transfer/MAcromolecular Design via the Interchange of Xanthates (RAFT/MADIX). The use of dithiocarbonates (xanthates) as chain transfer agent (CTA) allows controlling the length of the polymer chain and its dispersity. In this case, dithiocarbonate derivative of cholesterol was used as CTA.



Scheme 1. Synthesis path of dithiocarbonate derivative of cholesterol.

Different homopolymers, block copolymers and random polymers with cholesteryl moiety at the chain end were prepared. Its thermosensitivity (thermo-variable UV), differential scanning calorimetry (DSC) and thermogravimetry analysis (TGA) were performed and will be presented.

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Analyses were performed in Centre of Synthesis and Analyses BioNanoTechno of University of Bialystok.
The equipment in the Centre of Synthesis and Analysis BioNanoTechno of University of Bialystok was funded by EU, as a part of the Operational Program Development of Eastern Poland 2007-2013, project: POPW.01.03.00-20-034/09-00 and POPW.01.03.00-20-004/11.

Synthesis of block copolymers with cholesteryl acrylate block made by RAFT/MADIX polymerization

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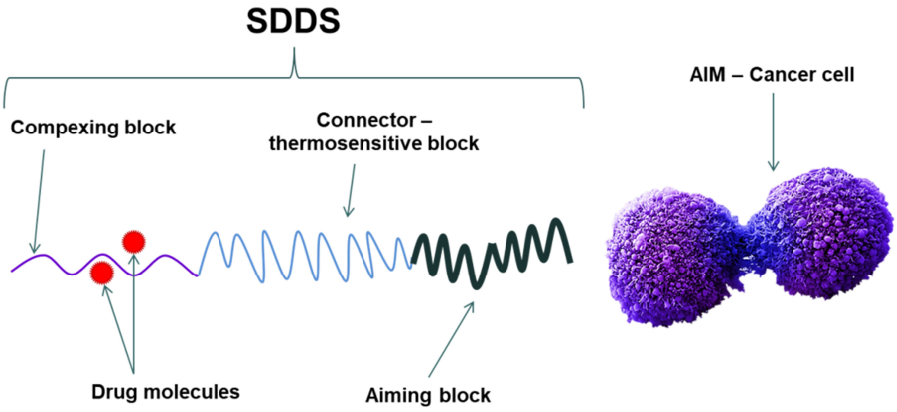
We will present the synthesis and characterization of polymers and copolymers made up of cholesteryl acrylate and vinyl monomers carrying specific functions (eq. thermosensitivity and chelating). The goal is to obtain smart drugs delivery systems (SDDS) consisted of three parts of block polymers (Scheme 1): aiming block, thermosensitive block and chelating block. Such systems change their physicochemical properties after exceeding the critical solution temperature (CST). The selection of an appropriate phase transition temperature may allow the release of drug molecules within diseased cells, e.g. cancer cells. [1]



Scheme 1. Construction of smart drug delivery system.

Block copolymers were synthesized via radical polymerization method – RAFT/MADIX (Reversible Addition-Fragmentation chain Transfer/MAcromolecular Design via the Interchange of Xanthates) with dithiocarbonate as chain transfer agent. The physicochemical properties of obtained polymers will be discussed.

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Acknowledgments: NCN, nr2016/21/B/ST5/01365
Analyses were performed in Centre of Synthesis and Analyses BioNanoTechno of University of Bialystok.
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Approaches to synthesize of adaline and its cyclic analogues with 7- and 8-carbon skeleton

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Adaline is one of approximately 50 alkaloids (Figure 1) isolated from the two-spot ladybird (*Adalia bipunctata*) secretions, and acting on the nervous system [1]. The hemolymph, that is rich in alkaloids, is produced by the insect leg joints under stress. The secretion is poisonous and protects ladybird from predators.

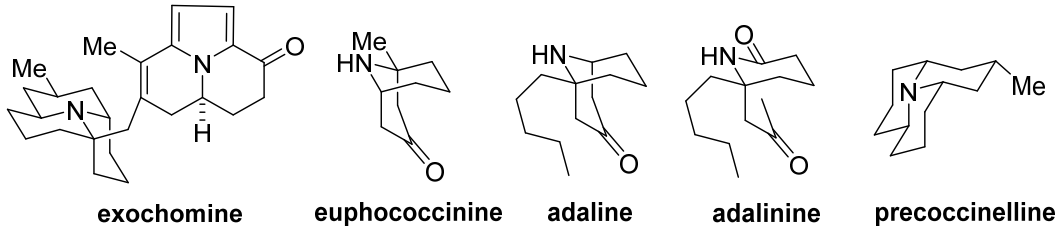
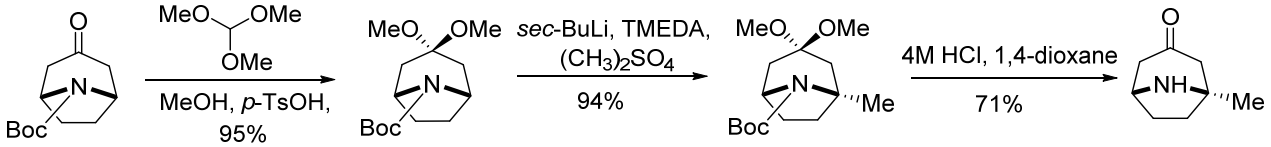


Figure 1. Selected alkaloids isolated from the ladybird secretion [2].

Our interest focused on compounds containing 9-azabicyclo[3.3.1]nonane structure, i.e. adaline, and its analogue euphococcinine isolated from *Euphorbia atoto* (Australian coastal plant) in 1967 [3]. Both alkaloids repel ants and spiders [4], and as such could be active ingredients of repellents. Also acting on the cholinergic receptors, may be used as potential lead to create new drugs against neurodegenerative diseases such as Alzheimer's and Parkinson's.

The previous syntheses of these compounds (i.e. adaline) were multi-step, relatively inefficient, and required elaborate substrates [5].

In our group we have successfully developed short (4 stages) and simple synthetic route for a new homologue of the known alkaloid euphococcinine – 1-methylnortropinone (Scheme 1). The obtained compound is an attractive representative of unnatural tropane alkaloids because of the potential biological activity and uncommon bicyclic structure.



Scheme 1. Synthesis of 1-methylnortropinone.

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Synthesis of 1,2- and 1,3-diamines from cholesterol as ligands for transition metal ions

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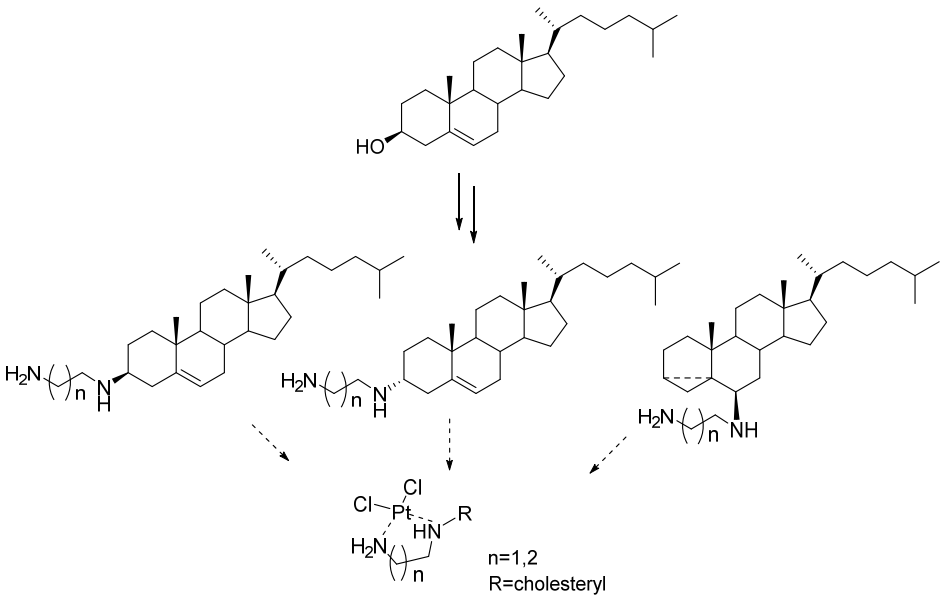
Cytotoxic complexes of platinum (II) are still the leading group of drugs used in anticancer therapy. Current research concentrates on designing drugs, which act more selectively and cause lower toxic side-effects than cisplatin and its derivatives.

The research project is based on experimental approach and involves the fundamental study associated with designing, preparation, and biological activity evaluation of steroidal platinum(II) complexes.

We designed and obtained a series of steroid *vic*-diamines based on cholesterol (Scheme 1) as potential ligands for platinum ions. The prepared complexes will be subjected to biological tests for their anti-cancer properties. We also plan to perform syntheses of other diamine systems based on elaborated methods starting from diosgenin, lithocholic acid etc.



Scheme 1. Steroidal ligands for Pt(II) complexation.

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Can we improve solasodine? Synthesis of new F-homo solasodine derivatives

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Many plants in the *Solanaceae* family contain steroidal alkaloids based on C27 cholestane skeleton, known as *Solanum* alkaloids. This group of compounds have a nitrogen atom at C26 in the ring F, like solasodine, tomatidine, solanidine; they are aza-analogs of steroidal sapogenins (*e.g.* diosgenin) (Fig. 1). Steroidal alkaloids are known to possess a variety of biological properties such as: antiproliferative, neurogenic, anticonvulsant and antiinflammatory. The interesting biological features and low natural occurrence (*Solanum* alkaloid content in plant is about 0.03%) of this compounds have inspired chemists to design synthetic analogs.

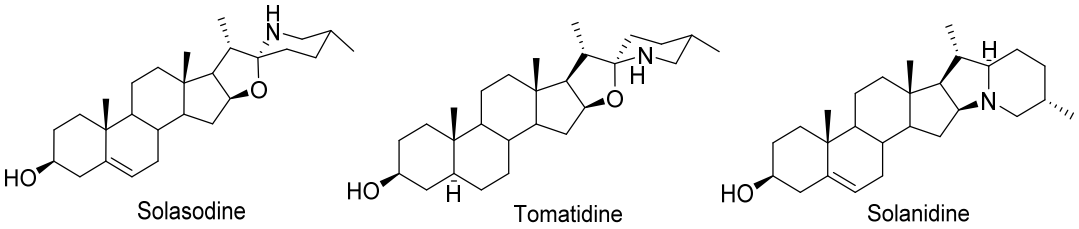

Fig. 1. Solanum alkaloids.

My research is focused on designing, synthesis and evaluation of biological activity of solasodine and its analogs. The main goal of this project is the synthesis of new F-homo derivatives of solasodine with nitrogen atom in different positions of the F-ring. The diosgenin seems to be perfect starting material for this purpose (Fig. 2).

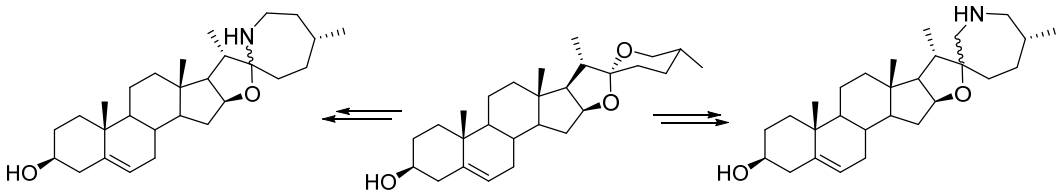

Fig. 2. New F-homo solasodine derivatives.

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Biosynthesis of sesquiterpene lactones in *Artemisia glabella* Kar. et Kir.

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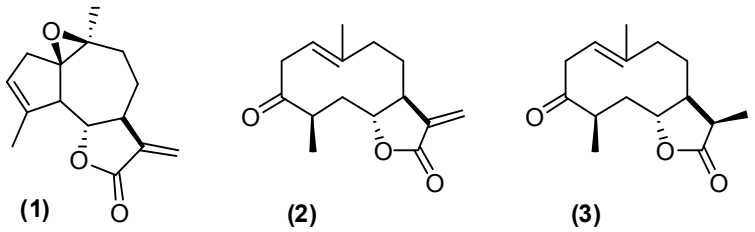
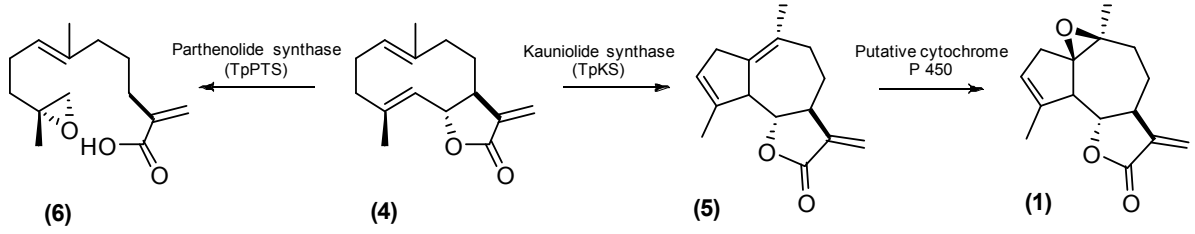
Terpenoid-containing organs in *Artemisia glabella* Kar. et Kir. are leaves and flower buds with the highest content (within 0.34 - 1.38%) of sesquiterpene lactone arglabin (**1**) which is observed during the budding phase; while the best solvents for its quantitative extraction are chloroform and carbon dioxide in the supercritical state (see data in Tables below). Besides arglabin, germacranolides argolide (**2**) and ketopelenolide B (**3**) have also been found in *Artemisia glabella* Kar. et Kir.

The solvent used	Arglabin yield, % of air-dry raw materials
water	0.002
diethyl ether	0.002
ethyl acetate	0.110
ethanol	0.180
chloroform	0.340
liquid CO_2	1.380

Plant organ	Arglabin content, % of air-dry raw materials	
	regrowth	budding
leaves	0.34	0.61
buds or flowers	-	0.60
stems	-	0.08



Recently, biosynthetic pathways of various sesquiterpene lactones have been researched in plants of the family *Asteraceae* (costunolide in *Cichorium intybus*, parthenolide in *Tanacetum parthenium*, artemisinin in *Artemisia annua*, etc.). As a result, key genes involved in the biosynthesis of some lactones have been identified which are responsible for control or to some extent for regulation of their accumulation in the plant body. In *Artemisia glabella* Kar. et Kir., the guaianolide kauniolide (**5**) formed from costunolide (**4**) under the influence of kauniolide synthase (TpKS) is considered a possible precursor of arglabin (**1**).



This biosynthetic pathway of arglabin is very likely, since the structures of molecules (**5**) and (**1**) differ only by the presence of an epoxy function at C1-C10 in molecule (**1**), and the same difference is true for (**4**) and (**6**) at C5-C6 bond. As is shown in the scheme, parthenolide (**6**) is formed from molecule (**4**) under the influence of parthenolide synthase (TpPTS), whereas the biosynthetic pathway of kauniolide has also been determined and successfully reconstructed in *Nicotiana benthamiana* by means of transient co-expression of the required biosynthetic genes.

New chloro-derivatives of grosheimin

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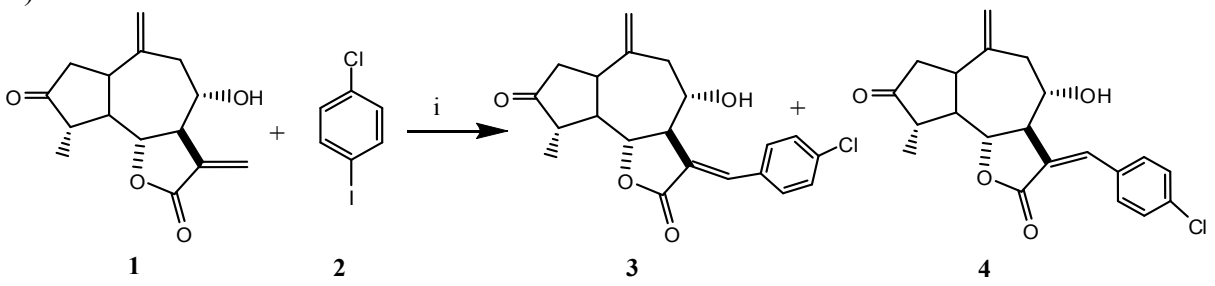
Grosheimin **1**, $C_{15}H_{18}O_4$, mp 200-202^{0}C, $[\alpha]_D^{20}$+159,9º (c 1.144; $CHCl_3$), is one of the most common guaiane-type sesquiterpene lactones in the natural flora isolated from 16 plant species of the family *Asteraceae*.

As a polyfunctional compound, 3-keto-8α-hydroxy-4,6,8β(*H*),7α(*H*)-guai-10(14),11(13)-dien-6,12-olide **1** has four reactive centers in its molecule: α-methylene group of γ-lactone, a hydroxyl functional group, keto- and exocyclic methylene groups.

Thus, in the course of a reaction of compound **1** with 1-chloro-4-iodobenzene **2** according to the Heck reaction catalyzed by the system $Pd(OAc)_2$-(*o*-tolyl)phosphine in DMF in the presence of triethylamine as a base, the following compounds form: (*E*)-(13-(4-chlorophenyl)-8α-hydroxy-3-oxo-5,7α(*H*)-guai-10(14),11(13)-dien-12,6β-olide **3** (with a yield of 15%) and (*Z*)-(13-(4-chlorophenyl)-8α-hydroxy-3-oxo-5,7α(*H*)-guai-10(14),11(13)-dien-12,6β-olide **4** (with a yield of 13%).



1 **2** **3** **4**

i) - $Pd(OAc)_2$, (*o*-Tol)$_3$P, Et_3N, TBAB, DMF, 100-110 ºC, 16 h;

The structures of the synthesized compounds **3**, **4** have been determined using physico-chemical constants and spectral data (IR-, UV-, ^{1}H and ^{13}C NMR, two-dimensional ^{1}H-^{1}H NMR spectra (COSY, NOESY), and ^{13}C-^{1}H (COSY, COLOC)). X-ray analysis has been performed to positively confirm the structure of **3**.

Thus, two new chloro-derivatives have been synthesized based on grosheimin **1**. The arylation of grosheimin **1** proceeds with the formation of derivatives by C11-C13 exocyclic coupling.

Terpenoids from plant essential oils of the family *Lamiaceae* growing in Kazakhstan

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In the territory of Kazakhstan, the family *Lamiaceae* comprises 233 species incorporated in 45 genera. Among them are plants of *Thymus, Ocimum, Origanum, Lavandula, Hyssopus, Nepeta, and Mentha* genera which are considered promising sources of essential oils.

The following genera take the leading positions in terms of the species variety: *Scutellaria* L. (35 species), *Thymus* L. (22 species), *Dracocephalum* L. (20 species), *Lagochillus* L. (16 species), and *Nepeta* L. (14 species).

Using Gas Chromatography-Mass Spectrometry (GC-MS), we have investigated the compositional analysis of essential oils from 47 plant species of the family *Lamiaceae*, namely *Dracocephalum integrifolium* Bge, *Dracocephalum grandiflorum* L, *Galeopsis bifida* Boenn., *Hyssopus ambiguus (*Trautv.) Ilji, *Hyssopus macranthus* Boriss., *Hyssopus* cuspidatus Boriss., *Lavandula angustifolia* Mill., *Lagochilus diacanthophyllus* (Pall.) Benth., *Lophantus schrenkii* Levin., *Leonurus quinquelobatus* Gilib., *Mentha micrantha* Litv, *Mentha piperita* L., *Mentha longifolia* L., *Mentha asiatica* L., *Melissa officinalis* L., *Nepeta cataria* L., *Nepeta pannonica* L., *Nepeta ucranica* L., *Origanum vulgare* L., *Origanum tyttanthum* Gonttsch., *Salvia macrociphon* Boiss, *Salvia stepposa* Schost., *Scutellaria baicalensis* L., *Scutellaria subcaespitosa* Pavl., *Scutellaria immaculata* Lam., *Scutellaria Sieversii* Bge., *Scutellaria krylovii* Juz., *Thymus crebrifolius* Klok., *Thymus dmitrivae* Gamajun, *Thymus karatavicus* A. Dmitr. ex Gamajun, *Thymus lavrenkoanus* Klok., *Thymus marschallianus* Willd., *Thymus minussinensis* Serg., *Thymus mugodzharicus* Klok. et Schost, *Thymus petraeus* Serg, *Thymus rasitatus* Klok., *Thymus roseus* Schipz., *Thymus serpyllum* L., *Thymus stepposus* Klok. et Schost, *Thymus sibiricus* (Serg.) Klok. et Schost, *Thymus vulgaris* L., *Teucrium scordioides* L., *Ziziphora interrupta* Juzz., *Ziziphora Vichodceviana* V. Tkatsch. ex Tuljaganova, *Ziziphora clinopodioides* Lam., *Ziziphora tenuiur* L., and *Ziziphora bungeana* Juz.. The main determined constituents are mono- and sesquiterpenoids such as 1,8-cineole, menthol, menthone, thymol, carvacrol, pulegone, nepetalactone, germacrene D, caryophyllene, overall 33 compounds. The extracted essential oils and their components have been screened for a biological activity.

Based on the biological activity screening data of the isolated samples, we have determined and proposed for further pharmacological studies the essential oils from the following plants: *Thymus rasitatus* Klok., *Thymus petraeus* Serg, *Nepeta ucranica* L. with a relatively high antiviral activity; *Thymus rasitatus* Klok. with a comparatively high analgesic activity; *Thymus vulgaris* L., *Thymus dmitrivae* Gamajun, *Thymus crebrifolius* Klok having a pronounced cytotoxic effect, and essential oils from *Thymus* vulgaris L, *Thymus dmitrivae* Gamajun, *Thymus sibiricus* (Serg.) Klok. et Schost, *Thymus crebrifolius* Klok, *Thymus roseus* Schipz with a high antimicrobial and antioxidant activities.

The plants listed below are considered prospective for isolation and production of the main essential oil constituents which can serve as starting reagents for the synthesis of new biologically active compounds: *Thymus crebrifolius* Klok., *Thymus dmitrivae* Gamajun, *Thymus lavrenkoanus* Klok., *Thymus petraeus* Serg, *Thymus serpyllum* L., *Scutellaria immaculata* Lam, *Nepeta ucranica* L., *Dracocephalum grandiflorum* L., *Origanum tettanthum* Gontsch., *Salvia macrosiphon* Boiss., *Melissa officinalis* L., *Lophantus schrenkii* Levin., *Galeopsis bifida* Boenn., and *Teucrium scordioides* L.

Accordingly, plant essential oils of the family *Lamiaceae* in the flora of Kazakhstan, containing a complex of substances with a broad spectrum of pharmacological activities, are undoubtedly of interest for multidimensional research and design of new effective drugs.

Orthodox Church of the Holy Spirit, Białystok

Eastern Orthodox Church of St. Nicholas, Białystok

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View of Białystok with Basilica of the Assumption of the Blessed Virgin Mary

Basilica of the Assumption of the Blessed Virgin Mary, Białystok

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