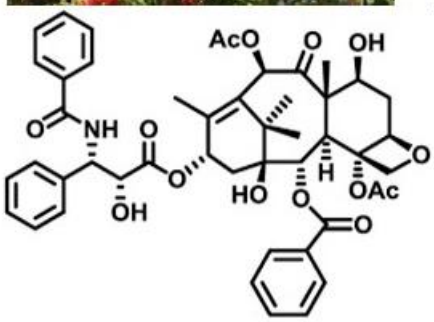
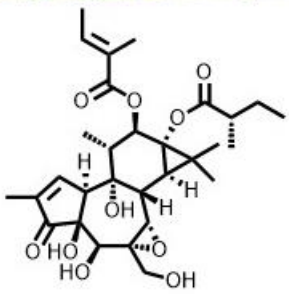
25th CONFERENCE ON ISOPRENOIDS

18-20 SEPTEMBER 2024 NAPLES





BOOK OF ABSTRACTS

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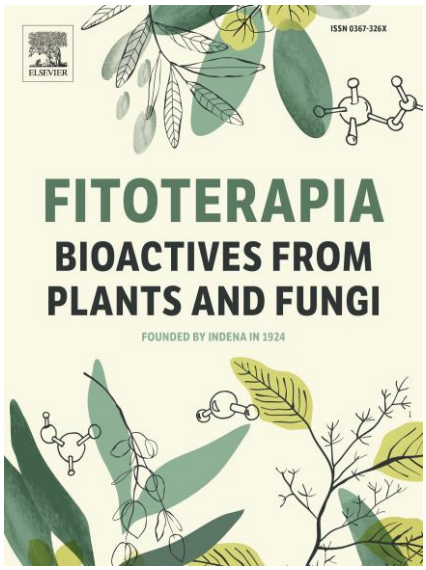

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Dear Colleagues,

On behalf of the Isoprenoid Society, we are very pleased to host you at the 25th Conference on Isoprenoids, September 18th -20th 2024, Naples, Italy.

This Conference series, started at the early 1960s, is one of the longer-running scientific events focused on natural products. The main purposes of the Conferences on Isoprenoids throughout these sixty years have been to promote research in the isoprenoid field at the interface of chemistry, biology, and medicine, with a great attention to industrial applications. The role of science is to contribute to the human progress and to overcome barriers. To this aim, the Isoprenoid conferences facilitate the networking and cooperation between researchers from different countries and provide a forum for the exchange of knowledge among scientists at all career stages in a friendly environment. The conferences have hosted many eminent scientists, including the late Kenji Mori and the Nobel Price laureates Derek H. R. Barton and Ryoji Noyori.

All the previous Conferences in this series have been organized in Eastern Europe countries; thus, this is the first time that Italy hosts this event. The Italian scientific community has a long tradition in the study of natural products, and several highly active groups, with worldwide network of collaborations, are based in Naples and in the Campania Region.

The conference venue, with a beautiful location on the Naples seaside, close to the city center, will allow participants to enjoy a warm hospitality, the outstanding food and wine and the beauty of the Neapolitan coast. In summary, we are confident that the Naples Congress on Isoprenoids will be unforgettable, and we hope you will have a great time in Naples.

The Organizing Committee

25th CONFERENCE ON ISOPRENOIDS
18-20 SEPTEMBER 2024 NAPLES

Scientific Program

Wednesday, 18 September (location: Hotel Royal Continental)	
Starting 09.00	Registration
10.00 – 10.40	**Opening Ceremony**
	Session 1 – Chairpersons: Prof. Orazio Taglialatela-Scafati; Prof. Nunziatina De Tommasi
10.40 - 11.20	**PL1** – Giovanni Appendino (University of Eastern Piedmont, Italy) "*Hidden gems from the early studies on isoprenoids*"
11.20 – 12.00	**PL2 –** Asaph Aharoni (Weizmann Institute of Science, Israel) "*How do plants evolve specialized metabolites and pathways*"
12.10 – 12.20	**O1 –** Tian Ma (Shenzhen Institute of Advanced Technology, China) *"Spacing engineering for terpenoid overproduction"*
12.20 – 12.40	**O2 -** Francisco Javier Ortiz-López (Fundacion Medina, Spain) "*Crossiellidines A-F: unprecedented pyrazine-alkylguanidine, prenylated metabolites with broad-spectrum antibacterial activity from Crossiella sp*."
12.40 – 14.00	**Snack Lunch**
	Session 2 – Chairpersons: Prof. Giovanni Appendino; Prof. Ren Xiang Tan
14.00 – 14.40	**PL3 –** Scott Snyder (University of Chicago, USA) *"Strategies and tactics for the rapid synthesis of molecular complexity"*
14.40 – 15.00	**O3 –** Georges Massiot (Université Reims-Champagne-Ardenne, France) *"Partial synthesis of tagitinin C derivatives as proteasome inhibitors"*
15.00 – 15.20	**O4 –** Alberto Minassi (University of Eastern Piedmont, Italy) *"Back to the future: rediscovering classic photochemistry in triterpene classes interconversion"*

15.20 – 15.40	**O5 –** Daniele Fiorito (Polytechnic University of Milan, Italy) *"Enzymatic catalysis for the sustainable synthesis of terpenoids"*
15.40 – 16.00	**O6 -** Agnieszka Wojtkielewicz (University of Bialystok, Poland) *"Synthesis of 25-hydroxyprovitamin D3 by direct hydroxylation of protected 7-dehydrocholesterol"*
16.00 – 16.30	**Coffee Break**
	Session 3 – Chairpersons: Prof. Martino Forino; Prof. Alessandra Braca
16.30 – 16.50	**O7 –** Ivana Kuzminac (University of Novi Sad, Serbia) "*Synthesis and antitumor activity of 19-modified steroidal D-homo lactones*"
16.50 – 17.10	**O8 –** Carmina Sirignano (University of Naples Federico II, Italy) *"Hedgehog pathway inhibiting activity of hydroxylated cyclopamine analogues from Veratrum californicum"*
17.10 – 17.30	**O9 -** Barbara Bednarczyk-Cwynar (Poznan University of Medical Science, Poland) *"When 2+2 is more than 4: oleanolic acid dimers - synthesis, spectral characteristics, cytotoxic and antioxidant activity"*
17.30 – 17.50	**O10 –** Antonella Porrello (University of Palermo, Italy) *"Synthesis of oxofunctionalized oleananes with antibacterial activities"*
17.50 – 18.10	**O11 -** Dorota Czajkowska-Szczykowska (University of Bialystok, Poland) *"One-step synthesis of hydroquinone linked 3,3'-steroid dimers using a modified Mitsunobu protocol"*
18.10 – 18.30	**O12 –** Valentina Parisi (University of Salerno, Italy) *"New diterpenes with potential anti-inflammatory activity from Lavandula pubescens Decne"*
Starting 19.00	**Welcome cocktail**

Thursday, 19 September (Location: Centro Congressi Partenope)	
	Session 4 – Chairpersons: Prof. Jeroen Dickschat; Prof. Agnieszka Wojtkielewicz
9.00 – 9.40	**PL4 –** Ikuro Abe (University of Tokyo, Japan) *"Unusual enzyme reactions in natural product biosynthesis"*
9.40 – 10.00	**O13 -** Dan T. Major (Bar-Ilan University, Israel) "*Making sense of substrate sensing in terpene synthases from plants and microorganisms. Insights from structural, bioinformatic, and EnzyDock analyses"*
10.00 – 10.20	**O14 -** Tomáš Pluskal (Czech Academy of Sciences of Prague, Czech Republic) *"Highly accurate discovery of terpene synthases powered by machine learning reveals functional terpene cyclization in Archaea"*
10.20 – 10.40	**O15 -** Guangkai Bian (Shenzhen Institute of Advanced Technology, China) *"Efficient genome mining of terpenoids from filamentous fungi"*
10.40 – 11.00	**O16 -** Sergazy M. Adekenov (Research and production center "Phytochemistry",

	Kazakhstan) *"New plant sources of sesquiterpene γ-lactones"*
11.00 – 11.30	**Coffee break**
	Session 5 – Chairpersons: Prof. Ikuro Abe; Prof. Carmen Formisano
11.30 – 12.10	**PL5 –** Dean Guo (Shanghai Institute of Materia Medica, China) *"Natural products chemistry and phytochemical profiling of isoprenoids from Chinese herbal medicines: taking Dendrobium nobile as an example"*
12.10 – 12.30	**O17 –** Antonietta Cerulli (University of Salerno, Italy) "*Guaiane-type sesquiterpenoids from the leaves of Cynara cardunculus L. var. scolymus, source of 'Carciofo bianco di Pertosa', and evaluation of their tyrosinase inhibitory activity"*
12.30 – 12.50	**O18 –** Antonio Cala Peralta (University of Cadiz, Spain) *"Recent advances on sesquiterpenes and diterpenes against parasitic weeds"*
12.50 – 13.10	**O19 –** Simona Piccolella (University of Campania L. Vanvitelli, Italy) *"Triterpene saponins from cilentan Calendula arvensis (Vaill.) L.: UHPLC-HRMS-based identification, occurrence, and oral bioaccessibility"*
13.10 - 14.40	**Lunch and**
	Poster Session 1 (Posters from P1 to P20)
	Session 6 – Chairpersons: Prof. Angela Bisio; Prof. Maurizio Bruno
14.40 – 15.20	**PL6 –** Paola Dugo (University of Messina, Italy) *"Innovative chromatographic methods for the analysis of isoprenoids in essential oils"*
15.20 – 15.40	**O20 –** Massimo Tacchini (University of Ferrara, Italy) *"Comprehensive analysis of Hyssopus officinalis L. essential oils: chemotype characterization, bioactivity assessment, and quality control implications"*
15.40 – 16.00	**O21 -** Hamid Mushtaq (University of Campania L. Vanvitelli, Italy) *"Supercritical CO_2 extraction and characterization of neuroprotective terpenes from the leaves of Olea europaea L. cultivars from Campania region"*
16.00 – 16.20	**O22 -** Marina Morozovová (Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic) *"Advances in steroid compound analysis: The role of ELSD detection"*
16.20 – 16.40	**O23** – Giuseppe Amato (University of Salerno, Italy) *"Chemical composition and anti-enzymatic activity of the volatile fraction of four species belonging to the Origanum genus"*
starting 17.15	**Social Activity**
20.30	**Social Dinner**

Friday, 20 September (Location: Centro Congressi Partenope)	
	Session 7 – Chairpersons: Prof. YueWei Guo; Prof. Marialuisa Menna
9.00 – 9.40	**PL7** – Ren Xiang Tan (Nanjing University, China) *"Discovery, biosynthesis, and regeneration of bioactive fungal metabolites"*
9.40 – 10.00	**O24 -** Eva Kudová (Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic) ***"****Neurosteroids drug development in pain signaling – Computational modelling and medicinal chemistry interplay"*
10.00 – 10.20	**O25 -** Monica Scognamiglio (University of Campania L. Vanvitelli, Italy) *"Dihydro-β-agarofuran sesquiterpenoids from Maytenus senegalensis"*
10.20 – 10.40	**O26-** Mariia Vodolazhenko (Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Czech Republic) *"Emerging opportunity: reshaping of steroid molecules"*
10.40 – 11.00	**O27 –** Alessandra Capuano (University of Salerno, Italy) *"Uncovering natural myrianthic acid targets with label-free proteomics and mass spectrometry"*
11.00 – 11.30	**Coffee break**
	Session 8 – Chairpersons: Prof. Guangkai Bian; Prof. Sonia Piacente
11.30 – 12.10	**PL8** - Jeroen S. Dickschat (University of Bonn, Germany) *"The mysterious case of sodorifen and beyond: mechanistic investigations on terpene synthases"*
12.10 – 12.30	**O28 –** Teo Hebra (Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, Czech Republic) *"The analytical challenge to uncover the first archaeal terpene synthases"*
12.30 – 12.50	**O29 -** Henrik Toft Simonsen (University Jean Monnet, Saint-Etienne, France) *"Fragrant terpenoids from plants you normally disregard"*
12.50 – 13.10	**O30 -** Adam Jozwiak (University of California Riverside, USA) *"Deciphering the mechanism and evolutionary origins of stereodivergent production of steroidal glycoalkaloids in Solanaceae"*
13.10 – 14.40	**Lunch and**
	Poster Session 2 (Posters from P21 to P40)
	Session 9 – Chairpersons: Prof. Asaph Aharoni; Prof. Giuseppina Chianese
14.40 – 15.20	**PL9** – Luca Senaldi (Indena spa, Italy) *"Synthesis, conformational behaviour and occurrence of cannabidiol isomers"*
15.20 – 15.40	**O31** – Paula Berman (Weizmann Institute of Science, Israel) *"Mapping the cannabinoid highway: exploring biosynthetic pathways in Helichrysum umbraculigerum"*
15.40 – 16.00	**O32 –** Giulia Mazzoccanti (University of Rome La Sapienza, Italy) *"Enantiomeric dominance and optical purity of cannabichromene (CBC) are affected*

	by plant chemovar and thermal configurational stability"
16.00 – 16.20	**O33 –** Nadia Benedetto (University of Basilicata, Italy) *"Humulus lupulus L. phytochemicals, docking and obesity: do bitter acids play a beneficial role?"*
16.20 – 16.50	**Coffee Break**
	Session 10 – Chairpersons: Prof. Pavel Drasar; Prof. Georges Massiot
16-50 – 17.20	**O34 - Winner of the Kenji Mori award** Miroslav Kvasnica (Institute of Experimental Botany, the Czech Academy of Science & Palacký University, Czech Republic) *"Neuroprotective activity of synthetic plant-inspired oxysterols"*
17.20 – 17.40	**O35 -** Yaroslava Bukhonska (National Academy of Sciences, Kyiv, Ukraine) *"The effect of exogenous 24-epicastasteron application on phytohormone content in soybean and Arabidopsis thaliana"*
17.40 – 18.00	**O36** – Maria Michela Salvatore (University of Naples Federico II, Italy) *"In vitro antiviral activity of sphaeropsidins towards bovine coronavirus: A translational study"*
18.00 – 18.20	**O37 -** Joanna Fiedor (University of Krakow, Poland) *"Interactions of carotenoids with red blood cells: insight into their stability and oxygen binding properties"*
18.20 – 18.40	**O38 –** Rui Tan (Southwest Jiaotong University, China) *"The active ingredients and mechanism from Tibetan medicine prescriptions mediating stem cells to repair nerve injury in ischemic stroke"*
Starting 18.40	**Announcement of the Winners of the Wiley Best Poster Award** **Announcement of the Winner of the Senatore Award** **Announcement of next Isoprenoids conference** **Closing Remarks**

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ABSTRACTS
PLENARY LECTURES

Hidden Gems from the Early Studies on Isoprenoids

Giovanni Appendino

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Because of their accumulation in specific biomasses, the isoprenoids camphor and cholesterol were among the first organic compounds obtained in pure form, a remarkable feat since these compounds lack ionisable groups, and formation of salts remained the main method of purification of organic compounds until the early 19th century [1]. Just like the study of alkaloids provided the foundation for modern pharmacology, so the one of isoprenoids spurred the development of organic chemistry. Optical activity, photochemistry, pericyclic reactions, cationic rearrangements and retrosynthetic analysis are among the many innovative developments first observed in isoprenoids or inspired by their chemistry, and the recent discovery by Baran of an unprecedented form of stereoiomerism in the marine cyclopeptide triptorubin A [2] suggests that the potential of natural products to provide contributions of general relevance to organic chemistry is not yet exhausted.

I will provide an overview of the early chemistry of isoprenoids, describing how it spurred, often in a serendipitous and unexpected way, critical developments in organic chemistry. Because of their pharmaceutical relevance and/or easy availability, the monoterpenes camphor and α-pinene, and the sesquiterpenoids santonin and β-caryophyllene dominated the early studies on lower isoprenoids. These investigations were recapped by the work of Otto Wallach (1847-1931), whose isoprenoid investigations were honoured by the 1910 Nobel Prize for chemistry. A similar leading figure is missing for the early investigations on higher isoprenoids, a class of compounds, which, on account of their hormonal and vitamin bioactivity, became dominant in the 20th century.

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PL2

HOW DO PLANTS EVOLVE SPECIALIZED METABOLITES AND PATHWAYS?

Asaph Aharoni

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Specialized metabolites represent a generous portion of the plant metabolic repertoire counting thousands in an individual plant. Generating such chemical complexity requires continues evolution of genes encoding proteins producing novel metabolites with selective advantage in a particular environmental niche. Genes with new function in specialized metabolism often arise following duplication of genes involved in primary/core metabolites formed across all species. In the presentation, I will portray several different molecular mechanisms wherein genes of core, primary metabolic pathways were 'hijacked' providing a template for the evolution of new enzymatic functions as well as new pathways. I will highlight our recent findings that protein of the cellulose synthase family (i.e., Cellulose Synthase-Like; CSLs) evolved novel activities. While we initially identified CSLs to be involved with small molecules glucuronidation, recent results showed that members of this protein family serve as structural, scaffolding elements critical for the function of proteins associated with the same metabolic pathway (i.e., metabolons). Thus, I will be highlighting several such evolutionary mechanisms that represent merely a small portion of nature's processes that create an extraordinary chemical diversity.

PL3

STRATEGIES AND TACTICS FOR THE RAPID SYNTHESIS OF MOLECULAR COMPLEXITY

Scott A. Snyder

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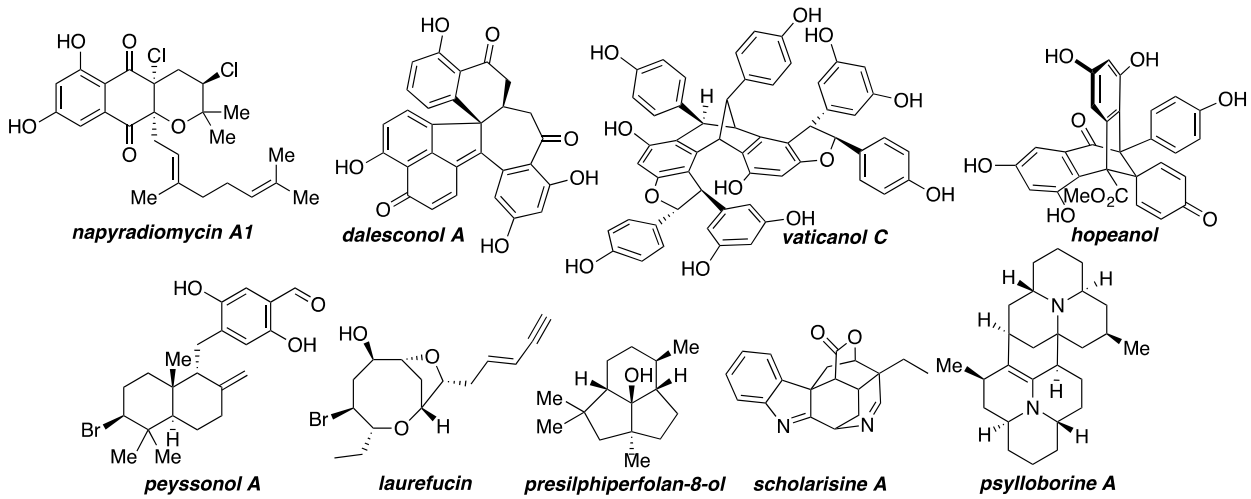
The total synthesis of natural products has long served as a principal driving force for discovering new chemical reactivity, evaluating physical organic theories, testing the power of existing synthetic methods, and enabling biology and medicine. Research in our group continues in that tradition, with efforts focused on developing reactions, reagents, and strategies to rapidly assemble entire collections of natural products in hopes of gaining a fuller understanding of their biochemical potential. Over the past decade, these efforts have afforded access to numerous classes of compounds, including halogenated materials, diverse polycyclic and stereochemically dense alkaloids, non-functionalized terpenes, and oligomeric polyphenols.[1-5] This talk will present recent advances and discoveries, focused particularly on structurally unique terpene targets.



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PL4

UNUSUAL ENZYME REACTIONS IN NATURAL PRODUCT BIOSYNTHESIS

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Nonheme iron-dependent enzyme reactions play crucial roles in building and modifying bioactive organic molecules in all major classes of natural product pathways. While the enzymes have evolved to use a limited repertoire of protein folding and metal binding sites, the oxidation reactions they catalyze are astonishingly diverse, spanning from complex rearrangements to uncommon bond formation and cleavage reactions. This presentation will summarize recent discoveries that have significantly expanded our understanding of unusual nonheme iron enzyme catalysis in natural product biosynthesis. [1, 2]

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2. Awakawa, T., Mori, T., Ushimaru, R., Abe, I. (2023). *Nat. Prod. Rep.*, **40**, 46-61

NATURAL PRODUCTS CHEMISTRY AND PHYTOCHEMICAL PROFILING OF ISOPRENOIDS FROM CHINESE HERBAL MEDICINES: TAKING *DENDROBIUM NOBILE* AS AN EXAMPLE

De-an Guo, Jiayuan Li, Yaling An and Hanze Wang

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Dendrobium species, as both the traditional Chinese herbal medicine and tonic dietary supplement, have attracted much attention due to its diverse chemical components and pronounced pharmacological activities. Systematically chemical isolation, identification, and diastereomers differentiation of the characteristic sesquiterpenoids from *Dendrobium nobile* and their phytochemical profiling were conducted.

Totally 83 sesquiterpenoids, including 40 new structures were isolated from the stems of *Dendrobium nobile*, which contains diverse structural types, such as picrotoxane-type with a lactone moiety, cyclocopacamphane-type with a C_{15}-ketone group, copacamphane-type with a tetracyclic system, and allo-aromadendrane-type with 5/7/3 carbon skeleton. Their structures were accurately elucidated by comprehensive spectroscopic analysis, chemical derivatization, quantum chemical calculations, and X-ray diffraction analysis. For the stereochemistry determination of cyclocopacamphane-type sesquiterpenoid glycoside diastereomers, we identified their structures by enzymatic hydrolysis using *β*-cellulase to obtain aglycone followed by recrystallization for the first time.

Additionally, a thorough phytochemical profiling of the isoprenoids and other components from several officially recorded *Dendrobium* species by Chinese Pharmacopoeia (2020 edition) were performed and lead to a set of new sesquiterpenoids characterized.

This research provided a reference for the confirmation of the stereochemistry of sesquiterpene analogs with polycyclic and polychiral centers and laid an activity research foundation for the further development of *Dendrobium* medicinal herbs.

INNOVATIVE CHROMATOGRAPHIC METHODS FOR THE ANALYSIS OF ISOPRENOIDS IN ESSENTIAL OILS

Paola Dugo,[1,2] Danilo Sciarrone,[1] Luigi Mondello[1,2]

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Essential oils may be considered medium to highly complex samples, which are formed of a volatile fraction (and a non-volatile fraction in the case of cold-pressed citrus essential oils) containing numerous isoprenoids. Gas chromatography (GC) is the analytical technique that has given the major contribution towards the determination of volatile profiles in essential oils. On the other hand, the study of the non-volatile fraction is generally achieved through high performance liquid chromatography (HPLC).

The development of different chromatographic methods has allowed the determination of the composition of essential oils, with information on authenticity, geographic origin, possible contamination and adulteration. A series of limitations, though, must be considered:

- single column chromatography often lacks the necessary resolving power to separate the components of a complex matrix in an acceptable analytical time. Furthermore, extensive peak overlapping is a hinderance towards reliable MS structural elucidation.
- conventional chromatographic methods, while sometimes ensuring satisfactory separations on essential oils samples, are also characterized by a substantial disadvantage: the cost in analytical time. This becomes a limiting factor especially for laboratories with a high sample throughput and/or where there is a need for quick and correct results.

In the past years, there has been an increasing interest, within the chromatographic community, towards the development of more effective separation methods, to obtain advantages in terms of time and separation power.

The present contribution will present an overview on the most advanced monodimensional and multidimensional chromatographic techniques today employed in essential oil analysis. Selected applications on different essential oil samples will be described, in order to demonstrate the effectiveness of the different approaches.

DISCOVERY, BIOSYNTHESIS, AND REGENERATION OF BIOACTIVE FUNGAL METABOLITES

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Fungal metabolites (FMs) account for 61.5% of hitherto described microbial natural products including drugs such as penicillin, lovastatin, and cyclosporin. FMs are structurally more diverse than commercial compound libraries, and some contain unique substructures not possessed by other compounds in established data sets (e.g., ChEMBL), thereby being a promising source of lead compounds that are pressingly required for developing high-value products such as medicines and agrochemicals (e.g., pesticides and herbicides). However, the past decades have witnessed a remarkable deceleration in hitting structurally unforeseeable FMs with potent bioactivity and sample scalability, two prerequisites for the follow-up research and development, primarily because such undescribed FMs are exceedingly low in abundance and tend to be overshadowed by a variety of co-produced known natural products. This talk will present the discovery protocol, biosynthetic pathway, and regeneration strategy of low-abundance bioactive FMs such as immunosuppressive isoprenoids.

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THE MYSERIOUS CASE OF SODORIFEN AND BEYOND: MECHANISTIC INVESTIGATIONS ON TERPENE SYNTHASES

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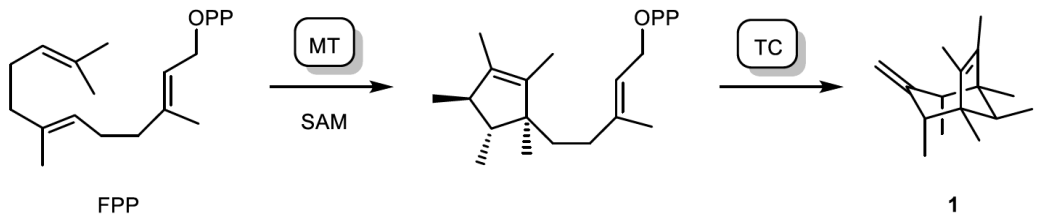
Terpene synthases catalyse the conversion of acyclic and achiral oligoprenyl diphosphate precursors into usually (poly)cyclic, chiral and enantiomerically enriched terpene hydrocarbons or alcohols. These reactions proceed with multiple carbon-carbon bond formations and changes of the hybridisation of often more than half of the precursor carbons in just one enzymatic step. The mechanisms of terpene synthases can be investigated through isotopic labelling experiments,[1] structure based site-directed mutagenesis, [2,3] computational chemistry, [4] and, ideally, combinations thereof.[5] The lecture will discuss the intricate biosynthesis of the unusual methylated sesquiterpene sodorifen [6,7] (**1**) and other examples currently investigated in our laboratory.



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SYNTHESIS, CONFORMATIONAL BEHAVIOUR AND OCCURRENCE OF CANNABIDIOL ISOMERS

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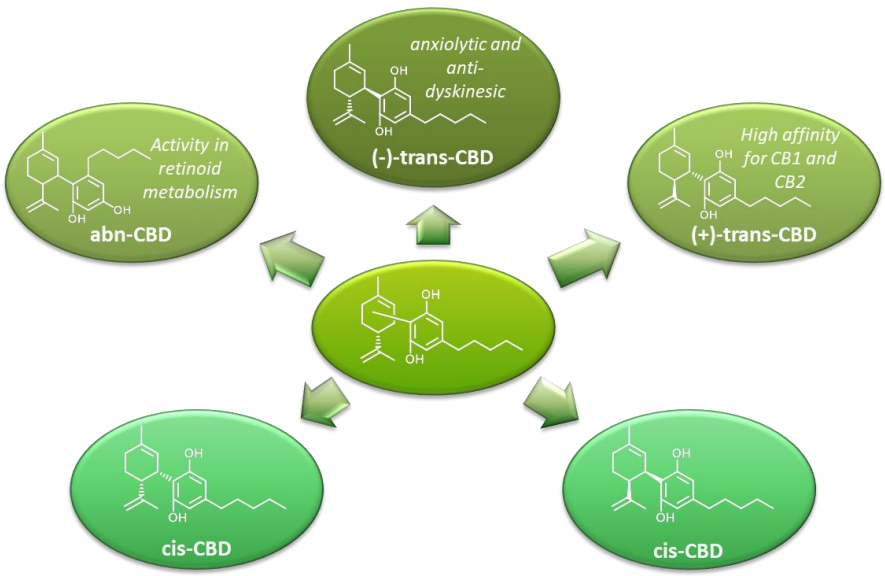
(-) *trans*-Cannabidiol (CBD) is a non-narcotic natural phytocannabinoid first isolated from hemp (*Cannabis sativa* L.) in 1940 by Adams. CBD is the active constituent of Epidiolex, a drug used to treat some rare forms of epilepsy otherwise refractory to pharmacological treatment, like the Lennox-Gastaut syndrome. CBD has a broad pharmacological profile, which include anxiolytic and anti-dyskinesic activity. [1] (-)-CBD has modest affinity for CB1 and CB2 receptors, while its unnatural enantiomer [(+)-CBD] is a high-potency ligand for both receptors.[2] Furthermore, a cannabidiol regioisomer, namely abnormal-CBD, is a first-in-class candidate to affect retinoid metabolism by targeting CRBPs (Cellular Retinoid Binding Proteins) and suppress its retinal overproduction, the hallmark of degenerative eye diseases like macular degeneration. [3] The regio- and stereochemical configuration of the cannabidiol framework seems therefore to play a key role on bioactivity. In order to fully elucidate this aspect, the synthesis and analytics of *cis*-CBD isomers were developed and provided samples of the pure isomers. Their conformational behaviour was investigated by NMR and the occurrence in *C. sativa* systematically investigated.



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ABSTRACTS
ORAL COMMUNICATIONS

SPACING ENGINEERING FOR TERPENOID OVERPRODUCTION

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Natural enzymes in their native metabolic pathway are tightly regulated on where to exert their activities, and on the concentration of metabolites to produce. This regulation is often lost or at least weakened when enzymes are cloned and expressed in an unfamiliar community of a heterologous host cell. Here, we firstly developed a pair of short peptide tags (RIAD and RIDD) to create two enzyme assemblies complex to control the flux of metabolites to increases carotenoid production by 5.7 folds[1]. Then, we developed a strategy named mimic PKS enzyme assembly line (mPKSeal) that assembles multiple key cascade enzymes to enhance biocatalytic efficiency and increase target production by recruiting cascade enzymes tagged with docking domains from type I *cis*-AT PKS to increase astaxanthin production by 2.4-fold[2]. Further, artificial membraneless organelles were constructed to increase nerolidol production by 7.8-fold. This study addresses the challenge of cascade catalytic efficiency and highlights the potential of continuous catalysis by spacial engineering.

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CROSSIELLIDINES A-F: UNPRECEDENTED PYRAZINE-ALKYLGUANIDINE, PRENYLATED METABOLITES WITH BROAD-SPECTRUM ANTIBACTERIAL ACTIVITY FROM *CROSSIELLA* SP.

Francisco Javier Ortiz-López,[1] Daniel Oves-Costales,[1] Daniel Carretero-Molina,[1]Jesús Martín,[1] Caridad Díaz,[1] Mercedes de la Cruz,[1] Fernando Román-Hurtado,[1] María Álvarez Arévalo,[2] Tue Sparholt Jørgensen,[2] Fernando Reyes,[1] Tilmann Weber [2] and Olga Genilloud [1]

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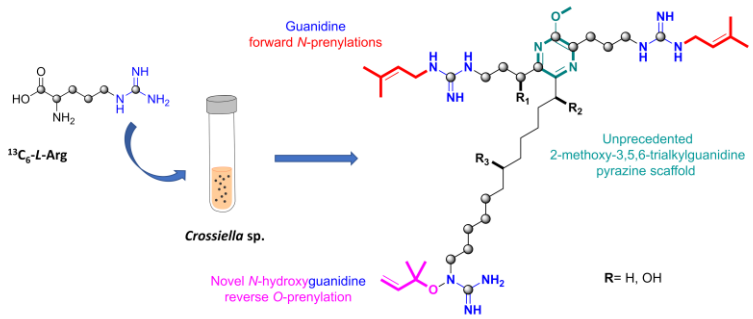
Unexplored bacterial taxa are a potential gold mine for the discovery of new bioactive compounds. Crossiellidines A-F, a family of novel antibacterial pyrazine-alkylguanidine metabolites, were isolated from the minor actinomycete genus *Crossiella*.[1] The intriguing structures of these new natural products were determined by 2D NMR spectroscopy and shown to be derived from an unprecedented 2-methoxy-3,5,6-trialkylguanidine pyrazine scaffold, further decorated with highly unusual “on-heteroatom” prenylations, such as forward *N*-prenylation of guanidine moieties and a previously unreported reverse, *O*-prenylation on an *N*-hydroxyguanidine group. The novel substitution pattern of the 2-methoxypyrazine core inaugurates a new class of naturally occurring pyrazine compounds, the biosynthetic implications of which are discussed in this work. Stable isotope-guided metabolomics combined with genome analysis allowed us to propose a biosynthetic pathway to these metabolites from arginine. Crossiellidines showed remarkable, broad-spectrum antibacterial activity, including relevant Gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumoniae*.[2]



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PARTIAL SYNTHESIS OF TAGITININ C DERIVATIVES AS PROTEASOME INHIBITORS

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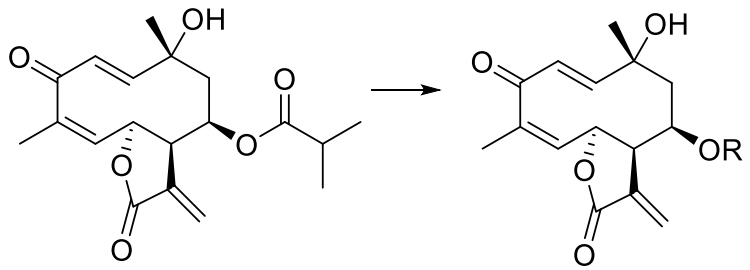
Tagitinin C is the major sesquiterpene lactone from *Tithonia diversifolia*, known as Mexican sunflower. It is a highly reactive germacradiene comprising cross-conjugated dienone, α-methylene-γ-lactone, tertiary alcohol, and ester functionalities. It is photochemically unstable and sensitive to acids, bases, and nucleophiles. It possesses a fair level of cytotoxic activity, and it was detected as a hit in a high throughput screening assay for proteasome inhibition. In order to improve the activity level, the stability and to secure an intellectual property, it was decided to prepare unnatural derivatives of tagitinin C. The communication will describe several routes to this goal, some of them could be generalized to other lactone-esters.



tagitinin C R = alkyl, acyl

Reference

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BACK TO THE FUTURE: REDISCOVERING CLASSIC PHOTOCHEMISTRY IN TRITERPENE CLASSES INTERCONVERSION

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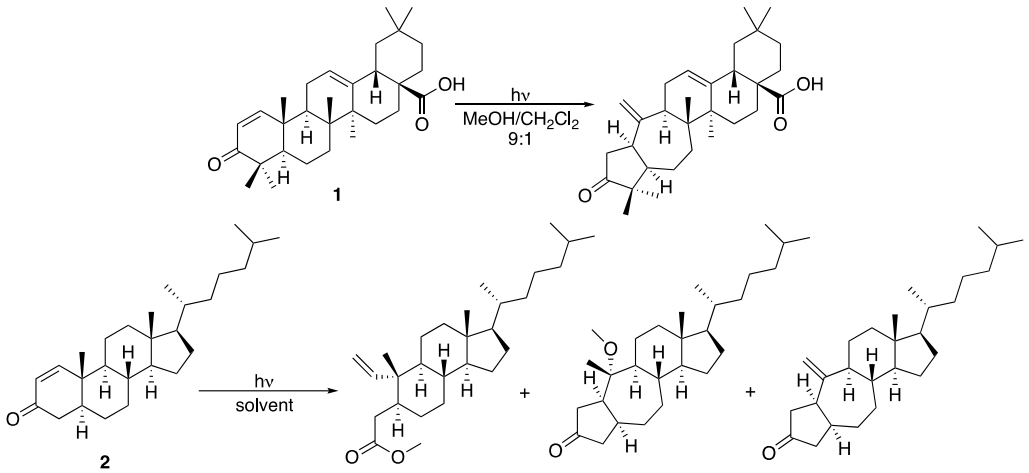
Natural product chemistry is constantly challenged by newly discovered complex molecules with unprecedented frameworks frequently characterized by interesting biological activity. Unfortunately, they are usually produced in small amounts and, for their total synthesis, a limit of what is considered practical in terms of steps and yield is frequently reached. As an alternative, semisynthetic approach could be a solution to provide answers to the pressing demand for sustainable, facile, and concise routes even to the most complex targets.[1]

Photochemistry has proven to be a highly efficient tool in organic synthesis for the construction of complex architectures through the cleavage and the formation of new carbon-carbon bonds.[2]

In this field, we rediscovered a classic photochemical approach exploring the photoreactivity of overlooked Δ^1-3-oxo-oleanolic acid (**1**) and Δ^1-3-oxo-dihydrocholesterol (**2**), obtaining new *lumi*-derivatives through a stereoselective rearrangement of the A/B rings. While Δ^1-3-oxo-oleanolic acid reacts in a defined way remodeling the oleanane skeleton into the justicane one,[3] the photoreactivity of Δ^1-3-oxo-dihydrocholesterol can be easily tuned by varying the reaction solvent.



The mechanism of the rearrangement along with the possible applications to the synthesis of complex natural compounds will be discussed.

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ENZYMATIC CATALYSIS FOR THE SUSTAINABLE SYNTHESIS OF TERPENOIDS

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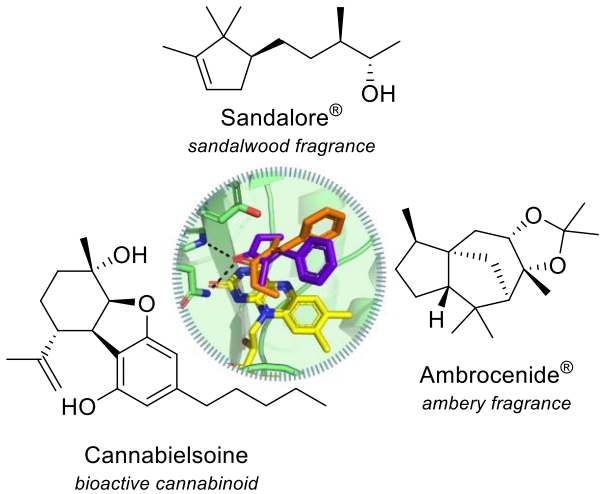
Terpenes represent a large class of natural products with applications in both pharma and fragrance industry. They are characterized by enormous structural diversity and stereochemical complexity, which ensure a broad range of biological properties.[1] Therefore, stereochemical control is paramount in their synthesis and derivatization.[2] In this context, biocatalytic reduction and oxidation reactions offer an opportunity for both selectivity and sustainability.[3] Herein, we present redox approaches in the synthesis and functionalization of terpenes by enzymatic catalysis. In particular, we will present the enzymatic C=C and C=O bond reduction for the stereoselective preparation of sandalwood fragrances;[4] and a stereoselective chemoenzymatic approach to the fragrance Ambrocenide®.[5] We will also present a chemoenzymatic approach to bioactive derivatives of Cannabidiol, itself recovered from agricultural biomass waste.[6]



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Acknowledgments This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

SYNTHESIS OF 25-HYDROXYPROVITAMIN D_3 BY DIRECT HYDROXYLATION OF PROTECTED 7-DEHYDROCHOLESTEROL

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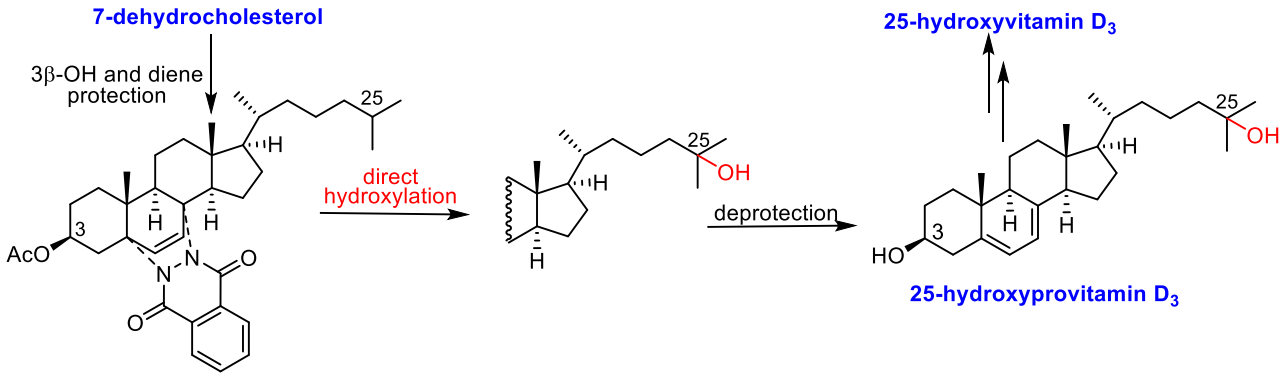
25-Hydroxyvitamin D_3, the metabolite of vitamin D_3, is used as an oral drug to replenish vitamin D deficiency in cases when cholecalciferol supplementation is not effective (e.g. vitamin D-resistant rickets, familial hypophosphatemia, hypoparathyroidism, hypocalcemia, renal osteodystrophy).[1] It is also a prescription medicine to treat secondary hyperparathyroidism in patients with chronic kidney disease. Moreover, recent studies proved its potential in preventing and treating cancer and COVID-19.

The current approaches to the synthesis of 25-hydroxyvitamin D_3 consist of enzymatic hydroxylation of vitamin or provitamin D_3 or multi-step transformations using raw materials such as 3β-hydroxy-5-cholenoic acid, ergosterol, stigmasterol, desmosterol, or vitamin D_2.[2] We elaborated the alternative strategy for preparing 25-hydroxyprovitamin D_3, a key-intermediate for the synthesis of calcifediol (25-hydroxyvitamin D_3).[3] The target cholesta-5,7-diene-3β,25-diol was obtained from readily available 7-dehydrocholesterol in a concise synthesis benefiting from successful direct 25-hydroxylation (Scheme 1). The proposed method could be a beneficial alternative to the multi-step and expensive approaches to the synthesis of 25-hydroxyprovitamin D_3 described so far.



Scheme 1. Synthesis of 25-hydroxyprovitamin D_3

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SYNTHESIS AND ANTITUMOR ACTIVITY OF 19-MODIFIED STEROIDAL D-HOMO LACTONES

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Since their discovery at the beginning of the 20th century, steroids have found use in important medicinal applications. They are irreplaceable for the treatment of certain types of hormone-dependent cancers, such as breast and prostate cancer. With this in mind, we have synthesized a series of steroidal D-homo lactones with modifications on C19. Multiple-phase synthesis was conducted starting from dehydroepiandrosterone, and after seven to ten synthetic steps 19-hydroxy derivatives were obtained. Its further chemical transformations afforded aldehyde, carboxylic, oxyimino, nitrile, and halogenated derivatives. All synthesized compounds showed favorable ADMET properties in *in silico* models. Antiproliferative activity was tested on six tumor and one healthy cell line. The best antiproliferative activity was detected against breast (MCF-7 and MDA-MB-231), colon (HT-29), and lung (A549) cancer cell lines, for some compounds in low micromolar and submicromolar values. Tested steroid derivatives displayed weak or no binding affinity for ligand-binding domains of estrogen receptor α, estrogen receptor β or androgen receptor expressed in-frame with yellow fluorescent protein in *Sacharomyces cerevisiae,*[1,2] suggesting lack of potential hormonal activity and hormone-related side effects. Aldo-keto reductases (AKRs) are involved in the metabolism of steroid hormones and may be overexpressed in various human diseases, so in this study selected synthesized compounds were also evaluated for inhibition potential against human recombinant AKR1C4 isoform by monitoring consumption of NADPH fluorometrically. Resuts from *in vitro* assays demonstrate the therapeutic potential of new 19-modified steroidal D-homo lactones as valuable anticancer drug candidates.

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The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200125) and Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: New steroid derivatives - potential chemotherapeutics, No. 142-451-3463/2023-01].

HEDGEHOG PATHWAY INHIBITING ACTIVITY OF HYDROXYLATED CYCLOPAMINE ANALOGUES FROM *VERATRUM CALIFORNICUM*

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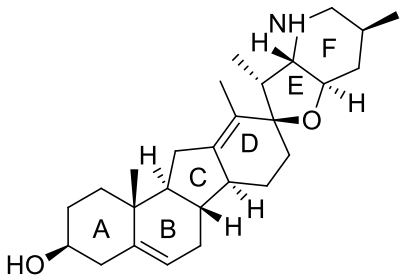
Cyclopamine is a hexacyclic isosteroidal alkaloid including fused tetrahydrofuran and piperidine rings obtained from corn lily, *Veratrum californicum*, belonging to the Melanthiaceae family.

This natural product is a specific Hedgehog (Hh) signalling pathway antagonist of Smoothened protein.[1] Since aberrant activation of the Hh signalling has been identified as a causal factor in the development of several tumors, inhibitors of this pathway can have a significant anticancer effect. Thus, cyclopamine and its semisynthetic derivatives have shown a marked anticancer activity on pancreatic cancer, on basal-cell carcinoma and other solid and liquid cancers.[2]

A chemical analysis of mother liquors obtained from the crystallization of cyclopamine, extracted from roots and rhizomes of *V. californicum*, led to the isolation of two unprecedented cyclopamine analogues, representing the first compounds of this class to show modifications on rings D-F. The detailed stereostructures of these new natural compounds have been established based on HRMS and 1D/2D NMR experiments. The isolated metabolites were evaluated with the dual-luciferase bioassay for their inhibition of the Hedgehog pathway in comparison to cyclopamine, providing new perspectives into the structure–activity relationships for this class of compounds.



Cyclopamine

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WHEN 2 + 2 IS MORE THAN 4: OLEANOLIC ACID DIMERS - SYNTHESIS, SPECTRAL CHARACTERISTICS, CYTOTOXIC AND ANTIOXIDANT ACTIVITY

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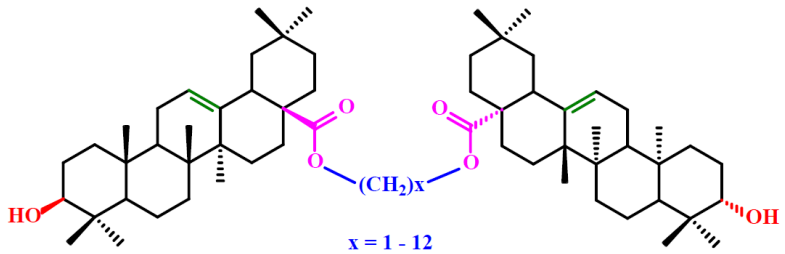
The presented work aimed to obtain a set of oleanolic acid derivatives with high level of cytotoxic and antioxidant activities and low level of toxicity and with the application of economical method. All derivatives of oleanolic acid had a dimer structure.

Oleanolic acid was alkylated with α,ω-dihalogenoalkane / α,ω-dihalogenoalkene. All of the newly obtained compounds were subjected to QSAR computational analysis to evaluate the probability of the occurrence of different types of pharmacological activities. All dimers were tested for cytotoxicity activity and for their antioxidant potential (on the SKBR-3, SKOV-3, PC-3, and U-87 cancer cell lines with MTT assay). HDF cell line was applied to evaluate the tested compounds' Selectivity Index. The antioxidant test was performed with a DPPH assay.



IC_{50} < 10 microMol

Almost all triterpene dimers showed a high level of cytotoxic activity towards selected cancer cell lines, with an IC50 value below 10 μM. The synthesized derivatives of oleanolic acid exhibited varying degrees of antioxidant activity, surpassing that of the natural compound in several instances. Conclusions: Joining of two oleanolic acid residues through their C-17 carboxyl group using α,ω-dihalogenoalkanes / α,ω-dihalogenoalkenes results in the synthesis of highly potent cytotoxic agents with favourable SI and of high level of antioxidant activity.

SYNTHESIS OF OXOFUNCTIONALIZED OLEANANES WITH ANTIBACTERIAL ACTIVITIES

Antonella Porrello,[1], Natale Badalamenti,[1,2] Maurizio Bruno,[1,2] Mario Varcamonti,[3] Sara Pio,[3] Giusy Castagliuolo,[3] Anna Zanfardino,[3] Gianfranco Fontana[1]

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Oleanolic acid (OA) belong to the class of natural triterpenes and are endowed with a wide range of biological activities, including cytotoxicity against several cancer cell lines.[1,2] The aim of this study is to investigate the antibacterial activity of a series of oleanolic acid derivatives with different modifications of the substitution at C-3. Some of the molecules in the set were already available from previous studies and some were designed and synthesized ex novo, providing a panel of compounds with an oleanolic carbon skeleton modified by the addition of a three carbon side chain at C-3 with varying lipophilic, hydrogen bond ability and flexibility.

Specifically, semisynthetic triterpenic acids were obtained from oleanolic acid as previously reported;[3] carbomethoxy derivatives were obtained by methylation of the corresponding acid by diazomethane. Finally, the spyro-tetrahydrofuryl derivative was obtained by a new method. The microbiology experiments carried out involved Kirby and Bauer assays to determine whether terpenes possess antimicrobial activity. They were conducted against two strains *E. coli* as Gram

negative and *S. aureus* as Gram positive model. Subsequently, experiments were performed using the microdilution method to determine the minimum inhibitory concentration for bacterial growth (MIC) on the model strains used previously.

The results showed that only derivatives with reduced hydrogen-bonding capacity on the A-ring possess significant activity towards *E. coli*. Furthermore, conversion from acid to methyl ester displays a lower activity, probably due to a reduced affinity for the bacterial membrane.

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ONE-STEP SYNTHESIS OF HYDROQUINONE LINKED 3,3'-STEROID DIMERS USING A MODIFIED MITSUNOBU PROTOCOL

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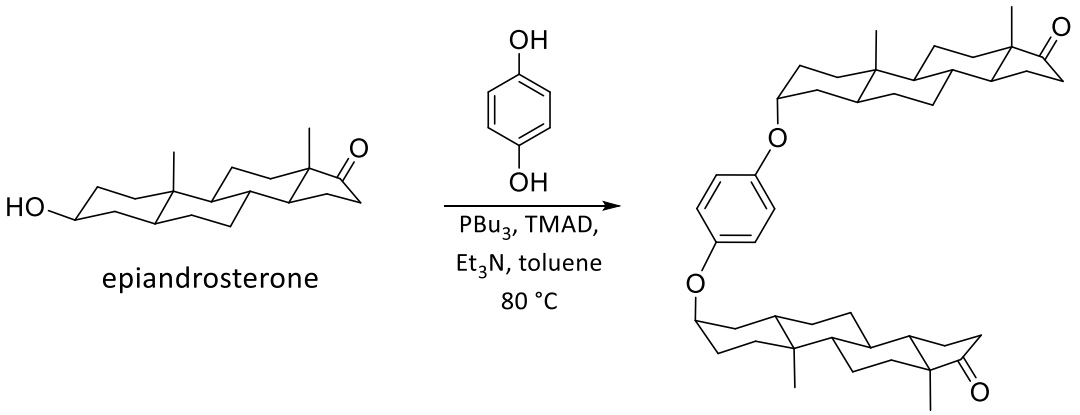
Steroid dimers are a specific group of compounds due to their wide profile of biological activity, such as cytotoxic, antimicrobial, antimalarial or antifungal activities.[1] They may also be of interest for material science because they exhibit interesting properties as crystalline molecular rotors or porous crystals.[2] Dimerization of steroids can occur in two different ways: a direct connection between steroid frameworks or a connection *via* a linker (spacer). However, introducing a linker, such as hydroquinone, between the C-3 carbon atoms of two steroid molecules to form ether bonds proved to be a challenge. The successful application of the Mitsunobu reaction to the synthesis of steroid dimers linked by the *O*,*O*-1,4-phenylene group and a new procedure for achieving this goal in one step will be presented.[3]



Scheme. Synthetic route to the bis-steroidal 1,4-phenylene diethers *via* modified Mitsunobu protocol

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NEW DITERPENES WITH POTENTIAL ANTI-INFLAMMATORY ACTIVITY FROM *LAVANDULA PUBESCENS* DECNE

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Lavandula ssp (Lamiaceae) are indigenous to the regions bordering the Mediterranean Sea, from southern Europe through north and east Africa, and Asia. Several *Lavandula* species have been subjected to chemical investigation, which has yielded the isolation of triterpenes, sesquiterpenes, diterpenes and phenolic compounds which are understood to be the genus secondary metabolites[1-3]. This genus comprises species which are traditionally employed in the treatment of a variety of ailments. Specifically, *Lavandula pubescens* is used in Saudi Arabia for its antibacterial and anti-inflammatory properties. Although preliminary studies have been carried out on the essential oil of the plant, the surface exudate has not been investigated to date. In this study, the extract of the surface exudate of *L. pubescens* was separated by BIOTAGE, MPLC, and HPLC, then the isolates were characterised as diterpenoids by NMR and HRESIMS analyses. So far, six new isopimarane, several new unusual secoabietanes and spiro-pimaranes have been identified. The compounds are decorated with hydroxy, epoxy and carbonyl groups. Configurational and conformational arrangement elucidation of the diterpenes was carried out by the application of experimental (i.e., NMR spectroscopy) and in *silico* methodologies. (i.e. QM/NMR). The latter represents one of the most powerful ways to elucidate the stereochemical features thanks to the calculation of chemical parameters (^{13}C and ^{1}H NMR chemical shifts, homo and heteronuclear *J* coupling constants), and their comparison with experimental ones using statistical parameters for the analysis. Since proinflammatory cytokines displayed a key role in the inflammatory process, their modulation by surface exudate and isolates in THP-1 derived M0 macrophages stimulated with LPS was investigated. IL-6 secretion was inhibited by 90, 75, and 65% in cells exposed for 6 h to 50, 25 and 12.5 µg/mL, respectively, of the extract. Dihydroxy-iso-pimar-8(9)-ene and 15,16,17-trihydroxypimar-8(9)-ene were able to downregulate IL-6 in THP1 cell lines with EC_{50} of 40.86±0.03 and 19.42±0.04 µg/mL.

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MAKING SENSE OF SUBSTRATE SENSING IN TERPENE SYNTHASES FROM PLANTS AND MICROORGANISMS. INSIGHTS FROM STRUCTURAL, BIOINFORMATIC, AND ENZYDOCK ANALYSES

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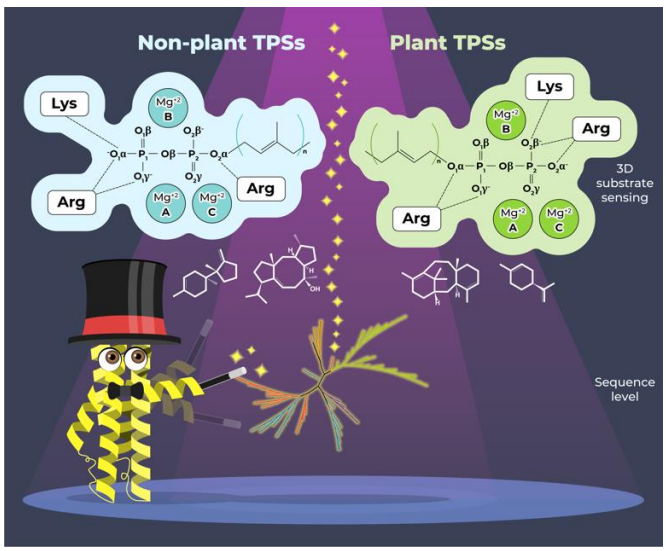
Terpene synthases (TPS) catalyze the first step in the formation of terpenoids, which comprise the largest class of natural products in nature. TPS employ a family of universal natural substrates, composed of isoprenoid units bound to a diphosphate moiety. The intricate structures generated by TPS are the result of substrate binding and folding in the active site, enzyme-controlled carbocation reaction cascades, and final reaction quenching. A key unaddressed question in class I TPS is the asymmetric nature of the diphosphate-$(Mg^{2+})_3$ cluster, which forms a critical part of the active site. In this asymmetric ion-cluster, two diphosphate oxygens protrude into the active site pocket. The substrate hydrocarbon tail, which is eventually molded into terpenes, can bind to either of these oxygens, yet to which is unknown. Here, we employ structural, bioinformatics, and EnzyDock docking tools to address this enigma.[1,2] We bring initial data suggesting that this difference is rooted in evolutionary differences between TPS. We hypothesize that this alteration in binding, and subsequent chemistry, is due to TPS originating from plants or microorganisms. We further suggest that this difference can cast light on the frequent observation that the chiral products or intermediates of plant and bacterial terpene synthases represent opposite enantiomers.



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HIGHLY ACCURATE DISCOVERY OF TERPENE SYNTHASES POWERED BY MACHINE LEARNING REVEALS FUNCTIONAL TERPENE CYCLIZATION IN ARCHAEA

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Terpene synthases (TPSs) generate the scaffolds of the largest class of natural products, including several first-line medicines. A wealth of knowledge on the complexity of TPS rearctions has been accummulated through decades of scientific research. However, millions of uncharacterized TPS sequences can be found in genomic databases and accurate computational prediction of their function remains an unsolved challenge. We curated a dataset of 2,500 characterized TPS reactions and developed a method to devise highly accurate machine-learning models for functional annotation in a low-data regime. Our models significantly outperform existing methods for TPS detection and substrate prediction. By applying the models to large protein sequence databases, we discovered and experimentally validated a number of new TPS enzymes previously undetected by state-of-the-art bioinformatic tools, including the first reported TPSs in Archaea. Furthermore, we described a new TPS structural domain and distinct subtypes of previously known domains. Our results demonstrate the potential of machine learning to speed up the discovery and characterization of novel enzymes.

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Preprint of the article related to this communication https://doi.org/10.1101/2024.01.29.577750

EFFICIENT GENOME MINING OF TERPENOIDS FROM FILAMENTOUS FUNGI

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Terpenoids are an important source of natural product drugs, and efficient genome mining and elucidation of unusual biosynthetic mechanisms are the focus of research in the field of terpenoid biosynthesis. However, difficult product acquisition, low throughput, and repeated product discovery are significant challenges that limit efficient genome mining of terpenoids. We have developed an innovative strategy for genome mining of terpenoids, based on an "automated biofoundry and efficient microbial chassis"[1]. This strategy has effectively solved the bottlenecks of difficult product acquisition and low research throughput and have resulted in a series of interesting discoveries including: 1) Unleashing the potential of terpene synthase to produce series of unusual sesqui-, di-, sester-, and triterpene skeletons[2-4]. 2) Achieving automatic and high-throughput genome mining of terpene synthases and gene clusters, characterizing the biosynthetic mechanism, and achieving efficient production of newly discovered anti-inflammatory sesterterpenoid mangicol J[1]. 3) Discovering a non-squalene-derived triterpene biosynthetic pathway that is catalyzed by class I terpene synthase[5]; uncovering the cryptic function of class I terpene synthases as aromatic prenyltransferases[6]; and determining their catalytic mechanism and biological significance.

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NEW PLANT SOURCES OF SESQUITERPENE γ-LACTONES

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More than 6000 plant species grow on the territory of the Republic of Kazakhstan, of which 667 endemic species and more than 1400 species are medicinal. Plants of the Asteraceae family (more than 1000 species) are widely represented in the natural flora of Kazakhstan, which are considered potential sources of biologically active terpenoid compounds. In terms of searching for new promising plants - sources of biologically active sesquiterpene γ-lactones, the Research and Production Centre 'Phytochemistry' has been conducting systematic resource and chemical studies of plants growing in the territory of the Republic of Kazakhstan for a number of years.

We have studied 527 species of plants of the family Asteraceae, of which 67 are representatives of the tribe Anthemideae, 35 belong to the tribe Supageae and 17 species - to the tribe Cichorieae. At the same time, more than 30 endemic plant species have been identified in the surveyed territories of Kazakhstan. 131 plant species of the family Asteraceae were studied for the content of sesquiterpene γ-lactones.

According to the results of chemical study of the above taxa, the presence of sesquiterpene lactones was found in 78 plant species. At the same time, more than 100 sesquiterpene γ-lactones were isolated from the sum of extractive substances of 65 plant species and their molecular structures were determined.

Thus, as a result of systematic phytochemical studies of plants of the flora of Kazakhstan we have identified promising sources of biologically active sesquiterpene lactones, including plants of the genera *Achillea* L, *Ajania* Poljak., *Artemisi*a L., *Centaurea* L., *Chartolepis* Boiss., *Cousinia* Cass., *Inula* L., *Jurinea* Cass., *Lepidolopha* C. Winnkl., *Ligularia* Cass., Pulicaria Gaeth., *Rhaponticum* Adans., *Saussurea* DC., *Stizolophus* Cass., *Tanacetopsis* R.Br., *Tanacetum* L.

It was determined that the majority of practically available sesquiterpene γ -lactones of plants of the Asteraceae family can be a renewable material for chemical modification and synthesis of new compounds - potential sources of original antiviral, antibacterial, immunomodulatory, anti-inflammatory, antitumour, antiparasitic agents.

GUAIANE-TYPE SESQUITERPENOIDS FROM THE LEAVES OF *CYNARA CARDUNCULUS* L. VAR. SCOLYMUS, SOURCE OF 'CARCIOFO BIANCO DI PERTOSA', AND EVALUATION OF THEIR TYROSINASE INHIBITORY ACTIVITY

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Globe artichoke (*Cynara cardunculus* L. var. scolymus), belonging to the family Asteraceae, is a perennial plant famous for its edible bracts[1]. "Carciofo di Pertosa", a traditional product of the Campania region (Italy), is known as the "white" artichoke of Pertosa for the pale green color of the flower head [2]. During the harvesting and industrial process, the leaves of artichoke represent the principal waste material. In order to evaluate the opportunity to use artichoke by-products for the development of nutraceutical and/or cosmetic formulations, the phytochemical investigation of "Carciofo di Pertosa" leaves was carried out. EtOH: H_2O (75:25) extract of the leaves obtained by SLDE-Naviglio extraction was preliminarily submitted to liquid chromatography coupled to high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS), in negative ion mode, allowing the identification of polar fatty acids and specialized metabolites belonging to flavonoids, phenylpropanoids, and sesquiterpenoids of which the structural elucidation was performed by 1D- and 2D-NMR experiments as well as FIA-MS analysis. In this way, in addition to the most well-known caffeoyl-, dicaffeoyl quinic acid derivatives and flavonoids, eleven sesquiterpenoids lactones, belonging to the guaiane-type class, were unambiguously characterized. Among these, one compound is here described for the first time and two compounds were never identified before in *Cynara* genus. Based on the ability to prevent skin photoaging processes reported for cynaropicrin[3], the most abundant sesquiterpenoids identified in the extract, the evaluation of the tyrosinase inhibitory activity of extract and isolated sesquiterpenoids is in progress.

Acknowledgments
This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022).

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RECENT ADVANCES ON SESQUITERPENES AND DITERPENES AGAINST PARASITIC WEEDS

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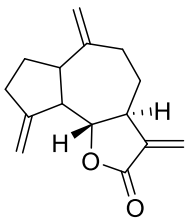
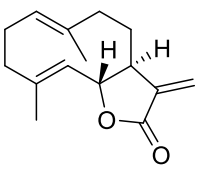
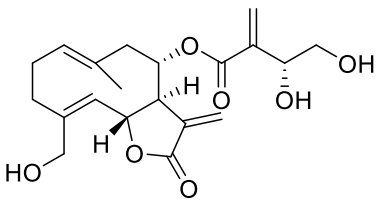
Parasitic weeds, such as broomrapes (Orobanchaceae), which encompass various *Orobanche* and *Phelipanche* species pose a significant threat to important agricultural crops, with limited methods available for their control. In the last few years, we investigated the structure-activity relationships of natural terpenoids and their derivatives, finding clues to design compounds to deal with these weeds.[1-4] Some of them, such as dehydrocostus lactone and costunolide (isolated from *Saussurea costus*), have been found to stimulate the germination of seeds of broomrapes. Some others, such as cnicin and salonitenolide (isolated from *Centaurea cineraria*), have been found to inhibit radicle growth of these weeds. In contrast, diterpenoids such as gibberellic or kaurenoic acid, were found initially inactive, to turn bioactive after chemical derivatization. From the isolation of bioactive terpenes to the synthesis of new strigolactone analogues and mimics, this communication summarizes the previous results, presents the most recent ones and offers insights into the development of new potential herbicides to deal with parasitic weeds.



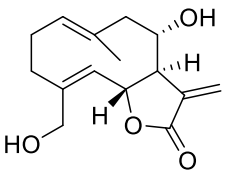
Dehydrocostus lactone



Costunolide



Cnicin



Salonitenolide

Acknowledgements

A.C.P. and J.G.Z. express their sincere gratitude to the “Plan Propio UCA 2022-2023 and 2023-24” for the financial support in the projects “Investigadores Noveles, Proyectos para impulsar su Carrera Científica” (Ref. PR2022-043 and PR2023-026). A.C.P. also thanks the support of the “Programa de Fomento e Impulso de la actividad Investigadora” of the University of Cádiz, funding his participation in this congress.

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TRITERPENE SAPONINS FROM CILENTAN *CALENDULA ARVENSIS* (VAILL.) L.: UHPLC-HRMS-BASED IDENTIFICATION, OCCURRENCE, AND ORAL BIOACCESSIBILITY

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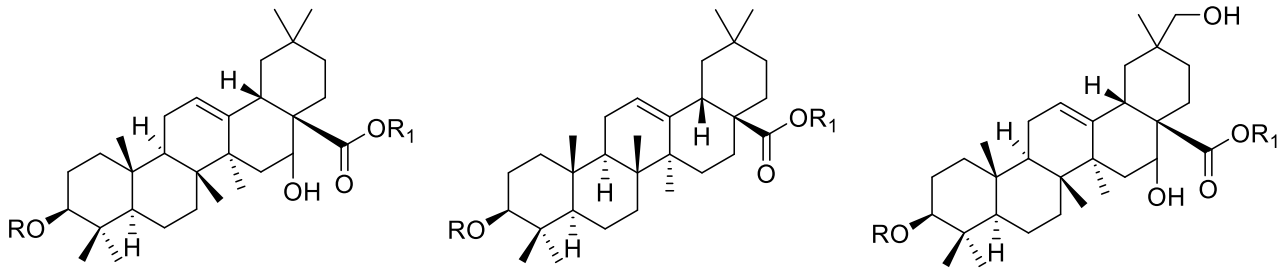
In the framework aimed at promoting and valorizing the functional value of *Calendula arvensis* (Vaill.) L. as a source of bioactive molecules, a systematic organ-specific chemical investigation was performed by a UHPLC-HRMS/MS untargeted approach, thus unraveling the chemical complexity of the specialized metabolites from its organs. In this context, the abundance and diversity of triterpene saponins in florets were highlighted, with oleanolic and echinocystic acid derivatives, glycosylated at C-3 and/or C-28 positions, as the most representative ones (Figure 1). The rationalization of fragmentation patterns from the deep study of HR-MS/MS spectra represented a great and challenging opportunity to draw guidelines that could feasibly favor the rapid identification of this class of compounds in complex mixtures.[1] After freeze-drying encapsulation into maltodextrin, their preservation was valued during *in vitro* simulated digestion. It was found that acylation and oxidation reactions, and deglycosylation impacted the triterpene saponins, which, however, kept intact the aglycone core.[2]



R = hex-hex-hex; R_1 = hex
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R = hex-hex; R_1 = hex
R = hexu; R_1 = hex
R = HMG-hex-hexu; R_1 = H
R = HMG-hexu; R_1 = H

R = hex-hex-hex; R_1 = hex
R = hex-hexu; R_1 = hex
R = hex-hex; R_1 = hex
R = hex; R_1 = hex
R = hexu; R_1 = hex
R = hexu; R_1 = Achex
R = HMG-hexu; R_1 = hex

R = hexu; R_1 = hex

Figure 1. Chemical structures of triterpene saponins (36 – 49) tentatively identified in the C. arvensis alcoholic extracts. Hex = hexose; Hexu = hexuronic acid; Achex = acetylhexose; HMG = Hydroxymethylglutaric acid.

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COMPREHENSIVE ANALYSIS OF *HYSSOPUS OFFICINALIS* L. ESSENTIAL OILS: CHEMOTYPE CHARACTERIZATION, BIOACTIVITY ASSESSMENT, AND QUALITY CONTROL IMPLICATIONS

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Hyssopus officinalis L. (Lamiaceae) essential oil (EO), known for its complex chemical composition and various chemotypes, has been extensively studied for its pharmacological properties [1]. This complexity enhances its value in traditional and modern medicine, aromatherapy, pharmaceuticals, and as a natural preservative. This research analysed 20 samples of commercial EOs obtained from the aerial parts of *Hyssopus officinalis* subsp. *officinalis* (var. *decumbens* has been considered a synonym since 2022), provided and certified by producers or retailers based in Italy, Spain, France, and Bulgaria. The chemical characterisation, performed by GC-MS-FID and by 1H NMR-based metabolomics supported by further 2D NMR experiments, revealed two primary chemotypes characterised by eucalyptol and pinocamphones (*cis*- and *trans*-) and a third linalool chemotype. The pinocamphones chemotype can be further divided into two groups, one of which is characterised by a high content of β-phellandrene. The evaluation of the biological properties of the EOs was focused on checking the antifungal activity against two dermatophytes (*Trichophyton menthagrophytes*, CBS 120356, and *T. violaceum*, CBS 459.61) and the antioxidant potential (DPPH test). The chemotype containing pinocamphones and a high concentration of beta-phellandrene exhibited the highest antioxidant activity (IC_{50} = 3.24 µl/ml). The same subgroup also exhibited the most significant antifungal activity, with an interesting growth inhibition of *T. mentagrophytes* (28%) and *T. violaceum* (15%). The eucalyptol chemotype, however, exhibited negligible antifungal activity, except for one sample that showed 55% growth inhibition of *T. mentagrophytes* growth, distinguished by its high aromatic or olefinic compound concentration. The supervised and unsupervised multivariate data analysis overviews the global phytochemical and biological results. This work underscores the need for detailed quality control procedures on commercial essential oils, especially when the plant of interest expresses extremely diversified chemotypes with different bioactivities and organoleptic properties. In this context, the predictive OPLS-DA model provided in this work offers a valuable tool for EOs quality control.

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SUPERCRITICAL CO_2 EXTRACTION AND CHARACTERIZATION OF NEUROPROTECTIVE TERPENES FROM THE LEAVES OF *OLEA EUROPAEA* L. CULTIVARS FROM CAMPANIA REGION

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Olive leaves by the pruning and harvesting of olive trees (*Olea europaea* L.) represent one of the olive oil industry by-products, whose valorization needs to be pursued also in an innovative food and nutraceutical scenario. In this work, leaves from five different *Olea europaea* L. cultivars (named Caiazzana, Carolea, Itrana, Leccino, and Frantoio) some of which autochthonous were collected from Campania region, Southern Italy with the consideration of cultivar biodiversity. After freeze drying and crushing, an innovative green extraction supercritical CO_2 fluid extraction (SFE) process was applied for selectively obtaining terpene-rich extracts. The SFE conditions were: 330 bar, 60°C, and 9 mL/min CO_2 flow rate. The extraction kinetic was studied by plotting the extraction yield curve considering total yield (%) vs. extraction time. The obtained terpene enriched extracts from different cultivars were analyzed by gas chromatography mass spectrometry (GC-MS) and liquid chromatography diode array detector (LC-DAD) to get insight into compounds belonging to each terpene class [1]. The relative content in tri- and tetraterpenes suggested the extracts could be neuroprotective. To this purpose, their acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) inhibitory activities were analyzed [2]. The antioxidant activity was also studied by using different methods (ABTS, FRAP). The obtained data highlighted that the selective extraction of terpene was improved by the application of the SFE extraction method in comparison to the other conventional methods. Moreover, all the extracts were capable of exerting neuroprotective effects, and this bioactivity was correlated to their high terpene content.

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ADVANCES IN STEROID COMPOUND ANALYSIS: THE ROLE OF ELSD DETECTION

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An evaporative light scattering detector (ELSD) is a destructive detector that is highly recommended for the analysis of compounds with weak or no UV absorption, such as steroids. ELSD works on the principle of light scattering by particles in an aerosol after evaporation of the mobile phase. It is a semi-universal detector that does not require the derivatization of analytes, which is a major advantage over UV detectors commonly used in high-performance liquid chromatography (HPLC).[1]

In our research, the determination of purity is carried out after the HPLC separation and the subsequent detection by means of a single quadrupole MS and ELSD. This setup of HPLC-MS with ELSD makes it a convenient technique, that allows us to record fingerprint spectra of studied steroidal compounds and confirmation their purity. In comparison, the identification of impurities by UV detection alone is deficient and may provide misleading information. It will be presented as an example of HPLC-UV spectra compared with HPLC-MS-ELSD and NMR.

In addition, we have also evaluated the potential of ELSD detection for the analysis of biological samples. Our comprehensive study aims to analyse the ADME of endogenous steroids and neurosteroids (stability, permeability, solubility, etc.) for further structure-activity relationship studies. The results of the comparative analysis of HLPC-ELSD versus HPLC-MS detection for the evaluation of steroid stability in rat plasma will be presented.

This work was supported by the Technology Agency of the Czech Republic (TACR), National Centers of Competence, Personalised Medicine: From Translational Research into Biomedical Applications, PERMED: T2BA No. TN02000109 and the Research Project of the CAS RVO 61388963.

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CHEMICAL COMPOSITION AND ANTI-ENZYMATIC ACTIVITY OF THE VOLATILE FRACTION OF FOUR SPECIES BELONGING TO THE *ORIGANUM* GENUS.

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The *Origanum* genus belongs to the Lamiaceae famly. *O. vulgare* L. is one of the most renowned aromatic species, with a strong traditional background as both a spice and medicinal plant [1]. *O. majorana* L., thanks to its rich chemical profile in terms of essential oil and plant extracts, is considered a plant with valuable pharmacological activities [2]. *O. dictamnus* L., known as 'Dittany of Crete' possesses numerous medicinal uses, such as antibacterial and antifungal properties[3]. *O. heracleoticum* L., also known as with the name of 'Greek oregano', has been used in traditional herbal medicine in the treatment of cough and toothache[4]. The aims of this study were: I) to determine the chemical composition of *O. vulgare, O. majorana, O. dictamnus,* and *O. heracleoticum* essential oils (EOs) obtained from their aerial parts using GC coupled to GC/MS; II) to evaluate their anti-enzymatic activity on enzymes involved in metabolism (α-amylase, α-glucosidase and lipase) and in the Central Nervous System (acetyl- and butyryl-cholinesterase and tyrosinase); III) to determine the mechanism of inhibition through Michaelis–Menten kinetics. *O. vulgare* and *O. dictamnus* EOs were mainly composed of carvacrol and *p*-cymene. They were the most active against butyrylcholinesterase (IC_{50}: 260 and 190 µg/mL, respectively) and α-glucosidase (IC_{50}: 520 and 460 µg/mL, respectively). *O. majorana* EO was predominantly characterized by γ-terpinene and terpinen-4-ol, resulting the only sample active against α-amylase and the most active against tyrosinase (IC_{50}: 250 µg/mL). *O. heracleoticum* EO was mainly composed of carvacrol, thymol and γ-terpinene and it was the most active against acetylcholinesterase (IC_{50}: 0.51 µg/mL) and lipase (IC_{50}: 14.94 µg/mL). The results obtained highlighted the differences between the four *Origanum* species, in terms of chemical composition and biological activities. These findings draw attention to the potential use of EOs of *Origanum* in the health sector, particularly in the management of metabolic and Central Nervous System disorders.

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NEUROSTEROIDS DRUG DEVELOPMENT IN PAIN SIGNALING - COMPUTATIONAL MODELLING AND MEDICINAL CHEMISTRY INTERPLAY

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Neuropathic pain remains a difficult-to-treat condition that remains a high unmet medical need. Neurosteroids (NS) offer therapeutic opportunities for treatment by altering inhibitory or excitatory signaling through interaction with ligand-gated ion channels and other cell surface receptors. [1] Indeed, the antinociceptive properties of NS have been in humans and animals. [2]

Our traditional approach to drug development targets GABAergic signaling. Unfortunately, the rational design of novel compounds using molecular docking at the binding site(s) is severely limited because NS bind to an overlapping subset of specific sites on GABAA receptors, with their net functional effect on channel gating being the sum of their independent effects at each site. [3] We have created a library of over 100 in-house and literature 3D structures. Multiple ranking and binary QSAR models were built based on these 3D structures to predict activities. The feasibility of our model for neurosteroid drug development will be discussed.

We have synthesized novel positive allosteric modulators of GABAA receptors. Their *in vitro* activity was evaluated in our novel model of acute dorsal root ganglia neuronal cultures. The most promising compound NS10 was then tested in a model of knee joint osteoarthritis. We have shown that our novel compound NS-10 exhibits a robust *in vivo* effect for further development in the treatment of neuropathic pain.

This work was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104), Funded by the European Union - Next Generation EU, and by the Czech Academy of Sciences institutional supports RVO:61388963.

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DIHYDRO-β-AGAROFURAN SESQUITERPENOIDS FROM *MAYTENUS SENEGALENSIS*

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Dihydro-β-agarofuran sesquiterpenoids are characterized by a polyoxygenated tricyclic skeleton core, bearing from as few as two to as many as nine ester groups [1, 2]. The family Celastraceae is the major source of these sesquiterpenoids [2]. Previously reported dihydro-β-agarofurans have shown a great potential as anticancer compounds [1].

In this study, a metabolomics-based approach was combined with the ethnobotanical tradition, in the search for anti-leukemia terpenoids from the root bark of a plant belonging to the Celastraceae family commonly used in traditional medicine in Botswana, namely *Maytenus senegalensis.*

The root bark of this plant was extracted and analysed by NMR-based metabolomics. Preliminary tests showed that this extract was active against the human U937 leukemia cell line. The extract was then partitioned using several chromatographic steps to afford pure compounds. The structural elucidation of the pure compounds was carried out by extensive 1D and 2D NMR analyses.

The main compound, shown in the figure below, was reported for the first time to the best of our knowledge. Most of the isolated compounds were characterized by the same macrolide structure. However, NMR data suggested that different organic acids were involved both in the formation of this structural unit and in the esterification at the other hydroxyl groups.

Further studies are currently dedicated to the complete structural elucidation of the isolated compounds and to the evaluation of their bioactivity against leukemia cell lines.

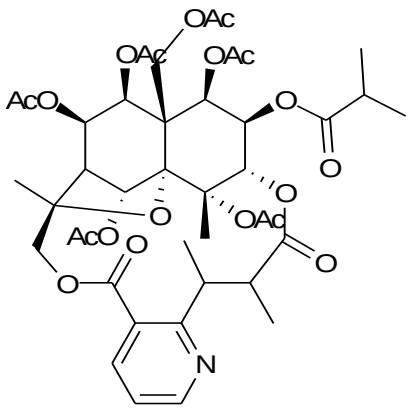

Figure 1. The main dihydro-β-agarofuran sesquiterpenoid isolated from *Maytenus senegalensis*

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EMERGING OPPORTUNITIES: RESHAPING OF STEROID MOLECULES

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Neurosteroids are endogenous compounds synthesized in and for the brain and play one of the most significant roles in the human body. Not only can they participate in our metabolism and be effective drugs with different types of activity, but they are also involved in synaptic plasticity, age-related neurodegenerative diseases, learning, and memory function, or disturbances associated with certain neuropsychiatric disorders[1-3] through interaction with ion channel receptors, e.g. gamma-amino-butyric acid ($GABA_A$) and *N*-methyl-*D*-aspartate receptors (NMDA).[4,5] The presence of multiple stereocentres in the steroid skeleton has a significant influence on the overall shape of the molecules and therefore their biological activity.

In this work, we have modified two relevant stereocentres to reshape the molecule starting from dehydroepiandrosterone. This approach allowed us to prepare new steroids with modified stereochemistry at positions C-13 and C-14. As a result, these compounds have an atypical shape of the skeleton. The structures and shapes of skeletons were confirmed by the X-ray analysis. Variations of the main functional groups were prepared for both series. The activity of the synthesised compounds on the NMDA receptor was tested and hit molecules were identified. In addition, their analogs with classical stereochemistry at C-13 and C-14 were prepared. For all synthesized compounds, their stability, solubility, and permeability were evaluated in comparison to each other. The results of this study define new skeletons for the further development of novel neurosteroid drug-like compounds.

This work was supported by the Czech Science Foundation GACR, No. 23-04922S and Research Project of the Academy of Sciences of the Czech Republic: RVO grants 61388963 and 61388963.

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UNCOVERING NATURAL MYRIANTHIC ACID TARGETS WITH LABEL-FREE PROTEOMICS AND MASS SPECTROMETRY

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Natural products (NPs), with their diverse structural characteristics, represent a rich source of potential drug candidates, providing notable advantages in terms of application and biocompatibility. Identifying the protein targets of these compounds is crucial for understanding their mechanisms of action and advancing drug development. Chemical proteomics has become an essential tool for this purpose, offering various methods to pinpoint NP protein targets.

This study investigates the interactions between myrianthic acid (MA) [1], a natural triterpenoid with an ursane skeleton extracted from *Oenothera maritima* Nutt. (Onagraceae), and its potential protein targets. Using advanced mass spectrometry-based chemical proteomic techniques, including Drug Affinity Responsive Target Stability (DARTS)[2] and targeted Limited Proteolysis coupled with Mass Spectrometry (t-LiP-MS)[3], the research reveals the intricate molecular interactions underpinning MA's biological activities.

A significant finding of this study is the identification of fatty acid synthase (FAS) as a major target of MA. Detailed experimentation and validation have elucidated the MA/FAS complex, and in vitro assays demonstrate MA's inhibitory effects on FAS enzyme activity. This underscores MA's potential therapeutic relevance, particularly in addressing tumor proliferation.

Given FAS's critical role in various pathological conditions, especially cancer, the MA chemical moiety offers a promising foundation for developing targeted therapeutic strategies. Moreover, understanding how myrianthic acid interacts with FAS paves the way for designing and optimizing novel small-molecule inhibitors with improved efficacy and selectivity, marking a significant advancement in precision medicine for treating debilitating diseases.

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THE ANALYTICAL CHALLENGE TO UNCOVER THE FIRST ARCHAEAL TERPENE SYNTHASES

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Our lab has developed machine learning models that detect terpene synthases with high accuracy.[1] To challenge the predictions of the models, we decided to examine the predicted terpene synthase activity of 7,000 “dark matter” protein sequences from UniRef50 with no InterProScan signatures (excluding even “domain of unknown function”).

We manually selected and expressed 17 of those “dark matter” proteins in *Saccharomyces cerevisiae* strain JWY501, genetically modified to overproduce sesquiterpenes ($C_{15}H_{24}$) and diterpenes ($C_{20}H_{32}$).[2] To handle this medium-throughput experiment, we performed the first phenotypic expression of our yeast strains in 24-deep-well plates, therefore cultivating the yeast in 2 mL of culture medium.

Classical metabolic engineering to discover terpene synthases typically relies on GC-EI-MS as a detector of enzymatic activity. In most cases, the product is known or guessed based on literature. Therefore, the major drawback of electron ionization (low or no signal from the molecular ion) is circumvented by searching diagnostic fragments and NIST-EI library queries. However, using this strategy we were only able to confirm 3 enzymes as terpene synthases.

Electrospray ionization is not commonly used for terpenes, but we found it can ionize terpene scaffolds surprisingly well. Thus, we took advantage of our high-resolution mass spectrometer instrument (Orbitrap ID-X) to analyze our yeast extracts. Leveraging a dereplication strategy for untargeted metabolomics, we discovered that 4 additional enzymes were producing terpenes. Out of 7 enzymes with confirmed activity, 3 were detected by both GC-MS and LC-HRMS, 2 only by GC-MS and 2 only by LC-HRMS.

Among the 7 confirmed terpene synthases, 3 were of archaeal origin. This work thus constitutes the first experimental evidence of active terpene biosynthesis in the Archaea kingdom.

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FRAGRANT TERPENOIDS FROM PLANTS YOU NORMALLY DISREGARD

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Bryophytes are the pillows in the forest with numerous ecological functions, and they produce a huge variety of specialized metabolites. It is well established, but maybe not so well known, that liverworts in particularly produce interesting terpenoids and bibisbenzyls. While some of these have interesting pharmacological functions, the liverworts also produce terpenoids with interesting fragrances, which attract microarthropods to help with sperm cell distribution. The knowledge about the production of fragrances among bryophytes is scarce, thus we have embarked on projects to identify novel terpene synthases and other enzymes part of the terpenoid profiles. In liverworts microbial-like terpene synthases plays a crucial role in the synthesis of smaller terpenoids. We have established the biosynthesis of several fragrant terpenoids from *Frullania tamariscii*, *Nardia scalaris, and Lophocolea bidentata*. In *F. tamarisci* we established the biosynthesis of tamariscol, a fragrance that was patented for the use in perfumes in 1985[1]. Based on a transcriptomic analysis followed by biochemical characterization seven MTPSLs where characterized, and one was shown to be tamariscol synthase.

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DECIPHERING THE MECHANISM AND EVOLUTIONARY ORIGINS OF STEREODIVERGENT PRODUCTION OF STEROIDAL GLYCOALKALOIDS IN SOLANACEAE

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Members of the genus *Solanum* produce steroidal glycoalkaloids, which are specialized metabolites known for their toxic properties. Some *Solanum* species, such as tomato and potato, accumulate glycoalkaloids with configuration 25*S*, while others, such as eggplant, accumulate 25*R* isomers. However, the source of this stereodiversity remained unknown. We hypothesized that GAME8 (cholesterol 26-hydroxylase), could be a source of stereodivergent production of glycoalkaloids. We first tested the GAME8 tomato and eggplant enzymes and found that they produce aglycones with different C25 chiralities. Phylogenetic analysis of GAME8 homologs from the *Solanaceae* family revealed that most plants in the genus *Solanum* contain two identical copies of GAME8, and majority of the species produce 25*R* isomer of the aglycone, while only tomato and potato produce the 25*S* counterparts. Additionally, we identified one clade (Morelloid) in the *Solanum* that produces both isomers and contains two different copies of *GAME8*. We used molecular docking and targeted mutagenesis to demonstrate that a change in just a few amino acids could change the stereospecificity of GAME8. Our results were confirmed *in vivo* by overexpressing the eggplant GAME8 in tomato and *vice versa*. Finally, we identified GAME8 enzymes in two wild tomato species (*Solanum pennellii* and *Solanum cheesmaniae*) that underwent parallel reverse evolution and began producing 25*R* alkaloids due to a change in only three to four amino acids. Analysis of metabolites and genomic information from 35 accessions of *S. cheesmaniae* from the Galapagos Islands, spanning from the easternmost to the westernmost islands, sheds light on the evolutionary processes that have shaped the stereospecificity of GAME8 and the metabolomic diversity of wild tomato species over the last 1 million years. Our findings provide insights to mechanisms generating the huge structural diversity of glycoalkaloids and mechanisms of cytochrome P450s stereospecificity. Moreover, it highlights the potential for altering the stereochemistry of glycoalkaloids in plants.

Mapping the Cannabinoid Highway: Exploring Biosynthetic Pathways in *Helichrysum umbraculigerum*

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In recent years, therapeutic applications of cannabinoids have surged, with emerging reports highlighting their medical potential. Cannabinoids, traditionally associated with *Cannabis sativa* L., have also been discovered in other plants like *Helichrysum umbraculigerum* Less (*H. umbraculigerum*). This South-African perennial, akin to *Cannabis*, produces cannabigerolic acid (CBGA), the precursor to major cannabinoids. Despite its promise as a source of cannabinoids, *H. umbraculigerum*'s chemical diversity and genetics remain largely unexplored. In our study, we combine *de novo* whole-genome sequencing data with unambiguous chemical structure annotation, enzymatic assays, and pathway reconstitution in *Nicotiana benthamiana* and in *Saccharomyces cerevisiae* to uncover the molecular and chemical features of this plant. We found that apart from CBGA, the plant synthesizes a plethora of terpenophenols, including known and novel cannabinoids, aralkyl cannabinoids (amorfrutins), prenyl-acyl-phloroglucinoids, prenylchalcones, and prenylflavanones via five parallel pathways. In addition to core biosynthetic enzymes, we uncovered tailoring enzymes generating novel cannabinoid metabolites, offering both an untapped cannabinoid source and tools for engineering in heterologous hosts. This exploration not only broadens our understanding of cannabinoids' sources but also paves the way for innovative therapeutic applications through metabolic engineering strategies.

ENANTIOMERIC DOMINANCE AND OPTICAL PURITY OF CANNABICHROMENE (CBC) ARE AFFECTED BY PLANT CHEMOVAR AND THERMAL CONFIGURATIONAL STABILITY

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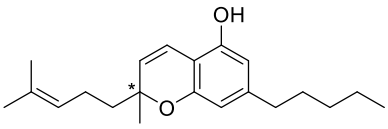
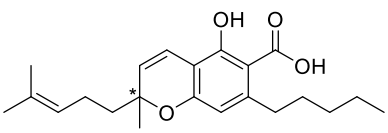
The chiral non-narcotic phytocannabinoid cannabichromene (CBC, **1a**) occurs in Cannabis (*Cannabis sativa* L.) as a scalemate, with the dominant enantiomer depending on the plant chemovar [1,2]. The enantiomeric state of CBC in different Cannabis strains is the presumably the result of the differential expression of cannabichromenic acid (CBCA)-synthase isoforms, and this genetic basis implies that the biological activities of the individual enantiomers of CBC need to be independently studied to fully understand their roles in Cannabis preparations fully.



1a



1b

We present evidence that the optical purity of CBC is also remarkably affected by the matrix in which it is thermally generated from its native carboxylated form (i.e., CBCA **1b**). Thus, thermolysis *in planta* significantly reduces the enantiomeric excess (ee) of CBC, while thermolysis *in extracto* leads to only a slight decrease in optical purity. To explain these observations, we examined the kinetics of thermal racemization of enantiopure CBC at 100 °C, using enantioselective ultra-high performance liquid chromatography, in solvents of different polarity and as a thin film on various solid surfaces. The results showed that contact as a thin film with solid surfaces, particularly glass, accelerates racemization, reducing the half-life ($t_{1/2}$) from 135 hours in decalin to just 6 hours. The optical stability of CBC was also lower in isopropanol compared to decalin, while acidification had no effect. Our findings underscore that the enantiomeric purity of natural CBC is not solely of biogenetic origin but is also significantly affected by the *milieu* where it is thermally generated from its native carboxylated form. This has practical implications for the production and application of CBC, highlighting the necessity of controlled conditions to maintain its optical purity and ensure consistency in its therapeutic effects. Understanding the interplay between genetic factors and matrix conditions is paramount for optimizing the use of CBC in medical and recreational Cannabis products.

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HUMULUS LUPULUS L. PHYTOCHEMICALS, DOCKING AND OBESITY: DO BITTER ACIDS PLAY A BENEFICIAL ROLE?

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Natural products such as *Humulus lupulus* L. (hops) have represented promising strategies against obesity[1] by targeting bitter taste receptors (Tas2rs). Since studies are lacking, the aim of this work is to identify which hop bitter compounds might be related of these effects. We have analyzed the phytochemical profile of hop inflorescence extract which reported the presence of 34 compounds including, polyphenols, their conjugates, and bitter acids. Moreover, hop extract stimulated the secretion of mediators related to satiety. Hop bitter acids, named α- (humulones) and β- (lupulones) acids (**Figure 1**), are chemical compounds found in the flowers of the plant that confer bitterness and flavor to beer. They are phloroglucinol derivatives that contain a 3-,4-,5-, or 6-carbon oxo-alkyl side chain however β- acids are less acidic than α-acids because the tertiary alcohol at C6 is replaced by another isoprene unit[2]. The results obtained suggest that β-acids increased the expression of bitter taste receptors and intracellular calcium concentration to a greater extent than α-acids, leading to the release of the orexigenic hormone GLP-1. Being very active on Tas2r138, molecular docking has allowed to define the main interactions between hop bitter compounds and the active site of mouse and human isoforms of the receptor. In conclusion, this study suggests that hop bitter acids might represent a new strategy in nutraceutical and pharmaceutical fields to prevent or treat obesity by targeting bitter taste receptors.

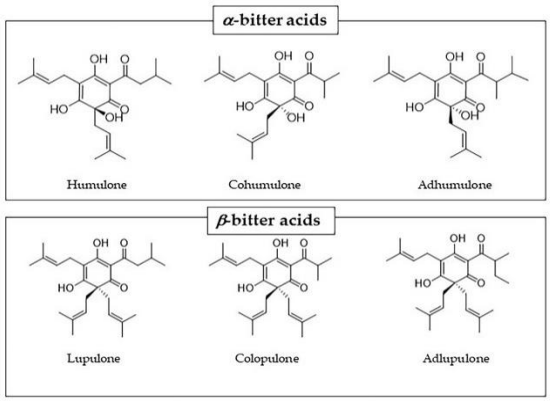

Figure 1. Chemical structure of main hop bitter compounds.

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NEUROPROTECTIVE ACTIVITY OF SYNTHETIC PLANT-INSPIRED OXYSTEROLS

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Oxysterols, oxygenated derivatives of sterols, are highly potent biologically active molecules[1] involved in various human biological processes, including cholesterol homeostasis, the hedgehog developmental pathway, immune responses, and transcriptional regulation through interaction with several nuclear receptors. Similarly, in plants, oxysterols play a crucial role as hormones, brassinosteroids, essential for normal plant growth and development.[2] The study of brassinosteroids and structurally similar compounds with effects at the molecular and cellular level is becoming an important approach in the selection of new substances with different biological activity in humans.

Nowadays, the development of disease-modifying agents towards neurodegenerative diseases such as Alzheimer's, Parkinson's, or Huntington's diseases and other related disorders has become even more urgent since there is a lack of efficient treatment.[3] Currently, we identified several neuroprotective brassinosteroid-inspired oxysterols of which we selected two structurally similar derivatives MK-238 and IG14 with high neuroprotective activity on the neuron-like cell line SH-SY5Y or neural stem cells (NSC). Both compounds show promising results on several models of neurodegeneration (e.g. glutamate, salsolinol, 6-hydroxydopamine, MPP+, L-BMAA). Further studies showed that both compounds mediated neuroprotection mainly by caspase-3,7 inhibition and the reduction of reactive oxygen species. IG14 had also significant results in maintenance of mitochondrial membrane potential in a glutamate-induced model of oxidative damage. Based on more detailed studies, it also appears that the given compounds modulate some nuclear receptors, e.g. LXR or PPAR. The exact mechanism of action is still being studied.

This work was supported financially by grant from the Czech Grant Agency (23-05389S).

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THE EFFECT OF EXOGENOUS 24-EPICASTASTERON APPLICATION ON PHYTOHORMONE CONTENT IN SOYBEAN AND *ARABIDOPSIS THALIANA*

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Our study aimed to examine the effects of exogenous 24-epicastasterone (ECS) and 24-epibrassinolide (EBL) treatment on the levels of indole-3-acetic (IAA), abscisic, salicylic, and jasmonic acids in soybean leaves and *Arabidopsis thaliana* seedling, respectively. ECS, a natural brassinosteroid phytohormone, is a direct biosynthetic precursor to 24-epibrassinolide (EBL), which is widespread in various plants. This research provides deeper insights into the intricate network of interactions between brassinosteroids (BRs) and other plant hormones.

Soybean seedlings (*Glycine max* L. cv. Terek) were cultivated in an artificial climate chamber, sprayed twice with ECS solutions on the 21st and 28th days after germination. Arabidopsis seedlings were grown in sterile conditions on solid ½ MS medium supplemented with EBL. Changes in the endogenous content of phytohormones have been quantified by LC/MS.

Our research showed that treatment with exogenous ECS significantly increased the IAA content in soybean leaves, while phenylacetic acid (PAA) and IAA ester with aspartate (IAA-Asp) levels remained unchanged. In soybean plants treated with ECS, salicylic acid (SA) levels decreased and benzoic acid levels significantly increased, with no notable difference between the ECS concentrations used, aligning with previous studies that suggest a role for BRs and SA in plant adaptation to abiotic stresses. The comparable results in endogenous hormones contents we obtained for Arabidopsis seedlings grown with EBL. EBL strongly induced levels of auxins in Arabidopsis and their conjugates - IAA, IAA-Asp, IAA-Glu, IAA-Ge, IAM. This induction was not repressed by BR biosynthesis inhibitor brassinazole (BRZ), while BRZ alone strongly supressed auxins levels. EBL induced levels of benzoic acid and decreased levels of SA in Arabidopsis as well.

These findings highlight the intricate crosstalk between BRs and other plant hormones, suggesting that ECS and EBL plays an important role

The research was supported by the EURIZON H2020 (EURIZON FELLOWSHIP PROGRAMME Remote Research Grants, grant #EU-3042-137) and Scholarship of the President of Ukraine for Young Scientists in 2023-2024.

*IN VITRO A*NTIVIRAL ACTIVITY OF SPHAEROPSIDINS TOWARDS BOVINE CORONAVIRUS: A TRANSLATIONAL STUDY

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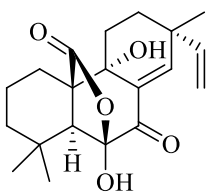
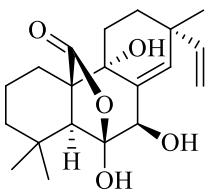
Sphaeropsidins (Sphx) are tetracyclic pimarane diterpenes isolated from different fungi. Although these secondary metabolites have shown a wide range of bioactivities with promising application in several biological fields [1,2], to date no targeted studies have been carried out to evaluate their antiviral properties. After the emergence of severe acute respiratory syndrome coronavirus (SARS-CoV-2), there is a growing interest in the scientific community in the search for new active compounds with antiviral properties. In this study the antiviral effects of sphaeropsidin A (SphA) and its analogue sphaeropsidin B (SphB) were evaluated on Madin Darby Bovine Kidney (MDBK) cell cultures infected with bovine coronavirus (BCoV), a betacoronavirus like SARS-CoV-2, responsible for enteric and respiratory disease in cattle. Sphx employed in this work were obtained from cultures of a *Diplodia corticola* strain isolated from *Quercus suber* in Algeria [3]. Our results showed that, following BCoV infection, non-cytotoxic concentrations of SphA and SphB significantly increased the viability of infected cells, and caused a reduction in virus yield as well as in viral spike S protein expression. In addition, in the presence of both SphA and SphB, we detected a regulation of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that modulates the host immune response to viral infections, upregulated by BCoV, but downregulated by Sphx. Thus, we first assessed the antiviral activity of both Sphx against BCoV, aimed at a possible translational study on SARS-CoV-2.



SphA



SphB

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INTERACTIONS OF CAROTENOIDS WITH RED BLOOD CELLS: INSIGHTS INTO THEIR STABILITY AND OXYGEN BINDING PROPERTIES

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Carotenoids are structurally and functionally a very diverse group of tetraterpenoids. They are produced by all photosynthetic organisms, some non-photosynthetic prokaryotes and fungi. They are also important dietary compounds related to human health[1]. To date, more than 700 carotenoids have been described, of which around 20 can be found in human blood and tissues[2].

Carotenoids can be dispersed in hydrophobic regions of red blood cells. In vitro studies showed, that they affect the physicochemical properties of model lipid bilayers[3]. Their location, orientation, and distribution was shown to strongly depend on the pigment type and concentration.

Here, we would like to present the results of experiments carried out to investigate the effect of structurally different carotenoids on the stability and functioning of erythrocytes. Blood samples were obtained from healthy donors, purified, and subjected to carotenoid treatment. To monitor cell response, microscopic technique and UV-VIS absorption and Mőssbauer spectroscopies were applied. Information on changes in the morphometric parameters and osmotic fragility of red blood cells, as well as the presence of various forms of hemoglobin, the Fe spin state, and their ability to bind oxygen was obtained[4].

Acknowledgements: The research project was partially supported by the programme “Excellence initiative – research university” for the AGH University of Krakow and the National Science Centre, Poland (grant 2019/33/B/NZ7/02724).

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THE ACTIVE INGREDIENTS AND MECHANISM FROM TIBETAN MEDICINE PRESCRIPTIONS MEDIATING STEM CELLS TO REPAIR NERVE INJURY IN ISCHEMIC STROKE

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Based on the screening model of "high-throughput targeted screening and targeted induction of bone marrow mesenchymal stem cells (bMSC) or neural stem cells (NSCs)", we constructed plasmid vectors containing luciferase reporter genes with nerve growth factor (NGF) or vascular growth factor (VEGF) as promoters. Stable cell lines were constructed and screened by lentivirus transfection, and the effects of Natural products（NPs） from Tibetan medicine on the proliferation, differentiation, migration and paracrine functions of bMSCs or NSCs were evaluated.[1-4] In addition, our laboratory further explored the nanoparticles that could modulate the physiological functions of bMSCs and verified the potential value of the "NP@bMSCs" complex in the treatment of ischemia-reperfusion injury.

Some NPs[5-8] have been found to enhance the effects of proliferation, differentiation and migration of bMSCs or NSCs and paracrine functions and play a therapeutic role in ischemia-reperfusion injury.

Ischemia-reperfusion injury is the main cause of acute tissue injury. Regulating bMSCs or NSCs by exogenous small molecules is a feasible way to reduce ischemia-reperfusion injury. Our study showed that some NPs could improve cerebral ischemia-reperfusion in rats by enhancing the proliferation, differentiation and migration of bMSCs or NSCs and paracrine function, or the "NP@bMSCs" complex could increase the expression of neurotrophic factors in bMSCs and glial cells through the CXCR4/SDF-1α axis signaling pathway. It suppressed inflammatory expression in microglia and promoted the migration of renewing cells. Taken together, these works highlight the promise and mechanism of some nanoparticles in combating ischemia-reperfusion injury.

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ABSTRACTS
POSTER COMMUNICATIONS

NEW 4-AZASTEROID 17-MODIFIED DERIVATIVES: SYNTHESIS AND BIOLOGICAL TESTING

Tijana Lj. Sestic,[1] Sofija S. Bekic,[1] Andjelka S. Celic,[2] Edward T. Petri,[2]
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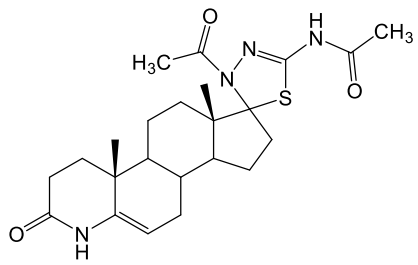
Introduction of a heterocyclic ring into the steroid structure could enhance the bioactivity, improve selectivity and reduce side effects in potential drugs for cancer therapy.[1] The present study describes the synthesis of new thiadiazoline and thiazolidinone steroid A-lactams. These steroidal hybrid molecules may be potential candidates for drug design, with improved biological activity and bioavailability. The starting androstenedione was modified by multi-phase synthesis into a 17-thiosemicarbazone androstane derivative, a direct precursor for the synthesis of heterocyclic androstane derivatives **1**-**3**. New compounds were evaluated for their relative binding affinities for the ligand-binding domains (LBDs) of estrogen receptor α (ERα), estrogen receptor β (ERβ), androgen receptor (AR) or glucocorticoid receptor (GR), using a fluorescent cell assay in yeast.[2,3] Furthermore, inhibition potential against human recombinant aldo-keto reductase 1C3 (AKR1C3) was evaluated by fluorescence spectroscopy. Results from *in vitro* assays suggest that 4-azasteroid 17-modified derivatives are active against hormone-dependent cancer drug targets and represent promising scaffolds for the development of novel therapeutics.



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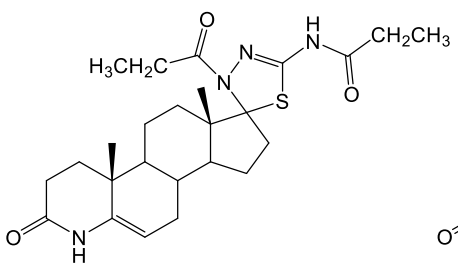

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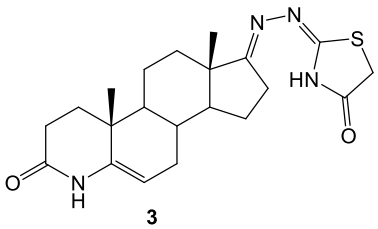

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The authors gratefully acknowledge the financial support of the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants No. 451-03-66/2024-03/ 200125 & 451-03-65/2024-03/200125) and Provincial Secretariat for Higher Education and Scientific Research of the Autonomous Province of Vojvodina [Project: New steroid derivatives - potential chemotherapeutics, No. 142-451-3463/2023-01].

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EXPLORING THE SELF-ASSEMBLY DYNAMICS OF NOVEL STEROID-COUMARIN CONJUGATES: A COMPREHENSIVE SPECTROSCOPIC AND SOLID-STATE INVESTIGATION

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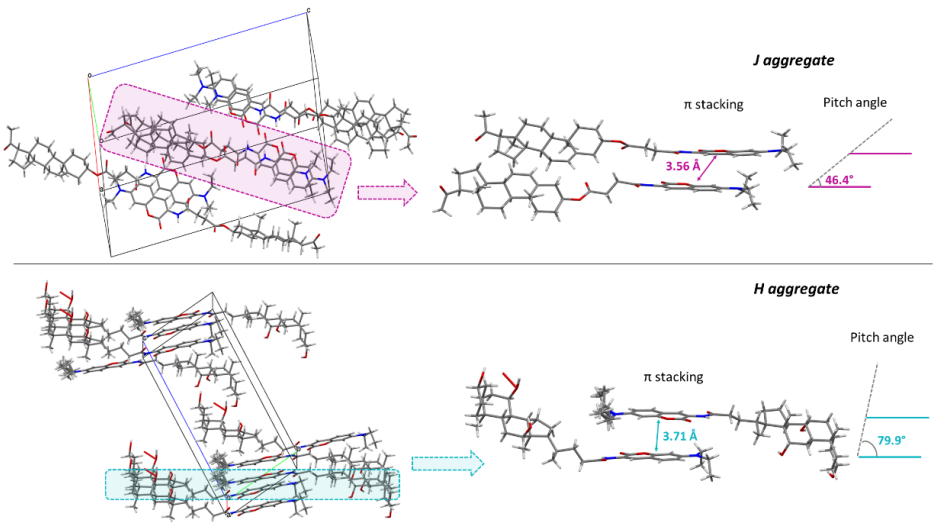
Fluorescent probes are chemical compounds that exhibit the optical phenomenon of fluorescence and are used for qualitative or quantitative detection of a broad range of analytes. Due to their high sensitivity, they have become effective tools for investigating biochemical and biological systems.[1] In the search for improving the properties of fluorescent probes, one interesting phenomenon that has caught attention is the process of aggregation which occurs when molecules spontaneously come together and form aggregates through non-covalent interactions.[2]

This work involves the synthesis, structural, and photophysical studies of a range of steroid-coumarin conjugates derived from pregnanolone, pregnenolone, cholic acid, 3β,19-dihydroxydehidroepiandros-5-en-17-one and estrone as well as its application as lipophilic fluorescent materials for bioimaging applications. Monocrystal X-ray diffraction analysis disclosed distinctive aggregation patterns exhibiting *J*- or *H*-aggregates in selected compounds. Bioimaging studies demonstrated selectivity for cell membrane and lipid droplets in some cases.[3]



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SYNTHESIS OF *N*-SUBSTITUTED ISOSTEVIOL-BASED 1,3-AMINOALCOHOLS WITH ANTIPROLIFERATIVE ACTIVITY

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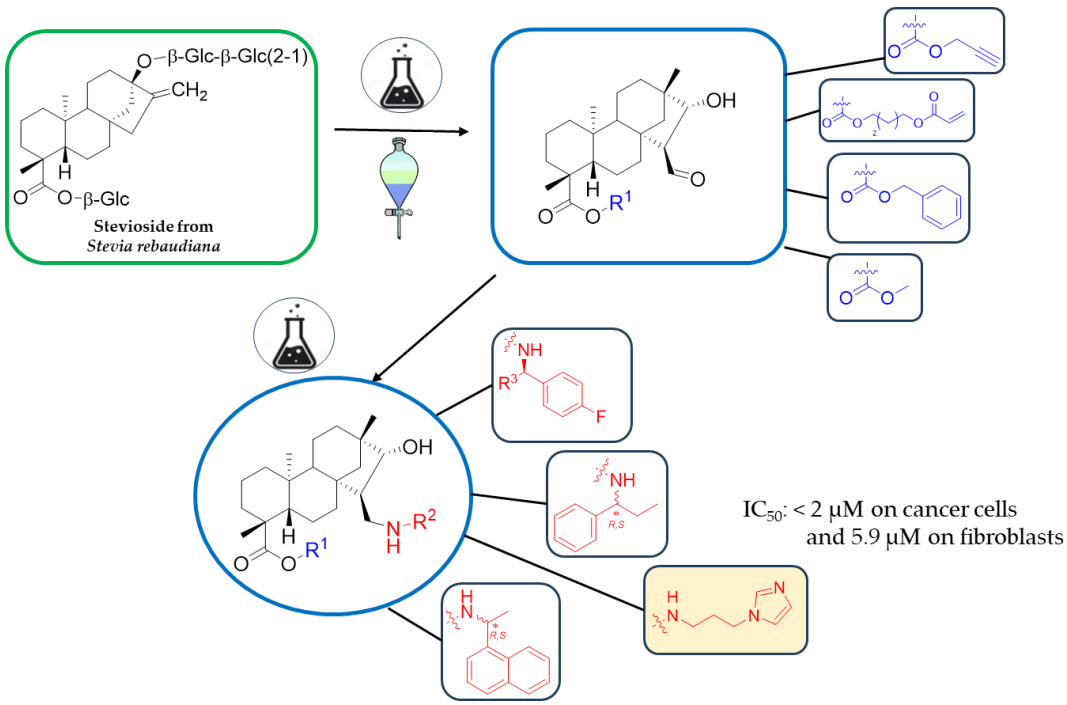
Nowadays, particular attention has been paid towards the glycosides of the plant *Stevia rebaudiana*, not only because they are used as artificial sweeteners, but because they have a wide range of biological activities including antibacterial, antiviral and anticancer properties.[1,2]

Based on our former result, a series of diterpenoid 1,3-aminoalcohol derivatives were prepared via stereoselective transformations.[2,3] Key intermediate keto alcohol was prepared in a three-step synthesis from stevioside. In the next step, a 1,3-aminoalcohol library was prepared by reductive amination. To study the effect of the carboxylate ester function at position 4, the free carboxylic acid, benzyl ester and acryloyl ester analogues were prepared as elongated derivatives in comparison with our earlier results in this field. The antiproliferative activity of compounds against human tumour cell lines (A2780, HeLa, MCF-7 and MDA-MB-231) was investigated.



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SYNTHESIS OF BIOACTIVE *ALLO*-GIBBERIC ACID-BASED 2,4-DIAMINOPYRIMIDINE DERIVATIVES

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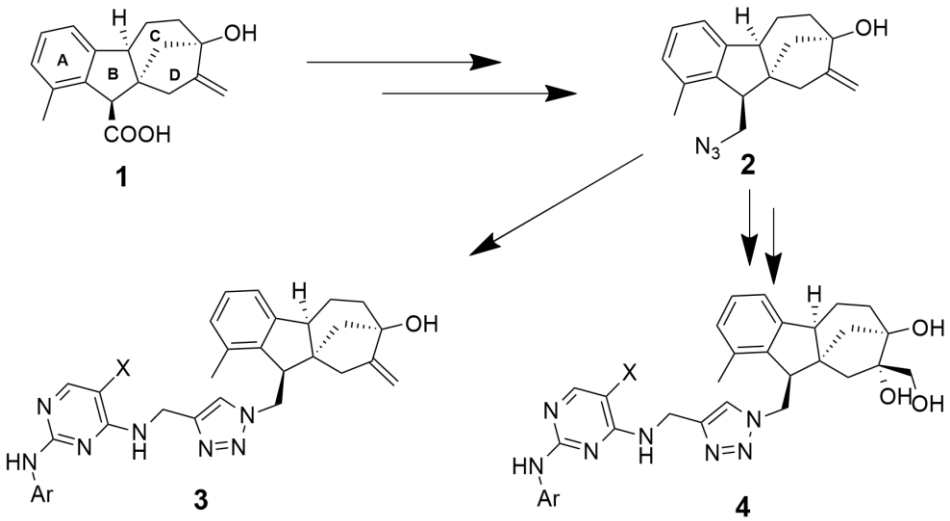
Resistant microorganisms are a major health concern nowadays and searching for new antimicrobials has become a need since the number of non-susceptible organisms is increasing gradually in comparison with the small number of new antimicrobials introduced to the market.

Allo-gibberic acid (**1**) is a tetracyclic diterpenoid and one of the well-known gibberellin derivatives in drug research.[1, 2] As a continuation of our previous work in this field, our aim was to prepare mono- and trihydroxy functionalized derivatives coupling the diterpene skeleton with 2,4-diaminopyrimidine moieties. The key-intermediate azide **2** was prepared in a four-step synthesis via esterification, followed by $LiAlH_4$-mediated reduction, tosylation and finally tosyl-azide exchange.[2][3] Moreover, the triol-azide derivative was obtained by oxidation of the double bond using OsO_4/NMO system. On the other hand, starting from propargyl amine and halogen-substituted pyrimidines, we were able to synthesize 5-fluoro-, 5-chloro-substituted,2,4-diaminopyrimidine derivatives with N-4-trifluoromethylphenyl-, and N-1-methyl-pyrazol-4-yl substituents within two steps. Afterwards, both azides underwent a CuAAC reaction to combine with the previously prepared alkynes in order to create a novel library of two series of *allo*-gibberic acid derived 2,4-diaminopyrimidines. The new compounds were tested for their antibacterial and antifungal activities to determine usable Structure-Activity Relationship.



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SESQUITERPENE QUINONE AVARONE: A MARINE NATURAL TOOL TO BE DEVELOPED AS A MULTITARGET AGENT FOR TYPE 2 DIABETES MELLITUS AND ITS PATHOLOGICAL COMPLICATIONS

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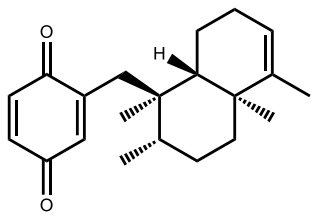
Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia due to an impaired glucose homeostasis. It may lead to the development of disabling and life-threatening health complications. First-line therapeutic options for T2DM treatment are monodrug therapies, often replaced by multidrug therapies to ensure that non-responding patients maintain target glycemia levels. The use of multitarget drugs instead of mono- or multidrug therapies has been emerging as a main strategy to treat multifactorial diseases, including T2DM[2]. Our study demonstrated that avarone[1], a sesquiterpene quinone isolated from the sponge *Dysidea avara*, is capable of inhibiting in vitro PTP1B, the main negative regulator of the insulin receptor, while it improves insulin sensitivity, and mitochondria activity in C2C12 cells. We observed that when avarone is administered alone, it acts as an insulin-mimetic agent. Moreover, avarone acts also as a tight binding inhibitor of aldose reductase, an enzyme involved in the development of diabetic complications. Overall, avarone represents a natural hit to be developed as a multitarget drug for diabetes and its pathological complications[3]. For this reason, the exploration of the chemical space of avarone has been performed by the synthesis of a chemical library around its quinone moiety and it represents a powerful tool to better understand the structural requirements responsible of the antidiabetic activity. Our data confirm, and reinforce, the idea that the use of natural molecules, with their unique chemical structures and the inherent ability to act against multiple targets, plays a crucial role in accelerating the discovery of new multitarget drugs.



avarone

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NATURAL AND SEMISYNTHETIC BILE ACIDS AS LEUKEMIA INHIBITORY FACTOR (LIF) RECEPTOR ANTAGONISTS

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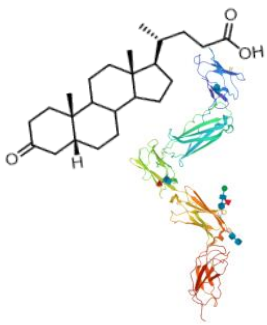
Leukaemia Inhibitory Factor (LIF) is a pleiotropic member of interleukine (IL)-6 cytokine family,[1] that regulates cell differentiation, proliferation and survival in embryo and adult cells and is involved in cancer growth and progression. LIF signalling is mediated via the heterodimeric LIF receptor (LIFR) complex, which is formed by LIFR and the glycoprotein (gp) 130. LIFR complex activates the downstream signalling pathways, which include JAK1/STAT3 axis; this signalling is over-regulated in several type of solid tumours, including PDAC, gastric cancer (GC) and hepatocellular carcinoma (HCC).[2] Several studies support the suppression of LIFR signalling as putative target to inhibit cell growth and tumour progression in several type of cancers.[3] Recently, has been demonstrated that mifepristone-mediate LIFR antagonism inhibits oncogenic signalling and might ameliorate chemoresistance.[4] Building on this background and based on steroidal structures of BAs, we performed a similarity screening of natural and semisynthetic bile acid using as query the structure of the LIFR antagonist, such as mifepristone and EC359.

Bile Acids (BAs) are steroid derivatives of cholesterol, synthesized in the liver and metabolized by microbiota in the intestine and reabsorbed through the enterohepatic circulation. BAs, especially secondary bile acids, have been reported to have a critical role in gastrointestinal carcinogenesis and breast cancers. However more increasing evidence described their anti-tumour effects.

In the present study, we shown that natural bile acid and their metabolites, especially the oxidized forms, are potential LIFR antagonists. Herein we demonstrated that among the various mechanisms of action of BAs as tumour suppressors, their role as onco-suppressors could be mediated by the antagonism on LIFR.



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A NEW SESQUITERPENE LACTONE FROM *CHAMAEMELUM NOBILE* L.

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Chamaemelum nobile (L.) All. (syn. *Anthemis nobilis* L. and *Chamomilla nobilis* Godr.) is a perennial herb of the Asteraceae family, known as Roman chamomile. Compared to common chamomile (*Matricaria chamomilla*, syn. *Matricaria recutita*), it results less studied in scientific literature, although it is also widely used in Traditional Chinese Medicine due to its antimicrobial, anti-inflammatory, antioxidant, mild astringent, mild sedative and antispasmodic properties [1]. Secondary metabolites of Roman chamomile are represented by sesquiterpene lactones belonging to the class of germacrane. Among these, nobilin (Fig. 1) and its derivatives are considered the main components.

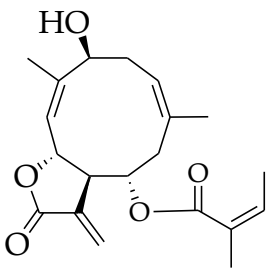

Figure 1. Nobilin

With the aim to perform a phytochemical investigation, the fresh aerial parts of *C. nobile* were extracted by solid-liquid dynamic (SLDE-Naviglio) extraction, a non-conventional extraction technique, using EtOH:H_2O 50:50. The extract was firstly analyzed by Liquid Chromatography/Mass Spectrometry (LCESI/QExactive/MS/MS) and subsequently, to unambiguously assign the structures of the detected compounds, it was fractionated on a Sephadex LH-20 column and further purified by semi-preparative HPLC-UV. The isolated compounds were characterised by 1D and 2D NMR spectroscopy in combination with Mass Spectrometry experiments, highlighting the presence of a derivative of nobilin, never reported before.

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P8

BIOMIMETIC MODIFICATION OF SESQUITERPENE γ-LACTONES

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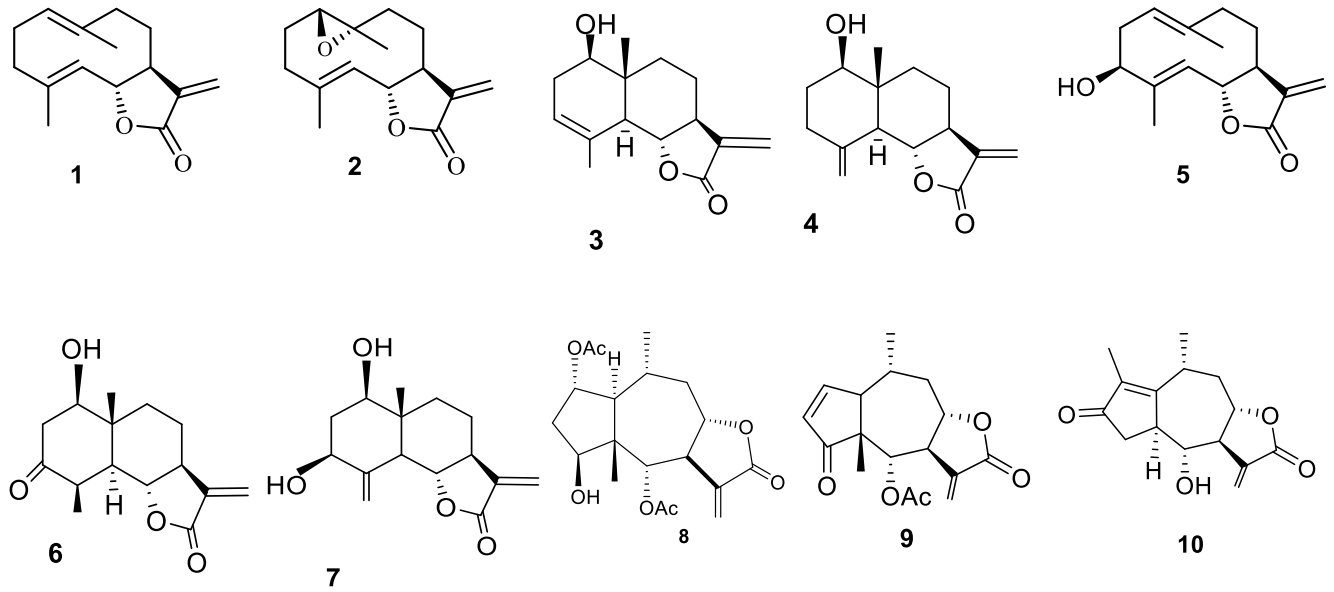
The interaction of *trans,trans*-germacranolide costunolide **1**, widely distributed in plant sources, with peracetic or m-chloroperbenzoic acids produces 1β,10α-epoxide **2**, unstable and isomerized, forming a mixture of natural eudesmanolides santamarin **3** and reynosin **4**. The transformation of **1** into **3** and **4** can be considered as a stereochemical effect of the epoxy function in **2**.



Stereochemically controlled 5,10-carbocyclization of hanphyllin **5**, a germacranolide characteristic of the species of the genera *Achillea* L., *Artemisia* L., *Handelia*, occurs under reaction conditions with peracetic and perbenzoic acids, leading to the formation of β-hydroxylated at C-1 position of natural trans-eudesmanolides: 4-epiartecalin **6** and ridentin-B **7**. This transformation of germacranolide hanphyllin **5** is considered as its biomimetic cyclization into 1β-hydroxy-eudesmanolides previously described from plants of the genera *Ambrosia* L., *Artemisia* L., *Chrysanthenum* L., *etc*., i.e. 1,10-epoxy-germacranolides are considered as intermediates in the biosynthesis of 1-hydroxy-eudesmanolides. The interaction of natural pseudoguaianolide brittanin **8** with pyridinium chlorochromate results in the formation of **9** containing a conjugated cyclopentanone fragment in its structure. Upon hydrolysis of **9** with a 4% potassium hydroxide solution, neohelenaline rearrangement is observed with the formation of **10**. The above structural modifications of brittanin **8** are considered as their biomimetic transformation with the formation of analogues of helenalin, characteristic of *Helenium* species a pseudoguaiane γ-lactone of the type.

MICROBIOLOGICAL TRANSFORMATION OF ARBORESCIN

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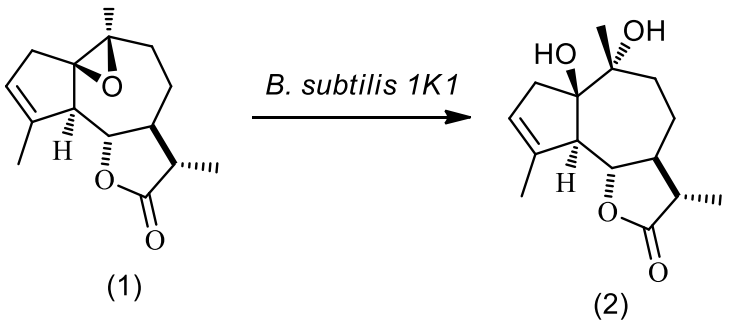
Biotransformational modification of natural compounds using them as substrates, as well as the study of their biocatalysis with the participation of bacterial strains and isolated enzymes, is considered a promising area of bioorganic chemistry and biotechnology. Therefore, the design of terpene biocatalysis systems based on bacterial genes for biodegradation of organic compounds is of particular relevance.

We carried out microbiological transformation of the sesquiterpene lactone arborescin (**1**) contained in *Artemisia arborescens* L., *A. austriaca* Jacq., *A. jacutica* Drob., *A. sieversiana* Willd.

As a result of our screening of industrial biotopes for the presence of terpene-utilizing bacterial strains, 3 strains were isolated - *Pseudomonas* sp. 1K1, 1K3 and 1K8, exhibiting the ability to biocatalyze terpenes.

It has been established that the genetic determinant responsible for the biocatalytic properties of the isolated strains is the biodegradation plasmid p1K1, which allows its subsequent use for the creation of biocatalysis systems for plant terpenoids.

The transformational transfer of the p1K1 plasmid into cells of the non-pathogenic *Bacillus subtilis* 168 strain resulted in the construction of a *Bacillus subtilis* 1K1 biocatalyst strain for the biomodification of terpenes. Using the recombinant strain Bacillus subtilis 1K1, the sesquiterpene lactone arborescin (**1**) was modified, resulting in the production of a new derivative - 1β,10α-dihydroxy-5,7α,6,11β(H)-guai-3(4)-en-6,12-olide (**2**), which has pronounced cytotoxicity.



(1) (2)

TERPENOIDS OF THE ESSENTIAL OIL *OF ARTEMISIA AUSTRIACA* JACQ.

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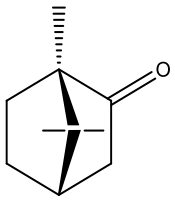
The report informs on the study of the qualitative composition and quantitative content of terpenoids in the essential oil of the above-ground part (buds, flower baskets, leaves) of *Artemisia austriaca* Jacq., collected in different phases of vegetation of the plant in Abay district of Karaganda region of Kazakhstan.

Essential oils were extracted by hydrodistillation and microwave extraction methods. At the same time, the yield of essential oil from the plant of regrowth phase was 0.63% and 0.66% respectively, and budding phase - 0.39% and 0.31%, respectively, per air-dry raw material.

According to chromatography-mass spectrometry data, in the essential oil extracted by hydrodistillation from the above-ground part of *Artemisia austriaca* Jacq. collected in the budding phase, 54 components were detected, of which 51 were identified, while microwave extraction detected 50 components, of which 47 were identified.



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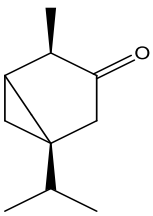

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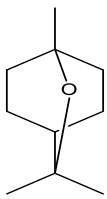

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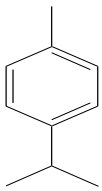

(4)

Thus, according to chromatography-mass spectrometry data, the essential oils isolated from the aboveground part of *Artemisia austriaca* Jacq. by hydrodistillation and microwave extraction methods are characterised by a quantitative content of camphor (1) (15.9-19.7%) in the regrowth phase, as well as α-thujone (2) (15.8-16.1%), 1,8-cineol (3) (13.3-16.2%), camphor (1) (10.9-14.40%) and para-cymol (4) (12.7%) in the budding phase of the plant.

Based on the results of pharmacological studies, it was determined that the substance based on the essential oil of *Artemisia austriaca* Jacq. is promising as an antimicrobial and anti-inflammatory agent.

EXPRESSION CHANGES OF GENES INVOLVED IN THE BIOSYNTHESIS OF SESQUITERPENE LACTONES IN DEVELOPING ARNICA FLOWERS

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Although the biological activity of secondary metabolites extracted from *Arnica spp.* has been well documented[1], there is a gap in the characterization of their biosynthesis. Among many valuable secondary metabolites present in arnica, sesquiterpene lactones (mainly helenalin, dihydrohelenalin, and their derivatives) are of great importance due to their pharmacological potential (e.g. cytotoxic, anti-inflammatory, antibacterial, and antifungal)[2]. This pilot study aimed at characterizing the transcriptional changes occurring in arnica flowers at various stages of development. The expression of the following genes encoding key enzymes involved in the biosynthetic pathway of sesquiterpene lactones was analyzed - FDS (farnesyl diphosphate synthase, EC 2.5.1.92), GAS (germacrene-A synthase, EC 4.2.3.23) and GAO (germacrene A oxidase, EC 1.14.14.95). Expression analysis of selected genes-of-interest was performed using the RT-qPCR technique. Prior to the analysis, the identification of reference genes showing stable expression in tested material was conducted. Obtained results provide valuable preliminary information on the transcriptional regulation of the final steps of sesquiterpene lactones biosynthesis in arnica flowers.

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EFFECT OF METHYL-JASMONATE, HIGH SUCROSE, AND *XYLELLA FASTIDIOSA* LYSATE ON THE PRODUCTION OF SECONDARY METABOLITES IN OLIVE (*OLEA EUROPAEA* L.) CALLI

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A more accurate knowledge of plant defence mechanisms and genes involved in resistance is necessary to improve the management of protection strategies for crops of economic interest. Our research aims to study secondary metabolic compounds possibly involved in olive tree defence mechanisms, such as polyphenolics and isoprenoids. Metabolites produced in the *callus* of the Ogliarola Salentina olive cultivar after treatment with the phytohormone methyl jasmonate (MeJA), a *Xylella fastidiosa* subsp. *pauca* lysate (XFL), and high sucrose as elicitors, at different exposure times and doses, were analysed. The total phenols analysis was performed by the COI method, while the individual phenols and terpenoids analysis was performed by LC-MS/MS.

The preliminary results showed that MeJA induced changes in the total biophenols based on doses and times. Higher doses apparently produced a later induction of total phenols. Analysis of individual compounds showed induction of verbascoside and its derivative 48 hours after treatment, whereas hydoxytyrosol was induced at 5 hours after treatment. The major effect of MeJA on isoprenoids was measured at 24 and 48 hours after treatment. Interestingly, XFL treatment induced accumulation of oleuropein, the main secoiridoid of olive. Oleuropein and its derivatives are known to be involved in defence response mechanisms to biotic stresses. High sucrose treatment induced total biophenols accumulation, while inhibiting the main olive triterpenoids such as oleanolic, maslinic and corosolic acids.

The expression analysis of genes involved in the biosynthetic pathways of these metabolites by Real-Time PCR is currently in progress to confirm the possible role of the tested elicitors on the regulation of olive polyphenols and terpenes and their involvement in the plant defence response to *Xylella fastidiosa*.

EFFECT OF *MYC2* CONSTITUTIVE EXPRESSION ON ISOPRENOIDS AND NATURAL RUBBER PRODUCTION OF *TARAXACUM KOK-SAGHYZ* L.E. RODIN

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Taraxacum kok-saghyz L.E. Rodin (TKS), the Russian dandelion, is a promising alternative species for natural rubber (NR) production. Like other dandelion species, TKS is also rich in additional isoprenoids and specialised metabolites in both leaves and roots [1]. The transcription factor MYC2 is a master regulator of plant physiological processes and specialised metabolite synthesis. Analysis of transcriptomic datasets showed that MYC2 expression correlates with NR biosynthesis genes and NR content in TKS [2, 3]. To further investigate the possible role of MYC2 in the regulation of NR and other isoprenoids biosynthesis, TKS plants expressing the *MYC2* gene under the control of the 35S Cauliflower Mosaic Virus promoter were generated by *Agrobacterium tumefaciens*-mediated transformation. NR from TKS plants was extracted by a combined solvent-sonication method and measured gravimetrically. TKS_35S:*MYC2* plants showed an increase in NR production compared to the wild type. The expression of isoprenoid-related genes was also evaluated, suggesting a role for MYC2 in their regulation. Finally, the major isoprenoids content was analysed by ultra-high-pressure liquid chromatography/tandem mass spectrometry (UHPLC-HRMS) technique.

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BIOACTIVE LABDANE-TYPE DITERPENOIDS FROM *MARRUBIUM ASCHERSONII* MAGNUS GROWING IN TUNISIA

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Since the earliest days of humanity, aromatic medicinal plants have formed all over the world, the basis of health care and have been considered of a paramount importance as a rich source of safe bioactive phytocompounds [1]. The Lamiaceae family comprises about 200 genera and 3200 species of aromatic herbs [2], including Marrubium genus widely described for its pharmaceutical uses such as antinociceptive antispasmodic, antioedematogenic and anti-inflammatory activities [2]. Based on the ethnobotanical and traditional use, and within the scope of searching for new bioactive principles, the current research focussed mainly on the chemical screening and biological activities of *Marrubium aschersonii* (Lamiaceae), collected from the Kroumiria region in north-west of Tunisia. A detailed phytochemical investigation of the aerial parts ethanol extract, led to the isolation of four diterpenoids. Importantly, the structure elucidation of the novel labdane-type diterpenoids: marrubaschs A (1) and B (2) was established on the basis of extensive NMR and HR-ESIMS spectroscopic techniques [2]. Moreover, the diterpenoids were evaluated for their inhibitory effects on the nitric oxide (NO) production induced by lipopolysaccharide in RAW 264.7 macrophage cells. marrubaschs B exhibited weak inhibition of NO production with an IC_{50} value of 35 ± 1.0 μM [2].

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CHEMICAL PROFILE AND BIOLOGICAL EFFECTS OF THE ESSENTIAL OIL EXTRACTED FROM *ERYNGIUM TRICUSPIDATUM* L. GROWING NATIVELY IN TUNISIA

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Since antiquity, natural products and essential oils have emerged as an excellent alternative to synthetic insecticides and fungicides. Natural volatile oils constitute golden liquids with multipurpose roles as health beneficial effects, flavors and fragrances [1]. Hence, the current research was undertaken to determine the interspecific chemical variability and evaluate the antifungal and insecticidal properties of the essential oils from *Eryngium tricuspidatum* subsp. *tricuspidatum* and subsp. *bovei* growing wildly in Tunisia. The phytochemical profiling demonstrated that both volatile oils were characterized by sesquiterpene hydrocarbons as the main constituents. Therefore, *β*-bisabolenal (17.8%), bicyclogermacrene (11.9%) and *trans*-muurola-3,5-diene (11.5%) were quantified as the dominant secondary metabolites of the essential oil extracted from *E. tricuspidatum* subsp. *tricuspidatum*. Moreover, bicyclogermacrene and *trans*-muurola-3,5-diene were quantified at higher percentages in *E. tricuspidatum* subsp. *bovei* (27.1% and 20.9% respectively). Regarding the bioactivity, both samples exhibited significant repellent activity towards the stored-product pest *Tribolium confusum* and a potent antifungal effect against *Candida albicans* [2].

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MULBERRY UNDIFFERENTIATED CELL SUSPENSIONS: SUSTAINABLE BIOFACTORIES FOR PRENYLATED FLAVONOIDS

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Among natural polyphenols, flavonoids are associated with a broad spectrum of health-promoting effects and are indispensable components in a variety of nutraceutical, pharmaceutical, and cosmetic applications[1]. Prenylated flavonoids are a subclass of flavonoids, which combine a flavonoid skeleton with a lipophilic prenyl side-chain. Prenylation usually ends in molecules with improved bioactivities, due to the increase of lipophilicity, resulting in a higher affinity to biological membranes and in a better interaction with the targets. While flavonoids are quite abundant in plants, prenylated flavonoids are characteristic of only some genera and in a pretty low concentration. Moreover, the complexity of the structures, ending in a difficult chemical synthesis, limits their application[2, 3].

Morus alba (mulberry), a common traditional plant belonging to the Chinese medicine and a functional food resource, is one of the main source of prenylated flavonoids, especially present in the root bark[4]. Being the roots a non-renewable source, the extraction leads to the damage of the plant, thus, alternative biotechnologies are needed for the production of this secondary metabolite class.

For this purpose, in this ongoing study, *in vitro* cell suspensions of *M*. *alba* have been established to evaluate the capability of undifferentiated cells to biotransform exogenous flavonoidic substrates into prenylated moieties. The prenylation ability of *M*. *alba* cells has been investigated adding directly the substrates in the cell suspension flasks as well as assessing the bioconversion capacity of the microsomal fraction.

The results obtained show an active and compound-selective prenylation activity, enhancing the cell culture of *M*. *alba* as alternative tool for obtaining prenylated flavonoids, pursuing a sustainable way of production and preserving biodiversity.

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IN VITRO PERMEATION OF SUPERCRITICAL CO2 EXTRACT OF *LAVANDULA ANGUSTIFOLIA* L. AS NEW POSSIBLE PHARMACEUTICAL INGREDIENT

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Lavandula angustifolia is used in traditional herbal medicine as *Lavandulae* herba, or as an extract based on essential oil (EO) obtained by hydrodistillation. EO is registered as traditional vegetal drug in Europe as mild sedative (1,2). In this work we compared supercritical carbon dioxide extract of L. angustifolia (LSCO2) extract with the essential oil (EO) formulated in drug with aim to identify its applicability as pharmaceutical ingredient. NMR and GC-MS were used to establish the composition of the extracts. Results revealed high amount of linalool, linalyl acetate and caryophyllene. Differences were observed with higher content of linalyl acetate in (LSCO2) compared to the EO, also lipid fraction is present in the LSCO2 while is absent in EO but is present in the commercial drug containing EO as excipient. To assess possible use of LSCO2 in new drug preparation, the LSCO2 extract and the EO formulated in lipid were subjected to *in vitro* intestinal permeability assay using a co-culture model composed by 2 intestinal cell lines, namely Caco-2 and HT29-MTX. Permeates were analysed by GC-MS. From both the extracts LSCO2 and EO the main compound that can be observed permeating through the in vitro intestinal tissue is linalool. Linalyl acetate that is largely present in LSCO2 is totally converted in the linalool during permeation and permeation achieved nearly 100%. The overall results demonstrated that the LSCO2 can be an alternative ingredient for the development of L. angustifolia based drugs and, comparatively to the essential oil, the LSCO2 contains lipidic fraction that is normally added to drugs as excipient offering a good alternative in drug production.

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ENZYME-DRIVEN PRENYLATION: UNLOCKING NEW HIGH-VALUE ISOPRENOIDS

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Prenylation significantly contributes to the diversity of natural products by utilizing simple five-carbon building blocks to synthesize various isoprenoids through the action of prenyl-converting enzymes (PCEs). Using the biocatalytic potential of three subclasses of PCEs, we introduced a green synthetic approach for producing pharmaceutically and industrially valuable isoprenoids and their non-natural derivatives.

Terpene synthases catalyze the intramolecular cyclization of geranyl (GPP), farnesyl (FPP), or geranylgeranyl diphosphate (GGPP). Screening limonene synthase from *Cannabis sativa* and 5-*epi*-aristolochene synthase from *Nicotiana tabacum* against 11 modified prenyl diphosphates resulted in the synthesis of five novel terpene analogs, including oxa- and thia-heterocycles and alkyne-modified derivatives. Extensive spectroscopic analysis revealed their structures that give access to potentially new fragrances. Docking studies highlight an on-off conversion of the unnatural substrates.[1]

Cis-prenyltransferases (CPTs) catalyze the sequential addition of isopentenyl diphosphate (IPP) to an allylic diphosphate, forming *cis*-configured C-C double bonds. Using a CPT from a thermophilic bacterium, we confirmed the enzymatic condensation of up to 8 halogenated IPP derivatives for the first time. This lays the groundwork for the enzymatic production of synthetic rubber.

Aromatic prenyltransferases (aPTs) add a prenyl or geranyl group to a phenolic substrate. Four aPTs from two *Hypericum* species were cloned and expressed in yeast and tobacco, catalyzing the prenylation of 1,3,6,7-tetrahydroxyxanthone (1367THX) and its 8-prenylated derivative to produce patulone, an anti-inflammatory compound. Based on their kinetic properties, each pair of enzymes sequentially added two prenyl groups to the carbon atom 8 of 1367THX. Coexpression experiments enhanced product formation and fluorescent protein fusions confirmed localization of the aPTs to the chloroplast envelope.[2] Despite their potential, the use of aPTs in biotechnology remains lagging. The newly established junior research group “PhenoPren” at MLU aims to create a library of technical aPTs for the biotechnological production of prenylated derivatives as drug leads, supported by AI for enzyme identification.

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SESTERTERPENOIDS IN MARINE AND TERRESTRIAL ORGANISMS: A RELATIONAL DATABASE

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Sesterterpenoids (SSTPs) are pentaprenyl terpenoids derived from the linear precursor geranylfarnesyl diphosphate, which have been isolated from fungi, bacteria, lichens, plants, insects, and various marine invertebrates, especially sponges. To define a schema of knowledge organization on this topic, it was necessary to collect skills from various disciplines such as chemistry, botany, biology, ecology, pharmacology and others. This joint effort led to the definition of a rather detailed conceptual model which was then instantiated in a logical diagram to define the database used in this project [1]. This database contains all the basic concepts, starting with the name of the molecule, the chemical class, the organisms from which it was first extracted, potential bioactivity properties, and major extraction method procedures. Bibliographic references are stored for each passage of information. A public website has been created to visualize and consult the collected material, to share database maintenance activities among managers, and to provide automatic links with other accredited databases for easy and timely updating of the information. Literature covers the years 1918 to 2023 and includes about 2000 SSTPs, classified according to their structural complexity, from linear pentaprenyl to carbocyclic and heterocyclic scaffolds. Marine and terrestrial organismal sources are reported, and also, when possible, the synthetic origin of their SSTPs repertoire. A variety of bioactivities were described, ranging from cytotoxicity against human cancer cells and inhibition of pathogenic microbes, to anti-inflammatory, antiprotozoal, antitubercular and antifeedant activities and modulation of neurodegenerative processes, as well as activity in the treatment of metabolic diseases such as type II diabetes, hypercholesterolemia and obesity, and as immunosuppressive molecules.

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NEW SESQUITERPENES FROM *DAPHNE LAUREOLA* (THYMELACEAE) ROOTS

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The genus *Daphne* (Thymelaceae family) comprises over 90 species, mainly shrubs, distributed in Europe, North Africa, and Asia, very well known for the presence of toxic diterpenes with the daphnane skeleton such as daphnetoxin;[1] although some species are poisonous others are used for medicinal and cosmetic purposes. Terpenoids, coumarins, flavonoids, and lignans, are among the most significant classes of compounds that were isolated from this genus.[2]

Daphne laureola L. (spurge laurel) is a Mediterranean evergreen shrub with glabrous, large, and glossy leaves used traditionally in South Italy as ethnoveterinary remedy,[3] pesticide, repellent,[4] and as potent purgative or emetic poison. In some rural areas of Sicily there is the practice to cover cottage cheese (ricotta) with the plant's leaves to avoid its theft. Although few preliminary chemical studies were carried out on the plant leaves, reporting the presence mainly of flavonoids[5] and coumarins,[6] the roots were not investigated to date.

In this study, *D. laureola* roots were subjected to static maceration using solvents of increasing polarity, *n*-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1), and MeOH. From the chloroform extract three new sesquiterpenes were isolated by means of Biotage® flash chromatography and RP-HPLC, then characterized through 1D and 2D NMR and electronic circular dichroism (ECD) spectroscopies and HRESIMS analyses as guaiane-type sesquiterpenoids with structure similarities to daphneaine G and stelleraterpenoid H. Moreover, some known biflavonoids, lignans, and coumarins were also purified. As future work, the pure isolates will be investigated for their antiangiogenic activity by two in vivo models, chick chorioallantoic membrane and zebrafish embryos.

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CHARACTERIZATION OF NOVEL *O*-ALKYLATED ANDROSTANE 3-OXIMES: CHROMATOGRAPHIC LIPOPHILICITY DETERMINATION IN C8-UHPLC SYSTEM

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Steroidal derivatives represent an outstanding basis for development and design of new anticancer drugs. The present study deals with the characterization of a new series of eighteen *O*-alkylated androstane 3-oximes in terms of determination of their lipophilicity. The representative structures are presented in Fig. 1. It was previously shown that these compounds possess significant anticancer activity [1,2]. The lipophilicity determination was done by using ultra-high performance liquid chromatography system (UHPLC) with C8 stationary phase and three mobile phases: two binary phases (methanol/water, acetonitrile/water) and one ternary phase (methanol/acetonitrile/water). The obtained chromatographic parameters (capacity factors, log*k*), determined by using different mobile phases, were correlated with *in silico* lipophilicity parameter (logP), estimated in ChemBioDraw v.13 software. High correlations between these parameter confirmed that the obtained chromatographic parameters in the applied UHPLC system with C8 stationary phase can be considered lipophilicity measure of the studied androstane 3-oximes.

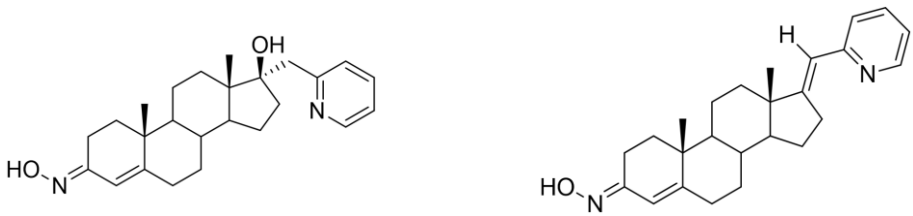

Figure 1. Representative structures of the studied compounds

The present research is supported by the project of Provincial Secretariat for Higher Education and Scientific Research of AP Vojvodina (Molecular engineering and chemometric tools: Towards safer and greener future, No. 142-451-3457/2023-01/01).

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CHEMOMETRICS OF CHROMATOGRAPHIC LIPOPHILICITY PARAMETERS OF NEWLY SYNTHESIZED ANDROSTANE 3-OXIME DERIVATIVES

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Physicochemical characterization of newly synthesized pharmacologically active compounds is an important step in their evaluation as potential medication. The present research is focused on chemometric evaluation of chromatographic lipophilicity of eighteen novel androstane 3-oxime derivatives[1,2] applying pattern recognition methods – hierarchical cluster analysis (HCA) and principal component analysis (PCA). The chromatographic lipophilicity was determined applying reversed-phase ultra-high performance liquid chromatography (RP-UHPLC) with C18 and C8 stationary phases and two different mobile phases, including methanol/water and acetonitrile/water mixtures, under isocratic conditions. The HCA and PCA revealed significant separation of two groups of compounds based on chromatographic lipophilicity parameters (capacity factors, log*k*) determined under different chromatographic conditions (various fractions of modifiers in mobile phases and different stationary phases). The results pointed out in which chromatographic parameters the analysed compounds differ the most and how different their behaviour is on different types of stationary phases. Furthermore, the determined retention parameters are a good basis for correlation with *in vitro* or *in silico* pharmacokinetic parameters of the studied derivatives considering their high predictive power in terms of biological activity of compounds.

The present research is supported by the project of Provincial Secretariat for Higher Education and Scientific Research of AP Vojvodina (Molecular engineering and chemometric tools: Towards safer and greener future, No. 142-451-3457/2023-01/01).

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QUANTITATIVE STRUCTURE-RETENTION RELATIONSHIP ANALYSIS OF LIPOPHILICITY OF A SERIES OF STEROIDAL DERIVATIVES IN RP(C8)-HPLC SYSTEM

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This study presents the quantitative structure-retention relationship analysis of chromatographic lipophilicity of a series of six groups of steroidal derivatives, including triazole, tetrazole, toluenesulfonylhydrazide, nitrile, dinitrile and dione compounds (25 compounds in total). The chromatographic lipophilicity was determined applying reversed-phase high performance liquid chromatography (RP-HPLC) system with C8 stationary phase and mobile phase consisting of 60% of acetonitrile and 40% of distilled water. The lipophilicity was expressed in the form of capacity factor, log*k*, and it was further correlated with molecular descriptor applying multiple-linear regression (MLR). The correlation analysis was based on a set of 10 *in silico* molecular descriptors (lipophilicity, pharmacokinetic and physicochemical descriptors as independent variables) and experimentally obtained chromatographic lipophilicity (dependent variable). The obtained MLR models were validated by internal and external validation (external test set contained 5 randomly selected compounds) and are based on lipophilicity and pharmacokinetic descriptors. The resulting models contribute to the understanding of retention mechanisms and pharmacokinetic properties of the analysed series of steroidal derivatives.

The present research is supported by the project of Provincial Secretariat for Higher Education and Scientific Research of AP Vojvodina (Molecular engineering and chemometric tools: Towards safer and greener future, No. 142-451-3457/2023-01/01).

LC-DAD-ESI-MS ANALYSIS OF OLEUROPEIN IN *SYRINGA* SPP. CULTIVATED IN POLAND

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Oleuropein is a glycosylated secoiridoid commonly present in the Oleaceae family. Pharmacological studies have demonstrated that oleuropein has anti-inflammatory, antitumor, antiarteriosclerosis, hypolipidemic and hepatoprotective activities. This compound is also the important active ingredient of *Syringa* spp [1]. With that in mind, the main aim of the study was to evaluate, by the means of LC-ESI-MS/MS, oleuropein profile in leaves and barks of 11 *Syringa* species cultivated in Institute of Dendrology of the Polish Academy of Sciences, Kórnik, Poland (*S. henryi*, *S. amurensis*, *S. josikaea*, *S. oblata*, *S. persica*, *S. pinetorum* Alba, *S. pubescens* (Manchuria), *S. villosa* var. *wolfii*, *S. pubescens* (Washington), *S. vulgaris* cv. Fale Bałtyku, *S. vulgaris* cv. Jutrzenka Pomorza). Some of them are not a part of the Polish flora, and we did not know whether they synthesize oleuropein or don't. The strong signal of ion with m/z-H 539.177, characteristic for oleuropein, was observed in all species. Considering the barks and leaves, the richest in oleuropein was the bark of *S. amurensis* (243.58 mg/g), and the leaves of *S. villosa var. wolfii* (152,9 mg/g). Cluster analysis demonstrated the distinctiveness of both *S. amurensis* and *S. villosa var. wolfii*. The group of species characterized by a high content of oleuropein in the bark and lower in the leaves comprises to *S. henryi*, *S. pinetorum* Alba, *S. pubescens* (Manchuria), *S. vulgaris* cv. Jutrzenka Pomorza. The other species are characterized by a lower content of the studied compound, particularly in the bark.

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NEUROSTEROID DISCOVERY PLATFORM FOR TREATMENT OF DISEASE-ASSOCIATED MUTATIONS IN NMDA RECEPTOR SUBUNITS

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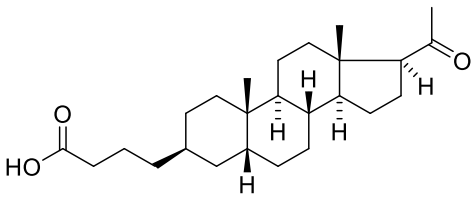
N-methyl-d-aspartate receptors (NMDARs) play a critical role in glutamate signaling, for example in brain function. As a result, even a small loss of function in these receptors can lead to severe pathological consequences. Missense and nonsense variants of genes (GRIN) encoding NMDA receptor subunits cause truncation of their C-transmembrane domains (CTDs) and are associated with neuropsychiatric disorders such as epilepsy, learning, and intellectual disabilities, autism spectrum disorders, etc.

We have prepared a series of C-3 carboxylic acid steroids by various C–C coupling reactions, esterification, or sulfation. Our studies have shown [1,2] that pregnane-based steroids can effectively modulate NMDARs, providing therapeutic opportunities for loss-of-function disease-associated receptor mutations. Furthermore, the effects of endogenous and synthesized steroids are additive. The compound EPA-But (I) with an atypical 3b5b stereochemistry was identified as the most promising candidate for further development. The mechanism of action of compound I was studied in detail. [1,2]

Our results improved the understanding of the molecular mechanisms of steroid activity on NMDAR CTD truncations and pointed the direction for the development of new therapeutic steroid ligands. The design and synthesis of such EPA-but analogs will be presented.



EPA-But (I)

This work was supported by the Czech Science Foundation (GACR): 23-04922S and the Research Project of the CAS RVO: 67985823 and 61388963.

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SELECTIVE AND SUSTAINABLE EXTRACTION OF THE TRITERPENOID OLEANOLIC ACID FROM GRAPE POMACE

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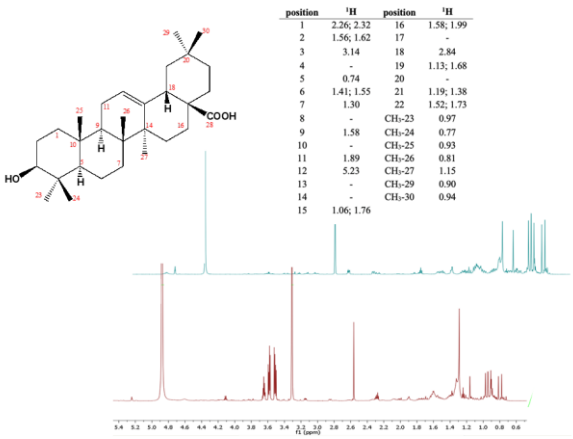
Grape pomace is one of the most abundant winery solid residue. Recently, this bulky by-product has attracted the interest of scientists and enologists since it has been characterized as a valuable source of high value-added compounds, including fibers, sugars and polyphenols. Many strategies have been implemented for its valorization worldwide. We have recently detected oleanolic acid in red grape pomace in relatively high amounts. Oleanolic acid is a triterpenoid with a plethora of biological activities, ranging from anticancer, anti-inflammatory to antidiabetic and antimicrobial ones. With the purpose of recovering this compound from pomaces, we have set up a selective extraction of oleanolic acid from grape pomace by using dimethyl carbonate (DMC), a recommended green solvent as a better alternative to fossil-based solvents. Hildebrand's solubility and Kamlet-Abboud-Taft parameters have been considered to drive the choice of DMC as a greener alternative to other organic solvents.

The extracts obtained from Aglianico (*Vitis vinifera* L.) grape pomace were characterized by means of NMR and LC-MS. Dimethyl carbonate allowed to recover oleanolic acid from grape pomace with a molar selectivity of 61% thus promoting the adoption of alternative green and sustainable technologies for biomass residues valorization.



position	^{1}H	position	^{1}H
1	2.26; 2.32	16	1.58; 1.99
2	1.56; 1.62	17	-
3	3.14	18	2.84
4	-	19	1.13; 1.68
5	0.74	20	-
6	1.41; 1.55	21	1.19; 1.38
7	1.30	22	1.52; 1.73
8	-	CH_3-23	0.97
9	1.58	CH_3-24	0.77
10	-	CH_3-25	0.93
11	1.89	CH_3-26	0.81
12	5.23	CH_3-27	1.15
13	-	CH_3-29	0.90
14	-	CH_3-30	0.94
15	1.06; 1.76		

Stereostructure of oleanolic acid and its chemical shifts in CD3OD; 1H-NMR spectrum of oleanolic acid (top) and of the extract in DMC of Aglianico grape pomace from Campania, Italy (bottom).

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TRITERPENES FROM *BOSWELLIA SERRATA* AS A NEW CHEMOTYPE OF LEUKAEMIA INHIBITORY FACTOR RECEPTOR ANTAGONISTS

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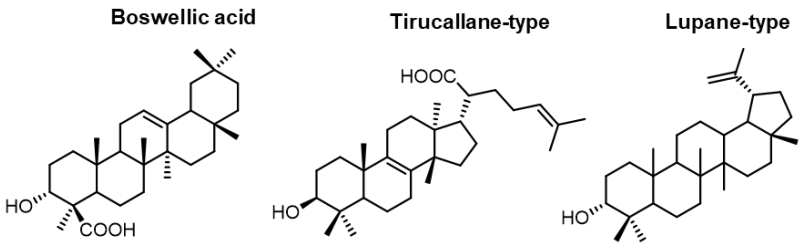
Boswellia serrata is considered a relevant medicinal plant, especially for its anti-inflammatory[1] effects, but also for its effectiveness in treating a wide range of conditions, including cancer[2,3] and diabetes. This plant, known as Indian frankincense, belongs to the Burseraceae family and grows in dry mountainous regions of India, Northern Africa and Middle East. Its extracts or resins contain many important bioactive phytometabolites, with boswellic acids recognized as the main components.

As part of our interest on plants for the identification of possible new anticancer drugs, we proceeded in the isolation of secondary metabolites from *Boswellia serrata.* This resulted in the identification of different pentacyclic and tetracyclic triterpenes belonging to the ursane, oleanane, lupane and tirucallane groups.



We demonstrated for the first time that boswellic and tirucallic acids are antagonists of leukaemia inhibitory factor receptor (LIFR), a relatively new target for cancer[4].

The primary ligand for LIFR is the leukaemia inhibitory actor (LIF), and LIF/LIFR axis is implicated in tumor growth and progression by acting on multiple aspect of cancer biology.

The discovery of plant-derived LIF/LIFR antagonists expands the understanding of the intricate LIF/LIFR pathway and highlights the potential use of boswellic acids in cancer treatment.

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EXPLORING THE MULTIFACETED TERPENYL MOIETY OF NATURAL PHYTOCANNABINOIDS

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Phytocannabinoids are meroterpenoids structurally characterized as isoprenylated resorcinyl polyketides, which represent the signature of the secondary metabolism of *Cannabis sativa* L.

To date, almost 200 phytocannabinoids have been isolated from natural sources but this number grows up day by day. The chemical, biological and medicinal research has been mainly focused on Δ^9-tethrahydrocannabinol (THC) and cannabidiol (CBD) for their easier availability. Regarding minor phytocannabinoids, main variations occur on the terpenyl moiety.[1]

Here, we analyse and compare the chemical and biological space of minor phytocannabinoids found in two different chemotypes (III and IV) of *C. sativa* L. obtained thanks to the application of our expeditious workflow for the rapid identification of phytocannabinoids.[2,3]

Among CBD derivatives, we isolated and completely characterised three naturally occurring hydroxylated CBD derivatives, namely 1,2-dihydroxycannabidiol, 3,4-dehydro-1,2-dihydroxycannabidiol, and hexocannabitriol. In addition to cannabimovone, a derivative with an unprecedented structural modification of the *p*-menthane moiety, previously reported by our research group, we isolated its derivative anhydrocannabimovone, and the biogenetically related dihydrobenzofuran derivatives cannabifuranols A and B. The most undershadowed major phytocannabinoid is cannabigerol (CBG). To date, only few natural derivatives have been identified and most of them presented variations on the isoprenyl moiety. Newly isolated phytocannabinoids included mono- or dihydroxylated CBGA/CBG analogs, a congener with a truncated side chain, cyclocannabigerol B, and, characterized by unprecedented structural architecture within phytocannabinoids chemical family.

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DISCOVERY OF A DEAROMATIZATIVE TAUTOMERISM IN CANNABINOQUINONE DERIVATIVES

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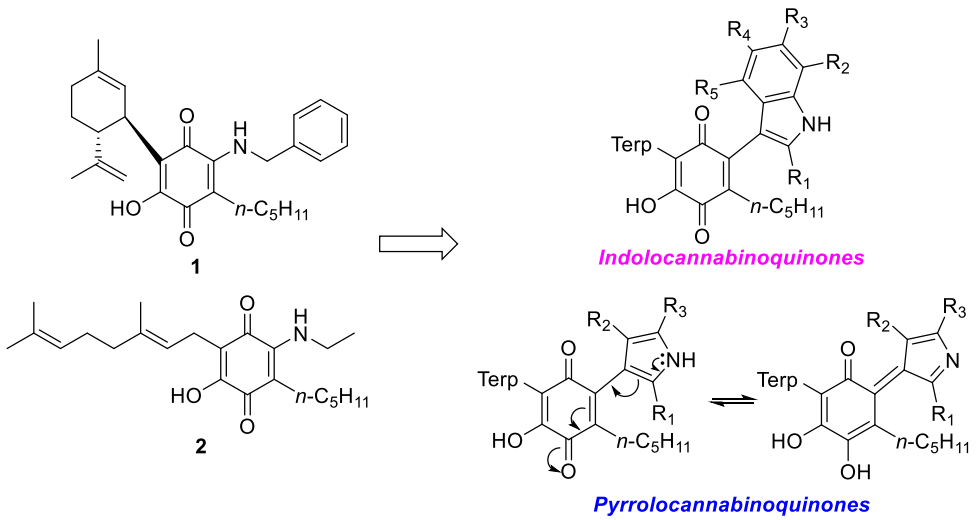
Emerald Health Pharmaceuticals has gained recognition for developing the cannabinoquinone derivatives etrinabdione (EHP-101, **1**) and EHP-102 (**2**) for the management of various immuno-inflammatory diseases, including systemic scleroderma, a condition for which **1** received orphan drug status in USA and EU.[1] These compounds are obtained by treatment of the oxidized quinoid form of phytocannabinoids with primary amines, a late-stage tandem amino-Michael - dehydrogenative modification which increases both stability and potency.[1]

Within this context, we have started a program of systematic exploration of the chemical and biological space of these compounds, starting with the replacement of primary amines in the late-stage functionalization reaction. A selective reactivity with electron-rich aromatics was discovered, identifying pyrroles and indoles, but not phenolics, as suitable amine replacements. The adducts from these compounds exhibited distinct chromatic signatures, a trend previously observed for *ortho*- and *para*-quinone tautomers,[2] with indole derivatives being violet and pyrrole derivatives deep blue. This colour differences were rationalized in terms of the lower kinetic stability of pyrrole compared to indole, which leads to the formation of an aza-fulvenic tautomeric forms. The biological translation of this modification of the aminocannabinoquinone pharmacophore showed a distinct biological profile.



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THE NON-PSYCHOACTIVE PHYTOCANNABINOIDS CANNABIDIOLIC AND CANNABIGEROLIC ACIDS AS MULTI-TARGET LIGANDS AGAINST ALZHEIMER'S DISEASE

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The multifactorial nature of Alzheimer's disease (AD), a neurodegenerative disorder accounting for over 60% cases of dementia, represents a great challenge for the research community. The multi-target directed ligand (MTDL) strategy, where a single molecule is able to modulate more than a single relevant molecular target, represents a valuable approach to develop effective drugs to alleviate and slow down the progression of the disease. The negatively-charged phytocannabinoids cannabidiolic (CBDA) and cannabigerolic (CBGA) acids are naturally-occurring molecules from the plant *Cannabis sativa*, previously identified by us as dual PPARα/γ agonists[1]. The increasing relevance of these nuclear receptors in AD animal models[2,3] prompted us to extend the evaluation of their potential activity on a panel of a enzymes involved in the modulation of the cholinergic tone and/or the beta-amyloid production, two hallmarks of AD. Here we report the identification and characterization of CBDA and CBGA as dual cholinesterase/beta-secretase inhibitors, active in a low micromolar range by a combined computational and experimental approach. Molecular docking and molecular dynamics simulations have been used to elucidate their binding modes at acetylcholinesterase (AChE), butyrylcholinesterase (BuChE) and beta-secretase (BACE-1) enzymes, also providing a rationale for the different inhibitory profile toward the two cholinesterase enzymes. These compounds also show anti-aggregation properties of β-amyloid fibrils and are able to improve the cognitive performance in AD mouse models. We also show that GPR109A is not a direct target for CBDA and CBGA. Instead, both compounds restore the physiological expression level of trpm7 gene, upregulated in mice treated with β-amyloid peptide. This study intends to broaden the activity profile of both CBDA and CBGA as novel anti-AD MTDLs and to suggest the use of negatively charged compounds as non-canonical multitarget ChE/BACE-1 inhibitors.

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METABOLIC SYNDROME MODULATION PROFILE OF TWO *CENTAUREA* SPECIES SESQUITERPENOIDS

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The genus *Centaurea* has always been a rich source of bioactive secondary metabolites, with a long history of use in traditional herbal remedies to treat indigestion, headaches, infections, inflammation and diabetes. Widespread in the Mediterranean region, these plants are characterised by a complex phytochemical profile that includes polyphenols, alkaloids and sesquiterpenoids, the latter being the hallmark of this genus. From our recent investigations of less studied *Centaurea* species,[1,2] we have highlighted the differences in their sesquiterpenoid composition, along with their activity on different targets related to metabolic syndrome. In fact, recent investigations have shown the potential benefits of the CH_2Cl_2 extracts of different *Centaurea* species, but the compounds responsible for the activity have rarely been identified. Our detailed spectroscopic analysis (NMR and HRMS) of the organic extracts of *C. kotschyi* and *C. sicula* led us to the isolation of several sesquiterpenoids belonging to different structural families and different types of polyphenols. Moreover, we have also characterised six previously unreported sesquiterpenes. All compounds were evaluated for their glucose uptake activity and α-glucosidase inhibitory activity, which helped us to identify the bioactive constituents of the extracts. In particular, the lignan salicifoliol from *C. sicula* showed the highest potential as a GLUT stimulator, together with the germacranolide dihydrocnicin and the guaianolide rhizantholide A from *C. kotschyi*, while all elemanes and eudesmanolides were inactive, indicating that structural features are required. Furthermore, evaluation of inhibitory effect against α-glucosidase showed that several sesquiterpene lactones have promising activity, with the elemane melitensin showing the highest potential.

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EXPLORING THE PHYTOCHEMICAL PROFILE AND ANTIMICROBIAL ACTIVITY OF *SALVIA DISCOLOR* AGAINST PHYTOPATHOGENS

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In the present study, we carried out phytochemical characterisation and antimicrobial activity of dichloromethane extract from aerial parts of *Salvia discolor* Kunth (Lamiaceae). This research aimed to fill the gap in understanding the potential applications of this underexplored species. The dichloromethane extract obtained from the plant surface of *S. discolor* after repeated chromatographic separation yielded a novel compound with clerodane diterpene skeleton and various known compounds, 8,3'-dihydroxy-6,7,4'-trimethoxyflavone[1], 5,7-dihydroxy-3,4'-dimethoxyflavone[2], divinatorin A[3] and patagonic acid[4], which were identified through spectroscopic NMR analysis, including DEPT, TOCSY, HSQC, HMBC, and ROESY experiments. Furthermore, we assessed the antimicrobial potential of the ground extract against three strains of phytopathogenic bacteria (*Clavibacter michiganesis* subsp*. michiganesis*, *Pectobacterium carotovorum* subsp*. carotovorum*, *Pseudomonas syringae* pv. *tomato*) and nine strains of phytopathogenic fungi *(Alternaria solani*, *Botrytis cinerea*, *Colletotrichum lindemuthianum*, *Fusarium solani*, *Fusarium oxysporum* fsp. *lactucae* race 1, *Phoma betae, Phaemoniella chlamydospora*, *Pythium dissocotum*, *Stemphylium* sp.), selected among the more common pathogens of agricultural interest. Our results showed that the extract was only effective at concentrations above 1000 µg/mL, showing a low antibacterial activity. Conversely, the extract showed a significant antifungal activity against all the fungi tested at different concentrations (100, 250, 500, 750 and 1000 µg/mL). The extract inhibited more than 80% of mycelial growth of *F. solani*, *P. chlamydospora* and *P. dissotocum*, and showed more than 50% inhibition against *A. solani*, *B. cinerea*, *P. betae* and *Stemphylium* sp. On the other hand, there was minimal inhibition (<30%) against *C. lindemuthianum* and *F. oxysporum* fsp. *lactucae* race 1.

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SOLID DISPERSION BASED ON LEUCOMISIN

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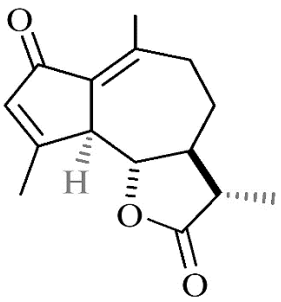
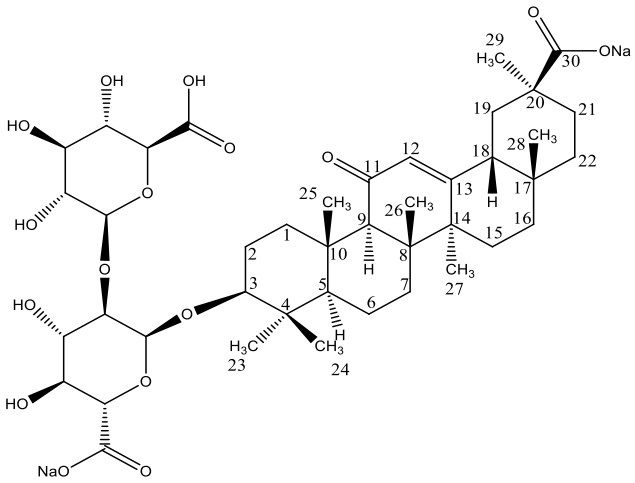
The solid-phase synthesis of the sesquiterpene lactone leucomisin (**1**) with the dynatrium salt of glycyrrhizic acid (Na_2GA) is reported in the paper.

The sesquiterpene lactone leucomysin (**1**), a colourless crystalline substance of composition $C_{15}H_{18}O_3$, m.p. 196-199°C (ethanol), $[\alpha]_D^{20}$ +55.9° (*c* 2.86; chloroform), isolated from white wormwood (*Artemisia leucodes* Schrenk.), and has antioxidant, hypolipidemic and antiatherosclerotic activities[1]. However, this guaiyanolide is practically insoluble in water, which significantly reduces the bioavailability of the substance in the body and, consequently, its pharmacological action. One of the ways to solve this problem could be a solid-phase synthesis based on leucomisin (**1**) using Na_2GA (**2**). The “Solid dispersion method” allows to significantly increase both the solubility and the release of a number of active substances from different dosage forms.



(1)



(2)

We have synthesised leucomisin (**1**) with Na_2GA (**2**) by the methods: ‘solvent removal’, ‘simple mixing’. The solubility of leucomisin from the synthesised solid dispersions (SD) increases up to 18.6 times, the dissolution rate up to 98.7 times, in comparison with the original natural leucomisin (**1**), which is probably due to the surface-active properties of glycyrrhizic acid as a saponin, while it envelops the leucomisin molecule and leads to the breakage of intermolecular hydrogen bonding between water molecules.

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EFFECT OF SESQUITERPENE LACTONES ON PANCREATIC TUMOR CELLS

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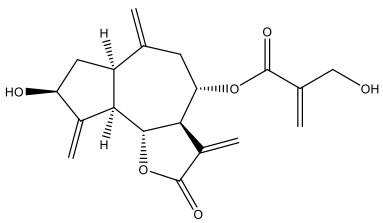
Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive disease. Drugs use for its treatment are characterized by the lack of effectivity and selectivity[1]. Sesquiterpene lactones are natural C15 terpenoid compounds with many biological activities reported[2]. Among them, the antitumor potential of this type of compounds stands out. The inhibition of the enzyme farnesyl protein transferase, as well as modulation of the Ras signaling pathway have been described as potential mechanisms of action of this type of compounds[3]. In this sense, the aim of this work was to evaluate the activity of 25 sesquiterpene γ-lactones, isolated from Kazakhstan plant species of the Apiaceae and Asteraceae families, on pancreatic tumor cells, in the search of novel drugs to treat PDAC. The effect of the compounds on the metabolic and proliferation activity was evaluated *in vitro* on MIAPaCa-2 and PANC-1 tumor cell lines.



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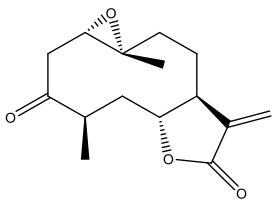

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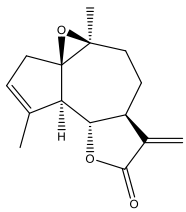

(**3**)

Sesquiterpene γ-lactones **1**-**3** were the most active compounds showing a significant effect on the metabolic activity and in the proliferation assay. The 50% inhibitory concentrations (IC_{50}) of the compounds on the proliferation assay in MIAPaCa-2 and PANC-1 cell lines were 12.46 and 6.56, 7.68 and 3.80, 7.15 and 6.40 μM for compounds 1-**3**, respectively. On the metabolic activity assay, these sesquiterpene lactones showed IC_{50}s of 14.95 and 7.62, 8.3 and 2.25, 12.51 and 5.57 μg/ml, respectively in MIAPaCa-2 and PANC-1 cell lines. These results showed the potential of sesquiterpene lactones as promising antitumor agents. Further studies including the evaluation of the toxicity on normal cells are in progress.

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ANTIPARASITIC ACTIVITY OF SESQUITERPENOIDS

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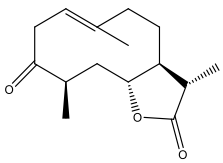
According to the World Health Organization, more than 1 billion people is affected by neglected diseases. Among them, parasitic diseases such as African trypanosomiasis, Chagas disease and leishmaniasis, can be mentioned[1]. In terms of searching for effective antiparasitic agents, natural terpenoids, which have a wide range of therapeutic effects, are considered promising sources[2]. The aim of this study was to evaluate the antiparasitic activity against *Trypanosoma brucei brucei*, *T. cruzi* and *Leishmania amazonensis* of 25 sesquiterpenoids isolated from Kazakhstan plant species of the Apiaceae and Asteraceae families.



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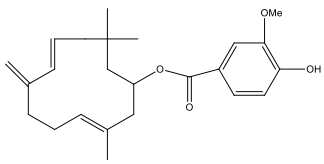

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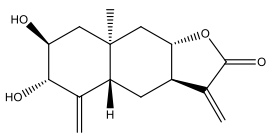
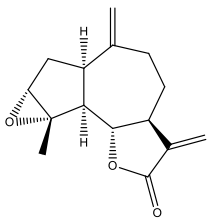


(**3**)

Sesquiterpenoids **1**-**3** were active against *T. b. brucei* and *T. cruzi*. The 50% inhibitory concentration (IC_{50}) values were 10.51±1.72, 3.73±5.26 and 2.51±1.13 µg/ml on *T. b. brucei* trypomastigotes and 7.50±0.23, 13.37±0.42 and 6.51±0.14 µg/ml on *T. cruzi* amastigotes, for compounds **1**-**3,** respectively. Compound **2** showed selectivity indexes of 8.94 and 2.50 on *T. b. brucei* and *T. cruzi*, respectively.



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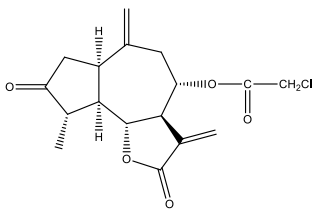

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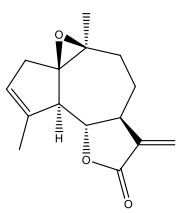

(**6**)

On *L. amazonensis* promastigotes, compounds **4**-**6** were the most actives with IC_{50} values of 0.16±0.13, 0.35±0.18 and 0.58±0.11 µg/ml, respectively. On the intracellular form of the parasite compounds **4** and **6** showed IC_{50}s of 0.68±0.007 and 1.13 ±0.006 µg/ml, respectively. Further studies will be conducted to determine the possible mechanism of action of the active compounds.

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BIOLOGICAL ACTIVITY OF AMINO ACID CONJUGATED BRASSINOSTEROID ANALOGUES

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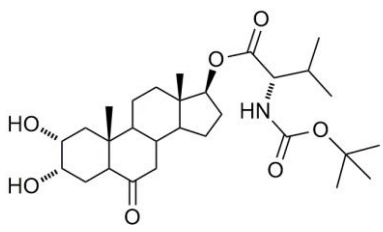
Brassinosteroids (BR) are phytohormones that play an important role not only in plants, but also show interesting antitumor activity *in vitro*. Antiproliferative activity of natural BRs was proved in breast cancer cell line, where their influence on steroid receptors was examined with positive results for estrogen receptors[1].

Brassinosteroid analog BR4848 (2α,3α-dihydroxy-6-oxo-5α-androstan-17β-yl *N-(tert*-butoxycarbonyl)-D,L-valinate) was cytotoxic in various cancer cell lines and not in normal human fibroblast. Its antiangiogenic activity, effect on interleukin-6 production and influence on migration were tested in human umbilical vein endothelial cells (HUVECs) with inhibitory effect[2]. This is reason why stereoisomers of this substance were prepared: L-valin-2β,3β-diol (RN6A), D-valin-2β,3β-diol (RN7A), L-valin-2α,3α-diol (RN6B) and D-valin-2α,3α-diol (RN7B) and investigated in more detail. Cytotoxicity of these isomers in cancer cell lines as well as anti-inflammatory activity in HUVECs was tested with different results. The most effective isomer was RN6B with L-valin and α,α configuration. The mechanism of action was examined by immunoblotting where changes in expression of steroid receptors, TNFR1 and apoptotic markers were observed also depending on the configuration.

This study affirmed how the configuration of compounds influences the biological activity.



RN-6B

This work was supported financially by grant from the Czech Grant Agency (23-05389S).

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ANTITUMOR EFFECTS OF SYNTHETIC DERIVATIVES FROM *ENT*-KAURANE ATRACTYLIGENIN

Natale Badalamenti[1,2], Gianfranco Fontana[1,2], Antonella Maggio[1,2], Marianna Lauricella[3], Antonella D'Anneo[1], Maurizio Bruno[1,2]

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Carlina gummifera L. is a species, belonging to the Asteraceae family, recommended for the treatment of several health problems such as ulcers, hydrops, snake-bite poisoning, and drowsiness.[1] It is also known for the lethal toxicity of two diterpenoid glucoside compounds contained in the roots: atractyloside and carboxyatractyloside.[2] However, the antitumor activity of different synthetic *ent*-kaurane diterpenes has been extensively studied[3,4], and several investigations have demonstrated the excellent antitumor activity of synthetic derivatives of the diterpene atractyligenin.[3,4] In this research, a series of new synthetic compounds of atractyligenin, the aglycone skeleton of atractyloside and carboxyatractyloside, have been synthesized. Several chemical modifications, such as oxidation, bromination, elimination, amidation, and rearrangement, on C-2, C-15, C-17, and C-19 positions, have been performed in order to explore the biological properties of new derivatives. The synthesized compounds were structurally elucidated by MS, 1D- and 2D-NMR experiments and were assayed for in vitro antiproliferative activity against a panel of human cancer cells, namely, malignant melanoma cells (A375), human colon epithelial cancer cells (CaCo2), colon cancer cells (HCT116 and Caco-2), and human keratinocyte (HaCaT). In particular, using different concentrations of the obtained compounds (10-300 μM) a reduction of cell viability of HCT116 colon cancer cells was observed at 48 h of treatment. All the oxidized compounds were more effective than their alcoholic precursors. The oxidized compounds reduced the viability of two colon cancer cells (HCT116 and Caco-2) already at 24 h when used at low doses (2.5-15 μM), while they turned out to be poorly effective in differentiated Caco-2 cells, a model of polarized enterocytes. The data obtained provided evidence that oxidized compounds induced apoptotic cell death as demonstrated by the appearance of condensed and fragmented DNA in treated cells as well as the activation of caspase-3 and fragmentation of its target PARP-1.

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UNDERSTANDING CAROTENOIDS: AVOIDANCE OF SYMMETRY EFFECTS *IN SILICO* STUDY

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The symmetry-based approach has been widely used to explain the puzzling photophysics of carotenoids, the linear polyunsaturated isoprenoids, by assuming that they all possess the C_{2h} symmetry. In our previous studies we have shown that such a high symmetry of carotenoid molecules is unrealistic, much due to the methyl side groups[1,2]. In the present study we undertook to assess the effects of symmetry in carotenoid molecules using an *ab initio* approach. In particular, we addressed the question of what are the implications and the energetic costs, on the molecular level, of imposing specific symmetries on these molecules? In order to reveal the effects of molecular symmetry on the properties of carotenoids the computations were carried out on a model series of closely related carotenoid molecules in which the symmetry is gradually reduced: *all-trans*- and 15-*cis*-β-carotene, and zeaxanthin. In parallel, the *ab initio* approach was applied to estimate the effects of imposing specific point symmetry (C_1, C_2, C_i and C_{2h}) on the ground state properties of β-carotene molecule. The computational results were then confronted with the relevant observables obtained through the electronic absorption and circular dichroism spectroscopies. Our major conclusion is that the combination of the plane of symmetry together and the inversion center, the two symmetry elements that define the C_{2h} symmetry, induces a very high steric strain in carotenoids and such symmetric conformations are thermodynamically very unfavorable. The most strain is caused by the plane of symmetry that requires a simultaneous flattening of various fragments of the isoprenoid skeleton. Hence, the C_{2h} symmetry is unavailable to these molecules. Furthermore, our computations reveal that native conformations of achiral carotenoids in various pigment-protein complexes are highly chiral (C_1 symmetry). These results shed new light on the conformational properties and geometry of isoprenoid chromophores.

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A NEW HOPE IN HOP: ANALYTICAL AND SEMI-PREPARATIVE METHODS FOR *HUMULUS LUPULUS* L. BITTER COMPOUNDS AND THEIR BIOLOGICAL ACTIVITY

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Humulus lupulus L., commonly known as hop, is a rich source of bitter compounds mainly used in the brewing industry to impart the bitter taste to beer and to protect against microbes. The most abundant hop bitter acids are *α*-acids (humulone, ad-humulone, co-humulone) and *β*-acids (lupulone, ad-lupulone, co-lupulone). Hop extract is reported for its activity in glucose homeostasis regulation and body weight reduction. One of the mechanisms involved might be the activation of bitter taste receptors (Tas2rs). These have been recently located in extra-oral tissues such as brain and gastrointestinal tract. They are not only key sensors against the ingestion of potentially poisonous agents, but they are also implicated in gut hormone release such as GLP-1 or CCK influencing appetite, therefore representing a strategy against obesity[1]. Hence, the present study aims to investigate the implications of *α*- and *β*-acids as novel satiety-inducing agents through intestinal activation of Tas2rs. The extraction of hop inflorescences was conducted by maceration using a hydroalcoholic mixture (55% EtOH). Quali-quantitative HPLC-DAD analysis reported the presence of *α*-acids (cohumulone 6.14±0.19 mg/g DW; adhumulone+humulone 2.04±0.11 mg/g DW) and *β*-acids (colupulone 7.04±0.54 mg/g DW; adlupulone+lupulone 2.10±0.02 mg/g DW).

Because of the lack of knowledge in these compounds, reproducible and sensitive analytical methods are necessary to recover and isolate them. Semi-preparative HPLC-DAD and UHPLC-DAD-ESI-Orbitrap Exploris^{TM}120 Mass Spectrometer analysis[2] were used to obtain pure *α*- and *β*-acids to be used in the following studies. These demonstrated that the five identified compounds showed different behaviour in secreting anorexigenic hormones from intestinal STC-1 cell line targeting different isoforms of Tas2rs probably due to their structural difference. Further studies are needed to attribute the pharmaceuticals and nutraceuticals activities to the diverse molecular scaffolds of *α*- and *β*-acids.

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DRIMANE SESQUITERPENES FROM *ASPERGILLUS XEROPHILUS* WITH INHIBITIVE EFFECTS AGAINST THREE PHYTOPATHOGENIC FUNGI OF AGRARIAN CROPS

Maria Michela Salvatore[1,2], Stefany Castaldi[2], Maria Teresa Russo[1], Moustafa Bani[3], Marina DellaGreca[1], Ivana Staiano[2], Alessio Cimmino[1], Rachele Isticato[2], Anna Andolfi[1], Marco Masi[1]

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Phytopathogenic fungi are able to cause plant diseases with tremendous impact on the crop yield and quality[1]. Among them *Fusarium oxysporum* f. sp. *pisi* (Fop) induce Fusarium wilt disease affecting field pea crops, *Botrytis cinerea* induce Botrytis disease that affects a wide range of crops, including grapes, strawberries, and tomatoes while *Alternaria alternata* can cause diseases like leaf spots, blights, and rots[2-4]. Chemical control based on synthetic products is the main method employed to manage these pathogens. However, most of these formulations determine harmful effects on human, animal, and environmental health. Thus, new and safe antifungal agents are needed to avoid these problems and secondary metabolites produced by microorganisms may represent a suitable alternative to discovery new lead structures for agricultural applications[2].

In the present study, the ability of a strain of *Aspergillus xerophilus* to produce specialized metabolites with potential antifungal activity was investigated for the first time. The fungus was cultured using two different liquid media and several drimane sesquiterpenes have been detected by GC-MS analysis of the organic extracts obtained from its mycelia and culture filtrates. Furthermore, from these organic extracts showing antifungal activity against *B. cinerea*, *A. alternata* and *F. oxysporum* f. sp. *pisi,* some of the detected drimane sesquiterpenes were also isolated using chromatographic techniques. Finally, these isoprenoids have been identified by spectroscopic and optical rotation methods and tested against the same pathogens to evaluate their potential inhibitive effects.

This communication will give an overview on the work carried out on the isolation and biological characterization of the isoprenoids produced by *A. xerophilus* in two cultural conditions and will illustrate some structure-activity relationships.

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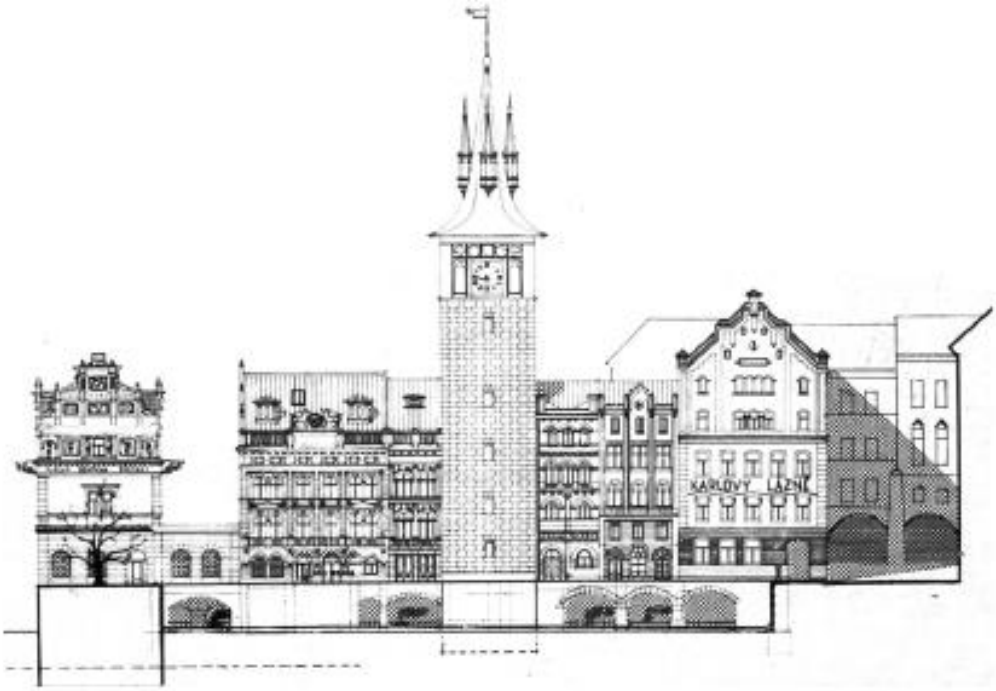
26TH CONFERENCE ON ISOPRENOIDS
SEPTEMBER 15-17, 2026
PRAGUE



The 26th Conference on Isoprenoids will take place on September 15-17, 2026 at the very downtown Prague at the historical buildings of the Czech Association of Scientific and Technical Societies (and the Czech Chemical Society), close to the Prague Charles Bridge.

Note the date and come to Prague to celebrate the flourishing development of the isoprenoid chemistry.

INDEX OF THE AUTHORS