

Retreat

of

Research Training Group 2158

“Natural products and analogs against therapy-resistant tumors and microorganisms”

Program

31.03.-01.04.25

**Naples - Facoltà di Biotecnologie - Policlinico di Napoli Via
Tommaso de Amicis, 95**

Table of Contents

- Introduction** 3
- Program** 5
 - 31st of March, 2025..... 5
 - 1st of April, 2025 7
- Abstracts** 9
 - 31st of March, 2025..... 9
 - 1st of April, 2025 19
- Participants** 30



Dear colleagues,

I am thrilled about the March 2025 GRK 2158 retreat. It will be our last retreat in the ninth year of GRK 2158 funding, and I am delighted that it will take place in Naples, the university city of our Mercator fellow, Prof. Maria Chiara Monti. This will allow us to conclude the excellent exchange of ideas we always valued when Prof. Monti was participating in our events in Düsseldorf.

I am excited to see the great interest of our Italian colleagues in the GRK's research topic centered around natural products and their analogs against therapy-resistant tumors and microorganisms, and I am grateful for their plentiful contributions to the retreat's program. This retreat will offer ample opportunities to interact, discuss results, and exchange or develop ideas that will help unravel and overcome molecular resistance mechanisms against pharmacologically active compounds.

It is also a privilege to visit and explore Naples, the economic and cultural center of southern Italy, with its manifold historic sites and cultural monuments.

I am grateful for the invaluable support and helpful local organization we experienced in the run-up to the retreat. I would like to thank Prof. Monti and her team as well as our German coordinator, Dr. Martina Holz, for making this retreat possible!

I wish all of us exciting, fruitful, and productive days during the retreat.

A handwritten signature in blue ink that reads 'Holger Gohlke'.

Holger Gohlke

Dear colleagues and friends,

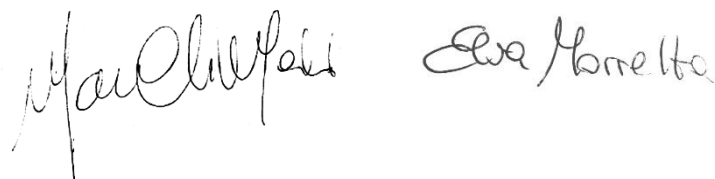
It is a great pleasure for us to welcome you here in Naples to the GRK 2158 Retreat 2025, an excellent opportunity for an in-depth sharing between Italian and German researchers on common research topics.

Our teamwork and joint efforts resulted in the creation of an exciting program, full of innovative topics that we hope will be stimulating for everyone. We designed this retreat to encourage dialogue and interaction between different experts in a wide range of scientific fields, which will be explored with a focus on Natural Products, and to offer an opportunity to reflect together on the challenges and opportunities that lie ahead of us.

We hope that these days will stimulate new ideas, collaborations and, above all, the desire to continue to grow together.

We wish you an active and fruitful participation,

Maria Chiara Monti and Elva Morretta



Program

31st of March, 2025

Time	Speaker	Topic
09:00	Get together	
09:15	Angelo Antonio Izzo	Institutional greeting of the Director of the Department of Pharmacy of the University of Naples Federico II
09:20	Valeria Costantino	Presentation of the International Summer School of Natural Products VI edition summer 2025
09:25	Maria Chiara Monti	Welcome
Session 1 - Chairs: Ivana Bello & Violetta Krisilia		
09:30-10:00	Carmen Festa	Marine natural products: potentiality and challenge in drug discovery
10:00-10:30	Jörg Pietruszka	Chemoenzymatic natural product synthesis
10:30-10:45	Matthias Bleser	Improvements in synthesis, isolation and derivatization of cyclic prodigiosin derivatives.
10:45-11:15	Elva Morretta	Proteasome activity modulation by natural products' inspired compounds
11:15-11:45	Thomas J. J. Müller	Angular anellated pentacyclic indoles – Synthesis and activity against <i>Toxoplasma gondii</i>
11:45-12:00	Alae-Eddine Moubait	Diversity-oriented Sonogashira-Cacchi synthesis of substituted indole derivatives
12:30-13:30	Lunch Break	
Session 2 - Chairs: Elva Morretta & Jingyu Li		
13:30-14:00	Sebastian Wesselborg	Targeting mitochondrial metabolism by the mitotoxin bromoxib in leukemia and lymphoma cells
14:00-14:15	Tobias Wassenberg	Rapid synthesis of highly cytotoxic and apoptosis inducing pyrimidolins via Masuda borylation-Suzuki coupling sequence
14:15-14:30	Karina Krings	Characterization of apoptosis inducing natural compounds for the treatment of therapy resistant tumors with focus on CDK inhibitors

Time	Speaker	Topic
14:30-15:00	Elisabetta Panza	Erucin, the major isothiocyanate from <i>Arugula (Eruca sativa, Mill.)</i> , as a promising therapeutic agent against triple-negative breast cancer
15:00-15:15	Ivana Bello	From the depths of the gulf of Naples sea to the bench: <i>Sidnyum elegans</i> extract as a novel promising therapeutic tool against triple-negative breast cancer
15:15-15:30	Alina Deipenbrock	Immunocompetent 3D pancreatic adenocarcinoma (PDAC) biochip model for preclinical drug testing
15:30	Coffee Break	
Session 3 - Chairs: Karina Krings & Claudia Finamore		
16:00-16:30	Holger Golke	Data-driven enabling technologies to guide targeted covalent inhibitor development
16:30-16:45	Jitheesh Joy	Multi-targeting Inhibitors on the sphingolipid pathway by introducing histamine H3 receptor pharmacophore
16:45-17:00	Christian Mammen	BceAB-type ABC transporters – Conservation, function & organization
17:00-17:15	Gürbüz Önder	Understanding NsrFP-NsrK resistance mechanism
17:15-17:45	Maria Laura Bellone	Multidisciplinary approach to unravel the biological space of cannabidiolic acid in glioblastoma cells
18:30	Free time for shopping	

1st of April, 2025

Time	Speaker	Topic
09:15	Get together	
Session 4 - Chairs: Marisa Conte & Tobias Wassenberg		
09:30-10:00	Gerardo della Sala	Novel peptides and alkaloids from marine extremophiles with activity against drug-resistant pathogens
10:00-10:15	Carmine Buonocore	Antimicrobial properties and potential applications of <i>Pseudomonas gessardii</i> M15 rhamnolipids
10:15-10:30	Violetta Krisilia	Repurposing the antispasmodic drug otilonium bromide and exploring its derivative as potential treatment options for tuberculosis
10:30-10:45	Di He	Isolation and mode-of-action study of the anti-MRSA lead compound pestalotic acid A from <i>Pestalotiopsis chamaeropsis</i>
10:45-11:00	Raffaella Nocera	Labdane diterpene manool as a new promising antimicrobial molecule
11:00-11:15	Martina Pannetta	Comparative purification strategies and functional characterization of extracellular vesicles from <i>Lactocaseibacillus rhamnosus</i> GG
11:15-11:45	Sanil Bhatia	Selective targeting of class-specific HDACs in leukemia
12:00	Lunch Break	
Session 5 - Chairs: Carmine Buonocore & Alina Deipenbrock		
13:00-13:15	Christian Anzenhofer	Development of class IIa HDAC inhibitors
13:15-13:30	Franziska Kinnen	Synthesis and Development of dual HDAC-LSD1 inhibitors
13:30-13:45	Jingyu Li	New drug combinations in lung cancer
13:45-14:00	Jia-Wey Tu	Targeting HDAC6 in therapy-resistant leukemia
14:00-14:15	Adarsh Nair	Modulation of RNASET2 by HDAC6 and its effect on tumor-associated macrophages

Time	Speaker	Topic
14:15-14:30	Lukas Biermann	Hydrazide-based HDAC inhibitors completely reverse chemoresistance synergistically in platinum-resistant solid cancer cells
14:30-14:45	Yu Lin Ho	Development of selective HDAC inhibitors targeting the CoREST protein complex
15:00	Coffee Break	
Session 6 - Chairs: <i>Martina Pannetta & Adarsh Nair</i>		
15:30-16:00	Björn Stork	Identification of autophagy inhibitors selectively targeting the ATG13-ATG101 protein-protein interaction
16:00-16:15	Céline David	Characterization of autophagy-modulating HSP90 inhibitors for the elimination of therapy-resistant tumor cells
16:15-16:45	Alfredo Ambrosone	Plant extracellular vesicles: a new frontier in natural nanomedicine
16:45-17:00	Marisa Conte	Cardoon cell cultures as a biofactory for extracellular vesicles with antisteatotic activity
17:00-17:30	Stefan D'Errico	A journey into the chemistry of cyclic adenosine diphosphate ribose (cADPR), a second messenger isolated from sea urchin eggs with potent Ca ²⁺ -modulating activities
17:30	Holger Gohlke	Closing Remarks
18:00	Free time for shopping	

Abstracts

31st of March, 2025

1 Marine natural products: potentiality and challenge in drug discovery

Carmen Festa

Organic Chemistry, Department of Pharmacy, University of Naples Federico II, UNINA

Natural products have historically played a major role in the pharmaceutical industry, either directly as drug substances or as inspiration for synthesis or semi-synthesis of novel analogues. Natural products have significant advantages compared to libraries of conventional synthetic molecules, characterized by enormous scaffold diversity and structural complexity that have been 'optimised' by evolution to function in biological systems.

The marine environment is extremely multiform, representing an important source of diversity and an enormous reserve of new bioactive compounds. The chemistry of these molecules is a challenge considering their structural novelty and complexity, however increasing attentions are drawn in understanding their biological activity, aiming to explore their potential application in various areas of biomedicine.

In the search for novel metabolites from marine sources, the combination of isolation procedures, dereplication step, MS- and bioassay-guided isolation, represent a useful approach for the discovery of new bioactive natural products. Examples will include metabolites from marine invertebrates, mainly sponges, and microbial sources, such as fungi.

2 Chemoenzymatic natural product synthesis

Jörg Pietruszka

Institute of Bioorganic Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Biocatalytic approaches towards new building blocks in organic synthesis have increasingly emerged as an important tool in recent years. Nowadays, a number of biotransformations are applied in the chemical and pharmaceutical industries delivering fine chemicals or drugs. The mild reaction conditions, also triggering high stereo-, regio-, and chemoselectivity, and the often-elegant short-cuts might lead to advantageous transformations. The focus of our projects is on natural product syntheses. Selected examples for the chemoenzymatic synthesis of versatile building blocks and their application in the total synthesis, e.g., of pyrroloindole alkaloids, will be presented.

3 Improvements in synthesis, isolation and derivatization of cyclic prodigiosin derivatives

Matthias Bleser

Institute of Bioorganic Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Bacterial infections were considered a solved problem after the discovery and widespread use of antibiotics in the population. However, due to excessive and preventive use, more and more multi-resistant strains are formed. Cycloprodigiosin shows antibiotic activity against a variety of multidrug-resistant bacterial strains. However, the severe side effects against mammalian cells hinder its use as antibiotic. With this in mind, a more attractive synthetic strategy was developed, improved and tested to synthesize derivatives of cycloprodigiosin and test those against multidrug resistant bacterial strains.

4 Proteasome activity modulation by natural products' inspired compounds

Elva Moretta

Organic Chemistry, Pharmacy Department, University of Naples Federico II

Quinazolinone are considered privileged structures bearing valuable pharmacophores, whose stability and relatively easy preparation methods are a core reason for scientists' continuous interest in such compounds. In this scenario, we sought to investigate the bioactivity potential of a focused set of quinazolinone (i.e., QUIN) derivatives. At first, the molecules cito-permeability was assessed through a parallel artificial membrane permeability assay (PAMPA), showing a very good permeation profile for all the tested compounds. Then, their cytotoxic potential was evaluated on both the breast MDA-MB-231 and MCF-7 and on the prostate DU-145 cancer cell lines, which allowed us to select QUIN-12 as the most cytotoxic compound and DU-145 as the most responsive cell system. To then disclose QUIN-12 interactome profile in DU-145 lysates, we performed Drug Affinity Responsive Target Stability (DARTS), a label-free functional proteomics strategy based on limited proteolysis. Thus, DARTS experiments coupled with high resolution mass spectrometry analyses allowed us to identify β 2 and β 5 proteasomal subunits as well as the proteasomal activator PA28 as QUIN-12 putative binding partners. Since our DARTS data showed QUIN-12 capability of interacting with both the proteasome activator PA28 and with β 2 and β 5 subunits, we sought to evaluate an eventual QUIN-12 induced modulation of the 20S-PA28 catalytic activity, through *in-vitro* and *in cell*-based assays

5 Angular anellated pentacyclic indoles – Synthesis and activity against *Toxoplasma gondii*

Thomas J. J. Müller

Institute of Organic Chemistry and Macromolecular Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Glyoxylation-alkynylation-cyclocondensation or activation-alkynylation-cyclocondensation sequences offer in a one-pot fashion concise accesses to 3-(hetero)aryl-2-alkynylphenazines, which can be cyclized to indolo[3,2-*a*]phenazines by gold-catalyzed cycloisomerization with concomitant 1,2-silyl shift. These angular anellated pentacyclic indoles are readily transformed by consecutive one-pot synthesis into 6-(hetero)aryl-indolo[3,2-*a*]phenazines, which are remarkably active against the parasite *Toxoplasma gondii*. In addition, the target compounds display remarkable redshifted fluorescence. While the initial series of indolo[3,2-*a*]phenazines not only revealed activity against the parasite, but also considerable cytotoxicity, a second series allowed identifying a suitably substituted derivative with both activity and diminished cytotoxicity, opening a therapeutic window. Congeners of indolo[3,2-*a*]phenazines, which are tetracyclic or lack a nitrogen atom, are also synthesized for comparison by modified strategies. Apparently, the indolo[3,2-*a*]phenazine is particularly favorable for the activity against *Toxoplasma gondii*. The conceptual synthetic approach and the photophysical and biological properties of the title compounds will be presented and discussed.

6 Diversity-oriented Sonogashira-Cacchi synthesis of substituted indole derivatives

Alae-Eddine Moubait

Institute of Organic Chemistry and Macromolecular Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Indole is a prevalent bicyclic heterocycle in a vast number of organisms, occurring as tryptophan in peptides and proteins, as structural element in secondary metabolites, such as alkaloids, as well as a constituent of many natural and non-natural pharmacologically active derivatives. In past decades, particularly indole alkaloids with enormous pharmacological potential have been isolated from organisms (sponges, plants) in various habitats. Some of these natural products were proven to exhibit for instance anti-microbial, anti-inflammatory as well as apoptosis inducing properties. Therefore, establishing modular, concise methodologies for the synthesis of variously substituted indole derivatives opens new avenues for addressing known or discovering novel biological activities. Here, we present a concise, consecutive one-pot synthesis of substituted indoles employing a Sonogashira alkynylation-Cacchi cyclization sequence based upon a sequentially palladium-catalyzed process. This access represents a catalyst economical use of a single employed catalyst system. Variation of the coupling agents used for synthesis set the stage for providing a diversity-oriented library of substituted indoles.

7 Targeting mitochondrial metabolism by the mitotoxin bromoxib in leukemia and lymphoma cells

Laura Schmitt, Karina S. Krings, Sebastian Wesselborg

Institute for Molecular Medicine I, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf

Targeting mitochondrial metabolism represents a promising approach for cancer treatment. Here, we investigated the mitotoxic potential of the polybrominated diphenyl ether bromoxib, a natural compound isolated from the marine sponge *Dysidea* family. We could show that bromoxib comprised strong cytotoxicity in different leukemia and lymphoma cell lines (such as HL60, HPBALL, Jurkat, K562, KOPTK1, MOLT4, SUPB15 and Ramos), but also in solid tumor cell lines (such as glioblastoma cell lines SJ-GBM2 and TP365MG). Bromoxib activated the mitochondrial death pathway as evidenced by the rapid translocation of Bax to the mitochondria and the subsequent mitochondrial release of Smac. Accordingly, bromoxib-induced apoptosis was blocked in caspase 9 deficient Jurkat cells and Jurkat cells overexpressing the anti-apoptotic protein Bcl-2. In addition, we could show that bromoxib functioned as an uncoupler of the electron transport chain with similar rapid kinetics as CCCP in terms of dissipation of the mitochondrial membrane potential ($\Delta\Psi_m$), processing of the dynamin-like GTPase OPA1 and subsequent fragmentation of mitochondria. Beyond that, bromoxib strongly abrogated ATP production via glycolysis as well as oxidative phosphorylation (OXPHOS) by targeting electron transport chain complexes II, III, and V (ATP-synthase) in Ramos lymphoma cells. Thus, bromoxib's potential to act on both cytosolic glycolysis and mitochondrial respiration renders it a promising agent for the treatment of leukemia and lymphoma.

8 Rapid synthesis of highly cytotoxic and apoptosis inducing pyrimeriolins via Masuda borylation-Suzuki coupling sequence

Tobias Wassenberg

Institute of Organic Chemistry and Macromolecular Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Pyrimeriolins are a subclass of the *meriolins*, which were originally synthetic hybrids of the naturally occurring alkaloids *variolin* and *meridianin*. Their structural framework is based 3-(pyridin-4-yl)-7-azaindoles and they show a plethora of biological activity in tests. They are highly cytotoxic in lymphoma und leukemia cell lines and predominantly target cyclin-dependent kinases (CDKs). Additionally, they potently induce apoptosis and promising *pyrimeriolins* have even shown IC_{50} values in the low two-digit nanomolar range. Due to hinge binding through the 7-azaindol moiety, further functionalization primarily targets the 4-position or allows for some variation of the heterocycle. Previous research indicated that alkoxy substitution is the most beneficial in increasing the biological activity and was thus thoroughly investigated. After prior functionalization, novel *pyrimeriolins* were synthesized concisely in two steps *via* two separate one-pot syntheses. *Masuda* borylation-Suzuki coupling sequence (MBSC) was used as the key reaction step, allowing cross coupling between the functionalized azaindole and the substituted pyridine.

9 Characterization of apoptosis inducing natural compounds for the treatment of therapy resistant tumors with focus on CDK inhibitors

Karina Krings

Institute for Molecular Medicine I, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf

Inducing apoptosis in cancer cells is a critical therapeutic strategy that selectively eliminates malignant cells and inhibits tumor progression without inducing inflammation. The application of meriolins has demonstrated their capacity to function as pan-specific kinase inhibitors, predominantly targeting CDKs, thereby inducing apoptosis. The study indicates that these substances block the cell cycle and trigger apoptotic signaling pathways, resulting in a substantial decrease in cell growth and survival of leukemia cells. The mechanisms underlying this apoptosis induction are investigated in greater detail using specific CDK inhibitors of the cell cycle and transcription. We could demonstrate that the inhibition of transcription, especially through CDK9, is particularly cytotoxic in leukemia cells. We could further show that by targeting CDK9, RNA polymerase II is hindered from its function, consequently preventing the transcription of anti-apoptotic proteins such as Mcl-1. This disruption in the equilibrium between pro- and anti-apoptotic proteins results in programmed cell death.

10 Erucin, the major isothiocyanate from arugula (*Eruca sativa*, Mill.), as a promising therapeutic agent against triple-negative breast cancer

Ivana Bello ¹, Martina Smimmo ¹, Camilla Esposito ¹, Martina Barile ¹, Mariarosaria Bucci ¹, Giuseppe Cirino¹, Ambra A Grolla ², Cristina Travelli ³, **Elisabetta Panza** ¹

¹ *Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, 80131 Naples, Italy.*

² *Department of Pharmaceutical Science, University of Eastern Piedmont, Novara, Italy.*

³ *Department of Drug Sciences, Università degli Studi di Pavia, Pavia, Italy.*

Breast cancer is the most frequent form of cancer occurring in women of any age. Among the different types, the triple-negative breast cancer (TNBC) subtype is recognized as the most severe form, being associated with the highest mortality rate. Currently, there are no effective treatments for TNBC. For this reason, the research of novel therapeutics is urgently needed. The latter may include also dietary prevention and treatment¹. Epidemiological studies demonstrated that the consumption of Brassicaceae, a rich source of biologically active isothiocyanates (ITCs), effectively reduces the risk of cancer ². In this study, we explored the potential anti-cancer effects of Erucin (ERU), the most abundant H₂S-releasing isothiocyanate present in arugula (*Eruca sativa*) on the most aggressive phenotype of human TNBC cells (MDA-MB-231) *in vitro* and in 4T1-bearing mice *in vivo*. Our results revealed that ERU inhibits the proliferation of MDA-MB-231 cells in a time (24-72h) and concentration (1–100 µM) -dependent manner by promoting: 1) apoptosis, through activation of caspase-3 and PARP; 2) autophagy, as reflected by the increased expression of key regulatory genes, including ULK1, ATG13, BECN1, and BNIP3. Additionally, ERU prevented intracellular ROS generation promoting the expression of key antioxidant genes (GCLC, GCLM, SOD1 and HMOX-1) and halted MDA-MB-231 cell migration, invasion, and colony formation. *In vivo* studies revealed that daily oral

administration of ERU (10 mg/kg) significantly reduced tumour volume and weight in 4T1 mice. Moreover, ERU affected the NF- κ B signalling pathway, reducing the expression of several inflammatory mediators and enzymes, such as inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumour necrosis factor-alpha (TNF α) and interleukin-6 (IL-6). Furthermore, ERU inhibited angiogenesis in 4T1 tumours as revealed by the reduced expression of vascular endothelial growth factor (VEGF). In conclusion, our results show that the consumption of ERU could be a useful dietary support intervention to prevent and treat TNBC.

¹ Li, Y.; Zhang, H.; Merkher, Y.; Chen, L.; Liu, N.; Leonov, S.; Chen, Y. Recent Advances in Therapeutic Strategies for Triple-Negative Breast Cancer. *J. Hematol. Oncol.* 2022, 15, 121.

² Citi, V., et al., (2019). Anticancer properties of Erucin, an H₂S-releasing isothiocyanate, on human pancreatic adenocarcinoma cells (AsPC-1). *Phytotherapy Research: PTR*, 33(3), 845–855. <https://doi.org/10.1002/ptr.6278>

11 From the depths of the gulf of Naples sea to the bench: *Sidnyum elegans* extract as a novel promising therapeutic tool against triple-negative breast cancer

Ivana Bello¹, Martina Barile¹, Camilla Esposito¹, Marcello Casertano¹, Concetta Imperatore¹, Marialuisa Menna¹, Raffaella Sorrentino¹, Emma Mitidieri¹, Roberta d'Emmanuele di Villa Bianca¹ and Elisabetta Panza¹.

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Substances derived from marine organisms such as sponges, mollusks, and ascidians exhibit a wide range of biological activities, including anticancer, antioxidant, antiviral, antibacterial, and neuroprotective effects [1]. However, the properties of the Mediterranean ascidian *Sidnyum elegans* remain largely unexplored [2]. This study aims to investigate, for the first time, the potential therapeutic effects of *Sidnyum elegans* extract on MDA-MB-231 human triple-negative breast cancer (TNBC) cells. Fresh specimens of *Sidnyum elegans*, collected from the Gulf of Naples (Pozzuoli), were exhaustively extracted and partitioned between H₂O, ethyl acetate, and n-butanol, yielding three extracts of varying polarity. Medium-pressure liquid chromatography (MPLC) with an increasing gradient from 100% H₂O to 100% MeOH was performed on the butanol-soluble material, resulting in nine fractions (SEB1–9). These fractions were tested for their ability to inhibit MDA-MB-231 cell proliferation at concentrations ranging from 10–50 μ g/mL. Among them, the SEB5 fraction, eluted with a MeOH/H₂O 7:3 (v/v) solution, was the most active, inducing apoptosis in MDA-MB-231 cells, as demonstrated by cytofluorimetric analysis with Annexin V/PI dual staining. Further analysis revealed a time-dependent arrest in the G₀/G₁ phase of the cell cycle, accompanied by a decrease in the S phase population, as shown by FACS analysis. This was linked to reduced expression of cyclin D1, cyclin B1, CDC25A, and CDK1. Additionally, we explored whether SEB5 could modulate autophagy in the same cell model. Our results showed that SEB5 promoted autophagy, indicated by increased expression of key regulatory genes (ULK1, ATG5, ATG7, ATG13) and protein (LC3II). Furthermore, SEB5 reduced several malignant cellular features, including migration and colony formation of MDA-MB-231 cells. In conclusion, our findings suggest that *Sidnyum elegans* extract may represent a novel therapeutic approach for TNBC.

References

- [1] Fakhri, S., Abdian, S., Moradi, S. Z., Delgadillo, B. E., Fimognari, C., & Bishayee, A. (2022). Marine Compounds, mitochondria, and malignancy: a therapeutic nexus. *Marine Drugs*, 20(10), 625.
- [2] Imperatore, C., Luciano, P., Aiello, A., Vitalone, R., Irace, C., Santamaria, R., Li, J., Guo, Y., & Menna, M. (2016). Structure and Configuration of Phosphoeleganin, a Protein Tyrosine Phosphatase 1B Inhibitor from the Mediterranean Ascidian *Sidnyum elegans*. *Journal of Natural Products*, 79(4), 1144–1148.

12 Immunocompetent 3D pancreatic adenocarcinoma (PDAC) biochip model for preclinical drug testing

Alina Deipenbrock

Institute of Pharmaceutical Biology and Biotechnology, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Pancreatic ductal adenocarcinoma (PDAC) is the most common and lethal form of pancreatic cancer. One major cause for a fast disease progression is the presence of a highly fibrotic tumor microenvironment (TME) mainly composed of cancer-associated fibroblasts (CAF), and various immune cells, especially tumor-associated macrophages (TAM). To conclusively evaluate drug efficacy, it is crucial to develop in vitro models that can recapitulate the cross talk between tumor cells and the surrounding stroma. Here, we constructed a fit-for-purpose biochip platform which allows the integration of PDAC spheroids (composed of PANC-1 cells and pancreatic stellate cells (PSCs)). Additionally, the chip design enables dynamic administration of drugs or immune cells via a layer of human umbilical vein endothelial cells (HUVEC). As a proof-of-concept for drug administration, vorinostat, an FDA-approved histone deacetylase inhibitor for cutaneous T cell lymphoma (CTCL), subjected via continuous flow for 72 h, resulted in a significantly reduced viability of PDAC spheroids without affecting vascular integrity. Furthermore, dynamic perfusion with peripheral mononuclear blood cells (PBMC)-derived monocytes resulted in an immune cell migration through the endothelium into the spheroids. After 72 h of infiltration, monocytes differentiated into macrophages which polarized into the M2 phenotype. The polarization into M2 macrophages persisted for at least 168 h, verified by expression of the M2 marker CD163 which increased from 72 h to 168 h, while the M1 markers CD86 and HLA-DR were significantly downregulated. Overall, the described spheroid-on-chip model allows the evaluation of novel therapeutic strategies by mimicking and targeting the complex TME of PDAC.

13 Data-driven enabling technologies to guide targeted covalent inhibitor development

Holger Gohlke

Institute for Pharmaceutical and Medicinal Chemistry, Heinrich Heine University Düsseldorf & Institute of Bio- and Geosciences (IBG-4: Bioinformatics), Forschungszentrum Jülich

Targeted covalent inhibitors and covalent ligand-first approaches have become pivotal in drug design, with cysteines being key targets due to their nucleophilicity and rarity. Despite the abundance of structural biology and chemoproteomics data on cysteine ligandability, these datasets have remained largely disconnected. To address this, we developed

TopCysteineDB, a resource integrating structural data from the PDB with chemoproteomics data from activity-based protein profiling. Using *TopCovPDB*, an automated classification pipeline with manual curation, we identified 787 covalent cysteines and categorized other functional roles, such as metal- and cofactor-binding, and disulfide bonds. Structural data were mapped to sequence space, enabling a unified view of cysteine ligandability by cross-referencing with proteomics datasets. To complement this, we developed *TopCySPAL*, a machine learning model that combines structural features and proteomics data, achieving high predictive performance (AUROC: 0.964, AUPRC: 0.914) and generalizing well to novel cases. *TopCysteineDB* and *TopCySPAL* are accessible via the *TopCysteineDBApp* (<https://topcysteinedb.uni-duesseldorf.de>), which enables exploration of cysteine sites in the human proteome. By unifying previously fragmented datasets, this resource enhances the systematic analysis and prediction of cysteine ligandability, advancing the design of targeted covalent inhibitors.

14 Multi-targeting inhibitors on the sphingolipid pathway by introducing histamine H3 receptor pharmacophore

Jitheesh Joy

Institute for Pharmaceutical and Medicinal Chemistry, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

The sphingolipid pathway offers a possibility into targeting various disease implications including cancer. Sphingosine-1-phosphate and ceramides are key metabolites. Regulation of these metabolites could open ways to overcome cancer by inducing apoptosis and/or immunomodulation.

Here, we are targeting spinster homolog 2 (Spns2) as well as ceramide synthase 6 (CerS6) selectively via small molecule inhibition. Spns2 is an ABC transporter protein involved in the efflux of sphingosine-1-phosphate out of the cell, while specific ceramide synthases (CerSs) are involved in the biosynthesis of different ceramides from sphingosine. In a multi-targeting approach, we simultaneously target histamine H3 receptor by merging its antagonist neuroprotective and procognitive pharmacophore into the inhibitory sphingolipid motifs.

We are focusing on different lead structures having benzothiazole-based compounds as Spns2 inhibitors and oxy-fingolimod derivatives as CerS6 inhibitors

15 BceAB-type ABC transporters – Conservation, function & organization

Christian Mammen

Institute of Biochemistry I, Faculty of Mathematics and Natural Sciences, Heinrich Heine University Düsseldorf

Multidrug-resistance of human pathogens is a rising problem and finding new treatment options for these strains is of critical importance. Here, antimicrobial peptides such as nisin and bacitracin, which inhibit peptidoglycan synthesis by binding to lipid II or undecaprenyl-pyrophosphate, are very promising candidates. However, pathogens like *Streptococcus agalactiae* already developed resistance against these compounds by the expression of resistance proteins. These are the nisin resistance protein (NSR), an BceAB-type ABC-transporter SaNsrFP and a two-component system (TCS) (Khosa et al. (2016)). BceAB-

type transporters are found in many firmicutes (Ditner et al. (2011)) and feature an extracellular domain (ECD), that facilitates substrate specificity (Cho et al. (2021)).

16 Understanding NsrFP-NsrK resistance mechanism

Gürbüz Önder

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Antimicrobial peptides (AMPs) serve as a crucial defense mechanism by targeting and disrupting bacterial cells. However, many bacteria have evolved sophisticated resistance mechanisms to counteract these peptides. A well-characterized example is the BceAB-BceS system in *Bacillus subtilis*, which consists of a dedicated ABC transporter (BceAB) and a two-component regulatory system (BceSR). In this system, AMP recognition by BceAB induces conformational changes that are sensed by BceS, the histidine kinase, triggering a signal that leads to transporter upregulation.

In *Streptococcus agalactiae*, a homologous resistance mechanism is represented by NsrFP-NsrK, which confers resistance against a range of AMPs, including nisin and bacitracin. However, the molecular mechanism underlying resistance following AMP recognition remains unclear.

In this study, molecular modeling and molecular dynamics simulations were employed to elucidate the mechanism of AMP resistance, with a particular focus on how the signal is transmitted to the kinase. This will reveal the structural mechanism of signal transmission, thereby helping understanding the overall AMP resistance in *Streptococcus agalactiae*.

17 Multidisciplinary approach to unravel the biological space of cannabidiolic acid in glioblastoma cells

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Glioblastoma is an aggressive, fast-growing tumor refractory to conventional therapies. Since genetic and epigenetic variations confers aggressiveness increasing and therapeutic resistance, a combination of targeted therapies is strongly required. To this end, natural compounds are currently used to elucidate their mechanism of action thus allowing the identification of new potentially druggable proteins. Phytocannabinoids, a large class of plant metabolites, showed a wide panel of biological activities. Recent discoveries have revealed the anti-tumor properties of these molecules, thus qualifying them as privileged structures for biomedical research. However, the molecular mechanisms underlying their effects are still largely undescribed, particularly for acidic phytocannabinoids. The present work is focused on the identification of eukaryotic initiation translation factor 2A (EIF2A) as protein target of cannabidiolic acid (CBDA) in glioblastoma cells. Multidisciplinary approach consisting of cell-based (DARTS, CETSA, Co-IP, LiP, SILAC) and cell-free (SPR, Molecular Docking) assays was carried out. First, DARTS assay led to identification of the protein Eukaryotic Initiation Translation Factor 2A (EIF2A) as a potential protein target of CBDA. CETSA assay confirmed EIF2A-CBDA molecular interaction, thus demonstrating that the binding of EIF2A to CBDA is able to induce the stabilization of EIF2A towards thermal denaturation. Using SPR approach the direct interaction was confirmed, as inferred

from the dissociation constant (KD) of 6.3 μ M measured for the EIF2A/CBDA complex. To investigate the protein region involved in CBDA binding, MS-LiP and molecular dynamics approaches were performed thus showing that CBDA engages stable interactions with a stretch of residues in the C-terminal region, thereby inducing a significant conformational change in EIF2A through anchoring to the flexible α -helix 482–502 to the WD domain. Interestingly, Co-IP assay demonstrated that CBDA-EIF2A interaction is able to induce an increase of affinity interaction of EIF2A towards the main eukaryotic translation factors. Additionally, since EIF2A has been widely described as the main initiation translation factor, pulsed-SILAC based on AHA-click chemistry was carried out to investigate the impact of CBDA on the nascent proteome. Interestingly, CBDA treatment appeared to be able to induce a proteome remodeling by increasing the synthesis of molecular chaperones involved in the UPR response, intracellular vesicle formation/trafficking process and the ubiquitination process. These findings demonstrated that CBDA is able to interact with EIF2A by activating it and modulating its biological function in glioblastoma cells.

1st of April, 2025

18 Novel peptides and alkaloids from marine extremophiles with activity against drug-resistant pathogens

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The continuous outbreak of drug-resistant bacterial and viral infections imposes the need to search for new drug candidates. In this scenario, natural products from marine bacteria may still inspire the design of pharmaceuticals. Indeed, marine bacteria, especially extremophiles, have developed unique metabolic flexibility to inhabit each ecological niche, thus expanding their biosynthetic ability to assemble unprecedented molecules.

Genome mining and network analysis of metabolomes has emerged as a valuable strategy to enhance the bioassay-guided fractionation workflow and enable discovery of novel natural products from bacteria, as reducing the rediscovery rate, prioritizing isolation of new chemical entities and giving access to the biosynthetic tools to create sustainable sources of rare bioactive compounds.

Herein, we will show top-down and bottom-up strategies leading to the identification of antiviral and antibacterial molecules from marine extremophilic bacteria.

Integration of high-throughput screening, untargeted metabolomics and genome mining enabled the isolation of the antibiotic nobilamides from a deep-sea *Bacillus* sp., showing activity against multidrug-resistant *Staphylococcus* strains and other Gram-positive pathogens, including the foodborne pathogen *Listeria monocytogenes* [1].

Combining the OSMAC approach, the tandem mass spectrometry molecular networking analysis and the bioassay-guided fractionation unveiled the presence of novel imidazolium-containing phenethylamine derivatives, namely shewazoles, from *Shewanella aquimarina*. The shewazole mixture showed potent activity against drug-resistant bacteria and viruses [2].

Finally, the case study of lasso-peptides from *Novosphingobium* sp. PP1Y highlighted the bottom-up synthetic biology approach for improving antiviral peptide synthesis in heterologous host.

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19 Antimicrobial properties and potential applications of *Pseudomonas gessardii* M15 rhamnolipids

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Biosurfactants are amphiphilic compounds produced by microorganisms that are able to lower the surface tension between two phases. Rhamnolipids (RLs) are biosurfactants mainly produced by the *Pseudomonas* genus, possessing a huge range of different bioactivities, such as antimicrobial, antifungal, and antibiofilm. *Pseudomonas gessardii* M15 was isolated from Antarctic marine sediment, and its RLs mixture (M15RL) was characterized by mass spectrometry, revealing the presence of new RLs. The antimicrobial activity of M15RL was investigated against a panel of bacterial and viral human pathogens, exhibiting high activity against enveloped viruses, such as herpes simplex type 1 and SARS-CoV-2, and against Gram-positive bacteria, such as MRSA and other clinically isolated *Staphylococcus aureus* strains. Finally, M15RL was successfully used to functionalize cotton swabs and plastic surfaces as proofs of concept in applications for wound dressing and surface disinfectants against MRSA and enveloped viruses, respectively.

20 Repurposing the antispasmodic drug otilonium bromide and exploring its derivative as potential treatment options for tuberculosis

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Treating bacterial infections like tuberculosis has become increasingly challenging due to the growing emergence of multi-drug resistant strains. Consequently, new antibiotics with novel mechanisms of action are urgently needed to address this global threat. Drug repurposing offers a faster and more efficient strategy for discovering new antimicrobial agents. In this study, we found that the spasmolytic agent otilonium bromide, representing a quaternary ammonium compound, has promising anti-tubercular activity and very low resistance frequency. Further studies into the antimycobacterial activity of otilonium bromide revealed that the compound caused changes to the permeability of the bacterial membrane and membrane damage, indicating its potential mechanism of action. Additionally, whole genome sequencing of spontaneous otilonium bromide-resistant mutants unveiled the possible role of the *rv3160c-rv3161c* operon in its metabolism. Screenings of derivatives led to the discovery of a structurally related compound, with 64-fold higher antimycobacterial activity than otilonium bromide, minimal cytotoxicity to human cells, and possibly a different mechanism of action. Our findings support that otilonium

bromide and its derivative could be new antitubercular agents to combat the fight against tuberculosis.

21 Isolation and mode-of-action study of the anti-MRSA lead compound pestalotic acid A from *Pestalotiopsis chamaeropsis*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a serious challenge to global public health because of its resistance to common antibiotics. In this study, we isolated nine secondary metabolites from the endophytic fungus *Pestalotiopsis chamaeropsis*. Among them, Pestalotic Acid A showed strong anti-MRSA activity and low cytotoxicity, making it a promising lead compound. Preliminary mode-of-action tests suggest that Pestalotic Acid A may interfere with key bacterial processes, though more research is needed to clarify its exact mechanism. These findings highlight the potential of endophytic fungi as a source of novel anti-infective agents and show that Pestalotic Acid A has good prospects for developing new drugs to combat MRSA.

22 Labdane diterpene manool as a new promising antimicrobial molecule

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The scientific community is working to discover new antibiotics that target previously unexplored pathways (Lomenick et al., 2009). Manool is a labdane diterpene found in many plants (Moreira, M. R., et al., 2013) and showed a promising antimicrobial activity against *Streptococcus mutans*. To understand how this works, the bacteria treated with manool were studied, using chemical proteomics and Drug Affinity Responsive Target Stability. These methods revealed manool-interacting proteins of the ATP-binding cassette (ABC) superfamily, which is involved in the transport of molecules across cell membranes. To confirm this, amino acid and sugar uptake assays were performed using LC-MS and NMR analyses. Inhibition of ABC transporters by manool could improve the efficacy of other antibacterial drugs. To test this, the fractions were analyzed using LC-MS and FICI values for manool and kanamycin were measured. Studies on *S. mutans* treated with manool identified the primary pathways affected by diterpene treatment, opening up further research into how it works.

23 Comparative purification strategies and functional characterization of extracellular vesicles from *Lactobacillus rhamnosus* GG

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Bacterial extracellular vesicles (bEVs) are nano-sized, lipid membrane-delimited particles that transport bacterial-derived biological cargo. bEVs have attracted considerable interest across biomedical fields, with potential applications as vaccines, cancer immunotherapy agents, and drug delivery vehicles. This study aims to investigate the biological and functional implications of different isolation methods used to purify EVs from the Gram-positive bacterium *Lactobacillus rhamnosus* GG (LGG). Given the challenges associated with bEVs purification, two methods were compared: a widely used protocol based on precipitation from cell-free supernatant and a sequential approach combining size-based and density-based separation techniques. The isolated LGG EVs were then characterized using electron microscopy (EM), nanoparticle tracking analysis (NTA), TLR2-based reporter assay, and a comparative evaluation of their effect on *Helicobacter pylori*. Ultimately, this study aims to elucidate how the choice of purification method influences the biological and functional properties of EVs derived from this probiotic bacterium.

24 Selective targeting of class-specific HDACs in leukemia

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Currently approved histone deacetylase inhibitors (HDACis) for the treatment of hematological malignancies are predominantly pan-HDAC inhibitors, which non-selectively target multiple HDAC isoforms involved in essential cellular processes. This lack of specificity contributes to a broad spectrum of adverse effects, including myelosuppression, cardiotoxicity, and gastrointestinal disturbances, some of which can be severe or life-threatening. Therefore, the development of isoform-specific HDAC inhibitors represents a significant therapeutic advancement. Recently, we introduced novel isoform-selective HDAC inhibitors, 4d and 4m, specifically designed to overcome therapy resistance in leukemia cells while minimizing off-target toxicity. These inhibitors demonstrated superior anti-leukemic efficacy compared to clinically approved pan-HDAC inhibitors. Notably, 4d exhibited strong synergy with standard chemotherapeutic agents, favorable pharmacokinetic properties, and potent leukemia suppression in preclinical models. Further refinement of 4d's *in vivo* pharmacokinetics through a lead optimization program will be critical to maximizing its therapeutic efficacy and overcoming existing limitations, ultimately supporting its advancement as a promising candidate for the treatment of therapy-resistant acute myeloid leukemia (AML).

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25 Development of class IIa HDAC inhibitors

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Dysregulation in the expression of class IIa histone deacetylases (HDACs) has been linked to various forms of cancer, yet no drug specific for this subclass has been approved to date. Therefore, the development of novel inhibitors for these targets is of great importance.

Trifluoromethyl-1,2,4-oxadiazoles are an emerging class of HDAC inhibitors, that demonstrate high potencies in vitro and have an intrinsic subclass selectivity towards class IIa HDACs. Additionally, they show promising synergistic effects with proteasome inhibitors, inducing apoptosis in different cancer cell lines. The two lead structures for this project are NT160, the most potent, literature-known class IIa HDAC inhibitor, and FFK24, a very potent and selective inhibitor that has shown significant synergistic effects with the proteasome inhibitor bortezomib in leukemia cell lines.

Based on these two structures we aim to develop inhibitors with increased isoform selectivity to target specific class IIa HDACs, while retaining the potency and synergistic properties in an effort to create new candidates for further preclinical development.

26 Synthesis and development of dual HDAC-LSD1 inhibitors

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Cancer remains one of the most lethal diseases, with its vast diversity making it difficult to develop standardized treatments. Despite significant progress in understanding cancer's origins and hallmarks, individual and selective therapies are still limited. Epigenetic regulation of the genome and its malfunctioning plays a critical role in cancer development. Histone Deacetylases (HDACs) are epigenetic erasers whose misregulation is closely linked to the development of cancer. Overexpressed in various cancers, HDACs are a key target in cancer therapy. The currently five FDA-approved HDAC inhibitors lack isoform selectivity which leads to therapy limiting side effects and the urge for isoform selective HDAC inhibitors. Another promising target is Lysine-specific demethylase 1 (LSD1), an epigenetic actor whose dysregulation is linked to carcinogenesis and tumor progression. LSD1 is overexpressed in cancers such as breast, prostate, and lung cancer. Given cancer's complexity, new therapeutic strategies like dual inhibition—targeting two pharmacological targets simultaneously—have shown promise. Lead structures as JBI-097 and Corin are dual HDAC-LSD1 inhibitors and demonstrated promising results in solid and hematological tumors. The synthesis and development of dual HDAC-LSD1 inhibitors focuses on modifying the HDAC pharmacophore, particularly the zinc-binding group, to enhance isoform selectivity toward specific HDAC subclasses especially HDAC1 and HDAC6.

27 New drug combinations in lung cancer

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Lung cancer is divided into 2 main subtypes: around 80% non-small cell lung cancer and 20% small cell lung cancer. The 5-year survival rate is rather low (less than 15% for non-small cell lung cancer and 5% for small cell lung cancer), mainly due to intrinsic or acquired resistance against conventional and targeted therapy. Besides genetic aberrances such as activating mutations in receptor-tyrosine kinases, it is now widely known that epigenetic dysregulation is involved in the development of cancer and chemoresistance. Thus, the discovery of novel drug combinations is of high medical need to overcome therapy-limiting drug resistance. This project investigates novel combinations of epigenetic and targeted inhibitors, such as histone-deacetylase inhibitors (e.g. entinostat), inhibitors of bromodomain and extraterminal motif (BET) proteins (e.g. OTX15), the KRAS inhibitor BI2865, and EGFR-family targeting kinase inhibitors (e.g. afatinib). Synergistic effects were found and investigated and underlying mechanisms were analyzed, such as increase in p21 expression. In conclusion, a combination of epigenetic and non-epigenetic inhibitors may eventually increase treatment efficacy of lung cancer.

28 Targeting HDAC6 in therapy-resistant leukemia

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HDAC is often found overexpressed in different leukemia subtypes. In addition, epigenetic dysregulation of HDAC6 is highly associated with leukemogenesis.¹ The involvement of HDAC6 in deacetylating non-histone substrates and chaperone proteins closely correlates to cell migration, cell cycle and protein degradation, which has made HDAC6 an attractive antileukemic target. However, the emergence of resistance and poor prognosis to standard chemotherapy have been frequently reported.^{2,3} Thus it is critical to dissect the correlation between HDAC6 and oncogenic processes. To this end, on the one hand, we generated HDAC6 gene knockout leukemic cell line and perform functional assays in *in vitro* and *in vivo* set ups, on the other hand, we pre-clinically testing and targeting HDAC6 in therapy resistant leukaemia in mouse models. Outlook on our future research would be investigation of the immunoregulatory potential of HDAC6 deactivation *in vitro* and *in vivo*, as well as looking for synergistic partners of HDAC6 inhibition.

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29 Modulation of RNASET2 by HDAC6 and its effect on tumor-associated macrophages

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In eukaryotic cells, transcriptional activity depends on DNA's packaging state. In its resting state, DNA is tightly compacted as condensed chromatin with restricted access for transcription factors. Accessibility to the DNA is increased through nucleosome modifications, such as the addition of methyl or acetyl groups, which influence gene expression. Histone deacetylases (HDACs) play a crucial role by working in the opposite direction, removing acetyl groups and returning the chromatin to its more condensed state. Among these, HDAC6 is unique as it predominantly resides in the cytoplasm, where it deacetylates cytoplasmic proteins and is implicated in various cancers.

Recent work by our collaborators revealed that HDAC6 knockout (KO) in K562 Chronic Myelogenous Leukemia (CML) cells leads to elevated expression of RNaseT2, a tumor suppressor protein. RNaseT2, suppresses tumorigenesis by recruiting monocyte/macrophage lineage cells to the tumor microenvironment (TME). While RNaseT2's role in macrophage migration is known, its influence on macrophage polarization and associated mechanisms remains unclear.

30 Hydrazide-based HDAC inhibitors completely reverse chemoresistance synergistically in platinum-resistant solid cancer cells

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This project investigated a set of peptoid-based histone deacetylase inhibitors (HDACi) with different linkers and zinc-binding groups obtained from our collaboration partner AG Finn Hansen, Bonn. In the first part, compounds were tested for inhibition of recombinant HDAC enzymes. Subsequently, all compounds were investigated for their cytotoxic activity alone and in combination with cisplatin in native and cisplatin-resistant cancer cell lines. Interestingly, whereas the ethyl-substituted hydrazide derivative DS-103 showed nanomolar potency at HDAC1-3 and HDAC6, an extension of the alkyl linker to propyl or butyl (resulting in compounds 5 and 6) led to nanomolar potent class I (HDAC1-3)-selective HDACi. Furthermore, compounds DS-103, 5 and 6 outperformed entinostat in cytotoxicity assays and were not only able to reverse chemoresistance in cisplatin-resistant A2780CisR (ovarian) and Cal27CisR (head-neck) cancer cell lines, but also increased cisplatin potency in the native cell lines. DS-103, 5 and 6 showed the strongest cisplatin resistance reversal observed by our groups so far. Effects were highly synergistic as analyzed by Chou-Talalay, resulting in combination index values of < 0.2 , again outperforming entinostat. In addition, DS-103, 5 and 6 increased synergistically DNA damage, and increased p21 and pro-apoptotic BIM expression thus leading to caspase-mediated apoptosis and cell death. In conclusion, compounds DS-103, 5 and 6 represent promising lead structures for developing new HDACi capable of reversing chemoresistance in platinum resistant cancer cells.

31 Development of selective HDAC inhibitors targeting the CoREST protein complex

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Histone deacetylases (HDACs) are major drug targets for treating cancer, neurodegenerative diseases, cardiac hypertrophy, and numerous other medical disorders. Since most HDAC inhibitors (HDACis) exhibit an unfavorable side effect profile due to their lack of selectivity towards isoforms, the development of selective inhibitors will be a distinct step innovation. However, due to the structural similarity of the HDAC's zinc-binding pockets, the development of isoform-selective HDACis has turned out to be challenging. To overcome this limitation, we targeted the protein-protein interaction surfaces where HDACs bind to their protein partners in complexes. We focused on the CoREST complex, which contains both HDACs and lysine-specific demethylase 1 (LSD1). Using computational approaches, we systematically characterized the protein-protein interaction interfaces in the CoREST complex to identify potential binding sites. These interaction interfaces have unique structural and chemical features that should enable the design of highly specific inhibitors that disrupt critical protein-protein interactions. This approach could improve HDAC-targeted therapies by increasing drug selectivity and reducing side effects. Our findings lay the groundwork for developing more effective and safer HDAC inhibitors that target the CoREST complex.

32 Identification of autophagy inhibitors selectively targeting the ATG13-ATG101 protein-protein interaction

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The dysregulation of autophagy promotes the development of various diseases like neurodegeneration, infections, metabolic syndromes or cancer. In order to maintain their metabolic needs under nutrient- and/or oxygen-depleted conditions typically found in the tumour microenvironment, cancer cells can upregulate autophagy autonomously or within the tumour microenvironment. Therefore, inhibition of autophagy is desirable in these settings. To date, however, there are few drugs that selectively target autophagy. The autophagy-inducing ULK1 complex comprises ULK1, a heterodimer consisting of ATG13 and ATG101, and FIP200. Our group has previously reported that the protein-protein interaction (PPI) between ATG13 and ATG101 is essential for both the assembly of the ULK1 complex and cellular autophagy activity. Here, we describe the identification of compounds that selectively inhibit autophagy by targeting this PPI. We propose that these compounds represent useful tools for basic research as well as lead structures for the development of therapeutically valuable small molecules

33 Characterization of autophagy-modulating HSP90 inhibitors for the elimination of therapy-resistant tumor cells

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Resistance to cancer therapy has become one of the most important problems in the treatment of cancer. Since the identification of heat shock protein 90 (HSP90) as potential cancer target, HSP90 inhibition has emerged as a promising therapeutic strategy. Up to date, mainly HSP90 inhibitors (HSP90i) of the N-terminal domain have been developed and evaluated in clinical trials. However, they display a limited efficacy due to dose-limiting cytotoxicity and the induction of a cytoprotective heat-shock response (HSR). Accordingly, inhibitors that target the C-terminal domain (CTD) of HSP90 and do not trigger an HSR have been proposed. In this study, we report that the novel small-molecule inhibitor VWK147 targets HSP90 CTD dimerization and causes the destabilization of classical HSP90 client proteins without inducing an HSR. Treatment with VWK147 triggers cell death in both cisplatin-sensitive and cisplatin-resistant urothelial carcinoma cells (UCCs). Furthermore, we demonstrate that VWK147 re-sensitizes resistant UCCs to cisplatin. We hypothesize that these cytotoxic effects of VWK147 are also mediated in part by the inhibition of late stages of autophagy.

Recently, an additional HSP90 CTD dimerization inhibitor (VWK141) has been included in the study. We were able to show that both inhibitors induce the conjugation of ATG8 proteins to single membranes (CASM). For VWK141, we could confirm that the induction of CASM is associated with lysosomal dysfunction. Hence, we hypothesize that the anti-cancer effect of both novel small-molecule inhibitors are partly related to their autophagy-modulating properties. In the case of VWK141, these are mainly mediated by the disruption of lysosomal function.

34 Plant extracellular vesicles: a new frontier in natural nanomedicine

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Plant extracellular vesicles (PEVs) are emerging as a promising non-mammalian alternative in nanomedicine, offering a natural and efficient system for drug delivery. Beyond their essential role in plant physiology (e.g. plant defence, cell wall remodelling and cross-kingdom communication), PEVs have the remarkable ability to transport bioactive molecules into human cells, opening new avenues for therapeutic applications. Furthermore, advances in bioengineering could enhance their capacity to deliver nucleic acids and selectively target specific cells, expanding their potential in precision medicine. However, the biomolecular composition of PEVs can vary significantly due to factors such as soil conditions, agricultural treatments, and fertilization practices, as well as genetic differences among plant species. This variability presents a major challenge in standardizing PEV isolation and application.

To overcome these hurdles, our research team has adopted plant biotechnology platforms to establish reliable protocols for isolating, characterizing, and analysing PEVs at the biophysical and multiomics levels. Our studies focus on EVs derived from plant cell cultures

and hairy roots (HRs) of various species, including both medicinal plants and crops. Our findings reveal that PEVs, particularly those derived from HRs, possess significant health-promoting properties. Notably, PEVs from *Salvia dominica* have demonstrated potent anticancer effects, effectively suppressing the growth of pancreatic and breast cancer cells by inducing apoptosis. Additionally, these vesicles exhibit strong neuroprotective properties, showing promise in models of Parkinson's disease. Ongoing research aims to optimize these therapeutic capabilities through bioengineering, enhancing their effectiveness and specificity. Overall, these discoveries mark a major step forward in utilizing PEVs for nanobiotechnology, paving the way for sustainable and eco-friendly innovations in nanomedicine.

35 Cardoon cell cultures as a biofactory for extracellular vesicles with antisteatotic activity

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Extracellular vesicles (EVs) are membrane-bound nanoparticles naturally released by cells, playing a crucial role in intercellular communication. While mammalian-derived EVs have been extensively studied for biomedical applications, challenges related to safety, scalability, and production costs have driven the search for alternative sources. Plant-derived EVs (PDEVs) have emerged as a promising solution, offering advantages such as biocompatibility, low immunogenicity, and ease of large-scale production.

This study investigates *Cynara cardunculus* var. *Atilis* cell suspension cultures (CSCs) as a novel biotechnological platform for EV production. Proteomic and metabolomic analyses revealed an enrichment of bioactive proteins and secondary metabolites with antioxidant and anti-inflammatory properties. In a HepG2 cell model of non-alcoholic fatty liver disease (NAFLD), cardoon EVs reduced reactive oxygen species (ROS) and nitric oxide (NO) levels, improved cell viability, and decreased lipid accumulation, demonstrating effects comparable to metformin. These findings position cardoon-derived EVs as a promising natural therapeutic for metabolic disorders. By advancing the understanding of plant-derived EVs, this study underscores their potential applications in nanomedicine, biotechnology, and functional food development.

36 A journey into the chemistry of cyclic adenosine diphosphate ribose (cADPR), a second messenger isolated from sea urchin eggs with potent Ca²⁺-modulating activities

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In biological systems, metal ions play pivotal roles as catalysts and structural regulators. Among them, calcium ions (Ca²⁺) regulate a wide array of intracellular processes in all living organisms. A key second messenger involved in Ca²⁺ mobilization is cyclic adenosine diphosphate ribose (cADPR), an 18-membered cyclic nucleotide first isolated from sea urchin egg extracts. Disruptions in cADPR production and calcium homeostasis have been

implicated in conditions such as diabetes, airway hyper-responsiveness, and autism. Moreover, cADPR-mediated Ca^{2+} dysregulation plays a significant role in neuroblastoma, a malignant tumor arising from neural crest cells. However, the chemical instability of cADPR in neutral aqueous solutions limits its full characterization. To address this challenge, stable cADPR analogues have been synthesized through chemo-enzymatic and total synthetic approaches, many of which exhibit promising Ca^{2+} -mobilizing properties. In this study, we report further chemical improvements of the cADPR structure and provide new insights into structure-activity relationship (SAR) studies. [1–3]

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