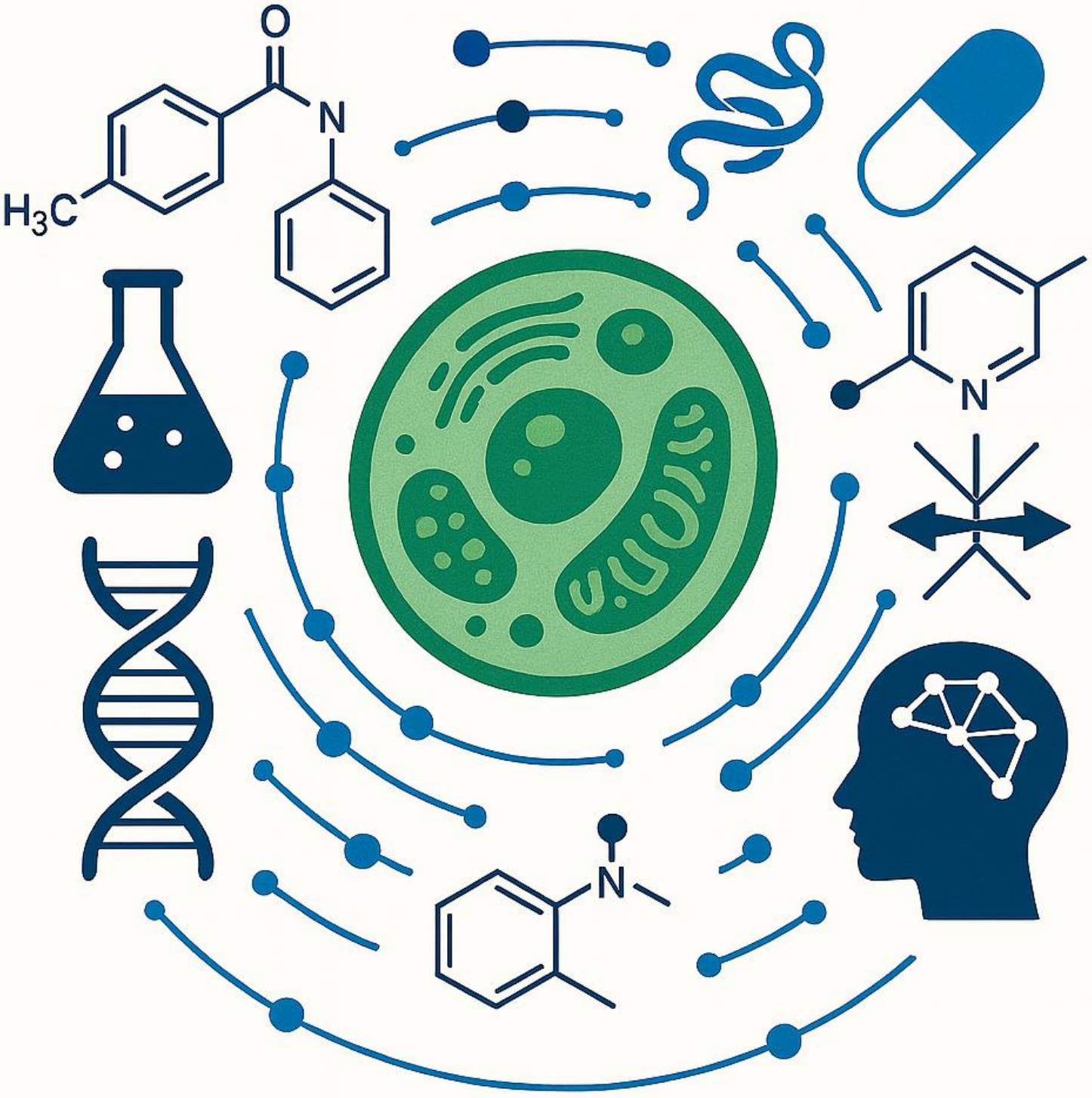
BOOK OF ABSTRACTS

CROSSING THE BOUNDARIES OF MEDICINAL CHEMISTRY



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Welcome to the Crossing the Boundaries of Medicinal Chemistry minisymposium

The Section for Medicinal Chemistry of the Slovenian Pharmaceutical Society, the University of Ljubljana, Faculty of Pharmacy, and EATRIS (Node Slovenia) are delighted to welcome you to the second edition of the minisymposium on medicinal chemistry and related disciplines, Crossing the Boundaries of Medicinal Chemistry!

It has been both a privilege and a pleasure to design this year's scientific programme and carefully select the speakers who will join us. The symposium brings together a broad spectrum of topics, showcasing the latest advances in medicinal chemistry as well as insights from neighboring research areas. Our aim is to foster dynamic discussions, spark new ideas, and create opportunities for meaningful exchange. We hope the programme will not only capture your interest but also inspire and support your future endeavors.

The symposium takes place in person, offering the invaluable chance to connect face-to-face, share ideas directly, and strengthen professional and personal ties. Beyond the lectures, we are particularly excited about the poster session - accompanied by refreshments - which we envision as a welcoming space for dialogue, collaboration, and networking.

On behalf of the organizing team, we extend a warm welcome to the Faculty of Pharmacy, University of Ljubljana. We look forward to engaging with you and making this symposium a memorable and fruitful experience for all.

With best regards,
Žiga and Tihomir

Timeline	Lecturer and title of talk
9.00 – 9.05	*Opening Address* **Žiga Jakopin and Tihomir Tomašič** Faculty of Pharmacy, University of Ljubljana, Slovenija
9.05 – 9.30	*Extracellular Flux-Based Metabolic Profiling in Pharmacology: Quantifying Cellular Bioenergetics in Real-Time* **Lovro Žiberna** Faculty of Pharmacy, University of Ljubljana, Slovenia
9.30 – 9.55	*Carbamoyl fluorides - from activity-based protein profiling to enzyme inhibitors* **Anže Meden** Faculty of Pharmacy, University of Ljubljana, Slovenia
9.55 – 10.25	*In vitro cellular models for investigating neuroinflammation and neurodegeneration: A case study for evaluating molecules with potential therapeutic action* **Selena Horvat, Anja Pišlar** Faculty of Pharmacy, University of Ljubljana, Slovenia
10.25 – 10.50	*Beyond Beta: the clinical potential of alpha emitting radiopharmaceuticals* **Marko Krošelj** Faculty of Pharmacy & Faculty of Medicine, University of Ljubljana, Slovenia
10.55 – 12.00	*Coffee break, snacks and poster presentation*
12.00 – 12.25	*Adventures in Fluorescence: New Probes, Unexpected Discoveries and Bright Innovations* **Stane Pajk** Faculty of Pharmacy, University of Ljubljana, Slovenia
12.25 – 12.50	*Nanomolar inhibitor of the galectin-8 N-terminal domain binds via a non-canonical cation-π interaction* **Edvin Purić** Faculty of Pharmacy, University of Ljubljana, Slovenia
12.50 – 12.55	*Closing Address*

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Invited Lectures

EXTRACELLULAR FLUX-BASED METABOLIC PROFILING IN PHARMACOLOGY: QUANTIFYING CELLULAR BIOENERGETICS IN REAL TIME

Lovro Žiberna

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Cellular energy metabolism lies at the core of all physiological and pharmacological processes, integrating mitochondrial oxidative phosphorylation and glycolysis to sustain ATP production and metabolic homeostasis. Pharmacological agents, whether designed to target metabolism or not, frequently influence these pathways, altering cellular bioenergetics in ways that can reveal mechanisms of action, identify novel therapeutic targets, or predict toxicological liabilities. In this lecture, I will present the application of extracellular flux analysis, primarily through Seahorse XF technology, as a quantitative, real-time tool to study drug-induced effects on cellular energy metabolism in intact living cells.

The Seahorse XF platform simultaneously measures two key extracellular fluxes: the extracellular acidification rate (ECAR) and the oxygen consumption rate (OCR). ECAR is expressed as change in milli-pH units per minute, and was historically used as a surrogate for glycolytic activity, reflecting the acidification of the extracellular medium due to the excretion of lactic acid and protons during anaerobic glycolysis. However, ECAR also includes contributions from (i) CO_2-derived acidification resulting from mitochondrial respiration, as CO_2 is rapidly hydrated to bicarbonate; and (ii) protons extruded through other metabolic and transport processes. To obtain a more biochemically accurate measure of glycolytic flux, ECAR must be corrected for buffering capacity and respiratory acidification. This correction yields the proton efflux rate (PER), which can be further partitioned into glycolytic PER (attributable to lactate/H^+ co-export) and mitochondrial PER (reflecting CO_2 hydration). OCR is expressed as change in picomoles of O_2 per minute per well (typically normalized to cell number or protein content), and reflects the rate of mitochondrial oxygen consumption, primarily driven by electron transport chain activity and coupled to ATP production. When used in parallel, OCR and PER enable quantitative evaluation of glycolytic versus oxidative contributions to cellular bioenergetics. Standardized perturbation protocols, such as the mitochondrial stress test and glycolytic rate assay, allow derivation of discrete bioenergetic parameters including basal respiration, ATP-linked respiration, proton leak, spare respiratory capacity, maximal glycolytic capacity, and glycolytic reserve. These high-resolution flux measurements provide dynamic, pathway-specific insights into cellular energy metabolism under both basal and drug-modulated conditions.

In pharmacological research, extracellular flux-based profiling provides mechanistic resolution into diverse drug effects on energy metabolism. For oncological drug candidates, Seahorse assays are used to screen compounds that exploit the Warburg phenotype, such as glycolytic inhibitors (e.g., 2-deoxyglucose, LDH inhibitors) that reduce glycolytic flux and induce energetic stress in glycolysis-addicted tumor cells. Conversely, mitochondria-targeting agents (e.g., complex I inhibitors, mitochondrial ROS inducers, or Bcl-2 antagonists) are assessed for their capacity to impair mitochondrial respiration, trigger mitochondrial permeability transition, and induce apoptosis. Seahorse profiling is also instrumental in characterizing metabolic modulators, such as PPAR agonists, AMPK activators, and substances that rewire substrate oxidation patterns (e.g., shifting from glucose to fatty acid oxidation or enhancing glutamine metabolism). Furthermore, real-time flux analysis reveals off-target effects of drugs

not originally developed as metabolic modulators—such as psychotropic agents and antivirals—with consequences for therapeutic activity and safety. These insights directly inform pharmacodynamic characterization, and support revealing the compound mechanisms of action in phenotypic screens.

In toxicology research, extracellular flux assays have become increasingly central to early drug safety evaluation, particularly for identifying compounds with potential to induce mitochondrial dysfunction, a key contributor to adverse drug reactions including drug-induced liver injury (DILI), myopathies, and cardiotoxicity. Mitochondria are highly susceptible to pharmacological insult due to their dependance on finely tuned respiratory chain activity, membrane potential maintenance, and substrate oxidation pathways. In this perspective, Seahorse-based assays allow detection of both acute mitochondrial impairments (e.g., inhibition of electron transport complexes, uncoupling, ATP synthase blockade) and subacute or adaptive changes (e.g., loss of spare respiratory capacity, increased proton leak, compensatory glycolysis). For instance, compounds such as valproate, chlorpromazine, or amiodarone showed characteristic alterations in OCR and ECAR consistent with mitochondrial toxicity, even at subcytotoxic concentrations. By incorporating dose–response profiling and testing in metabolically relevant cell types (e.g. hepatocytes, cardiomyocytes), these assays offer functional biomarkers for safety classification. Importantly, mitochondrial liabilities can be mechanistically subclassified, e.g., complex I inhibition, uncoupling, impaired substrate import. This information can facilitate rational structure-based optimization by medicinal chemists to mitigate risk. This functional approach complements traditional cell viability assays and is increasingly recommended as part of integrated preclinical safety testing pipelines by several regulatory and industry consortia. Moreover, the ability to correlate early flux-based indicators with long-term outcomes (e.g., cell death, ROS generation, ATP depletion) makes Seahorse XF a powerful mechanism-based toxicology platform that bridges *in vitro* safety assessment with anticipated *in vivo* responses.

In conclusion, extracellular flux-based metabolic profiling provides a functional bridge between molecular pharmacology and whole-cell physiology. Its capacity to deliver mechanistic, quantitative, and translational insights makes it as an essential tool in modern drug discovery, pharmacodynamic profiling, and preclinical safety assessment.

CARBAMOYL FLUORIDES - FROM ACTIVITY-BASED PROTEIN PROFILING TO ENZYME INHIBITORS

Anže Meden,[1] Damijan Knez,[1] George Randall,[2] Kaveri Vaidya,[3] Maša Zorman,[1] Anja Pišlar,[1] Siddhesh S. Kamat,[3] Matthias Fellner,[2] Stanislav Gobec[1]

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Carbamoyl fluorides (CFs) are organofluorine compounds with promising and untapped potential in medicinal chemistry and chemical biology, which is hampered by the scarcity of straightforward and benign synthetic methods. We recently disclosed a mild three-step procedure for their synthesis that avoids exotic, corrosive, and highly toxic reagents.[1] As expected, these carbamate-like compounds were potent inhibitors of cholinesterases. Next, CF-containing biotinylated probe was used to determine other proteomic targets of this warhead. Among others, a poorly-characterized serine hydrolase (SH) ABHD14B with unique acetyltransferase activity[2] was identified. Since no small-molecule inhibitors or pharmacological tools exist for ABHD14B, a medicinal chemistry project towards identification and optimization of CF inhibitors of ABHD14B was initiated. In recent years, intense research has focused on bacterial SHs, which are largely unexplored but hold large potential for diagnostics or treatment of infections.[3] CFs from our in-house compound library were identified as potent inhibitors of FphI SH from *Staphylococcus aureus* by biochemical assays and X-ray crystallography. Last but not the least, the screening of the CF library against proinflammatory caspase-1 surprisingly revealed that some CFs inhibit this catalytic Cys-containing protease, which is involved in neurodegeneration. Although being reactive electrophiles, these examples show that CFs can developed into useful enzyme inhibitors and activity-based probes.

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IN VITRO CELLULAR MODELS FOR INVESTIGATING NEUROINFLAMMATION AND NEURODEGENERATION: A CASE STUDY FOR EVALUATING MOLECULES WITH POTENTIAL THERAPEUTIC ACTION

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Neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis are driven by shared pathological mechanisms, notably chronic neuroinflammation and progressive neuronal loss. Translating novel chemical entities into clinically relevant neurotherapeutics requires preclinical testing in models that are both mechanistically informative and physiologically relevant. To this end, we have developed a panel of *in vitro* cellular systems that enable the targeted evaluation of compounds with potential neuroprotective and anti-inflammatory activity. Our models combine differentiated neuronal cultures with glial cells, including activated microglia, reactivated astrocytes, and differentiated oligodendrocytes. Mono- and co-culture approaches enable the controlled characterization of neuron–glia crosstalk under inflammatory or degenerative stressors, such as amyloid-β oligomers. As a proof-of-concept, we evaluated γ-enolase, a neuron-specific glycolytic enzyme with neurotrophic properties, and its regulation by cathepsin X, a cysteine protease that removes its C-terminal neuroprotective motif. Two candidate therapeutic approaches were tested: γ-enolase C-terminal mimetic peptides and the cathepsin X inhibitor AMS36. Under inflammatory and amyloid-β exposure, both treatments enhanced neurite outgrowth, improved neuronal survival, and reduced the release of pro-inflammatory mediators. These experimental models provide mechanistic, high-content screening platforms that support the design and optimization of neuroprotective agents. By directly linking molecular targets to cellular phenotypes, they facilitate structure–activity relationship analyses, accelerate hit-to-lead development, and minimize reliance on animal experimentation in early-stage neurodegenerative drug discovery.

BEYOND BETA: THE CLINICAL POTENTIAL OF ALPHA EMITTING RADIOPHARMACEUTICALS

Marko Krošelj

Faculty of Pharmacy & Faculty of Medicine, University of Ljubljana, Slovenia

Radiopharmaceuticals have become essential tools in both diagnostic imaging and targeted radionuclide therapy, offering a unique approach to managing various malignancies. This presentation will provide an overview of the foundational principles of radiopharmaceuticals, followed by an introduction to the theranostic paradigm that underpins modern nuclear medicine. Theranostic molecules enable the integration of diagnostic and therapeutic capabilities within a single molecular platform, paving the way for personalized, precision oncology.

To date, beta-emitting radiopharmaceuticals—such as those labelled with lutetium-177 (^{177}Lu) or yttrium-90 (^{90}Y)—have demonstrated considerable clinical success in treating neuroendocrine tumours and prostate cancer, among others. Their relatively long particle range and moderate energy deposition allow treatment of larger or less well-defined lesions, though often at the cost of increased toxicity to surrounding healthy tissue. Limitations in their efficacy against micro metastatic or hematologic disease, along with the potential for off-target effects, have prompted the exploration of alternative approaches.
Alpha-emitting radiopharmaceuticals represent a promising advancement in targeted radionuclide therapy. Characterized by high linear energy transfer (LET) and a short tissue penetration range, alpha particles can deliver highly cytotoxic doses to tumour cells with minimal collateral damage. Clinical and preclinical studies have shown encouraging results in hematologic malignancies, bone metastases, and micro metastatic disease. Radiopharmaceuticals, labelled with actinium-225 (^{225}Ac), astatine-211 (^{211}At) and lead-212 (^{212}Pb), have demonstrated proof-of-concept for effective tumour control in preclinical and some also in clinical setting.

However, the clinical integration of alpha therapies faces several challenges. These include the limited availability of suitable alpha-emitting radionuclides, complex radiochemistry, short half-lives, recoil daughter effects, and the need for more comprehensive dosimetric models. Regulatory, logistical, and production barriers further complicate routine adoption in most healthcare settings.
This talk will explore the biological and physical rationale for alpha therapy, contrast it with established beta radiotherapies, and examine the current clinical landscape, research frontiers, and future directions in the field. As nuclear medicine continues to evolve, alpha-emitters may redefine the standard of care for selected patient populations, particularly those with minimal residual disease or refractory tumours.

ADVENTURES IN FLUORESCENCE: NEW PROBES, UNEXPECTED DISCOVERIES AND BRIGHT INNOVATIONS

Stane Pajk,[1] Natalija Trunkelj,[1] Janez Mravljak,[1] Aljoša Bolje,[1] Jakob Kljun,[2] Hana Kokot,[3] Andrej Emanuel Cotman,[1] Urša Pečar Fonović,[1] Tihomir Tomašič,[1] Andrej Emanuel Cotman,[1] Lucija Peterlin Mašič[1]

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In recent years, multiplexing in fluorescence imaging has become a major focus. Multiplexing involves the simultaneous use of several specific fluorescent probes with distinct excitation and/or emission properties on the same sample. While a broad palette of probes now spans the entire visible and near-infrared spectrum, there remains a shortage of probes with a large Stokes shift. Such probes enable, for example, the simultaneous excitation of two fluorophores and their selective detection using different emission filters, provided they emit at sufficiently separated wavelengths.

Unlike modern drug discovery, where rational design prevails, fluorophore development still depends heavily on serendipity. This was the case in our discovery of nitrogen bridgehead-fused pyridine fluorophores, which were first obtained as side products during the synthesis of an antitubercular drug. Their true structure was revealed by X-ray diffraction. After optimization and synthesis of analogues, we found these compounds to possess large Stokes shifts, pH sensitivity, and utility as lysosome turn-on probes. However, their limited functionalization prompted a shift toward coumarins, a class with well-established structure–photophysical relationships. Starting from 3-acetylcoumarin, we succeeded in red-shifting excitation/emission maxima and introducing functional handles. To our surprise, X-ray analysis revealed that the products were merocyanines, arising from an unanticipated coumarin rearrangement. Several analogues were prepared, with some proving excellent lipid droplet probes.

Because these merocyanines displayed only modest Stokes shifts, we next explored coumarins bearing electron-withdrawing groups at position 4, especially CF_3. As predicted, these provided sufficiently large Stokes shifts. After evaluating synthetic strategies, sulfonamide proved the most effective functionalization handle. This platform enabled the development of a series of probes for mitochondria, lysosomes, membranes, and other cellular targets.

NANOMOLAR INHIBITOR OF THE GALECTIN-8 N-TERMINAL DOMAIN BINDS VIA A NON-CANONICAL CATION-π INTERACTION

Edvin Purić,[1] Tihomir Tomašič,[1] Mojca Pevec,[2] Jurij Lah,[2] Hakon Leffler,[3] Anders Sundin,[4] Ulf J. Nilsson,[4] Derek T. Logan,[5] Marko Anderluh[1]

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Galectin-8 plays an important role in both innate and adaptive immune responses as well as in the regulation of cancer progression and metastasis [1], making it an attractive target for the development of highly selective and potent galectin-8 inhibitors. We have designed focused libraries of 2-*O*-substituted D-galactosides that bind to the N-terminal domain of galectin-8, starting from lead **1** (***Figure 1A***) [2,3]. All compounds were docked to the selected galectin-8N crystal structure (PDB ID: 7AEN) using Glide to explore the possibility of 2-*O*-substituents that can make favourable contacts with the protein and help select candidates for synthesis and biochemical evaluation. Binding affinities were determined by a competitive fluorescence polarisation (FP) assay and isothermal titration calorimetry (ITC). This approach led us to selective nanomolar inhibitors of galectin-8N. We solved a co-crystallized structure of a selected galectin-8 inhibitor **11** (***Figure 1B***) in complex with the protein (PDB ID: 9FYJ), providing structural insights into the interactions of the substituents at position 2. Further thermodynamic analysis revealed important differences in enthalpic and/or entropic contributions to binding. Finally, an energy decomposition analysis was performed, which showed that a bonding interaction between galectom-8 Arg45 and acetylene of **11** occurs via a new molecular orbital, indicating the presence of an unanticipated non-canonical cation-π interaction.

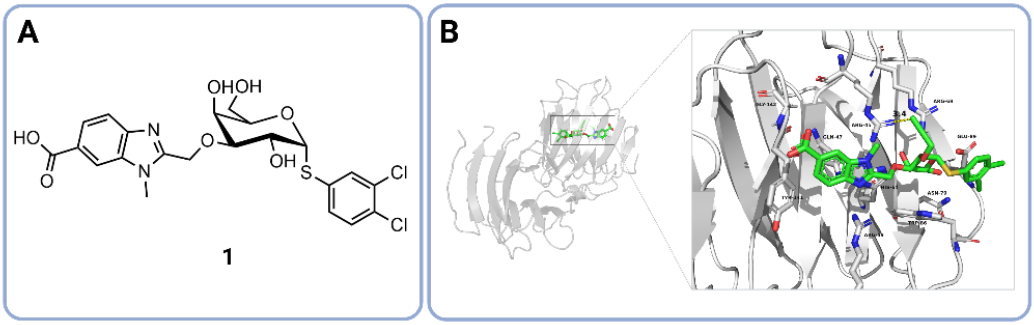

Figure 1. *(**A**) The structure of lead **1**. (**B**) A crystal structure of **11** in complex with galectin-8N.*

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Poster Presentations

STEREOSELECTIVE SYNTHESIS OF FLUORINATED CYCLIC SULFAMIDATES AND THEIR APPLICATIONS

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In recent decades, the synthesis of fluorinated organic compounds has experienced remarkable growth[1]; however, the asymmetric synthesis of chiral fluorinated molecules with multiple stereogenic centers remains a major challenge. One of the most promising methods for the stereoselective preparation of such compounds is asymmetric transfer hydrogenation (ATH), coupled with dynamic kinetic resolution (DKR), using ruthenium(II) or rhodium(III) Noyori–Ikariya catalysts.

Using soluble Noyori–Ikariya catalysts, formic acid as a hydrogen source, and a weak organic base as an epimerization promoter, we have developed an efficient method for the preparation of enantiomerically pure CF_3-substituted cyclic sulfamidates. The reaction conditions were first optimized on a model 4-phenyl-substituted oxathiazole and subsequently extended to a range of substrates, including variously substituted aromatic and heteroaromatic compounds as well as more challenging aliphatic derivatives. The strong electron-withdrawing effect of the CF_3 group enabled efficient epimerization even with a weak base, which significantly facilitated the DKR process. The synthetic route is versatile and can be applied in different contexts. It enables the preparation of dipeptides as well as analogues containing an amide group. The sequence proceeds through the opening of the sulfamidate ring with an azide, followed by reduction of the azide to the corresponding amine using palladium on carbon. The resulting amine is then alkylated with a suitable bromo derivative of an amino acid. As a demonstration, we successfully prepared a glycine derivative by employing ethyl bromoacetate as the electrophile.

The chiral backbones can be also used to prepare the analogues of established catalysts for asymmetric synthesis such as the Takemoto's thiourea catalyst[2], Dixon's iminophosphorane superbase catalyst,[3] Birman's benzotetramisole acyl-transfer catalyst[4,5] and Noyori–Ikariya catalyst for asymmetric transfer hydrogenation[6]. In particular, as a proof of concept, an analogue of the archetypal RuCl(p-cymene)(Ts-DPEN) with our new backbone outperformed the parent catalyst for the reduction of acetophenone in terms of enantioselectivity and retained its turnover frequency. The presented method provides a robust platform for the preparation of complex, chiral fluorinated building blocks with potential applications in the development of bioactive molecules and pharmaceutical agents.

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EXPLORING OSMI-4-DERIVED PROTACS FOR THE DEGRADATION OF O-GLCNAC TRANSFERASE AND REGULATION OF O-GLCNACYLATION LEVELS IN CELLS

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Glycosylation represents the most prevalent and structurally diverse post-translational modification (PTM), playing a crucial role in numerous cellular processes.[1] The addition of *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) to serine and threonine residues on cytosolic, nuclear, and mitochondrial proteins is a key PTM. This reversible process is tightly and dynamically regulated by the enzymes *O*-GlcNAc transferase (OGT) and *O*-GlcNAcase (OGA).[2] Elevated OGT activity has been associated with several pathological conditions, including cancer, diabetes, and neurodegenerative diseases, which has spurred interest in the development of small-molecule OGT inhibitors.[2,3] OSMI-4, a low nanomolar OGT inhibitor, failed to effectively inhibit OGT due to rebound enzyme expression after prolonged treatment, hindering the potential for a sustainable therapeutic effect.[4] Thus, robust molecular tools—such as fluorescent probes or PROTAC-based degraders—are needed to further dissect OGT function.

PROTACs are bifunctional molecules that harness the ubiquitin–proteasome system to trigger selective protein degradation. They consist of three elements: an E3 ligase ligand, a ligand that binds the protein of interest (POI), and a linker connecting the two.[5] Their mechanism of action allows PROTACs to degrade target proteins with high potency and selectivity, often at low concentrations. In this work, we designed and synthesized three sets of chimeric molecules, each coupling an OSMI-4 derivative to ligands for cereblon, von Hippel–Lindau (VHL), or inhibitor of apoptosis (IAP) proteins. We investigated their binding affinity toward OGT as well as their capacity to promote OGT degradation. Furthermore, the proteome-wide effects, influence on cell growth, and impact on *O*-GlcNAcylation were assessed for an IAP-recruiting PROTAC.

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DESIGN AND OPTIMIZATION OF *N*-BENZYLPIPERIDINES AS ALLOSTERIC HSP90 INHIBITORS WITH ANTICANCER ACTIVITY

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Triple-negative breast cancer (TNBC) accounts for approximately 15% of all breast cancer cases and remains a major therapeutic challenge due to the lack of effective targeted treatments. Despite extensive research, current therapeutic options are largely limited to cytotoxic drugs. Heat shock protein 90 (Hsp90), a molecular chaperone critical for the stability and function of numerous oncogenic proteins, is frequently overexpressed in cancer cells, making it an attractive therapeutic target in various cancers, including TNBC.

Most Hsp90 inhibitors target the N-terminal domain (NTD), but often induce a compensatory heat shock response, which in turn enhances Hsp90 expression, suppresses apoptosis and promotes cancer cell survival. An alternative strategy targeting the C-terminal domain (CTD) of Hsp90 could help circumvent these drawbacks by avoiding the induction of a heat shock response while still disrupting chaperone activity.

Our research team used a combination of molecular docking and pharmacophore screening to identify novel allosteric inhibitors targeting the CTD of Hsp90. Among these, the compound TVS-21 emerged as a promising lead compound that exhibited an IC_{50} of 44.8 ± 3.6 μM in the MCF-7 breast cancer cell line and was selected for further optimization. To validate Hsp90 as a target of TVS-21 and its analogues, several biophysical and biochemical assays were performed, including microscale thermophoresis to measure affinity, fluorescence thermal shift assay to rule out NTD binding, and NMR techniques (STD and trNOESY) to gain insight into the binding conformation in the absence of a co-crystal structure of the Hsp90-CTD inhibitor complex [1].

Compound **89** and related analogues showed potent low micromolar activity in MCF-7 and MDA-MB-231 cells, highlighting their potential for further optimization. Structural modifications, emphasizing the importance of an aromatic ring and a cation centre, will be presented along with further exploration of the binding site.

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SOLVENT-SENSITIVE THROUGH-SPACE $^{19}F–^{19}F$ COUPLING IN DIASTEREOMERIC FLUORINATED SYSTEMS

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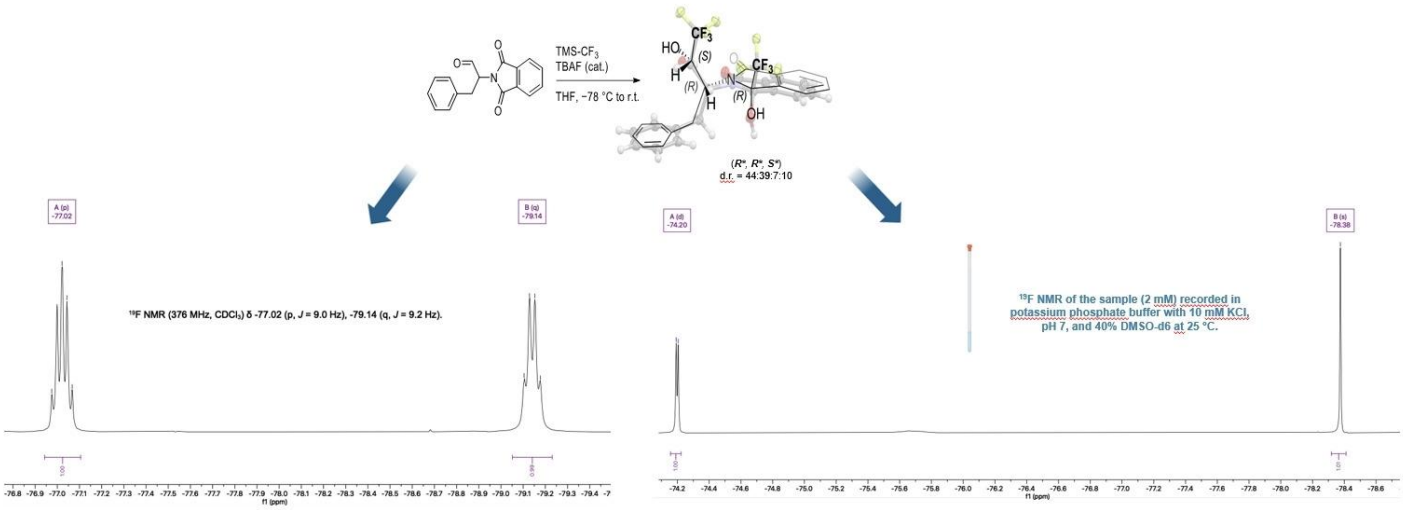
^{19}F NMR is a powerful technique for studying the structure, conformation, and intermolecular interactions of fluorinated compounds relevant to medicinal chemistry, materials science, and beyond.[1] Here, we report an intriguing ^{19}F NMR pattern observed in a molecule with three chiral centers bearing two CF_3 groups at tertiary and quaternary carbons. During the addition of the Rupert–Prakash reagent to the phthalimide-protected phenylalanine-derived aldehyde, a selective mono-alkylation can be achieved at −78 °C, whereas double alkylation occurs at higher temperatures.[2] The double-alkylated product was prepared on a gram scale with a diastereomeric ratio of 44:39:7:10. One of the diastereomers displayed a pentet and a quartet in the ^{19}F NMR spectrum, corresponding to the tertiary and quaternary carbon-bound CF_3 group, and the remaining diastereomers showed the expected doublet and singlet pattern. The observed ^{19}F NMR pattern can be attributed to through-space scalar $^{19}F–^{19}F$ *J*-coupling (J_{FF}). While through space J_{FF} has been documented in the literature,[3] the reports of intramolecular stereospecific long-range J_{FF} are scarce. The diastereomers were readily separated by flash chromatography on silica. Further structural elucidation by single-crystal X-ray diffraction analysis confirmed the (*R**,*R**,*S**) relative configuration with the CF_3 groups in close proximity stabilized by intramolecular H-bond. Solvent-dependent ^{19}F NMR experiments demonstrated that disruption of this hydrogen bond (in DMSO-*d*6/K^+ phosphate buffer) results in the disappearance of the scalar coupling pattern, confirming the dynamic and solvent-sensitive nature of the system. This solvent-induced conformational behavior may offer opportunities for the development of molecular sensors or switching devices based on intramolecular interaction monitoring via ^{19}F NMR. Finally, this study contributes to a deeper understanding of ^{19}F NMR and highlights its remarkable sensitivity to subtle stereochemical environments.



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NEW C-TERMINAL HSP90 INHIBITORS: DISRUPTING PROTEIN AND CALCIUM HOMEOSTASIS LEADS TO *IN VIVO* ANTICANCER ACTIVITY

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Heat shock protein 90 (Hsp90) is a molecular chaperone responsible for protein folding and maturation of several proteins. Both the decreased stability of mutated oncogenic proteins and stressful cancer microenvironment make cancer cells dependent on Hsp90. Therefore, Hsp90 inhibition has been a promising approach for anticancer therapy since the 90's. However, the limitations of N-terminal ATP-competitive Hsp90 inhibitors in clinical trials have halted the progression of such agents into the clinic. Luckily, with some of the new Hsp90 inhibitors with diverse modes of action the Hsp90 community was able to circumvent the induction of cytoprotective heat shock response (HSR), which was one of the main drawbacks of the classical Hsp90 inhibitors. Among these compounds, the development of C-terminal Hsp90 inhibitors was the focus of our work in recent years.

Recently, we evaluated *in vitro* anticancer activity of our Hsp90 inhibitors with antiviral activity, which highlighted TLK-6[1] as the starting point for structure-activity relationship optimization, as the compound displayed a low micromolar antiproliferative IC_{50} value (13.7 ± 0.0 μM) in MCF-7 breast cancer cells. The core 4,5,6,7-tetrahydrobenzo[*d*]thiazole was retained, while the nature and spatial orientations of substituents at positions 2 and 6 of the core ring were varied. In this way, we were able to improve the anticancer potency of the compounds for almost 60-fold. Some of our most promising compounds discovered in this study have also displayed *in vivo* efficacy in xenograft model of Ewing sarcoma in zebrafish larvae[2]. Among these compounds, TJD-268 appeared to be our most interesting and potent C-terminal Hsp90 inhibitor, therefore we used it for further biophysical and biochemical characterization.

We have uncovered that TJD-268 binds to both cytoplasmic isoforms of Hsp90 in the low micromolar range and the binding appears to be mediated by Glu489 in Hsp90β. Our Western blot and proteomics analysis also show a strong concentration and time-dependent effect of this compound on the proteins that rely on Hsp90 for folding and maturation. Moreover, through the effect on several proteins like IP3R, calnexin and calumenin, the compound disrupts calcium homeostasis thus leading to cell death. Along with the cytotoxic effects the compound induces cell cycle arrest in M/G2 phase *in vitro* and its *in vivo* application leads to a significant inhibition of tumor growth in an orthotopic 4T1 murine breast cancer model.

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BENZAMIDE-BASED MITOCHONDRIAL K_V1.3 INHIBITORS WITH POTENT ANTICANCER ACTIVITY

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Mitochondrial K_V1.3 channel is located in the mitochondrial inner membrane and belongs to the voltage-gated potassium channel K_V1.x subfamily. MitoK_V1.3 are involved in mitochondrial potassium cycle and in the regulation of the intrinsic apoptotic pathway.[1] It has emerged as a promising target for anticancer therapy due to a correlation between overexpression of mitoK_V1.3 and cancer development. [2,3]

In our research, we identified, as a starting point, a parent compound with potent cytotoxic activity. It presents a benzamide scaffold[4,5], a four carbon linker chain and triphenylphosphonium (TPP^+) as mitochondria-targeting moiety (MTM). We explored structural modifications to enhance its activity, we synthesized and evaluated different analogues with variations in the linker chain length (three to six carbons) and modifications in the mitochondria-targeting moiety (MTM), specifically TPP^+ and its substituted derivatives. The new inhibitors were screened for antiproliferative activity in pancreatic cancer COLO-357 and Ewing sarcoma SK-N-MC cell lines, demonstrating significant cytotoxicity and cell viability reduction. Among all tested compounds, *cis*-**24** emerged as the most promising one. Patch-clamp experiments confirmed its potent inhibition on mitoK_V1.3. Moreover, it caused mitochondrial membrane depolarization and induced apoptosis via caspase 3/7 activation in COLO-357 cell line. Further investigations revealed that *cis*-**24** selectively inhibited tumour cell growth without affecting growth of non-tumour hTERT-RPE1 cells, activated the permeability transition pore rather than significantly increasing reactive oxygen species, distinguishing it from other mitoK_V1.3 inhibitors. To assess the compound's efficacy beyond 2D cultures, *cis*-**24** was evaluated in 3D spheroids models of PANC-1 and COLO-357. It induced strong anti-proliferative and cytotoxic effects, particularly in COLO-357 spheroids, where significant impact was observed even at low micromolar concentrations. Finally, in an in vivo zebrafish xenograft model of Ewing sarcoma SK-N-MC, *cis*-**24** significantly inhibited tumour growth at 2 μM, demonstrating promising efficacy in an in vivo model. These findings highlight the potential of *cis*-**24** as a novel mitochondrial K_V1.3 inhibitor for cancer therapy, with significant anticancer activity. The observed efficacy in both in vitro and in vivo models suggested that *cis*-**24** could serve as valuable lead compound for further development in this field.

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OPTIMIZED SYNTHESIS OF SPYDYE FLUORESCENT CORE, CATIONIC AND BIOCONJUGATION DERIVATIVES

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The 3-aryl-4-trifluoromethylcoumarin-cored fluorescent dyes bearing a mesylamino derivatization handle (SpyDye) were recently developed at UL FFA.[1] We optimized the key concerted acylation–Knoevenagel condensation reaction step by switching from *N*,*N*'-dicyclohexylcarbodiimide (DCC) to 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). This simple adjustment significantly streamlined the synthesis, enabling gram-scale preparation of SpyDye405 in a single step from commercially available reagents.

Conjugates with selected permanent nitrogen-based cations were prepared as tool compounds to study organelle-localization ability of substituted pyridinium[2] and quinolinium moieties[3] by confocal microscopy through colocalization with established lysosome- and mitochondria-targeted fluorescent probes.

A bioconjugation variant of the dye was prepared by attaching an *N*-hydroxy(sulfosuccinimide) (SulfoNHS) ester through alkyl linker on the mesylamino handle. To facilitate the synthesis of SulfoNHS ester with SpyDye-derived carboxylic acid in organic solvent, a crown ether of the SulfoNHS sodium salt was used.[4] Preliminary staining experiments of bacteriophages demonstrated sufficient electrophilicity of the SulfoNHS group to label surface lysines, though probe solubility at working concentrations remained limited.

These results showcase SpyDye as a versatile and readily accessible platform with strong potential for the development of targeted imaging agents and bioconjugation probes in chemical biology, with future efforts directed toward enhancing solubility to further broaden its applicability.

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EXPLORING THE BINDING MODES OF TETRAHYDROPYRAN-BASED INHA INHIBITORS

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Tuberculosis, driven by multidrug-resistant *Mycobacterium tuberculosis*, remains a major global health challenge. The enzyme InhA (enoyl-[acyl-carrier-protein] reductase), a main component of the fatty acid synthase II (FAS-II) pathway, is a validated drug target. Although the frontline drug isoniazid inhibits InhA, its prodrug nature requires activation by KatG, an enzyme frequently mutated in resistant strains. Direct inhibition of InhA circumvents this resistance mechanism and provides a promising therapeutic strategy.

Through high-throughput screening in collaboration with GlaxoSmithKline, tetrahydropyran-based lead compound was identified. High-resolution crystal structures of this lead bound to InhA revealed a distinctive U-shaped binding mode, characterized by hydrophobic interactions within the substrate pocket and stabilizing hydrogen bonds. Guided by these structural insights, we designed and synthesized a series of analogues, retaining the biaryl system (rings D and E) while systematically varying substituents on rings A and B. Crystal structures demonstrated how larger groups on ring A disrupted binding, whereas a hydroxyl substituent on ring B improved physicochemical properties without loss of enzymatic activity. Furthermore, replacing the tetrahydropyran ring (C) with other cyclic scaffolds preserved the binding conformation observed crystallographically, confirming its role in maintaining inhibitor geometry.

Structural analysis was complemented by enzymatic assays and antibacterial evaluations against *M. tuberculosis*, *M. avium*, and *M. abscessus*. Newly solved InhA–inhibitor crystal structures provided critical insight into binding modes, directly shaping the design of next-generation inhibitors. Together, these findings serve as a promising starting point for developing novel antitubercular agents targeting InhA.

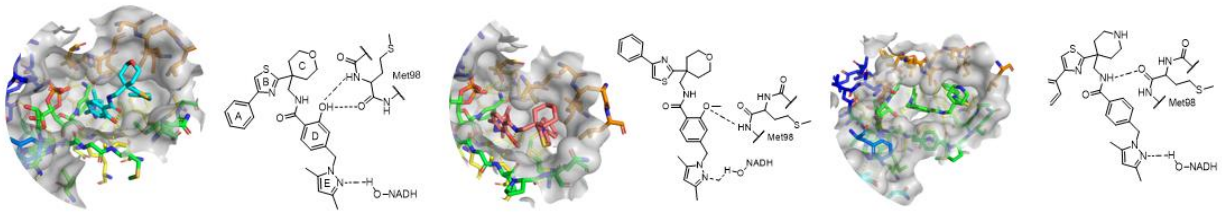

Figure 1: Crystal structures of our inhibitors in the InhA binding site and their structures with marked hydrogen bonds. From left to right: our lead compound, a derivative with substitution on ring D and a derivative with piperidine in place of tetrahydropyran

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POTENTIAL POTENT NOVEL BACTERIAL TOPOISOMERASE INHIBITORS WITH REDUCED HERG TOXICITY

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Life-threatening hospital bacterial infections are caused by increasing resistance to Gram-positive and Gram-negative pathogens which resulted in multiple emerging classes of antibacterial compounds against well-validated bacterial targets, such as DNA gyrase and topoisomerase IV[1,2]. Novel bacterial topoisomerase inhibitors (NBTIs) consist of the left-hand-side (LHS) moiety which intercalates in DNA, the linker that ensures a correct spatial orientation and appropriate physicochemical properties of the ligand, and the right-hand-side (RHS) moiety, which interacts with the GyrA subunits of the enzymes. NBTIs suffer from the class-related hERG blockage tendency, which is clinically manifested as ventricular tachyarrhythmia and prevents more potent NBTIs from progressing to clinical trials. Consequently, the reduction of hERG inhibitory activity by tuning of NBTI's physicochemical properties is necessary to develop potential clinical candidates. NBTIs tend to be less potent against Gram-negative bacteria, such as *Escherichia coli*, due to their relatively high substrate specificity for the bacterial efflux pumps, which is another potential direction of development of effective NBTI chemotypes[2,3].

We aim to develop DNA gyrase inhibitors with high on-target potency and antibacterial activity against Gram-negative bacteria with an acceptable hERG inhibitory profile. For this purpose, we selected some of the most promising NBTIs from the literature and conducted serial substitution of their RHS moieties with RHSs proposed by a machine-learning-based model DeepFrag. The generated RHS moieties were converted to aldehydes and carboxylic acids and filtered to resemble physicochemical properties of existing RHS moieties from known potent and toxicologically safe NBTIs. Virtual combinatorial reactions between aldehydes and carboxylic acids and known NBTI scaffolds were enumerated and the products were filtered with drug-likeness filters (Lipinski/Veber rule sets). Their inhibitory activities on hERG were predicted utilising Pred-hERG. Molecular docking calculations of the selected NBTIs with acceptable hERG profiles were conducted by using the available DNA gyrase structural data originating from *E. coli* (PDB ID: 6RKS) and *Staphylococcus aureus* (PDB ID: 6Z1A). All systems were further subjected to molecular dynamics (MD) simulations and their free energies of binding were predicted by the linear interaction energy (LIE) method. This drug discovery workflow resulted in selecting novel NBTI chemotypes with predicted nanomolar inhibitory potencies on *E. coli* and *S. aureus* DNA gyrase with a computationally predicted acceptable on-target potency/hERG toxicity ratio.

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FRAGMENT-BASED DISCOVERY OF COVALENT CASPASE-1 INHIBITORS REVEALS SULFONYL FLUORIDES AS PROMISING WARHEADS

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Neuroinflammation contributes to the pathophysiology of many neurodegenerative diseases and offers targets for therapeutic intervention, particularly in conditions such as Alzheimer's disease. Caspase-1 is a key protease involved in the maturation of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), converting them into their active forms and thereby amplifying the inflammatory response. Additionally, caspase-1 facilitates the cleavage of gasdermin D, triggering pyroptosis – a form of programmed cell death linked to inflammation.

We focussed on identifying covalent inhibitors of caspase-1 using a fragment-based drug discovery (FBDD) approach. The initial screening utilized a covalent fragment library of 462 small fragments with electrophilic warheads designed to react with the nucleophilic residues – such as catalytic Cys285 of caspase-1. The library included 20 chemically distinct covalent warheads, such as acrylamides, boronic acids, epoxides, and sulfonyl fluorides, enabling broad exploration of covalent interaction space. To evaluate inhibitory activity, a well-established fluorometric biochemical assay was used, in which caspase-1 activity was quantified by measuring the increase in fluorescence following substrate (Ac-YVAD-AMC) cleavage. Among the fragments tested, sulfonyl fluorides emerged as the most promising class of inhibitors, exhibiting the lowest residual caspase-1 activity. The top three sulfonyl fluoride hits were subsequently characterized by determining their IC_{50} values.

The ultimate goal is to optimise these hit compounds through a fragment growing strategy to enhance binding interactions within the S1 and S4 subsites of the caspase-1 active site. This approach aims to increase binding affinity from the micromolar to nanomolar range, ultimately yielding potent and selective inhibitors for therapeutic application in neuroinflammatory disorders.

STRUCTURE–ACTIVITY RELATIONSHIP STUDY OF ST6GAL1 INHIBITORS FOR ENHANCED CELL PERMEABILITY AND POTENCY

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Sialyltransferases transfer sialic acid to glycoproteins and glycolipids in the Golgi apparatus. α-2,6-sialyltransferase (ST6Gal1) does so via an α-2,6-linkage and plays an important role in cancer progression. Rillahan et al. discovered a series of glycosyltransferase inhibitors using high-throughput screening, of which the compound JFD00458 (Figure 1) showed the strongest inhibitory activity against ST6Gal1 with an IC50 of 10.8 μM. However, this inhibitor is not expected to cross the cell membranes and reach the Golgi apparatus in vitro or in vivo due to its high polarity [1]. In this work, we designed and synthesized JFD00458 derivatives with the goal of increasing cell permeability. Additionally, we synthesised a series of compounds to further explore structure-activity relationship in order to improve potency. We also used a recently developed biochemical assay to evaluate ST6Gal1 inhibitors [2]. Our structure-activity study demonstrates that potent inhibition can be maintained with simultaneous cellular permeation.

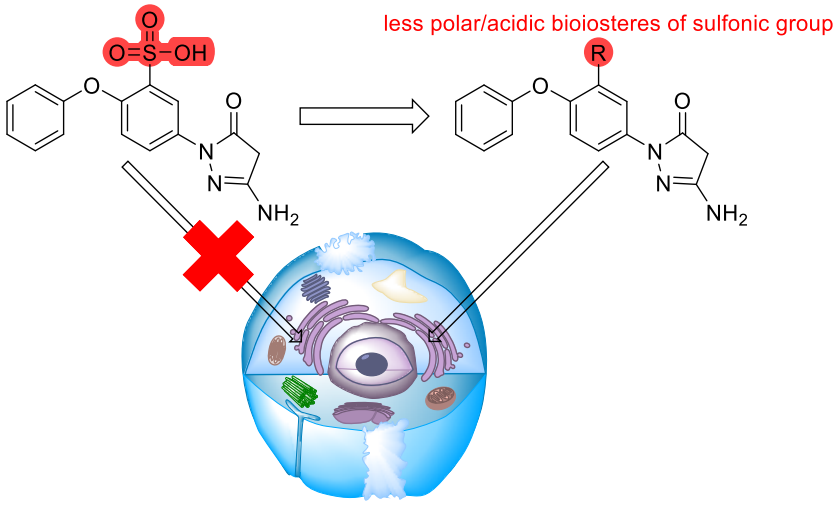

Figure 1. JFD00458 and derivatives – expected cell permeability

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DESIGN AND SYNTHESIS OF ETHYNYLANILINE AMIDES AS POTENTIAL COVALENT INHIBITORS OF LDTB

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The bacterial cell wall, predominantly composed of peptidoglycan (PG), plays a vital role in preserving cell morphology and preventing lysis due to osmotic stress. The structural integrity of this layer is ensured by enzymatic cross-linking, primarily mediated by two classes of enzymes: penicillin-binding proteins (PBPs) and L,D-transpeptidases (LDTs).[1] While PBPs catalyze the classical 4→3 cross-links, LDTs are responsible for forming 3→3 cross-links and for the covalent attachment of Braun's lipoprotein (Lpp) to the PG. This linkage represents the only known covalent connection between the outer membrane and the peptidoglycan layer and is therefore essential for maintaining the mechanical stability of the Gram-negative cell envelope. In *E. coli*, LDTs are upregulated in response to β-lactam exposure, enabling the bacteria to bypass PBP inhibition and adapt to stress, thereby enhancing survival under otherwise lethal conditions.[2]

This study aims to develop a small library of potential inhibitors targeting *E. coli* LdtB, incorporating an ethynyl aniline amide moiety, with the goal of achieving LdtB IC_{50} values in the low micromolar to possibly nanomolar range. A library of 74 compounds featuring 2-, 3-, or 4-ethynylaniline moieties was synthesized through a variety of synthetic routes, with amide formation using HATU and DIPEA being the most employed reaction. This method was selected based on its high yield and compatibility with a range of carboxylic acids, including both aryl and alkyl derivatives, as confirmed during the initial screening of reaction conditions. Of the 74 compounds synthesized, 10 demonstrated inhibitory activity against LdtB in micromolar range (43.8 – 270.2 μM, 6 best compounds shown in **Figure 1**). Future studies will focus on synthesizing and evaluating additional derivatives incorporating alternative aryl alkyne moieties, such as secondary amines, alkyl or aryl ethers, and alkyl amides.

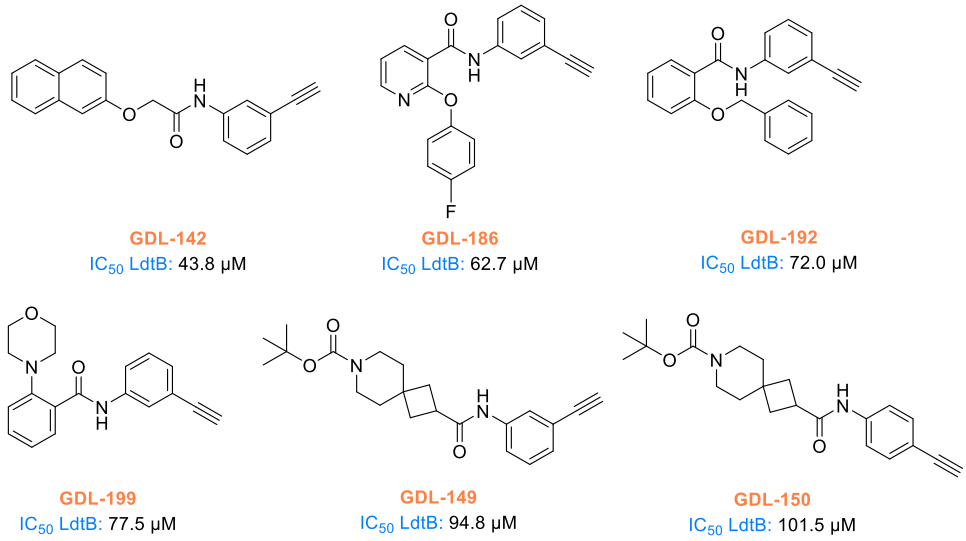

Figure 1: Most potent LdtB inhibitors from ethynyl aniline amides class

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Q&D - QUICK DOCKING OF KINASE INHIBITOR LIBRARY INTO HBCHE: DISCOVERY OF NOVEL MTDLS TO ADDRESS NEUROINFLAMMATION AND CHOLINERGIC DEFICIT IN NEURODEGENERATION

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Neurodegenerative diseases (NDDs) comprise a diverse group of disorders characterized by progressive neuronal loss in both the central and peripheral nervous system, posing substantial health challenges to aging populations and imposing major economic burdens.
Their defining pathological features include abnormal protein aggregation and impaired proteostasis.[1] Protein misfolding and aggregation frequently result from aberrant phosphorylation, positioning kinases as attractive therapeutic targets.[2,3] However, the development of CNS-penetrant kinase inhibitors remains challenging due to the need for kinome-wide selectivity and the risk of off-target effects. In contrast, specifically modulating another protein implicated in disease pathology can provide additional therapeutic benefit. A notable example is butyrylcholinesterase (BChE), an enzyme frequently overexpressed in neurodegenerative conditions.[4] A selective kinase inhibitor that also inhibits BChE could combine disease-modifying effects on neuroinflammation with additional cognition-enhancing properties.

To design such dual-acting molecules, we first conducted a thorough literature review to identify core kinases involved in NDDs. Ligand data were retrieved from ChEMBL, merged in KNIME, and filtered to remove inactive compounds, poor BBB penetrants, and PAINS. The resulting library was imported into Schrödinger Maestro and docked into the hBChE crystal structure (PDB 6QAA). Docking hits were clustered and prioritized for purchase based on their binding poses, docking scores, and availability from the Chemspace and Molport databases.

Of the 24 compounds purchased and assayed for hBChE inhibition in vitro, 11 showed activity (RA < 50%), with the most potent displaying IC_{50} values in the low micromolar range. An additional 12 compounds have been ordered and await testing. This strategy highlights a promising avenue for the discovery of novel lead compounds for multitarget therapy in NDDs.

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LINKER CHEMISTRY MODULATES SOLUBILITY AND IMMUNE RESPONSE IN CONJUGATED NOD2/TLR4 DUAL AGONISTS

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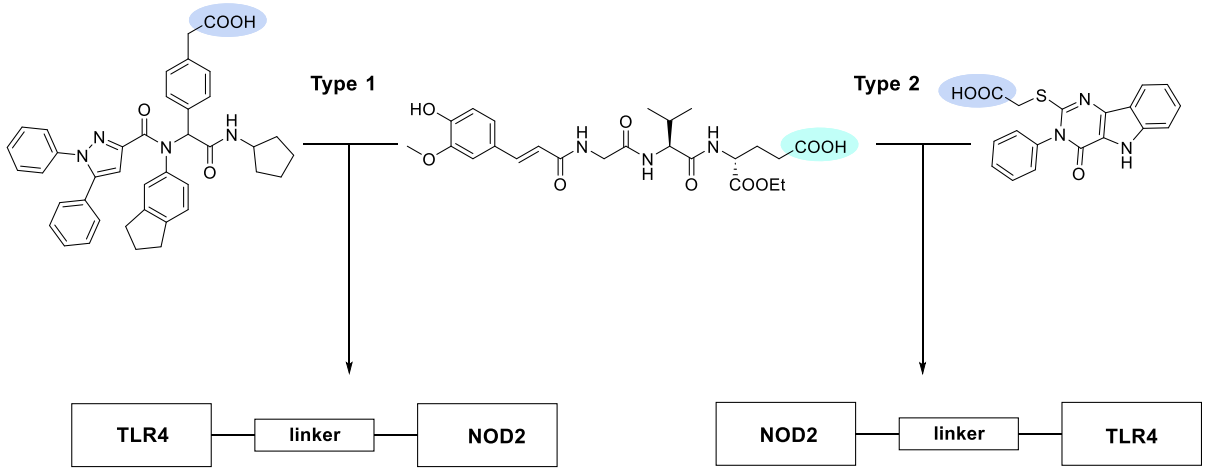
Simultaneous activation of multiple pattern recognition receptors represents a promising strategy for developing next-generation vaccine adjuvants. Here, we report the design, synthesis, and biological evaluation of conjugated NOD2/TLR4 dual agonists to investigate the immunological consequences of dual receptor activation and establish structure-activity relationships within this emerging class of immunomodulators. First, a series of conjugates was synthesized by covalently linking a proprietary NOD2 agonist[1] to two structurally distinct TLR4 agonists[2,3] using either flexible or rigid linkers. While conjugation generally attenuated dual receptor agonism compared to parent ligands administered separately, linker architecture profoundly influenced both physicochemical properties and biological activity. Notably, amide-to-amide coupling strategies provided superior kinetic solubility compared to previously employed amide-to-phenol ester linkages. Site-selective conjugation at the γ-carboxylic group of the NOD2 agonist and carboxylic acid substituents of TLR4 agonists proved optimal, establishing rational attachment points for future design efforts. Biological evaluation in human primary peripheral blood mononuclear cells revealed that conjugates induce distinct immunomodulatory profiles characterized by Th2-polarized cytokine responses, including elevated MCP-1 secretion and suppression of proinflammatory mediators. Remarkably, conjugate **21** demonstrated the highest immunomodulatory potency despite exhibiting the lowest solubility in the series, highlighting the complex relationship between physicochemical properties and biological activity. These findings establish foundational design principles for conjugated NOD2/TLR4 agonists with "tunable" solubility and immunological profile.



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SMALL-MOLECULE INHIBITORS OF H_V1 CHANNELS: A NEW ANTICANCER APPROACH

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Voltage-gated proton channels (H_V1) are proton-selective voltage-dependent channels that have been found in various mammalian and cancer cells, playing a key role in preventing intracellular acidification. They have been linked to tumours development, neuroinflammatory diseases, immune disorders and infertility.[1] In particular, H_V1 overexpression disrupts pH homeostasis and is closely associated with cancer progression. Indeed, in physiological conditions, at the resting membrane potential, the channels are closed; however, pathological conditions can trigger their opening even at the resting membrane potential, creating an acidic microenvironment in which tumour cells can adapt extremely well, while immune cells functions are impaired.[2]

The aim of our work is to discover and evaluate new H_V1 inhibitors. Currently, there are no selective inhibitors specific for H_V1 channels. A selective inhibitor would allow us to modulate the acidic tumor microenvironment and to better understand the role of H_V1 in tumours and other relevant diseases. An open structure of the human H_V1 channel was used to perform virtual screening (VS) of commercial and in-house libraries of compounds.[3] Compounds were docked to the binding site of some known guanidine derivatives, such as 2-guanidinobenzimidazole (2GBI), on the voltage-sensing domain.[4] VS results were evaluated, and a series of most promising hits were selected to be tested by manual patch-clamp electrophysiology in Chinese Hamster Ovary (CHO) cells transiently transfected with human H_V1. With an IC_{50} value of 8.5 μM, compound 13 exhibited a significant block of the proton current. Six additional compounds were found to block channel activity by more than 50% at 50 μM concentration when twenty-three analogues of compound 13 were biologically evaluated. These results enabled structure-activity relationship studies and led to the identification of a new series of molecules with a 5-phenyl-2-aminoimidazole core as a new structural class of inhibitors of hH_V1 channels.[5] Moreover, the antiproliferative activity of the seven hits was investigated by testing the compounds in two tumour cell lines, MDA-MB-231 and THP-1, in which H_V1 channels are highly expressed. Most of the molecules were found to be potent growth inhibitors, with compound 13 showing the lowest IC_{50} values (MDA-MB-231 IC_{50} = 9.0 ± 1.0 μM, THP-1 IC_{50} = 8.1 ± 4.3 μM).[5]

By bringing together the knowledge and the results from ligand- and structure-based drug design, biophysical and pharmacological characterization, and medicinal chemistry methods, we have obtained promising hits that can be used for further hit-to-lead optimization and serve as chemical tools to better understand the role of H_V1 in tumors and other relevant diseases.

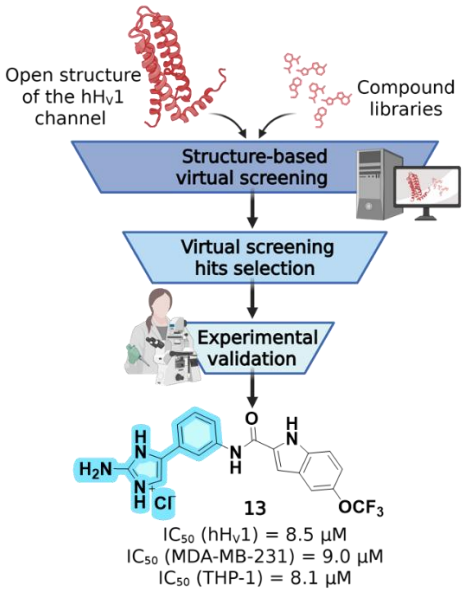

Figure 1. Graphical representation of the research workflow, highlighting the key steps of the study.

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ASSESSMENT OF BIOFILM FORMATION IN *PSEUDOMONAS AERUGINOSA* STRAINS AND ANTIBIOFILM ACTIVITY OF THE FACULTY OF PHARMACY COMPOUND LIBRARY

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Pseudomonas aeruginosa (*P. aeruginosa*) in an opportunistic pathogen notorious for causing persistent infections due to strong biofilm forming capability (1). It is frequently associated with hospital-acquired infections and poses significant threat to immunocompromised patients (1). Biofilms are structured bacterial communities embedded in a protective extracellular matrix that provides resistance to antibiotics and immune clearance (2).Within biofilms, *P. aeruginosa* can survive in a hypoxic atmosphere or other extremely harsh environmental conditions (1). Chronic biofilm-related infections represent a major clinical challenge, as cells in biofilms can be up to 1000 times more resistant to antibiotics than planktonic cells (3). The discovery and development of antibiofilm compounds aim to disrupt or inhibit biofilm formation and enhance infection treatment efficacy. Novel antibiofilm compounds are a promising strategy to overcome biofilm-mediated drug resistance and improve patient outcomes (4).

The aim of this study was to evaluate the biofilm-forming ability of approximately 80 *P. aeruginosa* isolates from diverse clinical and milk sources, and to screen a Faculty of Pharmacy compound library for small molecules capable of inhibiting biofilm formation in the reference strain PAO1.
Biofilm formations was quantified using a microtiter crystal violet staining assay. Negative controls and PAO1 reference strain (positive control) were included in every experiment. The biofilm formation index was calculated according to the method of Stepanović et al (5). Isolates were categorized as strong, moderate, weak, or non-biofilm formers. In parallel, a focused compound library was screened for antibiofilm activity against PAO1. Nearly 50% of the tested isolates demonstrated strong biofilm formation capabilities, while the remaining strains exhibited moderate, weak, or no biofilm forming phenotype. Several compounds from the Faculty of Pharmacy's chemical library significantly inhibited PAO1 biofilm formation, thereby identifying promising antibiofilm candidates.

The biofilm forming potential is highly variable among clinical *P. aeruginosa* isolates. The identification of small molecules with antibiofilm activity against PAO1 highlights the value of targeted screening approaches for discovering novel antibiofilm agents.

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TARGETING THE IMMUNOPROTEASOME: MODULATING PLATELET–CANCER CELL CROSSTALK IN BREAST CANCER

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The ubiquitin-proteasome system plays a pivotal role in maintaining protein homeostasis and regulating immune response[1]. Inflammatory cytokines can induce the constitutive catalytic subunits of the proteasome to be replaced by immunoproteasome counterparts (β1i, β2i, β5i), thereby modulating cellular function under stress or disease conditions[1,2]. While proteasome inhibitors are well established in the treatment of hematologic malignancies[3], their role in solid tumours, particularly in shaping tumour-microenvironment interactions, remains poorly understood.

Platelets are increasingly recognized as active participants in cancer progression by protecting tumour cells from immune attack, promoting adhesion to endothelial cells, and fostering an immunosuppressive microenvironment. To investigate whether immunoproteasome modulation influences crosstalk between platelets and cancer cells, we established direct and indirect co-culture models of breast cancer cell lines (MCF-7, SK-BR-3, MDA-MB-231) with platelets. Selective immunoproteasome inhibitors (KZR-504, M3258) and the broad-spectrum inhibitor carfilzomib were used to investigate their effects on platelet function and cytokine secretion.

Our results show that platelets constitutively express immunoproteasome subunits and that pharmacologic modulation alters their interaction with cancer cells. Pre-treatment with KZR-504 resulted in delayed platelet aggregation and a marked suppression of pro-inflammatory cytokines (IL-6, IL-8, IP-10) after co-culture. Interestingly, despite the reduced inflammatory signals, breast cancer cells showed phenotypic changes associated with increased migratory capacity or endothelial attachment, suggesting a complex balance between immune regulation and tumour-promoting effects.

These findings highlight the immunoproteasome as a potential regulator of platelet-mediated cancer progression and provide new insights into the tumour-microenvironment crosstalk. Ongoing studies aim to validate chemokine signatures in multiple donors, extend the analysis to 3D culture systems, and investigate macrophage polarization in response to conditioned media. Overall, these findings suggest that the immunoproteasome is an attractive therapeutic target to reshape the tumor microenvironment and potentially restraining metastatic spread.

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EXPLORATION OF LINKER ATTACHMENT POINTS OF THREE CDK1 INHIBITORS FOR PROTAC DESIGN

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Cyclin-dependent kinase 1 (CDK1) is a serine/threonine kinase possessing a crucial role in the regulation of cell cycle progression, particularly the G2/M transition. Abnormal CDK1 activity has been connected to uncontrolled cell proliferation and development of various malignancies, serving as evidence of this target's potential in anticancer therapy. Recent efforts have shifted from traditional inhibitor-based approaches toward the exploitation of targeted protein degradation strategies to modulate oncogenic drivers. Proteolysis Targeting Chimeras (PROTACs), a class of heterobifunctional molecules, have emerged as a transformative approach by harnessing the cell's ubiquitin-proteasome system to selectively degrade disease-associated proteins. In this context, CDK1-directed PROTACs offer the potential for increased selectivity and efficacy over traditional ATP-competitive CDK1 inhibitors, while reducing the likelihood of acquired resistance.[1,2,3]

Compounds that selectively inhibit CDK1 are scarce, with only few examples reported to date (such as Ro-3306, avotaciclib, and phthalazinone derivatives). Because having a target binder is a crucial element in PROTAC design, we decided to pursue all three compound classes. Without co-crystal data available, we focused on known structure-activity relationships and molecular modeling to establish the appropriate linker-attachment point and subsequent modification into heterobifunctional degraders. Using Kinase-Glo® assays, we determined that linker-modified phthalazinones (including the described inhibitor **8g**) fail to inhibit CDK1 even at 10 μM. These results urged us to not consider this compound class any further in CDK1 PROTAC development. In the case of Ro-3306 inhibitors, our findings revealed that both the position and chemical type of linkage have a vital role in retaining CDK1 inhibitory properties. For example, sterically bulky linking moieties, such as amides resulted in diminished inhibition as opposed to ether-based linking bonds, that still proved inhibitory, albeit to a smaller extent compared to parent Ro-3306. With the third CDK1 inhibitor, avotaciclib, we succeeded in preparing a derivative containing a solvent-exposed piperazine acting as a possible linker attachment point, which retained a similar inhibition profile as its starting compound. With this initial information, our current work is focused on the preparation of R0-3306- and avotaciclib-based heterobifunctional compounds. Initial series of cereblon-recruiting PROTACs showed potent cytotoxic effects in an acute lymphoblastic leukemia cell line.

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DESIGN AND EVALUATION OF TAK1 PROTACS FOR CANCER AND AUTOIMMUNE DISEASE THERAPY

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Transforming growth factor-β-activated kinase 1 (TAK1), a member of the MAP3K family, regulates multiple signaling pathways such as TNF-α, IL-1, TLR, and TGF-β.[1] Due to its pivotal involvement in immune regulation and cell growth, abnormal TAK1 activity has been associated with both tumor progression and autoimmune conditions.[2] In 2017, Totzke et al. identified takinib as a potent and selective TAK1 inhibitor with an IC_{50} of 9.5 nM, providing a foundation for further exploration of TAK1-targeting therapeutics.[3]

In recent years, proteolysis-targeting chimeras (PROTACs) have emerged as an innovative drug discovery approach. These bifunctional molecules induce targeted protein degradation by recruiting the ubiquitin–proteasome system.[4]

Building on takinib as the TAK1-binding ligand, we synthesized and tested a series of eighteen PROTACs featuring varied linkers and E3 ligase ligands (VHL, CRBN, and IAP). All compounds effectively inhibited TAK1, with IC_{50} values ranging from 3 nM to 1 μM. Within the first set, PROTAC **11**, designed to hijack IAP, successfully degraded TAK1 in TNF-α-stimulated MDA-MB-231 cells. In the second series of compounds, we introduced rigid linkers, leading to the development of compound **16**, a CRBN-hijacking PROTAC. Compound **16** showed effective depletion of TAK1 in both MDA-MB-231 and THP-1 cells, as well as a significant effect on the viability of TNF-α stimulated THP-1 cells. It also induced TAK1 depletion in peripheral blood mononuclear cells (PBMCs) and led to a significant decrease in the production of inflammatory cytokines in LPS-stimulated PBMCs.[5]

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SYNTHESIS OF 2-PYRROLAMIDO-BENZOTHIAZOLE INHIBITORS OF BACTERIAL DNA GYRASE WITH ALCOHOLS AND KETONES AS CARBOXYLIC ACID BIOISOSTERES

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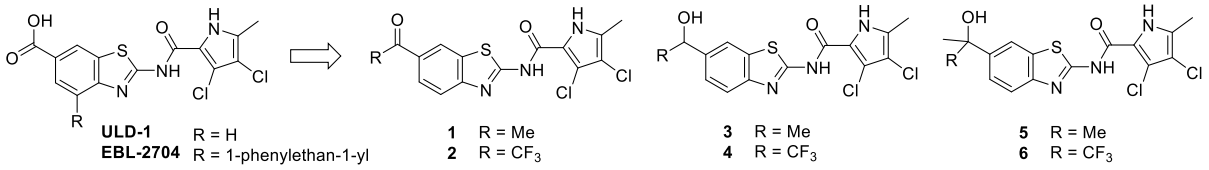
Increasing emergence of drug-resistant pathogenic bacteria has resulted in higher human mortality and increased healthcare costs. The 2-pyrrolamido-benzothiazole-based ATP-competitive Gyrase B inhibitor **EBL-2704** displayed outstanding activity against multidrug-resistant *Acinetobacter baumanii*, however, its further development was hampered by high plasma-protein binding.[1] Alcoholic bioisosteres of carboxylic acid have proven successful in resolving the plasma-protein binding issues of structurally related compounds.[2] Encouragingly, the tertiary alcohol motif is also present in the clinically evaluated Gyrase B inhibitor SPR720.[3]

We successfully synthesized a series of six bioisosteres: four alcohol derivatives (two secondary and two tertiary) and two ketone-containing analogues. The commercially available 2-amino-6-acetylbenzo[*d*]thiazole was coupled to 3,4-dichloro-5-methylpyrrole-2-carbonyl chloride to get **1**. 2-amino-6-trifluoroacetylbenzo[*d*]thiazole was prepared from the corresponding aniline and the pre-formed thiocyanogen in acetic acid, then readily converted to **2**. The synthesis of the alcohols **3**–**6** was first attempted by final-step acylation with pyrrole-2-carbonyl chloride, but elimination of both secondary and tertiary alcohols was observed at the rather harsh reaction conditions. We therefore resorted to late-stage $NaBH_4$-mediated reductions of **1** and **2** to access **3** and **4**, respectively. The tertiary alcohols **5** and **6** were also prepared by late-stage diversification i.e. by reacting excess methyl magnesium bromide with **ULD-1** ethyl ester or with the trifluoromethyl ketone **2**.



The compounds **1** and **3** were already tested in the *E. coli* gyrase supercoiling assay and display promising inhibitory activity: residual activities were 17% (1 μM compound **1**) and 6% (1 μM compound **3**). The study will be continued by evaluating the on-target activity of all 6 compounds, determination of their antibacterial activity (also in the presence of 50% human serum), and determination of key physicochemical properties (solubility, logD, plasma-protein binding).

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COMPARATIVE EVALUATION OF CARFILZOMIB-INDUCED CARDIOTOXICITY IN IPSC-DERIVED AND AC16 CARDIOMYOCYTES

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While advances in cancer therapy have markedly extended patient survival, cardiotoxicity remains a critical limitation, particularly with proteasome inhibitors (PIs) used in multiple myeloma treatment.[1] Carfilzomib, a selective β5/β5i PI, is strongly associated with adverse cardiovascular events, yet the underlying mechanisms are still poorly defined. Current knowledge is limited in part by the use of animal models, which may not fully capture human cardiomyocyte responses.[2–4]

To address this gap, we directly compared the human AC16 cardiomyocyte cell line with induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs), a model increasingly valued for its human-relevant physiology and ability to reflect patient-specific variation. We assessed proteasome subunit expression and activity, alongside the cytotoxic effects of PIs, to establish the strengths and limitations of each system for cardiotoxicity research.[5] In addition, transcriptomic data were leveraged to identify key gene expression changes triggered by carfilzomib.[6] Chemical perturbation analysis (SigCom Lincs) highlighted four candidate compounds—methotrexate, SMER28, tozasertib, and etomoxir—that may counteract carfilzomib-induced transcriptional signatures. These compounds are currently being evaluated for their ability to reduce cytotoxicity and exert cardioprotective effects.

Our findings contribute to a deeper understanding of PI-induced cardiotoxicity, while underscoring the value of iPSC-CMs as a platform for mechanistic studies and for developing strategies to mitigate cardiovascular side effects in oncology treatment.

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