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SIGMA-1 EUROPE

European research network on sigma-1 receptors CA23156

6th European Symposium
2nd Meeting of SIGMA-1 EUROPE

Physiopathology of Sigma-1 Receptors

Collegium Novum Jagiellonian University, Kraków

June 9-11, 2026

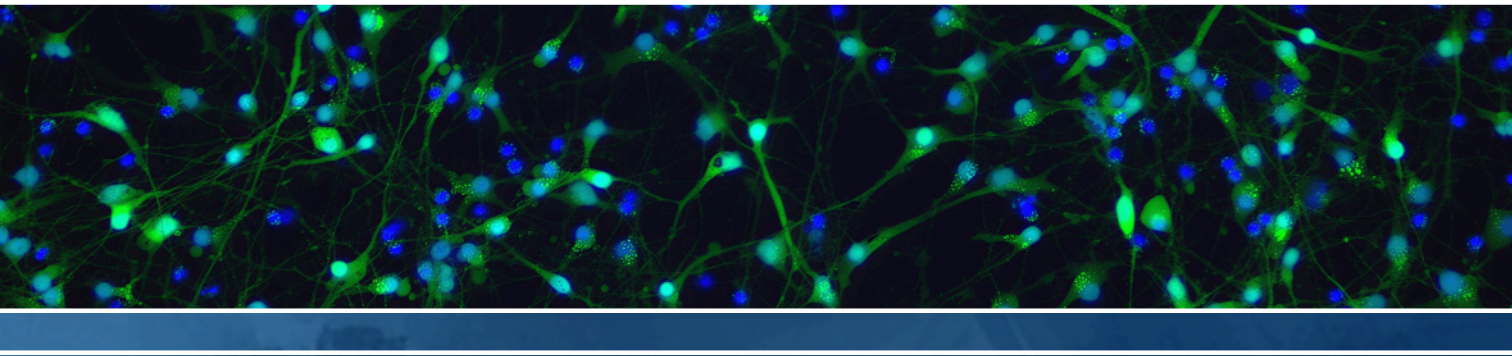
Keynote Speakers

Hongmei Jia
Beijing Normal University, China

Xavier Codony
Fundación Kaertor & CiMUS, Spain

Kenji Hashimoto
Chiba University, Japan

Philippe De Deurwaerdère
University of Bordeaux, France



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Physiopathology of Sigma-1 Receptors

6th European Symposium/ 2nd Meeting of the
European Network for Sigma-1 Receptor as a Therapeutic Opportunity
(SIGMA-1EUROPE - CA23156)

9-11 June 2026

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9:00-9:30 Opening ceremony

Karolina Pytka, on behalf of the Scientific Committee and Local Organizers

Tangui Maurice, Chair of SIGMA-1EUROPE

Aleksander Mendyk, Vice Dean for Science and Development, Faculty of Pharmacy JU MC

9:30-10:15 Keynote lecture

From molecule design to clinical translation: development of highly specific sigma-1 receptor radioligands for *in vivo* imaging

Hongmei Jia, Beijing Normal University, China

Chair: Carmen Abate, University of Bari Aldo Moro, Italy

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10:45-13:05 Scientific session 1 - Medicinal chemistry and drug discovery

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2. **Design of dual sigma-1/Cb2 receptor ligands as a therapeutic strategy for neurodegenerative disorders**
   Giovanni Graziano, University of Bari, Italy
3. **Towards multi-target ligands for pain therapy: design and biological evaluation of compounds directed at sigma-1, histamine H_3, and μ-opioid receptors**
   Katarzyna Szczepańska, Maj Institute of Pharmacology Polish Academy of Sciences, Poland
4. **Machine learning and artificial intelligence tools for the rational design of novel sigma-1 receptor modulators**
   Domenico Alberga, National Research Council, Italy
5. **Ligand-driven allosteric rearrangements of the sigma-1 receptor revealed by molecular dynamics**
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Chair: Carmen Abate, University of Bari Aldo Moro, Italy

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3. **Rare SIGMAR1 gene variants in a cohort of neurodevelopmental disorders**
   Gül Ünsel-Bolat, Balıkesir University, Türkiye
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   Gulay Sezer, Erciyes University, Türkiye

Chair: Enrique J Cobos, University of Granada, Spain

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Kenji Hashimoto, Chiba University, Japan

Chair: Enrique J Cobos, University of Granada, Spain

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   Charlyne Barry-Simonnet, University of Montpellier, France
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   Nil Oge, Ege University, Türkiye
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   Alicia Porro Pérez, Institute of General Organic Chemistry, Spain
5. **The sigma-1 receptor agonist fluvoxamine alleviates endotoxin-induced acute lung injury in mice**
   Akos Toth, Semmelweis University, Hungary
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   Gabriella Rosanna Musillo, University of Bari Aldo Moro, Italy
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Chair: Karolina Pytka, Jagiellonian University Medical College, Poland

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Targeting sigma-2 receptors as a promising therapy for the treatment of neurodegenerative diseases

Xavier Codony, Fundación Kaertor & CiMUS, Universidad de Santiago de Compostela, Spain

Chair: Karolina Pytka, Jagiellonian University Medical College, Poland

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2. **Pridopidine, a potent and selective therapeutic sigma-1 receptor (S1R) agonist for the treatment of neurodegenerative and neurodevelopmental diseases**
   May Meltzer, Prilenia Therapeutics B.V., Netherlands
3. **Preclinical development of RC-106: a brain-permeant sigma receptors modulator against glioblastoma**
   Giacomo Rossino, University of Pavia, Italy
4. **Sigma-1 receptor modulation as a potential missing link in ONC201 mechanism of action in diffuse intrinsic pontine glioma (DIPG)**
   Anselma Liturri, University of Bari Aldo Moro, Italy
5. **Sigma-1 receptor-targeted nanofibrous biomaterial systems for modulating cellular stress and enhancing wound healing**
   Hayrani Eren Bostancı, Sivas Cumhuriyet University, Türkiye

Chair: Liga Zvejniece, Latvian Institute of Organic Chemistry, Latvia

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Serotonergic psychedelics and the brain neurochemical tissue

Philippe De Deurwaerdère, University of Bordeaux, France

Chair: Liga Zvejniece, Latvian Institute of Organic Chemistry, Latvia

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Thursday, 11 June 2026

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(Establishment of SOPs for in-vitro/in-vivo models to assess sigma-1 receptor ligands)

11:00-11:30 Coffee break

11:30-13:00 WG5 meeting

(Dissemination, Communication and Outreach)

13:00-14:00 Lunch

14:00-16:00 Management Committee meeting

KEYNOTE LECTURE

From molecular design to clinical translation: Development of highly specific sigma-1 receptor radioligands for *in vivo* imaging

Jia, H.[1], Huang, H.[2]

[1]*Key Laboratory of Radiopharmaceuticals (Beijing Normal University), Ministry of Education, College of Chemistry, Beijing Normal University, Beijing 100875, China*
[2]*Yale PET Center, Department of Radiology and Biomedical Imaging, Yale University School of Medicine, New Haven, Connecticut 06520-8048, United States*

The sigma-1 receptor (S1R) is a unique “ligand-operated receptor chaperone” that facilitate protein functions. It is involved in a myriad of physiological functions and pathophysiology of diseases including CNS disorders, cardiac diseases, and cancers. The availability of optimal radioligands for positron emission tomography (PET) imaging of the S1R in humans will provide tools to monitor disease progression and drug development. This talk will share a story on development of highly specific S1R radioligands for *in vivo* imaging with an emphasis on the requirements of optimal radioligands to image the S1R in the human brain. First, we summarize the progress of ^{18}F-labeled S1R radioligands from the spirocyclic piperidine class, which is in good agreement with the Glennon’s pharmacophore model. In this part, we found sub-nanomolar affinity lead to slow kinetics in non-human primate brain, and thus not suitable for translation to humans for neuroimaging. Two radioligands with nanomolar affinity have fast kinetics in non-human primate brain, and are appropriate for neuroimaging and quantification of the S1R. Moreover, radioligand with lower lipophilicity and higher plasma free fraction displayed higher specific binding. Inspired by the above results, we then modify the S1R pharmacophore for ligand development and evaluate a series of radioligands without benzen ring at the primary hydrophobic region. Among these radioligands with lower lipophilicity, (*R*)- and (*S*)-[^{18}F]FBFP fulfill the criteria for optimal PET neuroimaging agents and emerge as the most promising radioligands for PET imaging of the S1R in the primate brain. Clinical translation of these two radioligands are currently ongoing.

Keywords: *Sigma-1 receptor, fluorine-18, positron emission tomography, brain, clinical translation*

Acknowledgments: This work was supported by the National Natural Science Foundation of China (grant numbers 22576020 and 22276016).

An optimized lead compound for treating dementia targeting sigma-1, sigma-2, and 5-HT_{2A} receptors and butyrylcholinesterase

Košak, U.[1], Strašek Benedik, N.[1], Knez, D.[1], Žakelj, S.[1], Trontelj, J.[1], Pišlar, A.[1], Horvat, S.[1], Bolje, A.[1], Žnidaršič, N.[2], Grgurevič, N.[2], Švara, T.[3], Kljun, J.[4], Shahid, M.[5], Skrzypczak-Wiercioch, A.[6], Lv, B.[7], Xiong, Y.[7], Wang, Q.[8], Bian, R.[7], Shao, J.[7], Dias, J.[9], Nachon, F.[9], Brazzolotto, X.[9], Stojan, J.[10], Sun, H.[7], Sałat, K.[11], Gobec, S.[1]

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Dementia is an umbrella term for progressive disorders characterized by cognitive decline, often caused by multifactorial pathophysiological mechanisms that may require combination therapies rather than monotherapy [1]. In the dementia brain, multiple neurotransmitter systems are disrupted, offering a range of therapeutic targets for prevention and intervention. Sigma-1 (σ_1) and sigma-2 (σ_2) receptor ligands [2], serotonin (5-HT) 2A receptor antagonists [3], and butyrylcholinesterase (BChE) inhibitors [4,5] have all shown therapeutic potential in the treatment of dementia.

We developed a novel multifunctional compound, **(*R*)-(–)-1** (Figure 1), which acts as a σ_1 and σ_2 receptor ligand, a 5-HT_2A receptor antagonist, and a BChE inhibitor. Compound **(*R*)-(–)-1** exhibits a high brain-to-plasma ratio in mice and demonstrates significant procognitive effects in two established mouse models of dementia: scopolamine-induced amnesia and amyloid-β_{1-42}-induced cognitive impairment. Notably, **(*R*)-(–)-1** does not induce cholinergic side effects, motor impairments, or acute toxicity in mice, supporting its potential as a safe and effective multitarget lead compound for treating dementia [5].

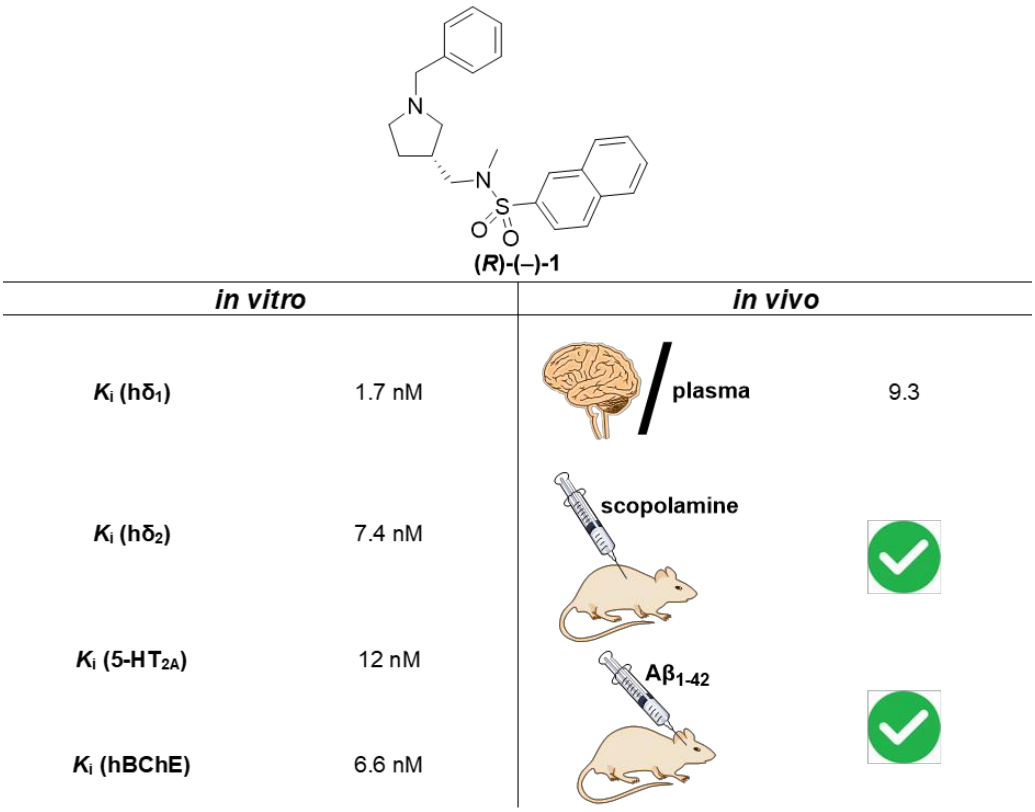

Figure 1. *In vitro* and *in vivo* pharmacological properties of compound **(*R*)-(–)-1**.

Keywords: *Dementia, lead optimization, multitarget compound, sigma-1 receptor, sigma-2 receptor, serotonin 2A receptor, butyrylcholinesterase, in vivo disease models*

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Design of dual sigma-1/CB2 receptor ligands as a therapeutic strategy for neurodegenerative disorders

Graziano, G.[1], Musillo, G.R.[1], Mammone, M.[1], Brea, J.[2], Loza, M.I.[2], Navarro, G.[3], Barry-Simonnet, C.[3], Meunier, J.[4], Maurice, T.[4], Stefanachi, A.[1], Colabufo, N.A.[1], Mangiatordi, G.F.[5], Laghezza, A.[1], Contino, M.[1], Abate, C.[1]

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Neurodegenerative disorders are characterized by multifactorial pathogenesis involving neuroinflammation, oxidative stress, and progressive synaptic and neuronal loss. Thus, therapeutic strategies able to modulate multiple targets simultaneously may offer synergistic neuroprotection. In this context, the sigma-1 receptor (S1R) and the Cannabinoid subtype-2 receptor (CB2R) have emerged as promising targets due to their complementary roles in regulating inflammation and neuroinflammation, neuroprotection, and cellular homeostasis. Altered levels of S1R and CB2R were found in AD patients' brain while some S1R and CB2R agonists have been shown to exert an important neuroprotection in preclinical models of neurodegenerative diseases. Notably, S1R agonists have also entered clinical phases for the treatment of neurodegenerative disorders. Building on our previously reported scaffolds with affinity for S1R and CB2R [1,2], we designed and synthesized novel structurally diverse dual-target ligands. Rational design strategies were employed to integrate key pharmacophoric elements required for the interaction with each receptor, aiming to achieve a balanced dual-target profile [3]. The synthesized compounds demonstrated significant affinity (nanomolar range) for both targets with further biological and computational investigations in progress to evaluate the extend of the synergistic effects induced by dual targeting of CB2R and S1R.

Keywords: *Dual-target strategy, cannabinoid 2 receptor, S1 receptor, neurodegeneration*

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Towards multi-target ligands for pain therapy: design and biological evaluation of compounds directed at sigma-1, histamine H_3, and μ-opioid receptors

Szczepańska, K.[1], Karcz, T.[2], Mogilski, S.[3], Pietruś, W.[1], Dichiara, M.[4], Ruiz-Cantero, M.C.[5], Obara, I.[6], Amata, E.[4], Cobos, E.J.[5], Bojarski, A.J.[1]

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Chronic pain, including neuropathic pain as one of its key forms, remains a major clinical challenge due to limited efficacy and adverse effects of current pharmacotherapies. Multi-target-directed ligands (MTDLs) represent a promising strategy to enhance analgesic efficacy through simultaneous modulation of complementary pain-related targets.[1,2]

In this study, we investigated a multi-target approach combining histamine H_3 receptor (H_3R) antagonism, sigma-1 receptor (σ_1R) antagonism, and μ-opioid receptor (MOR) agonism. An integrated computational workflow involving similarity analysis, machine learning models, and structure-based virtual screening was applied to identify candidate compounds, followed by experimental evaluation using radioligand binding assays. This strategy enabled efficient prioritization of compounds with a high probability of engaging multiple targets while maintaining favorable drug-like properties. As a result, a leading structure was identified that demonstrates the ability to simultaneously interact with all three receptors. This compound exhibited the highest affinity toward σ_1R, whereas its binding to H_3R and MOR remains moderate and will require further optimization to achieve a balanced multi-target profile. Importantly, this compound represents a first-in-class multi-target ligand acting simultaneously at H_3R, σ_1R, and MOR. Moreover, several dual-acting compounds were also discovered, supporting the validity of the applied design strategy. Notably, one compound exhibited one of the highest affinities for σ_1R and demonstrated antagonist properties at this receptor *in vivo*, confirming its functional relevance. These findings highlight the potential of multi-target modulation and identify a promising lead compound for further optimization and evaluation in animal models of chronic pain.

Keywords: *Chronic pain, multi-target-directed ligands, histamine H3 receptors, sigma-1 receptors, μ-opioid receptors*

Acknowledgments: This research was funded by the National Science Centre, Poland (2022/45/B/NZ7/03101).

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Machine learning and artificial intelligence tools for the rational design of novel sigma-1 receptor modulators

Alberga, D.[1], Nanna, V.[1], Papadopoulos, M.G.E.[1], Ancona, A.[1,2], Lomuscio, M.C.[3], Abate, C.[1,4], Mangiatordi, G.F.[1]

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The sigma-1 receptor (S1R) has emerged as a promising therapeutic target for neurodegeneration, cancer, pain, and viral infections. Despite growing interest, the computational toolbox for the rational design of S1R modulators remains limited. Herein, we present two complementary artificial intelligence (AI) platforms addressing distinct stages of the S1R drug discovery pipeline: bioactivity prediction and de novo molecular design.

SIGMAP[1] is an explainable AI tool for S1R affinity prediction. Twenty-five classifiers were trained on 2,018 curated compounds from ChEMBL v33, combining five machine-learning algorithms with five molecular fingerprint representations. The best model, based on support vector machines with Morgan fingerprints, achieved an AUC of 0.90. Two explainable AI approaches — SHAP and Contrastive Explanation — were implemented to enhance interpretability and guide medicinal chemistry optimization. SIGMAP is freely accessible as a web platform (https://www.ba.ic.cnr.it/softwareic/sigmap/).

ALCHIMIA[2] is an interpretable hybrid framework for de novo design combining reinforcement learning (RL) with a genetic algorithm (GA), built on 33 medicinal chemistry-inspired molecular transformations. The RL component prioritizes sequences optimizing synthetic accessibility and drug-likeness, while the GA leverages this policy within docking-guided optimization. Applied to S1R across three scenarios — hit identification, scaffold-constrained lead optimization, and dual CB2R/S1R modulator design — ALCHIMIA generated valid molecules with improved drug-likeness and synthetic accessibility compared to baselines and state-of-the-art models. ALCHIMIA is openly available (https://github.com/alberdom88/ALCHIMIA).

Together, SIGMAP and ALCHIMIA provide a comprehensive, interpretable, and freely accessible AI-driven workflow for the discovery and optimization of novel S1R modulators.

Keywords: *Machine learning, explainable artificial intelligence, de novo drug design, reinforcement learning, molecular fingerprints, in silico drug discovery*

Acknowledgments: This work was performed within the project: "Potentiating the Italian Capacity for Structural Biology Services in Instruct Eric (ITACA.SB)" (Project No.IR0000009), call MUR 3264/2021 PNRR M4/C2/L3.1.1.

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Ligand-driven allosteric rearrangements of the sigma-1 receptor revealed by molecular dynamics

Nanna, V.[1], Alberga, D.[1], Paternoster, C.[2,3], Bartocci, A.[2,3], Lattanzi, G.[2,3], Abate, C.[1,4], Mangiatordi, G.F.[1]

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Crystallographic data have revealed a trimeric architecture for the sigma-1 receptor (S1R), yet biochemical studies have identified higher-order oligomeric states that appear to play distinct functional roles [1,2]. Interestingly, S1R ligands, both agonists and antagonists, differently alter the oligomerization pattern. Moreover, changes in assembly have been associated with neurodegenerative diseases such as amyotrophic lateral sclerosis, suggesting a connection between supramolecular organisation and S1R functional response. Together, these observations establish oligomerization as a central regulatory mechanism and a promising therapeutic target in S1R-related pathologies, including paediatric diffuse midline glioma (DIPG), where S1R overexpression has been documented [3,4].

To investigate this mechanism, we performed all-atom molecular dynamics simulations of the S1R trimer embedded in a physiologically relevant MAM-mimicking membrane containing cholesterol, with the receptor bound to either haloperidol (antagonist) or pentazocine (agonist). We show that cholesterol stabilises the trimeric assembly independently of ligand identity, while the two ligands remodel intramolecular communication pathways in a distinct manner. Although both occupy the same binding pocket, the antagonist engages the β6 strand more deeply and shifts W136 — a residue that anchors interprotomer contacts and whose mutation is known to impair S1R multimerization [2]. This differential contact propagates through the protein network: haloperidol-bound S1R displays a communication pathway running coherently from E172 through the β-barrel to W136 and onward to the adjacent monomer, while pentazocine-bound S1R features a more fragmented architecture in which signal propagation across the intermonomeric interface is disrupted.

These findings provide an atomistic basis for ligand-dependent modulation of S1R assembly and identify W136 as a key hotspot bridging ligand recognition and interprotomer communication. By mapping the allosteric routes that connect the binding site to oligomeric interfaces, our computational framework offers a foundation for the virtual screening of drug candidates capable of selectively modulating S1R oligomerization.

Keywords: *Sigma-1 receptor, molecular dynamics, cholesterol, haloperidol, pentazocine, allostery, oligomerization*

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A Vintage-integrated modeling approach: From 2D-QSAR to molecular dynamics in search for new σ_1-receptor ligands

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The sigma-1 receptor (σ_1R) is an emerging target in neuropsychiatric disorders, where ligand-specific modulation drives distinct pharmacological effects. Here, we integrate chemometric modeling, molecular docking, and molecular dynamics (MD) simulations to elucidate structure–affinity relationships and binding properties of a series of novel arylpiperazine-based selenoethers toward σ_1.

A classical 2D-QSAR analysis revealed a parabolic relationship between lipophilicity (logD) and affinity, defining an optimal physicochemical window for σ_1R binding. Principal Component Analysis (PCA) further showed that the dataset is organized along orthogonal axes describing steric/lipophilic properties (PC1) and polarity (PC2).

To rationalize these findings at the molecular level, docking studies were performed to identify the key structural chemical cliches governing ligand recognition. Subsequently, MD simulations, carried out in comparison with reference σ_1R ligands, provided a dynamic description of ligand–receptor interactions. In particular, the most promising compound displayed a stable binding mode that explains its observed pharmacological profile.

Overall, this integrated approach offers a coherent framework for the rational design of novel σ_1R modulators.

***Keywords**: Selenoethers, σ_1R binding, chemometric, molecular modelling*

Investigation of σ1 receptor structure and function

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The human Sigma-1 receptor (hS1R) is an endoplasmic reticulum-resident transmembrane protein involved in neuroprotection and neuroplasticity, and is therefore an attractive therapeutic target for neurodegenerative diseases, including amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and Huntington's disease. Activation of hS1R promotes calcium transfer from the endoplasmic reticulum to mitochondria, sustains the unfolded protein response via IRE1 signaling, and stimulates autophagy, thereby counteracting endoplasmic reticulum stress. Although high-resolution three-dimensional structures of hS1R in complex with ligands have been determined, key aspects of its function remain unresolved [1]. These include the identity of endogenous ligands, the mechanism of ligand entry into the binding site, and the structural basis of hS1R oligomerization and its interaction with protein partners. Under basal conditions, hS1R forms a complex with the chaperone BiP, which dissociates upon agonist binding. In addition, hS1R oligomerization is dynamically regulated and disrupted by ligand engagement. To address these questions, we determined the cryo-EM structure of hS1R in its apo form and performed molecular dynamics simulations in both ligand-free and ligand-bound conditions [2,3]. In parallel, we investigated the mechanism of action of iloperidone, identified as a novel hS1R agonist [3], using both *in vitro* mass photometry and neurons derived from induced pluripotent stem cells from juvenile Huntington's disease patients. Our results show that iloperidone acts as a functional hS1R agonist, promoting receptor de-oligomerization. This is associated with reduced mutant huntingtin toxicity, including decreased apoptosis and oxidative stress, and activation of neuroprotective pathways such as the unfolded protein response [4].

Keywords: *Sigma1 receptor, cryo-EM structure, iloperidone, antagonist/agonist induced polymerization/depolymerization mechanisms*

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A standardized protocol for assessing the binding and selectivity of sigma-1 receptor-targeted ligands leveraging spectrofluorometry and cytofluorimetry: Experiences from the 1st COST ACTION SIGMA1-EUROPE training school

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Increasing evidence suggests that the Sigma-1 receptor (S1R) is critically involved in several physiological and pathological processes. Consequently, extensive research is underway to investigate, develop, and validate novel selective S1R ligands capable of modulating its multifaceted functions. Demonstrating target selectivity in living cells is a pivotal step that could accelerate the identification and development of promising S1R-targeted compounds.

By leveraging previously validated S1R ligands, we developed a standardized protocol to demonstrate target selectivity in living cells. First, the fluorescent S1R-selective ligands **MA3** ($K_{i\sigma1}$ = 20 nM, $K_{i\sigma2}$ = 140 nM) and **LM1** ($K_{i\sigma1}$ = 5.2 nM, $K_{i\sigma2}$ = 45.6 nM)[1] were utilized to measure target engagement via spectrofluorometry and cytofluorimetry in HEK 293T cells. Subsequently, a competitive binding assay was performed by co-incubating these fluorescent ligands with **(R/S)-RC33**, a potent and selective non-fluorescent S1R agonist ($K_{i\sigma1}$ = 0.7 nM, $K_{i\sigma2}$ = 103 nM)[2], which demonstrated efficient displacement of the fluorescent probes.

Finally, we established stable HEK 293T cell lines in which S1R was downregulated via lentiviral vector-mediated expression of an S1R-targeted shRNA (HEK S1R-KD), using a scramble shRNA (HEK.sc) as a control. By analyzing the internalization of fluorescent ligands through flow cytometry, we confirmed that the HEK S1R-KD cell model is suitable to demonstrate target selectivity.

Overall, this standardized protocol can be easily adapted to various cell lines and utilizes instrumentation commonly available in most laboratories, thereby accelerating the validation of novel S1R ligands.

Keywords: *Sigma-1 receptor ligands, fluorescent ligands, target-engagement, binding assays*

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Rare *SIGMAR1* gene variants in a cohort of neurodevelopmental disorders

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To date, the effect of sigma-1 receptor has been pointed out in previous studies on neurodevelopmental disorders. However, there is limited information about the rare variants in the *SIGMAR1* gene that encodes the sigma-1 receptor protein. In this study, we investigated *SIGMAR1* variants in a cohort of 318 individuals presenting with neurodevelopmental phenotypes.

Genetic analyses were performed using next-generation sequencing, and identified variants were evaluated according to ACMG/AMP guidelines. Two heterozygous missense variants in *SIGMAR1* were detected. The first variant, c.23C>T (p.Pro8Leu), identified in a male patient, was extremely rare (allele frequency: 6.5×10^{-6}) and classified as a variant of uncertain significance (VUS) (PM2, BP4). This variant has not been previously reported in the ClinVar database. The second variant, c.208C>T (p.Pro70Ser), detected in a female patient, was also rare (allele frequency: 1.3×10^{-5}) and classified as VUS (PM2) with moderate pathogenic evidence (CADD, PROVEAN). Both patients presented with similar phenotypes, including epilepsy and intellectual disability, consistent with the known phenotypic spectrum of *SIGMAR1*-related disorders.

Our findings highlight the importance of the rare *SIGMAR1* variants in epilepsy and intellectual disability and underscore the need for functional studies and larger datasets to clarify variant interpretation.

Previous studies in neurodevelopmental disorders related to Fragile X syndrome and Rett syndrome have demonstrated the potential therapeutic effects of sigma-1 receptor agonists in animal models, supported by limited clinical trials. We need future studies investigating effect of sigma-1 receptor agonists in patients with rare variants in the *SIGMAR1* gene.

Keywords: *Intellectual disability, epilepsy, rare genetic variations, SIGMAR1 gene*

Table 1: Summary of gene variants detected in our study

Case no	Age	Gender	Gene transcript	Variation/ (inheritance)	Exon	Amino acid variation	Variant type	gnomAD Frequency (genomes)	ACMG pathogenicity criteria	ClinVar	Conservation Scores phyloP100:	CADD	AlphaMissense	DANN	Zygosity	Associated phenotype
1	8	Male	SIGMAR1 (NM_001282206.2)	c.23C>T	2	p.(Pro8Leu)	missense	0.000006569	VUS/ BP4,BP1,PM2	Not Reported	9.725	Benign Strong/14.93	–	–	Heterozygous	epilepsy,ID
2	6	Female	SIGMAR1 (NM_005866.4)	c.208C>T	2	p.(Pro70Ser)	missense	0.00001314	VUS/ BP1,PP3,PM2	Reported	9.725	Pathogenic Moderate /29.1	Uncertain/ 0.4447	Uncertain/ 0.9986	Heterozygous	epilepsy,ID

Modulation of macrophage polarization via sigma-1 receptors

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Macrophage polarization is traditionally defined by pro-inflammatory (M1) and anti-inflammatory (M2) phenotypes; however, this binary classification does not fully reflect macrophage functional plasticity. Sigma-1 receptor (Sig-1R) has been implicated in immune regulation, yet its role in macrophage polarization remains unclear.

To investigate whether Sigma-1 receptor activation induces a classical M1-to-M2 transition or a context-dependent modulation of macrophage phenotype.

THP-1-derived macrophages were differentiated into M0, M1 (LPS + IFN-γ), and M2 (IL-4 + IL-13) phenotypes. Cells were treated with the Sigma-1 receptor agonist PRE-084 at increasing concentrations (2.5–15 μM), alone or in combination with the antagonist BD1047 (2.5–10 μM). Expressions of CD80, CD86, and CD206 were analyzed by flow cytometry, and mean fluorescence intensity (MFI) values were used for quantitative assessment. Morphological changes observed by microscopy were in line with flow cytometry findings.

Under M1 conditions, PRE-084 induced a dose-dependent increase in CD206 expression while CD80 and CD86 expression remained preserved, with no consistent downregulation observed. In M2 conditions, CD206 responses were heterogeneous and did not follow a consistent dose-dependent pattern. Co-treatment with BD1047 partially attenuated PRE-084–induced CD206 expression, although this effect varied across polarization conditions. In post-polarization experiments, PRE-084 increased CD206 expression, whereas co-treatment with BD1047 reduced this effect.

Sigma-1 receptor activation does not induce a classical M1-to-M2 transition but rather promotes a context-dependent modulation of macrophage activation. These findings support a dynamic model of macrophage plasticity and may help explain inconsistencies reported in Sigma-1 receptor–related immune responses.

Keywords: *Sigma 1 receptors, monocyte, macrophage, immune cells*

KEYNOTE LECTURE

Classical psychedelic DMT and ketamine enantiomers as rapid-acting antidepressants: Sigma-1 receptor signaling and beyond

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Depression is a leading cause of disability worldwide, and a substantial proportion of patients respond inadequately to currently available antidepressants. Effective pharmacological strategies that reliably prevent suicide in severe depression also remain limited, representing a critical unmet medical need. In this context, rapid-acting antidepressant approaches have received intense attention, particularly classical serotonergic psychedelics and the dissociative anesthetic ketamine. Among classical psychedelics, *N,N*-dimethyltryptamine (DMT) is being explored as a fast-acting option for treatment-resistant depression. Beyond its well-known serotonergic actions, DMT has been proposed as an endogenous agonist of the sigma-1 receptor, raising the possibility that sigma-1 receptor signaling contributes to its neurobiological and therapeutic effects. In parallel, ketamine is an established rapid-acting antidepressant, and esketamine nasal spray is widely used in clinical practice for treatment-resistant depression and related depressive symptoms. Accumulating preclinical evidence suggests that arketamine may produce more robust and longer-lasting antidepressant-like effects than esketamine in several rodent models. Notably, arketamine appears to show higher affinity for sigma-1 receptors than esketamine, supporting renewed interest in sigma-1 receptor–related mechanisms within rapid antidepressant pharmacology. In this keynote lecture, I will highlight recent advances in DMT and ketamine enantiomers as rapid-acting antidepressants, emphasizing sigma-1 receptor–dependent signaling alongside additional mechanisms relevant to efficacy, safety, and translational development.

Keywords: *Psychedelics, dimethyltryptamine, ketamine, arketamine, esketamine, sigma-1 receptors*

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Spirocyclic dual-acting soluble epoxide hydrolase inhibitors and sigma-1 receptor antagonists as non-opioid analgesics

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The identification of novel non-opioid analgesics remains a major health challenge. Given the multifactorial nature of pain, **multitarget approaches** have emerged as a promising alternative. Among arising therapeutic targets, **soluble epoxide hydrolase (sEH)** and the **sigma-1 receptor (S1R)** have attracted increasing interest due to their complementary roles in pain modulation. sEH inhibition enhances the levels of anti-inflammatory and analgesic epoxy fatty acids,[1] while S1R antagonism modulates neuronal excitability through protein–protein interactions at key pain-processing sites in the central nervous system.[2]

Based on previous validation of this dual-target approach and the identification of the preclinical candidate UB-EPB-117,[3,4] here we report the design, synthesis, and optimization of a second family of dual sEH inhibitors and S1R antagonists. The new series was rationally designed through replacement of the amide pharmacophore with a urea moiety to expand chemical space and explore alternative previously validated sEH-binding motifs. A systematic structure–activity relationship study was performed, focusing on aromatic substitution patterns and on the replacement of piperazine motifs by **spirocyclic and bicyclic bioisosteres**.[5] The introduction of spirocyclic systems proved to be a successful strategy, leading to a marked **improvement in metabolic stability** while preserving dual target activity.

As a result of this optimization campaign, several compounds with balanced *in vitro* activity and improved DMPK-related properties were identified. The most advanced candidates are currently under *in vivo* evaluation as potential **back-up molecules to UB-EPB-117**. Overall, this work highlights spirocyclic dual sEH/sigma-1 ligands as a promising medicinal chemistry strategy for developing innovative non-opioid analgesics.

Keywords: *Multitarget, dual, spirocyclic, soluble epoxide hydrolase, sigma-1 receptor, analgesia, non-opioid analgesics*

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Enhanced neuroprotection by the σ_1 receptor agonist PRE-084 against type 2 diabetes complication in an Alzheimer's disease mouse model

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Type 2 diabetes mellitus (T2DM) is increasingly recognized as a major risk factor for Alzheimer's disease (AD), with mounting evidence highlighting shared pathophysiological mechanisms. In particular, we previously showed that σ_1 receptor agonists are neuroprotective in AD preclinical models, a therapeutic strategy recently validated by the positive phase 3 clinical trial results obtained for Blarcamesine. σ_1 receptor agonists have also been proposed to alleviate diabetes-associated cognitive dysfunction. We therefore developed a pharmacological murine model of AD associated with type 2 diabetes and analyzed the protective effect of the σ_1 receptor agonist PRE-084. Mice were fed with a high fat (24%) high sugar (22% + 20% fructose in water) diet (HFHFD) for 14 weeks and where then intracerebroventricularly injected with oligomerized $A\beta_{25\text{-}35}$ peptide. Control regimen included 5% fat and 4% sugar + tap water (ND). Weight gain, sucrose and insulin tolerance were altered following HFHFD. Mice showed memory deficits in spontaneous alternation and active avoidance in the rectangular water-maze. The PRE-084 treatment (0.3-3 mg/kg IP) alleviated the weight gain and memory deficits but failed to restore the sucrose and insuline response. Neuroinflammation was observed in the brain of HFHFD mice and deregulation of the insuline receptor-mediated pathway (IRS1, GSK3β, AKT was measured by Western blot in the mouse hippocampus, that was alleviated by PRE-084 (3 mg/kg). HFHFD mice treated with increasing doses of $A\beta_{25\text{-}35}$ peptide showed a significant alteration of short-term memory and an additive effect on both short- and long-term memories. Treatment with PRE-084 (0.1-3 mg/kg IP) totally prevented the $A\beta_{25\text{-}35}$-induced memory deficits in HFHFD mice with a minimally active dose of 0.1 mg/kg vs. 0.3 mg/kg in ND mice. Recognition memory and anxiety were also ameliorated with PRE-084. These observations suggested that a therapeutic intervention through σ_1 receptor agonism is a shared and particularly effective strategy in patients with AD and comorbid diabetes.

Empagliflozin modulates ER stress and mitochondria-associated membrane signaling in the brain of type 2 diabetic rats: Involvement of sigma-1 receptors

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Background: Type 2 diabetes mellitus (T2DM) is associated with progressive neurodegeneration characterized by chronic endoplasmic reticulum (ER) stress, disrupted calcium handling, and impaired mitochondrial function. Mitochondria-associated membranes (MAMs) play a central role in coordinating calcium transfer and metabolic signaling between the ER and mitochondria. Key regulatory components of this interface, including the Sigma-1 receptor (Sig-1R), glucose-regulated protein 78 (GRP78), and sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), are essential for maintaining neuronal homeostasis. Dysregulation of these pathways contributes to insulin resistance, oxidative stress, and neuronal dysfunction in diabetes. Empagliflozin, a selective SGLT2 inhibitor, has demonstrated metabolic and neuroprotective benefits, yet its effects on ER–mitochondrial signaling and MAM integrity remain unclear.

Objective: This study aimed to investigate the impact of empagliflozin treatment on ER stress markers, MAM-associated signaling proteins, and calcium regulation in the brains of rats with experimentally induced type 2 diabetes.

Methods: T2DM was induced using a high-fat diet combined with streptozotocin. Animals were assigned to control, diabetic, and empagliflozin-treated diabetic groups. Brain tissues were analyzed for Sig-1R, GRP78, and SERCA2 protein levels by immunoblotting, while reactive oxygen species (ROS) levels were measured using luminometry. Additional indicators of ER stress and mitochondrial function were evaluated to assess changes in MAM-related pathways.

Results: Diabetic animals exhibited elevated GRP78 expression, decreased SERCA2 levels, and altered Sig-1R expression, indicating increased ER stress, impaired calcium homeostasis, and disrupted MAM integrity. Empagliflozin treatment attenuated ER stress, improved calcium regulatory capacity, and modulated Sig-1R-associated pathways. A reduction in ROS levels was also observed in the empagliflozin-treated group.

Conclusions: These findings suggest that empagliflozin may exert neuroprotective effects in T2DM by modulating ER stress and restoring MAM-related signaling. Regulation of the Sig-1R–GRP78–SERCA axis may represent a key mechanism underlying the central benefits of SGLT2 inhibition.

Keywords: *Empagliflozin, SGLT-2 inhibitors, ER stress, reactive oxygen species*

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Pharmacological evaluation of new multi-target ligands against sigma-1 and histamine H_3 receptors for the treatment of Alzheimer's disease

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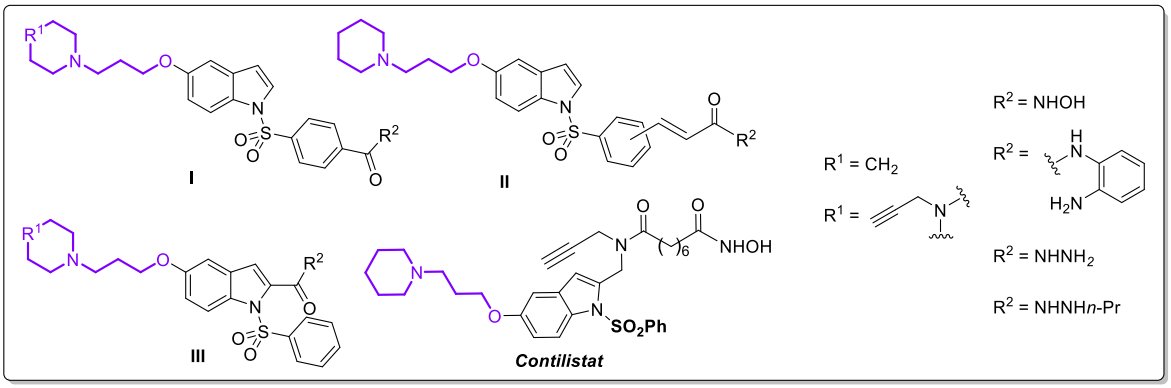
Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects the elderly and for which there is currently no effective treatment. This is largely due to the highly complex nature of the disease, as multiple biological targets are involved. Consequently, one of the most promising strategies under investigation is the development of multi-target drugs capable of simultaneously modulating several targets within a single molecule, potentially leading to improved therapeutic outcomes.

Contilisant,[1] is a multitarget-direct ligand (MTDL), designed and developed by our research group, that inhibits monoamine oxidase and cholinesterase enzymes, while also modulating histamine H_3 receptor (H_3R) and sigma-1 receptor (S1R). It is able to cross the blood-brain barrier and exhibits neuroprotective and antioxidant activities. In this context, our aim is to optimise *Contilisant*'s pharmacological profile by incorporating new pharmacophores capable of interacting with the serotonin 5-HT_6 receptor and histone deacetylase enzymes.

In this study, we present a series of compounds analogous to *Contilisant,* characterised by the presence of the piperidinopropoxy pharmacophore in their structure. These novel derivatives have been pharmacologically evaluated against S1R and H_3R.[3] Several of these synthesised compounds have demonstrated promising results and, in the future, they will be further investigated with the aim of developing a treatment for AD.



Keywords: *Alzheimer's disease, MTDL, S1R agonists, H_3R antagonists*

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The sigma-1 receptor agonist fluvoxamine alleviates endotoxin-induced acute lung injury in mice

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are severe inflammatory conditions with high mortality, characterized by extensive immune cell infiltration, increased vascular permeability, and respiratory failure. We previously demonstrated the antifibrotic effects of the Sigma1 receptor (S1R) agonist fluvoxamine (FLU) in different organs; therefore, this study aimed to investigate its protective mechanisms via S1R activation in an LPS-induced acute pneumonitis model.

C57BL/6J (wild-type) and S1R knock-out (S1R-/-) mice were randomized into 1) PBS+vehicle, 2) LPS+vehicle, 3) LPS+FLU or 4) LPS+dexamethasone groups. Acute pneumonitis was induced by intratracheal administration of endotoxin (lipopolysaccharide, LPS; 0.5 mg/kg) or PBS as control. Respiratory function was assessed by non-invasive restraint plethysmography 24 h later. Lung weight, inflammatory cell infiltration and cytokine mRNA expressions were determined by histopathology and qPCR.

LPS significantly reduced tidal volume, minute ventilation, peak expiratory, inspiratory and mid-tidal expiratory flows, which were counteracted by FLU in wild-type animals in similarly to the reference compound dexamethasone, but not in S1R-/- mice. Furthermore, LPS-induced inspiratory and expiratory time decreases were aggravated in FLU-treated S1R-/- mice. Fluvoxamine reduced the number of CD68+ macrophages 24h after LPS administration. FLU downregulated IL-1, IL-6, TNFα and monocyte chemoattractant protein-1 expression in wild-type, but not in S1R-/- mice.

Fluvoxamine reduces acute pneumonitis and related functional deteriorations mainly via S1R, suggesting its potential to be an effective and safe alternative to glucocorticoids for lung injury.

Keywords: *Sigma1 receptor, fluvoxamine, acute lung injury, lipopolysaccharide*

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Development of positron emission tomography (PET) and fluorescent ligands for the imaging of sigma-1 receptor (S1R)

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The sigma-1 receptor (S1R), a pluripotent chaperone protein highly expressed in the central nervous system, is involved in neurological disorders, inflammation and neuropathic pain. Consequently, research has focused on studying this receptor using techniques such as positron emission tomography (PET) and fluorescence-based imaging. PET imaging of S1R holds potential for early diagnosis, while fluorescence-based approaches allow investigation of its mechanisms of action. Among the synthesized compounds, the phenoxyalkylpiperidine class emerged as the most promising. In particular, compound L6 (1-(2-(4-chlorophenoxy)ethyl)-4-methylpiperidine) exhibited subnanomolar affinity and high selectivity (K_iS1R 0.86nM, K_iS2R 239nM)[1]. Therefore, several L6 derivatives were synthesized and evaluated to identify the most suitable candidate for radiolabeling with fluorine-18. One compound bearing fluoroethoxy chain demonstrated nanomolar S1R affinity with remarkable selectivity against S2R, highlighting its potential as an imaging agent. It was successfully labeled with fluorine-18, achieving high radiochemical yield and molar activity. Its *in vitro* stability and S1R specificity were evaluated through autoradiography on mouse brain sections. For fluorescent ligands, L6 scaffold was modified by introducing alkyl chains linked to fluorescent tags spanning from green to Near-InfraRed. Promising compounds with high selectivity, affinity and low lipophilicity were obtained and studied by flow cytometry on HEK293 cells overexpressing S1R. They demonstrated dose-dependent uptake and specific binding, suggesting ability to replace radioligands in binding assays. Confocal microscopy studies revealed co-localization with S1R, mitochondria and endoplasmic reticulum, with some off-target binding. In conclusion, the phenoxyalkylpiperidine scaffold is promising for developing PET and fluorescent S1R ligands, supporting further studies on S1R as a biomarker and a therapeutic target.

Keywords: *Sigma-1 receptor, PET ligands, fluorescent ligands, imaging*

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Brain region- and sex-specific transcriptome of sigma-1 receptor knock-out mice

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Activation of the stress-regulated chaperone Sigma-1 receptor (Sigmar1) has shown neuroprotective potential, while recessive *Sigmar1* mutations have been linked to several neurodegenerative diseases. However, gene expression changes in *Sigmar1* knockout (*Sigmar1*−/−) animals—particularly across brain regions and between sexes—remain poorly characterized. Through comprehensive transcriptomic analyses of specific cerebral regions, our study aimed to reveal how *Sigmar1* deletion influenced gene expression, regulatory pathways, and potential functional outcomes, as well as to characterize its association with pathways implicated in neurodegenerative diseases.

Wild-type (WT) and *Sigmar1*−/− mice of both sexes were used in this study. Total RNA was isolated from ten brain regions, followed by RNA sequencing with the DNBSEQ-G400 platform. Genome alignment and differential gene expression analysis were performed, and functional interpretation was conducted using Gene Ontology (GO) enrichment analyses.

Principal Component Analysis identified brain region as the primary driver of transcriptomic variation. Differential expression analysis revealed significant alterations in genes including *Vcp, Ccl27a, Il11ra1, Robo3, Nudt2, Car8, Fancg*, and *Rasgrp1*, with marked region- and sex-specific differences. Consistent with previous findings, reduced *Gaba-b R2* expression was observed in the hippocampus and habenula of male *Sigmar1*−/− mice, along with decreased *Gfap* expression in the cerebellum. Enriched pathways included protein processing, cellular stress responses, DNA repair, synaptic and neuronal functions, signaling pathways, immune responses, and pain-related mechanisms.

These findings highlight region- and sex-dependent roles of *Sigmar1* and its involvement in pathways underlying neurodegenerative disorders. Ultimately, these findings provide a foundation for more targeted research and the development of therapeutic interventions for neurodegenerative diseases.

Keywords: *Sigma-1 receptor, RNA-seq, transcriptomics, sex differences, neurodegenerative pathways*

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Design, synthesis, and photopharmacological evaluation of piperazine-based photoswitchable ligands targeting the chaperone protein sigma-1

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The sigma-1 receptor (S1R) is a ligand-regulated chaperone protein involved in multiple physiopathological processes, including neurodegenerative disorders. Accumulating evidence indicates that S1R agonists exert neuroprotective effects, particularly in the context of Alzheimer's disease. In this framework, the development of molecular tools, such as photoswitchable ligands, represents a promising strategy to achieve optical control of S1R activity with high spatial and temporal precision. Here, we report the design, synthesis, and biological evaluation of the first set of photoswitchable S1R ligands. Among the compounds synthesized, two (11d and 15a) displayed light-dependent activity in radioligand binding as well as in the Bip assay. Remarkably, the compounds also restored physiological locomotion in wfs1ab knockout zebrafish larvae in their trans-isoform, while showing no effect in the cis-isoform. Moreover, both compounds were evaluated in MK-801-induced amnesia Swiss mouse model, and they exhibited a "trans-on" anti-amnesic effects characteristic of S1R agonists at doses of 1 mg/kg (compound 15a) and 3 mg/kg (compound 11d), respectively.

Keywords: *Chaperone protein, light, photopharmacology, sigma-1*

Oligomerization dynamics of sigma-1 receptor: trimer, hexamer and nonamer revealed by Cryo-EM and mass photometry

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The Sigma-1 receptor (S1R) is an evolutionarily conserved ER-resident membrane protein acting as a pluripotent chaperone and signalling modulator. It maintains cellular homeostasis by orchestrating ER-mitochondria communication at MAMs, sensing lipid microenvironments, calcium flux, and protein quality control as a stress-response hub[1].

Structurally, S1R is a ~25 kDa single-pass transmembrane protein forming homo-oligomers. Crystallography reveals a trimeric architecture with a transmembrane helix and β-barrel-like ligand-binding domain (PDB: 5HK1, 6DK1)[2]. Ligand binding triggers conformational changes that regulate oligomerization and localization, linking chaperone activity to signalling.

S1R interacts with the ER chaperone BiP; under stress or ligand binding, S1R dissociates from BiP to stabilize client proteins at MAMs, enhancing cellular resilience.

We used mass photometry to investigate S1R oligomerization states in detergent solution, identifying distinct peaks with masses consistent with S1R monomer, trimer, hexamer, and nonamer, along with undefined higher molecular weight species.

Single-particle cryo-EM analysis of S1R in detergent enabled reconstruction of three 3D volumes: a trimer matching prior crystallographic structures, plus hexamer and nonamer forms composed of related trimers with two-fold and three-fold symmetry, respectively. We resolved the trimer and hexamer structures to 2.7 Å and 3.3 Å, respectively. Notably, no density appears in the hexamer active site, while density for an undefined ligand appears in the trimer volume.

Mass photometry on BiP characterized its oligomerization and ATP-hydrolysis cycle, revealing monomerization upon ATP binding with an apparent K_d of 0.6 μM.

We are now employing mass photometry to biophysically probe BiP-S1R interactions across the BiP ATP-hydrolysis cycle and varying Ca^{++} concentrations.

Keywords: *Sigma 1 receptor (σ1R), cryo-electron microscopy (Cryo-EM), hexameric/nonameric form, ligand free form, protein-protein interaction, mass photomtery, BiP, chaperone*

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Endoplasmic reticulum stress marker GRP78/BiP alterations in APOE ε4 positive Alzheimer's disease patients

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Endoplasmic reticulum (ER) stress and maladaptive activation of the unfolded protein response (UPR) may contribute to neurodegenerative pathways in Alzheimer's disease (AD). UPR mediators including GRP78/BiP may be used as indicators of misfolded-protein burden in neuronal tissue. The sigma-1 receptor (S1R), an ER-resident chaperone, modulates proteostasis by interacting with BiP/GRP78 and regulating ER–mitochondrial signaling. The study aimed to evaluate GRP78/BiP in patients with mild cognitive impairment (MCI) and Alzheimer's dementia, and to assess whether its relationship with tau pathology is influenced by APOE ε4 status. A total of 39 individuals with MCI or AD dementia were included. Plasma and cerebrospinal fluid (CSF) GRP78/BiP concentrations were quantified using an ELISA assay. Standard AD CSF biomarkers (t-Tau/Aβ42 and p-Tau/Aβ42 ratios) and APOE ε4 genotype were determined. Group comparisons used Mann–Whitney U tests, and associations between ER-stress markers and tau ratios were assessed with Spearman correlations. Absolute GRP78/BiP levels did not significantly differ between diagnostic groups (CSF $p = 0.756$; plasma $p = 0.847$; CSF/plasma ratio $p = 0.877$). However, among APOE ε4+ individuals, the CSF/plasma GRP78 ratio correlated positively with the t-Tau/Aβ42 ratio ($\rho = 0.485$, $p = 0.041$). No correlation was observed in ε4− individuals ($p = 0.889$). These findings provide preliminary evidence that ER-stress dysregulation, reflected by altered GRP78/BiP dynamics, is linked to tau pathology specifically in APOE ε4 carriers. Given the functional interaction between GRP78/BiP and the S1R, our results support the hypothesis that impaired sigma-1 receptor signaling may amplify ER-stress sensitivity and downstream tau pathology.

Keywords: *Endoplasmic reticulum stress, GRP78/BiP, t-Tau/Aβ42 ratio, APOE ε4 genotype, Alzheimer's disease*

In silico identification of dual-target ligands modulating TAAR1 and SIGMAR1 for Parkinson's disease with comorbid depression

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Central nervous system polypharmacology represents a promising therapeutic strategy for the treatment of neurodegenerative disorders accompanied by mood disturbances, such as Parkinson's disease with comorbid depression. Concurrent modulation of multiple monoaminergic systems may improve both motor and non-motor symptoms [1]. Among these, trace amine-associated receptor 1 (TAAR1) and the sigma-1 receptor (SIGMAR1) play key roles in the regulation of dopaminergic and serotonergic neurotransmission, and their simultaneous targeting may constitute an effective therapeutic approach [2–3].

In this study, we performed virtual screening of a compound library developed within our research group to identify ligands with potential dual-target activity at TAAR1 and SIGMAR1. The screening workflow combined molecular docking and molecular dynamics simulations, enabling the selection of compounds exhibiting favorable predicted interaction profiles with both targets. The identified candidates provide a foundation for further structural optimization and experimental validation in the context of Parkinson's disease accompanied by comorbid depressive symptoms.

Keywords: *Parkinson's disease, depression, polypharmacology, TAAR1, SIGMAR1*

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KEYNOTE LECTURE

Targeting sigma-2 receptors as a promising therapy for the treatment of neurodegenerative diseases

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Sigma-2 receptors (σ2R/TMEM97) have recently emerged as promising targets for treating neurodegenerative diseases, given their roles in cholesterol handling, endo-lysosomal function and the processing of proteins such as amyloid-β and α-synuclein. Studies in cells and animal models show that modulating σ2R activity can restore synaptic function, enhance clearance of misfolded proteins and dampen neuroinflammatory responses, positioning σ2R as a key regulator of neuronal resilience in age-related brain disorders.

Building on this biology, several σ2R-focused drug programmes have advanced into late preclinical or early clinical development, supported by phase 1–2 data in Alzheimer's disease, dementia with Lewy bodies and dry age-related macular degeneration alongside detailed measures of target engagement and cerebrospinal fluid proteomic change.

In this presentation, I will show new, unpublished findings on a next-generation σ2R-targeting lead compound, including data from multiple experimental paradigms spanning *in vitro* and *in vivo* settings. Results will be discussed in terms of their pharmacological relevance and translational value, providing a first look at the therapeutic potential of this compound series in neurodegeneration.

Keywords: *Sigma-2 receptors, neurodegeneration, small-molecule ligands, target validation, lead optimization, drug discovery*

SIGMAR1-related functional EEG signatures of Alzheimer's disease: Insights from theta/alpha ratio and cognitive performance

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Background: Synaptic dysfunction is a hallmark of Alzheimer's disease (AD). The Sigma-1 receptor (SIGMAR1) is involved in synaptic stability, calcium signaling, and neuroprotection. EEG spectral slowing may reflect synaptic disruptions linked to SIGMAR1 pathways.

Objective: To evaluate whether EEG-derived features, particularly the theta/alpha ratio, discriminate AD from controls and relate to cognitive performance within a SIGMAR1-relevant framework.

Methods: EEG data from an open-access OpenNeuro dataset (BIDS format) were analyzed, including 65 participants (36 AD, 29 controls). Spectral features (delta, theta, alpha, beta) and the theta/alpha ratio were extracted. Group differences were assessed using FDR correction. Logistic regression and ROC analyses evaluated classification performance. Cognitive function (MMSE) was analyzed using linear regression with and without adjustment for diagnosis.

Results: The theta/alpha ratio was significantly higher in AD (FDR-adjusted $p < 0.001$) and predicted AD status (OR = 2.41, $p = 0.001$; AUC = 0.766). Sensitivity improved to 75% after threshold optimization (F1 = 0.74). The ratio was associated with lower MMSE scores in unadjusted analysis ($p = 0.002$), but not after controlling for diagnosis or within the AD group.

Conclusion: The theta/alpha ratio is a robust EEG biomarker for distinguishing AD from controls but does not reflect within-group cognitive severity. Findings support the role of SIGMAR1-related synaptic dysfunction in AD.

Keywords: *SIGMAR1, Alzheimer's disease, EEG spectral analysis, theta/alpha ratio, synaptic dysfunction*

Pridopidine, a potent and selective therapeutic sigma-1 receptor (S1R) agonist for the treatment of neurodegenerative and neurodevelopmental diseases

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Pridopidine, a selective S1R agonist, is in development for Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and other neurodegenerative and neurodevelopmental diseases.

In preclinical models including HD, ALS, Vanishing White Matter (VWM), retinal degeneration, and Rett Syndrome, pridopidine activation of the S1R restores commonly impaired cellular pathways. Pridopidine exerts protective effects by restoring the integrity of the mitochondrial associated membrane (MAM) and improving calcium homeostasis, mitochondrial function, and autophagy while simultaneously alleviating oxidative and ER stress. Furthermore, S1R activation leads to improvements in dendritic spine density, synaptic function, and BDNF transport and signaling. All of these together contribute to improved neuronal health and survival.

Human brain PET imaging confirms pridopidine target engagement, with > 90% S1R occupancy at the clinically relevant dose (45 mg bid). In clinical trials, pridopidine consistently demonstrated a favorable safety profile comparable to placebo. In the Phase 3 PROOF-HD trial (NCT04556656) in patients with HD, and its open-label extension, pridopidine showed benefit in key independent features of HD including function, motor, cognition, and progression in subjects not using antidopaminergic medications (ADMs). The clinically meaningful benefits were significant and sustained through 2 years as compared to propensity score matched cohorts from two natural history studies (ENROLL-HD and TRACK-HD). These findings are being further evaluated in a pivotal Phase 3 (PRECISE-HD) clinical trial to initiate in 2026.

The Phase 2 Healey ALS Platform Trial (NCT0461592) showed improvements with pridopidine over 24 weeks in faster progressing subjects, slowing disease progression, respiratory decline, bulbar function, speech, and increasing time-to-median survival. Based on these findings, the PREVAiLS (NCT07322003) pivotal Phase 3 study was designed to prospectively evaluate pridopidine in an ALS population and is currently ongoing.

Pridopidine is also being assessed in an expanded access program (EAP) for VMW in 2 subjects, whom showed no decline in disease progression or change in cognition and other disability assessments. Furthermore, plasma neurofilament light chain (NfL) biomarker levels in these subjects were markedly reduced with pridopidine treatment; and BDNF was simultaneously increased suggesting improved trophic support and neuronal protection.

Overall, pridopidine continues to be developed in late-stage clinical trials as a highly-attractive, novel S1R therapeutic candidate for a spectrum of neurodegenerative diseases.

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Preclinical development of RC-106: A brain-permeant sigma receptors modulator against glioblastoma

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Glioblastoma (GBM) is the most aggressive primary brain tumor in adults, with a 5-year survival rate of only 6.6%, highlighting the urgent need for new therapeutic strategies.[1,2] In this context, Sigma receptors (SRs) have emerged as promising targets for the development of innovative therapeutic approaches: both Sigma-1 (S1R) and Sigma-2 receptors (S2R) are overexpressed in GBM and play key roles in tumor proliferation, survival, and malignancy.[3,4]

In this study, RC-106, a S1R antagonist and S2R agonist,[5,6] was investigated for its therapeutic potential against GBM. The compound was synthesized on a ten-gram scale using green chemistry approaches. *In vitro* evaluation using 2D and 3D patient-derived GBM cell lines confirmed its anticancer activity, with IC_{50} values ranging from 44 to 54 μM. Pharmacokinetic studies demonstrated that RC-106·HCl effectively crosses the blood–brain barrier and accumulates in brain tissue. Clearance was moderate in mouse microsomes and low in human microsomes (Cli = 44.0 and 11.8 μL/min/mg protein, respectively). *In vivo* efficacy was assessed using a patient-derived orthotopic xenograft (PDOX) mouse model. Daily intraperitoneal administration of RC-106·HCl (20 mg/kg) was well tolerated, with no observable systemic or neurological toxicity. Although tumor growth was not completely halted, a significant, time-dependent reduction in intracranial tumor burden was observed.

Overall, these findings support the viability of SRs as pharmacological targets in oncology and provide a first *in vivo* proof-of-concept for RC-106 in GBM treatment. Further optimization, including improved formulations, delivery, and/or dosing regimens, will be necessary to enhance exposure and fully realize its clinical potential.

Keywords: *Sigma-1 receptor, sigma-2 receptor, glioblastoma, sustainable synthesis, 2D and 3D cell cultures, PDOX, in vivo experiments*

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Sigma-1 receptor modulation as a potential missing link in ONC201 mechanism of action in diffuse intrinsic pontine glioma (DIPG)

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Mitochondrial dysfunction represents a critical vulnerability in diffuse intrinsic pontine glioma (DIPG), a highly aggressive pediatric brain tumor with limited therapeutic options. **ONC201**, an imipridone currently under clinical evaluation in DIPG, has emerged as a promising agent; however, its mechanism of action remains only partially understood. Although **ONC201** is known to induce mitochondrial stress through activation of the mitochondrial protease ClpP, this mechanism alone does not fully explain its biological activity, as other ClpP activators fail to reproduce comparable antitumor effects, suggesting a broader multitarget profile. In this context, we investigated the potential involvement of the Sigma-1 receptor (σ1R), a chaperone localized at mitochondria-associated ER membranes (MAM), which regulates Ca^{2+} signaling and proteostasis at the ER-mitochondria interface. The spatial proximity and functional interplay between σ1R and mitochondrial pathways support a possible contribution of σ1R to **ONC201**-induced mitochondrial dysfunction. Surface plasmon resonance experiments confirmed **ONC201** interaction with ClpP (K_D = 610 ± 70 nM). In parallel, preliminary binding studies indicate that **ONC201** also engages σ1R (Ki ≈ 293 nM) within a pharmacologically relevant range (Figure 1). Haloperidol, used as a reference ligand due to its dopaminergic D2 antagonism similarly to **ONC201**, also displays σ1R binding, supporting a potential shared pharmacological space. Notably, Western blot analysis revealed co-expression of σ1R and ClpP across CNS-derived cell lines, (BV-2 microglia cells, SH-SY5Y neuroblastoma cells, SU-DIPG-36 and SU-DIPG-50 patient-derived cells) suggesting a convergent vulnerability that may be therapeutically exploitable.

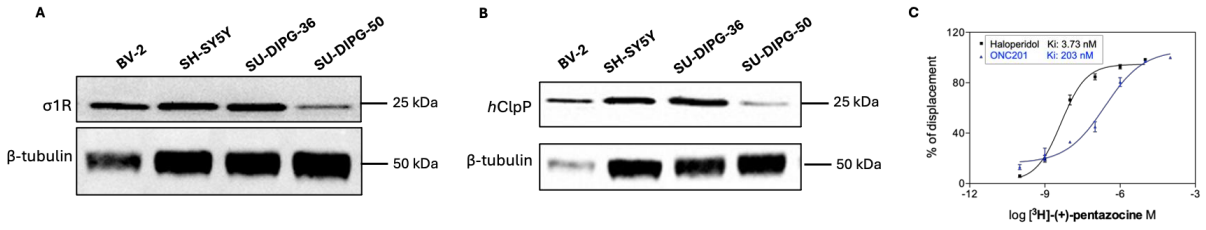

Figure 1. Sigma-1 receptor (σ1R) and ClpP expression across CNS-derived cell lines and σ1-binding profile of **ONC201** and haloperidol.

Ongoing studies aim to elucidate the functional impact of σ1R modulation on **ONC201**-induced mitochondrial stress, to determine whether σ1R represents an additional component of its mechanism of action and a potential target for multitarget therapeutic strategies in DIPG.

Acknowledgments: This work was supported by LorEdo per la Vita ODV, which funded a research fellowship awarded to Anselma Liturri.

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Sigma-1 receptor-targeted nanofibrous biomaterial systems for modulating cellular stress and enhancing wound healing

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The development of multifunctional biomaterials capable of modulating cellular stress responses represents a promising strategy in regenerative medicine and translational therapeutics. In this context, Sigma-1 receptor (Sig-1R), an endoplasmic reticulum (ER)-resident chaperone protein, has emerged as a key regulator of cellular homeostasis, calcium signaling, and stress adaptation, particularly under conditions of inflammation and hypoxia.

In the present study, we propose the development of quercetin-loaded polyvinyl butyral/polyethylene glycol (PVB/PEG)-nanoclay-based nanofibrous biomaterial systems, fabricated via solvent casting and/or electrospinning approaches, for potential application as bioactive wound dressings. The project is based on the hypothesis that incorporation of quercetin, a well-known antioxidant and anti-inflammatory flavonoid, into nanostructured biomaterials may synergistically interact with Sigma-1 receptor-mediated pathways to enhance cellular survival, reduce oxidative stress, and accelerate wound healing processes.

The designed biomaterial systems aim to provide controlled drug release, improved mechanical stability, and enhanced biocompatibility. *In vitro* evaluations will be conducted using L929 fibroblast cells to assess cytotoxicity, cell proliferation, and wound closure dynamics through scratch assays. In parallel, the potential involvement of Sigma-1 receptor-related pathways in mediating cellular responses to the biomaterials will be explored conceptually, with future studies planned to include molecular analyses such as gene expression and protein-level validation.

This study introduces a novel conceptual framework integrating biomaterial-based drug delivery systems with Sigma-1 receptor biology. The findings are expected to contribute to the development of next-generation smart wound dressings and may open new avenues for targeting cellular stress pathways in both regenerative medicine and disease contexts, including cancer and neurodegeneration.

Keywords: *Sigma-1 receptor, quercetin, nanofiber, biomaterial, wound healing, cellular stress*

KEYNOTE LECTURE

Serotonergic psychedelics and the brain neurochemical tissue

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Classical psychedelics bind some serotonergic receptors (5-HTR) and alter brain functions. Despite the clinical interests, brain mechanisms triggered by psychedelics are still elusive. They likely involve changes of neurotransmitters function alone or in interaction at brain scale, but it is never studied and cannot be addressed *in vivo*. The tissue measurement (post mortem) of neurotransmitters allows for quantitatively approximating presynaptic content of neurotransmitters in multiple brain regions. Using correlative approaches across brain regions and/or between neurotransmitters, it informs on a form of connectivity of neurotransmitters in the brain that would correspond to the "neurochemical connectivity". The lecture will address this evolving concept from its origin to the study of psychedelics. The acute action of one psychedelic (TCB-2; 0.3, 3, and 10 mg/kg) and/or the 5-HT2AR antagonist MDL-100,907 (0.2 mg/kg) was studied on the organization of neurochemical systems, including monoamines and amino acids neurotransmitters in 28 mouse brain regions in a forced exploration procedure (unconditioned behavior). The specific correlation profiles of neurotransmitters (distinct for neurotransmitters) imposed by the forced exploration was absent in the groups treated by the psychedelic and/or the antagonist, sometimes without any significant changes of the quantitative (mean +/- SD) data. More recently, this neurochemical approach was used in the study of the behavioral deficits induced by chronic restrained stress in mice, and the beneficial effect of the psychedelic 5-methoxy-dimethyltryptamine. Overall, this type of studies, by combining neurotransmitters and metabolism network analyses, offers another perspective on brain function and the mechanism of psychotropic drugs including psychedelics.

Keywords: *Neurochemistry, neuropharmacology, multivariate analysis, 5-HT2A receptors, connectivity, neurotransmitters, behavior*

PROGRAM - POSTER SESSION 1

No	Presenting Author	Title
1	Tadeusz Karcz	Multifunctional ligands targeting sigma-1, μ-opioid, and histamine H3 receptors: Design, synthesis, and biological evaluation of novel analgesic agents
2	Carlo Reale	Design and synthesis of novel, structurally simplified σ/HDAC dual-target prodrugs
3	Adam Hosszu	Sigma-1 receptor as a novel therapeutic target in diabetic kidney disease
4	Miriam Santos-Caballero	Analgesic effect of the combined action on sigma-1 and sigma-2 receptors: A preclinical study in capsaicin-induced sensitization and postoperative pain
5	Albrecht Clement	Partners in Flux? – The potential relationship of S1R and WFS1 in neuronal autophagy
6	Gniewomir Latacz	The drug-like properties assessment of a new selenoorganic σ1-receptor ligand
7	Giuliana Costanzo	Two-step synthesis of enantiomerically pure morphans from (R)-carvone
8	Diana Dias da Silva	Sigma-1 receptor as a translational lens for serotonergic hallucinogens in depression: A scoping review
9	Santiago Vázquez	Design and synthesis of dual soluble epoxide hydrolase inhibitors / sigma-1 receptor antagonists with analgesic activity
10	Andrew M. Tan	Pridopidine in Huntington's disease: PROOF-HD trial findings and 2-year follow-up
11	May Meltzer	Pridopidine protects ALS patient-derived neural progenitor cells by reducing ER stress and restoring mitochondrial function via sigma-1 receptor activation
12	Andrew M. Tan	Pridopidine, a sigma-1 receptor agonist, modulates cellular stress and survival pathways across neurodegenerative models
13	May Meltzer	Pridopidine, a selective and potent sigma-1 receptor for the treatment of ALS: Rationale for the Phase 3 trial
14	Kinga Czarnota-Łydka	Synthesis and neuroprotective profiling of a new phenylsulfanylpropyl-piperazine derivative as a potential antipsychotic agent

PROGRAM - POSTER SESSION 2

No	Presenting Author	Title
15	Tangui Maurice	Neuroprotection by Amylovis-201, an anti-amyloidogenic agent with sigma-1 receptor agonist activity, is enhanced in enriched environment conditions
16	Xavier Codony	Annotated sigma receptor ligand library for drug discovery
17	Anna Czader	ADMET in silico studies for 1,3,5-triazine-derived 5-HT6 serotonin receptor ligands with expected action of σ1- receptor agonists
18	Ewa Szymańska	Arylpiperazine revisited: New multitarget directions for CNS-active ligands
19	Angelika Jagielska	HBK-15, a multimodal compound activating sigma-1 receptors, modulates cognitive performance in aged mice
20	Klaudia Lustyk	Sigma-1 receptor engagement by HBK-10 alleviates cognitive deficits under NMDA receptor hypofunction
21	Kinga Sałaciak	HBK-15 prevents MK-801-induced memory consolidation deficits via sigma-1 receptor-dependent mechanisms
22	Elżbieta Żmudzka	The study of novel salicylamide derivative and its potential antipsychotic activity in rats
23	Joanna Nowak-Włodyga	Histamine H3 receptor antagonist with dual sigma-1 receptor activity attenuates pain and reverses memory impairments
24	Srećko Gajović	Modifying neuroinflammation as a preclinical intervention strategy after ischemic lesion in relation to sigma-1 receptor signaling
25	Tugba Agbektas	The relationship between *SIGMAR1* and purinergic signaling: Stage-dependent expression changes in diabetic retinopathy
26	Jens Loncke	Wolfram syndrome-associated protein CISD2 as a regulator of ER–mitochondrial calcium transfer and cell fate
27	Burçin Kurt	A SIGMAR1-centered network signature of synaptic adhesion in Alzheimer's disease
28	Michal Cagalinec	Perturbations in mitochondrial respiration in WFS1-deficient SH-SY5Y neuronal cells despite unaltered mitochondrial morphology

Multifunctional ligands targeting sigma-1, μ-opioid, and histamine H_3 receptors: Design, synthesis, and biological evaluation of novel analgesic agents

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Pain, as defined by the International Association for the Study of Pain, is a complex sensory and emotional experience associated with actual or potential tissue damage, encompassing both nociceptive and neuropathic components. Among the key molecular targets involved in pain signaling, μ-opioid receptors (MOR), sigma-1 receptors (σ_1R), and histamine H_3 receptors (H_3R) have attracted increasing interest. Current pharmacological strategies rely mainly on MOR agonists, σ_1R antagonists, and H_3R antagonists, while recent preclinical studies suggest that multifunctional ligands acting on multiple targets may offer improved therapeutic outcomes.[1,2]

This study aimed to design and synthesize novel piperidine and piperazine derivatives with potential dual or triple activity at MOR, H_3R, and σ_1R, and to evaluate their selected biological properties. Particular focus was placed on σ_1R affinity, membrane permeability assessed using the PAMPA assay, and interaction with P-glycoprotein (P-gp), a key efflux transporter influencing central nervous system drug distribution.

Numerous compounds were synthesized and evaluated *in vitro*. The PAMPA assay revealed variable membrane permeability, with fluoro-substituted analogs showing the highest permeability. All compounds demonstrated strong σ_1R affinity and the ability to activate P-gp. Among them, KSK_OP19 exhibited the most favorable profile, combining high permeability, strong σ_1R binding, and relatively low P-gp substrate activity.

These results support the potential of multifunctional ligands as promising candidates for the development of novel analgesics, particularly for the treatment of neuropathic pain.

Keywords: *Chronic pain, multi-target-directed ligands, histamine H_3 receptors, sigma-1 receptors, μ-opioid receptors*

Acknowledgments: This research was funded by the National Science Centre, Poland (2022/45/B/NZ7/03101).

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Design and synthesis of novel, structurally simplified σ/HDAC dual-target prodrugs

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Aberrant angiogenesis plays a critical role in the metastatic spread of uveal melanoma (UM).[1] Dual-target pharmacological strategies combining histone deacetylase (HDAC) inhibition with σ receptor modulation have emerged as highly promising therapeutic tools for the development of novel anticancer agents. Previously, the racemic prodrug (±)-MRJF22 and its (*S*)-(−)-enantiomer demonstrated potent antiangiogenic and antimetastatic properties, significantly reducing endothelial cell viability and UM cell migration.[2] Building upon these outcomes, a novel series of structurally simplified σ/HDAC prodrugs was rationally designed to reduce excessive steric hindrance while maintaining dual-target efficacy. These new derivatives feature the removal of the fluorophenyl ring, modifications to the methylene chain length, and esterification with butyric, 4-phenylbutyric, or valproic acid. The derivatives are designed to undergo hydrolysis to release two distinct active molecules, preserving both HDAC inhibitory activity and σ1 antagonist/σ2 agonist properties. Preliminary radioligand binding assays revealed that these simplified compounds retain strong target profiles, with several derivatives exhibiting single-digit nanomolar affinity for σ1 receptor alongside appreciable affinity for σ2 receptor. Furthermore, stability assays were conducted on the most promising candidates to verify their susceptibility to hydrolysis, supporting the effective *in vivo* release of the active molecules from the prodrugs. In conclusion, the rational reduction of steric hindrance preserves dual-target efficacy, establishing these novel prodrugs as viable synergistic candidates for the development of innovative antimetastatic therapies, with further biological evaluations currently underway.

Keywords: *Uveal melanoma, sigma receptors, HDAC inhibitors, dual-target prodrugs, (S)-(−)-MRJF22*

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Sigma-1 receptor as a novel therapeutic target in diabetic kidney disease

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Background and aims: Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease. Current treatments cannot halt the progression of renal injury, highlighting an urgent need for therapies targeting key disease mechanisms. Previously, we described the protective role of Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) in acute kidney injury. Thus, we hypothesized that a similar protective effect would be observed in DKD.

Methods: Diabetes mellitus was induced in male Wistar rats using streptozotocin, followed by a seven-week FLU treatment. Metabolic and renal parameters were assessed along with a histological analysis of glomerular damage (PAS) and tubulointerstitial fibrosis (Masson's trichrome). The effects of inflammation and hypoxia were tested in lipopolysaccharide (LPS)- or hypoxia-induced human proximal tubular cells (HK-2). Cells were treated with FLU. Inflammatory and hypoxia markers were measured by RT-qPCR.

Results: FLU improved renal function and urinary biomarkers of tubular damage (KIM-1, NGAL) in DKD rats. In parallel, FLU reduced glomerular damage and fibrosis. FLU decreased LPS-induced TLR2, NFKB1, and IL6 expressions, as well as hypoxia-induced HIF1A, SLC2A1, VEGFA, and TGFB1 elevation in HK-2 cells.

Conclusions: S1R activation can slow DKD progression by modulating critical inflammatory, hypoxic, and fibrotic pathways. Thus, S1R may serve as a promising novel therapeutic target in DKD.

Keywords: *Diabetic kidney disease, fibrosis, sigma-1 receptor*

Acknowledgments: LP2021-3/2021, TKP2021-EGA-24, 2024-1.2.3-HU-RIZONT-2024-00056

Analgesic effect of the combined action on sigma-1 and sigma-2 receptors: A preclinical study in capsaicin-induced sensitization and postoperative pain

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Two types of sigma receptors have been described, namely sigma-1 and sigma-2 (S1R and S2R). While S1R antagonists have been reported to produce analgesia [1], the role of S2R ligands in pain remains poorly explored, and the effects of targeting both receptors simultaneously are unknown. Capsaicin-induced secondary mechanical hypersensitivity (allodynia) is driven by central sensitization, a key mechanism in pathological pain [2], and is also a common feature of clinically relevant conditions such as postoperative pain, which remains inadequately managed [3].

In this study, we evaluated the analgesic effects of S2R ligands, alone and in combination with S1R ligands, in female CD-1 mice. Mechanical allodynia was induced by intraplantar injection of capsaicin (1 µg) and assessed using a punctate mechanical stimulus (0.5 g). To study postoperative pain we performed a transverse laparotomy, and the mechanical threshold was determined using von Frey filaments (up-and-down method). All drugs were administered subcutaneously.

S1R antagonists BD-1063 and S1RA dose-dependently reduced capsaicin-induced allodynia, an effect reversed by the S1R agonist PRE-084 but not by the S2R antagonist WLB-89462. Conversely, the S2R agonist siramesine produced dose-dependent antiallodynia, reversed by WLB-89462 but not by PRE-084, confirming target engagement. Importantly, the combination of ineffective doses of S1R antagonists and siramesine synergistically abolished allodynia. This synergistic effect was also observed in laparotomized mice and was reversed by both PRE-084 and WLB-89462, indicating the involvement of both receptors.

These findings suggest that S2R agonism, alone or combined with S1R antagonism, may represent a promising therapeutic strategy for pain management.

Keywords: *Mechanical allodynia, laparotomy, sigma-2 receptor, sigma-1 receptor, synergy*

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Partners in Flux? – The potential relationship of S1R and WFS1 in neuronal autophagy

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Autophagy is a fundamental cellular pathway required also for neuronal function. A deficiency in this process is particularly critical in non-mitotic cells such as neurons and result in a variety of pathologic conditions, including neurodegenerative diseases. The sigma-1 receptor (S1R) and wolframin (WFS1) are implicated in the regulation of autophagy. Both proteins are localized at the mitochondria-associated membranes (MAM) of the endoplasmic reticulum (ER), additionally regulating calcium transfer and modulating the ER stress response. Previous studies, including ours, demonstrated that S1R agonists enhance autophagic flux. Notably, S1R agonists rescued impaired autophagy in wolframin-deficient cells. Mutations of S1R or WFS1 are associated with disorders such as ALS and Wolfram syndrome, respectively.

To explore their functional relationship, we are generating NSC34 cell lines expressing APEX2 fused to either S1R or WFS1, including their disease-associated variants. Autophagic activity and mitochondrial function were characterized to confirm proper functionality of the fusion proteins. Using proximity biotin labeling followed by mass spectrometry, we aim to map the luminal and cytosolic *proximatomes* of WFS1 and S1R to identify potential novel interacting partners. Moreover, we will analyze changes in these proximatomes under different conditions, such as S1R activation, and compare wild-type proteins with their pathogenic variants. This approach will enable the identification of pathways underlying their functional roles in both, physiological and disease contexts. Ultimately, we aim to elucidate the mechanisms by which S1R and WFS1 modulate neuronal autophagy and thus gain deeper insight into the pathogenic consequences of their mutations.

Keywords: *Autophagy, wolframin, S1R, APEX*

Acknowledgments: ANR/DFG-Project “SIRWOLFPHAGY” to BD, CB (BE1475/12-1)

The drug-like properties assessment of a new selenoorganic σ_1-receptor ligand

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PPK-059 is a ligand of σ_1-receptor (σ_1R) with confirmed *in vitro* neuroprotective and antioxidative properties as well as *in vivo* antipsychotic-like efficacy in mouse. Within this study, we used a several *in vitro* assays to determine the drug-like properties of this new favorable selenoether derivative.

The analysis of hepatotoxicity and neurotoxicity was performed using the MTS assay on HepG2 *(hepatoma*) and SH-SY5Y (*neuroblastoma*) cell lines, respectively. To this purpose, cells were seeded in a multi-well plate and incubated with the tested compound for 72h. A possible mechanism of toxicity *via* phospholipidosis was also tested on HepG2 line with the LYSO-ID Red Cytotoxicity assay. To define oral absorption potential, the Corning® Pre-Coated PAPMA Plate System was used. The PK parameters (Cl_{int} and $t_{0.5}$) and the most probable metabolic pathways were determined in mouse liver microsomes (MLMs). Additionally, the effect of the compound on the activity of the CYP3A4 enzyme was measured for assessment of possible drug-drug interactions (DDIs).

Cell based results show moderate toxicity, as PPK-059 has a significantly toxic influence on SH-SY5Y cells at 25 μM concentration and higher. What is more, 25 μM concentration caused the development of phospholipidosis in HepG2 cells. The permeability *via* passive mechanism in PAMPA test was satisfying, however, high membrane retention of compound PPK-059 was observed. Very law risk of DDIs was determined, as PPK-059 began to markedly inhibit CYP3A4 at a 10 μM concentration and higher. The compound was metabolised in MLMs very extensively with Cl_{int}=1308 mL/min/kg and $t_{0.5}$= 4 min, mainly by reaction of demethylation.

In conclusion, although PPK-059 showed favourable *in vitro/in vivo* pharmacological properties and satisfying safety, the structure still requires optimization in order to improve drug-like properties, especially metabolic stability.

Keywords: *Organoselenium compounds, toxicity, bioavailability, cell-based in vitro models*

Acknowledgments: Studies were carried out with the use of research infrastructure co-financed by the Smart Growth Operational Programme POIR 4.2 project no. POIR.04.02.00–00-D023/20.4.

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Two-step synthesis of enantiomerically pure morphans from *(R)*-carvone

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The bicyclic morphan scaffold (2-azabicyclo[3.3.1]nonane) represents a relevant structural motif in medicinal chemistry, occurring in natural alkaloids such as morphine and strychnine as well as in clinically used compounds like pentazocine and dextromethorphan. Its rigid three-dimensional framework enables a well-defined spatial disposition of pharmacophoric elements, making it a suitable scaffold for the design of ligands targeting central nervous system receptors, including the σ1 receptor.[1,2] However, efficient access to enantiomerically pure morphans remains limited.[3] Herein, we describe a chiral pool strategy for the synthesis of enantiomerically pure 4-hydroxymorphan-7-ones starting from *(R)*-carvone. The synthetic route involves a concise two-step sequence: epoxidation of the isopropenyl moiety followed by nucleophilic ring opening with primary amines, leading to formation of the bicyclic core. This approach leads to the isolation of enantiomerically pure compounds by crystallization, offering practical and scalable advantages. The scaffold was further functionalized at key positions, including variation of the *N*-substituent, modification of the C4 hydroxyl group, and introduction of aromatic or heterocyclic moieties at the 6/7 positions. Binding affinities toward σ1, σ2, and opioid receptors were evaluated.[4] While no significant affinity was observed at opioid receptors, selected derivatives, particularly indole- and quinoline-annulated morphans, exhibited nanomolar affinity for the σ1 receptor with high selectivity. Overall, this work provides an efficient route to structurally diverse, enantiomerically pure morphans and supports the development of selective σ1 receptor ligands.

Keywords: *Sigma receptor ligands, enantiomerically pure morphans, two step synthesis*

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Sigma-1 receptor as a translational lens for serotonergic hallucinogens in depression: A scoping review

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Serotonergic hallucinogens, including psilocybin, have shown rapid and sustained antidepressant effects. However, their mechanistic integration across molecular, cellular and clinical domains remains incomplete[1-6]. The sigma-1 receptor (SIGMAR1), a chaperone protein involved in cellular stress modulation, calcium signalling and neuroplasticity, has emerged as a potential translational node relevant to depression [7-10].

This study present and critically appraise the extent, nature and strength of evidence linking SIGMAR1 to serotonergic hallucinogens in depression, distinguishing direct, indirect and speculative mechanistic relationships.

As such, a scoping review was designed following established frameworks. PubMed/MEDLINE, Embase, Scopus and Web of Science were searched from inception to March 2026, combining terms related to SIGMAR1, serotonergic hallucinogens (psilocybin/psilocin, DMT, ayahuasca, 5-MeO-DMT) and depressive disorders. Preclinical, translational and clinical studies were included. Data were charted by compound, model, mechanistic pathway and level of evidence linking outcomes to SIGMAR1.

Preliminary mapping suggests a heterogeneous and uneven evidence landscape. Direct evidence linking SIGMAR1 to antidepressant-relevant effects is strongest in preclinical models, particularly for DMT, where receptor binding and functional modulation have been reported[11,12]. For psilocybin, evidence remains predominantly indirect, with convergence on downstream processes, e.g., neuroplasticity, synaptic remodelling and network-level changes, that overlap with known SIGMAR1-mediated pathways but lack direct receptor-level confirmation. Clinical studies of psychedelics in depression rarely assess SIGMAR1-related biomarkers.

Overall, SIGMAR1 provides a plausible but underexplored translational framework for organising mechanistic hypotheses in psychedelic-assisted therapy for depression. Current evidence supports its relevance as a modulatory axis rather than a unifying mechanism, highlighting key gaps for future mechanistic and biomarker-driven studies.

Keywords: *Sigma-1 receptor, psilocybin, psychedelics, depression, neuroplasticity, translational research*

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Design and synthesis of dual soluble epoxide hydrolase inhibitors / sigma-1 receptor antagonists with analgesic activity

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Pain affects over 40% of the global population, yet current treatments often provide inadequate relief. Therefore, there is a clear need for novel analgesics with improved efficacy, reduced side effects, and lower abuse potential. This project aims to develop a first-in-class dual-acting analgesic for pain management by targeting soluble epoxide hydrolase (sEH) and the sigma-1 receptor (S1R), two targets independently involved in pain modulation. Inhibition of sEH elevates epoxyeicosatrienoic acids, which exhibit analgesic effects in multiple pain models. In parallel, S1R plays a key role in pain signaling, and its antagonism has been shown to reduce hypersensitivity[1]. Notably, we recently discovered that dual modulation of these targets produces a synergistic enhancement of analgesic activity[2]. Subsequently, we designed, synthesized and biologically evaluated two series of dual-acting compounds and performed structure–activity relationship studies leading to compounds that inhibit sEH and antagonize S1R in the low nanomolar range, while displaying good selectivity and DMPK properties[3,4].

Keywords: *Dual-target therapy, non-opioid analgesia, pain, sigma-1 receptor, synergia*

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Pridopidine in Huntington's disease: PROOF-HD trial findings and 2-year follow-up

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Background: Huntington's disease (HD) is a progressive neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin gene (HTT), that leads to worsening motor function, cognitive decline, and loss of daily functioning over time. Currently, available treatments address motor symptoms but do not slow disease progression. Sigma-1 receptor (S1R) activation has been linked to pathways relevant to HD, including cellular stress responses, calcium homeostasis, and mitochondrial function. Pridopidine, a selective S1R agonist, was evaluated in the Phase 3 PROOF-HD (NCT04556656) study in early manifest HD. In the overall study population , pridopidine did not meet the primary endpoint (change in Total Functional Capacity [TFC]) or key secondary endpoints during the double-blind period. However, in a predefined subgroup of participants not receiving antidopaminergic medications (ADMs), pridopidine showed favorable effects across multiple measures including disease progression, cognition, motor function and quality of life.

Objective: To determine whether the effects observed in PROOF-HD off-ADM participants from the double-blind phase were sustained with longer-term treatment, up to 2-years in the open-label extension (OLE).

Methods: PROOF-HD was a randomized, double-blind, placebo-controlled Phase 3 trial evaluating pridopidine, followed by an OLE in which all participants received active treatment. Long-term outcomes (up to 2-years) in patients not receiving ADMs were compared to matched external natural history cohorts (ENROLL-HD and TRACK-HD), using propensity score weighting. Disease progression was evaluated across multiple clinical endpoints, including function (Total Functional Capacity [TFC]), global disease progression (composite Unified Huntington's Disease Rating Scale [cUHDRS]), cognition (Stroop Word Reading [SWR]), and motor symptoms (Quantitative motor [Q-Motor] and Total Motor Score [TMS]). Analyses were designed to assess whether treatment effects observed during the double-blind period were maintained over longer-term follow-up.

Results: At 2 years (Week 104), findings from the PROOF-HD off-ADM subgroup were extended by comparison with matched natural history cohorts, showing consistent and clinically meaningful slowing of disease progression in pridopidine-treated participants. This included:

- Functional decline (TFC): 62-65% slowing of progression ($p<0.005$)
- Global disease progression (cUHDRS): 59–63% slowing of progression ($p<0.0001$)
- Cognition (SWR): 81–89% slowing of progression ($p<0.0004$)
- Q-Motor (finger-tapping): 78% slowing of progression ($p<0.0001$)
- Motor worsening (TMS): 37–63% slowing of progression ($p=0.02$)

Overall, pridopidine treatment was associated with slower progression across all measures of clinical outcome.

Conclusions: Taken together, these findings suggest that pridopidine benefits across key measures of the disease were sustained across two years of follow-up, with clinical trajectories differing from those estimated from matched external controls. These findings are being further evaluated in a pivotal Phase 3 (PRECISE-HD) clinical trial to initiate in 2026.

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Pridopidine protects ALS patient-derived neural progenitor cells by reducing ER stress and restoring mitochondrial function via sigma-1 receptor activation

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Background: Endoplasmic reticulum (ER) stress and disrupted ER–mitochondria signaling are central drivers of ALS pathogenesis. The sigma-1 receptor (S1R), an ER-resident chaperone protein enriched at mitochondria-associated membranes (MAMs), regulates ER homeostasis, mitochondrial function, and cell survival. Pridopidine, a selective S1R agonist, has demonstrated neuroprotective effects in preclinical ALS models and potential improvement of clinical measures in a subgroup of ALS patients.

Objective: To evaluate whether pridopidine reduces ER stress and enhances neuronal survival in sporadic ALS patient-derived neural progenitor cells (NPCs).

Methods: Human iPSC-derived NPCs from a sporadic ALS patient were exposed to tunicamycin (1.25 µM, 16 h) to induce ER stress. Cells were treated with pridopidine (0.1–1 µM) ± the S1R competitive antagonist NE-100. ER stress markers (BiP, CHOP), mitochondrial membrane potential (MMP), apoptotic signaling (BAX expression, caspase-3 activation), and cell viability were assessed using flow cytometry, qPCR, and confocal microscopy.

Results: ER stress increases expression of BiP and CHOP, key regulators of the unfolded protein response. In ALS NPCs experiencing ER stress, pridopidine (1uM) significantly reduced BiP (57%, $p<0.0001$) and CHOP (50%, $p<0.0001$) expression levels. Pridopidine also restored mitochondrial membrane potential (up to 47% increase), reduced apoptotic signaling (decreased BAX expression and caspase-3 activation), and improved survival (58% increase, $p<0.0001$) relative to controls. These effects are attenuated by the S1R antagonist NE-100.

Conclusion: These findings highlight pridopidine's potential to enhance neuronal survival in a human ALS model. The potential clinical benefit of pridopidine is being evaluated in PREVAiLS, a randomized, placebo-controlled Phase 3 study in subjects with early (<18 months), definite/probable ALS.

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Pridopidine, a sigma-1 receptor agonist, modulates cellular stress and survival pathways across neurodegenerative models

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Background: Pridopidine is a potent and selective sigma-1 receptor (S1R) agonist in clinical development for Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). The S1R is an endoplasmic reticulum-resident chaperone enriched at mitochondria-associated membranes (MAMs), where it regulates key processes involved in neuronal survival, including ER stress responses, mitochondrial function, calcium signaling, and protein homeostasis.

Objective: To evaluate how pridopidine modulates key cellular pathways involved in neurodegenerative diseases.

Methods: The effects of pridopidine were evaluated across multiple experimental systems, including *in vitro* neuronal models, patient-derived cells, and *in vivo* models of neurodegenerative disease, as well as clinical studies in HD and ALS.

Results: Across multiple experimental systems, S1R activation by pridopidine modulates cellular pathways disrupted in neurodegenerative diseases. In HD models, including YAC128 mouse neurons, HD patient-derived neural stem cells, and human HD lymphoblasts, pridopidine reduced ER stress, restored calcium homeostasis, improved mitochondrial membrane potential and respiration, and reduced oxidative stress. In ALS-related systems, including motor neuron-like cells expressing C9orf72 repeat expansions and SOD1 models, pridopidine enhanced autophagy, improved neuromuscular junction activity, rescued BDNF axonal transport, and supported motor neuron survival. Pridopidine has also shown to improve synaptic function, increase BDNF secretion, restore synaptic plasticity, and preserve of dendritic spine density. *In vivo*, neuroprotective effects were observed in HD mouse models (YAC128 and R6/2) and in the ALS SOD1 mouse model. Across these systems, pridopidine showed the biphasic dose-response characteristic of S1R agonists. Clinical studies in HD and ALS provide translational support for the relevance of these pathways to disease-related outcomes.

Conclusion: These findings support a central role for S1R activation in modulating interconnected cellular pathways underlying neurodegeneration. Pridopidine's multimodal mechanism of action supports its continued evaluation as a potential disease-modifying therapy across neurodegenerative and neurodevelopmental disorders.

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Pridopidine, a selective and potent sigma-1 receptor for the treatment of ALS: Rationale for the Phase 3 trial

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Background: Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by a loss of motor neurons from the cerebral cortex and spinal cord, leading to muscle weakness, respiratory failure, and death. ALS is a biologically heterogeneous neurodegenerative disease that includes both sporadic and familial forms, with disease biology involving protein misfolding, mitochondrial dysfunction, impaired axonal transport, and cellular stress pathways. Sigma-1 receptor (S1R) biology is directly relevant to ALS, as rare loss-of-function S1R variants are associated with severe forms of disease, and S1R activation regulates pathways involved in ALS including ER-mitochondrial signaling, calcium homeostasis, autophagy, and neuronal survival. Pridopidine, a selective S1R agonist, has demonstrated neuroprotective effects in preclinical ALS models. Pridopidine was evaluated as Regimen D in the Phase 2 HEALEY ALS Platform Trial (NCT05163860).

Objective: To evaluate the efficacy and safety of pridopidine in patients with early, definite/probable ALS.

Methods: A posthoc analysis of ALS subjects that had definite or probable ALS (El Escorial Criteria) and early in their disease (symptom onset <18mo) was performed. Disease progression was assessed using the revised ALS Functional Rating Scale (ALSFRS-R) and measures of respiration, bulbar, speech, through 24 weeks of the double-blind phase. Survival was assessed through 48 weeks.

Results: Pridopidine was well tolerated, consistent with its prior safety profile. Participants treated with pridopidine showed a potential slowing of decline compared to placebo in measures of disease progression including: ALSFRS-R (Δ2.90; 32%, p=0.03), respiratory (Δ1.20; 62%, p=0.03) and bulbar (Δ0.92; 40%, p=0.06) subdomains, speaking rate (Δ0.43; 70%, p=0.002) and articulation rate (Δ0.43; 93%, p=0.0007), reduced neurofilament light levels (-9%, p=0.75), and median survival time (from 300 to 600 days; p=0.069).

Conclusion: In the Phase 2 HEALEY ALS Platform Trial, pridopidine demonstrated clinically meaningful, nominal improvements at 24 weeks vs placebo in patients with definite/probable ALS and <18 months since onset. PREVAiLS (NCT07322003), is an ongoing pivotal Phase 3 study designed to prospectively evaluate pridopidine in a defined ALS population, informed by Phase 2 findings and supported by the mechanistic rationale of S1R activation in preclinical models. The study assesses the impact of pridopidine on clinical measures of ALS progression and relevant outcomes over a sustained treatment period.

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Synthesis and neuroprotective profiling of a new phenylsulfanylpropyl-piperazine derivative as a potential antipsychotic agent

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The development of more effective antipsychotic drugs requires a shift toward multi-target directed ligands (MTDLs) capable of addressing dopaminergic dysregulation alongside the neurodegenerative aspects associated with chronic neuropsychiatric disorders. In this study, we report the synthesis and pharmacological evaluation of compound **KCH-5**, a novel phenylsulfanylpropyl-piperazine derivative designed to engage a strategic triad of targets: serotonin 5-HT_{1A}, dopamine D_2, and σ1 receptors. The synthesis was achieved through a two-stage protocol utilizing sequential nucleophilic substitution (S_N2) reactions, consisting of an initial S-alkylation to form a haloalkyl intermediate followed by an N-alkylation step to assemble the final compound. Radioligand binding assays confirmed high-affinity profiles, with K_i values of 9 nM for 5-HT_{1A}, 9.5 nM for σ1, and 39 nM for D_2 receptors. The biological profile was extended by evaluating the cytotoxicity and neuroprotective properties of the compound in the HT-22 hippocampal neuronal cell line. **KCH-5** demonstrated a high safety profile and exhibited potent neuroprotective activity against glutamate-induced toxicity. It robustly attenuated neuronal damage, reducing membrane leakage and significantly suppressing intracellular calcium dysregulation. These results position the unique pharmacological profile of **KCH-5**, combining primary antipsychotic targets with potent neuroprotection, as a promising lead for the next generation of antipsychotics offering both symptomatic relief and disease-modifying potential.

Keywords: *Antipsychotic drugs, σ1-receptor, multi-target directed ligands (MTDL), neuroprotection*

Neuroprotection by Amylovis-201, an anti-amyloidogenic agent with sigma-1 receptor agonist activity, is enhanced in enriched environment conditions

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Amylovis-201 (CNEURO-201) is a multitarget ligand with potent neuroprotective activity in Alzheimer's disease (AD) models, acting through anti-Aβ aggregation and sigma-1 receptor (S1R) agonism. Its demonstrated efficacy in pharmacological and transgenic 3xTg rodent models of AD (middle-aged and aged) may modify disease progression by protecting neuronal and glial viability while actively reducing amyloid burden and, consequently, attenuating its toxicity in cellular metabolism. Currently, cognitive reserve, conceptualized as the capacity of the central nervous system to optimize cerebral processing through the efficient recruitment of alternative neural networks, is recognized as a critical modulating factor of individual vulnerability to neurodegenerative processes associated with Alzheimer's disease. This reserve emerges as a dynamic property shaped by non-pharmacological interventions, opening promising avenues for combined neuroprotection strategies. We aimed to determine whether the therapeutic efficacy of Amylovis-201 in AD treatment is potentiated through the stimulation and enhancement of cognitive reserve. Therefore, we combined Amylovis-201 administration with an experimental model of environmental enrichment (EE) using training in the Hamlet apparatus, and analyzed the synergistic effects of the combined treatment-EE intervention on brain plasticity and the development of resilience/protection against memory deficits induced in a pharmacological model of AD. We implemented a combined protocol of repeated oral administration of Amylovis-201 (0.1 and 1 mg/kg) and environmental enrichment (EE) over 14 days. EE consisted of daily 4-hour exposure to the Hamlet apparatus, a complex environment with five functionalized zones, at a frequency of 5 days/week. The combination of EE through Hamlet training with Amylovis-201 (0.1 mg/kg PO), as S1R agonist, produced additive effects on hippocampal neurogenesis. Furthermore, it protected against scopolamine (0.5 mg/kg IP)-induced spatial amnesia and mitigated $A\beta_{25-35}$ (ICV)-induced cognitive deficits at both doses tested (0.1 and 1 mg/kg PO). Given that EE is known to upregulate S1R expression, the combination of EE with Amylovis-201, acting as an S1R agonist, yields significant impacts on brain plasticity and neuroprotection. As EE is known to increase S1R expression, the combination between EE and Amylovis-201, acting as a S1R agonist results in major impacts on brain plasticity and neuroprotection.

Annotated sigma receptor ligand library for drug discovery

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Sigma receptors have rapidly emerged as valuable therapeutic targets across CNS disorders, oncology, pain, and other complex diseases. Their growing relevance has intensified the need for well-characterized small molecules that can accelerate target validation and reduce risk in the earliest stages of drug discovery. To address this challenge, Innopharma has developed an annotated, drug-like chemical library specifically enriched in sigma receptor ligands and comprehensively profiled across multiple decision-critical data layers.

Each compound in the collection has been evaluated for receptor affinity and selectivity, *in vitro* ADME properties, safety, and toxicity parameters, and—when appropriate—*in vivo* pharmacokinetics, safety, and efficacy. By integrating these pharmacological and developability-relevant insights, the dataset enables streamlined hit identification and supports efficient triage, prioritization, and iterative design of follow-up compounds.

This sigma-focused chemical collection provides a powerful, ready-to-use resource for academic groups, biotech innovators, and pharmaceutical discovery teams seeking validated, high-quality starting points for screening, hit expansion, and lead optimization. Together, the chemical matter and the associated data package form a robust platform to advance sigma receptor research and accelerate collaborative drug discovery efforts. In this poster, some data of the sigma library and data for some selected compounds will be presented.

Keywords: *Sigma-1 receptors, sigma-2 receptors, chemical library, small-molecule ligands, target validation, hit identification, lead optimization, drug discovery*

ADMET *in silico* studies for 1,3,5-triazine-derived 5-HT$_6$ serotonin receptor ligands with expected action of σ$_1$- receptor agonists

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The 5-HT$_6$ receptor (5-HT$_6$R) ligands have attracted considerable interest as potential therapeutics for Alzheimer's Disease (AD), due to their role in cognition, learning and memory processes. The unique distribution of 5-HT$_6$R, almost exclusively limited to the central nervous system (CNS), further supports their relevance as targets for CNS disorders. However, despite promising preclinical data, none of the 5-HT$_6$R ligands has successfully reached the pharmaceutical market, thus suggesting that modulation of this single target is insufficient. This highlights the need for multi-target approaches in search of effective AD therapy. In this context, activation of the σ$_1$ receptor (σ$_1$R), involved in neuroprotection and maintenance of cellular homeostasis, represents a promising complementary strategy [1]. In this work, an in-house library of over 200 triazine-based 5-HT$_6$R ligands [2] was analyzed for compatibility with the σ$_1$R ligand pharmacophore model proposed by Glenon [3]. Selected compounds were further evaluated *in silico* for CNS-oriented ADMET properties using *pkCSM* bioinformatics tool. A particular attention was paid to parameters such as lipophilicity, blood-brain barrier penetration, and overall pharmacokinetic profile. The identified structures exhibiting favorable CNS drug-like properties may serve as promising candidates for further experimental evaluation toward σ$_1$R activity and development of dual-target agents for AD therapy.

Keywords: *5-HT$_6$ receptor, σ$_1$ receptor, 1,3,5-triazine, Alzheimer's disease*

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Arylpiperazine revisited: New multitarget directions for CNS-active ligands

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Among the commonly considered biological targets for the treatment of both neurological and neuropsychiatric disorders are components of monoamine neurotransmitter systems, metabolic enzymes, ion channels and proteins that regulate signaling processes in the brain. Of particular interest in the latter field are σ_1 receptors, a class of intracellular proteins expressed in neurons and glia cells throughout the central nervous system and peripheral organs. Interaction between σ_1 receptors and systems of other neurotransmitters has been widely reported. For instance, the effects of selective serotonin reuptake inhibitors such as fluvoxamine or sertraline, seem to be partially exerted through agonism of σ_1 receptors, which most probably contributes to the antidepressant action of this drugs.

The object of the current project is to search for new compounds with therapeutic potential in neuropsychiatric diseases among multitarget – serotonin/dopamine/σ_1 receptors ligands. As part of our project, we revisited the general scaffold of arylpiperazines, well-known GPCR ligands, introducing innovative modifications, such as the incorporation of a selenium atom into the structure. Organic Se-compounds have been reported to show beneficial biological properties of therapeutic importance, and, in addition to their desired pharmacological activity, such compounds frequently manifest neuroprotective and antioxidative effects, along with a satisfactory pharmacokinetic profile. In the current work neuroprotective and antioxidative properties for the series of new selenoether piperazine derivatives, acting as triple 5-HT_{1A}R/D_2R/σ_1R agents, has been presented.

Keywords: *Arylpiperazines, serotonin receptors, dopamine receptors, sigma-1 receptor, neuroprotection*

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HBK-15, a multimodal compound activating sigma-1 receptors, modulates cognitive performance in aged mice

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Age-related cognitive decline is a fundamental aspect of aging and a major contributor to the growing burden of neurodegenerative disorders, with effective therapeutic options still remaining limited. In this context, HBK-15, a multimodal compound acting as a sigma-1 receptor agonist, has previously demonstrated rapid antidepressant-like and anti-amnestic effects in pharmacological and stress-induced models of cognitive impairment, suggesting its potential relevance for aging-associated cognitive deficits.

Building on this background, the present study investigated the effects of HBK-15 in a natural aging model using 12–16-month-old male BALB/c mice. Animals were assessed in a battery of behavioral tests targeting distinct cognitive domains, including recognition memory, working memory and cognitive flexibility.

In the novel object recognition task, HBK-15 administered at a dose of 0.625 mg/kg was associated with improved recognition memory, with performance exceeding chance level. This analysis was based on comparison to chance, while discrimination index calculations are currently ongoing and will provide a more detailed assessment of effect magnitude. In the Y-maze test, HBK-15 at 2.5 mg/kg improved spontaneous alternation performance, indicating enhanced working memory.

In parallel, the effects of HBK-15 on cognitive flexibility are currently being investigated using a translational visual discrimination reversal task based on a two-choice paradigm.

Overall, the current results suggest that HBK-15 may modulate selected aspects of cognitive performance in aging. Further studies are required to confirm these observations and to better characterize the extent and mechanisms of its action.

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Sigma-1 receptor engagement by HBK-10 alleviates cognitive deficits under NMDA receptor hypofunction

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Cognitive impairment represents a clinically relevant yet insufficiently treated dimension of major depression. Glutamatergic dysfunction, particularly NMDA receptor hypofunction, disrupts hippocampal plasticity and ERK-BDNF signaling, contributing to memory and learning deficits. The sigma-1 receptor has been implicated in modulation of glutamatergic signaling and cognition, making it a potential target for anti-amnesic interventions.

We characterized HBK-10, a methoxyphenylpiperazine derivative, for sigma-1 receptor engagement (radioligand binding, cellular activity assay), neuroprotection in SH-SY5Y cells exposed to amyloid-$\beta_{25\text{-}35}$ toxicity, anti-amnesic efficacy in mice subjected to NMDA receptor blockade with MK-801 (short-delay object recognition, Morris water maze, step-through passive avoidance), and downstream signaling (ERK1/2, *p*ERK1/2, BDNF, CREB, *p*CREB).

HBK-10 bound to the sigma-1 receptor with submicromolar affinity and acted as a potent agonist. The compound was non-cytotoxic and conferred sigma-1 receptor-dependent neuroprotection against amyloid-$\beta_{25\text{-}35}$ toxicity. *In vivo*, HBK-10 selectively rescued memory: it protected MK-801-induced deficits in the object recognition test and improved spatial learning and retrieval in the Morris water maze, while remaining inactive in the step-through passive avoidance. The behavioral effects were sigma-1 receptor-dependent and paralleled by restoration of hippocampal *p*ERK1/2 and upregulation of BDNF.

These findings indicate that HBK-10 engages the sigma-1 receptor and rescues NMDA hypofunction-induced cognitive impairment, in association with behaviorally relevant hippocampal ERK/BDNF signaling.

Keywords: *Sigma-1 receptor, NMDA receptor hypofunction, cognitive impairment, anti-amnesic effect, ERK/BDNF*

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HBK-15 prevents MK-801-induced memory consolidation deficits via sigma-1 receptor-dependent mechanisms

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Memory consolidation is a crucial stage of memory processing during which newly acquired information is stabilized for long-term storage. Its disruption is particularly relevant in disorders associated with glutamatergic dysfunction and impaired synaptic plasticity, where cognitive symptoms remain insufficiently treated. Although memory-enhancing compounds are increasingly explored, their effects on specific stages of memory formation remain poorly understood. HBK-15, a multimodal compound with sigma-1 receptor agonist activity, has previously demonstrated protective effects against MK-801-induced memory deficits during acquisition and retrieval in a sigma-1 receptor-dependent manner. However, whether this protective effect extends to memory consolidation remains unknown.

HBK-15 prevented MK-801-induced impairment of memory consolidation at both early (4 h) and late (24 h) time points, demonstrating efficacy at distinct temporal phases of consolidation. Pharmacological blockade of sigma-1 receptors abolished this effect, indicating that the protective action of HBK-15 is sigma-1 receptor-dependent.

To complement the behavioral findings, ongoing hippocampal analyses aim to identify molecular correlates of the observed effects, focusing on proteins involved in synaptic plasticity and memory consolidation, including BDNF, ERK1/2, and CaMKII.

Taken together, these findings position HBK-15 as a promising anti-amnesic candidate capable of preventing memory consolidation deficits through sigma-1 receptor-dependent mechanisms. These findings also strengthen the rationale for targeting sigma-1 receptors in cognitive disorders characterized by impaired memory processing.

Keywords: *HBK-15, sigma-1 receptor, NMDA receptor hypofunction, memory consolidation*

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The study of novel salicylamide derivative and its potential antipsychotic activity in rats

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Treatment of schizophrenia, which is one of the common psychiatric disorders, still remains insufficient. Unfavorable safety profile of available drugs and their limited efficacy are the most problematic during the therapy. Therefore, there is a huge need to develop novel antipsychotics.

As our previous research of novel salicylamide derivative JJGW07 in mice demonstrated its potential antipsychotic-like effects as well as anti-amnesic, antidepressant- and anxiolytic-like activity, in this study we aimed to explore the potential antipsychotic effects of the compound in rats.

We performed the hyperlocomotion test in the open field using male Wistar rats. To induce hyperlocomotion we used MK-801and as a reference compound we tested clozapine. The forward locomotion (cm/30 min) was registered in 10-min intervals for 30 min and analyzed with the aid of a computerized video tracking system.

The tested compounds at the range of doses 3.0-30.0 mg/kg did not reduce the hyperlocomotion induced by MK-801 during 30 minutes of the test, while clozapine reduced the hyperlocomotion at the doses 10 and 30 mg/kg by 53 and 98%, respectively (one-way ANOVA: $F(3,28)=13.830$, $p<0.0001$).

The obtained results did not confirm potential antipsychotic activity of JJGW07 in another animal species. Further studies are necessary to be able to verify the actual possible effects of novel compound in psychotic disturbances as well as in other psychiatric disorders.

Keywords: *Antipsychotic, schizophrenia, rats, salicylamide, open field test, hyperlocomotion*

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Histamine H_3 receptor antagonist with dual sigma-1 receptor activity attenuates pain and reverses memory impairments

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Background: Histamine H3 receptors (H_3R) and sigma-1 receptors (σ1R) are important modulators of nociception, neurotransmission, and cognition. H3R antagonism is generally associated with enhanced histaminergic, cholinergic, and noradrenergic signaling, supporting both analgesic and procognitive effects. In contrast, σ1R pharmacology appears highly state-dependent: σ1R antagonism is beneficial in pain, where it reduces sensitization, whereas σ1R agonism may promote memory by enhancing cholinergic/glutamatergic transmission and neuroplasticity. DL-76 was originally synthesized as an H3R antagonist. Many H_3R antagonist inherently possess nanomolar affinity at sigma receptors.

Methods: Radioligand binding studies were performer in Eurofins Cerep Laboratories. The functional activity of DL-76 at σ1R was evaluated in the BiP assay. Its *in vivo* effects were examined in animal models of pain, including the formalin test, hot-plate test, oxaliplatin-induced neuropathic pain, and streptozotocin-induced diabetic neuropathy. Cognitive activity was assessed in scopolamine-induced memory impairment using the passive avoidance task and Morris Water Maze. Pharmacological interaction studies included zolantidine, JNJ7777120, scopolamine, and PRE-084.

Results: DL-76 showed high affinity for σ1R and σ2R. In the BiP assay, DL-76 showed a dual σ1R profile, behaving as a weak agonist under basal conditions while also antagonizing the effect of the full σ1R agonist PRE-084. *In vivo*, DL-76 produced significant antinociceptive activity across inflammatory and neuropathic pain models. This effect was attenuated by zolantidine, JNJ7777120, scopolamine, and PRE-084, indicating indirect involvement of H2, H4, muscarinic, and direct σ1R-dependent mechanisms. DL-76 also reversed scopolamine-induced memory deficits in both behavioral paradigms.

Conclusions: DL-76 appears to be a pleiotropic, context-dependent ligand whose analgesic and procognitive activities may arise from adaptive, state-dependent interplay between H_3R antagonism and σ1R modulation. We hypothesize that it acts functionally as a σ1R antagonist in pain states, where σ1R activation contributes to sensitization, but as a weak σ1R agonist or positive modulator under mnemonic challenge, where σ1R activation supports cognition.

Modifying neuroinflammation as a preclinical intervention strategy after ischemic lesion in relation to sigma-1 receptor signaling

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Stroke represents a major health problem and its ischemic variant is the most prevalent. The neuroprotective and neurorestorative strategies remain elusive despite a significant body of research in this direction. Sigma-1 receptors are involved in both brain damage after ischemic injury and the subsequent repair. The resulting signaling cascades influence almost all aspects of cellular responses to the ischemic injury. From them the neuroinflammation was in our particular focus.

As clinically relevant model, transient middle cerebral artery occlusion (tMCAO) was used to achieve ischemia reperfusion injury corresponding to ischemic stroke successfully treated by recanalization. Subsequently, structural, cellular, and functional consequences of stroke were monitored by *in vivo* imaging and functional tests.

The structural brain changes were assessed by *in vivo* magnetic resonance imaging (MRI). The cellular processes in the brain were visualized by *in vivo* bioluminescence imaging (BLI), using the transgenic animals where luciferase expression was driven with specific promoters. The transgenic mouse used to monitor neuroinflammation was TLR2-luc, where TLR2 promoter drived luciferase reporter. The functional stroke consequences are monitored by behavioral tests resulting in a neurological score.

The proteomic analysis was performed to compare the proteomic signatures between early and late phase of ischemic injury and to the healthy mice. This allowed to characterize the Sigma-1 receptor related pathways and identify novel potential biomarkers. In conclusion, the described system of evaluating brain damage and repair represents a useful way to get insight in the dynamic of Sigma-1 receptor activities after ischemic brain injury.

Keywords: *Ischemic stroke, neuroinflammation, autophagy, proteomic analysis*

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The relationship between *SIGMAR1* and purinergic signaling: Stage-dependent expression changes in diabetic retinopathy

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Background: Diabetic retinopathy (DR) is characterized by inflammation, oxidative stress, and hyperglycemia-induced neurovascular damage. Purinergic signaling (*PANX1–P2X7–NLRP3-CASP1-IL-1β*) and the *Sigma-1 receptor* (*SIGMAR1*) regulate cellular stress responses. This study aimed to evaluate stage-dependent gene expression changes in this pathway in DR.

Methods: A total of 120 participants were enrolled: control (n=30), diabetes mellitus without retinopathy (DM; n=30), non-proliferative DR (NPDR; n=30), and proliferative DR (PDR; n=30). The gene expression levels of *SIGMAR1*, *PANX1*, *P2RX7*, *NLRP3*, *CASP1*, and *IL-1β* were analyzed using Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR). Group comparisons, fold-change, ROC curve, and correlation analyses were conducted.

Results: Demographic variables were comparable among groups (*p>0.05*), whereas BMI and body weight were higher in diabetic groups and correlated with HbA1c and glucose *(p<0.01*). *CASP1* was the most discriminative marker (*p<0.001*). *P2RX7* and *PANX1* were associated with DM, while *NLRP3* was increased in PDR (*p<0.05*). *SIGMAR1* showed significant variation (*p<0.05*) with consistent downregulation (0.17–0.20-fold) and was associated with *NLRP3*, particularly in PDR. Fold-change analysis showed *CASP1* upregulation in PDR (5.81-fold) and DM (3.54-fold), but downregulation in NPDR (0.27-fold), while *P2RX7* and *PANX1* were markedly reduced (0.03–0.06-fold). ROC analysis showed highest performance for *CASP1* in NPDR (AUC=0.761, *p<0.001*) and significance for *NLRP3* in PDR (AUC=0.629, *p=0.034*); *SIGMAR1* showed moderate performance (AUC=0.635, *p<0.05*). Correlation analysis showed strong positive associations of *P2RX7* with glucose and HbA1c, while *CASP1* was positively correlated with *IL-1β* and negatively with glucose and HbA1c; *SIGMAR1* showed a positive correlation with *NLRP3*.

Conclusion: *SIGMAR1* associated *PANX1–P2RX7–NLRP3–CASP1* signaling is differentially regulated across the stages of DR. *CASP1* and *NLRP3* may serve as stage-specific biomarkers, and *SIGMAR1* may act as a regulatory modulator of purinergic inflammasome signaling in DR.

Keywords: *Diabetic retinopathy, SIGMAR1, purinergic signaling, inflammasome*

Wolfram syndrome-associated protein CISD2 as a regulator of ER-mitochondrial calcium transfer and cell fate

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Calcium (Ca^{2+}) signaling is a fundamental regulator of cellular homeostasis, integrating metabolic control, autophagy, and apoptosis. At mitochondria-associated membranes (MAMs), tightly regulated Ca^{2+} exchange between the endoplasmic reticulum (ER) and mitochondria sustains bioenergetics and determines cell survival. Disruption of this interface contributes to neurodegeneration and Wolfram syndrome (WS). This work investigates the role of CISD2, a redox-active MAM-localized protein, in orchestrating inter-organellar Ca^{2+} signaling and downstream cellular responses.

We identify CISD2 as a macromolecular complex partner of Ca^{2+} release channels inositol 1,4,5-trisphosphate receptors (IP_3R1) and ryanodine receptors (RyRs). CISD2 loss disrupts ER–mitochondrial contacts and impairs Ca^{2+} transfer, with striking cell-type specificity: neurons exhibit suppressed cytosolic Ca^{2+} signaling, mitochondrial dysfunction, and heightened apoptotic susceptibility, whereas proliferative HeLa cells remain comparatively resilient. These findings highlight a unique neuronal dependence on tightly regulated ER–mitochondrial Ca^{2+} flux and suggest impaired Ca^{2+}-dependent cell death regulation as a driver of selective vulnerability. CISD2 deficiency may further exacerbate neurodegeneration by deregulating calpain-dependent proteolysis.

Beyond Ca^{2+} signaling, CISD2 modulates autophagic flux and mitochondrial quality control, consistent with emerging links to BCL-2–Beclin 1 and Pink1/Parkin pathways. We further demonstrate that CISD2 interacts with the BH4 domain of BCL-2, selectively counteracting BCL-2–mediated reduction of ER–mitochondrial tethering without affecting its canonical anti-apoptotic or IP_3R-inhibitory functions.

In future work, we aim to further mechanistically define how CISD2 functions as a central integrator of Ca^{2+} signaling, mitochondrial metabolism, autophagy, and neuronal survival at ER–mitochondrial contact sites. Moreover, given the convergence of CISD2- and WFS1-dependent pathways, we will explore pharmacological stimulation of the Sigma-1 receptor as a potential therapeutic strategy to restore MERCS homeostasis and Ca^{2+} signaling in Wolfram syndrome–related neurodegeneration.

A SIGMAR1-centered network signature of synaptic adhesion in Alzheimer's disease

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Background: The sigma-1 receptor (SIGMAR1) is a multifunctional chaperone protein involved in neuroprotection, endoplasmic reticulum stress regulation, and synaptic function. However, its transcriptional role in Alzheimer's disease (AD) remains unclear at the systems level.

Objective: This study aimed to investigate the transcriptional landscape associated with SIGMAR1 in AD using a network-based approach.

Methods: Gene expression data from the GEO dataset GSE33000, including postmortem prefrontal cortex samples (310 AD and 157 controls), were analyzed. Differential expression analysis was performed using a linear modeling framework with Benjamini–Hochberg correction. High-confidence differentially expressed genes (DEGs) were defined using adjusted $p < 0.01$ and $|logFC| > 0.3$. SIGMAR1 expression differences were assessed using Welch's t-test. Pearson correlation-based co-expression analysis was conducted using a stringent threshold (top 1% and $|r| > 0.75$), followed by Gene Ontology enrichment analysis.

Results: A total of 511 high-confidence DEGs were identified. SIGMAR1 (annotated as OPRS1) was not differentially expressed between AD and control groups ($p = 0.504$). Co-expression analysis revealed a network of 92 genes strongly correlated with SIGMAR1. No overlap was observed between DEGs and the SIGMAR1-associated gene set, indicating that its functional relevance is not captured by conventional differential expression approaches. Enrichment analysis showed a strong association with synaptic adhesion processes, particularly protocadherin-mediated cell adhesion (adjusted $p < 10^{-16}$).

Conclusion: Although SIGMAR1 is not differentially expressed in AD, it is embedded within a co-regulated network enriched for synaptic adhesion pathways, suggesting a network-level role in neuronal connectivity.

Keywords: *SIGMAR1, Alzheimer's disease, synaptic adhesion, gene co-expression, neurodegeneration*

Perturbations in mitochondrial respiration in WFS1-deficient SH-SY5Y neuronal cells despite unaltered mitochondrial morphology

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Wolfram syndrome (WS) is a rare neurodegenerative disorder characterized by diabetes insipidus, diabetes mellitus, optic nerve atrophy, and deafness. Majority of the WS cases are caused by mutations in the WFS1 gene, which encodes the protein wolframin (WFS1). WFS1 is highly expressed in the brain, pancreas, and heart [1]. WFS1 is a transmembrane endoplasmic reticulum (ER) resident protein present in the mitochondria-associated membranes, similarly as the Sigma-1 receptor (S1R). Although the direct interaction between WFS1 and S1R has not yet been demonstrated, they share their physiological function through ER–mitochondria calcium fluxes. Beyond this role, WFS1 regulates ER stress [2] and impacts mitochondrial function [3]. Therefore, in this work, we aimed to evaluate mitochondrial respiration and how this is associated with mitochondrial morphology in WFS1 knock-out (KO) model in the SHSY-5Y neuronal cell line developed in our laboratory using CRISPR-Cas9 technology.

Implementing Seahorse XFe96 extracellular flux analyzer we have found that the WFS1 KO cells exhibited a significant reduction in basal oxygen consumption rate, suggesting compromised mitochondrial bioenergetics under steady state. Additionally, ATP-linked respiration, and maximal respiration, were both significantly depressed in the KO group. Intriguingly, by implementing confocal microscopy quantitative image analysis, we observed no changes in the mitochondria morphological parameters of the WFS1 KO cells, including mitochondrial fragmentation, mitochondria total count, and area occupied by the mitochondria.

In conclusion, these findings suggest that functional mitochondrial impairment, rather than structural disruption, represents one of the pathological features associated with this WS cellular model.

Keywords: *Mitochondria, respiration, morphology, wolframin, Wolfram syndrome*

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