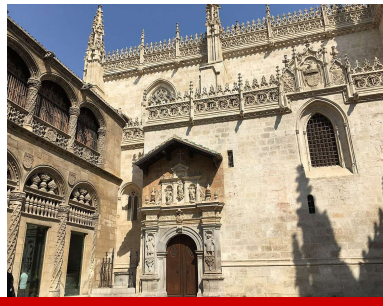




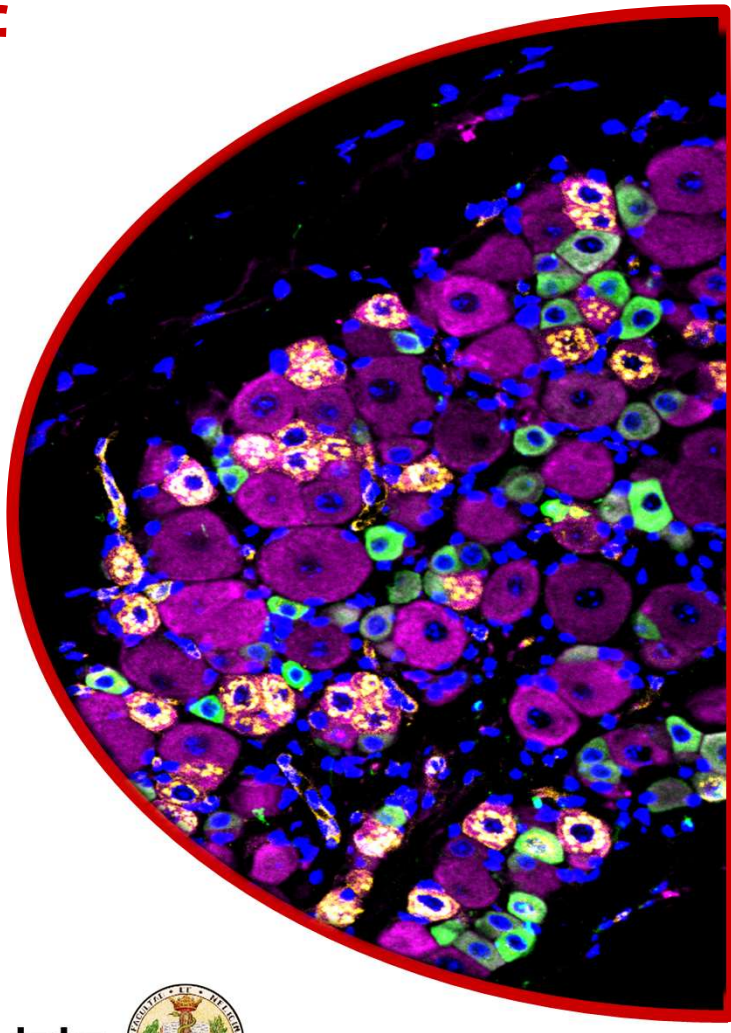
5th European Symposium
1st Meeting of SIGMA-1 EUROPE

SIGMA-1 EUROPE
European research network on sigma-1 receptors CA23156

ABSTRACT BOOK

Physiopathology of Sigma-1 Receptors

Faculty of Medicine, Universidad de Granada, Granada
June 25-27, 2025



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Physiopathology of Sigma-1 Receptors

5th European Symposium/ 1st Meeting of the European Network for Sigma-1 Receptor as a Therapeutic Opportunity (SIGMA-1EUROPE - CA23156)

25-27 june 2025

Organizers

Scientific Committee

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MMDN, University of Montpellier, EPHE, INSERM, Montpellier, France

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Latvian Institute of Organic Chemistry, Riga, Latvia

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25th June Wednesday
Facultad de Medicina – Faculty of Medicine
Salón de Actos - Assembly Hall

8.30 Registration

9.00 Opening ceremony. Enrique Cobos (on the behalf of the scientific committee), Tanqui Maurice (Chair SIGMA-1EUROPE) and Liga Zvejniece (vice Chair SIGMA-1EUROPE), José Juan Jiménez Moleón (Dean of the Faculty of Medicine, University of Granada)

09.30 Opening lecture.

Anti-amnesic and neuroprotective effects of sigma-1 receptor agonists. **Tangui Maurice**, University of Montpellier, Montpellier, France

Chair: Liga Zvejniece, Latvian Institute of Organic Synthesis, Riga, Latvia

10.15 Coffee (with cookies/sandwiches) + posters 1 (odd numbers)

11.00 Medicinal Chemistry 1

Development of sigma-1 receptor ligands: past, present and future perspectives. **Carmen Abate**, Università degli Studi di Bari Aldo Moro, Bari, Italy (25 min)

Modulation of Sigma-1 receptor and TSPO through bivalent ligands: exploring a new approach against neuroinflammation and neurodegeneration. **Giacomo Rossino**, University of Pavia, Pavia, Italy (10 min)

Discovery of novel class of multi-target ligands for sigma-1 receptor and selected GPCRs among selenoether derivatives of piperazine. **Patryk Pyka**, Jagiellonian University, Kraków, Poland (10 min)

Unlocking the Power of Inhibiting Soluble Epoxide Hydrolase and Sigma-1 Receptor Antagonism through Dual Mechanism Analgesic Compounds. **Celia Escriche** Molina, Universitat de Barcelona, Barcelona, Spain (10 min)

Debate and questions: 15 min

Chair: Francisco R Nieto, Universidad de Granada, Granada, Spain

12.15 Medicinal Chemistry 2

Development of sigma receptor modulators for the treatment of neuropathic pain. **Emanuele Amata**, Università degli Studi di Catania, Catania, Italy (25 min)

Sigma-1 receptor fluorescent ligands: development and use of powerful pharmacological tools. **Gabriella Rosanna Musillo**, Università Degli Studi di Bari Aldo Moro, Bari, Italy (10 min)

Structural and Dynamic Determinants of Sigma-1 Receptor Oligomerization: Insights from Molecular Dynamics Simulations. **Vittoria Nanna**, National Research Council - Institute of Crystallography, Bari, Italy (10 min)

Tackling Neurodegenerative Diseases by Developing Light-Activated Drugs through an Integrated Multiscale Approach. **Giacomo Salvadori**, Institute for Computational Biomedicine, Jülich,Germany (10 min)

Debate and questions: 15 min

Chair: Rafael González Cano, Universidad de Granada, Granada, Spain

13.30 Lunch (60 min) – Canteen of the PTS

14.30 Coffee + posters 2 (odd numbers)

15.30 Plenary
The Sigma-1 Receptor - Enigmatic?
Arnold Ruoho, University of Wisconsin-Madison, Madison, USA
Chair: Carmen Abate, Università degli Studi di Bari Aldo Moro, Bari, Italy

16.15 Neurodegenerative and other neurological diseases
Sigma-1 as a target for antiseizure and disease-modifying effects? Preclinical validation in mouse models of epilepsy. **Eva-Lotta von Rüden**, Ludwig-Maximilians-Universität München, Munich, Germany. (25 min)
Targeting Sigma-1 Receptor for Neurodegenerative Diseases: Insights into Physiopathology and Therapeutic Potential. **Kanika Verma**, Chulalongkorn University, Bangkok, Thailand (10 min)
Pridopidine, a Sigma-1 Receptor Agonist, Offers Therapeutic Potential for Huntington's Disease and Amyotrophic Lateral Sclerosis. **Andrew Tan**, Prilenia Therapeutics B.V., Naarden, The Netherlands (10 min)
Allosteric positive modulator of Sigma-1 receptor: a new weapon to mitigate disease progression in amyotrophic lateral sclerosis. **Hugo Mourier**, University of Montpellier, INSERM, Montpellier, France (10 min)
Debate and questions: 15 min
Chair: Enrique J Cobos. University of Granada, Granada, Spain

17.30-18.00 Break (coffee)

18.00 Cognition, depression, anxiety and other psychiatric disorders
Neuroprotective Potential of 6-Hydroxy-L-Nicotine: Modulating Memory, Oxidative Stress, and Neuroinflammation. **Lucian Hritcu**, Alexandru Ioan Cuza University of Iasi, Iasi, Romania (25 min)
Targeting Sigma-1 Receptors for Improved Executive Flexibility Under Glutamatergic Dysfunction. **Kinga Sałaciak**, Jagiellonian University, Kraków, Poland (10 min)
SIGMA1R play an indispensable role in Th9 differentiation. **Michel Mickael**, Institute of Genetics and Animal Biotechnology, Garbatka, Poland (10 min)
Reduced levels of the sigma-1 receptor in the brains of Alzheimer's disease patients. **Ruiqing Ni**, University of Zurich, Zurich, Switzerland (10 min)
Debate and questions: 15 min
Chair: Tangui Maurice, University of Montpellier, Montpellier, France

19.30-21.00 Welcome reception (same building)

26th June Thursday
Facultad de Medicina – Faculty of Medicine
Salón de Actos - Assembly Hall

09.00 Plenary
Sigma-1 Receptor Controls Glycolysis via Mitochondrial GRIM19 and Glucose Uptake. **Tsung Ping Su**. National Institute on Drug Abuse, Baltimore, USA
Chair: Tangui Maurice, University of Montpellier, Montpellier, France

09.45 Coffee (with cookies/sandwiches) + posters 3 (even numbers)

10.30 Sigma-1 receptor biochemistry and cellular biology: structure, protein targets and basic mechanisms
TIRF Microscopy-Based Assay for Detecting and Colocalizing Membrane Proteins. **Ago Rinken**, University of Tartu, Tartu, Estonia (25 min)
Conserved LIR-specific interaction of sigma-1 receptor and GABARAP. **Fazilet Bekbulat**, Johannes Gutenberg University Mainz, Mainz, Germany (10 min)
Investigation of σ1 receptor mechanism of activity and identification of novel agonists. **Andrea Ilari**, Institute of Molecular Biology and Pathology (IBPM), National Research Council of Italy (CNR), Rome (10 min)
Accelerated mutant huntingtin aggregation and reduction of ER stress by the S1R agonist pridopidine. **Gerardo Lederkremer**, Tel Aviv University, Tel Aviv, Israel (10 min)
Debate and questions: 15 min
Chair: Andrea Fekete, Semmelweis University and SigmaDrugs, Budapest, Hungary

11.45 Pain, inflammation and non-central diseases
Effects of S1RA (E-52862), a selective sigma-1 antagonist, in neuropathic pain: Two randomized, double-blind, phase 2 studies in patients with chronic postsurgical pain and painful diabetic neuropathy. **Víctor Mayoral**, Bellvitge University Hospital, Barcelona, Spain (25 min)
The application of AI tools in analyzing pain behavior in mice: examples on the study of sigma-1 receptor function. **Rafael Gonzalez-Cano**, University of Granada, Granada, Spain (10 min)
Dual-Targeting Histamine H3 and Sigma-1 Receptor Ligands as Candidates for the Treatment of Neuropathic Pain. **Katarzyna Szczepańska**, Maj Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland (10 min)
Fluvoxamine mitigates bleomycin-induced pulmonary fibrosis in mice. **Adam Hosszu**, Semmelweis University, Budapest, Hungary (10 min)
Debate and questions: 15 min
Chair: M Carmen Ruiz Cantero, University of Barcelona, Barcelona, Spain

13.00 Lunch (Same building)

14.00 Coffee + posters 4 (even numbers)

15.00 Other diseases and disease models

Identifying and Characterizing Novel Small Molecule-Sigma-1 Ligands using *Caenorhabditis elegans*. **James Brimson**, Chulalongkorn University, Bangkok, Thailand (25 min)

The role of sigma-1 receptor in the anticancer activity of thiosemicarbazones. **Bianca Stiller**, Medical University of Vienna, Vienna, Austria (10 min)

Regulation of Sigma-1 Receptor in Cancer Progression and Drug Resistance. **Ioannis Trougakos**, National and Kapodistrian University of Athens, Athens, Greece (10 min)

Fluvoxamine, eyedrop, glaucoma, intraocular pressure, fibrosis. **Judit Hodrea**, Semmelweis University, Budapest, Hungary (10 min)

Debate and questions: 15 min

Chair: Miguel Ángel Tejada, University of Granada, Granada, Spain

16.15 Closing lecture

Sex-Specific Role of Sigma-1 Receptor in Cardiac and Metabolic Function in Mice

Liga Zvejniece, Latvian Institute of Organic Synthesis, Riga, Latvia

Chair: Enrique J Cobos, University of Granada, Granada, Spain

17.00 Excursion to the city (GranaDown) – please follow the local committee to the bus

Closing dinner 21.00-23.00 (Carmen de la Victoria)

27th June Friday
Facultad de Medicina – Faculty of Medicine
Salón de Grados B - Graduation Hall B

9.00-10.30 Management Committee meeting 1

10.30-11.00
Coffee (with cookies/sandwiches)

11.00-12.00 Management Committee meeting 2

12.00-14.00 WG3 (Establishment of SOPs for in-vitro/in-vivo models to assess sigma-1 receptor ligands) meeting

14.00-15.30 Lunch + Coffee (Same building)

15.30-17.30 WG4 (Industry strategies requirements for drug development) meeting

17.30-17.45 Break

17.45-19.45 WG5 (Dissemination, Communication and Outreach) meeting

Poster Presentations - 25th June Wednesday (odd numbers)

Medicinal Chemistry

N	Presenting author name		Title of the communication
1	I	Barvik	Design of sigma-1 receptor agonists and antagonists using computer modeling
3	X	Codony	Innopharma Sigma Chemical Library for hit identification and tool compound discovery
5	G	Cosentino	Design, synthesis, and in silico studies of novel tetrahydropyrrolo[3,4-c]pyrazoles sigma-1 receptor ligands
7	G	Cosentino	Synthesis of new benzylpiperazines as selective sigma-1 receptor ligands with in vitro antiproliferative activity against cancer cells
9	AT	Lisi	Structure-activity relationship studies on phenoxyalkylpiperidines reveal the structural determinants for potent sigma1 receptor agonist activity and antineurodegenerative properties.
11	M	Mammone	Exploiting the Therapeutic Potential of Sigma and Cannabinoid subtype 2 Receptors (CB2R) as Dual Targets approach in Inflammatory Diseases
13	S	Obradović Jovčić	Vildagliptin: insight into SIGMA-1 receptor profile
15	C	Rodriguez-Tanty	Amylovis-201 is a potent agonist of the s1 chaperone protein with anti-amylodogenic activity for treatment of Alzheimer´s disease
17	J	Sánchez Sánchez-Corral	Targeting Sigma-1 for Pain Relief: Selective Antagonists with Dual Mechanism of Action

Neurodegenerative diseases, psychiatric disorders and pain

N	Presenting author name		Title of the communication
19	T	Aparicio Mescua	Antagonism of Sigma-1 inhibits binge ethanol drinking in adolescence
21	A	Gazzano	Leveraging Multi-Targeted Direct Ligands To target TSPO and S1R crosstalk in microglia.
23	MA	Huerta	Sigma-1 Receptor Inhibition Reduces Arthritis Progression and Pain
25	KK	Kumaree	Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuron loss, oxidative stress, and mitochondrial dysfunction.
27	A	López-Ruiz	Multi-Target Directed Ligands binding the sigma-1 receptor: promising therapeutic strategy for pain management
29	M	Meltzer	Pridopidine exerts neuroprotective effects, via activation of the Sigma-1 receptor (S1R)
31	M	Meltzer	The Phase 3 PROOF-HD Trial Demonstrates Meaningful Benefits of Pridopidine on Function, Cognition, and Motor in Huntington Disease (HD)
33	D	Svob Strac	DHEA(S) in dementia: from cellular and animal models to patients

Sigma-1 receptor biochemistry and cellular biology: structure, protein targets and basic mechanisms, and other diseases and disease models

N	Presenting author name		Title of the communication
35	AP	Cherciu	The hidden effects of Metformin on oxidative stress
37	J	Jonane	Sigma-1 receptor is associated with tetraspanin-labelled extracellular vesicles: insights from single particle analysis
39	I	Pantic	Concept of a random forest machine learning model for detection of chromatin structural rearrangements induced by Sigma-1 receptor ligands
41	A	Rozsahegyi	Sigma-1 receptor agonist fluvoxamine is protective against hyperglycemia-induced fibrosis in human trabecular meshwork cells
43	W	Trein	The interplay of S1R and Wolframin in neuronal autophagy

Poster Presentations - 26th June Thursday (even numbers)

Medicinal Chemistry

N	Presenting author name		Title of the communication
2	D	Arella	Development of Sigma-1 Receptor Photowitchable Ligands as Molecular Tools
4	V	Cavalloro	Sigma-1 receptor: the hidden target of traditional preparations for neurodegeneration and neuropathic pain
6	G	Costanzo	Synthesis and biological evaluation of novel S1R ligands
8	J	Handzlik	In vivo evaluation on antipsychotic-like activity for the first-in-class arylselenoether-piperazine derivatives targeting sigma-1/D2/5-HT1A receptors in mice
10	MC	Lomuscio	SIGMAP: an explainable artificial intelligence tool for SIGMA-1 receptor affinity prediction
12	F	Mastropasqua	DEVELOPMENT OF POSITRON EMISSION TOMOGRAPHY RADIOTRACERS FOR IMAGING SIGMA-1 RECEPTORS IN CNS DISEASES
14	S	Salvi	RC-106, a promising therapeutic agent against glioblastoma. Scale up synthesis, solid-state characterization and in vivo evaluation
16	E	Szymanska	Novel multi-target ligands of sigma-1 receptor as potential agents for treatment of neuropsychiatric disorders

Neurodegenerative diseases, psychiatric disorders and pain

N	Presenting author name		Title of the communication
18	F	Bekbulat	ni2n: a networking project to promote progressive, multidimensional research concepts
20	M	Cagalinec	Endoplasmic reticulum stress, integrity and mitochondrial morphology in a cellular model of Wolfram syndrome
22	A	Jagielska	HBK-15, a multimodal compound activating sigma-1 receptors, reverses memory and executive function deficits in neuropsychiatric disorder models
24	AM	Jiménez-García	Modulation of Frustration through Sigma-1 Receptors: Effects of PRE-084 and S1RA in Wild-Type and Knockout Rats Using the CSNc Paradigm
26	M	Meltzer	Pridopidine For the Treatment of ALS (Healey ALS Platform Trial)
28	MC	Ruiz Cantero	Sigma-1 receptor antagonism and soluble epoxide hydrolase inhibition: synergistic effect in the reduction of capsaicin-induced and surgical incision-induced tactile allodynia
30	M	Santos	Sigma-1 receptor antagonism enhances endogenous and drug-induced opioid analgesia: promising strategies for postoperative pain treatment

Sigma-1 receptor biochemistry and cellular biology: structure, protein targets and basic mechanisms, and other diseases and disease models

N	Presenting author name		Title of the communication
32	T	Lakat	Transcriptomic profiling of Sigma-1 receptor agonism in bleomycin-induced pulmonary fibrosis: Insights into therapeutic mechanisms
34	T	Medveczki	Age-specific anti-glaucomatous effect of a Fluvoxamine-containing eye drop
36	K	Palikaras	High-content drug screening for Sigma-1 Receptor activators using the nematode Caenorhabditis elegans
38	J	Paunovic Pantic	Potential of support vector machine learning in Sigma-1 receptor physiology
40	A	Toth	Protective effects of a novel Sigma-1 receptor agonist in renal ischemia/reperfusion injury

Anti-amnesic and neuroprotective effects of sigma-1 receptor agonists

Maurice, T[1]

[1] MMDN, University of Montpellier, EPHE, INSERM, 34095 Montpellier, France.

The sigma-1 receptor (S1R) is an atypical transmembrane protein expressed in numerous cells, such as, in the brain, neurons and glial cells. Highly expressed in the endoplasmic reticulum (ER) membrane, its main function consists in chaperoning a variety of partner proteins allowing direct modulations of numerous intracellular pathways at the ER. For instance, it facilitates the dialogs between ER and mitochondria (being particularly enriched in mitochondria aposed ER membranes known as MAMs), ER and cell nucleus or ER and plasma membrane. This chaperone activity can be triggered by small molecules acting as agonists or positive modulators and blocked by antagonist (that's why it retains the denomination of a receptor). S1R activity targets numerous client proteins and is thus involved in major cellular functions such as calcium or lipoproteins exchange, neurotransmitter release, ion fluxes, inflammation, autophagy or apoptosis. In particular, S1R efficiently alleviates cellular defect observed in and responsible for neurodegeneration in pathologies such as Alzheimer, Parkinson, Huntington, amyotrophic lateral sclerosis or several genetic or metabolic diseases. This talk will particularly describe the role of S1R and pharmacological effects of S1R agonists in physiological processes like learning and memory and in pathological indications such as Alzheimer's disease. At the precise time when the S1R activity of non-selective medicines is uncovered and first generation selective S1R agonists are in phase 3 clinical trials in several indications, we will propose a general overview of the current potentialities in the field.

Development of sigma-1 receptor ligands: past, present and future perspectives

Abate, C[1]

[1] Department of Pharmacy-Pharmaceutical Sciences, Università degli Studi di Bari ALDO MORO, Via Orabona 4, 70125, Bari, Italy.

Sigma receptor story begins in 1976, when Martin and colleagues, while studying a series of opiates, identified different classes of opioid receptors based on the ligands that activated them: the 'mu' receptor, modulated by morphine; the 'kappa' (k) receptor, activated by ketocyclazocine; and the 'sigma' receptor, activated by the benzomorphan compound SKF-10,047.[1] In the following years, it became evident that sigma receptors had been mistakenly classified as a type of opioid receptor. This misconception was challenged by several findings: sigma receptors were not bound by classical opioid antagonists such as naloxone and naltrexone; they exhibited high-affinity binding with several non-opioid ligands; and the stereochemistry of benzomorphan binding to sigma receptors was the opposite of that preferred by mu and kappa opioid receptors. Subsequent discoveries showed that benzomorphans also bound to phencyclidine (PCP) sites, and vice versa, PCP could bind sigma receptors. This led to the temporary classification of these receptors as PCP/sigma sites. In the early 1990s, the development of new ligands allowed the distinction of two sigma receptor subtypes: sigma-1 and sigma-2. Shortly thereafter, the sigma-1 receptor was successfully cloned from various sources. However, it was not until 2016 that its crystal structure was finally elucidated [2], revealing an unusual folding pattern and clarifying the receptor's binding site, putting to rest the various structural hypotheses proposed over the years. The story of sigma-2 receptor identification was longer and more complex. Its identity was only confirmed in 2017 as the TMEM97 protein, with its crystal structure unveiled in 2021. Interestingly, despite their very different overall structures, both sigma subtypes appear to have evolved convergent binding sites, allowing many ligands to interact with both receptors. This peculiarity has hampered the development of selective ligands in the early years. The long-standing absence of the protein structural data has not deterred medicinal chemists from the development of high-affinity sigma-1 receptor ligands during the decades. Notably, a pharmacophore model for the sigma-1 receptor was proposed as early as 1994, summarizing the key structural features necessary for high-affinity receptor binding.[3] Importantly, the binding site identified through structure-affinity relationship (SAfiR) studies, based on the analysis of hundreds of developed ligands, strictly matches the binding site revealed by the crystal structure. Over the years, this pharmacophore has guided the development of several classes of high-affinity sigma-1 ligands. The most promising among these have been thoroughly studied across various physiopathological contexts in which the sigma-1 receptor is implicated. These investigations have deepened our understanding of the receptor's roles, and contributed to its designation as a ligand-operated "pluripotent chaperone." The different structural classes of sigma-1 receptor ligands will be examined, highlighting both their limitations and the key advancements in the sigma-1 receptor they have facilitated. Particular attention will be given to the goals that remain to be achieved, objectives that may become attainable through the 'Sigma-1 Europe' initiative.

Keywords: medicinal chemistry, drug design

References

1. The effects of morphine- and nalorphine- like drugs in the nondependent and morphine-dependent chronic spinal dog. Martin WR, Eades CG, Thompson JA, Huppler RE, Gilbert PE. J Pharmacol Exp Ther. 1976, 197, 517-32. Doi: 10.1016/S0022-3565(25)30536-7
2. Crystal structure of the human σ1 receptor. Schmidt HR, Zheng S, Gurpinar E, Koehl A, Manglik A, Kruse AC. Nature 2016, 532, 527-530. doi: 10.1038/nature17391.
3. Structural features important for sigma 1 receptor binding. Glennon RA, Ablordeppey SY, Ismaiel AM, el-Ashmawy MB, Fischer J B, Howie K B. J Med Chem 1994, 37, 1214-1219. doi: 10.1021/jm00034a020.

Modulation of Sigma-1 receptor and TSPO through bivalent ligands: exploring a new approach against neuroinflammation and neurodegeneration.

Rossino, G[1], Gazzano, A[2], Linciano, P[1], Rossi, D[1], Schepmann, D[3], Wünsch, B[3], Crouzier, L[4], Tangui, M[4], Caballero, J[5], Peviani, M[2], Collina, S[1]

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[3] Institute of Pharmaceutical and Medicinal Chemistry, University of Münster, Corrensstraße 48, 48149, Münster, Germany.
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[5] University of Talca, Center for Bioinformatics and Molecular Simulations, 2 Norte 685, Talca, Chile.

Neurodegeneration is a multifaceted condition involving the combination of several mechanisms, such as neuronal cell death, inflammation, aberrant proteostasis, altered energy homeostasis, DNA and RNA defects. In this context, multi-target directed ligands (MTDLs) offer a promising strategy for achieving improved therapeutic outcomes. Additionally, they can help validate novel, unexplored protein-protein interactions and crosstalk. To probe novel approaches against neuroinflammation and neurodegeneration we envisaged to use MTDLs directed toward two promising targets: the Sigma-1 receptor (S1R) and the 18kDa Translocator Protein (TSPO). S1R has been correlated to many diseases of the central nervous system (CNS), and its agonists are known to promote neuroprotection and neuroplasticity. TSPO is a transmembrane protein involved in various pathways that influence cell survival. While it is clinically utilized for neuroinflammation imaging, its therapeutic potential remains largely unexplored. Given these considerations and the convergence of S1R and TSPO pathways in regulating mitochondrial function, targeting both receptors could enhance therapeutic outcomes. To assess the viability of this approach for managing neurological disorders, we designed and synthesized dual S1R-TSPO ligands, incorporating our in-house developed S1R agonist, RC-33, and the clinically validated TSPO ligand, PK-11195. Their design and synthesis were guided by our experience in mono- and bi-valent S1R ligands. Their interaction with the targets was assessed through binding assays and their functional profile was evaluated in vitro. Computational studies are ongoing to rationalize the experimental results. To date, our findings confirm the value of these compounds as pharmacological tools and establish a foundation for developing drug-like molecules able to target both S1R and TSPO, paving the way for an innovative approach to promote neuroprotection.

Keywords: Sigma-1 receptor, TSPO, neuroinflammation, neurodegeneration, multi-target directed ligands

References

1. Ryskamp, D. A.; Korban, S.; Zhemkov, V.; Kraskovskaya, N.; Bezprozvanny, I. Front. Neurosci. 2019, 13.
2. Guilarte, T. R. Pharmacol. Ther. 2019, 194, 44–58.
3. Rossino, G.; Rui, M.; Linciano, P.; Rossi, D.; Boiocchi, M.; Peviani, M.; Poggio, E.; Curti, D.; Schepmann, D.; Wünsch, B.; González-Avendaño, M.; Vergara-Jaque, A.; Caballero, J.; Collina, S. J. Med. Chem. 2021, 64 (20), 14997–15016.

Discovery of novel class of multi-target ligands for sigma-1 receptor and selected GPCRs among selenoether derivatives of piperazine

Pyka, P[1,2], Satała, G[3], Deuther-Conrad, W[4], Lippolis, A[5], Carrieri, A[5], Pytka, K[6], Handzlik, J[1], Szymańska, E[1]

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6. Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Krakow, Poland

Initially misclassified as an opioid receptor, sigma-1 receptor (S1R) is a chaperone protein located in the endoplasmic reticulum, modulating signaling pathways of G protein-coupled receptors (GPCR) and ion channels. Increased interest in S1R functions has led to intensive research into its significance in various diseases. In recent years, research has shown that modulation of this receptor holds therapeutic potential for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis, as well as for depression, schizophrenia, and pain management. The literature describes many examples of polypharmacological agents whose mechanism of action is based on simultaneous interaction with S1Rs and the serotonin system. A well-known example are serotonin reuptake inhibitors (SSRIs) – e.g. fluvoxamine, for which the affinity for the sigma-1 receptor has been reported. However, little information is available on ligands that interact simultaneously with S1R and individual GPCRs, particularly in the context of serotonin receptors. On the other hand, lines of evidence indicate that the presence of selenium in organic compounds, due to its antioxidant and neuroprotective properties, is an additional asset in the search for new therapies for CNS diseases and disorders. In our previous studies, we have identified a series of phenyl-selenoether derivatives of phenylpiperazine acting as anticancer agents [1, 2]. Recently, the affinity of some of these compounds for selected serotonin and dopamine GPCRs has been confirmed. The structural similarity of these agents to the well-known S1R agonist, cutamesine, followed by preliminary S1R affinity studies, led us to design, synthesize and biologically evaluate a new series of arylpiperazine derivatives containing a selenoether group, with potential simultaneous affinity for the selected GPCRs and S1Rs. The new compounds were obtained in multiple-step synthesis and evaluated for their affinity towards the selected GPCRs and S1R. Based on in vitro screening results a number of multi-target compounds with high affinity towards 5-HT1AR, D2R and S1R have been identified. The relationship between structure and affinity for individual biological targets has been studied and analyzed. Additionally, molecular modeling was performed to highlight possible binding modes for the selected receptors, providing insights into affinity and potential key interactions at the binding site. Given the polypharmacological affinity profile, the compounds may have a potential therapeutic effect in the treatment of schizophrenia through the modulation of key neurotransmitter pathways that are involved in the disorder. Therefore, one of the obtained compounds, possessing nanomolar affinity towards these receptors, was selected for further in vivo assessments to evaluate its potential antipsychotic, antiamnesic, anxiolytic and proactive coping

properties. This study was funded by the National Science Centre, Poland; grant no. 2024/53/B/NZ7/03768.

Keywords: Selenoethers, Arylpiperazines, GPCRs, Sigma-1, Schizophrenia,

References

1. Ali, W. et.al. (2020). Eur. J. Med. Chem. 200. 112435. [2] Ali, W. et.al. (2022). Eur. J. Med. Chem. 243. 114761.

Unlocking the Power of Inhibiting Soluble Epoxide Hydrolase and Sigma-1 Receptor Antagonism through Dual Mechanism Analgesic Compounds

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Pain affects over 40% of the global population, but current therapies often lack effectiveness. Innovative analgesics with better efficacy, fewer side effects, and lower abuse potential are urgently needed. With a pressing need for non-abusable analgesics, recent research has identified new potential targets1. This project aims to develop a first-in-class dual analgesic compound to address the management of pain. Our strategy centers on the design of compounds that inhibit soluble epoxide hydrolase (sEH) and antagonize the sigma-1 receptor (S1R). Inhibition of sEH enhances epoxyeicosatrienoic acids, showing beneficial therapeutic effects in neuropathic pain. Moreover, S1R is involved in pain signaling, and preclinical studies suggest that its antagonism may relieve hypersensitivity1. Of relevance, we recently discovered that the concurrent modulation of both targets amplifies the analgesic effects, offering a synergistic approach to alleviating pain2. Therefore, our group firstly designed, synthesized and biologically evaluated a series of dual-acting compounds and performed a structure–activity relationship study of the left-hand side of the scaffold. Next, as disclosed in the present work, a hit-to-lead optimization of the right-hand side of the molecule has been performed. Herein, we present the first dual acting compounds that inhibit sEH and antagonize S1R in the low nanomolar range3. After performing a screening cascade, ten compounds emerged as the most promising, displaying favorable characteristics, such as good DMPK properties in in vitro assays.

Keywords: dual-target therapy, non-opioid analgesia, pain, sigma-1 receptor, synergy

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Development of sigma receptor modulators for the treatment of neuropathic pain

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Neuropathic pain (NP) is a serious health problem affecting millions of people around the world, with significant costs to the healthcare system.1 Current treatments often provide only limited relief and are associated with undesirable side effects, limiting their long-term use. Despite extensive research, the need for safer, more effective non-narcotic analgesics remains an urgent challenge. Sigma receptors (S1R and S2R) have gained increasing attention in recent years for their involvement in pain modulation. Substantial evidence supports S1R's role in pain treatment, primarily based on the pain-attenuating phenotype observed in S1R knockout (KO) mice and the antinociceptive effects of S1R antagonists.2 Similarly, S2R agonists have demonstrated efficacy in reducing mechanical hypersensitivity in the spared nerve injury (SNI) model, exhibiting greater potency than gabapentin.3 These findings have driven the development of molecules with an S1R antagonist and/or S2R agonist profile as a promising strategy for identifying potent analgesic SR candidates. Over the years, piperidine and piperazine derivatives, as well as spirocyclic, diazabicyclo and tetrahydropyrrole compounds, have garnered significant interest in the development of bioactive compounds. The incorporation of these moieties into a molecule – taking into account the variable N–N distance dictated by specific structures – provides a unique spatial arrangement that can significantly influence key parameters such as potency, selectivity, and physicochemical properties. Using a multidisciplinary approach based on synthesis, computational and spectroscopic studies, and in vitro and in vivo assays, we have rationally designed, synthesized, and tested a series of derivatives able to bind SR in the nanomolar range and exhibit potent analgesic effects in animal models. The most promising candidates have undergone in vitro toxicity screening and in vivo functional assessment. Progress towards the identification of lead SR compounds will be fully disclosed, along with strategies for minimizing off-target effects, including hERG inhibition. Both challenges and successes encountered throughout the journey from hit identification to validation in animal models will be presented.

Keywords: Neuropathic pain, Sigma-1 Receptor, K+hERG, Drug discovery

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Sigma-1 receptor fluorescent ligands: development and use of powerful pharmacological tools

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Growing interest in sigma-1 receptor subtype (S1R) stems from its involvement in several pathologies such as neurodegenerative disorders, inflammatory diseases, cancer and neuropathic pain. This pluripotent chaperone protein acts through protein-protein interactions. Its relationship with proteins and factors is linked to its ability to form monomers or oligomers, which is closely related to ligand binding and responses to stress conditions. However, the underlying mechanisms of action are still unclear (1). The need to better understand S1R functions led our attention towards the development of fluorescent probes as useful tools in biological assays. Additionally, these probes may represent a green and safe alternative to the use of radioligands. With this aim, we previously designed green and red emitting S1R fluorescent ligands based on the S1R selective lead compound PB212 (2). Despite the promising results obtained with fluo-ligands LM1 and MM482 in flow-cytometry, non-specific binding was detected in confocal microscopy studies, likely due to the high lipophilicity. Thus, our goal is to obtain less lipophilic S1R-selective fluorescent compounds validated also in microscopy studies. For this reason, we focused on S1R reference compounds less lipophilic than PB212. In particular, 1-[□-(4-methoxyphenoxy)ethyl]-4-methylpiperidine, L13, was modified in the p-position, where different linkers were inserted for functionalization with fluorescent tags, such as 4,4-dimethylaminophthalimide (4-DMAP), 4-nitrobenzo-oxadiazole (NBD) cyanine 5 (Cy-5), or 4-nitrobenzo-selenadiazole (Se-NBD), which emit in the green and red regions of the light spectrum. Preliminary data for these compounds indicate low nanomolar affinity at S1R and appreciable selectivity towards the S2R subtype. Among these compounds, MA3 was studied further through flow-cytometry analysis performed in MCF7 cells overexpressing S1R, showing a selective interaction towards S1R and the ability to replace radioligands in binding assays. In conclusion, the phenoxyalkylpiperidine scaffold has been confirmed as promising for the development of S1R fluorescent ligands with reduced lipophilicity, whose fluorescent properties span from the green to the red emission wavelengths of the light spectrum. Some of these promising compounds are currently being validated using fluorescence-based techniques in cellular models overexpressing the S1R, holding the potential to generate tools to study S1R mode of action. This work was supported by PNRR - Missione 4, componente 2 “Dalla Ricerca all'Impresa” - Investimento 4.1 “Estensione del numero di dottorati di ricerca e dottorati innovativi per la pubblica amministrazione e il patrimonio culturale” (D.M.N. 118).

Keywords: sigma-1 receptor, fluorescent ligand, phenoxyalkylpiperidine

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Structural and Dynamic Determinants of Sigma-1 Receptor Oligomerization: Insights from Molecular Dynamics Simulations

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In recent years, great advances have transformed our understanding of the sigma-1 receptor (S1R). Beyond the receptor trimeric architecture revealed by crystallographic studies, biochemical approaches have identified dynamic higher-order oligomeric states that appear to serve distinct functional roles (1). The differential effects of agonists and antagonists on S1R oligomerization patterns strongly suggest that these supramolecular assemblies are not merely structural artifacts, but critical functional states directly linked to physiological signaling and pathological mechanisms. This relationship between ligand-induced oligomeric remodeling and downstream cellular effects establishes oligomerization as a central regulatory mechanism and promising therapeutic target in S1R-associated clinical conditions. For example, juvenile amyotrophic lateral sclerosis (ALS) has been linked to the S1R mutation E102Q. In this condition, it has been observed a disruption of higher-order S1R oligomers, suggesting that aberrant oligomerization dynamics directly contribute to neurodegenerative pathology (2). In our work, we employ comparative molecular dynamics (MD) simulations to investigate the structural and dynamic features governing S1R oligomerization. We examined three point mutations (E102Q, F191G, W136G), chosen for their positions at potential oligomerisation interfaces, and compared their behaviour with that of the wild-type (WT) protein. As mentioned before, the E102Q mutation, located at the base of the alpha1-helix, has been implicated in ALS and is hypothesized to disrupt critical intersubunit interactions. The F191G and W136G mutations were selected based on previous in vitro studies suggesting their involvement in oligomer stability. We analysed interface stability, residue-level interactions, and the protein network that may regulate oligomer formation. Our findings reveal that specific mutations alter the flexibility of transmembrane regions and interfacial contacts. In particular, mutations in the binding pocket induce allosteric changes that propagate to oligomerisation interfaces, suggesting a mechanistic link between ligand binding and higher-order assembly. Furthermore, we explored the influence of membrane composition on S1R stability. Comparison of simulations in POPC-only and cholesterol-enriched membranes indicate that lipid composition plays a crucial role in stabilizing of the trimeric state of the receptor. These insights provide a structural framework for understanding S1R-linked pathologies and offer new perspectives for designing therapeutic modulators that target S1R oligomerization dynamics. By identifying protein hotspots for communication between different regions of the protein, our computational approach potentially enables virtual screening of drug candidates that could selectively stabilize or disrupt specific oligomeric interfaces, redirecting aberrant oligomerization patterns observed in clinical conditions.

Keywords: Molecular dynamics simulations, point mutations, S1R oligomerization

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Tackling Neurodegenerative Diseases by Developing Light-Activated Drugs through an Integrated Multiscale Approach

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Photopharmacology is revolutionizing medicinal chemistry by enabling the precise spatial and temporal control of drug-receptor interactions with light. In the realm of neurodegenerative diseases, the sigma-1 receptor (S1R) has emerged as a compelling target due to its chaperone activity and demonstrated neuroprotective functions. Yet, the molecular basis underlying S1R activation, specifically its interplay with the chaperone BiP, remains elusive. Here, we outline two complementary approaches for modulating S1R. First, we aim to develop a red light-responsive, photoswitchable ligand by incorporating an azobenzene-based chromophore. This ligand is designed as an "affinity switch": the cis isomer exhibits high affinity for S1R, while the trans isomer remains largely inactive. Red light is particularly advantageous for such applications, as it penetrates deeper into biological tissues and mitigates phototoxicity. Second, we aim to design a dualsteric ligand, which simultaneously engages two distinct sites on S1R to stabilize a desired conformation and enhance neuroprotective signaling. Although the dualsteric ligand does not require photoswitching, it represents a crucial parallel strategy to achieve more robust and selective modulation of S1R function. To pursue these goals, we combine cutting-edge computational and experimental techniques. Our multiscale simulations, which include QM/MM nonadiabatic dynamics, molecular dynamics with enhanced sampling techniques, enable precise tuning of both photochemical properties (e.g., absorption maxima, photostationary states) and pharmacodynamic parameters (e.g., receptor affinity, conformational selectivity). These computational predictions are then compared and refined through collaborative experimental validation in Prof. Decker's laboratory in Würzburg, ensuring that our in silico insights translate into tangible lead compounds. By elucidating the link between S1R activation and neuroprotection, our integrated approach has the potential to accelerate therapeutic innovation for a range of neurodegenerative disorders. Moreover, the methodologies we develop, encompassing photoswitchable ligand design, dualsteric strategies, and multiscale simulation, are broadly applicable to other protein families, including ion channels and G protein-coupled receptors.

Keywords: sigma 1, allosteric molecule, molecular dynamics, drug design, photopharmacology

The Sigma-1 Receptor Chaperone: Enigmatic?

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My presentation will address the following: 1) Aspects of the ligand operated Sigma-1 Receptor Chaperone (S1R) crystal structure with relevance to endogenous regulators including the lipids Sphingosine (Sph) and Dimethylsphingosine (DMS); 2) Possible biochemical structural and functional linkages between the S1R and small heat shock protein chaperones (sHSPs); 3) Characterization of the binding of selected psychoplasmogenic tryptamine based psychedelics to the S1R monomer binding site using in silico modeling.

Keywords: Structure, Lipids, Endogenous, Psychedelics, Psychoplasmogenic

Sigma-1 as a target for antiseizure and disease-modifying effects? Preclinical validation in mouse models of epilepsy

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Chronic antiseizure medication therapy is a cornerstone of epilepsy therapy in human medicine. Most available drugs focus on seizure suppression rather than targeting underlying pathophysiological mechanisms or offering disease-modifying effects. Developing such approaches could improve treatment efficacy and help overcome drug resistance. In this context, Sigma-1 as a chaperone protein seems to serve an important protective and regulatory function, when homeostasis is threatened by pathophysiological mechanisms in disease states. Therefore, Sigma-1 is considered as a promising target with the potential to mediate disease-modifying effects. Thus, we addressed the hypothesis that genetic and pharmacological targeting of Sigma-1 affects seizure thresholds, seizure parameters, and the development of a hyperexcitable network in two chronic mouse epilepsy models, the amygdala kindling paradigm with repeated seizure induction and the intrahippocampal kainate (IHK) model with development of spontaneous recurrent seizures. Genetic Sigma-1 targeting was explored by comparison between Sigma-1 knockout and wildtype mice. For pharmacological targeting we evaluated the efficacy and tolerability of the selective positive allosteric Sigma-1 modulator E1R and Fenfluramine (FEN), which has a combined mechanism of action with effects on serotonergic signaling and Sigma-1, and NE-100 as a selective antagonist. Furthermore, we aimed to determine the compound's interaction with Sigma-1 for E1R and the relative contribution of the interaction between FEN and Sigma-1 by combination experiments with NE-100. In fully kindled mice, E1R exhibited dose-dependent antiseizure effects at well-tolerated doses, whereas FEN exerted only limited effects without a clear dose-dependency. The Sigma-1 antagonist NE-100 reduced the response of kindled mice to E1R and partially abolished the effect of FEN. In contrast, FEN showed antiseizure effects in the IHK model, while E1R did not affect electrographic seizure activity. Surprisingly, in the IHK model, pre-exposure to NE-100 rather enhanced and prolonged the antiseizure effects of FEN. Moreover, data provide evidence that E1R affects kindling progression by delaying the onset of generalized seizures and reduced motor and electroencephalographic seizure duration during the early kindling phase. Unexpectedly, neither the co-treatment of NE-100 and E1R nor the genetic deficiency had any impact on the E1R-mediated effects on kindling progression. Genetic Sigma-1 targeting in this model revealed that Sigma-1 deficiency had no impact on kindling progression but resulted in prolonged motor and electroencephalographic seizure durations. In conclusion, our findings reveal a different preclinical efficacy profile of both E1R and the antiseizure medication FEN. The findings from the IHK model indicate that it may be of interest to further evaluate the potential broad-spectrum effect of FEN. The relative contribution of FEN's interaction with Sigma-1 seems to vary depending on the preclinical model. Moreover, the results indicate that E1R delays the generation of a hyperexcitable neuronal network. As the E1R-mediated effect was not completely abolished either by the antagonist or genetically, further studies with highly selective Sigma-1 modulators are needed. Further research is planned to evaluate disease-modifying and preventive effects of Sigma-1 modulators in a model with spontaneous

recurrent seizures. Acknowledgements: Funded by DFG PO681/12-1. Epilepsie-Stiftung Wolf granted a scholarship to D. P.-P. UCB/Zogenix and LIOS supplied FEN and E1R, respectively.

Keywords: Antiseizure medication, Epileptogenesis, E1R, Fenfluramine, Temporal lobe epilepsy

Allosteric positive modulator of Sigma-1 receptor: a new weapon to mitigate disease progression in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is characterized by a degeneration of motor neurons, leading to muscle weakness and progressive paralysis. Currently, no treatment is available to halt or reverse the progression of the disease. Oxidative stress, mitochondrial dysfunction, accumulation of unfolded proteins and inflammation are interconnected key actors involved in ALS. A potent therapeutic strategy would be to find molecules that break this vicious circle leading to neuronal dysfunction and death. Targeting Sigma-1 receptor (S1R) could meet this objective, as this chaperone protein modulates many cell survival mechanisms. So far, the impact of S1R activation in ALS was studied using specific agonists and mostly on the SOD1 mutation that represents only 2% of patients. Our objectives are to determine how much S1R activation is protective in the ALS context and to better understand the mechanisms by which S1R acts. In our study, we compared the impact of two different S1R activators: the reference agonist PRE-084 and the positive modulator OZP002. Both S1R activators, significantly alleviated locomotor deficit of zebrafish models expressing mutations of two ALS key genes: TDP43 or C9orf72. Moreover, we provided direct evidence that S1R-induced beneficial effects were in part mediated by NRF2 signaling pathway. NRF2 cascade is known to regulate defense against oxidative stress and inflammation. More importantly, we studied the impact of PRE-084 or OZP002 on mice expressing mutant TDP43 (TDP43A315T). Administration of PRE-084 or OZP002 by intraperitoneal injection was able to ameliorate locomotor performances and coordination of TDP43A315T mice. Moreover, S1R activation reduced the motor neuron loss and the glial reaction in the spinal cord. Our data further demonstrate the therapeutic value of S1R to counteract ALS pathology. In addition, we showed that the use of S1R positive modulator achieves the same efficacy as agonists.
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Keywords: TDP43, C9orf72, Sigma-1 receptor, NRF2, ALS

Targeting Sigma-1 Receptor for Neurodegenerative Diseases: Insights into Physiopathology and Therapeutic Potential

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The Sigma-1 receptor (S1R) is an intracellular chaperone protein that is essential for neuroprotection, synaptic plasticity, and cellular stress responses, making it a promising therapeutic target for neurodegenerative diseases (NDs) such as Alzheimer's disease (AD) and Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS). This study explores the pharmacological modulation of S1R through the use of agonists and antagonists, its interaction with the S1R–Binding Immunoglobulin Protein (BiP) complex, and the application of in silico approaches to identify novel small-molecule ligands for S1R-targeted therapy. To elucidate the molecular interactions involving S1R, ligand docking studies were conducted to compare the binding affinities of various agonists and antagonists to both the S1R monomer and the S1R–BiP complex. Computational analyses indicated that S1R antagonists exhibited high binding affinity for S1RA and NE100. Additionally, comparative docking studies of orthologous S1R proteins across different species suggested evolutionary changes in ligand binding pockets, influencing receptor-ligand interactions. To identify novel S1R-targeting small molecules, a virtual screening approach was employed, focusing on N-benzylcinnamamide analogs, a bioactive compound derived from Piper submultinerve. Screening was conducted using the PubChem database, generating specific drug library a targeting S1R. considering the established relationship between S1R and the cholinergic system, we also screened M1–M4 muscarinic acetylcholine receptors, which are associated with cognitive functions and the pathology of neurodegenerative diseases. To prioritize potential hits, we employed SIGMAP, an explainable artificial intelligence (AI) tool, to predict S1R binding affinities and the neuroprotective effects induced by ligands. This AI-driven structure-activity relationship (SAR) analysis facilitated the identification of promising S1R ligands with therapeutic potential. This study provides new insights into physiopathology of S1R, the interactions between receptors and ligands, and its functional relationship with muscarinic receptors. It highlights the potential of computational drug discovery in developing novel neuroprotective therapies. Further validation of these computationally identified compounds through in vitro and in vivo studies is necessary to evaluate their therapeutic effectiveness and translational potential in neurodegenerative diseases.

Keywords: Sigma-1 receptor, neurodegeneration, Alzheimer's disease, muscarinic receptors, artificial intelligence, virtual screening, drug discovery

Pridopidine, a Sigma-1 Receptor Agonist, Offers Therapeutic Potential for Huntington's Disease and Amyotrophic Lateral Sclerosis

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Pridopidine, a highly selective sigma-1 receptor (S1R) agonist, is a promising candidate for Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS), neurodegenerative diseases with no disease-modifying treatments. The S1R, a chaperone in mitochondrial-associated membranes (MAMs), regulates cellular stress, calcium homeostasis, mitochondrial function, autophagy, and synaptic integrity. Dysfunction in these pathways contributes to neuronal degeneration in HD and ALS. In preclinical studies pridopidine activation of the S1R contributed to a restoration in MAM integrity, calcium homeostasis, and improvements in mitochondrial function. Pridopidine reduces oxidative and ER stress, promoting neuronal survival. In ALS models, pridopidine reduces mutant SOD1 aggregation and ER stress, both key contributors to motor neuron degeneration. In HD models, early-treatment upregulates neuroprotective pathways, including cAMP and brain-derived neurotrophic factor (BDNF) expression/signaling. The Phase 3 PROOF-HD trial evaluated pridopidine (45 mg bid) in a global, randomized, placebo-controlled study over 78 weeks, with an open-label extension up to 104 weeks. In HD patients not receiving antidopaminergic medications (ADMs) (pridopidine n=96, placebo n=112), pridopidine demonstrated sustained, clinically meaningful benefits across different measures of HD. Total Functional Capacity (TFC), a key measure of daily function d the composite Unified Huntington's Disease Rating Scale (cUHDRS) scores showed clinically meaningful slower progression Cognitive performance, particularly on the Stroop Word Reading Test (SWR) showed no worsening from baseline, while the placebo group declined. Motor function was assessed by the objective, rater independent measures of Q-Motor (quantitative motor) showing significant benefits over placebo throughout the 78 weeks of the study. Importantly, pridopidine showed persistence of efficacy in all endpoints over two years, outperforming matched cohorts from natural history studies (Enroll-HD/TRACK-HD). In ALS, pridopidine was evaluated in the Phase 2 Healey ALS Platform Trial, a large-scale, shared placebo-controlled study. Subgroup analysis in a population of faster-progressing ALS participants (<18 months post-onset) revealed significant benefits. Pridopidine slowed disease progression with a 32% reduction in ALS Functional Rating Scale-Revised (ALSFRS-R) score decline at 24 weeks, suggesting a meaningful impact on disease trajectory. Patients exhibited a slower decline in respiratory function and dyspnea measures, along with stabilization of articulation and speaking rates—critical indicators of disease burden in ALS. Additionally, Kaplan-Meier analysis showed an 85% survival probability at approximately 10-months for pridopidine-treated patients, as compared with around 50% in the placebo group, suggesting a potential survival advantage. In all studies, pridopidine was well tolerated, with no major safety concerns. These findings support an ongoing regulatory discussion for approval of pridopidine in HD, and support planning of a global Phase 3 ALS trial. In summary, pridopidine holds promise as a first-in-class therapy for neurodegenerative diseases, with over seven-years of safety data in HD supporting chronic use. Beyond HD and ALS, it is also being studied in Wolfram syndrome and Alexander disease. In an expanded access program for Vanishing White Matter Disease, two subjects showed no disease progression, stable cognition, and reduced plasma NfL-

biomarker levels, suggesting neuronal protection. As research advances, pridopidine remains a strong candidate for addressing unmet needs in multiple neurodegenerative conditions, with future studies set to evaluate its disease-modifying potential.

Keywords: Huntington's disease, amyotrophic lateral sclerosis, sigma-1 receptor, neuroprotection, pridopidine

Neuroprotective Potential of 6-Hydroxy-L-Nicotine: Modulating Memory, Oxidative Stress, and Neuroinflammation

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Neurodegenerative disorders, including Alzheimer's and Parkinson's disease, are characterized by progressive cognitive decline, oxidative stress, and neuroinflammation. In search of novel neuroprotective compounds, this study evaluates the effects of 6-hydroxy-L-nicotine (6HLN) using both in vivo and in vitro models. Behavioral assessments, including the Y-maze and novel object recognition tests, were conducted to investigate the impact of 6HLN on memory function in an experimental model of cognitive impairment. Additionally, oxidative stress markers, antioxidant enzyme activity, and neuroinflammatory mediators were analyzed to determine the compound's role in modulating neuronal homeostasis. Complementary in vitro experiments using a neuronal cell line further explored the cytoprotective properties of 6HLN against oxidative stress-induced damage and inflammation. Our findings reveal that 6HLN enhances cognitive performance, reduces oxidative stress, and attenuates neuroinflammatory responses, suggesting its potential as a promising neuroprotective agent. These results provide valuable insights into the therapeutic applications of 6HLN for neurodegenerative disease prevention and treatment.

Keywords: 6-Hydroxy-L-nicotine, Neuroprotection, Memory, Oxidative stress, Neuroinflammation

Targeting Sigma-1 Receptors for Improved Executive Flexibility Under Glutamatergic Dysfunction

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Currently, due to increased life expectancy, there is a significant rise in cognitive disorders that vary from mild impairments to severe dementia. Moreover, memory deficits often co-occur with psychiatric disorders such as depression and schizophrenia, worsening clinical presentations and complicating treatment. An intriguing therapeutic target for cognitive dysfunctions is sigma-1 receptors—transmembrane chaperone proteins localized in the endoplasmic reticulum—involved in the pathogenesis of neuropsychiatric and neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, depression, and schizophrenia. Sigma-1 receptor agonists have shown anti-amnesic potential, reversing memory impairments regardless of etiology. However, the role of sigma-1 receptors in modulating higher cognitive processes, particularly cognitive flexibility, working memory, and attention, is not fully explained. Here, we aimed to evaluate the role of sigma-1 receptors in MK-801-induced cognitive flexibility deficits linked to glutamatergic receptor hypofunction in male rats. We tested the activity of two sigma-1 receptor agonists—PRE-084, a model selective sigma-1 receptor agonist, and fluvoxamine, a compound known both as a selective serotonin reuptake inhibitor and sigma-1 receptor agonist in highly translatable cognitive paradigm – a two-choice visual discrimination task with reversal learning. Additionally, the behavioral data will be supported by the protein level analysis, e.g., synaptic proteins, including BDNF and PSD-95, and signaling molecules, such as ERK1/2 and CaMKII, in brain regions critical for executive functions. We observed that PRE-084 and fluvoxamine improved accuracy in the reversal phase compared to the MK-801-treated group, demonstrating improved cognitive flexibility and learning efficiency. Both compounds increased the percentage of correct responses, reduced correction trials, and decreased the number of perseverative errors, suggesting better adaptation to new contingencies and improved executive functioning. MK-801 moderately decreased latency to correct and incorrect choices and to reward collection, indicating impulsive responding and altered motivation. However, neither PRE-084 nor fluvoxamine reversed these latency changes, suggesting that sigma-1 receptor activation primarily enhances cognitive flexibility without directly affecting impulsivity or reward-seeking behavior. These findings highlight the sigma-1 receptor involvement in cognitive flexibility and glutamatergic dysfunction, therefore allowing the identification of novel therapeutic approaches effective in reversing not only memory impairments but also executive function deficits associated with neuropsychiatric and neurodegenerative disorders. This study has been conducted as part of a research project financed by the Jagiellonian University Medical College (grant number N42/DBS/000409).

Keywords: cognitive disorders, cognitive flexibility, sigma-1 receptors, MK-801

SIGMA1R play an indispensable role in Th9 differentiation

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T helper 9 (Th9) cells are a distinct subset of $CD4^+$ T cells, alongside Th17 and Treg cells, characterized by their production of interleukin-9 (IL-9). Th9 cells play key roles in allergic inflammation, autoimmune diseases, and tumor immunity. While transcription factors such as PU.1 and IRF4 have been identified as essential regulators of Th9 differentiation, the full network of genes controlling Th9 function and IL-9 production remains unclear. To investigate novel molecular regulators of Th9 differentiation, we performed RNA sequencing (RNA-seq) on stimulated and unstimulated Th9 cells activated with anti-CD3. Our analysis identified SIGMAR1 (Sigma-1 receptor) as significantly upregulated in Th9 cells upon activation. To determine whether this upregulation was exclusive to Th9, we conducted a comparative RNA-seq analysis of activated Th17 and Treg cells relative to Th0 controls. Interestingly, SIGMAR1 upregulation was unique to Th9 cells, with no significant expression changes observed in Th17 or Treg subsets. The selective upregulation of SIGMAR1 in Th9 cells suggests a novel role for this receptor in Th9 differentiation and IL-9 production. As SIGMAR1 is known to modulate cellular stress responses and calcium signaling, its involvement in Th9 differentiation may represent a previously unrecognized regulatory mechanism. These findings provide new insights into Th9 biology and may have implications for therapeutic strategies targeting Th9-mediated diseases.

Keywords: SIGMAR1, Th9, CD4, immune cells

Reduced levels of the sigma-1 receptor in the brains of Alzheimer's disease patients

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Background: The sigma-1 receptor (σ1R) has emerged as a critical player in Alzheimer's disease (AD), serving as both a potential biomarker and a therapeutic target. This study aimed to investigate alterations in regional σ1R expression in relation to amyloid-beta and tau pathologies, synaptic loss, gliosis, and autophagy in the brain of patients with AD. Methods: Immunohistochemical staining for σ1R was conducted on postmortem tissue slices from the frontal, temporal, and entorhinal cortices, and the hippocampus, from 45 sporadic AD patients and 44 nondemented controls (NCs). Correlation analyses were performed to examine the relationship between σ1R expression and key pathological markers, including amyloid-beta (4G8), phospho-tau (AT-8), synaptic markers (synaptic vesicle glycoprotein 2A, synaptophysin), as well as astrocytic (glial fibrillary acidic protein) and microglial (ionized calcium-binding adapter molecule 1) markers. Results: In comparison to NCs, AD patients exhibited significantly reduced σ1R expression in the hippocampus and frontal cortex, but not in the entorhinal or temporal cortices. Correlations revealed negative associations between σ1R levels and synaptic marker synaptic vesicle glycoprotein 2A with amyloid-beta, phospho-tau, and Braak stages, while positive correlations were observed between SV2A and astrocytic and microglial markers in the hippocampus and entorhinal cortex. Conclusions: Our findings provide postmortem evidence of reduced σ1R expression in the hippocampus and frontal cortex of AD patients. Furthermore, σ1R expression correlates with key pathological features, including amyloid-beta, tau, synaptic loss, and gliosis, highlighting its potential as both a biomarker and therapeutic target in AD.

Keywords: Alzheimer's disease, glia, sigma 1 receptor, synaptic dysfunction, tau

Sigma-1 Receptor Controls Glycolysis via Mitochondrial GRIM19 and Glucose Uptake

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Sigma-1 receptor (S1R) is a molecular chaperone protein residing mainly on the mitochondrion-endoplasmic reticulum (ER) interface called the MAM (i.e., Mitochondria-Associated ER Membrane). The S1R can maintain the Ca2+ efflux from the ER into the mitochondria by chaperoning IP3 receptor type3 (Ip3R3) at the MAM, thus ensuring the Ca2+ homeostasis for ATP production in mitochondria. S1R ligands therefore in part have been shown to be effective in treating neurodegenerative diseases in animal models and in humans (Macfarlane et al., JPAD, 2025). Glycolysis has been reported to relate to neuroprotection and is well known to bear an intrinsic relation with oxidative phosphorylation in mitochondria. Interestingly, although the S1R is related to mitochondrial reactivity, the role of S1R on glycolysis, in particular in the brain, is not well understood. Here, we addressed this question firstly by comparing the glycolysis and mitochondrial oxidative phosphorylation in WT vs S1R-KO Neuro2a (N2A) cells and similarly in cultured cortical primary neurons. In addition, we used PET brain imaging to examine if glucose uptake or utilization may differ in WT vs S1R-KO mouse brain. Results show that while no difference in mitochondrial oxidative phosphorylation was seen in WT vs S1R-KO samples, glycolysis was largely reduced in S1R-KO samples. In other words, results suggest that the S1R promotes glycolysis without compromising oxidative phosphorylation in mitochondria. Interestingly, western blots showed that most mitochondrial protein levels remain unaffected in S1R-KO samples except for the mitochondrial complex I component GRIM19 that was largely increased in S1R-KO samples. This result suggests that GRIM19 is normally suppressed by S1R in WT brains and that GRIM19 is anti-glycolysis. To provide a causal relation between GRIM19 and glycolysis, we knocked down GRIM19 in Sig1R-KO samples and demonstrated that the glycolysis was thence restored back up. In the PET study examining the dynamics of glucose in the living mouse brain, we found that the S1R apparently increases the glucose uptake into the brain. Those results, when taken together, suggest that the S1R promotes glycolysis in the brain by controlling GRIM19 of mitochondrial complex I without compromising the innate function of oxidative phosphorylation in mitochondria. Results also suggest that the S1R may regulate glucose uptake into the brain and that this action, in concert with the suppression of GRIM19, may orchestrate a perfect glycolysis for the action of brain when in need.

Keywords: Glycolysis, GRIM19, MAM, NAD/NADH, PET Imaging

TIRF Microscopy-Based Assay for Detecting and Colocalizing Membrane Proteins

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The development of novel fluorescent dyes and advanced microscopy techniques has significantly enhanced the study of cellular processes. Super-resolution microscopy enables the acquisition of highly detailed images, but it also necessitates compromises between sensitivity, resolution, and speed—the "eternal triangle" of fluorescent microscopy. This study focuses on the capabilities of Total Internal Reflection Fluorescence (TIRF) and Highly Inclined and Laminated Optical sheet (HILO) microscopy in examining various membrane proteins, including tetraspanins, G protein-coupled receptors (GPCRs), and sigma-1 receptors (Sig1R) in cells and extracellular vesicles (EV-s). In cells, the dynamics of proteins can be monitored if they are labeled with specific ligands, as is the case for GPCRs, although ligand binding properties and their biochemical activity set clear limits here [1]. An alternative approach is to use membrane proteins fused with suitable fluorescent proteins. Using the MultiBacMam transfection system ensures that up to four different proteins of interest are expressed in the same cell, and by selecting spectrally appropriate fluorescent proteins, their relative localization can be characterized. This approach demonstrated that Sig1R-YFP conjugate colocalizes with the endoplasmic reticulum marker KDEL-mRFP, and this overlap decreases with receptor activation [2]. Coexpression of Sig1R conjugates with fluorescent-tagged tetraspanins revealed high Sig1R-CD63 colocalization in EVs, suggesting that these originate from intracellular compartments such as multivesicular bodies. For the detection and characterization of EV-s, we have developed a TIRFM-based assay system [3], which enables the detection of EV-s with particular membrane proteins with single particle sensitivity and determination of colocalisation and ratio of different proteins within EV-s. The assay was implemented to detect different tetraspanins (CD9, CD63 and CD81) and other proteins in EV-s from different sources using fluorescent-labelled specific antibodies. It has been shown that the number of different tetraspanins, their mutual ratio, and their degree of colocalisation depend on the parent cells and give corresponding fingerprints, which are essential for developing EV-based tests for characterising different cells and tissues. Uncovering the dynamic localization and trafficking of regulatory membrane proteins like the Sig1R may offer new opportunities for diagnostic and research applications.

Keywords: TIRF microscopy; extracellular vesicles; sigma 1 receptor; tetraspanins, MultiBacMam

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Conserved LIR-specific interaction of sigma-1 receptor and GABARAP

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The sigma-1 receptor (σ1R), among its various functions, has been reported to modulate macroautophagy in mammalian cells and in C. elegans. The details of how this relationship is mediated has yet to be identified. To elucidate the connection between σ1R and autophagy, we analyzed σ1R biochemically and in terms of structure and phylogeny. Our analyses identified several putative motifs (LC3-interacting-regions, LIRs) that mediate interactions with proteins of the ATG8 family, which are known to promote autophagosome biogenesis, autophagic cargo reception, and lysosome fusion. Six putative LIR motifs in human σ1R are evolutionarily conserved. One of these, hLIR5, features a typical motif for interaction with a specific ATG8 protein, namely GABARAP. Employing a peptide array analysis, we demonstrated a direct σ1R-GABARAP interaction and, in addition, confirmed it in cellulo via proximity ligation assay and immunoprecipitation. Further, we verified a LIR-dependent presence of σ1R in isolated native autophagic vesicles. A loss-of-function mutation in hLIR5 resulted in massively decreased levels of autophagosome-associated σ1R. Our results establish a direct functional connection between σ1R and GABARAP, which are both associated with autophagosome-lysosome fusion. Supporting the physiological relevance of hLIR5 for σ1R function, two point mutations within this LIR-motif have previously been linked to autosomal-recessive distal spinal muscular atrophy 2.

Keywords: Sigma-1 receptor, autophagy, LIR motif, GABARAP, ATG8 proteins

Investigation of σ1 receptor mechanism of activity and identification of novel agonists

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The human sigma 1 receptor (HsS1-R) is an endoplasmic reticulum (ER) resident transmembrane protein implicated in a number of biological activities and, consequently, an attractive therapeutic target for several pathologies. Indeed, HsS1-R antagonists have analgesic properties, whereas agonists have neuroprotective activity and are being actively investigated for their ability to cure neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease and Huntington's disease. For these reasons, HsS1R structure and function have been intensively investigated. On the one hand, the 3D structure of HsS1-R bound to either agonists or antagonists has been elucidated by X-ray crystallography. On the other hand, HsS1-R activation has been shown to promote calcium flux from the ER to mitochondria, sustain prolonged unfolded protein response by the serine/threonine-protein kinase/endoribonuclease IRE1, and stimulate autophagy, counteracting ER stress. Additionally, agonists are well known to promote dissociation both of HsS1R polymers and of the complex between HsS1-R and the molecular chaperone BiP, which is formed under resting conditions. Nevertheless, several important questions regarding HsS1-R function are still unanswered: i) the identity of the receptor endogenous ligand(s); ii) the molecular mechanism of ligand access to the binding site; iii) the structural features underlying the biological activities. To try and answer these questions, we have: i) Solved the first 3D structure of HsS1-R in the apo form by cryo-electron microscopy, and compared it with the structures solved in complex with ligands; ii) Performed Molecular Dynamics simulations of HsS1-R structure in the presence and absence of ligands [1]; iii) Demonstrated that an FDA-approved drug, previously disclosed to be a HsS1-R agonist by virtual screening, in vitro surface plasmon resonance experiments, and ability to increase growth and growth rate of HD patients derived fibroblasts [2], exerts its activity by stimulating neuroprotection mechanisms such as autophagy and unfolding protein response. iv) Identified low molecular weight peptides (length ≤ 16 a.a.) mapping on the HsS1R solvent accessible surface and able to directly bind BiP and inhibit the HsS1R-BiP interaction, thereby mimicking the action of HsS1R agonists. Both the FDA-approved drug and the BiP-binding peptides are potential candidates and/or lead compounds for the development of therapeutic compounds for neurodegenerative diseases.

Keywords: Sigma1 receptor structure, ligand entry pathways; BiP binding peptides, Sigma1 Receptor agonists, Molecular Dynamics simulations

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Accelerated mutant huntingtin aggregation and reduction of ER stress by the S1R agonist pridopidine

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Activation of the Sigma-1 receptor (S1R) is neuroprotective in various neurodegenerative diseases, including Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), frontotemporal dementia and Alzheimer's disease (AD). In these diseases protein misfolding and aggregation lead to endoplasmic reticulum (ER) stress and cytotoxicity. Growing evidence, including our research, indicates that this occurs at early stages, through soluble oligomeric forms, before large aggregates develop. We had seen that ER stress is reduced upon sequestration of mutant huntingtin (mHtt) oligomers into large aggregates. Pridopidine (a potent S1R agonist) reduced ER stress in HD cellular models, primarily through the PERK branch of the unfolded protein response (UPR). Pridopidine increased toxic oligomeric mHtt recruitment into less toxic large SDS-insoluble aggregates, suggesting that this in turn reduces ER stress and cytotoxicity. To better understand when ER stress is induced during mHtt aggregation, we now employed TET-inducible expression of WT Htt and mHtt exon 1 linked to fluorescent proteins. We then used novel optical sorting techniques (pulse-shape FACS) to sort cells carrying or not large mHtt aggregates, combined with RNAseq analysis, which allowed us to identify early ER stress-induced genes. Indeed, known UPR genes and novel hits increased upon mHtt expression only prior to the appearance of large aggregates.

Keywords: endoplasmic reticulum (ER) stress, Huntington disease, neurodegeneration, Sigma-1 receptor, unfolded protein response

E-52862-A selective sigma-1 receptor antagonist, in peripheral neuropathic pain: Two randomized, double-blind, phase 2 studies in patients with chronic postsurgical pain and painful diabetic neuropathy

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Background: We report the efficacy and safety of E-52862-a selective, sigma-1 receptor antagonist-from phase 2, randomized, proof-of-concept studies in patients with moderate-to-severe, neuropathic, chronic postsurgical pain (CPSP) and painful diabetic neuropathy (PDN). Methods: Adult patients (CPSP [N = 116]; PDN [N = 163]) were randomized at a 1:1 ratio to 4 weeks of treatment with E-52862 (CPSP [n = 55]; PDN [n = 85]) or placebo (CPSP [n = 61]; PDN [n = 78]) orally once daily. Pain intensity scores were measured using a numerical pain rating scale from 0 (no pain) to 10 (worst pain imaginable). The primary analysis population comprised patients who received study drug with ≥1 baseline and on-treatment observation (full analysis set). Results: In CPSP, mean baseline average pain was 6.2 for E-52862 vs. 6.5 for placebo. Week 4 mean change from baseline (CFB) for average pain was -1.6 for E-52862 vs. -0.9 for placebo (least squares mean difference [LSMD]: -0.9; p = 0.029). In PDN, mean baseline average pain was 5.3 for E-52862 vs. 5.4 for placebo. Week 4 mean CFB for average pain was -2.2 for E-52862 vs. -2.1 for placebo (LSMD: -0.1; p = 0.766). Treatment-emergent adverse events (TEAEs) were reported in 90.9% of E-52862-treated patients vs. 76.7% of placebo-treated patients in CPSP and 34.1% vs. 26.9% in PDN. Serious TEAEs occurred in CPSP only: E-52862: 5.5%; placebo: 6.7%. Conclusions: E-52862 demonstrated superior relief of CPSP vs. placebo after 4 weeks. Reductions in pain intensity were seen in PDN with E-52862; high placebo response rates may have prevented differentiation between treatments. E-52862 had acceptable tolerability in both populations. Significance statement: These proof-of-concept studies validate the mode of action of E-52862, a selective sigma-1 receptor antagonist. In CPSP, E-52862 resulted in clinically meaningful pain relief. In PDN, reductions in pain intensity were seen with E-52862; high placebo response rates may have prevented differentiation between E-52862 and placebo. These findings are clinically relevant given that neuropathic pain is highly incapacitating, lacking effective treatments and representing a significant unmet medical need, and support further development of sigma-1 receptor antagonists for peripheral neuropathic pain.

Keywords: Peripheral neuropathic pain, sigma-1 receptor antagonist, E-52862

Fluvoxamine mitigates bleomycin-induced pulmonary fibrosis in mice

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Introduction & Aims: Idiopathic pulmonary fibrosis is the most common form of chronic inflammatory disorders characterized by severe scarring of the lungs. This results in declining pulmonary function and eventually death through respiratory failure. Incidence is constantly rising, and median survival after diagnosis is only 2-3 years. Current therapies (nintedanib and pirfenidone) may ameliorate pulmonary functional decrement but do not inhibit the progression of fibrosis or significantly reduce mortality. Thus, novel, effective anti-fibrotic therapies are desperately needed. Our recent results directed at using Sigma-1 receptor (S1R) agonists in treating progressive fibrosis in various organs have been patented worldwide. Based on these findings, here we investigated whether Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) can also ameliorate pulmonary fibrosis. Methods: Fibrotic processes were induced with TGF-β or PDGF in A549 lung epithelial cells and primary fibroblasts isolated from the lungs of S1R+/+ and S1R-/- mice. Cells were treated with FLU. Pulmonary fibrosis was induced in S1R+/+ and S1R-/- mice by oropharyngeal bleomycin (BLM) administration. Mice were treated daily with FLU and sacrificed after 21 days. Results: After pro-fibrotic factor induction, FLU mitigated α-SMA production and F-actin formation in both A549 cells and primary lung fibroblasts. The effect of FLU was not observed in fibroblasts isolated from S1R-/- mice. In mice, expressions of ECM components were elevated 21 days after fibrosis induction. FLU reduced the expressions of collagen I, collagen III, and fibronectin to control levels in S1R+/+ BLM+FLU, but not in S1R-/- BLM+FLU mice. Using multi-photon microscopy, the second-harmonic generation signal emission of collagen fibers was exploited. Intense collagen accumulation and fibrotic plaques were detected in the S1R+/+ BLM and S1R-/- BLM+FLU groups, that was nullified by FLU in S1R+/+ mice. Evaluation of Masson's trichrome-stained lung sections coupled with mapping the topographical structure of lungs using atomic force microscopy underlined the massive anti-fibrotic effect of FLU. In vivo MicroCT showed more preserved aerated tissue area in S1R+/+ BLM+FLU vs. S1R+/+ BLM and S1R-/- BLM+FLU. Conclusion: Here, we demonstrated that S1R agonist FLU diminishes the development and progression of lung fibrosis in mice with BLM-induced pulmonary fibrosis. With the use of S1R-/- mice, we showed that activation of S1R-mediated pathways is responsible for FLU's observed anti-fibrotic effect. Based on our preclinical data S1R may be a novel, effective drug target in treating pulmonary fibrosis. Funding: LP2021-3/2021, TKP2021-EGA-24, STAGE 2024-1.2.3-HU-RIZONT-2024-00056, SigmaDrugs Research Ltd.

Keywords: lung, fibrosis, fluvoxamine

Dual-Targeting Histamine H3 and Sigma-1 Receptor Ligands as Candidates for the Treatment of Neuropathic Pain

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The treatment of complex, multifactorial diseases with single-target therapies often fails to achieve satisfactory efficacy. Consequently, the pharmaceutical industry and academia have turned to strategies that modulate multiple biological targets simultaneously. Given the well-documented interplay between histamine H3 (H3R) and sigma-1 (σ1R) receptors, there is significant potential for developing dual-acting ligands for managing various pain conditions. In previous studies, we synthesized a series of dual ligands targeting both H3R and σ1R, identifying the key structural elements responsible for their dual activity.1,2 We further optimized their selectivity towards sigma-2 receptors and confirmed their antagonistic effects on both H3R and σ1R. Subsequent in vitro assays allowed us to evaluate the drug-like properties of these new ligands, leading to the selection of the most promising compounds for in vivo studies. We tested the activity of our compounds in both nociceptive and neuropathic pain models. Interestingly, compound KSK107 demonstrated the highest efficacy in the Chronic Constriction Injury (CCI) model, significantly outperforming the reference ligands, including the clinically tested σ1R antagonist, compound S1RA. This research supports the growing evidence that multi-target ligands can offer a more integrative and effective approach to pain management by modulating multiple pathways. Such compounds have the potential to deliver improved efficacy and safety profiles compared to current monotherapies. Acknowledgements. We gratefully acknowledge the National Science Center, Poland, for funding this research under grants 2020/36/C/NZ7/00284 and 2022/45/B/NZ7/03101.

Keywords: Sigma-1 receptor antagonists, histamine H3 receptor antagonists, neuropathic pain, dual-targeting ligands

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The application of AI tools in analyzing pain behavior in mice: examples on the study of sigma-1 receptor function

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Pain is highly prevalent, especially in the older adults. Non-evoked (ongoing and spontaneous) pain constitutes the core of the human pain phenotype, but its evaluation in rodents is challenging. We used Artificial Intelligence (AI) algorithms to determine the effects of sigma-1 receptor inhibition on this aspect of the pain phenotype in mice. We trained a convolutional neural network based on Google's InceptionV3 using images from mice with facial expressions reflecting normal (pain-free) states or clear pain features (e.g. orbital tightening, nose and cheek bulge), to detect the presence of ongoing pain. Our AI system detected increased facial pain expressions 3.5h after laparotomy, which were reversed by the subcutaneous administration of morphine. This effect of morphine was enhanced by the administration of the sigma-1 receptor antagonist S1RA. We also obtained 48-h recordings of mice 7 days after partial sciatic nerve transection, in the Spared Nerve Injury (SNI) model of chronic neuropathic pain. We used K-Means algorithm to cluster the positions of the mice into distinct behavioral groups in an unsupervised manner. We selected asymmetrically directed behaviors that involved the approach of the head to the injured limb as spontaneous pain-like behaviors. The duration of these responses significantly increased in SNI mice in comparison to uninjured (naïve or sham) animals. However, SNI sigma-1 knockout mice did not experienced such an increase in pain-like behaviors. Our results using AI algorithms support a prominent role of sigma-1 receptors in the modulation of non-evoked pain during acute (laparotomy-induced postoperative pain) or chronic pain (neuropathic pain) conditions. Grants IJC2020-046118-I, PID2019-108691RB-I00, PID2023-150747OB-I00 and C-CTS-226-UGR23 (funded by MICIU/AEI/10.13039/ 501100011033), Junta de Andalucía (CTS-109) and ERDF funds.

Keywords: pain, Artificial Intelligence, behavior, mice

Identifying and Characterizing Novel Small Molecule-Sigma-1 Ligands using Caenorhabditis elegans

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Neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's, represent a major challenge in drug development due to their complex pathophysiology and the difficulty of translating preclinical findings into effective therapies. Given the limitations of traditional in vitro and mammalian models, the use of alternative model systems is crucial for advancing our understanding of these diseases and identifying potential therapeutic targets. One such model organism that has proven invaluable in neurodegenerative disease research is Caenorhabditis elegans (C. elegans), a genetically tractable nematode that bridges the gap between cell culture studies and more complex mammalian models. With approximately 60-80% gene homology to humans and a fully mapped nervous system consisting of 302 neurons, C. elegans provides a powerful platform for investigating neuronal function and degeneration at the single-cell level. Among the promising molecular targets for neurodegenerative diseases, the sigma-1 receptor (σ1R) has gained increasing attention due to its role in neuroprotection, mitochondrial function, and calcium signaling. Dysregulation of σ1R activity has been implicated in the pathogenesis of multiple neurodegenerative disorders, making it an attractive target for drug discovery efforts. C. elegans serves as an ideal model for studying σ1R-related mechanisms due to its genetic manipulability, allowing researchers to explore the functional consequences of σ1R modulation in vivo. Additionally, C. elegans enables high-throughput screening of potential σ1R modulators, making it a cost-effective and scalable system for drug discovery. A key advantage of C. elegans in neurodegenerative disease research is its short lifespan of approximately 2–3 weeks, which allows for rapid assessment of disease progression and drug efficacy compared to rodent models. Moreover, its transparency facilitates real-time imaging of key pathological hallmarks, including protein aggregation, oxidative stress, and neuronal integrity. These features provide an unparalleled opportunity to study the molecular and cellular effects of σ1R ligands in a whole-organism context, overcoming the limitations of traditional cell culture models, which lack physiological complexity. Another significant advantage of using C. elegans in neurodegenerative research is its ability to mitigate ethical concerns associated with vertebrate research. Since C. elegans is a non-vertebrate organism, it offers a viable alternative for early-stage drug discovery studies while reducing the reliance on mammalian models. This not only accelerates the drug development process but also aligns with the growing emphasis on ethical considerations in biomedical research. We have explored the effects of different σ1R agonists and antagonists in C. elegans models of neurodegeneration. Notably, we have investigated the σ1R agonists PRE084 and fluoxetine, as well as the antagonists BD1047 and haloperidol, for their effects on neuronal health and function. Additionally, we have identified dipentylammonium (DPA) as a novel σ1R agonist with potential therapeutic properties for Alzheimer's disease. Furthermore, we have identified and tested two other potential σ1R ligands, N-benzylcinnamide and N-benzylbenzamide, in C. elegans models, demonstrating their potential role in neuroprotection. By integrating genetic, cellular, and behavioral analyses, C. elegans enhances our understanding of σ1R-associated neurodegenerative disease mechanisms. This model system can provide a powerful

platform for identifying novel therapeutic strategies, ultimately contributing to the development of effective treatments for neurodegenerative disorders.

Keywords: Sigma-1R Model organisms, C.elegans, Drug Development, Dipentylamine

The role of sigma-1 receptor in the anticancer activity of thiosemicarbazones

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α-N-Heterocyclic thiosemicarbazones (TSCs) are a class of small molecules with metal-chelating ability that also show excellent antineoplastic activity. The most prominent and best-studied drug candidate is 3-aminopyridine-2-carboxaldehyde TSC, also known as Triapine, which is currently tested in a clinical phase III trial against late stage vaginal and cervical cancer. During the last years, several new TSC derivates have been synthesized, including from our group the double-dimethylated Triapine derivative Me2NNMe2. This derivative was found to exhibit several hallmarks of paraptotic cell death, including appearance of endoplasmic reticulum (ER)-derived vesicles and mitochondrial swelling caused by increased Ca2+ influx as well as caspase-independent cell death via the MAPK signaling pathway. Interestingly, the stress-activated chaperon sigma-1 receptor (Sig-1R), encoded by the SIGMAR1 gene, is known for its role in regulating the Ca2+ signaling at the mitochondrial-associated membrane of the ER. Consequently, we hypothesized that Sig-1R might play a role in the mode of action of TSCs in cancer cells, especially in case of Me2NNMe2. Indeed, whole-genome gene expression analysis of human colon adenocarcinoma SW480 cells treated with Me2NNMe2 (100 nM, 1 µM) for 15 h showed significant downregulation of SIGMAR1 mRNA levels compared to control. In contrast, SIGMAR1 expression levels remained unchanged in 1 µM Triapine-treated cells. Subsequently, we investigated, on the one hand, the effects of TSC treatment in Sig-1R-overexpressing MCF7 cells in comparison to a vector control line and, on the other hand, the effects of the Sig-1R agonist (+)-pentazocine on the anticancer activity of Me2NNMe2 or Triapine. In conclusion, first indications were found that Me2NNMe2 but not Triapine influence Sig-1R-regulated mitochondrial Ca2+ homeostasis, which might be connected to Me2NNMe2-induced paraptosis.

Keywords: thiosemicarbazones, Triapine, Me2NNMe2, sigma-1 receptor, cancer

Regulation of Sigma-1 Receptor in Cancer Progression and Drug Resistance

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The Sigma-1 receptor (SIGMAR1) is a unique, ligand-regulated chaperone protein localized primarily in the endoplasmic reticulum (ER), where it plays a crucial role in maintaining cellular homeostasis by modulating (among others) calcium (Ca^{2+}) exchange between the ER and mitochondria. Beyond its fundamental role in intracellular signaling, SIGMAR1 has emerging significance in cancer biology, influencing cellular survival, stress response and apoptosis. Despite growing interest in its oncogenic and tumor-suppressive properties, its precise involvement in different malignancies remains unclear. To better understand its role in tumorigenesis, we analyzed its differential expression during carcinogenesis using genetic cell models of stepwise carcinogenesis and cell lines (near normal versus malignant) from different tissues (e.g. bladder, breast). Our findings indicate that SIGMAR1 expression varies across different types of cancer and grades suggesting a role in cancer progression. Furthermore, we examined SIGMAR1 alterations in doxorubicin-resistant osteosarcoma cancer cell lines alongside their chemo-sensitive parental cells, to explore potential links between SIGMAR1 expression and drug resistance mechanisms. By integrating multiple cellular systems that reflect different stages of tumorigenesis and therapeutic response, we aimed to uncover the complex regulatory patterns of SIGMAR1 and its potential implications in cancer progression and treatment resistance. Taken together, our data highlight SIGMAR1 dysregulation in cancer, which may provide new insights into its role as a biomarker or therapeutic target, paving the way for its potential application in cancer diagnosis and treatment. Acknowledgments: IPT acknowledges funding from NKUA SARG (C.S. 19067).

Keywords: Sigma-1 Receptor, cancer, chemoresistance

A newly developed fluvoxamine eyedrop for glaucoma treatment

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Purpose: Glaucoma is the leading cause of irreversible vision loss worldwide; effective therapies are still lacking. Elevated intraocular pressure (IOP), which results from trabecular meshwork (TM) fibrosis in the anterior segment, is the only modifiable risk factor for glaucoma. We recently reported that the sigma-1 receptor (S1R) agonist fluvoxamine (FLU) effectively reduces fibrotic processes in primary TM cells. To further prove the applicability of S1R as a novel anti-glaucoma drug target we designed a FLU containing eyedrop and tested its tolerability and efficacy in various glaucoma models. Methods: In vivo, tolerability and toxicity were tested using a fluorescein test with a slit lamp. Two glaucoma rodent models were applied: Model 1- Dexamethasone acetate (Dex, 10 mg/mL) was injected into the eyes of C57BL/6J wild-type (WT) and S1R knock-out (KO) mice then FLU containing eye drops (100 mM) were administered bilaterally twice daily after IOP elevation- for two weeks. Model 2- Glaucoma was induced in Brown-Norway rats by hypertonic saline injection into the episcleral vein then FLU eyedrop was applied for four weeks. IOP was measured weekly using the Icare Tonolab and at the end of the protocol, anterior segment tissues were collected for further molecular analysis. In rats visual acuity and contrast sensitivity were was assessed noninvasively by the OptoMotry computerized system. Results: The newly formulated FLU eyedrop is well-tolerated and non-toxic. Dex increased IOP both in WT and in S1R KO mice after two weeks. Of note, IOP elevation started one week earlier in KO mice. FLU treatment lowered IOP in glaucomatous WT mice to a control level but had no effect in KO mice. Increased levels of TGFb2 and fibronectin were also reduced by FLU eyedrop. In glaucomatous rats, four weeks of FLU eyedrop treatment restored IOP to control levels and remarkably improved visual acuity (13%) and contrast sensitivity (36%). Conclusion: We have developed a tolerable, non-toxic FLU-containing that effectively reduces high IOP, preserves visual function and ameliorates the fibrosis of the anterior segment in the eye. These findings support its eligibility as a novel antiglaucoma compound, which can be next used in a Phase 1 clinical trial. Grants: LP2021-3/2021, TKP2021-EGA-24, STAGE 2024-1.2.3-HU-RIZONT-2024-00056, SigmaDrugs Research

Keywords: fluvoxamine, eyedrop, glaucoma, intraocular pressure, fibrosis

Sex-Specific Role of Sigma-1 Receptor in Cardiac and Metabolic Function in Mice

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Sigma-1 receptors (Sig1R) are ligand-regulated chaperone proteins localized at the nuclear envelope, endoplasmic reticulum, and mitochondria-associated membranes. While Sig1R has been extensively studied in the context of neurodegenerative diseases, its role in peripheral organs such as the liver, kidney, and heart remains underexplored. Notably, recent studies have produced conflicting results, showing that Sig1R agonists may elicit both protective and pro-fibrotic effects in heart. This study aimed to elucidate the sex-specific physiological role of Sig1R in cardiac and metabolic functions in mice.
Male Sig1R-/- mice exhibited significantly reduced left ventricular ejection fraction and fractional shortening compared to WT controls, with no such changes in females. Mitochondrial assays revealed increased reactive oxygen species (ROS) generation in male Sig1R-/- mice during anoxia-reoxygenation, an effect not observed in females. Despite these changes, no cardiac fibrosis was detected in either sex. Ultrastructural analysis showed sex- and genotype-dependent alterations in cardiomyocyte morphology: males displayed uniform mitochondrial narrowing, while females exhibited cristae disruption, vacuolization, thinner myofibrils, and increased pseudomyelin figures, but fewer lipid inclusions. Neither glucose nor insulin tolerance was affected in Sig1R-/- mice under normal or high-fat diets, and infarct size remained unchanged across genotypes and diets.
Transcriptomic analysis revealed a striking sex difference: 142 differentially expressed genes (DEGs) in males versus 1,096 in females. Only 56 genes were commonly altered in both sexes, primarily associated with mitochondrial function, vesicle trafficking, protein folding, and muscle cell contractility. Pathway analysis highlighted mitochondrial dysfunction and altered oxidative phosphorylation, with links to both neurodegenerative and cardiometabolic diseases.
These findings demonstrate a sex-specific role of the Sigma-1 receptor in maintaining cardiac mitochondrial structure and function. While male Sig1R deficiency led to impaired cardiac performance and increased oxidative stress, females showed more extensive transcriptomic changes without functional decline. The absence of fibrosis and unchanged infarct sizes suggest a non-fibrotic mechanism underlying the observed alterations. This study underscores the importance of considering sex as a biological variable in cardiometabolic research and supports further exploration of Sig1R as a potential therapeutic target.

Keywords: transcriptome, metabolism, cardiomyocyte

Development of Sigma-1 Receptor Photowitchable Ligands as Molecular Tools

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The Sigma-1 receptor (S1R) is a chaperone protein implicated in various physiopathological processes, including neurodegenerative diseases. Recent studies have demonstrated that S1R agonists exhibit neuroprotective effects, particularly in the context of Alzheimer's disease. In this light, the development of photoswitchable ligands targeting S1R offers a promising strategy to optically modulate receptor activity, enabling precise "on-off" control. Herein, we report the design, synthesis, photophysical characterization, and in vitro evaluation of a novel series of S1R-targeting compounds. Applying an azoextension approach and based on structure–activity relationship (SAR) insights derived from the chromen-4-one scaffold, the distal benzene ring was replaced by an azobenzene, allowing reversible photoactivation. The aims of this study is to provide a toolset for spatiotemporal regulation of S1R activity, helping to understand better the mode of action of this receptor.

Keywords: Photoresponsive ligands, Azobezene, Affinity-switch

Design of sigma-1 receptor agonists and antagonists using computer modeling

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The sigma-1 receptor conformational space was mapped using the Colabfold software package. Selected sigma-1 receptor conformers were used as targets during the virtual synthesis of ligands using the open-source software package AutoGrow4. It enables semi-automated computer-aided drug discovery using a genetic algorithm evolving entirely novel ligands (i.e., it is not limited to a virtual library of pre-enumerated compounds). For complexes of sigma-1 receptor and ligands with the best AutoDock Vina scores, all-atom MD simulations were produced. The phospholipid membrane, water envelope, and ions were included in the simulated systems. Binding free energies of individual ligands were calculated using the BFEE2 software package.

Keywords: sigma-1 receptor, agonist, antagonist, molecular dynamics, binding free energy

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Sigma-1 receptor: the hidden target of traditional preparations for neurodegeneration and neuropathic pain

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Many traditional preparations have been developed over the years to alleviate pathological conditions associated with neurodegeneration and neuropathic pain. The mechanism of action of these preparations has been postulated considering different targets, such as Interleukins, matrix metalloproteinases, and vascular endothelial growth factors, to name a few. However, another target may play a relevant role in these pathologies: the Sigma 1 receptor (S1R). While many S1R agonists are under investigation both in Academia and Industry, their role in the effectiveness of traditional preparations has not been systematically investigated. Numerous traditional herbal preparations, particularly those from Chinese Traditional Medicine, contain S1R agonists like the benzylisoquinoline alkaloid berberine. This compound is present in several Chinese preparations: Huanglian Wendan's decoction (used against Alzheimer's disease-AD)1, Jiao-tai-wan (used as an antidepressant)2, or Huang-Lian-Jie-Du (used against dementia)3. These preparations often contain other alkaloids with potential additive effects and therefore the S1R-modulating effect may not be limited to berberine alone. For instance, Gegen Qinlian tablets (used against Alzheimer's disease)4 contain berberrubine, demethyleneberberine, and epiberberine, and Corydalis Rhizome (used against neuropathic pain)5 contains glaucine, palmatine, and corydaline, among others. In this study, we investigated the potential involvement of S1R in the efficacy of extracts used in folk medicines to treat neurodegeneration and neuropathic pain. Following a chemotaxonomic approach, we associate botanical families with classes of metabolites that may interact with S1R. Particular attention has been paid to plants characterizing the Italian biodiversity in line with a wider national project (National Biodiversity Future Center) whose final aim is the bioprospection of the plants growing in the selected territory. Next, computational studies allowed the identification of new metabolites potentially able to bind to S1R. Comprehensive results from both the chemotaxonomic analysis and computational studies will provide valuable insights into the role of S1R in traditional medicines and open new avenues for drug discovery in neurological disorders.
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Keywords: Traditional medicine, Secondary metabolites, Plant extract

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Innopharma Sigma Chemical Library for hit identification and tool compound discovery

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Innopharma drug discovery platform, a high-capacity site within the EU-OPENSCREEN ERIC, offers advanced early in vitro screening capabilities, including both biochemical and cell-based assays. The platform has technologies available for compound screening running assays in 96 to 1536-well format and with different readouts (fluorescence, luminescence, radioactivity, FRET, BRET, HTRF, FLIPR, automated patch-clamp, nephelometry, microelectrode arrays or High Content Screening). Our extensive small molecule chemical library comprises over 130,000 compounds, including an exclusive collection of sigma ligands, an ideal resource for hit identification and tool compound discovery. This structurally diverse library boasts availability in liquid form and the vast majority in solid form. The collection exhibits drug-like properties, with most compounds adhering to Lipinski's rule, ensuring favourable solubility, permeability, and absorption. The library is characterized by acceptable planarity disruption, fewer than three aromatic rings, and MPO scores exceeding 4 in most compounds, being thus good CNS drug candidates. Furthermore, a significant percentage of the sigma ligand library is annotated with comprehensive physicochemical, pharmacological, and ADMET data, readily accessible for data mining within our internal relational database. The library represents both selective sigma-1 and sigma-2 ligands, as well as sigma-1/2 dual compounds, with readily identifiable compounds having additional affinities for other targets of interest (e.g., mu-opioid receptor, norepinephrine transporter, alpha-2-delta calcium channel, etc). High-throughput screening (HTS) campaigns can leverage our robust sigma-1 and sigma-2 binding assays, with customization readily available for tailored assays in any screening cascade, including phenotypic assays when needed. Innopharma's advanced chemoinformatics capabilities enable efficient data processing, statistical analysis, and the application of intelligent methodologies to model and predict appropriate chemotypes and biological profiles, including cell painting in multiple cell phenotypes, facilitating the identification of promising drug candidates.

Keywords: sigma-1, sigma-2, chemical library, drug discovery, in vitro screening

Design, synthesis, and in silico studies of novel tetrahydropyrrolo[3,4-c]pyrazoles sigma-1 receptor ligands

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Sigma receptors (SRs) consist of two subtypes, sigma-1 (S1R) and sigma-2 (S2R), and are chaperone transmembrane proteins categorized as non-G protein-coupled receptors [1]. SRs have garnered growing interest due to their emerging roles in neurodegenerative disorders and neuropathic pain (NP) [2,3]. In this study, novel tetrahydropyrrolo [3,4-c] pyrazole-based compounds have been designed as S1R ligands, optimizing affinity and reducing off-target effects. The rigid bicycle fused ring improves the crucial ligands' properties, particularly their interaction with S1R, by refining molecular conformation and electronic distribution. Structure-activity relationship (SAR) analysis identified modifications in the nitrogen atoms that play a pivotal role in influencing S1R binding affinity. The most interesting result was swapping the nitrogen substituents in compound AD417, which exhibited a high affinity for S1R (Ki = 75 nM) and significant selectivity over S2R (Ki = 431 nM). Furthermore, AD417 showed minimal inhibition of the hERG channel (IC50 = 5.8 μM), suggesting a reduced risk of cardiotoxicity. Molecular modeling studies provided deeper insights into the SAR, clarifying the critical interactions that drive ligand affinity and selectivity and corroborating the experimental binding data. These findings underscore the potential of tetrahydropyrrolo[3,4-c]pyrazole-based compounds as selective S1R modulators, inspiring optimism about the future of S1R research and the development of more effective and safer therapies targeting S1R.

Keywords: Medicinal chemistry; Molecular modeling; Sigma-1 receptor; Tetrahydropyrrolo[3,4-c]pyrazole; hERG inhibition.

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Synthesis and biological evaluation of novel S1R ligands

Costanzo, G[1], Cosentino, G[1], Patamia, V[1], Grasso, M[2], Marrazzo, A[1], Amata, E[1], Parenti, C[1], Rescifina, A[1], Pasquinucci, L[1]

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Inflammatory pain represents one of the unmet clinical needs for patients, as conventional therapies cause several side effects. Recently, new targets involved in inflammatory pain modulation have been identified, including sigma-1 receptor (S1R). Selective S1R antagonists, indeed, have demonstrated analgesic efficacy in acute and chronic inflammatory pain models. In medicinal chemistry research, the normetazocine structure has been considered a versatile scaffold for developing new drugs. It has been established that the (–)-(2R,6R,11R)-configuration of the normetazocine nucleus is favored for opioid receptors interaction. Conversely, its (+)-(2S,6S,11S)-enantiomer exhibits different activity and affinity for S1R. So, we have designed and synthesized a series of novel (+)-(2S,6S,11S)-normetazocine derivatives. The most promising ligand was the S1R selective compound (+)-LP2 (Ki = 26.61 nM). It decreased the second phase of the formalin test and showed a S1R antagonist profile.1 Moreover, (+)-LP2 significantly attenuated neuropathic pain by reducing central gliosis and pro-inflammatory cytokine expression levels in the ipsilateral spinal cord tissues of animals undergoing sciatic nerve chronic constriction injury.2 A series of analogs was also synthesized because of (+)-LP2's interesting pharmacological profile. In vitro, the affinity profile of new ligands versus S1R was evaluated, and the most interesting (+)-LP2 analog showed a relevant S1R affinity (Ki = 27.67 ±8.49 nM). Its chemical stability was assessed in vitro at 37 °C and in mouse plasma, showing desirable results. This compound also significantly reduced the second phase of the mouse formalin test. Furthermore, molecular modeling studies were performed to analyze the binding mode and the key interactions between the new ligands and S1R.3

Keywords: drug discovery, binding assay, docking studies, sigma receptor antagonists, inflammatory pain.

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Amylovis-201 is a potent agonist of the s1 chaperone protein with anti-amylodogenic activity for treatment of Alzheimer´s disease

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Aggregation of beta-amyloid peptide (Aβ) is associated with neurodegeneration in Alzheimer's disease (AD) and represents a pharmacological target. The Sigma-1 receptor (S1R) is known to be associated with improved cognitive function due to its anti-amnestic and neuroprotective effects. S1R density decreases in the early stages of AD, and its activation by S1R agonists can significantly reduce cognitive dysfunction in AD. Amylovis-201 (CNEURO-201) has been identified as a compound capable of forming thermodynamically stable complexes with Aβ1-42 peptides and fibrils. This molecule exhibits high potency in inhibiting in vitro Aβ1-42 aggregation in human microglia, reducing in vivo Aβ load in the brains of 3xTg-AD mice, and attenuating cognitive deficits in these animal models. Structurally, Amylovis-201 aligns with the pharmacophore of S1R proposed by Glennon. In cells overexpressing S1R protein, the drug dissociates S1R-associated protein (BiP) with an IC50 of 362 nM and its effect is blocked by the S1R antagonist NE-100. In vivo, Amylovis-201 prevents dizocilpine and Aβ25-35 peptide-induced learning deficits in mice and the hyperlocomotor response in wfs1abKO zebrafish larvae, in a NE-100-sensitive manner, confirming its role as a S1R protein agonist. Its σ1 agonist activity was further validated in SH-SY5Y cells exposed to Aβ25-35 peptide-induced toxicity. Light scattering assays demonstrated that Amylovis-201 prevents Aβ25-35 aggregation, a capacity not observed with PRE-084 or NE-100. Moreover, all three compounds were able to disaggregate Aβ25-35 peptide after complete oligomerization, but Amylovis-201 more efficiently. In vivo evaluations in aged 3xTg-AD mice, Amylovis-201 is able to alleviate neuroinflammation, produced by glial activation, thanks to its dual action, by decreasing the toxicity caused by Aβ plaques. In summary, Amylovis-201 behaves as a potent S1R agonist, but with a unique anti-aggregating ability. The drug may there be highly effective in long-term treatment against amyloid-induced toxicity in AD.

Keywords: Alzheimer's disease, Amyloid-ß aggregation, neuroprotection, σ1 receptor, drug discovery.

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In vivo evaluation on antipsychotic-like activity for the first-in-class arylselenoether-piperazine derivatives targeting sigma-1/D2/5-HT1A receptors in mice

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Sigma-1 receptor (S1R), which represents chaperone protein located at the endoplasmic reticulum (ER)-mitochondrion interface, plays important roles in various physiological functions by modulating ER-nucleus cross talk and ER-mitochondrion signaling The high expression of S1R is confirmed in both central and peripheral nervous systems, in the areas associated with neuroprotection and neuroinflammation. Upon activation with contribution of agonists, S1R can interact with different ion channels and G-protein-coupled receptors (GPCRs) in the plasma membrane as well as is able to upregulate secretion of BDNF that is involved in neuronal survival, neurogenesis, and long-term potentiation. Thus, S1R agonists have potential therapeutic effects in various neurological and psychiatric disorders including neurodegenerative diseases, stroke, depression, pain, which have been confirmed in animal models, while S1R antagonists are confirmed to have analgesic effects and to enhance opioid mediated analgesia. [1] On the other hand, the crucial role for both, the 5-HT1A serotonin and D2 dopamine GPCRs in CNS associated with neuropsychiatric and neurodegenerative processes has been and still is under great interest in search for new antipsychotics and effective therapies of Parkinson's (PD) and other neurodegenerative diseases. From chemical point of view, the significant therapeutic success, expressed by FDA approval of new antipsychotics and antidepressants acting via D2 and/or 5-HT1A receptors has been attributed to arylpiperazine-containing agents for last 50-years (ziprasidone, vortioxetine, quetiapine, aripiprazole, clozapine, trazodone, etc.). Furthermore, recent lines of evidence indicate the continuously increasing importance of selenium-containing compounds in the search for effective and safe therapies for CNS diseases, due to their versatile neuroprotective effects confirmed in numerous preclinical studies. In this context, we have designed and synthesized a small series of phenylselenoether-derived arylpiperazines [2], based on simplest pharmacophore features of 5-HT1A receptor ligands that also displayed a potent affinity for S1R in the radioligand binding assays. Rational modifications within their aromatic rings provided the highly potent triple-target agent, compound PPK-059, with low-nanomolar affinity constants for all S1R, 5-HT1AR and D2R (Ki<10 nM) together with beneficial ADMET profile in vitro. In the research presented here, PPK-059 has been subjected to in vivo tests in mouse model to evaluate its antipsychotic-like properties. Initial results obtained demonstrated antipsychotic-like activity in the MK-801-induced hyperlocomotion test for PPK-059 at doses of 2.5, 5, and 10 mg/kg. Further

studies exploring its efficacy in cognitive enhancement, anxiety modulation, and coping mechanisms are currently in progress. This study was funded by the National Science Centre, Poland; grant no. 2024/53/B/NZ7/03768 and by Jagiellonian University in Krakow, Poland; grant no. N42/DBS/000080.

Keywords: Selenoethers, sigma-1, antipsychotic, in vivo

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Structure-activity relationship studies on phenoxyalkylpiperidines reveal the structural determinants for potent sigma1 receptor agonist activity and antineurodegenerative properties.

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According to a systematic analysis for the Global Burden of Disease Study of 2021, disorders affecting the nervous system, including Alzheimer's disease (AD) and Parkinson's disease (PD), are the leading cause of the overall disease burden worldwide. The pathogenesis of these neurological disorders is attributed to various factors and central-level dysfunctions, including alterations in sigma1 receptor, ubiquitous pluripotent chaperone involved in multiple functions and interactions with other human proteins1. In the central nervous system (CNS), the sigma1 receptor is implicated in neuroprotection, neuroinflammation, neurotransmission, and neuroplasticity and is therefore associated with advanced brain functions such as memory, cognition, mood, pain, and neurodegeneration. For this reason, it is now an actively pursued pharmacological target for various neurological and neuropsychiatric disorders. Indeed, the activation of the sigma1 receptor by agonists promotes neuronal survival and restores neuronal plasticity to slow disease progression through mechanisms including the modulation of calcium homeostasis and glutamate activity, attenuation of oxidative and nitrosative stress, maintenance of endoplasmic reticulum and mitochondrial integrity, and reduction of inflammation2. Background: Previous studies have shown that derivatives bearing a 4-methylpiperidine bound via alkyl linkers to a primary hydrophobic moiety, such as tetralin, naphthalene, or phenoxy rings, exhibit high affinity and selectivity for the sigma1 receptor. Moreover, the anti-amnesic properties of two phenoxyalkylpiperidines, L6 and L13, with excellent agonist activity and lack of cytotoxicity3 have been proven in mice models. Aim: Given the confirmed drug-like properties and in vivo potency of sigma1-binding phenoxyalkylpiperidines, a structure affinity relationship (SAfiR) study was initiated to introduce structural modifications to the lead compound L6 including i) decorations on the benzene ring with groups of varying electronic properties and steric hindrance; ii) isosteric replacement on the benzene ring and the linker; and iii) introduction of other cyclic amines in place of the 4-methylpiperidine. The goal is to develop new phenoxyalkylpiperidines as high-affinity and selective σ1 receptor ligands with antineurodegenerative properties and neuroprotective functions. Conclusions: Among the compounds obtained, there are subnanomolar affinity sigma1 ligands with notable selectivity towards the sigma2 subtype. The most promising ligands were also investigated for their functional activity as sigma1 agonists and are under investigation in preclinical models of AD.

Keywords: σ1 ligands, phenoxyalkylpiperidines, CNS, neurodegenerative diseases

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SIGMAP: an explainable artificial intelligence tool for SIGMA-1 receptor affinity prediction

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Developing modulators for the sigma-1 receptor (S1R) represents a promising strategy to counteract neurodegenerative diseases, cancer progression (1), and viral infections (2). In this context, reliable in-silico tools capable of accurately predicting S1R binding affinity are highly valuable. Here, we introduce a panel of 25 machine learning classifiers trained on a curated dataset of high-quality bioactivity data for small molecules experimentally assessed as potential S1R binders. The dataset was sourced from ChEMBL v33, and the models were built using multiple machine learning algorithms and five distinct molecular fingerprints. Most of the classifiers demonstrated strong predictive capability. Notably, the best-performing model, developed using the Support Vector Machine algorithm with Morgan fingerprints as molecular descriptors, achieved an AUC of 0.90. To enhance the model transparence and trustworthiness, we employed two Explainable Artificial Intelligence (XAI) techniques: Shapley Additive Explanations (SHAP) and Contrastive Explanation. The resulting top-performing model, combined with these XAI analysis, is accessible through SIGMAP (3) (https://www.ba.ic.cnr.it/softwareic/sigmap/), a user-friendly web platform specifically designed for S1R binding prediction. With its intuitive interface, strong predictive capabilities, and integrated XAI features, SIGMAP serves as a powerful tool to support the rational design of novel S1R modulators.

Keywords: S1R, Affinity Prediction, Machine Learning, Ligand-based classifiers, Explainable AI

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Exploiting the Therapeutic Potential of Sigma and Cannabinoid subtype 2 Receptors (CB2R) as Dual Targets approach in Inflammatory Diseases

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The sigma-1 (σ1R) and sigma-2 (σ2R) receptors, along with the cannabinoid subtype 2 receptor (CB2R), represent promising therapeutic targets for inflammatory-based diseases as neurodegenerative diseases and cancer. Notably, CB2R, a key component of the endocannabinoidome, is predominantly expressed in immune cells, offering a pathway for immunomodulation without the psychoactive effects associated with CB1R. While primarily peripheral, CB2R overexpression in neurodegenerative diseases and cancer highlights its potential as a pivotal biomarker for early-stage inflammation. Similarly, σ1R and σ2R play important roles in cancer and neuroinflammation. Their elevated expression in proliferating cancer cells suggests their involvement in tumor growth and metastasis, and their anti-inflammatory properties offer therapeutic potential in neurodegeneration. Specifically, σ2R ligands have demonstrated cytotoxic effects on cancer cells, including those resistant to conventional treatments, and can be used for targeted drug delivery. Furthermore, the potential of σ1R and σ2R as biomarkers for cancer diagnosis and prognosis is supported by their high expression in various tumors. Given the multifactorial nature of diseases such as neurodegenerative disorders and cancer, a polypharmacological approach is often necessary. Developing MultiTarget Directed Ligands (MTDLs) capable of simultaneously modulating σ1R/σ2R and CB2R represents a promising strategy. This approach aims to enhance the therapeutic efficacy and overcome the pharmacokinetic challenges associated with the administration of multiple drugs. The synergistic combination of CB2R agonists with σ1R/σ2R ligands holds promise for contrasting inflammatory-related pathologies. This project will identify novel selective ligands targeting these receptors and evaluate their therapeutic potential for preclinical studies. By exploring the synergistic interactions between these receptors, we aim to pave the way for innovative multi-target therapies.

Keywords: CB2R, neurodegeneration, inflammation

Development of positron emission tomography radiotracers for imaging sigma-1 receptors in CNS diseases

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The "pluripotent chaperone" Sigma-1 receptor (S1R) is highly expressed in the central nervous system (CNS) and is involved in the onset and progression of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases (PD and AD) [1]. Its imaging through Positron emission tomography (PET) holds great potential for the early diagnosis of such pathologies [2]. My research group has identified the class of phenoxyalkylpiperidines as high affinity S1R ligands. The p-Cl derivative, named L6 (1-(2-(4-chlorophenoxy)ethyl)-4-methylpiperidine), emerged as the most promising compound, displaying subnanomolar and high selectivity (0.86 nM at S1R vs. 239 nM at S2R). With the purpose to contribute to the development of S1R PET ligands, building on L6, we synthesized several analogs to identify the most suitable candidate for 18F-radiolabeling. One compound, in particular, stood out for its high affinity for S1R and remarkable selectivity (5.36 nM at S1R vs. 3009.5 nM at S2R). 18F-radiolabeling is currently in progress, and will be followed by the evaluation of the compound through in vitro autoradiography and PET imaging in a mouse model.

Keywords: PET, 18F, CNS, Phenoxyalkylpiperidines, sigma-1

Vildagliptin: insight into SIGMA-1 receptor profile

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The Sigma-1 receptor (SIGMAR1) plays a key role in CNS disorders, with both agonists and antagonists exhibiting therapeutic relevance. Vildagliptin, a DPP-4 inhibitor for type 2 diabetes, has been suggested to interact with SIGMAR1 in computational target prediction, prompting an investigation into its binding properties. We performed docking using AutoDock Vina, preparing ligands and receptor with MGLTools, and assessed its binding affinity compared to known SIGMAR1 agonists (fluvoxamine, sertraline, fluoxetine, etc.) and the antagonist haloperidol. Molecular dynamics (MD) simulations revealed a binding pattern and interaction networks more similar to antagonists than agonists, thus suggesting that vildagliptin could act as a SIGMAR1 antagonist. Altogether, these findings provide a preliminary computational basis for further experimental validation and highlight a potentially new pharmacological role for vildagliptin in neurodegenerative and CNS disorders.

Keywords: Vildagliptin, SIGMA-1, CNS profile, diabetes mellitus

Synthesis of new benzylpiperazines as selective sigma-1 receptor ligands with in vitro antiproliferative activity against cancer cells

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The overexpression of sigma receptors in various tumor types has driven efforts to elucidate their role in cancer pathophysiology. As a result, several studies proved that sigma receptor ligands can inhibit the survival, migration, and proliferation of cancer cells, highlighting their potential as anticancer agents [1]. In this work, we described the synthesis and binding properties of twenty-two benzylpiperazine derivatives as novel selective sigma-1 receptor ligands. Molecular modeling studies and in silico prediction were performed to analyze the binding mode and the drug-likeness properties of newly synthesized ligands. Among them, we identify compounds AL3 and MR10 which showed the best affinity and selectivity profile towards the sigma-1 receptor. Also, both analogs were able to significantly inhibit cell viability of SK-MEL-5 melanoma cells in an in vitro test of antiproliferative activity with a greater potency compared to the reference sigma-1 receptor antagonist haloperidol.

Keywords: sigma-1 receptor; selective sigma-1 ligands; benzylpiperazine derivates; antiproliferative activity; anticancer agents

RC-106, a promising therapeutic agent against glioblastoma. Scale up synthesis, solid-state characterization and in vivo evaluation

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Glioblastoma multiforme (GBM) is recognized as the most observed and malignant primary brain tumor in adults, with an extremely unfavourable prognosis and a median survival of 12-16 months. The current treatment involves a multimodal approach combining surgical resection, radiotherapy, and chemotherapy, using temozolomide (TMZ) as golden standard. Despite this aggressive strategy, GBM remains highly resistant and exhibits a high rate of recurrence. Hence, the development of novel and effective therapeutic strategies represents an urgent and unfulfilled medical need1. In this context, our research group has focused on the design and evaluation of new potential anticancer agents, leading to the identification of RC-106, a pan-Sigma receptors (SRs) modulator demonstrating a promising antitumor activity in vitro against various cancer cell lines, including GBM2,3. To enable its in vivo evaluation, we carried out a scale-up synthesis of RC-106, aiming to obtain a multigram-scale quantity of the compound through safer reactions and more efficient and green purification methods, preferring, for instance, crystallization over flash chromatography. Subsequently, we prepared different salts of RC-106 and assessed their solubility in water and in physiological solution, to identify the most suitable form for in vivo administration. The hydrochloride emerged as the best candidate, and it was subjected to further characterization. Specifically, we performed FT-IR analysis, X-ray diffractometry, SEM analysis, and two thermoanalytical techniques (TGA and DSC) on RC-106*HCl, providing a comprehensive solid-state characterization. Moreover, we optimized the formulation for intravenous administration of a solution of RC-106*HCl 0.5M, resulting in a composition of 5% DMSO, 5% Tween 80, and 90% water. Currently, in vitro experiments are ongoing, and the results will guide the administration conditions for the in vivo proof of concept.

Keywords: Glioblastoma, Pan-Sigma receptors, Scale-up synthesis, solid-state characterization, in vivo evaluation

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Targeting Sigma-1 for Pain Relief: Selective Antagonists with Dual Mechanism of Action

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Chronic pain is a major global health issue, with neuropathic pain (NP) affecting up to 40% of chronic pain patients. The limitations of conventional analgesics, alongside the opioid crisis, underscore the urgent need for alternative therapeutic strategies. This study presents the development of a first-in-class dual-acting analgesic compound acting as Sigma-1 receptor (S1R) antagonist and inhibitor of soluble epoxide hydrolase (sEH), two relatively underexplored targets associated with pain. S1R is a key modulator of ion channels and receptor function in pain pathways, particularly by regulating calcium influx and influencing the activity of various pain-related signalling proteins, making it an attractive target for pain treatment. Additionally, sEH inhibition contributes to the modulation of neuroinflammation, further enhancing the compound's analgesic profile. Moreover, dual inhibition of S1R and sEH potentiates analgesic effects, offering a synergistic and "opioid-free" approach to pain management.1 Herein, we disclose the design, synthesis, and structure-activity relationship (SAR) studies of a new family of compounds that led to the identification of a lead compound with low nanomolar potency, high selectivity, and favorable drug metabolism and pharmacokinetics (DMPK) properties.2 This compound exhibits selectivity for S1R over S2R, a crucial aspect for minimizing off-target effects. Additionally, S1R antagonism was confirmed through the inhibition of phenytoin-induced calcium signalling and suppression of S1R oligomerization. Moreover, optimized synthetic methodologies enabled multigram-scale preparation with high yields. The lead compound demonstrated potent analgesic efficacy in vivo, supporting its potential as a promising non-opioid alternative for pain management. This dual approach not only amplifies analgesic effects but also underscores an innovative approach for addressing the complexities of pain, and it may signify a promising frontier in therapeutic development in light of the current opioid crisis.

Keywords: dual-target therapy, non-opioid analgesia, pain, sigma-1 receptor, synergia.

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Novel multi-target ligands of sigma-1 receptor as potential agents for treatment of neuropsychiatric disorders

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According to recent data, mental disorders represent one of the greatest health challenges of modern times. The latest 2022 report of WHO on mental health and people with mental health conditions [1] states that currently approx. 13% of global population - 970 million people - live with mental disorders, including anxiety, depression, developmental disorder, attention-deficit/hyper-activity disorder, bipolar disorder, conduct disorder, autism and schizophrenia. Among the most commonly considered biological targets for the treatment of these disorders are components of monoamine neurotransmitter systems, metabolic enzymes, ion channels and proteins that regulate signaling processes in the brain. Of particular interest in this field are sigma receptors, a class of intracellular proteins expressed in neurons and glia cells throughout the central nervous system and peripheral organs. Accumulating evidence suggests that particularly sigma-1 subtype receptor plays a role in the pathophysiology of neuropsychiatric disorders. Modulation of this receptor by small-molecule ligands have been proposed to be useful in several therapeutic fields such as amnesic and cognitive deficits, depression, anxiety or schizophrenia. Interaction between sigma-1 receptors and systems of other neurotransmitters has been widely reported. It was found that sigma-1 receptors can regulate glutamatergic and GABAergic neurotransmission and the release of neurotransmitters, such as serotonin or dopamine. Numerous studies revealed that sigma-1 receptors increase 5-HT neurotransmission exerting antidepressant effects through various mechanisms. The effects of selective serotonin reuptake inhibitors such as fluvoxamine or sertraline, seem to be partially exerted through agonism of sigma-1 receptors, which is suggested to contribute 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 serotonin and dopamine receptor ligands. New organosulfur and selenium compounds from our recent series of arylpiperazine derivatives have been subjected to radioligand binding tests with selected serotonin and dopamine receptors and, due to their structural similarity to some known sigma-1 receptor ligands, to binding tests in this direction. The results of in vitro screening revealed a number of innovative, multi-target ligands with high simultaneous affinity towards 5 HT1AR, D2R and sigma-1 receptors. Functional profile of selected ligands at all three receptors has been determined. Compounds with such a combined mechanism of action

have great potential in the treatment of neuropsychiatric conditions. In the present work the impact of atom type of the chalcogen and other elements of the structure on the affinity for individual receptors in the series of compounds is discussed. The discussion is supported by the results of molecular docking to sigma-1 receptors.

Keywords: neuropsychiatric disorders, multi-target ligands, serotonine receptors, dopamine receptors, sigma-1 receptors

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ni2n: a networking project to promote progressive, multidimensional research concepts

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Today's aging societies face a myriad of health challenges; neurodegenerative diseases such as Alzheimer's and Parkinson's Disease range among the top of the list, contributing significantly to cognitive decline, disability, and mortality. Devastating to the affected individual and their family and caregivers, the prevalence of cases also poses a serious socioeconomic burden. While diagnostic methods driven by more and more advanced techniques have vastly improved over the last decade, the main challenge remains the lack of viable treatment options that can prevent, or at least significantly decrease pathological decline or improve symptoms. Here, the pitfall lies in the multifactorial causality and complexity of most neurodegenerative disorders and the fact that their exact individual pathogenesis is still far from understood, rendering prevention as well as therapy difficult. Advancements in research have also sparked a debate about disease definition, i.e. whether complex neurodegenerative diseases such as Alzheimer's Disease (AD) or Parkinson's disease should rather be considered a syndrome than a single disease entity. Many AD cases for example carry a (variable) genetic component, while many more display aging and lifestyle as the main risk factor, showcasing the complex disease nature and limited treatment options. These facts pose an urgent need for more interdisciplinary communication, collaboration, and innovative research. NI2 N, the Network for Interdisciplinarity and Innovation in Neurodegeneration Research (ni2n.org), is a Think Tank Project funded by the Dr. Eberhardt Strebel-Stiftung of the Stifterverband für die Deutsche Wissenschaft that aims to help reshape the necessary research landscape around AD and related neuropathologies by "expanding the box". Through offering a networking platform beyond the mainstream, NI 2 N strives to facilitate innovative global collaborations, enabling established as well as young scientists to share novel insights, challenge and expand existing hypotheses, and propose progressive approaches to target neurodegeneration. Further, this project aims to pinpoint existing knowledge gaps to stimulate new research directions. The network plans to promote early-career researchers through seed funding for innovative approaches and by offering collaborative opportunities. Through this initiative, NI²N encourages a progressive multidimensional approach to understanding, diagnosing, and treating neurodegenerative disorders.

Keywords: neurodegeneration, Alzheimer's disease, networking, funding, "breaking silos"

Endoplasmic reticulum stress, integrity and mitochondrial morphology in a cellular model of Wolfram syndrome

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Wolfram syndrome (WS; OMIM #222300) is a rare neurodegenerative disorder characterized by diabetes insipidus, diabetes mellitus, optic nerve atrophy and sensorineural hearing loss – deafness. Other symptoms may be present such as neurological issues (cerebellar ataxia, atrophy of the pons, epilepsy), psychiatric symptoms (anxiety, bipolar disorder, aggressiveness…) and urological problems. Currently, there is no effective treatment available, and patients typically die of central dysphagia or respiratory failure around the age of 40. Two types of WS have been described in the literature, WS type 1 and 2. WS1 is caused by mutations in the WFS1 gene, which encodes the protein wolframin (WFS1). WFS1 is highly expressed in the brain, pancreas, heart and in lesser amounts in other organs [1]. WFS1 is a transmembrane resident endoplasmic reticulum (ER) protein present in the mitochondria associated membranes, similarly as the Sigma-1 receptor (S1R). Although the direct interaction of WFS1 and S1R has not been demonstrated yet, they share their physiological function (at least) through ER – mitochondria calcium fluxes via the IP3R-GRP75-VDAC pathway. Beyond this role, WFS1 plays a role in the regulation of ER stress through the unfolded protein response (UPR) [2] and impacts mitochondrial function [3]. Therefore, specifically in this work, we aimed to evaluate ER stress, ER network integrity and mitochondrial morphology in WFS1 knock-out (KO) model in the SHSY-5Y neuronal cell line developed in our laboratory using CRISPR-Cas9 technology. Implementing transmission electron microscopy we have found, that in the WFS1 KO cells the integrity of the ER network was compromised. In detail, the ER network was less prominent and fragmented in the WFS1 KO cells when compared to scrambled controls. Moreover, the ER membrane with attached ribosomes, i.e. the rough ER areas were less abundant. Intriguingly, WFS1 KO cells have shown no ER stress changes, as assessed by the ER stress marker CHOP expression. Subsequently, by using transmission electron microscopy approach we observed in the SHSY-5Y WFS1 KO cells we have not observed any substantial changes in mitochondrial population in the WFS1 KO cells as fragmented mitochondria, cristae disruption or swollen mitochondria. This was in line with confocal microscopy approach, where we did not observe any significant mitochondrial fragmentation, loss in mitochondria total count and area occupied by mitochondria as visualized by MitoBrightGreen fluorescence and subsequently quantified by ImageJ software. In conclusion, in the WS cellular model developed in our laboratory by CRISPR-Cas9 approach, most probably the morphological perturbations in the ER network integrity play the most prominent role in the WFS1 knock-out induced pathology of the SH-SY5Y neuroblastoma cells. This work was supported by the project APVV-21-0473.

Keywords: wolframin, endoplasmic reticulum, mitochondria, SHSY-5Y cell line

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Leveraging Multi-Targeted Direct Ligands To target TSPO and S1R crosstalk in microglia.

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Amyotrophic Lateral Sclerosis (ALS) is the most common form of Motor Neuron diseases, characterized not only by selective loss of Motor Neurons (MNs) in the cerebral cortex, brainstem and spinal cord but also by the activation of both astrocytes and microglial cells along the disease progression, which reflects the extent of neuronal demise, as well as the variable engagement of glial cells in order to cope the with neuronal damage. Our previous studies on SOD1G93A transgenic rats highlighted a significantly increased expression and altered distribution of Translocator Protein 18 kDa (TSPO) in clusters of reactive microglial cells at different disease stages, sign of a heterogeneous inflammatory response corresponding to areas of severe neurodegeneration. Moreover, we confirmed the expression of Sigma-1 Receptor (S1R) in neuronal cells in non-transgenic and presymptomatic SOD1G93A rats. However, as the disease progressed up to the terminal stages, we observed that S1R is preserved in the surviving neuronal cells and it is upregulated in glial cells. In this context, enhancing neuroprotective pathways in motor neurons while modulating a neurosupportive microenvironment, by targeting glial cells, could represent a novel strategy to tackle this disease. S1R agonists can restore locomotor function and promote neuronal survival in presymptomatic and early symptomatic SOD1G93A mice. Similar effects were seen in wobbler mice treated with PRE-084, improving neuronal survival, grip strength, and increasing neuro-restorative microglia/macrophages (CD68+/CD206+), while reducing reactive astrocytes. On the other hand, the role of TSPO+ microglial cells is not yet fully understood. Thus, we hypothesized that Multi-Targeted Direct Ligands (MTDLs) against Sigma-1R (S1R) and TSPO could be a new promising pharmacological tool. However, the complexity of the mechanisms underlying neurodegeneration and the heterogeneity of the inflammatory responses make it necessary to further investigate the role of S1R and TSPO at the single cell level. MTDLs were generated by combining a S1R agonist (RC-33) with PK11195, a well-known TSPO-ligand. In this project we assessed: i) the selectivity of MTDLs for both TSPO and S1R, ii) the effects exerted by MTDLs on inflammatory responses and neuronal plasticity in vitro, iii) the association of S1R and TSPO in cells exposed to different stimuli and iv) the transcriptomic signature of cells treated with MTDLs through scRNA-seq. Pregnenolone production, assessed by ELISA assay, was used as a parameter to measure the selectivity for TSPO and S1R in microglial cell lines, highlighting a synergic effect of the MTDLs. The efficacy of MTDLs in promoting neuronal differentiation was investigated in differentiated PC12 neurite outgrowth assay while the anti-inflammatory effect was evaluated by RT-qPCR in HMC3 microglia cell lines. Indirect immunofluorescence on different cell lines and confocal microscopy helped us to understand the close connection between the two target proteins. In the end, a new single-cell RNA sequencing protocol has been recently developed in our laboratory

in order to describe the genetic modulation that occurs in microglial cells after MTDLs treatment.

Keywords: Sigma-1 receptor, TSPO, neuroinflammation, neurodegeneration, multi-target directed ligands

Sigma-1 Receptor Inhibition Reduces Arthritis Progression and Pain

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Introduction: Despite the arrival of several new pharmacological treatments in recent years (disease-modifying antirheumatic drugs, DMARDs), particularly biologics, therapeutic outcomes for rheumatoid arthritis (RA) remain often unsatisfactory and are sometimes accompanied by adverse effects. Furthermore, a significant number of patients who achieve adequate disease control with current DMARDs continue to experience residual chronic pain, which severely impacts their quality of life. Sigma-1 receptor blockade has demonstrated analgesic efficacy in animal models of chronic pain of different etiologies, and in patients with neuropathic pain. However, it had never been evaluated in a rheumatoid arthritis (RA) model. Methods: The efficacy of sigma-1 receptor blockade was evaluated using a collagen-induced arthritis (CIA) model in rats. Peripheral inflammation, joint pathology, functional impairments, and pain behaviors were comprehensively assessed through histology, magnetic resonance imaging, behavioral tests, and flow cytometry. Sigma-1 receptor knockout (KO) rats and pharmacological sigma-1 antagonists (S1RA, BD10633) were compared to saline and standard treatments (pregabalin, methotrexate) administered via subcutaneous osmotic pumps for 7 days (days 11–18 of CIA time course). Results: Both genetic deletion (KO) and pharmacological blockade of the sigma-1 receptor robustly reduced peripheral inflammation and joint degeneration, with efficacy comparable to the first-line DMARD methotrexate. Moreover, sigma-1 antagonism improved arthritis-associated functional deficits and pain behaviors, demonstrating superior efficacy compared to the first-line neuropathic pain treatment pregabalin or methotrexate. Importantly, the analgesic effects were not solely attributable to reduced inflammation and joint pathology, as acute treatment with sigma-1 antagonists also produced consistent analgesic effects. Conclusion: Sigma-1 receptor blockade emerges as a promising new strategy for the treatment of rheumatoid arthritis, with the added benefit of independently alleviating arthritis-associated pain.

Keywords: pain, inflammation, arthritis, drug discovery, pharmacology

HBK-15, a multimodal compound activating sigma-1 receptors, reverses memory and executive function deficits in neuropsychiatric disorder models

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Cognitive impairments encompass a wide range of deficits that differ in etiology, severity, and manifestation. They can result from neurodegenerative diseases, psychiatric disorders, or chronic stress, significantly affecting cognitive functions such as memory and executive functions, ultimately reducing overall quality of life. The lack of effective therapies in neurodegenerative and neuropsychiatric disorders stems from the limited efficacy of available drugs (e.g., AChEIs) and unaddressed deficits, especially in neuropsychiatric conditions. Additionally, the diverse etiology of these disorders complicates the development of universal therapies effective across various forms of cognitive impairments. Given their profound impact, the search for effective therapeutic strategies remains ongoing. HBK-15, a multimodal compound with sigma-1 receptor agonist properties, has demonstrated rapid antidepressant-like and antiamnesic properties. While previous studies have suggested its potential to ameliorate cognitive deficits, its impact on long-term recognition memory impairments remained unclear. Therefore, this study aimed to evaluate the effects of HBK-15 on long-term memory deficits in mice induced by MK-801, scopolamine, and unpredictable chronic mild stress (UCMS), focusing on recognition memory assessed using the object recognition test. Furthermore, given that cognitive dysfunction in neuropsychiatric disorders often involves both memory impairments and deficits in executive functions, we also sought to determine whether HBK-15 could improve cognitive flexibility under stress conditions. To enhance the translational value of our research, we employed a touchscreen-based two-choice pairwise visual discrimination reversal task in mice, allowing for a precise assessment of cognitive flexibility. Our results indicate that HBK-15 effectively reversed recognition memory deficits following a single administration in three different impairment models in mice: MK-801, scopolamine, and UCMS. Furthermore, HBK-15 significantly improved cognitive flexibility in UCMS-exposed mice. Specifically, the two lowest tested doses (0.625 mg/kg and 1.25 mg/kg) enhanced choice accuracy in the reversal phase, reduced task completion, and shortened decision-making time. Interestingly, HBK-15 did not influence animals' perseverative behavior, indicating a selective enhancement of cognitive processes rather than a general alteration of behavior. These results suggest that HBK-15 not only improves recognition memory in mice, as demonstrated in the object recognition test but also alleviates stress-induced cognitive flexibility deficits. Notably, HBK-15 has proven effective across various types of memory impairments, including those induced by scopolamine, MK-801, and stress. This versatility underscores its unique potential compared to other treatments, positioning HBK-15 as a promising candidate for future research targeting a wide range of cognitive deficits associated with neuropsychiatric conditions. This study has been conducted as part of a research project financed by the National Science Centre, Poland (grant number 2019/34/E/NZ7/ 00454) and Jagiellonian University Medical College (grant number U1C/W42/NO/28.26).

Keywords: Hbk-15, cognitive flexibility, memory, sigma-1 receptor, mice

Modulation of Frustration through Sigma-1 Receptors: Effects of PRE-084 and S1RA in Wild-Type and Knockout Rats Using the CSNc Paradigm

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Frustration resulting from reward devaluation is a fundamental affective response, with relevance to emotional regulation and decision-making. The consummatory successive negative contrast (cSNC) paradigm provides a robust model to study this phenomenon by comparing sucrose intake between shifted and unshifted subjects. Sigma-1 receptors (Sig1R), which are implicated in mood regulation and cognitive processes, have been proposed as potential modulators of frustration-related behavior. The present study evaluated the effects of the Sig1R agonist PRE-084 (4 mg/kg) and the antagonist S1RA (64 mg/kg) in wild-type (WT) rats, as well as the effects of PRE-084 in Sig1R knockout (KO) rats. Both KO and WT animals were also administered a control solution (saline). All treatments were administered systemically 30 minutes prior to the first post-shift session. The experimental groups included WT+vehicle (n=8 unshifted, n=8 shifted), WT+PRE-084 (n=8 unshifted, n=9 shifted), WT+S1RA (n=8 unshifted, n=8 shifted), KO+vehicle (n=5 unshifted, n=5 shifted), and KO+PRE-084 (n=8 unshifted, n=9 shifted). Behavioral responses were analyzed across pre- and post-shift sessions using t-tests, polynomial trend analyses (linear, quadratic, cubic), and the Drug Effect Ratio (DER) to control for baseline variability. Vehicle-treated WT rats displayed the expected cSNC effect, with the shifted group showing a significant reduction in sucrose intake following reward devaluation across three sessions. Administration of PRE-084 in WT animals eliminated this contrast on the first post-shift day, as indicated by a significant quadratic trend ($F(1,7) = 11.346$, $p < 0.01$) and convergence in consumption between shifted and unshifted groups. S1RA treatment produced significant group differences during the preshift phase (day 9: $t = 5.252$, $p < 0.001$), but only partial modulation of the contrast post-shift, with complex response patterns including a significant cubic trend ($F = 17.858$, $p = 0.003$). In KO rats, a disrupted contrast response was observed, resembling the pattern seen with S1RA treatment in WT animals. Notably, administration of PRE-084 failed to prevent the expression of frustration, with shifted animals showing a similar decline in sucrose intake to KO controls. The findings suggest that activation of Sig1R through PRE-084 does not primarily interfere with the emotional impact of reward loss, but rather enhances hedonic processing. The effective suppression of contrast in shifted animals may reflect an increased baseline of positive affect or reward sensitivity, rather than a blunting of the negative emotional response to devaluation. This pattern supports the hypothesis that the sigma-1 system selectively modulates hedonic tone without disrupting the formation of expectations or the affective response to their violation. From a translational perspective, this indicates that a sigma-1 agonist could elevate basal well-being while preserving the ability to respond emotionally to frustrating or adverse events. Such a dissociation between hedonic enhancement and affective reactivity warrants

further investigation into the neurobiological mechanisms underlying this functional separation.

Keywords: Sigma-1 receptor, Frustration, cSNC paradigm, Reward devaluation, Affective modulation

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopaminergic neuron loss, oxidative stress, and mitochondrial dysfunction

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The Sigma-1 receptor (Sig-1R) is a key molecular chaperone involved in neuroprotection, calcium regulation, and survival signaling. Modulating Sig-1R has emerged as a potential strategy to counteract neurodegeneration. Previous studies on Areca catechu ethyl-acetate extract (AC.ea) have shown its potential in upregulating Sigma-1 receptor expression and activating pro-survival pathways. However, the specific compounds responsible for this activity remain unidentified. This study aimed to fractionate and purify bioactive compounds from the ethyl acetate extract of Areca catechu and evaluate their ability to modulate Sigma-1 receptor expression and provide neuroprotection in a Parkinson's disease model. The role of Sig-1R in mediating the neuroprotective effects of these pure compounds will also be investigated. Molecular docking was performed to predict the binding affinity of the bioactive compounds againstSigma-1 receptor. In vitro studies were conducted using SH-SY5Y dopaminergic neurons treated with oxidopamine to mimic PD-like neurotoxicity, followed by treatment with the purified compounds. Western blot and qPCR analyses were performed to assess Sigma-1 receptor expression. The purified compounds significantly upregulated Sigma-1 receptor expression. The study provides new insights into the role of Sigma-1 receptor modulation in neuroprotection and suggests that these bioactive compounds from Areca catechu may serve as potential candidates for PD therapy.

Keywords: Sigma-1 Receptor Modulation, Neuroprotection, Parkinson's Disease, Plant Bioactive Compounds

Multi-Target Directed Ligands binding the sigma-1 receptor: promising therapeutic strategy for pain management

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The multi-targeted strategy simultaneously modulates multiple disease mechanisms. Among the different multi-targeted mechanisms, we focus on multimodal drugs formed by the direct fusion of two or more pharmacophores into a single chemical molecule. This strategy offers multiple advantages such as lower risk of drug-drug interactions, simpler pharmacokinetics and greater efficacy. This approach is very promising for pain management, which is an unmet need since it affects 20% of the world's population and the analgesics available on the market are ineffective in more than 50% of patients and have side effects that limit their use. The sigma-1 receptor (S1R) is a calcium-sensitive ligand-regulated chaperone capable of modulating the function of several ion channels and receptors involved in several biochemical pathways including pain. Specifically, S1R antagonists have antiallodynic and antihyperalgesic properties in pathological conditions. S1RA (E-52862) is the only S1R antagonist that has successfully completed phase 2 clinical trials in different types of pain. Of note, some classes of S1R antagonists with additional analgesic mechanisms have been described. The most studied family is dual mu opioid receptor (MOR) agonists and S1R antagonists. Although opioids effectively treat severe pain, their side effects such as constipation, nausea, respiratory depression, and addiction, limit their use. It is known that S1R antagonism enhances opioid analgesia without affecting its side effects. The most representative example is EST73502 (WLB-73502, ADV-502), that has shown efficacy in several acute and chronic pain models and is ready to enter phase 2 clinical trials.1 Another class of dual S1R antagonists targets the histamine H3 receptor (H3R), a GPCR primarily expressed in nociceptive pathways in the CNS. The H3R acts as a presynaptic autoreceptor and as a heteroreceptor, modulating the release of neurotransmitters implicated in pain transmission. Preclinical studies demonstrate the efficacy of H3R antagonists/inverse agonists in reducing hypersensitivity to mechanical and thermal pain in neuropathic pain models. Szczepańska et al. discovered dual H3R/S1R antagonists that potentiate loperamide analgesia in vivo.2 A third group of dual compounds, in addition to S1R antagonism, releases hydrogen sulfide (H2S), an endogenous gasotransmitter involved in inflammation, nociception, and vascular function. Exogenous H2S administration has demonstrated antinociceptive effects in inflammatory, visceral, and neuropathic pain models. Amata et al. reported the synergy resulting from the combination of S1R antagonism with H2S release and have developed dual compounds with analgesic activity in capsaicin-induced allodynia model.3 As part of our collaborative research with Prof. Enrique J. Cobos' group, we discovered a new family of S1R antagonists that also inhibit soluble epoxide hydrolase (sEH), a key metabolic enzyme of epoxyeicosatrienoic acids, which exhibit anti-inflammatory and vasodilator activity. sEH inhibition constitutes a promising analgesic strategy, fact demonstrated by EC5026, that is currently in clinical trials for neuropathic pain. We found that combining sEH inhibition with S1R antagonism produces a strong synergistic effect on certain types of pain, such as postoperative and rheumatoid arthritis pain. In addition, we have developed two families of compounds endowed with excellent potency in both targets and promising in vivo activities.

Keywords: sigma-1, multi-targeted strategy, dual, pain, analgesia.

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Pridopidine exerts neuroprotective effects, via activation of the Sigma-1 receptor (S1R)

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Pridopidine, a selective and potent S1R agonist in clinical development for ALS and Huntington Disease (HD), demonstrated neuroprotective effects in preclinical models of HD and ALS. The S1R is located in brain areas relevant to ALS and HD, and mutations cause juvenile and adult ALS.
Here, we report on studies in ALS patient derived iPSCs subjected to ER-stress and evidence for potential combination therapy with Sigma-2 receptor (S2R) antagonists. A hallmark of neurodegenerative diseases including ALS is prolonged endoplasmic reticulum (ER) stress. ER stress activates the unfolded protein response (UPR) through key mediators, including upregulation of BiP and CHOP. In neural progenitor cells from ALS patient derived iPSCs, pridopidine significantly reduces ER stress markers (BiP: 72%, $p<0.0001$; CHOP: 52%, $p<0.0001$). This reduction correlated with improved cell viability and growth (50% increase, $p<0.0001$).
Emerging evidence shows that complementary mechanisms that modulate the S1R may enhance neuroprotection. For instance, pharmacological antagonism of the S2R activates complementary neuroprotective mechanisms, suggesting that a dual-modality approach may enhance therapeutic benefits of pridopidine. Combining pridopidine with FA10 (selective S2R antagonist) enhances pridopidine's neuroprotection in a mouse primary neuron model of HD (74% reduced neuronal death, $p<0.0001$), greater than individual treatments (pridopidine, 51% $p<0.0001$; FA10, 46% r, $p<0.0001$).
These studies are advancing our understanding of targeted S1R therapies and provide novel therapeutic approaches for intractable neurodegenerative diseases.

Keywords: Pridopidine, S1R agonist, S2R antagonist

Pridopidine For the Treatment of ALS (Healey ALS Platform Trial)

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Pridopidine was evaluated for efficacy and safety in the phase 2 HEALEY ALS Platform Trial (NCT04615923).
Background: Pridopidine, a selective and potent S1R agonist, has demonstrated notable neuroprotective effects in ALS models. In preclinical models of ALS, pridopidine restores calcium homeostasis, maintains MAM integrity, and reduces ER stress. Moreover, pridopidine enhances survival pathways by upregulating brain-derived neurotrophic factor (BDNF), further supporting its neuroprotective potential.
Methods: A subgroup analysis of El Escorial definite+probable ALS and early (symptom onset <18mo) subjects assessing endpoints of ALSFRS-R, and measures of respiration, bulbar, speech, and quality-of-life (QoL) over 24 weeks.
Results: Pridopidine was well tolerated, consistent with its prior safety profile. Pridopidine (n=37) shows 32% slowing of decline vs placebo (n=35) in ALSFRS-R (wk24 Δ2.9, p=0.03). Benefits are observed in ALSFRS-R respiratory (wk24 Δ1.20, p=0.03), and bulbar (wk24 Δ0.93, p=0.06) domains. Pridopidine shows no worsening in dyspnea (wk24 Δ0.62, p=0.04). Benefits in speaking rate (Δ0.39, p=0.005) and articulation rate (Δ0.40, p=0.002) are observed. Pridopidine shows less decline in QoL (Δ-10.83, p=0.018), notably in eating & drinking (Δ-19.18, p=0.015) and communication (Δ-13.03, p=0.12) subdomains.
A Kaplan-Meier survival analysis shows a prolongation of median survival time (~300 to 600 days) compared to the delayed-start (168 days) placebo-to-pridopidine participants (n=12)(log rank p=0.069). The Cox Proportional Hazard Ratio (HR), adjusted for baseline characteristics is 0.429 (p=0.052).
Conclusion: Pridopidine demonstrated beneficial effects across multiple clinical measures of ALS, including survival benefits in definite+probable ALS and early participants. These encouraging observations support and inform planning for a Ph3 study.

Keywords: ALS, Pridopidine, Survival

The Phase 3 PROOF-HD Trial Demonstrates Meaningful Benefits of Pridopidine on Function, Cognition, and Motor in Huntington Disease (HD)

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Pridopidine is a highly selective and potent S1R agonist. PROOF-HD was a Ph3, global, double-blind, placebo-controlled trial assessing pridopidine (45 mg bid) in early manifest HD. Double-blind (DBP) duration was up to 78wk, followed by 26wk open-label period (OLE) (total 104 weeks). Key endpoints were change through wk104 in functional capacity (TFC), progression (cUHDRS), cognition (SWR), motor (Q-Motor) and QoL Prespecified subgroup analysis excluded participants on antidopaminergics (ADMs; VMAT2 inhibitors and neuroleptics).
Pridopidine was well tolerated with a safety profile comparable to placebo. Pridopidine did not show significant benefits in all patients.
In patients off ADMs, pridopidine was superior to placebo across key independent measures of HD progression, at all timepoints through 78 weeks. Pridopidine showed unprecedented significant improvements from baseline for 1 year in cUHDRS (wk52 Δ0.43, p=0.04); SWR (wk52 Δ4.22, p=0.02), Q-Motor finger tapping (FT) inter-onset interval (IOI)(wk52 (Δ-22.84, p=0.04) and preservation of QoL.
Pridopidine demonstrated persistence of benefits through wk104, across all endpoints compared to a propensity matched control from TRACK-HD observational study. Pridopidine showed improvements vs TRACK-HD in cUHDRS (Δ1.19, p<0.0001), TFC (Δ0.71, p=0.0004), SWR (Δ8.16, p=0.0003), and Q-Motor FT IOI (Δ-77.12 ms, p<0.0001).
Pridopidine inhibits CYP2D6 and its concomitant use with certain ADMs (CYP2D6 metabolized) increases exposure of ADMs. Participants on recommended doses of ADMs (per regulatory label guidance) maintain positive benefits of pridopidine.
Pridopidine shows consistent, sustained, and clinically meaningful benefits across multiple endpoints of HD progression in subjects off&on recommended ADM doses, and is currently under MAA review for the treatment of HD.

Keywords: HD, Pridopidine, PROOF-HD

Sigma-1 receptor antagonism and soluble epoxide hydrolase inhibition: synergistic effect in the reduction of capsaicin-induced and surgical incision-induced tactile allodynia

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Tactile allodynia is a feature of pain hypersensitivity common in many clinically-relevant circumstances, such as postoperative pain. Three quarters of postoperative patients experience moderate, severe, or even extreme pain in the immediate postoperative period despite pharmacological treatments, even with opioids. Both sigma-1 receptor (S1R) antagonism and soluble epoxide hydrolase (sEH) inhibition are promising pharmacological tools for pain treatment, although the effects of their association are unknown. Association of structurally unrelated S1R antagonists (BD-1063, S1RA and NE-100) and three representative sEHIs (AS2586114, EC-5026, and UB-SCG-54) induced a robust synergistic effect reversing capsaicin-induced tactile allodynia in mice, used as a model of central sensitization. We then selected the association of the clinical candidates S1RA and EC-5026 for further experiments in laparatomized animals, and found that they also exerted a synergistic full reversal of tactile allodynia. We also developed a dual compound which binds in the nM range both S1R and sEH, named EPB-117. This compound was able to abolish tactile allodynia induced by either capsaicin or laparotomy. The effects of the drug associations or the dual compound were reversed by either the S1R agonist PRE-084 or the inhibitor of microsomal CYP450s MS-PPOH, confirming that both S1R and sEH are necessary for the antiallodynic effects in both experimental situations. An analgesic dose of this compound did not affect motor coordination or gastrointestinal transit, which are common side effects of other widely used analgesics, as opioids or gabapentinoids, suggesting a favorable safety profile. Finally, we used a continuous delivery of EPB-117 using an Alzet mini-pump to emulate analgesic drug administration in a clinically relevant surgical setting, and found that it was able to block the development of tactile allodynia during the drug administration period (24h). In conclusion, S1R antagonism and sEH inhibition can be associated in separate drugs or in a dual compound as a novel strategy for pain treatment. Our results might have therapeutic applications, such as during postoperative pain. Funding: grants PID2019-108691RB-I00 and PID2023-150747OB-I00 (fom MICIU/AEI/10.13039/501100011033), ERDF funds, CaixaResearch Consolidate 2022-CC22-10176 and Junta de Andalucía (CTS-109). M Santos-Caballero and A Puerto-Moya are funded by FPI contracts (PRE2020-096203 and PRE2023-001628, respectively), and M Robles-Funes by a FPU contract (FPU23/03287).

Keywords: Tactile allodynia, soluble epoxide hydrolase, laparotomy, sigma-1 receptor, synergy.

Antagonism of Sigma-1 inhibits binge ethanol drinking in adolescence

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The consumption of ethanol during adolescence poses a significant health concern, yet there are limited pharmacological treatments available to curb adolescent binge drinking. This study evaluated whether sigma-1 receptor (S1-R) antagonists, S1RA or BD-1063, could disrupt ethanol consumption in male and female adolescent Wistar rats. The rats were administered S1-R antagonists three times a week for two weeks. Thirty minutes later, they were exposed to a bottle containing 8% or 10% v/v ethanol for 2 hours. Following these procedures, a 24-hour, two-bottle ethanol intake test was conducted. A subset of these rats underwent a recognition memory test using the novel object recognition test. Rats administered 64mg/kg S1RA consumed significantly less ethanol during each binge session compared to vehicle-treated counterparts. Male rats given 4 or 16mg/kg S1RA drank significantly less than those given 0mg/kg in session 3 or in sessions 1 and 2, respectively; whereas female rats given 4 or 16mg/kg consumed significantly less than those given 0mg/kg in sessions 2–5 or in sessions 2–6, respectively. Administration of 32mg/kg, but not 2 or 8mg/kg, BD-1063 consistently reduced ethanol consumption across sessions. S1-R antagonism decreased absolute ethanol consumption in the two-bottle choice post-test. Recognition memory was not impacted by ethanol exposure. These findings suggest that S1-R antagonism could be promising pharmacological strategy to prevent increased ethanol intake during adolescence. The lasting effect of S1-R antagonism on free-choice drinking implies that modulation of the S1-R system may lead to plastic effects associated with ethanol drinking behaviors.

Keywords: Ethanol, Sigma-1 receptor, Rat, Adolescence, Binge drinking

Sigma-1 receptor antagonism enhances endogenous and drug-induced opioid analgesia: promising strategies for postoperative pain treatment

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Most patients who undergo surgery experience pain in the immediate postoperative period, despite analgesic treatment. Therefore, new analgesic strategies are needed. The sigma-1 receptor is a neuromodulatory chaperone protein, known for its role on pain modulation, yet its impact on postoperative pain remains unexplored. Laparotomy is the initial step for many abdominal surgeries, which are among the most common types of surgery. Here, we used a laparotomy in mice to study the role of sigma-1 receptors in postoperative pain. We used the sigma-1 antagonists S1RA and BD-1063, as well as morphine, a standard opioid analgesic. The von Frey test was used to measure mechanical allodynia, with the response to the drugs assessed 3.5 hours post-laparotomy. Our results revealed a dose-dependent effect of S1RA (8-32 mg/kg) and BD-1063 (4-16 mg/kg), with both compounds fully reversing pain hypersensitivity. Morphine (0.13-0.5 mg/kg) also exhibited a dose-dependent and full antiallodynic effect. Administration of the sigma-1 receptor agonist PRE-084 (32 mg/kg) was able to reverse the effect of the sigma-1 antagonists without altering the effect of morphine, indicating the selectivity of this pharmacological approach. The antiallodynic effect induced by sigma-1 antagonism was also successfully reversed by the administration of the peripherally-restricted opioid antagonist naloxone methiodide (2 mg/kg), suggesting the involvement of peripheral opioid pathways. To further investigate the involvement of the opioid system in the antiallodynic effects of S1RA, we selectively blocked the three main opioid receptor subtypes by administering specific antagonists: the μ-opioid receptor antagonist cyprodime (15 mg/kg), the δ antagonist naltrindole (5 mg/kg), and the κ antagonist nor-binaltorphimine (10 mg/kg). Only cyprodime was able to reverse the antiallodynic effect of S1RA, indicating that the activation of μ-opioid receptors plays a predominant role in this sigma-1-mediated analgesic response. We then searched for the source of peripheral opioids, and found that neutrophils recruited to the injured site express the precursor of ß-endorphin (a μ-opioid agonist) but not the precursor of dynorphin (a δ-opioid agonist) or enkephalins (κ-opioid agonists). In fact, the antiallodynic effect of S1RA and BD-1063 was dependent on the presence of neutrophils in the injured site, as demonstrated through neutrophil depletion experiments with an anti-Ly6G antibody (8 μg). The combined administration of S1RA and morphine (a prototypic μ-opioid analgesic) each at doses that individually failed to produce a significant analgesic effect (8 mg/kg and 0.13 mg/kg, respectively), successfully restored the mechanical threshold to baseline levels, demonstrating a synergistic interaction between sigma-1 receptor antagonism and opioid-mediated analgesia. Interestingly, S1RA did not modify the rewarding properties of morphine or its effect in gastrointestinal transit inhibition. Our results support the potential of sigma-1 antagonism as a valuable addition to postoperative pain management

strategies by the potentiation of immune-driven endogenous opioid analgesia or by increasing the analgesic effect of opioid drugs without increasing their adverse events.

Keywords: Postoperative pain, morphine, sigma-1 antagonism, neutrophils

DHEA(S) in dementia: from cellular and animal models to patients

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Neurosteroids dehydroepiandrosterone (DHEA) and its sulfate (DHEAS) exert a broad range of effects by acting through several major receptor systems in the brain, including GABA-A, glutamate NMDA, and sigma receptors. Preclinical studies suggest these neurosteroids might have beneficial effects in various neuropsychiatric and neurodegenerative disorders. Since dementia represents one of the greatest medical and economic challenges, we have investigated the complex role of DHEA(S) in dementia, by combining cellular and animal models, as well as dementia patients. To model Alzheimer's disease (AD) and vascular dementia (VaD) in vitro, we have exposed primary mouse neurons and human SH-SY5Y neuroblastoma cells to toxic Aβ oligomers or oxygen-glucose deprivation (OGD). Moreover, as genetic and pharmacological animal model of AD, we have utilized 3xTg-AD triple-transgenic mice and C57BL/6 mice injected icv. with Aβ oligomers, respectively. Finally, our study also enrolled dementia patients and individuals with mild cognitive impairment (MCI), as comparative group. In cellular and animal models of dementia, despite differences observed between various research approaches, DHEA(S) demonstrated neuroprotective actions and homeostatic effects on expression of genes and proteins involved in PI3K/Akt and Bcl-2 signaling network, also shown to be regulated via sigma receptors. Together with reduced DHEAS plasma levels observed in dementia patients, these findings suggest that DHEA(S) supplementation should be further studied as a novel option for the prevention and/or treatment of dementia.

Keywords: dementia, dehydroepiandrosterone (sulfate), neuroprotection

The hidden effects of Metformin on oxidative stress

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Introduction: The sigma-1 receptor (σ1R) agonist metformin, which is frequently prescribed for type 2 diabetes, provides benefits beyond regulating blood sugar levels. The neuroprotective and anti-inflammatory actions of metformin are mediated via the σ1R, which is essential for protein folding, cellular stress responses, and homeostasis. σ1R activation regulates mitochondrial activity and ER stress, which are important in neurodegenerative diseases and problems from diabetes. This talk examines how a patient's treatment has changed over time, emphasizing metformin's wider therapeutic potential in treating stress and cellular malfunction and providing fresh approaches for a range of ailments. Methods: In this presentation, the evolution of a 69-year-old patient diagnosed with type 2 diabetes in 2015 is presented. data from blood tests (hemoglobin, white blood cell count, glycosylated hemoglobin, cholesterol) was collected and the macroscopic structure of the pancreas was monitored. The patient has been undergoing treatment with metformin since 2023, taking 2 capsules of 1000 mg per day. from the tests done in August 2023, the following results were obtained: HbA1C: 6.57, WBC: 7.37, cholesterol: 180, TGO: 20, TGP: 17, LDL: 105, HDL: 63.4. The following tests were done in February 2024: HbA1C: 6.07, WBC: 6.98, cholesterol: 179, AST: 20 ALT: 20, LDL: 103, HDL: 64. the latest tests were done in November 2024, following the reduction of the metformin dosage from 2 capsules to 1 capsule: HbA1C: 6.76, WBC: 8.32, cholesterol: 163, AST: 26, ALT: 28, LDL: 82, HDL: 70. . comparing an ultrasound from 2022 with one from 2025, an increase in hyperechogenicity is observed. Results: Results: HbA1C and WBC dropped when the patient was taking 2000 mg of metformin daily, indicating a decrease in oxidative stress and glycemic levels during this time. These readings rose in the subsequent month, which showed a poor progression, when the daily dose of metformin was decreased and a suitable diet was abandoned. It is emphasized how crucial metformin is to getting a favorable outcome.This data aided in tracking the less obvious consequences of improper treatment follow-up. Disscusion: The patient's development under the original medication and with just 1000 mg of metformin differs. Furthermore, the quantity of leukocytes can rise for a number of reasons. Some of the novel blood test results are related to pancreatic tissue degeneration, which is suggested by imaging.

Keywords: metformin, HbA1C, WBC, inflammation, pancreas

References

1. The Pharmacology of Sigma-1 Receptors
2. The mechanisms of action of metformin

High-content drug screening for Sigma-1 Receptor activators using the nematode Caenorhabditis elegans

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The sigma-1 receptor (σ1R) is an evolutionarily conserved chaperone protein implicated in neuroprotection, autophagy, and cellular homeostasis. Its role in neurodegenerative diseases, including Alzheimer's and Parkinson's disease, has attracted significant research interest. However, the mechanisms governing its regulation remain largely unexplored. Here, we report the generation of transgenic Caenorhabditis elegans strains expressing both transcriptional and translational σ1R reporters. Preliminary findings indicate that the hlh-30/TFEB transcription factor, a key regulator of autophagy, modulates σ1R expression, suggesting a potential link between σ1R and cellular clearance pathways. To further investigate the therapeutic potential of σ1R activation, we will utilize C. elegans genetics in combination with the SydLab One analyzer, the first fully automated organism-on-chip platform, to conduct a high-content screening of 770 FDA-approved drugs. Positive hits from this screen will be assessed for their neuroprotective properties in C. elegans models of Alzheimer's and Parkinson's disease, as well as during ageing. Our study aims to elucidate the regulatory mechanisms of σ1R and identify small-molecule activators with potential neuroprotective properties, providing insights for future therapeutic applications in neurodegenerative diseases.

Keywords: Ageing, C. elegans, neurodegeneration, neuroprotection, high-content screening

Sigma-1 receptor is associated with tetraspanin-labelled extracellular vesicles: insights from single particle analysis

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The Sigma-1 receptor (S1R) is considered to play a role in modulating the cellular secretory pathway by influencing vesicle formation and protein trafficking. S1R has been detected in blood plasma and is known to indirectly affect extracellular vesicle (EV) release. Additionally, recent findings suggest that modulating S1R activity with its ligands could serve as a novel strategy for altering EV content and production in cancer cells. However, direct evidence confirming the presence of S1R on EVs has yet to be verified. To validate the presence of S1R on EVs, MultiBacMam system was used for the simultaneous co-expression of fluorescent protein tagged S1R (S1R-EYFP or S1R-mCherry) and marker of EVs (tetraspanins EGFP-CD9, CD63-mCherry or CD81-miRFP670) in human ovarian adenocarcinoma SK-OV-3 cells. Widefield epifluorescence live cell imaging was performed to evaluate the expression pattern of generated constructs and cell phenotype. Tangential flow filtration was used to isolate EVs from conditioned SK-OV-3 cell culture media following cell transduction for 5 days. EVs were precipitated and concentrated using 8% polyethylene glycol (PEG6000). Emission spectra measurements were used to confirm the presence of specific fluorescent proteins in isolated samples while SDS-PAGE and Western blot was used to quantify the expression levels. A multi-well total internal reflection fluorescence (TIRF) microscopy system was used for the analysis of isolated EVs at a single particle level. Colocalization analysis on multi-channel TIRF images was performed with the Statistical Object Distance Analysis workflow. Fluorescent protein-labelled S1R-EVs were observed in the extracellular space during live-cell imaging and confirmed in isolated EV samples through emission spectra analysis, WB, and TIRF imaging. High-resolution characterization of S1R-EVs using TIRF microscopy revealed that 17% of Sig1R-enriched particles were associated with CD81, while 40% colocalized with CD9. The strongest association was observed between S1R and CD63-labelled particles, with 60% of S1R-EYFP-labeled spots overlapping with CD63-mCherry. Analysis of tetraspanin-labelled spots revealed that CD63 had the highest coupling ratio with S1R, with 27% of CD63-labelled spots colocalizing with S1R. Overexpression of S1R in SK-OV-3 cells had no significant effect on the endogenous levels of tetraspanins CD63 and CD9, suggesting that it does not promote their production to facilitate EV generation and release. This study provides the direct confirmation of S1R presence on EVs, with a strong association observed between S1R and CD63. The findings suggest that S1R may play a role in EV subpopulation dynamics without altering endogenous tetraspanin levels. Further research is needed to explore the functional implications of S1R-EVs in intercellular communication and their potential applications in disease diagnostics and therapeutics.

Keywords: Tetraspanins, extracellular vesicles, MultiBacMam, TIRF microscopy, single particles

Transcriptomic profiling of Sigma-1 receptor agonism in bleomycin-induced pulmonary fibrosis: Insights into therapeutic mechanisms

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Introduction & Aims: Idiopathic pulmonary fibrosis (IPF) is a chronic lung disease characterized by progressive scarring of lung tissue, leading to a decline in pulmonary function with a median survival of only 2–3 years following diagnosis. IPF represents a significant unmet medical need since current therapies, such as nintedanib and pirfenidone, slow the decline in lung function, but they are unable to stop disease progression or significantly improve survival outcomes. Our team has pioneered the development of S1R-targeted therapies, securing patents in the EU, US, and other regions for their application in fibrosis treatment. Building on this, we investigated the anti-fibrotic efficacy of the S1R agonist fluvoxamine (FLU) in a bleomycin-induced pulmonary fibrosis model, and to elucidate underlying mechanisms through bulk mRNA sequencing (mRNA-seq). Methods: Pulmonary fibrosis was induced in C57BL/6 mice via single oropharyngeal bleomycin (BLM 1mg/bwkg) administration. Mice were treated daily with FLU (20mg/bwkg) administered intraperitoneally (BLM + FLU). After 21 days, the mice were euthanized, and lung tissues were collected, and mRNA sequencing (mRNA-seq) was performed using the Illumina platform. Differentially expressed genes (DEGs) were identified through comparative analysis of transcriptomic profiles. To further explore the biological significance of the DEGs, gene set enrichment analysis (GSEA) and gene set variance analysis (GSVA) were conducted using the Hallmark gene set database. Protein-protein interaction (PPI) network was constructed using DEGs (BLMF vs. BLM) to identify key molecular interactions. Genes with a node degree greater than 10 were selected for overrepresentation analysis (ORA) to uncover enriched biological pathways. Results: We identified 880 DEGs ($|log2FC|>1$, $p<0.05$) between BLM and Control groups and 378 DEGs between BLMF and BLM groups. GSEA demonstrated that Epithelial Mesenchymal Transition (EMT) and Inflammatory Response pathways were significantly enriched in BLM induced pulmonary fibrosis, while FLU treatment reduced the enrichment scores of these pathways. PPI analysis identified 33 hub genes with node degree greater than 10. ORA of these hub genes revealed their association with cytokine and chemokine activity. Conclusion: Our findings demonstrate that treatment with the S1R agonist FLU effectively attenuates the upregulation of genes associated with EMT and inflammatory response pathways in a bleomycin-induced pulmonary fibrosis model. These findings underlines that S1R agonism represents a promising therapeutic strategy for mitigating key pathological processes driving pulmonary fibrosis. Funding: LP2021-3/2021, TKP2021-EGA-24, STAGE 2024-1.2.3-HU-RIZONT-2024-00056, SigmaDrugs Research Ltd.

Keywords: pulmonary fibrosis, bulk mRNA sequencing, transcriptomics, sigma-1 receptor, fluvoxamine

Age-specific anti-glaucomatous effect of a Fluvoxamine-containing eye drop

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Introduction The Sigma-1 receptor (S1R) is a multifunctional chaperone protein involved in cellular stress responses, neuroprotection, and modulation of ion channels. Recent studies have highlighted its role in ocular health, particularly in regulating trabecular meshwork (TM) function and reducing fibrosis. Glaucoma, the second leading cause of blindness worldwide, currently affects 80 million people, with projections exceeding 110 million by 2040. Elevated intraocular pressure (IOP) is a major risk factor, primarily driven by TM fibrosis, which compromises aqueous humor outflow. Current treatments are often insufficient and associated with significant side effects. Our previous studies demonstrated that S1R activation in TM cells significantly reduces fibrosis, suggesting a novel therapeutic approach. Based on these findings, we developed a fluvoxamine (FLU)-containing eye drop as an S1R agonist and confirmed its ocular safety. Objective This study aims to evaluate the IOP-lowering and anti-fibrotic effects of the novel FLU eye drop in aged mice and to investigate age-dependent transcriptional changes in primary TM cell cultures. Methods In vivo: Glaucoma was induced in 6-8-month-old C57BL/6J wild-type and S1R knockout mice using dexamethasone. Mice were treated twice daily for two weeks with either vehicle or FLU-containing eye drops. IOP was measured weekly over a four-week period. In vitro: Primary TM cells were isolated from 6-month-old and 2-year-old mice. Morphological characteristics were assessed, and following TGF-β2 and TNF-α stimulation, the expression of extracellular matrix proteins and inflammatory cytokines was analyzed. Results By the end of the two-week treatment, FLU eye drops reduced IOP by nearly 10% (from 18.69 mmHg to 16.97 mmHg) in wild-type mice, whereas no effect was observed in S1R knockout mice, confirming the role of S1R in this process. In primary TM cells derived from aged mice, the expression of extracellular matrix components (F-actin, fibronectin) was elevated. FLU treatment mitigated TGF-β2-induced F-actin accumulation and TNF-α-induced IL-6 gene expression in TM cells from aged mice. Conclusion FLU eye drops effectively, and S1R specifically reduce IOP, supporting the potential therapeutic role of S1R agonists in glaucoma. Our in vitro findings further suggest that fibrotic processes contributing to glaucoma are more pronounced with aging and that the effects of S1R agonists may be age-dependent. Grants OTKA- K135398, LP2021-3/2021, TKP2021-EGA-24, STAGE 2024-1.2.3-HU-RIZONT-2024-00056, SigmaDrugs Research. „SUPPORTED BY THE 2.1.1-2024-00004 UNIVERSITY SCHOLARSHIP PROGRAMME OF THE MINISTRY FOR CULTURE AND INNOVATION FROM THE SOURCE OF THE NATIONAL RESEARCH, DEVELOPMENT AND INNOVATION FUND."

Keywords: glaucoma, eye drop, fluvoxamine, aging, S1R

Concept of a random forest machine learning model for detection of chromatin structural rearrangements induced by Sigma-1 receptor ligands

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Both Sigma-1 receptor agonists and antagonists can induce changes in chromatin structure and distribution within the nucleus. These changes are often related to DNA damage, epigenetic modifications, and early phases of programmed cell death. During light microscopic evaluation, in many cell populations, the changes can be so discrete that they are undetectable to the human eye, even when the evaluation is performed by an expert in the field of cell physiology. Here, we propose a programming code and a concept for a hypothetical advanced machine learning model based on the random forest algorithm to detect such discrete alterations in the cell nucleus. The model would apply inputs in the form of fractal and textural parameters obtained after analysis of nuclear ROIs, namely fractal dimension, lacunarity, as well as a set of gray-level co-occurrence matrix features such as inverse difference moment and textural variance. The output of the model would be the presence or absence of Sigma-1 receptor ligands in the extracellular environment. We discuss the advantages and limitations of this machine learning approach, with a special focus on potential alternatives such as gradient boosting and other decision tree–based algorithms. Finally, we present our recently developed code for the random forest model, which has the potential to be included in future AI-based systems for Sigma-1 receptor research. Acknowledgements: This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant 451-03-66/2024-03/200110 (subgrant entitled "Development of artificial intelligence models based on the random forest algorithm for the detection of discrete structural changes in the cell nucleus").

Keywords: AI, Chromatin, Model, Microscopy, Cell Physiology

Potential of support vector machine learning in Sigma-1 receptor physiology

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Support Vector Machine (SVM) is a supervised machine learning algorithm with potential applications in molecular physiology research. When used for classification, it constructs a hyperplane in a multidimensional data space to optimally separate data points. Contemporary Sigma-1 receptor research often generates high-dimensional datasets where traditional classification models may struggle. These datasets may include concentrations of different chemical mediators, parameters of signal analysis, and electrochemical or imaging data. Compared to other AI-based approaches, SVM offers the advantage of mapping non-linearly separable data into a higher-dimensional space where separation becomes feasible. This is achieved through the introduction of various kernel functions, such as the linear kernel, polynomial kernel, sigmoid kernel, or radial basis function (RBF) kernel. In this work, we propose an advanced Support Vector Machine Python code that can be used for model training and testing in contemporary Sigma-1 receptor physiology research. The model could be applied to drug discovery and ligand prediction, biomarker identification and disease classification, signal transduction and functional prediction, as well as EEG and neurophysiological signal classification. We highlight the potential advantages of such a model, including its robustness against small sample sizes, while also addressing its limitations, such as challenges related to hyperparameter tuning and computational cost.

Keywords: AI, Machine Learning, Sigma-1 physiology, Signal, Computer

Sigma-1 receptor agonist fluvoxamine is protective against hyperglycemia-induced fibrosis in human trabecular meshwork cells

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Primary open-angle glaucoma (POAG) is a common ocular complication of diabetes. It is associated with elevated intraocular pressure (IOP) due to dysfunction and fibrotic changes of the trabecular meshwork (TM). While the exact molecular mechanisms remain unclear, hyperclicaemia in the aqueous humor significantly contributes to TM fibrosis. Previously we showed the antifibrotic effect of the Sigma-1 receptor (S1R) agonist fluvoxamine (FLU) in human trabecular meshwork (HTM-5) cells. Here, we investigated whether FLU is effective in the prevention of hyperglycemia-induced fibrotic-like changes in HTM-5 cells. Cells were exposed to high glucose (HG, 25 or 30 mM) alone or in combination with FLU (15 μM) for 24 or 48 hours. FLU treatment upregulated S1R protein levels, while reducing the expression of profibrotic factor TGF-β2 and extracellular matrix component fibronectin (Fn). Hyperglycemia also induced F-actin accumulation, indicating cytoskeletal remodeling, while FLU treatment mitigated this effect. Moreover, FLU significantly suppressed high glucose-induced cell proliferation. Fluorescence-based assays were performed to measure reactive oxygen species (ROS) and intracellular nitric oxide (NO) production. FLU treatment effectively enhanced intracellular NO levels and reduced ROS generation, demonstrating its antioxidant capacity in preventing oxidative stress caused by hyperglycemia. These findings highlight S1R activation by its agonist FLU as a promising therapeutic approach for alleviating hyperglycemia-induced fibrotic changes in the eye by modulating various molecular pathways. Grants:, LP2021-3/2021, TKP2021-EGA-24, SigmaDrugs Research Ltd, STAGE 2024-1.2.3-HU-RIZONT-2024-00056

Keywords: HTM-5, Sigma-1 receptor, fibrosis, fluvoxamine, glaucoma

Protective effects of a novel Sigma-1 receptor agonist in renal ischemia/reperfusion injury

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Introduction and Aims Renal ischemia/reperfusion injury (IRI)-induced acute kidney injury is associated with high mortality and morbidity, and effective therapies are lacking. We previously showed that Sigma-1 receptor (S1R) agonist fluvoxamine treatment is protective against renal IRI and improves organ preservation during kidney transplantation. In this study, we developed a new fluvoxamine analog compound (VCC) with limited blood-brain barrier penetration to minimize potential psychoactive effects. We aimed tested the renoprotective effect of the VCC compound in murine models of renal IRI and graft static cold storage. Furthermore, we identified the molecular mechanisms triggered by S1R activation. Methods In silico calculations were performed to explore blood-brain barrier penetration and determine the polar surface area of VCC. In the in vivo experiment, 6 week-old male C57BL/6J mice were subjected to 25 min of unilateral ischemia with contralateral nephrectomy. 30 min before the ischemic insult mice were treated i.p. as follows: isotonic saline as vehicle (IRI); or 20 mg x bwkg-1 VCC (IRI+VCC). Sham-operated animals that underwent the same surgical procedures without clamping were used as controls (Sham). Renal functional parameters (blood urea nitrogen (BUN) and serum creatinine (Scr)) and tubular injury markers (kidney injury molecule 1 (Kim1), neutrophil gelatinase-associated lipocalin (Ngal)) were measured. Structural damage was assessed on PAS-stained kidney sections. Expressions of inflammatory (Il1β, Tnfα) and apoptotic (Bax, P53, Caspase 3) genes were investigated. In the ex vivo experiment, kidneys were perfused and then stored at 0°C in a preservation solution supplemented with vehicle or the VCC compound. Structural injury was evaluated on PAS-stained kidney sections. Results In silico calculations showed that VCC has a lower brain-to-plasma concentration ratio and a higher polar surface area compared to fluvoxamine, indicating minimal blood-brain barrier penetration while maintaining a low affinity for the hERG channel. Impaired kidney function and structural damage after IRI were ameliorated by VCC. Expressions of early and sensitive tubular injury markers Kim1 and Ngal were less elevated in VCC-treated mice. S1R activation by VCC modulated apoptotic processes by inhibiting the p53-Bax pathway. Moreover, VCC regulated CaMKII-NF-κB signaling, leading to reduced proinflammatory cytokine expression. In the cold storage model, kidneys perfused and stored in VCC solution showed reduced tubular dilatation are more preserved structure. Conclusion S1R activation by VCC alleviates renal IRI by suppressing apoptotic and inflammatory pathways. Supplementation of the preservation solution with VCC reduces structural injury during cold storage before transplantation. Thus, graft quality may be improved and the number of transplantable organs may be increased. These findings underscore the potential of S1R activation in mitigating ischemic injury and enhancing transplant outcomes. Funding LP2021-3/2021, TKP2021-EGA-24, STAGE 2024-1.2.3-HU-RIZONT-2024-00056, Sigma Drugs Research Ltd.
Keywords: Sigma1 receptor, kidney, ischemia/reperfusion, inflammation, apoptosis

The interplay of S1R and Wolframin in neuronal autophagy

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Since autophagy is an essential cellular pathway for cell survival and proper protein homeostasis, mutations within or absence of proteins involved in this process contribute to several pathogenic conditions, like neurodegeneration. Sigma1-Receptor (S1R) and Wolframin (WFS1) are two proteins that are involved in autophagy. Both are transmembrane proteins located at the mitochondria-associated membranes (MAM) of the endoplasmic reticulum (ER), where they additionally influence the calcium flux from ER to mitochondria and modulate the ER stress response. We and others have shown that activation of S1R positively modulates the autophagic flux. Interestingly, treatment of Wolframin-deficient cells with S1R agonists had a rescuing effect on dysregulated autophagy. Deficiency or mutations of S1R or WFS1 are linked to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and Wolfram syndrome (WS), respectively. To investigate the functional connection between these two proteins, especially in terms of autophagy within a neuronal context, we are establishing different NSC34 cell lines expressing APEX2 fused to either S1R or WFS1 and their disease associated mutants. By using proximity biotin labeling followed by mass spectrometry, we aim to map the luminal and cytosolic proximatomes of WFS1 and S1R to identify prospective new interactors. Furthermore, we will examine changes in these proximatomes under various conditions, such as WFS1 knockout or S1R activation and compare the proximatomes of wild-type proteins to those of their pathogenic variants. This will help us to identify potential pathways that are involved in their mode of action in health and disease. Ultimately, our goal is to use these methods to uncover the mechanisms through which S1R and WFS1 influence neuronal autophagy and to better understand the pathogenic consequences of their mutant variants. Funding: ANR-DFG Project “SIRWOLFPHAGY” to BD and CB

Keywords: autophagy, wolframin, sigma-1-receptor, proximatome