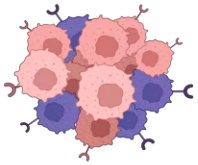
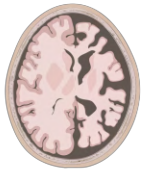
WE STUDY CONDITIONS SUCH AS

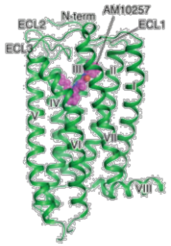


CANCERS

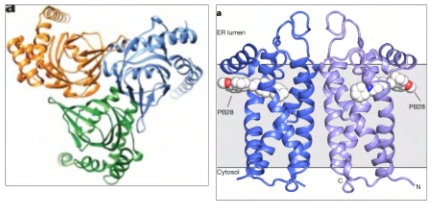


NEURODEGENERATIVE DISEASES

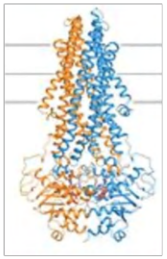
HITTING TARGETS SUCH AS



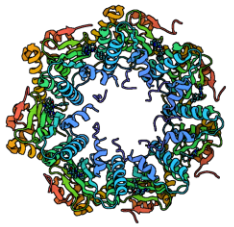
CANNABINOID 2 RECEPTOR (CB2R)



SIGMA 1 AND 2 RECEPTORS σ1 and σ2

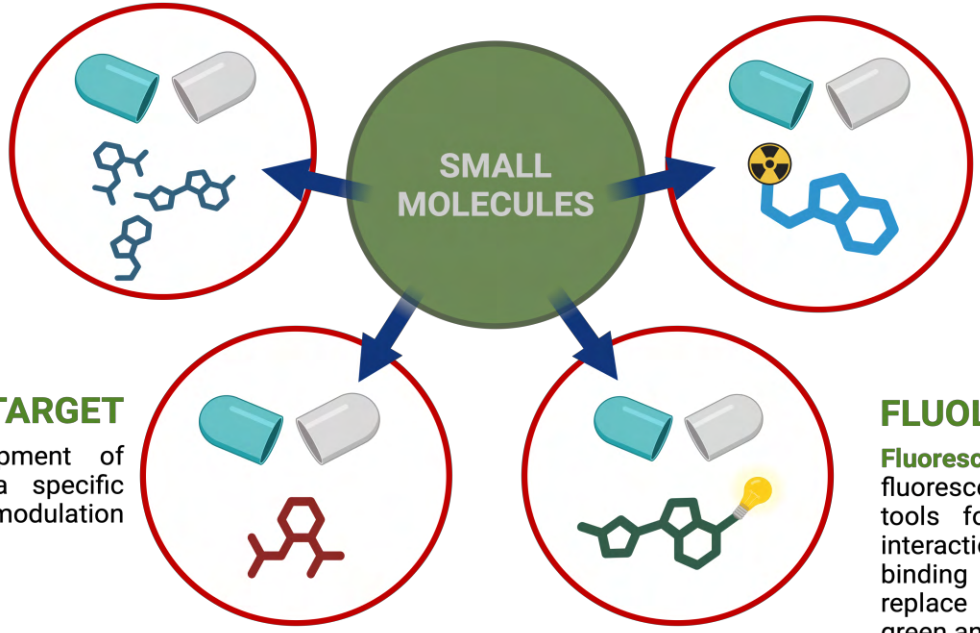


P-GLYCOPROTEIN 1 Pg-P



CASEINOLYTIC PROTEASE P ClpP

THROUGH THESE APPROACHES



MTDL

Multitarget Directed Ligand approach (MTDL): development of single molecules to simultaneously modulate multiple biological targets as a therapeutic option in multifactorial diseases.

PET

PET ligands: synthesis of "cold" ligands and the corresponding precursors for radiolabelling as tools for the in vivo diagnosis of pathologies.

SINGLE TARGET

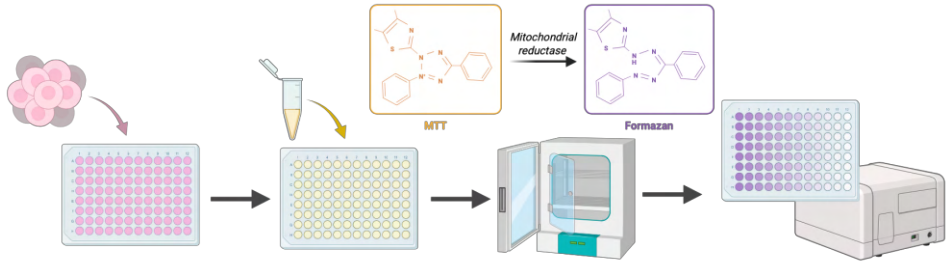
Single target approach: development of ligands highly selective towards a specific biological target for its therapeutic modulation with reduced off-target effects.

FLUOLIGANDS

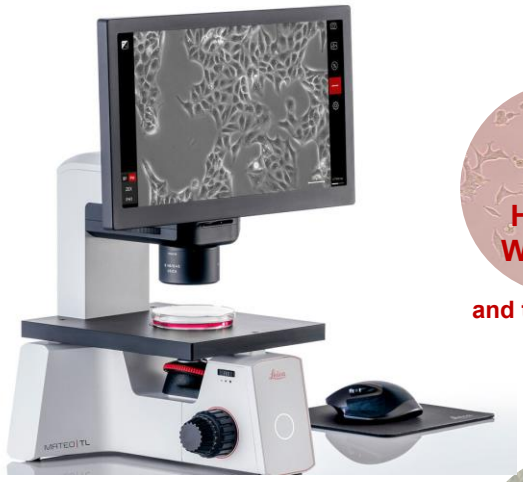
Fluorescent ligands: synthesis of fluorescent small molecules as powerful tools for the study of drug-receptor interactions, receptor localization, and binding kinetics. These ligands may replace the use of radioligands as a green and safe alternative.

WE CULTURE DIFFERENT CELL LINES

TO EVALUATE THE EFFECT OF COMPOUNDS



MTT /CCK-8 assay is useful to assess citotoxicity following compounds treatment



SH-SY5Y

BV-2

DIPG36

and DIPG50, DIPG25, DIPG38, SF8628, DIPG-48, DIPG-XIII and DIPG-VI

NSC34

HepG2

MCF-7 Wild type

MCF-7 Sigma-1

HEK293 Wild type

and transfected

Panc-1

HGC-S

HGC-R

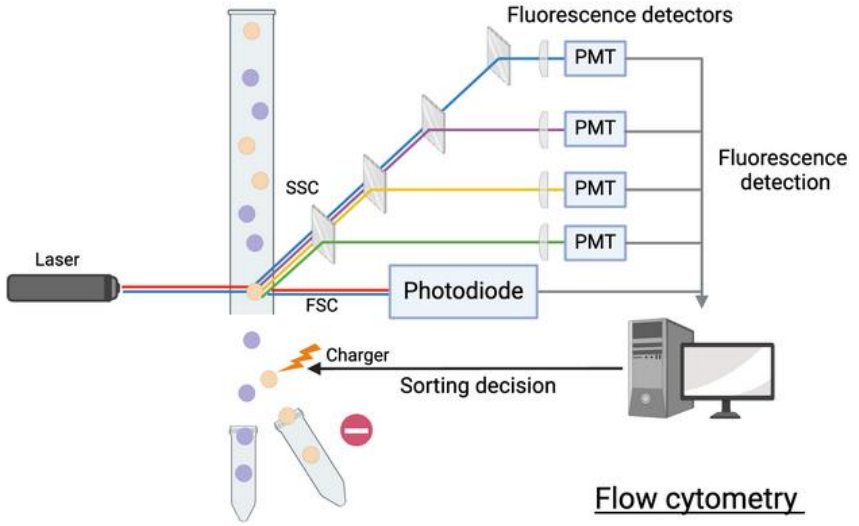
and AGS

SKOV-3

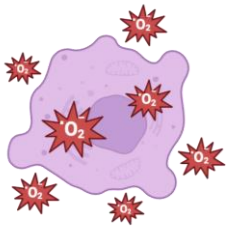
OVCAR-3

Caco-2

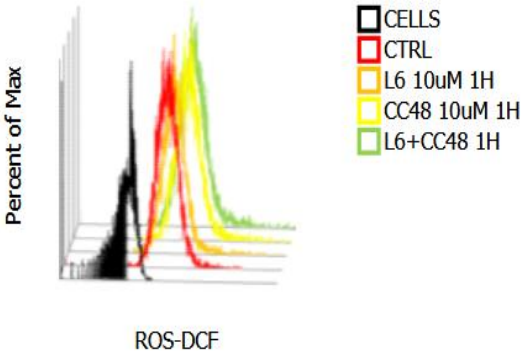
FLOW CYTOMETER

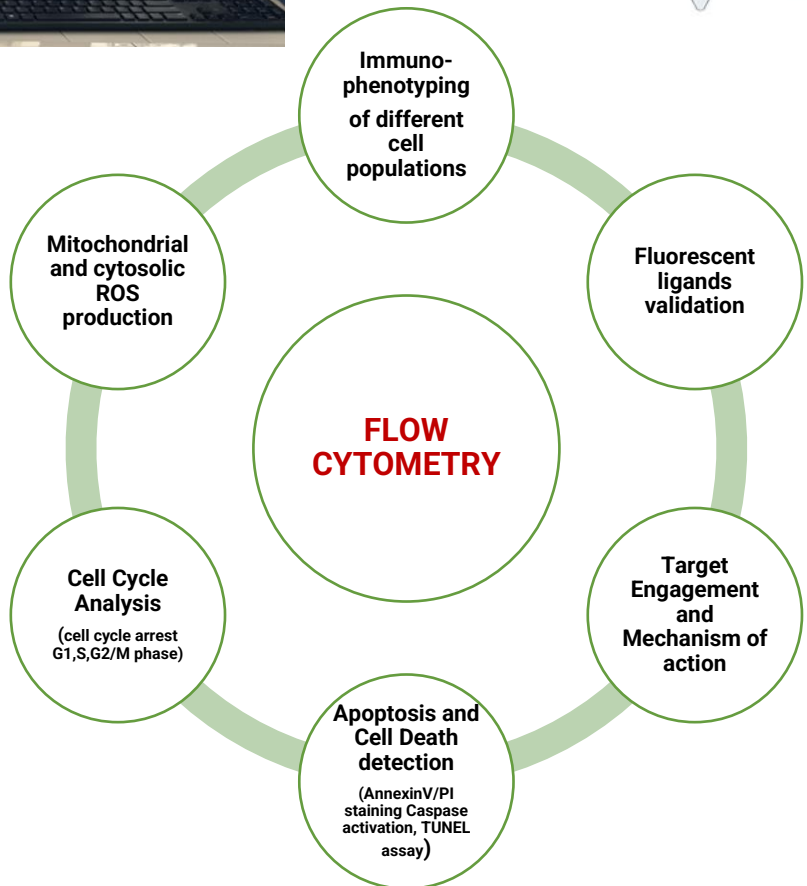


Flow cytometry

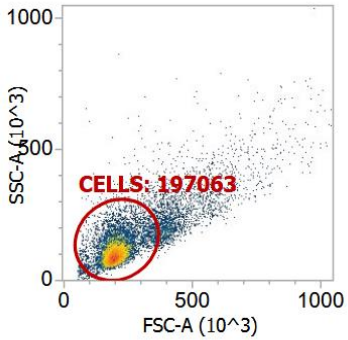


BV2 ACTIVATED - 1H

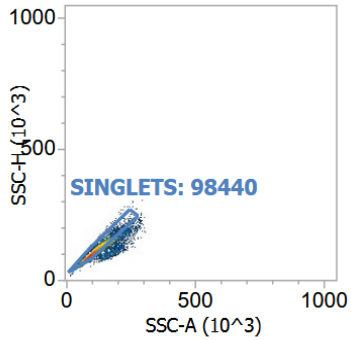




All Events - L6 10uM 1H

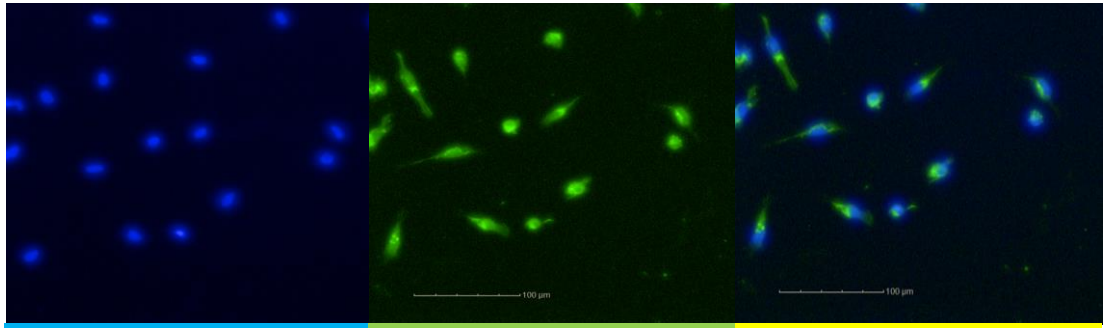


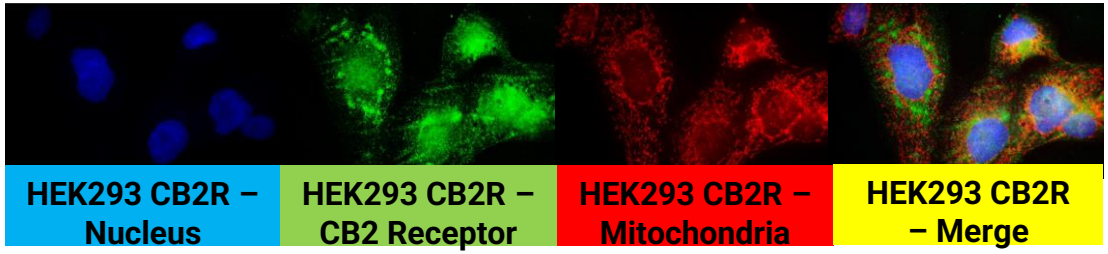
CELLS - L6 10uM 1H



WESTERN BLOTTING and IMMUNOFLUORESCENCE

Target Engagement and Validation (drug expression or activity modulation of its intended protein target)

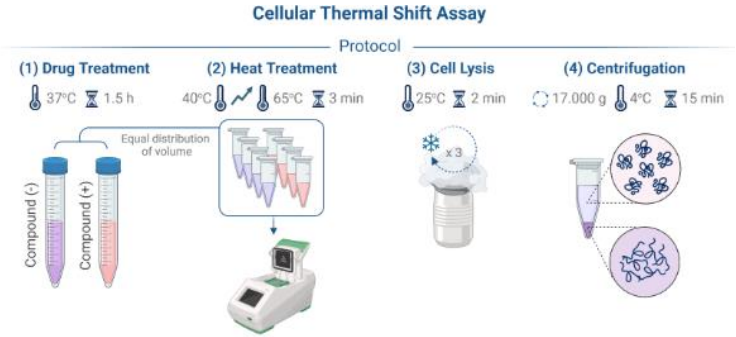


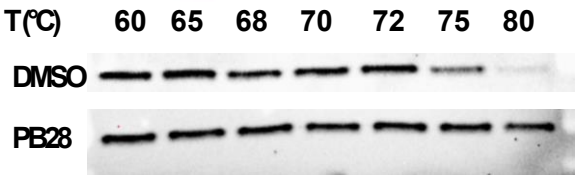


Operetta CLS-Perkin-Elmer for High Content Imaging

CETSA

TARGET ENGAGEMENT





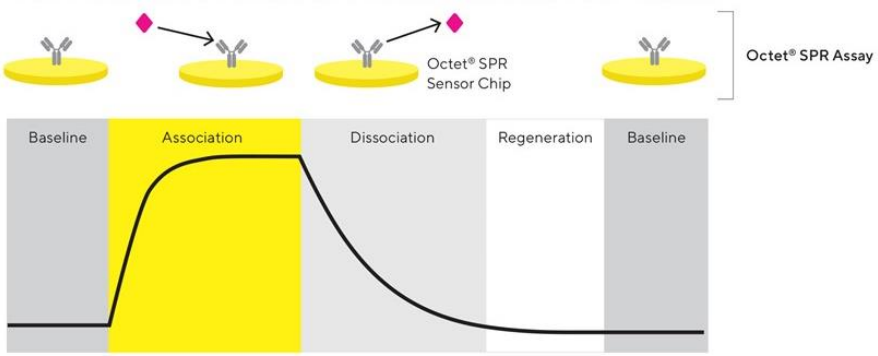
NSC-34 cell lines

WE ALSO EVALUATE TARGET BINDING USING

RADIOLIGAND DISPLACEMENT ASSAYS

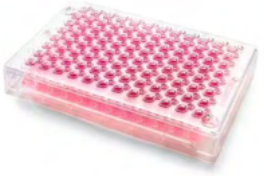
AND

SURFACE PLASMON RESONANCE (SPR)

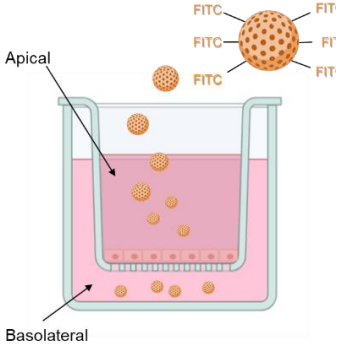


PHARMACOKINETIC PROPERTIES OF COMPOUNDS such as

- **Permeability through phisiological membranes (Caco-2/MDCK-MDR1))**

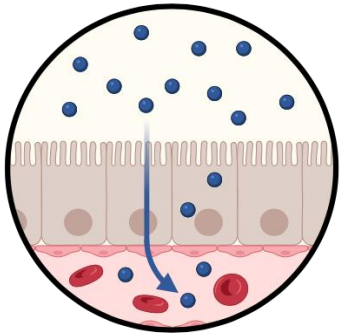


Millicell® 96 well Plate

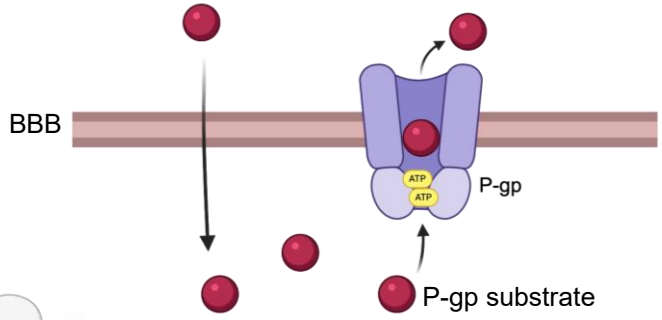


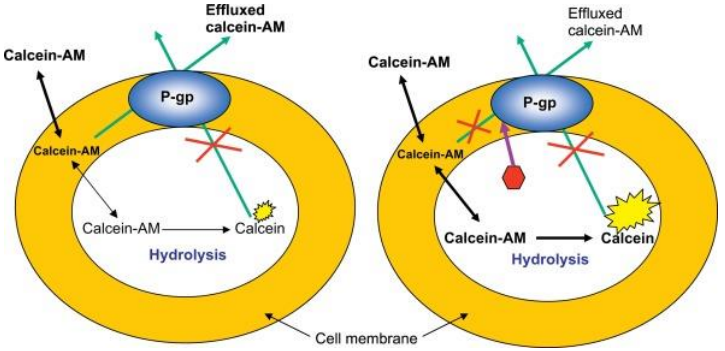
Caco-2 cell monolayer
(after 21 days growing)

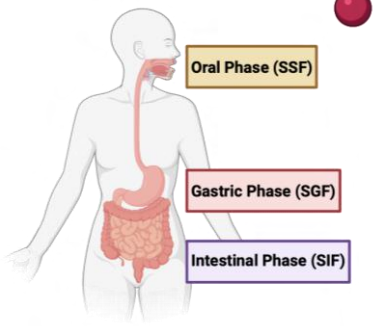
UV-Vis Spectrophotometer /HPLC to detect the amount of compound that crosses the cellular monolayer



- **Interaction with P-Glycoprotein (P-gp)**







CHEMICAL AND METABOLIC STABILITY USING STATIC MODELS OF HUMAN DIGESTION such as Simulated Salivary, Gastric and Intestinal Fluids.

Department of Drug and Health Sciences
University of Catania

Laboratory of Pharmaceutical Biotechnology
Viale Andrea Doria 6, 95125 Catania, Italy

Prof. Emanuele Amata; Email: eamata@un
Tel: +390-0957384102

Quantitative Characterization of Ligand-Receptor Interactions: Radioligand Binding Assays

Purpose

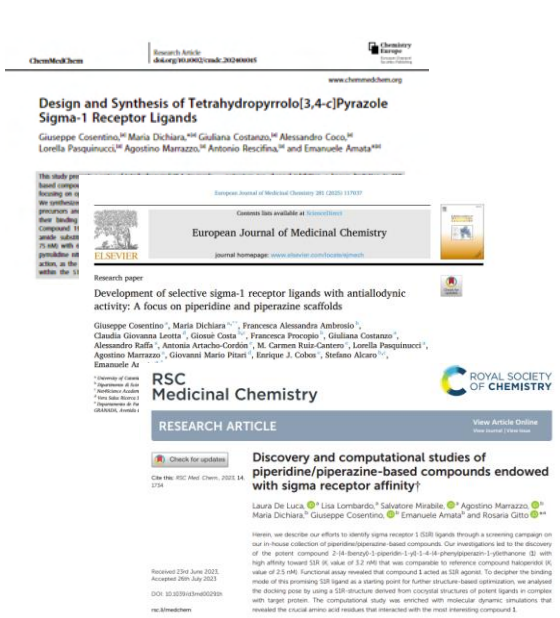
- Evaluate the affinity of test compounds *vs* S1R and S2R
- Assess selectivity (S1R *vs* S2R binding preference)
- Functional classification at S1R: (agonist/antagonist)

Main Parameters

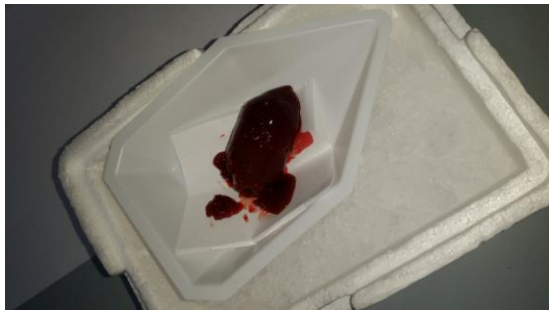
- K_i: Binding affinity (inhibition constant)
- IC_{50}: Concentration inhibiting 50% of radioligand binding
- Selectivity Index : S1R *vs* S2R K_i ratio
- % Displacement: Radioligand displacement by test compound at given concentration

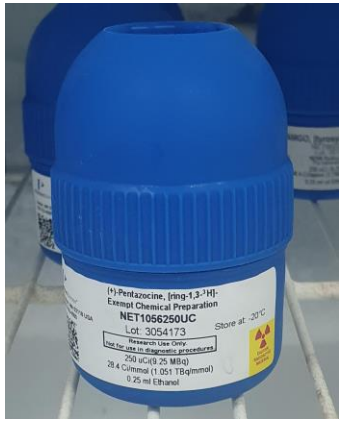
How It Works

- Use of a radiolabeled ligand with known affinity for the target
- Competitive displacement by test compounds
- Measurement of bound *vs* free ligand by scintillation counting



Receptor Sources





S1R BINDING ASSAY

Assay Conditions

Tris Buffer (50 mM), pH= 8; Volume (0.5 mL);

Temp: 25 °C; Incubation time (120 min);

Non-specific binding: 10 µM unlabeled (+)-Pentazocine.

Materials

Radioligand: [^{3}H]-Pentazocine (2 nM; K_d = 2.9 nM);

Biological Material: Rat liver homogenates.

Detection Filtration

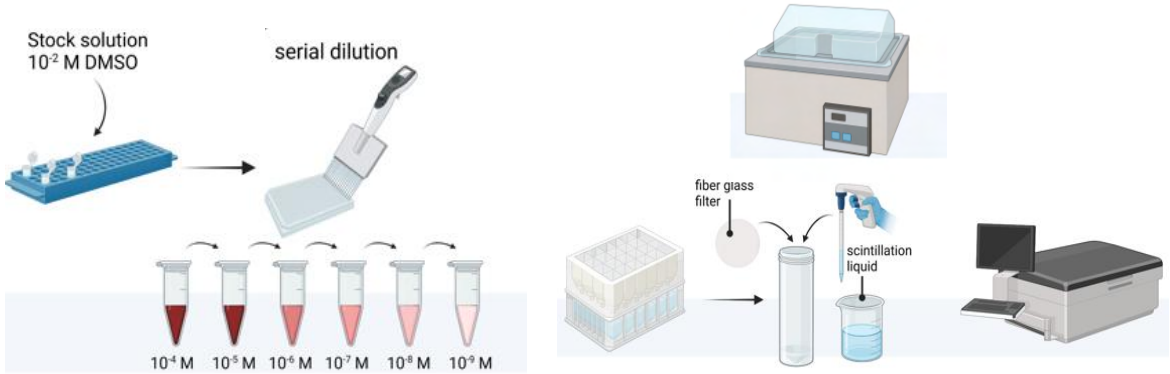
Whatman GF/6 filters, presoaked in 0.5% PEI;

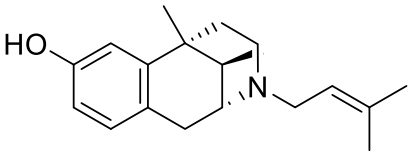
Washing: 3 × 3 mL Tris buffer (Scintillation cocktail: 3 mL Ultima Gold).

Counting and Data Analysis

MV Counting: Beckman LS 6500 scintillation counter;

Data analysis: nonlinear regression, Cheng-Prusoff equation.



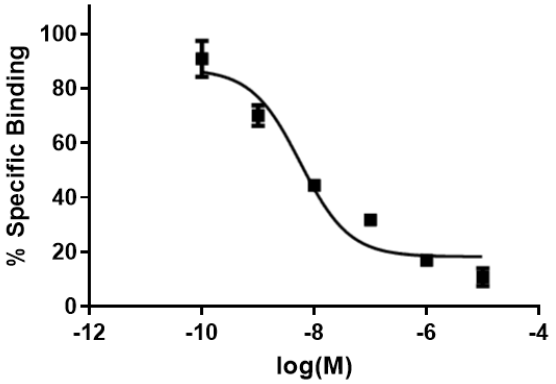


(+)-Pentazocine

K_i S_1R = 4.3 ± 0.5

K_i S_2R = 1465 ± 224

10 µM	7,58	14,00
1 µM	18,71	15,19
100 nM	33,86	29,60
10 nM	44,00	44,90
1 nM	73,70	66,21
0.1 nM	84,14	97,42



Cosentino et al., Eur J Med Chem, 2024, 281, 117037.

S2R BINDING ASSAY

Assay Conditions

Tris Buffer (50 mM), pH= 8; Volume (0.5 mL);

Temp: 25 °C; Incubation time (120 min);

Non-specific binding: 10 µM unlabeled DTG.

Materials

Radioligand: [^{3}H]-DTG (2 nM; K_d= 17.9 nM);

(+)-Pentazocine (5 µM) used to block S1R sites;

Biological Material: Rat liver homogenates.

Detection Filtration

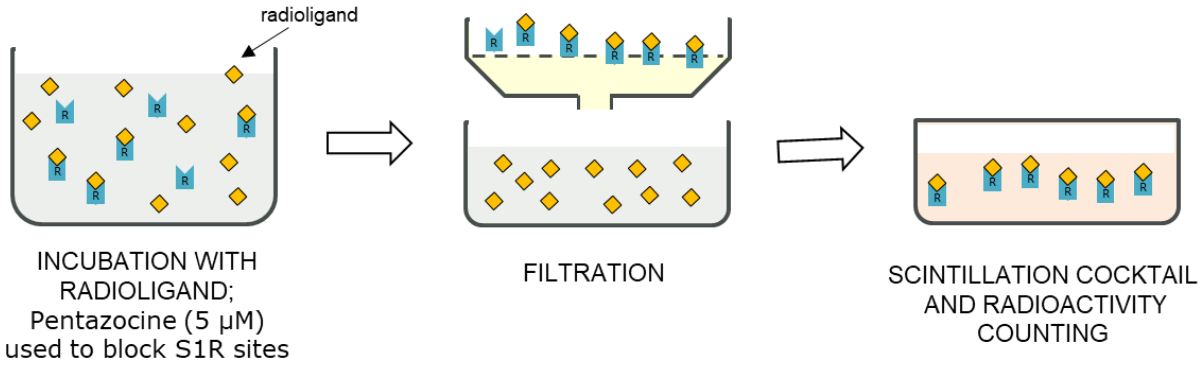
Whatman GF/6 filters, presoaked in 0.5% PEI;

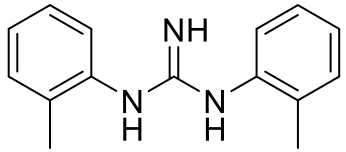
Washing: 3 × 3 mL Tris buffer (Scintillation cocktail: 3 mL Ultima Gold).

Counting and Data Analysis

MV Counting: Beckman LS 6500 scintillation counter;

Data analysis: nonlinear regression, Cheng-Prusoff equation.



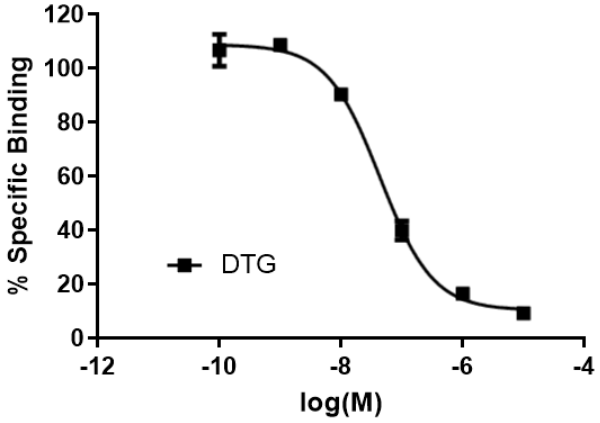


DTG

K_i S_1R = 124 ± 19

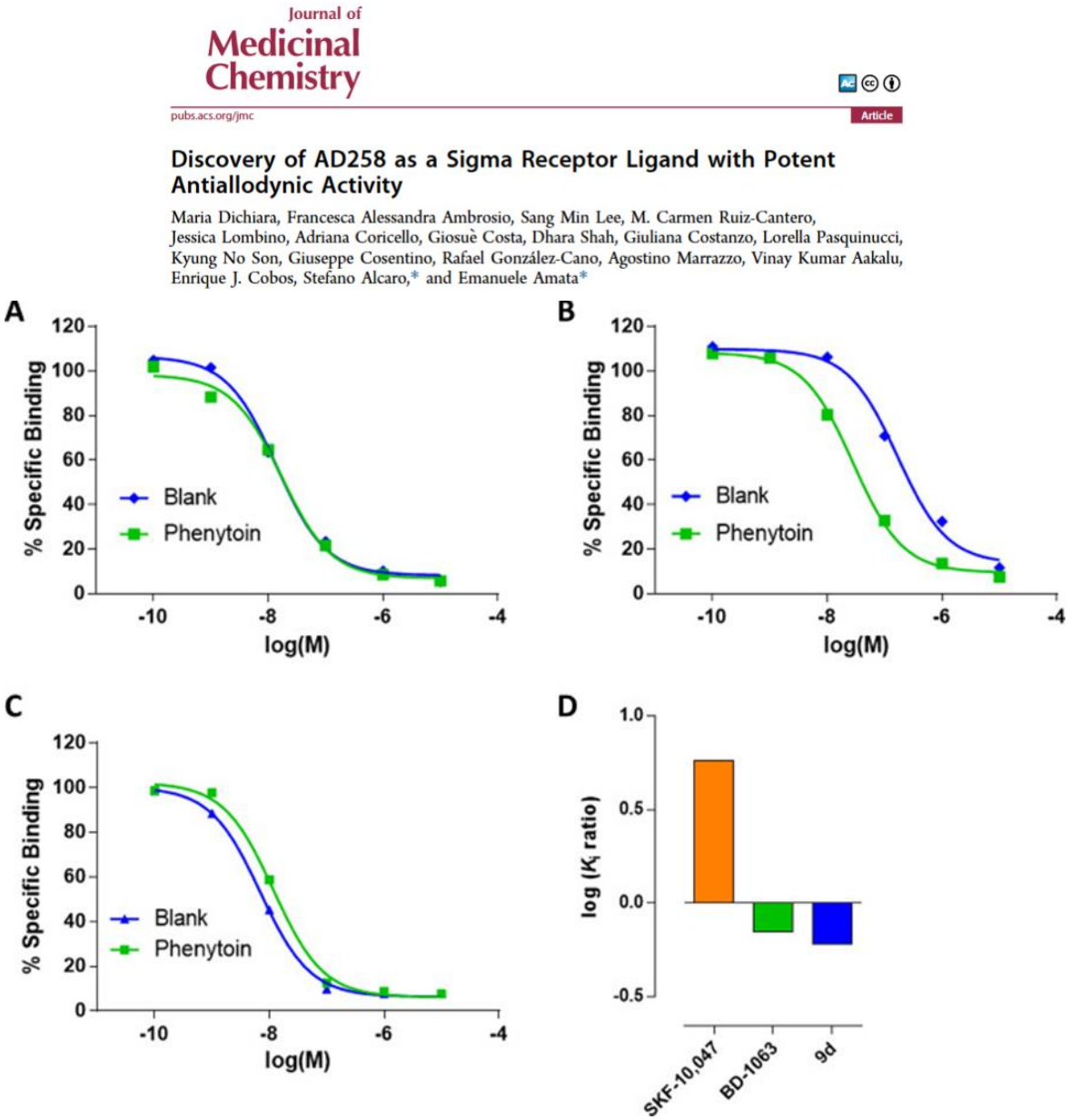
K_i S_2R = 18 ± 1

10 µM	8,27	10,14
1 µM	15,49	17,55
100 nM	43,50	36,44
10 nM	90,90	89,79
1 nM	108,35	108,95
0,1 nM	112,65	100,74



Cosentino et al., Eur J Med Chem, 2024, 281, 117037.

Functional Assay: Agonist or Antagonist Profile?



✓ **Objective:** Identify S1R agonists *vs* antagonists using phenytoin-induced binding shift.

✓ **Method:**

Liver homogenates incubated at 37 °C for 2 hours. Radioligand binding in the presence of phenytoin (1 mM) or vehicle (0.3 M NaOH).

Binding assay follows the same protocol as standard S1R binding.

✓ **Functional Classification:**

- **Agonist:** K_i ratio (without / with phenytoin) > 1;
- **Antagonist:** K_i ratio ≤ 1.

✓ **Data Analysis:**

K_i values calculated using GraphPad Prism 9.0.

Dichiara et al., J Med Chem, 2023, 66 (16), 11447−11463.

SIGMA-1EUROPE WG3
June 27, 2025

Biosensing Sigma-1 receptor signalling

Prof. Francisco Ciruela

fciruela@ub.edu

Grup de Neurofarmacologia i Dolor

Unitat de Farmacologia (Lab. 4104)

Departament de Patologia i Terapèutica Experimental

Facultat de Medicina i Ciències de la Salut

Universitat de Barcelona

Outline

1. **Introduction:** A collaborative project with Laboratorios Dr. Esteve

2. **First generation of Sigma-1 receptor biosensor:** FRET-based

3. **Second generation of Sigma-1 receptor biosensor:** Light-based

Introduction

A long collaboration with Laboratorios Esteve…..

5/2009-12/2020 **External Consultant and Collaborative Projects with**

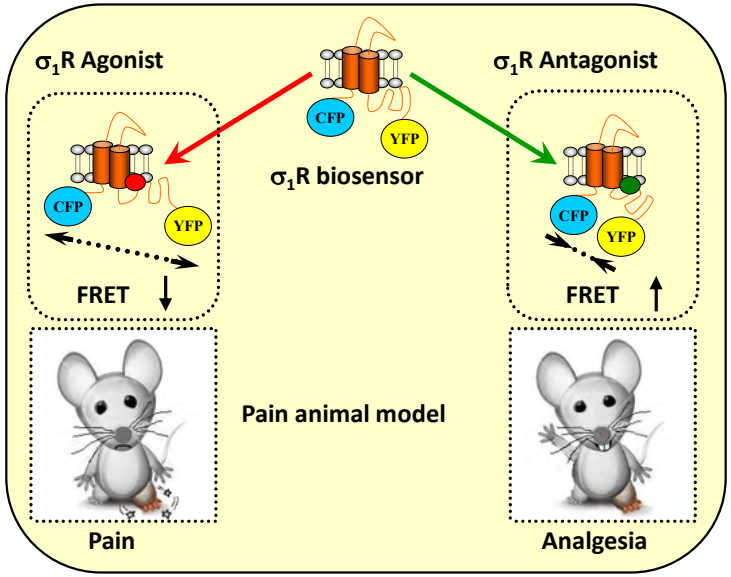
Laboratorios Dr. Esteve

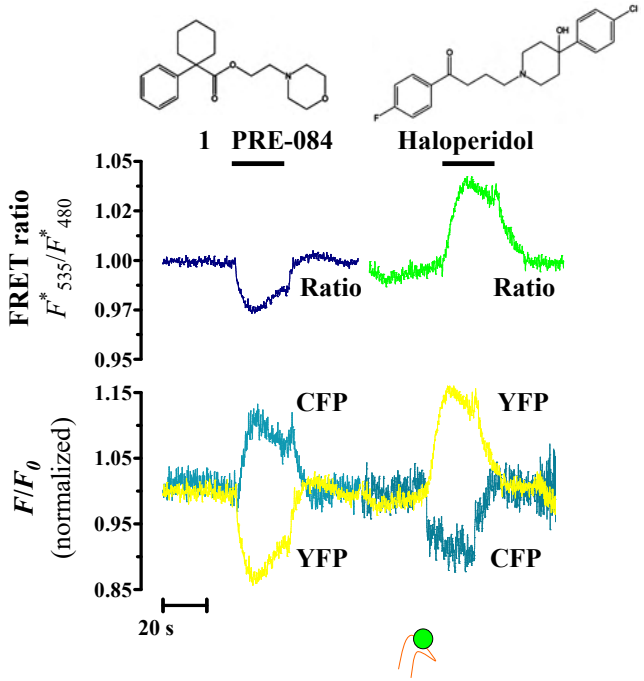
Av. Mare de Déu de Montserrat, 221
08041 Barcelona
Spain

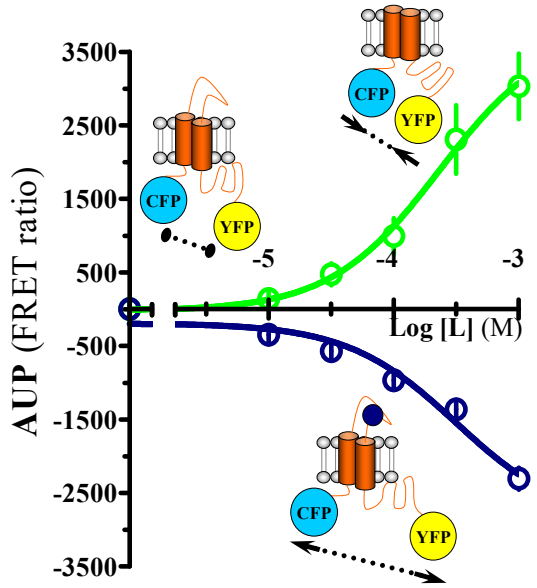
- ►Company/entity: Laboratorios del Dr. Esteve, S.A - FBG310613.
  Title: Refining sigma-1 receptor screening assays and sigma-2 protein-protein interaction discovery.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 1/1/2020-30/6/2020
- ►Company/entity: Laboratorios del Dr. Esteve, S.A - FBG310048.
  Title: Developing sigma-1 receptor screening assays and sigma-2 protein-protein interaction discovery.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 14/12/2018-30/12/2019
- ►Company/entity: Laboratorios del Dr. Esteve, S.A - FBG309628
  Title: Propelling sigma-1 receptor screening for ligands: biosensors and protein-protein interactions.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 14/12/2017-30/12/2018
- ►Company/entity: Laboratorios del Dr. Esteve, S.A
  Title: Caracteritzation of a of sigma-1 receptor biosensor based on the BRET technology.
  Duration: 14/9/2016-30/12/2016
- ►Company/entity: Laboratorios del Dr. Esteve, S.A
  Title: Caracteritzation of a of sigma-1 receptor biosensor based on the FRET technology, miniaturization and techno transfer.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 15/1/2011-31/7/2011
- ►Company/entity: Laboratorios del Dr. Esteve, S.A
  Title: Generation of a of sigma-1 receptor biosensor based on the FRET technology.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 12/05/2010-26/09/2010
- ►Company/entity: Laboratorios del Dr. Esteve, S.A
  Title: Detection by the membrane yeast two-hybrid (MYTH) system of with sigma-1 receptor interacting proteins.
  Principal investigator: Francisco Ciruela Alférez
  Duration: 15/3/2010-15/9/2010

The FRET-based biosensor

Monitoring Sigma-1 receptor intramolecular FRET in response to ligands







The FRET-based biosensor

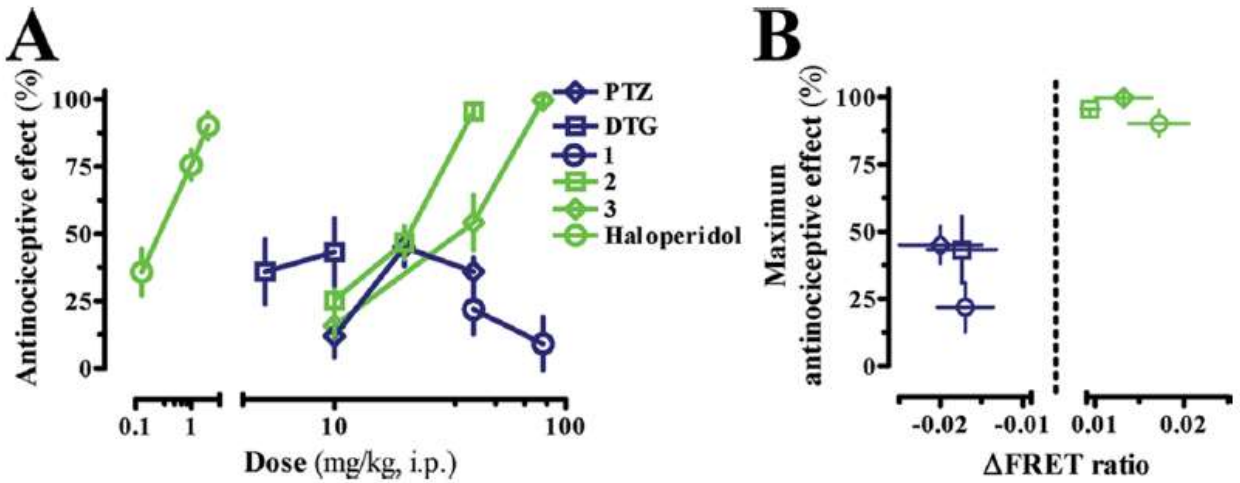


Figure 2. Antinociceptive effect of σ_1R ligands. (A) Dose–response relationship for the antinociceptive effect of several σ_1R ligands in the formalin test. Haloperidol, **1**, **2**, **3**, 1,3-di-*o*-tolylguanidine (DTG), or pentazocine was administered ip 15 min before the ipl injection of formalin, and the time spent licking or biting the injected paw was recorded. The antinociceptive effect in the phase II formalin test was calculated as the percentage of the maximum possible effect (mean ± SEM, $n = 8–10$). Note that the σ_1R antagonists (i.e., haloperidol, **1**, and **2**) inhibited the behavioral response to formalin in a dose-dependent manner and with a similar maximal efficacy (~100%). (B) Plot of the antinociceptive effect of the σ_1R ligands versus the changes that they induced in the normalized FRET ratio F^*_{535}/F^*_{480}. The ΔFRET ratio $(A - 1)$ induced by each single σ_1R ligand at 100 μM ($n = 5$) is represented vs the maximum antinociceptive effect achieved ($n = 8–12$) for the same ligand.

The FRET-based biosensor

Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

Predicting the Antinociceptive Efficacy of σ_1 Receptor Ligands by a Novel Receptor Fluorescence Resonance Energy Transfer (FRET) Based Biosensor

Maricel Gómez-Soler,[†] Víctor Fernández-Dueñas,[†] Enrique Portillo-Salido,[‡] Pilar Pérez,[‡] Daniel Zamanillo,[‡] José Miguel Vela,[‡] Javier Burgueño,[‡] and Francisco Ciruela*[,†]

[†]Unitat de Farmacologia, Departament Patologia i Terapèutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, 08907 Barcelona, Spain

[‡]Drug Discovery and Preclinical Development, ESTEVE, 08028 Barcelona, Spain

Supporting Information

ABSTRACT: We have developed a novel methodology for monitoring the σ_1 receptor activation switch in living cells. Our assay uncovered the intrinsic nature of σ_1 receptor ligands by recording the ligand-mediated conformational changes of this chaperone protein. The change triggered by each ligand correlated well with its ability to attenuate formalin induced nociception in an animal model of pain. This tool may assist in predicting the antinociceptive efficacy of σ_1 receptor ligands.

J. Med. Chem. 2014, 57, 238−242

► **"METHOD TO IDENTIFY LIGANDS FOR SIGMA-1 RECEPTORS"**

(Pub. No.: WO/2013/104648; International Application No.: PCT/EP2013/050265; Publication Date:18.07.2013; International Filing Date: 09.01.2013; Priority: EP/10.01.12/EPA12382003)

Inventors: Javier Burgueño-Hurtado, Francisco Ciruela-Alférez & José Miguel Vela-Hernández.

Applicant: Laboratorios del Dr. Esteve, S.A.

(http://patentscope.wipo.int/search/en/detail.jsf?docId=WO2013104648&recNum=1&office=&queryString=FP%3A%28ciruela%29&prevFilter=&sortOption=Pub+Date+Desc&maxRec=21)

The NanoBit-based biosensor

ACS Chemical Neuroscience

pubs.acs.org/chemneuro Research Article

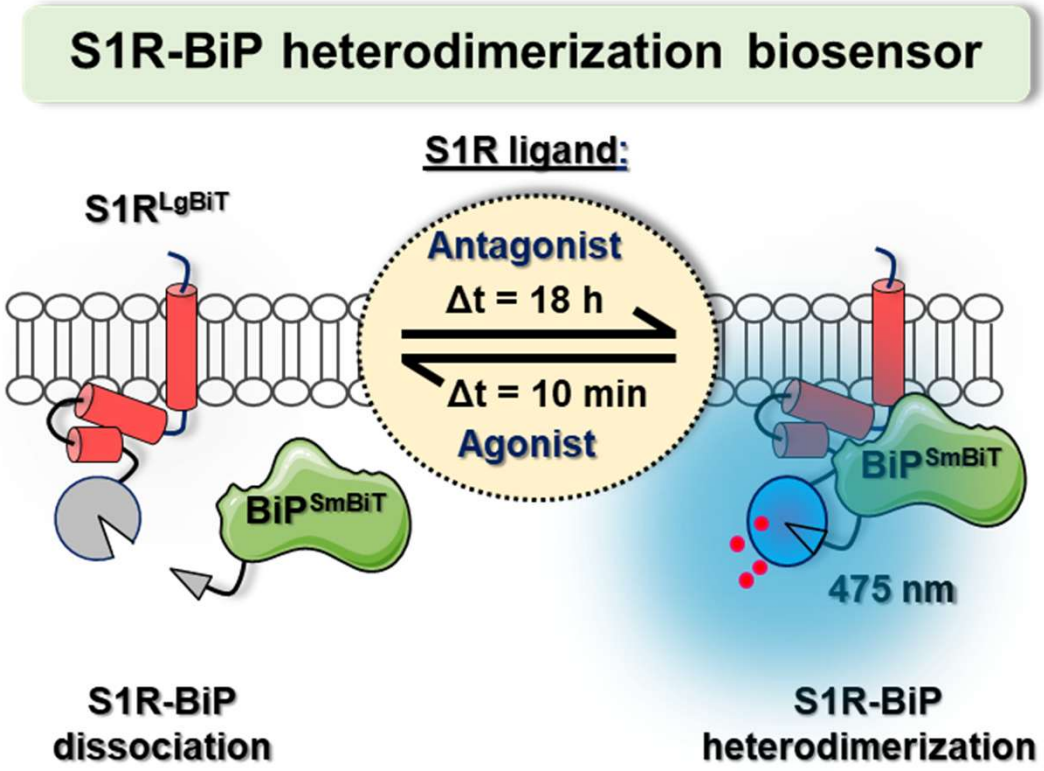
Development of a Novel σ_1 Receptor Biosensor Based on Its Heterodimerization with Binding Immunoglobulin Protein in Living Cells

Published as part of the ACS Chemical Neuroscience special issue "Monitoring Molecules in Neuroscience 2023".

Xavier Morató, Víctor Fernández-Dueñas, Pilar Pérez-Villamor, Marta Valle-León, José Miguel Vela, Manuel Merlos, Javier Burgueño,* and Francisco Ciruela*

ACS Chem. Neurosci. 2023, 14, 2201−2207

The NanoBit-based biosensor



The NanoBit-based biosensor

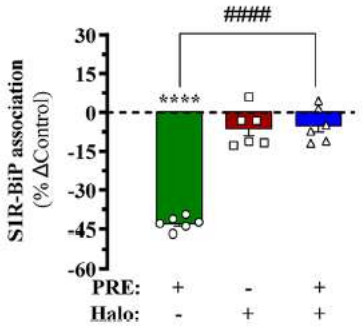
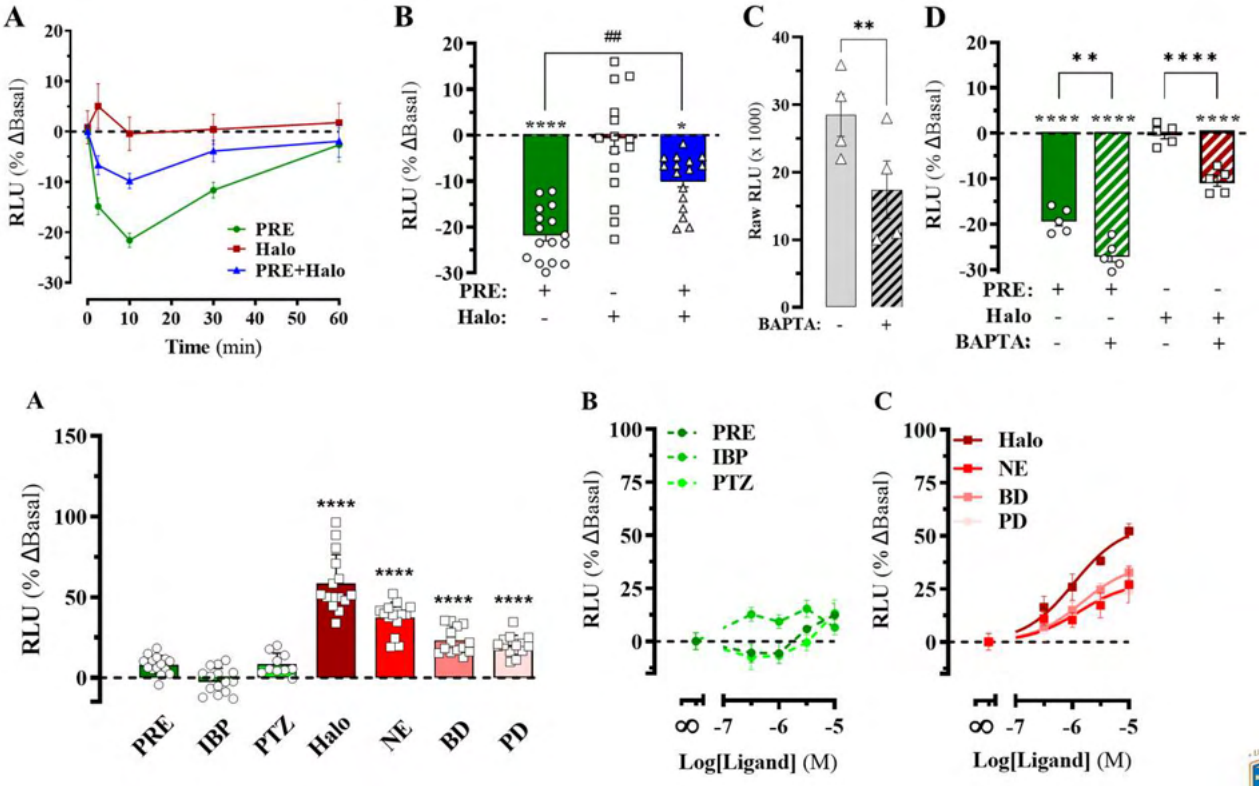


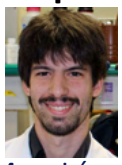
Figure 1. Effect of S1R ligands on receptor-BiP association in CHO cells. CHO cells were incubated with PRE-084 (10 μM, PRE) in the absence or presence of haloperidol (10 μM, Halo) for 30 min. The endogenous S1R and BiP were coimmunoprecipitated using a S1R antibody, and BiP levels were measured by ELISA. The results are represented as percentage of difference against CoIP-ELISA values determined in vehicle treated cells (% ΔBasal) and expressed as the mean ± SEM (n = 6): $^{****}p < 0.0001$ one-way ANOVA with Dunnett's post hoc test when compared to vehicle-treated cells (dashed line) and $^{\#\#\#\#}p < 0.0001$ with Tukey's post hoc test.

USCNK Life Sciences, Hubei, China, SEC343Mu



Paco's Team 2025

Neuropharmacology & Pain


Marc López


Paula Álvarez


Glòria Salort


Thiago Carnaval


Laura Sarasola


Marina Marsa

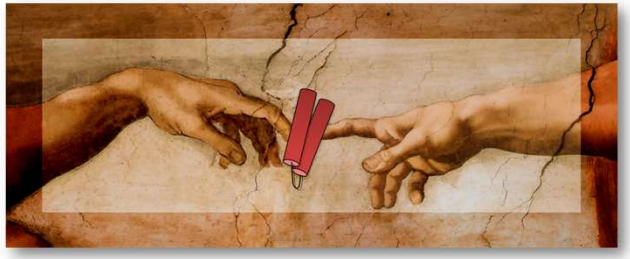
Collaborators

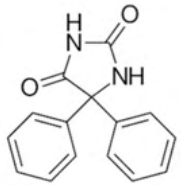
Ken Jacobson (*NIH, Bethesda, USA*)
Amadeu Llebaria (*CSIC, Barcelona, Spain*)
Dirk Trauner (*UPenn, Pennsylvania, USA*)
Jordi Hernando (*UAB, Barcelona, Spain*)
Sergi Ferré (*NIDA, NIH, Baltimore, Usa*)
Leonardo Pardo (*UAB, Barcelona, Spain*)
Rodrigo Cunha (University of *Coimbra, Coimbra, Portugal*)
Per Svenningsson (*KI, Stockholm, Sweden*)
Rafael Luján (*UCLM, Albacete, Spain*)
Ross Cheloha (*NIH, Bethesda, USA*)
Carla Tasca (*UFSC, Santa Catarina, Brasil*)
Kristoffer Sahlholm (University of Umeå, Umeå, Sweden)
Julie Le Merrer/Jérôme Becker (University of Tours, Nouzilly, France)
John A. Rogers (Northwestern University, Evanston, USA)
Janos Vörös (ETH, Zürich, Switzerland)

Sponsors

Proyecto PID2020-118511RB-I00 financiado por:

ibs.GRANADA
INSTITUTO DE INVESTIGACIÓN BIOSANITARIA





First paper on sigma receptors in 2005

Phenytoin differentially modulates the affinity of agonist and antagonist ligands for sigma -1 receptors of guinea pig brain. Cobos EJ, Baeyens JM, Del Pozo E. Synapse. 2005; 55(3):192-5. doi: 10.1002/syn.20103.

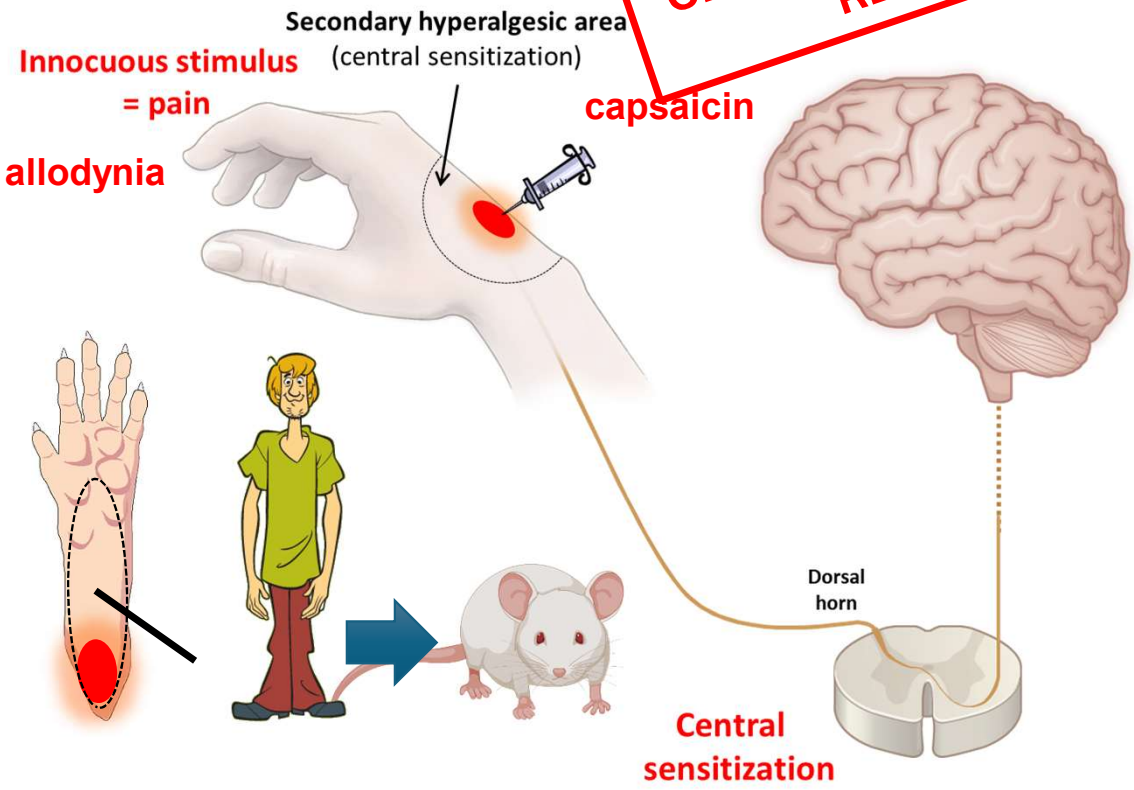
46 papers on sigma receptors

“FAST” METHODS FOR TESTING SIGMA-1 ANTAGONISTS

- **Capsaicin-induced allodynia (central sensitization)**

Predictive for effects in neuropath

CLEAR AGONIST-ANTAGONIST RELATIONSHIP



PAIN® 143 (2009) 252–261

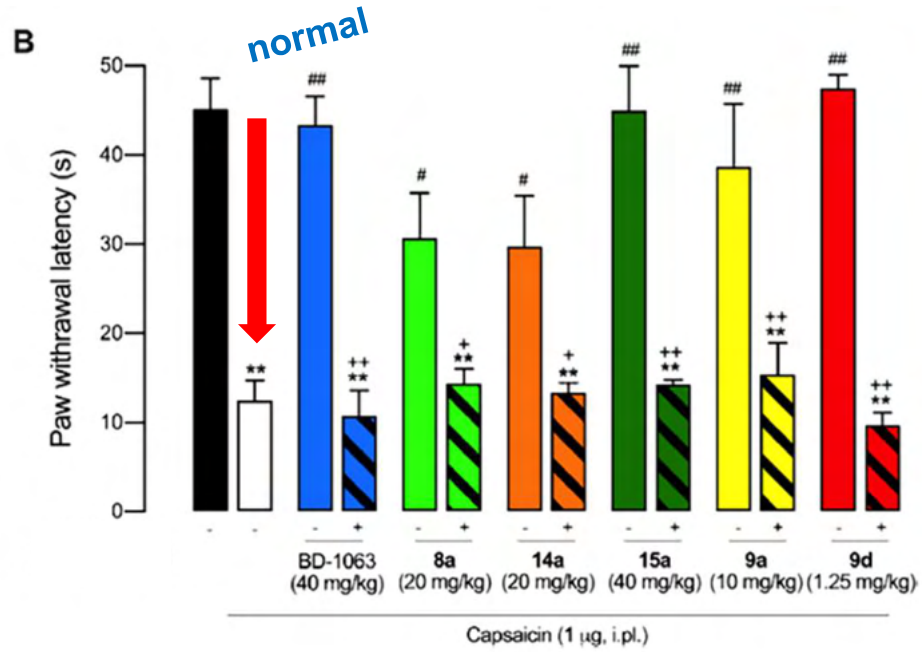
PAIN®

www.elsevier.com/locate/pain

ptors are essential for capsaicin-induced mechanical
ersensitivity: Studies with selective sigma-1 ligands and sigma-1 knockout mice

José Manuel Entrena [a,b,1], Enrique José Cobos [a,b,1], Francisco Rafael Nieto [a], Cruz Miguel Cendán [a], Georgia Gris [c], Esperanza Del Pozo [a], Daniel Zamanillo [c], José Manuel Baeyens [a,*]

[a] Department of Pharmacology and Institute of Neuroscience, Faculty of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain
[b] Biomedical Research Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, Armilla, 18100 Granada, Spain
[c] Laboratorios Dr. Esteve S.A., Avenida Virgen de Montserrat 221, 08041 Barcelona, Spain



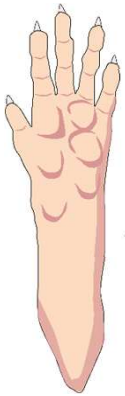
Dichiara et al., J Med Chem 2023

“FAST” METHODS FOR TESTING SIGMA-1 ANTAGONISTS

- **Capsaicin-induced allodynia (central sensitization)**
- **Potentiation of peripheral opioid antinociception**

CLEAR AGONIST-ANTAGONIST RELATIONSHIP

Tiempo de latencia forcejeo



Local effect - No metabolism (should be soluble in water)

Testing compounds (5 min pretest)

Loperamide (peripheral opioid agonist)

Contents lists available at SciVerse ScienceDirect

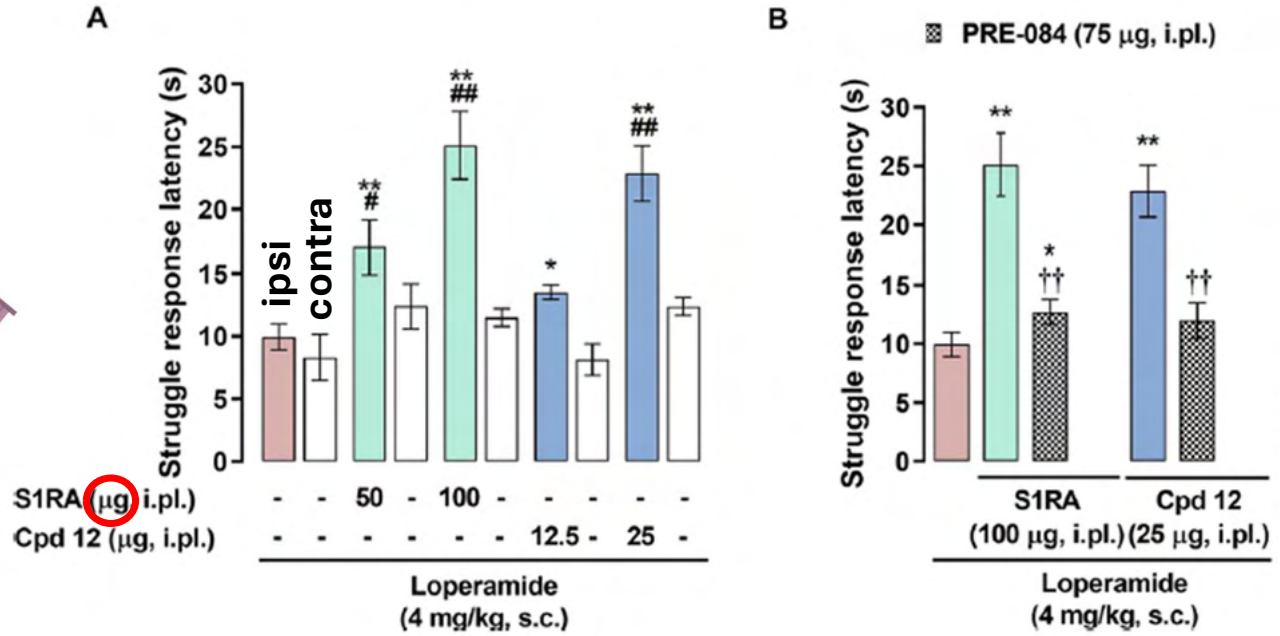
Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

of morphine-induced mechanical antinociception by σ_1 receptor ...ition: Role of peripheral σ_1 receptors

Cristina Sánchez-Fernández [a,b], Francisco Rafael Nieto [a,b], Rafael González-Cano [a,b], Antonia Artacho-Cordón [a], Lucía Romero [a], Ángeles Montilla-García [a], Daniel Zamanillo [d], José Manuel Baeyens [a,b], José Manuel Entrena [b,c], Enrique José Cobos [a,b,*]

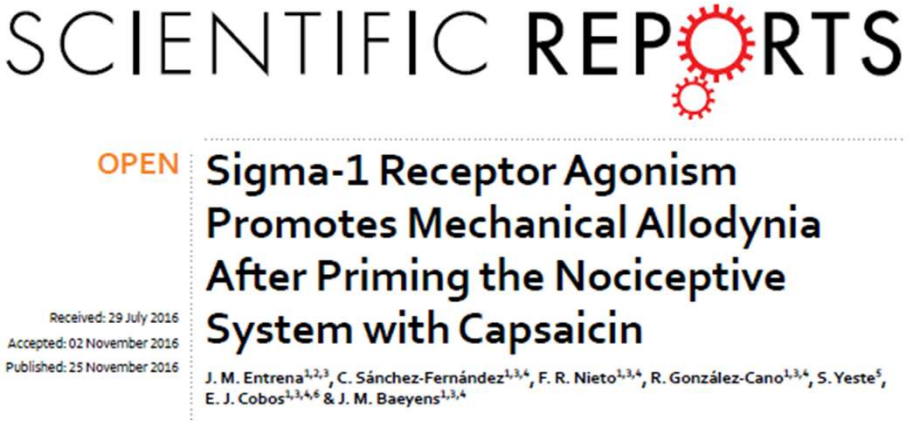
[a] Department of Pharmacology, School of Medicine, University of Granada, Avenida de Madrid 11, 18012 Granada, Spain
[b] Institute of Neuroscience, Biomedical Research Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain
[c] Animal Behavior Research Unit, Scientific Instrumentation Center, University of Granada, Parque Tecnológico de Ciencias de la Salud, 18100 Armilla, Granada, Spain
[d] Drug Discovery and Preclinical Development, Esteve, Avenida Mare de Déu de Montserrat 221, 08041 Barcelona, Spain



Szczepańska et al., J Med Chem 2023

“FAST” METHODS FOR TESTING SIGMA-1 AGONISTS

Reverse the effect of sigma-1 antagonists, and… are PRONOCICEPTIVE after priming of the nociceptive system (low inactive dose of capsaicin or PGE2 – or plantar incision)



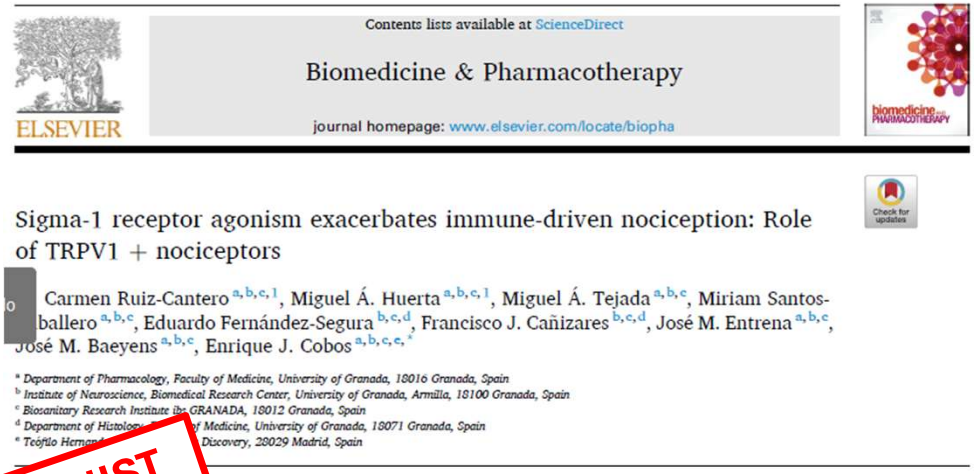
SCIENTIFIC REPORTS

OPEN

Sigma-1 Receptor Agonism Promotes Mechanical Allodynia After Priming the Nociceptive System with Capsaicin

Received: 29 July 2016
Accepted: 02 November 2016
Published: 25 November 2016

J. M. Entrena[1,2,3], C. Sánchez-Fernández[1,3,4], F. R. Nieto[1,3,4], R. González-Cano[1,3,4], S. Yeste[5], E. J. Cobos[1,3,4,6] & J. M. Baeyens[1,3,4]



Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Sigma-1 receptor agonism exacerbates immune-driven nociception: Role of TRPV1 + nociceptors

Carmen Ruiz-Cantero[a,b,c,1], Miguel Á. Huerta[a,b,c,1], Miguel Á. Tejada[a,b,c], Miriam Santos-Caballero[a,b,c], Eduardo Fernández-Segura[b,c,d], Francisco J. Cañizares[b,c,d], José M. Entrena[a,b,c], José M. Baeyens[a,b,c], Enrique J. Cobos[a,b,c,e,*]

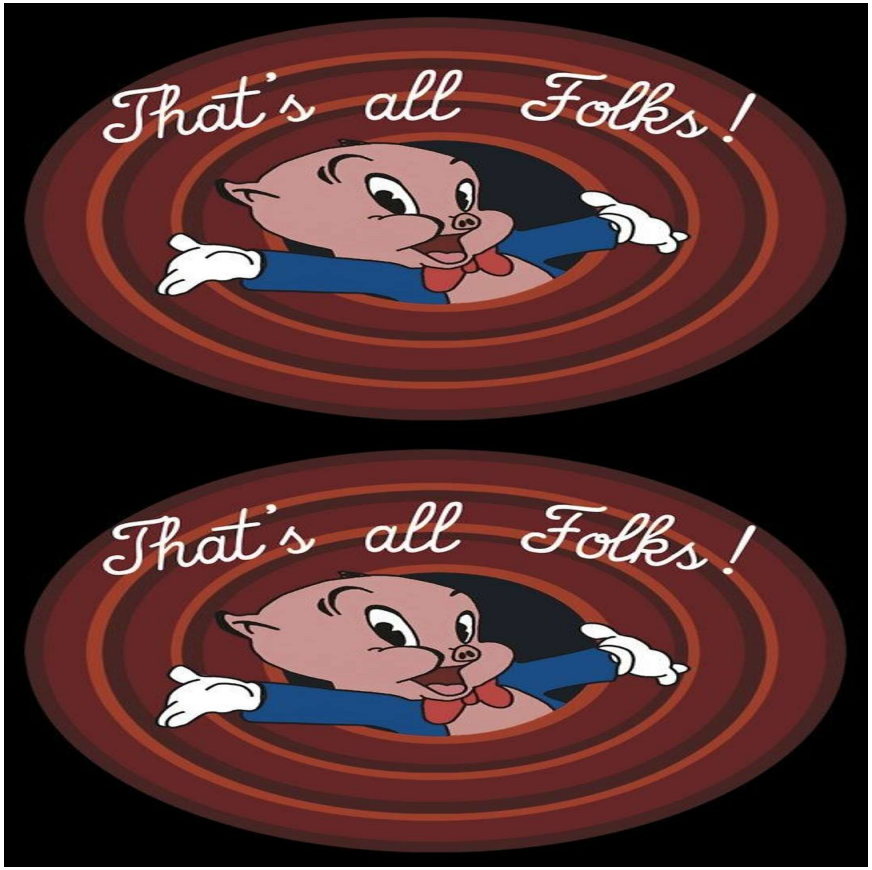
[a] Department of Pharmacology, Faculty of Medicine, University of Granada, 18016 Granada, Spain
[b] Institute of Neuroscience, Biomedical Research Center, University of Granada, Armilla, 18100 Granada, Spain
[c] Biosanitary Research Institute ibs.GRANADA, 18012 Granada, Spain
[d] Department of Histology [illegible] of Medicine, University of Granada, 18071 Granada, Spain
[e] Teófilo Hernando [illegible] Discovery, 28029 Madrid, Spain

CLEAR AGONIST-ANTAGONIST RELATIONSHIP

REPERTOIRE OF PAIN MODELS

MOUSE
Neuropathic pain (chemotherapy or mechanical injury – SNI)
Inflammatory pain (carrageenan, CFA)
Visceral pain (i.cl. algogens or cystitis by cyclophosphamide)
Postoperative pain

RAT
Rheumatoid arthritis pain



Innopharma
Drug Discovery

Xavier Codony, PhD

xavier.codony@usc.es
xavier.codony@kaertorfoundation.org

1991-2020

Welab BARCELONA

2020 - 2024

Kaertor Foundation

July 2024 →

CiMUS
Center for Research in Molecular Medicine and Chronic Diseases

February 2025 →

E-5842; E-52862/S1RA; E-73502…

Langa *et al.*, 2003

Phenotyping of Sigma-1 receptor knock-out rodents

PhD tesis, 2022

Innopharma
Drug Discovery

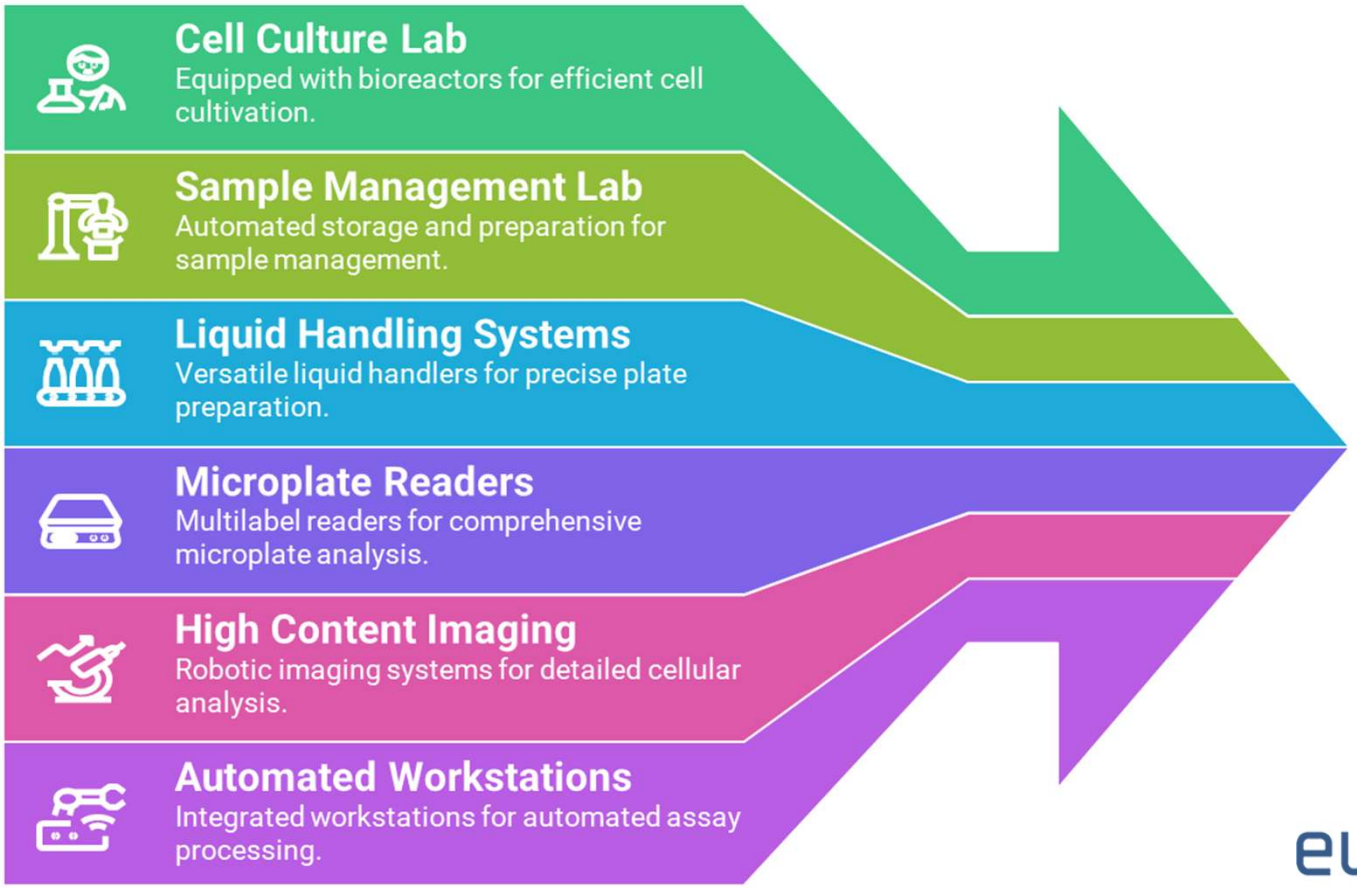
USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

CiMUS
Center for Research in **Molecular Medicine** and **Chronic Diseases**

Innopharma
Drug Discovery

Innopharma
Drug Discovery

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



Comprehensive Screening Capabilities

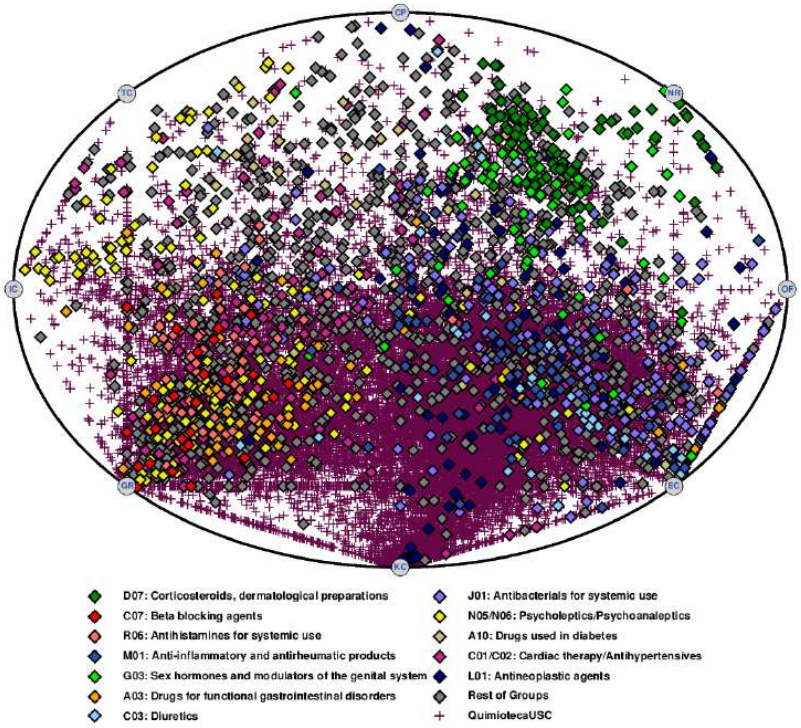
in vitro high-capacity sites of

eu::openscreen

Innopharma
Drug Discovery

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

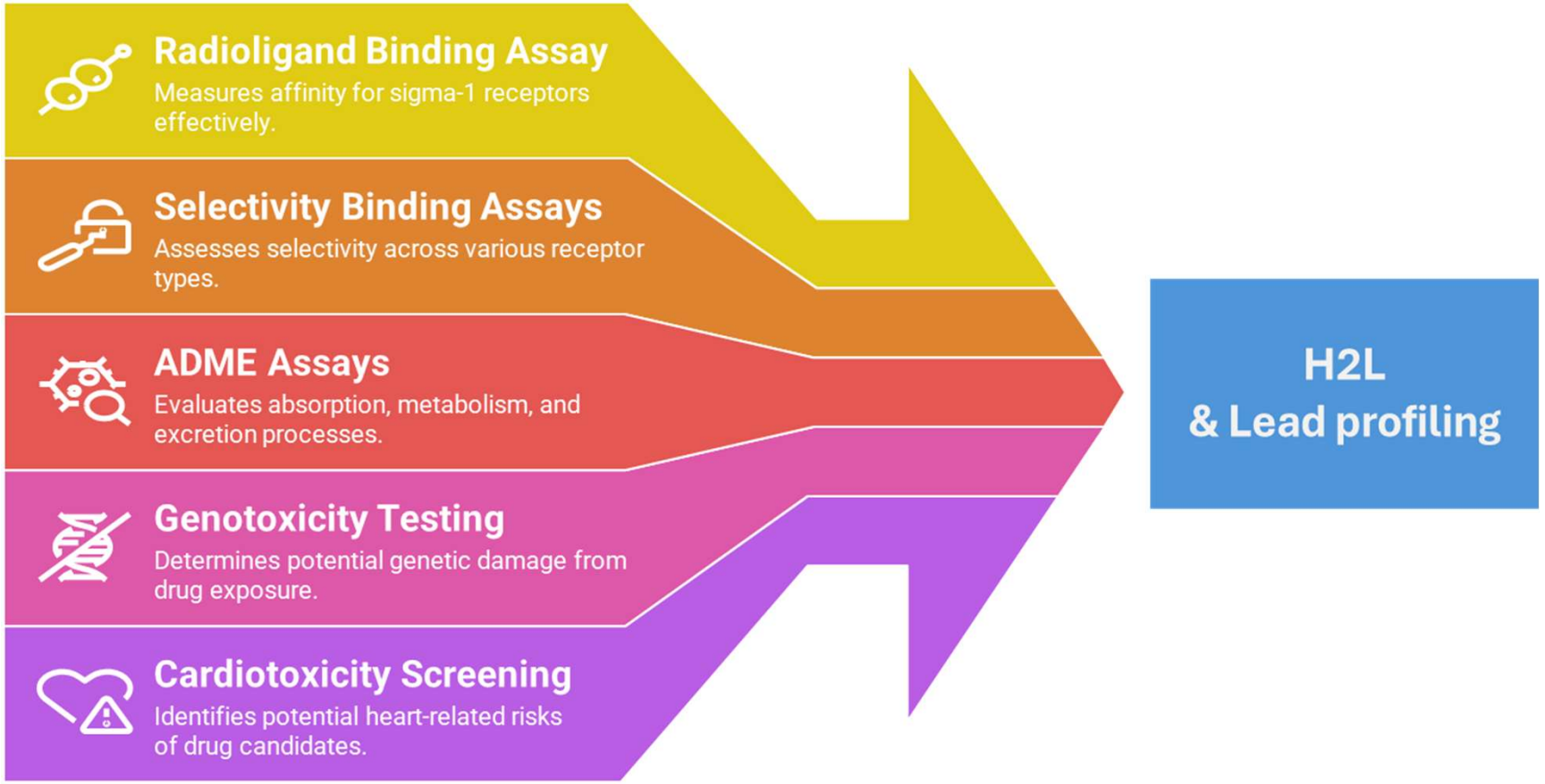
Chemical library of highly annotated compounds (135000) with high chemical and biological diversity. Enriched in sigma ligands coming from the former Esteve and Welab libraries.



HIT finding

Innopharma
Drug Discovery

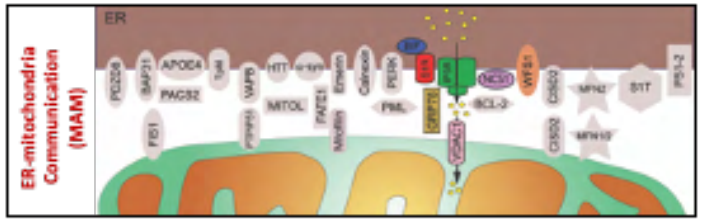
SIGMA-1 EUROPE
European research network on sigma-1 receptors CA23156



The team project

Pathologies

- Alzheimer's Disease
- Tauopathies
- Amyotrophic lateral sclerosis
- Huntington's disease
- Wolfram syndrome
- Spastic paraplegia 7



Pharmacological and transgenic models


Drosophila


Zebrafish


Mice

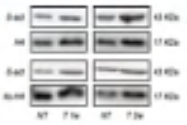

Patient fibroblasts, LCR, blood

Technological approaches

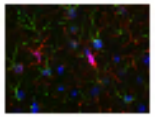

Behavioral phenotyping (zebrafish)

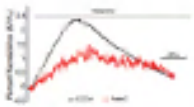

Behavioral tests (mice)


Metabolics


Biochemistry


Molecular biology


Histology & IHC

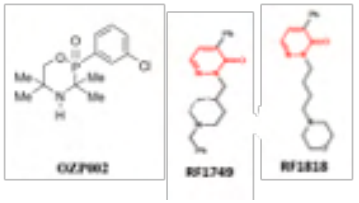

Ca2+ imaging *Coll. P. Pinton*


Electrophysiology


Human genetics

Library repositioning

New drug discovery (medicinal chemistry)



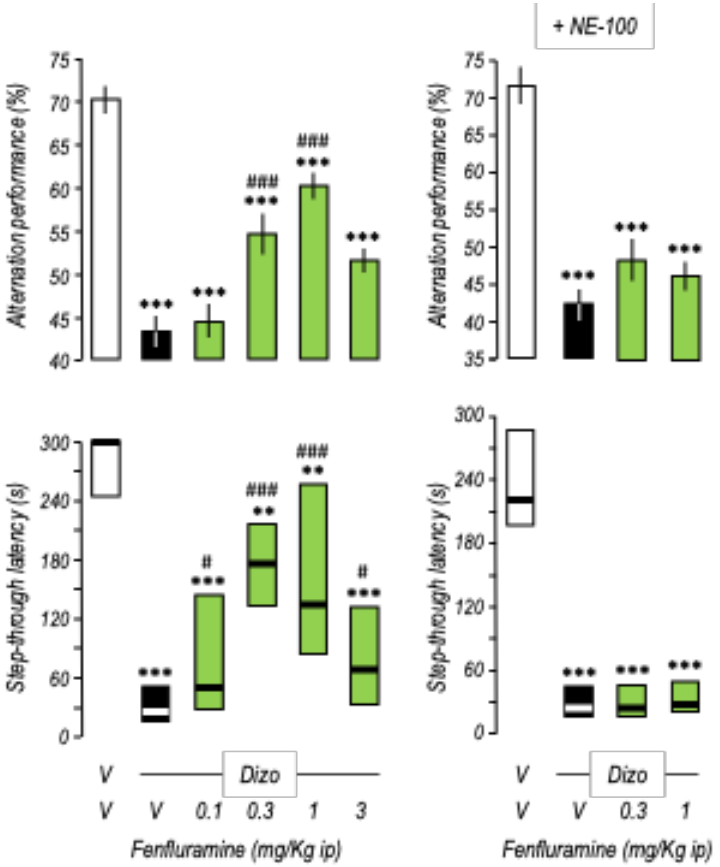
Addressing S1R pharmacology

In vivo

Agonist (PAM) induced prevention of (+)MK-801-induced amnesia

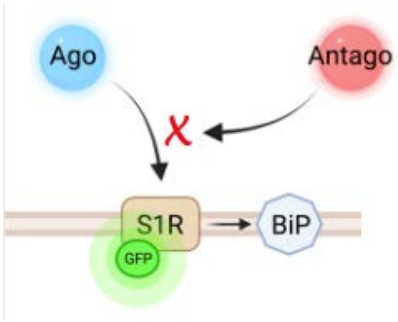
Spontaneous alternation

Passive avoidance

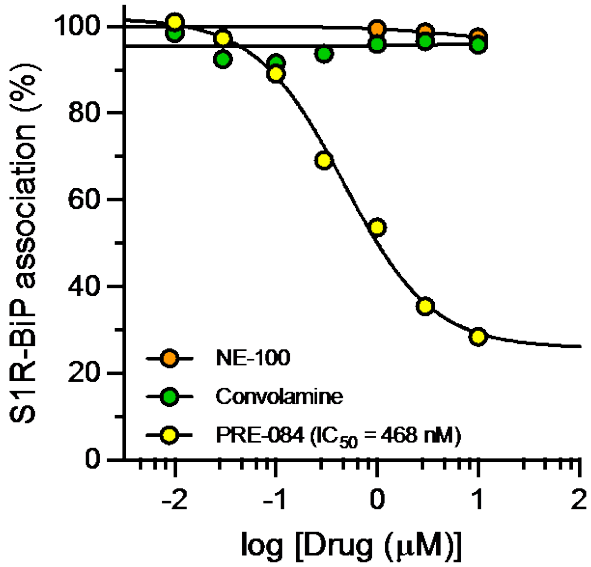


in vitro

Agonist (PAM) induced dissociation of BiP/S1R complex



In S1Roe CHO cells (provided by Drs Y. Yasui & TP Su)



UNIVERSITY OF BELGRADE
INSTITUTE OF PHYSICS | BELGRADE
NATIONAL INSTITUTE OF THE REPUBLIC OF SERBIA

Identification of the most appropriate in vitro and in vivo methods to test Sigma-1 Receptor (S1R) ligands

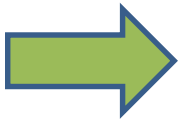
Biomimetics Laboratory,
Institute of Physics Belgrade, National Institute of the Republic of Serbia

1. Darija Obradović Jovčić (PhD, pharmacist)
2. Saša Lazović (PhD, physicist)
3. Vladimir Vlatković (PhD student, biologist)

Biomimetic approach

Biomimetic simulations

Imitation of the key molecular interactions that govern behaviour of molecules in the experimental system, modeled by analogy to fundamental mechanisms found in biological environments.



Biomimetic properties

Clinical outomes

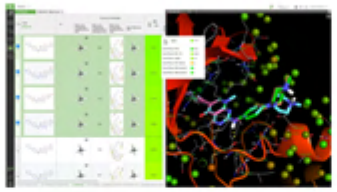
1. physico-chemical characteristics
2. pharmacokinetic data
3. ecotoxicological data
4. molecular mechsnisms

Methodologies

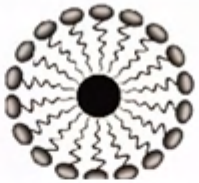
Biomimetic
chromatography

+

Biomimetic artificial
immobilized membranes

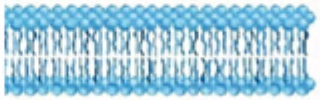


AI



hydrophobicity

plasma protein binding



cell membrane
transport

Clinical outcomes

(blood brain barrier permeability, GIT characterization (site of absorption, duration of absorption, permeability-flux etc.)

Vildagliptin: insight into SIGMA-1 receptor profile

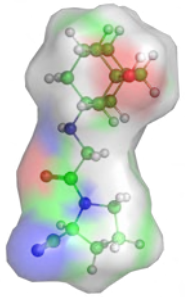


Fig.1. The chemical structure of vildagliptin

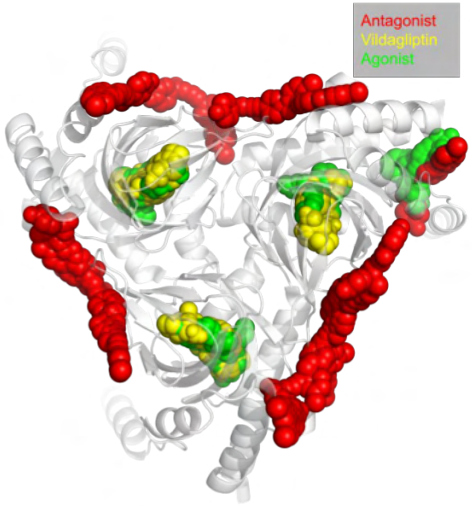


Fig.2. Molecular docking analysis reveals that vildagliptin is competing for the dominant agonist binding site.

Stefan Milenković, Physics Department,
University of Cagliari, Italy

Main Application Areas

- Biomarker discovery
- Target engagement studies
- In vivo and in vitro toxicology

Research focus:

- Neuroinflammation
- In vivo models of neurodegenerative and other brain-related disorders
- Nanoparticles and drugs biodistribution

Marco Peviani (marco.peviani@unipv.it)
Lab contact: cmnlab@unipv.it
Website: https://pevianilab.unipv.it/

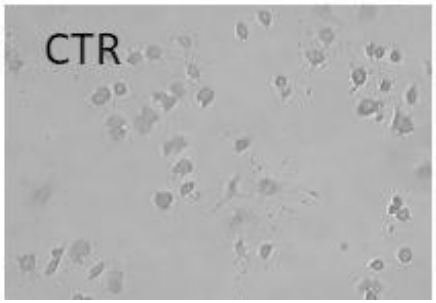
CMN LAB
Cellular and Molecular Neuropharmacology Lab

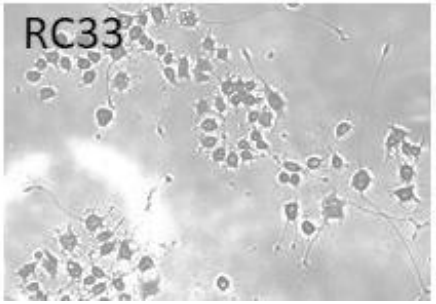
Investigation of the pharmacological profile of S1R-ligands

Collina's PAVIA MED CHEM lab
Medicinal Chemistry laboratorium
University of Pavia, Department of Drug Sciences

NGF-induced neurite outgrowth assay on PC12 cells

- Identify S1R agonists (potency and efficacy)
- Study neuroplasticity induced by S1R ligands





Bioorganic & Medicinal Chemistry 19 (2011) 6210–6224

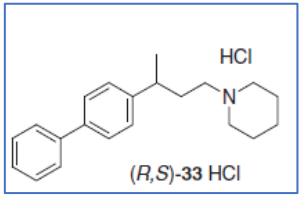
Contents lists available at SciVerse ScienceDirect

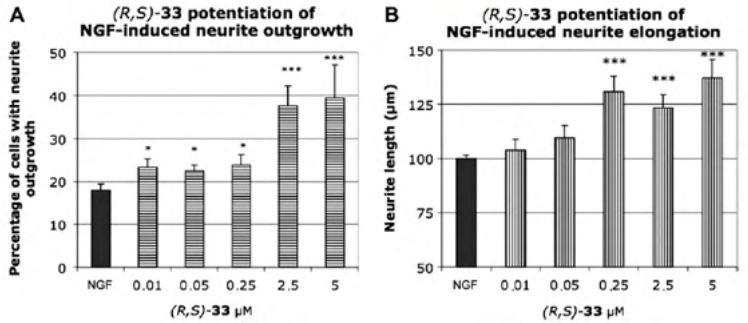
Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Identification of a potent and selective σ_1 receptor agonist potentiating NGF-induced neurite outgrowth in PC12 cells

Daniela Rossi [a], Alice Pedrali [a], Mariangela Urbano [a], Raffaella Gaggeri [a], Massimo Serra [a], Leyden Fernández [b], Michael Fernández [c], Julio Caballero [d], Simone Ronsisvalle [e], Orazio Prezzavento [e], Dirk Schepmann [f], Bernhard Wuensch [f], Marco Peviani [g], Daniela Curti [g], Ornella Azzolina [a], Simona Collina [a,*]





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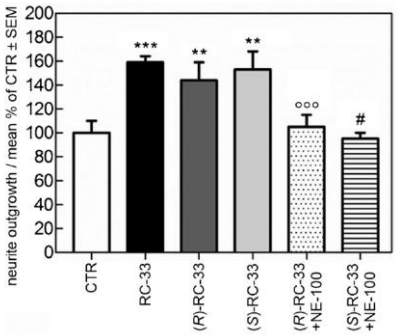
FULL PAPERS
www.chemmedchem.org

DOI: 10.1002/cmdc.201300218

Chemical, Pharmacological, and in vitro Metabolic Stability Studies on Enantiomerically Pure RC-33 Compounds: Promising Neuroprotective Agents Acting as σ_1 Receptor Agonists

Daniela Rossi,[a] Alice Pedrali,[a] Raffaella Gaggeri,[a] Annamaria Marra,[a] Luca Pignataro,[b] Erik Laurini,[c] Valentina Dal Col,[c] Maurizio Fermeglia,[c] Sabrina Pricl,*[c, d] Dirk Schepmann,[e] Bernhard Wünsch,[e] Marco Peviani,[f] Daniela Curti,[f] and Simona Collina*[a]



Contents lists available at ScienceDirect

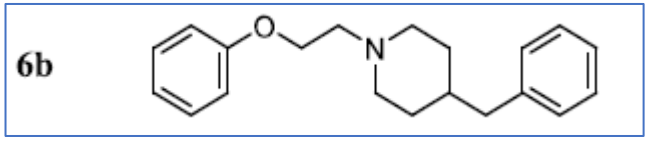
European Journal of Medicinal Chemistry

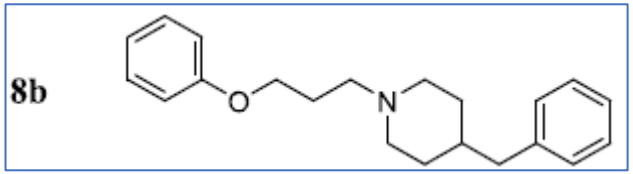
journal homepage: www.elsevier.com/locate/ejmech

Research paper

Novel S1R agonists counteracting NMDA excitotoxicity and oxidative stress: A step forward in the discovery of neuroprotective agents

Pasquale Linciano [a], Claudia Sorbi [b], Giacomo Rossino [a], Daniela Rossi [a], Andrea Marsala [c], Nunzio Denora [d], Martina Bedeschi [e], Noemi Marino [e], Giacomo Miserocchi [e], Giulio Dondio [f], Marco Peviani [c], Anna Tesei [e], Simona Collina [a], Silvia Franchini [b,*]





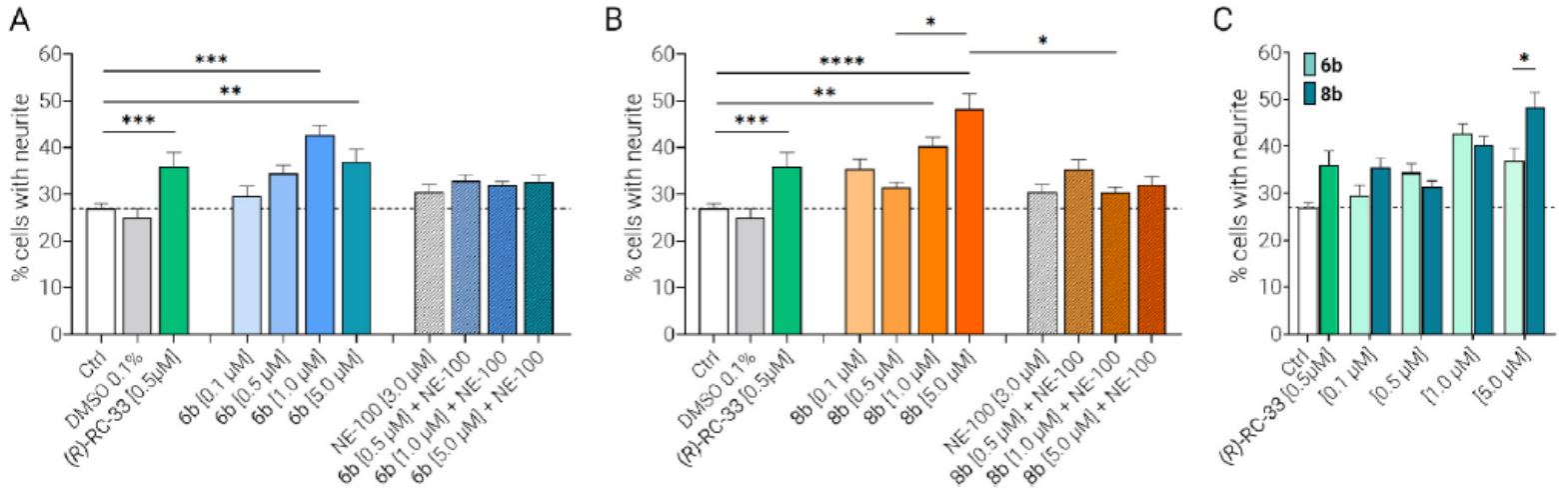
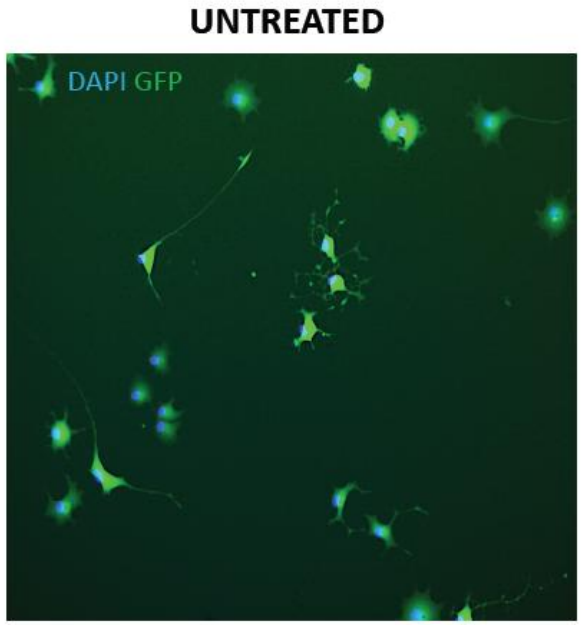
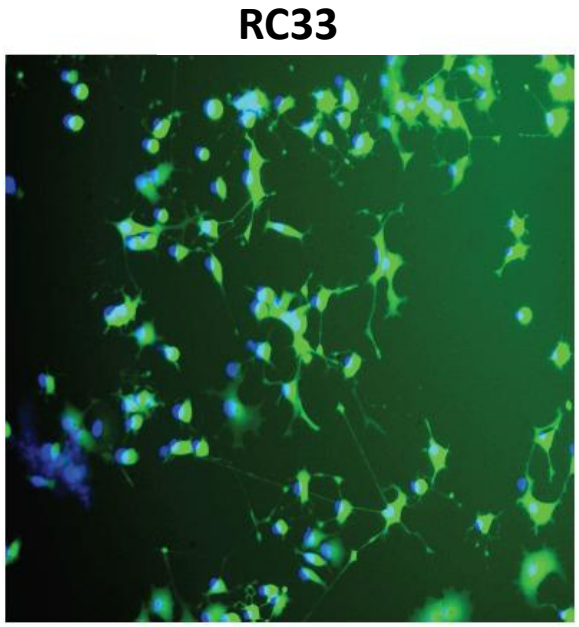


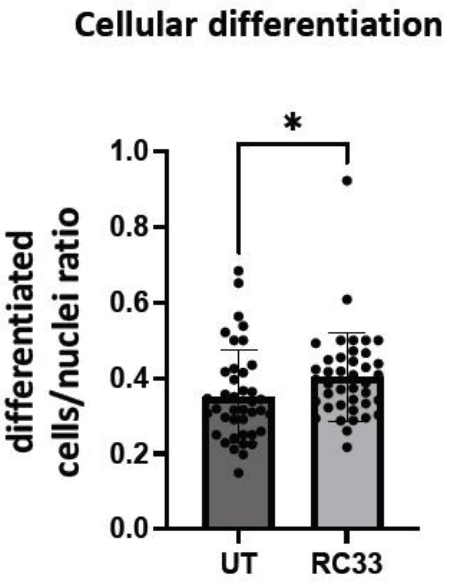
Fig. 6. Quantification of the neurite outgrowth in PC12 cells exposed to NGF and treated with compounds **6b** (A) and **8b** (B) alone or pre-treated with the S1R antagonist NE-100 (at 3 μM). The neurite outgrowth induced by *(R)*-RC-33, used as reference compound, has been included for comparison. (C) Comparison of the neurite outgrowth after treatment with compound **6b** and **8b**. Each bar represents the percentage of cells with neurite sprout. The results are expressed as mean ± SEM of three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ determined by Kruskal Wallis followed by Dunn's post-hoc test.

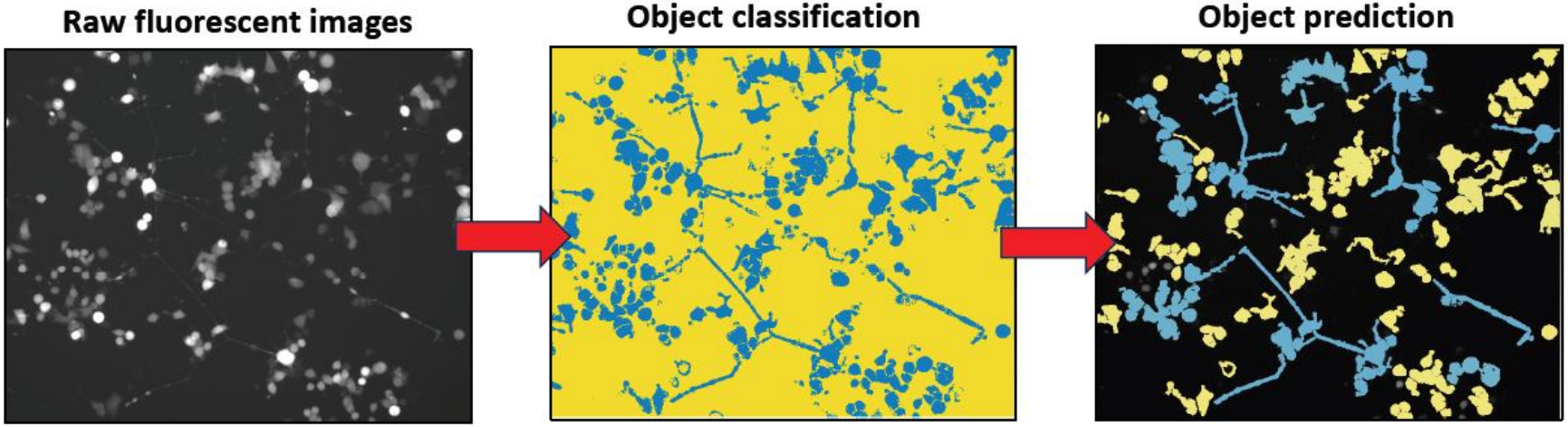
ilastik

- machine learning -
Towards an unbiased assessment of neurite outgrowth



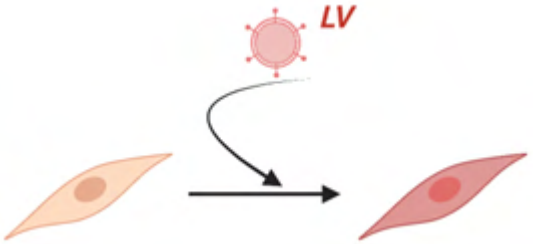






Gazzano et al., unpublished

Manipulation of target gene expression through lentiviral vectors to aid functional validation of novel ligands



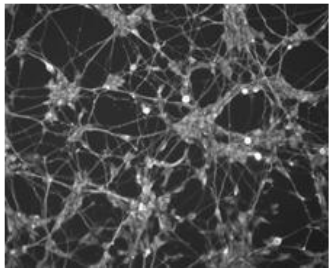
Stable cell lines

S1R-knock-down microglia and glioma cell lines
S2R-knock-down glioma cell lines
TSPO-GFP tagged microglia cell lines
TSPO-knock-down microglia cell lines

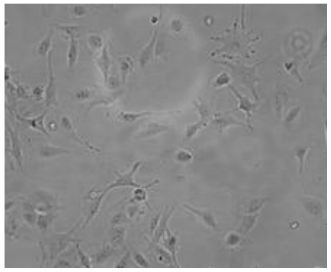
Human FLAG-tagged S1R constructs available:

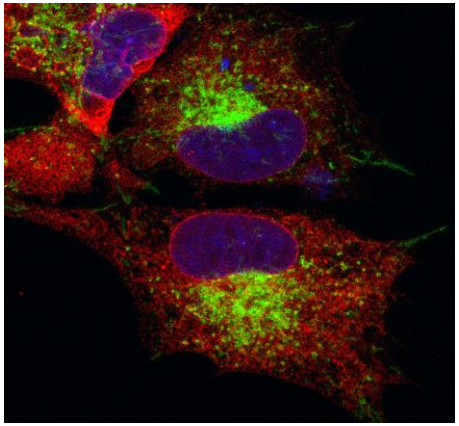
SIGMAR1 WT
SIGMAR1 E102Q (linked to juvenile ALS)

Differentiated SHSY-5Y (neuronal model)

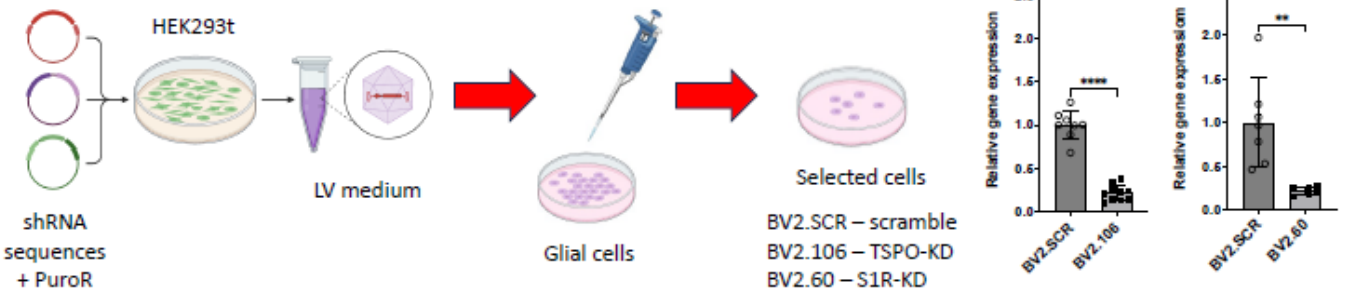


BV2 and HMC3 (microglia model)





Nuclei hTSPO S1R



Gazzano et al., unpublished

Institute of Chemical Biology, ISU:

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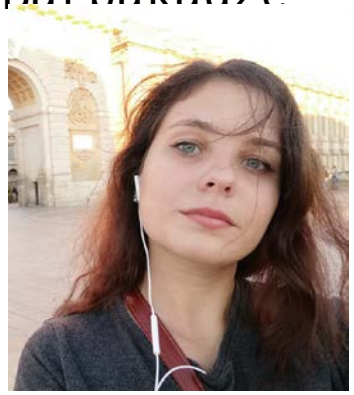
Dr. Tamar Barbakadze

Dr. Lali Shanshiashvili

Dr. Nana Narmania

Dr. Maia Sepashvili

Dr. Elene Zhuravliova



PhD student Natalia Kiknadze

PhD student Natia

PhD student Marika

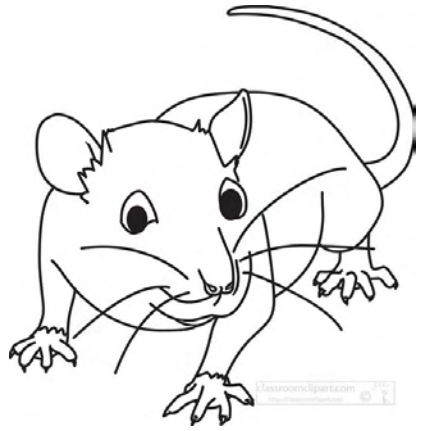
PhD student Nino

Our love story with Sigma-1

- Natsvlishvili, N.I., Abutidze, K.D. & Mikeladze, D.G. **Dextrorphan-binding proteins in the hippocampus of audiosensitized rats genetically predisposed to epilepsy.** Bull Exp Biol Med (1997) 124: 770.
- E. Zhuravliova, T. Barbakadze, N. Natsvlishvili, D.G. Mikeladze. **Haloperidol induces neurotoxicity by the NMDA receptor downstream signaling pathway, alternative from glutamate excitotoxicity,** Neurochem Int (2007), 50: 976-982.
- Natsvlishvili N, Goguadze N, Zhuravliova E, Mikeladze D. **Sigma-1 receptor directly interacts with Rac1-GTPase in the brain mitochondria.** *BMC Biochem*. (2015)16:11.
- Koriauli S, Natsvlishvili N, Barbakadze T, Mikeladze D. **Knockdown of interleukin-10 induces the redistribution of sigma1-receptor and increases the glutamate-dependent NADPH-oxidase activity in mouse brain neurons**. *Biol Res*. (2015) 48:55.
- Natsvlishvili. N. Shanshiashvili L, Mikeladze, D **Sigma-receptor-1 and mGluR5 may participate in Rac-dependent oncogenesis through modulation of macrophage activity.** Biopolymers & Cell (2015) Suppl. 5, p18-18.
- Goguadze, N., Zhuravliova, E., Morin, D. et al. **Sigma-1 Receptor Agonists Induce Oxidative Stress in Mitochondria and Enhance Complex I Activity in Physiological Condition but Protect Against Pathological Oxidative Stress.** Neurotox Res (2019) 35: 1

Animal models – rats

(mostly hypocampus, NAc, cortex, hypothalamus)



- Myelin basic peptides and multiple sclerosis
- P-cresol and depression
- Prenatal exposure to DEHP and neurodevelopment disorders
- Hypoxia, preconditioning
- Hypothyroidism in CNS
- Audiogenic epilepsy-prone rats
- Sleep deprivation
- STZ-induced diabetes (in past)

Cell cultures

- PC12 cells
- Raw macrophages
- Jurkat cells
- AML cells from patients

Compounds

- Sigma-1 agonists
- P-cresol and other gut microbiome metabolites
- Flavonoids and other plant-derived compounds
- Myelin basic proteins
- Phthalates and other environmental pollutants

Plasma membrane

- Receptor phenotypes and subunit composition (NMDA, DRs, mGluR, Sigma-1, GABA, MOR)
- DAT/PICK1 system and other transporters
- Interactome in heteroreceptor complex
- Synaptic vs Extrasynaptic systems

Intracellular pathways

- Small GTP-ases (Ras, Rac and others)
- ERK, Akt, mTOR kinases
- Tyrosine kinases
- CAMKII/calcineurin

Cytoskeleton in CNS cells

G/F actin upstream signaling

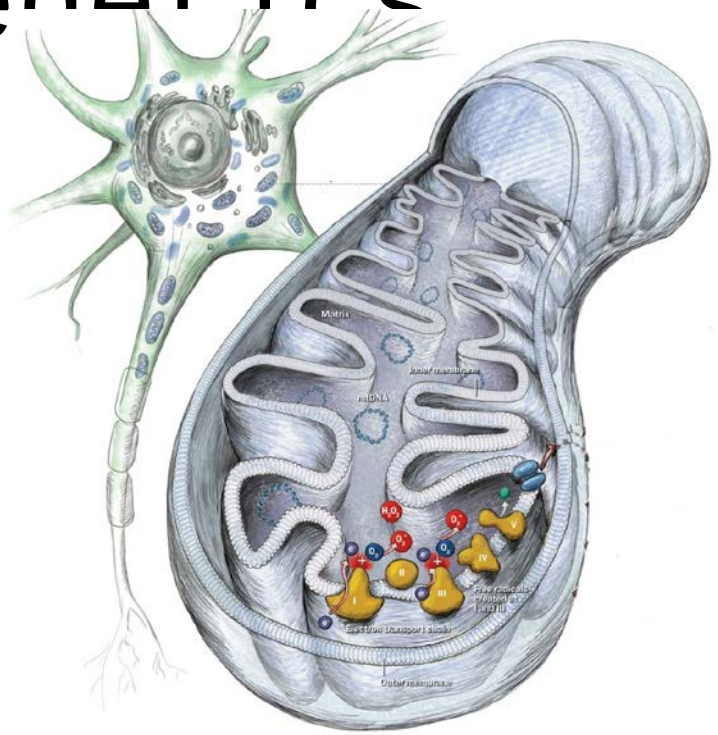
Mitochondria and Bioenergetics

Bioenergetics (OCR, ATP Production, Membrane Potential, ROS production, Ca2+ influx)

MDH, Hexokinase activity,

Fusion, fission and balance

Protein in MAM (IP3, BiP)



Post-translation modifications

- Nitrosylation
- Farnesylation
- Palmitoylation
- Methylation (CARM)
- Deamination/citrulination

Cycles/Shuttles

- Methyl cycle
- Glutamate

Neuroinflammation

- M1/M2 transition
- HMGB1 pathway

Transcriptional activity

- CREB
- FOS
- cMyc
- ATF4

Local Collaboration

- Sleep-wake cycle Research Lab
- Institute of Medicine and Public Health
- Tbilisi Heart and Cardiovascular Hospital
- I. Beritashvili Center of Experimental Biomedicine
- Georgian Association of Endocrine and Metabolic Disorders
- Institute of Neurology and Neuropsychology

and others …..