Imidazole-Linked Heterocycles with anti-Alzheimer properties

Carmen Escolano [a], Carla Barbaraci[a], Andrea Bagán [a], Christian Griñán-Ferré [b], Mercè Pallàs [b], José Ángel Morales-García [c], José Brea [d], Mabel Loza [d], Caridad Diaz [e], Rosario Fernández-Godino [e], Olga Genilloud [e]

[a] Laboratory of Medicinal Chemistry (Associated Unit to CSIC), Department of Pharmacology, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institute of Biomedicine (IBUB), University of Barcelona, Av. Joan XXIII, 27-31, E-08028, Barcelona, Spain. [b] Pharmacology Section, Toxicology and Medicinal Chemistry, Faculty of Pharmacy and Food Sciences, and Institut de Neurociències, University of Barcelona, Av. Joan XXIII, 27-31. E-08028, Barcelona, Spain. [c] Department of Cell Biology. School of Medicine. Complutense University (UCM), 28040 Madrid, Spain. [d] Drug Screening Platform/ Biofarma Research Group, CIMUS Research Center, University of Santiago de Compostela (USC), Santiago de Compostela 15782, Spain. [e]Fundación MEDINA Centro de Excelencia en Investigación de Medicamentos Innovadores en Andalucía, Avda. del Conocimiento 34, 18016 Armilla, Spain.

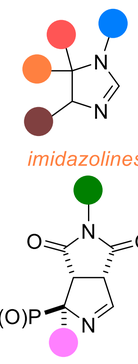
cescolano@ub.edu

INTRODUCTION

I_2 receptors (I_2-IR) are widely distributed in the brain [1].

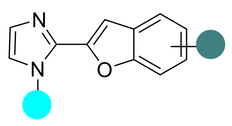
I_2-IR are found in bigger proportion in the brain of Alzheimer Disease (AD) patients.

Our group provided with several lines of evidence that demonstrate that the I_2-IR's modulation with original structurally selective ligands, from the families of (2-imidazolin-4-yl)phosphonates [2], bicyclic α-iminophosphonates [3] improves behavioural and psychological symptoms of dementia, including fear-anxiety, depressive-like behaviour, and memory decline, and ameliorates AD pathological features in well-stablished animal models of neurodegeneration and AD [4].



bicyclic α–iminophosphonates

In a more conservative fashion, having considered structural patterns of known ligands, we explored modifications on the benzofuranyl-2-imidazole nucleus of compound LSL60101 (Fig.1), which was proposed by our group as a disease-modifying treatment in an AD mouse model and compared with the gold-standard anti-AD donepezil [5].



benzofuranyl-2-imidazole

OBJECTIVES

Discover selective I_2-IR ligands to provide valuable tools to define the pharmacological characterization and molecular implications of these receptors.

Prepare imidazoline-linked heterocycles (Fig. 1) **LSL29**, **LSL33**, **LSL34**, **LSL35**, **LSL39** and **LSL42,** based on the observation/combination of key structural features of standard and well-established I_2-IR ligands, endowed with high affinity upon I_2-IR and anti-AD properties [6].

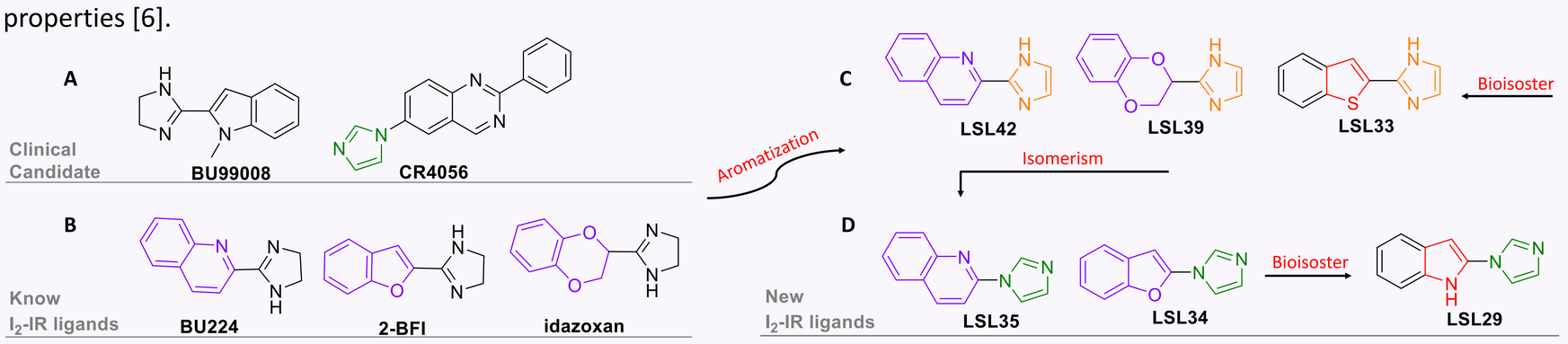
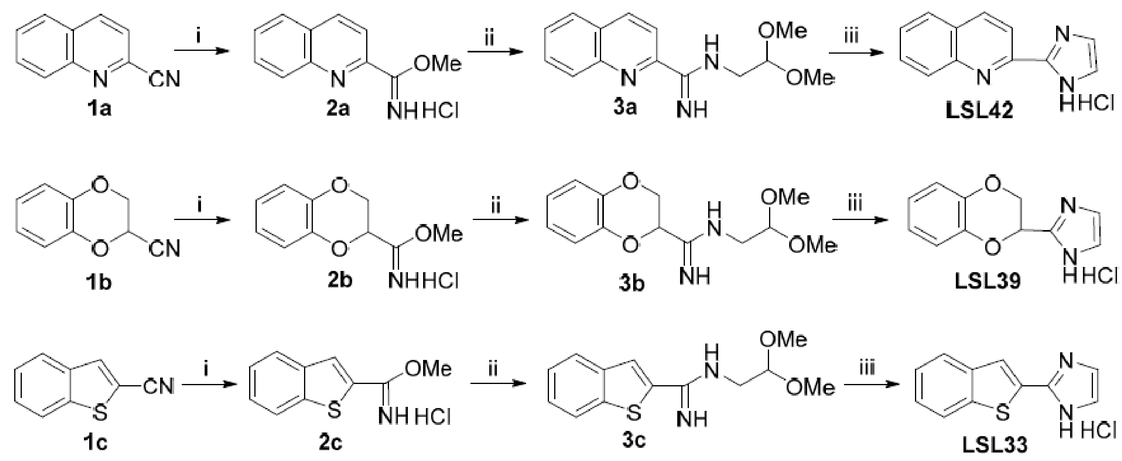
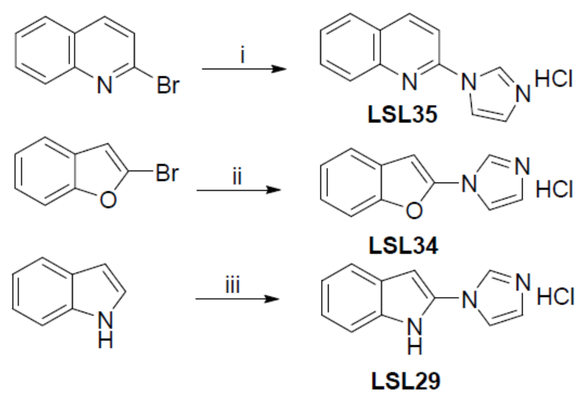


Figure 1. (A) Structure of clinical candidates CR4056 and [^{11}C]BU99008. (B) Structure of standard I_2-IR ligands BU224, idazoxan, and 2-BFI (C) Structure of compounds **LSL42**, **LSL39**, and **LSL33**, with the imidazole nucleous highlighted in orange connected by the C-2. (D) Structure of compounds **LSL35**, **LSL34** and **LSL29**, with the imidazole nucleous highlighted in green connected by the nitrogen atom. In red, the thiophene ring in **LSL33** and the pyrrole ring in **LSL29**. The aromatization, isomerism, and bioisoster relationships are indicated.

SYNTHESIS



Scheme 1: Condition reactions and yields: (i) Et_2O/HCl 2 M (0.25 mmol/mL), MeOH, 4 °C, 48 h, **2a**, 77%; **2b**, 94%; **2c**, 85% yield, respectively. (ii) 2,2-Dimethoxyethylamine (1.1 equiv.), MeOH, 60 °C, 16 h, quantitative for **3a**, **3b**, and **3c**. (iii) 2 M HCl (0.1 mmol/mL), 60 °C, 16 h, **LSL42**, 58%; **LSL39**, 64%; **LSL33**, 81% yield, respectively.



Scheme 2. Condition reactions and yields: (i) 2-Bromoquinoline (0.5 mmol), imidazole (0.25 mmol), HOBt (0.025 mmol), CuI (0.013 mmol), KOtBu (0.38mmol), DMSO, reflux, 24 h, **LSL35**, 48% yield. (ii) 2-Bromobenzofurane (1 mmol), imidazole (1.5 mmol), CuI (0.2 mmol), Cs_2CO_3 (2 mmol), acetonitrile, reflux, 24 h, **LSL34**, 55% yield. (iii) Indole (0.5mmol), imidazole (4 mmol), HCO_2NH_4, I_2 (1.25 mmol), dioxane, rt, 24 h, **LSL29**, 32% yield.

RESULTS

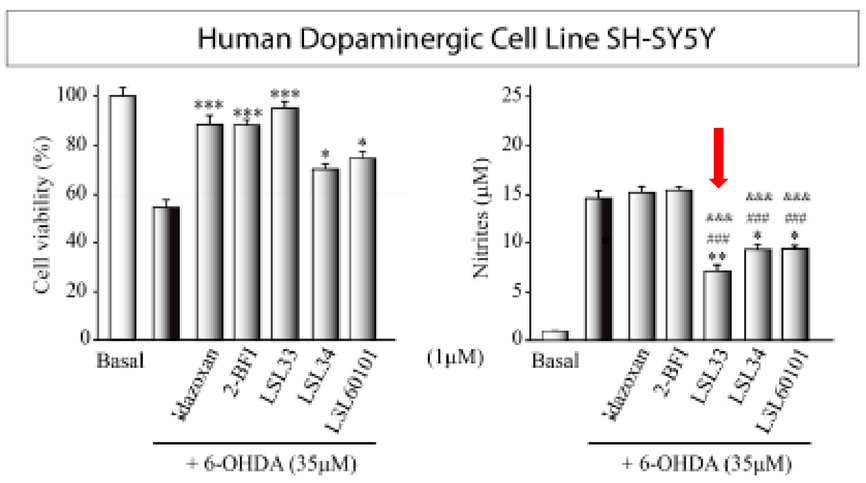


Figure 2. Assessment of the neuroprotective and anti-inflammatory effects of the indicated compounds on SH-SY5Y dopaminergic cells damaged with 6-hydroxydopamine (6-OHDA; 35 μM). Cell viability was evaluated using the MTT assay, and nitrite production in the cell supernatant was quantified using the Griess reaction. The reported values represent the mean ± SD obtained from triplicate determinations repeated at least three times. Statistical analysis was performed: * p ≤ 0.05, ** p ≤ 0.01, ***p ≤ 0.001 (versus 6-OHDA-treated cultures).

LSL33 and **LSL34** displayed excellent affinity for I_2-IR in human brain samples and good permeation of the BBB (PAMPA).

Selected compounds showed **neuroprotective properties and beneficial effect** in an *in vitro* model of Parkinson's disease. Furthermore, compounds rescued the human dopaminergic cell line SH-SY5Y from death after treatment with 6-hydroxydopamine (Fig. 2) and showed crucial **anti-inflammatory effect** in a celular model of neuroinflammation.

Cognitive studies of LSL33 in the familial AD murine model 5xFAD

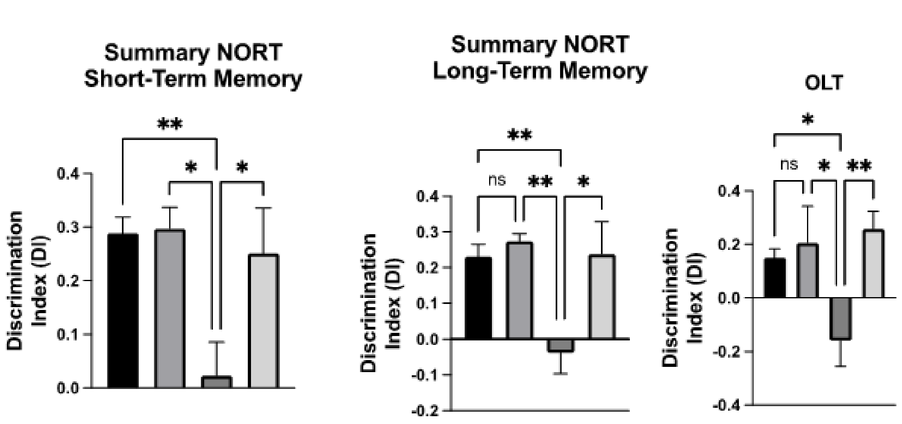
- **LSL33** reduces cognitive impairment presented in 5xFAD.
- **LSL33** ameliorates synaptic plasticity in 5xFAD.
- **LSL33** attenuates neuroinflammation presented in 5xFAD.

Female and male 4 months-old

In drinking water at 2 mg/Kg for 4 weeks

WT 5xFAD mice (n=32)

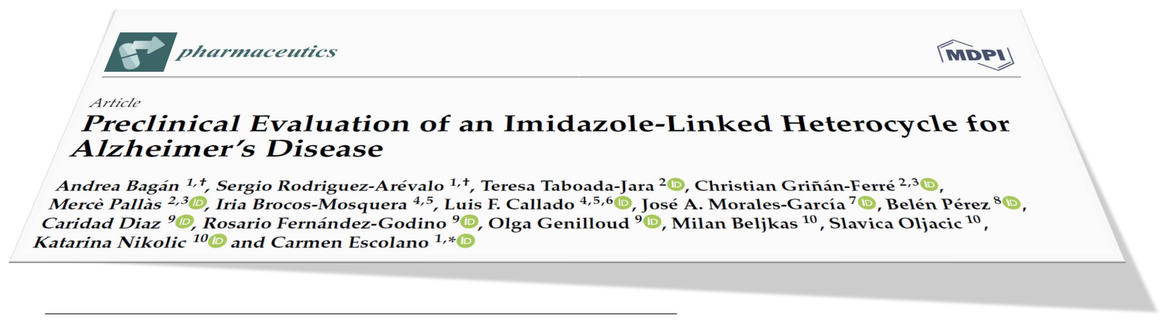
LSL 33



Conclusions

- ✓ Selected compounds demonstrate a **neuroprotective role** in the human dopaminergic cell line SH-SY5Y and they were shown to be **anti-inflammatory agents** even better than other known ligands.
- ✓ A preliminary pharmacokinetic study was performed with **LSL33**
- ✓ **LSL33** ameliorated 5xFAD cognitive impairment and synaptic plasticity, as well as reduced neuroinflammation markers.

I_2-IR ligands may be a suitable therapeutic strategy for AD



References: 1. Li, J.X. *Pharmacol. Ther.* **2017**, *178*, 48-56; Bousquet, P. *et al*. *Pharmacol. Rev.* **2020**, *72*, 50-79. Our research group: 2. *ACS Chem. Neurosci*. **2017**, *8*, 737; 3. *J. Med. Chem.* **2020**, *7*, 3610-3633. 4. *Eur. J. Med. Chem*. **2021**, *222*, 113540; 5. *Neurotherapeutics* **2019**, *16*, 416. *Pharmaceutics* **2020**, *12*, 475. *GeroScience* **2020**, *43*, 956; *Br. J. Pharmacol*. **2021**, 1-17. *Front. Pharmacol*. **2022**, *13*, 826837; *Int. J. Mol. Sci*. **2022**, *23*, 5408; 6. *Pharmaceutics* **2023**, *15*, 2381.

Webpage: @MCP_UB

MCP - Medicinal Chemistry and Pharmacology

FACULTAT DE FARMÀCIA

IBUB Institut de Biomedicina de la Universitat de Barcelona

Acknowledgements

This work was supported by PDC2022-133441-I00 (MCIN/AEI/ 10.13039/501100011033 and by the "European Union Next Generation EU/PRTR) and Proyecto PID2022-139180OB-I00 financiado por MCIN/AEI /10.13039/501100011033/ y por FEDER Una manera de hacer Europa.