Novel bicyclic α-iminophosphonates targeting the imidazoline I_2 receptor as neuroprotective agents

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INTRODUCTION

Imidazoline receptors (IR)

- Imidazoline receptors (IR) is a family of non-adrenergic binding sites with high affinity for imidazoline containing-compounds.
- 3 subtypes: I_1-IR, I_2-IR and I_3-IR.
- I_2-IR are widely distributed in the brain.
- I_2-IR are involved in analgesia, inflammation, glial tumors and human brain disorders [Alzheimer (AD) and Parkinson (PD) Diseases][1].

Density of I_2-IR significantly higher in Alzheimer Disease patients' brain[2]

AD impact

50M people affected around the world	Every 3s a new case is diagnosed
1 in 10 seniors suffer from AD	138M cases by 2050

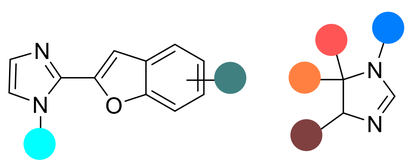
AD therapeutics

- *Scarce*
- *Limited efficacy*
- *No new cognitive enhancer drug*

Inappropriate *therapeutic target*?

I_2-IR new therapeutic target for neurodegeneration

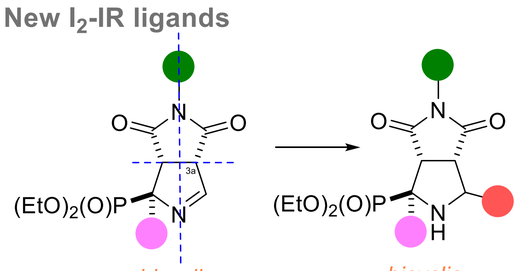
Previous work



imidazole *imidazolines*

- High affinity/selectivity I_2-IR
- Optimal ADME/BBB
- Amelioration of AD symptoms/hallmarks in murine models

New I_2-IR ligands

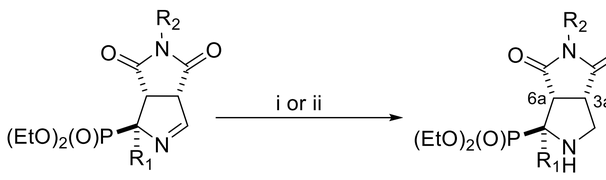


bicyclic α–iminophosphonates *bicyclic α–phosphoprolines*

- Synthetic strategy/ Bicyclic phosphoprolines
- I_2-IR Pharmacological evaluation
- *In vitro* cell models of PD and inflammation
- Anti-AD properties/ *Caenorhabditis elegans*

SYNTHESIS

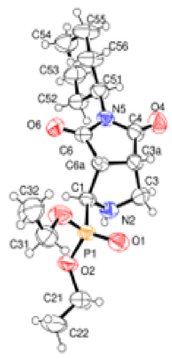
Starting bicycles (Schemes 1 and 2) resulted from a diastereoselective [3+2]cycloaddition reaction[3]. Reduction of the imine group led to bicyclic α-phosphoprolines (Scheme 1). The indol was added to the imine group by an aza Friedel Crafts reaction (Scheme 2). Opening of the imide ring led to a proline derivative (Scheme 3). The configuration of new compounds was unequivocally disclosed by X-ray crystallography.



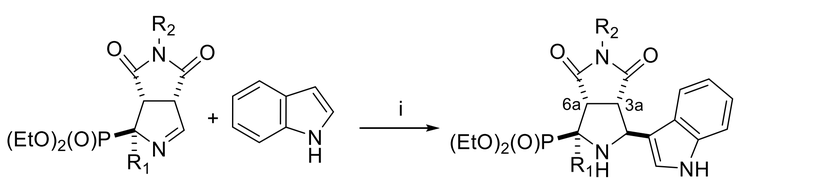
R_1 = H, R_2 = Me, **B03**
R_1 = H, R_2 = cyclohexyl, **B24**
R_1 = H, R_2 = Ph, **B02**
R_1 = Ph, R_2 = 3-Cl,4-FPh, **B06**

R_1 = H, R_2 = Me, **B03-red**
R_1 = H, R_2 = cyclohexyl, **B24-red**
R_1 = H, R_2 = Ph, **B02-red**
R_1 = Ph, R_2 = 3-Cl,4-FPh, **B06-red**

X-ray B24-red



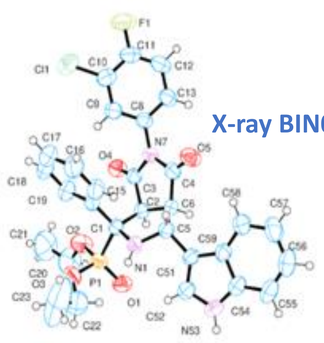
Scheme 1. *Reagents and conditions.* (i) H_2, Pd/C 5%, methanol, rt, 24h, 84%, **B03-red**; 90%, **B24-red**, and 87%, **B02-red** and for **B06** (ii) $NaBH_3CN$, acetonitrile, water and acetic acid, rt, 1h, 84%, **B06-red**.HCl. X-Ray crystallographic structure of **B24-red**.



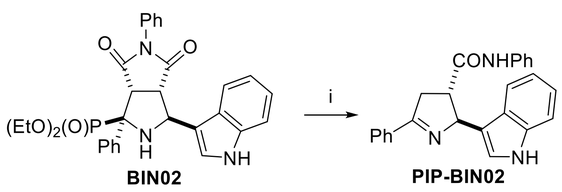
R_1 = H, R_2 = Ph, **B02**
R_1 = R_2 = Ph, **B16**
R_1 = Ph, R_2 = 3-Cl,4-FPh, **B06**
R_1 = Ph, R_2 = 3-ClPh, **B53**

R_1 = H, R_2 = Ph, **BIN01**
R_1 = R_2 = Ph, **BIN02**
R_1 = Ph, R_2 = 3-Cl,4-FPh, **BIN05**
R_1 = Ph, R_2 = 3-ClPh, **BIN06**

X-ray BIN06

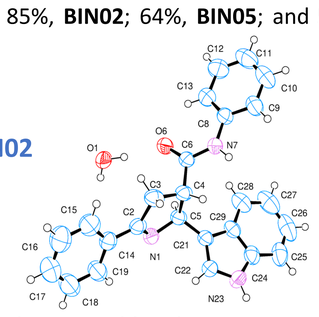


Scheme 2. *Reagents and conditions:* (i) $AgBF_4$, tetrahydrofurane, r.t., 48 h, 63%, **BIN01**; 85%, **BIN02**; 64%, **BIN05**; and 59%, **BIN06**. X-Ray crystallographic structure of **BIN05**.



BIN02 **PIP-BIN02**

X-ray PIP-BIN02



Scheme 3. *Reagents and conditions:* (i) NaOH 0.05 M, tetrahydrofuran: water (2:1), r.t., 2.5 h, 80% yield and X-ray structure of **PIP-BIN02**.

RESULTS

The new bicyclic α-iminophosphonates showed **promising activities as I_2-IR ligands** in human brain tissues and **good BBB permeation capabilities**. After ***in silico* ADME prediction studies**, we assessed the neuroprotective properties of selected compounds and beneficial effect in an *in vitro* model of Alzheimer´s and Parkinson's disease.[4]

HT-22 Mouse Hippocampal Neuronal Cell Line | **Primary astroglial cultures** | **Primary microglial cultures**

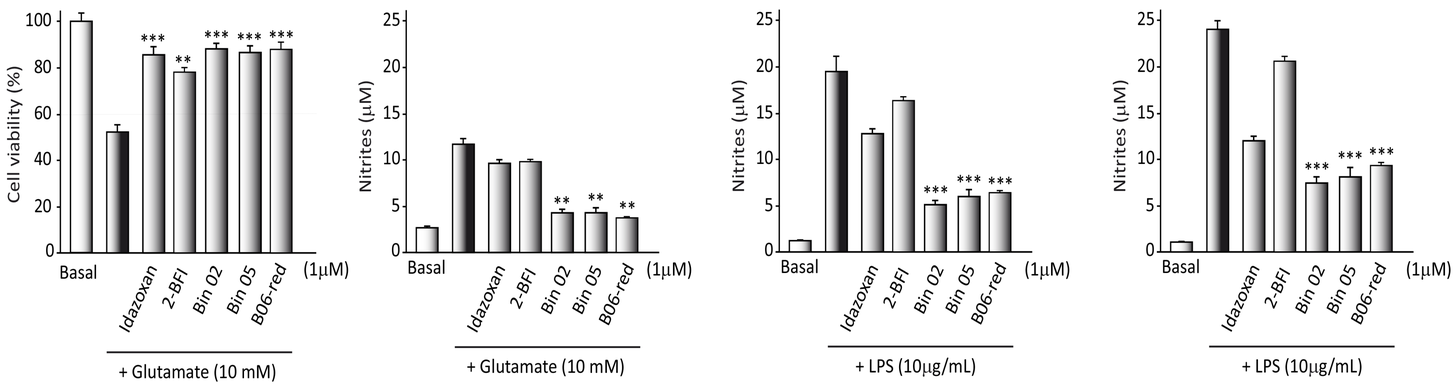
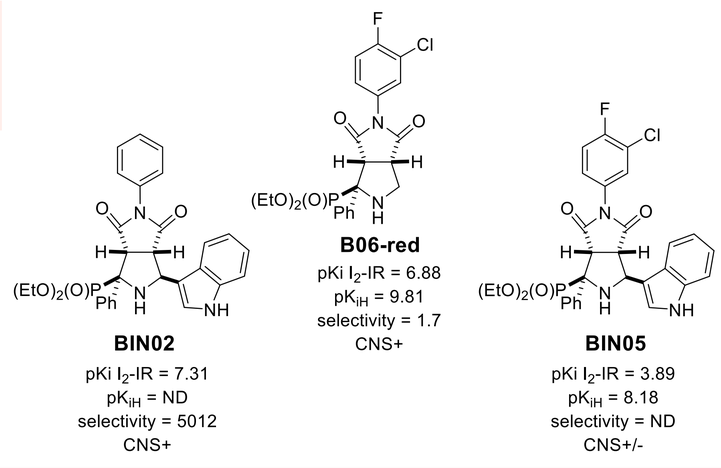


Figure 1. ***In vitro* neuroprotective and anti-inflammatory effect.** HT-22 mouse hippocampal neuronal cell line was exposed to glutamate (10 mM) for 24h. To evaluate the neuroprotective role of the bicyclic α-phosphoprolines, some cultures were pre-treated with the compounds (1 μM) for 1 h before damage. Cell viability was determined by MTT assay. *In vitro* inflammatory status was determined by nitrite production measurement (Griess reaction) in the supernatant of the HT-22 cell line and in murine-derived primary astroglial and microglial cultures treated for 24h with lipopolysaccharide (LPS, 10 μg/mL), in the presence or not of the different compounds (1 μM). Values represent the mean ± SD from triplicate determinations repeated at least three times. ** p ≤ 0.01, *** p ≤ 0.001 versus insult-treated cultures.

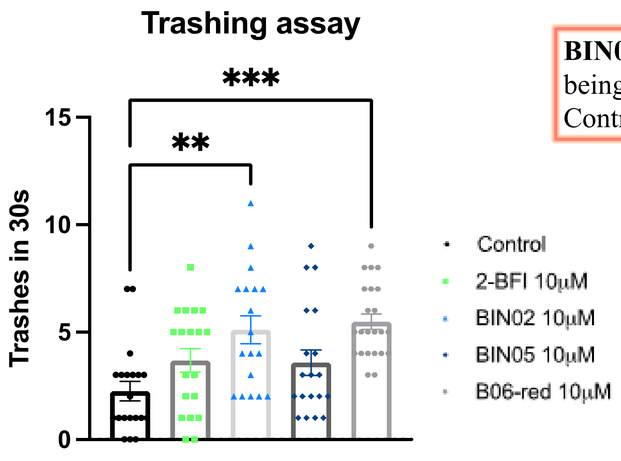


BIN02
pKi I_2-IR = 7.31
pK_{iH} = ND
selectivity = 5012
CNS+

B06-red
pKi I_2-IR = 6.88
pK_{iH} = 9.81
selectivity = 1.7
CNS+

BIN05
pKi I_2-IR = 3.89
pK_{iH} = 8.18
selectivity = ND
CNS+/-

BIN02, **BIN05** and **B06-red** are able to protect neurons from cytotoxic damage in two well-known preclinical models of AD and PD.



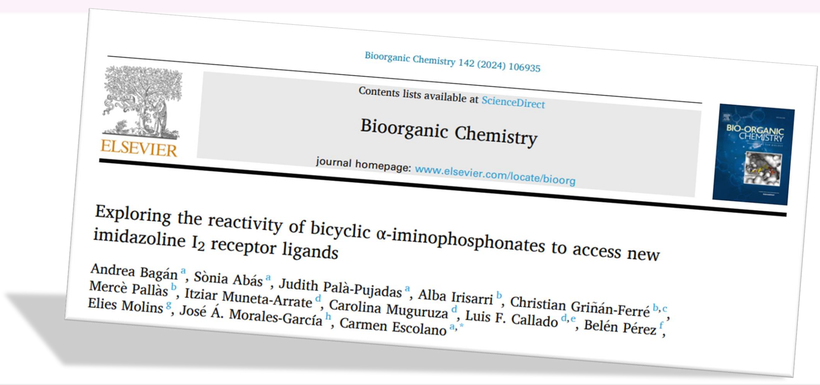
BIN02 and **B06-red**, showed higher number of thrashes, being at least 2-fold more compared to the CL2006 Control group, suggesting **neuroprotective effects**.

Cognitive improvement in *C. elegans* after treatment with I_2-IR ligands

Figure 2. ***In vivo* efficacy of 2-BFI, B06-red, BIN02, BIN05 on transgenic AD *C. elegans* model.** Study of motility on CL2006 *C. elegans* strain as estimated by thrashing assay (n= 20-30 worms/group; One-Way ANOVA and post-hoc Tukey's test:**p<0.01;***p<0.001). Values represented are mean ± Standard error of the mean (SEM).

CONCLUSIONS

- ✓ Bicyclic α-phosphoprolines showed **promising activities as I_2-IR ligands** in human brain tissues and **good BBB permeation** capabilities.
- ✓ The **neuroprotective** and **anti-inflammatory** properties of selected compounds and its beneficial effect have been evaluated *in vitro* using a pre-clinical model of AD.
- ✓ The treatment of a transgenic AD *C. elegans* with I_2-IR ligands rescued the neurodegenerative condition presented by CL2006 strain at the behavioural phenotype level. Thus, ***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; 2. García-Sevilla, J.A. *et al*. *Neurosci. Lett*. **1998**, *247*, 95-98; 3. S. Abás, S. Rodríguez-Arévalo *et al. J. Med. Chem.* **2020**, *7*, 3610-3633; 4. A. Bagán, S. Abás *et al. Bioorg. Chem*. **2024**, 142, 106935.

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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.