The 8th International Electronic Conference on Medicinal Chemistry (ECMC 2022)
01–30 NOVEMBER 2022 | ONLINE

Synthesis, Pharmacological, and Toxicological Evaluation of Potent Melanostatin Peptidomimetics Incorporating Chiral β-Amino Acids as Proline Surrogates

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pharmaceuticals MDPI

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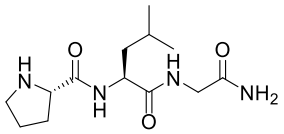
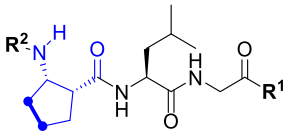
Organic Synthesis



Melanostatin (MIF-1)

Design



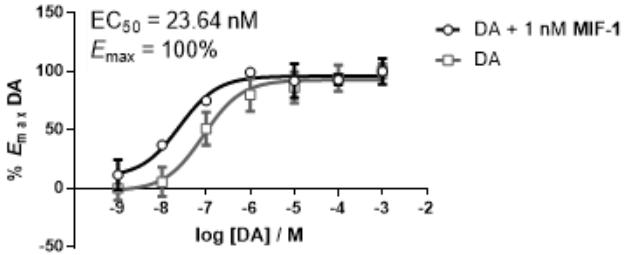
MIF-1 Peptidomimetics

C-C or C=C

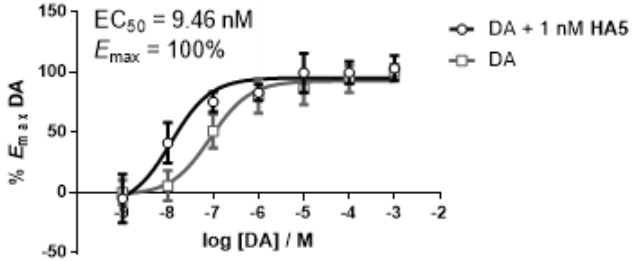
$R^1 = NH_2$, OMe

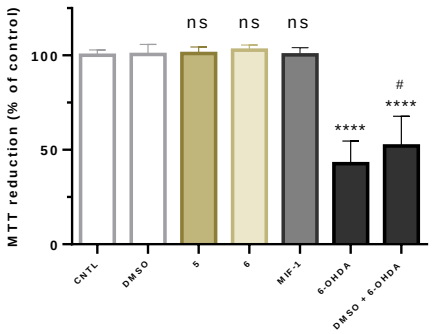
R^2 = H, Boc

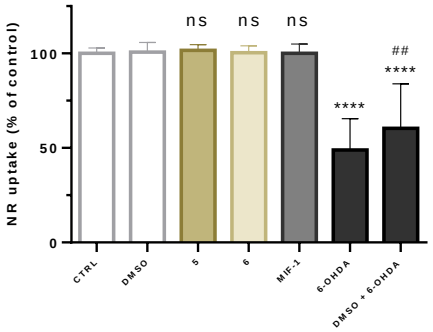
Pharmacological & Toxicological Evaluation



Higher Potency







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Abstract:

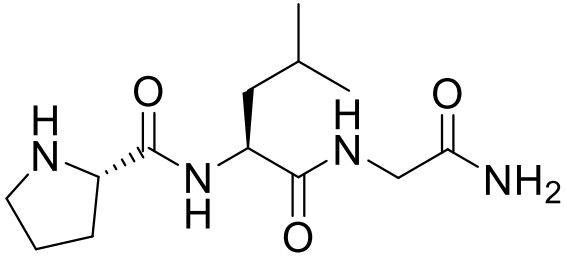
Melanostatin (MIF-1) is an endogenous tripeptide (Pro-Leu-Gly-NH_2) with several functions within the central nervous system (CNS). It has been widely recognized as a potent and selective positive allosteric modulator (PAM) of the dopamine D_2 receptors (D_2R), with potential biomedical applications in neurological diseases such as Parkinson's disease (PD). Upon binding to their allosteric binding sites, PAM induce conformational changes that increase binding affinity of orthosteric ligands to the receptor. Comparatively to orthosteric drugs, PAM present several advantages such as high specificity and reduced side effects since they are only effective in the presence of the orthosteric ligand.

Despite its undeniable pharmacological potential in PD, MIF-1 exhibits reduced stability towards CNS-derived peptidases and low gastrointestinal bioavailability, hampering oral administration. In this work, the bioisosteric replacement of prolyl residue with non-proteinogenic amino acids is disclosed as a strategy to overcome the unfavorable pharmacokinetic profile of MIF-1 without compromising its PAM activity.

Six novel MIF-1 proline mimetics bearing cyclic β-amino acids were designed, synthesized, and pharmacologically evaluated. In functional assays at D_2R, one of these peptidomimetics exhibits a superior performance (lower EC_{50}) than MIF-1 neuropeptide at 1 nM. Furthermore, no cytotoxic effect was observed for this compound at 100 µM using differentiated human neuronal SH-SY5Y cells. Further studies are currently underway to determine its chemical stability and *in vitro* biological permeability. The discovery of a new potent PAM of the D_2R with a safe cytotoxic profile opens new directions for the development of complementary anti-Parkinson therapies.

Keywords: Cyclic β-amino acids; Melanostatin; Neuropeptides; Parkinson's Disease; Peptidomimetics.

Introduction – Melanostatin and its Biological Activities

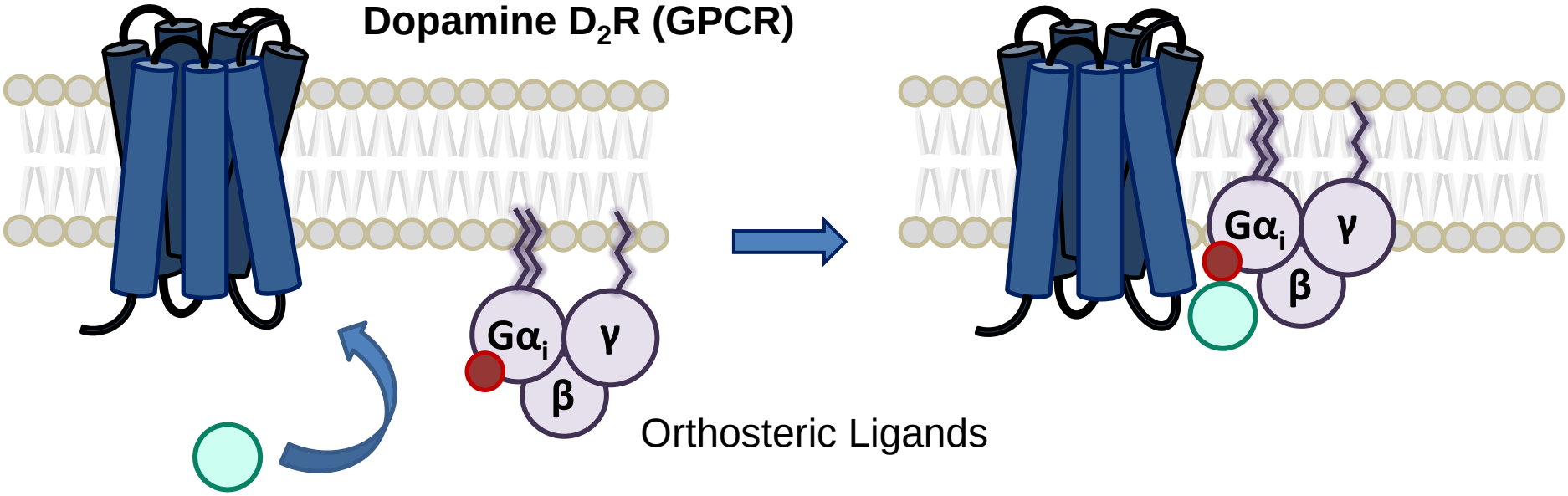


Melanostatin (MIF-1)

Acts as Positive Allosteric Modulator of Dopamine D_2 Receptors (D_2R)

- **Improvement** of both PD and severe depression patients
- Tremors **Reduction**
- **Prevents** stereotypy
- Levodopa **Potentiation**
- **Reduces** morphine dependence
- **Potentiates** dopamine agonists activity and binding

Introduction – Allosteric Modulators



- Receptor **subtype selectivity**
- **Combination** therapies

Ratio **high** affinity/low affinity state of receptors

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Introduction – MIF-1 Pharmacokinetic Problems

A Low absorption

D Rapidly distributed and directed to the CNS

M Hydrolysis by peptidases, short half-life

E Renal excretion or incorporation into the amino acids pool

T No meaningful toxicity at pharmacological dosages

Unsuitable for oral administration

Peptidomimetics!

Improve pharmacokinetics

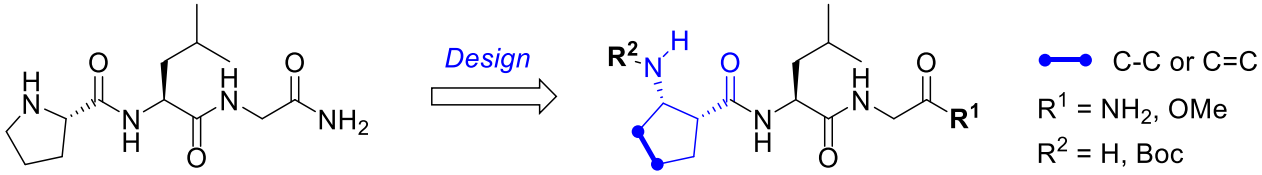
Potency

Objectives

1. Synthesize MIF-1 Peptidomimetics bearing cyclic β-amino acids;

2. Pharmacological evaluation;

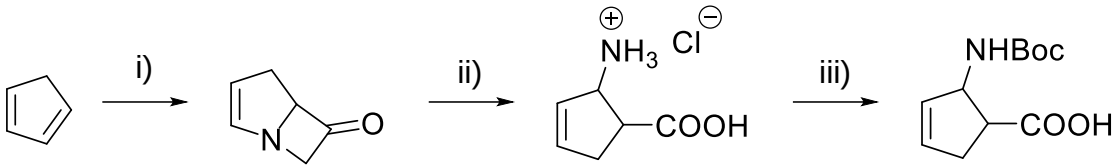
3. Toxicological evaluation.



Melanostatin (MIF-1) **MIF-1 Peptidomimetics**

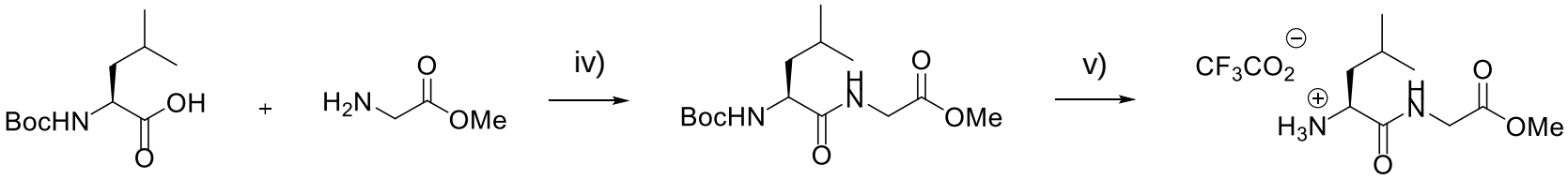
Results and discussion

β-amino acid synthesis



44.2% 82.9% 82.2%

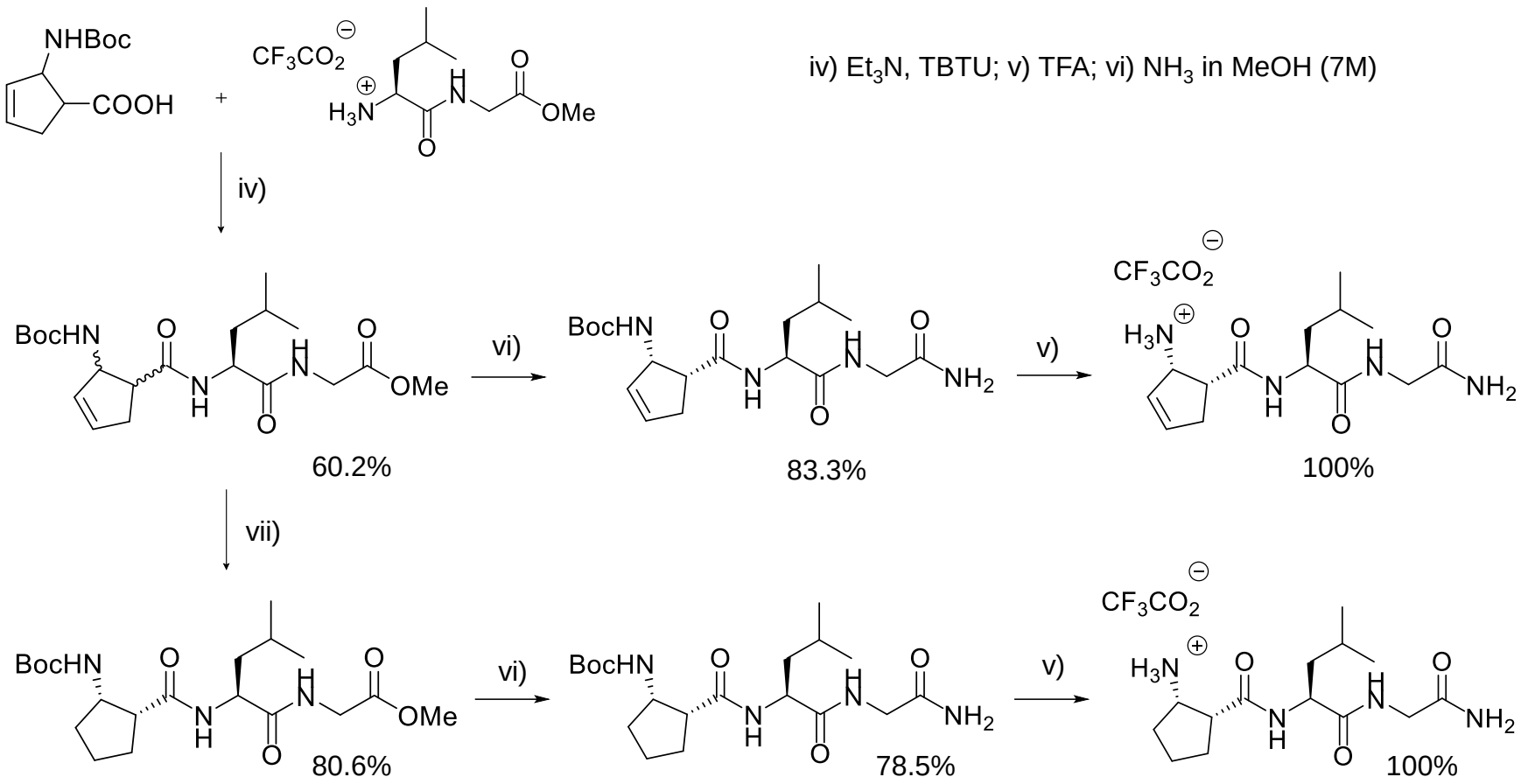
C-terminal dipeptide synthesis



75.2% 100%

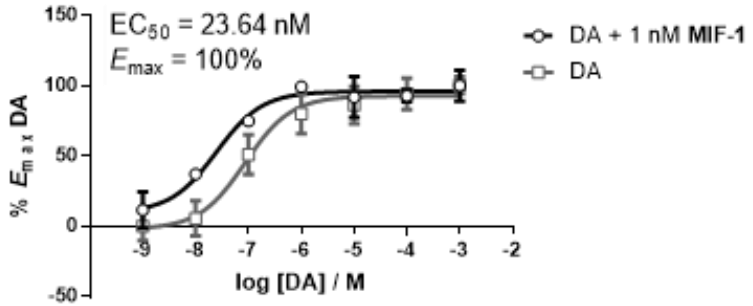
i) CSI, followed by KI, Na_2SO_4; ii) HCl; iii) Boc_2O, $NaHCO_3$, followed by HCl; iv) Et_3N, TBTU; v) TFA

Results and discussion

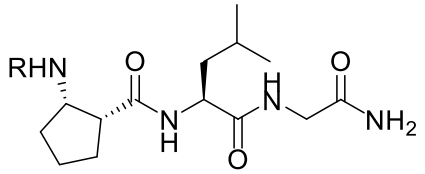


iv) Et_3N, TBTU; v) TFA; vi) NH_3 in MeOH (7M)

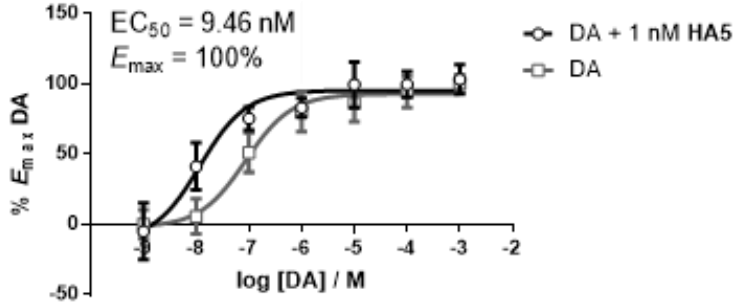
Pharmacological Evaluation – Concentration-Response Curve of Dopamine (DA) at D_2R



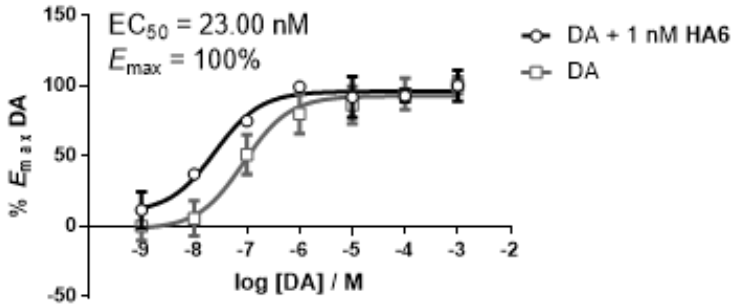
EC_{50} (DA) = 88.49 nM



HA05: R = Boc
HA06: R = H



EC_{50} (DA) 9.4x



EC_{50} (DA) 3.8x

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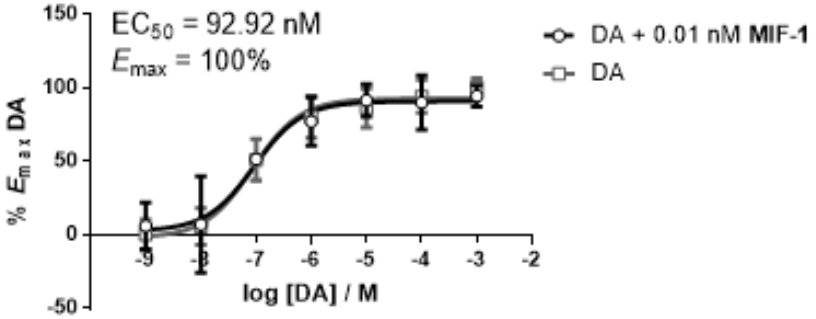
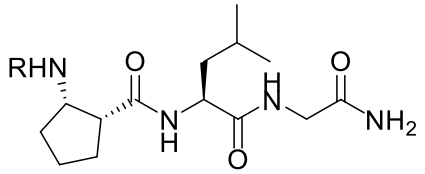
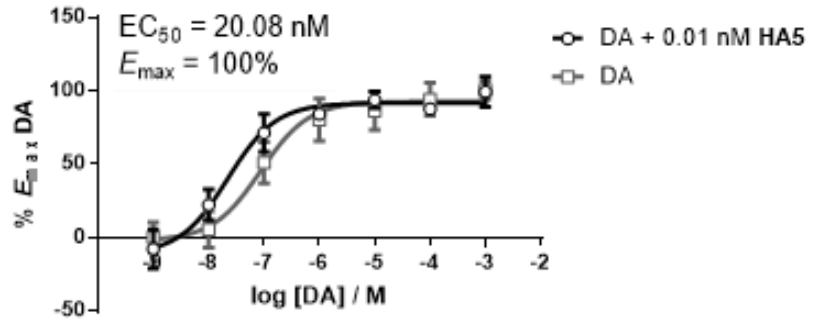
Pharmacological Evaluation – Concentration-Response Curve of Dopamine (DA) at D_2R



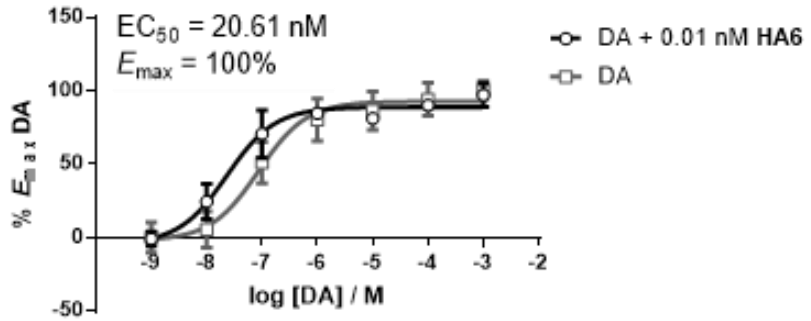
EC_{50} (DA) = 88.49 nM



HA05: R = Boc
HA06: R = H



EC_{50} (DA) 4.4x



EC_{50} (DA) 4.3x

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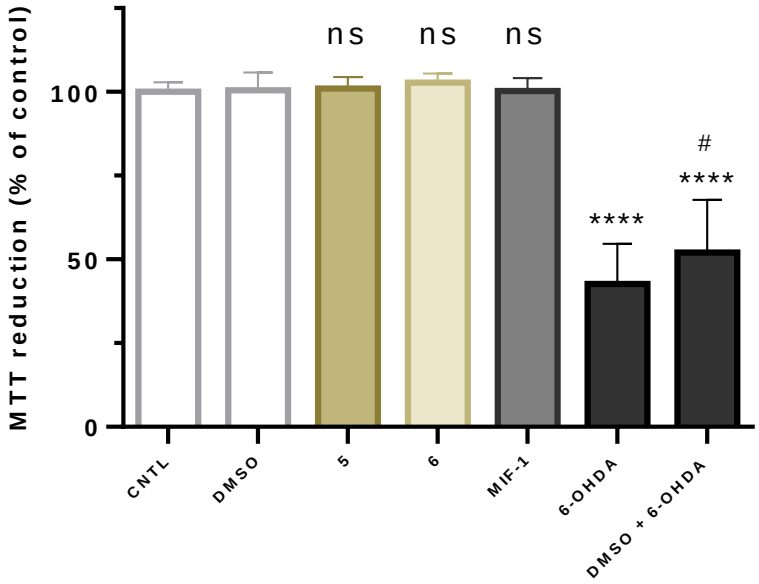
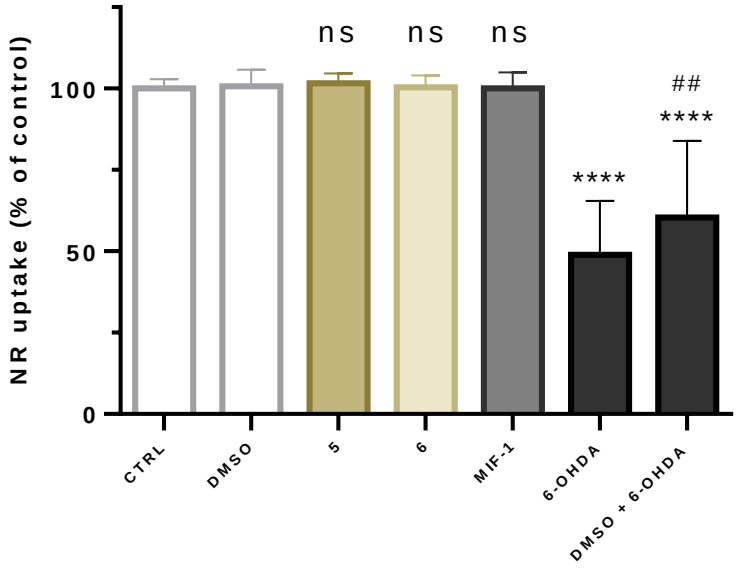
Toxicological Evaluation in differentiated SH-SY5Y (MTT)



Neurotoxicity was evaluated by the MTT reduction assay in differentiated SH-SY5Y neuronal cells incubated for 48 h with 5, 6, MIF-1, DA, and 6-OHDA at 200 μM. 6-OHDA = 6-hydroxydopamine, a neurotoxic used as model of Parkinson Disease. Data are expressed as a percentage of control and are presented as mean ± standard deviation. The results were obtained from 8−12 wells and 2−3 independent experiments. Statistical analyses were performed using the analysis of variance (ANOVA) test followed by the Tukey *post hoc* test (****$p < 0.0001$ vs control; $^{\#}p < 0.05$ vs the same concentration of 6-OHDA).

Toxicological Evaluation in differentiated SH-SY5Y (NR)



Neurotoxicity was evaluated by the NR uptake assay in differentiated SH-SY5Y neuronal cells incubated for 48 h with 5, 6, MIF-1, DA, and 6-OHDA at 200 μM. Data are expressed as a percentage of control and are presented as mean ± standard deviation. The results were obtained from 8−12 wells and 2−3 independent experiments. Statistical analyses were performed using the analysis of variance (ANOVA) test followed by the Tukey post hoc test (****$p < 0.0001$ vs control; ##$p < 0.01$ vs the same concentration of 6-OHDA).

Conclusions and Future Perspectives

- Two series of MIF-1 peptidomimetics were synthesized with global yields of **11.4 %** (8 steps, unsaturated derivatives) and **8.6 %** (9 steps, saturated counterparts);
- Pharmacological assays showed that two of the synthesized peptides **decrease the EC_{50} of dopamine** by **3 – 10x** at concentrations of **0.01 and 1 nM**;
- Toxicological evaluations showed **no meaningful toxicity** at concentrations up to 200 nM for both peptidomimetics.
- In the future, **permeability assays** and **chemical and biochemical stability assays** are envisioned in order to further study the ADMET properties of these potent PAMs.

Acknowledgments

SCSR and IESD thank FCT for the PhD grant SFRH/BD/147463/2019 and funding through the Individual Call to Scientific Employment Stimulus (Ref. 2020.02311.CEECIND/CP1596/CT0004), respectively. VMC acknowledges FCT for her grant (SFRH/BPD/110001/2015), under the Norma Transitória – DL57/2016/CP1334/CT0006. XG-M thanks Xunta de Galicia for financial funding with references GPC2017/GI-1597 and REDES2017-REGID.

This work received financial support from PT national funds (FCT/MCTES, Fundação para a Ciência e a Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the project UIDB/50006/2020.

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