Synthesis, pharmacological, and biological evaluation of pyridine-based MIF-1 peptidomimetics as positive allosteric modulators of D_2R

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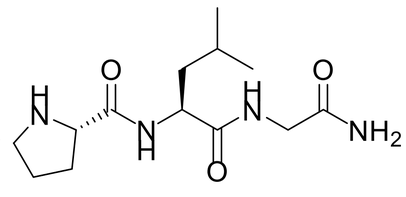
INTRODUCTION

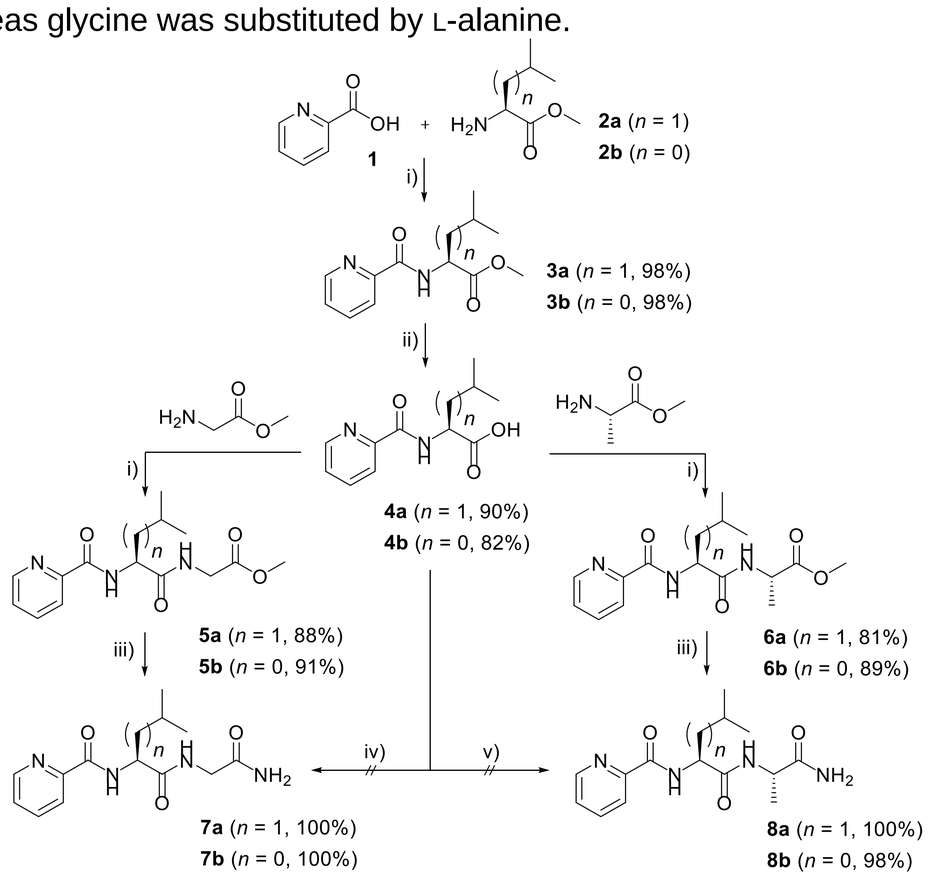
In the last few decades, investigators deal with synthesizing structurally modified peptides in order to improve the stability and biological activity of these compounds, with the aim of obtaining new potential pharmaceuticals. This project describes the synthesis, pharmacological and biological evaluation of new class of mimetic compounds for neuropeptide MIF-1 (L-prolyl-L-leucylglycinamide) which perform important roles in central nervous system. The main objective was to explore the replacement of L-proline by heteroaromatic system such as picolinic acid (**1**), which is naturally synthesized in the body from L-tryptophan (TRP). Picolinic acid plays an important neuroprotective role since it protects against quinolinic acid, and kainic acid, inductors of neurotoxicity in the brain, which may contribute for the design of potent peptidomimetics with intrinsic neuroprotection.



MIF-1

ORGANIC SYNTHESIS

The synthesis of pyridine-based peptidomimetics were prepared based on DIPEA/TBTU peptide coupling strategy in solution-phase (as depicted in **Scheme 1**), following a diversity-oriented synthesis (DOS) to create a set of structural-related amino acid combinations at the central and C-terminal positions. In some peptidomimetics, L-leucine was replaced by L-valine whereas glycine was substituted by L-alanine.



Scheme 1. Synthesis of picolinoyl-based peptidomimetics **5-8(a,b)**. Reagents and conditions: (i) DIPEA, TBTU, CH_2Cl_2; (ii) LiOH, MeOH/H_2O followed by H_2SO_4 1 M; (iii) NH_3 (g), MeOH; (iv) DMF, TBTU, glycinamide hydrochloride, CH_2Cl_2; (v) DMF, TBTU, L-alaninamide hydrochloride, CH_2Cl_2.

PHARMACOLOGY

Dopamine D_2 receptor Binding Assay

Peptidomimetics **5-8(a,b)** were tested to their ability to modulate dopamine D_{2S} receptors by enhancing the binding of radiolabeled [^{3}H]-NPA ligand. A statistically significant enhancement ($P < 0.05$) of the [^{3}H]-NPA response was observed for the peptidomimetic **6b,** as depicted in **Scheme 2.**

Scheme 2. Modulation of [^{3}H]-NPA binding exerted by MIF-1 and **6b** at three different concentrations.

PHARMACOLOGY

Dopamine D_2 receptor Functional Assay

A functional study was performed for peptidomimetic **6b** at human D_2R expressed in CHO cells by analysis of cyclic adenosine monophosphate (cAMP) mobilization using homogeneous time resolved fluorescence measurements in presence of dopamine. The results are shown in **Figure 1A**. Compound **6b** produced a typical bell-shaped dose-response curve and demonstrated a better performance than the parent neuropeptide (18.3 ± 7.1% for **6b** *vs*. 15.4 ± 5.5% for MIF-1, both at 0.1 nM), without intrinsic agonism effect (**Figure 1B**).

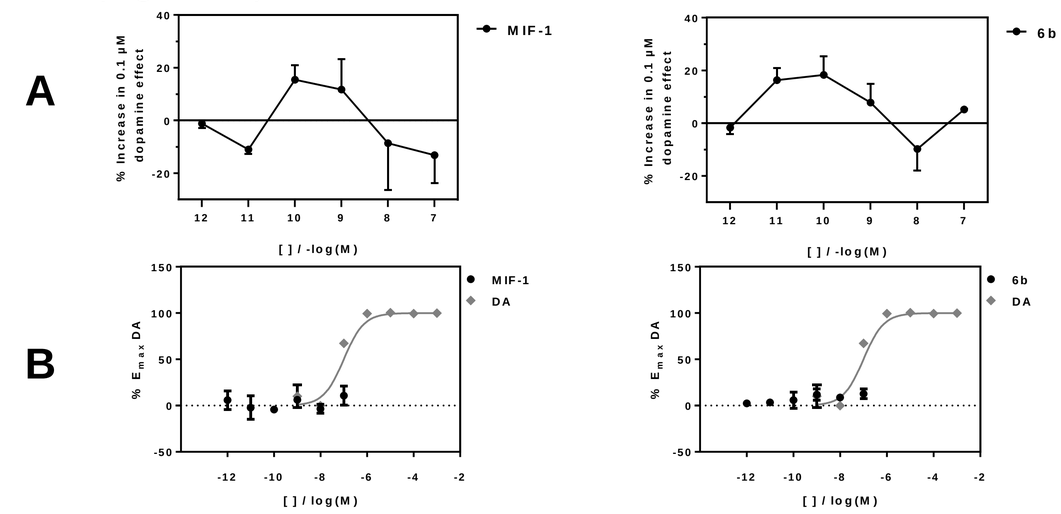


Figure 1. **A**: Dopamine effect exerted by peptidomimetic **6b** and MIF-1 (positive control) at different concentrations; **B**: Concentration-response curves of the compounds and dopamine (DA) at human D_2R

ENERGY MINIMIZATION

In silico studies by energy minimization in gas-phase for compound **6b** shows that this peptidomimetics exhibits an extended conformation placing the ester moiety outwards (**Figure 2**). The bioactive peptidomimetic **6b** cannot adopt a type-II β-turn conformation since the postulated C-terminal carboxamide pharmacophore is absent, supporting existence of a secondary bioactive conformation (extended) for MIF-1.

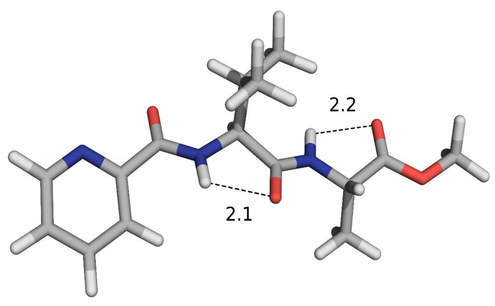


Figure 2. Optimized geometry, at the M06-2X/6-31G(d,p) level of theory, for peptidomimetic **6b**.

CYTOTOXICITY

Peptidomimetic **6b** was evaluated on HEK 293T cells for 8 days up to 120 µM and cell viability was estimated based on MTT reduction (**Figure 3**). The results show that absence of cytotoxicity under the conditions tested.

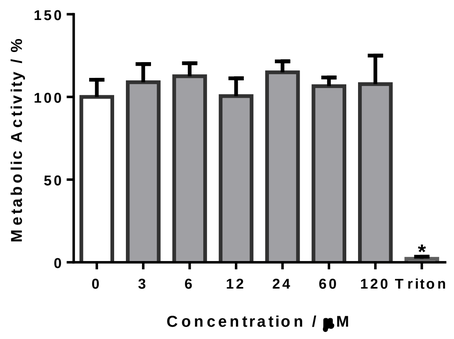


Figure 3. Cell viability of HEK 293T cells tested by the MTT assay. HEK 293T cells were treated with peptidomimetic **6b** at different concentrations (0, 3, 6, 12, 24, 60 and 120 µM) for 8 days. Following treatment with MTT reagent, viable cells were quantified by measuring the OD_{490} of sample wells. *Level of significance $P < 0.05$.

CONCLUSION

The pharmacological data obtained from binding and functional assays on human D_2R shows that peptidomimetic **6b** acts as a positive allosteric modulator of D_2R. The absence of the postulated C-terminal carboxamide pharmacophore and the minimization energy performed for **6b** indicates that it cannot adopt the postulated type-II β-turn bioconformation. This finding suggests that heteroaromatic scaffolds at prolyl position of MIF-1 may have a significant impact in the folding of the these tripeptidomimetics, inducing an extended conformation rather than type-II β-turn.

Overall, the pharmacological and toxicological profile of peptidomimetic **6b** and its structural simplicity makes it a potential lead compound for further development and optimization of novel potent MIF-1 analogues.

Acknowledgements

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