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Design-Oriented Synthesis and Biological Evaluation of Melanostatin Neuropeptide Derivatives with Improved Pharmacokinetic Profiles

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INTRODUCTION & AIM

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons, leading to motor and non-motor dysfunctions.[1] Current treatments primarily enhance dopamine levels using levodopa and enzyme inhibitors.[2] Although initially effective, long-term levodopa use often causes severe side effects that exacerbate PD symptoms.[2] Melanostatin (MIF-1, Figure 1), an endogenous tripeptide and positive allosteric modulator (PAM) of dopamine D_2 receptors (D_2R), has emerged as a promising alternative therapy.[3] However, its poor pharmacokinetic profile, including low gastrointestinal absorption, limits clinical application.[3,4] Structural optimization efforts, such as substituting L-proline with L-pipecolic acid, have improved bioactivity (compound **I**, Figure 1).[5] Here, we report the synthesis and biological evaluation of bridged MIF-1 derivatives incorporating a fused L-proline/L-pipecolic acid scaffold, (1*R*,3*S*,4*S*)-2-azanorbornane-3-carboxylic acid, compound (1*R*,3*S*,4*S*)-**1** (Figure 1).

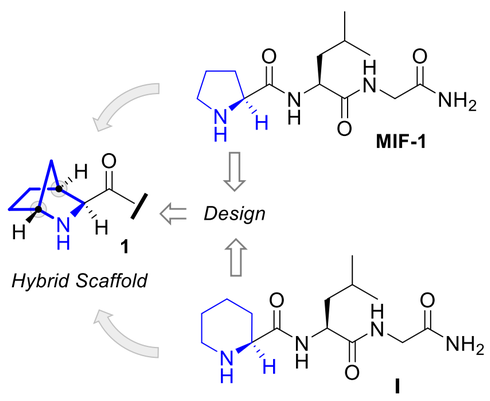
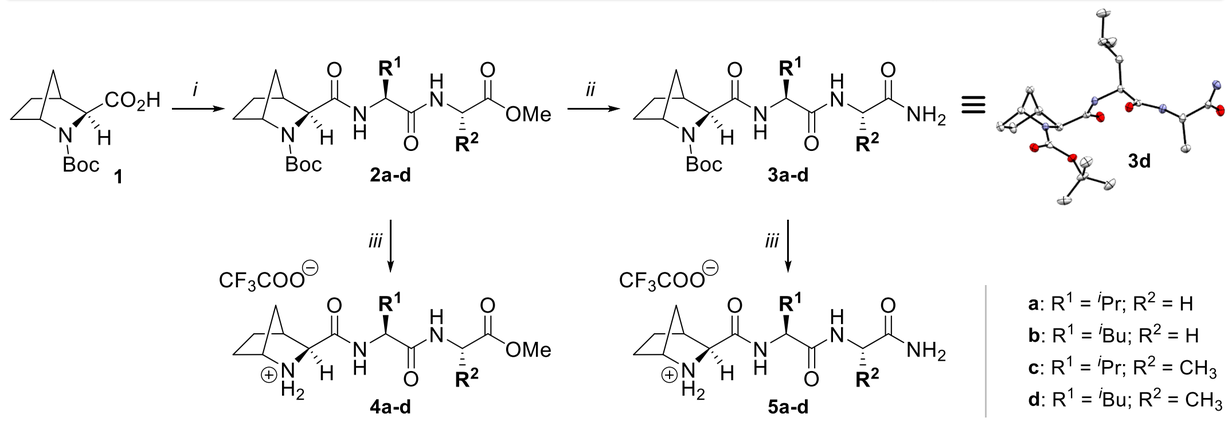


Figure 1. Rational design of bridged MIF-1 derivatives using (1*R*,3*S*,4*S*)-**1**.

METHOD

The synthesis began with the peptide coupling of (1*R*,3*S*,4*S*)-**1** with methyl L-valylglycinate (**a**), methyl L-leucylglycinate (**b**), L-valyl-L-alaninate (**c**), or methyl L-leucyl-L-alaninate (**d**) using TBTU as the coupling reagent, affording tripeptides **2(a-d)** in excellent yields (91.8-99.8%, Scheme 1). Ammonolysis of methyl esters **2(a-d)** produced primary carboxamides **3(a-d)** in 92.3-98.3% yield. The structure of **3d** was confirmed by single-crystal X-ray diffraction (Scheme 1). Subsequent acidolytic removal of the *N*-Boc group from **2(a-d)** and **3(a-d)** with trifluoroacetic acid (TFA) afforded **4(a-d)** and **5(a-d)**, respectively, in 88.2-98.4% yield. Pharmacological evaluation was conducted on human D_2R expressed in CHO cells using a cAMP assay with homogeneous time-resolved fluorescence in the presence of dopamine.[3,4] Dopamine alone displayed an EC_{50} of 0.53 μM (Table 1). Four bridged MIF-1 derivatives enhanced dopamine potency by 5.3-6.6-fold at 0.01 nM, demonstrating strong PAM activity (Table 1). Cytotoxicity of **2c**, **3d**, **4b**, and **4d** was assessed in differentiated SH-SY5Y neuroblastoma cells at 100 and 200 μM using the MTT reduction assay, with 6-hydroxydopamine (6-OHDA) as a positive control.[3] Compound **4b** was cytotoxic at both concentrations, while **2c** showed toxicity only at 200 μM; MIF-1 and the other derivatives were non-cytotoxic (Figure 2). P-glycoprotein (P-gp) inhibition, evaluated via the calcein-AM assay,[6] revealed no interference with P-gp-mediated transport, indicating lack of interaction with the efflux transporter (Table 2). Permeability studies in Caco-2 monolayers[7] showed all compounds exhibited lower efflux ratios (BA/AB) than MIF-1, suggesting enhanced permeability relative to the parent neuropeptide (Table 2).

RESULTS & DISCUSSION



Scheme 1. Synthesis of bridged MIF-1 derivatives **2-5(a-d)**. Reagents and conditions: i) Et_3N, TBTU, dipeptides **a-d**, anhydrous CH_2Cl_2; (ii) 7 M ammonia in MeOH; iii) TFA, anhydrous CH_2Cl_2. The ORTEP diagram of **3d** is shown at 50% probability level.

Table 1. Pharmacological evaluation of bridged MIF-1 and compounds **2c**, **3d**, **4b**, and **4d** by functional assays at 0.01 nM.

Compound	0.01 nM	
	EC_{50} of DA (μM)	E_{max} of DA (%)
DA	0.53	100
DA + 2c	0.09	97
DA + 3d	0.09	98
DA + 4b	0.08	89
DA + 4d	0.10	92
DA + MIF-1	0.17	94

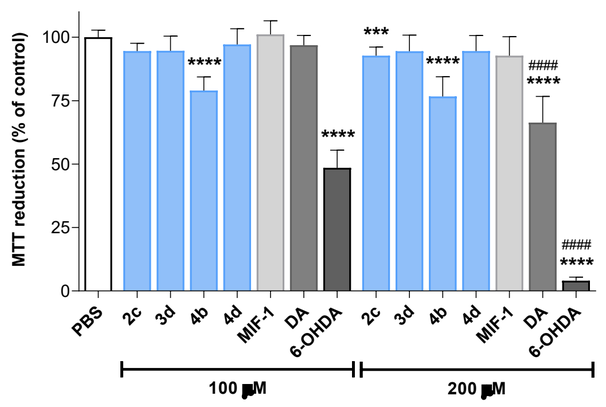


Figure 2. Neurotoxicity was evaluated by the MTT reduction assay in differentiated SH-SY5Y neuronal cells incubated for 48 h at 100 or 200 μM of test compounds. Data are expressed as a % of control (mean ± standard deviation, 12 wells, 3 independent experiments). Statistical analyses were performed using ANOVA test, followed by the Tukey *post hoc* test (***p < 0.001, ****p < 0.0001 vs. control; ####p < 0.0001 vs. the lowest concentration of the same drug).

Table 2. EC_{50} values in the calcein-AM assay and Caco-2 cell monolayer permeability.

Compound	EC_{50} P-gp (100 μM)	P_{app}BA (nm/s)	P_{app}AB (nm/s)	BA/AB
MIF-1	45%	2367	788	3.00
2c	39%	2352	959	2.45
3d	12%	1977	737	2.68
4b	2%	2331	796	2.92
4d	15%	2367	865	2.73

CONCLUSION

The replacement of L-proline with a (1*R*,3*S*,4*S*)-**1** proved to be a feasible strategy with the discovery of bridged MIF-1 derivatives with potent PAM activity at the D_2R, enhancing dopamine potency up to 6.6-fold at 0.01 nM. Most compounds showed minimal cytotoxicity and limited P-gp interaction, with Caco-2 assays indicating improved permeability compared with MIF-1. These findings highlight bridged MIF-1 analogues as promising leads for developing next-generation anti-Parkinson's agents with enhanced pharmacological efficacy and favorable pharmacokinetic profiles.

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