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Constrained Glypromate® Analogues Incorporating a Bicyclic Proline Surrogate

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Abstract: Neurodegenerative diseases affecting the central nervous system, such as Alzheimer's and Parkinson's Diseases, result from progressive degeneration and/or death of neurons, without curative treatments currently available. Glypromate® is a neuroprotective tripeptide obtained by the N-terminal cleavage of insulin-like growth factor 1 (IGF-1), which is found in brain tissue. In vitro and in vivo studies have demonstrated that this neuropeptide is capable of stimulating the release of acetylcholine and dopamine and acting as neuroprotective.3 However, the clinical trials with this neuropeptide failed in phase III.

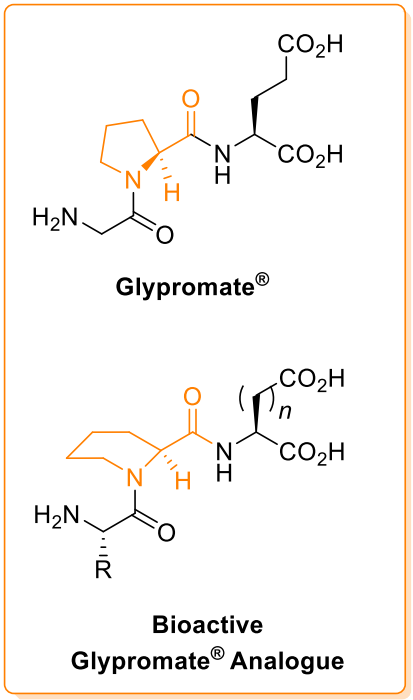
Constrained proline mimetics of Glypromate®, such as Trofinetide®, whereupon the alpha-proton of proline was substituted by a methyl group, is currently undergoing clinical trials for Rett and Fragile X syndrome, proving that highly constrained proline mimetics may be beneficial for the activity of this peptide. Also, a Glypromate® analogue with pipecolic acid instead of L-proline demonstrated good stability in comparison with Glypromate®.

In this work, the design, synthesis, and biological evaluation of Glypromate® peptidomimetics using 2-azanorbornane as a proline surrogate is described. Following a diversity-oriented synthesis approach, four novel highly constrained Glypromate® analogues were synthesized in excellent global yields (75-84%) using a one-pot protocol in peptide synthesis.

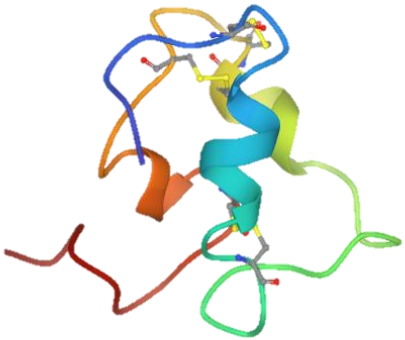
Neuroprotective assays performed in human neuroblastoma SH-SY5Y cells using 6-hydroxydopamine (6-OHDA) as stress inducer demonstrated that Glypromate® analogues display superior neuroprotection in comparison with the parent peptide (100 mM concentration) and a remarkable percentage of recovery (29.7-40.0%) after 6-OHDA injury in contrast with 12.8% found for Glypromate®.

Keywords: Glypromate; Hybrid Scaffolds; Neuroprotection; Peptidomimetics.

Constrained Glypromate® Analogues Incorporating a Bicyclic Proline Surrogate



Introduction

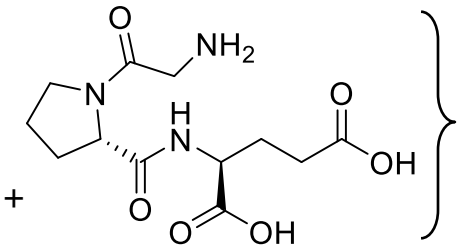


IGF-1

(PDB: 1B9G)

acid protease

des-IGF-1 +



Glypromate®

- Possible interaction with the *N*-methyl-D-aspartate (NMDA) receptors.
- Stimulates the release of potassium-induced acetylcholine in the cerebral cortex and dopamine in the striatum.

Neuroprotective effects in animal models of neurodegenerative conditions, such as Huntington's, Parkinson's, and Alzheimer's diseases.

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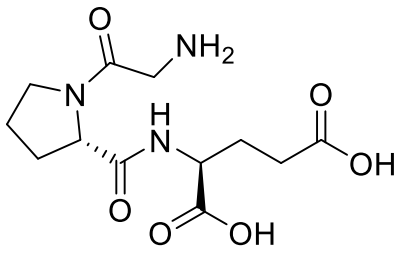
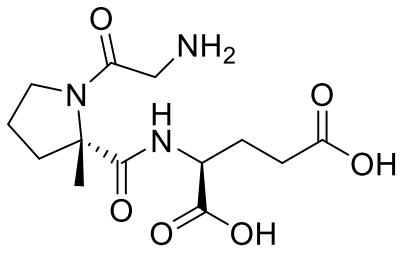
Introduction



Glypromate®



Trofinetide ®

Trofinetide® exhibits increased half-life in blood and brain with the retention of the neuroprotective efficacy of Glypromate.

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Introduction

Constrained proline mimetics:

- ✓ increase potency either by promoting a better fitting in a target molecule or by inducing an optimal conformation;
- ✓ improve stabilization toward enzymatic systems by a lack of or poorer recognition;
- ✓ increase specificity for a particular molecular target.

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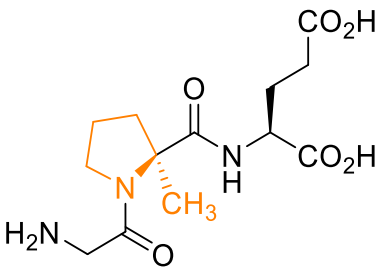
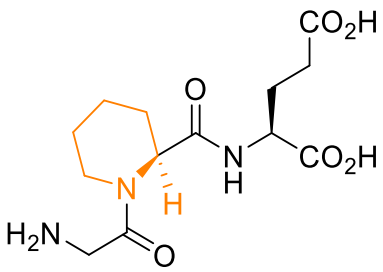
Results and discussion

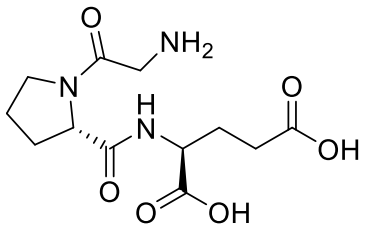


Trofinetide®

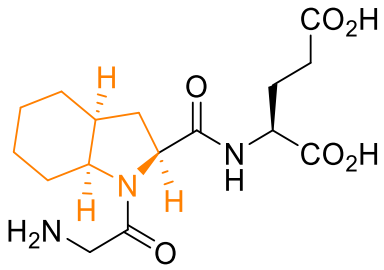


L-pipecolic acid derivative

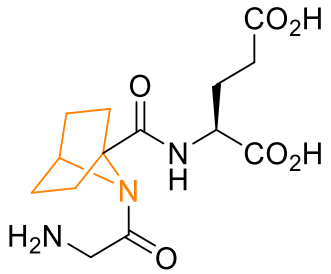
+



Glypromate®

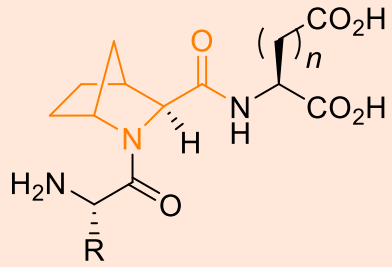


L-octahydroindole-2-carboxylic acid (Oic) derivative



7-azanorbornane-1-carboxylic acid (b_7Pro) derivative

This work:



Glypromate® constrained analogues

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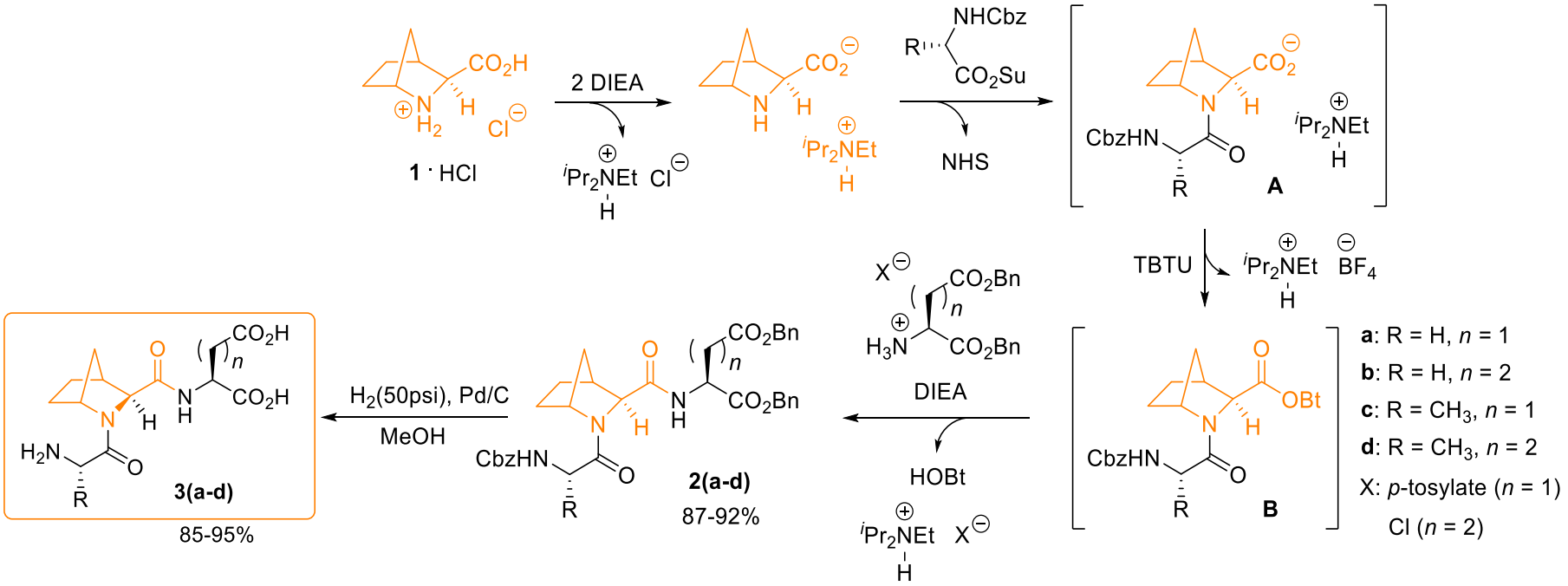
Results and discussion



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Results and discussion

Cytotoxicity in hAd-MSCs cells (MTS assay)

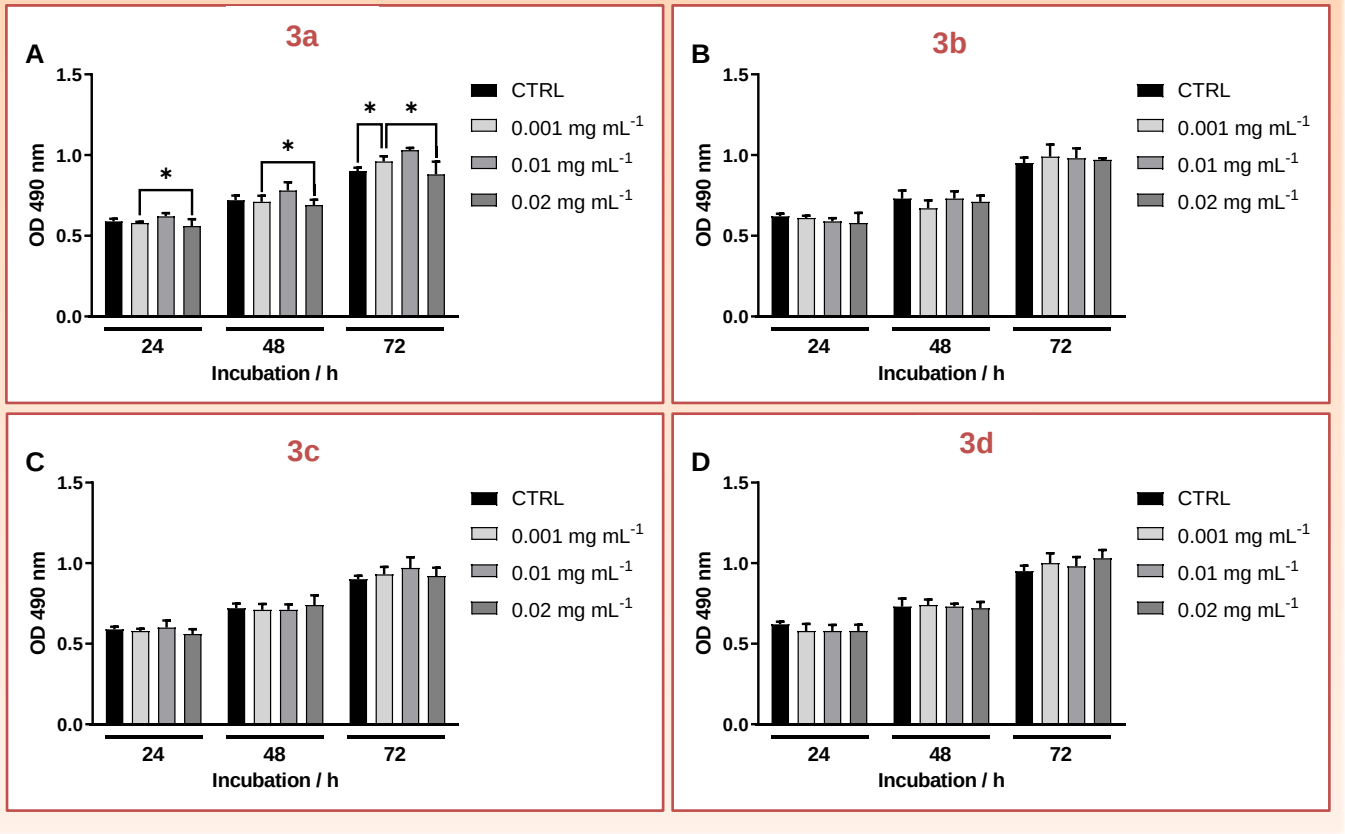

Figure 1. Cell viability of hAd-MSCs (MTS assay) after 24, 48, and 72 h incubation with peptidomimetics **3(a-d)** at different concentrations (0.001, 0.01, and 0.02 mg mL^{-1}). Standard culture medium was used as control (CTRL).

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Results and discussion

Neurotoxicity and Neuroprotection in SH-SY5Y cells (MTT assay)

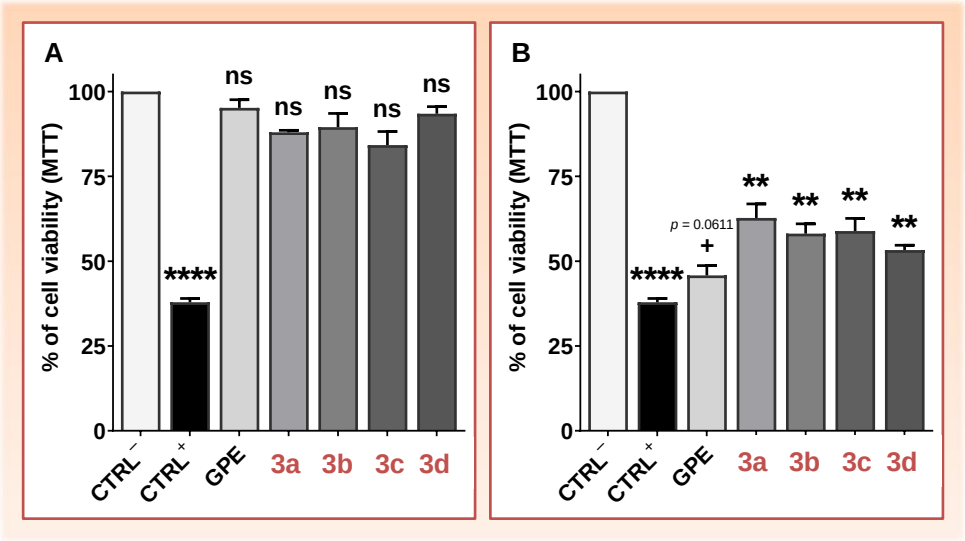

Figure 2. (A) SH-SY5Y cell viability (MTT assay) after 24 h period of incubation with Glypromate® and peptidomimetics **3(a-d)** at 100 μM concentration. **(B)** SH-SY5Y cell viability (MTT assay) after 1 h incubation with Glypromate® and peptidomimetics **3(a-d)** at 100 μM concentration, followed by further 24 h incubation period in the presence of 25 μM of 6-OHDA.

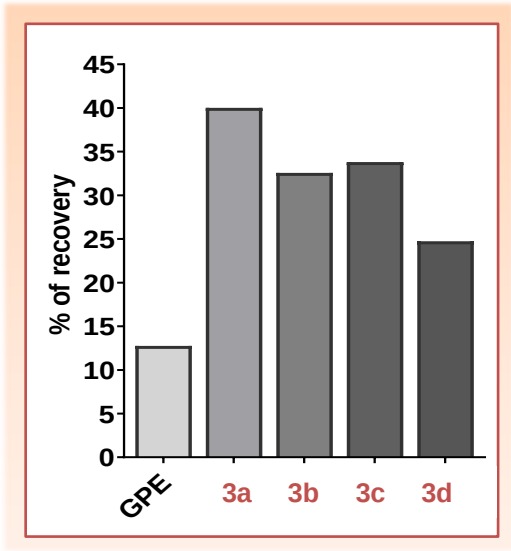

Figure 3. Percentage (%) of SH-SY5Y cells recovery after exposure to 25 μM 6-OHDA in the presence of GPE and **3(a-d)** at 100 μM.

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Conclusions

- A small library of Glypromate® peptidomimetics incorporating chiral bicyclic construct as a constrained L-proline and L-pipecolic acid surrogate was successfully synthesized and biologically evaluated.

- None of the tested peptidomimetics display cytotoxic effects on hAd-MSCs up to 0.02 mg mL^{-1}.

- A statistically significant increase in cell proliferation was observed for **3a** at the lowest concentration (0.001 mg mL^{-1}).

- Peptidomimetics **3(a-d)** display better neuroprotective profiles than the parent neuropeptide, being bioactive in the micromolar range (while Glypromate® only exhibits neuroprotection at 1 mM concentration), with recovery values above 20-40%.

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Design, Synthesis, and Biological Evaluation of Hybrid Glypromate Analogues Using 2-Azanorbornane as a Prolyl and Pipecolyl Surrogate

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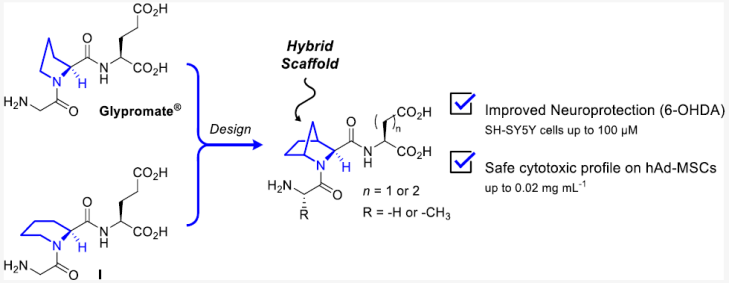

ABSTRACT: Neurodegenerative disorders of the central nervous system are a class of heterogeneous pathologies affecting millions of people worldwide and represent a global health burden in developed and developing countries. Without restorative treatments currently available, research on neuroprotective drugs is considered a health priority. In this study, new analogues of the glycyl-L-prolyl-L-glutamic acid (Glypromate) neuropeptide were designed, synthesized, and biologically evaluated using (1*R*,3*S*,4*S*)-2-azanorbornane-3-carboxylic acid as a hybrid construct of L-proline and L-pipecolic acid. Neuroprotection assays carried out in human neuroblastoma SH-SY5Y cells using 6-hydroxydopamine as a stress inducer showed great percentage of recovery (29.7−40.0%) at 100 μM. Among this series, [(1*R*,3*S*,4*S*)-2-glycyl-2-azanorbornane-3-carbonyl]-L-aspartic acid (**2a**) stands out with a remarkable percentage of recovery (40.0%, at 100 μM) and safe toxicological profile in SH-SY5Y and human adipose mesenchymal stem cells.

KEYWORDS: *glypromate, GPE, hybrid scaffolds, neuroprotective drugs, peptidomimetics*

Acknowledgments

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