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Development of Neurotensin(8-13) Analogs as Potent NTS1/NTS2 Receptor Ligands with Enhanced Effects on a Mouse Model of Parkinson's Disease

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Introduction

The tridecapeptide Neurotensin (NT, **1**) is widely expressed throughout the central nervous system (CNS) and periphery [1,2]. NT exerts its neurophysiological effects mainly by interactions with the human NT receptors type 1 (hNTS1) and 2 (hNTS2) [1]. In the brain, NT modulates directly or indirectly dopamine (DA) neurotransmission through different neuronal mechanisms, including antagonistic NT/D_2 receptor interaction and second messenger-dependent receptor alteration [3,4]. Moreover, both NTS1 and NTS2 receptors are also found in high concentrations on dopaminergic neurons in substantia nigra (SN), suggesting that the activation of both NTS1 and NTS2 receptors in this brain region by NT may stimulate the DA release [3,4]. Accordingly, the main effect of NT binding in SN is a D_2 autoreceptor inhibition with a subsequent increase of DA signaling [5,6]. Therefore, both receptor subtypes are promising targets for the development of novel NT-based analogs for the treatment of Parkinson's disease (PD).

In the current study we aim at:

- Developing NT(8-13) peptidomimetics by using virtually guided two-step protein structure homology/molecular modelling approach (Fig. 1 and 2, Scheme 1, and Table 1) [2].
- Identifying of NTS1/NTS2 dual-specific NT(8-13) analogues with *in vitro* (by radioligand binding studies) and *in vivo* (in a MPTP-induced mouse model of PD) confirmed efficacy against PD (Fig. 3).

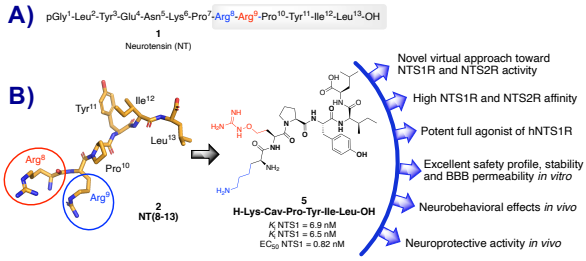


Figure 1: A) Amino acid sequences of neurotensin (NT); B) NT(8-13) (**2**, left), the biologically active fragment of neurotensin (NT, **1**), and the most potent C-terminal hexapeptide H-Arg⁸-Arg⁹-Pro¹⁰-Tyr¹¹-Ile¹²-Leu¹³-OH as dual NTS1R/NTS2R ligand (middle) as well as the investigated herein in vitro and in vivo properties (right).

Homology modeling of hNTRs and synthesis

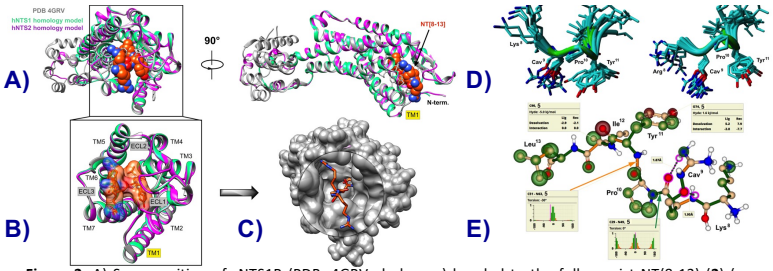
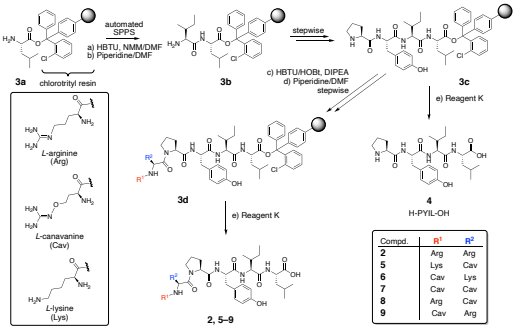


Figure 2: A) Superposition of rNTS1R (PDB: 4GRV, dark grey) bonded to the full agonist NT(8-13) (**2**) (orange spheres) with the homology models of hNTS1R (green) and hNTS2R (magenta). B) Extracellular receptor side of rNTS1R and hhNTRs with the binding side of **2**. C) Space filling model of the superimposed receptors (dark grey) with **2** (orange sticks) [5]. D) NMR structures of **5** (left) and **8** (right). E) HYdrogen DEsolvation (HYDE) visual assessment of **5** into the hhNTS1R. HYDE coloring and torsion analysis: **green** = good, **red** = bad for affinity [6].



Scheme 1: Synthesis of H-PYIL-OH (**4**), NT(8-13) (**2**) and its analogs **5–9**.

Prediction *vs.* Experiment

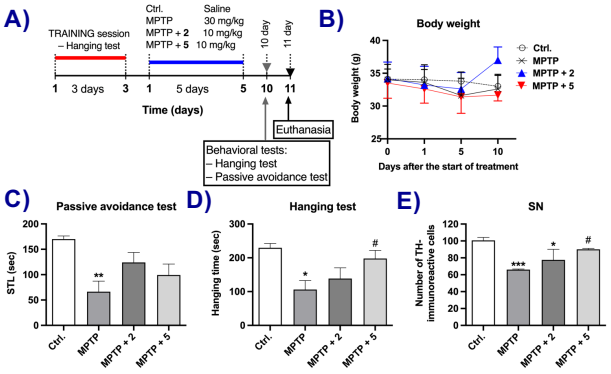


Figure 3. A) Experimental design of the *in vivo* studies in a mice model of MPTP-induced PD. B) Body weight change in mice. C) Effects of **2** and **5** on the memory and motor (D) disturbances. E) Attenuation of the MPTP-induced nigrostriatal neuronal damage in SN [2].

Peptide	K_i binding (nM ± SEM) [1] (experiment)		$K_{i\,HYDE}$ ranges (nM) (SeeSAR/HYDE prediction)		SI [4]	G-protein activation [5]
	*hh*NTS1	*hh*NTS2	*hh*NTS1	*hh*NTS2		EC_{50} (nM ± SEM
NT(8-13) (**2**)	0.68 ± 0.04 [2] 1.07 ± 0.05 [3]	1.8 ± 0.17 [2] 6.6 ± 2.18 [3]	0.13–12.6	4.28–425	0.38 0.16 [3]	0.88 ± 0.10
4	>50,000	>50,000	n.a.	n.a.	n.a.	3,200 ± 320
5	**6.9 ± 0.59**	**6.5 ±0.63**	**0.10–10.3**	**3.23–321**	**1.06**	**0.82 ± 0.16**
6	15 ± 4.5	14 ± 4.4	0.15–15.0	0.31–30.5	1.07	0.85 ± 0.06
7	60 ± 5.4	54 ± 11	0.35–34.7	3.47–344	1.11	1.30 ± 0.26
8	5.5 ± 1.7	5.6 ± 1.0	2.35–233	2.94–292	0.98	0.74 + 0.19
9	4.2 ± 0.62	5.8 ± 0.65	1.19–118	1.56–154	0.72	0.89 ± 0.15

Table 1. Receptor binding data for human (h) and HYDE estimated *binding affinity* for human homolog (hh) NTS1 and NTS2 receptor of NT(8-13) (**2**) and ligands **4–9** [2].
[1] Determined by radioligand competition binding with [^{3}H]**2** at HEK293T cells (N = 3). [2] K_D value in nM ± SEM.[3] Data are from Ref. [6]. [4] Selectivity index: SI = K_i (hNTS1R)/K_i (hNTS2R). [5] IP-One® assay (Ciscbio) with HEK293T cells transiently transfected with hNTS1R [2]. n.a. = not applicable.

Conclusions

SeeSAR/HYDE concept: An innovative drug design concept combining a homology modeling of hNTS1/2 receptors with the molecular modelling platform SeeSAR/HYDE was used to predict the binding affinities ($K_{i\,HYDE}$ ranges) towards both hNTRs of a small set of NT(8-13) (**2**) analogs (peptides **5–9**).

Proof-of-concept: Consequently, we synthesized such series of NT(8-13) analogs **5–9** with modifications at positions 8 and/or 9 in NT(8-13) and determined the 3D-structures of **5** and **8** using 2D-NMR. The experimental binding affinities (K_i values) for peptides **5–9** reproduced their predicted $K_{i\,HYDE}$ ranges. I*n vivo* evaluation of **5** suggested that dual NTS1/NTS2-acting NT(8-13) peptidomimetics represent an attractive strategy for the treatment of PD and possibly other CNS disorders.

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