Neurotensin(8-13) Analogs Targeting NTS1 and NTS2 Receptors: A Comparative *In vitro* and Molecular Modeling Study

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The simultaneous activation of both neurotensin type 1 and 2 receptors (NTS1R and NTS2R) through the neuronal peptide neurotensin (NT), activating the dopamine (DA) release and DA signaling within the dopaminergic system in the brain, suggest that NTS1R/NTS2R dual-specific NT analogs may represent an attractive tool in the treatment of Parkinson's disease (PD) and/or other related conditions.[1,2] Herein, we report *in silico* exploration of NTS1R and NTS2R driven by *in vitro* pharmacological evaluation of the linear hexapeptide NT analogs **3** (sequence Lys^{8}-Cav^{9}-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}) and **6** (Arg^{8}-Cav^{9}-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}), both active towards the human NTS1R and NTS2R. Compared to the parent peptide NT(8-13), compounds **3** and **6** showed improved *in vitro* human plasma stability and BBB permeability (Figure 1). Moreover, *in silico* ADMET evaluation indicated that both NT-analogs have strong pharmacological properties combined with good safety profiles, highlighting their potential for further structural improvements. Furthermore, we applied an AI-based approach to generate the homology models of hNTS1R and hNTS2R, followed by MD simulations of their ligand-free state and molecular docking in order to estimate the most probable protein–ligand complexes of peptides **3** and **6**. Binding interaction/affinity analysis of the best-ranked docking modes, obtained with selected time-frames from the respective MD trajectories, suggest that the receptor activation occurs via a ligand-receptor binding into the initial "entry" conformation of hNTS1R and hNTS2R (Figure 2). This assumption is supported by additional HYDE analysis confirming the binding affinities of peptides **3** and **6** towards hNTS1R and hNTS2R obtained by radioligand binding experiments (Figure 3).[3] The reported study may serve as a ready-to-use *in silico* approach for further development of therapeutic options against PD and potentially other neurological disorders.

		NT(8-13)	Lys[8]-Cav[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13] (3)	Arg[8]-Cav[9]-Pro[10]-Tyr[11]-Ile[12]-Leu[13] (6)
Binding Affinity Data				
EXPERIMENT K_i binding (nM)	**hNTS1R**	0.68 ± 0.04 (K_d)	**6.9 ± 0.59**	**5.5 ± 1.7**
	hNTS2R	1.8 ± 0.17 (K_d)	**6.5 ± 0.63**	**5.6 ± 1.0**
Selectivity K_i (hNTS1R) / K_i hNTS2R)		0.38	1.06	0.98
PREDICTION $K_{i\ HYDE}$ range (nM)	**hNTS1R**	0.13–12.6	**0.43–43.2** (MD, „entry" pose 1)	**2.95–293** (MD, „entry" pose 1)
	hNTS2R	4.28–42.5	**6.13–609** (MD, „entry" poase 1)	**1.16–115** (MD, „entry" pose 1)
ADME Data				
Peptide stability (@50 µM)				
% remaining @24 h, pH 2.0/7.4		100 / 100	100 / 100	100 / 100
% remaining >30 d, pH 2.0/7.4		> 97 / 100	> 97 / 100	> 94 / > 95
Plasma stability (@30 min, after 5 min @37°C)				
% remaining @100 µM		0.02	0.65	0.22
$t_{1/2}$ (min) @100 µM		< 2.5	< 7.3	< 3.7
PAMPA permeability				
P_e (×10^{-6} cm/s) @pH 7.4		0.64 ± 0.20	**2.81 ± 0.45**	0.89 ± 0.10
–logP_e		6.12 ± 0.13	**5.55 ± 0.07**	6.05 ± 0.03

Figure 1. *In vitro* data for NT(8-13) and selected peptides **3** and **6**.[2,3]

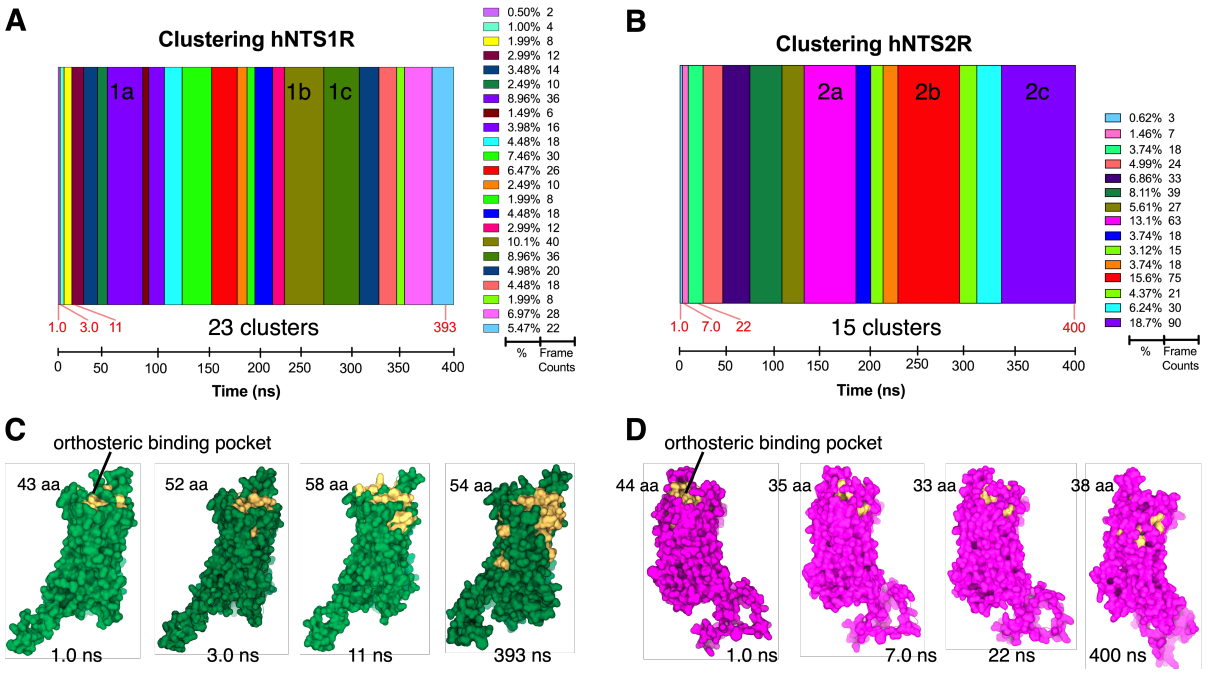


Figure 2. Molecular dynamic simulations with apo-state of hNTSRs showing the most distributed clusters (**1a–1c**) for hNTS1R (**A**) and (**2a–2c)** for hNTS2R (**B**). Flexible *vs.* more "closed" conformational extracellular changes of the actives site from 43 to 54 aa for hNTS1R (**C**) and from 44 to 38 for hNTS2R (**D**), respectively.[3]

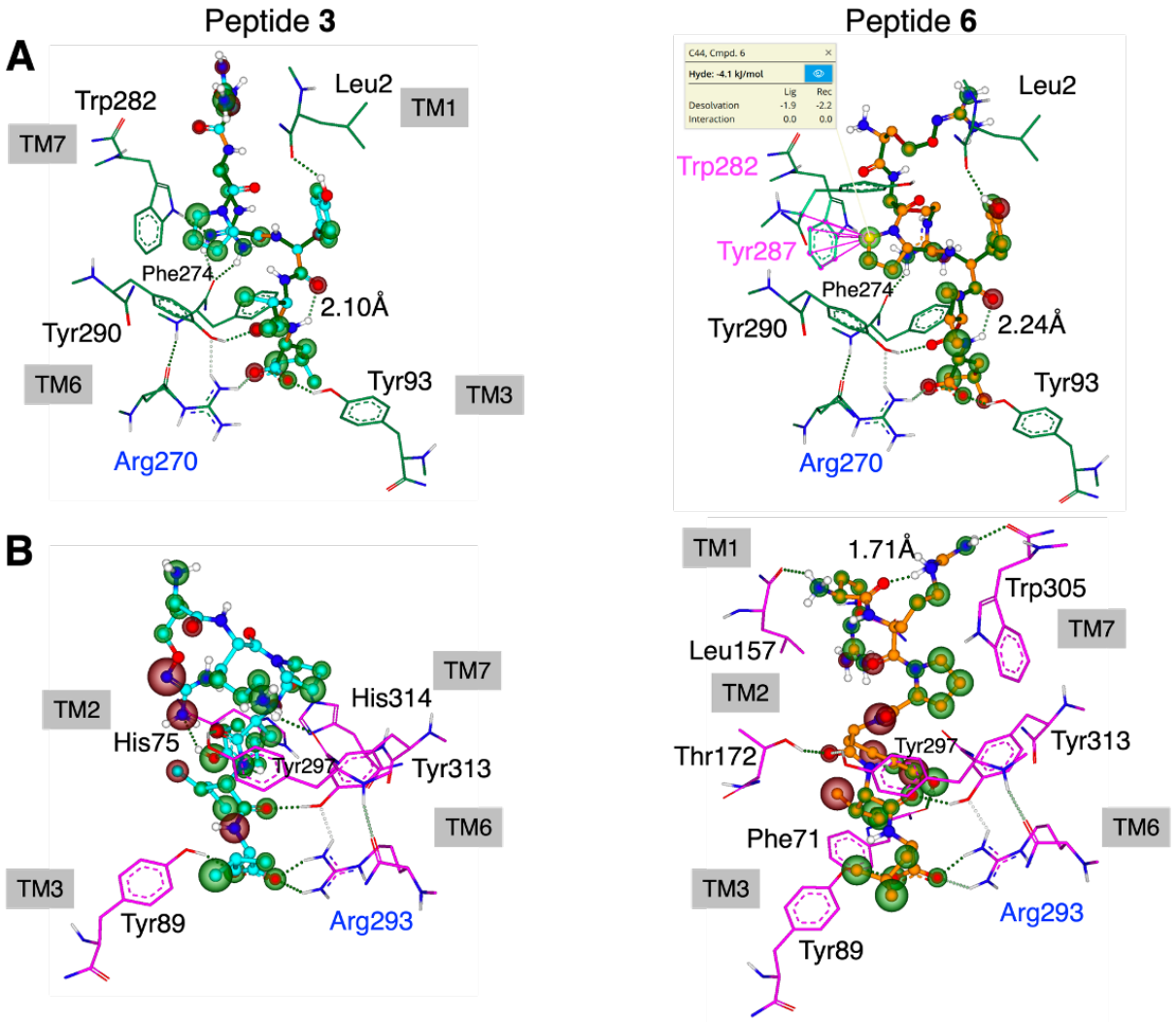


Figure 3. Docking models for the best-ranked poses of peptides **3** and **6** using ”entry” conformations at 1.0 ns MD with hNTS1R (**A**) and hNTS2R (**B**).

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