Journal of Computational Methods in Molecular Design, *2017, 7 (1):11-16*

ISSN : 2231- 3176
CODEN (USA): JCMMD

Docking Studies of the New Neurotensin Analogues

Tatyana Dzimbova*, Tamara Pajpanova

Institute of Molecular Biology "Roumen Tsanev" Bulgarian Academy of Sciences, 21 acad. G. Bonchev str., Sofia, 1113, Bulgaria

Corresponding Email: tania_dzimbova@abv.bg

ABSTRACT

Neurotensin (NT) is a neurohormone and/or neuromodulator containing 13 amino acid residues. Neurotensin receptors (NTR) are transmembrane receptors that bind neurotensin. The active fragment of neurotensin is 8-13 (RRPYIL). The main drawback in the use of NT or any other endogenous peptide as a drug is extremely short half-life as a result of their rapid degradation by the action of peptidases. To overcome this problem, various neurotensin analogues were synthesized, which include linear peptides, cyclic peptides and nonpeptide molecules. Chemical modification of the native peptide, however, may result in a radical change in the receptor affinity and specificity. The purpose of this work is to identify interactions between newly synthesized neurotensin analogues and neurotensin receptor (NTR1) and to establish the relationship between structure and action using computational methods. All new analogues were synthesized using SPPS. The results of docking shows that the tested analogues of neurotensin bind in the active site of the receptor, but significantly weaker than NT. Replacement of arginine residue in the second position with canavanine strongly increases the total energy of the ligand-receptor complex, therefore it is more unstable than the complex NT/NTR1.

Keywords: Neurotensin, Neurotensin receptor, Docking, GOLD, Canavanine

INTRODUCTION

Neurotensin (NT) is a 13-amino-acid peptide originally isolated in 1973 from bovine hypothalamus [1]. This neuropeptide is found in the central nervous system [2], as well as in the gastrointestinal tract [3,4]. Neurotensin (NTS) functions both as a neurotransmitter and a hormone through the activation of the neurotensin receptor NTR1, a G-protein-coupled receptor (GPCR) [5]. In the brain, NTR1 modulates the activity of dopaminergic systems, opioid-independent analgesia, and the inhibition of food intake; in the gut, NTR1 regulates a range of digestive processes [6]. The tridecapeptide neurotensin (NT) mediates its central and peripheral effects through interaction with three identified receptor subtypes. In the periphery, NT induces hypotension [7], decreases gastric acid secretion [8], activates lipid digestion and protects pancreatic beta cells from cytotoxic agent-induced apoptosis [9]. Activation of NTR1 is probably responsible for the observed effects of NT on cancer cell proliferation and food intake. However, the most convincing implication of NTR1 is related to the NT-dopamine interactions in the brain. Indeed, NT modulates dopamine transmission in the nigro-striatal and mesocorticolimbic pathways through NTR1, indicating that NT analogues specifically targeting this receptor might represent a new class of antipsychotic drugs [10].

It is well-known that the active fragment of neurotensin is 8-13 (RRPYIL). The main drawback in the use of NT or any other endogenous peptide as a drug is extremely short half-life as a result of their rapid degradation by the action of peptidases. To overcome this problem, various neurotensin analogues were synthesized, which include linear peptides, cyclic peptides and non-peptide molecules. Chemical modification of the native peptide, however, may result in a radical change in receptor affinity and specificity. The design of new biologically active peptide is successful when interactions with the receptor are known. Particularly important interactions in the binding site of NTR1 are hydrogen bonds formed between free carboxyl group of neurotensin and Arg327 and Tyr146, interactions between tyrosine hydroxyl group and Leu55 and guanidino-group of the second arginine residue and Asp336 and Phe331 [11] (Figure 1).

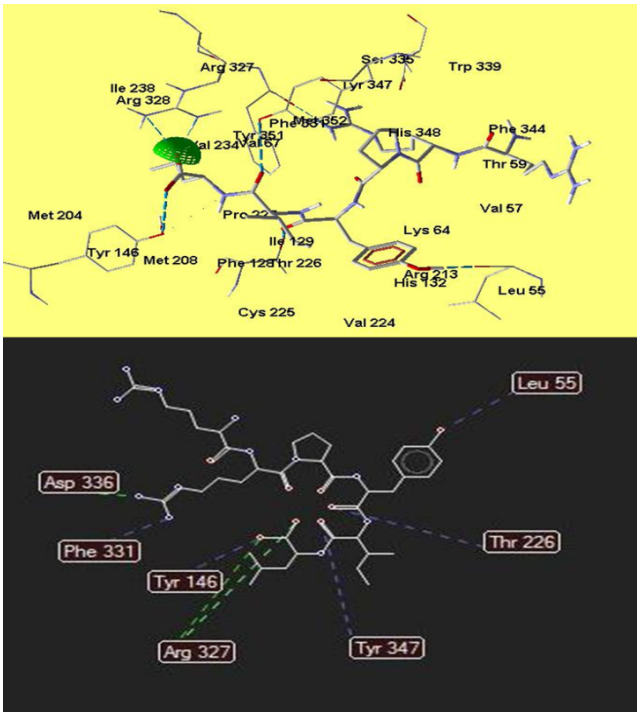


Figure 1: The interactions in the binding site of neurotensin receptor (NTR1) with neurotensin

The aim of the presented work is to study the interactions of the newly synthesized neurotensin analogues and NTR1 using docking and determine some relationships between their structures and biological activity.

MATERIALS AND THEORETICAL METHODS

Crystal structure of the neurotensin receptor (NTR1) was obtained from RCSB (PDB id: 4grv) [11]. Ligand preparation was done with Avogadro (an open-source molecular builder and visualization tool – Version 1.0.3) [12]. Docking studies were performed by using GOLD 5.2 (Genetic Optimization for Ligand Docking) [13], run on Scientific LINUX 5.5 operating system. Search space was 10 Å around the residue Arg327. ChemPLP scoring function was used:

$$fitness_{PLP} = -(w_{PLP} \cdot f_{PLP} + W_{lig\text{-}clash} \cdot f_{lig\text{-}clash} + W_{lig\text{-}tors} \cdot f_{lig\text{-}tors} + f_{chem\text{-}cov} + w_{prot} \cdot f_{chem\text{-}prot} + w_{cons} \cdot f_{cons})$$

$$fitness_{CHEMPLP} = fitness_{PLP} - (f_{chem\text{-}hb} + f_{chem\text{-}cho} + f_{chem\text{-}met})$$

PLP and CHEMPLP are empirical fitness functions optimized for pose prediction. CHEMPLP is the default scoring function in GOLD. In both cases, the Piecewise Linear Potential (f_{PLP}) is used to model the steric complementarity between protein and ligand, while for CHEMPLP additionally the distance and angle-dependent hydrogen and metal bonding terms from ChemScore are considered ($f_{chem\text{-}hb}$, $f_{chem\text{-}cho}$, $f_{chem\text{-}met}$). The internal score of the ligand consists of the heavy-atom clash potential ($f_{lig\text{-}clash}$) as well as the torsional potential used within ChemScore ($f_{lig\text{-}tors}$). Both fitness functions are capable of covalent docking ($f_{chem\text{-}cov}$), considering flexible sidechains ($f_{chem\text{-}prot}$) and explicit water molecules as well as handling constraints (f_{cons}). Parameters for both fitness functions can be altered by changing the files plp.params and chemplp.params for PLP and CHEMPLP, respectively. CHEMPLP parameters are used by default as they show on average an improved performance in pose prediction and virtual screening applications [14].

GOLD 5.2 was run independently 10 times and the best score for each ligand was calculated. For generation figures Molegro Molecular Viewer was used [15].

RESULTS

The docking of newly synthesized analogues of neurotensin with NTR1 was performed using GOLD 5.2. After ten independent runs of the program the best pose for every ligand was chosen and the total energy of the formed complex with NTR1 was calculated using Molegro Molecular Viewer. They are presented in the Table 1.

All analogues bind in the active site of the receptor and their interactions are presented in several pictures. The

interactions of NTCA with the receptor active site are shown on Figure 2. The free carboxylic group of NTCA interacts electrostatically with His348. Oxyguanidino-group forms hydrogen bonds with residues Tyr324 and Asp150. Guanidine-group bind to Phe128 and His348.

Table 1: The total energies of ligand receptor complexes of neurotensin analogues with NTR1

Ligand	Total energy of ligand-receptor complex
NT	-192.986
NTAC	-161.894
NTCA	-166.024
NTCC	-149.059
NTCL	-160.481
NTLC	-179.656

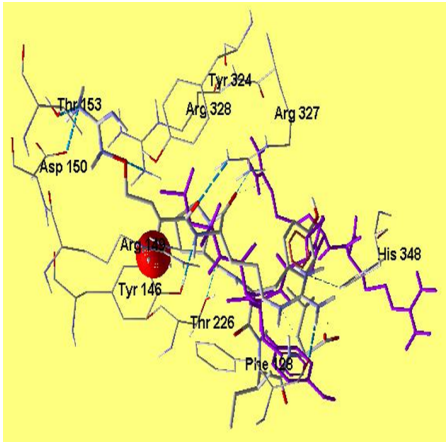


Figure 2: The interactions in the binding site of NTR1 with NTCA. Neurotensin is presented in purple

In the case of NTAC oxyguanidino-group forms hydrogen bonds with Tyr351, His348 and Tyr324, guanidine-group with Arg328, and free carboxyl group interacts electrostatically with Arg213 (Figure 3A). In the case NTCC there is no interaction of the first oxyguanidino-group with receptor amino acid residue, the second bind to Tyr351 and Tyr146, and the free carboxyl group is interacting again with Arg213 (Figure 3B).

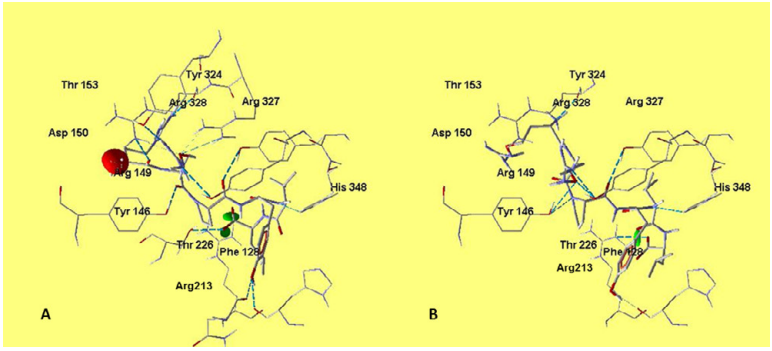


Figure 3: The interactions in the binding site of NTR1 with NTAC (A) and NTCC (B)

When NTLC interacts with NTR1, oxyguanidino-group binds by forming hydrogen bonds with the receptor through residues Tyr324 and Asn355. The free carboxyl group is not interacting with Arg327, but with His348. The free amino-group in the side chain of the lysine residue of the analogue is not involved in interactions with the receptor (Figure 4A). In the case of NTCL Arg327 together with Arg149 and Asn355 forms hydrogen bonds with amino group of the side chain of Lys, oxyguanidino-group binds to Val234 and Thr207 and Arg213 interacts electrostatically with free carboxyl group (Figure 4B).

DISCUSSION

In our work we use newly synthesized analogues of NT (8-13), where arginine residues are replaced with two different amino acids. One of them is antimetabolite of arginine canavanine (Figure 5). This is a natural nonproteinogenic

amino acid from some plants [16]. Difference in the structure is that the last CH2 group in the side chain is replased by oxygen atom. This change leads to reducing the pKA value of the side chain and if in arginine it is 12.48, in canavanine it is 7.04. Replacement of arginine by lysine does not lead to the critical reducing of the basicity as the pKA of lysine side chain is 10.53, but number of nitrogen atoms that can bind to the receptor is reduced from 3 to 1.

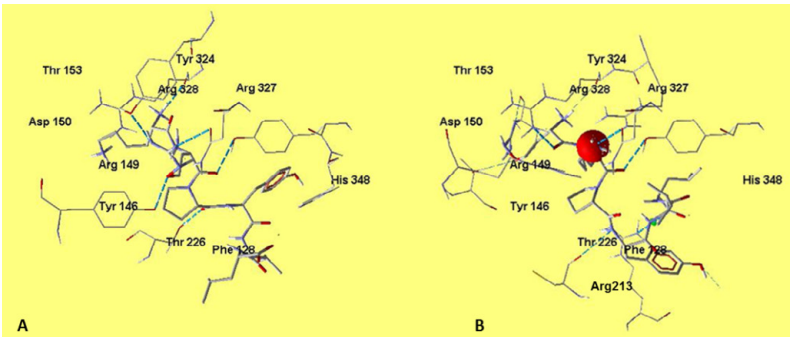


Figure 4: The interactions in the binding site of NTR1 with NTLC (A) and NTCL (B)

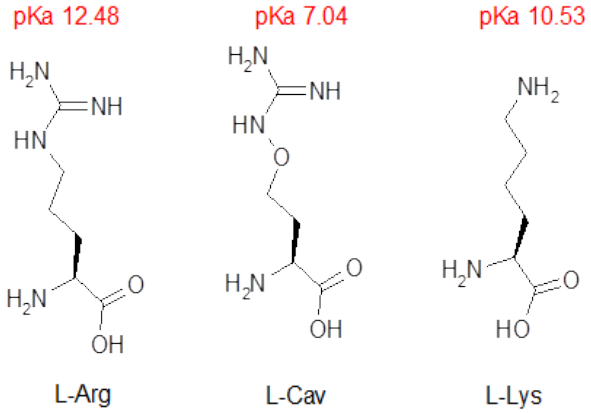
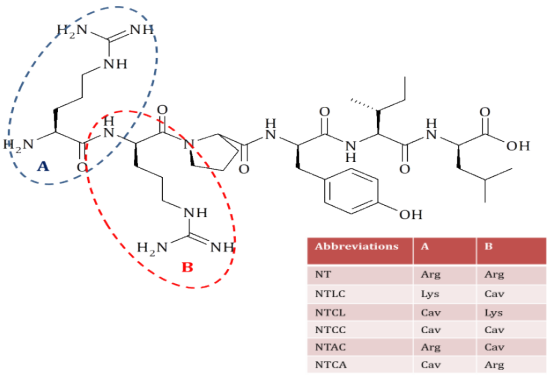


Figure 5: L-Arginine, L-canavanine and L-lysine structures and pK_A values of their side chains

There are two arginine residues in the molecule of neurotensin and we made changes on a both positions (Figure 6).



Abbreviations	A	B
NT	Arg	Arg
NTLC	Lys	Cav
NTCL	Cav	Lys
NTCC	Cav	Cav
NTAC	Arg	Cav
NTCA	Cav	Arg

Figure 6: Modifications in the active fragment of neurotensin

As can be seen from the data in the Table 1, NT the most tightly bound to the receptor. The energy of the complex that it forms with NTR1 is the lowest in the series of the tested compounds. Of particular importance for the recognition of the ligand by the receptor is the arginine residue in the second position. It interacts electrostatically with its guanidino group in the side chain with a carboxyl group of an aspartic acid residue Asp336. Furthermore, very important is a forming of a hydrogen bond with the nitrogen atom of the guanidino group and the carbonyl oxygen atom of Phe331. The free carboxyl group of neurotensin binds electrostatically with Arg327 and Tyr146 which is very important for recognition of the bioactive peptide.

All analogues bind in the active site of the receptor. In all of them the carboxyl group remains unchanged and it is expected to have typical interactions with the receptor. None of them interacts with Arg327 and Tyr146. NTAC, NTCC and NTCL bind to Arg213 and NTCA and NTCL – to His348. These bindings are crucial for the disposal of the molecule of the analogue in the active site of NTR1 thus changing all interactions with the receptor.

Only in one other analogue there is unchanged arginine residue in the second position. This is NTCA. In this case, however, interactions with characteristic amino acid residues of the receptor are not observed. The analogue molecule is positioned differently than NT in the binding site of NTR1 and hence binds to the different amino acid residues. There is missing electrostatic interaction of the guanidino group with Asp336, and the free carboxylic group of NTCA interacts electrostatically with His348, and not with Arg327 (Figure 2).

When arginine in the second position was replaced by canavanine (NTAC, NTCC and NTLC) oxyguanidino-group cannot play the role of guanidino-group because of its reduced basicity. The interactions of guanidino-group are very important for the activation of the receptor and their lack provokes changes in the biological action of the analogues. As it is expected replacement of the arginine in the second position with lysine leads to the analogue (NTCL) that interacts with the receptor less than NT.

Analyzing the results of the docking can be clearly seen that the tested analogues of neurotensin bind in the active site of the receptor, but significantly weaker than NT. The substitution of arginine residues in the second position with canavanine greatly increases the total energy of the ligand-receptor complex; therefore it is more unstable than the complex NT/NTR1. The result of this study shows that if the effects of the new neurotensin analogues only depend on their ability to bind to NTR1, it would be significantly lower than the NT. On the other hand, however, it is necessary to carry out the docking studies of the stability of the analogues in respect of peptidases as we published previously for kyotorphin analogues [17]. For example, the fact that the arginine was replaced with canavanine will likely increase their stability. The increased stability means that the compound will have the ability to provide a longer time its effect.

CONCLUSION

There are four key conclusions from presented study:

1. In various modifications of amino acids and at different positions in neurotensin (8-13) molecule, the resulting analogs can bind in the active site of the neurotensin receptor;

2. In case of replacement of arginine residues in a second position of the active fragment of neurotensin (8-13) with another basic amino acid, for example lysine, typical interactions with the active site of the receptor is retained, albeit weaker;

3. Replacement of the arginine residue with canavanine affords analogue with reduced basicity which in turn strongly influences the ability to bind to the receptor;

4. The studied analogues of neurotensin receptor form complexes with higher total energy than that of the neurotensin with the receptor, i.e., formed complexes are unstable; therefore these analogues are not antagonists of the neurotensin receptor.

ACKNOWLEDGEMENT

This work is partially supported by the project of the Bulgarian National Science Fund, entitled: “Bioinformatics research: protein folding, docking and prediction of biological activity”, code NSF I02/16, 12.12.14.

REFERENCES

[1] Carraway, R. and Leeman, S.E., *J Biol Chem,* **1973**. 248: p. 6854.

[2] St-Gelais, F., Jomphe, C. and Trudeau, L., *J Psychiatry Neurosci,* **2006**. 31: p. 229.

[3] Eysselein Klin, V.E., *Wochenschr,* **1984**: 62: p. 523.

[4] Kulinska-Niedziela, I. and Paluszak, J., *Postepy Hig Med Dosw,* **1997**. 51: 329.

[5] Vincent, J.P., Mazella, J. and Kitabgi, P., *Trends Pharmacol Sci*, **1999**. 20: p. 302.

[6] Kleczkowska, P. and Lipkowski, A.W., *Eur J Pharmacol*, **2013**: 716: p. 54.

[7] Zogovic, B. and Pilowsky, P.M., *Br J Pharmacol*, **2012.** 166: p. 378.

[8] Fletcher, D.R., Shulkes, A. and Hardy, K.J., *Regul Pept*, **1985**. 11: p. 217.

[9] Wang, J.G., et al., *Neuropeptides*, **2011**. 45: p. 151.
[10] Binder, E.B., et al., *Pharmacol Rev*, **2001**. 53: p. 453.
[11] White, J.F., et al., *Nature*, **2012**. 490: p. 508.
[12] Hanwell, M.D., et al., *J Cheminf*, **2012**. 4: p. 17.
[13] Jones, G., et al., *J Mol Biol*, **1997**. 267: p. 727.
[14] Korb, O., Stutzle, T. and Exner, T.E., *Springer Verlag*, Berlin, **2006.**
[15] Thomsen, R. and Christensen, M.H., *J Med Chem,* **2006**. 49: p. 3315.
[16] Rosenthal, G.A. and Dahlman, D.L., *Proc Natl Acad Sci*, **1986**. 83: p. 14.
[17] Dzimbova, T.A., Georgiev, K.D. and Pajpanova, T.I., *Eur J Biotechnol Biosci,* **2014**. 2: p. 21.