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Theoretical and biological studies of oxytocin containing conformationally fixed analogue of Tyrosine Tic(OH) (1,2,3,4-Tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid)

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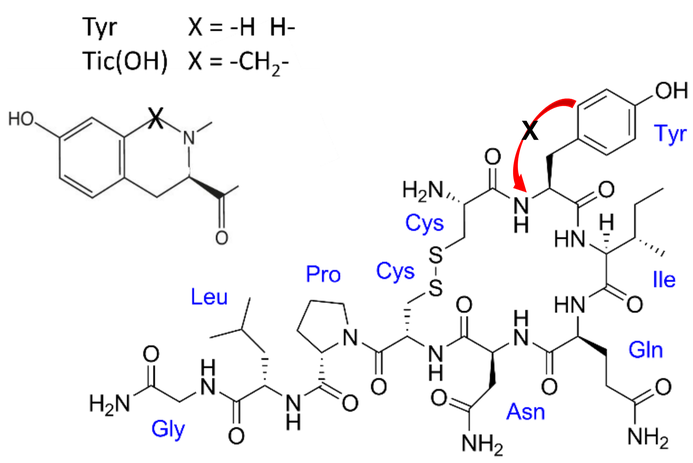


Introduction:

The most efficient tool for studying the effect of conformational stabilization of peptides is the cyclization of the peptide chain or replacement of the natural amino acid with an analog which has limited flexibility. While the cyclization inevitably modifies the functionality of the side chains and results in drastic conformational changes, introduction of modified amino acids brings less disturbance of the peptide chain. One of the example of conformationally stabilized amino acids is Tic (tetrahydroisoquinoline carboxylic acid) as a replacement of Phe. We studied earlier the effect of replacement of Phe by Tic on the biological activity of analogs of oxytocin (OXT)[1]. However, just the replacement of the native Tyr in position 2 of OXT by Phe has very pronounced effect on the biological activity of this hormone and therefore the effect of the stabilization of the conformation of this analog does not necessarily predict the effect of the same type of stabilization in the native molecule of OXT. We have developed the strategy to prepare the stabilized Tyr – 7-hydroxy-tetrahydroisoquinoline carboxylic acid (Tic(OH))[2] and made this amino acid commercially available (https://www.5z.com/spyderinstitute/unnaturals.html).

Earlier we speculated[3] about the interaction of the aromatic side chain of Tyr in OXT with the sulfur atoms in the cysteine in the disulfide bridge of this cyclic peptide based on the NMR studies of OXT carba analogs and about steric hindrance of the analogs with methyl groups attached to the cysteine methylene side chain in penicillamine containing analogs of OXT. In this study we wanted to answer the fundamental question whether the addition of one CH_2 group into the molecule of OXT will turn this hormone onto its antagonist.

1. Lebl, M.; Hill, P.; Kazmierski, W.; Karaszova, L.; Slaninova, J.; Fric, I.; Hruby, V. J. Conformationally restricted analogs of oxytocin; stabilization of inhibitory conformation. Int. J. Peptide Prot. Res. 1990, 36 (4), 321-330.
2. Verschueren, K.; Toth, G.; Tourwe, D.; Lebl, M.; Van Binst, G.; Hruby, V. J. Facile synthesis of 1,2,3,4-tetrahydro-7-hydroxyisoquinoline-3-carboxylic acid, a conformationally constrained tyrosine analogue. Synthesis-Stuttgart 1992, 458-460.
3. Lebl, M.; Sugg, E. E.; Hruby, V. J. Proton NMR spectroscopic evidence for sulfur-aromatic interactions in peptides. Int. J. Peptide Prot. Res. 1987, 29 (1), 40-45.

Strategy:

Here we present the alternative approach to our earlier structure activity studies of the neurohypophysial hormones:

- Computational modelling of solution structure of two proposed analogs of oxytocin - [L-Tic(OH)[2]]OXT and [D-Tic(OH)[2]]OXT
- Docking of the selected conformations into oxytocin receptor
- Synthesis of an analog with higher probability of interaction with the receptor
- Biological evaluation of oxytocin receptor response.

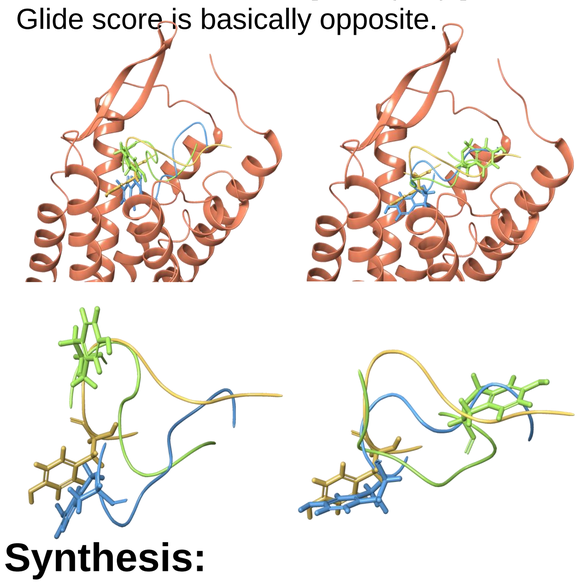
Computational approach:

The approach for in silico screening of the structure and binding activity of oxytocin ligands involves molecular dynamic simulations of free ligands (oxytocin and its variants) and subsequent docking of the ligands to the oxytocin receptor. Finally, the estimated free binding energies for each ligand to the receptor were used to compare the binding activity of oxytocin with its analogues. The NMR solution structure of oxytocin (pdb code 2MGO) was used as a template for the ligand structures. The first five structures stored in the pdb file were used.

The prepared ligands were surrounded by explicit waters (TIP3P) in an orthorhombic periodic box and subjected to 100 ns MD simulations in Desmond. The OPLS4 force field was used to define the ligand atoms. Ten thousand ligand geometries were regularly sampled during the simulation. The obtained MD trajectories (5) were analyzed using the Desmond Trajectory Clustering tool and the sampled ligand geometries were clustered according to their backbone conformation. Furthermore, only the twenty most abundant clusters were considered. For each cluster, a representative structure (5 trajectories x 20 clusters = 100 total / ligand) was stored and further used in docking.

The 100 stored geometries for each ligand were then docked into the oxytocin receptor using Glide. The binding site in the oxytocin receptor was defined according to the 7RYC pdb structure as a centroid within 10 Å of oxytocin. More than 1000 ligand-receptor poses (ligand conformations/orientations within the binding site) were docked and ranked according to their Glide SP score for oxytocin ligand. All poses were then rescored by estimating their binding energies (DG_{bind}) according to the MM-GBSA approach in Prime at the OPLS4/VSGB level.

Figure below displays [L-Tic(OH)[2]]OXT and [D-Tic(OH)[2]]OXT docked into the oxytocin receptor. As for oxytocin, two different poses (selection based on the Glide score or DG_{bind}) are shown for each ligand. The best oxytocin pose according to DG_{bind} is shown for comparison. We can see from Figure 4 that both considered poses of [L-Tic(OH)[2]]OXT in the receptor differ from the best pose of oxytocin, while the best pose of [D-Tic(OH)[2]]OXT selected according to DG_{bind} is relatively similar. Note that the orientation of [D-Tic(OH)[2]]OXT in the best pose according to the Glide score is basically opposite.



Zoom in on the two best poses (according to the Glide score in green, according to the MM-GBSA DG_{bind} in blue) of [L-Tic(OH)[2]]OXT (A) and [D-Tic(OH)[2]]OXT (B) in the oxytocin receptor (A). The best predicted structure of oxytocin in the receptor (according to the MM-GBSA DG_{bind}), shown for comparison, is in yellow.

Synthesis:

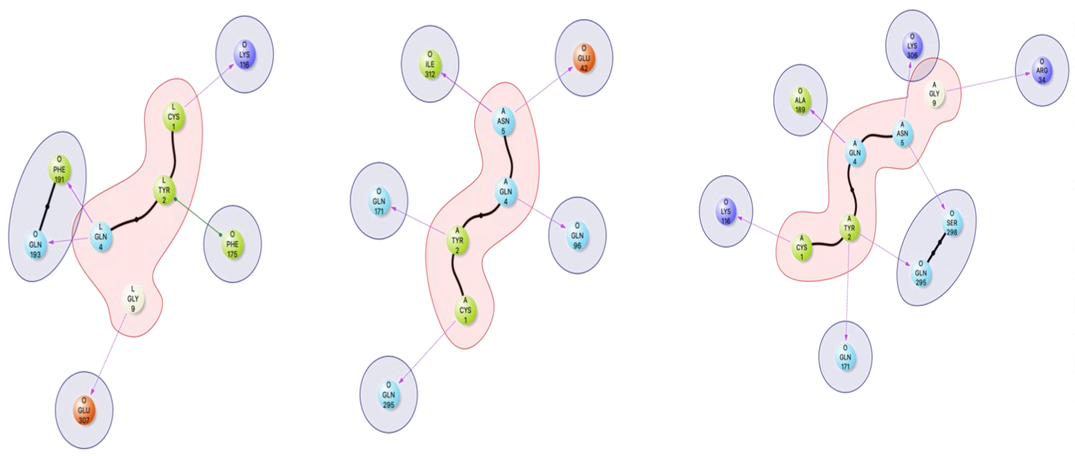
Since [D-Tic(OH)[2]]OXT showed higher probability of interaction with the oxytocin receptor, it was synthesized using classical solid phase strategy on an automatic centrifugal solid phase synthesizer Spyder Mark IV (https://www.5z.com/spyderinstitute/synthesizer.html).

Biological activity:

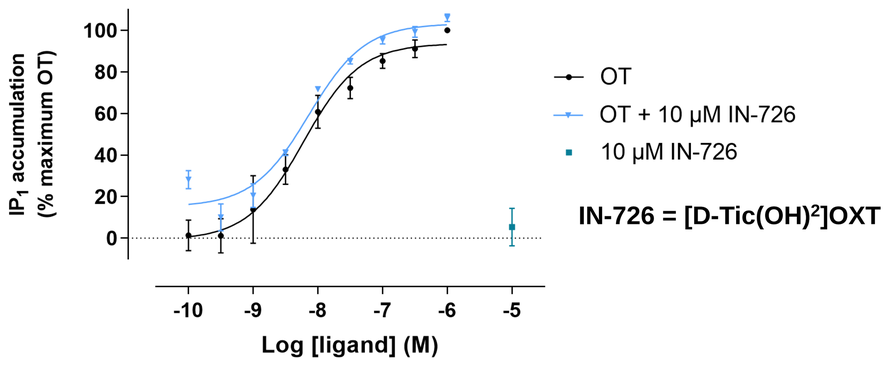
[D-Tic(OH)[2]]OXT was tested at 10 µM concentration as agonist (no activation) and as antagonist (the whole dose-response, no right-shift of the curve hence no displacement of oxytocin), both n=2.

Since the compound doesn't displace OXT in the antagonist assay it seems that the compound doesn't interact with the orthosteric binding pocket of the receptor. Based on the antagonist curve it doesn't seem to be an allosteric modulator either.

Finally, we can compare the predicted structures of all studied ligands in the oxytocin receptor in terms of energy and ligand-receptor interactions. Figure below compares interactions between amino acids of the receptor and the ligand for the best ligand-receptor poses selected according to DG_{bind}. We can see four H-bonding and one p-p interaction between oxytocin and the receptor. In the case of the modified oxytocins, we see five H-bonds for [L-(TIC(OH)2]OXT and even seven H-bonds for [D-Tic(OH)[2]]OXT. No p-p stacking was observed for the modified analogues. Most of the interactions involve different amino acids of the receptor. The binding energies DG_{bind} of individual oxytocin ligands in the oxytocin receptor are -63.4 kcal/mol for oxytocin, -41.2 kcal/mol for [L-TIC(OH)[2]]OXT and -53.9 kcal/mol for [D-Tic(OH)[2]]OXT. By definition, the more negative the binding energy, the stronger the affinity of the ligand for the receptor. Although MM-GBSA DG_{bind} energies are often overestimated in absolute values, they can usually be used for qualitative comparison of individual ligands. Thus, oxytocin seems to have the highest affinity towards the oxytocin receptor, followed by [D-Tic(OH)[2]]OXT and the weakest [L-Tic(OH)[2]]OXT.



Hydrogen bonding (magenta) and p-p (green) interactions of oxytocin (left), [L-Tic(OH)[2]]OXT (middle) and [D-Tic(OH)[2]]OXT (right) with the oxytocin receptor. The best predicted poses according to their MM-GBSA DG_{bind} energies were used.



Conclusion:

Even though the theoretical studies predicted possible interaction of an analog of oxytocin containing only one additional carbon atom to interact with the oxytocin receptor, the prepared analog did not show any interaction with the oxytocin receptor.