

# Rational Design, Synthesis and Evaluation of Modified Natural Peptides from *Boana pulchella* (anura) as Inhibitors of Human Cholinesterases <sup>†</sup>

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**Abstract:** Cholinesterase inhibitors are the most exploited compounds in the fight against Alzheimer's disease (AD), and those acting on both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are of special interest to drug developers. Some peptides with this property have been previously reported. In this work, we designed and synthesized substitution derivatives of a natural cholinesterase inhibitor 10-mer peptide (LL) to increase its inhibitory activity. The design was guided by a computational study of the inhibitor-enzyme complex, including flexible docking and molecular dynamics simulations. Specific residues of LL peptide were replaced by aromatics to introduce aromatic-aromatic interactions since LL was known to act near key aromatic residues of cholinesterases. Peptides were synthesized through solid Fmoc synthesis and tested against both recombinant human AChE and BChE. Substitution of proline with tryptophan resulted in an impressive 100-fold increase of the IC<sub>50</sub> of AChE (97.89±7.13µM to 0.99±0.02µM) and a 12-fold of BChE IC<sub>50</sub>. This compound thus becomes the most active peptide against cholinesterases ever reported. Another interesting derivative with two tryptophans presented an AChE of 1.70±0.05µM and BChE IC<sub>50</sub> of 9.40±0.48µM, an over 20-fold increase for this latter. We propose these sequences as potential scaffold candidates for developing new anti-AD drugs.

**Keywords:** drug design; peptides; inhibitors; cholinesterases; Alzheimer's disease.

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## **Conflicts of Interest**

The authors declare no conflict of interest.