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Novel AChE Inhibitory Peptides with Application in Aquaculture: A Bioinformatic Approach Through QSAR

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Introduction

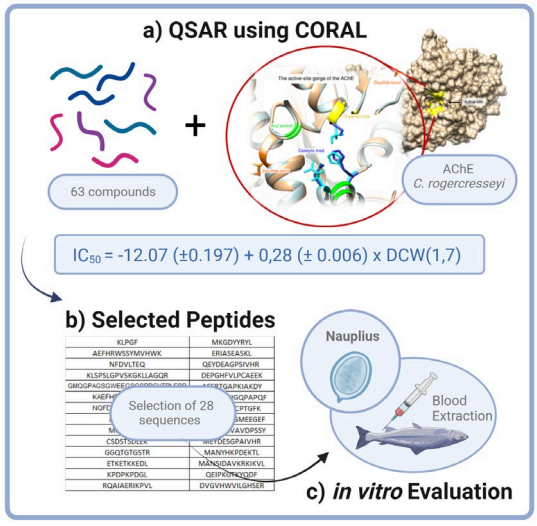


Fig. 1. a) QSAR design using CORAL. b) Selection of peptides with a promising IC_{50}. c) Antiparasitic activity and hemolytic activity.

In Chile, the salmon aquaculture industry is highly vulnerable to infections by ectoparasites, such as *Caligus rogercresseyi* [1], that greatly affect the fish's immune system making salmon susceptible to many diseases, thus causing large economic losses. Control of the sea louse relies on chemical treatments with organophosphates as Azamethiphos, able to inhibit the acethylcholinesterase (AChE) enzyme in the parasite; however, in 2014 reduced sensitivity was detected in Chile [2]. For this reason, in the search for new strategies for parasites elimination the use of new molecules like peptides able to inhibit the AChE in this parasite is proposed [3].

Up to now, studies of peptides in fish have focused on their bactericidal or antiviral properties, with little evidence of their participation in the control of parasites [4], so the use of antiparasitic peptides (APPs) is proposed to control or eliminate this kind of pest.

For this reason, bioinformatics tools were used under the application of Quantitative Structure-Activity Relationship (QSAR) [5] procedures in order to identify the main properties and structural characteristics responsible for antiparasitic activity, performing a simulation with active peptides described in the literature, followed by evaluation and construction of models with good statistical quality for validation.

The results allowed us to obtain 21 sequences with a promising IC_{50}. These sequences were synthesized by solid phase peptide synthesis using the Fmoc strategy and they were evaluated with the larval stage, nauplius of *C. rogercresseyi*. Four of those peptides showed antiparasitic activity with no hemolytic activity at the concentrations tested.

Results and Discussion

Initially a homology model was built for the *C. rogercresseyi* AChE 3D structure with ITasser server [6], and compared with the *Homo sapiens* structure (PDB ID 4EY4). High similarity was obtained, with conservation of the amino acids in the active site of the catalytic triad: SER203, GLU334, HIS447 are in blue color, and SER171, GLU299, HIS413 are in cyan color in Fig 2, referring to *H. sapiens* and *C. rogercresseyi* respectively. As can also be seen, the acyl pocket (in green), oxyanion hole (in yellow) and disulfide bonds (in orange) are superimposed. This similarity allows us to use the information of compounds directed against the human enzyme, taking advantage of the information obtained in databases and literature (Figure 2).

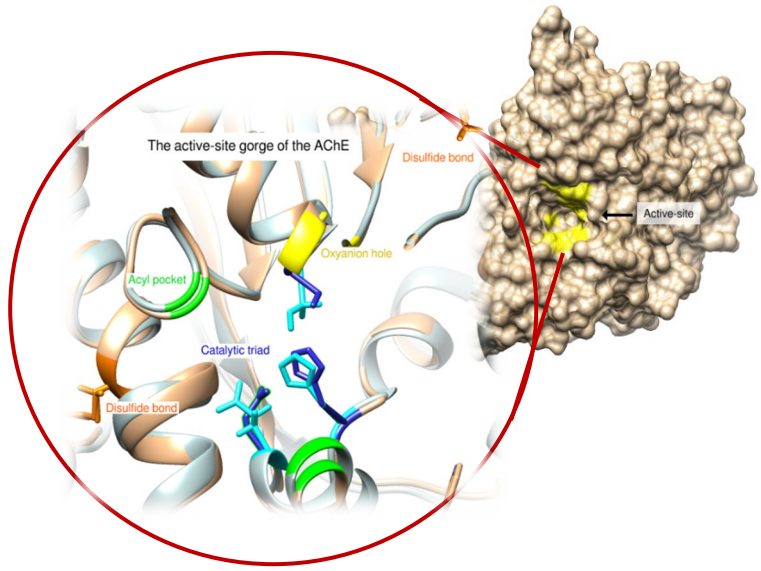


Fig. 2. Surface of the predicted 3D structure of C. rogercresseyi and the active-site gorge of the AChE.

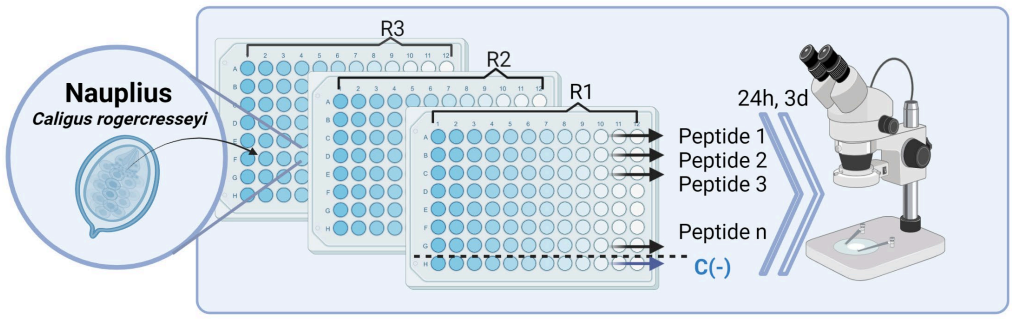
The construction of the QSAR model was carried out with the CORAL software. The compounds for the construction of the model were obtained from databases and literature with specific quantitative information about their activity. Compounds SMILES (Simplified Molecular Input Line Entry Specification) were used for their characterization. We used a total of 63 compounds and evaluated a set of 956 peptides obtained from *Salmo salar* mucus. The obtained model (Equation 1) resulted in the selection of 21 peptides with potential inhibitory activity of the enzyme AChE.

$$\text{Equation 1: } IC50 = -12.07\ (\pm 0.197) + 0.28(\pm 0.006) * DCW(1,7)$$

The selected peptide sequences were synthesized by the Fmoc/tBu standard solid phase strategy using a “tea-bag” protocol [7]. All peptides were characterized by electrospray-mass spectrometry (ESI–MS) in an LCMS-2020 ESI–MS equipment (Shimadzu Corp., Kyoto, Japan) and by high-performance liquid chromatography (HPLC) in a JASCO system (JASCO Corp., Tokyo, Japan).

The antiparasitic activity of the synthesized peptides were evaluated through screening of lethality. Lethality was determined considering death when they remain immobile lying at the bottom of the well. These larval stages were recorded using an optical microscope (Olympus model BX41) fitted with a 10 X objective. Through this assay, it was possible to obtain four peptide sequences (AS4525, AS4528, AS4531 and AS4532) with a lethality effect greater than 90% against the nauplius stage of *C. rogercresseyi* after 60 hours of exposure at a peptide concentration of 100 µM (Figure 3).

a)



b)

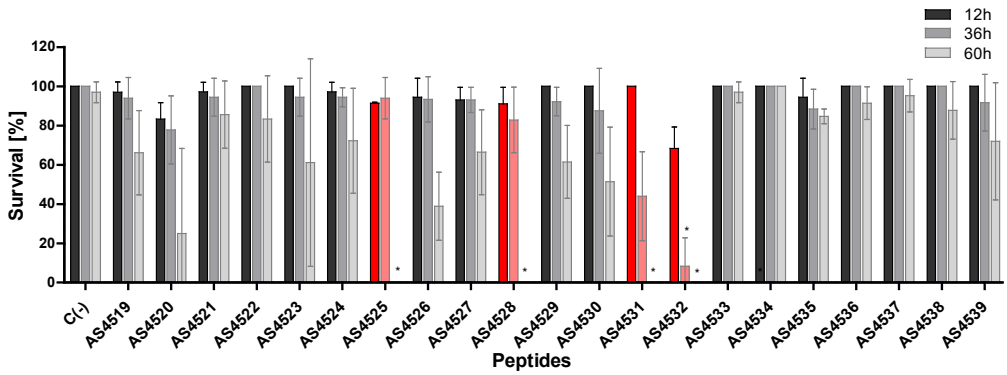


Fig. 3. a) Methodological design to assess lethality of Caligus rogercresseyi using 12 replicates, in triplicate for each peptide and with a photoperiod of 11:13 (light: dark) during 3 days b) Effect of peptides on lethality of the nauplius stage of C. rogercresseyi.

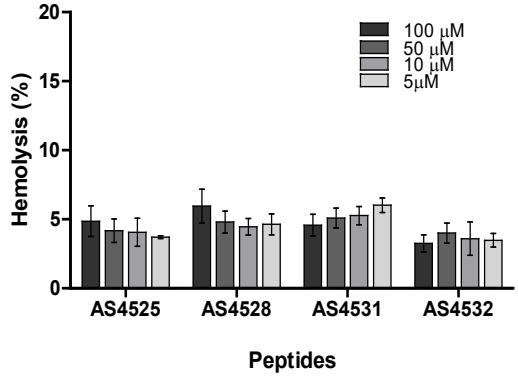


Fig. 4. Evaluation of cytotoxicity of selected peptides using different peptide concentrations.

On the other hand, the measurement of the hemolytic activity of the four selected peptides showed an effect of less than 6% at a concentration of 100 µM, showing no toxicity (Figure 4). These results are promising, placing peptides as alternatives in the treatment of the parasite for the replacement of organophosphates compounds by less toxic and environmentally friendlier compounds.

It is further necessary to verify if the target of action is the AChE enzyme and determine its mechanism of action. These aspects will be addressed in future works.

Acknowledgments

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References

1. Carvajal, J., González, L., George-Nascimento, M. *Aquaculture* **166**, 241-246 (1998), https://doi.org/10.1016/S0044-8486(98)00301-9
2. Agusti-Ridaura, C., Dondrup, M., Horsberg, T.E., Leong, J.S., Koop, B.F., Bravo, S., Mendoza, J., and Kaur K. *Parasites Vectors* m11, 570 (2018), https://doi.org/10.1186/s13071-018-3151-7
3. Helgesen, K.O., Bravo, S., Sevatdal, S., Mendoza, J. and Horsberg T.E. *J Fish Dis*. **37**, 877-890 (2014), https://doi.org/10.1111/jfd.12223
4. Masso-Silva, J.A. and Diamond, G. *Pharmaceuticals* **7**, 265-310 (2014), https://doi.org/10.3390/ph7030265
5. Patel, H.M., Noolvi, M.N., Sharma, P., Jaiswal, V., Bansal, S., Lohan, S., Kumar, S.S., Abot, V., Dhimna, S. and Bhardwaj V. *Med Chem Res* **23**, 4991-5007 (2014), https://doi.org/10.1007/s00044-014-1072-3
6. Yang, J., Yan, R., Roy, A., Xu, D., Poisson, J., Zhang, Y. *Nat Methods* **12**(1), 7-8 (2015), https://doi.org/10.1038/nmeth.3213
7. Guzmán, F., Gauna, A., Luna, O., Román, T., Álvarez, C., Albericio, F., et al. *Amino Acids* **52**(8),1201-1205 (2020), https://doi.org/10.1007/s00726-020-02883-8