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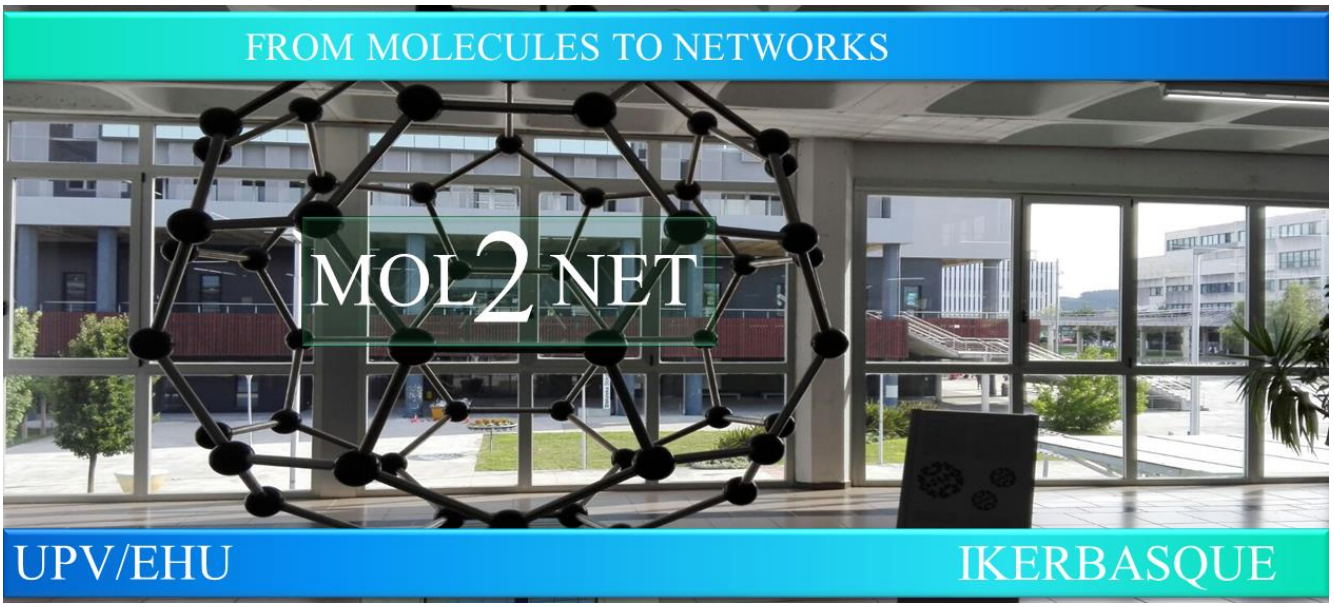

Computational study of the interaction of santhemoidin C and 2-oxo-8-deoxyligustrin on TcTS

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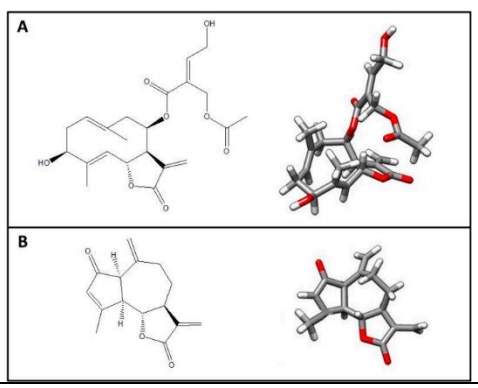
Graphical Abstract



Abstract.

Sialidases are increasingly used in the production of sialyloligosaccharides**.** Interestingly, several sialidases displayed significant conformational transition and formed a new cleft in the simulations. Santhemoidin C inhibited oligopeptidase activity when tested against recombinant Tc80 using a fluorometric assay, reaching an IC50 of 34.9 µM. Molecular docking was performed to study the interaction between santhemoidin C and the Tc80 protein, reaching high docking energy levels.

Keywords: TctS; protein; Sialidases; Docking; sialic acid.

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Introduction

Chagas disease, caused by the parasite *Trypanosoma cruzi*, represents a worldwide epidemiological, economic, and social problem. In the last decades, the trans-sialidase enzyme of *Trypanosoma cruzi* has been considered an attractive target for the development of new agents with potential trypanocidal activity. TcTS from *Trypanosoma cruzi* has received particular interest as a highly stereospecific trans-sialidase [1]. *Trypanosoma cruzi* is incapable of synthesizing sialic acid (SA) de novo. Consequently, the expression of the trans-sialidase (TcTS) enzyme allows the cleavage of terminal SA residues present in glycoconjugates of host tissues. Sialidases catalyze trans-sialylation reactions via a classical ping-pong mechanism [2].

The SA obtained from this process is afterwards transferred onto mucins on the parasite surface, creating a protective and adhesive coat against the immune system. Additionally, TcTS shedding into the bloodstream induces alterations in the sialylation pattern of host cells, generating immune dysfunction and hematological alterations. TcTS represents a potentially attractive drug target against *T. cruzi* since it is absent in mammalian hosts and because of its role in parasite survival [4].

Molecular docking computationally predicts the conformation of a small molecule when binding to a receptor. Scoring functions are a vital piece of any molecular docking pipeline as they determine the fitness of sampled poses. Molecular docking results revealed that the new cleft may serve to accommodate the glycosyl acceptor. In this paper, we aim to study the anti-T. cruzi properties of two STLs isolated from Stevia species. In this sense, in vitro activities against different parasite forms and possible molecular mechanisms of parasite inhibition are explored [3].

Materials and Methods

- Dockin Molecular

Molecular Modeling of Tc80 as a Proposed Target The 3D structure of the prolyl endopeptidase Tc80 from T. cruzi has not yet been elucidated. In this sense, a homology modelling of the primary sequence Q71MD6 entry of Uniprot https://www.uniprot.org/ (accessed on 1 November 2022)—the prolyl endopeptidase of 80 kDa from T. cruzi, with organism identifier OX = 5693 and gene name GN = TCPO — was performed in the Swiss Model server using the 1H2W PDB entry (https://www.rcsb.org/ accessed on 1 November 2022) as the selected template. The selected template is a prolyl oligopeptidase from the porcine brain with 43.6% sequence identity with the Tc80 protein. To achieve a reliable model, the global model quality and QMEANDistCo parameters were considered, and the rotamer outliers observed in the Ramachandran plot were corrected.

- Molecular Modeling of Proposed Ligands for Tc80

The structures of compounds A and B were built and checked with MarvinSketch version 21.17.0 (Chemaxon, Budapest, Hungary) and GaussView softwares (Gaussian, Inc., Wallingford, CT, USA). The ligands ground state employed in the docking experiments were obtained by performing a geometry optimization with a semi-empirical AM1 method and then, were refined within the density functional theory with use of the B3LYP [38] functional and 6-31G* basis set. The partial atomic charges were obtained through a single point at HF/6-31G* using Gaussian 03 and the Merz–Singh–Kollman protocol

- Docking Studies

A blind docking was carried out by mapping the entire surface of the Tc80 protein with the Autodock Vina software (The Scripps Research Institute, La Jolla, CA, USA). The grid box with the corresponding coordinates of the box's spatial origin was checked in the AutoDockTools 1.5.6 software (The Scripps Research Institute, La Jolla, CA, USA) for both ligands. The protein surface was mapped with two boxes of 52 Å in the search for the docking site. The site of higher docking binding energy was re-gridded with a smaller box centered in each ligand and each of them was re-docked with a higher exhaustiveness (=1000) to thoroughly evaluate the best docking pose for both ligands. Default values for the other configuration parameters were used. Interaction energy results were expressed as ΔGdock, in Ikcal/mol. Interactions of the ligands with the target were evaluated with the Pymol 2.4.0 software (Schrödinger, Inc., New York, NY, USA).

- Statistical Analysis

The results are expressed as means ± SEM. The GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) was employed to determine IC50 and CC50 values by non-linear regression. Student's t

test, or one-way ANOVA and Dunnett's post-test were used for the determination of statistical significance. p-values < 0.05 were considered significant.

Results and Discussion

A molecular docking study of santhemoidin C and 2-oxo-8-deoxyligustrin on the prolyl oligopeptidase of 80 kDa of T. cruzi (Tc80) was performed. Primary sequence homology modeling of Tc80 was performed with SwissModel server using a porcine brain prolyl oligopeptidase, with 43,6% sequence identify to the Tc80 target, as a template (Figure 1A). The model had a Global Model Quality Estimate of 0.81and a QMEANDistCo of 0.71 ± 0.05. 93.65% of the residues were distributed in Ramachandran's favored regions, and the rotamer outliers were corrected (Figure 1B).

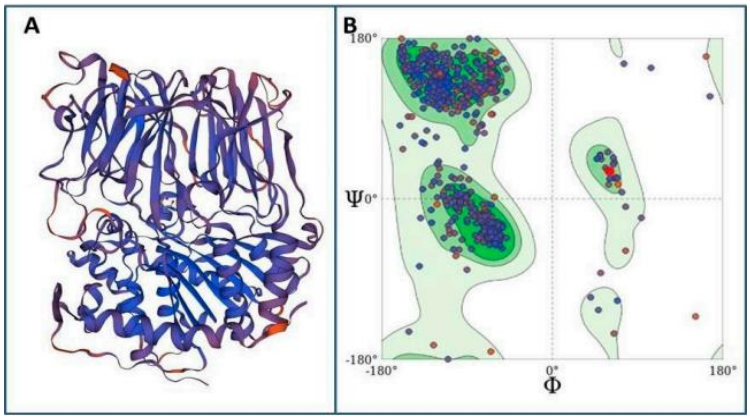

Figure 1. Homology modeling of Tc80. (A) Graphic representation of the 3D structure of the Tc80 protein of T. cruzi. The color pattern is in accordance with the quality of the estimated local model: in blue the similarity with the template is higher, and in red, the similarity is lower. (B) Ramachandran plot showing the ϕ and Ψ dihedral values for each amino acid of the Tc80 model.

A blind molecular docking performed on the entire surface of the Tc80 model of *T. cruzi* identified a preferential docking site for both ligands, as was show in figure 2A and 2B.

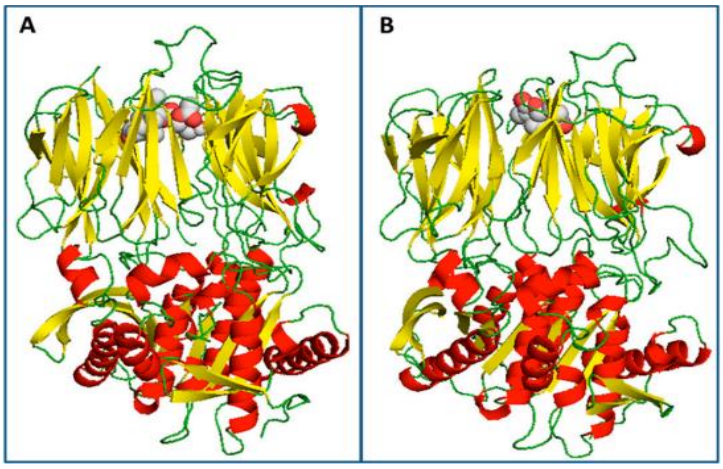

Figure 2. (A) Best interaction pose for santhemoidin C (light gray and red spheres) with Tc80 (B) Best interaction pose for 2-oxo-8-deoxyligustrin (light grey and red spheres) with Tc80. The secondary structure of the protein is rendered in cartoon representation, as: α-helices (red), β-sheets (yellow) and loops and turns (green).

Results showed ΔG_{dock} energy values for santhemoidin C and 2-oxo-8-deoxyligustrin of -8.5 and -7.1 kcal/mol, respectively. The best poses of interaction for each compound resulted in the formation of five hydrogen bonds for the first compound (Figure 3A) and only one for the last one (Figure 3B).

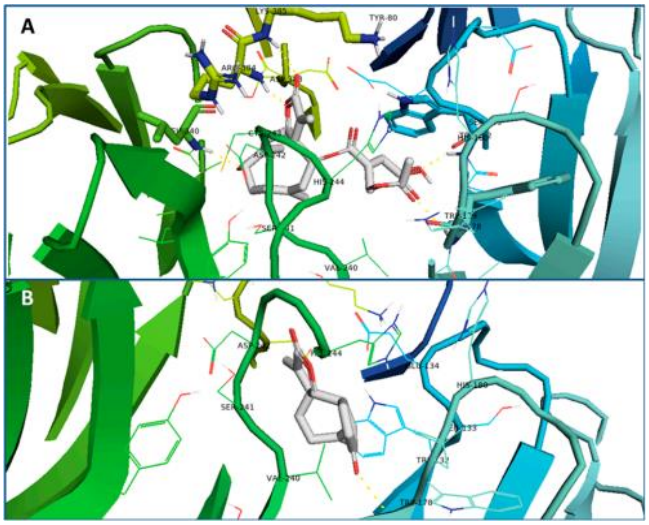

Figure 3. (A) Best interaction pose for santhemoidin C (in gray) with target Tc80. Five hydrogen bonds can be observed (yellow lines) with residues Trp 178, Trp 132, Lys 385, Leu 340 and Arg 384. Best interaction pose for 2-oxo-8-deoxyligustrin (in gray) with target Tc80 one hydrogen bond formed can be observed with residue Trp178 (yellow lines).

The best interaction of santhemoidin C and 2-oxo-8-deoxyligustrin with the enzyme depends on stability. That is, stability comes with the amount of hydrogen bonding. The stability conferred is related to the reaction and competition studies with sialyllactose.

Conclusions

In this work, the anti-T. cruzi effect of the two natural STLs, sesquiterpene lactones, santhemoidin C and 2-oxo-8-deoxyligustrin isolated from *Stevia spp*., was evaluated. Santhemoidin C was shown to be more active and selective than 2-oxo-8-desoxyligustrin on amastigotes. The first showed inhibition of Tc80 and promoted plasma membrane shedding by the parasites. These results show the relevance and importance of natural products in the discovery and identification of novel trypanocidal hit compounds for the development of possible therapies for Chagas disease.

ESSA CONCLUSÃO SE REFERE AOS 2 ARTIGOS ESCOLHIDOS? – Sim!

References

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