COLLEGE OF SCIENCE
THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

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In Silico Identification of Oropouche Virus Protease Inhibitors

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Overview

Oropouche virus is a tropical Bunyavirus transmitted by mosquitoes and other biting insects that is responsible for an infection with symptoms similar to that of chikungunya, dengue, and Zika viruses. It is among several arboviruses that fall under the classification of Neglected Tropical Diseases (NTD) and is prevalent in the Amazon region of Brazil, Ecuador, Panama, Peru and the Carribbean islands. The viral genome is comprised of three single-stranded RNA sequences that encode large multifunction proteins, one of which is a negative sense ssRNA polymerase that is essential for viral replication.

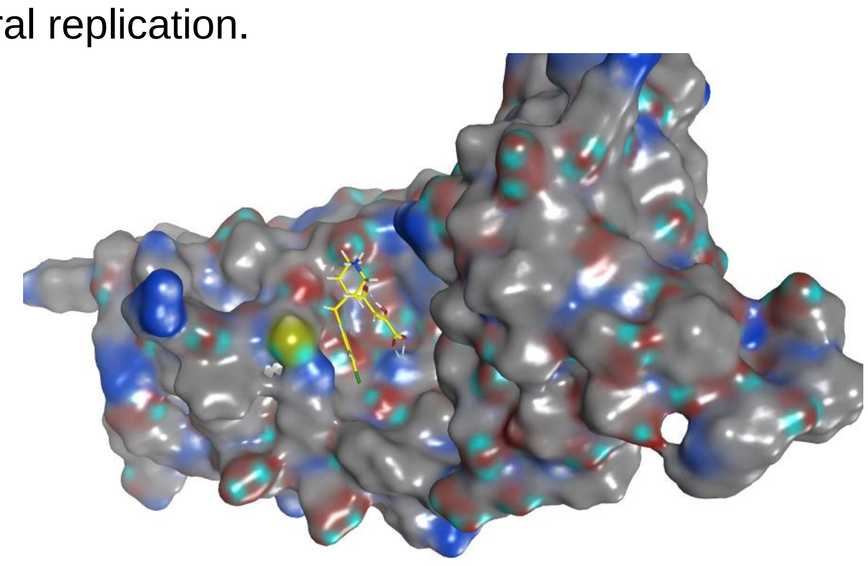

Figure 1. Homology model of endonuclease (space-filling) with bound reference ligand, L-742,001.

Methods

A crystal structure with high sequence similarity from the PDB database was identified using NCBI's BLAST and used to construct a homology model in Molecular Operating Environment.[1]

•La Crosse virus (5AMR) endonuclease used as template for homology model (86.7% local similarity)

•H1N1 Influenza virus (5D8U) in complex with inhibitor used in minimization (56.3% local similarity)

A library of 2,174 plant natural product structures was docked to the site of inhibition using Molegro and the similarity between docked poses and the inhibitor was used to scale the docking scores.[3] This was followed by 400 ps simulated annealing using NAMD to assess retention of conformation upon heating and cooling.[2]

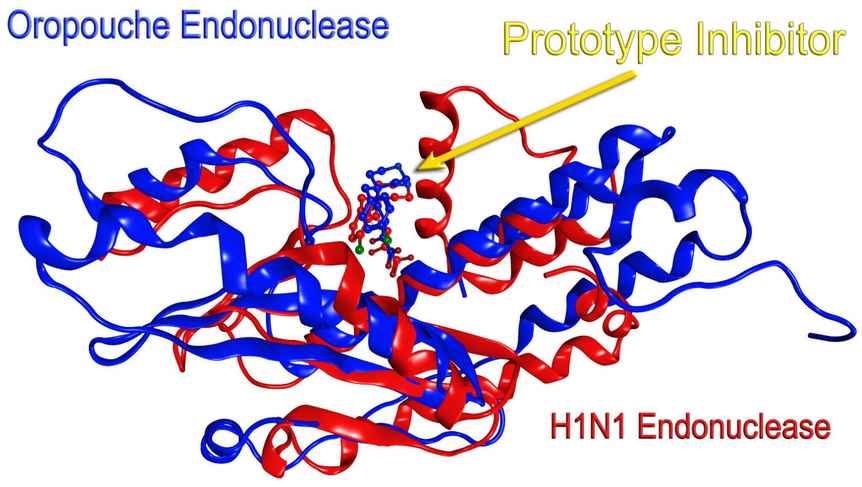

Figure 2. Superposition of H1N1 endonuclease and La Crosse endonuclease with the prototype inhibitor bound to each.

Results

An analysis of sequences in Refseq shows that the amino acid residues in the vicinity of the putative inhibitor are highly conserved across Bunyaviruses, more so than those of Influenza H1N1. The interaction between Lys92 and the inhibitor (26% of interaction energy) in the homology model is not present in the H1N1 pocket, but replaces Lys134, which is in the same region. Five binding site residues are homologous between H1N1 and the homology model.

Figure 3. Homology model sequence showing highly conserved residues (*in purple*) in the binding site (*red boxes*).

After docking, five structures were selected for evaluation with molecular dynamics. Since the binding energy fluctuates over time, the cosine distance of per-residue binding interactions was used as a measure of binding mode retention. The cosine distances show little diminution along the MD trajectories, and suggest these structures will exhibit strong binding interactions *in vitro*.

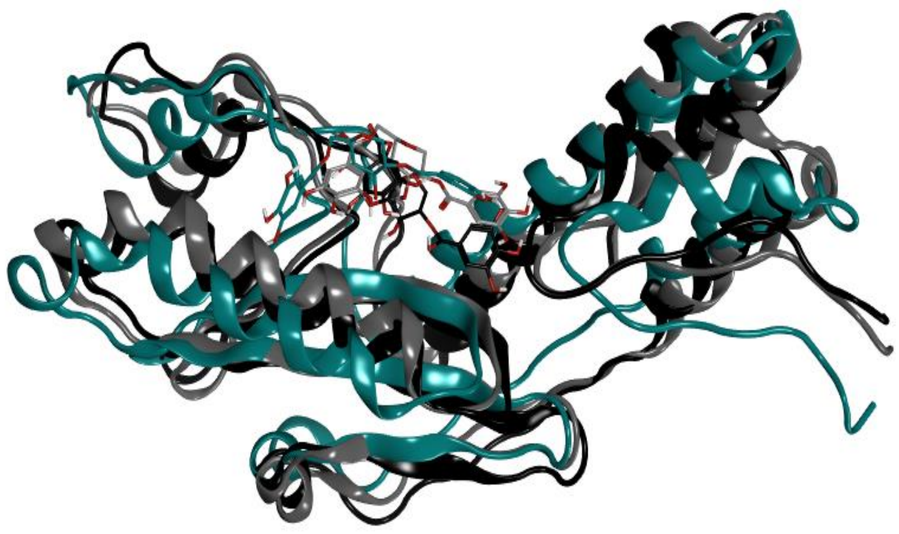

Figure 4. Superposition of La Crosse Virus endonuclease with aceritannin bound in frames 0 (black), 138 (gray), and 1391 (teal) from the molecular dynamics simulation.

Conclusions

Pharmacophore-Scaled Docking with Simulated Annealing (PSD/SA) is a computational method used to identify molecular structures with high binding affinity for selected receptor structures with co-crystallized reference ligands and has been used here to select five candidate structures for *in vitro* / *in vivo* screening against Oropouche protease as inhibitors.

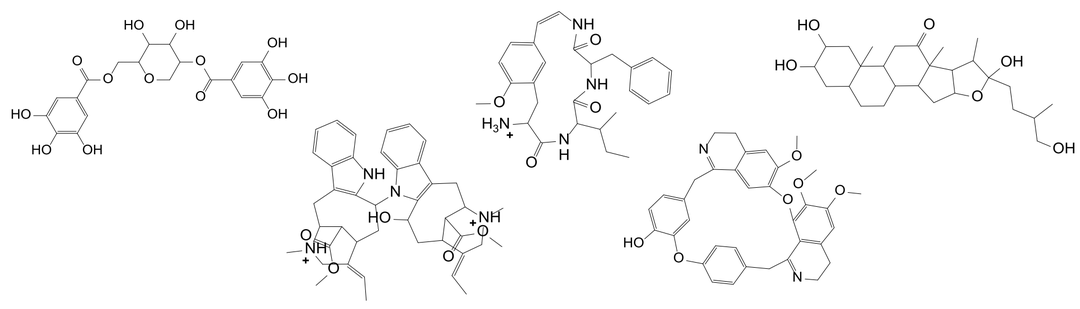

Figure 5. Structures of the five natural product structures identified by PSD/SA to be tight binders (*left to right*): aceritannin, bisnicalaterine, puertogaline, mucronine, and tribufuroside.

References

1. **Molecular Operating Environment (MOE), 2014.09; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2017.**
2. **Phillips, J. C.; Braun, R.; Wang, W.; Gumbert, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. J. Comp Chem. 2005, 26, 1781 – 1802.**
3. **Thomsen, R.; Christensen, M. H. MolDock: a new technique for high accuracy molecular docking. *J. Med. Chem*. 2006, 49, 3315-3321.**

Acknowledgements

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