"Novel leads and drugs for vector borne diseases: Targets and off targets (toxicity and ecotoxicity) and mechanism of action"

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One Health Approach in Drug Discovery for Leishmaniasis by Targeting Calpain cys-protease.

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Leishmaniasis plague millions in impoverished regions, causing disfiguring skin lesions and potentially fatal organ damage. Despite the urgent need, research and development for new treatments remain underfunded, leaving those affected with limited options. To protect human health, a One Health approach is critical, tackling the disease at its environmental and animal roots. MS proteomic study[1] revealed an up-regulation of a protein known as Calpain-like Cysteine Peptase (Uniprot: A0A6L0WUS2). We started this project with this sequence, which consists of 115 residues. Unfortunately, very few references regarding this protein are available. The lack of information and the absence of a catalytic site in this sequence forced us to focus on the full-length sequence of Calpain (GenBank: XP_001468282.2) for our virtual screening study. The cys-protease protein was already known as a drug target for antitrypanosomatidic drug discovery, but unclear aspects about the on target studies prompted our interest. Within the One Health framework, we started to study the protein and compared the sequence by alignment with calpain sequences from other species to ensure that this is an environmentally friendly target with low impact on other species. Bioinformatics tools[2-4] were then used to compare the calpain sequence with counterparts from various species, revealing significant homology with trypanosomatidic sequences and very low homology with other species, making calpain a suitable target for the development of new selective drugs against leishmaniasis. We constructed and validated a homology model for the calpain protein from *Leishmania infantum*[5], which laid the groundwork for a comprehensive virtual screening campaign. Using libraries of compounds from Enamine and ChemDiv, thorough screening methods found 4000 compounds that might be able inhibit calpain enzymatic activity. Further refinement prioritized 400 compounds based on ΔG binding calculations and selectivity against human calpain. SwissADME and ADMETlab 3.0 have screened the top 100 compounds to evaluate their pharmacokinetics and eco-tox profile, resulting in a more environmentally friendly selection of the top 50 candidates. These candidates will undergo in vitro tests to evaluate their efficacy, selectivity, and safety profiles. Furthermore, a collaborative work within structural biologists from Diamond Light Source and from the University of Siena, started to develop a plasmid that will be used to produce the catalytic domain of the protein for X-ray crystallographic studies and

for the development of an in vitro assay with the recombinant protein, aiming to validate the data obtained from the virtual screening study and progress with the medicinal chemistry program. Advances of the project will be presented.

References

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