STRUCTURAL CHARACTERIZATION OF INSECT TREHALASE AND ASSESSMENT OF POTENTIAL INHIBITORS.

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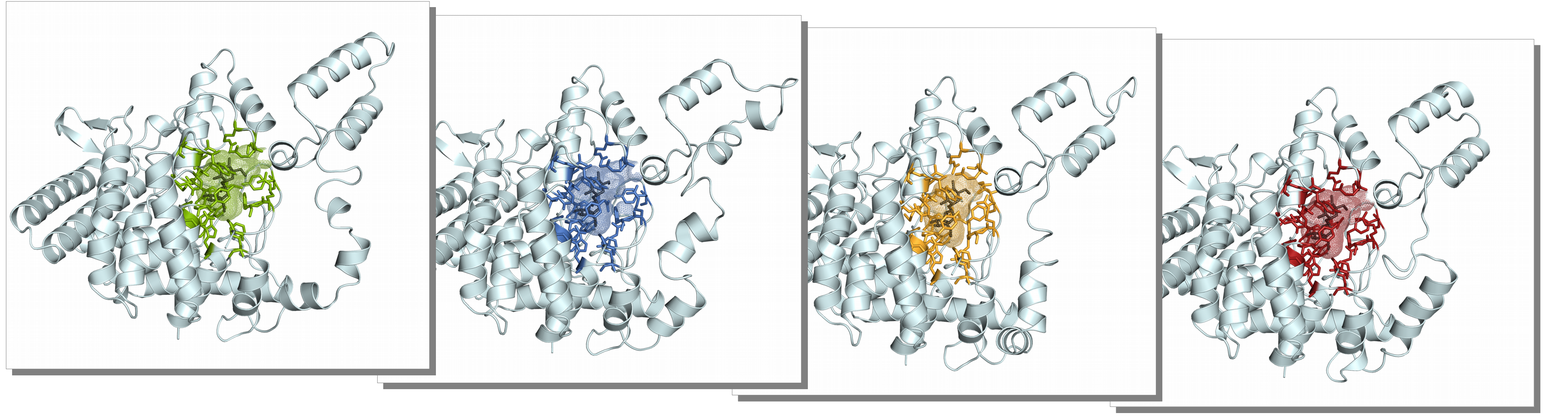
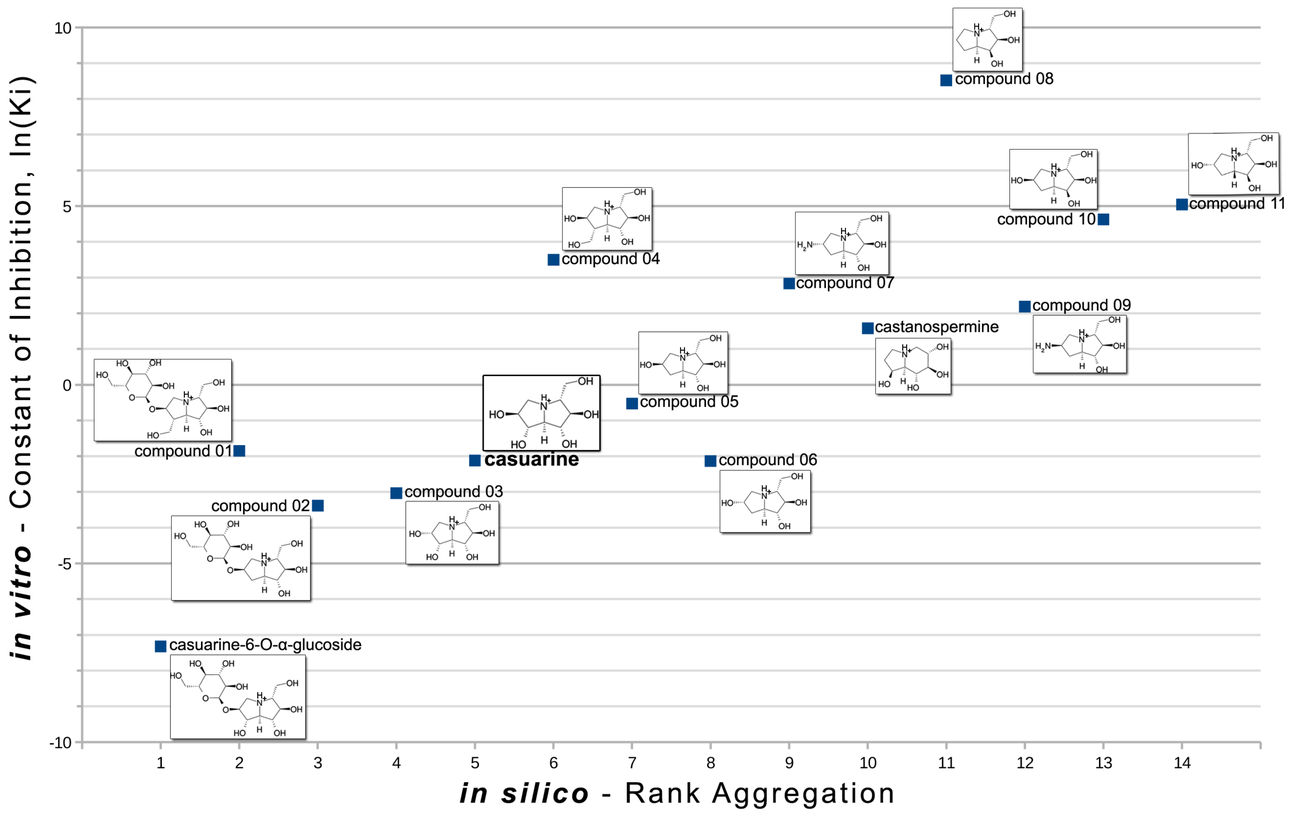
Trehalase is a glycosidase that catalyses the hydrolysis of trehalose into two molecules of glucose. This enzyme is vital for insects since trehalose is the principal hemolymph sugar and its catabolism results pivotal for physiological processes like energy production and macromolecular biosynthesis. By contrast, the biological relevance of trehalase in mammals has not been elucidated yet since the trehalose intake is occasional. Starting from such observations, trehalase represents an attractive molecular target for the development of inhibitors that can act as novel and selective insecticides.

1 *"Build a structural model of the insect variant of trehalase."*

In this work we have realized a **homology model** for the *Chironomus riparius* threalase, since this insect is a suitable animal model for *in vivo* experiments. To predict the binding modes of potential inhibitors we adopted an **ensemble docking** strategy, where the dynamic behavior of the binding site and its surroundings is taken into account during the ligand placement by docking to multiple (4) receptor conformations [1].



2 *"Propose binding modes and assess the agreement of binding affinities."*

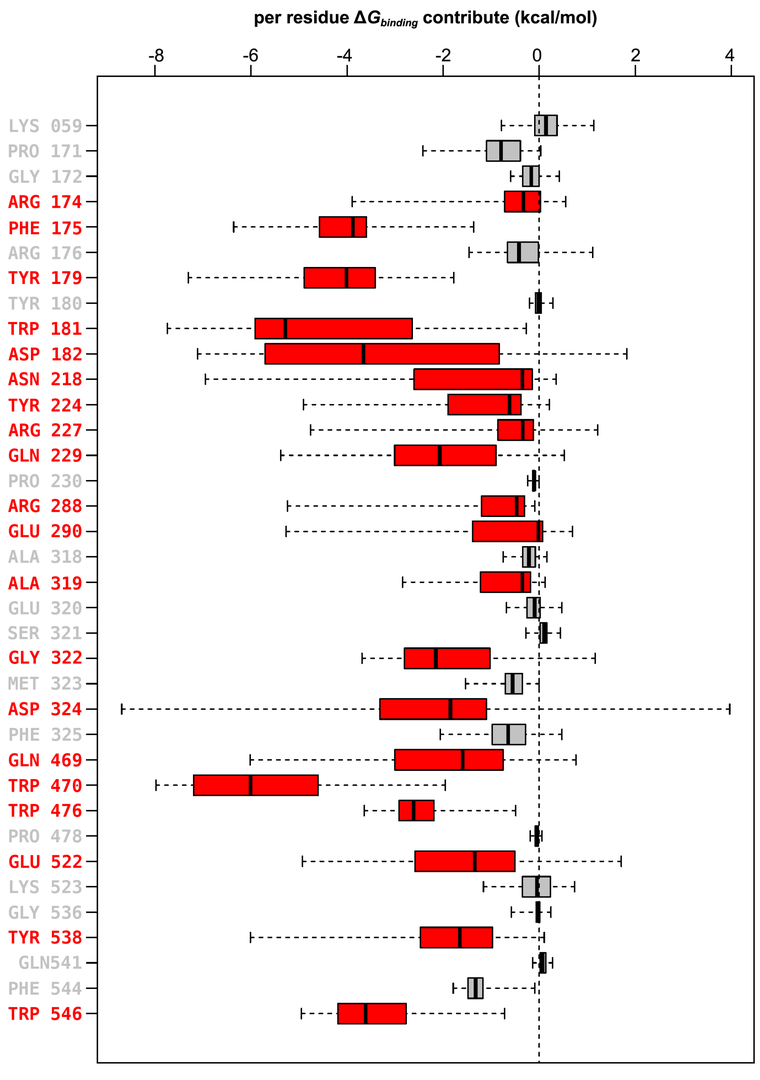


The computational ranking proposed is in good agreement with experimental values (Spearmann's ρ value 0.815) and it allows to discriminate a potent inhibithor of threalase enzyme from a weaker one.

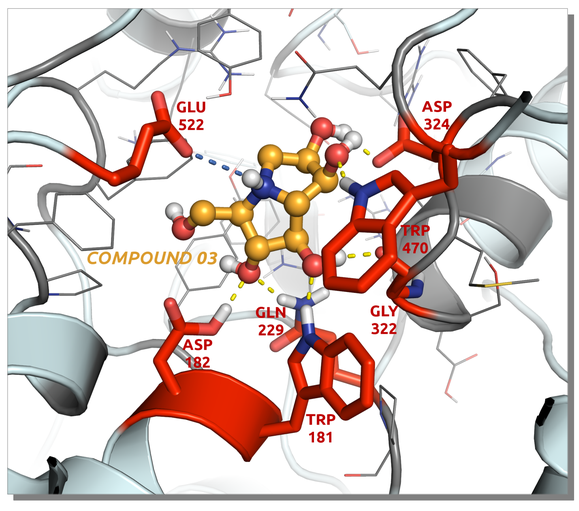
We have analized a set of ligands comprising casuarine, castanospermine and 11 casuarine derivates. For all of these compounds experimental IC_{50} were measured [2-4], defining a panel of inhibitors with a wide range of affinities. The best poses obtained were sorted using a **consensus ranking** algorithm that aggregates affinity evaluations provided from diverse criteria based on empirical docking scores and $\Delta G_{binding}$ calculations [5].

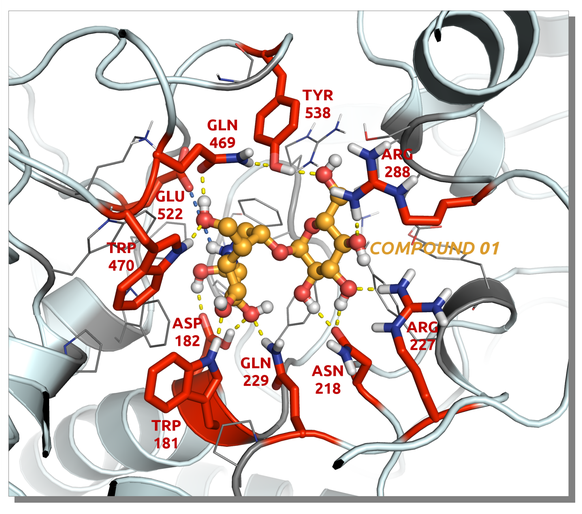
LIGAND	GlideXP score	Emodel score	MM-GBSA ΔG_{bind}	MM-GBSA ΔG_{bind} [NS]	Rank	ln(Ki)
casuarine-6-α-D-glucoside	-15.910	-112.970	-115.200	-153.018	1	-7.323
compound 01	-15.540	-100.970	-129.842	-159.397	2	-1.852
compound 02	-15.070	-92.690	-117.915	-139.217	3	-3.381
compound 03	-10.760	-76.610	-92.207	-105.510	4	-3.037
casuarine	-9.550	-74.160	-92.178	-102.090	5	-2.120
compound 04	-9.710	-65.760	-84.568	-99.331	6	3.497
compound 05	-11.890	-61.870	-78.915	-85.413	7	-0.528
compound 06	-7.720	-59.920	-87.512	-97.545	8	-2.137
compound 07	-7.910	-58.790	-87.832	-97.237	9	2.833
castanospermine	-6.380	-64.710	-67.426	-90.558	10	1.581
compound 08	-7.050	-56.870	-83.932	-91.405	11	8.517
compound 09	-7.140	-56.350	-78.345	-87.267	12	2.186
compound 10	-8.290	-51.990	-71.123	-80.661	13	4.625
compound 11	-7.490	-51.160	-58.374	-74.422	14	5.043

3 *"Rationalize the molecular determinants of inhibition."*



Most of the binding geometries of our poses resemble the topology of receptor/ligand interactions found in the crystallographic structures of *E. coli* trehalase complexes. **Energy Decomposition** analysis was performed in order to evaluate the per-residue contribution to $\Delta G_{binding}$ and a pattern of 20 amminoacids was identified as relevant for energy stabilization [6]. Such residues demonstrated differential role and importance in the binding modes of different ligands. This fine characterization shed light on how modifications of casuarine derivates can improve or disrupt the complex framework of non-bonding interactions, providing us the basis for future rational drug design tasks.





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