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Nature-Inspired Gallinamides Are Potent Antischistosomal Agents: Inhibition of the Cathepsin B1 Protease Target and Binding Mode Analysis

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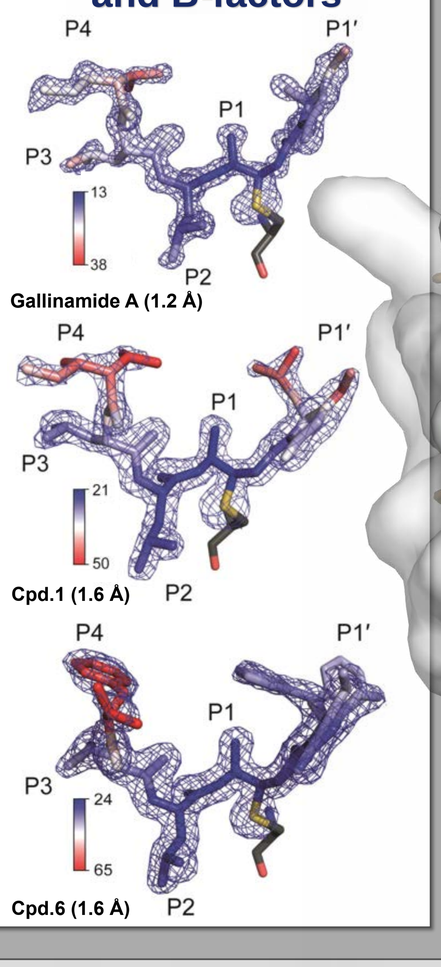
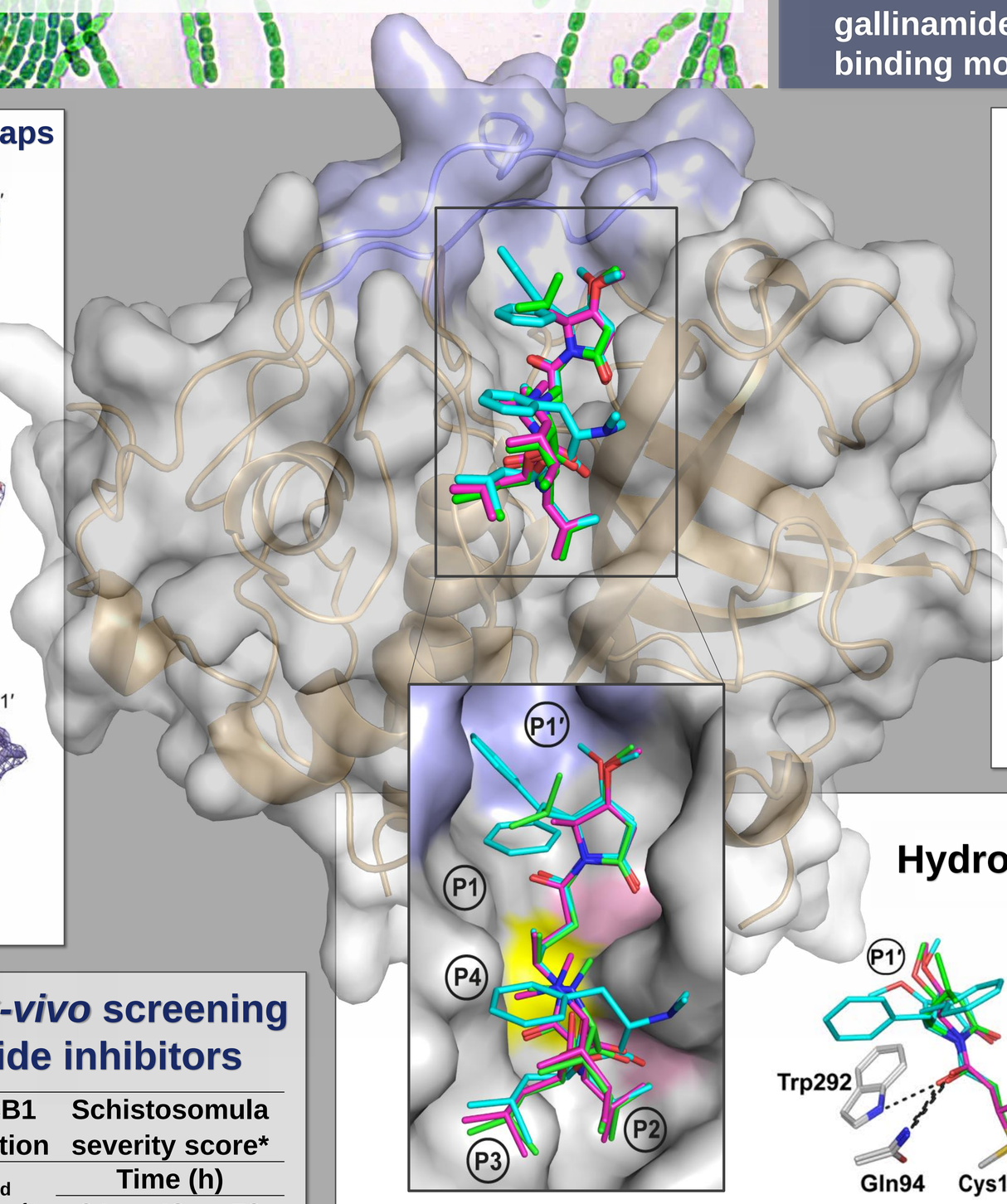
Introduction

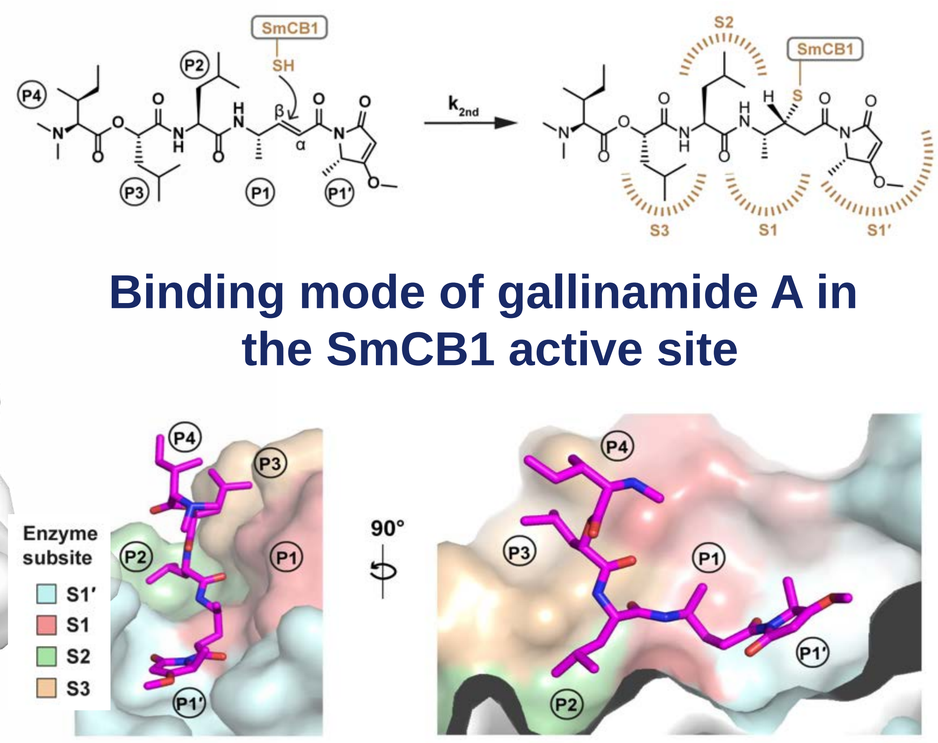
Gallinamide A is a natural product of marine cyanobacteria. This compound and its derivatives can bind cysteine proteases in a covalent-irreversible manner. Here we analyze the interaction of gallinamides with *Schistosoma mansoni* cathepsin B1 (SmCB1), a central digestive protease, which has recently been validated as molecular target against schistosomiasis. This parasitic disease is caused by blood flukes of the genus *Schistosoma* and is a global health problem with over 240 million people infected. Treatment relies on just one drug and new therapies are needed. Adult schistosomes live in the blood vessels and host blood proteins are the main source of nutrients.

- ***Schistosoma mansoni*** **cathepsin B1 (SmCB1) is a validated drug target against schistosomiasis**
- **We screened a library of synthetic analogues of gallinamide A for inhibition of SmCB1 and identified inhibitors with low nanomolar potency.**
- **Gallinamides exhibit a strong suppression effect on live schistosomes in culture.**
- **We solved the first high-resolution crystal structures of gallinamides in complex with SmCB1 and determined their binding mode in the active site.**

Electron density maps and B-factors







Binding mode of gallinamide A in the SmCB1 active site

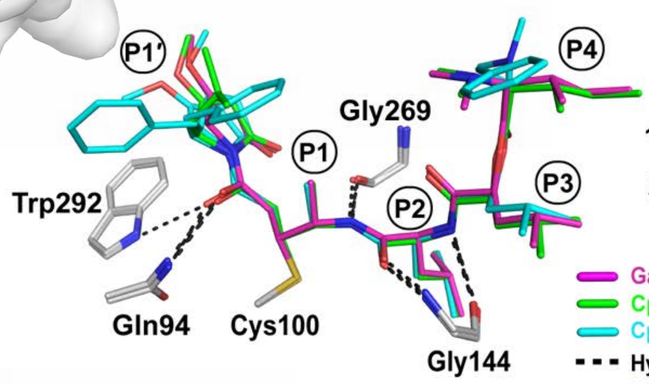
In-vitro and *ex-vivo* screening of gallinamide inhibitors

Compound name	SmCB1 Inhibition k_{2nd} ($M^{-1}\cdot s^{-1}$)	Schistosomula severity score* Time (h) 24	48	72
Gallinamide A	6644	0	2	4
1	3394	2	4	4
2	3334	0	2	4
3	2902	0	0	2
4	2894	0	0	4
5	2792	0	4	4
6	1930	3	4	4
7	942	0	3	4
8	886	1	4	4
9	793	0	1	4
10	537	0	2	4
11	295	0	3	4
12	212	1	4	4
13	109	0	3	4
14	55	0	4	4
15	41	0	3	4
16**	36	0	0	0
17	18	0	2	4
18	16	0	0	3

*Induction of phenotypic alterations by the inhibitors was determined with newly transformed schistosomula of *S. mansoni*. The inhibitors were tested at 1 μM concentrations for 3 days and the resulting phenotypes were graded by severity score from 0 to 4 being the most severe.

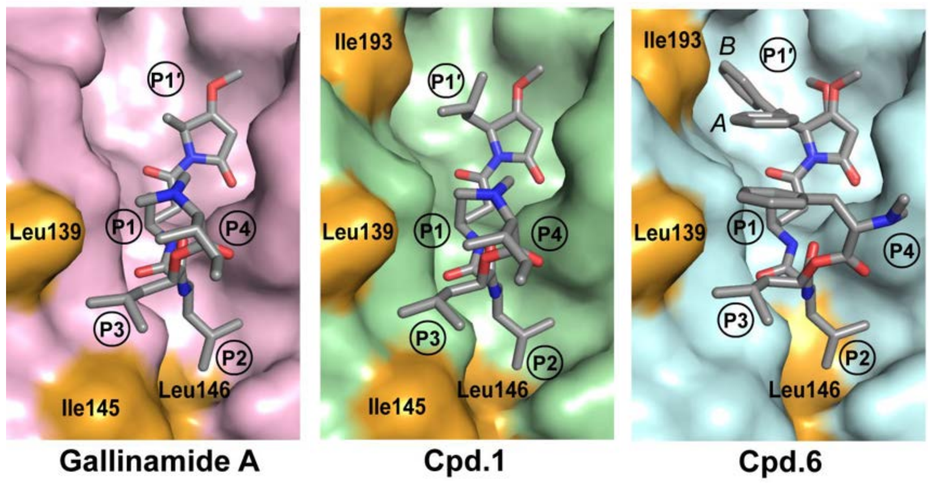
**Gallinamide A without warhead

Hydrogen bonds

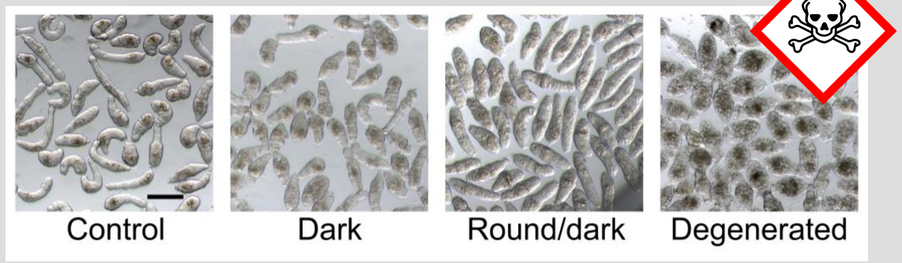


Water bridges

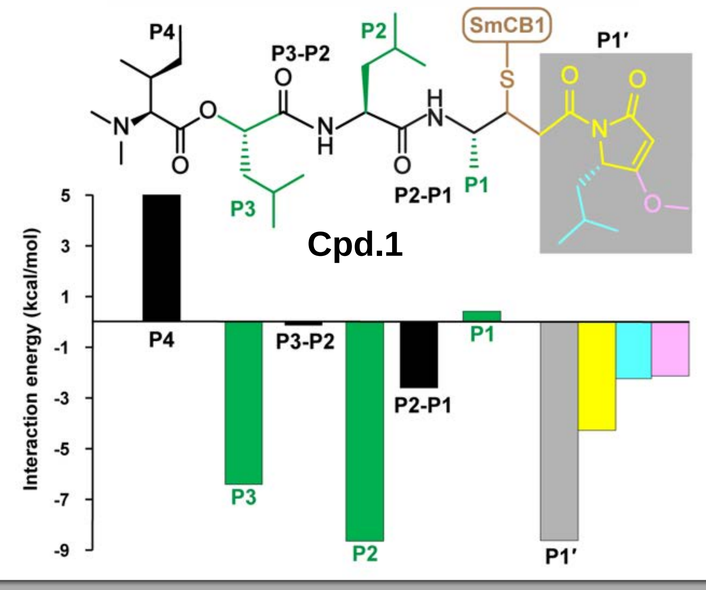
Hydrophobic interactions



Parasite phenotypic screening



Quantum chemical calculation of interaction energy



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