Supplementary Material

Screening the Pathogen box to discover and characterize new cruzain and *Tbr*CatL inhibitors

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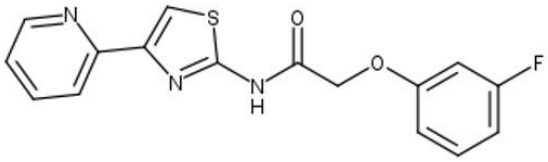
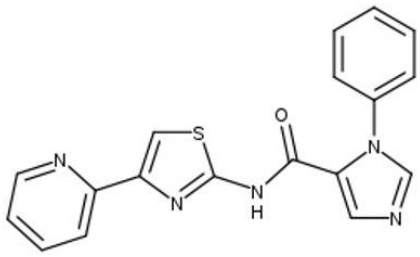
Table S1 – Effect of **MMV676409** and **MMV676512** in the inicial velocitities of the enzymatic reactions, after 10 minutes of pre-incubation of the enzyme and the compound. All effects were calculated in respect to the DMSO control.

Compound	Increase in the initial velocity of the reaction (%)					
	5 µM using PB samples[a]		100 µM using solid samples[b]		5 µM using salid samples[b]	
	Cruzain	*Tbr*CatL	Cruzain	*Tbr*CatL	Cruzain	*Tbr*CatL
 **MMV676409**	510 ± 216	772 ± 382	0 ± 6	*ND*	-4 ± 2	*ND*
 **MMV676512**	84 ± 54	109 ± 71	24 ± 5	*ND*	14 ± 9	*ND*

[a]: experiments performed using the solutions from the PB compound library. [b]: experiments performed using solutions prepared from the solid samples. *ND*: not determined.

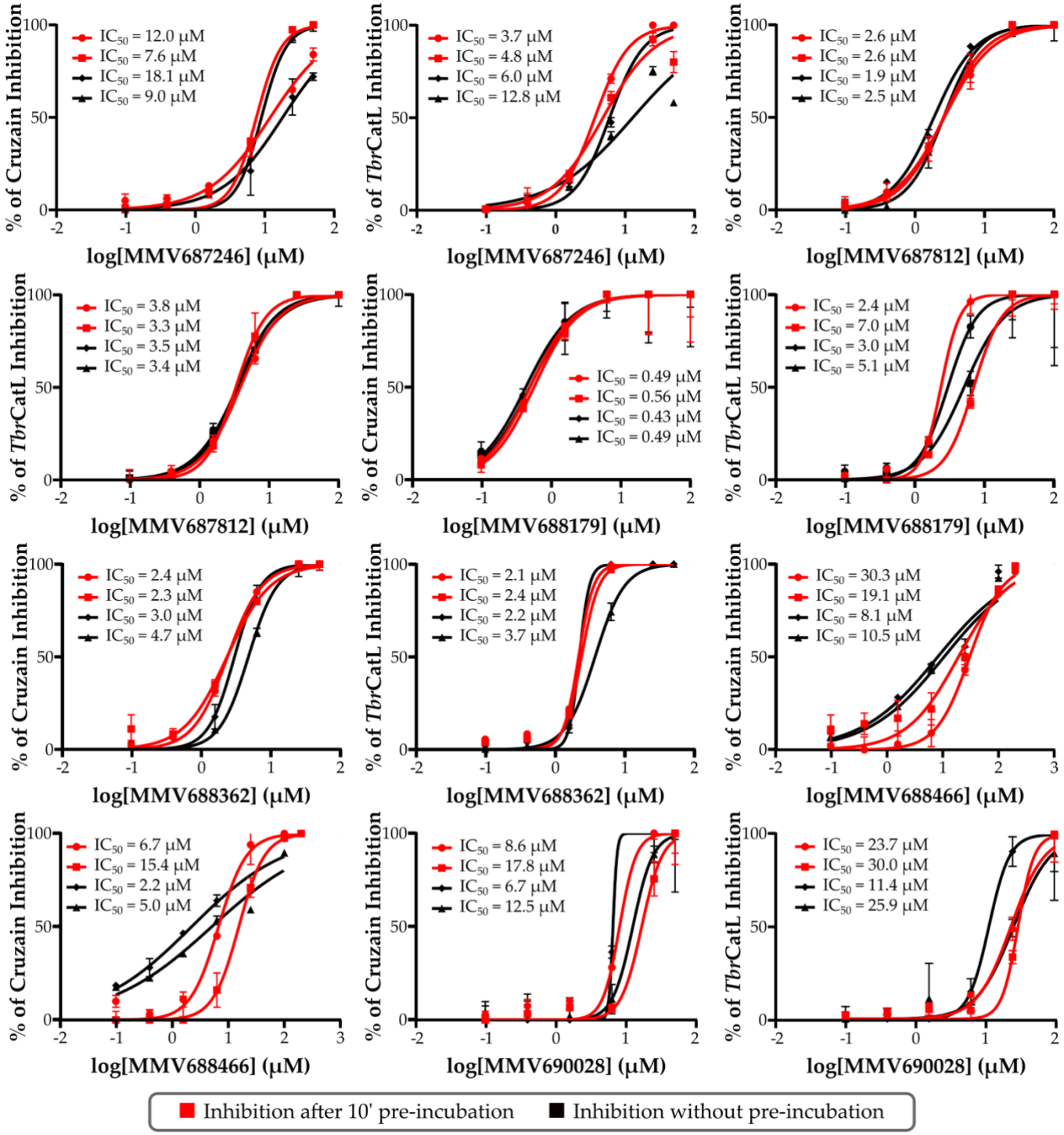


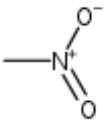
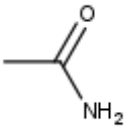
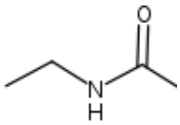
Figure S1 – IC_{50} curves for compounds **MMV687246**, **MMV687812**, **MMV688179**, **MMV688362**, **MMV688466**, **MMV690028** against cruzain and *Tbr*CatL. For each compound/enzyme pair, four curves are reported, two obtained in experiments with a ten minute pre-incubation of the compounds with the enzymes (red) and two obtained in experiments without pre-incubation (black curves). Each curve was determined based on at least seven compound concentrations, in triplicate. All percentages of inhibition were calculated based on a DMSO control.

Table S2 – Cruzain inhibition by hits from the initial screening, at varying assay conditions.

Compound	Concentration of the compound evaluated (μM)	% Cruzain Inhibition (Mean ± SEM) [a]			
		0.1% Triton X-100	0.01% Triton X-100	0.001% Triton X-100	0.001% Triton + BSA 1 mg/ mL
MMV687246	12.5	32 ± 2	51 ± 0	62 ± 3	45 ± 1
MMV687812	3.125	54 ± 1	95 ± 0	92 ± 0	85 ± 1
MMV688179	1.563	82 ± 13	80 ± 1	85 ± 6	89 ± 8
MMV688362	3.125	25 ± 2	41 ± 2	59 ± 2	29 ± 3
MMV688466	6.25	82 ± 11	32 ± 5	83 ± 3	96 ± 3
MMV690028	25	100 ± 0	100 ± 0	69 ± 14	91 ± 7

[a]: mean over two independent experiments, in triplicates.

Table S3 – Cruzain inhibition by analogues of compound **MMV688179.**

Compound	Structure			% Cruzain Inhibition at 100 μM (Mean ± SEM) [a]	Comments
	R1	**R2**	**R3**		
1	H	H	H	ND	Solubility issues
2	H		H	0 ± 6	-
3	H		H	ND	Solubility issues
4				ND	Fluorescence, interference with the assay readout
5	H		H	ND	Fluorescence, interference with the assay readout
6	H		H	57 ± 4	Fluorescence, interference with the assay readout
7	H		H	65 ± 4	Insoluble at concentrations higher than 100 μM

ND = not determined [a]: mean over two independent experiments, in triplicates.