EV-D68 3C pro vs GC376 IC50

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Experiment Started:
Projects: **Activity Assay**;**Small Molecule Libraries**;**ASAP**
Related Pages:
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Tags:

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I wanted to repeat the experiment using a fifferent dilution factor (1.5 instead of 2) to have more data point in the curve slope.
Top FAC is 20uM

Protocol

Fluorescence assay for EV-D68 3C Pro activity measurement

This method is intended to measure the activity of viral proteases by using a specific labelled-peptide that allows the detection of the cleaved product. The substrate contains the cleavage-sequence specific to the tested protease and is labeled in C-terminal by the fluorophore Edans (abs: 336 nm; em: 490 nm) and in N-ternimal by the quencher Dabcyl (abs 472 nm). In the case of a non-cleaved substrate, the proximity of Dabcyl to Edans prevents the emission and the detection of the fluorescence at 490 nm. The cleavage of the peptide by the protease allows Edans' fluorescence emission and detection.

Reagents

Assay buffer: 50 mM Tris pH 7.0, 150 mM NaCl, 10% glycerol and 0.5 mM DTT (optional).

Incubation: 1 hour at room temperature.

EV-D68 3C: protein stocks were stored at -80C and used as 2x solution (1 µM, 0.5 µM final assay concentration) in assay buffer.

Substrate: Dabcyl-KEALFQGPPQFE-Edans (LifeTein, USA) prepared as a stock solution at 5 mM in DMSO and used at 2x solution (40 µM, 20 µM final concentration assay concentration) in assay buffer.

Positive control: GC376 (Pubchem CID 71481119), 20 µM top final assay concentration, dilution factor = 1.48; dilution = 0.7.

Plates: ProxiPlate-384 Plus, white, Greiner cat# 6008280.

Liquid handler: Echo® acoustic liquid handler (Beckman Coulter, USA)

Plate reader: Pherastar FS, BMG Labtech (Germany), 350-490 FI optic module, the plate is read every 30 s for 2 hours and shacked during 5 s before the first reading.

Protocol EV-D68 3C Pro

Ten µL of 2x protein solution were added to each well containing the compounds to be tested previously dispensed onto the plate and incubate with the enzyme for one hour at room temperature. Enzymatic reaction

was initiated by the addition of 10 uL of 2x (40 µM) substrate solution using the plate reader injector. The fluorescence intensity at 490 nm was read every 30 seconds for 2 hours in kinetic mode.

The IC50 was calculated by plotting the initial velocity against various concentrations of tested inhibitor by using a four parameter dose–response curve in Prism (v8.0) software.

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Reac. buffer 50 mM Tris pH 7.0, 150 mM NaCl, 10% glycerol
Plate ProxiPlus 384, white, PerkinElmer

Mix	2 ml		Disp	10 uL/well
Protein	**[Final] uM**	**[Stock] uM**	**Use (ul)**	**Buffer uL**	**Dil. Factor**
EV68-2A	10	30	1333.33	667	2
EV71-2A	10	344	116.28	1884	2
EV68-3C	0.5	1000	**2.00**	1998	2
EV71-3C	10	340	117.6	1882	2

Dispense 10uL per well on previously dispensed compound incubated 1 hour at RT

Mix	1 ml		Disp	10 uL/well
Dabcyl-Edans S	**[Final] uM**	**[Stock] uM**	**Use (ul)**	**Buffer uL**	**Dil. Factor**
EV68-2A	20	2000	20.0	980	2
EV71-2A	20	5000	8.0	992	2
EV68-3C	20	5000	**8.0**	992	2
EV71-3C	20	5000	8.0	992	2

Add 10 uL of substrate and read the plate for 30 min (FS at RT, ex 350, em 450; gain 500; kinetic 250 x 30s, focal height 11.5, shaking 5s before the first reading)

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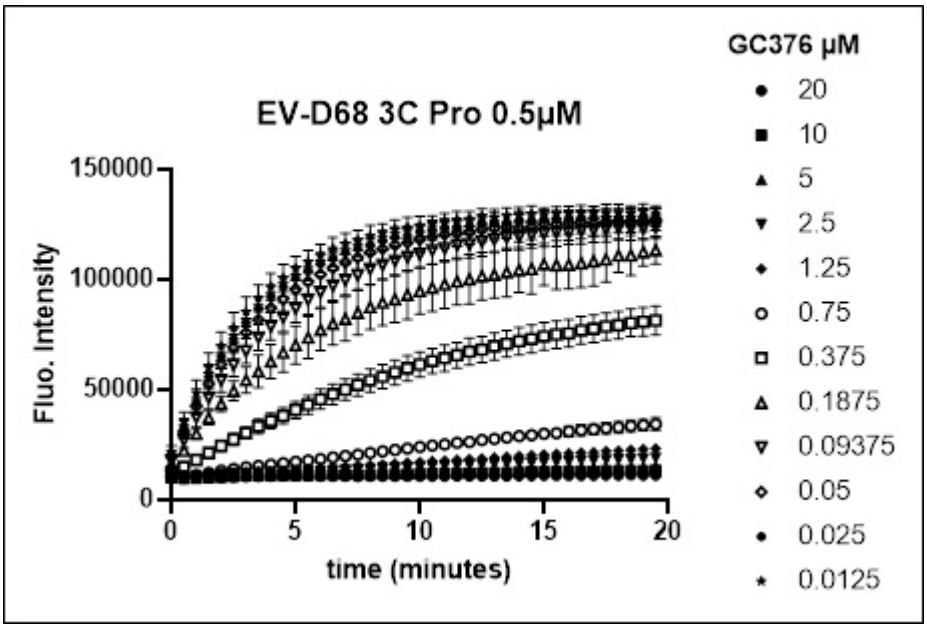

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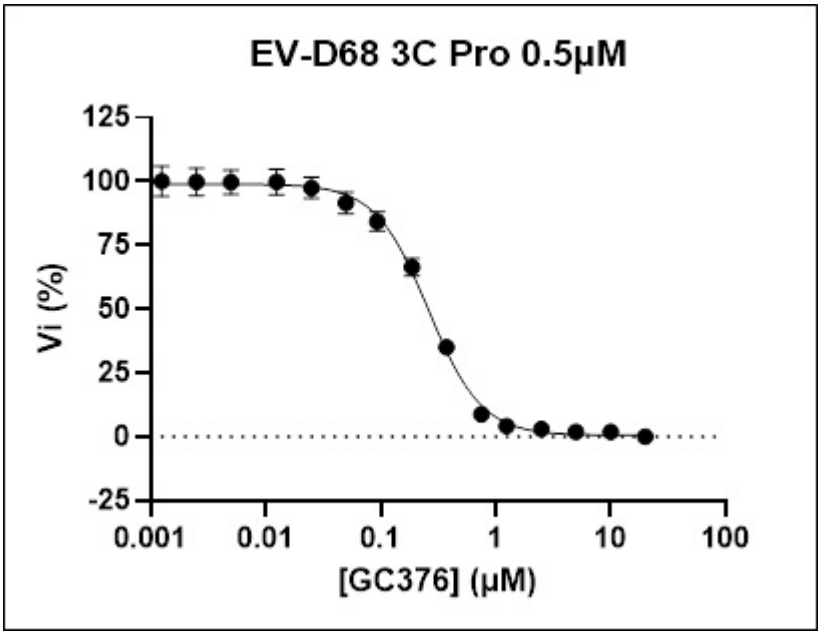

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log(inhibitor) vs. response -- Variable slope (f	
Best-fit values	
Bottom	0.5278
Top	98.95
LogIC50	-0.5838
HillSlope	-1.883
IC50	0.2607
Span	98.42
95% CI (profile likelihood)	
Bottom	-1.671 to 2.666
Top	96.98 to 101.0
LogIC50	-0.6186 to -0.5493
HillSlope	-2.158 to -1.655
IC50	0.2406 to 0.2823
Goodness of Fit	
Degrees of Freedom	11
R squared	0.9985
Sum of Squares	43.15
Sy.x	1.981
Number of points	
# of X values	15
# Y values analyzed	15
S to N	937.271
Z'	0.795

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2023 03 17 E5XX-1322_Transfer_1679053021.csv
2023 03 17 picklist evotec 10mM 6 cpds.csv
2023 03 17 processed Transfer_1679053021.csv
2023 04 17 EV-D68 3C vs GC376.pzfx
2023 04 17 EV-D68 3S vg GC376 raw data.xls
2023 04 17 EV-D68 3S vg GC376 table.xlsx

data location

S:\Biophysics\People\Charline\EV-protease\2023 04 17 EV-D68 3C IC50 GC376