Fluorescence assay for EV-A71 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-71 3C: protein stocks were stored at -80C and used as 2x solution (20 µM, 10 µM final assay concentration) in assay buffer.
Substrate: Dabcyl-IEALFQGPPKFRE-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), 50 µM top final assay concentration.
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-A71 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 µL 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.