

POSTER 20**DISCOVERY AND CHARACTERIZATION OF NOVEL INHIBITORS OF FOOT AND MOUTH DISEASE VIRUS REPLICASE**

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Foot-and-Mouth Disease Virus (FMDV) is a plus-strand RNA picornavirus that infects cloven-hoofed animals, disturbing agricultural economies during outbreaks. Seven distinct FMDV serotypes have been identified worldwide. Vaccination against one serotype will not protect against viruses from other serotypes. Further, vaccination does not protect animals until weeks after administration. Prophylactic vaccination is not used in US and there are currently no chemical treatments. The RNA-Dependent-RNA-Polymerase (3D) of FMDV is an attractive target because of its role in replication and amino acid conservation among FMDV serotypes. In this study, we are screening the Maybridge HitFinder library to identify FMDV 3D inhibitors. All HitFinder compounds follow Lipinski's rule of five for druglikeness.^[1] The target enzyme (3D) was cloned into a pet15 vector, expressed in Rosetta 2 bacteria and purified by immobilized metal affinity chromatography followed by mono Q anion exchange chromatography. Its enzymatic activity was characterized and replication reaction conditions were optimized by adjusting pH, salt, temperature, and substrate conditions. Screening for 3D replicase inhibitors was carried out using a luciferase-based assay^[2] that follows production of luminescence linked to release of pyrophosphate from the polymerization reaction. After optimizing conditions the assay was validated using primer extension gel-based polymerization assays. Using this assay we screened for compounds that suppressed production of luminescence by ~90% at 20 μ M. Nine compounds were validated to inhibit FMDV 3D with IC₅₀ < 10 μ M in a secondary gel-based primer extension assay. Cytotoxicity of the compound hits was evaluated by incubation of the compounds with BHK-21 cells and measurement of the release of cell death-associated proteases (CytoTox-Glo kit, Promega). Two compounds that are chemically related were shown to have negligible cytotoxicity at 100 μ M while they inhibited FMDV 3D at IC₅₀s of 20nM and 3 μ M. Steady-state kinetics characterization revealed that both compounds are noncompetitive with respect to NTP and template/primer concentrations. These are the first reported FMDV 3D inhibitors *in vitro*, and they are currently being evaluated for *in vivo* antiviral activity.

[1] Christopher A Lipinski, Franco Lombardo, Beryl W Dominy, Paul J Feeney. *Advanced Drug Delivery Reviews* 23 (1997) 3-25.

[2] Lahser & Malcolm. *Analytical Biochemistry* 325 (2004), 247-254.