Identifying Initiation and Elongation Inhibitors of Dengue Virus RNA Polymerase in a High-throughput Lead-finding Campaign

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Background/Objective

Dengue virus (DENV) is the most significant mosquito-borne viral pathogen in the world and is the cause of dengue fever. The DENV RNA-dependent RNA polymerase (RdRp) is conserved among the four viral serotypes and is an attractive target for antiviral drug development. During initiation of viral RNA synthesis, the polymerase switches from a "closed" to "open" conformation to accommodate the viral RNA template. Inhibitors that lock the "closed" or block the "open" conformation would prevent viral RNA synthesis. Herein, we describe a screening campaign that employed a biochemical assay to identify inhibitors of RdRp initiation and elongation. Using a DENV subgenomic RNA template that promotes RdRp de novo initiation, the assay measures cytosine nucleotide analogue (Atto-CTP) incorporation. Liberated Atto fluorophore allows for quantification of RdRp activity via fluorescence. Active compounds were evaluated in a renilla luciferase-based DENV replicon cell-based assay to monitor cellular efficacy. Assays described herein are medium to high throughput, are robust and reproducible, and allow identification of inhibitors of the open and closed forms of DENV RNA polymerase.

Method

Cloning, protein expression, de novo FAPA fluorescence & dengue replicon cell-based assays

Result

The de novo FAPA was used as the primary screening at the initial stage to test 257,000 cpds tested against DENV4 RdRp. After passing various filters, 8133 reconfirmed hits were profiled in the DSF assay. A total of 300 DSF-active compounds (thermal shift $\geq 1.5^{\circ}C$) were then assessed for cellular activity in the DENV-Renilla Luciferase-replicon assay & 42 cpds were found to be active (EC50 <30uM).

Conclusion

Assays described herein are medium to high throughput, are robust and reproducible, and allow identification of inhibitors of the open and closed forms of DENV RNA polymerase