

A high-throughput screening assay against Bluetongue virus

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Introduction

Arthropod borne viruses (arboviruses) are important human pathogens that cause acute virus infections with severe diseases and/or death, including those listed in NIAID Priority Pathogen, Dengue virus (DENV) and Rift Valley Fever virus (RVFV) in Category A; West Nile virus (WNV), LaCrosse virus (LCV), Venezuela Equine encephalitis virus (VEEV) and Japanese encephalitis virus (JEV) in Category B; Yellow Fever virus (YFV) and Tick-borne encephalitis virus (TBEV) in Category C. Arboviruses are also important animal pathogens, such as Bluetongue virus (BTV) and Africa Horse Sickness virus (AHSV), listed as the "USDA High Consequence Livestock Pathogens". For many arbovirus based diseases, there is no effective vaccine or therapeutics currently available. The need to develop antiviral drugs is critical in treating and managing these diseases.

Arboviruses are unique because they are transmitted to their vertebrate hosts by arthropod vectors, therefore, they must be capable of replicating in two very divergent host taxa—vertebrates and insects. Although arboviruses replicate efficiently in both vertebrate and insect cells, the respective host responses are quite different in these cells. BTV is a non-enveloped, multi-layered, double-stranded RNA virus of *Reoviridae* family. Bluetongue virus (BTV) is transmitted to vertebrates by biting midges (*Culicoides*), and replicates in both vertebrate and insect hosts, but only causes severe disease in vertebrate hosts, such as sheep, goats and deer. BTV is one of the most important diseases of domestic livestock, causing \$3 billion/year loss worldwide.

MATERIALS AND METHODS

Cell Culture: BSR cells were cultured in DMEM with 2 mM L-glutamine, and 5% FBS. The cells are maintained at 37 °C, 5.0% CO₂ to 90% confluence.

Virus: The BTV-10 virus stock was propagated in BSR cells. The virus stocks from the cells were titrated in BSR cells using the TCID₅₀ method. The final titer was at 1 X 10⁸ TCID₅₀/ml.

Control and Drug Preparation: Carrier Control consisted of DMSO diluted in assay media to 0.6% and 5 μl was dispensed to both cell and virus control wells of 384 well black clear-bottom tissue culture treated plates. Test compounds were diluted in media to 60 μM with a DMSO concentration of 0.6% and 5ul was dispensed to the assay plate for a final test concentration of 10μM and final DMSO concentration of 0.1%.

Cell Plating: Once controls and compounds had been dispensed to the assay plates, 5,000 cells/well were plated in 15μl using a Matrix WellMate. Plates were then transferred to the Virology HTS lab and incubated 2-4 hrs at 37 °C, 5.0% CO₂ and high humidity.

Virus Addition: BTV was diluted in media to 5,000 pfu/ml and 10 μl was added to the compound wells and the virus control wells (MOI of 0.01). Plates were then incubated for 72 hrs at 37 °C, 5.0% CO₂ and high humidity.

Endpoint Read: The assay plates were equilibrated to room temperature for 30 minutes and then an equal volume of CellTiter-Glo reagent (Promega Inc.) was added to each well. Plates were incubated for 10 min at room temperature and luminescence was measured using a Perkin Elmer Envision multi-label reader with an integration time of 0.1 s. This step was also performed within the BSL-2 facility.

Data Analysis: Thirty two control wells containing cells only and 32 wells containing cells and virus were included on each assay plate and used to calculate Z' value for each plate and to normalize the data on a per plate basis. Results were expressed as percent inhibition of CPE where 100% inhibition of CPE was equal to the cell only control.

Abstract

Arthropod borne viruses (arboviruses) are important human/animal pathogens that cause acute virus infections with severe diseases and/or death. Several recent human/animal epidemics were caused by arboviruses, including DNV in Asia, WNV in North America and BT in Europe. There are no anti-viral drugs available against these diseases. We have designed, developed, optimized and validated a cytopathic effect (CPE) based, high-throughput screening (HTS) assay using the viability endpoint CellTiter Glo (Promega, Madison, WI) to identify novel anti-viral drugs against bluetongue virus infection in BSR cells. The 72h assay against Bluetongue virus was validated in 384-well plates with average Z factor of 0.7 ± 0.06, average cell CV of 9.84 ± 1.66, average S/N: 3.88 ± 0.56 respectively. The compound library provided by the NIH Molecular Libraries Small Molecule Repository (MLSMR) has been screened using this assay. A number of compounds have been identified with IC₅₀ value ranging from 0.1μM up to 100μM. Mechanism of action studies will be undertaken on the hits to help prioritize them for drug development and for additional studies in other flaviviruses including DNV, WNV, JEV, TBEV and YFV infections.

Results

The criteria for determining compound activity are based on percent inhibition of CPE. Of the substances demonstrating activity, they have been scored using a tiered range based on their relative percent inhibition. We have identified a number of compounds, as listed below (top 20 hits) in the table. These compounds are active in the above assay by inhibiting BTV-induced CPE. The hits were identified based on percent inhibition of CPE where 100% inhibition of CPE was equal to the cell only control. The follow up dose response assay will eliminate the false positives and help prioritize the hits. Mechanism of action studies for these compounds will be undertaken to help prioritize them for drug development and for additional studies against other arboviruses infection, including RVFV, DNV, WNV, YFV, LCV, VEEV, AHSV and BT infection.

Figure 1 The Average Z Factor for the CPE-Based Assay

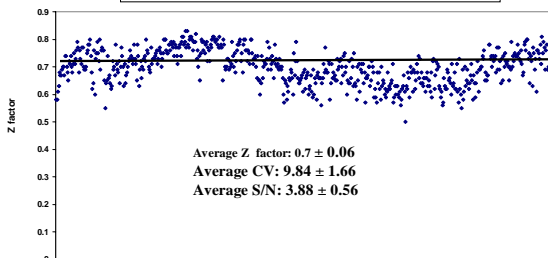


Figure 2 HTS Screening of the MLSMR library

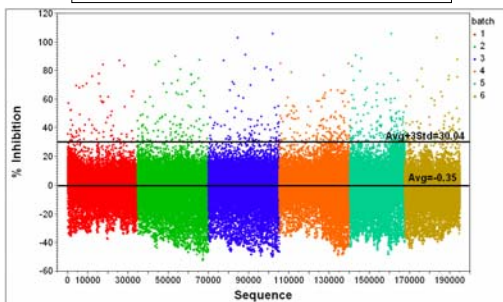


Table 1. Dose-Response and IC₅₀ of the Top-20 Hits from the Screening

VP0001:AB00550966 IC50 = 0.11	VP0001:AB00553939 IC50 = 0.17	VP0001:AB00407428 IC50 = 0.16	VP0002:AB00531539 IC50 = 0.12
VP0001:AB00532480 IC50 = 0.12	VP0002:AB00542636 IC50 = 0.10	VP0002:AB00419405 IC50 = 0.15	VP0001:AB00611360 IC50 = 0.18
VP0002:AB00563476 VP0002:AB005634	VP0002:AB00550890 IC50 = 0.10	VP0001:AB00593052 IC50 = 0.15	VP0002:AB00557362 IC50 = 4.00
VP0002:AB00148482 IC50 = 0.10	VP0002:AB00382987 IC50 = 0.15	VP0001:AB00168995 IC50 = 0.10	VP0002:AB00292130 IC50 = 5.00
VP0002:AB00562659 IC50 = 5.00	VP0002:AB00128688 IC50 = 0.30	VP0002:AB00074987 VP0002:AB000744	VP0001:AB00601602 VP0001:AB006016

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