Establishment of a medium-throughput drug-screening for Chikungunya Virus

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BACKGROUND

Newly emerging and reemerging viruses are posing a threat to public health worldwide. So far, no efficient therapeutics or anti-viral drugs are available for most of these viruses. This is particularly worrisome since most of the highly pathogenic and emerging viruses are, and will likely continue to be, RNA viruses. The instability of RNA viral genomes results in frequent mutations in viral strains, which leads to significant challenges for developing effective therapeutics. To overcome the hurdle, host-directed and broad-spectrum drug discovery using approved drugs has been considered a promising solution. The repurposing drug discovery workflow for infectious diseases is a time- and cost-effective platform for not only drug discovery but also for the understanding of pathogenesis mechanisms of the host pathways that the pathogens hijack. These viruses can cause acute and severe illness, including joint pain, respiratory diseases, and hemorrhagic fever with a high case fatality rate. So, it is important to have a potent and safe drug at hand that can be used for the treatment or prophylaxis of such infections. Chikungunya Virus (CHIKV) is used as a model system to develop a repurposing drug discovery workflow, and is a single-stranded positive-sense RNA alphavirus. Still, no FDA-licensed vaccines or therapeutics are available to combat these pathogens. Here we established a process workflow to perform medium-throughput screening of antiviral activity assessments using a commercially available small molecule library. Inhibition of viral titer, and quantitation of viral genomic RNA were performed by qPCR and plaque assay. A total of ninety two host-directed inhibitors against CHIKV were screened and six top inhibitors significantly targeted the viral protease and integrase when compared with DMSO. Further studies of *in vitro*, *in vivo,* and transcriptomics, will be followed to understand the inhibition mechanism clearly to select and optimize drugs.

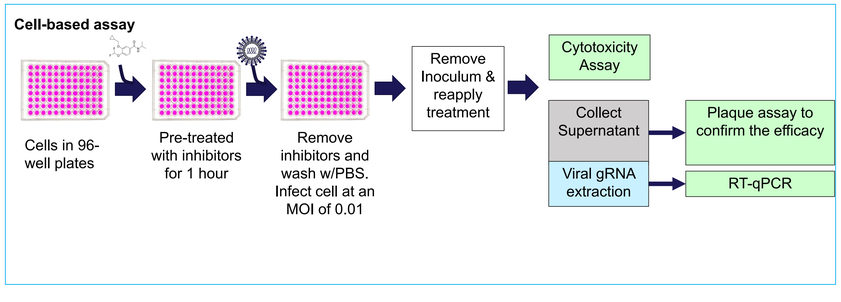
METHODS

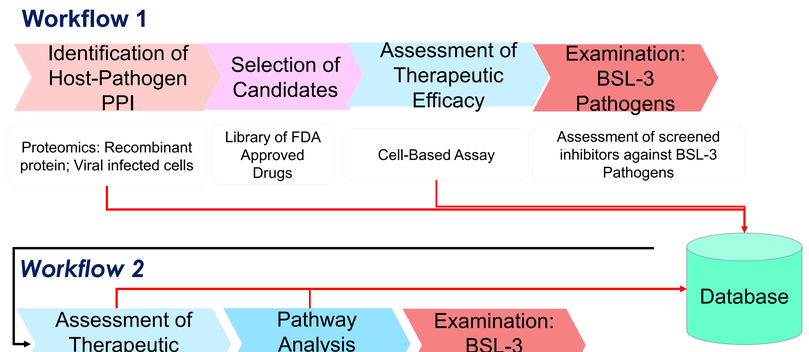
Two workflows of repurposing drug discovery against Chikungunya Virus were designed and executed in *in vitro* cell-based efficacy studies.

Vero Cells were seeded ~10K in 96 well plate, pre- treated with inhibitors (30μM each) for 1hr. Inhibitors were removed and cells were washed with PBS and cells were infected at MOI of 0.01. Inoculum was removed and treatment was reapplied. After 18 hrs. of post infection supernatants were collected for efficacy by plaque assay and the cells were tested for cell cytotoxicity by Cell titer Glow assay.

Workflow 1: Screening a commercially available protein-protein interaction library to identify inhibitors against CHIKV with Plaque assay.

Workflow 2: Medium throughput screening of approved drugs against CHIKV was performed without pre-selection by proteomics studies. The results will be followed by pathway analysis with additional *in vitro* assays, *in vivo* assays, and transcriptomics and Inflammatory Cytokines & Receptors array---- by RT² Profiler™ PCR Kit





RESULTS

Fig: 1 Cell Viability assay in ATCC CCL-81™ (Vero cells) in response to treatment with 72-92 compounds.

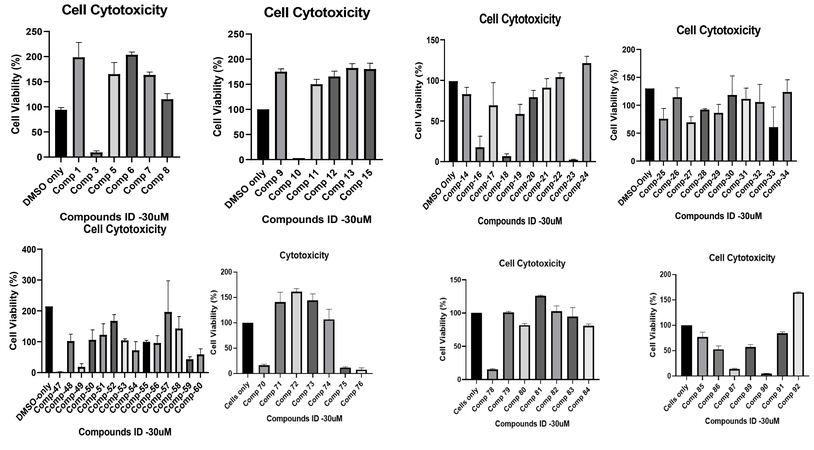
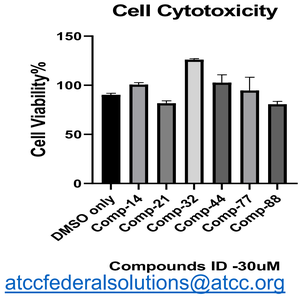


Fig: 2 Cell-Viability Assay from Selected compounds -30μM Conc.



RESULTS

Fig. 3 Efficacy by Plaque assay.

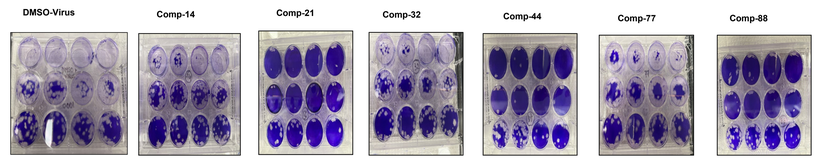
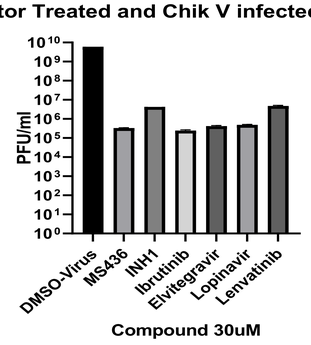


Fig: 4. Efficacy from Selected compounds



Inhibitors			Conc.	Target
DMSO			30uM	Control
Comp-14	s1164	Lenvatinib	30uM	Tyrosine Kinase Ihibitor-VEGFR
Comp-21	s1380	Lopinavir	30uM	HIV Protease
Comp-32	s2001	Elvitegravir	30uM	HIV Integrase
Comp-44	s2680	Ibrutinib	30uM	Brutons tyrosine kinase
Comp-77	s7493	INH1	30uM	Disrupts the Hec1/Nek2 interaction
Comp-88	s7305	MS436	30uM	BET bromodomain inhibitor inhibits I(HHV)

CONCLUSION

- The cell-based assays of both workflows screened inhibitors against CHIKV infection from a library of FDA approved drugs.
- In the study, we identified 6 inhibitors for host system which reduced CHIKV titer by at least 3 log scale.
- Viral proteases and integrase could be ideal target for broad spectrum drug discovery

ACKNOWLEDGEMENTS

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