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A549-ACE2 SARS-CoV-1 antiviral effect screening assay

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Nick Lynch^{1,2}

¹Curlew Research; ²ASAP Discovery Consortium

ASAP Discovery



Nick Lynch

Curlew Research, ASAP Discovery Consortium

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Protocol status: Working

We use this protocol and it's working

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Abstract

To evaluate the antiviral efficacy of compounds against SARS-CoV-1 infection using an engineered A549-ACE2 cell line model. A549 human lung adenocarcinoma cells stably transfected to express ACE2 receptors were used as the host cell system. Cells were pre-treated with a range of test compounds prior to SARS-CoV-1 infection at a multiplicity of infection levels.

The regular P-GP inhibitor used was Elacridar and positive control was Remdesivir

Viral replication was assessed at post-infection using detection methods. Control compounds/vehicle controls were included for comparison.

Materials

2uM Elacridar

Positive control = 50uM Remdesivir

Safety warnings

- ⚠ Always wear appropriate PPE for this protocol
Samples contain BSL-3 level high risk agents (viral) with infectious aerosol transmission potential.

Before start

Always wear appropriate PPE for this protocol

Refer to Material Safety Data Sheets for additional safety and handling information.

Summary

- 1 SARS-CoV-1 utilizes the angiotensin-converting enzyme 2 (ACE2) receptor for cellular entry, making ACE2-expressing cell lines valuable models for antiviral drug screening. The A549-ACE2 system provides a standardized platform for evaluating compound efficacy against coronavirus infection.

To screen potential antiviral compounds for inhibitory activity against SARS-CoV-1 replication using engineered A549 lung epithelial cells expressing ACE2 receptors.

- 2 A549-ACE2 cells were treated with test compounds in the presence of 2 μ M elacridar (efflux pump inhibitor) to enhance intracellular drug accumulation. Following compound treatment, cells were infected with SARS-CoV-1 and viral replication was monitored. Remdesivir (50 μ M) served as the positive control for antiviral activity. Viral replication inhibition was measured through cytopathic effect (CPE) reduction. Cell viability and viral inhibition were assessed to determine compound efficacy and cytotoxicity profiles.

Analysis

- 3 All statistical tests were executed using GraphPad Prism. The EC_{50} values were defined as the concentration at which there was a 50% decrease in viral replication relative to vehicle alone (0% inhibition) and for EC_{90} which there was a 90% decrease in viral replication relative to vehicle alone. Curves were fitted based on four parameter non-linear regression analysis.



Protocol references

A Newly Engineered A549 Cell Line Expressing ACE2 and TMPRSS2 Is Highly Permissive to SARS-CoV-2, Including the Delta and Omicron Variants

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<https://pmc.ncbi.nlm.nih.gov/articles/PMC9318744/>

Cell culture model system utilizing engineered A549 cells to express high levels of 1 ACE2 and TMPRSS2 for investigating SARS-CoV-2 infection and antivirals

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<https://doi.org/10.1016/j.antiviral.2025.106212>

Remdesivir Inhibits SARS-CoV-2 in Human Lung Cells and Chimeric SARS-CoV Expressing the SARS-CoV-2 RNA Polymerase in Mice

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