In a search for an effective, orally administered treatment for COVID-19, the *in vitro* activity of AT-527, the free base of the antiviral drug candidate AT-527, a modified guanosine nucleotide prodrug known to inhibit the hepatitis C virus NS5B polymerase (1), was evaluated against several coronaviruses, including SARS-CoV-2, the virus responsible for COVID-19. Additionally, the inhibition of replication of SARS-CoV-2 by AT-527 was compared to that by N4-hydroxycytidine, a nucleoside recently reported to be active against SARS-CoV-2 (2).

**Methods**

Viruses. Seasonal human coronaviruses (HCoV-229E and HCoV-OC43) were obtained from ATCC (Manassas, VA). MERS-CoV (EMC), SARS-CoV (Überlingen), and SARS-CoV-2 (武汉2020) were supplied by The Centers for Disease Control and Prevention, Atlanta, GA.

Concentration and infection of HBE-21, Hu-7, and RD cells. The original activity of AT-527 was evaluated against human coronavirus aegro (229E), boletus (OC43), MERS (Überlingen) and SARS-CoV-2 (武汉2020) and was compared to that of a standard dilution of a diluted standard air of HAE before or after confluent Human (EC80) in coronavirus (ECoV, HCoV-229E, HCoV-OC43, MERS and SARS, respectively). A virus yield reduction (VYR) assay (4) which calculated virus titer using a standard end point dilution assay was used. The equation was used as a second, independent determination of the inhibition of viral replication.

Chromopeptide (di-mannose) (Manck-Chern, Palo Alto, CA) was also tested against HCoV-229E in Hu-7 cells. Schisandra (Pharmacia Sia, Ciy, NC, up to 100 µM) was tested against HCoV-222E in Hu-7 in confluent HBE-21 cells.

SARS-CoV-2 infection and treatment of HAE cells. Differenntiated normal human airway epithelial (HAE) cells (EpithAir® ARH-100 or ARH-112) were prepared by MyAir Corporation (Auburn, MA) from a single donor. The cells form palisaded monolayer, the apical apical side of which is exposed to air and a creating a lumen. SARS-CoV-2 virus was diluted in ARH-100/HAE medium before being inoculated to a MOI when added to cultures of 0.005% CPE. Cellular solutions at dilutions of 1:1 N-4-hydroxydrin (Gilead, Menlo Park), Foscarnet were applied to the cells (120 µl) to the basal side, while virus (120 µl) was applied only to the apical side. As a control, some cells were treated with cell culture medium only. After a 2 h incubation, the apical medium was removed, and the basal medium was replaced with fresh culture medium (or 1% FCS). The cell viability was monitored using a visual inspection. Virus released into the apical compartment of the HAE cells was harvested by the addition of Medium, incubated for 20 min, mixed and plated onto 96-well plates. All plates were analyzed using the Cytopathic Effect Assay (CPE). Viral yield, produced CPE was quantified by the number of plaque forming units (PFU) per well. The activity of AT-527 and other antiviral compounds was measured in cells infected with different viruses, using the neutral red assay and/or the virus yield reduction (VYR) assay method. The effective concentration required to achieve a 50% reduction (EC50) of the viral-induced cytopathic effect (CPE); the concentration of virus yield to 1 log EC50, and the cytostatic concentration of the drug to be killed to 50% of viable cells without virus (CDD). Values represent rates from single or multiple (mean ± SD) experiments.

**Background**

**Results**

<table>
<thead>
<tr>
<th>Virus (genus)</th>
<th>Cell line</th>
<th>Compound</th>
<th>Cytopathic Effect Assay</th>
<th>Virus Yield Reduction Assay EC50 (µM)</th>
<th>Selectivity (CDD/EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCoV-229E (alpha)</td>
<td>BMK-21</td>
<td>AT-527</td>
<td>1.8 ± 0.3 (2) &gt;100</td>
<td>ND</td>
<td>&gt;55 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Hu-7</td>
<td>chloroquine</td>
<td>1.7 ± 0.3 (2) &gt;100</td>
<td>ND</td>
<td>&gt;2 ± 0.2 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydroxychloroquine</td>
<td>7.1 ± 0.8 (2) 80</td>
<td>ND</td>
<td>&gt;0.05 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>AT-527</td>
<td>2.8 ± 0.6 (2) &gt;86</td>
<td>ND</td>
<td>&gt;2 ± 0.3 (3)</td>
</tr>
<tr>
<td>SARS-CoV-2 (b)</td>
<td>Hu-7</td>
<td>AT-527</td>
<td>ND</td>
<td>&gt;60</td>
<td>&gt;2.5 ± 0.3 (3)</td>
</tr>
<tr>
<td></td>
<td>RD</td>
<td>AT-527</td>
<td>2.8 ± 0.6 (2) &gt;86</td>
<td>ND</td>
<td>&gt;2 ± 0.3 (3)</td>
</tr>
<tr>
<td></td>
<td>HAE</td>
<td>N4-hydroxycytidine</td>
<td>ND</td>
<td>&gt;192</td>
<td>&gt;25 ± 0.3 (3)</td>
</tr>
</tbody>
</table>

The activity of AT-527 and other antiviral compounds was measured in cells infected with different viruses, using the neutral red assay and/or the virus yield reduction (VYR) assay method. The effective concentration required to achieve a 50% reduction (EC50) of the viral-induced cytopathic effect (CPE); the concentration of virus yield to 1 log EC50, and the cytostatic concentration of the drug to be killed to 50% of viable cells without virus (CDD). Values represent rates from single or multiple (mean ± SD) experiments.

**In vitro Potency and Cytotoxicity of AT-527 and Other Oral Drugs Against Several Coronaviruses**

- **AT-527** exhibited a mean EC50 value of 0.5 µM against SARS-CoV-2 and similar potencies against HCoV-229E, HCoV-OC43 and MERS and no cytotoxicity up to a concentration of 100 µM.
- **N4-hydroxycytidine** inhibited SARS-CoV-2 replication with an EC50 value of 3.9 µM in the same HAE cell model.

**Conclusions**

- The substantial formation of the active TP metabolite in infections with human bronchial and nasal epithelial cells is consistent with the sub-micromolar potency of the free base of AT-527 against SARS-CoV-2 replication in the HAE tissue model.
- Considering that these cell types are actual targets of infection in COVID-19 patients, the potency of AT-527, combined with its favorable safety profile previously established in HCV subjects, suggests that AT-527 may be highly efficacious in treating COVID-19.

**References**


**Acknowledgements**

Disclosures: SS, ALG and JPS are employees of Atea Pharmaceuticals. We thank Dr. Kerry-Win de Costa for her assistance in preparing this poster presentation.

**Intracellular Half-life of AT-527 in Primary Human Bronchial and Nasal Epithelial Cells after 8-h Exposure to 10 µM AT-527

About 3-fold more AT-9010 was formed in human bronchial compared to nasal epithelial cells.

AT-9010 half-life was similar (38-39 h) in both human cell types.