Discovery of potent small molecule inhibitors of RSV Fusion protein

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Background

Respiratory syncytial virus (RSV) infections in infant, elderly, and immunocompromised patients represent substantial unmet medical need.1,2 Tractable options for the development of anti-RSV therapies include inhibition of RSV-encoded fusion (F) protein.3 We report the discovery of orally bioavailable RSV F inhibitors exhibiting highly potent and balanced activities against diverse RSV isolates, large cytotoxicity indices, and promising in vivo pharmacokinetics. The profile of a front-running candidate from this program (‘Lead 9’) is presented below.

Program development

Lead optimization was instigated on three novel 6,6-bicyclic cores (series 1-3) with the aim of selecting a candidate drug capable of sustaining therapeutic drug exposures against a broad range of RSV infections in humans. Inhibitors synthesized early in the lead optimization campaign achieved potencies <10 nM against a primary RSV A screening strain (RSV A2) but were often associated with lower activities against additional RSV strains and non-optimal ADMET profiles. Subsequent optimizations resulted in several promising molecules from series 3 with picomolar EC50 values against both RSV A and B subtypes, cytotoxicity indices >50,000, favorable ADMET properties, and encouraging in vivo PK profiles in rat and dog. Lead 9 was identified as one of several special interest molecules from series 3 and was profiled extensively.

Methods

Established virology, molecular/structural biology, and drug metabolism/pharmacokinetic assays were used to screen and profile F inhibitors generated from the internal chemistry program.

Results

Mechanism of action for series 1-3 molecules

Time-of-addition studies and the generation of specific resistance-associated substitutions in the F protein using series 1-3 examples indicated the mechanism of action for these molecules was mediated by targeting the RSV F protein. Co-crystallization of series 1-3 examples with pref revealed compounds bound in a pocket of pref created at the intersection of the 3 monomeric subunits:

• Medivir example compounds from all 3 series bind in the same pocket with the same stoichiometry: 1 inhibitor per pref trimer
• Binding pocket contains residues involved in conferring resistance to fusion inhibitors e.g. L141 and D489.
• The inhibitors are likely ‘triggering antagonists’: they tether and stabilize 2 structurally labile regions of F (heptad repeat B and fusion peptide) to prevent release of the fusion peptide during the conformational change required to initiate the membrane fusion process.

Co-crystal structure of a series 1 inhibitor (yellow) bound to pref trimer. L141 and D489 residues are highlighted in pink.

In vitro safety assessments for Lead 9 revealed benign safety profiles

<table>
<thead>
<tr>
<th>Assay</th>
<th>Description</th>
<th>Result Lead 9</th>
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<tbody>
<tr>
<td>Cytotoxicity evaluations</td>
<td>Hep3B/HUV17/MT4 cell lines</td>
<td>CC50 &gt;50µM</td>
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<tr>
<td>High content multi-parameter</td>
<td>HepG2 and rat primary hepatocytes</td>
<td>No significant effects on any parameter tested (top concentration 200 µM)</td>
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<td>toxicity assessment (Cell/cpH4)</td>
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<td>Secondary pharmacology target screen (87 targets)</td>
<td>In vitro binding to GPCR, ion channels, transporters, nuclear receptors, kinases and other non-kinase enzymes.</td>
<td>No hits (tested at 10 µM)</td>
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Conclusions

• A lead optimization campaign directed upon three novel 6,6-bicyclic cores (series 1-3) resulted in the identification of Lead 9, which demonstrated:
  ✓ Biology data consistent with inhibition of RSV Fusion protein.
  ✓ Balanced picomolar EC50 against a broad range of RSV A and B isolates.
  ✓ Favourable human in vitro DMPK properties.
  ✓ Excellent oral bioavailability in rat and dog.
  ✓ A robust antiviral effect in the cotton rat model for human RSV infection.
  ✓ A benign in vitro safety profile.

• The profile of Lead 9 supports progression to preclinical development with the aim of developing a safe and effective treatment against RSV infections in humans.

References