Structure-based discovery of potent and selective SARS-CoV-2 3-chymotrypsin-like cysteine protease inhibitors using a multiplex screening platform

Koen Vandyck[1], Jerome Deval[2], Dorothée Bardiot[5], Leonid Beigelman[1,2], Lawrence M. Blatt[1,2], Sandro Boland[5], Patrick Chaltin[3,5], Zachary A. Gurard-Levin[6], Cheng Liu[2], Michael D. Scholle[6], Arnaud Marchand[5]; Pierre Raboisson[1], Antitsa Stoycheva[2], Julian A. Symons[2], and Johan Neyts[4]

[1]Aligos Belgium BV, Leuven, Belgium; [2]Aligos Therapeutics, Inc., South San Francisco, CA; [3]Centre for Drug Design and Discovery (CD3), KU Leuven, Leuven, Belgium; [4]Rega Institute for Medical Research, KU Leuven, Leuven, Belgium; [5]CISTIM Leuven vzw, Leuven, Belgium; [6]SAMDI Tech, Inc., Chicago, USA.

Background:

The 3-chymotrypsin-like cysteine protease (3CLpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is considered a major target for the discovery of direct antiviral agents. Currently, at least two 3CLpro inhibitors are in (pre)clinical development for the treatment of COVID-19: GC-376 and PF-07304814 (a prodrug of PF-00835231). Both agents exhibit different degrees of cathepsin L inhibition. Cathepsin L has been shown to block SARS-CoV-2 infection of human and monkey cells by proteolytic cleavage of the SARS-CoV-2 spike protein. Therefore, it can be hypothesized that the cell-based activity of several 3CL protease inhibitors described in literature is at least partially mediated by inhibition of host cathepsin L.

Methods:

We have developed a novel self-assembled monolayer desorption ionization mass spectrometry (SAMDI-MS) enzymatic assay to measure the dual inhibition of SARS-CoV-2 3CLpro and rhinovirus HRV3C protease, in parallel with human cathepsin L inhibition. This novel multiplex assay was used to profile protease potency and selectivity of a diverse set of reference protease inhibitors, including GC-376, PF-00835231, boceprevir, and rupintrivir. X-ray structure analysis of SARS-CoV-2 3CLpro and cathepsin L binding sites provides key information for rational drug design of selective SARS-CoV-2 3CLpro inhibitors.

Results:

Selectivity profiling in the multiplex assay indicated that GC-376 inhibits all three proteases, whereas PF-00835231, boceprevir, and rupintrivir appear to be selective for at least one enzyme. Structural analysis showed that the combination of promiscuous cysteine-targeted warheads, minimal specific interactions, and limited bulk allows GC-376 to potently inhibit all three enzymes. In contrast, selection of an appropriate warhead and optimized side chains interacting more tightly with P2, P3, and P4 pockets led to selective SARS-CoV-2 3CLpro inhibitors with no cathepsin L activity. Our lead compound ALG-009711 exhibits a potent SARS-CoV-2 3CLpro activity (IC_{50} = 0.009 μM, Ki = 1 nM) with no associated cathepsin L inhibition (IC_{50} > 10 μM). Although PF-00835231 suffers from instability in simulated intestinal fluid (with pancreatin), ALG-009711 has been shown to be stable in these conditions ($T_{1/2}$ > 480 minutes).

Conclusions:

Our lead optimization effort led to the identification of potent and selective SARS-CoV-2 3CLpro inhibitors with promising drug-like characteristics.