Broad-spectrum therapeutics against SARS-CoV-2 3CL protease

Ladislau C. Kovari[1], Franck Amblard[2], Keivan Zandi[2], Peng Liu[2], Shaoman Zhou[2], Kendall M. Muzzarelli[1], Iulia A. Kovari[1], Tami McBrayer[2], Raymond F. Schinazi[2]

[1]Department of Biochemistry, Microbiology, and Immunology, School of Medicine, Wayne State University, Detroit, MI 48201, USA
[2]Laboratory of Biochemical Pharmacology, Department of Pediatrics, School of Medicine, Emory University and Children's Healthcare of Atlanta, Atlanta, GA 30322, USA

Background

Since the beginning of January 2020, over 1,200 clinical trials have been initiated, aiming to treat and prevent COVID-19 (clinicaltrials.gov). These unprecedented efforts have resulted in only modest results, and repurposed drugs may not achieve full therapeutic means to prove effective drug treatment options. Out of several ongoing clinical trials, two treatment options are effective, remdesivir and dexamethasone, to date, but the clinical outcomes are controversial. Given the current gap in coronavirus (CoV) therapy, novel drugs and vaccines development are needed. Our objective is to design and develop a safe and effective antiviral agent targeting the SARS-CoV-2 3CL protease (3CLpro) for the treatment of coronavirus disease 2019 (COVID-19) and human coronavirus infections in general. The central hypothesis is that inhibition of SARS-CoV-2 polyprotein cleavage results in inhibition of virus and that the drug can be used to prevent or treat COVID-19. Our research targets the 3CLpro, a key enzyme for SARS-CoV-2 polyprotein cleavage and viral replication using our established and proven drug discovery expertise.

Methods

Our drug discovery platform includes *(1)* our proprietary libraries and medicinal chemistry expertise of viral protease inhibitors; *(2)* molecular modeling and virtual screening capabilities of millions of compounds using the Schrödinger programs and other drug discovery suites; *(3)* cell-based coronavirus assays in our in house BSL-2* and BSL-3 facility; *(4)* recombinant protein-based enzyme assays and X-ray crystallographic studies of SARS-CoV-2 3CLpro, and; *(5)* the capability of evaluating candidate inhibitors for bioavailability and ADME-T characteristics, and efficacy through animal models of COVID-19 such as golden Syrian hamsters and non-human primates.

Results

We selected the nsp7/nsp8 cleavage site as representative for the SARS-CoV-2 3CLpro cleavage employing a covalent inhibitor design strategy. We decided to proceed with a P-side (P4-P1) inhibitor design. To further enhance inhibitor binding, we added an electrophilic warhead designed to react with the nucleophilic thiol group of Cys145 of the SARS-CoV-2 3CLpro. To develop highly potent and specific lead molecules, we targeted the catalytic Cys145-His41 dyad and other essential residues within the binding pocket which are important in the proteolytic process of SARS-CoV-2. We identified compound **3150**, a unique, non-toxic, small peptidomimetic molecule that inhibits structurally related viral 3CL proteases (*e.g.*, norovirus and enterovirus) with an EC_{50} of 1 to 20 nM in cell culture, and SARS-CoV-2 replication (in Vero cells - EC_{50} = 0.6 μM) with no apparent cytotoxicity up to 100 μM.

Conclusions

The expected outcome of this work is the discovery of submicromolar to low nanomolar COVID-19 inhibitors. The ultimate goal of the proposed studies is to speed-up and advance an anti-COVID-19 drug candidate to the stage of filing an investigational new drug (IND) application. The results will have a significant positive impact because they lay the groundwork for the clinical development of COVID-19 antiviral therapy and the potential to combine a potent and selective protease inhibitor with a nucleoside analog and anti-inflammatory drugs such as a JAK-STAT inhibitor with antiviral activity (*e.g.*, baricitinib) first in culture and then in an animal model.