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Crystallographic and Kinetic Studies of Novel SARS-CoV 3CLpro Protease Inhibitors that Inhibit SARS-CoV and MHV-A59 Replication. Andrew Mesecar¹, Kiira Ratia¹, Bernard Santarsiero¹, Kai Xi¹, Dalia Jukneliene², Brian Harcourt³, Paul Rota³, Susan Baker², Arun Ghosh¹. ¹Univ. of Illinois at Chicago, ²Loyola Univ. of Chicago Stritch School of Medicine, ³Centers for Disease Control and Prevention, National Center for Infectious Diseases.

Severe Acute Respiratory Syndrome (SARS) is a life-threatening, acute, atypical pneumonia caused by the SARS coronavirus (SARS-CoV). The genome of SARS-CoV is composed of a single RNA strand with positive polarity and encodes a polyprotein that must be cleaved by two virally encoded proteases, PLpro and 3CLpro, for viral replication. We have initiated structure-based drug design studies on SARS 3CLpro using the rhinovirus 3C-protease inhibitor, AG7088, as a starting template. The SARS 3CLpro enzyme was over-expressed, purified, and crystallized without the use of affinity-tags. A high throughput, FRET-based fluorescence assay was developed to measure the kinetic parameters of the wild-type and two mutant enzymes, as well as to screen our library of 10,000 compounds. Six compounds were synthesized and tested as inhibitors of SARS 3CLpro in vitro. Two of the compounds that inhibit SARS 3CLpro activity also show antiviral activity against SARS-CoV and MHV-A59 infected cells with EC₅₀s <100 μ M, and one was more effective at reducing viral titer than the protease inhibitor E64-D. The crystal structures of wild type and mutant SARS 3CLpro enzymes in complex with these inhibitors and others have been determined to between 1.9 and 2.1 Å resolution. These structures should serve as important drug-design templates for the development of anti SARS-CoV therapeutics.