Kinetic and Crystallographic Analyses of SARS Coronavirus 3CLpro Inhibitors

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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. Ten 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 infected cells with EC50s <100 μM, 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.

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