

ORAL PRESENTATION O2

SARS Coronavirus Major Protease Substrate Specificity and Inhibitor Development

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Severe acute respiratory syndrome (SARS) is an emerging infectious disease associated with a high rate of mortality. The SARS-associated coronavirus (SARS-CoV) has been identified as the etiological agent of the disease, and concern remains about the possibility of a recurrence. Here we report a complete description of the tetrapeptide substrate specificity of 3Clpro using fully degenerate peptide libraries consisting of all 160,000 possible naturally occurring tetrapeptides. The substrate specificity data show the expected P1-Gln P2-Leu specificity and elucidates a novel additional P1 preference. These data were then used to develop optimal substrates for a high-throughput screen of a 2000 compound small-molecule inhibitor library consisting of known cysteine protease inhibitor scaffolds. We also report the 1.8 Å X-ray crystal structure of 3Clpro bound to an irreversible inhibitor. This inhibitor, an α,β -epoxyketone, inhibits viral replication with an IC_{50} of 10 μ M in a tissue culture assay and inhibits 3Clpro with a k_3/K_i of 0.1 μ M⁻¹s⁻¹. Furthermore, our crystal structure of the inhibitor-enzyme complex has enabled us to develop a 2-dimensional HSQC NMR assay to determine the mode of binding of other identified inhibitors. Finally, we demonstrate the feasibility of rational improvement of this scaffold using a second generation inhibitor with a k_3/K_i of 0.5 μ M⁻¹s⁻¹. These data provide the foundation for a rational small-molecule drug design effort based upon our identified inhibitor scaffold, our crystal structure of the complex, and our more complete understanding of substrate specificity.