

IDENTIFICATION AND MECHANISTIC CHARACTERIZATION OF NOVEL INHIBITORS OF SARS-COV ENTRY

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Severe acute respiratory syndrome (SARS) is an infectious and highly contagious disease that is caused by SARS-associated coronavirus, SARS-CoV. Virus entry is a key target step for therapies because it can prevent the propagation of virus at an early stage of the disease. We used a cell-based assay to identify inhibitors of SARS-CoV entry. We prepared HIV-luc/SARS *env* pseudotypes and infected, in the presence or absence of potential inhibitors, 293T cells that had been transfected with a plasmid expressing the receptor for SARS-CoV (angiotensin-converting enzyme 2, ACE2). Using this assay we screened >2000 compounds and identified three compounds that specifically inhibit entry of the HIV-luc/SARS *env*, but not of the HIV-luc/ Vesicular Stomatitis Virus *env* pseudotypes. The compounds had strong potencies (EC_{50} s were 2.9, 4.8 and 5.8 μ M) and low cytotoxicities (high CC_{50} s), resulting in promising Selectivity Indices (CC_{50}/EC_{50} were >175, >65, and >86, respectively). Only one of the compounds was a moderate inhibitor of cathepsin L, a cellular protease whose activity is required to process the SARS-CoV *env* glycoprotein (Spike) and allow viral entry. Moreover, none of the compounds affects the cleavage activity of furin, another host protease who may also be involved in SARS-CoV entry. Using a flow cytometry binding assay, we found that all three compounds decrease binding of the SARS-CoV Spike receptor binding domain to ACE2 receptor expressed on the surface of 293T cells. Hence, we have identified three promising compounds as the first small molecule inhibitors that can block receptor-dependent entry of SARS-CoV.