Discovery and Mechanistic Characterization of Novel SARS Coronavirus Inhibitors that Block Viral 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). Viral entry is a key target step for therapies because it can prevent the propagation of virus at early stages of the disease. We used a cell-based assay to identify inhibitors of SARS-CoV entry. We prepared a pseudotyped virus in which the core is from HIV and envelop is from the SARS-CoV (HIV-luc/SARS *env)*. This pseudotyped virus was used to infect, 293T cells expressing the receptor for SARS-CoV, Aangiotensin-converting enzyme-2 (ACE2).

Using this assay we screened a chemical library of more than 2000 compounds and identified three compounds that specifically inhibit entry of the HIV-luc/SARS *env*. These compounds did not inhibit another pseudotyped virus which had same core from HIV but envelop was from Vesicular Stomatitis Virus. The compounds had strong potencies (EC_{50}s were 2.9, 4.8 and 5.8 µM) and low cytotoxicities (high CC_{50}s) resulting in promising Selectivity Indices (CC_{50}/EC_{50} were >175, >65, and >86, respectively). Importantly, the compounds were found to have excellent antiviral activities, blocking SARS-CoV replication at low nM concentrations. 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, which 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 293Tcells.

Hence, we have discovered three promising compounds as the first small molecule inhibitors that can block receptor-dependent entry of SARS-CoV.