NMR OF SARS-COV-2 MAIN PROTEASE (3CLPRO) FOR DRUG DEVELOPMENT

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The main protease (3CLp) of the SARS-CoV-2, the causative agent for the COVID-19 pandemic, is one of the main targets for drug development, as this enzyme is essential for viral replication and highly conserved among coronaviruses. To be active as a homodimer, 3CLp relies on a complex interplay between dimerization, active site conformational flexibility, and allosteric regulation. These molecular mechanisms are not fully resolved and their deciphering is a crucial step to enable the search for inhibitors of 3CLp activity. In this context, using NMR spectroscopy, we studied the conformation of dimeric 3CLp from the SARS-CoV-2 and monitored ligand binding, based on the backbone NMR signal assignments. To identify small compounds that can be used for antiviral development, we performed a fragment-based screening using both NMR ligand- and protein-observed methods, which led to the identification of 38 fragment hits that bind 3CLp. Analysis of the binding sites showed three hotspots on 3CLp, two located in the substrate binding pocket and one at the dimer interface. Further analysis showed that F01 is a non-covalent reversible inhibitor of the 3CLp and has antiviral activity in SARS-CoV-2 infected cells. This study sheds light on the complex structure-function relationships of 3CLp, and constitutes a strong basis to assist in developing potent 3CLp inhibitors, opening new perspectives to fight the COVID-19 pandemic or a next coronaviruses outbreak.