Structural studies towards the development of an oral protease (Mpro) inhibitor to treat SARS-CoV-2 infection

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Despite progress in vaccine development, antivirals targeting SARS-Co-2 are needed for those who are immunocompromised, and for future outbreaks. Proteases cleave peptide bonds of a very specific sequence making them strong drug targets. Antivirals that target proteases are already used clinically to treat HIV and Hepatitis C virus. We have developed inhibitors of the SARS-CoV-2 protease to prevent the main protease (M^{pro} or $3CL^{pro}$) from cleaving the viral polypeptide and subsequent viral replication in cells. We developed novel α-acyloxymethylketone warhead peptidomimetic compounds with a 6-membered lactam glutamine mimic in P1. Compounds with potent SARS-CoV-2 3CL protease and *in vitro* viral replication inhibition were identified with low cytotoxicity and good plasma and glutathione stability. α-Acyloxymethylketone compounds also exhibited antiviral activity against an *alpha*- and non-SARS *beta*-coronavirus strains with similar potency and better selectivity index than remdesivir. X-ray crystallography revealed the mechanism of inhibition, and has helped the optimisation of new derivatives. Moving forward, these inhibitors will be tested with variant proteases, followed up by studies in animals to determine efficacy and pharmacokinetics in preparation for clinical trials.

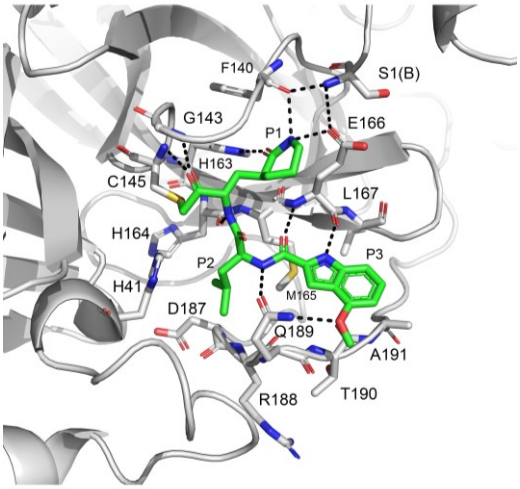

Figure 1. Cocrystal structure of chain C covalent adduct from 15l reacting with SARS-CoV-2 3CLP (PDB: 7MBI).

[1] Bai, B., Belovodskiy, A., Hena, M., Kandadai, A.S., Joyce, M.,Saffran, H., Shields, J., Khan, M.B., Arutyunova, E., Lu, J.; Bajwa, S., Hockman, Darren; Fischer, Conrad; Lamer, Tess; Voung, Wayne; Van Belkum, Marco; Gu, Zhengxian; Lin, Fusen; Du, Yanhua; Xu, Jia; Rahim, Mohammad; Young, Howard; Vederas, John; Tyrrell, D. Lorne; Lemieux, M Joanne; Nieman, James. Peptidomimetic α-acyloxymethylketone warheads with 6-membered lactam P1 glutamine mimic: SARS-CoV-2 3CL protease inhibition, coronavirus antiviral activity and in vitro biological stability. *J Med Chem in press*

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