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Challenges of short substrate analogues as SARS-CoV-2 main protease inhibitors

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ABSTRACT

Specific anti-coronaviral drugs complementing available vaccines are urgently needed to fight the COVID-19 pandemic. Given its high conservation across the betacoronavirus genus and dissimilarity to human proteases, the SARS-CoV-2 main protease (M^{pro}) is an attractive drug target. SARS-CoV-2 M^{pro} inhibitors have been developed at unprecedented speed, most of them being substrate-derived peptidomimetics with cysteine-modifying warheads. In this study, M^{pro} has proven resistant towards the identification of high-affinity short substrate-derived peptides and peptidomimetics without warheads. 20 cyclic and linear substrate analogues bearing natural and unnatural residues, which were predicted by computational modelling to bind with high affinity and designed to establish structure–activity relationships, displayed no inhibitory activity at concentrations as high as 100 μM. Only a long linear peptide covering residues P_6 to P_5' displayed moderate inhibition (K_i = 57 μM). Our detailed findings will inform current and future drug discovery campaigns targeting M^{pro}.

With over 200 million reported cases and 4 million deaths,[1] the ongoing COVID-19 pandemic is among the most devastating pandemics in human history.[2] Specific antiviral drug candidates targeting SARS-CoV-2 are urgently needed to complement available vaccines and prepare for future coronavirus outbreaks.[3] Inspired by the successful discovery of HIV and HCV protease inhibitors and their development into drugs,[4] coronaviral proteases are currently among the most promising targets.[5–7]

The betacoronavirus RNA genome encodes two proteases, the papain-like protease (PL^{pro}) and the main protease (M^{pro} or $3CL^{pro}$), which process the viral polyproteins pp1a and pp1ab into smaller non-structural proteins that assemble the replisome.[8] M^{pro} is structurally conserved across SARS-CoV-1, MERS-CoV and SARS-CoV-2, which may allow the development of pan-coronaviral drugs.[7,9] The majority of polyprotein cleavage events are performed by M^{pro}, making it an attractive drug target. M^{pro} forms a homodimer and is a cysteine protease with distinct substrate specificity ranging from P_4 to P_1' (using the nomenclature of Schechter and Berger),[10] with a particularly strong preference for glutamine in P_1.[7,11] No human host proteases with similar substrate recognition are known, rendering M^{pro} an ideal drug target with respect to off-target effects.[5–7]

Before the emergence of SARS-CoV-2, M^{pro} had already attracted attention as a potential drug target against the related coronaviruses SARS-CoV-1 and MERS-CoV,[12] which emerged in 2002 and 2012, respectively. None of the small molecules and peptidomimetics reported to inhibit M^{pro} of SARS-CoV-1 and MERS-CoV were developed further into antiviral drugs.[13,14] Substrate-derived peptidomimetics relied on electrophilic reactive groups modifying the catalytic cysteine residue (commonly known as cysteine warheads) to achieve sufficient affinity. Inhibitors included Michael acceptors,[15–20] aldehydes,[20–24] aldehyde prodrugs,[25,26] α-ketoamides,[27] epoxides and aziridines,[28,29] and α-halomethyl ketones.[30,31]

Since the emergence of SARS-CoV-2 in late 2019, several more M^{pro} inhibitors have been discovered at unprecedented speed. The same dependence on reactive warheads prevails for substrate-derived peptides and peptidomimetics. Warheads employed include Michael acceptors,[32] aldehydes,[33,34] aldehyde prodrugs,[35] α-ketoamides,[36] vinylsulfones,[11] azanitriles,[37] and ketones.[38] In April 2021, Pfizer revealed the orally available M^{pro} inhibitor PF-07321332, which is a short substrate analogue featuring a C-terminal nitrile warhead.[39] Very recently, a cyclic peptide has been reported, which binds to SARS-CoV-2 M^{pro} without forming a covalent bond.[40] With an IC_{50} value of about 160 μM, however, the activity of this compound is many orders of magnitudes below those of substrate-based analogues with warheads.

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