SARS Coronavirus 3CL Protease Inhibitors with an Electrophilic Arylketone Warhead

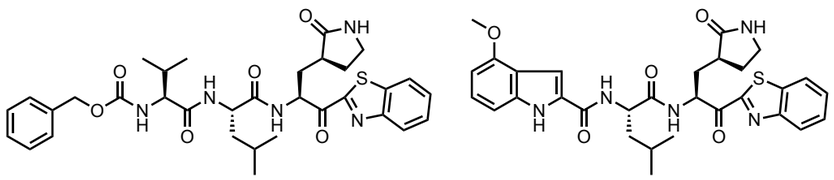
The novel coronavirus, SARS-CoV-2, identified as the pathogen for the coronavirus disease-19 (COVID-19), has 3CL protease (3CLpro), a cysteine protease that plays a pivotal role in the replication of the virus. We have developed peptidomimetic 3CLpro inhibitors with a unique benzothiazolyl ketone as a warhead group, which display potent inhibitory activity against SARS-CoV-2 3CLpro. X-ray structural analysis revealed that these inhibitors establish multiple hydrogen bond interactions with backbone amino acids and a covalent bond with the active site of 3CLpro. It is suggested that one of the most potent inhibitors, YH-53, is a high potential lead compound in COVID-19 drug discovery.

The coronavirus outbreak that began in 2019 caused a pandemic and devastated public healthcare systems and the global economy. The pathogen of COVID-19 was then identified as a novel coronavirus, SARS-CoV-2. This coronavirus is an enveloped, single-stranded, positive-sense RNA virus, and its replicase gene encodes for two large overlapping polyproteins, pp1a and pp1ab. The functional proteins are released from these polyproteins by extensive proteolytic processing, predominantly by the 3C-like protease (3CLpro), also referred to as the Main protease (Mpro), and the papain-like protease (PLpro). 3CLpro, a cysteine protease that hydrolyzes at 11 conserved sites within the polyproteins, is essential for viral replication and multiplication. Since there is no closely related human homolog, 3CLpro is an ideal target for the development of antiviral agents for coronavirus diseases.

Since the outbreak of SARS in 2002, we have been developing a series of substrate-derived SARS-CoV-1 3CLpro inhibitors that contain an electron-withdrawing benzothiazolyl-ketone warhead [1-3]. It was believed that the electrophilic ketone warhead forms a covalent bond reversibly with the nucleophilic thiolate of the active site Cys145 in 3CLpro, resulting in a hemithioketal intermediate that transiently inactivates the enzyme. This reversibility reduces the chances of the nonspecific irreversible reaction of numerous mammalian thiols and the risk of undesirable side effects or immune reactions. The developed compounds, SH-5 and YH-53 (**Fig. 1**), were shown to be potent inhibitors of SARS-CoV-2 $3CL^{pro}$ with K_i values in the nanomolar range and their inhibition mode was competitive [3].

The crystal structures of the 3CLpro-YH-53 and -SH-5 complexes were determined at 1.65 and 2.15 Å resolutions, respectively [**Fig. 2(A, B)**] using the BL-17A beamline [3]. The data clearly indicated that both inhibitors interact with 3CLpro with extended conformations, and the main chains of the inhibitors interact with the 12th β-strand of the 3CLpro in an antiparallel manner. Besides, Cys145 forms a tetrahedral hemithioketal bond with the ketone warhead carbon. This suggests that these inhibitors interact tightly and reversibly at respective S-pockets in the active site of 3CLpro. More specifically, since the chemical structures of P1' (benzothiazole), P1 (pyrrolidin-2-one) and P2 (isobutyl) moieties of two inhibitors are identical, the C-terminal portion of the inhibitors interacts with the 3CLpro in the same manner [**Fig. 2(C)**]. While the benzothiazole, pyrrolidine-2-one, and isobutyl groups are well accommodated in the S1', S1, and S2 pockets, respectively [**Fig. 2(D, E)**], only the pyrrolidin-2-one group is completely buried in the protein. Since the chemical structures of the N-terminal part of YH-53 and SH-5 are different, the inhibitors provoke different interactions with the S3 and S4 specificity pockets of the target. In the SH-5 complex, the isopropyl group at the P3 position is exposed to the bulk solvent [**Fig. 2(D)**]. The benzyloxy group at the P4 position interacts with Gln189, Thr190, and Ala191, and one of its faces is exposed to the solvent [**Fig. 2(B)**]. At the P3 and P4 residues, only small conformational changes were observed when the structure was compared with the inhibitor-free form (PDB ID: 6M2Q). On the other hand, YH-53 binding induced a larger conformational change in a loop region (residues 188–194) of 3CLpro, and the residues of the loop region were shifted towards the inhibitor by approximately 2.5 Å [**Fig. 2(C)**]. As a result, Thr190, Gln189, and the backbone Glu166 cover the 4-methoxyindole group at the P3 position of YH-53, which is important for the enhanced inhibitory activity. The side-chain carbonyl of Gln189 forms a hydrogen bond with the main-chain amide group at the P2 position. As a result, the active site in the YH-53 complex has a more closed conformation [**Fig. 2(E)**], since the methoxy group on the P3 indole group is not involved in strong interactions with the protein. Notably, the observed conformational change of the loop region is rather rare among the 3CLpro structures that have been characterized to date. Of the 345 3CLpro coordinates that were selected with RMSD values less than 2.0 Å for the YH-53 complex, only twelve structures have similar conformations of the loop region [**Fig. 2(F)**].

Further comprehensive characterizations such as in vitro ADME, toxicity, pharmacokinetics and metabolic analysis have shown YH-53 to be an attractive lead compound in the development of anti-SARS-CoV-2 agents.



SH-5

SARS CoV-1 3CLpro: K_i = 4.1 nM
SARS CoV-2 3CLpro: K_i = 14.5 nM

YH-53

SARS CoV-1 3CLpro: K_i = 6.3 nM
SARS CoV-2 3CLpro: K_i = 34.7 nM

Figure 1: Structures of SARS-CoV 3CLpro inhibitors.

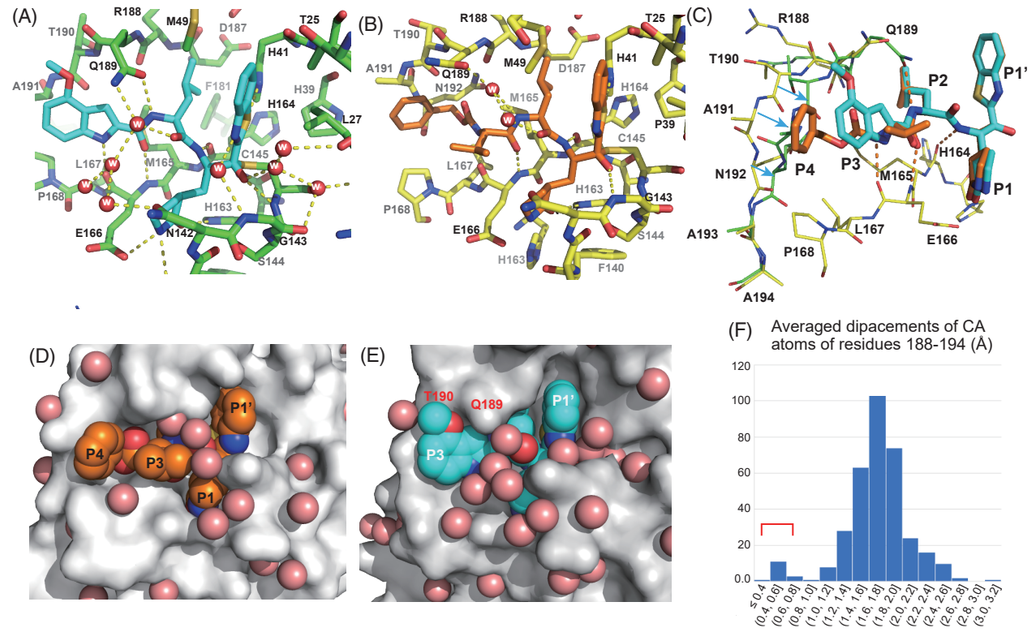


Figure 2: (A, B) Inhibitor binding sites of 3CLpro for YH-53 **(A)** and SH-5 **(B)**. Protein carbons for YH-53 and SH-5 complexes are shown in green and yellow, respectively. YH-53 **(A)** and SH-5 **(B)** are in cyan and orange, respectively. This notation is the same in panels **C–E**. Water molecules are labeled with "w". **(C)** LSQ superposition of the YH-53 and SH-5 complexes using 281 CA atoms (RMSD = 0.594Å). Cyan arrows show the shifts of loop residues. The atom color scheme is the same as panels A and B. **(D, E)** Surface representations of YH-53 and SH-5 binding sites. Water molecules are in salmon pink. **(F)** Distribution of RMSD values for the YH-53 complexes with 3CLpro coordinates in PDB. In total, 345 3CLpro PDB coordinates with RMSD values less than 2.0Å for the YH-53 complex were used in this analysis. The twelve structures indicated with the red bracket have loop structures similar to the YH-53 complex; the others have loop structures similar to the inhibitor free form. PDB codes for SARS-CoV-2 3CLpro with bound YH-53 and SH-5 are 7E18 and 7E19, respectively.

REFERENCES

[1] S. Konno, P. Thanigaimalai, T. Yamamoto, K. Nakada, R. Kakiuchi, K. Takayama, Y. Yamazaki, F. Yakushiji, K. Akaji, Y. Kiso, Y. Kawasaki, S. -E. Chen, E. Freire and Y. Hayashi, *Bioorg. Med. Chem.* **21**, 412 (2013).
[2] P. Thanigaimalai, S. Konno, T. Yamamoto, Y. Koiwai, A. Taguchi, K. Takayama, F. Yakushiji, K. Akaji, S. -E. Chen, A. Naser-Tavakolian, A. Schön, E. Freire and Y. Hayashi, *Eur. J. Med. Chem.* **68**, 372 (2013).
[3] S. Konno, K. Kobayashi, M. Senda, Y. Funai, Y. Seki, I. Tamai, L. Schäkel, K. Sakata, P.Thanigaimalai, A. Taguchi, A. Taniguchi, M. Gütschow, C. E. Müller, K. Takeuchi, M. Hirohama, A. Kawaguchi, M. Kojima, T. Senda, Y. Shirasaka, W. Kamitani and Y. Hayashi, *J. Med. Chem.* **65**, 2926 (2022).

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BEAMLINE

BL-17A

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