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SARS Coronavirus 3CL Protease Inhibitors with an Electrophilic Aryl-Ketone Warhead

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Introduction

The novel coronavirus, SARS-CoV-2, has been identified as the pathogen for the current coronavirus disease (COVID-19) outbreak. 3CL protease (3CLpro), a viral cysteine protease, plays a pivotal role in the SARS-CoV-2 replication, which is therefore the imperative target for the development of therapeutic agents. Since the outbreak of SARS in 2002, we have been studying 3CLpro inhibitors based on its substrate sequence (Figure 1) [1, 2] and developed a potent tripeptide-type inhibitor, Z-Val-Leu-Ala(oxopyrrolidin-3-yl)-2-benzothiazole (**SH-5**, Ki = 4 nM) [3] via early designed inhibitor **1**. **SH-5** contains an electron-withdrawing reactive warhead, benzothiazolyl-2-ketone (P1'-moiety), to capture the SH group at the catalytic center of 3CLpro. Then, based on **SH-5**, a series of low-molecular-weight dipeptidomimetics were also developed by truncating the P3-Val unit, and the 4-methoxy-indole-2-carbonyl unit was identified as one of the best P3-scaffolds, resulting in the development of a potent dipeptide-type inhibitor, 4-methoxyindole-2-carbonyl-Leu-Ala((S)-2-oxopyrrolidin-3-yl)-2-benzothiazole (**YH-53**, Ki = 6 nM) in 2013 [4, 5].

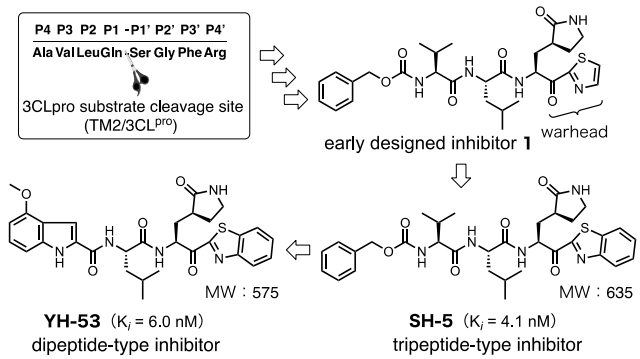

*Fig. 1. Development of SARS-CoV 3CL protease inhibitor **YH-53** with an electrophilic aryl-ketone warhead.*

Results and Discussion

Our substrate-derived SARS-CoV-1 3CLpro inhibitor **YH-53** showed a potent inhibitory activity against SARS-CoV-2 3CLpro with a Ki value of 34.7 nM, and the inhibition mode was competitive [6]. This suggests that aryl ketone-type 3CLpro inhibitors developed for SARS-CoV-1 are effective

against that of SARS-CoV-2 with a clear mechanism of action. This result is largely attributed to the quite high similarity in the entire amino acid sequence and three-dimensional structure, particularly 100% identity at the active site in 3CLpro between SARS-CoV-1 and SARS-CoV-2. The reversible mode of action is enzymologically supported by the analysis of progress curves which did not show time-dependent inhibition. We also carried out an NMR titration experiment, which confirmed that **YH-53** binds to SARS-CoV-2 3CLpro in 1:1 stoichiometry and with slow exchange kinetics [6]. Then, crystal structures of the SARS-CoV-2 3CLpro-**YH-53** at 1.65 Å resolutions was determined. It revealed the binding mode of **YH-53** with multiple hydrogen bond interactions to backbone amino acids (Figure 2A) and a covalent bond to the active site thiol group of 3CLpro (Figure 2B). These data confirm the predicted mechanism of action, a tight and reversible binding, and interaction with the active site in the S4-S1'-pockets.

YH-53 completely blocked the viral proliferation *in vitro* (Vero cells) and the $TCID_{50}$ value was ~5 μM. This antiviral activity of **YH-53** was enhanced by adding CP-100356, which is a potent inhibitor of MDR-1 efflux transporter, suggesting that **YH-53** acts as a substrate of the p-glycoprotein efflux pump. Safety and toxicological evaluations suggested that **YH-53** has a high safety index with low cytotoxicity, no or moderate but tolerable CYP inhibition, no mutagenicity and no cardiotoxicity. By means of the simultaneous analysis of intravenous and oral pharmacokinetic data, the bioavailability of **YH-53** in rats was estimated to be approximately 3.6%. This low bioavailability is generally caused by poor absorption and/or significant first-pass metabolism. Interestingly, *in vitro* assessment of intestinal permeability of **YH-53** using a Caco-2 cell monolayer system exhibited excellent cell permeability, close to that of metoprolol, a completely absorbed drug (Fa ≥ 90%). Moreover, an *in vitro* metabolism study using cryopreserved hepatocytes revealed that **YH-53** is mainly metabolized by hydrolytic enzymes that cleave it at the *C*-terminal of Leu (P2-moiety). Taking these results into account, it is reasonable to consider that the low bioavailability of **YH-53** in rats may be mainly due to the first-pass metabolism catalyzed by hydrolytic enzymes in the intestine and/or liver. These results suggest that **YH-53** has high potential as a lead compound for the development of COVID-19 therapeutics.

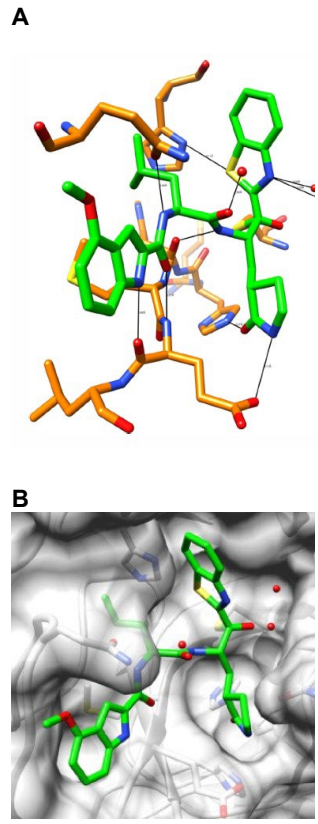

*Fig. 2. Crystal structures of the SARS-CoV-2 3CLpro-**YH-53**.*

Acknowledgments

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