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Crystal structure of SARS-CoV-2 main protease in complex with the natural product inhibitor shikonin illuminates a unique binding mode

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Almost everyone is susceptible to the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an RNA virus, which can cause many symptoms and even death among high-risk individuals [1,2]. The main protease (Mpro, also known as 3CLpro) is a cysteine protease essential for producing infectious virions and thus, an attractive target for drug development. Up to now, many studies using either in silico ligand docking or drug discovery based on available structures have been performed to discover new Mpro-inhibiting agents [3,4]. However, most studies have used either peptidomimetics or covalent inhibitors for Mpro, which may introduce non-specific interactions with host proteins. Here, we presented the structure of shikonin in a non-covalent binding configuration with Mpro and compared it with covalent bonding structures in pursuit of novel scaffolds capable of inhibiting the main protease.

As shown in Fig. 1, the crystal structure of Mpro in complex with shikonin (ShiMpro) is resolved at 2.45 Å (Fig. 1a and Table S1 online), and shikonin binds to only one of the protomers (i.e., protomer A) despite their overall structural similarity (Fig. S1 online, Supplementary materials and methods online). ShiMpro shows the same overall fold as for the apo structure of Mpro at pH 7.5 (apoMpro) [5]. The root mean square (RMS) difference of equivalent Ca positions between apo and ShiMpro is -0.3 Å (Fig. 1b).

An overlay of the ShiMpro structure with the previously solved inhibitor-bound structures shows high spatial conservation (Fig. 1b and Fig. S2 online). The inhibitor binding pocket is surrounded by S1–S4 subsites, and shikonin forms multiple interactions with them (Fig. 1b). First, shikonin forms a hydrogen bond network with the protease polar triad Cys145 and His164 located on the S1 subsite. Second, shikonin forms H-bonds with Arg188 and Gln189 in the S3 pocket (Fig. 1d and e). Third, the imidazole group of His41 points toward the binding pocket in covalent-bonding structures, but it flips outward in the current structure, opening a way for the entry of shikonin.

Superimposing ShiMpro with other inhibitor-bound structures reveals a striking difference in the arrangement of the catalytic dyad His41-Cys145 and smaller, but substantial, differences in Phe140 and Glu166. First, in covalent-bonding structures, the inhibitor binds to the Sy atom of Cys145, but in the current structure, the side chain of Cys145 adopts a different configuration to form a hydrogen bond with shikonin (Fig. 1c and d). Second, shikonin forms H-bonds with Arg188 and Gln189 in the S3 pocket (Fig. 1d and e). Third, the imidazole group of His41 points toward the binding pocket in covalent-bonding structures, but it flips outward in the current structure, opening a way for the entry of shikonin. Fourth, the distance between His41 Ni2 and Cys145 Sy is 5.3 Å in ShiMpro structure, significantly longer than those observed in other Mpro structures (Fig. 1c) [6–9]. Fifth, the phenyl ring of Phe140 in ShiMpro moves outward to the solvent and no longer has π-π interaction with His163. Lastly, the side chain of Glu166
is flexible in ShiMpro structures but is well ordered in covalent inhibitor binding structures (Fig. 1c). Glu166 is strictly conserved among all Mpro and is critical for forming a hydrogen bond with peptidomimetic inhibitors and N terminal residues from the other protomer [9]. The conformational change of Glu166 in the current ShiMpro structure may explain how the non-covalent binding of shikonin can inhibit protease activity.

Additionally, the apoMpro structure has two water molecules in the substrate-binding site (Fig. S3a online). Water 1 forms a hydrogen bond network involving Phe140, His163, and Glu166 located in the S1 pocket, stabilizing the oxyanion hole in the apo state structure [5]. Water 2 hydrogen-bonded with His41 and Cys145. However, these two water molecules are not observed in the ShiMpro structure, and the space for water 2 in the apo structure is now occupied by shikonin (Fig. S3b online), suggesting water molecule displacement may be part of the inhibitor mechanism.

Unlike ShiMpro, covalent and peptidomimetic inhibitors bind to the S1/S2/S4 site, carmofur binds to the S2 subsite, and baicalein binds the S1/S2 pocket (Fig. 1f) [6–10]. Therefore, the ShiMpro structure highlights a new mode of binding, and may serve as an invaluable resource to improve the design of novel antiviral drugs.

Conflict of interest

The authors declare that they have no conflict of interest.

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Author contributions

Jian Li, Xuelan Zhou, Yan Zhang, Fanglin Zhong, and Cheng Lin made constructs for expression and determined the conditions used to enhance protein stability. Huan Zhou and Qisheng Wang carried out X-ray experiments, including data acquisition and processing. Jian Li and Jin Zhang built the atomic model. Jin Zhang, Jian Li, Yan Zhang, Yang Fu, Jun Luo, Feng Jiang, Peter J. McCormick, and Jingjing Duan drafted the manuscript. Jin Zhang and Jian Li supervised the research.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2020.10.018.

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