Supplemental information

Chemical screen uncovers novel structural classes of inhibitors of the papain-like protease of coronaviruses

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Figure S1. Optimization of fluorescence-based PLpro protease assay (related to Figure 1 and Figure 3). (A, B) Identification of optimal substrate and MERS-PLpro (A) and SARS-PLpro (B) enzyme concentrations for screening at room temperature. (C, D) Validation of selected substrate and enzyme concentrations using a known PLpro inhibitor 6-TG on MERS-PLpro (C) and SARS-PLpro (D). Each data point represents the mean of duplicate assays with ±SEM.
Figure S2. Z’ factor of the compound screen and exclusion of cytotoxic compounds (related to Figure 1).
(A) Z’-factors of 60 assay plates. Average Z’-factor of 60 assay plates was calculated to be 0.91±0.01. (B) Cytotoxicity profile of primary hits. A total of 81 compounds from 257 primary hits were excluded by the cytotoxicity test.
Figure S3. Efficacy measurement of thiophenes (compound 1) against MERS-CoV using immunofluorescence-based infection assay in cell model (related to Figure 3).
Efficacy and cytotoxicity measurements of 1 (A) and its analogs (B-I). Green dots represent relative infection rate, while blue dots represent relative cytotoxicity at various compound concentrations. Each data point represents the mean of duplicate assays with ±SD.
Figure S4. Efficacy measurement of compound 1 and 1e in SARS-CoV infection cell model (related to Figure 3). 
(A, B) Efficacy measurement of compound 1 (A) and 1e (B) against SARS-CoV infection using immunofluorescence-based infection assay in cell model. Green and blue curve show efficacy and toxicity, respectively. Each data point represents the mean of duplicate assays with ±SD. (C) Calculation of IC\textsubscript{50} and EC\textsubscript{50}. IC\textsubscript{50} values were calculated from data generated by in vitro PLpro protease assay, while EC\textsubscript{50}, CC\textsubscript{50}, and SI were calculated from the data obtained by immunofluorescence-based infection assay.
Figure S5. Efficacy measurement of compound 1 and 1e against HCoV-229E or HCoV-OC43 infection in cell model (related to Figure 3).

(A, B) Efficacy of compound 1 against HCoV-229E (A) and HCoV-OC43 (B) infection measured by quantification of viral RNA. (C, D) Efficacy of compound 1e against HCoV-229E (C) and HCoV-OC43 (D) infection measured by quantification of viral RNA. Each data point represents the mean of duplicate assays with ±SEM. *p<0.5, **p<0.1, ***p<0.001 compared to no compound (0 μM) control by Student’s t-test.
Figure S6. Determination of top 3 binding poses upon docking compound 1 to MERS-PLpro using different residues as a center (related to Figure 3).

(A, B) Top 3 binding poses generated by docking compound 1 to MERS-PLpro with T251 of the region near BL2 loop as a center (A) or C112 of the catalytic site as a center (B). Results demonstrate that compound 1 does not bind to the catalytic site even when the docking was performed with the catalytic site as a center. The catalytic site is marked by a red circle. The numbers represent the binding score of each pose. (C, D) Result of molecular dynamics simulation (MDS) performed with MERS-PLpro and compound 1 using Cresset’s Flare 6.0.1. MDS was performed for 80 ns. Root mean square deviation (RMSD) during the MDS was plotted (C) and docking pose at 80 ns was presented (D).
Figure S7. Efficacy measurement of furans (compound 2) against MERS-CoV infection using infection assay in cell model (related to Figure 4).

Efficacy and cytotoxicity measurements of 2 (A) and its analogs (B-E). Green dots represent relative infection rate, while blue dots represent relative cytotoxicity at various compound concentrations. Each data point represents the mean of duplicate assays with ±SD.
Figure S8. Determination of top 3 binding poses upon docking compound 2 to MERS-PLpro (related to Figure 4).

(A, B) Top 3 binding poses generated by docking compound 2 to MERS-PLpro with T251 of the region near BL2 loop as a center (A) or C112 of the catalytic site as a center (B). Results demonstrate that compound 2 does not bind to the catalytic site even when the docking was performed with the catalytic site as a center. The catalytic site is marked by a red circle. The numbers represent the binding score of each pose. (C, D) Results of MDS (molecular dynamics simulation) performed with MERS-PLpro and compound 2 for 80 ns using Cresset’s Flare 6.0.1. The profile of RMSD (root mean square deviation) during MDS was plotted (C) and the docking pose at 80 ns was presented (D).
Figure S9. Efficacy measurement of triazoloquinazolines (compound 3) against MERS-CoV infection using immunofluorescence-based infection assay in cell model (related to Figure 5).

Efficacy and cytotoxicity measurements of 3 (A) and its analogs (B-P). Green dots represent relative infection rate, while blue dots represent relative cytotoxicity at various compound concentrations. Each data point represents the mean of duplicate assays with ±SD.
Figure S10. Determination of top 3 binding poses upon docking compound 3 to MERS-PLpro (related to Figure 5).

(A, B) Top 3 binding poses generated by docking of compound 3 to MERS-PLpro with T251 of the region near BL2 loop as a center (A) or C112 of the catalytic site as a center (B). Results demonstrate that compound 3 does not bind to the catalytic site. The catalytic site is marked by a red circle. The numbers represent the binding score of each pose. (C, D) Results of MDS (molecular dynamics simulation) performed with MERS-PLpro and compound 3 for 80 ns using Cresset’s Flare 6.0.1. (C) The RMSD (root mean square deviation) during MDS was calculated and plotted. (D) The docking pose of compound 3 at 80 ns was presented.
Figure S11. Mutation in T251 residue of MERS-PLpro resulted in loss of enzyme activity and reactivity to thiophene (related to Figure 3, Figure 4, and Figure 5). (A) Enzyme activity of MERS-PLpro with mutation at T251 residue (MERS-PLpro T251A). Substitution of T251 with alanine resulted in a significant loss of its enzyme activity when compared with the wild-type control MERS-PLpro (MERS-PLpro wt). (B-D) Potency of compounds against MERS-PLpro T251A. Compounds 1 (B), compound 2 (C), and compound 3 (D) were tested at 0, 3.12, 6.25, 12.5, 25, and 50 μM for their potency against MERS-PLpro T251A. Each data point represents the mean of triplicate assays with ±SEM. *p<0.5, **p<0.01, ***p<0.001 compared to wild-type MERS-PLpro by Student’s t-test.
Figure S12. Efficacy and cytotoxicity of remdesivir measured by virus infection assay (related to Figure 6). Ten different concentrations of remdesivir were tested by the immunofluorescence assay, and IC₅₀ and cytotoxicity were calculated through curve fitting analysis using Prism 6. Each data point represents the mean of duplicate assays with ±SD.
| Compound | Vendor ID       | Vendor        |
|----------|----------------|---------------|
| 1        | Lead compound  | Screen library|
| 1a       | 7114072        | ChemBridge    |
| 1b       | 7119694        | ChemBridge    |
| 1c       | D304-0025      | ChemDiv       |
| 1d       | 5900099        | ChemBridge    |
| 1e       | 5889373        | ChemBridge    |
| 1f       | 7112485        | ChemBridge    |
| 1g       | MolPort-004-297-411 | MolPort |
| 1h       | 5895218        | ChemBridge    |
| 2        | Lead compound  | Screen library|
| 2a       | 7744029        | ChemBridge    |
| 2b       | 7934758        | ChemBridge    |
| 2c       | 7937442        | ChemBridge    |
| 2d       | 7940498        | ChemBridge    |
| 3        | Lead compound  | Screen library|
| 3a-1     | E543-0745      | ChemDiv       |
| 3a-2     | E543-0755      | ChemDiv       |
| 3a-3     | E543-0737      | ChemDiv       |
| 3a-4     | E543-0773      | ChemDiv       |
| 3a-5     | E543-0740      | ChemDiv       |
| 3a-6     | E543-0733      | ChemDiv       |
| 3a-7     | E543-0763      | ChemDiv       |
| 3a-8     | E543-0766      | ChemDiv       |
| 3a-9     | C200-8702      | ChemDiv       |
| 3b       | E543-0720      | ChemDiv       |
| 3c-1     | E543-0394      | ChemDiv       |
| 3c-2     | E543-0720      | ChemDiv       |
| 3c-3     | E543-0443      | ChemDiv       |
| 3c-4     | E543-0198      | ChemDiv       |
| 3d       | E543-0001      | ChemDiv       |

Figure S13. Lead compounds and their analogues used for structure-activity relationship (SAR) studies (related to STAR* methods). List of the vendor ID’s and source libraries of the lead compounds (compounds 1-3) and their analogs.