Supplementary Material

Identification of Highly Effective Inhibitors Against SARS-CoV-2 Main Protease: From Virtual Screening to In Vitro Study

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**Table S1.** The drugs targeting SARS-CoV-2-3CLpro

|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Company** | **Delivery** | **States** |
| PF-07321332 (Paxlovir) | Pfizer | Oral | Approved |
| S-217622 | Shionogi | Oral | Phase III |
| PF-07304814 | Pfizer | IV | Phase I |
| EDP-235 | Enanta | Oral | Phase I |
| JTT-705 RG-1658(Dalcetrapib) | DalCor Pharmaceuticals | – | Phase II |
| FB2001/11a (DC402234) | Frontier | IV | Phase I |
| SIM0417 (SSD8432) | Simcere | Oral | Phase II |
| PBI-0451 | Pardes | Oral | Phase I |

**Table S2.** The drug-like filter properties of identified hits in virtual screen.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Lipinski’s filter** | **Hit 1** | **Hit 2** | **Hit 3** | | | **Hit 4** |  |
| mol\_MW | 580.21 | 584.09 | | 544.23 | 493.15 | |  |
| nHA | 7.00 | 7.00 | | 7.00 | 6.00 | |  |
| nHD | 1.00 | 1.00 | | 1.00 | 1.00 | |  |
| LogS | -4.75 | -5.20 | | -4.67 | -4.57 | |  |
| LogP | 5.84 | 4.55 | | 5.46 | 4.47 | |  |

mol\_MW: molecular weight, optimal:100~600; nHA: number of hydrogen bond acceptors, optimal: 0~10; nHD: number of hydrogen bond donors, optimal: 0~5; LogS: Log of the aqueous solubility, optimal: -6~0.5; LogP: log of the octanol/water partition coefficient, optimal: 0~6.

**Table S3.** Hydrogen bond and its occupancy formed in the binding of each hit and 3CLpro during the MD simulation.

|  |  |  |  |
| --- | --- | --- | --- |
| **Acceptor** | **Hydrogen** | **Donor** | **Occupancy%** |
| **Hit 1** | | | |
| Hit 1@O2 | Glu166@H | Glu166@N | 74.4 |
| **Hit 2** | | | |
| Hit 2@O2 | Glu166@H | Glu166@N | 77.1 |
| **Hit 3** | | | |
| Hit 3@O2 | Glu166@H | Glu166@N | 93.1 |
| **Hit 4** | | | |
| Hit 4@O2 | Glu166@H | Glu166 @N | 67.0 |

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**Figure S1.** The RMSD trajectories of each individual hit during 50 ns simulations.



**Figure S2.** Binding affinity of hits 1-4 and ML300 to 3CLpro.



**Figure S3.** 3CLpro concentration−response curves for hits 1-4 and ML300.

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**Figure S4.** Dynamics of evolution of secondary structure elements of the 3CLpro complex with hit 1 (A), hit 2 (B), hit 3 (C), and hit4 (D) during the MD simulations.



**Figure S5.** Structural superimposition of the top ranked cluster representative of 3CLpro-hit complexes (shown in green) obtained from MD simulation as compared to the 3CLpro alone (Yellow) without any ligand. (A) Secondary structural changes of 3CLpro protein upon binding to hit 1. (B) Secondary structural changes of 3CLpro protein upon binding to hit 2. (C) Secondary structural changes of 3CLpro protein upon binding to hit 3. (D) Secondary structural changes of 3CLpro protein upon binding to hit 4.