**SUPPORTING INFORMATION**

# Discovery of Novel Inhibitors of SARS-CoV-2 Main Protease

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SGFRKMAFPS GKVEGCMVQV TCGTTTLNGL WLDDVVYCPR HVICTSEDML NPNYEDLLIR
KSNHNFLVQA GNVQLRVIGH SMQNCVLKLK VDTANPKTPK YKFVRIQPGQ TFSVLACYNG
SPSGVYQCAM RPNFTIKGSF LNGSCGSVGF NIDYDCVSFC YMHHMELPTG VHAGTDLEGN
FYGPFVDRQT AQAAGTDTTI TVNVLAWLYA AVINGDRWFL NRFTTTLNDF NLVAMKYNYE
PLTQDHVDIL GPLSAQTGIA VLDMCASLKE LLQNGMNGRT ILGSALLEDE FTPFDVVRQC
SGVTFQ

**Figure S1. Sequence of the $M^{pro}$ protein.**

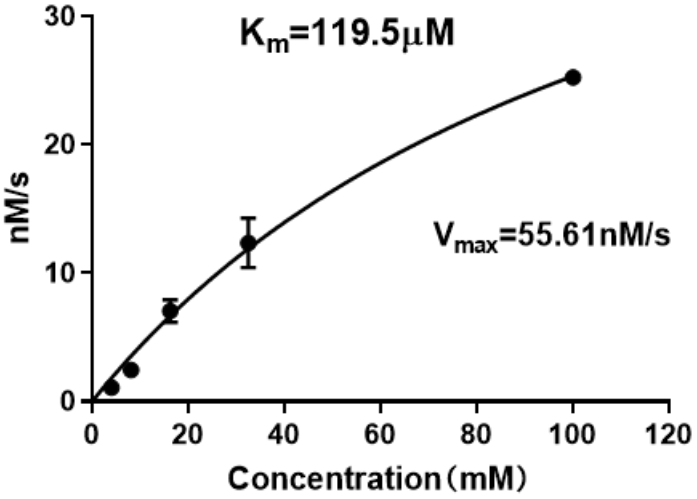



Figure S2. The biological activity of $M^{pro}$ with the FRET-based substrate Dabcyl-KTSAVLQ/SGFRKME.

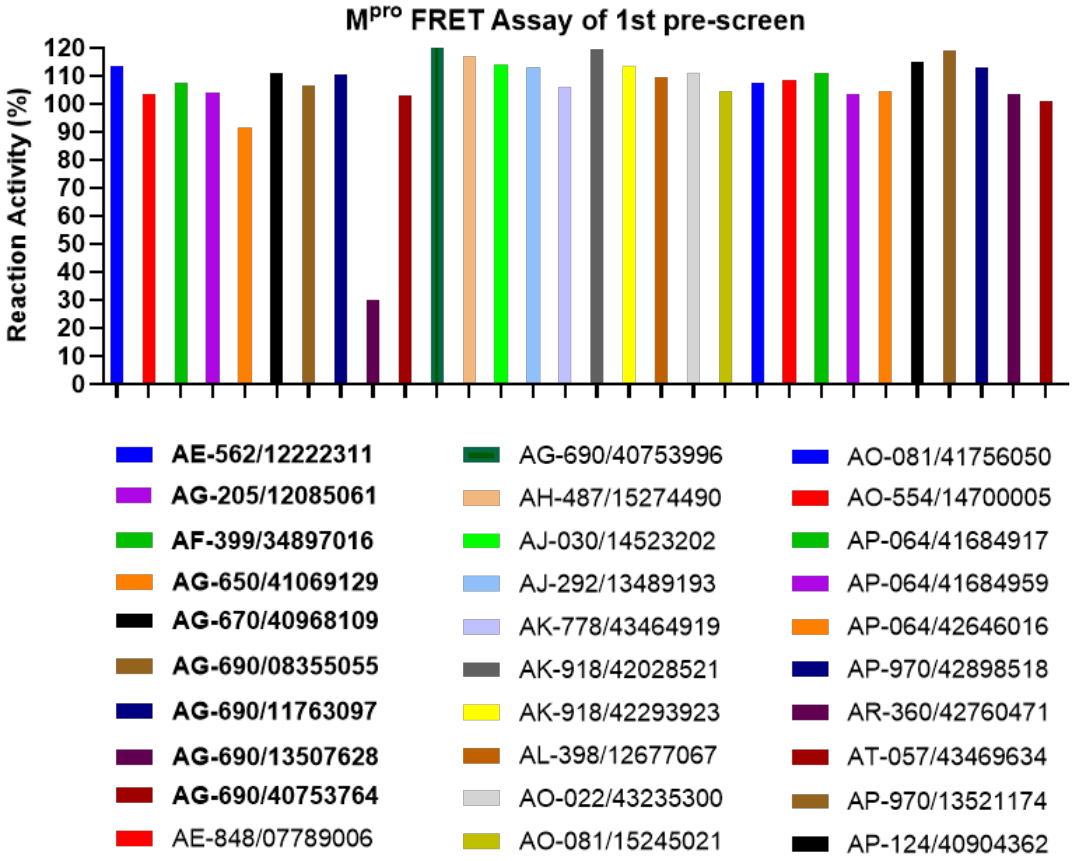



Figure S3. FRET Assay of $M^{pro}$ for the first round pre-screening at 10μM.

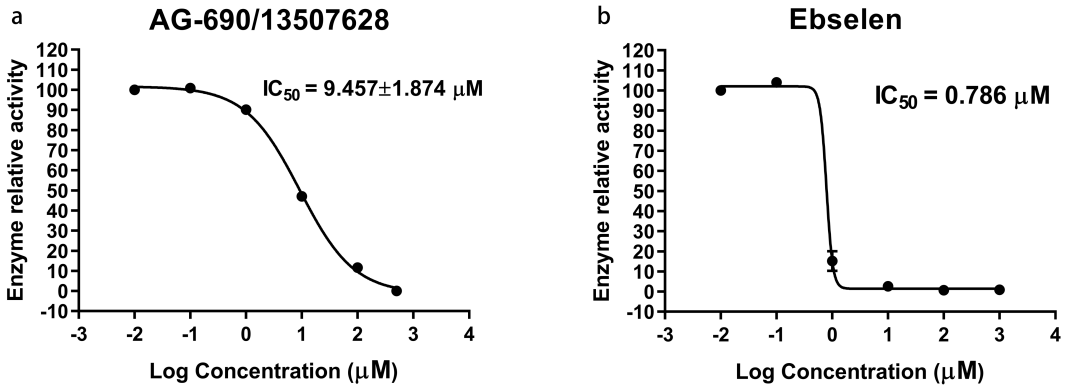



Figure S4. (a) The $IC_{50}$ of AG-690/13507628 from 2 measurements (95% confidence interval). (b) The $IC_{50}$ of the inhibitor Ebselen as a control from 2 measurements.

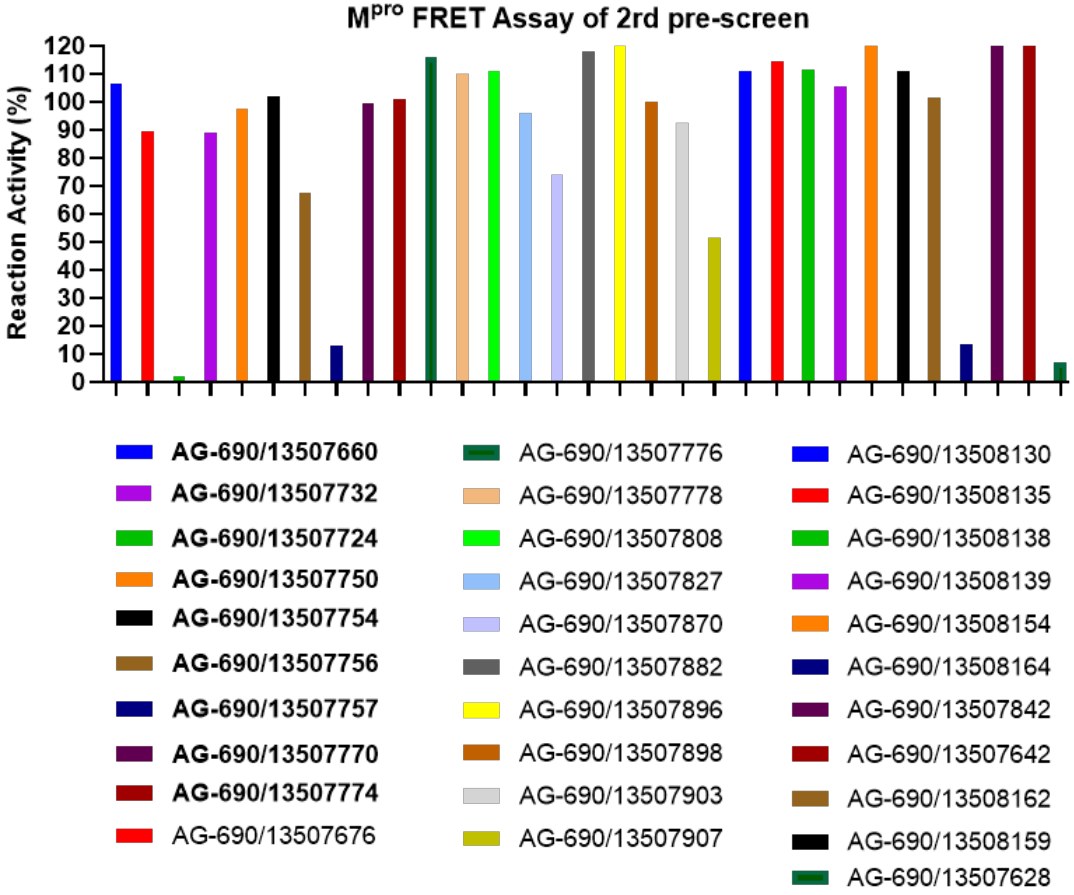



Figure S5. FRET Assay of $M^{pro}$ for the second round of pre-screening at 10μM.

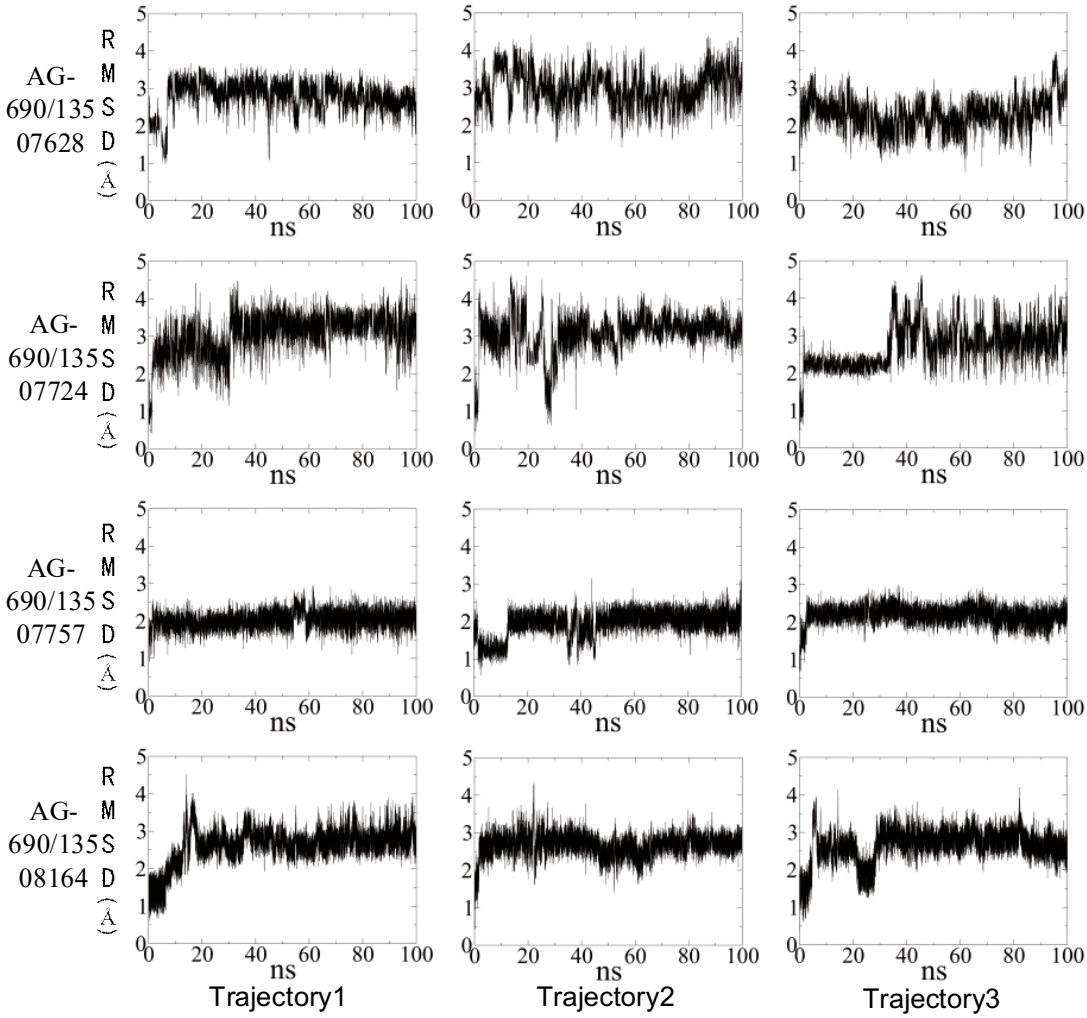



Figure S6. The RMSD for ligands of four compounds for three trajectories.

Table S1. The structures of the non-active compounds for comparison with the active compounds.

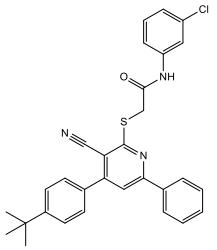
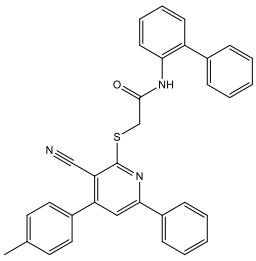
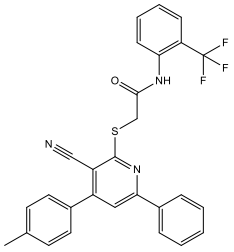
| Specs ID | 2D-structure | Reaction Activity 50 (μM) | Reaction Activity 10 (μM) | IC50 (μM) |
|---|---|---|---|---|
| AG-690/13 507750 |  | 99.8 | 97.6 | ~ |
| AG-690/13 507754 |  | 75.8 | 101.8 | ~ |
| AG-690/13 507732 |  | 90.7 | 89.0 | ~ |

Table S2. Structural similarity between known $M^{pro}$ inhibitors and the inhibitors discovered in this study.

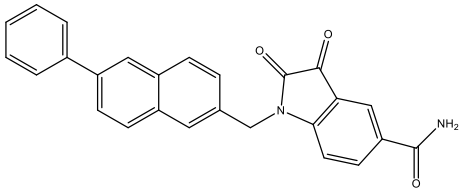
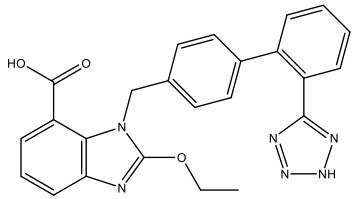
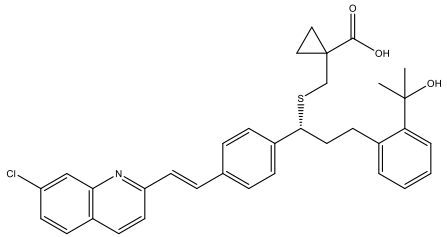
| Rank | 3Dstruture | smiles | name | AG-690/13507628 | AG-690/13507724 | AG-690/13507757 | AG-690/13508164 |
|---|---|---|---|---|---|---|---|
| 1 |  | O=C(N)c(c1)ccc(c12)N(C(=O)C2=O)Cc(cc3)cc(c34)ccc(c4)-c5ccccc5 | N-substuted isatin 28[1] | 0.408 | 0.513 | 0.503 | 0.472 |
| 2 |  | OC(=O)c1cccc(c12)nc(OCC)n2Cc3ccc(cc3)-c4c(cccc4)-c5n[nH]nn5 | Candesartan [2] | 0.394 | 0.474 | 0.455 | 0.408 |
| 3 |  | Clc(c1)ccc(c12)ccc(n2)\C=C\c(ccc3)cc3[C@H](SCC4(CC4)CC(=O)O)CCc5c(C(O)(C)C)cccc5 | Montelukast Sodium[2] | 0.341 | 0.411 | 0.468 | 0.407 |

**Table S3**. The ADMET properties of the inhibitors predicted with ADMETlab2.0 [3].

| Property | Range for good drug candidates | AG-690/13507628 | AG-690/13507724 | AG-690/13507757 | AG-690/13508164 |
|---|---|---|---|---|---|
| Physicochemical Property | | | | | |
| Molecular Weight | 100 to 600 | **523.19** | **465.15** | **511.15** | **549.13** |
| # Hydrogen bond acceptors | 0 to 12 | **6** | **5** | **4** | **6** |
| # Hydrogen bond donors | 0 to 7 | **1** | **1** | **1** | **1** |
| Topological polar surface area | 0 to 140 | **84.24** | **75.01** | **65.78** | **84.24** |
| LogS | -4 to 0.5 | -6.819 | -6.874 | -6.846 | -7.485 |
| LogD | 1 to 3 | 4.298 | 4.372 | 5.014 | 4.291 |
| LogP | 0 to 3 | 6.115 | 5.639 | 7.304 | 5.446 |
| Absorption | | | | | |
| Human intestinal absorption | 0 to 0.3 | **0.007** | **0.004** | **0.006** | **0.005** |
| human oral bioavailability 20% | 0 to 0.3 | **0.005** | **0.295** | **0.135** | **0.002** |
| human oral bioavailability 30% | 0 to 0.3 | **0.269** | **0.018** | **0.003** | **0.004** |
| Caco-2 Permeability | > -5.15 | -5.275 | -4.998 | -5.288 | -5.222 |
| Distribution | | | | | |
| BBB Penetration | 0 to 0.3 | **0.014** | **0.11** | **0.056** | **0.059** |
| Metabolism | | | | | |
| CYP1A2 inhibitor | 0 to 1, less is better | **0.13** | **0.482** | **0.462** | **0.255** |
| CYP1A2 substrate | 0 to 1, less is better | 0.96 | 0.501 | 0.175 | 0.894 |
| CYP2C19 inhibitor | 0 to 1, less is better | 0.729 | 0.885 | 0.749 | 0.912 |
| CYP2C19 substrate | 0 to 1, less is better | **0.088** | **0.062** | **0.053** | **0.065** |

| | | | | | |
|---|---|---|---|---|---|
| CYP2C9 inhibitor | 0 to 1, less is better | 0.947 | 0.958 | 0.933 | 0.963 |
| CYP2C9 substrate | 0 to 1, less is better | 0.912 | 0.894 | 0.859 | 0.926 |
| CYP2D6 inhibitor | 0 to 1, less is better | **0.003** | **0.009** | **0.02** | **0.011** |
| CYP2D6 substrate | 0 to 1, less is better | 0.923 | 0.72 | 0.211 | 0.743 |
| CYP3A4 inhibitor | 0 to 1, less is better | 0.552 | 0.703 | 0.252 | 0.809 |
| CYP3A4 substrate | 0 to 1, less is better | 0.916 | 0.629 | 0.789 | 0.508 |
| Excretion | | | | | |
| clearance | ≥ 5 | **8.573** | **8.364** | **6.324** | **8.958** |
| half-life | 0 to 0.3 | **0.161** | **0.13** | **0.023** | **0.125** |
| Toxicity | | | | | |
| hERG Blockers | 0 to 0.3 | **0.217** | **0.242** | 0.326 | **0.166** |
| human hepatotoxicity | 0 to 0.3 | 0.777 | 0.917 | 0.665 | 0.947 |
| Carcinogenicity | 0 to 0.3 | **0.26** | 0.63 | **0.261** | **0.123** |
| Eye Corrosion | 0 to 0.3 | **0.003** | **0.003** | **0.003** | **0.003** |
| Eye Irritation | 0 to 0.3 | **0.285** | 0.748 | 0.894 | **0.11** |
| Respiratory Toxicity | 0 to 0.3 | 0.823 | 0.848 | 0.574 | 0.9 |

## REFERENCE

[1] P. Liu, H. Liu, Q. Sun, H. Liang, C. Li, X. Deng, Y. Liu, L. Lai, Potent inhibitors of SARS-CoV-2 3C-like protease derived from N-substituted isatin compounds, Eur J Med Chem, 206 (2020) 112702.

[2] Z. Li, X. Li, Y.Y. Huang, Y. Wu, R. Liu, L. Zhou, Y. Lin, D. Wu, L. Zhang, H. Liu, X. Xu, K. Yu, Y. Zhang, J. Cui, C.G. Zhan, X. Wang, H.B. Luo, Identify potent SARS-CoV-2 main protease inhibitors via accelerated free energy perturbation-based virtual screening of existing drugs, Proc Natl Acad Sci U S A, (2020).

[3] G. Xiong, Z. Wu, J. Yi, L. Fu, Z. Yang, C. Hsieh, M. Yin, X. Zeng, C. Wu, X. Chen, T. Hou, D. Cao, ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties.Nucleic Acids Res, 2021, in press.