

**Supporting Information:**  
**Hit expansion of a non-covalent SARS-CoV-2**  
**main protease inhibitor**

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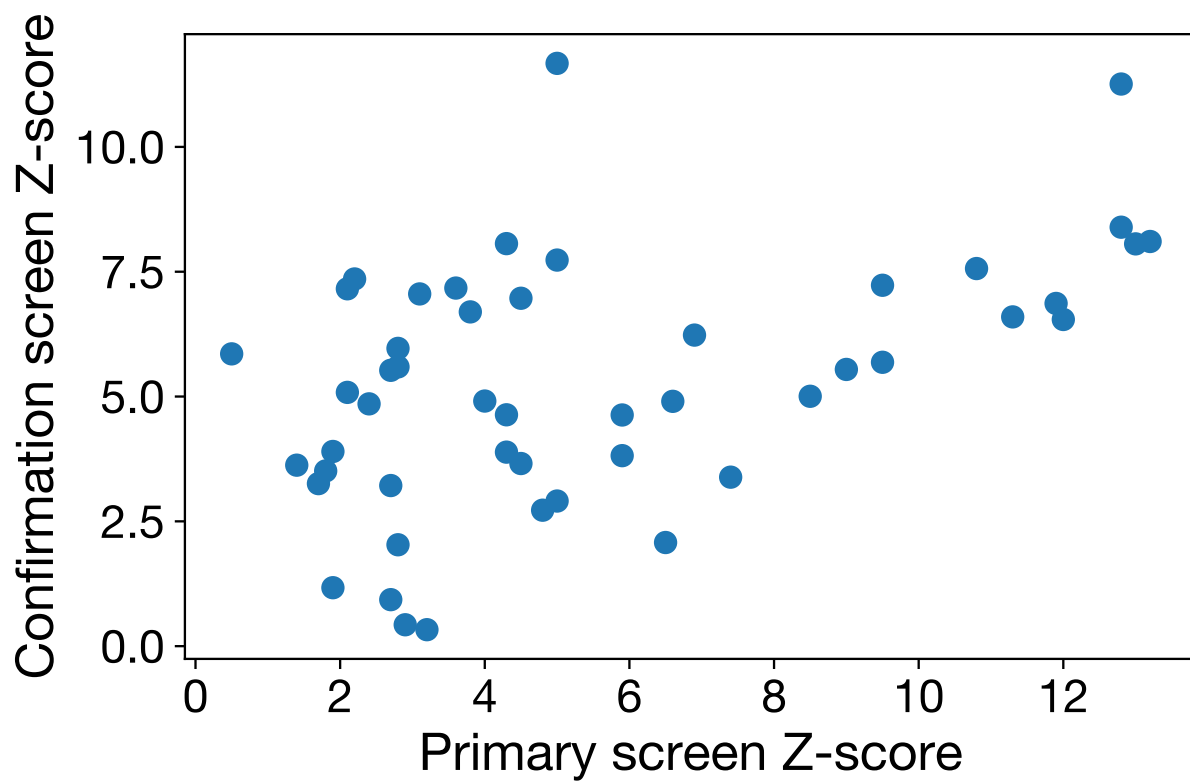


Figure S1: Validation of the enzymatic assay. Affinity data (Z-scores) for M<sup>pro</sup> inhibitors at 20  $\mu$ M for confirmation screen *vs.* primary screen.

Table S1: Crystallographic data collection and refinement statistics for room-temperature structures of M<sup>pro</sup> in complex with compound 12, compound 19, and compound 21. Values in parenthesis indicate highest resolution shell.

PDB ID	M <sup>pro</sup> - <b>compound 12</b> 7S3K	M <sup>pro</sup> - <b>compound 19</b> 7S4B	M <sup>pro</sup> - <b>compound 21</b> 7S3S
Data Collection:	X-ray (in-house)		
Diffractometer	Rigaku HighFlux Eiger R 4M		
Space group	I2	I2	I2
Wavelength (Å)	1.5406	1.5406	1.5406
Cell dimensions:			
a, b, c (Å)	45.23, 54.59, 114.99	45.37, 54.41, 114.28	45.66, 54.72, 114.97
$\alpha$ , $\beta$ , $\gamma$ (°)	90, 101.17, 90	90, 101.00, 90	90, 101.44, 90
Resolution (Å)	56.41 – 1.90 (1.97-1.90)	56.09 – 2.00 (2.07-2.00)	56.33 – 2.00 (2.07-2.00)
No. of reflections unique	21 813 (2174)	18 177 (1770)	18 686 (1834)
$R_{\text{merge}}$	0.078 (0.498)	0.078 (0.438)	0.059 (0.552)
$R_{\text{pim}}$	0.040 (0.282)	0.045 (0.241)	0.030 (0.281)
CC <sub>1/2</sub>	0.986 (0.762)	0.992 (0.806)	0.991 (0.728)
$I/\sigma_I$	13.39 (1.73)	12.51 (1.92)	18.40 (1.98)
Completeness (%)	99.9 (99.5)	97.5 (95.0)	98.6 (96.6)
Redundancy	5.0 (4.0)	4.1 (4.2)	4.6 (4.6)
<b>Refinement:</b>			
$R_{\text{work}}/R_{\text{free}}$	0.1751 / 0.2039	0.1717 / 0.2098	0.1832 / 0.2220
B-factors			
Protein	36.43	37.87	42.76
Ligand	31.08	34.55	35.23
Water	40.65	42.44	43.71
R.M.S. deviations			
Bond lengths (Å)	0.003	0.005	0.003
Bond angles (°)	0.591	0.743	0.650
All atom clashscore	0.84	0.00	0.21

Table S2: List of M<sup>Pro</sup> crystal structures (PDB IDs) used for the hydration analysis.

5R7Z, 5R80, 5RE4, 5RE5, 5RE6, 5RE7, 5RE8, 5RE9, 5REA, 5REB, 5REC, 5RED, 5REE, 5REF, 5REG, 5REH, 5REI, 5REJ, 5REK, 5REL, 5REM, 5REN, 5REO, 5REP, 5RER, 5RES, 5RET, 5REU, 5REV, 5REW, 5REX, 5REY, 5REZ, 5RF0, 5RF1, 5RF2, 5RF3, 5RF4, 5RF5, 5RF6, 5RF7, 5RF8, 5RF9, 5RFB, 5RFC, 5RFD, 5RFE, 5RFF, 5RFG, 5RFH, 5RFI, 5RFJ, 5RFK, 5RFL, 5RFM, 5RFN, 5RFO, 5RFP, 5RFQ, 5RFR, 5RFS, 5RFT, 5RFU, 5RFV, 5RFW, 5RFX, 5RFY, 5RFZ, 5RG1, 5RG2, 5RG3, 5RGG, 5RGH, 5RGJ, 5RGK, 5RGL, 5RGN, 5RGO, 5RGP, 5RGQ, 5RGR, 5RGS, 5RGT, 5RGU, 5RGV, 5RGW, 5RGX, 5RGY, 5RGZ, 5RH1, 5RH2, 5RH3, 5RH4, 5RH5, 5RH6, 5RH7, 5RH8, 5RH9, 5RHA, 5RL0, 5RL1, 5RL2, 5RL3, 5RL4, 5RL5, 6LU7, 6LZE, 6M03, 6M0K, 6M2N, 6M2Q, 6W63, 6WNP, 6WQF, 6WTJ, 6WTK, 6WTM, 6WTT, 6XB0, 6XB1, 6XB2, 6XBG, 6XBI, 6XCH, 6XFN, 6XG2, 6XHU, 6XKF, 6XKH, 6XOA, 6XQS, 6XQT, 6XQU, 6XR3, 6Y2E, 6Y2F, 6Y2G, 6YB7, 6YNQ, 6YT8, 6YVF, 6YZ6, 6ZRT, 7A1U, 7AEG, 7AF0, 7AGA, 7AHA, 7ALH, 7ALI, 7AMJ, 7ANS, 7AOL, 7AP6, 7AQE, 7AQI, 7AQJ, 7AR5, 7AR6, 7AVD, 7AWR, 7AWS, 7AWU, 7AWW, 7AX6, 7AXM, 7AXO, 7AY7, 7B3E, 7B83, 7BAJ, 7BB2, 7BE7, 7BFB, 7BGP, 7BQY, 7BRO, 7BRP, 7BRR, 7BUY, 7C2Q, 7C2Y, 7C7P, 7C8R, 7C8T, 7CB7, 7COM, 7CUU, 7CWB, 7D1M, 7D1O, 7D3I, 7DJR, 7DK1, 7DPU, 7DPV, 7JFQ, 7JKV, 7JOX, 7JOY, 7JP1, 7JPY, 7JPZ, 7JQ0, 7JQ1, 7JQ2, 7JQ3, 7JQ4, 7JQ5, 7JST, 7JT7, 7JU7, 7JUN, 7JVZ, 7JW8, 7JYC, 7K0F, 7K3T, 7K40, 7K6D, 7K6E, 7KFI, 7KFJ, 7KHP, 7KPH, 7KVG, 7KVL, 7KVR, 7KW5, 7L0D, 7LB7, 7LBN, 7LCO, 7LCS, 7LCT, 7LDX, 7LFE, 7LKD, 7LKR, 7LKS, 7LMC, 7LTJ, 7LYH, 7LYI, 7LZT, 7LZX, 7MBG, 7MGS, 7MHF, 7MHG, 7MHH, 7MHI, 7MHJ, 7MHK, 7MHL, 7MHM, 7MHN, 7MHO, 7MHP, 7MHQ, 7MNG, 7MPB, 7MRR, 7N5Z, 7N6N, 7N89, 7N8C, 7NBY, 7NF5, 7NG3, 7NG6, 7NT1, 7NT2, 7NT3, 7NTV, 7NUK, 7NW2, 7S3K, 7S3S, and 7S4B