Supporting Information for: Optimization of Potential Non-covalent Inhibitors for SARS-CoV-2 Main Protease Inspected by a Descriptor of Subpocket Occupancy

Yujia Sun [a], Bodi Zhao [a], Yuqi Wang [a], Zitong Chen [a], Huaiyu Zhang [b], Lingbo Qu [a], Yuan Zhao *[c], Jinshuai Song *[a]

[a] Green Catalysis Center, and College of Chemistry, Zhengzhou University, No. 100 Science Avenue, Zhengzhou, Henan 450001, P. R. China

[b] Institute of Computational Quantum Chemistry, College of Chemistry and Materials Science, Hebei Normal University, Shijiazhuang, Hebei 050024, P. R. China

[c] The Key Laboratory of Natural Medicine and Immuno−Engineering, Henan University, Kaifeng, Henan 475000, P. R. China

E−mail: jssong@zzu.edu.cn ; zhaoyuan@henu.edu.cn

Content

Part1: Functions of 13 Marketed Drugs

Adrafinil, a psychostimulant that activates the postsynaptic α1 adrenergic receptors of the central nervous system. Bromebric acid, a medicine for the treatment of neurological disorders. Fusidic acid is an antibiotic with steroidal skeleton, which is highly sensitive to various gram-positive cocci, especially staphylococci. LSN-2463359 is a positive allosteric modulator of metabotropic glutamate 5 (mGlu5), which can attenuate behavioral response after the administration of competitive NMDA receptor antagonists. MUT056399 is a highly potent inhibitor of the FabI enzyme of both Staphylococcus aureus and Escherichia coli. Necrostatin-1 (Nec-1) effectively inhibits TNFα-induced necrotic death of L929 cells and prevents radiocontrast media (RCM)-induced dilation of peritubular capillaries. Nec-1 is a potent necroptosis inhibitor, RIP1 kinase inhibitor and IDO inhibitor. Polydatin, the glycoside of Resveratrol, can inhibit ICAM-1 expression, elevate Ca^{2+}, weaken white blood cell−endothelial cell adhesion, and activate KATP channels in the myocardial cell, white blood cell, vascular smooth muscle cell, and endothelial cell. SEN-1269, a novel inhibitor of amyloid-beta toxicity. AZD6482 is a selective inhibitor that blocks the interaction of ATP with PI3Kβ and inhibits platelet aggregation induced by low concentrations of agonists. SUN-B-8155 is a non−peptide agonist of the calcitonin receptor which selectively mimics the biological effects of calcitonin. Clonidine is an alpha 2-adrenergic agonist and it can suppress the firing activity of neurons. UNC2327 is an allosteric inhibitor of protein arginine methyltransferase 3 (PRMT3). Tretazicar, an antitumor prodrug, is highly selective against the Walker 256 rat tumour line which has sensitivity for retrovirally transduced AB22 (AB22-nr) cells.

Part 2: Docking Results

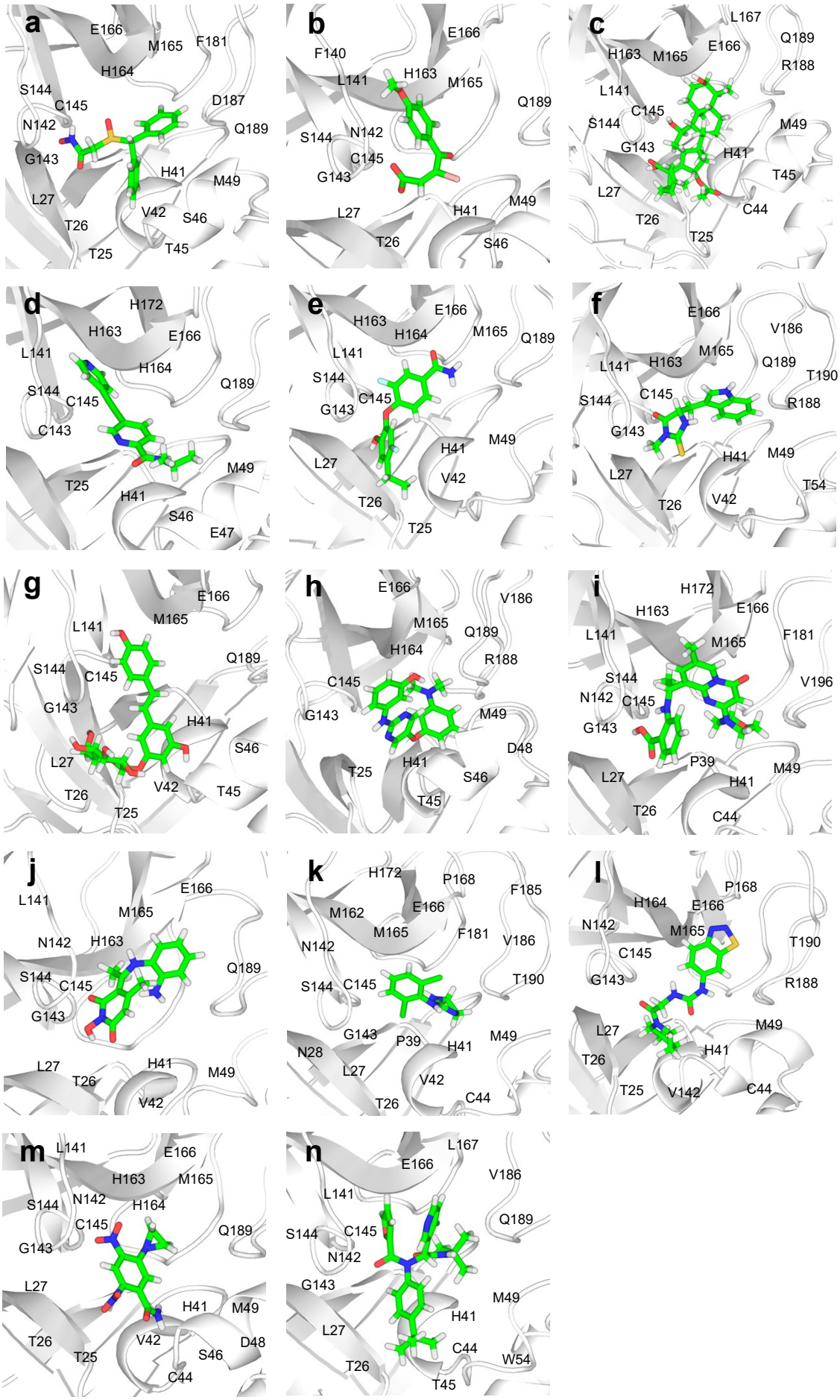

Fig. S1 Docking of 13 selected compounds and inhibitor ML188 in the pocket of the Mpro: (a) adrafinil; (b) bromebric; (c)Fusidic Acid; (d) LSN-2463359; (e) MUT056399; (f) necrostatin-1; (g) polydatin; (h) polydatin; (i) AZD6482; (j) SUN-B-8155; (k) clonidine; (l) UNC-2327; (m) tretazicar;(n) ML188

Part 3: Molecular Dynamics Simulations Results and Free−Energy Calculations

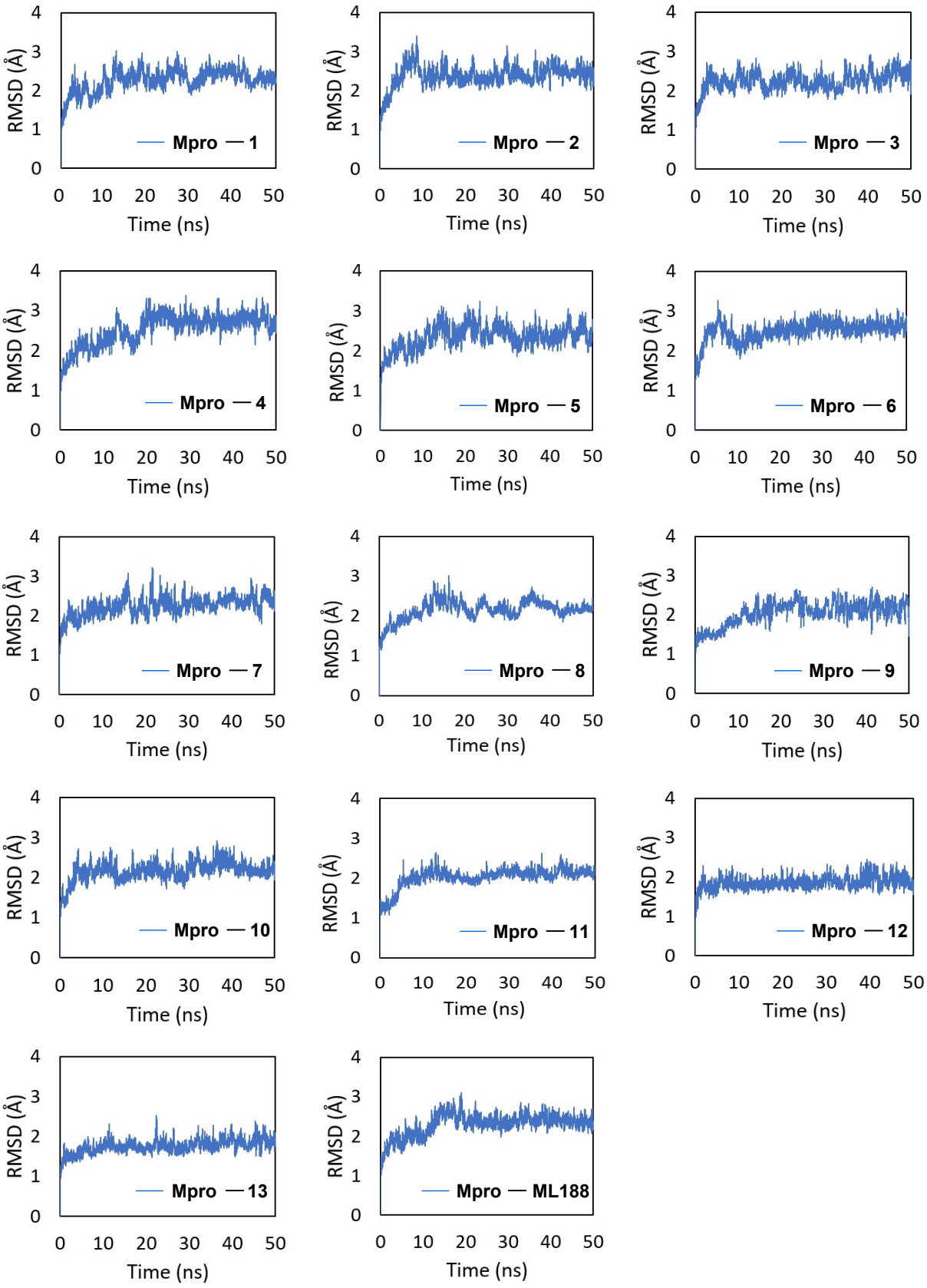

Fig. S2 Graphical representation of the RMSD for for each ligand in complex with Mpro protein during 50 ns molecular dynamics simulations.

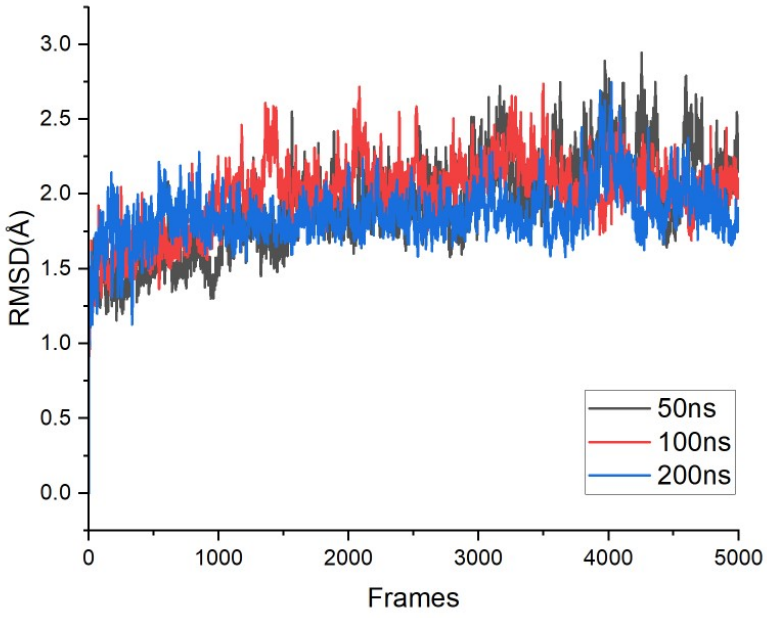

Fig. S3 Graphical representation of the RMSD during 50 ns,100 ns and 200ns of MD simulation for compound **12**.

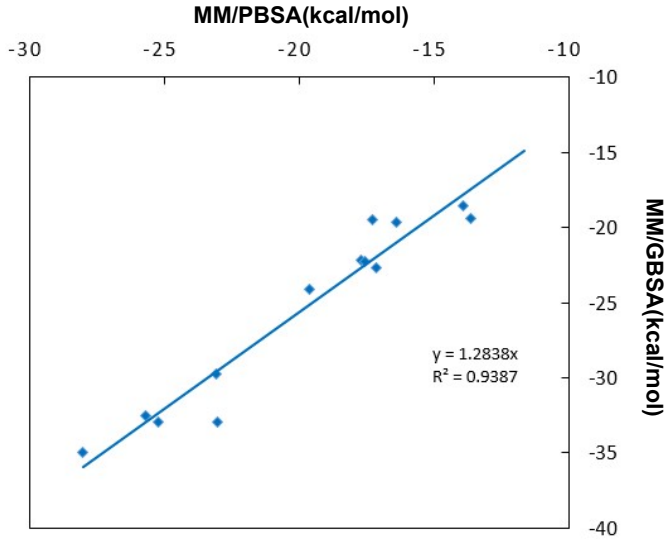

Fig. S4 The linear relationship between binding energies of $\Delta G_{MM/PBSA}$ and $\Delta G_{MM/GBSA}$.

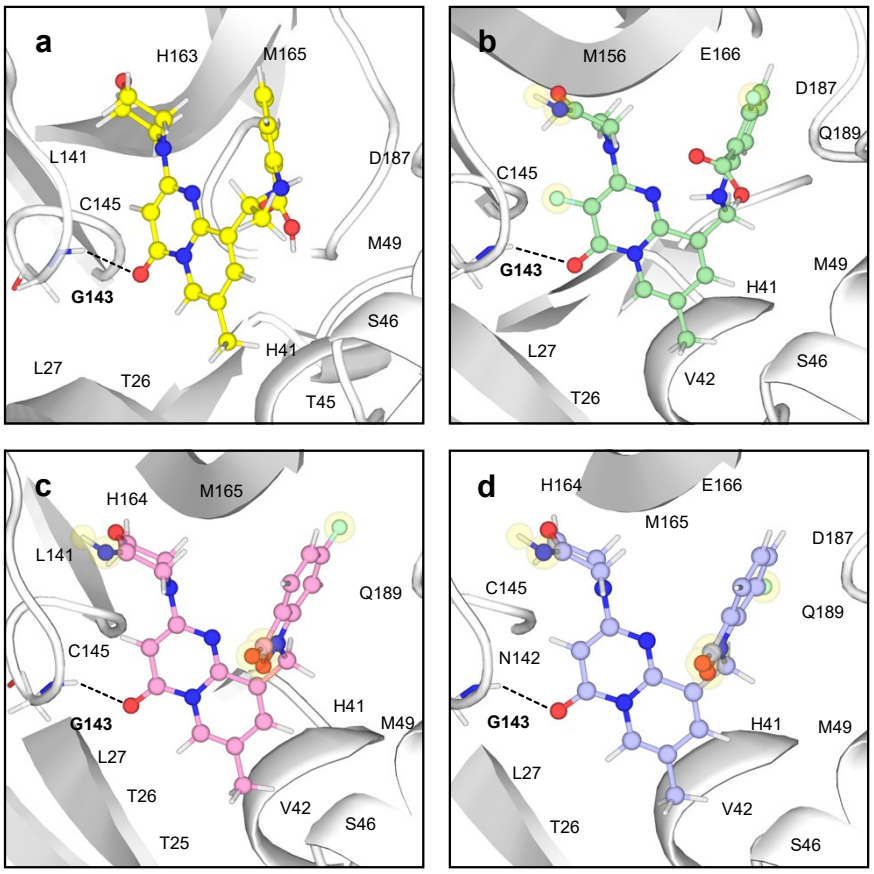

Fig. S5 Representative snapshot of 3 Mpro-novel ligand complexes. Compared with **9**, the optimized groups in the novel lead compounds are highlighted in yellow sphere.

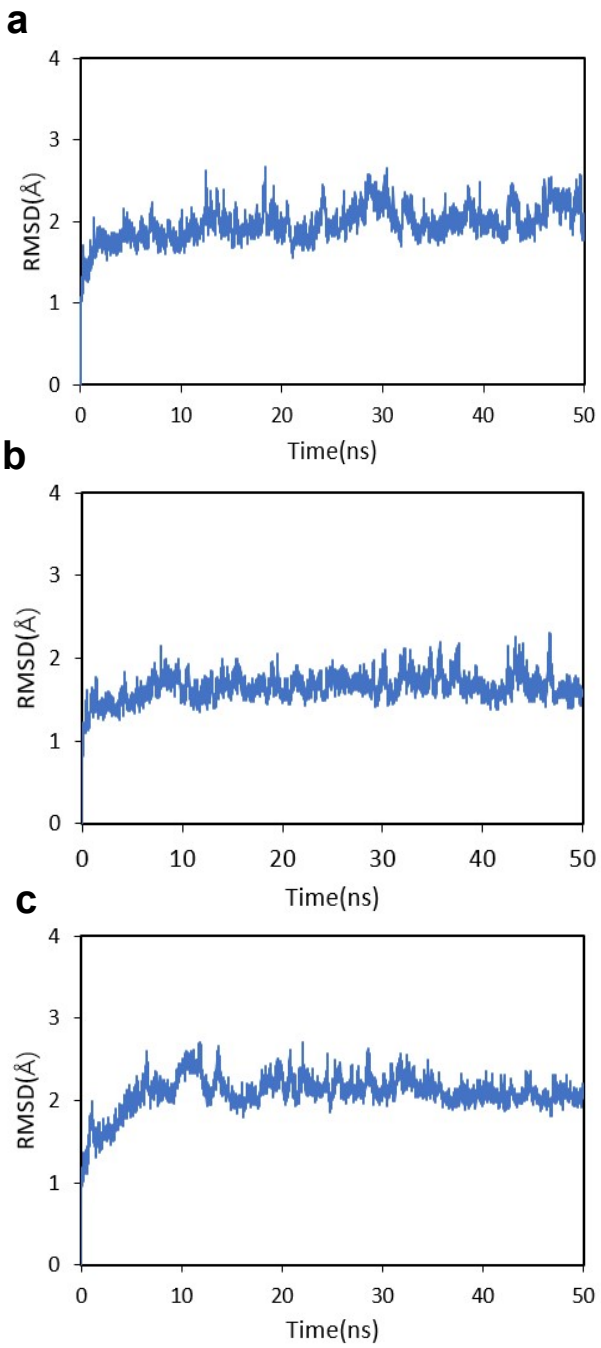

Fig. S6 Graphical representation of the RMSD during 50 ns of MD simulation for Mpro with lead compound (a) **L1** (b) **L2** (c) **L3** complexes.

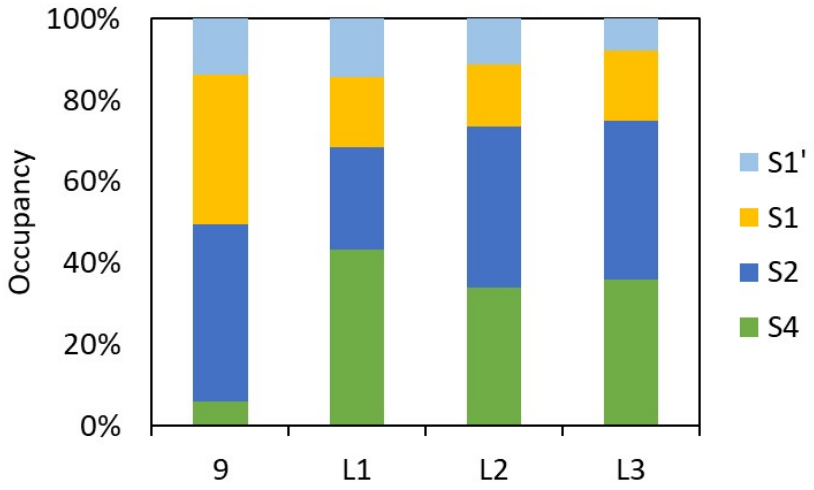

Fig. S7 Occupancy percentage of compound **9** and three novel lead compound in four subpockets.

Table S1 Average RMSD values (Å) for 13 selected compounds.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**L1**	**L2**	**L3**
RMSD(Å)	2.5	2.1	2.1	1.8	2.3	2.1	2.4	2.1	1.8	2.2	2.0	2.3	2.0	2.0	1.6	2.1

Table S2 Relative binding energies of the compound **12** using different frames (energies in kcal/mol).

Frames	**ΔE_{vdw}**	**ΔE_{EEL}**	**ΔE_{EPB}**	**$\Delta E_{ENPOLAR}$**	**$\Delta G_{MM/PBSA}$**	**$\Delta G_{MM/GBSA}$**
50	-41.57	-16.01	30.75	-3.24	-30.08	-38.23
100	-40.43	-13.27	28.68	-3.26	-28.27	-36.11
200	-40.21	-13.60	28.82	-3.24	-28.24	-35.91
500	-40.38	-13.24	28.73	-3.24	-28.13	-35.82

Table S3 Relative binding energies of compound **12** during 50 ns,100 ns and 200ns of MD simulation (energies in kcal/mol).

Time(ns)	**ΔE_{vdw}**	**ΔE_{EEL}**	**ΔE_{EPB}**	**$\Delta E_{ENPOLAR}$**	**$\Delta G_{MM/PBSA}$**	**$\Delta G_{MM/GBSA}$**
50	-40.43	-13.27	28.68	-3.26	-28.27	-36.11
100	-39.84	-13.99	28.70	-3.18	-28.31	-35.52
200	-39.18	-14.03	28.65	-3.16	-28.06	-34.80

Table S4 MM/PB(GB)SA binding free energies along with its constituent energies for the selected compounds and three novel lead compounds in eight MD simulations (energies in kcal/mol).

Compound	ΔE_{vdw}	ΔE_{EEL}	ΔE_{EPB}	$\Delta E_{ENPOLAR}$	$\Delta G_{MM/PBSA}$	$\Delta G_{MM/GBSA}$
1	−29.20	−9.07	26.76	−3.03	−14.53	−16.62
	−29.35	−11.72	30.81	−3.07	−13.34	−17.06
	−31.08	−21.80	43.26	−3.07	−12.69	−18.87
	−29.72	−17.75	38.13	−3.09	−12.43	−17.86
	−25.65	−39.50	53.64	−2.98	−14.49	−18.45
	−32.41	−20.10	40.93	−3.19	−14.76	−19.55
	−29.45	−39.01	55.65	−3.17	−15.98	−21.98
	−30.57	−11.81	32.40	−3.15	−13.12	−17.75
2	−23.00	−0.39	11.25	−2.44	−14.58	−18.43
	−23.75	−8.51	6.68	−2.28	−10.83	−15.90
	−22.46	−6.56	19.57	−2.31	−11.76	−19.52
	−24.25	−4.94	10.00	−2.40	−11.71	−17.07
	−11.15	−19.35	17.95	−1.60	−14.15	−17.63
	−24.75	−12.35	23.16	−2.47	−16.40	−24.35
	−25.54	−9.56	20.47	−2.48	−17.12	−23.05
	−24.59	−2.09	16.63	−2.35	−12.40	−19.22
3	−42.45	−6.41	27.57	−4.08	−25.36	−34.43
	−44.69	−2.62	23.05	−4.00	−28.26	−34.03
	−45.97	−5.74	28.53	−4.41	−27.60	−34.66
	−34.40	−9.59	24.43	−3.61	−23.17	−27.01
	−50.96	−1.47	26.91	−4.61	−30.13	−39.46
	−47.67	−6.54	28.46	−4.47	−30.22	−37.91
	−47.59	−5.23	28.71	−4.43	−28.54	−36.87
	−45.59	−1.68	20.88	−4.43	−30.82	−35.57
4	−32.44	−2.97	18.16	−2.67	−19.92	−26.91
	−25.01	−6.48	18.53	−2.41	−15.38	−19.23
	−21.71	−5.22	15.01	−2.11	−14.03	−16.77
	−30.50	−7.63	19.18	−2.76	−21.72	−25.92
	−29.20	−5.20	18.26	−2.74	−18.89	−23.43
	−23.78	−4.37	15.88	−2.34	−14.62	−17.38
	−23.33	−5.42	16.61	−2.22	−14.35	−17.78
	−33.23	−9.68	23.46	−3.31	−22.77	−29.77
5	−27.73	−9.92	24.30	−2.87	−16.23	−18.79
	−30.70	−28.89	44.50	−3.11	−18.20	−27.76
	−21.03	−15.29	22.51	−2.29	−16.10	−17.49
	−32.93	−8.22	23.63	−3.12	−20.63	−24.86
	−31.60	−9.38	27.30	−3.21	−16.89	−23.44
	−28.51	−9.11	21.19	−2.79	−19.23	−24.69

	−29.36	−8.91	24.25	−3.11	−17.13	−21.75
	−24.72	−11.81	23.57	−3.15	−16.11	−19.20
6	−32.52	−16.66	30.85	−2.79	−21.12	−26.98
	−29.69	−8.05	22.73	−2.67	−17.67	−22.61
	−25.82	−9.57	23.44	−2.87	−14.81	−19.61
	−31.36	−14.22	28.17	−2.79	−20.18	−26.38
	−29.74	−10.70	27.02	−2.93	−16.35	−23.06
	−27.08	−12.47	26.53	−2.87	−15.89	−21.40
	−25.06	−8.18	21.18	−2.57	−14.63	−19.25
	−29.57	−7.53	23.66	−2.89	−16.33	−22.04
7	−47.81	−17.14	45.51	−4.14	−23.59	−38.28
	−39.43	−9.39	28.48	−3.45	−23.78	−33.50
	−32.56	−10.25	25.91	−3.18	−20.08	−27.39
	−38.76	−7.88	24.56	−3.55	−25.63	−34.51
	−47.56	−13.61	37.61	−4.05	−27.60	−39.18
	−39.79	−18.46	40.96	−3.82	−21.12	−32.53
	−38.08	−13.93	36.55	−3.80	−19.27	−30.19
	−35.37	−11.39	27.04	−3.40	−23.13	−28.00
8	−36.12	−9.71	25.56	−3.23	−23.50	−30.06
	−39.62	−4.47	22.76	−3.52	−24.85	−32.21
	−38.94	−9.23	25.38	−3.44	−26.22	−32.73
	−37.42	−8.32	24.40	−3.34	−24.68	−31.31
	−37.44	−9.25	24.40	−3.20	−25.50	−31.69
	−38.75	−3.64	21.24	−3.43	−24.58	−31.29
	−44.34	−7.66	26.02	−3.67	−29.65	−36.61
	−41.67	−6.82	25.62	−3.72	−26.60	−34.47
9	−42.22	−16.84	39.95	−3.83	−22.93	−29.66
	−48.34	−7.73	31.69	−4.27	−28.65	−36.00
	−38.72	−18.78	40.31	−3.84	−21.04	−27.80
	−42.91	−13.63	37.83	−3.90	−22.62	−31.18
	−39.96	−12.36	34.37	−3.51	−21.45	−29.20
	−39.54	−11.76	31.92	−4.05	−23.44	−28.77
	−38.71	−12.69	31.48	−3.88	−23.80	−27.61
	−34.04	−26.37	43.52	−3.66	−20.54	−27.83
10	−27.92	−13.15	27.11	−2.91	−16.87	−19.47
	−27.21	−11.63	27.28	−2.90	−14.46	−18.62
	−24.09	−11.99	23.48	−2.84	−15.43	−16.75
	−26.85	−7.57	22.90	−2.78	−14.30	−16.98
	−24.55	−10.97	21.79	−2.58	−16.31	−18.17
	−29.32	−23.33	32.33	−3.03	−23.36	−26.15
	−25.80	−16.71	24.13	−2.82	−21.20	−23.63
	−23.64	−10.06	20.24	−2.74	−16.21	−16.38

11	−29.65	−3.29	18.02	−2.69	−17.61	−25.46
	−27.78	−3.83	13.46	−2.43	−20.59	−24.42
	−27.22	−4.43	15.06	−2.44	−19.03	−23.91
	−26.02	−2.91	11.73	−2.38	−19.58	−22.72
	−27.70	−3.45	11.49	−2.40	−22.06	−25.66
	−24.20	−3.39	12.27	−2.31	−17.64	−21.12
	−26.40	−2.29	12.58	−2.46	−18.57	−23.04
	−28.89	−4.19	13.89	−2.45	−21.65	−26.53
12	−38.44	−7.62	24.58	−3.12	−24.60	−31.02
	−40.43	−13.27	28.68	−3.26	−28.27	−36.11
	−37.21	−8.85	27.26	−3.32	−22.13	−30.41
	−31.46	−11.90	23.20	−3.13	−23.29	−27.11
	−42.59	−18.07	34.55	−3.41	−29.52	−37.42
	−39.44	−16.48	35.29	−3.40	−24.03	−34.38
	−37.62	−7.75	24.69	−3.27	−23.94	−31.62
	−40.21	−18.73	36.30	−3.36	−25.99	−35.50
13	−26.18	−11.87	26.28	−2.55	−14.33	−18.05
	−30.30	−27.38	41.00	−2.54	−19.22	−21.11
	−26.60	−13.65	27.18	−2.64	−15.70	−19.35
	−23.89	−16.86	29.17	−2.50	−14.08	−16.63
	−26.30	−12.68	25.90	−2.53	−15.61	−18.37
	−31.21	−13.82	28.83	−2.59	−18.79	−22.54
	−28.17	−12.40	26.27	−2.75	−17.05	−21.06
	−29.06	−9.95	25.23	−2.49	−16.28	−19.95
L1	−47.60	−13.78	34.60	−4.16	−30.94	−40.74
	−49.48	−16.24	38.52	−4.20	−31.39	−43.32
	−50.58	−14.77	35.65	−4.28	−33.98	−43.15
	−52.83	−14.35	38.20	−4.33	−33.31	−45.11
	−53.15	−16.38	38.48	−4.44	−35.49	−45.29
	−46.38	−15.14	33.62	−4.16	−32.06	−38.52
	−46.39	−15.43	32.79	−3.85	−32.87	−41.42
	−50.90	−15.89	37.97	−4.29	−33.10	−43.90
L2	−44.59	−10.40	31.22	−3.77	−27.55	−38.42
	−52.73	−9.64	34.57	−4.32	−32.12	−44.09
	−53.65	−9.43	34.99	−4.34	−32.42	−44.92
	−50.76	−8.82	32.79	−4.22	−31.02	−42.07
	−49.55	−5.43	28.25	−4.06	−30.79	−40.54
	−47.78	−10.85	34.00	−4.07	−28.70	−39.32
	−51.70	−8.22	33.90	−4.32	−30.34	−41.98
	−45.20	−5.65	27.34	−4.03	−27.54	−36.85
L3	−42.49	−9.25	24.46	−3.39	−30.67	−37.21
	−48.38	−15.55	37.39	−4.14	−30.68	−40.24

	−51.31	−10.00	31.01	−3.97	−34.27	−45.88
	−52.01	−9.46	34.27	−4.35	−31.55	−42.61
	−51.54	−12.27	35.56	−4.26	−32.52	−44.03
	−54.35	−13.36	36.69	−4.32	−35.33	−47.30
	−49.71	−9.84	33.80	−4.13	−29.89	−39.77
	−52.53	−10.44	35.57	−4.29	−31.69	−43.45

Table S5 Relative binding energies of compound **12** with entropy contributions during 50ns (energies in kcal/mol).

Frames	ΔE_{vdw}	ΔE_{EEL}	ΔE_{EPB}	$\Delta E_{ENPOLAR}$	$T\Delta S$	$\Delta G_{MM/PBSA}$	$\Delta G_{MM/GBSA}$
50	−41.57	−16.01	30.75	−3.24	−	−30.08	−38.23
50	−41.57	−16.01	30.75	−3.24	−16.36	−13.71	−21.86
100	−40.43	−13.27	28.68	−3.26	−	−28.27	−36.11
100	−40.43	−13.27	28.68	−3.26	−19.66	−8.61	−16.45

We calculated the atomic partial charges before docking using the AM1-BCC charges and restrained electrostatic potential (RESP). Then their binding energies were calculated separately, and the results were not much different.

Table S6 Comparison of MM/PB(GB)SA binding free energies assigning AM1-BCC charges and RESP charges for compound **12** (energies in kcal/mol).

Compound	ΔE_{vdw}	ΔE_{EEL}	ΔE_{EPB}	$\Delta E_{ENPOLAR}$	$\Delta G_{MM/PBSA}$	$\Delta G_{MM/PBSA}$
	−38.44	−7.62	24.58	−3.12	−24.60	−31.02
	−40.43	−13.27	28.68	−3.26	−28.27	−36.11
	−37.21	−8.85	27.26	−3.32	−22.13	−30.41
12	−31.46	−11.90	23.20	−3.13	−23.29	−27.11
(BCC)	−42.59	−18.07	34.55	−3.41	−29.52	−37.42
	−39.44	−16.48	35.29	−3.40	−24.03	−34.38
	−37.62	−7.75	24.69	−3.27	−23.94	−31.62
	−40.21	−18.73	36.30	−3.36	−25.99	−35.50
	−38.27	−16.13	33.29	−3.37	−24.48	−31.12
	−37.95	−17.69	31.45	−3.57	−27.75	−32.89
	−36.71	−8.67	26.50	−3.31	−22.19	−30.10
12	−37.20	−9.90	27.10	−3.29	−23.29	−29.48
(RESP)	−37.39	−16.92	31.99	−3.51	−25.83	−31.05
	−33.03	−8.47	22.57	−3.00	−21.94	−26.93
	−35.37	−6.67	24.09	−3.21	−21.15	−26.76
	−32.89	−14.49	28.33	−3.16	−22.21	−27.09

Table S7 Hydrogen bond interactions in the Mpro-ligand complexes

compound	Hydrogen Bond	Lifetime(%)	Distance(Å)
3	(UNK307)@O5…H−N(G143)	14.78	2.53
	(UNK307)@O5…H−N(E166)	60.22	2.37
8	(UNK307)@O2…H1−OG1(T25)	19.28	2.08
	(UNK307)@O1…HD1−ND1(H41)	85.10	2.67
	(UNK307)@N2−H2…OG(S46)	15.84	2.61
9	(UNK307)@O2…H−N(G143)	53.08	2.35
12	(UNK307)@N5−H51…O (L141)	8.04	2.46
	(UNK307)@O2…H−N(G143)	16.68	2.41
	(UNK307)@O2…H−N(G145)	21.98	2.25

Table S8 Protein−ligand binding energy decomposition of critical residues involved in the formation of Mpro binding pocket (energies in kcal/mol).

residue	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
T 25	−1.29	−0.10	−1.27	0.01	−1.36	−0.74	−0.92	−1.14	−0.23	−0.45	−0.11	−0.35	−0.43
T 26	−0.07	−0.01	−0.16	0.01	−0.05	−0.26	−0.50	−0.04	−0.01	−0.80	0.00	−0.03	−0.10
L 27	−0.27	−0.24	−0.71	−0.02	−0.40	−0.65	−0.52	−0.24	−0.45	−0.43	−0.37	−0.22	−0.63
P 39	−0.02	−0.04	−0.22	0.00	−0.15	−0.27	−0.12	−0.13	−0.15	−0.05	−0.16	−0.11	−0.10
R 40	−0.06	−0.07	−0.34	0.01	−0.03	−0.44	−0.08	−0.31	−0.25	0.01	−0.21	−0.20	−0.05
H 41	−2.02	−1.25	−1.18	−0.01	−1.65	−2.06	−1.71	−2.24	−1.60	−1.38	−1.48	−1.96	−1.52
V 42	−0.09	−0.01	−0.30	0.00	−0.11	−0.21	−0.10	−0.22	−0.11	−0.07	−0.11	−0.21	−0.18
C 44	−0.29	−0.02	−1.09	0.00	−0.77	−0.51	−0.18	−1.62	−0.81	−0.12	−0.12	−0.40	−0.08
T 45	−1.05	−0.05	−0.88	0.00	−0.33	−0.40	−0.11	−1.84	−1.03	−0.17	−0.07	−0.39	−0.10
S 46	−0.56	−0.17	−1.13	−0.06	−0.68	−0.55	−0.10	−0.97	−0.50	−0.41	−0.30	−0.30	−0.16
D 48	−0.01	0.03	−0.19	0.00	−0.15	−0.07	−0.04	−0.38	−0.26	0.00	−0.06	−0.11	−0.03
M 49	−0.36	−0.64	−1.75	−0.20	−1.71	−1.17	−0.70	−2.75	−2.12	−1.63	−1.52	−1.65	−1.02
P 52	0.00	−0.01	−0.25	0.00	−0.05	−0.09	−0.14	−0.32	−0.17	−0.09	−0.15	−0.03	−0.01
F 140	−0.03	−0.13	0.00	−0.33	0.00	−0.02	−0.12	0.00	−0.24	0.00	−0.01	−0.12	−0.08
L 141	−0.05	−0.26	−0.02	−0.54	0.00	−0.07	−0.16	0.00	−0.27	−0.02	0.00	−0.32	−0.03
N 142	−2.55	−2.34	−0.04	−1.19	0.06	−0.33	−0.39	0.05	−0.97	−0.18	0.02	−0.50	−0.49
G 143	−1.71	−1.36	−0.28	−0.11	0.00	−0.27	−0.19	−0.02	−0.20	−0.20	−0.04	−0.18	−0.50
S 144	−0.83	−0.76	−0.11	−0.40	−0.05	−0.24	−0.15	−0.02	−0.23	−0.05	−0.05	−0.25	−0.47
C 145	−1.46	−1.20	−0.71	−0.22	−0.90	−0.81	−0.63	−0.15	−0.77	−0.48	−0.49	−0.76	−1.17
H 163	−0.51	−0.29	−0.07	−0.48	−0.04	−0.21	−0.09	−0.05	−0.95	−0.07	−0.07	−0.18	−0.29
H 164	−0.13	−0.32	0.16	−0.14	−0.22	−0.30	−0.22	−0.07	−0.22	−0.31	−0.31	−0.92	−0.29
M 165	−0.92	−2.09	−0.86	−1.02	−0.69	−0.84	−1.56	−0.83	−2.25	−1.66	−1.02	−2.70	−1.48
E 166	−0.22	−0.52	0.18	−0.81	0.05	−0.18	−0.18	0.09	−0.41	−0.49	0.03	−0.72	−0.39
L 167	0.04	−0.02	−0.03	−0.18	−0.01	0.00	−0.47	−0.04	−0.17	−0.14	−0.03	−0.06	−0.06
P 168	0.00	0.00	0.02	−0.40	−0.01	0.00	−0.60	0.00	−0.24	−0.08	−0.01	−0.04	−0.03
H 172	−0.11	−0.31	−0.01	−0.40	0.00	−0.05	−0.21	−0.01	−0.26	−0.02	−0.02	−0.17	−0.08
D 187	0.07	−0.20	−0.27	−0.01	−0.46	−0.11	−0.26	−0.02	−0.02	−0.15	−0.77	−0.83	−0.33
R 188	−0.01	−0.25	−0.35	−0.04	−0.69	−0.08	−0.27	−0.37	−0.28	−0.28	−0.23	−0.40	−0.24
Q189	−0.04	−0.46	−0.62	−0.52	−0.35	−0.30	−0.86	−0.41	−0.40	−0.66	−0.53	−0.69	−0.34

Table S9 Protein−ligand binding energy decomposition of secondary structures (SS) involved in the formation of Mpro binding pocket (energies in kcal/mol).

SS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Sheet4	−1.63	−0.35	−2.14	0.00	−1.81	−1.66	−1.94	−1.42	−0.68	−1.68	−0.48	−0.59	−1.17
Helix2	−2.48	−1.38	−3.12	0.00	−2.70	−3.50	−2.19	−4.52	−2.93	−1.61	−2.09	−2.89	−1.94
ASL1	−1.98	−0.85	−4.20	−0.27	−3.69	−2.27	−1.09	−6.26	−4.09	−2.31	−2.10	−2.48	−1.32
Loop1	−6.63	−6.05	−1.15	−2.80	−0.89	−1.74	−1.65	−0.15	−2.68	−0.94	−0.56	−2.13	−2.74
Sheet12	−1.75	−3.24	−0.62	−2.63	−0.91	−1.53	−2.52	−0.89	−4.00	−2.67	−1.40	−4.57	−2.50
ASL2	−0.08	−1.21	−1.24	−1.38	−1.51	−0.53	−2.19	−0.82	−1.19	−1.20	−1.56	−2.14	−1.03

Table S10 Pocket occupancy of 13 selected compounds and generated 3 novel lead compounds

Compound	S1'	S1	S2	S4
1	29.80%	35.79%	34.64%	−0.23%
2	28.45%	47.98%	16.67%	6.89%
3	27.90%	12.48%	50.48%	9.14%
4	9.21%	63.96%	14.65%	12.18%
5	25.63%	15.84%	45.70%	12.84%
6	30.81%	17.82%	47.04%	4.33%
7	27.11%	29.34%	26.44%	17.11%
8	8.77%	12.92%	72.67%	5.64%
9	13.79%	36.86%	43.38%	5.98%
10	24.34%	27.77%	36.53%	11.36%
11	15.25%	21.95%	45.12%	17.69%
12	14.24%	38.39%	34.06%	13.31%
13	33.52%	29.73%	27.86%	8.88%
L1	14.41%	17.06%	25.14%	43.38%
L2	11.14%	15.40%	39.62%	33.84%
L3	7.91%	17.06%	39.24%	35.79%

Part 4: Physicochemical properties and ADME analysis

Physicochemical and ADME characteristics of these inhibitors for Mpro, derived using the software SwissADME:

Table S11 Physicochemical properties of these compounds.

Ligand	MW	Natoms	Nrotb	H-bond acceptor	H-bond donor	Molar Refractivity	TPSA	LogP	LogS	Lipinski
1	289.4	20	6	3	2	77.5	85.6	1.5	−2.8	+
2	285.1	16	4	4	1	61.9	63.6	1.8	−3.0	+
3	516.7	37	6	6	3	146.3	104.1	0.8	−6.1	+
4	265.3	20	3	3	1	77.1	54.9	2.4	−3.1	+
5	293.3	21	4	5	2	72.8	72.6	2.4	−3.7	+
6	259.3	18	2	1	2	81.9	80.2	2.0	−2.9	+
7	390.4	28	5	8	6	100.0	139.8	1.8	−2.9	+
8	322.4	24	5	4	2	94.3	70.5	2.9	−4.2	+
9	408.5	30	5	5	2	117.7	96.2	2.9	−3.7	+
10	273.3	20	2	4	3	78.1	100.8	2.1	−2.0	+
11	230.1	14	2	1	2	67.0	36.4	1.8	−2.4	+
12	319.4	22	6	4	2	88.7	115.5	2.6	−2.5	+
13	252.2	18	4	5	1	67.2	137.7	0.0	−1.4	+
L1	477.4	35	4	8	2	127.0	126.1	2.9	−3.8	+
L2	423.4	31	3	7	1	115.5	105.8	3.0	−3.8	+
L3	423.4	31	3	7	1	115.5	105.8	2.9	−3.8	+

Table S12 ADME characteristics of these compounds.

Ligand	GI	BBB	P-gp	CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4	LogKp (cm/s)
1	high	−	+	−	−	−	−	+	−6.91
2	high	+	−	+	+	−	−	−	−6.45
3	high	−	+	−	−	−	−	+	−5.54
4	high	+	−	+	−	−	−	−	−6.35
5	high	+	−	+	+	+	+	−	−5.98
6	high	−	−	+	+	−	−	−	−6.56
7	high	−	+	−	−	−	−	−	−7.95
8	high	+	−	+	+	+	+	+	−5.87
9	high	−	+	−	−	+	−	+	−7.40
10	high	−	−	−	−	−	−	−	−7.80
11	high	+	−	−	−	−	−	−	−6.59
12	high	−	+	−	−	−	−	−	−7.37
13	high	−	−	−	−	−	−	−	−7.85
L1	high	−	+	−	−	+	−	−	−7.49
L2	high	−	+	−	−	+	−	−	−7.68
L3	high	−	+	−	−	+	−	−	−7.68

Part 5: Simplified Molecular Input Line Entry System

Table S13 SMILES of 13 input compounds.

Compound	SMILES
1	O=C(CS(=O)C(c1ccccc1)c1ccccc1)NO
2	BC(=CC(=O)O)C(=O)c1ccc(OC)cc1
3	CC(=O)OC1CC2(C)C(CC(O)C3C4(C)CCC(O)C(C)C4CCC32C)C1=C(CCC=C(C)C)C(=O)O
4	CC(C)NC(=O)c1ccc(C#Cc2ccncc2)cn1
5	CCc1cc(O)c(Oc2ccc(C(N)=O)cc2F)cc1F
6	CN1C(=O)C(Cc2c[nH]c3ccccc23)NC1=S
7	OCC1OC(Oc2cc(O)cc(C=Cc3ccc(O)cc3)c2)C(O)C(O)C1O
8	CN(C)c1cccc(Oc2cnc(Nc3cccc(O)c3)nc2)c1
9	Cc1cc(C(C)Nc2ccccc2C(=O)O)c2nc(N3CCOCC3)cc(=O)n2c1
10	CC(=Nc1ccccc1N)c1c(C)cc(=O)n(O)c1O
11	Clc1cccc(Cl)c1NC1=NCCN1
12	O=C(NCC(=O)N1CCCCC1)Nc1ccc2nnsc2c1
13	NC(=O)c1cc(N2CC2)c([N+](=O)[O-])cc1[N+](=O)[O-]
L1	Cc1cc(CNc2cccc(F)c2C(=O)O)c2nc(N3CCOC(=N)C3)c(F)c(=O)n2c1
L2	Cc1cc(Cn2oc(=O)c3ccc(F)cc32)c2nc(N3CCOC(=N)C3)cc(=O)n2c1
L3	Cc1cc(Cn2oc(=O)c3cccc(F)c32)c2nc(N3CCOC(=N)C3)cc(=O)n2c1