Supporting Information

Structure-Based Design, Synthesis, and Biological Evaluation of a Series of Novel and Reversible Inhibitors for the Severe Acute Respiratory Syndrome-Coronavirus Papain-Like Protease

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Purity. HPLC system was used Agligent 1100 series. Column and flow rate were employed XDB-C18 5µm 4.6 x 150mm and 1.5 mL/min. **Solvent system A** = linear gradient from 25% acetonitrile, 75% water to 90% acetonitrile, 10% water in 15 min. **Solvent system B** = linear gradient from 30% methanol, 70% water to 100% methanol in 18 min. **Solvent system C** = linear gradient from 20% acetonitrile, 80% 25mM NH_4OAc in water (pH 4.8) to 80% acetonitrile, 20% 25mM NH_4OAc in water (pH 4.8) in 15 min.

Table S1. Purity of inhibitors

Inhibitor	Solvent system	Retention Time (min)	Purity (%)	Column
5a	A	10.9	99.3	XDB-C18 5µm 4.6 x 150mm
5b	A	10.8	99.7	XDB-C18 5µm 4.6 x 150mm
5c	A	11.0	98.3	XDB-C18 5µm 4.6 x 150mm
5d	A	10.2	99.6	XDB-C18 5µm 4.6 x 150mm
5e	A	9.9	98.0	XDB-C18 5µm 4.6 x 150mm
5f	A	10.7	96.7	XDB-C18 5µm 4.6 x 150mm
5g	A	11.6	98.7	XDB-C18 5µm 4.6 x 150mm
5h	A	10.5	99.7	XDB-C18 5µm 4.6 x 150mm
5i	A	10.7	98.5	XDB-C18 5µm 4.6 x 150mm
8	A	11.5	96.5	XDB-C18 5µm 4.6 x 150mm
9	A	7.9	99.3	XDB-C18 5µm 4.6 x 150mm
14	A	11.3	99.8	XDB-C18 5µm 4.6 x 150mm
17	A	12.5	99.7	XDB-C18 5µm 4.6 x 150mm
21	A	12.4	99.9	XDB-C18 5µm 4.6 x 150mm
23	A	7.8	99.3	XDB-C18 5µm 4.6 x 150mm
24	A	8.1	99.2	XDB-C18 5µm 4.6 x 150mm
25	A	7.7	98.9	XDB-C18 5µm 4.6 x 150mm
29	A	8.6	98.2	XDB-C18 5µm 4.6 x 150mm

32	B	13.1	96.0	XDB-C18 5µm 4.6 x 150mm
33	B	11.2	99.9	XDB-C18 5µm 4.6 x 150mm
40	B	10.8	95.3	XDB-C18 5µm 4.6 x 150mm
47	A	12.9	99.8	XDB-C18 5µm 4.6 x 150mm
49	C	5.0	98.6	XDB-C18 5µm 4.6 x 150mm
2	C	4.7	98.3	XDB-C18 5µm 4.6 x 150mm

(Flow rate 1.5 mL/min)

Table S2. HRMS for inhibitors

5a	HRMS (*m/z*) calcd for $C_{20}H_{19}NO$ $[M]^+$ 289.1467, found 289.1468
5b	HRMS (*m/z*) calcd for $C_{20}H_{19}NO$ $[M]^+$ 289.1467, found 289.1469
5c	HRMS (*m/z*) calcd for $C_{20}H_{19}NO_2$ $[M]^+$ 305.1416, found 305.1414
5d	HRMS (*m/z*) calcd for $C_{20}H_{19}NO_2$ $[M]^+$ 305.1416, found 305.1417
5e	HRMS (*m/z*) calcd for $C_{20}H_{19}NO_2$ $[M]^+$ 305.1416, found 305.1419
5f	HRMS (*m/z*) calcd for $C_{21}H_{21}NO$ $[M]^+$ 303.1623, found 303.1624
5g	HRMS (*m/z*) calcd for $C_{19}H_{17}NO_2$ $[M]^+$ 291.1259, found 291.1261
5h	HRMS (*m/z*) calcd for $C_{20}H_{19}NO$ $[M]^+$ 289.1467, found 289.1468
5i	HRMS (*m/z*) calcd for $C_{20}H_{18}N_2O_3$ $[M]^+$ 334.1317, found 334.1323
8	HRMS (*m/z*) calcd for $C_{24}H_{26}N_2O_3$ $[M]^+$ 390.1943, found 390.1942
9	HRMS (*m/z*) calcd for $C_{19}H_{18}N_2O$ $[M]^+$ 290.1419, found 290.1424
14	HRMS (*m/z*) calcd for $C_{21}H_{21}NO$ $[M]^+$ 303.1623, found 303.1624
17	HRMS (*m/z*) calcd for $C_{25}H_{21}NO$ $[M]^+$ 351.1623, found 351.1618
21	HRMS (*m/z*) calcd for $C_{21}H_{21}NO$ $[M]^+$ 303.1623, found 303.1627
23	HRMS (*m/z*) calcd for $C_{19}H_{18}N_2O$ $[M]^+$ 290.1419, found 290.1422
25	HRMS (*m/z*) calcd for $C_{22}H_{22}N_2O_2$ $[M]^+$ 346.1681, found 346.1682
29	HRMS (*m/z*) calcd for $C_{21}H_{22}N_2O$ $[M]^+$ 318.1732, found 318.1729
32	HRMS (*m/z*) calcd for $C_{20}H_{18}INONa$ $[M+Na]^+$ 438.0331, found 438.0333
33	HRMS (*m/z*) calcd for $C_{21}H_{18}N_2O$ $[M]^+$ 314.1419, found 314.1424
40	HRMS (*m/z*) calcd for $C_{21}H_{22}N_2O_2$ $[M]^+$ 334.1681, found 334.1679
47	HRMS (*m/z*) calcd for $C_{26}H_{30}N_2O_3$ $[M]^+$ 418.2256, found 418.2252
49	HRMS (*m/z*) calcd for $C_{22}H_{24}N_2O$ $[M]^+$ 332.1889, found 332.1891
2	HRMS (*m/z*) calcd for $C_{21}H_{22}N_2O$ $[M]^+$ 318.1732, found 318.1734

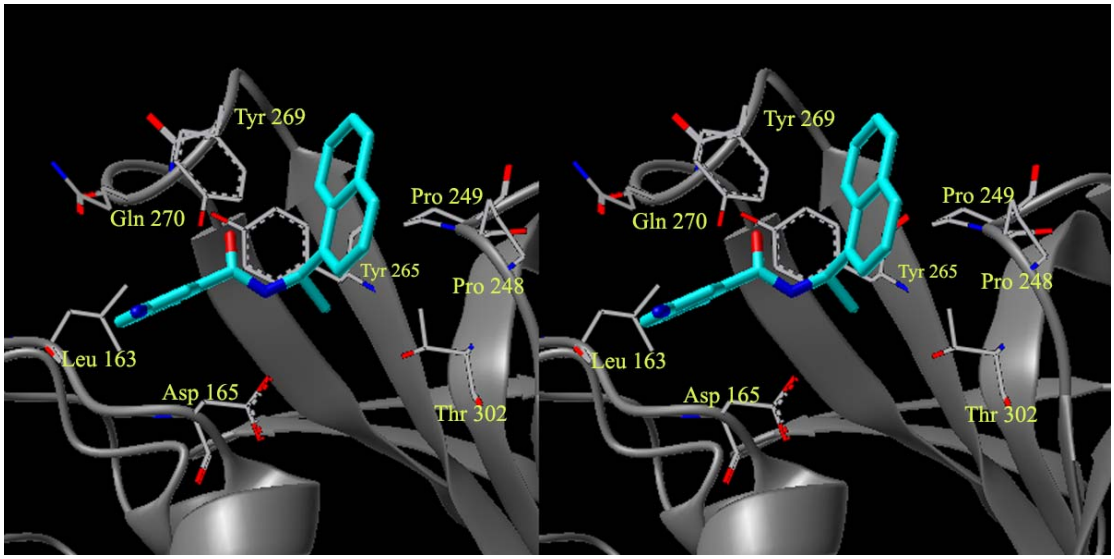

Figure 1. Stereoview of the X-ray structure of inhibitor **24**-bound SARS-CoV PLpro. Interactions of inhibitor **24** (cyan) with the surrounding residues within the S3 and S4 subsites are shown. For clarity, no water molecules are shown here. Critical waters are shown and discussed in conjunction with Figure 2.

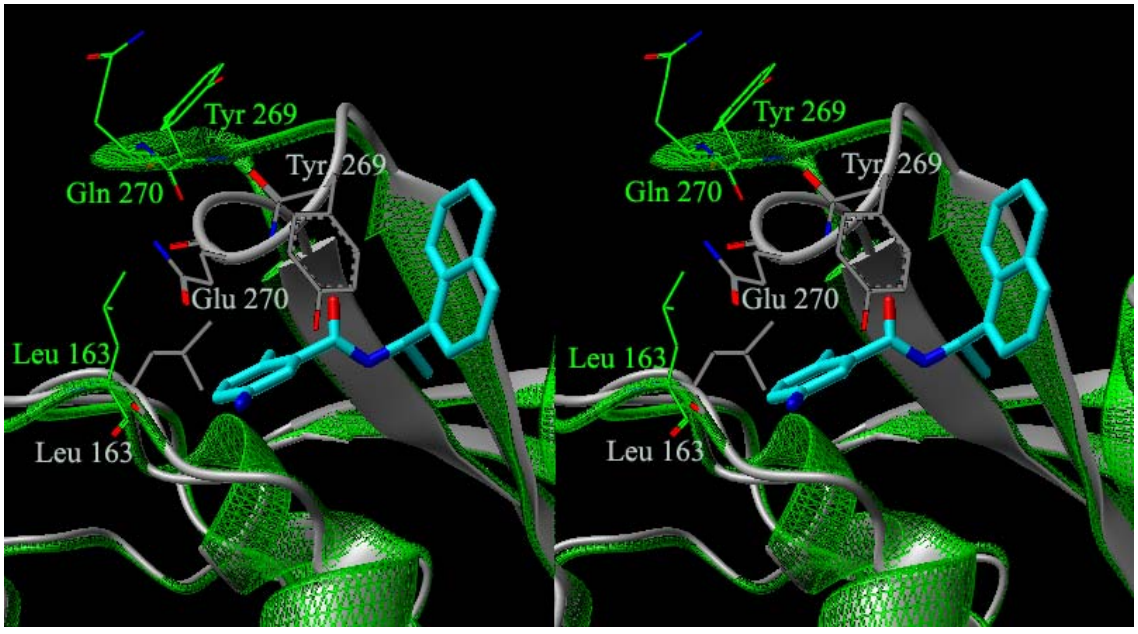

Figure 22. Inhibitor **24** (cyan) induces loop closure. A mesh representation of apo PLpro (green) superimposed on the ribbon diagram of the X-ray of inhibitor-bound enzyme (in grey). For clarity, no water molecules are shown here. Critical waters are shown and discussed in conjuction with Figure 2.