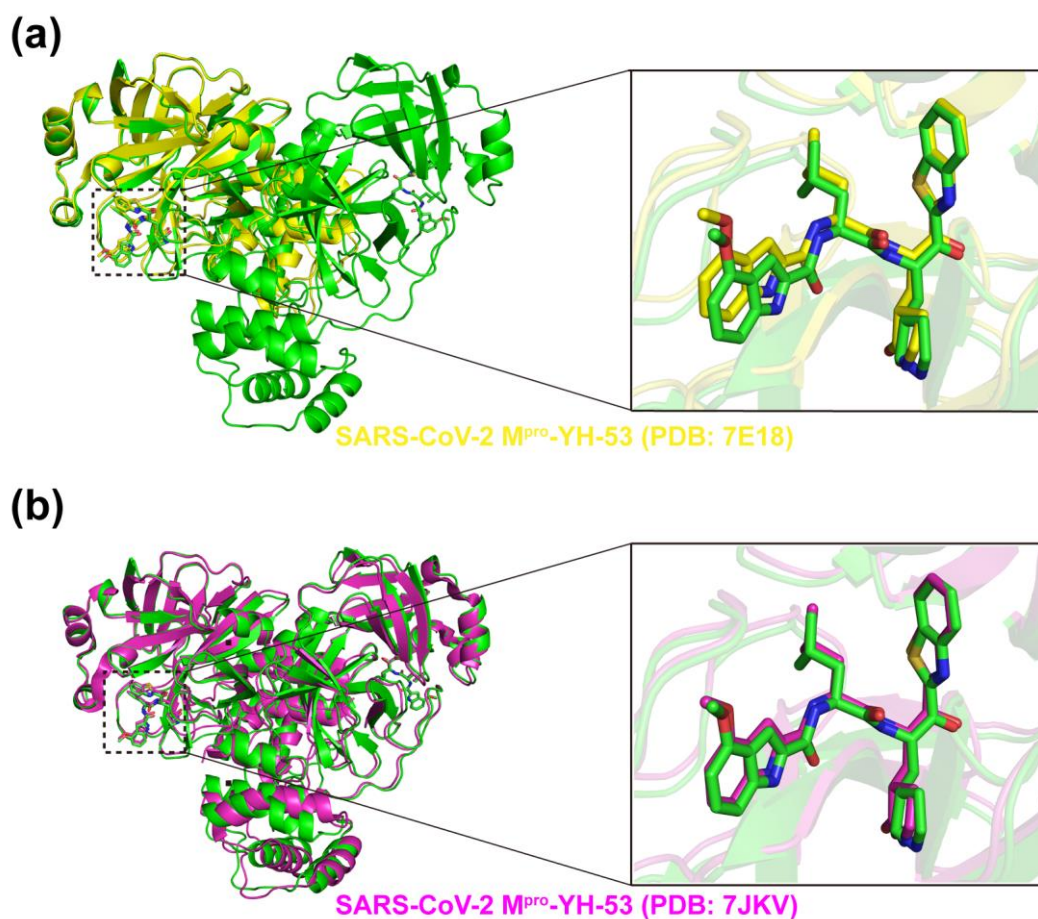
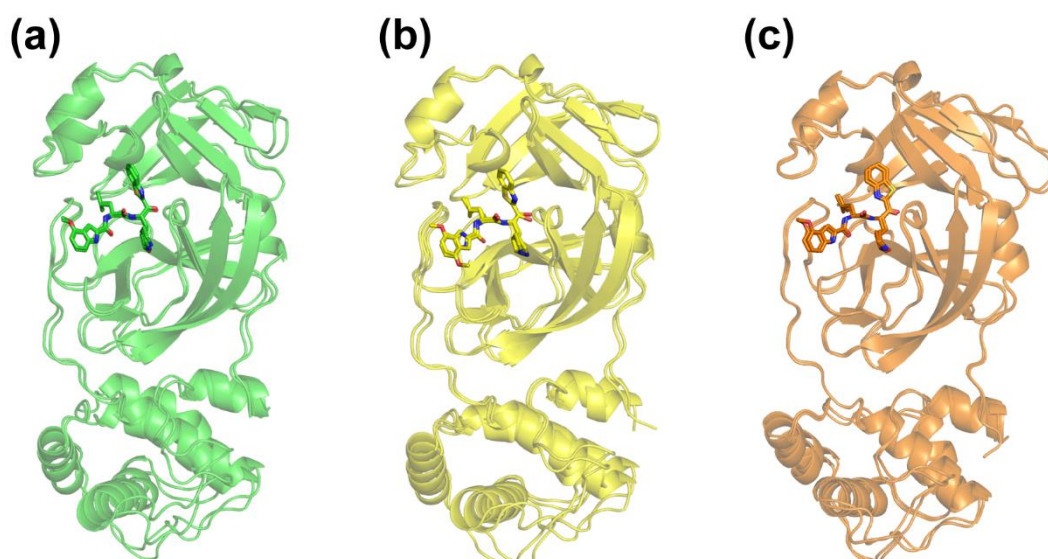


**Figure S1.** Enzymatic inhibition activities of YH-53 and PF-07321332. (a) Inhibition of PF-07321332 against main protease of SARS-CoV-2. (b) Inhibition of PF-07321332 against main protease of MERS-CoV. (c) Inhibition of PF-07321332 against main protease of SARS-CoV. (d) Inhibition of YH-53 against main protease of SARS-CoV. Main proteases were preincubated in the reaction buffer with various concentrations of YH-53 or PF-07321332 at room temperature for 30 minutes before reacting with the FRET substrate.



**Figure S2.** Comparison of the SARS-CoV-2 M<sup>pro</sup>-YH-53 structure we reported in this study to SARS-CoV-2 M<sup>pro</sup>-YH-53 structures reported previously. **(a)** Comparison of our structure (green) to the SARS-CoV-2 M<sup>pro</sup>-YH53 structure (PDB ID 7E18, yellow). **(b)** Comparison of our structure (green) to the SARS-CoV-2 M<sup>pro</sup>-YH53 structure (PDB ID 7JKV, magentas).



**Figure S3.** Structural comparison of protomer A and B of SARS-CoV-2, SARS-CoV and MERS-CoV M<sup>pro</sup>s bound to YH-53. Structural comparison of protomer A and protomer B of SARS-CoV-2 M<sup>pro</sup> (a), SARS-CoV M<sup>pro</sup> (b) and MERS-CoV M<sup>pro</sup> (c) bound to YH-53. Protomers are shown as cartoons, while ligands are shown as sticks.

SARS-CoV-2	1	10	20	30	40	50	60
SARS-CoV-2 Alpha variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2 Gamma variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2 Delta variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2 Beta variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2 Omicron variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2 Lambda variant	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
HCoV-HKU1	SGILVKMVS	PTSKIEP	CVSVTV	YGSMTLNG	LWLDV	KVYCPRHVICT	SSNMNEPDYSALLCR
HCoV-OC43	SGILVKMVS	PTSKIEP	CVSVTV	YGSMTLNG	LWLDV	KVYCPRHVICT	SSNMNEPDYSALLCR
MERS-CoV	SGILVKMVS	PTSKIEP	CVSVTV	YGSMTLNG	LWLDV	KVYCPRHVICT	SSNMNEPDYSALLCR
HCoV-229E	AGLRKMAQ	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
HCoV-NL63	SGFRKMAF	PSGKVEG	CMVQVT	CGTTTLNG	LWLDV	VYCPRHVICT	SEDMLNPNYEDLLIR
SARS-CoV-2	70	80	90	100	110		
SARS-CoV-2 Alpha variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV-2 Gamma variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV-2 Delta variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV-2 Beta variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV-2 Omicron variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV-2 Lambda variant	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
SARS-CoV	KSNHNF	LVQAGN	...VQLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
HCoV-HKU1	VTLDG	FTIMSGR	...MSLTIVSY	QMOGCOLV	TVSLQNP	YTPKYK	FGVVKPGET
HCoV-OC43	VTSSD	FTVLFDR	...LSLTIVSY	QMOGCOLV	TVSLQNP	YTPKYK	FGVVKPGET
MERS-CoV	MTNHS	FVQKHIGAP	ANLRVIGH	SMQNCVLK	KLKVD	TANPKTPKYK	FVRIQPGQTF
HCoV-229E	MRLHNF	SIISGT	...AFILGVVG	ATMHGVT	LKIKVS	QSNVHTPK	KHVFKTLKPGD
HCoV-NL63	MRLHNF	SIISGT	...AFILGVVG	ATMHGVT	LKIKVS	QSNVHTPK	KHVFKTLKPGD
SARS-CoV-2	120	130	140	150	160	170	
SARS-CoV-2 Alpha variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV-2 Gamma variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV-2 Delta variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV-2 Beta variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV-2 Omicron variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV-2 Lambda variant	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
SARS-CoV	YNGSP	SGVYQCA	MRPNFTIK	GSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
HCoV-HKU1	YNGRP	QGAHVT	MRSSYTI	KGSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
HCoV-OC43	YNGRP	QGAHVT	MRSSYTI	KGSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
MERS-CoV	YNGRP	QGAHVT	MRSSYTI	KGSFLNGS	CGSVGFNI	DY	DCVSTFCYMH
HCoV-229E	YDCA	QGVFGVN	MRTNWT	IRGSFING	ACGSB	GYNLKN	GEVEFVYMH
HCoV-NL63	YDCA	QGVFGVN	MRTNWT	IRGSFING	ACGSB	GYNLKN	GEVEFVYMH
SARS-CoV-2	180	190	200	210	220	230	
SARS-CoV-2 Alpha variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV-2 Gamma variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV-2 Delta variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV-2 Beta variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV-2 Omicron variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV-2 Lambda variant	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
SARS-CoV	LEGNF	YGPFFV	DRQTAQA	AGTDTT	ITVNV	LAWLYAAV	INGDRWFLN
HCoV-HKU1	FTGNF	YGPYRDA	QVQVLP	VKDYQ	TVNV	LAWLYAAV	INGDRWFLN
HCoV-OC43	FNGDF	YGPYKDA	QVQVLP	IQDYI	QSVNF	LAWLYAAV	INGDRWFLN
MERS-CoV	FDGTM	YGA	FMDKQV	HQVQL	TDKY	CSNV	VAVLYAAV
HCoV-229E	FDGVM	YGFED	QPNLO	VESAN	LM	LSDNV	VAVLYAAV
HCoV-NL63	FTGSV	YGNF	DOP	SLQV	ESAN	LM	LSDNV
SARS-CoV-2	240	250	260	270	280	290	
SARS-CoV-2 Alpha variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV-2 Gamma variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV-2 Delta variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV-2 Beta variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV-2 Omicron variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV-2 Lambda variant	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
SARS-CoV	YNYEP	LTQDH	VDI	LGPLSA	QTGIA	VLD	MCA
HCoV-HKU1	NGFSQ	VKADLV	..	LDALAS	MTGVS	IETLLAA	IKR
HCoV-OC43	NGFSQ	VKADLV	..	LDALAS	MTGVS	IETLLAA	IKR
MERS-CoV	NQFTE	FVGTQS	..	VDM	LAVK	TGVA	IEQLLYAI
HCoV-229E	NGFTAM	NGEDA	..	FSI	LAAK	TGV	VERLLHAI
HCoV-NL63	NGYTS	VSSVE	..	YSI	LAAK	TGV	VERLLHAI
SARS-CoV-2	300						
SARS-CoV-2 Alpha variant	VRQCS	GVT	FQ				
SARS-CoV-2 Gamma variant	VRQCS	GVT	FQ				
SARS-CoV-2 Delta variant	VRQCS	GVT	FQ				
SARS-CoV-2 Beta variant	VRQCS	GVT	FQ				
SARS-CoV-2 Omicron variant	VRQCS	GVT	FQ				
SARS-CoV-2 Lambda variant	VRQCS	GVT	FQ				
SARS-CoV	VRQCS	GVT	FQ				
HCoV-HKU1	YQQLAG	VKL	Q				
HCoV-OC43	YQQLAG	VKL	Q				
MERS-CoV	NMIM	GVM	Q				
HCoV-229E	VKMF	GVL	Q				
HCoV-NL63	VKMF	GVL	Q				

**Figure S4.** Sequence alignment of M<sup>pro</sup>s from different coronaviruses. The M<sup>pro</sup> amino acid sequences of SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, SARS-CoV-2 Alpha variant, SARS-CoV-2 Beta variant, SARS-CoV-2 Gamma variant, SARS-CoV-2 Delta variant, SARS-CoV-2 Lambda variant, and SARS-CoV-2 Omicron variant were retrieved from Genbank or GISAID. ClustalX (1.83) software was used for sequence alignment and ESPrpt 3.0 was used to generate a graphical representation. The key residues involved in interacting with inhibitors were labeled with blue dashed boxes.