

1 **Supplementary Table 1. PK dataset of compounds 1A, 1B, 2 and 3.**

Preclinical PK Properties										
Compound	1A		1B		2		3			
Species	Mouse		Mouse		Mouse		Mouse		Dog	
PK Parameters	IV n=3 5 mpk	PO n=3 20 mpk	IV n=3 1 mpk	PO n=3 20 mpk	IV n=3 1 mpk	PO n=3 100 mpk	IV n=3 1 mpk	PO n=3 100 mpk	IV n=6 2 mpk	PO n=6 20 mpk
C _{max} (ng/mL)	-	205 ± 59	-	1219 ± 263	-	1845 ± 448	-	9498 ± 4281	-	2040 ± 608
Vd _{ss} (L/kg)	1.61 ± 0.27	-	7.61 ± 1.28	-	4.41 ± 0.55	-	1.37 ± 0.10	-	1.83 ± 0.43	-
CL (mL/min/kg)	112 ± 23	-	171 ± 8	-	80 ± 4	-	150 ± 7	-	36 ± 7	-
T _{max} (h)	-	0.25 ± 0.00	-	0.25 ± 0.00	-	0.50 ± 0.00	-	0.30 ± 0.00	-	0.88 ± 0.63
T1/2 (h)	0.21 ± 0.03	0.80 ± 0.09	0.64 ± 0.15	1.07 ± 0.18	0.64 ± 0.11	1.90 ± 0.20	0.13 ± 0.01	0.60 ± 0.22	0.98 ± 0.32	1.60 ± 1.05
AUC _{0-last} (ng.h/mL)	646 ± 120	225 ± 103	95 ± 5	1938 ± 275	131 ± 6	8168 ± 1951	105 ± 6	7522 ± 1850	969 ± 238	4710 ± 1740
AUC _{0-inf} (ng.h/mL)	646 ± 120	232 ± 103	98 ± 4	1952 ± 269	134 ± 6	15833 ± 10853	111 ± 5	7535 ± 1848	973 ± 241	4720 ± 1740
Bioavailability (%)	-	9	-	101.68	-	75.8	-	72.0	-	49.0

2 IV = intravenous, PO = oral administration. Data are presented as mean values ±

3 SD. Source data are provided as a Source Data file.

4

5

6

7 Supplementary Table 2. Single-dose (10 µM) protease profiling of ISM3312

No	Target	% Enzyme Activity (relative to DMSO controls)		Control Compound IC50 (M)	Control compound ID
		Activity% 1	Activity% 2		
1	ACE1	102.98	103.75	2.30E-07	Captopril
2	ACE2	103.96	103.8	9.58E-10	ACE 2 inhibitor
3	Activated Protein C	99.25	98.75	1.57E-07	Gabexate mesylate (GM)
4	ADAM10	102.62	99.32	7.76E-07	GM6001
5	BACE1	115.04	112.32	1.23E-07	B-Secretase inhibitor IV
6	Calpain 1	95.67	93.68	4.16E-08	E64
7	Caspase 1	96.27	95.28	2.76E-08	IETD-CHO
8	Caspase 2	97.75	96.82	2.48E-07	IETD-CHO
9	Caspase 3	108.69	105.8	1.78E-09	DEVD-CHO
10	Caspase 4	95.65	95.68	1.68E-06	IETD-CHO
11	Caspase 5	91.93	93.78	1.30E-08	IETD-CHO
12	Caspase 6	101.86	102.24	5.10E-08	DEVD-CHO
13	Caspase 7	110	107.2	2.62E-09	DEVD-CHO
14	Caspase 8	97.52	96.23	2.71E-09	IETD-CHO
15	Caspase 9	91.42	94.44	4.48E-08	IETD-CHO
16	Caspase 10	103.38	102.8	9.27E-09	IETD-CHO
17	Caspase 11	93.62	94.9	1.67E-07	IETD-CHO
18	Caspase 14	99.68	99.52	7.27E-08	WEHD-CHO
19	Cathepsin B	106.64	106.38	7.28E-09	E64
20	Cathepsin C	98.78	98.4	9.60E-07	E64
21	Cathepsin D	102.79	99.24	7.29E-11	Pepstatin A
22	Cathepsin E	104.89	104.09	3.47E-11	Pepstatin A
23	Cathepsin G	102.48	102.14	4.19E-06	Chymostatin
24	Cathepsin H	103.87	105.59	3.37E-08	E64
25	Cathepsin K	120.35	130.37	1.45E-09	E64
26	Cathepsin L	96.1	98.51	2.21E-09	E64
27	Cathepsin S	107.59	103.55	1.78E-09	E64
28	Cathepsin V	99.35	109.2	8.04E-09	E64
29	Chymase	92.02	93.09	7.77E-09	Chymostatin
30	Chymotrypsin	96.26	97.12	1.02E-09	Chymostatin
31	DPP IV	95.63	95.78	7.63E-08	P32/98
32	DPP VIII	101.77	103.09	6.95E-07	P32/98
33	DPP IX	98.66	97.58	3.62E-07	P32/98
34	Elastase	102.84	102.24	1.06E-08	Sivelestat
35	Factor VIIa	100.4	98.73	3.50E-08	PCI 27483
36	Factor IXa	99.19	93.29	8.60E-08	Nafamostat mesylate
37	Factor Xa	99.35	97.81	7.90E-07	Gabexate mesylate (GM)
38	Factor Xla	103.63	104.06	1.87E-07	Gabexate mesylate (GM)
39	Factor XIIa	102.04	101.32	3.26E-08	Nafamostat mesylate
40	Furin	99.08	100.37	2.96E-09	Furin Inhibitor I
41	Granzyme B	105.33	106.7	1.85E-07	IETD-CHO
42	HIV-1	127.86	120.04	1.24E-05	Pepstatin A
43	Kallikrein 1	106.01	105.75	3.90E-06	Leupeptin
44	Kallikrein 2	103.11	102.65	4.26E-07	Gabexate mesylate (GM)
45	Kallikrein 5	100.2	99.75	8.29E-06	Gabexate mesylate (GM)
46	Kallikrein 7	101.93	99.41	1.56E-05	Gabexate mesylate (GM)
47	Kallikrein 12	103.88	103.07	3.57E-08	Gabexate mesylate (GM)
48	Kallikrein 13	103.66	104.44	1.22E-05	Gabexate mesylate (GM)
49	Kallikrein 14	103.8	101.73	6.41E-07	Gabexate mesylate (GM)
50	Matriptase 2	109.79	110.98	4.12E-07	Gabexate mesylate (GM)
51	MMP 1	99.45	99.62	4.18E-10	GM6001
52	MMP 2	99.43	93.11	2.22E-10	GM6001
53	MMP 3	99.98	101	1.25E-08	GM6001
54	MMP 7	98.45	100.57	2.94E-09	GM6001
55	MMP 8	105.87	105.89	8.76E-10	GM6001
56	MMP 9	101.94	99.91	3.39E-10	GM6001
57	MMP 10	102.08	101.55	9.78E-09	GM6001
58	MMP 12	101.92	100.01	3.28E-10	GM6001
59	MMP 13	102.04	105.81	1.29E-10	GM6001
60	MMP 14	98.85	96.06	1.37E-09	GM6001
61	Nepriylsin	103.29	103.99	5.75E-07	Phosphoramidon
62	Papain	103.49	114.56	1.59E-10	E64
63	Plasma Kallikrein	102.72	102.5	8.64E-08	Gabexate mesylate (GM)
64	Plasmin	100.08	101.42	3.16E-07	Gabexate mesylate (GM)
65	Proteinase A	104.43	105.27	1.19E-04	Leupeptin
66	Proteinase K	101.07	99.62	3.59E-07	Proteinase K inhibitor
67	SARS-CoV2-Mpro	0.28	-0.24	1.03E-08	GC376
68	SARS-CoV2-Plpro	98.24	100.61	1.65E-06	Plpro inhibitor
69	TACE	102.08	101.44	3.84E-08	GM6001
70	Thrombin a	98.71	100.46	5.88E-07	Gabexate mesylate (GM)
71	TMPSRS2	101.48	100.24	1.07E-09	Camostat mesylate
72	TMPSRS11D	102.99	102.42	2.08E-08	Camostat mesylate
73	Trypsin	92.61	96.82	8.88E-09	Gabexate mesylate (GM)
74	Trypsin beta II	100.63	103.81	4.28E-09	Gabexate mesylate (GM)
75	Trypsin gamma I	110.77	111.66	1.99E-06	Gabexate mesylate (GM)
76	Urokinase	97.59	99.54	2.36E-08	Gabexate mesylate (GM)

9 **Supplementary Table 3. Data collection and refinement statistics of M^{pro} crystal**
10 **structures.**

	M ^{pro} + ISM3312 (PDB: 8WTS)
Data collection	
Space group	C 2
Cell dimensions	
a, b, c (Å)	98.46 80.98 51.34
a, b, g (°)	90.00 115.69 90.00
Resolution (Å)	46.27 (1.56) *
R _{merge}	0.065 (0.832)
I / σI	12.9 (2.1)
Completeness (%)	97.4 (95.8)
Redundancy	6.8 (6.9)
CC*	0.999(0.949)
CC _{1/2}	0.996 (0.818)
Refinement	
Resolution (Å)	1.56
No. reflections	51607
R _{work} / R _{free}	0.179/0.207
No. atoms	
Protein	2400
Ligand/ion	45
Water	251
B-factors	
Protein	28.96
Ligand/ion	35.55
Water	38.54
R.m.s. deviations	
Bond lengths (Å)	0.015
Bond angles (°)	1.97

11 Source data are provided as a Source Data file.

12

13

14 **Supplementary Table 4. 50% cytotoxic concentration (CC₅₀) of cell lines and**
15 **human proximal airway organoid**

Drug	Vero E6 (μM, SD)	Huh7 (μM, SD)	2D human proximal airway organoid (μM, SD)
ISM3312	86.8±6.3	140±4.5	> 1000
Nirmatrelvir	249.9±48.6	> 1000	
Ensitrelvir	92.4±1.8	335.6±79.2	
ISM3312 + Elacridar	93.0±5.7		
Nirmatrelvir + Elacridar	189.9±25.7		
Ensitrelvir + Elacridar	107.9±9.8		

16
17
18
19
20

21 **Supplementary Table 5. Selection index (SI) values of ISM3312, Nirmatrelvir,**
22 **Ensitrelvir against various SARS-CoV-2 strains**

SI	ISM3312	Nirmatrelvir	Ensitrelvir	ISM3312 + Elacridar	Nirmatrelvir + Elacridar	Ensitrelvir + Elacridar
WT	873.9±651.8	340.0±160.8	748.8±338.4	1007.3±636.5	15995.0±6525.5	2621.1±760.9
Delta	1309.2±773.5	334.3±89.1	571.0±159.4	1755.3±1209.5	15995.0±6525.5	1860.5±325.1
BA.2.3	1587.2±639.0	198.3±92.0	397.3±93.7	1380.7±314.5	12343.3±4217.5	1245.5±248.6
BA.5	2656.9±495.1	412.1±297.0	713.6±53.2	2599.4±597.6	15341.7±3826.1	2046.2±809.8
XBB.1	2295.6±761.1	312.5±224.5	1168.4±159.2	4132.2±924.5	9496.7±1283.3	3596.7±326.3
EG.5	1092.9±112.0	152.2±33.1	362.9±212.2	2170.0±296.3	7280.0±1271.8	2025.8±52.2

23 Data are presented as mean values ± SD.

24

25

26

27

28

29

30 **Supplementary Table 6. M^{pro} Enzymatic Assay Conditions for Human**
 31 **Coronaviruses**

32
 33
 34
 35

Coronavirus	Protease (nM)	Substrate (μM)	Peptide Sequence
SARS-CoV-2 WT	25	25	Dabcyl-KTSAVLQSGFRKM-(Edans)
SARS-CoV-2 Omicron	25	25	Dabcyl-KTSAVLQSGFRKM-(Edans)
229E	50	12.5	Dabcyl-YGSTLQAGLRKM-(Edans)
OC43	25	12.5	Dabcyl-KTSAVLQSGFRKM-(Edans)
SARS-CoV-1	25	25	Dabcyl-KTSAVLQSGFRKM-(Edans)
MERS	100	25	Dabcyl-KTSAVLQSGFRKM-(Edans)
NL63	50	12.5	Dabcyl-YNSTLQSGLKKM-(Edans)
HKU1	12.5	12.5	Dabcyl-KTSAVLQSGFRKM-(Edans)

36 **Supplementary Table 7. FRET Assay Conditions for SARS-CoV-2 M^{pro} Mutants**

SARS-CoV-2 M ^{pro} mutants	Protease (nM)	Substrate (μM)	Reaction time (min)
T21I	25	6.25	30
L50F	25	6.25	60
P252L	25	6.25	50
T21I+L50F	25	6.25	30
50 [^] NP+P252L	25	12.5	30
L50F+P252L	25	6.25	30
Y54A	100	25	120
N133H	100	25	120
F140A	25	12.5	60
E166A	100	12.5	120
E166V	100	25	120
L167F	50	12.5	120
H172Y	100	25	120
L50F+E166A+L67F	100	25	120

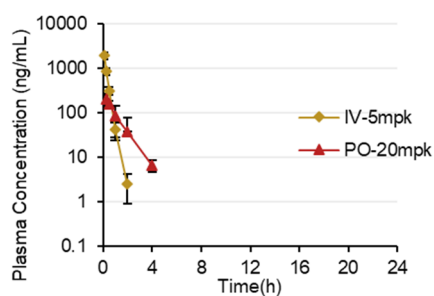
37

38

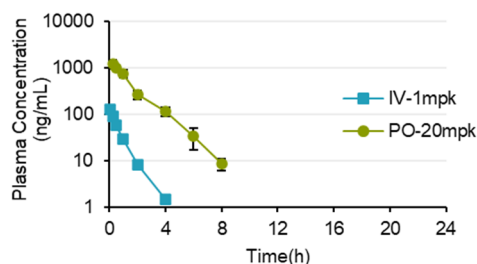
39 **Supplementary Table 8. Primers used for quantitative PCR analysis of**
40 **coronavirus**

Primer	Sequence
SARS-CoV-2	The testing kit was purchased from DaAn Gene (Cat: DA0930)
MERS E-Forward	5'-GCAACGCGCGATTTCAGTT-3'
MERS E-Reverse	5'-GCCTCTACACGGGACCCATA-3'
MERS E- FAM probe	5'-FAM-CTCTTCACATAATCGCCCCGAGCTCG-3'-BHQ1
229E-N-Forward	5'-CGCAAGAATTCAGAACCAGAG-3'
229E-N-Reverse	5'-GGGAGTCAGGTTCTTCAACAA-3'
229E-N-FAM probe	5'-CCACACTTCAATCAAAAGCTCCCAAATG-3'-BHQ1
NL63-N-Forward	5'-AGGACCTTAAATTCAGACAACGTTCT -3'
NL63-N-Reverse	5'-GATTACGTTTGCGATTAC CAAGACT-3'
NL63-N-FAM probe	5'-FAM-TAACAGTTTTAGCACCTTCCTTAGCAACCCAAACA-3'-BHQ1
OC43-N-Forward	5'-CGATGAGGCTATTCCGACTAGGT-3'
OC43-N-Reverse	5'-CCTTCCTGAGCCTTCAATATAGTAACC-3'
OC43-N-FAM probe	5'-FAM-TCCGCCTGGCACGGTACTCCCT-3'-BHQ1
HKU1-RDRP-Forward	5'-CTTGAGCACATTCATTGCAAG-3'
HKU1-RDRP-Reverse	5'-GGTTGGGATTATCCTAAATGTGA-3'

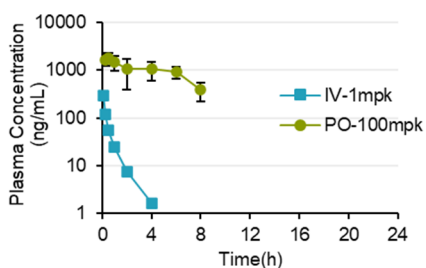
a. PK Curve of Compound **1A** in Mouse



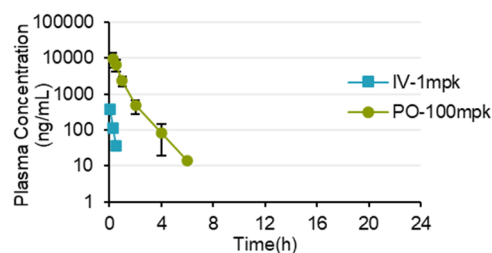
b. PK Curve of Compound **1B** in Mouse



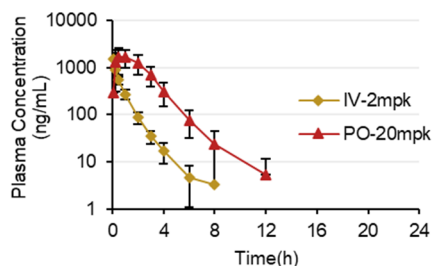
c. PK Curve of Compound **2** in Mouse



d. PK Curve of Compound **3** in Mouse



e. PK Curve of Compound **3** in Dog



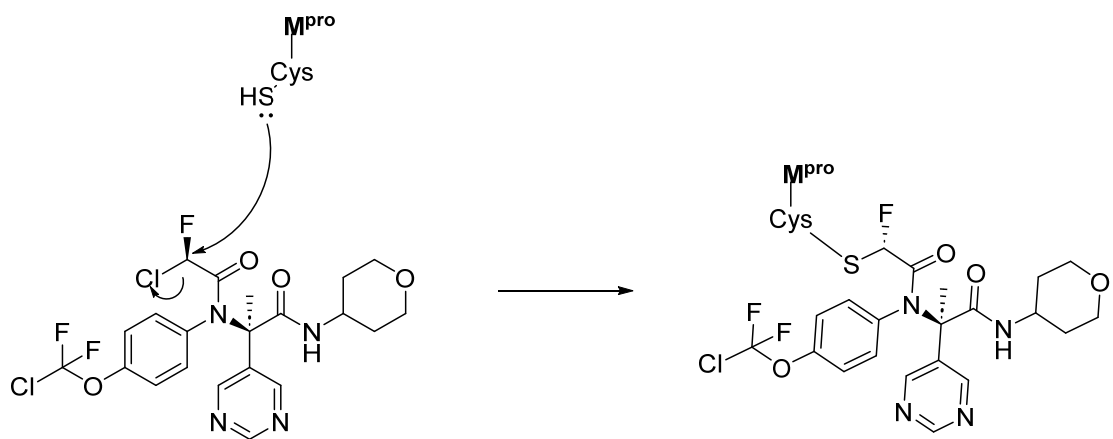
47

48 **Supplementary Fig. 1. PK curve of compounds 1A, 1B, 2 and 3.** a. PK Curve of
 49 Compound **1A** in Mouse. b. PK Curve of Compound **1B** in Mouse. c. PK Curve of
 50 Compound **2** in Mouse. d. PK Curve of Compound **3** in Mouse. e. PK Curve of
 51 Compound **3** in Dog. N=3 both for mouse IV and PO PK; N=6 both for dog IV and PO
 52 PK. Data are presented as mean values $\pm$ SD. Source data are provided as a Source
 53 Data file.

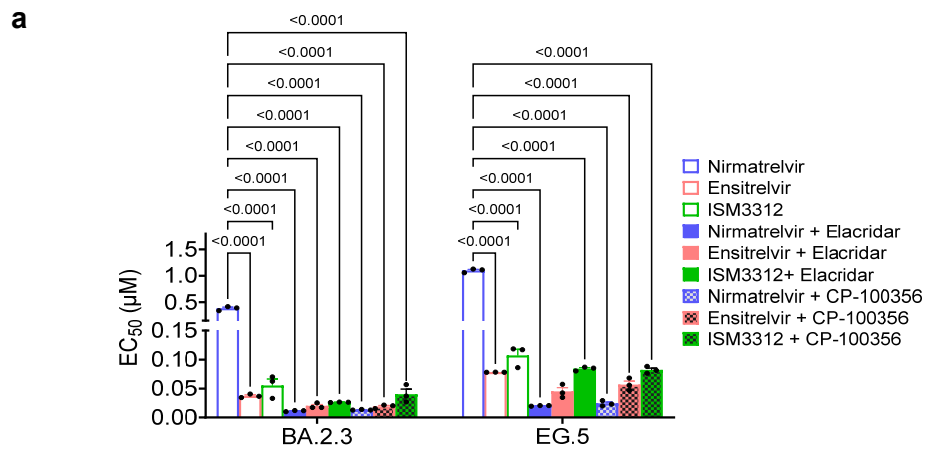
54

55

56



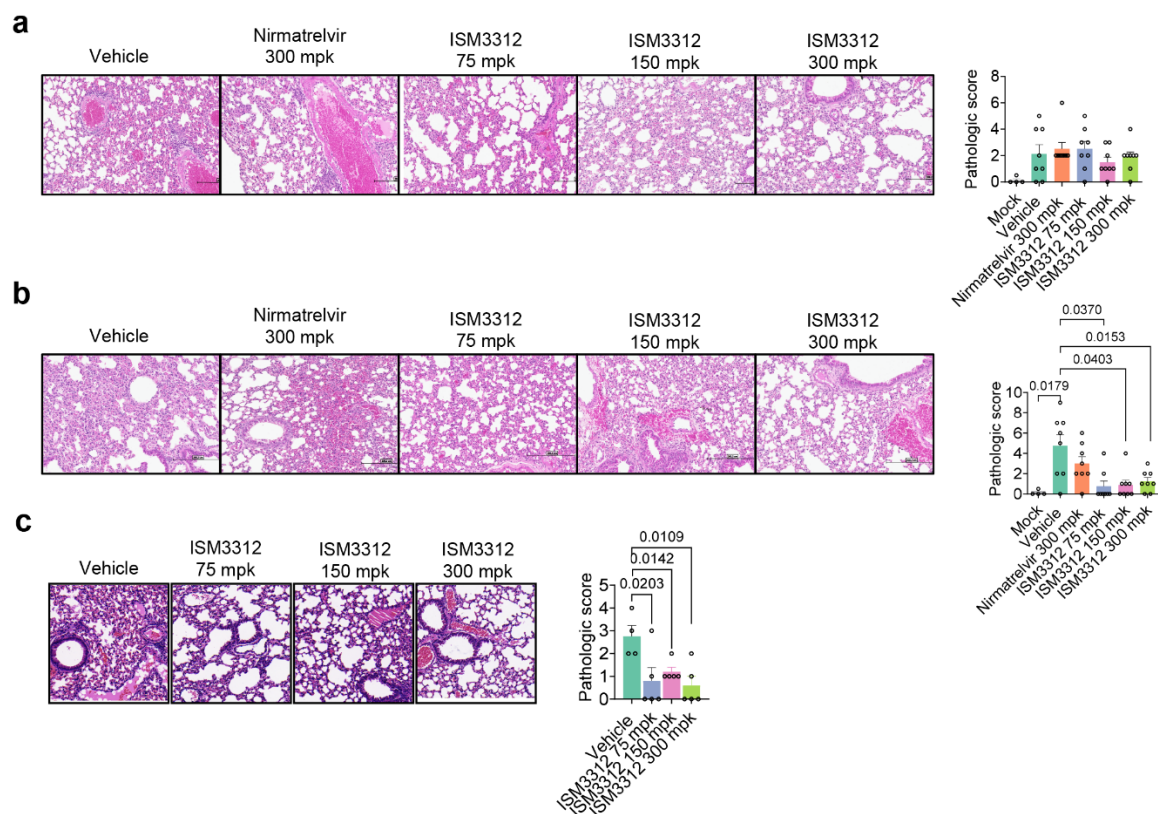
Supplementary Fig. 2. A scheme for covalent inhibition mechanism. The cystine of M^{pro} undergoes SN_2 attack to the CFA warhead of ISM3312, with the chlorine as leaving group and covalently binds with ISM3312.



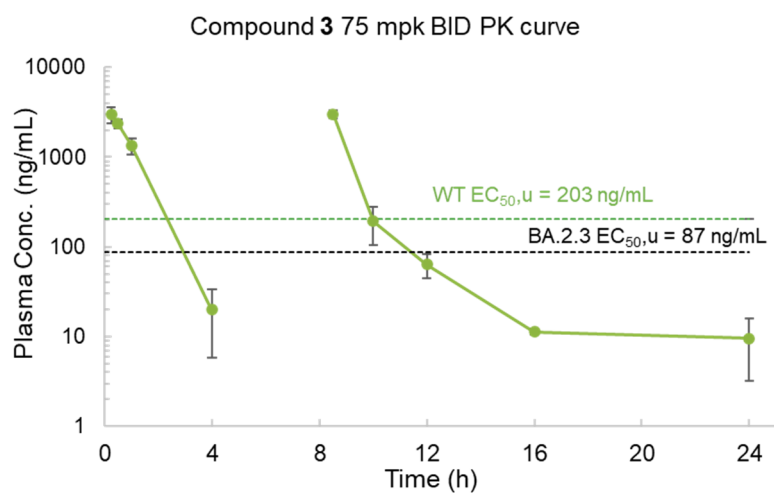
b

Group	EC ₅₀ (μM)	
	BA.2.3	EG.5
Nirmatrelvir	0.38	1.1
Ensitrelvir	0.04	0.08
ISM3312	0.06	0.11
Nirmatrelvir + Elacridar	0.01	0.02
Ensitrelvir + Elacridar	0.02	0.04
ISM3312 + Elacridar	0.03	0.08
Nirmatrelvir + CP-100356	0.01	0.02
Ensitrelvir + CP-100356	0.02	0.06
ISM3312 + CP-100356	0.04	0.08

Supplementary Fig. 3. Comparison of antiviral effects ISM3312, Nirmatrelvir, Ensitrelvir combined with Elacridar or CP-100356 against SARS-CoV-2. a. Comparison of EC₅₀ of ISM3312, Nirmatrelvir, Ensitrelvir combined with Elacridar or CP-100356 against SARS-CoV-2 BA.2.3 or EG.5 by IFA. **b.** EC₅₀ values of respective compounds against infection with SARS-CoV-2 variants. Data are presented as mean values ± SEM. Data were analyzed by using two-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.



Supplementary Fig. 4. Lung pathology caused by SARS-CoV-2 and MERS-CoV infection. **a-b.** Presentative hematoxylin/eosin staining pictures and pathological scores from SARS-CoV-2 ancestral (**a**) and BA.2.3 (**b**) strain infected H11-K18-hACE2 C57BL/6 mouse lung (Scale bar= 200 μ m). **c.** Presentative hematoxylin/eosin staining pictures and pathological scores from MERS-CoV MA30 strain infected hDPP4 KI C57BL/6 mouse lung, magnification=200 $\times$. Data are presented as mean values $\pm$ SEM. Data was analyzed by using one-way ANOVA followed by Dunnett's T3 multiple comparisons test. Source data are provided as a Source Data file.



97

98 **Supplementary Fig. 5 75 mpk BID dosing of ISM3312 vs *in vitro* unbound EC_{50} of**

99 **two strains.** Female C57BL/6J mice were dosed 75 mpk BID ISM3312 (totally 4 days,

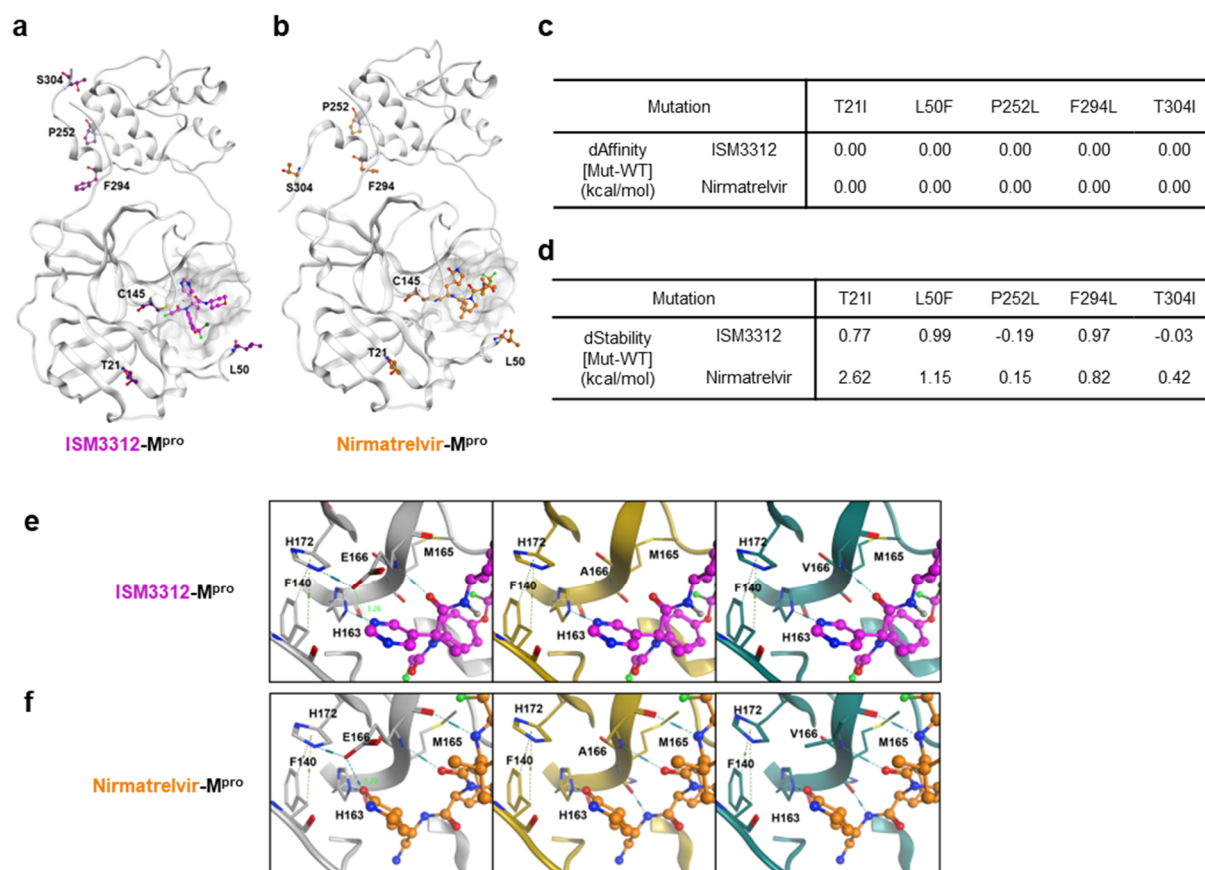
100 8 doses) and the plasma concentration was compared with *in vitro* antiviral activity of

101 two strains. N=3. Data are presented as mean values $\pm$ SD. Source data are provided

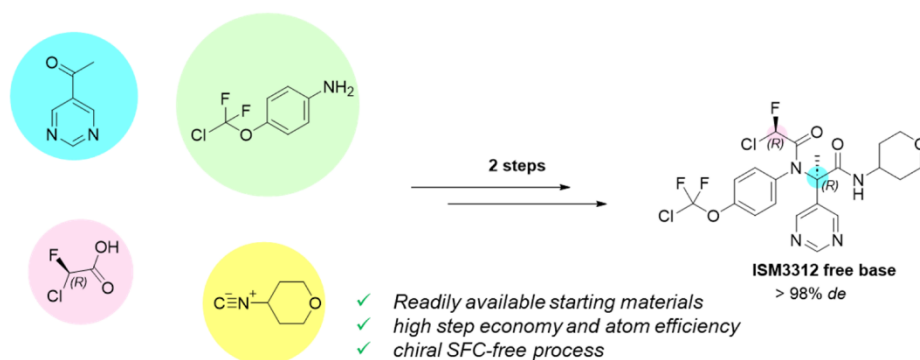
102 as a Source Data file.

103

104



Supplementary Fig. 6. The key mutated residues and virtual mutations. a-b. The key mutated residues (T21, L50F, P252, F294 and T304) are far away from ligand-bound active sites. **c.** The dAffinity values for 5 mutations, including T21I, L50F, P252L, F294L, T304I. **d.** The dStability values for 5 mutations, including T21I, L50F, P252L, F294L, T304I. The key residues near E166, A166, and V166 in ISM3312-M^{pro} complex (**e**) and Nirmatrelvir-M^{pro} complex (**f**).



Supplementary Fig. 7. Key features of ISM3312 manufacturing process. ISM3312

was successfully synthesized from the bench scale to the kilogram scale from cheap starting materials in high step economy and atom efficiency in an overall yield of 15% with excellent chemical (98.1%) and optical purity (>98% de) without chromatographic purification and chiral SFC.