In Vitro and *in Vivo* Antiviral Activity of S-892216, a Second Generation Oral 3CL^{pro} Inhibitor against SARS-CoV-2

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Background and Purpose

COVID-19 caused by SARS-CoV-2 remains a global public health concern. Although oral direct-acting antivirals for COVID-19 (such as molnupiravir, nirmatrelvir/ritonavir, ensitrelvir [approved in Japan and Singapore]) were approved for clinical use, there are concerns about drug-drug interactions (DDI) and patient eligibility, so development of new therapeutics is needed. In this study, we describe enzyme inhibitory and antiviral activity of S-892216, a second generation small molecular 3C-like protease (3CL^{pro}) inhibitor.

Methods

The 3CL^{pro} enzymatic assay was conducted by mass spectrometry system. *In vitro* antiviral activity was evaluated using VeroE6/TMPRSS2 cells and human airway epithelial cells (hAEC) following infection with several SARS-CoV-2 variants. *In vivo* efficacy was evaluated using Balb/c mice, intranasally infected with SARS-CoV-2, and S-892216 was orally administered.

Results

S-892216 showed high 3CL^{pro} inhibitory activity (IC_{50} = 0.655 nmol/L) and exhibited *in vitro* antiviral activity against several SARS-CoV-2 strains, including Omicron variants (EC_{50} = 2.27-12.5 nmol/L in VeroE6/TMPRSS2 cells, EC_{90} = 2.31-2.41 nmol/L in hAECs). Furthermore, S-892216 suppressed lung virus titer in Balb/c mice infected with SARS-CoV-2 in a dose-dependent manner.

Table 1 3CL^{pro} inhibitory activity

	IC_{50} (nmol/L)		
	S-892216	Ensitrelvir	Nirmatrelvir
wild-type	0.655 ± 0.062	14.3 ± 1.3	13.3 ± 0.3
P132H	0.767 ± 0.041	16.5 ± 1.1	11.6 ± 1.1

Mean±SD (n=3)

Table 2 Selectivity of S-892216 against mammalian and HIV protease

Protease	IC_{50} (nmol/L)
Human Caspase 2	>10,000
Human Chymotrypsin	>10,000
Human Cathepsin B/D/G/L	>10,000
Human Thrombin	>10,000
HIV-1 Protease	>10,000

Table 3 Anti-SARS-CoV-2 activity against various variants in VeroE6/TMPRSS2 cells

SARS-CoV-2		EC_{50} (nmol/L)		
		S-892216	Ensitrelvir	Remdesivir
Ancestral	A	8.77 ± 1.92	345 ± 102	1910 ± 140
Gamma	P.1	3.85 ± 1.41	316 ± 38	2140 ± 390
Delta	B.1.617.2	4.91 ± 1.29	338 ± 46	1550 ± 220
Omicron	BA.1.1	2.63 ± 0.18	136 ± 16	1010 ± 50
	BA.2.12.1	2.27 ± 0.26	198 ± 75	495 ± 198
	BE.1 (BA.5-like)	6.54 ± 3.49	259 ± 66	1250 ± 540
	XBB.1.5.19	7.40 ± 1.08	570 ± 74	1040 ± 180
	XBB.1.9.1	12.5 ± 0.9	986 ± 111	3020 ± 310

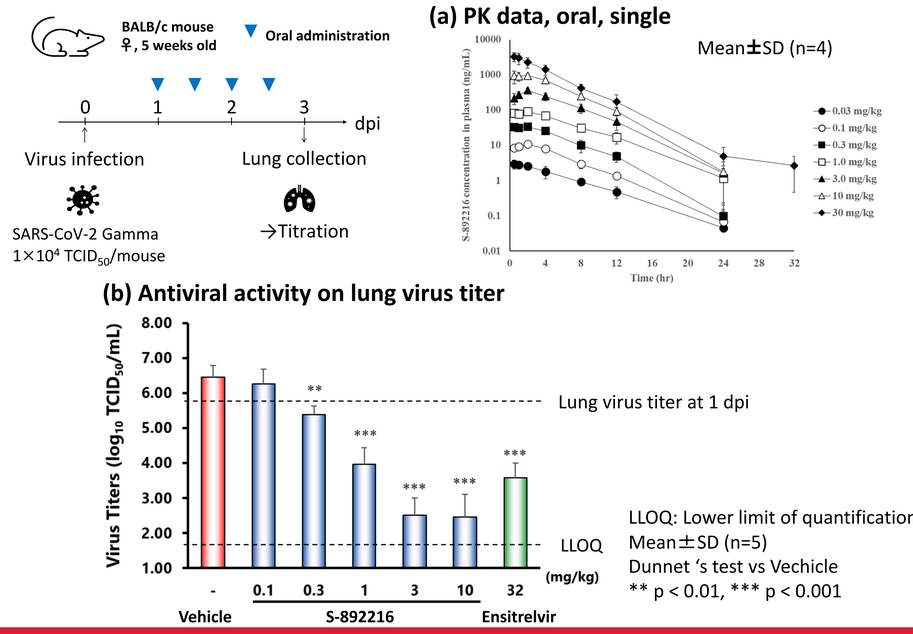
Mean±SD (n=3)

Table 4 Anti-SARS-CoV-2 activity in various cells

Cells	SARS-CoV-2			S-892216	Ensitrelvir	Nirmatrelvir	Remdesivir	NHC*2
VeroE6/TMPRSS2 cell	Ancestral	A	EC_{50} (nmol/L)	8.77 ± 1.92	345 ± 102	4880 ± 2030	1910 ± 140	360 ± 49*3
VeroE6/TMPRSS2 cell with P-gp inhibitor*1	Ancestral	A	EC_{50} (nmol/L)	3.36 ± 0.42	94.9 ± 38.6	68.1 ± 16.2	56.5 ± 15.0	677 ± 9*3
A549/ACE2-TMPRSS2 cell	Delta	B.1.617.2	EC_{50} (nmol/L)	2.21 ± 0.63	73.1 ± 16.4	47.5 ± 12.3	51.7 ± 22.4	2300 ± 330
Human airway epithelial cells (hAEC) MucilAir™ nasal	Omicron	BE.1 (BA.5-like)	EC_{90} (nmol/L)	2.41 ± 1.61	60.1 ± 32.8	45.1 ± 21.2	21.1 ± 9.0*4	3660 ± 920*4
		XBB.1.5.19		2.31 ± 1.28	78.4 ± 36.1	75.2 ± 20.1		

Mean±SD (n=3)
*1 0.75 μmol/L CP-100356
*2 NHC: Parent compound of molnupiravir
*3 Delta, Ref 1)
*4 Omicron BA.1, Ref 2)

Figure 1 In vivo efficacy of S-892216 in a mouse infected model



Conclusions

S-892216 has stronger 3CL^{pro} inhibitory and antiviral activity than approved 3CL^{pro} inhibitors and has been confirmed to be effective *in vivo* without the need of a pharmacoenhancer. Due to the strong antiviral activity of S-892216, it is suggested to be effective at low doses in clinical settings. DDI and safety will be evaluated in clinical trials.

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Reference

1) M. Sasaki et al, *Sci Transl Med*. 2023; 15 (679), eabq4064
2) T. Kuroda et al, *J Antimicrob Chemother.* 2023, 78 (4), 946-952.