ゾコーバ錠 125mg

第 2 部 (モジュール 2) : CTD の概要 (サマリー)

2.6 非臨床試験の概要文及び概要表

2.6.3 薬理試験概要表

塩野義製薬株式会社

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略号及び用語定義一覧表

略号	英語
3CL	3C-like
ACE2	angiotensin-converting enzyme 2
CPE	cytopathic effect
DMSO	dimethyl sulfoxide
$EC_{50\,(90)}$	50% (90%) effective concentration
FC	fold change
GISAID	Global Initiative on Sharing All Influenza Data
hAEC	human airway epithelial cells
IC_{50}	50% inhibitory concentration
MC	methylcellulose aqueous solution
nsp5	non-structual protein 5
RT-PCR	reverse transcription polymerase chain reaction
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SD	standard deviation
$TCID_{50}$	50% tissue culture infectious dose
TMPRSS2	transmembrane protease, serine 2

2.6.3 薬理試験概要表

2.6.3.1 薬理試験：一覧表

Test Article: Ensitrelvir fumaric acid

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in CTD
Primary Pharmacodynamics					
Inhibitory Effect of Ensitrelvir on Cytopathic Effect in SARS-CoV-2 Infected Cells	Virus: hCoV-19/USA/MI-UM-10052670540/2023 hCoV-19/Japan/RB23-006-81/2023 hCoV-19/Japan/RB24-001-48/2024 Cell: VeroE6/TMPRSS2	In vitro	Shionogi & Co., Ltd.	S-217622-EB-219-N	4.2.1.1-01
Inhibitory Effect of Ensitrelvir on SARS-CoV-2 replication in Primary Human Nasal Epithelial Cells	Virus: hCoV-19/Japan/23-018/2022 Cell: MucilAir™ Nasal epithelial cells, Human	In vitro	Shionogi & Co., Ltd.	[redacted]-EB-086-N	4.2.1.1-02
Cytotoxicity of Ensitrelvir fumaric acid in Primary Human Nasal Epithelial Cells	Cell: MucilAir™ Nasal epithelial cells, Human	In vitro	Shionogi & Co., Ltd.	S-217622-EB-217-X	4.2.1.1-03
Sanger Sequencing Analysis to Investigate the Nsp5 Region of Reverse Genetics-derived SARS-CoV-2	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region With reporter gene ([redacted]) wild type, nsp5/M49L, S144A, M49L/S144A, D48G, P52S Without reporter gene wild type, nsp5/L87F, T198I, A234S, H246Y, T21I, E166A, H172Y, T21I+E166V, L50F+E166V, L50F, P252L, T25A, T25I, D48Y, M49I	-	Shionogi & Co., Ltd.	S-217622-EB-222-X	4.2.1.1-04
Drug Susceptibility Testing of Reverse Genetics-derived SARS-CoV-2 Mutants to Ensitrelvir in Cultured Cell (1)	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region T21I, T25A, T25I, D48Y, M49I, L50F, E166A, H172Y, P252L, T21I+E166V, L50F+E166V Cell: VeroE6/TMPRSS2	In vitro	Shionogi & Co., Ltd.	S-217622-EB-214-N	4.2.1.1-05

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in CTD
Drug Susceptibility Testing of Reverse Genetics-derived SARS-CoV-2 Mutants to Ensitrelvir in Cultured Cell (2)	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region T25N, F140L, L167F, A173V*, T304I, T21I+S144A, T21I+A173V, T21I+T304I, L50F+E166A, F140L+A173V, A173V+T304I, T21I+A173V+T304I, L50F+E166A+L167F Cell: VeroE6/TMPRSS2	In vitro	Shionogi & Co., Ltd.	S-217622-EB-221-N	4.2.1.1-06
Drug Susceptibility Testing of Reverse Genetics-derived SARS-CoV-2 Mutants to Ensitrelvir in Cultured Cell at [redacted]	Virus: Recombinant SARS-CoV-2 with NanoLuc reporter gene in place of ORF7a/b and with the following nsp5 mutations: T21I, L50F, E166V, A173V, P252L, T304I, T21I + S144A, T21I + E166V, T21I + A173V, T21I + T304I, L50F + E166V, T21I + A173V + T304I Cell: Huh7-ACE2	In vitro	[redacted]	S-217622-EF-194-N	4.2.1.1-07
Polymorphism of Amino Acid Associated with Ensitrelvir in SARS-CoV-2 3CL Protease	Database: GISAID	-	Shionogi & Co., Ltd.	S-217622-EB-215-X	4.2.1.1-08
Prophylactic Effect of Ensitrelvir against Lethal Infection with SARS-CoV-2 in Mice	Animal: Mouse Virus: SARS-CoV-2 MA-P10	Subcutaneous administration	Shionogi & Co., Ltd.	S-217622-EB-171-N	4.2.1.1-09
Inhibitory Effect of Prophylactic Treatment with Ensitrelvir on Virus Replication in Mice Infected with SARS-CoV-2	Animal: Mouse Virus: SARS-CoV-2 MA-P10	Subcutaneous administration	Shionogi & Co., Ltd.	S-217622-EB-189-N	4.2.1.1-10
Sanger Sequencing Analysis to Investigate the Nsp5 Region of Reverse Genetics-derived SARS-CoV-2 (2)	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region Without reporter gene wild type, nsp5/T25N, F140L, L167F, A173V*, T304I, T21I+S144A, T21I+A173V, T21I+T304I, L50F+E166A, F140L+A173V, A173V+T304I, T21I+A173V+T304I, L50F+E166A+L167F, M49L, D48G, P52S, S144A, M49L+S144A	-	Shionogi & Co., Ltd.	S-217622-EB-223-X	4.2.1.1-11
Inhibitory Effect of Ensitrelvir on Cytopathic Effect in SARS-CoV-2 Infected Cells (2)	Virus: hCoV-19/France/BRE-RELAB-IPP06405/2024 hCoV-19/France/IDF-RELAB-IPP09137/2024	In vitro	Shionogi & Co., Ltd.	S-217622-EB-231-N	4.2.1.1-12

Type of Study	Test System	Method of Administration	Testing Facility	Study Number	Location in CTD
	hCoV-19/Japan/TY/WK-521/2020 [reference strain] Cell: VeroE6/TMPRSS2				

3CL = 3C-like; GISAID = Global Initiative on Sharing All Influenza Data (https://www.gisaid.org/), nsp5 = non-structual protein 5, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, sfGFP= superfolder GFP, TMPRSS2 = transmembrane protease, serine 2
Amino acid substitutions are abbreviated as following.
A173V = alanine-to-valine at amino acid position 173, A234S = alanine-to-serine at amino acid position 234, D48G = aspartic acid-to-glycine at amino acid position 48, D48Y = aspartic acid-to-tyrosine at amino acid position 48, E166A = glutamic acid-to-alanine at amino acid position 166, E166V = glutamic acid-to-valine at amino acid position 166, F140L = phenylalanine-to-leucine at amino acid position 140, H172Y = histidine-to-tyrosine at amino acid position 172, H246Y = histidine-to-tyrosine at amino acid position 246, L50F = leucine-to-phenylalanine at amino acid position 50, L87F = leucine-to-phenylalanine at amino acid position 87, L167F = leucine-to-phenylalanine at amino acid position 167, M49I = methionine-to-isoleucine at amino acid position 49, M49L = methionine-to-leucine at amino acid position 49, P52S = proline-to-serine at amino acid position 52, P252L = proline-to-leucine at amino acid position 252, S144A = serine-to-alanine at amino acid position 144, T198I = threonine-to-isoleucine at amino acid position 198, T21I = threonine-to-isoleucine at amino acid position 21, T25A = threonine-to-alanine at amino acid position 25, T25I = threonine-to-isoleucine at amino acid position 25, T304I = threonine-to-isoleucine at amino acid position 304

* Sanger sequencing analysis (Study S-217622-EB-223-X [4.2.1.1-11]) revealed that T21T/I was introduced into the nsp5 sequence of the A173V virus.

2.6.3.2 効力を裏付ける試験

2.6.3.2.1 SARS-CoV-2 感染細胞に対する細胞変性抑制効果

Test Article: Ensitrelvir fumaric acid

Species/Biological material	VeroE6/TMPRSS2 cells infected with SARS-CoV-2 clinical isolate (hCoV-19/USA/MI-UM-10052670540/2023 [Pango Lineage: BA.2.86], hCoV-19/Japan/RB23-006-81/2023 [Pango Lineage: EG.5.1], hCoV-19/Japan/RB24-001-48/2024 [Pango Lineage: JN.1])
Final concentration as free form (μmol/L)	Viral replication inhibition assay: 0.00457, 0.0137, 0.0412, 0.123, 0.370, 1.11, 3.33, 10.0
Reference substance (Final concentration [μmol/L])	Remdesivir Viral replication inhibition assay: 0.00914, 0.0274, 0.0823, 0.247, 0.741, 2.22, 6.67, 20.0
Vehicle	0.5% DMSO
Evaluation Items	EC_{50}: The concentration achieving 50% of inhibition on CPE induced by SARS-CoV-2
Evaluation Method	Viral replication inhibition assay: One hundred microliters of serially diluted substance solutions were added to a 96-well plate. SARS-CoV-2 virus stock solutions were diluted to appropriate concentrations in advance, and each 50 μL/well of diluted SARS-CoV-2 solution was dispensed on the 96-well plate containing substance solutions. The infectious dose of virus was 300 $TCID_{50}$/well. Then, each 50 μL/well of VeroE6/TMPRSS2 cells which were adjusted to 3.00×10^5 cells/mL was dispensed on the plate. The plate was mixed with a plate mixer and was incubated at 37°C, 5% CO_2 for 4 days. The inhibitory effects of the test or reference substance on CPE induced by SARS-CoV-2 were measured by cell viability. Measurement of cell viability: After 4 days incubation of the plate prepared in the viral replication inhibition assay, 60 μL of CellTiter-Glo® 2.0 Reagent was added into each well. After mixing with a plate mixer, the plate was incubated at room temperature for approximately 30 minutes, and each 200 μL of mixture was transferred to a new white 96-well plate. Luminescent signal was measured by a multiplate reader EnVision (PerkinElmer Japan Co., Ltd.).

Results

Strains	Pango Lineage	EC_{50} (μmol/L)	
		Ensitrelvir fumaric acid	Remdesivir
hCoV-19/USA/MI-UM-10052670540/2023	BA.2.86	0.261 ± 0.021	0.877 ± 0.022
hCoV-19/Japan/RB23-006-81/2023	EG.5.1	0.612 ± 0.066	1.59 ± 0.27
hCoV-19/Japan/RB24-001-48/2024	JN.1	0.196 ± 0.101	0.879 ± 0.085

CPE = cytopathic effect; EC_{50} = 50% effective concentration; $TCID_{50}$ = 50% tissue culture infectious dose; TMPRSS2 = transmembrane serine protease 2

Data are expressed as the mean ± standard deviation (SD) in 3 independent experiments.

Study Number [Location in CTD]	S-217622-EB-219-N [4.2.1.1-01]

2.6.3.2.2 初代ヒト鼻腔上皮細胞における SARS-CoV-2 の増殖性に対するエンシトレルビルフマル酸の阻害効果

Test Article: Ensitrelvir fumaric acid

Species/Biological material	MucilAir™ (Nasal epithelial, Human) infected with SARS-CoV-2 clinical isolates [hCoV-19/Japan/23-018/2022 (Pango Lineage: XBB.1.5.19)].
Final concentration as free form (μmol/L)	Viral replication inhibition assay: Ensitrelvir fumaric acid fumaric acid and Nirmatrelvir 0.0185, 0.0556, 0.167, 0.500
Vehicle	0.5% DMSO
Evaluation Items	Viral replication inhibition assay: EC_{90}, The concentration achieving 90% of inhibition against SARS-CoV-2 by viral titers in the apical supernatant sample Cytotoxicity assay: % of cytotoxicity
Evaluation Method	Viral replication inhibition assay: MucilAir™ (about 5.0×10^5 cells/well) seeded in transwells on a 24-well plate was infected with hCoV-19/Japan/23-018/2022 strain at 5000 $TCID_{50}$/well (multiplicity of infection of about 0.01). The plate was incubated at 37°C, 5% CO_2 for 2 hours. After incubation, the cells were washed with the viral assay medium to remove unadsorbed viruses, followed by transferring the transwells to a 24 well plate containing 700 μL of serially diluted substance solutions. Afterwards, the plate was incubated at 37°C, 5% CO_2. At 3 days post infection, the viral medium was added into apical side of transwells and collected apical supernatant sample. All collected samples were stored in a deep freezer until use. The collected samples were subjected to the viral titration. The assay was performed duplicate for each substance concentration. Measurement of viral titers: The collected samples were diluted with the viral assay medium a 2-fold dilution. The 2-fold diluted samples were diluted a 10-fold dilution serial dilution scheme. Then, 25 μL of serially diluted collected samples were added to the 96 well plates, and 175 μL of VeroE6/TMPRSS2 cell suspension (0.857×10^5 cells/mL) were dispensed on the plates. The plates were incubated at 37°C in a 5% CO_2 incubator for 4 days. After incubation, the presence of virus-induced CPE was observed under a microscope. The viral titers were expressed as log $TCID_{50}$/mL. Cytotoxicity assay: In transwells seeded with MucilAir™ on a 24-well plate was added viral assay medium. The plate was incubated at 37°C, 5% CO_2 for 2 hours. After incubation, the cells were washed with the viral assay medium. Transwells were transferred to a 24 well plate containing 700 μL of substance solutions. Afterwards, the plate was incubated at 37°C, 5% CO_2 for 3 days. The cytotoxicity of the substance solutions in MucilAir™ were measured by cell viability. The cytotoxicity assay was performed in duplicate.

Results

Strains	Pango Lineage	EC_{90} μmol/L	
		Ensitrelvir fumaric acid	Nirmatrelvir
hCoV-19/Japan/23-018/2022	XBB.1.5.19	0.0784 ± 0.0362	0.0752 ± 0.0201

Data are expressed as the mean ± SD in 3 independent experiments.

Study Number [Location in CTD]	[redacted]-EB-086-N [4.2.1.1-02]

2.6.3.2.3 初代ヒト鼻腔上皮細胞におけるエンシトレルビルフマル酸の細胞毒性

Test Article: Ensitrelvir fumaric acid

Species/Biological material	MucilAir™ (Nasal epithelial, Human)
Final concentration as free form (μmol/L)	Cytotoxicity assay: 50
Vehicle	0.5% DMSO
Evaluation Items	% of cytotoxicity
Evaluation Method	In transwells seeded with MucilAir™ on a 24-well plate was added viral assay medium. The plate was incubated at 37°C, 5% CO_2 for 2 hours. After incubation, the cells were washed with the viral assay medium. Transwells were transfered to a 24 well plate containing 700 µL of substance solutions. Afterwards, the plate was incubated at 37°C, 5% CO_2 for 3 days. The cytotoxicity of the substance solutions in MucilAir™ were measured by cell viability. The cytotoxicity assay was performed in duplicate. The % of cytotoxicity was calculated with 3 independent experiments.
Results	
Cells	% of cytotoxicity of ensitrelvir fumaric acid at 50 µmol/L
MucilAir™ (Nasal epithelial, Human)	1.75 ± 4.11
Data are expressed as the mean ± SD in 3 independent experiments.	
Study Number [Location in CTD]	S-217622-EB-217-X [4.2.1.1-03]

2.6.3.2.4 リバースジェネティクス由来 SARS-CoV-2 変異体の配列確認

Species/Biological material	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region With reporter gene ([redacted]) wild type, nsp5/M49L, S144A, M49L + S144A, D48G, P52S Without reporter gene wild type, nsp5/L87F, T198I, A234S, H246Y, T21I, E166A, H172Y, T21I+E166V, L50F+E166V, L50F, P252L, T25A, T25I, D48Y, M49I
Evaluation Item	Sequence data
Evaluation Method	To analyze nucleotide mutation of nsp5 region of recombinant SARS-CoV-2, viral RNA was extracted from the virus stock and the nsp5 region of viral genome was amplified by reverse transcription polymerase chain reaction (RT-PCR). Sequencing of the RT-PCR products were entrusted to Eurofins Genomics sequencing service at Eurofins Genomics K.K. The obtained sequence of the nsp5 region site was compared with the sequence registered sequence of hCoV-19/Japan/TY/WK-521/2020 (Accession ID: EPI_ISL_408667) in the Global Initiative on Sharing All Influenza Data (GISAID) database. Then, nucleotide and amino acid substitutions were identified. When the second peak is identified at more than 10% of the first peak in the sequencing data, the nucleotide was judged as mixed base.
Results	Sanger sequencing analysis detecting more than 10% of the peak in the sequencing data revealed that only targeted amino acid substitutions were introduced into the nsp5 sequences of the evaluated recombinant viruses.
Study Number [Location in CTD]	S-217622-EB-222-X [4.2.1.1-04]

2.6.3.2.5 リバースジェネティクス由来 SARS-CoV-2 変異体に対する薬剤感受性試験

Test Article: Ensitrelvir fumaric acid

Species/Biological material	VeroE6/TMPRSS2 cells infected with SARS-CoV-2, generated by reverse genetics (rgSARS-CoV-2/Hu/DP/Kng/19-020 (wild-type), rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25A, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25I, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/D48Y, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/M49I, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/E166A, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/H172Y, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/P252L, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+E166V, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F+E166V)
Final concentration as free form (nmol/L)	4.57, 13.7, 41.2, 123, 370, 1111, 3333, 10000
Reference substance (Final concentration [nmol/L])	Remdesivir: 22.9, 68.6, 206, 617, 1852, 5556, 16667, 50000 Nirmatrelvir: 91.4, 274, 823, 2469, 7407, 22222, 66667, 200000
Vehicle	0.5% DMSO
Evaluation Items	EC_{50}: The concentration achieving 50% of inhibition on CPE induced by SARS-CoV-2 FC: The relative EC_{50} to wild-type virus
Evaluation Method	Viral replication inhibition assay: One hundred microliters of serially diluted substance solutions were added to a 96-well plate. SARS-CoV-2 virus stock solutions were diluted to appropriate concentrations in advance, and each 50 µL/well of diluted SARS-CoV-2 solution was dispensed on the 96-well plate containing substance solutions. The infectious dose of virus was 1000 $TCID_{50}$/well. Then, each 50 µL/well of VeroE6/TMPRSS2 cells which were adjusted to 3.0×10^5 cells/mL was dispensed on the plate. The plate was mixed with a plate mixer and was incubated at 37 °C, 5% CO_2 for 3 days. The inhibitory effects of the test or reference substance on CPE induced by SARS-CoV-2 were measured by cell viability. Measurement of cell viability: After 3 days incubation of the plate prepared in the viral replication inhibition assay, 60 µL per well of CellTiter-Glo® 2.0 Reagent was added into each well. After mixing with a plate mixer, the plate was incubated at room temperature for approximately 30 minutes, and each 200 µL of mixture was transferred to a new white 96-well plate. Luminescent signal was measured by a multimode plate reader EnVision (PerkinElmer Japan Co., Ltd.).

Results

Strains	Ensitrelvir fumaric acid			Remdesivir			Nirmatrelvir		
	EC_{50} (nmol/L)		FC	EC_{50} (nmol/L)		FC	EC_{50} (nmol/L)		FC
rgSARS-CoV-2/Hu/DP/Kng/19-020 (wild-type)	119 ± 14		N/A	2010 ± 51		N/A	2970 ± 401		N/A
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I	132 ± 22		1.11	2300 ± 262		1.14	5420 ± 1530		1.82
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25A	1710 ± 340		14.4	2630 ± 443		1.31	3150 ± 429		1.06
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25I	340 ± 92		2.86	1490 ± 642		0.741	1860 ± 361		0.626
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/D48Y	264 ± 46		2.22	1950 ± 330		0.970	3570 ± 441		1.20
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/M49I	625 ± 154		5.25	1470 ± 110		0.731	738 ± 134		0.248
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F	140 ± 40		1.18	1150 ± 358		0.572	3970 ± 356		1.34
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/E166A	403 ± 141		3.39	1560 ± 448		0.776	3590 ± 835		1.21

rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/H172Y	92.9	±	37.0	0.781	834	±	313	0.415	1710	±	347	0.576
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/P252L	129	±	20	1.08	1830	±	321	0.910	3420	±	352	1.15
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+E166V	328	±	173	2.76	1760	±	118	0.876	107000	±	39900	36.0
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F+E166V	334	±	144	2.81	1350	±	156	0.672	129000	±	11800	43.4

FC = fold change; TMPRSS2 = transmembrane serine protease 2; N/A = not applicable.
Data are expressed as the mean ± SD in 3 independent experiments.

Study Number [Location in CTD]	S-217622-EB-214-N [4.2.1.1-05]

2.6.3.2.6 リバースジェネティクス由来 SARS-CoV-2 変異体に対する薬剤感受性試験 (2)

Test Article: Ensitrelvir fumaric acid

Species/Biological material	VeroE6/TMPRSS2 cells infected with SARS-CoV-2, generated by reverse genetics (rgSARS-CoV-2/Hu/DP/Kng/19-020 [wild-type], rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25N, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/F140L, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L167F, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/A173V* , rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T304I , rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+S144A, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+A173V, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+T304I, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F+E166A, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/F140L+A173V , rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/A173V+T304I, rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+A173V+T304I , rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/ L50F+E166A+L167F)
Final concentration (nmol/L)	9.14, 27.4, 82.3, 247, 741, 2222, 6667, 20000
Reference substance (Final concentration [nmol/L])	Remdesivir: 22.9, 68.6, 206, 617, 1582, 5556, 16667, 50000 Nirmatrelvir: 91.4, 274, 823, 2469, 7407, 22222, 66667, 200000
Vehicle	0.5% DMSO
Evaluation Items	EC_{50}: The concentration achieving 50% of inhibition on CPE induced by SARS-CoV-2 FC: The relative EC_{50} to wild-type virus
Evaluation Method	Viral replication inhibition assay: One hundred microliters of serially diluted substance solutions were added to a 96-well plate. SARS-CoV-2 virus stock solutions were diluted to appropriate concentrations in advance, and each 50 μL/well of diluted SARS-CoV-2 solution was dispensed on the 96-well plate containing substance solutions. The infectious dose of virus was 1000 $TCID_{50}$/well. Then, each 50 μL/well of VeroE6/TMPRSS2 cells which were adjusted to 3.00×10^5 cells/mL was dispensed on the plate. The plate was mixed with a plate mixer and was incubated at 37 °C, 5% CO_2 for 3 days. The inhibitory effects of the test or reference substance on CPE induced by SARS-CoV-2 were measured by cell viability. Measurement of cell viability: After 3 days incubation of the plate prepared in the viral replication inhibition assay, 60 μL/well of CellTiter-Glo® 2.0 Reagent was added into each well. After mixing with a plate mixer, the plate was incubated at room temperature for approximately 30 minutes, and each 200 μL of mixture was transferred to a new white 96-well plate. Luminescent signal was measured by a multimode plate reader EnVision (PerkinElmer Japan Co., Ltd.).

Results

Strains	Ensitrelvir fumaric acid			Remdesivir			Nirmatrelvir		
	EC_{50} (nmol/L)		FC	EC_{50} (nmol/L)		FC	EC_{50} (nmol/L)		FC
rgSARS-CoV-2/Hu/DP/Kng/19-020 (wild-type)	64.9 ± 26.2		N/A	1770 ± 211		N/A	2280 ± 526		N/A
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T25N	314 ± 138		4.84	486 ± 110		0.275	485 ± 184		0.213
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/F140L	< 34.4 [a]		< 0.530	262 ± 35		0.148	654 ± 427		0.287
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L167F	232 ± 107		3.57	2000 ± 39		1.13	3020 ± 474		1.32
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/A173V*	25.3 ± 19.2		0.390	1700 ± 288		0.960	2830 ± 868		1.24
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T304I	55.1 ± 25.0		0.849	1910 ± 332		1.08	2410 ± 478		1.06
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+S144A	473 ± 253		7.29	2070 ± 80		1.17	6800 ± 1480		2.98

rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+A173V	30.4	±	18.3	0.468	1690	±	235	0.955	3000	±	856	1.32
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+T304I	86.2	±	37.4	1.33	2160	±	181	1.22	4800	±	622	2.11
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/L50F+E166A	400	±	171	6.16	1780	±	250	1.01	7230	±	1050	3.17
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/F140L+A173V	28.3	±	12.6	0.436	400	±	282	0.226	2480	±	2010	1.09
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/A173V+T304I	< 11.3 [a]			< 0.174	474	±	43	0.268	966	±	537	0.424
rgSARS-CoV-2/Hu/DP/Kng/19-020-nsp5/T21I+A173V+T304I	< 20.9 [a]			< 0.322	2040	±	101	1.15	3330	±	863	1.46
rgSARS-CoV-2/Hu/DP/Kng/19-020 -nsp5/L50F+E166A+L167F	3860	±	2970	59.5	1180	±	223	0.667	22300	±	8070	9.78

EC_{50} values are expressed as the mean ± SD in 3 independent experiments. FC against each SARS-CoV-2 strain were calculated as relative EC_{50} to wild-type virus.
a. In the case of the EC_{50} of ensitrelvir fumaric acid is less than 9.14 nmol/L in one of 3 independent experiments, only the mean is calculated and the SD is not calculated.
* Sanger sequencing analysis (Study S-217622-EB-223-X [4.2.1.1-11]) revealed that T21T/I was introduced into the nsp5 sequence of the A173V virus.

Study Number [Location in CTD]	S-217622-EB-221-N [4.2.1.1-06]

2.6.3.2.7 [redacted]におけるリバースジェネティクス由来 SARS-CoV-2 変異体に対する薬剤感受性試験

Test Article: Ensitrelvir fumaric acid

Cell line	Huh7-ACE2
Final concentration (μmol/L)	0.0046, 0.0137, 0.0412, 0.1235, 0.3704, 1.1111, 3.3333, 10
Reference Article (Concentration)	Nirmatrelvir, Remdesivir (0.0046, 0.0137, 0.0412, 0.1235, 0.3704, 1.1111, 3.3333, 10 μmol/L)
Vehicle	DMSO (0.1% final concentration or less)
Evaluation Items	IC_{50} (50% inhibitory concentration)
Evaluation Method	20,000 Huh7-ACE2 cells were seeded in 96 well-plates in DMEM supplemented with 10% fetal calf serum and penicillin/streptomycin (DMEM + FCS + P/S) and incubated for 24 hours at 37 °C under 5% CO_2. Cells were then overlaid with the test or reference article diluted in DMEM + FCS + P/S and infected with 0.05 MOI of the appropriate virus diluted in DMEM + FCS + P/S. Cells were further incubated for an additional 24 hours at 37 °C under 5% CO_2 before being lysed with diluted lysis buffer (Luciferase Cell Culture Lysis 5X Reagent, Promega). The substrate (Nano-Glo® Luciferase Assay Substrate, Promega) was then added and luminescence was quantified on a plate reader. Inhibition was calculated in proportion to mock-treated virus-only wells and untreated/uninfected wells. IC_{50} values were derived by nonlinear regression in GraphPad Prism. Mutants were considered significantly resistant if they exhibited greater than 5-fold resistance.

Results

Virus[a]	IC_{50} (μmol/L)					
	Ensitrelvir fumaric acid		Nirmatrelvir		Remdesivir	
	Mean ± SD	Fold change[b]	Mean ± SD	Fold change[b]	Mean ± SD	Fold change[b]
Wild-type	0.0281 ± 0.0108	N/A	0.0305 ± 0.0181	N/A	0.00301 ± 0.00211	N/A
T21I	0.0129 ± 0.00471	0.458	0.0133 ± 0.00382	0.435	0.00173 ± 0.000954	0.575
L50F	0.0301 ± 0.00885	1.07	0.0647 ± 0.00308	2.12	0.00171 ± 0.00115	0.570
E166V	0.0292 ± 0.0216	1.04	1.70 ± 0.890	55.8	0.00181 ± 0.00116	0.602
A173V	0.00456 ± 0.00593	0.162	0.0170 ± 0.00970	0.557	0.00199 ± 0.000907	0.662
P252L	0.0329 ± 0.00611	1.17	0.208 ± 0.144	6.82	0.00233 ± 0.000921	0.773
T304I	0.0200 ± 0.00829	0.713	0.117 ± 0.0114	3.84	0.00214 ± 0.000325	0.711
T21I + S144A	0.0581 ± 0.0239	2.07	0.0959 ± 0.0230	3.14	0.00313 ± 0.00135	1.04
T21I + E166V	0.0390 ± 0.0247	1.39	8.48 ± 12.4	278	0.0143 ± 0.000310	4.75
T21I + A173V	0.00219 ± 0.00181	0.0779	0.0250 ± 0.0307	0.820	0.00840 ± 0.00520	2.79
T21I + T304I	0.0182 ± 0.00826	0.648	0.216 ± 0.0600	7.08	0.00178 ± 0.000511	0.590
L50F + E166V	0.00730 ± 0.00166	0.260	0.985 ± 0.376	32.3	0.0135 ± 0.000226	4.49
T21I + A173V + T304I	0.000546 ± 0.000302	0.0194	0.0181 ± 0.0113	0.594	0.00755 ± 0.0000460	2.51

Each value represents the mean ± SD of 3 independent experiments.
a. All viruses are in the background of USA-WA1/2020 strain with ORF7a/b deleted and replaced with a NanoLuc reporter gene. Denoted mutations are in nsp5.
b. Fold change was calculated as relative IC_{50} to wild-type.

Study Number [Location in CTD]	S-217622-EF-194-N [4.2.1.1-07]

2.6.3.2.8 SARS-CoV-2 3CL プロテアーゼの遺伝子多型解析

Evaluation Item	GISAID data, downloaded on 20 Jan 2025, meeting the following criteria was included in the analysis as a dataset. The first criterion was that the amino acid sequence of Nsp5 did not include any ambiguity codes. The second criterion was that the length of amino acid sequence of Nsp5 was 306 amino acids. The third criterion was that isolates were from humans. The fourth criterion was that the collection date was after 01 Oct 2022 and the submission to GISAID date was 31 Dec 2024. The fifth criterion was that the header information did not include unreadable character by python. A total of 2,627,268 amino acid sequences met these criteria.
Evaluation Method	The prevalence of polymorphisms of SARS-CoV-2 M^{pro} in each amino acid was calculated as the ratio of registered mutation counts to the total registered count. The total number of sequences was 17,113,541 amino acid sequences.
Results	The prevalence of polymorphism of amino acids in 3CL protease responsible for low susceptibility to ensitrelvir and amino acids that contact or are located in the proximity (≤5 Å) of ensitrelvir was less than 1%, except for the prevalence of M49L in Japan. The highest prevalence of M49L was 1.554% in Oct 2023. However, the prevalence of M49L in Japan has been consistently declining since Dec 2023. The prevalence of polymorphisms of other investigated amino acids was less than 1%.
Study Number [Location in CTD]	S-217622-EB-215-X [4.2.1.1-08]

2.6.3.2.9 SARS-CoV-2 感染マウスモデルにおけるエンシトレルビル予防投与による治療効果

Test Article: Ensitrelvir

Species/Strain	Mouse/ BALB/cAJcl				
Gender/Number of animals	Female/15 per group				
Age of animal	37-57 weeks old (on the day of drug administration)				
Virus	SARS-CoV-2 MA-P10 (mouse-adapted hCoV-19/Japan/TY/WK-521/2020)				
Vehicle/Formulation	0.5 w/v% MC				
Method of Administration	Subcutaneously administration under inhalation anesthesia with isoflurane (Single administration with two administration sites)				
Dose (mg/kg)	0, 32, 64, 96, or 128				
Treatment	The mice were subcutaneously administered with ensitrelvir or vehicle 24 hours before lethal viral infection under inhalation anesthesia with isoflurane. Mice were intranasally inoculated with 3.00×10^2 $TCID_{50}$ (50 μL/mouse) of the virus suspensions under anesthesia.				
Evaluation Item	The survival and the body weight of mice were examined for 14 days after virus infection.				
Evaluation method	Mice were examined daily for survival through 14 days after infection. Mice were euthanized and regarded as dead when their body weights in the morning were less than 80% of the initial body weights (in the morning on the day of virus infection) according to humane endpoints.				
Results					
Day after virus infection	Survived/total per group (Survival rate [%])				
	Vehicle	Ensitrelvir (mg/kg)			
		32	64	96	128
Day 1	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Day 2	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Day 3	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Day 4	8/15 (53%)	9/15 (60%)	15/15 (100%)	15/15 (100%)	15/15 (100%)
Day 5	2/15 (13%)	2/15 (13%)	12/15 (80%)	14/15 (93%)	15/15 (100%)
Day 6	1/15 (6.7%)	2/15 (13%)	12/15 (80%)	14/15 (93%)	15/15 (100%)
Day 7	1/15 (6.7%)	1/15 (6.7%)	11/15 (73%)	13/15 (87%)	15/15 (100%)
Day 8	0/15 (0%)	1/15 (6.7%)	10/15 (67%)	13/15 (87%)	15/15 (100%)
Day 9	0/15 (0%)	1/15 (6.7%)	10/15 (67%)	13/15 (87%)	15/15 (100%)
Day 10	0/15 (0%)	1/15 (6.7%)	9/15 (60%)	13/15 (87%)	15/15 (100%)
Day 11	0/15 (0%)	1/15 (6.7%)	9/15 (60%)	13/15 (87%)	15/15 (100%)
Day 12	0/15 (0%)	1/15 (6.7%)	9/15 (60%)	12/15 (80%)	15/15 (100%)
Day 13	0/15 (0%)	1/15 (6.7%)	9/15 (60%)	12/15 (80%)	15/15 (100%)
Day 14	0/15 (0%)	1/15 (6.7%)	9/15 (60%)	12/15 (80%)	15/15 (100%)
P value vs vehicle	-	0.5715	< 0.0001	< 0.0001	< 0.0001
Mice were subcutaneously administered (10 mL/kg) with ensitrelvir or vehicle (0.5 w/v% methylcellulose solution) 24 hours before mouse-adapted hCoV-19/Japan/TY/WK-521/2020 (SARS-CoV-2 MA-P10) infection. The P values were calculated by the log-rank test (multiplicity was adjusted by fixed-sequence procedure).					

Day after virus infection	Body weight proportion to initial body weight (%)				
	Vehicle	Ensitrelvir (mg/kg)			
		32	64	96	128
Day 1	97.2 ± 2.2	96.9 ± 1.7	97.6 ± 1.5	97.3 ± 1.9	97.7 ± 2.0
Day 2	93.5 ± 3.3	94.0 ± 1.9	95.8 ± 1.6	95.4 ± 3.6	97.2 ± 2.7***
Day 3	86.4 ± 4.6	86.5 ± 1.6	90.3 ± 2.9**	93.0 ± 4.6***	94.9 ± 3.5***
Day 4	82.6 ± 5.0	80.8 ± 1.2	86.8 ± 4.7*	91.0 ± 5.4***	93.9 ± 5.0***
Day 5	81.1 ± 4.1	80.6 ± 1.6	86.3 ± 6.2**	91.5 ± 6.6***	94.2 ± 6.0***
Day 6	80.7 ± 2.9	80.6 ± 1.7	87.8 ± 7.2***	93.0 ± 6.3***	95.6 ± 6.5***
Day 7	80.2 ± 0.9	80.5 ± 2.0	87.0 ± 6.4***	90.4 ± 5.7***	95.4 ± 5.2***

Mice were subcutaneously administered (10 mL/kg) with ensitrelvir or vehicle (0.5 w/v% methylcellulose solution) 24 hours before mouse-adapted hCoV-19/Japan/TY/WK-521/2020 (SARS-CoV-2 MA-P10) infection.
*, ** and *** denote respectively $P < 0.05$, $P < 0.01$, and $P < 0.001$ vs vehicle, using ANOVA-test (multiplicity was adjusted by fixed-sequence procedure).
Values are mean body weight proportion ± SD at each day (AM). When the mice died before the analysis time point or the body weight proportion to initial body weight was less than 80%, the body weight proportion of the mouse was imputed as 80% at all time point after that time point.

Species/Strain	Mouse/ BALB/cAJcl
Gender/Number of animals	Female/4 per group
Age of animal	37-57 weeks old (on the day of drug administration)
Virus	None
Vehicle/Formulation	0.5 w/v% MC
Method of Administration	Subcutaneously administration under inhalation anesthesia with isoflurane (Single administration with two administration sites)
Dose (mg/kg)	32, 64, 96, or 128
Treatment	The mice were subcutaneously administered with ensitrelvir or vehicle under inhalation anesthesia with isoflurane, and the blood was taken 0.5, 2, 4, 8, 24, 48, 72, and 168 hours postdose.
Evaluation Item	The concentrations of ensitrelvir in plasma (0.5, 2, 4, 8, 24, 48, 72, and 168 hours postdose)
Evaluation method	The plasma concentrations of ensitrelvir were determined by high performance liquid chromatography with tandem mass spectrometry (LC/MS/MS) and were calculated by Chromeleon (Thermo Fisher Scientific).

Results

Time (hour)	Plasma concentrations of ensitrelvir (ng/mL)											
	32 mg/kg			64 mg/kg			96 mg/kg			128 mg/kg		
0.5	425	±	167	570	±	88	610	±	114	689	±	91
2	1820	±	760	3150	±	1020	3390	±	460	4000	±	960
4	3100	±	1070	4550	±	1390	5490	±	960	6050	±	1030
8	3560	±	1060	4750	±	980	6510	±	1130	6710	±	810
24	1740	±	670	2990	±	550	3110	±	820	3370	±	900
48	1130	±	240	1720	±	520	1400	±	400	1840	±	470
72	946	±	306	1310	±	390	951	±	185	1230	±	230
168	584	±	59	1100	±	260	1110	±	290	1250	±	170

Mice were subcutaneously administered (10 mL/kg) with ensitrelvir or vehicle (0.5 w/v% methylcellulose solution).

Study Number [Location in CTD]	S-217622-EB-171-N [4.2.1.1-09]

2.6.3.2.10 SARS-CoV-2 感染マウスモデルにおけるエンシトレルビル予防投与による肺内ウイルス力価への影響

Test Article: Ensitrelvir

Species/Strain	Mouse/ BALB/cAJcl			
Gender/Number of animals	Female/10 per in each group			
Age of animal	36-56 weeks old (on the day of drug administration)			
Virus	SARS-CoV-2 MA-P10 (mouse-adapted hCoV-19/Japan/TY/WK-521/2020)			
Vehicle	0.5 w/v% MC			
Method of Administration	Subcutaneous administration under inhalation anesthesia with isoflurane (Single administration with two administration sites)			
Dose (mg/kg)	0, 64, or 128			
Treatment	The mice were subcutaneously administered with ensitrelvir or vehicle 24 hours before viral infection under inhalation anesthesia with isoflurane. Mice were intranasally inoculated with 3.00×10^2 $TCID_{50}$ (50 µL/mouse) of the virus suspensions under anesthesia.			
Evaluation Items	The lung virus titers 1, 2, 4, and 9 days after infection.			
Analytical method	Virus titers in lungs were measured by standard 50% tissue culture infectious dose ($TCID_{50}$) method using VeroE6/TMPRSS2 cells (virus titers in lungs) For the pairwise comparison of the virus titers in lung homogenates between ensitrelvir groups and the vehicle group for the overall time, the two-way analysis of variance model was applied. Additionally, for the pairwise comparison of the virus titers in lung homogenates between ensitrelvir groups and the vehicle group at each time point, the one-way analysis of variance model was applied by time point. The multiplicity of statistical tests was adjusted using the Dunnett method.			
Results				
Test substance		Vehicle	Ensitrelvir	
Dose (mg/kg)		-	64	128
Virus titers (log_{10} $TCID_{50}$/mL) (survived/total mice)	Sampling: 1 day after infection	6.90 ± 0.31 (10/10)	6.13 ± 0.43** (10/10)	5.50 ± 0.48** (10/10)
	Sampling: 2 days after infection	7.30 ± 0.29 (10/10)	6.97 ± 0.33 (10/10)	6.78 ± 0.47* (10/10)
	Sampling: 4 days after infection	5.94 ± 0.41 (10/10)	5.60 ± 0.41 (10/10)	5.26 ± 0.34* (10/10)
	Sampling: 9 days after infection	1.80 ± 0.00 (3/10)	1.80 ± 0.00 (8/10)	1.84 ± 0.11 (9/10)
The lower limit of quantification of virus titers is 1.80 log_{10} $TCID_{50}$/mL. Data are expressed as the mean ± SD of survived mice in each group. The following *p*-values were calculated by one-way ANOVA at each time point (multiplicity was adjusted using the Dunnett method). * $p < 0.01$, ** $p < 0.001$ vs vehicle-treatment group The observations 9 days after infection were excluded from the analysis set of the ANOVA model because only 3 mice survived 9 days after infection in vehicle-treated group.				
Study Number [Location in CTD]	S-217622-EB-189-N [4.2.1.1-10]			

2.6.3.2.11 リバースジェネティクス由来 SARS-CoV-2 変異体の配列確認 (2)

Species/Biological material	Virus: Recombinant viruses based on hCoV-19/Japan/TY/WK-521/2020 (rgSARS-CoV-2/Hu/DP/Kng/19-020) with amino acid substitutions in the nsp5 region Without reporter gene (used for ensitrelvir related pharmacology studies) wild type, nsp5/T25N, F140L, L167F, A173V, T304I, T21I+S144A, T21I+A173V, T21I+T304I, L50F+E166A, F140L+A173V, A173V+T304I, T21I+A173V+T304I, L50F+E166A+L167F, M49L, D48G, P52S, S144A, M49L+S144A
Evaluation Item	Sequence data
Evaluation Method	To analyze nucleotide mutation of nsp5 region of recombinant SARS-CoV-2, viral RNA was extracted from the virus stock and the nsp5 region of viral genome was amplified by RT-PCR. Sequencing of the RT-PCR products were entrusted to Eurofins Genomics sequencing service at Eurofins Genomics K.K. The obtained sequence of the nsp5 region site was compared with the sequence registered sequence of hCoV-19/Japan/TY/WK-521/2020 (Accession ID: EPI_ISL_408667) in the GISAID database. Then, nucleotide and amino acid substitutions were identified. When the second peak is identified at more than 10% of the first peak in the sequencing data, the nucleotide was judged as mixed base.
Results	Sanger sequencing analysis revealed that in addition to A173V*, T21T/I was introduced into the nsp5 sequence of the A173V virus, whereas only targeted amino acid substitutions were introduced into the nsp5 sequences of the evaluated recombinant viruses other than A173V virus. *The recombinant virus corresponding to A173V+T21T/I was used as A173V in Study S-217622-EB-221-N [4.2.1.1-06].
Study Number [Location in CTD]	S-217622-EB-223-X [4.2.1.1-11]

2.6.3.2.12 SARS-CoV-2 感染細胞に対する細胞変性抑制効果 (2)

Test Article: Ensitrelvir fumaric acid

Species/Biological material	VeroE6/TMPRSS2 cells infected with SARS-CoV-2 clinical isolate (hCoV-19/France/BRE-RELAB-IPP06405/2024 [Pango Lineage: KP.3.1.1], hCoV-19/France/IDF-RELAB-IPP09137/2024 [Pango Lineage: XEC], hCoV-19/Japan/TY/WK-521/2020 [Pango Lineage: A] [reference strain])
Final concentration as free form (μmol/L)	0.00457, 0.0137, 0.0412, 0.123, 0.370, 1.11, 3.33, 10.0
Reference substance (Final concentration [μmol/L])	Remdesivir: 0.00914, 0.0274, 0.0823, 0.247, 0.741, 2.22, 6.67, 20.0
Vehicle	0.5% DMSO
Evaluation Items	EC_{50}: The concentration achieving 50% of inhibition on CPE induced by SARS-CoV-2
Evaluation Method	Viral replication inhibition assay: One hundred microliters of serially diluted substance solutions were added to a 96-well plate. SARS-CoV-2 virus stock solutions were diluted to appropriate concentrations in advance, and each 50 μL/well of diluted SARS-CoV-2 solution was dispensed on the 96-well plate containing substance solutions. The infectious dose of virus as follows:300 $TCID_{50}$/well for hCoV-19/France/BRE-RELAB-IPP06405/2024, hCoV-19/France/IDF-RELAB-IPP09137/2024, 1000 $TCID_{50}$/well for hCoV-19/Japan/TY/WK-521/2020 (reference strain). Then, each 50 μL/well of VeroE6/TMPRSS2 cells which were adjusted to 3.00×10^5 cells/mL was dispensed on the plate. The plate was mixed with a plate mixer and was incubated at 37°C, 5% CO_2 for 3 or 4 days. The inhibitory effects of the test or reference substance on CPE induced by SARS-CoV-2 were measured by cell viability. Measurement of cell viability: After 3 or 4 days incubation of the plate prepared in the viral replication inhibition assay, 60 μL of CellTiter-Glo® 2.0 Reagent was added into each well. After mixing with a plate mixer, the plate was incubated at room temperature for approximately 30 minutes, and each 200 μL of mixture was transferred to a new white 96-well plate. Luminescent signal was measured by a multiplate reader EnVision (PerkinElmer Japan Co., Ltd.).

Results

Strains	Pango Lineage	EC_{50} (μmol/L)					
		Ensitrelvir fumaric acid			Remdesivir		
hCoV-19/France/BRE-RELAB-IPP06405/2024	KP.3.1.1	0.387	±	0.206	1.090	±	0.200
hCoV-19/France/IDF-RELAB-IPP09137/2024	XEC	0.309	±	0.091	0.946	±	0.121
hCoV-19/Japan/TY/WK-521/2020(reference strain)	A	0.412	±	0.102	2.550	±	0.560

Data are expressed as the mean ± SD in 3 independent experiments.

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2.6.3.3 副次的薬理試験

該当する試験なし.

2.6.3.4 安全性薬理試験

該当する試験なし.

2.6.3.5 薬力学的薬物相互作用試験

該当する試験なし.