KU LEUVEN

REGA INSTITUTE KU LEUVEN

Cistim
Center for Innovation and Stimulation of Drug Discovery

ALIGOS THERAPEUTICS

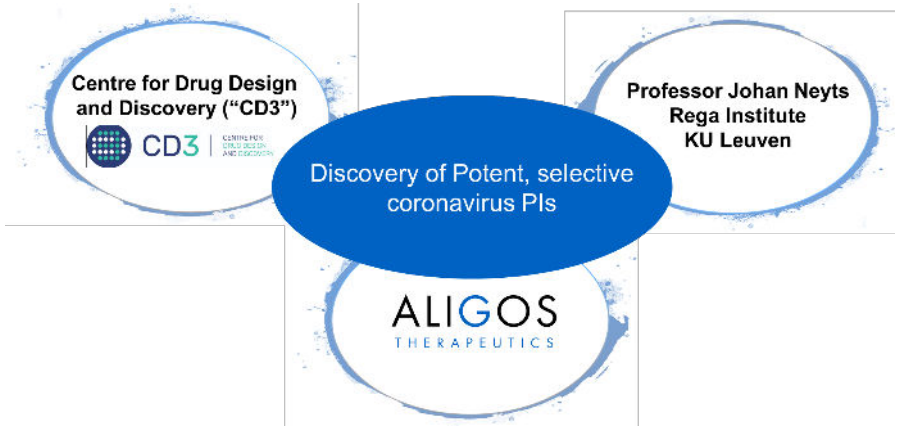
CD3 | CENTRE FOR DRUG DESIGN AND DISCOVERY

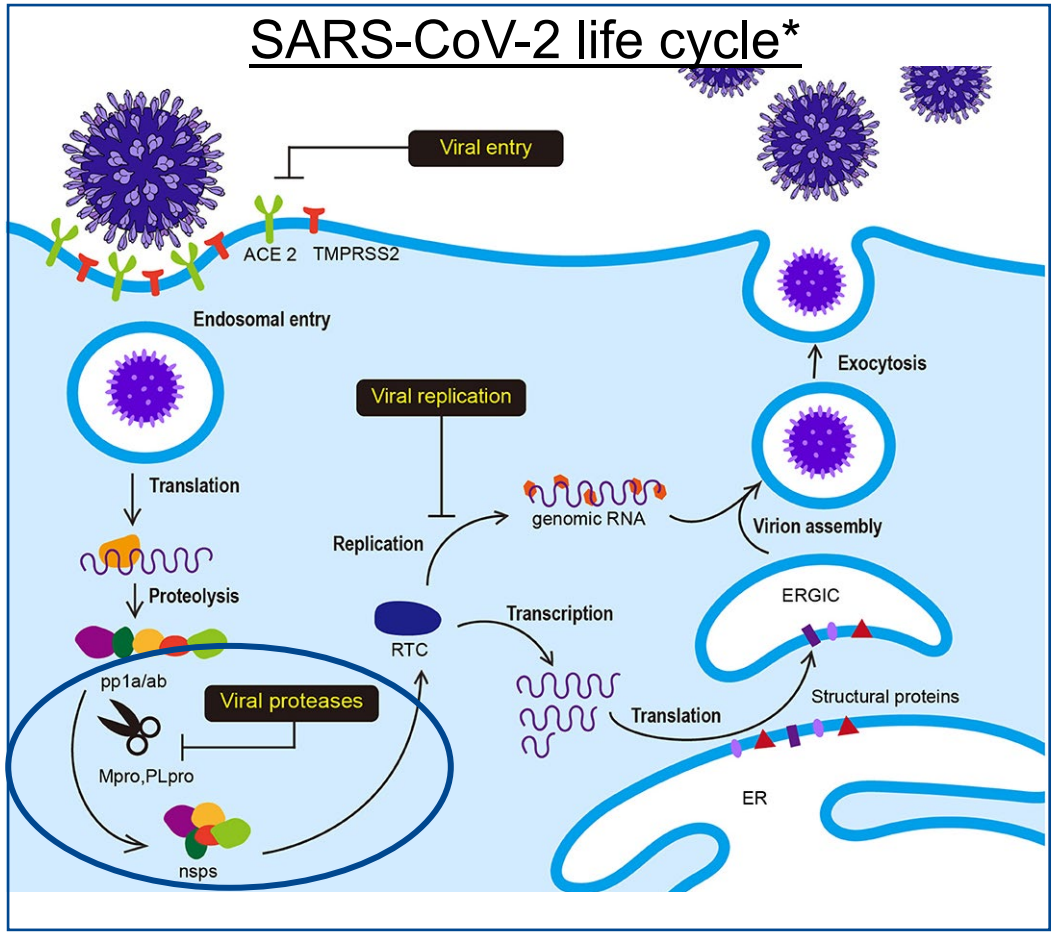
Oral 3CL-Protease Inhibitors for Treating Coronaviral Infections

Discovery on Target
October 20th 2022, Boston

From the Start of the SARS-CoV-2 Pandemic, SARS-CoV-2 3CLpro Represented an Attractive Target…

- Oral PIs are clinically validated (HIV/HCV)
- 3CLpro is conserved across CoV's,
  - Pan-CoV coverage for future variants and pandemics
- Amenable to structure-based drug design
- No human homolog of 3CLpro
- Collaboration on Aligos and CD3/Rega at KU Leuven established in 2020

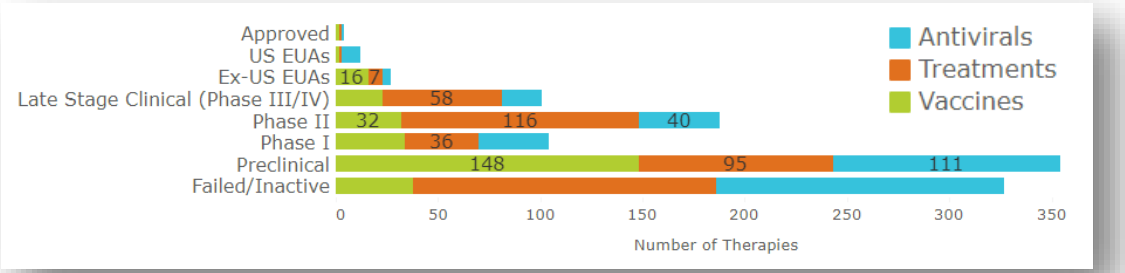




*https://www.frontiersin.org/articles/10.3389/fmicb.2020.01723/full

…Triggering Intensive 3CLpro Targeting R&D Activities

As with the unprecedented monumental effort to combat Covid-19 with Vaccines/antivirals/treatments…



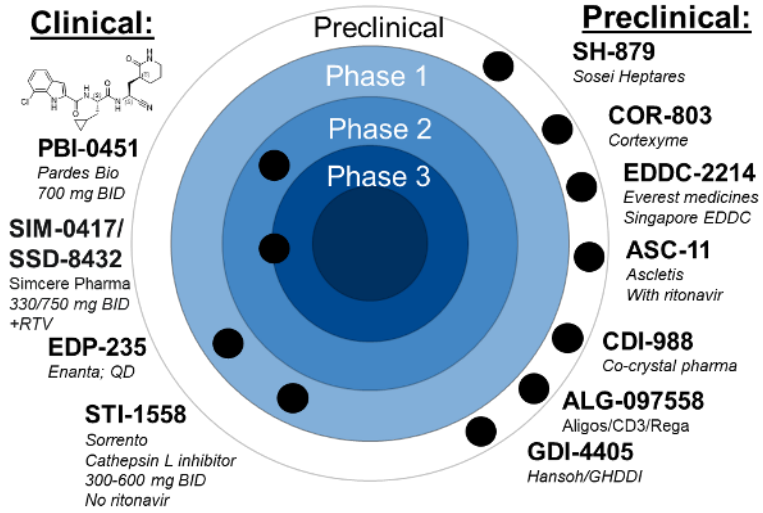
Subselection of oral 3CLpro inhibitors in (pre)clinical development:

US and Europe

Nirmatrelvir
Pfizer
(Nirma. / PF-07321332)
PAXLOVID™
(Nirma. + ritonavir)
Oral 300 mg/100mg RTV
(3 pill) BID dosing

Filed for approval in Japan

Ensitrelvir
Shionogi
Xocova™
(S-217622)
Oral (1 pill) QD dosing

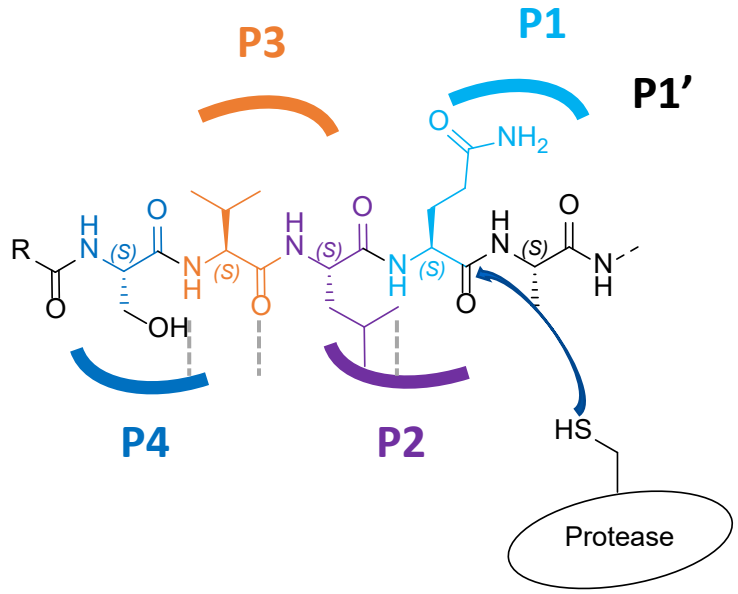


- *Repurposed/purpose build*
- *(Non)Selective*
- *IV/nasal/oral administration*
- *(non)Covalent*
- *(Ir)Reversible*
- *QD/BID dosing*
- *+/- ritonavir*

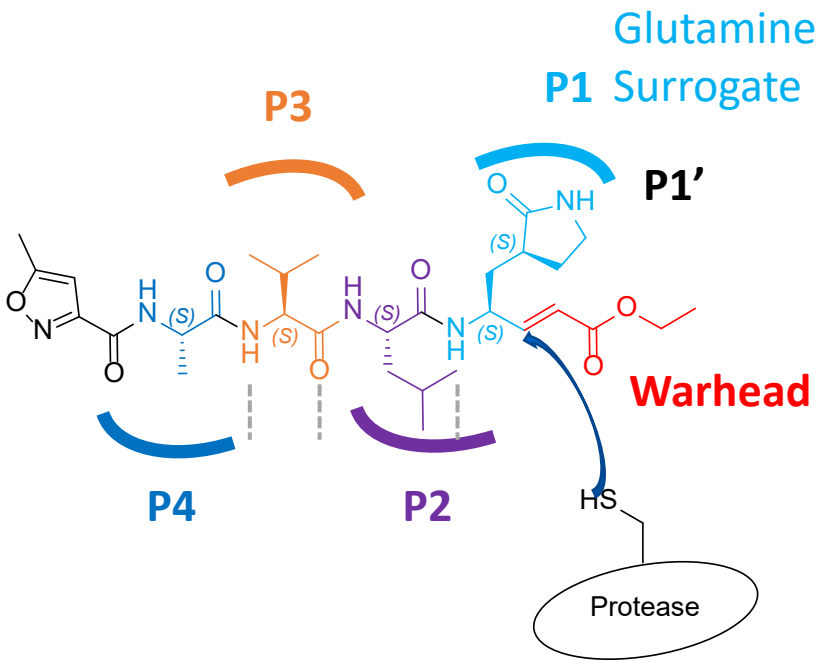
… a broad array of 3CLpro inhibitors were investigated and are currently in active (pre)clinical development

https://www.bio.org/policy/human-health/vaccines-biodefense/coronavirus/pipeline-tracker

SARS-CoV-2 Protease Substrate and Inhibitor



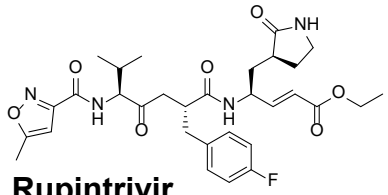
SARS-CoV-2 **Polyprotein Substrate**



SARS-CoV-2 **Protease Inhibitor**

Lessons from Pre-Covid-19 3C(L) Protease Inhibitors

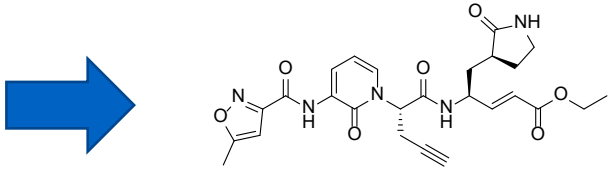
- Orally available 3C-inhibitors for Human Rhinovirus are described, indicating feasibility for oral administration of the P1-glutamine surrogate peptidomimetic chemotype



Rupintrivir

1999

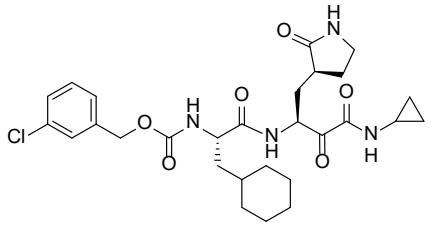
Clinical stage non-PO



AG-7404

2005

Clinical stage PO



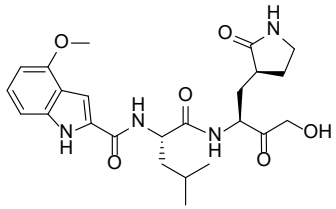
PO F(%)~20% (rat)
Kansas State University

2016*

Discovery stage PO

- P1-glutamine peptidomimetic inhibitors were described for SARS-CoV-1 (and were a steppingstone to the first clinical 3CLpro SARS-CoV-2 inhibitors)

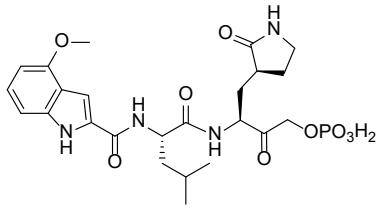
SARS-CoV-1



PF-00835231
PO F(%): <0.1-1.4%
(monkey/rat)

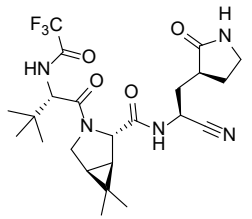
2004-2005

SARS-CoV-2



IV-dosing
PF-07304814

2020



PO-dosing
PF-07321332

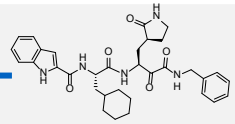
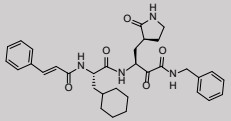
2021

*http://dx.doi.org/10.1016/j.antiviral.2015.11.010

Our Effort Towards 3CLpro Inhibitors…

- Preference for designing a 3CLpro inhibitor selective over host targets, aiming for a highly efficacious pan-corona antiviral with a high tolerability.
  - › Not targeting host proteases (cysteine/serine) involved in SARS-CoV-2 entry (Cathepsin/TMPRSS2)
  - › Observed discrepancy between activity in enzymatic 3CL-protease activity and cellular activity on SARS-CoV-2, singling out cathepsin L inhibition as a possible culprit

10 human proteases tested: inhibiting Cathepsin L/B and calpain

ID	SARS-CoV-2			Cathepsin L
	3CLpro IC_{50} (μM)	Vero E6 + CP EC_{50} (μM)	Huh-7 EC_{50} (μM)	IC_{50} (μM)
 **Cpd A1** WO202003014	0.58	0.094 *SI = 481*	< 0.005 *SI = 2380*	<0.0005
 **Cpd 11r** J. Med. Chem. 2020, 63, 9, 4562–4578	1.2	0.074 *SI =451*	0.001 *SI > 1000*	<0.0005

- Target product profile evolved with emerging new information on the SARS-CoV-2 pandemic and 3CLpro inhibitor landscape

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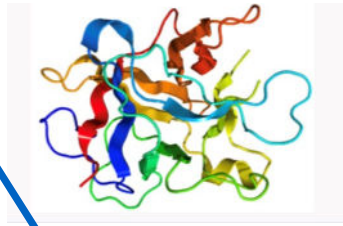
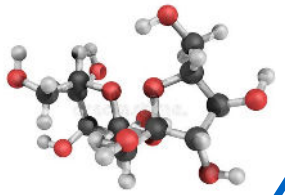
SARS-CoV-2 Protease Inhibitor Screening Cascade

Structure based drug design ⟷ **Medicinal Chemistry LLO**





3CL Protease IC_{50}
Cathepsin L selectivity
SARS-Cov-2/OC43 cell-based EC_{50}
Cell Toxicity CC_{50}
In vitro ADME

In vitro antiviral potency and selectivity

SARS-CoV-1/229E/SARS-CoV-2 variants
In vivo rodent IV/PO PK,
SARS-CoV-2 ALI assay

Hamster efficacy study
Tox Profiling

Candidate

ADME Tox and DMPK
Animal efficacy

Biochemical Cathepsin L assay early in the screening cascade

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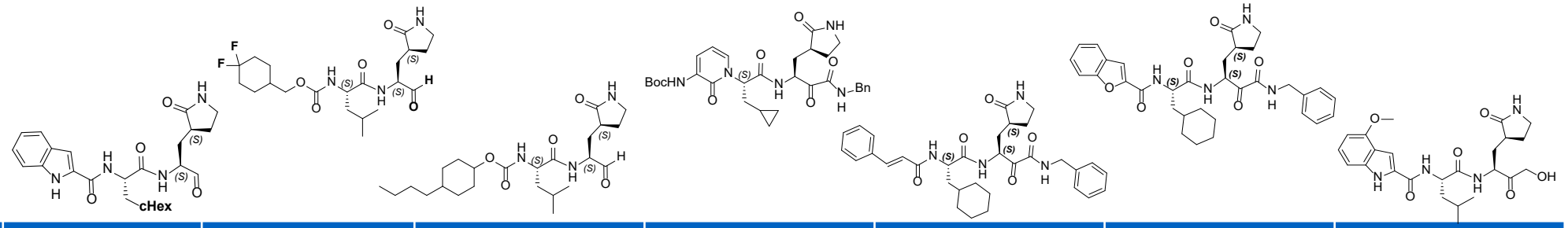
Early Reported SARS-CoV-2 3CLpro Inhibitors are also Cathepsin L Inhibitors



compound	Cpd 11a	6j	6e	Cpd 13b	Cpd 11r	A9	PF-00835231
Reference	W Dai et al, Science (2020)	Rathnayake, A. et al Science Translational Medicine (2020)		L. Zhang et al., Science (2020)	L. Zhang et al J.Med Chem (2020)	WO2020030143	J. Med. Chem. (2020)
Cathepsin L IC_{50} (nM)	0.21	<0.5	<0.5	290	<0.5	<0.5	168
SARS-CoV-2 3CLpro IC_{50} (nM)	9	8	10	472	1154	4891	5
HRV 3Cpro IC_{50} (nM)	9	170	90	2590	58	294	1675
SARS-CoV-2 Huh-7 EC_{50} (nM)*	54	53	242	3676	1	<0.5	952

*EC_{50} E64D: 80 nM; Z-FA-FMK <50 nM

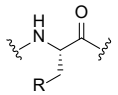
Impact of Cathepsin L and 3CLpro Activity on SARS-CoV-2 Inhibition in Huh-7 Cells

High cellular SARS-CoV-2 potency was observed for two types of compounds:

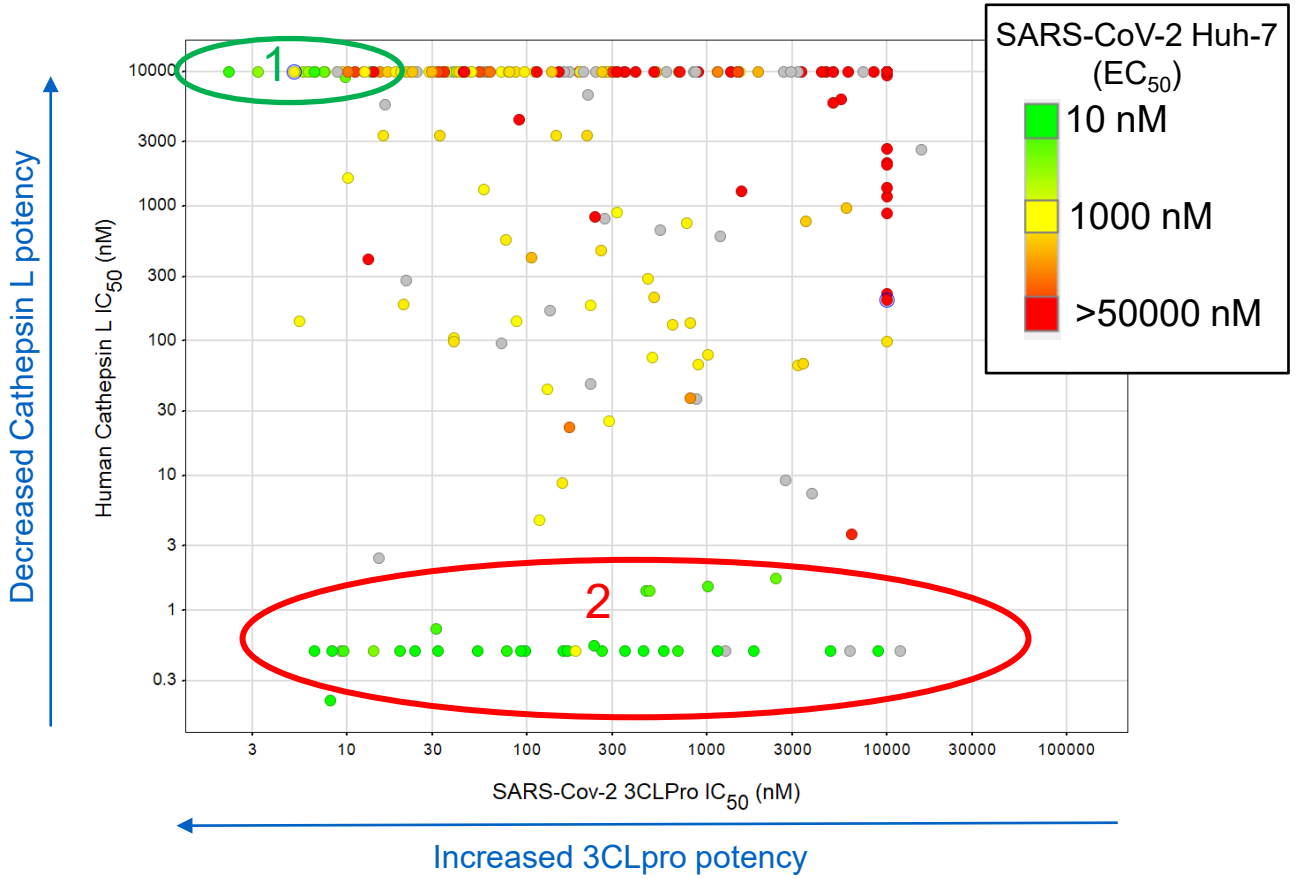
1) **Desired profile**: Highly selective and potent 3CLpro compounds

2) **Undesired profile**: Cathepsin L inhibitors shows high potency in Huh-7, independent of their 3CLpro activity
   → results in overestimation of cellular potency

Unselective inhibitors all have:

1) P2: 

2) Aldehyde or ketoamide warhead



Cathepsin L inhibitors shows high potency against SARS-CoV-2 in Huh-7, independent of 3CLpro activity

Highly selective and potent 3CLpro compounds also show good potency in Huh-7

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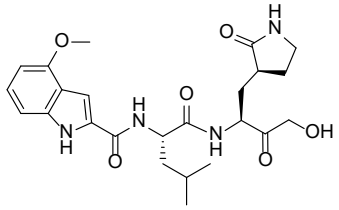
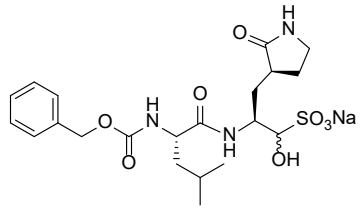
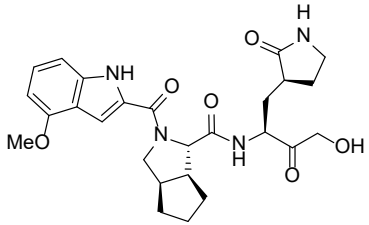
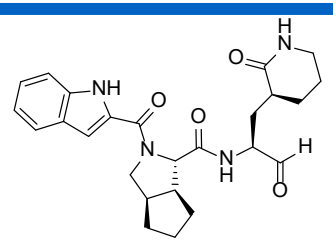
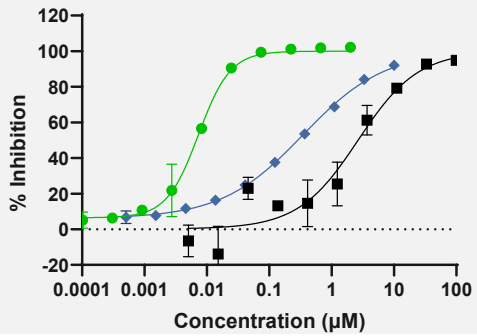
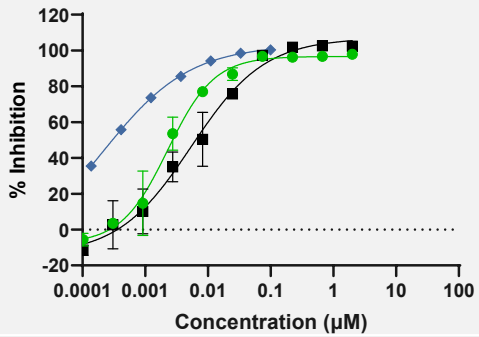
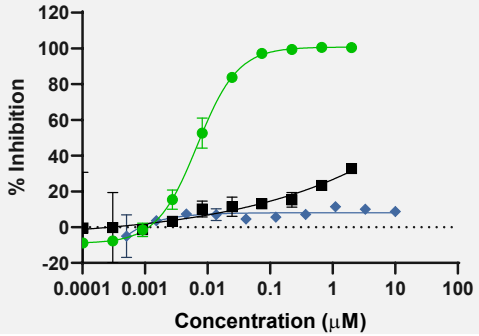
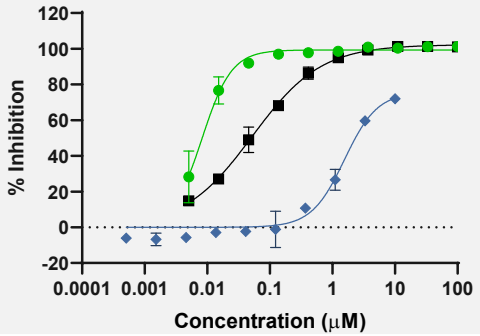
Biochemical Activity ALG-097111 and ALG-097070 vs. PF-00835231 and GC-376









	PF-00835231	GC-376	ALG-097111	ALG-097070
IC_{50} (μM)				
3CLpro	0.005	0.025	0.007	0.010
HRV3C	1.82	0.042	>2	0.049
Cathepsin L	0.155	<0.0005	>10	1.63

Higher SARS-CoV-2 3CLpro selectivity over CatL observed for ALG-097111 and ALG-097070

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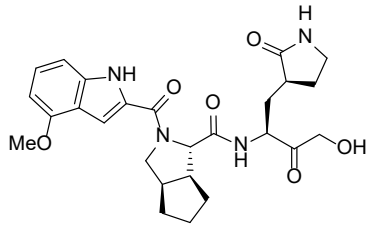
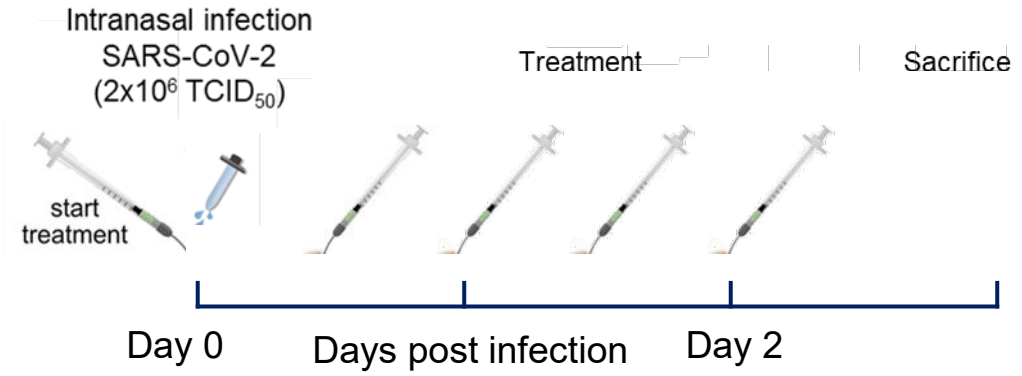
SARS-CoV-2 Hamster Model with ALG-097111 and ALG-097200

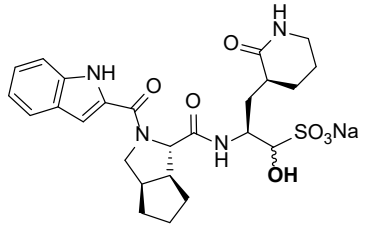


ALG-097111

SARS-CoV-2 (A549+ACE2):
EC_{50}= 200 nM



Group	N	Treatment	mg/kg/dose	Dosing Frequency	Dose route
Group 1	5	**Vehicle/RTV**	50	BID	SC/PO
Group 2	5	**Molnupiravir**	200	BID	PO
Group 3	5	**ALG-097111/RTV**	200/50	BID/BID	SC/PO
Group 4	5	**ALG-097200/RTV**	200/50	BID/BID	SC/PO



ALG-097200
Prodrug of
ALG-097070

SARS-CoV-2 (A549+ACE2):
EC_{50}= 757-1122 nM

ALG-097111 and ALG-097200 were dosed SC in a SARS-CoV-2 Hamster model in a prophylactic setting with molnupiravir as control

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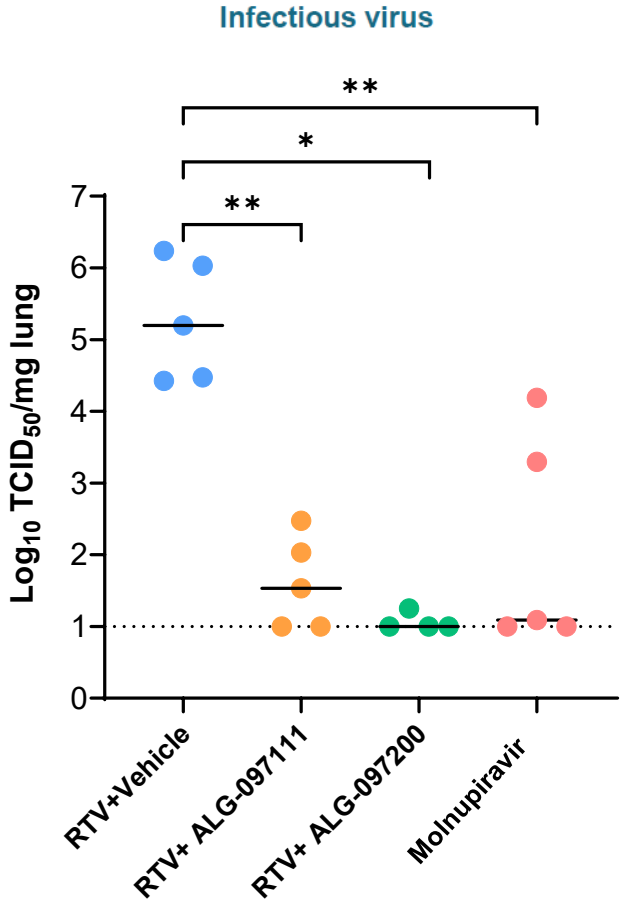
Vandyck K, et al. Biochem Biophys Res Commun. 2021 May 28;555:134-139.
doi: 10.1016/j.bbrc.2021.03.096

SARS-CoV-2 Hamster Model with ALG-097111 and ALG-097200

- ALG-097111 and ALG-097200 significantly reduced infectious virus titers in the lungs of treated hamsters
- Plaque assay confirmed with reduction in viral RNA loads (not shown)
- Hamster plasma and lung C_{trough} (based on PK in uninfected hamster):

  ALG-097111: 5.5 and 3-fold SARS-CoV-2 A549-ACE2 EC_{50}

  ALG-097070: 7-10 and 11-16 fold SARS-CoV-2 A549-ACE2 EC_{50}
- Molnupiravir reduced the infectious virus titers by 3.3 log with some variations among treated hamsters.
- ALG-097111 and ALG-097200 were tool-compounds and optimization continued to improve oral availability and potency, resulting in the selection of **ALG-097558**



First report of SARS-CoV-2 inhibition in SARS-CoV-2 hamster model for a selective protease inhibitor

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Vandyck K, et al. Biochem Biophys Res Commun. 2021 May 28;555:134-139. doi: 10.1016/j.bbrc.2021.03.096.

ALG-097558
Superior Biochemical Potency Against SARS-CoV-2

SARS-CoV-2 3CLpro	IC_{50} (nM)[1]	Hillslope	K_i (nM)
Nirmatrelvir	2.92	0.91	2.03
PBI-0451	3.6	1.74	3.4
Ensitrelvir	4.0	1.31	2.6
ALG-097558	**0.26**	**1.99[2]**	**0.074**

ALG-097558, a reversible covalent binder, K_i is 27-46 fold more potent vs. competitors in the 3CLpro biochemical assay

[1]The assays were performed with 0.3 nM 3CLpro.
[2]High hillslope (>1.5) is indicative of enzyme titration.

ALG-097558
Superior Cell-Based Potency Against SARS-CoV-2 and CoV

	Virus	Variant/Cell line	EC_{50} (μM)			
			PBI-0451	Ensitrelvir	Nirmatrelvir	ALG-097558
Beta	SARS-CoV-2	**Wuhan** Vero E6+CP	n.d.	n.d.	0.116	0.010
		B.1.1.7 (alpha) A549-ACE2-TMPRSS2	0.038	0.023	0.099	0.012
		B.1.617.2 (delta) A549-ACE2-TMPRSS2	0.126	0.141	0.217	0.013
		B.1.1.529 (omicron) VeroE6-GFP+CP	0.136	0.095	0.059	0.008
	SARS-CoV-1	Vero-E6+CP	0.323	0.154	0.173	0.022
	OC43 (β-hCoV)	HeLa	0.168	n.d.	0.047	0.009
Alpha	229E (α-hCoV)	Huh-7	0.281	6.30	0.476	0.017

ALG-097558 demonstrates pan-coronavirus antiviral activity
ALG-097558 is more active than PF-07321332, PBI-0451 and S-217622 across all CoV's tested

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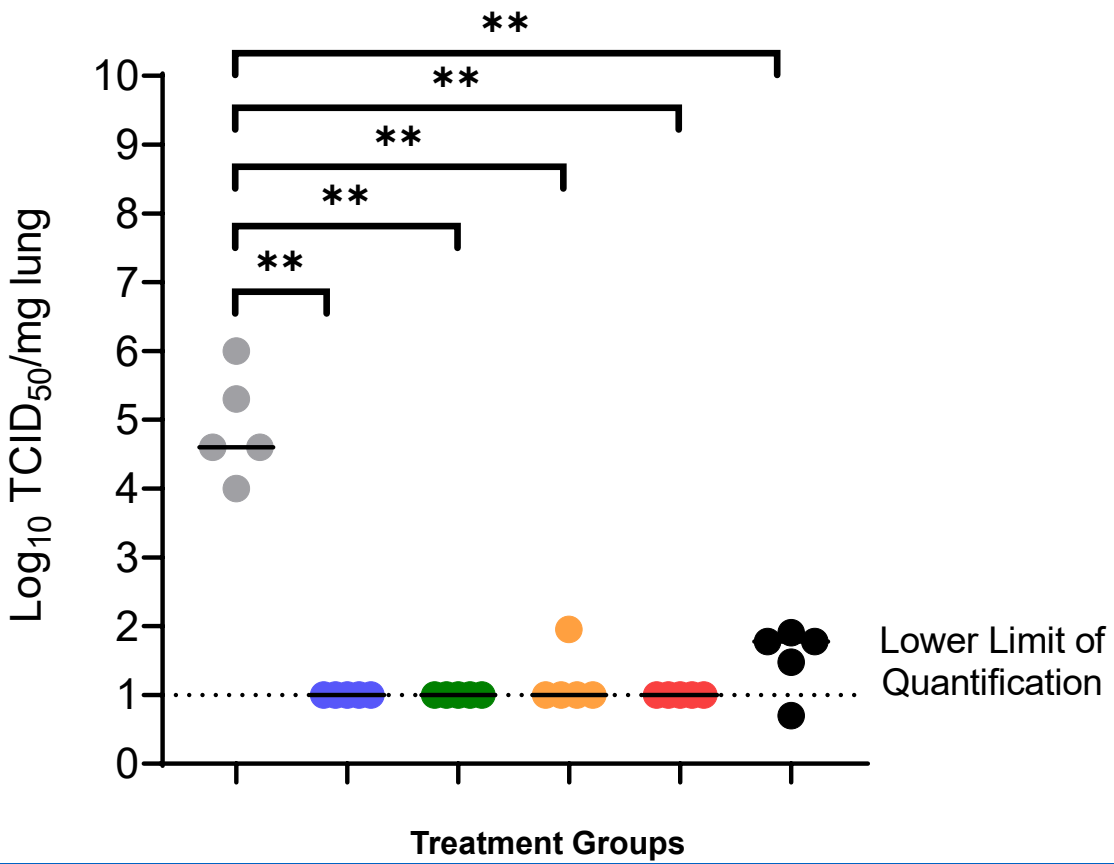
Aligos data.

ALG-097558
Oral Prophylactic Treatment in the SARS-CoV-2 Hamster Model

Amount of Infectious Virus at Day 4



Dosing (BID)

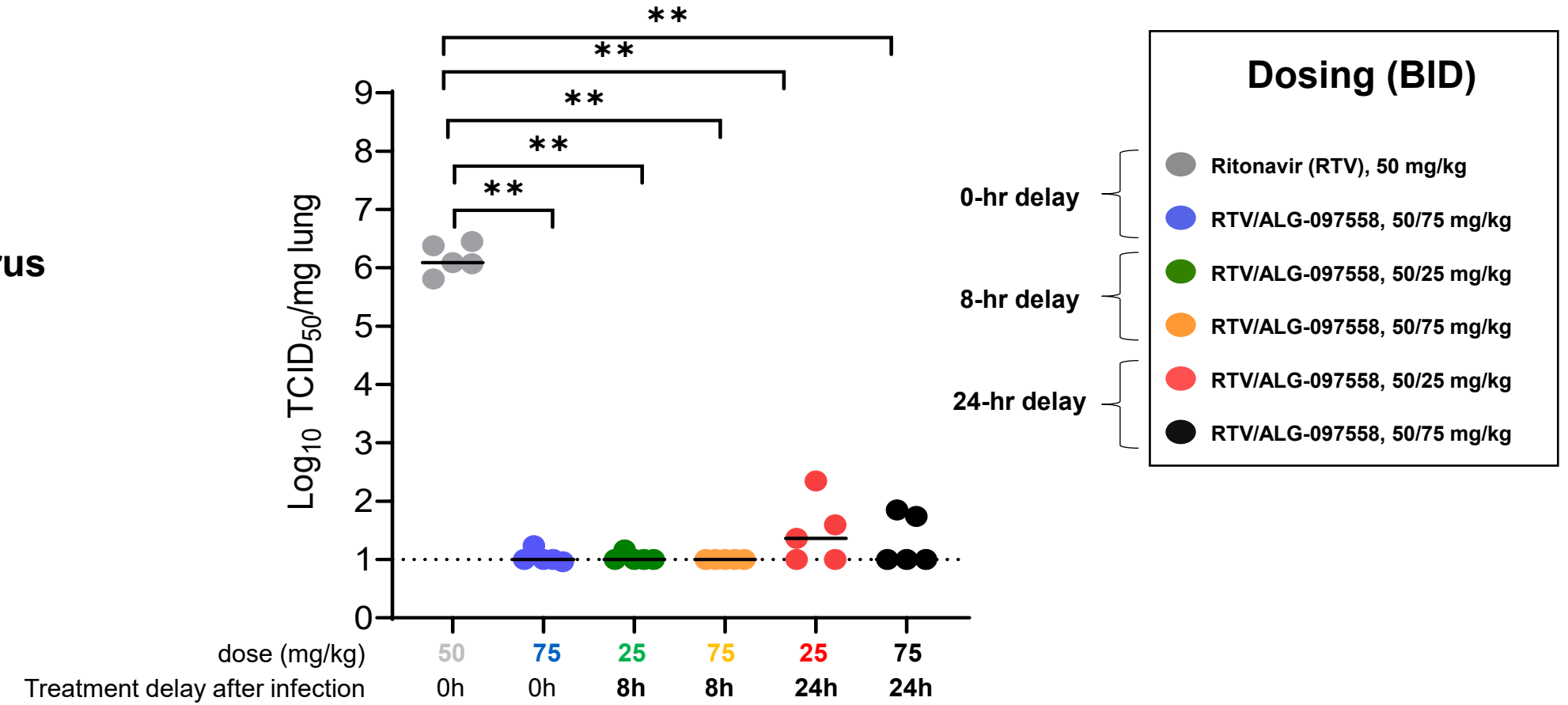
- Ritonavir (RTV), 50 mg/kg
- RTV/ALG-097558, 50/25 mg/kg
- RTV/ALG-097558, 50/50 mg/kg
- RTV/ALG-097558, 50/100 mg/kg
- RTV/ALG-097558, 50/150 mg/kg
- Nirmatrelvir, 250 mg/kg

Significant reduction in infectious virus titers after **oral prophylactic treatment** with ALG-097558
Use of ritonavir is only needed in the hamster model

5 Hamsters / group, intranasal infection, B.1.617.2 (1 x 10^4 $TCID_{50}$).
Treatment started before infection, administered by oral gavage BID over 3-days.
RTV administered 1 h before the ALG cpd, RTV and ALG-097558 formulated as a solution in 43% Ethanol + 27% PG in water.
Sacrifice and assessment of infectious virus in lung at day 4.

ALG-097558
Oral Therapeutic Treatment in the SARS-CoV-2 Hamster Model

Amount of Infectious Virus at Day 4



Significant reduction in infectious virus titers after **oral therapeutic treatment** with ALG-097558
Use of ritonavir is only needed in the hamster model

Treatment started before or after infection as indicated, administered by oral gavage BID over 3-days.
RTV administered 1 h before the ALG cpd, RTV and ALG-097558 formulated as a solution in 43% Ethanol + 27% PG in water.
Sacrifice and assessment of infectious virus in lung at day 4.

Summary and Next Steps

- Collaboration with KU Leuven/Rega Institute/CD3, resulted in the identification of highly potent and selective 3CL protease inhibitors with broad anti-Coronaviral activity
- **ALG-097111** and **ALG-097200** were used as tool compounds to confirm *in vivo* activity of selective SARS-CoV-2 3CLpro inhibitors in a hamster model.
- **ALG-097558**
  - › Shows high potency *in vitro* in biochemical and cell-based assays
  - › High potency *in vivo* in a SARS-CoV-2 hamster model (prophylactic and therapeutic oral dosing)
  - › Broadly active against a diverse range of coronaviruses
  - › PK profile in preclinical species predicts a projected human oral efficacious dose of 350-600 mg BID without ritonavir
  - › Phase 1 enabling nonclinical studies ongoing, Phase 1: H1 2023

Centre for Drug Design and Discovery (“CD3”)

Thank you!

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KU Leuven

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