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Structure-based discovery of potent and selective SARS-CoV-2 3-chymotrypsin-like cysteine protease inhibitors using a multiplex screening platform

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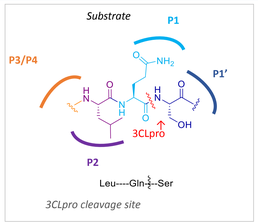
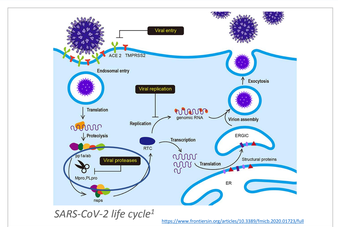
Background:

The 3-chymotrypsin-like cysteine protease (3CLpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is considered a major target for the discovery of direct antiviral agents. Currently, at least two 3CLpro inhibitors are in (pre)clinical development for the treatment of COVID-19: GC-376 and PF-07304814 (a prodrug of PF-00835231). Both agents exhibit different degrees of cathepsin L inhibition. Cathepsin L has been shown to block SARS-CoV-2 infection of human and monkey cells by proteolytic cleavage of the SARS-CoV-2 spike protein. Therefore, it can be hypothesized that the cell-based activity of several 3CL protease inhibitors described in literature is at least partially mediated by inhibition of host cathepsin L.

SARS-CoV-2 3CLpro (Mpro) Represents an Attractive Target

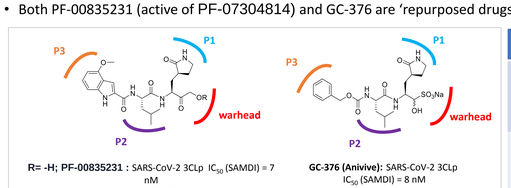




- 3CLpro is conserved within the group of CoVs, and in a related form, by members of the Enterovirus genus (picornavirus)
- 3CLpro operates at multiple sites on the large polyprotein 1ab
- No human homologue of 3CLpro

SARS-CoV-2 3CLpro (Mpro) Inhibitors

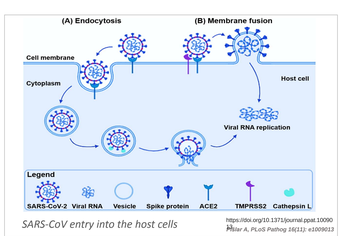
- At least two 3CLpro inhibitors have been reported in early development for treating SARS-CoV-2 infection
- Both PF-00835231 (active of PF-07304814) and GC-376 are 'repurposed drugs' for SARS-CoV-2

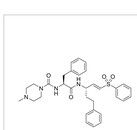


IV	F%	$t_{1/2}$
PF-231[2]	<0.1-1.4% (monkey/ rat)	1-1.6h (Rat/dog/ Monkey)
GC-376[3]	3% (rat)	1.3 h (rat)

- Both PF-07304814 and GC-376 are injectable drug candidates with limited oral bioavailability and short half-life

Cathepsin L Regulates SARS-CoV-2 Entry into Host Cells





Protease activity	Ki (μM)
Cathepsin L	0.005
Cathepsin B/K/S	3.0/0.4/0.002
SARS-CoV-2 3Cl Pro/PL	>100

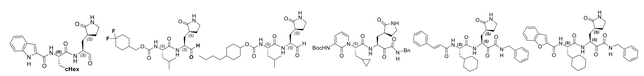
SARS-Cov-2 activity	EC_{50} (μM)
VeroE6	0.07-0.62
A549/Hela-ACE2	<0.08/0.004
Calu-3	3.7

https://doi.org/10.1101/2020.10.23.347534

Biochemical and cellular activity of K11777[4]

- Cathepsin L is involved in SARS-CoV-2 entry into host cells
- Cathepsin L inhibitors have demonstrated cellular potency versus SARS-CoV-2 in different cell lines
- Monitoring Cathepsin L inhibition of SARS-CoV-2 $3CL_{pro}$ inhibitors, is considered essential to analyze their intrinsic cellular potency related to on target $3CL_{pro}$ inhibition

Reported SARS-CoV-2 3CLpro Inhibitors vs. Cathepsin L

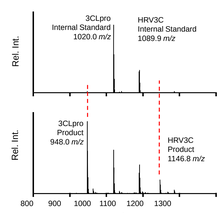


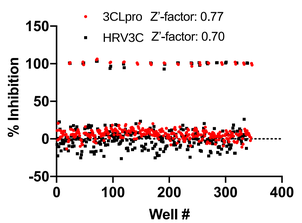
	Cpd 11a[5]	6j[6]	6e[6]	Cpd 13b[7]	Cpd 11r[8]	A9[9]
	W Dai et al, Science (2020)	Rathnayake, A. et al Science Translational Medicine (2020)		L. Zhang et al., Science (2020)	L. Zhang et al J.Med Chem (2020)	WO2020030143
Cathepsin L IC_{50} (nM)	<0.5	<0.5	<0.5	214	<0.5	<0.5
SARS-CoV-2 IC_{50} (nM)	7	11	9	510	1290	7118
SARS-CoV-2 Huh-7 EC_{50} (nM)*	<50	<50	242	3676	2	<46

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

- The majority of reported SARS-CoV-2 Mpro inhibitors are also cathepsin L inhibitors

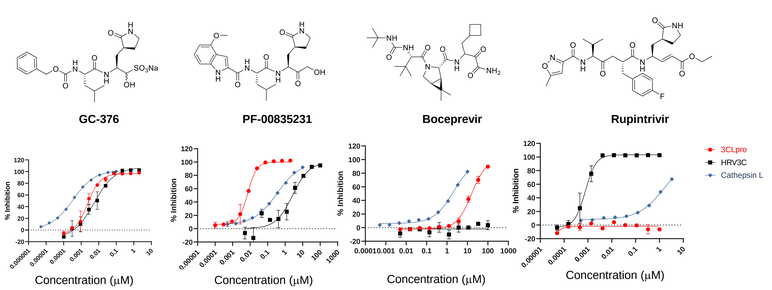
Development of a Multiplex 3CLpro/HRV3C Protease Assay





- The 3CLpro/HRV3C protease assay is based on mass spectrometry relies on high enzyme/substrate specificity
- Each compound was tested against 3CLpro/HRV3C and cathepsin L (FRET assay)

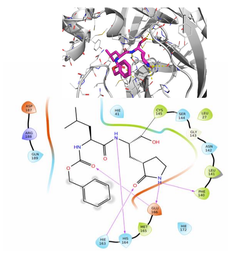
Activity of Selected Compounds vs. SARS-Cov-2 3CLpro, HRV3C and Cathepsin L



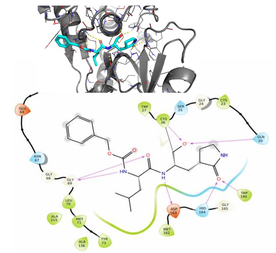
- GC-376 inhibits cathepsin L more potently than 3CLpro and HRV3C
- PF-00835231, boceprevir, and rupintrivir all show some degree of 3CLpro selectivity

Structure Analysis of GC-376 Bound to SARS-CoV-2 3CLpro/Cathepsin L

XRD of GC-376 in SARS-CoV-2 $3CL_{pro}$ (7c6u)



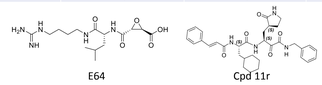
Docking of GC-376 in Cathepsin L (based on 3OF8)



- Despite very low identity overlap (<10%), GC-376 is active on both SARS-CoV-2 3CLpro and cathepsin L

SARS-CoV-2pro Inhibitors can Achieve a High Degree of Selectivity towards Cathepsin L

Compound ID	Cathepsin L 84 pM Substrate 10 uM IC_{50} (nM)	Cathepsin L 50 pM Substrate 10 uM IC_{50} (nM)	Cathepsin L 50 pM Substrate 20 uM IC_{50} (nM)	Cathepsin L 10 pM Substrate 2 uM IC_{50} (nM)	Cathepsin L 10 pM Substrate 2 uM IC_{50} (nM)
E64	1.9	4.4	4.1	4.9	5.6
Cpd 11r	<0.5	0.21	0.17	0.18	0.35
PF-00835231	2,250	1,308	1,085	456	427
ALG-097111	>>10,000	>>10,000	>>10,000	>>10,000	>>10,000

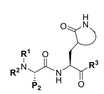


E64 Cpd 11r PF-00835231 ALG-097111/CIM412

- Unlike all SARS-CoV-2_{pro} tested benchmarks, ALG-097111 did not inhibit Cathepsin L protease activity

Conclusions and Perspectives

	ALG-097111/CIM412	ALG-161/CIM780
SARS-CoV-2 3CLp IC_{50} (SAMDI)/Ki(FRET) (μM)	0.008/0.001	0.008/-
Cathepsin L IC_{50} (μM)	>10	>10
SARS-Cov2 Huh-7 EC_{50}/CC_{50} (μM)	1.3 (n=2)/ >100	ongoing
OC43(Hela) EC_{50} /CC_{50} (μM)	0.134 (n=3)/>100	0.057 (n=2)/>100
HLM $t_{1/2}$ (min)	>60	49.5
Human Hepatocyte $t_{1/2}$ (min)	>360	ongoing
Kin Sol (μM) PBS/Fassif/FeSSIF/pH2	>500	ongoing
CYP inhibition at 10 uM	All <50%	ongoing
Plasma stability (R/H) $t_{1/2}$ (min)	>480 ea	ongoing
SIF + Pancreatin/SGF $t_{1/2}$ (min)	>480 ea	ongoing



- SARS-CoV-2 3CLpro (Mpro) represents an attractive target
- Cellular potency of reported SARS-CoV-2 3CLpro is at least partially mediated by Cathepsin L protease activity
- We have developed a multiplex protease assay that was used to optimize our series of SARS-CoV-2 3CLpro inhibitors
- Our lead compound ALG-097111 is a potent SARS-CoV-2 3CLpro inhibitor, which did not inhibit Cathepsin L protease activity up to the highest concentration tested (IC_{50}>10μM)
- ALG-097111 exhibits promising drug-like properties

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