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Molecular Basis for the Antiviral Action of EDP-235: A Potent and Selective SARS-CoV-2 3CLpro Inhibitor

Anand Balakrishnan

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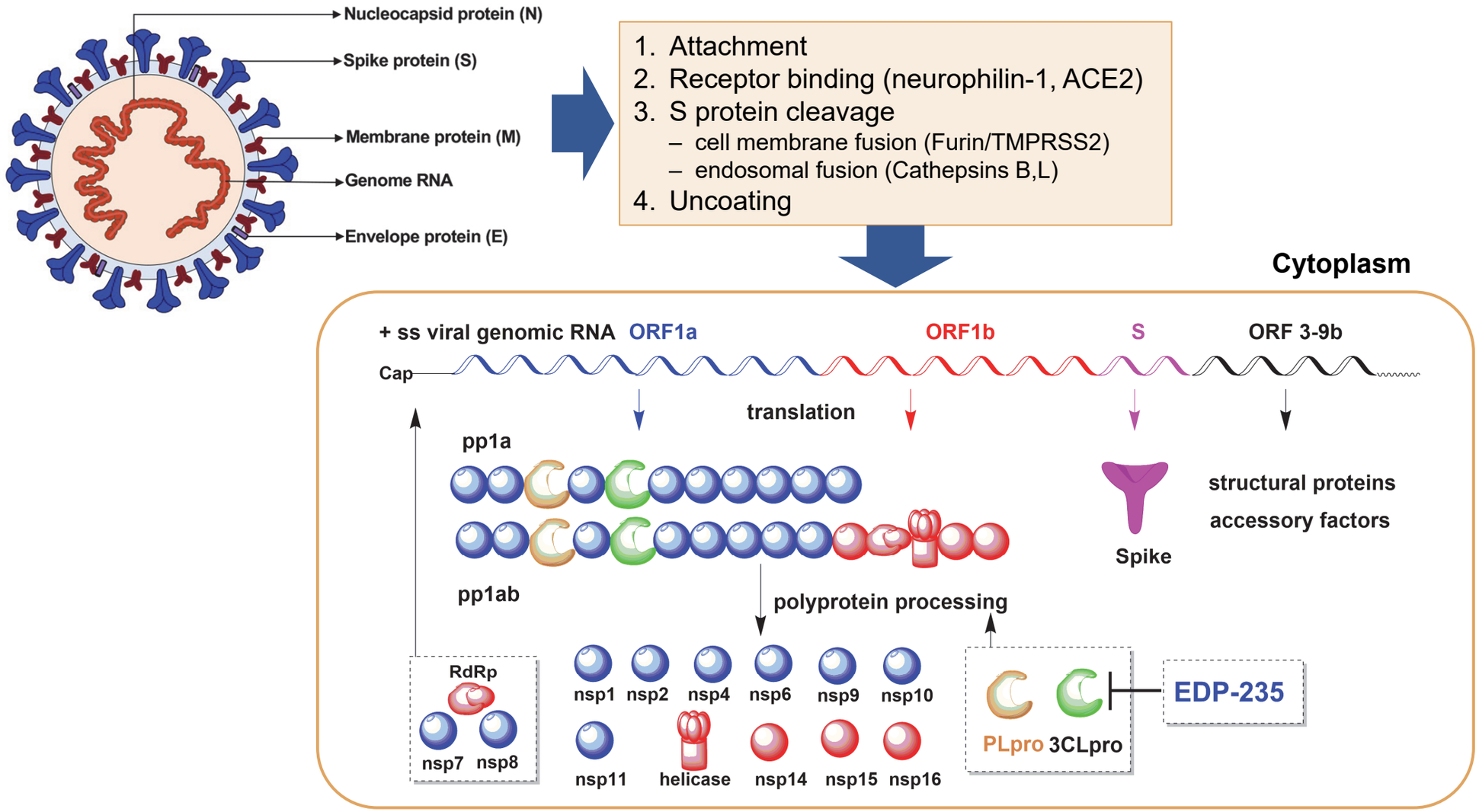
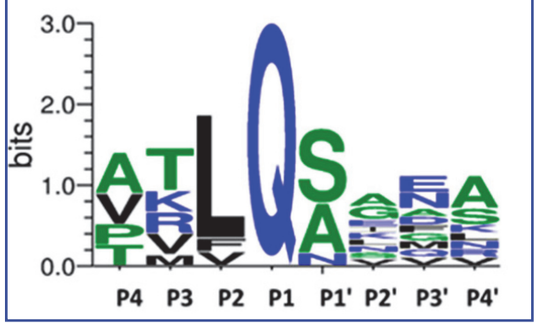
SARS-CoV-2 life cycle and enzyme targets for antiviral development

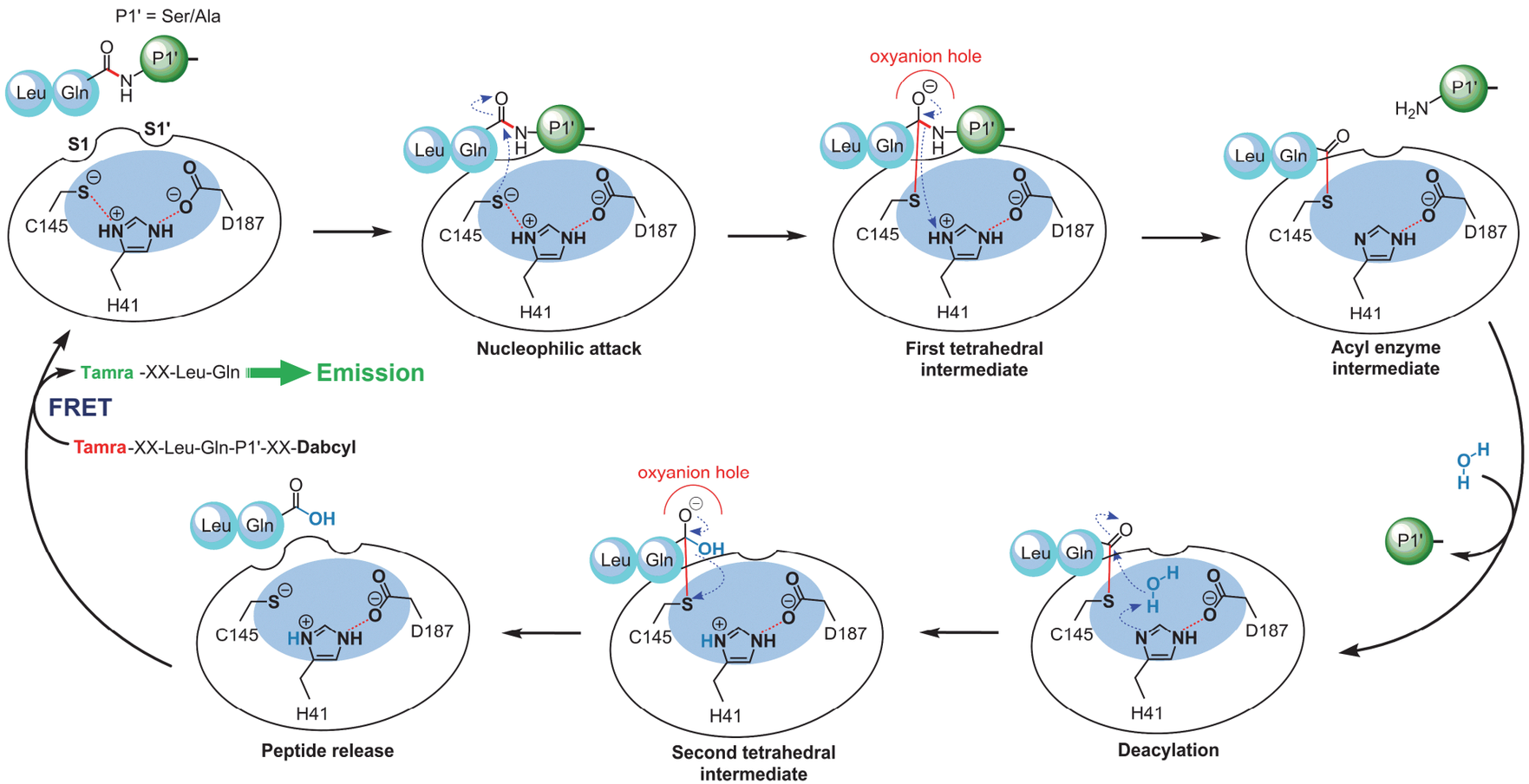


Signal Transduction and Targeted Therapy (2021). 6:233

Catalytic cycle of 3C-like protease from SARS-CoV-2

3CLpro Consensus Site



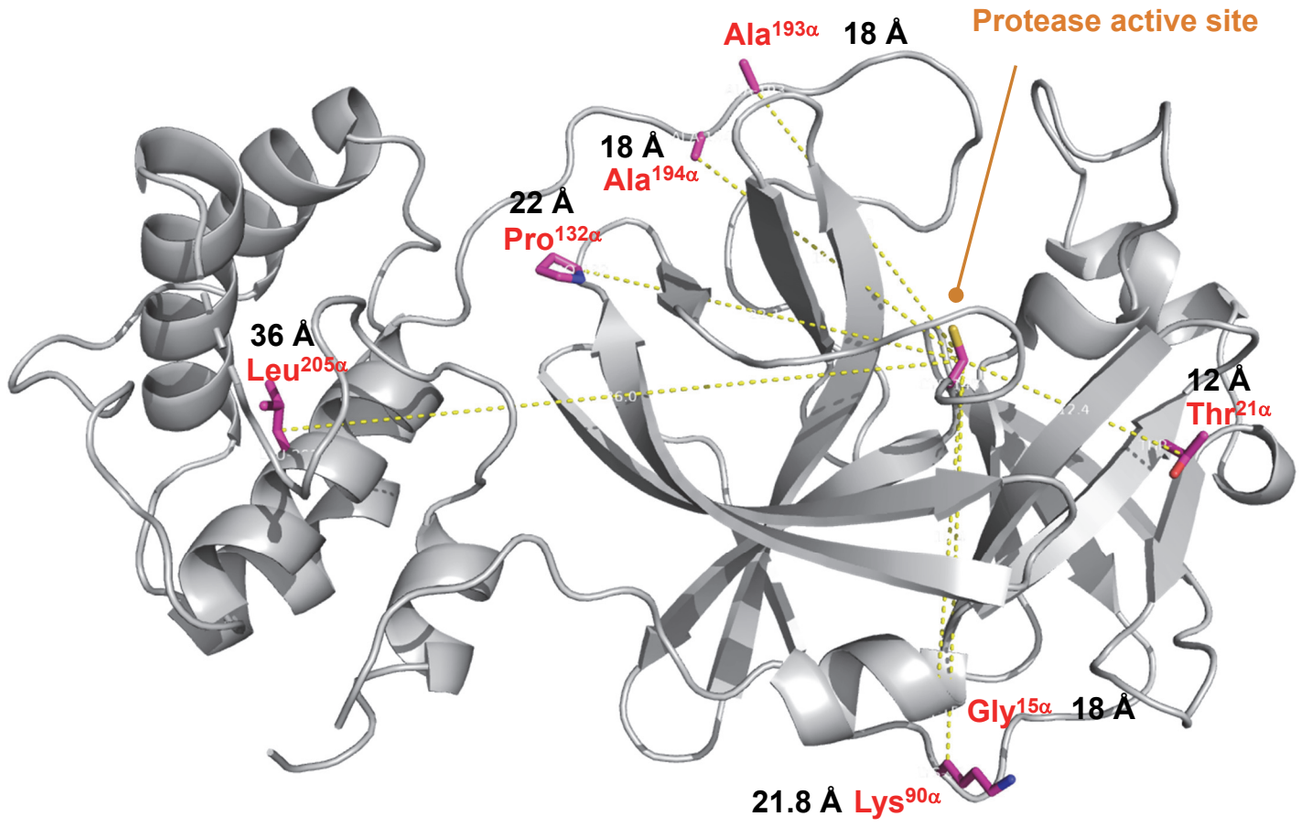


Catalytic activity monitored with FRET [fluorescence resonance energy transfer] assay

Int. J. Mol. Sci. (2020), 21(24), 9523

EDP-235 is a highly potent 3CLpro inhibitor and retains activity against SARS-CoV-2 variants

- 3CLpro is highly conserved across SARS-CoV-2 variants
- All variant enzymes were active in protease biochemical assays



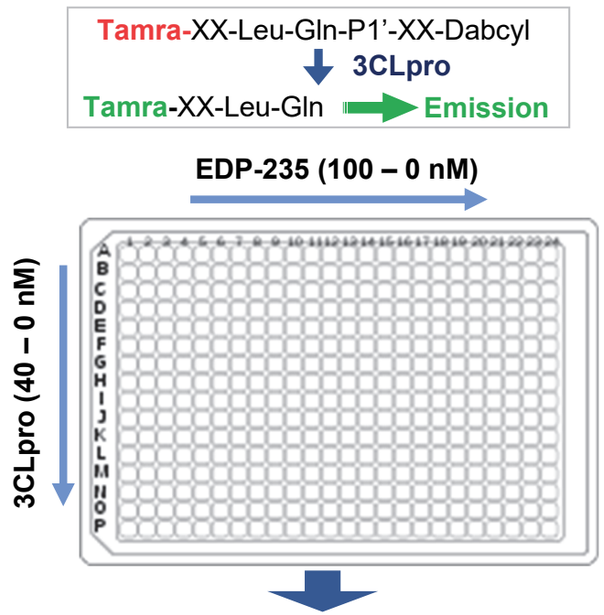
3CLpro Enzyme Assay			
SARS-CoV-2 Lineage	WHO Classification	3CLpro Mutation	EDP-235 IC_{50} (nM)
A	n/a	-	5.8 ± 3.7
B.1.351	Beta	K90R	2.8 ± 0.9
B.1.351.2	Beta	K90R/A193V	5.4 ± 1.0
P.2	Zeta	L205V	3.4 ± 1.0
B.1.617.3	n/a	A194S	5.7 ± 0.5
B.1.1.318	n/a	T21I	2.0 ± 0.1
C.36.3, C.37	n/a	G15S	4.7 ± 2.5
B.1.1.529	Omicron	P132H	4.1 ± 0.8

Live Virus			
SARS-CoV-2 Lineage	WHO Classification	3CLpro Mutation	EDP-235 EC_{50} (nM)
A	n/a	-	5.1
B.1.617.2	Delta	-	4.3
B.1.1.529	Omicron	P132H	7.3

IC_{50} = half-maximal inhibitory concentration. *The 3CLpro sequences for the ancestral A lineage and B.1.617.2 (Delta) variant are identical. Antiviral activity determined in the presence of a p-glycoprotein inhibitor.

EDP-235 is a tight-binding inhibitor of SARS-CoV-2 3CLpro

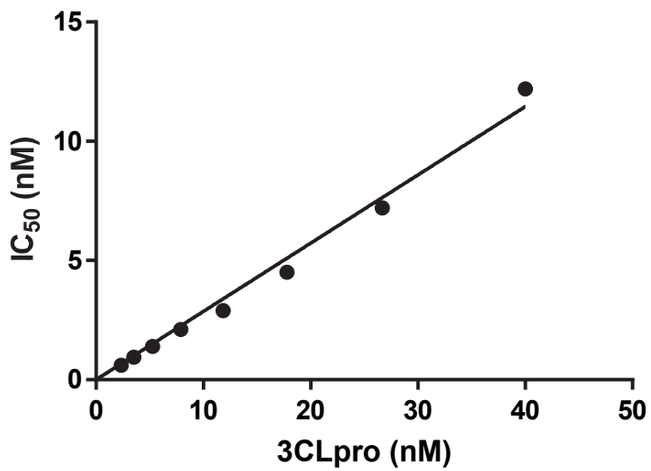
Experimental Setup



1. Incubate at RT for 30 minutes
2. Add substrate
3. Kinetic readout over 1 hour

RT = room temparture

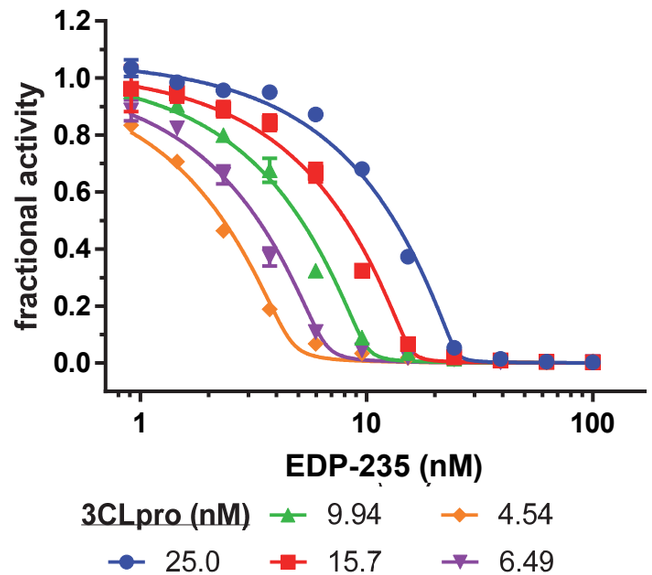
IC_{50} vs. 3CLpro Concentration



The IC_{50} of EDP-235 increases linearly with increasing 3CLpro

$$IC_{50} = 0.5*[3CLpro] + K_i^{app}$$

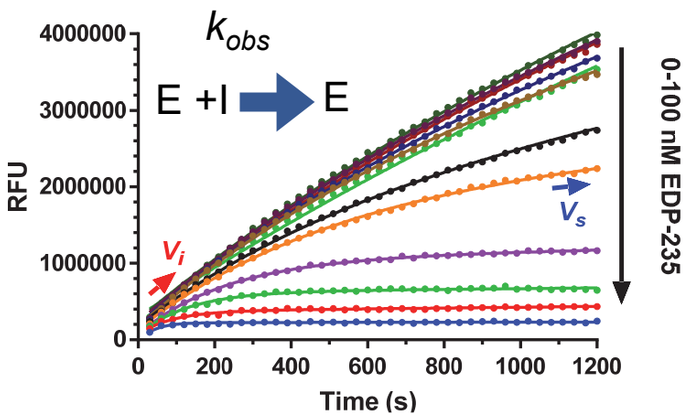
Morrison Fit Analysis for K_i^{app}



$$K_i^{app} = 0.1 \pm 0.06 \text{ nM}$$

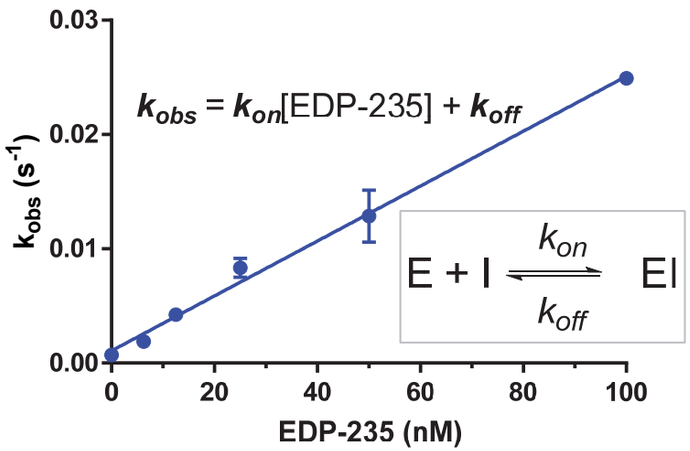
EDP-235 is a time-dependent inhibitor of SARS-CoV-2 3CLpro

Progress curves



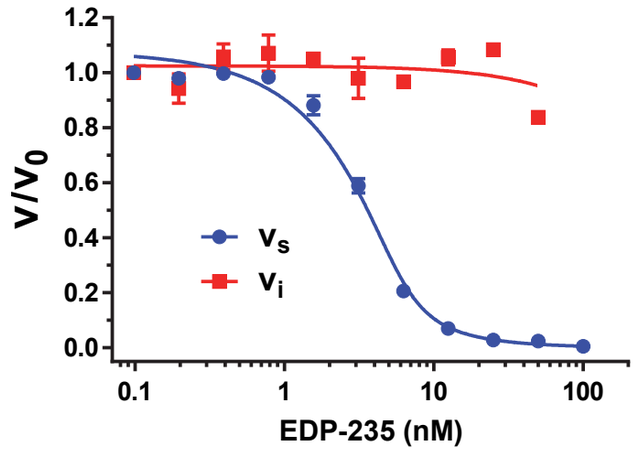
Slow onset of inhibition

k_{obs} vs. EDP-235 concentration



One-step reversible model

Velocity vs. EDP-235 concentration



v_s decreases with [EDP-235]

Parameter	Value Mean ± SD, n=3
k_{on}	(2.6 ± 1.6) x 10^5 $M^{-1}s^{-1}$
k_{off}	(9 ± 1) x 10^{-4} s^{-1}
K_i^{app}	4.7 ± 2.4 nM

~ k_{cat}/K_m = 2.1 x10^5 $M^{-1}s^{-1}$

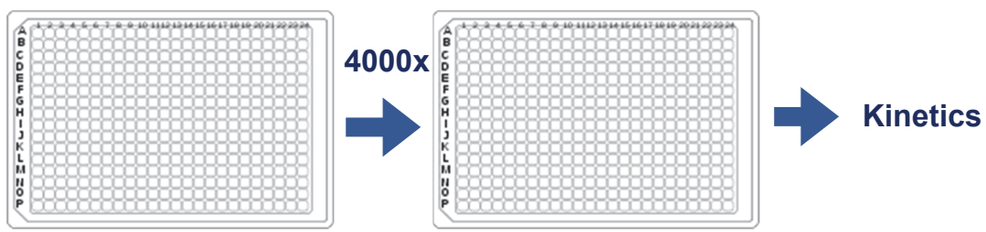
Antimicrobial Agents and Chemotherapy (2021). 65(4):e02577-20

EDP-235 is a reversible inhibitor of SARS-CoV-2 3CLpro

Experimental Setup

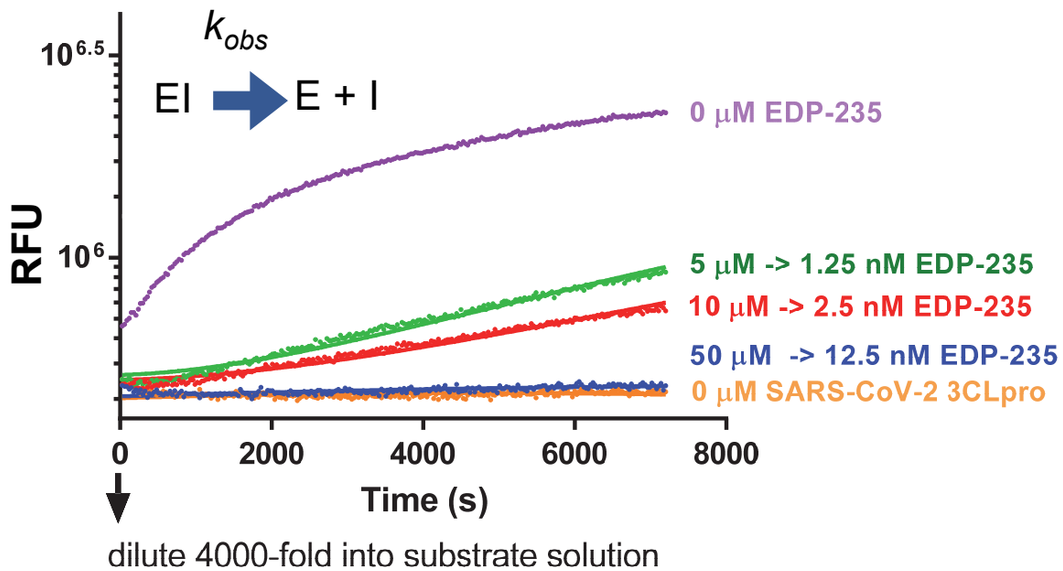
Preincubate enzyme with inhibitor

Rapid dilution into substrate solution



1. 3CLpro (5 µM)
2. EDP-235 (0, 5, 10, 50 µM)
3. 2 h at RT to form EI complex

Dilution Phase and Recovery of Activity



RT = room temparture
E = Enzyme
I = Inhibitor

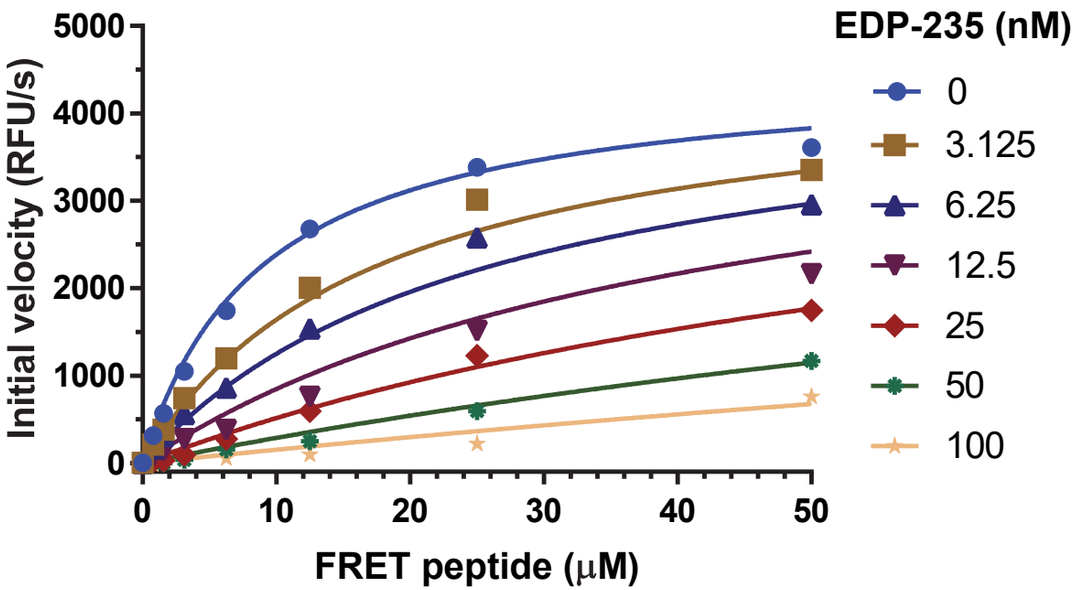
$$EI \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} E + I$$

$k_{obs} = (8.7 \pm 2.6) \times 10^{-4}\ s^{-1}$

corresponds to residence time ~ 19 min

EDP-235 is a substrate competitive inhibitor of SARS-CoV-2 3CLpro

Kinetic Analysis with Respect to Substrate



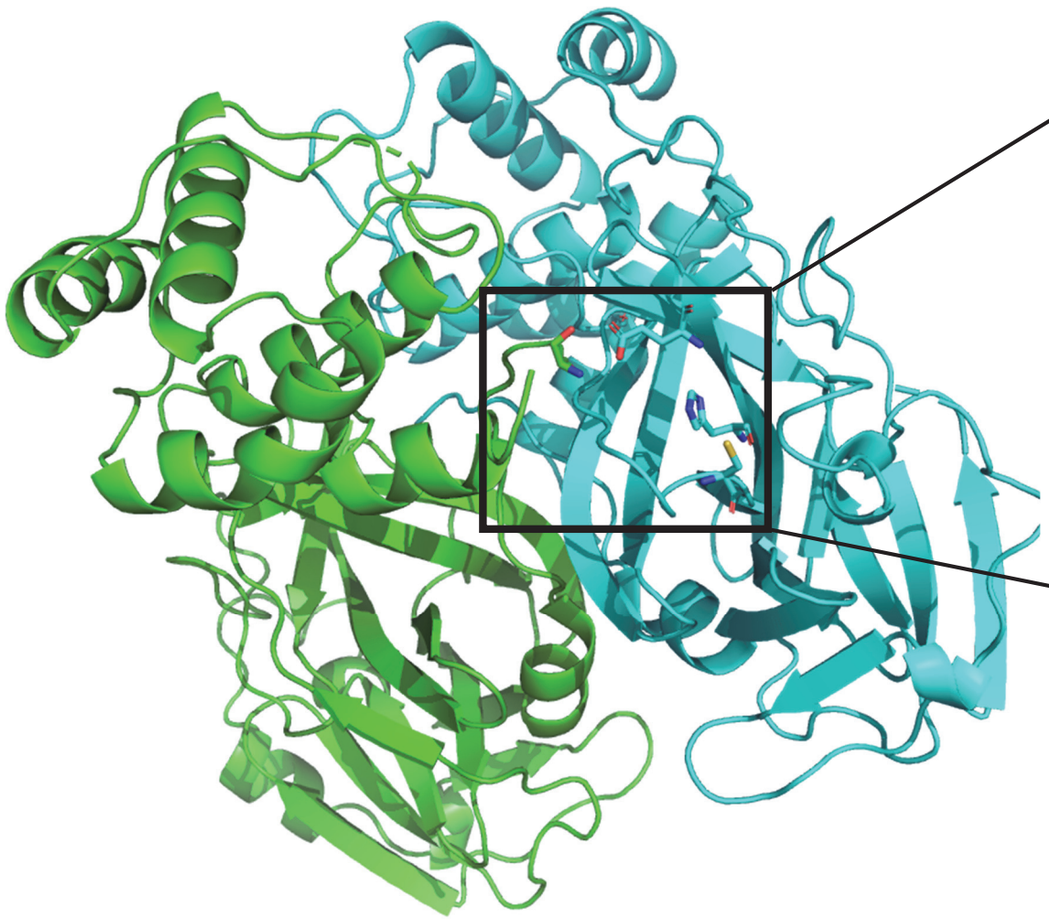
EDP-235 is a competitive inhibitor with respect to the FRET peptide substrate

Summary of Kinetic Mechanism Characterization

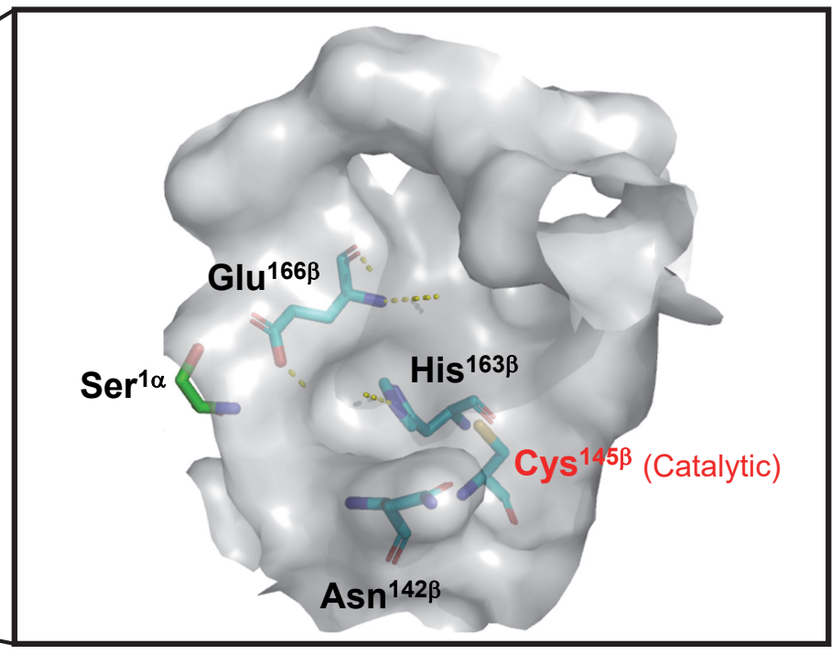
Parameter	Value
IC_{50}	5.8 nM
K_i	3.0 ± 1.6 nM
k_{on}	(2.6 ± 1.6) x 10^5 $M^{-1}s^{-1}$
k_{off}	(8.7 ± 2.6) x10^{-4} s^{-1}
Residence time	~ 19 min
Kinetic Mechanism	Time-dependent, reversible, Substrate competitive inhibition

EDP-235 analog binds at the active site of SARS-CoV-2 3CLpro

Quarternary structure of SARS-CoV-2 3CLpro dimer



Compound binding site with key residues



- Crystal structures of apo 3CLpro and co-structures of key compounds were obtained at 2.5-2.8 Å.
- 3CLpro crystallized as a dimer and active site residues make essential polar contacts with compounds.
- Structures provide support for mechanism of inhibition of EDP-235.

EDP-235 shows antiviral activity against all human coronaviruses

Virus	3CLpro Enzyme Assay IC_{50} (nM)	Live Virus Assay				
		Cell Type	Endpoint	EC_{50} (nM)	CC_{50} (nM)	SI
SARS-CoV-2	**5.8**	Vero E6*	CPE	**5.1**	>10,000	>1,960
HCoV-229E	**5.4**	MRC-5	CPE	**3.6**	>50,000	>13,889
HCoV-HKU1	**3.8**	-	-	-	-	-
HCoV-NL63	**1.8**	LLC-MK2	RT-qPCR	**6.1**	-	-
HCoV-OC43	**3.4**	HCT-8*	RT-qPCR	**56**	-	-
SARS-CoV	**1.9**	Vero E6*	CPE	**24**	>3,000	>125
MERS-CoV	**70**	Vero 76	CPE	**150**	>26,000	>173
			Viral Yield	**130 [EC_{90}]**	>26,000	>200

*Assay performed in the presence of a P-glycoprotein inhibitor (CP-100356, 2 μM) to prevent transporter-mediated efflux. CPE = cytopathic effect; P-gpi = P-glycoprotein inhibitor CP-100356 (2 μM); qPCR = quantitative polymerase chain reaction. HCoV-229E, HCoV-HKU1, HCoV-OC43, HCoV-NL63 = human coronavirus 229E, HKU1, OC43, and NL63, respectively; SARS-CoV = severe acute respiratory syndrome; MERS-CoV = Middle East respiratory syndrome. Vero 76 and Vero E6 cells are derived from African green monkey kidney epithelia, MRC-5 are human lung fibroblasts, HCT-8 are derived from a human ileocecal adenocarcinoma, and LLC-MK2 are Rhesus monkey epithelial cells. pHAEC = primary human airway epithelial cells.

EDP-235 shows highly selective inhibition of 3CLpro compared to human proteases

Cysteine Proteases
- SARS CoV 2 – PLpro, 3CLpro
- Caspases 1 – 11, 14
- Cathepsins B, C, D, E, G, H, K, L, S, V
- Papain, Calpain 1

Serine Proteases
- Trypsin
- TMPRSS 2
- Furin

Aspartyl protease
- BACE1

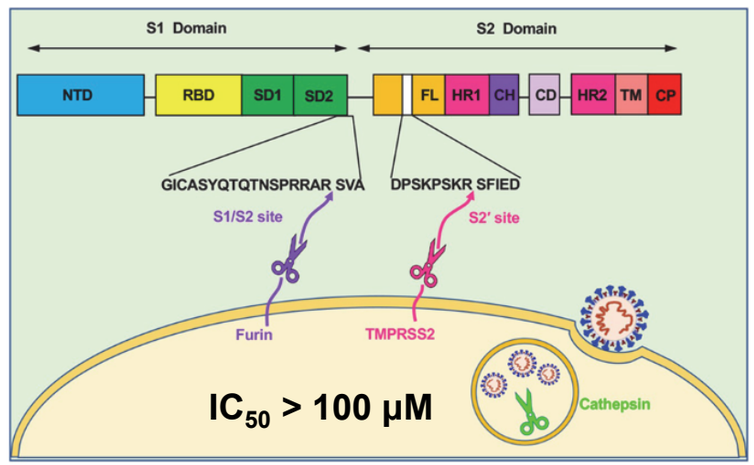
Zn metalloprotease
- ACE 1, 2

- Tested against 31 host proteases of diverse classes
- IC_{50} > 100 µM against 23 out of 31 including host proteases relevant to viral infection

Target	EDP-235 IC_{50} (µM)
Caspase 2	4.6
Caspase 3	4.2
Caspase 6	2
Caspase 7	4.7
Caspase 8	22.5
Caspase 9	4.7
Caspase 14	9
Cathepsin K	18.5
SARS-CoV-2 3CLpro	0.0058
Selectivity Index	**> 340**

Cysteine Proteases

Host proteases in viral entry/fusion



- ACE 2
- Cathepsin B
- Cathepsin L
- Trypsin
- TMPRSS 2
- Furin

1. *Signal Transduction and Targeted Therapy* (2021). 6:233
2. *Int. J. Mol. Sci.* (2020), 21(24), 9523

Molecular mechanism of EDP-235 action and its pharmacological effect

Molecular Observations	*In vitro* Pharmacology
Time-dependence/on-target residence time	High potency and antiviral efficacy
Substrate competitive /active site binder	Broad spectrum anti-coronaviral
Strong interactions with highly conserved active-site residues	High barrier to resistance
High degree of selectivity over other mammalian proteases	On target pharmacology and low off-target effects

Preclinical Properties		EDP-235[1]
Mechanism		**Protease Inhibitor**
Potency	Enzyme IC_{50} (nM)	**5.8**
	Vero Cell EC_{50} (nM)	**5.1**
Oral Bioavailability[2]		**95%**
Lung/Plasma ratio[3]		**4.1**
Projected Efficacious Dose		**100 – 500mg QD**

1. Jiang *et al.*, ISIRV Poster #120, Oct 19, 2021
2. Oral bioavailability in rats for EDP-235
3. AUC lung to plasma ratio in rats for EDP-235

Acknowledgements

Virology

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Guoqiang Wang

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