Broad-spectrum therapeutics against SARS-CoV-2 3CL protease

Ladislau C. Kovari[1], Franck Amblard[2], Keivan Zandi[2], Peng Liu[2], Shaoman Zhou[2], Kendall M. Muzzarelli[1], Iulia A. Kovari[1], Tami McBrayer[2], Raymond F. Schinazi[2]

[1]Department of Biochemistry, Microbiology, and Immunology, School of Medicine, Wayne State University, Detroit, MI 48201, USA

[2]Laboratory of Biochemical Pharmacology, Department of Pediatrics, School of Medicine, Emory University and Children's Healthcare of Atlanta, Atlanta, GA 30322, USA

BACKGROUND

Since the beginning of January 2020, over 1,200 clinical trials have been initiated, aiming to treat and prevent COVID-19 (clinicaltrials.gov). These unprecedented efforts have resulted in only modest results, and repurposed drugs may not achieve full therapeutic means to prove effective drug treatment options. Out of several ongoing clinical trials, two treatment options are effective, remdesivir and dexamethasone, to date, but the clinical outcomes are controversial. Given the current gap in coronavirus (CoV) therapy, novel drugs and vaccines development are needed. Our objective is to design and develop a safe and effective antiviral agent targeting the SARS-CoV-2 3CL protease (3CLpro) for the treatment of coronavirus disease 2019 (COVID-19) and human coronavirus infections in general. The central hypothesis is that inhibition of SARS-CoV-2 polyprotein cleavage results in inhibition of virus and that the drug can be used to prevent or treat COVID-19. Our research targets the 3CLpro, a key enzyme for SARS-CoV-2 polyprotein cleavage and viral replication using our established and proven drug discovery expertise.

METHODS

Our drug discovery platform includes *(1)* our proprietary libraries and medicinal chemistry expertise of viral protease inhibitors; *(2)* molecular modeling and virtual screening capabilities of millions of compounds using the Schrödinger programs and other drug discovery suites; *(3)* cell-based coronavirus assays in our in house BSL-2* and BSL-3 facility; *(4)* recombinant protein-based enzyme assays and X-ray crystallographic studies of SARS-CoV-2 3CLpro, and; *(5)* the capability of evaluating candidate inhibitors for bioavailability and ADME-T characteristics, and efficacy through animal models of COVID-19 such as golden Syrian hamsters and non-human primates.

RESULTS

We selected the nsp7/nsp8 cleavage site as representative for the SARS-CoV-2 3CLpro cleavage employing a covalent inhibitor design strategy. We decided to proceed with a P-side (P4-P1) inhibitor design. To further enhance inhibitor binding, we added an electrophilic warhead designed to react with the nucleophilic thiol group of Cys145 of the SARS-CoV-2 3CLpro. To develop highly potent and specific lead molecules, we targeted the catalytic Cys145-His41 dyad and other essential residues within the binding pocket which are important in the proteolytic process of SARS-CoV-2. We identified compound **3150**, a unique, non-toxic, small peptidomimetic molecule that inhibits structurally related viral 3CL proteases (*e.g.*, norovirus and enterovirus) with an EC_{50} of 1 to 20 nM in cell culture, and SARS-CoV-2 replication (in Vero cells - EC_{50} = 0.6 µM) with no apparent cytotoxicity up to 100 µM.

CONCLUSIONS

The expected outcome of this work is the discovery of submicromolar to low nanomolar COVID-19 inhibitors. The ultimate goal of the proposed studies is to speed-up and advance an anti-COVID-19 drug candidate to the stage of filing an investigational new drug (IND) application. The results will have a significant positive impact because they lay the groundwork for the clinical development of COVID-19 antiviral therapy and the potential to combine a potent and selective protease inhibitor with a nucleoside analog and anti-inflammatory drugs such as a JAK-STAT inhibitor with antiviral activity (*e.g.*, baricitinib) first in culture and then in an animal model.

ACKNOWLEDGEMENTS

WSU-SoM generously funded this project for LCK, a Vera Fay Righthand Fellowship in Virology to KMM, Emory University funding for FA, and a CFAR grant for RFS.

RESULTS

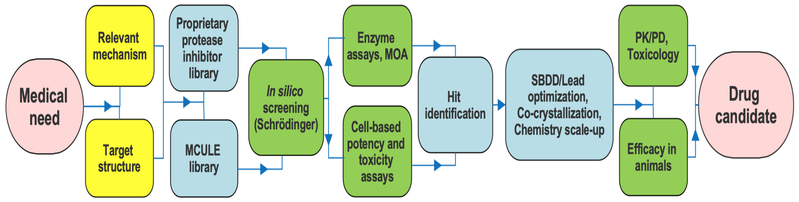


Fig. 1. Drug discovery diagram. The figure illustrates the activities and decision-making process for the project. MOA = mechanism of action. The MCULE library contains about 45 million compounds.

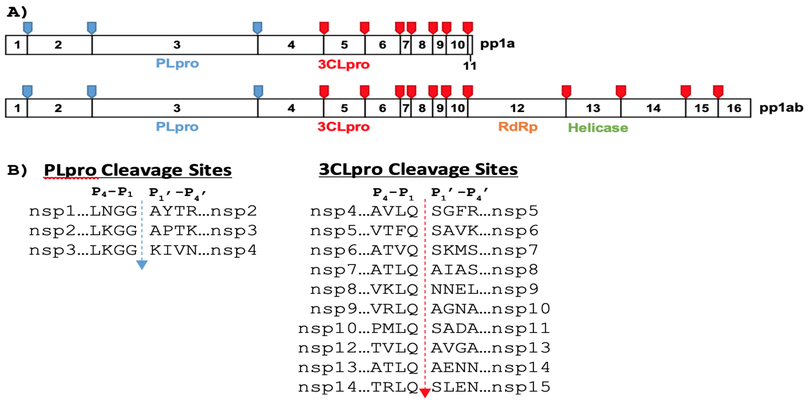


Fig. 2. SARS-CoV-2 replicase protein products. A) Translation of the replicase gene from the genomic RNA results in two polyproteins (pp1a and pp1ab) due to ribosomal frameshifting. The proteolytic processing of pp1a and pp1ab by PLpro and 3CLpro results in nsp1 through nsp16 proteins. Nsp11 is an oligopeptide generated when ribosomal frameshifting does not occur. The drug targets PLpro (part of nsp3), 3CLpro (nsp5), RNA-dependent RNA polymerase (nsp12), and RNA helicase (nsp13) are shown in blue, red, orange, and green color. PLpro cleavage sites are shown in blue color and 3CL cleavage sites in red. **B)** The three amino acid sequences of the predicted 2019-nCoV PLpro cleavage sites are shown on the left side, and the ten 3CLpro cleavage sites are listed on the right side of panel B.

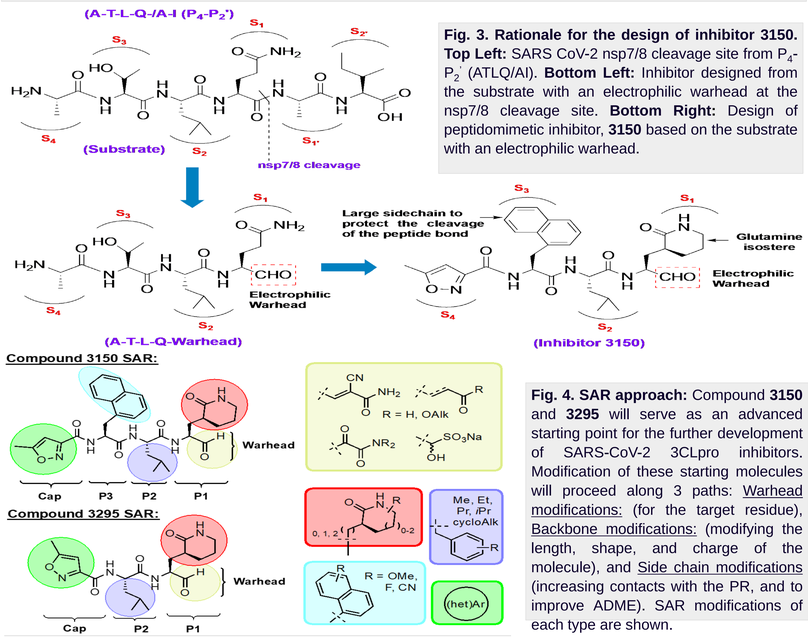


Fig. 3. Rationale for the design of inhibitor 3150. Top Left: SARS CoV-2 nsp7/8 cleavage site from P_4-P_2' (ATLQ/AI). **Bottom Left:** Inhibitor designed from the substrate with an electrophilic warhead at the nsp7/8 cleavage site. **Bottom Right:** Design of peptidomimetic inhibitor, **3150** based on the substrate with an electrophilic warhead.

Fig. 4. SAR approach: Compound **3150** and **3295** will serve as an advanced starting point for the further development of SARS-CoV-2 3CLpro inhibitors. Modification of these starting molecules will proceed along 3 paths: Warhead modifications: (for the target residue), Backbone modifications: (modifying the length, shape, and charge of the molecule), and Side chain modifications (increasing contacts with the PR, and to improve ADME). SAR modifications of each type are shown.

Table 1: Antiviral and toxicity profile of 3150, 3762, and related inhibitors. Remdesivir is used as a positive control. ND: Not Determined; mean of replicates shown. [a]maximal concentration tested, no inhibition observed.

Compound	Anti-SARS-CoV-2 activity in Vero cells (µM)		Anti-OC43 activity in Huh 7 cells (µM)		Cytotoxicity: MTT/MTS CC_{50} (µM) in			Therapeutic Index (TI) in Vero cells
	EC_{50}	EC_{90}	EC_{50}	EC_{90}	Huh7	Vero	PBM	
3150	**0.6**	**2.3**	**0.8**	**1.4**	72	**> 100**	20	**> 167**
3762	**1**	**2.3**	**0.4**	**0.6**	44.5	> 100	ND	> 100
3294	**1.3**	**3.0**	**8.9**	**> 10**	> 100	> 100	> 100	> 77
3114	**0.3**	**4.5**	**0.8**	**1.4**	11.2	**> 100**	43.8	**> 333**
3295	**3**	**4.6**	**8**	**10**	> 100	> 100	> 100	> 33
Remdesivir	1.2	3.6	0.04	0.09	2.1	> 100	4.5	> 80
Lopinavir	9.4	> 20	7.6	34.2	ND	13.1	> 100	~ 1
Ritonavir	> 2[a]	> 2[a]	> 2[a]	> 2[a]	ND	13.2	28.3	ND

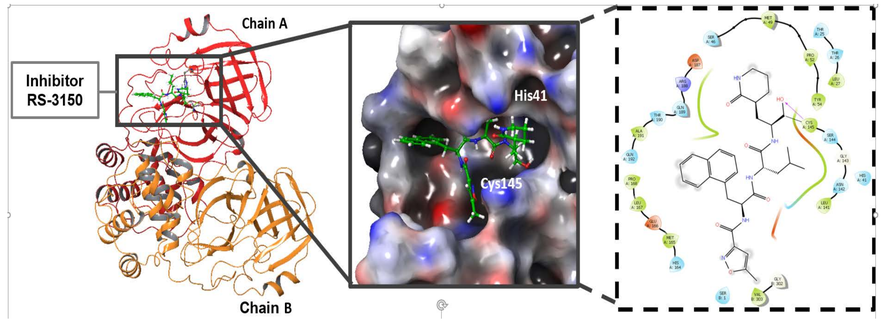
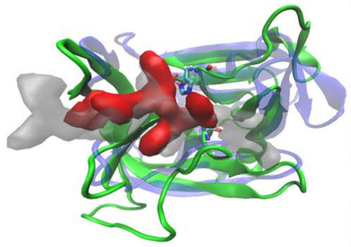
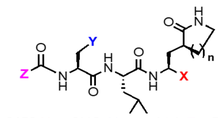


Fig. 5. Covalent docking of compound 3150 in the active site of the recently reported crystal structure of the SARS-CoV2 3CLpro (6Y2G.pdb). Left: Chain A is in red and chain B in orange. The inhibitor is shown in green. **Center:** The inhibitor (green) is docked in the active site of SARS-CoV2 3CLpro. The inhibitor is covalently attached to the active site residue Cys145. The catalytic residue His41 is also shown. **Right:** 2D interaction diagram of the inhibitor with key active site residues.



Compound	NV Inhibition (µM)		EV71 Inhibition (µM)	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
Rupintrivir*	0.30	1.5	0.014	0.032
RS-3115	0.60	1.7	0.03	0.12
RS-3150	0.020	0.090	0.001	0.007

Fig. 6. Broad-spectrum inhibitors of the viral 3CLprotease. Left: Superposition of the EV and NoV 3C-like protease (blue and green); bound substrate in red. **Right:** Potency data for the top compounds. *Rupintrivir data from Rocha-Pereira *et. al.*[1]



3150 X = -CHO, Y = naphtyl, Z = 5-Me-isoxazole, n = 1
3114 X = -CH(OH)SO_3Na, Y = naphtyl, Z = -OBn, n = 1
3115 X = -C=C(CN)$CONH_2$, Y = naphtyl, Z = -OBn, n = 1
3116 X = -CH(OH)CF_3, Y = naphtyl, Z = -OBn, n = 1
3117 X= -CH(OH)SO_3Na, Y= 4-F-phenyl, Z= -OBn, n = 1
3163 X = 1,3-dioxolane, Y = naphtyl, Z = -OBn, n = 0
3292 X = (*R*)-CH(OH)CN, Y = naphtyl, Z = -OBn, n = 1
3293 X = (*S*)-CH(OH)CN, Y = naphtyl, Z = -OBn, n = 1
3294 X = -CHO, Y = 4-F-phenyl, Z = 5-Me-isoxazole, n = 1
3441 X = -C=C-COOEt, Y = naphtyl, Z = 5-Me-isoxazole, n = 1
3133 X = -C(O)-CF_3, Y = naphtyl, Z = -OBn, n= 1

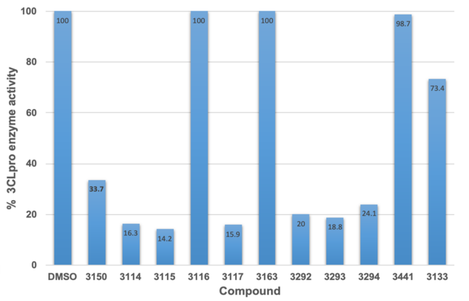


Fig. 7. Screening of inhibitors from our proprietary library against 3CLpro using the FRET assay. 10 µM of compound was incubated with 500 nM of SARS-CoV-2 3CLpro for 150 min at 23 ºC, and then 50 µM FRET substrate was added to the reaction mixture to initiate the reaction.