0712

Novel Class of Small Molecules Inhibits SARS-CoV-2 PLpro Through Molecular Glue-Induced Dimerization

Amanda M. Nevins[1*], Orville Pemberton[1*], Thomas E. Frederick[1*], Emily Nicholl[1], Myron Srikumaran[1], Jun Chen[1], Alla Korepanova[1], Vincent Stoll[1], Andrew Petros[1], Sujatha Gopalakrishnan[1], Justin Dietrich[1], Liliam Rios Cordero[1], **David J. Hardee[1]**, Teresa I. Ng[1], Chaohong C. Sun[1]

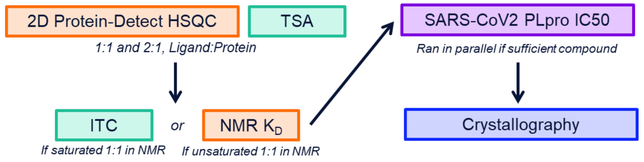
[1]AbbVie Inc., 1 North Waukegan Road, North Chicago, IL, USA , [*]equal contributors to this work

BACKGROUND

- SARS-CoV-2 papain-like protease (PLpro) represents a promising target for novel COVID-19 therapeutics.
- It has essential roles in viral replication and dysregulation of host innate immunity.
- Currently, there are no SARS-CoV-2 PLpro inhibitors in clinical development.

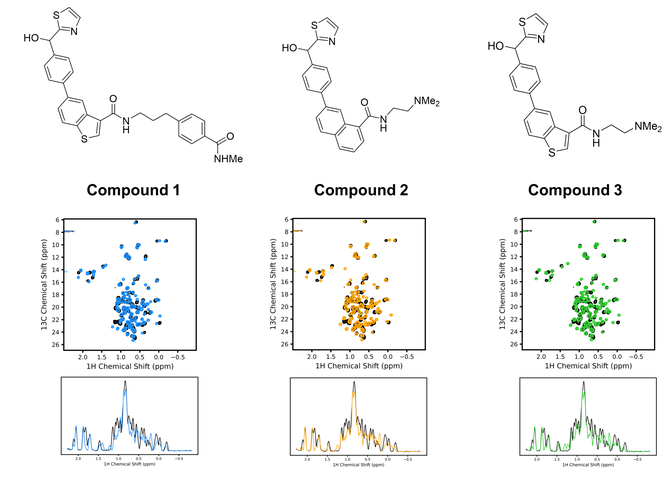
METHODS

- Identified non-covalent PLpro-binding small molecules containing diarylmethanol substructures from a DNA-encoded library (DEL) screen.
- Small molecules were evaluated in biochemical and virological assays to characterize antiviral potencies.
- Lead molecules were also evaluated in X-ray crystallography and 2D-NMR for MOA.



RESULTS

Verification of Diarylmethanol Binding by Protein-Detect NMR



X-Ray Crystal Structure Reveals Molecular-Glue Induced Dimer

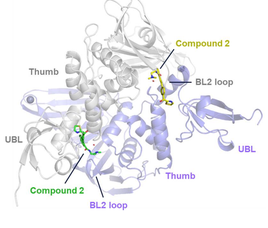


Figure 1. Two monomers of PLpro (colored purple and gray) with compound **2** bound. Compound **2** is shown in sticks and colored green and yellow.

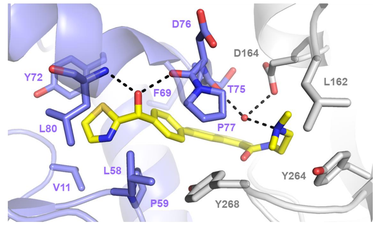


Figure 2. Molecular interactions of compound **2** with PLpro dimer. Compound **2** is shown in sticks and colored yellow. Hydrogen bonds are represented as black dashed lines.

A novel class of SARS-CoV-2 PLpro-binding small molecules demonstrates biochemical (IC_{50} of 271 nM) and anti-viral activity (EC_{50} of 33 nM). Crystal structures reveal molecules inhibit PLpro through simultaneous engagement of two PLpro monomers through the BL2-loop region and a cryptic pocket, ***mechanistically distinct*** from that of known SARS-CoV/SARS-CoV-2 PLpro inhibitors.

Compounds are Potent and Selective Inhibitors of SARS-CoV-2 PLpro

Biochemical Activity Assay – Compound 1

- Inhibitor was preincubated for 30 minutes with 10 nM recombinant PLpro in assay buffer. Reaction was initiated by addition of 100 nM Ubiquitin-Rho110.
- Fluorescence was measured after 120 minutes.
- IC_{50} was calculated based on 11 points at various inhibitor concentrations.

PLpro IC_{50} (μM)			
SARS-CoV-2	SARS-CoV-1	MERS	OC43
0.357	>10	>10	>10

Table 1. Activity of Compound 1 against a panel of recombinantly purified PLpro enzymes

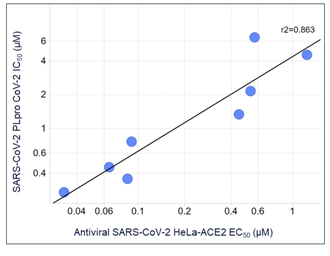


Figure 3. A strong correlation (r^2 = 0.86) is observed for PLpro inhibition in the biochemical assay and SARS-CoV-2 antiviral activity in HeLa-ACE2 cells

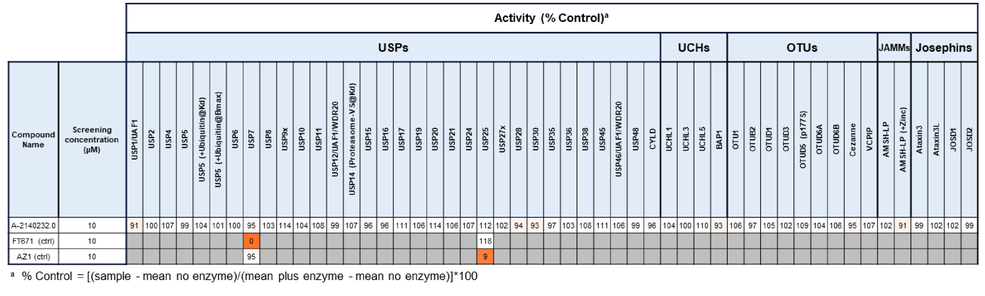
Cell-Based Assays – Compound 3

- Anti-SARS-CoV-2 (strain USA-WA1/2020) activity was measured in two cell types: HeLa-ACE2 and HBEC.
- Cytotoxicity measured by CellTiter-Glo luminescent cell viability assay
- Cell-based protease assay used Flip-GFP format in transfected cells

Compound 3	Antiviral Assay, EC_{50} (μM)		Cytotoxicity Assay, EC_{50} (μM)	Cell-based Protease Assay EC_{50} (μM)
Human Coronavirus	SARS-CoV-2	SARS-CoV-2	None	SARS-CoV-2 (PLpro only)
Cells	HBEC[a]	HeLa-ACE2[b]	HeLa-ACE2	HEK-293T
EC_{50} (μM)	0.032	0.033	>20	0.70

[a] HBEC: Primary human bronchial epithelial cells
[b] HeLa-ACE2: HeLa cells that stably express ACE2, the cellular receptor of SARS-CoV-2

Table 2. Activity of Compound 3 in cell-based antiviral and cytotoxicity assays

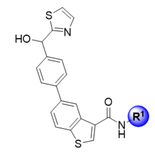


[a] % Control = [(sample - mean no enzyme)/(mean plus enzyme - mean no enzyme)]*100

Figure 4. Compound 3 is inactive against DUB enzymes representative of human DUB family (DUBprofiler™)

- Highly selective for SARS-CoV-2 PLpro.

Medicinal Chemistry and SAR for Diarylmethanol Series



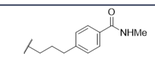
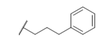
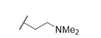
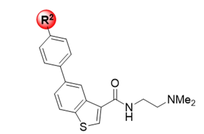
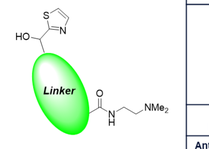
Compound	R[1]	SARS-CoV-2 PLpro IC_{50} (μM)	Antiviral SARS-CoV-2 EC_{50} (μM)
Cmpd 1		0.357	0.085
4		1.32	-
5		>10	-
Cmpd 3		0.271	0.033

Figure 5. SAR Table – Benzamide Arm. Early SAR studies truncated the benzamide arm of Compound **1** which led to reduced activity. The benzamide functionality could be successfully replace with a tertiary amine to yield a potent molecule with increased ligand efficiency (Compound **3**). The basic amine also improved solubility relative to Compound **1** and was utilized for further SAR exploration.



Compound	R[2]	SARS-CoV-2 PLpro IC_{50} (μM)	Antiviral SARS-CoV-2 EC_{50} (μM)
Cmpd 1		0.271	0.085
6		0.763	0.090
7		2.16	0.535
8		>10	-
9		6.49	0.568

Figure 6. SAR Table – Thiazole Alcohol. The thiazole and secondary alcohol of Compound **1** were both crucial for activity. Excising either of these groups significantly reduced activity and even minor modifications led to a loss in potency.



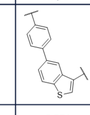
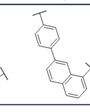
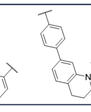
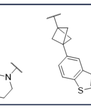
Compound	Cmpd 3	Cmpd 2	10	11
Linker				
SARS-CoV-2 PLpro IC_{50} (μM)	0.271	0.455	>10	>10
Antiviral SARS-CoV-2 EC_{50} (μM)	0.033	0.065	-	-

Figure 7. SAR Table – Central Aromatic Region. The benzothiophene in the central aromatic region could be converted to a naphthalene with minimal loss in activity, however, multiple attempts to introduce saturation were not tolerated.

CONCLUSIONS

There is a great interest in the discovery of inhibitors for SARS-CoV-2 PLpro to complement currently available SARS-CoV-2 therapeutics. This study reveals a class of small molecule inhibitors targeting SARS-CoV-2 PLpro through the novel mechanism of PLpro dimerization. These compounds are proposed to mediate inhibition through the molecular glue-induced self-association of PLpro. This mechanism is different from those of other PLpro inhibitors which mediate inhibition through a single interaction with monomeric PLpro near the substrate.

Disclosure: All authors are employees of AbbVie. The design, study conduct, and financial support for this research were provided by AbbVie. AbbVie participated in the interpretation of data, review, and approval of the publication. No honoraria or payments were made for authorship.