Compendium of HTS of SARS-CoV-2 Targets to Prepare for the Next Pandemic

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Abstract

Since late 2019, the outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has had an enormous negative impact on the world. The emergence of new variants is constantly challenging scientists to produce more effective vaccines and identify specific anti-viral drugs. Here at the High-Throughput Molecular Screening Center at UF Scripps Biomedical Research, we have been screening of variety of SARS-CoV-2 targets, including virus entry, helicase (nsp13), Mpro, and papain-like protease (PLpro) against selected libraries using both cell and biochemical based methods. All the assays were optimized to 1536-well format and were evaluated against >15,000 small molecule drugs. The outcomes have identified four clinically relevant drugs from screening PLpro and eleven lead compounds from screening of SARS-CoV-2 entry. The Mpro HTS, as a gain of function assay, identified several interesting hits that are currently under investigation. Helicase also completed assessing a 100K diversity library and hit validation is underway. All these outcomes will be described here-in.

Screening Libraries

ReFRAME library – ~13 K Compounds

CALIBR partnered with the Bill and Melinda Gates Foundation to form consolidated set of drug candidates. ReFRAME contains approximately 13,000 purchased or resynthesized FDA-approved/registered drugs (~40%), as well as investigational new drugs currently or previously used in any phase of clinical development (~60%).

Pathogen Box library – ~400 compounds

The Pathogen Box library contains 400 diverse, drug-like molecules active against neglected diseases of interest provided by the Medicines for Malaria Venture.

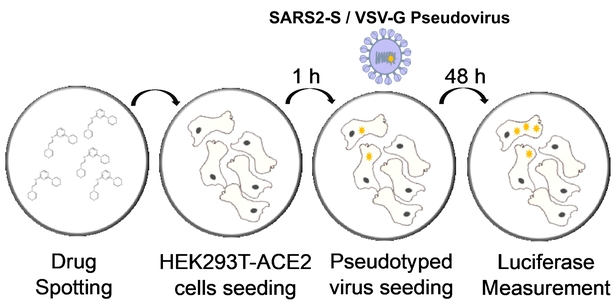
TargetMol library – ~1.1 K compounds

TargetMol performed a CADD docking study using the Swiss-Model Homology process to generate a library based on 3D protein structures of RBD of Spike protein, ACE2, viral papain like protease (PLpro) and main protease (3CLpro, also named 3-chymotrypsin-like protease). Based on these protein structures, TargetMol selected the ~1.1K top-ranked docked molecules into PLpro-Targeted compound library (CADD) by molecular docking virtual screening against 15,376 compound structures.

Cathepsin-L library – ~500 compounds

Cathepsin L1 (CL) blocks viral fusion by inhibiting host endosomal CL which is one pathway used for SARS-CoV entry. Hence, we used a criteria of having a Tanimoto score greater than 80% matched vs our SDDL to identify ~ 450 compounds. These compounds were cherry-picked and registered into source plate for further HTS.

Virus Entry – Assay Principle



Compounds were pre-spotted in 1536-well plates. Next, 2000 HEK293T-ACE2 cells in 2.5uL were added to each well and pre-incubated with each compound for 1 h, followed by infection with MOI 0.1 - 0.5 of MLV reporter luciferase virus pseudotyped with the SARS-CoV-2 Spike protein (SARS2-S) or VSV-G protein (VSV-G). Luciferase was measured 48 h later .

References

Mediouni S, Mou H, Otsuka Y, Jablonski JA, Adcock RS, Batra L, et al. Identification of potent small molecule inhibitors of SARS-CoV-2 entry. SLAS Discov. 2022;27(1):8-19.

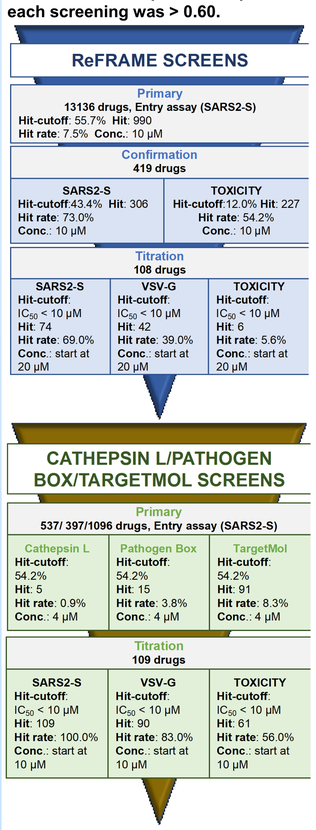
Moghadasi SA, Esler MA, Otsuka Y, Becker JT, Moraes SN, Anderson CB, et al. Gain-of-Signal Assays for Probing Inhibition of SARS-CoV-2 M(pro)/3CL(pro) in Living Cells. mBio. 2022;13(3):e0078422.

Smith E, Davis-Gardner ME, Garcia-Ordonez RD, Nguyen TT, Hull M, Chen E, et al. High-Throughput Screening for Drugs That Inhibit Papain-Like Protease in SARS-CoV-2. SLAS Discov. 2020;25(10):1152-61.

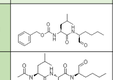
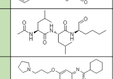
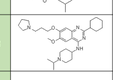
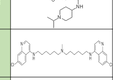
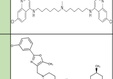
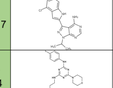
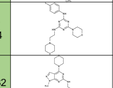
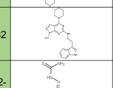
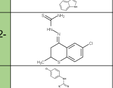
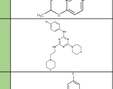
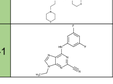
Smith E, Davis-Gardner ME, Garcia-Ordonez RD, Nguyen TT, Hull M, Chen E, et al. High Throughput Screening for Drugs that Inhibit 3C-Like Protease in SARS-CoV-2. SLAS Discov. 2023.

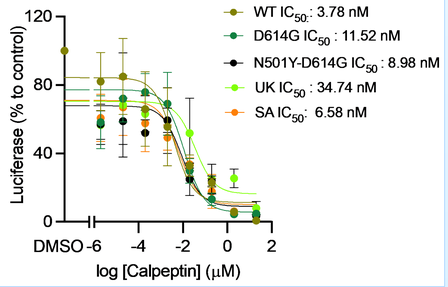
Virus Entry – Screening Summary

Summary of the lead compounds that identified from the Cathepsin L, Pathogen box and TargetMol libraries in this study. Activity of the selected compounds against the different MLV pseudotyped viruses in HEK293-ACE2 cells and their respective cytotoxicity. Values for SARS2-S, VSV-G and toxicity are mean ± SEM of 2–4 independent experiments. TI: therapeutic index. ∗ n = 1. Assay was robust and average Z' of each screening was > 0.60.



TI > 20

	SR ID	Drug	Structure	SARS2-S (IC_{80}; nM)	VSV-G (IC_{80}; µM)	Toxicity (CC_{50}; µM)	TI	VSV-G / SARS-2
TARGETMOL	SR-914	Calpeptin		10.7 ± 1.4	> 20.0 ± 0.0	> 20.0 ± 0.0	> 1612.9	> 1612.9
	SR-372	Calpain inhibitor I		4.0 ± 1.3	7.9 ± 1.2	8.6 ± 1.3	795.7	645.2
	SR-452	UNC0638		92.9 ± 4.6	0.9 ± 0.2	1.9 ± 0.2	20.5	9.7
	SR-510	DC661		1.4 ± 0.8	0.2 ± 0.0	1.2 ± 0.3	857.1	142.9
	SR-700	Ataxin		565.0 ± 25.0	> 20.0 ± 0.0	> 20 ± 0.0	> 35.4	> 35.4
CATHEPSIN L/PATHOGEN BOX	SR-629*	MMV688417		472.6	5.4	15.2	32.2	11.4
	SR-603	ChemDiv K890-0084		489.5 ± 13.4	5.3 ± 1.1	36.5 ± 5.1	74.7	10.8
	SR-542	MMV676382		372.5 ± 84.2	4.4 ± 0.1	13.8 ± 2.1	37.0	11.8
	SR-728	Molport-002-922-080		406.5 ± 67.2	>100.0 ± 0.0	>100.0 ± 0.0	246.0	246.0
	SR-580	–		147.8 ± 9.5	3.6 ± 0.9	3.7 ± 0.2	25.0	24.4
	SR-687	Cruzain-IN-1		281.8 ± 154.8	33.9 ± 4.6	>100.0 ± 0.0	354.9	120.3



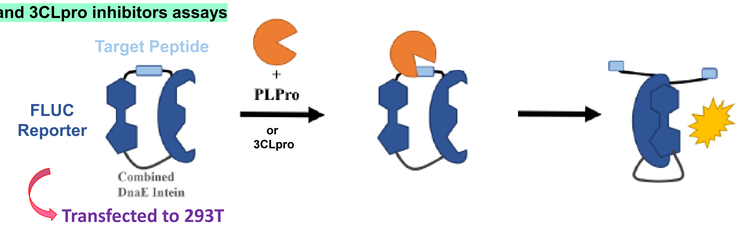
Activity of calpeptin activity against crucial mutations present in the S protein of the new emergent strains. Shown is the mean ± SEM of n = 2–5 independent experiments. WT: wild type, SA: South Africa, UK: United Kingdom

Mpro and PLpro – Assay Principle

Mpro Gain of function (GOF) assay

Tat is sequestered in the cytoplasm by an Mpro-cleavable cytosolic membrane anchor such as the N-terminal myristoylation domain from the Src kinase, then Mpro-catalyzed cleavage during infection would cause relocalization of Tat to the nucleus to activate expression of a reporter construct. Auto-proteolytic activity of Mpro causes reduction of luciferase

PLpro and 3CLpro inhibitors assays



The reporter consists of a split firefly luciferase protein connected by a cleavable peptide for the tested protease. Upon cleavage of the peptide, the luciferase protein undergoes dimerization for an active state. DnaE intein helps in this dimerization.

Mpro and PLpro– Screening Summary

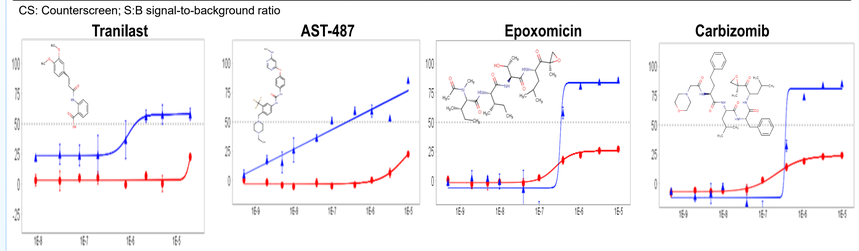
Mpro GOF screening

Libray	Stage	Drug concentration (µM)	# Samples	# Replicates	# Plates	Z'	S:B	Hit Cutoff	# Hits
8.7 K library	Confirmation	10	8700	3	24	0.55±0.11	89.95±21.86	4.76%	145
	Titration	20	145	3	6	0.56±0.01	82.54±5.81	IC50 < 10 µM	105

PIpro screening

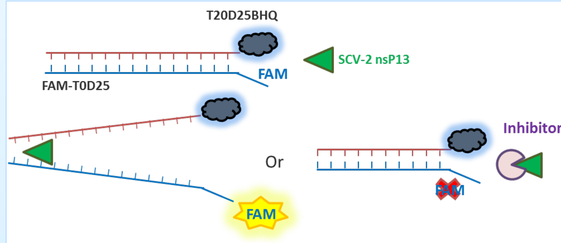
Libray	Stage	Drug concentration (µM)	# Samples	# Replicates	# Plates	Z'	S:B	Hit Cutoff	# Hits
ReFRAME	Primary	10	13,104	1	11	0.71±0.04	11.38±1.57	35.1%	212
	Confirmation	10	235	3	1	0.75	15.48	27.5%	210
	Titration	20	210	3	6	0.72±0.03	18.39±1.30	IC50 < 10 µM	164
	Titration CS	20	210	3	6	0.76±0.02	3.02±0.15	IC50 < 10 µM	185
Pathogen Box	Primary	4	398	3	3	0.70±0.02	10.28±0.47	28.0%	16
	Confirmation	4	12	3	3	0.72±0.02	10.98±0.98	27.3%	3
	Counterscreen	4	12	3	3	0.68±0.04	2.58±0.19	21.3%	8
TargetMol	Confirmation	4	1097	3	6	0.75±0.05	17.23±1.46	34.3%	27
	Counterscreen	4	1097	3	6	0.75±0.04	3.04±0.04	10.10%	132
	Titration	10	27	3	3	0.71±0.09	11.27±0.86	IC50 < 4 µM	19
	Titration CS	10	27	3	3	0.86±0.01	28.61±1.12	IC50 < 4 µM	24

CS: Counterscreen; S:B signal-to-background ratio



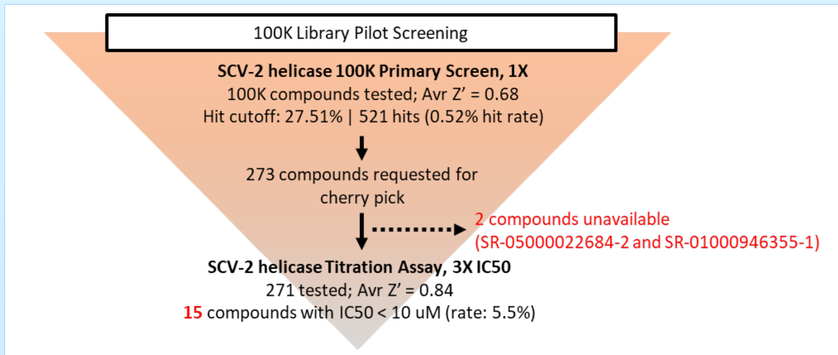
Hits obtained from the 3CLpro inhibitors assay: One compound from the ReFrame library, Tranilast, as well three compounds from the Target Mol library, AST-487, Epoxomicin, and Carfilzomib appeared to be nontoxic (red) and active in the 3CLpro assay (blue).

Helicase – Assay Principle



The assay measures the fluorescence intensity of FAM, which caused by interaction between dsDNA and SCV-2 nsp13. Use excitation: 480 nm and emission: 540 nm filters to find SCV-2 nsP13 inhibitors.

Helicase – Screening Summary



We screened the Helicase assay against 100K compound library of diverse drug like molecules from the UF Scripps 665K Drug Discovery Library.

Conclusion and On-going Screening

At The Herbert Wertheim UF Scripps Institute for Biomedical Innovation & Technology we managed to optimize and scale 5 assays that targeted either SARS2-CoV Entry, proteases or helicase in 1536 well-plate format. The cell-based and biochemical based assays identified many interesting lead compounds. MOA studies and further investigation is currently on-going. These studies proved the capability of these assays and now the Mpro GOF and Helicase assays have been integrated into the Midwest Antiviral Drug Discovery program (AViDD: 1U19AI171954) for large library screening to identify and develop first-in-class drugs that target current and emerging viral pathogens.