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Midwest AViDD Center

DISCOVERY OF A NOVEL SARS-CoV2 HELICASE INHIBITOR FROM A HTS CAMPAIGN

Project 5: Antiviral Helicase

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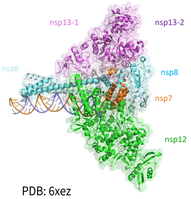
Abstracts

- Viral RNA helicase is a critical component of the viral replicase complex.
- Viral RNA helicases can serve as a novel antiviral target for RNA viruses with a high barrier to drug resistance.
- An HTS-compatible enzymatic assay was developed that can measure the unwinding activity of double strand DNA by recombinant SARS-CoV2 helicase (nsP13) using a FRET-based readout.
- An HTS campaign with 100K small molecule compounds of Scripps Drug Discovery Library in 1536-well format identified 521 primary hit compounds.
- Following dose-response and cheminformatics studies, a total of 18 compounds emerged and were then tested for antiviral activity with live SARS-CoV2.
- Bioinformatical approaches identified a potential binding site of the hit compound near the 3' RNA groove of SARS-CoV2 helicase model.
- A scaled-up HTS with a 660K library has conducted and promising hits are being followed with various antiviral assays.
- Overall our study shows that SARS-CoV2 helicase does serve as a novel antiviral target and our HTS approach can discover novel antiviral compounds targeting viral helicases.

SARS-CoV2 nsP13 as an antiviral target

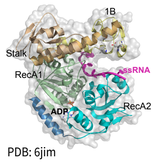
SCV2 nsP13 in the replicase complex



PDB: 6xez

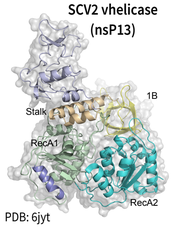
Viral homologs

Alphavirus vhelicase (nsP2)



PDB: 6jim

SCV2 vhelicase (nsP13)



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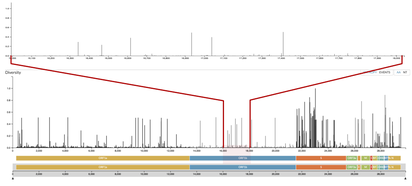
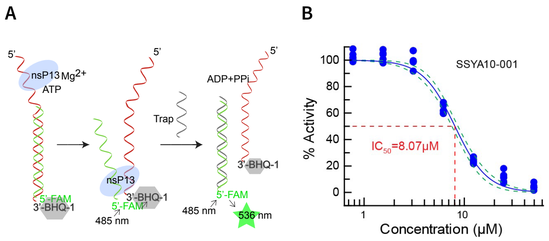
Comparison of viral helicases

	Alphavirus	CoV	Flavivirus	Human orth. (e.g.)
Gene	nsP2	nsP13	NS3	RecQ
Helicase family	SF1	SF1	SF2	SF2
Substrate	RNA	DNA or RNA	DNA or RNA	DNA
Translocation	5' > 3'	5' > 3'	5' > 3'	5' > 3'

High genetic barrier

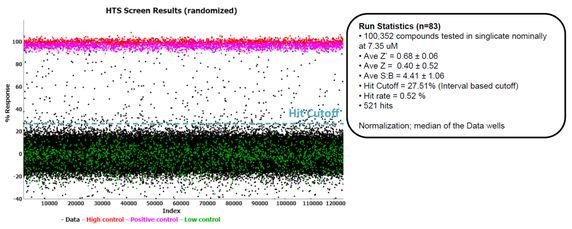


Approach



A. Principle of the nsP13 ds-DNA unwinding assay. Unwinding of 5' overhang dsDNA with FAM/BHQ by nsP13 restores the fluorescence from FAM. **B.** Validation of the assay with a positive control compound SSYA10-001.

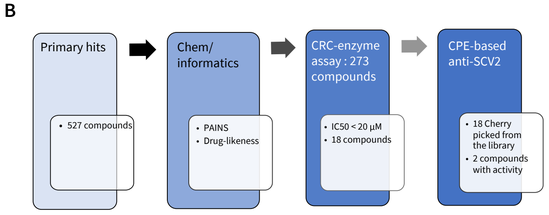
A Pilot HTS with 100 K compounds



High control = Trap DNA + dsDNA substrate + DMSO

Low control = Trap DNA + nsP13 + dsDNA substrate + DMSO

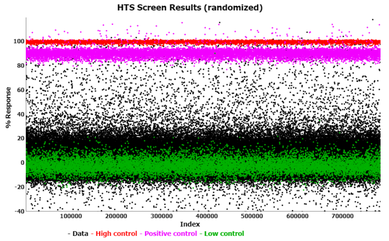
Positive control = all reagents + Stop solution (after pinning compounds)



A. Implementation and a pilot HTS with a 100K compounds library. Assay was miniaturized to the 1536-well format in a reaction volume of 5 μL. His-tagged SCV2 nsP13 expressed in E. coli was used for the assay. **B.** Strategy to identify hit compounds from the pilot scree.

uHTS on 665K compounds

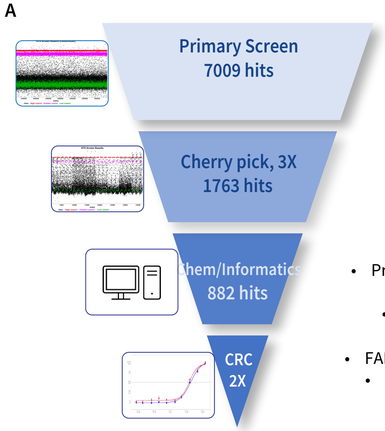
Primary screen



- Assay in 1536wpf
- Run Statistics (n=522)
- 649,568 compounds tested in singlicate nominally at 6.95 uM
- Ave Z' = 0.86 ± 0.05
- Ave Z = 0.61 ± 0.72
- Ave S:B = 5.14 ± 0.55
- Hit Cutoff = 22.29% (Interval based cutoff)
- Hit rate = 1.08 %
- 7,009 hits

Recombinant SCV2 nsP13 (no His-tag) expressed in E.coli was used for the assay (75 pM). The primary screen at 6.95 μM in singlicate showed a robust assay performance with a high confidence.

Hit Identification

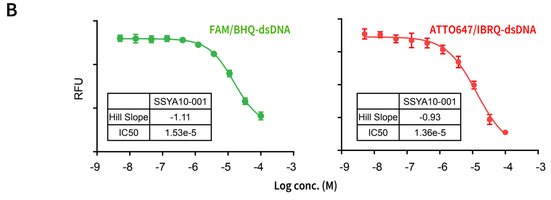


- 650K comp.
- Single conc. @ 6.95 μM
- Cutoff: 22.29%

- 6994 compounds
- Single conc. @6.95 μM
- Triplicates/average
- Cutoff: 22.29%

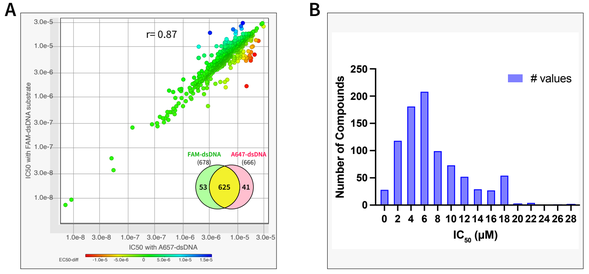
- MW : 142 < MW < 700
- Promiscuity : ≤ 1 PAINS filter, < 6% FL Screen
- Activity > 29% @ 6.95 μM

- FAM-dsDNA, ATTO647-dsDNA
- 10 points / 2-fold / 17.4 μM
- Hits : 625 @ 10 μM



A. uHTS funneling progress for identification of nsP13 inhibitors. **B.** Concentration response curve analysis (CRC) were conducted with FAM-tagged and ATTO647-tagged DNA substrate to remove potential false positives interfering the fluorescence readout of FAM. Both substrates have been validated with a positive control compound, SSYA10-001

uHTS Summary



A. High performance and reproducibility of uHTS. The selected compounds were tested with FAM-tagged and ATTO647-tagged dsDNA substrate indipendently and IC50s were compared. r, Pearson corelation coefficience. **B.** Distribution of IC50s against SCV2 nsP13 of the selected compounds. **C.** Activity-structure landscape analysis. **D.** Potential compound interaction sites identified via *in silico* approaches using FTmap. Yellow, 3' RNA groove; tan, 5' RNA binding site; pink, ATP binding pocket.

Conclusions

1. We developed a robust, HTS-compatible dsDNA unwinding assay with bacterial expressed SCV-2 nsP13.
2. The assay implemented in uHTS with the 1536-well format. A pilot screen performed well with a high confidency.
3. An uHTS on a 665K library was conducted with an excellent performance.
4. The initial hits have been followed up with various confirmatory and secondary assays, resulting 625 compounds with IC50 < 10 μM.
5. The initial hit compounds collection includes diverse structres centered around several traceable core structures.
6. Biological and other follow-up assays are in progress with resupplied compounds.

Acknowledgements

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