Identification of SARS-CoV-2 helicase inhibitors by large-scale virtual screening

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Introduction

❑ **Facts**: The Rapidly Emerging Antiviral Drug Discovery Initiative – Antiviral Drug Discovery Center (**READDI-AC**) aims to expedite the discovery and preclinical development of orally bioavailable, direct-acting antivirals with broad-spectrum activity against Alphaviruses, Flaviviruses, Coronaviruses, and Filoviruses, all possessing high pandemic potential.[1]

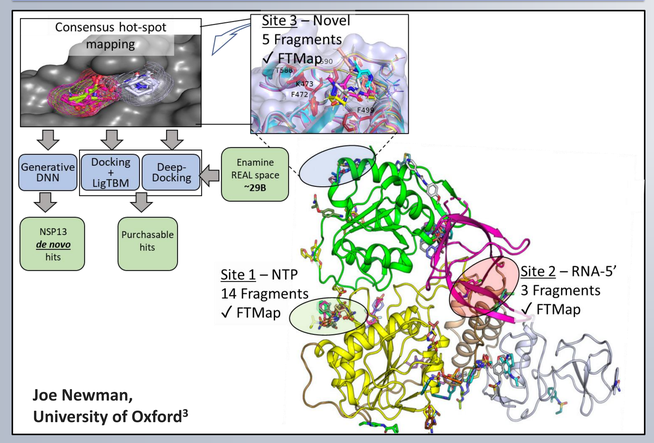
❑ **Goals**:

- ➢ Employ an integrated, evidence-based approach to design allosteric inhibitors targeting SARS-CoV-2's NSP13 helicase.
- ➢ Assess the potential of NSP13 helicase inhibitors as COVID-19 therapeutic agents.

❑ **Viral Helicases**:

- ➢ Potential drug targets regulating RNA metabolism
  - Involved as key motor enzymes
- ➢ Key functions of NSP13 helicase:
  - RNA unwinding & ATP hydrolysis[2]
  - Required for viral replication
  - NSP13 is highly conserved across coronaviruses[3]
- ➢ Druggability of Helicases: Limited evidence[4,5]
  - Data from phenotypic screenings
  - X-ray structures

Rational design of allosteric helicase inhibitors



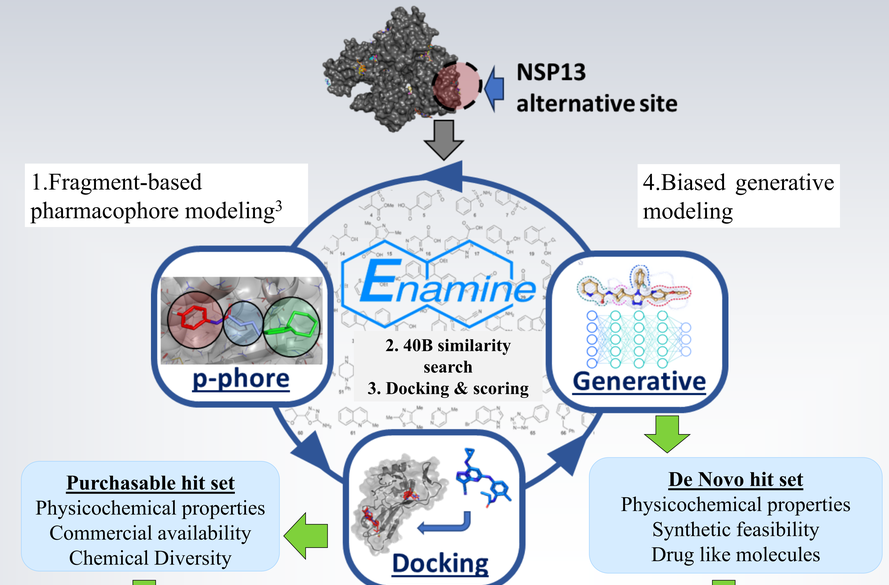
Is NSP13 Helicase druggable?[3,6]

Site#1: NTP binding site soaked with 14 fragments

Site#2: RNA binding site soaked with 3 fragments; potential for PPI design

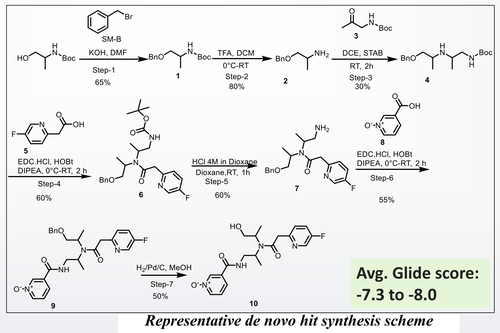
Site#3: A novel site soaked with multiple fragments. Potential for allosteric inhibitors?

Large-Scale Virtual Screening



Parameter	Range of Values	Property Compliance
MW	260 to 460	✓
cLogP	-1.9 to 2.5	✓
cLogS	-6.8 to -3.5	✓
tPSA	75 to 130	✓
HBA	6 to 10	✓
HBD	1 to 3	✓

Physicochemical properties of selected hits

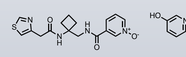


Representative de novo hit synthesis scheme

Compound screening

Purchased hit compounds DeNovo Synthesis hits → SARS-COV-2 ATPase & SPR MERS SPR Assay → Antiviral Assay, Crystallography → - New Analogs - New SAR Design

Results: NSP13 SPR and ATPase

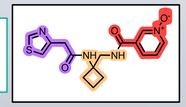


ID	RA-0001264	RA-0001263
SARS-CoV-2 SPR KD (μM)	6	11
MERS SPR KD (μM)	5	11
SARS-CoV-2 ATPase IC_{50} (μM)	48	12

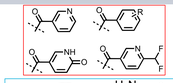
Parameter	RA-0001264	RA-0001263
MW	346.41	342.35
cLogP	0.14	-0.32
Kinetic Solubility (μM)	> 180	> 195

Approaches to validate the hits:

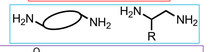
1. X-ray crystallography
2. Synthesis of a small set to generate SAR



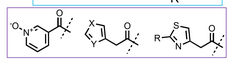
Study importance of presence of *N*-oxide

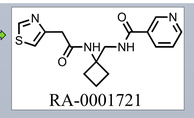


Explore the impact of other diamines



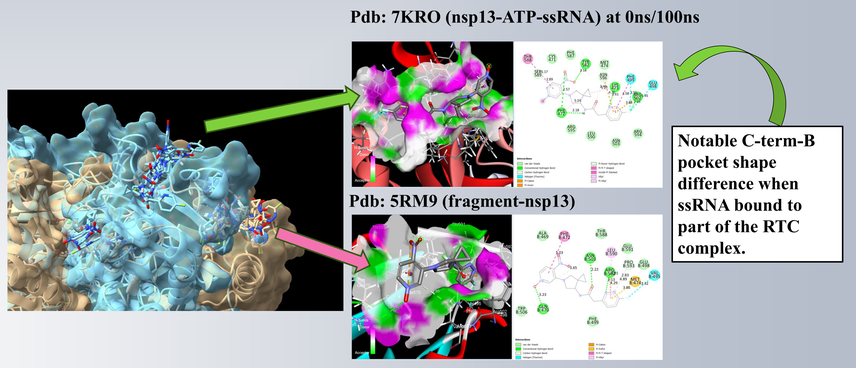
Thiazole replacement





ATPase IC_{50} = 14 μM
Kinetic Solubility = 142 μM

MD Simulation of De Novo Hits



100 ns AMBER simulation:

- ssRNA interaction with nsp13 induces deeper pocket formation at site#3
- Ligand is highly flexible with numerous binding modes.
- Weak (pi-pi, pi-alkyl, and pi-cation) interactions frequently occur → pyridinium-*N*-oxide interacts with an electron-rich region by pi interactions

Challenges

- NSP13 is a motor enzyme which makes it difficult to design inhibitors using static X-ray structures
- No positive control for the NSP13 ATPase assay
- No known helicase inhibitors active in SPR or other biophysical assays

Conclusions

- Viral helicases are promising therapeutic targets
- Novel NSP13 helicase binding site (#3) was targeted as a unique allosteric pocket
- Micromolar NSP13 helicase inhibitors were identified in an ATPase assay
- MD simulations revealed how RNA impacts ligand binding
- Hit expansion in the READDI-AViDD program is ongoing
- Further optimization is needed due to the lack of cell-based antiviral activity

References

1. https://www.readdi.org/
2. Tanner, et al., J. Biol. Chem. 2003, 278, 39578–39582
3. Newman et al., Nat. Commun., 2021, 12, 4848
4. Birkman et al., J. Med. Chem., 2022, 65, 20, 13614-628
5. Chen et al., eLife, 2021, 10:e65339
6. Diamond X-ray, fragalysis.diamond.ac.uk

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Explore more at *SGC Open Chem for NSP13*

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