Table S1. Summary of all helicases present in Heli-SMACC

Helicase Name	Organism(s)	Super Family	DNA/RNA	Entries
DNA2 Helicase/Nuclease	Homo sapiens	SF1	DNA	3
DNA Helicase/Primase	Human herpesvirus 1, Herpes simplex virus type 1	SF1	DNA	33
Helicase/NTPase	SARS-CoV-1	SF1	RNA	20
NSP13 Helicase	SARS-CoV-1	SF1	RNA	111
NS3 Helicase/NTPase	Hepatitis C, Dengue Virus, West Nile virus, Japanese encephalitis virus	SF2	RNA	875
ATP-dependent DNA helicase Q1	Homo sapiens	SF2	DNA	5352
ATP-dependent RNA helicase DDX1	Homo sapiens	SF2	RNA	11
ATP-dependent RNA helicase DDX3X	Homo sapiens	SF2	RNA	28
BRR2 Helicase	Homo sapiens	SF2	RNA	6
Bloom syndrome protein helicase	Homo sapiens	SF2	DNA	4080
SUV3 Helicase	Homo sapiens	SF2	RNA	40
Werner syndrome ATP-dependent helicase	Homo sapiens	SF2	DNA	2546
elF4A3 helicase	Homo sapiens	SF2	RNA	2
Helicase	BK polyomavirus, Human poliovirus 1, JC polyomavirus	SF3	DNA, RNA	70
Helicase/ATPase	Enterovirus A71	SF3	RNA	1
E1 DNA Helicase/ATPase	Human papillomavirus type 11	SF3	DNA	52
DNA Helicase	Bacillus anthracis, Staphylococcus aureus	SF4	DNA	158
Helicase IV	Escherichia coli	SF4	DNA	6
DNAc Helicase	Staphylococcus aureus	SF4	DNA	18
DNAb Helicase	Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa	SF4	DNA	52

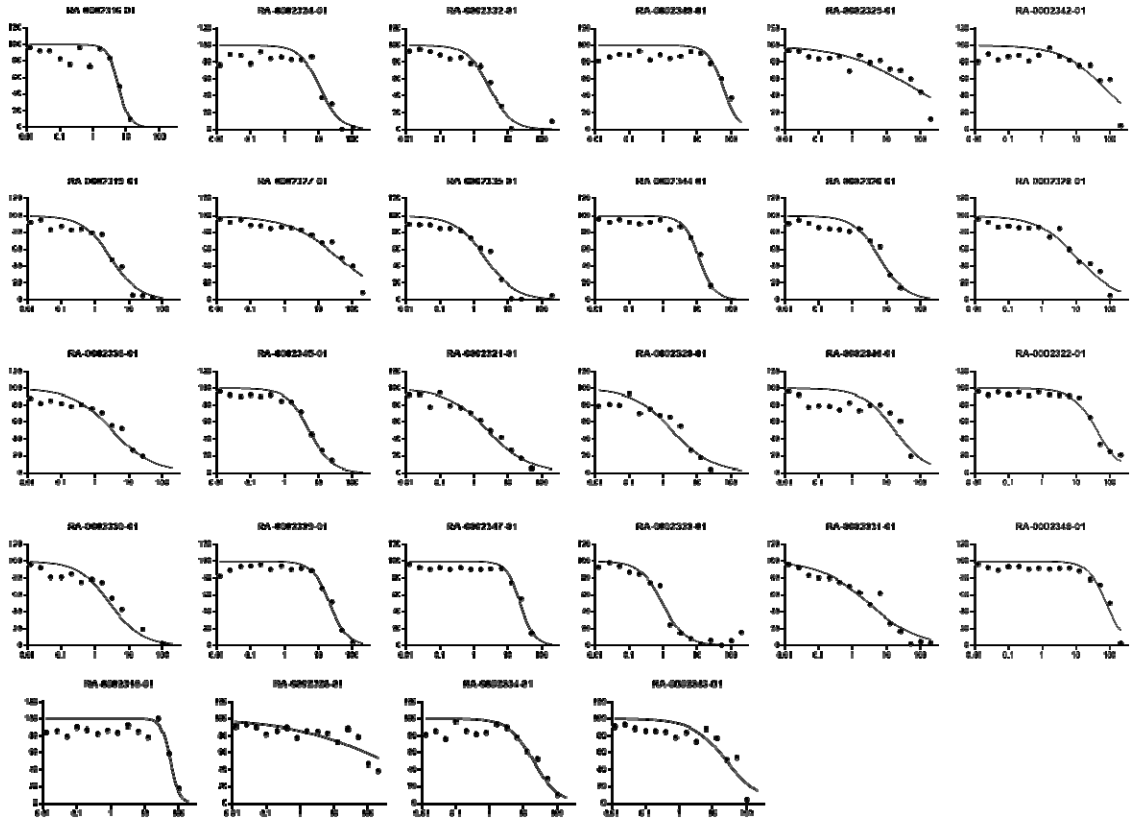
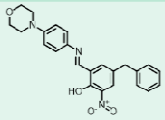
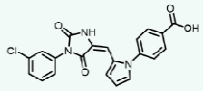
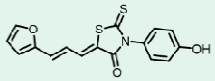
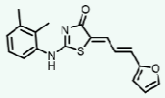
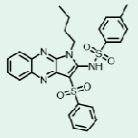
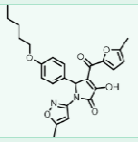
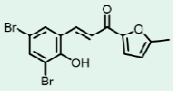
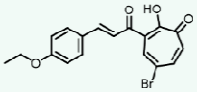
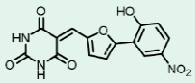


Figure S1. Dose-response of selected viral helicase inhibitors in NSP13 ATPase assay (kinase-glo).

Table S2: Calculated physicochemical properties and experimental kinetic solubility of the selected NSP13 ATPase assay hits from the viral helicase inhibitors.

ID	Structure	cLogP	tPSA	NSP13 ATPase (IC_{50}) µM	Kinetic Solubility (µM)
RA-0002319-01		4.57	96.87	3	78
RA-0002321-01		4.05	89.95	3	83
RA-0002323-01		3.16	49.77	3	112
RA-0002332-01		4.87	50.69	3	39
RA-0002335-01		5.98	108.27	3	11
RA-0002345-01		4.29	97.66	5	77
RA-0002336-01		4.47	46.53	5	NT
RA-0002320-01		3.19	63.6	6	NT
RA-0002328-01		1.47	156.54	10	85

SARS-CoV-2 NSP13 ATPase assay protocol:

The final reaction mixtures consisted of 50 mM HEPES, pH 7.5, 5% Glycerol, 5 mM magnesium acetate, 5 mM DTT, and 0.01% BSA, 0.1 nM nsp13, 3.5 nM ssDNA and 2.5 µM ATP. The reactions were started by the addition of substrates and incubated for 60 min at room temperature. The level of enzyme activity was then measured using a luciferase reagent, and the data were analyzed using GraphPad Prism 9.

Solubility assay protocol:

Kinetic solubility was performed by the CRO, Analiza. The reported methods for sample preparation and analysis as follows:

Sample Preparation: 50-fold dilutions of each DMSO stock solution were prepared in singleton by combining 6µL of DMSO stock with 294µL of the appropriate media in a Millipore solubility filter plate with 0.45µM polycarbonate filter membrane using Hamilton Starlet liquid handling. The final DMSO concentration is 2.0% and maximum theoretical compound concentration is 200µM (assuming stock concentration of 10mM). The filter plate was heat sealed for the duration of the 24-hour incubation period.

Buffer Preparation: 1XPBS, pH 7.4: Phosphate Buffered Saline solution 10X, PBS (Fisher Bioreagent part number BP399-500). 50mL was added to approximately 450mL HPLC grade H2O. The volume of the solution was then adjusted to 500mL for a total dilution factor of 1:10 and a final PBS concentration of 1X.

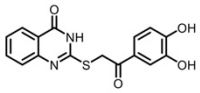
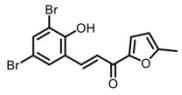
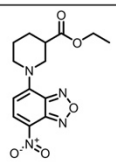
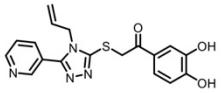
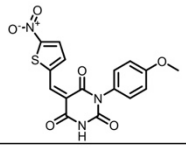
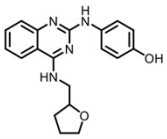
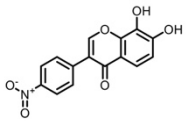
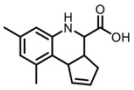
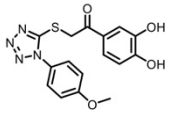
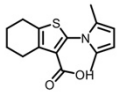
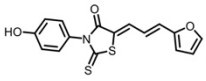
Solubility Analysis: The samples were placed on a rotary shaker (200RPM) for 24 hours at ambient temperature (20.3–22.3°C) then vacuum filtered. All filtrates were injected into the nitrogen detector for quantification on Analiza's Automated Discovery Workstation. The results are reported here in µg/ml and µM.

Calculation of Results: The equimolar nitrogen response of the detector is calibrated using standards which span the dynamic range of the instrument from 0.08 to 4500 µg/ml nitrogen. The

filtrates were quantified with respect to this calibration curve. The calculated solubility values are corrected for background nitrogen present in Analiza's in-house DMSO and the media used to prepare the samples. Three separate on-board performance indicating standards were assayed in triplicate from 10mM stock solutions at 2.0% DMSO with the University of North Carolina supplied compounds, and all results were within the acceptable range. A comments field contains notes pertinent to the assay of each compound, such as below LOQ or measured solubility is greater than 75% of the dose concentration, the actual solubility may be higher.

Multi-helicase Inhibitors

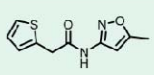
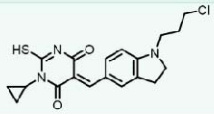
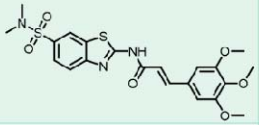
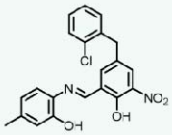
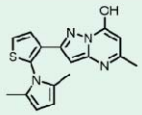
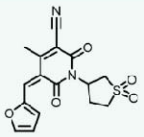
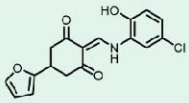
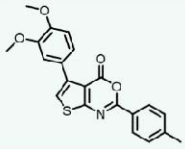
There were 151 compounds selected which demonstrated activity at multiple helicases (examples in **Figure S2**). The majority of these multi-helicase inhibitors targeted a combination of Bloom syndrome protein helicase, Werner syndrome ATP-dependent helicase, and ATP-dependent DNA helicase Q (examples in **Figure S2**). There was also a subset of compounds that showed activity at the viral Hepatitis C NS3 Helicase/NTPase as well as one of the human helicases (Bloom syndrome protein helicase, Werner syndrome ATP-dependent helicase, or ATP-dependent DNA helicase Q1). These pan helicase inhibitors are intriguing as a tool molecule to investigate the role of helicase and underscore the homology of helicase across the species. All the helicases contain RecA type domain, and there is possibility of similar modes of action of ligands.

Chemical Structure	Data*	Chemical Structure	Data*	Chemical Structure	Data*
	**ChEMBL ID** Helicase Name(s) Organism Super family		**CHEMBL240331** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1549566** Werner, NS3 Helicase/NTPase Homo sapiens, Hepatitis C SF2
	**CHEMBL1502002** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1576140** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1545427** Bloom, Werner, NS3 Helicase/NTPase Homo sapiens, Hepatitis C SF2
	**CHEMBL530280** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1505222** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1334412** Werner, NS3 Helicase/NTPase Homo sapiens, Hepatitis C SF2
	**CHEMBL254255** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1364573** Bloom, Werner, Q1 Homo Sapiens SF2		**CHEMBL1493191** Bloom, Werner, NS3 Helicase/NTPase Homo sapiens, Hepatitis C SF2

Bloom=Bloom syndrome protein helicase, Werner= Werner syndrome ATP-dependent helicase, Q1= ATP-dependent DNA helicase Q1

Figure S2. Examples of compounds active at two or more helicases nominated for experimental testing.

Table S3: SARS-CoV-2 NSP13 ATPase inhibition of selected SF2 series compounds.

ID	Structure	cLogP	tPSA	NSP13 ATPase (IC_{50}) µM
RA-0002650-01		1.62	50.69	29
RA-0002647-01		3.23	52.98	29
RA-0002643-01		306	106.53	33
RA-0002661-01		5.66	104.63	45
RA-0002626-01		4.28	57.12	57
RA-0002655-01		5.28	51.43	74
RA-0002641-01		-0.54	104.54	84
RA-0002654-01		3.17	75.63	86