Supplementary Figure legends

Supplementary Figure S1. (**A**) Schematic diagram illustrating the FRET-based primer extension assay. The RNA substrate is composed of a Cy3 fluorophore-containing template strand and an annealed primer strand. The substrate is incubated with RdRp, which extends the primer strand generating duplex RNA. After incubation, a quencher strand is added, which can only anneal to the template strand and quench Cy3 fluorescence if RNA synthesis did *not* occur. (**B**) Results of the FRET-based primer-extension assay shown in (A). The quencher strand was added after 60 min of RdRp and primed-substrate incubation and fluorescence was measured after another 35 min. (**C-E**) Strand displacement assay using the indicated concentrations Sf nsp12-F/7H8 in the absence (C) or presence of 0.5 mM Mn^{2+} (D) or 2 mM Mn^{2+} (E). (**F**) Strand displacement assay using Sf nsp12-F/7H8 in the presence (+NTPs) or absence (no NTP) of 300 μM NTPs.

Supplementary Figure S2. (**A**) Strand displacement assay using the indicated concentrations of insect cell (Sf)-expressed nsp12-HF/7/8. This experiment was performed in parallel with the experiment shown in Figure 1E.

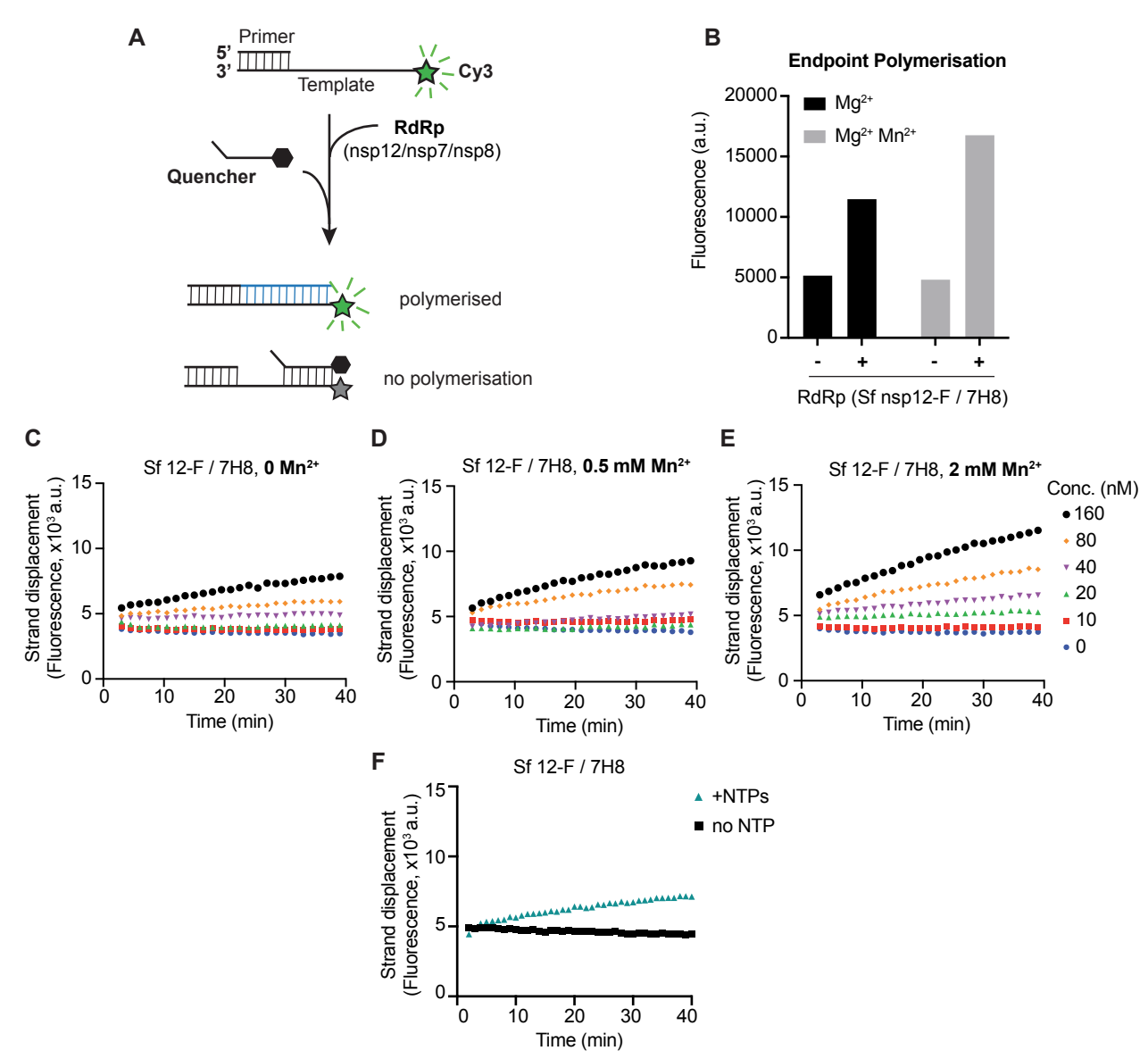
Supplementary Figure S3. (**A**) Strand displacement assay using 150 nM Sf nsp12-HF/7L8 premixed with Sf 7H8 (1:5 ratio) in the presence (+) or absence (-) of 5% DMSO.

Supplementary Figure S4. High-throughput SARS-CoV-2 RdRp inhibitor screen. (**A**) Representative kinetic data from control reactions in the HTS screen. For screen analysis, reaction velocities were extracted from the slope. (**B**) Z-score distribution for the screen performed at 3.75 μM compound concentration. (**C-D**) Analysis of the screen by endpoint signal. Normalised endpoint signal is plotted against compound number for the screen performed at 1.25 μM (C) or 3.75 μM (D) compound concentration.

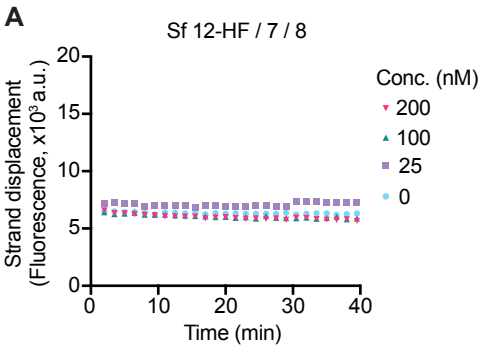
Supplementary Figure S5. Concentration-response curves for validation of selected compounds identified in SARS-CoV-2 RdRp screen. (**A**) The experiment was performed using 150 nM RdRp, 100 nM RNA substrate, 300 μM of each NTP and in the presence (+Triton) or absence (-Triton) of 0.01% Triton X-100. Fluorescence quenching properties of the compounds were tested to identify false positives that interfered with the fluorescent substrate (quenching). (**B**) Quenching controls for the experiment shown in Figure 5A. IC_{50} values were calculated using Prism software.

Supplementary Figure S6. Comparative dose–response curves of selected antiviral compounds against SARS-CoV-2 in cell culture. (A) Anti-SARS-CoV-2 activities of GSK-650394, C646, BH3I-1, MDK-83190 and Cefsulodin in combination with 0.5 μM remdesivir following protocol detailed in **Figure 6A**. Representative overlaid images showing N protein immunofluorescence (green) and DRAQ7 nuclei staining (red). **(B)** Dose-response curve analysis. Viral infection values represent the area of virus infected cells visualised by N protein staining (green curves) and, simultaneously, cell viability was measured as the area of

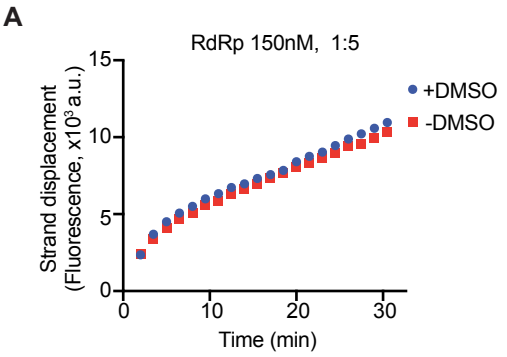
cells stained for DRAQ7 (red curves). Data is plotted as percentage normalised to 0.5 μM remdesivir only treated wells (100%). Values represent mean and standard deviation (SD) of 3 replicates. Areas were calculated using FIJI software and EC_{50} values were calculated using Prism software.



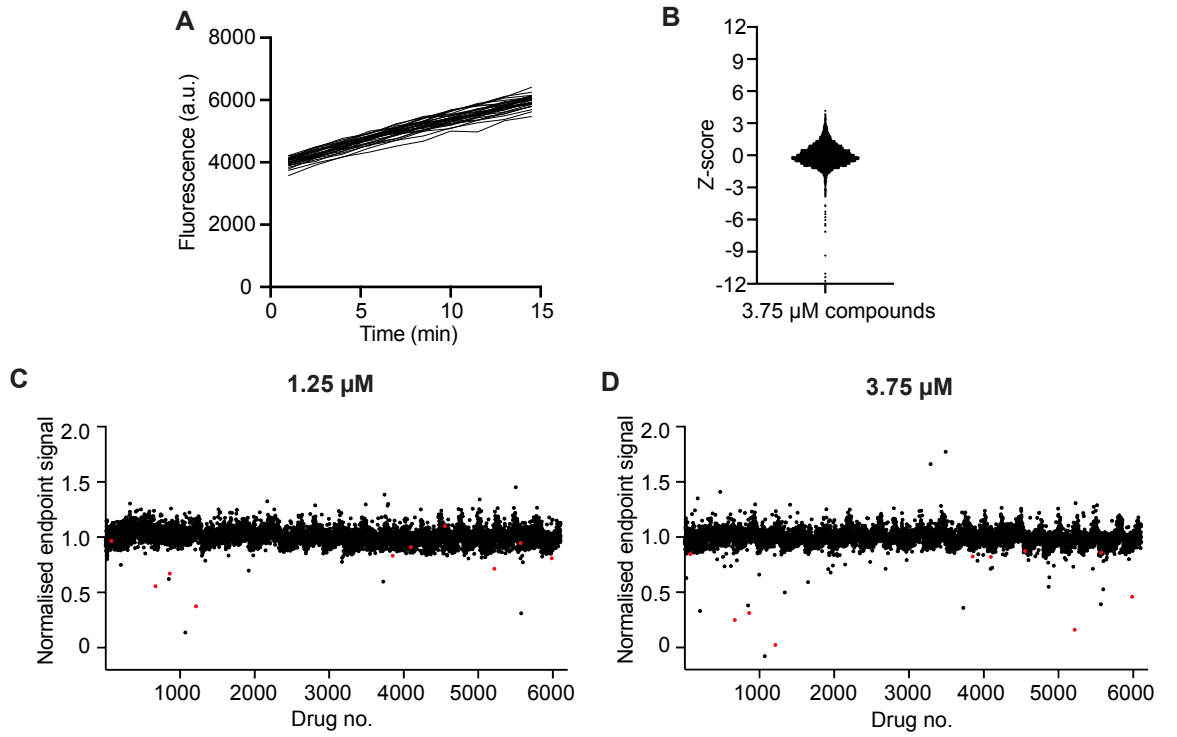
Supplementary Figure 1



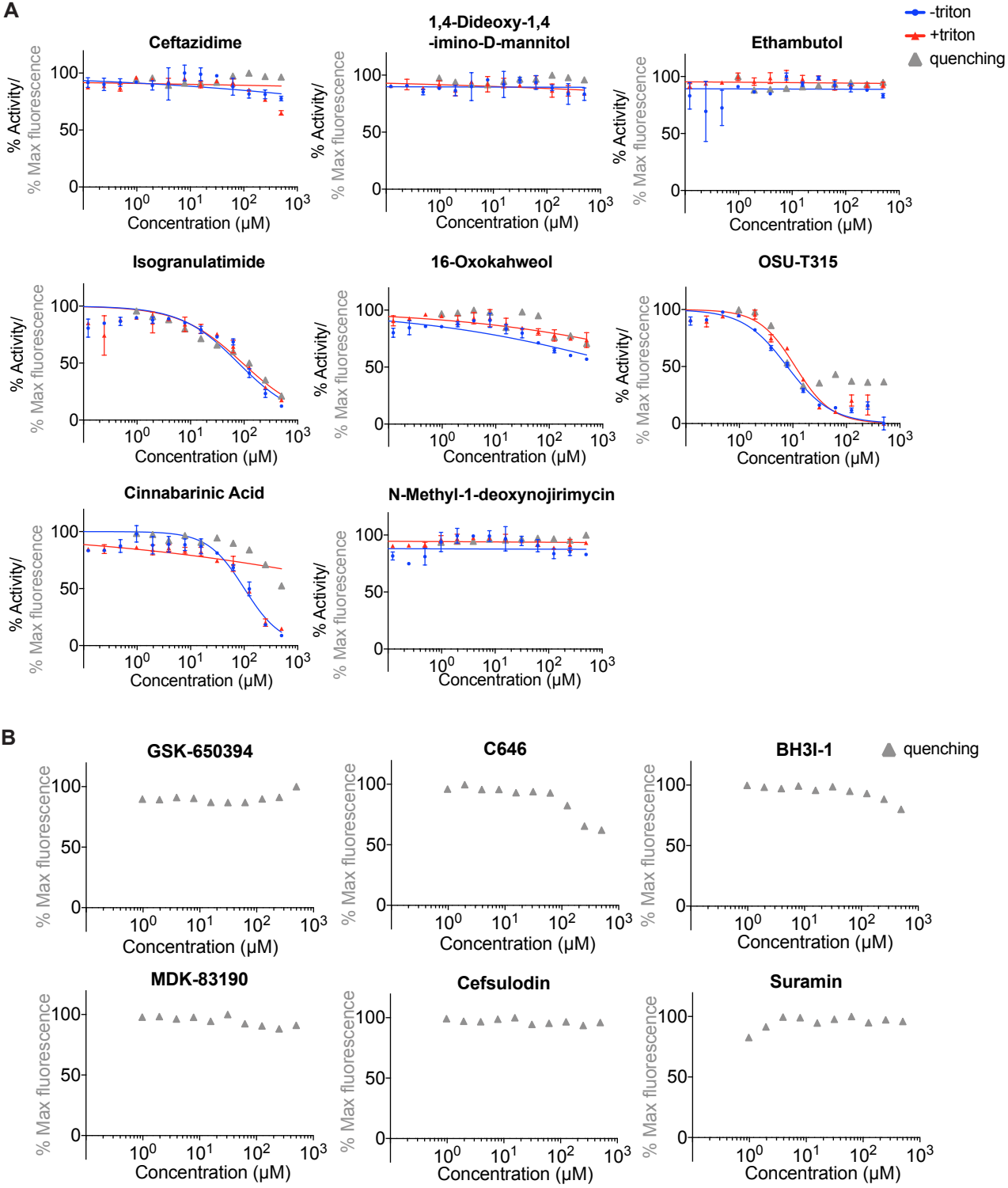
Supplementary Figure 2



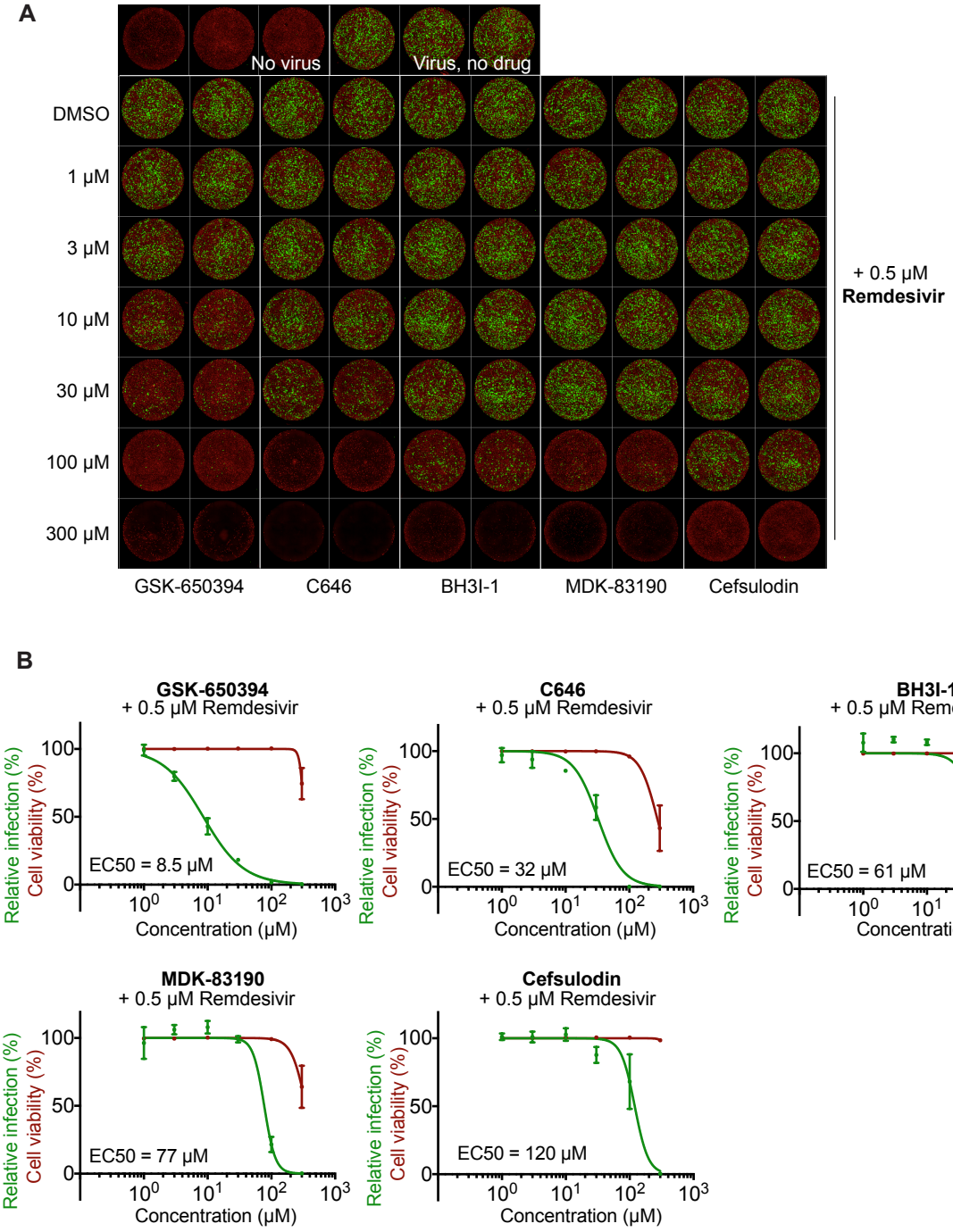
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplementary Table S1. Overview of purified SARS-CoV-2 RdRp proteins including yields

Protein preparation	Expression system	Yield (mg)	Yield (nmol)	Construct	Purification
7H8	Baculovirus-insect cell	19.5[a]	**607[a]**	nsp7-His6-nsp8	Ni-NTA, MonoQ, S200
nsp12-HF	Baculovirus-insect cell	1.92	17.3	nsp12-His6-3xFlag	Ni-NTA, MonoQ, S200
nsp12-F/7H8	Baculovirus-insect cell	0.084	0.59	nsp12-3xFlag/nsp7-His6-nsp8	FLAG M2, Heparin, S200
nsp12-HF/7L8	Baculovirus-insect cell	17.0[a]	**119[a]**	nsp12-His6-3xFlag/nsp7-GGSGGS-nsp8	Ni-NTA, MonoQ, S200
nsp12-HF/7/8	Baculovirus-insect cell	0.053	0.37	nsp12-His6-3xFlag/nsp7/nsp8	FLAG M2, Heparin, S200
nsp7	*E. coli*	1.96	213	14His-Sumo-nsp7	Ni-NTA, Ulp1, Ni-NTA, MonoQ, S200
nsp8	*E. coli*	5.34	244	14His-Sumo-nsp8	Ni-NTA, Ulp1, Ni-NTA, MonoQ, S200
nsp12	*E. coli*	0.65	6.1	14His-Sumo-nsp12	Ni-NTA, Ulp1, Ni-NTA, MonoQ, S200

[a] Combined yield from 2 preparations.

Supplementary Table S2. Predicted aggregation propensity of 18 HTS hit compounds

Compound name	LogP	Structural Similarity Index
NF 023	-6.1	-
Suramin	-5.7	-
Cefsulodin	-5.7	-
Ceftazidime	-5	-
PPNDS	-3.3	-
Evans Blue	-2.7	-
Diphenyl Blue	-2.7	-
1,4-Dideoxy-1,4-imino-D-mannitol	-2.6	-
N-Methyl-1-deoxynojirimycin	-2.2	-
Ethambutol	0.4	-
Cinnabarinic Acid	1.1	-
Isogranulatimide	2	-
BH3I-1	3.1	-
16-Oxokahweol	3.3	-
MDK 83190	3.8	-
C646	4.7	-
OSU-T315	5.2	-
GSK-650394	5.8	-

Compounds analysed using the open access tool Aggregator Advisor See (Irwin et al., 2015)

Supplementary Table S3. Compounds that showed only weak or no clear activity against SARS-CoV-2 RdRp *in vitro* or interfered with the fluorescent substrate by quenching

Compound name	IC_{50} (µM)		Data figure
	No detergent	+ 0.01% Triton X-100	
Cinnabarinic acid	>100	>100	S4A
Ceftazidim	no clear inhibition	no clear inhibition	S4A
1,4-Dideoxy-1,4-imino-D-mannitol	no clear inhibition	no clear inhibition	S4A
Ethambutol	no clear inhibition	no clear inhibition	S4A
16-Oxokahweol	no clear inhibition	no clear inhibition	S4A
N-Methyl-1-deoxynojirimycin	no clear inhibition	no clear inhibition	S4A
OSU-T315	[a]	[a]	S4A
Isogranulatimide	[a]	[a]	S4A

[a] IC_{50} could not be assessed due to compound-related quenching effects on fluorescent substrate

Supplementary Table S4. Purchased drugs for *in vitro* validation and viral inhibition experiments

Compound name	CAS Number	Company	Catalog Number
Suramin	129-46-4	Sigma	S2671
Cefsulodin	52152-93-9	Sigma	C 8145
Cinnabarinic Acid	606-59-7	Sigma	SML0096
1,4-Dideoxy-1,4-imino-D-mannitol	114976-76-0	Sigma	D 8390
C646	328968-36-1	Sigma	SML0002
OSU-T315	2070015-22-2	MedChem Express	HY-18676
MDK 83190	79183-19-0	MedChem Express	HY-18633
Ceftazidime	72558-82-8	MedChem Express	HY-B0593
BH3I-1	300817-68-9	MedChem Express	HY-100383
Remdesivir	1809249-37-3	MedChem Express	HY-104077
Isogranulatimide	244148-46-7	Calbiochem/Millipore	371957
16-Oxokahweol	108664-99-9	Cayman Chemicals	CAY30117
N-Methyl-1-deoxynojirimycin	69567-10-8	Biovitica	BVT-0130-M001
Ethambutol	1070-11-7	Selleck Chemicals	S4004
GSK-650394	890842-28-1	Tocris	3572