

SUPPORTING INFORMATION for

Targeting RNA-Protein interactions within the HIV-1 lifecycle.

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SUPPORTING RESULTS AND DISSCUSION

The small molecule screen uses the increase in fluorescence intensity to monitor the rearrangement of an RNA molecular beacon substrate **FQ WT** (based on SL3 within the Ψ -packaging domain), upon binding of the Gag protein¹. Small molecules that can prevent the unwinding of **FQ WT** either through the inhibition of Gag binding to or unwinding of the **FQ WT** will be detected by the low fluorescence intensity when compared to the negative control. Addition of unlabeled SL3 RNA to **FQ WT** prior to the addition of the gag polypeptide showed the **FQ WT**-Gag interaction could be inhibited and fluorescence intensity comparable to that of the **FQ WT** molecular beacon alone was achieved. For these reasons the **FQ WT** alone as well as SL3 were used as positive controls, (with SL3 being a “small molecule like” positive control set at 100% inhibition), DMSO alone was used as the negative control for the SM screen set at 0% inhibition.

The SM screening data was normalized as percentage inhibition of **FQ WT** relative to the positive and negative controls (Supp. Fig. 1); positive hits were defined as small molecules showing inhibition greater than three standard deviations (21%) from the mean negative control. On each plate, the controls were used to calculate the Z' score and any plate which failed to gain a Z' greater than 0.5 was rejected and the plate was rescreened. Confirmation of the primary hits was performed in triplicate under the original screening conditions.

SUPPORTING FIGURES

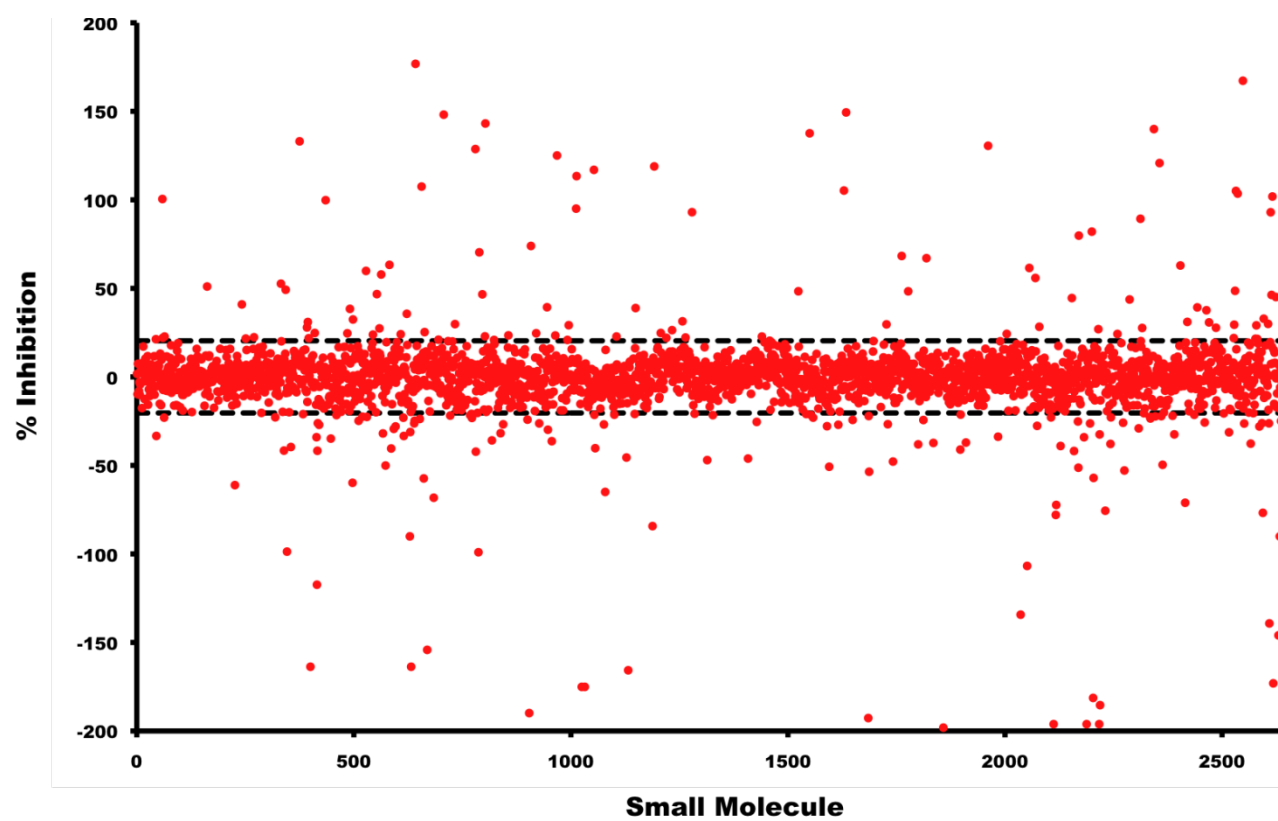


Figure S1: Results from the initial small molecule screen. Hits showing a positive inhibition of three standard deviations or more, from the negative control (dotted line), were validated by rescreening under the original conditions in triplicate.

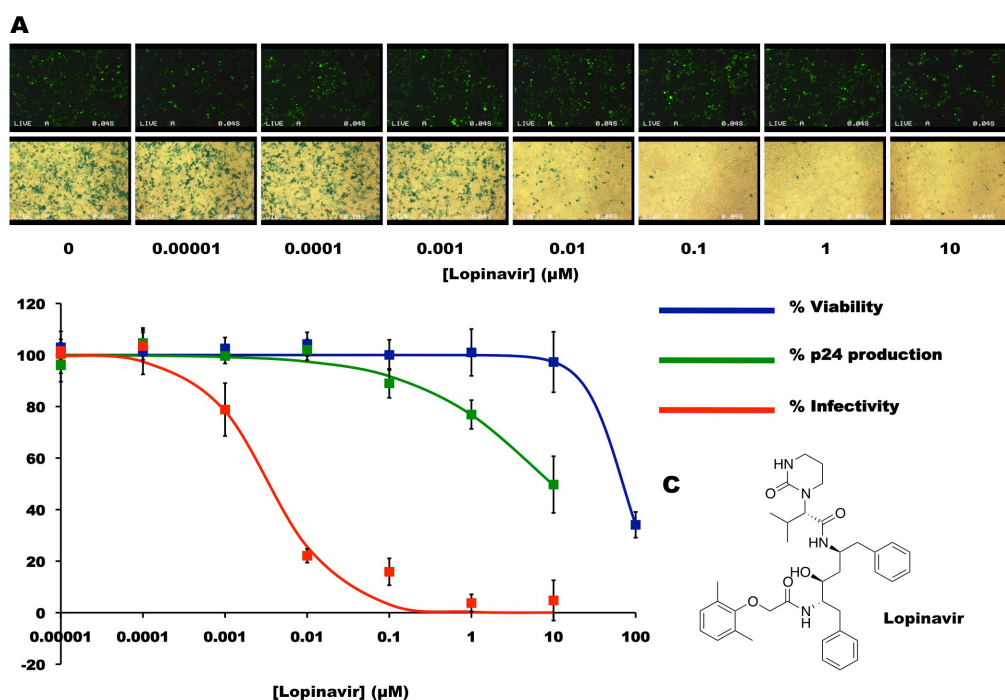


Figure S2 Biological activity of Lopinavir. **A)** 24 h post-infection the expression of β -galactosidase within the TZM-bl cell was imaged using X-Gal (bottom row). **B)** Viability of the 293T cells (blue line, 50% inhibition (CC_{50}) = $69.1 \pm 15.3 \mu\text{M}$), viral production from transfected 293T (green line, 50% inhibition ($p24_{50}$) = $9.6 \pm 3.2 \mu\text{M}$) and infectivity of harvested viral particles (red line 50% inhibition (IC_{50}) = $0.035 \pm 0.008 \mu\text{M}$) in the presence of different concentrations of Lopinavir. **C)** Structure of Lopinavir

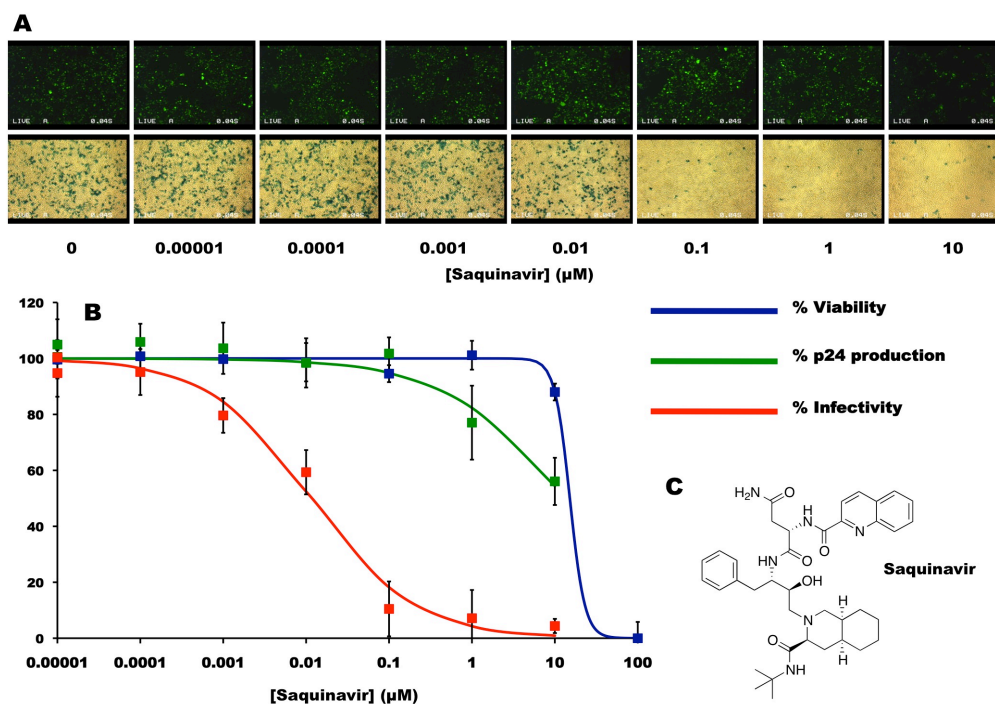


Figure S3: Biological activity of Saquinavir. **A)** 24 h post-infection, the expression of β -galactosidase within the TZM-bl cell was imaged using X-Gal (bottom row). **B)** Viability of the 293T cells (blue line, 50% inhibition ($CC_{(50)} = 15.1 \pm 4.5 \mu\text{M}$)), viral production from transfected 293T (green line, 50% inhibition ($p24_{(50)} = 13.5 \pm 4.2 \mu\text{M}$)) and infectivity of harvested viral particles (red line 50% inhibition ($IC_{(50)} = 0.011 \pm 0.005 \mu\text{M}$)) in the presence of different concentrations of Saquinavir. **C)** Structure of Saquinavir

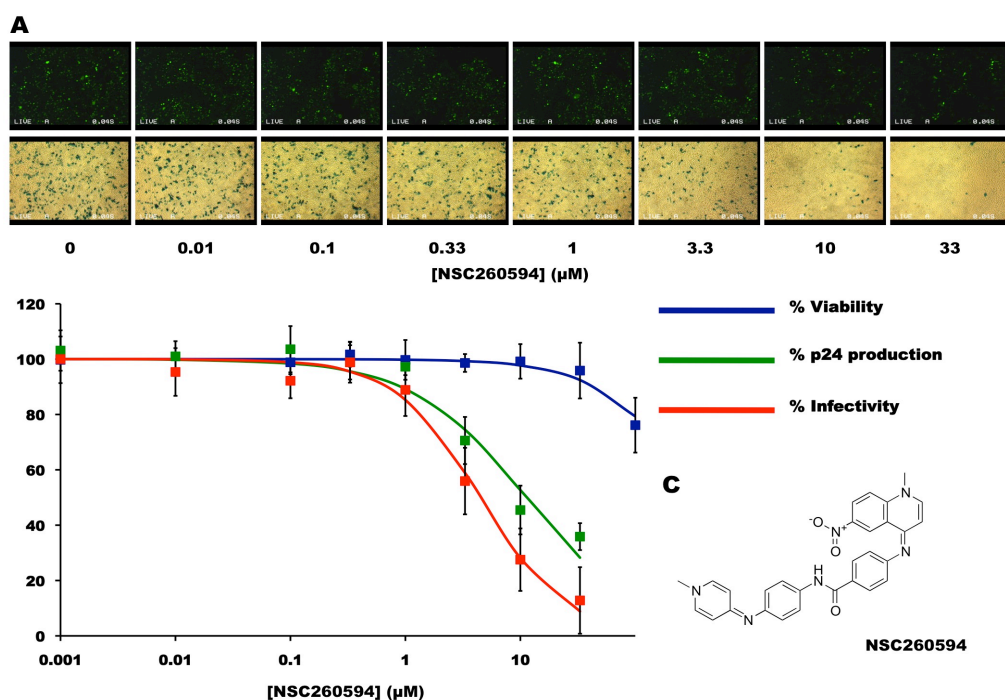


Figure S4: Biological activity of NSC260594. **A)** 24 h post-infection, the expression of β -galactosidase within the TZM-bl cell was imaged using X-Gal (bottom row). **B)** Viability of the 293T cells (blue line, 50% inhibition ($CC_{(50)}$ = N/A)), viral production from transfected 293T (green line, 50% inhibition ($p24_{(50)}$ = $11.3 \pm 3.4 \mu\text{M}$)) and infectivity of harvested viral particles (red line 50% inhibition ($IC_{(50)}$ = $4.5 \pm 1.8 \mu\text{M}$)) in the presence of different concentrations of NSC260594. **C)** Structure of NSC260594

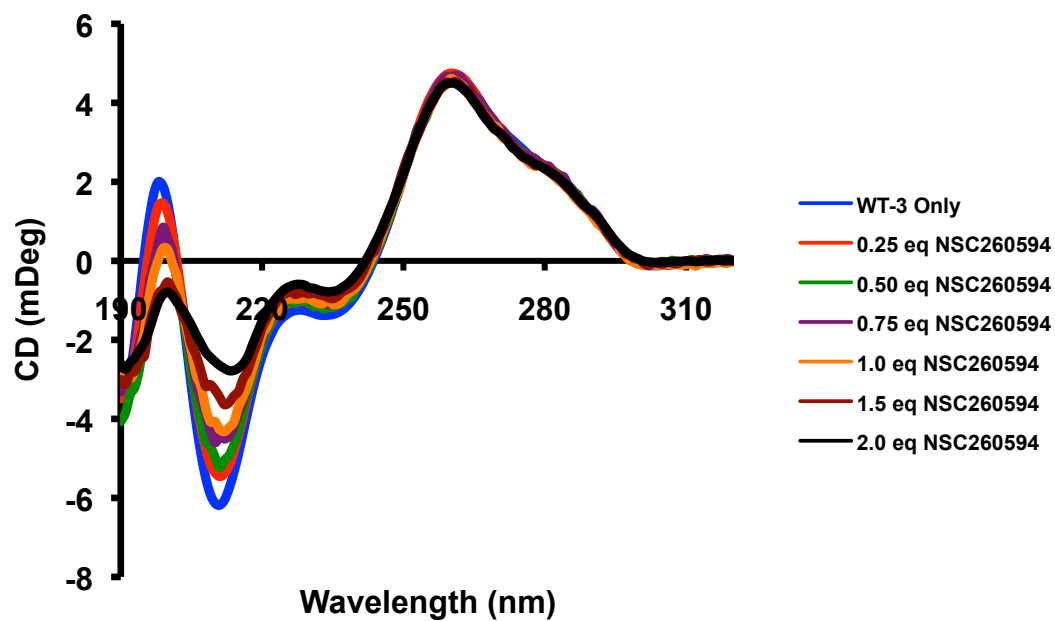


Figure S5: CD spectra of **WT-3** titrated against increasing amount of NSC260594. The decrease in CD signal at 210 nm and 195 nm with increasing concentrations of NSC260594 indicates a direct interaction between the molecule and the SL3 RNA.

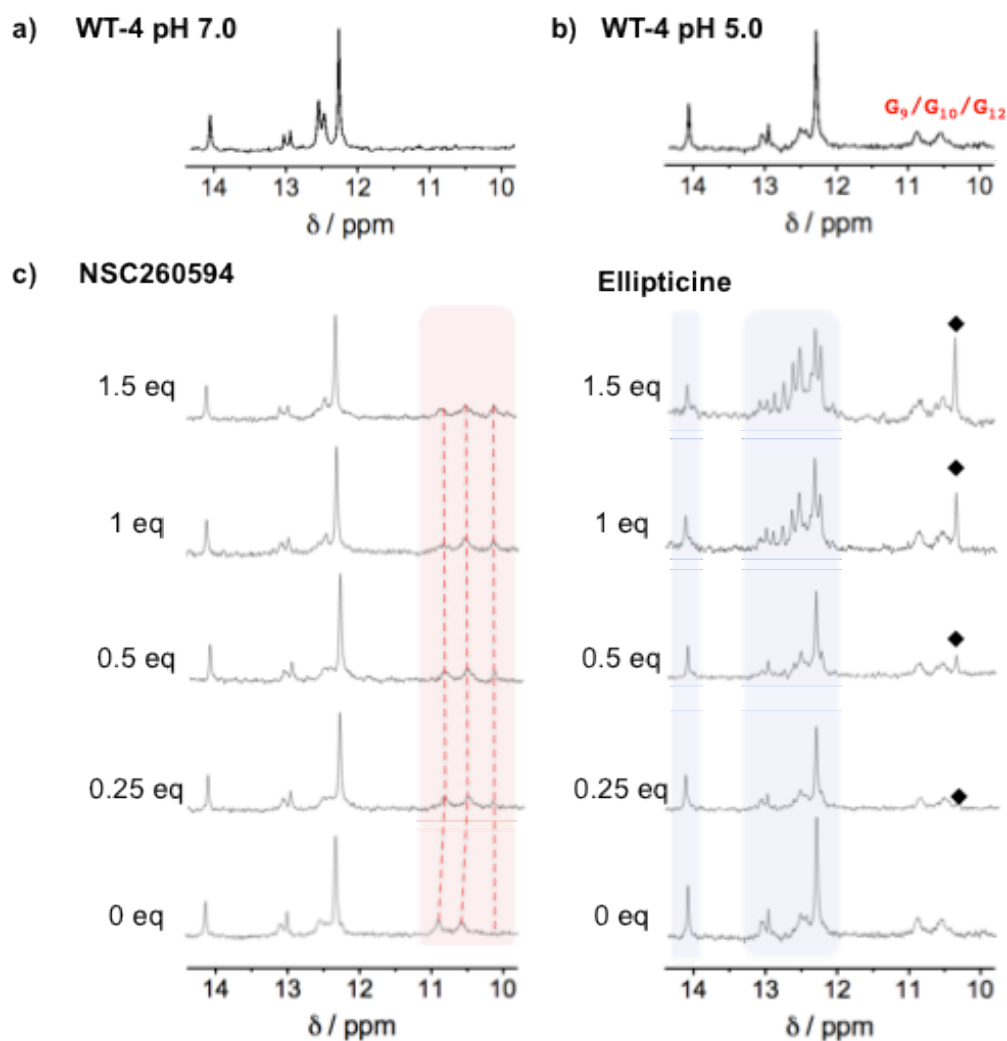


Figure S6: Imino proton NMR spectra of the titrations of a) NSC260594 and b) ellipticine against **WT-3** done at pH 5.0. The imino protons between 12 and 14.5 p.p.m (blue region) are attributed to the Watson-Crick H-bonded base pairs of the stem of the hairpin structure and imino protons between 10 - 11 p.p.m (red region) are attributed to the **WT-3** loop G bases. The black diamond ($\blacklozenge$) highlights a peak attributed to ellipticine.

SUPPORTING TABLES

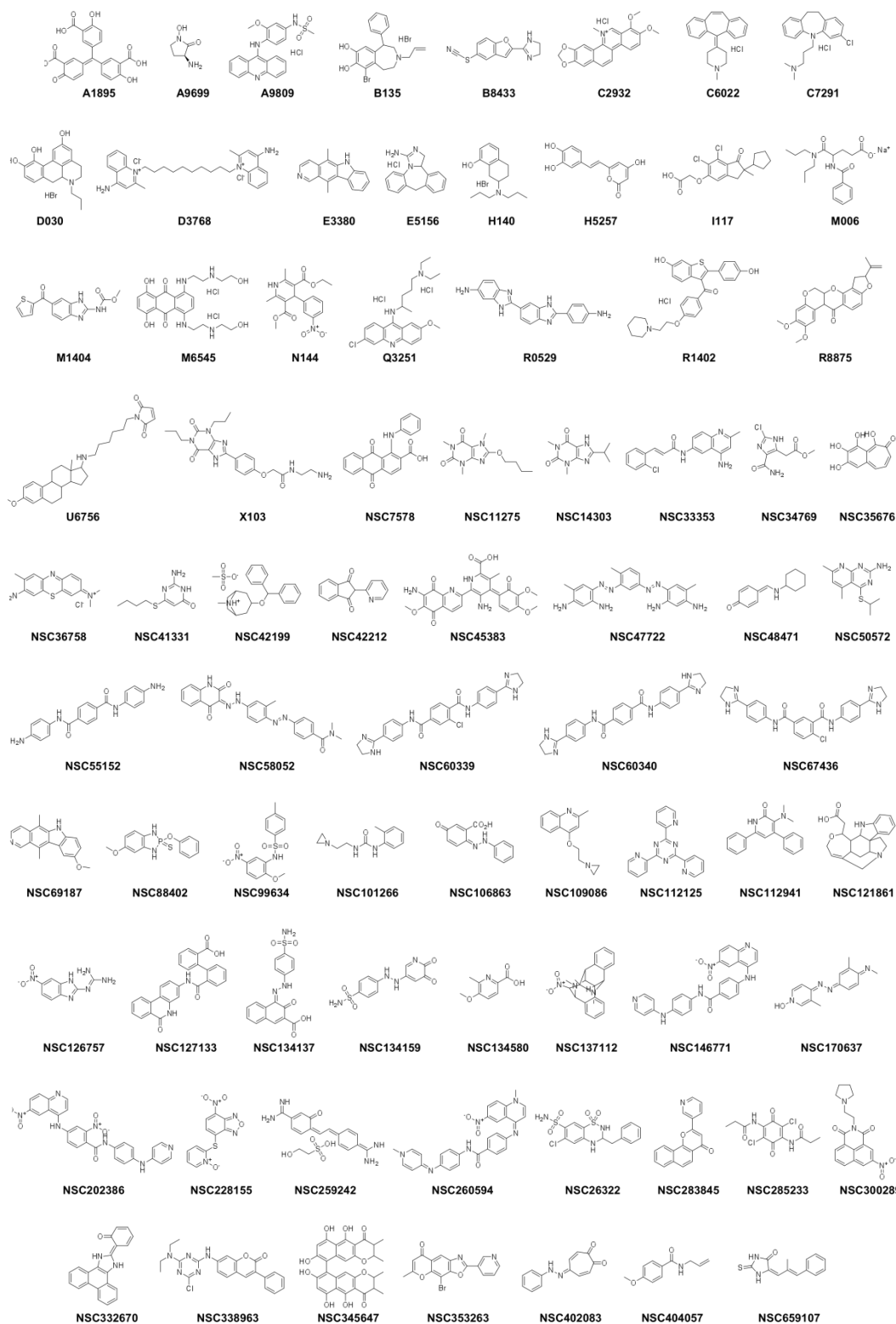


Table S1: Small molecules taken forward from the *in vitro* destabilisation assay to the viral replications assays.

Compound	CC ₍₅₀₎	SD	IC ₍₅₀₎	SD	Compound	CC ₍₅₀₎	SD	IC ₍₅₀₎	SD
A1895	-	-	0.2	0.1	NSC50572	-	-	-	-
A9699	-	-	-	-	NSC55152	-	-	-	-
A9809	3.9	3.3	-	-	NSC58052	48.5	12.0	-	-
B135	24.2	3.4	-	-	NSC60339	-	-	68.6	21.2
B8433	37.9	4.9	6.7	3.5	NSC60340	-	-	56.1	25.1
C2932	2.7	0.5	-	-	NSC67436	-	-	92.0	14.3
C6022	16.6	2.2	-	-	NSC69187	11.2	2.2	3.3	1.5
C7291	20.7	11.3	8.4	5.2	NSC88402	-	-	-	-
D030	19.4	12.9	3.8	2.2	NSC99634	-	-	-	-
D3768	26.7	15.8	-	-	NSC101266	-	-	56.1	9.8
E3380	27.0	12.9	22.7	12.2	NSC106863	-	-	-	-
E5156	77.9	9.4	-	-	NSC109086	42.2	5.5	-	-
H140	0.6	0.4	-	-	NSC112125	-	-	47.7	17.9
H5257	27.9	3.2	7.4	2.8	NSC112941	-	-	-	-
I117	58.9	7.5	-	-	NSC121861	-	-	-	-
M006	-	-	-	-	NSC126757	-	-	57.0	23.4
M1404	-	-	-	-	NSC127133	-	-	-	-
M6545	0.9	0.4	-	-	NSC134137	-	-	-	-
N144	32.4	10.6	-	-	NSC134159	10.3	2.9	-	-
Q3251	3.0	1.6	-	-	NSC134580	-	-	-	-
R0529	-	-	13.9	8.0	NSC137112	-	-	-	-
R1402	17.1	2.3	-	-	NSC146771	-	-	46.2	19.3
R8875	3.0	0.9	-	-	NSC170637	11.9	3.8	-	-
U6756	29.2	14.5	-	-	NSC202386	-	-	20.5	2.4
X103	-	-	-	-	NSC228155	13.8	1.2	-	-
NSC7578	55.0	8.0	24.2	7.2	NSC259242	-	-	41.5	20.9
NSC11275	-	-	-	-	NSC260594	-	-	4.5	1.8
NSC14303	-	-	-	-	NSC263220	-	-	-	-
NSC33353	3.1	1.6	-	-	NSC283845	44.2	4.6	-	-
NSC34769	-	-	-	-	NSC285233	1.4	0.6	-	-
NSC35676	63.7	7.7	-	-	NSC300289	-	-	-	-
NSC36758	4.6	2.7	-	-	NSC317605	5.0	2.9	-	-
NSC41331	-	-	72.8	21.0	NSC332670	17.0	2.5	4.7	2.0
NSC42199	36.8	5.8	-	-	NSC338963	-	-	83.6	1.1
NSC42212	-	-	21.6	12.3	NSC345647	11.2	2.7	-	-
NSC45383	0.4	0.2	-	-	NSC353263	-	-	-	-
NSC47722	20.5	5.2	-	-	NSC402083	32.0	6.9	-	-
NSC48471	-	-	-	-	NSC404057	-	-	-	-
					NSC659107	-	-	73.4	52.3

Table S2: Results from the viral replication assays to determine initial cytotoxicity and efficacy of potential hits from the initial small molecule screen.

[NSC260594] μM	0	0.5	1	5	10	20	30	40	50	100
T_m ($^{\circ}\text{C}$)	85.7	85.8	85.5	86.1	86.7	88.4	<95	<95	<95	<95
SD	1.0	0.9	1.2	0.5	0.2	1.0	-	-	-	-
ΔT_m ($^{\circ}\text{C}$)	-	0.11	-0.23	0.57	0.57	1.71	-	-	-	-

Table S3: Fluorescence melting titration of compound NSC260594 (0-100 μM) against the oligo **FQ WT** that was used in the initial *in vitro* screen. Above 20 μM of NSC260594 **FQ WT** becomes stabilised and FQ WT can not be melted even at 95 $^{\circ}\text{C}$.

SUPPORTING REFERENCES

- (1) Bell, N. M.; Kenyon, J. C.; Balasubramanian, S.; Lever, A. M. *Biochemistry* **2012**, *51*, 3162.
- (2) Wei, X. P.; Decker, J. M.; Liu, H. M.; Zhang, Z.; Arani, R. B.; Kilby, J. M.; Saag, M. S.; Wu, X. Y.; Shaw, G. M.; Kappes, J. C. *Antimicrob Agents Ch* **2002**, *46*, 1896.
- (3) Akari, H.; Uchiyama, T.; Fukumori, T.; Iida, S.; Koyama, A. H.; Adachi, A. *J Gen Virol* **1999**, *80* (Pt 11), 2945.
- (4) Aiken, C. *J Virol* **1997**, *71*, 5871.