



Figure S1. Antiviral activity comparison of HCI and PR assay. Z' (A), S/B (B) and CV% (C) of HCI (used for HTS) and plate-reader (PR = whole-well fluorescence – used for hit confirmation) antiviral assays. The average Z' factors are 0.90 in HCI and 0.92 in PR; signal-to-background ratio are 8960 in HCI and 362 in PR; percentage of coefficient of variation of infected control are 3.4% in HCI and 2.7% in PR. The violin plot graphs show data from 13 plates. (D) Anti-RABV activity of ribavirin in BHK-21 cells in HCI and PR assay. Serial dilutions of ribavirin were added together with mCherry-RABV (MOI 0.019 in HCI assay, MOI 0.01 in PR assay) to BHK-21 cells. Antiviral activity and cell viability were determined by HCI analysis for 384-well assay, or by using a standard plate reader (PR) for 96-well assay. Fitting the dose-response curves results in the calculation of EC₅₀ of ribavirin of 9.5 μ M and 3.9 μ M, and CC₅₀ of 30 μ M and 28 μ M, respectively. Averages and standard deviations of at least 3 independent experiments are presented.

Table S1. Effect of selected molecules on *in vitro* RABV replication.

Compounds	Screening antiviral activity at 10 μ M		Dose response confirmation		
	% infection	% viability	EC ₅₀ (μ M) ^a	CC ₅₀ (μ M) ^b	SI ^c
Cyproheptadine	21	78	5.6 \pm 0.95	39 \pm 13	6.9
Levamlodipine	5.3	60	7.9 \pm 3.1	24 \pm 2.0	3.1
Dasatinib	7.0	65	2.0 \pm 2.6	5.8 \pm 4.8	2.9
Salinomycin	20	63	<0.080	3.0 \pm 0.8	>38
Ribavirin ^d	40	92	5.1 \pm 1.6	36 \pm 18	7.0

a. Concentration at which present virus infection was reduced by 50%.

b. Concentration at which cell viability was reduced by 50%.

c. Selectivity index (ratio of CC₅₀ to EC₅₀).

d. Included as reference compound.