One pill to control them all: Identification of the thiazole-5-carboxamide GPS491 as an inhibitor of HIV-1, adenovirus, and coronavirus replication

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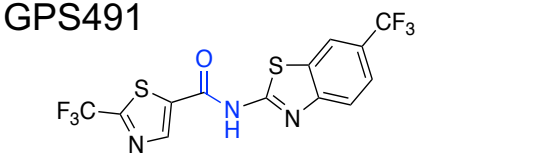
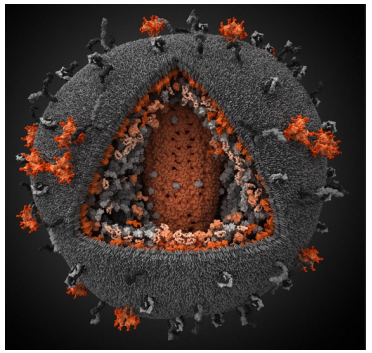
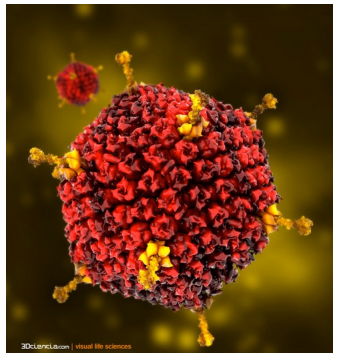
Most current antivirals target discrete stages in the virus replication cycle (entry, genome replication, proteolytic cleavage of viral proteins) by inhibiting the activity of virus-encoded proteins or their interactions with host cell machinery. However, as obligate parasites, viruses are dependent upon a common subset of host cell processes for their replication. Consequently, altering the ability of a virus to effectively use a host cell process may provide an alternative strategy to impede virus replication, generating a more robust barrier to virus resistance, and affect a broader spectrum of viruses with a single agent. Medicinal chemistry optimization of a previously described stilbene inhibitor of HIV-1, 5350150 (2-(2-(5-nitro-2-thienyl)vinyl)quinoline), led to the identification of the thiazole-5-carboxamide derivative (GPS491) which retained potent anti-HIV-1 activity with reduced toxicity. In this report, we demonstrate that the block of HIV-1 replication by GPS491 is accompanied by a drastic inhibition of viral gene expression (IC_{50} ~0.25 µM), and alterations in the production of unspliced, singly-spliced and multiply spliced HIV-1 RNAs. GPS491 also inhibited the replication of adenovirus and multiple coronaviruses. Low µM doses of GPS491 reduced adenovirus infectious yield ~1000 fold, altered virus early gene expression/viral E1A RNA processing, blocked viral DNA amplification, and inhibited late (hexon) gene expression. Loss of replication of multiple coronaviruses (229E, OC43, SARS-CoV2) upon GPS491 addition was associated with the inhibition of viral structural protein expression and the formation of virus particles. Consistent with the observed changes in viral RNA processing, GPS491 treatment induced selective alterations in the accumulation/phosphorylation/function of splicing regulatory SR proteins and their kinases. Our study establishes that a compound which impacts the activity of cellular factors involved in RNA processing can prevent the replication of several viruses with minimal effect on cell viability.

GPS491

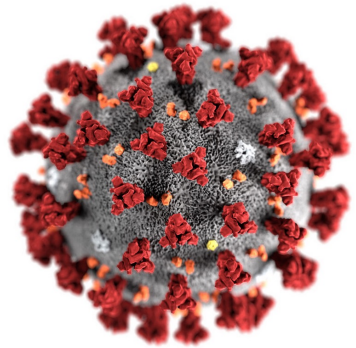




HIV-1



Adenovirus



Coronavirus

GPS491 inhibits HIV-1 replication by altering viral RNA accumulation/protein expression

Table 1. Effect of GPS491 on replication of multiple mammalian viruses.

Virus	Strain	EC_{50}	CC_{50}
HIV-1 Assayed in CEM-GXR cell line	Clade A	176 nM	12,052 nM
	Clade B	248 nM	12,052 nM
	NL43 E0043 RTI	235 nM	12,052 nM
	NL43 2918 PI	230 nM	12,052 nM
	NL43 11845 INI	203 nM	12,052 nM
	IIIB MVC res	216 nM	12,052 nM
HIV-1 Assayed in PBMCs	HIV-1 Bal (average of 3 donors)	248 nM	2,509 nM
	HIV-1 IIIB (average of 3 donors)	738 nM	2,509 nM
Adenovirus Assayed in A549 cell line	HAdV-C5	1000 nM	>40,000 nM
Coronavirus Assayed in Huh7 cell line	229E	250 nM	>10,000 nM
	OC43	250 nM	>10,000 nM
	SARS-Cov2	100 nM	>10,000 nM

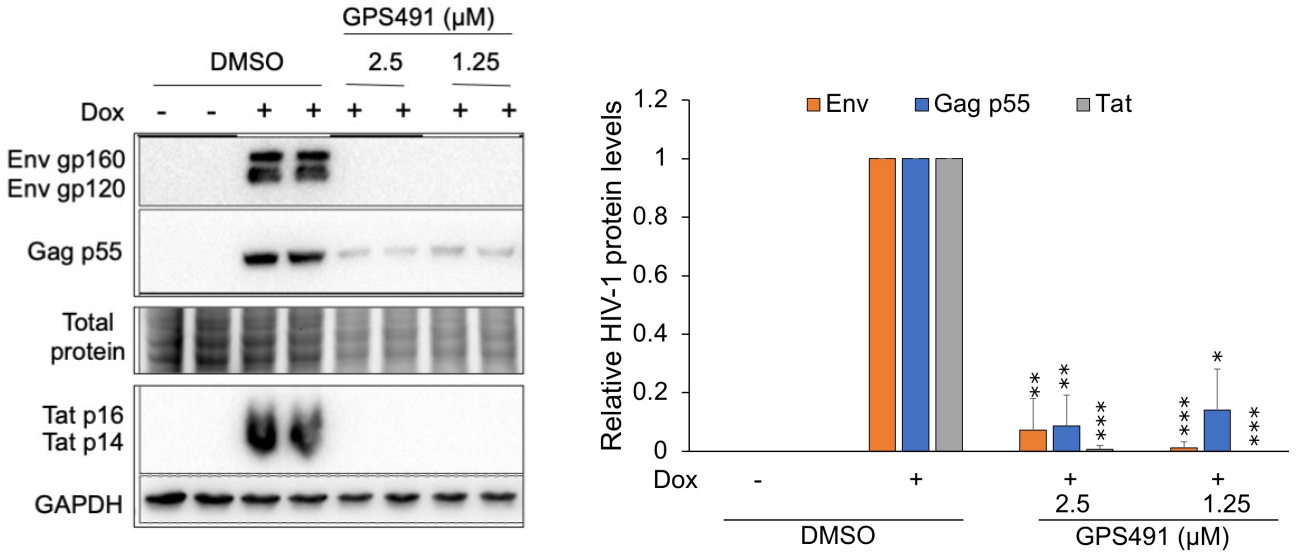


Figure 1. GPS491 inhibits expression of multiple HIV-1 proteins. HeLa rtTA HIVΔmls cells were treated with either 2.5 μM or 1.25 μM GPS491. Cells grown with or without doxycycline (Dox) with 1% DMSO served as positive and negative controls, respectively. Cells were harvested for HIV-1 protein and RNA analyses after 24 h of induction. Shown at left are representative Western blots showing the effects of compounds on HIV-1 Gag, Env, and Tat levels. On the right is a summary of $n = 3$ independent experiments; data are indicated as mean ± SD; * $p \le 0.05$, ** $p \le 0.01$, and *** $p \le 0.001$.

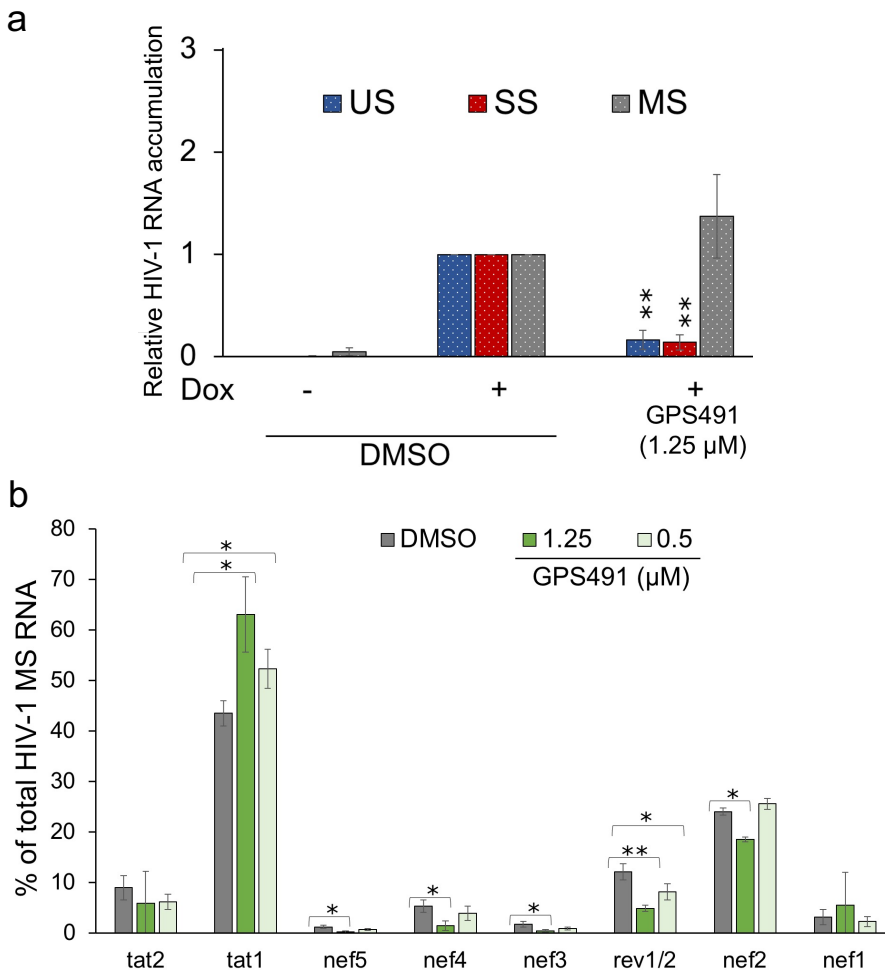


Figure 2. Effects of GPS491 on HIV-1 RNA accumulation and processing. HeLa rtTA HIVΔmls cells were treated with DMSO or varying concentrations of GPS491 +/− doxycycline (Dox) for 24 h. Cells were then harvested, total RNA was extracted, and cDNA was generated. Samples were subsequently analyzed (**a**) by RT-qPCR to measure levels of HIV-1 US, SS, and MS RNA levels or (**b**) by RT-PCR to measure levels of different MS RNA isoforms. Shown is a summary quantitation of results from $n > 3$ independent assays. Data are indicated as mean ± SD, * $p \le 0.05$, ** $p \le 0.01$, ns—not significant

GPS491 inhibits adenovirus replication by altering E1A RNA splicing/early & late viral protein expression

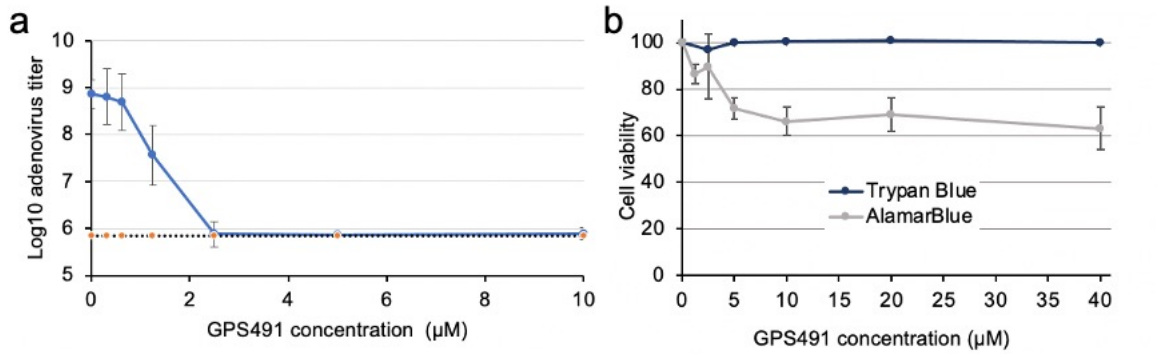


Figure 3. GPS491 inhibits adenovirus (HAdV-C5) replication in A549 cells. (**a**) A549 cells were uninfected or infected with human adenovirus serotype 5 (HAdV-C5) at an input MOI of 100 IU/cell then virus inoculum was removed and replaced with media containing DMSO or the indicated concentrations of GPS491. After 24 h, virus was collected for titration by endpoint dilution (blue line). T0 (dotted line, orange dots) is a sample harvested immediately after the 1 h adsorption period. (**b**) A549 cells were treated with the indicated doses of GPS491 for 24 h and then assessed for changes in metabolic activity by alamarBlue (grey line) or for viability by staining with trypan blue (blue line).

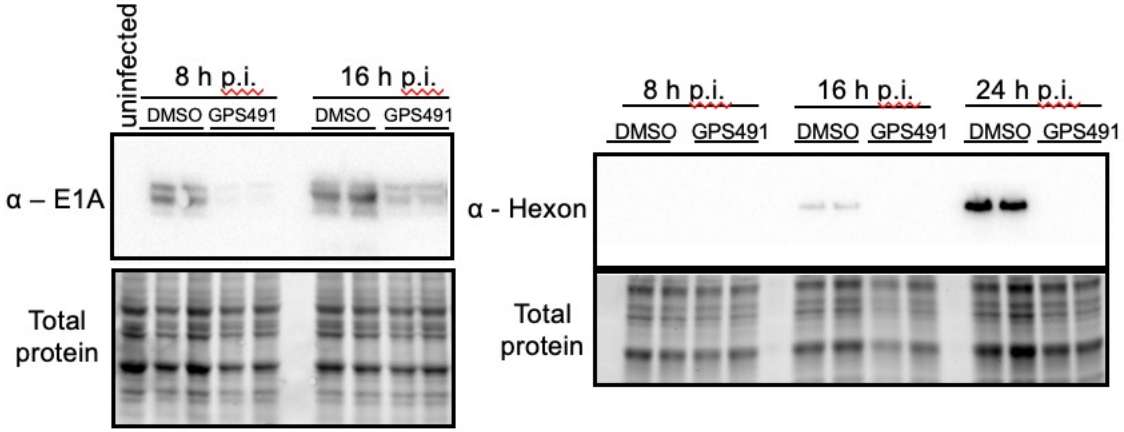


Figure 4. GPS491 delays E1A production and inhibits adenovirus hexon expression. A549 cells were infected with HAdV-C5 at an input MOI of 100 IU/cell for 1 h after which the virus inoculum was removed and replaced with media containing DMSO or GPS491 (2.5 μM). Cells were harvested 8 h, 16 h, or 24 h p.i. and lysates fractionated on SDS-PAGE gels. Shown are the representative Western blots showing the expression levels of E1A and hexon proteins in the control versus treated lysates.

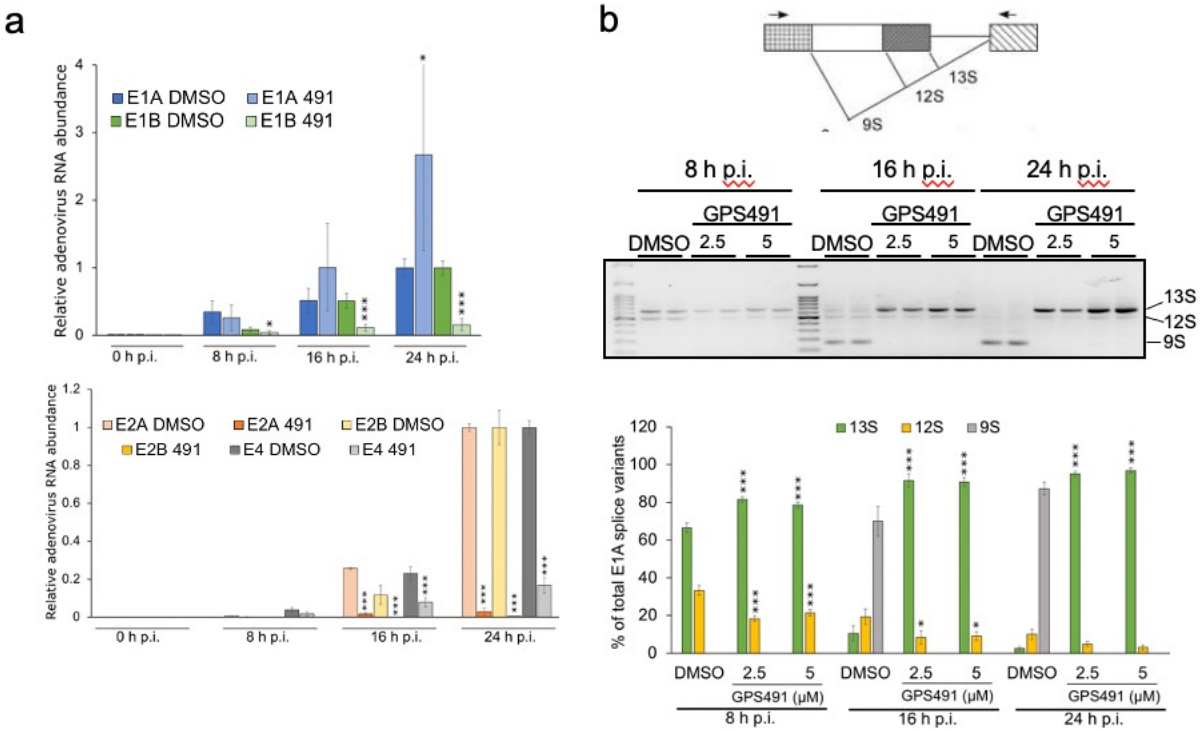


Figure 5. GPS491 alters adenovirus RNA expression/processing. A549 cells were infected with HAdV-C5 at an input MOI of 100 IU/cell for 1 h after which virus inoculum was removed and replaced with media containing DMSO or GPS491 (2.5 or 5 μM). (**a**) Total RNA was extracted at 8 h, 16 h, or 24 h after virus infection. RT-qPCR was performed using primers for E1A, E1B, E2A, E2B, and E4 RNAs. Values are expressed relative to RNA abundance observed 24 h p.i. in the presence of 1% DMSO. Shown are data from samples treated with 2.5 μM GPS491. (**b**) The effect of GPS491 on E1A RNA processing. Shown on the top is the schematic diagram of E1A RNA processing indicating the major E1A mRNA isoforms generated by alternative splicing. In the middle is a representative gel of the E1A RNA amplicons generated from cDNA. The graph on the bottom represents quantitation of amplicons generated across $n > 3$ independent assays.. Data are indicated as mean ± SD, * $p \leq 0.05$, ** $p \leq 0.01$, and *** $p \leq 0.001$.

GPS491 inhibits replication of multiple coronaviruses post-entry

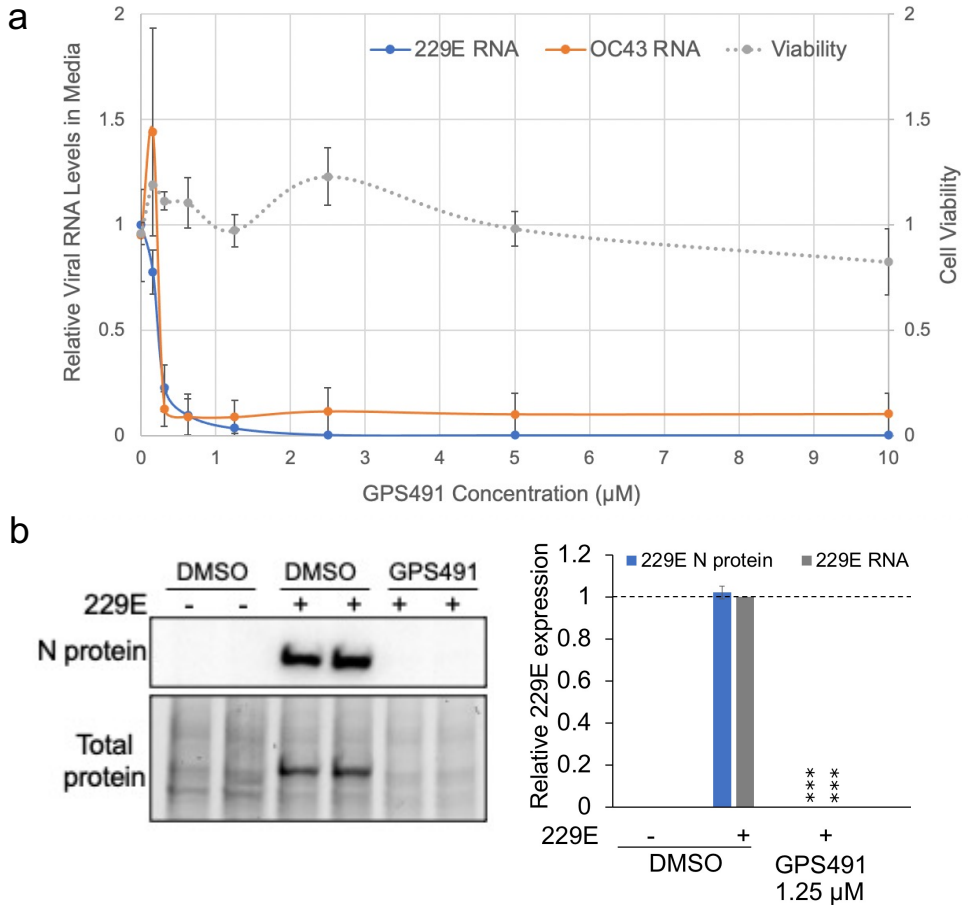


Figure 6.GPS491 inhibits replication of 229E, and OC43 coronaviruses. (**a**) Huh7 cells were infected with either 229E or OC43 at an input MOI of 0.1 and 1, respectively, for 1 h. Virus inoculum was removed, and replaced with media containing 1% DMSO or varying concentrations of GPS491. Then, 2 days p.i (229E) or 4 days p.i. (OC43), media were harvested to quantitate viral genomic RNA levels by RT-qPCR assay Values are expressed relative to those detected in virus-infected DMSO-treated samples. Effect of GPS491 on cell viability was assessed 2 days post compound addition using alamarBlue at the indicated doses of GPS491. Data are indicated as mean ± SD, ** $p \leq 0.01$, and *** $p \leq 0.00$. (**b)** Cells and media were harvested 2 days (229E) p.i. and the levels of viral proteins (N) were determined by Western blot and virus production assessed by RT-qPCR of the media (229E RNA).

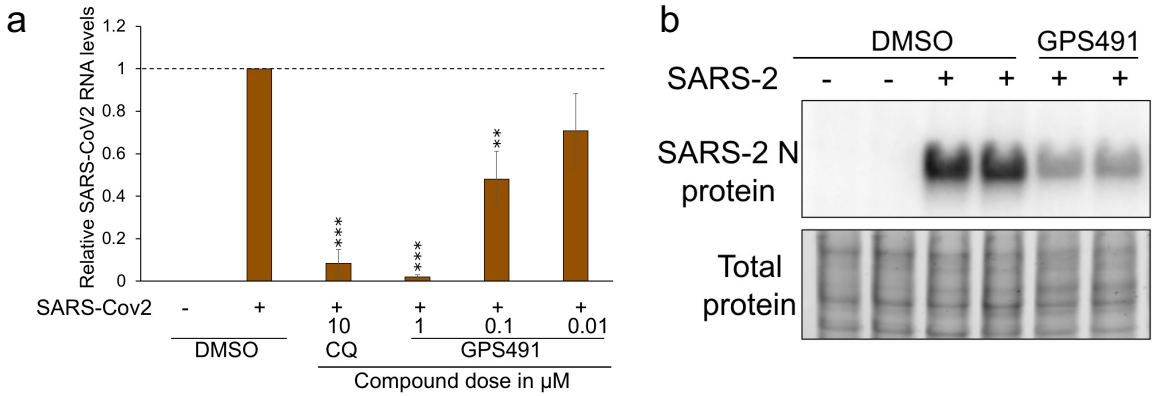


Figure 7. GPS491 inhibits replication of SARS-CoV2 coronaviruses. Huh7 cells were infected with SARSCoV2 at an input MOI of 1 for 1 h. Virus inoculum was removed, cells washed, and fresh media containing 1% DMSO or varying concentrations of GPS491. Cells and media were harvested 2 days p.i., and the levels of (a) virus production assessed by RT-qPCR of the media and (b) viral proteins (N) determined by Western blot.

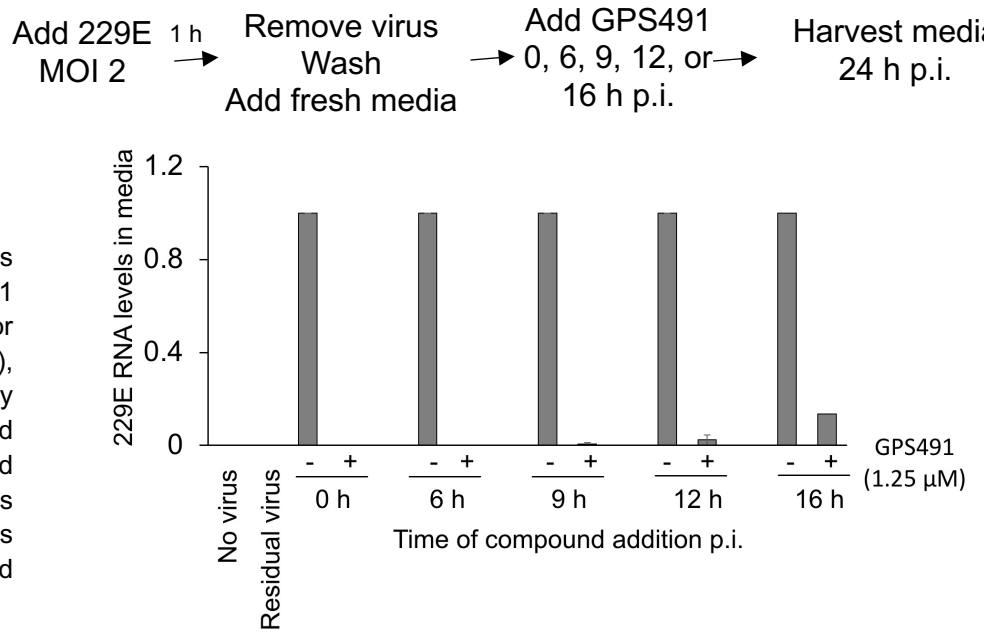


Figure 8. GPS491 inhibits 229E virus replication post entry. Huh7 cells were infected at an input MOI of 2. After incubation of cells for 1 h, cells were washed and fresh media added. At indicated times post infection (p.i.), DMSO or GPS491 was added at a final concentration of 1.25 μM and incubation continued. At 24 h post-virus infection, media were harvested and the abundance of viral RNA in media was determined by RTqPCR

GPS491 alters expression of select SR proteins/SR kinases

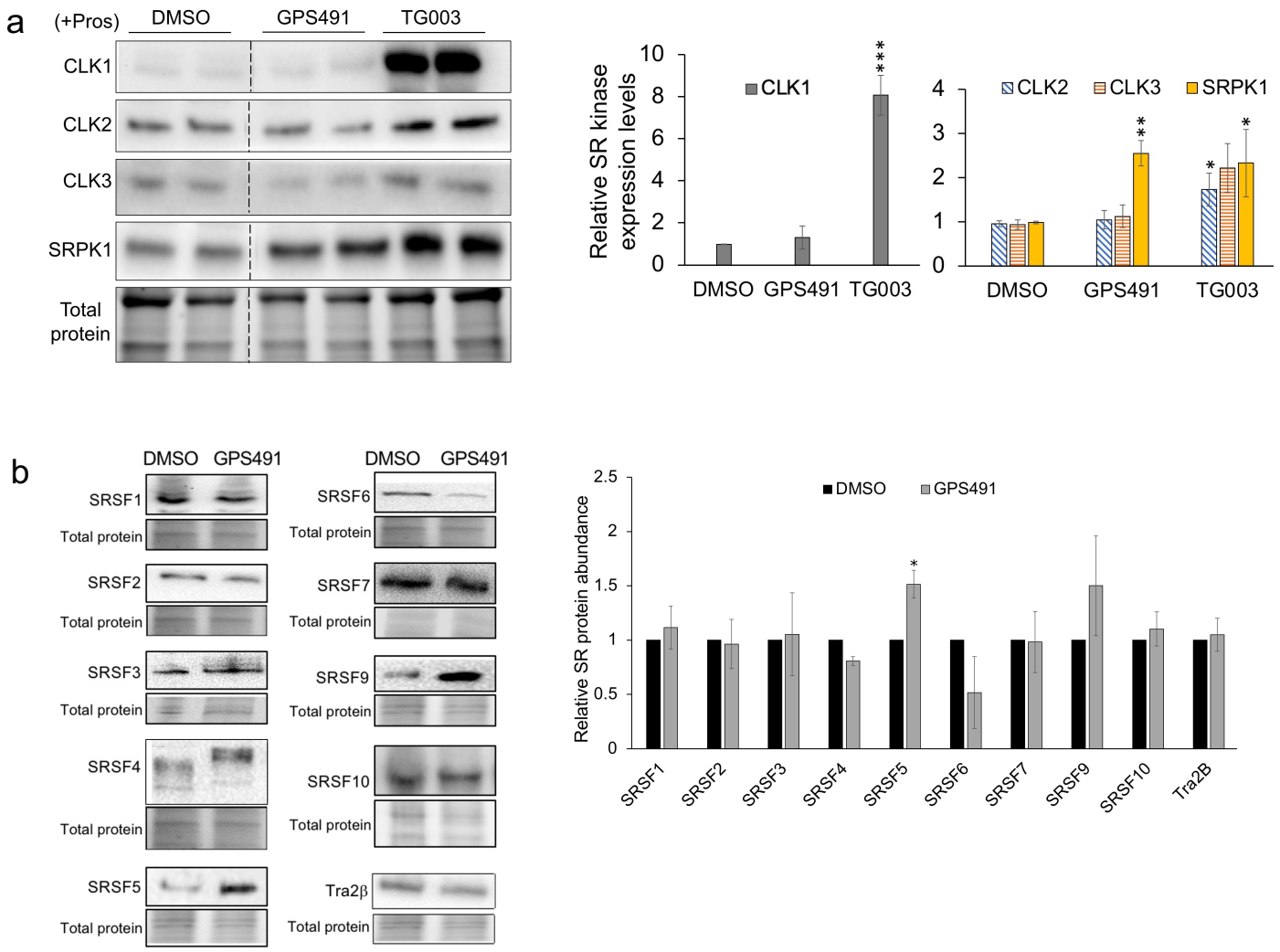


Figure 9. GPS491 alters expression of select SR kinases and proteins. Cells were treated with DMSO or GPS491 (2.5 μM). Twenty hours later, cells were harvested and analyzed by western for expression of (a) SR kinases (CLK1-3, SRPK1) (b) SR proteins. At the right, a summary of Western blots from three independent assays measuring the effect of GPS491 on protein levels relative to DMSO-treated cells. Asterisks (*) represent changes in expression level at $p < 0.05$.

Conclusions

- Demonstrated that GPS491 is a broad-spectrum inhibitor of multiple unrelated viruses (HIV-1, adenovirus, coronavirus) at doses that had minimal effect of cell viability.
- GPS491 induced changes in viral RNA splicing (HIV-1, adenovirus) that ultimately affected viral structural protein expression and virion formation.
- GPS491 induced changes in expression of select SR proteins/SRPK1 that regulate RNA processing.

Funding

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