Cell-dependent activation of ProTide prodrugs and its implications in antiviral studies

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Cytotoxicity assay

We seeded cells at a density of 15,000 cells/well in a white-wall clear-bottom 96-well plate (Corning) and incubated the cells at 37°C in a CO_2 cell culture incubator. When cells reached 70-80% confluence, we added a serial of concentrations (0, 1, 5, 10, 25, 30 μM) of TAF or SOF in the culture medium to each well and incubated the cells for 24 hours at 37°C. Cytotoxicity was determined using the MTS Assay Kit (ab197010) according to the manufacturer's instruction. No cytotoxicity was observed for both compounds at the tested concentrations.

Mass spectrometer parameters of the LC-MS/MS assay

The following MS parameters were adopted for all compounds: nebulizer gas: 15 psi; curtain gas: 15 psi; collision gas: 6 psi; ion spray voltage: 5500 V; source temperature: 550°C. Other compound-specific MS parameters, including M/Z transitions, delustering potential (DP), focusing potential (FP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP), were summarized in Table S1.

Table S1. Mass spectrometer parameters for TAF, TFV, TFV-DP, SOF, SOF-MP, SOF-TP, and the internal standards 2-chloroadenosine and adenosine-^{15}N5 5'-triphosphate

Compound	MRM (*m/z*)	DP(eV)	FP(eV)	EP(eV)	CE(eV)	CXP (eV)
TAF	477.2→176.1	30	40	10	50	15
TFV	288.1→176.1	15	28	6	30	15
TFV-DP	448.0→270.1	15	29	6	26	15
SOF	530.3→243.0	14	30	6	35	15
SOF-MP	341.3→243.0	20	28	5	22	6
SOF-TP	501.2→243.1	17	24	7	34	15
2-Chloroadenosine	302.3→170.1	15	20	6	30	15
Adenosine-^{15}N5 5'-triphosphate	513.0→141.0	15	30	6	30	15