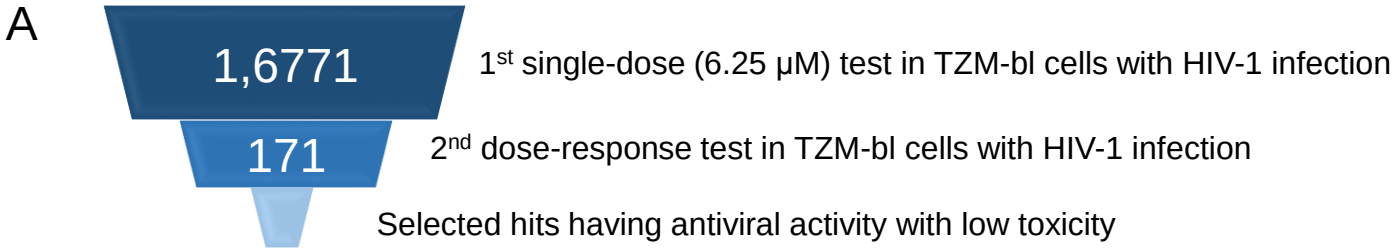
Supplementary Fig. S1



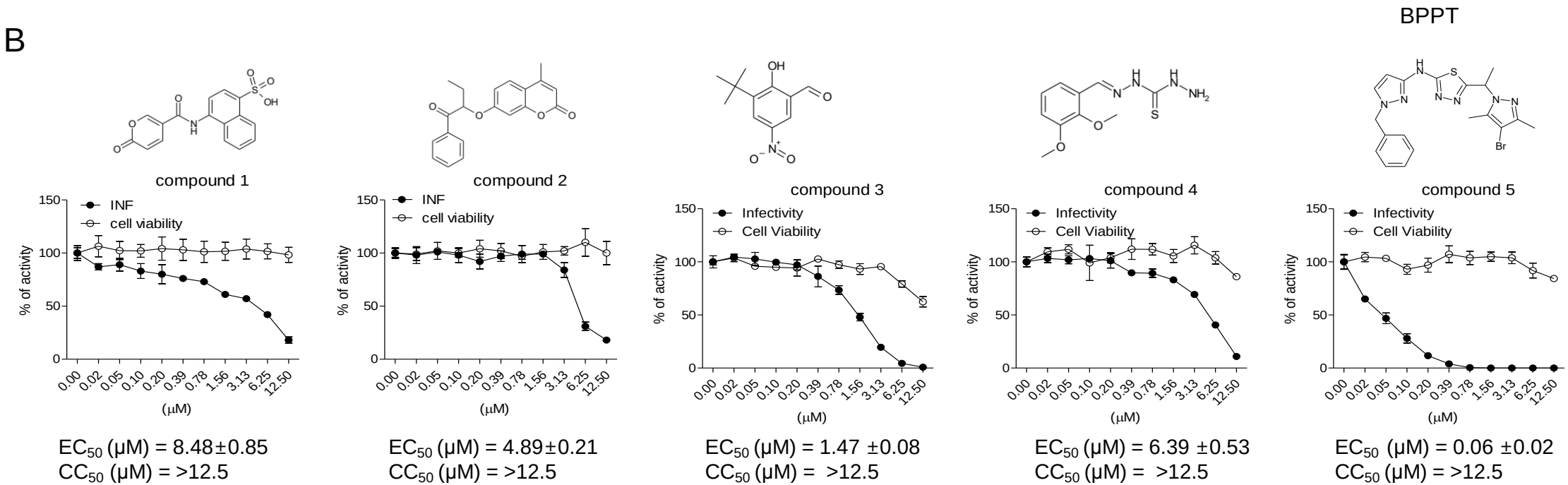
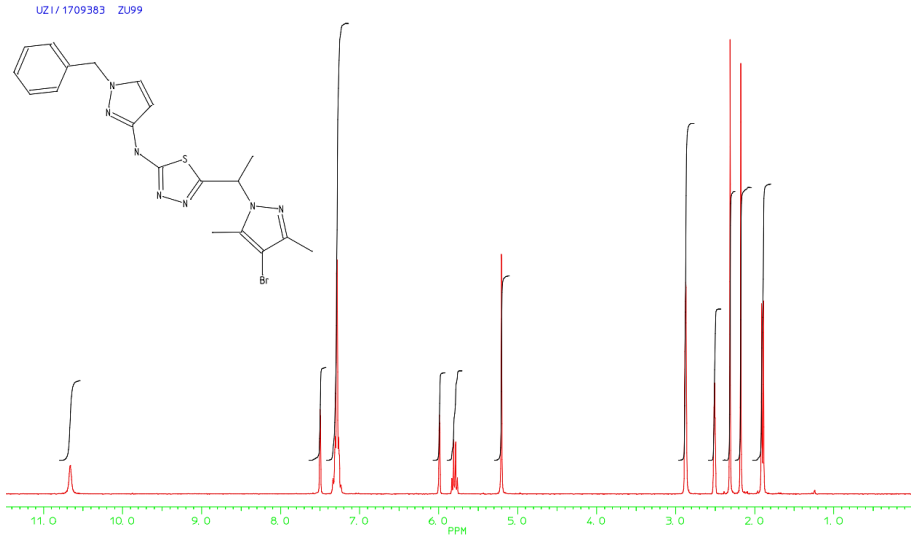


FIGURE S1. (A) A schematic funnel for screening hit compounds having the anti-HIV-1 activity. **(B)** Antiviral effects of hit compounds. TZM-bl cells (1×10^4) were treated with two-fold serial dilutions of hit compounds (0–12.5 μM) and then infected with HIV-1_{NL4-3} at an MOI 0.5. After 48 h of infection, the firefly luciferase (F-Luc) activity indicating viral infectivity was determined using the Bright-Glo luciferase assay kit, and cell viability was determined using PrestoBlue Cell Viability Reagent kit. The dose-response curves show the relative activity compared with that of the DMSO control (mean ± SD; *n* = 3). The upper graphic data present the chemical structure of hits and each EC_{50} value of hits is presented in the bottom of each graphic data.

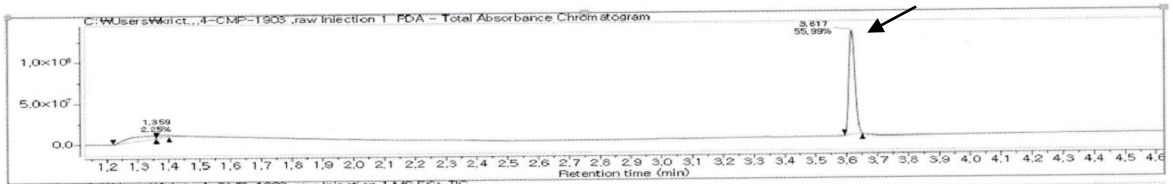
Supplementary Fig. S2

A



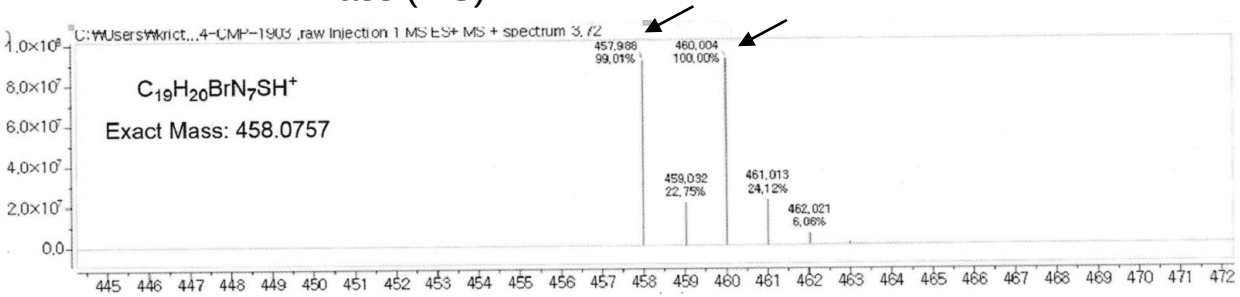
B

Liquid chromatography (LC)



The LC data indicates that purity of BPPT is > 95 %

Mass (MS)



Mass spectra : the exact mass is 458.0757, including two peaks by Br isotopes and addition of an H^+

C

Data : 12-4-002 Date : 04-Dec-2024 16:56
Instrument : MStation
Sample : 1903
Note :
Inlet : Direct Ion Mode : EI+
RT : 1.90 min Scan# : 58
Elements : C 19/0, H 20/0, Br 1/0, N 7/0, S 1/0
Mass Tolerance : 1000ppm, 5mmu if m/z < 5, 10mmu if m/z > 10
Unsaturation (U.S.) : -0.5 - 20.0

	Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
1	457.0684	27.57	-0.1 / -0.0	14.0	C19 H20 Br N7 S

HRMS (EI) for $C_{19}H_{20}BrN_7S$ *m/z*: calculated, 457.0684; found, 457.0684 $[M]^+$

Figure S2. **(A)** ^{1}H NMR spectra of synthesized N-(1-benzyl-1H-pyrazol-3-yl)-5-[1-(4-bromo-3,5-dimethyl-1H-pyrazol-1-yl)ethyl]-1,3,4-thiadiazol-2-amine (BPPT) provided by manufacturer. **(B)** LC/MS was used to determine the purity of the compounds and was carried out with a Waters ACQUITY Micromass (SQD2) equipped with a BEH C18 column, 2.1 x 50 mm, 1.7 µm. **(C)** High-resolution mass spectrometry (HRMS) analysis was performed using a MStation JMS-700 spectrometer (JEOL, Tokyo, Japan).

Supplementary Fig. S3

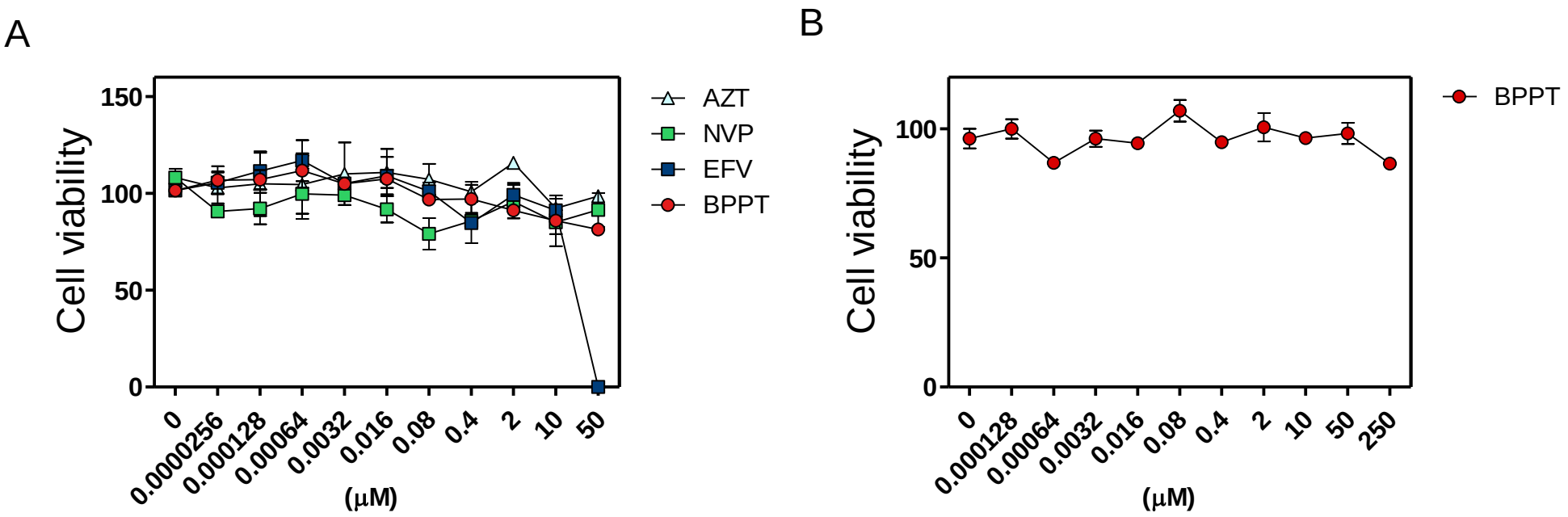


FIGURE S3. Cytotoxicity of BPPT and antiretrovirals (ARVs) in TZM-bl cells. TZM-bl cells (1×10^4) were treated with five-fold serial dilutions of BPPT and ARVs, starting from 50 μM **(A)** or 250 μM **(B)**. After 48 h of treatment, the cell viability was determined using the PrestoBlue Cell Viability Reagent. The dose-response curves show the relative activity compared with that of the dimethyl sulfoxide (DMSO) control (mean ± SD; *n* = 3).

Supplementary Fig. S4

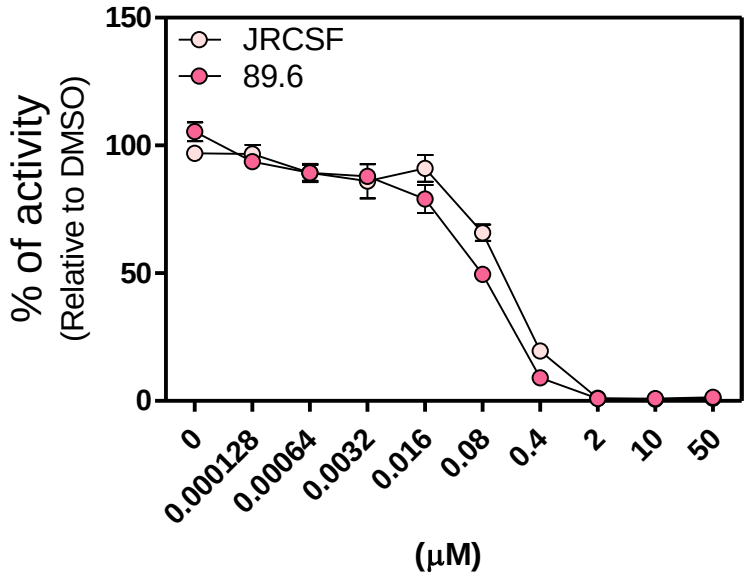


FIGURE S4. Antiviral activity of BPPT in various HIV-1 strains. TZM-bl cells (1×10^4) infected with HIV-1_{JR_CSF} or HIV-$1_{89.6}$ at an MOI of 0.5 were treated with five-fold serial dilutions of BPPT. After 48 h of infection, the firefly luciferase (F-Luc) activity indicating viral infectivity was determined using the Bright-Glo luciferase assay kit. The dose-response curves show the relative activity compared with that of the DMSO control (mean ± SD; *n* = 3).

Supplementary Fig. S5

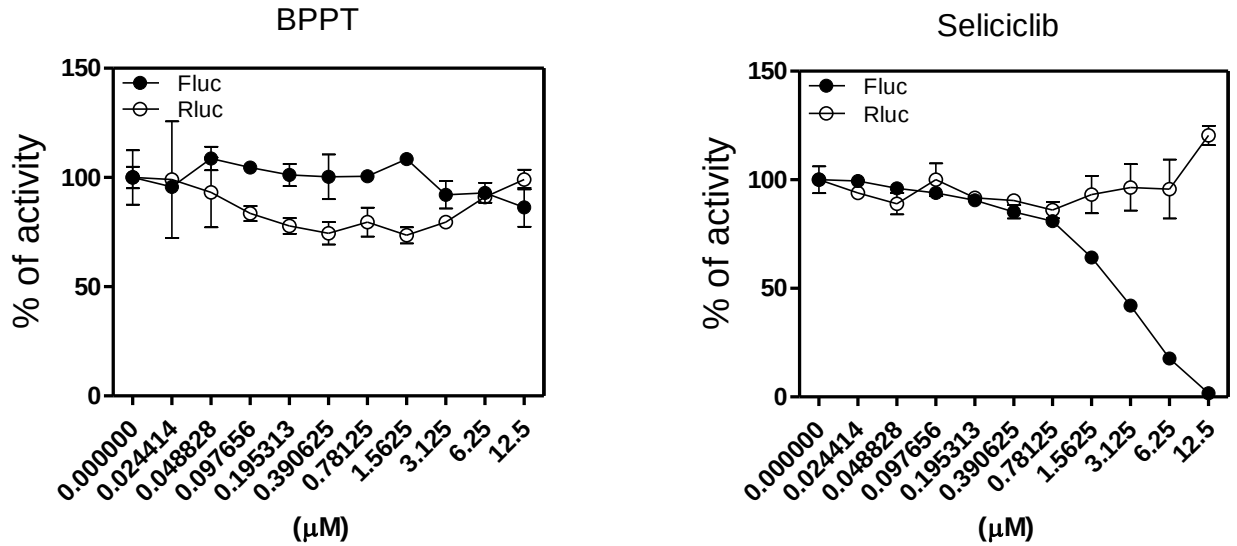
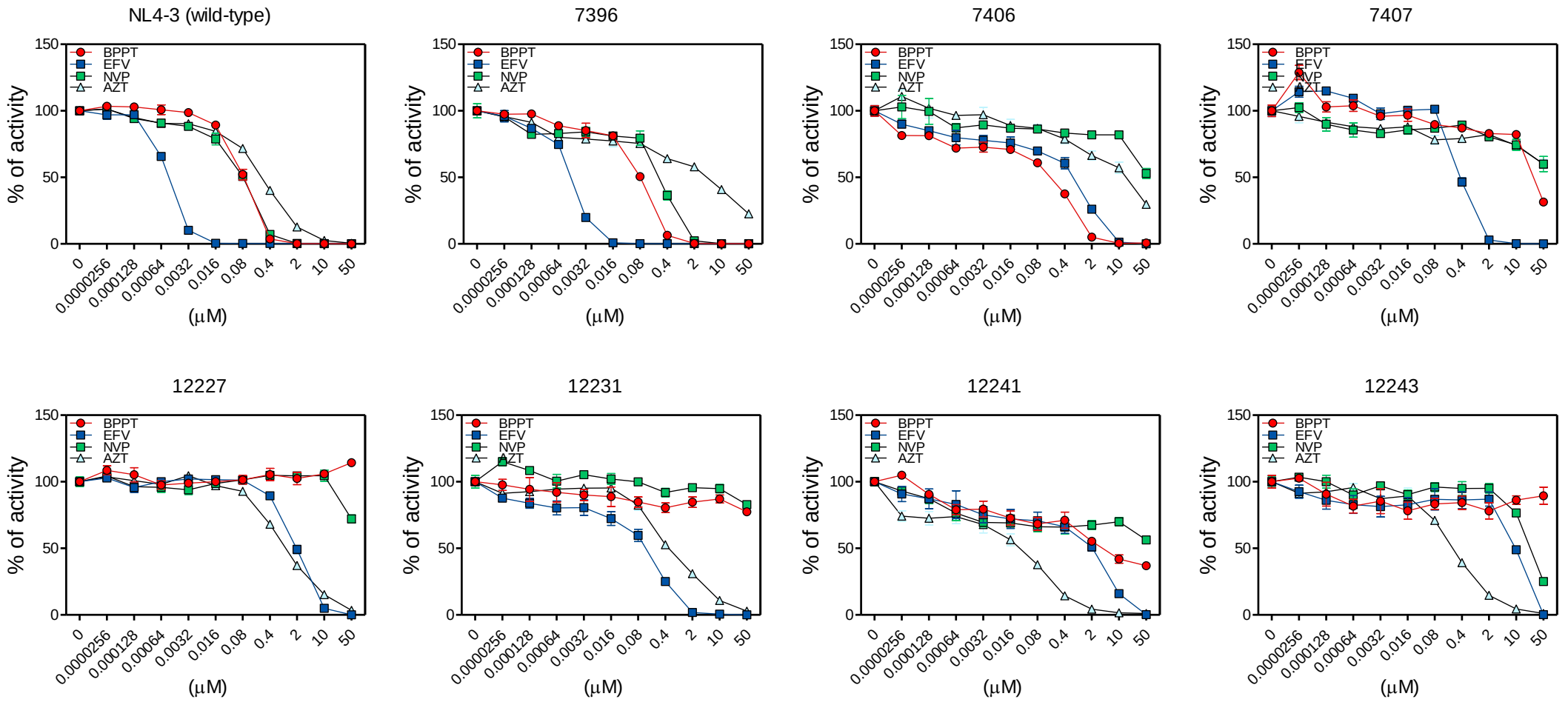


FIGURE S5. Inhibitory effect of BPPT on viral transcription. bl-DTR cells (1×10^4) were treated with two-fold serial dilutions of BPPT (left panel) or seliciclib (right panel) upon doxycycline (Dox) treatment (final concentration, 50 ng/mL). After 24 h of treatment, the activities of firefly luciferase (F-Luc, red line) and Renilla luciferase (R-Luc, blue line) were determined using the Dual-Glo™ Luciferase assay system. The dose-response curves show the relative activity compared with that of the DMSO control (mean ± SD; *n* = 3).

Supplementary Fig. S6

A Clinically derived (N)NRTI resistance-associated variants



B NNRTI resistance-associated single mutations

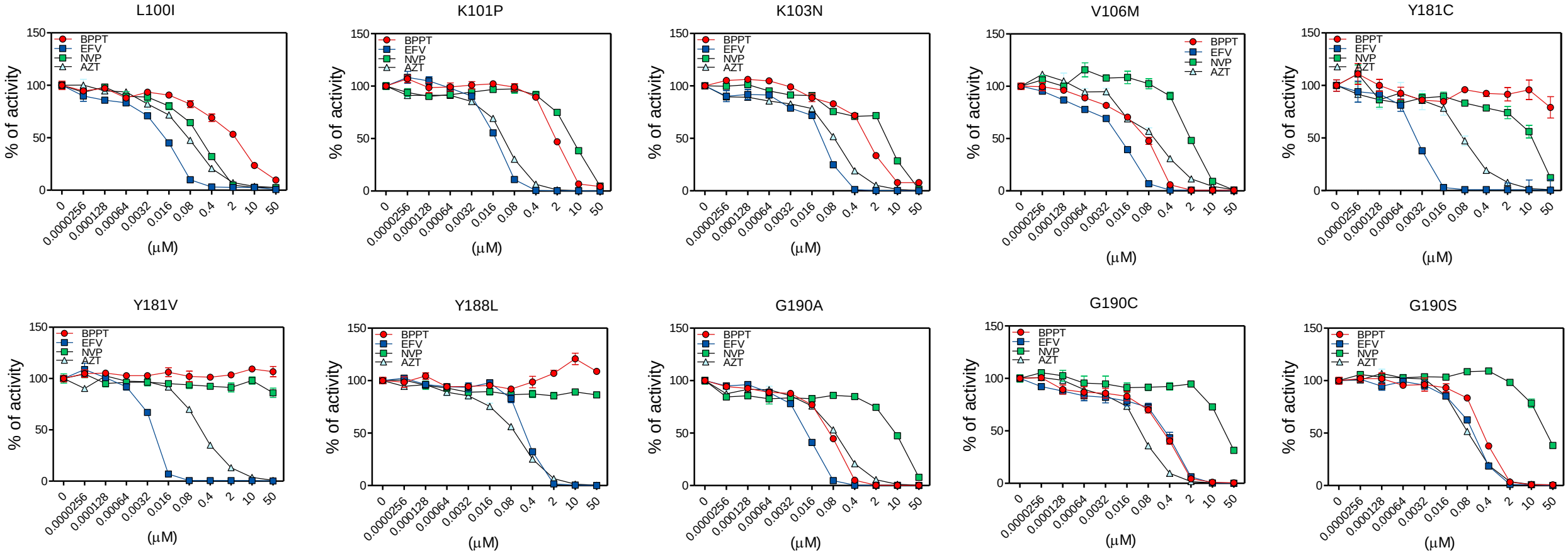
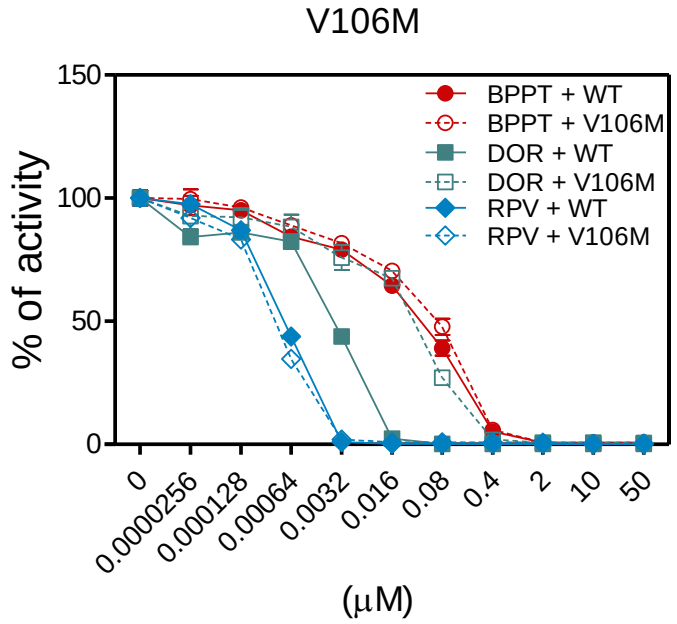


FIGURE S6. Antiviral effect of BPPT on (N)NRTI-resistant HIV-1 variants. TZM-bl cells (1×10^4) infected with clinically derived (N)NRTI-resistant variants **(A)** or variants harboring NNRTI-associated single mutations **(B)** were treated with five-fold serial dilutions of BPPT or ARVs. After 48 h of infection, the firefly luciferase (F-Luc) activity indicating viral infectivity was determined using the Bright-Glo luciferase assay kit. The dose-response curves show the relative activity compared with that of the DMSO control (mean ± SD; *n* = 3).

Supplementary Fig. S7

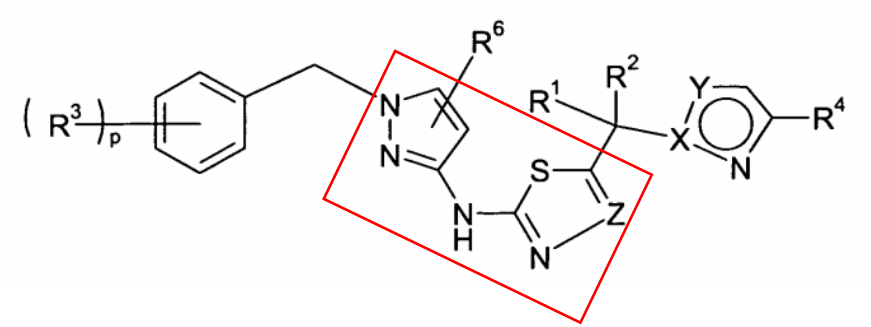


	EC_{50} (μM)		Fold Chang (FC)*
	Wild type (WT)	V106M	
BPPT	0.06 ± 0.01	0.065 ± 0.01	1.1
DOR	0.003 ± 0.0002	0.037 ± 0.001	12.3
RPV	0.0006 ± 0.00004	0.0005 ± 0.00003	0.83

FIGURE S7. Comparison of antiviral effect BPPT and Doravirine (DOR) on V106M variants. TZM-bl cells (1×10^4) infected with V106M were treated with five-fold serial dilutions of BPPT or ARVs. After 48 h of infection, the firefly luciferase (F-Luc) activity indicating viral infectivity was determined using the Bright-Glo luciferase assay kit. The dose-response curves show the relative activity compared with that of the DMSO control (mean ± SD; *n* = 3) (left panel). The half maximal effective concentration (EC_{50}) of left panel (right table). * The fold-change (FC) values were calculated by dividing the V106M EC_{50} by the wild type EC_{50}

Supplementary Fig. S8

Mtb-inhibitory compounds reported in WO 010/118852



(pyrazol-3-YL)-1,3,4-thiadiazol-2-amine and (pyrazol-3-YL)-1,3,4-thiazol-2-amine compounds

Figure S8. (pyrazol-3-YL)-1,3,4-thiadiazol-2-amine and (pyrazol-3-yl)-1,3,4-thiazol-2-amine compounds identified as inhibitor of Mtb. The core structure is highlighted with red-box, which is same to that of BPPT