Supporting Information

Targeting HIV-1 Reverse Transcriptase using a Fragment-Based Approach

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Table S1. Binding site, chemical structure, and potency of HIV-1 RT fragments hits discovered by Bauman et al. [7].

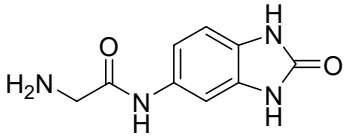
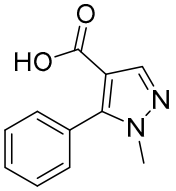
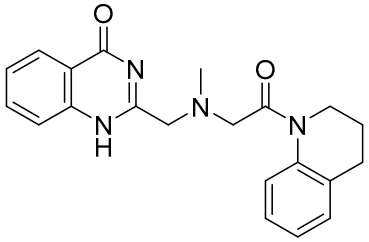
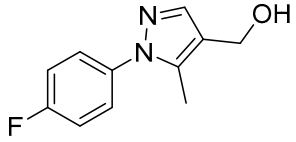
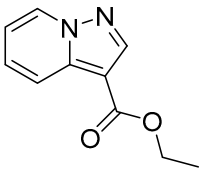
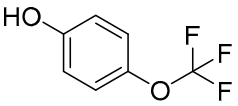
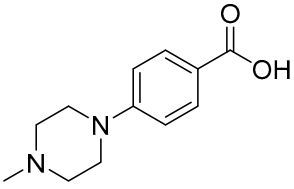
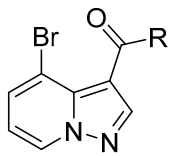
Site	IC_{50} (μM)	Compound	Structure
428	N/A	7	
RNase H Primer Grip Adjacent	N/A	9	
507	150	8	
399	N/A	6	
NNRTI Adjacent	350	**B-1**	
Knuckles	600	3	
Incoming Nucleotide Binding	200	5	

Table S2. HIV-1 RT inhibition, dissociation constants and ligand efficiencies of Series 2 compounds.



#	R_1	RT Inhibition - IC_{50} ± SEM (μM)[a]	SPR - K_D (μM)[b]		
		WT	WT	K103N	Y181C
17	-OH	>1000	NB	NB	NB
18	-OCH3	>1000[c]	-	-	-
19	$-OCH_2CH_3$	>1000[c]	-	-	-
20	$-NHCH_2CH_3$	>1000	-	-	-
21	$-N(CH_2CH_3)_2$	-	-	-	-
22	$-NHCH_2CH_2PhOH$	111.4[c]	-	-	-

[a] The 50% inhibitory concentration (IC_{50}) values were determined by assessing inhibition of HIV-1 RT DNA-dependent DNA Polymerase (DDDP) activity using the nonradioactive PicoGreen or ^{33}P radiolabeled assay. IC_{50} values were determined from at least $n \geq 2$ independent assays. [b]Dissociation constants (K_D) were measured using surface plasmon resonance (SPR). [c]RT DDDP inhibitory activity determined using the PicoGreen assay. NB denotes no binding observed. – denotes not determined.

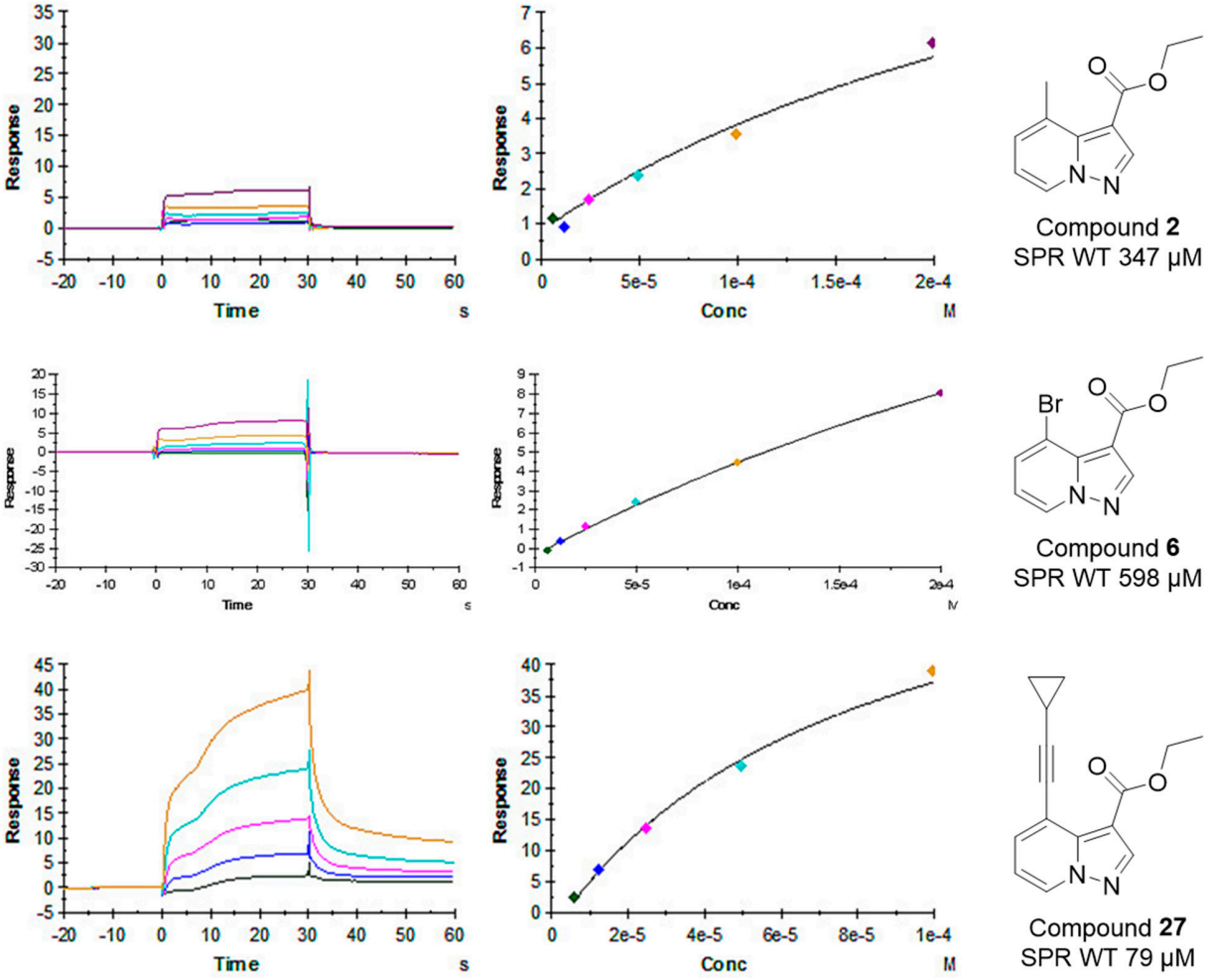


Figure S1. SPR curves for compounds **2**, **6** and **27.**

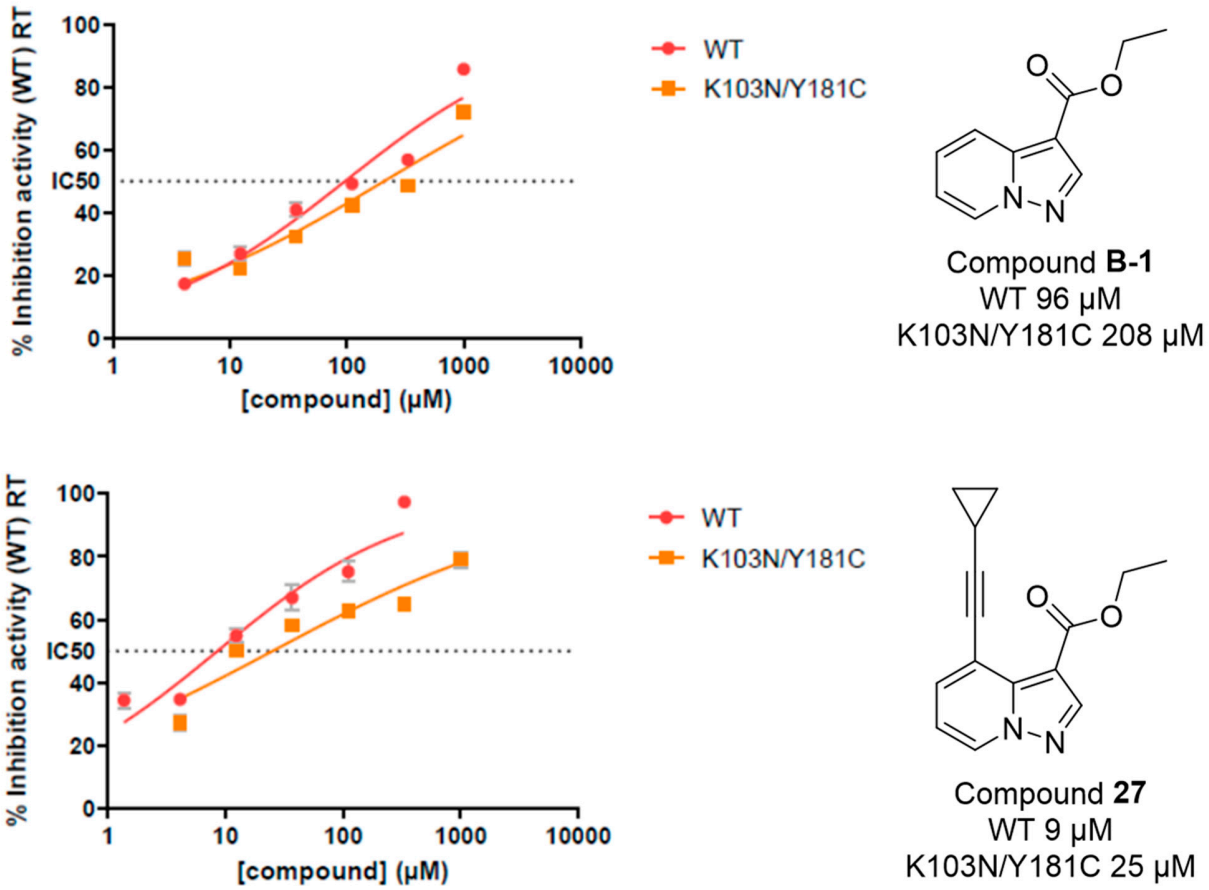


Figure S2. Dose response curves for compounds **B-1** and **27** determined using the Picogreen RT DDDP assay.

Table S3. X-ray data and refinement statistics for PDB 8FFX.

Protein Data Bank (PDB) accession code	8FFX
Wavelength (Å)	1.033
Resolution range (last shell) (Å)	48.29 - 2.42 (2.51 - 2.42)
Space group	C2
Cell constants (a, b, c in Å; α, β, γ in °)	161.95, 73.38, 107.97, 90.0, 100.1, 90.0
Total reflections (last shell)	95241 (9331)
Unique reflections (last shell)	47666 (4692)
Multiplicity (last shell)	2.0 (2.0)
Completeness (last shell) (%)	98.98 (95.54)
Mean I/sigma(I) (last shell)	9.75 (1.62)
Wilson B-factor	54.47
R-merge (last shell)	0.088 (-17.150)
R-meas	0.124 (-24.260)
R-pim	0.088 (-17.150)
CC1/2	0.425 (0.288)
CC*	0.772 (0.668)
Reflections used in refinement	47358 (4556)
Reflections used for R-free	1989 (193)
R-work	0.214 (0.335)
R-free	0.266 (0.394)
CC(work)	0.951 (0.374)
CC(free)	0.923 (0.549)
Number of non-hydrogen atoms	8124
macromolecules	7951
ligands	40
solvent	123
Protein residues	971

Nucleic acid bases	N/A
RMS(bonds)	0.005
RMS(angles)	0.70
Ramachandran favored (%)	96.06
Ramachandran allowed (%)	3.73
Ramachandran outliers (%)	0.23
Rotamer outliers (%)	0.46
Clashscore	6.79
Average B-factor	82.23
macromolecules	82.54
ligands	91.00
solvent	58.49
Number of TLS groups	1

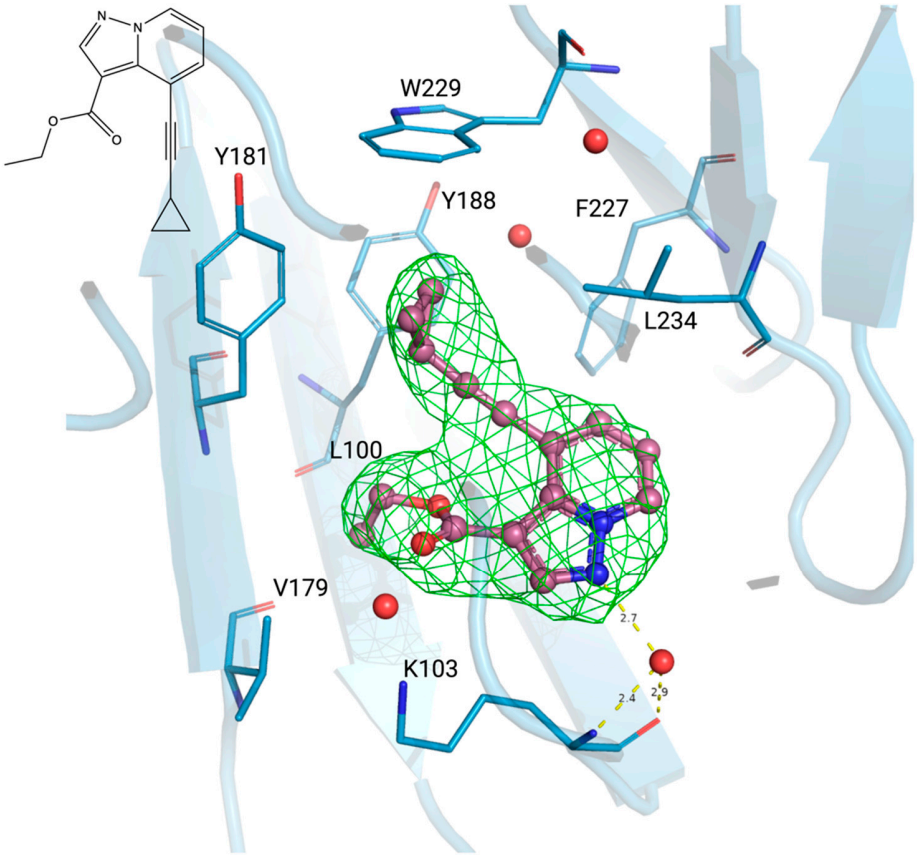


Figure S3. Interactions of compound **27** with neighboring waters in the NNIBP (PDB ID 8FFX). Atomic model of **27** (white) bound to HIV-1 RT residues (pale cyan). Pocket residues forming hydrophobic interactions shows as sticks. Polder OMIT mFo–DFc map density (green mesh, 3σ) of **27**. Water molecules shown as red spheres. Created using Pymol and BioRender.com.

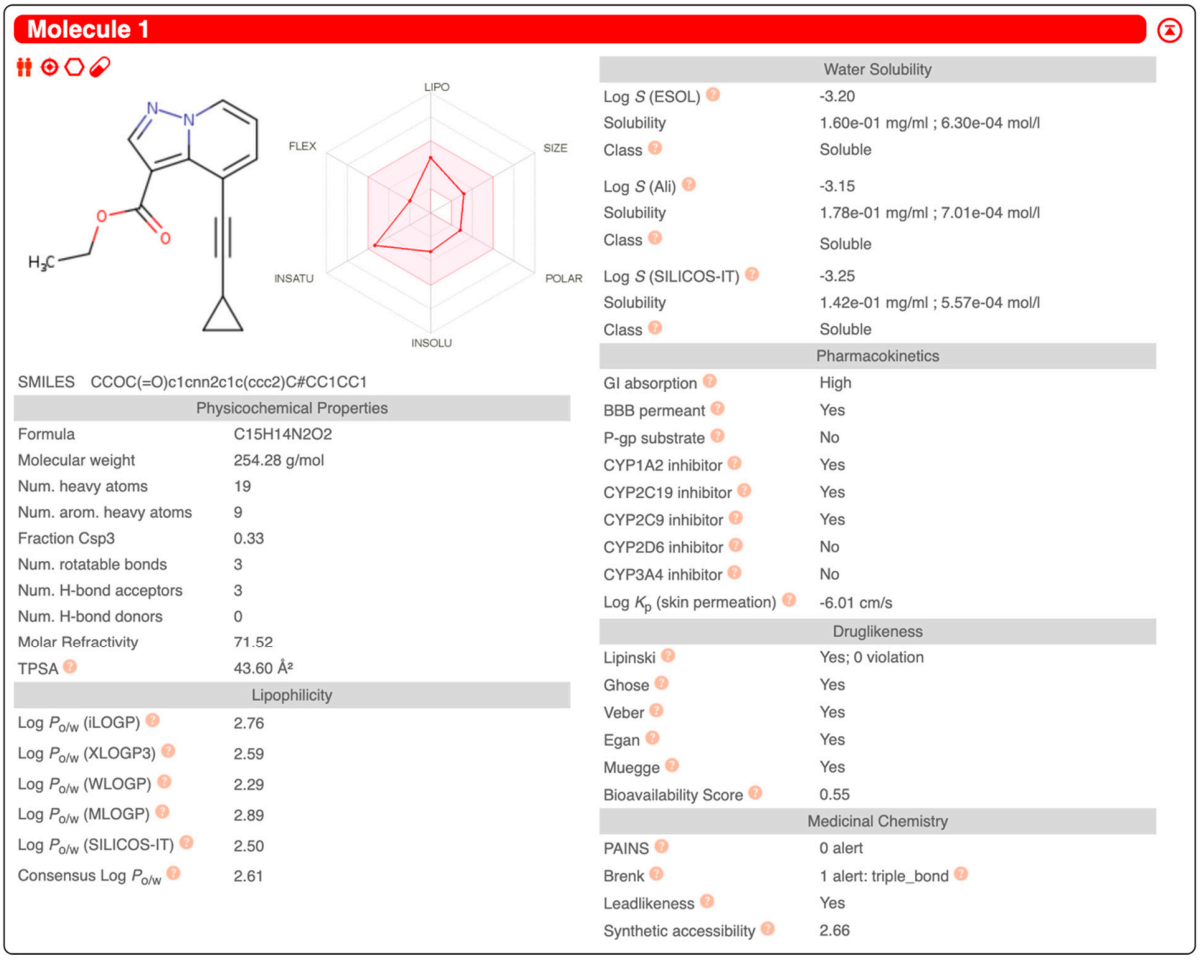


Figure S4. SWISSADME analysis (http://www.swissadme.ch/) summary of compound **27**.

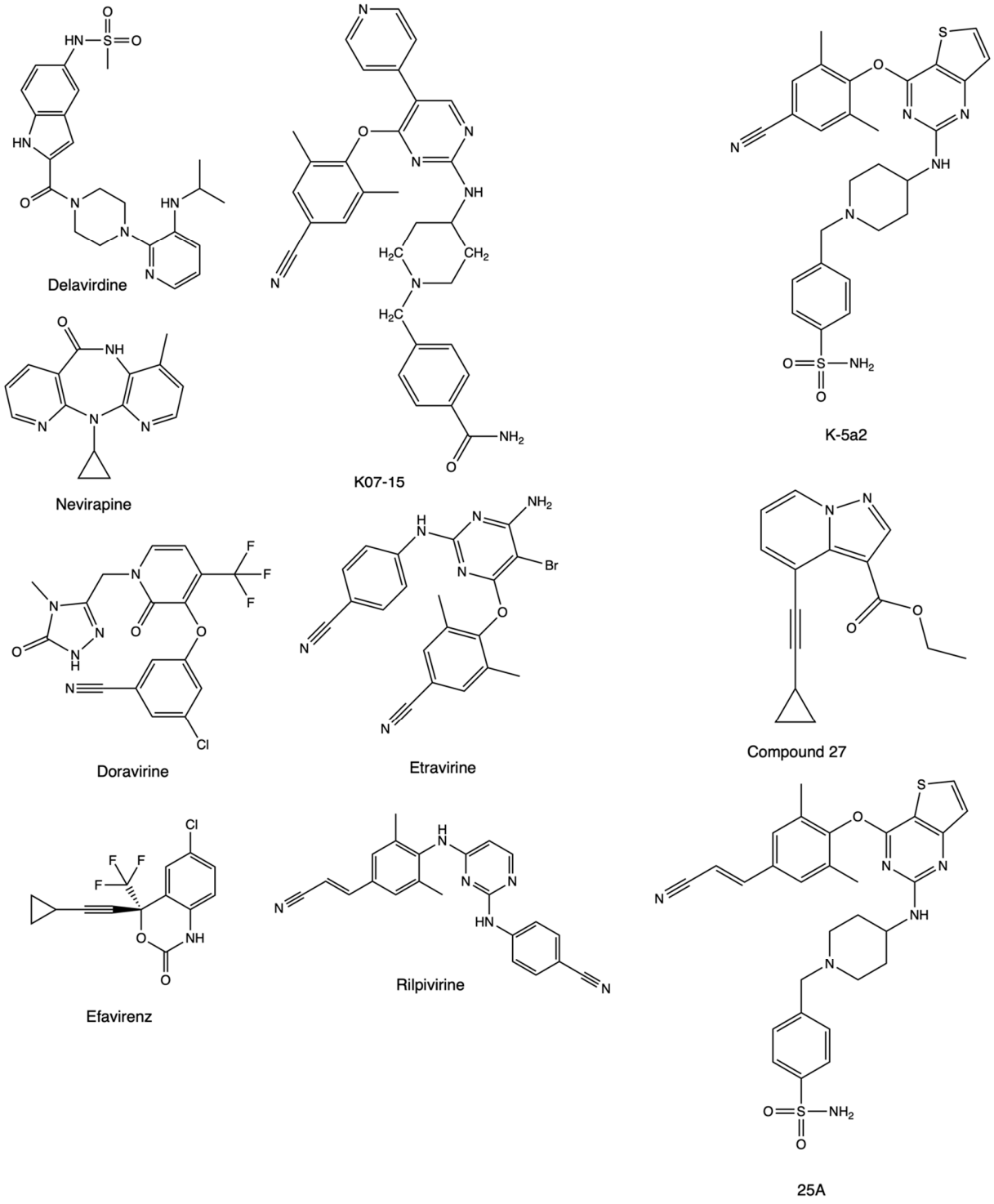


Figure S5. Chemical structures of NNRTIs and compound **27** described in this study.