Supporting Information

Inhibiting HTLV-1 Protease: A Viable Antiviral Target

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SUPPLEMENTARY FIGURES

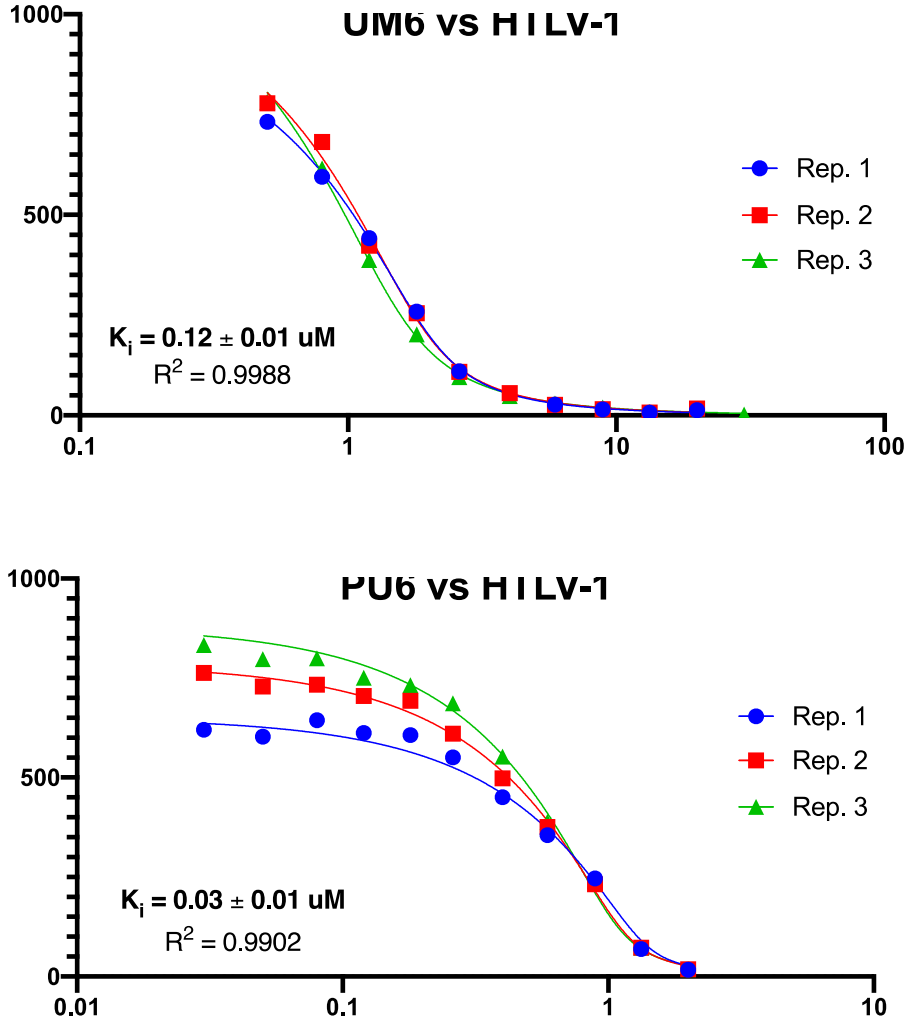


Figure S1. Representative dose-response curves. Initial velocity (V_i) as a function of inhibitor concentration was globally fit for 3 replicates (Rep 1–3) to obtain the inhibition constants (K_i).

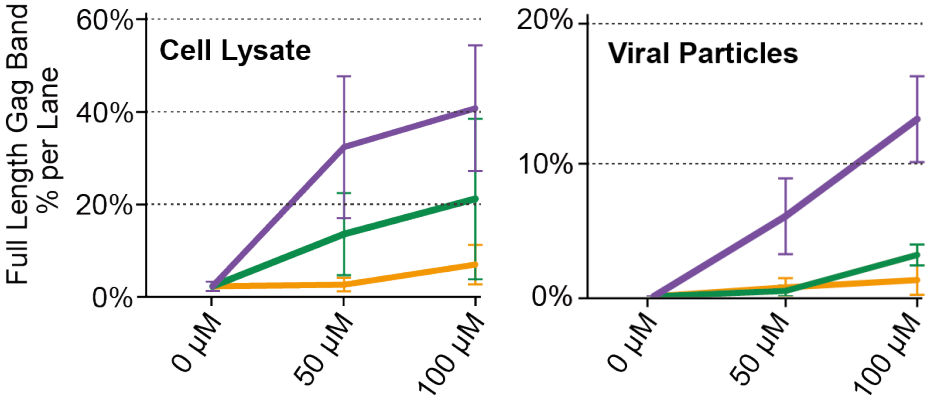


Figure S2: Immunoblot quantification of full-length (FL Gag) band as a percentage of total bands in each lane for the different SP cell drug treatments reported as the mean ± SEM (n=3).

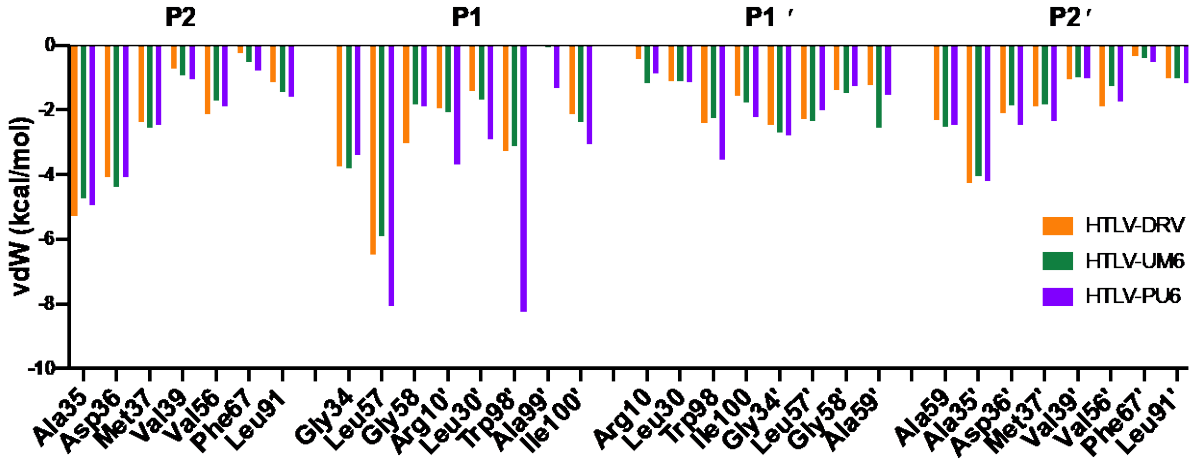


Figure S3: Per-residue vdW contacts between protease and inhibitor, grouped by inhibitor moiety.

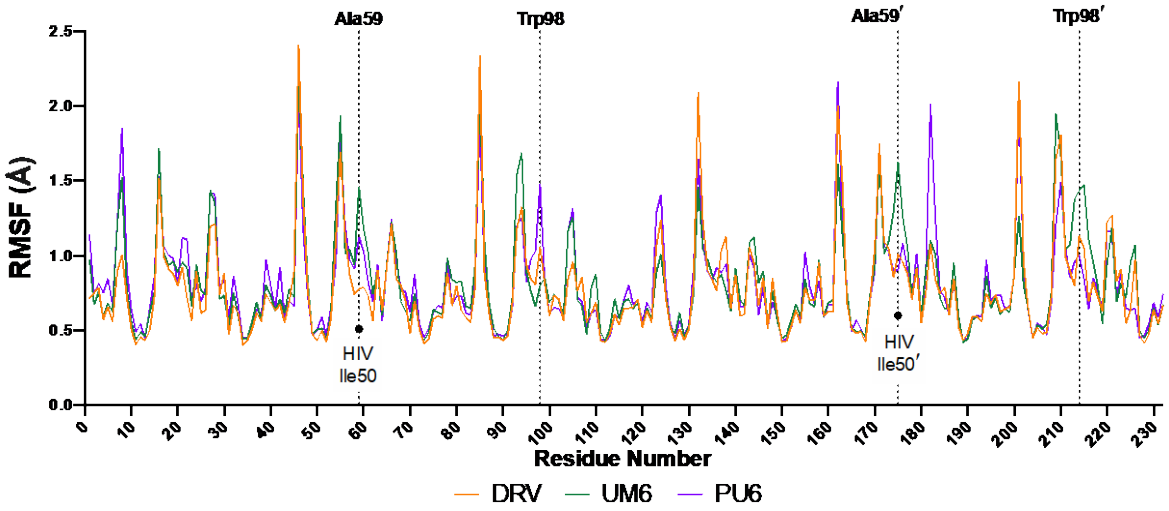


Figure S4: Root-Mean-Square Fluctuation (RMSF) of Cα atoms for DRV, UM6 and PU6 in complex with HTLV-1 protease from molecular dynamics simulations. Ala59/59′ residues are at the tips of the flaps in HTLV-1 protease (corresponding to Ile50/50′ in HIV-1 protease), and Trp98/98′ are at the P1/P1′ subsites of the active site.

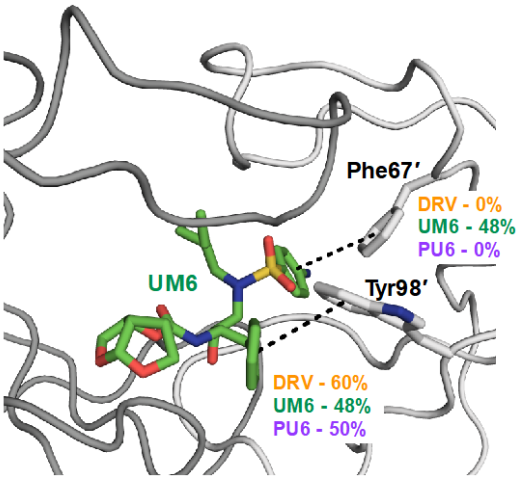


Figure S5. Aromatic side chains in HTLV-1 active site can form π-π stacking interactions, primarily in edge-to-face configuration, with the P1 phenylalanine and P2′ aniline of DRV analogs. Frequency of the π-π stacking interactions (dashed lines) during MD simulations is indicated.

SUPPLEMENTARY TABLES

Table S1. HIV-1 protease and HTLV-1 protease viral polyprotein cleavage sites.

Cleavage Site	HIV-1 (P4-P1/P1′-P4′)	HTLV-1 (P4-P1/P1′-P4′)
MA/CA	SQNY/PIVQ	PQVL/PVMH
CA/NC	-	TKVL/VVQP
Gag/PR	SFNF/PQIT	ASIL/PVIP
PR/Pol	TLNF/PISP	PVIL/PIQA
Pro/RT	-	PAVL/GLEL
RT-RH/IN	RKIL/FLDG	VLQL/SPAD

Table S2. Enzymatic activity of HTLV-1 protease measured using natural substrate sequences.

Cleavage Site	Sequence (P4-P1/P1′-P4’)	K_M (μM)	k_{cat} (s^{-1})	k_{cat} / K_M ($\mu M^{-1}s^{-1}$)
MA/CA	PQVL/PVMH	101.3 ± 1.9	21.6 ± 0.1	0.21 ± 0.02
CA/NC	TKVL/VVQP	31.6 ± 5.9	1.9 ± 0.1	0.06 ± 0.01

Table S3. Inhibition constants (K_i) against HTLV-1 protease.

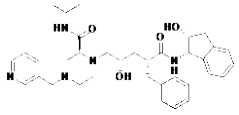
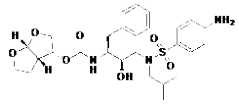
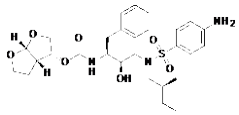
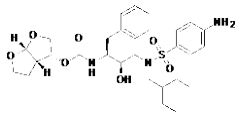
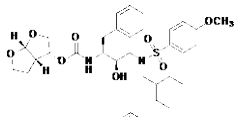
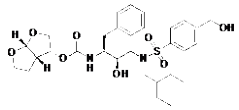
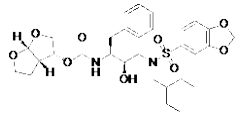
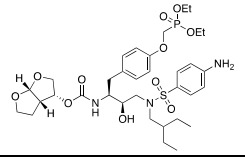
Inhibitor	2D Structure	K_i (μM)
Indinavir (IDV)		63 ± 9
Darunavir (DRV)		**0.8 ± 0.1**
UM1		0.28 ± 0.02
UM6		**0.12 ± 0.01**
UM7		1.2 ± 0.1
UM8		1.9 ± 0.4
UM9		0.4 ± 0.1
PU6		**0.03 ± 0.01**

Table S4. X-ray data collection and crystallographic refinement statistics.

Protease-Inhibitor	**HTLV-DRV**	**HTLV-UM6**	**HTLV-PU6**	**HIV-PU6**
PDB ID	6W6Q	6W6R	6W6S	6W6T
Data Collection				
Space group	$P6_322$	$P6_322$	$P6_322$	$P2_12_12_1$
Cell dimensions:				
a (Å)	78.5	77.9	76.6	51.1
b (Å)	78.5	77.9	76.6	58.0
c (Å)	160.6	160.1	157.3	61.7
α, β, *γ* (°)	90, 90, 120	90, 90, 120	90, 90, 120	90, 90, 90
Resolution (Å)	40.1 - 2.10 (2.18 - 2.10)	41.9 - 2.05 (2.12 - 2.05)	39.3 - 2.29 (2.37 - 2.29)	21.9-1.84 (1.91-1.84)
Unique reflections	17823 (1728)	18768 (1804)	12954 (1244)	16226 (1420)
Total reflections	162887 (16621)	173500 (15456)	114508 (11155)	104007 (4828)
R_{merge} [a]	0.09 (2.00)	0.10 (3.49)	0.24 (3.89)	0.06 (0.29)
R_{pim}	0.03 (0.68)	0.04 (1.25)	0.09 (1.36)	-
CC1/2	1.00 (0.37)	1.00 (0.23)	0.99 (0.15)	-
CC*	1.00 (0.73)	1.00 (0.62)	1.00 (0.50)	-
Completeness (%)	99.8 (100)	99.8 (99.6)	99.9 (99.9)	98.4 (88.0)
Redundancy	9.1 (9.6)	9.2 (8.6)	8.8 (9.0)	6.4 (3.4)
Average I/σ	12.6 (1.2)	12.2 (0.7)	5.9 (0.9)	22.7 (3.3)
Wilson *B*-factors (Å^2)	55.2	50.6	61.3	25.2
Refinement				
R_{factor} (%)[c]	22.9	22.1	26.6	20.0
R_{free} (%)[d]	26.2	24.3	30.5	22.7
RMSD[b] in:				
Bond lengths (Å)	0.007	0.012	0.006	0.003
Bond angles (°)	0.81	1.32	0.82	0.59
Ramachandran:				
Favored	96.05	96.49	96.05	99.48
Allowed	3.95	3.07	3.95	0.52
Outliers	0.00	0.44	0.00	0.00

[a] $R_{sym} = \Sigma \mid I - \langle I \rangle \mid / \Sigma I$, where I = observed intensity, $\langle I \rangle$ = average intensity over symmetry equivalent; values in parentheses are for the highest resolution shell.
[b] RMSD, root mean square deviation.
[c] $R_{factor} = \Sigma \mid\mid F_o \mid - \mid F_c \mid\mid / \Sigma \mid F_o \mid$.
[d] R_{free} was calculated from 5% of reflections, chosen randomly, which were omitted from the refinement process.