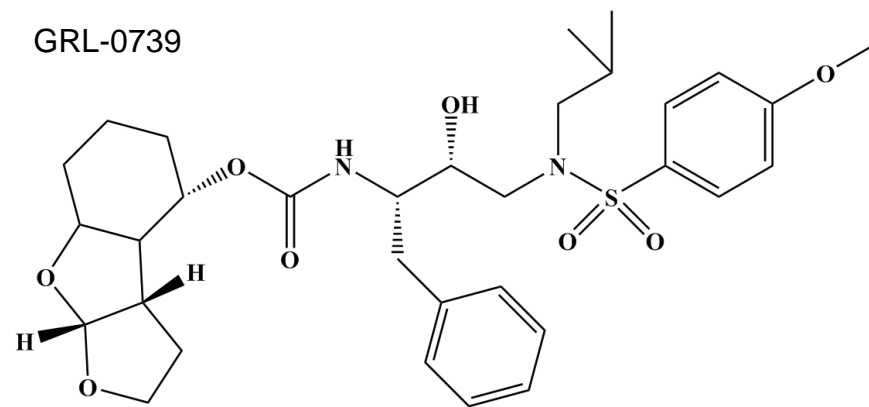Supplemental Figure S1. Structures of GRL-0739, -04810, and -05010
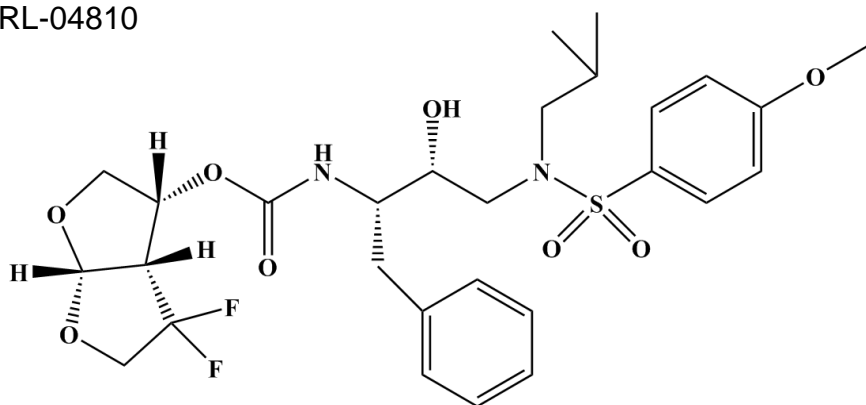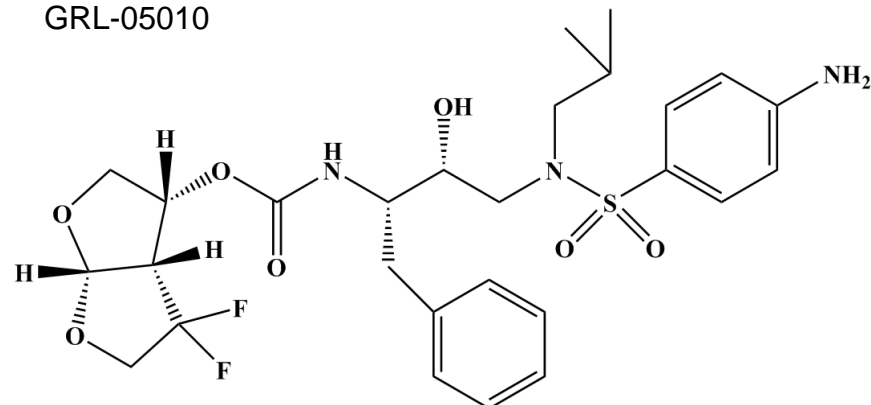
GRL-0739

M.W. : 616.8
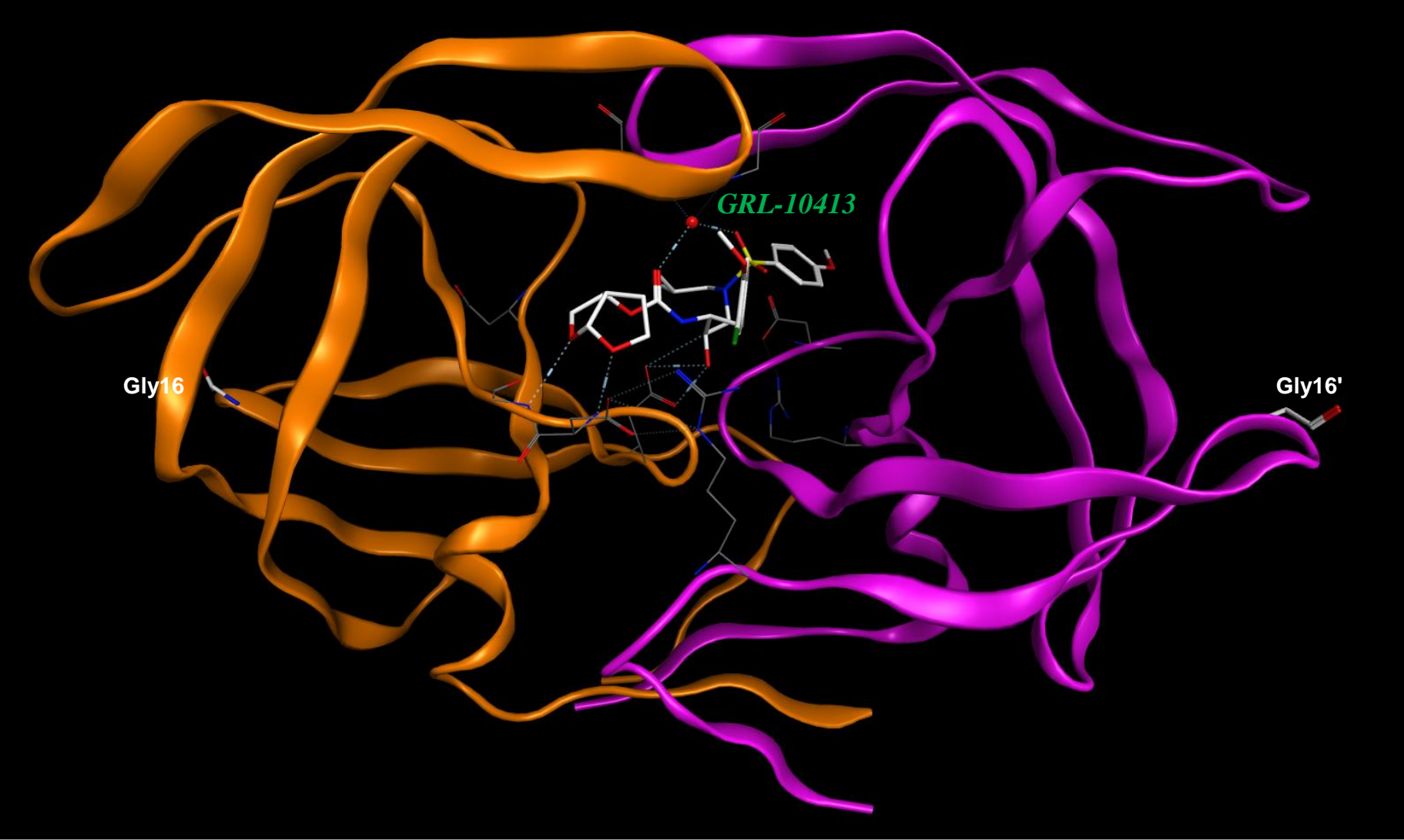
Cyclohexyl-*bis*-THF

GRL-04810

M.W. : 598.7

GRL-05010

M.W. : 583.7

Supplemental Figure S2. Locations of amino acid substitution accumulated in protease in the presence of increasing concentrations of GRL-10413

```
NL4-3     MGARASVLSGGELDKWEKIRLRPGGKKQYKLKHIVWASRELERFAVNPGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTIAVLYCVHQRIDVKDTKEA    100
104 30P   ....................................................................................................    100
104 40P   .........................................................H..........................................    100
104 50P   .........................................................H..........................................    100
                                                             p17 | p24
NL4-3     LDKIEEEQNKSKKKAQQAAADTGNNSQVSQNYPIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQM    200
104 30P   .................................................................................................I    200
104 40P   .................................................................................................I    200
104 50P   .................................................................................................I    200

NL4-3     LKETINEEAAEWDRLHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTHNPPIPVGEIYKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRF    300
104 30P   ........................................................E...........................................    300
104 40P   ........................................................E.....T.....................................    300
104 50P   ........................................................E.....T.....................................    300
                                                                          p24 | p2        p2 | p7
NL4-3     YKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALGPGATLEEMMTACQGVGGPGHKARVLAEAMSQVTNPATIMIQKGNFRNQRKTVKCFNCGKEGH    400
104 30P   ....................................................................................................    400
104 40P   ....................................................................................................    400
104 50P   ....................................................................................................    400
                                      p7 | p1        p1 | p6
NL4-3     IAKNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPSHKGRPGNFLQSRPEPTAPPEESFRFGEETTTPSQKQEPIDKELYPLASLRSLFGSDPSSQ    500
104 30P   ...............E....................................................................................    500
104 40P   ....................................................................................................    500
104 50P   ....................................................................................................    500
```

Supplemental Table S1. Determination of apparent BBB permeability coefficients of GRL-04810, -05010, and -0739 using a novel *in vitro* model.

Compound	Class	Initial luminal tracer concentration (μM)	Final abluminal tracer concentration (μM)	Papp (10^{-6} cm/s)
GRL-04810	PI	100	$3.16 \pm 0.48^*$	$47.8 \pm 8.8^*$
GRL-05010	PI	100	$4.08 \pm 0.65^*$	$61.8 \pm 12.1^*$
GRL-0739	PI	100	$1.80 \pm 0.66^*$	$27.3 \pm 10.1^*$

In the *in vitro* model using a triple co-culture of rat astrocytes, pericytes and monkey endothelial cells, GRL-04810, -05010, or -0739 (all 100 μM) were added to the luminal interface (termed blood side) of duplicate wells. The mathematical formula used for the calculation of Papp is described in Materials and Mehtods section. Results show average values ± 1 S.D. of duplicated determinations. *All data in this table were previously reported by us and indicated here as references.

Supplemental Table S2. Antiviral activity of GRL-10413 against R5 tropic subtype-A strain or subtype-C strain in PHA-PBMs.

Virus[a]	EC_{50} (μM)[b]			
	GRL-10413	APV	ATV	DRV
HIV-1$_{92UG037}$ (subtype A, R5)	0.00032 ± 0.00002	0.051 ± 0.007	0.0038 ± 0.0004	0.009 ± 0.001
HIV-1$_{97ZA003}$ (subtype C, R5)	0.00040 ± 0.00003	0.029 ± 0.005	0.0028 ± 0.0003	0.0055 ± 0.0001

[a]R5 denote R5-tropic HIV-1 strain.

[b]The EC_{50} (50 % effective concentration) values were determined by using PHA-PBM as target cells and the inhibition of p24 Gag protein production by each drug was used as an endpoint. All assays were conducted in duplicate or triplicate, and the data shown represent mean values ($\pm$ 1 S.D.) derived from the results of two independent experiments. PHA-PBMs were derived from a single donor in each independent experiment.

Supplemental Table S3. X-ray diffraction data processing details and structure refinement statistics for the X-ray crystal structure of PR_{WT} in complex with GRL-10413.

	PR_{WT} + GRL-10413
PDB entry	5KAO
Data processing parameters	
Resolution range (Å)	50.00 - 1.68
Unit cell - a (Å)	50.650
b (Å)	57.675
c (Å)	61.558
α (°)	90.00
β (°)	90.00
γ (°)	90.00
Space group	$P2_12_12_1$
Solvent content (%)	54.20
No. of unique reflections	39,782
Mean ($I/\sigma(I)$)	16.01 [a](1.8)
[b]R_{merge}	0.093 (0.331)
Data redundancy	2.1 (1.5)
Completeness (%)	89.4 (75.6)
Refinement	
Resolution range (Å)	32.37 - 1.80
No. of reflections used	15,506

$^{c}R_{cryst}$	0.1838
R_{free}	0.2209
No. of protein atoms per ^{d}AU	1514
No. of ligand atoms per AU	50
No. of water molecules	140
Mean temperature factors - protein ($\mathring{A}^{2}$)	18.703
Main chains ($\mathring{A}^{2}$)	16.392
Side chains ($\mathring{A}^{2}$)	21.238
Ligand ($\mathring{A}^{2}$)	16.585
Waters ($\mathring{A}^{2}$)	30.290
RMSD bond lengths ($\mathring{A}$)	0.009
RMSD bond angles ($\mathring{A}$)	1.203
Ramachandran plot - Most favored (%)	98.46
Additional allowed (%)	1.54
Generously allowed (%)	0
Disallowed (%)	0

[a]Values in parentheses are for the highest resolution shell

[b]$R_{merge} = \Sigma \, |I - <I>| \, / \, \Sigma \, I$

[c]$R_{cryst} = \Sigma \, ||F_{obs}| - |F_{calc}|| \, / \, \Sigma |F_{obs}|$

[d]AU - Asymmetric unit