# Design of HIV-1 Protease Inhibitors with Amino-bis-tetrahydrofuran Derivatives as P2-Ligands to Enhance Backbone-Binding Interactions: Synthesis, Biological Evaluation, and Protein-Ligand X-ray Studies

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*J. Med. Chem.*, **2015**, *58*, 6994–7006.

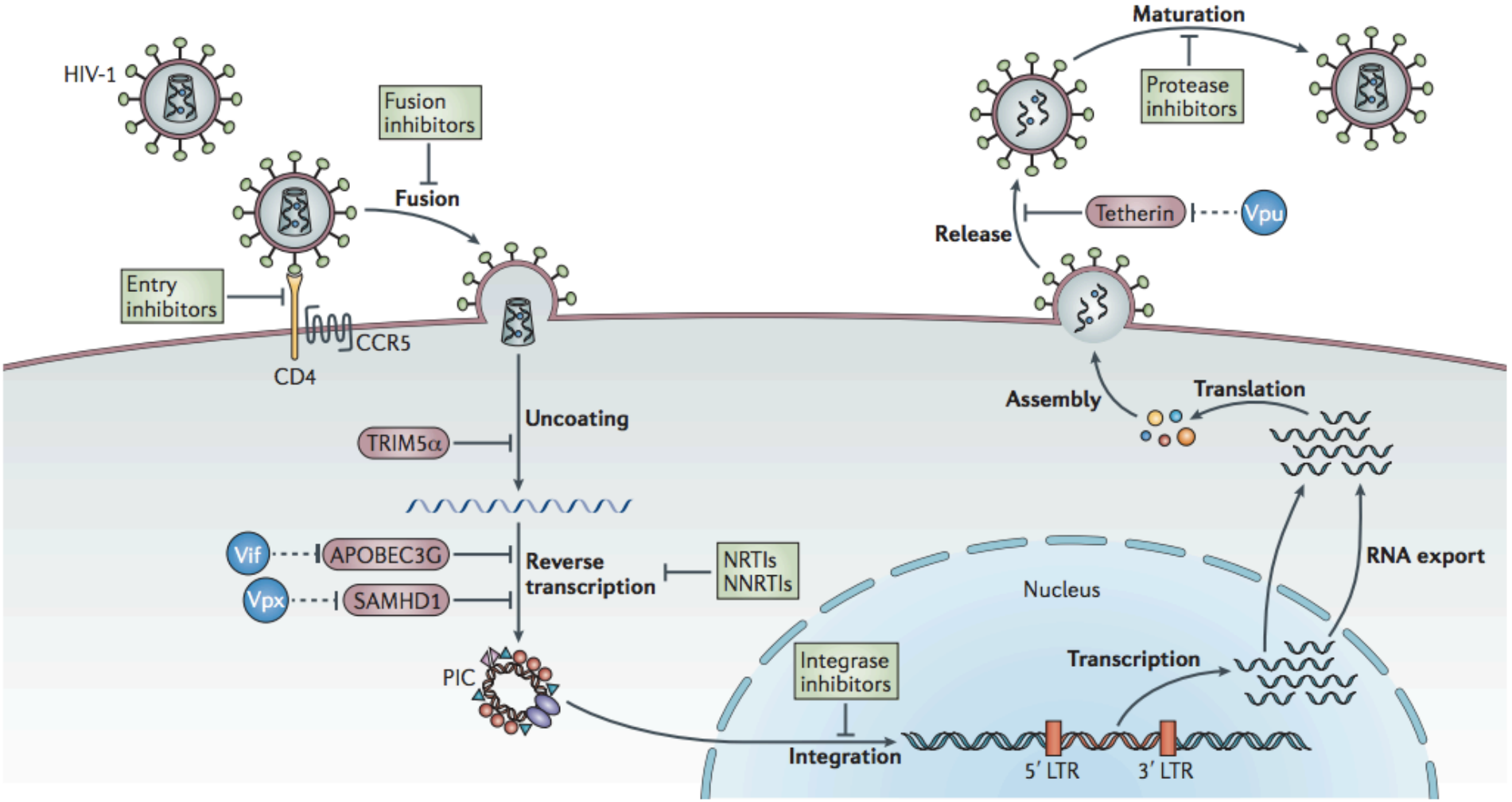
Wipf Group Current Literature

**Chaemin Lim**

09/26/2015

# Schematic overview of the HIV-1 replication cycle





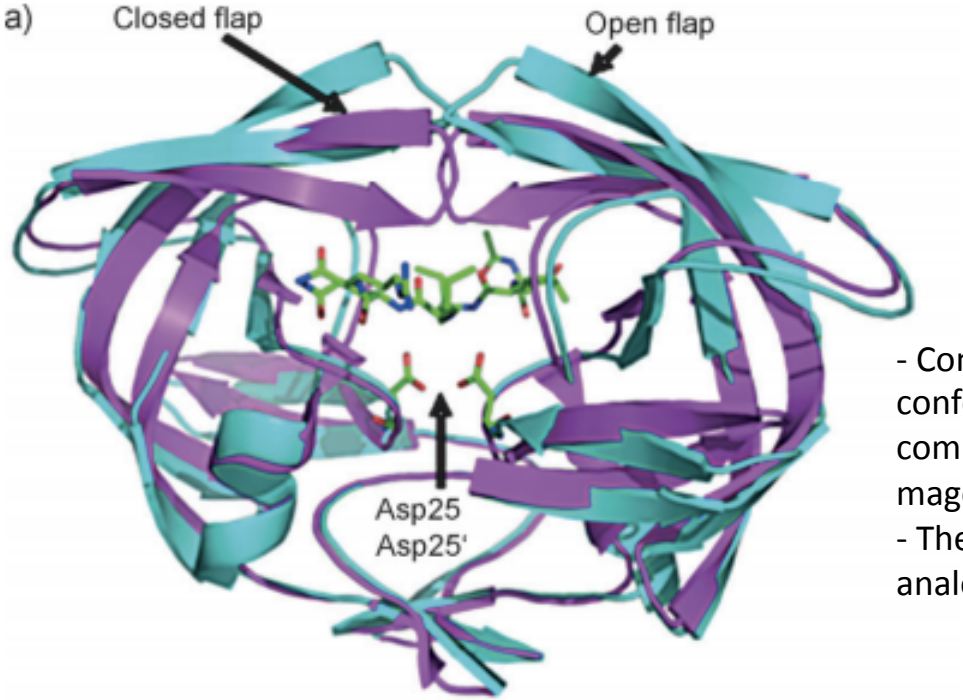

*Nat. Rev. Microbiol.* **2013**, *11*, 877.

# HIV-1 protease, the most effective target



- ***Essential enzyme critical to viral maturation and infectivity***

- Inhibition of HIV-1 protease results in immature, noninfectious viral particles
- A member of the aspartyl proteinase family of enzymes
- The active form of the protease is a homodimer
- For its activation, two PR monomers must interact to create the catalytic site at their dimerization interface
- The active site formed by this interaction consists of a pair of Asp residues, on from each monomer, and a water molecule to mediate the hydrolysis of peptide bonds.

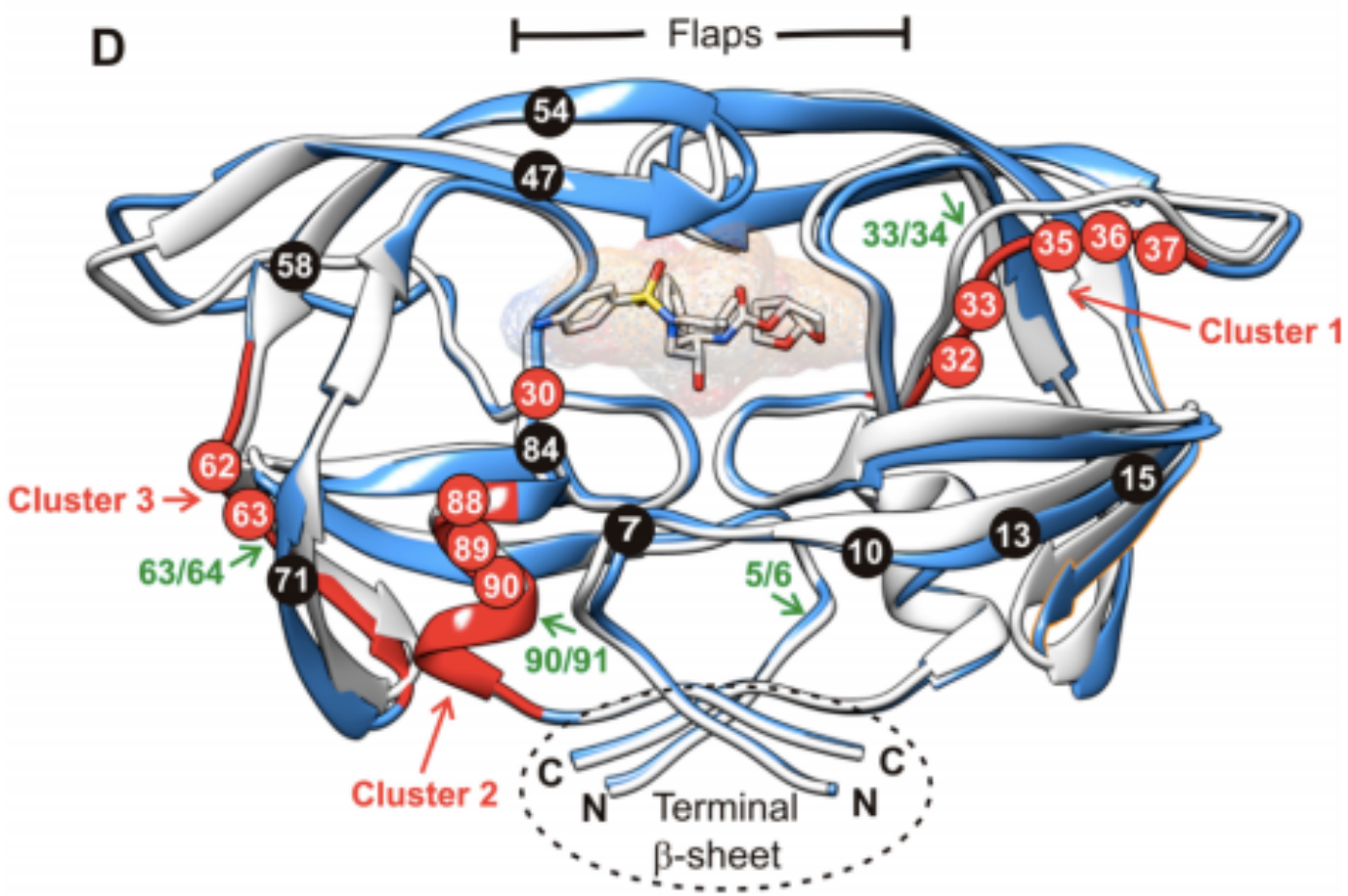



- Comparison of unliganded protease with open conformation flaps (PDB code 1HHP in cyan) and protease in complex with a substrate analogue (PDB code 2AOD in magenta) showing closed conformation flaps.
- The catalytic residues Asp25 and Asp25' and the peptide analogue are shown as stick models.

*Angew. Chem. Int. Ed.* **2012**, *51*, 1778.

# Main obstacle: drug resistant mutant of HIV-1 protease





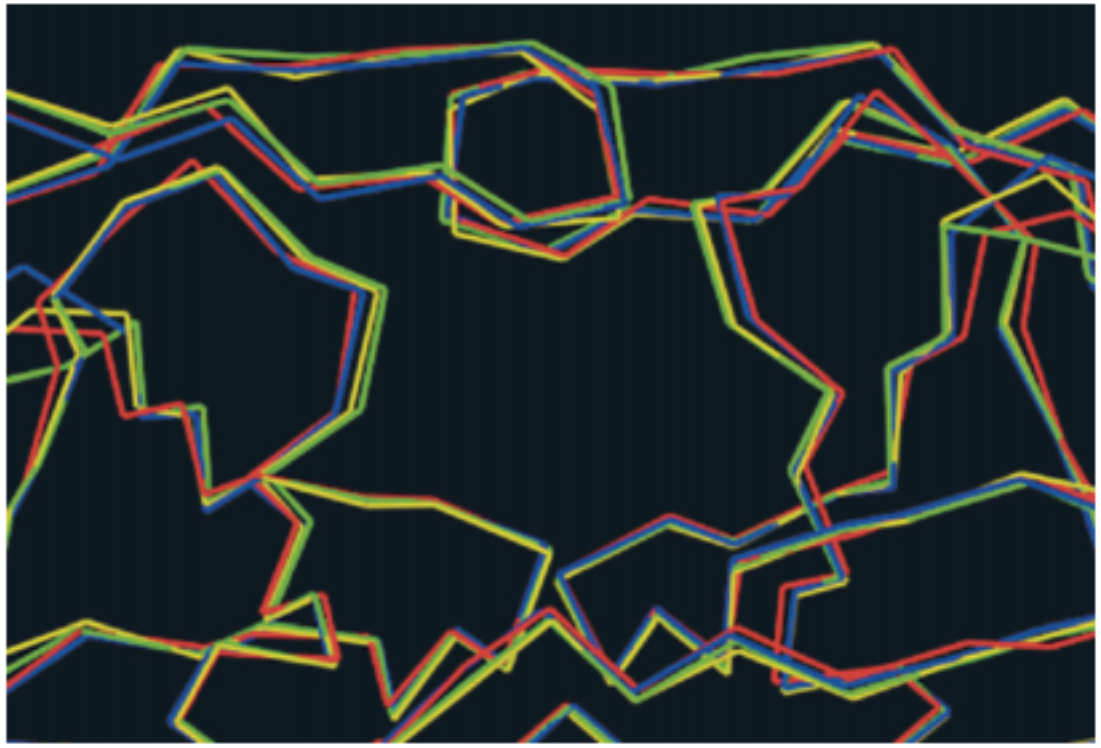

*Biochemistry* **2015**, *54*, 5414.

# Backbone binding concept



- ***Development of new PIs with enhanced active-site interactions, in particular inhibitor-backbone H-bonding interactions***

- Superimposition of multiple mutant structures indicates only a minimal deviation in the backbone atoms around the active site.

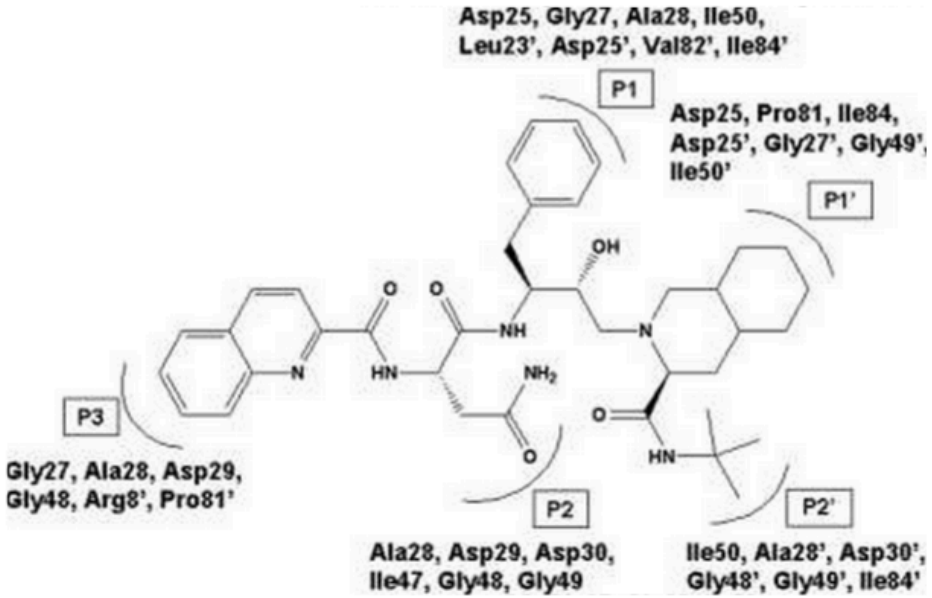


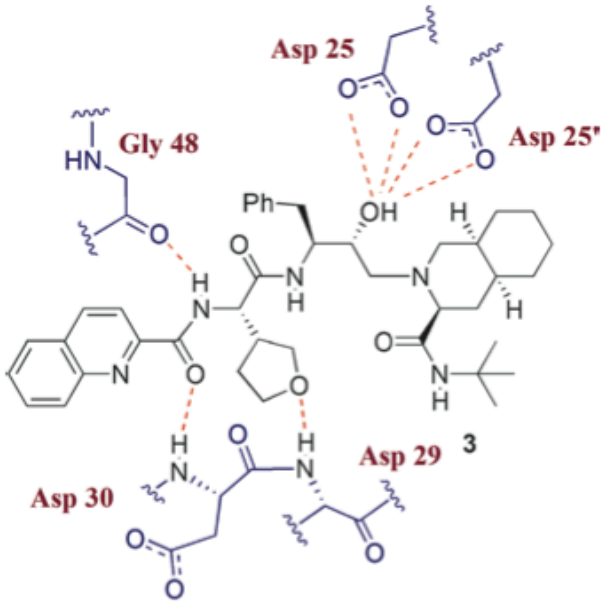
Overlay of HIV-1 protease with multiple mutants (green: PDB 2FDD yellow: PDB 1SGU), HIV-2 protease (red: PDB 1HSH) with HIV-1 protease (blue: PDB 2IEN) showing minimal backbone deviation.

*Angew. Chem. Int. Ed.* **2012**, *51*, 1778.

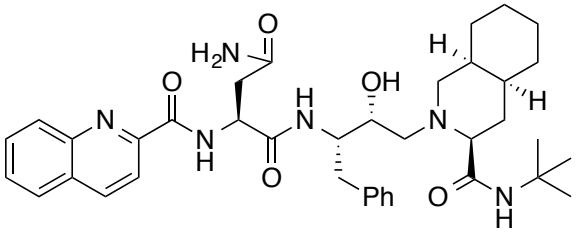
# Structure-based modifications from Saquinavir





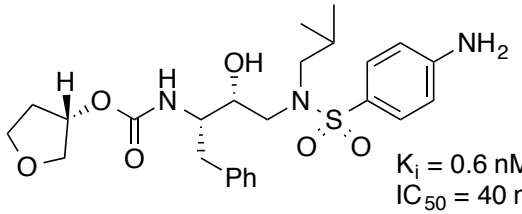

- Replacement of the P2 asparagine of saquinavir with 3*R*-tetrahydrofuryl glycine resulted **3**



**Saquinavir**
(FDA Approval in 1995)

- Poor oral bioavailability: the presence of multiple amide/peptide bonds

Proteins: Structure, Function, and Bioinformatics 2007, 67, 232




**Amprenavir**
(FDA Approval in 1999)

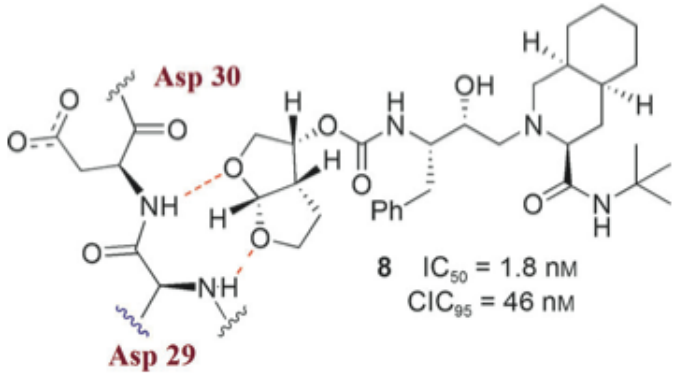
- Introduction of 3*S*-THF urethane in the hydroxyethylamine sulfonamide isostere resulted in Amprenavir

*Angew. Chem. Int. Ed.* **2012**, *51*, 1778.

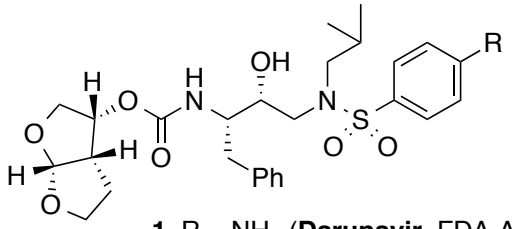
# Design of bis-THF: an inspiration from bioactive natural products



- Incorporation of a stereochemically defined and conformationally constrained cyclic ether template that could replace peptide bonds and mimic their biological mode of action by retaining critical interaction in the active site.

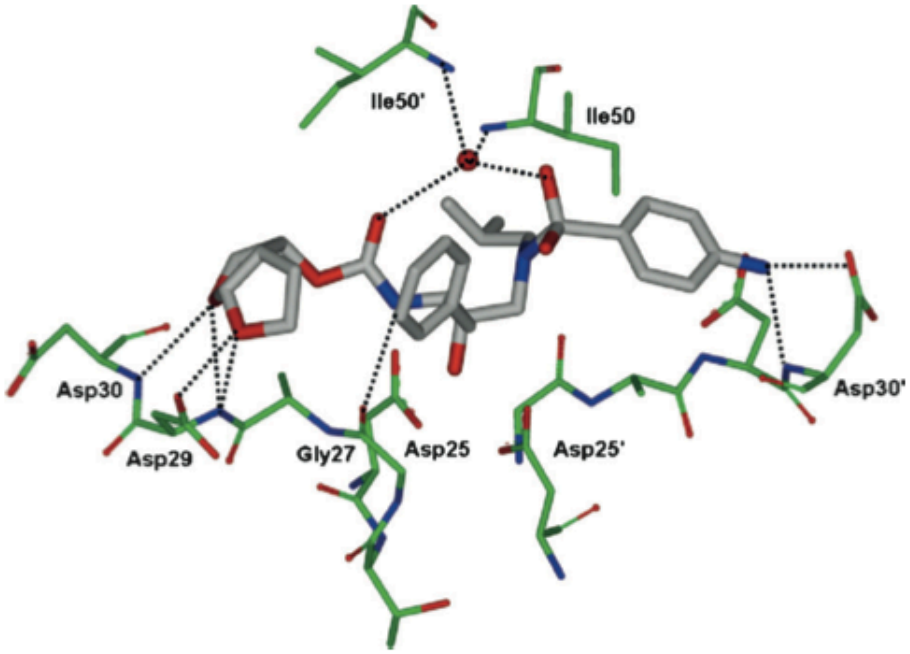



- Design of PIs containing a bis-THF ligand



**1**, R = $NH_2$ (**Darunavir**, FDA Approval in 2006)
$K_i$ = 16 pM; $IC_{50}$ = 4.1 nM

**2**, R = OMe (TMC-126)
$K_i$ = 14 pM; $IC_{50}$ = 0.22 nM




- Darunavir binding to HIV-1 protease like a "molecular crab" (PDB 2IEN)

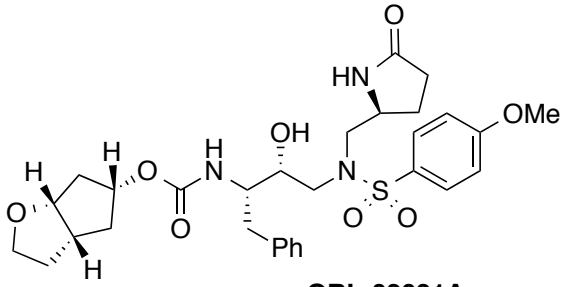
*J. Mol. Biol.* **2006**, *363*, 161.

*Angew. Chem. Int. Ed.* **2012**, *51*, 1778.

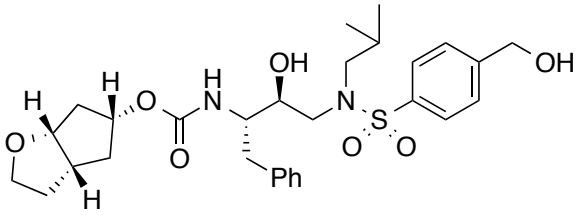
# Enhancing protein backbone binding



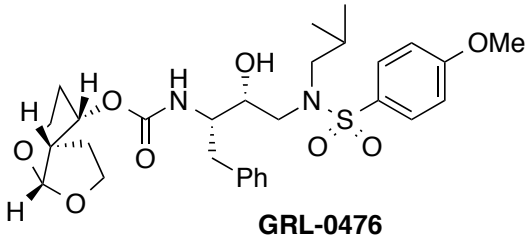
- The combination of the 'backbone binding' strategy and molecular design novelty culminated in the development of Darunavir, GRL-0519, and a variety of exceedingly potent HIV-1 protease inhibitors with intriguing features.



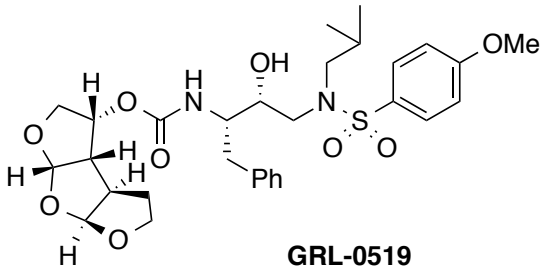
**GRL-02031A**
$K_i$ = 160 pM, $IC_{50}$ = 28 nM



**GRL-07965**
$K_i$ = 4.5 pM, $IC_{50}$ = 1.8 nM



**GRL-0476**
$K_i$ = 2.7 pM, $IC_{50}$ = 0.5 nM

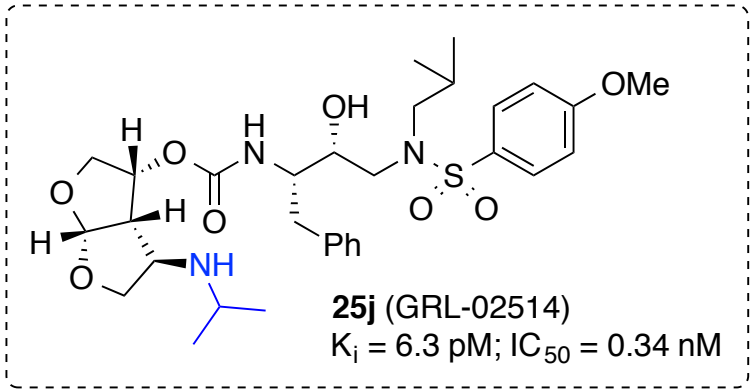


**GRL-0519**
$K_i$ = 5.9 pM, $IC_{50}$ = 1.8 nM

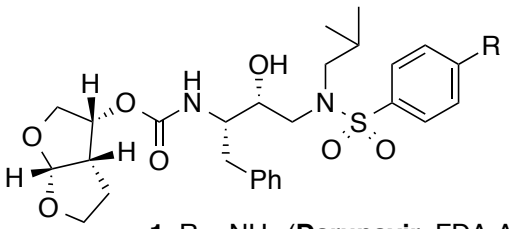
# In this study



- In a continuous effort to further enhance ligand-binding site interactions
: design and synthesis of the C4-amine-derived bis-THF ligands



**25j** (GRL-02514)
$K_i$ = 6.3 pM; $IC_{50}$ = 0.34 nM



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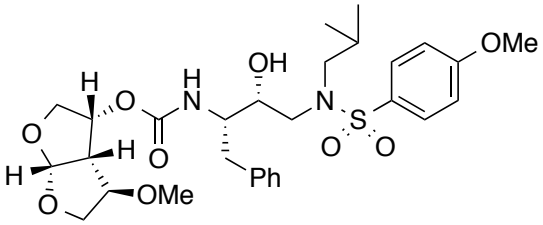
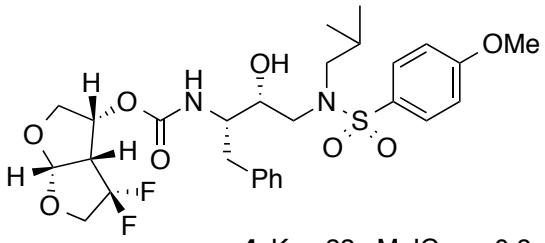


**3**, $K_i$ = 2.9 pM; $IC_{50}$ = 2.4 nM

*ACS Med. Chem. Lett.* **2011**, *2*, 298.

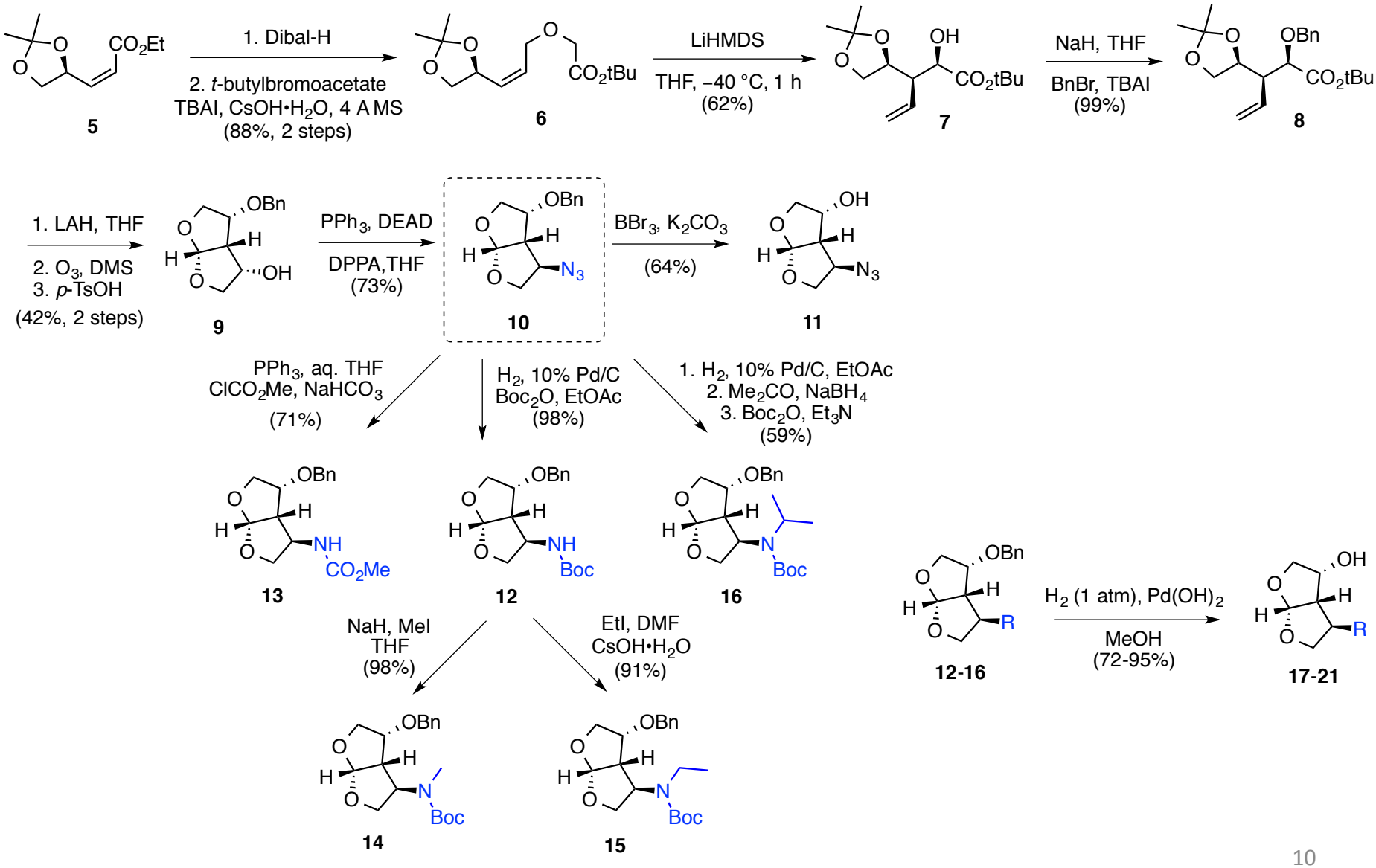


**4**, $K_i$ = 22 pM; $IC_{50}$ = 0.8 nM

*ChemMedChem.* **2015**, *10*, 107.

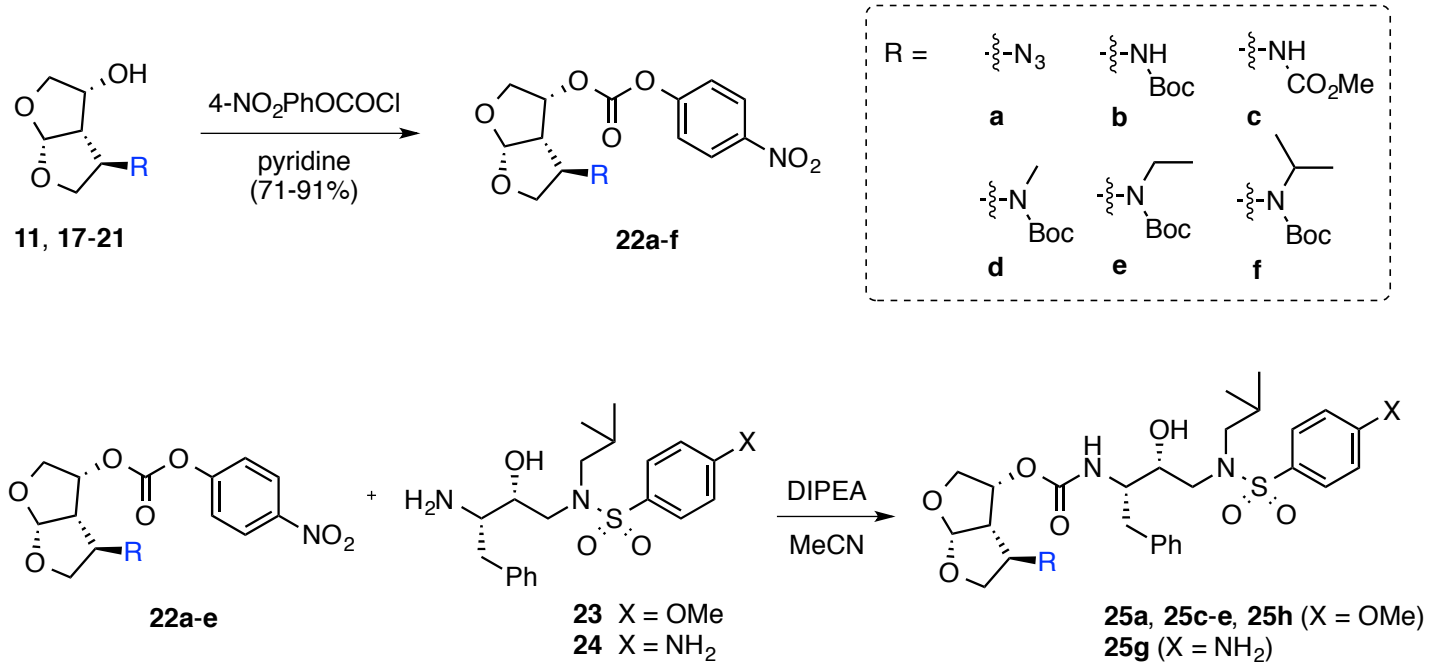
# Scheme 1: Synthesis of C4-amino bis-THF derivatives





# Scheme 2: Synthesis of HIV-1 protease inhibitors

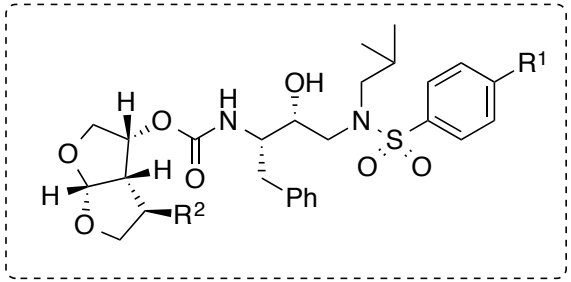





11

# Enzymatic inhibitory and antiviral activity of inhibitors





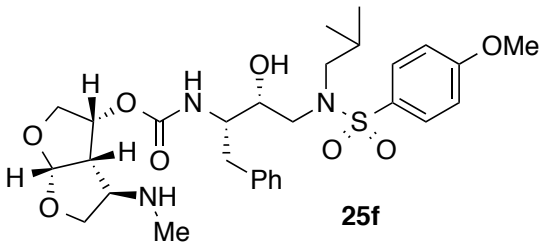

| Entry | Inhibitor | $K_i$ (nM) | $IC_{50}$ (nM)[a,b] | Entry | Inhibitor | $K_i$ (nM) | $IC_{50}$ (nM)[a,b] |
|---|---|---|---|---|---|---|---|
| 1 (**25a**) | $R^1$ = OMe<br>$R^2$ = $N_3$ | 0.0039 | 4.4 ± 0.5 | 7 (**25g**) | $R^1$ = $NH_2$<br>$R^2$ = NHMe | 0.0099 | 480 ± 31 |
| 2 (**25b**) | $R^1$ = OMe<br>$R^2$ = $NH_2$ | 0.53 | 48 ± 6.5 | 8 (**25h**) | $R^1$ = OMe<br>$R^2$ = NEtBoc | 0.048 | nt |
| 3 (**25c**) | $R^1$ = OMe<br>$R^2$ = NHBoc | 0.014 | 4.4 ± 0.9 | 9 (**25i**) | $R^1$ = OMe<br>$R^2$ = NHEt | 0.027 | 22 ± 1.1 |
| 4 (**25d**) | $R^1$ = OMe<br>$R^2$ = $NHCO_2Me$ | 0.024 | 3.7 ± 0.5 | 10 (**25j**) | $R^1$ = OMe<br>$R^2$ = NHiPr | **0.0063** | **0.34 ± 0.2** |
| 5 (**25e**) | $R^1$ = OMe<br>$R^2$ = NMeBoc | 0.012 | 37 ± 7.7 | 11 (**25k**) | $R^1$ = OMe<br>$R^2$ = $NMe_2$ | 0.014 | 4.4 ± 0.7 |
| 6 (**25f**) | $R^1$ = OMe<br>$R^2$ = NHMe | 0.0015 | 35 ± 2.8 | 12 (**25l**) | $R^1$ = OMe<br>$R^2$ = $NEt_2$ | 0.014 | 7.2 ± 0.4 |

[a]Human T-lymphoid (MT-2) cells were exposed to 100 $TCID_{50}$ values of HIV-$1_{LAI}$ and cultured in the presence of each PI, and $IC_{50}$ values were determined using the MTT assay. Darunavir exhibited $K_i$ = 16 pM, $IC_{50}$ = 3.0 nM. [b]nt = not tested.

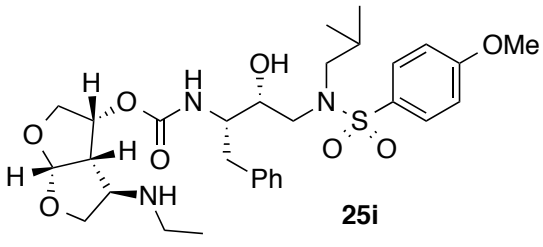
12

# Antiviral activities against DRV-resistant HIV-1 variants

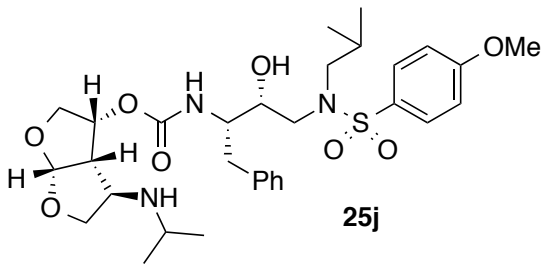





**25f**




**25i**




**25j**

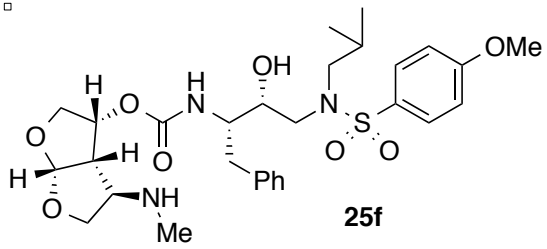
**Table 2. Antiviral Activity of 25f, 25i, and 25j against Multidrug Resistant HIV-1 Variants**

| | | $EC_{50} \pm SD$ ($\mu$M) (fold change)[a,b] | | |
|---|---|---|---|---|
| virus | DRV | inhibitor **25f** | inhibitor **25i** | inhibitor **25j** |
| HIV-$1_{NL4-3}$ | 0.0026 ± 0.0006 | 0.029 ± 0.003 | 0.015 ± 0.003 | 0.001 ± 0.0033 |
| HIV-$1_{DRV^{R}P10}$ | 0.031 ± 0.005 (12) | nd | 0.039 ± 0.009 (2.6) | 0.021 ± 0.004 (21) |
| HIV-$1_{DRV^{R}P20}$ | 0.097 ± 0.051 (37.3) | 0.032 ± 0.003 (1.1) | nd | 0.024 ± 0.013 (23) |
| HIV-$1_{DRV^{R}P51}$ | 3.5 ± 1.4 (1346) | nd | 0.23 ± 0.08 (15) | 0.26 ± 0.12 (260) |

[a]MT-4 cells ($1 \times 10^4$) were exposed to 50 $TCID_{50}$s of wild-type HIV-$1_{NL4-3}$, HIV-$1_{DRV^{R}P10}$, HIV-$1_{DRV^{R}P20}$, or HIV-$1_{DRV^{R}P51}$ and cultured in the presence of various concentrations of each PI, and the $IC_{50}$ values were determined using the XTT assay. The amino acid substitutions identified in protease of HIV-$1_{DRV^{R}P10}$, HIV-$1_{DRV^{R}P20}$, and HIV-$1_{DRV^{R}P51}$ compared to HIV-$1_{NL4-3}$ include L10I/I15V/K20R/L24I/V32I/M36I/M46L/L63P/V82A/L89M; L10I/I15V/K20R/L24I/V32I/M36I/M46L/L63P/A71T/V82A/L89M, and L10I/I15V/K20R/L24I/V32I/L33F/M361/M46L/I54M/L63P/K70Q/V82I/I84V/L89M, respectively. [b]All assays were conducted in triplicate, and the data shown represent mean values (±1 standard deviation) derived from the results of two independent experiments. nd, not determined.

# X-ray structures of inhibitor bound HIV-1 protease






**25f**

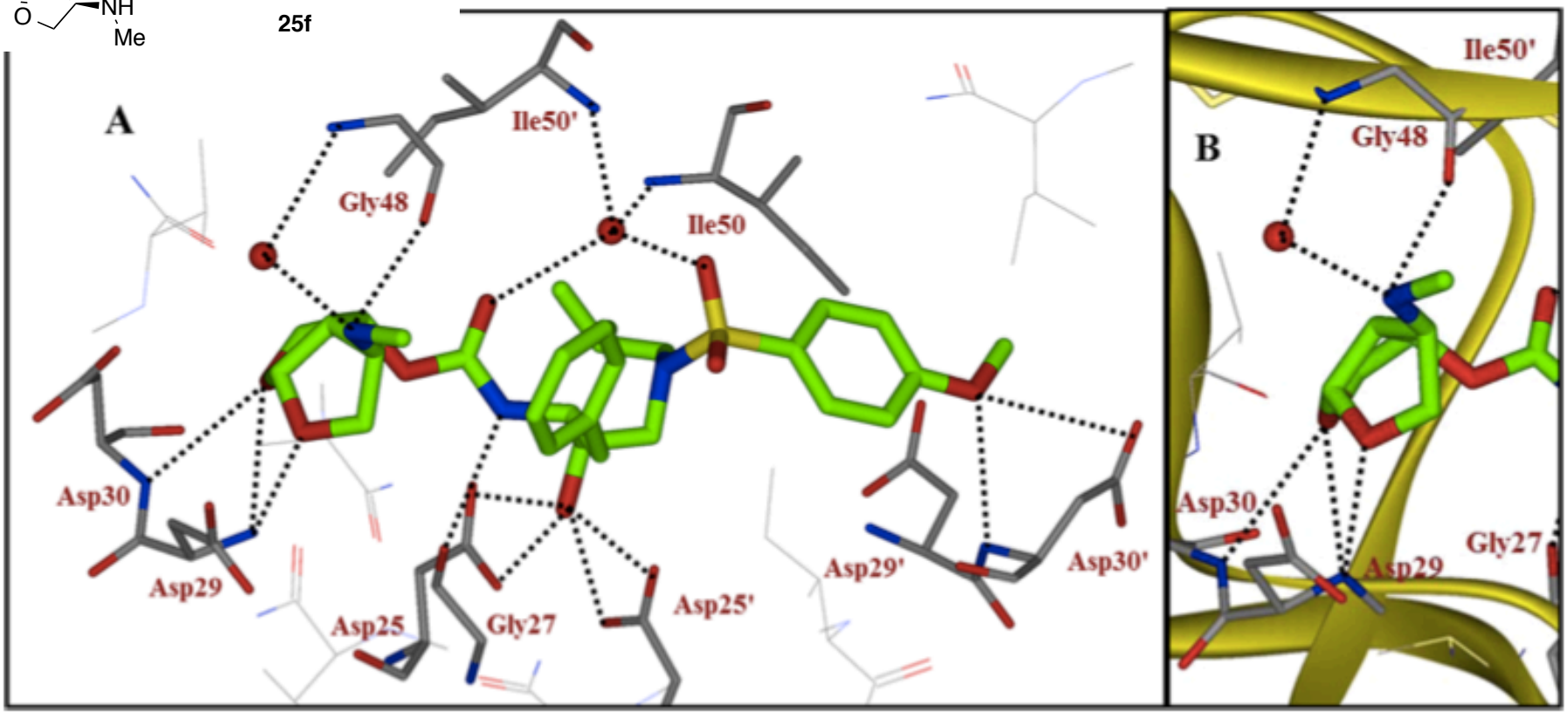
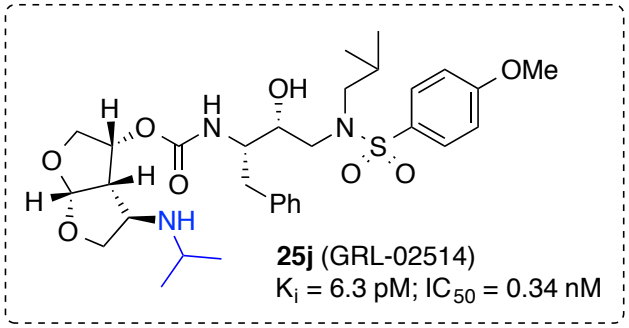



Figure 2. (A) X-ray structure of inhibitor **25f**-bound HIV-1 protease (PDB code: 5BRY). Hydrogen bonding interactions are shown as dotted lines. (B) The main backbone interactions with Gly48 in the flap region and Asp29 and Asp30 in the S2 subsite are shown.

# Summary






**25j** (GRL-02514)
$K_i$ = 6.3 pM; $IC_{50}$ = 0.34 nM

- Inhibitor **25j** (GRL-02514) with a C4-isopropylamine functionality exhibited the best result

: Marked antiviral activity with an $IC_{50}$ value of 0.34 nM, showing over 10-fold improvement compared to darunavir.

- Inhibitors **25f**, **25i**, and **25j** were evaluated against a panel of multidrug-resistant HIV-1 variants and these inhibitors showed improved antiviral activity over darunavir.

- High resolution X-ray crystal structures of **25f** and **25g** bound to HIV-1 protease have been obtained.

- Further studies aimed at optimization of ligand-binding site interactions and improving drug-like properties of these inhibitors are currently in progress.