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Scaffold rearrangement of dihydroxypyrimidine inhibitors of HIV integrase: Docking model revisited

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ABSTRACT

A series of dihydroxypyrimidine (DHP) derivatives were designed as inhibitors of HIV integrase (IN) based on known homology models. Through chemical synthesis and biochemical assays it was found that the activity profile of these compounds largely deviates from predictions with existing models. With the recently disclosed IN crystal structure of prototype foamy virus (PFV), a new HIV IN homology model was constructed featuring a critical IN/DNA interface previously lacking. With this new model, docking results completely corroborated observed biological activities. This new model should provide a more accurate and improved platform for the design of new inhibitors of HIV IN.

Integration of viral DNA into host genome is a defining step during the replication of human immunodeficiency virus (HIV). This process is catalyzed by a virally encoded enzyme integrase (IN) involving two distinct enzymatic activities: a sequence-specific endonuclease activity for 3′ processing (3′-P) and a non-specific polynucleotidyltransferase for strand transfer (ST).[1,2] Integration allows HIV to establish stable viral latency[3,4] which creates a difficult hurdle to complete viral eradication and profoundly impacts the chemotherapy of infected hosts. Stalling this process offers an appealing venue for the design and development of anti-HIV drugs. Due to the lack of cellular counterpart, IN represents a particularly attractive target for the design of novel antivirals. Efforts in this line have led to the discovery of a few major inhibitor chemotypes[5] represented by quinolone compound **1** (elvitegravir)[6,7] and dihydroxypyrimidine (DHP) compound **2** (raltegravir)[8,9] (Fig. 1), with the latter being the only FDA approved IN inhibitor.

Notably most IN inhibitors under development selectively inhibit ST.[10,11] Catalysis of ST most likely involves a well-coordinated high-order architecture consisting of IN, viral and host DNA substrates as well as certain cellular factors.[12] Inhibitors are believed to be binding to and stabilizing DNA/IN interface.[2] Nevertheless, despite tremendous efforts in elucidating IN structure[13–19] crystallization of DNA bound HIV IN has proved to be a daunting challenge due to substantial technical barriers. As a result detailed mechanism of IN catalysis remains elusive, which greatly hinders structure-based design for improved inhibitors. To facilitate new inhibitor design various pharmacophore models have been developed.[20–29] These models entail two common structural components for IN binding: a diketoacid (DKA) or its bioisosteric chelating triad capable of binding two Mg^{2+} ions, and a spatially properly aligned hydrophobic benzyl moiety. Based on these models, scaffold rearrangement involving repositioning the terminal benzyl moiety from one end to the other could yield new inhibitors with retained binding ability. This operation is exemplified in Figure 2. Naphthyridine compound **3** (L-870,810, Merck)[30] and naphthyridinone compound **4** (GSK)[31,32] represent two major IN inhibitor chemotypes, both exhibiting exceptional inhibitory activity against ST. A quick structural examination reveals that these two share an identical two-metal chelating functionality (highlighted in red) constructed on a very similar bicyclic ring system, whereas the terminal benzyl groups (marked in blue) are positioned at the opposite end of the scaffold (Fig. 2).

This observation prompted us to explore the possibility of generating new chemotypes from the DHP scaffold, the most successful molecular backbone for IN inhibitors as manifested by raltegravir (**2**, Fig. 1). In this event, we designed two types of new inhibitors from a model DHP inhibitor **5**[33]: compound **6** where the benzyl group is transposed from the amide end to N-3 site,

Abbreviations: HIV, human immunodeficiency virus; IN, integrase; 3′-P, 3′ processing; ST, strand transfer; DHP, dihydroxypyrimidine; DKA, diketoacid; PDB, protein data bank; PFV, prototype foamy virus; CCD, catalytic core domain.

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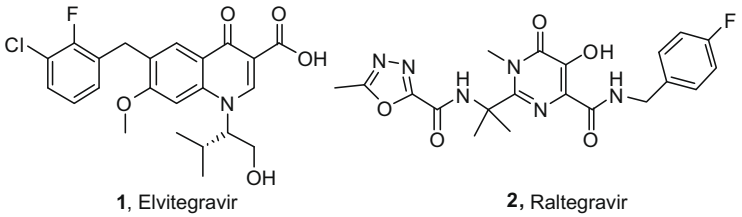


Figure 1. Representative IN ST inhibitors.

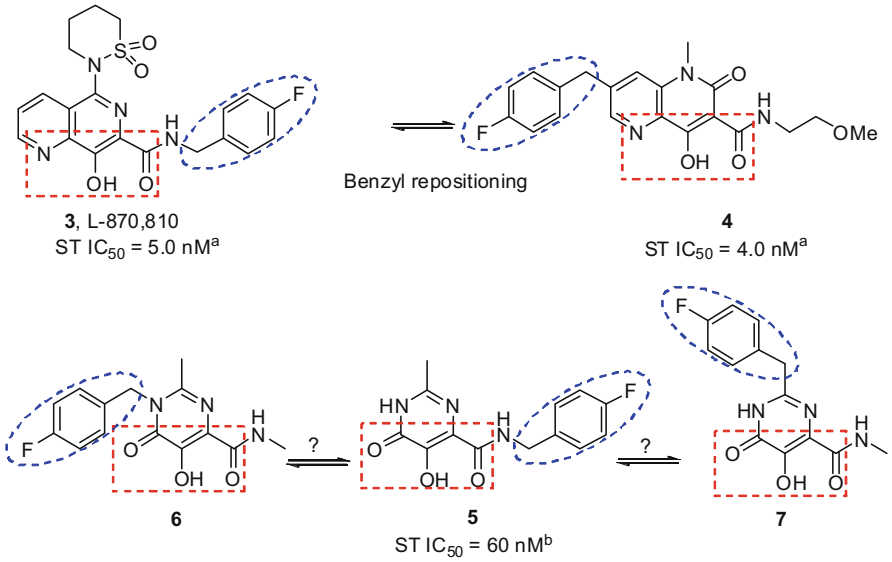


Figure 2. Scaffold rearrangement of known ST inhibitors. Potent inhibitors **3** and **4** reflect a benzyl group repositioning on a common naphthyridine scaffold. The same operation within DHP inhibitor scaffold **5** could generate two new type of IN inhibitors **6** and **7**. Ref: a[31] and b.[33]

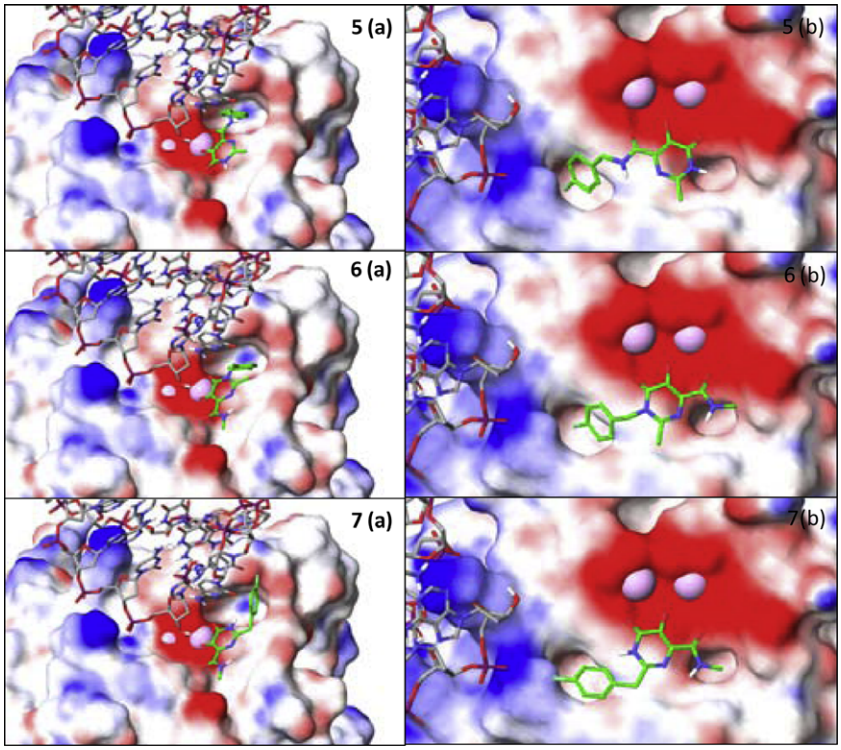


Figure 3. Docking of compounds **5**–**7** into previously reported homology models of HIV IN. Both models of Chen et al. (a)[34] and Chimirri et al. (PDB: 1WKN) (b)[35] allowed reasonable mode of binding of compounds **5** and **6** in which the parafluorobenzyl substituent binds into each of their proposed hydrophobic binding pocket.

and compound **7** where the benzyl group is repositioned to the C-2 site.

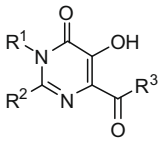
To validate this design, we performed molecular docking using two known homologous docking models[34,35] constructed based on Tn5 transposes. In these models, the ligands were placed manually into the potential binding site and the final models were validated via direct correlation with site direct mutagenesis studies.[34,35] Docking study was carried out by overlaying each of the compounds onto the bound ligand within the already validated binding site of the reported modeled complex followed by restraint energy minimization using OPLS 2005 forcefield[36] with generalized born solvent accessible (GB/SA) implicit solvent model.[37] In line with the common pharmacophore model, these docking models suggest that the chelating triad of the inhibitor interacts with Mg^{2+} ions in a relatively hydrophilic region, anchoring the inhibitor onto the protein surface, whereas the benzyl moiety fits in a highly hydrophobic cavity near the active site. Direct interaction is not observed between inhibitor and viral DNA. As shown in Figure 3, compounds **5** and **6** are perfectly docked into both homologous models. The docking of compound **7** is slightly off in model (a) though the terminal benzyl still makes contact with the hydrophobic cavity, whereas in model (b) compound **7** seems to fit well in this cavity. Overall, these docking results support scaffold rearrangements described in Figure 2.

Chemical synthesis of these compounds was achieved based on reported strategy (Scheme 1).[31,33] The key DHP carboxylate intermediate **13** was prepared from commercially available nitrile **12** in three steps. Direct amidation of ester **13** under microwave condition provides an efficient access to compounds **7**–**9**. The saponification of intermediate **13** also led to the preparation of DHP carboxylic acid **10**. The preparation of N-3 alkylated analogues was effected through a 5-OH protected intermediate. In this event, **13** was benzoylated to give intermediate **14**, which was methylated with methyl iodide followed by a microwave-assisted amidation to produce compound **11**.

Compound **6** was prepared in a similar manner from compound **15** via intermediate **16** (Scheme 2).

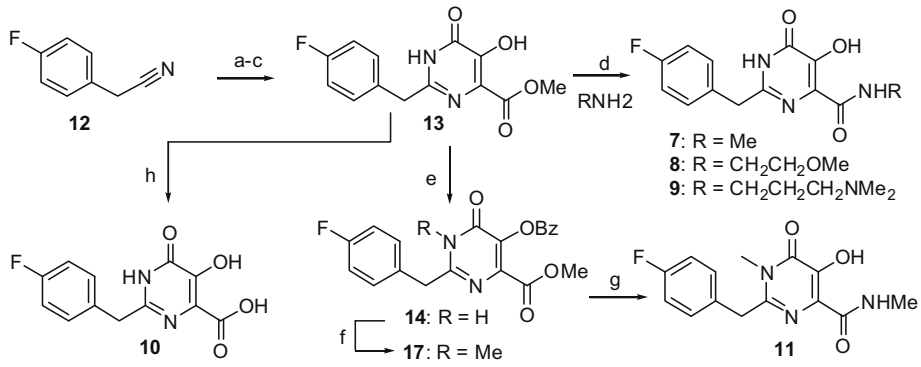
All final compounds were evaluated for their ability to inhibit the two distinct steps of integration: 3′-P and ST. The assay results are summarized in Table 1. In our assay compound **5** shows selective inhibitory activity against ST at low micromolar concentration, whereas all other compounds exhibit a 40 to 100-fold higher IC_{50} value against ST. Although a general trend of selective inhibition against ST can still be observed with compounds **6**–**11** (Table 1), their strikingly low inhibitory activity against ST when compared to compound **5** is extremely intriguing given that these compounds

Table 1
Assay results of compounds **6**–**11** against HIV IN

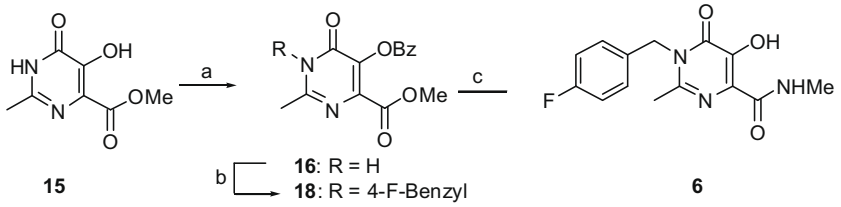


Compds	R^1	R^2	R^3	3′-P IC_{50} (μM)	ST IC_{50} (μM)
5[a]	H	Me	NHBn-4-F	111 ± 18	2.6 ± 0.1
6	4-F-Bn	Me	NHMe	>333	111
7	H	4-F-Bn	NHMe	>333	169
8	H	4-F-Bn	$NH(CH_2)_2OMe$	>333	206
9	H	4-F-Bn	$NH(CH_2)_3NMe_2$	>333	>333
10	H	4-F-Bn	OH	>333	243
11	Me	4-F-Bn	NHMe	>333	241

[a] Reported ST IC_{50} was 0.06 μM.[33]



Scheme 1. Synthesis of compounds **7**–**11**. Reagents and conditions: (a) NH_2OH, MeOH, 60 °C, 7 h, 65%; (b) dimethylacetylenedicarboxlate, MeOH, rt, quantitative yield; (c) xylene, MW, 140 °C, 40 min, 23%; (d) RNH_2, MW, 150 °C, 4 min, 50–76%; (e) benzoyl chloride, pyridine, 66%; (f) MeI, Cs_2CO_3, THF, 40 °C, 57%; (g) $MeNH_2$ (33% solution in EtOH), MW, 150 °C, 4 min, 55%; (h) NaOH, MeOH, rt, overnight, 27%.



Scheme 2. Synthesis of compound **6**. Reagents and conditions: (a) benzoyl chloride, pyridine, 89%; (b) 4-F-benzyl bromide, Cs_2CO_3, THF, 80 °C, 30%; (c) $MeNH_2$, MW, 150 °C, 4 min, 50%.

appear to be binding to IN according to both homologous docking models. Apparently to gain better understanding on HIV IN binding it is imperative to introduce a new docking model significantly different from the existing ones.

The recent publication[38] of the first crystal structures of DNA-bound retroviral IN allowed us to construct new docking models for HIV IN. As these structures suggested an induced fit mechanism of inhibition by the displacement of 3′adenosine, two models were constructed based on the original X-ray crystal structures of IN from prototype foamy virus (PFV) (PDB: 3L2T and 3L2U),[38] a retro-lentivirus belonging to the same viral genus as HIV. All modeling was carried out using the Schrödinger modeling suite package.[39] The models of the HIV catalytic core domain (CCD)/viral DNA complex were homology modeled using Prime v3.1 in silico by replacing the viral DNA and IN enzyme sequences of PFV with those of HIV (GenBank: AAC37875.1). Since the catalytic core domain of the HIV-1 IN was previously solved,[13,19] the sequence alignment used for the homology modeling was based on the secondary structure alignment of the PFV and HIV-1 IN CCD to identify the structure conserve regions (Fig. S1A). The backbone RMSD between the two CCD's was 1.13 Å showing a highly conserved protein fold. All side chains were optimized by standard side chain

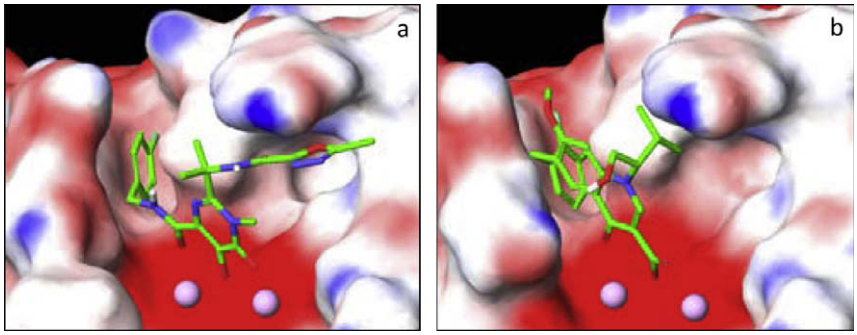


Figure 4. A new HIV IN model docked based on 3L2T with raltegravir (a) and 3L2U with elvitegravir (b). The observed modes of binding were consistent with that of PFV IN X-ray structures.[36]

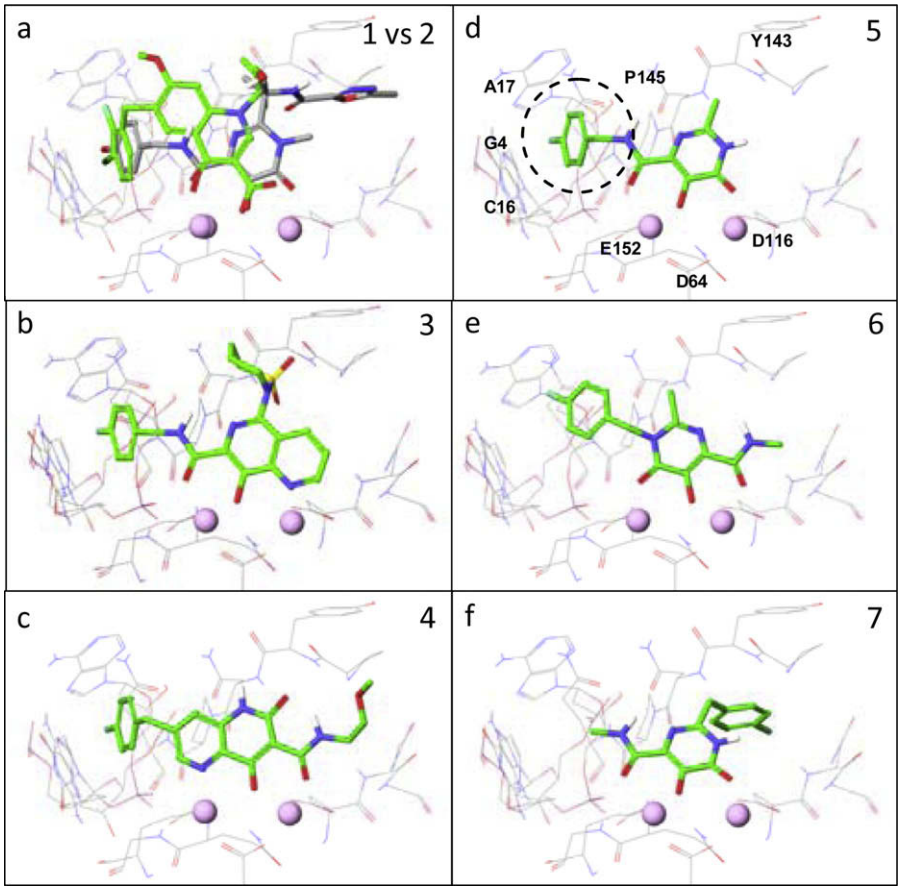


Figure 5. Docking of compounds **1**–**7** into the new homology model of HIV IN (a–f). Overlay of compounds **1** and **2** showed two distinct modes of binding to the Mg^{2+} ions while retaining reasonable overlap of the halobenzyl group within the hydrophobic binding pocket (a). Major protein and viral DNA residues which formed the interfacial DNA/protein hydrophobic pocket (A17, G4 and C16, P145) (in dotted circle) and the E152-D64-D116 motif which chelates to the two Mg^{2+} ions within the binding pocket are highlighted (d).

optimization protocol using PrimeX. The final models were further refined by restraint energy minimization using OPLS 2005 forcefield[36] with GB/SA implicit solvent model. Docking of all compounds in the new homology model was carried out using Glide v2.5 at Standard Precision[40] with both Mg^{2+} ions and the interfacial hydrophobic pocket between the HIV IN and DNA defined as required constraints. Validation of both models were carried out by docking of either raltegravir (**2**, model a) or elvitegravir (**1**, model b) into the ligand binding site and structure comparison with the original X-ray structure of PFV IN (Fig. 4). Consistent with observations from the original crystallographic studies, in these models inhibitors seem to adopt an orientation perpendicular to the protein surface with the chelating group binding to both Mg^{2+} ions. This chelation then allows the terminal benzyl group to induce a pocket by taking the space originally occupied by the terminal adenosine on the 3′ end of viral DNA. Overall, the binding of IN inhibitors conforms to a previously proposed interfacial inhibition mechanism[2] as they bind all three key elements: the enzyme by hydrophobic and Van der Waals interactions; the metals by chelation; and the DNA by π–π stacking. Further docking of compounds **1–7** was conducted with the model based on PDB: 3L2T (model a). Remarkably all active compounds **1–5** are docked similarly with the terminal benzyl group optimally oriented to fill in the newly created pocket (Fig. 5a–d). As a result, close contacts are observed between this terminal benzyl group and both viral DNA and neighboring IN amino acids residues (Fig. 5 d). By contrast, the terminal benzyl group of compound **6** is skewed away from the pocket (Fig. 5 e) and compound **7** adopts a conformation in which the benzyl group is pointing to the opposite direction of the pocket (Fig. 5 f). These docking results are in agreement with observed inhibitory activities against HIV IN and prove that this new docking model is robust.

In conclusion, we have designed and synthesized a series of new DHP type IN inhibitors based on known pharmacophore and homologous docking models. The unexpected assay results led us to construct a new docking model for HIV IN binding, which provides docking results that strongly corroborate observed biological activities. We expect this model to serve as a useful platform for the design and discovery of novel HIV IN inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.048.

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