Substrate Envelope Designed Potent HIV-1 Protease Inhibitors
to Avoid Drug Resistance

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Summary

The rapid evolution of HIV under selective drug pressure has led to multi-drug resistant (MDR) strains that evade standard therapies. We designed highly potent HIV-1 protease inhibitors (PIs) using the substrate envelope model, which confines inhibitors within the consensus volume of natural substrates, providing inhibitors less susceptible to resistance as a mutation impacting such inhibitors will simultaneously affect viral substrate processing. The designed PIs share a common chemical scaffold but utilize various moieties that optimally fill the substrate envelope, as confirmed by crystal structures. The designed PIs retain robust binding to MDR protease variants, and display exceptional antiviral potencies against different clades of HIV as well as a panel of 12 drug resistant viral strains. The substrate envelope model proves to be a powerful strategy to develop potent and robust inhibitors that avoid drug resistance.

Introduction

Drug resistance is a major problem in the treatment of patients with HIV/AIDS. Resistance in HIV occurs when the target enzyme mutates leading to less efficient drug binding, but maintains biological function. Currently, there are 25 FDA approved drugs targeting different stages in the life cycle of HIV, including 9 protease inhibitors (PIs). These drugs, especially when used in combination in the highly active antiretroviral therapy (HAART), have improved the quality and life expectancy of HIV-infected patients (Hogg, et al., 1998; Palella, et al., 1998). However, the very high replication rate of the virus and the lack of an error-proof mechanism in HIV reverse transcriptase promote the emergence of drug resistant viral strains in patients undergoing therapy. New patients are also infected with already
resistant viruses, which is an added challenge in the treatment of HIV infection. Thus novel potent drugs targeting the drug-resistant viral ensemble are needed for effective treatment of HIV/AIDS.

Various strategies have been used to develop new antiviral PI therapies against drug resistant HIV, including increasing the plasma levels of existing PIs by using a boosting agent (Kempf, et al., 1997; Youle, 2007; Zeldin and Petruschke, 2004) and developing new PIs using structure-based drug design (Ghosh, et al., 2008; Gulnik and Eisenstat, 2008; Nalam and Schiffer, 2008; Wensing, et al., 2010). The design strategy to maximize the number of hydrogen bonds with the protease backbone led to the development of highly potent PIs active against drug-resistant HIV (Ghosh, et al., 2011; Ghosh, et al., 2008; Ghosh, et al., 2009). PIs with improved resistance profiles were also developed using a solvent anchoring approach (Cihlar, et al., 2006), and utilizing a new lysine sulfonamide-based molecular core (Stranix, et al., 2003). A more comprehensive strategy is to incorporate the substrate envelope constraints in the structure-based drug design. This strategy is based on the observation that substrates with high sequence diversity adopt a conserved shape when bound to HIV-1 protease (Prabu-Jeyabalan, et al., 2002). The consensus volume shared by the bound substrates defines the substrate envelope.

Drug resistance mutations often occur at sites where the inhibitor protrudes outside the substrate envelope (King, et al., 2004). Similar observations have recently been demonstrated to be valid for the HCV NS3/4A protease as well (Romano, et al., 2012; Romano, et al., 2010), supporting the generality of the substrate envelope model. Inhibitors designed to fit within this envelope are likely to be less susceptible to drug resistance, as a mutation that impairs inhibitor binding will simultaneously affect substrate recognition and protease function.

Previously, we designed and synthesized series of PIs with and without substrate envelope constraints (Ali, et al., 2006; Ali, et al., 2010; Altman, et al., 2008; Chellappan, et al., 2007; Nalam, et al., 2010; Nalam and Schiffer, 2008). These studies revealed that in contrast to inhibitors designed without any constraint, inhibitors designed to fit within the substrate envelope retain binding affinity to drug resistant HIV-1 protease variants, thereby having flatter resistance profiles. A careful examination of Darunavir (DRV) binding to HIV-1 protease by free energy analysis suggested that the interactions in the S1′ and S2′ sites can be improved by using more hydrophobic P1′ and P2′ groups to sustain van der Waals contacts to variants with active site mutations (Cai and Schiffer, 2010). In previous structure-activity relationship (SAR) studies of protease inhibitor libraries based on the (R)-(hydroxyethylamino)sulfonamide dipeptide isostere, several high affinity moieties were identified for the P2 and P2′ positions, including the bis-tetrahydrofuranyl (bis-THF) group in DRV (Ghosh, et al., 1998). However, most of these studies had the isobutyl group at the P1′ position. We have identified other P1′ ligands capable of more extensive van der Waals contacts with the protease due to enhanced size and flexibility, while still staying within the substrate envelope (Altman, et al., 2008; Nalam, et al., 2010). The challenge is combining these moieties into a single inhibitor that retains extremely high potency and stays within the volume of the substrate envelope.

We used the substrate envelope strategy to optimize PIs based on the DRV scaffold by incorporating ligands at the P1′ and P2′ positions that fit within the substrate envelope and therefore are predicted as promising candidates to be potent inhibitors of MDR HIV-1 protease variants. In this study, we report the structure-guided design and synthesis of a series of such potent inhibitors and their subsequent structural, biochemical, antiviral and pharmacokinetic evaluation. Two new chemical moieties at the P1′ position and five chemical moieties at the P2′ position were incorporated to optimize inhibitor flexibility and
interactions with the protease. The resulting 10 inhibitors (Fig. 1) were evaluated for their inhibitory activity against wild-type (WT) and MDR HIV-1 protease variants, antiviral activity against a panel of WT and patient-derived drug resistant HIV strains, and pharmacokinetic properties. The new compounds retain high inhibitory activity against MDR protease variants, display exceptional potency against a panel of twelve MDR strains in antiviral assays, and overall perform better than the most potent FDA-approved inhibitor, DRV. Crystal structures of complexes with WT HIV-1 protease reveal that the inhibitors indeed fit within the substrate envelope as designed, and support the use of substrate envelope constraints in the design of potent inhibitors evading resistance.

Results

Inhibitor Design and Synthesis

The design strategy for high affinity inhibitors against drug resistant HIV-1 protease variants was based on three criteria: The inhibitors should (1) fit within the substrate envelope (2) be based on the scaffold of a known high affinity inhibitor (3) have optimized P1′ and P2′ groups to retain contacts with the protease with drug resistance mutations.

The primary constraint in the design of inhibitors was proper fit within the substrate envelope of HIV-1 protease. This approach aims to minimize susceptibility to resistance, as a mutation that affects inhibitor binding will simultaneously affect substrate affinity (King, et al., 2004). In accord with the second criterion, the design was based on the \((R)-(\text{hydroxyethylamino})\) sulfonamide dipeptide isostere, the shared core scaffold of APV and DRV. Finally, new chemical moieties were introduced at the P1′ and P2′ positions. As the isobutyl P1′ moiety in DRV loses van der Waals contacts with the protease variants containing drug resistance mutations I50V, V82A, and I84V (King, et al., 2004), two new P1′ ligands, \((S)-2\)-methylbutyl (isopentyl) and 2-ethyl-n-butyl (isohexyl), with enhanced flexibility and steric volume were probed to enable sustained contacts with the protease. Combination of these two P1′ ligands with five P2′ ligands that satisfy the substrate envelope constraints yielded 10 new inhibitors (Fig. 1). The P2′ moieties were selected based on the previous structure-activity studies in the DRV series and our computational library designs, and include 4-aminobenzene, 4-methoxybenzene, 4-(hydroxymethyl)benzene, 1,3-benzodioxolane, and benzothiazole (Altman, et al., 2008; Ghosh, et al., 2008; Surleraux, et al., 2005; Surleraux, et al., 2005). The designed protease inhibitors were prepared following the synthetic route illustrated in Figure 2. Briefly, ring opening of chiral epoxide 1 with primary amines 2a-b provided the amino alcohols 3 and 4. Reactions of sulfonyl chlorides 5a-e with 3 and 4 gave the intermediate \((R)-(\text{hydroxyethylamino})\) sulfonamides 6-7. Boc deprotection followed by the reactions of the free amines with bis-THF carbonate 9, prepared from chiral bis-THF alcohol 8, provided the target compound series 10a-e and 11a-e.

The Designed Compounds Inhibit WT and Drug-Resistant Protease Variants

The enzyme inhibition constants \((K_i)\) values of the designed inhibitors were determined against WT and three drug resistant HIV-1 protease variants, along with all nine FDA-approved PIs (Fig. 3 and Table S2). Two MDR protease variants (M1: L10I/G48V/I54V/L63P/V82A, M2: L10I/L63P/A71V/G73S/I84V/L90M) represent the pattern of resistance mutations that occur under the selective pressure of three or more currently prescribed PIs in HIV-1 infected patients (Wu, et al., 2003). The third variant (M3: I50V/A71V) is a signature resistant variant of APV/DRV, which share the same scaffold used in the design of the ten inhibitors.
All designed inhibitors were highly potent in inhibiting enzyme activity with $K_i$ values in the 0.2–30 pM range against WT protease. Five PIs had sub-pM $K_i$ values (PIs 10c, 10d, 11b, 11c, 11d, $K_i = 0.2–0.9$ pM). Seven of the nine FDA approved drugs are 2-3 orders of magnitude weaker inhibitors of WT protease ($K_i = 46–284$ pM), while two are low picomolar binders (LPV and DRV, $K_i = 5$ pM). The precise measurements of $K_i$ values in the low pM range using standard biochemical assays are limited (Gulnik and Eissenstat, 2008; KuzmiČ & Caron, 1996; Miller et al., 2006). This makes direct comparison of new PIs with DRV, and each other, challenging, as all have $K_i$ values in the low pM range. With those caveats, these novel PIs have $K_i$ values against WT protease an order of magnitude lower than the most potent FDA-approved drug DRV ($K_i = 5$ pM).

The novel PIs also retained potency against MDR variants of HIV-1 protease. The $K_i$ values for all PIs against the three MDR proteases (M1, M2 and M3) were in the pM range. Most FDA-approved PIs lose significant potency against M1 and M2, except DRV and tipranavir (TPV) (Fig. 3). All the new PIs retained potent activity against the M1 variant with $K_i$ values comparable to or lower than DRV ($M1K_i = 25$ pM), in particular three PIs (10b, 11b and 11c) had $K_i$ values lower than DRV. Similarly, most new PIs inhibited the M2 variant with potency comparable to DRV. The M3 protease variant contains signature mutations of DRV resistance (I50V, A71V). Compared to WT protease, M3 susceptibility to DRV was 50-fold decreased, with a $K_i$ of 245 pM. In contrast, the designed inhibitors exhibited better potency against M3, with $K_i$ values consistently lower than that of DRV, and as low as 6 pM for PIs 10a and 11d (Fig. 3). Thus, the new design strategy yielded highly potent PIs that retain enzyme inhibition activity against multi-drug resistant variants of HIV-1 protease.

Overall, replacement of the P1′ isobutyl group of DRV with larger, more flexible isopentyl and isohexyl groups resulted in improved inhibition of drug resistant protease variants. This is evident from better potency of PIs 10a and 11a, which incorporate the same 4-aminobenzene group at P2′ as DRV. The modifications at the P1′ position also proved to be advantageous when combined with other groups at the P2′ position. In particular, PIs 10c and 11c with the 4-(hydroxymethyl)benzene P2′ group were highly potent inhibitors of WT protease and retained low pM activity against resistant variants. Thus incorporation of optimized ligands that can retain interactions with drug resistance protease variants coupled with the substrate envelope constraint proved to be a useful strategy for designing robust inhibitors.

The Inhibitors Fit within the Substrate Envelope with Enhanced Protease Contacts

The crystal structures of the ten novel potent inhibitors were determined in complex with WT HIV-1 protease. The crystals diffracted to 1.45–1.95 Å, yielding high-resolution complex structures. The crystallographic and refinement statistics, as well as PDB IDs for all the structures are listed in Table S1.

The co-crystal structures of all ten PIs in complex with the protease superimpose with each other extremely well (Fig. 1b). The protease backbone is very similar in all the complexes, with an RMSD of 0.11–0.16 Å for Cα atoms. Even the protease side chains, including those in the active site, have similar conformations in all the crystal structures, except minor local changes in side chain conformations surrounding the different P1′ and P2′ groups. These tight-binding PIs “lock” into the active site and induce a tightly shared rigid protease conformation with little variability.

Except for the chemically diverse P1′ and P2′ groups, the inhibitors superimpose very well in all the crystal structures, and fit well within the substrate envelope (Fig. 1c). Despite inherent flexibility, the P1′ isopentyl and isohexyl groups adopt the same conformation in all the complexes, similar to the isobutyl group in DRV bound to protease (Fig. 4a). The key
difference is the enhanced van der Waals contacts of the longer isopentyl (displayed for PI 10a in Fig. 4b) and isohexyl (displayed for PI 11a in Fig. 4c) groups within the S1′ pocket of the protease active site, compared to those of the isobutyl group of DRV. The P1′ group makes vdW interactions with the hydrophobic residues I50, P81, V82 and I84 in the active site of the protease. With the exception of P81, these residues are key sites of multi-drug resistance, and mutate to those with smaller side chains (Ile to Val, Val to Ala) to confer resistance, such as in the case of I50V mutation in the DRV signature resistance variant M3. The isopentyl and isohexyl groups in the novel PIs fill the S1′ pocket better compared to the isobutyl group of DRV, and their flexibility makes them capable of adapting and forming van der Waals contacts even with the mutated shorter side chain residues. These enhanced contacts are consistent with retained affinities to drug resistant HIV protease variants with mutations around the S1′ pocket (Fig. 3 and Table S2).

Improved Antiviral Activity against WT and Drug-Resistant HIV Strains

The new PIs were tested for antiviral activity against WT HIV from clades A, B and C, as well as a diverse panel of 12 clinically relevant patient-derived drug resistant viruses using PhenoSense™ HIV assays (Monogram Biosciences, San Francisco, CA), and compared with DRV (Fig. 5 and Table S3, Fig. S1). The average EC₅₀ values against all twelve viral variants tested, and fold-changes in potency with respect to WT strain were used to evaluate PI resistance profiles (Table S3).

All ten inhibitors exhibited sub-nanomolar potency against WT virus from three different clades (EC₅₀ = 0.04–0.4 nM). PIs 10b, 10d and 11b are more potent against WT clade B virus compared to DRV (EC₅₀ = 0.4 nM).

The novel PIs even retained antiviral potency against MDR strains, further validating the enzymatic assays. The average EC₅₀ values against 12 different drug resistant strains were lower than DRV for all 10 PIs (Table S3 and Fig. 5). Overall, the new PIs were 1.5-4.5-times more potent than DRV against drug resistant HIV strains tested. There was no significant difference in the EC₅₀ values of PIs with the isopentyl (10a-d) versus the isohexyl (11a-d) group at the P1′ position. However, the P2′ phenylsulfonamide groups appear to have more effect on the potency against drug resistant strains. Thus, consistent with previous SAR results in the DRV series (Surleraux, et al., 2005), compounds with the 4-methoxybenzene (10b and 11b) and 1,3-benzodioxolane (10d and 11d) groups at P2′ position exhibited better potency than the corresponding 4-aminobenzene analogues 10a and 11a. However, in contrast to the corresponding DRV analogue (Ghosh, et al., 2008), PIs with the 4-(hydroxymethyl)benzene P2′ group (10c and 11c) also exhibited highly potent activity, likely due to the increased lipophilicity In a previous study, HIV PIs with cLogP values in the 3.7–5.0 range exhibited better antiviral potencies against drug resistant HIV variants than DRV (cLogP = 2.88) (Miller, et al., 2006). As the cLogP values of the new PIs are all in the desired range (Table S4), the increased lipophilicities, in addition to other factors, may be contributing to the improved antiviral potencies compared to DRV.

DRV retains low nM activity against most MDR strains, but loses significant potency against two MDR strains with the I50 V protease mutation. However, a number of the new analogues retain better potency against these MDR strains, particularly analogues 10b and 10d with the 4-methoxybenzene and 1,3-benzodioxolane groups at P2′ position. In fact, compound 10b retains sub nM antiviral potency against 10 of the 12 MDR strains tested and low nM potency against the strains with the I50V protease mutation, exhibiting the lowest average EC₅₀ of 1.33 nM compared to 5.98 nM for DRV. Thus, the combination of isopentyl group at P1′ and 4-methoxybenzene group at P2′ provided the compound (10b) with the best resistance profile.
The excellent resistance profiles of the PIs indicate that the design strategy of filling the subsites without violating the substrate envelope constraints was successful in optimizing the inhibitor for robustness against resistance mutations.

**In Vitro Pharmacokinetic Evaluation**

The exciting PhenoSense™ resistance profile data prompted further evaluation of the new PIs as potential drug candidates using in vitro pharmacokinetic assays. The aqueous solubility, plasma protein binding, plasma stability, and microsomal stability in dog, rat, and human liver microsomes of all new PIs were determined by Apredica (Watertown, MA) using DRV as a control (Tables 1 and S4).

The oral bioavailability of a drug depends in part on water solubility and lipophilicity. The latter is estimated by the calculated partition coefficient cLogP, which is optimized to balance between aqueous solubility and transport through the hydrophobic cell membrane (Miller, et al., 2006). Compounds with cLogP values in the 3.5–5.0 range have been reported to have the best antiviral activity as HIV PIs (Miller, et al., 2006). The cLogP values of the new PIs are all in the desired range (Table S4). In fact, they have higher lipophilicities compared to DRV, which may enhance cell penetration. Despite the enhanced lipophilicity, the PIs also have good aqueous solubility except the PIs 10b and 11b with the methoxybenzene group in the P2'. Overall the increased lipophilicity and reasonable aqueous solubility likely contribute to the improved antiviral activity profiles (EC_{50} values), which depend on both the intrinsic binding affinity to the target (K_{i}), and the transport efficiency into the cell.

The metabolic stability of PIs was evaluated by measuring the microsomal stability, plasma stability and plasma protein binding (Tables 1 and S4). Assays were performed in rat and human microsomes to determine the stability of PIs at 15, 30, and 60 min time points and in the absence and presence of CYP-450 inhibitor ritonavir (RTV) (Table 1). Similar to DRV, RTV boosting considerably enhances the microsomal stability for majority of the new PIs, which are otherwise not stable in the presence of liver microsomes at 60 min. Interestingly, two inhibitors with the benzothiazole P2' moiety (10e and 11e) showed excellent stability in both rat and human microsomal tests even without RTV boosting. Overall, the new PIs have good pharmacokinetic properties, encouraging their further development as drug candidates.

**Discussion**

With the emergence of resistance, many drugs become obsolete in therapies against rapidly evolving disease targets, such as in the case of HIV/AIDS. In HIV infection, error-prone reverse-transcriptase and the high replication rates of the virus result in a diverse viral population, among which drug-resistant viral strains are selected under the pressure of drug therapy. To avoid spending time and resources in developing drugs that rapidly becomes obsolete, strategies at the initial design stage need to be implemented to minimize the chances of subsequent drug resistance.

The *substrate envelope* provides a framework for implementing drug resistance considerations into structure-based drug design. For drug resistance to occur, the target needs to be able to carry out its biological function while at the same time avoiding drug binding. The chances of this happening are diminished if the designed drug binds the target similarly to the natural substrates. Protrusions beyond the substrate envelope cause vulnerable sites for drug resistance mutations (King, et al., 2004), as mutations into bulkier side chains impact drug binding without affecting the substrates.
The results presented here demonstrate that optimally filling the substrate envelope is key to developing potent inhibitors. In cases where the designed drug occupies a smaller volume than the substrates, mutations into shorter side chains may diminish van der Waals interactions with the inhibitor, while still maintaining sufficient interactions with the larger natural substrates. In the DRV signature resistant HIV-1 protease, the I50V mutation causes loss of contacts with the P1′ isobutyl group. The new longer isopentyl and isohexyl moieties introduced here to the P1′ site optimally fill the substrate envelope and have flexibility, which enables sustaining van der Waals interactions even when resistant mutations occur. Thus, the inhibitors have improved van der Waals contacts with the protease while still staying within the substrate envelope, and retain potency against a wider variety of HIV-1 protease mutants.

The improved binding affinities of PIs against drug resistant protease variants correlate with their exceptional antiviral potencies against a panel of twelve patient-derived drug resistant HIV strains in PhenoSense™ HIV assays. The new PIs retain better antiviral potency against MDR HIV variants less susceptible to DRV, with EC$_{50}$ values in the low nM range. However, MDR viruses insensitive to DRV are also relatively less sensitive to new PIs, implying the same factors may render the inhibitors less effective in the antiviral assays. The MDR viruses insensitive to DRV and the new PIs contain a combination of protease secondary mutations outside the active site, which may interdependently confer resistance to all these inhibitors. Such secondary mutations may alter the processivity of the protease to favor substrate processing over inhibitor binding, thereby decreasing susceptibility to inhibitors (Chang and Torbett, 2011; Muzammil, et al., 2003; Xie, et al., 1999). Thus, the compensatory mutations outside the protease active site may also be contributing towards the observed resistance profiles.

All ten inhibitor-protease complex structures superimpose extremely well with each other, despite chemical diversity at the P1′ and P2′ sites of the inhibitors. The exceptionally high affinity of the designed sub- and low-picomolar inhibitors may be locking the protease structure, and not allowing much conformational variability. Future studies of protease-inhibitor complex dynamics will provide insights into the effect of sub-picomolar binders on the protease conformational and dynamic behavior.

In conclusion, in quickly evolving therapeutic targets, restricting inhibitors to fit within the substrate envelope is a key filter in minimizing the susceptibility to drug resistance.

**Significance**

Overcoming resistance is crucial in designing robust drugs against HIV/AIDS. Resistance to direct-acting antiviral drugs, including HIV-1 protease inhibitors, occurs when the target mutates to avoid drug binding, while maintaining activity on substrates. Our design strategy of optimizing inhibitor fit within the substrate envelope yielded high affinity inhibitors of multi-drug resistant HIV protease variants, which exhibited improved antiviral potencies than DRV against a large panel of drug resistant HIV strains. Some of these potent inhibitors also have promising pharmacokinetic profiles, even in the absence of a boosting agent, encouraging their potential development as drugs.

This study provides strong rationale for incorporating substrate envelope constraints in the initial stages of drug design as a powerful strategy to design potent inhibitors that evade resistance. Drug resistance extends well beyond HIV, thwarting the success of many drugs to quickly evolving targets in pathogens and cancer. This strategy is broadly applicable to the design of inhibitors against such quickly evolving disease targets, facilitating a more rational and rapid selection of drug candidates that are robust and thus less susceptible to resistance.
Experimental Procedures

Protein Crystallography

The expression, isolation, and purification of wild-type and mutant HIV-1 proteases used for crystallization and binding experiments were carried out as previously described.(King, et al., 2002) Co-crystals of the inhibitors with the wild-type protease were grown at room temperature by hanging drop vapor diffusion method. Protease concentration of 1.6 mg/ml with 3-fold molar excess of inhibitors was used to set the crystallization drops with the reservoir solution consisting of 126 mM phosphate buffer at pH 6.2, 63 mM sodium citrate and 24-29% ammonium sulfate.

The crystals used for data collection were mounted in Mitegen Micromounts and flash frozen over a nitrogen stream. Intensity data for complex structures of 10a, 10b, 10d, 10e, 11b, 11c and 11d were collected at -80°C on an in-house Rigaku X-ray generator equipped with an R-axis IV image plate. 180 frames were collected per crystal with an angular separation of 1° and no overlap between frames. The data processing of the frames was carried out using the programs DENZO and ScalePack respectively.(Minor, 1993; Otwinowski, et al., 1997) Intensity data for 10c, 22a and 11e were collected under cryogenic conditions at BioCARS 14-BMC beamline at Argonne National Laboratory (Advanced Photon Source, Chicago, IL). Diffraction images were indexed and scaled using the program HKL2000.(Otwinowski, et al., 1997)

The crystal structures were solved and refined with the programs within the CCP4 interface. Structure solutions for all the wild-type protease-inhibitor complexes were obtained with the molecular replacement package AMoRe(Navaza, 1994) using 1F7A(Prabu-Jeyabalan, et al., 2000) as the starting model. Upon obtaining solution, the molecular replacement phases were further improved using ARP/wARP(Morris, et al., 2002) to build solvent molecules into the unaccounted regions of electron density. Model building was performed using the interactive graphics program Coot.(Emsley and Cowtan, 2004) Conjugate gradient refinement using Refmac5(Murshudov, et al., 1997) was performed by incorporating Schomaker and Trueblood tensor formulation of TLS (translation, libration, screw-rotation) parameters.(Kuriyan and Weis, 1991; Schomaker and Trueblood, 1968; Tickle and Moss, 1999) The working $R(R_{facto})$ and its cross validation ($R_{free}$) were monitored throughout the refinement. The data collection and refinement statistics are shown in Table S5.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Design of HIV-1 protease inhibitors (PIs) using the substrate envelope model.
- Crystal structures show PIs chemical moieties fill the substrate envelope.
- PIs display exceptional enzymatic and antiviral potency to drug resistant HIV.
- The design strategy is applicable to other quickly evolving disease targets.
Figure 1.
Structure of the designed protease inhibitors. (a) The chemical scaffold, and P1′ and P2′ groups of the 10 inhibitors. (b) Superimposed x-ray crystal structures of all 10 inhibitors in complex with WT HIV-1 protease. See Table S1 for crystallographic statistics. (c) Fit of inhibitors within the substrate envelope. The substrate envelope is in blue space filling representation, and the superimposed inhibitors are displayed as sticks. Protrusions beyond the substrate envelope are in red.
Figure 2.
Synthesis of protease inhibitors. Reagents and Conditions: (a) R1NH2, EtOH, 80 °C, 3 h; (b) aq. Na2CO3, CH2Cl2, 0 °C to rt, overnight; (c) CH2Cl2, Et3N, 0 °C to rt, overnight; (d) NaBH4, MeOH, 0 °C, 15 min; (e) TFA, CH2Cl2, 1 h; (f) Py, p-NO2-PhOCOCl, 0 °C to rt, 24 h; (g) DIEA, CH3CN, 0 °C to rt, 24 h; (h) SnCl2.2H2O, EtOAc, 70 °C, 3 h. See Supplemental experimental procedures for more details.
Figure 3.
Binding affinities of FDA-approved drugs and the 10 designed protease inhibitors to WT and drug resistant variants of HIV-1 protease; the $K_i$ values for the FDA-approved drugs are from Nalam et al. (Nalam, et al., 2010) Inhibitory activity was determined by a FRET-based enzymatic assay; $K_i$ values are the average of at least three independent measurements. See also Table S2.
Figure 4.
Comparison of van der Waals contacts with the protease for P1’ groups in PIs. (a) Superposition of DRV, PI 10a and PI 11a structures bound to WT HIV-1 protease. (b) Isobutyl P1’ group in DRV (c) Isopentyl P1’ group in PI 10a (d) Isohexyl P1’ group in PI 11a. The longer P1’ groups make enhanced van der Waals contacts with the protease.
Figure 5.
Resistance profile of DRV and the 10 PIs designed. Antiviral potencies ($EC_{50}$ values) were obtained for wild-type HIV from clades A, B and C, and 12 different patient-derived drug-resistant variants of the virus. The PhenoSense™ HIV assays were performed by Monogram Biosciences. See also Table S3 and Figure S1.
Table 1

Microsomal stability profile of protease inhibitors, related to Table S4.

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<td>66 20</td>
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<td>64 79</td>
<td>69 57</td>
<td>26 27</td>
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<td>+</td>
<td>55 21</td>
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<tr>
<td>11e</td>
<td>−</td>
<td>100 84</td>
<td>83 87</td>
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<td>+</td>
<td>96 62</td>
<td>95 44</td>
<td>3 44</td>
</tr>
<tr>
<td>Compound</td>
<td>RTV</td>
<td>Microsomal Stability (Avg. % Fraction Remaining)</td>
<td>Microsomal CL\textsubscript{int} (μL min\textsuperscript{-1} mg\textsuperscript{-1})</td>
<td>Microsomal T\textsubscript{1/2} (min)</td>
</tr>
<tr>
<td>----------</td>
<td>-----</td>
<td>-----------------------------------------------</td>
<td>---------------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>at 30 Minutes Human</td>
<td>Rat</td>
<td>at 60 Minutes Human</td>
</tr>
<tr>
<td>DRV</td>
<td>−</td>
<td>37</td>
<td>40</td>
<td>13</td>
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<td>+</td>
<td>100</td>
<td>84</td>
<td>100</td>
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</tbody>
</table>

RTV: ritonavir; CL\textsubscript{int}: microsomal intrinsic clearance; T\textsubscript{1/2}: half-life