Cyclic HIV protease inhibitors capable of displacing the active site structural water molecule

George V. De Lucca, Susan Erickson-Viitanen and Patrick Y.S. Lam

A new era has begun in the chemotherapeutic treatment of AIDS with the marketing of the first generation of peptidomimetic HIV protease inhibitors from Roche, Merck and Abbott. Owing to the potential drug resistance problems associated with HIV mutations, a main emphasis in the search for the next generation of HIVPR drugs is in the area of structurally different cyclic nonpeptides. This article reviews the different approaches that have been undertaken to find or design cyclic nonpeptides that are capable of displacing the structural water molecule usually associated with the peptidomimetic class of inhibitors. The favorable entropic effect from water displacement and preorganization can lead to smaller and more potent inhibitors with improved therapeutic potential.

The human immunodeficiency virus (HIV), the causative agent of Acquired Immunodeficiency Syndrome (AIDS), encodes an essential aspartic protease (PR)[1] that processes the viral Gag and Gag–Pol polyproteins into structural and functional proteins. Inhibition of HIVPR *in vitro* results in the production of progeny virions, which are immature and noninfectious[2,3]. The abundance of structural information available on HIVPR has made the enzyme an attractive target for computer-aided drug design strategies[4–6]. As a consequence, HIVPR has emerged as a prime target for the development of therapeutics for the treatment of HIV diseases[7–12].

Intense world-wide research in the area of HIVPR inhibition has produced a variety of potent compounds with inhibition constants, K_i, in the nanomolar or subnanomolar range[13–20]. Most of these potent inhibitors are pseudopeptides (containing one or more amide bonds), or molecules containing substrate-like linear scaffolds. Despite many elegant structure–activity studies, the combination of relatively high molecular weight (>600 daltons), poor solubility and substantial peptide character have generally resulted in limited oral bioavailability. The difficulty of combining adequate potency with good oral bioavailability is reminiscent of the two decades of research focused on identifying orally available inhibitors of the aspartic protease renin. In the case of HIVPR, this intense effort has produced several compounds with sufficiently adequate overall properties to justify further clinical evaluation[21–29].

In recent clinical studies, several HIVPR inhibitors have been shown to reduce the viral load and increase the number of

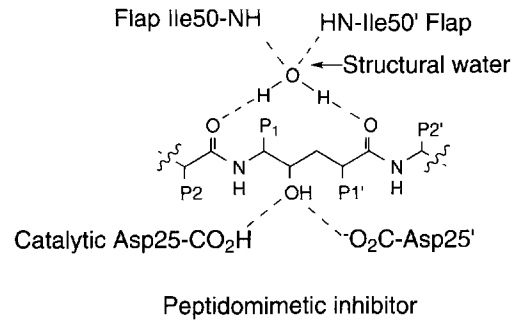


Figure 1. *Peptidomimetic inhibitor binding at HIVPR active site via a bridging structural water.*

George V. De Lucca, **Susan Erickson-Viitanen** and **Patrick Y.S. Lam***, Dupont Merck Pharmaceutical Company, Experimental Station, PO Box 80500 Wilmington, DE 19880-0500, USA. tel: +1 302 695 2387, fax: +1 302 695 9673, *e-mail: lampy@carbon.dmpc.com

  1359-6446/97/$17.00. PII: S1359-6446(96)10048-9

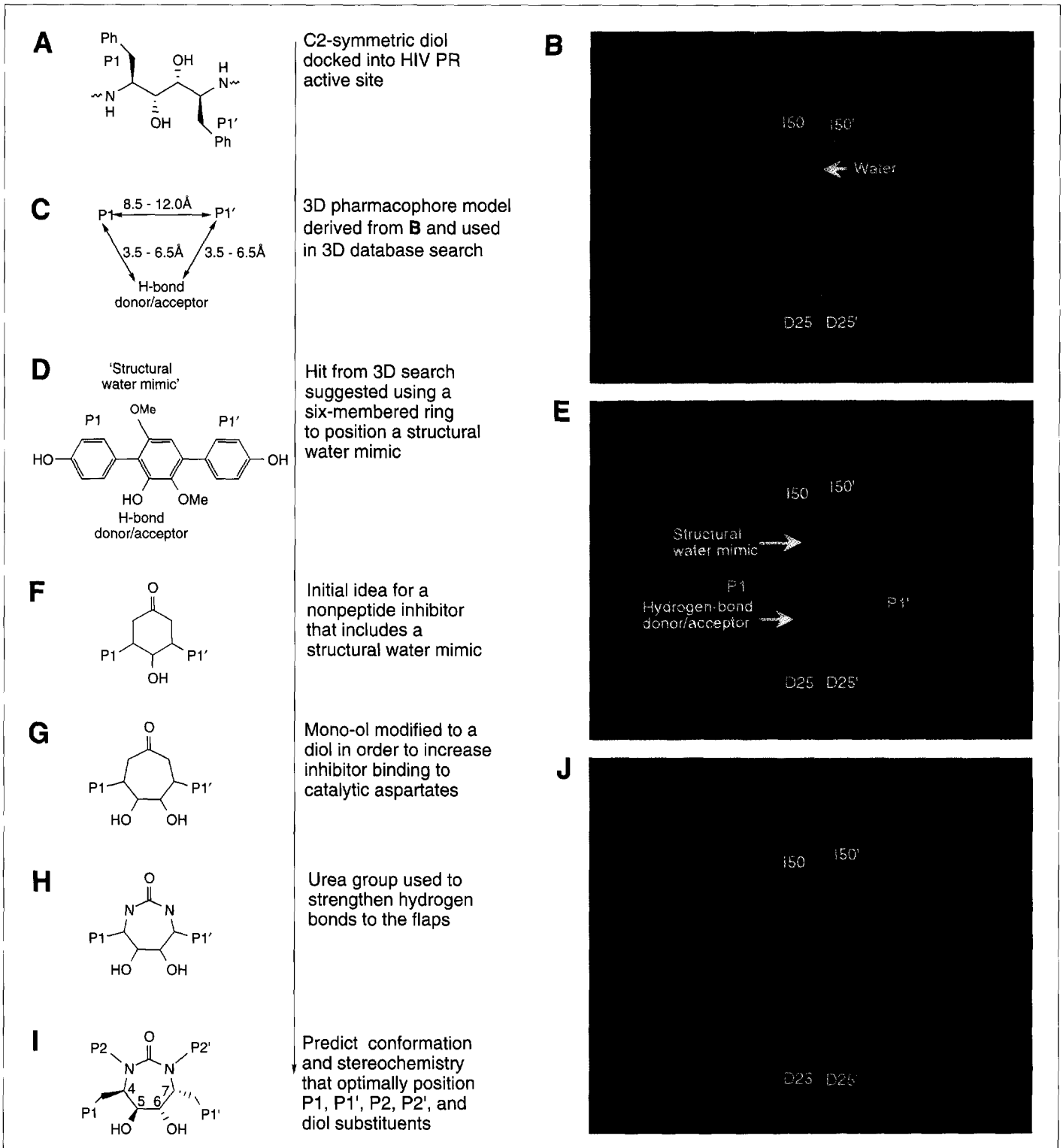


Figure 2. *Strategy and steps involved in the design of cyclic urea inhibitors of HIVPR. Reprinted with permission from Ref. 45.*

$CD4^+$ lymphocytes in HIV infected patients[21–29]. Saquinavir, Ritonavir and Indinavir have recently been approved by the FDA and are being used in AIDS therapy in combination with reverse transcriptase (RT) inhibitors. However, the daunting ability of the virus to rapidly generate resistant mutants[30–34] suggests that there is an ongoing need for new

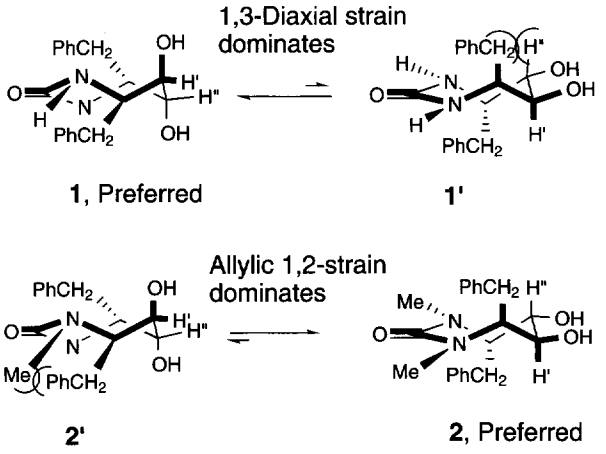


Figure 3. *Conformational analysis of designed cyclic ureas predicting that* ***1*** *is preferred when the nitrogens are not substituted, whereas conformation* ***2*** *is preferred when the nitrogens are substituted due to* $A_{1,2}$ *strain.*

HIVPR inhibitors with superior pharmacokinetic and efficacy profiles.

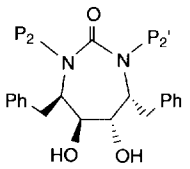
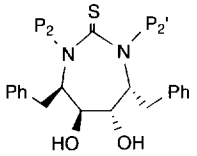
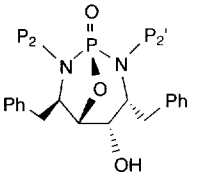
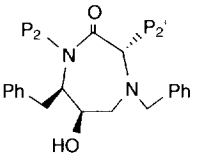
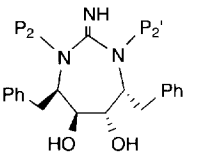
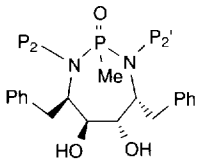
The different approaches to the discovery of the various types of HIVPR inhibitors have been extensively reviewed[35–40] and are outside the scope of this review. However, to summarize, most of the structures can be classified into two categories: transition-state analogs (for example linear peptidomimetics) and nonpeptides, which are often rigid cyclic structures. Lead structures for both classes of inhibitors have been identified through random screening and rational drug design. Regardless of how the leads were generated a main feature of current work in HIV protease inhibitors is the extensive use of structural information and computational/computer modeling techniques to optimize initial lead structures.

Unique structural water of inhibitor–HIVPR complexes

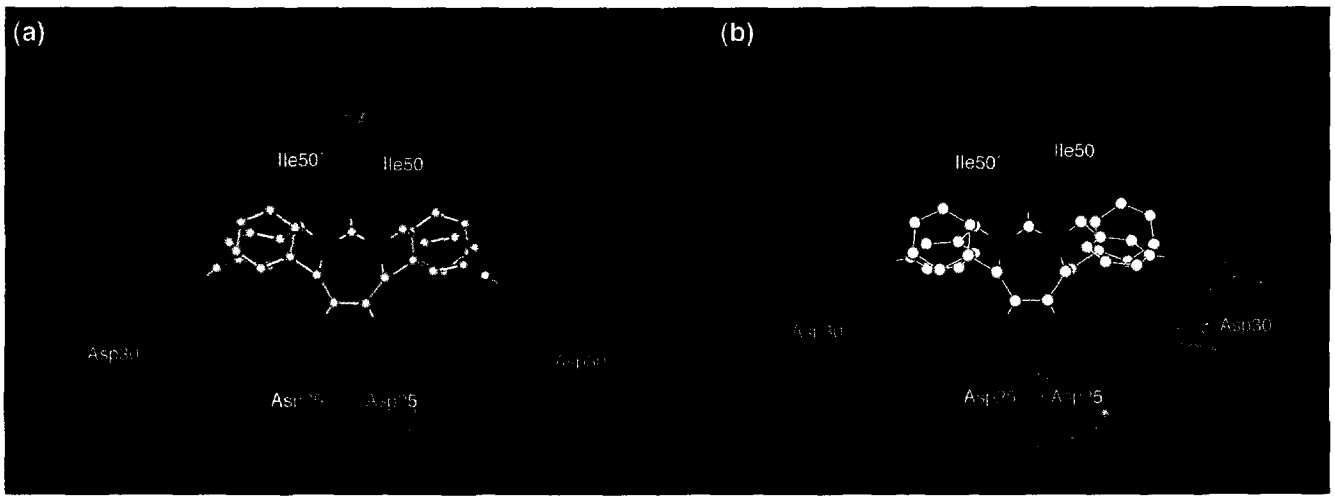
High resolution X-ray structures of many linear inhibitors complexed with the C-2 symmetric, dimeric HIVPR-1 have been reported[4–6]. A common feature among these structures is the presence of a tetracoordinated structural water molecule linking the bound inhibitor to the flexible glycine-rich β-strands or 'flaps' of the HIVPR dimer (Figure 1). This structural water molecule accepts two hydrogen bonds from the backbone amide hydrogens of symmetry-related isoleucine residues Ile50 and Ile50′, and donates two hydrogen bonds to the carbonyl oxygens flanking the transition state mimetic of the inhibitor molecule. Its relevance to the generation of HIVPR inhibitors had been previously noted[8,9].

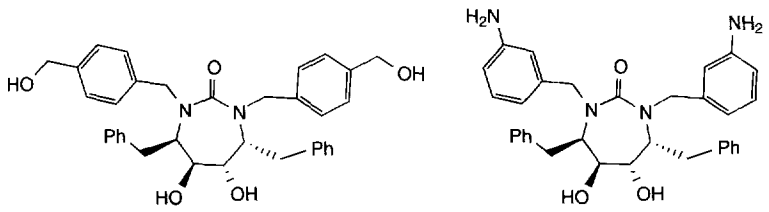
An important key design element in many approaches has been to use the C-2 symmetrical nature of the enzyme[4,35] and the presence of the structural water molecule in the active site to build in specificity and potency. In this paper we wish to review compounds and design strategies that incorporate the structural water into the inhibitor framework. This covers mainly rigid, nonpeptide, cyclic structures that are known to or that are likely to displace the structural water molecule usually found in all linear HIVPR/inhibitor complexes. The main emphasis in the search for the next generation HIVPR drug candidate appears to be in this cyclic area.

Table 1. Comparison of cyclic urea analogs

No.	Structure[a]	K_i (nM)
3		3.6
4		3.1
5		44
6		180
7		220
8		1,100

[a]P2/P2′ = Benzyl





9, DMP323 **10**, DMP450

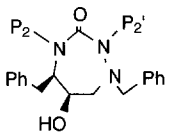
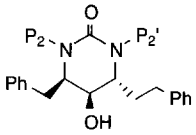
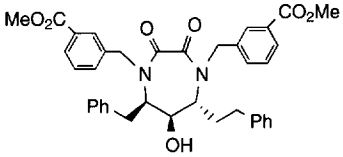
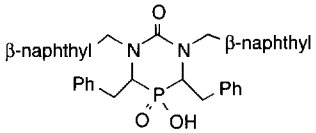
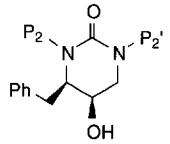
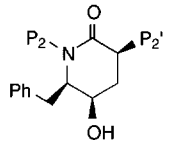
Figure 4. *X-ray structures of the complexes of DMP323 (a) and DMP450 (b) with HIV-1PR (green/orange) showing that the urea oxygen accepts hydrogen bonds from the protease flaps Ile50/50′ with the exclusion of the intervening structural water commonly found in linear peptidomimetic inhibitors. X-ray structures solved and graphics provided by Dr Chong-Hwan Chang of DMPC.*

Design of the cyclic ureas

The technique of searching databases containing 3D molecular structures using a 3D pharmacophore model has been used to identify synthetic frameworks that can serve as the starting point for the design of nonpeptide inhibitors[41–44]. This technique was incorporated as part of the design strategy that led to the discovery of cyclic ureas as nonpeptide inhibitors by the Dupont Merck team[45–47] and is summarized in Figure 2.

This process began by using the available structural information[9] and the structure–activity relationships (SAR) established for linear C-2 symmetric diols[48–51] to generate several pharmacophore models (Figures 2a and 2b). The simplest model (Figure 2c) was based on two key intramolecular distances: that between symmetric P1 and P1′ hydrophobic groups, and that from P1 and P1′ to hydrogen bond donor/acceptor group(s) that bind to the catalytic aspartates. A 3D database search[52–54] with this pharmacophore model yielded the 'hit' shown in Figure 2d. Because a phenyl ring might not properly position all substituents of the inhibitor, a cyclohexanone ring (Figure 2f) was chosen as the initial synthetic scaffold with the ketone oxygen as the structural water mimic. The cyclohexanone ring was enlarged to a seven-membered ring (Figure 2g) to incorporate a diol functionality because the SAR established for linear C-2 symmetric diols indicated that the diol imparts significant potency compared with corresponding mono-ol transition state analogs[49,50]. This synthetic target was further modified to a cyclic urea (Figure 2h) based on two considerations. First, cyclic ureas have precedent as excellent hydrogen bond acceptors both in nature[55] and in synthetic systems[56,57]. Second, it was realized that the seven-membered cyclic urea was synthetically accessible by cyclizing the precursor used in the linear C-2 symmetric diol series[48–51].

Table 2. Cyclized linear HIVPR inhibitors

No.	Structure[a]	K_i (nM)	Company
11		8.7	Abbott Ciba-Geigy DMPC
12		15	DMPC
13		40	DMPC
14		260	Hoechst
15		2,500	DMPC
16		9,400	DMPC

[a]P2/P2′ = Benzyl

Ring conformations of cyclic ureas

Critical to the design strategy is the qualitative prediction of the conformation[46] of the cyclic ureas. The seven-membered ring cyclic ureas can exist in two pseudo chair conformations (Figure 3). When the nitrogens are unsubstituted, 1,3-diaxial strain[58] dominates and conformer **1** with pseudo diequatorial benzyl groups is preferred. When the two nitrogens are substituted, the partial double bond character of the urea C–N bond introduces severe allylic 1,2-strain[59,60] between the benzylic groups and the nitrogen substituents. This allylic 1,2-strain overcomes the 1,3-diaxial strain, and conformer **2** with pseudo diaxial benzyl groups is preferred. This conformational prediction was subsequently confirmed by small molecule X-ray crystallography[46].

Using this type of conformational analysis the predicted optimal stereochemistry for cyclic ureas with substituents on the nitrogens is 4*R*,5*S*,6*S*,7*R* (Figure 2i), which is derived from unnatural (D) phenylalanine. This is in contrast to the linear C-2 symmetric diol inhibitors where natural (L) phenylalanine provides the optimal stereochemistry[48–51]. The design insights and predictions were confirmed by the biological results and subsequent structural studies[45–47]. The D-phenylalanine-derived cyclic urea **3**, with benzyl groups on the nitrogens (Table 1, K_i = 3.6 nM) is a potent inhibitor of HIVPR and is 1000-fold more potent than the corresponding L-phenylalanine derived cyclic urea. Further optimization of P1 (Ref. 47) and P2 (Ref. 46) led to increased potency and resulted in two clinical candidates, DMP323 and DMP450 (see below). The high resolution X-ray structures of the complexes of DMP323 (**9**)[46] and DMP450 (**10**)[61] with HIVPR-1 have been solved (Figure 4) and show that the urea oxygen directly hydrogen bonds to the flap residues Ile50/50′, with the exclusion of the intervening water molecule, consistent with the design. The displacement of the structural water was further confirmed by NMR experiments[62–65].

Other seven-membered ring cyclic inhibitors

Several factors are responsible for the potency of nitrogen-substituted cyclic ureas:

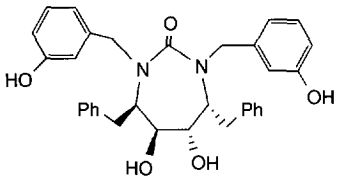
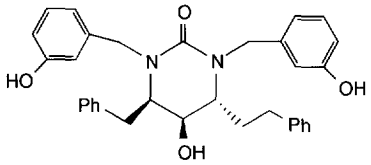
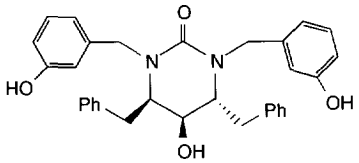
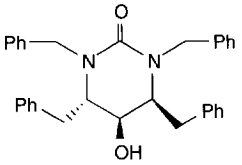
- the cyclic ureas are preorganized[66,67] for highly complementary binding to HIVPR, with the conformational entropic penalties typically associated with binding a linear, flexible inhibitor being 'prepaid' during synthesis rather than during binding;
- displacement of the water molecule is probably thermodynamically favorable[68,69]; and,
- hydrophobic interactions between the cyclic urea and the S1/S1′ and the S2/S2′ subsites of HIVPR are optimized with the preferred conformation and stereochemistry.

These factors can also be accommodated, to one degree or another, by other types of cyclic structures to yield potent inhibitors.

Tables 1–4 summarize all the cyclic structures that are discussed in this review. When possible, benzyl groups as P1/P2 substituents are used to make direct comparison easier. However, it should be noted that the benzyl group may or may not be the optimal substituent for each compound and with another substituent the rank order may change.

The use of cyclizing agents other than carbonyldiimidazole (CDI) to cyclize the diamino diol core leads to the formation of other cyclic structures capable of interacting with the enzyme. The use of thiocarbonyldiimidazole

Table 3. Importance of the P1 phenethyl for the potency of tetrahydropyrimidinones

No.	Structure	K_i (nM)
17		0.12
18		0.28
19		33
20		80,000

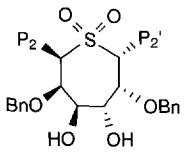
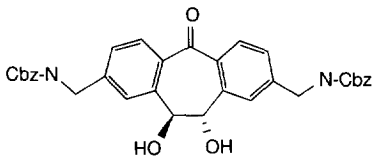
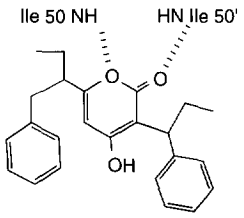
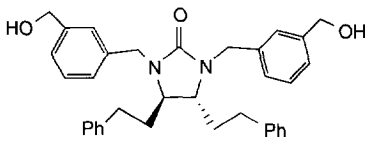
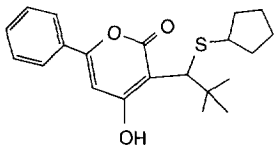
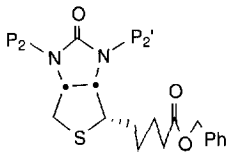
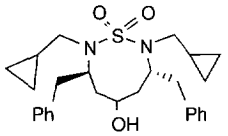
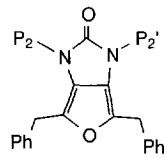
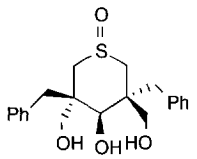
(TCDI) instead of CDI as the cyclizing agent leads to the corresponding thiourea **4** (Table 1) (P.Y.S. Lam, unpublished), which has the same overall conformation. Although the carbon–sulfur double bond is 40% longer (than the urea carbonyl) and thus shortens the hydrogen bonds to the flap residues, the strength of a sulfur–hydrogen bond is weaker. The overall effect is that the thiourea has the same potency as the corresponding cyclic urea analog.

The use of a guanidinating reagent as the cyclizing agent yields the corresponding seven-membered ring cyclic hydroxy-guanidines[70–72]. The hydroxy-guanidine analog has a K_i of 42 nM. The parent guanidine **7** (J. Rodgers and B. Johnson, unpublished) is the weakest inhibitor. This may be due to high desolvation penalty associated with the protonated guanidine compared with the urea.

The phosphordiamide analog **8** can also be obtained using the appropriate cyclization reagent to give weak inhibitors. The bicyclic phosphordiamidate **5**, however, is a fairly potent compound, although not very stable to hydrolysis[73,74].

The azalactam **6** was synthesized and found to be a modest inhibitor of HIVPR (Ref. 75). The azalactam can be viewed as an analog of the cyclic urea in which the

Table 4. Other cyclic inhibitors from *de novo* design or screening

No.	Structure[a]	K_i (nM)	Company
21		0.6	Gilead
22		5	Bayer
23		38	Upjohn
24		55	DMPC
25		58	Parke-Davis
26		570	DMPC
27		1,500	DMPC
28		3,700	DMPC
29		7,000	SKB

[a]P2/P2′ = Benzyl

nitrogen is displaced. It can also be viewed as a cyclic hydroxyethylamine dipeptide isostere analog (discussed below).

Cyclization of linear HIVPR inhibitors

Re-analysis of the cyclic ureas showed that, in practice, they are obtained by a process that first inverts the stereochemistry of the core diamino diol linear inhibitor followed by cyclization as shown in Figure 5. In order to obtain other heterocyclic cores that may also be nonpeptide HIVPR inhibitors, it is possible to use other cyclizing reagents, as discussed above, or to start with different linear core structures. The generality of using this cyclization process with other linear inhibitors and the benefits of cyclizing any linear inhibitor has been shown to be significant. However, it is important in this process of cyclizing linear inhibitors, as outlined in Figure 5, not only to get the correct stereochemistry, but also to understand the conformation of the resulting ring system and its interaction with the enzyme.

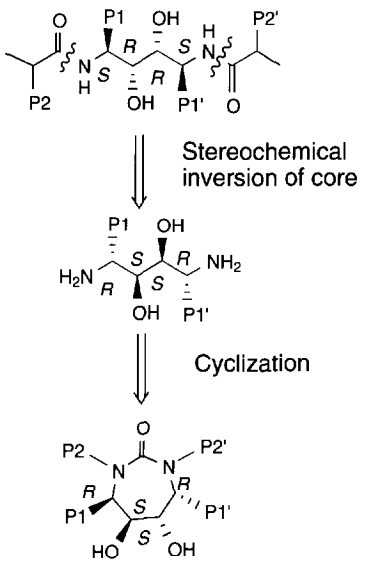


Figure 5. *General outline of the steps needed to convert a linear HIVPR inhibitor into a cyclic HIVPR inhibitor.*

Tetrahydropyrimidinones

As an example let us examine in detail the closely related linear diamino-alcohol HIVPR inhibitors that were first developed by Abbott[53]. Inversion of the stereocenters and cyclization gives the corresponding tetrahydropyrimidinone (Figure 6). In this six-membered ring the 1,3-*trans*-dibenzyl P1 substituents cannot both be axial. If one benzyl is axial then the other has to be equatorial. Thus one of the benzyl groups cannot interact with the enzyme as it does in the seven-membered ring cyclic urea (where both P1 benzyl groups are axial).

However, if one changes the equatorial P1′ benzyl group into a phenethyl group, then, although this substituent is still equatorial, the benzyl portion can take an axial-like position and will again be able to interact with the enzyme in the same way as the seven-membered ring cyclic urea. This becomes apparent when computer models are examined (Figure 7). The tetrahydropyrimidinone **12** (Table 2) was synthesized and found to be a potent inhibitor[76–78].

The importance of this structural analysis is shown in Table 3. Whereas the P1′ phenethyl tetrahydropyrimidinone **18** is nearly equipotent to the seven-membered ring cyclic urea **17**, the P1/P1′ benzyl/benzyl analog **19** is found to be about 100 times less potent than the P1/P1′ benzyl/phenethyl analog[76–78]. If, in addition, the wrong stereochemistry is used, as exemplified by the tetrahydropyrimidinone **20** (Ref. 79), the potency drops an additional three orders of magnitude.

Other cyclic inhibitors from linear inhibitors

Cyclic structures, other than a cyclic urea, with the diamino-alcohol core can also be envisioned. For example, following the same strategy used for tetrahydropyrimidinones, the diamino-alcohol core can be cyclized using oxalyl chloride instead of CDI to yield the seven-membered cyclic oxamide **13** (Table 2; Ref. 80), which is a potent inhibitor of HIVPR.

Recently there have been other examples of linear HIVPR inhibitors that have been cyclized to give potent cyclic compounds. Researchers at Abbott[81], Ciba[82] and Dupont Merck[75] (P.Y.S. Lam *et al.*, unpublished) have disclosed the cyclization of lincar hydrazine inhibitors to give the azacyclic ureas (**11**; Table 2). Elegant work published by Abbott has shown these to be potent inhibitors of HIVPR. In addition, an X-ray structure of a complex with HIVPR shows that the azacyclic ureas have a similar conformation and binding mode as the cyclic ureas[81].

Researchers at Hoechst have cyclized their linear diaminophosphinic acids to give the six-membered ring urea phosphinic acids (**14**; Table 2)[83]. Taking into account the fact that these exist as mixtures of isomers and do not have the optimal phenethyl P1′ substituent, they show surprisingly good activity.

Cyclization of peptide isosteres

In some cases the cyclization of the linear inhibitor yields a compound that cannot properly interact with the enzyme. An interesting example is the cyclization of the common hydroxyethylene isostere found at the core of many protease

inhibitors. Inversion of the stereocenters and cyclization gives the lactam **16** (G.V. De Lucca, unpublished). Computer models of this lactam show that the P1′ substituent in the linear structure is now the P2′ substituent in the cyclic structure (Figure 8). Thus, the cyclic analog lacks a P1′ substituent and is not a good inhibitor. The importance of the P1′ substituent[84] is evident by comparing the corresponding tetrahydropyrimidinone analogs that possess (for example **12**) or lack (for example **15**) the P1′ substituent.

One of the most common and successful dipeptide isosteres in linear HIVPR inhibitors is the hydroxyethylamine isostere. There are two ways of making cyclic analogs based on this isostere. Cyclizing only the core leads to the tetrahydropyrimidinone analog **15** (Table 2) and cyclizing the dipeptide isostere leads to the azalactam **7** (Table 1). In both cases the potency is significantly less in the cyclic analogs. In the former, the loss of potency is due to the lack of an S1′ interaction, and in the latter, it is probably the result of unfavorable conformational energetics.

Other cyclic inhibitors from *de novo* design or screening

From random screening of sample collections the hydroxycoumarins[85–89] and 4-hydroxy-2-pyrones[90–93] were identified as lead inhibitors. Extensive structure-based design work led to potent inhibitors of HIVPR (**23**, **25**; Table 4) with antiviral activities in the μM range. These have been shown by X-ray analysis of complexes with HIVPR, to be able to hydrogen bond directly to the flap residues and displace the structural water molecule. These compounds have extremely attractive pharmacokinetic profiles in various animal models with C_{max} reaching 120 μM (Ref. 88), which is unprecedented in the HIVPR inhibitor area.

Many other cyclic structures have been disclosed that attempt to incorporate the structural water into the inhibitor design. The seven-membered ring cyclic sulfone **21** (Table 4) has recently been disclosed by researchers at Gilead Sciences. These cyclic sulfones are extremely potent inhibitors of HIVPR (Ref. 94).

The eight-membered ring sulfamide **27** (Table 4)[95] was shown to be a fairly weak inhibitor, probably because of the unfavorable conformation of the ring. Searching 3D databases for compounds that could displace the structural water molecule followed by further design work led to the sulfoxide **29** (Ref. 96), which is a μM inhibitor of HIVPR. The dibenzosuberone core was developed by computer-aided design to give an inhibitor (**22**)[97,98] with IC_{50} of 5 nM, but it was found to be a non-competitive inhibitor.

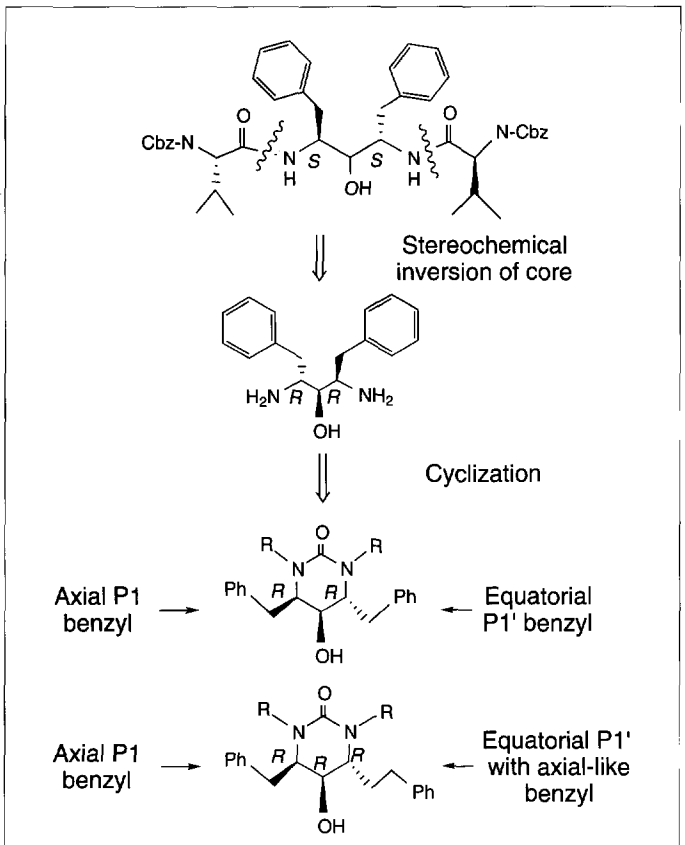


Figure 6. *Outline of steps needed to convert the linear diamino-alcohol class of HIVPR inhibitors to the tetrahydropyrimidinone class of HIVPR inhibitors.*

Five-membered ring cyclic ureas

Several types of five-membered ring cyclic ureas have been designed to displace the structural water molecule. For example the use of biotin as a naturally occurring scaffolding to build inhibitors led to a series of inhibitors (**26**) with modest activity[99]. If one considers that it lacks both a P1 substituent and a hydrogen bond donor to interact with the catalytic aspartic acids, the activity is surprisingly good. The related furanylimidazolone **28** (J.M. Smallheer and R.J. McHugh, unpublished), which also lacks a substituent to interact with the aspartic acids, is a low μM inhibitor of the enzyme. The best five-membered ring urea seems to be the imidazolidinone **24** (G.V. De Lucca, unpublished), which gives inhibitors with K_i's in the nM range. The low K_i is unexpected because this compound does not hydrogen bond to the catalytic aspartates. However, it appears to be very complementary to the enzyme's S1/S2 pockets because the corresponding six- or seven-membered ring cyclic urea analogs, lacking the same interaction, have comparable

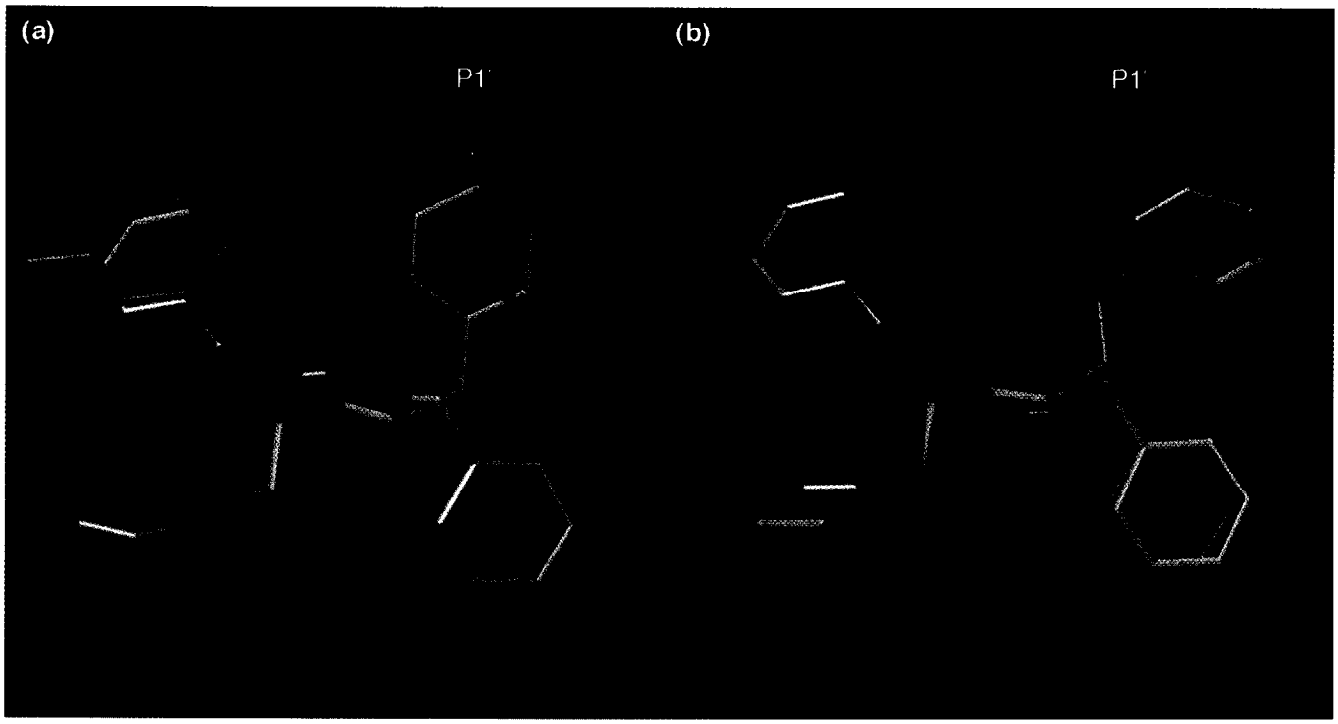


Figure 7. *The bound conformation of DMP450 (green), obtained from X-ray analysis of the complex with HIVPR, (a) overlapped with a model of the tetrahydropyrimidinone inhibitor having a P1′ benzyl substituent (white); (b) overlapped with a model of the tetrahydropyrimidinone inhibitor having a P1′ phenethyl substituent (white).*

binding affinities, as shown in Figure 9 (G.V. De Lucca, unpublished results).

Oral bioavailability and clinical trials

As described above, the requirements for a potentially useful HIVPR inhibitor include combining inhibitor potency with good oral bioavailability and low cross resistance to other HIVPR inhibitors. The recent clinical failure of SC52151, which was ultimately linked to a high level of binding by the plasma protein alpha-1-acid glycoprotein, has heightened the awareness of the fact that it is the 'free' or unbound drug that enters cells and interacts with the intracellular protease dimer target[100,101]. Together, these requirements suggest that a durable, efficacious HIV protease inhibitor will be one that can maintain plasma levels that meet or exceed the concentrations of free drug necessary to inhibit both wild-type and mutant viruses. Pharmacokinetic properties of the three approved drugs, along with two phase II clinical compounds are shown in Table 5. Except for Indinavir, the extent of human plasma protein binding of current HIVPR inhibitors is large (>90%), so that the effective concentration of 'free' drug will be 10–30 times lower for the different inhibitors.

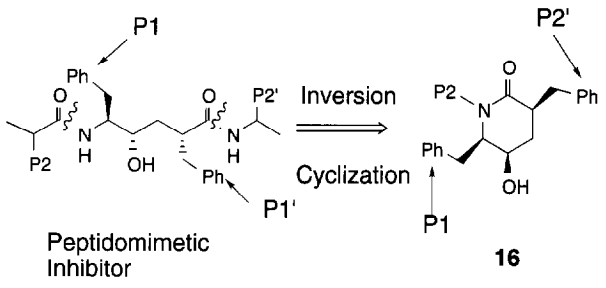
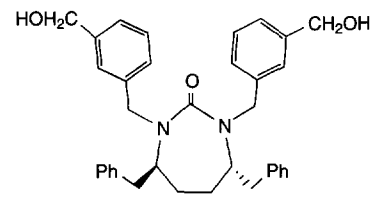


Figure 8. *The P1′ substituent of linear HIVPR inhibitors corresponds to the P2′ substituent in the cyclic inhibitors.*

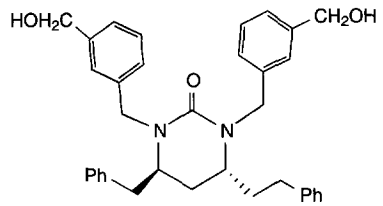
The measurement of oral bioavailability of candidate inhibitors has thus become a crucial hurdle in the development of new series of compounds. The factors that limit oral bioavailability of peptide-like inhibitors include poor absorption, high first-pass metabolism by the liver and gastrointestinal degradation of drug substance. These parameters, in turn, are influenced by the physicochemical properties of

the compounds, including molecular weight, nature of substituents, solubility and log P. Part of the design strategy of the cyclic ureas was that a non-peptidic, low-molecular-weight scaffold that could optimally direct substituents into the corresponding enzyme subsites would obviate the need to occupy all of the subsites to yield adequate potency. In general, fewer substituents and lack of peptide bonds would translate to lower total molecular weight and enhanced stability, which might improve oral bioavailability.

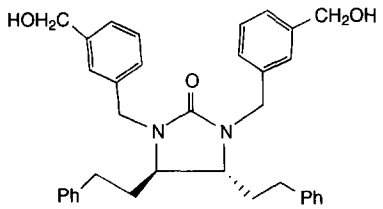
As a class, the cyclic ureas are significantly bioavailable[45–47,61,102]. Table 6 summarizes the pharmacokinetic parameters in various species for a number of substituted benzylic cyclic ureas. Indeed, the favorable pharmacokinetics in the rat and dog led to the identification of a first cyclic urea clinical candidate (DMP323)[46] shortly after its initial design and synthesis. Unfortunately, poor solubility (7 μg/ml at pH 7), first-pass metabolism and limited formulation possibilities resulted in poor performance in Phase I studies in seronegative volunteers.



K_i =170 nM



K_i = 99 nM



K_i = 55 nM

Figure 9. *Five-, six- and seven-membered ring cyclic ureas show comparable affinities, suggesting a similar mode of interaction with the S1/S2 sites of the HIVPR enzyme.*

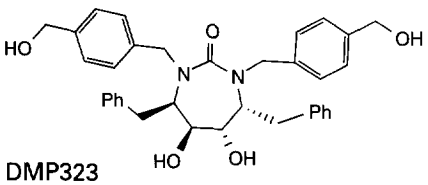
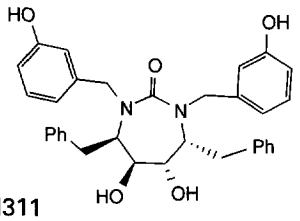
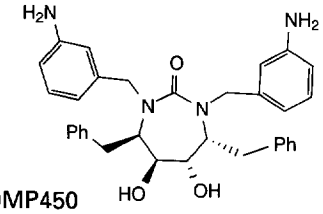
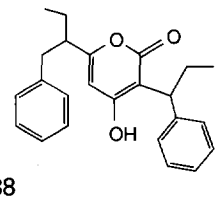
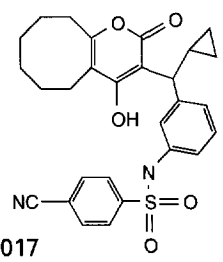
Improvement of physical properties and pharmacokinetics without loss of potency towards HIV led to the design and synthesis of weakly basic cyclic urea analogs. DMP450, the bis-*m*-aminobenzyl-substituted cyclic urea, combines antiviral potency (average concentration required to inhibit laboratory strains and patient isolates of HIV-1 = 144 nM)[61] with superior physical properties and was chosen as a second clinical candidate. As shown in Figure 10, DMP450 yields plasma levels in man that are six times higher than those of DMP323 at peak, and 535 times higher at 8 hours. The effects of plasma protein binding on DMP450's antiviral potency are minimal, and it maintains significant antiviral activity against a variety of mutant viruses with single amino acid changes within the protease. Avid Parmaceutical Co. (Philadelphia, PA, USA) is currently in the process of licensing DMP450 to continue the clinical trials. Current efforts are aimed at identifying new non-symmetric cyclic-urea clinical candidates that will retain the desirable physical and pharmacokinetic properties of

Table 5. Pharmacokinetic properties of five HIV protease inhibitors in man

Inhibitor	Dosage	C_{max} (μM)	IC_{90} (μM)	Trough level (μM)	Reference
Indinavir	800 mg[a]	12.6	0.025–0.10[e]	0.25	23, 24, 106
Ritonavir	600 mg[b]	15.6	0.004–0.153[f]	4.2	25, 26, 28, 107
Saquinavir	600 mg[a]	0.079	0.009	<<0.079	27, 108
Viracept	750 mg[a]	Pending[d]	0.008–0.120[g]	Pending[d]	103, 104
Vx-478	1200 mg[b,c]	18.2[c]	0.012–0.019[h]	2.06[c]	101, 105
DMP450	750 mg (single)	6.49	0.144	0.3	61

[a] Administered three times daily.
[b] Administered twice daily.
[c] Single dose data. Multiple dose data have not been reported outside of oral presentations, but the long half life reported for single doses suggests significant accumulation at trough occurs.
[d] Pharmacokinetic parameters for multiple doses have not been reported outside of oral presentations. The latter have indicated that micromolar levels are present at trough with multiple dosing.
[e] Range of IC_{95} values for laboratory and clinical isolates.
[f] IC_{50} reported for a range of laboratory and clinical isolates.
[g] Range of IC_{90} values for laboratory and clinical isolates.
[h] Range of IC_{50} values for laboratory and clinical isolates.

Table 6. Pharmacokinetic properties of cyclic inhibitors

Inhibitor	K_i (nM)	IC_{90}	Species	C_{max} (μM)[a]	$T_{1/2}$ (h)	F%[d]
 DMP323	0.26	57 nM	Rat Dog Man	0.78 2.61 0.82	1.5 1.1 1	27 37 –
 XM311	0.10	54 nM	Rat Dog	0.86 1.98	3.3 1.1	30 16
 DMP450	0.22	125 nM	Rat Dog Man	2.25 11.19 6.49	1.3 3.6 4	71 79 –
 U96988	38	3 μM[b]	Rat[c] Dog	– >50	4 6	76 45
 U103017	0.8	1 μM[b]	Rat[c] Dog	24 156	3 6	42 77

[a] Peak plasma concentrations observed after oral gavage at 10 mg/kg in rats or dogs, or after single 750 mg oral doses in seronegative human volunteers.
[b] Concentration required to inhibit p24 production by 50% in acute HIV-1 infections.
[c] Oral gavage at 5 mg/kg in rats.
[d] Oral bioavailability (F%) was determined by the ratio AUC PO/AUC IV, where AUC is the area under the plasma concentration–time curve from time zero to infinity and is normalized for dose.

DMP450, while offering greater potency towards wild-type and mutant forms of HIV.

Several other cyclic inhibitors have recently entered clinical trials. The pseudo symmetric hydroxy-2-pyrone U96988 (Table 3, **20**) has been examined in the rat and the dog[85], where clearance was moderate and half life after IV dosing was several hours. Oral doses resulted in substantial plasma levels of compound, with levels at 6 hours exceeding the IC_{50} (Table 6). Phase I studies were undertaken, but further development was abandoned in favor of more potent

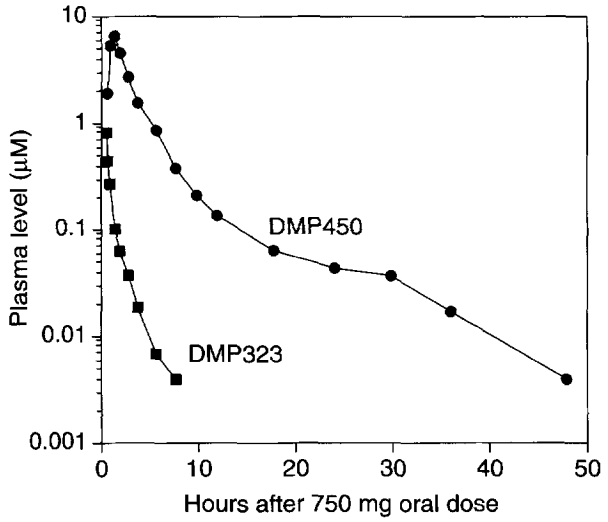


Figure 10. *Pharmacokinetics of DMP323 and DMP450 after oral dosing. HIV-1 seronegative human subjects were given DMP323 in a liquid formulation (750 mg) or DMP450 as a neat powder in capsules (750 mg). Blood samples were withdrawn at the indicated times, and quantities of compound determined on extracts of plasma using HPLC.*

analogs. Continued work in the pyrone template led to several series of more potent inhibitors including the cyclooctylpyranone sulfonamide[88]. As shown in Table 6, such derivatives are extremely bioavailable, with estimated levels at 8 hours far exceeding the level required for inhibition of wild-type HIV-1. One compound, U103017, was taken into Phase I trials in man[88]. Unfortunately, the clinical trial was terminated as a result of elevated liver enzyme activity. A superior third candidate, U140690, with an IC_{90} of 100 nM will be entering clinical trials at the beginning of 1997. The above reported animal pharmacokinetic studies, although promising, do not take into account the potential effects of plasma protein binding or loss of activity towards mutant variants of HIV.

Conclusions

The challenges facing the development of new HIV protease inhibitors that will provide improvements in overall efficacy compared with currently approved inhibitors are substantial. The design of new and improved inhibitors requires the simultaneous optimization of potency, efficacy against mutant proteases, propensity for plasma protein binding, physical properties, ease of synthesis and pharmacokinetics. Despite these sizeable challenges, the cyclic inhibitors provide one of the most promising approaches to identify second generation inhibitors, and will provide important clinical benefit.

The cyclic inhibitors displace a critical structural water molecule and are highly preorganized and complementary to the HIVPR active site. The resultant entropic and enthalpic gain has in many cases translated to nanomolar or subnanomolar potency towards the enzyme, and nanomolar potency towards HIV in cell culture. This optimization has allowed for such potency at a relatively lower molecular weight. Lower molecular weight is, in general, associated with better oral bioavailability.

The design of inhibitors with low plasma protein binding or increased binding to mutant forms of the protease remains enigmatic, although the abundance of crystal structures for wild-type and mutant protease–inhibitor complexes has begun to shed light on the most critical enzyme–inhibitor interactions. The need for more potent and more durable therapies for AIDS is well documented. Optimization for each of the parameters described here within the cyclic HIVPR inhibitors may provide the next generation of drug candidate(s) that can effectively answer that need. Many of the more potent series, for example cyclic ureas, cyclic thioureas, azacyclic ureas and cyclic sulfones await further optimization in order to yield drug candidates.

ACKNOWLEDGEMENT

The authors would like to acknowledge the DuPont Merck HIVPR team for their dedication and contribution in the discovery of the cyclic HIVPR inhibitors.

REFERENCES

1 For recent reviews see: Katz, R.A. and Skalka, A.M. (1994) *Annu. Rev. Biochem.* 63, 133–173
2 Kohl, N.E. *et al.* (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 4686–4690
3 Peng, C. *et al.* (1989) *J. Virol.* 63, 2550–2556
4 Wlodawer, A. and Erickson, J.W. (1993) *Annu. Rev. Biochem.* 62, 543–585
5 Appelt, K. (1993) *Perspect. Drug Des. Discovery* 1, 23–48
6 Ringe, D. (1994) *Methods Enzymol.* 241, 157–177
7 Wlodawer, A. *et al.* (1989) *Science* 245, 616
8 Erickson, J. *et al.* (1990) *Science* 249, 527–532
9 Swain, A.L. *et al.* (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 8805–8809
10 De Clercq, E. (1995) *J. Med. Chem.* 38, 2491–2517
11 Boehme, R.E. *et al.* (1995) *Annu. Rep. Med. Chem.* 30, 139–149
12 Chong, K.T. (1996) *Expert Opin. Invest. Drugs* 5, 115–124
13 Babine, R.E. *et al.* (1992) *Bioorg. Med. Chem. Lett.* 2, 541–546

14 Ikeda, S. *et al.* (1992) *J. Am. Chem. Soc.* 114, 7604–7606
15 Rich, D.H. *et al.* (1992) *J. Med. Chem.* 35, 3803–3812
16 Yarchoan, R., Mitsuya, H. and Broder, S. (1993) *Trends Pharmcol. Sci.* 14, 196–202
17 Ghosh, A.K. *et al.* (1993) *J. Med. Chem.* 36, 292–294
18 Getman, D.P. *et al.* (1993) *J. Med. Chem.* 36, 288–291
19 Thompson, W.J. *et al.* (1993) *J. Am. Chem. Soc.* 115, 801–803
20 Holmes, D.S. *et al.* (1993) *Bioorg. Med. Chem. Lett.* 3, 503
21 Vella, S. (1994) *AIDS* 8, Suppl. 3, S25–29
22 Pollard, R.B. (1994) *Pharmacotherapy* 14, 21S–29S
23 Vacca, J.P. *et al.* (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 4096–4100
24 Wei, X. *et al.* (1995) *Nature* 373, 117–122
25 Kempf, D. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 2484–2488
26 Ho, D.D. *et al.* (1995) *Nature* 373, 123–126
27 Kitchen, V.S. *et al.* (1995) *Lancet* 345, 952–955
28 Danner, S.A. *et al.* (1995) *New Engl. J. Med.* 333, 1528–2533
29 Kim, E.E. *et al.* (1995) *J. Am. Chem. Soc.* 117, 1181–1182
30 Jacobsen, H. *et al.* (1995) *Virology* 206, 527–534
31 Markowitz, M.M. *et al.* (1995) *J. Virol.* 69, 701–706
32 Condra, J.H. *et al.* (1995) *Nature* 374, 569–571
33 Ridky, T. and Leis, J. (1995) *J. Biol. Chem.* 270, 29621–29623
34 Perelson, A.S. *et al.* (1996) *Science* 271, 1582–1586
35 Kempf, D. (1994) *Methods Enzymol.* 241, 334–354
36 Vacca, J.P. (1994) *Methods Enzymol.* 241, 311–334
37 Wlodawer, A. (1994) *Pharmacotherapy* 14, 9S–20S
38 Erickson, J. and Kempf, D. (1994) *Arch. Virol.* Suppl. 9, 19–29
39 Drake, P.L. and Huff, J.R. (1995) *Adv. Pharmacol.* 25, 399–454
40 Romines, K.R. and Chrusciel, R.A. (1995) *Curr. Med. Chem.* 2, 825–838
41 Kuntz, I.D. *et al.* (1982) *J. Mol. Biol.* 161, 269–276
42 DesJarlais, R.L. *et al.* (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 6644–6654
43 VanDrie, J.H. (1989) *J. Comput.-Aided Mol. Design* 3, 225
44 Bures, M.G. *et al.* (1990) *Tetrahedron Comput. Methodol.* 3, 673–680
45 Lam, P.Y.S. *et al.* (1994) *Science* 263, 380–384
46 Lam, P.Y.S. *et al.* (1996) *J. Med. Chem.* 39, 3514–3525
47 Nugiel, D.A. *et al.* (1996) *J. Med. Chem.* 39, 2156–2169
48 Jadhav, P.K. and Woerner, F.J. (1992) *Bioorg. Med. Chem. Lett.* 2, 353–356
49 Kempf, D.J. *et al.* (1992) *J. Org. Chem.* 57, 5692–5700
50 Kempf, D.J. *et al.* (1993) *J. Med. Chem.* 36, 320–330
51 Jadhav, P.K. *et al.* (1994) US Patent 5,294,720
52 Ripka, W.C. and Eyermann, C.J. (1993) in *Proceedings of the 2nd International Conference on Chemical Information* (W. Warr, ed.), pp 303–314, Springer-Verlag
53 Martin, Y.C. (1992) *J. Med. Chem.* 35, 2145–2154
54 Allen, F.H. *et al.* (1991) *J. Chem. Inf. Comput. Sci.* 31, 187
55 Weber, P.C. *et al.* (1989) *Science* 243, 85–88
56 Cram, D.J. and Lam, P.Y.S. (1986) *Tetrahedron* 42, 1607–1615
57 Cram, D.J. *et al.* (1984) *J. Am. Chem. Soc.* 106, 7150–7167
58 March, J. (1985) *Advanced Organic Chemistry* (3rd edn), Wiley, p. 125
59 Johnson, F. (1968) *Chem. Rev.* 68, 375–413
60 Hoffmann, R.W. (1992) *Angew. Chem. Int. Ed.* 31, 1124–1134
61 Hodge, C.N. *et al.* (1996) *Chemistry & Biology* 3, 301–314
62 Grzesiek, S. *et al.* (1994) *J. Am. Chem. Soc.* 116, 1581–1582
63 Yamazaki, T. *et al.* (1994) *J. Am. Chem. Soc.* 116, 10791–10792
64 Nicholson, L.K. *et al.* (1995) *Struct. Biol.* 2, 274–280
65 Yamazaki, T. *et al.* (1996) *Protein Sci.* 5, 495–506
66 Cram, D.J. (1986) *Angew. Chem. Int. Ed.* 25, 1039–1057
67 Cram, D.J. (1988) *Science* 240, 760–767
68 Timasheff, S.N. (1992) *Biochemistry* 31, 9857
69 Dunitz, J.D. (1994) *Science* 264, 670
70 Jadhav, P.K. (1995) Society of Chemical Industry Conference on 'Chemical Lead Generation in Drug Discovery', 25–27 June, Cambridge, UK
71 Lam, P.Y.S. and Jadhav, P.K. *et al.* (1994) PCT Patent Application WO 9419329
72 Jadhav, P.K. *et al.* (1993) 205th ACS National Meeting, 28 March–2 April, Division of Med. Chem., 107 (Abstr.)
73 Smallheer, J.M. and Seitz, S.P. (1995) 209th ACS National Meeting, 2–9 April, Anaheim, CA, USA, Division of Med. Chem., 110 (Abstr.)
74 Smallheer, J.M. and Seitz, S.P. *Heterocycles*, in press
75 Hogde, C.N. *et al.* (1994) PCT Patent Application WO 9422840
76 De Lucca, G.V. *et al.* (1995) 209th ACS National Meeting, 2–9 April, Anaheim, CA, USA, Division of Med. Chem., 004 (Abstr.)
77 De Lucca, G.V. *et al.* (1995) International Chemical Congress of Pacific Basin Societies, 17–22 December, Honolulu, HI, USA, Org. Chem., 631 (Abstr.)
78 De Lucca, G.V. *et al.* (1995) 209th ACS National Meeting, 2–9 April, Anaheim, CA, USA, Division of Org. Chem., 514 (Abstr.)
79 Randad, R.S. *et al.* (1994) *Bioorg. Med. Chem. Lett.* 4, 1247–1252
80 Jadhav, P.K. and Man, H-W. (1996) *Tetrahedron Lett.* 37, 1153–1156
81 Sham, H.L. *et al.* (1996) *J. Med. Chem.* 39, 392–397
82 Bold, G. *et al.* (1995) PCT Patent Application WO 9502582-A
83 Peyman, A. *et al.* (1994) *Bioorg. Med. Chem. Lett.* 4, 2601–2604
84 Nugiel, D.A. and Reese, K. (1995) *Drug Des. Discov.* 13, 82–86
85 Thaisrivongs, S. *et al.* (1994) *J. Med. Chem.* 37, 3200–3204
86 Romines, K.R. *et al.* (1995) *J. Med. Chem.* 38, 1884–1891
87 Thaisrivongs, S. *et al.* (1995) *J. Med. Chem.* 38, 3624–3637
88 Skulnick, H.I. *et al.* (1995) *J. Med. Chem.* 38, 4968–4971
89 For review see: Romines, K.R. and Chrusciel, R.A. (1995) *Curr. Med. Chem.* 2, 825–838
90 Lunney, E.A. *et al.* (1994) *J. Med. Chem.* 37, 2664–2677
91 Vara Prasad, J.V.N. *et al.* (1994) *J. Am. Chem. Soc.* 116, 6989–6990
92 Vara Prasad, J.V.N. *et al.* (1995) *J. Med. Chem.* 38, 898–905
93 Vara Prasad, J.V.N. *et al.* (1995) *J. Am. Chem. Soc.* 117, 11070–11074
94 Kim, C.U. *et al.* (1996) *J. Med. Chem.* 39, 3431–3434
95 Jadhav, P.K. and Woerner, F.J. (1995) *Tetrahedron Lett.* 36, 6383–6386
96 Chenera, B. *et al.* (1993) *Bioorg. Med. Chem. Lett.* 3, 2717–2722
97 Wild, H. *et al.* (1994) European patent 589322–A1
98 Wild, H., Hansen, J. and Lautz, J. (1993) 9th International Conference on AIDS, June 7–11, Berlin, Germany
99 Han, Q. *et al.* (1996) *Bioorg. Med. Chem. Lett.* 6, 1371–1374
100 Bryant, M. *et al.* (1995) *Antimicrob. Agents Chemother.* 38, 2229–2234
101 Livingston, D.J. *et al.* (1995) *J. Infect. Dis.* 172, 1238–1245
102 Wong, Y.N. *et al.* (1994) *Biopharm. Drug Dispos.* 15, 535–544
103 Reich, S.H. *et al.* (1995) *Proc. Natl. Acad. Sci. U. S. A.* 92, 3298–3302
104 Shetty, B. *et al.* (1996) *Antimicrob. Agents Chemother.* 40, 110–114
105 St Clair, M.H. *et al.* (1996) *Antiviral Res.* 29, 53–56
106 Package Insert for Indinavir
107 Package Insert for Ritonavir
108 Package Insert for Saquinavir