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# DE NOVO DESIGN OF NONPEPTIDIC HIV-1 PROTEASE INHIBITORS: INCORPORATION OF STRUCTURAL WATER.

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The design of inhibitors of HIV-1 protease (HIV PR) is an important therapeutic goal in the treatment of AIDS. Numerous classes of potent peptidic inhibitors of HIV PR have been designed using the natural cleavage site of the precursor polyproteins as a starting point [1]. $C_2$ symmetry based inhibitors have recently been designed to mimic the two-fold symmetry of the active site of the enzyme as determined by X-ray crystal structure analysis of the inhibitor/enzyme complex [2]. Recently, two novel computational approaches to the problem of designing non-peptidic HIV PR inhibitors have led to the discovery of haloperidol[3] and benzophenones[4]. These approaches employed procedures that search three dimensional databases for compounds that display surface complementarity to a potential binding pocket[3] or that possess a defined, three-dimensional substructure; i.e., atomic patterns related by geometric constraints[4]. The determination of numerous X-ray crystal structures of HIV PR/inhibitor complexes has made it possible to evaluate the detailed interactions that govern inhibitor binding to the target enzyme[5]. In this report we describe our initial effort in the *de novo* structure based design of HIV PR inhibitors. Our goal in this study was to design a $C_2$ symmetric template that contains a replacement for the buried water molecule found in the structure of HIV PR inhibitor complexes.

Our design of a non-peptidic inhibitor began with an analysis of the key hydrogen bond interactions observed in the crystal structure of the $C_2$ symmetric inhibitor, A-74704, bound to HIV PR[6]. We focused on several sets of interactions that appeared to be necessary for strong binding (**Fig. 1**). The central hydroxyl group of the inhibitor, which interacts with the carboxylate side chains of the Asp25 and Asp125 in the catalytic site of the enzyme, is common to most potent inhibitors of HIV PR reported so far. A buried water molecule, $H_2O401$, stabilizes the interaction of inhibitor with the "flaps" of HIV PR through a bridging network of hydrogen bonds between the carbonyl groups of the inhibitor and the amide hydrogens of Ile50 and Ile150 of the flap. This water molecule has been observed in all crystal structures of HIV PR/inhibitor complexes and is unique to the retroviral proteases. Replacement of this water by an appropriate functional group(s) on the inhibitor has been hypothesized to lead to a favorable entropic effect for inhibitor binding[6]. SAR studies show that $P_1/P_1'$ benzyl groups provide favorable contacts with the $S_1/S_1'$ subsites[4]. The interactions between amide hydrogens of the inhibitors and the carbonyl oxygens of Gly27 and Gly127 have distorted geometries with lengths varying between 3.1 to 3.8 Å, and thus, were considered less significant in the present design strategy.

In all the crystal structures of HIV PR/Inhibitor complexes the central OH group and $H_2O401$ are positioned *anti* to one another (similar to hydroquinone) and are 5.6Å apart, suggesting that a cyclic ring with *para* related oxygens could make desired interactions. We realized that cyclic ureas **1-3** would provide $C_2$ symmetric templates which could incorporate all three basic interacting elements described above. The strong

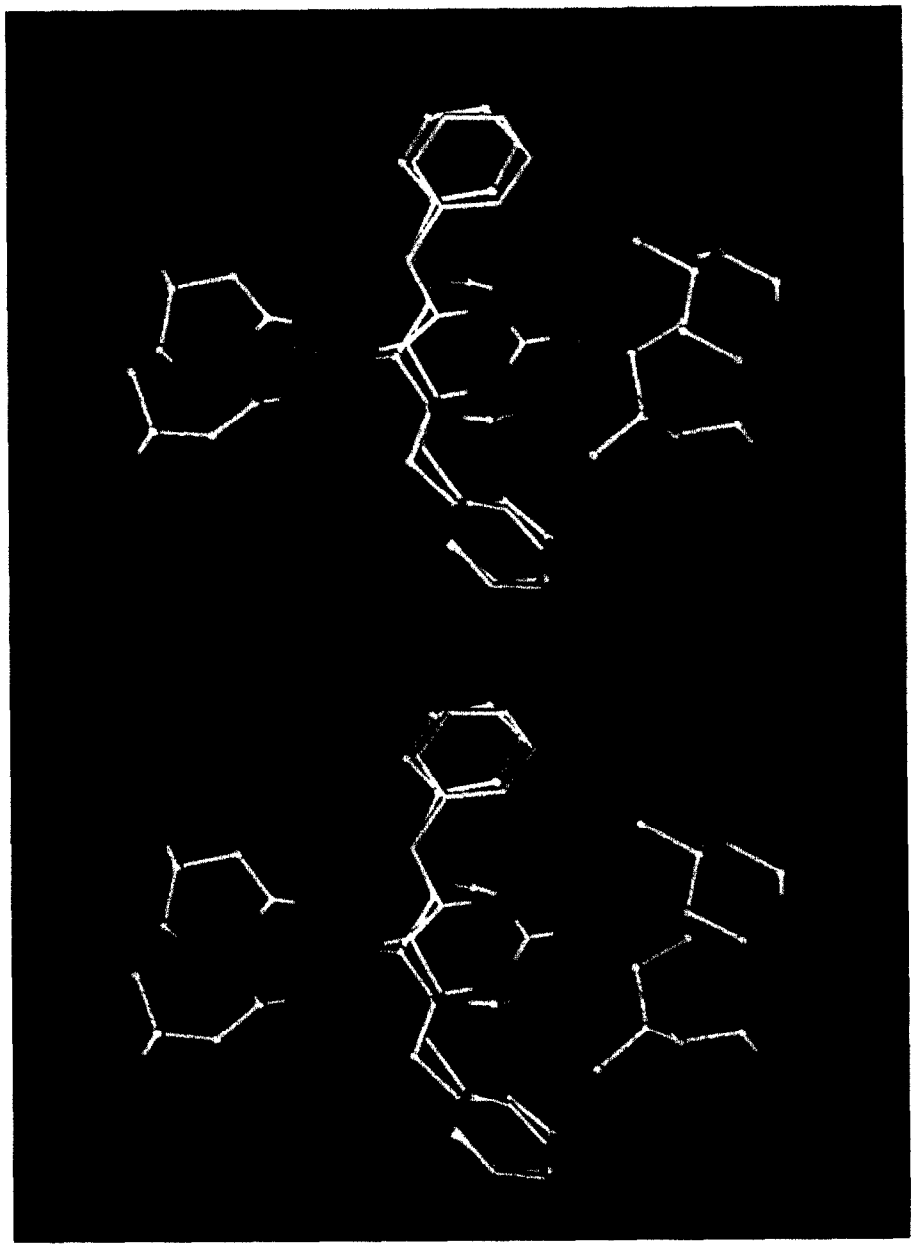
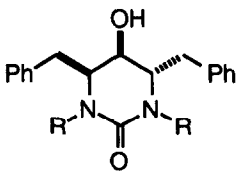


**Figure 1.** Stereo view of modeled six-membered cyclic urea **1** (capped stick) superposed onto the A-74704 core structure (ball-and-stick) in the HIV PR active site. Key hydrogen bonds between the core of A-74704 and the enzyme active site are shown as dashed lines; distances are in Å.

hydrogen bond-accepting urea C=O moiety[7] should replace the bridging water molecule found in the crystal structure of the complex. An OH group positioned opposite to the C=O moiety should interact favorably with the active site aspartates. Finally, we noted that C-substituted benzyl groups would overlap well with the $P_1/P_1'$ benzyl groups in A-74704.



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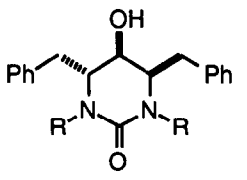

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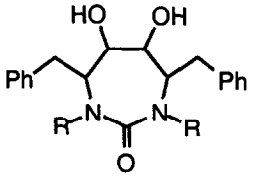

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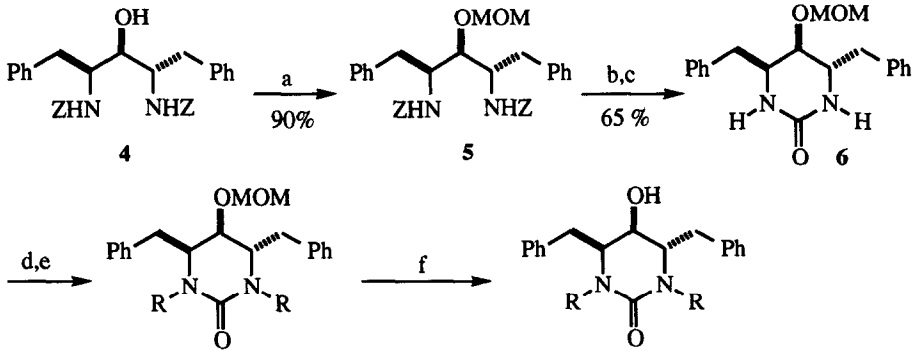
We initially modeled the six-membered (S,S) cyclic urea derivative **1**, by modifying the S,S-2,4-diamino-1,5-diphenyl-3-hydroxypentane core of A-74704 from the crystal structure of HIV PR/A-74704 complex[11]. Compound **1** was energy minimized using the program SYBYL after first tethering the side chain benzyl and –OH groups. Minimization produced the relaxed, half-chair conformer for the six-membered pyrimidone ring **1**. Next, **1** was docked into the HIV PR active site by superposing the hydroxy groups of A-74704 and **1**, the C=O oxygen of **1** with that of $H_2O401$, and the respective aromatic *p*-carbon atoms of the benzyl side chains of **1** and A-74704. The complete structure of compound **1** was then energy minimized while keeping the geometry of the protease fixed. The final minimized structure of **1** superposed onto A-74704, using the above four reference atoms, to within 0.57 Å RMS deviation. In the minimized structure, the -OH group is positioned to interact with the active site aspartates 25/125, the benzyl groups make hydrophobic contacts with the $S_1/S_1'$ subsites on the enzyme, and the urea C=O is within 0.35 Å of $H_2O401$ (**Fig. 1**).

To evaluate our *de novo* design and modeling efforts, we synthesized urea derivatives of general formula **1**, because of the ready availability of the starting material, S,S-2,4-diamino-1,5-diphenyl-3-

**Scheme 1**



**7a**, R = Bn (89%)
**7b**, R = β-naphthyl methyl (85%)
**7c**, R = allyl (80%)
**7d**, R = cyclopropyl methyl (60%)

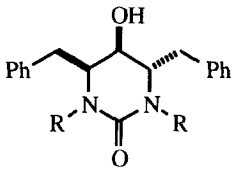
**1a**, R = Bn (94%)
**1b**, R = β-naphthyl methyl (92%)
**1c**, R = allyl (80%)
**1d**, R = cyclopropyl methyl (84%)
**1**, R = H (95% from **6**)

(a) NaH, MOMCl; (b) $H_2$, Pd/C; (c) $Im_2CO$; (d) NaH, DMF, 2h; (e) RBr; (f) TFA, $CH_2Cl_2$, 2-5 h.

hydroxypentane. The synthesis of the target compound is shown in **Scheme 1**. The N–Cbz alcohol **4**[7a] was reacted with MOMCl in the presence of sodium hydride to provide MOM ether **5** in 90% yield. Hydrogenation of **5** with catalytic Pd/C in methanol gave the diamine, which on reaction with 1,1'-carbonyldiimidazole in THF at concentration of 1 mg/mL, provided the 6-membered cyclic urea **6** in 65% overall yield from MOM ether **5**. Urea **6** was alkylated after reacting with sodium hydride in anhydrous DMF at room temperature for 2 hr, and then with alkyl bromide at room temperature for 12 hr. In the case of cyclopropyl methyl bromide, the reaction mixture was heated at 70°C for 12 h to provide **7d** in 60% yield. Finally, deprotection of the MOM group with TFA in $CH_2Cl_2$ at room temperature gave the N,N'-dialkyl SS-4,6-dibenzyl-5-hydroxy-2-pyrimidone derivatives **1a-d** in 80-95% yield. The structure of all new compounds were confirmed by $^1H$ NMR spectroscopy and mass spectral (FAB or/and HRMS) analysis.

The good agreement between the key pharmacophoric groups on compound **1** and A-74704 suggested that, despite its small size (MW=297), the former should possess some inhibitory activity. The inhibition of HIV PR by several six-membered cyclic urea inhibitors is shown in **Table 1**. Compound **1** showed weak inhibition ($IC_{50}$ >100 μM). Nitrogen substitution by alkyl or aryl -alkyl groups generally improved potency (compounds **1a-d**). Compound **1d** possessing cyclopropyl methyl groups was the most potent inhibitor with an $IC_{50}$ of 9 μM.

**Table 1.** Inhibition of HIV-1 Protease by Cyclic Urea Inhibitors[a]




| Compound | R | $IC_{50}$ (μM) |
|---|---|---|
| **1** | H | > 100 [b] |
| **1a** | $PhCH_2$- | 89 |
| **1b** | β-naphthyl-$CH_2$- | - [c] |
| **1c** | $CH_2$=$CHCH_2$- | 53 |
| **1d** | cycopropyl methyl- | 9 |

[a]Inhibition of HIV PR was measured using a flurogenic substrate under assay conditions described previously[12]. [b]35% inhibition at 100 μM. [c]6% inhibition at 10μM, insoluble at 50μM.

Comparison with the linear $C_2$ symmetry-based monols[8] indicates that unsubstituted compound **1** and the acetylated monol core [(2S,4S)-2,4-Bis-(((N-tertbutyloxy)carbonyl)amino)-1,5-diphenyl-3-hydroxy-pentane] exhibits weak inhibition. However, the N-substituted ureas (**1a-d**) were somewhat less potent than the $P_2/P_2'$ substituted monols; for example, the (2S,4S)-2,4-Bis-(N-valine)amino)-1,5-diphenyl-3-hydroxypentane[2] compound exhibited an $IC_{50}$ of 590 nM. In an effort to understand the weak inhibition of **1a-d** we performed comparative molecular modeling analysis on both SS and RR compounds, **1** and **2.** The enantiomeric derivative **2** (R,R) was obtained from **1** by inversion of chiral centers and was minimized and docked using similar procedures as for **1**. Examination of the docked structures of **1** and **2** in the enzyme active site suggested that both pyrimidone rings should bind in a low energy, half-chair conformation when R=H. In

this conformation, the N–substituents in **2** appear to be oriented towards the $S_2/S_2'$ subsite of the enzyme, whereas in the S,S-compound **1** they were twisted by 70° with respect to the R,R–isomer (**Fig. 2**). As a consequence, the ring conformation in N-substituted derivatives of **1** is predicted to be in a higher energy, twisted half-chair[9] conformation in order for these compounds to achieve extended contacts with both the $S_1/S_1'$ and $S_2/S_2'$ subsites, simultaneously. On the other hand, both the N–substituted and unsubstituted

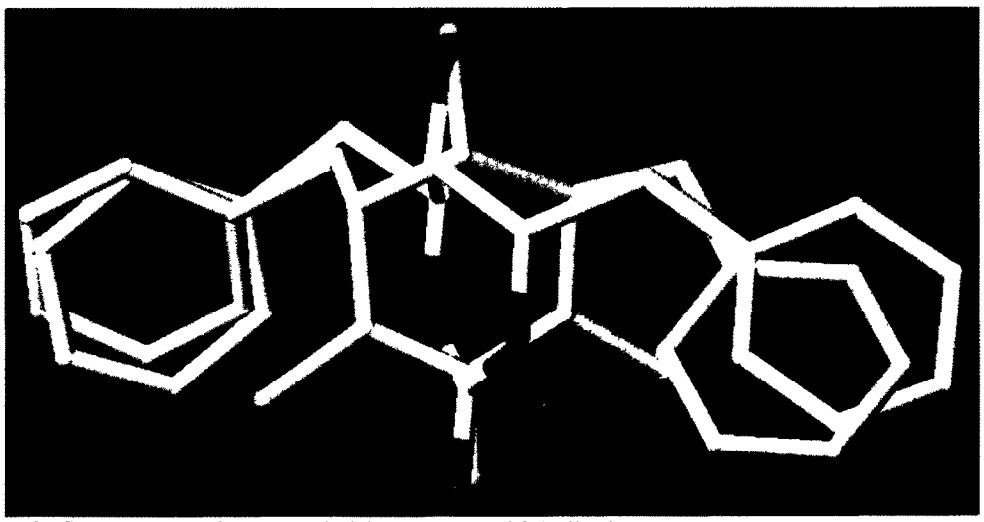


**Figure 2.** Superposition of compounds **1** (atom type) and **2** (yellow).

R,R–isomers should be able to bind in the lower energy half chair conformation. Therefore, it appears that the R,R six-membered cyclic urea **2** should bind more favorably with the enzyme. This conclusion was confirmed by colleagues at DuPont-Merck who independently designed and synthesized the R,R–isomer **2** and found that it possesses activity in the submicromolar range[10].

Our design confirms earlier proposals that the buried water may be replaced by a suitable hydrogen bond acceptor group and extends the paradigm of $C_2$ symmetric inhibitor design to cyclic templates. The importance of designing templates that can bind productively in low energy conformations is exemplified by the stereochemical dependence of inhibition of the synthesized cyclic ureas. Proof that the cyclic ureas bind in the proposed manner awaits crystallographic analysis. Recently, however, N-substituted derivatives of **3**, designed using three dimensional substructure search procedures, have been independently shown to inhibit HIV PR with nanomolar potency, and preliminary crystallographic analysis confirms the replacement of water by the urea carbonyl group[11].

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**Supplementary Material Available:** Experimental details and spectroscopic data for compounds reported here are available from the authors.

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