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DESIGN, SYNTHESIS, AND CONFORMATIONAL ANALYSIS OF A NOVEL SERIES OF HIV PROTEASE INHIBITORS

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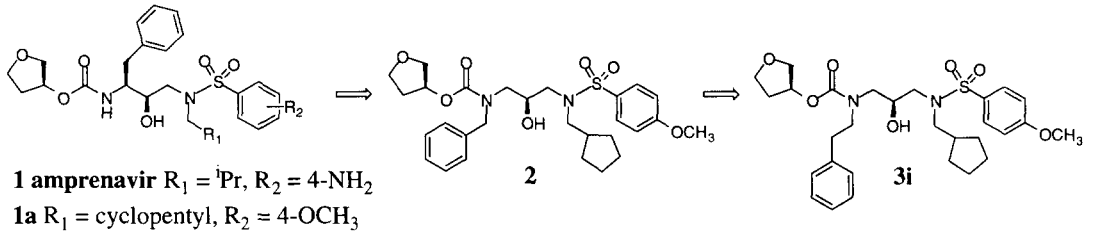
Abstract: A combination of structure-based design and both solution, and solid-phase synthesis were utilized to derive a potent (nM) series of HIV-1 protease inhibitors bearing a structurally novel backbone. Detailed structural analysis of several inhibitors prepared in this series has suggested that rigidification of the P_1/P_2 region of this class of molecules may result in compounds with improved potency. © 1998 Elsevier Science Ltd. All rights reserved.

Figure 1



1 amprenavir $R_1 = {}^{i}Pr$, $R_2 = 4\text{-}NH_2$
1a R_1 = cyclopentyl, $R_2 = 4\text{-}OCH_3$

2

3i

Introduction: The inhibition of the aspartyl protease encoded by human immunodeficiency virus type 1 (HIV-1) has undergone a great deal of scrutiny over the last ten years and has emerged as a promising therapeutic target in the treatment of HIV infection.[1] As a result of the research this past decade, several inhibitors of this enzyme have demonstrated in vivo efficacy. Four of these agents have recently been approved as marketed drugs and others are in late-stage clinical trials including amprenavir (**1**), which has resulted from our own structure based research efforts.[2] This new line of therapy, in combination with reverse-transcriptase inhibitors, has revolutionized the treatment of HIV infection and AIDS and offers new hope that HIV may be managed in the future as a chronic infection rather than a necessarily fatal one.[2] While these breakthroughs have generated a great deal of optimism, the need for new drugs against the protease continues to be of critical importance. The currently approved agents suffer from various drawbacks including dose-limiting and potentially long-term idiosyncratic toxicities, complex dosing regiments, often with dietary restrictions, in combination with multiple agents for effective viral suppression, and increasingly, the emergence of drug resistance.[3,4] While with **1** we hope to have addressed many of these issues,[2] the need for new, structurally diverse protease inhibitors remains. During the past several years we have been engaged in a research program aimed at the development of novel

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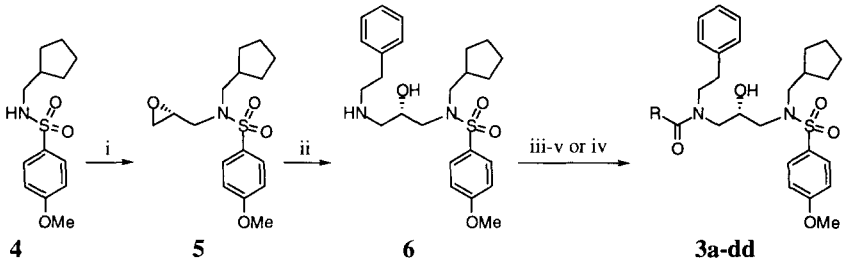
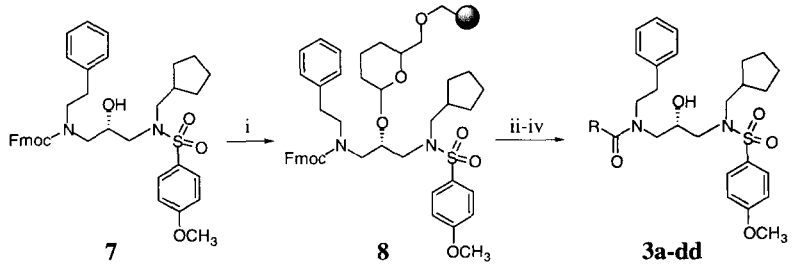
HIV-1 protease inhibitors, with the goal of enhancing even further the potency and overall usability profile of these agents. Early results in this program, partially driven by combinatorial chemistry approaches, have yielded a set of potent (nanomolar K_i) novel compounds as well as vital structural information on a set of lead compounds. Here we describe the synthesis, P2 SAR, and computational analysis of a subset of this series.



Scheme 1. (i) NaH, (*R*)-(–)-epichlorohydrin, DMF; (ii) Phenethylamine, EtOH; (iii) TMS-Cl, Et_3N, CH_2Cl_2; (iv) RC(O)X, Et_3N, CH_2Cl_2; (v) TBAF, THF.

Experimental: Compounds were synthesized via either a conventional solution phase method (Scheme 1) or a parallel solid-phase method (Scheme 2). In Scheme 1 the synthesis of key intermediate **6** was achieved in two steps from the readily available sulfonamide **4**, the P_1[5] moiety being set by the amine used to open epoxide **5** (step ii). Reaction of intermediate **6** with isocyanates, chloroformates, or activated carboxylic acids yielded the desired final products **3**. In some cases the hydroxyl of intermediate **6** was transiently protected with trimethylsilyl in order to avoid side reactions. To expedite the synthesis of a large number of derivatives, a combination of solution-phase and solid-phase techniques was utilized (Scheme 2). This approach resulted in the rapid synthesis of several dozen compounds of type **3**. In Scheme 2, intermediate **7**, readily available from compound **6**, was linked to a dihydropyran derivatized Merrifield resin through the central hydroxyl group.[6] Subsequent deprotection of the Fmoc group followed by coupling and deprotection from the resin yielded final products **3**. Representative examples of amide, carbamate and urea based compounds with P2 variations are shown in Table 1. The K_i values for HIV-1 protease inhibition were determined using an HPLC assay.[7]



Scheme 2. (i) DHP resin, PPTS, dichloroethane; (ii) piperidine; (iii) RC(O)X; (iv) TFA.

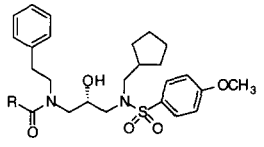
Results and Discussion: Transposition of the benzyl P_1 group in compound **1** (K_i < 0.1 nM) to the carbamate nitrogen resulted in compound **2** (K_i = 600 nM, Figure 1) which lost approximately three orders of magnitude in potency relative to parent compound **1**. Modeling analysis of this analog suggested that extending the P_1 side chain to a phenethyl group could result in the more active analog, thus compound **3i** was prepared and found to have substantially enhanced activity (K_i = 40 nM). This promising result on a structurally simple compound encouraged us to further explore a series of P2 analogs where the P1 group was fixed as phenethyl. It has been well established in the literature that P_2 (and/or P_2') variations in HIV-1 protease inhibitors have profound effects on the binding potency to the enzyme. For example, moieties such as the tetrahydrofuran (**3i**) bicyclic bistetrahydrofuran (**3l**), sulphones (**3n, 3p**), and indanol (**3dd**) have been incorporated into a variety of inhibitor backbone scaffolds resulting in low to subnanomolar inhibition by virtue of specific interactions established with residues which line the P_2 pocket of the protease.[8] It was our hope that establishing such interactions within this series would result in similar enhacements in binding potency.

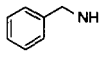
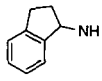
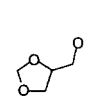
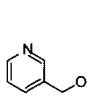
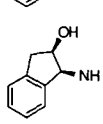
Inhibition constants on these compounds suggest that potent inhibitors can be obtained within this novel three carbon based backbone; however the P_2 SAR revealed for this series, as detailed in Table 1, is relatively flat, the best compounds remaining 10 to 100-fold less potent than amprenavir. This finding is in contrast to results observed in other series where significant increases in potency are observed when, for example, the P_2 moiety is elaborated from a Boc group to a more highly functionalized P_2 as described above. In comparison, the relative difference in potency between Boc compound **3q** and more highly functionalized P_2 compounds (i.e., **3i**, **3l**, and **3p**) is unfortunately small. In order to better understand the binding mode and the fundamental reasons for the relatively flat SAR observed in this series of compounds, we co-complexed and solved the bound crystal structures of several inhibitors with HIV-1 protease. The results of one study, utilizing compound **3t**, are illustrated in Figures 2 and 3.

The bound X-ray crystal structure of compound **3t** overlayed onto the bound conformation of amprenavir is shown in Figure 2. Comparative analysis with amprenavir suggests that important interactions with the catalytic aspartic acids, flap water, and P_2 through P_2' binding pockets are maintained, but only as a result of severe intramolecular distortions of the N-carbonyl bond and of the backbone conformation. Measurements of the torsion angles reveals that the N-C amide torsion angle is twisted from planarity by approximately 60° (132° vs 180° energy minimum for an amide bond). Although not readily apparent from the Figure 2, the backbone N-C-C-OH torsion angle, measured at 13°, is twisted from the calculated energy minimum and results in a high energy conformation in which the backbone nitrogen (bearing the phenethyl group) and the central hydroxyl group are eclipsed. In addition, the backbone distortion results in a nonplanar placement of the central hydroxyl group with the catalytic aspartic acids. This result, in part, may explain the relatively small difference in potency observed with *S* vs *R* hydroxyl groups in this series.[9] These observations have been consistent for several compounds co-crystallized with the enzyme (**3c**, **3g**, **3h**, and **3bb**), and have included compounds in the

amide and urea class as well as carbamates. We have postulated that, in total, these collective distortions translate into a 1 to 2 order of magnitude loss in binding to HIV-1 protease relative to Amprenavir (K_i < 1 nM). The high-energy conformation of **3t** is perhaps better illustrated from Figure 3, which overlays the bound conformation of **3t** (color coded) with its calculated energy minimum (blue). Analysis of this overlay reveals that a proper vector for interaction of the P_2 carbonyl with the enzyme flap water would not be established within an energy minimum conformation and that the P_1 moiety would occupy a position beyond the allowed region of this pocket. Interestingly, these studies have suggested that rigidification of the P_1/P_2 portion of these molecules could lead to compounds with significantly enhanced potency.[10]

Table 1



Compd	R	K_i (nM)	Compd	R	K_i (nM)	Compd	R	K_i (nM)
3a		20	**3k**		15	**3u**		200
3b*		29	**3l**		50	**3v**		38
3c*		27	**3m**		300	**3w**		3,400
3d*		220	**3n**		1400	**3x**		>10,000
3e*		680	**3o**		104	**3y**		2,100
3f		66	**3p**		38	**3z**		>20,000
3g*		76	**3q**		125	**3aa**		>10,000
3h	Cbz-Val-	7	**3r**		20	**3bb**		22
3i		40	**3s**		173	**3cc**		>10,000
3j		65	**3t**		17	**3dd**		43

* m-aminosulfonamide

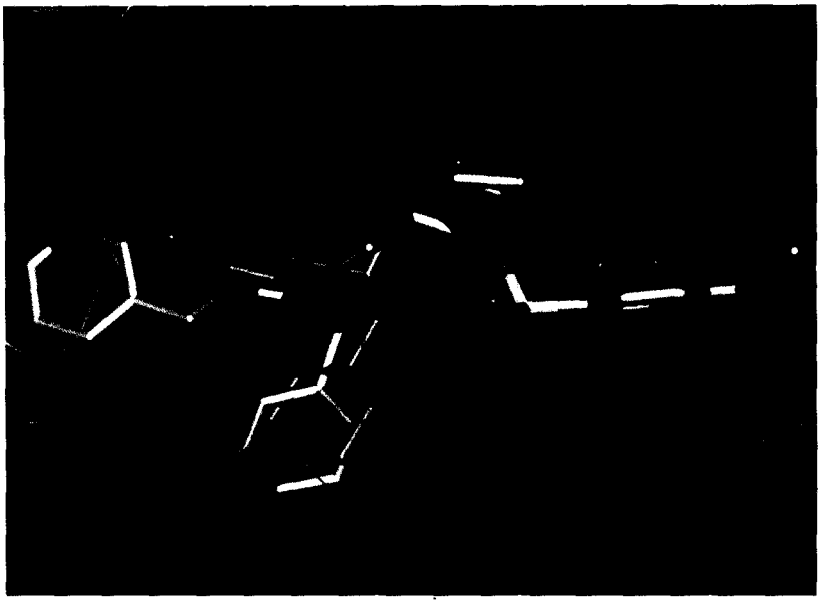


Figure 2. Overlay of the bound crystal structure of compound **1** (amprenavir, pink) with the bound structure of compound **3t** (color coded).

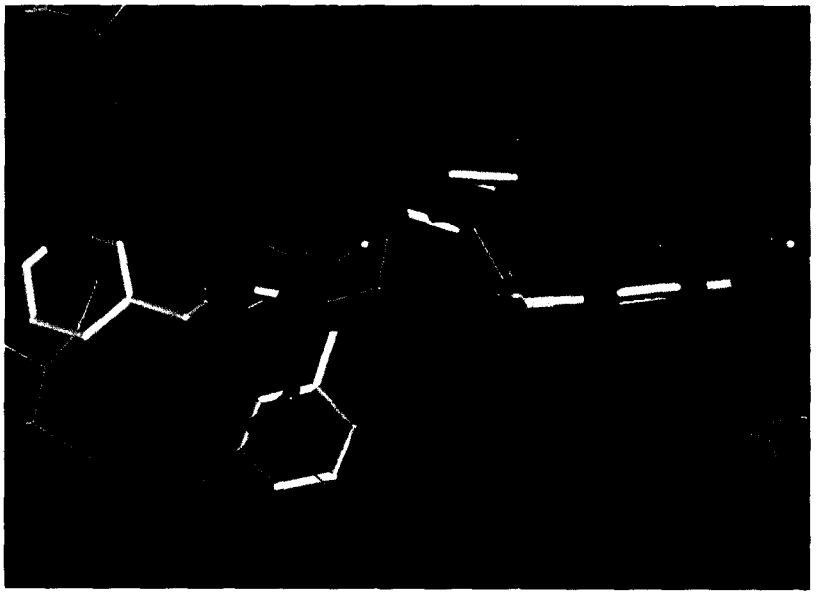


Figure 3. Overlay of the bound conformation of compound **3t** (color coded) with the calculated energy minimum structure of **3t** (blue).

Conclusion: A large number of P_2 amide, carbamate, and urea based compounds of type **3** have been prepared, several examples of which are shown in Table 1. Overall these compounds are one to two orders of magnitude weaker inhibitors than amprenavir. We have postulated that the intramolecular energy costs associated with optimum binding of these compounds to HIV-1 protease appears to have placed a formidable barrier to the realization of compounds with enzyme potency rivaling compound **1a** or amprenavir. This hypothesis is supported by the analysis of several solved co-crystal complexes; one example utilizing compound **3t** is illustrated above. Similar structures have been observed with amide and urea linked compounds. Finally, based on the structural data obtained, we have postulated that rigidification of these compounds could result in compounds with increased potency; the results of these studies are reported in the following paper.

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