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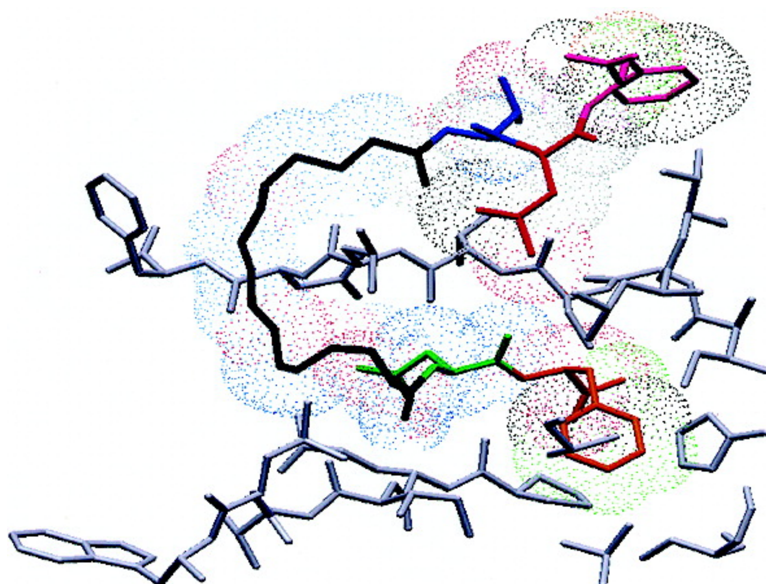

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Small-Molecule Dimerization Inhibitors of Wild-Type and Mutant HIV Protease: A Focused Library Approach

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The active site of HIV protease has been the object of close scrutiny for inhibitor design, resulting in many classes of potent inhibitors and six agents approved for clinical use.[1] Although potent therapeutics are available, their individual use has been limited due to the high mutation rate of HIV that leads to drug resistance.[2] Inhibitors of HIV that are impervious to mutations within the virus would be extremely powerful tools to fight viral infection. With this in mind we targeted the four-stranded, β-sheet dimerization interface of HIV-1 protease for inhibition,[3] as this region has been relatively free of mutations.[4]

Designed agents based on cross-linked interfacial peptides of protease had good potency against HIV-1 protease dimerization and activity, but high molecular complexity.[5] Efforts to identify the minimal structure necessary for activity led to an agent (**1**) that functioned as a dimerization inhibitor, but with reduced potency.[6] On the basis of a model of the complex of inhibitor **1** and a monomer of HIV-1 protease, we designed a focused library of agents in an effort to regain high potency. The results of these efforts and the activity of these compounds against drug-induced mutants of HIV-1 protease are disclosed herein.

A model of compound **1** bound in the dimerization interface of a folded HIV-1 protease monomer[7] indicates a number of structural features for inhibitor modification (Figure 1). Phe-1 (purple) of inhibitor **1**, for instance, lies on an extended hydrophobic surface and may be replaced with larger aromatic side chains, whereas Phe-5 (yellow) occupies a well-defined hydrophobic cavity that may not accept larger aromatics. With these considerations in mind and the placement of the other three side chains, we devised a focused library containing 68 single modifications to the parent compound **1** (Table 1). These compounds were synthesized in parallel using a solid-phase approach, purified to homogeneity by HPLC, and analyzed by mass spectrometry and amino acid analysis.

The activity of each library component against HIV-1 protease was initially screened at a single concentration (5.0 μM) using the fluorescent assay of Toth and Marshall.[8] From this assay 54 compounds of the library were chosen for further study. IC_{50} values were obtained, and the mechanism of inhibition was determined for the best inhibitors (25 compounds) using the kinetic method of Zhang and Poorman.[9] Of the library more than 50% of the compounds were more potent than the parent compound **1**, and a number of the modifications led to significantly enhanced efficacy against HIV-1 protease (Figure 2).

At each side-chain position within inhibitor **1** a range of interesting trends were discerned from the library data (Table 1). For instance, the Phe residue at position 1 was not successfully replaced with charged aromatics, such as His, but extended aromatics, such as biphenyl (Bip) or the biphenyl ether moiety of Thx were well accommodated, leading to approximately 10- to 40-fold increases in inhibitor potency. Although hydrophobic moieties

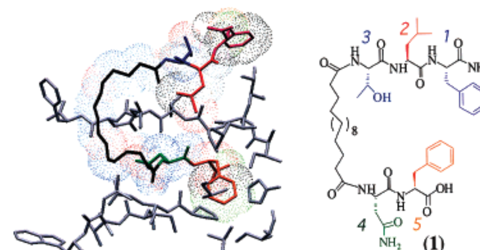

Figure 1. Model of compound **1** bound to the dimerization interface of an HIV-1 protease monomer.

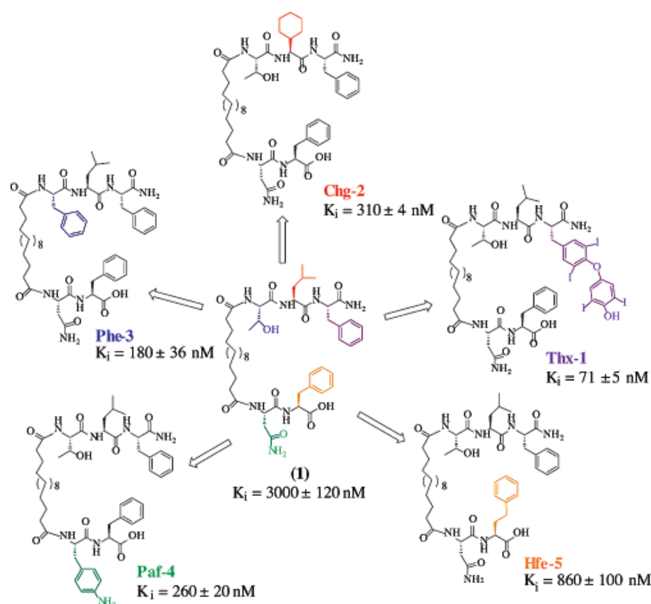

Figure 2. Results from focused library.

were found to predominate in many of the more potent inhibitors, the nature of the hydrophobic group was found to play a key role. At position 2, for instance, butyl side-chain geometry was found to be significant with the efficacy trend *sec*-butyl > isobutyl > *n*-butyl > *tert*-butyl. Amide side-chain functionality could be modified to nitro- or methylsulfone-moieties in position 4 (Asn) with 3- to 4-fold increases in potency, whereas a methylsulfoxide group led to a 2-fold decrease in inhibition. The Phe residue at position 5 was much less accommodating for larger aromatic groups as compared to position 1, but addition of a methylene unit (Hfe) or replacement of phenyl with cyclohexyl (Cha) led to approximately 3- to 4-fold increases in potency. These data are supported by modeling in which position 5 occupies a confined hydrophobic cavity.

This focused library was composed of single amino acid replacements, but one could envision an extensive library composed

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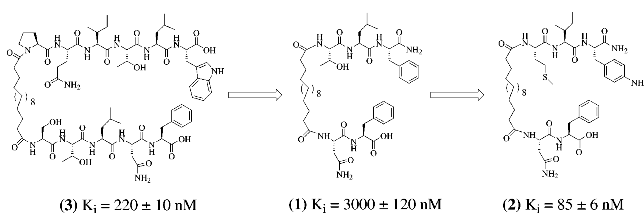
Table 1. Activity of Focused Library of Single Position Modifications (**1**−**5**) of Inhibitor **1**[a]

1		*2*		*3*		*4*		*5*	
Thx	71 nM	Chg	310 nM	Phe	180 nM	Phe	240 nM	Hfe	860 nM
Bip	228 nM	Ile	500 nM	Leu	370 nM	Paf	260 nM	Cha	1.1 μM
Bpa	495 nM	Cha	690 nM	Val	890 nM	ANO_2	830 nM	1-Nal	1.2 μM
Chg	1.1 μM	Hfe	820 nM	Met	1.6 μM	Leu	840 nM	Pbf	(1.2 μM)
Paf	1.4 μM	Val	1.3 μM	Paf	(1.0 μM)	$CmeO_2$	905 nM	Bip	(1.2 μM)
Hfe	2.2 μM	Phe	(4.6 μM)	$MetO_2$	(1.9 μM)	Val	930 nM	Bpa	(2.6 μM)
1-Nal	(3.1 μM)	Met	(5.0 μM)	CmeO	(2.0 μM	Met	(2.7 μM)	2-Nal	(5.2 μM)
Pbf	(3.4 μM)	Tyr	(7.2 μM)	Tyr	(2.7 μM)	Tyr	3.0 μM	Trp	(5.6 μM)
2-Nal	(3.6 μM)	Nle	(11 μM)	Pro	50%	$HANO_2$	(3.6 μM)	Leu	(12 μM)
Tpi	(6.1 μM)	Tbg	(13 μM)	Asn	30%	GlnO	(4.1 μM)	Tyr	(30 μM)
Tic	(32 μM)	Pro	15%	Gln	30%	AsnO	(4.4 μM)	Met	5%
His	(125 μM)	Asn	5%	Hyp	20%	$MetO_2$	(5.3 μM)		
Tyr	40%			Asp	20%	CmeO	(12 μM)		
Leu	25%					MetO	(13 μM)		
Pro	15%					Gln	35%		
Met	15%					Lys	35%		

[a] Each position is indicated with activity for the individual modification. Activity is reported as K_i, IC_{50} (in parentheses), and percent inhibition at 5.5 μM. Values with error bars are in Supporting Information. Nonstandard amino acids are the following: Thx/thyroxine, Bip/biphenylalanine, Chg/cyclohexylglycine, Paf/4-aminophenylalanine, Hfe/homophenylalanine, Nal/naphthylalanine, Pbf/4-bromophenylalanine, Tpi/tryptoline-3-carboxylic acid, Bpa/*p*-benzoylphenylalanine, Tic/1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, Cha/cyclohexylalanine, Nle/norleucine, Tbg/*tert*-butylglycine, Hyp/hydroxyproline, MetO/methioninesulfoxide, $MetO_2$/methioninesulfone, CmeO/methylcysteine sulfoxide, $CmeO_2$/methylcysteine sulfone, $HANO_2$/3-nitroalanine, ANO_2/4-nitro-2-aminobutyric acid, AsnO/*N*-hydroxyasparagine, GlnO/*N*-hydroxyglutamine.

of multiple modifications based on the best individual changes. In this way it may be possible to discover particularly potent agents. To test this hypothesis we prepared compound **2** containing three fairly subtle modifications within the northern tripeptide (F1Paf, L2I, T3M). Although these are not the most potent modifications, the changes to the side chains would be minimal. Individually these changes were found to provide modest 1.9- to 6-fold increases in potency, whereas their combination resulted in an agent that was 35-fold more potent at half the molecular weight than the starting inhibitor **1**, and 2.5-fold more potent than a cross-linked agent (**3**)



(3) K_i = 220 ± 10 nM **(1)** K_i = 3000 ± 120 nM **(2)** K_i = 85 ± 6 nM

containing the full length HIV protease interfacial peptides. Interestingly, this result demonstrated that the combined changes with inhibitor **2** resulted in an additive increase in binding energy as compared to the individual modifications. An extensive library containing multiple mutations will demonstrate if this additivity trend holds true for all positions.

An important consideration with dimerization inhibitors of HIV-1 protease is what potency these agents would have against mutant proteases derived from drug resistant strains of the virus. To evaluate this we tested five dimerization inhibitors with a range of potency from this study (Thx1, Chg1, Bip1, Ile2, and Phe3) on a recombinant HIV-1 protease containing multiple protease inhibitor resistant mutations (L10I, K45R, I54V, L63P, A71V, V82T, L90M, I93L). This set of resistant mutations was derived from protease inhibitor resistant HIV-1 obtained from a patient who had been heavily treated with active-site inhibitors, including indinavir, ritonavir, saquinavir, and amprenavir.[10] In each case, the inhibitors had equipotent activity with wild-type and mutant proteases, with the exception of Phe3 which was 80% as active with the mutant protease as compared to the wild type.

In conclusion, we have demonstrated that a limited focused library produced effective dimerization inhibitors of HIV-1 protease. By combining individual changes of the library into a single compound, we obtained a significantly more potent agent and found that an additive increase in inhibitor efficacy was obtained. The good activity of library members against an active-site drug-resistant protease mutant bodes well for dimerization inhibition as a complementary method to targeting the active site.

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Supporting Information Available: Inhibition values with error bars, amino acid analysis, and mass spectrometry. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Ren, S.; Lien, E. J. *Antiviral Agents* **2001**, 1−34. (b) Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249−284.
(2) (a) Erickson, J. W. *Infect. Dis. Ther.* **2002**, *25*, 1−25. (b) Little, S. *New Engl. J. Med.* **2002**, *347*, 385−394. (c) Flexner, C. *New Engl. J. Med.* **1998**, *338*, 1281−1292.
(3) (a) Berg, T. *Angew. Chem., Int. Ed*. **2003**, *42*, 2462−2481. (b) Bowman, M.; Chmielewski, J. *Biopolym.: Pept. Sci.* **2002**, *66*, 126−133. (c) Toogood, P. *J. Med. Chem.* **2002**, *45*, 1543−1558.
(4) (a) Gustchina, A.; Weber, I. T. *Proteins: Struct., Funct., Genet.* **1991**, *10*, 325−339. (b) Miller, V. *JAIDS, J. Acquired Immune Defic. Syndr.* **2001**, *26*, S34−S50.
(5) Zutshi, R.; Franciskovich, J.; Shultz, M.; Schweitzer, B.; Bishop, P.; Wilson, M.; Chmielewski, J. *J. Am. Chem. Soc.* **1997**, *119*, 4841−4845.
(6) Shultz, M. D.; Bowman, M. J.; Ham, Y.-W.; Zhao, X.; Tora, G.; Chmielewski, J. *Angew. Chem., Int. Ed*. **2000**, *39*, 2710−2713.
(7) Ishima, R.; Torchia, D. A.; Lynch, S. M.; Gronenborn, A. M.; Louis, J. M. *J. Biol. Chem*. **2003**, *278*, 43311−43319.
(8) Toth, M. V.; Marshall, G. R. *Int. J. Pept. Protein Res.* **1990**, *36*, 544−550.
(9) Zhang, Z.; Poorman, R.; Maggiora, L.; Heinrikson, R.; Kezdy, F. *J. Biol. Chem.* **1991**, *266*, 15591−15594.
(10) Yoshimura, K.; Kato, R.; Yusa, K.; Kavlick, M. F.; Maroun, V.; Nguyen, A.; Mimoto, T.; Ueno, T.; Shintani, M.; Falloon, J.; Masur, H.; Hayashi, H.; Erickson, J.; Mitsuya, H. *Proc. Natl. Acad. Sci. U.S.A*. **1999**, *96*, 8675−8680.

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