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Structure Activity Studies on Pseudo-symmetrical HIV-1 Protease Inhibitors

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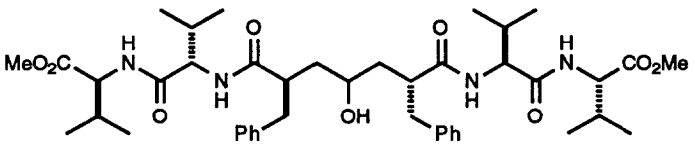
Abstract: Lead compound **1**, obtained from a previously reported structure-assisted design approach, was optimized to **17** using a traditional medicinal chemistry approach.

We have previously reported [1] on the structure-assisted design, synthesis and enzyme inhibiting properties of a pseudo-symmetrical [2] HIV-1 protease inhibitor **1**. We now wish to report on structure-activity relationships for this series of bis-dipeptide derived inhibitors, extension of this work to less peptidyl inhibitors, and the anti-viral properties of these compounds.



1

As shown in Scheme 1 our synthesis is quite flexible for evaluating structure activity relationships. Diacids **2** are prepared in three steps as described previously [1] from the appropriate chiral oxazolidinone [3]. Treatment of **2** with various amines (R_2NH_2) in the presence of amide coupling reagents afford bis-amides **3**. We used dipeptides in our early work for the preliminary evaluation of terminal groups. Bis-amides **3** were converted into alcohols **5** as described previously. Table 1 lists IC_{50} values as enzyme inhibitors [4a] for various dipeptide derived secondary alcohols. Of the derivatives surveyed, those with an isopropyl group in the P_2 position are most potent.

A late branch point in our synthesis was provided by the ease of manipulation of the exocyclic olefin. This allowed us to explore various options. Specifically, oxidation using either ozone or catalytic OsO_4 in the presence of sodium periodate afforded ketones **4**. Reduction of the ketone afforded alcohols **5**. Catalytic osmylation of **3** afforded diols **6**. Treatment of **3** with *m*-chloroperoxybenzoic acid afforded epoxides **7**. Table 2 lists IC_{50} values [4a] as enzyme inhibitors for these various derivatives. As expected [1], secondary alcohols are much more potent than the corresponding ketones, diols and epoxides in a direct comparison. We have not been able to obtain any evidence that epoxide **14** acts as an irreversible enzyme inhibitor.[5]

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Scheme 1

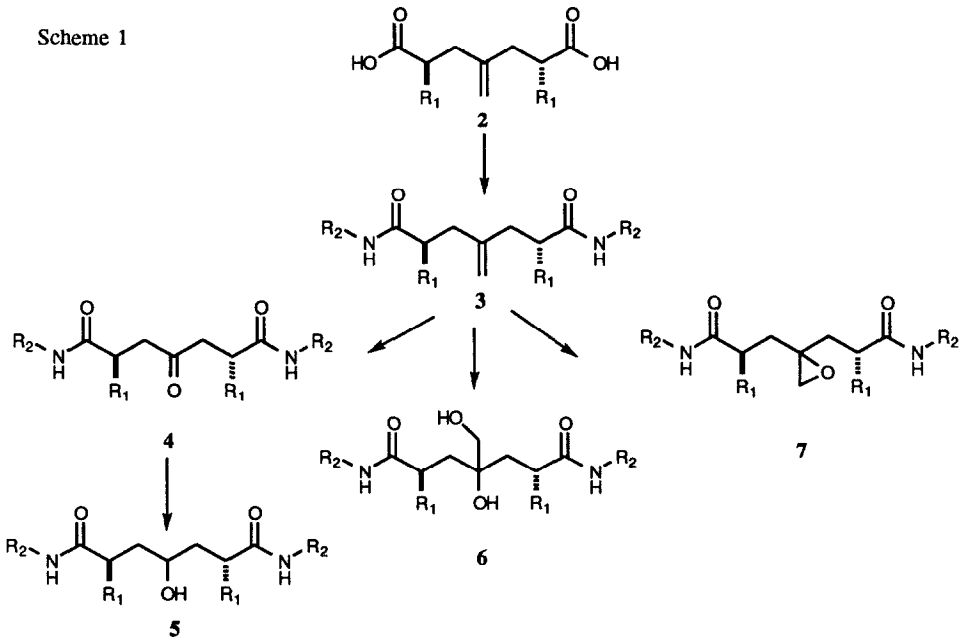
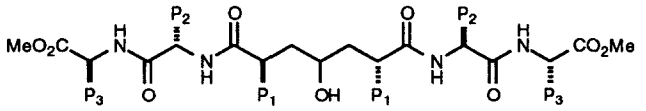
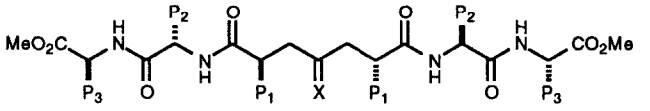


Table 1 Enzyme inhibiting properties of various dipeptide derived inhibitors of HIV-1 protease.



Compound	P_1	P_2	P_3	IC_{50} (nM)[4a]
8	*n*-Bu	Me	Me	75
9	*n*-Bu	*i*-Bu	Bn	90
10	*n*-Bu	*i*-Pr	*i*-Pr	5
11	Bn	*i*-Bu	Bn	30
1	Bn	*i*-Pr	*i*-Pr	5
12	Bn	*i*-Pr	Bn	10

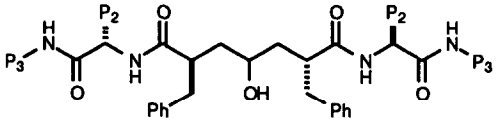
Table 2 Enzyme inhibiting properties of various dipeptide derived inhibitors of HIV-1 protease. The effect of central group.



Compound	P_1	P_2	P_3	X	IC_{50} (nM)[4a]
9	*n*-Bu	*i*-Bu	Bn	H,OH	90
13	*n*-Bu	*i*-Bu	Bn	OH,CH_2OH	2,000
14	*n*-Bu	*i*-Bu	Bn	-CH_2O-	400
15	Bn	*i*-Pr	*i*-Pr	O	>10,000

The results of Table 1 and 2 allowed us to define the central unit (4-hydroxy-2,6-bis-(phenylmethyl) heptanediamide) of a pseudo-symmetrical lead structure. At this point there were two divergent strategies for lead optimization: *de novo* design of non-peptidyl end groups [6] and traditional medicinal chemistry optimization of **1**. Using the data in Table 1 and 2 as well as structure-activity information which was reported in the literature [7] we prepared a variety of less peptidyl compounds. Several examples of these compounds are reported in Table 3. Of these compounds, **17** proved to be the most potent inhibitor in each of our enzyme assays.[4]

Table 3 Enzyme inhibiting properties of various monopeptide derived inhibitors of HIV-1 protease.



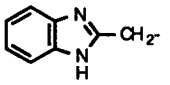
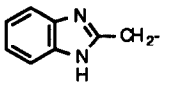
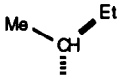
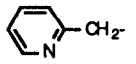
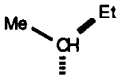
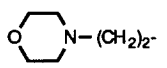
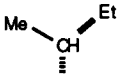
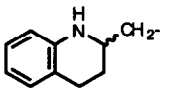
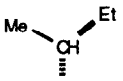
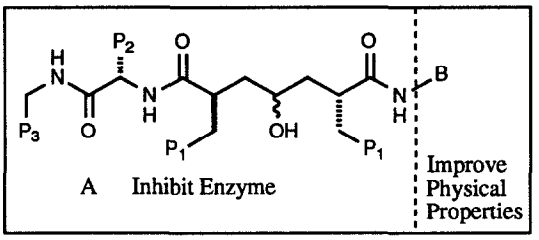
Compound	P_3	P_2	IC_{50} (nM)[4a]	IC_{50} (nM)[4b]	K_i (nM)[4c]
16		i-Pr	10		
17			3	40	3.5
18				450	
19				850	
20				1,200	

Table 4 Ability of compounds to inhibit growth of HIV-1 IIIB

Compound	IC_{50}(μM)	Compound	IC_{50}(μM)	Compound	IC_{50}(μM)
1	1.6	**16**	2.1	**19**	>10
11	2.3	**17**	0.4	**20**	7.5
12	2.4	**18**	0.5	**Ro 31-8959**[4b]	0.003

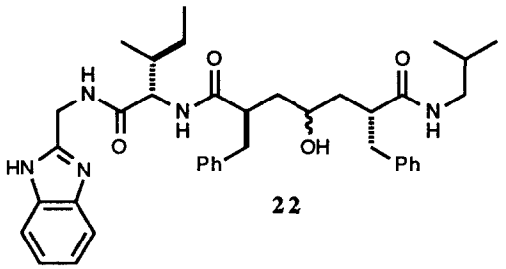
Many of the compounds reported in Tables 1 and 3 were evaluated for their ability to act as antiviral agents in cell culture.[8] This data is reported in Table 4. Of interest is the lack of a direct correlation between enzyme inhibition (relative potency: **17** > **16** > **18** > **19** > **20**) and antiviral activity (relative potency: **17** > **18** > **16** > **20** > **19**). The compounds reported in Table 4 have rather high molecular weights, poor solubility properties in all solvents examined, and a poor ratio of antiviral activity to enzyme inhibiting activity. This information led us to speculate that the relatively modest antiviral activity of these compounds was due to low cellular bioavailability as a consequence of their poor physical properties. In addition, compound **17** shows no oral bioavailability in mice.[9]

In an attempt to change the physical properties of **17**, unsymmetrical compounds of the general structure **21** were prepared. In **21** "A" represents a group to impart potent enzyme inhibiting properties while "B" represents a group to change physical properties. While these unsymmetrical compounds are expected to be less potent enzyme inhibitors due to lack of P_3 groups, it was hoped that improved physical properties might allow these compounds to have comparable or enhanced antiviral activities.

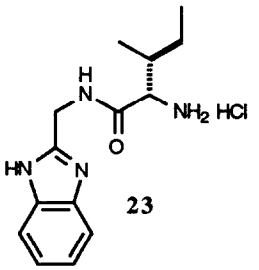


21

In a striking example, compound **22** was prepared and in direct comparison to **17** found to be ca. 8 times less potent (IC_{50} = 300 nM)[4b] as an enzyme inhibitor, have significantly improved solubility in several solvents (solubility of **17**: 0.02 mg/ml in $CHCl_3$, < 0.002 mg/ml in H_2O; solubility of **22**: 0.5 mg/ml in $CHCl_3$, 0.02mg/ml in H_2O) and be equipotent (IC_{50} = 0.55 μM) as an antiviral agent.



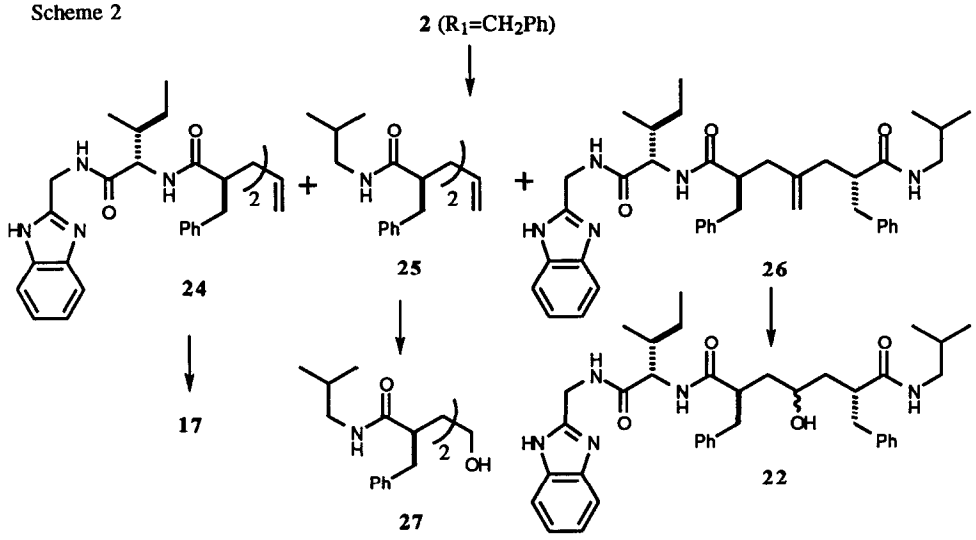
22



23

The synthesis of **22** is described in Scheme 2. Diacid **2** (R_1= CH_2Ph) was teated successively with 1.0 mol-equiv. of iso-butyl amine in the presence of the BOP reagent [10], followed by treatment with an excess of **23** in the presence of BOP. This generated a statistical mixture of symmetrical products **24** and **25** and unsymmetrical product **26**. The mixture was separated and **26** was converted in two steps (1. O_3 ; 2. $NaBH_4$) into **22** (as an inseparable, 1:1 mixture of diastereomers). Symmetrical olefin **25** had been previously converted into alcohol **27** which was shown to be a weak inhibitor (IC_{50} > 1000 nM)[4a] of HIV-1 protease. Other low molecular weight (< 500) symmetrical alcohols, lacking terminal P_3 groups and connecting amides, also proved to be weak inhibitors of the enzyme.

Scheme 2



The potent antiviral activity of unsymmetrical compound **22** is consistent with the hypothesis that poor physical properties, of high (> 700) molecular weight symmetrical compounds, manifests itself in less than optimal antiviral activity. This result gives credence to a strategy to prepare compounds of general structure **21**, and related strategies, where one part of the molecule is designed to inhibit the enzyme while other part is designed to improve physical properties. Studies which address this strategy will be reported in the future.[11]

References and Notes

† Department of Medicine, Hematology/Oncology Research Laboratory, New England Deaconess Hospital, Boston, MA 02215

1. Babine, R. E.; Zhang, N.; Jurgens, A. R.; Schow, S. R.; Desai, P. R.; James, J. C.; Semmelhack, M. F. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 541-6

2. (a) For references to symmetrical HIV-1 protease inhibitors see Ref 1 Footnotes 5b-5g. For more recent work see: (b) Askin, D.; Wallace, M. A.; Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1992**, *57*, 2771-3 (c) Jadhav, P. K.; Woerner, F. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 353-6 (d) Humber, D. C.; Cammack, N.; Coates, J. A. V.; Cobley, K. N.; Orr, D. C.; Storer, R.; Weingarten, G. G.; Weir, M. P. *J. Med. Chem.* **1992**, *35*, 3080-1

3. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737-9

4. We used two different enzyme assays in this work:

(a) Assay A. These enzyme inhibition studies were performed using HIV-1 protease obtained from Medigenics, Omaha, NE. using a modification of the procedure in Tomaselli , A. G.; Olsen, K. K.; Hui, J. O.; Staples, D. J.; Sawyer, T. K.; Heinrikson, R. L.; Tomich, C-S. C. *Biochem.* **1990**, *29*, 264-9. As a literature control A-74704 (Erickson, J.; Neidhart, D. J.; VanDrie, J.; Kempf, D. J.; Wang, X. C.; Norbeck, D. W.; Plattner, J. J.;

Rittenhouse, J. W.; Turon, M.; Wideburg, N.; Kohlbrenner, W. E.; Simmer, R.; Helfrinch, R.; Paul, D. A.; Knigge, M. *Science* **1990**, *249*, 527-33) was determined to have an IC_{50} = 25 nM and Ki = 10 nM (literature report of IC_{50} = 3 nM and K_i = 4.5 nM).

(b) Assay B. These enzyme inhibition studies were performed using recombinant HIV-1 protease prepared and purified by Drs. S. Plotch and E. Baum. The enzyme assay was an ^{125}I-SPA (scintillation proximity assay) using a kit provided by Amersham International plc, Bucks, England. Using the procedure described by the manufacturer, enzyme and test compounds were incubated with the bead suspension for 5 min., following termination of the reaction the radioactivity of the assay mixture was determined. Enzyme inhibition was determined as a function of enzyme concentration. As a literature control Ro 31-8959 (Roberts, N. A.; Martin, J. A.; Kinchington, D.; Broadhurst, A. V.; Craig, J. C.; Duncan, I. B.; Galpin, S. A.; Handa, B. K.; Kay, J.; Krohn, A.; Lambert, R. W.; Merrett, J. H.; Mills, J.S.; Parkes, K. E. B.; Redshaw, S.; Ritchie, A. J.; Taylor, D. L.; Thomas, G. L.; Machin, P. J. *Science* **1990**, *248*, 358-61) was determined to have an IC_{50} = 30 nM and Ki = 3 nM (literature report of IC_{50} < 0.4 nM).

(c) Apparent Ki values using assay B were determined by performing a Dixon plot at two different substrate concentrations. Substrate concentration was varied by using different amounts of beads.

5. For an example of an epoxide acting as an irreversible inhibitor of this enzyme, see: Kaldor S. W.; Schevitz, R. W.; Wery, P.; Clawson, D. K.; Burck, P. J.; Berg, D. H.; Kaiser, R. E.; Lai, M. T.; Lai, M. T.; Munroe, J. E.; Jones, N. D. Abstracts of Papers, 203rd ACS National Meeting, San Francisco, CA, April 1992, MEDI 178

6. Babine, R. E.; Zhang, N. et. al. , manuscript in preparation.

7. (a) Vacca, J. P.; Guare, J. P.; DeSolms, S. J.; Sanders, W. M.; Giuliani, E. A.; Young, S. D.; Darke, P. L.; Sigal, I. S.; Schleif, W. A.; Quintero, J. C.; Emini, E. A.; Anderson, P. S.; Huff, J. R. *J. Med. Chem.* **1991**, *34*, 1225-8 (b) Thaisrivongs, S.; Tomasselli, A. G.; Moon, J. B.; Hui, J.; McQuade, T. J.; Turner, S. R.; Strohbach, J. W.; Howe, W. J.; Tarpley, W. G.; Heinrikson, R. L. *J. Med. Chem.* **1991**, 34, 2344-56 (c) DeSolms, S. J.; Giuliani, E. A.; Guare, J. P.; Vacca, J. P.; Sanders, W. M.; Graham, S. L.; Wiggins, J. M. Darke, P. L.; Sigal, I. S.; Zugay, J. A.; Emini, E. A.; Schleif, W. A.; Quintero, J. C.; Anderson, P. S.; Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2852-7 For recent reviews see: (d) Norbeck, D. W.; Kempf, D. J. *Annu. Rep. Med. Chem.* **1991**, *26*, 141-50 (e) Petteway, S. R., Jr.; Dreyer, G. B.; Meek, T. D.; Metcalf, B. W.; Lambert, D. M. *AIDS Res. Rev.* **1991**, *1*, 267-88 (f) Huff, J. R. *J. Med. Chem.* **1991**, *34*, 2305-14

8. The anti-viral assay consists of treating MT-2 cells (human T lymphoid cells) that are infected with HIV-1 IIIB virus with test compounds. After an incubation period of 5 days at 37°C, the number of viable cells was determined using an MTT dye assay. Compound concentration that provides 50% inhibition (IC_{50}) of cell death, relative to untreated contol, is reported in Table 4.

9. Pickett, W. C., unpublished observations.

10. Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, *16*, 1219-1222.

11. Trova, M. P.; Babine, R. E.; Byrn, R. A.; Casscles, W. T.; Hastings, R. C.; Hsu, G. C.; Jirousek, M. R.; Johnson, B. D.; Kerwar, S. S.; Schow, S. R.; Wissner, A.; Zhang, N. , next paper in this issue.

12. Note added in proof: A similar strategy to break up symmetry has been reported: Norbeck, D. W. *et. al.* VII th International Conference on AIDS, III STD World Congress, Amsterdam, the Netherlands, July 19-24, 1992; ThA 1507; Norbeck, D. W. *et. al.* 32 nd I.CAAC., Anaheim, CA, 1992, session 56.