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Nonpeptide HIV Protease Inhibitors Designed to Replace a Bound Water

Balan Chenera,[§] Renee L. DesJarlais,*[,¶] Joseph A. Finkelstein,[§]
Drake S. Eggleston,[¶] Thomas D. Meek,[§] Thaddeus A. Tomaszek, Jr.,[§] and Geoffrey B. Dreyer*[,§]

Departments of [§]Medicinal Chemistry and [¶]Physical & Structural Chemistry
SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, PA 19406

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Abstract: Cyclic nonpeptide molecules were designed and synthesized with the goal of displacing the conserved flap-associated water of HIV-1 protease. Several such molecules were competitive inhibitors with micromolar inhibition constants, and their structure-activity relationships were consistent with the design hypothesis.

Inhibitors of HIV-1 protease are under study as potential AIDS therapeutics based on promising *in vitro* antiviral properties.[1,2,3] HIV protease inhibitors are generally peptide analogues whose cardinal features include a hydroxyl group that interacts with the catalytic aspartate residues of the enzyme (Asp25, Asp125), amide hydrogens that form polar contacts with the carbonyl oxygens of Gly27 and Gly127, and hydrophobic groups that occupy the enzyme specificity pockets.[4,5,6] X-ray structures also show that Ile50 and Ile150 in the flaps of the enzyme are hydrogen-bound to inhibitors via a tetrahedrally coordinated water molecule.[4-6] We report our preliminary efforts to design nonpeptide inhibitors which could displace this coordinated water.

Using a modification[7] of the DOCK 1.0 program,[8] we searched a company database for molecules that were complementary to the active site of HIV protease, and which contained one oxygen that could displace the flap-associated water and a second oxygen that could interact with Asp25 and Asp125. Several hits contained a *trans*-1,4-cyclohexanediol or hydroquinone, suggesting that a six membered ring with *para* related oxygens could make the desired interactions. This core structure was then elaborated to include hydrophobic groups for filling the S1 and S1' enzyme pockets and hydroxyl groups that could hydrogen bond with Gly27 and Gly127 (Figure 1).

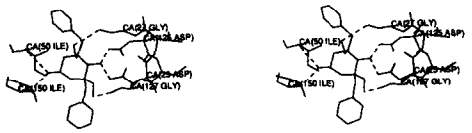
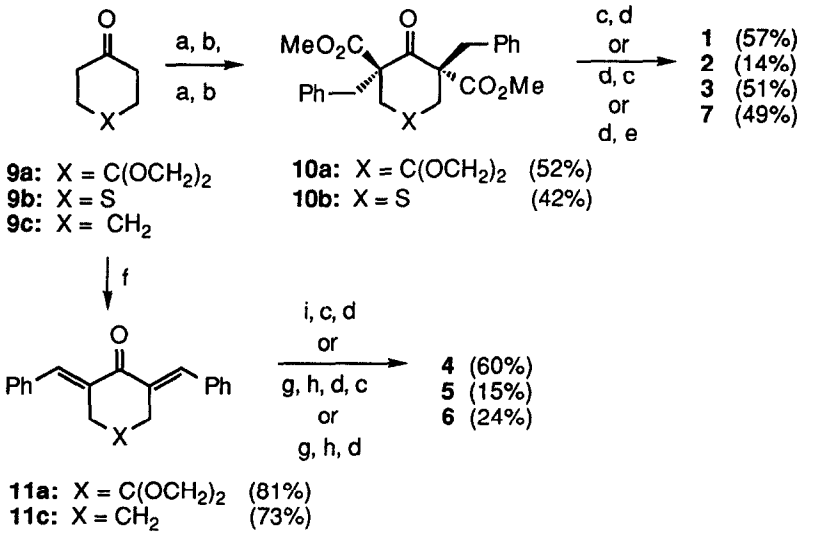


Figure 1. Model of the complex of **1** with HIV protease, showing only the enzyme residues expected to hydrogen-bond with the inhibitor.

Scheme I outlines the synthesis of several compounds resulting from our design (Table 1). Sequential carboxymethylation[9] and alkylation of ketones **9a** and **9b** led exclusively to **10a** and **10b**. Deketalization and reduction of **10a** gave epimeric 1,4-diols **1** and **2** as verified by ^{1}H NOE studies,[10] while reduction prior to deprotection afforded the 4-keto analogue **3**. Hydride reduction of **10b** and oxidation of the sulfur yielded sulfoxide **7**, which was resolved by chiral HPLC. X-ray analysis of (-)**7** established the (R) absolute configuration of its two quaternary carbons and the *trans* relationship of the ring hydroxyl and sulfoxide oxygens (see Figure 2).[11] Aldol adducts **11a** and **11c** were converted to **4-6** in a straightforward manner via their diepoxide derivatives.

Scheme I [a]

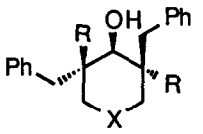


[a] Reagents and conditions: (a) $LiN(Me_3Si)_2$, MeO_2CCN, THF, -78 → 0 °C; (b) NaH, BnBr, THF, 0 → 25 °C; (c) 60% HCO_2H, 60 °C; (d) $LiAlH_4$, Et_2O, 25 °C; (e) $NaIO_4$, MeOH; (f) PhCHO, NaOH, MeOH-H_2O; (g) $(iBu)_2AlH$, CH_2Cl_2, -78 °C; (h) m-CPBA, CH_2Cl_2; (i) H_2 (1 atm), 10% Pd/C.

Inhibition constants for the compounds in Table 1 with HIV-1 protease were determined initially by Dixon analysis, assuming competitive inhibition. Competitive inhibition was verified for compounds **1** and **5** by using double reciprocal plots (rate^{-1} vs [substrate]$^{-1}$ at several inhibitor concentrations). These are relatively weak inhibitors,[12] but the structure-activity relationships are broadly consistent with the design hypothesis. The enhanced activity of compound **1** (K_i = 48 μM) vs its 4-hydroxy epimer **2** is consistent with the model in Figure 1, in which an axial 4-hydroxyl group would be poorly positioned to interact with the flaps. The 4-hydroxyl group can be replaced by 4-keto without loss of activity (compound **3**), as expected if the function of the oxygen is to accept hydrogen bonds from Ile50 and Ile150. Consistent with their intended role as hydrogen-bond donors to the carbonyls of Gly27 and Gly127, removal of the primary hydroxyl groups from **1** results in total loss of

inhibition (compound **4**), and the analogue **5** with tertiary hydroxyl groups is more potent (10 μM). The contribution of the C_4 oxygen is again shown by the desoxy analogue **6**, which is not an inhibitor. The 4-keto group can, however, be replaced by a sulfoxide. Curiously, both enantiomers of **7** are low micromolar inhibitors, although the more active antipode, (+)**7**, is the one anticipated by our binding model. The model in Figure 3a shows a good match between (+)**7** and the inhibitor A74704 (((S)-CbzValNHCHBn)$_2$CHOH) in its bound conformation with HIV protease.[5] A possible explanation for the similar activity of (-)**7** is shown in Figure 3b: (-)**7** can be reasonably superimposed with A74704 in a binding mode in which its hydroxyl groups may hydrogen bond with Asp25 and Asp125, rather than with Gly27 and Gly127.

Table 1. Inhibition of HIV protease[a]



Compound	R	X	K_i (μM)
1	CH_2OH	CH····OH	48±6
2	CH_2OH	CH—OH	830±130
3	CH_2OH	C=O	62±3
4	H	CH····OH	>2000
5	OH	C=O	10±1
6	OH	CH_2	>2000
(-)**7**	CH_2OH	S→O	19±1
(+)**7**	CH_2OH	S→O	7±2

[a] Inhibition of recombinant HIV-1 protease was determined at 37 °C, pH 6.0 (0.2 M NaCl) using the substrate Ac-Arg-Ala-Ser-Gln-Asn-Phe-Pro-Val-Val-NH_2 as previously described.[13] K_i values were determined by Lineweaver-Burk plots (for **1**, **5**) or Dixon plots (**2-4**, **6-7**). All compounds except **7** are racemates with the indicated relative configurations. (+)**7** is (S) at the quaternary carbons.

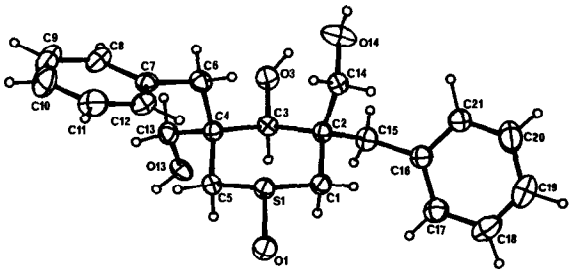


Figure 2. ORTEP view of the (-)**7** structure[11] as determined by single crystal X-ray diffraction. Non-hydrogen atoms are drawn as principal ellipses at the 50% probability level, hydrogen atoms as spheres of arbitrary size.

The molecules described here lack certain of the features that would be expected for high potency, most notably hydrophobic groups that can occupy the S2 and S2' pockets.[6a,12] It seems likely that by incorporating judicious additional hydrophobicity and polarity, potency could be significantly increased.[14]

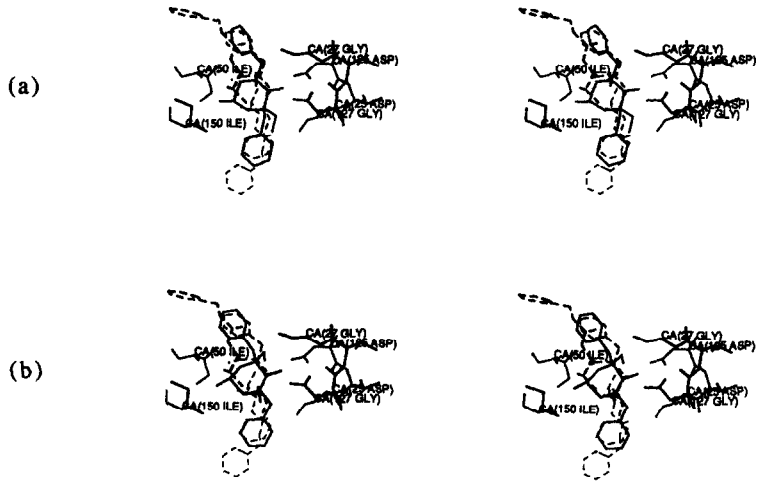


Figure 3. Molecule (+)**7** (a) and molecule (-)**7** (b) overlaid with the crystal structure of the HIV-1 protease/A74704 complex.[5] The bond lengths and angles for (+)**7** and (-)**7** were derived from the crystal structure of (-)**7**. The torsion angles for the benzyl and hydroxymethyl side chains were adjusted to obtain the best overlap with A74704 and to maximize favorable interactions with the protein.

Supplementary Material Available: For (-)**7**, additional crystallographic details, tables of fractional atomic coordinates, anisotropic thermal parameters for non-hydrogen atoms and tables of further metrical details, as well as listings of structure factors (12 pages). These data are available from the authors and have been deposited at the Cambridge Crystallographic Data Centre.

Acknowledgment: We thank Jeffrey C. Boehm for initial synthetic studies, Karl F. Erhard for chiral HPLC resolution of **7**, Priscilla H. Offen for NOE studies, Edith A. Reich for optical rotation measurements, and J. Scott Dixon for contributions to the modification of DOCK. We thank Brian W. Metcalf and John G. Gleason for their support.

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[10]In **1** each of the CH_2Ph and CH_2OH methylenes exhibits a distinct pair of doublets (hence **1** lacks a σ plane): ^1H NMR (250 MHz, CD_3OD): δ (4.23(d), 3.60(d); J = 11 Hz), (3.38(d), 2.94(d); J = 10 Hz), (3.20(d), 2.95(d); J = 14 Hz), (3.17(d), 2.86(d); J = 13 Hz). Ring methine H_1 gives +tive NOE to axial ring protons H_{3a}, H_{5a} (δ 0.76, 1.17), while methine H_4 (δ 4.12) gives +tive NOE to equatorial H_{3e}, H_{5e} (δ 1.59).

[11](a) For (-)**7**, $[\alpha]_D$ = -17.9° (c = 1.00, methanol). Crystallographic data for (-)**7** (at -50 °C): a=9.855(1)Å, b=10.124(2)Å, c=10.132(1)Å, β=109.55(2)°, V=952.6(4)Å^3, Z=2, space group $P2_1$. Data (4615) were measured on an Enraf Nonius CAD4 diffractometer using an ω-2θ scan mode (2θ max = 50°) with graphite monochromated moblybdenum radiation. The structure was solved with SHELXS[11b], and refined by full-matrix least squares to final agreement factors of R(F) = 0.025, Rw(F) = 0.035, G.O.F. = 2.235 for 1710 observations

($I \geq 3\sigma(I)$) and 234 variables. The absolute configuration assignment was based on Hamilton's R-factor ratio test[11c] (Rw for the antipode = 0.041) and corroborated by examination of Friedel pairs. (b) Sheldrick, G.M. In *Crystallographic Computing 3*; Sheldrick, G.M., Kruger C., Goddard, R., Eds.; Oxford University Press: London, **1985**; pp 175-189. (c) Hamilton, W.C. *Acta Cryst.* **1965**, *18*, 502-510.

Table 2. Positional Parameters and Their Estimated Standard Deviations for (-)**7**[a]

Atom	x	y	z	B(Å^2)
S1	0.79227(4)	0.546	0.84668(4)	1.971(8)
O1	0.9093(1)	0.5604(2)	0.7831(1)	2.73(3)
O3	0.5056(1)	0.2727(2)	0.9824(1)	2.36(3)
O13	0.8277(1)	0.1563(2)	1.0963(1)	2.36(3)
O14	0.3373(2)	0.4644(2)	0.8443(2)	3.44(3)
C1	0.6593(2)	0.4411(2)	0.7278(2)	2.04(4)
C2	0.5416(2)	0.3919(2)	0.7834(2)	1.70(3)
C3	0.6115(2)	0.3171(2)	0.9250(2)	1.83(4)
C4	0.7379(2)	0.3836(2)	1.0421(2)	1.75(3)
C5	0.8552(2)	0.4244(2)	0.9824(2)	1.94(4)
C6	0.6877(2)	0.5011(2)	1.1131(2)	2.06(4)
C7	0.8032(2)	0.5708(2)	1.2279(2)	1.96(4)
C8	0.8391(2)	0.5334(3)	1.3682(2)	2.59(4)
C9	0.9416(3)	0.6007(3)	1.4722(2)	3.58(5)
C10	1.0096(3)	0.7083(3)	1.4406(2)	3.56(5)
C11	0.9736(2)	0.7507(3)	1.3035(2)	3.19(5)
C12	0.8721(2)	0.6819(2)	1.1991(2)	2.31(4)
C13	0.8029(2)	0.2780(2)	1.1557(2)	2.10(4)
C14	0.4452(2)	0.5091(2)	0.7912(2)	1.94(4)
C15	0.4491(2)	0.2876(2)	0.6765(2)	2.26(4)
C16	0.3946(2)	0.3310(2)	0.5262(2)	2.16(4)
C17	0.4688(2)	0.2982(3)	0.4344(2)	2.73(4)
C18	0.4206(3)	0.3403(3)	0.2964(2)	3.42(5)
C19	0.2988(3)	0.4170(3)	0.2459(2)	3.84(6)
C20	0.2221(3)	0.4487(3)	0.3355(3)	3.88(6)
C21	0.2684(2)	0.4051(3)	0.4720(2)	2.92(5)

[a] Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as: $Beq = (8\pi^2/3)\ \Sigma_i\Sigma_j Uij a_i^* a_j^* a_i a_j$

[12] The potencies of the better inhibitors in Table 1 are similar to those of many peptidyl inhibitors of a similar size. For example, the pseudosymmetric peptide analogue ((S)-AcNHCHBn)$_2$CHOH inhibits HIV-1 protease with $IC_{50} > 10\ \mu M$,[12b] and the hydroxyethylene isostere (2R, 4S, 5S)-BocNHCHBnCH(OH)CH$_2$CHBnCONH$_2$ has $K_i = 3.2\ \mu M$.[6a] (b) Kempf, D. L.; Norbeck, D. W.; Codacovi, L.; Wang, X. C.; Kohlbrenner, W. E.; Wideburg, N. E.; Paul, D. E.; Knigge, M .F.; Vasavanonda, S.; Craig-Kennard, A.; Saldivar, A.; Rosenbrook, W., Jr.; Clement, J. J.; Plattner, J. J.; Erickson, J. *J. Med. Chem.* **1990**, *33*, 2687.

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[14]A structurally related group of highly potent nonpeptide HIV protease inhibitors, derived by a similar design strategy, has been reported: Lam, P. Y.-S. *et al.*, Abst. 205th ACS Natl Meeting, MEDI 96 (March 31, 1993); WO 93/07128. As shown by X-ray crystallography, these molecules displace the flap water, and in addition, possess side chains that extend into the S2/S2' pockets.