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INVESTIGATING THE STEREOCHEMISTRY OF BINDING TO HIV-1 PROTEASE WITH INHIBITORS CONTAINING ISOMERS OF 4-AMINO-3-HYDROXY-5-PHENYLPENTANOIC ACID

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Summary: *A series of inhibitors containing all possible isomers of 4-amino-3-hydroxy-5-phenylpentanoic acid was synthesized and tested for inhibition of HIV-1 protease. Incorporation of the (3S,4S) isomer of the t-butyloxycarbonyl protected amino acid into the sequence Glu-Phe resulted in a potent inhibitor of HIV-1 protease (K_i = 63 nM). This inhibitor is at least 47- times more potent than the inhibitors containing other isomers of 4-amino-3-hydroxy-5-phenylpentanoic acid, indicating that the (3S,4S) isomer is the preferred isomer for binding to HIV-1 protease.*

HIV-1 protease is an essential enzyme in the reproductive cycle of the HIV-1 virus (1). HIV-1 PR cleaves the precursor polyprotein to functional proteins needed for production of mature HIV-1 virus. Cell culture studies using HIV-1 protease inhibitors of HIV-1 PR were have shown that the protease is necessary for viral reproduction (2). Thus, inhibition of HIV-1 protease is regarded as a promising approach for treatment of AIDS and related retroviral diseases.

Based on the principles elaborated for inhibiting renin and related aspartyl proteases, potent inhibitors of HIV-1 protease have been synthesized. Most of the tight binding inhibitors of HIV-1 protease were developed by replacing the scissile peptide bond in substrate analogs by a transition state element. Contrary to other aspartyl proteases, a transition state element with either (R) or (S) configuration at the hydroxyl bearing carbon atom is tolerated by HIV-1 protease (3,4). Rich *et al.* (5) have proposed that the configuration of the hydroxyl group necessary for maximal inhibitory potency is dependent upon the peptide framework.

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Abbreviations
AHPPA: 4-amino-3-hydroxy-5-phenylpentanoic acid, or commonly known as phenylstatine.
AHPBA: 3-amino-2-hydroxy-4-phenylbutanoic acid, or phenylnorstatine.
Boc: *t*-butyloxycarbonyl. BOP: benzotriazolyl N-oxytri-dimethylamino-phosphonium hexafluorophosphate. LAH: lithium aluminum hydride.

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We reported a series of inhibitors containing phenylnorstatine as the transition state element for HIV-1 protease inhibitors (6). For the phenylnorstatine inhibitors, we found that the (2S,3S) isomer of phenylnorstatine is preferred over other isomers as the transition state element. Also, Class C substrate analog inhibitors (6) were found to be superior to either Class A or Class B substrate analog inhibitors. In this communication, we report incorporation of all four isomers of 4-amino-3-hydroxy-5-phenylpentanoic acid (AHPPA, or commonly referred to phenylstatine) into Class C type substrate analogs and, their inhibitory properties for HIV-1 protease.

Experimental

i) Synthesis: Synthesis of the four possible isomers of Boc-AHPPA was accomplished by a slight modification of the reported procedure (7). N^{α}-(*t*-butoxycarbonyl)-L-phenylalanyl-N-methoxy-N-methylamide, obtained by coupling *t*-Butoxycarbonyl-L-phenylalanine with N,O-dimethylhydroxylamine hydrochloride using BOP, was reduced with LAH in anhydrous THF to give *t*-Butoxycarbonyl-L-phenylalinal (8). The amino aldehyde thus obtained was reacted with lithium-ethylacetate at -70° to yield a mixture of diastereomeric esters. Separation of the diastereomers by column chromatography (silica gel). The stereochemistry of the individual diastereomers was establised by comparing the 400 MHz ^{1}H-NMR spectra with those reported (7). Basic hydrolysis of the individual diastereomers resulted in N-protected-ß-hydroxy-γ-amino acids. The N-protected amino acids were incorporated in peptide sequences by solution phase techniques. Homogeneity of the peptides was determined by TLC, HPLC, ^{1}H-NMR (400 MHz), FAB-MS and amino acid analysis.

ii) HIV-1 protease inhibition assay: The HPLC based assay for inhibition of HIV-1 protease (9) was performed by using cloned HIV-1 protease (10). Reactions were carried out at ambient temperature. The reagents used in the assay were as follows: HIV-1 protease, (50 µg/ml) was obtained from The AIDS Reference and Reagent Program (Rockville, MD) (10); HIV-1 protease substrate (Val-Ser-Gln-Asn-Phe(4-NO_2)-Pro-Ile-Val), 4.04 mM solution in double distilled water; buffer, 50 mM NaOAc (pH 5.8) containing 1 mM EDTA, 2.5 mM DTT, 10% glycerol, 0.2% NP-40.

The total volume of the reaction mixture was 100 µl. Ten µL of the enzyme stock solution were mixed with varying concentrations of the inhibitor and the buffer. Stock solutions of inhibitors were prepared by using a mixture of DMSO and double distilled water. The final concentration of DMSO in the assay was less than 8% in all cases. Substrate (20 µl) was added to the reaction mixture. The reaction was quenched after 20 min by addition of 20 µl of TFA.

Hydrolysis of the substrate was quantitated by injecting 80 µl of the reaction mixture on a SYSTEM GOLD™ HPLC. Percent inhibition was plotted against inhibitor concentrations to determine the IC_{50} values. K_i values were calculated form corresponding IC_{50} values by using the equation of Cha *et al.* (11).

Results and Discussion

In the preceding communication (6), we reported a series of HIV–1 protease inhibitors containing phenylnorstatine as the transition state element. Based on that series, it was shown that Class C type substrate analogs are preferred over Class A and Class B type substrate analogs. We thus decided to explore the inhibitory potencies of Class C type substrate analog inhibitors with phenylstatine, a transition state element which possesses one more backbone carbon atom than does phenylnorstatine.

The sequences of phenylstatine containing inhibitors and their K_i values are shown in Table I. Incorporation of phenylstatine into a Class C type substrate analog resulted in inhibitors of HIV–1 protease. The (3S,4S) isomer of phenylstatine is the preferred isomer for binding to HIV–1 protease (K_i = 63 nM). Boc–(3S,4S)AHPPA–Glu–Phe is at least 47– times more potent than inhibitors containing the other isomers of AHPPA.

Moore *et al.* (12) reported inhibitory property of a compound containing phenylstatine as the transition state element (Ac–Ser–Gln–Asn–(3RS,4S)AHPPA–Val–Val–NH_2; K_i = 39.0 µM). This inhibitor may be classified as either a Class A, or a Class B type inhibitor. A comparison between the inhibitor reported by Moore *et al.* and inhibitor #1 (Table I), indicates that Class C substrate analog inhibitors may be superior to either Class A or Class B type inhibitors. This result is consistent with that obtained with inhibitors containing phenylnorstatine as the transition state element (6).

Changing the transition state element from phenylnorstatine to phenylstatine had a significant effect on inhibitory potencies of Class C inhibitors. The best inhibitor obtained with the phenylnorstatine series, Boc–(2S,3S)AHPBA–Glu–Phe, had a K_i = 3.3 µM (6). The best inhibitor obtained with phenylstatine (inhibitor #1) is about 50– times more potent than the best

Table I. Phenylstatine containing inhibitors and their K_i values

Inhibitor#	Sequence	K_i(µM)
1	Boc-(3S,4S)AHPPA - Glu - Phe	0.063
2	Boc-(3S,4R)AHPPA - Glu - Phe	7
3	Boc-(3R,4S)AHPPA - Glu - Phe	4
4	Boc-(3R,4R)AHPPA - Glu - Phe	3

AHPPA = 4-Amino-3-hydroxy-5-phenylpentanoic acid

phenylnorstatine inhibitor. The increased potency may be attributed to the extra C–atom phenylstatine inserts into the inhibitor backbone. In compound #1, the C_α of Glu is shifted about 1.0 Å away from the hydroxyl group, when compared to the corresponding phenylnorstatine inhibitor. Preliminary modeling studies with HIV–1 protease/inhibitor complex suggest that such a shift may give rise to favorable interactions between the enzyme and the inhibitor.

The stereochemistry of the hydroxyl group necessary for maximal inhibitory potency is also reversed. In phenylnorstatine series, the stereochemistry of the hydroxyl group is *S* (equivalent to *R* in phenylstatine), while in the phenylstatine series the stereochemistry is *S* (equivalent to *S* in phenylnorstatine). This observation supports the hypothesis of Rich *et al.* (5) that the stereochemistry of the hydroxyl group necessary for good inhibition is dependent on the peptide framework.

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References

1. Skalka, A.M. (1989) Cell 56, 911.
2. McQuade, T., Tomasselli, A., Liu, L., Karacostas, V., Moss, B., Sawyer, T., Heinrickson, R., Tarpley, W. (1990) Science 47, 454.
3. Rich, D., Green, J., Toth, M., Marshall, G., Kent, S.B.H. (1990) J. Med. Chem. 33, 1285.
4. Roberts, N., Martin, J., Kinchington, D., Broadhurst, A., Craig, C., Duncan, I., Galpin, S., Handa, B., Kay, J., Krohn, A., Lambert, R., Merrett, J., Mills, J., Parkes, K., Redshaw, S., Ritchie, A., Taylor, D., Thomas, G., Machlin, P. (1990) Science 248, 358.
5. Rich, D.H., Sun C.Q., Prasad, J.V.N.V., Pathiasseril, A., Toth, M.V., Marshall, G.R., Clare, M., Mueller, R.A. and Houseman, K. (1991) J. Med. Chem. 34, 1222.
6. B. Raju, Deshpande, M. (1991) Biochem. Biophys. Res. Comm. 180, 181-186.
7. Rich, D., Sun, E.T.O., (1980) J. Med, Chem. 23, 27.
8. Fehrentz, J.A. and Castro, B. (1983) Synthesis 676.
9. Deshpande, M.S., Raju, B. and Manly, S.P. Int. J. Pep. Protein Res. (Submitted for publication).
10. Boutelje, J., Karlstorm, A., Hartmanis, M., Holmgren, E., Sjogren, A., Levine, R. (1990) Arch. Biochem. Biophys. 15, 141.
11. Cha, S., Agarwal, R.P. and Parks, R.E. Jr. (1975) Biochemical Pharmacology 24, 2187.
12. Moore, M., Bryan, W., Fakhoury, S., Magaard, V., Huffman, W., Dayton, B., Meek, T., Hyland, L., Dreyer, G., Metcalf, B., Strickler, J., Gorniak, J., Debouck, C. (1989) Biochem. Biophys. Res. Comm. 159, 420.