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NOVEL PSEUDOSYMMETRIC INHIBITORS OF HIV-1 PROTEASE

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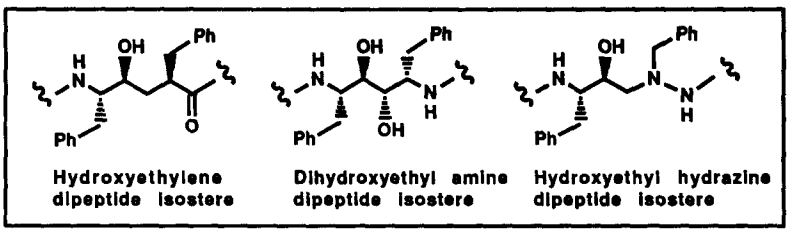
Abstract: Compounds containing the easily accessible Phe[CH(OH)CH_2N(NH)]Phe dipeptide isostere as a non-hydrolyzable replacement of the scissile amide bond in the natural substrate are potent inhibitors of HIV-1 protease. The expected symmetric binding pattern of the most potent inhibitor in this series (CGP 53820, IC_{50} = 9 nM) is illustrated by the X-ray analysis performed with the corresponding enzyme-inhibitor complex.

The human immunodeficiency virus type 1 (HIV-1) is considered to be a causative agent for the spread of AIDS[1]. HIV-1 encodes a unique aspartyl protease, responsible for the processing of the polyprotein products of the gag and pol genes to produce the structural and core proteins, and the enzymes essential for viral replication[2]. Inactivation of this protease by site-directed mutagenesis of the catalytic site leads to formation of immature and non infectious virus particles[3]. For this reason inhibition of HIV-1 protease represents a major target for potential chemotherapy of AIDS[4].

The enzyme has been shown to function as a C-2 symmetric dimer with each subunit contributing an amino acid triad Asp-Thr-Gly to the active site[2a, 5]. This particular degree of symmetry has spurred the design of C-2 symmetric inhibitors that might provide higher specificity and reduced peptide character than non-symmetric inhibitors based on a peptide substrate. On the basis of the structural type of the hydroxyethylene core as a tetrahedral transition-state replacement for the peptide substrate, various potent C-2 symmetric inhibitors have been introduced[6, 7]. Recently, a similar concept has been extended to a class of compounds with sulfur[8] or phosphorus[9] containing central units. Our own molecular modeling studies as well as the synthetic accessibility and variability have prompted us to explore a novel pseudo-symmetric type of dipeptide isostere containing a hydroxyethyl hydrazine moiety[10]. The carbon atom bearing the P_1' substituent in a hydroxyethylene or dihydroxyethyl amine isostere is replaced by a nitrogen atom of a hydrazine group. This functionality eliminates one stereogenic center and allows simultaneous symmetric acylation of the mimic as with the known dihydroxyethyl amine isostere[6a, c]. We wish to report that this concept led to a series of potent HIV-1 protease inhibitors with high antiviral activity and good specificity.

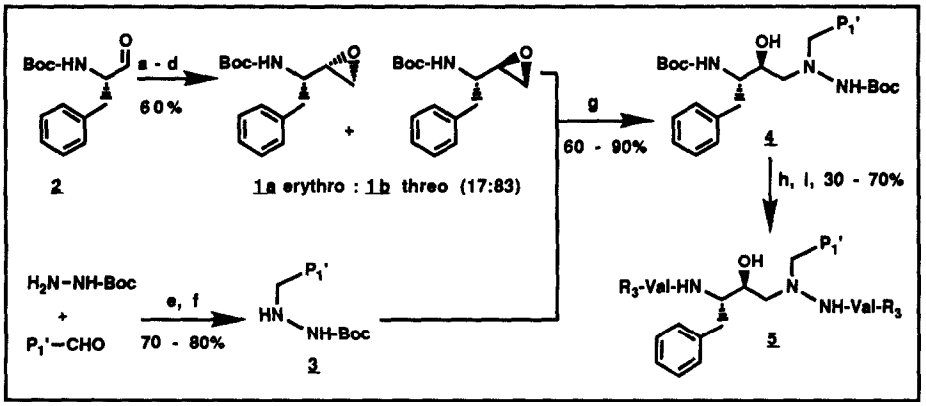
Figure 1: Comparison of Phe-Phe dipeptide isosteres



Hydroxyethylene dipeptide isostere | Dihydroxyethyl amine dipeptide isostere | Hydroxyethyl hydrazine dipeptide isostere

The general synthesis applied is illustrated in Scheme 1. Epoxides **1a,b**[11] were formed via olefination[12] of Boc-phenylalaninal **2**[13], followed by epoxidation with 3-chloroperbenzoic acid (mCPBA)[11b] and chromatography on silica gel as a (17:83) erythro/threo mixture in 89 % yield (60 % from **2**). All four possible stereoisomers could be separated on a *Chiralcel OD* analytical HPLC column[14]. The enantiomeric excess of both the desired threo isomer **1b**, and the erythro isomer **1a** was >95 %. Nucleophilic epoxide ring opening was achieved with the respective t-butyl alkylcarbazates **3** in refluxing methanol to afford the crystalline Boc-protected dipeptide isosteres **4** in 60 to 90 % yield. The required t-butyl alkylcarbazates were readily accessible by catalytic hydrogenation (Pt on carbon, 3 atm H_2, THF, RT, 70 to 80 %) of the hydrazones formed with t-butylcarbazate and the appropriate aldehyde[15]. Simultaneous cleavage of the Boc-protecting groups (4N HCl (g) in dioxane, 1h, RT) followed by coupling with the various acylated or carbamoylated valine derivatives according to standard procedures (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), 1-hydroxybenzotriazole (HOBt), 4-methylmorpholine (NMM) in DMF, 16h, RT) furnished the target inhibitors **5** in moderate yields from 30 to 70 %. Not unexpected under the conditions required, coupling with acylated valine derivatives (e.g. acetyl-L-valine) occurred with some degree of racemization of the valyl side chains, giving rise to up to 20 % of diastereomeric side products, which could be removed by column chromatography on silica gel or repeated precipitation of the final products from methylene chloride/diisopropyl ether.

Scheme 1: Synthesis of the substituted hydrazine derivatives

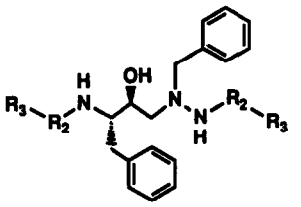


a) $ClMgCH_2SiMe_3$, Et_2O, -60°C to RT, 2h, **b)** $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0°C to RT, then RT, 3h, **c)** $(Boc)_2O$, CH_2Cl_2, RT, 2h, **d)** mCPBA, CH_2Cl_2, 0°C, 18h, **e)** EtOH, 80°C, 2h, **f)** H_2, Pt, C, MeOH, RT, 1-6h, **g)** MeOH, 80°C, 6h, **h)** HCl, dioxane, RT, 1h, **i)** 6 equivalents R_3-Val-OH, BOP, HOBt, NMM, DMF, RT, 16h.

Compounds containing the central dipeptide mimic symmetrically acylated with L-valine or even Boc-L-valine are only weak inhibitors of HIV-1 protease (compounds **7** and **8** in Table 1). Replacement of the bulky tert-butoxycarbonyl substituents obviously interfering with the P_3 and P_3' subsites of the enzyme with acetyl groups results in a 1700-fold increase in potency (compound **9**). Introduction of the benzyloxycarbonyl or 2-quinolinoyl substituents intended to bind to the P_3 and P_3' subsites instead of the aforementioned acetyl groups has little effect *in vitro* (compounds **12** and **13**). This result indicates that hydrogen bonding of the carbonyl functions of the acylated valine residue contributes more to

binding than the lipophilic interactions with the P_3 and P_3' subsites of the enzyme. However, as shown in Table 2, the antiviral potency is greatly enhanced by increasing the overall lipophilicity of the inhibitor (compounds **9** and **13**, **17** and **21** respectively). The complete loss of *in vitro* activity upon exchange of L-valine with D-valine demonstrates that geometry of the P_2 and P_2' residues is crucial for binding (compounds **13** and **14** respectively).

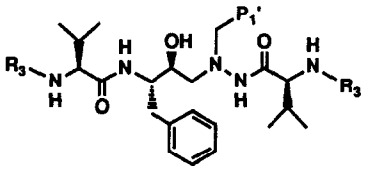
Table 1: Influence of the R_2 and R_3 substituents *in vitro*



compound	R_3	R_2	IC_{50} [μM] [a]
6	-	Boc	~ 50
7	HCl x H	L-Val	>100 [b]
8	Boc	L-Val	52
9	acetyl	L-Val	0.03
10	phenyl acetyl	L-Val	0.36
11	3-pyridyl acetyl	L-Val	0.5
12	2-quinolinoyl	L-Val	0.065
13	benzyloxycarbonyl	L-Val	0.033
14	benzyloxycarbonyl	D-Val	>100 [c]
15	4-morpholino-carbonyl	L-Val	2

[a] The IC_{50} against HIV-1 protease was determined in 20 mM morpholineethanesulfonic acid buffer at pH 6 using 164 μM peptide substrate (H-Arg-Arg-Ser-Asn-Gln-Val-Ser-Gln-Asn-Tyr-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Arg-Arg-OH). The cleavage was monitored by HPLC. Boc-Phe[$CH(OH)CH_2$]Phe-Val-Phe-morpholine with an IC_{50} of 20.2 nM served as a reference compound. The standard error was determined to be ±13 %. [b] 32 % inhibition at 100 μM; [c] 40 % inhibition at 100 μM.

Table 2: Variation of the P_1' substituent

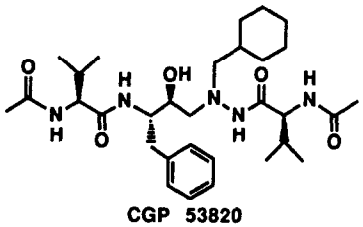


cpd.	R_3	P_1'	IC_{50} [μM]	ED_{90} [μM] a)
9	acetyl	phenyl	0.03	10
16		4-fluorophenyl	0.028	10
17		4-cyanophenyl	0.05	>10
18		cyclohexyl	0.009	1
19		isopropyl	0.045	>1 b)
13	benzyloxycarbonyl	phenyl	0.033	0.1
20		4-fluorophenyl	0.022	1
21		4-cyanophenyl	0.024	0.1
22		cyclohexyl	0.095	1
23		isopropyl	0.01	>1 b)

a) The tests were performed using MT-2 cells, seeded in 96-well round bottom plates at 4 x 10^4 per well in complete RPMI medium. The compounds and the HIV-1_{LAV} virus (360 $TCID_{50}$/ well) were added to a final volume of 200 μL. The assays were performed in triplicates. After 4 days of incubation, 10 μL samples of the culture supernatants were collected and assayed for reverse transcriptase activity. AZT with an ED_{90} of 0.3 μM served as a reference compound in this assay.
b) highest concentration tested.

Further improvement of the overall profile is achieved by variation of the P_1' substituent, especially in the acetyl-L-valine substituted series. Replacement of the the phenyl substituent in compound **9** occupying the P_1' subsite by the more lipophilic cyclohexyl group further increases activity and yields a potent HIV-1 protease inhibitor (CGP 53820, compound **18**) with good selectivity against human aspartic proteases.

Table 3: Selectivity profile of CGP 53820 (compound 18)



CGP 53820

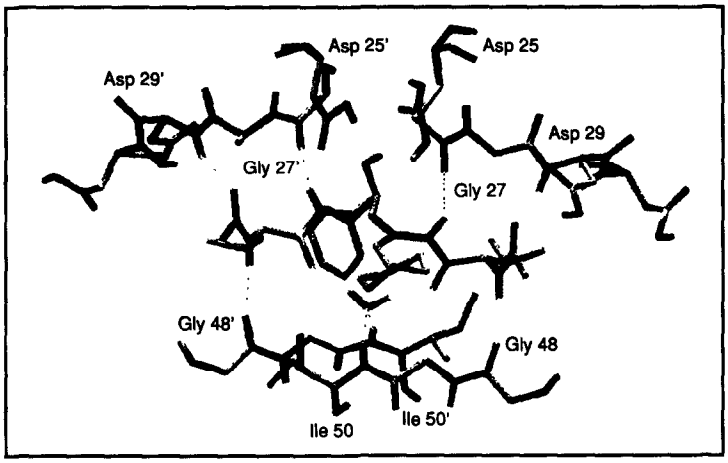
human protease	IC_{50} [μM] a)
HIV-1 protease	0.009
renin	>58
gastricsin	1
cathepsin D	7.5
cathepsin E	2
pepsin	1

a) The IC_{50} values for the human enzymes were determined as described in reference 16.

The conformation of CGP 53820 bound to the active site of HIV-1 protease has been determined by the refined complex crystal structure at 2.0 Å resolution[17]. Figure 2 shows that the inhibitor binds in a extended conformation with the hydroxyl group positioned symmetrically between the two catalytic aspartates of the enzyme active site. The phenyl and cyclohexyl groups are accommodated by the respective P_1 and P_1' specificity pockets. As observed in previously reported X-ray structures[6b, 18], a tightly bound water molecule bridges between the amide bonds of the Ile-50 and Ile-150 residues of the flap and the two carbonyl functions adjacent to the mimic.

CGP 53820 as a potent HIV-1 protease inhibitor with a novel, easily accessible pseudosymmetric transition-state dipeptide mimic illustrates with its crystal structure that it provides an optimal framework for both the hydrogen bond formation between enzyme and inhibitor as well as favorable hydrophobic interactions especially in the P_1, P_2 and P_1', P_2' subsites.

Figure 2: Hydrogen bonding pattern of CGP 53820 in the enzyme-inhibitor complex



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14 HPLC analysis of the epoxides **1a,b**: stationary phase: *Chiralcel OD*, mobile phase: hexane/i-propanol (98:2), detection at 210 nm, retention times in min.: (2S,3R)-isomer 12.2, (2R,3S)-isomer 13.7, (2R,3R)-isomer 15.5, (2S,3S)-isomer 16.6.

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