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STRUCTURE-ACTIVITY RELATIONSHIPS FOR MACROCYCLIC PEPTIDOMIMETIC INHIBITORS OF HIV-1 PROTEASE.

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Abstract : A series of appended macrocycles were synthesized and tested as inhibitors of HIV-1 protease (HIV PR). The macrocycle structurally mimics an N-terminal tripeptide component of peptide substrates. Structure-activity relationships explore steric limitations to the size and shape of the substituents and provide evidence for functional mimicry of substrate components.

Introduction: A rational approach to developing inhibitors of proteolytic enzymes is the systematic modification of their peptide substrates to proteolytically stable, low molecular weight, nonpeptidic inhibitors. Replacing the scissile amide bond with a noncleavable transition state isostere usually gives potent protease inhibitors, but the remaining hydrolysable amide bonds render the inhibitors unstable to peptidases generally. Attempts to replace them with retention of inhibitor potency has proved difficult[1,2] due to the unpredictable cooperative influences of such variations on the conformations of both neighbouring inhibitor groups and enzyme residues. We recently described a method[3] for *regioselectively* fixing the conformation of inhibitor *components* and now show effects on activity of varying both the 'free' and 'fixed' components.

We have reported[3] a hydrolytically stable macrocycle that mimics the protease-bound conformation of Leu-Asn-Phe in the inhibitor Ac-Leu-Asn-Phe-{*S*-CHOH-CH_2}-Pro-Ile-Val-NH_2 (**4**).[4] An X-ray crystal structure of **1a** bound to HIV PR showed a similar conformation with similar hydrogen bonds to the enzyme as the acyclic inhibitor **4** (Fig. 1).

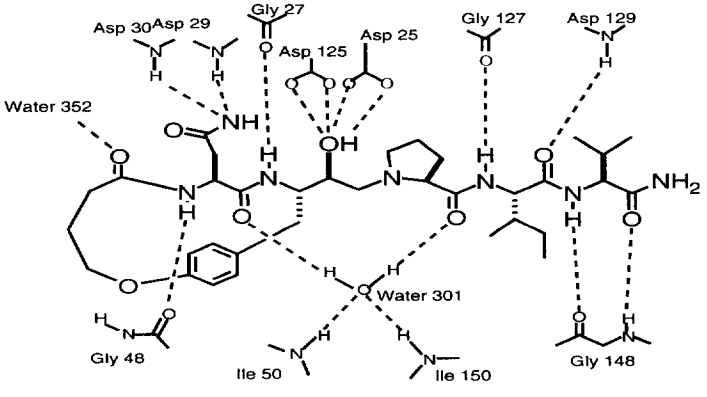
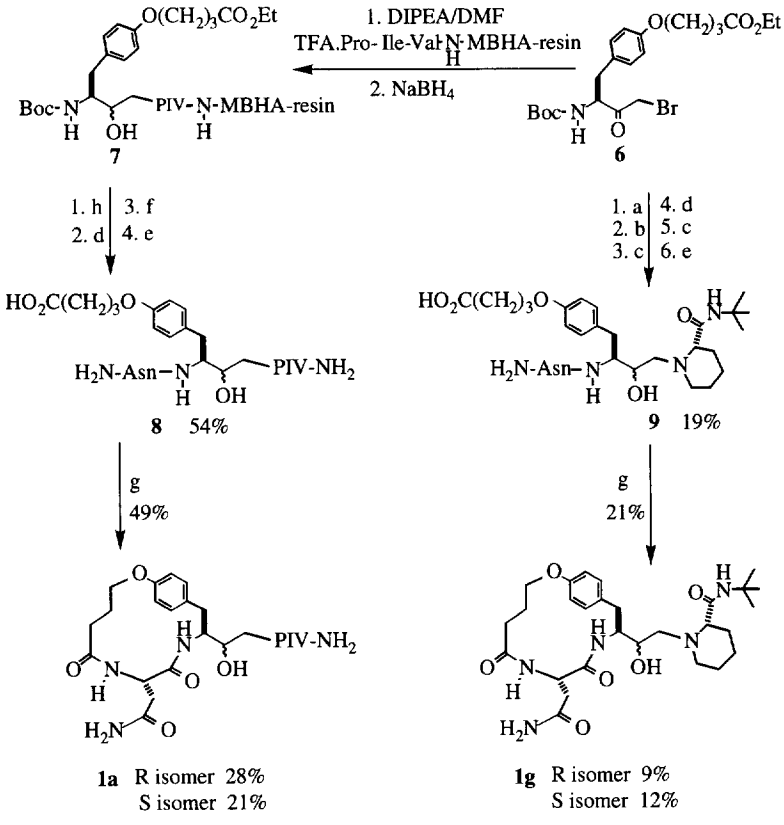


Figure 1

This structural mimicry resulted in functional mimicry (K_i = 12 nM (**1a**), 3 nM (**4**)), with the N-terminal macrocycle (Fig. 1) possessing conformational rigidity[5] due to the presence of one aromatic and two *trans* amide planes. Because this cycle fixes the position and orientation of the amino acid side chains at P1, P2, and P3, it becomes theoretically possible to independently vary the P1′, P2′, and P3′ subsites of the inhibitor C-terminus without affecting interactions between the macrocyclic N-terminus and enzyme. We now report preliminary observations of such regioselective structure-activity optimisation of macrocyclic inhibitors of HIV PR.

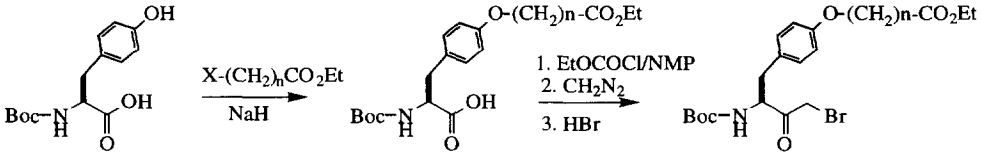


(a) HCl.PIP-NHtBu/DIPEA/CH_2Cl_2, (b) $NaBH_4$, (c) HCl, (d) HBTU/DIPEA/DMF/Boc-Asn-OH, (e) NaOH, (f) HF, (g) BOP/DIPEA, (h) TFA

Scheme 1

The cyclic inhibitors of HIV PR reported in Table 1 were synthesized via two similar procedures outlined in Scheme 1. Cycles attached to the C-terminal fragment Pro-Ile-Val-NH_2 were prepared via solid-

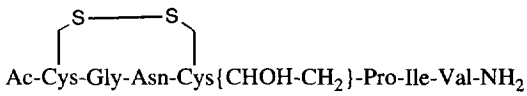
phase synthesis by our reported method,[3] shown here for compound **1a**. Other macrocyclic analogues were synthesized in solution via the strategy illustrated for **1g**. Both procedures require the synthesis of *O*-alkylated tyrosine ketobromide derivatives which can be obtained (yield 70-90%) by direct alkylation of Boc-tyrosine with either ethyl 4-bromobutyrate (-$(CH_2)_3$- derivatives),[3] ethyl 5-bromopentanoate (-$(CH_2)_4$- derivatives) or ethyl chloroacetate (-CH_2- derivatives)[6] in the presence of NaH (Scheme 2). These modified amino acids were converted to α-bromoketones via reported procedures.[3]



Scheme 2 n = 1 X = Cl ; n = 3, 4 X = Br .

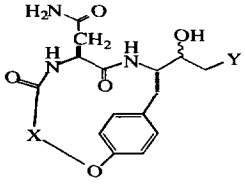
The α-bromoketones reacted with various nitrogen nucleophiles to form amino methyl ketones which were reduced with $NaBH_4$ to give a diastereomeric mixture (typically 1:1) of hydroxyethylamine derivatives. These intermediates were elaborated to the linear precursors (**8**,**9** Scheme 2), which were cyclised in dilute solution (10^{-4} M, DMF) using BOP/DIPEA to macrocycles (yields ~20-50 %). Diastereomers were separated and purified by rp-HPLC.

Derivatives of **1a** were designed, synthesised, and assayed (Table 1) to optimise inhibitor potency. Increasing the size of the 15-membered macrocycle of **1a** (e.g., **1b**) only marginally increased inhibitor potency, while the 17-membered cycle **1i** was two times less active than **1h** suggesting that a 16-membered cycle is essentially optimised. Incorporating an extra amino acid in the cycle and using a one methylene spacer unit gave the 16-membered cycles (**2a-c**). With respect to **1b**, the extra amide bond was expected to further constrain the cycle and provide interaction with Arg 8 (via the carbonyl oxygen) while the side chain of the extra amino acid could interact with either Asp 29 and/or Asp 30, at the S3 subsite of the enzyme. There is some indication of success for **2c**, although we were unable to separate the diastereomers, for which the (*S*)-diastereomer should be even more active. This is in agreement with modelling studies[7] that predicted the D-Gln side chain would interact through hydrogen bonding with Asp 30 and Asp 29 of HIV PR. By contrast **2d**, which has a 14-membered macrocycle, was a much weaker inhibitor of HIV PR, (K_i 8 μM (*S*), 36 μM (*R*)).

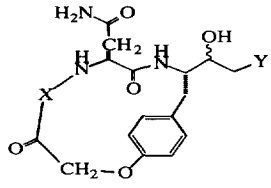


2d

Table 1 : Inhibition of HIV-1 Protease by Cyclic Peptidomimetics.



1



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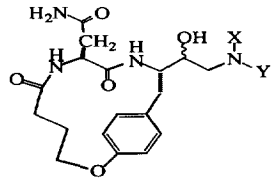
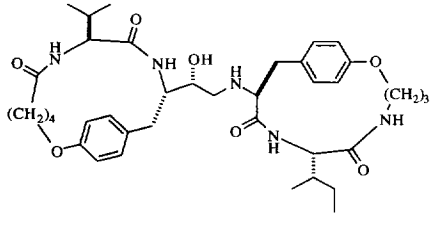


3

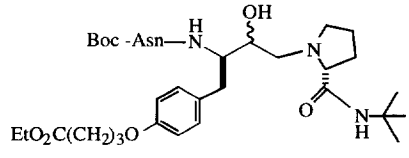
Compound	X	Y		Ki (nM)[a]
1a	-$(CH_2)_3$-	PIV-NH_2	R	470
			S	12
1b	-$(CH_2)_4$-	PIV-NH_2	R	74
			S	8
1c	-$(CH_2)_3$-	PI-NH_2	R	2,800
			S	250
1d	-$(CH_2)_3$-	P-NH_2	R	16,000
			S	36,000
1e	-$(CH_2)_3$-	Pro-*N*-tBu	R	57
			S	145
1f	-$(CH_2)_3$-	Pro-*N*-diisopropyl	R	31,000
			S	86,000
1g	-$(CH_2)_3$-	Pip-*N*-tBu	R	5
			S	1,660
1h [b]	-$(CH_2)_4$-	Pip-*N*-tBu	R	17
1i [b]	-$(CH_2)_5$-	Pip-*N*-tBu	R	50
1j	-$(CH_2)_3$-	Phe-*N*-tBu	R/S	10,300
1k [b]	-$(CH_2)_3$-	Phe-*N*-isopropyl	R/S	6,200
1l [b]	-$(CH_2)_3$-	Phe-*N*-isobutyl	R/S	800
1m	-$(CH_2)_4$-	cyclec	R	3
2a	-Gly-	PIV-NH_2	R	200
			S	11
2b	-Tyr-	PIV-NH_2	R	165
			S	11
2c	-(D)Gln-	PIV-NH_2	(R/S)	4
2d	Ac-Cys-Gly-Asn-Cys(HEA)-Pro-Ile-Val-NH_2		R	36,000
	|_____|		S	8,100
3a	isobutyl	-$CH_2C(O)NH$tBu	R	250
			S	380
3b	benzyl	-$CH_2C(O)NH$tBu	R	440
			S	13,600
3c	isoamyl	-SO_2-Phenyl	R	0.6
3d [b]	isoamyl	-SO_2-Phenyl	R	4
4	Ac-LeuAsnPhe(HEA)ProIleVal-NH_2		R	18
			S	3

[a] Assay conditions in Ref. 8. [b] Val replacing Asn in cycle. [c] Tripeptide macrocycle, see text.

As amino acids are removed from the C-terminal end of **1a**, there is a marked loss in inhibitor potency. Deletion of the Val residue (**1c**) decreases activity 20 times, further loss of isoleucine (**1d**) reduces activity 100 times more. In contrast to **1a** and **1c**, the smaller **1d** is preferred by the enzyme as the (*R*)-diastereomer, the (*S*)-isomer being half as potent. Replacing the primary amide of **1d** with the bulkier t-butyl amide (**1e**) substantially increased inhibitor potency, the (*R*)-diastereomer being more active (K_i = 57 nM vs. 145 nM). This is consistent with trends observed for peptidic analogues of JG-365[9] where bulky substituents are directed into the P2′ pocket of the enzyme reversing the stereochemical preference for the hydroxyl group. Incorporating two bulky diisopropyl substituents at P2′ (**1f**) dramatically decreased inhibition consistent with steric interference with the enzyme. Replacing proline with (*L*)-pipecolinic acid (**1g**) increased inhibition 10-fold over **1e** due probably to more efficient fitting of this larger residue in the S1′ subsite. As expected the (*R*)-diastereomer is the more active isomer for all truncated analogues reported in Table 1. Compounds **1j-l**, which contain a phenylalanine at P1′ and a bulky substituent at P2′, are evidently sterically impeded from fitting the substrate-binding groove. Molecule **1m** is a bicycle,[5] in which the C-terminal substituent (Y) is a macrocycle that mimics Phe-Ile-Val, that potently inhibits HIV-1 protease.



1m



5

To simplify the P1′ and P2′ substituents, analogues **3a-d** were synthesized and tested. Replacing the proline ring with an *N*-alkylated glycine derivative (**3a**) resulted in a dramatic loss of activity, while replacing the isobutyl chain with the bulkier benzyl substituent (**3b**) was even worse. This attenuated activity is attributed to either incorrect orientation of the carbonyl oxygen or the latter is too far away to hydrogen bond with water '301' of the enzyme. A similar compound SC52151 lacking the methylene unit is very active.[1,2] In contrast the insertion of *N*-isoamyl benzene sulfonamide, a component of inhibitior VX-478,[2] into P1′-P2′ positions gave very active inhibitors **3c** and **3d**. **3c** is 2-5 times more potent than reference acyclic inhibitor **4**.[3] Compound **3d** with Asn replaced by Val is less active than **3c**, due to lack of two hydrogen-bonds known to be made with the enzyme by the Asn side chain (Fig. 1).

The linear precursor **5** (K_i = 2,100 nM) is ~37 times less potent than cycle **1e**, providing an indication of the entropic advantage brought by the cycle to inhibitor binding. This may under-estimate the effect, since the linear compound can potentially make additional hydrogen bonds with enzyme via its ester group and its Boc substituent may make hydrophobic contact with enzyme. Other cyclic inhibitors of HIV PR have been reported before[10] but their protease-bound structures are not known. By contrast, crystal structures for inhibitors **1a**, **1g**, **1m**, and an analogue of **3d** all bound to HIV-1 protease have been deposited in the Brookhaven data bank and demonstrate optimal structural mimicry of the receptor-bound conformation of the tripeptide. Elsewhere we show that the cycles confer not only conformational rigidity to half of the inhibitor but also stability toward peptidases.[3,5] This method of regioselectively constraining peptide segments may find more general applications for enzyme inhibition.

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