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SHORT AND UNEXPECTEDLY POTENT DIFLUOROSTATONE TYPE INHIBITORS OF HIV-1 PROTEASE

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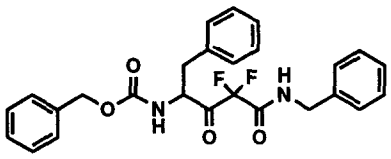
Abstract. Structure-activity relationship and concept evaluation studies have yielded short and unexpectedly potent inhibitors of HIV-1 protease of the difluorostatone type.

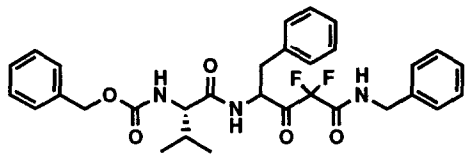
HIV-1 protease, a member of the aspartyl protease family of enzymes, represents an extremely attractive target in the treatment of AIDS[1]. Several groups have demonstrated that its inactivation by mutation of the essential active site aspartate residue[2a] or its inactivation with synthetic inhibitors[2b] produces non-infectious virions and inhibition of the spread of viral infection in susceptible cells.

Our approach to its inhibition, like that of most other groups, was based on the transition state analogue concept[3]. Screening of our protease inhibitor library resulted in the intriguing discovery of a small and potent inhibitory structure (1), a difluorostatone type peptide mimetic. That concept, first described by Abeles and coworkers in 1985, has yielded potent mechanism based inactivators of aspartyl proteases (such as pepsin[4] or renin[5]), in which hydration of the central fluorinated ketone moiety generates mimics of the tetrahedral intermediate.

This communication describes SAR studies related to ketone 1 and a comparative evaluation of the concept itself. As a result, an extremely efficient inhibitor (2) of limited size of HIV-1 protease has been discovered.



1

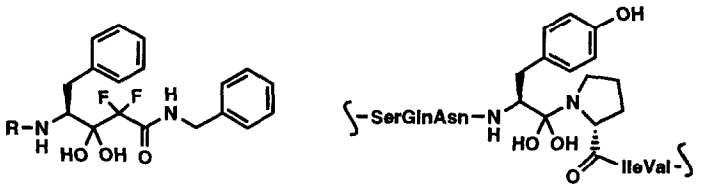


2

Lead structure (1)[6], 4-benzyloxycarbonylamino-2,2-difluoro-3-oxo-5-phenylpentanoic acid benzylamide, easily prepared in five steps from N-benzyloxycarbonylphenylalanine, inhibits HIV-1 protease with a K_I of 0.6 μM (Table I). The unexpected and interesting high affinity of this phenylalanine analogue is attributed to its difluorostatone type nature.

Furthermore, since the P_1[7] benzyl side chain of 1 matches the known P_1 recognition selectivity[8] of HIV-1 protease, the hydrated form closely resembles the postulated tetrahedral intermediate of several of the known sites cleaved by the protease, as for instance the *gag* p17/p24 cleavage site (Figure I).

FIGURE I



1 R = Cbz

2 R = CbzVal

Gag p17 / p24 cleavage site

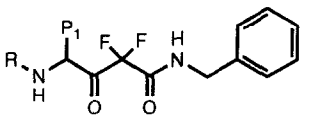
TETRAHEDRAL INTERMEDIATE

In order to optimize the affinity of 1 for the target enzyme, a systematic SAR was performed and selected results are presented below.

P_1 residue

As shown in Table I , the replacement of the P_1 benzyl substituent of ketone 1 by either a 4-hydroxyphenylmethylene (6) or a trimethylsilylmethylene (3 or 7) side chain is tolerated. However, introduction of a more sterically demanding group such as 1-naphthylmethylene (4) results in loss of almost all activity.

TABLE I



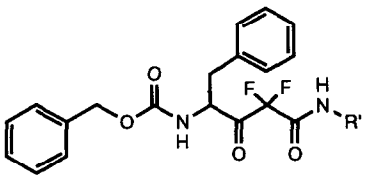
N°	R	P_1	K_i or IC_{50}^* (10^{-6} M)[9]
1	Cbz	$CH_2C_6H_5$	0.6
3	Cbz	$CH_2Si(CH_3)_3$	12
4	Cbz	CH_2(1-Naphthyl)	>>1000
5	Boc	$CH_2C_6H_5$	4*
6	Boc	CH_2(4-OH)C_6H_5	0.3
7	Boc	$CH_2Si(CH_3)_3$	2.5

Carboxy terminus

Benzamide (1), neopentylamide (8) and trimethylsilylmethyl amide (9) exhibit comparable HIV-1 protease inhibitory activity. However, removal of the

nitrogen amide substituent R'(ketone 10) results in a dramatic decrease in potency (Table II).

TABLE II

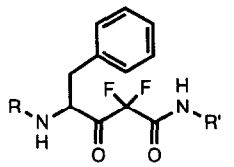


N°	R'	K_i or IC_{50}^* (10^{-6}M) [9]
1	$CH_2C_6H_5$	0.6
8	$CH_2C(CH_3)_3$	0.65
9	$CH_2Si(CH_3)_3$	0.6
10	H	300*

Amino terminus

Inhibition of HIV-1 protease occurs at comparable concentrations with carbamates 1 or 5 (Table I). The addition of one more residue in position P_2 does not improve the inhibition constants (Table III, ketones 11 and 12) until a β-disubstituted amino acid residue is introduced. The *tert*-leucine dipeptide 13, and more spectacularly, the valine derivative 2 inactivate the target protease in the low nanomolar concentration range. This result is extremely intriguing in that dipeptide 2 is about 160 times more potent than the hexapeptide difluorostatone analogue 14, described by Dreyer and coworkers[10].

TABLE III



N°	R	R'	K_i or IC_{50}^* (10^{-9} M) [9]
1	Cbz	$CH_2C_6H_5$	600
11	Cbz-n-Val*	$CH_2C_6H_5$	400
12	CbzLeu	$CH_2C_6H_5$	750*
2	CbzVal	$CH_2C_6H_5$	1
13	Cbz-t-Leu*	$CH_2C_6H_5$	27*
14[10]	BocSerAlaAla	ValValOCH$_3$	160

* n-Val represents (S)-propylglycine and t-Leu, (S)- t-butylglycine.

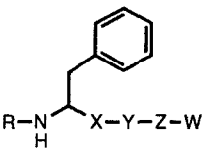
Concept evaluation

In order to gain more insight into the essential components of our inhibitors, we prepared and tested some closely related analogues. α-Diketoamide 15[11] and fluoroketoneretroamide 16 (a true isostere of phenylalanyl glycine)[12] are respectively 10 and 100 times less potent than lead structure 1 (Table IV).

Moreover alcohol 17[13] and difluoromethylene-1,3-diketone 18[14] are respectively 5000 and 100 times less efficient in inhibiting HIV-1 protease than dipeptide 2.

From these studies it can be concluded that both the ketone and the amide functionalities of inhibitors 1 and 2 are essential[15]. The electronic effects[16], hydrogen bonding potential[17], suitable geometry[16] and internal distances[18] contribute to some extent to the strong *in vitro* inhibition of HIV-1 protease by 2[21].

TABLE IV



N°	R	X	Y	Z	W	K_i or IC_{50}* (10^{-9} M) [9]
1	Cbz	CO	CF_2	CO	$NHCH_2C_6H_5$	600
15	Cbz	CO	CO	CO	$NCH_3CH_2C_6H_5$	5000
16	Cbz	CO	CF_2	CH_2	$NHCOCH_2C_6H_5$	60000
2	CbzVal	CO	CF_2	CO	$NHCH_2C_6H_5$	1
17	CbzVal	CHOH	CF_2	CO	$NHCH_2C_6H_5$	5000*
18	CbzVal	CO	CF_2	CO	$(CH_2)_2C_6H_5$	100*

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6) All new compounds were characterized by ^1H NMR and ^{19}F NMR, MS and /or combustion analysis. Compound 1 was prepared according to procedures described in : Thaisrivongs, S.; Pals, D.T.; Kati, W.M.; Tuner, S.R.; Thomasco, L.M.; Watt, W., *J. Med. Chem.* **1986**, *29*, 2080.

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9) HIV-1 protease assay:
Protein source: recombinant enzyme (E. Coli); substrate: H-SerGlnAsnTyrProIleValNH$_2$ ($K_M \approx 1$ mM); buffer: 0.1 M Mes-tri acetate, 0.2 M NaCl, pH 5.5-6.0 (EDTA, PMSF, DTT 1 mM and 0.5% BSA), 37°C; kinetic analysis: HPLC analysis of the two products.

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13) Surprisingly alcohol 17 is much less potent than ketone 2 in contrast to the results obtained with a similar series of difluorostatine or difluorostatone derivatives on pepsin[4].

14) Difluoromethylene-1,3-diketone 18 was prepared as follows in 17 % yield.
2-(Benzyloxycarbonylvalylamino)-4,4-difluoro-1,7-diphenyl-3-hydroxy-5-keto-heptane. To a suspension of activated zinc (0.196 g, 3 mAtg) and titanium tetrachloride (0.019g, 0.1 eq) in anhydrous tetrahydrofuran (3 mL) was added, under nitrogen at 0°C, a mixture of N-benzyloxycarbonylvalylphenylalaninal (0.420 g, 1.1 mmol) and 1-chloro-1,1-difluoro-2-oxo-4-phenylbutane (0.218 g, 1 mmol) in anhydrous tetrahydrofuran (2 mL). The temperature was allowed to rise to room temperature and stirring was continued for 15 hours. The mixture was hydrolyzed with saturated aqueous ammonium chloride and extracted with diethyl ether (2 x 15 mL). The combined organic layers were washed with brine and dried over anhydrous magnesium sulphate. Filtration and removal of the solvent *in vacuo* yielded a white solid recrystallized from ethyl acetate/pentane mixture. 0.381 g (67 %) of the expected alcohol was obtained.
Oxidation to 1,3 diketone 18. To a solution of oxalyl chloride (89.7 mg, 4 eq) in methylene chloride (1 mL) at - 55°C, under nitrogen, was added dropwise a solution of dimethylsulphoxide (110.4 mg, 8 eq) in methylene chloride (0.5 mL). The mixture was stirred at - 55°C for 10 min before the addition of a solution of 2-(benzyloxycarbonylvalylamino)-4,4-difluoro-1,7-diphenyl-3-hydroxy-5-keto-heptane (100 mg, 1 eq) in methylene chloride (2 mL). The mixture was stirred at -55°C for 2 hours. The temperature was then allowed to rise to - 20°C. Triethylamine (107 mg, 6 eq) was added dropwise. The mixture was stirred for an additional few minutes. The solution was diluted with ethyl acetate (10 mL). The organic phase was washed with 0.1 N hydrochloric acid (3 x 3 mL) and saturated aqueous ammonium chloride. The organic layer was dried over anhydrous magnesium sulfate, filtered and the solvent removed *in vacuo*. The crude residue was recrystallized from ethyl acetate/pentane. 26 % of the desired ketone 18 was isolated as a white solid.

15) A series of related structures lacking the difluoromethylenamide functionality has been published by Sham, H.L. *et al.* in *Biochem. Biophys. Res. Comm.* **1991**, *175*, 914. These potent fluoromethyleneketones being more flexible, do apparently interact with both S_1 and S'_1 binding pockets (as mentioned by Sham, H.L. *et al*), in contrast to our 1,3-dicarbonyl inhibitors 2 and 18.

16) Difluoromethyleneketones 1 and 2 act as hydrates at the level of the active site. Thus their carboxyterminal amides are adjacent to two sp^3 hybridized carbon atoms. α-diketoamide 15 has been shown by NMR to be hydrated only at the central carbonyl[11].

17) Hydrates of ketone 1 and 2 present four hydrogen bonding possibilities (two OH groups and two F atoms)[19] next to the carboxy terminal amide, in contrast to diketoamide hydrate 15 (one CO and two OH groups)[11].

18) Distances between benzamide carbonyl and the Cbz or P_2 valine carbonyls of inhibitors 1 and 2 respectively are suitable for optimal interaction with the essential water[20] molecule (W301), found in the complexes formed by the inhibitors with HIV-1 protease.

19) X-ray analysis of a complex formed by Porcine Pancreatic Elastase with a difluorostatone derivative[22] reveals hydrogen bond formation between the fluorine atoms and a hydrogen bond donor of the active site of the enzyme. Recent X-ray data led to similar observations for some of our inhibitors with HIV-1 protease (J.-M. Rondeau *et al.*, manuscript in preparation).

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21) The ability of compound 2 to inhibit virus replication in HIV-1 chronically or acutely infected cell systems has been established (Tyms, S.A.; Taylor, D.L; MRC Collaborative Centre, London, U.K). Chronic infection (H_9 cells, RF HIV-1 strain): ED_{50} = 3μM, determined by analysis for p24 antigen (p24 ELISA method). Acute infection (MT4, C_{8166} and JM cell lines, RF HIV-1 strain): ED_{50} = 2-5 μM; CD_{50} = 20-30 μM, determined by the MTT viability assay.

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