Volume 329, number 1,2, 144–146 FEBS 12882 August 1993

Pseudo-symmetrical difluoroketones

Highly potent and specific inhibitors of HIV-1 protease

Hing L. Sham, David A. Betebenner, Norman Wideburg, Ayda C. Saldivar, William E. Kohlbrenner, Adrienne Craig-Kennard, Sudthida Vasavanonda, Dale J. Kempf, Jacob J. Clement, John E. Erickson, Jacob J. Plattner and Daniel W. Norbeck

Abbott Laboratories, Anti-infective Research, D-47D, Abbott Park, IL 60064-3500, USA

Received 8 June 1993; revised version received 5 July 1993

A series of novel, pseudo-symmetrical difluoroketones which are highly potent inhibitors of the HIV-1 protease (IC_{50} = 1.55–0.02 nM) were synthesized. These compounds also possess good antiviral activity by inhibition of the cytopathic effect of HIV-1_{3B} in MT-4 cells in vitro.

HIV-1; Protease inhibitor; Difluoroketone; Anti-viral

1. INTRODUCTION

The human immunodeficiency virus (HIV), which is the causative agent of AIDS, encodes a protease which proteolytically processes the *gag* and *gag-pol* polyproteins to form the mature proteins needed for the production of infectious viral particles [1]. The HIV-1 protease is a member of the aspartic acid protease family and has been shown by X-ray crystallography to be a C-2 symmetrical dimer [2,3]. Replacement of the aspartic acid residues at the catalytic active site by site-directed mutagenesis leads to the formation of immature, noninfective virions [4,5]. For this reason, the HIV protease is considered as an important target for the development of agents for the treatment of HIV infection. Towards this end, potent and selective inhibitors of HIV protease have been shown to inhibit the spread of HIV infection in vitro [6–14].

We wish to report the synthesis and the biological evaluation of a novel series of pseudo-symmetrical difluoroketones which complements the symmetric, dimeric nature of the HIV-1 protease. This series, which is represented by the generic structure **1** in Scheme 1, displays highly potent and selective activity against the HIV protease. The activities of these compounds against a series of other aspartic acid proteases such as renin, pepsin or cathepsin D are at least 5 to 6 orders of magnitude less potent. The hydrolyzable amide bond of the -Phe(S_1)-Pro(S_1')-cleavage site of the substrate of HIV protease is replaced by a non-hydrolyzable difluoroketone transition state mimic [15] in these inhibitors. The P_1 and P_1' side chains are kept identical (benzyl or isobutyl). The N-terminal blocking group represented by T (see Scheme 1) can be benzyloxycarbonyl, 2-pyridyl-carbamate, 2-pyridyl-urea or 2-pyridyl-sulfonamide to provide a range of different aqueous solubilities to the inhibitors [16].

Correspondence address. H.L. Sham, Abbott Laboratories, Anti-infective Research, D-47D, Abbott Park, IL 60064-3500, USA.

2. MATERIALS AND METHODS

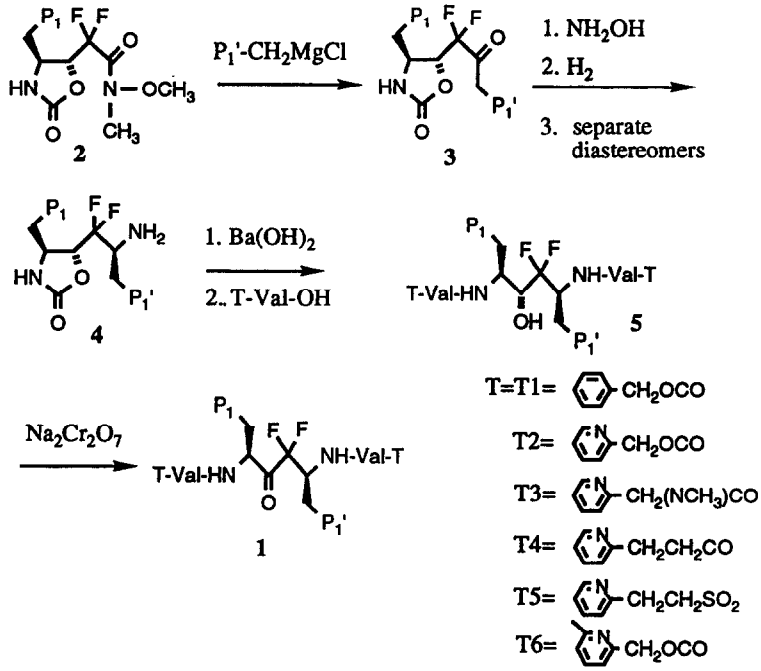
2.1. *Synthesis*

The synthesis of the novel series of difluoroketones represented by the generic structure **1** is outlined in Scheme 1. The addition of benzyl or isobutyl magnesium chloride to the known amide **2** [17] by the procedure of Weinreb [18] provided intermediate ketone **3**. Oxime formation and catalytic hydrogenation provided the corresponding amine **4** (as a mixture of *S* and *R* configuration) which can be chromatographically separated and the *S*-amine was used for further reaction. The assignment of the stereochemistry was based on X-ray crystallography [17]. Opening of the oxazolidinone ring in **4** by basic hydrolysis and coupling with the protected L-valine fragment using DCC/HOBt coupling conditions provided compound **5**. Oxidation of **5** with sodium dichromate in acetic acid [19] provided the series of difluoroketones **1**.

2.2. *Protease inhibition assay*

HIV-1 protease inhibition was measured by a fluorescence assay [20] using recombinant HIV-1 protease, isolated as described previously [21]. Reactions were carried out at 30°C in reaction mixtures containing the following in a final volume of 300 μl: 125 mM sodium acetate, pH 4.5; 1 M sodium chloride; 0.5 mM dithiothreitol; 0.5 mg/ml bovine serum albumin; 1.3 μM fluorogenic substrate [20]. The fluorogenic substrate used is Dabcyl-Ser-Gln-Asp-Tyr-Pro-Ile-Val-Gln-EDANS wherein DABCYL = 4-(4-dimethylaminophenyl)azobenzoic acid and EDANS = 5-((2-aminoethyl)amino)- naphthalene-1-sulfonic acid. Inhibitors, when present, were added from stock solutions made in dimethylsulfoxide. The final dimethylsulfoxide concentration was adjusted to 2% in all cases. The reactions were initiated

 Published by Elsevier Science Publishers B.V.



Scheme 1.

by the addition of approximately 1 nM HIV-1 protease and rates were determined by following the change in fluorescence intensity (excitation 340 nM, emission 490 nM) that accompanies the cleavage of the fluorogenic substrate. The % inhibition of enzyme activity in the presence of inhibitor was determined by comparing inhibition rates with uninhibited control reactions. All IC_{50} values were determined with a range of inhibitor concentrations using the relation IC_{50} = (100 : % inhibition − 1) × inhibitor concentration. IC_{50} values should closely approximate true K_i values since the substrate concentration is much less than the K_m determined previously [20].

2.3. *Antiviral assays*

The in vitro antiviral activities of the difluoroketones is determined by the inhibition of the cytopathic effect (CPE) of HIV-1_{3B} in MT-4 cells [22]. MT-4 cells (1×10^4) were infected with 10 $TCID_{50}$ of HIV-1_{3B} for 1 h, washed, and cultured at 37°C in RPMI 1640 + 10% FBS medium containing inhibitors (fluoroketones) in triplicate. Cytopathic effect and toxicity were monitored at day 5 by MTT uptake in 96-well microtiter plates using a Biotek model EL 320 at a wavelength of 570–650 nM. The IC_{50} and TC_{50} values are shown in Table III.

3. RESULTS AND DISCUSSION

A series of pseudo-symmetrical difluoroketones that complements the C-2 symmetric nature of the HIV-1 protease was synthesized and their inhibitory potency of recombinant HIV-1 protease was determined. The results expressed in IC_{50} values are shown in Table I. These compounds were represented by the generic structure **1**. Compound **1a** can be considered the parent compound of the series with T = T_1 = benzyloxycarbonyl, a common N-terminal protecting group for amino acids. The P_1 and P_1' side chains are benzyl groups. It is a potent inhibitor of the HIV-1 protease (IC_{50} = 0.1 nM). Changing the phenyl ring of the benzyloxycarbonyl protecting group to the 2-pyridyl (T_2) increased the inhibitory potency by 5-fold. Changing the P_1 and P_1' side chain from benzyl to isobutyl (**1g**) resulted in a decrease in potency of more than 10-fold. Variation of the linking group from a carbamate (T_1, T_2) to that of *N*-methyl-urea (T_3); amide (T_4); or sulfonamide (T_5) maintained the inhibitory potency below 1 nM, while affording a variation of aqueous solubilities to the compounds. It is also important to note that all the compounds have high specificity against HIV-1. As shown in Table II, there are at least 5 to 6 orders of magnitude difference in inhibitory potency against HIV-1 and a panel of other aspartic proteases.

The in vitro antiviral activities of the difluoroketones against HIV-1_{3B} in MT-4 cells are summarized in Table III. Compound **1a**, though quite non-toxic to the cells, did not show any antiviral activity. This may be due to the highly lipophilic nature of **1a**. The proper balance

Table I

Inhibition of HIV-1 protease by fluoroketones **1**

Compound no.	T	P_1	P_1'	IC_{50} (nM)
1a	T_1	phenyl	phenyl	0.10
1b	T_2	phenyl	phenyl	0.02
1c	T_3	phenyl	phenyl	0.40
1d	T_4	phenyl	phenyl	0.33
1e	T_5	phenyl	phenyl	0.24
1f	T_6	phenyl	phenyl	0.05
1g	T_3	isopropyl	isopropyl	1.55

For T_1 to T_6, see Scheme 1 for structures.

Table II

Inhibition of other aspartic acid proteases by fluoroketones **1** (IC_{50})

Compound no.	Renin	Pepsin	Cathepsin D
1a	>100 μM	90 μM	>100 μM
1b	>100 μM	407 μM	94 μM
1c	>100 μM	165 μM	77 μM
1d	>100 μM	127 μM	>100 μM
1e	>100 μM	256 μM	>100 μM
1f	>100 μM	210 μM	>100 μM
1g	>100 μM	165 μM	98 μM

between lipophilicity and hydrophilicity is important for antiviral activity because of cell membrane permeability. Compounds **1b–1g**, with 2-pyridyl groups incorporated to increase their hydrophilicity, all showed good antiviral activity. The selectivity index (SI = TC_{50}/IC_{50}) which is an indication of the compounds' effectiveness in inhibition of viral spread relative to their cell toxicity showed a fairly wide range. Compound **1f**, which inhibited the HIV-1 protease with an IC_{50} = 0.05 nM, exhibited a good antiviral activity (IC_{50} = 7 nM) and an excellent SI (>14,000).

In conclusion, a novel series of pseudo-symmetrical difluoroketones which are highly potent inhibitors of the HIV-1 protease and exhibit good antiviral activities in vitro are described. With further refinement, it is feasible to synthesize inhibitors with even greater potency and hopefully oral bioavailability. Inhibition of the retroviral HIV-1 protease may provide a novel and potentially useful therapeutic approach for the treatment of AIDS and related diseases.

Acknowledgements: We are grateful to H. Stein and J. Cohen for performing the renin assays.

Table III

Inhibition of HIV-1 in vitro (MT-4 cells) by fluoroketones **1**

Compound no.	IC_{50} (μM)	TC_{50} (μM)	SI (TC_{50}/IC_{50})
1a	no effect	>100	N.D.
1b	0.04	>100	>2,500
1c	0.035	56	1,600
1d	0.05	>100	>2,000
1e	0.56	42	75
1f	0.007	>100	>14,285
1g	2.3	63	27
AZT	0.016	>10	>639

REFERENCES

[1] Kramer, R.A., Schaber, M.D., Skalka, A.M., Ganguly, K., Wong-Staal, F. and Reedy, E.P. (1986) Science 231, 1580–1583.
[2] Navia, M.A., Fitzgerald, P., McKeever, B., Leu, C.T., Heimbach, J., Herber, W., Sigal, I. and Darke, P. (1989) Nature 337, 615–618.
[3] Wlodawer, A., Miller, M., Jaskolski, M., Sathyanarayana, B.K., Baldwin, E., Weber, I.T., Selk, L.M., Clawson, L., Schneider, J. and Kent, S.B.H. (1989) Science 245, 616–618.
[4] Seelmeier, S., Schmidt, H., Turk, V. and von der Helm, K. (1988) Proc. Natl. Acad. Sci. USA 85, 6612–6616.
[5] Kohl, N.E., Emini, E.A., Schleif, W.A., Davies, L.J., Heimback, J.C., Dixon, R.A.F., Scolnick, E.M. and Sigal, I.S. (1988) Proc. Natl. Acad. Sci. USA 85, 4686–4690.
[6] Moore, M.L., Bryan, W.M., Fakhoury, S.A., Magaard, V.W., Huffman, W.F., Dayton, B.D., Meek, T.D., Hyland, L., Dreyer, G.B., Metcalf, B.W., Strickler, J.E., Gorniak, J.G. and Debouck, C. (1989) Biochem. Biophys. Res. Commun. 159, 420–425.
[7] Dreyer, G.B., Metcalf, B.W., Tomaszek Jr., T.A., Carr, T.J., Chandler, A.C., Hyland, L., Fakhoury, S.A., Magaard, V.W., Moore, M.L., Strickler, J.E., Debouck, C. and Meek, T.D. (1989) Proc. Natl. Acad. Sci. USA 86, 9752–9756.
[8] Sigal, I.S., Huff, J.R., Darke, P.L., Vacca, J.P., Young, S.D., deSolms, J.S., Thompson, W.J., Lyle, T.A., Graham, S.L. and Ghosh, A.K. (1988) European Patent Application no. 033714.
[9] McQuade, T.J., Tomasselli, A.G., Lui, L., Karacostas, V., Moss, B., Sawyer, T.K., Heinickson, R.L. and Tarpley, W.G. (1990) Science 247, 454–456.
[10] Robert, N.A., Martin, J.A., Kinchington, D., Broadhurst, A.V., Craig, J.C., Duncan, I.B., Galpin, S.A., Handa, B.K., Kay, J., Krohn, A., Lambert, R.W., Merrett, J.H., Mills, J.S., Parkes, K.E.B., Redshaw, S., Richie, A.J., Taylor, D.L., Thomas, G.J. and Muchin, P.J. (1990) Science 248, 358–361.
[11] Rich, D.H., Green, J., Tota, M.V., Marshall, G.R. and Kent, S.B.H. (1990) J. Med. Chem. 33, 1285–1288.
[12] Kempf, D.J., Norbeck, D.W., Codacovi, L., Wang, X.C., Kohlbrenner, W.E., Wideburg, N.E., Paul, D.A., Knigge, M.F., Vasavanonda, S., Craig-Kennard, A.C., Saldivar, A., Rosenbrook Jr., W., Plattner, J.J. and Erickson, J. (1990) J. Med. Chem. 33, 2687–2689.
[13] Thompson, W.J., Fitzgerald, P., Holloway, M.K., Emini, E.A., Darke, P.L., McKeever, B.M., Schleif, W.A., Quintero, J.C., Zugag, J.A., Tucker, T.J., Schwering, J.E., Homnick, C.F., Nunberg, J., Springer, J.P. and Huff, J.R. (1992) J. Med. Chem. 35, 1685–1701.
[14] Tucker, T.J., Lumma, W.C., Payne, L.S., Wai, J.M., deSolms, S.J., Giuliani, E.A., Darke, P.L., Heimback, J.C., Zugag, J.A., Schleif, W.A., Quintero, J.C., Emini, E.A., Huff, J.R. and Anderson, P.S. (1992) J. Med. Chem. 35, 2525–2533.
[15] For the concept and application of fluoroketones as inhibitors of hydrolytic enzymes, see Gelb, M.H., Svaren, J.P. and Abeles, R.H. (1985) Biochemistry 24, 1813–1820.
[16] Kempf, D.J., Norbeck, D.W., Erickson, J.E., Codacovi, L., Plattner, J. and Sham, H. (1990) European Patent Application no. EP 0402646A.
[17] Sham, H.L., Wideburg, N.E., Spanton, S.G., Kohlbrenner, W.E., Betebenner, D.A., Kempf, D.J., Norbeck, D.W., Plattner, J.J. and Erickson, J.E. (1991) J. Chem. Soc. Chem. Commun. 110–112.
[18] Nahm, S. and Weinreb, S.M. (1981) Tet. Lett. 22, 3815–3818.
[19] Gallina, C. and Giordano, C. (1989) Synthesis 466–467.
[20] Matayoshi, E.D., Wang, G.T., Kraft, G.A. and Erickson, J.E. (1990) Science 247, 954–958.
[21] Rittenhouse, J., Turon, M.C., Helfrich, R.J., Albrecht, K.S., Weigl, D., Simmer, R.L., Mordini, F., Erickson, J. and Kohlbrenner, W.E. (1990) Biochem. Biophys. Res. Commun. 171, 60–66.
[22] Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewin, P., Desmyter, J. and DeClercq, E. (1988) J. Virol. Methods 20, 309–317.