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Antiviral activity and cross-resistance profile of P-1946, a novel human immunodeficiency virus type 1 protease inhibitor

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Abstract

The HIV protease inhibitor P-1946 is a member of a novel family of L-Lysine derivatives. The compound is a specific HIV-1 protease inhibitor that has potent and selective in vitro antiviral activity (EC_{50} 152 nM) against a range of isolates resistant to commercially available protease inhibitors. The presence of at least four primary and four secondary drug resistance mutations is required to achieve greater than four-fold resistance to P-1946. P-1946's favorable resistance profile makes it a good lead for the development of new agents active against existing PI-resistant virus in treatment-experienced patient.

Keywords: Protease inhibitors; P-1946; HIV-1 resistance

HIV-1 protease is an excellent therapeutic target since its inhibition prevents proteolytic processing of the Gag and Gag-Pol polyproteins, thereby blocking viral maturation (Debouck, 1992; Katz and Skalka, 1994; Kohl et al., 1988). Protease inhibitors (PIs), along with reverse transcriptase inhibitors (RTIs) are the basis of the Highly Active Anti-Retroviral Therapy (HAART), which has led to a significant reduction in HIV-associated morbidity and mortality since its widespread availability in 1996 (Hogg et al., 1999; Palella et al., 1998). Unfortunately, long-term use of HAART often leads to the development of drug resistance, which occurred in more than 75% isolates evaluated in AIDS patients in the United States during the last few years (Richman et al., 2001, 2004). One of the highest priorities in antiretroviral drug research today is the development of new HIV inhibitors that exhibit distinct resistance profiles to provide patients with more alternatives in combination therapy. In this study, we present the enzyme inhibition properties and antiviral potency of P-1946, a representative compound of a novel family of L-lysine derivatives (Stranix et al., 2003) targeting HIV protease (Fig. 1). These compounds containing a highly flexible inner backbone are proposed to have a facilitated binding adaptation to HIV protease mutations.

The potency of P-1946 to inhibit wild-type HIV-1 protease enzyme was assessed by measuring the inhibitory constant (K_i) using recombinant, highly purified HIV-1 protease and a fluorogenic peptide substrate (Matayoshi et al., 1990; Williams and Morrison, 1979). For comparison, inhibitory constants for the commercially available PIs amprenavir (APV), lopinavir (LPV), indinavir (IDV), nelfinavir (NFV), ritonavir (RTV) and saquinavir (SQV) were also determined in parallel. P-1946 and IDV showed a K_i of 2.6 and 1.9 nM, respectively, while the other PIs scored under 1 nM (Table 1). Specificity of P-1946 was assessed by evaluating its ability to inhibit two cellular aspartyl proteases, human cathepsin D (Calbiochem Cat. No. 219401, >95%, >300 units/mg protein) and porcine pepsin (Roche, Cat. No. 100911, ~2500 units/mg protein) and the results were compared to the activity of APV since both compounds share the same aminobenzenesulfonamide moiety. P-1946 showed weak affinity for these two proteases as indicated by IC_{50}s greater than 30 μM, while APV displayed significant inhibition of pepsin (Table 2). Thus, P-1946 represents a potent and highly specific inhibitor of HIV-1 protease.

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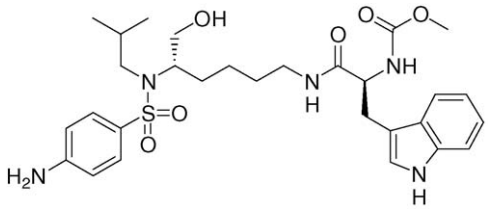

Fig. 1. Chemical structure of P-1946.

Table 1
Inhibition of HIV-1 aspartyl protease by PIs

PI	$K_i \pm$ S.D. (nM)[a]
P-1946	2.600 ± 0.076
APV	0.638 ± 0.077
LPV	0.477 ± 0.096
IDV	1.945 ± 0.358
NFV	0.661 ± 0.153
RTV	0.489 ± 0.078
SQV	0.510 ± 0.073

[a] Mean of three experiments. Each determination was done in triplicate.

Table 2
Inhibitory activity of APV and P-1946 against cellular aspartyl proteases

PIs	IC_{50}[a] (μM)	
	Cathepsin D	Pepsin
APV	15.2	0.28
P-1946	30	40

[a] IC_{50} values are a mean of experiments performed twice in duplicate.

Cytotoxicity and inhibition of viral replication by P-1946 and other PIs were determined in parallel and expressed as 50% cytotoxic concentration (CC_{50}) and 50% effective concentration (EC_{50}), respectively (Table 3). The antiviral activity was determined based on inhibition of the cytopathic effects induced by wild-type and PI-resistant HIV strains in MT-4 cells cultured for 6 days (Pauwels et al., 1988). In this cell culture system, P-1946 exhibited potent antiviral activity against the laboratory wild-type strains NL4-3 and RF with EC_{50}s of 152 nM and 136 nM, respectively. Comparative studies in cell culture against wild-type virus showed a 4- to 14-fold difference between the antiviral activity of P-1946 and that of more potent, commercially available PIs. Cytotoxicity determinations using the same culture system showed that P-1946 does not exhibit significant cytotoxicity with a CC_{50} of 40 μM, yielding a selective index (SI) of 263. In comparison, only APV and IDV were less cytotoxic than P-1946 with CC_{50} values higher than 100 μM.

Table 3
Cytotoxicity and selectivity index of P-1946 and marketed PIs

Agent	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
P-1946	0.152	40	263
APV	0.030	>100	>3333
LPV	0.015	22	1467
IDV	0.041	>100	>2439
NFV	0.015	6.0	400
RTV	0.042	27	643
SQV	0.011	14	1273

[a] Antiviral activity using the molecular clone HIV-1 NL4-3 in MT-4 cells. Mean of at least three experiments.
[b] Cytotoxic concentration : drug concentration that kills 50% of MT-4 cells.
[c] Selectivity index is the ratio CC_{50}/EC_{50}.

We next evaluated the antiviral activity of P-1946 and five FDA-approved PIs using two panels of recombinant viruses carrying a range of mutations in the protease gene (Table 4). The recombinant HIV-1 strains in the first panel contained two to five amino acid substitutions in the protease-encoding region that have been reportedly associated with resistance to various PIs (Boden and Markowitz, 1998; Condra et al., 1995; Jacobsen et al., 1995; Kaltenbach et al., 2001; Otto et al., 1993). The panel included mutated laboratory-adapted strain NL4-3 showing resistance to SQV (mutations G84V, L90M) or IDV (M46I, V82T, I84V), and a RF mutant carrying mutations V82F and I84V, which arose in HIV-infected cells exposed to increasing doses of cyclic urea DMP 323 (Boden and Markowitz, 1998; Condra et al., 1995; Jacobsen et al., 1995; Kaltenbach et al., 2001; Otto et al., 1993). This first panel employed a multi-cycle assay over 6 days in MT-4 cells. The second panel, obtained through the Phenosense™ assay, included eight isolates showing loss of susceptibility to one or more PIs (ViroLogic Inc., South San Francisco, CA). It included mutants specifically resistant to NFV (D30N) and APV (I50V), isolates with low-level resistance to various PIs, and three isolates showing resistance to four or more PIs with higher than 10-fold loss of susceptibility (Table 4). For the purpose of analysis, taking into account the differences between whole-cell antiviral assays used, an isolate was considered resistant when a loss of susceptibility greater than four-fold was recorded. The antiviral activity of P-1946 was not affected by mutations 82F and 84V (strain RF2802) and by additional mutations at positions 10, 46 and 63 (strain 4596). In these assays, strain 4596 scored slightly over the four-fold loss in drug susceptibility to IDV, NFV and LPV. Typical mutations 48V/90M associated with SQV resistance did not result in resistance to P-1946 (strain SaqR), while the activity of IDV, NFV and SQV was affected by the mutations. Resistance to P-1946 was further evaluated using the second panel of eight resistant strains. P-1946 maintained its activity against viral strains V2002 and V2033 carrying key mutations associated with resistance to NFV (D30N) or APV (I50V), respectively, as well as against strain V2064, having primary mutations 46L/82A/90M. In addition, no significant reduction in P-1946 susceptibility to virus strains with primary mutations 84V/90 and 46I/84V/90M was observed, despite a background of three additional secondary mutations (strains V2066 and V2089, respectively). Loss of susceptibility to P-1946 required a minimum of four secondary mutations combined with four or more primary mutations (strains V2057, V2059 and V2031). It is noteworthy that maximum change in susceptibility to P-1946 (28-fold change) was observed with strain V2031, which carried five primary mutations (48M/53L/54V/82A/90M) and five secondary mutations (10I/63P/71I/77I/93L). This same multi-drug resistant strain showed high-level (FC > 100) resistance to IDV, NFV, LPV and SQV. Only APV scored better than P-1946 against this strain with a 11-fold loss of activity. In summary,

Table 4
Susceptibility of PI-resistant HIV strains to P-1946 and other PIs

Viral strains	Mutations in protease gene[a]	Fold decrease in susceptibility[b]					
		P-1946	IDV	NFV	LPV	APV	SQV
SaqR	**48V**, **90M**[c,d]	2.6 (395)	6.2 (254)	7.6 (106)	1.2 (15.6)	1.8 (56)	24.7 (271)
4596	**46I**, **82T**, **84V**, 10R, 63P[c,d]	3.6 (957)	5.1 (209)	4.1 (57)	5.3 (69)	3.2 (99)	1.3 (14.3)
RF2802	**82F**, **84V**[c,e]	0.9 (122)	2.4 (134)	3.6 (68)	2.3 (16.1)	2.5 (115)	1.8 (14.4)
V2002[f,g]	**30N**, **46I**, 64V, 77I	1.2 (127)	1.2 (9.6)	30.1 (203)	0.8 (4.7)	0.8 (16.7)	0.4 (1.6)
V2033[f]	**50V**, 15V, 36I, 57K, 63P	1.3 (136)	0.6 (5.2)	1.4 (9.7)	3.6 (22.5)	7.8 (157)	1.1 (4.2)
V2064[f]	**46L**, **82A**, **90M**, 12S, 19I, 64V	2.8 (295)	1.3 (11)	1.1 (7.3)	0.8 (4.8)	0.6 (12)	0.6 (2.2)
V2066[f]	**84V**, **90M**, 10I, 63P, 71T	1.7 (182)	2.6 (21)	4.8 (32)	2.4 (15)	2.9 (59)	8.0 (30)
V2089[f]	**46I**, **84V**, **90M**, 10I, 63P, 71T	2.0 (215)	3.5 (29)	6.0 (41)	3.8 (23)	3.5 (70)	5.7 (22)
V2057[f]	**46I**, **82T**, **90M**, 10I, **53L**, 63P, 71V, 73S	4.5 (474)	31.1 (255)	30.0 (203)	18.1 (112)	3.9 (79)	25.4 (97)
V2059[f]	**46I**, **84V**, **88D**, **90M**, 10I, 63P, 71V, 72V, 77I	9.3 (973)	29.4 (240)	48.6 (327)	22.3 (139)	9.9 (200)	91.4 (349)
V2031[f]	**48M**, **82A**, **90M**, 10I, 12A, 14R, 21Q, 41K, **53L**, **54V**, 63P, 71I, 77I, 93L	28.4 (2981)	⋙[h] (>1500)	143.6 (969)	100.7 (623)	11.0 (223)	⋙ (>1.5)

[a] Primary mutations associated with resistance to PIs are listed in bold type.
[b] Numbers in parentheses represent EC_{50} in nM; all the assays were performed at least in duplicate.
[c] Amino acid sequence of recombinant virus reported by NIH. EC_{50} determined using 6-day multi-cycle assay in MT-4 cells.
[d] EC_{50} (nM) for the corresponding wild-type virus were: P-1946 (152), IDV (41), NFV (14), LPV (13), APV (31), SQV (11).
[e] EC_{50} (nM) for the corresponding wild-type virus were: P-1946 (136), IDV (56), NFV (19), LPV (7), APV (46), SQV (8).
[f] V*xxxx* denomination of strains from ViroLogic's repository. Determined using Phenosense™ assay.
[g] EC_{50} (nM) for the corresponding wild-type virus were: P-1946 (106), IDV (8), NFV (7), LPV (6), APV (20), SQV (4).
[h] ⋙, over 100-fold.

P-1946 retained full activity against 8 of the 11 resistant strains tested in this study. Based on the number of strains that remained fully sensitive to the drugs, the following ranking may be suggested for the PIs: P-1946/APV > LPV > IDV > SQV > NFV. Despite the apparent similarity between APV and P-1946 cross-resistance profiles, the two compounds displayed distinct properties since strain V2033 carrying the APV signature mutation I50V remained fully sensitive to P-1946, while a 7.8-fold loss of susceptibility to APV was observed. These results suggest that P-1946 has a favorable cross-resistance profile making it a good lead for the development of new potent anti-HIV agents, offering therapeutic alternatives for individuals carrying HIV isolates resistant to current PIs. Lead optimization is underway to improve the antiviral potency while maintaining a favorable resistance profile.

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References

Boden, D., Markowitz, M., 1998. Resistance to human immunodeficiency virus type 1 protease inhibitors. Antimicrob. Agents Chemother. 42 (11), 2775–2783.

Condra, J.H., Schleif, W.A., Blahy, O.M., Gabryelski, L.J., Graham, D.J., Quintero, J.C., Rhodes, A., Robbins, H.L., Roth, E., Shivaprakash, M., et al., 1995. In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. Nature 374 (6522), 569–571.

Debouck, C., 1992. The HIV-1 protease as a therapeutic target for AIDS. AIDS Res. Hum. Retroviruses 8 (2), 153–164.

Hogg, R.S., Yip, B., Kully, C., Craib, K.J., O'Shaughnessy, M.V., Schechter, M.T., Montaner, J.S., 1999. Improved survival among HIV-infected patients after initiation of triple-drug antiretroviral regimens. CMAJ 160 (5), 659–665.

Jacobsen, H., Yasargil, K., Winslow, D.L., Craig, J.C., Krohn, A., Duncan, I.B., Mous, J., 1995. Characterization of human immunodeficiency virus type 1 mutants with decreased sensitivity to proteinase inhibitor Ro 31-8959. Virology 206 (1), 527–534.

Kaltenbach III, R.F., Trainor, G., Getman, D., Harris, G., Garber, S., Cordova, B., Bacheler, L., Jeffrey, S., Logue, K., Cawood, P., Klabe, R., Diamond, S., Davies, M., Saye, J., Jona, J., Erickson-Viitanen, S., 2001. DPC 681 and DPC 684: potent, selective inhibitors of human immunodeficiency virus protease active against clinically relevant mutant variants. Antimicrob. Agents Chemother. 45 (11), 3021–3028.

Katz, R.A., Skalka, A.M., 1994. The retroviral enzymes. Annu. Rev. Biochem. 63, 133–173.

Kohl, N.E., Emini, E.A., Schleif, W.A., Davis, L.J., Heimbach, J.C., Dixon, R.A., Scolnick, E.M., Sigal, I.S., 1988. Active human immunodeficiency virus protease is required for viral infectivity. Proc. Natl. Acad. Sci. U.S.A. 85 (13), 4686–4690.

Matayoshi, E.D., Wang, G.T., Krafft, G.A., Erickson, J., 1990. Novel fluorogenic substrates for assaying retroviral proteases by resonance energy transfer. Science 247 (4945), 954–958.

Otto, M., Garber, S., Winslow, D., Reid, C., Aldrich, P., Jadhav, P., Patterson, C., Hodge, C., Cheng, Y., 1993. In vitro isolation and identification of human immunodeficiency virus (HIV) variants with reduced sensitivity to C-2 symmetrical inhibitors of HIV type 1 protease. PNAS 90 (16), 7543–7547.

Palella Jr., F.J., Delaney, K.M., Moorman, A.C., Loveless, M.O., Fuhrer, J., Satten, G.A., Aschman, D.J., Holmberg, S.D., 1998. Declining morbidity and mortality among patients with advanced human immunodeficiency

virus infection. HIV Outpatient Study Investigators. N. Engl. J. Med. 338 (13), 853–860.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., De Clercq, E., 1988. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20 (4), 309–321.

Richman, D.D., Bozzette, S., Morton, S., Chien, S., Wrin, T., Dawson, K., Hellmann, N., 2001. The prevalence of antiretroviral drug resistance in the U.S. In: 41st International Conference on Antimicrobial Agents and Chemotherapy, Chicago, December 18 (abstract LB-17).

Richman, D.D., Morton, S.C., Wrin, T., Hellmann, N., Berry, S., Shapiro, M.F., Bozzette, S.A., 2004. The prevalence of antiretroviral drug resistance in the United States. Aids 18 (10), 1393–1401.

Stranix, B.R., Sauvé, G., Bouzide, A., Coté, A., Sévigny, G., Yelle, J., 2003. Lysine sulfonamides as novel HIV-protease inhibitors: optimization of the Nε-acyl-phenyl spacer bio-org. Med. Chem. Lett. 13, 4289–4292.

Williams, J.W., Morrison, J.F., 1979. The kinetics of reversible tight-binding inhibition. Methods Enzymol. 63, 437–467.