Stabilization from Autoproteolysis and Kinetic Characterization of the Human T-cell Leukemia Virus Type 1 Proteinase*

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We have developed a system for expression and purification of wild-type human T-cell leukemia virus type 1 (HTLV-1) proteinase to attain sufficient quantities for structural, kinetic, and biophysical investigations. However, similar to the human immunodeficiency virus type 1 (HIV-1) proteinase, HTLV-1 proteinase also undergoes autoproteolysis rapidly upon renaturation to produce two products. The site of this autoproteolytic cleavage was mapped, and a resistant HTLV-1 proteinase construct (L40I) as well as another construct, wherein the two cysteine residues were exchanged to alanines, were expressed and purified. Oligopeptide substrates representing the naturally occurring cleavage sites in HTLV-1 were good substrates of the HTLV-1 proteinase. The kinetic parameters \( k_{\text{cat}} \) and \( K_m \) were nearly identical for all the three enzymes. Although three of four peptides representing HTLV-1 proteinase cleavage sites were fairly good substrates of HIV-1 proteinase, only two of nine peptides representing HIV-1 proteinase cleavage sites were hydrolyzed by the HTLV-1 proteinase, suggesting substantial differences in the specificity of the two enzymes. The large difference in the specificity of the two enzymes was also demonstrated by inhibition studies. Of the several inhibitors of HIV-1 or other retroviral proteinases that were tested on HTLV-1 proteinase, only two inhibit the enzyme with a \( K_i \) lower than 100 nm.

The human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that has been etiologically associated with human adult T-cell leukemia (1, 2), HTLV-1-associated myelopathy, tropical spastic paraparesis (3, 4), and a number of other chronic diseases (5). Although it has not been shown to be directly linked to the development of leukemia, several recent studies indicate that viral replication is critical for the development of HTLV-1-associated myelopathy. Initial studies have reported the treatment of this syndrome using 5′-azidothymidine (6).

All replication-competent retroviruses including HIV and HTLV-1 code for an aspartic proteinase (PR). Unlike HIV-1, HTLV-1 PR is coded through a mechanism of expression in separate gag-pro and gag-pro-pol open reading frames (7). The level of expression of the Gag and Gag-Pro polyproteins in HTLV-1 is comparable with that of Gag and Gag-Pol polyproteins in HIV-1. The function of the mature 125-amino acid-long HTLV-1 PR is crucial for virion replication (for a review, see Ref. 8). The HIV-1 PR proved to be a promising target of antiretroviral therapy of AIDS, and various PR inhibitors are now in clinical use (for a review, see Ref. 9).

As in the case of treatment of patients with reverse transcriptase inhibitors, selection of viral variants that are resistant to inhibitors of PR also develops rapidly (9). Some of the amino acid changes in the HIV-1 PR that are responsible for drug resistance are found in equivalent positions of other retroviral proteinases, including HTLV-1 (see Fig. 1). A comparative study of various retroviral proteinases is expected to reveal the common features of their specificity. These studies will aid in the rational design of inhibitors effective against different retroviral proteinases, which may reduce the frequency of occurrence of drug-resistant mutants. In this respect, many inhibitors designed against HIV-1 PR have much reduced potency against the closely related HIV-2 PR (10) as well as against the PR of lentiviral equine infectious anemia virus (11). Furthermore, a recent study demonstrated that all four HIV-1 PR inhibitors currently used in therapy failed to block HTLV-1 Gag processing in vitro (12).

Although several studies report expression of HTLV-1 PR in Escherichia coli (13–17) as well as its chemical synthesis (18), its biochemical and kinetic properties and specificity have not been well described. This can be attributed to the difficulty in protein purification and to the autoproteolysis that is known to occur with native retroviral proteinases (19, 20). Peptides representing some of the natural cleavage sites of HIV-1 are hydrolyzed by HTLV-1 PR (16, 18), although no kinetic parameters were reported for these peptides.

The crystal structures of HIV-1 and several other retroviral proteinases have been determined (21–23). However, to date no structural information is available for the HTLV-1 protease. Furthermore, insight into the mechanism of autocatalytic maturation of the mature PR from the precursor is limited to HIV-1 (24, 25). Here we report the high level expression in E. coli and rapid purification of wild type and autoproteolysis-resistant HTLV-1 proteinases. The specificities of these two enzymes were compared utilizing synthetic peptides that correspond to known Gag and Pol cleavage sites of HIV-1 and HTLV-1. Sev-
eral potent inhibitors of HIV-1 PR and other retroviral proteases have been assessed for their inhibition of the HTLV-1 PR. We also report the effect of substituting the two cysteine residues in HTLV-1 PR and define a chromogenic substrate for assaying this enzyme.

MATERIALS AND METHODS

Cloning, Expression, and Site-directed Mutagenesis of the HTLV-1 Proteinase—The PR coding region of the Sucl infectious HTLV-1 clone, pCS-HTLV-1 (26), derived by polymerase chain reaction amplification was cloned at the NdeI and BamHI sites of pet-11a vector (Novagen, WI). Mutagenesis of the Leu40 to Ile was performed by using the overlap extension method (27). The C90A and C109A mutations were introduced into the L40-I mutated HTLV-1 PR using the Quick-Change mutagenesis protocol (Stratagene, CA). Triple mutant L40I,C90A,C109A PR was prepared by using the L40I,C109A double mutant as the template.

Cells bearing the construct for the expression of the wild-type HTLV-1 PR and its mutants were grown in Luria-Bertani medium supplemented with 1% glucose at 37 °C, 30% PO2, pH 7.0, in a 3-l Braun fermenter. The culture was harvested when the cell suspension reached an absorbance of 0.5–0.6 at 600 nm, protein expression was induced by the addition of 25 mM isopropyl β-D-thiogalactopyranoside for 3–4 h. Cells (4 g) were washed twice by resuspension in buffer A containing 1 M urea and 1% guanidine HCl to give a final concentration of 6 M. The solution was acidified by addition of trifluoroacetic acid, and an aliquot was injected onto a Nova-Pak C18-reversed-phase chromatography column (3.9 × 150 mm, Waters Associates, Inc.) using an automatic injector. Substrates and the cleavage products were separated using an increasing water-acetonitrile gradient (0–100%) in the presence of 0.05% trifluoroacetic acid. Cleavage products were identified by amino acid analysis. Kinetic parameters were determined by fitting the data obtained at less than 20% substrate hydrolysis to the Michaelis-Menten equation by using the Fig. P program [Fig. P Software Corp.]. To assay the inhibitors, the volume of substrate KTVKLV 2 J. M. Louis, unpublished data.

Expression and Purification of HTLV-1 Proteinase—The 375-base pair coding region derived from an infectious clone of HTLV-1 by polymerase chain reaction was cloned into pET-11a vector and expressed in E. coli. Fig. 2 (lanes 1 and 2) shows the analysis of the total cell lysate before and after induction. Two products corresponding to the full-length mature PR and a smaller product were observed, the larger molecular weight product corresponding to the full-length mature PR and a smaller product.

Results and Discussion

Expression and Purification of HTLV-1 Proteinase—The 375-base pair coding region derived from an infectious clone of HTLV-1 by polymerase chain reaction was cloned into pet-11a vector and expressed in E. coli. Fig. 2 (lanes 1 and 2) shows the analysis of the total cell lysate before and after induction. Two products were observed, the larger molecular weight product corresponding to the full-length mature PR and a smaller product with an apparent size of roughly 7 kDa. The latter product was expected to be the result of self-degradation (autoproteolysis) of the mature PR rather than by premature termination during expression.

As in the case of HIV-1 PR (28, 36), expression of HTLV-1 PR results in its accumulation in the form of inclusion bodies. Lanes 3, 4, and 5 show the supernatant upon lysis of cells and the supernatant and the pellet derived after 1 h urea treatment of the initial insoluble pellet, respectively. A comparison of lanes 4 and 5 shows that 1 h urea treatment of the inclusion bodies (see lane 5) was solubilized in guanidine hydrochloride and subjected to size-exclusion chromatography under denaturing conditions. The peak fractions of both the full-length PR and the truncated protein were further subjected to reverse-phase HPLC chromatography. The two proteins that were >98% pure prepared as described previously (34). Active-site titration for the HIV-1 PR was performed with Compound 3 (33). The enzyme concentrations for HTLV-1 PR preparations were determined by amino acid analysis of the stock solutions, and K values were calculated by assuming 100% activity for the PR dimers. The PR assays were initiated by mixing 5 μl of HTLV-1 or HIV-1 PR (8–140 nM), 10 μl of 2× incubation buffer (0.5 mM potassium phosphate buffer, pH 5.6, containing 10% glycerol, 2 mM EDTA, 4 mM NaCl), and 5 μl of 0.1 to 0.4 mM substrate. The range of substrate concentration was selected depending on the approximate K values. The reaction mixture was incubated at 37 °C for 1 h and terminated by the addition of guanidine HCl to give a final concentration of 6 M. The solution was acidified by the addition of trifluoroacetic acid, and an aliquot was injected onto a Nova-Pak C18-reversed-phase chromatography column (3.9 × 150 mm, Waters Associates, Inc.) using an automatic injector. Substrates and the cleavage products were separated using an increasing water-acetonitrile gradient (0–100%) in the presence of 0.05% trifluoroacetic acid. Cleavage products were identified by amino acid analysis. Kinetic parameters were determined by fitting the data obtained at less than 20% substrate hydrolysis to the Michaelis-Menten equation by using the Fig. P program [Fig. P Software Corp.]. To assay the inhibitors, the volume of substrate KTVKLV was reduced to 4.8 μl (0.25 ml final concentration), and 0.2 μl of inhibitor (in water or in Me SO) was added. Inhibitors were initially tested at 10 μl or higher concentration. Me SO was also added at the same concentration to the appropriate control experiments. When inhibition was observed in initial testing, K values were determined according to Dixon (35). A competitive mode of inhibition was observed, and the standard error for K values was estimated to be below 20%.
Analyses of total cell lysate prepared from *E. coli* bearing the construct for the expression of the wild-type HTLV-1 PR before and after isopropyl β-d-thiogalactopyranoside induction are shown in lanes 1 and 2, respectively. Lane 3 shows the soluble supernatant derived after lysis of induced cells in 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 10 mM DTT, and 100 μg/ml lysozyme. The insoluble pellet attained from this step was treated with 1 M urea solution in buffer containing 50 mM Tris-HCl, pH 8.0, 10 mM EDTA, 10 mM DTT, 0.5% Nonidet P-40. The soluble supernatant and the pellet (inclusion bodies) that were derived from this step are shown in lanes 4 and 5, respectively. The inclusion bodies were solubilized, and the mature PR was purified to homogeneity by size-exclusion followed by reverse-phase HPLC chromatography. The purified PR that was renatured from 50 mM formic acid into 50 mM sodium acetate buffer, pH 5.5, 1 mM DTT, and 0.05% Triton X-100 is shown in lane 6. Proteins were electrophoresed on 10–20% gradient polyacrylamide gels (Novex, CA), and bands were visualized by Coomassie staining. M denotes molecular mass standards in kDa. Mature PR and truncated PR 41–125 were confirmed by N-terminal sequencing and mass spectrometry. Arrows indicate the truncated PR 41–125 before its characterization.

were analyzed for their mass and N-terminal sequence. The full-length mature PR clearly corresponded to the expected N-terminal sequence and mass (m/z = 13475). The truncated protein showed its N-terminal sequence to start with residues Pro-Ile-Ala-Leu-Phe-Ser-Ser, and the expected mass was close to the experimental value (m/z = 9171). From these results, we confirmed our prediction that the truncated protein is derived via autoproteolysis between residues Leu-40/Pro-41 of the full-length mature PR with a sequence spanning amino acids 41 through 125, now termed PR 41–125. The pure full-length protein was also renatured from 25 mM formic acid into 50 mM sodium acetate buffer, pH 5.5, 1 mM DTT, and 0.05% Triton X-100 at a final concentration of 6.7 μM and subjected to SDS-polyacrylamide gel electrophoresis (Fig. 2, lane 6). As expected, the full-length PR gave rise to two products, with apparent mobilities corresponding to 9 and 4.5 kDa. The latter protein is termed PR 1–40. Also, similar to the HIV-1 PR, autoproteolysis of the HTLV-1 PR proceeds roughly to about 50%. It is likely that either one or both products inhibit further autoproteolysis. This site of autoproteolysis in HIV-1 PR is analogous to a less susceptible site in HIV-1 PR (Leu33-Glu34). The major cleavage in HIV-1 PR occurs between residues Leu5 and Trp6 (19). Recently the feline immunodeficiency virus PR was also shown to undergo autoproteolysis at four primary sites in its 116-amino acid sequence (20).

**Autoproteolysis-resistant HTLV-1 Proteinase**—To prevent autoproteolysis of the mature HTLV-1 PR, the Leu40 residue was substituted with an Ile. This substitution was chosen because it has been shown by using synthetic peptides and proteins as substrates that HIV-1 and other retroviral proteinases cannot process at the C-terminal side of β-branched residues (37, 38). Furthermore, autoprocessing at an equivalent position (Leu83) and another position (Leu81) in HIV-1 was effectively reduced by substituting these residues with Ile without impairing the k_cat/K_m for several substrates (36). The expression of the HTLV-1 PR bearing the mutation L40I compared with the wild-type PR is shown in Fig. 3. Clearly as observed in duplicate clones, there is a single product that accumulates with a substantially higher yield corresponding to the size of the full-length PR. 1.6 g (wet weight) of cells derived from 0.4 liters of culture yields ~19 and 14 mg of PR after size-exclusion and reversed-phase HPLC chromatography, respectively.

**Kinetic Characterization of the Wild-type and L40I HTLV-1 Proteinases**—Based on the sequence comparison with HIV-1 PR and homologous molecular modeling, the Leu40 residue of HTLV-1 PR is not expected to interact with the substrate or inhibitor. Therefore we predicted that substituting this residue with Ile would not change the kinetic parameters but would prevent its autolysis as compared with the wild-type enzyme. To verify this assumption and to characterize the HTLV-1 PR specificity, kinetic analysis was performed using oligopeptide substrates. The proteinases were renatured by quenching the denatured protein in 50 mM formic acid into 100-fold excess of PIPES buffer at pH 7.0 under conditions similar to those previously described for refolding HIV-2 and equine infectious anemia virus proteinases (39). Kinetic data for the wild type and L40I mutant PR-catalyzed hydrolysis of synthetic substrates are shown in Table I. Both enzymes exhibit nearly identical kinetic parameters. The nearly identical values of k_cat for the wild-type and mutant enzymes also suggest that under

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3. P. Bagossi, G. Zahuczky, and J. Tozser, unpublished results.
these refolding and assay conditions there is no significant autoproteolysis of the wild-type PR. Stabilization of the enzyme can be attributed to 1) the refolding procedure, which involves quenching the denatured protein with a large excess of buffer (pH 7) to a final concentration of 0.1 mM as compared with dialyzing the protein to pH 5.0 at a higher protein concentration and 2) presence of Nonidet P-40 and glycerol in the quench buffer.

**Mutation of the Cysteine Residues of the Stabilized HTLV-1 Proteinase**—Because one of our major objectives was to produce quantities of enzyme suitable for structural studies by solution NMR and x-ray crystallography, we next addressed the role of the two cysteines (Cys90, Cys109) on enzymatic activity and NMR and x-ray crystallography, we next addressed the role of

| Cleavage site | Substrate sequence | Enzyme | $K_m$ [mM] | $k_{cat}$ [s$^{-1}$] | $k_{cat}/K_m$ [msec$^{-1}$] |
|--------------|--------------------|--------|-------------|---------------------|--------------------------|
| MA/CA        | APQVL↓PVMHP        | HTLV-1 (wt) | 0.115 ± 0.018 | 8.67 ± 0.60 | 75.4 |
|              |                    | HTLV-1 (L40I) | 0.098 ± 0.020 | 6.78 ± 0.50 | 69.2 |
| CA/NC        | KTKVL↓VQPK         | HTLV-1 (wt) | 0.033 ± 0.004 | 5.15 ± 0.10 | 156.1 |
|              |                    | HTLV-1 (L40I) | 0.032 ± 0.004 | 5.18 ± 0.12 | 161.8 |
| TF1/PR       | DPASIL↓PVIP        | HTLV-1 (wt) | 0.230 ± 0.035 | 0.70 ± 0.05 | 3.0 |
|              |                    | HTLV-1 (L40I) | 0.244 ± 0.033 | 0.62 ± 0.04 | 2.5 |
| PR/p3        | KGPPVIL↓PIQAP      | HTLV-1 (wt) | 0.030 ± 0.006 | 6.91 ± 0.39 | 230.3 |
|              |                    | HTLV-1 (L40I) | 0.034 ± 0.005 | 6.26 ± 0.30 | 184.2 |

**Kinetic Characterization of the Stabilized C2A-PR Mutant HTLV-1 Proteinase and Comparison of Its Specificity with HIV-1 Proteinase**—To characterize the specificity of C2A-PR, we used oligopeptide substrates that represent naturally occurring cleavage sites in HTLV-1 Gag-Pol polyprotein. Comparison of the kinetic parameters using C2A-PR (Table I) to those obtained with wild-type and L40I mutant enzymes (Table II) suggest that substitution of the Cys residues to Ala does not alter its catalytic activity. Three of the four peptides tested were excellent substrates of HTLV-1 PR, with catalytic constants comparable with the best naturally occurring HIV-1 cleavage site substrates with HIV-1 PR (42). Thus HTLV-PR is an enzyme with high specific activity, similar to HIV-1 PR but unlike avian myeloblastosis virus PR, which has much lower $k_{cat}$ for its specific substrates. This interpretation is consistent with the mechanisms that regulate the level of expression of the Gag and Gag-Pol products. HIV-1 and HTLV-1, wherein the PR is coded in a Pol open reading frame at −10-fold lower level than Gag proteins, have higher specific activity for the PR as compared with avian myeloblastosis virus PR, which is coded in a Gag open reading frame. Another interesting similarity with the HIV-1 and HTLV-1 proteases is that among the least efficient sites for hydrolysis is the one that yields the free N terminus of PR (42). Studies with an analog of the HIV-1 PR precursor indicate that the cleavage at the N terminus of the PR occurs intramolecularly, concomitant with a large increase in enzymatic activity, and this cleavage has been suggested to be the rate-limiting step in the maturation cascade of Gag-Pol polyprotein (24, 43). When HTLV-1 cleavage site substrates were used as substrates for HIV-1 PR (Table II), three of four peptides were found to be hydrolyzed. The kinetic parameters of HIV-1 PR-catalyzed hydrolysis of these HTLV-1 substrates were in the same range as those for substrates that correspond to analogous sites in HIV-1 (42). On the other hand, only two of the nine HIV-1 cleavage site substrates were cleaved by the HTLV-1 PR (Table III) but with substantially lower values of $k_{cat}/K_m$ than those obtained with HTLV-1 substrates.

The lack of cleavage of HIV-1 Gag substrates by HTLV-1 PR is in good agreement with previous findings that HTLV-1 PR was not able to process HIV-1 Gag precursor in a vaccinia virus expression system (44). In the same expression system, HIV-1
PR was also not able to process HTLV-1 Gag (44) even though the peptides representing HTLV-1 Gag cleavage sites were hydrolyzed by HIV-1 PR in vitro. This result in vitro may at least partially be because of reduced specificity of this enzyme toward these sites as compared with HTLV-1 PR.

Hydrolysis of Chromogenic Substrates by HTLV-1 Proteinase—To develop a more convenient chromogenic assay, we synthesized and assayed analogs of the HTLV-1 CA/NC cleavage site substrate that contained a Phe (NO2) substitution in either the P1 or P1' positions (Table III). Although the substrate with P1' substitution was an inefficient substrate, the substrate containing the P1 substitution was hydrolyzed to about 4% the level of the native substrate. This substrate may serve to screen for ideal-folding conditions of C2A-PR in simple buffer systems for structural studies. In good agreement with the lack of hydrolysis of the HIV-1 CA/p2 peptide by HTLV-1 PR, its chromogenic derivative, which is a widely used chromogenic substrate of HIV-1 PR was not hydrolyzed (Table III).

Daenke et al. (16) reported kinetic parameters for peptides representing naturally occurring cleavage sites of HTLV-1 in which the P1' residue were substituted by a chromogenic reporter, Phe (NO2). They have reported Km and kcat values within a range of 0.003–0.031 mM and 14–33 s⁻¹, respectively. These Km values are lower than our observed values (0.030–0.230 mM, see Table I) using entirely natural peptides with no residue in the P1 position. This may also be the reason why the naturally occurring cleavage site sequences representing the autocatalytic maturation sites (TF1/PR and PR/p3) in HTLV-1 as compared with the corresponding chromogenic derivatives do not have substantially higher affinities toward HTLV-1 PR than the peptides representing other sites, as noted for the chromogenic derivatives by Daenke et al. (16). The reason for the differences in the reported kcat values is less obvious. When we introduced Phe(NO2) group in either the P1 or P1' positions of the naturally occurring cleavage site sequence, a substantial decrease in hydrolytic rate was observed instead of the expected increase (see Table III). Our observations which suggest that bulky residues at the site of cleavage are unfavorable are in accordance with the interpretation of Pettit et al. (12) that lack of inhibition by HIV-1 PR inhibitors on in vitro HTLV-1 Gag processing might be because of the presence of Trp98 in HIV-1 rather than Val in equivalent position of HIV-1 reducing the size of S1 binding pocket. A two-fold increase in NaCl concentration used in our assay conditions as compared with that reported (16) does not account for this large difference in kcat values. The specific activity reported using the chromogenic substrate representing the CA/NC site (1 μmol/min/mg; Ref. 16) suggests a much smaller catalytic rate.

Inhibition of the HTLV-1 Proteinase—Several inhibitors of HIV-1 PR were assayed as inhibitors of the HTLV-1 PR. DMP 323, KH 164, and Compound 3 have previously been shown to be potent inhibitors of the HIV-1 PR. Only Compound 3 inhibited HTLV-1 PR with a Ki of 80 nM, whereas the others did not inhibit up to 10 μM (Table VI). Another potent inhibitor of HIV-1 PR, Ro-31-8959 was also inactive against HTLV-1 PR (16). These results also emphasize the large differences in specificity of the two enzymes. Interestingly, Compound 3 was also a good inhibitor of HIV-2 PR (45), equine infectious anemia virus PR (39), murine leukemia virus PR, and mouse mammary tumor virus proteases (46) and, therefore, may serve as a lead compound for general inhibitors of retroviral proteinases. However, the most potent inhibitor of the enzyme in our assay system was a statine-based inhibitor, which was based on the MA/CA cleavage site sequence with a Ki of 50 nM (Inhibitor 10 in Table IV). Potent, active-site inhibitor of Mason-Pfizer monkey virus PR (Inhibitor 9 in Table IV; Ref. 47) and avian myeloblastosis virus PR (Inhibitor 8 in Table IV; Ref. 50) also

### Table II

Kinetic parameters for hydrolysis of naturally occurring HTLV-1 cleavage site peptides by C2A-PR and wild-type HIV-1 PR

| Cleavage site | Substrate sequence | Enzyme     | Km     | kcat  | kcat/Km |
|---------------|--------------------|------------|--------|-------|---------|
| MA/CA         | APQVL | PVMP      | C2A-PR  | 0.069 ± 0.020 | 5.88 ± 0.59 | 85.2 |
| HIV-1 PR      | 0.023 ± 0.004 | 0.37 ± 0.01 | 16.1 |
| CA/NC         | KTKVL | VVQP      | C2A-PR  | 0.051 ± 0.005 | 7.68 ± 0.17 | 150.6 |
| HIV-1 PR      | 0.182 ± 0.033 | 2.60 ± 0.20 | 14.3 |
| TF1/PR        | DPASIL | PVIP      | C2A-PR  | 0.221 ± 0.031 | 0.84 ± 0.06 | 3.8 |
| HIV-1 PR      | ND     |           |         |       |         |
| PR/p3         | KGPPVIL | PIPQ      | C2A-PR  | 0.041 ± 0.010 | 11.82 ± 0.89 | 288.3 |
| HIV-1 PR      | 0.025 ± 0.002 | 0.21 ± 0.01 | 8.4 |

### Table III

Hydrolysis of oligopeptides representing naturally occurring HIV-1 cleavage sites and chromogenic substrates by the C2A-PR

| Cleavage site | Substrate sequence | Km     | kcat  | kcat/Km |
|---------------|--------------------|--------|-------|---------|
| HIV-1 MA/CA   | VSQNY | PIVQ     | ND     |         |         |
| CA/p2        | KARVL | AEMAS    | ND     |         |         |
| p2/NC        | TATTIM | MQRGN   | ND     |         |         |
| NC/p1        | EIKQAN | FLGK    | ND     |         |         |
| p1/p6        | RPCNF | LQSRRP   | ND     |         |         |
| in p6         | DKELY | PLTS     | >0.5  | 0.02   |         |
| TF/PR        | VSFNF | PQITL    | ND     | ND     |         |
| PR/reverse transcriptase | CTLNF | PISP | ND     |         |         |
| Reverse transcriptase/IN | IKTL | FDLO | 0.283 ± 0.038 | 0.21 ± 0.01 | 8.4 |
| HIV-1 CA/p2   | KARVL | ΦEAM     | ND     |         |         |
| HTLV CA/NC   | KTKVL | ΦVQP     | >0.3 | 0.01   | 0.04   |
| HTLV CA/NC   | KTKVL | ΦVQP     | 0.188 ± 0.013 | 1.23 ± 0.04 | 6.5 |
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| PR Inhibitor | HTLV-1 PR $K_i$ | HIV-1 $K_i$ |
|--------------|----------------|------------|
| Compound 3   | 0.080          | 0.0004$^a$ |
| DMP 323      | >10            | 0.0003$^b$ |
| Ro-31-8959   | >10            | 0.0004$^b$ |
| UK 88,947    | >10            | 0.012$^c$  |
| KH 164       | >10            | 0.400$^d$  |
| LP-149       | >10            | 0.0017$^e$ |
| N-1460       | >10            | 0.50$^f$   |
| Pepstatin    | >10            | >10$^g$    |
| Pro-Cys-Val-Phc-Sta-Ala-Met-Thr-Met$^h$ | >10 | Not determined |
| Peo-Cys-Val-Sta-Ala-Met-Thr-Met$^i$ | >10 | 12.5$^j$ |
| Al-Pre-Gln-Sta-Sta-Met-Met-Pre$^j$ | 0.050 | 0.13 |
| Lys-Pre-Lys-Sta-Sta-Sta-Pre$^k$ | >10 | >10 |

$^a$ Compound 3 inhibits HIV-2, equine infectious anemia virus, murine leukemia virus, and mouse mammary tumor virus proteases, but does not inhibit HTLV-1 PR up to 10 μM.

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TABLE IV

Inhibition of C2A-PR and HIV-1 PR by inhibitors specific to HIV-1 and other retroviral proteases and by stannate-based HTLV-1 substrate analogs
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