Title
Characterization of a Novel Human Immunodeficiency Virus Type 1 Protease Inhibitor, A-790742

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Running Title
A-790742, a novel HIV-1 protease inhibitor

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Abstract

A-790742 is a potent human immunodeficiency virus type 1 (HIV-1) protease inhibitor with an EC$_{50}$ value ranging between 2 and 7 nM against wild-type HIV-1. The activity of this compound is lowered by approximately 7-fold in the presence of 50% human serum. A-790742 maintained potent antiviral activity against lopinavir-resistant variants generated in vitro as well as a panel of molecular clones containing proteases derived from HIV-1 patient isolates with multiple protease mutations. During in vitro selection, A-790742 selected two primary mutations (V82L and I84V) along with L23I, L33F, K45I, A71V/A, and V77I in the pNL4-3 background, and two other mutations (A71V and V82G) accompanied by M46I and L63P in the HIV-1 RF background. HIV-1 pNL4-3 clones with a single V82L or I84V mutation were phenotypically resistant to A-790742 and ritonavir. Altogether, A-790742 displays a favorable anti-HIV-1 profile against both wild type and a large number of mutants resistant relative to other protease inhibitors. The selection of the uncommon V82L and V82G mutations in protease by A-790742 suggests the potential for an advantageous resistance profile with this protease inhibitor.
**Introduction**

Human immunodeficiency virus type 1 (HIV-1) protease inhibitors (PIs) are potent and effective antiretroviral agents. The current standard of care for acquired immunodeficiency syndrome (AIDS) patients involves the combination of reverse transcriptase inhibitors (RTIs) and/or PIs with other HIV inhibitors, which can reduce viremia to an undetectable level for an extended period of time (22, 37, 41, 42). However 5% to 68% of patients ultimately fail therapy due to non-adherence to drug schedules, insufficient drug exposure and resistance development (3, 8, 10, 18, 36, 38, 39). In addition, there are many cases reporting the isolation of PI-resistant HIV-1 from patients who have not received any treatment with currently available PIs, indicating that transmission of PI-resistant HIV-1 can occur, particularly during primary infection when the viral burden is high (1, 19, 20, 37).

Lopinavir (LPV), an HIV-1 PI, maintains reasonable potency in the presence of human serum and demonstrates favorable pharmacokinetics when co-formulated with a low dose of ritonavir (RTV) (5, 34). Kaletra (LPV/r) has displayed efficacy in both PI-naïve and PI-experienced patients (5, 6, 14, 15, 24, 26). However, treatment with PIs, including Kaletra, is associated with lipid elevations in humans (23, 32). In addition, PIs such as atazanavir (ATV) and indinavir (IDV) have been shown to induce hyperbilirubinemia (17, 43). To avoid the undesirable metabolic side effects of HIV PIs, there is a need to identify new PIs with high potency, increased genetic barrier to the development of resistance, as well as reduced metabolic side effects. In an effort to address this need, we investigated the biological properties, antiviral activity, and resistance profile of a novel HIV-1 PI, A-790742. A-790742 had high oral bioavailability in rats and dogs, with 500-fold enhancement in AUC upon co-dosing with RTV (7). This compound also exhibited fewer proteasome gene changes than RTV.
when analyzed in an *in vivo* model for hyperlipidemia associated with PI therapy (7, 21, 40).

In this study, we characterized A-790742 in a tissue culture system against wild type (WT) and mutant HIV-1 viruses. In addition, we performed *in vitro* selection and characterization of A-790742 resistant variants using two different HIV-1 strains. Finally, we describe the results of cross-resistance studies of A-790742 resistant variants with commercially available PIs.
Materials and Methods

Generation of HIV-1 resistant to A-790742 by in vitro selection

Generation of resistant virus by in vitro passages has been described previously (2, 4, 9, 25, 27, 35). Briefly, 2x10^6 MT-4 cells were infected with WT HIV-1 strains pNL4-3 or RF separately at a multiplicity of infection (MOI) of 0.003 for 2 hours, washed, and then cultured in the presence of A-790742 at an initial concentration of 1.4 nM. Viral replication was monitored by determination of p24 antigen levels in the culture supernatants by ELISA every 3-4 days. When p24 antigen levels exceeded 10 ng/ml, the viral supernatants were filtered and used to infect fresh MT-4 cells in the presence of an increasing concentration of A-790742, leading to the generation of virus with increased resistance to A-790742. Samples of viral supernatants from each passage were frozen at –80ºC for later analyses.

Titration, EC_{50} and TD_{50} determination using MTT assay

All viruses used for the MTT assay were titered by infecting 2x10^5 MT-4 cells for 1 hr at 37ºC with serial half-log dilutions of virus. Following infection, cells were washed and plated into a 96-well plate at a concentration of 10^4 cells per well in the presence of 0.5% dimethyl sulfoxide (DMSO). Five days post-infection, the level of virus-induced cytopathic effect (CPE) was measured using an MTT colorimetric assay (2, 12, 16, 31) and the tissue culture infectious dose (TCID_{50}) of each virus was calculated using the Sperman-Karber method.

For 50% effective concentration (EC_{50}) determinations, 10^6 MT-4 cells were infected with viral stock at an MOI of 0.003 (2, 16, 27, 29, 31). One hour post-infection, cells were washed and plated into 96-well plates at concentrations of 10^4 cells per well in the presence of eight serial dilutions of each tested compound in triplicate. The final concentration of DMSO in all wells...
was 0.5%. Five days post-infection, an MTT assay was done and the EC$_{50}$ for each compound was calculated using Prism software (version 4) as described previously.

50% toxic dose (TD$_{50}$) determination was done in MT-4 cells. Cells were plated into 96-well plates at concentrations of $10^4$ cells per well in the presence of eight serial dilutions of each tested compound in duplicate. The final concentration of DMSO in all wells was 0.5%. Five days later an MTT assay was done and the TD$_{50}$ for each compound was calculated using Prism software (version 4) as described before.

**DNA sequence analyses and construction of recombinant HIV-1 molecular clones**

Culture supernatants from virus passages and plasma samples from patients who failed LPV/r clinical trials were analyzed by DNA sequence analysis. RNA from these samples was isolated using the QIAamp RNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Subsequently, it was processed by RT-PCR and nested PCR to amplify the HIV-1 protease genes. The amplified samples were sequenced using the ABI Prism dye terminator cycle sequencing ready reaction kit, and were analyzed on an Applied Biosystem 3100 Genetic Analyzer.

HIV-1 molecular clones containing mutations in the protease gene were constructed for study in single cycle assays. For LPV-resistant HIV-1 patient samples, the protease coding region was first RT-PCR-amplified with primers that incorporated ApaI and XmaI restriction sites, and then cloned into a pNL4-3 replication-defective viral vector with a luciferase gene (pNL4-3-Luc) which contains an ApaI site upstream and a XmaI site downstream of the protease coding region (27, 28). In addition, HIV-1 molecular clones with mutations selected by A-790742 in the protease gene were constructed by generating PCR fragments from the protease region of different mutant viruses derived from passage in the presence of A-790742, cloning
the fragments into the pNL4-3-Luc vector as ApaI-XmaI fragments, and selecting individual clones containing the mutation(s) of interest. The RF-Luc constructs contained the WT or mutant RF protease gene inserted into the pNL4-3-Luc vector as an ApaI-XmaI fragment.

**Antiviral activity of PIs against HIV-1 molecular clones with mutations in the protease gene**

Antiviral activities of HIV-1 PIs against HIV-1 molecular clones with and without mutations in the protease gene were measured using a single-cycle assay. Human embryonic kidney 293 (HEK-293) cells were cotransfected with HIV-1 molecular clones containing a luciferase reporter gene plus a VSV-G envelope protein expression vector using Lipofectamine 2000 and PLUS reagent (Invitrogen). The transfection conditions were optimized to achieve >90% transfection efficiency according to the manufacturer’s instructions. Four hours after transfection, cells were trypsinized and 2x10^4 cells per well were plated into 96-well plates containing serial dilutions of each tested protease inhibitor in triplicate. Forty-eight hours post transfection, pseudotyped viral stocks generated in the presence of inhibitors were used to infect fresh HEK-293 cells plated in 96-well plates at a density of 10^4 cells per well. Replication in each well was monitored by measuring luciferase expression in infected target cells forty-eight hours after infection. Percent inhibition of luciferase signal in cells treated with inhibitors relative to that of the untreated cells was calculated. EC_{50} values were determined using Prism software (11, 30, 33). Replication capacities (RC) of mutant constructs were calculated by comparing the luciferase activity generated by mutant to that generated by the WT. The RC values were expressed as percentages of the replication of the WT construct.
Results

Antiviral activity of A-790742 against WT HIV-1 and LPV-resistant HIV-1 mutant viruses

The anti-HIV activity of A-790742 (Figure 1) against WT HIV-1 and HIV-1 mutant viruses with LPV-resistant mutations was compared to those of LPV, ATV, darunavir (DRV) and tipranavir (TPV). A-790742 had an EC\textsubscript{50} of 3 nM against WT pNL4-3 and 5 nM against WT RF in the absence of human serum (HS) (Table 1). Its activity was thus similar to or slightly better than that of ATV against pNL4-3 and RF. It was six times more active than LPV against both viruses and four times better than DRV against pNL4-3. A-790742 was 61 and 41 times more active than TPV against pNL4-3 and RF, respectively. In the presence of 50% human serum (HS), A-790742 was approximately seven times less potent than without HS, with an EC\textsubscript{50} value of 20 nM. It was 7.5 and 392 times more potent than LPV and TPV respectively, under the same conditions (Table 1).

The activities of A-790742, ATV, LPV, DRV and TPV were evaluated against a panel of passaged viruses derived from \textit{in vitro} selection with LPV. These viruses had key mutations in protease at the following positions: 32, 46, 47 and 84 for A17; 46, 50, 54 and 82 for B26; and 32, 46, 47 and 84 for P25 (Table 2). The EC\textsubscript{50} values of A-790742 against A17, B26 and P25 passaged viruses were 3- to 15-fold higher than that of the WT pNL4-3. A-790742 was the least active against the P25 virus with a 15-fold change in its EC\textsubscript{50} value. ATV, on the other hand, was 13 to 89 times less active against the same panel of viruses than against WT pNL4-3. It was more potent against the B26 virus and less potent against the A17 and P25 viruses. DRV lost 28- to 71-fold of its potency against this panel of viruses compared to WT pNL4-3. It was more active against the A17 than the B26 and P25 viruses. TPV was tested only against
A17 and was two times less potent than against WT. As reported before, all of these mutant viruses were highly resistant to LPV, with 59- to more than 500-fold reduction in susceptibility relative to the WT virus (2, 27). Taken together, A-790742 displayed anti-HIV activity superior to LPV, ATV, DRV and TPV for WT pNL4-3 and RF, as well as LPV-resistant HIV-1 mutants.

**Antiviral activity of A-790742 against HIV-1 molecular clones with protease genes derived from patient isolates**

To determine if A-790742 could effectively inhibit different PI resistant viruses, it was tested along with LPV and ATV in single cycle assays against HIV molecular clones containing protease genes derived from HIV-1 isolates from patients who failed different PI treatments (Table 3). The first three constructs in Table 3 were derived from samples from patients who failed LPV treatment. The rest of the constructs were from a panel of molecular clones displaying reduced susceptibility to more than one PI. Each of these tested isolates contained a mixture of primary and secondary mutations as designated by the International AIDS Society-USA-resistance panel (11, 13). In general, all clones were less resistant to A-790742 than LPV and ATV. A-790742 was active against clones with V82A/F/T mutations. However, combined mutations at positions 71 and 84 appeared to confer some degree of resistance to A-790742. Of note, mutations at positions 71 and 84 were selected during the passage of pNL4-3 in the presence of A-790742 as described below. Other secondary mutations also contributed to the generation of resistance to all tested PIs.

**Selection of A-790742 resistant virus by in vitro passages**

In order to understand the importance of genetic background in the selection of viruses resistant to A-790742, two different strains of HIV-1 (pNL4-3 and RF) were passaged in the
presence of increasing concentrations of A-790742 (Table 4, 5). Viral replication was monitored every 3-4 days by detection of viral antigen (p24) before increasing the concentration of the inhibitor. pNL4-3 and RF were initially grown in the presence of 1.4 nM A-790742 (1/2 EC$_{50}$ value for pNL4-3 and ¼ EC$_{50}$ value for RF virus). There was also an attempt to grow RF virus at an initial concentration of 3 nM A-790742 (1/2 EC$_{50}$ value), but viral replication was very low (data not shown). During the selection process, the A-790742 concentration was increased gradually to a final concentration of 2 µM (pNL4-3) (Table 4) or 0.5 µM (RF) (Table 5). It took approximately three and half months to complete the selection. The selection with pNL4-3 progressed faster than with RF virus. A total of twenty-one passages were performed for pNL4-3, but only fifteen passages for RF. This difference might be due to the different genetic backgrounds of the two viruses, and suggests that resistance in the RF background developed less rapidly than in the pNL4-3 background.

Sequence analyses of the protease-coding region from HIV-1 passaged in A-790742

To monitor the protease mutations selected by A-790742, RNA was isolated from the culture supernatant of passages 5, 6, 7, 8, 9, 10, 13, 15, 18 and 21 for pNL4-3 selection (Table 4), as well as from those of passages 6, 8, 10, 13 and 15 for RF selection (Table 5). The RNA was amplified by RT-PCR and nested PCR, and then the population sequences of the PCR products were determined. In the selection with pNL4-3, two primary mutations were initially observed: I84V (passages 5, 6, 7, 8, 9, 10 and 13) and V82L (passages 8, 9, 10 and 13). These two mutations were also detected in passages 15, 18 and 21, suggesting that they were important for the generation of viral resistance. In passages 15, 18 and 21, additional mutations L23I, L33F, K45I, A71V and V77I were also detected. The A-790742 resistance selection using RF virus produced a different pattern of mutations. The mutation V75I was found in passage 8, but
this mutation became undetectable in passage 10. Instead, mutations A71V and V82G were found in passage 10. In passages 13 and 15, additional mutations M46I and L63P were observed. The cleavage sites of viruses derived from passage 21 of pNL4-3 selection and passage 15 of RF selection were sequenced, and no mutations were detected.

**Susceptibility of HIV-1 selected by A-790742 to different PIs**

To determine the susceptibility of HIV-1 mutants selected by A-790742 to different PIs, several pNL4-3 passage viruses (P9, P15 and P21) and one RF passage virus (P13) were titered, and their levels of resistance to A-790742 and a number of commercially available PIs such as ATV, LPV, saquinavir (SQV), RTV, amprenavir (APV), IDV and nelfinavir (NFV) were determined using an MTT assay (Table 6). Virus from pNL4-3 passage 9 was 12.7-fold less susceptible to A-790742 than the WT pNL4-3 virus. This passage was the most resistant to RTV, with a 26.7-fold increase in its EC\textsubscript{50} value as compared to the WT pNL4-3 virus. ATV, LPV, SQV, APV, IDV, and NFV demonstrated 3- to 9-fold increases in their EC\textsubscript{50} values at passage 9. Virus selected in pNL4-3 passage 15 had a similar level of resistance to A-790742 and RTV, with a 28-fold change in EC\textsubscript{50} value. In addition, ATV and APV had 13.6- and 10.5-fold changes in their respective EC\textsubscript{50}s to virus from passage 15. LPV, SQV, IDV and NFV had about 5- to 6- fold change in their EC\textsubscript{50} values with this passaged virus. pNL4-3 passage 21 virus had the highest degree of resistance to all tested PIs. It was highly resistant to A-790742 (>2500-fold resistance), RTV (>141-fold resistance), ATV (145-fold resistance) and NFV (>93-fold resistance). However, it had only a low to moderate level of resistance to the other tested PIs. In particular, it retained most of its sensitivity to SQV, LPV and IDV.

Virus from passage 13 selected in the RF background had a similar level of resistance to A-790742, RTV and NFV with 72-, 79- and 67- fold change in EC\textsubscript{50} values, respectively, as
compared to WT RF virus. APV, ATV and IDV had about 31- to 41- fold changes in their EC\textsubscript{50} values with this passaged virus. In addition, the passage 13 virus displayed the greatest resistance to LPV, with >301-fold increase in its EC\textsubscript{50} value, and was the least resistant to SQV, with a 21-fold increase in its EC\textsubscript{50} value as compared to the WT RF virus.

**Phenotypic analyses of HIV-1 molecular clones resistant to A-790742**

To determine the effect of genotypic changes found in the passaged variants on the susceptibility of HIV-1 to A-790742 and commercially available PIs, HIV-1 molecular clones with protease mutations selected by A-790742 in pNL4-3 and RF were engineered in the pNL4-3-Luc vector (Table 7). These constructs permitted rapid and sensitive quantification of drug susceptibility during a single round of viral replication in the presence of increasing concentration of drugs (30, 33).

Molecular clones containing the single mutation V82L or I84V in the pNL4-3 background displayed resistance only to A-790742 and RTV (Table 7). In particular, clone 1, which contained an I84V mutation, was 2- and 3-fold less susceptible to A-790742 and RTV, respectively, as compared to the WT pNL4-3-Luc. In contrast, clone 2, which harbored a V82L mutation, displayed 22-fold resistance to A-790742, but only 7-fold resistance to RTV. Mutant clone 3, which had secondary mutations L33F and K45I in addition to primary mutations V82L and I84V, was 23-fold less susceptible to A-790742. Clone 3 was more resistant than clone 2 to all other tested PIs except SQV (Table 7). Clone 4 contained secondary mutations L33F, A71V, G73S and V77I in addition to primary mutations V82L and I84V. Mutations L33F, A71V, and V77I were secondary mutations detected by population sequencing in the passaged variants (Table 4). However, G73S was not detected by population sequencing, but was identified by sequencing individual TA clones derived from pNL4-3 passaged in the presence...
Clone 4 was more resistant to A-790742 (158-fold change in the EC$_{50}$ value) but less resistant to all tested PIs than clone 3 (Table 7). The difference in the resistance profiles of clone 3 and 4 suggests that K45I is important for the resistance to all PIs except A-790742 and SQV. Furthermore, individual or combined A71V, G73S and V77I mutations contributed to the increase in resistance to A-790742. Among these 3 mutations, A71V was detected in both pNL4-3 and RF after selection using A-790742, and probably contributed most of the additional resistance to A-790742. It is of note that molecular clone 3, which contained most of the mutations of passaged HIV-1 P21 (Table 6), showed the same trend of resistance as P21. Both clone 3 and P21 were more resistant to A-790742, ATV and RTV than the other tested PIs.

Clone 5, which contained A-790742 selected PI mutations in the RF background, was also evaluated in the single cycle assay (Table 7). This clone contained the primary mutation V82G and other mutations L63P and A71V. It was 72-fold less susceptible to A-790742 than the WT construct, and highly resistant to LPV and RTV, with more than 1300-fold resistance to these two PIs. However, it had only moderate levels of resistance to the other tested PIs. Importantly, it was only 28 times more resistant to SQV than the WT construct. Interestingly, this construct conferred overall a higher level of resistance to all tested PIs than all molecular clones in the pNL4-3 background (Clones 1, 2, 3 and 4, Table 7).

During phenotypic testing, significant differences in replication efficiencies between molecular clones with wild-type and mutant protease genes in the RF background were observed (Figure 2). Constructs with the combined mutations L63P, A71V, and V82G or M46I, A71V, and V82G replicated 99% less efficiently than the wild-type HIV-RF-luc construct based on the difference in luciferase signal. The luciferase signal of the RF molecular clone harboring the
mutations M46I, A71V, V82G was so low that its phenotype could not be determined in the single cycle assay. One of the possible reasons for the lower replication capacity of these constructs with the V82G mutation might be due to the negative impact of this mutation on the fitness of the constructs. The presence of this mutation might also explain the slow growth of virus during the selection with A-790742 in the RF background. All other clones with mutations in the protease gene replicated at the same level as the virus with WT protease (pNL4-3-Luc).
Discussion

The current standard of care for AIDS patients involves the combination of drugs with different mechanisms of action against HIV-1. Due to the metabolic side effects induced by some PIs, there is a significant need for novel HIV-1 PIs with superior potency against both WT and mutant viruses, as well as less metabolic side effects. A-790742 is a novel protease inhibitor that exhibited fewer proteasome gene changes that could lead to lipid elevation than RTV (7) and only showed modest elevation of bilirubin as compared to ATV and IDV (43). In this study we characterized the antiviral activity, resistance profile and development of resistance to A-790742. A-790742 is a potent protease inhibitor with activity ranging between 2 and 7 nM against wild-type HIV-1. With few exceptions, this compound retained its activity against LPV-resistant viruses as well as molecular clones with protease genes derived from LPV-resistant viruses and a panel of HIV-1 patient isolates with multiple mutations in the protease gene.

Based on the activity of A-790742 against HIV-1 molecular clones containing protease derived from PI-resistant patient isolates (Table 3), we speculate that A-790742 is likely to retain most of its antiviral activity against patient isolates with multiple mutations in the protease gene. Multiple combinations of mutations could confer resistance to A-790742, but their degree of resistance to A-790742 appeared to be less than that to LPV and ATV. In particular, patient isolates with mutations A71V combined with I84V displayed resistance to A-790742, whereas mutations V82A/F/T did not seem to confer much resistance to A-790742 (Table 3). Consistent with these results, mutations A71V and I84V were also detected in selection of resistant viruses with pNL4-3 in the presence of A-790742 in vitro (Table 4).
To better understand the resistance profile of A-790742, we performed selection of HIV-1 mutant viruses in two different genetic backgrounds with this PI. Passage in a pNL4-3 background initially selected the I84V mutation (Table 4). Further passages selected an additional primary mutation V82L (passage 8), and a number of other mutations, e.g. A71V, in later passages. In contrast, the V75I mutation was first detected in the selection in the RF background (Table 5). However, the combination of A71V and V82G mutations was later observed in the RF selection at passage 10, accompanied by the appearance of other mutations in passage 13. In two studies conducted previously, passage of viruses in the presence of LPV in a pNL4-3 background resulted in the selection of mutant viruses with different mutation patterns including primary mutations I84V and I50V/M46I (2, 27).

The selection of different major mutations with A-790742 in different strains of HIV-1 points to the importance of genetic background for selection of resistant mutants. In addition, the selection with pNL4-3 progressed faster than with RF virus in the presence of A-790742, suggesting that resistance developed more readily in the pNL4-3 than the RF background. Slower selection with the RF than with the pNL4-3 strain is consistent with the observation that molecular clones with the RF protease gene containing mutations A71V plus V82G had greatly reduced replication capacities compared to the molecular clone containing the wild-type RF protease (Figure 2). Our results are consistent with previous reports demonstrating that the development of resistance was affected by the viral strain used in the selection (4, 9).

Primary mutations V82L and V82G are not commonly selected with existing PIs. The single mutation V82L (clone 2, Table 7), and probably the V82G mutation, did not confer a significant degree of resistance to A-790742. In contrast, mutants harboring V82L or V82G, in combination with other mutations, e.g. A71V, were highly resistant to A-790742. These
mutants were mainly cross-resistant to RTV and LPV as well as ATV (Tables 6 and 7). However, they were only moderately resistant to most other tested PIs, and remained quite susceptible to SQV, APV, IDV, DRV and TPV.

In summary, A-790742 displays favorable anti-HIV-1 potency against both WT and most PI-resistant mutants. In vitro passage of HIV-1 with A-790742 led to the selection of the primary mutation I84V, as well as the uncommon mutations V82L and V82G in protease. Molecular clones harboring these primary mutations, in combination with other mutations, were highly resistant to A-790742, as well as to LPV and RTV. These clones, however, were only moderately resistant to most other PIs and retained their susceptibility to SQV. In addition, mutation V82G in the RF background appeared to have a significant negative impact on the replication capacity of the virus. A-790742 thus has promising antiviral activity and a potentially advantageous resistance profile.

**Acknowledgment**

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References


preclinical model for assessing the potential for unconjugated hyperbilirubinemia
produced by human immunodeficiency virus protease inhibitors. Antimicrob Agents

18. Ledergerber, B., M. Egger, M. Opravil, A. Telenti, B. Hirschel, M. Battegay, P.
Clinical progression and virological failure on highly active antiretroviral therapy in

19. Little, S. J. 2000. Transmission and prevalence of HIV resistance among treatment-

20. Little, S. J., E. S. Daar, R. T. D'Aquila, P. H. Keiser, E. Connick, J. M. Whitcomb,
N. S. Hellmann, C. J. Petropoulos, L. Sutton, J. A. Pitt, E. S. Rosenberg, R. A.
Koup, B. D. Walker, and D. D. Richman. 1999. Reduced antiretroviral drug

Fong, R. Gum, L. Jin, G. E. Adamson, C. J. Roberts, D. B. Olsen, D. J. Hazuda,
and R. G. Ulrich. 2007. Gene expression profiling of rat liver reveals a mechanistic

Kozal, M. van den Berg-Wolf, C. Henely, B. Schmetter, and M. Dehlinger. 2006. A
comparison of three highly active antiretroviral treatment strategies consisting of non-
nucleoside reverse transcriptase inhibitors, protease inhibitors, or both in the presence


Tables.
Table 1. Activity of A-790742, ATV, LPV, DRV and TPV against WT HIV-1

<table>
<thead>
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<th>Inhibitor</th>
<th>EC$_{50}$ ± SD (nM)</th>
<th>TD$_{50}$ ± SD (uM)</th>
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<tr>
<td></td>
<td>0% HS</td>
<td>50% HS</td>
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<tr>
<td>A-790742</td>
<td>3 ± 1</td>
<td>20 ± 7</td>
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<tr>
<td>ATV</td>
<td>4 ± 2</td>
<td>11 ± 3</td>
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<td>LPV</td>
<td>18 ± 4</td>
<td>151 ± 12</td>
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<tr>
<td>DRV</td>
<td>12 ± 4</td>
<td>40 ± 15</td>
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<tr>
<td>TPV</td>
<td>184 ± 42</td>
<td>7839 ± 888</td>
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</table>

Values shown are representatives of at least ten experiments for EC$_{50}$ of A-790742, ATV and LPV against pNL4-3, three experiments for EC$_{50}$ of DRV and TPV against pNL4-3, and three experiments for all tested PIs against RF.

ND - Not determined
Table 2. Activity of A-790742, ATV, LPV, DRV and TPV against LPV-resistant HIV-1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>EC\textsubscript{50} ± SD (nM)</th>
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<tr>
<td></td>
<td>A 17 \textsuperscript{a}</td>
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<tr>
<td>A-790742</td>
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<tr>
<td>ATV</td>
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<td>LPV</td>
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<td>DRV</td>
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<td>TPV</td>
<td>360 ± 15</td>
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</table>

Values shown are representatives of at least three experiments.

A17, B26 and P25 were derived from \textit{in vitro} passage with LPV.

\textsuperscript{a}A17: L10F, V32I, M46I, I47V, Q58E and I84V

\textsuperscript{b}B26: L33F, K45I, M46I, I50V, I54V, A71V and V82F

\textsuperscript{c}P25: L10F, G16E, V32I, M46I, I47A, H69Y, I84V and T91S

ND - Not determined
Table 3. Activity of A-790742, ATV, and LPV against HIV-1 molecular clones containing proteases derived from PI-resistant patient isolates

<table>
<thead>
<tr>
<th>Virus</th>
<th>Mutations</th>
<th>Fold change in EC&lt;sub&gt;50&lt;/sub&gt;</th>
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<tr>
<td>HIV-Luc</td>
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<tr>
<td>3</td>
<td>L10I, E35D, N37D, M46I, I54V, L63P, A71V, T74P, I84V, L90M, I93L</td>
<td>33</td>
</tr>
<tr>
<td>4</td>
<td>N37T, M46I, Q58E, I64V, T74K, V77I, V82F, L90M</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>L10F, I15V, M46I, L63P, A71V, I72L, V82T, I84V, I85V, L90M</td>
<td>2</td>
</tr>
</tbody>
</table>

Fold resistance relative to the EC<sub>50</sub> of the wild-type molecular clone pNL4-3 Luc.
### Table 4. *In vitro* selection and genotype of mutant variants during passage of HIV-1 pNL4-3 in the presence of A-790742

<table>
<thead>
<tr>
<th>Passage number</th>
<th>A-790742 concentration&lt;sup&gt;a&lt;/sup&gt; (nM) (Fold above EC&lt;sub&gt;50&lt;/sub&gt;)</th>
<th>Mutation&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P5</td>
<td>12 (4)</td>
<td>I84V</td>
</tr>
<tr>
<td>P6</td>
<td>16 (5.3)</td>
<td>I84V</td>
</tr>
<tr>
<td>P7</td>
<td>32 (10.7)</td>
<td>I84V</td>
</tr>
<tr>
<td>P8</td>
<td>50 (16.7)</td>
<td>V82V/L, I84V</td>
</tr>
<tr>
<td>P9</td>
<td>80 (26.7)</td>
<td>V82V/L, I84V</td>
</tr>
<tr>
<td>P10</td>
<td>140 (46.7)</td>
<td>V82V/L, I84V</td>
</tr>
<tr>
<td>P13</td>
<td>400 (133.3)</td>
<td>V82L, I84V</td>
</tr>
<tr>
<td>P15</td>
<td>560 (186.7)</td>
<td>L33L/F, K45I, V82L, I84V</td>
</tr>
<tr>
<td>P18</td>
<td>900 (300)</td>
<td>L33F, K45I, A71A/V, V77V/I, V82L, I84V</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration of A-790742 was increased each passage based on p24 value of previous passage.

<sup>b</sup>Mutation detected by population sequencing.
Table 5. *In vitro* selection and genotype of mutant variants during passage of HIV-1 RF in A-790742

<table>
<thead>
<tr>
<th>Passage number</th>
<th>A-790742 concentration(^a) (nM) (Fold above EC(_{50}))</th>
<th>Mutation(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P6</td>
<td>16 (3.2)</td>
<td>WT</td>
</tr>
<tr>
<td>P8</td>
<td>52 (10.4)</td>
<td>V75I</td>
</tr>
<tr>
<td>P10</td>
<td>160 (32)</td>
<td>A71V, V82G</td>
</tr>
<tr>
<td>P13</td>
<td>340 (68)</td>
<td>M46I, L63P, A71V, V82G</td>
</tr>
<tr>
<td>P15</td>
<td>500 (100)</td>
<td>M46I, L63P, A71V, V82G</td>
</tr>
</tbody>
</table>

\(^a\)Concentration of A-790742 was increased each passage based on p24 value of previous passage.

\(^b\)Mutation detected by population sequencing.
Table 6. Susceptibility of A-790742-passaged HIV-1 to different PIs

<table>
<thead>
<tr>
<th>Virus</th>
<th>Fold change in EC_{50}</th>
<th>A-790742</th>
<th>ATV</th>
<th>LPV</th>
<th>SQV</th>
<th>RTV</th>
<th>APV</th>
<th>IDV</th>
<th>NFV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNL4-3\textsuperscript{a}</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>P9\textsuperscript{b}</td>
<td>13 ±4</td>
<td>6 ±2</td>
<td>4 ±1</td>
<td>3 ±1</td>
<td>26.7 ±3</td>
<td>9 ±2</td>
<td>3 ±1</td>
<td>5 ±2</td>
<td></td>
</tr>
<tr>
<td>P15\textsuperscript{c}</td>
<td>29 ±3</td>
<td>14 ±3</td>
<td>6 ±2</td>
<td>4 ±1</td>
<td>29 ±3</td>
<td>11 ±3</td>
<td>5 ±2</td>
<td>6 ±2</td>
<td></td>
</tr>
<tr>
<td>P21\textsuperscript{d}</td>
<td>&gt;2500 ±250</td>
<td>145 ±10</td>
<td>14 ±4</td>
<td>5 ±2</td>
<td>&gt;141 ±22</td>
<td>34 ±6</td>
<td>14 ±4</td>
<td>&gt;93 ±15</td>
<td></td>
</tr>
<tr>
<td>RF\textsuperscript{e}</td>
<td>72 ±6</td>
<td>31 ±3</td>
<td>&gt;301 ±31</td>
<td>21 ±3</td>
<td>79 ±6</td>
<td>41 ±4</td>
<td>31 ±3</td>
<td>67 ±4</td>
<td></td>
</tr>
<tr>
<td>P13\textsuperscript{f}</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Fold resistance relative to the wild type pNL4-3 or RF EC_{50} and represent average values of three experiments.

P9, P15 and P21 were derived from \textit{in vitro} passage of pNL4-3 with A-790742.0.

P13 derived from \textit{in vitro} passage of RF with A-790742.0.

\textsuperscript{a} pNL4-3: WT

\textsuperscript{b} P9: V82V/L, I84V

\textsuperscript{c} P15: L33L/F, K45I, V82L, I84V

\textsuperscript{d} P21: L23I, L33F, K45I, A71A/V, V77I, V82L, I84V

\textsuperscript{e} RF: WT

\textsuperscript{f} P13: M46I, L63P, A71V, V82G
Table 7. Activity of A-790742 and other PIs against HIV-1 molecular clones containing protease mutations selected by A-790742

<table>
<thead>
<tr>
<th>Clone #</th>
<th>A-790742</th>
<th>ATV</th>
<th>LPV</th>
<th>SQV</th>
<th>RTV</th>
<th>APV</th>
<th>IDV</th>
<th>NFV</th>
<th>DRV</th>
<th>TPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pNL4-3-Luc&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clone 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2 ±0.1</td>
<td>1 ±0.4</td>
<td>1 ±0.4</td>
<td>1 ±0.2</td>
<td>3 ±1</td>
<td>1 ±0.2</td>
<td>1 ±0.1</td>
<td>1 ±0.1</td>
<td>1 ±0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Clone 2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22 ±2</td>
<td>1 ±0.2</td>
<td>1 ±0.1</td>
<td>1 ±0.2</td>
<td>7 ±2</td>
<td>1 ±0.1</td>
<td>1 ±0.2</td>
<td>1 ±0.7</td>
<td>1 ±0.1</td>
<td>ND</td>
</tr>
<tr>
<td>Clone 3&lt;sup&gt;d&lt;/sup&gt;</td>
<td>23 ±3</td>
<td>34 ±4</td>
<td>10 ±3</td>
<td>0.4</td>
<td>79 ±5</td>
<td>12 ±2</td>
<td>5 ±2</td>
<td>3 ±0.8</td>
<td>12 ±3</td>
<td>ND</td>
</tr>
<tr>
<td>Clone 4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>158 ±11</td>
<td>16 ±3</td>
<td>4 ±1</td>
<td>1 ±0.3</td>
<td>14 ±2</td>
<td>2 ±0.3</td>
<td>2 ±0.2</td>
<td>2 ±0.3</td>
<td>7 ±1.4</td>
<td>6 ±1.4</td>
</tr>
<tr>
<td>RF-Luc&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Clone 5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>72 ±8</td>
<td>39 ±3</td>
<td>&gt;1788 ±0.1</td>
<td>28 ±4</td>
<td>1386 ±161</td>
<td>41 ±6</td>
<td>76 ±65</td>
<td>203 ±33</td>
<td>59 ±18</td>
<td>ND</td>
</tr>
</tbody>
</table>

Fold change for clones 1-4 relative to the wild type HIV pNL4-3-Luc EC<sub>50</sub> and represent average values of three experiments.

Fold change for clone 5 relative to the wild type HIV RF Luc EC<sub>50</sub> and represent average values of three experiments.

<sup>a</sup>pNL4-3-Luc: Wild-type pNL4-3

<sup>b</sup>Clone 1: I84V

<sup>c</sup>Clone 2: V82L

<sup>d</sup>Clone 3: L33F, K45I, V82L, I84V

<sup>e</sup>Clone 4: L33F, A71V, G73S, V77I, V82L, I84V

<sup>f</sup>RF-Luc: Wild-type RF

<sup>g</sup>Clone 5: L63P, A71V, V82G

ND - Not determined
Figure Legends

Figure 1. Structure of A-790742.

Figure 2. Replication capacity of pNL4-3-Luc and RF-Luc molecular clones containing different protease mutations. Replication capacity of mutant molecular clones was calculated by comparing the median of luciferase signal generated by mutants to that generated by WT pNL4-3-Luc and RF-Luc respectively, in at least three experiments. The RC values were expressed as percentages of the WT and reflected the levels of replication for mutant clones compared to that of the WT control. Replication capacity of WT pNL4-3-Luc molecular clone was approximately two times higher than WT RF-Luc molecular clone. Black boxes represent pNL4-3 and pNL4-3-derived mutants, and gradient boxes represent RF and RF-derived mutants. Error bars represent SD.
Figure 2

The bar chart shows the replication capacity of different variants of the NL4-3 virus. The x-axis represents different virus strains, including pNL4-3 Luc, I84V, V82L, L33L, K45I, V82L, I84V, L33L, A71V, G73S, V77L, V82L, I84V, RF-Luc, L63P, A71V, V82G, M46I, A71V, V82G. The y-axis represents the replication capacity in percentage, ranging from 0.1 to 100.0. The chart indicates that the replication capacity varies significantly among different strains.