Published in final edited form as:
AIDS. 1999 April 16; 13(6): 661–667.

Highly drug-resistant HIV-1 clinical isolates are cross-resistant to many antiretroviral compounds in current clinical development

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Abstract

Objectives—To assess the in-vitro drug susceptibility of a panel of five well-characterized drug-resistant HIV variants to recently developed anti-HIV compounds including seven reverse transcriptase (RT) inhibitors and seven protease inhibitors.

Methods—Drug-resistant viral strains were selected on the basis of the prevalence of these mutants in patient samples from local area HIV clinics. The isolates included one multinucleoside-resistant virus containing the Q151M mutation, and four clinical isolates containing multiple RT and protease resistance mutations. The activity of the experimental compounds against these isolates was determined using drug susceptibility assays and measuring the viral antigen p24 end-point.

Results—These clinically relevant highly drug-resistant viruses were resistant to many of the new compounds in clinical development. In most cases the resistance mutations of the clinical isolate were different from those selected *in vitro* for the particular experimental compound.

Conclusions—It is critical to expand the preclinical development of new drugs to include the assessment of their activity against currently circulating highly drug-resistant clinical strains, in order to develop appropriate salvage therapies for patients harboring resistant strains.

Keywords

antiviral therapy; HIV-1; protease; resistant; reverse transcriptase

Introduction

Potent and rationally designed combination chemotherapy can dramatically slow the progression of HIV infection in patients. Multiple-drug therapies containing three or four drugs active against both the protease and reverse transcriptase (RT) enzymes of HIV have been shown to decrease plasma HIV-RNA levels, increase patient CD4 cell counts, and reduce patient mortality [1-3]. The capacity of HIV to develop drug resistance mutations, however, presents a major obstacle to long-term effective anti-HIV therapy [4-7]. As a result of viral cross-resistance, the accumulation of multiple resistance mutations may reduce the effectiveness of subsequent retroviral treatments [8-10].

The increased clinical use of combination retroviral treatment for HIV-1 infection has led to the selection of viral strains resistant to multiple drugs, including strains resistant to all licensed nucleoside analog RT inhibitors and protease inhibitors (PIs) [10-13]. The treatment options for patients harboring these highly drug-resistant viral strains are limited. New anti-HIV

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compounds in clinical development may offer these patients alternative treatment strategies, however, it is important to determine their activity against highly drug-resistant clinical isolates.

In the present study, we assessed the in-vitro drug susceptibility of four highly drug-resistant HIV-1 clinical isolates and one recombinant virus to 14 experimental anti-HIV compounds. The genotypic profiles of these five isolates were frequently detected in heavily treated patients with persistent viremia attending HIV clinics in the San Francisco Bay area (Shafer RW, Zolopa A, Merigan TC, personal communication, 1998).

Materials and methods

A molecular clone of HIV-1 containing four nucleoside resistance mutations (V75I, F77L, F116Y, Q151M) observed in patients receiving zidovudine (ZDV) and didanosine (ddI) combination chemotherapy was previously generated [14]. The clinical isolates were obtained from four patients who had received extensive and long-term multidrug antiretroviral therapy, and the resistance mutations of one of these isolates has recently been reported [10].

Cell culture and drug compounds

Peripheral blood mononuclear cells (PBMCs) combined from two seronegative donors were isolated by centrifugation on Ficoll-Paque and cultured in Roswell Park Memorial Institute (RPMI) medium containing 15% heat-inactivated fetal calf serum, IL-2 and antibiotics. Cells were stimulated for 2 to 3 days with phytohemagglutinin (PHA) (Sigma Chemical Co., St Louis, MO, USA) before use. Drugs were made available from the manufacturers as mentioned in the acknowledgements section.

Virus isolation

Primary isolates of HIV-1 were generated and expanded to high titers by the co-cultivation of infected patient PBMCs with PHA-stimulated donor PBMC (Virology manual for ACTG HIV Laboratories, NIH 1997). The cell-free supernatants were harvested, sequenced, and stored in aliquots at -70°C for drug susceptibility assays.

Sequence analysis of HIV-1 strains

A 1.3 kb fragment of cDNA encompassing HIV-1 protease and the first 300 codons of RT was sequenced from each cultured supernatant as previously described [9]. Briefly, purified viral RNA (Qiagen Viral RNA Extraction Kits Qiagen, Chatsworth, CA, USA) was reverse-transcribed and polymerase chain reaction (PCR) amplified using Superscript-One-Step RT-PCR reagent (Life Technologies, Gaithersburg, MD, USA) using the two primers, MAW-26 and RT21 [15]. A 5 μl aliquot of the first PCR reaction was used for a second round nested PCR reaction using primers PRO-1 [16] and RT20 [15]. Approximately 70 ng of the 1.3 kb product was sequenced using dye-labelled dideoxyterminator cycle sequencing (Applied Biosystems, Foster City, CA, USA). Isolate sequences were compared with both patient plasma sequences and the consensus B sequence from the Los Alamos HIV Sequence Database [17].

Characterization of viral strains

The five viral strains included in this study represent six separate HIV-1 resistance profiles: two for the RT gene, and four for the protease gene. The sequences from two of the five isolates have previously been studied and were consistent with sequence results obtained in this study (Table 1) [10,14,18].

For the purpose of this analysis we characterized several mutations as ‘signature mutations’. The selected signature mutations were known to cause resistance and were used in

distinguishing the different isolates. These signature mutations were prevalent among patients with persistent viremia that attending physicians considered failing long-term multidrug therapies. Among 102 patient referrals, all of which had received at least four RT inhibitors, 54% were found to have all three of the signature mutations at positions M41L, M184V, and T215Y or the Q151M signature mutation. Among 49 patient referrals, all of which had received at least three PIs, 46% had one of the three protease signature genotypic profiles. Therefore, increases in the percentage of the three protease signature genotypic profiles relate closely to increases in the number of PIs utilized in treatment (Table 2). The signature mutations of each isolate are depicted as subscripts.

Drug susceptibility assays

In-vitro drug susceptibility assays were performed using a modified ACTG/Division of AIDS consensus method (virology manual for ACTG HIV laboratories). PBMCs were preinfected with titrated viral stocks for 4 h at 37°C in a humidified atmosphere of 5% carbon dioxide. After 4 h incubation, infected cells were washed twice in media and pipetted into a microtiter plate with eight serial drug dilutions (see Table 3 for drug nomenclature and concentrations). Each well contained 100 000 preinfected PBMCs and all drug dilutions were made in cell culture media except for the PIs, which were first dissolved in dimethyl sulfoxide (DMSO) before being diluted in cell culture media. The drug dilutions were chosen to span the 50% inhibitory concentration (IC_{50}) for each single drug [19-33]. Control wells containing cells and virus were co-incubated on each plate.

Each assay also contained a titration of the virus-infected cells, obtained by using serial 10-fold dilutions to determine the viral 50% tissue-infective dose ($TCID_{50}$). To enable assay standardization and comparison, the $TCID_{50}$ of each isolate was maintained between 30 and 100. After a 7 day incubation at 37°C in a humidified atmosphere of 5% carbon dioxide, viral growth was determined using a p24 antigen assay on supernatants (Abbott Laboratories Inc., Chicago, IL, USA). The percentage inhibition of viral growth compared with the control wells without drugs was calculated. Results were expressed as the mean IC_{50} of four to six values obtained in two to three different experiments per isolate.

Results

Susceptibility of recombinant isolate SR151(RT)$_{151}$ to reverse transcriptase inhibitors

Table 4 shows the drug susceptibility of SR151(RT)$_{151}$ to four different classes of RT inhibitors. The recombinant virus SR151(RT)$_{151}$ had high-level resistance (15-fold to > 100-fold) to the approved nucleoside analogs ZDV, stavudine (d4T), ddI, and lamivudine (3TC) compared with the wild-type isolate NL_{4-3}. The susceptibility of SR151(RT)$_{151}$ to the experimental nucleoside analog abacavir (1592U89) and the pyrophosphate analog (PFA; foscarnet) was reduced 16-fold, and over sixfold, respectively. This virus showed minor reduced susceptibility to the nucleotides 9-(2-phosphonylmethoxyethyl) adenine (PMEA; adevovir dipivoxil) and (R)-9-(2-phosphonylmethoxypropyl) adenine (PMPA) (threefold and five-fold, respectively) and to the experimental nucleoside analog F-ddA (threefold). The IC_{50}s of the non-nucleoside RT inhibitors for isolate SR151(RT)$_{151}$ were similar to the wild type, indicating that the mutant isolate SR151(RT)$_{151}$ retained susceptibility to these compounds.

Susceptibility of clinical isolate 807(RT)$_{41,184,215}$ to reverse transcriptase inhibitors

The clinical isolate 807(RT)$_{41,184,215}$ exhibited fivefold to over 100-fold resistance to the approved nucleoside analogs ZDV, d4T, ddI, and 3TC compared with the wild-type isolate NL_{4-3} (Table 4). The susceptibility of isolate 807(RT)$_{41,184,215}$ to the experimental nucleoside analogs F-ddA and 1592U89 was reduced fourfold and sixfold, respectively, compared with

the wild type. Isolate 807(RT)$_{41,184,215}$ retained susceptibility to PMEA, PMPA, PFA and to all the non-nucleoside RT inhibitors assayed: the IC_{50}s of PMEA, PMPA, PFA and the non-nucleoside RT inhibitors for the mutant virus 807(RT)$_{41,184,215}$ were similar to or less than their IC_{50}s for the wild-type isolate NL_{4-3}.

Susceptibility of clinical isolates 807(PR)$_{48,54,82}$, 3761(PR)$_{46,84,90}$, 1385(PR)$_{46,54,82,90}$ and 769 (PR)$_{46,54,82,84,90}$ to protease inhibitors

The susceptibilities of mutant clinical isolates to three different classes of protease inhibitors are shown in Table 5. Clinical isolates 807(PR)$_{48,54,82}$, 3761(PR)$_{46,84,90}$, and 769 (PR)$_{46,54,82,84,90}$ had high-level resistance (11-fold to 91-fold) to the approved peptidomimetic inhibitors indinavir (IDV), saquinavir (SQV), and nelfinavir (NFV), compared with the wild-type isolate NL_{4-3}. Isolate 1385(PR)$_{46,54,82,90}$ also had high-level resistance to IDV and NFV (15-fold and 41-fold, respectively) but was found to be sensitive to inhibition by SQV. All isolates showed from fourfold to over 100-fold reduced susceptibility to the experimental peptidomimetic inhibitors (Vx-478, BMS 232632, DG-35, DG-43, and palinavir), and the cyclic sulfone inhibitor (GS 3333) compared with the wild type. The IC_{50}s of the cyclic urea amide inhibitor, SD146, for all isolates were similar to the wild type indicating that SD146 maintains activity against these mutant clinical isolates. With the exception of NFV, DG-35, and BMS 232632, isolate 3761(PR) appeared to be more resistant (higher IC_{50} values) to PIs than the other isolates analysed.

Discussion

We assessed the activity of recently developed anti-HIV compounds against three highly drug-resistant patient isolates. The RT and protease mutations present in these isolates were found in a high proportion of heavily treated patients with persistent detectable plasma HIV-1 levels attending clinics in the San Francisco Bay area (Table 2) (Shafer RW, Zolopa A, Merigan TC, personal communication, 1998). Our findings show for the most part that these isolates demonstrated some degree of resistance to experimental anti-HIV agents (Tables 4 and 5). In some cases, however, we also identified drugs to which these prototypical isolates retained susceptibility.

The IC_{50}s of the experimental compounds for the wild-type NL_{4-3} in the present study were similar to those reported previously for other wild-type strains [19-33]. In contrast, the recombinant virus SR151(RT)$_{151}$ had high-level resistance to all licensed nucleoside analogs (Table 4) [18]. The Q151M signature mutation of isolate SR151(RT)$_{151}$ forms part of the template grip of the RT, and this single mutation has been shown to cause resistance to multiple nucleoside analogs [34]. Moreover, isolate SR151(RT)$_{151}$ showed varying levels of decreased susceptibility to many of the new compounds in this study: F-ddA, 159289U, PMEA, PMPA and PFA. The RT resistance mutations of isolate SR151(RT)$_{151}$ (Table 1) are different from the RT gene mutations selected *in vitro* that confer HIV-1 resistance to F-ddA (P119S, V179D, and L214F), 159289U (K65R, L74V, M184V), PMEA (K65R, K70E), PMPA (K65R), and PFA (W88G, E89G/K, L92I, S156A, Q161L, H208Y) [19,35-40]. This finding indicates that the presence of the Q151M mutation associated with ZDV and ddI combination therapy can alter susceptibilities to different degrees across a number of classes of new drug compounds. The clinical importance of the three-to-five-fold level of reduced susceptibility to F-ddA, PMEA and PMPA measured for the virus SR151(RT)$_{151}$ is unclear at this time.

The clinical isolate 807(RT)$_{41,184,215}$ had reduced susceptibility to F-ddA (fourfold) and 1592U89 (sixfold) but retained susceptibility to PMEA and PMPA (Table 4). This isolate contained five ZDV-resistance mutations (M41L, D67N, L210W, T215Y, K219N) and one 3TC-resistance mutation (M184V) as well as other changes from consensus B [17]. It is difficult to correlate individual mutations precisely with resistance to each of the experimental

drugs tested. The M184V together with the ZDV-resistance mutations may, however, explain the level of viral resistance to the experimental nucleoside analog 1592U89 [35]. Conversely, the M184V RT mutation in the presence of ZDV-resistance mutations has been reported to increase viral susceptibility to PMEA and PMPA [41,42]. In accordance with these reports, isolate 807(RT)$_{41,184,215}$ harboring the M184V mutation together with multiple ZDV-resistance mutations was susceptible to inhibition by PMEA and PMPA.

In agreement with our earlier studies, clinical isolate 807(PR)$_{48,54,82}$ containing signature mutations G48V, I54T, and V82A had high-level resistance to the approved peptidomimetic inhibitors IDV, SQV and NFV (Tables 2 and 5) [10]. The clinical isolates 3761(PR)$_{46,84,90}$ and 769(PR)$_{46,54,82,84,90}$ with different signature mutations also exhibited phenotypic resistance to these approved compounds. Moreover, sequence analysis of five biological clones of isolate 769(PR)$_{46,54,82,84,90}$ revealed that the signature resistance mutations of this isolate were located on one viral strain.

The clinical isolate 1385(PR)$_{46,54,82,90}$ containing signature mutations M46I, I54I/V, V82T, and L90M was resistant to IDV and NFV but sensitive to SQV. Previous studies have shown that molecular clones containing mutations M36I, I54V and V82T are susceptible to inhibition by SQV, whereas primary isolates containing mutations M46I, I54V, V82A or F, and L90M are between fourfold and eightfold resistant to SQV [5,6]. The specific amino acid change at position 82 may influence the activity of SQV against these different mutant viruses.

The structural basis for the fourfold to over 100-fold resistance of all the clinical isolates to the experimental peptidomimetic compounds (Vx-478, BMS 232632, DG-35, DG-43, and palinavir) and the cyclic sulfone inhibitor (GS 3333) is unknown. The genotypic profiles of these isolates may be related to their drug resistance. Differences in drug bioavailability and tolerance *in vivo* may, however, reduce the significance of in-vitro resistance.

The protease gene mutations at codons G48V, V82A/T/F, and I84V alter the active site of the protease enzyme and mutations at codons M46I and I54T and modify the flap region of the enzyme [43,44]. The resistance mutations of the clinical isolates in this study are located in important regions of the protease enzyme and may interfere with binding and activity of the experimental compounds. To date, PIs are typically designed to inhibit the protease enzyme of wild-type viral strains [44]. On the basis of the findings of the present study, PIs should be designed to inhibit clinically relevant drug-resistant viral strains as well as the wild type.

The new PI that was found to be active against all the isolates was the cyclic urea amide, SD 146. This compound forms 14 hydrogen bonds to the backbone of the protease enzyme, with four of these bonds binding to the catalytic aspartates (25/25′) [32]. In a recent study [32], SD 146 has been shown to retain activity against a number of molecularly constructed protease-resistant viruses. SD 146 thus appears to inhibit both recombinant and clinical viral isolates effectively, which express a broad range of protease resistance mutations. To date, however, no soluble formulation of SD 146 has been developed and this compound has not progressed to clinical testing.

Conclusion

The results of this in-vitro study underline the importance of determining the activity of new compounds against highly drug-resistant patient isolates. Many of the current anti-HIV compounds in clinical development were found to be less potent against the highly drug-resistant patient isolates as compared with the wild type. This was the case even in the absence of specific RT and protease gene mutations previously shown to decrease the susceptibility of HIV-1 to these new compounds. Although the in-vitro assays utilized in this study are limited to one cell type and do not take into account such factors as patient viral load, drug dosage,

drug uptake and drug metabolism, these assays may indicate the efficacy of prospective drug therapy. Such preclinical assessments could aid in the development of appropriate clinical trials and salvage therapies for patients harboring resistant HIV strains.

Acknowledgements

The authors would like to thank the following companies and individual for generously providing their compounds for this study: Astra Arcus AB, Sweden (PFA); Boehringer Ingelheim, Canada (palinavir); Bristol Myers Squibb, USA (BMS 232632); Dupont Merck, USA (DMP 266, SD146); Gilead Sciences, USA (PMEA, PMPA, GS 3333); Glaxo Wellcome Inc., USA (1592U89, Vx-478); Hoechst, Germany (HBY 097); Narhex Operations, Australia (DG-35, DG-43); and Dr Hiroaki Mitsuya, NCI (F-ddA). We especially wish to thank Darcy Levee and Kristi Cooley for excellent technical assistance with the sequencing.

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Table 1

Reverse transcriptase gene and protease gene mutations of highly resistant patient isolates

Isolate	Gene	Resistance mutations	Other changes from consensus B
SR151	RT	V751, F77L, F116Y, **Q151M**[*]	V13I, K102Q, E122K, S162C, E207Q
807	RT	**M41L**, D67N, **M184V**, L210W, **T215Y**, K219N	K43N, V60I, A98S, V118I, I135V, G196E, R211K
807	PR	L10I, **G48V**, **I54T**, L63Q, A71V, **V82A**	V31I, R41K, I62V, T74A, V77I, I93L
3761	PR	L10I, **M46I**, **I84V**, L63P, A71I, **L90M**	T12A, V13I, I15V, L19I, K20I, I64P, G73T, P79S, I93L
1385	PR	L10I, M36I, **M46I**, **I54I/V**, L63P, A71V, **V82T**, **L90M**	V13I, N37D, R57K, L89M, I93L
769	PR	L10I, M36M/V, **M46I**, **I54V**, L63P, A71V, **V82A**, **I84V**, **L90M**	V13I, D60E, I62V

[*] Bold figures represent the signature mutations of the gene.

Table 2

Prevalence of reverse transcriptase and protease gene mutational patterns in sequenced samples from Stanford area HIV clinics

Signature* RT gene mutations	Percentage after 4 RTIs
151	6% (6/102)
41+184+215	48% (49/102)

		Percentage after PI/s treatment		
Signature protease gene mutations	**Untreated (n = 278)†**	**1 Drug**	**2 Drugs**	**3-4 Drugs**
48+54+82	0	1.5% (1/65)	10% (5/48)	20% (10/49)
(46 and/or 54)+82+90	0	5% (3/65)	19% (9/48)	18% (9/49)
(46 and/or 54)+84+90	0	0	4% (2/48)	8% (4/49)

PI, protease inhibitor; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor.

*Selected signature mutations were known to cause resistance and were used in distinguishing the different isolates.

†The sequences for these data drawn from Los Alamos Data Bank for untreated patients and Stanford area HIV clinics.

Table 3

Abbreviations, names and concentrations of anti-HIV compounds included in antiviral assay

Abbreviation/company nomenclature	Generic name	Proprietary name	Drug concentration (μM)
Nucleoside/nucleotide analogs			
ZDV	Zidovudine	Retrovir	5-0.0039
d4T	Stavudine	Zerit	5-0.031
ddI	Didanosine	Videx	50-0.05
3TC	Lamivudine	Epivir	25-0.039
F-ddA	-	-	50-0.20
1592U89	Abacavir	Ziagen	50-0.20
PMEA	Adefovir dipivoxil	Preveon	50-0.39
PMPA	-	-	50-0.39
Pyrophosphate analog			
PFA	Foscarnet	Foscavir	200-2.0
Non-nucleoside inhibitors			
NVP	Nevirapine	Viramune	0.5-0.0039
DLV	Delavirdine	Rescriptor	0.5-0.0039
HBY 097	-	-	0.13-0.00098
DMP 266	Efavirenz	Sustiva	0.13-0.00025
Protease inhibitors			
IDV	Indinavir	Crixivan	50-0.039
SQV	Saquinavir	Invirase	10-0.020
NFV	Nelfavir	Viracept	10-0.020
Vx-478	Amprenavir	-	4.0-0.0078
BMS 232632	-	-	1.0-0.00078
DG-35	-	-	10-0.0078
DG-43	-	-	10-0.00078
Palinavir	Palinavir	-	10-0.0039
GS3333	-	-	10-0.0078
SD146	-	-	0.125-0.00098

Table 4

Susceptibility of a wild-type laboratory isolate and highly resistant viruses to reverse transcriptase inhibitors

Drug	WT_{NL4-3}	$SR151(RT)_{75,77,116,151}$ *		$807(RT)_{41,67,184,210,215,219}$	
Nucleoside analogs	**IC_{50} (μM)†**	**IC_{50} (μM)**	**Fold change‡**	**IC_{50} (μM)**	**Fold change**
Approved					
ZDV	0.04 ± 0.01	4.9 ± 2.3	> 100	1.5 ± 0.50	37
d4T	0.16 ± 0.004	2.3 ± 0.72	15	0.74 ± 0.05	5
ddI	0.32 ± 0.1	62 ± 4.5	> 100	3.6 ± 0.47	11
3TC	0.27 ± 0.04	11 ± 1.1	40	37 ± 2.4	> 100
Experimental					
F-ddA	6.4 ± 1.5	20 ± 0.50	3	24 ± 5.5	4
1592U89	1.4 ± 0.55	22 ± 6.9	16	7.8 ± 2.6	6
Nucleotide analogs					
PMEA	2.8 ± 1.37	9.5 ± 1.60	3	4.5 ± 1.94	2
PMPA	1.4 ± 0.52	6.9 ± 0.08	5	1.2 ± 0.76	0.9
Pyrophosphate analog					
PFA	24 ± 12	> 150	> 6	19 ± 11	0.8
Non-nucleoside inhibitors					
NVP	0.06 ± 0.01	0.12 ± 0.01	2	0.04 ± 0.01	0.7
DLV	0.10 ± 0.03	0.16 ± 0.02	2	0.02 ± 0.002	0.2
HBY 097	0.008 ± 0.002	0.015 ± 0.001	2	0.005 ± 0.001	0.6
DMP 266	0.004 ± 0.001	0.004 ± 0.001	1	0.003 ± 0.002	0.8

* Resistance mutations.

† PMBCs were exposed to 30-100 $TCID_{50}$ of each viral isolate for 4 h. Drugs and infected cells were then incubated in microtiter plates for 7 days. The p24 content of each well was measured and the IC_{50} for each drug was calculated. Results are expressed as the means of four to six values obtained in two to three different experiments ± standard deviation.

‡ Fold changes compared with wild-type NL_{3-4} isolate. For abbreviations see Table 3.

Table 5

Susceptibility of a wild-type laboratory isolate and highly resistant patient isolates to protease inhibitors

Drug	**WT_{NL4-3}**	**807$(PR)_{48,54,82}$***		**3761$(PR)_{46,84,90}$**		**1385$(PR)_{46,54,82,90}$**		**769$(PR)_{46,54,82,84,90}$**	
Inhibitors Peptidomimetic	**IC_{50} (μM)†**	**IC_{50} (μM)**	**Fold change‡**	**IC_{50} (μM)**	**Fold change**	**IC_{50} (μM)**	**Fold change**	**IC_{50} (μM)**	**Fold change**
Approved									
IDV	0.035 ± 0.02	2.3 ± 1.7	66	3.2 ± 0.64	91	0.52 ± 0.04	15	2.2 ± 0.4	63
SQV	0.030 ± 0.01	1.9 ± 0.02	63	2.3 ± 0.03	77	0.03 ± 0.02	1	1.3 ± 0.2	43
NFV	0.15 ± 0.07	3.3 ± 0.47	22	1.7 ± 0.78	11	6.1 ± 1.2	41	7.0 ± 1.4	47
Experimental									
Vx-478	0.023 ± 0.01	0.20 ± 0.09	9	1.1 ± 0.07	47	0.1 ± 0.02	4	0.31 ± 0.06	14
BMS 232632	0.004 ± 0.002	0.13 ± 0.05	33	0.26 ± 0.05	65	0.04 ± 0.006	10	0.32 ± 0.1	80
DG-35	0.058 ± 0.007	8.6 ± 1.5	> 100	1.9 ± 0.03	> 100	ND§		ND	
DG-43	0.001 ± 0.0001	1.4 ± 0.65	> 100	1.9 ± 0.03	> 100	0.04 ± 0.02	40	0.50 ± 0.08	> 100
Palinavir	0.027 ± 0.01	5.0 ± 0.94	> 100	5.6 ± 1.2	> 100	1.9 ± 0.4	70	> 10	> 100
Cyclic sulfone									
GS 3333	0.030 ± 0.01	0.38 ± 0.10	13	7.3 ± 3.8	> 100	1.0 ± 0.2	33	3.4 ± 0.2	> 100
Cyclic urea amide									
SD 146	0.013 ± 0.001	0.031 ± 0.01	2	0.032 ± 0.004	2	0.010 ± 0.003	1	0.035 ± 0.001	3

*Resistance mutations.

†PMBCs were exposed to 30-100 $TCID_{50}$ of each viral isolate for 4 h. Drugs and infected cells were then incubated in microtiter plates for 7 days. The p24 content of well was measured and the IC50 for each drug was calculated. Results are expressed as the means of four to six values obtained in two to three different experiments ± each standard deviation.

‡Fold changes compared with wild-type NL3-4 isolate.

§Not determined. For abbreviations see Table 3.