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Synthesis and Biological Evaluation of Novel Allophenylnorstatine-based HIV-1 Protease Inhibitors Incorporating High Affinity P2-ligands

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Abstract

A series of stereochemically defined cyclic ethers as P2-ligands were incorporated in an allophenylnorstatine-based isostere to provide a new series of HIV-1 protease inhibitors. Inhibitors **3b** and **3c**, containing conformationally constrained cyclic ethers, displayed impressive enzymatic and antiviral properties and represent promising lead compounds for further optimization.

Keywords

HIV protease; Inhibitors; Darunavir; Allophenylnorstatine; Design; Synthesis

The introduction of protease inhibitors into highly active antiretroviral treatment (HAART) regimens with reverse transcriptase inhibitors represented a major breakthrough in AIDS chemotherapy.[1] This combination therapy has significantly increased life expectancy, and greatly improved the course of HIV management. Therapeutic inhibition of HIV-1 protease leads to morphologically immature and noninfectious viral particles.[2] However, under the selective pressure of chemotherapeutics, rapid adaptation of viral enzymes generates strains resistant to one or more antiviral agents.[3] As a consequence, a growing number of HIV/AIDS patients harbor multi-drug-resistant HIV strains. There is ample evidence that such strains can be readily transmitted.[4] Therefore, one of the major current therapeutic objectives has been to develop novel protease inhibitors (PIs) with broad-spectrum activity against multidrug-resistant HIV-1 variants. In our continuing interest in developing concepts and strategies to combat drug-resistance, we have reported a series of novel PIs including

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Darunavir, TMC-126, GRL-06579 and GRL-02031.[5–8] These inhibitors have shown exceedingly potent enzyme inhibitory and antiviral activity as well as exceptional broad spectrum activity against highly cross-resistant mutants. Darunavir, which incorporates (*R*)-(hydroxymethyl)-sulfonamide isostere and a stereochemically defined *bis*-tetrahydrofuran (*bis*-THF) as the P2-ligand, was initially approved for the treatment of patients with drug-resistant HIV and more recently, it has been approved for all HIV/AIDS patients including pediatrics.[9]

Darunavir was designed based upon the 'backbone binding' concept developed in our laboratories. Darunavir-bound X-ray structure revealed extensive hydrogen bonding with the protease backbone throughout the enzyme active site.[10] The P2-*bis*-THF ligand is responsible for its superior drug-resistance properties. The *bis*-THF ligand has been documented as a privileged ligand for the S2-subsite. Incorporation of this ligand into other transition-state isosteres also resulted in significant potency enhancement.[11] Besides 3(*S*)-THF, [3*aS*,5*S*,6*R*]-*bis*-THF, we have designed a number of other novel cyclic ether-based high affinity ligands. Incorporation of these ligands in (*R*)-(hydroxyethyl)-sulfonamide isosteres provided PIs with excellent potency and drug-resistance properties.[6–8] We have then investigated the potential of these structure-based designed P2-ligands in KNI-764-derived isostere designed by Mimoto and co-workers.[12] This PI incorporates an allophenylnorstatine isostere. Interestingly, KNI-764 has maintained good activity against HIV-1 clinical strains resistant to several FDA-approved PIs. The flexible *N*-(2-methyl benzyl) amide P2′-ligand may have been responsible for its activity against drug-resistant HIV-1 strains as the flexible chain allows better adaptability to mutations.[12,13] The *bis*-THF and other structure-based designed P2-ligands, make several critical hydrogen bonds with the protein backbone, particularly with Asp-29 and Asp-30 NH's.[11] Therefore, incorporation of these ligands into the KNI-764-derived isostere, may lead to novel PIs with improved potency and efficacy against multidrug-resistant HIV-1 variants. Furthermore, substitution of P2-phenolic derivative in KNI-764 with a cyclic ether-based ligand could result in improved metabolic stability and pharmacological properties since phenol glucuronide is readily formed when KNI-764 is exposed to human hepatocytes in vitro.[12]

The synthesis of target compounds **3a–e** was accomplished as described in Scheme 1. Our synthetic plan for the synthesis of carboxylic acid **7** (Scheme 1) involved the preparation of the key intermediate **5** which was prepared through two different synthetic pathways. In the first approach, known optically active azidodiol **4**[14] was first hydrogenated in the presence of Boc_2O. The resulting diol was converted to **5** by selective acylation of the primary alcohol with acetic anhydride in the presence of pyridine and a catalytic amount of DMAP at 0 °C for 4 h to provide **5** in 77% overall yield. As an alternative approach, commercially available optically active epoxide **6** was exposed to lithium acetate, formed *in situ* from lithium carbonate and acetic acid in DMF. This resulted in the regioselective opening[15] of the epoxide ring and afforded compound **5** in 62% yield. The alcohol **5** thus obtained was protected as the corresponding acetonide by treatment with 2-methoxypropene in the presence of a catalytic amount of CSA. The acetate group was subsequently hydrolyzed in the presence of potassium carbonate in methanol to afford the corresponding alcohol. This latter was subjected to an oxidation reaction using ruthenium chloride hydrate and sodium periodate in a mixture of aqueous acetonitrile and CCl_4 at 23 °C for 10 h. This resulted in the formation of target carboxylic acid **7** in 61% yield. Amine **9a** was prepared by activation of carboxylic acid **7** into the corresponding mixed anhydride by treatment with *iso*-butylchloroformate followed by reaction with amine **8a**.[16,17]

Synthesis of various inhibitors was carried out as shown in Scheme 2. Deprotection of Boc and acetonide groups was carried out by exposure of **9** to 1 M solution of hydrochloric acid in methanol at 23 °C for 8 h. This provided amine **10** in quantitative yield. Reaction of **11a**

with amine **10** in CH_2Cl_2 in the presence of Et_3N at 23 °C for 6 h, provided inhibitor **3a** in 62% yield. The 3(S)-tetrahydrofuranyl carbonate **11a** was prepared as described previously.[18] Similarly, allophenylnorstatine-based inhibitors **3b–e** were synthesized. As shown, carbonates **11b**[19]**, 11c**[7], and **11d–e**[19] were prepared as previously described. Reaction of these carbonates with amine **10** furnished the desired inhibitors **3b–e** in 45–62% yield.

The syntheses of inhibitors **14a,b** and **16a–c** were carried out as shown in Scheme 3. Inhibitors **14a,b**, containing hydroxyethylamine isostere were prepared by opening of epoxide **6** with amine **8a** in the presence of lithium perchlorate in diethyl ether at 23 °C for 5 h to provide amino alcohol **12** in 64% yield. Removal of Boc-group by exposure to 1M HCl in MeOH at 23 °C for 12 h afforded amine **13**. Reactions of amine **13** with activated carbonates **11a** and **11b** afforded urethane **14a** and **14b** in 44% and 59% yields, respectively. For the synthesis of inhibitors **16a–c**, commercially available (*R*)-5,5-dimethyl-thiazolidine-4-carboxylic acid was protected as its Boc-derivative. The resulting acid was coupled with amines **15a–c** in the presence of DCC and DMAP in CH_2Cl_2 to provide the corresponding amides. Removal of Boc-group by exposure to 30% trifluoroacetic acid afforded **8b–d**. Coupling of these amines with acid **7** as described in Scheme 1, provided the corresponding products **9b–d**. Removal of Boc group and reactions of the resulting amine with activated carbonate **11b** furnished inhibitors **16a–c** in good yields (55–60%).

Inhibitors **3a–e** were first evaluated in enzyme inhibitory assay utilizing protocol described by Toth and Marshall.[20] Compounds that showed potent enzymatic K_i values were then further evaluated in antiviral assay. The inhibitor structure and potency are shown in Table 1. As shown, incorporation of a stereochemically defined 3(*S*)-tetrahydrofuran ring as the P2-ligand provided inhibitor **3a**, which displayed an enzyme inhibitory potency of 0.2 nM and antiviral IC_{50} value of 20 nM. The corresponding derivative **14a** with a hydroxyethylamine isostere exhibited over 400-fold reduction in enzyme inhibitory activity. Introduction of a stereochemically defined bis-THF as the P2-ligand, resulted in inhibitor **3b**, which displayed over 40-fold potency enhancement with respect to **3a**. Inhibitor **3b** displayed a *K*i of 5.2 pM in the enzyme inhibitory assay. Furthermore, compound **3b** has shown an impressive antiviral activity with an IC_{50} value of 9 nM. Inhibitor **14b** with hydroxyethylamine isostere is significantly less potent than the corresponding norstatine-derived inhibitor **3b**. Inhibitor **3c** with a (3a*S*, 5*R*, 6a*R*)-5-hydroxy-hexahydrocyclopenta[*b*]furan as the P2-ligand has displayed excellent inhibitory activity, and particularly, antiviral activity, showing an IC_{50} value of 13 nM. Other structure-based designed ligands in inhibitors **3d** and **3e** have shown subnanomolar enzyme inhibitory activity. However, inhibitor **3b** with a *bis*-THF ligand has shown most impressive activity.

To obtain molecular insight into the possible ligand-binding site interactions, we have created energy-minimized models of a number of inhibitors based upon protein-ligand X-ray structure of KNI-764 (**2**).[21] An overlayed model of **3b** with X-ray structure of **2**-bound HIV-1 protease is shown in Figure 2. This model for inhibitor **3b** was created from the X-ray crystal structure of KNI-764 (**2**)-bound HIV-1 protease (KNI-764, pdb code 1MSM[21]) and the X-ray crystal structure of darunavir (pdb code 2IEN[22]), by combining the P2-end of the darunavir structure with the P2′-end of the KNI-764 structure, followed by 1000 cycles of energy minization. It appears that both oxygens of the bis-THF ligand are suitably located to form hydrogen bonds with the backbone atoms of Asp-29 and Asp-30 NH's, similar to darunavir-bound HIV-1 protease.[10] Furthermore, the KNI-764-X-ray structure-derived model of **3b** suggested that the incorporation of appropriate substituents on the phenyl ring could interact with Asp-29′ and Asp-30′ in the S2′-subsite. In particular, it appears that a 4-hydroxymethyl substitutent on the P2′-phenyl ring could conceivably interact with backbone Asp-30′ NH in S2′-subsite. Other substituents such as a methoxy group or an amine

functionality also appears to be within proximity to Asp-29′ and Asp-30′ backbone NHs. Based upon these speculations, we incorporated *p*-MeO, *p*-NH_2 and *p*-CH_2OH substituents on the P2′-phenyl ring of inhibitor **3b**. As shown in Table 1, neither *p*-MeO nor *p*-NH_2 groups improved enzyme inhibitory potency compared to inhibitor **3b**. Of particular note, compound **16a,** displayed a good antiviral potency, possibly suggesting a better penetration through the cell membrane. Inhibitor **16c** with a hydroxymethyl substituent showed sub-nanomolar enzyme inhibitory potency but its antiviral activity was moderate compared to unsubstituted derivative **3b**. As it turned out, inhibitor **3b** is the most potent inhibitor in the series. We subsequently examined its activity against a clinical wild-type X_4-HIV-1 isolate (HIV-$1_{ERS104pre}$) along with various multidrug-resistant clinical X_4- and R_5- HIV-1 isolates using PBMCs as target cells.[5b] As can be seen in Table 2, the potency of **3b** against HIV-$1_{ER104pre}$ (IC_{50} = 31 nM) was comparable to FDA approved PI amprenavir with IC_{50} value of 45 nM. Darunavir and atazanavir on the other hand, are significantly more potent with IC_{50} values of 5 nM and 3 nM respectively. Inhibitor **3b**, while less potent than darunavir, maintained 5-fold or better potency over amprenavir against HIV-$1_{MDR/C}$, HIV-$1_{MDR/G}$, HIV-$1_{MDR/TM}$ and HIV-$1_{MDR/MM}$. It maintained over 2-fold potency against HIV-$1_{MDR/JSL}$. In fact, inhibitor **3b** maintained comparable potency to atazanavir against all multidrug-resistant clinical isolates tested. The reason for its impressive potency against multidrug-resistant clinical isolates is possibly due to its ability to make extensive hydrogen-bonds with protease backbone in the S2 subsite and its ability to fill in the hydrophobic pockets in the S1′-S2′ subsites effectively.

In conclusion, incorporation of stereochemically defined and conformationally constrained cyclic ethers into the allophenylnorstatine resulted in a series of potent protease inhibitors. The promising inhibitors **3b** and **3c** are currently being subjected to further in-depth biological studies. Design and synthesis of new classes of inhibitors based upon above molecular insight are currently ongoing in our laboratories.

Acknowledgments

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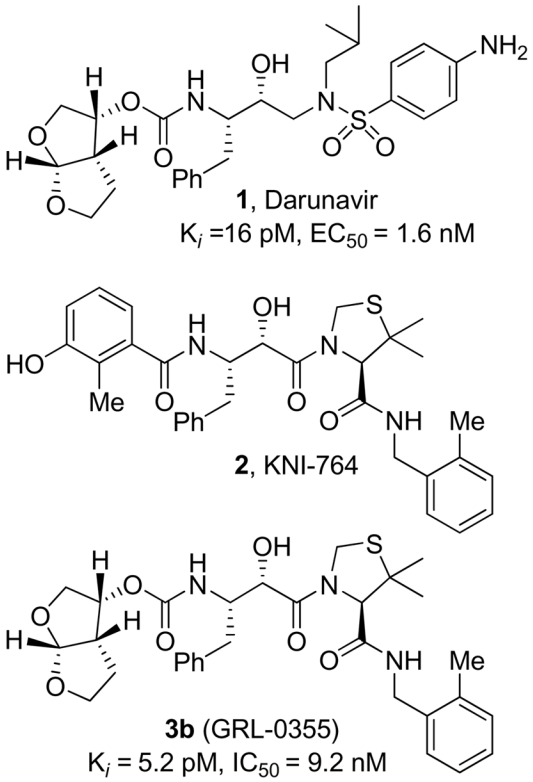


Figure 1.
Structures of inhibitors**1**, **2**, and **3b**

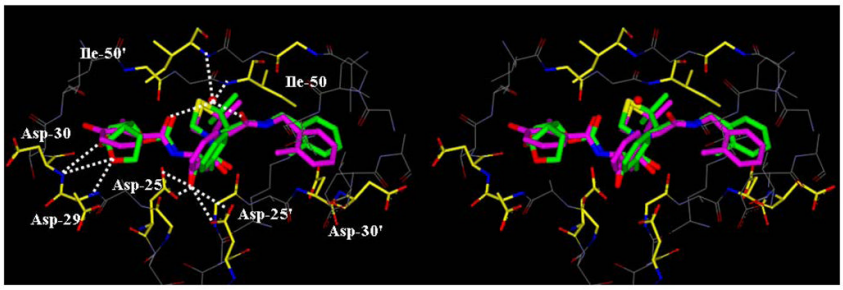
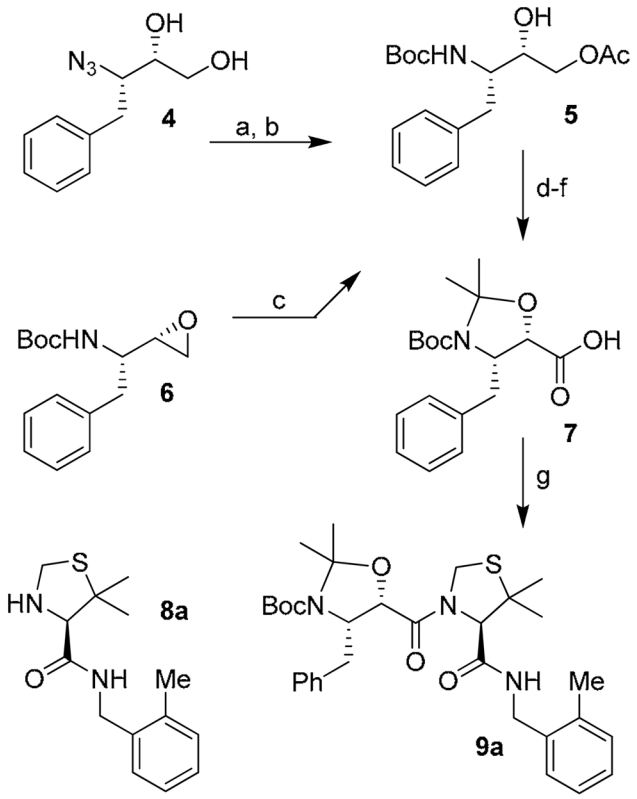


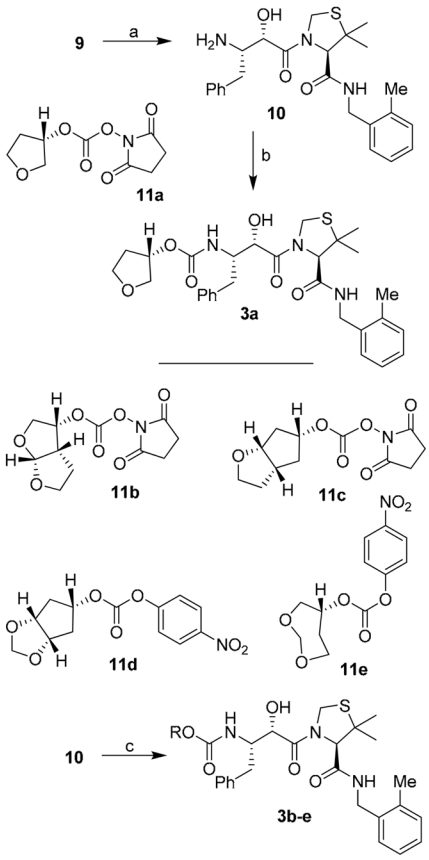
Figure 2.

Structure of inhibitor **3b**, modeled into the active site of HIV-1 protease, superimposed on the X-ray crystal structure of KNI-764. Inhibitor **3b** carbons are shown in green and KNI-764 carbons are shown in magenta.

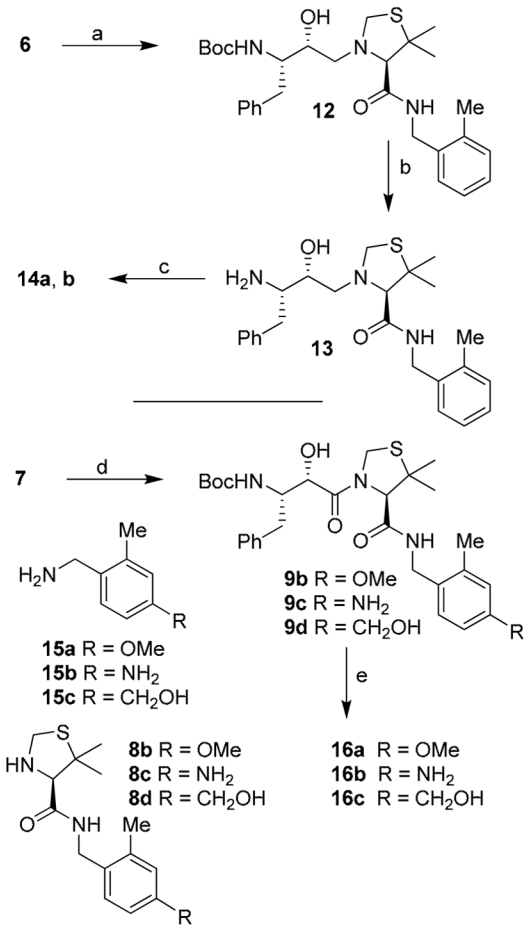


Scheme 1.

Reagents and conditions: (a) H_2, Pd/C, Boc_2O, EtOAc; (b) Ac_2O, Pyr, DMAP; (c) $LiCO_3$, AcOH, DMF; (d) 2- methoxypropene, CSA, DCM; (e) K_2CO_3, MeOH; (f) $RuCl_3$, $NaIO_4$, CCl_4-MeCN-H_2O (2:2:3); g) *N*-methylmorpholine, *i*BuOCOCl, **8a**, THF.



Scheme 2.
(a) 1 M HCl, MeOH; (b) **11a**, Et3N, CH2Cl2; (c) **11b,c** Et3N, CH2Cl2; or, **11d,e**, DIPEA, THF.

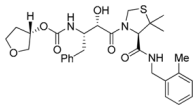
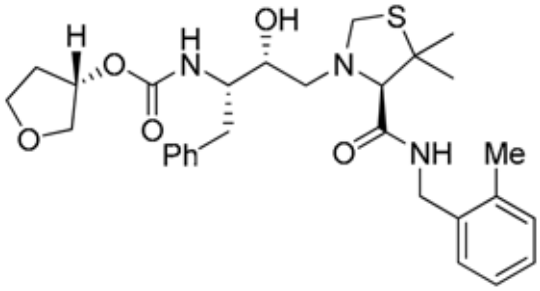
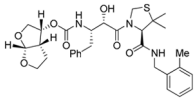
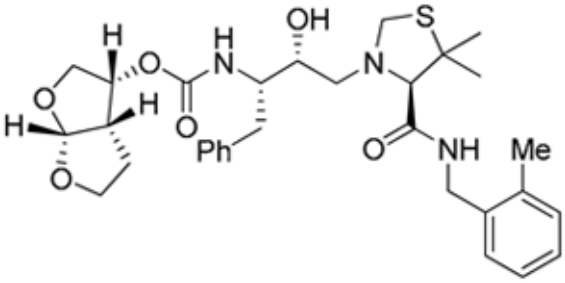
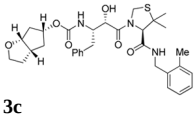
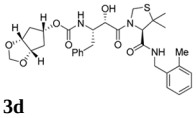
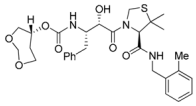
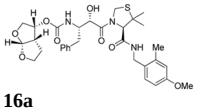


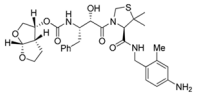
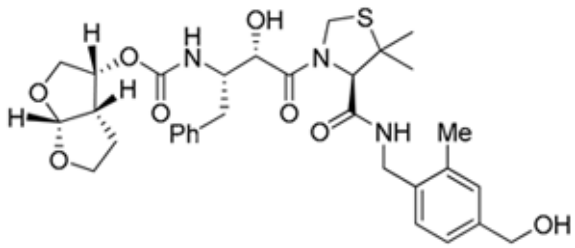
Scheme 3.

Reagents and conditions: (a) **8a**, Li(ClO4), Et2O; (b) CF3CO2H, CH2Cl2; (c) **11a** or, **11b**, Et3N, CH2Cl2; (d) *N*-methylmorpholine, *iso*-butylchloroformate, **8b–d**, THF; (e) CF3CO2H, CH2Cl2, then **11b**, Et3N, CH2Cl2.

Table 1

Enzymatic inhibitory and antiviral activity of allophenylnorstatine-derived inhibitors

Entry	Inhibitor	K_j(nM)	IC_{50}(μM)[a,b]
1.	 **3a**	0.21	0.02
2.	 **14a**	86.2	nt
3.	 **3b** (GRL-0355)	0.0052	0.009
4.	 **14b**	2.6	nt
5.	 **3c**	0.29	0.013
6.	 **3d**	0.65	nt
7.	 **3e**	0.78	nt
8.	 **16a**	2.03	0.051

Entry	Inhibitor	K_j(nM)	IC_{50}(μM)[a,b]
9.	 **16b**	1.01	0.53
10.	 **16c**	0.31	0.23

[a] Values are means of at least three experiments.

[b] Human T-lymphoid (MT-2) cells were exposed to 100 $TCID_{50}$ values of HIV-1_{LAI} and cultured in the presence of each PI, and IC_{50} values were determined using the MTT assay. Darunavir exhibited K_j = 16 pM, IC_{50} = 1.6 nM.

Table 2

Antiviral activity of **3b** (GRL-0355) against multi-drug resistant clinical isolates in PHA-PBMs

Virus	IC_{50} (μM)			
	3b (GRL-0355)	**APV**	**ATV**	**DRV**
HIV-$1_{ERS104pre}$ (wild-type: X4)	0.031 ± 0.002	0.045 ± 0.014	0.003 ± 0.003	0.005 ± 0.001
HIV-$1_{MDR/C}$ (X4)	0.061 ± 0.005 (2)	0.346 ± 0.071 (8)	0.045 ± 0.026 (15)	0.010 ± 0.006 (2)
HIV-$1_{MDR/G}$ (X4)	0.029 ± 0.002 (1)	0.392 ± 0.037 (9)	0.029 ± 0.020 (10)	0.019 ± 0.005 (4)
HIV-$1_{MDR/TM}$ (X4)	0.064 ± 0.032 (2)	0.406 ± 0.082 (9)	0.047 ± 0.009 (16)	0.007 ± 0.003 (1)
HIV-$1_{MDR/MM}$ (R5)	0.042 ± 0.001 (1)	0.313 ± 0.022 (7)	0.040 ± 0.002 (13)	0.027 ± 0.008 (5)
HIV-$1_{MDR/JSL}$ (R5)	0.235 ± 0.032 (8)	0.531 ± 0.069 (12)	0.635 ± 0.065 (212)	0.028 ± 0.008 (6)

The amino acid substitutions identified in the protease-encoding region of HIV-$1_{ERS104pre}$, HIV-1_{C}, HIV-1_{G}, HIV-1_{MM}, HIV-1_{JSL} compared to the consensus type B sequence cited from the Los Alamos database include L63P; L10I, I15V, K20R, L24I, M36I, M46L, I54V, I62V, L63P, K70Q,V82A, L89M; L10I, V11I, T12E, I15V, L19I, R41K, M46L, L63P, A71T, V82A, L90M; L10I, K14R, R41K, M46L, I54V, L63P, A71V, V82A, L90M; L10I, K43T, M46L, I54V, L63P, A71V, V82A, L90M, Q92K; and L10I, L24I, I33F, E35D, M36I, N37S, M46L, I54V, R57K, I62V, L63P, A71V, G73S, V82A, respectively. HIV-$1_{ERS104pre}$ served as a source of wild-type HIV-1. The IC_{50} values were determined by using PHA-PBMs as target cells and the inhibition of p24 Gag protein production by each drug was used as an endpoint. The numbers in parentheses represent the fold changes of IC_{50} values for each isolate compared to the IC_{50} values for wild-type HIV-$1_{ERS104pre}$. All assays were conducted in duplicate, and the data shown represent mean values (± 1 standard deviations) derived from the results of two or three independent experiments. Amprenavir = APV; Atazanavir = ATV; Darunavir = DRV.