

# Cross-Resistance Profile of the Novel Lysine-Containing HIV-1 Protease Inhibitor PL-100

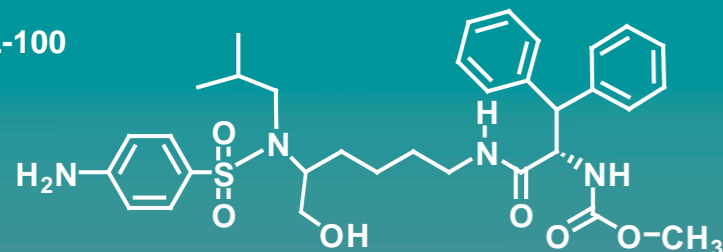
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## Introduction

The rapid emergence of drug-resistant strains of HIV is a major issue in HIV/AIDS treatment. New viral inhibitors with distinct structural properties are urgently needed to address this problem. Our approach has been to synthesize a series of protease inhibitors (PI) based on an L-lysine scaffold. The cross-resistance profile of one selected compound, PL-100, was determined using a selected series of 14 HIV isolates with known reduced susceptibility to other protease inhibitors. PL-100 is currently in pre-clinical development. PL-100 showed no evidence of mutagenicity (genotoxicity study) and it was safe in single and 7-day studies in rat and dog at concentration up to 2 g/kg/day (rat) and 1 g/kg/day (dog).

## PL-100



## Methods

A series of L-lysine derivatives were first evaluated using recombinant HIV protease enzymatic assays and then in cell culture antiviral assays using wild type HIV-1 (NL4-3) grown in MT4 cells. One of the most active compounds of the series, PL-100, was further characterized. Activity against a panel of 14 multi-PI resistant strains was evaluated using the PhenoSense™ assay (ViroLogic). For comparison, saquinavir (SQV), indinavir (IDV), nelfinavir (NFV), amprenavir (APV), and lopinavir (LPV) were tested in parallel.

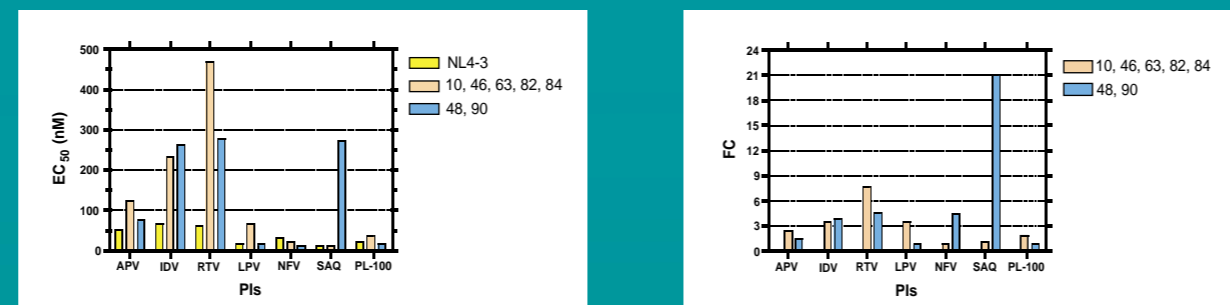
## Results

**Table 1: Activity of PL-100 against purified HIV-1 protease**

PI	Ki (nM)
PL-100	0.051
APV	0.117
LPV	0.014
IDV	1.945
NFV	0.306
RTV	0.034
SQV	0.080

PL-100 is a novel L-lysine derivative, which inhibited purified HIV-1 protease with a mean Ki of 51 pM. With the exception of indinavir (IDV) (Ki~2nM), all other PIs tested demonstrated Ki values in the same picomolar range.

**Figure 1: Antiviral activity of PL-100 against site-directed mutant viruses in cell culture assay**



PL-100 was as or more potent than the approved PIs when tested in MT-4 cells against the recombinant wild-type HIV-1 strain NL4-3 with an EC<sub>50</sub> of 22 nM. PL-100 retained potent activity against site-directed mutant viruses carrying primary mutations 46I, 82T and 84V or 48V and 90M (Figure 1). These strains showed marked reduced susceptibility to RTV and SQV but remained fully susceptible to PL-100.

**Table 2: Phenotypic susceptibility (FC) and genotype profile of clinical isolates**

Virus	APV	IDV	NFV	SQV	LPV	PL100	Primary mutations	Secondary mutations
11	2.8	7.2	1.6	1.0	8.1	1.2	46I, 82T, 84V	10L/I, 63P, 71V, 93L
28	1.8	2.3	4.2	6.0	1.2	1.4	30N, 88D, 90M	63P, 77I, 93L
26	3.9	53	68	1000	70	1.9	48V, 54V, 82A, 90M	10I, 63P, 71V, 93L
21	9.1	8.1	1.2	3.7	8.5	2.4	84V, 90M	10F, 63A, 71V
13	6.0	1.3	5.2	3.2	2.0	2.5	46I, 82T, 84V	10F, 20K/R, 63P, 71V, 77V/I/M, 93L
24	3.7	1.3	3.6	1.7	1.3	2.7	50V	10I, 33F, 36L, 63L/P
1	1.3	1.0	3.1	8.5	4.3	4.2	24I, 54V, 82A, 84V	10I, 33F, 36I, 63P, 71V
15	4.5	3.1	4.0	10.6	1.7	4.8	46I, 82T, 84V, 90M	10I, 20I, 36I, 63P, 71V, 73G/A
16	8.7	3.9	7.2	193	2.9	6.3	46I, 84V, 88D, 90M	10F, 33I, 36I, 63P, 71I
3	7.7	3.3	8.1	1.5	1.7	6.4	24I, 46L, 54L, 82A	10I/V, 20R, 33F, 36I, 63C
5	9.7	2.9	3.3	125	4.7	7.0	54V, 82A, 84V, 90M	10I, 20I, 36I, 60E, 63P, 71I
10	5.0	2.8	2.7	4.7	2.2	7.0	46I, 53L, 82T, 90M	10I, 63P, 71V, 73S
7	2.1	1.1	3.1	6.1	6.0	1.8	46I, 54V, 82A, 84V, 90M	10I, 33F, 63P, 71V
6	69	3.3	172	1000	130	3.7	54V, 82A, 84V, 90M	10V, 20T, 33F, 36I, 60E, 63P, 71V, 73S

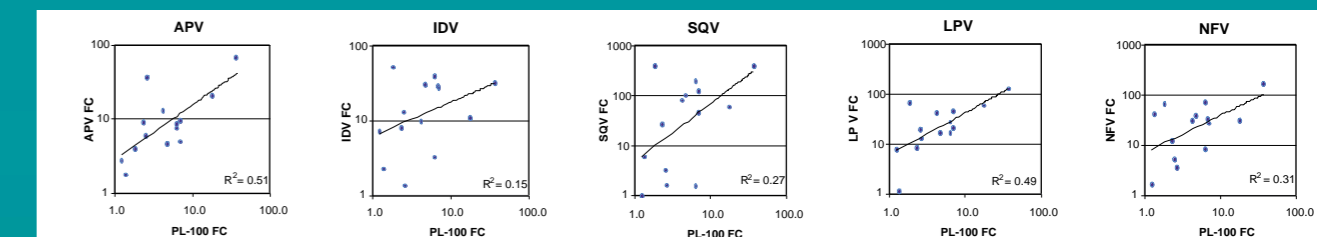
FC < 2.5   FC 2.5-10   FC 10-50   FC > 50

The cross-resistance to five marketed PIs was evaluated using a panel of 14 constructs containing HIV clinical isolates-derived protease sequences from highly PI-experienced patients. Selection criteria included the variety of primary mutations and high-level loss of susceptibility to one or more specific PIs. The antiviral activity and the fold change (FC) in susceptibility were evaluated using the PhenoSense™ assay (ViroLogic). Broad cross-resistance among the approved PIs was observed with a range of median FC of 8.2- to 54-fold. In comparison, PL-100 had a median FC of 4.5-fold.

Isolates containing mutations at positions 54, 82, 84 and 90 had the higher loss of susceptibility to PL-100 and had a markedly reduced susceptibility to all other PIs. Strains containing signature mutation for APV, SQV and NFV remained fully susceptible to PL-100 suggesting a different cross-resistance pattern.

No PL-100 FC over 50-fold was observed despite the presence of many mutation combinations required for the development of high level loss of susceptibility to PIs. This suggests a genetic barrier to high level reduced susceptibility to PL-100.

**Figure 2: Pairwise Combination of PIs' FC**



**Table 3: Non-parametric comparison (Spearman rank correlation)**

	APV	IDV	LPV	NFV	SQV
Rho	<b>0.626</b>	0.363	<b>0.641</b>	0.32	0.419
P-Value	<b>0.024</b>	0.191	<b>0.021</b>	0.25	0.13

Pairwise combination of PIs' FC showed r<sup>2</sup> values ranging from 0.15 to 0.51 indicating a divergence of PI cross-resistance patterns. The best correlation was seen between PL-100 and APV or LPV. Non-parametric comparison using the Spearman rank correlation corroborated this relationship (Table 3). On the other hand, individual FC data point out that isolates with reduced sensitivity to LPV generally retained susceptibility to PL-100, and that APV FC was never lower than the FC observed for PL-100. This might indicate possible resistance to APV or LPV in virus that has developed resistance to PL-100, though the reverse relationship seems less probable.

**Table 4: Human serum protein effect of PL-100 antiviral activity**

Culture condition	EC50 nM (Fold change)	
	PL-100	APV
10% FBS	21	52
10% FBS + 40% HS	128 (6.1)	110 (2.1)

The antiviral activity of PIs is largely affected by extensive plasma protein binding, which decreases the effective free drug concentration. Supplementation of human serum to the cell culture media was used to evaluate the *in vitro* effect of protein binding on PL-100' antiviral activity in MT-4 cells infected with NL4-3. The addition of 40% human serum to the cellular antiviral assay resulted in a 6-fold reduction of PL-100 potency, increasing the EC<sub>50</sub> to 128 nM.

## Conclusion

- Human serum protein binding has limited effect on PL-100 antiviral activity.
- Isolates investigated in this study were generally more susceptible to PL-100 than to the five marketed PIs tested.
- In a background of several secondary mutations, primary amino acid substitutions at positions 54, 82, 84 and 90 together are required to cause significant loss of susceptibility to PL-100.
- Isolates carrying key mutations for the loss of susceptibility to APV (50V), NFV (30N) and SQV (48V and 90M) remained fully susceptible to PL-100.