

Pharmacological and cross-resistance profiling of PL-100 and its pro-drug, PPL-100

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Background

We reported previously that PL-100 has a favorable cross-resistance profile against 14 resistant HIV isolates. However, some of the pharmacological parameters of this novel protease inhibitor (PI) in animal models were deemed not to be optimal. In the current study, a phosphorylated pro-drug (PPL-100) has been developed to improve solubility and pharmacokinetics (PK) of the parent compound (PL-100). We have also further characterized the cross-resistance profile of PL-100 against 49 additional HIV isolates with reduced susceptibility to all approved PIs including atazanavir.

Methods

PL-100 and PPL-100 were evaluated for pharmacokinetic profiling in vivo. Solubility was determined using a standard LC-MS method. Antiviral activity of PL-100 was determined against 49 diverse multi-PI resistant strains using PhenoSense assay (ViroLogic Inc). For comparison, atazanavir, saquinavir, indinavir, nelfinavir, amprenavir, and lopinavir were tested in parallel.

Results

Table 1. In vitro antiviral activity and cytotoxicity

Protease Inhibitor	EC ₅₀ (nM)	CC ₅₀ (nM)	SI (CC ₅₀ /EC ₅₀)
ATV (atazanavir)	4	55,000	13,750
APV (amprenavir)	47	>100,000	>2,128
IDV (indinavir)	67	>100,000	>1,493
LPV (lopinavir)	19	28,000	1,474
NFV (nelfinavir)	29	8,000	276
RTV (ritonavir)	61	25,000	410
SQV (saquinavir)	12	19,000	1,583
PL-100 (Procyon)	18	33,000	2,063

- The antiviral activity of PL-100 was determined using MT4 cells infected with a laboratory adapted HIV strain (NL4-3). For comparison, seven approved PIs were tested in parallel. The data suggest that the antiviral activity of PL-100 is comparable to that of the other PIs.
- Cytotoxicity of PL-100 and other PIs was evaluated in the same cell culture system. The selectivity index (SI) is the ratio of cytotoxicity (CC₅₀) to antiviral activity (EC₅₀). This result and other data indicate that PL-100 is a potent, selective and non-cytotoxic PI.

Table 2. Protein binding adjusted antiviral activity of PL-100

Culture condition	EC ₅₀ (nM) (Fold-Change)
10% FBS	18 (1.0)
10% FBS + 40% HS	106 (5.9)

The addition of 40% human serum to the cellular antiviral assay results in a 6-fold reduction in the antiviral activity of PL-100.

Table 3. Phenotypic susceptibility results against 63 diverse, multi-PI-resistant strains

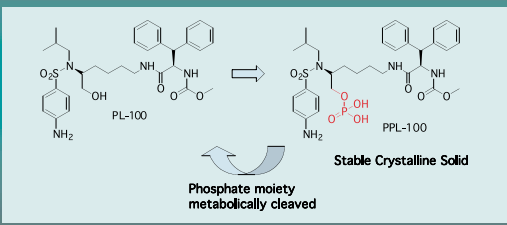
	ATV	APV	IDV	LPV	NFV	SQV	PL-100
Median FC	15.6	9.7	8.1	17.9	15	23.2	3.6
Mean FC	25.7	18.5	16.5	31.3	31.7	85	8.7
%FC < 2.5	16	14	13	10	10	27	37
%FC < 10	38	52	54	37	27	40	76
%FC > 50	22	8	5	19	10	37	3

A panel of 63 viral strains were selected based on the following rationale: 1) High-level loss of susceptibility to specific PIs; 2) High-level loss of susceptibility to multiple PIs; 3) Good representation of the primary mutations. This panel consists of resistant viruses from highly PI-experienced patients with high-level PI resistance. Susceptibility for each PI was measured by Fold Change (FC) in EC₅₀s against references. The genotype of these viruses encompasses a wide variety of mutational patterns. We particularly evaluated the following primary PI mutations: D30N, L33F/I, M46I/L, G48V, I50L/V, V82A/F/S/T, I84V and L90M as defined by the International AIDS Society (IAS)-USA.

Strength of the chosen panel

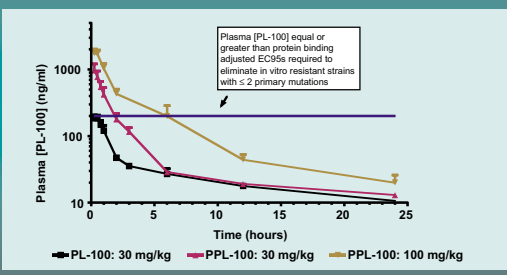
# primary PI Mutations	# viral strains
0	3
1	6
2	11
3	22
4	17
5	3
6	1

Figure 1. Phosphorylated Pro-Drug: PPL-100



- Pro-drug strategy has previously been applied to PIs to improve chemical stability, aqueous solubility or PK of parent compounds.
- Various pro-drugs of PL-100 were designed, synthesized and purified. These pro-drugs were evaluated for their PK profiles in rats. Phosphorylated pro-drug, designated as PPL-100, was selected for further evaluation and development due to its stability, solubility and oral bioavailability.

Figure 2. PPL-100 has an improved PK profile

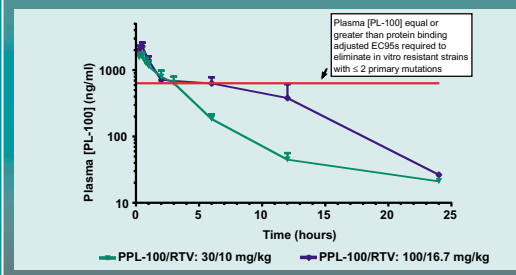


# primary PI Mutations	# viral strains with EC95s ≤ 200 ng/ml
0	100
1	100
2	36
3	23
4	0
5-6	0

In our cross-resistance profiling studies against 63 resistant strains, EC₉₅ of PL-100 was determined against each strain. This table illustrates the percentage of resistant strains tested with protein-binding adjusted EC₉₅s ≤ 200 ng/ml. The resistant strains are grouped by # primary mutations they have.

- Comparison of PK profiles in rats between PL-100 and PPL-100. PL-100 or PPL-100 was administered orally at doses indicated above. Each time point in the figure represents the average plasma [PL-100] (ng/ml) of 6 female rats at a given dose.
- Absolute oral bioavailability for PL-100 and PPL-100 at 30 mg/kg is 8.7 and 23%, respectively. The bioavailability of PPL-100 at 100 mg/kg is 23%.
- The blue line in the figure represents plasma [PL-100] of 200 ng/ml. The PK profile of PPL-100 shows that the time (t) > 200 ng/ml is approximately 6 hours in rats at 100 mg/kg, suggesting PPL-100 has a potential as a twice daily drug to maintain such plasma [PL-100] in man at an equivalent dose.
- Water solubility of PL-100 and PPL-100 was compared. At pH 7.5, solubility is 0.079 and 145 mg/ml for PL-100 and PPL-100, respectively.

Figure 3. PK profile of PPL-100 when boosted by RTV

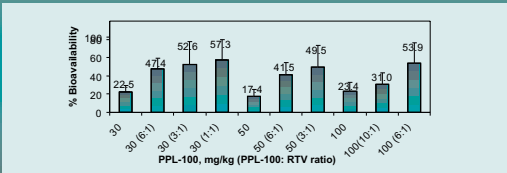


# primary PI Mutations	# viral strains with EC95s ≤ 630 ng/ml
0	100
1	100
2	82
3	55
4	29
5-6	0

In cross-resistance profiling studies against 63 resistant strains, EC₉₅ of PL-100 was determined against each strain. This table illustrates the percentage of resistant strains tested with protein-binding adjusted EC₉₅s ≤ 630 ng/ml. The resistant strains are grouped by # primary mutations they have.

- PK profiles of PPL-100 in rats when boosted by RTV. PPL-100 and RTV were co-administered orally at doses indicated above. Each time point in the figure represents the average plasma [PPL-100] (ng/ml) of 6 female rats at a given dose.
- The red line in the figure represents plasma [PL-100] of 630 ng/ml. The PK profile shows that the time (t) > 630 ng/ml is approximately 6 hours in rats at 100 mg/kg PPL-100 and 16.7 mg/kg RTV, suggesting PPL-100 has a potential as a twice daily drug to maintain such plasma [PL-100] in man at an equivalent dose.

Figure 4. Boosting with various PPL-100:RTV ratios



- Absolute oral bioavailability of PPL-100 was determined under various conditions as indicated in the figure.
- Sufficient oral bioavailability, relative to protein binding adjusted EC₉₅s against resistant strains in the cross-resistance profiling study, achieved when boosted at a ratio of 6 (PPL-100) to 1 (RTV).

Table 4. Pharmacokinetic parameters of PL-100 and PPL-100 in rats**

	Dose mg/kg	Route	λz (1/hr)	T _{1/2} (hr)	T _{max} (hr)	C _{max} (ng/mL)	T _{last} (hr)	C _{last} (ng/mL)	AUC _{last} (hr ng/mL)	AUC _{inf} (hr ng/mL)	Vz_F (L/kg)	CL_F (L/hr/kg)
PL-100*	5	i.v.	0.52	1.2	0.1	1804	6	44	1369	1434	6.1	3.5
PL-100*	50	p.o.	0.09	8.4	0.4	457	24	15	1101	1284	549	44
PPL-100*	50	p.o.	0.10	9.4	0.5	1216	24	13	2226	2416	282	23

* Each treatment group had at least 6 female rats.

** PK analysis was done using the non-compartmental method with WinNonLin Professional (version 4.0). AUC was calculated using the linear-up/log-down method.

λz	elimination rate constant	AUC _{last}	area under the plasma conc-time curve from zero to the last measurable concentration
t _{1/2}	plasma elimination half-life	AUC _{inf}	area under the plasma concentration-time curve from zero extrapolated to infinity
T _{max}	time of maximum concentration	Vz_F	apparent volume of distribution
C _{max}	maximum concentration	CL_F	apparent oral clearance
T _{last}	time of last measurable concentration		
C _{last}	last measurable concentration		

Conclusions

- PL-100 is a potent, specific and non-cytotoxic novel PI and has a favorable cross-resistance pattern compared to all approved PIs.
- PPL-100, pro-drug of PL-100, is >1800-fold more water soluble than PL-100 and has a 2 to 3-fold improved oral bioavailability over PL-100.
- PPL-100 has a great potential as a novel PI for the treatment of PI-naïve patients as well as PI-experienced patients infected with drug-resistant HIV strains bearing two or less primary PI mutations.
- PPL-100, when boosted by ritonavir, has a potential for the treatment of patients infected with drug-resistant HIV strains bearing two to four primary PI mutations as defined in the current cross-resistance profiling study. The ratio of PPL-100 to ritonavir required to achieve adequate plasma trough levels to suppress PI-resistant strains was determined to be 6:1.