

# PL-100, a Next Generation Protease Inhibitor against Drug-Resistant HIV: *In Vitro* and *In Vivo* Metabolism

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## Abstract

## BACKGROUND:

PL-100 is a novel HIV-1 protease inhibitor (PI) with a favorable cross-resistance profile and high genetic barrier. We have developed a phosphorylated pro-drug of PL-100, namely PPL-100 which is currently in Phase I human clinical trials.

## METHODS:

*In vitro* metabolism of PL-100 in human and rat liver microsomes was carried out according to standard methods. In induction studies, freshly isolated human primary hepatocytes were incubated with PL-100 for 48 h at final concentrations of 0.5, 5 or 25  $\mu$ M. Activity of CYP2C9 and CYP3A4 was assayed according to standard methods. *In vivo* metabolism and pharmacokinetics were carried out in normal and portal vein-cannulated rats. PL-100 or PPL-100 was orally dosed. Plasma samples were analyzed by LC-MS.

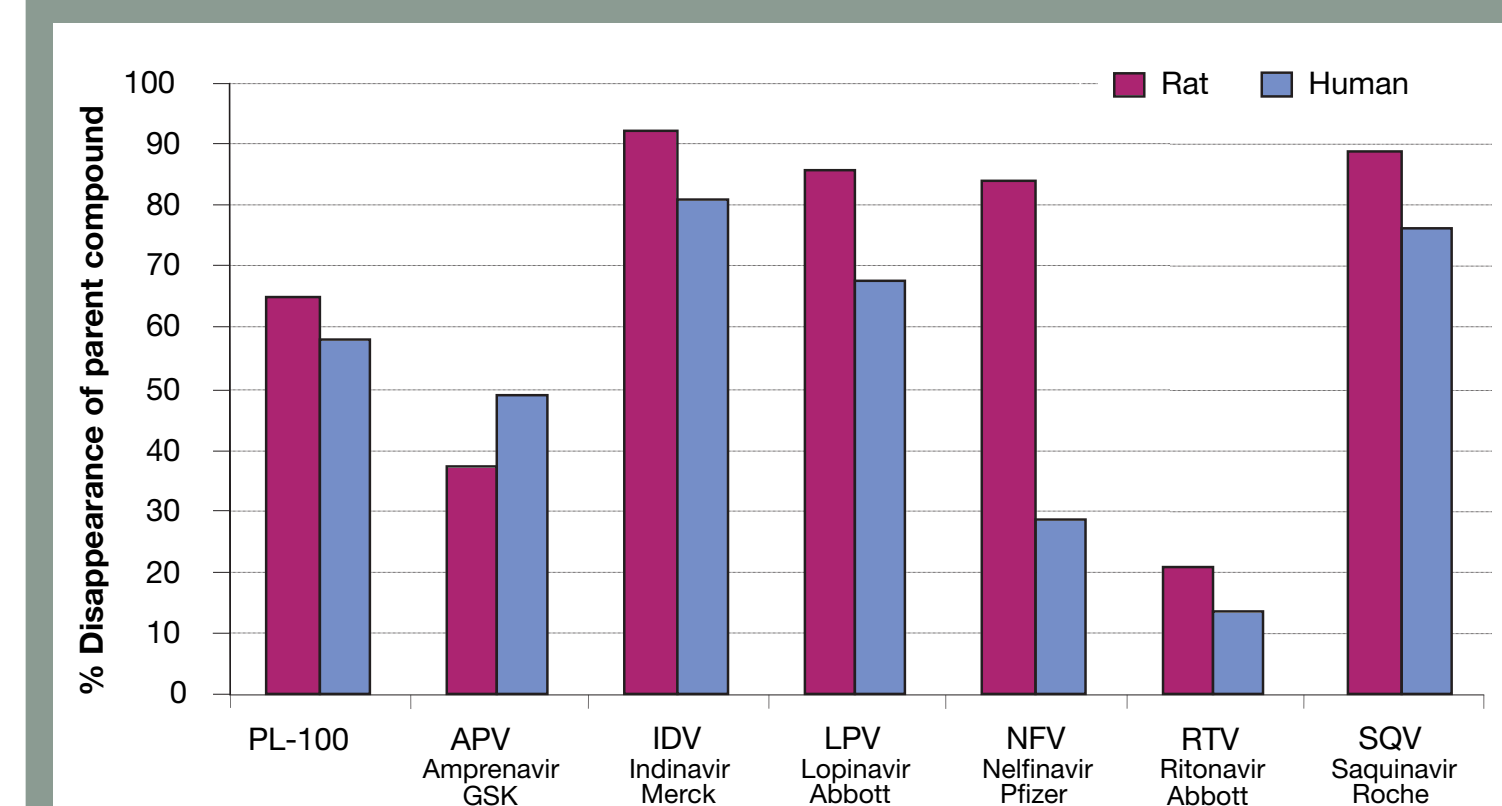
## RESULTS:

*In vitro* metabolism of PL-100 in human and rat liver microsomes was compared with amprenavir, lopinavir, saquinavir, indinavir, nelfinavir and ritonavir. PL-100 was more stable than lopinavir, saquinavir, indinavir. Metabolism of PL-100 in human liver microsomes was significantly inhibited by ritonavir, which was consistent with *in vivo* data showing that pharmacokinetics of this PI was significantly boosted by ritonavir. PL-100 did not inhibit CYP2C9 and CYP2D6 but did inhibit CYP3A4 *in vitro*. PL-100 did not induce the activity of CYP2C9 and CYP3A4 in the freshly isolated primary human hepatocytes. Pro-drug PPL-100 was found to be >1000-fold more water soluble than PL-100 and had shown 2- to 3-fold improvement over PL-100 in key pharmacokinetic parameters in rats. The studies in cannulated rats demonstrate that PL-100 was the major metabolite found in the portal vein and systemic circulation when pro-drug PPL-100 was orally dosed.

## CONCLUSION:

Our data suggest that PL-100 is both a substrate and an inhibitor of CYP3A4. Pro-drug PPL-100, with significantly improved aqueous solubility and oral bioavailability, is a promising drug for the treatment of PI-naïve and experienced HIV/AIDS patients.

## Results and Discussion

*In vitro* metabolism in liver microsomes

PLs (10  $\mu$ M) were incubated with Human or Rat liver microsomes for 1 hr in the presence of the co-factors NADPH and UDPGA. Human microsome (male pool) or Rat microsomes (male S/D) = 1.6 mg/mL, NADPH = 1.5  $\mu$ M, UDPGA = 1.5  $\mu$ M. Samples were analyzed by a LC/MS method.

PL-100 Metabolism *in vitro*

## Rat liver microsomes

Compound	Elution time (min)	MH+	% Parent
PL-100	6.8	625.3	60.6
Metabolite 1	6.6	641.3	5.6
Metabolite 2	6.3	641.3	12.9

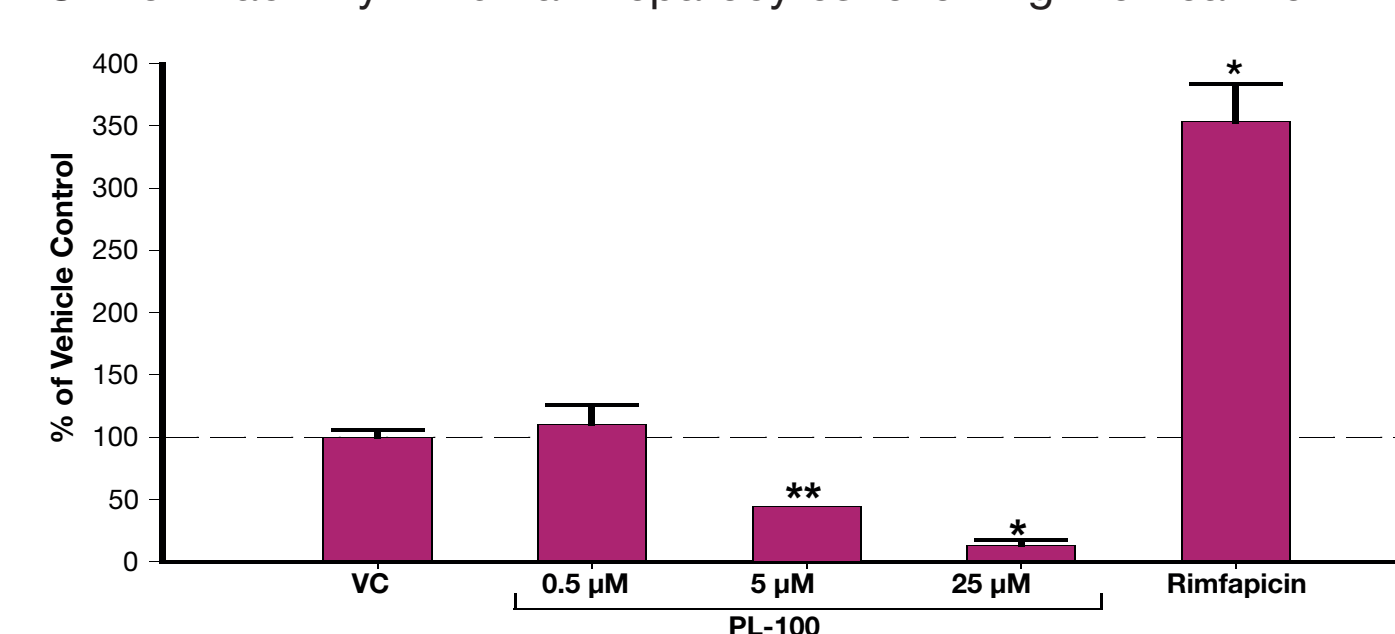
## Human liver microsomes

Compound	Elution time (min)	MH+	% Parent
PL-100	6.8	625.3	70.7
Metabolite 1	6.6	641.3	1.8
Metabolite 2	6.3	641.3	8.5
Metabolite 3	4.0	641.3	1.1
Metabolite 4	3.5	801.2	1.1
Metabolite 5	3.0	641.3	1.1

PL-100 (10  $\mu$ M) were incubated with Human or Rat liver microsomes for 1 hr in the presence of the co-factors NADPH and UDPGA. Human Microsome (male pool) or Rat microsomes (male S/D) = 1.6 mg/mL, NADPH = 1.5  $\mu$ M, UDPGA = 1.5  $\mu$ M. Samples were analyzed by a LC/MS method.

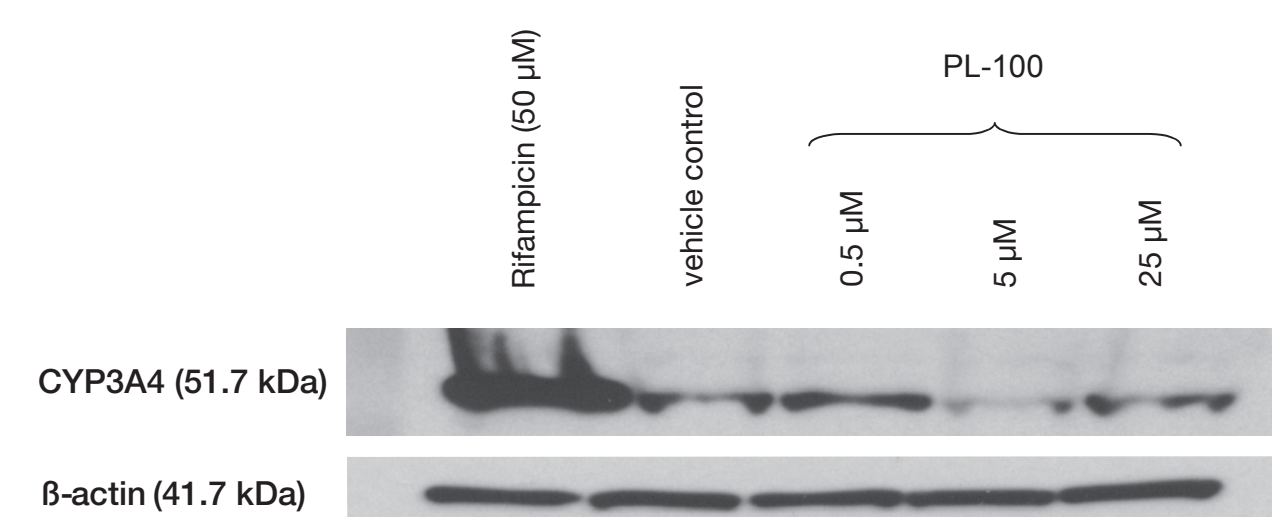
## Potential of PL-100 to induce CYP3A4 activity in primary culture of human hepatocytes

## A - CYP3A4 activity in human hepatocytes following the treatment with PL-100



Hepatocytes were cultured for 48 hours in the absence (VC; vehicle control) or in the presence of 0.5, 5, or 25  $\mu$ M PL-100, or 50  $\mu$ M rifampicin (positive control). At the end of the treatment period, terfenadine carboxylation was measured by LC-MS/MS. Each value represents the mean of triplicate wells with the SD indicated by the vertical bars. A Dunnett's post hoc test was used to identify the group mean values that were significantly different from the vehicle control.  
 \* indicates  $P < 0.01$ ; \*\* indicates  $P < 0.05$   
 PL-100 did not induce CYP2C9 activity in human hepatocytes (data not shown).

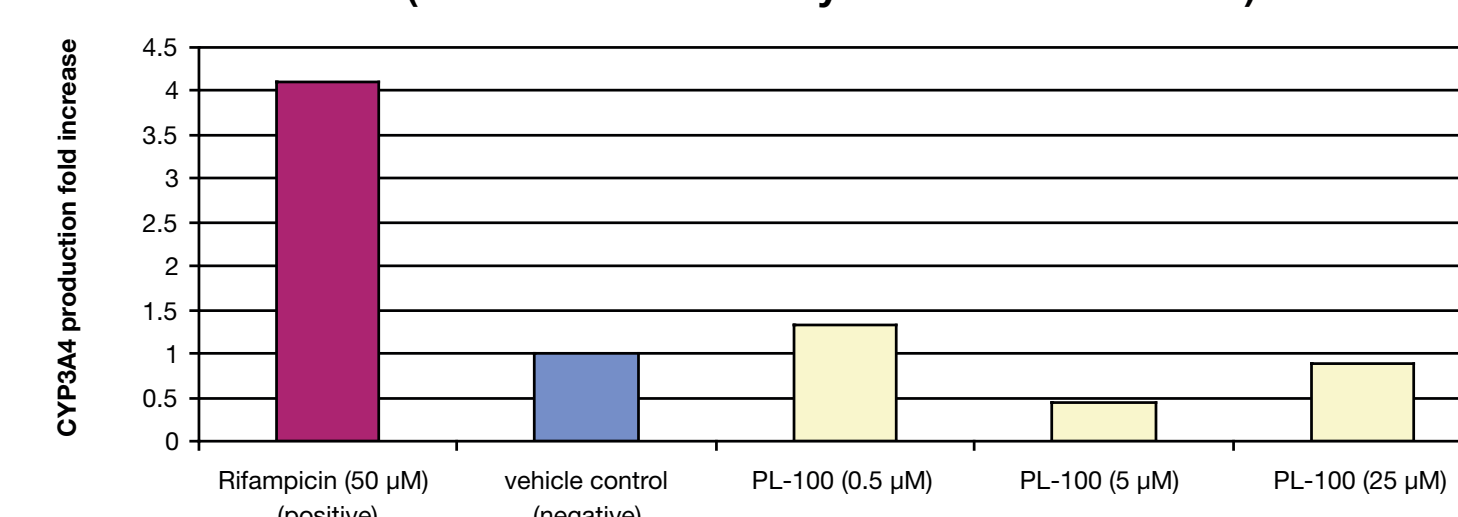
## B - Western blot for CYP3A4 in human hepatocytes following the treatment with PL-100



Equal volumes of the same primary hepatocyte extracts used for the terfenadine carboxylation quantitation were lyophilized, resuspended in PBS and loaded on a NuPAGE Bis-Tris gel for electrophoresis. After transfer to Immobilon PVDF membrane, antibodies against CYP3A4 and  $\beta$ -actin were used.

## C - Impact of PL-100 on CYP3A4 expression in primary culture of human hepatocytes

## (densitometric analysis of Western blot)



Densitometric analysis was performed to quantify the relative fold-change in expression of CYP3A4.

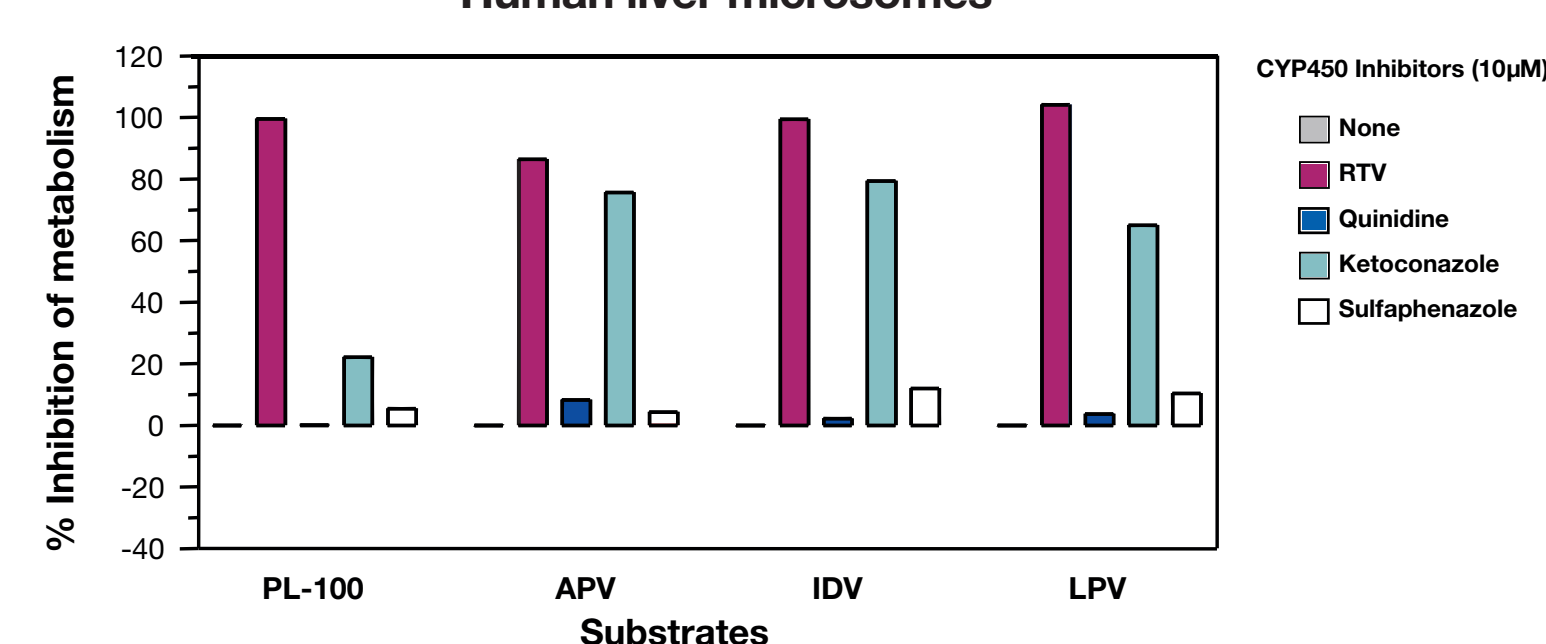
*In vitro* CYP inhibition

Enzyme	Substrate	Metabolic reaction	Ki ( $\mu$ M)	
			PL-100	Ritonavir
CYP 2C9	Tolbutamide	Tolbutamide	13.3 $\pm$ 1.7	13.9 $\pm$ 2.0
		Methyl-Hydroxylation		
CYP 2D6	Dextromethorphan	Dextromethorphan	27.8 $\pm$ 2.3	2.7 $\pm$ 0.3
		O-Demethylation		
CYP 3A4/5	Testosterone	Testosterone	0.445 $\pm$ 0.050	0.033 $\pm$ 0.005
		6 $\beta$ Hydroxylation		

Pooled human liver microsomes (15 donors) were utilized to measure the activities associated with tolbutamide methyl hydroxylation (CYP2C9), dextromethorphan O-demethylation (CYP2D6) and testosterone 6 $\beta$ -hydroxylation (CYP3A4/5) in the presence of various concentrations of PL-100 and Ritonavir. Selective inhibitors of the corresponding cytochromes P450 were also tested at one concentration to confirm the sensitivity of the assays. The enzyme kinetic parameters ( $K_m$  and  $K_i$ ) were determined for each assay from Michaelis-Menten plots using nonlinear regression and the types of inhibition determined from Lineweaver-Burk plots.

## Effect of CYP450 inhibitors on the metabolism of PL-100

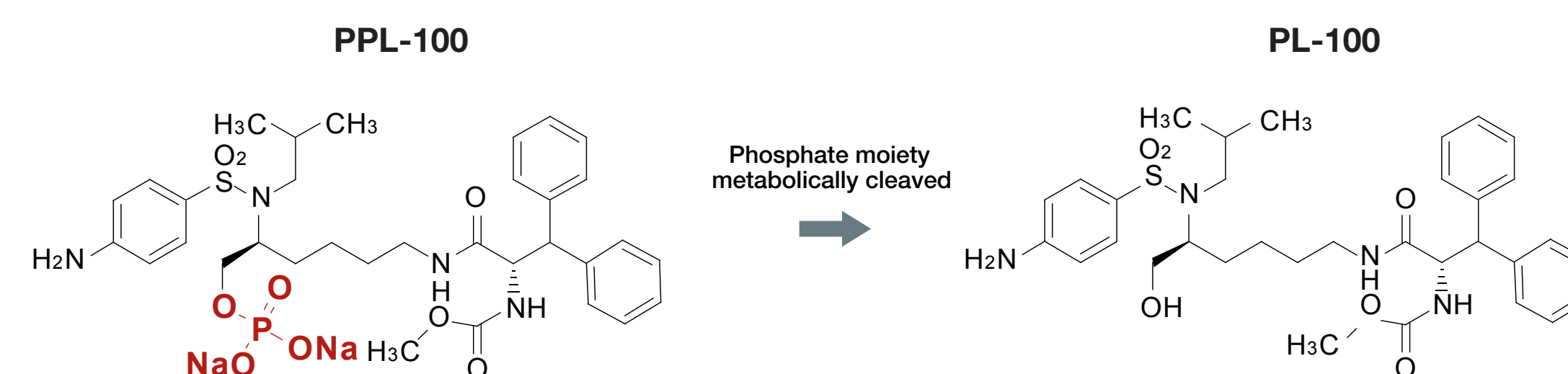
## Human liver microsomes



A standard protocol was developed using commercially available reagents. Briefly, the HIV protease inhibitors (10  $\mu$ M) were incubated with Human liver microsomes for 1h in the presence or absence of the co-factors NADPH and UDPGA, and the CYP inhibitors (10  $\mu$ M). The quenched supernatants were assayed by LC/MS for the Protease inhibitor. A control consisting of the above incubation mixture quenched at 0 min served as a reference.

## PPL-100 vs. PL-100

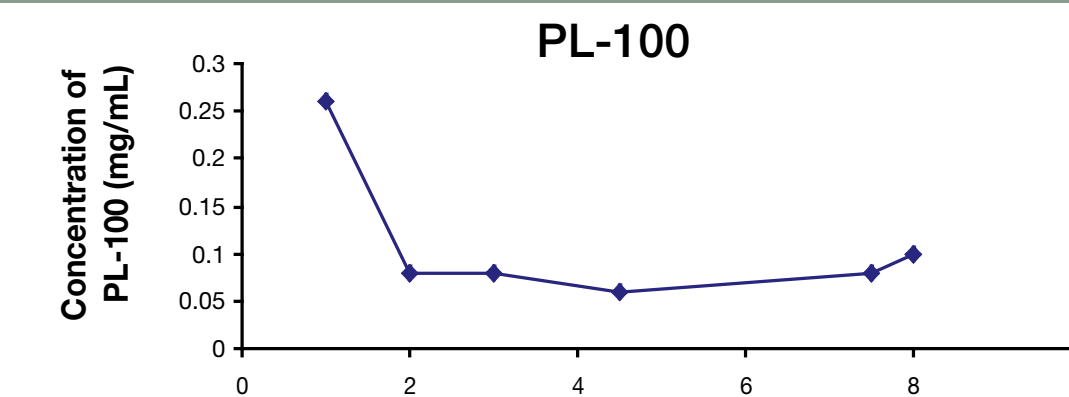
## PPL-100: a phosphorylated pro-drug of PL-100



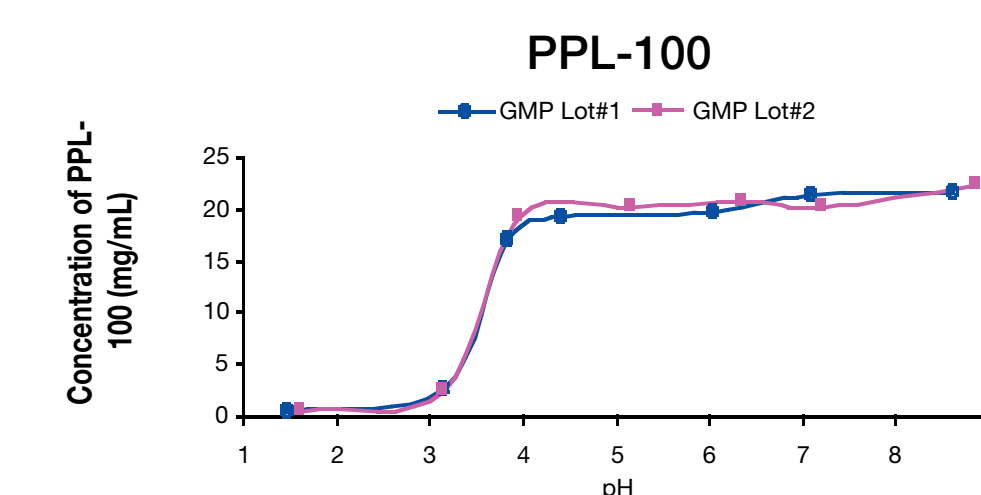
- PPL-100 (>145 mg/ml) is 1800 fold more water soluble than PL-100
- PPL-100 is 2 to 3-fold more orally bioavailable than PL-100
- Not active against HIV-1 protease

- Ki = 36 pM against HIV-1 protease
- EC50 = 5.3  $\pm$  1.75 nM against wild-type virus in PhenoSense assays

## Aqueous Solubility



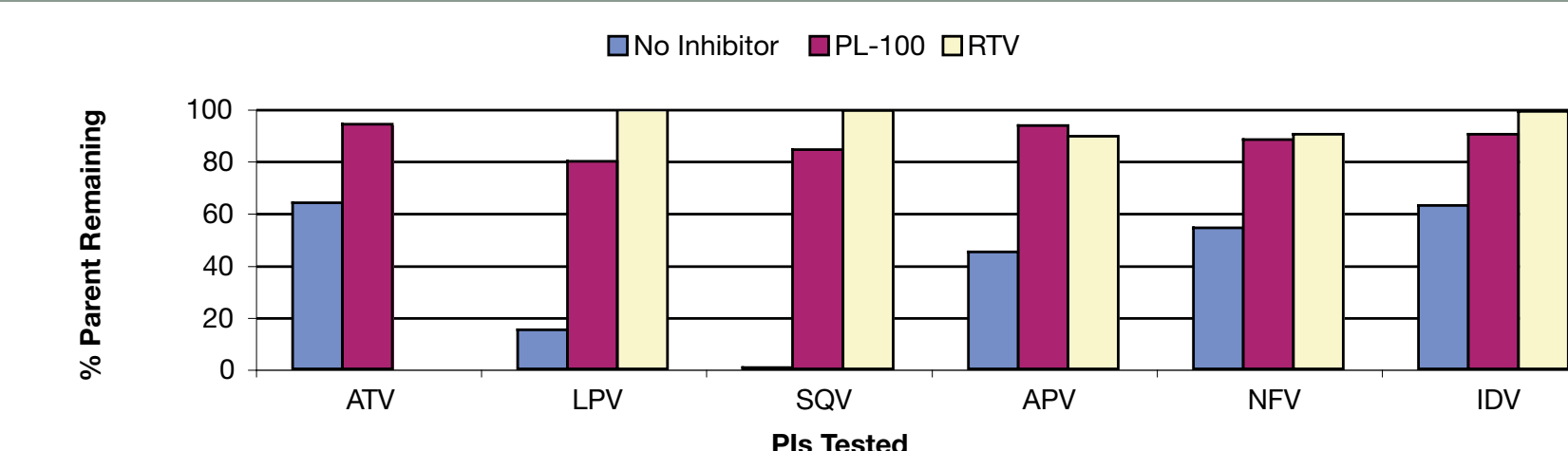
PL-100 was initially prepared at 1.0 mg/mL in buffers with different pH. After shaking for 18 hrs, samples were centrifuged and analysed by a HPLC/UV method.



PPL-100 was initially prepared at 20 mg/mL in water. Drops of 1 N HCl were added to obtain desired pH, and 300  $\mu$ L was aliquoted out, centrifuged and analyzed by a HPLC/UV method.

PPL-100 as a PK Enhancer: *In Vitro* Data

## Effect of PL-100 and RTV on metabolism of marketed PIs

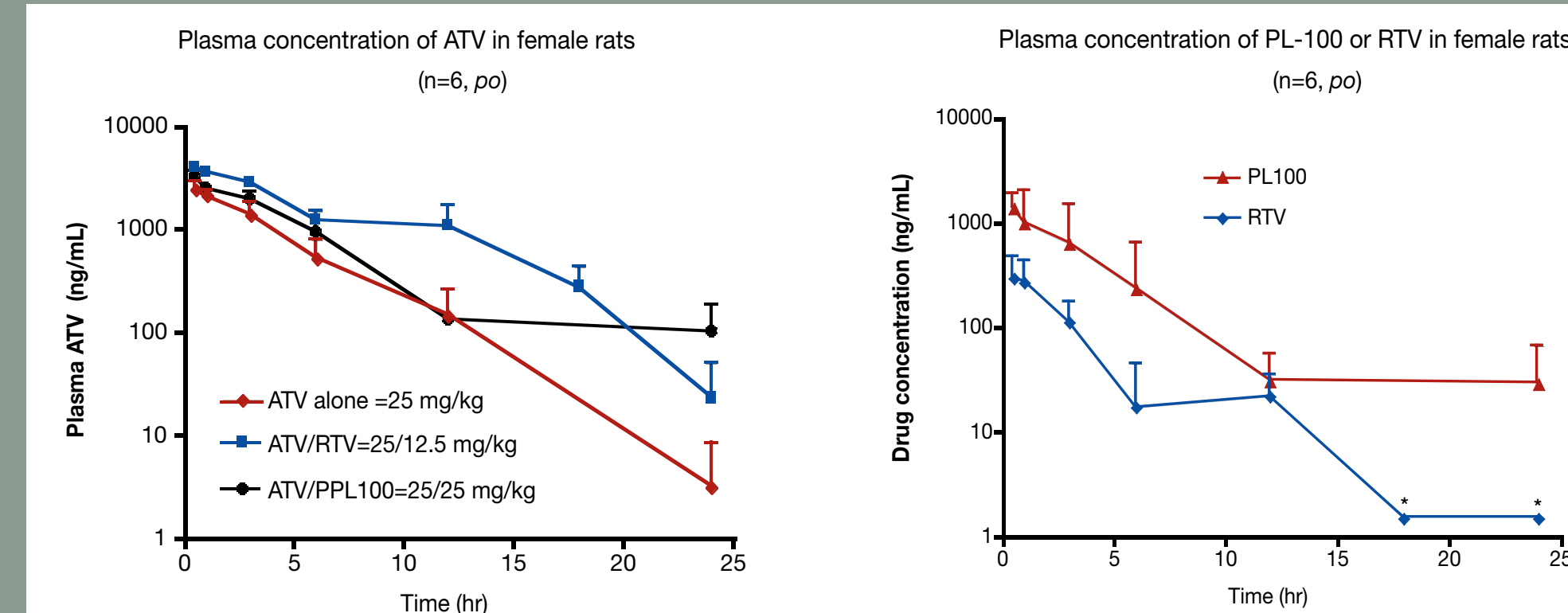


The mixture: human liver microsomes (mixed sex pool) = 1.6 mg/ml, NADPH = 1.5  $\mu$ M, inhibitor = 10  $\mu$ M, was preincubated at 37°C for 10 minutes. The reaction was initiated by the addition of the substrate (10  $\mu$ M). The incubation time was 60 mins.

ATV\*: not available for the data of RTV's effect. ATV = 1  $\mu$ M, Inhibitor (PL-100) = 2  $\mu$ M, Microsomes = 1.0 mg/mL

PPL-100 as a PK Enhancer: *In Vivo* Data

## Pharmacokinetics of ATV when orally co-administered with either PPL-100 or RTV



\* Below the lower limit of quantification

## Conclusions

- PL-100 is predominately metabolized by CYP3A4.
- PL-100 does not induce activities of CYP3A4 and 2C9.
- PL-100 is an inhibitor of CYP3A4/5 and PPL-100 is a rare, long-lasting PK enhancer of marketed PIs.
- PPL-100, a prodrug of PL-100, has the potential to be a once daily, un-boosted PI.