

β -D-2'-Deoxy-2'-fluoro-2'-C-methyluridine Phosphoramidates are Potent and Selective Inhibitors of HCV RNA Replication



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Abstract

β -D-2'-Deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) is a potent inhibitor of HCV RNA replication in an HCV replicon assay whereas the uridine congener, β -D-2'-Deoxy-2'-fluoro-2'-C-methyluridine (PSI-6206) is inactive in the replicon assay because it can not be phosphorylated. However, PSI-6206 triphosphate (PSI-7409) is a potent inhibitor of the HCV RdRp. Since the monophosphate of PSI-6206 can be anabolized to the corresponding triphosphate, we explored nucleoside 5'-phosphoramidates as a method of delivering the monophosphate of PSI-6206. EC_{90} values for seven key compounds ranged from 0.04 μ M to 0.86 μ M without detectable cytotoxicity. Rats dosed orally with (50 mg/kg) PSI-7672, PSI-7851, and PSI-8118 gave the highest liver triphosphate C_{max} and AUC values. A multiple dose PK study performed in rats, dogs and monkeys showed a significant concentration of PSI-7409 in the liver. In vitro phosphorylation studies using primary human hepatocytes and HPLC analysis showed that the highest level of PSI-7409 was achieved with PSI-7851 compared to those achieved with PSI-7672 and PSI-8118. Based on these studies we have determined that it is possible to deliver high concentrations of PSI-7409 to liver cells with selectively modified phosphoramidate prodrugs. In these studies, PSI-7851 demonstrated the most favorable characteristics while maintaining potent inhibition of HCV RNA replication with no cytotoxicity or mitochondrial toxicity. These favorable characteristics make PSI-7851 a candidate for further preclinical testing.

Introduction

Hepatitis C is a viral infection of the liver. Nearly 2% of the US population and an estimated 170 million people worldwide are HCV carriers. The current standard of care, a combination of pegylated interferon and ribavirin, has limited efficacy, consequently, there exists significant effort to develop novel direct acting antivirals as either alternative therapies or for use in combination with the standard of care. β -D-2'-deoxy-2'-fluoro-2'-C-methylcytidine (PSI-6130) has been shown to be a potent and non-cytotoxic inhibitor of HCV in the subgenomic replicon assay (1) and it has been demonstrated that the triphosphate of PSI-6130 is a potent inhibitor of the HCV NS5B polymerase (2). Cell metabolism studies have shown that PSI-6130 is converted to its uridine metabolite (PSI-6206) via cytidine deaminase. It has also been demonstrated that PSI-6206 is not an inhibitor of HCV in the replicon assay and is not metabolized to its monophosphate derivative, however, its triphosphate is a potent inhibitor of the HCV NS5B polymerase (3). Further metabolism studies have shown that the monophosphate of PSI-6130 is partially metabolized to the uridine monophosphate and that this PSI-6206 monophosphate can be converted to the triphosphate derivative via YMPK and NDPK (Figure 1 and reference (3)). To investigate the potential for utilizing PSI-6206 as an inhibitor of HCV replication required that we bypass the first phosphorylation step. This was accomplished by the preparation of phosphoramidate derivatives at the 5'-position. Such a strategy has produced potent and safe inhibitors of HCV replication.

Figure 1: Metabolism of PSI-6130

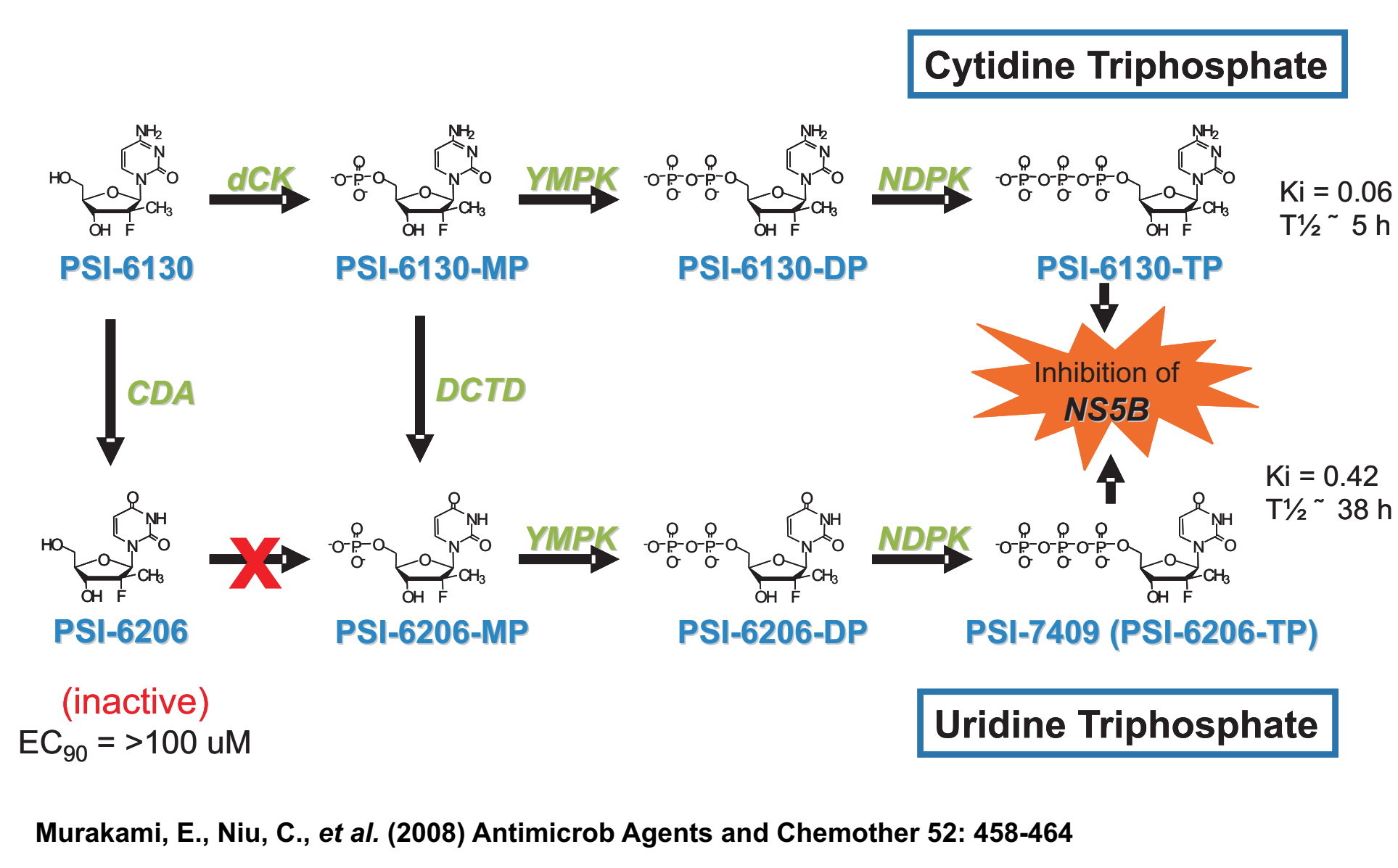
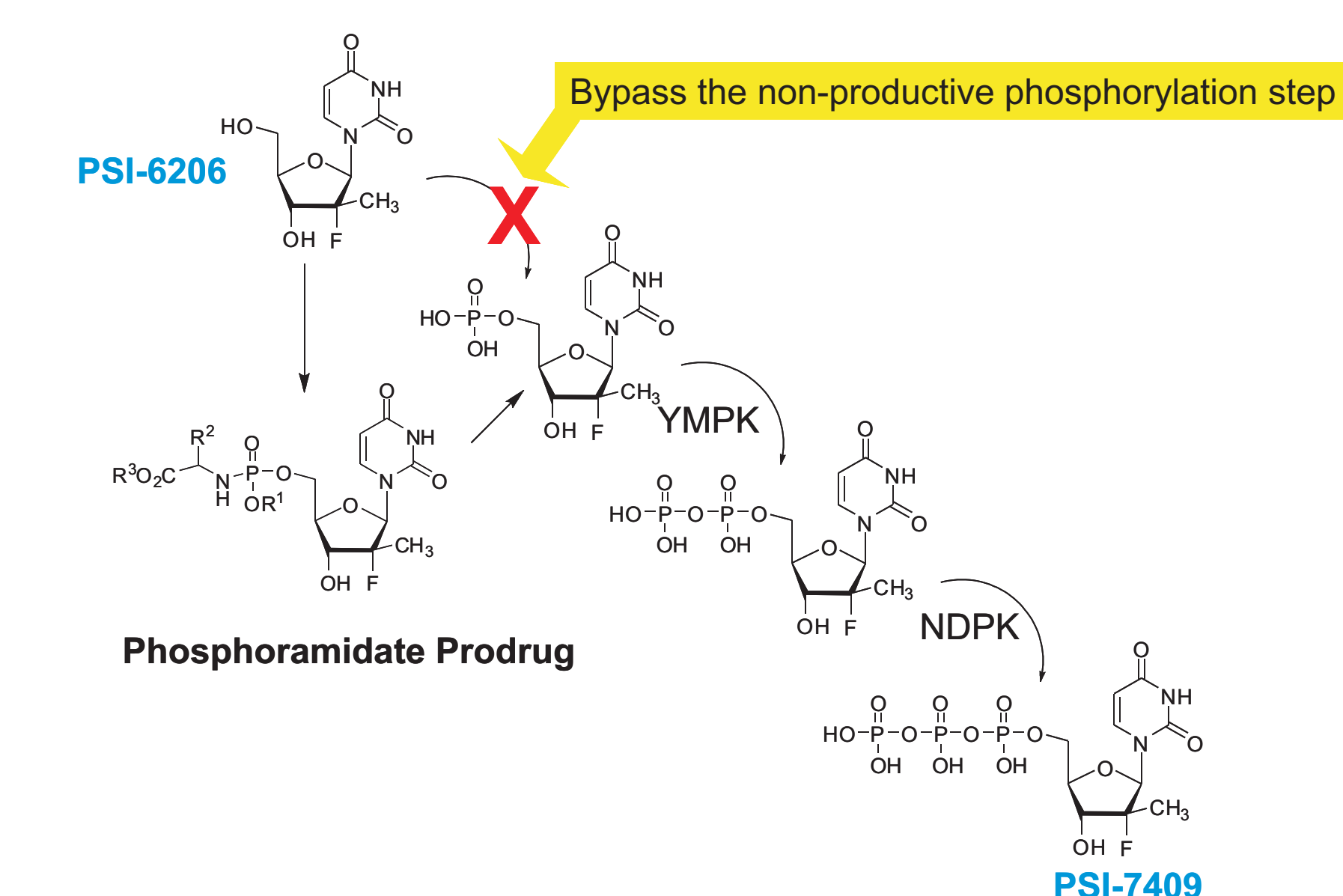


Figure 2: Phosphoramidate Prodrug Strategy



Results

Table 1: Antiviral activity, cytotoxicity, and mitochondrial toxicity

Cmpd	Activity Replicon EC_{50} (μ M)	Cytotoxicity CC_{50} (μ M)				Mitochondrial toxicity CC_{50} (μ M)			
		Huh7	BxBC3	HepG2	CEM	Huh7	BxBC3	HepG2	CEM
PSI-6130	4.8 \pm 2.0	>100	>100	>100	>100	>100	>100	>100	>100
PSI-8118	0.04	>100	>100	70	>100	>100	85.3	ND	ND
PSI-8028	0.14	>100	>100	>100	>100	33.5	71.8	98.6	91.7
PSI-7951	0.47	>100	>100	>100	>100	>100	>100	>100	>100
PSI-7851	0.52	>100	>100	>100	>100	>100	>100	>100	>100
PSI-7994	0.75	>100	>100	>100	>100	>100	>100	>100	>100
PSI-7672	0.86	>100	>100	>100	>100	>100	>100	>100	>100
PSI-7950	0.66	>100	>100	>100	>100	ND	ND	ND	ND

ND: Not determined.

Phosphoramidate prodrugs of PSI-6206 showed potent activity in the Clone A replicon assay.

Table 2: Stability

Compound	Stability [$t_{1/2}$, Hours]			
	SGF	SIF	Plasma	S9
PSI-6130	20	>20	>24	0.18
PSI-8118	17	>20	>24	1.4
PSI-8028	>20	>20	>24	0.35
PSI-7951	22	>24	>24	0.57
PSI-7851	>20	>20	>24	0.42
PSI-7994	15.5	>20	16.7	0.18
PSI-7672	17	>20	>8	0.23

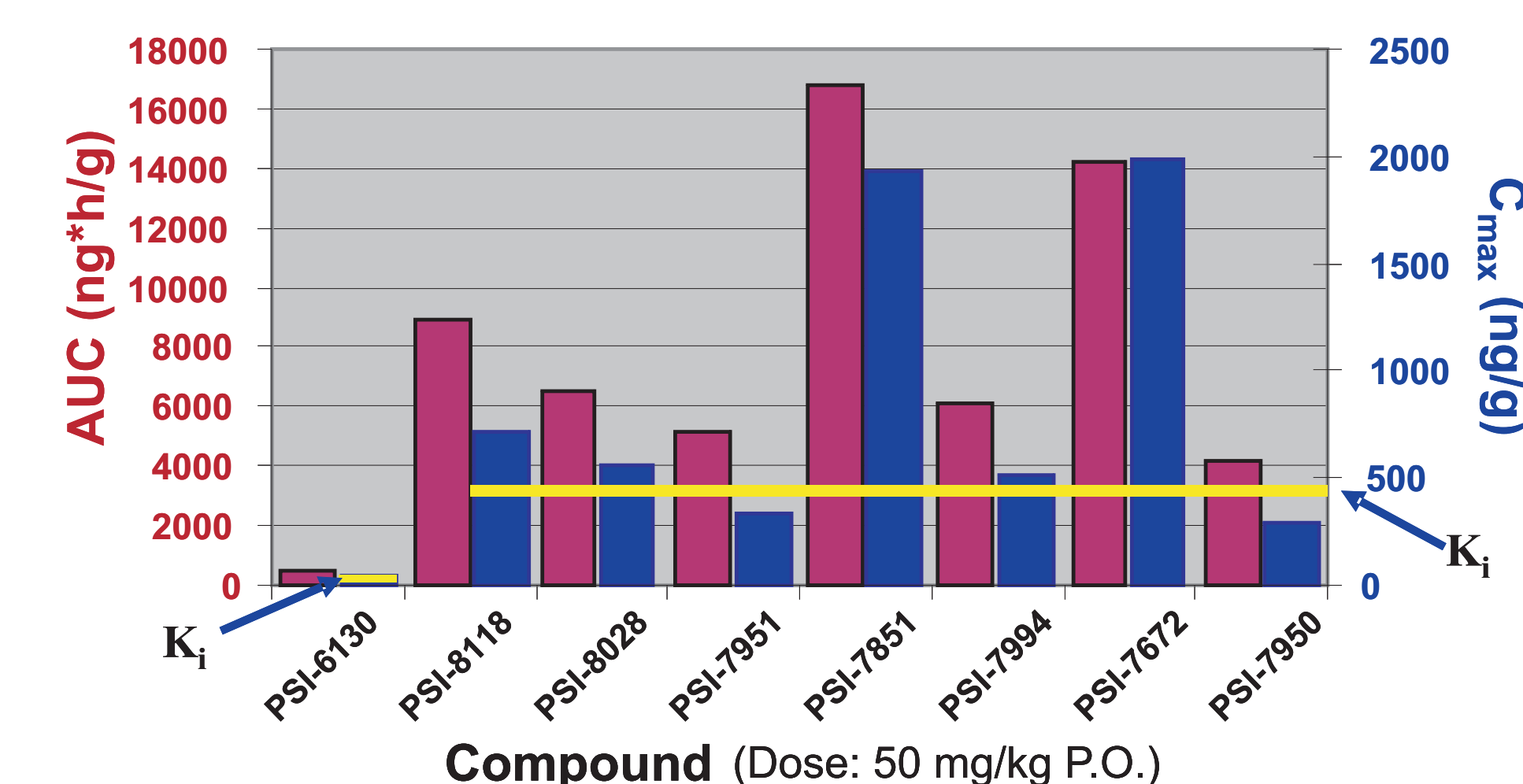
Desired stability profile: SGF >8 hr, SIF >8 hr, Plasma >8 hr, Liver S9 <1 hr

Results, continued

Table 3: Liver levels of PSI-7409 in rats

Prodrug	C_{max} (ng/g)	t_{max} (h)	AUC(0-t) (ng·h/g)	AUC(inf) (ng·h/g)
PSI-8118	837.00	4.00	10456.00	11568.00
PSI-8028	634.00	2.00	7395.00	10067.00
PSI-7951	382.00	1.00	5811.00	9567.00
PSI-7851	2030.00	4.00	17735.00	18984.00
PSI-7994	564.00	6.00	6692.00	8038.00
PSI-7672	1985.30	6.00	14206.58	18968.21
PSI-7950	308.00	4.00	4442.00	5748.00

Figure 3: Relationship between the liver levels of PSI-7409 and its K_i for NS5B RdRp



Extracts prepared from the livers of rats (3 per time points) dosed with 50 mg/kg were analyzed by LC-MS/MS (4). PSI-7851 and PSI-7672 gave the highest liver concentrations of PSI-7409.

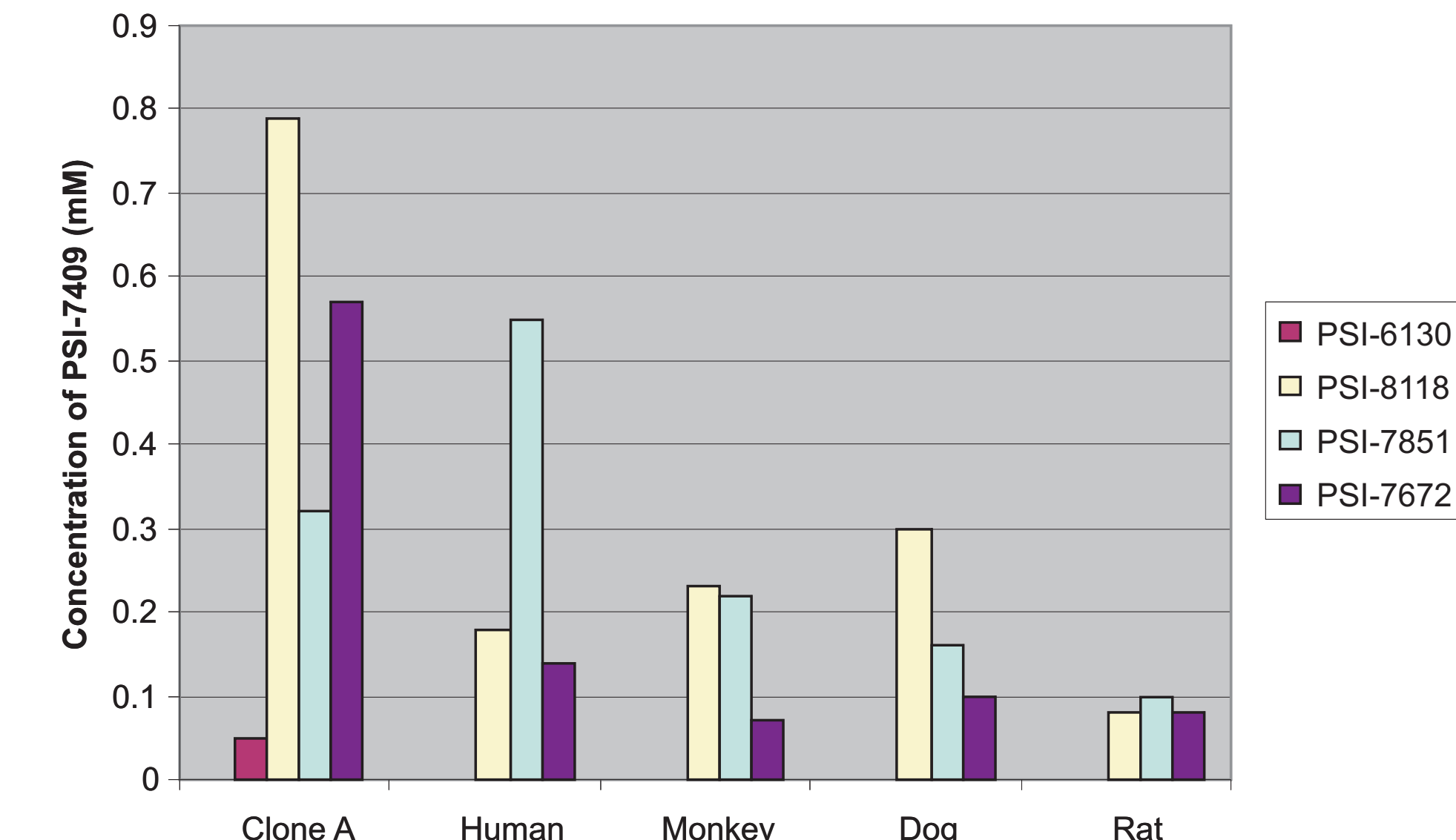
Table 4: PSI-7409 levels in dog and monkey liver

	Compound	Mean Concentration (ng/g)
PSI-7409 in Dog Liver (ng/g liver)	PSI-7672	4960
	PSI-7851	10560
	PSI-8118	476
PSI-7409 in Monkey Liver (ng/g liver)	PSI-7672	26.06
	PSI-7851	28.6
	PSI-8118	ND

ND: Not detected.

Animals were dosed with 50 mg/kg and liver samples were collected 4 hours post-dose. Extracts were prepared and analyzed by LC-MS/MS (4). PSI-7409 levels were highest in dogs dosed with PSI-7851.

Figure 3: Concentration of PSI-7409 after treating primary hepatocytes for 48 Hours



Methanol extracts prepared from cells incubated with each compound (100 μ M) were analyzed by HPLC. In primary human hepatocytes, PSI-7851 gave the highest level of PSI-7409.

Conclusions

- PSI-6130 is a potent, selective and safe HCV polymerase inhibitor with unique properties relative to competitor nucleosides and has completed Phase-I clinical trials.
- 5'-Phosphoramidate derivatives of PSI-6206 are potent inhibitors of HCV in the subgenomic replicon assay.
- Selected phosphoramidates of PSI-6206 are as much as 120X more potent than the cytidine analog PSI-6130.
- Selected PSI-6206 phosphoramidate derivatives show good in vitro safety and stability characteristics.
- Primary human hepatocytes incubated with PSI-7851 gave the highest level of PSI-7409.
- In vivo PK studies support the liver targeting potential of the phosphoramidate class of HCV inhibitors.
- PSI-7851 was nominated as the preclinical candidate.
- PSI-7851 is currently progressing through preclinical development.

References

- Stuyver, L.J., Mc Brayer, T.R., et al. (2006) Antiviral Chem and Chemother 17: 79-87
- Murakami, E., Bao, H., et al. (2007) Antimicrob Agents and Chemother 51: 503-509
- Murakami, E., Niu, C., et al. (2008) Antimicrob Agents and Chemother 52: 458-464
- Hecker, S. J., Raja Reddy, K., et al. (2007) J Med Chem 50: 3891-3896

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