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

Poster # P-259

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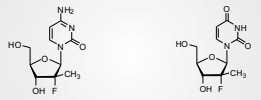
Introduction

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 need to develop novel direct acting antivirals as either alternative therapies or for use in combination with the standard of care. Pharmasset and Roche are currently developing R7128 (RO4048), a prodrug of β-D-2'-deoxy-2'-fluoro-2'-C-methylcytidine, PSI-6130, for the treatment of chronic hepatitis C. 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. Cell metabolism studies have shown that PSI-6130 is converted to its uridine metabolite (PSI-6206) via cytidine deaminase (2). 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. 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). 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 (3). Such a strategy has produced potent and safe inhibitors of HCV replication.

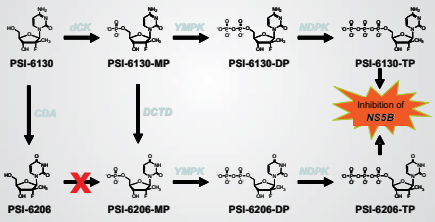
Figure 1: PSI-6130 and PSI-6206



PSI-6130 (RO4048) EC_{90} = 4.6 ± 2.0 μM

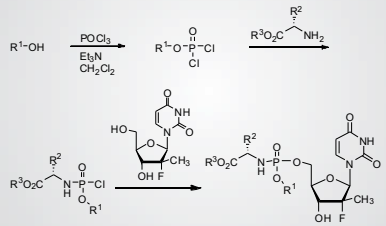
PSI-6206 (RO2433) EC_{90} = >100 μM

Figure 2: Proposed Intracellular Mode of Action of PSI-6130

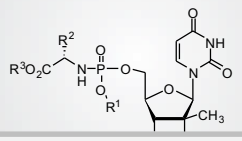


Methods & Results

Scheme 1: Preparation of Phosphoramidates



PSI-6206 Phosphoramidate



SAR Results

Table 1: Amino Acid Side Chain (R^2) SAR

Cmpd No.	EC_{90} CloneA Cells (μM)
PSI-7672	0.90
PSI-7673	>50
PSI-7823	19.0
PSI-7834	60.1
PSI-7894	>50

Table 2: Phosphorus Ester (R^1) SAR

Cmpd No.	EC_{90} CloneA Cells (μM)
PSI-7672	0.90
PSI-7694	2.11
PSI-7831	0.69
PSI-7832	0.09
PSI-7840	0.69
PSI-7847	0.58
PSI-7848	0.45

Table 3: Amino Acid Ester (R^3) SAR

Cmpd No.	EC_{90} CloneA Cells (μM)
PSI-7672	0.90
PSI-7818	0.98
PSI-7838	0.09
PSI-7839	0.13
PSI-7851	0.52
PSI-7849	0.06

Table 4: Base Modifications

Cmpd No.	Base	EC_{90} CloneA Cells (μM)
PSI-7672	Uracil	0.90
PSI-7693	Cytosine	14.55

HCV replicon containing cells wee seeded in 96-well plates and test compounds added immediately. After incubating for 4 days, total cellular RNA was extracted and HCV replicon RNA levels were quantitated by Q-RT-PCR.

Table 5: Cytotoxicity [CC_{50} (μM)] Evaluated Against Several Cell Lines

Cmpd No.	EC_{90} CloneA Cells (μM)	Huh7	HepG2	BxPC3	CEM
PSI-6130	4.6	>100	>100	>100	>100
PSI-7672	0.90	>100	>100	>100	>100
PSI-7831	0.69	80	>100	>100	>100
PSI-7838	0.09	>100	>100	>100	>100
PSI-7839	0.13	30	>100	75	80

Cytotoxicity in Huh7, HepG2, BxPC3 and CEM cells were determined after 8 days by determining the absorbance after addition of MTS dye.

Table 6: Compound Stability in Simulated Gastric Fluid (SGF), Simulated Intestinal Fluid (SIF) and Liver S9 Fraction

Cmpd No.	EC_{90} CloneA Cells (μM)	SGF $T_{1/2}$ (h)	SIF $T_{1/2}$ (h)	Liver S9 $T_{1/2}$ (min)
PSI-7672	0.90	15.5	>20	14
PSI-7831	0.69	15.5	13	19
PSI-7838	0.09	19	>20	4.8
PSI-7839	0.13	21.75	>20	8.3

Simulated gastric fluid (pH = 1.4 ,without pepsin) and simulated intestinal fluid (pH = 7.5, without pancreatin) were obtained from Fisher Scientific. Compound was dissolved in acetonitrile and diluted to 50 μg.ml in 2mL of SGF or SIF and incubated at 37ºC over 24 h period. Samples were analyzed by LC/MS/MS. The % remaining is calculated based on peak area and half-lives are calculated.

Conclusions

1. 5'-Phosphoramidate derivatives of PSI-6206 are potent inhibitors of HCV in the subgenomic replicon assay.
2. Selected phosphoramidates of PSI-6206 are as much as 100X more potent than the cytidine analog PSI-6130.
3. Selected PSI-6206 phosphoramidate derivatives show no cytotoxicity across several different cell lines.
4. PSI-6206 phosphoramidates show good stability under simulated gastrointestinal conditions and have the potential to be rapidly released at the target organ, the liver.
5. Clear SAR was demonstrated for PSI-6206 phosphoramidates with a 1000x range in potency.
6. Several PSI-6206 phosphoramidates have demonstrated stability profiles that are attractive for further development.
7. β-D-2'-Deoxy-2'-fluoro-2'-C-methyluridine phosphoramidates have potential as therapeutic agents for the treatment of HCV infection
8. Conducting in vivo studies to select a candidate for clinical development

References

1. Stuyver, L. J., *etal.*, *Antiviral Chem and Chemother*, 2006, 17, 79-87.
2. See Poster P-262