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

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 [P-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 cytldine 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 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

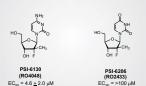
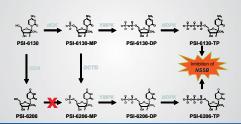
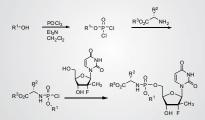


Figure 2: Proposed Intracellular Mode of Action of PSI-6130



Methods & Results

Scheme 1: Preparation of Phosphoramidates



PSI-6206 Phosphoramidate

SAR Results

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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 1: Amino Acid Side Chain Table 2: Phosphorus Ester (R¹) SAR (R²) 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 (R3) 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 (μ M)
PSI-7672	Uracil	0.90
PSI-7693	Cytosine	14.55

Table 5: Cytotoxicity [CC 50 (μΜ)] 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

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

Simulated Intestinal Fluid (SII				
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

Conclusions

References

