

# Characterization of 2'-C-methyl C-nucleoside HCV polymerase inhibitors

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# Nucleotide Inhibitors in HCV Therapy

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Phase 1	Phase 2a	Phase 2b
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GS-6620

INX-08189

PSI-7977

RG7432

PSI-938

IDX-184

Mericitabine

## ◆ Class Related Properties:

- High barrier to resistance
- Pan genotype activity
- Potential for potent antiviral activity

# 2' CMe N- and C-Nucleosides

## Pyrimidine

## Purine

N-Nuc

1 2

2' CMeU

2' CMeC

R = N; 2' CMeA  
R = CH; MK608

2' CMeG

Walton E. *et al.* J Med Chem 1969  
Olson DB. *et al.* Antimicrob Agents chemo 2004

7

9  
4

C-Nuc

1 2

2' CMepseudoU

2' CMepseudoC

R = N; 2' CMe-4-aza-9-deazaA  
R = CH; 2' CMe-4-aza-7,9-dideazaA (1)

2' CMe-4-aza-9-deazaG (2)

7

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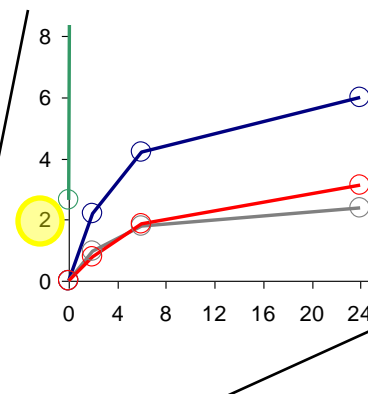
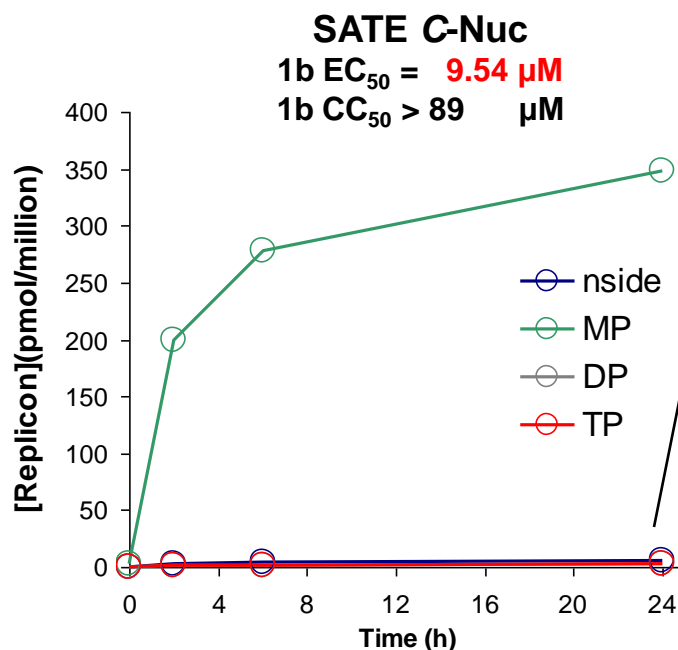
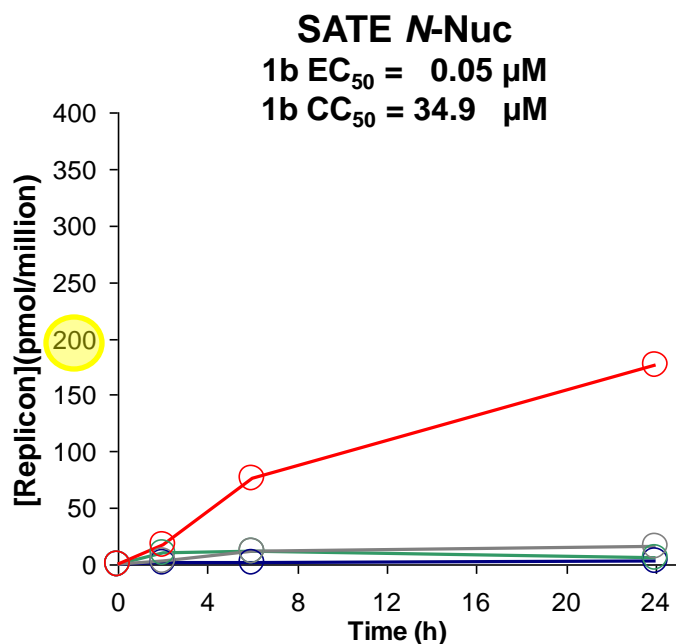
# Anti-HCV Activity *in vitro*

Base	Nucleoside	Genotype 1b Replicon EC <sub>50</sub> (μM)	Huh-7 CC <sub>50</sub> (μM)	Genotype 1b NS5B Triphosphate IC <sub>50</sub> (μM)
U	2'CMeU	15.2	>89	1.32
	<b>2'CMepseudoU</b>	<b>&gt;89</b>	<b>&gt;89</b>	<b>68.3<sup>a</sup></b>
C	2'CMeC	4.07	>89	0.73
	<b>2'CMepseudoC</b>	<b>&gt;89</b>	<b>&gt;89</b>	<b>13.4<sup>a</sup></b>
G	2'CMeG	3.25	>89	0.25
	<b>C-Nuc (2)</b>	<b>&gt;89</b>	<b>&gt;89</b>	<b>0.19</b>
A	2'CMeA	0.35	66	6.83
	<b>2'CMe-4-aza-9-deazaA</b>	<b>7.45</b>	<b>&gt;89</b>	<b>2.10</b>
7-deazaA	MK608	0.08	>89	0.30
	<b>C-Nuc (1)</b>	<b>2.50</b>	<b>85.0</b>	<b>0.31</b>

<sup>a</sup> No incorporation observed for triphosphates by NS5B.

- ◆ **Pyrimidine:** C-Nucs lack replicon activity and their triphosphates are poor inhibitors of NS5B
- ◆ **Purine:** C-Nucs showed selective replicon activity, although reduced relative to corresponding N-Nuc, and potent NS5B inhibition as triphosphates

# Prodrug of C-Nuc 2' CMeG (C-Nuc (2))

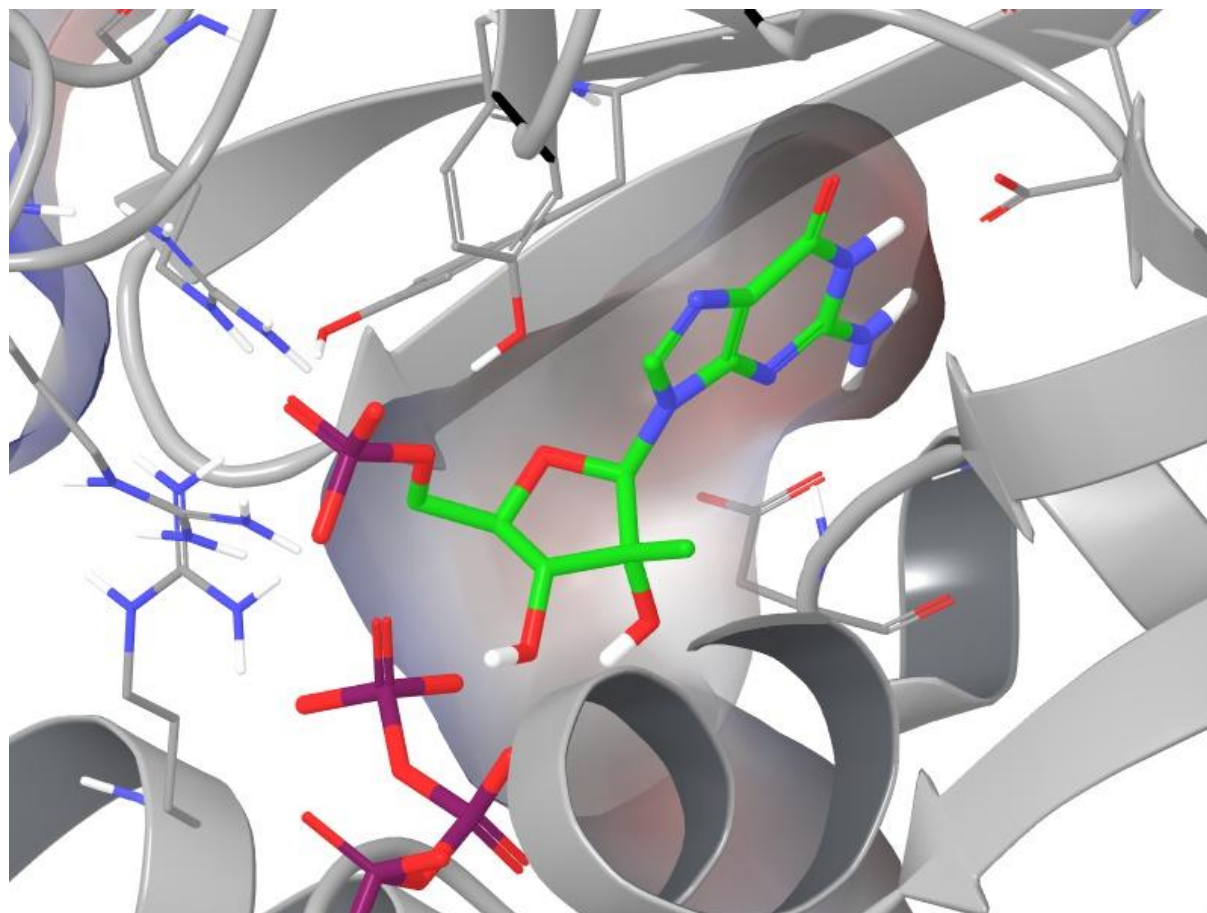


- ◆ SATE prodrug only partially rescued C-Nuc activity (200-fold less potent than N-Nuc)
- ◆ In contrast to the N-Nuc, high levels of the monophosphate accumulate after incubation of cells with the C-Nuc prodrug

Results from a single side-by-side experiment done in duplicate. Prodrugs continuously incubated at 10 μM.

# Homology Model of Human GMP Kinase

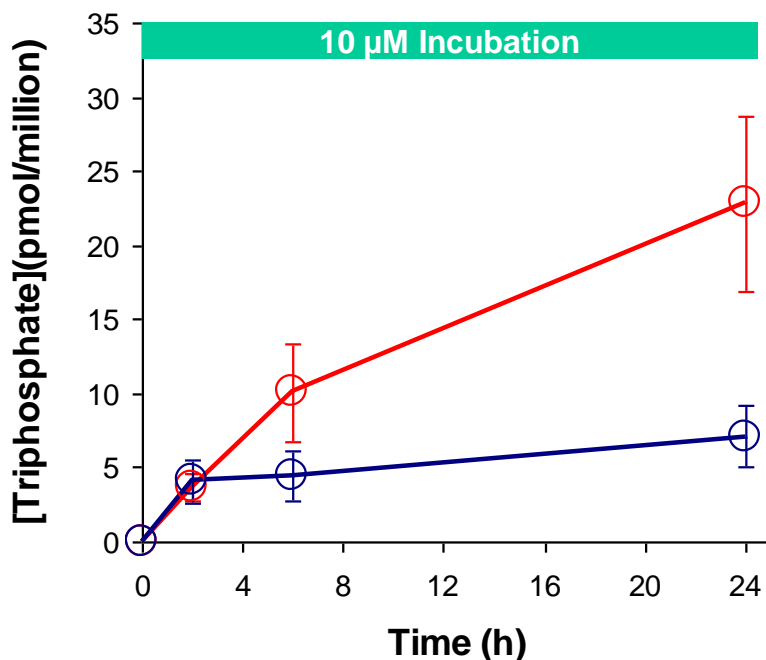
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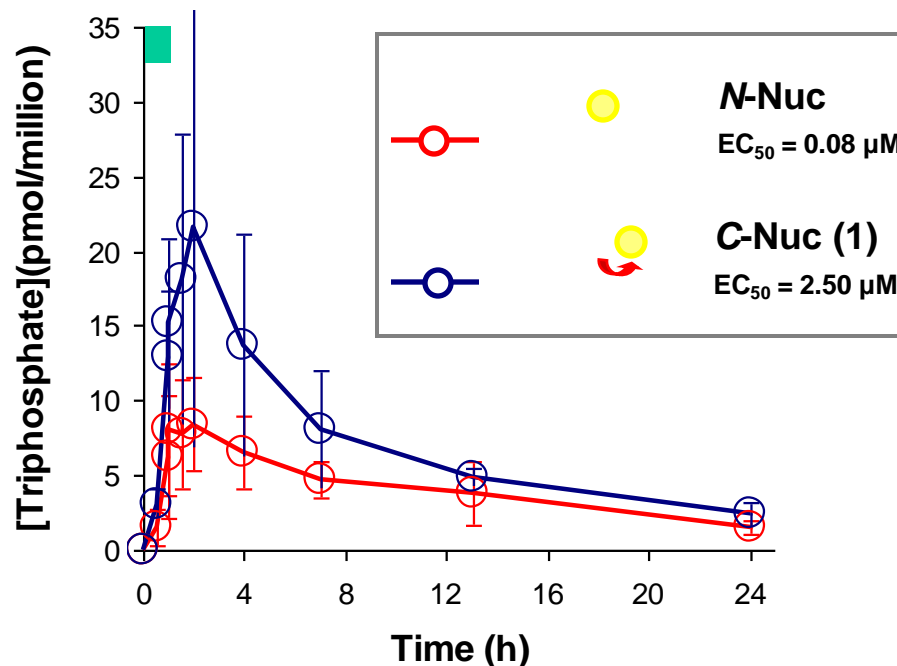
- ◆ Tight spatial and hydrogen bond constraints may make GMP Kinase highly sensitive to base modifications

# Intracellular Metabolism: 7-deaza-Adenosine Analogs

## Replicon



## Primary Human Hepatocytes



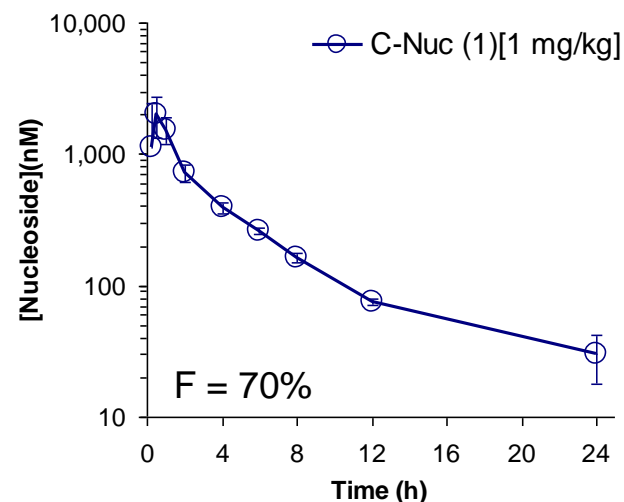
- ◆ Consistent with reduced replicon activity, C-Nuc is phosphorylated less efficiently in replicon cells
- ◆ However, uptake and phosphorylation of C-Nuc is more efficient in primary human hepatocytes

Results from two independent experiments done in duplicate. Primary hepatocytes isolated from two separate donors.

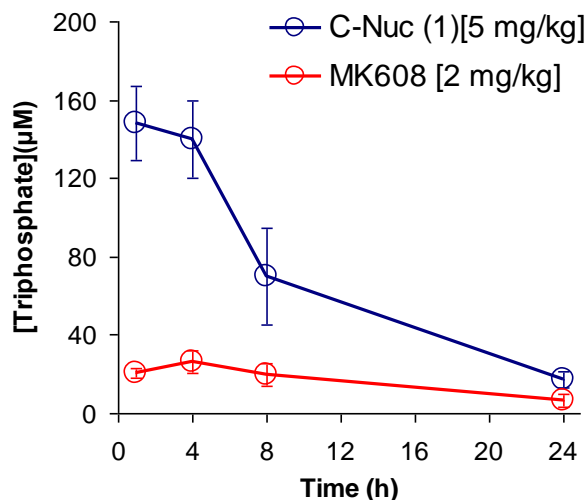
# Oral Pharmacokinetics

## Plasma

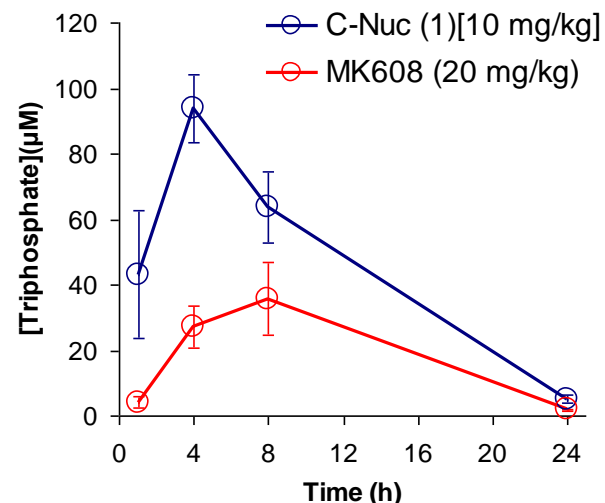
## Liver



Dog



Hamster



Rat

- ◆ Similar to what has been reported for *N*-Nucs, the 7-aza containing C-Nuc was found to be a substrate for adenosine deaminase but C-Nuc (1) was not<sup>a</sup>
- ◆ C-Nuc (1) had oral bioavailability of 50 to 70% in rat and dog
- ◆ Higher dose normalized liver triphosphate levels were observed than MK608 in rodents (hamsters [2-fold] and rats [5-fold])

# Conclusions

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- ◆ Characterization of a series of 2'-C-Me C-nucleosides has identified nucleoside analogs with potent anti-HCV activity
- ◆ The 2'CMe 7-deazaA C-nucleoside (**1**) was found to have potent and selective anti-HCV activity *in vitro* and a favorable pharmacokinetic profile *in vivo*
- ◆ C-nucleosides may hold promise as potential future therapies for HCV infection