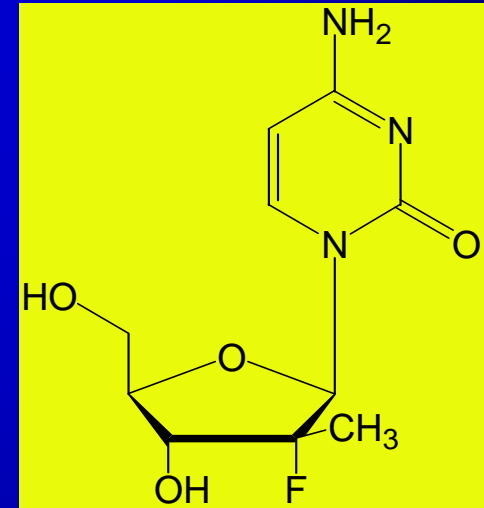


Inhibition of HCV Replication by PSI-6130: Mechanism of Biochemical Activation and Inhibition

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PSI-6130

- Cytidine analog
- Potent and selective activity *in vitro*
- Little or no cytotoxicity or mitochondrial toxicity
- Activation via deoxycytidine salvage pathway
- Potent inhibitor of the NS5B polymerase



***In Vitro* Activity of PSI-6130 against HCV and BVDV**

Compound	HCV EC ₉₀ (μM)	BVDV EC ₉₀ (μM)
PSI-6130	4.6 ± 2.0	>100
2'-C-Methylcytidine	21.9 ± 4.3	2.3 ± 0.1
2'-C-Methyladenosine	2.1 ± 0.27	2.0 ± 0.08
2'-F-Cytidine	6.5 ± 1.6	>100

PSI-6130 showed little or no activity against dengue virus, West Nile virus, and yellow fever virus (data provided by R. Sidwell, D. Smee, and J. Morrey, Utah State University, Utah State University). The compound also was found to be inactive against HIV and HBV.

Cytotoxicity of PSI-6130 Compared with other Nucleoside Analogs

Compound	Clone A (CC ₅₀ , μM)	Huh7 (CC ₅₀ , μM)	HepG2 (CC ₅₀ , μM)	CEM (CC ₅₀ , μM)	PBM (CC ₅₀ , μM)
PSI-6130	>100	>100	>100	>100	>100
2'-C-Methyl-C	>100	>100	>100	29.4	24.5
2'-C-Methyl-A	30.5	50.2	31.2	>100	>100
2'-F-Cytidine	>100	>100	>100	ND	ND

ND = Not Determined

14-Day Mitochondrial Toxicity Assay Using HepG 2 Cells

Compound	MitCoxII DNA IC ₅₀ ± S.D., (μM)	rDNA IC ₅₀ ± S.D. (μM)
Dideoxycytidine	<10	<10
PSI-6130	>100	71.80 ± 33.6
2'-C-Methylcytidine	32.5 ± 11.7	43.5 ± 9.5

Reversal of the Anti-HCV Activity of PSI-6130 by Exogenously Added Nucleosides

Competing Nucleoside (50 μ M)	% Anti-HCV Activity
None	100
Cytidine	44.2
Uridine	101
Adenosine	94.9
Guanosine	95.7
2'-Deoxycytidine	0.0
2'-Deoxyuridine	99.9
Thymidine	106
2'-Deoxyadenosine	100
2'-Deoxyguanosine	98.8

PSI-6130 concentration was 5 μ M (the approximate EC₉₀)

Anabolism of PSI-6130

First step: PSI-6130 → PSI-6130-MP

Enzyme	Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1}\mu\text{M}^{-1}$)
dCK	2'-Deoxycytidine	0.4 ± 0.1	0.025	6.3×10^{-2}
	Cytidine	6.6 ± 1.0	0.009	1.4×10^{-3}
	PSI-6130	81.2 ± 8.0	0.016	1.9×10^{-4}
	2'-C-Me-C	914 ± 209	0.011	1.2×10^{-5}
UCK-1	Cytidine	131 ± 27	0.50 ± 0.04	3.8×10^{-3}
	Uridine	407 ± 52	0.51 ± 0.03	1.3×10^{-3}
	2'-Deoxycytidine	No activity observed up to 1 mM		
	PSI-6130	No activity observed up to 3 mM		
	2'-C-Me-C	No activity observed up to 3 mM		

All kinase assays were performed spectrophotometrically using a coupled assay with pyruvate kinase and lactate dehydrogenase following NADH oxidation.

Anabolism of PSI-6130 Cont'd.

Second step: PSI-6130-MP → PSI-6130-DP

Enzyme	Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1}\mu\text{M}^{-1}$)
UMP-CMPK	UMP	151 ± 31	81 ± 5	0.54
	CMP	56 ± 10	30 ± 2	0.54
	dCMP	837 ± 69	30 ± 1	0.036
	PSI-6130-MP	282 ± 88	3.3 ± 0.4	0.012

Anabolism of PSI-6130 Cont'd.

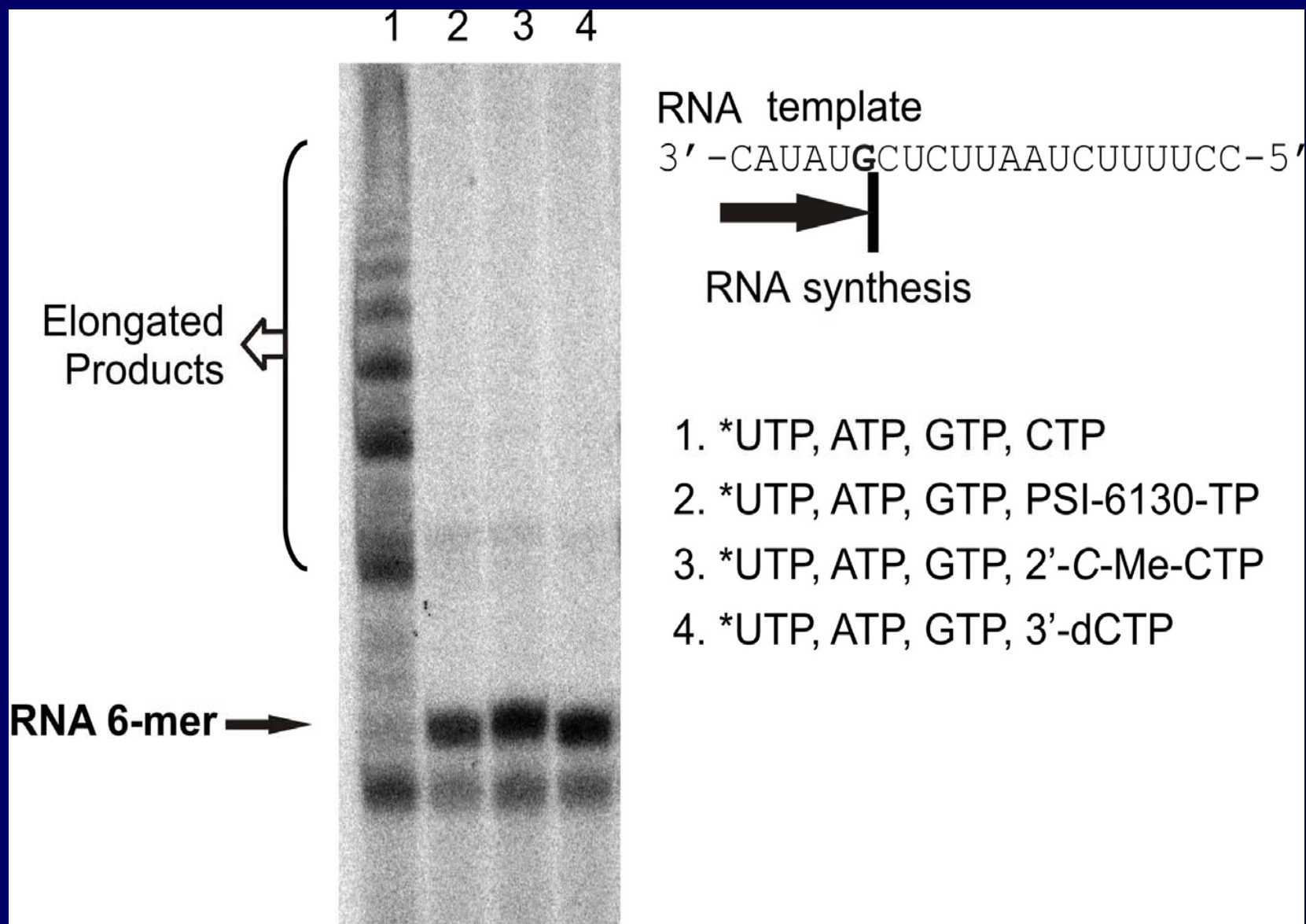
Third step: PSI-6130-DP → PSI-6130-TP

Enzyme	Substrate	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\text{s}^{-1}\mu\text{M}^{-1}$)
NDPK	CDP	307 ± 62	183 ± 14	0.60
	PSI-6130-DP	1380 ± 310	21 ± 3	0.015

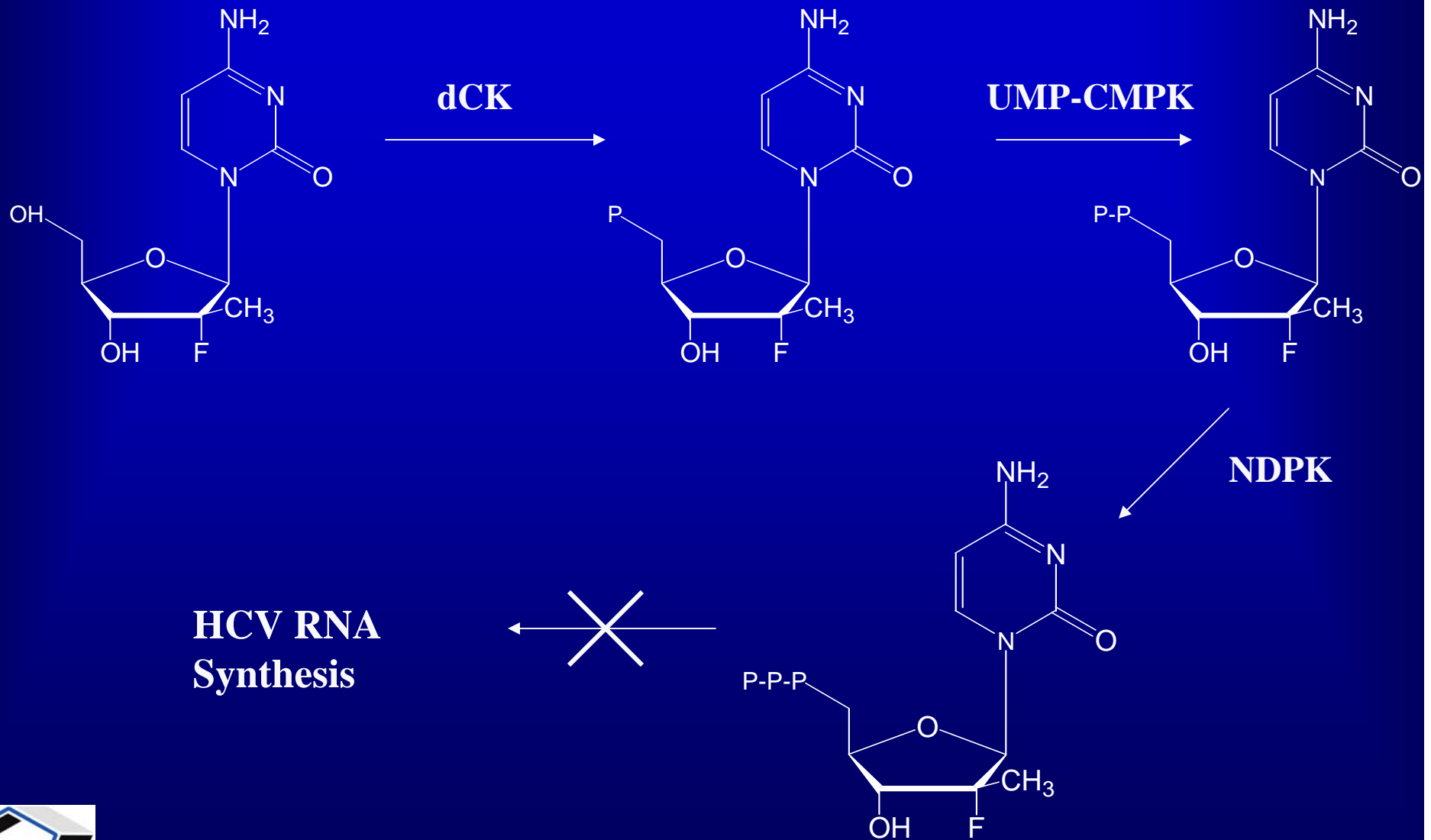
Inhibition of HCV NS5B by PSI-6130-TP

Nucleotide Analog	K_m (μM)*	K_i (μM)	K_i/K_m
PSI-6130-TP	13.7 ± 2.59	4.3 ± 0.7	0.31
2'-C-Me-CTP	13.7 ± 2.59	1.6 ± 0.3	0.12
2'-C-Me-ATP	2.7 ± 0.66	1.5 ± 0.4	0.56

HCV (-) IRES RNA was used as template and ^{32}P -UTP was used to follow RNA synthesis
* K_m for CTP is shown with PSI-6130-TP and 2'-C-methyl-CTP and K_m for ATP with 2'-C-methyl-ATP



Metabolism of PSI-6130 to PSI-6130TP



Conclusions

1. PSI-6130 is both a potent and selective inhibitor of HCV replication.
2. PSI-6130, unlike 2'-C-MeC and 2'-C-MeA, is a specific inhibitor of HCV. These results suggest that the fluorine in the 2' position of PSI-6130 confers specificity to this molecule.
3. Cytotoxicity assays using several different cell types indicate that little or no toxicity is associated with PSI-6130.
4. PSI-6130 has no effect on mitochondrial DNA content or lactic acid production.
5. The antiviral effect was inhibited strongly by deoxycytidine.
6. Enzyme studies indicate that PSI-6130 is phosphorylated by human deoxycytidine kinase. Phosphorylation to the di- and triphosphate forms is catalyzed by UMP-CMP kinase and NDPK, respectively.
7. PSI-6130 5'-triphosphate is a potent inhibitor of the HCV RdRp and appears to terminate chain elongation.

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