Efficient Liver Targeting and Uptake by Novel Tenofovir Prodrug, CMX157, For the Treatment of Hepatitis B

R Rush[1], J Greytok[2], T Matkovits[2], R Driz[2], JZ Sullivan-Bólyai[2], and D Standring[3]
[1]Allon Preclinical Consulting LLC, USA; [2]ContraVir Pharmaceuticals Inc., USA; and [3]David Standring Consulting, USA.

Lipid Conjugates Mimic Natural Phospholipids

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Intestine

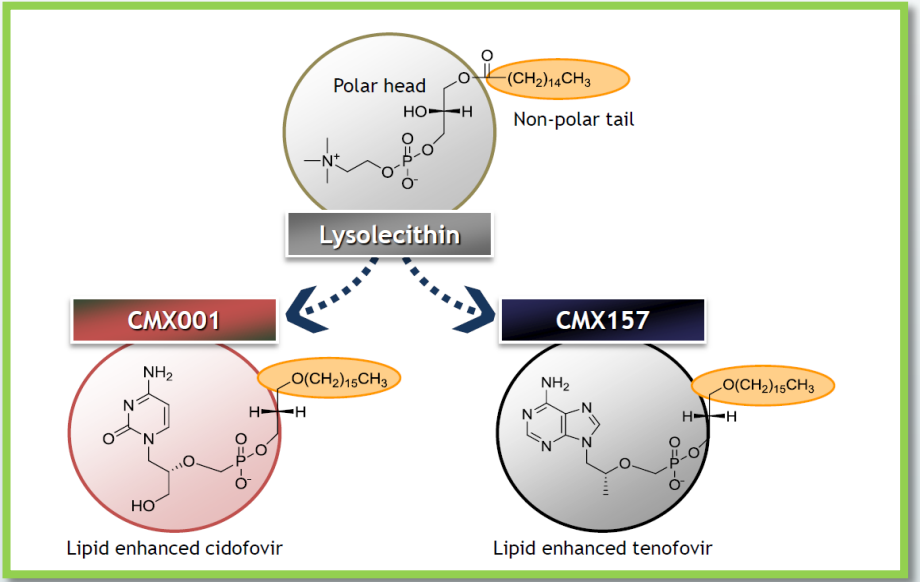
- Efficiently absorbed

Peripheral Circulation

- Not cleaved in periphery

Target Cells

- Facilitate uptake and are converted to active drug inside cells
- Significantly more active drug in target cells



CMX157

Novel and Highly Potent Prodrug of Tenofovir

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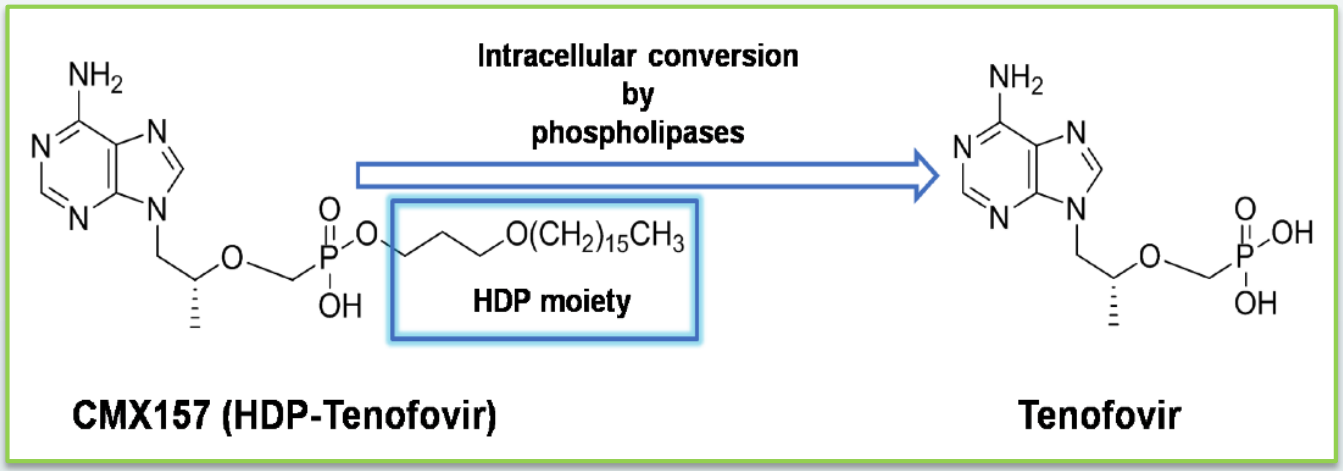
Enhanced Efficacy and Safety

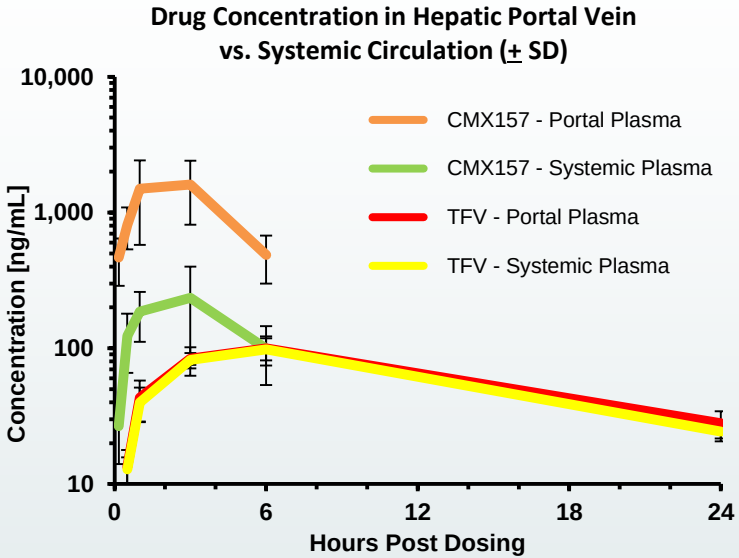
- Increased bioavailability by harnessing lipid uptake mechanisms
- Enhanced target tissue penetration
- Decreased renal and bone toxicity by reducing circulating TFV

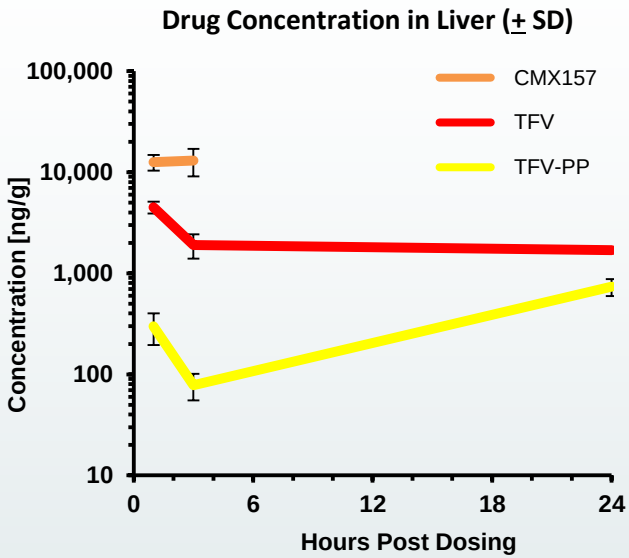
Enhanced Potency

- 97 fold more potent in HBV than TFV in vitro
- 200 fold more potent in HIV than TFV in vitro



CMX157: Liver Extraction

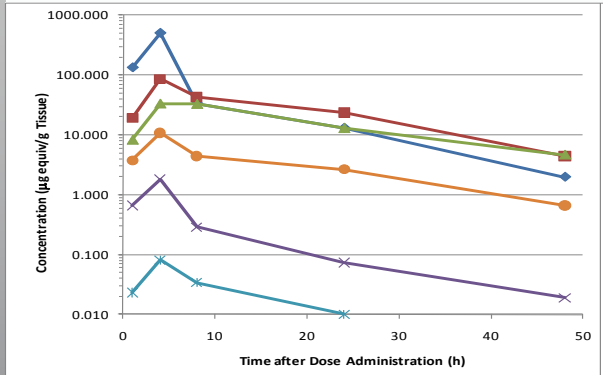




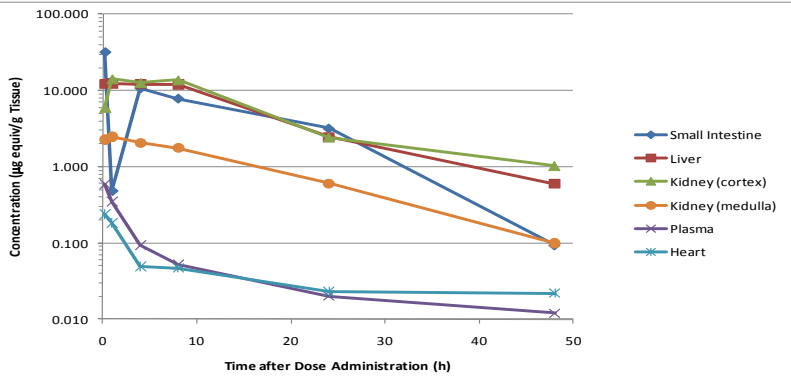
- Rapid, efficient extraction and conversion to high liver levels of the active antiviral, TFV-PP after single dose in rat
  - 86% first pass extraction by the liver
  - Portal vein levels of CMX157 peaked at 1-3 hours and declined rapidly
  - Same pattern observed in liver
  - Low systemic levels of TFV

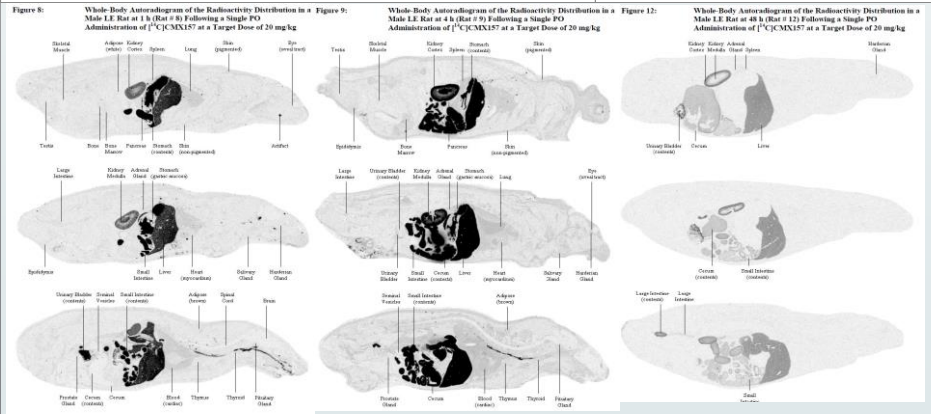
CMX157: Tissue Distribution

Tissue Concentrations of Radioactivity After Oral Administration of 20 mg/kg ^{14}C-CMX157 to Rats



Tissue Concentrations of Radioactivity After IV Administration of 2 mg/kg ^{14}C-CMX157 to Rats





- **Highly liver targeted**
- **No substantial accumulation or retention in the heart.**

CMX157: Metabolism in Primary Human Hepatocytes

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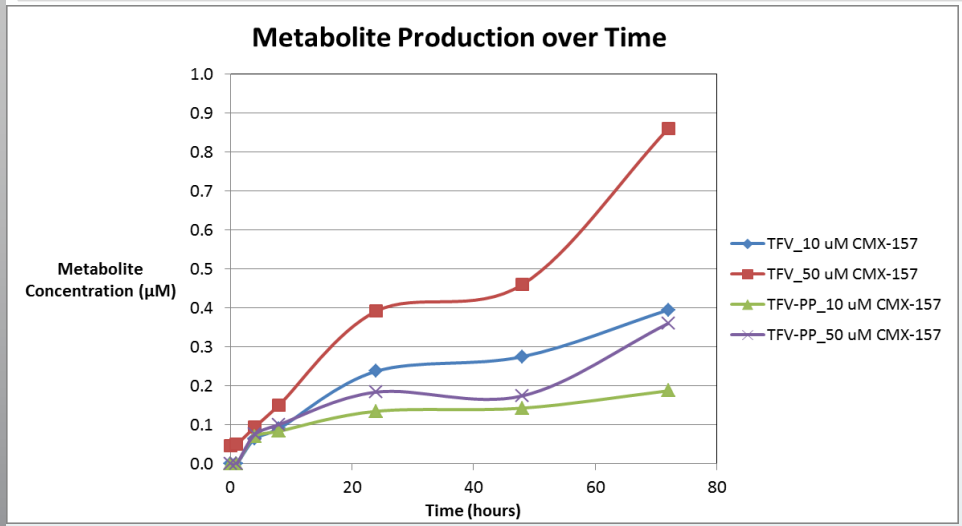

Time Point	CMX157 10 µM		CMX157 50µM	
	TFV, nM	TFV-PP, nM	TFV, nM	TFV-PP, nM
0	0	0	46.0	0
1	0	0	50.3	0
4	65.1	70.7	94.9	76
8	91.3	84.3	152	101
24	238	135	392	185
48	275	143	460	175
72	395	188	860	361

- Rapid, efficient conversion to TFV-PP
- Hepatocytes fully viable
- Conversion is time and concentration dependent

CMX157: Metabolism in Stem-Cell Derived Human Cardiomyocytes

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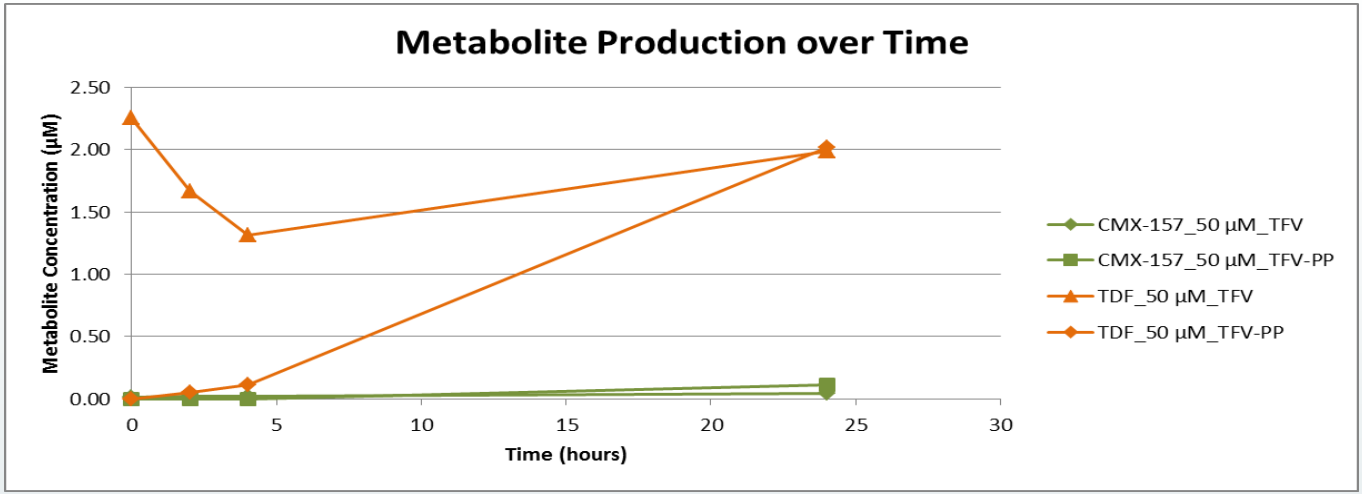

Substrate	Concentration (µM)	Time (hr)	TFV (nM)	TFV-PP (nM)
CMX-157	50	0	10.8	0
		2	18.1	0
		4	22.9	0
		24	47.4	115
TDF	50	0	2260	0
		2	1670	54.1
		4	1320	116
		24	1990	2020

- **Minimal CMX157 metabolism by cardiomyocytes**
- **Cardiomyocytes fully viable**

CMX157: Antiviral Activity *in vitro*

- 97 fold more potent than TFV
  - HepG2.2.15 cells in a standard assay
  - Real time qPCR (Taqman) HBV DNA quantitation

Test Article	EC_{50} (nM) Mean ± SD*	CC_{50} (nM) Mean ± SD	Selectivity Index CC_{50}/ EC_{50}
CMX157	15.03 ± 4.31	18,110 nM	1205
TFV	1460± 1,127	>100,000 µM	>68

*Mean ± standard deviation

- Supported by experiments in HIV systems
  - 200 fold more potent than TFV *in vitro*

CMX157: Cytotoxicity Evaluation

Cell Line	Tissue Origin	CMX157 CC_{50} (μM)	TFV CC_{50} (μM)	TDF CC_{50} (μM)	
Proliferating					3 Day Incubation
HepG2	Liver	>100.0	>100.0	12.5	
Caki-1	Kidney	>100.0	>100.0	>100.0	
SNB-78	Brain	>100.0	>100.0	>100.0	
COLO-205	Colorectal	28.0	>100.0	40.2	
CCRF-CEM	PBMC	86.0	>100.0	16.0	
SJCRH30	Muscle	57.7	>100.0	46.6	
Non-proliferating					
Cardiomyocyte	Heart	>100.0	>100.0	61.6	
Proliferating					6 Day Incubation
CEM-SS	Lymph	>10.0	>100.0	>10.0	
HeLa	Cervical	>10.0	>100.0	>10.0	
ME 180	Cervical	>10.0	>100.0	>10.0	
Huh-7	Liver	>10.0	>100.0	>10.0	
HepG2	Liver	>10.0	>100.0	8.8	
PBMC (stimulated)	Blood	>10.0	>100.0	>10.0	
Non-proliferating					
PBMC (unstimulated)	Blood	>10.0	>100.0	7.4	
Monocyte Macrophage	Blood	>10.0	>100.0	>10.0	
Dendritic	Brain	>10.0	>100.0	>10.0	
Hepatocyte	Liver	>10.0	>100.0	>10.0	
PBMC (unstimulated)	Blood	>10.0	>100.0	7.4	

CMX157: Cytotoxicity Evaluation

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- Low potential for cytotoxicity
  - Tested in a large panel of proliferating or non-proliferating cell lines for up to 14 days
  - Tissues represented include liver, brain, heart, kidney, lymph, muscle, cervix, blood, bone marrow and gut

Cell Line	Tissue Origin	CMX157 CC_{50} (µM)	TFV CC_{50} (µM)	TDF CC_{50} (µM)	
Proliferating					12 or 13 Day Incubation
COLO-205	Colorectal	36.0	>100.0	12.9	
SJCRH30	Muscle	17.4	>100.0	9.1	
Non-proliferating					
Cardiomyocyte	Heart	>100.0	>100.0	12.8	

Cell Line	Tissue Origin	CMX157 CC_{50} (µM)	TFV CC_{50} (µM)	TDF CC_{50} (µM)	
GM-CFU	Bone Marrow	2.46	4.72	1.89	14 Day Incubation
E-BFU		4.62	3.48	0.5	

CMX157: Mitochondrial Toxicity Evaluation in the HepG2 Liver Cell Line

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	Viable Cells (% vehicle)		SDH-A:COX-I Ratio	
Compound (µM)	**Glucose**	**Galactose**	**Glucose**	**Galactose**
Medium	117.3	102.8	2.3	2.2
Vehicle	100.0	100.0	2.0	1.9
Chloramphenicol				
30	20.7	14.0	10.1	6.8
CMX157				
0.1	83.2	106.5	1.9	2.1
1	88.3	83.2	2.2	2.0
10	70.4	65.4	2.2	1.9
100	*	*	*	*
TDF				
0.1	72.2	94.4	2.4	1.9
1	61.5	29.0	2.3	2.1
10	*	*	*	*
100	*	*	*	*
TFV				
0.1	76.0	68.2	2.1	1.7
1	69.3	61.7	2.3	1.8
10	62.0	57.0	2.0	2.0
100	54.7	59.8	2.3	1.8

* Insufficient cells for analysis

- **Low potential for mitochondrial toxicity**
- **Confirms previous experiments**

CMX157: Impact on Transporters

- Low potential for Drug-Drug Interactions

	% Inhibition	
Compound	**CMX157 (3 µM)**	**TFV (1 µM)**
BSEP	0	0
MRP2	0	0
BCRP	< 10	11
	CMX157 (15 µM)	**TFV (1 µM)**
OATP1B1	93*	< 10
OATP1B3	32	< 10
	CMX157 (0.15 µM)	**TFV (0.5 µM)**
OAT1	< 10	< 10
OAT3	< 10	0
OCT2	11	0
MATE1	< 10	0
MATE2-K	< 10	0
	CMX157 (0.15 µM)	**TFV (1 µM)**
P-gp	0	0

* > 26,000 fold above protein-adjusted Cmax at 400 mg SD

CMX157: Low Potential for CYP450 Inhibition

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In Vitro Evaluation of CMX157 as an Inhibitor of Human CYP Enzymes		
	Direct Inhibition	Time-Dependent Inhibition
Enzyme	Zero-Minute Preincubation	30-Minute Preincubation
	IC_{50} (μM)[a]	IC_{50} (μM)[a]
CYP1A2	39	32
CYP2B6	18	15
CYP2C8	4.4	3.3
CYP2C9	11	15
CYP2C19	31	28
CYP2D9	27	26
CYP3A4/5	12	12
CYP3A4/5	24	19
CYP3A4/5	18	16

[a]Average data(i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.

*** Value for CYP2C8 is > 3,500 fold above the protein-adjusted C_{max} at 400 mg SD**

CMX157: Low Potential for CYP450 Induction

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In Vitro Evaluation of CMX157 as an Inducer of Human CYP Enzymes

		Fold Increase[a]		
Treatment	Concentration	Phenacetin O-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl Sulfoxide	0.1% (v/v)	1.00 ± 0.19	1.00 ± 0.26	1.00 ± 0.09
CMX157	1 µM	1.03 ± 0.24	0.954 ± 0.117	1.15 ± 0.09
CMX157	10 µM	1.05 ± 0.16	0.974 ± 0.098	1.10 ± 0.09
CMX157	100 µM	1.08 ± 0.20	0.849 ± 0.356	1.40 ± 0.09†
Omprazole	100 µM	44.9 ± 24.5*	12.8 ± 9.0	2.56 ± 0.42
Phenobarbital	750 µM	2.60 ± 0.59	20.1 ± 7.8	7.02 ± 0.66*
Rifampin	10 µM	2.04 ± 0.27	10.3 ± 4.5	6.32 ± 0.33*

[a]Values are the mean ± standard deviation of three determinations (human hepatocyte preparations H893, H895 and H899).
*Significantly different from the vehicle control (DMSO) as a result of One-way Analysis of Variance ($p<0.05$) with all treatment groups included in the statistical analysis.
† Significantly different from the vehicle control (DMSO) as a result of One-way Analysis of Variance ($p<0.05$) with the positive control groups (omeprazole, phenobarbital and rifampin) excluded from the statistical analysis.

CMX157 Potent HBV Antiviral Activity

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Key HBV IND/CTA Enabling Data

Test Article	EC_{50} (nM) Mean ± SD*	CC_{50} (nM) Mean ± SD	Selectivity Index CC_{50}/ EC_{50}
CMX157	15.03 ± 4.31	18,110 nM	1205
TFV	1460± 1,127	>100,000 µM	>68

*Mean ± standard deviation

CMX157 is 97 fold more potent than TFV *in vitro* systems

- Equal, possibly better efficacy at low dose vs. TDF
- Potential for improved safety profile due to less circulating TFV

Favorable *in vitro* safety profile

- ✓ **Low potential for QT interval cardiac arrhythmias**
  - No inhibition of hERG potassium channel at clinically relevant concentrations
- ✓ **No genotoxicity in standard assays**
- ✓ **Low potential for cytotoxicity**
  - Tested in a large panel of proliferating or non-proliferating cell lines for 3, 6 or 13 days
  - Tissues represented include liver, brain, heart, kidney, lymph, muscle, cervical, blood bone marrow and gut
- ✓ **Low potential for mitochondrial toxicity***
  - Tested in HepG2 cells incubated with glucose or galactose

*Characteristic toxicity of the nucleoside class