



# Antiviral and Pharmacokinetic Properties of 3'-Azido-2',3'-Dideoxypurines that Exhibit Potent Activity against Drug-Resistant HIV-1

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## Abstract

**Background:** Currently approved NRTI have significant limitations including the selection of drug-resistant HIV-1 variants that are cross-resistant to other NRTI. Based on knowledge of both resistance mechanisms and structural components of NRTI that affect sensitivity to these resistance mechanisms, we have identified a lead class of 3'-azido-2',3'-dideoxypurines (ADPs) that retain potent activity against NRTI-resistant HIV-1.

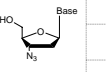
**Methods:** 3'-Azido-dideoxyguanosine (3'-azido-ddG) and 3'-azido-2',3'-dideoxyadenosine (3'-azido-ddA) were subjected to extensive *in vitro* evaluations of antiviral potency, activity against purified HIV-1 reverse transcriptase (RT), cytotoxicity and cellular pharmacology.

**Results:** 3'-Azido-ddA and 3'-azido-ddG showed excellent antiviral activity in primary human mononuclear cells, HeLa and T-cell lines (EC50 ranged from 0.19 to 2.1 μM for 3'-azido-ddG and from 0.36 to 10 μM for 3'-azido-ddA). Both 3'-azido-ddGTP and 3'-azido-ddATP were incorporated by HIV-1 RT as efficiently as the natural dGTP or dATP substrates. Furthermore, both 3'-azido-ddA and 3'-azido-ddG retained activity against viruses containing K65R, L74V or M184V (EC50 fold-change < 2.0) and against those containing 3 or more thymidine analog mutations (EC50 fold-change < 3.5). Even at high concentrations of drug (100 μM), 3'-azido-ddG did not exhibit cytotoxicity in primary lymphocytes, epithelial or T-cell lines, and did not decrease the mitochondrial DNA content of HepG2 cells. 3'-Azido-ddG was efficiently phosphorylated to 3'-azido-ddGTP in primary human mononuclear cells with an intracellular half-life of the nucleoside triphosphate of 9 h.

**Conclusions:** The ADPs exhibit a favorable activity profile against both wild-type and NRTI-resistant viruses. 3'-Azido-ddG also exhibited minimal cytotoxicity and favorable cellular pharmacologic profile. Based on these findings, additional preclinical studies are warranted to assess the potential of ADPs for treatment of HIV-1 infection.

## ADPs are Active Against AZT-Resistant HIV-1 and HIV-1 Containing K65R.

**Table 1:** 3'-azido-2',3'-dideoxypurines (ADPs) are ACTIVE against HIV-1 containing D67N/K70R/T215F/K219Q (AZT<sup>R</sup>) or K65R

Nucleoside	Base	EC <sub>50</sub> , μM (MT-2 cells)		Fold-R	EC <sub>50</sub> , μM (P4/R5 or MT-2 cells)		Fold-R
		WT	AZT <sup>R</sup>		WT	K65R	
 3'-azido-2',3'-dideoxy	3'-azido-2',3'-dideoxypyrimidines						
	C	15.6 ± 10.7	160.8 ± 24.1	10.7	15.6 ± 10.7 <sup>b</sup>	37.8 ± 28.5	2.5
	T	0.031 ± 0.02	0.36 ± 0.2	11.7	0.2 ± 0.12 <sup>a</sup>	0.22 ± 0.16	1.1
	3'-azido-2',3'-dideoxypurines						
A	7.90 ± 3.4	8.6 ± 5.4	1.1	3.9 ± 1.1 <sup>a</sup>	3.7 ± 0.3	0.9	
G	7.90 ± 4.63	10.3 ± 8.3	1.3	7.90 ± 4.63 <sup>b</sup>	15.8 ± 9.8	2.0	

Data adapted from: Sluis-Cremer et al. (2005) J. Biol. Chem. 280, 29047-52; Parikh et al. (2005) Antimicrob. Agents Chemother. 49, 1139-44.

## Cellular Toxicity

**Table 2:** Cytotoxicity of ADPs in four different cell lines.

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye was used a probe for cell viability

NRTI	CC <sub>50</sub> , μM			
	PBMC	MT-2	Vero <sup>a</sup>	CEM <sup>b</sup>
AZT	>100	150	>100	86.2
3'-azido-ddA	74.3	200	50.6	50.6
3'-azido-ddG	> 100	140	> 100	> 100

<sup>a</sup> kidney epithelial cells from the African Green monkey; <sup>b</sup> human T-cell derived cell line

## Mitochondrial Toxicity

**Table 3:** Quantification of mitochondrial DNA content in HepG2 cells treated with 3'-azido-ddG, ddC and 3TC using the comparative C<sub>T</sub> method.

NRTI	ΔC <sub>T</sub> (average ± SD)		ΔΔC <sub>T</sub> (average ± SD)	Fold-Change (range)
	mtDNA	rDNA		
Media	0.00±0.09	0.00±0.25	0.00±0.28	1.0 (0.82-1.22)
10 μM 3TC	1.45±1.37	1.81±0.49	0.03±1.02	0.98 (0.49-1.98)
10 μM ddC	11.86±0.03	1.88±0.39	9.98±0.42	0.0010 (0.00074-0.0013)
10 μM 3'-azido-ddG	0.22±1.04	0.70±0.40	-0.09±0.81	1.06 (0.60-1.87)
100 μM 3'-azido-ddG	0.09±0.12	0.47±0.27	-0.38±0.36	1.30 (1.01-1.67)

## Antiviral Activity in Different Cell Lines

**Table 4:** Anti-HIV activity of ADPs and FDA-approved NRTI in three different cell lines.

NRTI	EC <sub>50</sub> , μM		
	P4/R5	MT-2	PBMC
3'-azido-ddA	2.1 ± 0.9	1.73 ± 1.06	0.19 ± 0.19
3'-azido-ddG	10.0 ± 4.9	6.43 ± 3.10	0.36 ± 0.14
Abacavir (ABC)	6.21 ± 1.66	4.76 ± 1.63	0.91-0.98 <sup>a</sup>
Tenofovir (TNV)	4.67 ± 2.31	0.54 ± 0.61	N/A
Didanosine (ddI)	2.32 ± 0.98	0.49 ± 0.32	0.1
Lamivudine (3TC)	0.78 ± 0.48	0.60 ± 0.46	0.03-0.15 <sup>a</sup>
Emtricitabine (FTC)	0.17 ± 0.07	0.044 ± 0.036	0.001-0.69 <sup>a</sup>
Zalcitabine (ddC)	1.35 ± 0.70	0.13 ± 0.18	0.2 <sup>b</sup>
Zidovudine (AZT)	0.19 ± 0.11	0.031 ± 0.024	0.01-0.09 <sup>b</sup>
Stavudine (d4T)	5.72 ± 3.34	1.87 ± 0.76	0.1 <sup>b</sup>

Data adapted from: <sup>a</sup> Schinazi et al. (2006) Antiviral Res. 71, 322-334; <sup>b</sup> Sharma et al. (2004) Curr. Top. Med. Chem. 4, 1035-44

## Activity Against NRTI-resistant HIV-1

**Table 5:** Anti-HIV activity of ADPs against a panel of drug resistant HIV-1.

Virus	AZT	Fold-R	3'-azido-ddA	Fold-R	3'-azido-ddG	Fold-R
	EC <sub>50</sub> , μM		EC <sub>50</sub> , μM		EC <sub>50</sub> , μM	
WT	0.19 ± 0.11	-	10.7 ± 4.9	-	2.1 ± 0.9	-
K65R	0.21 ± 0.15	1.1	9.8 ± 8.4	0.9	5.0 ± 1.3	2.3
L74V	0.21	1.1	13.7 ± 5.7	1.2	2.9 ± 0.9	1.4
M184V	0.18 ± 0.16	1.0	8.9 ± 2.3	0.8	1.7 ± 0.1	0.8
<sup>a</sup> AZT2	10.4 ± 8.9	54.4	24.2 ± 3.7	2.2	5.2 ± 2.3	2.5
<sup>b</sup> AZT3	11.9 ± 11.6	62.7	19.4 ± 8.1	1.8	3.7 ± 1.4	1.8
<sup>c</sup> AZT7	96.7 ± 29.3	507.4	31.6 ± 3.7	2.9	7.6 ± 2.2	3.5
<sup>d</sup> AZT9	58.6 ± 9.2	307.4	37.5 ± 6.2	3.5	7.9 ± 4.9	3.7
<sup>e</sup> Q151M	213.7	1124	70.1 ± 10.8	6.5	72.9 ± 29.5	34.7
<sup>f</sup> 69 insertion	204.6	1076	29.9 ± 5.9	2.8	26.4 ± 8.8	12.5

<sup>a</sup> M41L210W/T215Y

<sup>b</sup> D67N/K70R/T215F/K219Q

<sup>c</sup> M41L/D67N/K70R/T215F/K219Q

<sup>d</sup> M41L/D67N/K70R/L210W/T215Y/K219Q

<sup>e</sup> A62V/V75I/F77L/F116Y/Q151M

<sup>f</sup> M41L/S insert between 69 and 70; L210W/T215Y

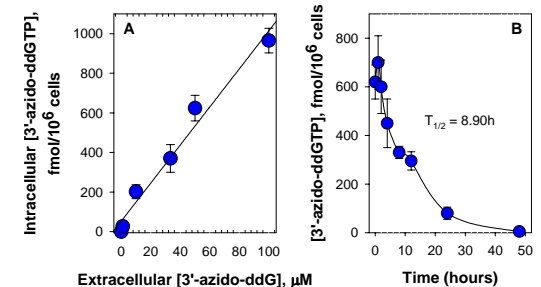
## Activity Against HIV-1 RT

**Table 6:** Pre-steady-state kinetic parameters determined for the incorporation of clinically approved NRTI-TP and 3'-azido-ddATP and 3'-azido-ddGTP by WT HIV-1 RT.

dNTP/NRTI-TP	k <sub>pol</sub> s <sup>-1</sup>	K <sub>d</sub> μM	k <sub>po</sub> /K <sub>d</sub> μM <sup>-1</sup> .s <sup>-1</sup>	NRTI-TP			Selectivity
				k <sub>pol</sub> s <sup>-1</sup>	K <sub>d</sub> μM	k <sub>po</sub> /K <sub>d</sub> μM <sup>-1</sup> .s <sup>-1</sup>	
dNTP							
dATP/ddATP	24.4 ± 3.9	3.2 ± 1.2	7.6	5.4 ± 2.3	5.3 ± 1.7	1.0	7.5
dATP/TNV-DP				6.2 ± 1.0	4.3 ± 1.3	1.4	5.3
dATP/3'-azido-ddATP				23.2 ± 0.5	2.3 ± 0.8	10.2	0.7
dCTP/ddCTP	1.2 ± 0.7	1.1 ± 0.4	1.1	0.4 ± 0.1	2.7 ± 1.3	0.2	6.7
dCTP/3CTP				0.023 ± 0.004	0.37 ± 0.20	0.06	17.8
dGTP/CVBTP	7.5 ± 1.1	4.2 ± 1.4	1.8	0.6 ± 0.2	4.0 ± 1.8	0.16	11.2
dGTP/3'-azido-ddGTP				4.8 ± 1.1	2.8 ± 0.9	1.7	1.1
TTP/dATTP	9.2 ± 1.3	2.2 ± 0.8	4.2	4.3 ± 1.2	2.7 ± 1.1	1.6	2.6
TTP/AZTTP				8.8 ± 0.2	2.0 ± 0.1	4.4	1.0

<sup>a</sup> Selectivity is defined as: (k<sub>po</sub>/K<sub>d</sub>)<sup>NRTI</sup> / (k<sub>po</sub>/K<sub>d</sub>)<sup>dNTP</sup>

## Anabolism of 3'-azido-ddG



**Figure 1A:** Dose-response of cellular uptake of 3'-azido-ddG and conversion to 3'-azido-ddGTP in PHA-stimulated human PBMC.

**Figure 1B:** Decay of 3'-azido-ddGTP in PBMC after incubation with 50 μM 3'-azido-ddG for 12 h. The half-life of 3'-azido-ddGTP is calculated to be 8.9 ± 0.14 h.

## Acknowledgements

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