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Novel 2'-Fluoro Substituted Nucleotide HIV Reverse Transcriptase Inhibitor GS-9148 Exhibits Low Potential for Mitochondrial Toxicity *In Vitro*

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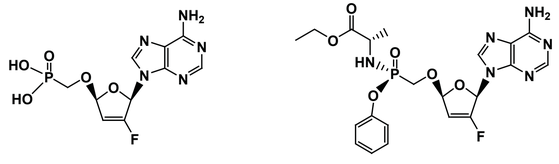
Introduction

- Mitochondrial toxicity is an important etiological factor associated with various adverse symptoms observed in patients treated with some nucleoside HIV reverse transcriptase inhibitors (NRTIs)[1,2].
- GS-9148 (Fig. 1) is a novel nucleotide HIV RT inhibitor with *in vitro* antiretroviral activity against both wild-type and NRTI-resistant strains (M184V, K65R, L74V, multiple TAMs)[3]. It has been rationally designed to minimize the mitochondrial toxicity of its structural precursor d4AP[4] by incorporating a 2'-fluoro (2'-F) substitution into its 2',3'-dideoxy-2', 3'-didehydro-ribose ring.
- Here we report on the *in vitro* mitochondrial toxicity profile of GS-9148 and its orally bioavailable prodrug GS-9131 (Fig. 1) that is currently undergoing clinical evaluation.

Figure 1. Structure of GS-9148 and its Prodrug GS-9131



GS-9148 GS-9131

Methods

- Composer homology modeling module in Sybyl v.7.3 was used to optimize the sequence alignment and 3D model of human DNA polymerase γ based on the X-ray structure of T7 DNA polymerase[5].
- The steady-state inhibition of DNA polymerase γ by active di-/triphosphate metabolites was characterized using the incorporation assay with [^{3}H]dATP and activated calf-thymus DNA[6].
- Relative levels of mitochondrial DNA (mtDNA) in HepG2 cells treated with tested compounds were determined by sequential DNA hybridization[7] using ^{32}P-labeled cytochrome *c* and β-actin DNA probes and/or by quantitative PCR using $\Delta\Delta C_T$ method[8] for cytochrome *b* and β -actin gene fragment amplification.
- Lactate production by treated HepG2 cells was quantified using a colorimetric assay (Trinity Biotech, St. Louis, MO).

Results

Figure 2. Rationale for the Design of GS-9148

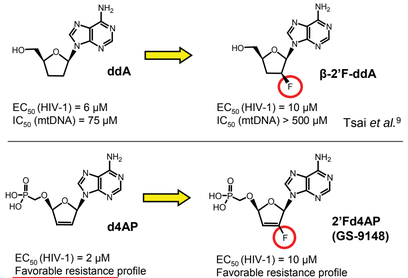
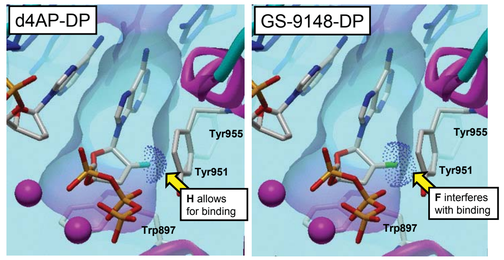


Figure 3. Binding of d4AP-DP and GS-9148-DP in the Active Site of Human DNA Polymerase γ



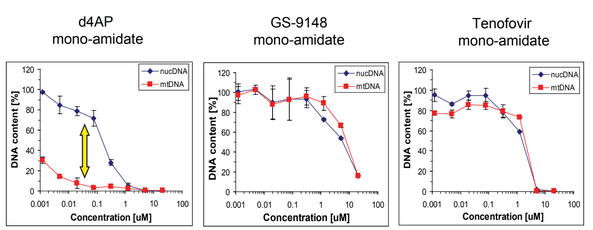
- Diphosphates of d4AP and GS-9148 were docked into the active site of DNA Pol γ that was modeled based on its homology with T7 DNA Pol[5].

Table 1. Inhibition of HIV RT and Human DNA Polymerases

Inhibitor	IC_{50} [μM][a]				
	HIV-1 RT	Pol α	Pol β	Pol γ	
ddA-TP	0.2 ± 0.1	> 100	2.4 ± 0.6	1.0 ± 0.4	5-fold
Tenofovir-DP	0.6 ± 0.1	61 ± 23	51 ± 3.5	> 300	> 500 fold
d4AP-DP	0.8 ± 0.4	-	-	21.0 ± 1.0	25-fold
GS-9148-DP	1.8 ± 0.8	43 ± 21	> 150	> 300	> 160-fold

IC_{50} values represent mean ± SD from n ≥ 2.

Figure 4. Effect of d4AP and GS-9148 Amidate Prodrugs on mtDNA Levels



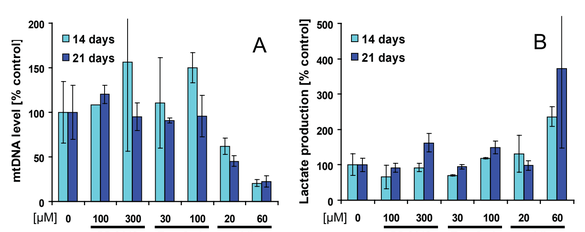
- HepG2 cells were incubated with tested prodrugs for 14 days and the levels of nucDNA and mtDNA were quantified by DNA hybridization. Data are from a representative experiment performed in triplicates.

Table 2. Effect of GS-9131 and GS-9148 on the Levels of mtDNA (Quantitative PCR Analysis)

Compound	Concentration [μM]	mtDNA [% control][a]
None (control)	-	100.0 ± 15.1
GS-9131	5	205.3 ± 0.4
	50	139.1 ± 22.1
GS-9148	100	106.7 ± 19.1
	300	178.4 ± 28.2
Tenofovir	100	110.5 ± 15.9
	300	190.6 ± 45.7
ddI	100	9.7 ± 0.0
	300	6.9 ± 0.8
ddC	10	4.7 ± 1.9
	30	8.1 ± 3.2
d4T	100	35.7 ± 5.8
	300	34.4 ± 8.9

a. Samples were analyzed by quantitative PCR following a 10-day treatment of HepG2 cells with tested compounds. Results represent means ± SD from n = 2.

Figure 5. Correlation of Effects on mtDNA and Lactate Production



- HepG2 cells were incubated with tested compounds for 14 and 21 days. The relative levels of mtDNA and production of lactate were determined by DNA hybridization and a spectrophotometric quantification, respectively. Results are means ± SD from a representative experiment performed in duplicate.

Conclusions

- **GS-9148 was rationally designed to minimize the mitochondrial toxicity of its precursor d4AP**
- **Similar to previously characterized 2'-F-2',3'-dideoxynucleosides[9], the substitution of d4AP with 2'-F decreased the inhibiting potential towards DNA pol γ, reducing the potential for mitochondrial toxicity**
- **Unlike d4AP and some marketed NRTIs (ddI, d4T, ddC), neither GS-9148 nor its prodrugs deplete mtDNA in HepG2 liver cells**
- **A minimal effect of GS-9148 on the production of lactate by HepG2 cells was observed. In contrast, treatment with ddI increased lactate production**
- **The present *in vitro* study suggests that similar to tenofovir, GS-9148 and its prodrug GS-9131 have low potential for mitochondrial toxicity**

References

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