1007 Metabolically Stable ω-Functionalized Lipid Prodrugs of Tenofovir Have the Potential to Serve as Safer, Long-Acting Antiretrovirals for the Treatment of HIV.

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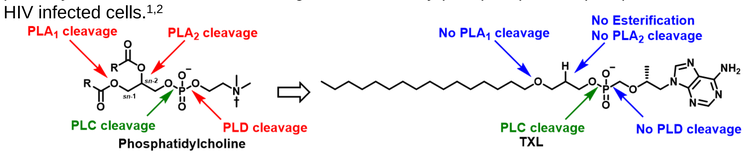
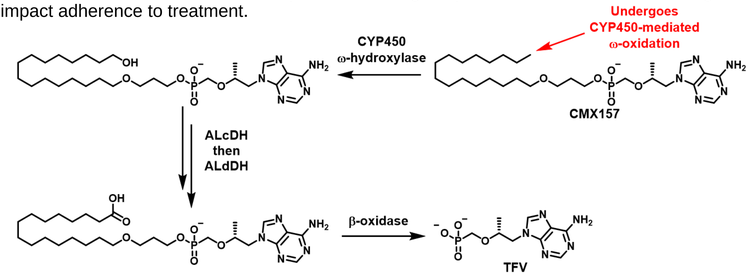
Introduction

Tenofovir (TFV) is an acyclic nucleoside reverse transcriptase inhibitor (NRTI) that forms a major component of most prophylactic and therapeutic regimens for the treatment of HIV-1 world-wide. However, TFV itself demonstrates poor oral bioavailability and cellular uptake due to the presence of an intrinsic phosphonate which is negatively charged at physiological pH. A potent, plasma stable and orally bioavailable prodrug of TFV, tenofovir exalidex (TXL) (formerly CMX-157) is able to remedy this issue by disguising the charged phosphonate as a lysophospholipid mimic (shown in the figure below). Moreover, TXL is designed to target natural lipid uptake pathways and an intracellular cleavage mechanism by phospholipase C (PLC) to deliver TFV to HIV infected cells.[1,2]

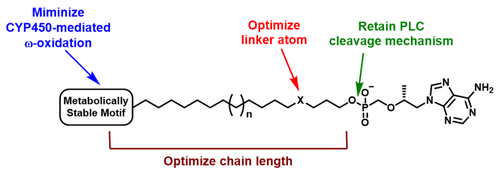


However, like tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF), which are phosphonate diester and phosphoramidate prodrugs of TFV, respectively, the utility of TXL is limited due to considerable metabolism by the liver, namely by CYP450-mediated ω-oxidation at the terminus of the lipid prodrug, as illustrated below.[3] This undesired metabolism by the liver not only compromises the amount of prodrug available to access HIV infected cells, but also, due to chronic use, increases the potential for organ-specific toxicities which can negatively impact adherence to treatment.



Objective

To overcome the limitations exhibited by TXL, TAF and TDF, a series of TXL lipid analogues were designed to mitigate undesired CYP450-mediated ω-oxidation by replacing the labile terminal methyl group with various structural motifs with diminished sensitivity to hepatic metabolism (illustrated in the figure below). It was hypothesized that the introduction of these metabolically stable motifs would, in turn, minimize TFV-associated toxicities of the bone, kidney and liver. In addition, these prodrugs have the potential to improve the efficacy of TFV-based regimens by directing a larger fraction of the administered dose to HIV-infected cells. Herein, we describe the pharmacological evaluation of these novel TXL analogues featuring variable lipid chain lengths, linker atoms and terminal motifs. Several of these analogues demonstrated improved human liver microsomal (HLM) stability *in vitro* and enhanced pharmacokinetic properties *in vivo*, relative to TXL.



References

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2. Painter, R.; Trost, C.; Lampert, M.; Almond, R.; Buller, M.; Kern, E.; Painter, G.; Robertson, A.; Frazer, N.; Mahony, R., *Drugs of the Future* **2008,** *33*, 655.
3. Painter, G. R.; Almond, M. R.; Trost, L. C.; Lampert, B. M.; Neyts, J.; De Clercq, E.; Korba, B. E.; Aldern, K. A.; Beadle, J. R.; Hostetler, K. Y., *Antimicrob Agents Chemother* **2007,** *51*, 3505-3509
4. Wan, W. B.; Beadle, J. R.; Hartline, C.; Kern, E. R.; Ciesla, S. L.; Valiaeva, N.; Hostetler, K. Y., *Antimicrob Agents Chemother* **2005,** *49*, 656-662.
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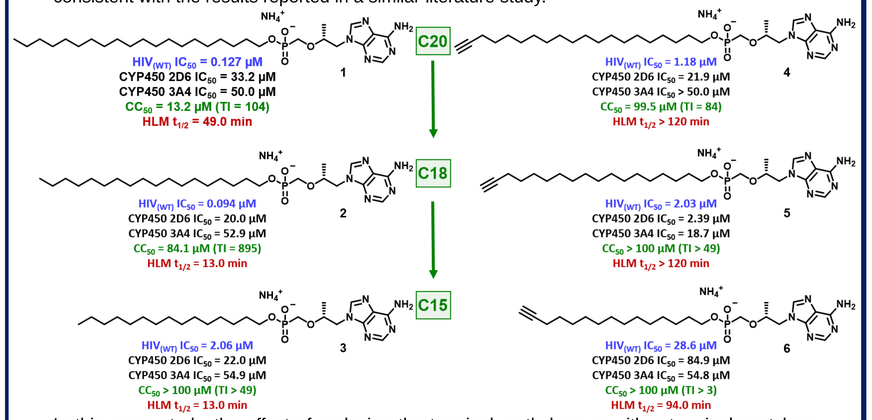
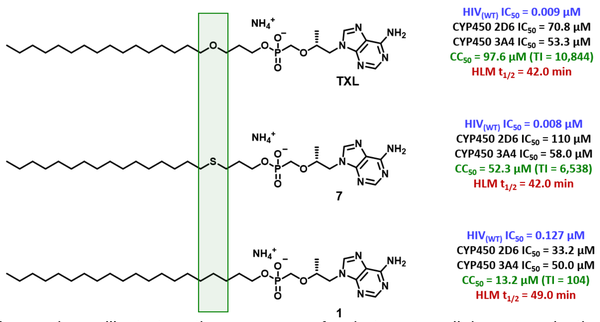
SAR on Chain Length

The figure below illustrates the results of a structure-activity-relationship (SAR) study to determine the effect of lipid chain length on HIV antiviral activity and metabolic stability in HLMs. Overall, TXL analogues featuring a lipid chain of 20 atoms in length exhibited greater efficacy relative to TXL analogues featuring shorter lipid chains of 15 atoms in length. This trend is consistent with the results reported in a similar literature study.[4]



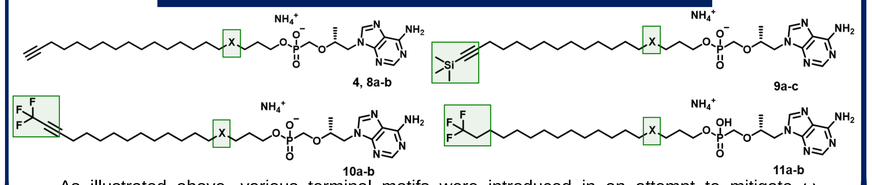
In this same study, the effect of replacing the terminal methyl group with a terminal acetylene was explored. It was hypothesized that the terminal acetylene would demonstrate greater HLM stability due to the higher C-H bond dissociation energy (133.3 kcal/mol) of acetylene relative to that of ethane (101.1 kcal/mol).[5] As anticipated, acetylene compounds **4** - **6** demonstrated improved half-life ($t_{1/2}$) relative to terminal methyl analogues **1** - **3**. However, the terminal acetylene was also found to have deleterious effects on antiviral activity.

SAR on Linker Atom



As the figure above illustrates, the presence of a heteroatom linker atom in the lipid chain significantly improved the efficacy of these analogues when assessed in an HIV whole cell assay. However, the nature of the linker atom did not have any obvious effect on the metabolic stability of these analogues.

SAR on Terminal Motif



As illustrated above, various terminal motifs were introduced in an attempt to mitigate ω-oxidation. Overall, terminal motifs reported herein demonstrated improved HLM stability with a $t_{1/2} \geq 120$ minutes (**Table 1**). Moreover, analogues featuring TMS and CF_3 motifs not only improved HLM stability but also retained potent antiviral activity. In addition, the inclusion of a heteroatom linker further boosted antiviral activity for almost all TXL analogues.

Table 1. Summary of SAR Exploring TXL Analogues with Variable Terminal Motifs and Linker Atom

Compound	X	HIV IC_{50} (μM)[a]	CYP450 IC_{50} (μM)[a]		CC_{50} (μM)[a]	Therapeutic Index[c]	HLM $t_{1/2}$ (min)[a]
			2D6	3A4			
4	CH_2	1.18	21.9	>50	99.5	84	> 120
8a	O	0.132	>20	>20	>100	> 758	>120
8b	S	0.028	*ND*	*ND*	>100	> 3,570	96
9a	CH_2	0.086	27.1	27.0	68.1	792	>120
9b	O	0.069[b]	21.7	>20	>100	>1,450	>120
9c	S	0.024	*ND*	*ND*	>100	>4,170	>120
10a	CH_2	0.186	>20	>20	78.7	423	>120
10b	O	0.023[b]	>20	12.0	>100	> 4,350	>120
11a	CH_2	0.120	>20	>20	72.4	603	>120
11b	O	0.049[b]	>20	>20	>100	>2,040	>120

[a]n = 2. [b]n = 4. [c]Therapeutic index = CC_{50} / HIV IC_{50}.

TXL and analogues **9b**, **10b**, and **11b** were selected for further evaluation in an *in vivo* mouse PK study. Male C57BL/6 mice (n = 3 per time point) were administered a single oral dose (10 mg/kg) of TFV prodrug using 90:10 olive oil: EtOH as a vehicle. Levels of prodrug and TFV as the metabolite were quantified using LC-MS/MS. The results of this study are shown below.

In Vivo Mouse PK Plasma Results

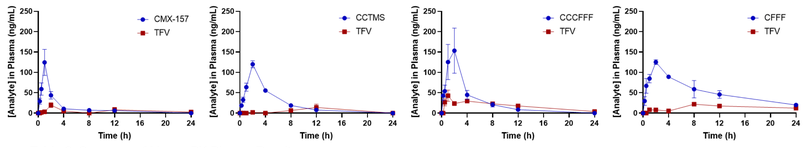


Table 2. Summary of Mouse PK Plasma Results

Parameter	TXL (CMX-157)	9b (CCTMS)	10b (CCCFFF)	11b (CFFF)
T_{max} (h)	1.00	2.00	2.00	2.00
C_{max} (ng/mL)	125	120	153	125
$AUC_{0\text{-}24h}$ (h*ng/mL)	**301**	**550**	**643**	**1280**
$t_{1/2}$ (h)	**2.92**	**2.49**	**2.69**	**8.66**
TFV T_{max} (h)	2.00	12.0	1.00	8.00
TFV C_{max} (ng/mL)	19.6	14.1	42.8	22.1
TFV $AUC_{0\text{-}24h}$ (h*ng/mL)	132	142	425	337
Prodrug $AUC_{0\text{-}24h}$ / TFV $AUC_{0\text{-}24h}$	**2.29**	**3.87**	**1.51**	**3.80**

In mouse plasma, higher systemic exposure levels were observed for compounds **9b**, **10b** and **11b** relative to TXL as demonstrated by the reported AUC values in **Table 2**.

In Vivo Mouse PK Liver Results

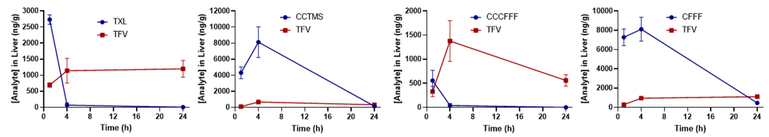


Table 3. Summary of Mouse PK Liver Results

Parameter	TXL (CMX-157)	9b (CCTMS)	10b (CCCFFF)	11b (CFFF)
T_{max} (h)	1.00	4.00	1.00	2.00
C_{max} (ng/mL)	2730	8130	558	8710
$AUC_{0\text{-}24h}$ (h*ng/mL)	**4970**	**101000**	**1310**	**109000**
TFV T_{max} (h)	**17.3**	4.00	4.00	**24.0**
TFV C_{max} (ng/mL)	1460	671	1380	1120
TFV $AUC_{0\text{-}24h}$ (h*ng/mL)	26100	11200	21900	22600
Prodrug $AUC_{0\text{-}24h}$ / TFV $AUC_{0\text{-}24h}$	**0.190**	**9.06**	**0.060**	**4.81**

In mouse liver (**Table 3**), **9b** and **11b** exhibited superior metabolic stability relative to TXL. In the plasma and the liver, relative levels of prodrug to the metabolite TFV, were far superior for **9b** and **11b** relative to TXL and **10b**, which were readily metabolized to TFV.

Conclusion

The *in vitro* and *in vivo* results herein serve as a proof-of-concept that supports the hypothesis that introducing metabolically stable motifs at the terminus of lipid prodrugs of TFV would mitigate undesired metabolism by the liver. Furthermore, the observed enhanced systemic exposure levels of these terminally substituted TXL analogues and their relatively superior stability in mouse plasma and liver indicate that these prodrugs have the potential to act as safer and long-acting therapeutics for the treatment of HIV.

Acknowledgements

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