



Dioxolane -Thymine Nucleoside (DOT) is Active Against a Variety of NRTI Drug Resistant HIV-1 Mutants

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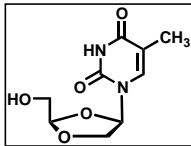
ABSTRACT

Background: The majority of HIV-infected individuals are currently taking nucleosides as part of combination therapy. Since the emergence of nucleoside-resistant HIV-1 mutants is a serious problem for the management of this infection, new drugs that are effective against common mutants are needed

Methods: The finding that DAPD (Amdoxovir) is active against AZT- and 3TC-resistant mutants, prompted the synthesis of several other nucleosides with a dioxolane moiety and their anti-HIV activity against drug sensitive and drug-resistant mutants determined. Furthermore, their molecular mechanisms have also been studied by molecular modeling.

Results: Among the series of dioxolane nucleosides,¹ the thymidine analog, 1-(β-D-dioxolane)thymine (DOT) showed significant and promising anti-HIV activity without cytotoxicity (IC₅₀ > 100 μM, in human PBM cells) against variety of clinically relevant nucleoside-resistant mutants, as shown below. It was found from the molecular modeling studies that the dioxolane moiety plays a significant role in stabilizing the binding between the mutant HIV RT and the nucleoside.

Conclusions: DOT was markedly effective against numerous clinically relevant drug resistant mutants, including virus containing the T69S insert mutation in HIV-RT. Thus, additional biological studies are warranted to determine the full potential of DOT as a potential clinical candidate (Supported by NIH AI32351, AI25899 and Veterans Affairs).



(-)-(2R,4R)-1-(2-Hydroxymethyl-1,3-dioxolan-4-yl)thymine (Dioxolane-Thymine or DOT)

Conformational search of D-dioxolane thymine by Monte Carlo method² in 50,000 steps found the global minimum as 3'-endo sugar conformation, but the 3'-exo conformation was only 0.4 kcal/mol less stable than the global minimum.

Results and Discussion

DOT in WT-HIVRT (Figure 1)

- DOT conformation is 3'-exo, and this conformation is stabilized by the formation of a H-bond between 3'-oxygen and Tyr115.
- Arg72, Asp113, Ala114 and Lys65 hold the triphosphate moiety of DOT in place, by forming a network of H-bonds.
- Dioxolane ring of DOT stacks on top of the Tyr115, thereby providing additional stability.

DOT in K65R-HIVRT Mutant (Figure 2)

- Mutation of Lys65 to Arg65 results in a significant enhancement in the H-bond network around β and γ-phosphates of the triphosphate moiety.
- Additionally, Arg65 stabilizes this geometry by forming another H-bond with the adjacent Asp113 residue.

DOT in L74V-HIVRT Mutant (Figure 3)

- Residue 74 in HIVRT provides additional stabilizing contact to Arg72, which in turn holds β & γ-phosphate of DOT-TP in proper geometry and forms H-bond with base moiety of DOT-TP. Also, the 3'-oxygen atom in the D-dioxolane allows the stabilization of DOT-TP by its interaction with NH backbone of Tyr115 (not shown, in order to maintain clarity).
- Mutation of residue 74 from leucine to valine, doesn't alter the van der Waals interaction between Val74 and Arg72, but it increases the net favorable electrostatic interactions in the triphosphate region, significantly.

Table 1. Biological Activity of DOT in Clinically Relevant Multi-Drug Resistant HIV-1 Strains

HIV-RT	dTTP			DOT		DOT-TP	
	K ₅₀ (μM)	FI	Binding Energy	EC ₅₀ ^a	FI ^a	Binding Energy	ΔE _{tot} ^b
WT	0.80 ^c 4.72 ± 0.83 ^b 6.60 ± 2.10 ^c 0.11 ± 0.01 ^d 4.80 ^e 6.50 ± 0.50 ^f	-	-5366	0.43 ± 0.07	-	-5393	-27
K65R (ddC/ddI/ABC/TFV/DXG)	0.60 ^c 3.06 ± 0.87 ^b 0.085 ± 0.017 ^d	0.75 ^c 0.65 ^b 0.77 ^d	-5449	0.21 ± 0.55	0.5	-5487	-121 (-385)
L74V (ddC/ddI/ABC/TFV/DXG)	2.73 ± 0.40 ^b 0.12 ± 0.02 ^d	0.57 ^c 1.10 ^d	-5375	0.33 ± 0.93	0.8	-5437	-71 (-62)
K103N (NRTI)	2.18 ± 0.52 ^b 4.10 ^c	0.46 ^b 0.85 ^c	-5380	1.40	3.2	-5305	+61 (+75)
M184V (3TC)	0.81 ^c 4.70 ± 0.71 ^b 0.085 ± 0.014 ^d	1.00 ^c 1.00 ^b 0.77 ^d	-5378	0.20 ± 0.16	0.5	-5377	-11 (+1)
T215Y (AZT)	0.093 ± 0.03 ^d	0.84 ^c	-5411	0.27	0.6	-5431	-65 (-20)
K65R/Q151M (DAPD/DXG)	4.40 ± 1.22 ^b	0.90 ^b	-5389	-	-	-5392	-26 (-3)
M184V/T215Y (3TC/AZT)	-	-	-5384	0.23 ± 0.20	0.5	-5414	-48 (-30)
D67N/K70R/T215Y/K219Q (AZT/ddI/ddI)	6.00 ± 1.18 ^b 6.90 ± 2.40 ^c	1.30 ^b 1.10 ^c	-5371	0.49 ± 0.21	1.1	-5363	+3 (+8)
V75I/F77L/F116Y/Q151M (Multi-Drug Resistant)	6.30 ± 1.01 ^c	1.01 ^c	-5402	ND	ND	-5419	-53 (-17)
A62V/V75I/F77L/F116Y/Q151M (Multi-Drug Resistant)	0.16 ± 0.03 ^b 12.0 ± 3.0 ^c	1.4 ^b 1.8 ^c	-5414	ND	ND	-5423	-57 (-9)

^aMiller, M. D.; Lamy, P. D.; Fuller, M. D.; Mulato, A. S.; Margot, N. A.; Cihlar, T.; Cherrington, J. M. *Molecular Pharmacology*, 1998, 54, 291-297

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^gThe EC₅₀ Data is for DOT in PBM cells.

^hΔE_{tot} = Binding Energy (mutant-Mutant) - Binding Energy (WT-WT). Values have been rounded off to remove any decimal places. Values in parenthesis is ΔE_{tot} = Binding Energy (mutant-Mutant) - Binding Energy (dTP-Mutant)

DOT in M184V-HIVRT Mutant (Figure 4)

The natural substrates, 2'-deoxyribonucleoside triphosphates (dNTPs), escape from the steric stress from the bulky side chain of Val184 by virtue of the D-sugar conformation as well as the interaction of its 3'-OH group with Tyr115, which prevents the dNTP's out of the clashing distance from Val184.

As can be seen in figure 4b & 4c, the D-dioxolane sugar moiety of DOT-TP acquires enough distance from Val184 due to the specific interaction of its 3'-oxygen atom with the nearby enzyme residues such as Tyr115 and Arg72.

DOT in T215Y-HIVRT Mutant (Figure 5)

- Threonine to tyrosine mutation at residue 215, causes an indirect effect. The bulky sidechain of tyrosine fills up the empty gap which is otherwise present in Arg72 (Figure 5c & 5d).
- It makes favorable van der Waals contacts with Asp113 and Ala114 residues.

DOT in D67N-K70R-T215Y-K219Q-HIVRT Mutant (Figure 6)

- Asp67, Pro217 and Lys219 forms a network of H-bonds in WT-HIVRT (Figure 6c).
- In the case of D67N-K70R-T215Y-K219Q-HIVRT this network is broken, and the Gln219 sidechain points inwards towards the triphosphate region. This results in the formation of a new set of H-bonds and increase in electrostatic energy, but is also accompanied by slight steric clashes between lys65 and β-phosphate (Figure 6b).
- This steric clash is also seen between Arg70 and Gln219.

DOT in V75I/F77L/F116Y/Q151M-HIVRT Mutant (Figure 7)

- F116Y and Q151M play the major role in this mutation pattern. F116Y mutation results in the stabilization of the binding pocket geometry by the formation of H-bond between the Tyr116 and backbone amide of Lys73.
- Q151M mutation results in the better packing inside the binding pocket, especially in the case of DOT (Figure 7a & 7b).
- The reasons for the effect of V75I and F77L mutations are not clear but since these mutations are located in the template strand binding region, it may be possible that it can have some effect on the polymerization rate of the mutant polymerase.

DOT in A62V/V75I/F77L/F116Y/Q151M-HIVRT Mutant (Figure 8)

- The only difference between A62V/V75I/F77L/F116Y/Q151M-HIVRT mutant and V75I/F77L/F116Y/Q151M-HIVRT mutant is the addition of A62V in the former mutation pattern. The binding mode of DOT-TP and dTTP doesn't vary significantly from that observed in V75I/F77L/F116Y/Q151M-HIVRT mutant.
- Arg72 plays a very vital role in maintaining the binding geometry of incoming nucleotide by forming H-bonds with β- and γ-phosphate (Figure 1). Val62 doesn't directly interact with the incoming nucleotide but it plays the role of stabilizing the conformation Arg72. Since, Val62 is located right adjacent to the Arg72 so it stacks well to the Arg72 and holds it in the right conformation (Figure 8).

Preliminary Pharmacokinetic Data

Preliminary pharmacokinetic studies showed that bioavailability of DOT in rat and rhesus monkeys is 100%. Detail pharmacokinetic studies are in progress.

Figure 1. Binding Mode of DOT-TP in WT-HIVRT

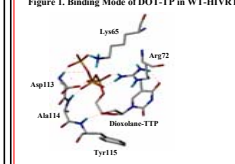


Figure 4. Binding Mode of DOT-TP and 3TC-TP in WT-HIVRT and M184V-HIVRT Mutant

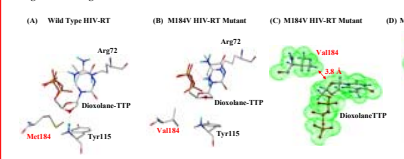


Figure 2. Binding Mode of DOT-TP in WT-HIVRT and K65R-HIVRT Mutant

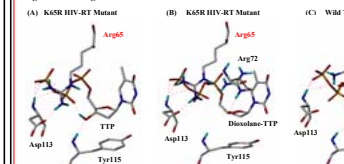


Figure 5. Binding Mode of DOT-TP in WT-HIVRT and T215Y-HIVRT Mutant

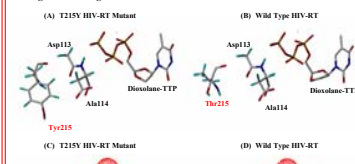


Figure 3. Binding Mode of DOT-TP in L74V-HIVRT Mutant

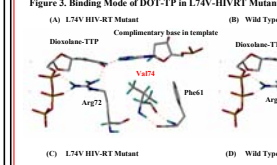


Figure 6. Binding Mode of DOT-TP in WT-HIVRT & D67N-K70R-T215Y-K219Q HIVRT Mutant

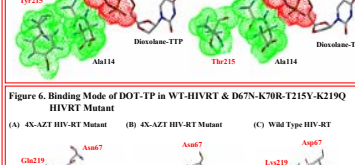


Figure 7. Binding Mode of DOT-TP in WT-HIVRT & V75I-F77L-F116Y-Q151M HIVRT Mutant

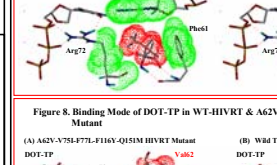
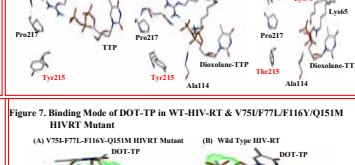


Figure 8. Binding Mode of DOT-TP in WT-HIVRT & A62V/V75I-F77L-F116Y-Q151M HIVRT Mutant



Summary

DOT is the first thymidine kinase activated nucleoside that is significantly active against all of the commonly found NRTI-resistant HIV-1 mutants, including K65R and M184V. The main reason for this interesting antiviral activity profile is the presence of dioxolane ring. The 3'-oxygen atom in the D-dioxolane allows the stabilization of DOT-TP by its interaction with either Arg72 or NH backbone of Tyr115. In addition, 3'-oxygen atom is also capable of having favorable electrostatic interaction with other enzyme residues to mimic the 3'-OH of dNTPs. Preliminary pharmacokinetic data showed that bioavailability of DOT is 100% in rat and rhesus monkey studies. In light of these findings, additional biological studies are warranted to determine the full potential of DOT as an anti-HIV agent.

References

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