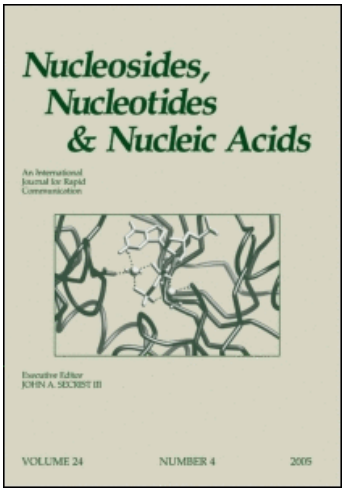

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BORANO-NUCLEOTIDES: NEW ANALOGUES TO CIRCUMVENT HIV-1 RT-MEDIATED NUCLEOSIDE DRUG-RESISTANCE

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▫ *α-Boranophosphates suppress RT-mediated resistance when the catalytic rate of incorporation (k_{pol}) of the analogue 5′-triphosphate is responsable for drug resistance, such as in the case of K65R mutant and ddNTPs, and Q151M toward AZTTP and ddNTPs. This suppression is also observed with BH_3-d4T and BH_3-3TC toward their clinically relevant mutants Q151M and M184V. Moreover, the presence of the borano (BH_3^-) group renders the incorporation of the analogue independent from amino-acid substitutions in RT. To our knowledge, this is the first example of rescue of polymerase activity by means of a nucleotide analogue.*

INTRODUCTION

Nucleoside analogues (ddN, AZT, d4T, or 3TC) have been extensively used as antiviral drugs targeting HIV reverse transcriptase (RT).[1] Each of 5′-triphosphate analogue compete for DNA incorporation with its natural counterpart. The lack of a 3′ hydroxyl group promote viral DNA chain termination. However, under therapeutic pressure, the viral RT gene mutates and specifies enzymes bearing substitutions responsible for the loss of drug efficacy. Resistance-associated mutations map at the RT active site and resistance mechanisms are now well characterized.[2,3] Three major mechanisms have been described: repair of the analogue-terminated DNA, discrimination of the analogue by a decreased affinity

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(K_d) for RT, or decreased polymerization rate (k_{pol}).[4] One strategy to circumvent resistance is to develop nucleoside analogues that are still potent against mutant RTs.

α-Boranophosphate nucleosides (α-BH_3-dNTPs) are nucleotide analogues on which one non-bridging oxygen on the α phosphate is replaced by a borano BH_3^- group.[5,6] Here, we summarize results obtained with BH_3-nucleotide analogues as inhibitors of HIV-1 RT, emphasizing their capacity to overcome RT-mediated drug resistance.[4,7–10]

RESULTS AND DISCUSSION

(Rp)-α-BH_3-AZT and (Rp)-α-BH_3-d4T act as DNA chain terminators for RT.[7] The BH_3^- group improves both phosphorylation by nucleotide diphosphate kinase and chain termination efficiency.[7] We investigated whether α-boranophosphate nucleosides have the capability to affect nucleotide drug resistance through their affinity (K_d) or catalytic rate of incorporation (k_{pol}). We have extented our studies to other interesting available α-boranophosphate nucleosides.

We evaluated the capability of resistance suppression of α-BH_3-ddATP and α-BH_3-AZTTP towards $Q151M_{complex}$ RT,[8] and the α-BH_3-ddATP toward K65R RT.[9] We also evaluated the capability of resistance suppression of α-BH_3-d4TTP and α-BH_3-3TCTP toward Q151M and M184V RTs, respectively (Table 1).[10]

Incorporation of these analogues into DNA by purified RTs were measured comparatively using pre-steady-state kinetics. The nucleotide affinity K_d is calculated as the nucleotide concentration giving half of the maximum incorporation rate k_{pol}. The incorporation efficiency of the nucleotide into DNA (k_{pol}/K_d) is used to calculate the selectivity factor: $(k_{pol}/K_d)_{dNTP}/(k_{pol}/K_d)_{analogue}$. A selectivity factor greater than one means that the enzyme discriminates the analogue over the natural nucleotide. Finally, the resistance of RT to the inhibitor is the ratio between the selectivity of the mutant over the selectivity of the wild-type enzyme.

Data presented in Table 1 indicate that the impact of the BH_3 modification is larger on k_{pol} than on K_d. Using $Q151M_{complex}$ RT, k_{pol} increases up to 70- and 13-fold using α-BH_3-ddATP and α-BH_3-AZTTP, respectively, but K_d remains unchanged and suppression of resistance is observed. α-BH_3-ddATP is a 2-fold better substrate than dATP and inhibits DNA synthesis by K65R RT 153-fold better than ddATP. This complete suppression of resistance is due to an important increase of the catalytic rate constant k_{pol} (20-fold) associated with a better binding affinity K_d (7-fold). α-BH_3-d4TTP is well incorporated (high k_{pol} values) regardless of the enzyme used and there is no discrimination of α-BH_3-d4TTP relative to dTTP. As a consequence, there is no resistance arising from the $Q151M_{complex}$ RT toward borano-phosphate analogues. Similarly, there is a limited resistance to α-BH_3-3TCTP by K65R and M184V. Is it quite remarkable that the incorporation rate of α-BH_3-3TCTP is > 11-fold higher than that of 3TCTP in the case of wild-type RT. The presence of the BH_3^- group on 3TCTP compensates the initial

TABLE 1 Pre-Steady State Kinetic Constants of dATP/ddATP/BH_3-ddATP/dTTP/AZTTP/BH_3-AZTTP Incorporation by WT, $Q151M_{complex}$ and K65R Mutant RTs, of dTTP/d4TTP/BH_3-d4TTP Incorporation by WT, Q151M and $Q151M_{complex}$ RT mutants, of dCTP/3TCTP/BH_3-3TCTP Incorporation by WT, M184V and K65R/M184V RT Mutants

RT	Nucleotide	Kd (μM)	Kpol (s^{-1})	Kpol/Kd	Selectivity[f]	Resistance[g]
WT	dATP[a]	7.5	50	6.7	7.4	
	ddATP[b,h]	8.0	7.2	0.91		
	BH_3-ddATP[a]	29.9	22.9	0.75	8.94	
$Q151M_{complex}$	dATP[b]	41	99	2.3	63.8	8.62
	ddATP[b]	11	0.38	0.036		
	BH_3-ddATP[b]	7.6	28	3.7	0.62	0.084
WT	dTTP[a]	17	13	0.75	0.41	
	AZTTP[b]	7.1	13	1.8		
	BH_3-AZTTP[e]	7.6	18.4	2.4	0.31	
$Q151M_{complex}$	dTTP[b]	9.7	7.6	0.79	7.18	17.51
	AZTP[b]	9.9	1.1	0.11		
	BH_3-AZTP[b]	13	14	1.1	0.72	1.76
WT	dATP[a]	7.47	50.16	6.71	34.95	
	ddATP[a,h]	33.8	6.49	0.192		
	BH_3-ddATP[a]	29.9	22.9	0.75	8.94	
K65R	dATP[a]	6.89	11.63	1.69	112.7	3.2
	ddATP[a]	47.54	0.71	0.015		
	BH_3-ddATP[a]	6.5	14.9	2.3	0.73	0.0816
WT	dTTP[a]	17	13	0.75	1.5	
	d4TTP[d]	21	11	0.51		
	BH_3-d4TTP[d]	19	16	0.85	0.88	
Q151M	dTTP[b]	14	17	1.2	4.1	2.8
	d4TTP[d]	23	6.7	0.29		
	BH_3-d4TTP[d]	14	20	1.4	0.86	1.0
$Q151M_{complex}$	dTTP[b]	9.7	7.6	0.79	7.0	4.7
	d4TTP[d]	19	2.2	0.12		
	BH_3-d4TTP[d]	14	10	0.77	1.0	1.2
WT	dCTP[b]	7.9	7.3	0.93	50	
	3TCTP[c]	2.5	0.047	0.019		
	BH_3-3TCTP[d]	4.1	0.54	0.13	7	
M184V	dCTP[c]	21	9.5	0.45	1500	30
	3TCTP[c]	88	0.026	0.0003		
	BH_3-3TCTP[d]	46	0.36	0.0078	58	8.3
K65R/M184V	dCTP[c]	19	5.7	0.3	8900	180
	3TCTP[c]	89	0.003	0.000034		
	BH_3-3TCTP[d]	82	0.49	0.006	50	6.8

[a]Values from,[4] [b]from,[8] [c]from,[9] [d]from,[10] [e]from,[7] [f]Selectivity = $[k_{pol}/K_d$ (nucleotide)$]/[k_{pol}/K_d$ (analogue)], [g]Resistance = $selectivity_{mutant}/selectivity_{WTRT}$), [h]$K_d$ values for ddATP differ for these two sets of experiments because the assay conditions are different. When slow incorporation rates are measured, an excess of enzyme is required to avoid the influence of the off-rate due to multiple turnovers. The latter gives artificially high K_d values such as for ddATP in ref [4]. However, In ref. [4] artificially high K_d(ddATP) values were measured for both WT and K65R, and thus, the general conclusion regarding the cause of resistance is still valid. In subsequent works, this artifact was eliminated using a 2-fold excess of RT over primer/template.[8-10]

discrimination by K65R and M184V, mostly through an increase of the catalytic rate k_{pol}. Overall, resistance due to M184V or K65R/M184V is 2.5- to 25-fold reduced when α-BH_3-3TCTP replaces 3TCTP.

Until a counter-example is found, the borano modification acts as a general suppressor of resistance whenever k_{pol} promotes resistance. Indeed, the presence of the BH_3^- group does not influence K_d, but provides (or restores) a high k_{pol} value specifically. The α-boranophosphate group at the RT active site renders incorporation independent from the nature of catalytic amino acid side chains.[10]

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