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PREPARATION AND ANTI-HIV ACTIVITY OF N-3 AMINO SUBSTITUTED THYMIDINE NUCLEOSIDE ANALOGS

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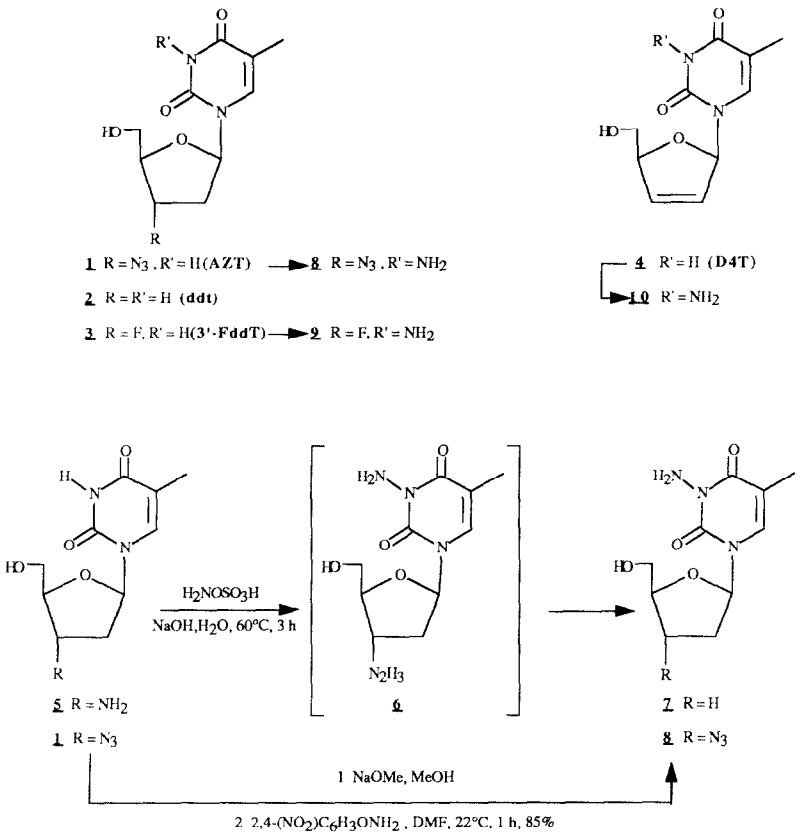
Abstract: *The N-3 amino derivatives* ***7-10*** *of ddT, AZT, 3'-FddT, and D4T were prepared by electrophilic amination of the parent compounds. Although compounds* ***7***, ***9***, *and* ***10*** *were essentially inactive, N-3 amino AZT* ***8*** *(RP67042) maintained activity and displayed lower toxicity and a longer plasmatic halflife compared to AZT..*

A wide range of 2',3'-dideoxynucleoside analogs have been prepared.[1] in the search for new, long acting, highly selective, and non toxic anti-HIV agents to replace AZT.[2] As an outcome essentially four modifications of the sugar component, as in AZT **1**, ddT **2**, 3'-FddT **3**, and D4T **4**, result in potent anti-HIV activity.[2,3,4,5] Alterations of the pyrimidine and purine base component have also been studied. However, the influence of substitution at the N-3 position of the thymine base on HIV replication has not been evaluated, which may not seem suprizing in view of the importance of this position in base pairing to adenosine in DNA.[6] In this communication we report the preparation of the N-3 amino derivatives **7-10** of ddT, AZT, 3'-FddT, and D4T, and describe the anti-HIV activity determined for N-3 amino AZT **8** (RP67042).[7]

This study was initiated by the observation that reaction of 3'-amino-3'-deoxythymidine **5** with hydroxylamine-O-sulfonic acid (HOSA) led to direct formation of the N-3 amino derivative **7** of ddt (37%).[8,9] It is probable that both N-3 amination, and deamination of the C-3' amino substituent are a consequence of having to employ an excess of HOSA and highly basic conditions in order to detect any reaction of **5**. For the latter process it has been suggested that, in the presence of excess base, HOSA is converted to a nitrene which "oxidizes" the initially formed hydrazine **6** to an unstable diazene that decomposes to give **7** and nitrogen.[10] Under these conditions amination at the N-3 position of AZT was also observed. However, higher yields (85%) of **8** were obtained by reacting the N-3 anion of **1** with O-(2,4-dinitrophenyl)hydroxylamine



(DNPA) in DMF at room temperature.[9,11] In the same way 3'-FddT, and D4T were converted in >80% yields to their corresponding N-3 amino derivatives **9** and **10**, respectively. As expected only minor alterations were observed in the ^{1}H and ^{13}C NMR spectra of the N-3 aminated products relative to the parent compounds. The occurence of a strong parent ion and fragments for N-3-aminothymine + 2H (m/z 142) and the sugar component were characteristic in the CI mass spectra for this series of analogs.

Biological Results and Discussion

The anti-HIV activity of compounds **7-10** were studied *in vitro* against the LAV_{Bru}[12] strain of HIV-1 in CEM c113 cells. The 3-amino analogue **7** ddT was inactive at doses up to 100 μM, and compound **9**, 3-NH_2- 3'-FddT, proved to be highly cytotoxic, killing the cells at doses as low as 0.1 μM. Analog **10** displayed a cytotoxicity similar to D4T, but provided only partial protection (40%) against HIV induced

cytopathicity at 1 μM. In contrast, the corresponding AZT derivative **8** (RP67042) displayed a clear inhibitory effect with an EC_{50} of 0.03 μM for reduction of HIV-induced cytopathic effect in CEM cells 7 days after infection, as measured by the MTT method. This indicates that compound **8** has a large selectivity index (SI = IC_{50}/EC_{50} = 2,500) comparable to that of AZT (SI = 1,666). On the other hand, under the same conditions an EC_{50} of 0.01 μM for effective inhibition of reverse transcriptase production in CEM supernatant corresponds to an SI of 750 (for AZT; SI = 1000). Viral replication was evaluated measuring both the HIV-induced cytopathic effect and the production of reverse transcriptase (RT) in culture supernatants. The cellular toxicities were evaluated by running parallel experiments in uninfected cultures with the same concentrations of compound. Essentially the same activities were observed in U937 and MT4 cells and these activities were maintained in 14 day incubation experiments.

In order to further explore the potential interest of RP67042, its anti-HIV potency was compared to AZTs' in PBMC. The effective concentration inducing 90% inhibition of virus replication (EC_{90}) as the amount of p24 antigen and of RT activity in cell supernatants was found to be 2.5 μM for RP 67042 versus 35 nM for AZT at 14 day post infection. As observed in the CEM cell line, the toxicity of RP67042 for PBMC growth was reduced compared to the AZT impact (EC_{50} superior to 100 μM and 25 μM respectively).

In view of the reduced toxicity of RP67042 compared to AZT in several cell systems, and the fact that one of the major drawbacks of AZT for AIDS patients is a pronounced bone marrow toxicity and anemia[13], a comparative study of the effect of both compounds in the *in vitro* human bone marrow progenitor cell culture assay (colony-forming unit of granulocyte-macrophage (CFU-GM) and burst forming unit for erythrocyte (BFU-E) progenitors) was undertaken.[14] The results displayed in Table I illustrate the bone marrow toxicity of the two tested compounds against cells from two different donors (three cultures for each donor and for each concentration of drug). Taken together these results allowed us to calculate an IC_{50} of 150 μM and 300 μM for RP67042 and an IC_{50} of 1 μM and 7 μM for AZT in CFU-GM and BFU-E respectively. If the selectivity index for both compounds is calculated as the ratio of IC_{50} for CFU-GM or BFU-E, versus the EC_{90} in CEM the values 60 and 120 respectiviely were obtained for RP67042 and 27 and 194 for AZT. These values suggest a distinct advantage for RP67042 over AZT for the problem of bone marrow supression.

A comparison of the anti-viral activities and pharmacokinetics of RP67042 and AZT in mice was also made. Initially, the pharmacological activities of both compounds

Table I : Bone marrow cell toxicity of AZT (**1**) and RP 67042 (**8**)

Compound	Concentration mM	CFU-GM Exp.1	CFU-GM Exp.2	BFU-E Exp.1	BFU-E Exp.2
Control		39 ± 8.7[(a)]	13 ±1[(a)]	131 ±39[(a)]	243 ± 25[(a)]
1	0.3		10.7 ± 1.5		143 ± 8
	1	33.4 ± 5	4.7 ± 2.1	120 ± 15	132 ± 6
	3	18.7± 4.7	4.5 ± 3.6	88 ± 13	79 ± 11
	10	4.3 ± 2.9	1 ± 1	76 ± 4	79 ±27
	30		0	46 ±8	59 ± 1
	100	1 ± 1		4 ± 3	
8	0.036	48.3 ± 13.6		94 ± 15	10
	0.36	31.3 ± 11		142 ± 12	
	1		12.3 ± 2.9		249 ± 20
	3.6/3	31.3 ± 14.6	14.0 ± 2.8	140 ± 8	218 ± 29
	10		10.0 ± 2		170 ± 11
	36/30	28.3 ± 9.3	11.3 ± 1.5	98 ± 19	198 ±14
	100		9.3 ± 2.5		158 ± 27
	300		0.3 ± 0.6		117 ± 18
	360	0.7 ± 2		111 ± 23	

[(a)] Number of clones

Table II : Effect of AZT and RP 67042 on F-MuLV induced splenomegaly

Compound	Dose (mg/Kg)	Virus	Spleen Weights (mg)[b]	Inhibition (%)
None		-	107 ±12	
Placebo		-	103 ± 9	
		+	1225 ± 51	0
AZT	40	+	357 ± 27	78
	12	+	669 ± 18	50
	4	+	706 ± 21	46
RP 67042	40	+	607 ± 27	55
	12	+	715 ± 38	46
	4	+	907 ± 50	28

[a] The compounds were administered twice daily at the indicaed dose in mL of PBS.
[b] The values represented the mean of 10 organs.
[c] 0.25 mL administered twice daily

in mice infected with Friend Complex Murine Leukemia Virus (F-MuLV) were investigated. It was found that approximately 25 mg/kg orally of RP67042 were required to reduce spleenomegaly by 50%. The same effect was illicited at 9 mg/kg using AZT. Subsequently, the evolution of the level of RP67042 and AZT in serum after one single oral administration of both drug at 60 mg/kg to mice was analyzed. The compounds were extracted and quantified by HPLC [C18 ultrabase column (5µ, 250 x 4.6 mm), eluant

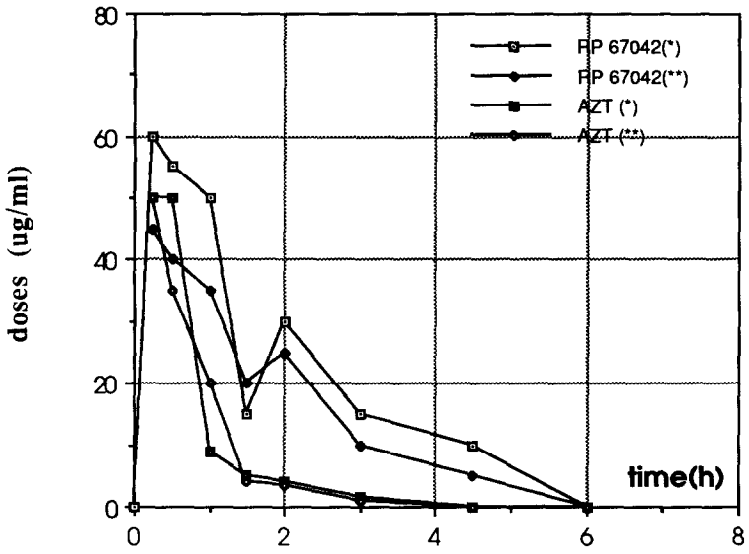


Figure 1

(93.75/6.25 (v/v) 20mM pH 7.0 phosphate buffer-CH_3CN; flow rate = 1 ml/min.]. As shown in figure 1, the peak concentration for both compounds were similar. However, RP67042 displayed an increased plasmatic half life. This characteristic of RP67042 may explain the reduced difference between it and AZT observed in the *in vivo* anti-viral effect. (1/3-1/4 ratio) compared to the 1/100 ratio in the HIV-1 infected PBMC experiment.

In conclusion, 3-amino-3'-azido-3'-deoxythymidine **8** (RP67042) displayed reduced anti-viral activity compared to the parent compound AZT **1** in *in vitro* anti-HIV assay and in *in vivo* anti Fr-MuLV assay. However, this compound possesses the advantage over AZT in that it is potentially less toxic *in vivo*, and has a longer plasmatic half-life in mice.

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