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ARYL PHOSPHATE DERIVATIVES OF AZT INHIBIT HIV REPLICATION IN CELLS WHERE THE NUCLEOSIDE IS POORLY ACTIVE

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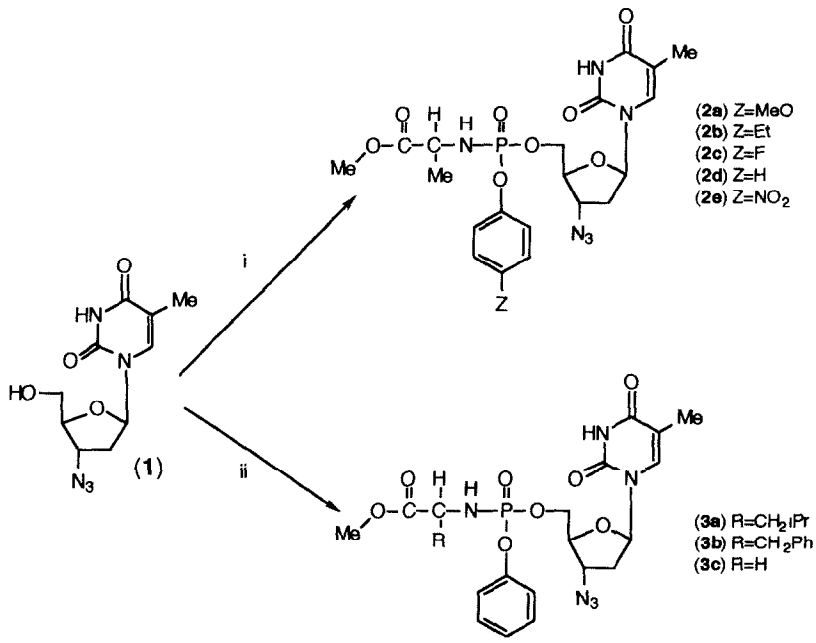
Abstract. Phosphate derivatives of the anti-HIV nucleoside analogue AZT were prepared as potential pro-drugs of the bio-active free nucleotide. In marked contrast to the parent nucleoside AZT, several of the derivatives are active inhibitors of HIV in kinase-deficient cells. The precise activity varies greatly with the phosphate structure, data consistent with a mode of action involving intracellular hydrolysis to release the bio-active nucleotide forms.

Recently, there has been much interest in 2',3'-dideoxynucleosides as inhibitors of HIV-1, the causative agent of AIDS.[1] The dideoxy analogue AZT (**1**) is firmly established as a useful treatment for HIV infection and AIDS.[2] However, it suffers from an absolute dependence on (host cell) kinase-mediated activation, which can lead to poor activity, the emergence of drug resistance, and clinical toxicity.[3] In an effort to circumvent this dependence, we,[4] and others[5] have suggested the use of masked phosphate pro-drugs of the bio-active nucleotide forms of several chemotherapeutic nucleoside analogues. We now report the preparation and biological evaluation of various aryloxy phosphoramidate derivatives of AZT, designed to act as intracellular sources of the free 5'-monophosphate AZTMP. In particular, we report herein that certain derivatives retain activity against HIV in a cell line deficient in thymidine kinase, within which the activity of AZT is extremely poor.[6] It is possible that such cells may be a model for HIV infection of macrophages, also known to be poor in thymidine-phosphorylating capability. If so, enhancement of the activity of AZT therein may be of clinical significance.

The synthetic strategy followed that we have previously developed for alkyl analogues of the present aryl.[7] Thus, p–methoxyphenol reacted with phosphoryl chloride in diethyl ether to give the aryl phosphorodichloridate (85%, δ_P +6). This was allowed to react with alanine methyl ester hydrochloride in dichloromethane in the presence of triethylamine to give p-methoxyphenyl methoxyalaninyl phosphorochloridate (98%, δ_P +9). This reacted with AZT (**1**) in THF in the presence of N-methyl imidazole to give the target compound (2a) in very good yield (Scheme). As

anticipated,[8] this material displayed two closely-spaced signals in the ^{31}P NMR corresponding to the two diastereomers about the phosphate centre (δp ca. +4),[9] and considerable splitting in the H-decoupled ^{13}C spectrum.[10] Similarly prepared were the p-ethyl- (**2b**), and p-fluorophenyl (**2c**) analogues. Similarly, the recently reported[11] phenyl (**2d**) and p-nitrophenyl (**2e**) were prepared for comparative purposes.

Besides studying the effect of substitution in the aryl moiety, it was of interest to probe the effect of modification in the amino acid region. Thus, the parent (phenyl) phosphorodichloridate was allowed to react with the methyl ester hydrochlorides of leucine, phenylalanine and glycine. The corresponding phosphorochloridates were then allowed to react with AZT to give the target series (**3a-c**).



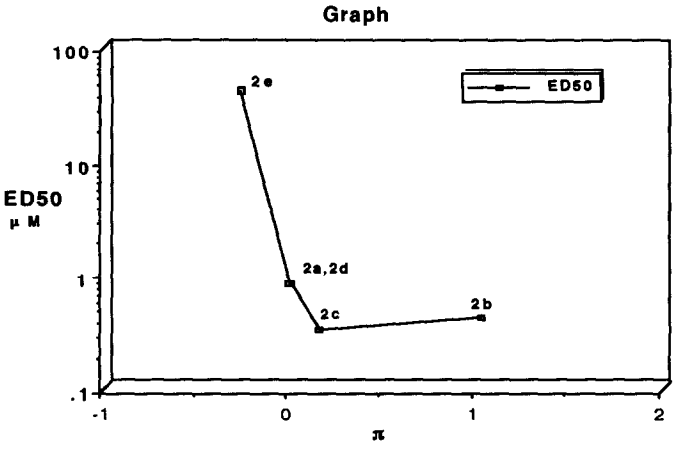
i. [MeOC(O)CHMeNH][p-ZPhO]POCl, THF, N-methylimidazole, 20 °C, 12-24h, 91-96%
ii. [MeOC(O)CHRNH][PhO]POCl, THF, N-methylimidazole, 20 °C, 5-44h, 83-91% (56% for **3b**)

The parent nucleoside (**1**), the alanine compounds (**2a-e**), and the other amino acid derivatives (**3a-c**) were tested for their ability to inhibit the replication of HIV-1 in JM cells, a human T-lymphoblastoid cell line known to be poorly effective at phosphorylating thymidine nucleosides, and in which AZT is relatively inactive.[6] The results are displayed in the Table.[12] It is notable that several of the compounds are potent inhibitors of viral proliferation, in marked contrast to the very low activity of AZT in this cell line. Moreover, the activity of the derivatives varies greatly with variation in the structure. As noted[11] the p-nitro group is highly deleterious to activity in the alanine series;

Table.[12]

Compound	Z	AA	ED50 (μM)	TD50 (μM)
1	-	-	100	>1000
2a	MeO	Ala	0.8	100
2b	Et	Ala	0.4	40
2c	F	Ala	0.32	>200
2d	H	Ala	0.8	500
2e	NO_2	Ala	40	300
3a	H	Leu	8	80
3b	H	Phe	1.6	100
3c	H	Gly	100	1000

comparing (**2d**) to (**2e**). The p-methoxy group has no effect on activity, whilst the p-ethyl and p-fluoro groups both enhance activity 2-3 fold. There appears to be no simple correlation between electron donating or withdrawing properties of the aryl substituent and activity. However, there may be some correlation between activity in this series and the π, hydrophobicity parameter,[13] as shown in the Graph; in general there is increasing activity with increasing lipophilicity.



Lastly, it is notable that changes in the amino acid have a major effect on antiviral activity.[7] Thus, the alanine and phenylalanine compounds (**2d**, **3b**) are of rather similar activity, whilst the leucine compound (**3a**) is rather less active. The glycine compound (**3c**) is rather poorly active, being no more active than the parent nucleoside, AZT (**1**). This observation is entirely consistent with our suggested mode of action,[7,11] which involves P-N cleavage; although the agent catalysing this breakdown remains unclear.

Although the data herein reported are obtained from *in vitro* assay, and as such may be a poor measure of the *in vivo* or clinical properties of such drugs, the greatly enhanced activities in JM cells,

of (**2b-c**) in particular, suggest that such pro-drugs warrant further biological study.

Acknowledgements

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10. Data for (2a): $\delta_P(CDCl_3)$ 4 3, 4.1 (1:2), $\delta_C(CDCl_3)$ (starred peaks are split due to diastereoisomers) 174.1*($\underline{C}O_2Me$),164.0(C2), 157.0(Ph-para), 150.5(C2), 144.0(d, Ph-ipso, J=7Hz), 135.5(C6), 121.1*(m, Ph-ortho), 114.7*(Ph-meta), 111.5(C5), 85.0*(C1'), 82.4*(m, C4'), 65.8*(m, C5'), 60.5*(C3'), 55.8(Ph-OMe), 52.7*(Ala-OMe), 50 4*(Ala-CH*), 37.4(C2'), 21.1(d, Ala-Me, J=5Hz), 12 6*(5-Me); $\delta_H(CDCl_3)$ 9.5(1H, sb, NH), 7.4(1H, s, H6), 6.8-7.2(4H, 2xd, Ph), 6.2(1H, 2xt, H1'), 3.8-4.4(6H, m, H3', H4', H5', Ala-CH*, Ala-NH), 3 8(6H, m, OMe), 2.4(2H, m, H2'), 1.8(3H, s, 5-Me), 1.4(3H, m, Ala-Me); EIMS m/e 539(MH^+, 17%), 370(19), 290(22), 230(35), 154(45), 136(45), 81(C_5H_5O, 100); HPLC retention time 21.99, 22.17 min (1:2) [ACS quaternary system, using an ODS5 column and an eluant of water / acetonitrile, with 82% water 0-10 min, then a linear gradient to 20% water at 30 min, with a flow rate of 2 ml/min and detection by UV at 265 nm].

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12. Z is the para-phenyl substituent, AA is the amino acid code, EC_{50} is the concentration of compound that decreases antigen production in infected cells to 50% of control, and TC_{50} is the concentration of compound which causes 50% cytotoxicity to uninfected cells. Data are shown for HIV-1 infected JM cells. For full details see (11).

13. See for example: Taylor, J.B.; Kennewell, P.D. "Introductory Medicinal Chemistry", Ellis Horwood, Chichester, 1981, Ch. 3, and references cited therein