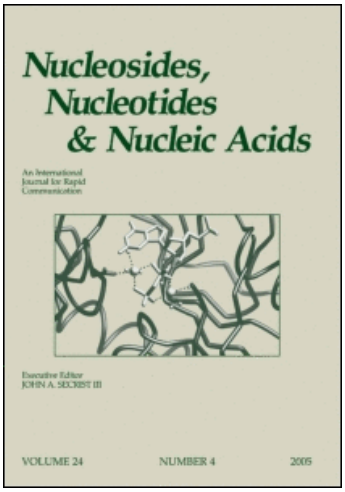

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Synthesis of Bis(Aziridinyl) Phosphinic Amide Derivatives of Thymidine as Potential Anticancer Agents

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SYNTHESIS OF BIS(AZIRIDINYL) PHOSPHINIC AMIDE DERIVATIVES OF THYMIDINE AS POTENTIAL ANTICANCER AGENTS

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Abstract

3'- and 5'-Bis(aziridinyl)phosphinic amide derivatives of thymidine have been synthesized as potential anticancer agents from 3'-amino-3'-deoxythymidine and 5'-amino-5'-deoxythymidine, respectively. The aziridine-containing compounds were tested for their cytotoxic action in vitro against the L1210 leukemia; the 3'-bis(aziridinyl)phosphinic amide derivative was found to be about 11-times more active than its 5'-counterpart in inhibiting the replication of these leukemic cells, with ED_{50} values of 0.6 and 7 μM, respectively, being obtained.

Introduction

A variety of compounds possessing a bis(aziridinyl)phosphinoyl moiety have been synthesized as possible alkylating agents.[1-5] Compounds of this type have been evaluated against both transplanted animal neoplasms[3,6,7] and human tumors,[8-10] and have been shown to have anticancer activity in several experimental systems. Some of these bis(aziridinyl)phosphinoyl compounds appeared to potentiate the effects of x-irradiation against animal and human neoplasms[8,10-14] and, in addition, have been reported to exhibit lower hematological toxicity compared to other kinds of alkylating agents.[6,8-10]

To determine the impact of including a bis(aziridinyl)phosphinoyl group on a thymidine antimetabolite, which could direct the alkylating activity of this moiety to the level of DNA, on cytotoxicity, we have synthesized PP-bis(aziridinyl)phosphinic N-3'-thymidinylamide and PP-bis(aziridinyl)phosphinic N-5'-thymidinylamide and have demonstrated their capacities to inhibit the replication of L1210 leukemia cells in culture.

Chemistry

3'- and 5'-Bis(aziridinyl)phosphinic amide analogues of thymidine (compounds 4 and 7) have been synthesized as potential anticancer agents with alkylating ability (Scheme I). Treatment of 5'-amino-5'-deoxythymidine in DMF with a solution of bis-ethylenimine phosphinic chloride (3) and triethylamine in THF gave the 5'-bis(aziridinyl)phosphinic amide derivative 4. Treatment of 3'-amino-3'-deoxythymidine with bis-ethylenimine phosphinic chloride under the same reaction conditions, however, did not produce the desired product 7. It is conceivable that this was due to the presence of the 3'-amino group in a sterically hindered position in the thymidine molecule such that the electrophilic attack by the relatively bulky bis-ethylenimine phosphinic chloride (3) was prevented. The 3'-bis(aziridinyl)phosphinic amide derivative of thymidine 7 was obtained by reaction of 3'-amino-3'-deoxythymidine (5) with phosphorus oxychloride and triethylamine in THF-DMSO followed by treatment of the resulting dichlorothymidinyl phosphoramide intermediate (6) with ethylenimine. The key starting compounds, 5'-amino-5'-deoxythymidine (2) and 3'-amino-3'-deoxythymidine (5), were fabricated by methodology described previously.[15]

Biological Activity:

Compounds 4 and 7 were evaluated for their cytotoxic potential against cultured L1210 leukemia cells. Both compounds demonstrated significant growth inhibitory activity in this system (Table I). The 3'-bis(aziridinyl)phosphinic amide derivative of thymidine (7) was found to be about 11 times more potent than its 5'-counterpart 4, with estimated ED_{50} values of 0.6 and 7 μM, respectively.

Experimental Section:

Melting points were taken on a Thomas-Hoover Unimelt apparatus and are not corrected. The thin-layer chromatography was performed on EM silica gel 60 F_{254} sheets

Scheme 1

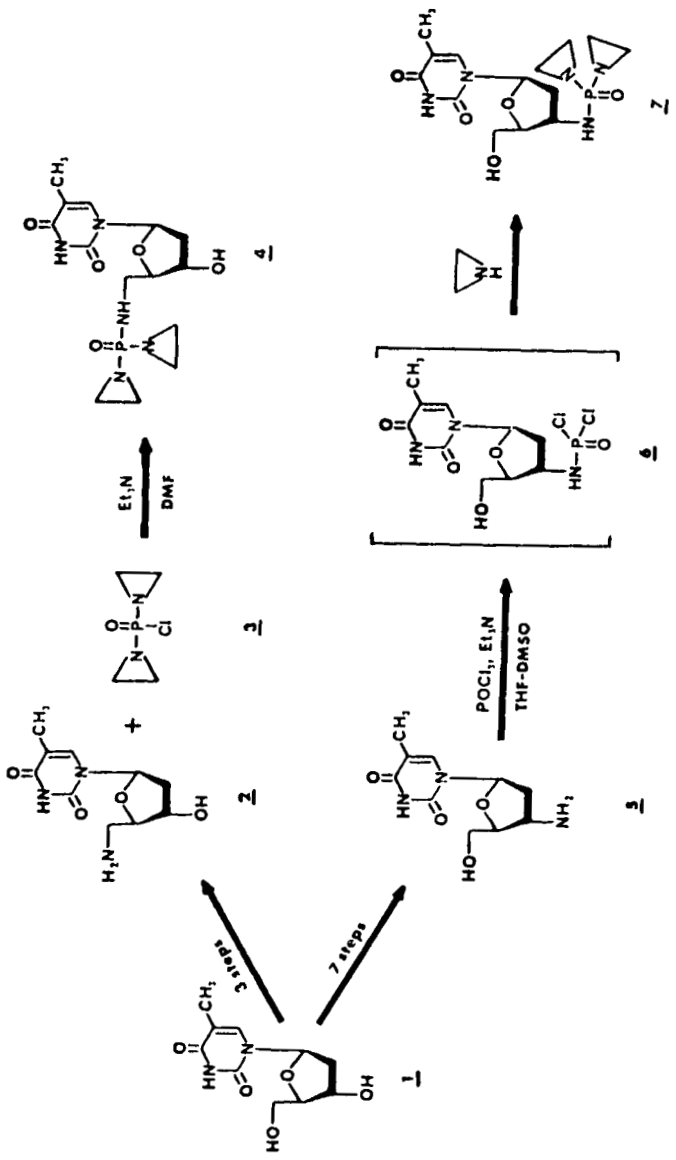

Table I. The effect of 3'- and 5'-bis(aziridinyl)phosphinic amide derivatives of thymidine on the replication of L1210 leukemia cells in vitro. ED_{50} values were estimated from dose-response curves and represent the drug concentration required to inhibit replication of L1210 cells by 50%.

Compound	Concn (μM)	% Inhibition	ED_{50} (μM)
4	1	17	7
	5	33	
	10	65	
	15	82	
7	1	69	0.6
	5	77	
	10	80	
	20	83	

(0.2 mm). NMR spectra were obtained with a Bruker WM500 spectrometer at 500 MHz using Me_4Si as an internal reference. The mass spectra were generated by the Yale University Chemical Instrumentation Center at 70 eV.

PP-Bis(aziridinyl)phosphinic N-5'-thymidinylamide (4).

A solution of ethylenimine (1.72 g, 40 mmol) in 30 mL of tetrahydrofuran (THF) was added slowly over a period of 1.5 h via a dropping funnel to a well-stirred solution of phosphorus oxychloride (1.86 mL, 20 mmol) and triethylamine (9.26 mL, 66 mmol) in 150 mL of tetrahydrofuran chilled to -40°C under nitrogen. The reaction mixture was stirred for an additional 2 h and then filtered to remove the precipitated triethylamine hydrochloride salt. 5'-Amino-5'-deoxythymidine (2, 1.10 g, 4.60 mmol) in 30 mL of DMF was added at a moderate rate from a dropping funnel to the resulting THF solution of bis-ethylenimine phosphinic chloride (3) and triethylamine. The reaction mixture was permitted to warm gradually to 0°C over a period of 1 h and then was stirred at 0°C for another 3 h. The resulting reaction mixture was stored at 4°C overnight and then filtered to remove the insoluble salt. The filtrate was concentrated at reduced pressure and chromatographed through a silica gel column ($CHCl_3$-EtOH, 6:1, v/v) to give 0.75 g (44%)

of a glassy product: NMR (Me_2SO-d_6) δ 1.76 (s, 3H, 5-CH_3), 1.95-2.07 (m, 10H, 2'-H, $\triangleright$N-P(=O)-N$\triangleleft$) 3.50 (m, 2H, 5'-H), 4.20 (m, 1H, 3'-H), 4.52 (br s, 1H, NH), 4.72 (br s, 1H, 3'-OH, D_2O exchangeable), 5.18 (m, 1H, 4'-H), 6.12 (t, 1H, 1-H'), 7.92 (s, 1H, 6-H), 11.2 (br s, 1H, 3-NH, D_2O exchangeable); MS: m/e 372 (M+1), 246 (M-B), 210 (M-$\triangleright$N-P(=O)(NH-)-N$\triangleleft$ -CH_3), 174 (M-B-2 N$\triangleleft$-Oxygen); B=thymin-1-yl. UV(EtOH)λ_{max}266nm (E9,130),λ_{min}236nm. TLC:R_f0.7 ($CHCl_3$-EtOH,1:1,v/v). Anal for $C_{14}H_{22}N_5O_5P \cdot 2DMF \cdot 2.5H_2O$: Calcd C,42.69; H,6.45; N,17.43. Found C,42.60; H,6.74; N,18.09. The NMR spectrum of this compound indicated the presence of 2 moles of DMF based on the integration of the $(CH_3)_2N$-signals in the DMF molecule: 2.74 (s, CH_3-N a or b), 2.90 (s, CH_3-N a or b).

PP-Bis(aziridinyl)phosphinic N-3'-thymidinylamide (7).

A solution of 0.1 mL of phosphorus oxychloride and 0.42 mL of triethylamine in 8 mL of THF was chilled to -40°C and treated over a 1 h period with a solution of 0.24 g (1 mmol) of 3'-amino-3'-deoxythymidine (5) in 6 mL of DMSO. The resulting slurry was gradually warmed to 0°C over a period of 2 h. Ethylenimine (0.5 mL) was added to the solution and the reaction mixture was stored overnight at 4°C and then stirred at room temperature for another 24 h. The solution was filtered and the filtrate concentrated in vacuo. The resulting residue was chromatographed on preparative TLC plates (Analtech, 2 mm) (R_f 0.38, CH_2Cl_2-EtOH, 1:1, v/v) to yield 0.1 g (27%) of a glassy product: NMR (Me_2SO-d_6) δ 1.76 (s, 3H, 5-CH_3), 2.07 (m, 2H 2'-H), 2.50 (m, 8H, $\triangleright$N-P(=O)-N$\triangleleft$), 3.45-3.65 (m, 4H, 3'-, 4'- and 5'-H), 4.35 (br s, 1H, NH, D_2O exchangeable), 5.16 (br s, 1H, 5'-OH, D_2O exchangeable), 6.15 (t, 1H, 1'-H), 7.72 (s, 1H, 6-H); MS: m/e 372 (M+1), 356 (M-CH_3), 225 (M-$\triangleright$N-P(=O)(NH-)-N). UV(EtOH)λ_{max}267nm(E9,311),λ_{min}237nm. Satisfactory elemental analysis could not be obtained due to the hydroscopic and non-crystalline-like properties of this compound. The compound was homogeneous on TLC (R_f 0.38, CH_2Cl_2-EtOH, 1:1, v/v).

Biological Evaluation:

Murine L1210 leukemia cells were maintained in suspension culture in Fischer's medium supplemented with 10% horse serum at 37°C in a humidified atmosphere of 5% CO_2--95% air. Under these conditions, the generation time for L1210 cells was approximately 12 h. Each compound was added to exponentially growing L1210 cells (2 x 10^4 cells/mL) at various concentrations. The increase in cell numbers of cultures not exposed to drugs (controls), as well as those of L1210 cultures supplemented with the test compounds, was determined after 24, 48, and 72 h of cellular growth.

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