New Biologically Active Cyclophosphazene Derivatives

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There has been considerable recent interest in the use of inorganic ring systems as anticancer agents. The study of the aziridino derivatives of hexachlorocyclotriphosphazene, $N_3P_3Cl_6$ [1], and pentachlorothiatriazadiphosphorine 1-oxide, $[(NPCl_2)_2(NSOCl)]$ [2], has been the principal focus of this effort with the fully substituted derivatives, $N_3P_3(NC_2H_4)_6$ [3] and $[NP(NC_2H_4)_2]_2NSONC_2H_4$ [4] being the most active species. Both of these materials appear to act as complex alkylating agents which interact with DNA at both adenine sites and the oxygen atoms of the ribose backbone [5]. Given the excitement which these materials have generated, we have begun to explore the synthesis and biological activity of cyclotriphosphazenes with various alkylating groups as substituents. This communication is concerned with β-haloethylamino derivatives of $N_3P_3Cl_6$.

The series of β-haloethylaminocyclotriphosphazenes is prepared by the reaction of the amine hydrohalide with $N_3P_3Cl_6$ in the presence of two equivalents of triethylamine.

$$N_3P_3Cl_6 + nHX\cdot NH_2CH_2CH_2X + 2n(C_2H_5)_3N \rightarrow$$

$$\rightarrow N_3P_3Cl_{6-n}(NHCH_2CH_2X)_n + 2n(C_2H_5)_3N\cdot HX$$

(X = Cl, Br; n = 1, 2, 4)

The chromatographic separation of isomers is severely limited by the irreversible binding of the products to the silica surface. None of the products could be converted to the corresponding aziridino derivative. The structures of all products were unambiguously established using mass spectrometry and ^{31}P NMR spectroscopy. Details of the synthesis and characterization of these and related compounds will be presented in a forthcoming publication [6].

TABLE I. Activity of the Non-Geminal Rich Mixture, $N_3P_3Cl_4(NHCH_2CH_2Br)_2$ against L1210 Leukemia in Mice.[a]

Dose (mg/Kg)	Mean Survival Time (days)	% T/C[b]
0	8.2	100
25[c]	9	110
50[c]	9	110
100[c]	11	134
250[d]	12.5	152

[a]$6BDF_1$ mice per group were inoculated with 1×10^6 leukemia cells intraperitoneally. Treatment was started 24 hours later (day 1). The phosphazene was administered intraperitoneally as a suspension in corn oil. [b]% T/C = 100 × mean survival time of drug treated animals/mean survival time of control animals that received injections of corn oil vehicle but no drug. [c]Treatment on days 1, 2, 5, 6 and 9 following inoculation of tumor. [d]Treatment on day 1 following inoculation of tumor; two mice died from acute drug toxicity.

Initially, all compounds were screened for antitumor activity against L1210 leukemia cells in culture**. If one employs an $I.D._{50}$ value of 10 μg/ml as an upper limit for activity of interest, the following compounds proved to be inactive: $N_3P_3Cl_5(NHCH_2CH_2X)$, geminal-$N_3P_3Cl_4(NHCH_2CH_2X)_2$ and geminal-$N_3P_3Cl_2(NHCH_2CH_2X)_4$ (X = Cl, Br). The non-geminal bis isomers could not be completely separated from the geminal isomer, however non-geminal (2,4-$N_3P_3Cl_4(NHCH_2CH_2X)_2$) rich samples showed moderate activity: X = Cl, $I.D._{50}$ = 4.8 μg/ml; X = Br, $I.D._{50}$ = 5.2 μg/ml. There were no significant changes in activity when a microsome suspension was added to the cell culture medium.

The non-geminal rich sample was administered to mice bearing the L1210 tumor and the results of

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**L1210 leukemic cells are maintained in a suspension culture consisting of McCoy's 5A medium (GIBCO, Grand Island, N.Y.) supplemented with 10% horse serum, 100 μg/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 10% CO_2/90% air at 37 °C. The drug is added to 4 ml of cell suspension (10^5 L1210 cells/ml) at final drug concentrations of 0.01, 0.1 and 10 μg/ml. After 72 hours of incubation, cell concentration is measured using a Coulter counter model ZB_f (Coulter Electronics, Hialeah, FL) and percent growth inhibition calculated. The $I.D._{50}$ value in these studies refers to the drug concentration required to inhibit cell growth by 50%.

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this experiment are given in Table I. Both the *in vitro* and *in vivo* experiments indicate that the non-geminal $N_3P_3Cl_4(NHCH_2CH_2X)_2$ derivatives exhibit moderate antitumor activity which, at high dose levels, is accompanied by toxic effects. The absence of activity of the mono substituted, compared to the disubstituted derivatives, is common for β-haloethylamine derivatives and suggests that these materials function as bifunctional alkylating agents. The isomeric selectivity of activity is of particular interest in that this degree of subtle structural control has not been previously observed with anticancer agents derived from inorganic ring systems. The origin of this behavior may be related to the proposed bifunctional alkylation mechanism. The distance between the alkylating centers in the geminal isomer may not fit the geometrical requirements for binding to the DNA molecule while the distance in the non-geminal isomer permits interaction with the target nucleic acid. The reason for the lack of activity of the tetra substituted material is less easy to rationalize.

Our studies demonstrate that other alkylating substituents, in addition to the aziridino moiety, are capable of producing biologically active phosphazenes. Of greater interest is the observation that the biological activity can be modified by the geometrical disposition of substituents about the phosphazene ring, thus allowing one to employ the structural complexities of the phosphazenes in the design of new anticancer agent.

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