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Synthesis and Antitumor Evaluation of 4-Ethoxycarbonyl Cyclophosphamide Analogs

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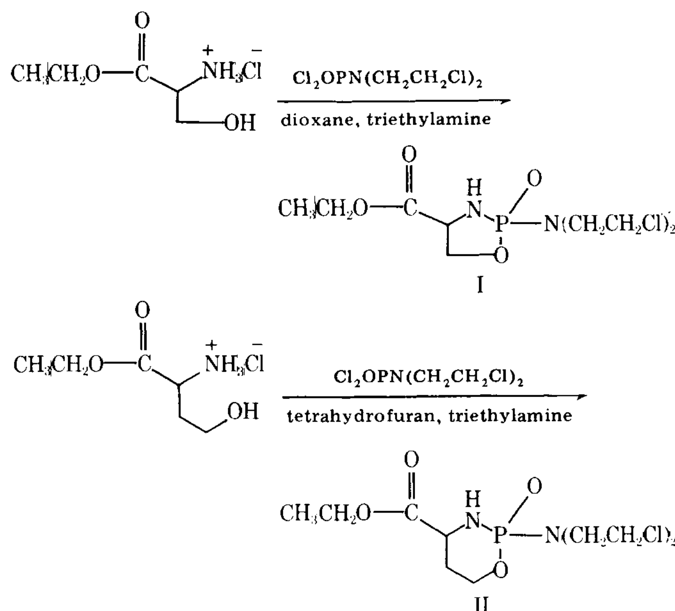
Abstract □ 4-Ethoxycarbonyl analogs of cyclophosphamide and its five-membered ring homolog were synthesized utilizing the cyclization method previously described. *N,N*-Bis(2-chloroethyl)-4-ethoxycarbonyl-1,3,2-oxazaphospholidin-2-amine 2-oxide demonstrated activity against L-1210 lymphoid leukemia whereas *N,N*-bis(2-chloroethyl)-4-(ethoxycarbonyl)tetrahydro-2*H*-1,3,2-oxazaphosphorin-2-amine 2-oxide did not. The oxazaphosphorin-2-amine was evaluated against human epidermoid carcinoma of the nasopharynx (cell culture). The results again were negative: $ED_{50} = 2.8 \times 10$.

Keyphrases □ Cyclophosphamide analogs—synthesized, antitumor activity evaluated □ Antitumor activity—evaluated in cyclophosphamide analogs □ Structure–activity relationships—4-ethoxycarbonyl cyclophosphamide analogs evaluated for antitumor activity

Previous studies demonstrated that cyclophosphamide is an active antitumor agent (1). The active principle responsible for significant inhibitory activity against Yashida sarcoma in rats and L-1210 leukemia in mice is 4-hydroxycyclophosphamide (1, 2). Compounds such as *N,N*-bis(2-chloroethyl) -4- ethoxycarbonyl -1,3,2- oxazaphospholidin-2-amine 2-oxide (I) and *N,N*-bis(2-chloroethyl)-4-(ethoxycarbonyl)tetrahydro -2*H*- 1,3,2-oxazaphosphorin-2-amine 2-oxide (II) were readily synthesized, as described previously (3), by reaction of *N,N*-bis(2-chloroethyl)phosphinamide dichloride (III) with DL-serine ethyl ester hydrochloride and DL-homoserine ethyl ester hydrochloride, respectively, in the presence of triethylamine (Scheme I). All products were isolated as oils. Elemental analysis data of I and II are shown in Table I. The antitumor activity of I and II was evaluated on the basis of survival[1] (Table II).



Scheme I

Table I—Physical Data for the 4-Ethoxycarbonyl Cyclophosphamide Analogs

Compound	Yield, %	Formula		Analysis, % Calc.	Found
I	35.4	$C_9H_{17}Cl_2N_2O_4P$	C	33.87	33.42
			H	5.37	5.43
			Cl	22.71	22.38
			N	8.77	8.41
			P	9.70	9.60
II	23.1	$C_{10}H_{19}Cl_2N_2O_4P$	C	36.05	36.04
			H	5.75	5.21
			Cl	21.28	21.13
			N	8.40	8.68
			P	9.29	9.45

EXPERIMENTAL[2]

Compound I—DL-Serine ethyl ester hydrochloride, 1.25 g (0.002 mole), was dissolved in warm dioxane and 4 ml (0.029 mole) of triethylamine and then added slowly to a solution of 2.43 g (0.009 mole) of III in dioxane while being stirred under a nitrogen atmosphere. The mixture was stirred overnight at room temperature.

The reaction mixture was then filtered, and the solvent was evaporated under reduced pressure. Separation on magnesium silicate[3] gave 1.5 g

[1] National Cancer Institute Drug Research and Development, National Institutes of Health, Bethesda, MD 20014.
[2] IR spectra were run on a Perkin-Elmer 247 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tenn.
[3] Florisil.

Table II—Screening Data Summary

Compound	Dose Number	Dosage, mg/kg	T/C[a], %	NSC Number
Cyclophosphamide	1	500	168	26271
	2	250	310	
	3	135	201	
	4	62.5	144	
I	1	750	129	252152
	2	500	124	
	3	330	123	
	4	250	106	
	5	220	105	
	6	62.5	99	
II	1	500	130[b]	266053
	2	500	118	
	3	500	110	
	4	333	106	
	5	333	111	
	6	250	114	
	7	222	108	
	8	222	111	
	9	62.5	108	

[a] Ratio of median survival time of tested animal to median survival time of control animals; expressed as percent. [b] Could not be confirmed.

(35.4%) of I as an oil. The product was eluted by benzene followed by methanol. It showed IR bands in mineral oil at 5.75, 7.75–8.30, 8.85–9.1, and 13.9 μm.

Compound II—Approximately 3.0 g (0.016 mole) of DL-homoserine ethyl ester hydrochloride and 4.14 g (0.016 mole) of III were suspended in tetrahydrofuran and heated. Triethylamine, 6.60 ml (0.047 mole), in tetrahydrofuran was added slowly. After addition was complete, the reaction mixture was kept overnight at room temperature.

The reaction was worked up as previously described to give 1.26 g (23.1%) of II as an oil. It showed IR bands in mineral oil at 5.75 and 13.9 μm. Other IR bands appeared but were broad and not well resolved.

RESULTS

Compounds I and II showed minimum activity relative to that demonstrated by cyclophosphamide. Generally speaking, an increase in survival of treated animals compared to control animals resulting in a test/control percent value of 125 or more is required to merit further investigation[1]. An attempt to increase the antitumor activity of cyclophosphamide by testing II against L-1210 lymphoid leukemia proved to be negative. The same can be said for I.

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Simple and Rapid High-Pressure Liquid Chromatographic Simultaneous Determination of Aspirin, Salicylic Acid, and Salicyluric Acid in Plasma

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Received April 14, 1977, from the *Department of Pharmacy and the Clinical Pharmacokinetics Laboratory, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60612.* Accepted for publication August 3, 1977.

Abstract ☐ A rapid and sensitive high-pressure liquid chromatographic assay was developed for aspirin, salicylic acid, and salicyluric acid in plasma. The procedure involves the solvent extraction of these compounds from plasma and separation using a reversed-phase column eluted by acidified aqueous acetonitrile. Small quantities of aspirin can be assayed directly in the presence of a large quantity of salicylic acid. The assay is also free from blank interference.

Keyphrases ☐ Aspirin—high-pressure liquid chromatographic analysis in plasma simultaneously with salicylic acid and salicyluric acid ☐ Salicylic acid—high-pressure liquid chromatographic analysis in plasma simultaneously with aspirin and salicyluric acid ☐ Salicyluric acid—high-pressure liquid chromatographic analysis in plasma simultaneously with aspirin and salicylic acid ☐ High-pressure liquid chromatography—analyses, aspirin, salicylic acid, and salicyluric acid simultaneously in plasma ☐ Analgesics—aspirin, salicylic acid, and salicyluric acid, simultaneous high-pressure liquid chromatographic analyses in plasma

Many procedures have been reported for quantifying salicylates in dosage forms and biological media because of the frequent and popular use of these drugs and their various pharmacological properties. The standard colorimetric (1) and fluorometric (2) determinations of aspirin and salicylic acid are not specific and measure aspirin only by difference after hydrolysis to salicylic acid. The differential spectrophotometric assay of the two salicylates based on the pH-dependent shift in their UV absorbances may require corrections because of overlap of their absorption spectra (3). Furthermore, when applied to biological samples, such as plasma, these procedures may yield high and variable blank values.

Several GLC determinations have been reported for aspirin and salicylic acid in dosage forms (4–6) and biological fluids (7–9). These methods are specific and sensitive and permit the assay of both compounds simultaneously without conversion of aspirin to salicylic acid. However, chemical derivatizations, such as silylations, are necessary to make these compounds suitable for GLC (7, 10). The chemical derivatizations are inherently time consuming (requiring up to 60 min) and can be complicated by hydrolysis of aspirin to salicylic acid (10) and multiple-product formation (ester and/or ether) for the latter (7).

TLC (11) and liquid chromatographic (12) methods for salicylates also were reported. Recently, an automated high-pressure liquid chromatographic (HPLC) analysis of aspirin, phenacetin, and caffeine in dosage forms was