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Synthesis and Biological Activity of Potential Antimetabolites

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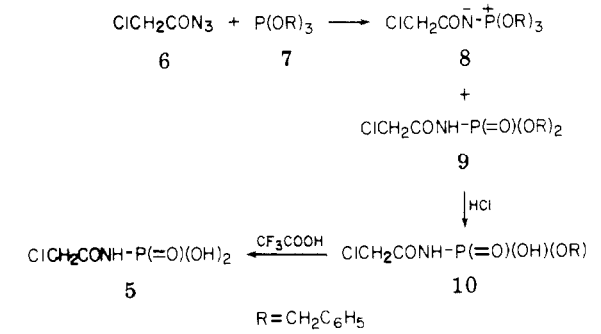
Several known α-amino acid analogues and a new compound, *N*-chloroacetylphosphoramidate, a carbamyl phosphate analogue, were screened as antitumor agents. All gave 50% growth inhibition of cultures of human epidemeroid carcinoma of the nasopharynx at dosage levels of 2–8 μg/ml while showing no activity against L1210 lymphoid leukemia in vivo in BDFi mice.

In view of the potential of compounds containing the alkylating functional group, $-COCH_2Cl$, as antimetabolites, *N*-chloroacetylphosphoramidate (**5**) was prepared as an analogue of carbamyl phosphate, and it and α-amino-chloromethyl ketones (compounds **1–4**) containing this functional group in lieu of the carboxyl of tyrosine,[1] phenylalanine,[2] leucine,[3] and lysine[4] were screened as antitumor agents.

Chemistry. The chloromethyl ketones **1–4** were prepared as referenced above. The monobenzylcarbamyl phosphate analogue **10** was prepared by modification of the reported method for synthesis of diethyl *N*-chloroacetylphosphoramidate.[5] In the synthesis of the diethyl ester of **5**, it was necessary to treat the intermediate triethoxyphosphonium salt (**8** with R = ethyl) with hydrogen chloride to remove one of the ethyl groups. The crude



product from the reaction of **6** with **7** was a mixture of **8**, **9**, and benzyl chloride rather than **8** alone. After treatment of the mixture with hydrogen chloride gas we isolated **10**, which is the monobenzyl ester of **5**. This ester was crystalline and all spectral and analytical data are in agreement with the structure proposed. Since the first benzyl group was so labile, we treated **10** with trifluoracetic acid under mild conditions at 25 °C giving complete removal of the blocking group. The product was isolated as the dilithium salt which had IR and NMR absorptions consistent with the structure even though the analysis for carbon was not quite acceptable.

Biological Results and Discussion. Compounds **1–5** were submitted to Drug Research and Development, National Cancer Institute, and were screened in their routine preliminary program in vitro in cell culture using human epidemeroid carcinoma of the nasopharynx (90 KB system) and in vivo using BDFi mice as the host for L1210 lymphoid leukemia (2 LE system).[6] No activity was noted in the latter assay while all the compounds showed growth inhibition in the in vitro assay at concentrations in the 2–8 μg/ml range. Compounds **1**, **2**, **4**, and **5** are sufficiently active to warrant secondary screening. These systems are routinely employed by the NCI for synthetics and extensive testing has been limited by the present availability of the compounds.

Table I. Antitumor Activity of Potential Antimetabolites[a]

Compd	Structure[b]	ED_{50}, μg/ml[c]
1	R = *p*-OH-C_6H_4	2.1
2	R = C_6H_5	4
3	R = $(CH_3)_2CH$	7.6
4	R = $H_2N(CH_2)_3$	2.7
5	$Li_2(O_3PNHCOCH_2Cl)$	2.5

[a] Compounds were assayed in cell cultures of human epidemeroid carcinoma of the nasopharynx (90 KB system).[6] [b] Compounds 1-4 have the general structure $RCH_2CH(NH_2)COCH_2Cl$, administered as the hydrochloride salts. [c] ED_{50} is the concentration giving 50% growth inhibition compared to untreated controls.

The amino acid analogues **1–4** could function by inhibiting a variety of enzymes that bind the corresponding amino acids as substrates, but irreversible inhibition of transport of essential amino acids in malignant cells could also lead to growth inhibition. The latter mode of action is suggested by other work. Phenylalanine-deficient diets in rats lead to decreased tumorigenesis and to decreased growth rates of already formed tumors.[7] Treatment of murine leukemic (L5178Y) lymphoblasts with phenylalanine ammonia lyase inhibits the growth of these cells also by depriving them of phenylalanine.[8] Growth inhibition of SV40-transformed 3T3 cells by dibutyryl cAMP is accompanied by decreased capability for transport of leucine.[9]

The analogue **5** may be functioning as an antagonist of carbamyl phosphate. The reaction between 5 mM aspartate and 4 mM carbamyl phosphate catalyzed by 0.1 μg/ml of aspartate transcarbamylase from *Escherichia coli* is 60% inhibited by 66 mM **5** while the same concentration of phosphate (a weak competitive inhibitor) gave only 24% inhibition. No irreversible inhibition of this enzyme by **5** was noted even on prolonged incubations in the presence and absence of aspartate, but this does not preclude the possibility of irreversible inhibition by **5** of mammalian enzymes that bind carbamyl phosphate.

Experimental Section

NMR spectra were recorded on a Varian T-60 spectrometer using tetramethylsilane as an internal reference. Infrared spectra were obtained using a Perkin-Elmer 237B grating spectrophotometer. Melting points were obtained in capillary tubes and are reported uncorrected. Elemental analyses were within 0.4% of theoretical values except where noted and were performed by Galbraith Laboratories. *Note* that hydrazoic acid is toxic and

care should be taken in its preparation and handling (see ref 10). Aspartate transcarbamylase was the generous gift of Dr. G. A. O'Donovan; the enzyme activity was measured colorimetrically.[11]

Benzyl Hydrogen *N*-Chloroacetylphosphoramidate (10). A solution of *N*-chloroacetyl azide (6) (1.35 M in chloroform) was prepared from hydrazoic acid[10] and chloroacetyl chloride in the presence of triethylamine.[5] The acyl azide solution, 34.3 ml (46 mmol), was allowed to react with 15.9 g (46 mmol) of tribenzyl phosphite (7) according to a method for the preparation of triethyl *N*-chloroacetylphosphorimidate.[5] The reaction evolved 78% of the theoretical amount of nitrogen. The triethylamine hydrochloride was removed by evaporating the chloroform in vacuo and distributing the oily residue between water and carbon tetrachloride. The organic layer was washed with one portion of water and with 5% sodium bicarbonate solution and dried over anhydrous sodium sulfate. The solution was cooled to 0 °C and saturated with anhydrous hydrogen chloride and a small amount of thick oil formed in a few minutes, followed by a white precipitate. After 3.5 h the small amount of oil was skimmed off and the precipitate was collected by filtration. Two recrystallizations from acetonitrile yielded 1.92 g (24% based on the yield of nitrogen): mp 132–133 °C dec; IR (Nujol) 1687 cm^{-1} (CO); NMR (Me_2SO-d_6) δ 4.30 (2 H, s, $COCH_2Cl$), 5.15 (2 H, d, J_{HP} = 4 Hz, benzylic), 7.55 (5 H, s, Ph). Anal. ($C_9H_{11}ClNO_4P$) C, H, N.

Dilithium Salt of *N*-Chloroacetylphosphoramidate (5). To 0.74 g (2.8 mmol) of 10 was added 3 ml of trifluoroacetic acid. After several minutes at 25 °C, the trifluoroacetic acid was removed in vacuo and the residue dissolved in 12 ml of 95% ethanol. Two equivalents of a solution of lithium hydroxide in 90% ethanol (1.32 M) was added; the fine white precipitate was centrifuged, washed with absolute ethanol, and dried in vacuo to yield 0.4 g (77%) of the desired salt: IR (Nujol) 1630, 1476 cm^{-1}; NMR (D_2O) δ 4.40 (s). Anal. Calcd for $Li_2(C_2H_3ClNO_4P)$: C, 12.9; H, 1.6; N, 7.55. Found: C, 12.34; H, 1.94; N, 7.28.

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Narcotic Antagonists. Synthesis and Evaluation of Some Substituted 1,2,3,4,5,6-Hexahydro-1,4:2,6-dimethano-3-benzazocines

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A series of (±)-N-substituted 6-ethyl- or -methyl-8-hydroxy-1,2,3,4,5,6-hexahydro-1,4:2,6-dimethano-3-benzazocines has been prepared from 6-hydroxytropinone. The *N*-cyclopropylmethyl compounds **10a** and **10b** were found to be strong narcotic antagonists approximately equivalent to nalorphine. Only slight analgetic activity was found in any of these compounds including the two *N*-methyl analogues.

Synthetic investigations on the 1,2,3,4,5,6-hexahydro-2,6-methano-3-benzazocines[1] have produced many compounds possessing interesting profiles with respect to narcotic antagonist and analgetic activities.[2] The structure–activity effects of altering the substituents at positions 3, 6, 8, and 11 have been intensively studied, because of their close structural resemblance to morphine and the morphinans. Only recently has attention been directed toward modification of other positions on the ring system. Ziering et al.[3] have introduced methyl groups into the 1α and 9 positions and phenyl into the 1α position on the 3-benzazocine nucleus. Equatorial methyl groups have been introduced into the 4 and 5 positions,[4] and these modifications have produced compounds with interesting analgetic properties, although the 8 position lacks a phenolic hydroxyl substituent. Another interesting series has been prepared by benzylic oxidation to give 1-keto derivatives possessing mixed analgetic and narcotic antagonist activities.[5] Both isomeric 3-benzazocin-1-ols have also been prepared.[6]

In order to investigate further the structure–activity relationships in the 3-benzazocine series at positions which have attracted only limited attention, we chose to introduce a methylene group connecting the 1 and 4 positions. This modification creates a very rigid bridged [5,6,7] tricyclic ring system and should affect the steric environment around the nitrogen and above the aromatic ring. Lewis[7] has proposed that this area may be important for binding on the receptor site. The synthesis of several (±)-1,2,3,4,5,6-hexahydro-1,4:2,6-dimethano-3-benzazocines and their activities are reported in this paper.

Chemistry. The synthesis of the title compounds from 6-hydroxytropinone (1) is outlined in Scheme I. Treatment of 1 with ethylene glycol in the presence of *p*-toluenesulfonic acid gave the ketal 2 in high yield. Pfitzner–Moffit[8] oxidation smoothly converted 2 into the 6-keto derivative 3. It is worthy of note that the use of the Collins reagent[9] for this conversion was not satisfactory because of extensive oxidation of the *N*-methyl group to *N*-formyl. Treatment of 3 with *p*-methoxyphenylmagnesium bromide gave the endo alcohol 4 in good yield. The stereochemical assignment of 4 is based on steric and electronic factors as they strongly favor exo addition of the organometallic reagent. The highly hindered nature of the