observation that an equilibrium does not exist between the liquid surface and the film for water vapor. The resulting equation readily leads to the calculation of a permeability coefficient for the film; the units of *P* are the same as the diffusion coefficient, square centimeters per second. A steady state was assumed to exist within the diffusion cell following the lag time. This was justified by the linearity of the graphical data in Fig. 1. The theory was tested with films of unplasticized methylhydroxypropoxyl ethers of cellulose cast from water and an organic solvent; and the permeability coefficients were calculated from the slope of 1/rate *versus* film thickness. This provides a direct experimental method for easily determining permeability coefficients and, by maintaining constant cell geometry, relative permeability values can be assigned to any film or series of films.

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Potential Anticancer Agents VI: 5-Substituted Pyrimidine-6-carboxaldehydes

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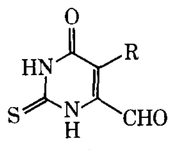
Abstract ☐ A series of 5-substituted pyrimidine-6-carboxaldehydes and their derivatives were synthesized and tested for inhibition of growth of the Ehrlich ascites carcinoma and Ehrlich carcinoma. Further studies included inhibition of incorporation of L-phenylalanine-1-^{14}C and formate-^{14}C into proteins, and orotic acid -5-^{3}H, thymidine-2-^{14}C, and formate-^{14}C into nucleic acids of the ascitic tumor cells *in vitro*. The following compounds were found to be particularly active as inhibitors: 2-mercapto-4-hydroxy-5-(3-phenylpropyl)pyrimidine-6-carboxaldehyde (VIII-2), 2-mercapto-4-hydroxy-5-(4-phenylbenzyl)pyrimidine-6-carboxaldehyde (VIII-3), and 2-mercapto-4-hydroxy-5-(α-naphthylmethyl)pyrimidine-6-carboxaldehyde (I*c*). The best compounds of this series are equally as effective as 5-fluorouracil and 2-mercapto-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (I*b*) in inhibiting formate incorporation into DNA and growth of the ascitic tumor. They are more effective than 5-fluorouracil in inhibiting incorporation of formate and orotic acid into RNA, thymidine into DNA, and phenylalanine into proteins. The active compounds also showed a strong inhibitory activity against respiration of the ascitic tumor. Compounds VIII-2 and VIII-3 also inhibited growth of the Ehrlich carcinoma as a solid tumor after subcutaneous transplantation, but in these tests the drugs were more toxic to the host when injected intraperitoneally since the drugs were not preferentially absorbed by the tumor cells in contrast to the tests *versus* the ascites form of the carcinoma.

Keyphrases ☐ Pyrimidine-6-carboxaldehydes, 5-substituted—synthesis ☐ Anticancer activity—5-substituted pyrimidine-6-carboxaldehydes ☐ Protein synthesis inhibition—5-substituted pyrimidine-6-carboxaldehydes ☐ Nucleic acid synthesis inhibition—5-substituted pyrimidine-6-carboxaldehydes ☐ Tumor aerobic respiration inhibition—5-substituted pyrimidine-6-carboxaldehydes

In earlier studies (1–3) on 5-substituted derivatives of Compound I, it was observed that 5-fluoro (I*a*) and 4-(4-chlorobenzyl) (I*b*) substituents resulted in derivatives possessing strong inhibitory activity against the Ehrlich ascites carcinoma.



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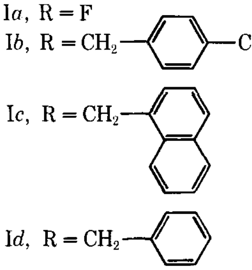
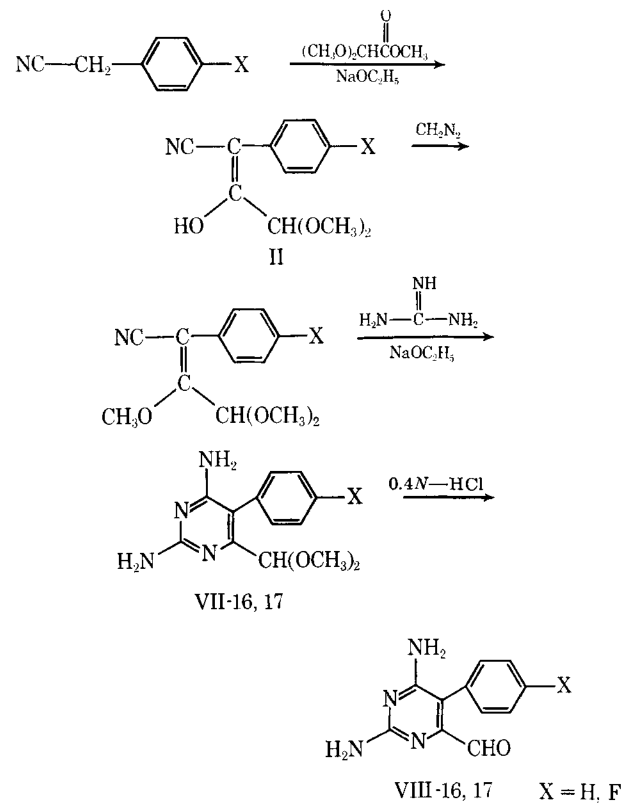


It was further observed that this series of 5-substituted pyrimidine-6-carboxaldehydes inhibited incorporation of amino acids and formate into proteins, and of orotic acid, thymidine, and formate into nucleic acids of the tumor cells *in vitro* (3). Compound I*b* was equally as effective as 5-fluorouracil (FU) in inhibiting formate incorporation into DNA and growth of the tumor and more effective than FU in inhibiting incorporation of formate and orotic acid into RNA, thymidine into DNA, and phenylalanine and glycine into proteins. However, Compound I*b* had only negligible inhibitory activity against the folate reductases.

The fact that an enhanced inhibitory activity was obtained by the introduction of benzyl substituents in the 5-position led to the synthesis of further derivatives of pyrimidine-6-carboxaldehydes containing bulky substituents in the 5-position in order to study the structure–activity relationship of these analogs in the test system.

RESULTS AND DISCUSSION

Chemistry—The majority of the α-substituted β-keto esters (VI) were prepared by the alkylation reaction of ethyl γ,γ-dimethoxyacetoacetate (IV) with an aralkyl (benzyl or naphthylmethyl) halide



Scheme I

in the presence of sodium ethoxide in 70% yield. Claisen condensation of isopropyl ester (V) with methyl dimethoxyacetate (III) in benzene in the presence of sodium hydride afforded the α-alkyl (*n*-hexyl and *n*-dodecyl) and α-aralkyl (2-phenylethyl and 3-phenylpropyl) β-keto esters in 95% yield. The isopropyl esters were employed to reduce self-condensation (4). Due to extensive decomposition during fractionation, the crude esters were used without purification. The condensation of the β-keto ester with thiourea gave the pyrimidine acetal (VII) in 30% yield. With guanidine hydrochloride, the yield was 45%.

When the guanidine reaction was performed utilizing guanidine hydrochloride and sodium methoxide in boiling ethanol, the intermediate guanide (2-methoxyacetyl-2-substituted acetyl guanide) separated during the 1st hour (5). This guanide was redissolved and cyclized to the pyrimidine acetal (VII). Hydrolysis of the acetals (VII) was accomplished in dioxane with 10% H_2SO_4 or 10% HCl, resulting in a 60% yield of the pyrimidine-6-carboxaldehydes (VIII). Dioxane was used as a solvent for those acetals which did not dissolve in acid solution, and hydrolysis was completed in about 1 hr. Claisen condensation of the phenylacetonitriles with methyl dimethoxyacetate and ethanolic sodium ethoxide gave α-acylphenylacetonitriles (II) in 50% yield. Compound II was converted to the enol ether by diazomethane and then condensed with guanidine to give Compounds VII-16 and VII-17. These pyrimidine acetals were then hydrolyzed with aqueous 0.4 *N* HCl to the aldehydes (VIII-16,17) (Scheme I).

The pyrimidine aldehydes (VIII) contained a molecule of ethanol when recrystallized from ethanol or ethanol–water. This molecule of ethanol was removed by heating at 100° for several hours *in vacuo*. Although a molecule of ethanol could be removed, some compounds were found to have one or one-half molecule of water, which was difficult to remove. The Cannizzaro reaction of Compound VIII-2 with 25% KOH yielded the corresponding alcohol (XI) and acid (XII) in good yield. The alcohol was precipitated at pH 7–8, and the acid was precipitated at lower pH (1–3). The thiosemicarbazones (IX), hydrazones (X), and Schiff bases (X) were prepared in the usual manner in good yields. Compound XII was decarboxylated by heating in tetralin, resulting in Compound XIII. Scheme II shows the sequence of reactions discussed.

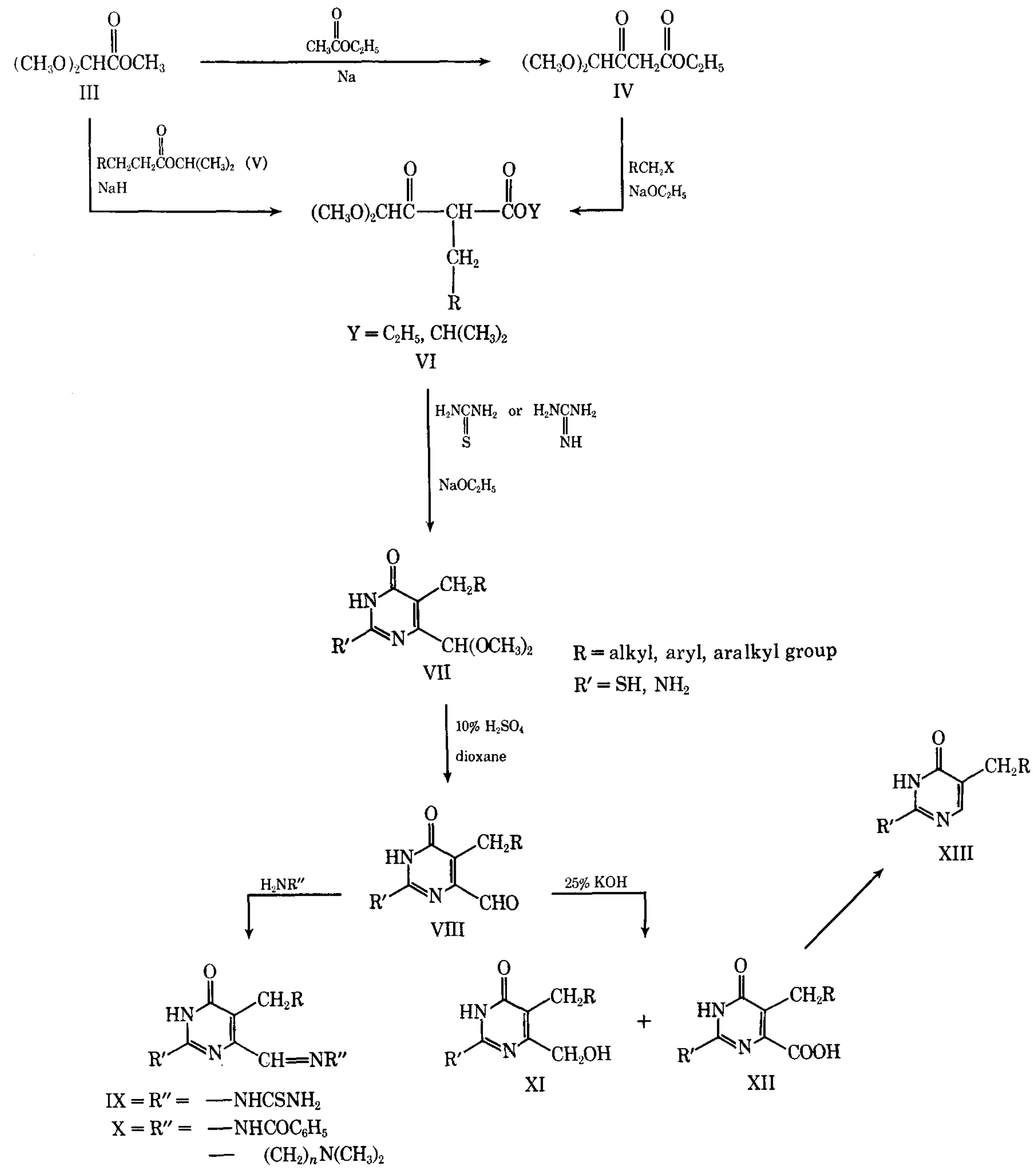
Screening Tests—*Test* versus *Ehrlich Ascites Carcinoma*—The compounds were tested *versus* the Ehrlich ascites carcinoma in Swiss-Webster white mice by a slight modification of procedures described previously (6, 7). Twenty-four hours after the inoculation, each control mouse received an intraperitoneal injection of 0.2 ml. of dimethyl sulfoxide (DMSO), and each experimental mouse received a solution of the tested compound in DMSO. The intraperitoneal injections of control and experimental mice were continued twice daily for 6 days (one injection on the last day; total of 11 injections). On the 7th day, all surviving mice in control and experimental groups were sacrificed. The volume of ascitic fluid was measured for each sample of ascitic fluid by centrifugation in heparinized capillary tubes. The total packed-cell volume (TPCV) of tumor cells was calculated in each case together with average values and standard deviations.

The results of tests of representative compounds are recorded in Table I. The results showed that almost all compounds had an inhibitory activity and low toxicity at the dosage of 50–100 mg./kg./day. Compounds I*c*, VIII-2, VIII-3, VIII-10, X-4, and XII showed strong activity against the tumor growth at the dosage of 10–60 mg./kg./day. Compounds I*c*, VIII-2, VIII-3, and VIII-10 were as effective as Compound I*b*, but they showed greater toxicity than I*b*. Since VIII-6 showed less activity than I*c*, the α-substituted naphthalene derivative seemed to be superior to the β-substituted compound. Also, the unsubstituted naphthylmethyl derivative (I*c*) was found to be a better compound than the methyl-substituted derivatives (VIII-7 and VIII-8).

The 5-dodecyl-pyrimidine-6-carboxaldehyde (VIII-10) showed greater antitumor activity than the 5-hexyl derivative (VIII-9), but both compounds were quite toxic. Since Compound I*c* was found to be more active than VIII-13, the SH group at the pyrimidine 2-position seemed to be a better functional group than NH_2 for anticancer activity in this system. A similar observation was noticed for Compound I*b* in a previous study (3). The aldehyde group at the pyrimidine 6-position also seemed to be very important for activity, because Compounds I*b* and VIII-2 showed superior activity when compared with corresponding acetal (VII-2), thiosemicarbazone (IX-2), hydrazones (X-4 and X-5), and Schiff bases (X-1 and X-2). Furthermore, the corresponding alcohol and carboxylic derivatives (XI and XII) were found to be less active than the corresponding aldehyde derivative (VIII-2).

Test versus *Ehrlich Carcinoma* (*Solid Tumor*)—The compounds were also tested *versus* the Ehrlich solid tumor in Swiss-Webster white mice by a modification of a procedure described previously (8). Each mouse (initial weight approximately 30 g.) received a subcutaneous injection of 0.2 ml. of 10% ascitic fluid (containing 1.4×10^7 carcinoma cells) into the inguinal region or the back shoulder region on one side. For each assay the mice were divided into a control group of eight mice and several experimental groups of eight mice each. Seventy-two hours after the inoculation, each control mouse received an intraperitoneal or a subcutaneous (tumor area) injection of 0.2 ml. DMSO, and each experimental mouse received a solution of the tested compound in DMSO. The intraperitoneal or subcutaneous injections of control and experimental mice were continued once daily for 9 days (total of nine injections). On the 12th day after inoculation, all surviving mice in control and experimental groups were sacrificed. The solid tumors were carefully taken out and weighed. The average weight of solid tumor was calculated in each case together with standard deviations. The results of the more active pyrimidine analogs on solid tumor growth are listed in Table II.

These compounds showed some activity and high toxicity against the solid tumor as opposed to a strong activity and low toxicity against the ascites tumor. Compound VIII-2 showed 74% inhibition with no mortality at a dosage of 16.46 mg./kg./day on subcutaneous injection, while this compound showed 25% inhibition with 3/8 mortality at a similar dosage on intraperitoneal injection. A similar result was obtained with Compound VIII-3. Compounds I*b* and I*c* showed 20% inhibition at a dosage of 15–20 mg./kg./day. FU showed 50% inhibition at a dosage of 16.06 mg./kg./day on intraperitoneal injection. However, subcutaneous injection did not enhance the inhibitory activity for FU and I*b*. Administration of 31.6 mg./kg./day of I*b* resulted in complete mortality. The higher toxicity of these compounds in the tests *versus* the solid tumor in comparison with the tests *versus* the ascites tumor can be explained on the basis that in the latter tests the compounds are immediately in contact with the carcinoma cells upon intraperitoneal injection, and the rapid uptake by these cells minimizes the concentrations attained in normal tissues of the host animal. On the other hand, when the compounds are injected intraperitoneally in the tests *versus* the



Scheme II

solid tumor, the tumor cells do not have a preferential opportunity to absorb the drugs, and higher concentrations are attained in normal tissues of the host. The lower toxicity upon subcutaneous injection in the region of the tumor also can be explained upon the basis of preferential uptake by tumor cells, as well as a slower rate of rise in drug concentration in the peripheral circulation.

Inhibition of Protein Synthesis—The effects of the compounds upon protein synthesis were studied by determining the inhibition of incorporation of L-phenylalanine-1-^{14}C and formate-^{14}C into the proteins of Ehrlich ascites carcinoma cells, which were incubated aerobically for 1 hr. with labeled substrate and the compound *in vitro* in Krebs-Ringer phosphate (KRP) buffer at 37 ± 1° by a procedure described previously (1). After the incubation, the acid-soluble fraction (ASF) and the total proteins were isolated and freed of lipids and nucleic acids. Each protein preparation was dissolved in 2.0 ml. of 0.3 *N* NaOH, and 0.2 ml. of the protein solution was transferred to a glass scintillation vial. To each vial, 0.2 ml. of hyamine hydroxide 10-X and 16 ml. of POPOP-PPO tyloxapol[1] scintillation fluid were added for determination of radioactivity in a Packard liquid scintillation spectrometer, model 3320.

The effects of the compounds upon incorporation of L-phenylalanine-1-^{14}C and formate-^{14}C into proteins of the carcinoma cells are recorded in Tables III and V. Compounds I*c*, VIII-1, VIII-2, VIII-3, VIII-6, VIII-7, VIII-9, VIII-10, IX-2, and X-4 almost completely inhibited the incorporation of L-phenylalanine-1-^{14}C into proteins at the concentrations of 0.48–0.96 m*M*. Compounds I*c*, VIII-2, and VIII-3 showed 90–95% inhibition of formate-^{14}C incorporation into proteins at 0.77 m*M*.

The effects of the homologous 5-aralkylpyrimidine-6-carboxaldehydes (I*d*, VIII-1, and VIII-2) are shown in Fig. 1. The phenylpropyl derivative (VIII-2) was found to be the most active compound among them, and the activity decreased in the order: $C_6H_5CH_2CH_2CH_2 > C_6H_5CH_2CH_2 > C_6H_5CH_2$. However, the difference in activity was not significant; only a 5–10% difference in inhibition existed at a concentration range of 0.24–0.48 m*M*. These compounds lost their inhibitory activity at a concentration of 0.12 m*M*. The difference in activity might be explained from the binding difference by

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[1] In 1 l. of toluene, 5 g. of 2,5-diphenyloxazole (PPO) and 0.3 g. of 1,4-bis(4-methyl-5-phenyloxazolyl)benzene (dimethyl-POPOP) were dissolved. Two liters of this POPOP–PPO solution and 1 l. of tyloxapol (Triton X-100) were mixed.

Table I—Results of Screening Tests *versus* Ehrlich Ascites Carcinoma[a]

Compound	Dose,[b] mg./kg./day	Mortality C	Mortality T	Average Weight Change, T/C, g.	Average TPCV T/C, ml.	Average TPCV *SD* T ±ml.	Average TPCV T as % of C
I*c*	9.5	0/8	0/8	6.3/3.7	1.1/2.6	0.58	42
	31.7	0/8	1/8	3.2/5.2	0.4/1.8	0.16	22
	59.8	0/8	1/8	−0.1/5.2	0.3/1.8	0.78	17
	115.9	3/8	3/8	6.1/1.0	0.1/1.6	0.06	6
VII-2	57.4	0/8	2/8	−0.6/3.2	0.8/2.4	0.44	35
VII-3	64.2	0/8	1/8	2.3/3.2	0.5/2.3	0.19	22
VIII-1	58.4	0/8	2/8	4.4/5.2	1.3/1.8	0.38	72
	93.1	0/8	3/8	4.0/3.2	0.5/2.3	0.30	22
VIII-2	9.3	0/8	1/8	6.6/3.7	1.3/2.6	0.81	50
	16.2	0/8	1/8	9.1/3.2	0.5/2.3	0.17	22
	30.2	0/8	1/8	4.2/5.2	0.3/1.8	0.10	17
	55.8	0/8	0/8	1.0/5.2	0.16/1.8	0.22	9
	91.7	0/8	1/8	3.1/3.2	0.17/2.3	0.15	7
VIII-3	8.6	0/8	2/8	1.9/3.7	0.6/2.6	0.05	24
	16.3	0/8	0/8	3.7/3.2	0.5/2.3	0.18	22
	31.9	0/8	1/8	0.4/3.2	0.4/2.3	0.33	17
	59.8	0/8	2/8	0.4/5.2	0.15/1.8	0.29	8
	93.3	0/8	3/8	0.3/3.2	0.3/2.3	0.16	13
VIII-4	68.5	0/8	3/8	7.0/3.2	0.67/2.3	0.29	29
VIII-6	59.8	0/8	0/8	0.8/5.2	0.7/1.8	0.79	39
	98.8	0/8	4/8	10.2/3.7	0.9/2.6	0.34	35
VIII-7	58.4	0/8	3/8	4.1/5.2	1.7/1.8	0.99	94
VIII-8	55.8	0/8	1/8	2.3/5.2	0.9/1.8	0.64	50
VIII-9	95.8	0/8	3/8	6.1/3.7	0.9/2.6	0.21	35
VIII-10	63.5	0/8	4/8	5.0/3.2	0.2/2.3	0.06	9
VIII-11	61.0	0/8	2/8	9.0/3.2	0.8/2.3	0.59	35
VIII-12	59.4	0/8	0/8	4.7/5.2	0.7/1.8	0.41	39
	88.9	0/8	0/8	4.0/3.2	0.6/2.3	0.43	26
VIII-13	64.0	0/8	0/8	0.3/3.2	0.7/2.3	0.31	30
VIII-16	60.0	0/8	0/8	6.2/5.2	0.6/1.8	0.29	33
VIII-17	63.1	0/8	1/8	3.7/5.2	0.4/1.8	0.09	22
IX-1	31.6	0/8	0/8	0.9/5.2	0.4/1.8	0.19	22
IX-2	57.4	0/8	0/8	−1.8/5.2	0.5/1.8	0.27	28
IX-5	40.5	1/8	1/8	4.1/8.2	1.4/1.9	0.51	74
X-1	58.7	0/8	0/8	−4.0/5.2	0.4/1.8	0.21	22
X-2	58.3	0/8	0/8	−1.6/5.2	0.7/1.8	0.34	39
X-4	57.2	0/8	0/8	−5.3/5.2	0.3/1.8	0.79	17
X-5	60.4	0/8	0/8	3.3/5.2	0.4/1.8	0.17	22
XI	61.6	0/8	1/8	7.5/3.2	0.5/2.3	0.34	22
XII	49.6	0/8	2/8	2.9/3.2	0.3/2.3	0.09	13
I*b*[c]	8.9	0/8	0/8	1.6/3.7	0.7/2.6	0.29	28
	30.0	0/8	0/8	3.4/8.1	0.3/2.4	0.10	12
	48.2	0/8	0/8	3.1/9.1	0.1/3.0	0.23	3
FU[d]	27.5	0/8	0/8	−0.3/5.3	0.15/1.5	0.10	10

[a] T = treated group; C = controls; and TPCV = total packed-cell volume of tumor cells on final day of assay. The average standard deviation for TPCV of all control groups was ±0.44 ml. [b] The compound, 5, 10, 25, 50, 100, or 150 mg., was dissolved in 20 ml. of DMSO. Each mouse (initial weight approximately 30 g.) received two intraperitoneal injections of 0.2 ml. of the drug solution daily. [c] Data from *Reference 5*. [d] 5-Fluorouracil; data from *Reference 3*.

hydrophobic bonding with these groups to the enzyme which might be involved at the inhibitory site. This hypothesis seems to be plausible, because the 5-hydrocarbon chain alkylpyrimidine-6-carboxaldehydes (VIII-9 and VIII-10) showed 95–98% inhibition at a concentration of 0.96 m*M*, while 2-thioorotic aldehyde (TOA) showed no activity at 0.65 m*M* and only 70% inhibition at 1.96 m*M* (1). The aldehyde group at pyrimidine 6-position appears to be essential, since Compound VIII-2 showed an increased inhibitory activity

Table II—Results of Screening Tests *versus* the Ehrlich Carcinoma (Solid Tumor)[a]

Compound	Injection	Dose,[b] mg./kg./day	Mortality C	Mortality T	Average Tumor Weight T/C, g.	Average Tumor Weight *SD* T ± g.	Average Tumor Weight T as % of C
I*b*	Intraperitoneal	14.16	0/8	2/8	2.86/3.95	0.73	72
	Intraperitoneal	15.5	1/8	0/8	3.31/4.02	0.14	83
	Intraperitoneal	21.4	1/8	4/8	3.05/4.02	0.07	75
	Intraperitoneal	31.6	1/8	8/8	—	—	—
	Subcutaneous	16.0	1/8	2/8	2.64/3.00	0.25	88
I*c*	Intraperitoneal	15.0	1/8	4/8	3.26/4.02	0.25	81
	Intraperitoneal	16.3	1/8	4/8	3.46/4.02	0.05	86
VIII-2	Intraperitoneal	16.0	1/8	3/8	3.03/4.02	0.33	75
	Subcutaneous	16.6	0/8	0/8	0.36/1.35	0.39	26
VIII-3	Intraperitoneal	16.3	1/8	5/8	3.57/4.02	0.06	89
	Subcutaneous	16.7	0/8	0/8	0.76/1.35	0.51	56
FU[c]	Intraperitoneal	16.0	1/8	3/8	2.05/4.02	0.33	51
	Subcutaneous	15.6	1/8	2/8	3.05/3.00	0.26	100

[a] T = treated group; C = control. The average standard deviation for the control group was ±0.21 g. for the intraperitoneal injection and ±0.95 g. for the subcutaneous injection. [b] The compound, 50, 70, or 100 mg., was dissolved in 20 ml. of DMSO. The drug solution (0.2 ml.) was injected intraperitoneally or subcutaneously into the mouse (initial weight approximately 30 g.) once daily. [c] FU = 5-fluorouracil.

Table III—Effects of Compounds on Incorporation of L-Phenylalanine-1-^{14}C[a] into Proteins of Ehrlich Ascites Carcinoma Cells

Compound	Concentration of Compound, m*M*	Average Experimental Values, % of Control Protein	ASF[b]
None	0	100[c]	100[d]
I*c*	0.96	6	96
VIII-1	0.12	113	124
	0.24	26	205
	0.48	10	231
	0.96	4	70
VIII-2	0.12	100	136
	0.24	17	202
	0.48	6	216
	0.96	0	18
VIII-3	0.96	3	45
VIII-6	0.96	5	30
VIII-7	0.96	3	31
VIII-9	0.96	2	30
VIII-10	0.96	5	11
VIII-13	0.96	34	123
VIII-14	0.96	30	94
IX-1	0.48	73	152
IX-2	0.96	6	157
X-2	0.48	22	209
X-4	0.48	3	48
XI	0.96	28	—
XII	0.96	127	—
I*b*[e]	0.38	5	—
	0.96	5	67
FU[f]	0.68	87	83
	2.0	81	85

[a] 0.1 μmole/ml. (0.2 μCi). [b] ASF = acid-soluble fraction. [c] 1.122 × 10^3 counts/min./mg. [d] 1.751 × 10^3 counts/min. [e] Data from *Reference 5.* [f] FU = 5-fluorouracil; data from *Reference 3.*

over the alcohol (XI) and the acid (XII) derivatives. The 5-naphthylmethyl-pyrimidine-6-carboxaldehydes were also found to be very effective compounds against the incorporation of L-phenylalanine-1-^{14}C into proteins. However, the 2-amino-5-naphthylmethyl derivatives (VIII-13 and VIII-14) showed 70% inhibition at a concentration of 0.96 m*M*, while the corresponding 2-mercaptopyrimidine derivatives showed 95–98% inhibition at the same concentration.

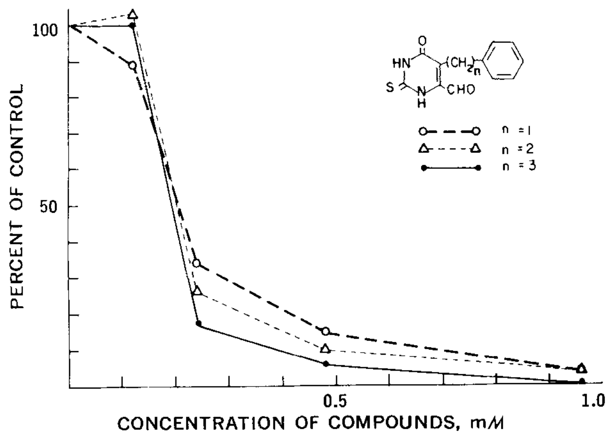


Figure 1—*Effects of phenylalkylpyrimidine-6-carboxaldehydes on incorporation of* L-*phenylalanine-1-^{14}C into proteins of Ehrlich ascites carcinoma cells* in vitro. L-*Phenylalanine-1-^{14}C: 0.1 μmole/ml.; 0.2 μCi.*

Table IV—Effects of Compounds on Incorporation of Thymidine-2-^{14}C and Orotic Acid-5-^{3}H into Nucleic Acids of Ehrlich Ascites Carcinoma Cells[a]

Compound	Concentration of Compound, m*M*	Average Experimental Values, % of Control DNA	RNA
None	0	100[b]	100[c]
I*c*	0.96	39	30
	1.92	5	4
VIII-2	0.96	4	42
	1.92	8	3
VIII-3	0.96	21	11
	1.92	6	7
VIII-10	0.96	107	101
	1.92	74	85
XII	0.96	190	91
XIII	0.96	23	—
I*b*[d]	0.96	4[e]	24
	1.92	0	13
FU[f]	0.57	—	90
	1.60	330	—

[a] Thymidine-2-^{14}C: 0.1 μmole/ml. (1 μCi); orotic acid-5-^{3}H: 0.04 μmole/ml. (10 μCi). [b] 3.26 × 10^5 c.p.m./mg. [c] 1.43 × 10^5 c.p.m./mg. [d] Data from *Reference 5.* [e] New result. [f] FU = 5-fluorouracil; data from *Reference 3.*

When an amino acid is concentrated in a cell, it rapidly equilibrates with exogenous amino acids and is competitively displaced by certain structurally related analogs (9). The radioactivity of the ASF obtained after 1 hr. incubation of a suspension of Ehrlich ascites cells and L-phenylalanine-1-^{14}C is due to unchanged L-phenylalanine-1-^{14}C and other compounds produced metabolically from L-phenylalanine-1-^{14}C. Inhibition of L-phenylalanine-1-^{14}C transport into the cell interior or relatively faster biochemical reactions involving L-phenylalanine-1-^{14}C may reduce the total radioactivity in the ASF of the cell. The inhibition of L-phenylalanine-1-^{14}C incorporation into the ASF by the compounds listed in Table III may be the result of such effects. However, inhibition of incorporation into protein was always greater than inhibition of incorporation into the ASF. Compounds VIII-2, VIII-6, VIII-7, VIII-9, and VIII-10 gave 70–90% inhibition of accumulation in the ASF at a concentration of 0.96 m*M*. Compounds VIII-1 and VIII-2, however, stimulated L-phenylalanine-1-^{14}C incorporation into the ASF to 220% of controls at 0.48 m*M* concentration. This increase in accumulation of radioactivity in the ASF could result in part from the inhibition of incorporation of phenylalanine into protein, which would produce a "pile-up" of phenylalanine-1-^{14}C in the ASF.

Inhibition of Nucleic Acid Synthesis—The effects of the compounds upon nucleic acid synthesis were studied by determining the inhibition of incorporation of orotic acid-5-^{3}H and formate-^{14}C into RNA and of thymidine-2-^{14}C and formate-^{14}C into DNA of Ehrlich ascites carcinoma cells. These cells were incubated aerobically for 1 hr. with the labeled substrate and the compound *in vitro* in KRP buffer at 37 ± 1° by a procedure described previously (3).

Incorporation of Orotic Acid-5-^{3}H and Thymidine-2-^{14}C into Nucleic Acids—Each incubation flask contained 5 ml. of a 20% suspension of tumor cells in KRP buffer (pH 7.1–7.2), 1 ml. of KRP buffer containing orotic acid-5-^{3}H (0.04 μmole/ml., 10 μCi), thymidine-2-^{14}C (0.1 μmole/ml., 1 μCi), glucose (5 mg./ml.), and 0.2 ml. of the compound in DMSO.

After incubation, the acid-insoluble residue was obtained and washed as previously described (1). The acid-insoluble residue then was suspended in 5 ml. of 0.5 *N* $HClO_4$ and heated for 30 min. at 95°. At the end of heating, the tubes were centrifuged, and the supernatant was neutralized with KOH. To each glass scintillation

Table V—Effects of Compounds on Incorporation of Formate-^{14}C into Nucleic Acids and Proteins of Ehrlich Ascites Carcinoma Cells[a]

Compound	Concentration of Compound, m*M*	Average Experimental Values, % of Control RNA	DNA	Protein
None	0	100[b]	100[c]	100[d]
I*c*	0.77	9	15	9
VIII-2	0.77	4	8	6
VIII-3	0.77	2	2	4
XIII	0.77	62	59	—
I*b*[e]	0.77	4.4	5.3	2.2
	1.54	0.4	0.09	0.2
FU[f]	0.64	126	7	—

[a] Formate-^{14}C: 0.318 μmole/ml. (8 μCi). [b] 1.57 × 10^4 c.p.m./mg. of RNA. [c] 2.26 × 10^4 c.p.m./mg. of DNA. [d] 1.88 × 10^4 c.p.m./mg. [e] Data from *Reference 5.* [f] FU = 5-fluorouracil; data from *Reference 3.*

Table VI—Effect of Compounds on Respiration of Ehrlich Ascites Carcinoma Cells

Compound	Concentration of Compound, m*M*	Q_{O_2},[a] µl. O_2/mg. cells/hr. Control	Experiment	Inhibition, %
I*b*	1.0	3.50	1.57	55
	2.0	3.50	1.30	63
I*c*	1.0	3.50	1.31	63
	2.0	3.50	1.44	60
VIII-1	1.0	4.00	1.48	63
	2.0	4.00	1.51	63
IX-2	1.0	4.00	3.79	5
	2.0	4.00	3.31	17
X-1	1.0	4.00	2.23	44
	2.0	4.00	2.14	47
X-2	1.0	4.00	1.38	66
	2.0	4.00	1.06	72
X-4	1.0	4.00	3.79	5

[a] For calculation of Q_{O_2} value, 2 ml. of 10% cell suspension was considered as equivalent to 32 mg. dry weight.

vial, 0.2 ml. of the neutralized solution, 0.2 ml. of hyamine hydroxide 10-X, and 16 ml. of the scintillation fluid were added for determining radioactivity. The radioactivities of ^{14}C and ^{3}H were determined according to Kabara *et al.* (10), and the exact channel ratios of ^{14}C and ^{3}H were determined (1). The concentration of RNA was determined by the orcinol reaction and corrected for the interference by DNA. The amount of DNA was determined by the diphenylamine reaction (11). The specific activities were calculated as c.p.m. (^{3}H)/mg. of RNA and c.p.m. (^{14}C)/mg. of DNA.

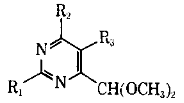
The effects of the compounds upon incorporation of orotic acid-5-^{3}H into RNA and thymidine-2-^{14}C into DNA of the carcinoma cells are recorded in Table IV. Compounds I*b* and VIII-2 showed complete inhibition of thymidine-2-^{14}C incorporation into DNA–thymine at a concentration of 0.96 m*M*. A recent study with Compound I*b* (12) showed that the inhibition of thymidine incorporation into DNA was possibly due to the inhibition of thymidine monophosphate (TMP) kinase by this compound.

Compounds I*b*, I*c*, VIII-2, and VIII-3 showed 80–95% inhibition of incorporation of orotic acid-5-^{3}H into RNA at a relatively high concentration (1.92 m*M*) of the compounds.

The previous work showed that the conversion of orotic acid to orotidylic acid was inhibited by some of the 5-fluoroorotic aldehyde derivatives (1). The 5-substituted pyrimidine derivatives might also inhibit this conversion. The possibility that Compound XII is acting as an antimetabolite of orotic acid does not seem to be valid. While this compound was quite active as a growth inhibitor in this system, it showed no inhibition against incorporation of L-phenylalanine into proteins and of orotic acid into RNA at a concentration of 0.96 m*M*.

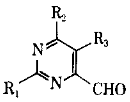
Incorporation of Formate-^{14}C into Nucleic Acids—Each incubation flask contained 5 ml. of a 40% suspension of tumor cells in KRP buffer, 1 ml. of glucose in KRP buffer (3 mg./ml.), 1 ml. of formate-^{14}C in KRP buffer (0.318 µmole/ml., 8 µCi), and 0.2 ml. of the compound in DMSO. After incubation, nucleic acids were isolated as sodium nucleates. The proteins also were isolated for evaluation of inhibition of formate incorporation into protein. RNA was separated from DNA by hydrolyzing with 0.3 *N* KOH at 37° for 16–18 hr. After purification, the radioactivities were counted for each nucleic acid by a procedure described previously (1). The effects of the compounds upon incorporation of formate-^{14}C into the nucleic acids of ascites cells are recorded in Table V. Compounds I*c*, VIII-2, and VIII-3 showed almost complete inhibition of incorporation of formate into DNA and RNA at a concentration of 0.77 m*M*, and they were as active as 2-mercapto-5-(4-chlorobenzyl) pyrimidine-6-carboxaldehyde (I*b*) as previously reported (3). These compounds are practically identical in activity with FU in inhibiting formate incorporation into DNA, but they are more active than FU in inhibiting formate incorporation into RNA. Compound XIII was less active than Compound VIII-2, which further indicates the im-

Table VII—6-(Dimethoxymethyl)-5-substituted Pyrimidines



No.	R_1	R_2	R_3	Yield, %	M.p.	Formula	Anal. Calcd.	Anal. Found
1	SH	OH	$CH_2CH_2C_6H_5$	40	175–177°	$C_{15}H_{18}N_2O_3S$	C, 58.82 H, 5.88	C, 58.96 H, 6.01
2	SH	OH	$CH_2CH_2CH_2C_6H_5$	12	118–120°	$C_{16}H_{20}N_2O_3S$	C, 60.00 H, 6.25	C, 60.06 H, 6.32
3	SH	OH	$CH_2C_6H_4$-C_6H_5(4)	33	210–212°	$C_{20}H_{20}N_2O_3S$	C, 65.25 H, 5.44	C, 65.17 H, 5.41
4	SH	OH	$CH_2C_6H_3(CH_3)_2$(2,5)	34	185–187°	$C_{16}H_{20}N_2O_3S$	C, 60.00 H, 6.25	C, 60.34 H, 6.32
5	SH	OH	$CH_2C_6H_3(OH)$(2)NO_2(5)	10	238–240° dec.	$C_{14}H_{15}N_3O_6S$	C, 47.59 H, 4.25	C, 47.53 H, 4.21
6	SH	OH	$CH_2(\beta)C_{10}H_7$	16.3	163–165°	$C_{18}H_{18}N_2O_3S$	C, 63.16 H, 5.26	C, 63.09 H, 5.18
7	SH	OH	$CH_2(\alpha)C_{10}H_6CH_3$(2)	23	262–263°	$C_{19}H_{20}N_2O_3S$	C, 64.04 H, 5.62	C, 64.28 H, 5.59
8	SH	OH	$CH_2(\alpha)C_{10}H_6CH_3$(4)	10	183–185°	$C_{19}H_{20}N_2O_3S.H_2O$	C, 61.00 H, 5.89	C, 61.11 H, 6.01
9	SH	OH	$CH_2(CH_2)_4CH_3$	28	99–100°	$C_{13}H_{22}N_2O_3S$	C, 54.54 H, 7.69	C, 54.62 H, 7.96
10	SH	OH	$CH_2(CH_2)_{10}CH_3$	36	81.5–82.5°	$C_{19}H_{34}N_2O_3S$	C, 61.62 H, 9.19	C, 61.48 H, 9.22
11	NH_2	OH	$CH_2C_6H_4Br$(4)	26.2	242–243° dec.	$C_{14}H_{16}BrN_3O_3$	C, 47.46 H, 4.52	C, 47.34 H, 4.43
12	NH_2	OH	$CH_2CH_2C_6H_5$	47	219–221°	$C_{15}H_{19}N_3O_3$	C, 62.28 H, 6.58	C, 62.26 H, 6.74
13	NH_2	OH	$CH_2(\alpha)C_{10}H_7$	19.1	243–245°	$C_{18}H_{19}N_3O_3$	C, 66.46 H, 5.85	C, 66.48 H, 6.03
14	NH_2	OH	$CH_2(\beta)C_{10}H_7$	22	222–224° dec.	$C_{18}H_{19}N_3O_3$	C, 66.46 H, 5.85	C, 66.34 H, 6.27
15	NH_2	OH	$CH_2(\alpha)C_{10}H_6CH_3$(4)	32.7	264–265° dec.	$C_{19}H_{21}N_3O_3$	C, 67.25 H, 6.19	C, 67.07 H, 5.96
16	NH_2	NH_2	C_6H_5	22.3	247–248°	$C_{13}H_{16}N_4O_2$	C, 60.00 H, 6.16	C, 59.97 H, 6.15
17	NH_2	NH_2	C_6H_4F(4)	18.7	259–260°	$C_{13}H_{15}FN_4O_2$	C, 56.11 H, 5.40	C, 56.15 H, 5.55

Table VIII—5-Substituted Pyrimidine-6-carboxaldehydes



No.	R_1	R_2	R_3	Yield, %	M.p.	Formula	Anal.	
							Calcd.	Found
1	SH	OH	$CH_2CH_2C_6H_5$	40	201–202°	$C_{13}H_{12}N_2O_2S$	C, 60.00 H, 6.16	C, 59.97 H, 6.15
2	SH	OH	$CH_2CH_2CH_2C_6H_5$	93.5	168–170°	$C_{14}H_{14}N_2O_2S$	C, 61.31 H, 5.11	C, 60.98 H, 5.04
3	SH	OH	$CH_2C_6H_4\text{-}C_6H_5(4)$	74	234–235° dec	$C_{18}H_{14}N_2O_2S$	C, 67.10 H, 4.35	C, 67.24 H, 4.28
4	SH	OH	$CH_2C_6H_3(CH_3)_2(2,5)$	47	254–256° dec.	$C_{14}H_{14}N_2O_2S$	C, 61.31 H, 5.11	C, 62.06 H, 5.09
5	SH	OH	$CH_2C_6H_3(OH)(2),NO_2(5)$	75	263–265° dec.	$C_{12}H_{19}N_3O_5S$	C, 46.90 H, 2.92	C, 47.00 H, 3.01
6	SH	OH	$CH_2(\beta)C_{10}H_7$	75	204–205°	$C_{16}H_{12}N_2O_2S \cdot {}^1/_2H_2O$	C, 62.95 H, 4.26	C, 63.07 H, 4.18
7	SH	OH	$CH_2(\alpha)C_{10}H_6CH_3(2)$	69	239–240° dec.	$C_{17}H_{14}N_2O_2S$	C, 65.80 H, 4.51	C, 66.05 H, 4.57
8	SH	OH	$CH_2(\alpha)C_{10}H_6CH_3(4)$	71	247–249° dec.	$C_{17}H_{14}N_2O_2S \cdot {}^1/_2H_2O$	C, 63.95 H, 4.70	C, 64.07 H, 4.79
9	SH	OH	$CH_2(CH_2)_4CH_3$	100	123–125°	$C_{11}H_{16}N_2O_2S$	C, 55.00 H, 6.67	C, 55.04 H, 6.79
10	SH	OH	$CH_2(CH_2)_{10}CH_3$	100	75–77°	$C_{17}H_{28}N_2O_2S$	C, 62.96 H, 8.64	C, 62.56 H, 8.51
11	NH_2	OH	$CH_2C_6H_4Br(4)$	72.5	>300° dec.	$C_{12}H_{10}BrN_3O_2$	C, 46.75 H, 3.24	C, 47.10 H, 3.32
12	NH_2	OH	$CH_2CH_2C_6H_5$	95	>330° dec.	$C_{13}H_{13}N_3O_2$	C, 64.20 H, 5.35	C, 63.91 H, 5.40
13	NH_2	OH	$CH_2(\alpha)C_{10}H_7$	94	293–294° dec.	$C_{16}H_{13}N_3O_2 \cdot {}^1/_2H_2O$	C, 66.66 H, 4.86	C, 66.40 H, 4.90
14	NH_2	OH	$CH_2(\beta)C_{10}H_7$	47	>300° dec	$C_{16}H_{13}N_3O_2$	C, 68.82 H, 4.66	C, 68.89 H, 4.95
15	NH_2	OH	$CH_2(\alpha)C_{10}H_6CH_3(4)$	96	>300° dec.	$C_{17}H_{15}N_3O_2 \cdot {}^1/_2H_2O$	C, 67.55 H, 5.30	C, 67.78 H, 5.55
16	NH_2	NH_2	C_6H_5	93.5	>300° dec.	$C_{11}H_{10}N_4O \cdot {}^1/_2H_2O$	C, 59.19 H, 4.93	C, 59.65 H, 4.78
17	NH_2	NH_2	$C_6H_4F(4)$	90	>300° dec.	$C_{11}H_9FN_4O \cdot {}^1/_2H_2O$	C, 54.77 H, 4.14	C, 55.03 H, 4.11

portance of the aldehyde group at the 6-position.

In the initial studies by Chae (13), the effects of the 5-fluoroorotic aldehydes on the incorporation of labeled formate into DNA–purines were not investigated because of the low radioactivity in the purines of DNA; the radioactivity could be mainly accounted as DNA–thymine. In the present experiments, the total radioactivity of DNA was counted without separation of each base. Therefore, the authors did not determine whether the radioactivity was counted mainly from DNA–thymine or whether the DNA–purines made a significant contribution. However, since previous studies (13) indicated a low radioactivity from DNA–purines, the authors assumed that the radioactivity was counted mainly from DNA–thymine. This assumption indicated that probably the inhibition of incorporation of formate-^{14}C into DNA by the 5-substituted pyrimidine derivatives might be attributable to inhibition of thymidylate synthesis by these compounds.

A recent study (12) also indicated that these compounds inhibited TMP kinase, and the inhibition of formate incorporation into DNA might be due to these two inhibitions. It was also observed (12) that Compound I*b* inhibited the incorporation of formate-^{14}C into guanine of RNA 2–4 times more than the formate incorporation into adenine of RNA. Furthermore, Compound I*b* showed 75–80% inhibition of incorporation of labeled 5-amino-4-imidazolecarboxamide (AICA) and hypoxanthine (Hx) into guanine of RNA at a concentration of 0.25 m*M*, while this compound showed practically no inhibition against incorporation of AICA and Hx into adenine of RNA at the same concentration. These results would indicate that one of the possible steps inhibited by I*b* is the conversion of xanthosine monophosphate (XMP) to guanosine monophosphate (GMP). Since the incorporation of formate-^{14}C into adenine of RNA was also inhibited by I*b*, there must be other possible inhibition steps which utilize formate or THFA coenzymes. However, the 5-substituted pyrimidine derivatives were essentially inactive as folate reductase inhibitors when compared with accepted antifols such as aminopterin, pyrimethamine, and trimethoprim (3). Therefore, the inhibition of formate incorporation into DNA and RNA by these compounds could not be attributed to inhibition of the folate reductases.

Inhibition of Aerobic Respiration of the Tumor—The effects of the compounds upon aerobic respiration of the Ehrlich ascites tumor were studied by measuring oxygen consumption by means of the Warburg apparatus. To each Warburg flask was added 2 ml. of the 10% cell suspension in KRP buffer, and 0.2 ml. of 20% KOH was added in the center well which contained a small piece of filter paper. Three-tenths milliliter of drug solution in DMSO (or 0.3 ml. of DMSO for the control) and 0.3 ml. of 0.5 *M* glucose solution were then added to each sidearm. For the thermobarometer, 2.6 ml. of distilled water was added to the flask and 0.2 ml. of 20% KOH in the center well. The flasks were equilibrated in a 37° water bath for 15 min. after gassing with oxygen (10 min.). After equilibration the stopcocks were closed, and the contents of the flask were then mixed. Readings were taken every 10 min. thereafter for 1-hr. For each experiment, at least two controls and one thermobarometer were needed.

The oxygen uptake for respiration was calculated by the method described previously (14). The change in readings, corrected for thermobarometer, was multiplied by the flask constant (K_{O_2}), resulting in the oxygen uptake ($\mu l.\ O_2$/hr.).

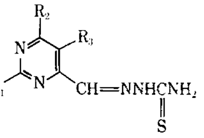
Two milliliters of the 10% cell suspension contained 32 mg. dry weight of cells. Therefore, Q_{O_2} (microliter O_2 taken up per milligram dry weight of cells per hour) was calculated by dividing the oxygen uptake by 32 mg. The inhibitory activity was calculated from the mean value (Q_{O_2}) of the duplicate experiments of each sample.

The effects of the compounds upon aerobic respiration of the Ehrlich ascites tumor are recorded in Table VI.

Compounds I*b*, I*c*, VIII-1, and X-2 showed 55–80% inhibition of respiration at a concentration of 1 m*M* in the presence of added glucose. Most of these compounds did not show an enhanced inhibitory activity at an increased concentration (2 m*M*).

There may be two possible explanations for the inhibition of respiration by these compounds. Since preliminary data seem to indicate that these analogs inhibit the synthesis of AMP and GMP

Table IX—Thiosemicarbazones of 5-Substituted Pyrimidine-6-carboxaldehydes



No.	R_1	R_2	R_3	Yield, %	M.p.	Formula	Anal.	
							Calcd.	Found
1	SH	OH	$CH_2C_6H_5$	62.5	200–201°	$C_{13}H_{13}N_5OS_2$	C, 48.90 H, 4.07	C, 48.56 H, 4.17
2	SH	OH	$CH_2C_6H_4Cl(4)$	51	245–246° dec.	$C_{13}H_{12}ClN_5OS_2$	C, 44.13 H, 3.39	C, 44.16 H, 3.30
3	C_2H_5S	OH	$CH_2C_6H_4Cl(4)$	63	231–233° dec.	$C_{15}H_{16}ClN_5OS_2$	C, 47.18 H, 4.19	C, 46.99 H, 4.11
4	SH	OH	$CH_2C_6H_4$-$C_6H_5(4)$	51	215–217°	$C_{19}H_{17}N_2OS_2 . {}^1/_2H_2O$	C, 56.43 H, 4.46	C, 56.70 H, 4.74
5	NH_2	NH_2	C_6H_4Cl-4	62	270–275° dec.	$C_{12}H_{12}ClN_7S$	C, 44.79 H, 3.73	C, 44.86 H, 4.02
6	OH	OH	Br	68.5	>300° dec.	$C_6H_6BrN_5O_2S$	C, 24.65 H, 2.05	C, 24.33 H, 2.31

(12), such inhibition could result in inhibition of oxidative phosphorylation and synthesis of the coenzymes which participate in glycolysis, the tricarboxylic acid cycle, and the electron-transport system. The second possibility may be a direct inhibition of the oxidative enzymes themselves by the compounds. However, these compounds have less inhibitory effect upon respiration than upon the synthesis of nucleic acids and proteins. Consequently, it seems likely that the inhibition of growth of the carcinoma cells by these compounds is attributable to the latter rather than to the former. The elucidation of the sites of inhibition will be the subject for further research.

EXPERIMENTAL[2]

Melting points were taken in capillary tubes in a Mel-Temp apparatus and have been corrected. The designation of a roman numeral followed by an arabic numeral indicates a specific compound number in the table indicated by the roman numeral (Tables I–VI).

Isopropyl-5-phenylvalerate (V)—This compound was prepared by a known procedure (4) from 5-phenylvaleric acid and isopropyl alcohol; yield 79% of crude product; b.p. 102–105° (0.4 mm.).

The isopropyl-4-phenylbutyrate (b.p. 100–102° at 0.4 mm.), isopropyl octanoate, and isopropyl myristate were all prepared in an analogous manner.

Isopropyl α-(3-Phenylpropyl)-γ,γ-dimethoxyacetoacetate (VI)—This compound was prepared by a known procedure (4) from isopropyl 5-phenylvalerate and methyl dimethoxyacetate; yield 99% of crude product.

The isopropyl α-(2-phenylethyl)-γ,γ-dimethoxyacetoacetate, isopropyl α-(*n*-hexyl)-γ,γ-dimethoxyacetoacetate, and isopropyl α-(*n*-dodecyl)-γ,γ-dimethoxyacetoacetate were all prepared by a similar procedure.

2-Bromomethylnaphthalene—A mixture of 56.8 g. (0.4 mole) of 2-methylnaphthalene, 71.2 g. (0.4 mole) of *N*-bromosuccinimide, and 0.2 g. of benzoyl peroxide in 500 ml. of carbon tetrachloride was refluxed with magnetic stirring for 10 hr. The hot reaction mixture was filtered, and the filtrate was evaporated *in vacuo*. The crude product was recrystallized from petroleum ether (30–60°); yield 70.7 g. (80%) of white crystals, m.p. 55–56°.

Anal.—Calcd. for $C_{11}H_9Br$: C, 59.75; H, 4.10. Found: C, 59.73; H, 4.09.

The other chloromethylnaphthanes used were commercially available.

Ethyl α-(4-Phenylbenzyl)-γ,γ-dimethoxyacetoacetate (VI)—To a sodium ethoxide solution prepared from 2.88 g. (0.125-g. atom) of sodium in 75 ml. of absolute ethanol, 23.8 g. (0.125 mole) of ethyl γ,γ-dimethoxyacetoacetate (15) and 25.34 g. (0.125 mole) of *p*-phenylbenzyl chloride were added. The brown solution was refluxed gently with magnetic stirring for 9 hr., during which time sodium chloride separated. The neutral reaction mixture was filtered, and the sodium chloride was washed with 20 ml. of absolute ethanol. The combined filtrate was dried with anhydrous magnesium sulfate for 3 hr., and the ethanol was evaporated *in vacuo* at 80–90°. A light-brown oil was obtained; yield 44 g.

The α-benzyl-β-keto esters and α-naphthylmethyl-β-keto esters were prepared in an analogous manner. Because of the extensive decomposition that occurred during distillation, the crude esters were used for the next step.

2-Mercapto-6-(dimethoxymethyl)-5-(3-phenylpropyl)-4-pyrimidol (VII-2)—To a sodium ethoxide solution prepared from 5.72 g. (0.248 g. atom) of sodium in 200 ml. of absolute ethanol, 80 g. (about 0.248 mole) of crude isopropyl α-(3-phenylpropyl)-γ,γ-dimethoxyacetoacetate and 18.9 g. (0.248 mole) of dried thiourea were added. The mixture was refluxed with magnetic stirring for 12 hr. During the refluxing, a yellowish-brown precipitate separated. The solvent was evaporated *in vacuo*, and the residue was dissolved in 600 ml. of water. The aqueous mixture was filtered, and the filtrate was extracted twice with 200 ml. portions of ether. The aqueous solution was acidified with 10% aqueous HCl, resulting in the separation of a brown oil. This oil solidified gradually in an ice bath after several hours. The brown precipitate was filtered and washed with cold water. Recrystallization from ethanol gave 9.5 g. (12%) of white crystals; m.p. 118–120°. Table VII lists the compounds prepared in an analogous manner.

2-Amino-6-(dimethoxymethyl)-5-(2-phenylethyl)-4-pyrimidol (VII-12)—A mixture of 41 g. (about 0.133 mole) of crude isopropyl α-(2-phenylethyl)-γ,γ-dimethoxyacetoacetate and a solution of guanidine [from 12.7 g. (0.133 mole) of guanidine hydrochloride and 3.06 g. (0.133 g. atom) of sodium in 100 ml. of absolute ethanol] was refluxed with stirring for 6 hr. The white precipitate, which separated during the 1st hour, gradually dissolved to yield a reddish-brown solution. The solution was evaporated to dryness under reduced pressure, and 500 ml. of water was added. The mixture was acidified with acetic acid. After cooling at 0° for several hours, the gummy precipitate was filtered and washed with cold ethanol and ether. The product was recrystallized from ethanol; yield 18 g. (47%) of white crystals, m.p. 219–221°. Table VII lists the compounds prepared by a similar procedure.

2,4-Diamino-6-(dimethoxymethyl)-5-(4-fluorophenyl)pyrimidine (VII-17)—This compound was prepared by a known procedure (3) from guanidine hydrochloride and α-(dimethoxyacetyl)-*p*-fluorophenylacetonitrile; yield 18.7% of white crystals, m.p. 259–260°.

2,4-Diamino-6-(dimethoxymethyl)-5-phenylpyrimidine (VII-16) was prepared in an analogous manner.

2-Mercapto-4-hydroxy-5-(3-phenylpropyl)pyrimidine-6-carboxaldehyde (VIII-2)—A mixture of 2 g. (6.25 mmoles) of VII-2, 20 ml. of 10% aqueous HCl, and 30 ml. of dioxane was refluxed for 1 hr. The cooled yellow solution was diluted with ethanol–water (1:1) to turbidity. After being cooled at 0° for several hours, the crystals were filtered and washed with water. The crude product was recrystallized from ethanol and dried at 100°; yield 1.6 g. (93.5%) of yellow crystals, m.p. 168–170°. Table VIII lists the compounds prepared in an analogous manner.

2-Amino-4-hydroxy-5-(2-phenylethyl)pyrimidine-6-carboxaldehyde (VIII-12)—A mixture of 5 g. (17.3 mmoles) of VII-12, 30 ml. of

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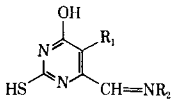
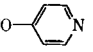
[2] Analyses were performed by M-H-W Laboratories, Garden City, MI 48135

Table X—Schiff Base and Hydrazones of 5-Substituted Pyrimidine-6-carboxaldehydes



No.	R_1	R_2	Yield, %	M.p.	Formula	Anal. Calcd.	Anal. Found
1	$CH_2C_6H_4Cl(4)$	$CH_2CH_2N(CH_3)_2$	80.5	181–183° dec.	$C_{16}H_{19}ClN_4OS$	C, 54.78 H, 5.42	C, 54.71 H, 5.36
2	$CH_2C_6H_4Cl(4)$	$CH_2CH_2CH_2N(CH_3)_2$	62.5	161.5–162.5° dec.	$C_{17}H_{21}ClN_4OS$	C, 55.97 H, 5.76	C, 56.13 H, 5.51
3	$CH_2CH_2C_6H_5$	$CH_2CH_2CH_2N(CH_3)_2$	100	177–178° dec.	$C_{18}H_{24}N_4OS$	C, 62.79 H, 6.97	C, 63.17 H, 6.97
4	$CH_2C_6H_4Cl(4)$	$NHCOC_6H_5$	80	262–264°	$C_{19}H_{15}ClN_4O_2S$	C, 57.21 H, 3.76	C, 57.37 H, 3.92
5	$CH_2C_6H_4Cl(4)$	NHCO— 	100	335–338° dec.	$C_{18}H_{14}ClN_5O_2S$	C, 54.03 H, 3.50	C, 54.05 H, 3.68

10% aqueous HCl, and 50 ml. of dioxane was refluxed for 1 hr. The solvent was evaporated *in vacuo*, and the residue was triturated with 25 ml. of cold water and then dissolved in warm 2-methoxyethanol. The solution was decolorized with charcoal and then poured into 100 ml. of saturated aqueous $NaHCO_3$. The pale-yellow powder was collected on a filter and washed with water. Recrystallization from ethanol–water (1:1) and drying at 100° gave 4 g. (95%) of yellow crystals, m.p. > 300° dec. Table VIII lists the compounds prepared by a similar procedure.

Thiosemicarbazone of 2-Mercapto-4-hydroxy-5-benzylpyrimidine-6-carboxaldehyde (IX-1)—To a warm solution of 0.616 g. (2.5 mmoles) of I*d* in 30 ml. of ethanol was added a warm solution of 0.23 g. (2.5 mmoles) of thiosemicarbazide in 15 ml. of 80% ethanol. The solution was refluxed for 20 min. and filtered while warm. After cooling at 0° for 1 hr., the yellow crystals were filtered and washed with cold ethanol. The crude product was recrystallized from ethanol; yield 0.5 g. (62.5%) of yellow crystals, m.p. 200–201°. Table IX lists the compounds prepared by a similar procedure.

***N*-[2-Mercapto-4-hydroxy-5-(4-chlorobenzyl)-6-pyrimidylmethylidene]-*N*,*N*-dimethylethylenediamine (X-1)**—A stirred mixture of 0.7 g. (2.5 mmoles) of I*b* in 30 ml. of absolute ethanol and 0.22 g. (2.5 mmoles) of *N*,*N*-dimethylethylenediamine was refluxed for 30 min. White to yellow crystals separated. This was filtered, washed with cold ethanol, and recrystallized from ethanol; yield 0.7 g. (80.5%), of yellow crystals, m.p. 181–183° dec. Table X lists the compounds prepared by a similar procedure.

Hydrazones of the Pyrimidine Aldehydes (X-4 and X-5)—To a solution of 2.5 mmoles of the aldehyde I*b* in 50 ml. of 50% aqueous acetic acid was added a solution of 2.5 mmoles of the respective hydrazide in 20 ml. of 50% aqueous acetic acid. It was heated at 80–90° for 10 min., during which time yellow crystals precipitated. After cooling at 0° for 1 hr., the crystalline precipitate was collected by filtration and recrystallized from ethanol. Table X lists these derivatives.

2-Mercapto-6-hydroxymethyl-5-(3-phenylpropyl)-4-pyrimidol (XI) and 2-Mercapto-4-hydroxy-5-(3-phenylpropyl)pyrimidine-6-carboxylic Acid (XII)—To a cold solution of 1.5 g. of KOH in 6 ml. of water was added 1.5 g. of VIII-2. The mixture was left standing at room temperature for a total of 48 hr. The yellow solution was diluted with 20 ml. of water and filtered. To the filtrate, 10% aqueous HCl was added to bring the mixture to pH 7–8, resulting in the precipitation of white crystals (XI). After cooling at 0° for 1 hr., the white crystals were filtered and washed thoroughly with cold water. The filtrate was further acidified with 10% aqueous HCl, resulting in precipitate of yellow crystals (XII). After cooling at 0° for 1 hr., the yellow crystals were filtered and washed with cold water. Both products were recrystallized from ethanol:

The alcohol (XI); yield 0.65 g. (86%), m.p. 214–215°.

Anal.—Calcd. for $C_{14}H_{16}N_2O_2S$: C, 60.87; H, 5.80. Found: C, 61.07; H, 5.84.

The acid (XII); yield 0.65 g. (82%), m.p. 271–273° dec.

Anal.—Calcd. for $C_{14}H_{14}N_2O_3S$: C, 57.93; H, 4.83. Found: C, 57.88; H, 4.98.

2-Mercapto-5-(3-phenylpropyl)-4-pyrimidol (XIII)—A mixture of 0.3 g. (1.03 mmoles) of XII and 20 ml. of tetralin was refluxed for 5 hr. The brown solution was cooled at 0° for 1 hr. The precipitate was filtered and washed with toluene. The crude product was dissolved in 10 ml. of 10% aqueous NaOH and acidified with 10% aqueous HCl to pH 7, resulting in a white precipitate. Recrystallization from ethanol gave 62 mg. (24.5%), m.p. 172–174°.

Anal.—Calcd. for $C_{13}H_{14}N_2OS$: C, 63.41; H, 5.69. Found: C, 62.96; H, 5.86.

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