Synthesis of Potential Anticancer Agents. Pyrido[4,3-*b*][1,4]oxazines and Pyrido[4,3-*b*][1,4]thiazines

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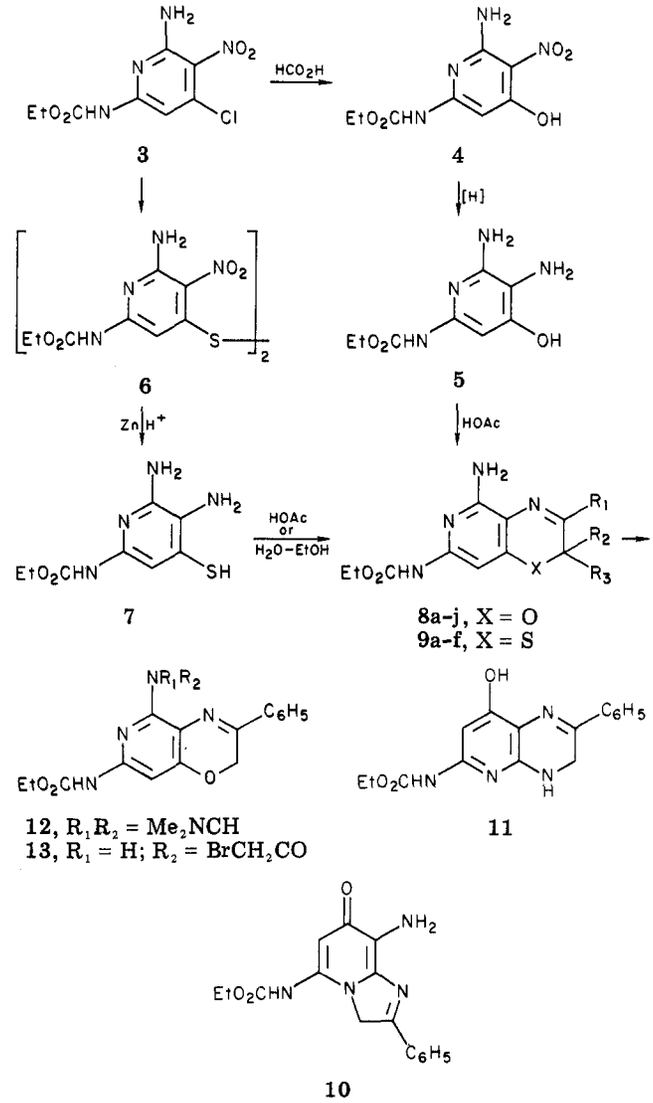
Hydrolysis of the chloro group of ethyl (6-amino-4-chloro-5-nitropyridin-2-yl)carbamate (**3**) with formic acid gave the corresponding 4-hydroxypyridine **4**. Catalytic hydrogenation of the nitro group of **4** gave the 5-amino-4-hydroxypyridine **5**, which was reacted with α-halo ketones in acetic acid at room temperature to give a series of 3- and 2,3-substituted ethyl (5-amino-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl)carbamates **8**. Treatment of **8** with hot concentrated hydrochloric acid regenerated the pyridine synthon **5**. In the reaction of **3** with thioacetate, the product underwent hydrolysis and air-oxidation to give the corresponding disulfide **6**. Simultaneous reduction of both the nitro group and disulfide linkage of **6** gave the 5-amino-4-mercaptopyridine **7**, which was reacted with α-halo ketones either in acetic acid at room temperature or in a mixture of ethanol and water at reflux to give a series of 3-, 2,3-, and 2,2,3-substituted ethyl (5-amino-2*H*-pyrido[4,3-*b*][1,4]thiazin-7-yl)carbamates **9**. The effects of these pyridooxazines and pyridothiazines upon the proliferation and the mitotic index of cultured L1210 cells and upon the survival of mice bearing P388 leukemia were determined.

The vinca alkaloids (vincristine and vinblastine) and the podophyllotoxin derivative VP-16 are valuable clinical antitumor agents.[1] These compounds are thought to act primarily by binding with tubulin,[2] which causes the accumulation of cells at mitosis. This biological action is similar to that produced by colchicine, griseofulvin, and certain substituted benzimidazoles (e.g., nocodazole)[3] and pyrimidines (e.g., 1-propargyl-5-chloropyrimidin-2-one).[4] Recently, a number of 1,2-dihydropyrido[3,4-*b*]pyrazines were identified as antimitotic inhibitors with antitumor activity against several experimental neoplasms.[5-7] As part of a program to investigate the structural features of the 1,2-dihydropyrido[3,4-*b*]pyrazines that are necessary for activity, a series of 2*H*-pyrido[4,3-*b*][1,4]oxazines and 2*H*-pyrido[4,3-*b*][1,4]thiazines was prepared.

In previous work it was demonstrated that oxidation of 1,2-dihydropyrido[3,4-*b*]pyrazines to the corresponding heteroaromatic system destroyed antitumor activity.[6] In order to block in vivo inactivation by this type of transformation, it was desirable to prepare and evaluate some pyridooxazines and pyridothiazines.

Chemistry. The formation of a pyrido[4,3-*b*][1,4]oxazine is limited to one report in which 3-amino-4-hydroxypyridine was reacted with ethyl 2-chloroacetoacetate in a mixture of ethanol and pyridine.[8] In contrast, several groups have investigated the preparation and evaluation of pyrimido[4,5-*b*][1,4]oxazines as potential antifolates.[9] The formation of pyrimido[4,5-*b*][1,4]oxazines from polyfunctional pyrimidines (e.g., 2,4,5-triamino-6-hydroxypyrimidine) and α-halo carbonyl compounds in a mixture of water and ethanol has been studied

Scheme I



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in detail by Wood and co-workers.[10] Although these reactants might possibly generate a number of bicyclic ring systems, only dihydropteridines were observed either as a minor or major byproduct.[10] Apparently, pyrimido[4,5-*b*][1,4]oxazines are only formed from pyrimidines in which the hydroxy group is conjugated with an electron-donating group containing a lone pair of electrons.[10,11]

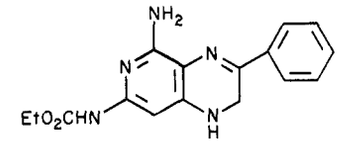
The 5,6-diamino-4-hydroxypyridine **5**,[12] prepared by catalytic hydrogenation of the known nitropyridine **4**,[13] appeared to be an ideal synthon for the formation of pyrido[4,3-*b*][1,4]oxazines. Although 2,4,5-triamino-6-hydroxypyrimidine reacted with α-chloroacetophenone to give the corresponding pyrimidooxazine,[10] treatment of **5** with α-bromoacetophenone under the same conditions gave an unidentified product. The latter was insoluble in weak base and showed a mass peak at *m/e* 310, which would indicate the formation of an oxidized (dehydro) derivative of **8a** or **10**.

The formation of a byproduct in water–ethanol and the instability of **5** toward air prompted the use of other solvents for this reaction. Treatment of **5** with α-bromoacetophenone in acetic acid under nitrogen at room temperature gave a product with the correct mass ion and elemental analysis for the pyrido[4,3-*b*][1,4]oxazine **8a**. The formation of isomeric bicyclic ring systems is limited to **10** and **11**. The ^{1}H NMR spectrum of the product showed an amino group (δ 6.21) and ring methylene moiety (δ 5.20), which is inconsistent with the dihydropteridine **11**. Further support for the presence of an amino group was provided by the condensation of the product with *N,N*-dimethylformamide dimethyl acetal to give **12** and with bromoacetic anhydride to give **13**. In addition, the product was insoluble in aqueous base, which would be unexpected for **11** and possibly **10**, which could form an acidic tautomer. Furthermore, treatment of the product with hot acid regenerated **5**, which is only likely to occur with **8a**. Further experiments showed that the yield of **8a** could be increased by the addition of sodium acetate as a strong acid scavenger. The pyridooxazines **8c,f,h–j** were prepared by hydrogenation of **4** and by reaction of the resulting crude **5** with acetate and the appropriate α-halo ketone. The 2-oxopyridooxazine **8e** was prepared in this manner using methyl benzoylformate. In contrast, the preparation of **8b,d,g** by this procedure was unsuccessful. Reasonable yields of these products were obtained, however, by using **5** that was dried to remove excess ethanol and water. The structures assigned to **8b–f** and **8h–j** were confirmed by cleavage of the oxazine ring with hot acid to give **5**.

Two reports have appeared on the alkylation of 4-mercapto-3-nitropyridine with an α-halocarbonyl compound, followed by reductive cyclization of the resulting thioether to give a pyrido[4,3-*b*][1,4]thiazine.[14,15] Also, pyrimido[4,5-*b*][1,4]thiazines have been prepared by this procedure[16] and by treatment of 5-amino-4-mercaptopyrimidines with α-halocarbonyl compounds.[10] Because of the nucleophilicity of sulfur, relative to oxygen, the formation of isomeric ring systems in either the pyrido or pyrimido systems has not been encountered.

In initial studies directed toward the preparation of **7**,[12] treatment of **3**[17] with sodium hydrogen sulfide gave no identifiable product. In contrast, reaction of **3** with thiourea gave the isothiuronium salt, which was decomposed in base to give the corresponding 5-nitro-4-mercaptopyridine. The latter, however, underwent air-oxidation readily to generate the disulfide **6**. A more convenient method for the preparation of **6** involved the reaction of **3** with potassium thioacetate in ethanol. Simultaneous reduction of the nitro group and disulfide linkage of **6** was effected in acetic acid with zinc dust to give the zinc salt of **7**. The latter, without purification, was treated with α-bromoacetophenone in acetic acid at room temperature to give the pyrido[4,3-*b*][1,4]thiazine **9a**. Similar methods were used for the preparation of **9d–f**. Although the formation of a pyridooxazine from **5** and α-bromoacetophenone in refluxing ethanol and water was unsuccessful, treatment of **7** with α-halo ketones under these conditions provided reasonable yields of **9b** and **9c**.

Biological Evaluation. Certain derivatives of 1,2-dihydropyrido[4,3-*b*]pyrazines (e.g., **1** and **2**) were shown to



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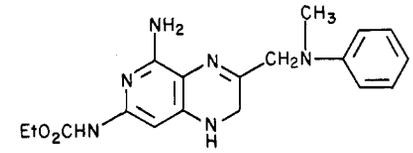

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inhibit the proliferation of cultured L1210 cells and to give significant activity against experimental neoplasms in mice.[5,6] In general, these activities correlated with the number of cells in mitosis in cultured L1210 cells, which suggested that the primary mode of action of this type of compound was binding with tubulin.[18] In **1** and **2**, antitumor activity was promoted by electron-donating groups in the benzene ring and diminished or destroyed by replacement of the 5-amino group, oxidation at the 1,2-bond, and substitution of methyl at N-1.[6] Although the 1-methyl derivative of **1** was prepared to prevent oxidation at the 1,2-bond, the decrease in activity relative to **1** suggested that the methyl group interfered with binding to tubulin. The 2*H*-pyrido[3,4-*b*]oxazines and -thiazines retain the reduced moiety at the 1,2-bond and remove from consideration the steric effect of a substituent at this position.

Preliminary biological data for the pyridooxazines and pyridothiazines are reported in Table III. In the latter (**9a–f**), no significant antitumor activity was observed. These results are consistent with the higher concentrations required to inhibit the proliferation of cells (ID_{50}) and to cause the accumulation of cells at mitosis ($MI_{0.5}$) relative

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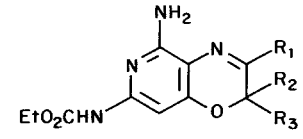
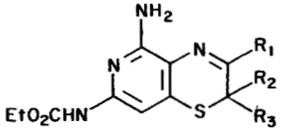
Table I. Ethyl (5-Amino-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl)carbamates



compound	reaction time, h	reaction yield, %	mp, °C	*m/e*[a]	^{1}H NMR[b], chem shift, δ, of ring protons: 2	8	UV[c] (0.1 N HCl) λ_{max}, nm (ε × 10^{-3})	formula	anal.
8a, $R_1 = C_6H_5$; $R_2 = R_3 = H$	24	40	191–193 dec	312	5.20 (s)	6.67 (s)	297 (7.00), 303 sh (6.81), 362 sh (22.1),[d] 372 (23.5)	$C_{16}H_{16}N_4O_3 \cdot 0.26AcOH \cdot 0.5HCl$[e]	C, H, N
8b, $R_1 = C_6H_5$; $R_2 = H$, $R_3 = CH_3$	72	21	235–238 dec	326	6.05 (q)	6.48 (s)	299 (7.07), 306 sh (5.76), 358 sh (21.8), 371 (23.8)	$C_{17}H_{18}N_4O_3 \cdot HCl$	C, H, N
8c, $R_1 = R_2 = C_6H_5$; $R_3 = H$	72	8	187–190 dec	388	6.65 (s)	6.77 (s)	296 (7.09), 306 sh (6.76), 358 sh (20.9),[d] 374 (24.0)	$C_{22}H_{20}N_4O_3 \cdot C_2H_5OH$[f]	C, H, N
8d, R_1, R_2 = 3,2-(2-$C_6H_4CH_2CH_2$); $R_3 = H$	24	31	248–255 dec	338	5.13 (dd)	6.36 (s)	302 sh (7.12), 309 (7.26), 367 sh (22.2),[d] 380 (25.4)	$C_{18}H_{18}N_4O_3 \cdot 1.42HCl$	C, H, N
8e, $R_1 = C_6H_5$; $R_2R_3 = O$	48	74	205–210[h]	326		7.00 (s)	305 sh, 323, 396[g]	$C_{16}H_{14}N_4O_4 \cdot 0.1H_2O$	C, H, N
8f, R_1 = 3-CH_3O-C_6H_4; $R_2 = R_3 = H$	64	52	160–163 dec	342	5.17 (s)	6.68 (s)	300 sh (6.87), 305 (6.93), 361 sh (21.8),[d] 374 (24.9)	$C_{17}H_{18}N_4O_4$	C, H, N
8g, R_1 = 3,5-$(CH_3O)_2$-C_6H_3; R_2 = H; $R_3 = CH_3$	48[i]	22	177–179	386	5.77 (q)	6.72 (s)	302 sh (7.75), 307 (7.97), 359 sh (21.7), 373 (23.5)	$C_{19}H_{22}N_4O_5$	C, H, N
8h, R_1 = 3,4,5-$(CH_3O)_3$ C_6H_2; $R_2 = R_3 = H$	24	70	160–163 dec	402	5.17 (s)	6.70 (s)	302 sh (7.79), 308 (7.93), 367 sh (25.0),[d] 379 (26.8)	$C_{19}H_{22}N_4O_6$	C, H, N
8i, R_1 = 4-ClC_6H_4; $R_2 = R_3 = H$	24	63	>345 dec	346	5.17 (s)	6.69 (s)	298 (7.28), 307 sh (6.96), 367 sh (21.3),[d] 376 (26.1)	$C_{16}H_{15}ClN_4O_3 \cdot 0.23AcOH \cdot 0.23H_2O$	C, H, N
8j, R_1 = 4-$O_2NC_6H_4$; $R_2 = R_3 = H$	16	82	>300 dec	357	5.24 (s)	6.68 (s)	254 (15.7), 310 sh (5.66), 398 (22.9)[d]	$C_{16}H_{15}N_5O_5$	C, H, N

[a] Mass spectra were determined with a Varian MAT 311A spectrometer. [b] ^{1}H NMR spectra were determined in $(CD_3)_2SO$ solutions with tetramethylsilane as an internal reference with a Varian XL-100-15 spectrometer. [c] Cary Model 17 spectrophotometer. Samples were dissolved in 8% methanolic Me_2SO and diluted with 0.1 N HCl. [d] The ε value for the high-wavelength peak decreased slowly with time. [e] ^{1}H NMR spectrum showed the methyl of the acetate at δ 1.93. [f] The presence of solvents was confirmed by the ^{1}H NMR spectra. ^{1}H NMR (H_2O) δ 3.31 (br s). ^{1}H NMR (CH_3CH_2OH) δ 1.06 (t), 3.45 (q). [h] Solidified and remelted at 365–370 °C dec. [i] See Experimental Section. [j] ^{1}H NMR spectrum showed the methyl of the acetate at δ 1.64 and water at δ 3.59.

Table II. Ethyl (5-Amino-2*H*-pyrido[4,3-*b*][1,4]thiazin-7-yl)carbamates



compound	reaction time, h	reaction yield, %	method	mp, °C	*m/e*[a]	^{1}H NMR[b], chem shift, δ, of ring protons: 2	8	UV (0.1 N HCl)[c] λ_{max}, nm ($\epsilon \times 10^{-3}$)	formula	anal.
9a, $R_1 = C_6H_5$; $R_2 = R_3 = H$	40	40	I	197–199 dec	328	3.88 (s)	7.04 (s)	258 (27.0), 393 (19.0)	$C_{16}H_{16}N_4O_2S \cdot 0.09CHCl_3 \cdot 0.33H_2O$[d]	C, H, N
9b, $R_1 = C_6H_5$; $R_2 = H$; $R_3 = CH_3$	1	35	II	>214 dec	342	4.94 (q)	6.80 (s)	258 (26.8), 391 (19.8)	$C_{17}H_{18}N_4O_2S \cdot 0.4C_2H_5OH \cdot HCl$	C, H, N
9c, $R_1 = C_6H_5$; $R_2 = R_3 = CH_3$	8	25	II	170–172 dec	356		6.78 (s)	251 (26.1), 373 (16.5)	$C_{18}H_{20}N_4O_2S \cdot HCl$	C, H, N
9d, $R_1 = 3\text{-}CH_3OC_6H_4$; $R_2 = R_3 = H$	48	27	I	169–173	358	3.87 (s)	7.04 (s)	256 (25.5), 304 sh (8.28), 395 (18.9)	$C_{17}H_{18}N_4O_3S$	C, H, N
9e, $R_1 = 4\text{-}CH_3OC_6H_4$; $R_2 = R_3 = H$	60	28	I	194–196 dec	358	3.84 (s)	7.03 (s)	250 (23.1), 291 (19.6), 399 (23.2)	$C_{17}H_{18}N_4O_3S \cdot 0.05CHCl_3$[c]	C, H, N
9f, $R_1 = 2\text{-}C_{10}H_7$; $R_2 = R_3 = H$	40	40	I	174–177 dec	378	4.04 (s)	7.08 (s)	254 (38.2), 289 (16.8), 297 (16.7), 401 (21.7)	$C_{20}H_{18}N_4O_2S$	C, H, N

[a] Mass spectra were determined with a Varian MAT 311A spectrometer. [b] ^{1}H NMR spectra were determined in $(CD_3)_2SO$ solutions with tetramethylsilane as internal reference with a Varian XL-100-15 spectrometer. [c] Cary Model 17 spectrophotometer. Samples were dissolved in 8% methanolic Me_2SO and diluted with 0.1 N HCl. [d] The presence of solvents was confirmed by the ^{1}H NMR spectra. ^{1}H NMR (H_2O) δ 3.31 (br s). ^{1}H NMR (CH_3CH_2OH) δ 1.06 (t), 3.45 (q). ^{1}H NMR ($CHCl_3$) δ 8.31.

Table III. Biological Data for Ethyl (5-Amino-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl)carbamates and Ethyl (5-Amino-2*H*-pyrido[4,3-*b*][1,4]thiazin-7-yl)carbamates

compd	L1210[a] ID_{50}, μM	L1210[b] $MI_{0.5}$, μM	P388[c] 10^6 tumor cell implant, ip schedule	% ILS (mg/kg)[d]
8a	0.225	0.56	1–5	44 (100)
				80 (200)[e]
8b	0.11	0.49	1–5	49 (100)
8c	0.88	3.0	1–5	2 (200)
8d	>0.3		1–5	32 (300)
8e	3		1–5	0 (300)
8f	0.052	0.10	1–5	73 (50)[f]
8g	1.25	2.9	1–5	
8h	>3		1–5	0 (300)
8i	>3		1–5	0 (300)[f]
8j	>3		1–5	0 (300)
12	0.31	0.78	1–5	1 (100)[g]
13	2.2	11		
9a	6.1	>10	1–5	0 (300)
9b	1.6	9.6	1–5	10 (150)
9c	>10			
9d	0.53	2.0	1–5	3 (75)
9e	0.34	4.9	1–5	3 (300)
9f	0.58	3.0	1–5	5 (300)

[a] Concentration of agent that inhibits proliferation of cultured lymphoid leukemia L1210 cells to 50% control growth during 48 h (ref 7). [b] Concentration of agent that causes a mitotic index (fraction of cells in mitosis divided by total cells) of 0.5 for cultured lymphoid leukemia L1210 cells during an exposure period of 12 h (see ref 7). [c] Lymphocytic leukemia P388 (ref 19). [d] Increase in life span at the highest nontoxic dose tested. [e] Toxic by weight change at a dose of 400 mg/kg. [f] Toxic by weight change at this dose. [g] Toxic by weight change at a dose of 200 mg/kg.

to 1. Of particular interest was the proliferation data for **9a–c**. Inhibition decreased in the order 2-methyl (**9b**) > parent (**9a**) > 2,2-dimethyl (**9c**), which suggested that disubstitution at the 2-position was detrimental to activity.

In the pyridooxazine series, significant antitumor activity was observed for **8a,b,d,f** but not for **8c,e,g–j**. In general, activity was observed for those compounds that gave low concentrations for the ID_{50} and $MI_{0.5}$ (Table III). Although the 3-methoxyphenyl compound **8f** was active, the presence of three adjacent electron-donating methoxy groups on the benzene ring as in **8h** diminished activity. Previously, substitution of a methyl group at the 2-position of **1** was shown to increase activity.[6] The diminished activity of **8d** in which the ortho position of the 3-phenyl group is joined via an ethylene group to the 2-position of the pyrazine ring probably indicates that the ring must be free to assume a preferred orientation before binding to tubulin. This might also be reflected in the lack of activity of the 2,3-diphenyl derivative **8c** in which the 2-phenyl group interferes with free rotation of the 6-phenyl group.

The ID_{50} and $MI_{0.5}$ results obtained with **12** are similar with those observed for **8a**, which probably indicates that **12** is hydrolyzed to **8a** under the test conditions. In the bromoacetylated compound **13**, higher concentrations are required to reach the ID_{50} and $MI_{0.5}$ relative to **8a**. This result probably can be attributed to a decrease in the basicity of the 5-amino group.

The absence of antitumor activity in the pyridothiazines and the higher doses required for activity in the pyridooxazine analogues of the 1,2-dihydropyrido[4,3-*b*]pyrazines indicate that the 1-NH of the latter plays an important role in binding to tubulin. Previous results indicated that the 5-amino group and a 3-substituent containing a

benzene ring were necessary for activity. Presumably, these groups are also important for binding to tubulin.

Experimental Section

Typical procedures are given for the preparation of the compounds listed in Tables I and II. The α-halo ketones and methyl benzoylformate were purchased from commercial sources, except for α-bromo-3,4,5-trimethoxyacetophenone,[20] 2-bromo-1-tetralone,[21] and α-bromo-3,5-dimethoxypropiophenone,[22] which were prepared by bromination of the corresponding ketones. Raney nickel catalyst no. 28 was obtained from W. R. Grace and Co. Melting points were determined with a Mel-Temp apparatus and are uncorrected. Thin-layer chromatograms were performed on Analtech precoated (250 μm) silica gel G(F) plates, which were usually developed with mixtures of $CHCl_3$ and MeOH.

Ethyl (5,6-Diamino-4-hydroxypyridin-2-yl)carbamate (5). A solution of the nitropyridine **4** (6.7 g, 28 mmol)[13] in a 1:1 mixture of EtOH–H_2O (600 mL) containing Raney nickel (6 g, weighed wet, washed with H_2O and then EtOH) was stirred under hydrogen at room temperature and atmospheric pressure until the theoretical volume of hydrogen was absorbed (1.5 h). The catalyst was removed by filtration (Celite), and the filtrate was acidified with concentrated HCl (2.3 mL) and evaporated to dryness in vacuo. The residue was triturated with EtOH (50 mL), the mixture was cooled, and the product was collected by filtration and dried in vacuo over P_2O_5: yield 7.0 g (92%); mp ~210 °C with foaming; ^1H NMR (Me_2SO-d_6, 5% w/v) δ 1.27 (t, 3, CH_3), 4.22 (q, 2, CH_2), 6.63 (s, 1,3-CH), 8.56 (br s, NH, H_2O), 11.03 (br s, 1 H, NH). Anal. ($C_8H_{12}N_4O_3$·1.68HCl·0.14H_2O) C, H, Cl, N.

Diethyl 4,4′-Dithiobis[(6-amino-5-nitropyridin-2-yl)carbamate] (6). A. A solution of the 4-chloropyridine **3** (2.6 g, 10 mmol)[17] and potassium thioacetate (1.7 g, 15 mmol) in EtOH (50 mL) was refluxed for 2 h. The yellow solid that precipitated was collected by filtration, washed with EtOH (50 mL), and stirred in H_2O (100 mL) for 16 h. The yellow-orange product was collected by filtration and dried in vacuo: yield 2.0 g (78%); mp ~255 °C dec; mass spectrum, m/e 514 (M^+); ^1H NMR (Me_2SO-d_6, 6%, w/v) δ 1.18 and 1.20 (2 t, 3, 3, CH_3), 4.08 and 4.11 (2, q, 2, 2, CH_2), 7.66 and 7.74 (2 s, 1, 1, 2-CH, 2′-CH), 7.74 and 8.14 (2 br s, 2, 2, NH_2), 10.4 (s, 2 H, NH). Anal. ($C_{16}H_{18}N_8O_8S_2$·0.15C_2H_6O) C, H, N.

B. A solution of **3** (2.6 g, 10 mmol) and thiourea (3.1 g, 40 mmol) in EtOH (50 mL) was refluxed under N_2 for 5 h, cooled to 25 °C, treated with 1 N NaOH (10 mL, 10.0 mmol), and stirred (exposed to air) at room temperature for 24 h. The pale green precipitate was collected by filtration, washed with H_2O, and heated in refluxing EtOH (60 mL) to give crude **6**: yield 2.0 g (~79%); mass spectrum, m/e 514 (M^+).

Ethyl [5-Amino-3-(4-chlorophenyl)-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl]carbamate (8h). A solution of **4** (1.0 g, 4.1 mmol) in 1:1 H_2O–EtOH (100 mL) was hydrogenated as described above for the preparation of **5**. The filtrate was acidified with 1 N HCl (8.5 mL) and evaporated to dryness in vacuo. A solution of this residue in acetic acid (22 mL) containing α-bromo-*p*-chloroacetophenone (1.0 g, 4.3 mmol) and potassium acetate (1.2 g, 12 mmol) was stirred under N_2 at room temperature for 24 h. The yellow precipitate was collected by filtration, washed with acetic acid (15 mL), and suspended in water (50 mL). This mixture was adjusted to pH 8.5 with 1 N NaOH, and the product was collected by filtration. A similar procedure was used to prepare **8a,c,e,f,h–j**. In order to obtain the yields reported in Table I for **8b,d,g**, it was necessary to use **5**, which had been washed with ethanol and dried in vacuo over P_2O_5. In addition, reaction of a dried sample of **5** with α-bromoacetophenone increased the yield of **8a** from 40 to 77%.

In the preparation of **8g**, the reactants were heated at 55 °C for 48 h, which also resulted in the generation of an unidentified byproduct (TLC). The mixture was eluted from a short column of EM silica gel 60 (45 × 65 mm, 230–400 mesh) with $CHCl_3$ to remove unreacted α-bromoketone and with $CHCl_3$–CH_3OH (99:1) to give **8g** containing a trace amount of impurity.

Cleavage of the Oxazine Ring of 2*H*-Pyrido[4,3-*b*][1,4]oxazines with Hydrochloric Acid. A solution of **8a** (100 mg, 0.32 mmol) in concentrated HCl (5 mL) was heated at ~100 °C for 48 h and evaporated to dryness. TLC (7:3 $CHCl_3$/MeOH, 5% HOAc) showed the formation of **5** (R_f 0.48): mass spectrum, m/e 212 (M^+). Under similar conditions, **8b–f** and **8h–j** gave **5**.

Ethyl (5-Amino-3-phenyl-2*H*-pyrido[4,3-*b*][1,4]thiazin-7-yl)carbamate (9a). Method I. To a solution of the disulfide **6** (6.00 g, 11.5 mmol) in acetic acid (240 mL) under N_2 at 80 °C was added zinc dust (23 g) portionwise over 0.5 h. After the mixture was heated to reflux and then cooled, the insoluble material was removed by filtration and washed with acetic acid (30 mL). The filtrate and wash were combined and evaporated to dryness, and the resulting brownish oil was triturated with an aqueous solution of 0.1 M KH_2PO_4 (200 mL) to give the crude zinc salt of **7** as a light, blue-tinted solid: yield 6.5 g. A portion of this solid (2.0 g) and α-bromoacetophenone (3.1 g, 15 mmol) in acetic acid (30 mL) was stirred under N_2 for 40 h. The yellow solid was collected by filtration, washed well with H_2O (50 mL) and EtOH (50 mL) and dissolved in $CHCl_3$. The solution was applied to a short column of EM silica gel 60 (65 × 35 mm, 230–400 mesh) and eluted with $CHCl_3$, which retained unreacted **7**. The fractions containing product were combined, evaporated to dryness in vacuo, and dried in vacuo: yield 0.95 g.

Ethyl (5-Amino-2-methyl-3-phenyl-2*H*-pyrido[4,3-*b*]-[1,4]thiazin-7-yl)carbamate (9b). Method II. The zinc salt of **7** (2.0 g), prepared as described in method I, was dissolved in H_2O (12 mL) and a solution of α-bromopropiophenone (3.4 g, 15 mmol) in EtOH (12 mL) was added with stirring over 5 min. The resulting solution was refluxed for 1 h and evaporated to dryness in vacuo. The dried residue was dissolved in $CHCl_3$ and eluted from a short column of EM silica gel 60 (65 × 40 mm, 230–400 mesh) with $CHCl_3$. The fractions containing product were combined and evaporated to dryness in vacuo. The residue was dissolved in EtOH and acidified with concentrated HCl (0.25 mL) to precipitate the hydrochloride: yield 0.85 g.

Ethyl [5-[[(Dimethylamino)methylene]amino]-3-phenyl-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl]carbamate (12). A solution of **8a** (165 mg, 0.530 mmol) and *N,N*-dimethylformamide dimethyl acetal (630 mg, 5.30 mmol) in *N,N*-dimethylformamide (4 mL) was stirred under N_2 at room temperature for 24 h. The solvent was removed in vacuo on a warm water bath at 35 °C, and the resulting semicrystalline yellow residue was triturated with Et_2O and collected by filtration: yield 100 mg (52%); mp 158–161 °C; mass spectrum, m/e 367 (M^+); ^1H NMR ($CDCl_3$, 3.75%, w/v) δ 1.30 (t, 3 H, OCH_2CH_3), 3.10 (s, 3 H, N–CH_3), 3.19 (s, 3 H, N–CH_3), 4.23 (q, 2 H, OCH_2CH_3), 5.04 (s, 2 H, 2-CH_2), 7.21 (br s, 1 H, $NHCO_2$), 7.20 (s, 1 H, 8-CH), 7.44 (m, 3 H, aromatic CHs), 7.91 (m, 2 H, aromatic CHs), 8.28 [s, 1 H, $CHN(CH_3)_2$]. Anal. ($C_{19}H_{21}N_5O_3$) C, H, N.

Ethyl [5-[(Bromoacetyl)amino]-3-phenyl-2*H*-pyrido[4,3-*b*][1,4]oxazin-7-yl]carbamate (13). A heterogeneous slurry of bromoacetic anhydride (7 g) and **8a** (350 mg, 1.12 mmol) was heated at ~50 °C for 2 h. The cooled solution was diluted to twice its volume with anhydrous ether and stirred for 5 min. The hydrobromide was collected by filtration (450 mg), suspended in H_2O (50 mL), and adjusted to pH 7 with 1 N NaOH. The solid was collected by filtration, washed with H_2O (15 mL), and dried. An incomplete solution of this material (340 mg) in $CHCl_3$ was eluted with $CHCl_3$ from a short column of EM silica gel 60 (32 × 35 mm, 230–400 mesh). The fractions containing product were evaporated to give a light yellow solid: yield 200 mg (41%); mp >230 °C with dec; ^1H NMR (CF_3COOD, 5%, w/v) δ 1.45 (t, 3 H, OCH_2CH_3), 4.36 (s, 2 H, CH_2Br), 4.49 (q, 2 H, OCH_2CH_3), 5.63 (s, 2 H, 2-CH_2), 6.65 (s, 1 H, 8-CH), 7.63 and 8.0 (2 m, 3 H, 2 H, aromatic CHs). Anal. ($C_{18}H_{17}BrN_4O_4$·0.4H_2O) C, H, Br, N.

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Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations.

Registry No. **3**, 6506-86-1; **4**, 86970-41-4; **5**, 86970-42-5; **6**, 86970-43-6; **7**, 86970-44-7; **8a**·$^1/_2$HCl, 86970-45-8; **8b**·HCl, 86970-46-9; **8c**, 86970-47-0; **8d**·HCl, 86970-48-1; **8e**, 86970-49-2; **8f**, 86970-50-5; **8g**, 86970-51-6; **8h**, 86970-52-7; **8i**, 86970-53-8; **8j**, 86970-54-9; **9a**, 86970-55-0; **9b**·HCl, 86970-57-2; **9c**·HCl, 86970-58-3; **9d**, 86970-59-4; **9e**, 86970-60-7; **9f**, 86970-61-8; **12**, 86970-62-9; **13**, 86970-63-0; **13**·HBr, 86970-64-1; α-bromo-3,4,5-trimethoxyacetophenone, 51490-01-8; α-bromo-3,5-dimethoxypropiophenone, 72661-28-0; 2-bromo-1-tetralone, 13672-07-6; potassium thioacetate, 10387-40-3; thiourea, 62-56-6; α-bromo-*p*-chloroacetophenone, 536-38-9; α-bromoacetophenone, 70-11-1; α-bromopropiophenone, 2114-00-3; *N*,*N*-dimethylformamide dimethyl acetal, 4637-24-5; bromoacetic anhydride, 13094-51-4; **7**·Zn, 86970-65-2; **9b**, 86970-56-1.

Potential Antitumor Agents. 38. 3-Substituted 5-Carboxamido Derivatives of Amsacrine

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The synthesis and biological evaluation of a series of 3-substituted 5-carboxamido derivatives of amsacrine (*m*-AMSA) are described. This series was developed as the result of previous quantitative structure–activity relationship (QSAR) studies of the antitumor activity of 9-anilinoacridine derivatives. In agreement with these studies, this class of compounds, possessing a variety of small nonpolar groups at the 3-position, together with very hydrophilic carboxamido groups at the 5-position, have high in vivo activity against animal leukemia models.

The clinically useful antileukemic agent amsacrine (*m*-AMSA)[1-3] is a member of the 9-anilinoacridine class of antitumor agents. Compounds of this class bind tightly to double-stranded DNA[4,5] by intercalation, as shown by helix-unwinding studies with closed circular DNA[6] and by high-field NMR studies with very short DNA fragments.[7] In common with other DNA-binding agents, such as the anthracyclines and the anthracenediones, amsacrine and other 9-anilinoacridines cause DNA breaks[8] and chromosome damage.[9] Studies of 9-anilinoacridines[10] have shown a significant correlation between antitumor activity and DNA association constants, determined in vitro by using either calf thymus DNA or poly(dA-dT). Thus, the DNA-binding ability of these compounds is thought to be an important component in the mechanism of their biological activity.

Work over the last several years on the general class of 9-anilinoacridine antitumor agents has shown the extraordinarily wide range of structural variations permitted to the parent structure while still retaining biological activity.[3] these structural variations, by modulating various aspects of the binding of the drug to DNA, can have a significant effect on biological activity, both in vivo and in vitro. For the several hundred structures examined so far, in vivo potency against the L1210 leukemia (the dose required to give a standard response of a 40% increase in life span) varies by greater than 3000-fold,[11] while in vitro potency against the same tumor varies by more than 60 000-fold.[12]

Recently, we have published an extensive QSAR study for the in vivo antileukemic (L1210) activity of a large number of 9-anilinoacridine derivatives.[11] While overall drug lipopohilicity and pK_a values (determined by summation of the electronic contributions of all substituent groups on the molecule) were both found to influence antitumor potency, by far the most significant influence was the steric effects of groups placed at various positions on the 9-anilinoacridine skeleton. The current[3,4,11] model for the binding of 9-anilinoacridines to DNA was based on the published[12] X-ray crystallographic data for the pseudosymmetric orientation found for 9-aminoacridine bound to iodo-dCpG and assumes intercalation of the acridine chromophore with the 9-anilino group lying in the minor groove and the 4- and 5-positions of the acridine ring oriented toward the major groove. Crystallographic studies of the DNA intercalator ethidium bromide complexed with dinucleotides have shown a similar orientation of the phenyl ring of this compound.[13] X-ray studies of several

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