10- to 20-fold by DEAE-cellulose and ammonium sulfate precipitation. The inhibition test medium was 2.1 mM in ATP, 6 mM in magnesium acetate, 20 μM in ferrous ion, 6 mM in dithiothreitol, 0.25 μM in thioredoxin (*Escherichia coli*), 8 mM in potassium phosphate (pH 7), and either 80 μM in [^{32}P]CDP (cytidine diphosphate) or 170 μM in [^{14}C]CDP and contained sufficient enzyme to reduce the concentration of CDP by 12.5–25 μM in 30 min. The reaction product was separated as dCMP (deoxycytidine monophosphate) on Dowex 50^{10} or as dCDP and dCTP on boronate columns.[17]

The inhibitors were dissolved in water and neutralized, except for **3**, which was dissolved in dimethyl sulfoxide and diluted with water to give less than 1% dimethyl sulfoxide in the final solution. The enzyme was added to ice-cold mixtures of the substrates and inhibitors. The solutions were immediately incubated at 37 °C for 30 min. Each inhibitor was tested in four separate runs.

^{15}N NMR Studies. The *N*-hydroxyguanidine derivatives were synthesized as described elsewhere.[4] The ^{15}N NMR spectra were obtained with a Bruker WH-180 spectrometer operating at 18.25 MHz in the FT mode. The ^{15}N NMR spectra of 2-[2-pyridylmethylene)diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**1**), 2-[[(3-methyl-2-thienyl)methylene]diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**2**), and 2-[[(3-iodophenyl)methylene]diazanyl]-*N*-hydroxymethanamidinium 4-methylbenzenesulfonate (**3**) were taken at the natural-abundance level in 25-mm sample tubes, with broad-band decoupling, a repetition rate of 5 s, and a pulse width of 35 μs (34° flip angle). The concentrations were about 0.3 M.

The ^{15}N NMR chemical shifts are reported in parts per million upfield from an external standard made up to be 1 M $H^{15}NO_3$ in D_2O.

The pH dependence of the ^{15}N NMR chemical shifts of the salts of **1**–**4** were determined in water solution, with the pH values were adjusted by addition of small increments of concentrated sodium hydroxide or hydrochloric acid, with the pH being measured directly in the NMR sample tube with the aid of a Radiometer pH meter.

Registry No. **1**, 85894-16-2; **2**, 85894-18-4; **3**, 85894-20-8; ribonucleoside diphosphate reductase, 9047-64-7.

(17) Moore, E. C.; Peterson, D.; Yang, L.-Y.; Yeung, C.-Y.; Neff, N. F. *Biochemistry* **1974**, *13*, 2904–2907.

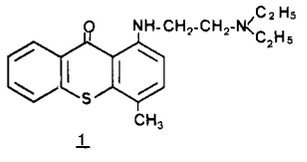
Aza Analogues of Lucanthone: Synthesis and Antitumor and Bactericidal Properties

Martine Croisy-Delcey and Emile Bisagni*

Laboratoire de Synthèse Organique, Institut Curie, Section de Biologie, Bâtiments 110-112, 91405 Orsay Cedex, France.
Received December 30, 1982

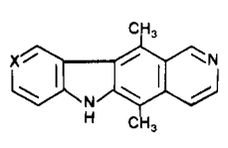
Three types of aza analogues of lucanthone were synthesized for evaluation as antitumor drugs. None of the compounds was found to have significant cytotoxic effects either on Friend tumor cells or on L1210 leukemia cells. However, one of the target compounds, 5,10-dihydro-10-oxo-1-[[3-(diethylamino)propyl]amino]-3-methylpyrido[4,3-*b*]quinoline, was shown to have noticeable antibiotic properties.

Lucanthone (**1**, Miracil D) is an important schistosomicidal agent whose antiparasitic properties have been known for a long time.[1,2] Besides this clinical property, **1** is also effective against a wide range of experimental laboratory tumors[3,4] and shows a strong affinity for nucleic acids,[5,6] including DNA, with which it interacts through intercalation.[7,8] A large variety of lucanthone analogues have been synthesized and tested for antitumor activity,[3,9,10] the most potent compound being the 7-hydroxy derivative of **1**.[9]

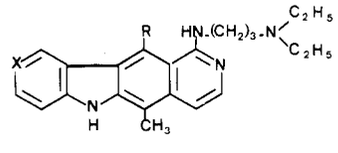


1

Several members of the ellipticine series, another family of intercalating drugs, are known to have antitumor properties. As observed with lucanthone derivatives, ring-hydroxylated compounds, such **2b**, as well as the methoxy analogue **2c**, are more potent than the unsubstituted ellipticine **2a**.[11,12]



2a. X = C-H
2b. X = C-OH
2c. X = C-OCH$_3$
2d. X = N



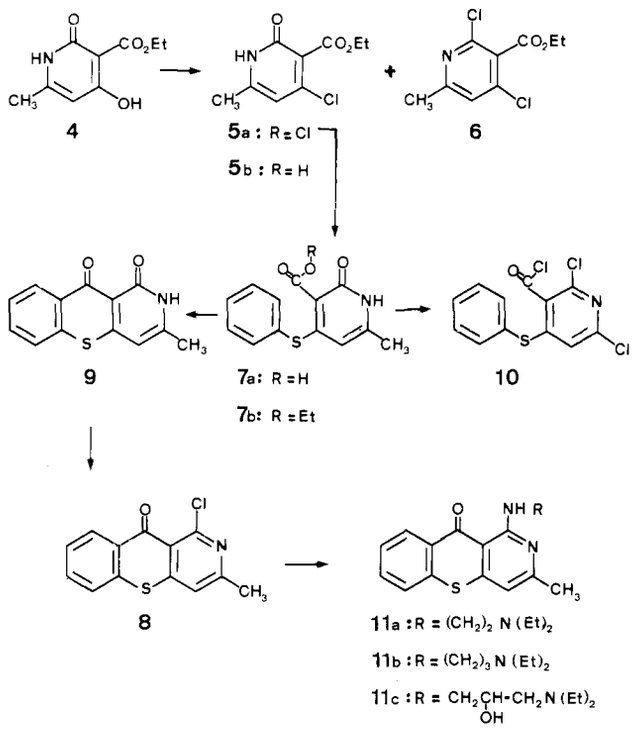
3a. X = C-OCH$_3$ R = CH$_3$
3b. X = N R = H

On the one hand, studies carried out in this laboratory on 9-azaellipticine (**2d**) have shown that replacement of

(1) Kikuth, W.; Gonnert, R.; Mauss, H. *Naturwissenschaften* **1946**, *33*, 253.
(2) Mauss, H. *Chem. Ber.* **1948**, *81*, 19.
(3) Blanz, E. J.; French, F. A. *J. Med. Chem.* **1963**, *6*, 185.
(4) Hirschberg, E.; Gellhorn, A.; Murray, M. R.; Elslager, E. F. *J. Natl. Cancer Inst.* **1959**, *22*, 567.
(5) Hirschberg, E.; Weinstein, B.; Gersten, N.; Marner, E.; Finkelstein, T.; Carckman, R. *Cancer Res.* **1968**, *28*, 601.
(6) Waring, M. *J. Mol. Biol.* **1970**, *54*, 247.
(7) Sarma, M. H.; Mitra, C. K.; Sarma, R. H.; Miller, K. J.; Archer, S. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1285.
(8) Gale, E. E.; Cundliffe, E.; Reynold, P. E.; Richmond, M. H.; Waring, M. J. "The Molecular Basis of Antibiotic Action"; Wiley: New York, 1972; p 188.
(9) Archer, S.; Miller, K. J.; Rej, R.; Periana, C.; Fricker, L. *J. Med. Chem.* **1982**, *25*, 220.
(10) Archer, S.; Rej, R. *J. Med. Chem.* **1982**, *25*, 328.
(11) Le Pecq, J. B.; Gosse, C.; Xuong, N. D.; Paoletti, C. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1975**, *281*, 1365.
(12) Le Pecq, J. B.; Gosse, C.; Xuong, N. D.; Cros, S.; Paoletti, C. *Cancer Res.* **1976**, *36*, 3067.

0022-2623/83/1826-1329$01.50/0

Scheme I

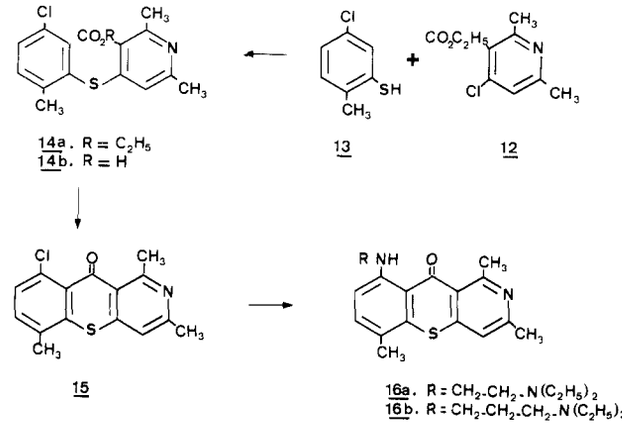


carbon 9, which is the target for metabolic activation,[13] by a nitrogen atom significantly increases the antitumor properties.[14] The same effect was observed upon introduction of a [(dialkylamino)alkyl]amino side chain on carbon 1 of the ellipticine skeleton, as shown by the high antineoplasic activity of compound **3a** and, as a result of the combination of both modifications, of the nitrogen analogue **3b** (known as BD 40),[15] which is presently under clinical trials. On the other hand, affinity for DNA appears to depend on the pK of the intercalating system.[16] These reasons led us to undertake the synthesis of various compounds bearing a nitrogen atom in one of the positions (2 or 7) of the benzene rings of the tricyclic system of lucanthone, as well as the synthesis of two aza analogues resulting from the replacement of the sulfur atom by a nitrogen.

Chemistry. Chlorination of ethyl 1,2-dihydro-2-oxo-4-hydroxy-6-methylpyridine-3-carboxylate (**4**)[17] in refluxing phosphorus oxychloride afforded a mixture of ethyl 1,2-dihydro-2-oxo-4-chloro-6-methylpyridine-3-carboxylate (**5a**) and ethyl 2,4-dichloro-6-methylpyridine-3-carboxylate (**6**).

The structure of compound **5a** has been proved through dehalogenation to the ester **5b**, which shows a characteristic AB system in its NMR spectrum. This compound reacted with benzenethiol in refluxing ethyl alcohol to give directly acid **7a**, resulting from concomitant hydrolysis of the intermediary **7b** by hydrochloric acid liberated in situ.

Scheme II



Acid **7a** was converted to the chloro derivative 8 following two different procedures.

Method A involved cyclization of **7a** to 3-methyl-10H-[1]benzothiopyrano[3,2-c]pyridine-1,10(2H)-dione (**9**) with polyphosphoric acid at 120 °C and subsequent chlorination. However, in spite of various attempts, using various halogenating agents ($POCl_3$ alone or with PCl_5, $SOCl_2$, and PBr_3 with or without solvent), only phosphorus oxychloride allowed us to obtain the expected compounds. Under these conditions, compound 8 was obtained in very low yield (10 to 15%) mixed with a high proportion of byproducts.

Method B involved chlorination of **7a** with phosphorus pentachloride in phosphorus oxychloride and cyclization of the resulting chloride of 2-chloro-4-(phenylthio)-6-methylpyridine-3-carboxylic acid (**10**) with stannic chloride in benzene solution. In this case, the overall yield of 8 was 25% from **7a**, and purification occurred without difficulty (Scheme I).

Then, the aza analogues of lucanthone (**11a-c**) were easily obtained through substitution of the intermediate chloro derivative by the appropriate amine in refluxing ethyl alcohol.

Ethyl 2,6-dimethyl-4-chloropyridine-3-carboxylate (**12**)[18] reacted with 2-methyl-5-chlorobenzenethiol (**13**)[19] to give the ester **14a**, which was directly converted to the corresponding acid **14b** by alcoholic potassium hydroxide. Cyclization by means of polyphosphoric acid at 120 °C then afforded 1,3,6-trimethyl-9-chloro-10H-[1]benzothiopyrano[3,2-c]pyridin-10-one (**15**), which was easily converted to the lucanthone analogues **16a** and **16b** by heating at 120 °C in the appropriate [(dialkylamino)alkyl]amine (Scheme II).

Chloropyridinone **5a** also reacted with aniline in ethyl alcohol in the presence of triethylamine to form ethyl 1,2-dihydro-2-oxo-4-anilino-6-methylpyridine-3-carboxylate (**17a**). In refluxing 2-ethoxyethanol (bp 135 °C) only the anilino pyridinone **17b** was obtained, probably through acidic hydrolysis of the ester **17a** and subsequent thermal decarboxylation.

As expected, cyclization of **17a** in refluxing diphenyl oxide gave 1,2,5,10-tetrahydro-1,10-dioxo-3-methylpyrido[4,3-b]quinoline (**18**), which upon treatment with phosphorus oxychloride furnished a low yield of a mixture of 1,2-dihydro-1-oxo-10-chloro-3-methylpyrido[4,3-b]-

(13) Braufman, A. R.; Bruni, R. J.; Reinhold, V. N.; Silveira, D. M.; Chaudwick, M.; Yesair, D. W. *Drug. Metab. Disp.* **1978**, *6*, 542.
(14) Lidereau, R.; Chermann, J. C.; Gruest, J.; Montagnier, L.; Ducrocq, C.; Rivalle, C.; Bisagni, E. *Bull. Cancer* **1980**, *67*, 1.
(15) Ducrocq, C.; Wendling, F.; Tourbez-Perrin, M.; Rivalle, C.; Tambourin, P.; Pochon, F.; Bisagni, E.; Chermann, J. C. *J. Med. Chem.* **1980**, *23*, 1212.
(16) Le Pecq, J. B.; Xuong, N. D.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(17) E. Knoevenagel and K. Fries, "Beilsteins Handbuch der Organischen Chemie", 4th ed.; Springer: Berlin, 1935; Vol 22, p 258.
(18) Bachman, G. B.; Barker, R. S.; *J. Org. Chem.* **1949**, *14*, 97.
(19) Sharp, T. S. *J. Chem. Soc.* **1951**, 2961.
(20) Koogman, E. C.; Wibault, J. P. *Recl. Trav. Chim. Pays-Bas* **1946**, *65*, 10.

Table I. Antibiotic Activity of 5,10-Dihydro-10-oxo-1-[[3-(diethylamino)propyl]amino]-3-methylpyridyl[4,3-*b*]quinoline (21)[a]

dose, mol	*C. albicans*	*S. aureus* ATCC6358P	*B. subtilis* ATCC6633	*E. coli* ATCC10536
1.8×10^{-8}	–	+/–	–	+
3.6×10^{-8}	–	+/– (b)	+	+
7.2×10^{-8}	– (a)	+/–	+	++

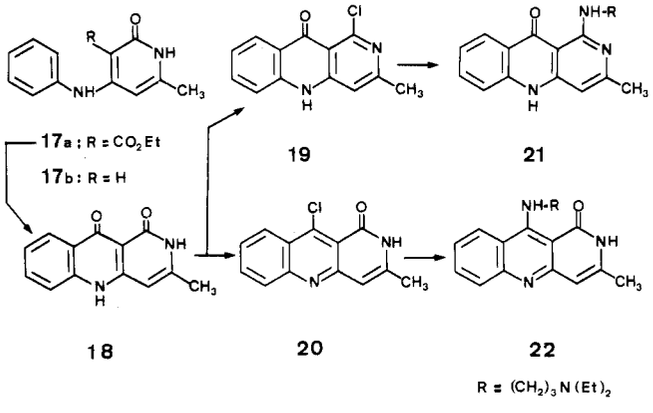
[a] Growth inhibition test (see Experimental Section): –, negative result; +/–, slightly positive result; +, inhibition zone 6-10 min; ++, (a) slight constraint, (b) transient positive result.

Table II. Substituted Pyridines and Tricyclic Intermediate Compounds

no.	formula	mp, °C	recrystn solvent	yield, %	anal.
5a	$C_9H_{10}ClNO_3$	135-137	EtOH	38	C, H, N, Cl
5b	$C_9H_{11}NO_3$	243[a]	benzene-cyclohexane (1:1)	88	C, H, N
6	$C_9H_9Cl_2NO_2$	58[b]	hexane	33	C, H, N, Cl
7a	$C_{13}H_{11}NO_3S$	257	EtOH	80	C, H, N
8	$C_{13}H_8ClNOS$	210	cyclohexane	14.8 (A), 25 (B)	C, H, N, Cl
9	$C_{13}H_9NO_2S$	280	EtOH	65	C, H, N
14b	$C_{15}H_{14}ClNO_2S \cdot H_2O$	155	EtOH-H_2O (1:1)	78	C, H, N
15	$C_{15}H_{12}ClNOS$	192	EtOH	74	C, H, N
17a	$C_{15}H_{16}N_2O_3$	219	EtOH	83	C, H, N
17b	$C_{12}H_{12}N_2O$	307	EtOH	70	C, H, N
18	$C_{13}H_{10}N_2O_2 \cdot H_2O$	>350	HOAc	58	C, H, N
19	$C_{13}H_9ClN_2O$	260	EtOH	5	Cl
20	$C_{13}H_9ClNO \cdot 0.5EtOH$	>300	EtOH	25	C, H, N, Cl

[a] Sublimes 235 °C. [b] Literature[20] mp 56 °C.

Scheme III



quinoline (**20**) and 5,10-dihydro-10-oxo-1-chloro-3-methylpyrido[4,3-*b*]quinoline (**19**), which were isolated by column chromatography on silica gel.

Substitution of both chloro derivatives **19** and **20** was accomplished by heating at 140 °C in an excess of 3-(diethylamino)propylamine to afford the [3-(diethylamino)propyl]amino compounds **21** and **22** (Scheme III).

Biological Assays. In vitro cytotoxicity of [(dialkylamino)alkyl]amino compounds **11a–c** on Friend tumor cells was studied by using the standard procedure developed in this Institute;[15] none was found significantly cytotoxic until 10^{-3} M concentrations. Additionally, compounds **11a**, **16a**, and **16b** were tested in vitro on the L1210 leukemia system, without any positive results.

All the compounds were also submitted to a general screening for antibiotic activity using four strains of microorganisms, *Candida albicans*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*, and using the standard assay of growth inhibition in the solid phase, each compound being absorbed within a 2 to 10×10^{-8} range on a paper disk, as discussed under Experimental Section. All tested compounds, except **22**, were found active on at least one strain at the maximum tested dose (8 to 10×10^{-8} M). Thus, **11a** was slightly active (±) on *B. subtilis* at 9×10^{-8} M, **11b** presented some antibacterial activity on *S. aureus* (+) and *B. subtilis* (+) at 7.3×10^{-8} M, **11e** showed a positive result (+) on *S. aureus* at 9.2×10^{-8} M, **16a** inhibited *B. subtilis* (+) at 8.3×10^{-8} M, and **16b** was active (+) on both *S. aureus* and *B. subtilis* at 9.7×10^{-8} M. However, as shown in Table I, acridone (**21**) had significant antibiotic properties, especially on *E. coli*. No growth inhibition was detected for any of these substances on *Candida albicans*.

Table III. Aza Analogues of Lucanthone 11

no.	formula	mp, °C	recrystn solvent	yield, %	anal.
11a	$C_{19}H_{23}N_3OS \cdot 0.5H_2O$	95	hexane	81.5	C, H, N
11b	$C_{20}H_{25}N_3OS$	56	hexane	79	C, H, N
11c	$C_{20}H_{25}N_3O_2S$	90	cyclohexane	58	C, H, N
16a	$C_{21}H_{27}N_3OS$	105	hexane	40	C, H, N
16b	$C_{22}H_{29}N_3OS$	70	hexane[a]	30	C, H, N
21	$C_{20}H_{26}N_4O$	198	benzene	78	C, H, N
22	$C_{20}H_{26}N_4O \cdot 0.5H_2O$	179	benzene	50	C, H, N

[a] After purification by chromatography on alumina ($CHCl_3$).

Experimental Section

Chemistry. All melting points were determined with a Reichert hot-stage microscope and are uncorrected. ^1H NMR spectra were recorded on a Varian XL-100 (100 MHz) spectrometer, using FT or a Varian EM360 spectrometer (60 MHz) in the CW mode. Chemical shifts are given in parts per million related to tetramethylsilane (Me_4Si = 0 ppm). Abbreviations used are: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; br s, broad singlet. All elemental analyses (CNRS, Paris) are within ±0.4% of the theoretical values for the mentioned elements. Mass spectra were recorded on a AEI MS9 spectrometer.

Ethyl 1,2-Dihydro-2-oxo-4-chloro-6-methylpyridine-3-carboxylate (5a) and Ethyl 2,4-Dichloro-6-methylpyridine-3-carboxylate (6). Ethyl 1,2-dihydro-2-oxo-4-hydroxy-6-methylpyridine-3-carboxylate (4; 10 g, 51 mmol) was refluxed in phosphorus oxychloride (100 mL) for 1 h. Excess of $POCl_3$ was removed under reduced pressure, and the residue was poured in ice-cold water. After neutralization with sodium carbonate, extraction with chloroform, and evaporation of the solvent, the residue was extracted in refluxing hexane (200 mL). Filtration of the hot suspension and recrystallization of the solid afforded **5a**: NMR (acetone-d_6, 60 MHz) δ 1.33 (t, 3 H, ethyl CH_3), 2.33

(s, 3 H, CH_3), 4.3 (q, 2 H, ethyl CH_2), 6.25 (s, 1 H, H5).

The filtrate was concentrated to 50 mL from which ethyl 2,4-dichloro-6-methylpyridine-3-carboxylate (**6**) crystallized.

Ethyl 1,2-Dihydro-2 oxo-6-methylpyridine-3-carboxylate (5b). Catalytic hydrogenation of compound **5a** (4.3 g, 20 mmol) in ethyl alcohol (150 mL), in the presence of sodium hydroxide (0.8 g dissolved in the minimum amount of water), over palladium on charcoal (30% Pd, 0.5 g) was conducted under atmospheric pressure and at room temperature.

After absorption of the theoretical volume of hydrogen (1.5 h to 2 h) and filtration, the solvent was removed under reduced pressure, and the residual solid was recrystallized: NMR ($CDCl_3$, 60 MHz) δ 1.4 (t, 3 H, ethyl CH_3), 2.5 (s, 3 H, CH_3), 4.4 (q, 2 H, ethyl CH_2), 6.23 (d, 1 H, H_5, $J_{4,5}$ = 7 Hz), 8.21 (d, 1 H, H_4).

1,2-Dihydro-2-oxo-4-(phenylthio)-6-methylpyridine-3-carboxylic Acid (7a). The chloro derivative **5a** (2.6 g, 12 mmol) and thiophenol (2.2 g, 20 mmol) were refluxed in ethyl alcohol (20 mL) for 24 h. After the mixture was cooled, the solid was collected by filtration and recrystallized.

3-Methyl-10*H*-[1]benzothiopyrano[3,2-*c*]pyridine-1,10-(2*H*)-dione (9). The acid **7a** (2.6 g, 10 mmol) was heated with stirring in polyphosphoric acid (70 g) at 120 °C for 30 min. After cooling, the reaction mixture was poured into ice-cold water (100 mL), and the solid formed was collected by filtration and crystallized: NMR (Me_2SO, 60 MHz) δ 2.2 (s, 3 H, CH_3), 6.30 (s, 1 H, H_4), 7.68 (m, 3 H, H_6, H_7, and H_8), 8.35 (m, 1 H, H_9), 12.12 (br s, 1 H exchangeable with D_2O, NH).

1-Chloro-3-methyl-10*H*-[1]benzothiopyrano[3,2-*c*]pyridin-10-one (8). Method A. Compound **9** (2.43 g, 10 mmol) was refluxed in phosphorus oxychloride (200 mL) for 2 h. After the usual workup, the red residue was purified by chromatography over silica gel (CH_2Cl_2).

Method B. Acid **7a** (2 g, 7.6 mmol) and phosphorus pentachloride (1.6 g, 7.6 mmol) were refluxed in phosphorus oxychloride (20 mL) for 3 h. After the excess of phosphorus oxychloride was removed, the reaction mixture was taken up with benzene (30 mL), and stannic chloride (2.5 g, 9.5 mmol) was slowly added to the solution under stirring. After refluxing for 1 h, the mixture was poured in cold hydrochloric acid (1 N). The organic layer was washed with water, dried over magnesium sulfate, and evaporated under vacuum. Chromatography over silica gel of the red residue, eluting with dichloromethane, afforded compound **8** identical with that obtained by method A.

1-[[(Diethylamino)alkyl]amino]-3-methyl-10*H*-[1]benzothiopyrano[3,2-*c*]pyridin-10-one (11a–c). Method C. Chlorothiopyrone **8** (261 mg, 1 mmol) and the appropriate diethylaminoalkylamine (2 mmol) were refluxed in ethyl alcohol (5 mL). The reaction was monitored by TLC (alumina) until complete disappearance of compound **8** (2 h). Ethanol was evaporated, and the residual material was taken up with dilute sodium hydroxide and extracted with chloroform. After the solvent was removed, compounds **11a–c** were recrystallized: NMR of **11a** (representative compound) ($CDCl_3$, 100 MHz) δ 1.10 (t, 6 H, ethyl CH_3), 2.38 (s, 3 H, CH_3), 2.66 (q, 4 H, ethyl CH_2), 2.77 (q, 2 H, β-CH_2), 3.71 (m, 2 H, α-CH_2), 6.43 (s, 1 H, H_4), 7.47 (m, 3 H, H_6, H_7, and H_8), 8.51 (m, 1 H, H_9), 10.31 (br s, 1 H, NH).

2,6-Dimethyl-4-[(2-methyl-5-chlorophenyl)thio]pyridine-3-carboxylic Acid (14b). Ethyl 2,6-dimethyl-4-chloropyridine-3-carboxylate (**12**) and 2-methyl-5-chlorothiophenol (**13**) were reacted in refluxing ethanol as for the preparation of acid **7a**, but for 48 h. The reaction mixture was then treated with 3 equiv of potassium hydroxide dissolved in the minimum amount of water and refluxed for a further 3 h. Ethyl alcohol was removed in vacuo, and the residue was taken up in water. Acid **14b** was precipitated upon acidification with 6 N hydrochloric acid and recrystallized.

9-Chloro-1,3,6-trimethyl-10*H*-[1]benzothiopyrano[3,2-*c*]pyridin-10-one (15). Cyclization of **14b** following the same procedure used for the preparation of compound **9** afforded thiopyrone **15** as colorless needles.

9-[[(Diethylamino)alkyl]amino]-1,3,6-trimethyl-10*H*-[1]benzothiopyrano[3,2-*c*]pyridin-10-one (16a,b). A mixture of compound **15** (525 mg, 2 mmol) and the appropriate (diethylamino)alkylamine (4 mL) was heated at 120 °C for 6 h. After cooling, the solution was poured in 1 N sodium hydroxide (50 mL), and the oil formed was extracted with chloroform. The organic phase was dried over magnesium sulfate and evaporated in vacuo. The residue was taken up with dilute acetic acid (10%, 100 mL), and the clear solution obtained upon filtration was alkalinized by addition of 1 N sodium hydroxide. **16a**: NMR ($CDCl_3$, 100 MHz) δ 1.11 (t, 6 H, ethyl CH_3), 2.31 (s, 3 H, 6-CH_3), 2.56 (s, 3 H, 3-CH_3), 2.64 (q, 4 H, ethyl CH_2), 2.78 (m, 2 H, β-CH_2), 3 (s, 3 H, 1-CH_3), 3.31 (m, 2 H, α-CH_2), 6.57 (d, 1 H, H_8, $J_{7,8}$ = 8.7 Hz), 7.10 (s, 1 H, H_4), 7.22 (d, 1 H, H_7), 9.64 (br s, 1 H, NH).

Ethyl 1,2-Dihydro-2-oxo-4-anilino-6-methylpyridine-3-carboxylate (17a). Chloropyridone **5a** (2.6 g, 12 mmol) and aniline (1.8 g, 20 mmol) were refluxed in ethyl alcohol (20 mL) in the presence of triethylamine (0.5 mL) for 24 h. After cooling, the reaction mixture was diluted with water (100 mL) and extracted with chloroform. The organic phase was dried over magnesium sulfate and evaporated in vacuo to afford **17a**.

If the condensation was conducted in refluxing 2-ethoxyethanol (bp 135 °C) and in the absence of triethylamine, only **1,2-dihydro-2-oxo-4-anilino-6-methylpyridine (17b)** was obtained: mass spectrum, m/z 200 (100, 199 (82.5); NMR (Me_2SO, 60 MHz) δ 2.05 (s, 3 H, CH_3), 5.4 (d, 1 H, H_6, $J_{4,6} \simeq$ 3 Hz), 5.56 (m, 1 H, H_4), 7.06 (m, 5 H, phenyl), 8.38 (s, 1 H, NH phenyl or NHCO), 10.53 (br s, 1 H, NHCO or NH phenyl).

1,2,5,10-Tetrahydro-1,10-dioxo-3-methylpyrido[4,3-*b*]quinoline (18). The ester **17a** (1.09 g, 4 mmol) was added to a refluxing solution of phenyl ether (10 mL), and reflux was maintained for 20 min. After cooling, the reaction mixture was diluted with petroleum ether (200 mL), and the solid obtained was collected by filtration and crystallized.

5,10-Dihydro-10-oxo-1-chloro-3-methylpyrido[4,3-*b*]quinoline (19) and 1,2-Dihydro-1-oxo-10-chloro-3-methylpyrido[4,3-*b*]quinoline (20). The dioxo compound **18** (1.13 g, 5 mmol) was added to phosphorus oxychloride (100 mL) at 60 °C, and the solution was refluxed for 3 h. After treatment as usual, the residual solid was taken up with boiling toluene.

The resulting solution was evaporated in vacuo to afford **19** as yellow needles: NMR ($CDCl_3$, 100 MHz) δ 2.36 (d, 3 H, CH_3) $J_{H_4,CH_3} \simeq$ 1 Hz), 6.56 (m, 1 H, H_4), 7.65 (m, 1 H, H_8), 7.85 (m, 1 H, H_7), 8.07 (m, 1 H, H_6), 8.53 (m, 1 H, H_9).

The insoluble material isolated after treatment with toluene was crystallized from ethyl alcohol to afford **20** as colorless needles: mass spectrum, m/z 246 (^{37}Cl, 34), 244 (^{35}Cl, 100); NMR (Me_2SO, 100 MHz) δ 2.45 (d, 3 H, CH_3, $J_{4,CH_3} \simeq$ 0.75 Hz), 7.15 (d, 1 H, H_4), 7.30 (m, 1 H, H_7), 7.47 (m, 1 H, H_9), 7.74 (m, 1 H, H_8), 8.16 (m, 1 H, H_6).

The deshielding of H_4 under the effect of the pyridinic nitrogen N5 allowed unambiguous structure confirmation of **19** and **20**.

5,10-Dihydro-10-oxo-1-[[3-(diethylamino)propyl]amino]-3-methylpyrido[4,3-*b*]quinoline (21) and 1,2-Dihydro-1-oxo-10-[[3-(diethylamino)propyl]amino]-3-methylpyrido-[4,3-*b*]quinoline (22). Heating a mixture of 3-(diethylamino)-propylamine (2 mL) and either **19** or **20** (50 mg, 0.2 mmol) at 140 °C for, respectively, 2 and 4 h afforded, respectively, **21** and **22**, which crystallized upon cooling. Treatment of the reaction mixture as for compounds **11a–c** and recrystallization gave pure amines. Compound **21**: NMR ($CDCl_3$, 100 MHz) δ 1.02 (t, 6 H, CH_3), 1.96 (m, 2 H, β-CH_2), 2.3 (s, 3 H, 3-CH_3), 2.55 (q, 4 H, ethyl CH_2), 2.64 (m, 2 H, γ-CH_2), 3.99 (m, 2 H, α-CH_2), 6.4 (s, 1 H, H_4), 7.24 (m, 1 H, H_8), 7.64 (m, 1 H, H_7), 7.84 (m, 1 H, H_6), 8.3 (m, 1 H, H_9), 8.5 (s, very broad, 1 H, 5-NH), 11.1 (br s, 1 H, N*H*CH_2).

Compound **22**: NMR ($CDCl_3$, 100 MHz) δ 1.04 (t, 6 H, ethyl CH_3), 1.8 (m, 2 H, β-CH_2), 2.37 (s, 3 H, 3-CH_3), 2.57 (q, 4 H, ethyl CH_2), 2.62 (m, 2 H, γ-CH_2), 3.64 (m, 2 H, α-CH_2), 6.08 (s, 1 H, H_4), 7.18 (m, 1 H, H_9), 7.25 (m, 1 H, H_7), 7.58 (m, 1 H, H_8), 8.35 (m, 1 H, H_6), 8.05 (br s, 1 H, 2 NH), 10.1 (br s, 1 H, N*H*CH_2).

Biological Assays. (a) In vitro cytotoxicity studies on Friend tumor cells and on L1210 leukemia cells were performed as described earlier.[14,15]

(b) Antibiotic activity was measured as follows: the tested compounds were dissolved in ethyl alcohol ($3–5 \times 10^{-6}$ mol/mL), and aliquots of 5, 10, and 20 μL, respectively, were applied on 6-mm filter disks and allowed to dry under a slight stream of nitrogen. Then the disks were placed on the top of the agar overlay, previously inoculated with the appropriate germ, and the diameters of inhibition zones were measured after incubation at 37 °C for 24 h. Germs used were *Escherichia coli* (ATCC 10536); *Staphylococcus aureus* (ATCC 6358P); *Bacillus subtilis* (ATCC

6633), from the Pasteur Institue; and *Candida albicans*, grown by the same Institute (Mycology Department).

Acknowledgment. The authors acknowledge support of this work by Fondation pour la Recherche Médicale (M.D.C.). We thank J. C. Chermann to whom we are indebted for in vivo biological studies, and Mrs. M. Charon for in vitro cytotoxicity determination.

Registry No. **4**, 10350-10-4; **5a**, 86129-62-6; **5b**, 51146-04-4; **6**, 86129-63-7; **7a**, 86129-64-8; **8**, 86129-65-9; **9**, 86129-66-0; **11a**, 86129-67-1; **11b**, 86129-68-2; **11c**, 86129-69-3; **12**, 70271-80-6; **13**, 18858-06-5; **14b**, 86129-71-7; **15**, 86129-71-7; **16a**, 86129-72-8; **16b**, 86129-73-9; **17a**, 86129-74-0; **17b**, 86129-80-8; **18**, 86129-75-1; **19**, 86129-76-2; **20**, 86129-77-3; **21**, 86129-78-4; **22**, 86129-79-5; thiophenol, 108-98-5; 2-(diethylamino)ethanamine, 100-36-7; 3-(diethylamino)propanamine, 104-78-9; 3-(diethylamino)-2-hydroxypropanamine, 6322-01-6; aniline, 62-53-3.

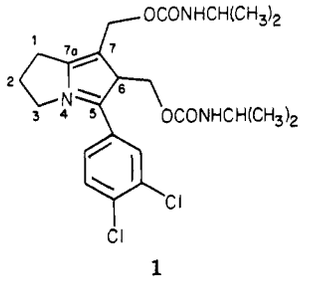
Synthesis, Evaluation of Chemical Reactivity, and Murine Antineoplastic Activity of 2-Hydroxy-5-(3,4-dichlorophenyl)-6,7-bis(hydroxymethyl)-2,3-dihydro-1*H*-pyrrolizine Bis(2-propylcarbamate) and 2-Acyloxy Derivatives as Potential Water-Soluble Prodrugs[1]

Wayne K. Anderson,* Chiung-Pin Chang, and Howard L. McPherson, Jr.

Department of Medicinal Chemistry, School of Pharmacy, State University of New York at Buffalo, Buffalo, New York 14260.
Received July 9, 1982

2-Hydroxy-5-(3,4-dichlorophenyl)-6,7-bis(hydroxymethyl)-2,3-dihydro-1*H*-pyrrolizine bis(2-propylcarbamate) (**11**) was prepared in a multistep synthesis. The 2-hydroxy group was used to prepare ester prodrugs **14** and **15**, and the antineoplastic activities of **11**, **14**, and **15a** were compared to **1** (the 2-deoxy analogue of **11**) in murine P388 lymphocytic leukemia and B16 melanocarcinoma. The alcohol **11** showed comparable activity to **1**, while **14** was less active and **15a** showed very low activity. The hydrolytic rates of **1**, **11**, **14**, **15a**, and **15b** were compared, and it was found that the two carbamate moieties were much more susceptible toward hydrolysis than the C-2 esters. The salts **15a** and **15b** exhibited good water solubility, 3.0×10^{-2} and 3.88×10^{-2} M, respectively.

In a series of recent publications we have described the design, synthesis, and biological activity of a new class of antitumor agents which we refer to as acylated vinylogous carbinolamines.[2] The continuing antitumor evaluation of selected agents in this class has resulted in the emergence of several compounds that exhibit outstanding activity against a broad spectrum of experimental murine neoplasias and human tumor xenografts in nude mice.[3,4] The pyrrolizine bis(carbamate) **1** is one such compound and is one of the members of this class that has been selected for more extensive preclinical studies.



1

One potential difficulty with **1** is the exhibited lack of any significant water solubility. Thus, the development of water-soluble compounds in this class has emerged as an important goal, and several approaches to this problem are being pursued simultaneously in our laboratories. One approach involves the development of water-soluble prodrugs, in which the hydrophilic moiety can be separated from the parent drug in vivo. This report will describe an approach to a water-soluble prodrug.

The first step in the design of water-soluble analogues of **1** is to determine where changes in the parent compound can be made. The parent compound can be subdivided into four parts: these are the heteroaromatic nucleus, the C-5 aryl moiety, the 6,7-bis[(carbamoyloxy)methyl] groups, and the aliphatic ring (C-1, C-2, and C-3). We chose, as our first point of attack, to modify the aliphatic ring, since this region of the molecule, unlike the other three, is not directly involved as a chemically reactive site in the molecule and it is not implicated in the stabilization of reaction transition states.[2a] Within the aliphatic ring, C-2 was chosen for modification because of its distal location relative to the reactive electrophilic sites at C-6 and C-7 and because of the minimal steric effect C-2 substituents would have upon the C-5 phenyl substituent.

The next step in the design of the prodrug involved selection of an appropriate means to attach the hydrophilic residue to C-2. We chose the hydroxyl group because it is neutral, easily derivatized, and synthetically accessible. Alcohol derivatives, such as carboxylic and phosphoric acid esters, as well as glycosides, can be converted to the parent alcohol in vivo by a variety of enzymatic and/or nonenzymatic processes.

Chemistry. The 2-hydroxypyrrolizine **11** was synthesized from 4-hydroxyproline (**2**) in 19% overall yield (Scheme I). N-Benzoylation of **2**, followed by acetylation of the secondary alcohol in **3**, gave the requisite α-amido acid precursor **4** for the ensuing cycloaddition reaction. It

(1) Vinylogous Carbinolamine Tumor Inhibitors. 11. For part 10 in this series, see Anderson, W. K.; McPherson, H. L., Jr. *J. Med. Chem.* **1982**, *25*, 84.
(2) (a) Anderson, W. K.; Corey, P. F. *J. Med. Chem.* **1977**, *20*, 812. (b) Anderson, W. K.; Corey, P. F. *Ibid.* **1977**, *20*, 1691. (c) Anderson, W. K.; Halat, M. J. *Ibid.* **1979**, *22*, 977. (d) Anderson, W. K.; Halat, M. J.; Rick, A. C. *Ibid.* **1980**, *23*, 87. (e) Anderson, W. K.; New, J. S.; Corey, P. F. *Arzneim.-Forsch.* **1980**, *30*(1), 765.
(3) (a) Anderson, W. K. *Cancer Res.* **1982**, *42*, 2168. (b) Anderson, W. K.; Chang, C.-P.; Corey, P. F.; Halat, M. J.; Jones, A. N.; McPherson, H. L., Jr.; New, J. S.; Rick, A. C. *Cancer Treat. Rep.* **1982**, *66*, 91.
(4) Lomax, N. R.; Narayanan, V. L. "Chemical Structures of Interest to the Division of Cancer Treatment", Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute, January 1983; Vol III.

0022-2623/83/1826-1333$01.50/0