and 2 × CH_2O), 6.38 (2 H, s, 2-NH_2), 7.69 (1 H, s, 8-H), and 10.58 (1 H, br s, 1-H). Anal. ($C_{20}H_{31}N_5O_5$) C, H, N.

9-[4-(Acyloxy)-3-(hydroxymethyl)but-1-yl]guanines 30-33. To a solution of **23** (0.80 g, 1.0 mmol) in pyridine (4 mL) was added acyl chloride (3.0 mmol), and the solution was stirred for 20-30 min. The mixture was precipitated in water (40 mL) and the resulting precipitate stirred in 80% acetic acid (10 mL) at 80 °C for 0.5-2.0 h. The solvent was removed and the residue purified by column chromatography on silica gel eluting with chloroform-methanol mixtures.

9-[4-Acetoxy-3-(hydroxymethyl)but-1-yl]guanine (30): yield 64%; mp 201-205 °C; IR (KBr) ν_{max} 3330, 3170, 2930, 1730, 1690, 1660, 1610, and 1565 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.6-1.8 (3 H, m, 2'-H and 3'-H), 1.98 (3 H, s, CH_3), 3.39 (2 H, br s, D_2O exchange gives d, J = 5 Hz, CH_2OH), 3.9-4.1 (4 H, m, 1'-H and CH_2OCO), 4.61 (1 H, br t, D_2O exchangeable, OH), 6.44 (2 H, s, D_2O exchangeable, 2-NH_2), 7.68 (1 H, s, 8-H), and 10.59 (1 H, s, D_2O exchangeable, 1-H); FABMS (positive ion, thioglycerol) 318 (MNa^+), 296 (MH^+). Anal. ($C_{12}H_{17}N_5O_4 \cdot 0.3CH_3CO_2H$) C, H, N.

9-[4-(Hexanoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (31): yield 38%; mp 179-181 °C; IR (KBr) ν_{max} 2960, 2930, 1730, 1690, 1630, and 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.84 (3 H, t, J = 6.9 Hz, CH_3), 1.24 (4 H, m, $CH_3(CH_2)_2$), 1.50 (2 H, quintet, J = 7.3 Hz, CH_2CH_2CO), 1.6-1.8 (3 H, m, 2'-H and 3'-H), 2.26 (2 H, t, J = 7.3 Hz, CH_2CO), 3.40 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 3.9-4.1 (4 H, m, 1'-H and CH_2OCO), 4.60 (1 H, t, J = 5.1 Hz, D_2O exchangeable, OH), 6.38 (2 H, s, D_2O exchangeable, 2-NH_2), 7.67 (1 H, s, 8-H), and 10.49 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{16}H_{25}N_5O_4 \cdot 0.25H_2O$) C, H, N.

9-[4-(Hexadecanoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (32): yield 52%; mp 183-191 °C; IR (KBr) ν_{max} 3340, 3160, 2920, 2850, 1740, 1690, and 1605 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.85 (3 H, t, J = 6.6 Hz, CH_3), 1.23 (24 H, m, $CH_3(CH_2)_{12}$), 1.49 (2 H, m, CH_2CH_2CO), 1.6-1.8 (3 H, m, 2'-H and 3'-H), 2.26 (2 H, t, J = 7.3 Hz, CH_2CO), 3.39 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 3.9-4.1 (4 H, m, 1'-H and CH_2OCO), 4.60 (1 H, t, J = 5.2 Hz, D_2O exchangeable, OH), 6.38 (2 H, s, D_2O exchangeable, 2-NH_2), 7.67 (1 H, s, 8-H), and 10.50 (1 H, s, D_2O exchangeable, 1-H). Anal. ($C_{26}H_{45}N_5O_4$) C, H, N.

9-[4-(Benzoyloxy)-3-(hydroxymethyl)but-1-yl]guanine (33): yield 25%; mp 160-169 °C; UV (MeOH) λ_{max} 231 (ϵ 15100) and 254 (13100) nm; IR (KBr) ν_{max} 1715, 1690, 1625, and 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.75-1.90 (3 H, m, 2'-H and 3'-H), 3.50 (2 H, t, J = 5 Hz, D_2O exchange leaves d, CH_2OH), 4.07 (2 H, t, J = 6.9 Hz, 1'-H), 4.2-4.35 (2 H, AB of ABX, CH_2OCO), 4.68 (1 H, t, J = 5.1 Hz, D_2O exchangeable, OH), 6.39 (2 H, s, D_2O exchangeable, 2-NH_2), 7.5-8.0 (6 H, m, C_6H_5 and 8-H), and 10.53 (1 H, s, D_2O exchangeable, 1-H); FABMS (positive ion, thioglycerol) 380 (MNa^+), 358 (MH^+). Anal. ($C_{17}H_{19}N_5O_4 \cdot 1.5H_2O$) C, H, N.

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Registry No. 1, 39809-25-1; 2, 7459-46-3; 3, 6482-32-2; 4, 102147-75-1; 5, 97845-58-4; 6, 97845-59-5; 7, 108970-72-5; 8, 108970-73-6; 9, 108970-74-7; 10, 97845-64-2; 11, 97845-68-6; 12, 97845-65-3; 13, 97845-66-4; 14, 97845-87-9; 15, 108970-75-8; 16, 97845-88-0; 17, 97845-67-5; 18, 97845-69-7; 19, 97845-70-0; 20, 97845-71-1; 21, 104227-86-3; 22, 97845-78-8; 23, 97845-77-7; 24, 97845-75-5; 25, 97845-72-2; 26, 97845-73-3; 27, 97845-74-4; 28, 97866-91-6; 29, 97845-79-9; 30, 97845-80-2; 31, 97845-82-4; 32, 97845-83-5; 33, 97845-81-3; 2-amino-6-chloropurine, 10310-21-1; 6-chloropurine, 87-42-3; 6-chloro-7-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine, 108970-76-9; 2-amino-9-[2-(2,2-dimethyl-1,3-dioxan-5-yl)ethyl]purine, 104227-89-6.

1-Amino-Substituted 4-Methyl-5*H*-pyrido[3',4':4,5]pyrrolo[3,2-*c*]pyridines: A New Class of Antineoplastic Agents

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In an attempt to find new anticancer agents, a series of pyrido[3',4':4,5]pyrrolo[3,2-c]pyridines were synthesized and evaluated in the standard NCI screening. Among these new compounds, which are structurally related to 9-azaellipticines but differ by deletion of a cycle, those that have a 4-methyl group and a $NHCH_2CH_2CH_2NR_2$ side chain at the 1-position show significant cytotoxicity on L1210 cultured cells and antitumor properties in the in vivo P388 leukemia system. The in vivo antineoplastic activity of the most potent compounds were confirmed on the L1210 leukemia model.

The antineoplastic activity in the ellipticine series and tetracyclic analogues is now well-documented.[1-10] A common feature of this class of compounds is their intercalative binding to DNA. Nevertheless, in various series of closely related derivatives, many compounds having the same apparent affinity for DNA differ in their biological properties.[2,7] It was concluded that a good affinity for DNA is a necessary, but not a sufficient, condition.

Ellipticine treatment of cells in culture causes DNA strand breaks and formation of DNA protein crosslinks probably through the interaction with topoisomerases (Pommier et al.[11,12]). The role of the binding to DNA is currently unknown.

Studies of DNA binding vs. antitumor potency of a series of *m*-AMSA analogues, another family of intercalating antitumor compounds, suggest that these parameters are related, but other parameters are clearly also involved in

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(1) Dalton, L. K.; Demerac, S.; Elmes, B. C.; Loder, J. W.; Swass, J. M.; Teitei, T. *Aust. J. Chem.* **1967**, *20*, 2715.
(2) Le Pecq, J. B.; Dat Xuong, N.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(3) Mathé, G.; Hayat, M.; De Vassal, F.; Schwarzenberg, L.; Schneider, M.; Schlumberger, J. R.; Jasmin, C.; Rosenfeld, C. *Rev. Eur. Etud. Clin. Biol.* **1970**, *15*, 541.
(4) Le Pecq, J. B.; Gosse, C.; Dat Xuong, N.; Paoletti, C. *C. R. Seances Acad. Sci., Ser. D* **1975**, *281*, 1365.
(5) Chermann, J. C.; Gruest, J.; Montagnier, L.; Wendling, F.; Tambourin, P.; Perrin, M.; Pochon, F.; Ducrocq, C.; Rivalle, C.; Bisagni, E. *C. R. Seances Acad. Sci., Ser. D* **1977**, *285*, 945.
(6) Lidereau, R.; Chermann, J. C.; Gruest, J.; Montagnier, L.; Ducrocq, C.; Rivalle, C.; Bisagni, E. *Bull. Cancer* **1980**, *67*, 1.
(7) Ducrocq, C.; Wendling, F.; Tourbez-Perrin, M.; Rivalle, C.; Tambourin, P.; Pochon, F.; Bisagni, E. *J. Med. Chem.* **1980**, *23*, 1212.

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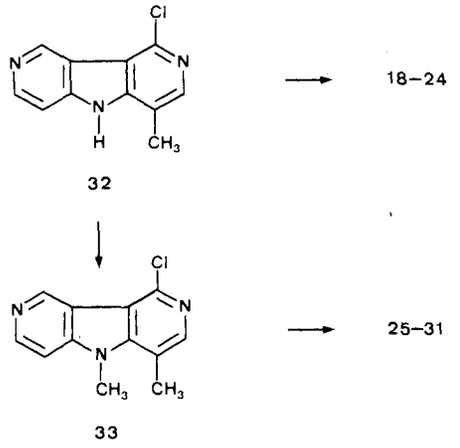
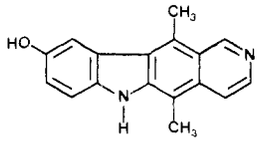
Scheme I

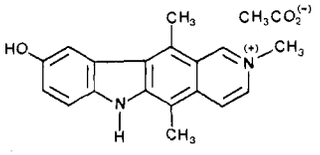


the action of this series.[13] Therefore, it is important to search for a clearer understanding of the exact mechanism of action of compounds that intercalate into DNA and to know if DNA binding plays a minor or a determinant role. In this prospect, extension of our knowledge of the structure–activity relationships of a defined series could provide a fruitful contribution. Moreover, it is possible that highly cytotoxic metabolites are produced in vivo, which can explain cell death. Simplified active molecules which could be involved in a metabolic activation process might then allow an easier way to study this problem.

A typical example of a possible metabolic activation of a drug is the oxidative formation of a quinone imine derived from 9-hydroxyellipticine (**1**).[1]



1

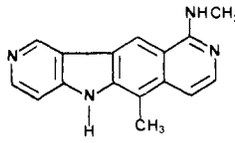


2

Recently, it has been shown that after its oxidation, the antineoplastic agent ellipticinium acetate **2** can bind covalently to biological molecules such as ribonucleosides and RNA.[15,16] In the presence of iron(III) salts, EDTA, and H_2O_2, it degrades guanosine,[17] and other various adducts derived from **1** and **2** have been isolated or characterized.[18]

However, whether such intermediates are actual cytotoxic species that induce cell death or, on the contrary, lead to detoxification and elimination of these drugs (in the case of glutathione adduct)[18] remains an open question.

The case of compound **3** (BD40), the intercalative binding of which to DNA is very high,[19] is also interesting.



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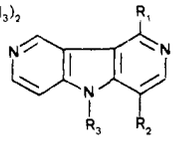
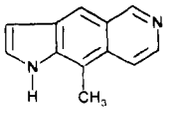


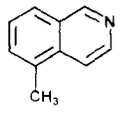
6

R_1 = $NH(CH_2)_nN(R)_2$

R_2 = H or CH_3; R_3 = H, CH_3 or R_2



4



5

It is a potent antitumor agent[5,6,10] currently under investigation in clinical trials that has not yet provided characterized adduct(s) with possible biological target(s). However, it has been shown that it requires the presence of oxygen for exhibiting cytotoxic properties in yeast[20] and probably an activation step before revealing its cytotoxicity on mouse cultured cells.[21]

Therefore, the possibility that a common bioactivation process involves the pyrrolo[3,2-*g*]isoquinoline **4** (or 5-methylisoquinoline (**5**)) part of compounds **1** and **3** cannot be ruled out.

In order to estimate the importance of the DNA intercalative binding properties, with respect to the required structural characteristics, it seemed to us of interest to synthesize new molecules structurally related to **3** but having an a priori minorated intercalative propensity.

Chosen tricyclic systems seemed suitable in this prospect.

Moreover, we thought that the difficult problem of the study of the possible isoquinoline (or pyrrolo[3,2-*g*]isoquinoline) nucleus oxidative biotransformation, which could involve the methyl group of **3**, should be easier to approach if new series of simplified antitumor derivatives were available.

Compounds related to **3**, having or not having a methyl group on a convenient position, were thus required.

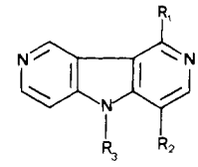
Taking into account these considerations, we designed the tricyclic system **6**, which is related to antineoplastic compound **3** by deletion of a ring.

This paper presents data concerning various pyrido-[3′,4′:4,5]pyrrolo[3,2-*c*]pyridine derivatives **6**, which have been studied in vitro on cultured tumor cells and screened in vivo on the P388 leukemia system.

Chemistry. We reported recently two general ways for the synthesis of 1-functionalized 5*H*-pyrido[3′,4′:4,5]-pyrrolo[3,2-*c*]pyridines.[22,23] Compounds **7–13** and **15–17**

(8) Rivalle, C.; Wendling, F.; Tambourin, P.; Lhoste, J. M.; Bisagni, E.; Chermann, J. C. *J. Med. Chem.* **1983**, *26*, 181.
(9) Juret, P.; Tanguy, A.; Girard, A.; Le Talaer, J. Y.; Abbatucci, J. S.; Dat Xuong, N.; Le Pecq, J. B.; Paoletti, C. *Eur. J. Cancer* **1978**, *14*, 205.
(10) Marty, M.; Jasmin, C.; Pouillard, P.; Gisselbrecht, C.; Gouvenia, G.; Magdelainat, H. *Annu. Meet. Am. Soc. Clin. Oncol., 17th* 1981, C-108.
(11) Pommier, Y.; Schwartz, R. E.; Swelling, L. A.; Kohn, K. W. *Biochemistry* **1985**, *24*, 6406.
(12) Pommier, Y.; Minford, J. K.; Schwartz, R. E.; Swelling, L. A.; Kohn, K. W. *Biochemistry* **1985**, *24*, 6410.
(13) Ross, W. E.; Glaubiger, D. L.; Kohn, K. W. *Biochim. Biophys. Acta* **1978**, *519*, 23.
(14) Auclair, C.; Paoletti, C. *J. Med. Chem.* **1981**, *24*, 289.
(15) Dugue, B.; Paoletti, C.; Meunier, B. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 416.
(16) Bernadou, J.; Meunier, B.; Meunier, G.; Auclair, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1297.
(17) Dugue, B.; Meunier, B. *Biochem. Biophys. Res. Commun.* **1985**, *133*, 15.
(18) Meunier, B.; Auclair, C.; Bernadou, J.; Meunier, G.; Mafouth, M.; Cros, S.; Monsarat, B.; Paoletti, C. *Structure–Activity Relationships of Antitumor Agents*; Martinus Nijhoff: 1983; 149.
(19) Tourbez-Perrin, M. S.; Pochon, F.; Ducrocq, C.; Rivalle, C.; Bisagni, E. *Bull. Cancer* **1980**, *67*, 3.
(20) Moustacchi, E.; Favaudon, V.; Bisagni, E. *Cancer Res.* **1983**, *43*, 3700.
(21) Vilarem, M. J.; Charcosset, J. Y.; Primaux, F.; Gras, M. P.; Calvo, F.; Larsen, C. J. *Cancer Res.* **1985**, *45*, 3906.
(22) Bisagni, E.; Nguyen, C. H. *Tetrahedron* **1986**, *42*, 2311.
(23) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1986**, *42*, 2303.

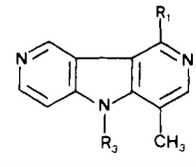
Table I. 5*H*-Pyrido[3′,4′:4,5]pyrrolo[3,2-c]pyridines 7–17 Already Described: Structure and Biological Data



compd	salt[a]	R_1	R_2	R_3	K_{aff}[b]	ID_{50},[c] μM	in vivo tests, P388: T/C (mg/kg), (D1–5 ip), |survivors|
7	3M	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	H	0.03 (±0.006)	0.34	135 (10), 148 (20), 155 (40)[d]
8	3HCl	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	CH_3	0.04 (±0.005)	0.13	147 (10), 164 (20), 172 (40), 207 (50) |3|
9	4HCl	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	CH_2CH_3	0.017 (±0.004)	1.4	inactive (0.3–40); toxic (60)
10	3M	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	$CH_2C_6H_5$	0.002 (±0.0006)	6.4	inactive (2.5–40)[d]
11	3M	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	H	H	0.015 (±0.0002)	46	inactive (2.5–20); toxic (40)
12	3M	$NHCH_2CH_2CH_2N(CH_2CH_3)_2$	H	CH_3	0.017 (±0.004)	1.6	inactive (5–50)[d]
13	3HCl	$NHCH_2CH_2CH_2N(CH_3)_2$	CH_3	H	0.02 (±0.005)	0.16	156 (10), 186 (20), 184 (40), 181 (50) |1|[d]
14[e]	2M	$NHCH_2CH_2CH_2N(CH_3)_2$	H	CH_3	0.02 (±0.003)	1.2	inactive (5–60)[d]
15	3M	$N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	H	0.006 (±0.005)	224	inactive (0.30–60)[d]
16	4HCl	$N(CH_3)CH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	CH_3	0.003 (±0.001)	52	inactive (1.25–40); toxic (80)
17	FB	$NHCH_2CH_2CH_2N{=}(CH_2CH_2)_2NCH_3$	CH_3	H	0.027 (±0.005)	59	inactive (1.25–80)[d]
3					0.46 (±0.1)	0.018	136 (0.75), 238 (24) |4|
5-FU[f]							175 (20) |0|

[a] 3M = trimaleate; 3HCl = trihydrochloride; FB = free base. [b] K_{aff} = values of the apparent DNA affinity constant in 10^6 M^{-1} with (±) standard deviation (6 values determined by ethidium bromide displacement in two independent experiments). [c] ID_{50} = the micromolar concentration of drug that, when added to cultures of L1210 cells for a period of 48 h, reduces the counted cells to 50% of the controls (mean of two values obtained in two independent experiments). [d] Nontoxic at the highest dose tested. [e] The single compound of this table that was not described: yield, 90%; mp 155 °C. Anal. ($C_{16}H_{21}N_5 \cdot 2C_4H_4O_4 \cdot 1.2H_2O$) C, H, N. [f] 5-Fluorouracil.

Table II. 1-Amino-Substituted 4-Methyl-5*H*-pyrido(3′,4′:4,5)pyrrolo(3,2-*c*)pyridines 18–31: Physical and Biological Data



no.	R_1	R_3	yield, %	mp, °C	formula	anal.[a]	K_{aff}[b]	ID_{50},[b] μM	in vivo tests, P388: T/C (mg/kg), (D1–5 ip)
18	$NHCH_2CH_2CH_2NHCH_2CH_3$	H	65	>260	$C_{16}H_{21}N_5 \cdot 3HCl \cdot 1.2H_2O$	CHNCl	0.025 (±0.006)	17.4	inactive (2.5–40); toxic (80)
19	$NHCH_2CH_2N(CH_2CH_3)_2$	H	34	>260	$C_{17}H_{23}N_5 \cdot 3.1HCl$	CHNCl	0.012 (±0.003)	19.5	inactive (2.5–20); toxic (40)
20	$NHCH_2CH_2CH_2CH_2N(CH_2CH_3)_2$	H	51	>260	$C_{19}H_{27}N_5 \cdot 3HCl \cdot 0.5H_2O$	CHNCl	0.030 (±0.003)	12.0	inactive (5–20); toxic (50)
21	$NHCH_2CH_2CH_2N(CH_2CH_2CH_3)_2$	H	25	240	$C_{20}H_{29}N_5 \cdot 3HCl \cdot 2H_2O$	CHNCl	0.008 (±0.001)	3.3	inactive (1.25–20)[c]
22	$NHCH_2CH_2CH_2N{=}(CH_2)_5$	H	42	>260	$C_{19}H_{25}N_5 \cdot 3HCl \cdot H_2O$	CHNCl	0.04 (±0.002)	7.8	inactive (1.25–40)[c]
23	$NHCH_2CH_2CH_2N{=}(CH_2)_4$	H	44	>260	$C_{18}H_{23}N_5 \cdot 3HCl \cdot H_2O$	CHNCl	0.04 (±0.003)	4.2	inactive (1.25–40)[c]
24	$NHCH_2CH_2CH_2N{=}(CH_2CH_2)_2O$	H	82	>260	$C_{18}H_{23}N_5O \cdot 3HCl \cdot 2H_2O$	CHNCl	0.02 (±0.003)	19.2	inactive (1.25–40)[c]
25	$NHCH_2CH_2CH_2NHCH_2CH_3$	CH_3	90	140	$C_{17}H_{23}N_5 \cdot 3.5C_4H_4O_4 \cdot 1.5H_2O$	CHN	0.05 (±0.004)	3.8	127 (10), 145 (20), 138 (40), 149 (80)
26	$NHCH_2CH_2CH_2N(CH_3)_2$	CH_3	61	>260	$C_{17}H_{23}N_5 \cdot 3HCl \cdot 1.25H_2O$	CHNCl	0.04 (±0.004)	0.30	136 (5), 150 (10), 167 (20), 173 (40)
27	$NHCH_2CH_2CH_2CH_2N(CH_2CH_3)_2$	CH_3	62.5	250	$C_{20}H_{29}N_5 \cdot 3HCl \cdot 1.5H_2O$	CHNCl	0.04 (±0.003)	2	inactive (5–100)[c]
28	$NHCH_2CH_2CH_2N(CH_2CH_2CH_3)_2$	CH_3	30	220	$C_{21}H_{31}N_5 \cdot 3HCl \cdot 1.5H_2O$	CHNCl	0.016 (±0.002)	8.6	inactive (2.5–20)[c]
29	$NHCH_2CH_2CH_2N{=}(CH_2)_5$	CH_3	43	>260	$C_{20}H_{27}N_5 \cdot 3HCl \cdot 1.5H_2O$	CHNCl	0.06 (±0.002)	3.6	inactive (2.5–40)[c]
30	$NHCH_2CH_2CH_2N{=}(CH_2)_4$	CH_3	75	>260	$C_{19}H_{25}N_5 \cdot 3HCl \cdot 1.5H_2O$	CHNCl	0.06 (±0.002)	1.6	inactive (2.5–40)[c]
31	$NHCH_2CH_2CH_2N{=}(CH_2CH_2)_2O$	CH_3	80	>260	$C_{19}H_{25}N_5O \cdot 3HCl \cdot 1.5H_2O$	CHNCl	0.03 (±0.004)	5.3	inactive (2.5–40)[c]
3							0.46 (±0.1)	0.018	136 (0.75), 238 (24) |4|

[a] Microanalyses are within ±0.4% of the theoretical values for the elements corresponding to the mentioned empirical formulas. [b] K_{aff} and ID_{50} are expressed as in Table I. [c] Nontoxic at the highest dose tested.

were already described in these papers (Table I). 1-[[3-(Dimethylamino)propyl]amino]-5-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridine (14) was obtained by substitution of 1-chloro-5-methyl-5*H*-pyrido[3′,4′:4,5]-pyrrolo[3,2-*c*]pyridine[21] (Table I). 1-Amino-substituted 5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines **18–31** were prepared by starting from 1-chloropyridopyrrolopyridines **32** and **33**[20,21] (Scheme I), which were substituted by the

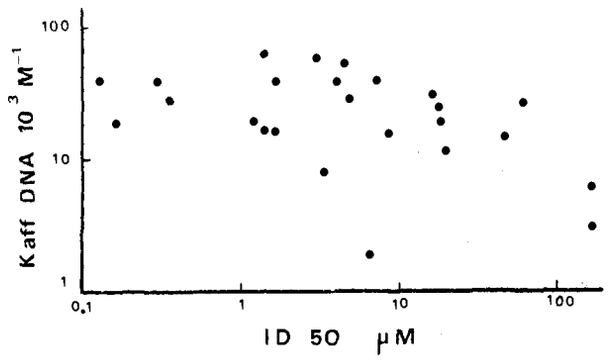


Figure 1. Lack of correlation between the apparent affinity for DNA and the cytotoxicity for L1210 cells in the series of pyridopyrrolopyridines.

required diamines at 150–170 °C, worked up in standard conditions, and purified by usual methods (Table II).

DNA Binding Measurements. According to the intercalation model first described by Lerman,[24] compounds **1–3** have been shown to bind to DNA in vitro with high affinity.[2,7,19] Since biological activities of this type of drug were admitted to be related to their DNA affinity,[2] it was of interest to study the new series of tricyclic derivatives from this point of view. Three experimental evidences suggested the intercalative binding of compounds **7**, **8**, **12**, and **13** to DNA: (1) hypsochromic and bathochromic changes in absorption spectra in the presence of DNA; (2) length increase of sonicated DNA; and (3) competition with ethidium bromide (not shown, manuscript in preparation). As it can be seen in the K_{aff} entries of Tables I and II, the deletion of one cycle decreased the DNA affinity by 1 order of magnitude (compare **3** and **7**). Two modifications had a drastic effect on the affinity: the introduction of a benzyl group on the N-5 position ($R_3 = CH_2C_6H_5$, compound **10**) and the NH substitution of the side chain by a methyl group (**15** and **16**). The other changes had only slight effects on the affinity.

Biological Studies. (a) In Vitro Cytotoxicity Determination. Cytotoxicity toward tumor cells grown in vitro was studied as described in the Experimental Section. For all the compounds, the ID_{50} values are reported in Tables I and II. As in the case of DNA affinity, the deletion of one cycle of compound **3** significantly decreased the cytotoxicity (compare **3** and **7**). However, cytotoxicity was sensitive to slight modifications of the side chain (**15–20**) and to the presence of methyl groups at the 4- and 5-positions (**11**).

In the series of pyridopyrrolopyridines, it must be emphasized that all the compounds having a very low affinity toward DNA (**10**, **15**, **16**, **21**) are devoid of cytotoxicity. Thus, the observation of Le Pecq et al.[2] that DNA affinity is a necessary but not a sufficient condition for cytotoxicity seems valid in this series despite the lack of correlation between the apparent affinity for DNA and the cytotoxicity toward L1210 cells, as shown by Figure 1.

(b) In Vivo Antitumor Effects. 1-Amino-substituted pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines were screened in vivo against P388 leukemia, according to the protocol of Geran et al.[25] In this test, five compounds were active: **7**, **8**, **13**, **25**, and **26** (see Table I and II). Moreover, the most potent antitumor compounds against the P388 tumor system (**8** and **13**) showed a good activity on the L1210 leukemia tumor model (see Table III). The comparison of activities emphasized that the most efficient compounds in vivo were the most cytotoxic in vitro (compare **8** and **13** vs. **25**).

In addition, as for DNA affinity and cytotoxicity, the deletion of one cycle of compound **3** decreased the antitumor activity (compare **3** and **7**).

Table III. In Vivo Antitumor Properties of Compounds 8 and 13 on L1210 Leukemia Model

compd	doses, mg/kg	weight changes, g	median survival day	T/C, %	survivors
8	2.5	+0.7	10.9	118	0/10
	5	−0.3	12.75	138	0/10
	15	+0.8	13.75	149	0/10
	30	+0.2	17.25	187.5	0/10
	60	−2.3	18	196	1/10
	80	−2.3	9.1	99	0/10
13	1.25	+0.3	11.75	127.5	0/10
	2.5	+0.3	12.9	140	0/10
	5	+0.3	14.0	152	0/10
	10	+0.5	16.25	177	3/10
	20	−1.7	15.6	169.5	0/10
	40	−1.1	9.25	100	0/10
	50	−2.4	8.2	89	0/10
5-fluoro-uracil	20	−0.1	18	196	0/10
3	3	−0.7	18.3	198	4/10
control		+0.6	9.2		0/30

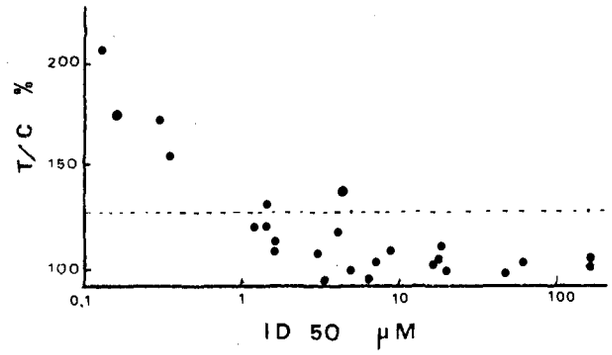


Figure 2. Correlation between the antitumor activity (P388, D1–5, ip) and the cytotoxicity (L1210 cells) in the series of pyridopyrrolopyridines.

Results and Discussion

Physicochemical and biological data for 25 examples of the new 1-amino-substituted 5*H*-pyrido[3′,4′:4,5]pyrrolo-[3,2-*c*]pyridine class of antitumor agents are recorded in Tables I and II. As expected for tricyclic compounds, pyridopyrrolopyridines **7–31** are poor intercalators, compared to their tetracyclic analogue **3**. Their DNA affinity constants, measured by ethidium bromide displacement, were at least 1 order of magnitude lower. As already mentioned, no clear correlation between DNA affinity and cytotoxicity seemed to exist in this series even if DNA affinity is a necessary condition (Figure 1). On the other hand, there is a good correlation between cytotoxicity (ID_{50}) and antitumor activity (Figure 2).

Among the 25 compounds evaluated, only five of them show antitumor properties in the P388 in vivo model. They are **7**, **8**, **13**, **25**, and **26**. Except for 1-[[3-(ethylamino)propyl]amino]-4,5-dimethyl-5*H*-pyrido[3′,4′:4,5]-pyrrolo[3,2-*c*]pyridine (**25**), which has a mean ID_{50} of 3.8 μM, these products are the most cytotoxic in this series ($ID_{50} < 1$ μM).

The evaluation of compounds **8** and **13** in the in vivo L1210 leukemia system confirms the antitumor properties of this new class of antineoplastic derivatives (Table III).

(24) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18.
(25) Geran, R. I.; Greenburg, N. H.; Mac Donald, M. M.; Schumacher, A. M.; Abott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 9.

The following is a summary of the structure–activity relationships.

(1) When the 1-substituent is the $NHCH_2CH_2CH_2N(Et)_2$ group, transformation of 5-NH to 5-NCH_3 increases both in vitro and in vivo biological activities (compare **7** vs. **8**). However, only minor changes at the N-5 position are allowed in order to maintain the in vivo activity. This restriction is shown by the low cytotoxicity and the total in vivo inefficiency of compounds **9** (5-NEt) and **10** (5-$NCH_2C_6H_5$).

(2) By itself, the structure of the (aminoalkyl)amino side chain plays also a crucial role.

(i) There are no drastic changes in the activity levels when the terminal diethylamino substituent is replaced by a dimethylamino group (compare compounds **13** and **26** vs. compounds **7** and **8**, respectively). The activity of compound **25** shows that antitumor properties can be exhibited by compounds having a terminal NHR. Nevertheless, the replacement of dimethylamino or diethylamino groups by the more bulky dipropylamino substituent (**21** and **28**) as well as by cyclic amino groups (compounds **17**, **22–24**, and **29–31**) abolishes in vivo activity and also greatly decreases in vitro cytotoxicity. Since the K_{aff} values of these last compounds are comparable to those of the active compounds **7**, **8**, **13**, **25**, and **26**, the role played by DNA affinity seems to be minor, with regard to the structural requirements.

(ii) In contrast to the structure–activity relationships recently observed in other series of antitumor drugs,[26] 1-[[2-(diethylamino)ethyl]amino]-4-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridine (**19**), which has a C_2 unit between the two nitrogen atoms of the side chain, results in a total loss of biological activity. Insertion of an additional methylene unit in the side chain, as performed with 1-[4-(diethylamino)butyl]amino derivatives **20** and **27**, also results in unexpectedly inactive products. Here again, it must be pointed out that the K_{aff} values of the compounds are still similar to those of active derivatives.

(iii) Compared to **7** and **8**, compounds **15** and **16**, which have equivalent structures but differ by their 1-N substituents ($N(CH_3)R$ in place of NHR), establish the importance of this NH group for cytotoxicity and antitumor activity of the series.

(3) With respect to the closely related active compounds **7** and **8**, pyridopyrrolopyridines **11**, **12**, and **14**, which do not contain the 4-methyl group present in the former products, also result in low cytotoxicity and total loss of in vivo activity, without significant changes in the DNA affinity. The fact that such a minor modification strongly decreased cytotoxicity and in vivo antitumor properties underlines the critical role of this substituent for the antineoplastic activity of the new series of antitumor pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridine derivatives.

Conclusions

Our results indicate that tricyclic analogues of compound **3**, namely, 1-amino-substituted 4-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines, which retain the convenient structural characteristics, exhibit good cytotoxicity on L1210 cultured cells and significant antitumor properties in P388 and L1210 in vivo leukemia systems with a good correlation between dose potency in in vitro and in vivo models.

With respect to antitumor activity of compound **3**, further studies will be necessary in order to establish if the antineoplastic activity of the pyridopyrrolopyridine series **6** is revealed through common target and mechanism. At the present time, however, the analogy between compounds **3** and **6**, which is obtained through deletion of a cycle but while maintaining the key substituents, seems to be justified.

DNA affinity, cytotoxicity, and antitumor activity are lowered when a tetracyclic chromophore like BD40 (**3**) is replaced by an equivalent tricyclic one. In this series, antitumor activity is retained only if key substituents are present.

Structure–activity relationships, performed with 25 analogues, showed that there is no direct correlation between pyridopyrrolopyridine DNA affinity and cytotoxicity, or between DNA affinity and in vivo antitumor properties. For example, considering two compounds with similar DNA affinity, corresponding cytotoxicities can be strikingly different. However, DNA interaction might be an important parameter for biological activities in this series, since compounds having the lowest DNA affinity are totally inactive.

As already shown in other series,[2] these findings seem to show that, in addition to DNA association constants, structural requirements also play an important role. The radical changes in biological activites when minor structural modifications are introduced in the side chain are particularly significant in this respect.

Moreover, as removal of the 4-methyl moiety results in compounds totally inactive in vivo, it seems thus possible that this group could be involved in a metabolic activation process, as was postulated by us in a recent paper.[22]

Finally, the main remark that can be drawn from this initial structure–activity study of the new pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridine class of antitumor agents is the necessity to have a $NHCH_2CH_2CH_2NR_1R_2$ unit, a methyl substituent, and an NH or NCH_3 group at the 1-, 4-, and 5-positions, respectively.

Experimental Section

Chemistry. All melting points were determined with a Kofler apparatus and are uncorrected. ^1H NMR spectra were recorded in $(CD_3)_2SO$ or D_2O with either a Varian XL100 or a Brucker WP80 spectrometer. Me_4Si was used as internal standard, and chemical shifts are reported on the δ scale, with peak multiplicities. Only NMR spectra of some typical examples are recorded, but all others are consistent with the reported structures.

Techniques for substitution of chloro derivatives **32** and **33** are given below. Purification of products was followed by thin-layer chromatography on silica gel and alumina, and further details of new compounds are noted in Table II. Elemental analyses were performed by the Service Central de microanalyses du CNRS, 91190 Gif/Yvette.

As noted in Table II in the formula entries, we observed that after conventional treatment 1-amino-substituted pyrido[3′,4′:4,5]pyrrolo[3,2-c]pyridines or their salts were frequently associated with solvation water.

General Procedure for the Preparation of 1-Amino-Substituted Pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines 14, 18–31. Chloro derivative **32** or **33** (1 g) in free amine (10 g) was heated under nitrogen in an oil bath at 160–170 °C, with the disappearance of the chlorinated derivative being followed by thin-layer chromatography on silica gel, with methylene chloride–ethanol, 9/1, as eluent. Then, the excess amine was removed under reduced pressure, and the residue was taken up in 50 mL of 3 N solution of sodium hydroxide and extracted with methylene chloride. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The crude product was then chromatographed on a silica gel column, eluting with methylene chloride–ethanol, 95/5 and 9/1. By evaporation of the fractions containing the expected product, the pure base was thus obtained.

For prepataion of maleate salts, the bases were dissolved in acetone and poured into an acetone solution of at least 3 molar

(26) Zee-Cheng, R. K. Y.; Cheng, C. C. *J. Med. Chem.* **1985**, *28*, 1216.

equiv of maleic acid, and the reaction mixture was kept at the boiling point for 2 min and cooled to afford the trimaleate precipitates, which were collected and air-dried. When the pure bases were taken up in ethanol solutions of hydrochloric acid, usual workup led to the crystalline trihydrochlorides.

Chemical shifts of compounds **20**, **23**, **26**, and **27** are given as typical examples of ^1H NMR spectra in D_2O.

20: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-$CH_2\delta$-N(CH_2CH_3)$_2$) δ 1.50 (t, 2 × 3 H, CH_2CH_3), 1.97–2.25 (m, 2 × 2 H, CH_2-β + CH_2-γ), 2.53 (s, 3 H, CH_3-4), 3.31–3.62 (m, 3 × 2 H, CH_2CH_3 + CH_2-δ), 3.68–3.97 (m, 2 H, CH_2-α), 7.94 (s, 1 H, H-3), 8.20 (d, 1 H, H-6, J_{6-7} = 8 Hz), 8.88 (d, 1 H, H-7), 9.95 (s, 1 H, H-9).

23: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-N(CH_{2a}-CH_{2b})$_2$) δ 2.20–2.35 (m, 3 × 2 H, (CH_2b)$_2$ + CH_2-β), 2.45 (s, 3 H, CH_3-4), 3.2–3.7 (m, 3 × 2 H, (CH_{2a})$_2$ + CH_2-γ), 3.82 (t, 2 H, CH_2-α), 7.85 (s, 1 H, H-3), 7.89 (d, 1 H, H-6, J_{6-7} = 6 Hz), 8.73 (d, 1 H, H-7), 9.70 (s, 1 H, H-9).

26: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-N(CH_3)$_2$) δ 2.35–2.59 (m, 2 H, CH_2-β), 2.87 (d, 3 H, CH_3-4, $J_{CH_3\text{-}4\text{-}H\text{-}3}$ = 1 Hz), 3.07 (s, 2 × 3 H, N(CH_3)$_2$), 3.46–3.66 (m, 2 H, CH_2-γ), 3.94 (t, 2 H, CH_2-α), 4.44 (s, 3 H, CH_3-5), 8.0 (d, 1 H, H-3), 8.38 (d, 1 H, H-6, J_{6-7} = 7 Hz), 8.88 (d, 1 H, H-7), 10.0 (s, 1 H, H-9).

27: (R_1 = NH-$CH_2\alpha$-$CH_2\beta$-$CH_2\gamma$-$CH_2\delta$-N(CH_2CH_3)$_2$) δ 1.40 (t, 2 × 3 H, CH_2CH_3), 1.81–2.12 (m, 2 × 2 H, CH_2-β + CH_2-γ), 2.84 (d, 3 H, CH_3-4, $J_{CH_3\text{-}4\text{-}H\text{-}3}$ = 1 Hz), 3.23–3.53 (m, 3 × 2 H, CH_2CH_3 + CH_2-δ), 3.72–3.94 (m, 2 H, CH_2-α), 4.39 (s, 3 H, CH_3-5), 7.89 (d, 1 H, H-3), 8.33 (d, 1 H, H-6, J_{6-7} = 9 Hz), 8.84 (d, 1 H, H-7), 9.86 (s, 1 H, H-9).

DNA Binding Measurements. The DNA binding constants were determined by competition with ethidium bromide, as described,[27] in 20 mM Na_2NPO_4/NaH_2PO_4, 0.1 M NaCl, 1 mM EDTA, pH 7.4. DNA (calf thymus, Sigma) concentration was 10 μg/mL.

L1210 Cytotoxicity Determination. L1210 cells in exponential phase of growth were grown in RPMI 1640 medium supplemented with 2 mM glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10% fetal calf serum, in a 5% CO_2 atmosphere at 37 °C. The drugs were dissolved in distilled water and added to the cells (0.8×10^5 cells/mL) for 48 h. The cells were then counted, and results were expressed as the drug concentration that inhibited by 50% the cell proliferation (ID_{50}). The ID_{50}'s were estimated by regression analysis of the dose–response data.

In Vivo Murine Tumor Models. The murine tumors were provided by Prof. G. Atassi (Institut Jules Bordet, Bruxelles, Belgium), and NCI protocols[25] were used throughout the drug evaluation tests.

P388 and L1210 leukemia viable cells, respectively 10^6 and 10^5, were inoculated ip on day 0 in CDF1 hybrid male or female mice (10 mice for test group). Compounds, dissolved in distilled water, were administered ip (0.1 mL/10 g of body weight), at various doses, for 5 days (D1–5). Vehicle, 5-fluorouracil (positive control of test), and BD40 (reference compound) were administered under the same conditions. The antitumor activity (T/C) was evaluated, according to the formula T/C = (median day of survival of treated animals at a given dose of product/median day of survival of "negative control" animals) × 100. Mice surviving for 30 days were considered as cured; they were included in the calculation of the median survival time.

The NCI criteria for activity in the in vivo murine models were used:

tumor model	act. criterion	good act. criterion
P388 leukemia	T/C > 127%	T/C > 175%
L1210 leukemia	T/C > 125%	T/C > 150%

Acknowledgment. We acknowledge financial support of this work by Institut National de la Santé et de la Recherche Médicale (CRE 842001), Institut Curie, and Société Sanofi.

(27) Le Pecq, J. B.; Paoletti, C. *J. Mol. Biol.* **1967**, *27*, 87.

Aldosterone Antagonists. 2. Synthesis and Biological Activities of 11,12-Dehydropregnane Derivatives

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Several steroid derivatives having the Δ^{11}-pregnane skeleton with a 17-γ-spirolactone function were synthesized to evaluate their antialdosterone activity and to elucidate the relation between their binding affinity to mineralocorticoid receptor (MR) and their mineralo- and/or antimineralocorticoid activity. Although many of the synthesized compounds showed strong binding affinity for the MR and aldosterone agonist activity, 3-(17β-hydroxy-3-oxoandrosta-1,4,6,11-tetraen-17α-yl)propionic acid γ-lactone (**12**) exhibited good aldosterone antagonist activity in an in vivo assay. Its in vivo antiandrogenic activity was also found to be relatively weak.

Antimineralocorticoid therapy has been considered to be effective for treating edematous diseases and essential hypertension with primary and secondary aldosterone excess.[1] Aldosterone (Ald), the most potent mineralocorticoid hormone,[2] is synthesized in the zona glomerulosa cells of the adrenal cortex from deoxycorticosterone (DOC) via corticosterone and 18-hydroxycorticosterone by stepwise hydroxylations mediated by cytochrome P-450.[3] This hormone regulates the electrolyte balance of body fluids by promoting excretion of potassium and retention of sodium ions.[4] Treatment of aldosterone excess has been attempted with spironolactone (Sp)[5] and potassium canrenoate (soldactone),[6] well-established aldosterone antagonists, but their clinical usefulness is often limited by

(1) Ochs, H.; Geenblatt, D. J.; Bodam, G.; Smith, T. W. *Am. Heart J.* **1978**, *96*, 389.

(2) (a) Simpson, S. A.; Tait, J. F.; Wettstein, A.; Neher, R.; Von Euw, J.; Reichstein, T. *Experientia* **1953**, *9*, 333. (b) Gomez-Sanchez, C.; Holland, O. B.; Higgins, J. R.; Mathieu, R.; Gruber, G. M.; Milewich, L.; Kaplan, N. M. *J. Lab. Clin. Med.* **1976**, *88*, 571.

(3) Kojima, I.; Inano, H.; Tamaoki, B. *Biochem. Biophys. Res. Commun.* **1982**, *106*, 617.

(4) Leuetscher, J. A., Jr.; Johnson, B. B. *J. Clin. Invest.* **1954**, *23*, 1441.

(5) (a) Cella, J. A.; Kagawa, C. M. *J. Am. Chem. Soc.* **1957**, *79*, 4808. (b) Kagawa, C. M. *Endocrinology (Baltimore)* **1960**, *67*, 125.

(6) Kagawa, C. M.; Bouska, D. J.; Anderson, M. L.; Krol, W. F. *Arch. Int. Pharmacodyn. Ther.* **1964**, *149*, 8.

0022-2623/87/1830-1647$01.50/0