# 1-Amino-Substituted 4-Methyl-5*H*-pyrido[4,3-*b*]indoles (γ-Carbolines) as Tricyclic Analogues of Ellipticines: A New Class of Antineoplastic Agents

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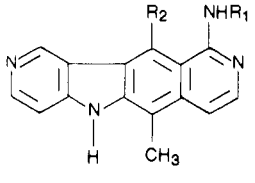
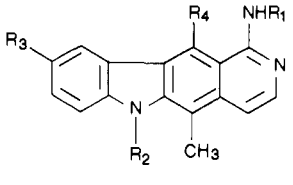
A series of 1-amino-substituted 4-methyl-5*H*-pyrido[4,3-*b*]indoles that are structurally related to ellipticines by deletion of a ring have been synthesized in order to evaluate their DNA affinity, their in vitro cytotoxicity on L1210 cultured cells, and their in vivo antitumor activity. Among 24 derivatives that have been prepared and studied for the structure–activity relationship in this new class of antineoplastic agents, those that have a $NH(CH_2)_3N(R)_2$ side chain (R = $CH_3$ or $C_2H_5$) at their 1-position, a 4-methyl group, and an 8-OH substituent, either with a 5-NH or with a 5-$NCH_3$ group, show the most potent cytotoxicities on L1210 cultured cells and in vivo antitumor properties in P388 and L1210 leukemia systems. In vivo antineoplastic activity of the most potent products was confirmed on other mouse experimental tumors from the standard NCI screening:B16 melanoma and C38 adenocarcinoma.

The precise mode of action of antitumor intercalating drugs, like ellipticine derivatives, remains unknown. It was first suggested that a high affinity for DNA is a necessary but not sufficient condition for antitumor activity in this series.[1] Among the different pharmacological properties of these drugs, at the cellular level the induction of DNA breaks was recently proposed as the basis for their cytotoxic and antitumor properties.[2] Moreover, the mechanism of action, at both the molecular and cellular levels, of ellipticine analogues seems to be significantly different[3] when a nitrogen atom is present at the 9-position (9-azaellipticines **1**, whose most active compound is BD 40, **1a**)[4,5] instead of a carbon atom (9-C ellipticines **2**, whose most active compound is BD 84, **2a**).[6] It is therefore important to determine more precisely the exact mechanism of action of these compounds, and first to know whether DNA binding plays a minor or a determinant role for their cytotoxic and antitumor properties. In this respect, extension of our knowledge of the structure–activity relationships with defined series could provide a fruitful contribution. In addition, the availability of two series of simplified analogues of 9-C ellipticines and 9-azaellipticines could give interesting information on differences in their mode of action.



**1**

**1a** (BD 40): $R_1$ = $(CH_2)_3N(C_2H_5)_2$, $R_2$ = H



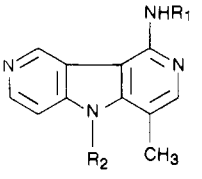
**2**

**2a** (BD 84): $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = H, $R_3$ = $OCH_3$, $R_4$ = $CH_3$

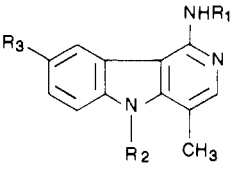
In a recent paper,[7] we reported the synthesis and the study of a series of pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines **3**, which appeared as a new class of antineoplastic agents structurally related to 9-azaellipticines **1** by deletion of a ring.

Our continuing interest in the search for new antitumor drugs and the results from the study of structure–activity relationships devoted to the preceding tricyclic analogues **3** of 9-azaellipticines **1** led us to the synthesis of other tricyclic derivatives, namely the pyrido[4,3-*b*]indoles **4** (γ-carbolines), which are closely related to both the ellipticine series **2** and pyridopyrrolopyridines **3**.

Thus, with these new simplified analogues of the more typical antitumor ellipticine derivatives **1a** and **2a** (BD 40[4,5] and BD 84[6,8]), studies on the importance of DNA intercalative binding, with respect to the structural characteristics required, and other parameters probably involved in antitumor properties of these compounds, as well as the problem of a possible bioactivation process, should be easier to approach.



**3**



**4**

Moreover, new antitumor agents derived from the acridine chromophore and having DNA intercalative binding properties appeared recently.[9-11] Thus, in view of better structure–activity relationship knowledge, further studies in the area of aromatic tricyclic compounds supposed to display some DNA intercalative binding propensity seem to be justified.

This paper presents data concerning various pyrido[4,3-*b*]indole derivatives **4**, which have been synthesized, studied for their apparent DNA affinity and for their in vitro cytotoxicity on L1210 cultured cells, and evaluated in vivo in some models of the standard NCI screening.

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[‡] Sanofi Recherche.

(1) LePecq, J. B.; Dat Xuong, N.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(2) (a) Pommier, Y.; Schwartz, R. E.; Zwelling, L. A.; Kohn, K. W. *Biochemistry* **1985**, *24*, 6406. (b) Pommier, Y.; Minford, J. K.; Schwartz, R. E.; Zwelling, L. A.; Kohn, K. W. *Biochemistry* **1985**, *24*, 6410.
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(7) Nguyen, C. H.; Bisagni, E.; Pepin, O.; Pierré, A.; de Cointet, P. *J. Med. Chem.* **1987**, *30*, 1642.
(8) Rivalle, C.; Wendling, F.; Tambourin, P.; Lhoste, J. M.; Bisagni, E.; Chermann, J. C. *J. Med. Chem.* **1983**, *26*, 181.
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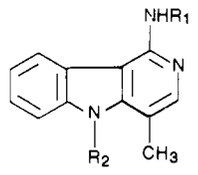
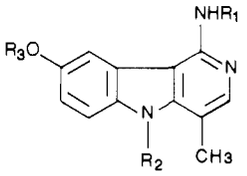
## Chemistry

Pyrido[4,3-*b*]indole derivatives **5a–c** and **6a–g** have been already described by us in a recent paper.[12] They were obtained by substitution of the corresponding chloro derivatives, which were synthesized by starting from phenylhydrazine and 4-hydroxy-5-methyl-1*H*-pyrid-2-one for the 8-H series **5**, and according to a six-step sequence for the 8-OR series **6**, by using 4-hydrazino-5-methyl-1*H*-pyrid-2-one (**7a**) and 3,3-dimethyl-1,5-dioxaspiro[5.5]undecan-9-one (**8**) as starting materials.[12]



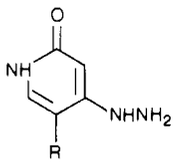
**5**

**5a**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = H
**b**: $R_1$ = $(CH_2)_3N(CH_3)_2$; $R_2$ = H
**c**: $R_1$ = $(CH_2)_2NH(CH_2)_2OH$; $R_2$ = H
**d**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = $CH_3$
**e**: $R_1$ = $(CH_2)_3N(CH_3)_2$; $R_2$ = $CH_3$



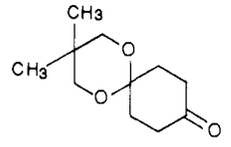
**6**

**6a**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = H; $R_3$ = $CH_3$
**b**: $R_1$ = $(CH_2)_3NHC_2H_5$; $R_2$ = H; $R_3$ = $CH_3$
**c**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = $R_3$ = H
**d**: $R_1$ = $(CH_2)_3N(CH_3)_2$; $R_2$ = $R_3$ = H
**e**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = $R_3$ = $CH_3$
**f**: $R_1$ = $(CH_2)_3N(C_2H_5)_2$; $R_2$ = $CH_3$, $R_3$ = H
**g**: $R_1$ = $(CH_2)_3N(CH_3)_2$; $R_2$ = $CH_3$; $R_3$ = H

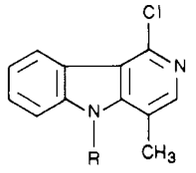


**7**

**7a**: R = $CH_3$
**b**: R = H



**8**



**9**

**9a**: R = H
**b**: R = $CH_3$

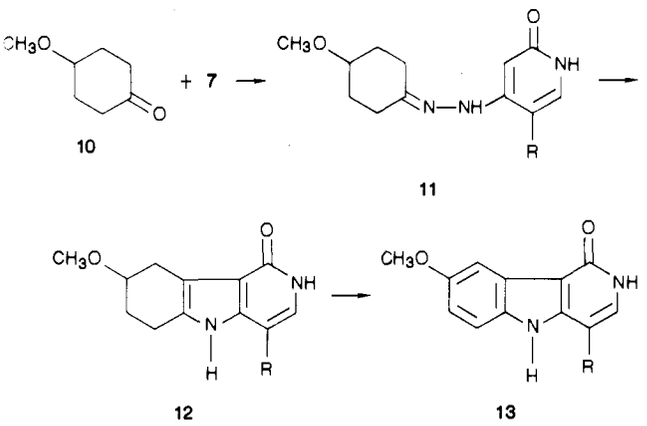
Whereas methylation of 4-methyl-5*H*-pyrido[4,3-*b*]-indole **9a** to 4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole **9b** and subsequent substitution by the required diamines led to 5-methyl derivatives of the 8-H series, **5d** and **5e**, which were not previously described, it appeared to us that our initial method for the obtention of the 8-OR series could be improved. Both in order to simplify the synthesis of compound **6** and to more easily obtain larger quantities of the various derivatives that were necessary to study the in vivo biological properties in this series, we then decided to work out a new pathway, avoiding the use of the expensive ketone **8** and the protection and deprotection steps of the 8-OH function.

Thus, replacement of ketone **8** by 4-methoxycyclohexanone (**10**) and its condensation with hydrazinopyridone **7a** gave hydrazone **11**. Thermal Fischer indole cyclization with subsequent aromatization of the intermediate 4-methyl-8-methoxy-6,7,8,9-tetrahydro-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (**12a**) gave 4-methyl-8-methoxy-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (**13a**) in 85% overall yield (Scheme I).

As phosphorus oxychloride transformation of pyrido-[4,3-*b*]indolone **13a** to 1-chloro-4-methyl-8-methoxy-5*H*-pyrido[4,3-*b*]indole (**14**) was previously performed,[12] it was then easy to obtain sufficient quantities of all the required derivatives of the 8-OR series, by following the transformations summarized in Scheme II. Moreover, this new pathway allowed us to prepare new derivatives **6h-q**, which

(12) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1987**, *43*, 535.

Scheme I

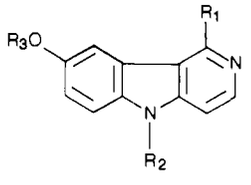


**10** **11** **12** **13**

**7**, **11**, **12**, **13**: **a**, R = $CH_3$; **b**, R = H

were suitable for our structure–activity relationship study of the series.

Finally, in order to appreciate the importance of the presence of the 4-methyl group, we have also prepared compounds **18e** and **18f**, by substitution of the corresponding chloro derivatives **18b** and **18d**. These last products were obtained by starting from 8-methoxy-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (**13b**), which was synthesized as summarized in Scheme I and transformed into **18a** and subsequently into **18b** or **18c** and then **18d**.



**18**

**18a**: $R_1$ = Cl; $R_2$ = H; $R_3$ = $CH_3$
**b**: $R_1$ = Cl; $R_2$ = $R_3$ = H
**c**: $R_1$ = Cl; $R_2$ = $R_3$ = $CH_3$
**d**: $R_1$ = Cl; $R_2$ = $CH_3$; $R_3$ = H
**e**: $R_1$ = $NH(CH_2)_3N(CH_3)_2$, $R_2$ = $R_3$ = H
**f**: $R_1$ = $NH(CH_2)_3N(CH_3)_2$, $R_2$ = $CH_3$, $R_3$ = H

## Results and Discussion

Physicochemical and biological data for 24 examples of the new 1-amino-substituted pyrido[4,3-*b*]indole class of antitumor agents are recorded in Tables I and II. As specified in the Table II footnotes, in vivo evaluations were performed according to the Geran et al. protocol[13] and the determinations of $K_{aff}$ and $ID_{50}$ on L1210 cultured cells were performed by using standard methods, as previously described for pyridopyrrolopyridine studies.[7]

The intercalative binding to DNA of compounds **6c**, **6d**, **6f**, and **6g** was demonstrated by three experimental results: (1) hypsochromic and bathochromic changes in absorption spectra induced by DNA; (2) competitive binding with ethidium bromide, as shown by the Scatchard analysis of the binding data (the curves were perfectly linear and intercepted the abscissa axis at an $R$ value of 0.20); and (3) length increase of sonicated DNA measured by viscosimetry.

Among these experimental evidences, the latter is the most relevant to demonstrate the intercalation into DNA. When the viscosimetric data are plotted according to Cohen and Eisenberg[14] $[(N/N_0)^{1/3}$ as a function of $R]$, a value

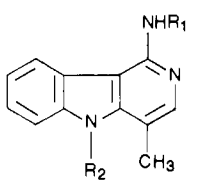
(13) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 9.

**Table I.** 1-Amino-Substituted 5*H*-Pyrido[4,3-*b*]indoles: Physical Data

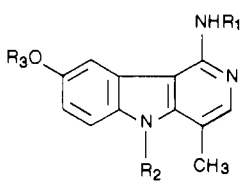
| no. | mp, °C (ref) free base | mp, °C (ref) salt | salt[a] | yield, % | meth[b] | formula[c] | anal. |
|---|---|---|---|---|---|---|---|
| **5a** | | (4) | 2M | 57 | C | $C_{27}H_{34}N_4O_8$ | |
| **5b** | 163 | (4) | 2M | 50 | C | $C_{25}H_{30}N_4O_8$ | |
| **5c** | 165 | 205 | 2M | 47 | C | $C_{24}H_{28}N_4O_9$ | C, H, N |
| **5d** | | 222 | 2HCl | 39 | C | $C_{20}H_{30}Cl_2N_4$ | C, H, N, Cl |
| **5e** | | 250 | 2HCl | 63 | C | $C_{18}H_{26}Cl_2N_4 \cdot 1.5H_2O$ | C, H, N |
| **6a** | | (4) | 2M | 62 | C | $C_{28}H_{36}N_4O_9 \cdot H_2O$ | |
| **6b** | | (4) | 2M | 80 | C | $C_{26}H_{32}N_4O_9 \cdot 0.5H_2O$ | |
| **6c** | 125 | 208 | 2HCl | 41 | C + D | $C_{19}H_{28}Cl_2N_4O \cdot 2.4H_2O$ | C, H, N |
| **6d** | 235–245 | 210–220 | 2M | 78 | C + D | $C_{25}H_{30}N_4O_9 \cdot 0.5H_2O$ | |
| **6e** | 107–109 | (4) | 2M | 85 | C | $C_{29}H_{38}N_4O_9$ | |
| **6f** | 187–189 | 200 | 2HCl | 49 | C + D | $C_{20}H_{30}Cl_2N_4O \cdot 1.25H_2O$ | C, H, N |
| **6g** | subl >260 | (4) | 2M | 50 | C + D | $C_{26}H_{32}N_4O_9 \cdot 0.5H_2O$ | |
| **6h** | 108 | 200 | 2HCl | 61 | C | $C_{19}H_{28}Cl_2N_4O \cdot 2.25H_2O$ | C, H, N |
| **6i** | | 236 | 2HCl | 83 | C | $C_{20}H_{30}Cl_2N_4O \cdot 0.5H_2O$ | C, H, N, Cl |
| **6j** | | 220 | 2HCl | 66 | C | $C_{20}H_{30}Cl_2N_4O \cdot 1.75H_2O$ | C, H, N |
| **6k** | | 232 | 2HCl | 26 | C | $C_{22}H_{34}Cl_2N_4O \cdot 0.25H_2O$ | C, H, N |
| **6l** | | 160 | 2HCl | 61 | C | $C_{21}H_{30}Cl_2N_4O \cdot 0.75H_2O$ | C, H, N |
| **6m** | 180–181 | 250 | 2HCl | 59 | C | $C_{20}H_{30}Cl_2N_4O_2 \cdot 0.75H_2O$ | C, H, N |
| **6n** | | 260 | 2HCl | 75 | D | $C_{19}H_{28}Cl_2N_4O \cdot H_2O$ | C, H, N, Cl |
| **6o** | | 236 | 2HCl | 67 | D | $C_{19}H_{28}Cl_2N_4O \cdot 2.25H_2O$ | C, H, N |
| **6p** | | 180 | 2HCl | 44 | D | $C_{21}H_{32}Cl_2N_4O \cdot 2.75H_2O$ | C, H, N |
| **6q** | | 266 | 2HCl | 75 | D | $C_{20}H_{28}Cl_2N_4O \cdot H_2O$ | C, H, N |
| **18e** | 165 | 175 | 2M | 57 | C | $C_{24}H_{28}N_4O_9 \cdot 1.2H_2O$ | C, H, N |
| **18f** | 211 | 173 | 2M | 65 | C | $C_{25}H_{30}N_4O_9$ | C, H, N |
| BD84 | | (3) | 2M | | | | |

[a] 2M, dimaleate; 2HCl, dihydrochloride. [b] See Experimental Section for description of general methods. [c] For the hydrated compounds, titration of water was performed according to the Karl Fisher method. Results were within ±0.4% of the give nonstoichiometric numbers of water of hydration.

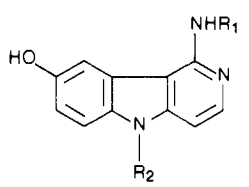
Table II. 1-Amino-Substituted 5*H*-Pyrido[4,3-*b*]indoles: Biological Data




**5**




**6**

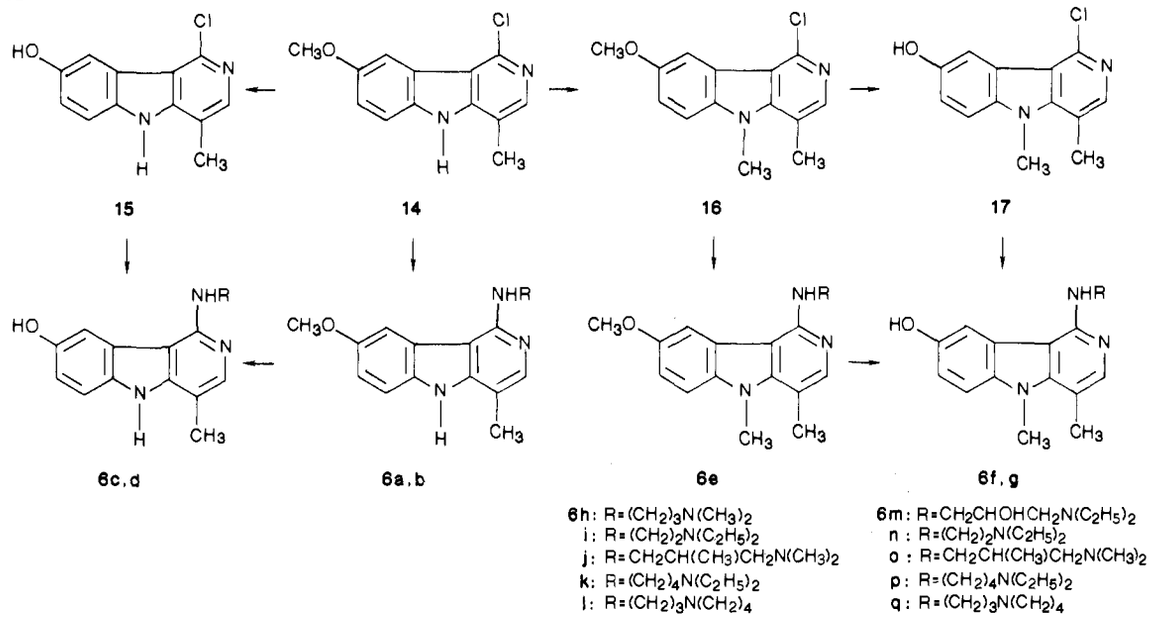



**18**

| no. | $R_1$ | $R_2$ | $R_3$ | $K_{aff}$[a] | $ID_{50}$,[b] μM | in vivo tests,[c] P388: T/C (mg/kg), $D_{1-5}$ ip |
|---|---|---|---|---|---|---|
| **5a** | $(CH_2)_3N(C_2H_5)_2$ | H | | 0.15 | 0.22 | 130 (10), 175 (50)[d] |
| **5b** | $(CH_2)_3N(CH_3)_2$ | H | | 0.13 | 0.16 | 129 (10), tox (50) |
| **5c** | $(CH_2)_2NH(CH_2)_2OH$ | H | | 0.05 | 1.14 | 109 (10), 100 (50)[d] |
| **5d** | $(CH_2)_3N(C_2H_5)_2$ | $CH_3$ | | 0.16 | 0.72 | 105 (6), 110 (12), 118 (24), 131 (50), tox (100) |
| **5e** | $(CH_2)_3N(CH_3)_2$ | $CH_3$ | | 0.15 | 0.35 | 107 (6), 114 (12), 131 (24), tox (50) |
| **6a** | $(CH_2)_3N(C_2H_5)_2$ | H | $CH_3$ | 0.22 | 0.30 | 147 (10), 149 (20), 176 (40), tox (80) |
| **6b** | $(CH_2)_3NHC_2H_5$ | H | $CH_3$ | 0.22 | 1.20 | 119 (5), 119 (10), 107 (20), tox (40) |
| **6c** | $(CH_2)_3N(C_2H_5)_2$ | H | H | 0.20 | 0.06 | 153 (1.25), 162 (2.5), 170 (5), 185 (10), 208 (15) \|3\|,[e] 238 (20) \|1\|, tox (30) |
| **6d** | $(CH_2)_3N(CH_3)_2$ | H | H | 0.21 | 0.09 | 139 (0.625), 150 (1.25), 145 (2.5), 186 (5) \|1\|, 148 (10), tox (20) |
| **6e** | $(CH_2)_3N(C_2H_5)_2$ | $CH_3$ | $CH_3$ | 0.25 | 0.10 | 107 (5), 115 (10), 125 (20), 115 (40), tox (80) |
| **6f** | $(CH_2)_3N(C_2H_5)_2$ | $CH_3$ | H | 0.24 | 0.03 | 149 (2.5), 166 (5), 205 (10), \|2\|, tox (20) |
| **6g** | $(CH_2)_3N(CH_3)_2$ | $CH_3$ | H | 0.27 | 0.01 | 164 (1.5), 184 (2.5), 236 (5), tox (10) |
| **6h** | $(CH_2)_3N(CH_3)_2$ | $CH_3$ | $CH_3$ | 0.36 | 0.13 | 134 (5), 160 (10), 150 (20), tox (40) |
| **6i** | $(CH_2)_2N(C_2H_5)_2$ | $CH_3$ | $CH_3$ | 0.08 | 1.20 | NT[f] |
| **6j** | $CH_2CH(CH_3)CH_2N(CH_3)_2$ | $CH_3$ | $CH_3$ | 0.15 | 1.0 | NT[f] |
| **6k** | $(CH_2)_4N(C_2H_5)_2$ | $CH_3$ | $CH_3$ | 0.46 | 1.30 | NT[f] |
| **6l** | $(CH_2)_3N(CH_2)_4$ | $CH_3$ | $CH_3$ | 0.30 | 0.50 | NT[f] |
| **6m** | $CH_2CHOHCH_2N(C_2H_5)_2$ | $CH_3$ | H | 0.23 | 0.06 | inactive (5), tox (10) |
| **6n** | $(CH_2)_2N(C_2H_5)_2$ | $CH_3$ | H | 0.11 | 0.15 | 142 (5), 153 (10), 182 (20), 137 (40)[d] |
| **6o** | $CH_2CH(CH_3)CH_2N(CH_3)_2$ | $CH_3$ | H | 0.14 | 0.01 | NT[g] |
| **6p** | $(CH_2)_4N(C_2H_5)_2$ | $CH_3$ | H | 0.35 | 0.31 | NT[f] |
| **6q** | $(CH_2)_3N(CH_2)_4$ | $CH_3$ | H | 0.37 | 0.05 | NT[g] |
| **18e** | $(CH_2)_3N(CH_3)_2$ | H | | 0.07 | 11.20 | 111 (2.5), 102 (5), 115 (10)[d] |
| **18f** | $(CH_2)_3N(CH_3)_2$ | $CH_3$ | | 0.12 | 0.19 | 137 (2.5), 145 (5), 169 (10), 173 (20)[d] |
| BD84 | | | | 0.70 | 0.10 | 171 (0.5), 178 (1), 188 (2), 245 (4), 273 (8) \|1\|, 283 (16) \|1\|, 113 (24) |

[a] $K_{aff}$: values of the apprent DNA affinity constant, in $10^6$ $M^{-1}$. [b] $ID_{50}$: the micromolar concentration of drug that, when added to cultures of L1210 cells for a 48-h period, reduces the counted cells to 50% of the controls (mean of two values obtained in two independent experiments). [c] In vivo tests were performed according to the Geran et al. protocol[13] by ip inoculation of $10^6$ P388 leukemia viable cells on day 0 in CDF1 hybrid male or female mice (10 mice per test group). Compounds dissolved in distilled water were administered ip for 5 days ($D_{1-5}$). BD 84 (reference compound) was 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/median day of survival of control mice) × 100. Only % T/C > 128 indicates significant activity. [d] Nontoxic at the highest dose tested. [e] The number within vertical bars represents the number of cured mice/10, at $D_{60}$, for the preceding dose (within parentheses). [f] NT: compound not tested in vivo. [g] NT: compound not yet tested in vivo.

Scheme II




of 2 is expected for the slope ($S$) of the straight line obtained, but experimental values are below this theoretical value. Compounds **5a**, **5b**, **6c**, **6d**, **6f**, and **6g** were subjected to viscosimetric analysis, and the $S$ values found were 1.2, 1.1, 1.3, 1.4, 1, 2, and 1.1, respectively. Adriamycin, included as a reference compound, gave an $S$ value of 1.3 in the same conditions. All these results strongly suggest the intercalative binding of these compounds. As it can be seen in the $K_{aff}$ entry of Table II, all compounds have been studied for their DNA binding capacity by displacement of ethidium bromide. All of the 24 substituted pyrido[4,3-*b*]indoles inhibited the binding of ethidium bromide in a competitive manner when the data were plotted according to Scatchard (not shown). These results strongly suggest that, as is unambiguously the case for **6c**, **6d**, **6f**, and **6g**, all of the substituted analogues intercalate into DNA helix. Most of the compounds present similar levels of DNA affinity, with $K_{aff}$ values near $0.2 \times 10^6$ $M^{-1}$ with exceptions varying from 0.05 to 0.46. As expected, these values are always lower than that determined for the parent tetracyclic ellipticine derivative, the reference compound BD 84 ($0.7 \times 10^6$ $M^{-1}$).

However, although compounds that present the lowest DNA affinity correspond to the less cytotoxic ones (**5c**, **6i**, and **18e**), once again it must be pointed out that there is no clear and direct correlation between DNA binding constants and in vitro cytotoxicity. Compound **6k**, which presents one of the highest $ID_{50}$ values (1.30 $\mu$M) and the highest $K_{aff}$ constant ($0.46 \times 10^6$ $M^{-1}$) in this series, significantly illustrates this observation.

On the contrary, in vivo P388 activity is generally well-correlated to a good cytotoxicity level on L1210 cultured cells. Since $ID_{50}$ values vary from 0.01 $\mu$M (**6g**, **6o**) to 11.2 $\mu$M (**18e**), positive results in the in vivo P388 leukemia system are generally observed for compounds that present an $ID_{50}$ equal to or lower than 0.3 $\mu$M, such as **6a**, **6c**, **6d**, **6f**, **6h**, and **18f**. As demonstrated by in vivo P388 activities of compounds **5b**, **6e**, and **6m**, there are, however, various exceptions to this rule.

When the results summarized in Table II are taken into account, four compounds come into view and appear as promising ones in this series of 1-amino-substituted pyrido[4,3-*b*]indoles. They are derivatives **6c**, **6d**, **6f**, and **6g**, which give T/C responses in the P388 leukemia system ($D_0$ inoculation/$D_{1-5}$ ip treatment) near 200%, with cases of survivors at $D_{60}$, pronounced in vivo antitumor activity at low doses [0.625–5 mg/kg], and good dose–effect relationship.

**Table III.** In Vivo Antitumor Properties of Compounds **6c**, **6d**, **6f**, and **6g** on P388 Leukemia Model Inoculated Ip at $D_0$ Followed by a Single Ip Administration of the Drugs at $D_1$

| no. | ip dose, mg/kg | wt changes, g | median survival day | T/C, % | survivors at $D_{30}$ |
|---|---|---|---|---|---|
| **6c** | 10 | +1.2 | 15.1 | 129 | 0/10 |
| | 20 | +0.2 | 16.25 | 139 | 0/10 |
| | 40 | −0.2 | 15.9 | 136 | 0/10 |
| | 80 | −1.7 | 2.3 | 20 | 0/10 |
| control | – | +1.1 | 11.7 | – | 0/20 |
| **6d** | 5 | −0.1 | 15.3 | 139 | 0/10 |
| | 10 | +0.3 | 15.25 | 139 | 0/10 |
| | 20 | −0.8 | 15.5 | 142 | 0/10 |
| | 40 | −1.4 | 15.3 | 139 | 0/10 |
| **6f** | 5 | +0.1 | 15.87 | 139 | 0/10 |
| | 10 | −0.6 | 17.75 | 156 | 0/10 |
| | 20 | −1.2 | 20.75 | 182 | 0/10 |
| | 40 | −5.2 | 23.0 | 202 | 1/10 |
| **6g** | 5 | −1.2 | 19.75 | 173 | 0/10 |
| | 10 | −1.4 | 19.0 | 167 | 1/10 |
| | 20 | −2.5 | 18.75 | 164 | 1/10 |
| control | – | −0.05 | 11.4 | – | 0/20 |

Only these selected drugs have then been submitted to complementary in vivo studies: (i) the P388 model where $10^6$ cells were inoculated ip at $D_0$, followed by a single ip administration of the drugs at the given doses (Table III); (ii) the B16 melanoma experimental tumor studied according to the usual protocol[13] (Table IV).

Although there are important differences in the responses with regard to the system studied and the protocol used, the results reported in Tables III and IV clearly confirm the potent antitumor activity of compounds **6c**, **6d**, **6f**, and **6g**. These four compounds were about equally effective on B16 melanoma (Table IV), but **6f** and **6g** appeared more potent than **6c** and **6d** on P388 leukemia when administered ip on day 1 (Table III). This latter result is in agreement with the higher cytotoxicity of **6g** and **6f** (Table II). For these reasons, we selected **6f** and **6g** for a more thorough in vivo evaluation.

(14) Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.

Table IV. In Vivo Antitumor Properties of Compounds **6c**, **6d**, **6f**, and **6g** on B16 Melanoma[a]

| no. | dose, mg/kg | wt changes, g | median survival day | T/C, % | survivors at $D_{60}$ |
|---|---|---|---|---|---|
| **6c** | 2.5 | +3.6 | 34 | 124 | 0/10 |
| | 5 | -0.1 | 48 | 174 | 0/10 |
| | 10 | +1.6 | 56.25 | 205 | 4/10 |
| | 20 | -0.8 | 58 | 211 | 5/10 |
| | 30 | -1 | 9 | 33 | 0/10 |
| **6d** | 1.25 | +2.8 | 32 | 116 | 0/10 |
| | 2.5 | +1.8 | 38.75 | 141 | 0/10 |
| | 5 | +2.4 | 44 | 160 | 0/10 |
| | 10 | +0.5 | 11.7 | 42 | 0/10 |
| **6f** | 1.25 | -2.5 | 32.25 | 133 | 0/10 |
| | 2.5 | +0.6 | 35.25 | 145 | 0/10 |
| | 5 | +0.2 | 41.25 | 170 | 3/10 |
| | 10 | -1 | 19.3 | 79 | 0/10 |
| **6g** | 1.25 | +1 | 29.75 | 123 | 0/10 |
| | 2.5 | +0.1 | 32.6 | 134 | 0/10 |
| | 5 | -0.2 | 50 | 206 | 1/10 |
| | 10 | -1.9 | 11.75 | 48 | 0/10 |
| control | - | +2.1 | 27.5 | - | 0/25 |

[a] Tests were performed according to the Geran et al. protocol.[13] Thus, hybrid BD F1 mice were inoculated ip at $D_0$ with 0.5 mL of a homogenate of B16 melanoma cells obtained from 1 g of tumor in 10 mL of physiological serum. Compounds under test were daily administered ip at the given doses for 9 days ($D_{1-9}$), and positive control was *cis*-platinum (0.5 mg/kg) administered in the same conditions. Survivors were counted at $D_{60}$, and a compound was considered as active when T/C > 125%.

Table V. In Vivo Antitumor Properties of Compounds **6f** and **6g** on P388 Leukemia Model ($10^6$ Cells) and on L1210 Leukemia Model ($10^5$ Cells) Inoculated Ip at $D_0$ Followed by a Single $D_1$ Iv Treatment

| no. | iv dose, mg/kg | wt changes, g | median survival day | T/C, % | survivors at $D_{30}$ |
|---|---|---|---|---|---|
| | | | P388 | | |
| **6f** | 50 | -0.7 | 19.0 | 174 | 0/10 |
| | 100 | -1.6 | 24.3 | 223 | 1/10 |
| **6g** | 50 | +0.5 | 17.75 | 163 | 1/10 |
| | 100 | -0.25 | 25.75 | 236 | 4/10 |
| control | - | +3.5 | 10.90 | - | 0/10 |
| | | | L1210 | | |
| **6f** | 20 | -0.4 | 11.8 | 132 | 0/10 |
| | 40 | -0.2 | 13.3 | 149 | 0/10 |
| **6g** | 20 | -1.1 | 14.0 | 157 | 0/15 |
| control | - | +0.8 | 8.9 | - | 0/15 |

In fact, the demonstration of significant activity in experimental models using iv administrations and/or solid tumors is a more valuable criterion by which to establish the antitumor properties of a drug. We thus tested **6f** and **6g**, administered by the iv route, on the P388 and L1210 leukemia and the solid tumor C38 colon adenocarcinoma models.

Significant activity was obtained in the L1210 leukemia model, with a single administration by the iv route at $D_1$ (Table V). Compound **6g** was the most active compound on P388 leukemia, giving a T/C of 236% and 40% survivors with a 100 mg/kg dose. The two compounds gave excellent results on the C38 colon adenocarcinoma: the tumor was not detectable at $D_{38}$ after two iv administrations of 25 or 50 mg/kg of **6g** at $D_2$ and $D_9$ (Table VI). Thus, the interest of **6f** and **6g** as potential antitumor drugs is strongly confirmed by the activities detected in these models with the iv administration of the drugs.

**Structure–Activity Relationship.** From the structure–activity relationship point of view, taking mainly into account in vitro cytotoxicity of the compounds ($ID_{50}$ entry of Table II), we must emphasize some general remarks.

Table VI. In Vivo Antitumor Properties of Compounds **6f** and **6g** Administered by the Iv Route on the C38 Adenocarcinoma Model

| no. | day of iv drug injectn | dose, mg/kg | median tumoral vol at $D_{38}$, $cm^3$ | % tumor vol T/C at $D_{38}$ | survivors at $D_{38}$ |
|---|---|---|---|---|---|
| **6f** | $D_1$ | 100 | 3.24 | 36 | 10/10 |
| **6g** | $D_1$ | 50 | 2.73 | 30 | 10/10 |
| control | - | - | 8.94 | 100 | 5/10 |
| | | | median tumoral vol at $D_{32}$ | % tumor vol T/C at $D_{32}$ | survivors at $D_{32}$ |
| **6f** | $D_2 + D_9$ | 50 | 1.38 | 18 | 10/10 |
| | | 75 | 0.97 | 13 | 10/10 |
| **6g** | $D_2 + D_9$ | 25 | 0.0 | 0 | 10/10 |
| | | 50 | 0.0 | 0 | 10/10 |
| control | - | - | 7.51 | 100 | 5/10 |

[a] These tests were performed according to the Geran et al. protocol,[13] with hybrid BD F1 mice (10 animals for every test) inoculated sc with a standard 3-$mm^3$ fragment of tumor followed by iv administration of the drugs at the given doses ($D_1$ or $D_2 + D_9$). Survivors were counted at $D_{38}$ ($D_1$ treatment), or $D_{32}$ ($D_2$ and $D_9$ treatment) and median tumoral volumes were measured the same day.

(1) With exceptions for compounds **5a** and **5b** vs **5d** and **5e**, whose $ID_{50}$ values were slightly increased by methylation of the 5-NH group, transformation of the 5-NH into 5-$NCH_3$ generally led to more cytotoxic derivatives: compare **6a** vs **6e**, **6c** vs **6f**, **6d** vs **6g**, and **18e** vs **18f**.

(2) When 4-methyl-8-methoxy-1-amino-substituted pyrido[4,3-*b*]indoles were transformed into corresponding 8-hydroxy derivatives, $ID_{50}$ values were lowered in all cases, but the values of the factor varied from 3 (**6e** vs **6f**), 4 (**6k** vs **6p**), 5 (**6a** vs **6c**), 8 (**6i** vs **6n**) to 100 (**6j** vs **6o**), without drastic changes in the affinity for DNA.

(3) Compared to the closely related active compound **6d**, the total loss of in vitro and in vivo activity of 1-[[3-(dimethylamino)propyl]amino]-8-hydroxy-5*H*-pyrido[4,3-*b*]indole (**18e**), which did not contain the 4-methyl group present in the former compound, as well as the important differences between both in vitro and in vivo activity levels of the weakly active 1-[[3-(dimethylamino)propyl]amino]-5-methyl-8-hydroxy-5*H*-pyrido[4,3-*b*]indole (**18f**) and of its potent 4-methyl analogue **6g**, clearly established the critical role of the 4-methyl group for the antineoplastic activity of the new series of antitumor pyrido[4,3-*b*]indole derivatives, despite the fact that such a minor modification did not induce drastic changes in the affinity for DNA.

(4) As shown by comparison of $ID_{50}$ values (Table II) recorded for the 8-hydroxylated compound **6f**, which had a three carbon unit side chain (30 nM), to those of its homologous C-2 shortened **6n** (150 nM) and C-4 lengthened **6p** (310 nM) analogues, the C-3 propylene unit joining the two amino functions obviously corresponded to the optimal side chain in this series. Same differences were found when we compared the cytotoxicity of corresponding 8-methoxylated derivatives **6e** (100 nM), **6i** (1200 nM), and **6k** (1300 nM).

(5) All the above-mentioned remarks correlate well with those recently reported for another tricyclic series of weak intercalators, namely, 1-amino-substituted pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines (**3**),[7] which are related to 9-azaellipticines by deletion of a cycle, as the present series of pyrido[4,3-*b*]indoles are toward pyrido[4,3-*b*]carbazoles (ellipticines).

Several conclusions can be drawn from this initial structure–activity relationship study of the new 1-amino-substituted pyrido[4,3-*b*]indole class of antitumor agents.

Like pyridopyrrolopyridines **3**, pyrido[4,3-*b*]indoles **4** are weaker DNA intercalators, compared to the tetracyclic reference compound BD 84 (**2a**). Structure–activity relationship studies performed with 24 analogues show that there is no direct correlation between pyrido[4,3-*b*]indole DNA affinity and cytotoxicity, or between DNA affinity and in vivo antitumor properties. For example, cytotoxicity of two compounds having similar DNA affinity can be strikingly different. DNA interaction, however, seems to be an important parameter for biological activity in this series since compounds having the lowest affinity for DNA (**5c**, **18e**) are totally inactive.

These findings prompt us to think that, in addition to DNA association constants, structural requirements also play an important role. The changes in biological activities when minor structural modifications are introduced either in the side chain or in the ring substituents are significant in this respect.

Finally, the key substituents in the pyrido[4,3-*b*]indole series are a [3-(dimethylamino)propyl]amino or [3-(diethylamino)propyl]amino side chain at the 1-position, a 4-methyl and an 8-hydroxy substituent, and a 5-NH or 5-$NCH_3$ group for high biological responses, as is the case for compounds **6c**, **6d**, **6f**, and **6g**.

Studies of the possible interaction of this new class of antineoplastic agents and other series of related drugs with topoisomerase II and the induction of DNA breaks in L1210 cells in culture are now in progress. This might contribute to establish if, with respect to antitumor activity of compounds **1** and **2**, the tricyclic products **3** and **4** reveal their biological properties through common target and mechanism. Moreover, the availability of various congeners in two closely related series of tricyclic intercalators, allowing structure–activity relationship studies, could provide information on the relation between the capacity to induce DNA breaks and the cytotoxicity for tumor cells in culture. At the present time, however, it can be emphasized that the analogy obtained through deletion of a cycle in related series of heterocyclic compounds leads to promising results and deserves special attention.

## Experimental Section

**Chemistry.** All melting points were determined with a Kofler apparatus and are uncorrected. $^1H$ NMR spectra were recorded with either a Varian XL 100 or a Brucker WP 80 spectrometer. $Me_4Si$ was used as internal standard, and chemical shifts are reported on the $\delta$ scale, with peak multiplicities. Only NMR spectra of some typical examples are recorded, but all others are consistent with the reported structures. Purification of products was followed by thin-layer chromatography on silica gel and alumina. Elemental analysis was performed by Service Central de microanalyses du CNRS, 91190 Gif-sur-Yvette, France. As noted in Table I in the formula entries, we observed that, after conventional treatment, 1-amino-substituted pyrido[4,3-*b*]indoles or their salts were frequently associated with solvation water.

**1-Chloro-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole (9b). Example of Method A.** A solution of 1-chloro-4-methyl-5*H*-pyrido[4,3-*b*]indole (**9a**)[12] (3.3 g, 15 mmol) in dimethylformamide (DMF, 100 mL) was treated with methyl iodide (4.7 g, 33 mmol) in the presence of dry potassium carbonate (11.5 g, 80 mmol) under $N_2$ and stirred for a 5-h period at ambient temperature. The mixture was evaporated to dryness at 25 °C under reduced pressure and taken up in 1 N hydrochloric acid (100 mL). Neutralization by ammonia gave a precipitate, which was collected and chromatographed on silica gel, eluting with ethyl acetate to give 2.07 g (59%) of colorless crystals, mp 214 °C. Anal. ($C_{13}H_{11}ClN_2$) C, H, N, Cl.

***N*-(4-Methoxycyclohexylidene)-*N'*-(5-methyl-1*H*-pyrid-2-on-4-yl)hydrazine (11a).** 4-Methoxycyclohexanone[15] (25 g, 0.193 mol) and 4-hydrazino-5-methyl-1*H*-pyrid-2-one (**7a**)[16] (25 g, 0.18 mol) were successively added to absolute ethanol (500 mL), and the mixture was heated at reflux for 4 h. After concentration to half of the volume, the heterogeneous mixture was cooled to 0 °C. The solid was collected and air-dried to give 38.8 g (86%) of colorless crystals, mp 270 °C. Anal. ($C_{13}H_{19}N_3O_2$) C, H, N.

***N*-(4-Methoxycyclohexylidene)-*N'*-(1*H*-pyrid-2-on-4-yl)-hydrazine (11b).** Starting from 4-hydrazino-1*H*-pyrid-2-one (**7b**)[16] (11 g, 88 mmol) and using the preceding technique (12.4 g, 96 mmol of 4-methoxycyclohexanone), we obtained hydrazone **11b** (16.7 g, 80.5%) as colorless crystals, mp >260 °C. Anal. ($C_{12}H_{17}N_3O_2$) C, H, N.

**6,7,8,9-Tetrahydro-8-methoxy-4-methyl-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (12a).** A suspension of hydrazone **11a** (20 g, 80 mmol) in diphenyl ether (450 mL) was stirred under $N_2$ and heated under reflux for 30 min. The resulting homogeneous mixture was allowed to cool to room temperature, and toluene (400 mL) was added. The white solid was filtered, washed with acetone and diethyl ether, and then air-dried to give 17.7 g (95%) of **12a**: mp >280 °C; $^1H$ NMR ($C_5D_5N$) $\delta$ 1.85–2.07 (m, 2 H, 7-$CH_2$), 2.21 (s, 3 H, 4-$CH_3$), 2.66–2.94 (m, 2 H, 6-$CH_2$), 3.35 (s, 3 H, $OCH_3$), 3.57–3.93 (m, 3 H, 8-H + 9-$CH_2$), 6.94 (s, 1 H, 3-H), 11.54 (s, 1 H, 2-NH), 11.94 (s, 1 H, 5-NH). Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

**4-Methyl-8-methoxy-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (13a).** The preceding compound (**12a**, 13.4 g, 58 mmol) was progressively added to a stirred suspension of 10% palladium on charcoal (3 g) in boiling diphenyl ether (200 mL) under $N_2$ and then heated under reflux for a further 1 h. Toluene (100 mL) was added to the cooled (50 °C) mixture, and the precipitate was collected. It was taken up in boiling acetic acid (150 mL), filtered, and cooled. The resulting solid was filtered, washed with acetone and diethyl ether, and then dried to give 9.5 g of colorless crystals of **13a**, mp >270 °C. Evaporation of acetic acid mother liquors afforded a residual solid, which was treated as described above to give an additional crop (1.56 g); total yield, 86%. Anal. ($C_{13}H_{12}N_2O_2$) C, H, N.

Remark: This compound was in all respects identical with that prepared by another route.[12] It was also obtained with an 80% yield by a one-pot procedure starting from hydrazone **11a**, which was transformed as described below for obtention of compound **13b**.

**8-Methoxy-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (13b).** A well-stirred mixture of hydrazone **11b** (7.4 g, 32 mmol) in diphenyl ether (150 mL) was heated under reflux in a nitrogen atmosphere for 30 min, and a suspension of 10% palladium on charcoal (2.2 g) in diphenyl ether (20 mL) was added dropwise. Reflux was maintained for 1.25 h, and hexane (300 mL) was added to the cooled mixture. The solid was collected, taken up in boiling acetic acid (400 mL), and filtered. The filter was washed with two 200-mL portions of acetic acid, and the filtrate was evaporated under reduced pressure. The resulting solid was boiled in ethanol (50 mL), allowed to cool, and filtered to give 4.9 g (73%) of colorless crystals, mp >280 °C. Anal. ($C_{12}H_{10}N_2O_2$) C, H, N.

**1-Chloro-8-methoxy-4-methyl-5*H*-pyrido[4,3-*b*]indole (14).** This key intermediate (mp 243–245 °C) was previously obtained from pyridoindolone **13a** and phosphorus oxychloride in a ratio **13a** (g)/$POCl_3$ (mL) = 1/83 by a 72-h reflux period and subsequent conventional treatment.[12] It was now prepared by the same technique but with a ratio of 1/28, without loss in yield.

**1-Chloro-8-hydroxy-4-methyl-5*H*-pyrido[4,3-*b*]indole (15). Example of Method B.** A mixture of compound **14** (200 mg) and concentrated hydrobromic acid (7 mL) was heated under reflux for 1 h and then evaporated to dryness under reduced pressure. The residual solid was taken up in a 10-mL portion of boiling water and alkalinized by ammonia. The precipitate was collected and chromatographed on a silica gel column (methylene chloride–ethanol, 98/2, as eluent) to give 141 mg (75%) of pale-yellow crystals, which sublimated above 265 °C. It was identical with that prepared by another previously described method.[12]

**1-Chloro-8-methoxy-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole (16).** This compound (mp 173–176 °C as already described[12]) has

(15) Cook, J. W.; Graham, W.; Cohen, A.; Lapsley, R. W.; Lawrence, C. A. *J. Chem. Soc.* **1944**, 332.

(16) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1986**, *42*, 2303.

now been obtained in an 89% yield by starting from **14** and using method A mentioned for the preparation of **9b**.

**1-Chloro-8-hydroxy-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole (17).** Demethylation of compound **16** according to the above-described method B led to **17** (mp >260 °C)[12] in a 65% yield.

**1-Chloro-8-methoxy-5*H*-pyrido[4,3-*b*]indole (18a).** A mixture of 8-methoxy-2*H*,5*H*-pyrido[4,3-*b*]indol-1-one (**13b**) (5.7 g, 26.5 mmol) and phosphorus oxychloride (150 mL) was heated under reflux for 24 h and evaporated under reduced pressure. Ice (100 g) and 3 N hydrochloric acid (1 L) were successively added to the residue. The mixture was heated at reflux for 1 h and filtered. When cooled, it was alkalinized with ammonia. The resulting precipitate was collected, dried, and chromatographed on a silica gel column (ethyl acetate as eluent) to give 3.8 g (61.3%) of a pale yellow powder, which sublimated above 250 °C: $^1$H NMR [$(CD_3)_2SO$] $\delta$ 3.91 (s, 3 H, $OCH_3$), 7.24 (d × d, 1 H, 7-H, $J_{7-6}$ = 8.7 Hz, $J_{7-9}$ = 2.7 Hz), 7.52 (d, 1 H, 4-H, $J_{4-3}$ = 5.7 Hz), 7.59 (d, 1 H, 6-H), 7.88 (d, 1 H, 9-H), 8.22 (d, 1 H, 3-H), 11.97 (br s, 1 H, NH). Anal. ($C_{12}H_9ClN_2O$) C, H, N, Cl.

**1-Chloro-8-hydroxy-5*H*-pyrido[4,3-*b*]indole (18b).** Demethylation of **18a** (700 mg) with concentrated hydrobromic acid (40 mL) was performed as for the preparation of pyridoindole **15** (method B, 1.5 h at reflux). The crude precipitate was recrystallized from a 1/1 acetonitrile–ethanol mixture (200 mL) that was concentrated to 50 mL to afford 550 mg (83%) of pale yellow microcrystals, mp >260 °C. Anal. ($C_{11}H_7ClN_2O$) C, H, N, Cl.

**1-Chloro-8-methoxy-5-methyl-5*H*-pyrido[4,3-*b*]indole (18c).** Methylation of **18a** (2 g, 8.6 mmol) with methyl iodide according to method A and subsequent chromatography on a silica gel column, with ethyl acetate as eluent, afforded 1.8 g (85%) of colorless needles: mp 136–138 °C; $^1$H NMR [$(CD_3)_2SO$] $\delta$ 3.91 + 3.92 (2 s, 2 × 3 H, $NCH_3$ + $OCH_3$), 7.3 (d × d, 1 H, 7-H, $J_{7-6}$ = 9 Hz, $J_{7-9}$ = 2.5 Hz), 7.66 (d, 1 H, 4-H, $J_{4-3}$ = 5.8 Hz), 7.69 (d, 1 H, 6-H), 7.89 (d, 1 H, 9-H), 8.28 (d, 1 H, 3-H). Anal. ($C_{13}H_{11}ClN_2O$) C, H, N.

**1-Chloro-8-hydroxy-5-methyl-5*H*-pyrido[4,3-*b*]indole (18d).** Compound **18c** (800 mg) was demethylated with hydrobromic acid (50 mL) as for obtention of **18b** (method B), thus giving 600 mg (86%) of the expected product **18d**, which sublimated above 250 °C (from a 1/1 dioxane–ethanol mixture). Anal. ($C_{12}H_9ClN_2O$·0.5$H_2O$) C, H, N, Cl.

**Preparation of 1-[[3-(Dimethylamino)-2-methylpropyl]amino]-8-methoxy-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole Dihydrochloride (6j). Example of Method C.** 1-Chloro-8-methoxy-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole (**16**) (2.6 g, 10 mmol) and 3-(dimethylamino)-2-methylpropylamine (25 mL) were heated in a steel vessel at 200–210 °C for 4 h. After cooling, excess diamine was evaporated under reduced pressure. The residue was taken up in a 50-mL portion of water, and an excess of ammonia was added. The mixture was extracted with 3 × 100 mL portions of methylene chloride, and evaporation of solvent afforded a residue, which was chromatographed on a silica gel column with a 9/1 methanol–triethylamine mixture as eluent. Evaporation of the pure free base containing fractions gave the free base, which was transformed into its dihydrochloride salt by dissolution in ethanol, treatment with a diethyl ether solution of hydrochloric acid in excess, and subsequent conventional treatment.

Remark: In cases where dimaleate salts were prepared, they were obtained by addition of the free base acetone solution to a solution of 2.2 equiv of maleic acid in the same solvent.

Compound **6j**-2HCl: $^1$H NMR ($D_2O$) $\delta$ 1.41 (d, 3 H, $CHCH_3$), 2.53 (s, 3 H, 4-$CH_3$), 2.61–2.93 (m, 1 H, $CHCH_3$), 3.22 (s, 2 × 3 H, $N(CH_3)_2$), 3.37–3.81 (m, 2 × 2 H, $CH_2$-$\gamma$ + $CH_2$-$\alpha$), 3.52 (s, 3 H, 5-$CH_3$), 4.09 (s, 3 H, $OCH_3$), 7.16 (q, 1 H, 7-H, $J_{7-6}$ = 9 Hz, $J_{7-9}$ = 2 Hz), 7.34 (d, 1 H, 6-H), 7.53 (d, 1 H, 9-H), 7.58 (s, 1 H, 3-H).

**Preparation of 1-[[2-(Diethylamino)ethyl]amino]-8-hydroxy-4,5-dimethyl-5*H*-pyrido[4,3-*b*]indole (6n). Example of Method D.** Compound **6i** as dihydrochloride (3 g) was refluxed in concentrated (48%) hydrobromic acid (30 mL) for 3 h. After evaporation under reduced pressure, the residue was washed with acetone and chromatographed on a silica gel column, eluting with a 9/1 methanol–triethylamine mixture. The resulting pure free base was transformed into its dihydrochloride salt as described above in method C: $^1$H NMR ($D_2O$) $\delta$ 1.47 (t, 2 × 3 H, $CH_2CH_3$), 2.40 (d, 3 H, 4-$CH_3$, $J_{4\text{-}CH_3\text{-}3\text{-}H}$ = 0.9 Hz), 3.34–3.62 (m, 3 × 2 H, $CH_2CH_3$ + $CH_2$-$\beta$), 3.47 (s, 3 H, 5-$CH_3$), 3.84–4.03 (m, 2 H, $CH_2$-$\alpha$), 6.86 (d × d, 1 H, 7-H, $J_{7-6}$ = 8.9 Hz, $J_{7-9}$ = 2.2 Hz), 7.02 (d, 1 H, 6-H), 7.19 (d, 1 H, 9-H), 7.33 (d, 1 H, 3-H).

**DNA Binding Measurements.** The DNA binding constants were determined by competition with ethidium bromide as described[17] in 20 mM $Na_2HPO_4/NaH_2PO_4$, 0.1 M NaCl, 1 mM EDTA, pH 7.4. DNA (calf thymus, Sigma) concentration was 10 $\mu$g/mL.

**Viscosimetry.** Viscosimetric titrations were conducted in 3 mL of buffer (20 mM $Na_2HPO_4/NaH_2PO_4$, 0.1 M NaCl, 1 mM EDTA, pH 7.4) containing 50 $\mu$g/mL of sonicated DNA, in an Ubbelohde microviscosimeter. The flow times were automatically measured, and data were plotted according to Cohen and Eisenberg.[14] The slope ($S$) of the straight line obtained when $(N/N_0)^{1/3}$ is expressed as a function of the ratio of bound drug per nucleotide ($R$) was calculated by regression analysis (correlation coefficient = 0.99). We calculated $R$ from a quadratic equation, using the $K_{aff}$ values measured in the same buffer as described above.

**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 $\mu$g/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[13] were used throughout the drug evaluation tests.

Viable P388 and L1210 leukemia cells, $10^6$ and $10^5$ respectively, were inoculated ip on day 0 in CDF1 hybrid male or female mice (10 mice per test group). Compounds, dissolved in distilled water, were administered ip (0.1 mL/10 g of body weight), at various doses, for 5 days ($D_{1-5}$). Vehicle, 5-fluorouracil (positive control), and BD 84 (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 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 > 128% | T/C > 175% |
| L1210 leukemia | T/C > 125% | T/C > 150% |

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**Registry No.** **5a**, 111380-12-2; **5a** (free base), 111380-11-1; **5b**, 111380-14-4; **5b** (free base), 111380-13-3; **5c**, 111380-16-6; **5c** (free base), 111380-15-5; **kd**, 111380-17-7; **5d** (free base), 111380-60-0; **5e**, 111380-18-8; **5e** (free base), 111380-61-1; **6a**, 111380-20-2; **6a** (free base), 111380-19-9; **6b**, 111380-22-4; **6b** (free base), 111380-21-3; **6c**, 111380-23-5; **6c** (free base), 111380-62-2; **6d**, 111380-25-7; **6d** (free base), 111380-27-9; **6e**, 111380-27-9; 6e (free base), 111380-26-8; **6f**, 111380-28-0; **6f** (free base), 111380-63-3; **6g**, 111380-30-4; **6g** (free base), 111380-29-1; **6h**, 111380-31-5; **6h** (free base), 111380-64-4; **6i**, 111380-32-6; **6i** (free base), 111380-65-5; **6j**, 111380-33-7; **6j** (free base), 111380-66-6; **6k**, 111380-34-8; **6k** (free base), 111380-67-7; **6l**, 111380-35-9; **6l** (free base), 111380-68-8; **6m**, 111380-36-0; **6m** (free base), 111380-69-9; **6n**, 111380-37-1; **6n** (free base), 111380-70-2; **6o**, 111380-38-2; 6o (free base),

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111380-71-3; **6p**, 111380-39-3; **6p** (free base), 111380-72-4; **6q**, 111380-40-6; **6q** (free base), 111380-73-5; **7a**, 106689-40-1; **7b**, 106689-41-2; **9a**, 111380-45-1; **9b**, 111380-47-3; **11b**, 111380-48-4; **12a**, 111380-49-5; **13a**, 111380-50-8; **13b**, 111380-51-9; **14**, 111380-52-0; **15**, 111380-53-1; **16**, 111380-54-2; **17**, 111380-55-3; **18a**, 111380-56-4; **18b**, 111380-57-5; **18c**, 111380-58-6; **18d**, 111380-59-7; **18e**, 111380-42-8; **18e** (free base), 111380-41-7; **18f**, 111380-44-0; **18f** (free base), 111380-43-9; $H_2NCH_2CH(CH_3)CH_2N(CH_3)_2$, 6105-72-2; $H_2N(CH_2)_3N(CH_3)_2$, 109-55-7; $H_2N(CH_2)_2N(C_2H_5)_2$, 100-36-7; $H_2N(CH_2)_3N(CH_2)_4$, 23159-07-1; $H_2N(CH_2)_4N(C_2H_5)_2$, 27431-62-5; $H_2NCH_2CHOHCH_2N(C_2H_5)_2$, 6322-01-6; 4-methoxycyclohexanone, 13482-23-0.

# Synthesis and Biological Evaluations of Certain 2-Halo-2′-Substituted Derivatives of 9-β-D-Arabinofuranosyladenine

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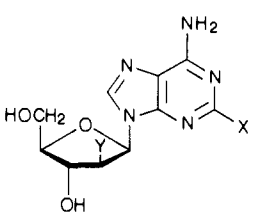
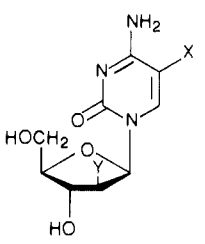
The synthesis of a series of 2-chloro- or 2-fluoro-9-(2-substituted-2-deoxy-β-D-arabinofuranosyl)adenines (**4g–n**) is described. New compounds were prepared from either 2-chloroadenosine or 2-fluoroadenosine by first blocking the 3′- and 5′-hydroxyls as the tetraisopropyldisiloxane derivatives. Activation of O-2′ by formation of a triflate followed by nucleophilic displacement allowed introduction of various groups in the proper configuration at C-2′. Fluoride ion treatment then produced the deblocked nucleosides. All of the new compounds were evaluated as cytotoxic agents against L1210 and H.Ep.-2 cells and as antiviral agents against herpes simplex viruses 1 and 2 and vaccinia virus in culture.

Certain arabinofuranosyl nucleosides have interesting and useful biological activities. Both 9-β-D-arabinofuranosyladenine (araA, **1a**)[1] and 1-β-D-arabinofuranosylcytosine (araC, **2a**)[2] are well known in the areas of viral and cancer chemotherapy, respectively. 9-β-D-



**1a**: X=H; Y=OH
**b**: X=F; Y=OH
**c**: X=H; Y=$N_3$



**2a**: X=H; Y=OH
**b**: X=I; Y=F

Arabinofuranosyl-2-fluoroadenine (2-F-araA, **1b**), administered as the 5′-monophosphate, has completed phase I clinical trials[3,4] and is presently in phase II trials as an anticancer agent,[5,6] and various other arabinofuranosyl nucleosides have received considerable attention. Certain arabinofuranosyl nucleosides with 2′-substituents other than a hydroxyl also have produced marked biological effects. Notable among these compounds are 9-(2-azido-2-deoxy-β-D-arabinofuranosyl)adenine (arazide, **1c**) and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC, **2b**). Arazide has pronounced cytotoxicity in cell culture[7-10] and also has in vivo activity against the P388 mouse leukemia when administered with the potent adenosine deaminase inhibitor pentostatin (2′-deoxycoformycin).[7] This combination is superior to the araA–pentostatin combination on a once-a-day schedule for 6 days, resulting in a significant number of mice that were long-term survivors. Without the pentostatin, arazide did not result in a significant increase in the life span of the leukemic mice. The reduced effectiveness of arazide without pentostatin is presumably caused by a significant deamination of arazide by adenosine deaminase, even though arazide is much less susceptible to deamination than araA itself. Arazide triphosphate has been found to be an inhibitor of DNA polymerase *a*.[11,12] FIAC has in vitro[3,4] and in vivo[15,16] activity against herpes simplex viruses and shows some phase I clinical activity against immunosuppressed patients with herpes simplex virus infections.[15,17]

All of the nucleosides mentioned above require activation in order to exert their effects. Generally, the arabinofuranosyl nucleosides are primarily activated (phos-

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