of the pyrimidine nucleus of I decreases the log P of I by 2.37 [for X = 3,4,5-$(OCH_3)_3$] or 2.61 (for X = H), and (2) $\pi_{X,obsd}$ from the benzene system for 3,4,5-$(OCH_3)_3$ has an unusually low value of −0.60, as compared with $\Sigma\pi_{X,calcd} = 3\pi_{OCH_3} = -0.06$; this same effect is seen for I where X = 3,4,5-$(OCH_3)_3$.

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Structure–Activity Relationships in a Series of Newly Synthesized 1-Amino-Substituted Ellipticine Derivatives

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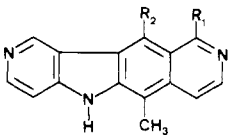
The synthesis of a series of 1-amino-substituted pyrido[4,3-*b*]carbazole derivatives, based on the substitution of corresponding 1-chloroellipticines, is reported. The cytotoxic properties on tumor cells grown in vitro, the in vivo acute toxicity of the most potent in vitro cytotoxic compounds, and the antitumor properties toward the L1210 leukemia system are described. No correlation between the apparent association constant to DNA and the in vitro cytotoxicity or the in vivo antitumor efficiency could be observed in this series. 9-Hydroxylated derivatives were more cytotoxic in vitro than the corresponding 9-methoxylated compounds. However, their antitumor efficiencies on the in vivo experimental systems do not confirm the advantage of demethylation. The presence of a [(dialkylamino)alkyl]amino side chain at the 1 position of ellipticines increases the antitumor potency: 1-[[3-(diethylamino)propyl]amino]-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**5**) is a very potent antitumor compound (% ILS of 134 on the L1210 leukemia system).

Recently, we have described the synthesis and the antitumor properties of pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline derivatives, which were formerly misnamed dipyrido[4,3-*b*][3,4-*f*]indoles. Amongst these compounds, those having a [(dialkylamino)alkyl]amino side chain at the 10 position of this new heterocyclic ring system (such as **1**) display a higher antitumor activity on the L1210



1, $R_1 = NH(CH_2)_3N(Et)_2$; $R_2 = H$
2, $R_1 = H$; $R_2 = CH_3$

leukemia system than the basic ellipticine analogue **2**.[1-5] Drugs in the ellipticine series are endowed with anticancer properties toward several experimental tumors. We then decided to examine whether various diamino side chains would also increase the biological activity in the ellipticine series, and an appropriate synthesis of required 1-chloropyrido[4,3-*b*]carbazoles, **3**, has been carried out in our laboratory.[6]

This report presents data concerning the preparation of new 1-[[(dialkylamino)alkyl]amino]ellipticine derivatives and the structure–activity relationships of these compounds studied in vitro on cultured tumor cells and in vivo on the L1210 leukemia system.

According to the intercalation model first described by Lerman,[7] the parent compounds **1** and **2** have been shown to bind to DNA in vitro with a high affinity.[4] Since biological activities of intercalating drugs were admitted to be related to their DNA affinity,[8,9] it was of interest to study if such a relation could also be shown with this new series of pyrido[4,3-*b*]carbazole derivatives. Thus, the apparent association constants for DNA of some compounds were also determined.

Chemistry. Compounds **4–9** were already described.[6] 1-Amino-substituted ellipticines **10–18** and **20–26** were obtained starting from chloroellipticines **3a–d**, which were substituted by their corresponding amines in an inert atmosphere. Substitutions were performed in boiling pure free amines, until complete disappearance of the starting material by monitoring with TLC on silica gel or alumina. 1-[[3-(Dimethylamino)propyl]amino]-5-methyl-9-hydroxypyrido[4,3-*b*]carbazole (**19**) was prepared by catalytic hydrogenation of benzylated derivative **18** as for

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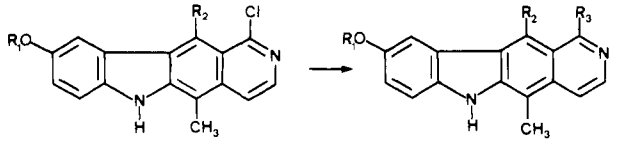
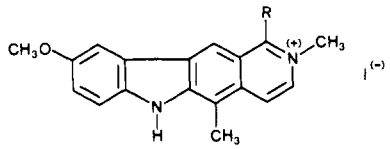


3 4-27

a, $R_1 = CH_3$; $R_2 = H$
b, $R_1 = R_2 = CH_3$
c, $R_1 = CH_2C_6H_5$; $R_2 = H$
d, $R_1 = CH_2C_6H_5$; $R_2 = CH_3$

obtaining parent compound 8.[6] The anilino-substituted ellipticine **27** was obtained from chloroellipticine **3a** (activated by hydrochloric acid) and *p*-methylsulfonamidoaniline.[10] Quaternarized derivatives **28** and **29** were prepared by N-2 quaternarization of **3a** with methyl iodide and subsequent substitutions with aniline and dimethylamine, respectively.



28, $R = NHC_6H_5$
29, $N(CH_3)_2$

Biological Results

In Vitro Cytotoxic Determination. Cytotoxicities toward tumor cells grown in vitro were studied as described under Experimental Section. Dose–effect relationships of the various compounds were determined from the regression lines drawn as the percentage of cell growth inhibition plotted as a function of the logarithm of the dose. The concentration of drug which lowered control cell growth by 50% (ID_{50}) was estimated from these curves. For the sake of clarity, regression lines are not presented in this paper. Only the ID_{50} values (μM) are given. In order to compare the in vitro cytotoxic potency of the various compounds, an arbitrary value (K in Table I) was assigned where an ID_{50} of 1 μM corresponds to $K = 1$. The more potent the compound, the higher is the K value. For example, in our culture system, the ID_{50} for actinomycin D, a very potent DNA binding agent, was 10^{-3} μM, which corresponds to a K value of 1000. Results are summarized in Table I.

Substitutions on the 5-methylpyrido[4,3-*b*]carbazole nucleus were performed mainly at the C-9, C-11 and, especially, C-1 positions. All the newly synthesized ellipticine derivatives possess a varying ability to decrease the cell growth rate, in a 0.2- to 50-fold range as compared to the 1000, 3, and 0.5 range for actinomycin, 2-methyl-9-hydroxyellipticinium acetate,[11] and 9-methoxyellipticine, respectively. The majority of them have a highly cytotoxic effect for concentrations of 10^{-8} to 10^{-6} μM (0.01 to 1 μg/mL).

Some general remarks can be inferred from the data: (a) Except for compounds having a 9-hydroxy substituent (**8** and **9**), the presence of a CH_3 group at the 11 position significantly increases the cytotoxic effect (compare **4** with **5** and **16** with **17**). (b) The transformation of the 9-OCH_3 to 9-OH groups considerably increases the cytotoxicity of the parent compounds. Thus, starting from K values of 0.2 and 3 for products **4** and **5**, the cytotoxic effect increases for the corresponding 9-hydroxylated derivatives **8** and **9**, up to K values of 50 and 30, respectively. (c) The presence of an $NH(CH_2)_nN$ side chain at the 1 position generally increases the biological activity. The highest effect was ascertained for a $-(CH_2)_3-$ chain between the two amino groups. (d) Substitution by a secondary amine, such as piperidine (compound **24**), at the C-1 position results in a striking decrease of the cytotoxic response. With a simple secondary amino group, such as dimethylamino (**29**), even quaternarization by a 2-methyl substituent, which is likely to result in an increase of antitumor effect for ellipticine derivatives,[12] does not yield highly cytotoxic compounds. (e) It has been stated that DNA affinity constants are related to the antitumor properties of various ellipticines.[9] In our series of new derivatives, 10 compounds have been studied for this purpose. If their apparent association constants (K_{app}) are generally of the same order of magnitude (2–4.5 $\times$ 10^{-7} M), the corresponding K values change in a range of 1.2 to 50. Thus, for 1-amino-substituted ellipticines which show higher apparent association constants to DNA, DNA binding does not seem to be directly related to the in vitro cytotoxicity.

In Vivo Antitumor Effects. For 12 compounds, the lethal dose (LD_{100}) and the highest nonlethal dose (LD_0) were determined after a single injection to mice by the intraperitoneal or intravenous route. When drugs were given ip, sometimes an inflammatory reaction developed, leading to a fibrous adhesion of the organs. However, similar LD_{100} and LD_0 were obtained when the compounds were intravenously injected, except for compounds having an NH_2 terminal group on their (aminoalkyl)amino side chain which were very toxic (Table II).

In order to compare if the in vitro cytotoxicity toward Friend tumor cells occurs on the same target cells grafted in vivo, mice received compounds 3 days after 10^6 cells inoculation. Results presented in Table III show that in vivo antitumor activity is weak but significant.

Antitumor properties of nine derivatives were determined on the L1210 leukemia system. All tests were performed at the highest nontoxic doses (LD_0). Drugs were injected ip. Results, in Table IV, show that 9-hydroxyellipticine derivatives **8**, **9**, and **19**, which were the best in vitro cytotoxic compounds (K = 50, 30, and 30, respectively) were active on the L1210 leukemia test, but the ILS did not exceed 60%. The most potent compound on the L1210 leukemia test was 1-[[γ-(diethylamino)propyl]amino]-5,11-dimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (**5**), which includes the same substituent as pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline (**1**). This compound gives an ILS of 134%.

Conclusion

Taking into account all of our results, some general conclusions can be pointed out. (1) On the in vitro model, 9-hydroxylated derivatives are more cytotoxic compounds than the corresponding 9-methoxylated ones, but their acute toxicity in mice is also increased and antitumor properties on in vivo models do not confirm the advantage of demethylation. Consequently, if the in vitro tests retain attention in order to sort out the most cytotoxic derivatives, all active compounds which present an in vitro cytotoxicity at concentration levels ≤ 0.5 μg/mL (10^{-6} μM) are worth studying on the in vivo models. (2) 9-Methoxyellipticine tested under the same conditions gives a % ILS of 24.[5] This ILS was 134% for compound **5**. This result clearly shows that the presence of a [(dialkylamino)alkyl]amino side chain at the 1 position of ellipticine

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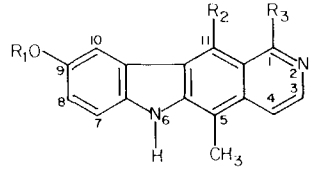
Table I. Pyrido[4,3-*b*]carbazole Derivatives



compd	R_1	R_2	R_3	ref or mp, °C	method (time)[a]	yield,[b] %	purifn proced[c]	formula	anal.[d]	ID_{50}, μM	K	K_{app}, 10^{-7} M^{-1}	B_{app}
AD (actinomycin D)										0.001	1000		
HUM	H	CH_3	H (+ 2-CH_3)							0.3	3		
9-OCH_3-E	CH_3	CH_3	H							5	0.2		
4	CH_3	H	$NH(CH_2)_3N(Et)_2$	ref 6						5	0.2	3.15	0.1
5	CH_3	CH_3	$NH(CH_2)_3N(Et)_2$	ref 6						0.3	3	3.15	0.16
6	$CH_2C_6H_5$	H	$NH(CH_2)_3N(Et)_2$	ref 6						2	0.5		
7	$CH_2C_6H_5$	CH_3	$NH(CH_2)_3N(Et)_2$	ref 6						1	1		
8	H	H	$NH(CH_2)_3N(Et)_2$	ref 6						0.02	50	3.7	0.11
9	H	CH_3	$NH(CH_2)_3N(Et)_2$	ref 6						0.03	30	2.0	0.16
10	CH_3	H	$NH(CH_2)_2NH_2$	180–185 dec	A (2 h)	79	I	$C_{19}H_{20}N_4O\cdot 0.5H_2O$	C, H, N	0.1	10	3.3	0.135
11	CH_3	H	$NH(CH_2)_3NH_2$	190 dec	A (1 h)	71	I	$C_{20}H_{22}N_4O\cdot CH_3CN$	C, H, N	0.2	5	3.05	0.07
12	CH_3	H	$NH(CH_2)_4NH_2$	230–238 dec	A (45 min)	48	I	$C_{21}H_{24}N_4O\cdot 0.5CH_3CN$	C, H, N	5	0.2	4	0.13
13	CH_3	H	$NH(CH_2)_5NH_2$	110–115	A (45 min)	82	I	$C_{22}H_{26}N_4O\cdot 1.5H_2O\cdot 0.5CH_3CN$	C, H, N	0.5	2	4.5	0.15
14	CH_3	H	$NH(CH_2)_6NH_2$	210–215 dec	A (45 min)	62	I	$C_{23}H_{28}N_4O\cdot 0.5H_2O$	C, H; N[e]	0.8	1.2	3.5	0.11
15	CH_3	CH_3	$NH(CH_2)_3NH_2$	210 dec	A (30 min)	38	II	$C_{21}H_{24}N_4O\cdot 2H_2O\cdot C_2H_5OH$	C, H, N	0.1	10		
16	CH_3	H	$NH(CH_2)_2N(Me)_2$	235–240 dec	A (19 h)	64	III	$C_{21}H_{24}N_4O\cdot 0.5H_2O$	C, H, N	0.8	1.2	2.8	0.11
17	CH_3	CH_3	$NH(CH_2)_3N(Me)_2$	218–227 subl	A (6 h)	52	IV	$C_{23}H_{28}N_4O\cdot C_2H_5OH$	C, H, N	0.3	3		
18	$CH_2C_6H_5$	H	$NH(CH_2)_2N(Me)_2$	228	A (22 h)	54	V	$C_{27}H_{28}N_4O$	C, H, N				
19	H	H	$NH(CH_2)_2N(Me)_2$	232	B	65	VI	$C_{20}H_{22}N_4O\cdot C_2H_5OH$	C, N; H[f]	0.03	30		
20	CH_3	H	$NH(CH_2)_3N(Me)_2$	195–200 dec	A (7 h)	85	III	$C_{22}H_{26}N_4O\cdot H_2O$	H, N; C[g]	1	1	3.15	0.09
21	CH_3	H	$NHCH(CH_3)(CH_2)_3N(Et)_2$	74–75	A (5 h)	40	V	$C_{26}H_{34}N_4O\cdot H_2O$	C, H; N[h]	0.5	2		
22	CH_3	H	$NH(CH_2)_3$-c-$N(CH_2CH_2)_2N(CH_2)_3NH_2$	190–195	C (1 h)	74	VI	$C_{27}H_{36}N_6O$	C, H, N	0.2	5		

23	CH_3	H	$NH(CH_2)_3$-$N(C_2H_5)$-$(CH_2)_3NH_2$	159	C (6 h)	42	VI	$C_{25}H_{33}N_5O$	C, H, N	0.2	5
24	CH_3	H	$N{=}(CH_2)_5$	220	A (15 h)	64	IV	$C_{22}H_{23}N_3O$	C, H, N	30	0.03
25	$CH_2C_6H_5$	H	$NH(CH_2)_3NH_2$	214 and 230	A (1.5 h)	45	VII	$C_{26}H_{26}N_4O$	C, H, N	0.3	3
26	CH_3	H		258–259	C (5 h)	47	IV	$C_{23}H_{23}N_5O \cdot 0.5H_2O$	C, H, N	0.5	2
27	CH_3	H	NH-C_6H_4-$NHSO_2CH_3$	290 dec	D	97	VIII	$C_{24}H_{22}N_4O_3S \cdot 3H_2O$	C, H, N	2	0.5
28	CH_3	H	NH-C_2H_5 (+ 2-CH_3)	275 dec	D	20	IX	$C_{24}H_{22}IN_3O$	H, N; C[i]	5	0.2
29	CH_3	H	$N(Me)_2$ (+ 2-CH_3)	280 dec	D	47	V	$C_{20}H_{22}IN_3O \cdot 0.5C_2H_5OH$	H, N; C[j]	>100	<0.01

[a] Methods: A, at reflux temperature in free amine for the indicated time; B, this compound was obtained by catalytic hydrogenation of 18 on Pd/C in ethanol, 3 h at 60 °C in a hydrogen atmosphere; C, reaction temperature 155 °C; D, see Experimental Section. [b] The reported yields are calculated on the purified materials. [c] Recrystallization solvents: I, 50% acetonitrile–methanol; II, 50% benzene–ethanol; III, 50% acetonitrile–ethanol; IV, ethanol; V, cyclohexane; VI, xylene; VII, 50% xylene–ethanol; VIII, purified in boiling ethylcellosolve (insoluble); IX, dimethylformamide. [d] Unless otherwise stated, microanalysis are within ±0.4% of the theoretical values for C, H, and N corresponding to the mentioned emperical formulas. [e] N: calcd, 14.54; found, 14.91. [f] H: calcd, 7.42; found, 6.91. [g] C: calcd, 69.47; found, 69.82. [h] N: calcd, 12.83; found, 12.41. [i] C: calcd, 58.06; found, 57.65. [j] C: calcd, 53.60; found, 53.15.

Table II. In Vivo Acute Toxicity

	ip		iv	
drug	LD_{100}, mg/kg	LD_0, mg/kg	LD_{100}, mg/kg	LD_0, mg/kg
4	30	15	NT[a]	NT
5	60	15	60	20
6	NT[b]	NT	NT	NT
7	NT[b]	NT	NT	NT
8	15	5	15	5
9	7	2.5	7	2
10	10	5	NT	NT
15	20	10	NT	NT
17	30	15	NT	NT
19	5	2.5	NT	NT
21	30	1.5	NT	NT
22	50	25	2.5	<1
23	15	5	NT	NT

[a] NT = not tested. [b] In the given conditions, these compounds form a jelly.

Table III. Antitumor Properties on Tumor Friend Cells Grafted in Vivo

drugs	dose, mg/kg	↓ iv 10^6 cells: median survival time, days	% ILS
controls		8.5 ± 0.5	
5	20	10.6 ± 0.7	24.7
8	5	11.0 ± 1.1	29.4
9	2.5	11.4 ± 1.9	34.1
10	5	10.1 ± 1.3	18.8

Table IV. Antitumor Properties of Compounds on L1210 Leukemia

drug	dose,[a] mg/kg	range of death, days	MST[b]	% ILS	level of signif (p)
4	15	12–19	14.3	24.6	<0.01
5	15	19–35	25	134	<0.001
8	5	13–18	16	49.5	<0.001
9	2.5	14–19	16.4	57.1	<0.001
10	5	11–20	13.8	8.7	NS
15	10	11–14	12.2	14	<0.05
17	15	13–18	15.3	33.3	<0.01
19	2.5	12–20	16	26	<0.01
21	15	10–14	12.5	8.7	NS

[a] Drugs were given ip at indicated doses 1 day after 10^5 L1210 leukemic cells. In these conditions, the death of control mice occurs between 9 and 11 days. [b] MST = mean survival time (days).

significantly increases the antitumor properties of the basic intercalating heterocyclic system. (3) If binding to DNA must be necessary to observe antitumor properties of intercalating drugs, this is probably not a sufficient condition.

Experimental Section

Chemistry. All melting points are uncorrected and were determined with a Reichert hot-stage microscope. ^1H NMR spectra were recorded in $(CD_3)_2SO$ with a Varian XL-100 apparatus. In this series of 1-aminoellipticine derivatives, we frequently observed that, after conventional treatment, resulting products were associated with recrystallization or reaction solvents and/or moisture. However, all compounds exhibited IR and NMR spectra consistent with the reported structures. Techniques for substitutions of chloro derivatives **3** are given below. Purification of products were followed by thin-layer chromatography on silica gel and alumina, and further details of the compounds are noted in Table I.

General Procedure for the Preparation of 1-Amino-Substituted Ellipticines (10–18 and 20–26). Chloro derivatives **3a–d** (500 mg) in free amine (10 g) were heated under nitrogen in an oil bath at the temperatures and times noted in Table I. After

elimination of excess amine under reduced pressure, the residue was taken up in water for compounds comprising an NH_2 terminal group or in 50 mL of 0.5 N sodium hydroxide solution in other cases. The resulting solid or oily substance was filtered or decanted, dried, and recrystallized to afford yellow crystals corresponding to the expected products.

1-Amino-substituted ellipticines show the more characteristic IR absorption bands in KBr pellets at ν_{max} 3400 (NH, weak), 1650 (NH, weak), and 1600 (strong) cm^{-1}. Chemical shifts of compounds **17**, **20**, and **21** are given as typical examples of ^1H NMR spectra in Me_2SO-d_6.

17 (R_3 = NH-CH_2^{α}-CH_2^{β}-CH_2^{γ}-$N(CH_3)_2$: δ 7.75 (d, 1, J_{3-4} = 6 Hz, H-3), 6.98 (d, 1, H-4), 7.44 (q, 1, J_{7-8} = 8.7 Hz, J_{7-10} = 0.3 Hz, H-7), 7.11 (q, 1, J_{8-10} = 2.4 Hz), 7.77 (q, 1, H-10), 2.63 (s, 3, CH_3-5), 3.33 (s, 3, CH_3-11), 3.88 (s, 3, OCH_3), 2.19 [s, 6, $N(CH_3)_2$], 10.93 (br, 1, NH-6), 6.50 (br, 1, NH-1), 3.51 (m, 2, CH_2-α), 1.84 (m, 2, CH_2-β), 2.40 (m, 2, CH_2-γ).

20: δ 7.81 (d, 1, J_{3-4} = 6.3 Hz, H-3), 7.02 (d, 1, H-4), 7.43 (q, 1, J_{7-8} = 8.7 Hz, J_{7-10} = 0.3 Hz, H-7), 7.09 (q, 1, J_{8-10} = 2.5 Hz, H-8), 7.67 (q, 1, H-10), 8.87 (s, 1, H-11), 2.69 (s, 3, CH_3-5), 3.89 (s, 3, OCH_3), 2.20 [s, 6, $N(CH_3)_2$], 10.96 (br, 1, NH-6), 7.40 (br, 1, NH-1), 3.55 (m, 2, CH_2-α), 1.84 (m, 2, CH_2-β), 2.39 (m, 2, CH_2-γ).

21 [as for **20** and R_3 = NH-$CH^{\alpha}(CH_3)$-CH_2^{β}-CH_2^{γ}-CH_2^{δ}-$N(CH_2CH_3)_2$]: δ 4.47 (m, 1, J_{H-NH} = 8.4 Hz, J_{H-CH_3} = 6.5 Hz, H-α), 1.27 (d, 2, CH_3-α), 1.60 (m, 4, CH_2-β,γ), 2.37 (m, 2, CH_2-δ), 2.41 (q, 4, CH_2CH_3), 0.90 (t, 6, CH_2CH_3), 6.87 (br, 1, NH-1).

1-[4′-(Methylsulfonamido)anilino]-5-methyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (27). Chloro derivative **3a** (0.2 mmol) and *p*-(methylsulfonamido)aniline (0.4 mmol) were dissolved in ethylcellosolve (3 mL). A solution of hydrochloric acid in dry ethyl ether (0.2 mmol equiv) was added and the mixture was heated at reflux under nitrogen for 3–5 h. The resulting precipitate was filtered, washed with boiling ethylcellosolve, and dried.

1-Anilino-2,5-dimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazolium Iodide (28) and 1-(Dimethylamino)-2,5-dimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazolium Iodide (29). 1-Chloro-5-methyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (**3a**) (200 mg) in dry acetonitrile (50 mL) was treated under nitrogen with methyl iodide (10 mL) at 65 °C for 3 h. After concentration to 25 mL, aniline (125 mg) or dimethylamine (5 mL of 40% aqueous solution) was added and the mixtures were left at ambient temperature for 2 h. Evaporation to dryness under reduced pressure afforded solid residues, which were recrystallized, giving **28** and **29**, respectively.

Biological Assays. (a) Cell Cultures and in Vitro Cytotoxicity Determination. Friend tumor cells were grown in suspension in Dulbecco-modified Eagle's medium (MBA) supplemented with 10% heat-inactivated fetal calf serum and L-glutamine (2 mmol/mL). Cultures were performed in the absence of antibiotics and were shown to be free of contamination by mycoplasma (Service des Mycoplasmes, Institut Pasteur). The doubling time of the tumor cell population is about 14–16 h.

On day 0, 10^5 cells were plated in a volume of 1 mL in sterile disposo trays limbro (Bio-Block). On day 1, cultures were in the exponential phase of growth, and increasing dilutions of the drugs, dissolved in acidified water (10 μL of acetic acid in 10 mL of H_2O), were given in duplicate cultures, in a volume of 10 μL (10^{-5}, 3×10^{-6}, 10^{-6}, 3×10^{-7}, 10^{-7}, 3×10^{-8}, 10^{-8}, 3×10^{-9}, 10^{-9} M). Thirty hours later (about two doubling times of the cell population) cell count was determined using a hemocytometer, and viability was estimated by the Trypan blue exclusion test. The growth rate of tumor cells exposed to the drugs was compared to the growth rate of unexposed cells, and the percent inhibition was calculated as follows: $[(A - B)/A]100$, where A is the mean cell number in control (day 2) minus mean cell number in control (day 1), and B is the mean cell number in treated cells (day 2) minus mean cell number in control (day 1).

In preliminary experiments, we confirmed that a 2 or 3 day duration of cell-drug contact does not significantly change the results. Therefore, the effects of drugs on cell multiplication seem to be immediate.

(b) Determination of in Vivo Acute Toxicity and Therapeutic Doses. The drugs were dissolved in acidified physiological saline (equimolar concentration of acetic acid) and administered ip or iv to five groups of six ICFW, C3H, or B6D2 F1 mice (age, 6–8 weeks; weight, 22–24 g) as a single dose of 2, 5, 10, 25, and 50 mg/kg. Mortality from toxicity was noted in each of the groups during a 30-day observation period. The lethal dose (LD_{100}) and the highest nontoxic dose (LD_0) were thus determined.

(c) In Vivo Antitumor Potency. For tests performed on Friend tumor cells, 10^6 cells were intravenously injected into syngeneic mice. Animals were randomized in groups of 16 mice, and the drugs were given 3 days later by iv route. Antitumor activity on L1210 leukemia was studied as previously described.[5] The therapeutic effect of drugs was measured as the percent increase in life span over controls (% ILS), evaluated as follows:

$$\% \text{ ILS} = [(\text{median survival time in treated} - \text{median survival time in controls})/\text{median survival time in controls}] \times 100$$

Binding Measurements. The association constants for binding to DNA were determined from the absorption changes of 6×10^{-7} M calf thymus DNA (expressed in mononucleotides concentration) in 50 mM Tris-HCl buffer, pH 7.4, 0.1 M NaCl. Measurements were performed in a Cary 118C in 5-cm light-path curvettes at 25 °C. Binding curves were plotted as previously described.[13] K_{app} is the apparent association constant and B_{app} the apparent number of binding site per base pair.

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