Synthesis and Evaluation of 9-Hydroxy-5-methyl-(and 5,6-dimethyl)-6*H*-pyrido[4,3-*b*]carbazole-1-*N*-[(dialkylamino)alkyl]carboxamides, a New Promising Series of Antitumor Olivacine Derivatives

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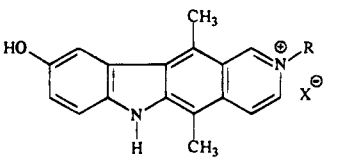
Starting from 2-(2-aminoethyl)-6-methoxy-1-methylcarbazole, ethyl 9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate was obtained through a three-step sequence. This compound and its 6-methyl derivative react with (dialkylamino)alkylamines to provide various 9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-(*N*-substituted carboxamides) whose boron tribromide demethylation afforded corresponding 9-hydroxy-1-(*N*-substituted carbamoyl)-olivacines. The same pathway but starting from 2-(2-aminoethyl)-6-methoxy-1,4-dimethylcarbazole led to ethyl 9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate which did not normally react with amines. It provided either the recovered starting material at 120 °C or 9-methoxyellipticine resulting from an unexpected decarboethylation in a steel vessel at 180 °C. Biological testing of the newly obtained 1-carbamoylolivacine derivatives showed that 9-hydroxylated compounds displayed high cytotoxicity for cultured L1210 and colon 38 cells (IC_{50} range 5–10 nM) and good antitumor activity in vivo in the P388 leukemia and colon 38 models when administered by the iv route. The most active compound in these series is 9-hydroxy-5,6-dimethyl-1-[*N*-[2-(dimethylamino)ethyl]carbamoyl]-6*H*-pyrido[4,3-*b*]carbazole which was selected for further evaluation on murine solid tumors and for toxicological studies.

In preceding papers, we described various series of heterocyclic derivatives bearing [(dialkylamino)alkyl]-amino side chains which display high antitumor properties. 1-[[(Dialkylamino)alkyl]amino]-substituted ellipticines,[1,2] -5*H*-pyrido[3′,4′:4,5]pyrrolo[3,2-*c*]pyridines,[3,4] -5*H*-pyrido[4,3-*b*]indoles (γ-carbolines),[5,6] 10-amino-substituted 5*H*-pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines,[7–10] and 11-amino-substituted 7*H*-benzo[*e*]pyrido-[4,3-*b*]indoles[11–13] are the most significant examples in this respect. Besides these series, other tricyclic intercalators have appeared as promising new antitumor drugs, especially acridine- and phenazine-*N*-[(dialkylamino)alkyl]carboxamide derivatives.[14–17]

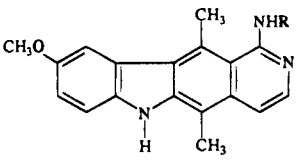
In all cases, the basic side chains seem to play a key role, either to increase or to confer the antitumor properties. For example, despite the lack of antitumor activity of unsubstituted acridine and phenazine, the basic *N*-[(dialkylamino)alkyl]carboxamide group was by itself sufficient to confer antitumor properties to these two series. Therefore, *N*-substituted carboxamide derivatives of a biological active chromophore such as ellipticine or olivacine seemed highly interesting in the search for new potential antitumor drugs.

This prompted us to undertake a study on the synthesis of the yet unknown 1-(*N*-substituted carbamoyl)-9-methoxy-(and 9-hydroxy)-6*H*-pyrido[4,3-*b*]carbazoles. Indeed, 9-methoxy- and 9-hydroxyellipticine derivatives are generally the most potent antitumor compounds in these series. Thus, 9-hydroxy-2-methylellipticinium acetate (**1**, elliptinium) has been used in the clinic,[18] its analogue (**2**, datelliptium) has been studied in phase I and II clinical trials,[19] and the antitumor activity of 1-[[3-(diethylamino)propyl]amino-9-methoxyellipticine (**3**, retelliptine, BD84) in rodents[20,21] has justified its preclinical study[22,23] and evaluation in phase I clinical trials.[24]



1 : R = CH_3, X = CH_3COO

2 : R = $CH_2CH_2N(Et)_2$, X = Cl



3 : R = $CH_2CH_2CH_2N(Et)_2$

Compound **1** has been shown to lead to the corresponding highly reactive quinone imine through a two-electron redox process.[25] Its antitumor activity was then mainly assigned to the adducts which could result from this highly reactive metabolite and various in vivo occurring nucleophiles, especially at the DNA level.[26]

More recently, another possible mechanism which could explain the key role of the 5-methyl group in the ellipticine series has been proposed by Archer et al.[27] It was based on what is observed for lucanthone, where the methyl group is oxidized into an hydroxymethyl one, the esters of which would be alkylating agents responsible for the biological activity. Experimental data supporting a covalent binding of 9-hydroxy-5-(hydroxymethyl)ellipticine derivatives to DNA were then presented.[27] At the same time, we presented a similar working hypothesis,[3] without experimental data, based on the reported observations of Archer et al. concerning the thioxanthone series. As these authors, we consid-

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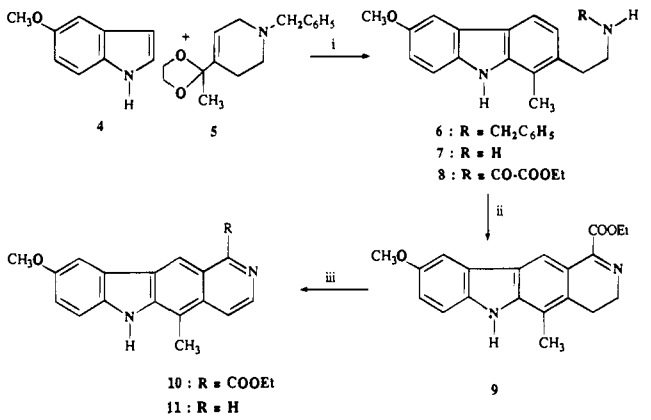
Scheme 1[a]



[a] (i) $CH_3COOH-H_2O$, reflux; (ii) $POCl_3$, 110 °C; (iii) Pd/C, 240 °C.

ered as likely the implication of the ellipticine 5-methyl group in the mechanism of action of this series. However, our recent results concerning the γ-carboline and 7*H*-benzo[*e*]pyrido[4,3-*b*]indole series[12,13] clearly show that the 4-methyl group is not essential for these series to display antitumor properties. This led us to consider that such interpretations did not perhaps correspond to the reality. In fact, it is now generally accepted that the antitumor activity of these compounds is due to their interaction with the enzyme topoisomerase 2, leading to the stabilization of the cleavable complex and then to cell death.[28]

The reactive quinone imines which probably result from the bioactivation of all the 9-methoxy- (after demethylation) and 9-hydroxyellipticine derivatives could be thus involved in the general toxicity of these compounds as could be the possible alkylating species generated from their 5-methyl group. However, on the basis of our knowledge of the ellipticines, the presence of a 9-OH group was required to maintain their in vivo antitumor properties. If this is correct, it could then be theoretically possible to reduce the general toxicity of ellipticine and olivacine derivatives by blocking their 6-NH-pyrrolic group by a methyl substituent.

These considerations and the key role played by the basic side chain as mentioned above led us to synthesize 9-OH-6-NH and 9-OH-6-NCH_3 pairs of compounds bearing a *N*-[(dialkylamino)alkyl]carboxamide side chain, in order to carefully study and compare their in vivo antitumor properties and toxicity. In this paper, we describe our results on the synthesis and biological evaluation of new 9-OCH_3 and 9-OH, 6-NH and 6-NCH_3 1-(*N*-substituted carbamoyl)olivacine derivatives.

Chemistry

According to the Besselievre and Husson biomimetic reaction,[29] 5-methoxyindole (**4**) was reacted with the ethylene glycol ketal of 4-acetyltetrahydropyridine (**5**) to provide 2-[2-(benzylamino)ethyl]-6-methoxy-1-methylcarbazole (**6**) in about 25% yield. Catalytic debenzylation then easily led to 2-(2-aminoethyl)-6-methoxy-1-methylcarbazole (**7**), which was previously obtained by a six-step sequence.[30]

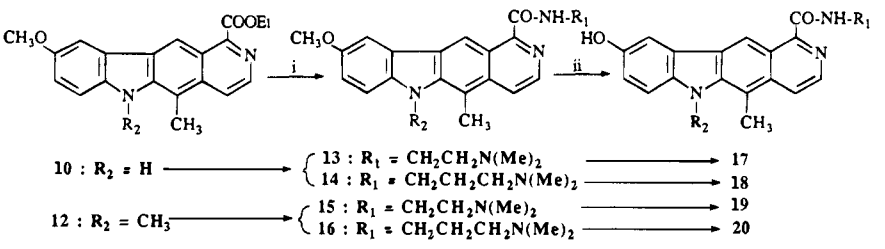
After transformation of compound **7** into 2-[2-(ethoxalylamino)ethyl]-6-methoxy-1-methylcarbazole (**8**) by reaction with diethyl oxalate, phosphorus oxychloride cyclization in boiling toluene gave ethyl 3,4-dihydro-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (**9**) and corresponding aromatized olivacine derivative **10** by deshydrogenation over 10% palladium on charcoal, in boiling diphenyl oxide (Scheme 1). Aromatization of **9** to **10** however, also afforded a byproduct which was identified as being 9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole (**11**). N-6-Methylation of **10** to give **12** was performed by using dimethyl carbonate in dimethylformamide in the presence of potassium carbonate and 18-crown ether. Both **10** and **12** were then reacted with 2-(dimethylamino)ethylamine and 3-(dimethylamino)propylamine to give the expected carboxamides **13**–**16**, and the corresponding 9-hydroxylated derivatives **17**–**20** resulted from subsequent boron tribromide demethylation (Scheme 2).

For comparison with **19**, the most active compound among **13**–**20** (see Biological Results), some additional examples were prepared. They were obtained from **12**, by demethylation into **21** and subsequent reaction with the required amines which directly led to hydroxy derivatives **22**–**25**. As we have verified, for the preparation of compound **19**, this protocol performing the demethylation prior to amidification provided the 9-hydroxy-1-(*N*-substituted carbamoyl)olivacines in better yields than the preceding one (Scheme 3). In order to determine the effect of the *N*-pyrrolic substituent, we also transformed compound **10** into its *N*-6-ethyl derivative, **26**, which was demethylated to give ethyl 9-hydroxy-6-ethyl-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (**27**) and then its *N*-substituted carboxame derivative, **28** (Scheme 3).

Finally, for a complete structure–activity relationships study of the series, it would be interesting to have some carbamoyl olivacine derivatives possessing in their structure the 11-methyl group of ellipticine. Therefore, we performed the various transformation **29** → **35** summarized in Scheme 4. Ethyl 9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (**35**) was thus obtained with a 21% overall yield.

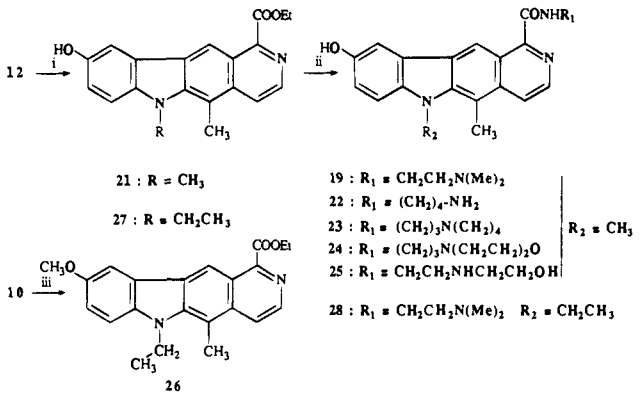
Whereas it has been demonstrated that 1-cyanoellipticines could normally be hydrolyzed to give corresponding *N*-unsubstituted carboxamides,[31] when **35** was reacted with 2-(dimethylamino)ethylamine and 3-(dimethylamino)propylamine at reflux temperature for a 8-h

Scheme 2[a]



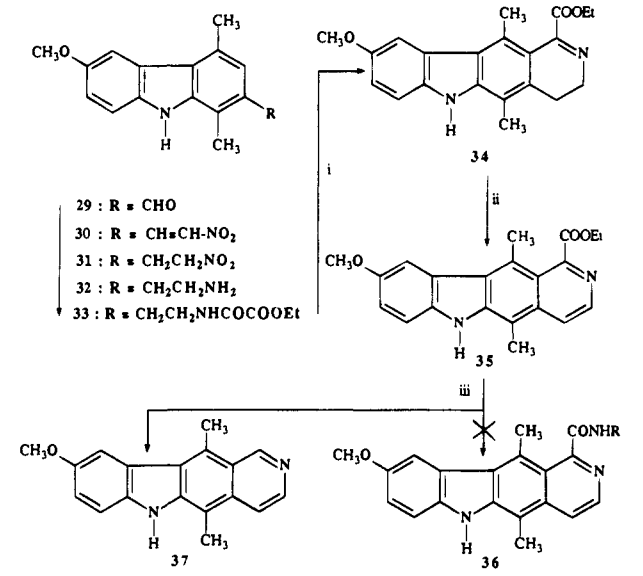
[a] (i) R_1NH_2, reflux; (ii) BBr_3.

Scheme 3[a]



[a] (i) BBr_3, (ii) R_1NH_2, reflux; (iii) $CO_3(Et)_2$.

Scheme 4[a]



[a] (i) $POCl_3$, 110 °C; (ii) Pd/C, 240 °C; (iii) RNH_2, 180 °C.

period (105 and 133 °C), starting material was recovered. Heating the same mixtures at 180 °C in a steel vessel did not gave the expected amides **36** but solely 9-methoxyellipticine (**37**) (Scheme 4). This result shows that for compound **35**, the 11-methyl group steric hindrance is sufficient to prevent the usual reaction of the ester function which obviously would involve a sp^2 to sp^3 carbon atom hybridization change. The mechanism of the ethoxycarbonyl group loss, however, remains unclear and unexplained.

Biological Results

The study of the biological properties of these new pyridocarbazole derivatives was carried out first in vitro on L1210 leukemia and, for the most cytotoxic derivatives, colon 38 cells, in comparison with adriamycin (ADR) and elliptinium acetate. The results (IC_{50}) are reported in Table 1. Compounds **17**, **19**, **20**, **23**, and **24** which bear a hydroxyl group at C9 were more cytotoxic for L1210 cells than ADR. As previously described for other ellipticine derivatives, these 9-OH compounds are generally more cytotoxic (36–233-fold) than their corresponding 9-OCH_3 counterparts (compare **19** and **15**, **20** and **16**). When tested on colon 38 cells, compounds **19**, **23**, and **24** were as cytotoxic as ADR.

The compound **19** was shown to accumulate L1210 cells in the G2 + M phase of the cell cycle (Figure 1, top), as is the case for ADR. Moreover, a good correlation was found in this series between cytotoxicity and potency in accumulating cells in the G2 + M phase (not shown). This relationship is illustrated in Figure 1, bottom, for the 9-OH derivative **19** and the corresponding 9-OCH_3 **15**, in comparison with ADR and elliptinium acetate.

In vivo, we chose to test these derivatives by iv administration, this route of administration being different from the site of tumor implantation (ip or sc), to better mimic the clinical situation. Important parameters, like distribution, for the biological activity of these compounds can be taken into account using this methodology. This approach is especially important in the case of ellipticine derivatives, which are often highly active only in the standard ip/ip models.[19a]

We used two schedules of administration, D1 or D1, 5, 9, on the P388 leukemia. Table 1 shows the results, in terms of percent *T/C* obtained at the optimal dosage, i.e., the dose giving the best therapeutic effect without toxicity. Compounds having the carboxame chain are more active when administered on D1, 5, 9, as opposed to adriamycin which is more active with the D1 schedule (Table 1). If one considers the optimum schedule of administration for each compound, the derivatives **19** and **20** (given D1, 5, 9) are more active than adriamycin (given D1), while **17** and **18** are about as active (Table 1).

The active compounds **17**, **19**, and **20** were selected and tested against the solid tumor colon 38 using both an early and a delayed schedule of administration. All three derivatives were active in this model, **20** being the most potent and active one. When administered using a delayed schedule, the three derivatives maintained a good activity at doses higher than 20 mg/kg (Table 2).

Discussion and Conclusion

The synthesis of 9-hydroxy-5-methyl-(and 5,6-dimethyl)-6*H*-pyrido[4,3-*b*]carbazole-1-*N*-[(dialkylamino)-alkyl]carboxamides was undertaken in order to evaluate their cytotoxic and antitumor activity and, more precisely, to assess the relative contribution of the side chain and the 6-NH-pyrrolic group. Our aim was to increase the antitumor activity of these compounds by grafting a new chain on the pyridocarbazole skeleton and to decrease their general toxicity by methylating the indolic nitrogen atom. This latter approach was based upon a double assumption: (i) the quinone imine, and thereby the covalent binding of these molecules, is involved in the toxicity rather than in the antitumor activity and (ii) a NCH_3 derivative would induce less quinone imine than a NH one, as suggested by the NCH_3 derivative of elliptinium acetate, for which no glutathione conjugate was found in the bile of treated rats.[32] The presence of such an adduct was considered to be proof of the two-electron oxidation of elliptinium acetate in vivo.[32]

In spite of the very limited number of molecules studied in this work, the NH derivatives **17** and **18** appeared to be more potent, but also more toxic, in vivo than their corresponding NCH_3 derivatives, **19** and **20**. Although we have no indication concerning the reactivity of these molecules, this experimental result is in agreement with our initial rationale. This toxicity

Table 1. Biological Activity of Compounds

compd	cytotoxicity IC_{50} (nM)[a] L1210	C38	antitumor activity against P388 leukemia[b] schedule	route	optimal dosage (mg/kg)[c]	T/C (%)[d] (median survival time)
adriamycin	26 ± 4	10 ± 2	DI	iv	15	241
			D1, 5, 9	iv	5	163
elliptinium acetate	73 ± 18	38 ± 8	D1	iv	10	113
			D1, 5, 9	iv	5	109
13	831 ± 257	NT	NT			
14	1496 ± 377	NT	NT			
15	574 ± 120	NT	NT			
16	1327 ± 493	NT	NT			
17	4.9 ± 0.9	22 ± 5	D1	iv	15	168
			D1, 5, 9	iv	5	220
18	53 ± 13	NT	D1	iv	10	159
			D1, 5, 9	iv	10	235
19	4.1 ± 0.6	6.9 ± 1.2	D1	iv	90	238
			D1, 5, 9	iv	60	356
20	5.7 ± 0.7	19 ± 7	D1	iv	40	158
			D1, 5, 9	iv	30	296
22	490 ± 65	NT	D1	iv	20	121
			D1, 5, 9	iv	10	120
23	11 ± 3	11 ± 2	D1	iv	40	143
			D1, 5, 9	iv	20	135
24	10 ± 2	11 ± 2	D1	iv	80	159
			D1, 5, 9	iv	80	214
25	27 ± 2	259 ± 13	D1	iv	20	144
			D1, 5, 9	iv	10	134
28	38 ± 6	72 ± 46	D1	iv	80	124
21	2498 ± 343	NT	NT			
27	19700 ± 6400	NT	NT			

[a] Inhibition of cell proliferation measured by the MTT assay (mean ± standard error of ≥3 values obtained in independent experiments. NT: not tested. [b] Mice were inoculated ip on day 0 with 10^6 P388 cells. [c] Dose (mg/kg/day) giving the optimal therapeutic effect without toxicity (no lethality and weight variation <10% of body weight). [d] On average (six experiments), 27.5% of mice treated by **19** (60 mg/kg, D1, 5, 9) survived for more than 60 days.

Table 2. Antitumor Activity against Early and Advanced Colon 38 Adenocarcinoma

compd	schedule	route	dose (mg/kg/day)	T/C (%)[a] (median tumor volume)
17	D2, 9	iv	10	35
			20	22
			30	10
	D9, 16	iv	10	59
			20	32
			30	8
19	D2, 9	iv	20	68
			40	16
			60	5
	D9, 16	iv	20	30
			40	25
			60	10
20	D2, 9	iv	20	0
			40	0
			60	0
	D9, 16	iv	20	16
			40	23
			60	21
ADR	D2, 9	iv	15	0

[a] The day of evaluation was D25 in the D2, 9 schedule and D26 in the D9,16 schedule.

prevents the administration of higher, more active doses of the NH derivatives **17** and **18**. However, **17** and **19** are equally cytotoxic, and surprisingly, **18** is 9-fold less cytotoxic than **20**. Hence, the higher toxicity of **17** and **18** might be due to the in vivo generation of toxic species.

The 6-N-Et derivative **28** was about 10-fold less cytotoxic than the analogues **17** and **19** and equally toxic in vivo as **19** but markedly less active. As already demonstrated in the series of benzo[*e*]pyrido[4,3-*b*]-indoles where the 4-methyl to 4-ethyl group change also led to an almost inactive compound,[13] this result which is probably due to the increasing bulkiness of the product clearly shows that the convenient size of the molecule is determinant for the compounds to display cytotoxic, antitumor, and topoisomerase 2 poisoning properties. The nature of the side chain is also important for the in vivo activity. Thus, compounds **17**–**20** which have either a $(CH_2)_2N(CH_3)_2$ or a $(CH_2)_3N(CH_3)_2$ side chain are the more active ones, and the pyrrolidinoethyl and [(hydroxyethyl)amino]ethyl derivatives **23** and **25** are clearly more toxic than **19** and thereby less active, while the morpholino-substituted compound **24** is not toxic but less active than the better ones. Moreover, the importance of the nature of the side chain is underlined by the ester derivative **21** which is 550-fold less cytotoxic than **19**, a carboxame-substituted derivative.

On the basis of results generally obtained with structurally related series, one would expect these molecules to interact with topoisomerase 2, the consequence of which would be the stabilization of the cleavable complex and the generation of DNA breaks. Preliminary results have shown that **19** effectively intercalated into DNA and stabilized the cleavable complex in an in vitro assay using purified, topoisomerase 2.[33] This property, currently under investigation, is supported by the accumulation of the treated cells in the G2 + M phase of the cell cycle. On the basis of its high cytotoxicity and excellent activity when administered by the iv route in the two animal models used in this work, **19** was selected for further evaluation on solid tumors in mice and for toxicological studies.

Experimental Section

Chemistry. All melting points were determined with a Kofler apparatus and are uncorrected. ^1H NMR spectra were

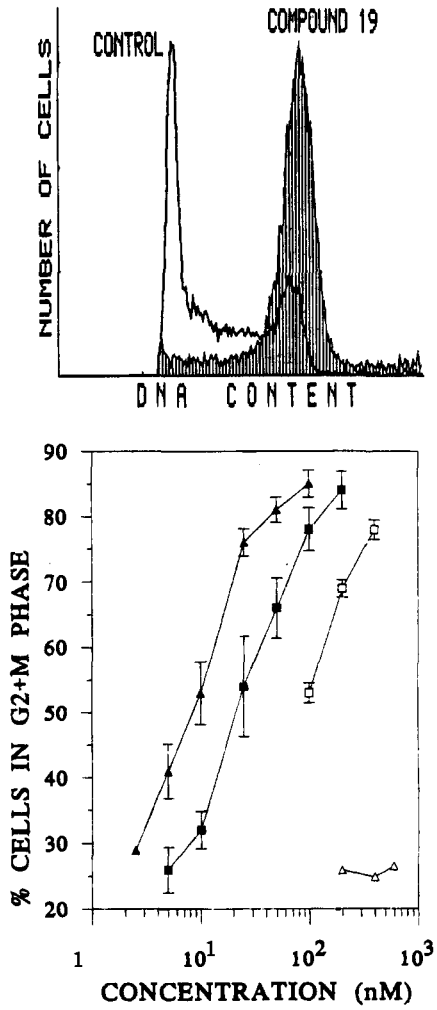


Figure 1. (Top) Modification of the typical DNA histogram of L1210 cells treated with 50 nM compound **19** for 21 h. (Bottom) Effect of compound **15** (△) and **19** (▲) compared to that of ADR (■), and ELP (□) on the G2 + M phase accumulation of L1210 cells.

recorded in the mentioned solvent, with a Bruker AC 200 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 described, but all others are consistent with the assigned structures. Analyses indicated by element symbols were within ±0.4% of the theoretical values for mentioned empirical formulas. They were performed by the Service Central de Microanalyses du CNRS, ICSN, 91190 Gif-sur-Yvette.

2-[2-(Benzylamino)ethyl)]-6-methoxy-1-methylcarbazole (6). 5-Methoxyindole (**4**) and 4-acetyl-1-benzyl-1,2,3,6-tetrahydropyridine ethylene glycol ketal (**5**) (bp_7 = 184–188 °C, obtained by sodium borohydride reduction of the corresponding crude 1-benzylpyridinium chloride in methanol) were refluxed in 50% acetic acid (1 L) for a 66-h period, and the mixture was poured in water (2 L). Extraction with methylene chloride, usual workup,[29] and evaporation of solvent provided an oily residue which was taken up in ethyl acetate. The solid which appeared progressively was filtered and washed with cold methylene chloride to give colorless crystals (30.2 g, 22%) of the acetate, mp 130 °C. Corresponding free base was crystallized from toluene to give colorless crystals, mp 150 °C. Anal. ($C_{23}H_{24}N_2O$) C, H, N.

2-[2-(Ethoxalylamino)ethyl]-6-methoxy-1-methylcarbazole (8). The acetate of 2-[2-(benzylamino)ethyl]-6-methoxy-1-methylcarbazole (**6**) (35.6 g, 88 mmol) was dissolved in acetic acid (400 mL), and 10% palladium on charcoal (10 g) was added. The mixture was heated at 50 °C, and maintaining this temperature, it was stirred under hydrogen at normal pressure until a theoretical volume of hydrogen was consumed (10 h). The catalyst was filtered and washed with acetic acid, and the solvent was evaporated under reduced pressure to provide a residue which was taken up in water (200 mL). After filtration and basification with ammonia, the resulting solid was collected, air-dried, and recrystallized from toluene to give beige crystals (18.16 g, 81.4%, mp 167–168 °C), in all respects identical to the compound already described.[30] This compound was heated at 100–110 °C in diethyl oxalate (70 mL) for 1 h, and the mixture was evaporated to dryness. The solid residue was taken up in cyclohexane, filtered, and recrystallized from ethyl acetate to give 23.92 g (94.6%) of the title compound, mp 144 °C. ^{1}H NMR ($CDCl_3$): δ 1.36 (t, 3H, CH_3CH_2), 2.5 (s, 3H, 1-CH_3), 3.06 (t, 2H, α-CH_2, $J_{CH_2-CH_2}$ = 7.6 Hz), 3.6 (m, 2H, β-CH_2), 3.93 (s, 3H, OCH_3), 4.34 (q, 2H, CH_2CH_3), 7.02 (d, 1H, 3-H, J_{3-4} = 8 Hz), 7.05 (q, 1H, 7-H), 7.35 (d, 1H, 8-H, J_{8-7} = 8.9 Hz), 7.43 (d, 1H, 5-H, J_{5-7} = 2.4 Hz), 7.83 (d, 1H, 4-H), 8.02 (br s, 1H, 9-H). Anal. ($C_{20}H_{22}N_2O_4$) C, H, N.

Ethyl 3,4-Dihydro-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (9). The preceding amide **8** (3.54 mg, 10 mmol) was dissolved in boiling toluene (300 mL) and treated dropwise with phosphorus oxychloride (30 mL). Reflux was continued for a 24-h period, and evaporation under reduced pressure afforded a residue which was taken up in water (200 mL). The solution was filtered and basified to pH 9–10 with sodium carbonate, and the solid was collected, washed with water, and air-dried. It was recrystallized from ethyl acetate to give yellow crystals (2.36 g, 70%), mp 233–234 °C. ^{1}H NMR ($CDCl_3$): δ 1.47 (t, 3H, CH_3CH_2), 2.41 (s, 3H, 5-CH_3), 2.77 (t, 2H, 4-CH_2, $J_{CH_2-CH_2}$ = 6.9 Hz), 3.88 (t, 2H, 3-CH_2), 3.94 (s, 3H, OCH_3), 4.52 (q, 2H, CH_2CH_3), 7.04 (dd, 1H, 8-H, J_{8-7} = 8.8 Hz, J_{8-10} = 2.54 Hz), 7.34 (dd, 1H, 7-H, J_{7-11} = 0.5 Hz), 7.51 (d, 1H, 10-H), 8.19 (br s, 1H, 11-H), 8.24 (br s, 1H, 6-NH). Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

Ethyl 9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (10). The preceding ester **9** (2.036 g, 6 mmol) was refluxed in diphenyl ether (40 mL) in the presence of 10% palladium on charcoal (300 mg) for 10 min. After filtration, the catalyst was washed with acetone and the acetone was evaporated. The residue was extracted with 1 N hydrochloric acid, and the aqueous solution was neutralized with sodium hydrogen carbonate. The resulting precipitate was extracted with methylene chloride, and evaporation of solvent provided a solid residue which was chromatographed on a silica gel column. Elution with methylene chloride gave traces of residual diphenyl ether, and using methylene chloride–ethyl acetate, 98–2 v/v mixture, as eluent then provided the expected compound (R_f = 0.69 on silica gel with methylene chloride–ethyl alcohol 95–5 v/v, as eluent). This compound was recrystallized from ethyl acetate to give yellow crystals (1.17 g, 58.3%), mp 255 °C. ^{1}H NMR ($CDCl_3$): δ 1.55 (t, 3H, CH_3CH_2), 2.73 (s, 3H, 5-CH_3), 3.96 (s, 3H, OCH_3), 4.66 (q, 2H, CH_2CH_3), 7.13 (dd, 1H, 8-H, J_{8-7} = 8.8 Hz, J_{8-10} = 2.4 Hz), 7.36 (d, 1H, 7-H), 7.67 (d, 1H, 10-H), 7.97 (dd, 1H, 4-H, J_{4-3} = 6 Hz, J_{4-11} = 1 Hz), 8.14 (br s, 1H, NH), 8.55 (d, 1H, 3-H), 9.27 (br s, 1H, 11-H). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

Continuing elution with ethyl acetate afforded a byproduct (R_f = 0.5 in conditions aforementioned for **10**) which was recrystallized from ethyl acetate to give yellow crystals corresponding to compound **11** (480 mg, 30%), mp 242 °C. ^{1}H NMR ($CDCl_3$): δ 2.80 (s, 3H, 5-CH_3), 3.97 (s, 3H, OCH_3), 7.16 (dd, 1H, 8-H, J_{8-7} = 8.6 Hz, J_{8-10} = 2.4 Hz), 7.32 (d, 1H, 7-H), 7.72 (d, 1H, 10-H), 7.86 (d, 1H, 4-H, J_{4-3} = 6.4 Hz), 8.06 (br s, 1H, NH), 8.49 (d, 1H, 3-H), 8.53 (s, 1H, 1-H), 9.40 (br s, 1H, 11-H). Anal. ($C_{17}H_{14}N_2O$) C, H, N.

Ethyl 9-Methoxy-5,6-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (12). A mixture of the ester **10** (334 mg, 1 mmol), fine powdered dry potassium carbonate (250 mg), dimethyl carbonate (5 mL), dimethylformamide (1 mL), and 18-crown-6 ether (1 drop) was heated at reflux under stirring for a 8-h period. After evaporation to dryness, the residue was taken up in water. The solid was collected, air-dried, and recrystallized from cyclohexane to give yellow crystals (270 mg, 77.5%), mp 162–164 °C. Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

1-Carbamoyl-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole Derivatives 13–16: General Technique. Compound **10** or **12** (1 mmol) was heated in the required diamine

(5 mL) at 110–120 °C for 8 h (**13** and **16**) or 18 h (**14** and **15**), and excess amine was evaporated under reduced pressure, giving a residue which was taken up in water. For compounds **13–15**, the resulting solid was collected, air-dried, and recrystallized from cyclohexane (**13** and **15**) or ethyl acetate (**14**) to give yellow crystals. Since compound **16** could not be crystallized, it was extracted with methylene chloride, the solvent was evaporated, and the residue was treated with an excess of maleic acid (2 equiv) in acetone to provide the solid dimaleate salt.

13: yield 88%; mp 215 °C. Anal. ($C_{22}H_{24}N_4O_2 \cdot 0.5H_2O$) C, H, N.

14: yield 93%; mp 206 °C. Anal. ($C_{23}H_{26}N_4O_2 \cdot H_2O$) C, H, N.

15: yield 60%; mp 139 °C. Anal. ($C_{23}H_{26}N_4O_2$) C, H, N.

16: yield 92.5%; mp 139 °C. Anal. ($C_{24}H_{28}N_4O_2 \cdot 2C_4H_4O_4$) C, H, N.

1-Carbamoyl-9-hydroxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole Derivatives 17–20: General Technique. The required methoxy carboxamide (1 mmol) was dissolved in dry methylene chloride (40 mL) under argon atmosphere, and the solution was cooled to −70 °C. Boron tribromide (10 molar equiv, 10 mL of a 1 M commerical solution in methylene chloride) was added dropwise, and the mixture was left to reach room temperature (18 h) and then poured into ice–water (100 mL). The resulting mixture was basified with triethylamine and stirred at room temperature for a 3-h period. Compounds **17** and **18** were almost insoluble in methylene chloride. They were filtered off and recrystallized from ethyl acetate. Compounds **19** and **20** were extracted with methylene chloride, and evaporation of solvent provided a residue which was crystallized from ethyl acetate in the case of **20**. Before crystallization of **19** in the same solvent, it was chromatographed on an alumina column, eluting with methylene chloride–ethanol 95–5. Via evaporation of the fractions containing the expected product, the pure base was thus obtained. In all cases, the compounds were yellow crystals.

17: yield 47%; mp >270 °C. Anal. ($C_{21}H_{22}N_4O_2$) C, H, N.

18: yield 56%; mp 218 °C. Anal. ($C_{22}H_{24}N_4O_2 \cdot 0.5H_2O$) C, H, N.

19: yield 43.6%; mp 256 °C. Anal. ($C_{22}H_{24}N_4O_2$) C, H, N.

20: yield 76.8%; mp 198 °C. Anal. ($C_{23}H_{26}N_4O_2 \cdot 0.5H_2O$) C, H, N.

Chemical shifts of compounds **14** and **20** are given as typical examples of ^1H NMR spectra, in $(CD_3)_2SO$.

14, NH-R_1 (R_1 = $CH_{2\alpha}CH_{2\beta}CH_{2\gamma}N(CH_3)_2$): δ 1.88 (m, 2H, β-CH_2), 2.29 (s, 6H, $N(CH_3)_2$), 2.47 (t, 2H, γ-CH_2), 2.96 (s, 3H, 5-CH_3), 3.56 (m, 2H, α-CH_2), 4.03 (s, 3H, OCH_3), 7.29 (dd, 1H, 8-H, J_{8-7} = 8.8 Hz, J_{8-10} = 2.5 Hz), 7.6 (d, 1H, 7-H), 7.92 (d, 1H, 10-H), 8.2 (d, 1H, 4-H, J_{4-3} = 6.1 Hz), 8.53 (d, 1H, 3-H), 8.98 (t, 1H, *NH*CH_2), 9.67 (s, 1H, 11-H), 11.36 (br s, 1H, 6-NH).

20, NH-R_1 (R_1 = $CH_{2\alpha}CH_{2\beta}CH_{2\gamma}N(CH_3)_2$): δ 1.75 (m, 2H, β-CH_2), 2.17 (s, 6H, $N(CH_3)_2$), 2.34 (t, 2H, γ-CH_2), 3.06 (s, 3H, 5-CH_3), 3.46 (m, 2H, α-CH_2), 4.11 (s, 3H, 6-NCH_3), 7.07 (dd, 1H, 8-H, J_{8-7} = 8.9 Hz, J_{8-10} = 2.4 Hz), 7.45 (d, 1H, 7-H), 7.53 (d, 1H, 10-H), 8.14 (d, 1H, 4-H, J_{4-3} = 6.2 Hz), 8.41 (d, 1H, 3-H), 8.87 (m, 1H, NH), 9.22 (br s, 1H, 9-OH), 9.47 (s, 1H, 11-H).

Ethyl 6-Ethyl-9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (26). Compound **10** and diethyl carbonate were reacted in the conditions described for the preparation of **12**. After usual workup, **26** was obtained in 76% yield as yellow crystals, mp 149 °C (from toluene). Anal. ($C_{22}H_{22}N_2O_3$) C, H, N.

Ethyl 9-Hydroxy-5,6-dimethyl-(and 6-ethyl-5-methyl)-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylates (21 and 27). The required ester (1 g) was dissolved in dry dichloromethane (150 mL), and the mixture was cooled to −78 °C, under stirring. A 1 M solution of boron tribromide in dichloromethane (10 equiv) was added at once under an argon atmosphere. After a further 2.5-h period (TLC monitoring) of stirring, the mixture was allowed to reach room temperature for 1 h and poured in ice–water. The resulting mixture was basified with concentrated ammonia, and the resulting precipitate was collected and air-dried. The pure compounds were obtained as yellow crystals by recrystallization from ethyl acetate (**21**) and toluene (**27**).

21: mp 216 °C (73%). Anal. ($C_{20}H_{18}N_2O_3$) C, H, N.

27: mp 260 °C dec (78%). Anal. ($C_{21}H_{20}N_2O_3$) C, H, N.

9-Hydroxy-1-carbamoylolivacines 19, 22–25, and 28 from 9-Hydroxy Esters: General Procedure. The hydroxy ester (0.5 g) was heated with an excess of the required amine (10 mL) under argon till disappearance of the starting compound: 27 h in a steel vessel at 115 °C for **19**, 3 h at 130 °C for **22**, 16 h at 120 °C for **23**, 19 h at 120 °C for **24**, 24 h at 120 °C for **25**, and 36 h at 115 °C for **28**. The excess of amine was evaporated, and the residue was taken up in water. The resulting precipitate was collected, and the aqueous layer was extracted with dichloromethane and then dried over magnesium sulfate. After evaporation of the solvent, the solid was added to the residue and recrystallized in the given solvent. To the purified compound (except for compounds **25** and **28** which did not recrystallize in these conditions) was then added an excess of a solution of hydrochloric acid in ethyl alcohol. The solid dihydrochloride salts were then filtered and dried under vacuum. For compound **28**, the solution was evaporated to dryness and the residue was taken up in dry acetone to give a solid which was dried under vacuum.

19: this protocol provided a sample which was in all respects identical to that obtained from **15**.

22: free base mp 95 °C dec (from absolute ethyl acohol); dihydrochloride salt mp 200 °C dec. Anal. ($C_{22}H_{24}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N.

23: free base mp 120 °C dec (from absolute ethyl alcohol); dihydrochloride salt mp 190 °C dec. Anal. ($C_{24}H_{26}N_4O_2 \cdot 2HCl \cdot 3H_2O$) C, H, N.

24: free base mp 110 °C (from absolute ethyl alcohol); dihydrochloride salt mp >260 °C. Anal. ($C_{25}H_{28}N_4O_3 \cdot 2HCl \cdot 0.5H_2O$) C, H, N.

25: dihydrochloride salt mp 165 °C dec. Anal. ($C_{22}H_{24}N_4O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N.

28: free base mp 115 °C dec. It was purified by chromatography over a silica gel column, eluting with dichloromethane–ethyl alcohol, 9–1 v/v, and 0.5% of triethylamine. Dihydrochloride salt: mp 140 °C dec. Anal. ($C_{23}H_{26}N_4O_2 \cdot 2HCl \cdot 2H_2O$) C, H, N.

***trans*-2-(6-Methoxy-1,4-dimethylcarbazol-2-yl)-1-nitroethylene (30).** A mixture of 2-formyl-6-methoxy-1,4-dimethylcarbazole (**29**)[1] (10.2 g, 40 mmol), nitromethane (100 mL), and ammonium acetate (8.5 g) was stirred and heated at reflux for 5 h under nitrogen. Evaporation to dryness under reduced pressure left a residue which was taken up in ethyl alcohol (120 mL). The solid was collected and washed with water and ethyl alcohol to provide the pure expected compound as yellow needles (8.73 g, 73%), mp 208–209 °C. ^1H NMR ($CDCl_3$) chemical shifts of vinylic protons appear as doublets at 7.64 and 8.52, with a coupling constant of 13.8 Hz, characteristic of a trans geometry. Anal. ($C_{17}H_{16}N_2O_3$) C, H, N.

6-Methoxy-1,4-dimethyl-2-(2-nitroethyl)carbazole (31). To the preceding compound (7.4 g, 23 mmol) dissolved in methanol (700 mL), was progressively added sodium borohydride (7 g) under stirring. The mixture was stirred 1 h further, acidified to pH 4 with acetic acid, and evaporated to dryness. The residue was triturated with water; the solid was collected and recrystallized from toluene to provide beige crystals (6.66 g, 90%), mp 178–179 °C. Anal. ($C_{17}H_{18}N_2O_3$) C, H, N.

2-[2-(Ethoxalylamino)ethyl]-6-methoxy-1,4-dimethylcarbazole (33). A mixture of the (nitroethyl)carbazole **31** (5.75 g, 18 mmol), acetic acid (250 mL), and Raney nickel catalyst (5 g) was stirred in a steel vessel at 50 °C for 6 h, under hydrogen at an initial pressure of 30 atm. The catalyst was filtered off, the solution was washed with acetic acid and ethyl alcohol, and the solvent was evaporated to dryness, under reduced pressure. The residue was taken up in water, and the solution was filtered. The filtrate was basified with an excess of ammonia to provide a solid which was collected, washed with water, and air-dried. The crude product **32** (mp 172 °C) was heated in diethyl oxalate (15 mL) at 120 °C for a 2-h period, under stirring. After cooling, the resulting solid was collected, washed with pentane, and recrystallized from

ethyl acetate to give 4.4 g (55.7%) of beige crystals, mp 187–188 °C. Anal. ($C_{21}H_{24}N_2O_4$) C, H, N.

Ethyl 3,4-Dihydro-9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (34). A mixture of [(ethoxalylamino)ethyl]carbazole **35** (4.6 g), toluene (400 mL), and phosphorus oxychloride (100 mL) was stirred and heated at reflux for 28 h and evaporated to dryness under reduced pressure. An additional 100-mL portion of toluene was evaporated, the residue was taken up in ethyl acetate, and the solid was collected. It was dissolved in water, basified with ammonia, and extracted with methylene chloride. The resulting solid from the usual workup was recrystallized from toluene to give 2.6 g (59.5%) of yellow crystals, mp 235–236 °C. Anal. ($C_{21}H_{22}N_2O_3$) C, H, N.

Ethyl 9-Methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole-1-carboxylate (35). The preceding compound (2.15 g, 6 mmol) was heated in diphenyl oxide (50 mL) at reflux for 5 min, in the presence of 30% palladium on charcoal (300 mg). The mixture was filtered when hot and allowed to cool, and the catalyst was washed with acetone. Acetone was evaporated, hexane (150 mL) was added to the mixture, and the precipitate was collected. Recrystallization from ethyl acetate provided 2.02 g (94.8%) of the title compound as yellow crystals, mp 217–218 °C. ^{1}H NMR ($CDCl_3$): δ 1.58 (t, 3H, CH_3CH_2), 2.27 (s, 3H, 11-CH_3), 2.93 (s, 3H, 5-CH_3), 3.91 (s, 3H, 9-OCH_3), 4.68 (q, 2H, CH_2CH_3), 7.12 (dd, 1H, 8-H, J_{8-7} = 8.7 Hz, J_{8-10} = 2.3 Hz), 7.38 (d, 1H, 7-H), 7.39 (d, 1H, 10-H), 7.7 (d, 1H, 4-H, J_{4-3} = 6.1 Hz), 8.37 (d, 1H, 3-H). Anal. ($C_{21}H_{20}N_2O_3 \cdot 0.25\ H_2O$) C, H, N.

Attempts To Convert Ester 35 to *N*-Substituted Carboxamides. When compound **35** (500 mg) was heated in 2-(dimethylamino)ethylamine and 3-(dimethylamino)propylamine (20 mL) at reflux (105 and 133 °C) for 8 h, it was totally recovered. In a steel vessel at 180 °C for the same time, it was transformed totally, giving 9-methoxyellipticine (**37**) (69%), identical in all respects to an authentic sample (Sanofi).

Cell Culture and Cytotoxicity. L1210 and colon 38 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described.[34] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for four doubling times (48 h for L1210 cells or 96 h for C38 cells). Results were expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to the density of untreated controls.

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNase and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10 000 cells were analyzed on an ATC3000 flow cytometer (Brucker, Wissembourg, France).

Antitumor Activity. The antitumor activity of the compounds was evaluated on two experimental murine models: the P388 leukemia and the colon 38 adenocarcinoma. P388 cells (NCI, Frederick) were inoculated ip (10^6 cells/mouse) into B6D2F1 mice (Iffa credo) on day 0. The drugs were dissolved in water and injected iv on day 1 or days 1, 5, and 9. The results are expressed in terms of percent *T/C* (median survival time of treated animals divided by median survival time of controls, × 100). The colon adenocarcinoma 38 (NCI, Frederick) was introduced by sc implantation of a tumor fragment into the dorsal flank. The drugs were administered by iv injection at days 2 and 9 (early treatment) or 9 and 16 (delayed treatment). The tumor volume was measured, and the results are expressed as percent *T/C* (median tumor volume in treated animals divided by median tumor volume of controls, × 100).

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