priately adjusting the incubation time and protein concentrations such that the hydrolysis of either cyclic nucleotide was limited to less than 25% under the assay conditions. None of the agents were found to alter the elution profile of the nucleotides and nucleosides during the polyacrylamide–boronate gel chromatography.

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Registry No. **1b**, 58-74-2; **2a**, 83633-12-9; **2b**, 83633-13-0; **3a**, 83633-14-1; **3a**·HCl, 83649-36-9; **3b**, 83633-15-2; **3b**·HCl, 83633-16-3; **4a**, 83633-17-4; **4a**·HCl, 83633-18-5; **4b**, 83633-19-6; **4b**·HCl, 83633-20-9; **5a**, 83633-21-0; **5a**·HCl, 83633-22-1; **5b**, 83633-23-2; **5b**·HCl, 83633-24-3; **6a**, 83633-25-4; **6a**·HCl, 83633-26-5; **6b**, 83633-27-6; **6b**·HCl, 83633-28-7; **7a**, 83633-29-8; **7b**, 83633-30-1; **7b**·HCl, 83633-31-2; **8a**, 83633-32-3; **8a**·HCl, 83633-33-4; **8b**, 83633-34-5; **8b**·HCl, 83633-35-6; **9**, 10268-50-5; **10**, 10268-35-6; **11**, 83633-36-7; **12**, 6309-18-8; **13**, 6924-15-8; **15**, 4230-93-7; **16**, 57542-90-2; **17**, 4722-08-1; **18**, 83633-37-8; **19**, 26193-61-3; **20**, 25932-34-7; **21**, 3052-50-4; **22**, 17081-97-9; **23a**, 83633-38-9; **23a**·HCl, 53633-39-0; **23b**, 83633-40-3; **23b**·HCl, 83633-41-4; 3,4-dimethoxyphenethylamine, 120-20-7; 4-nitrobenzoyl chloride, 122-04-3; 3,4-dimethoxybenzaldehyde, 120-14-9; nitromethane, 75-52-5; 4-nitrobenzeneacetyl chloride, 50434-36-1; acryloyl chloride, 814-68-6; chloroacetyl chloride, 79-04-9; ethylene oxide, 75-21-8; maleic anhydride, 108–31–6; phosphodiesterase, 9025-82-5.

Antitumor Amino-Substituted Pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines and Pyrido[4,3-*b*]carbazole Derivatives: Synthesis and Evaluation of Compounds Resulting from New Side Chain and Heterocycle Modifications

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New modifications of 10-[[3-(diethylamino)propyl]amino]-6-methyl-5*H*-pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline (**1b**) and 1-[[3-(diethylamino)propyl]amino]-9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**4b**), which display important antitumor properties, were performed either on the side chain or on the intercalating heterocycle. Side chains were introduced by direct substitution of the corresponding chloro derivatives and 6-*N*-methyl-9-hydroxypyrido[4,3-*b*]carbazoles analogues were prepared via 9-*O*-benzoyl-1-chloroellipticines. Evaluation of all new compounds shows no significant increase of in vitro cytotoxicity and percent ILS on the L1210 leukemia system by comparison with the model compounds **1b** and **4b**.

Recently, we reported the synthesis of various [[(dialkylamino)alkyl]amino]pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines (9-azaellipticines) (**1** and **2**) and 1-amino-substituted ellipticines (**3** and **4**) [Chart I; R = $NH(CH_2)_nN(R_2)R_3$].[1-5] Among these compounds, **1b** and **4b** exhibit high antitumor activities.[3,6]

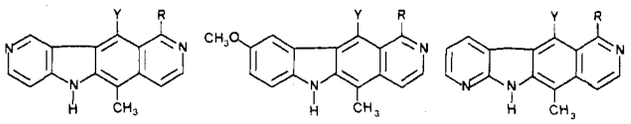
However, the biological results appeared closely dependent upon the nature of the side chain.[5] Furthermore, the biological properties could depend upon modifications of the heterocyclic intercalating ring, as suggested by the lack of antitumor activity of 7-azaellipticine derivatives (**5** and **6**).[7] Therefore, we decided to carry out further modifications of compounds **1–4** by introducing either new hydrophilic and lipophilic side chains or a 6-methyl group on the pyrido[4,3-*b*]carbazole heterocycle.

The synthesis and biological properties of these new compounds are reported in this paper.

Chemistry. Amino-substituted compounds **1–10** were obtained by substitution of the corresponding chloro derivatives, **1a–10a**, with appropriate amines. Bis(chloroethyl)amino derivative **31** was obtained from [bis(hydroxyethyl)amino]propylamino compound **3b** by a standard method.[8]

The key intermediates **1a**, **3a**, and **4a** were already described,[1,4] and compounds **7a** and **8a** were prepared by methylation of the corresponding **3a** and **4a** (Scheme I). However, preparation of 6-methyl-9-hydroxypyrido[4,3-*b*]carbazoles **9b** and **10b** required an appropriate route to 9-*O*-benzoylpyrido[4,3-*b*]carbazoles **19a** and **20a**. This route involves demethylation of **11** and **12** to **13** and **14**,

Chart I



1: Y=H
2: Y=CH_3

3: Y=H
4: Y=CH_3

5: Y=H
6: Y=CH_3

Substituent key:

a) R = Cl
b) R = $NH-(CH_2)_3-N(CH_2CH_3)_2$
c) R = $NH-(CH_2)_3-NH-CH_2CH_3$
d) R = $NH-CH_2-CH(OH)-CH_2-N(CH_2CH_3)_2$
e) R = $NH-CH_2-CH(OH)-CH_2OH$
f) R = $NH-(CH_2)_3-N$ (morpholino)
g) R = $NH-(CH_2)_3-N(CH_2CH_3)-(CH_2)_3-NH_2$
h) R = $NH-(CH_2)_3-N(CH_2CH_2OH)_2$
i) R = $NH-(CH_2)_3-N(CH_3)_2$
j) R = $NH-(CH_2)_3-N(CH_2CH_2CH_2CH_3)_2$
k) R = $NH-(CH_2)_2-N(CH_2CH_3)_2$
l) R = $NH-(CH_2)_3-N(CH_2CH_2Cl)_2$
m) R = NH-(C6H4)-$NH-SO_2CH_3$

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Scheme I

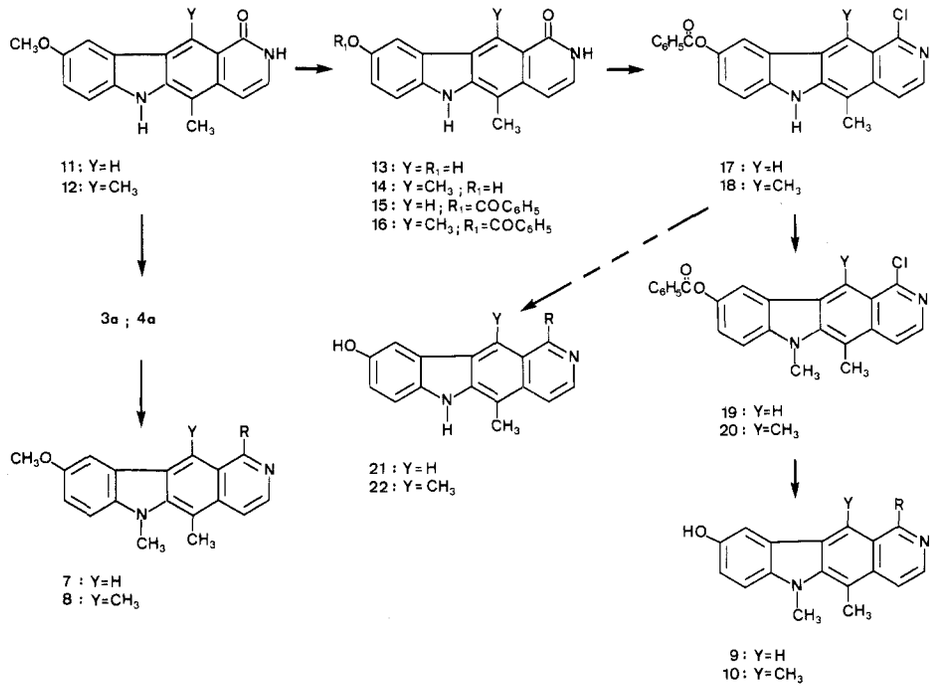

Table I. New Aminoalkyl- or Aminoaryl-Substituted Pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline and Pyrido[4,3-*b*]carbazole Derivatives

no.	mp, °C	method[a] (time)	yield,[b] %	formula[c]	anal.	purifn proced[d]
1c[e]	s 100, F = 140	A (6 h)	64	$C_{20}H_{23}N_5 \cdot 0.5H_2O$	C, H, N	I
1d[f]	180	A (6 h) and B	38	$C_{22}H_{27}N_5O \cdot (C_4H_4O_4)_3$	C, H, N	II
1e	>300	A (6 h)	73	$C_{18}H_{18}N_4O_2$	C, H, N	III
1g	110	A (7 h)	5	$C_{23}H_{30}N_6 \cdot 2H_2O$	C, H, N	IV
1h	220	A (6 h)	64	$C_{22}H_{27}N_5O_2 \cdot 0.5H_2O$	C, H, N	V
1j	180	A (5 h) and B	53	$C_{26}H_{35}N_5 \cdot 3C_4H_4O_4$	C, H, N	II
1m	>300	C (24 h)	41	$C_{22}H_{19}N_5O_2S \cdot H_2O \cdot 0.5C_2H_5OH$	C, H, N, S	VI
3c	198	A (4 h)	77	$C_{22}H_{26}N_4O$	C, H, N	VII
3e	246	A (5 h)	73	$C_{20}H_{21}N_3O_3$	C, H, N	VI
3f	100	A (6 h)	62	$C_{24}H_{26}N_4O_2$	C, H, N	VII
3h	190	A (6 h)	55	$C_{24}H_{30}N_4O_3$	C, H, N	VI
3l[g]	290	D	89	$C_{24}H_{28}Cl_2N_4O \cdot 2HCl$	C, H, N	III
4c	194	A (3 h)	55	$C_{23}H_{28}N_4O$	C, H, N	I
4d	185	A (6 h)	63	$C_{25}H_{32}N_4O_2$	C, H, N	I
4h	182	A (6 h)	46	$C_{25}H_{32}N_4O_3 \cdot 0.5C_2H_5OH$	C, H, N	IV
7b	138	A (4 h)	18	$C_{25}H_{32}N_4O$	C, H, N	VII
7i	143	A (6 h)	66	$C_{23}H_{28}N_4O$	C, H, N	VII
7k	123	A (7 h)	47	$C_{24}H_{30}N_4O \cdot 0.5H_2O$	C, H, N	VIII
8b	114	A (4 h)	57	$C_{26}H_{34}N_4O$	C, H, N	VIII
8i	88	A (4 h)	73	$C_{24}H_{30}N_4O$	C, H, N	VIII
8k	93	A (4 h)	58	$C_{25}H_{32}N_4O \cdot 0.5H_2O$	C, H, N	VIII
9b	200	A (3 h)	27	$C_{24}H_{30}N_4O$	C, H, N	VII
10b	110	A (3 h)	68	$C_{25}H_{32}N_4O \cdot H_2O$	C, H, N	I
10c	168	A (3 h)	76	$C_{23}H_{28}N_4O$	C, H, N	I
10i	158	A (5 h)	70	$C_{23}H_{28}N_4O$	C, H, N	I

[a] Methods: A = heating the chloroellipticine with the appropriate amine in an oil bath, at 160 °C, for the indicated time; B = maleate salt isolated by treatment of the aminoellipticine with maleic acid, in acetone; C = by boiling of starting compound 1a·HCl, with 2 molar equiv of amine in 2-ethoxyethanol; D = see Experimental Section. [b] The reported yields are calculated on the purified materials. [c] Unless otherwise stated, microanalyses are within 0.4% of the theoretical values for C, H, and N corresponding to the mentioned empirical formulas. [d] Recrystallization solvents: I = toluene; II = acetone; III = purified in boiling ethanol (insoluble); IV = purified by column chromatography on alumina and elution with ethanol plus 2% NH_4OH; V = chloroform, after a 3-day continuous extraction in a Kumagawa apparatus; VI = ethanol; VII = xylene; VIII = cyclohexane. [e] Anal. Calcd for $C_{20}H_{23}N_5 \cdot 0.5H_2O$: C, 70.18; Found, 70.63. [f] Anal. Calcd for $C_{34}H_{39}N_5O_{13}$: C, 56.27; H, 5.38. Found: C, 55.83; H, 5.80. [g] Heating compound 3h with thionyl chloride in boiling chloroform for 48 h.

benzoylation to **15** and **16**, chlorination of these last compounds by boiling in phosphorous oxychloride to give **17** and **18**, and, finally, methylation to **19** and **20** by methyl iodide in the presence of sodium hydride. Substitution of

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Table II. In Vitro and in Vivo Biological Properties of Amino-Substituted Pyrido[3',4':4,5]pyrrolo[2,3-*g*]isoquinoline and Pyrido[4,3-*b*]carbazole Derivatives

	in vivo cytotoxicity		in vivo acute toxicity ip		antitumor properties on L1210 leukemia system				
drug	ID_{50},[a] μM	K[a]	LD_{100},[a] mg/kg	LD_0,[a] mg/kg	dose, mg/kg	range of death, days	MST,[a] days	% ILS[a] (survivors/6)	level of signif[b]
1c	0.1	10	30	20	10	16-42	25	114	3
1d	0.05	20	40	25	12.5	12-27	16.3	45	3
1e	0.25	4	60	40	20	14-17	16	32	2
1g	0.2	5	40	20	20	15-19	17	45	3
1h	0.6	1.7	100	30	25	14-19	15.5	38	2
2b	0.007	143	20	10	10	16-20	16.8	46	2
2i	0.008	125	20	15	15	7-44	25.2	89 (1)	3
3b	5	0.2	40	20	15	12-19	14.3	24.6	1
3c	0.6	1.7	30	20	10	12-25	14.8	26	1
3l	0.2	5	20	15	10	12-14	13.6	26	1
4c	0.1	10	20	16	10	8-28	20	84 (2)	3
4h	0.4	2.5	15	10	3.75	15-21	18.4	78 (1)	3
7k	2	0.5	30	20	20	9-12	11	21	1
9b	0.06	16	25	12.5	12	12-14	13	34	3
10b	0.2	5	25	12	10	14-19	16.1	66.5	3
10c	0.02	50	25	10	2.5	12-19	14.5	49.5	3
10i	0.03	33	50	10	10	8-20	16.3	68	3
21b	0.02	50	7	2.5	2.5	14-19	16.4	56	3
22b	0.03	33	10	3	2.5	14-19	16.5	57	3
1b	0.02	50	30	25	20	14-25	20.5	85	3
4b	0.03	30	60	15	15	19-35	29.5	156 (4)	3
Act. D	0.001	1000							

[a] For definitions, see text. [b] Level of significance: 3, $p \leq 0.001$; 2, $p \leq 0.01$; 1, $p \leq 0.1$.

1-chloro-6-methyl-9-*O*-benzoylellipticines **19a** and **20a** with primary amines was then accompanied by the 9-*O*-benzoyl group elimination, giving directly the 9-hydroxylated derivatives **9** and **10** [R = $NH(CH_2)_nN(R_2)R_3$]. It should be pointed out that this new route leads to 9-hydroxy-6*H*-pyrido[4,3-*b*]carbazoles, **21** and **22**, more conveniently than that previously reported,[4] since it involves the direct substitution and debenzoylation of chloro derivatives **17** and **18**.

All newly synthesized amino-substituted ellipticines and ellipticine analogues are described in Table I.

Biological Results

In Vitro Cytotoxicity. Cytotoxicity toward tumor cells grown in vitro and dose–effect relationships for the various compounds were investigated as described in a preceding paper.[5] ID_{50} values (μM) correspond to concentrations of drugs that lowered cell growth by 50%. In order to compare more easily the in vitro cytotoxic activity, we defined an inverse value, K (μM^{-1}). For example, in this system, actinomycin D, which is a very potent DNA binding agent, corresponds to a ID_{50} value of 10^{-3} μM and a K value of 1000 (Table II).

(b) In Vivo Antitumor Effects. The lethal dose (LD_{100}) and the highest nontoxic dose (LD_0) were determined after a single intraperitoneal injection to mice. We then investigated antitumor properties on the L1210 leukemia system, using first the highest nontoxic dose, then lower doses when positive effects were observed. The percent ILS are given in Table II only for compounds that exhibited a significant in vitro cytotoxicity. Compounds **1b**[3,6] and **4b**[5] have been introduced in the table as references.

Discussion and Conclusion

By comparison with the reference compounds **1b** and **4b**, which have the 3-(diethylamino)propylamino side chain and exhibit both high cytotoxicity and antitumor properties, it appears that further modifications of the side chain do not improve significantly the biological properties. Often, the antitumor properties were lost and the cytotoxicity was lowered as in the case of **1j,m**, **3e,f,h**, **4d**, **5b**, **6b**, **7b,i**, and **8b,i,k**, which therefore have not been tabulated. However, the bis(chloroethyl)amino side chain (**3b**), as well as the hydroxylated side chains (**1d,h**, and **4h**) maintain some antitumor activity, though less cytotoxic. Only the 3-(ethylamino)propylamino derivatives (**1c**, **3c**, **4c**) are as active as the reference compounds **1b** and **4b**.

All compounds in which the heterocyclic nucleus was modified exhibited good cytotoxicity on cultured cells, except for the methoxyellipticines **3b** and **7b** in which position 11 is unsubstituted. The L1210 leukemia test, however, clearly shows that 7-azaellipticine derivatives **5b** and **6b**, as well as 6-*N*-9-*O*-dimethylpyrido[4,3-*b*]carbazoles **7b** and **8b**, are inactive as antitumor agents, whereas 9-hydroxylated 6-*N*-methylpyrido[4,3-*b*]carbazoles **9b** and **10b** exhibit significant antitumor properties.

These results could be compared to those of Le Pecq et al.[9,10] for various 1-unsubstituted 9-hydroxyellipticine derivatives. They confirm the importance of the 9-OH group for increasing both cytotoxic and antitumor activities in the pyrido[4,3-*b*]carbazole series. This was interpreted by Auclair and Paoletti[11] in terms of a one- or two-electron oxidizing activating process involving a quinone imine intermediate.

A parallelism between azaellipticine and hydroxyellipticine behavior can be observed for positions 9 and 7, corresponding, respectively, to active and unactive compounds in both series. However, the in vivo toxicity of pyrido[3',4':4,5]pyrrolo[2,3-*g*]isoquinolines **1** and **2** seems to be weaker than that of the parent ellipticines derivatives **21** and **22**. This suggests a possibility of two different metabolic pathways for toxicity and for antitumor activity. The former one could correspond to the quinone imine intermediate, which was shown by Auclair and Paoletti[11] to react with nucleophiles. The later one, which remains to be discovered, should be common to both series and

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could involve the pyrrolo[2,3-*g*]isoquinoline part of the molecules. The role of the [(dialkylamino)alkyl]amino side chains on the observed increase of antitumor properties of these classes of DNA intercalating drugs is unexplained. It could contribute to the transport of the drug as well as to its affinity and localization within DNA, thought to be the real target.

For potential applications, the present work demonstrates that hydrophilic substituents on the side chains do not increase, as a rule, the antitumor activity of 10-amino-substituted pyrido[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinolines (**1** and **2**) as well as that of their ellipticine analogues **3**, **4**, **9**, and **10**. The exceptions are the 3-[(ethylamino)propyl]amino substituents in **1**, **3**, and **4**, in which case they are found to maintain the activity, justifying further biological investigations.

On the other hand, the 6-N-methylation of 9-methoxyellipticines **3** and **4** leads to inactive compounds **7** and **8**, but the corresponding 9-hydroxylated derivatives **9** and **10** exhibit significant antitumor activities.

However, taking into account both toxic and antitumor properties, compounds **1b** and **4b** remain the most promising ones for clinical applications.

Experimental Section

Chemistry. All melting points are uncorrected and were determined by a Reichert hot-stage microscope. Purification procedures described in Table I were checked by thin-layer chromatography on silica gel (Kieselgel, 60 F 254 from Merck) or alumina (neutral, type E, 60 F 254 from Merck). The structure and purity were also systematically controlled by IR and ^{1}H NMR spectroscopy.

General Procedure for the Preparation of 1-Amino-Substituted Ellipticines and Ellipticine Analogues. Chloro derivatives (500 mg) in free amine (10 g) were heated under nitrogen in an oil bath at 160 °C for times noted in Table II. 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 compounds were filtered, dried, and recrystallized to afford yellow crystals corresponding to the expected products (see Table I). When crude compounds were oily substances, they were extracted with chloroform, dried with sodium sulfate, and evaporated, and the residues were taken up in the solvent listed in Table I. In some cases, this treatment afforded oily substances from which maleate salts were obtained and purified as indicated in Table I.

1-Chloro-5,6-dimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (7a). 1-Chloro-5-methyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (**3a**;[4] 1 g, 3.3 mmol) in dry dimethylacetamide (20 mL) was treated with sodium hydride (200 mg at 50% in mineral oil), under stirring, at ambient temperature for 15 h. To the resulting dark solution was added methyl iodide (0.52 g, 3.6 mmol), and the mixture was stirred for 1 h, during which time the solution turned yellow. Decomposition with water, methylene chloride extraction, and evaporation of solvent afforded a solid residue, which was recrystallized from ethyl acetate to give yellow crystals (680 mg, 65%), mp 206 °C. Anal. ($C_{18}H_{15}ClN_2O$) C, H, N, Cl.

1-Chloro-5,6,11-trimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (8a). 1-Chloro-5,11-dimethyl-9-methoxy-6*H*-pyrido[4,3-*b*]carbazole (**4a**;[4] 1 g, 3.2 mmol) was reacted with sodium hydride and subsequently with methyl iodide, as in the preceding case. The resulting solid was recrystallized from ethyl acetate to afford yellow crystals (947 mg, 90.5%), mp 133 °C. Anal. ($C_{19}H_{17}ClN_2O$) C, H, N, Cl.

9-Hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (14). 9-Methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (**12**; 5 g, 17.1 mmol) and anhydrous pyridine hydrochloride (80 g) were heated to reflux (220–230 °C) for 30 min. The mixture was poured into ice–water. The resulting solid was filtered off, washed with water, and purified in boiling dioxane to give beige crystals (2.9 g, 54%), mp >310 °C, corresponding approximately to the dihydrated expected compound. Anal. ($C_{17}H_{14}N_2O_2 \cdot 2H_2O$) C, H, N.

9-(Benzoyloxy)-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (15). Benzoic anhydride (2 g, 8.8 mmol) was added to 9-hydroxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (**13**)[4] in dry pyridine (10 mL), and the mixture was heated to reflux for 1 h. Pyridine was evaporated, the residue was treated with an excess of a sodium hydrogen carbonate solution, and the compound, taken up in boiling ethanol and filtered, afforded a solid (585 mg, 83.8%), mp >340 °C, corresponding to the expected compound, associated with 1 mol of ethanol. Anal. ($C_{23}H_{16}N_2O_3 \cdot C_2H_5OH$) C, H, N.

9-(Benzoyloxy)-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (16). Starting from 9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazol-1(2*H*)-one (**14**; 1 g, 3.6 mmol), the above treatment with benzoic anhydride afforded an oily compound, which was taken up in boiling ethanol for 10 min and filtered to give beige crystals (900 mg, 65%), mp >310 °C. Anal. ($C_{24}H_{18}N_2O_3$) C, H, N.

1-Chloro-9-(benzoyloxy)-5-methyl-6*H*-pyrido[4,3-*b*]carbazole (17). Compound (**15**; 500 mg, 1.4 mmol) was heated to reflux in phosphorous oxychloride (50 mL) for 3 h, and excess phosphorous oxychloride was evaporated under reduced pressure. The residue was taken up in water plus methylene chloride, treated with an excess of sodium acetate, and stirred at room temperature for 18 h. Extraction with methylene chloride and evaporation of the solvent afforded a solid residue, which was recrystallized from toluene to give yellow crystals (370 mg, 67%), mp 246 °C. Anal. ($C_{23}H_{15}ClN_2O_2 \cdot 0.5C_6H_5CH_3$) C, H, N, Cl.

1-Chloro-9-(benzoyloxy)-5,11-dimethyl-6*H*-pyrido[4,3-*b*]-carbazole (18). Starting from the benzoyloxy derivative (**16**), the above chlorination technique afforded the expected compound, which was recrystallized from toluene to give yellow crystals (72%), mp 230–232 °C. Anal. ($C_{24}H_{17}ClN_2O_2$) C, H, N, Cl.

1-Chloro-9-(benzoyloxy)-5,6-dimethyl-6*H*-pyrido[4,3-*b*]-carbazole (19). 1-Chloro-9-(benzoyloxy)-5-methyl-6*H*-pyrido-[4,3-*b*]carbazole (**17**; 200 mg, 0.5 mmol) in dimethylacetamide (10 mL) was treated for 30 min, at −15 °C, with sodium hydride (31 mg at 50% in mineral oil) under stirring. Methyl iodide (81 mg, 0.57 mmol) was added at the same temperature, and stirring was pursued for 15 h at −15 °C. The mixture was poured in ice–water. The resulting solid was filtered, air-dried, and recrystallized from toluene to afford yellow crystals (150 mg, 72.5%), mp 248 °C. Anal. ($C_{24}H_{17}ClN_2O_2$) C, H, N, Cl.

1-Chloro-9-(benzoyloxy)-5,6,11-trimethyl-6*H*-pyrido[4,3-*b*]carbazole (20). N-6-Methylation of pyrido[4,3-*b*]carbazole (**18**; 400 mg, 1 mmol) was performed as above. The expected compound was recrystallized from toluene to afford yellow crystals (365 mg, 88%), mp 270 °C. Anal. ($C_{25}H_{19}ClN_2O_2$) C, H, N, Cl.

1-[[3-(Diethylamino)propyl]amino]-9-hydroxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole (21b) and 1-[3-(Diethylamino)-propyl]amino]-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]-carbazole (22b). These compounds were already described.[4] They have now been obtained starting from chloro derivatives **17** and **18**, respectively, by the general technique (heating time 2 h). They were purified by column chromatography on alumina and with methylene chloride and subsequently methylene chloride–ethanol, 9:1, as eluents.

Biological Assays. (a) Cell cultures and in vitro cytotoxicity determination, (b) determination of in vivo acute toxicity and therapeutic doses, and (c) in vivo antitumor activity on L1210 leukemia were carried out following the procedures described previously.[5] Characteristics of the L1210 leukemia used in this study were also described.[3]

The therapeutic effect of drugs was measured as the percent increase in life span (% ILS) over controls, evaluated as follows: % ILS = (median survival time (MST) in treated) − (median survival time in controls)/(median survival time in controls) × 100.

Under our conditions, mice controls were inoculated with 10^5 leukemic cells on day 0, and death occurred within 10.5 days (range of death = 9–13). Mice were treated with a single intraperitoneal injection of indicated drug 1 day after the cells' inoculation.

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Registry No. **1a**, 69022-46-4; **1b**, 65222-35-7; **1c**, 83947-99-3; **1d**, 83948-00-9; **1e**, 83948-01-0; **1g**, 83948-02-1; **1h**, 83948-03-2; **1k**, 83948-04-3; **1m**, 83948-05-4; **2b**, 73323-33-8; **2i**, 73323-34-9; **3a**, 72237-96-8; **3b**, 72238-00-7; **3c**, 83948-06-5; **3e**, 83948-07-6; **3f**, 83948-08-7; **3h**, 83948-09-8; **3i**, 83948-10-1; **4a**, 72237-98-0; **4b**, 72238-02-9; **4c**, 83948-11-2; **4d**, 83948-12-3; **4h**, 83948-13-4; **7a**, 83947-90-4; **7b**, 83948-14-5; **7i**, 83948-15-6; **7k**, 83948-16-7; **8a**, 83947-91-5; **8b**, 83948-17-8; **8i**, 83948-18-9; **8k**, 83948-19-0; **9b**, 83948-20-3; **10b**, 83948-21-4; **10c**, 83948-22-5; **10i**, 83948-23-6; **12**, 72237-94-6; **13**, 72238-06-3; **14**, 83947-93-7; **15**, 83947-92-6; **16**, 83947-94-8; **17**, 83947-95-9; **18**, 83947-96-0; **19**, 83947-97-1; **20**, 83947-98-2; **21b**, 72238-04-1; **22b**, 72238-05-2.

Synthesis and Antileishmanial Activity of 6-Methoxy-4-methyl-*N*-[6-(substituted-1-piperazinyl)hexyl]-8-quinolinamines and Related Compounds[1,2]

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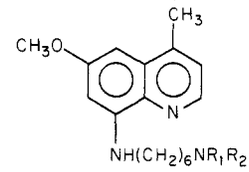
The 8-quinolinamine, 4-[6-[(6-methoxy-4-methyl-8-quinolinyl)amino]hexyl]-1-piperazineethanol (**1b**), has been shown to be highly effective against *Leishmania donovani* infections in hamsters. In an effort to obtain a more potent, less toxic 8-quinolinamine, a series of analogues (**2**) was prepared that examined particularly the structural requirements of the terminal piperazine moiety. Of the substituted piperazines and alternative heterocycles prepared, as well as those quinoline analogues with ring insertion of a methyl group in the 2-position or an aryloxy substituent in the 5-position, an increase in potency was achieved only with the 2-hydroxypropyl analogue (**2f**).

Leishmaniasis is a disease of tropical and subtropical areas caused by intracellular protozoan parasites of the genus *Leishmania* and is transmitted by the bite of phlebotomine flies (sandflies). It displays four general clinical forms: cutaneous, mucocutaneous, chiclero ulcer, and visceral, which are caused, respectively, by *L. tropica*, *L. braziliensis*, *L. mexicana*, and *L. donovani*. In the first three forms the parasites invade the cutaneous tissues in various parts of the body, causing disfiguring lesions of differing severity. In the visceral form of the disease, the phagocytic cells of the spleen, liver, and bone marrow are invaded, often resulting in death. Antimonials and the antibiotic Amphotericin B are used to treat the disease but are often of limited efficacy and the cause of host toxicity;[3,4] thus, the need for a better drug is real and ongoing.

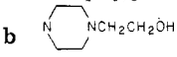
Although a multitude of 8-quinolinamines have been examined for antimalarial activity, relatively little information is available, and apparently only limited effort has been expended toward the development of an agent for *Leishmaniasis* from this class of compounds.

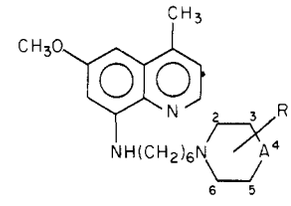
A series of 4-unsubstituted 8-quinolinamines containing side chains related to that in **1b** had been reported to be active against *L. donovani* and *L. tropica* infections in hamsters.[5-8] More recently, the presence of a methyl group in the 4-position has been shown to enhance activity, and *N*,*N*-diethyl-*N*-(6-methoxy-4-methyl-8-quinolinyl)-1,6-hexanediamine (**1a**) was the most potent compound examined, exhibiting a *G* index[9] of 474[3] against *L. donovani* infections in hamsters. Since an analogue with a piperazine side chain (**1b**) prepared in the course of our previous investigations proved to be quite active (*G* = 104) in this experimental model, we undertook the preparation of analogues with the aim of providing a more potent, less toxic 8-quinolinamine structure.



NR_1R_2

1a $N(C_2H_5)_2$

b 



2, A = NR', $S(O)_n$, O

Our primary goal was the modification of **1b** by variation of and substitution on the piperazine moiety as represented in structure **2**.

Recent efforts to develop a less toxic 8-quinolinamine for malaria therapy have revealed that the introduction of a 5-phenoxy group reduced toxicity while retaining activity in murine and primate antimalarial models.[10]

(1) This investigation was supported by the U.S. Army Medical Research and Development Command Contract DAMD 17-79-C-9122. This is contribution no. 1552 to the Army Research Program on Antiparasitic Drugs.
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