layer was extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate extracts were washed with 25 mL of 10% potassium bicarbonate, dried over sodium sulfate, filtered, and concentrated to ca. 2 mL. The concentrate was stored at −20 °C. A supernate was decanted from a crystalline solid, which was triturated with ether and collected by filtration. Further purification to TLC homogeneity was accomplished by recrystallization from acetone: yield 250 mg; mp 166–167 °C ^{1}H NMR (Me_2SO-d_6) δ 1.0–1.1 (t, 2, CH_2 in aziridine, 1.2–1.8 (complex m, 12, cyclohexyl $(CH_2)_5$ and CH_2 in aziridine), 2.2–2.4 (t, 2, CH_2N), 3.4–3.6 (t, 2, $CH_2N(C{=}O)_2$) 9.6 (s, 1, HN); FAB MS and EI MS identical with FAB MS and EI MS, respectively, of the metabolite; high-resolution MS (obsd, theory) 237.147, 237.148. Anal. ($C_{12}H_{19}N_3O_2$) C, H; N: calcd, 17.70; found, 16.89.

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Registry No. SHM, 56605-16-4; SHAZ, 102234-07-1; NH-$(CH_2CH_2Cl)_2$·HCl, 821-48-7; spirohydantoin, 702-62-5.

Basically Substituted Ellipticine Analogues as Potential Antitumor Agents

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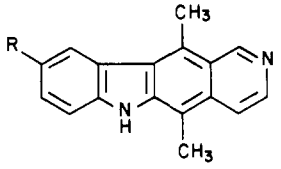
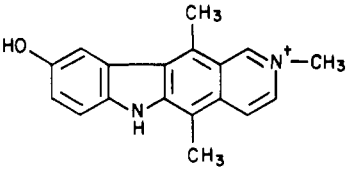
Installation of a basic side chain on the ring nitrogen of ellipticine did, as expected, improve the DNA binding properties of these molecules as measured by an ethidium displacement assay. In vivo antitumor activity was not, however, improved.

Ellipticine (**1a**) and its 9-methoxy analogue (**1b**) are constituents of plants of the Apocynacae family. In 1967, antitumor properties of ellipticine were revealed,[1] and in



1a: R=H
b: R=OCH_3
c: R=OH



2

1970 a study of **1b** against acute myeloblastic leukemia in man was reported.[2] More recent work has led to new derivatives such as **1c**[3] and the charged species **2**,[4] which has been said to have shown interesting results in phase II trials.[5]

The ellipticines are generally thought to exert their antitumor activity via their interaction with DNA. It was of interest, therefore, to prepare some examples of an ellipticine nucleus to which a basically substituted aliphatic side chain was appended, since such functionality has not infrequently been associated with improved DNA binding of planar molecules.

A basic side chain could be installed on the indole ring nitrogen of **1a** by alkylation of the anion formed with NaH with a corresponding alkyl halide in much the same way that the *N*-methyl analogue had been prepared.[1] Alternatively the basic side chain could be introduced on the pyridine nitrogen similar to the methodology used for the predecessor of **2**, the deshydroxy analogue.[6]

In this manner compounds 1–8 were prepared, and data on their properties are summarized in Table I.

Biology

The compounds were evaluated against the L1210 leukemia and a human colon tumor line in tissue culture. The data are shown in Table II. In vitro these analogues display cytotoxicity of the same order of magnitude as the parent, ellipticine. Against the murine P388 leukemia IPIP on a Q04DX02 schedule, however, only the quaternary analogues **7** and **8** showed a low order of activity (T/C of 145 at 25 mg/kg and 137 at 5 mg/kg, respectively), while all others were inactive at the highest toxic doses tested. In the same test system, ellipticine resulted in a T/C of 200 at 10 mg/kg. A recent patent[7] contains basically substituted quaternary analogues of **2** although the biological data presented reveals little advantage over the methyl quaternary **2**.

To ascertain whether the lack of in vivo activity was due to a reduction of the DNA binding capabilities of the analogues, they were subjected to an ethidium displacement assay. Data shown in Table III indicates the concentration at which the fluorescence of an ethidium–DNA complex was reduced by 50%.

All compounds tested were superior to ellipticine itself in DNA binding capability.

Conclusions

Addition of a basic side chain to the ellipticine structure has been demonstrated to improve DNA binding properties of the system. The poor in vivo antitumor response of the compounds despite the improved DNA binding capability must involve unfavorable pharmacokinetic, pharmacodynamic, or metabolic properties and must receive further attention.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus and are uncorrected. The ^{1}H NMR 90-MHz spectra were obtained with a Varian FM 390 or Brüker WH90

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(4) LePecq, J.-B.; Gosse, C.; Dat-Xuong, N.; Paoletti, C. *C. R. Acad. Sci. Paris, Ser. D* **1975**, *281*, 1365–1367.
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Table I. N-Substituted Ellipticine Analogues and Properties

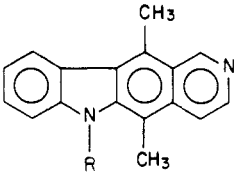
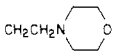
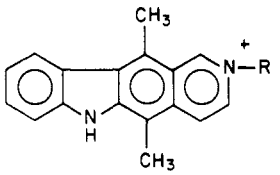
compd	R	mp, °C	recrystn solvent	yield, %	formula	anal.
						
1	CH_3	>300	EtOH	55	$C_8H_{16}N_2$·HCl	C, H, N
2	$(CH_2)_3NMe_2$	295–303 dec	EtOH	21	$C_{22}H_{25}N_3$·2HCl·0.3H_2O	C, H, N, H_2O
3		>300	MeOH	28	$C_{23}H_{25}N_3O$·2HCl·1.5H_2O	C, H, N, H_2O
4	$CH_2CH_2NEt_2$	310 dec	EtOH	22	$C_{23}H_{27}N_3$·2HCl	C, H, N, Cl
5	$CH_2CH_2N(CH_2)_5$	>300	EtOH	51	$C_{24}H_{27}N_3$·2HCl·1.8H_2O	C, H, N, H_2O
6	CH_2CONEt_2	194–197	EtOAc	2.5	$C_{23}H_{25}N_3O$·0.1$C_4H_8O_2$	C, H, N
						
7	CH_3	>300		100	$C_{18}H_{17}N_2I$	C, H, N
8	$CH_2CH_2NEt_2$	275 (dec)	EtOH	20	$C_{23}H_{28}N_3Br$·0.5HBr·0.4H_2O	C, H, N, Br, H_2O

Table II. Effects of Ellipticine and Analogues against L1210 Leukemia[a] in Tissue Culture and HCT8 Human Colon Adenocarcinoma[b]

compd	L1210 ID_{50}[c]	HCT8 ID_{50}[c]
1	4.3×10^{-7}	
2	1.1×10^{-7}	3.9×10^{-7}
3	3.2×10^{-7}	1×10^{-7}
4	1.9×10^{-7}	8×10^{-8}
5	1.7×10^{-7}	8.2×10^{-8}
6	inactive	
7	6×10^{-7}	inactive
8	4.1×10^{-8}	5.0×10^{-7}
ellipticine	1×10^{-7}	2.6×10^{-7}

[a] For a description of the assay see Baguley, B. C.; Nash, R. *Eur. J. Cancer* **1981**, *17*, 671. [b] For a description of the assay see Finlay, G. J.; Baguley, B. C. *Eur. J. Cancer Clin. Oncol.* **1984**, *20*, 947. [c] ID_{50} = the molar concentration of test drug required to reduce the number of cells by 50% after incubation for 2 days.

Table III. Effects of Ellipticine and Analogues in an Ethidium Binding Assay

compd	C_{50}, nM	compd	C_{50}, nM
1	454	5	122
2		6	
3	440	7	
4	106	8	159
		ellipticine	1039

spectrometer at 90 MHz or a Varian XL-200 spectrophotometer at 200 MHz. The IR spectra were obtained on Digilab DP-1-15 or Beckman IR-9 spectrophotometers. The IR and NMR spectra of all compounds were consistent with the assigned structures. Analyses for C, H, N, and Cl were within 0.4% and for H_2O (method of Karl Fisher) within 0.5% of calculated values, unless otherwise noted. A typical procedure follows.

5,11-Dimethyl-6-[2-(1-piperidinyl)ethyl]-6*H*-pyrido[4,3-*b*]carbazole Dihydrochloride (5). To a suspension of 4.19 g (0.017 mol) of ellipticine in 45 mL of dry DMF was added 0.84 g (0.021 mol) of a 60% oil suspension of NaH that had been triturated with hexane. The mixture was stirred at room temperature for 0.5 h, and a solution of 3.1 g (0.021 mol) of *N*-(2-chloroethyl)piperidine in 30 mL of DMF was added dropwise over about 5 min. The *N*-(2-chloroethyl)piperidine was prepared by dissolving 10 g of the hydrochloride salt in 90 mL of H_2O, adding a solution of 2.16 g of NaOH in 10 mL of H_2O and extracting 3 times with 100-mL portions of CH_2Cl_2. The extracts were dried over $MgSO_4$, and the solvent was removed in vacuo to provide 7.7 g of the liquid-free base. The reaction mixture was allowed to stir at room temperature for 17 h; water was added dropwise to destroy any residual NaH; and the mixture was poured into 500 mL of H_2O and extracted with CH_2Cl_2. The extracts were filtered through basic alumina (EM, activity II–III, 70–270 mesh), and the solvent was removed in vacuo. The resulting gum was warmed with 100 mL of Et_2O and filtered. The filtrate was cooled to provide 2.76 g of a yellow solid. This was dissolved in 50 mL of EtOH, and a solution of dry HCl gas in 2-PrOH was added. The resulting suspension was diluted with 25 mL of EtOH and filtered. The solid was washed with Et_2O and dried in vacuo at 70 °C to afford 3.0 g of yellow solid: mp >300 °C. Anal. Calcd ($C_{24}H_{27}N_3$·2HCl·1.8H_2O) C, H, N, H_2O.

2-[2-(Diethylamino)ethyl]-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide Hemihydrobromide (8). To a suspension of ellipticine (6.0 g, 0.024 mol) in 1 L of MeOH was added a solution of 6.8 g (0.026 mol) of (*N*,*N*-diethylamino)ethyl bromide hydrobromide in 100 mL of MeOH. A solution of triethylamine (3.65 mL, 0.026 mol) in 25 mL of MeOH was then added dropwise over 0.5 h, and the mixture was allowed to remain at room temperature. After 21 h an additional 1.6 g of (*N*,*N*-diethylamino)ethyl bromide hydrobromide in 10 mL of MeOH was added to the clear mixture, and then 0.83 mL of triethylamine in 5 mL of MeOH was added dropwise. After 5 h another 1.6 g of (*N*,*N*-diethylamino)ethyl bromide hydrobromide in 10 mL of MeOH was added followed by a solution of triethylamine (0.83 mL) in 5 mL of MeOH dropwise. An identical addition was made after another 2 h, and the mix was allowed to stir at room temperature overnight. The solvent was removed in vacuo, and the residual solid was triturated with 100 mL of H_2O and filtered. Recrystallization from 75 mL of 95% EtOH provided 2.3 g of orange solid: mp 275 °C dec. Anal. ($C_{23}H_{28}N_3Br$·0.5HBr·0.4H_2O) C, H, N, Br, H_2O.

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