Anticancer Specificity of Some Ellipticinium Salts against Human Brain Tumors *in Vitro*

Edward M. Acton,† Ven L. Narayanan,† Prabhakar A. Risbood,‡ Robert H. Shoemaker, David T. Vistica, and Michael R. Boyd*

Laboratory of Drug Discovery Research & Development, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Building 1052, Room 121, Frederick, Maryland 21702-1201, and Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland 20892

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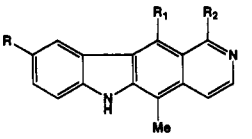
Novel structure–activity relationships (SAR) distinct from known SAR for ellipticines have been revealed for certain ellipticinium salts. In particular, ellipticiniums such as the prototypical 9-methoxy-2-methylellipticinium (I^- or OAc^-) were found to be preferentially cytotoxic to the brain tumor cell line subpanel of the NCI 60 cell-line screening panel. Similar specificity also was apparent with 9-unsubstituted ellipticiniums, or others bearing 9-methyl or 9-chloro substituents. In contrast, 9-hydroxy-substituted ellipticiniums, as well as all nonquaternized ellipticines tested, were devoid of brain tumor specificity. Therefore, it did not appear that this unusual preference was correlated with the relative availability of redox cycling mechanisms, since redox cycling presumably is blocked in 9-methyl- and 9-chloroellipticiniums. Indeed, related investigations have indicated that the brain tumor specificity is mediated by preferential uptake and intracellular accumulation of the specific ellipticiniums. The present study further supports that the NCI *in vitro* "disease-oriented" primary screen can facilitate the discovery of novel, selectively cytotoxic leads for *in vivo* and mechanistic investigations.

Introduction

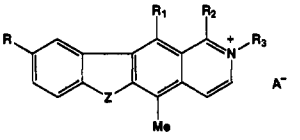
The ellipticine series[1,2] has been the subject of extensive efforts in preclinical development and analog synthesis, beginning with reports[3] of the antitumor properties of ellipticine (1), 9-methoxyellipticine (2), and olivacine[4] (6). Various structure–activity relationships have been pursued. Implicated[1,2] mechanisms of cytotoxicity include DNA intercalation, production of DNA strand breaks, inhibition of topoisomerase II, and redox cycling with the generation of free radicals and covalent alkylation of biological nucleophiles. RNA and protein as well as DNA have been suggested as molecular targets.[5] Both 1 and 2 are metabolized[2,6] to 9-hydroxyellipticine (3), which is an intermediate in the redox cycling process.



	R	R_1	R_2
1	H	Me	H
2	OMe	Me	H
3	OH	Me	H
4	Me	Me	H
5	Cl	Me	H
6	H	H	Me

The clinical development of ellipticine was deterred by problems with solubility and by hemolysis and hypotension as acute toxic side effects. The quaternary salt, 2-methyl-9-hydroxyellipticinium acetate (15) was more soluble, less toxic, and had entered clinical testing (as elliptinium, or Celiptium).[7,8] More recently, clinical attention shifted to the analogous salt, 2-(2-(diethylamino)ethyl)-9-hydroxyellipticinium chloride (16, Datelliptine).[9] However, to date neither compound has proven particularly impressive in clinical trials.

Upon preliminary assay in the NCI cell-line screen,[10–12] 15 did not show any indication of specificity for any of the tumor subpanels[13] contained therein. Therefore, it was especially surprising that the initial screening of 9-methoxy-2-methylellipticinium iodide (9) revealed one of the most striking examples of *in vitro* subpanel specificity—for the brain tumor subpanel—thus far observed in the new disease-oriented screen.[10] Hence, based on the preliminary results from 9, we initiated a more detailed exploration of structure–activity relationships among a selected group of compounds (1–22) to see if there were distinctions from previously observed relationships in the ellipticine series.[14–17]



	R	R_1	R_2	R_3	Z	A
7	H	Me	H	Me	NH	I
8	H	Me	H	Me	NH	OSO_2Me
9	OMe	Me	H	Me	NH	I
10	OMe	Me	H	Me	NH	OAc
11	Me	Me	H	Me	NH	OAc
12	Cl	Me	H	Me	NH	OAc
13	H	Me	H	$CH_2CH_2NEt_2$	NH	Br.1/2HBr
14	H	Me	H	$CH_2C_6H_4$-p-I	NH	Cl
15	OH	Me	H	Me	NH	OAc
16	OH	Me	H	$CH_2CH_2NEt_2$	NH	Cl.HCl
17	OH	Me	H	$CH_2CH_2NC_5H_{10}$	NH	OAc
18	OMe	Me	H	Me	NMe	I
19	H	Me	H	Me	$N(CH_2)_4CH=CH_2$	OAc
20	H	Me	H	Me	O	OAc
21	H	H	Me	Me	NH	OAc
22	H	Me	H	O⁻	NH	–

* Corresponding author.
† Drug Synthesis & Chemistry Branch.
‡ Current address: Starks Associates, Inc., Buffalo, NY 14213.
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Table 1. Summary of Analyses of Brain Tumor Subpanel Selectivity of Cytotoxic Ellipticines and Ellipticiniums (1–22)

compd no.	TGI concentration (μM) full panel [A]	TGI concentration (μM) brain tumor subpanel [B]	ratio A/B	TGI-Corr correlation coefficient[a]	selectivity rating[b]
1	3.0	2.9	1.0	<0.5	-
2	3.2	3.6	0.9	<0.5	-
3	2.8	3.4	0.8	<0.5	-
4	2.7	3.1	0.9	<0.5	-
5	17.	16.	1.1	<0.5	-
6	3.6	3.7	1.0	<0.5	-
7	12.	1.4	8.9	0.96	**
8	11.	1.0	11.	0.86	**
9	11.	1.1	9.8	1.00	**
10	18.	2.1	8.7	0.94	**
11	21.	6.9	3.0	0.83	*
12	22.	4.7	4.8	0.85	*
13	4.2	2.7	1.6	0.66	-
14	44.	54.	0.8	<0.5	-
15	78.	58.	1.3	<0.5	-
16	34.	16.	1.7	0.78	-
17	34.	20.	1.7	0.71	-
18	3.4	1.0	3.3	0.81	*
19	2.9	1.8	1.6	0.60	-
20	20.	5.5	3.7	0.89	*
21	32.	8.9	3.5	0.80	*
22	62.	41.	1.5	<0.5	-

[a] The TGI-based Compare analyses were performed using the TGI-centered mean graph profile of compound 9 as the benchmark or "seed" against a TGI mean graph database derived from the screening of compounds 1–22; each mean graph used in the analyses was the calculated average from at least quadruplicate tests of each compound in the full screen; standard-errors averaged less than 10% of the respective means. [b] Compounds were rated as "selective" (**) if (A/B) ≥ 6 and TGI-Corr ≥ 0.8; compounds were rated as "moderately selective" (*) if 3 ≤ (A/B) ≤ 6 and TGI-Corr ≥ 0.8; compounds meeting neither of these criteria were rated as nonselective (-).

Chemistry

Six nonquaternized ellipticines (1–6) were included for comparison with their ellipticinium counterparts. Quaternization at N-2 was generally with methyl groups, but examples of (alkylamino)alkyl[9,17,18] (13, 16, 17) and iodobenzyl (14) also were included. The *N*-oxide[19,20] (22) was chosen as a possibly interesting comparison with the quaternary salts. Changes at the carbazole N-6 included N-alkylation (18[3],19) and isosteric replacement with O (20).[21] Substitution of the ring system was at position 9 only. Most of these compounds may be substrates for oxidative metabolism at position 9, but in 4, 5, 11, and 12 that process is blocked.

Several ellipticinium salts were synthesized for the first time by quaternization of known ellipticines. 9-Chloroellipticine (5) was synthesized using the reaction sequence[3] for 9-bromoellipticine. As found previously[21-23] in the ellipticine series, some compounds, even after vacuum-drying, could not be characterized without the presence of fractional molar amounts of adhering solvents. Most of the ellipticinium salts were prepared as the acetates, in order to favor solubility in water; solubility was improved at least 100 times when the iodide 9 was converted[18] to the acetate (10).

Biological Results

For these contemporaneous, "side-by-side" comparisons, each compound was tested[24] in quadruplicate at each of three different concentration ranges (10^{-4}, 10^{-5}, and 10^{-6} M upper limits; five, $\log_{10}$-spaced concentrations in each range). This permitted statistically robust and reproducible comparisons of relative potency and cell line specificity within the study set. Data display and analyses were performed as described.[11,25]

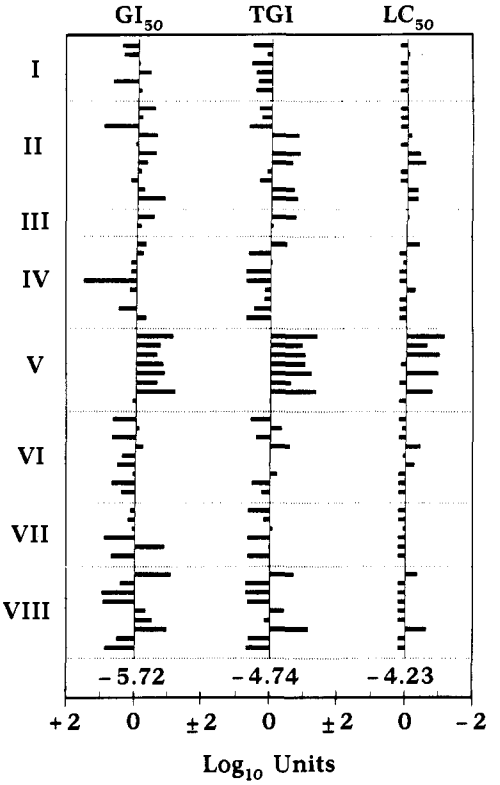

Figure 1. GI_{50}-, TGI-, and LC_{50}-based mean graphs[11,25] from screening of 9-methoxy-2-methylellipticinium acetate (10) in the NCI human tumor cell line panel. The tumor cell line subpanels are identified as follows: I (leukemia); II (lung, non-small-cell); III (lung, small-cell); IV (colon); V (brain); VI (melanoma); VII (ovary); VIII (kidney). The individual cell lines in all subpanels are identified in the Experimental Section. The $\log_{10}$ molar concentrations used as the reference point for each of the centerlines of each mean graph are shown at the bottom of each graph, along with the horizontal scaling marks in units of 1 $\log_{10}$ (negative to the right, and positive to the left of each centerline). Bars projecting to the right of the centerlines represent cell lines which are proportionately more sensitive to the compound, whereas bars projecting to the left of the centerlines represent those that are proportionately less sensitive.

The brain tumor specificity which was first noticed[10] in the routine screening of the 2-methyl-9-methoxyellipticinium iodide (9) was strikingly confirmed in the present study (Table 1). In remarkable contrast, Celiptium (15), as well as the related quaternary salts 16 and 17, all of which contain a 9-hydroxy substituent rather than the 9-methoxy, showed no evidence of brain tumor cell-line specificity (Table 1).

The differences between 9 and 15, 16, and 17 prompted further comparison of ellipticine analogs and an exploration of structure–activity relationships employing the NCI screen. Brain tumor specificity for the 2-methyl-9-methoxyellipticinium was confirmed with the much more water-soluble acetate (10) as well (Figure 1). Interestingly, the parent ellipticinium salts, 7 and 8, lacking any substitutent at position 9, also showed comparable specificity for the brain tumor subpanel.

Because all these compounds have access to ellipticine redox cycling mechanisms[5,6] (7 and 8 through metabolic hydroxylation, 9 and 10 through *O*-demethylation), it was of interest to test analogs that might be metabolically blocked to these mechanisms. The examples chosen, the 9-methyl (11) and 9-chloro (12), both showed moderate

Table 2. ^{1}H NMR Data

compound		chemical shifts (δ) in DMSO-d_6 at 500 MHz (ppm from Me_4Si)[a]										
		H-1	H-3	H-4	H-10	H-7	H-8	N-CH_3	11-CH_3	5-CH_3	OAc⁻	9-substit
(ellipticine)												
9-MeO	2[b]	9.69	8.41	7.91	7.89	7.49	7.20		3.27	2.77		3.91 s (MeO)
9-Me	4[c]	9.73 s	8.46 d	7.95 d	8.24 s	7.50 d	7.41 d		3.31 s	2.82 s		2.53 s (Me)
9-Cl	5[d,f]	9.76 s	8.49 d	7.97 d	8.38 s	7.60 m			3.28 s	2.83 s		
(quaternary)												
9-MeO	9	9.97 s	8.38 m		7.84 s	7.58 d	7.32 dd	4.42 s	3.25 s	2.79 s		3.98 s (MeO)
9-MeO	10[f]	9.87 s	8.27 m		7.86 s	7.65 d	7.26 dd	4.37 s	3.25 s	2.81 s	1.63 s	3.91 s (MeO)
9-Me	11[f]	9.89 s	8.31 s		8.14 s	7.60 d	7.46 d	4.42 s	3.21 s	2.81 s	1.70 s	2.57 s (Me)
9-Cl	12[f]	9.88 s	8.30 d	8.27 d	8.40 d	7.76 d	7.60 d	4.41 s	3.30 s	2.89 s	1.76 s	
6-hexenyl	19[e,f]	10.18 s	8.57 d	8.53 d	8.68 d	7.91 d	7.79 t	4.52 s	3.37 s	3.13 s	1.62 s	7.51 t (H)
6-oxa	20[f]	10.19 s	8.68 s		8.50 d	7.91 d	7.79 t	4.57 s	3.31 s	2.92 s	1.70 s	7.63 t (H)

[a] s = singlet, d = doublet, t = triplet, m = multiplet. [b] Literature data at 250 MHz, from G. Commenges and R. C. Rao, heterocycles **27**, 1395 (1988), and summarized in ref 3a. [c] $J_{3,4}$ = 6 Hz; $J_{7,8}$ = 8.1 Hz. Signals for DMF were observed at δ 8.00, 2.93, 2.79. [d] $J_{3,4}$ = 6 Hz. Signals for EtOAc were observed at δ 4.08 q, 2.04 s, 1.22 t. [e] Signals for the hexenyl side chain were δ 5.84 m (=CH), 5.0 m (=CH_2), 4.78 t (NCH_2), 2.15 m (allylic CH_2), 1.90 m (2-CH_2), 1.56 m (3-CH_2). A weak extraneous multiplet was present at δ 5.50. [f] Signals for H_2O were observed between δ 3.34 and 3.65.

brain tumor specificity. It therefore appeared that the unique specificity observed for these compounds in the NCI screen did not correlate with the presence or absence of structural opportunity for the widely studied redox cycling process.

Specificity was lost or considerably diminished by quaternization with more extended or bulkier groups (**13**, **14**) than methyl (**7**, **8**), or by alkylation of the carbazole NH with a bulkier group (**19**) than methyl (**18**). Moderate specificity was retained in a compound with isosteric replacement of the carbazole NH with O (**20**). As might be expected (e.g., in comparison with **7**, **8**), the olivacinium salt (**21**) retained moderate specificity. The semipolar bond in ellipticine *N*-oxide (**22**) presumably lent some electropositive character to N-2, but this compound showed no indication of the specificity seen with the corresponding ellipticinium salts (**7**, **8**). Finally, all of the parent ellipticines (**1**–**6**) were devoid of subpanel specificity, although they were quite indiscriminately cytotoxic, showing sharp dose–response curves uniformly against the full panel leading to cell kill at *ca.* 10^{-5} M and yielding relatively featureless mean graph profiles.

Discussion

The unusual brain tumor specificity described herein was entirely unanticipated from any previously known biological activity of ellipticinium salts or parent ellipticines. Recent mechanistic investigations[26] of the prototype, 2-methyl-9-methoxyellipticinium acetate (**10**), indicate that its selective cytotoxicity to brain tumor cell lines is due, at least in part, to its preferential transport and accumulation in the sensitive lines. Moreover, the transport mechanism appears similar to or the same as a physiological process which has been implicated in certain glial cell elements in brain tissue. In normal cells, the process may be involved in the transport of endogenous substances with which the ellipticiniums share certain structural similarities. Of further significance is that both the preferential accumulation and the cytotoxicity of **10** in brain tumor cells can be competitively inhibited by certain compounds (e.g., reserpine) which also contain some structural resemblances to the ellipticiniums.[26] The elucidation of such an unprecedented mechanism of brain tumor selectivity would squarely address the key rationale[10,12] underlying the NCI *in vitro* screen, i.e., that histiospecific antitumor agents likely exist, and may be discoverable by a screen comprised of diverse tumor cell lines that retain biochemical and biological attributes unique to their particular normal tissue(s) or cell(s) of origin.

In vivo xenograft studies have been initiated with some of the ellipticiniums of the present study, with interesting preliminary results. For example, when **10** was tested *in vivo* against sensitive lines such as SF-268 and U-251, no *in vivo* antitumor activity was detected; however, it was also concurrently shown (NCI Developmental Therapeutics Program, unpublished data) that **10** is rapidly demethylated *in vivo* to form the 9-hydroxy compound (**15**), which is neither selectively accumulated[26] nor preferentially cytotoxic (Table 1) to brain tumor cell lines *in vitro*. On the basis of those preliminary studies, subsequent attention has been focused upon further *in vivo* investigation of selective ellipticiniums containing 9-substituents which are less prone to metabolic alterations. In one such study, the 9-chloro derivative (**12**) has shown particularly promising indications of *in vivo* brain tumor xenograft activity.[27] The detailed investigations, which are continuing on this and other potentially active compounds *in vivo*, will be reported elsewhere.

Experimental Section

Compounds were obtained from the NCI repository, and/or from various donors, or synthesized as described. The products were solids which were dried *in vacuo*. Retention of small amounts of organic solvents in some samples was evidenced in ^{1}H NMR spectra and verified in elemental analytical data: DMF or EtOAc in **4**, EtOAc in **5**, DMF in **20**. The quaternary salts were hygroscopic and were isolated as partial hydrates as evidenced by elemental analysis and ^{1}H NMR. Reverse-phase HPLC analyses were run on a Zorbax-C8 column with 0.01 M sodium toluenesulfonate and 0.075 M HOAc in CH_3OH–H_2O (9:1) as the mobile phase; detection was in the UV at 310 or 320 nm. NMR and UV data are provided in Tables 2 and 3, respectively.

2-Methylellipticinium iodide[14,17] (**7**), the corresponding **methanesulfonate** (**8**), and **2-(2-(diethylamino)ethyl)ellipticinium bromide hemihydrobromide**[17] (**13**) were gifts from Dr. Roger Westland, Warner-Lambert Co.

2-(4-Iodobenzyl)ellipticinium chloride (**14**) was a gift from Prof. Ned Heindel, Department of Chemistry, Lehigh University.

9-Hydroxyellipticine[18] (**3**), **9-hydroxy-2-methylellipticinium acetate**[18] (**15**, Celiptium), **9-hydroxy-2-(2-piperidinylethyl)ellipticinium acetate**[18] (**17**), and **2-methylolivacinium acetate**[16] (**21**) were gifts from Dr. Dat Xuong, Centre Nationale Recherche Scientifique (CNRS), Gif-sur-Yvette.

9-Hydroxy-2-(2-(diethylamino)ethyl)ellipticinium chloride hydrochloride[9] (**16**, Datelliptine) was a gift from M. Olivier Gros, Sanofi.

Table 3. Comparisons of UV-Vis Spectral Data[a]

compound		λ_{max} (log ϵ)
(ellipticine)		
9-Me	4	242 (4.40), 280 sh, 289 (4.86), 298 (4.74), 334 (3.76), 389 br (3.63), 406 br (3.60)
9-Cl	5	249 sh, 272 sh, 278 sh, 288 (4.43), 333 (3.48), 348 (3.25), 388 (3.27), 406 (3.25)
(quaternary)		
9-MeO	10[b]	247 (4.37), 278 (4.33) 307 (4.56), 363 br (3.65), 379 (3.70), 443 br (3.49)
9-Me	11	244 (4.39), 253 (4.33), 278 sh, 314 (4.81), 362 (3.75), 3.75 (3.67), 437 br (3.65)
9-Cl	12	245 sh, 252 (4.55), 278 sh, 311 (4.93), 357 (3.83), 375 (3.89), 428 br (3.76)
6-hexenyl	19	243 (4.33), 254 (4.36), 286 sh, 314 (4.79), 364 (3.71), 380 (3.71), 432 (3.70)
6-oxa	20	290 (4.94), 336 (3.84), 370 br (3.64)

[a] In EtOH solution (10 was also run in H_2O); sh = shoulder, br = broad. [b] The EtOH spectrum of iodide 9 was as reported in ref 9.

A sample of **9-chloroellipticine (5)** was originally synthesized (unpublished) and provided to NCI by Dr. D. W. Henry, SRI International. We resynthesized **5** as follows. 6-Chloro-1,4-dimethylcarbazole[28] was prepared (59% yield) from 5-chloroindole (Aldrich) and hexane-2,5-dione in the presence of *p*-toluenesulfonic acid and was converted to 6-chloro-1,4-dimethylcarbazole-3-carboxaldehyde (mp 256-260 °C) using the reaction sequence[3] for 9-bromoellipticine, by treatment with phosphoryl chloride and DMF (Vilsmeyer reaction) in 1,2-dichlorobenzene at 100 °C for 6 h. The crude product was reacted with aminoacetaldehyde diethyl acetal in the presence of potassium carbonate at 100 °C to give 3-(((2,2-diethoxyethyl)imino)methyl)-6-chloro-1,4-dimethylcarbaxole (55%) (mp 144-146 °C). Ring closure using 91% H_3PO_4 (aqueous) at 120 °C for 15 min afforded **5** (21% yield): mp 319-322 °C; TLC on silica gel, R_f = 0.36 in $CHCl_3$-MeOH (10:1); EIMS *m/e* calcd for $C_{17}H_{13}ClN_2$ 280.08, found 280.01 (relative intensity 100%), 265 (12, M - Me), 245 (12, M - Cl). Anal. ($C_{17}H_{13}ClN_2 \cdot 0.7H_2O$) C, H, Cl, N.

6-(5-Hexen-1-yl)ellipticine, and **9-methoxy-2,6-dimethylellipticinium iodide (18)** were submitted by Dr. D. W. Henry, SRI International. Synthesis of 9-methoxy-6-methylellipticine, the precursor to **18**, has been described.[3] Specificity of conditions for N-6 alkylation with NaH in DMF as distinguished from N-2 quaternization has been discussed.[17]

General Procedure for Quaternization. A solution of the ellipticine analog (1-10 g) in DMF (30 mL/g) at room temperature was treated with excess iodomethane (10-20 molar equiv) and stirred for 1-2 h, usually with the formation of a yellow or orange precipitate. Ethyl ether (100-500 mL/g of starting compound) was added with stirring to complete the precipitation, and the ellipticinium salt was collected on a filter, washed, and dried. In most cases, the iodides were converted directly to the acetates.

Anion Exchange from Iodide to Acetate. A stirred suspension of the ellipticinium iodide in water-DMF (15:1, 200 mL/g) was treated with moist ion-exchange resin (Bio-Rad AG1-X8, acetate, 30 mL/g) 100-200 mesh. The resulting mixture was stirred for 1-2 h and filtered through a pad of the resin. The filtrate and washings were evaporated *in vacuo*, the residual yellow or orange solid was dissolved in ethanol (10 mL/g of starting compound), ether or an ether-hexane mixture was added, and the reprecipitated product was washed with ether and dried *in vacuo*.

9-Methoxy-2-methylellipticinium Acetate[18] (10). The yield of iodide[14-16] (**9**; from **2** plus 3 MeI) was 91%, and the yield of **10** was 96% as an orange solid, 99.0% pure by HPLC. Anal. ($C_{21}H_{22}N_2O_3 \cdot 1.9H_2O$) C, H, N.

2,9-Dimethylellipticinium Acetate (11). 9-Methylellipticine (4) (mp 295-299 °C; lit.[3] 295-296 °C) was dissolved in DMF at 60 °C. The solution was cooled to room temperature, and methyl iodide was added. After 3 h, the precipitated yellow product was collected and suspended in a mixture of DMF, water, and ion-exchange resin (Bio-Rad AG1-X8, acetate, 30 mL/g) 100-200 mesh. After 2 h, the resin was removed by filtration, and the solvents were evaporated. Precipitation from ethanolic solution with ether afforded **11** (84%), homogeneous by HPLC: EIMS *m/e* 275 (100, M - OAc), 274 (15, M - HOAc) 260 (35, M - OAc - Me), 245 (14, M - OAc - 2Me). Anal. ($C_{21}H_{22}N_2O_2 \cdot 1.3H_2O$) C, H, N.

9-Chloro-2-methylellipticinium Acetate (12). The yellow iodide (93% yield from **5**) gave the yellow-orange **12** (99% yield), homogeneous by HPLC. Anal. ($C_{20}H_{19}ClN_2O_2 \cdot 0.6H_2O$) C, H, Cl, N.

2-Methyl-6-oxaellipticinium Acetate (20). Precipitation of this iodide (86% from 6-oxaellipticine[21]) did not begin until ether was added to the DMF solution. The acetate **20** (85%) was a pink-brown solid: TLC on silica gel, R_f 0.23 in MeOH-HOAc (25:4). EIMS *m/e* 262 (70, M^+ - OAc), 247 (22, M^+ - OAc - Me), 232 (10, M^+ - OAc - 2Me). A weak NMR singlet at δ 2.86 may have been from a small amount of DMF. Anal. ($C_{20}H_{19}NO_3 \cdot 2.3H_2O$) C, H, N.

6-(5-Hexen-1-yl)-2-methylellipticinium Acetate (19). 6-(5-Hexen-1-yl)ellipticine (above) was dissolved in DMF-CH_2Cl_2 (5:1, 30 mL/g) for the quaternization. After anion exchange, crude **19** was dissolved in CH_2Cl_2 (12 mL/g of starting ellipticine) and precipitated with ether to give an 88% yield of yellow solid, homogeneous by HPLC. Anal. ($C_{26}H_{30}N_2O_2 \cdot 1.7H_2O$) C, H, N.

Ellipticine *N*-oxide[19,20] (22) was a gift from Dr. J. R. Price, CSIRO, Australia.

Biological Testing and Data Display and Analysis. Compounds were subjected to the NCI *in vitro* screening panel assay as described elsewhere.[10,11,24] Data calculations employed the three experimental measurements: control optical densities (C), test optical densities (T), and optical densities at time zero (T_0). If $T \geq T_0$, then the calculation for percentage growth (PG) was $100 \times [(T - T_0)/(C - T_0)]$. If $T \leq T_0$, the PG calculation was $100 \times [(T - T_0)/T_0)]$. For each cell line, a five point dose-response curve was created, and the three response parameters, GI_{50}, TGI, and LC_{50}, were calculated for each cell line. The GI_{50} was calculated for each line where PG = $100 \times [(1 - T_0)/(C - T_0)]$ = 50; this value corresponds to the drug concentration causing a 50% decrease in net cell growth during the drug incubation. The drug concentration resulting in total growth inhibition, or TGI, is calculated from $T - T_0$; this corresponds to the drug concentration yielding an amount of cellular protein at the end of the incubation that is the same as at the beginning of the incubation (PG = 0). Finally, the LC_{50} corresponds to the drug concentration causing a net 50% reduction in the measured protein at the end of the incubation compared with that at the beginning (i.e., a net loss of cells). Having all parameters thus calculated for each compound for each cell line, the construction of the respective mean graphs (Table 1) and performance of COMPARE analyses were performed as described elsewhere[11,25] by computer. The definitions of "selectivity" and "modest selectivity", as used in the present text, are defined in the footnotes to Table 1.

Screening Data Summary. The negative $\log_{10}$ GI_{50}, TGI, and LC_{50} values, respectively, are listed as follows for compound **10** with the individual cell line identifiers; the tumor-type subpanel identifiers (I-VIII) correspond directly to those shown in Figure 1 (see legend). [I]: CCRF-CEM (5.22, 4.15, >4.0), HL-60 TB (5.28, 4.59, 4.28), K-562 (5.77, 4.11, >4.00), MOLT-4 (6.10, 4.26, >4.00), RPMI-8226 (4.96, 4.32, >4.00), SR (5.82, 4.24, >4.00). [II]: A549/ATCC (6.24, 4.35, >4.00), EKVX (5.85, 4.43, >4.00), HOP-18 (4.70, 4.07, >4.00), HOP-62 (6.29, 5.57, 4.36), HOP-92 (5.64, 4.77, >4.00), NCI-H226 (6.26, 5.60, 4.62), NCI-H23 (6.00, 5.38, 4.77), NCI-H322M (5.82, 4.60, >4.00), NCI-H460 (5.51, 4.38, >4.00), NCI-H522 (5.92, 5.42, 4.55), LXFL 529 (6.54, 5.52, 4.55). [III]: DMS 114 (6.22. 5.48, 4.28). DMS 273 (5.85, 4.82, 4.21). [IV]: COLO 205 (6.00, 5.24, 4.62), DLD-1 (5.92, 4.08, >4.00), HCC-2998 (5.55, 4.80, 4.11), HCT-116 (5.55, >4.00, >4.00), HCT-15 (4.15, >4.00, >4.00), HT29 (5.51, 4.55, 4.49), KM12 (5.74, 4.54, 4.01), KM20L2 (5.17, 4.24, >4.00), SW-620 (6.00, >4.00, >4.00). [V]: SF-268 (6.82, 6.13, 5.36), SF-295 (6.44, 5.70, 4.85), SF-539 (6.35, 5.77, 5.22); SNB-19 (6.52, 5.77, 4.06), SNB-75 (6.57,

5.96, 5.17), SNB-78 (6.36, 5.35, >4.00), U251 (6.89, 6.09, 5.00), XF498 (5.60, 4.70, >4.00). [VI]: LXOX IMVI (5.02, 4.14, >4.00), MALME-3M (5.82, 5.09, 4.10), M14 (5.00, 4.30, >4.00), M19-MEL (5.96, 5.35, 4.68), SK-MEL-2 (5.32, 4.74, 4.14), SK-MEL-28 (5.18, 4.72, 4.49), SK-MEL-5 (5.64, 4.96, >4.00), UACC-257 (5.00, 4.19, >4.00), UACC-62 (5.30, 4.48, >4.00). [VII]: IGROV1 (5.57, 4.09, >4.00), OVCAR-3 (5.49, 4.54, >4.00), OVCAR-4 (5.62, 4.82, 4.15), OVCAR-5 (4.80, 4.07, >4.00), OVCAR-8 (6.60, 4.70, >4.00), SK-OV-3 (5.01, 4.07, >4.00). [VIII]: 786-0 (6.80, 5.46, 4.59), A498 (5.28, >4.00, >4.00), ACHN (4.74, >4.00, >4.00), CAKI-1 (4.77, 4.07, >4.00), RXF-393 (6.06, 5.17, >4.00), RXF-631 (6.24, 4.57, >4.00), SN12C (6.68, 5.89, 4.85), TK-10 (5.17, 4.09, >4.00), UO-31 (4.82, 4.02, >4.00).

Comments Concerning Alphanumeric and Graphical Presentation of Data Derived from the NCI Screen. An alphanumeric report of NCI screening data (see above) suffices fully for the reconstruction and display of mean graphs in the original or any desired modified format, and for related immediate or future structure–activity calculations, such as Compare analyses.[11,25] Moreover, in a structure–activity study, as exemplified herein, it is necessary to enumerate data in this detail only for the selected "benchmark" compound (compound **10** in this instance). The quantitative (e.g., potency) and qualitative (e.g., tumor-type subpanel or other response profile specificity) comparisons among other members of the study set can be adequately summarized and reported otherwise, for example as shown in Table 1.

In Figure 1 we provide an example of a simplified "mean graph" display format prepared from the above data for compound **10**. While this may reinforce the reader's ready appreciation of the unusual brain tumor subpanel preference exhibited within this series of cytotoxic ellipticiniums, we nonetheless suggest that such graphical presentation of data derived from the NCI screen is generally not at all necessary. In most cases, the alphanumeric format is the most efficient and effective means of reporting or documenting (e.g., as supplementary material) NCI screening data. In the very rare instances where a highly novel tumor response profile is discovered for a new or known compound or series, an abbreviated or other concise graphical display, such as in Figure 1 may, in the initial report thereof, serve usefully in supplement to, or in lieu of, the alphanumeric reporting.

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