N-Methylcarbamate Derivatives of Ellipticine and Olivacine with Cytotoxic Activity against Four Human Lung Cancer Cell Lines

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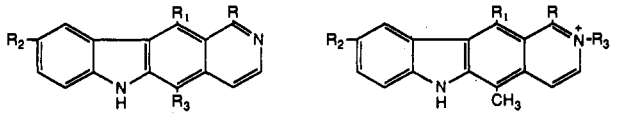
A series of analogues of the antitumor alkaloids ellipticine and olivacine were tested for cytotoxicity against four human lung cancer cell lines: H69, N417, H460, and H358. Adriamycin (doxorubicin), ellipticine, olivacine, and celiptinium were used as standards. Adriamycin was cytotoxic at 2 μM and celiptinium was inactive at the highest concentrations tested (IC_{50} > 48 μM). *N*-methylcarbamates of 9-methoxy-6*H*-pyrido[4,3-*b*]carbazole 1-, 5-, and 11-methanols gave IC_{50} values ranging from 0.02 to 0.11 μM against N417, H460, and H358 and were only slightly less effective against H69.

Introduction

Following the report of Dalton et al.[1] on the clinical activity of ellipticine (1) and related compounds, there has been a continued interest in the development of therapeutially useful compounds related to ellipticine and olivacine (3). Congeners of these alkaloids are cytotoxic in several in vivo and in vitro systems.[2] Earlier efforts were focused on analogues on 9-hydroxyellipticine (2).[2,3] This emphasis was based on the hypothesis that 2, which is a metabolite of 1, is then oxidized further to a highly reactive quinone imine which reacts covalently with DNA.[3] These studies led to the development of celiptinium (4),[4] dateliptinium (5),[5] and the 9-methoxy compound 6.[6] Archer and colleagues[7] proposed an alternate mechanism wherein it was suggested that ellipticine or its metabolic product 2 was enzymically hydroxylated at the 5-methyl group to give 7, followed by enzymic esterification to furnish either the phosphate or sulfate ester 8 which then alkylated DNA. Although hydroxylation at other methyl groups such as C-11 in ellipticine or C-1 in olivacine was not discussed, there is no apparent reason why metabolic hydroxylation at these positions cannot occur. The major difference between the Auclair–Paoletti mechanism and that of Archer et al. is that the latter group considered



1, R=R$_2$=H, R$_1$= R$_3$= CH$_3$, Ellipticine
2, R=H, R$_1$= R$_3$=CH$_3$, R$_2$=OH, 9-Hydroxyellipticine
2a, R=H, R$_1$=R$_3$=CH$_3$, R$_2$=OCH$_3$, 9=Methoxyellipticine
3, R=R$_3$=CH$_3$, R$_1$=R$_2$=H, Olivacine
4, R=H R$_1$=R$_3$=CH$_3$, R$_2$=OH
5, R$_1$= CH$_3$, R=R$_2$=H, R$_3$= CH$_2$CH$_2$N(C$_2$H$_5$)$_2$
6, R$_1$=CH$_3$, R$_2$=OCH$_3$ R$_3$=H, R =NH(CH$_2$)$_3$N(C$_2$H$_5$)$_2$
7, R=H, R$_1$=CH$_3$,R$_2$=OH, R$_3$=CH$_2$OH
8, R=H, R$_1$= CH$_3$, R$_2$=OH, R$_3$=CH$_2$OPO$_3$H$_2$ or CH$_2$OSO$_3$H

that the key oxidation occurred at the methyl groups, whereas Auclair and Paoletti[3] considered oxidation of the 9-hydroxyl group to be of prime importance.

Because of the possible chemical instability of esters such as 8, Archer et al.[7] prepared the *N*-methylcarbamate 9 as a surrogate for 8 for testing as an antitumor agent, a tactic that was used successfully in studies on the schistosomicide, hycanthone.[8] The in vivo antitumor activity vs murine P-388 lymphocytic leukemia of 9 was superior to that of ellipticine 1 and the alcohol 7.[7]

9-Methoxyellipticine 2a along with ellipticine was isolated from the stems of *Ochrosia acuminata*[9] and in vitro callus cultures derived from the stems of *Ochrosia elliptica*.[10,11] In in vitro assays, 2a appears to be equally

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(1) Dalton, L. K.; Demerac, S.; Elmes, B. C.; Swan, J. M.; Teitel, T. Synthesis of the Tumor-inhibitory Alkaloids, Ellipticine, 9-Methoxyellipticine and Related Pyrido[4,3-b]carbazoles. *Aust. J. Chem.* **1967**, *20*, 2715–2727.

(2) LePecq, J.-B.; Gosse, C.; Dat-Xuong, N.; Paoletti, C. Two New Antitumor Derivatives. 1-Hydroxy-9-methyl-2-ellipticinium Acetate and 9-Dimethyl-2,6-ellipticinium (chloride). Action on Lizio Leukemia of Mice. *C. R. Acad. Sci. Paris* **1975**, *281*, 1365–1367.

(3) Auclair, C.; Paoletti, C. Bioactivation of the Antitumor Drug 9-Hydroxyellipticine and Derivatives by a Peroxidase–Hydrogen Peroxide System. *J. Med. Chem.* **1981**, *24*, 289–295.

(4) Juret, P.; Tanguy, A.; Girard, A.; Letalaeu, J. Y.; Arbatucci, J. S.; Dat-Xuong, N.; LePecq, J.-B.; Paoletti, C. Preliminary Trial of 9-Hydroxy-2-methylellipticinium (NSC 264137) in Advanced Human Cancers. *Eur. J. Cancer* **1978**, *14*, 205–206.

(5) Kuayat, D.; Borel, C. H.; Merle, S.; Vu, C.; Myle, J. H.; Creton, R.; Oudart, S.; Bouloux, C.; Weil, M.; Piedbois, P.; Auclerc, G.; Thill, L.; Soubane, Cl.; Jacquillat, Cl. Phase I Study of a New Ellipticine Derivative SR 951568 (Dateliptinium) Using a 24 Hour Continuous Infusion. *Proc. AACR* **1989**, *30*, 996.

(6) Atassi, G.; Dumont, P.; Pepin, O.; Gros, O.; Gros, P. SR95325B, A New Ellipticine Derivative Highly Active Against Established Murine Solid Tumors. *Proc. AACR* **1989**, *30*, 2458.

(7) Archer, S.; Ross, B. S.; Pica-Mattoccia, L.; Cioli, D. Synthesis and Biological Properties of Some 6H-Pyrido[4,3-*b*]carbazoles. *J. Med. Chem.* **1987**, *30*, 1204–1210. Evidence, which tended to favor our mechanism over the Auclair–Paoletti mechanism, was the ineffectiveness of 9-hydroxy-6*H*-pyrido[4,3-*b*]carbazole as an antitumor agent in the P388 system. Chemically, this compound behaves like 9-hydroxyellipticine. Since it is devoid of both the 5- and 11-methyl groups, our mechanism would predict such as result while the Auclair–Paolitti mechanism would tend to suggest that it should be active. That blooxidation of the 5-methyl group in ellipticine in metabolically possible was shown by the transformation of ellipticine to 5-formylellipticine by *Choisya ternata*. Kouadio, K.; Rideau, M., Ganser, C.; Chenieux, J. C.; Viel, C. Biotransformation of Ellipticine to 5-Formylellipticine by *Choisya ternata*. Plant Cell Rep. **1984**, *3*, 203–205.

(8) Archer, S.; Pica-Mattoccia, L.; Cioli, D.; Seyed-Mozaffari, A.; Zayed, A.-H. Preparation and Antischistosomal and Antitumor Activity of Hycanthone and Some of its Congeners. Evidence for the Mode of Action of Hycanthone. *J. Med. Chem.* **1988**, *31*, 254–260.

(9) Lin, Y. M.; Juichi, M.; Wu, R. Y.; Lee, K. H. Antitumor Agents. LXIX. Alkaloids of Ochrosia Acuminata. *Planta Med.* **1985**, 545–6.

(10) Weber, J. F.; Garnier, S.; Trinn, T. H.; Viel, C. Formation of Antitumoral Pyridocarbazole Alkaloids by In Vitro Cultures of Ochrosia Elliptica Labill. *Plant Med. Phytother.* **1987**, *21*, 99–105.

(11) Kouadio, K.; Creche, J.; Chenieux, J. C.; Rideau, M.; Viel, C. Alkaloid Production of Ochrosia Elliptica Cell Suspension Cultures. *J. Plant Physiol.* **1985**, *118*, 277–284.

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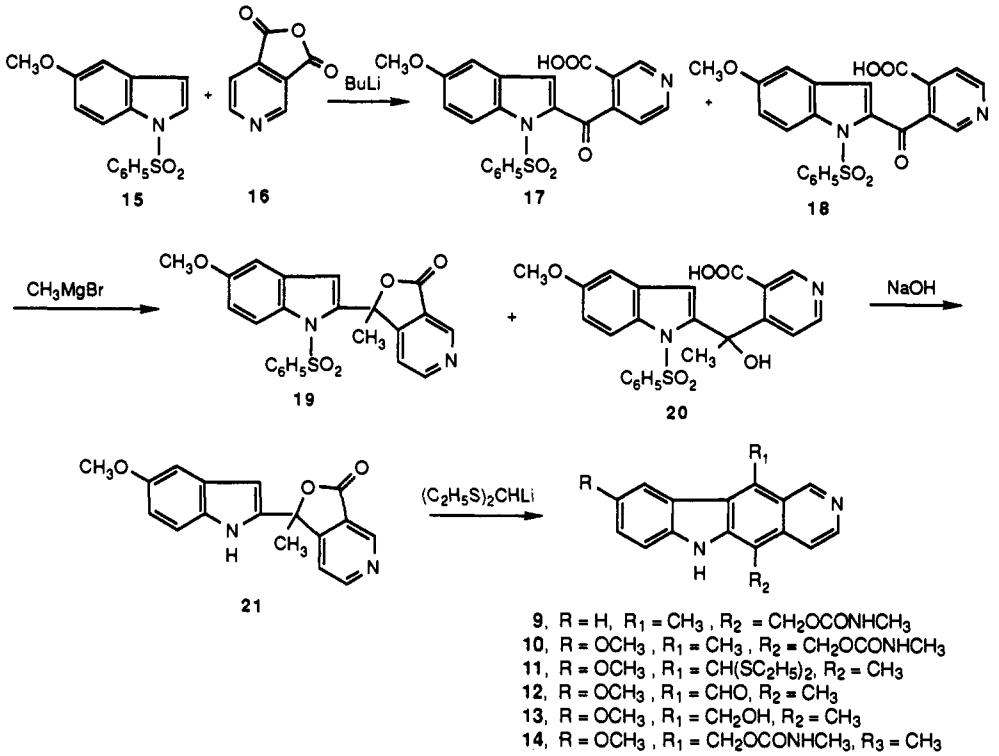
Scheme I



9, R = H, R_1 = CH_3, R_2 = $CH_2OCONHCH_3$
10, R = OCH_3, R_1 = CH_3, R_2 = $CH_2OCONHCH_3$
11, R = OCH_3, R_1 = $CH(SC_2H_5)_2$, R_2 = CH_3
12, R = OCH_3, R_1 = CHO, R_2 = CH_3
13, R = OCH_3, R_1 = CH_2OH, R_2 = CH_3
14, R = OCH_3, R_1 = $CH_2OCONHCH_3$, R_3 = CH_3

cytotoxic as ellipticine itself.[12] Thus it seemed reasonable to prepare and test 9-methoxy-6*H*-pyrido[4,3-*b*]carbazole derivatives such as **10**, **14**, and **26**.

Prediction of in vivo drug sensitivity has been the goal of several investigators. A bioassay developed by Salmon et al.[13] has been a standard method for in vitro drug selection since 1978. More recently, Weisenthal et al.[14] and Ruckdeschel and co-workers[15] developed in vitro bioassays which appear to overcome some of the problems associated with the Salmon assay.

In this paper we report the synthesis of 9-methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-11-methanol *N*-methylcarbamate (**14**) and in vitro bioassays of cytotoxicity of ten 6*H*-pyrido[4,3-*b*]carbazoles in four human lung cancer cell lines.[15] With the exception of **14**, the syntheses of the other 6*H*-pyrido[4,3-*b*]carbazoles were reported elsewhere.[7,16-18]

Chemistry

A general synthesis of 5-methyl-6*H*-pyrido[4,3-*b*]carbazole-11-methanols was reported earlier,[17,18] but the preparation of **14** was not described previously. Its synthesis is outlined in Scheme I.

N-(Phenylsulfonyl)-5-methoxyindole (**15**) was converted to its lithio derivative with the aid of BuLi and then condensed with pyridine-3,4-dicarboxylic anhydride (**16**) to give a mixture of isomers **17** and **18** in which the former largely predominated. Treatment of **17**, which was obtained pure by fractional crystallization, with methylmagnesium bromide, afforded a mixture of the lactone **19** and the acid **20**. Hydrolysis of **19** with NaOH followed by acidification with HCl gave the lactone **21**. Better yields of **21** were obtained by using the crude magnesium salt of **20**. The lithio derivative of formaldehyde diethyl mercaptal was condensed with **21** and the unisolated intermediate was reduced with $NaBH_4$ to give **11**. Oxidation with bis(trifluoroacetoxy)iodosobenzene as described previously[17,18] furnished the aldehyde **12** which, on reduction with $NaBH_4$, afforded **13**. Reaction of the latter with methyl isocyanate gave the target compound **14**.

Biological Results

The cytotoxicities of adriamycin (Doxorubicin), ellipticine (**1**), olivacine (**3**), celiptinium (**4**), and the newer 6*H*-pyrido[4,3-*b*]carbazoles against four human lung cancer cell lines are reported in Table I. Celiptinium is active in vivo against L1210 and P388 in mice[2] and was reported to be clinically active in a limited number of patients in a Phase I trial.[19] In the bioassays reported here, the drug was inactive against all four cell lines at the highest concentration tested (IC_{50} > 48 μM). A phase II study in 43 patients with non-small cell lung cancer was disap-

(12) Meunier, G.; de Montauzon, J.; Bernardou, J.; Grassy, G.; Bonnafous, M.; Cros, S.; Meunier, B. The Biooxidation of Cytotoxic Ellipticine Derivatives: A Key to Structure-Activity Relationship Studies? *Mol. Pharmacol.* **1988**, *33*, 93-102.

(13) Salmon, S. E.; Hamburger, L. W.; Soehnlein, B.; Quantitation of Differential Sensitivity of Human-Tumor Stem Cells to Anticancer Drugs. *N. Engl. J. Med.* **1978**, *298*, 1321-1327.

(14) Weisenthal, L. M.; Lippman, M. E. Clonogenic and Nonclonogenic In Vitro Chemosensitivity Assays. *Cancer Treat. Rep.* **1985**, *69*, 615-632.

(15) Ruckdeschel, J. C.; Carney, D. N.; Ole, H. K.; Russell, E. K.; Gazdar, A. F. In Vitro Chemosensitivity of Human Lung Cancer Cell Lines. *Cancer Treat. Rep.* **1987**, *71*, 697-704.

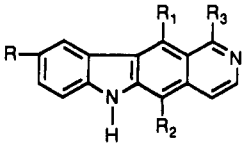
(16) Modi, S. P.; McComb, T.; Zayed, A.-H.; Oglesby, R. C.; Archer, S. Synthesis of 6H-Pyrido[4,3-b]carbazoles. *Tetrahedron* **1990**, *46*, 5555-5562.

(17) Modi, S. P.; Carey, J. J.; Archer, S. Synthesis of 5-Methyl-6H-Pyrido[4,3-*b*]carbazole-11-methanol. *Tetrahedron Lett.* **1990**, *41*, 5845-5848.

(18) Modi, S. P.; Michael, M. A.; Archer, S.; Carey, J. J. An Efficient Synthesis of C-11 Substituted 6H-Pyrido[4,3-*b*]carbazoles. *Tetrahedron* **1990**, *47*, 6539-6548.

(19) Einzig, A. I.; Gralla, R. J.; Leyland-Jones, B. R.; Kelson, D. P.; Cibas, I.; Lewis, E.; Greenberg, E. Phase I Study of Elliptinium (2-*N*-Methyl-9-hydroxyellipticinium). *Cancer Invest.* **1985**, *3*, 235-241.

Table I. IC_{50} Values for 6*H*-Pyrido[4,3-*b*]carbazoles in Four Human Lung Cancer Cells Lines



compound	R	R_1	R_2	R_3	IC_{50} (μM) H 69	N 417	H 460	H 358
adriamycin					0.87	0.23	0.23	0.62
1, ellipticine	H	CH_3	CH_3	H	1.80	0.87	0.51	0.20
2, 9-OH-ellipticine	OH	CH_3	CH_3	H	1.70	3.50	2.80	5.80
4, celiptinium					>48.0	>48.0	>48.0	>48.0
7[a]	H	CH_3	CH_2OH	H	1.60	1.80	0.95	1.50
9[a]	H	CH_3	$CH_2OCONHCH_3$	H	0.86	0.33	0.17	0.19
10[b]	CH_3O	CH_3	$CH_2OCONHCH_3$	H	0.22	0.09	0.06	0.10
14	CH_3O	$CH_2OCONHCH_3$	CH_3	H	0.17	0.04	0.02	0.09
3, olivacine	H	H	CH_3	CH_3	1.70	3.40	1.80	2.30
22[b]	CH_3O	H	CH_3	CH_3	1.20	1.80	2.10	2.10
23[b]	CH_3O	H	CH_3	CHO	1.80	0.90	1.80	1.50
24[b]	CH_3O	H	CH_3	CH_2OH	1.30	1.30	1.90	1.30
25[b]	CH_3O	H	CH_3	CH_2OAc	1.40	2.80	1.70	2.00
26[b]	CH_3O	H	CH_3	$CH_2OCONHCH_3$	0.14	0.10	0.07	0.11

[a] Reference 7. [b] Reference 16.

pointing. Celiptinium showed only one partial response,[20] a result that could have been expected on the basis of the present results.

9-Hydroxyellipticine (2), which LePecq et al.[2] reported to be more effective than ellipticine in L1210 murine lymphocytic leukemia, was less active than 1 in three of the lung cancer cell lines and equiactive in a fourth. It was reported[7] that 7 was slightly more active than 1 in P388-infected mice and that the carbamate 9 was more active than either. Roughly the same order activity was observed in the four lung cancer cell lines (Table I).

Since the 9-methoxy analogue 10 of the carbamate 9 was more cytotoxic than 9, only the 9-methoxy derivatives of *N*-methylcarbamate esters of the 6*H*-pyrido[4,3-*b*]-carbazole C-1 and C-11 methanols 26 and 14 were prepared. The trio, 10, 14, and 26, were the most cytotoxic members of the series. Against N417, H460, and H358, the IC_{50} values were in the 0.02–0.11 μM range and these compounds were only slightly less effective against H69.

Experimental Section

Adriamycin (doxorubicin) and ellipticine were obtained from commercial sources. Celiptinium was a gift from Dr. Bernard Meunier. Compounds to be tested for cytotoxicity were dissolved in DMSO and diluted with 10 volumes of bovine serum albumin to give stock solutions which were then diluted to proper concentrations with a phosphate buffer.

Melting points were determined on a Mel-Temp open capillary melting point apparatus and are uncorrected. ^1H NMR spectra were obtained in the solvents indicated on a Varian XL-200 (200 MHz) spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as the internal standard. Infrared spectra were recorded on a Perkin-Elmer Model 298 or a Perkin-Elmer 1800 Fourier transform infrared spectrophotometer. Mass spectra were obtained on a Hewlett-Packard HP 5987 GC/MS spectrometer using isobutane or methane as the CI gas. Elemental analyses were performed by Atlantic Microlab, Inc. Despite careful drying, some of the compounds retained water tenaciously. Proton signals attributed to water were observed in the NMR spectra.

Unless specified otherwise, for lithiation reactions, a three-neck round-bottom flask equipped with a magnetic stirring bar, an inlet adapter for nitrogen or argon, and a rubber septum were used. All reactions were carried out in an atmosphere of nitrogen or argon in flame-dried glassware. THF was distilled over sodium benzophenone ketyl before use. Methyl isocyanate and diisopropylamine were distilled before use. Low temperature reactions were carried out in liquid nitrogen or dry ice–methanol cooling baths.

3-Carboxy-4-pyridyl 1′-(Phenylsulfonyl)-5′-methoxy-2′-indolyl Ketone and 4-Carboxy-3-pyridyl 1′-(Phenylsulfonyl)-5′-methoxy-2′-indolyl Ketone (17 and 18). A 2-L flask equipped with a mechanical stirrer was cooled to −70 °C and charged with a solution of 15.5 mL (11.2 g) of diisopropylamine in 100 mL of THF. Then 44.5 mL of a 2.5 M solution of BuLi in hexane was placed in the dropping funnel, and the solution was added dropwise over a period of 15 min. After stirring for 30 min a solution of 31.7 g of *N*-(phenylsulfonyl)-5-methoxyindole in 80 mL of THF was added dropwise over a period of 20 min. The resulting clear yellow solution was stirred for 30 min during which time a yellow precipitate appeared. The reaction mixture was allowed to warm to −10 °C and stirred at that temperature for 30 min. The contents of the flask were cooled to −100 °C, and a solution of 16.5 g of pyridine-3,4-dicarboxylic anhydride in 100 mL of THF was added dropwise. After the addition was complete, the cooling bath was removed and the reaction mixture was allowed to stir overnight.

The resulting red-brown solution was concentrated to dryness, and the residue was treated with 1 L of cold H_2O. The aqueous solution was acidified to pH 2.0 with 10% HCl, stirred for 15 min, and filtered. The filter cake was washed thoroughly with H_2O and dried to give 41 g of a mixture of the isomeric keto acids which were readily separated by fractional crystallization from ethyl acetate. The less soluble isomer was the major product 17: mp 232–234 °C; IR (KBr) 3450, 1674, 1536, 1449, 1356, 1220, 1172, 1089, 1032, 726, 684 cm^{-1}; NMR (DMSO-d_6) δ 9.10 (1 H, s), 8.91 (1 H, d, J = 5.0), 8.15–8.08 (3 H, m), 7.74–7.67 (4 H, m), 7.55 (1 H, d, J = 5.0), 7.24–7.15 (3 H, m), 3.77 (3 H, s, OCH_3). Anal. ($C_{22}H_{16}N_2O_6S$) C, H, N.

The more soluble isomer melted at 246–247 °C, NMR (DMSO-d_6) δ 8.92 (1 H, d, J = 5.0), 8.80 (1 H, s), 8.09–8.02 (3 H, m), 7.79–7.60 (4 H, m), 7.19–7.18 (3 H, m), 3.73 (3 H, s, OCH_3). Anal. ($C_{22}H_{16}N_2O_6S$) C, H, N.

1-(3-Carboxy-4-pyridyl)-1-[1′-(phenylsulfonyl)-5′-methoxy-2′-indolyl]ethanol (20) and Its Lactone 19. A stirred solution of 2.05 g of the keto acid 17 in 80 mL of THF was cooled to 0 °C and treated with 8.1 mL of a 3.0 M solution of CH_3MgBr in ether. After being heated under reflux for 24 h, the mixture was cooled in ice and acidified with diluted H_2SO_4 to pH 1.0. The

(20) Anderson, G.; Clavel, M.; Smyth, J.; Giacgone, G.; Gracia, M.; Planting, A. S.; Dalesio, O.; Kirkpatrick, A.; McVie, G. Phase II Study of 9-Hydroxy-2-methyl Ellipticinium Acetate (Ellipticinium) in Patients with Advanced Carcinoma of the Lung, EORTC Lung Cancer Cooperative Group. *Eur. J. Cancer Clin. Oncol.* **1989**, *25*, 909–910.

solution was stirred at room temperature for 2 h and then concentrated in vacuo. The residue was extracted with $CHCl_3$, and the extract was washed with water, dried, and evaporated to dryness. The residual oil was chromatographed on silica gel using a gradient of ethyl acetate–hexane (20–60%). The lactone **19** was obtained as a foam which crystallized from MeOH: wt 750 mg (37%); mp 203–205 °C; IR (KBr) 1775, 1608, 1475, 1364, 1206, 1183, 1089, 1038, 1015, 927, 809 cm^{-1}; NMR ($CDCl_3$) δ 9.23 (1 H, s), 8.86 (1 H, d, J = 5.0), 8.16 (1 H, d, J = 9.2), 7.83 (1 H, d, J = 7.4), 7.48–7.25 (5 H, m), 7.01 (1 H, dd, J = 2.6, J = 9.2), 6.89 (1 H, d, J = 2.2), 6.79 (1 H, s), 3.81 (3 H, s, OCH_3), 2.79 (3 H, s, CH_3); MS m/e = 435 (M + 1). Anal. ($C_{23}H_{20}N_2O_6S$) C, H, N.

Further elution of the column with ethyl acetate–methanol (10%) gave the hydroxy acid **20**: mp 175–178 °C; IR (KBr) 3088 (br), 1702, 1599, 1478, 1448, 1369, 1210, 1179, 1094, 109, 755, 727, 687 cm^{-1}; NMR (DMSO-d_6) δ 8.76 (1 H, s), 8.32 (1 H, d, J = 5.2), 7.93–7.89 (3 H, m), 7.59–7.43 (3 H, m), 7.12 (1 H, d, J = 2.4), 6.93–6.84 (3 H, m), 3.76 (3 H, s, OCH_3); MS m/e 435 (base peak, M + 1), 452 (parent peak).

1-(3-Carboxy-4-pyridyl)-1-(5′-methoxy-2′-indolyl)ethanol Lactone (21). A suspension of the crude magnesium salt of the hydroxy acid **20** and 0.7 g of NaOH pellets in 75% ethanol was heated for 10 h. The resulting solution was concentrated, acidified to pH 2.0 with 20% HCl, and extracted with $CHCl_3$–CH_3OH (10%). The dried extract was evaporated and the residual foam was chromatographed on a silica gel column using an elution gradient of hexane–ethyl acetate 10–60%. The lactone **21** crystallized after trituration with ether: wt 210 mg (35%); mp 193–196 °C; IR (KBr) 3827, 1765, 1605, 1493, 1457, 1422, 1366, 1275, 1227, 1206, 1139, 1026, 914, 851, 793, 725 cm^{-1}; MS m/e = 295 (M + 1); NMR ($CDCl_3$) δ 11.11 (1 H, s), 9.17 (1 H, s), 8.31 (1 H, d, J = 5.2), 7.74 (1 H, d, J = 5.0), 7.17 (1 H, d, J = 8.0), 7.00 (1 H, d, J = 1.8), 6.74 (1 H, dd, J = 2.2, J = 9.0), 6.42 (1 H, d, J = 1.6), 3.71 (3 H, s, OCH_3), 3.33 (s, H_2O), 2.10 (3 H, s, CH_3). Anal. ($C_{17}H_{14}N_2O_3 \cdot 0.2H_2O$) C, H, N.

9-Methoxy-5-methyl-6*H*-Pyrido[4,3-*b*]carbazole-11-carboxaldehyde Diethyl Mercaptal (11). A solution of 14 mL of 2.5 M n-BuLi in hexane was added to a hexane solution of 460 mg of formaldehyde diethyl mercaptal at −55 °C. The solution was stirred for 1 h while the temperature was allowed to rise to −40 °C. The lactone **21** (130 mg) was added in one portion. The colorless solution turned orange-red and after 3 h became yellow. Three milliliters of water was added, and the solvent were removed in vacuo. The residue was dissolved in 50 mL of EtOH, and 106 mg of $NaBH_4$ was added. The mixture was heated under reflux for 5 h, cooled, and concentrated to small volume. Water was added, and the yellow insoluble solid was collected on a filter, washed with 2 × 2 mL of cold water, and dried, wt 70 mg (40%) of almost pure **11**. Chromatography of 30 mg of this material on a silica gel column using EtOAc–hexane as the eluant gave 28 mg of pure **11**: mp 222–224 °C; IR (KBr) 2925, 1602, 1482, 1370, 1217, 1146, 1043, 803 cm^{-1}; NMR ($CDCl_3$) δ 11.40 (1 H, s, NH), 10.23 (1 H, s, H_1), 8.40 (1 H, d, J = 6.4 H_3), 7.94 (1 H, d, J = 8.8 H_7), 7.79 (1 H, d, J = 2.4 H_{10}), 7.55 (1 H, d, J = 6.8 H_4), 7.27 (1 H, dd, J = 2.0, J = 8.9 H_8), 6.57 (1 H, s, $(EtS)_2CH$) , 3.90 (3 H, s, OCH_3), 3.34 (s, H_2O), 2.79 (3 H, s, CH_3), 2.75 (4 H, m, $(SCH_2CH_3)_2$), 1.55 (6 H, t, $(SCH_2CH_3)_2$); MS m/e 397 (M + 1). Anal. ($C_{22}H_{24}N_2OS_2 \cdot 0.3H_2O$) C, H, N.

9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-11-carboxaldehyde (12). To a solution of 185 mg of the mercaptal **11** in 80 mL of CH_3CN–H_2O (9:1) was added 60 mg of bis(trifluoroacetoxy)iodosobenzene in one portion. After 1 h at room temperature 50 mL of a saturated $NaHCO_3$ solution was added followed by CH_3CN–MeOH (9:1). The layers were separated and the organic layer was concentrated to dryness. Chromatography of the residue on a silica gel column using EtOAc–CH_3OH (95:5) as the eluant furnished 57 mg (42%) of pure orangered material, mp 290–291 °C, and 20 mg (18%) of darker material suitable for use in the next step: IR (KBr) 3432, 1673, 1599, 1485, 1384, 1224, 1035 cm^{-1}; NMR (DMSO-d_6) δ 1160 (1 H, s, NH), 11.48 (1 H, s, H_1), 10.35 (1 H, s, CHO), 8.52 (1 H, d, J = 6.0 H_3), 8.10 (1 H, d, J = 2.4 H_{10}), 8.03 (1 H, d, J = 6.0 H_4), 7.53 (1 H, d, J = 9.0 H_7), 7.29 (1 H, dd, J = 2.4, J = 8.8, H_8), 3.89 (3 H, s, OCH_3), 3.34 (s, H_2O), 2.86 (3 H, s, CH_3). Anal. ($C_{18}H_{14}N_2O_2 \cdot 0.5H_2O$) C, H, N.

9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-11-methanol (13). To an orange solution of 41 mg of the aldehyde **12** was added 110 mg of $NaBH_4$. The suspension turned to a yellow solution with a few minutes. After stirring at room temperature for 5 h, 40 mL of $CHCl_3$ was added and the solution was washed with H_2O and brine and dried. On concentration, a yellow crystalline solid separated, wt 25.6 mg (62%), mp 262–265 °C. Evaporation of the filtrate gave an additional 15 mg (36%) suitable for use in the next step: IR (KBr) 3428, 1660, 1484, 1401, 1296, 1220, 1147, 1036, 805 cm^{-1}; NMR (DMSO-d_6) δ 11.26 (1 H, s, NH), 9.77 (1 H, s, H_1), 8.40 (1 H, d, J = 5.8 H_3), 7.94 (1 H, d, J = 6.0 H_4), 7.89 (1 H, d, J = 2.0 H_{10}), 7.49 (1 H, d, J = 8.6 H_7), 7.19 (1 H, dd, J = 2.0, J = 8.7 H_8), 5.56 (3 H, s, CH_2OH, OH exchangeable with D_2O), 3.89 (3 H, s, OCH_3), 3.34 (s, H_2O), 2.80 (3 H, s, CH_3). Anal. ($C_{18}H_{16}N_2O_2 \cdot 0.25H_2O$) C, H, N.

9-Methoxy-5-methyl-6*H*-pyrido[4,3-*b*]carbazole-11-methanol *N*-Methylcarbamate (14). A mixture of 25 mg of **13**, 0.5 mL of methyl isocyanate, 0.2 mL of pyridine, and 20 mL of dry $CHCl_3$ (hydrocarbon stabilized) was stirred at room temperature for 12 h. The clear solution was taken to dryness and the residue chromatographed on a silica gel column using ethyl acetate as the developing solvent. The pure carbamate melted at 242–243 °C: wt 25 mg (84%); IR (KBr) 3300, 1685, 1480, 1260, 1140, 1130 cm^{-1}; NMR (DMSO-d_6) δ 11.72 (1 H, s, NH), 9.30 (1 H, s, H_1), 8.40 (1 H, d, J = 6.0 H_3), 7.90 (1 H, d, J = 5.8, H_4), 7.85 (1 H, d, J = 2.0 H_{10}), 7.20 (1 H, d, J = 8.8 H_7), 7.10 (1 H, dd, J = 2.0, J = 8.7 H_8), 6.20 (2 H, s, CH_2), 3.83 (3 H, s, OCH_3), 3.25 (s, H_2O), 2.80 (3 H, s, CH_3), 2.60 (3 H, s, NCH_3). Anal. ($C_{20}H_{14}N_3O_3 \cdot 0.5H_2O$) C, H, N.

Cell Lines. All cell lines for cytotoxicity studies were graciously provided by Dr. A. Gazdar of the NCI–Navy Medical Oncology Branch and had been studied in earlier assays of drug sensitivity.[15] H69 is a classic small cell lung cancer (SCLC) cell line and N417 is a "variant" SCLC line. H460 and H358 are non-SCLC cell lines with varying degrees of sensitivity to standard chemotherapeutic agents.[15] Cells were maintained in a 5% CO_2–95% air atmosphere at 37 °C and 85–100% humidity in RPMI 1640 medium (Gibco Lab., Grand Island, NY). Mycoplasma contamination was periodically evaluated by the New York State Department of Health Laboratories (Dr. Jerry Koratowski).

Cytotoxicity Assay. Adherent cell lines (H460, H358) were trypsinized and resuspended in RPMI-1640/2% FBS prior to counting. Floating cultures (H69, N417) were disaggregated by gentle pipetting and collected by sedimentation. Cells were plated onto half-well microliter plates (Corning) in concentrations previously determined to be optimal for assessing growth inhibition; 2500 cells/well for non-SCLC and 8000 cells/well for SCLC cell lines. Twenty microliters of the appropriate drug solution or control were added to each well and the plates were incubated for 1 h, centrifuged, washed with RPMI 1640 twice, and kept at 37 °C for 4 days. On the fourth day 50 μL of an aqueous solution of 5-(dimethylthiazol-2-yl)-2,3-diphenyltetrazolium bromide (2 mg/mL) (Sigma Chemical Co., St. Louis, MO) was added and the plates were incubated for an additional 4 h. The plates were centrifuged and after removal of the supernatant, the purple dye remaining was suspended in DMSO and the plates were shaken for 5 min. Absorbance at 540 nm was determined using a Biotek EL310 Microplate Autoreader. Appropriate concentrations of each drug was tested to permit the calculation of IC_{50} values, i.e., the concentration at which 50% inhibition of cell growth occurred. IC_{50} values were calculated using the Crick-Graph program.

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