Synthesis and Evaluation of a Series of Benzylaniline Hydrochlorides as Potential Cytotoxic and Antimitotic Agents Acting by Inhibition of Tubulin Polymerization

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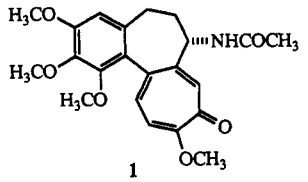
Received May 13, 1993•

Although certain substituted *cis*-stilbenes have displayed potent tubulin polymerization inhibitory activity and significant cytotoxicities in cancer cell cultures, these compounds have limited aqueous solubility and are therefore difficult to formulate for *in vivo* evaluation. A series of water-soluble *N*-(3,4,5-trimethoxybenzyl)aniline salts has therefore been synthesized in which the olefinic bridge of the stilbenes is replaced by an aminomethylene hydrochloride moiety. A relationship was found between the size of the substituent in the 4-position of the aniline ring and both antitubulin activity and cytotoxicity, such that the smaller the substituent, the greater the potency. The most promising of the newly synthesized compounds was 4-methyl-*N*-(3,4,5-trimethoxybenzyl)aniline hydrochloride, with an IC_{50} value of 3.5 μM for inhibition of tubulin polymerization and cytotoxicity for a wide variety of cancer cell lines. The cytotoxicities of the benzylaniline hydrochlorides correlated remarkably well with their antitubulin activities.

The design of inhibitors of tubulin polymerization is an appealing strategy in the search for potential anticancer agents. A variety of compounds, including colchicine (1),[1-6]



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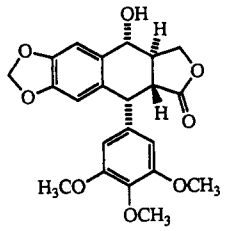

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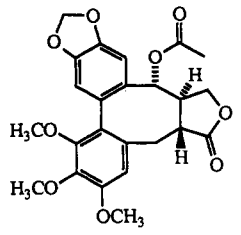

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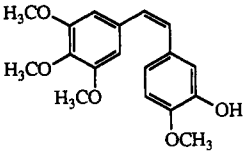

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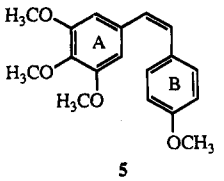

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podophyllotoxin (2),[7-11] steganacin (3),[8,12,13] and their synthetic analogues, inhibit tubulin polymerization by binding to the colchicine binding site. Numerous biphenyls,[14] diphenylmethanes,[15] benzopyrans,[16] and chalcones[17] display similar biological activities. The combretastatins are a family of recently discovered plant metabolites which also bind to the colchicine binding site of tubulin,[18] and the most potent representative has proven to be combretastatin A-4 (4), which has also shown activity against multidrug-resistant cancer cells.[19]

We recently studied a series of cytotoxic stilbenes related to the combretastatins. The most active of these as an inhibitor of tubulin polymerization was stilbene **5**, which displayed an IC_{50} value of 2.0 μM and was cytotoxic against an array of cancer cell cultures.[20,21] However, despite the significant *in vitro* cytotoxicities and antitubulin activities shown by these stilbenes in cancer cell cultures, a significant problem in their *in vivo* evaluation as anticancer agents has been their low aqueous solubilities. Thus, a variety of stilbenes were synthesized having acidic or basic groups appended to various locations of the stilbene structure.[20,21] Although the salts of these substances had satisfactory solubilities in water, their biological activities were generally disappointing. The exception was benzylaniline **9c** (Table I), which retained significant cytotoxicity in cancer cell cultures.[20]

The objective of the present investigation has been to utilize the knowledge gained in our prior studies of stilbenes and dihydrostilbenes to design and synthesize additional benzylanilines and their water-soluble salts in an attempt to obtain cytotoxic tubulin polymerization inhibitors that could be formulated for further testing. As described below, this has resulted in some remarkably simple and inexpensive compounds having potential therapeutic value in the treatment of cancer.

Chemistry. Our previous studies with stilbenes related to **5** had shown that both the number and location of the methoxyl groups on ring A were important for biological activity. The removal, replacement, or relocation of any of these substituents resulted in a substantial decrease in cytotoxicity and tubulin polymerization inhibitory activity.[20,21] The methoxy group on ring B was less critical. Accordingly, in the present study of benzylanilines, the 3,4,5-trimethoxy substitution pattern on the benzyl moiety was not altered, while effects of replacement of the methoxyl group in the aniline region were examined. Heating 3,4,5-trimethoxybenzaldehyde (**6**) with *p*-substituted anilines **7a-g** in refluxing toluene provided the Schiff

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• Abstract published in *Advance ACS Abstracts*, August 15, 1993.

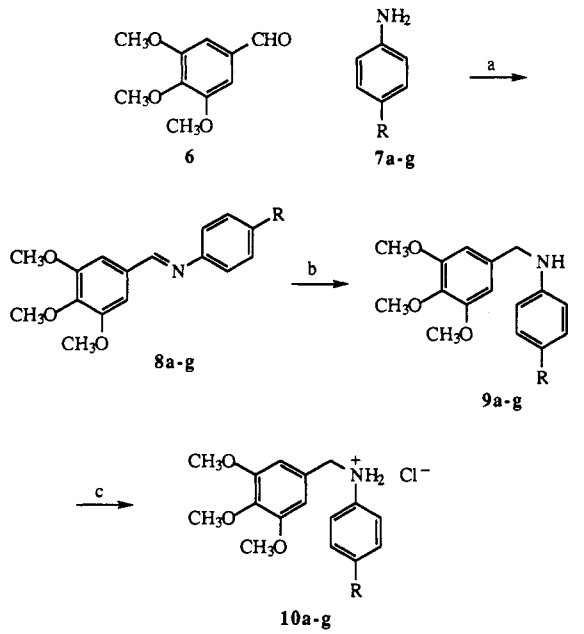
0022-2623/93/1836-2817$04.00/0

Table I. Cytotoxicites and Antitubulin Activities of Schiff Bases, Benzylanilines, and Benzylaniline Hydrochlorides

no.	cytotoxicity (GI_{50} in μM)[a] HL-60 (TB)	NCI-H522	DMS 273	COLO 205	SF-295	M14	OVCAR-3	CAKI-1	inhibn of tubulin polymn IC_{50} (μM((±SD)
8b	21.6	>100	>100	49.1	>100	>100	>100	>100	>40
8c	26.8	>100	33.0	28.6	52.7	83.8	>100	>100	>40
8d	>100	>100	>100	75.9	>100	>100	>100	>100	>40
8f	>100	>100	52.5	>100	>100	>100	>100	>100	>40
8g	22.6	>100	>100	>100	>100	>100	>100	>100	>40
9a	0.122	0.195	0.0926	0.152	0.163	0.150	0.184	0.192	6.0(±0.6)
9b	0.168	0.234	0.135	0.344	0.266	0.315	0.283	0.354	3.0(±0.4)
9c	0.115	0.412	0.141	0.235	0.266		0.363	0.458	4.6(±0.6)
9d	0.296	0.380	0.296	0.432	0.274	0.403	0.340	2.04	12(±1.0)
9e	3.70	4.68	3.09	3.88	7.28	5.26	5.93	32.0	>40
10a	0.722	0.262	0.104	0.219	0.233	0.292	0.214	0.162	3.5(±0.05)
10b	0.245	0.371	0.818	0.352	0.339	0.437	0.405		7.2(±0.4)
10c	0.834	2.76	0.534	1.66	2.32	2.38	1.96	2.50	8.9(±0.5)
10d	0.448	0.574	0.961	0.538	0.418	0.532	0.464	1.05	11(±0.6)
10e	3.29	2.95	4.91	3.60	3.41	4.84	4.85	16.3	16(±2)
10f	14.4	11.1	12.9	17.9	20.9	16.9	16.3	16.9	>40
10g	14.1	17.3	16.2	19.0	10.5	22.4	19.0		>40

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition, and they are the averages of two determinations.

Scheme I[a]



a R = CH_3
b R = C_2H_5
c R = OCH_3
d R = OC_2H_5
e R = SCH_3
f R = $CH(CH_3)_2$
g R = $CH_2CH_2CH_3$

[a] (a) Toluene, reflux (3 h); (b) $NaBH_4$, ethanol, reflux (2 h); (c) HCl, ether, 0–5 °C (30 min).

bases **8a–g**. Reduction of the imines **8a-g** with sodium borohydride in ethanol at reflux gave amines **9a-g**, which were converted to the corresponding hydrochloride salts **10a–g** with HCl gas in ether. All of the hydrochloride salts **10a–g** were isolated as stable, crystalline solids (Scheme I).

Biological Results and Discussion. The effects on cell growth and tubulin polymerization of five Schiff bases **8**, five amines **9**, and seven hydrochlorides **10** are summarized in Table I. These compounds were examined for cytotoxicity in the human cancer cell lines HL-60 (TB) leukemia, NCI-H522 non-small-cell lung cancer, DMS 273 small-cell lung cancer, COLO 205 colon cancer, SF-295 CNS cancer, M14 melanoma, OVCAR-3 ovarian cancer, and CAKI-1 renal cancer. Inhibition of tubulin polymerization was examined using electrophoretically homogeneous tubulin from bovine brain.

The five Schiff bases examined, **8b–d, f,** and **8g**, were all inactive as inhibitors of tubulin polymerization (IC_{50} > 40 μM), and this lack of activity was reflected in their very low cytotoxicities. The lack of activity of these imines probably derived from their trans (*E*) geometry.[22-26] Prior studies in the stilbene series have shown that a cis (*Z*) geometry about the double bond is required for activity.[20,21]

With the amines **9a–e** and the corresponding hydrochloride salts **10a–g**, potency as a tubulin polymerization inhibitor inversely correlated with the size of the R substituent in the C-4 position of the aniline ring. The smaller the substituent, the higher the potency. Among the highly soluble hydrochloride salts, 4-methyl-*N*-(3,4,5-trimethoxybenzyl)aniline hydrochloride (**10a**) was the most potent of the compounds studied (IC_{50} 3.5 μM), and the corresponding 4-isopropyl and 4-*n*-propyl analogues (**10f** and **10g**) were inactive (IC_{50} > 40 μM) as tubulin inhibitors. Intermediate values were observed for the 4-ethyl (**10b**, IC_{50} = 7.2 μM), 4-methoxy (**10c**, 8.9 μM), 4-ethoxy (**10d**, 11.0 μM), and 4-thiomethyl (**10e**, 16.0 μM) analogues. This trend between the potencies of the compounds as tubulin polymerization inhibitors and the size of the aniline substituent R was reflected remarkably well by the cytotoxicities in all of the cancer cell cultures studied. The smaller the substituent, the higher the cytotoxicity. These relationships generally held for the corresponding free bases **9a–e**, except that the compound with the ethyl substituent (**9b**) was more effective (IC_{50} = 3.0 μM) against tubulin polymerization than the analog with the methyl substituent (**9a**, IC_{50} = 6.0 μM).[27]

A more extensive analysis of the cytotoxicities of the most potent benzylaniline hydrochloride **10a** is detailed in Table II. A total of 55 cell lines from the leukemia, non-small-cell lung cancer, small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, and renal cancer panels were examined. As can be seen from the results in Table II, the cytotoxicity was broad in scope, although **10a** was clearly most cytotoxic (log GI_{50} < −7.00) in the HL-60 (TB) leukemia, K-562 leukemia, DMS 273 small-cell lung cancer, M14 melanoma, ACHN renal cancer, and CAKI-1 renal cancer cell cultures. It therefore appears that benzylaniline hydrochloride salt **10a** is an uncommonly simple, water-soluble tubulin polymerization

Table II. Cytotoxicities of 4-Methyl-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (**10a**)

panel/cell line	log GI_{50}	panel/cell line	log GI_{50}
	Leukemia		
CCRF-CEM	-6.55	MOLT-4	-6.37
HL-60 (TB)	-7.05	RPMI-8226	-6.36
K-562	-7.00		
	Non-Small-Cell Lung Cancer		
A549/ATCC	-6.29	NCI-H23	-6.39
EKCX	-6.13	NCI-H322M	-6.30
HOP-18	-4.43	NCI-H460	-6.67
HOP-62	-6.55	NCI-H522	-6.69
HOP-92	-4.73	LXFL 529	-6.20
	Small-Cell Lung Cancer		
DMS114	-6.37	DMS 273	-7.03
	Colon Cancer		
COLO 205	-6.67	HT29	-6.46
DLD-1	-6.24	KM12	-6.12
HCT-116	-6.24	KM20L2	-6.60
HCT-15	-6.61	SW-620	-6.96
	CNS Cancer		
SF-268	-6.04	SNB-75	-6.41
SF-295	-6.49	SNB-78	-5.96
SF-539	-6.50	U251	-6.48
SNB-19	-6.53	XF 498	-6.33
	Melanoma		
LOX IMVI	-6.07	SK-MEL-2	-6.88
MALME-3M	-6.33	SK-MEL-5	-6.04
M14	<-8.00	UACC-257	>-4.00
M19-MEL	-6.48	UACC-62	-6.24
	Ovarian Cancer		
IGROV1	-6.15	OVCAR-5	-5.87
OVCAR-3	-6.71	OVCAR-8	-6.12
OVCAR-4	-4.67	SK-OV-3	-6.57
	Renal Cancer		
786-0	-6.49	RXF-393	-6.95
A498	>-4.00	SN12C	-6.07
ACHN	-7.01	TK-10	>-4.00
CAKI-1	-7.88	UO-31	-6.08

inhibitor which is suitable for further *in vivo* examination in a variety of animal cancer models.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp apparatus and are uncorrected. Spectra were obtained as follows: CI mass spectra on a Finnegan 4000 spectrometer; FAB mass spectra and EI mass spectra on a Kratos MS50 spectrometer; ^{1}H NMR spectra on a Varian VXR-500S spectrometer with TMS as the internal standard in $CDCl_3$. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions.

4-Methyl-*N*-(3,4,5-trimethoxybenzylidene)aniline (8a). A mixture of compound **6** (6.0 g, 98%, 31.0 mmol) and **7a** (3.21 g, 30 mmol) in ethanol (150 mL) was heated at reflux under argon for 3 h. After evaporation of the solvent, the residual white solid was recrystallized from ethyl acetate and hexane to give **8a** (7.05 g, 82.4%) as white crystals: mp 74–6 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 7.20 (d, J = 8 Hz, 2 H), 7.16 (s, 2 H), 7.15 (d, J = 8 Hz, 2 H), 3.95 (s, 6 H), 3.92 (s, 3 H), 2.36 (s, 3 H); EIMS m/e 285 (M^+, 100).

4-Ethyl-*N*-(3,4,5-trimethoxybenzylidene)aniline (8b). A solution of **6** (6.0 g, 98%, 30 mmol) and **7b** (3.63 g, 30 mmol) in ethanol (150 mL) was heated at reflux under argon for 3 h. The solvent was evaporated and the residual oil was subjected to flash chromatography (silica gel, 230–400 mesh, ether/hexane, 4:6 by volume) to give **8b** (8.1 g, 90.3%) as an oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 7.23 (d, J = 8 Hz, 2 H), 7.16 (s, 2 H), 7.15 (d, J = 8 Hz, 2 H), 3.94 (s, 6 H), 3.91 (s, 3 H), 2.67 (q, J = 8 Hz, 2 H), 1.26 (t, J = 8 Hz, 3 H); EIMS m/e 299 (M^+, 100).

4-Methoxy-*N*-(3,4,5-trimethoxybenzylidene)aniline (8c). A mixture of 3,4,5-trimethoxybenzaldehyde (**6**) (19.6 g, 100 mmol) and 4-methoxyaniline (**7c**) (12.3 g, 100 mmol) in ethanol (100 mL) was heated at reflux under argon for 3 h. About half of the solvent was evaporated and the residual solution was filtered through a glass-wool pad. The filtrate was left at room temperature overnight to give the crystalline **8c** (28.2 g, 93.7%): mp 78–86 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 7.23 (d, J = 8 Hz, 2 H), 7.15 (s, 2 H), 6.93 (d, J = 8 Hz, 2 H), 3.94 (s, 6 H), 3.91 (s, 3 H), 3.83 (s, 3 H); CIMS (isobutane) m/e 302 (MH^+, 100). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

4-Ethoxy-*N*-(3,4,5-trimethoxybenzylidene)aniline (8d). From 3,4,5-trimethoxybenzaldehyde (**6**) (6.0 g, 30 mmol) and 4-ethoxyaniline (**7d**) (4.1 g, 30 mmol), a similar procedure as that described for **8a** gave **8d** (5.9 g, 97.4%) as yellow crystals: mp 75–7 °C after recrystallization from ethanol; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 7.21 (d, J = 8 Hz, 2 H), 7.14 (s, 2 H), 6.91 (d, J = 8 Hz, 2 H), 4.05 (q, J = 6 Hz, 2 H), 3.94 (s, 6 H), 3.91 (s, 3 H), 1.43 (t, J = 6 Hz, 3 H); EIMS m/e 315 (M^+, 93).

4-(Methylthio)-*N*-(3,4,5-trimethoxybenzylidene)aniline (8e). From compounds **7e** (5.0 g, 98%, 35.2 mmol) and **6** (7.04 g, 98%, 35.2 mmol), a similar procedure as that described for **8a** gave **8e** (11.0 g, 98.2%) as yellow solid. The analytical sample was obtained by preparative TLC (ether/hexane, 1:2 by volume, precoated silica TLC plate, 1000 μm): mp 86–88 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ 8.54 (s, 1 H), 7.31 (d, J = 8 Hz, 2H), 7.23 (d, J = 8 Hz, 2 H), 7.26 (s, 2 H), 3.85 (s, 3 H), 3.84 (s, 3 H), 3.73 (s, 3 H), 2.49 (s, 3 H); EIMS m/e 317 (M^+, 100).

4-Isopropyl-*N*-(3,4,5-trimethoxybenzylidene)aniline (8f). From compounds **7f** (4.2 g, 99%, 31.0 mmol) and **6** (6.0 g, 98%, 31.0 mmol), a similar procedure as that described for **8a** gave **8f** (8.2 g, 84.5%) as a yellow solid: mp 68–70; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.37 (s, 1 H), 7.25 (d, J = 8 Hz, 2 H), 7.16 (d, J = 8 Hz, 2 H), 7.16 (s, 2 H), 3.94 (s, 6 H), 3.91 (s, 3 H), 2.93 (sextet, J = 8 Hz, 1 H), 1.27 (d, J = 8 Hz, 6 H); EIMS m/e 313 (M^+, 100).

4-*n*-Propyl-*N*-(3,4,5-trimethoxybenzylidene)aniline (8g). From compounds **7g** (4.2 g, 99%, 31.0 mmol) and **6** (6.0 g, 98%, 31.0 mmol), a similar procedure as that described for **8a** gave **8g** (8.5 g, 87.6%) as a thick oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.36 (s, 1 H), 7.20 (d, J = 8 Hz, 2 H), 7.16 (s, 2 H), 7.14 (d, J = 8 Hz, 2 H), 3.93 (s, 6 H), 3.91 (s, 3 H), 2.60 (t, J = 8 Hz, 2 H), 1.64 (sextet, J = 8 Hz, 2 H), 0.95 (t, J = 8 Hz, 3 H); EIMS m/e (M^+, 100).

4-Methyl-*N*-(3,4,5-trimethoxybenzyl)aniline (9a). To a solution of **8a** (6.0 g, 21.1 mmol) in ethanol (100 mL) was added $NaBH_4$ (4.06 g, 98%, 105 mmol) in portions. The reaction mixture was stirred at reflux under argon for 2 h. The solvent was removed under reduced pressure. Saturated aqueous NaCl (30 mL) was added to the residue and the mixture extracted with ether (100, 40, and 40 mL). The combined ether layer was washed with saturated NaCl solution (30 mL) and dried over anhydrous Na_2SO_4. Evaporation of the filtrate gave **9a** (5.6 g, 92.7%) as white crystals: mp 94–6 °C after recrystallization from ethanol; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.00 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 6.58 (d, J = 8 Hz, 2 H), 4.23 (s, 2 H), 3.84 (s, 9 H), 2.24 (s, 3 H); CIMS (isobutane) m/e 288 (MH^+, 76).

4-Ethyl-*N*-(3,4,5-trimethoxybenzyl)aniline (9b). From **8b** (7.0 g, 23.4 mmol) and $NaBH_4$ (4.4 g, 117 mmol), a similar procedure as that described for **9a** gave **9b** (5.4 g, 76.6%) as white crystals: mp 64–6 °C after recrystallization from ethanol; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.03 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 6.62 (d, J = 8 Hz, 2 H), 4.24 (s, 2 H), 3.84 (s, 9 H), 2.55 (q, J = 8 Hz, 2 H), 1.19 (t, J = 8 Hz, 3 H); EIMS m/e 301 (M^+, 34).

4-Methoxy-*N*-(3,4,5-trimethoxybenzyl)aniline (9c). From **8c** (10.8 g, 35.8 mmol) and $NaBH_4$ (6.8 g, 179 mmol), a similar procedure as that described for **9a** gave **9c** (10.1 g, 93.5%) as pale purple crystals: ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.79 (d, J = 8 Hz, 2 H), 6.62 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 4.21 (s, 2 H), 3.84 (s, 9 H), 3.74 (s, 3 H); CIMS (isobutane) m/e 304 (MH^+, 20).

4-Ethoxy-*N*-(3,4,5-trimethoxybenzyl)aniline (9d). From the imine **8d** (5.6 g, 17.8 mmol) and $NaBH_4$ (3.4 g, 88 mmol), a similar procedure as that described for **9a** gave **9d** (4.6 g, 81.9%) as white crystals: mp 76–8 °C after recrystallization from ethanol; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.78 (d, J = 8 Hz, 2 H), 6.62 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 4.21 (s, 2 H), 3.96 (q, J = 6 Hz, 2 H), 3.84 (s, 9 H), 1.37 (t, J = 6 Hz, 3 H); CIMS (isobutane) m/e 318 (MH^+, 27).

4-(Methylthio)-*N*-(3,4,5-trimethoxybenzyl)aniline (9e). From **8e** (11.0 g, 35.0 mmol) and $NaBH_4$ (6.6 g, 175 mmol), a similar procedure as that described for **9a** gave **9e** (10.6 g, 94.9%) as an oil: ^1H NMR (200 MHz, DMSO-d_6) δ 7.07 (d, J = 8 Hz, 2 H), 6.67 (s, 2 H), 6.57 (d, J = 8 Hz, 2 H), 6.23 (t, J = 6 Hz, 1 H), 4.10 (d, J = 6 Hz, 2 H), 3.73 (s, 6 H), 3.62 (s, 3 H), 2.31 (s, 3 H); EIMS m/e 319 (M^+, 69).

4-Isopropyl-*N*-(3,4,5-trimethoxybenzyl)aniline (9f). From **8f** (8.2 g, 26.0 mmol), a similar procedure as that described for **9a** gave **9f** (7.4 g, 89.8%) as an oil: ^1H NMR (200 MHz, $CDCl_3$) δ 7.06 (d, J = 8 Hz, 2 H), 6.61 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 4.24 (s, 2 H), 3.84 (s, 9 H), 2.81 (h, J = 8 Hz, 1 H), 1.21 (d, J = 8 Hz, 6 H); EIMS 315 (M^+, 100).

4-*n*-Propyl-*N*-(3,4,5-trimethoxybenzyl)aniline (9g). From **8g** (8.5 g, 26.9 mmol), a similar procedure as that described for **9a** gave **9g** (6.0 g, 70.3%) as an oil: ^1H NMR (200 MHz, $CDCl_3$) δ 7.00 (d, J = 8 Hz, 2 H), 6.61 (s, 2 H), 6.60 (d, J = 8 Hz, 2 H), 4.23 (s, 2 H), 3.84 (s, 9 H), 2.84 (t, J = 8 Hz, 2 H), 1.60 (sextet, J = 8 Hz, 2 H) 0.92 (t, J = 8 Hz, 3 H); EIMS m/e 315 (M^+, 93).

4-Methyl-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10a). A solution of **9a** (3.9 g, 13.6 mmol) in ether (150 mL) was treated with HCl gas at 0–5 °C with stirring for about 0.5 h. Collection of the resulting pale purple crystals and recrystallization from ethanol and methanol gave **10a** (3.6 g, 81.8%) as tiny white crystals: mp 162–4 °C after recrystallization from ethanol; ^1H NMR (200 MHz, $CDCl_3$) δ 7.21 (d, J = 8 Hz, 2 H), 7.09 (d, J = 8 Hz, 2 H), 6.63 (s, 2 H), 4.26 (s, 2 H), 3.77 (s, 6 H), 3.76 (s, 3 H), 2.30 (s, 3 H). Anal. ($C_{17}H_{22}ClNO_3$) C, H, N.

4-Ethyl-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10b). From **9b** (4.0 g, 13.2 mmol), a similar procedure as that described for **10a** gave **10b** (3.17 g, 70.7%), as yellow crystals: mp 155–7 °C after recrystallization from ethanol and methanol; ^1H NMR (200 MHz, $CDCl_3$) δ 7.20 (s, br, 4 H), 6.86 (s, 2 H), 4.34 (s, 2 H), 3.72 (s, 6 H), 3.62 (s, 3 H), 2.55 (q, J = 8 Hz, 2 H), 1.13 (t, J = 8 Hz, 3 H). Anal. ($C_{18}H_{24}ClNO_3$).

4-Methoxy-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10c). From **9c** (9.0 g, 29.7 mmol), a similar procedure as that described for **10a** gave **10c** (8.14 g, 80.7%), as tiny white crystals: mp 182–4 °C; ^1H NMR (200 MHz, DMSO-d_6) δ 7.36 (d, J = 8 Hz, 2 H), 6.98 (d, J = 8 Hz, 2 H), 6.62 (s, 2 H), 4.36 (s, 2 H), 3.74 (s, 9 H), 3.63 (s, 3 H). Anal. ($C_{17}H_{22}ClNO_4 \cdot {}^1/_2H_2O$) C, H, N.

4-Ethoxy-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10d). From **9d** (4.0 g, 12.6 mmol), a similar procedure as that described for **10a** gave **10d** (3.4 g, 76.2%), as white crystals: mp 170–2 °C after recrystallization from ethanol and methanol; ^1H NMR (200 MHz, $CDCl_3$) δ 7.22 (d, J = 8 Hz, 2 H), 6.57 (d, J = 8 Hz, 2 H), 6.64 (s, 2 H), 4.25 (s, br, 2 H), 3.95 (q, J = 6 Hz, 2 H), 3.79 (s, 6 H), 3.77 (s, 3 H), 1.38 (t, J = 6 Hz, 3 H). Anal. ($C_{18}H_{24}ClNO_4$) C, H, N.

4-(Methylthio)-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10e). From **9e** (3.0 g, 9.4 mmol), a similar procedure as that described for **10a** gave **10e** (1.80 g, 53.9%), as yellow crystals: mp 194–6 °C after recrystallization from ethanol/methanol/water: ^1H NMR (200 MHz, DMSO-d_6) δ 7.23 (d, J = 8 Hz, 2 H), 7.07 (d, J = 8 Hz, 2 H), 6.83 (s, 2 H), 4.30 (s, 2 H), 3.73 (s, 6 H), 3.62 (s, 3 H), 2.40 (s, 3 H). Anal. ($C_{17}H_{22}ClNO_3S$) C, H, N.

4-Isopropyl-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10f). From **9f** (6.0 g, 18.9 mmol), a similar procedure as that described for **10a** gave **10f** (4.4 g, 65.9%), as yellow crystals: mp 160–2 °C after recrystallization from methanol/ethanol/water: ^1H NMR (200 MHz, $CDCl_3$) δ 7.35 (d, J = 8 Hz, 2 H), 7.29 (d, J = 8 Hz, 2 H), 6.92 (s, 2 H), 4.37 (s, 2 H), 3.73 (s, 6 H), 3.63 (s, 3 H), 2.88 (h, J = 6 Hz, 1 H), 1.17 (d, J = 6 Hz, 6 H). Anal. ($C_{19}H_{26}ClNO_3$) C, H, N.

4-*n*-Propyl-*N*-(3,4,5-trimethoxybenzyl)aniline Hydrochloride (10g). From **9g** (6.0 g, 19.0 mmol), a similar procedure as described for **10a** gave **10g** (5.5 g, 82.5%), as yellow crystals: mp 118–20 °C after recrystallization from ethyl acetate/methanol/hexane: ^1H NMR (200 MHz, $CDCl_3$) δ 7.21 (d, J = 8 Hz, 2 H), 7.08 (d, J = 8 Hz, 2 H), 6.58 (s, 2 H), 4.29 (s, 2 H), 3.78 (s, 3 H), 3.74 (s, 6 H), 3.52 (t, J = 8 Hz, 2 H), 1.56 (sextet, J = 8 Hz, 2 H), 0.86 (t, J = 8 Hz, 3 H). Anal. ($C_{19}H_{26}ClNO_3$) C, H, N.

Tubulin Polymerization Inhibition Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[28] Determination of IC_{50} values for the polymerization of purified tubulin was performed as described in detail elsewhere.[6] In brief, tubulin was preincubated at 37 °C with varying compound concentrations, reaction mixtures were chilled on ice, GTP (required for the polymerization reaction) was added, and polymerization was followed at 37 °C by turbidimetry at 350 nm in Gilford recording spectrophotometers equipped with electronic temperature controllers. Four instruments were used, and two control reaction mixtures were present in each experiment. The extent of polymerization after a 20-min incubation was determined (the values for the two controls were usually within 5% of each other). IC_{50} values were determined graphically. Active compounds were examined in at least three independent assays, while inactive compounds (defined as IC_{50} value >40 μM) were examined in at least two independent experiments.

Acknowledgment. This research was made possible by contract NO1-CM-17512, awarded by the National Cancer Institute, DHHS.

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(27) The tubulin polymerization assay used in the studies presented here employs 1 M monosodium glutamate and GTP to induce the assembly reaction. Although the reaction conditions are identical in the current studies to those described earlier (see refs 6, 20, 21) a different glutamate preparation was used. This modification has caused a reduction in all IC_{50} values obtained with antimitotic compounds. The reason for the change is presently not known. Several standard agents were evaluated for comparison with the new compounds described here. The following IC_{50} values were obtained: colchicine (**1**), 1.9 ± 0.2 μM; podophyllotoxin (**2**), 1.3 ± 0.06 μM; combretastatin A-4 (**4**), 1.0 ± 0.06 μM; and compound **5**, 1.2 ± 0.05 μM.
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