dimethylformamide (1 mL) was treated with 50% sodium hydride in oil (20 mg) for 20 min, and then with ethyl iodide (0.02 mL) for 20 min longer. The mixture was concentrated to dryness at 0.1 mm, taken up in ethyl acetate, washed with sodium bicarbonate solution, dried (Mg_2SO_4), concentrated to dryness, and triturated with ether to afford the ester as an off-white solid, mp 110–118 °C (30 mg, 31%). Similarly, **3s** was prepared from **3a** through the use of *tert*-butyl 4-(2-aminoethyl)phenylacetate.[28]

Acknowledgment. We wish to thank Karl Gunderson for NMR interpretation, Lia Raabis for NMR spectra, Natalie Cahoon and Michael Hatolski for IR spectra and rotations, and Magda Brzechffa for mass spectra. Statistical analyses were performed by Nancy Hall.

Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents That Inhibit Tubulin Polymerization

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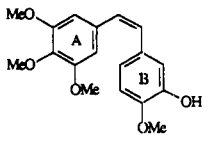
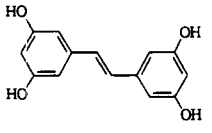
An array of *cis*-, *trans*-, and dihydrostilbenes and some *N*-arylbenzylamines were synthesized and evaluated for their cytotoxicity in the five cancer cell cultures A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. Several *cis*-stilbenes, structurally similar to combretastatins, were highly cytotoxic in all five cell lines and these were also found to be active as inhibitors of tubulin polymerization. The most active compounds also inhibited the binding of colchicine to tubulin. The most potent of the new compounds, both as a tubulin polymerization inhibitor and as a cytotoxic agent, was (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (**5a**). This substance was almost as potent as combretastatin A-4 (**1a**), the most active of the combretastatins, as a tubulin polymerization inhibitor. Compound **5a** was found to be approximately 140 times more cytotoxic against HT-29 colon adenocarcinoma cells and about 10 times more cytotoxic against MCF-7 breast carcinoma cells than combretastatin A-4. However, **5a** was found to be about 20 times less cytotoxic against A-549 lung carcinoma cells, 30 times less cytotoxic against SKMEL-5 melanoma cells, and 7 times less cytotoxic against MLM melanoma cells than combretastatin A-4. The relative potencies **5a** > **8a** > **6a** for the cis, dihydro, and trans compounds, respectively, as inhibitors of tubulin polymerization are in agreement with the relative potencies previously observed for combretastatin A-4 (**1a**), dihydrocombretastatin A-4 (**1c**), and *trans*-combretastatin A-4 (**1b**). The relative potencies **5a** > **8a** > **6a** were also reflected in the results of the cytotoxicity assays. Structure–activity relationships of this group of compounds are also discussed.

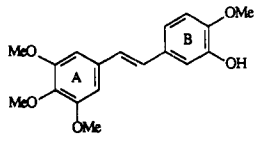
Interest in the synthesis and evaluation of polymethoxylated stilbenes and dihydrostilbenes as potential anticancer agents stems from the discovery of many such natural products as antimitotic and antileukemic agents.[1-12] This includes isolation of many stilbene derivatives, termed combretastatins, from the South African tree *Combretum caffrum*. Many of these combretastatins were found to be cytotoxic, with combretastatin A-4 (**1a**) the most potent.[7]



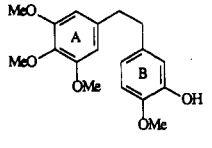
Combretastatin A-4 (1a)



Piceatannol (2)



trans-Combretastatin A-4 (1b)



Dihydrocombretastatin A-4 (1c)

This compound was found to cause mitotic arrest in L1210 murine leukemia cells, inhibit tubulin polymerization, and competitively inhibit the binding of radiolabeled colchicine to tubulin.[7] It is presently being investigated under the sponsorship of the Cancer Research Campaign Clinical Trails scheme.[13] A recent investigation of combretastatins revealed that combretastatin A-4 (**1a**) was active against multidrug resistant (MDR) cancer cell lines.[13] Combretastatin A-4 (**1a**) as well as its trans isomer **1b**, its dihydro derivative **1c**, and a number of related substances have been found to cause mitotic arrest in cells in culture at cytotoxic concentrations.[1,2,4-9,13-17] Several other *trans*-stilbenes and 1,4-diarylalkanes were also reported to be

† Purdue.
‡ NIH.

(1) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.
(2) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374.
(3) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119.
(4) Pettit, G. R.; Singh, S. B.; Cragg, G. M. *J. Org. Chem.* **1985**, *50*, 3404.
(5) Pettit, G. R.; Cragg, G. M.; Singh, S. B. *J. Nat. Prod.* **1987**, *50*, 386.
(6) Pettit, G. R.; Singh, S. B. *Can. J. Chem.* **1987**, *65*, 2390.
(7) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200.
(8) Pettit, G. R.; Singh, S. B.; Schmidt, J. M.; Nivin, M. L.; Hamel, E.; Lin, C. M. *J. Nat. Prod.* **1988**, *51*, 517.
(9) Itoh, Y.; Brossi, A.; Hamel, E.; Lin, C. M. *Helv. Chem. Acta* **1988**, *71*, 1199.
(10) Bai, R.; Pettit, G. R.; Hamel, E. *Biochem. Pharmacol.* **1990**, *39*, 1941.
(11) Ferrigni, N. R.; McLaughlin, J. L.; Powell, R. G.; Smith, C. R., Jr. *J. Nat. Prod.* **1984**, *47*, 347.
(12) Gill, M. T.; Bajaj, R.; Chang, C. J.; Nichols, D. E.; McLaughlin, J. L. *J. Nat. Prod.* **1987**, *50*, 36.
(13) McGown, A. T.; Fox, B. W. *Cancer Chemother. Pharmacol.* **1990**, *26*, 79.
(14) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984.
(15) Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, *32*, 3864.
(16) McGown, A. T.; Fox, B. W. *Anti-Cancer Drug Design* **1989**, *3*, 249.
(17) Jiang, J. B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. J.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 1721.

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cytotoxic agents.[7,18,19] Apart from these reports, many natural products possessing a trimethoxybenzene ring, e.g., colchicine, podophyllotoxin, and steganacin, were found to be potent cytotoxic agents and exert their antitumor property by their antitubulin character.[1,2,4-9,13-17] Therefore, there has been considerable interest in the development of new cytotoxic and/or antitubulin agents based on these products as structural leads.[7,9,20-22] Research in this direction has led to the discovery of several new anticancer agents and has provided insight into their structure–activity relationships.[7,9,20-23]

Our interest in stilbenes began with piceatannol (**2**), a cytotoxic inhibitor of protein-tyrosine kinases,[11,12,24] and the design and synthesis of superior inhibitors of such enzymes.[25,26] The present paper describes the synthesis of an array of methoxylated stilbenes and related compounds as potential cytotoxic agents and their evaluation for the inhibition of tubulin polymerization and inhibition of binding of radiolabeled colchicine to tubulin.

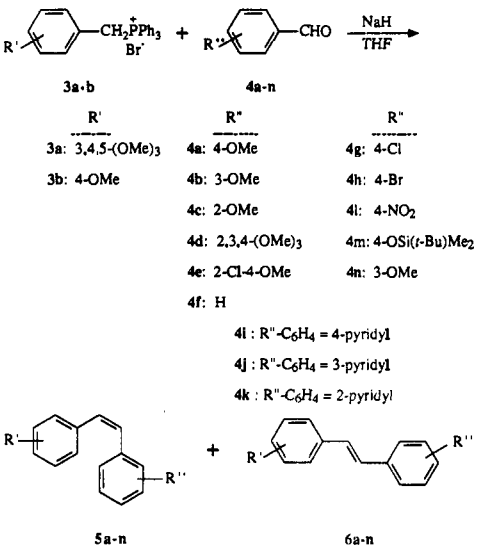
Our initial syntheses and evaluation of some *cis*- and *trans*-stilbenes as cytoxic agents showed that *cis*-stilbenes were much superior as cytotoxic agents when compared to the simultaneously obtained *trans*-stilbenes. Our new agents were thus clearly related to the potent antimitotic natural product derived from *Combretum caffrum*, combretastatin A-4.[1,13,14] We therefore evaluated these initial compounds as inhibitors of tubulin-dependent reactions, and the results supported the conclusion that their mechanism of action involved inhibition of microtubule assembly.

These findings have led us to begin an extensive synthetic program and biological and biochemical evaluation of *cis*-stilbenes and dihydrostilbenes derived from them, since a number of the latter agents also show significant toxic effects on cells in culture. Our goals are the further definition of structure–activity relationships among this class of compounds with their target protein, tubulin, and the development of effective agents for the treatment of cancer. Our initial results with 16 *cis*-stilbenes, 17 dihydrostilbenes, and 36 related compounds are presented here.

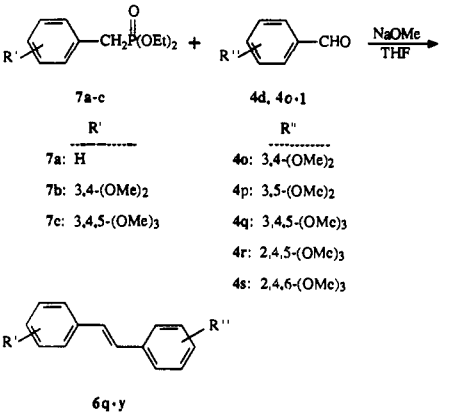
Chemistry

Wittig reaction of phosphonium bromides **3a,b** with aryl aldehydes **4a–n** in THF in the presence of sodium hydride followed by preparative thin layer chromatographic separation gave the corresponding *cis*-stilbenes **5a–n** and *trans*-stilbenes **6a–n** (Scheme I).[27] In general all these reactions gave the cis isomers as major components, and, except in a few cases, trans isomers were also isolated as minor products in yields of over 10%. However, in the case of aryl aldehydes with a substituent at the 2-position (**4c**, **4d**, and **4e**) and pyridine-2-carboxaldehyde, cis isomers were obtained in very high yields, and the trans isomers were obtained in poor yields. With 2,3,4-trimethoxybenzaldehyde (**4d**), an isolable amount of the *trans*-stilbene was not obtained. *trans*-Stilbenes **6q–y** were prepared by the Wittig–Horner reaction of the phosphonate esters **7a–c** with the aryl aldehydes **4d** and **4o–t** in DMF using sodium methoxide as the base (Scheme II).[28] Under these reaction conditions, trans isomers were obtained exclusively. 4′-Hydroxystibenes **5o** and **6o** were prepared from the corresponding O-silyloxylated stilbenes **5m** and **6m** by the

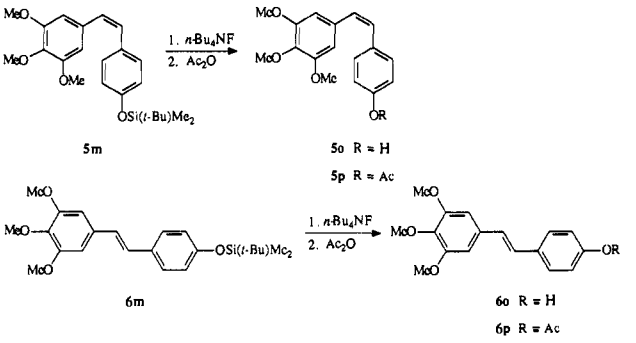
Scheme I



Scheme II

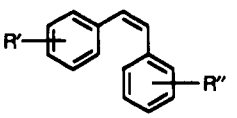


Scheme III



(18) Klaus, M.; Mohr, P.; Weiss, E. Eur. Pat. Appl. *EP* 331,983, 1989.
(19) Neiss, E. S.; Allen, L. M. Eur. Pat. Appl. EP 297,733, 1989.
(20) Jardine, I. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J. J., Ed.; Academic Press: New York, 1980; p 319.
(21) Batra, J. K.; Kang, G. J.; Jurd, L.; Hamel, E. *Biochem. Pharmacol.* **1988**, *37*, 2595.
(22) Wang, Z.-Q.; Kuo, Y.-H.; Schnur, D.; Bowen, J. P.; Liu, S.-Y.; Han, F.-S.; Chang, J.-Y.; Cheng, Y.-C.; Lee, K.-H. *J. Med. Chem.* **1990**, *33*, 2660.
(23) Denny, W. A. *Drug Design Delivery* **1988**, *3*, 99.
(24) Geahlen, R. L.; McLaughlin, J. L. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 241.
(25) Cushman, M.; Chinnasamy, P.; Chakraborti, A. K.; Jurayj, J.; Geahlen, R. L.; Haugwitz, R. D. *Int. J. Pept. Protein Res.* **1990**, *36*, 538.
(26) Cushman, M.; Nagarathnam, D.; Burg, D. L.; Geahlen, R. L. *J. Med. Chem.* **1991**, *34*, 798.
(27) Asakawa, Y.; Tanikawa, K.; Aratani, T. *Phytochemistry* **1976**, *15*, 1057.
(28) Bachelor, F. W.; Loman, A. A.; Snowdon, L. R. *Can. J. Chem.* **1970**, *48*, 1554.

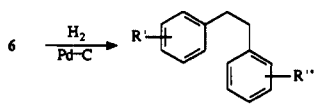
Table I. *cis*-Stilbenes



5

no.	R′	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C
5a	3,4,5-$(OMe)_3$	4-OMe	2.2×10^{-5}	1.2×10^{-6}	2.7×10^{-5}	9.7×10^{-7}	9.3×10^{-5}	oil
5b	3,4,5-$(OMe)_3$	3-OMe	1.3×10^{-1}	1.4×10^{-1}	9.0×10^{-2}	6.0×10^{-2}	1.4	oil
5c	3,4,5-$(OMe)_3$	2-OMe	1.1	1.3	8.7×10^{-1}	1.2	8.6	oil
5d	2,3,4-$(OMe)_3$	4-OMe	9.7×10^{-1}	2.3×10^{-1}	1.0	1.1	10.9	55–7
5e	3,4,5-$(OMe)_3$	2-Cl-4-OMe	5.1×10^{-2}	4.6×10^{-2}	6.6×10^{-2}	1.7×10^{-2}	1.4×10^{-1}	oil
5f	3,4,5-$(OMe)_3$	H	1.7×10^{-1}	2.5×10^{-1}	8.4×10^{-2}	1.2×10^{-1}	>25	oil
5g	3,4,5-$(OMe)_3$	4-Cl	8.0×10^{-2}	1.8×10^{-1}	5.0×10^{-2}	1.0×10^{-2}	1.7×10^{-1}	oil
5h	3,4,5-$(OMe)_3$	4-Br	1.1×10^{-2}	1.6×10^{-2}	8.2×10^{-3}	6.7×10^{-3}	1.4×10^{-2}	oil
5i	1-(4-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene		14.2	17.0	12.9	14.4	>25	oil
5j	1-(3-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene		13.7	14.7	9.8	6.0	>25	oil
5k	1-(2-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene		>25	>25	>25	>25	>25	oil
5l	3,4,5-$(OMe)_3$	4-NO_2	>25	>25	>25	>25	>25	140–2
5m	3,4,5-$(OMe)_3$	4-OSi(*t*-Bu)Me_2	10.95	7.29	10.60	7.59	17.23	oil
5n	3,4,5-$(OMe)_3$	4-NMe_2	4.1×10^{-3}	5.8×10^{-3}	8.1×10^{-3}	1.5×10^{-4}	9.4×10^{-3}	oil
5o	3,4,5-$(OMe)_3$	4-OH	12.70	5.70	1.75	2.27	12.60	148–50
5p	3,4,5-$(OMe)_3$	4-OAc	1.7	3.0×10^{-1}	6.0	6.0×10^{-1}	6.3	oil
1a	combretastatin A-4		1.2×10^{-6}	3.8×10^{-6}	1.2×10^{-5}	3.0×10^{-8}	1.4×10^{-5}	
	adriamycin		2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}	

Scheme IV



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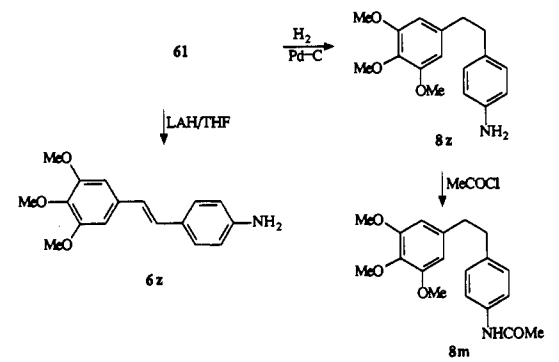
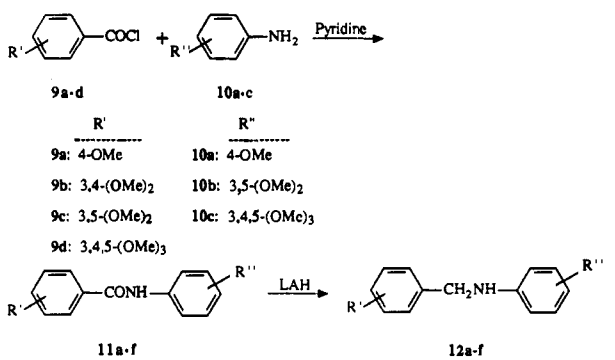
Scheme V



Scheme VI



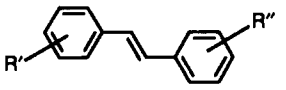
reaction of tetra-*n*-butylammonium fluoride in THF. In another set of experiments, 4′-acetoxystilbenes **5p** and **6p** were prepared by acetylation of 4′-hydroxystilbenes **5o** and **6o** (Scheme III). Cis or trans geometries of most of these compounds were confirmed by their characteristic coupling constants for the olefinic protons of about 12 Hz for cis and 16.0–16.5 Hz for trans isomers.[29] The two olefinic protons of compounds **5d**, **5m**, **6g**, and **6p** gave singlets and those of compounds **5o** and **6b** gave multiplets, and the geometries of these compounds were assigned relative to their isomers, which gave distinct doublets with characteristic coupling constants. Catalytic hydrogenation of (*E*)-stilbenes **6** at about 40 psi in the presence of 10% palladium on charcoal gave dihydrostilbenes **8** (Scheme IV). Lithium aluminum hydride reduction of (*E*)-4′-nitro-3,4,5-trimethoxystilbene (**6l**) provided (*E*)-4′-amino-3,4,5-trimethoxystilbene (**6z**). Catalytic hydrogenation of compound **6l** in EtOAc at 40 psi in the presence of 10% palladium on charcoal gave 4′-amino-3,4,5-trimethoxydihydrostilbene (**8z**), which on subsequent reaction with acetyl chloride gave the acetamido compound **8m** (Scheme V). Scheme VI describes the general method adopted for the preparation of amides **11a–f** and their subsequent reduction to substituted benzylamines **12a–f**.

Results and Discussion

In the present study we synthesized 16 *cis*-stilbenes, 24 *trans*-stilbenes, 17 dihydrostilbenes, six *N*-phenylbenzamides, and six *N*-phenylbenzylamines and tested them against the five cancer cell cultures A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. The cytotoxicity results are summarized in Tables I–IV. Among the 69 compounds tested in the present study, 11 of them, **5a**, **5b**, **5e–h**, **5n**, **6n**, **8a**, **8n**, and **12a**, were found to have significant cytotoxicity ($ED_{50} < 1$ μM in at least three cell lines). In general, *cis*-stilbenes were more potent than the other groups of compounds, and (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (**5a**) was the most potent of all. Taking compound **5a**, a close analogue of combretastatin A-4 (**1a**), as the model compound for structure–activity relationship discussion, the presence of a 4-methoxy group in the B ring plays a very important role for this compound to be highly cytotoxic. Transfer of the 4-methoxy group in the B ring to the 3- or 2-position (compounds **5b** and **5c**) or substitution of it with H, NO_2, OSi(*t*-Bu)Me_2, OH, OAc (compounds **5f**, **5l**, **5m**, **5o**, and **5p**) decreased the activity drastically. Similarly, intro-

(29) Silverstein, E. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley and Sons: New York, 1981; pp 264–265.

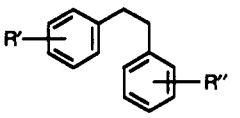
Table II. *trans*-Stilbenes



6

no.	R′	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C
6a	3,4,5-$(OMe)_3$	4-OMe	1.18	1.05	1.82	8.1×10^{-1}	2.07	152–5[38,39]
6b	3,4,5-$(OMe)_3$	3-OMe	9.8	12.2	7.3	10.5	>25	123–5
6c	3,4,5-$(OMe)_3$	2-OMe	12.2	18.0	12.1	13.5	>25	oil
6e	3,4,5-$(OMe)_3$	2-Cl-4-OMe	>25	>25	>25	>25	>25	oil
6f	3,4,5-$(OMe)_3$	H	>25	>25	>25	>25	>25	105–6[28]
6g	3,4,5-$(OMe)_3$	4-Cl	>25	>25	>25	>25	>25	147–9
6h	3,4,5-$(OMe)_3$	4-Br	6.47	9.14	12.69	6.53	5.13	155–6
6i	1-(4-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene		>25	>25	>25	>25	>25	247–8[47]
6j	1-(3-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene		>25	>25	>25	>25	>25	103–5[48]
6l	3,4,5-$(OMe)_3$	4-NO_2	>25	>25	>25	>25	>25	192–4[40]
6m	3,4,5-$(OMe)_3$	4-OSi(*t*-Bu)Me_2	>25	>25	>25	>25	>25	oil
6n	3,4,5-$(OMe)_3$	4-NMe_2	6.1×10^{-3}	8.2×10^{-2}	6.9×10^{-3}	4.6×10^{-3}	1.25×10^{-2}	114–5
6o	3,4,5-$(OMe)_3$	4-OH	>25	18.63	>25	11.55	24.15	188–90[8]
6p	3,4,5-$(OMe)_3$	4-OAc	9.7	9.6	5.4	4.6	13.0	oil
6q	3,4-$(OMe)_2$	H	>25	>25	>25	>25	>25	106–8[41]
6r	2,3,4-$(OMe)_3$	H	>25	>25	>25	>25	>25	79–82
6s	3,4,5-$(OMe)_3$	3,5-$(OMe)_2$	>25	>25	>25	>25	>25	132–4[49]
6t	3,4,5-$(OMe)_3$	2,3,4-$(OMe)_3$	12.5	14.72	10.27	10.64	23.86	87–8
6u	3,4,5-$(OMe)_3$	3,4,5-$(OMe)_3$	>25	>25	>25	>25	>25	174–5[50]
6v	3,4,5-$(OMe)_3$	2,4,5-$(OMe)_3$	>25	>25	>25	>25	>25	147–8
6w	2,4,5-$(OMe)_3$	H	>25	8.5	>25	>25	>25	81–2
6x	2,4,6-$(OMe)_3$	H	>25	>25	>25	>25	>25	57–9[51,52]
6y	3,4-$(OMe)_2$	3,5-$(OMe)_2$	>25	>25	>25	>25	>25	65–7[49]
6z	3,4,5-$(OMe)_3$	NH_2	>25	>25	>25	>25	>25	251–3
	adriamycin		2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}	

Table III. Dihydrostilbenes



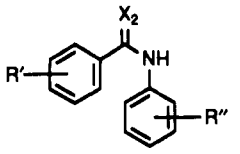
8

no.	R′	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C
8a	3,4,5-$(OMe)_3$	4-OMe	1.8×10^{-4}	1.6×10^{-4}	2.5×10^{-4}	1.4×10^{-4}	1.8×10^{-4}	73–5[39]
8b	3,4,5-$(OMe)_3$	3-OMe	11.7	12.4	7.6	9.2	>25	oil
8c	3,4,5-$(OMe)_3$	2-OMe	13.5	11.8	>25	20	>25	oil
8f	3,4,5-$(OMe)_3$	H	>25	>25	>25	>25	>25	oil
8i	1-(4-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethane		>25	>25	>25	>25	>25	oil
8j	1-(3-pyridyl)-2-(3,4,5-trimethoxyphenyl)ethane		12.2	>25	>25	>25	>25	oil[48]
8m	3,4,5-$(OMe)_3$	4-$NHCOCH_3$	>25	>25	>25	>25	>25	112–4
8n	3,4,5-$(OMe)_3$	4-NMe_2	8.3×10^{-2}	6.4×10^{-2}	7.7×10^{-2}	5.9×10^{-2}	1.2×10^{-1}	oil
8o	3,4,5-$(OMe)_3$	4-OH	>25	>25	>25	>25	>25	108–10[8]
8p	3,4,5-$(OMe)_3$	4-OAc	19.0	>25	>25	>25	>25	oil
8q	3,4-$(OMe)_2$	H	>25	>25	>25	>25	>25	oil
8r	2,3,4-$(OMe)_3$	H	>25	>25	>25	>25	>25	oil
8s	3,4,5-$(OMe)_3$	3,5-$(OMe)_2$	>25	>25	>25	>25	>25	76–7[49]
8t	3,4,5-$(OMe)_3$	2,3,4-$(OMe)_3$	>25	>25	>25	>25	>25	oil
8u	3,4,5-$(OMe)_3$	3,4,5-$(OMe)_3$	>25	>25	>25	>25	>25	137–8[53]
8v	3,4,5-$(OMe)_3$	2,4,5-$(OMe)_3$	>25	>25	>25	>25	>25	88–9
8z	3,4,5-$(OMe)_3$	4-NH_2	12.23	11.88	24.56	12.65	>25	84–5
1c	dihydrocombretastatin A-4		1.0×10^{-2}	3.3×10^{-1}	8.1×10^{-3}	2.1×10^{-3}	1.0×10^{-2}	
	adriamycin		2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}	

duction of a Cl group at the 2-position of **5a** (compound **5e**) decreased the cytotoxicity. However, when the methoxy group in the B ring was substituted with a Cl, Br, or NMe_2 group (compounds **5g**, **5h**, **5n**), although the potency decreased they were still highly cytotoxic ($ED_{50} < 10^{-1}$ μM). Rotating the three A-ring methoxy groups from the 3,4,5-positions to the 2,3,4-positions reduced the cytotoxicity by more than 5 orders of magnitude. In another modification we replaced the B ring of compound **5a** with 4-, 3-, or 2-pyridyl rings (compounds **5i**, **5j**, **5k**) and none of them were active ($ED_{50} > 10$ μM). These results show that the exact locations of the four methoxy groups are very important features for the pronounced cytotoxicity of compound **5a** and that changes in their locations result in decreased potency. In comparison with combretastatin A-4 (**1a**), compound **5a** was found to be approximately 140 times more cytotoxic against HT-29 cells and about 10 times more cytotoxic against MCF-7 cells than combretastatin A-4 (**1a**). However, **5a** was found to be about 20 times less cytotoxic against A-549 cells, 30 times cytotoxic against SKMEL-5, and 7 times less cytotoxic against MLM cells than combretastatin A-4 (**1a**).

Except for compound **6n**, *trans*-stilbenes were essentially inactive. This includes tetramethylated piceatannol (**6y**) and its methoxylated derivatives **6s**, **6t**, **6u**, and **6v**. Only two dihydrostilbenes (compounds **8a** and **8n**) were found

Table IV. Benzamides and Benzylamines



11, 12

no.	R'	R''	X_2	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C
11a	3,4,5-$(OMe)_3$	4-OMe	O	14.07	12.21	22.65	>25	>25	160–1[42]
11b	3,4-$(OMe)_2$	3,4,5-$(OMe)_3$	O	>25	>25	>25	>25	>25	155–6
11c	4-OMe	3,4,5-$(OMe)_3$	O	>25	>25	>25	>25	>25	159–160
11d	3,5-$(OMe)_2$	4-OMe	O	9.02	5.47	14.52	11.88	>25	104–5
11e	4-OMe	3,5-$(OMe)_2$	O	>25	>25	>25	>25	>25	105–6
11f	3,4,5-$(OMe)_3$	3,4,5-$(OMe)_3$	O	>25	>25	>25	>25	>25	211–2
12a	3,4,5-$(OMe)_3$	4-OMe	H_2	1.9×10^{-3}	2.4×10^{-3}	1.0×10^{-3}	7.0×10^{-4}	1.6×10^{-3}	73–4
12b	3,4-$(OMe)_2$	3,4,5-$(OMe)_3$	H_2	>25	>25	>25	>25	>25	oil
12c	4-OMe	3,4,5-$(OMe)_3$	H_2	8.3×10^{-1}	8.6×10^{-1}	2.24	1.13	>25	77–8
12d	3,5-$(OMe)_2$	4-OMe	H_2	5.71	6.08	17.84	2.41	>25	oil
12e	4-OMe	3,5-$(OMe)_2$	H_2	>25	>25	>25	>25	>25	oil
12f	3,4,5-$(OMe)_3$	3,4,5-$(OMe)_3$	H_2	>25	>25	>25	>25	>25	127–8
	adriamycin			2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}	

to be active, with **8a** being the second most cytotoxic agent we prepared (ED_{50} values about 2×10^{-4} μM). Compound **8a** was more cytotoxic than dihydrocombrestatin A-4 (**1c**) in all five cancer cell lines studied here. When the ethylene bridge in compound **8a** was replaced with an amide or an aminomethylene linkage (compounds **11a**, **11c**, **12a**, **12c**, and other analogues), none of the amides had significant activity ($ED_{50} > 1$ μM), but the *N*-benzylamine derivative **12a** possessing the closest structural analogy to **8a** was active. 3,4,5-Trimethoxy-*N*-(4-methoxyphenyl)benzylamine (**12a**) was only an order of magnitude less cytotoxic than **8a** (ED_{50} in the 10^{-3} μM range).

The mechanism of action of the combretastatins has been shown to be at the microtubule level, since they cause cells to accumulate in apparent metaphase arrest and inhibit in vitro microtubule assembly.[1,7] They bind specifically to tubulin, the major component of microtubules, at the colchicine binding site, since combretastatin A-4 (**1a**) has been shown to competitively inhibit the binding of radiolabeled colchicine to tubulin.[14]

Initial investigation of several of the synthetic compounds prepared here revealed that they do in fact cause mitotic arrest in cell culture. We therefore performed a detailed quantitative study of the effects of most of these substances on tubulin polymerization. With the exception of compounds **5p** and **12d**, noncytotoxic agents had minimal effects on polymerization (IC_{50} values > 50 μM), but significant inhibition of the reaction occurred with 10 of the 11 highly cytotoxic compounds and with compounds **5p** and **12d**. Tubulin polymerization and colchicine binding inhibition data of the compounds we prepared for this study are presented in comparison with simultaneously obtained inhibitory data for the effects of combretastatin A-4 (**1a**; cf. **5a**), its trans isomer (**1b**, cf. **6a**), and its dihydro derivative (**1c**; cf. **8a**) (Table V). Data are presented as well for podophyllotoxin, a potent tubulin inhibitor that binds at the colchicine site,[30] and for thiocolchicine, a particularly potent colchicinoid that has reproducibly yielded the lowest IC_{50} value in the polymerization assay for agents binding to the colchicine binding site.[31,32]

Table V. Effects of Compounds **5a**, **5b**, **5e**, **5f**, **5g**, **5h**, **5n**, **5p**, **6a**, **6n**, **8a**, **8n**, **12a**, **12c**, and **12d** on Tubulin Polymerization and on the Binding of Radiolabeled Colchicine to Tubulin[a]

compd	tubulin polymerization IC_{50} (μM) (± S. D.)	colchicine binding % inhibition
5a	2.2 (±0.07)	95
5b	8.8 (±1)	50
5e	3.5 (±0.3)	73
5f	36 (±1)	14
5g	4.8 (±0.3)	55
5h	3.1 (±0.1)	73
5n	3.4 (±0.1)	83
5p	29 (±5)	24
6a	>50	
6n	>50	
8a	7.9 (±0.8)	65
8n	29 (±1)	31
12a	23 (±0.5)	34
12c	>50	
12d	29 (±2)	39
combretastatin A-4 (**1a**)	1.9 (±0.2)	99
1b	>50	
1c	3.3 (±0.2)	79
podophyllotoxin	2.1 (±0.1)	88
thiocolchicine	1.4 (±0.08)	57

[a] The IC_{50} values for tubulin polymerization were determined as described in the text, with full details presented elsewhere.[31] For the colchicine binding assay, reaction mixtures (in triplicate) contained 1 μM tubulin, 5 μM [^{3}H]colchicine, and 5 μM inhibitor and were incubated for 10 min at 37 °C prior to analysis. Further details have been described previously.[32]

Compound **5a** was the most potent of the new agents as an inhibitor of tubulin polymerization, with an IC_{50} value (2.2 μM) essentially indistinguishable from those of combretastatin A-4 and podophyllotoxin and somewhat higher than that of thiocolchicine. This is in agreement both with **5a** being the most cytotoxic of the new compounds and with its close similarity to combretastatin A-4 (**1a**) in its overall effects on the cell lines we evaluated. The difference in IC_{50} values between the two dihydrostilbenes **1c** and **8a** was more noticeable. The combretastatin A-4 analogue **1c** had an IC_{50} value of 3.3 μM, only modestly lower than the IC_{50} value of combretastatin A-4, but the corresponding hydrogenation of **5a** to yield **8a** resulted in an almost 4-fold increase in the IC_{50} value, from 2.2 to 7.9 μM. Similarly the modest reduction in activity in the *cis*-stilbene **5n** as compared to combretastatin A-4 (**1a**) (3.5

(30) Wilson, L. *Biochemistry* **1970**, *9*, 4999.
(31) Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 567.
(32) Kang, G.-J.; Getahun, Z.; Muzaffar, A.; Brossi, A.; Hamel, E. *J. Biol. Chem.* **1990**, *265*, 10255.

versus 1.9 μM) was not reflected in the dihydrostilbene analogue **8n**, which had an IC_{50} value of 29 μM. *cis*-Stilbenes **5b**, **5e**, **5g**, and **5h** were also active as inhibitors of tubulin polymerization, while the other ten *cis*-stilbenes had little or no activity. It should be noted that, with the exception of the most potent agents (**1a** and **5a**), there was only qualitative agreement between the tubulin polymerization and cell culture assays. For example, while dihydrocombretastatin A-4 (**1c**) was more effective than compound **8a** as an inhibitor of tubulin polymerization, the latter agent was more cytotoxic with the cell lines studied here. Similarly, although the halogenated *cis*-stilbenes **5e**, **5g**, and **5h** were not much less active than **1a** and **5a** as inhibitors of tubulin polymerization, they were about 1000-fold less cytotoxic.

The cytotoxic compounds gave reproducible results in the tubulin polymerization assay with the exception of the *trans*-stilbenes **1b** and **6n**. Initial evaluation of these compounds in the tubulin polymerization assay yielded results concordant with the cytotoxicity data, although the apparent IC_{50} value obtained in the polymerization assay for **6n** was difficult to reproduce and that for **1b** initially obtained in the current experiments was lower than that obtained previously.[7] Compound **6a**, a close analogue of **1b**, did not inhibit tubulin polymerization at all, and this led us to reevaluate the inhibitory effects of **1b** and **6n**. We found that both **1b** and **6n** solutions increased in activity with storage, and that, when care was taken to evaluate the solutions immediately after their preparation, neither of the *trans*-stilbene was able to significantly inhibit tubulin polymerization. This suggested that both compounds were unstable in solution and that more active agents might be formed during their storage. The cytotoxic properties of these two agents may similarly result from chemical changes in solution. NMR analysis (500 MHz) of **6n** in solution demonstrated significant formation of the cis isomer **5n**. The ratio of **6n**:**5n** was 1:1 after 24 h of the dissolution of pure **6n** in DMSO at room temperature. In a separate analysis of the stability of compound **1b** in DMSO at room temperature (well protected from light), ^1H NMR analysis over a period of 1 month at frequent intervals confirmed the formation of about 3% and 10% of the cis isomer (compound **1a**) after 2 and 4 weeks, respectively.

Compounds **5a** and **8a** can be taken as standards for structure–activity comparisons of *cis*-stilbenes and dihydrostilbenes, respectively, in the tubulin polymerization assay. Without exception, when the same modified analogue was available in both series, a greater loss of activity occurred in the dihydrostilbene than in the analogous *cis*-stilbene (cf. **5b** and **6b**; **5f** and **6f**; **5n** and **6n**; **5p** and **6p**). No modification among the compounds we have prepared thus far improved on the antitubulin activity of either **5a** or **8a**.

In the *cis*-stilbene series, a shift of a single methoxy group in the A ring, from position 5 to position 2, yielded an inactive agent (**5d**). When the B-ring methoxy group was shifted from position 4′ to position 3′, there was a 4-fold drop in activity (compound **5b**; IC_{50}, 8.8 μM). When the B-ring methoxy group was eliminated, there was a much larger drop in activity (compound **5f**; IC_{50}, 36 μM), while its placement at 2′-position yielded the inactive compound **5c**. Addition of a Cl at position 2′ (compound **5e**) or replacement of the methoxy group with a Cl (**5g**), Br (**5h**), or NMe_2 (**5n**) group resulted in small reductions in antitubulin activity. Demethylation of the 4′-methoxy group led to loss of activity (compound **5o**), and its replacement with an acetoxy group yielded a weak inhibitor (compound **5p**; IC_{50}, 29 μM).

Turning to the dihydrostilbene series, replacement of the B-ring methoxy group with an amino group (compound **8z**) resulted in loss of activity, but some activity returned if the amino group was converted to a dimethylamino group (compound **8n**; cf. **5n**). Addition of one (compound **8s**) or two (compounds **8t–v**) additional methoxy groups to the B ring also resulted in loss of activity. The only enhancement of antitubulin activity in the **5a**/**8a** structure yet obtained by modification of the substituents on either phenyl ring has been addition of a single hydroxy group at position 3′ [as occurs in combretastatin A-4 (**1a**) and dihydrocombretastatin A-4 (**1c**)] or addition of two hydroxy groups in a vicinal diol arrangement at positions 2′ and 3′ (as occurs in combretastatin A-1 and B-1), as described previously.[3,7]

Replacement of the ethylene bridge connecting the two aromatic rings in **8a** with amide or aminomethylene units as represented by structures **11a**, **11c**, and **12c** resulted in loss of inhibitory activity in the tubulin polymerization assay. On the other hand, replacement of the ethylene bridge of **8a** with an aminomethylene unit with the alternative orientation shown in structure **12a** resulted in a 3-fold loss of activity (increase in the IC_{50} value from 7.9 μM for compound **8a** to 23 μM for compound **12a**). Comparing compound **12a** to compound **12d** indicates that only a small loss of activity occurs with elimination of the 4-methoxy group of the A ring (IC_{50} of 29 μM without the methoxy group as opposed to 23 μM).

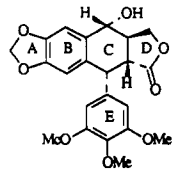
Combretastatin A-4 (**1a**), compound **1c**, and related compounds all inhibit the binding of radiolabeled colchicine to tubulin. We therefore evaluated the new active agents in this assay, too, and they were all found to inhibit the binding reaction. Relative activity as inhibitors of colchicine binding correlated well with their activity as inhibitors of tubulin polymerization. The mechanism of action of the new agents, like that of the combretastatins, thus appears to involve an interaction of the drug with the colchicine binding site of tubulin. Only compound **5a**, however, approached the nearly total inhibition of colchicine binding observed with equimolar combretastatin A-4 (**1a**).

With the compounds described here, as with the combretastatins[7] and other classes of antimitotic agents,[10,33] there is only partial agreement between cytotoxicity and effects on tubulin, the presumptive target molecule. Seven of the most cytotoxic agents (**5a**, **5b**, **5e**, **5g**, **5h**, **5n**, and **8a**) were strong inhibitors of tubulin polymerization, and, except for the *trans*-stilbene **6n**, no compound inactive as inhibitor of tubulin polymerization had significant cytotoxic activity. Nevertheless, compounds **8n** and **12a** were strongly cytotoxic yet had only modest inhibitory effects on tubulin polymerization. Similarly, the structural differences between compounds **12a** and **12d** yielded only minor differences in antitubulin activity but resulted in major changes in their cytotoxic properties.

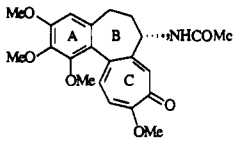
Besides the clear analogy of the compounds described here to the combretastatins, the activity observed in compound **5n**, and to a lesser extent in compound **8n**, suggests a relationship to the benzylbenzodioxole class of agents synthesized by Jurd.[34,35] Among the active tubulin inhibitors were compounds **13**, **14**, and **15** (Chart I), with the latter having the dimethylamino substituent in common

(33) Batra, J. K.; Powers, L. J.; Hess, F. D.; Hamel, E. *Cancer Res.* **1986**, *46*, 1889.
(34) Jurd, L.; Fye, R. L.; Morgan, J. J. *J. Agric. Food Chem.* **1979**, *27*, 1007.
(35) Jurd, L. *J. Heterocycl. Chem.* **1985**, *22*, 993.

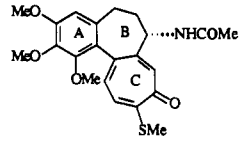
Chart I



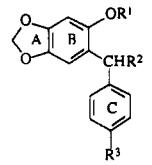
Podophyllotoxin



Colchicine



Thiocolchicine



13 R^1 = Et; R^2 = Me; R^3 = OMe

14 R^1 = H; R^2 =  ; R^3 = OMe

15 R^1 = H; R^2 =  ; R^3 = NMe_2

with **5n**.[36,37] This common substitution pattern in active compounds makes it likely that the B ring of combretastatin derivatives is analogous to the C ring of the benzylbenzodioxole group of compounds.

The relative potencies **5a** > **8a** > **6a** for these cis, dihydro, and trans compounds as inhibitors of tubulin polymerization are in agreement with the relative potencies previously observed[7] for combretastatin A-4 (**1a**) and dihydrocombretastatin A-4 (**1c**) and our finding in the present study that freshly dissolved *trans*-combretastatin A-4 (**1b**) has little or no activity. We assume that the flexibility of the dihydro compound **8a** allows it to adopt a conformation resembling the cis isomer **5a**, which explains why **8a** is more cytotoxic and potent as a tubulin polymerization inhibitor than the trans isomer **6a**. The relative potencies **5a** > **8a** > **6a** for these cis, dihydro, and trans compounds, respectively, as inhibitors of tubulin polymerization were also reflected in the results of the cytotoxicity assays. These relative potencies of **5a** > **8a** > **6a** in the cytotoxicity assays are also in agreement with the relative cytotoxicities of **1a** > **1c** previously reported for L1210 murine leukemia cells in the combretastatin series, although in that study **1b** was intermediate in cytotoxic activity between **1a** and **1c**.[7]

In summary, a series of stilbene derivatives has been prepared and tested for cytotoxicity in cancer cell cultures, for inhibition of tubulin polymerization, and for inhibition of colchicine binding to tubulin. The most potent of these substances was **5a**, which was found to be almost as potent as combretastatin A-4 (**1a**), the most active of the combretastatins, as a tubulin polymerization inhibitor. Compound **5a** was also determined to be slightly more cytotoxic than combretastatin A-4 (**1a**) in HT-29 colon adenocarcinoma and MCF-7 breast carcinoma cells and slightly less cytotoxic in A-549 lung carcinoma, SKMEL-5 melanoma, and MLM melanoma cells. It is clear from these results that the phenolic hydroxyl group of combretastatin A-4 (**1a**) is not a critical structural feature for either its anticancer activity or for its ability to inhibit tubulin polymerization. The relative potencies **5a** > **8a** > **6a** for these cis, dihydro, and trans compounds, respectively, are in agreement with the previously established potencies for combretastatin A-4 (**1a**), dihydrocombretastatin A-4 (**1a**), and *trans*-combretastatin A-4 (**1b**) when tested for inhibition of tubulin polymerization. These studies contribute knowledge about the structure–activity relationships in the combretastatin series that may eventually allow the design and synthesis of a stilbene derivative having superior anticancer activity.

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; ^1H NMR spectra on a Chemagnetics A-200 or Nicolet QE-300 or Varian VXR-500S spectrometers with TMS as an internal standard in $CDCl_3$ or DMSO-d_6; IR spectra on a Beckman IR-33 spectrophotometer. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated composition. All organic solvents were appropriately dried and/or purified prior to use. Diethyl benzylphosphonate (**7a**), aryl aldehydes **4a–t**, and a 1 M solution of tetra-*n*-butylammonium fluoride in THF were obtained from commercial sources. Compounds **7b–c** were prepared by the reaction of the corresponding benzyl bromides and triethyl phosphite.[28] Phosphonium bromides **3a,b** were prepared by stirring a mixture of triphenylphosphine and the corresponding benzyl bromides in toluene.[27] Combretastatin A-4 and its trans isomer were kindly provided by Prof. G. R. Pettit, Arizona State University. Compound **1c** was prepared as described previously.[7] Podophyllotoxin was obtained from Aldrich Chemical Co., and thiocolchicine was from Roussel-Uclaf. Preparative silica gel TLC plates (200 μm) were purchased from Analtech.

General Procedure for the Preparation of (*Z*)-Stilbenes 6a–n and (*E*)-Stilbenes 5a–n. Sodium hydride (72 mg, 3 mmol) was added in portions to a well-stirred suspension of phosphonium bromide **3a,b** (2.0 mmol) and aryl aldehyde (2.0 mmol) in benzene (20 mL) under an argon atmosphere at 0–5 °C, and the mixture was allowed to warm to room temperature. After an additional stirring for 16 h, excess sodium hydride was quenched by the addition of methanol (1 mL). Solvents from the reaction mixture were evaporated at reduced pressure, and the residue was purified by preparative thin layer chromatography using 5% EtOH in hexane as the eluent. Products **5d** and **5l** were obtained as solids, and all the other *cis*-stilbenes were obtained as viscous oils.

(*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene (5a): 400 mg; 66%; oil; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.25 (d, J = 9 Hz, 2 H), 6.80 (d, J = 9 Hz, 2 H), 6.53 (d, J = 12 Hz, 1 H), 6.51 (s, 2 H), 6.44 (d, J = 12 Hz, 1 H), 3.84 (s, 3 H), 3.79 (s, 3 H), 3.69 (s, 6 H); CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

(*Z*)-1-(3-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene (5b): 410 mg; 69%; oil; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.18 (t, J = 7.9 Hz, 1 H), 6.91–6.83 (m, 2 H), 6.78–6.72 (m, 1 H), 6.58 (d, J = 12.2 Hz, 1 H), 6.50 (d, J = 12.2 Hz, 1 H), 6.49 (s, 2 H), 3.83 (s, 3 H), 3.70 (s, 3 H), 3.67 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 159.94, 153.29, 139.63, 137.63, 132.84, 130.67, 130.22, 129.63, 121.79, 114.24, 113.46, 106.42, 61.06, 56.01, 55.26; CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

(*Z*)-1-(2-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-ethene (5c): 440 mg; 73%; oil; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.27–7.20 (m, 2 H), 6.90 (d, J = 8.4 Hz, 1 H), 6.82 (t, J = 8.4 Hz, 1 H), 6.65 (d, J = 12.2 Hz, 1 H), 6.54 (d, J = 12.2 Hz, 1 H), 6.47 (s, 2 H), 3.84 (s, 3 H), 3.82 (s, 3 H), 3.63 (s, 6 H); CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

(*Z*)-1-(4-Methoxyphenyl)-2-(2,3,4-trimethoxyphenyl)-ethene (5d): 460 mg; 77%; mp 55–7 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.19 (d, J = 8.9 Hz, 2 H), 6.94 (d, J = 8.7 Hz, 1 H), 6.75 (d, J = 8.9 Hz, 2 H), 6.52 (s, 2 H), 6.49 (d, J = 8.7 Hz, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.84 (s, 3 H), 3.78 (s, 3 H); CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

(*Z*)-1-(2-Chloro-4-methoxyphenyl)-2-(2,3,4-trimethoxy-

(36) Batra, J. K.; Jurd, L.; Hamel, E. *Mol. Pharmacol.* **1985**, *27*, 94.
(37) Batra, J. K.; Jurd, L.; Hamel, E. *Biochem. Pharmacol.* **1986**, *35*, 4013.

phenyl)ethene (5e): 420 mg; 63%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.21 (d, J = 8.5 Hz, 1 H), 6.96 (d, J = 2.6 Hz, 1 H), 6.67 (dd, 1 H), 6.59 (d, J = 12.1 Hz, 1 H), 6.57 (d, J = 12.1 Hz, 1 H), 6.42 (s, 2 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 3.66 (s, 6 H). Anal. ($C_{18}H_{19}ClO_4$) C, H.

(*Z*)-1-Phenyl-2-(3,4,5-trimethoxyphenyl)ethene (5f): 270 mg; 50%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.35–7.25 (m, 5 H), 6.61 (d, J = 12.2 Hz, 1 H), 6.50 (d, J = 12.2 Hz, 1 H), 6.47 (s, 2 H), 3.83 (s, 3 H), 3.65 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.27, 137.86, 137.12, 132.84, 130.48, 130.36, 129.28, 128.62, 127.51, 106.35, 61.09, 55.96; CIMS (isobutane) m/e 271 (MH^+, 100). Anal. ($C_{17}H_{18}O_3$) C, H.

(*Z*)-1-(4-Chlorophenyl)-2-(3,4,5-trimethoxyphenyl)ethene (5g): 307 mg; 50%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (s, 4 H), 6.55 (d, J = 12 Hz, H), 6.49 (d, J = 12 Hz, 1 H), 6.45 (s, 1 H), 3.84 (s, 3 H), 3.68 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.43, 137.77, 136.17, 133.19, 132.53, 131.20, 130.74, 128.96, 128.75, 106.26, 61.10, 56.05. Anal. ($C_{17}H_{17}ClO_3$) C, H.

(*Z*)-1-(4-Bromophenyl)-2-(3,4,5-trimethoxyphenyl)ethene (5h): 363 mg; 52%; oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.38 (d, J = 8.6 Hz, 2 H), 7.16 (d, J = 8.6 Hz, 2 H), 6.56 (d, J = 12.1 Hz, 1 H), 6.47 (d, J = 12.1 Hz, 1 H), 6.44 (s, 2 H), 3.84 (s, 3 H), 3.68 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.42, 137.77, 136.63, 132.49, 131.71, 131.28, 131.02, 128.98, 121.29, 106.25, 61.09, 56.05; CIMS (isobutane) m/e 350 (93) 348 (MH^+, 100). Anal. ($C_{17}H_{17}BrO_3$) C, H.

(*Z*)-1-(4-Pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene (5i): 277 mg; 51%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.49 (d, J = 6.0 Hz, 2 H), 7.18 (d, J = 6.0 Hz, 2 H), 6.69 (d, J = 12.2 Hz, 1 H), 6.48 (d, J = 12.2 Hz, 1 H), 6.42 (s, 2 H), 3.84 (s, 3 H), 3.66 (s, 6 H); CIMS (isobutane) m/e 272 (MH^+, 100). Anal. ($C_{16}H_{17}NO_3$) C, H.

(*Z*)-1-(3-Pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene (5j): 292 mg; 54%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.53 (s, 1 H), 8.43 (d, J = 4.8 Hz, 1 H), 7.60 (d, J = 7.9 Hz, 1 H), 7.18 (dd, J_1 = 4.8 Hz, J_2 = 7.9 Hz, 1 H), 6.67 (d, J = 12.2 Hz, 1 H), 6.53 (d, J = 12.2 Hz, 1 H), 6.41 (s, 2 H), 3.84 (s, 3 H), 3.67 (s, 6 H); CIMS (isobutane) m/e 272 (MH^+, 100). Anal. ($C_{16}H_{17}NO_3$) C, H.

(*Z*)-1-(2-Pyridyl)-2-(3,4,5-trimethoxyphenyl)ethene (5k): 351 mg; 65%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.64 (d, J = 4.7 Hz, 1 H), 7.58–7.54 (dt, J_1 = 7.5 Hz, J_2 = 1.8 Hz, 1 H), 7.32–7.30 (m, 1 H), 7.17–7.15 (m, 2 H), 6.79 (d, J = 12.4 Hz, 1 H), 6.58 (s, 2 H), 3.89 (s, 3 H), 3.74 (s, 6 H); CIMS (isobutane) m/e 272 (MH^+, 100). Anal. ($C_{16}H_{17}NO_3$) C, H.

(*Z*)-1-(4-Nitrophenyl)-2-(3,4,5-trimethoxyphenyl)ethene (5l): 170 mg; 27%; mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.15 (d, J = 8.6 Hz, 2 H), 7.56 (d, J = 8.6 Hz, 2 H), 7.05 (d, J = 12 Hz, 1 H), 6.95 (d, J = 12 Hz, 1 H), 6.71 (s, 2 H), 3.86 (s, 6 H), 3.82 (s, 3 H); CIMS (isobutane) m/e 316 (MH^+, 100). Anal. ($C_{17}H_{17}NO_5$) C, H.

(*Z*)-1-[4-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (5m): 429 mg; 53%; oil; IR (Neat) 2980, 2960, 1610, 1580, 1520, 1470, 1420, 1360, 1330, 1270, 1140 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (d, J = 8.0 Hz, 2 H), 6.73 (d, J = 8.0 Hz, 2 H), 6.51 (s, 2 H), 6.41 (s, 2 H), 3.84 (s, 3 H), 3.79 (s, 6 H), 0.99 (s, 9 H), 0.14 (s, 6 H). Anal. ($C_{23}H_{32}O_4Si$) C, H.

(*Z*)-1-[4-(Dimethylamino)phenyl]-2-(3,4,5-trimethoxyphenyl)ethenes (5n): 450 mg; 72%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.22 (d, J = 8.9 Hz, 2 H), 6.61 (d, J = 8.9 Hz, 2 H), 6.58 (s, 2 H), 6.41 (d, J = 12.1 Hz, 1 H), 6.34 (d, J = 12.1 Hz, 1 H), 3.85 (s, 3 H), 3.72 (s, 6 H), 2.93 (s, 6 H); CIMS (isobutane) m/e 314 (MH^+, 100). Anal. ($C_{19}H_{23}NO_3$) C, H.

(*E*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6a): 67 mg; 11%; mp 152–5 °C (lit.[38,39] oil); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.45 (d, J = 8.5 Hz, 2 H), 6.97 (d, J = 16.0 Hz, 1 H), 6.91 (d, J = 16.0 Hz, 1 H), 6.90 (d, J = 8.5 Hz, 2 H), 6.72 (s, 2 H), 3.87 (s, 6 H), 3.84 (s, 3 H), 3.82 (s, 3 H); CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{18}H_{20}O_4$) C, H.

(*E*)-1-(4-Nitrophenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6l): 280 mg; 44%; mp 192–4 °C (lit.[40] mp 186–187 °C).

(*E*)-1-[4-[(*tert*-Butyldimethylsilyl)oxy]phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6m): 218 mg, 27%; oil; IR (neat) 2980, 2960, 1605, 1585, 1520, 1470, 1420, 1340, 1320, 1270, 1170, 1130, 1010 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.89 (d, J = 16.5 Hz, 1 H), 6.81 (d, J = 16.5 Hz, 1 H), 7.31 (d, J = 8.5 Hz, 2 H), 6.76 (d, J = 8.5 Hz, 2 H), 6.64 (s, 2 H), 3.84 (s, 3 H), 3.79 (s, 6 H), 0.92 (s, 9 H), 0.14 (s, 6 H). Anal. ($C_{23}H_{32}O_4Si$) C, H.

(*E*)-1-[4-(Dimethylamino)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6n): 145 mg; 23%; mp 114–5 °C; IR (KBr) 3000, 2980, 2940, 2860, 1600, 1580, 1520, 1340, 1240, 1120, 960 cm^{-1}; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.42 (d, J = 8.85 Hz, 2 H), 7.10 (d, J = 16.3 Hz, 1 H), 6.92 (d, J = 16.3 Hz, 1 H), 6.86 (d, J = 8.8 Hz, 2 H), 6.61 (s, 2 H), 3.83 (s, 6 H), 3.67 (s, 3 H), 2.94 (s, 6 H); CIMS (isobutane) m/e 314 (MH^+, 100), 313 (72). Anal. ($C_{19}H_{23}NO_3$) C, H.

(*Z*)-1-(4-Hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (5o). A solution of n-Bu_4NF in THF (1 M, 2 mL, 2 mmol) was added to a stirred solution of silyl ether **5m** (372 mg, 1 mmol) in THF (5 mL) at room temperature and the stirring was continued for 30 min. Solvent was removed at reduced pressure, the resulting residue was treated with 20 mL of water, and the product was extracted with EtOAc (2 × 20 mL). The EtOAc solution was dried ($MgSO_4$) and concentrated and the residue was crystallized from EtOAc/hexane to give **5n** (217 mg, 76%): mp 148–150 °C; IR (KBr) 3440, 3020, 2940, 2840, 1610, 1580, 1510, 1420, 1330, 1230, 1160, 1120, 980, 790, 750 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40 (bs, 1 H), 7.24 (d, J = 8.1 Hz, 2 H), 7.10 (d, J = 8.1 Hz, 2 H), 6.60–6.30 (m, 4 H), 3.80 (s, 6 H), 3.76 (s, 3 H); CIMS (isobutane) m/e 287 (MH^+, 100). Anal. ($C_{17}H_{18}O_4$) C, H.

(*E*)-1-(4-Hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6o). With use of the same procedure as described for **5o**, compound **6o** was prepared from **6m** in 1-mmol scale (228 mg, 80%): mp 188–190 °C (lit.[8] mp 188–90 °C).

General Procedure for the Preparation of Acetates 5p and 6p. A solution of n-Bu_4NF in THF (1 M, 2 mL, 2 mmol) was added to a solution of stilbenes **5m**/**6m** (400 mg, 1 mmol) in THF (5 mL) and the mixture was stirred at 0 °C. After 30 min, acetic anhydride (0.5 mL) was added, and the stirring was continued at room temperature for 24 h. Solvents were evaporated at reduced pressure, and the residue was mixed with water (50 mL). The product was extracted with ether (2 × 25 mL), and the ether solution was washed with water (2 × 100 mL). Evaporation of the solvents and purification of the crude product by preparative TLC using 40% ethyl acetate in hexanes as the eluent afforded the desired products.

(*Z*)-1-(4-Acetoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (5p): 93 mg; 28%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.30 (d, J = 8.3 Hz, 2 H), 6.98 (d, J = 8.3 Hz, 2 H), 6.57 (d, J = 12.1 Hz, 1 H), 6.47 (d, J = 12.1 Hz, 1 H), 6.45 (s, 2 H), 3.83 (s, 3 H), 3.67 (s, 6 H), 2.29 (s, 3 H); CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{19}H_{20}O_5$) C, H.

(*E*)-1-(4-Acetoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6p): 114 mg; 34%; oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.51 (d, J = 8.7 Hz, 2 H), 7.09 (d, J = 8.7 Hz, 2 H), 6.99 (s, 2 H), 6.73 (s, 2 H), 3.92 (s, 6 H), 3.87 (s, 3 H), 2.31 (s, 3 H); CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{19}H_{20}O_5$) C, H.

General Procedure for the Preparation of 6q–y. A solution of phosphonate esters **7a–c** (12 mmol) in dry DMF (10 mL) was added to a magnetically stirred solution of NaOMe (0.65 g, 12 mmol) in dry DMF (10 mL) at 0 °C, and the solution was stirred for 30 min. A solution of aldehyde **4d**/**4o–t** (10 mmol) in dry DMF (10 mL) was added at 0 °C, and the reaction mixture was allowed to warm to room temperature over a period of 1.5 h. The mixture was heated at 95–100 °C for 1 h and left overnight at room temperature. The mixture was poured slowly onto crushed ice, and the precipitated solid was filtered, washed with water, dried, and crystallized from EtOAc–hexane.

(*E*)-1-(3,4-Dimethoxyphenyl)-2-phenylethene (6q): 1.64 g; 68%; mp 106–8 °C (lit.[41] mp 111 °C).

(*E*)-1-Phenyl-2-(2,3,4-trimethoxyphenyl)ethene (6r): 2.34 g; 87%; mp 79–82 °C; IR (KBr) 3020, 3000, 2940, 2840, 1600, 1510, 1470, 1420, 1300, 1260, 1230, 1090, 1030, 1000, 980 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.60–7.50 (m, 2 H), 7.40–7.20 (m, 5 H), 6.90

(38) Carroll, J. F.; Kulkowit, S.; McKervey, M. A. *J. Chem. Soc., Chem. Commun.* **1980**, 507.
(39) Letcher, R. M.; Nhamo, L. R. M.; Gumiro, I. T. *J. Chem. Soc., Perkin Trans. 1* **1972**, 206.
(40) Schiemenz, G. P.; Thobe, J. *Chem. Ber.* **1966**, *99*, 2663.
(41) Dey, B. B.; Row, K. K. *J. Indian Chem. Soc.* **1925**, *1*, 277.

(d, J = 16.5 Hz, 1 H), 6.70 (d, J = 16.5 Hz, 1 H), 3.91 (s, 3 H), 3.90 (s, 3 H), 3.89 (s, 3 H); CIMS (isobutane) m/e 271 (MH^+, 100). Anal. ($C_{17}H_{18}O_3$) C, H.

(*E*)-1-(4-Aminophenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6z). Lithium aluminum hydride (76 mg, 2 mmol) was added to a solution of nitro stilbene **6l** (270 mg, 0.87 mmol) in THF (25 mL), and the mixture was stirred at room temperature for 12 h. Solvent was evaporated at reduced pressure, and the residue was decomposed by careful addition of ice–water (20 mL) containing 2 mL of glacial acetic acid. The red solid formed was filtered and crystallized from CH_2Cl_2–ether to give **6z** (200 mg, 82%): mp 251–3 °C; IR (KBr) 3440, 3400, 3000, 2920, 2820, 1600, 1580, 1340, 1240, 1130, 990, 950, 830 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 7.87 (d, J = 8.3 Hz, 2 H), 7.59 (d, J = 8.3 Hz, 2 H), 7.08 (d, J = 16.0 Hz, 1 H), 7.01 (d, J = 16.0 Hz, 1 H), 6.71 (s, 2 H), 3.87 (s, 6 H), 3.82 (s, 3 H); CIMS (isobutane) m/e 286 (MH^+, 100). Anal. ($C_{17}H_{19}NO_3$) C, H.

General Procedure for the Preparation of Dihydrostilbenes 8. A solution of stilbene **5** and **6** (1 mmol) in EtOAc (25 mL) was hydrogenated at 40 psi in the presence of 10% Pd–C (30 mg) until the uptake of hydrogen ceased (4 h). The solution was filtered and concentrated to obtain the dihydrostilbenes **8** almost as single components.

1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (8a): 280 mg; 93%; mp 73–5 °C (lit.[39] oil); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.10 (d, J = 8.5 Hz, 2 H), 6.83 (d, J = 8.5 Hz, 2 H), 6.36 (s, 2 H), 3.83 (s, 3 H), 3.82 (s, 6 H), 3.79 (s, 3 H), 2.80–2.90 (m, 4 H); ^{13}C NMR $CDCl_3$, 50 MHz) δ 158.53, 153.62, 138.16, 136.68, 134.23, 129.96, 114.22, 105.88, 61.16, 56.31, 55.52, 38.85, 37.33; CIMS (isobutane) m/e 303 (MH^+, 100). Anal. ($C_{18}H_{22}O_4$) C, H.

1-[4-(Dimethylamino)phenyl]-2-(3,4,5-trimethoxyphenyl)ethane (8n): 265 mg; 84%; oil; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.08 (d, J = 8.7 Hz, 2 H), 6.72 (d, J = 8.7 Hz, 2 H), 6.38 (s, 2 H), 3.85 (s, 3 H), 3.83 (s, 6 H), 2.92 (s, 4 H), 2.82 (s, 6 H); CIMS (isobutane) m/e 316 (MH^+, 100%). Anal. ($C_{19}H_{25}NO_3$) C, H.

1-(4-Aminophenyl)-2-(3,4,5-trimethoxyphenyl)ethane (8z). A solution of nitrostilbene **6l** (250 mg, 0.8 mmol) in EtOAc (20 mL) was hydrogenated at 30 psi in the presence of 10% Pd–C (25 mg) at room temperature for 4 h, and the catalyst was filtered off. Evaporation of the solvent and crystallization of the residue from hexanes gave the amine **8z** (180 mg, 80%): mp 84–5 °C; IR (KBr) 3450, 3400, 3020, 2920, 2840, 1600, 1580, 1520, 1330, 1240, 1120, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, J = 8.2 Hz, 2 H), 6.63 (d, J = 8.2 Hz, 2 H), 6.37 (s, 2 H), 3.83 (s, 9 H), 3.75 (bs, 2 H), 2.80 (s, 4 H); CIMS (isobutane) m/e 288 (MH^+, 100). Anal. ($C_{17}H_{21}NO_3$) C, H.

1-(4-Acetamidophenyl)-2-(3,4,5-trimethoxyphenyl)ethane (8m). The amine **8z** (0.574 g, 2 mmol) was dissolved in dry benzene (10 mL) containing triethylamine (0.5 mL), and the solution was cooled to 0 °C. Acetyl chloride (320 mg, 4 mmol) was added dropwise, and the solution was stirred for 30 min. The contents were poured into ice-cold water, and the mixture was extracted with ether (25 mL). The organic layer was washed with water and 5% sodium bicarbonate solution, and dried ($MgSO_4$), and the solvent was evaporated. The residue was crystallized from EtOAc–hexane (0.52 g, 79%): mp 112–4 °C; IR (KBr) 3450, 3000, 2930, 2840, 1670, 1600, 1580, 1510, 1340, 1120 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.52 (bs, 1 H), 7.42 (d, J = 8.4 Hz, 2 H), 7.11 (d, J = 8.4 Hz, 2 H), 6.36 (s, 2 H), 3.82, (s, 3 H), 3.81 (s, 6 H), 2.85 (s, 4 H), 2.14 (s, 3 H); CIMS (isobutane) m/e 330 (MH^+, 100). Anal. ($C_{19}H_{23}NO_4$) C, H.

General Procedure for Preparation of Benzamides 11a–f. Aroyl chloride **9a–d** (20 mmol) was added to a stirred solution of substituted aniline **10a–c** (20 mmol) in pyridine (50 mL) at room temperature, and the reaction mixture was stirred for 4 h and poured into a mixture of ice (400 g) and hydrochloric acid (100 mL). The precipitated product was filtered, washed with water, dried and recrystallized from $CHCl_3$–hexane.

3,4,5-Trimethoxy-*N*-(4-methoxyphenyl)benzamide (11a): 5.83 g; 92%; mp 160–161 °C (lit.[42] mp 162 °C); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.22 (bs, 1 H), 7.50 (d, J = 8.1 Hz, 2 H), 7.03 (s, 2 H), 6.83 (d, J = 8.1 Hz, 2 H), 3.85 (s, 3 H), 3.80 (s, 6 H), 3.77 (s, 3 H); CIMS (isobutane) m/e 318 (MH^+, 100).

4-Methoxy-*N*-(3,4,5-trimethoxyphenyl)benzamide (11c): 5.60 g; 88%; mp 159–160 °C; IR (KBr) 3300, 2980, 2940, 1650, 1605, 1515, 1455, 1420, 1340, 1270, 1220, 1020, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 8.18 (bs, 1 H), 7.60 (d, J = 8.0 Hz, 2 H), 6.90 (s, 2 H), 6.88 (d, J = 8.0 Hz, 2 H), 3.92 (s, 3 H), 3.80 (s, 6 H), 3.76 (s, 3 H); CIMS (isobutane) m/e 318 (MH^+, 100). Anal. ($C_{17}H_{19}NO_5$) C, H.

General Procedure for Preparation of *N*-Benzylanilines 12a–f. A solution of benzamide **11a–f** (5 mmol) in THF (50 mL) was added to a well-stirred suspension of lithium aluminum hydride (0.285 g, 7.5 mmol) in dry THF (10 mL) at 0 °C under a nitrogen atmosphere, and the reaction mixture was allowed to warm to room temperature. After 4 h, the reaction mixture was poured onto ice (200 g), and the mixture was extracted with ether (3 × 20 mL). The combined extracts were washed with water and dried (K_2CO_3). Evaporation of ether from the solution afforded amines **12a–f** almost as single products. Analytical samples of solid products were prepared by crystallization from ether–hexane, and liquids were purified by preparative thin-layer chromatography using 2% methanol in $CHCl_3$ as eluent.

3,4,5-Trimethoxy-*N*-(4-methoxyphenyl)benzylamine (12a): 1.42 g; 94%; mp 73–4 °C; IR (KBr) 3400, 2990, 2920, 2220, 1600, 1510, 1460, 1420, 1330, 1260, 1230, 1120, 1110, 1030, 1000 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.78 (d, J = 8.6 Hz, 2 H), 6.62 (d, J = 8.6 Hz, 2 H), 6.61 (s, 2 H), 4.21 (s, 2 H), 3.86 (bs, 1 H), 3.84 (s, 9 H), 3.74 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.98, 152.88, 143.03, 137.50, 136.04, 115.37, 114.66, 104.77, 61.18, 56.39, 56.08, 50.00; CIMS (isobutane) m/e 304 (MH^+, 100). Anal. ($C_{17}H_{21}NO_4$) C, H.

4-Methoxy-*N*-(3,4,5-trimethoxyphenyl)benzylamine (12c): 1.42 g; 94%; mp 77–8 °C; IR (KBr) 3380, 2980, 2960, 2940, 2820, 1605, 1580, 1520, 1460, 1440, 1255, 1225, 1130, 1110, 1010, 990 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.29 (d, J = 8.6 Hz, 2 H), 6.88 (d, J = 8.6 Hz, 2 H), 5.87 (s, 2 H), 4.22 (s, 2 H), 3.82 (bs, 1 H), 3.80 (s, 3 H), 3.79 (s, 6 H), 3.76 (s, 3 H); CIMS (isobutane) m/e 304 (MH^+, 100). Anal. ($C_{17}H_{21}NO_4$) C, H.

Cytotoxicity Assays. An MTT colorimetric assay was employed according to the established procedure.[43,44] After the addition of the samples to the cell cultures, the cells were incubated for 6 days before the MTT reagent was added. The assays were performed in the Purdue Cell Culture Laboratory. All of the compounds were initially tested once in each of the cell lines listed in Tables I–IV. The active compounds (ED_{50} < 25 μM) were tested again, and the values shown for these cytotoxic substances are the averages of two determinations.

Tubulin Polymerization and Colchicine Binding Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[45] Determination of IC_{50} values for the polymerization of purified tubulin was performed as described in detail elsewhere.[31] 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 > 50 μM) were examined in at least two independent experiments. The effect of agents on the binding of [^3H]colchicine (obtained from Amersham) to tubulin was measured by the DEAE-filter technique as described previously.[32,46]

(42) Grammaticakis, P. *Compt. Rend., Ser. C* **1966**, *262*, 584.
(43) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
(44) Mossman, T. *J. Immunol. Meth.* **1983**, *65*, 55.
(45) Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173.
(46) Borisy, G. G. *Anal. Biochem.* **1972**, *13*, 373.
(47) Baker, B. R.; Gibson, R. E. *J. Med. Chem.* **1971**, *14*, 1057.
(48) Erdtman, H.; Rosengren, A. *Acta. Chem. Scand.* **1968**, *22*, 1475.

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Registry No. **3a**, 61240-20-8; **3b**, 1530-38-7; **4a**, 123-11-5; **4b**, 591-31-1; **4c**, 135-02-4; **4d**, 2103-57-3; **4e**, 54439-75-7; **4f**, 100-52-7; **4g**, 104-88-1; **4h**, 1122-91-4; **4i**, 872-85-5; **4j**, 500-22-1; **4k**, 1121-60-4; **4l**, 555-16-8; **4m**, 120743-99-9; **4n**, 120-21-8; **4o**, 120-14-9; **4p**, 7311-34-4; **4q**, 86-81-7; **4r**, 4460-86-0; **4s**, 830-79-5; **5a**, 134029-49-5; **5b**, 97399-87-6; **5c**, 134029-50-8; **5d**, 134029-51-9; **5e**, 134029-52-0; **5f**, 74809-44-2; **5g**, 134029-53-1; **5h**, 74809-51-1; **5i**, 134029-54-2; **5j**, 134029-55-3; **5k**, 134029-56-4; **5l**, 134029-57-5; **5m**, 134029-58-6; **5n**, 134029-59-7; **5o**, 134029-60-0; **5p**, 134029-61-1; **6a**, 134029-62-2; **6b**, 97399-88-7; **6c**, 134029-63-3; **6e**, 134054-80-1; **6f**, 74809-43-1; **6g**, 134029-64-4; **6h**, 74809-50-0; **6i**, 118160-75-1; **6j**, 134029-65-5; **6l**, 134029-66-6; **6m**, 134029-67-7; **6n**, 134029-68-8; **6o**, 116519-00-7; **6p**, 134029-69-9; **6q**, 3892-92-0; **6r**, 134029-70-2; **6s**, 89946-15-6; **6t**, 134029-71-3; **6u**, 61240-22-0; **6v**, 134029-72-4; **6w**, 129047-45-6; **6x**, 22887-34-9; **6y**, 83088-26-0; **6z**, 134029-73-5; **7a**, 1080-32-6; **7b**, 78055-65-9; **7c**, 134029-74-6; **8a**, 35450-81-8; **8b**, 108853-15-2; **8c**, 134029-75-7; **8f**, 133462-62-1; **8i**, 134029-76-8; **8j**, 20934-93-4; **8m**, 134029-77-9; **8n**, 134029-78-0; **8o**, 39499-95-1; **8p**, 39500-11-3; **8q**, 3892-93-1; **8r**, 134029-79-1; **8s**, 52961-91-8; **8t**, 117048-69-8; **8u**, 33284-74-1; **8v**, 134029-80-4; **8z**, 110997-90-5; **9a**, 100-07-2; **9b**, 3535-37-3; **9c**, 17213-57-9; **9d**, 4521-61-3; **10a**, 104-94-9; **10b**, 10272-07-8; **10c**, 24313-88-0; **11a**, 742-15-4; **11b**, 134029-81-5; **11c**, 134029-82-6; **11d**, 134029-83-7; **11e**, 134029-84-8; **11f**, 748-31-2; **12a**, 134029-85-9; **12b**, 134029-86-0; **12c**, 134029-87-1; **12d**, 134029-88-2; **12e**, 134054-81-2; **12f**, 134029-89-3.

Supplementary Material Available: Physical and spectral data of compounds **6b,c,e,g-j,s-y**, **8b,c,f,i,j,o-v**, **11b,d-f** and **12b,d-f** (6 pages). Ordering information is given on any current masthead page.

(49) Drews, S. E.; Fletcher, I. P. *J. Chem. Soc., Perkin Trans. 1* **1974**, 961.
(50) Sieber, R. H. *Justus Liebigs Ann. Chem.* **1969**, *730*, 31.
(51) Loudon, G. M.; Noyce, D. S. *J. Am. Chem. Soc.* **1969**, *91*, 1433.
(52) Molho, D.; Coillard, J. *Bull. Soc. Chim. Fr.* **1968**, 78.
(53) Iyoda, M.; Sakaitani, M.; Otsuka, H.; Oda, M. *Chem. Lett.* **1985**, 127.

Relaxant Activity of 6-Cyano-2,2-dimethyl-2*H*-1-benzopyran-4-carboxamides and -thiocarboxamides and Their Analogues in Guinea Pig Trachealis

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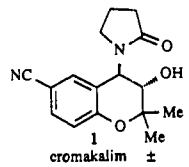
Structural modifications of the potassium channel activator cromakalim (1) are described in which the amide moiety at C-4 has been replaced by carboxamide and thiocarboxamide functions. Analogues in which the hydroxyl group at C-3 has been oxidized or removed are also disclosed. Such analogues display an interesting profile of smooth muscle relaxant activity in the guinea pig isolated trachea, not all of which appears to result from the opening of potassium channels, but few compounds retain useful in vivo activity. However, one compound in particular, 6-cyano-2,2-dimethyl-*N*-methyl-2*H*-1-benzopyran-4-thiocarboxamide (**13**) was shown to be a potent potassium channel activator in vitro and to provide prolonged protection to guinea pigs from the respiratory effects of inhaled histamine.

Introduction

Interest in the potassium channel activators has increased markedly over the past few years, having gained particular momentum following the classification of additional potassium channel subtypes and the identification of a number of blockers with subtype specificity.[1] Another major contributor to this growth was the discovery of cromakalim (**1**), a specific potassium channel activator,[2]



1
cromakalim ±
BRL 34227 (3S,4R)

which is thought to exert its smooth muscle relaxant activity through the opening of ATP-sensitive potassium channels and consequent hyperpolarization.[3,4] While such activity was initially found in the smooth muscle of the vasculature, and thus demonstrated in models of hypertension, it is now recognized that potassium channel activators also have potential application for the treatment of other diseases involving smooth muscle, such as asthma and urinary incontinence.[1]

Particular support for the utility of potassium channel activators in respiratory disorders results from the detailed study of cromakalim in animal models[5] and preliminary

(1) Robertson, D. W.; Steinberg, M. I. Potassium Channel Modulators: Scientific Applications and Therapeutic Promise. *J. Med. Chem.* **1990**, *33*, 1529-1541.
(2) Hamilton, T. C.; Weir, S. W.; Weston, A. H. Comparison of the Effects of BRL 34915 and Verapamil on Electrical and Mechanical Activity in Rat Portal Vein. *Br. J. Pharmacol.* **1986**, *88*, 103-111.
(3) Ashwood, V. A.; Buckingham, R. E.; Cassidy, F.; Evans, J. M.; Faruk, E. A.; Hamilton, T. C.; Nash, D. J.; Stemp, G.; Willcocks, K. Synthesis and Antihypertensive Activity of 4-(Cyclic amido)-2*H*-1-benzopyrans. *J. Med. Chem.* **1986**, *29*, 2194-2201.
(4) Standen, N. B.; Quayle, J. M.; Davies, N. W.; Brayden, J. E.; Huang, Y.; Nelson, M. T. Hyperpolarising Vasodilators Activate ATP-sensitive K^+ Channels in Arterial Smooth Muscle. *Science* **1989**, *245*, 177-180.
(5) Arch, J. R. S.; Buckle, D. R.; Bumstead, J.; Clarke, G. D.; Taylor, J. F.; Taylor, S. G. Evaluation of the Potassium Channel Activator Cromakalim (BRL 34915) as a Bronchodilator in the Guinea pig: Comparison with Nifedipine. *Br. J. Pharmacol.* **1988**, *95*, 763-770.

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