was collected and the radioactivity determined with 1 mL of eluate mixed with 10 mL of scintillation cocktail (3a70, Research Products International) in a scintillation counter. Data obtained for the methylene nucleoside analogues are presented in Table IX.

Determination of Kinetic Constants for Inactivation of AdoHcy Hydrolase. The inactivation constants, K_I and k_2 were determined by the method previously described.[32] For these determinations, AdoHcy hydrolase was preincubated with various concentrations of inhibitors for various amounts of time and the residual enzyme activity was measured. The enzyme activity was determined in the direction of synthesis of AdoHcy from adenosine and homocysteine by incubating 20 nM bovine liver AdoHcy hydrolase with 0.2 mM adenosine and 5 mM homocysteine for 5 min at 37 °C in 150 mM potassium phosphate buffer (pH 7.6) containing 1 mM EDTA (total reaction volume 0.5 mL). The amount of AdoHcy formed was measured by HPLC after the reaction was stopped by addition of perchloric acid (final concentration 0.25 M). An aliquot (100 μL) of the supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse phase column, Econosphere, Alltech, 25 cm × 4.6 mm) and analyzed with a gradient program at a flow rate of 1 mL/min [solvent A, acetonitrile; solvent B, 50 mM sodium phosphate buffer (pH 3.2) containing 10 mM heptanesulfonic acid; program, 8-15% A for 10 min, 50% A for 5 min]. The peak area was monitored at 254 nm to quantitate the AdoHcy.

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The pseudo-first-order rate of inactivation (k_{obs}) was determined from a plot of the residual activity versus preincubation time. K_I and k_2 were obtained from a plot of $1/k_{obs}$ versus 1/[inhibitor] ([I]) using the equation

$$\frac{1}{k_{obs}} = \frac{K_I}{k_2[I]} + \frac{1}{k_2}$$

The data for 2'-deoxy-2'-methyleneadenosine (**44**) are shown in Figures 3 and 4. For **44**, K_I and k_2 values of 13.1 μM and 0.195 min^{-1}, respectively, were calculated.

Acknowledgment. We thank the American Cancer Society (Grant No. CH-405), the Belgian "Fonds voor Geneeskundig Wetenschappelijk Onderzoek" (Project 3.0026.91) and "Nationaal Fonds voor Wetenschappelijk Onderzoek" (Project 7.0049.90), and the National Institutes of Health (Grant No. GM 29332) for generous support. We also thank Lizette van Berckelaer and Ann Absillis for excellent technical assistance and Mrs. Kathryn M. Rollins for assistance with the manuscript.

Registry No. **8**, 137058-86-7; **9**, 137058-88-9; **17**, 134660-23-4; **18**, 134660-26-7; **19**, 119410-95-6; **20**, 119804-96-5; **21**, 2004-07-1; **21** tris-*O*-TBDMS derivative, 141320-73-2; **22**, 133519-46-7; **23**, 133519-47-8; **24**, 141320-56-1; **25**, 141320-58-3; **26**, 141320-57-2; **27**, 141320-59-4; **28**, 141320-60-7; **29**, 141320-62-9; **30**, 141320-63-0; **31**, 141320-65-2; **32**, 141320-67-4; **33**, 141320-69-6; **34**, 141320-71-0; **35**, 78151-96-9; **36**, 137058-77-6; **37**, 141320-61-8; **38**, 141344-03-8; **39**, 141320-64-1; **40**, 141320-66-3; **41**, 141320-68-5; **42**, 141320-70-9; **43**, 141320-72-1; **44**, 104409-41-8; **45**, 137058-79-8; **46**, 69304-45-6; **47**, 90318-44-8; **48**, 104409-40-7; **49**, 104409-41-8; E.C. 3.3.1.1, 9025-54-1; methyltriphenylphosphonium bromide, 1779-49-3.

Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as Potential Cytotoxic and Antimitotic Agents

Mark Cushman,*,† Dhanapalan Nagarathnam,† D. Gopal,† Hu-Ming He,† Chii M. Lin,‡ and Ernest Hamel*,‡

Department of Medicinal Chemistry and Pharmacognosy, Purdue University, West Lafayette, Indiana 47907, and Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892. *Received November 22, 1991*

A series of stilbenes has been prepared and tested for cytotoxicity in the five human cancer cell lines A-549 non-small cell lung, MCF-7 breast, HT-29 colon, SKMEL-5 melanoma, and MLM melanoma. The cis stilbenes **6a-f** proved to be cytotoxic in all five cell lines, with potencies comparable to that of combretastatin A-4. These cytotoxic compounds were all potent inhibitors of tubulin polymerization. The corresponding trans stilbenes **7b-f** were inactive as tubulin polymerization inhibitors and were significantly less cytotoxic in the five cancer cell lines. In the dihydro series, **8b**, **8c**, and **8f** were inactive as tubulin polymerization inhibitors, while **8a**, **8d**, and **8e** were less active than the corresponding cis compounds **6a**, **6d**, and **6e**. The lack of tubulin polymerization inhibitory activity and cytotoxicity displayed by the phenanthrene **23b**, which was synthesized as a conformationally rigid analogue of the lead compound **1**, indicates that the activity of the stilbenes is not due to a totally planar conformation. Similarly, inactivity of the conformationally restricted analogue **26** suggests that the biologically active conformation of **1a** resembles that of the cis alkene **1**. Additional inactive compounds prepared include the benzylisoquinoline series **28-32** as well as the protoberberines **38** and **39**. Shortening the two-carbon bridge of **1a** to a one-carbon bridge in the diphenylmethane **20** resulted in a decrease in cytotoxicity and tubulin polymerization inhibitory activity. Although the corresponding benzophenone **18** was as active as **1a** as a tubulin polymerization inhibitor, it was less cytotoxic than **1a**, and the benzhydrol **19** was essentially inactive. With the exception of the amide **15c**, which displayed low antitubulin activity, all of the phenylcinnamic acid derivatives **14a-c** and **15a-f** were inactive in the tubulin polymerization inhibition assay. The acid **14b** and the ester **15a** were cytotoxic in several of the cancer cell cultures in spite of their inactivity as tubulin polymerization inhibitors.

The design of inhibitors of tubulin polymerization is an attractive strategy for the development of compounds useful in cancer chemotherapy. Ligands binding in the colchicine binding site of tubulin represent an array of antimitotic agents that inhibit cancer cell proliferation. Such compounds, including colchicine,[1-6] podophyllo-

† Purdue University.
‡ NIH.

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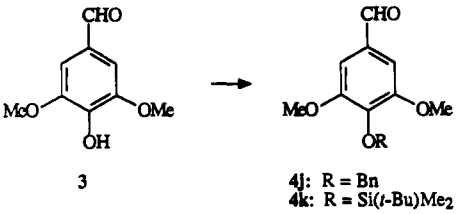
0022-2623/92/1835-2293$03.00/0

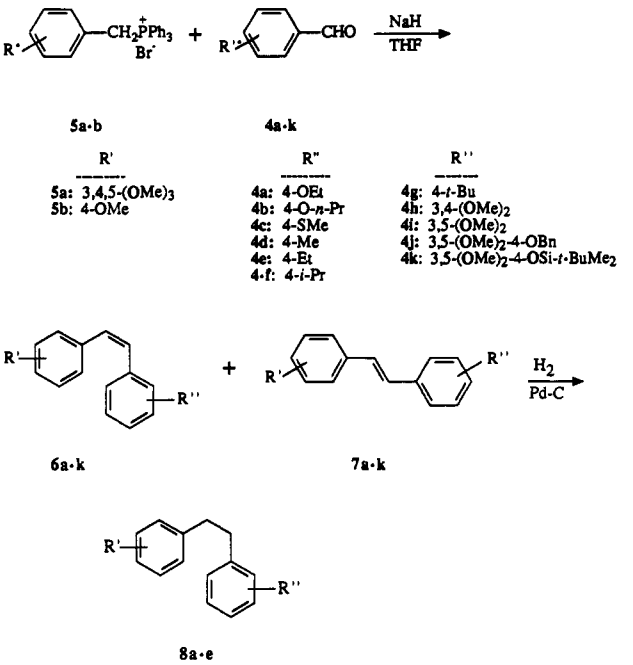
toxin,[7-10] steganacin,[8,11,12] and their synthetic analogues, inhibit tubulin polymerization. Structurally related biphenyls,[13] diphenylmethanes,[14] benzopyrans,[15] and chalcones[16] have also been prepared and found to possess similar activities. Recently, a new series of natural products termed combretastatins has been isolated and added to the list of substances which interact with the colchicine binding site of tubulin.[17] The most potent of these is combretastatin A-4 (2), which has been found to be a potent cytotoxic agent that is active against multidrug-resistant cancer cells.[18] These interesting features recently motivated us to prepare and evaluate an array of stilbene derivatives structurally related to the combretastatins.[19] This resulted in the identification of (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (1) as a cytotoxic tubulin polymerization inhibitor with potency comparable to that of combretastatin A-4 (2, Table I). The dihydro derivative 1a (Table III) was also found to be a potent cytotoxic tubulin polymerization inhibitor.

These results have encouraged the preparation of congeners of 1 and 1a in an effort to probe the structural features associated with their antitubulin and anticancer activities. As detailed in the present study, this has involved the synthesis of conformationally restricted analogues of 1a, the replacement of the methoxyl group on the B-ring of 1 and 1a with a variety of other substituents, and the determination of the effect of double bond isomerization on activity in this series. A variety of substituents were also introduced on the double bond of 1 in an attempt to prepare compounds having enhanced aqueous solubilities that could be more readily formulated. These stilbenes were tested for cytotoxicity in a variety of human

Scheme I



Scheme II



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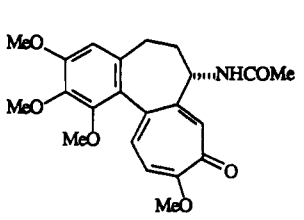
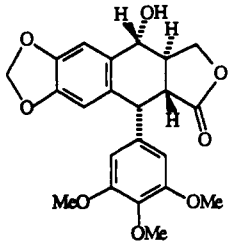
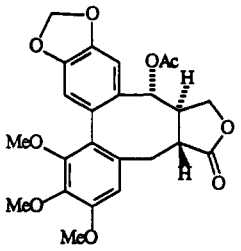


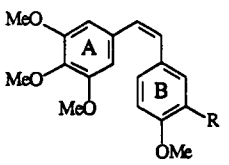
Colchicine



Podophyllotoxin

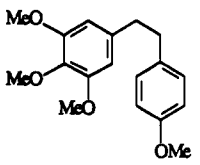


Steganacin



1 R = H

2 R = OH (Combretastatin A-4)



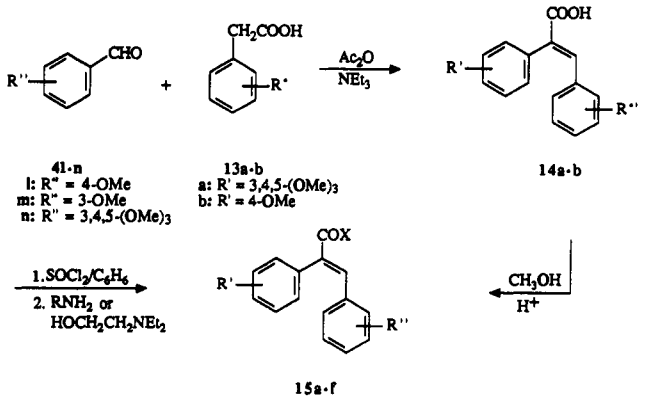
1a

cell lines, including A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. We confirmed that the most active new agents caused the accumulation of cells arrested in mitosis. All compounds were also analyzed for their inhibitory effects on tubulin polymerization, with a direct comparison to compounds 1 and 2.

Chemistry

4-(Benzyloxy)-3,5-dimethoxybenzaldehyde (4j)[20] was prepared by the reaction of syringaldehyde (3) with benzyl chloride in the presence of K_2CO_3 in boiling acetone. Similarly, reaction of *tert*-butyldimethylsilyl chloride with syringaldehyde (3) in DMF in the presence of *N,N*-diisopropylethylamine gave 4-[(*tert*-butyldimethylsilyl)oxy]-3,5-dimethoxybenzaldehyde (4k) (Scheme I). Wittig reaction[19,21] of phosphonium bromides 5a,b with benzaldehydes 4a–k in THF in the presence of sodium hydride followed by preparative thin-layer chromatographic separation of the crude products afforded the cis stilbenes 6a–k and trans stilbenes 7a–k (Scheme II, Tables I and II). Reaction of compounds 6k and 7k with tetra-*n*-butylammonium fluoride and in situ acetylation of the phenols with acetic anhydride gave the acetoxy compounds 6l and 7l (Tables I and II). The cis and trans geometries of the stilbenes were assigned by the characteristic ^1H NMR coupling constants of the olefinic protons. Catalytic hydrogenation of stilbenes 6 and 7 at about 40 psi in the presence of 10% palladium on charcoal gave the dihydrostilbenes 8a–e (Scheme II and Table III). The amino ethers 8f,g (Table III) were prepared by the reaction of 1-(4-hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (9)[19] with (dialkylamino)ethyl chlorides 10a,b in refluxing acetone in the presence of K_2CO_3. Compounds 8h and 8i (Table III) were prepared by the alkylation of 3,4,5-trimethoxyphenylacetonitrile (11a) and 4-methoxyphenylacetonitrile (11b) with 4-methoxybenzyl bromide (12a) and 3,4,5-trimethoxybenzyl bromide (12b), respectively, using LDA as the base. Similarly, alkylation of methyl 4-methoxyphenylacetate (11c) with 3,4,5-trimethoxybenzyl bromide 12b gave product 8j (Table III).

Scheme III



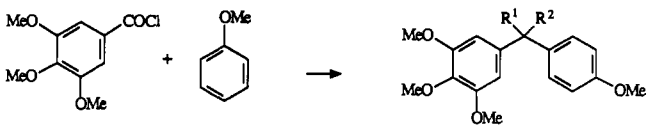
41-n
l: R'' = 4-OMe
m: R'' = 3-OMe
n: R'' = 3,4,5-(OMe)3

13a-b
a: R' = 3,4,5-(OMe)3
b: R' = 4-OMe

14a-b

15a-f

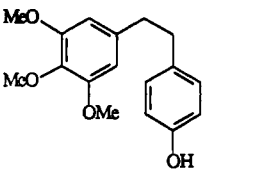
Scheme IV



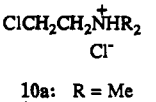
16

17

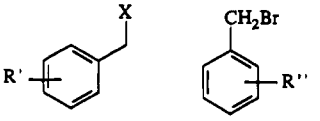
18: R1, R2 = O
19: R1 = H, R2 = OH
20: R1 = R2 = H



9



10a: R = Me
10b: R = Et



11a-c

12a-b

11a: R'= 3,4,5-(OMe)3, X = CN
11b: R'= 4-OMe, X = CN
11c: R'= 4-OMe, X = COOMe
12a: R''= 4-OMe
12b: R''= 3,4,5-(OMe)3

Several derivatives containing acidic and basic functional groups, including the previously mentioned amines 8f,g, were prepared in an attempt to make compounds that were more soluble in water and could therefore be formulated more easily. Base-catalyzed condensation of phenylacetic acids 13a,b with aryl aldehyde 4n in the presence of tri-

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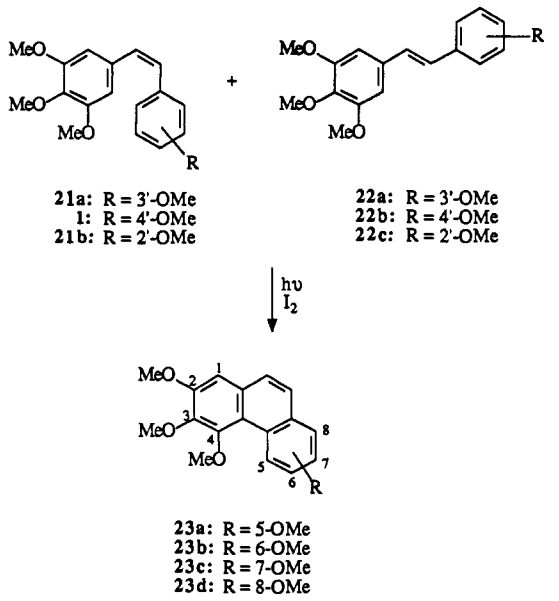
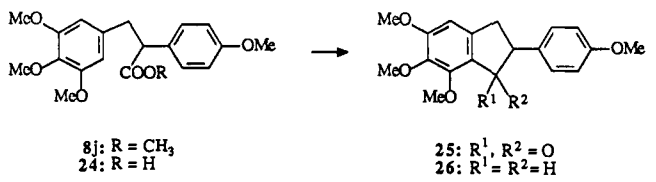
Scheme V



Scheme VI

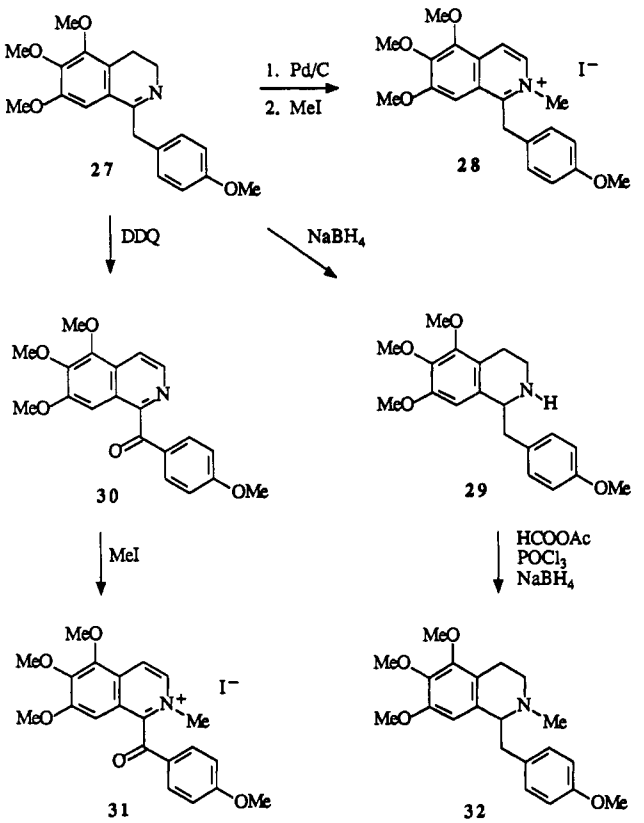


ethylamine gave the carboxylic acids **14a,b** (Scheme III, Table IV).[22] Esterification of compounds **14a,b** with methanol using a catalytic amount of H_2SO_4 gave products **15a,b** (Table IV). Reaction of thionyl chloride with the carboxylic acids **14a,b** in refluxing benzene gave the corresponding acid chlorides, which on subsequent reaction with appropriate amines, and (dialkylamino)ethanol gave compounds **15c–f** (Scheme III, Table IV).

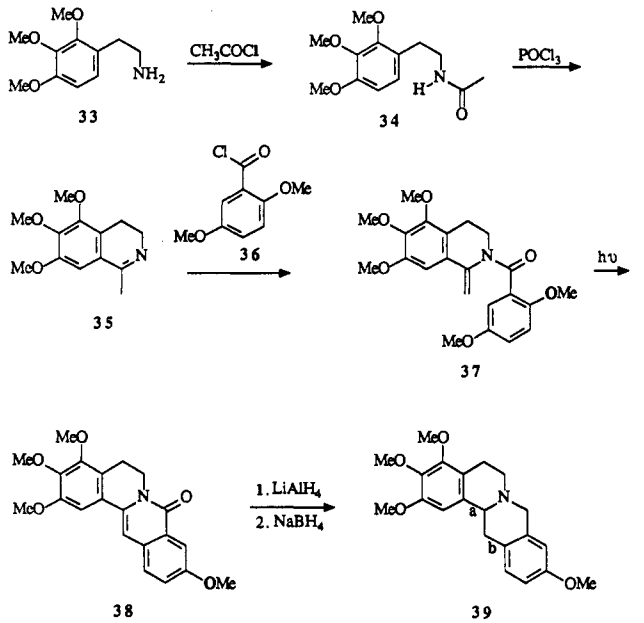
The effect of shortening the distance between the two aromatic rings was investigated by preparing compound **20**, having a methylene unit separating the rings. Friedel–Crafts acylation of anisole with 3,4,5-trimethoxybenzoyl chloride gave 3,4,4′,5-tetramethoxybenzophenone (**18**, Scheme IV). Sodium borohydride reduction of compound **18** in ethanol afforded (4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (**19**), which on catalytic hydrogenolysis in the presence of 10% palladium on charcoal gave (4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methane (**20**) (Scheme IV).

Several conformationally rigid analogues of the lead compound **1** were synthesized in an attempt to gain evidence concerning the biologically active conformation of this substance. Different conformations are available to **1** through rotation about the two bonds connecting the aromatic rings to the alkene unit. This question was investigated by forming a covalent bond between the two aromatic rings of several stilbenes, resulting in the phenanthrenes **23a–d** (Scheme V). Photocyclization of the cis–trans mixtures of stilbenes **1**, **21a,b**, and **22a–c**[19,23,24]

Scheme VII



Scheme VIII



in the presence of iodine afforded the desired phenanthrenes **23a–d**.

Conformationally restricted analogues of the active dihydrostilbene **1a** were also prepared. Synthesis of one such compound based on the indane system is detailed in Scheme VI. Hydrolysis of the methyl ester **8j** (Table III) under basic conditions gave the acid **24**. The indanone **25** was then prepared by an intramolecular Friedel–Crafts

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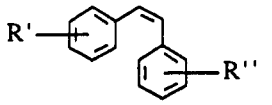
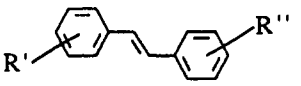
Table I. Cis Stilbenes



6

no.	R′	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
6a	3,4,5-$(OMe)_3$	4-OEt	1.6×10^{-3}	9.6×10^{-2}	1.8×10^{-3}	2.5×10^{-3}	2.9×10^{-2}	oil	2.7 (±0.2)
6b	3,4,5-$(OMe)_3$	4-OPr^n	3.9×10^{-2}	6.6×10^{-1}	2.8×10^{-2}	1.4×10^{-2}	6.5×10^{-2}	oil	6.0 (±0.8)
6c	3,4,5-$(OMe)_3$	4-SMe	1.9×10^{-4}	5.4×10^{-3}	1.8×10^{-5}	4.0×10^{-6}	3.3×10^{-3}	oil	6.2 (±0.5)
6d	3,4,5-$(OMe)_3$	4-Me	9.4×10^{-4}	2.4×10^{-2}	2.3×10^{-3}	8.3×10^{-4}	6.6×10^{-3}	oil	2.0 (±0.2)
6e	3,4,5-$(OMe)_3$	4-Et	1.2×10^{-2}	7.2×10^{-2}	2.7×10^{-3}	8.6×10^{-4}	7.5×10^{-3}	oil	3.4 (±0.3)
6f	3,4,5-$(OMe)_3$	4-Pr^i	6.6×10^{-3}	1.4×10^{-3}	2.4×10^{-3}	4.7×10^{-4}	7.0×10^{-2}	oil	12 (±2)
6g	3,4,5-$(OMe)_3$	4-Bu^t	1.02	1.57	8.8×10^{-1}	2.1×10^{-1}	4.32	oil	>40
6h	3,4-$(OMe)_2$	4-OMe	>25	>25	>25	>25	>25	oil	18 (±0.6)
6i	3,5-$(OMe)_2$	4-OMe	1.3×10^{-1}	1.6×10^{-1}	3.4×10^{-1}	4.2×10^{-1}	9.8×10^{-2}	oil	3.8 (±0.3)
6j	3,5-$(OMe)_2$; 4-OBn	4-OMe	1.04	1.92	9.5×10^{-1}	6.1×10^{-1}	>25	oil	>40
6k	3,5-$(OMe)_2$; 4-OSi(*t*-Bu)Me_2	4-OMe	>25	>25	9.0	>25	>25	oil	>40
6l	3,5-$(OMe)_2$; 4-OAc	4-OMe	21.5	>25	8.7	0.6	>25	oil	24 (±5)
1	3,4,5-$(OMe)_3$	4-OMe	3.7×10^{-4}	6.2×10^{-4}	2.6×10^{-4}	2.6×10^{-4}	1.6×10^{-3}	oil	2.5 (±0.1)
2	(combretastatin A-4)		3.2×10^{-4}	5.6×10^{-3}	1.0×10^{-2}	1.4×10^{-4}	4.3×10^{-4}		2.0 (±0.3)
adriamycin			2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}		

Table II. Trans Stilbenes



7

no.	R′	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
7a	3,4,5-$(OMe)_3$	4-OEt	1.7×10^{-1}	7.5×10^{-1}	1.49	1.17	2.2×10^{-1}	87–88	>40
7b	3,4,5-$(OMe)_3$	4-OPr^n	9.2	12.5	>25	>25	>25	82–83	>40
7c	3,4,5-$(OMe)_3$	4-SMe	4.7×10^{-1}	5.9×10^{-2}	8.3×10^{-2}	2.8×10^{-1}	7.3	109–111	>40
7d	3,4,5-$(OMe)_3$	4-Me	1.1	1.9	9.0×10^{-1}	8.0×10^{-1}	6.3	125–127	>40
7e	3,4,5-$(OMe)_3$	4-Et	1.3×10^{-1}	1.2	1.1×10^{-1}	1.7×10^{-1}	2.2×10^{-1}	98–100	>40
7f	3,4,5-$(OMe)_3$	4-Pr^i	9.8	18.4	6.8	11.1	>25	74–75	>40
7g	3,4,5-$(OMe)_3$	4-Bu^t	>25	>25	>25	>25	>25	127–128	>40
7h	3,4-$(OMe)_2$	4-OMe	11.7	>25	>25	>25	>25	135–137	>40
7i	3,5-$(OMe)_2$	4-OMe	7.5	9.7	6.9	8.8×10^{-1}	>25	55–56	>40
7j	3,5-$(OMe)_2$; 4-OBn	4-OMe	>25	>25	17.8	>25	>25	104–105	>40
7k	3,5-$(OMe)_2$; 4-OSi(*t*-Bu)Me_2	4-OMe	>25	>25	>25	>25	>25	118–120	>40
7l	3,5-$(OMe)_2$; 4-OAc	4-OMe	16.4	19.4	11.7	10.2	21	129–131	>40

acylation reaction using the acid chloride derived from **24**. The desired indane **26** was obtained by treatment of **25** with hydrogen in the presence of palladium on charcoal.

Several conformationally restricted congeners of the dihydrostilbene **1a** were prepared based on the 1-benzylisoquinoline ring system. In these compounds, the rotation about the bond connecting the trimethoxybenzene ring and the attached carbon of the dihydrostilbene moiety is restricted. Compounds **27–29** and **32** (Scheme VII) are known compounds that were resynthesized by a modification of the route originally published by Kupchan et al.[25] Treatment of **27** with DDQ gave derivative **30**, which was methylated using methyl iodide to afford compound **31**.

A conformationally rigid tetrahydroprotoberberine analogue of **1a** was also synthesized as shown in Scheme VIII. Acylation of the primary amino group of **33** with acetyl chloride gave the acetamide derivative **34**. A Bischler–Napieralski reaction involving the treatment of **34** with phosphorus oxychloride afforded the dihydroisoquinoline **35**. Reaction of **35** with the acid chloride **36** yielded **37**, which underwent the enamide photocyclization reaction to give the substituted protoberberine **38**.[26] Reduction of **38** by sequential treatment with lithium aluminum hydride and sodium borohydride yielded the desired tetrahydroprotoberberine **39**. In this compound, each of the three C–C bonds connecting the two aromatic rings of the 1,2-diphenylmethane moiety is conformationally restricted.

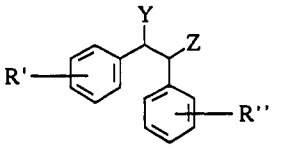
Biological Results and Discussion

The effects on cell growth and tubulin polymerization of 43 new stilbene analogues are summarized in Tables I–V. This group of compounds includes twelve cis stilbenes (**6a–l**), the corresponding 12 trans stilbenes (**7a–l**),

(25) Kubota, S.; Masui, T.; Fujita, E.; Kupchan, S. M. The Structure and Total Synthesis of Takatonine. *J. Org. Chem.* **1966**, *31*, 516–520.

(26) Ninomiya, I.; Naito, T.; Takasugi, H. Studies on the Synthesis of Heterocyclic Compounds. Part 698. An Alternative Protoberberine Synthesis; Total Synthesis of (±)-Xylopinine, (±)-Schefferine, (±)-Nandanine, (±)-Corydaline, and (±)-Thalictricavine. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1151–1155.

Table III. Dihydrostilbenes

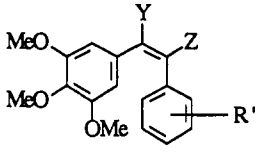


8

no.	R′	Y	Z	R″	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
8a	3,4,5-$(OMe)_3$	H	H	4-OEt	1.9×10^{-1}	1.9×10^{-1}	1.8×10^{-1}	1.7×10^{-1}	2.7×10^{-1}	oil	10 (±1)
8b	3,4,5-$(OMe)_3$	H	H	4-OPr^n	7.2	3.9	6.4	6.7	15.0	oil	>40
8c	3,4,5-$(OMe)_3$	H	H	4-SMe	1.5×10^{-1}	2.0×10^{-1}	4.0×10^{-1}	2.4×10^{-1}	1.3	52–54	>40
8d	3,4,5-$(OMe)_3$	H	H	4-Me	1.8×10^{-1}	2.2×10^{-1}	1.0×10^{-1}	2.7×10^{-1}	1.4	51–52	21 (±3)
8e	3,4,5-$(OMe)_3$	H	H	4-Et	8.8×10^{-2}	1.6×10^{-1}	1.6×10^{-2}	4.7×10^{-2}	2.7×10^{-1}	oil	18 (±1)
8f	3,4,5-$(OMe)_3$	H	H	4-$O(CH_2)_2NMe_2$	>25	10.3	9.8	11.4	>25	oil	>40
8g	3,4,5-$(OMe)_3$	H	H	4-$O(CH_2)_2NEt_2$	6.8	4.3	5.2	8.5	>25	oil	>40
8h	3,4,5-$(OMe)_3$	CN	H	4-OMe	9.6×10^{-3}	1.4×10^{-2}	7.5×10^{-3}	4.1×10^{-3}	1.6×10^{-2}	82–83	11 (±0.4)
8i	3,4,5-$(OMe)_3$	H	CN	4-OMe	11.5	14.3	9.4	6.4	21.1	102–103	>40
8j	3,4,5-$(OMe)_3$	H	COOMe	4-OMe	>25	>25	>25	>25	>25	84–85	>40
1a	3,4,5-$(OMe)_3$	H	H	4-OMe	1.8×10^{-4}	1.6×10^{-4}	2.5×10^{-4}	1.4×10^{-4}	1.8×10^{-4}	73–75	7.9 (±0.8)[a]

[a] Previously published value.[19] A different tubulin preparation was used in the earlier study.

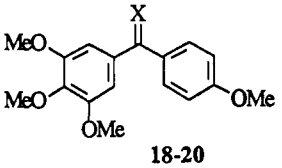
Table IV. Compounds 14a–c and 15a–f



14, 15

no.	Y	Z	R′	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
14a	COOH	H	4-OMe	13.9	12.8	8.4	9.1	>25	187–189	>40
14b	COOH	H	3-OMe	2.5×10^{-2}	>25	1.2×10^{-1}	5.0×10^{-2}	>25	178–180	>40
14c	H	COOH	4-OMe	5.2	1.9	5.9	2.3	>25	206–207	>40
15a	COOMe	H	4-OMe	1.1×10^{-2}	2.0×10^{-2}	9.5×10^{-3}	6.4×10^{-3}	9.6	74–75	>40
15b	COOMe	H	3-OMe	1.3	1.3	7.0×10^{-1}	1.5	15.5	87–88	>40
15c	CONHMe	H	4-OMe	2.4×10^{-2}	5.0×10^{-2}	2.6×10^{-2}	2.4×10^{-2}	9.3	172–174	35 (±2)
15d	CONHEt	H	4-OMe	3.4	3.7	1.8	7.05	>25	152–154	>40
15e	$COO(CH_2)_2NEt_2$	H	4-OMe	1.8	2.1	2.8	2.7	>25	oil	>40
15f	$COO(CH_2)_2NEt_2$	H	3-OMe	7.7	10.4	>25	6.7	>25	oil	>40
1	H	H	4-OMe	3.7×10^{-4}	6.2×10^{-4}	2.6×10^{-4}	2.6×10^{-4}	1.6×10^{-3}	oil	2.5 (±0.1)

Table V. Compounds 18–20



18-20

no.	X	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
18	O	1.1×10^{-2}	1.5×10^{-2}	1.3×10^{-2}	1.2×10^{-2}	1.3×10^{-2}	72–73	7.4 (±0.4)
19	H, OH	1.5	1.9	1.2	1.5	16.8	104–105	>40
20	H_2	1.5×10^{-1}	1.9×10^{-2}	1.3×10^{-2}	1.2×10^{-2}	1.3×10^{-1}	66–67	15 (±0.5)

10 dihydrostilbenes (**8a–j**), nine cis stilbene derivatives with substitution on the bridge connecting the two phenyl rings (compounds **14a–c** and **15a–f**), and three analogues of compound **1a** with a one-carbon bridge between the two phenyl rings (compounds **18–20**). In addition to these stilbenes, the activities of the conformationally restricted phenanthrenes **23a–d**, indanes **25** and **26**, 1-benzylisoquinolines **28–32**, and protoberberines **38** and **39** were determined and are detailed in Table VI.

In an earlier study, modifications were performed on (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (**1**) by rotating the four methoxy groups of both A- and B-rings to different positions, and it was established that their exact locations as in compound **1** were essential for

Table VI. Conformationally Restricted Analogues

no.	cytotoxicity (ED_{50} in μM) A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	inhibn of tubulin polym IC_{50} (μM) (±SD)
23a	5.7	1.8	1.9	1.3	21.4	-	>40
23b	>25	>25	1.1	12.5	>25	68–70	>40
23c	>25	>25	>25	>25	>25	142–4	>40
23d	>25	14.6	9.3	12.0	>25	80–2	>40
24	14.3	>25	9.4	7.4	>25	-	>40
25	>25	>25	>25	>25	>25	104–6	>40
26	>25	>25	12.8	19.5	>25	-	>40
28	19.5	>25	20.5	2.1	>25	180–2	>40
29	19.3	>25	20.2	>25	>25	-	>40
30	>25	>25	>25	>25	>25	-	>40
31	>25	>25	>25	>25	>25	-	>40
32	11.4	22.7	9.7	8.8	>25	-	>40
38	>25	>25	>25	>25	>25	196–8	>40
39	>25	>25	>25	>25	>25	104–6	>40

the pronounced cytotoxicity and antitubulin activity of 1.[19] As an extension of that investigation, two more cis stilbene derivatives were synthesized in which the 5-OMe or 4-OMe substituents were removed (compounds **6h** and **6i**, respectively) and these changes resulted in complete loss (**6h**: ED_{50} > 25 μM in all cell lines) or significant reduction (**6i**: ED_{50} in the 10^{-1} μM range) of cytotoxic activity. The ability of **6i** to inhibit tubulin polymerization (IC_{50} 3.8 μM) was not greatly reduced relative to that of 1 (IC_{50} 2.5 μM), while the activity of **6h** (IC_{50} 18 μM) was about 1 order of magnitude less than that of 1.

Next, we studied replacement of the 4-OMe group of the B-ring. Seven cis stilbenes were prepared with the methoxy group replaced by OEt, OPr^n, SMe, Me, Et, Pr^i, or Bu^t groups (compounds **6a**, **6b**, **6c**, **6d**, **6e**, **6f**, and **6g**, respectively). Substitution with the large Bu^t group on **6g** resulted in the reduction of cytotoxicity by approximately 3–4 orders of magnitude, and this modification greatly diminished ability to inhibit tubulin polymerization (IC_{50} > 40 μM). However, compounds **6a–f** were highly cytotoxic towards all five cancer cell lines, with potencies ranging from 100 times less to 100 times greater than that of combretastatin A-4. Replacement of the OMe of the B-ring with an SMe group (compound **6c**) resulted in a compound which was as cytotoxic as the parent compound 1 in the A-549 and MLM cell cultures. However, the thiomethyl compound was about 1 order of magnitude less cytotoxic than 1 in the MCF cell culture, while being about 1 order of magnitude more potent than 1 in HT-29 cells and 2 orders of magnitude more potent in SKMEL-5 cells. The thiomethyl compound **6c** is an analogue of thiocolchicine, which is more potent as a tubulin polymerization inhibitor and is more cytotoxic in certain cell cultures than colchicine.[2,5,6] Substitution with Pr^i (compound **6f**) decreased cytotoxicity somewhat (ED_{50} 7.0×10^{-2} to 4.7×10^{-4} μM range), as did substitution with an OPr^n group (compound **6b**). In addition to cytotoxicity, compounds **6a–f** retained significant activity as inhibitors of tubulin polymerization relative to 1. The decreased antitubulin activity of the 4-isopropyl compound **6f** and the lack of activity of the 4-*tert*-butyl compound **6g** demonstrates that an increase in steric bulk at this position results in a decrease in activity. Of particular interest is the enhancement of antitubulin activity which occurred with a reduction in size of the 4-substituent in the B-ring. The only new compound more effective than the parent compound 1 as an inhibitor of tubulin polymerization was **6d**, in which a methyl group replaced the 4-methoxy group of 1. The potency of this agent as a tubulin polymerization inhibitor was equivalent to combretastatin A-4 (**2**), the natural product, even though it lacks the adjacent hydroxyl group in the B-ring.

The potent cytotoxicities displayed by compound 1 and the related substances **6a–f** in Table I are of interest in relation to a recent QSAR study of combretastatins published by Lien and co-workers.[27] According to their analysis, the optimal Hansch–Fujita π constant $\Sigma\pi_b$, which is an estimation of the optimal lipophilicity of ring B, for cytotoxicity should be in the range of –0.69 to –0.71. This corresponds to the ring B substitution pattern found in combretastatin A-4 (**2**). The $\Sigma\pi_b$ value of ring B in compound 1 is only –0.02.[28] In spite of this, the cytotoxicity of 1 is not less than that of **2** in the cell cultures utilized in the present study. A wider diversity of structures would be useful in the QSAR analysis.

Consistent with earlier observations,[17,19] all the trans stilbenes (compounds **7a–l**) were less potent than their corresponding cis isomers. Compounds **7a**, **7c** and **7f** showed moderate cytotoxicity (in 1.0×10^{-1} μM range) in at least three cell lines, and the other compounds were less potent. None of these trans isomers significantly inhibited tubulin polymerization at concentrations up to 40 μM.

Turning to the cis stilbenes with substitution on the olefinic bridge (Table IV), introduction of substitutions on either the 1 or 2 position of the olefin reduced the cytotoxicity by from 1 to at least 5 orders of magnitude. In separate experiments, a COOH group was introduced on position 1 or 2 of the olefinic linkage, and this resulted in the formation of compounds **14a** and **14c** (ED_{50} 1.9 to > 25 μM). However, when the COOH group of compound **14a** was converted to the methyl ester (compound **15a**) or the *N*-methylamide (compound **15c**), the cytotoxicity increased 2–3 orders of magnitude in at least four cell cultures (as compared to **14a**). Compounds **15a** and **15c** had ED_{50} values of 5.0×10^{-2} to 6.4×10^{-3} μM in A-549, MCF-7, HT-29, and SKMEL-5 cell cultures. However, the (diethylamino)ethyl esters (compounds **15e** and **15f**) or the *N*-ethylamide (compound **15d**) of compound **14a** were minimally cytotoxic. Transfer of the B-ring methoxy group in compound **14a** to the 3-position (compound **14b**) resulted in about 10–100-fold increase in the cytotoxicity in three cell lines, and similar movement in compound **15a**

(27) Nandy, P.; Banerjee, S.; Gao, H.; Hui, M. B. V.; Lien, E. J. Quantitative Structure-Activity Relationship Analysis of Combretastatins: A Class of Novel Antimitotic Agents. *Pharm. Res.* **1991**, *8*, 776–781.

(28) Lien, E. J. Molecular Structure, Properties and States of Matter. In *Remington's Pharmaceutical Sciences*, 18th ed.; Mack: Easton, PA, 1990; pp 158–181.

(compound **15b**) reduced the cytotoxicity by 100–1000-fold. With the exception of **15c**, which inhibited tubulin polymerization with an IC_{50} of 35 μM, none of the cis stilbenes substituted on the olefin inhibited tubulin polymerization at concentrations up to 40 μM.

Among the dihydrostilbene analogues of **1a** (Table III), five compounds (**8a**, **8c–e**, and **8h**) had ED_{50} values of less than 1 μM in at least four cell lines, with 3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)propanenitrile (**8h**) being the most potent, both as a cytotoxic agent and as a tubulin polymerization inhibitor. However, this compound was about 10–100-fold less cytotoxic than 1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (**1a**), although its activity as a tubulin polymerization inhibitor (IC_{50} 11 μM) differed little from that of **1a** (IC_{50} 7.9 μM). While in the cis stilbene series, substitution of the B-ring methoxy with ethoxy, methyl, or ethyl reduced cytotoxicity by a maximum of 2 orders of magnitude, similar changes in the dihydrostilbene derivatives (compounds **8a**, **8d**, and **8e**) reduced cytotoxicity about 100–1000-fold. These dihydro compounds were also less potent as tubulin polymerization inhibitors. In the absence of the 3-hydroxyl group in the B-ring of combretastatin A-4 we have routinely observed a much larger loss of antitubulin activity upon reduction of the cis stilbene to the dihydrostilbene than the approximately 50% loss of activity that occurred when combretastatin A-4 was reduced.[17] Similarly, substitution with OPr^n, SMe, $O(CH_2)_2NMe_2$, or $O(CH_2)_2NEt_2$ groups (compounds **8b**, **8c**, **8f**, and **8g**) also decreased cytotoxicity.

Introduction of a CN group adjacent to the A-ring of **1a** (compound **8h**) merits further discussion. As noted above, this modification reduced cytotoxicity by 10–100-fold, while a similar introduction of a CN group adjacent to the B-ring (compound **8i**) reduced cytotoxicity by 10000-fold. Consistent with these cytotoxicity data, **8i** did not inhibit tubulin polymerization (IC_{50} > 40 μM). This relationship of **8h** and **8i** is identical to that observed when hydroxyl groups were introduced into corresponding positions in dihydrocombretastatin A-4.[17] Moreover, the data with the dihydrocombretastatin derivatives indicate that only one of the two stereoisomers with the hydroxyl at the bridge carbon adjacent to the A-ring is highly active.[17] If this is also the case when a nitrile group is placed at this position, then the active stereoisomer of **8h** would be nearly as active as **1a** as an inhibitor of in vitro tubulin polymerization. The active stereoisomer of **8h** would not, however, be more potent than the cis stilbene **1**.

Conversion of the cyano group in compound **8i** to a COOMe did not restore activity. This modification resulted in the formation of the inactive compound **8j**, (ED_{50} > 25 μM in all cell cultures, IC_{50} > 40 μM in the tubulin polymerization assay).

Several stilbenes and dihydrostilbenes containing acidic and basic groups were synthesized in an effort to obtain substances that could be more readily formulated. Examples of such compounds are **8f,g**, **14a–c**, and **15e,f**. None of these compounds inhibited tubulin polymerization, nor were they very cytotoxic.

In another set of modifications, the two-carbon bridge in 1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (**1a**) was reduced to a one-carbon bridge (compounds **18**, **19**, and **20**, Table V). All of these compounds were less potent than **1a**. 3,4,4′,5-Tetramethoxybenzophenone (**18**) was about 100 times less cytotoxic than **1a**, although its inhibitory effect on tubulin polymerization (IC_{50} 7.4 μM) was essentially identical to that of **1a** (IC_{50} 7.9 μM). Conversion of **18** to the alcohol **19** further reduced cytotoxicity 100 times and resulted in loss of inhibitory effect on tubulin polymerization (IC_{50} > 40 μM). Hydrogenolysis of alcohol **19** to 4-methoxyphenyl-(3,4,5-trimethoxyphenyl)methane (**20**) restored cytotoxic activity in the MCF-7, HT-29, and SKMEL-5 systems to levels comparable to those obtained with **18**, and also increased activity in the A-549 and MLM cell cultures. These enhanced effects on cytotoxicity were reflected in increased activity of **20** as an inhibitor (IC_{50} 15 μM) of tubulin polymerization.

The antitubulin activities of conformationally restricted analogues of the stilbene **1** and the dihydrostilbene **1a** are included in Table VI. These compounds, without exception, were not inhibitors of tubulin polymerization. Particularly striking is the inactivity of the phenanthrene **23b** (IC_{50} > 40 μM) in comparison to the antitubulin activity of the stilbene **1** (IC_{50} 2.5 μM). The data indicate that the active conformation of the stilbene **1** is not planar. In this context, it should be pointed out that the planar conformation of **1** is a high-energy species due to a nonbonded interaction between the protons of the two aromatic rings that are ortho to the bridge. Consequently, a totally planar conformation of **1** is not expected to exist to any appreciable extent. The X-ray structure of combretastatin A-1 reveals that the normals to the least squares planes of the two phenyl rings are inclined 66° to each other.[29] This likely represents a low energy conformation which may be involved in binding at the receptor site. Consistent with this hypothesis is the well-documented and recognized fact that the planes of the trimethoxybenzene ring and the other oxygen-substituted ring in podophyllotoxin,[30–32] colchicine,[33–36] steganacin,[8,12] and combretastatin A-4[29] exist in similar dihedral relationships, so that these natural products presumably resemble each other structurally to some extent when bound at the receptor site.

The results also imply that in the active conformation of **1a** the dihedral angle between the two bridge bonds connected to the aromatic rings approaches 0°, so that the conformation would resemble the structure of the cis alkene **1**. This might explain the inactivity of the indane derivative **26**, since in this case the dihedral angle between the relevant bonds would be closer to 120°. The inactivity

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of the benzylisoquinolines in Scheme VII is more difficult to rationalize on conformational grounds because the benzyl group is more conformationally mobile. However, the tetrahydroprotoberberine system **39** is more conformationally restricted, with a dihedral angle between the relevant bonds labeled "a" and "b" in structure **39** of about 165°.

The lack of activity of the compounds in Table VI as inhibitors of tubulin polymerization was reflected in their low cytotoxicities. None of these compounds had ED_{50} values of less than 1 μM in any of the cell lines.

In summary, only limited modifications can be made in the structures of combretastatin A-4 (**2**) and its tetramethoxy analogue (**1**) without substantially compromising cytotoxic and antitubulin activity. The cis-stilbene configuration confers optimal activity, and all bridge substituents that have been tried to date reduce activity. The methoxy groups at positions 3, 4, and 5 in the A-ring and at position 4 in the B-ring are all required, although a number of sterically small alternatives for the B-ring substituent yield compounds with good activity. In one case, with a methyl (compound **6d**) instead of a methoxy (compound **1**) substituent in the B-ring, a compound with enhanced antitubulin activity was obtained. It will be interesting to determine whether similar alterations in the A-ring substituents will enhance activity of combretastatin A-4 analogues.

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 Chemagnetics A-200, Nicolet QE-300, Varian VXR-500S, or Gemini 200 spectrometers with TMS as the internal standard in $CDCl_3$; ^{13}C NMR on a Gemini 200 spectrometer; IR spectra on a Beckman IR-33 spectrometer or on a Perkin-Elmer 1600 series FTIR. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values were within ±0.4% of the calculated compositions. 4-*tert*-Butylbenzaldehyde (**4g**) was prepared from *tert*-butylbenzene as reported in the literature.[37]

4-(Benzyloxy)-3,5-dimethoxybenzaldehyde (4j). A mixture of syringaldehyde (3.64 g, 20 mmol), benzyl chloride (2.52 g, 20 mmol), NaI (2 g), and potassium carbonate (2.76 g, 20 mmol) in anhydrous acetone (60 mL) was refluxed for 5 h and cooled to room temperature. The solid materials were removed by filtration, the filtrate was concentrated, and the residue was purified by chromatography on silica gel (230–400 mesh, 50 g) using 5% EtOAc in hexane as the eluent to obtain **4j** (4.3 g, 79%): mp 62–63 °C (lit.[20] mp 63 °C).

4-[(*tert*-Butyldimethylsilyl)oxy]-3,5-dimethoxybenzaldehyde (4k). To a well-stirred solution of syringaldehyde (3.64 g, 20 mmol) and *N,N*-diisopropylethylamine (4.87 g, 30 mmol) in dry DMF (30 mL) at 0 °C, *tert*-butyldimethylsilyl chloride (3 g, 20 mmol) was added, and stirring was continued for 2 h at 0 °C and at room temperature for 10 h. The mixture was poured into ice–water (500 mL), and the product was extracted with hexane (3 × 70 mL). The combined hexane extracts were washed with water (4 × 70 mL) and dried (Na_2SO_4). Evaporation of solvents gave compound **4k** as a white crystalline solid (5.17 g, 87%). An analytical sample was prepared by recrystallization from anhydrous ethanol: mp 70–71 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 9.81 (s, 1 H), 7.09 (s, 2 H), 3.85 (s, 6 H), 0.99 (s, 9 H), 0.14 (s, 6 H). Anal. ($C_{15}H_{24}O_4Si$) C, H.

General Procedure for the Preparation of Stilbenes 6a–k. Sodium hydride (0.2 g) was added to a well-stirred suspension of the phosphonium bromide **5a,b** (2 mmol) and the aldehyde **4a–k** (2 mmol) in THF (30 mL), and the mixture was stirred at room temperature for 24 h. The mixture was cooled to 0 °C, and the excess sodium hydride was quenched by careful addition of methanol (5 mL). Solvents were removed at reduced pressure, and the residue was subjected to preparative thin-layer chromatography on silica gel using 20% EtOAc in hexane as the eluent to yield the *Z* and *E* isomers in pure form.

(*Z*)-1-(4-Ethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6a): 313 mg, 44%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.8 Hz, 2 H), 6.78 (d, J = 8.8 Hz, 2 H), 6.52 (d, J = 12.1 Hz, 1 H), 6.51 (s, 2 H), 6.41 (d, J = 12.1 Hz, 1 H), 4.01 (q, J = 7.0 Hz, 2 H), 3.84 (s, 3 H), 3.69 (s, 6 H), 1.39 (t, J = 7.0 Hz, 3 H); CIMS (isobutane) m/e 315 (MH^+, 100). Anal. ($C_{19}H_{22}O_4$) C, H.

(*Z*)-1-(4-*n*-Propoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6b): 346 mg, 53%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.8 Hz, 2 H), 6.78 (d, J = 8.8 Hz, 2 H), 6.52 (s, 2 H), 6.52 (d, J = 12.2 Hz, 1 H), 6.41 (d, J = 12.2 Hz, 1 H), 3.88 (t, J = 6.6 Hz, 2 H), 3.84 (s, 3 H), 3.69 (s, 6 H), 1.79 (sextet, J = 6.6 Hz, 2 H), 1.02 (t, J = 6.6 Hz, 3 H); CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{20}H_{24}O_4$) C, H.

(*Z*)-1-[4-(Methylthio)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6c): 319 mg, 51%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.4 Hz, 2 H), 7.13 (d, J = 8.4 Hz, 2 H), 6.50 (bs, 2 H), 6.49 (s, 2 H), 3.84 (s, 3 H), 3.69 (s, 6 H), 2.46 (s, 3 H); CIMS (isobutane) m/e 317 (MH^+, 100). Anal. ($C_{18}H_{20}O_3S$) C, H.

(*Z*)-1-(4-Methylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6d): 294 mg, 50%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.20 (d, J = 8.0 Hz, 2 H), 7.07 (d, J = 8.0 Hz, 2 H), 6.56 (d, J = 12.2 Hz, 1 H), 6.49 (s, 2 H), 6.45 (d, J = 12.2 Hz, 1 H), 3.83 (s, 3 H), 3.67 (s, 6 H), 2.31 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 153.28, 137.56, 137.30, 134.77, 133.14, 130.35, 129.82, 129.22, 106.31, 61.09, 55.99, 21.27; CIMS (isobutane) m/e 285 (MH^+, 100). Anal. ($C_{19}H_{20}O_3$) C, H.

(*Z*)-1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6e): 321 mg, 54%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.21 (d, J = 8.1 Hz, 2 H), 7.00 (d, J = 8.1 Hz, 2 H), 6.57 (d, J = 12.1 Hz, 1 H), 6.48 (s, 2 H), 6.46 (d, J = 12.1 Hz, 1 H), 3.84 (s, 3 H), 3.66 (s, 6 H), 2.61 (q, J = 7.4 Hz, 2 H), 1.20 (t, J = 7.4 Hz, 3 H); CIMS (isobutane) m/e 299 (MH^+, 100). Anal. ($C_{19}H_{22}O_3$) C, H.

(*Z*)-1-[4-(2-Propyl)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6f): 340 mg, 55%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.2 Hz, 2 H), 7.13 (d, J = 8.2 Hz, 2 H), 6.60 (d, J = 12.2 Hz, 1 H), 6.46 (s, 2 H), 6.46 (d, J = 12.2 Hz, 1 H), 3.83 (s, 3 H), 3.65 (s, 6 H), 2.88 (septet, J = 7.0 Hz, 1 H), 1.27 (d, J = 7.0 Hz, 6 H); CIMS (isobutane) m/e 313 (MH^+, 100). Anal. ($C_{20}H_{24}O_3$) C, H.

(*Z*)-1-(4-*tert*-Butylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6g): 192 mg, 31%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.29 (d, J = 8.4 Hz, 2 H), 7.23 (d, J = 8.4 Hz, 2 H), 6.60 (d, J = 12.2 Hz, 1 H), 6.46 (d, J = 12.2 Hz, 1 H), 6.45 (s, 2 H), 3.83 (s, 3 H), 3.64 (s, 6 H), 1.29 (s, 9 H); CIMS (isobutane) m/e 327 (MH^+, 100%). Anal. ($C_{21}H_{26}O_3$) C, H.

(*Z*)-1-(4-Methoxyphenyl)-2-(3,4-dimethoxyphenyl)ethene (6h): 280 mg, 46%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.8 Hz, 2 H), 6.83–6.75 (m, 5 H), 6.46 (s, 2 H), 3.87 (s, 3 H), 3.79 (s, 3 H), 3.65 (s, 3 H); CIMS (isobutane) m/e 271 (MH^+, 100). Anal. ($C_{17}H_{18}O_3$) C, H.

(*Z*)-1-(3,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)ethene (6i): 241 mg, 45%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.22 (d, J = 8.8 Hz, 2 H), 6.77 (d, J = 8.8 Hz, 2 H), 6.54 (d, J = 12.2 Hz, 1 H), 6.46 (d, J = 2.3 Hz, 2 H), 6.44 (d, J = 12.2 Hz, 1 H), 6.32 (t, J = 2.3 Hz, 1 H), 3.79 (s, 3 H), 3.67 (s, 6 H); CIMS (isobutane) m/e 271 (MH^+, 100). Anal. ($C_{17}H_{18}O_3$) C, H.

(*Z*)-1-[4-(Benzyloxy)-3,5-dimethoxyphenyl]-2-(4-methoxyphenyl)ethene (6j): 249 mg, 33%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.52–7.45 (m, 2 H), 7.41–7.26 (m, 3 H), 7.21 (d, J = 8.7 Hz, 2 H), 6.78 (d, J = 8.75 Hz, 2 H), 6.52 (d, J = 12.1 Hz, 1 H), 6.49 (s, 2 H), 6.42 (d, J = 12.1 Hz, 1 H), 5.01 (s, 2 H), 3.79 (s, 3 H), 3.66 (s, 6 H); CIMS (isobutane) m/e 377 (MH^+, 100). Anal. ($C_{24}H_{24}O_4$) C, H.

(*Z*)-1-[4-[(*tert*-Butyldimethylsilyl)oxy]-3,5-dimethoxyphenyl]-2-(4-methoxyphenyl)ethene (6k): 277 mg, 35%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.23 (d, J = 8.8 Hz, 2 H), 6.76 (d, J = 8.8 Hz, 2 H), 6.49 (s, 2 H), 6.45 (s, 2 H), 3.78 (s, 3 H), 3.63 (s, 6 H), 1.02 (s, 9 H), 0.14 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 159.21, 151.90, 134.04, 129.63, 129.21, 113.95, 106.47, 55.86, 55.51, 26.06, 18.96, −4.49. Anal. ($C_{23}H_{32}O_4Si$) C, H.

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(*E*)-1-(4-**Ethoxyphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7a**): 127 mg, 20%; mp 87–88 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.44 (d, J = 8.7 Hz, 2 H), 6.97 (d, J = 16.2 Hz, 1 H), 6.90–6.80 (m, 3 H), 6.72 (s, 2 H), 4.34 (q, J = 7.2 Hz, 2 H), 3.91 (s, 6 H), 3.88 (s, 3 H), 1.38 (t, J = 7.2 Hz, 3 H); CIMS (isobutane) m/e 315 (MH^+, 100). Anal. ($C_{19}H_{22}O_4$) C, H.

(*E*)-1-(4-*n*-**Propoxyphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7b**): 187 mg, 28%; mp 82–83 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.44 (d, J = 8.8 Hz, 2 H), 6.95–6.87 (m, 4 H), 6.72 (s, 2 H), 3.93 (t, J = 6.6 Hz, 2 H), 3.91 (s, 6 H), 3.89 (s, 3 H), 1.82 (sextet, J = 6.6 Hz, 2 H), 1.04 (t, J = 6.6 Hz, 3 H); CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{20}H_{24}O_4$) C, H.

(*E*)-1-[4-(**Methylthio**)**phenyl**]-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7c**): 178 mg, 28%; mp 109–111 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.44 (d, J = 8.5 Hz, 2 H), 7.24 (d, J = 8.5 Hz, 2 H), 6.99 (s, 2 H), 6.73 (s, 2 H), 3.92 (s, 6 H), 3.87 (s, 3 H), 2.51 (s, 3 H); CIMS (isobutane) m/e 317 (MH^+, 100). Anal. ($C_{18}H_{20}O_3S$) C, H.

(*E*)-1-(4-**Methylphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7d**): 121 mg, 21%; mp 125–127 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.40 (d, J = 8.1 Hz, 2 H), 7.16 (d, J = 8.1 Hz, 2 H), 6.98 (s, 2 H), 6.73 (s, 2 H), 3.91 (s, 6 H), 3.87 (s, 3 H), 2.35 (s, 3 H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 153.84, 138.19, 137.90, 134.81, 133.68, 129.80, 128.50, 128.00, 126.71, 103.74, 61.12, 56.25, 21.30; CIMS (isobutane) m/e 285 (MH^+, 100). Anal. ($C_{18}H_{20}O_3$) C, H.

(*E*)-1-(4-**Ethylphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7e**): 182 mg, 30%; mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.44 (d, J = 8.1 Hz, 2 H), 7.20 (d, J = 8.1 Hz, 2 H), 7.00 (s, 2 H), 6.74 (s, 2 H), 3.92 (s, 6 H), 3.87 (s, 3 H), 2.66 (q, J = 7.4 Hz, 2 H), 1.26 (t, J = 7.4 Hz, 3 H); CIMS (isobutane) m/e 299 (MH^+, 100). Anal. ($C_{19}H_{22}O_3$) C, H.

(*E*)-1-[4-(2-**Propyl**)**phenyl**]-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7f**): 151 mg, 24%; mp 74–75 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.45 (d, J = 8.2 Hz, 2 H), 7.23 (d, J = 8.2 Hz, 2 H), 7.00 (s, 2 H), 6.74 (s, 2 H), 3.93 (s, 6 H), 3.87 (s, 3 H), 2.92 (septet, J = 7.0 Hz, 1 H), 1.27 (d, J = 7.0 Hz, 6 H); CIMS (isobutane) m/e 313 (MH^+, 100). Anal. ($C_{20}H_{24}O_3$) C, H.

(*E*)-1-(4-*tert*-**Butylphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethene** (**7g**): 143 mg, 23%; mp 127–128 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.46 (d, J = 8.7 Hz, 2 H), 7.38 (d, J = 8.7 Hz, 2 H), 7.0 (s, 2 H), 6.74 (s, 2 H), 3.92 (s, 6 H), 3.87 (s, 3 H), 1.34 (s, 9 H); CIMS (isobutane) m/e 327 (MH^+, 100%). Anal. ($C_{21}H_{26}O_3$) C, H.

(*E*)-1-(4-**Methoxyphenyl**)-2-(3,4-**dimethoxyphenyl**)**ethene** (**7h**): 110 mg, 20%; mp 135–137 °C (lit.[38] 133–135 °C).

(*E*)-1-(3,5-**Dimethoxyphenyl**)-2-(4-**methoxyphenyl**)**ethene** (**7i**): 123 mg, 23%; mp 55–56 °C (lit.[39] 55–56 °C).

(*E*)-1-[4-(**Benzyloxy**)-3,5-**dimethoxyphenyl**]-2-(4-**methoxyphenyl**)**ethene** (**7j**): 207 mg, 28%; mp 104–105 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.55–7.48 (m, 2 H), 7.45 (d, J = 8.8 Hz, 2 H), 7.40–7.25 (m, 3 H), 6.98 (d, J = 16.1 Hz, 1 H), 6.90 (d, J = 8.8 Hz, 2 H), 6.89 (d, J = 16.1 Hz, 1 H), 6.71 (s, 2 H), 5.03 (s, 2 H), 3.87 (s, 6 H), 3.83 (s, 3 H); CIMS (isobutane) m/e 377 (MH^+, 100). Anal. ($C_{24}H_{24}O_4$) C, H.

(*E*)-1-[4-[(*tert*-**Butyldimethylsilyl**)**oxy**]-3,5-**dimethoxyphenyl**]-2-(4-**methoxyphenyl**)**ethene** (**7k**): 224 mg, 28%; mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.42 (d, J = 8.8 Hz, 2 H), 6.91 (s, 2 H), 6.88 (d, J = 8.8 Hz, 2 H), 6.69 (s, 2 H), 3.84 (s, 6 H), 3.82 (s, 3 H), 1.01 (s, 9 H), 0.14 (s, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 159.67, 152.33, 130.97, 130.87, 127.98, 127.49, 127.04, 114.59, 103.93, 56.08, 55.61, 26.03, 18.54, −4.42. Anal. ($C_{23}H_{32}O_4Si$) C, H.

Preparation of Acetates 6l and 7l. A solution of *n*-Bu_4NF in THF (1 M, 2 mL, 2 mmol) was added to a solution of stilbenes **6k** and **7k** (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 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% EtOAc in hexane as the eluent afforded the desired products.

(*Z*)-1-(4-**Acetoxy**-3,5-**dimethoxyphenyl**)-2-(4-**methoxyphenyl**)**ethene** (**6l**): 111 mg, 33%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.24 (d, J = 8.6 Hz, 2 H), 6.78 (d, J = 8.6 Hz, 2 H), 6.55 (d, J = 12.1 Hz, 1 H), 6.53 (s, 2 H), 6.43 (d, J = 12.1 Hz, 1 H), 3.77 (s, 3 H), 3.64 (s, 6 H), 2.32 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.27, 159.27, 152.24, 136.00, 130.66, 130.53, 129.79, 128.84, 127.93, 113.91, 105.85, 56.11, 55.37, 20.51; CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{19}H_{20}O_5$) C, H.

(*E*)-1-(4-**Acetoxy**-3,5-**dimethoxyphenyl**)-2-(4-**methoxyphenyl**)**ethene** (**7l**): 137 mg, 41%; mp 129–131 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.45 (d, J = 8.8 Hz, 2 H), 6.97–6.88 (m, 4 H), 6.73 (s, 2 H), 3.87 (s, 6 H), 3.83 (s, 3 H), 2.35 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 169.35, 159.87, 152.70, 136.55, 130.18, 128.95, 128.13, 126.73, 114.48, 103.16, 56.28, 55.48, 20.55; CIMS (isobutane) m/e 329 (MH^+, 100). Anal. ($C_{19}H_{20}O_5$) C, H.

General Procedure for the Preparation of Dihydrostilbenes 8a–e. A mixture of *E*-stilbenes (**7**) and the corresponding *Z*-stilbenes (**6**) (1 mmol) in EtOAc was hydrogenated at 40 psi in the presence of 10% palladium on charcoal (50 mg) for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated, yielding the dihydrostilbene derivatives **8a–e**. Analytical samples were prepared by preparative thin-layer chromatography on silica gel using 20% EtOAc in hexane as the eluent.

1-(4-**Ethoxyphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8a**): 250 mg, 80%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.06 (d, J = 8.5 Hz, 2 H), 6.80 (d, J = 8.5 Hz, 2 H), 6.34 (s, 2 H), 4.32 (q, J = 7.3 Hz, 2 H), 3.81 (s, 3 H), 3.80 (s, 6 H), 2.82 (s, 4 H), 1.40 (t, J = 7.3 Hz, 3 H); CIMS (isobutane) m/e 317 (MH^+, 100). Anal. ($C_{19}H_{24}O_4$) C, H.

1-(4-*n*-**Propoxyphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8b**): 284 mg, 86%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.09 (d, J = 8.6 Hz, 2 H), 6.83 (d, J = 8.6 Hz, 2 H), 6.37 (s, 2 H), 3.90 (t, J = 6.6 Hz, 2 H), 3.82 (s, 9 H), 2.84 (s, 4 H), 1.80 (m, 2 H), 1.03 (t, J = 7.4 Hz, 3 H); CIMS (isobutane) m/e 331 (MH^+, 100). Anal. ($C_{20}H_{26}O_4$) C, H.

1-[4-(**Methylthio**)**phenyl**]-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8c**): 276 mg, 86%; mp 52–54 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.21 (d, J = 8.1 Hz, 2 H), 7.11 (d, J = 8.1 Hz, 2 H), 6.36 (s, 2 H), 3.82 (bs, 9 H), 2.86 (bs, 4 H), 2.47 (s, 3 H); CIMS (isobutane) m/e 319 (MH^+, 100). Anal. ($C_{18}H_{22}O_3S$) C, H.

1-(4-**Methylphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8d**): 247 mg, 86%; mp 51–52 °C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.09 (s, 4 H), 6.38 (s, 2 H), 3.83 (s, 3 H), 3.82 (s, 6 H), 2.85 (bs, 4 H), 2.32 (s, 3 H); CIMS (isobutane) m/e 287 (MH^+, 100). Anal. ($C_{18}H_{22}O_3$) C, H.

1-(4-**Ethylphenyl**)-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8e**): 261 mg, 87%, oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.12 (s, 4 H), 6.37 (s, 2 H), 3.83 (s, 3 H), 3.82 (s, 6 H), 2.86 (bs, 4 H), 2.63 (q, J = 7.6 Hz, 2 H), 1.23 (t, J = 7.6 Hz, 3 H); CIMS (isobutane) m/e 301 (MH^+, 100). Anal. ($C_{19}H_{24}O_3$) C, H.

General Procedure for the Preparation of Compounds 8f,g. A mixture of 1-(4-hydroxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (**9**)[19] (288 mg, 1 mmol), aminoalkyl chloride hydrochloride **10a,b** (1.1 mmol), and potassium carbonate (276 mg, 2 mmol) in acetone (15 mL) was heated at reflux for 12 h, and the solids were removed by filtration. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel using 5% methanol in $CHCl_3$ as the eluent. Both of these compounds were obtained as viscous oils.

1-[4-[2-(*N*,*N*-**Dimethylamino**)**ethoxy**]**phenyl**]-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8f**): 243 mg, 68%, oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.08 (d, J = 8.5 Hz, 2 H), 6.85 (d, J = 8.5 Hz, 2 H), 6.36 (s, 2 H), 4.09 (t, J = 5.5 Hz, 2 H), 3.82 (s, 3 H), 3.81 (s, 6 H), 2.85–2.80 (m, 6 H), 2.41 (s, 6 H); CIMS (isobutane) m/e 360 (MH^+, 100). Anal. ($C_{21}H_{29}NO_4$) C, H.

1-[4-[2-(*N*,*N*-**Diethylamino**)**ethoxy**]**phenyl**]-2-(3,4,5-**trimethoxyphenyl**)**ethane** (**8g**): 296 mg, 76%, oil; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.10 (d, J = 8.5 Hz, 2 H), 6.84 (d, J = 8.5 Hz, 2 H), 6.38 (s, 2 H), 4.08 (t, J = 6.2 Hz, 2 H), 3.85 (s, 3 H), 3.84 (s, 6 H), 2.94 (t, J = 6.2 Hz, 2 H), 2.86–2.82 (m, 4 H), 2.71 (q, J = 7.1 Hz, 4 H), 1.11 (t, J = 7.1 Hz, 6 H); CIMS (isobutane)

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m/e 388 (MH^+, 100). Anal. ($C_{23}H_{33}NO_4$) C, H.

Typical Procedure for Preparation of Compounds 8h–j. A solution of compound **11a** (2 mmol) in THF (20 mL) was added to a well-stirred solution of LDA (2 mmol) in THF (22 mL) at −78 °C and stirring continued for 30 min. To this 4-methoxybenzyl bromide (**12a**) (2 mmol) was added and stirring continued at −78 °C for 1 h and at room temperature for 6 h. The reaction mixture was quenched by the addition of glacial acetic acid (2 mL), and the solvents were distilled off at reduced pressure. The residue was treated with water (20 mL), and the product was extracted with ether (2 × 70 mL). The combined ether extracts were washed with water and dried (Na_2SO_4). Evaporation of the ether and recrystallization of the residue from CH_2Cl_2–hexane gave compound **8h**. Compounds **8i** and **8j** were prepared by using the same method.

3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-propanenitrile (8h): 320 mg, 49%; mp 82–83 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.05 (d, *J* = 8.5 Hz, 2 H), 6.83 (d, *J* = 8.5 Hz, 2 H), 6.41 (s, 2 H), 3.89 (t, *J* = 7.2 Hz, 1 H), 3.84 (s, 3 H), 3.81 (s, 6 H), 3.78 (s, 3 H), 3.12–3.07 (m, 2 H); CIMS (isobutane) *m/e* 328 (MH^+, 100). Anal. ($C_{19}H_{21}NO_4$) C, H.

2-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-propanenitrile (8i): 450 mg, 69%; mp 102–103 °C (lit.[22] mp 96.5–97.5 °C); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.13 (d, *J* = 8.5 Hz, 2 H), 6.85 (d, *J* = 8.5 Hz, 2 H), 6.28 (s, 2 H), 3.92 (t, *J* = 6.7 Hz, 1 H), 3.80 (s, 3 H), 3.76 (s, 3 H), 3.75 (s, 6 H), 3.06–3.00 (m, 2 H); CIMS (isobutane) *m/e* 328 (MH^+, 100). Anal. ($C_{19}H_{21}NO_4$) C, H.

Methyl 2-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-propanoate (8j): 533 mg, 74%; mp 84–85 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.22 (d, *J* = 8.5 Hz, 2 H), 6.85 (d, *J* = 8.5 Hz, 2 H), 6.29 (s, 2 H), 3.80 (s, 3 H), 3.78 (s, 6 H), 3.77 (s, 3 H), 3.62 (s, 3 H), 3.42–3.24 (m, 2 H), 3.00 (m, 1 H); CIMS (isobutane) *m/e* 361 (MH^+, 100%). Anal. ($C_{20}H_{24}O_6$) C, H.

General Procedure for the Preparation of Compounds 14a–c. A mixture of phenylacetic acid **13a,b** (2 mmol), benzaldehyde **41,m** (2 mmol), and triethylamine (0.5 mL) in acetic anhydride (5 mL) was heated at reflux for 12 h, poured into hot saturated sodium carbonate solution (50 mL), and left overnight. The mixture was extracted with ether (2 × 50 mL), and the ether extracts were discarded. The aqueous solution was acidified with dilute HCl, and the precipitated product was filtered and dried. Recrystallization from EtOAc–hexane gave pure product.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-prop-2-enoic acid (14a): 523 mg, 76%; mp 187–189 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 9.8 (bs, 1 H), 7.89 (s, 1 H), 7.07 (d, *J* = 8.9 Hz, 2 H), 6.73 (d, *J* = 8.9 Hz, 2 H), 6.47 (s, 2 H), 3.91 (s, 3 H), 3.79 (s, 6 H), 3.78 (s, 3 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.90, 161.31, 154.15, 142.79, 138.04, 133.26, 131.51, 129.09, 127.07, 114.19, 106.87, 61.14, 56.25, 55.43; CIMS (isobutane) *m/e* 345 (MH^+, 100). Anal. ($C_{19}H_{20}O_6$) C, H.

(*E*)-3-(3-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-prop-2-enoic acid (14b): 483 mg, 70%; mp 178–180 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.70 (bs, 1 H), 7.90 (s, 1 H), 7.15 (t, *J* = 8.1 Hz, 1 H), 6.85–6.76 (m, 2 H), 6.62 (bs, 1 H), 6.49 (s, 2 H), 3.88 (s, 3 H), 3.78 (s, 6 H), 3.55 (s, 3 H); CIMS (isobutane) *m/e* 345 (MH^+, 100). Anal. ($C_{19}H_{20}O_6$) C, H.

(*E*)-2-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)-prop-2-enoic acid (14c): 468 mg, 68%; mp 206–207 °C (lit.[22] mp 207–208 °C).

Preparation of Compounds 15a,b. Concentrated H_2SO_4 (0.5 mL) was added to a stirred solution of carboxylic acid **14a,b** (172 mg, 0.5 mmol) in absolute methanol (20 mL), and the mixture was heated under reflux for 6 h. About 90% of the excess methanol was removed by evaporation, and the residue was poured into ice–water (300 mL). The product was extracted with ether (2 × 40 mL), and the combined extracts were washed with 2% aqueous NaOH solution (2 × 50 mL) followed by water (200 mL). Evaporation of ether from the dried (Na_2SO_4) solution gave the desired products.

(*E*)-Methyl 3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enoate (15a): 316 mg, 88%; mp 74–75 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.77 (s, 1 H), 7.03 (d, *J* = 9.0 Hz, 2 H), 6.72 (d, *J* = 9.0 Hz, 2 H), 6.44 (s, 2 H), 3.91 (s, 3 H), 3.81 (s, 3 H), 3.78 (s, 6 H), 3.77 (s, 3 H); CIMS (isobutane) *m/e* 359 (MH^+, 100). Anal. ($C_{20}H_{22}O_6$) C, H.

(*E*)-Methyl 3-(3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enoate (15b): 308 mg, 86%; mp 87–88 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.79 (s, 1 H), 7.13 (t, *J* = 8.1 Hz, 1 H), 6.82–6.70 (m, 2 H), 6.59 (bs, 1 H), 6.46 (s, 2 H), 3.88 (s, 3 H), 3.83 (s, 3 H), 3.77 (s, 6 H), 3.54 (s, 3 H); CIMS (isobutane) *m/e* 359 (MH^+, 100). Anal. ($C_{20}H_{22}O_6$) C, H.

(*E*)-*N*-Methyl-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enamide (15c). A mixture of carboxylic acid **14a** (172 mg, 0.5 mmol) and thionyl chloride (1 mL) in benzene (10 mL) was refluxed for 6 h. The excess thionyl chloride and benzene were removed at reduced pressure, and the residue was kept under vacuum for 30 min. It was subsequently mixed with aqueous methylamine solution (40%, 5 mL) and kept at room temperature for 2 h. The precipitated product was filtered, washed sequentially with 2% NaOH solution and water, and dried. An analytical sample was prepared by recrystallization from EtOAc–hexane: 156 mg, 87%; mp 172–174 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.79 (s, 1 H), 6.99 (d, *J* = 8.8 Hz, 2 H), 6.71 (d, *J* = 8.8 Hz, 2 H), 6.46 (s, 2 H), 5.10 (bq, 1 H), 3.94 (s, 3 H), 3.81 (s, 6 H), 3.76 (s, 3 H), 2.87 (d, *J* = 4.8 Hz, 3 H); CIMS (isobutane) *m/e* 358 (MH^+, 100). Anal. ($C_{20}H_{23}NO_5$) C, H.

Preparation of Compounds 15d–f. A solution of ethylamine (0.5 mL) or the appropriate amino alcohol (0.5 mmol) in THF (5 mL) was added to a solution of the acid chlorides (prepared from **14a,b** in 0.5-mmol scale, as described above) in THF (10 mL). The mixture was stirred for 3 h. Solvents were removed at reduced pressure, and the residue was poured onto ice (200 g). The product was extracted with ether (2 × 20 mL), washed with water, and dried (Na_2SO_4). Evaporation of ether gave crude products. Product **15d** was purified by recrystallization from EtOAc–hexane, and the liquid products **15e** and **15f** were purified by column chromatography on silica gel using ether as the eluent.

(*E*)-*N*-Ethyl-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enamide (15d): 149 mg, 80%; mp 152–154 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.77 (s, 1 H), 6.99 (d, *J* = 8.4 Hz, 2 H), 6.70 (d, *J* = 8.4 Hz, 2 H), 6.46 (s, 2 H), 5.58 (bt, 1 H), 3.95 (s, 3 H), 3.80 (s, 6 H), 3.76 (s, 3 H), 3.36 (q, *J* = 7.1 Hz, 2 H), 1.11 (t, *J* = 7.1 Hz, 3 H); CIMS (isobutane) *m/e* 372 (MH^+, 100). Anal. ($C_{21}H_{25}NO_5$) C, H.

(*E*)-2-(*N*,*N*-Diethylamino)ethyl 3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enoate (15e): 192 mg, 87%, oil; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.77 (s, 1 H), 7.06 (d, *J* = 8.8 Hz, 2 H), 6.72 (d, *J* = 8.8 Hz, 2 H), 6.44 (s, 2 H), 4.28 (t, *J* = 6.1 Hz, 2 H), 3.90 (s, 3 H), 3.78 (s, 6 H), 3.77 (s, 3 H), 2.77 (t, *J* = 6.1 Hz, 2 H), 2.55 (q, *J* = 7.2 Hz, 4 H), 1.01 (t, *J* = 7.2 Hz, 6 H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 168.49, 160.90, 154.06, 140.53, 137.88, 132.88, 132.16, 130.19, 127.42, 114.10, 106.89, 63.94, 61.14, 56.25, 55.41, 50.98, 47.89, 12.04; CIMS (isobutane) *m/e* 444 (MH^+, 100). Anal. ($C_{25}H_{33}NO_6$) C, H.

(*E*)-2-(*N*,*N*-Diethylamino)ethyl 3-(3-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-enoate (15f): 201 mg, 91%, oil; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.78 (s, 1 H), 7.13 (d, *J* = 7.9 Hz, 1 H), 6.80–6.74 (m, 2 H), 6.61–6.59 (m, 1 H), 6.46 (s, 2 H), 4.30 (t, *J* = 6.1 Hz, 2 H), 3.87 (s, 3 H), 3.78 (s, 6 H), 3.54 (s, 3 H), 2.77 (t, *J* = 6.1 Hz, 2 H), 2.56 (q, *J* = 7.1 Hz, 4 H), 1.05 (t, *J* = 7.1 Hz, 6 H); CIMS (isobutane) *m/e* 444 (MH^+, 100). Anal. ($C_{25}H_{33}NO_6$) C, H.

3,4,4′,5-Tetramethoxybenzophenone (18). Anhydrous $AlCl_3$ (260 mg, 2 mmol) was added to a well-stirred solution of 3,4,5-trimethoxybenzoyl chloride (**16**) (461 mg, 2 mmol) and anisole (216 mg, 2 mmol) at 0 °C in CH_2Cl_2 (25 mL). The mixture was stirred while allowing it to warm to room temperature. After 6 h, the resultant dark reaction mixture was poured into ice-cold 5% HCl (20 mL), and the CH_2Cl_2 layer was separated. The aqueous layer was extracted with an additional 30 mL of CH_2Cl_2, and the combined CH_2Cl_2 solutions were washed with saturated sodium bicarbonate solution. Evaporation of solvents from the dried CH_2Cl_2 extract and purification of the residue by chromatography on a column of silica gel, using 5% EtOAc in hexane as eluent, gave product **18** (487 mg, 80%): mp 72–73 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.83 (d, *J* = 8.7 Hz, 2 H), 7.03 (s, 2 H), 6.98 (d, *J* = 8.7 Hz, 2 H), 3.94 (s, 3 H), 3.90 (s, 3 H), 3.88 (s, 6 H); CIMS (isobutane) *m/e* 303 (MH^+, 100). Anal. ($C_{17}H_{18}O_5$) C, H.

(4-Methoxyphenyl)(3,4,5-trimethoxyphenyl)methanol (19). Sodium borohydride (76 mg, 2 mmol) was added in small portions to a well-stirred solution of 3,4,4′,5-tetramethoxybenzophenone

(18) (302 mg, 1 mmol) in ethanol (15 mL) at 0 °C over 15 min, and the resultant mixture was stirred for 3 h at room temperature. The reaction was quenched by careful addition of glacial acetic acid (1 mL), and the solvents were removed at reduced pressure. The residue was poured into water, and the product was extracted with ether (2 × 50 mL). The combined ether extracts were washed with saturated $NaHCO_3$ solution, followed by water, and dried (Na_2SO_4). Evaporation of solvents and crystallization of the residue from EtOAc–hexane gave product **19** as a white crystalline solid (287 mg, 94%): mp 104–105 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.29 (d, J = 8.7 Hz, 2 H), 6.88 (d, J = 8.7 Hz, 2 H), 6.60 (s, 2 H), 5.73 (d, J = 3.2 Hz, 1 H), 3.82 (s, 9 H), 3.80 (s, 3 H), 2.32 (d, J = 3.2 Hz, 1 H); CIMS (isobutane) m/e 305 (MH^+, 100). Anal. ($C_{17}H_{20}O_5$) C, H.

(4-Methoxyphenyl)(3,4,5-trimethoxyphenyl)methane (20). A solution of **19** (304 mg, 1 mmol) in EtOAc (20 mL) was hydrogenated at 60 psi in the presence of 10% Pd–C (60 mg) for 12 h. The solution was filtered, and solvents were evaporated. The crude product was purified by crystallization from EtOAc and hexane (183 mg, 60%): mp 66–67 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.12 (d, J = 8.5 Hz, 2 H), 6.85 (d, J = 8.5 Hz, 2 H), 6.39 (s, 2 H), 3.87 (s, 2 H), 3.82 (s, 3 H), 3.81 (s, 6 H), 3.79 (s, 3 H); CIMS (isobutane) m/e 289 (MH^+, 100). Anal. ($C_{17}H_{20}O_4$) C, H.

2,3,4,5-Tetramethoxyphenanthrene (23a) and 2,3,4,7-Tetramethoxyphenanthrene (23c). A mixture of **21a** and **22a** (1.1 g, 3.6 mmol) was dissolved in cyclohexane (500 mL) containing iodine (60 mg) and acetophenone (0.22 mL). The solution was irradiated with a 450-W medium pressure mercury UV lamp for 6 h with stirring and cooling. TLC showed that the starting material had disappeared. The solvent was evaporated and the residue subjected to flash chromatography (ether–hexane, 30:70 by volume, silica gel 230–400 mesh) to give **23a** (460 mg, 42%) and **23c** (560 mg, 52%). **23a**: pale yellow oil; IR (neat) 836 (2 H adjacent), 760 cm^{-1} (3 H adjacent); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.30–7.50 (m, 3 H), 7.00–7.10 (m, 3 H), 4.00 (s, br, 9 H), 3.70 (s, 3 H); EIMS m/e 298 (M^+, 58), 283 (11). Anal. ($C_{19}H_{18}O_4$) C, H. Compound **23c**: mp 142–144 °C; IR (KBr) 866 (1 H), 831 cm^{-1} (2 H adjacent); ^1H NMR ($CDCl_3$, 500 MHz) δ 9.41 (d, 1 H), 7.60 (s, 2 H), 7.23–7.21 (m, 2 H), 7.08 (s, 1 H), 4.03 (s, 3 H), 4.01 (s, 3 H), 4.00 (s, 3 H), 3.96 (s, 3 H); EIMS m/e 298 (M^+, 100), 283 (41). Anal. ($C_{19}H_{18}O_4$) C, H.

2,3,4,6-Tetramethoxyphenanthrene (23b). Compound **23b** (460 mg, 58% yield) was prepared by irradiation of a mixture of **1** and **22b** (800 mg, 2.66 mmol) in hexane (500 mL) as described above: mp 68–70 °C; IR (KBr) 865 (1 H), 843 cm^{-1} (2 H adjacent); ^1H NMR ($CDCl_3$, 200 MHz) δ 9.06 (d, J = 4 Hz, 1 H), 7.75 (d, J = 8 Hz, 1 H), 7.60 (d, J = 8.8 Hz, 1 H), 7.47 (d, J = 8 Hz, 1 H), 7.22 (dd, J = 8.6 and 2.8 Hz, 1 H), 7.08 (s, 1 H), 4.02 (s, 6 H), 4.01 (s, 3 H), 4.00 (s, 3 H); EIMS m/e 298 (M^+, 100), 283 (45). Anal. ($C_{18}H_{18}O_4$) C, H.

2,3,4,8-Tetramethoxyphenanthrene (23d). The stilbene mixture containing **21b** and **22c** (1010 mg, 3.36 mmol) in cyclohexane (500 mL) containing iodine (53 mg) and acetophenone (1.71 mmol, 0.5 equiv) was irradiated as in the above synthesis of **23a** and **23c** to give **23d** (760 mg, 76%): mp 80–82 °C; IR (KBr) 846 (2 H adjacent), 790 cm^{-1} (3 H adjacent); ^1H NMR ($CDCl_3$, 200 MHz) δ 9.12 (d, J = 10 Hz, 1 H), 8.20 (d, J = 10 Hz, 1 H), 7.57 (m, 2 H), 7.10 (s, 1 H), 6.99 (d, J = 8 Hz, 1 H), 4.03 (s, 6 H), 4.02 (s, 3 H), 4.00 (s, 3 H); EIMS m/e 298 (M^+, 100), 283 (40). Anal. ($C_{18}H_{18}O_4$) C, H.

2-(4-Methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)propionic Acid (24). A mixture of the ester **8j** (3.0 g, 8.3 mmol) in ethanol (50 mL) and potassium hydroxide (4.0 g, 71 mmol) in ethanol–water (60 mL, 4:1 by volume) was heated at reflux under argon until most of the starting material had disappeared (about 24 h). The reaction mixture was poured into ice-cold water (500 mL) and acidified with 20% H_2SO_4 acid (200 mL), extracted with ether (2 × 100 mL and 1 × 50 mL), washed with water (50 mL) and saturated sodium chloride solution (50 mL), and dried over anhydrous Na_2SO_4. Evaporation of the filtrate and flash chromatography (ether–hexane, 7:3 by volume as eluent, silica gel 230–400 mesh) gave **24** as a yellow oil (1.97 g, 79%): IR (neat) 3231 (br), 3005, 2933, 1733, 1703, 1590, 1513, 1462, 1421, 1246, 1180, 1123 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.32 (d, J = 10 Hz, 2 H), 6.85 (d, J = 10 Hz, 2 H), 6.83 (s, 2 H), 3.80 (s, 3 H), 3.79 (s, 3 H), 3.77 (m, 1 H), 3.74 (s, 6 H), 3.31 (m, 1 H), 2.95 (m, 1 H); FABMS m/e 347 (MH^+, 39.2); HRFABMS m/e 347.1489 ($C_{19}H_{22}O_6$ requires 347.1495). Anal. ($C_{19}H_{22}O_6$) C, H.

2-(4-Methoxyphenyl)-4,5,6-trimethoxyindan-3-one (25). A solution of the acid **24** (0.5 g, 1.4 mmol) in $POCl_3$ (5 mL, 53.4 mmol) was heated at reflux for 3 min. The dark red solution was poured onto crushed ice (30 g) and extracted with ether (3 × 40 mL). The combined ether extracts were dried (Na_2SO_4), and the solvent was evaporated to afford a gray solid. Crystallization of this solid from EtOAc and hexane afforded pale gray crystals (0.32 g, 70%): mp 104–106 °C; IR (KBr) 3010, 2960, 1697, 1595, 1512, 1323, 1251, 1139 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.11 (d, J = 8 Hz, 2 H), 6.85 (d, J = 8 Hz, 2 H), 6.71 (s, 1 H), 4.03 (s, 3 H), 3.96 (s, 3 H), 3.87 (s, 3 H), 3.78 (s, 3 H), 3.78 (m, 1 H), 3.54 (m, 1 H), 3.10 (m, 1 H); EIMS m/e 328 (M^+, 98). Anal. ($C_{19}H_{20}O_5$) C, H.

2-(4-Methoxyphenyl)-4,5,6-trimethoxyindan (26). A mixture of the ketone **25** (250 mg, 0.74 mmol) and 10% Pd–C (100 mg) in acetic acid (40 mL) was subjected to hydrogenolysis at 42 psi hydrogen pressure until the uptake of hydrogen ceased. The catalyst was removed by filtration, and the solvent was evaporated from the filtrate to leave the crude product as an oil, which was purified by flash chromatography on silica gel (230–400 mesh) using ether–hexane (7:3) to yield a colorless oil (230 mg, 96%): ^1H NMR ($CDCl_3$, 500 MHz) δ 7.22 (d, J = 8 Hz, 2 H), 6.72 (d, J = 8 Hz, 2 H), 6.59 (s, 1 H), 3.90 (s, 3 H), 3.88 (s, 3 H), 3.84 (s, 3 H), 3.80 (s, 3 H), 3.63 (m, 1 H), 3.36 (m, 1 H), 3.26 (m, 1 H), 2.97 (m, 2 H). EIMS m/e 314 (M^+, 100). Anal. ($C_{19}H_{22}O_4$) C, H.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxyisoquinolinium Methiodide (Takatonine Iodide, 28). A solution of **27** (200 mg, 0.59 mmol) in anhydrous decahydronaphthalene (5 mL) containing palladium black (20 mg) was heated at reflux for 2 h under argon. The reaction mixture was filtered through a Celite pad, and the Celite pad was rinsed with $CHCl_3$ (10 mL). After the $CHCl_3$ was evaporated, the residue was dissolved in ether (10 mL), and MeI (0.5 mL) was added. The resulting solution was kept at room temperature overnight. The yellow crystalline precipitate was filtered and washed with ether (5 mL) to give takatonine iodide (**28**)[25] as yellow plates (174.1 mg, 61%): mp 180–182 °C (lit.[25] mp 181–182 °C); ^1H NMR ($CDCl_3$, 200 MHz) δ 8.72 (d, J = 6 Hz, br, 1 H), 8.32 (d, J = 6 Hz, 1 H), 7.40 (s, 1 H), 7.01 (d, J = 8 Hz, 2 H), 6.84 (d, J = 8 Hz, 2 H), 5.11 (s, 2 H), 4.61 (s, 3 H), 4.14 (s, 3 H), 4.10 (s, 3 H), 4.01 (s, 3 H), 3.77 (s, 3 H).

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (29). $NaBH_4$ (460 mg, 12.9 mmol) was added portionwise to a solution of **27** (460 mg, 1.36 mmol) in methanol (5 mL) over a period of 30 min, and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated under reduced pressure. The residue was dissolved in water (5 mL), basified with ammonium hydroxide solution, and extracted with ether (3 × 20 mL). The combined ether layer was dried (Na_2SO_4). Evaporation of the solvent from the filtrate and flash chromatography (silica gel 230–400 mesh) using ether as the eluent gave compound **29** as an oil that was recrystallized from acetone–petroleum ether (450 mg, 96%): mp 84–86 °C (lit.[25] mp 85–87 °C); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.18 (d, J = 8 Hz, 2 H), 6.87 (d, J = 8 Hz, 2 H), 6.49 (s, 1 H), 4.06 (m, 1 H), 3.86 (s, 3 H), 3.85 (s, 3 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 3.16 (m, 2 H), 2.87 (m, 2 H), 2.68 (t, 2 H, J = 6 Hz), 1.84 (s, br, 1 H); FABMS m/e 344 (MH^+, 41).

1-(4′-Methoxybenzoyl)-5,6,7-trimethoxyisoquinoline (30). A solution of **27** (250 mg, 0.73 mmol) and DDQ (188 mg, 0.81 mmol) in anhydrous THF (2 mL) was heated at reflux overnight. Preparative TLC purification (ether, precoated silica gel plate, 1000 μm) gave **30** as an oil (125 mg, 48%): IR (neat) 2924, 2851, 1659, 1560, 1475, 1260, 1159, 1122 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz) δ 8.48 (d, J = 6 Hz, 1 H), 7.98 (d, J = 6 Hz, 1 H), 7.95 (d, J = 8 Hz, 2 H), 7.37 (s, 1 H), 6.96 (d, J = 8 Hz, 2 H), 4.08 (s, 3 H), 4.03 (s, 3 H), 3.93 (s, 3 H), 3.88 (s, 3 H); CIMS (isobutane) m/e 354 (MH^+, 100).

1-(4′-Methoxybenzoyl)-5,6,7-trimethoxyisoquinolinium Methiodide (31). A solution of **30** (70 mg, 0.2 mmol) in anhydrous benzene (2 mL) and iodomethane (0.6 mL) was heated at reflux for 24 h under argon. The reaction mixture was evaporated to dryness, and the residue was partitioned between distilled water (10 mL) and $CHCl_3$ (10 mL). The $CHCl_3$ layer was extracted with

H_2O (2 × 5 mL), and the combined aqueous extracts were washed with ether (5 mL). Evaporation of the water from the aqueous solution gave **31** as a yellow solid (60 mg, 60%): ^1H NMR ($CDCl_3$, 200 MHz) δ 8.95 (d, J = 6 Hz, 1 H), 8.52 (d, J = 6 Hz, 1 H), 8.11 (d, J = 8 Hz, br, 2 H), 7.10 (d, J = 8 Hz, 2 H), 6.73 (s, 1 H), 4.53 (s, 3 H), 4.15 (s, 3 H), 4.14 (s, 3 H), 3.92 (s, 3 H), 3.80 (s, 3 H); FABMS calcd for $C_{21}H_{22}INO_5$ 368.1498 (cation), found 368.1489.

1-(4′-Methoxybenzyl)-5,6,7-trimethoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (Tetrahydrotakatonine, 32). A solution of **29** (400 mg, 1.2 mmol) in formic acetic anhydride (80 mL) was stirred at room temperature overnight. A clear yellow solution was obtained. The solvent was evaporated to dryness. To this residue was added water (5 mL), and the aqueous solution was extracted with CH_2Cl_2 (3 × 15 mL). The CH_2Cl_2 layer was washed successively with 10% NaOH solution (5 mL), water (5 mL), and saturated aqueous NaCl (5 mL), and dried (Na_2SO_4). Evaporation of solvent from the filtrate gave an oil (590 mg). A solution of this oil (450 mg) in anhydrous toluene (10 mL) containing $POCl_3$ (2 mL) was heated at reflux for 3 h under argon. After evaporation of the solvent, the resulting brown residue was dissolved in methanol (30 mL). $NaBH_4$ (1.6 g) was added over 0.5 h, and the reaction mixture was stirred at room temperature for 2 h. Evaporation of the solvent gave a residue which was extracted with CH_2Cl_2 (3 × 15 mL). The organic layer was washed successively with water (10 mL) and saturated aqueous NaCl solution (10 mL) and dried (Na_2SO_4). Evaporation of solvent from the filtrate and flash chromatography ($CHCl_3$, then $CHCl_3$–EtOH, 96:4 by volume, silica gel 230–400 mesh) gave **32** as an oil (225 mg, 52%): ^1H NMR ($CDCl_3$, 200 MHz) δ 7.02 (d, J = 8 Hz, 2 H), 6.80 (d, J = 8 Hz, 2 H), 5.87 (s, 1 H), 3.84 (s, 3 H), 3.83 (s, 3 H), 3.78 (s, 3 H), 3.67 (t, J = 6 Hz, 1 H), 3.55 (s, 3 H), 3.13 (m, 2 H), 2.75 (m, 4 H), 2.51 (s, 3 H); FABMS m/e 358 (MH^+, 100). The ^1H NMR spectrum of **32** was consistent with the previously reported ^1H NMR of tetrahydrotakatonine.[25]

***N*-(2,3,4-Trimethoxyphenethyl)acetamide (34).** Acetyl chloride (1.3 mL, 1.45 g, 18.2 mmol) was added dropwise to a stirred suspension of compound **33** (3 g, 12.1 mmol) in 2.0 N NaOH solution (27 mL, 54.0 mmol) cooled in an ice bath. The resulting solution was stirred at 0 °C for 1 h. The reaction mixture was extracted with $CHCl_3$ (3 × 30 mL), and the combined $CHCl_3$ layer was washed with saturated NaCl solution and dried (Na_2SO_4). Evaporation of the filtrate gave a pale yellow oil that was subjected to flash chromatography on silica gel (230–400 mesh), eluting with ether to give compound **34** as an oil (2.75 g, 89%): ^1H NMR ($CDCl_3$, 200 MHz) δ 6.83 (d, J = 8 Hz, 1 H), 6.62 (d, J = 8 Hz, 1 H), 5.84 (s, br, 1 H), 3.90 (s, 3 H), 3.87 (s, 3 H), 3.85 (s, 3 H), 3.44 (q, J = 6 Hz, 2 H), 2.76 (t, J = 6 Hz, 2 H), 1.93 (s, 3 H); EIMS m/e 253 (M^+, 72).

1-Methyl-5,6,7-trimethoxy-3,4-dihydroisoquinoline (35). A solution of the acetamide **34** (280 mg, 1.1 mmol) in toluene (5 mL) containing $POCl_3$ (0.8 mL, 8.5 mmol) was heated at reflux under argon for 2 h. The excess $POCl_3$ and the solvent were evaporated under reduced pressure. The black residue was washed with petroleum ether (10 mL), and the residue was dissolved in distilled water (10 mL) and made basic by 5% aqueous NH_4OH (10 mL). The aqueous solution was extracted with $CHCl_3$ (3 × 15 mL). The combined $CHCl_3$ layer was washed successively with water (10 mL) and saturated NaCl solution (10 mL) and dried (Na_2SO_4). Evaporation of the filtrate and chromatography on silica gel (230–400 mesh) using Et_2O–EtOH (98:2) as the eluent gave compound **35** as a pale brown oil (230 mg, 89%): ^1H NMR ($CDCl_3$, 200 MHz) δ 6.84 (s, 1 H), 3.92 (s, 3 H), 3.89 (s, 3 H), 3.85 (s, 3 H), 3.61 (t, J = 8 Hz, 2 H), 2.64 (t, J = 8 Hz, 2 H), 2.36 (s, 3 H); EIMS m/e 235 (M^+, 84).

2,5-Dimethoxybenzoyl Chloride (36). A mixture of 2,5-dimethoxybenzoic acid (25 g, 137 mmol) and thionyl chloride (35 mL, 471 mmol) was heated at reflux under argon for 4 h. The reaction mixture was evaporated to dryness, and the residue was purified by distillation at 127 °C (2 mmHg) to give compound **36** as a pale yellow oil (26.5 g, 96%) which solidified on standing: mp 36–38 °C.

2-(2′,5′-Dimethoxybenzoyl)-1-methylene-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline (37). A solution of compound **36** (746 mg, 3.7 mmol) in anhydrous benzene (2 mL) was slowly added at room temperature to a solution of compound **35** (880 mg, 3.7 mmol) in anhydrous benzene (10 mL) containing triethylamine (568 mg, 5.6 mmol, 0.78 mL). The resulting solution was heated at reflux with stirring under argon for 2 h and cooled. The white crystalline solid of triethylamine hydrochloride was removed by filtration and the solvent evaporated from the filtrate to leave the product as an oil. It was purified by flash chromatography on silica gel (230–400 mesh), eluting with Et_2O containing 1% Et_3N to give compound **37** as an oil (1.3 g, 87%): ^1H NMR ($CDCl_3$, 200 MHz) δ 6.87 (s, 1 H), 6.85 (d, J = 10 Hz, 1 H), 6.80 (d, J = 3 Hz, 1 H), 6.70 (d, J = 10 Hz, 1 H), 5.21 (s, br, 1 H), 4.55 (s, br, 1 H), 3.90 (s, 6 H), 3.88 (s, 3 H), 3.84 (s, 3 H), 3.75 (s, 3 H), 3.41 (s, br, 2 H), 2.88 (t, J = 6 Hz, 2 H); CIMS (isobutane) m/e 400 (MH^+, 100).

5,8-Dihydro-8-oxo-2,3,4,10-tetramethoxy-6*H*-dibenzo[*a*,*g*]quinolizine (38). A stirred solution of compound **37** (1.59 g, 4.0 mmol) in methanol (500 mL) containing triethylamine (0.5 mL) was irradiated with a 450-W medium pressure mercury lamp and cooled at room temperature for about 2 h. Evaporation of the solvent gave a yellow syrup that was subjected to flash chromatography (silica gel 230–400 mesh), eluting with ether, to give a yellow solid. Recrystallization of the solid from methanol gave compound **38** as yellow needles (350 mg, 24%): mp 196–198 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.84 (d, J = 4 Hz, 1 H), 7.51 (d, J = 8 Hz, 1 H), 7.26 (dd, J = 8 and 4 Hz, 1 H), 7.09 (s, 1 H), 6.89 (s, 1 H), 4.34 (t, J = 6 Hz, 2 H), 3.97 (s, 3 H), 3.94 (s, 6 H), 3.91 (s, 3 H), 2.96 (t, J = 6 Hz, 2 H); CIMS (isobutane) m/e 368 (MH^+, 100). Anal. ($C_{21}H_{21}NO_5$) C, H.

5,8,13,13a-Tetrahydro-2,3,4,10-tetramethoxy-6*H*-dibenzo[*a*,*g*]quinolizine (39). A suspension of $LiAlH_4$ (1.4 mL, 1.4 mmol, 5 equiv, 1.0 M in THF) was added dropwise to a solution of compound **38** (100 mg, 0.27 mmol) in anhydrous THF (15 mL) with stirring at room temperature under argon. The reaction mixture was stirred under reflux for 2 h. The excess $LiAlH_4$ was decomposed by adding water until no hydrogen bubbles appeared. The residue was extracted with ether–THF (7:3 by volume, 30, then 20 mL). The combined organic layer was filtered through a glass wool pad, and the filtrate was evaporated to dryness. The residue was dissolved in fresh methanol (10 mL), and $NaBH_4$ (125 mg, 3.28 mmol) was added in several portions. The reaction mixture was stirred at reflux under Ar for 1.5 h. The reaction mixture was evaporated to dryness under vacuum. The residue was dissolved in 10% HCl (5 mL), neutralized with solid K_2CO_3 to pH 8, extracted with $CHCl_3$ (3 × 15 mL), and dried (Na_2SO_4). Evaporation of solvent from the filtrate obtained after removal of the Na_2SO_4 gave a pale yellow oil. Preparative silica gel TLC (silica gel precoated plate, 1000 μm), eluting with ether, gave compound **39** (92 mg, 95%). Recrystallization of this product from methanol gave the analytical sample as pale yellow needles: mp 104–106 °C; ^1H NMR ($CDCl_3$, 200 MHz) δ 7.07 (d, J = 8 Hz, 1 H), 6.75 (dd, J = 8 and 2 Hz, 1 H), 6.62 (d, J = 2 Hz, 1 H), 6.57 (s, 1 H), 3.88 (s, 6 H), 3.87 (s, 3 H), 3.79 (s, 3 H), 3.79 (m, 3 H), 3.21 (m, 2 H), 2.85 (m, 3 H), 2.52 (m, 1 H); FABMS (glycerol) m/e 356 (MH^+, 47). Anal. ($C_{21}H_{25}NO_4$) C, H.

Cytotoxicity Assays. An MTT colorimetric assay was employed according to the established procedure.[40,41] Since compounds **1** and **6a–f** had very low aqueous solubilities, all dilutions involving these compounds were performed in DMSO prior to the addition of 0.5 μL aliquots to each well. 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–V. The active compounds (ED_{50} < 25 μM) were tested again, and the values shown for these cytotoxic substances are the averages of two determinations. Compounds **6a**, **6d**, and **6e** were also examined in L1210 leukemia cells, and all three agents caused the accumulation of cells arrested in metaphase at cytotoxic concentrations.

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Tubulin Polymerization Inhibition Assays. Electrophoretically homogeneous tubulin was purified from bovine brain as described previously.[42] 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.

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Registry No. **3**, 134-96-3; **4a**, 10031-82-0; **4b**, 5736-85-6; **4c**, 3446-89-7; **4d**, 104-87-0; **4e**, 4748-78-1; **4f**, 122-03-2; **4g**, 939-97-9; **4h**, 120-14-9; **4i**, 7311-34-4; **4j**, 6527-32-8; **4k**, 106852-80-6; **5a**, 61240-20-8; **5b**, 1530-38-7; **6a**, 141172-07-8; **6b**, 141172-05-6; **6c**, 141172-06-7; **6d**, 141172-08-9; **6e**, 141172-09-0; **6f**, 141172-10-3; **6g**, 141172-11-4; **6h**, 106053-26-3; **6i**, 94608-23-8; **6j**, 141172-12-5; **6k**, 141172-13-6; **6l**, 141172-14-7; **7a**, 141172-15-8; **7b**, 141172-16-9; **7c**, 141172-17-0; **7d**, 141172-18-1; **7e**, 141172-19-2; **7f**, 141172-20-5; **7g**, 141172-21-6; **7h**, 18513-95-6; **7i**, 22255-22-7; **7j**, 141172-22-7; **7k**, 141172-23-8; **7l**, 141172-24-9; **8a**, 141197-76-4; **8b**, 141172-25-0; **8c**, 141172-26-1; **8d**, 141172-27-2; **8e**, 141172-28-3; **8f**, 141172-29-4; **8g**, 119041-22-4; **8h**, 141172-30-7; **8i**, 141172-31-8; **8j**, 141172-32-9; **9**, 39499-95-1; **10a**, 4584-46-7; **10b**, 869-24-9; **11a**, 13338-63-1; **11b**, 104-47-2; **12a**, 2746-25-0; **12b**, 21852-50-6; **13a**, 951-82-6; **13b**, 104-01-8; **14a**, 141172-33-0; **14b**, 141172-34-1; **14c**, 141172-35-2; **15a**, 141172-36-3; **15b**, 141197-64-0; **15c**, 141172-37-4; **15d**, 141172-38-5; **15e**, 141172-39-6; **15f**, 141172-40-9; **16**, 4521-61-3; **18**, 109091-08-9; **19**, 141172-41-0; **20**, 101747-36-8; **21a**, 97399-87-6; **21b**, 134029-50-8; **22a**, 97399-88-7; **22b**, 134029-62-2; **22c**, 134029-63-3; **23a**, 99257-48-4; **23b**, 141172-42-1; **23c**, 97399-69-4; **23d**, 141172-43-2; **24**, 141172-44-3; **25**, 141172-45-4; **26**, 141172-46-5; **27**, 47439-73-6; **28**, 4668-06-8; **29**, 141172-47-6; **30**, 84716-73-4; **31**, 141172-48-7; **32**, 4668-07-9; **33**, 3937-16-4; **34**, 107485-77-8; **35**, 107485-76-7; **36**, 17918-14-8; **37**, 141172-49-8; **38**, 141172-50-1; **39**, 141172-51-2; syringaldehyde, 134-96-3; *tert*-butyldimethylsilyl chloride, 18162-48-6; anisole, 100-66-3; acetophenone, 98-86-2.

2-Acetylpyridine Thiocarbonohydrazones. Potent Inactivators of Herpes Simplex Virus Ribonucleotide Reductase

Todd A. Blumenkopf,*,† Joan A. Harrington,‡ Cecilia S. Koble,† Donald D. Bankston,† Robert W. Morrison, Jr.,† Eric C. Bigham,† Virgil L. Styles,† and Thomas Spector‡

Divisions of Organic Chemistry and Experimental Therapy, Wellcome Research Laboratories, Burroughs Wellcome Co., Research Triangle Park, North Carolina 27709. *Received November 20, 1991*

A series of 2-acetylpyridine thiocarbonohydrazones was synthesized for evaluation as potential antiherpetic agents. The compounds were prepared by the condensation of 2-acetylpyridine with thiocarbonohydrazide followed by treatment with isocyanates or isothiocyanates. Many were found that were potent inactivators of ribonucleotide reductase encoded by HSV-1 and weaker inactivators of human enzyme. Several thiocarbonohydrazones (e.g. **38** and **39**) inactivated HSV-1 ribonucleotide reductase at rate constants as much as seven times that of lead compound **2**. In general, those substituted with weak electron-attracting groups offered the best combination of potency and apparent selective activity against the HSV-1 enzyme. Seven new thiocarbonohydrazones (**21**, **25**, **31**, **36**, **38**, **39**, and **40**) were apparently greater than 50-fold more selective than **2** against HSV-1 ribonucleotide reductase versus human enzyme. The results indicated new compounds worthy of further study as potentiators of acyclovir in combination topical treatment of herpes virus infections.

Introduction

Recurrent labial and perioral herpes simplex virus type 1 infections (HSV-1), the common cold sore or fever blister, are the most frequent cutaneous virus infections encountered in immunocompetent patients.[1] HSV-1 encodes a unique ribonucleotide reductase (EC 1.17.4.1) in infected cells[2,3] that catalyzes the reduction of all four ribonucleoside diphosphates to 2′-deoxynucleoside diphosphates.[4,5] In marked contrast to the mammalian enzyme, which is highly regulated by nucleoside triphosphates,[6,7] the viral enzyme is insensitive to allosteric control.[8-10] Indeed, HSV-1 is able to replicate in the presence of thymidine at concentrations that are inhibitory to host cell DNA synthesis.[4] This insensitivity permits unrestricted synthesis of 2′-deoxynucleotides in HSV-1 infected cells, and thereby suggests that the reductase may have significance as a potential antiviral target.[10]

† Division of Organic Chemistry.
‡ Division of Experimental Therapy.

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