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_1 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 μ L) of the supernatant obtained by centrifugation of the reaction mixture was injected into an HPLC column (C-18 reverse phase column, Econosphere, Alltech, $25 \text{ cm} \times 4.6 \text{ mm}$) 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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$$\frac{1}{k_{\rm obs}} = \frac{K_{\rm I}}{k_2[{\rm I}]} + \frac{1}{k_2}$$

The data for 2'-deoxy-2'-methyleneadenosine (44) are shown in Figures 3 and 4. For 44, K_1 and k_2 values of 13.1 μ M and 0.195 min⁻¹, respectively, were calculated.

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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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toxin,⁷⁻¹⁰ steganacin,^{8,11,12} and their synthetic analogues, inhibit tubulin polymerization. Structurally related biphenyls,¹³ diphenylmethanes,¹⁴ benzopyrans,¹⁵ and chalcones¹⁶ 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

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4-0E





-t-Bu

82-6 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

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

derivative 1a (Table III) was also found to be a potent

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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 61 and 71 (Tables I and II). The cis and trans geometries of the stilbenes were assigned by the characteristic ¹H

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Scheme III



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 4methoxyphenylacetate (11c) with 3,4,5-trimethoxybenzyl bromide 12b gave product 8j (Table III).

20: $R^1 = R^2 = H$



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



 MeO
 MeO
 R^1 R^2

 8j: R = CH₃
 25: R¹, R² = O

 24: R = H
 26: R¹ = R² = H

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)(3,4,5trimethoxyphenyl)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 $22a-c^{19,23,24}$



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



				cytoto	xicity (ED ₅₀	in µM)			inhibn of tubulin polym ICro
no.	R′	R ″	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(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}	$6.6 imes 10^{-1}$	2.8×10^{-2}	$1.4 imes 10^{-2}$	$6.5 imes 10^{-2}$	oil	6.0 (±0.8)
6c	$3,4,5-(OMe)_3$	4-SMe	1.9×10^{-4}	$5.4 imes 10^{-3}$	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	$2.4 imes 10^{-2}$	2.3×10^{-3}	8.3 × 10⁴	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 × 10⁴	7.5 × 10⁻³	oil	3.4 (±0.3)
6 f	$3,4,5-(OMe)_3$	4-Pr	6.6 × 10 ⁻³	$1.4 imes 10^{-3}$	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
6ħ	$3,4-(OMe)_2$	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}	$4.2 imes 10^{-1}$	$9.8 imes 10^{-2}$	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
6 k	$3,5-(OMe)_2; 4-OSi(t-Bu)Me_2$	4-OMe	>25	>25	9.0	>25	>25	oil	>40
61	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	$5.6 imes 10^{-3}$	1.0×10^{-2}	1.4 × 10⁴	4.3×10^{-4}		2.0 (±0.3)
adri	amycin		2.9×10^{-2}	3.1×10^{-2}	5.5×10^{-2}	3.2×10^{-2}	1.3×10^{-1}		

Table II. Trans Stilbenes



				cytoto		inhibn of tubulin polym ICro			
no.	R′	R″	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(μM) (±SD)
7a	3,4,5-(OMe) ₃	4-0Et	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	10 9– 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
7ĥ	$3,4-(OMe)_2$	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
71	3,5-(OMe) ₂ ; 4-OAc	4-OMe	16.4	19.4	11.7	10.2	21	12 9- -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-1), the corresponding 12 trans stilbenes (7a-1),

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Table III. Dihydrostilbenes



					cytotoxicity (ED ₅₀ in μ M)						inhibn of tubulin polym ICro
no.	R′	Y	Z	R″	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(μM) (±SD)
- 8a	3,4,5-(OMe) ₃	Н	Н	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	$3,4,5-(OMe)_3$	Н	н	4-OPr ⁿ	7.2	3.9	6.4	6.7	15.0	oil	>40
8c	$3,4,5-(OMe)_3$	н	н	4-SMe	1.5×10^{-1}	2.0×10^{-1}	4.0×10^{-1}	2.4×10^{-1}	1.3	52-54	>40
8 d	$3,4,5-(OMe)_3$	Н	н	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	$3,4,5-(OMe)_3$	Н	н	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)
8 f	$3,4,5-(OMe)_3$	Н	н	$4-O(CH_2)_2NMe_2$	>25	10.3	9.8	11.4	>25	oil	>40
8g	$3,4,5-(OMe)_3$	н	н	$4-O(CH_2)_2NEt_2$	6.8	4.3	5.2	8.5	>25	oil	>40
8 h	$3,4,5-(OMe)_3$	CN	н	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	$3,4,5-(OMe)_3$	Н	CN	4-OMe	11.5	14.3	9.4	6.4	21.1	102-103	>40
8j	$3,4,5-(OMe)_3$	Н	COOMe	4-OMe	>25	>25	>25	>25	>25	84-85	>40
1a	3,4,5-(OMe) ₃	Н	Н	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



					cytoto			inhibn of tubulin polym ICro		
no.	Y	Z	$\mathbf{R'}$	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(μM) (±SD)
14a	COOH	Н	4-OMe	13.9	12.8	8.4	9.1	>25	187-189	>40
1 4b	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
1 5a	COOMe	Н	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	Н	3-OMe	1.3	1.3	7.0×10^{-1}	1.5	15.5	87-88	>40
15c	CONHMe	н	4-OMe	2.4×10^{-2}	5.0×10^{-2}	2.6×10^{-2}	2.4×10^{-2}	9.3	172 - 174	35 (±2)
15 d	CONHEt	н	4-OMe	3.4	3.7	1.8	7.05	>25	152 - 154	>40
1 5e	COO(CH ₂) ₂ NEt ₂	Н	4-OMe	1.8	2.1	2.8	2.7	>25	oil	>40
15 f	COO(CH ₂) ₂ NEt ₂	H	3-OMe	7.7	10.4	>25	6.7	>25	oil	>40
1	Н	Н	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



			inhibn of tubulin polym IC ₅₀					
no.	Х	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(μM) (±SD)
18 19 20	О Н, ОН Н ₂	1.1×10^{-2} 1.5 1.5×10^{-1}	1.5×10^{-2} 1.9 1.9×10^{-2}	1.3×10^{-2} 1.2 1.3×10^{-2}	1.2×10^{-2} 1.5 1.2×10^{-2}	1.3×10^{-2} 16.8 1.3×10^{-1}	72-73 104-105 66-67	7.4 (±0.4) >40 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

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Table VI. Conformatio	nally Restricted Analog	ues
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		cyte	otoxicity (ED ₅₀		tubulin polym ICro		
no.	A-549	MCF-7	HT-29	SKMEL-5	MLM	mp, °C	(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**: $ED_{50} > 25 \ \mu$ M in all cell lines) or significant reduction (**6i**: ED_{50} in the $10^{-1} \ \mu$ M range) of cytotoxic activity. The ability of **6i** to inhibit tubulin polymerization (IC₅₀ 3.8 μ M) was not greatly reduced relative to that of 1 (IC₅₀ 2.5 μ M), while the activity of **6h** (IC₅₀ 18 μ M) 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, OPrⁿ, SMe, Me, Et, Prⁱ, 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 (IC₅₀) > 40 μ M). 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 (ED₅₀ 7.0 \times 10⁻² to 4.7 $\times 10^{-4} \,\mu M$ range), as did substitution with an OPrⁿ 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–1) were less potent than their corresponding cis isomers. Compounds 7a, 7c and 7f showed moderate cytotoxicity (in $1.0 \times 10^{-1} \mu M$ 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 (ED₅₀ 1.9 to > 25 μ M). 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 $6.4 \times 10^{-3} \,\mu\text{M}$ 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

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(compound 15b) reduced the cytotoxicity by 100–1000-fold. With the exception of 15c, which inhibited tubulin polymerization with an IC₅₀ of 35 μ M, none of the cis stilbenes substituted on the olefin inhibited tubulin polymerization at concentrations up to 40 μ M.

Among the dihydrostilbene analogues of 1a (Table III), five compounds (8a, 8c-e, and 8h) had ED₅₀ values of less than 1 μ M in at least four cell lines, with 3-(4-methoxyphenvl)-2-(3.4.5-trimethoxyphenvl)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-(4methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (1a), although its activity as a tubulin polymerization inhibitor (IC₅₀ 11 μ M) differed little from that of 1a (IC₅₀ 7.9 μ M). 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 Se) 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 OPrⁿ, SMe, O(CH₂)₂NMe₂, or O(CH₂)₂NEt₂ 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 (IC₅₀ > 40 μ M). 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, $(ED_{50} > 25 \ \mu M$ in all cell cultures, $IC_{50} > 40 \ \mu M$ 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 (IC₅₀ 7.4 μ M) was essentially identical to that of 1a (IC₅₀ 7.9 μ M). Conversion of 18 to the alcohol 19 further reduced cytotoxicity 100 times and resulted in loss of inhibitory effect on tubulin polymerization (IC₅₀ > 40 μ M). 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 (IC₅₀ 15 μ M) 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 $(IC_{50} > 40 \ \mu M)$ in comparison to the antitubulin activity of the stilbene 1 (IC₅₀ 2.5 μ M). 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-4²⁹ 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 μ M 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; ¹H NMR spectra on Chemagnetics A-200, Nicolet QE-300, Varian VXR-500S, or Gemini 200 spectrometers with TMS as the internal standard in CDCl₃; ¹³C 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 $\pm 0.4\%$ 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 (Na₂SO₄). 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; ¹H NMR (CDCl₃, 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. (C₁₅H₂₄O₄Si) 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; ¹H NMR (CDCl₃, 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. (C₁₉H₂₂O₄) C, H.

(Z)-1-(4-*n*-Propoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6b): 346 mg, 53%, oil; ¹H NMR (CDCl₃, 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 = 6.6Hz, 2 H), 1.02 (t, J = 6.6 Hz, 3 H); CIMS (isobutane) m/e 329 (MH⁺, 100). Anal. (C₂₀H₂₄O₄) C, H.

(Z)-1-[4-(Methylthio)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6c): 319 mg, 51%, oil; ¹H NMR (CDCl₃, 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. (C₁₈H₂₀O₃S) C, H.

(Z)-1-(4-Methylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6d): 294 mg, 50%, oil; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₁₈H₂₀O₃) C, H.

(Z)-1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6e): 321 mg, 54%, oil; ¹H NMR (CDCl₃, 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. (C₁₉H₂₂O₃) C, H.

(Z)-1-[4-(2-Propyl)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (6f): 340 mg, 55%, oil; ¹H NMR (CDCl₃, 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. (C₂₀H₂₄O₃) C, H.

(Z)-1-(4-tert-Butylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (6g): 192 mg, 31%, oil; ¹H NMR (CDCl₃, 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. (C₂₁H₂₆O₃) C, H.

(Z)-1-(4-Methoxyphenyl)-2-(3,4-dimethoxyphenyl)ethene (6h): 280 mg, 46%, oil; ¹H NMR (CDCl₃, 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. (C₁₇H₁₈O₃) C, H.

(Z)-1-(3,5-Dimethoxyphenyl)-2-(4-methoxyphenyl)ethene (6i): 241 mg, 45%, oil; ¹H NMR (CDCl₃, 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. (C₁₇H₁₈O₃) C, H.

(Z)-1-[4-(Benzyloxy)-3,5-dimethoxyphenyl]-2-(4-methoxyphenyl)ethene (6j): 249 mg, 33%, oil; ¹H NMR (CDCl₃, 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. (C₂₄H₂₄O₄) C, H.

(Z)-1-[4-[(tert-Butyldimethylsily])oxy]-3,5-dimethoxyphenyl]-2-(4-methoxyphenyl)ethene (6k): 277 mg, 35%, oil; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₂₃H₃₂O₄Si) C, H.

⁽³⁷⁾ Smith, W. E. Formylation of Aromatic Compounds with Hexamethylene Tetramine and Trifluoroacetic Acid. J. Org. Chem. 1972, 37, 3972-3973.

(E)-1-(4-Ethoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (7a): 127 mg, 20%; mp 87-88 °C; ¹H NMR (CDCl₃, 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/e315 (MH⁺, 100). Anal. (C₁₉H₂₂O₄) C, H.

(E)-1-(4-*n*-Propoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (7b): 187 mg, 28%; mp 82-83 °C; ¹H NMR (CDCl₃, 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. (C₂₀H₂₄O₄) C, H.

(E)-1-[4-(Methylthio)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (7c): 178 mg, 28%; mp 109–111 °C; ¹H NMR (CDCl₃, 200 MHz) δ 7.44 (d, J = 8.5 Hz, 2 H), 7.24 (d, J = 8.5Hz, 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. (C₁₈H₂₀O₃S) C, H.

(E)-1-(4-Methylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (7d): 121 mg, 21%; mp 125–127 °C; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₁₈H₂₀O₃) C, H.

(E)-1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (7e): 182 mg, 30%; mp 98–100 °C; ¹H NMR (CDCl₃, 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. (C₁₉H₂₂O₃) C, H.

(E)-1-[4-(2-Propyl)phenyl]-2-(3,4,5-trimethoxyphenyl)ethene (7f): 151 mg, 24%; mp 74–75 °C; ¹H NMR (CDCl₃, 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/e313 (MH⁺, 100). Anal. (C₂₀H₂₄O₃) C, H.

(E)-1-(4-tert-Butylphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (7g): 143 mg, 23%; mp 127-128 °C; ¹H NMR (CDCl₃, 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. (C₂₁H₂₆O₃) 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; ¹H NMR (CDCl₃, 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. (C₂₄H₂₄O₄) C, H.

(E)-1-[4-[(tert-Butyldimethylsilyl)oxy]-3,5-dimethoxyphenyl]-2-(4-methoxyphenyl)ethene (7k): 224 mg, 28%; mp 118-120 °C; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₂₃H₃₂O₄Si) C, H.

Preparation of Acetates 61 and 71. A solution of n-Bu₄NF 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 \times 25 mL), and the ether solution was washed with water (2 \times 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 (61): 111 mg, 33%, oil; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₁₉H₂₀O₅) C, H.

(*E*)-1-(4-Acetoxy-3,5-dimethoxyphenyl)-2-(4-methoxyphenyl)ethene (71): 137 mg, 41%; mp 129–131 °C; ¹H NMR (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₁₉H₂₀O₅) 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; ¹H NMR (CDCl₃, 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. (C₁₈H₂₄O₄) C, H.

1-(4-*n***-Propoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethane** (**8b**): 284 mg, 86%, oil; ¹H NMR (CDCl₃, 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. (C₂₀H₂₈O₄) C, H.

1-[**4**-(Methylthio)phenyl]-2-(**3**,**4**,**5**-trimethoxyphenyl)ethane (8c): 276 mg, 86%; mp 52-54 °C; ¹H NMR (CDCl₃, 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. (C₁₈H₂₂O₃S) C, H.

1-(4-Methylphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (8d): 247 mg, 86%; mp 51-52 °C; ¹H NMR (CDCl₃, 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. (C₁₈H₂₂O₃) C, H.

1-(4-Ethylphenyl)-2-(3,4,5-trimethoxyphenyl)ethane (8e): 261 mg, 87%, oil; ¹H NMR (CDCl₃, 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/e301 (MH⁺, 100). Anal. (C₁₉H₂₄O₃) 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 CHCl₃ as the eluent. Both of these compounds were obtained as viscous oils.

1-[4-[2-(N,N-Dimethylamino)ethoxy]phenyl]-2-(3,4,5trimethoxyphenyl)ethane (8f): 243 mg, 68%, oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.08 (d, J = 8.5 Hz, 2 H), 6.85 (d, J = 8.5Hz, 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. (C₂₁H₂₉NO₄) C, H.

1-[4-[2-(N,N-Diethylamino)ethoxy]phenyl]-2-(3,4,5-trimethoxyphenyl)ethane (8g): 296 mg, 76%, oil; ¹H NMR (CDCl₃, 500 MHz) δ 7.10 (d, J = 8.5 Hz, 2 H), 6.84 (d, J = 8.5Hz, 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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⁽³⁹⁾ Nonomura, S.; Kanagawa, H.; Makimoto, A. Chemical Constituents of Polygonaceous Plants. I. Studies on the Components of Ko-gô-kon. (*Polygonum cuspidatum*. Sieb et Zucc.) Yakugaka Zasshi 1963, 83, 988-990.

m/e 388 (MH⁺, 100). Anal. (C₂₃H₃₃NO₄) 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 (Na₂SO₄). Evaporation of the ether and recrystallization of the residue from CH₂Cl₂-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 (CDCl₃, 200 MHz) δ 7.05 (d, J = 8.5 Hz, 2 H), 6.83 (d, J = 8.5Hz, 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. (C₁₉H₂₁NO₄) 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 (CDCl₃, 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. (C₁₉H₂₁NO₄) C, H.

Methyl 2-(4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)propanoate (8j): 533 mg, 74%; mp 84-85 °C; ¹H NMR (CDCl₃, 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. (C₂₀H₂₄O₆) 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₁₉H₂₀O₆) 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 (CDCl₃, 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. (C₁₉H₂₀O₆) 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 (Na₂SO₄) 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 (CDCl₃, 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. (C₂₀H₂₂O₆) 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 (CDCl₃, 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. (C₂₀H₂₂O₆) 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 (CDCl₃, 200 MH₂) 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. (C₂₀H₂₃NO₅) 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 (Na₂SO₄). 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 (CDCl₃, 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. (C₂₁H₂₆NO₅) 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 (CDCl₃, 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); ¹³C NMR (CDCl₃, 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. (C₂₅H₃₃NO₆) 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 (CDCl₃, 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. (C₂₅H₃₃NO₆) C, H.

3.4.4.5-Tetramethoxybenzophenone (18). Anhydrous AlCl₃ (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 CH₂Cl₂ (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 CH₂Cl₂ layer was separated. The aqueous layer was extracted with an additional 30 mL of CH₂Cl₂, and the combined CH₂Cl₂ solutions were washed with saturated sodium bicarbonate solution. Evaporation of solvents from the dried CH₂Cl₂ 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 (CDCl₃, 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. (C₁₇H₁₈O₅) 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 NaHCO₃ solution, followed by water, and dried (Na₂SO₄). 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 (CDCl₃, 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. (C₁₇H₂₀O₅) 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 (CDCl₃, 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. (C₁₇H₂₀O₄) 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 (CDCl₃, 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. (C₁₈H₁₈O₄) C₄ H. Compound 23c: mp 142-144 °C; IR (KBr) 866 (1 H), 831 cm⁻¹ (2 H adjacent); ¹H NMR (CDCl₃, 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. (C₁₈H₁₈O₄) 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 (CDCl₃, 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. (C₁₈H₁₈O₄) 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 (CDCl₃, 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. (C₁₈H₁₈O₄) 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 ethanolwater (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% H2SO4 acid (200 mL), extracted with ether $(2 \times 100 \text{ mL and } 1 \times 50 \text{ mL})$, washed with water (50 mL) and saturated sodium chloride solution (50 mL), and dried over anhydrous Na₂SO₄. 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 (CDCl₃, 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 (C₁₉H₂₂O₆ requires 347.1495). Anal. (C₁₉H₂₂O₆) 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 POCl₃ (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 (Na₂SO₄), 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 (CDCl₃, 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. (C₁₉H₂₀O₅) 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 (CDCl₃, 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. (C₁₉H₂₂O₄) 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 CHCl₃ (10 mL). After the CHCl₃ 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 (CDCl₃, 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). NaBH₄ (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 $(3 \times 20 \text{ mL})$. 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 (CDCl₃, 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 μ m) gave 30 as an oil (125 mg, 48%): IR (neat) 2924, 2851, 1659, 1560, 1475, 1260, 1159, 1122 cm⁻¹; ¹H NMR (CDCl₃, 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 CHCl₃ (10 mL). The CHCl₃ layer was extracted with

H₂O (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 (CDCl₃, 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 C₂₁H₂₂INO₅ 368.1498 (cation), found 368.1489.

1-(4'-Methoxybenzyl)-5,6,7-trimethoxy-2-methyl-1,2.3.4tetrahydroisoquinoline (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 POCl₃ (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). NaBH4 (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 (Na₂SO₄). Evaporation of solvent from the filtrate and flash chromatography (CHCl₃, then CHCl₃-EtOH, 96:4 by volume, silica gel 230-400 mesh) gave 32 as an oil (225 mg, 52%): ¹H NMR (CDCl₃, 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 CHCl₃ (3 × 30 mL), and the combined CHCl₃ layer was washed with saturated NaCl solution and dried (Na₂SO₄). 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 (CDCl₃, 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 POCl₃ (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 NH₄OH (10 mL). The aqueous solution was extracted with $CHCl_3$ (3 × 15 mL). The combined CHCl₃ 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 Et₂O-EtOH (98:2) as the eluent gave compound 35 as a pale brown oil (230 mg, 89%): ¹H NMR (CDCl₃, 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, 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 Et₂O containing 1% Et₃N to give compound 37 as an oil (1.3 g, 87%): ¹H NMR (CDCl₃, 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-8-0x0-2,3,4,10-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 (CDCl₃, 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. (C₂₁H₂₁NO₅) C, H.

5,8,13,13a-Tetrahydro-2,3,4,10-tetramethoxy-6H-dibenzo-[a.g.] guinolizine (39). A suspension of LiAlH₄ (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 LiAlH₄ 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 NaBH₄ (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 K₂CO₃ to pH 8, extracted with $CHCl_3$ (3 × 15 mL), and dried (Na₂SO₄). 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 μ m), 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 (CDCl₃, 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. (C₂₁H₂₅NO₄) 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 μ L 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 (ED₅₀ < 25 μ M) were tested again, and the values shown for these cytotoxic substances are the averages of two determinations. Compounds 6a, 6d, and 6w 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 μ M) 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

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[†]Division of Organic Chemistry.

[‡]Division of Experimental Therapy.