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Antineoplastic Agents. 548. Synthesis of Iodo- and Diiodocombstatin Phosphate Prodrugs

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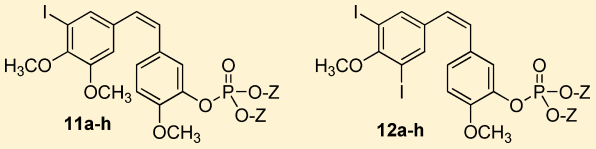
ABSTRACT: Toward the objective of designing a structurally modified analogue of the combretastatin A-4 phosphate prodrug (**1b**) with the potential for increased specificity toward thyroid carcinoma, synthesis of a series of iodocombstatin phosphate (**11a–h**) and diiodocombstatin phosphate prodrugs (**12a–h**) has been accomplished. The diiodo series was obtained via **8a** and **9c** from condensation of **4** and **6**, and the iodo sequence involved a parallel pathway. Both series of iodocombstatins were found to display significant to powerful inhibition of the growth of a panel of human cancer cell lines and of the murine P388 lymphocytic leukemia cell line. Of the diiodo series, **12a** was also found to markedly inhibit growth of pediatric neuroblastoma, and monoiodocombstatin **9a** strongly inhibited HUVEC growth. Overall, the strongest activity was found against the breast, CNS, leukemia, lung, and prostate cancer cell lines and the least activity against the pancreas and colon lines. Parallel biological investigations of tubulin interaction, antiangiogenesis, and antimicrobial effects were also conducted.



From our initial discoveries over 30 years ago of the combretastatins, which are constituents of the African tree *Combretum caffrum* (Combretaceae), knowledge of their chemistry, biology, and medical potential has continued to advance.[2] Preclinical and clinical developments over the last 12 years have been rapidly accelerating, especially for combretastatin A-4 (CA4, **1a**), in the form of the phosphate prodrug CA4P (**1b**), and combretastatin A-1 (CA1, **1c**) as promising cancer vascular-disrupting and ophthalmology drugs.[2,3] In turn, these encouraging developments have stimulated a variety of efforts devoted to synthesis and biological evaluation of combretastatin structural modifications, related primarily to CA4 (**1a**) and CA1 (**1c**). Recent reports include SAR studies that provide varying levels of cancer cell growth inhibition.[4]

For 2011, the estimated incidence of thyroid cancer in the United States is at about 48 000, including 1740 deaths.[5a] Most thyroid cancers are well differentiated papillary (about 80%) and follicular (about 14%) carcinomas. Both types of tumor cells are believed to be derived from follicular epithelial cells that produce thyroid hormone. Of the remaining thyroid malignancies, about 4% are medullary carcinoma (neuroendocrine) and about 2% are the exceptionally aggressive anaplastic carcinoma (median survival of 4–5 months and a near-100% lethal outcome).[2b,5b] Significantly, the incidence of both follicular and anaplastic carcinomas is increased in populations residing in areas of iodine deficiency. Radiation exposure represents the most general risk factor for thyroid cancer. In addition, excess production of the pituitary hormone thyroid-stimulating hormone (TSH), important in the regulation of thyroid gland growth and function, may be involved in the etiology of thyroid cancer. Previously used clinical treatments[5c,d] for thyroid cancer include surgery, suppression of TSH, ^{131}I-radiotherapy,[5e] and anticancer drugs. Thyroid cancer is the fastest increasing (240% increase in the past 30 years) cancer type in both men and women in the United States; most of the increase in incidence of papillary carcinomas is probably due to earlier detection,[5f] but the need for new and effective anticancer drugs, especially for the more aggressive carcinomas, is clear. Early in the human cancer phase I clinical trials of CA4 (**1a**), as the sodium phosphate prodrug **1b** (CA4P, Fosbretabulin, Zybrestat), evidence for activity against otherwise refractory anaplastic thyroid carcinoma was observed, and this was further supported in a phase II clinical trial.[2b,6,7] The pioneering clinical studies of Remick and colleagues[2b,7a] have led via phase II clinical trials of CA4P against anaplastic thyroid carcinoma to the current phase III level.[5b]

The most active thyroid hormone components are triiodothyronine (**2a**) and its tetraiodo derivative thyroxine

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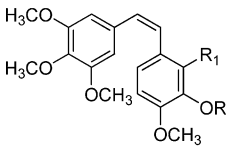
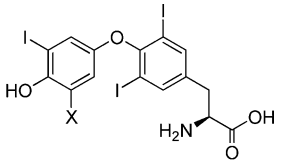
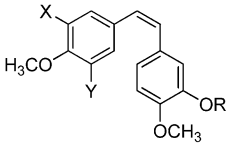
(**2b**). Both are derived from 3,5-diiodotyrosine, with release controlled by TSH. Consequently, the focus of the present investigation was evaluation of iodo modifications of CA4P (**1b**), with the ultimate goal of obtaining enhanced concentration of drug in the thyroid carcinoma tissue. Of the continuing CA4 (**1a**) structural modification studies,[4] those involving halogen substitutions have been primarily concerned with fluoro, chloro, bromo, and iodo derivatives.[4a,8] Our objective here was the synthesis and initial evaluation of the 3-iodo- and 3,5-diiodostilbene CA4 modifications, herein designated iodocombstatin (**3a**) and diiodocombstatin (**3b**), and of their phosphate prodrug modifications.



1a, R = R_1 = H (CA4)
b, R = P(O)(O^-Na^+)$_2$, R_1 = H (CA4P)
c, R = H, R_1 = OH (CA1)



2a, X = H
b, X = I



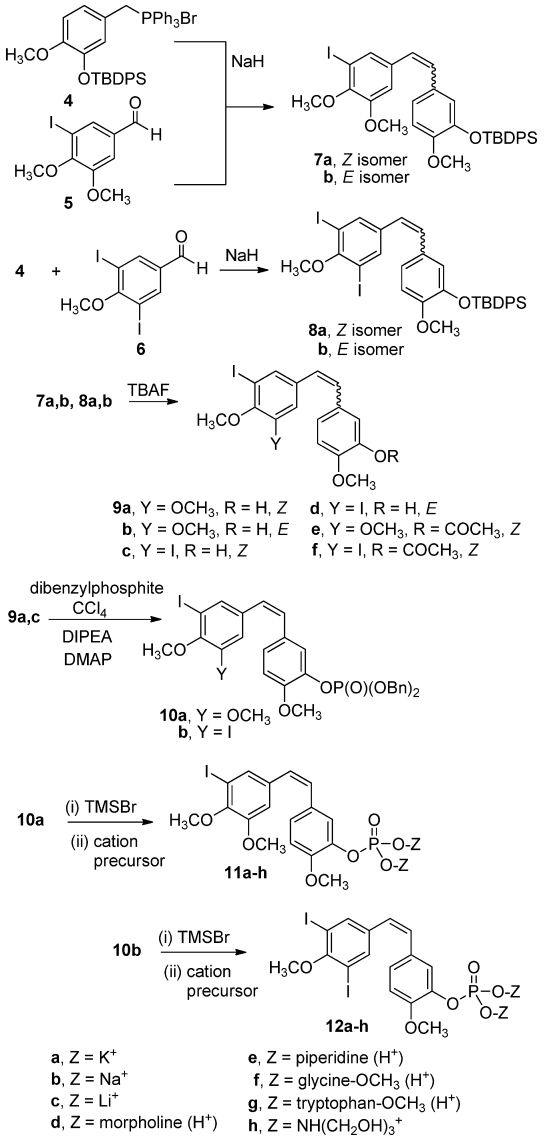
3a, X = I, Y = OCH_3, R = H
b, X = Y = I, R = H
c, X = OH, Y = OCH_3, R = H
d, X = F, Y = OCH_3, R = H

RESULTS AND DISCUSSION

The initial intermediates were synthesized by Wittig reaction of compound **4** with 3-iodo-4,5-dimethoxybenzaldehyde (**5**) and with 3,5-diiodo-4-methoxybenzaldehyde (**6**) to provide iodostilbenes **7** and **8**, respectively, as *Z*/*E* mixtures (Scheme 1). The isomers were separated by silica gel column chromatography (CC), and subsequent deprotection with tetrabutylammonium fluoride afforded the corresponding phenols **9a**–**d**. Iodocombstatins **9a** and **9c** were further characterized as their 3-acetyl derivatives **9e** and **9f**. The *Z*-isomers **9a** and **9c** were treated with dibenzylphosphite and CCl_4 in the presence of diisopropylethylamine and *N*,*N*-dimethylaminopyridine (DMAP) to provide bisbenzylphosphates **10a** and **10b**, from which the benzyl ester protecting groups were cleaved with bromotrimethylsilane.[3b,8b,9] Subsequent treatment of the resulting phosphoric acids with the appropriate cation precursor readily afforded prodrugs **11a**–**h** and **12a**–**h**. In this sequence, the debenzylation step proved to be the most challenging: in order to minimize conversion of the *Z*- to *E*-geometry, presumably due to available bromonium ion, cooling (0 °C) was required, as well as an aqueous sodium thiosulfate treatment. Once the potassium salts of the prodrugs were in hand, a selection of alkali metal, ammonium, and amino acid salts of the phosphates were synthesized by ion exchange chromatography to complete the **11a**–**h** and **12a**–**h** prodrug series.

The ability of the iodostilbenes to inhibit cancer cell growth was examined and compared to the activities of combretastatin A-3 (**3c**),[10] fluorcombstatin (**3d**),[8b] and combretastatin A-4 (**1a**) (Table 1). The iodostilbene phosphate salts all retained strong activity and, as expected, demonstrated markedly better

Scheme 1



aqueous solubility (Table 1) than the iodostilbene precursors (**9a**–**d**). Compound **12a** was also tested against three pediatric neuroblastoma cell lines (GI_{50} in μg/mL: SK-N-SH, 0.0075; SK-N-AS, 0.002; IMR-32, 0.081).

Against two cancer cell lines long believed to be human anaplastic thyroid carcinoma cells (KAT-4 and SW1736), we had earlier evaluated the potential for increased specificity by the new iodocombstatins. Diiodocombstatin **9c** proved to be more inhibitory than iodocombstatin (**9a**, Table 2), but both strongly inhibited growth of the ATC line KAT-4. However, phenols **9a** and **9c** were 100× less active against the SW1736 line. The potassium phosphates **11a** and **12a** as expected proved to be less inhibitory (10×). While growth of the KAT-4 cells was significantly reduced by phosphates **11a** and **12a**, the SW1736 cells proved to be resistant. It has since been reported that KAT-4 is one of 12 redundant cell lines of 40 presumptive human thyroid cancer cell lines tested for cross-contamination and is likely not of thyroid origin.[11a] Thus the KAT-4 cancer cell line data, while interesting, are not directly significant with respect to thyroid cancer but are a further indication of the cancer cell growth inhibitory activity of these compounds.

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Table 1. Human Cancer Cell Line Growth Inhibition (GI_{50}, μg/mL) and Murine P388 Lymphocytic Leukemia Inhibitory Activities (ED_{50}, μg/mL)

compound	solubility[a] (mg/mL)	leukemia P388	pancreas BXPC-3	breast MCF-7	CNS SF-268	lung-NSC NCI-H460	colon KM20L2	prostate DU-145
1a		0.0003	0.39		<0.001	0.0006	0.061	0.0008
1b		0.0004	0.23		0.036	0.029	0.034	0.0072
3c		0.26	2.3	0.49	0.0083	0.19	1.2	0.0043
3d		0.0020	0.745	0.0027	0.0016	0.0032	>1	0.0019
9a		0.0020	0.048	0.00022	0.00018	0.00029	0.328	0.00018
9c		0.0028	0.038	0.0027	0.0036	0.0034	0.15	0.0021
9b		0.189	2.7	0.18	0.55	0.21	1.7	0.27
9d		>10	3.0	0.94	3.3	3.4	>10	5.8
11a	14	0.0021	0.381	0.0064	0.0057	0.0043	0.41	0.0038
11b	2	0.0020	0.469	0.018	0.018	0.017	>1	0.011
11c	≥2.4	0.017	0.490	0.0038	0.0040	0.0039	>1	0.0043
11d		0.0032	0.21	0.0047	0.0037	0.0036	0.24	0.0026
11e	≥4	0.0026	0.32	0.0065	0.0044	0.0036	0.51	0.0029
11f	≥2	0.0026	0.16	0.0044	0.0033	0.0031	0.32	0.0021
11g		0.0022	0.26	0.035	0.0097	0.0034	0.59	0.0030
11h		0.0029	0.37	0.0048	0.0043	0.0040	0.40	0.0047
12a	22	0.0034	0.44	0.050	0.053	0.046	>1	0.028
12b	2	0.030	>1	0.066	0.051	0.327	>1	0.242
12c	≥4	0.021	0.37	0.051	0.050	0.050	>1	0.032
12d		0.014	0.35	0.066	0.054	0.033	>1	0.028
12e		0.011	0.33	0.070	0.041	0.025	>1	0.025
12f		0.011	0.36	0.10	0.054	0.030	>1	0.023
12g		0.017	0.37	0.22	0.086	0.033	>1	0.026
12h		0.026	0.33	0.047	0.040	0.025	0.94	0.021

[a]Solubility values represent solution in 1 mL of H_2O at 25 °C.

Table 2. Human Anaplastic Thyroid Carcinoma Cell Line Inhibition Values (GI_{50}, μg/mL)

compound	KAT-4	SW1736
9a	0.089	2.2
9c	0.039−0.063	1.2
11a	0.37−0.43	>10
12a	0.38−0.44	>10

Antiangiogenesis is now actively pursued as a promising antitumor strategy.[11b] Iodo- (**9a**) and diiodocombstatin (**9b**), as well as phosphate prodrugs **11a**,**b** and **12a**,**b**, were evaluated against human umbilical vein endothelial cells (HUVEC) in vitro (Table 3). These cells showed significant sensitivity to the new compounds. Iodocombstatin (**9a**) was the most active, with an ED_{50} value of 4×10^{-5} μg/mL, followed by diiodocombstatin (**9c**). A similar pattern was observed with phosphates **11a** and **12a**. With both the mono- and diiodocombstatins, **9a** and **9c**, cord lengths as well as junction numbers were markedly reduced at 0.001 μg/mL (see Table 4 and Figure 1B,D) but were similar to the untreated control (Figure 1A) at 0.0001 μg/mL (Figure 1C,E). At 0.001 μg/mL, **11a** (Figure 1F) showed a slightly larger reduction in the size of the cords as compared to **12a** (Figure 1G). Such inhibitory activity against the HUVECs is of considerable interest since endothelial cells play a central role in the angiogenic process. Moreover, combretastatin derivatives have been shown to be involved in vascular targeting therapy by destroying the existing vasculatures of a tumor, specifically staunching blood flow and inhibiting tumor growth through the disruption of the tubulin cytoskeleton of endothelial cells, which leads to thrombosis of the vasculature (see Table 5).[12]

Table 3. Human Umbilical Vein Endothelial Cell (HUVEC) Inhibition Values (GI_{50}, μg/mL)

compound	HUVEC
9a	0.000040
9c	0.00028
11a	0.00025
11b	0.00035
12a	0.0049
12b	0.051

The mono (**9a**) and diiodo (**9b**) analogues of CA4 (**1a**) and their sodium phosphate derivatives (**11b** and **12b**, respectively) were also evaluated and compared to **1a** for inhibitory effects on tubulin (experiments were performed contemporaneously, Table 5). Similar IC_{50} values were obtained for the non-phosphorylated compounds, with **9a** appearing to be somewhat less inhibitory than **1a**, and **9c** more active than **1a**. The two phosphorylated derivatives **11b** and **12b** had little or no inhibitory effect on tubulin assembly, as had been observed previously with CA4P (**1b**).

Combretastatin A-4 (**1a**) is a potent inhibitor of the binding of [^{3}H]colchicine to tubulin. In our standard assay with 1.0 μM tubulin and 5.0 μM [^{3}H]colchicine, the binding of the radiolabel to the protein has routinely been 95−100% inhibited by 5.0 μM **1a** and 75−85% inhibited when **1a** was present in the reaction mixture at 1.0 μM, equimolar with tubulin.[13] In the current experiments, this finding was obtained again (Table 5). We compared **9a** and **9c** with **1a** as inhibitors of colchicine binding in the standard reaction conditions. While **9a** was identical to **1a** in its inhibitory effect, the diiodo **9c** was more potent. Virtually complete inhibition of colchicine binding occurred with **9c** at 1 μM, making this compound the most potent inhibitor of colchicine binding we have yet examined.

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Table 4. Length of Cords Formed, Number of Junctions, and Relative Percent Growth of Cells

	drug conc (μg/mL)	lengths of cords[a]	junction no.[b]	rel % growth[c]
9a	0.01	−	−	14
	0.001	+	+	14
	0.0001	++(+)	++(+)	18
	0.00001			90
9c	0.01	−	−	4
	0.001	+	(+)	8
	0.0001	+++	+++	84
	0.00001			87
11a	0.01			1
	0.001	++	++(+)	10
	0.0001	+++	+++	77
	0.00001	+++	+++	95
11b	0.1	−	−	7
	0.01	−	−	14
	0.001	+	+	5
	0.0001			104
12a	0.1	−	−	15
	0.01	++(+)	++(+)	33
	0.001	+++	+++	88
	0.0001			96
12b	1	−	−	−7
	0.1	+	(+)	−2
	0.01	++(+)	++(+)	>100
	0.001			>100

[a]Lengths of cords: −, no cords; +, small; +(+), 25% control; ++, 50% control; ++(+), 75% control; +++, same as control. [b]Number of junctions: −, no junctions; +, few; +(+), 25% control; ++, 50% control; +(+), 75% control; +++, same as control. [c]Relative to control.

Combretastatin A-4 (**1a**) and the prodrug CA4P (**1b**) were previously shown to have marginal antimicrobial activity (minimum inhibitory concentration [MIC] = 25−100 μg/disk) against the Gram positive opportunist *Micrococcus luteus* and the Gram negative pathogen *Neisseria gonorrhoeae*.[14] The new CA4 analogues **9a**,**c**, as well as **9b**, and phosphate salts **11a**−**h** and **12a**−**h** were tested against a panel of bacteria and fungi. Phenols **9a** and **9c** were active against *M. luteus* (MIC: 2−4 and 4−16 μg/mL, respectively), and the diiodocombstatin phosphates **12a**−**12h** were very active against *N. gonorrhoeae* (MIC in μg/mL: **12a**, <0.5−4; **12b**, 32−64; **12c**, <0.5−2; **12d**, <0.5; **12e**, <0.5; **12f**, <0.5−1; **12g**, <0.5; **12h**, <0.5−2). Iodocombstatins **11d**, **11g**, and **11h** were also active against *N. gonorrheae* (MIC in μg/mL: **11d**, 16−32; **11g**, 4−8; **11h**, 32−64), and iodocombstatin **9a** had marginal activity against the pathogenic yeast *Cryptococcus neoformans* (MIC: 64 μg/mL).

From all evidence now in hand, diiodocombstatin (**9c**) appears especially suitable for further preclinical development. The very powerful biological properties of the 3-iodo- and especially the 3,5-diodocombstatins may be the result, in part, of increased halogen−protein bonding, as suggested by Ley and colleagues.[4a] 3,5-Diiodocombstatin (**9c**) and derived prodrugs offer promising candidates for further preclinical development.

EXPERIMENTAL SECTION

General Experimental Methods. All solvents were redistilled, and ether refers to diethyl ether. Isovanillin, 3-iodo-4,5-dimethoxybenzaldehyde, anhydrous CH_2Cl_2, anhydrous toluene, tetrabutylammonium fluoride [1.0 M solution in tetrahydrofuran (THF)], dibenzyl phosphate, 4-dimethylaminopyridine, CCl_4, Dowex-50W (HCR-W2), and L-tryptophan methyl ester hydrochloride were obtained from Sigma-Aldrich Chemical Co. 3,5-Diiodo-4-hydroxybenzaldehyde was obtained from Lancaster Synthesis Inc., and diisopropylethylamine from Avocado (Alfa Aesar, Ward Hill, MA, USA). The remaining reagents were purchased from Acros Organics (Fisher Scientific, Pittsburgh, PA, USA). All reactions were performed under an argon atmosphere, protected from bright light to avoid the potential for photochemical side reactions. The reactions and products were monitored by TLC using Analtech silica gel GHLF Uniplates visualized under long-wave and short-wave UV irradiation and stained by dipping into phosphomolybdic acid in EtOH followed by heating. All organic extracts of aqueous solutions were dried over anhydrous magnesium sulfate. Where appropriate, the crude products were separated by column chromatography, using flash (230−400 mesh ASTM) silica gel from E. Merck.

Melting points were measured with an electrothermal digital melting point apparatus and are uncorrected. All ^1H and ^{13}C NMR spectra were obtained using Varian Gemini 300 MHz or Varian Unity 400 or 500 MHz instruments with $CDCl_3$ (TMS internal reference) as solvent unless otherwise noted. The ^{31}P NMR spectra were obtained in $CDCl_3$ or D_2O solution with 85% H_3PO_4 as an external standard employing a Varian Unity 400 or 500 MHz instrument. Elemental analyses were performed by Galbraith Laboratories, Inc. Mass spectra were obtained in our institute with a JEOL LCmate instrument.

*3,5-Diiodo-4-methoxybenzaldehyde (**6**).* A solution of 3,5-diiodo-4-hydroxybenzaldehyde (5 g, 13.37 mmol) in anhydrous DMF (50 mL) was cooled to 0 °C, and sodium hydride (0.64 g, 16 mmol, 60% dispersion in mineral oil) was slowly added. Iodomethane was then added, and stirring was continued at rt in the dark for 19 h. The reaction was terminated by the addition of H_2O (50 mL), and the mixture was extracted with EtOAc−hexane (1:1, 3 × 50 mL). The combined organic extract was filtered and concentrated in vacuo. The residue was separated by CC on silica gel using EtOAc−hexane (1:9) as eluent. The product was a colorless solid (4.2 g, 80%) that crystallized from hexane: mp 121−123 °C (lit.[15] mp 124 °C); ^1H NMR ($CDCl_3$, 300 MHz) δ 3.93 (s, 3 H), 8.27 (s, 2 H), 9.81 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 60.35, 90.72, 140.65, 140.71, 187.51, 187.56; anal. C 24.86, H 1.58%, calcd for $C_8H_6I_2O_2$, C 24.77, H 1.56%.

*3-Iodo-4,4′,5-trimethoxy-3′-O-tert-butyldiphenylsilyl-Z-stilbene (**7a**) and 3-Iodo-4,4′,5-trimethoxy-3′-O-tert-butyldiphenylsilyl-E-stilbene (**7b**). Method A.* Phosphonium bromide **4**[10] (3.67 g, 5.13 mmol) was dissolved in DCM at 0 °C. Sodium hydride (60% dispersion in mineral oil, 0.41 g, 10.2 mmol) was added, and the mixture turned orange. Next, 3-iodo-4,5-dimethoxybenzaldehyde (**5**, 1 g, 3.42 mmol) was added, and stirring was continued for 21 h. The reaction was terminated by addition of H_2O (50 mL), and the mixture was extracted with DCM (3 × 50 mL). The organic phase was filtered and concentrated. The oil obtained was subjected to flash chromatography on silica gel and eluted with 0−3% EtOAc in hexane to afford *Z*-stilbene **7a** (0.86 g, 39%), which crystallized as a colorless solid from hexane: mp 122−124 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.07 (s, 9 H), 3.45 (s, 3 H), 3.55 (s, 3 H), 3.79 (s, 3 H), 6.21 (d, 1 H, *J* = 12 Hz), 6.31 (d, 1 H, *J* = 12 Hz), 6.59 (d, 1 H, *J* = 7.8 Hz), 6.72 (s, 2 H), 6.77 (dd, 1 H, *J* = 7.8, 1.5 Hz), 7.19 (d, 1 H, *J* = 1.8 Hz), 7.40−7.20 (m, 6 H), 7.64 (d, 4H, *J* = 7.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.68, 26.62, 55.05, 55.56, 60.33, 91.94, 111.72, 113.09, 120.78, 122.43, 126.73, 127.33, 129.32, 130.28, 130.93, 133.54, 135.17, 144.70, 149.82, 151.82; HRMS *m*/*z* 651.1474 $[M + H]^+$ (calcd for $C_{33}H_{36}IO_4Si$, 651.1428); anal. C, 60.79; H, 5.67%, calcd for $C_{33}H_{35}IO_4Si$, C 60.92, H 5.45%.

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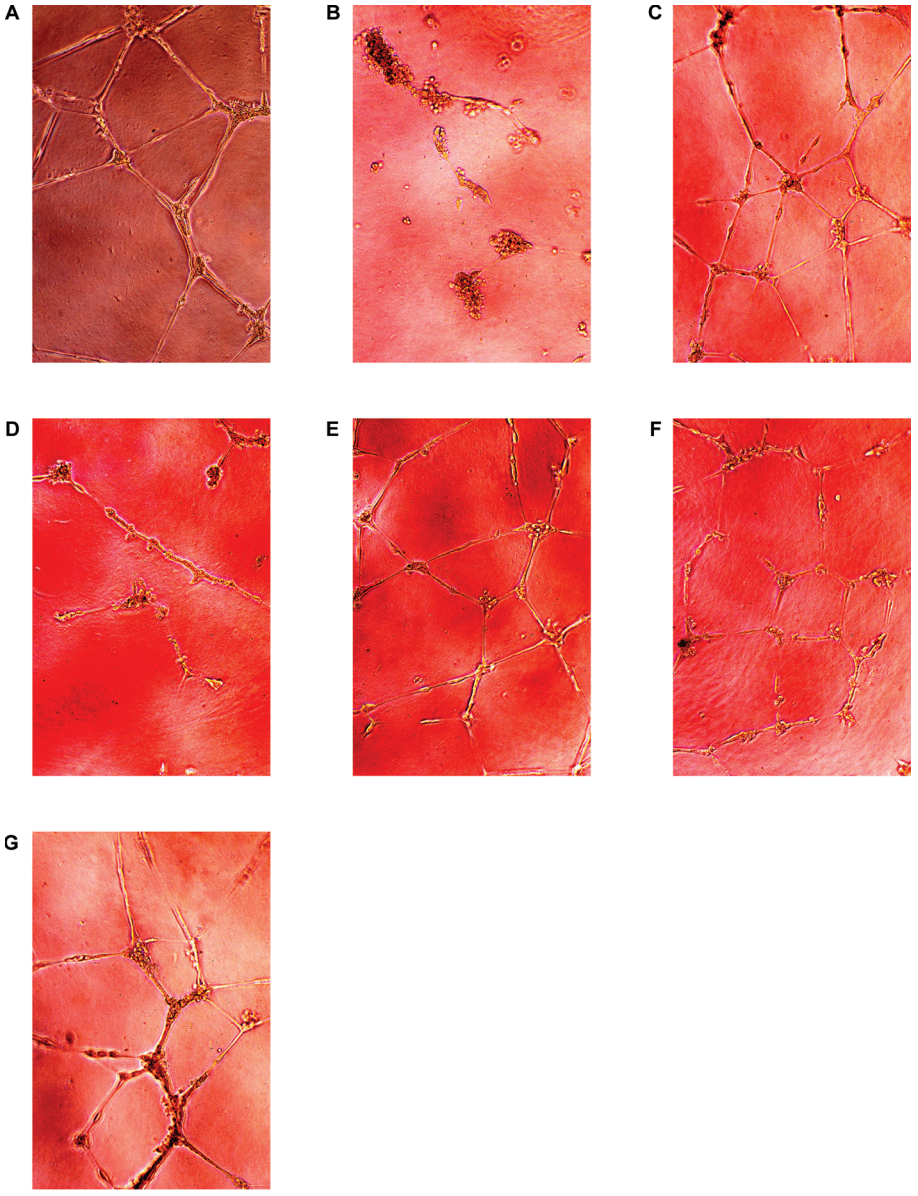

Figure 1. Treated human umbilical vein endothelial cells. Morphological appearance of the cords in HUVEC cells: (A) untreated control; (B) after exposure to **9a** (0.001 μg/mL for 24 h); (C) after exposure to **9a** (0.0001 μg/mL for 24 h); (D) after exposure to **9c** (0.001 μg/mL for 24 h); (E) after exposure to **9c** (0.0001 μg/mL for 24 h); (F) after exposure to **11a** (0.001 μg μg/mL for 24 h); (G) after exposure to **12a** (0.001 μg/mL for 24 h).

Table 5. Inhibitory Effects of Iodocombstatins on Tubulin Assembly and on Colchicine Binding to Tubulin in Comparison with Those of Combretastatin A-4 (1a)

	inhibition of tubulin assembly	inhibition of colchicine binding to tubulin	
		1.0 μM compound	10 μM compound
	IC_{50} (μM) ± SD	% inhibition ± SD	
1a	2.1 ± 0.03	81 ± 5	99 ± 0.9
1b	>40[a]		
9a	2.5 ± 0.04	78 ± 8	100 ± 0.8
9c	1.5 ± 0.3	95 ± 0.8	100 ± 0.3
11b	>40		
12b	>40		

[a]Data from previous evaluations, not repeated in current studies.

In a parallel series of Wittig reactions evaluating a variety of solvents (CH_2Cl_2, EtOAc, toluene, and $CHCl_3$), temperature (−70 to 0 °C to rt), time (2.5−9.25 h), and base (NaH, KOH/18-crown-6), production of the *Z*-isomer ranged from 42% to 52% yields, as judged by NMR analyses.

Further elution gave *E*-stilbene **7b** (0.96 g, 43%), which crystallized from hexane as a colorless solid: mp 98−99 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.14 (s, 9 H), 3.55 (s, 3 H), 3.82 (s, 3 H), 3.82 (s, 3 H), 3.89 (s, 3 H), 6.43 (d, 1 H, *J* = 15.9 Hz), 6.71−6.76 (m, 2 H), 6.86−6.95 (m, 3 H), 7.33−7.42 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.81, 26.67, 55.28, 60.50, 92.65, 110.22, 112.11, 117.70, 120.56, 124.58, 127.52, 128.45, 128.68, 129.60, 129.77, 133.64, 135.38, 135.82, 145.18, 148.13, 150.56, 152.49; HRMS *m*/*z* 651.1400 $[M + H]^+$ (calcd for $C_{33}H_{36}IO_4Si$, 651.1428); anal. C 60.88, H 5.63%, calcd for $C_{33}H_{35}IO_4Si$, C 60.92, H 5.42%.

Method B. Butyllithium (4.5 mL, 11.3 mmol) was added to a stirred and cooled (−70 °C) suspension of phosphonium bromide **4** in dry THF (100 mL). The solution was stirred for 30 min at −70 °C and then for 6 h at rt. Water (50 mL) was added, and the reaction mixture

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was extracted with EtOAc (3 × 100 mL). The organic phase was filtered and concentrated to an oil, which was subjected to flash chromatography on silica and eluted with 0−3% EtOAc in hexane to afford *Z*-stilbene **7a** (1.4 g, 21%) as a colorless solid: mp 122−124 °C.

*3,5-Diiodo-4,4′-dimethoxy-3′-O-tert-butyldiphenylsilyl-Z-stilbene (**8a**) and 3,5-Diiodo-4,4′-dimethoxy-3′-O-tert-butyldiphenylsilyl-E-stilbene (**8b**).* *Method A.* To a solution of phosphonium bromide **4**[10] (2.77 g, 3.87 mmol) in DCM at 0 °C was added sodium hydride (60% dispersion in mineral oil, 0.31 g, 7.7 mmol), and the mixture turned orange. After addition of aldehyde **6** (1.0 g, 2.57 mmol), stirring was continued for 7.5 h. The reaction was terminated by addition of H_2O (50 mL) and extracted with DCM (3 × 50 mL). The organic extract was filtered and concentrated. The oily residue was flash chromatographed on silica gel using hexane as eluent to give an isomeric mixture of **8a** and **8b** (71% yield, 1.35 g). Further elution gave the *E*-isomer **8b** (0.10 g, 5%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.14 (s, 9 H), 3.56 (s, 3 H), 3.84 (s, 3 H), 6.33(d, 1 H, J = 15.9 Hz), 6.72 (d, 1 H, J = 8.4 Hz), 6.73 (d, 1 H, J = 15.9 Hz), 6.72 (d, 1 H, J = 8.4 Hz, ArH), 6.85 (d, 1H, J = 2.1 Hz), 6.92 (dd, 1 H, J = 1.8 Hz and J 8.4 Hz), 7.34−7.46 (m, 6 H), 7.72−7.75 (m, 6 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 19.82, 26.69, 55.30, 60.77, 90.59, 112.09, 117.73, 120.83, 122.47, 127.55, 129.38, 129.65, 129.99, 133.58, 135.40, 137.15, 137.73, 145.22, 150.84, 157.55; HRMS m/z 747.0442 $[M + H]^+$ (calcd for $C_{32}H_{33}I_2O_3Si$, 747.0289).

Method B. Butyllithium (0.6 mL, 1.47 mmol) was added to a stirred and cooled (−10 °C) suspension of phosphonium bromide **4** (1.01 g, 1.4 mmol) in dry THF (80 mL). The orange-red solution was stirred for 10 min at rt. Aldehyde **6** (0.50 g, 1.33 mmol) was added, and the reaction mixture color changed from red to yellow. Stirring was continued at rt for 10 min before addition of ice water (100 mL) and extraction of the mixture with EtOAc (3 × 100 mL). The organic phase was washed with water (100 mL), filtered, and concentrated. The resulting oil was partially separated by flash chromatography on silica gel using hexane−EtOAc (100:1) as eluent to give an isomeric mixture in a ratio of approximately 1:1.9 (*Z*:*E*, 0.90 g, 90%).

*3-Iodo-4,4′,5-trimethoxy-3′-hydroxy-Z-stilbene (**9a**).* To a solution of silyl ether **7a** (1.30 g, 1.99 mmol) in THF was added tetrabutylammonium fluoride (2.2 mL, 2.2 mmol). The mixture was stirred under Ar in the dark for 10 min, and the reaction was terminated by the addition of H_2O (5 mL). The product was extracted with EtOAc (3 × 15 mL), and the organic phase was filtered and concentrated. The crude product was separated by silica gel CC using 1:4 EtOAc−hexane to give stilbene **9a** (0.70 g, 85%) as a colorless solid: mp 92−94 °C; IR (film) ν_{max} 3543, 3011, 2937, 2841, 1510, 1273, 1001, 908, 732 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.61 (s, 3 H), 3.81 (s, 3 H), 3.84 (s, 3 H), 6.32 (d, 1 H, J = 12 Hz), 6.34 (s, 1 H), 6.56 (d, 1 H, J = 12 Hz), 6.75 (s, 1 H), 6.83 (d, 1 H, J = 1.8 Hz), 6.85 (s, 3 H), 7.25 (d, 1 H, J = 1.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.56, 55.82, 60.33, 91.78, 110.50, 113.11, 115.00, 120.91, 126.96, 129.94, 130.28, 135.93 145.29, 146.10, 147.67, 151.79; HRMS m/z 413.0250 $[M + H]^+$ (calcd for $C_{17}H_{18}IO_4$, 413.0259); anal. C 49.38, H 4.24%, calcd for $C_{17}H_{17}IO_4$, C 49.53, H 4.16%.

*3-Iodo-4,4′,5-trimethoxy-3′-hydroxy-E-stilbene (**9b**).* The *E*-isomer **9b** (0.29 g, 98%) was obtained from silyl ether **7b** (0.46 g, 0.7 mmol) as described above for the synthesis of the *Z*-isomer **9a**. Separation by CC (7:3 hexane−EtOAc as eluent) gave *E*-isomer **9b** (0.29 g, 98%) as a colorless solid: mp 111−113 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3 H), 3.87 (s, 3 H), 3.88 (s, 3 H), 5.85 (bs, 1 H), 6.77 (d, 1 H, J = 16.5 Hz), 6.89 (d, 1 H, J = 16.5 Hz), 6.82 (s, 1 H), 6.96 (s, 1 H), 6.93 (d, 1 H, J = 2.4 Hz), 7.11 (d, 1 H, J = 1.5 Hz), 7.46 (d, 1 H, J = 1.5 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 55.85, 60.41, 92.56, 110.40, 110.63, 111.77, 119.28, 124.97, 128.36, 128.70, 130.15, 135.71, 145.71, 146.56, 148.11, 152.44: HRMS m/z 413.0250 $[M + H]^+$ (calcd for $C_{17}H_{18}IO_4$, 413.0257); anal. C 49.38, H 4.24%, calcd for $C_{17}H_{17}IO_4$, C 49.53, H 4.16%.

*3,5-Diiodo-4,4′-dimethoxy-3′-hydroxy-Z-stilbene (**9c**) and 3,5-Diiodo-4,4′-dimethoxy-3′-hydroxy-E-stilbene (**9d**).* Stilbenes **9c** and **9d** were obtained from a mixture of silyl ethers **8a** and **8b** (1.35 g, 1.81 mmol) as described above for the synthesis of *Z*-isomer **9a**. The oily mixture was separated by CC with 2:1 hexane−EtOAc as eluent to provide *Z*-isomer **9c** as an oil (0.45 g, 49%): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.85 (s, 3 H), 3.89 (s, 3 H), 5.54 (s, 1 H), 6.26 (d, 1 H, J = 12 Hz), 6.49 (d, 1 H, J = 12 Hz), 6.74 (s, 2H), 6.82 (s, 1 H), 7.67 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 55.98, 60.73, 89.98, 110.46, 114.87, 120.98, 125.08, 129.47, 131.57, 137.37, 139.96, 145.42, 146.21, 157.50; HRMS m/z 508.9111 $[M + H]^+$ (calcd for $C_{16}H_{15}I_2O_3$, 508.9113); anal. C 37.80, H 2.83%, calcd for $C_{16}H_{14}I_2O_3$, C 37.82, H 2.78%.

Further elution led to the *E*-stilbene **9d** (0.46 g, 50% yield) as a colorless solid, which crystallized from hexane: mp 127−129 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.86 (s, 3 H), 3.91 (s, 3 H), 5.62 (s, 1 H), 6.71 (d, 1 H, J = 16.5 Hz), 6.83 (d, 1 H, J = 8.1 Hz), 6.90 (d, 1 H, J = 17.1 Hz), 6.95 (d, 1 H, J = 8.4 Hz), 7.10 (d, 1 H, J = 2.4 Hz), 7.85 (s, 2 H, H-2); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 55.51, 60.30, 90.17, 100.17, 100.21, 110.18, 111.35, 119.17, 122.56, 129.63, 129.81, 136.82, 137.19, 146.34, 157.25; HRMS m/z 508.9119 $[M + H]^+$ (calcd for $C_{16}H_{15}I_2O_3$, 508.9113); anal. C 38.01, H 2.91%, calcd for $C_{16}H_{14}I_2O_3$, C 37.82, H 2.78%.

*3-Iodo-4,4′,5-trimethoxy-3′-acetyl-Z-stilbene (**9e**).* Phenol **9a** (0.45 g) was dissolved in pyridine (3 mL)−acetic anhydride (170 μL), and the mixture was stirred for 2 h. The mixture was concentrated under reduced pressure from toluene (3 × 10 mL). The residue was diluted with EtOAc (30 mL), and the solution was washed successively with H_2O (10 mL) and $NaHCO_3$ (10% aq sol, 10 mL), filtered, and concentrated. Flash chromatography on silica using 1:24 EtOAc−hexane yielded acetate **9e** (0.20 g, 41%) as a colorless solid that crystallized from hexane: mp 121−122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.29 (s, 3 H), 3.83 (s, 3 H), 3.85 (s, 3 H), 6.29 (d, 1 H, J = 12 Hz), 6.48 (d, 1 H, J = 12 Hz), 6.85 (d, 1 H, J = 8.7 Hz), 6.93 (d, 1 H, J = 2.43), 7.06 (d, 1 H, J = 1.5 Hz), 7.09 (d, 1 H, J = 2.4 Hz), 7.67 (s, 2 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.66, 55.94, 60.72, 90.11, 112.16, 123.25, 125.41, 127.47, 128.85, 130.64, 137.10, 139.54, 139.89, 150.69, 157.67, 168.79; HRMS m/z 582.9482 $[M + CH_3OH]^+$ (calcd for $C_{19}H_{20}I_2O_5$, 582.9479); anal. C 39.30, H 3.13%, calcd for $C_{18}H_{16}I_2O_4$, C 39.30, H 2.93%

*3,5-Diiodo-4,4′-dimethoxy-3′-acetyl-Z-stilbene (**9f**).* To a solution of phenol **9c** (0.1 g, 0.24 mmol) in 3 mL of anhydrous pyridine was added acetic anhydride (50 μL, 0.51 mmol) and a catalytic amount of DMAP. The mixture was stirred for 90 min, and the reaction was terminated by the addition of CH_3OH (5 mL). Toluene was added, and the solution was concentrated under reduced pressure prior to flash chromatography on silica gel. EtOAc−hexane (1:9) as eluent provided a colorless solid (0.1 mg, 91%) that crystallized from hexane: mp 103−104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.27 (s, 3 H), 3.61 (s, 3 H), 3.81 (s, 6 H), 6.38 (d, 1 H, J = 12 Hz), 6.48 (d, 1 H, J = 12 Hz), 6.77 (d, 1 H, J = 1.8 Hz), 6.83 (d, 1 H, J = 8.4 Hz), 6.96 (d, 1 H, J = 1.5 Hz), 7.09 (dd, 1 H, J = 8.4 Hz, J = 2.4 Hz), 7.26 (s, 1 H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 20.61, 55.67, 55.93, 60.44, 92.07, 112.07, 112.92, 123.17, 127.63, 127.74, 129.39, 129.65, 103.97, 134.96, 139.49, 147.99, 150.39, 152.05, 168.81; HRMS m/z 455.0356 $[M + H]^+$ (calcd for $C_{19}H_{20}IO_5$, 455.0355); anal. C 49.67, H 4.18%, calcd for $C_{19}H_{19}IO_5$, C 50.24, H, 4.22%.

*Dibenzyl 3-Iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**10a**).* A solution of *Z*-stilbene **9a** (0.68 g, 1.64 mmol) in acetonitrile (7 mL) was cooled to −10 °C before the addition of CCl_4 (1.6 mL, 16.4 mmol), and the mixture was stirred for 10 min at −10 °C in the dark. Diisopropylamine (0.57 mL, 3.28 mmol) and DMAP (20 mg, cat.) were added in rapid succession. After 1 min, dibenzylphosphite (0.44 mL, 1.96 mmol) was added, and the mixture was stirred for 20 min at −10 °C. The reaction was terminated by the addition of 0.5 M KH_2PO_4 (7 mL) and extracted with EtOAc (3 × 15 mL). The organic phase was filtered and concentrated. The oily residue was separated by CC using 4:1 hexane−EtOAc as eluent to yield 0.94 g (86%) of a pure oil: bp (dec) 274 °C (0.01 mmHg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.51 (s, 3 H), 3.65 (s, 3 H), 3.72 (s, 3 H), 5.04 (s, 2 H), 5.06 (s, 2 H), 6.36 (d, 1 H, J = 9 Hz), 6.42 (d, 1 H, J = 9 Hz), 6.77 (d, 1 H, J = 1.2 Hz), 6.89 (d, 1 H, J = 6 Hz), 7.02 (d, 1 H, J = 6 Hz), 7.01 (s, 1 H), 7.19 (d, 1 H, J = 1.2 Hz), 7.28−7.35 (m, 10 H); ^{13}C NMR ($CDCl_3$, 75

MHz) δ 56.20, 56.50, 60.73, 71.18, 71.24, 92.73, 113.72, 114.23, 122.58, 122.61, 128.10, 128.93, 129.50, 129.56, 130.18, 130.85, 130.86, 131.87, 136.26, 136.66, 136.73, 140.32, 140.39, 149.12, 151.21, 151.25, 153.26; HRMS *m/z* 673.0808 [M + H]$^+$ (calcd for $C_{31}H_{31}IO_7P$, 673.0852); anal. C 55.37, H 4.64%, calcd for $C_{31}H_{30}IO_7P$, C 55.37, H 4.50%.

*Dibenzyl 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**10b**).* Dibenzyl phosphate **10b** (0.38 g, 55% yield) was obtained from **9c** (0.46 g, 0.91 mmol), by the method described above for the synthesis of monoiodide **10a**, as a colorless oil: bp (dec) 220 °C; (0.01 mmHg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.78 (s, 3 H), 3.81 (s, 6 H), 5.13 (s, 2 H), 5.16 (s, 2 H), 6.28 (d, 1 H, *J* = 12 Hz), 6.42 (d, 1 H, *J* = 12 Hz), 6.78 (d, 1 H, *J* = 9 Hz), 7.00 (d, 1 H, *J* = 8.7 Hz), 7.07 (s, 1 H), 7.33 (s, 10 H), 7.64 (s, 2 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.96, 60.71, 69.83, 69.89, 90.15, 112.40, 122.23, 122.26, 125.60, 126.20, 126.21, 127.93, 128.49, 128.55, 130.66, 137.12, 139.92, 157.68; ^{31}P NMR ($CDCl_3$,162 MHz) δ −5.51; HRMS *m/z* 768.9699 [M + H]$^+$ (calcd for $C_{30}H_{28}I_2O_6P$, 768.9713).

General Procedures for Syntheses of the Phosphoric Acids and Derivatives. *Method A.* Each of the metal cation phosphate salts was obtained by the procedure outlined below for preparing the potassium salt **11a**, except for the metal counterions introduced by treatment of the phosphoric acid using either LiOH or NaOMe.

Method B. Dowex-50W (2 g, HCR-W2) was placed in a column and washed successively with CH_3OH (50 mL), 1 N HCl (until pH 1), water (until pH 7), base/amine/amino acid (until pH 7−14), and H_2O (until pH 7). The column was recycled. The potassium salt **11a** or **12a** (about 25 mg) was dissolved in deionized H_2O (1 mL) and applied to a Dowex-50W (HCR-W2) resin column (bearing the appropriate amine or amino acid methyl ester), eluted with approximately 40 mL of H_2O. The eluent was concentrated by freeze-drying to give the required cation derivative.

Method C. Amino Acid Methyl Esters. The amino acid methyl ester hydrochloride was neutralized in CH_3OH solution by addition of potassium carbonate. Ether was added to precipitate the potassium chloride, and the solution was filtered and concentrated. The amino acid methyl ester residue was then applied to the Dowex-50W (HCR-W2) resin column as described in method B.

*Potassium 3-Iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11a**).* Trimethylbromosilane (277 μL, 1.8 mmol) was added to a cooled (0 °C) solution of benzyl phosphate **10a** in DCM (40 mL), and the mixture was stirred for 90 min before addition of sodium thiosulfate (10% aq, 10 mL). After an additional 1 min of stirring, the phases were separated and the aqueous phase was extracted successively with DCM (20 mL) and EtOAc (2 × 20 mL). The combined organic extracts were filtered and concentrated to afford the phosphoric acid intermediate as a clear oil. After drying (high vacuum) for 1 h, the oil was dissolved in CH_3OH (10 mL) with cooling to 0 °C, KOH (1.8 mL, 1 N solution in CH_3OH) was added, and the mixture was stirred for 20 min. The precipitate was collected and triturated with ether to afford the potassium salt as a colorless solid: mp 197−198 °C (dec); ^{1}H NMR (D_2O, 300 MHz) δ 3.51 (s, 3 H), 3.64 (s, 3 H), 3,71 (s, 3 H), 6.33 (d, 1 H, *J* = 12 Hz), 6.51 (d, 1 H, *J* = 12 Hz), 6.70 (s, 2 H), 6.84 (s, 1 H), 7.22 (s, 2 H); ^{31}P NMR (D_2O, 162 MHz) δ 0.94.

*Sodium 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11b**):* colorless solid; mp 194−195 °C (dec); ^{1}H NMR (D_2O, 300 MHz) δ 3.50 (s, 3 H), 3.67 (s, 3 H), 3.68 (s, 3 H), 6.50 (d, 1 H, *J* = 12 Hz), 6.70 (d, 1 H, *J* = 12 Hz), 6.72 (s, 1 H), 6.77 (s, 1 H), 6.79 (s, 1 H), 7.01 (s, 1 H), 7.13 (s, 1 H).

*Lithium 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11c**):* colorless solid; mp 245−275 °C (dec); ^{1}H NMR (D_2O, 400 MHz) δ 3.50 (s, 3 H), 3.62 (s, 3 H), 3.66 (s, 3 H), 6.33 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.70 (s, 2 H), 6.83 (s, 1 H), 7.20 (s, 1 H), 7.22 (s, 1 H).

*Morpholine 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11d**):* colorless oil; ^{1}H NMR (D_2O, 300 MHz) δ 3.11−3.15 (m, 8 H), 3.50 (s, 3 H), 3.63 (s, 3 H), 3.68 (s, 3 H), 3.77−3.81 (m, 8 H), 6.33 (d, 1 H, *J* = 12 Hz), 6.50 (d, 1 H, *J* = 12 Hz), 6.73 (s, 2 H), 6.82 (s, 1 H), 7.18 (s, 1 H), 7.20 (s, 1 H).

*Piperidine 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11e**):* colorless oil; ^{1}H NMR (D_2O, 300 MHz) δ 1.51 (m, 4 H), 1.62 (m, 8 H), 3.00 (t, 8 H, *J* = 6 Hz), 3.51 (s, 3 H), 3.63 (s, 3 H), 3.67 (s, 3 H), 6.34 (d, 1 H, *J* = 12.6 Hz), 6.51 (d, 1 H, *J* = 12.6 Hz), 6.72 (s, 2 H), 6.83 (s, 1 H), 7.21 (s, 1 H).

*Glycine-OMe 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11f**):* colorless solid; mp 74−78 °C; ^{1}H NMR (D_2O, 300 MHz) δ 3.48 (s, 3 H), 3.61 (s, 3 H), 3.67 (s, 3 H), 3.68 (s, 3 H), 3.76 (s, 2 H), 6.30 (d, 1 H, *J* = 12 Hz), 6.46 (d, 1 H, *J* = 12 Hz), 6.69−6.77 (m, 3 H), 7.10 (s, 1 H), 7.16 (s, 1 H).

*Tryptophan-OMe 3-iodo-4,4′,5-trimethoxy-Z-stilbene 3′-O-phosphate (**11g**):* colorless solid; mp 108−112 °C; ^{1}H NMR (DMSO, 300 MHz) δ 3.19 (d, 2 H, *J* = 6.3 Hz), 3.56 (s, 3 H), 3.61 (s, 3 H), 3.66 (s, 3 H), 3.70 (s, 3 H), 4.09 (t, 1 H, *J* = 6 Hz), 6.35 (d, 1 H, *J* = 12 Hz), 6.47 (d, 1 H, *J* = 12 Hz), 6.81−6.85 (m, 2 H), 6.98 (t, 1 H, *J* = 7.2 Hz), 7.07 (t, 1 H, *J* = 8.1 Hz), 7.18 (s, 1 H), 7.22 (s, 1 H), 7.34 (d, 1 H, *J* = 8.1 Hz), 7.40 (s, 1 H), 7.46 (d, 1 H, *J* = 7.2 Hz).

*Tris(3-iodo-4,4′,5-trimethoxy-Z-stilbene) 3′-O-phosphate (**11h**):* colorless solid; mp 75−81 °C; ^{1}H NMR (DMSO, 300 MHz) δ 3.42 (s, 9 H), 3.57 (s, 3 H), 3.67 (s, 3 H), 3.70 (s, 3 H), 6.35 (d, 1 H, *J* = 12 Hz), 6.48 (d, 1 H, *J* = 12 Hz), 6.76 (d, 1 H, *J* = 8.4 Hz), 6.81 (d, 1 H, *J* = 8.7 Hz), 6.92 (s, 1 H), 7.22 (s, 1 H), 7.42 (s, 1 H).

*Potassium 3,5-diiodo-4,4′dimethoxy-Z-stilbene 3′-O-phosphate (**12a**).* Phosphate **12a** (0.20 g, 80%) was obtained from ester **10b** (0.29 g, 0.38 mmol) as described above for the synthesis of **11a**, except that the aqueous phase was extracted with butyl alcohol (3 × 25 mL) because the phosphoric acid was sparingly soluble in EtOAc and DCM. The potassium salt was a colorless solid: mp 210−215 °C (dec); ^{1}H NMR (D_2O, 300 MHz) δ 3.69 (s, 6 H), 6.27 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.64 (s, 2 H), 7.20 (s, 1 H), 7.62 (s, 2 H); ^{31}P NMR (D_2O, 162 MHz) δ 0.973.

*Sodium 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12b**):* colorless solid; mp 215−234 °C (dec); ^{1}H NMR (D_2O, 300 MHz) δ 3.69 (s, 3 H), 3.72 (s, 3 H), 6.29 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.69 (s, 2 H), 7.20 (s, 1 H), 7.64 (s, 2 H).

*Lithium 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12c**):* colorless solid; 250−270 °C (dec); ^{1}H NMR (D_2O, 300 MHz) δ 3.68 (s, 3 H), 3.71 (s, 3 H), 6.28 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.68 (s, 2 H), 7.19 (s, 1 H), 7.64 (s, 2 H); ^{31}P NMR (D_2O, 162 MHz) δ 0.96.

*Morpholine 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12d**):* colorless, waxy solid; mp 75−80 °C; ^{1}H NMR (DMSO, 300 MHz) δ 2.96−2.99 (m, 8 H), 3.74−3.77 (m, 8 H), 3.82 (s, 3 H), 3.83 (s, 3 H), 6.43 (d, 1 H, *J* = 12.5 Hz), 6.60 (d, 1 H, *J* = 12.5 Hz), 6.86 (d, 1 H, *J* = 8.2 Hz), 6.93 (d, 1 H, *J* = 8.2 Hz), 7.49 (s, 1 H), 7.78 (s, 2 H).

*Piperdine 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12e**):* colorless oil; ^{1}H NMR (DMSO, 300 MHz) δ 1.51 (br s, 12 H), 2.79−2.81 (m, 8 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 6.31 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.73 (d, 1 H, *J* = 8.4 Hz), 6.80 (d, 1 H, *J* = 8.4 Hz), 7.40 (s, 1 H), 7.61 (s, 1 H).

*Glycine-OMe 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12f**):* colorless solid; mp 90−97 °C; ^{1}H NMR (DMSO, 300 MHz) δ 3.61 (s, 4 H), 3.68 (s, 6 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 6.31 (d, 1 H, *J* = 12 Hz), 6.49 (d, 1 H, *J* = 12 Hz), 6.72 (d, 1 H, *J* = 9.6 Hz), 6.80 (d, 1 H, *J* = 8.1 Hz), 7.37 (s, 1 H), 7.67 (s, 1 H).

*Tryptophan-OMe 3,5-diiodo-4,4′-dimethoxy-Z-stilbene 3′-O-phosphate (**12g**):* colorless solid; mp 125−130 °C; ^{1}H NMR (DMSO, 300 MHz) δ 3.34 (d, 1 H, *J* = 6.5 Hz), 3.36 (d, 1 H, *J* = 6.5 Hz), 3.66 (s, 3 H), 3.70 (s, 3 H), 3.72 (s, 3 H), 4.32 (t, 1 H, *J* = 6.5 Hz), 6.31 (d, 1 H, *J* = 12 Hz), 6.48 (d, 1 H, *J* = 12 Hz), 6.78−6.81 (m, 2 H), 7.01 (s, 1 H), 7.05 (t, 1 H, *J* = 7 Hz), 7.13 (t, 1 H, *J* = 7 Hz), 7.39 (d, 1 H, *J* = 7.5 Hz), 7.47 (d, 1 H, *J* = 8 Hz), 7.60 (s, 1 H).

*Tris 3,5-diiodo-4,4′dimethoxy-Z-stilbene 3′-O-phosphate (**12h**):* colorless solid; mp 115−120 °C; ^{1}H NMR (DMSO, 300 MHz) δ 3.34 (s, 18 H), 3.69 (s, 3 H), 3.71 (s, 3 H), 6.30 (d, 1 H, *J* = 12 Hz), 6.47 (d, 1 H, *J* = 12 Hz), 6.70 (d, 1 H, *J* = 8.1 Hz), 6.78 (d, 1 H, *J* = 8.1 Hz), 7.37 (s, 1 H), 7.67 (s, 2 H).

Tubulin Evaluation Procedures. The methods used to measure inhibition both of tubulin assembly[16] and of [^{3}H]colchicine binding to tubulin[13] have been described in detail elsewhere. In brief, the assembly reaction was evaluated using purified tubulin at 10 μM in 0.8 M monosodium glutamate (pH 6.6 with HCl), varying compound concentrations, 0.4 mM GTP, and 4% (v/v) dimethyl sulfoxide (compound solvent). Extent of assembly after 20 min at 30 °C was measured. In the colchicine binding assay, the tubulin concentration was 1.0 μM, the [^{3}H]colchicine concentration was 5.0 μM, and compound concentrations were as indicated. Additional reaction components are detailed in ref 13. Incubation was for 10 min at 37 °C, and the tubulin−[^{3}H] colchicine complex was separated from unbound colchicine by retention of the complex by DEAE-cellulose filters.

Cancer Cell Line Procedures. Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay as previously described.[17] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium were inoculated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the plates were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated microplate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data with Immunosoft software.

Mouse leukemia P388 cells[18] were incubated for 24 h in a 10% horse serum/Fisher medium followed by a 48 h incubation with serial dilutions of the compounds. Cell growth inhibition (ED_{50}) was then calculated using a Z1 Beckman/Coulter particle counter.

Anti-angiogenesis Assays. In vitro Matrigel anti-angiogenesis assays were conducted according to the Developmental Therapeutics Program, NCI, protocols.[19] Growth inhibition and cord formation assays were performed using HUVECs purchased from GlycoTech. HUVECs were grown in EGM-2 medium (Cambrex). The standard sulforhodamine B assay (see above) was used to evaluate the activity against HUVECs. IC_{50} values (drug concentration causing 50% inhibition) were calculated from the plotted data.

Cord Formation Assay. Matrigel, a basement membrane matrix, was purchased from BD Biosciences. An aliquot of 60 μL was placed in each well of an ice-cold 96-well plate. The plates were left for 15 min at rt and incubated for 30 min at 37 °C to permit the Matrigel to polymerize. Meanwhile, HUVECs were harvested and diluted to a concentration of 2×10^5 cells/mL, and 100 μL of this preparation was added to each well. A second solution of 100 μL containing the compound to be tested was added. After a 24 h incubation, images were taken at each concentration using an inverted Nikon Diaphot microscope and D100 digital camera. Drug effect was assessed, compared to untreated controls, by measuring the length of cords formed and the number of junctions.

Antimicrobial Susceptibility Testing. The stilbenes (**9a−c**, **11a−h**, **12a−h**) were evaluated against the bacteria *Stenotrophomonas maltophilia* ATCC 13637, *Micrococcus luteus* Presque Isle 456, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 13047, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 6303, and *Neisseria gonorrheae* ATCC 49226 and the fungi *Candida albicans* ATCC 90028 and *Cryptococcus neoformans* ATCC 90112, according to established broth microdilution susceptibility assays.[20,21] Each substance was reconstituted in a small volume of sterile DMSO and diluted in the appropriate medium immediately prior to susceptibility experiments. The minimum inhibitory concentration was defined as the lowest concentration of compound that inhibited all visible growth of the test organism (optically clear). Assays were repeated on separate days.

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Notes

The authors declare no competing financial interest.

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DEDICATION

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