Combretastatin Dinitrogen-Substituted Stilbene Analogues as Tubulin-Binding and Vascular-Disrupting Agents[#]

Rogelio Siles,[†] J. Freeland Ackley,[†] Mallinath B. Hadimani,[†] John J. Hall,[†] Benon E. Mugabe,[†] Rajsekhar Guddneppanavar,[†] Keith A. Monk,[†] Jean-Charles Chapuis,[§] George R. Pettit,[§] David J. Chaplin,[⊥] Klaus Edvardsen,[‡] Mary Lynn Trawick,[†] Charles M. Garner,[†] and Kevin G. Pinney*[,†]

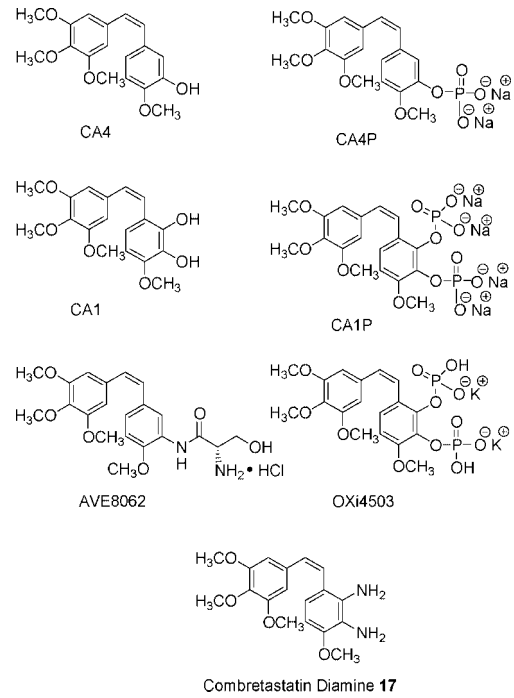
Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, Texas 76798-7348, Department of Cell and Molecular Biology, University of Lund, BMC 112, 22184, Lund, Sweden, Cancer Research Institute and Department of Chemistry and Biochemistry, Arizona State University, P.O. Box 872404, Tempe, Arizona 85287-2404, and Oxigene Inc., 230 Third Avenue, Waltham, Massachusetts 02451

Received July 27, 2007

Several stilbenoid compounds having structural similarity to the combretastatin group of natural products and characterized by the incorporation of two nitrogen-bearing groups (amine, nitro, serinamide) have been prepared by chemical synthesis and evaluated in terms of biochemical and biological activity. The 2′,3′-diamino B-ring analogue **17** demonstrated remarkable cytotoxicity against selected human cancer cell lines in vitro (average GI_{50} = 13.9 nM) and also showed good activity in regard to inhibition of tubulin assembly (IC_{50} = 2.8 μM). In addition, a single dose (10 mg/kg) of compound **17** caused a 40% tumor-selective blood flow shutdown in tumor-bearing SCID mice at 24 h, thus suggesting the potential value of this compound and its corresponding salt formulations as new vascular-disrupting agents.

The African bush willow tree [*Combretum caffrum* Kuntze (Combretaceae)] has proved to be an exceptionally rich source of stilbenoid natural products initially isolated and characterized by Pettit and colleagues.[1,2] Two of these compounds, combretastatin A4 (CA4)[3] and combretastatin A1 (CA1),[4] have pronounced activity as inhibitors of tubulin assembly and, in appropriate phosphate prodrug formulations (CA4P[5,6] and CA1P,[7] respectively), are clinically relevant examples of potent vascular-disrupting agents (VDAs).[8,9] Small-molecule VDAs are characterized by their ability to disrupt blood flow selectively in the tumor microenvironment, resulting in further hypoxia and ultimately necrosis for certain tumor types. CA4P is rapidly cleaved to CA4, which is a strong inhibitor of tubulin assembly. Microtubule disruption induces cytoskeletal rearrangements, leading to cell shape changes of endothelial cells in tumor microvasculature that results in vessel occlusion.[10–15]

Structure–activity relationship (SAR) studies around the *Z*-stilbenoid molecular template, inherent to a large combretastatin group of compounds, resulted in the discovery of a CA4 analogue substituted with an amine group at position 3′ of the B ring.[16–20] This compound is a potent inhibitor of tubulin assembly and also shows significant cytotoxicity against human cancer cell lines in vitro.[16,19] Formulated as a serinamide prodrug known as AVE8062, this compound is currently in human clinical trials.[16,21–23] Recently we reported examination of a small molecular library of combretastatins each substituted with one nitrogen entity (amine, nitro, serinamide, etc.) and extended the SAR around this stilbenoid molecular core by demonstrating that substitution at the 2′-position of the B ring with an amino moiety results in a modified CA4 analogue with impressive biochemical and biological activity.[24] The current study delineates our recent efforts of molecular design and synthesis along with biochemical and biological evaluation of dinitrogen-substituted combretastatins. Of special significance is the 2′,3′-diaminocombretastatin analogue **17**, which is the nitrogen variant of the diphenol CA1.[25] This molecule is especially noteworthy since both CA4P and the corresponding nitrogen analogue (AVE8062), along with the diphenol phosphate prodrug (CA1P, also known as Oxi4503), are all currently in human clinical trials.[13,22,23,26,27] Details of the syntheses of these new dinitrogen combretastatin analogues as well as preliminary biochemical and biological results are presented herein.



Results and Discussion

Design and Synthesis. The synthetic strategy that allowed the preparation of the combretastatin dinitrogen derivatives utilized a Wittig reaction as a key step to form the requisite *Z*-stilbenoid. Accordingly, the *Z*-stilbenoids were prepared by reacting 3,4,5-trimethoxybenzylphosphonium bromide **2** (Scheme 1)[19,28] with commercially available 3,5-dinitro-4-methoxybenzaldehyde along with aldehydes **6** and **7** (Scheme 2)[24a,29] using NaH as the base to generate the ylide (Scheme 3). The *Z*-alkenes were separated from

[#] Dedicated to Dr. G. Robert Pettit of Arizona State University for his pioneering work on bioactive natural products.

* To whom correspondence should be addressed. Tel: (254) 710-4117. Fax: (254) 710-4272. E-mail: Kevin_Pinney@baylor.edu.

[†] Baylor University.

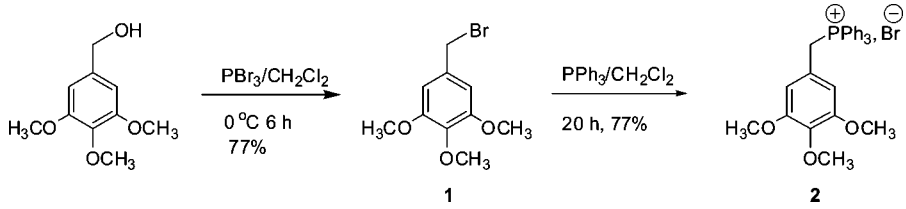
[‡] University of Lund.

[§] Arizona State University.

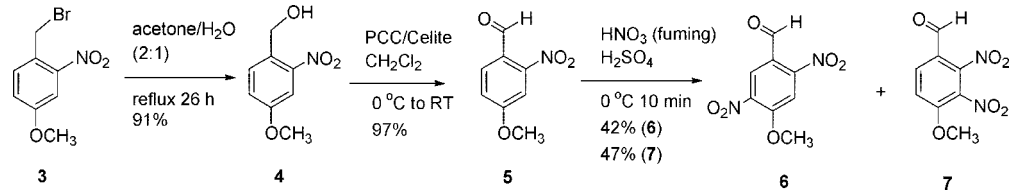
[⊥] Oxigene, Inc.

10.1021/np070377j CCC: $40.75
Published on Web 02/28/2008

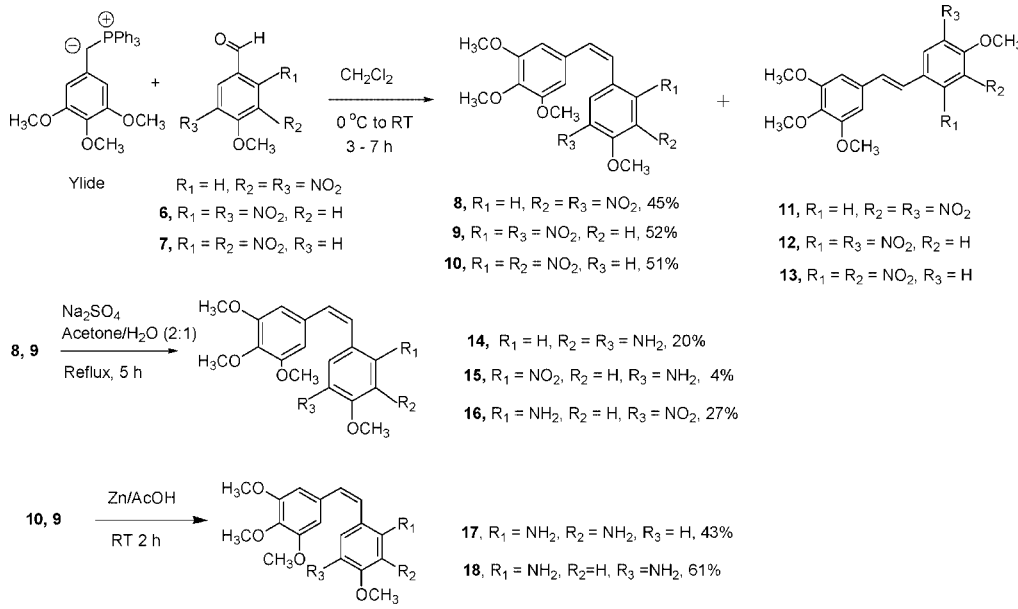
Scheme 1. Synthesis of 3,4,5-Trimethoxybenzyltriphenylphosphonium Bromide



Scheme 2. Synthesis of 4-Methoxy-2,5- and 2,3-Dinitrobenzaldehydes



Scheme 3. Synthesis of *Z*-CA1 Analogues



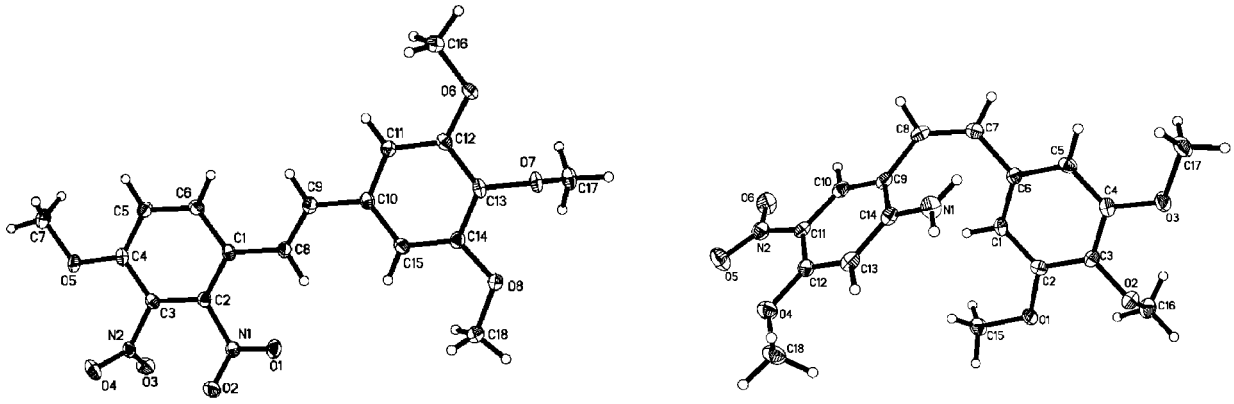
their corresponding *E*-isomers by flash chromatography to afford stilbenes **8**−**10** in moderate yield.

Bromide **3**[24a] was hydrolyzed to benzyl alcohol **4**,[30] which upon treatment with PCC, afforded the intermediate benzaldehyde **5**.[31] Nitration[29] of 4-methoxy-2-nitrobenzaldehyde (**5**) afforded both 2,5- and 2,3-dinitrobenzaldehydes (**6** and **7**, respectively),[25] which were separated by column chromatography. Yields of aldehydes **6** and **7** decreased considerably when the reaction was stirred for more than 10 min, due to the formation of carboxylic acids, which complicated product separation.

Reduction of the nitro groups on stilbenes **8** and **9** was carried out using sodium dithionite (Scheme 3). Interestingly, when the same number of molar equivalents of the reducing reagent was used for both isomers, only stilbene **8** was successfully converted to its corresponding diamine **14** (albeit in low yield), while stilbene **9** produced two isomeric monoamines, **15** and **16**. The formation of monoamine **15** resulted when the reduction took place at the 5′-position of the B ring while the nitro group at the 2′-position remained intact. Isomer **16** was formed when the 2′-nitro group was selectively reduced. The regioisomeric assignments for **15** and **16** were initially determined by NMR. Significant differences in the ^{1}H and ^{13}C NMR spectra for both isomers were noted, particularly in the vinylic region, where the vinyl hydrogens of isomer **15** appeared more downfield (δ 6.87 and 6.53 ppm) than the respective hydrogens of isomer **16** (δ 6.63 and 6.32 ppm). This was explained, in part, by resonance contributions that place a positive charge on one of the vinylic carbons of isomer **15**; thus rendering the respective hydrogens more deshielded. The effect was not seen in isomer **16** because the nitro group located at the 5′-position does not allow a resonance structure that can be stabilized by the carbon−carbon double bond. Additional spectroscopic support for these assignments was obtained from DEPT 45, COSY, and HETCOR NMR spectra of both isomers. Final confirmation of the structure resulted from single-crystal X-ray crystallographic analysis of monoamine **16** that indicated both the *Z* double bond configuration as well as the correct regioisomeric assignment of the amino group (Figure 1).[32] In addition, X-ray crystallographic analysis was carried out on compound **13**, confirming the *E* double bond configuration of this dinitro analogue (Figure 1).[32]

Successful reduction of both nitro groups of compounds **9** and **10** to their corresponding diamines **18** and **17**, respectively, was achieved using zinc powder in acetic acid (Scheme 3).[33] It is important to note that the 2′,5′-diamino analogue **18** partially decomposed at room temperature from an initial purity level after flash chromatography of approximately 95% (by NMR) to approximately 75%. However, the compound retains its original level of purity if stored at freezer temperature (approximately −20 °C).

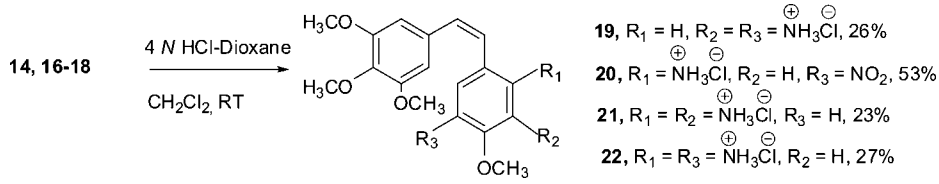
Conversion of monoamine **16** and diamines **14**, **17**, and **18** into their corresponding hydrochloride salts proceeded smoothly with a 4.0 N solution of hydrochloric acid in dioxane (Scheme 4).[16,33] 3′,5′-Diaminostilbene **14** was also converted to serinamide **24**



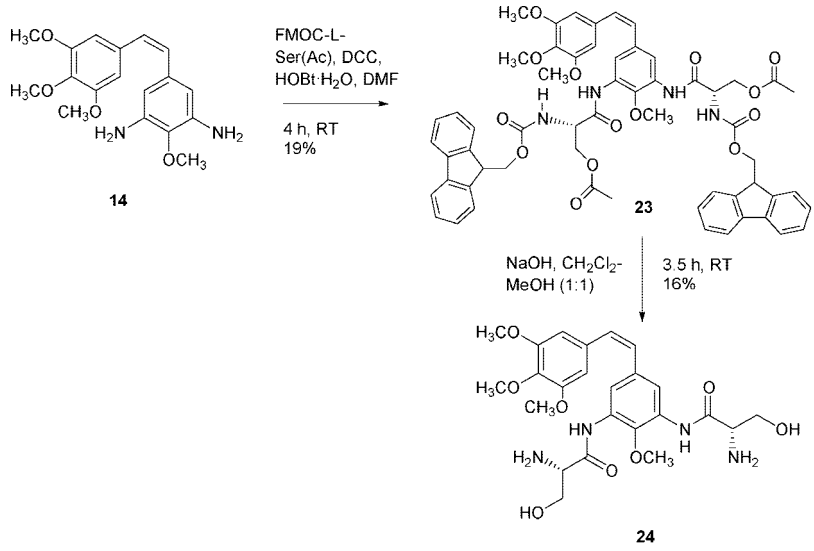
Compound **13** Compound **16**

Figure 1. Thermal ellipsoid plots at 50% probability for compounds **13** and **16**.

Scheme 4. Synthesis of Mono- and Diamine Hydrochloride Salts



Scheme 5. Synthesis of 3′,5′-Diserinamide CA1 Derivative



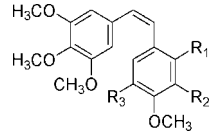
(Scheme 5). While the 2′,5′-diamino analogue **18** is not entirely stable at room temperature, its corresponding hydrochloride salt **22** is stable.

Biological Evaluation. A series of 15 new dinitrogen-substituted combretastatin-type derivatives, each with two nitrogen entities (amine, nitro, serinamide, etc.), have been evaluated for their ability to inhibit tubulin assembly and for cytotoxicity against human cancer cell lines. In addition, selected compounds were evaluated for their ability to impair blood flow to tumors in SCID mice.

A comparison of regioisomeric diaminocombretastatins **14** and **17**, along with hydrochloride salts **19**, **21**, and **22**, illustrates the significant differences in terms of biological activity among the 3′,5′-, 2′,3′-, and 2′,5′-substitution patterns. The 2′,5′-diaminohydrochloride salt **22** demonstrated moderate activity against six human cancer cell lines and had an IC_{50} for inhibition of tubulin polymerization of 14.1 μM, while the 3′,5′-diamino compound **14** had more limited cancer cell cytotoxicity. Although **14** exhibited some activity against the murine P388 lymphocytic leukemia cell line, it showed no ability to inhibit tubulin polymerization. In contrast, the 2′,3′-diamino analogue **17** demonstrated impressive biological activity. For example, compound **17** inhibits tubulin assembly with an IC_{50} value comparable to that of CA1. In addition, compound **17** and its dihydrochloride salt **21** showed outstanding cytotoxicity, with average GI_{50} values of 13.9 and 12.7 nM, respectively (Table 1), against all six human cancer cell lines in this study. This result was confirmed in the MTT assay (Table 3), in which compound **17** demonstrated cytotoxicity in both 1 h (IC_{50} = 2.4 μM) and 5 day (IC_{50} = 0.0043 μM) exposures. Furthermore, compound **17** demonstrated significant in vivo bloodflow shutdown in SCID mice at a dose of 10 mg/kg, which is intermediate between the two clinically relevant compounds, CA4P and CA1P (Table 3).

Of the six dinitro-substituted stilbenoids **8**−**13**, only compound **8** showed a significant ability to inhibit tubulin polymerization. In this group, compound **8** was the most cytotoxic against the six selected cell lines (Table 1). Despite their inability to inhibit tubulin assembly, the *Z*-analogues **9** and **10** have significant

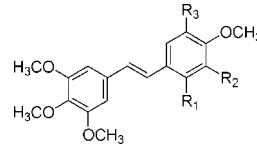
Table 1. Inhibition of Microtubule Formation and Cytotoxicity against Six Cancer Cell Lines by Compounds **8**−**10**, **14**−**22**, and **24**



compound	R_1	R_2	R_3	tubulin inhibition IC_{50} (μM)	GI_{50} (μM) (SRB assay) BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
CA4	H	OH	H	1.2	0.39[a]	na[b]	na	0.0006[a]	0.34[a]	0.0008[a]
CA1	OH	OH	H	1.9	4.4[a]	na	na	0.74[a]	0.061[a]	0.17[a]
8	H	NO_2	NO_2	7.4	0.79	0.41	0.68	0.37	0.52	0.26
9	NO_2	H	NO_2	>40	3.6	2.3	2.8	3.5	4.6	5.5
10	NO_2	NO_2	H	>40	2.1	2.3	2.5	0.37	0.33	0.2
14	H	NH_2	NH_2	>40	1.5	1.5	1.5	2.4	1.1	2.1
15	NO_2	H	NH_2	>40	2.7	2.6	2.8	2.5	3.5	3.3
16	NH_2	H	NO_2	31	3.2	2.6	2.6	3.6	3.5	3.6
17	NH_2	NH_2	H	2.8	0.0079	0.0062	0.0083	0.018	0.025	0.018
19	H	NH_3Cl	NH_3Cl	>40	na	na	na	na	na	na
20	NH_3Cl	H	NO_2	na	1.2	1.5	1.2	4.3	0.98	3.5
21	NH_3Cl	NH_3Cl	H	1.8	0.014	0.011	0.011	0.009	0.019	0.012
22	NH_3Cl	H	NH_3Cl	14.1	1.0	0.61	0.55	0.38	0.44	0.33
24	H	NH-Ser	NH-Ser	>40	na	na	na	na	na	na

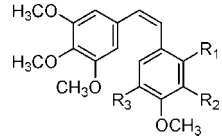
[a] Ref 34 [b] na = not analyzed in this study.

Table 2. Inhibition of Microtubule Formation and Cytotoxicity against Six Cancer Cell Lines by Compounds **11**−**13**



compound	R_1	R_2	R_3	tubulin inhibition IC_{50} (μM)	GI_{50} (μM) (SRB assay) BXPC-3	MCF-7	SF-268	NCI-H460	KM20L2	DU-145
11	H	NO_2	NO_2	>40	>10	>10	>10	>10	>10	>10
12	NO_2	H	NO_2	>40	>10	2.5	1.7	>10	>10	>10
13	NO_2	NO_2	H	>40	>10	>10	>10	>10	>10	>10

Table 3. Cytotoxicity and Blood Flow Reduction by Compounds **14**, **16**, **17**, **19**, and **20**



compound	R_1	R_2	R_3	P388 ED_{50}(μM)	in vivo blood flow shutdown (%) 10 mg/kg	100 mg/kg	MTT (IC_{50} in vitro cytotoxicity) (μM) 1 h	5 days
CA4P	H	OPO_3Na_2	H	0.0004[a]	10	88	0.8	0.0020
CA1P	OPO_3Na_2	OPO_3Na_2	H	<0.01[a]	70	99	3.2	0.0046
14	H	NH_2	NH_2	5.2	3.1	4.6	>44.8	>1.4
16	NH_2	H	NO_2	na[b]	0	0	37.7	>1.4
17	NH_2	NH_2	H	na	40	−[c]	2.4	0.0043
19	H	NH_3Cl	NH_3Cl	na	0	6	>44.8	>1.4
20	NH_3Cl	H	NO_2	6.6	0	13.7	na	na

[a] ref 34. [b] na = not analyzed in this study. [c] SCID mice did not tolerate this dose level.

cytotoxicity, with compound **10** demonstrating submicromolar activity against the NCI-H460, KM20L2, and DU-145 cell lines. The *E*-isomers were inactive in these evaluation systems with the exception of **12**, which demonstrated moderate activity against both the MCF-7 and SF-268 cell lines (Table 2). The mixed amino/nitro-substituted compounds **15**, **16**, and **20**, each containing one amino or amine hydrochloride substitution and one nitro substitution, showed comparable cytotoxicity toward human cancer cell lines (Table 1).

Conclusions

Expansion of our library of nitrogen-substituted combretastatin analogues to the disubstituted series has extended the SAR of these potent compounds and led to the discovery of the 2′,3′-diamino analogue **17** with exceptional biological activity including vascular disruption in a SCID mouse model. While substitution of the 5′-position of the B ring is tolerated, diamino substitution at the 2′- and 3′-positions is most effective. This result is particularly noteworthy in that the 2′,3′-diamino analogue is capable of forming the *ortho* di-imine species analogous to the *ortho* quinone derivative of CA1[35] that is postulated to make a major contribution to the anticancer activity of this vascular-disrupting agent. The most potent compounds in this series, **17**, **22**, and **8**, all have low IC_{50} values for inhibition of tubulin polymerization, thus providing evidence that a significant aspect of their activity is due to microtubule disruption. The *Z*-series is much more active compared to the corresponding *E*-isomers.

Experimental Section

General Experimental Procedures. [36] Reactions involving air- or moisture-sensitive reagents were performed in oven-dried glassware under inert atmospheric conditions (N_2). Solvents used for chromatography and reactions were purchased from commercial sources (e.g., Aldrich, Acros, Alfa Aesar) and used without further purification unless indicated. Column chromatography was performed on Merck silica gel 60, 0.040–0.063 mm, 230–400 mesh ASTM. Precoated silica gel plates (EM Science 60, F_{254}, 250 μm and 2 mm) were used for both analytical and preparative TLC. IR spectra were recorded either neat or as Nujol mulls with a Genesis II FTIR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained by using Bruker DPX (300 MHz for ^{1}H, 121 MHz for ^{31}P, and 75 MHz for ^{13}C), Bruker AMX (360 MHz for ^{1}H and 90 MHz for ^{13}C), and Varian (500 MHz for ^{1}H, and 125 MHz for ^{13}C) spectrometers in deuterated chloroform with 0.03% TMS as the internal reference unless otherwise specified. Chemical shifts are expressed in ppm (δ), coupling constants (J) are expressed in hertz (Hz), and peaks are reported as broad (b), singlets (s), doublets (d), triplets (t), quartets (q), or combinations of each. All ^{13}C spectra reported are proton decoupled. Additional COSY and HETCOR spectra were performed on the same spectrometers to verify molecular structure. Gas chromatography/mass spectrometry (Hewlett-Packard GCD system with electron-impact ionization) was used to monitor selected reactions and to characterize certain products.

Synthesis of Intermediates and Analogues. 3,4,5-Trimethoxybenzyl Bromide (1). [28] At 0 °C and under a nitrogen atmosphere, a solution of phosphorus tribromide (1.1 mL, 11.6 mmol) in CH_2Cl_2 (6.6 mL) was added to a well-stirred solution of 3,4,5-trimethoxybenzyl alcohol (3.22 g, 15.8 mmol) in anhydrous CH_2Cl_2 (15 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and for an additional 4 h at room temperature. At this point, the reaction mixture was slowly added to ice–water (100 mL) and neutralized with $NaHCO_3$. The product was extracted twice with CH_2Cl_2 from the aqueous phase, and the combined organic phase was washed twice with water and brine solution and dried over Na_2SO_4. The solvent was evaporated, yielding a pale brown solid, which was purified by recrystallization (hexanes/diethyl ether) to afford bromide **1** (3.15 g, 12.06 mmol, 77% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.62 (2H, s, H-2, H-6), 4.47 (2H, s, benzylic C*H*$_2$), 3.87 (6H, s, C-3, C-5 OC*H*$_3$), 3.85 (3H, s, C-4 OC*H*$_3$); EIMS *m*/*z* 260/262 (M^+, 1:1), 181 (base).

3,4,5-Trimethoxybenzyltriphenylphosphonium Bromide (2). [19] Bromide **1** (11.6 g, 44.5 mmol) and triphenylphosphine (11.8 g, 44.5 mmol) in CH_2Cl_2 (100 mL) were heated at reflux for 20 h, at which point water was added and the product was extracted from the aqueous phase with CH_2Cl_2. The organic phase was washed twice with water and brine solution and dried over Na_2SO_4. The crude material was triturated (Et_2O) at 0 °C to afford phosphonium salt **2** (32.4 g, 61.9 mmol, 77% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.74 (9H, m, Ar*H*), 7.60 (6H, m, Ar*H*), 6.43 (2H, d, J = 2.7 Hz, H-2, H-6), 5.43 (2H, d, J = 14.1 Hz, benzylic C*H*$_2$), 3.74 (3H, s, C-4 OC*H*$_3$), 3.48 (6H, s, C-3, C-5 OC*H*$_3$); ^{31}P NMR (acetone-d_6, 121 MHz) δ 23.37.

4-Methoxy-2-nitrobenzyl Alcohol (4). [30] Bromide **3** [19] (0.0937 g, 0.38 mmol) was dissolved in acetone/water (6 mL, 2:1 ratio) and heated at reflux for 26 h, at which point water was added and the product was extracted with CH_2Cl_2. The organic phase was washed twice with water and brine and dried over Na_2SO_4. After recrystallization (hexanes/EtOAc), alcohol **4** (0.0635 g, 0.35 mmol, 91% yield) was obtained: ^{1}H NMR ($CDCl_3$, 360 MHz) δ 7.60 (1H, d, J = 2.7 Hz, H-3), 7.58 (1H, d, J = 8.6 Hz, H-6), 7.19 (1H, dd, J = 8.6, 2.7 Hz, H-5), 4.86 (2H, d, J = 6.7 Hz, benzylic C*H*$_2$), 3.88 (3H, s, C-4 OC*H*$_3$), 2.53 (1H, t, J = 6.8 Hz, O*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 159.4 (C, C-4), 148.5 (C, C-2), 131.6 (CH, C-6), 128.7 (C, C-1), 120.5 (CH, C-5), 109.7 (CH, C-3), 62.4 (CH_2, C-1 *C*H$_2$OH), 55.9 (CH_3, C-4 O*C*H$_3$); EIMS *m*/*z* 183 (M^+, 12), 165 ($M^+ - 18$, 32), 135 (100), 106 (64), 77 (52).

4-Methoxy-2-nitrobenzaldehyde (5). [31] A suspension of PCC (3.18 g, 14.7 mmol) and Celite (3.2 g) in anhydrous CH_2Cl_2 (30 mL) was stirred at 0 °C under a nitrogen atmosphere for 30 min, at which point a CH_2Cl_2 solution (20 mL) of alcohol **4** (1.80 g, 9.82 mmol) was added. After stirring for 4.5 h at room temperature, ethyl ether (50 mL) was added, and the solution was filtered through Florisil and washed with ethyl ether (10 mL) followed by CH_2Cl_2 (10 mL). Purification by flash chromatography (EtOAc/hexanes, 20:80) afforded aldehyde **5** (1.73 g, 9.55 mmol, 97% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.28 (1H, s, C*H*O), 7.98 (1H, d, J = 8.7 Hz, H-6), 7.51 (1H, d, J = 2.5 Hz, H-3), 7.25 (1H, dd, J = 8.7, 2.5 Hz, H-5), 3.98 (3H, s, C-4 OC*H*$_3$); EIMS *m*/*z* 181 (M^+, 8), 151 (100), 134 (24), 106 (36), 63 (36).

4-Methoxy-2,5-dinitrobenzaldehyde (6) [25,29] **and 4-methoxy-2,3-dinitrobenzaldehyde (7).** [25,29] At 0 °C, aldehyde **5** (1.53 g, 8.45 mmol) was dissolved in concentrated sulfuric acid (25 mL), to which 7 mL of a precooled (0 °C) mixture of fuming nitric acid (5.32 g, 84.5 mmol) and concentrated sulfuric acid (5.29 g, 54.0 mmol) was slowly added. After stirring for 10 min, the resulting solution was added dropwise into ice–water (approximately 100 mL). After 2 h at 0 °C, the solution was filtered and the solid was rinsed with ice–water (10 mL). Purification by flash column chromatography (EtOAc/hexanes, 20:80) yielded isomers **6** (0.80 g, 3.54 mmol, 42% yield) and **7** (0.89 g, 3.94 mmol, 47% yield).

Spectroscopic characterization of isomer **6**: IR (CH_2Cl_2) ν_{max} 3054, 2987, 2950, 2908, 2855, 1703, 1622, 1552 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.31 (1H, s, C*H*O), 8.43 (1H, s, H-6), 7.73 (1H, s, H-3), 4.14 (3H, s, C-4 OC*H*$_3$); EIMS *m*/*z* 226 (M^+, 4), 196 (12), 179 (16), 149 (56), 121 (100), 75 (40), 63 (32).

Spectroscopic characterization of isomer **7**: IR (CH_2Cl_2) ν_{max} 3097, 3054, 3005, 2987, 2953, 2900, 2858, 1709, 1612, 1564 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.95 (1H, d, J = 0.5 Hz, C*H*O), 8.15 (1H, d, J = 8.9 Hz, H-6), 7.38 (1H, d, J = 8.9 Hz, H-5), 4.08 (3H, s, C-4 OC*H*$_3$); EIMS *m*/*z* 132 (40), 120 (48), 103 (84), 75 (100).

General Procedure for the Synthesis of *Z*-Stilbenes. A suspension of NaH (approximately 6.0 equiv) in anhydrous CH_2Cl_2 (approximately 0.7 M solution) at 0 °C under an inert (N_2) atmosphere was stirred for about 10 min, at which point 1.1 equiv of a previously prepared solution of 3,4,5-trimethoxybenzylphosphonium bromide (**2**, approximately 0.1 M in CH_2Cl_2) was added dropwise. After stirring for 20 min, 1.0 equiv of 4-methoxydinitrobenzaldehyde (0.1 M in CH_2Cl_2) was added, and the mixture was stirred at room temperature for 3–7 h. At this point, ice–water was slowly added until the hydrogen evolution stopped, indicating that all of the NaH had reacted. The product was extracted with CH_2Cl_2, washed twice with water and twice with brine, and dried over Na_2SO_4. The requisite *Z*-stilbenes were separated from their corresponding *E*-isomers by flash chromatography using the solvent system specified for each alkene. Numbering for the combretastatin analogues for spectroscopic analysis is as follows: the trimethoxy A ring is numbered 1–6, and the B ring is numbered 1′−6′. The atoms of the ethylene bridge are numbered 1a and 1a′, where 1a is bound to the A ring and 1a′ is bound to the B ring.

(*Z*)-2-(4′-Methoxy-3′,5′-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)-ethene (8). Flash chromatography (EtOAc/hexanes, 10:90) led to the product in a 45% yield: mp 112–115 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.91 (2H, s, H-2′, H-6′), 6.77 (1H, d, J = 12.1 Hz, H-1a), 6.44 (2H, s, H-2, H-6), 6.43 (1H, d, J = 12.0 Hz, H-1a′), 4.02 (3H, s, C-4′ OC*H*$_3$), 3.86 (3H, s, C-4 OC*H*$_3$), 3.74 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.6 (C, C-3, C-5), 145.7 (C, C-4′), 145.1 (C, C-1), 138.7 (C, C-4), 134.9 (C, C-3′, C-5′), 133.9 (C, C-1′), 130.4 (CH, C-1a′), 128.9 (CH, C-2′, C-6′), 124.5 (CH, C-1a), 105.9 (CH, C-2, C-6), 64.9 (CH_3, C-4 O*C*H$_3$), 61.1 (CH_3, C-4′ O*C*H$_3$), 56.2 (CH_3, C-3, C-5 O*C*H$_3$); EIMS *m*/*z* 390 (M^+, 100), 375 (36); *anal.* C 55.71%, H 4.69%, N 6.85%, calcd for $C_{18}H_{18}N_2O_8$, C 55.39%, H 4.65%, N 7.18%.

(*Z*)-2-(4′-Methoxy-2′,5′-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (9). Flash chromatography (EtOAc/hexanes, 20:80) led to the *Z*-isomer in a 52% yield: mp 90–92 °C; R_f 0.71 (EtOAc/hexanes, 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.72 (1H, s, H-6′), 7.71 (1H, s, H-3′), 6.74 (1H, d, J = 12.0 Hz, H-1a′), 6.68 (1H, d, J = 12.0 Hz, H-1a), 6.29 (2H, s, H-2, H-6), 4.03 (3H, s, C-4′ OC*H*$_3$,), 3.81 (3H, s, C-4 OC*H*$_3$), 3.66 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR (acetone-d_6, 75 MHz) δ 154.7 (C, C-3, C-5), 151.9 (C, C-4′), 150.9 (C, C-2′), 143.4 (C, C-5′), 140.3 (C, C-4), 135.4 (C, C-1), 132.9 (CH, C-1a′), 125.7 (CH, C-1a), 124.7 (CH, C-6′), 120.5 (C, C-1), 111.1 (CH, C-3′), 105.7 (CH, C-2, C-6), 60.7 (CH_3, C-4 O*C*H$_3$), 58.0 (CH_3, C-4′ O*C*H$_3$), 56.5 (CH_3, C-3, C-5 O*C*H$_3$); EIMS *m*/*z* 390 (M^+, 60), 196 (92), 181 (100); *anal.* C 55.32%, H 4.70%, N 7.00%, calcd for $C_{18}H_{18}N_2O_8$, C 55.39%, H 4.65%, N 7.18%.

(*Z*) + (*E*)-2-(4′-Methoxy-2′,3′-dinitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (10 and 13). Crystallization from CH_2Cl_2 at 4 °C afforded a pure sample of the *E*-isomer **13**: mp 226–228 °C; R_f 0.09 (EtOAc/hexanes, 50:50); ^{1}H NMR (acetone-d_6, 300 MHz) δ 8.18 (1H, d, J = 9.1 Hz, H-6′), 7.71 (1H, d, J = 9.1 Hz, H-5′), 7.31 (1H, d, J = 16.1 Hz, H-1a′), 7.08 (1H, d, J = 16.1 Hz, H-1a), 6.95 (2H, s, H-2, H-6), 4.10 (3H, s, C-4′ OC*H*$_3$), 3.86 (6H, s, C-3, C-5 OC*H*$_3$), 3.75

(3H, s, C-4 OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.6 (C, C-3, C-5), 150.9 (C, C-4), 139.2 (C, C-2′), 134.8 (CH, C-6′), 131.4 (C, C-4), 129.9 (C, C-1′, C-3′), 124.2 (C, C-1′), 118.7 (CH, C-1a, C-1a′), 116.1 (CH, C-5′), 104.3 (CH, C-2, C-6), 61.0 (CH_3, C-4 O*C*H$_3$), 57.4 (CH_3, C-4′ O*C*H$_3$), 56.5 (CH_3, C-3, C-5 O*C*H$_3$); *anal.* C 55.69%, H 4.58%, N 7.07%, calcd for $C_{18}H_{18}N_2O_8$, C 55.39%, H 4.65%, N 7.18%. Single-crystal X-ray diffraction further confirmed the *E*-configuration of **13**.[32]

The filtrate was subjected to flash column chromatography (EtOAc/hexanes, 50:50) to isolate a sample of the pure *Z*-isomer **10** in 51% yield as a yellow powder: mp 146–148 °C; R_f 0.21 (EtOAc/hexanes, 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.36 (1H, d, J = 8.9 Hz, H-6′), 7.09 (1H, d, J = 8.9 Hz, H-5′), 6.77 (1H, d, J = 11.8 Hz, H-1a′), 6.49 (1H, d, J = 11.8 Hz, H-1a), 6.30 (2H, s, H-2, H-6), 3.95 (3H, s, C-4′ OC*H*$_3$), 3.82 (3H, s, C-4 OC*H*$_3$), 3.69 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz); δ 153.2 (C, C-3, C-5), 150.9 (C, C-4′), 143.1 (C, C-2′), 138.0 (CH, C-6′), 135.2 (C, C-4), 134.4 (C, C-1), 130.7 (C, C-3′), 124.6 (CH, C-1a′), 121.6 (CH, C-1a), 115.9 (C, C-1′), 106.1 (CH, C-2, C-6, C-5′), 60.9 (CH_3, C-4 O*C*H$_3$), 57.3 (CH_3, C-4′ O*C*H$_3$), 56.0 (CH_3, C-3, C-5 O*C*H$_3$); *anal.* C 55.43%, H 4.58%, N 7.11%, calcd for $C_{18}H_{18}N_2O_8$, C 55.39%, H 4.65%, N 7.18%.

General Procedure A for the Reduction of CA-1 Analogues. To a round-bottomed flask containing approximately 1.0 equiv of *Z*-stilbene in acetone/water (0.05 M, 2:1 ratio), heated to 50 °C (approximately 15 min to achieve solution), was added sodium dithionite (approximately 11.9 equiv). The reaction mixture was heated at reflux for approximately 5 h, CH_2Cl_2 was added, and the organic phase was washed three times with brine and dried over Na_2SO_4. The solvent was evaporated at reduced pressure. The products were purified by flash chromatography using the solvents specified.

(*Z*)-2-(3′,5′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (14). Flash chromatography (EtOAc/hexanes, 40:60) led to a pure sample of diamine **14** in a 20% yield: mp 84–86 °C; ^{1}H NMR ($CDCl_3$, 360 MHz) δ 6.56 (2H, s, H-2, H-6), 6.41 (1H, d, J = 12.2 Hz, H-1a), 6.34 (1H, d, J = 12.2 Hz, H-1a′), 6.14 (2H, s, H-2′, H-6′), 3.82 (3H, s, C-4′ OC*H*$_3$), 3.72 (3H, s, C-4 OC*H*$_3$), 3.70 (6H, s, C-3, C-5 OC*H*$_3$), 3.69 (4H, b, N*H*$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.6 (C, C-3, C-5), 139.7 (C, C-4′), 137.0 (C, C-3′, C-5′), 134.1 (C, C-4), 133.8 (CH, C-1), 132.6 (CH, C-1′), 130.2 (CH, C-1a), 129.0 (CH, C-1a′), 106.7 (CH, C-2, C-6), 106.2 (CH, C-2′, C-6′), 60.9 (CH_3, C-4 O*C*H$_3$), 58.3 (CH_3, C-3, C-5 O*C*H$_3$), 55.8 (CH_3, C-4′ O*C*H$_3$); EIMS *m*/*z* 330 (M^+, 60), 315 (100); *anal.* C 65.49%, H 6.77%, N 8.40%, calcd for $C_{18}H_{22}N_2O_4$, C 65.44%, H 6.71%, N 8.48%.

(*Z*)-2-(5′-Amino-4′-methoxy-2′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (15) and (*Z*)-2-(2′-Amino-4′-methoxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (16). Purification by flash chromatography (EtOAc/hexanes, 20:80) afforded pure amines **15** (4% yield) and **16** (27% yield, mp 139–141 °C). Spectroscopic characterization of isomer **15**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.70 (1H, s, H-3′), 6.87 (1H, d, J = 12.0 Hz, H-1a′), 6.53 (1H, d, J = 12.0 Hz, H-1a), 6.46 (1H, d, J = 0.6 Hz, H-6′), 6.31 (2H, s, H-2, H-6), 4.36 (2H, b, N*H*$_2$), 3.92 (3H, s, C-4′ OC*H*$_3$), 3.79 (3H, s, C-4 OC*H*$_3$), 3.62 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8 (C, C-3, C-5), 145.0 (C, C-4′), 142.2 (C, C-5′), 137.5 (C, C-2′), 137.4 (C, C-4), 131.8 (C, C-1), 130.1 (CH, C-1a′), 129.5 (CH, C-1a), 127.9 (C, C-1′), 114.6 (CH, C-6′), 107.0 (CH, C-3′), 106.4 (CH, C-2, C-6), 60.9 (CH_3, C-4 O*C*H$_3$), 56.0 (CH_3, C-4′ O*C*H$_3$), 55.9 (CH_3, C-3, C-5 O*C*H$_3$); *anal.* C 59.51%, H 5.65%, N 7.17%, calcd for $C_{18}H_{20}N_2O_6$, C 59.99%, H 5.59%, N 7.77%.

Spectroscopic characterization of isomer **16**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.97 (1H, s, H-6′), 6.63 (1H, d, J = 12.0 Hz, H-1a), 6.47 (2H, s, H-2, H-6), 6.32 (1H, d, J = 12.0 Hz, H-1a′), 6.20 (1H, s, H-3′), 4.38 (2H, b, N*H*$_2$), 3.91 (3H, s, C-4′ OC*H*$_3$), 3.81 (3H, s, C-4 OC*H*$_3$), 3.65 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.5 (C, C-4′), 153.0 (C, C-3, C-5), 150.3 (CH, C-2′), 138.1 (C, C-4), 133.5 (C, C-1), 131.3 (CH, C-1a), 129.7 (CH, C-1a′), 129.3 (C, C-5′), 122.7 (CH, C-6′), 114.5 (C, C-1′), 105.9 (CH, C-2, C-6), 97.4 (CH, C-3′), 60.9 (CH_3, C-4 O*C*H$_3$), 56.4 (CH_3, C-4′ O*C*H$_3$), 55.9 (CH_3, C-3, C-5 O*C*H$_3$); *anal.* C 60.65%, H 5.88%, N 7.26%, calcd for $C_{18}H_{20}N_2O_6$, C 59.99%, H 5.59%, N 7.77%. Single-crystal X-ray diffraction further confirmed the *Z*-configuration of **16**.[32]

General Procedure B for the Reduction of CA-1 Analogues. To a well-stirred solution of *Z*-stilbene (1.0 equiv, 0.03 M in glacial acetic acid) was added zinc powder (221 equiv), and the resulting suspension was stirred for 2 h at room temperature. At this point, the solution was filtered through Celite and the filtrate was concentrated at reduced pressure. The desired diamine was purified by flash chromatography (EtOAc/hexanes, 50:50).

(*Z*)-2-(2′,3′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (17). The resulting brown-colored residue was subjected to flash chromatography (EtOAc/hexanes, 50:50) to isolate the desired diamine product as a brown oil in 43% yield: R_f 0.14 (EtOAc/hexanes, 50:50); IR (neat) ν_{max} 3433, 3351, 3004, 2942, 2840, 2363, 2257, 1616, 1582, 1505, 1467, 1428 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.66 (1H, d, J = 8.4 Hz, H-6′), 6.52 (1H, d, J = 12.1 Hz, H-1a′), 6.49 (2H, s, H-2, H-6), 6.48 (1H, d, J = 12.1 Hz, H-1a), 6.38 (1H, d, J = 8.4 Hz, H-5′), 3.82 (3H, s, C-4′ OC*H*$_3$), 3.80 (3H, s, C-4 OC*H*$_3$), 3.61 (6H, s, C-3, C-5 OC*H*$_3$), 3.41 (4H, s, N*H*$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.4 (C, C-3, C-5), 147.4 (C, C-4′), 137.0 (C, C-4), 132.7 (C, C-2′), 132.0 (C, C-1), 130.9 (CH, C-1a), 125.9 (CH, C-1a′), 123.0 (C, C-3′), 119.2 (CH, C-6′), 117.6 (C, C-1′), 105.7 (CH, C-2, C-6), 101.9 (C, C-5′), 60.5 (CH_3, C-4 O*C*H$_3$), 55.6 (CH_3, C-4′ O*C*H$_3$), 55.5 (CH_3, C-3, C-5 O*C*H$_3$); *anal.* C 65.19%, H 6.30%, N 7.82%, calcd for $C_{18}H_{22}N_2O_4$, C 65.44%, H 6.71%, N 8.48%.

(*Z*)-2-(2′,5′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (18). Flash column chromatography led to the product in 61% yield: R_f 0.34 (EtOAc/hexanes, 50:50); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.56 (1H, s, H-6′), 6.55 (2H, s, H-2, H-6), 6.46 (1H, d, J = 12.0 Hz, H-1a′), 6.41 (1H, d, J = 12.1 Hz, H-1a), 6.25 (1H, s, H-3′), 3.82 (3H, s, C-4′ OC*H*$_3$), 3.79 (3H, s, C-4 OC*H*$_3$), 3.66 (6H, s, C-3, C-5 OC*H*$_3$), 3.31 (4H, s, N*H*$_2$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.6 (C, C-3, C-5), 148.2 (C, C-4′), 137.2 (C, C-2′), 136.2 (C, C-4), 132.3 (C, C-1), 130.3 (CH, C-1a′), 128.1 (CH, C-1a), 126.0 (CH, C-5′), 116.1 (CH, C-6′), 115.7 (C, C-1′), 105.7 (CH, C-2, C-6), 99.8 (CH, C-3′), 60.8 (CH_3, C-4 O*C*H$_3$), 55.7 (CH_3, C-3, C-5, O*C*H$_3$), 55.4 (CH_3, C-4′ O*C*H$_3$). It is important to note that the 2′,5′-diamino analogue **18** partially decomposed at room temperature from an initial purity level after flash chromatography of approximately 95% (by NMR) to approximately 75%. However, the compound retains its original level of purity if stored at −20 °C.

General Procedure for the Synthesis of Mono- and Diamine Hydrochloride Salts. To 1.0 equiv of a well-stirred solution of a mono- or diamino stilbene (0.02 M in CH_2Cl_2) was added 5 equiv of HCl (4.0 N solution in dioxane), and the reaction mixture was stirred for 2–10 h at room temperature. At this point, the solvent was removed under reduced pressure and the resulting oil or solid was purified as described for each particular salt.

(*Z*)-2-(3′,5′-Diamine hydrochloride-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (19). The solid was recrystallized (CH_2Cl_2/MeOH) to obtain the product in 26% yield: mp 208–212 °C; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.08 (2H, s, H-2′, H-6′), 6.69 (1H, d, J = 12.1 Hz, H-1a), 6.54 (1H, d, J = 12.2 Hz, H-1a′), 6.51 (2H, s, H-2, H-6), 3.91 (3H, s, C-4 OC*H*$_3$), 3.73 (3H, s, C-4′ OC*H*$_3$), 3.69 (6H, s, C-3, C-5 OC*H*$_3$); *anal.* C 52.31%, H 5.96%, N 6.61%, calcd for $C_{18}H_{24}Cl_2N_2O_4$·0.5 H_2O, C 52.55%, H 6.11%, N 6.79%.

(*Z*)-2-(2′-Amine hydrochloride-4′-methoxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (20). The solid was filtered and rinsed with ethyl ether (5 mL) to obtain the salt in 53% yield: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.78 (1H, s, H-6′), 6.74 (1H, s, H-3), 6.73 (1H, d, J = 12.0 Hz, H-1a), 6.55 (2H, s, H-2, H-6), 6.40 (1H, d, J = 12.0 Hz, H-1a′), 3.92 (3H, s, C-4′ OC*H*$_3$), 3.72 (3H, s, C-4 OC*H*$_3$), 3.63 (6H, s, C-3, C-5 OC*H*$_3$).

(*Z*)-2-(2′,3′-Diamine hydrochloride-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (21). To the resulting oil was added anhydrous methanol, and the product crystallized over a period of 3–4 days at −20 °C. The brown-colored crystalline solid that formed was filtered, washed with methanol, and dried to afford the desired salt in 23% yield: mp 92 °C (dec); ^{1}H NMR (CD_3OD, 300 MHz) δ 7.05 (1H, dd, J = 8.5, 0.9 Hz, H-6′), 6.67 (1H, d, J = 11.9 Hz, H-1a′), 6.52 (1H, d, J = 8.6 Hz, H-5′), 6.50 (2H, s, H-2, H-6), 6.44 (1H, d, J = 11.9 Hz, H-1a), 3.90 (3H, s, C-4′ OC*H*$_3$), 3.70 (3H, s, C-4 OC*H*$_3$), 3.60 (6H, s, C-3, C-5 OC*H*$_3$); ^{13}C NMR (CD_3OD, 75 MHz) δ 154.1 (C, C-3, C-5), 153.6 (C, C-4′), 138.6 (C, C-4), 133.9 (C, C-2′, CH, C-6′), 133.7 (C, C-1′), 130.5 (C, C-1), 125.5 (CH, C-1a, C-1a′), 120.2 (C, C-3′), 107.4 (CH, C-2, C-6), 102.5 (CH, C-5′), 61.1 (CH_3, C-4 O*C*H$_3$), 56.8 (CH_3, C-4′ O*C*H$_3$), 56.3 (CH_3, C-3, C-5 O*C*H$_3$).

(*Z*)-2-(2′,5′-Diamine hydrochloride-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (22). The salt was recrystallized from anhydrous methanol. The resultant white solid was filtered, washed

with methanol, and dried to give the desired salt in 27% yield: ^{1}H NMR (CD_3OD, 300 MHz) δ 7.27 (1H, s, H-6′), 7.26 (1H, s, H-3′), 6.89 (1H, d, J = 11.9 Hz, H-1a′), 6.56 (1H, d, J = 11.8 Hz, H-1a), 6.47 (2H, s, H-2, H-6), 4.03 (3H, s, C-4′ OCH_3), 3.71 (3H, s, C-4 OCH_3), 3.63 (6H, s, C-3, C-5 OCH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ 154.5 (CH, C-3, C-5), 154.2 (C, C-4′), 139.2 (C, C-4), 136.4 (C, C-1), 133.8 (C, C-2′), 132.5 (CH, C-6′), 127.3 (C, C-1′), 125.7 (CH, C-1a′), 122.5 (CH, C-1a), 120.2 (CH, C-5′), 108.2 (C, C-3′), 107.7 (CH, C-2, C-6), 61.1 (CH_3, C-4 OCH_3), 57.5 (CH_3, C-4′ OCH_3), 56.5 (CH_3, C-3, C-5 OCH_3); *anal.* C 53.24%, H 6.02%, N 6.88%, calcd for $C_{18}H_{24}Cl_2N_2O_4$, C 53.61%, H 6.00%, N 6.95%.

(Z)-2-(3′,5′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethane-Fmoc-L-serinamide (23). A solution, at room temperature, of compound **14** (0.4407 g, 1.34 mmol), DCCI (0.6680 g, 3.21 mmol), FMOC (Ac) serine amino acid (1.196 g, 3.21 mmol), and HOBt·H_2O (0.501 g, 3.21 mmol), in anhydrous DMF (5 mL), was stirred for 4 h. At this point, EtOAc was added and the solution was filtered. The filtrate was washed five times with water and twice with brine and then dried over Na_2SO_4. The solvent was removed under reduced pressure, and the product was purified by flash chromatography (EtOAc/hexanes, 50:50) to afford the FMOC-L-serinamide **23** (0.2518 g, 0.24 mmol, 19% yield): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.44 (1H, b, C-3′ NH), 7.75 (4H, d, J = 7.6 Hz, FMOC H-4), 7.57 (4H, d, J = 7.5 Hz, FMOC H-1), 7.39 (4H, t, J = 7.5 Hz, FMOC H-3), 7.31 (4H, t, J = 7.2 Hz, FMOC H-2), 6.52 (2H, s, H-2, H-6), 6.50 (1H, s, H-2′), 6.49 (1H, s, H-6′), 6.46 (1H, d, J = 12.5 Hz, H-1a′), 6.41 (1H, d, J = 12.3 Hz, H-1a), 5.70 (1H, b, C-5′ NH), 4.36 (12H, m), 3.82 (3H, s, C-4′ OCH_3), 3.68 (6H, s, C-3, C-5 OCH_3), 3.64 (3H, s, C-4 OCH_3), 2.7 (2H, b, COCHNH), 2.09 (3H, s, CH_3CO_2), 2.03 (3H, s, CH_3CO_2).

(Z)-2-(3′,5′-Diamino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethane-L-serinamide (24). Fmoc-L-serinamide **23** (0.131 g, 0.226 mmol) dissolved in CH_2Cl_2/MeOH (6 mL, 1:1 ratio) along with a solution of 2 N sodium hydroxide (0.53 mL) were stirred at room temperature for 3.5 h. At this point, CH_2Cl_2 was added and the organic phase was washed once with water and twice with brine and dried with Na_2SO_4. The solvent was removed under reduced pressure. Purification by normal-phase preparative TLC (CH_2Cl_2/MeOH, 95:5) afforded serinamide **24** (19.1 mg, 0.04 mmol, 16% yield): ^{1}H NMR ($CDCl_3$, 360 MHz) δ 9.88 (1H, b, C-3′ NH), 7.66 (1H, d, J = 1.6 Hz, C-5′ NH), 6.52 (2H, s, C-2, C-6), 6.46 (1H, s, C-2′), 6.47 (1H, s, C-6′), 6.45 (1H, d, J = 12.5 Hz, C-1a′), 6.39 (1H, d, J = 12.3 Hz, C-1a), 3.98 (1H, dd, J = 10.7, 5.0 Hz, COCHNH$_2$), 3.81 (3H, s, C-4′ OCH_3), 3.77 (4H, m, CH_2OH), 3.73 (3H, s, C-4 OCH_3), 3.68 (6H, s, C-3, C-5 OCH_3), 3.62 (1H, t, J = 4.5 Hz, COCH), 2.40 (4H, b, CHNH_2CH_2OH); ^{13}C NMR ($CDCl_3$, 90 MHz) δ 171.6 (C, C=O), 152.7 (C, C=O), 139.4 (C, C-3, C-5), 137.1 (C, C-4′), 135.7 (C, C-1), 134.2 (C, C-1′), 132.5 (CH, C-1a′), 131.1 (CH, C-1a), 129.9 (C, C-3′), 129.6 (C, C-5′), 111.9 (CH, C-2′), 111.2 (CH, C-6′), 106.2 (CH, C-2, C-6), 65.0 (CH_2, CH_2OH), 60.9 (CH_3, C-4 OCH_3), 59.4 (CH_3, C-4′ OCH_3), 56.6 (CH, CHNH$_2$), 55.9 (CH_3, C-3, C-5 OCH_3).

X-ray Crystallographic Analysis of Compounds 13 and 16. [32] Crystallographic data were collected on crystals with dimensions 0.25 × 0.29 × 0.33 mm^3 for **13** and 0.119 × 0.137 × 0.242 mm^3 for **16**. Data were collected at 110 K on a Bruker X8 Apex using Mo Kα radiation (λ = 0.71073 Å). The structure was solved by direct methods after correction of the data using SADABS.[37] Crystallographic data and refinement details for the complexes mentioned herein are found in the Supporting Information (Table 4 and Table 5). The thermal ellipsoid plots at 50% probability for compounds **13** and **16** are displayed in Figure 1. All data were processed using the Bruker AXS SHELXTL software, version 6.10.[38] All hydrogen atoms were placed in calculated positions for **13**. For **16** the amine and ethene hydrogens were located in the difference map and refined isotropically; all other hydrogen atoms were placed in calculated positions. For both **13** and **16** all non-hydrogen atoms were refined anisotropically. The absolute structure of **16** could not be determined reliably.

Biological Assays. Tubulin Polymerization Assay. Tubulin was purified from calf brain according to the method of Hamel and Lin.[24a,39] Polymerization was followed turbidimetrically at 350 nm. IC_{50} values of the various analogues were determined from the data using nonlinear regression analysis with Prism software (GraphPad) 3.02 version.

MTT Assay. The MTT cell proliferation assay was used to quantify cell viability, measuring cell survival and proliferation spectrophotometrically.[40] Comparison of the cells treated with the drug to an untreated control group provided the relative cytotoxicity, reflecting the loss of cell viability as MTT reduction decreased. Heart endothelioma cells (MHEC5-T) from mice were exposed to serial dilutions of the reported compounds, and cell viability was determined after incubation at 37 °C at 1 h and at 5 days by the MTT method, yielding the drug concentration that reduced cell viability by 50% of the control (IC_{50}).

Blood Flow Reduction. In vivo experiments were performed in the MHEC5-T tumor model established by the injection of cultured MHEC5-T cells into the right flank of SCID mice.[15] When the established tumor reached the size of 300 mm^3 (a mass without the development of necrosis), mice were injected ip with doses of the various compounds at 100 or 10 mg/kg. At 24 h after injection, the animals were injected in the tail vein with 0.25 mL of diluted FluoSphere beads (1:6 in physiological saline). The mice were sacrificed 3 min thereafter, and cryosections at a thickness of 8 μm were removed from the tumor, heart, liver, spleen, and kidney. Three control animals were tested for blood flow reduction in tumor and control tissues only after being injected with the vehicle without any reduction in blood flow. These cryosections were directly examined under a fluorescent microscope, providing a blue fluorescence from the injected microbeads. The results were quantified from three sections of three tumors in each group and in each section, recording more than 70% of the area using a microscopic digital camera at 100× magnification. The computer program Stage Pro (Media Cybernetics, Bethesda, MD) was used to control the picture recording, and image analysis was performed using Image Plus software (Media Cybernetics).

SRB Assay.[41] Inhibition of human cancer cell growth was assessed using the National Cancer Institute's standard sulforhodamine B assay, as previously described.[41] Briefly, cells in a 5% fetal bovine serum/RPMI1640 medium solution 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.

P388 Assay. Cell growth inhibition (ED_{50}) of the murine P388 lymphocytic leukemia cell line was determined as previously described.[42]

Acknowledgment. The authors are grateful to Oxigene Inc. (Waltham, MA, grants to K.E., M.L.T., C.M.G., and K.G.P.), The Welch Foundation (grant no. AA-1278 to K.G.P. and AA-1395 to C.M.G.), grant (to G.R.P.) R-01 CA-90441 from the Division of Cancer Treatment and Diagnosis, NCI, DHHS for generous financial support of this project, and the National Science Foundation for funding both the 500 MHz NMR spectrometer (award CHE-0420802) and the Bruker X8 APEX diffractometer (grant CHE-0321214). The authors are grateful to F. Hung-Low and Dr. K. K. Klausmeyer for their valuable assistance with the X-ray crystallographic structures. The authors also thank H&B Packing (Waco, TX) for providing calf brain and Mr. L. Williams for other assistance.

Supporting Information Available: ^{1}H NMR spectra for compounds **16**−**21** and **24** along with X-ray crystallographic data for compounds **13** and **16**. This material is available free of charge on the Internet at http://pubs.acs.org.

References and Notes

(1) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1374–1376.
(2) Pettit, G. R.; Cragg, G. M.; Singh, S. B. *J. Nat. Prod.* **1987**, *50*, 386–391.
(3) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984–6991.
(4) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119–131.
(5) Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183–191.
(6) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299–309.
(7) Pettit, G. R.; Lippert, J. W., III *Anti-Cancer Drug Des.* **2000**, *15*, 203–216.

(8) Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, *32*, 3864–3867.
(9) Pettit, G. R.; Singh, S. B. *Can. J. Chem.* **1987**, *65*, 2390–2396.
(10) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829–1834.
(11) Iyer, S.; Chaplin, D. J.; Rosenthal, D. S.; Hamid Boulares, A.; Li, L.; Smulson, M. E. *Cancer Res.* **1998**, *58*, 4510–4514.
(12) Galbraith, S. M.; Chaplin, D. J.; Lee, F.; Stratford, M. R. L.; Locke, R. J.; Vojnovic, B.; Tozer, G. M. *Anticancer Res.* **2001**, *21*, 93–102.
(13) Young, S.; Chaplin, D. J. *Expert Opin. Investig. Drugs* **2004**, *13*, 1171–1182.
(14) Kanthou, C.; Tozer, G. M. *Blood* **2002**, *99*, 2060–2069.
(15) Sheng, Y.; Hua, J.; Pinney, K. G.; Garner, C. M.; Kane, R. R.; Prezioso, J. A.; Chaplin, D. J.; Edvardsen, K. *Int. J. Cancer* **2004**, *111*, 604–610.
(16) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. *J. Med. Chem.* **1998**, *41*, 3022–3032.
(17) Ohsumi, K.; Hatanaka, T.; Fujita, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Morinaga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3153–3158.
(18) Hatanaka, T.; Fujita, K.; Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Nihei, Y.; Suga, Y.; Akiyama, Y.; Tsuji, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3371–3374.
(19) Pinney, K. G.; Mejia, M. P.; Villalobos, V. M.; Rosenquist, B. E.; Pettit, G. R.; Verdier-Pinard, P.; Hamel, E. *Bioorg. Med. Chem.* **2000**, *8*, 2417–2425.
(20) Pinney, K. G.; Jelinek, C.; Edvardsen, K.; Chaplin, D. J.; Pettit, G. R. In *Anticancer Agents from Natural Products*; Cragg, G. R.; Kingston, D. G. I., Newman, D. J., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, 2005; pp 23–46..
(21) Ohsumi, K.; Hatanka, T.; Nakagawa, R.; Fukuda, Y.; Morinaga, Y.; Suga, Y.; Nihei, Y.; Ohishi, K.; Akiyama, Y.; Tsuji, T. *Anti-Cancer Drug Des.* **1999**, *14*, 539–548.
(22) Demers, B.; Vrignaud, P.; Bissery, M *J. Clin. Oncol. (2006 ASCO Ann. Meet. Proc., Part 1)* **2006**, *24*, 18S13074 (abstract).
(23) Hori, K; Saito, S. *Br. J. Cancer* **2003**, *89*, 1334–1344.
(24) (a) Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231–3244. (b) Subsequent to the publication noted in ref 24a, an additional paper appeared later: Chang, J. Y.; Yang, M. F.; Chang, C. Y.; Kuo, C. C.; Liou, J. P. *J. Med. Chem.* **2006**, *49*, 6412–6415.
(25) (a) Chaplin, D. J.; Garner, C. M.; Kane, R. R.; Pinney, K. G.; Prezioso, J. A.; Edvardsen, K. United States Patent US 6,919,324 B2, July 19 2005. (b) Chaplin, D. J.; Garner, C. M.; Kane, R. R.; Pinney, K. G.; Prezioso, J. A.; Edvardsen, K. PCT Patent Publication WO 2003035008 A2, 2003.
(26) Akerley, W. L.; Schabel, M.; Morrell, G.; Horvath, E.; Yu, M.; Johnsson, B.; Arbogast, K. *J. Clin. Oncol. (2007 ASCO Ann. Meet. Proc., Part 1)* **2007**, *25*, 18S, 14060 (abstract).
(27) Patterson, D. M.; Ross, P.; Koetz, B.; Saleem, A.; Stratford, M.; Stirling, J.; Padhani, A.; Asselin, M.; Price, P.; Rustin, G. J. *J. Clin. Oncol. (2007 ASCO Ann. Meet. Proc., Part 1)* **2007**, *25*, 18S, 14146 (abstract).
(28) Ohta, A.; Tonomura, Y.; Sawaki, J.; Sato, N.; Akiike, H.; Ikuta, M.; Shimazaki, M. *Heterocycles* **1991**, *32*, 965–973.
(29) Monk, K.; Siles, R.; Pinney, K. G.; Garner, C. M. *Tetrahedron Lett.* **2003**, *44*, 3759–3761.
(30) Gal'bershtam, M. A.; Budarina, Z. N. *Z. Organ. Khim.* **1969**, *5*, 953–956.
(31) Simonsen, J. L.; Rau, M. G. *J. Chem. Soc., Dalton Trans.* **1917**, *220*, 236.
(32) Crystallographic data for structure **13** (deposition number CCDC-654067) and structure **16** (deposition number CCDC-653756) reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
(33) Pettit, G. R.; Anderson, C. R.; Herald, D. L.; Jung, M. K.; Lee, D. J.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2003**, *46*, 525–531.
(34) Pettit, G. R.; Grealish, M. P.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2000**, *43*, 2731–2737.
(35) Chaplin, D. J.; Edvardsen, K.; Pinney, K. G.; Prezioso, J. A.; Wood, M. PCT Patent Publication WO 2004078126 A2, 2004.
(36) Portions of this work have appeared in the following dissertations: (a) Hadimani, M. B. Studies Toward the Discovery of New Classes of Privileged Molecules as Colchicine-Site Binding Ligands for Tubulin: Structure-Based Design, Synthesis, and Bioactivity of Small Ligands Targeted at Tumor Vasculature. Ph.D. Dissertation, Baylor University, Waco, TX, 2004. (b) Siles, R. Design, Synthesis and Biological Evaluation of New Anti-Cancer Nitrogen-Containing Combretastatins and Novel Cysteine Protease Inhibitors for the Treatment of Chagas Disease. Ph.D. Dissertation, Baylor University, Waco, TX, 2005. (c) Mugabe, B. E. Structure-activity Relationships and Thermodynamics of Combretastatin A-4 and A-1 Derivatives as Potential Inhibitors of Tubulin Polymerization. Ph.D. Dissertation, Baylor University, Waco, TX, 2005.
(37) Sheldrick, G. M. *SADABS*; University of Göttingen: Göttingen, Germany, 1997.
(38) Sheldrick, G. M. SHELXTL, 6.10 ed.; Bruker AXS, Inc: Madison, WI, 2000.
(39) Hamel, E.; Lin, C. M. *Biochemistry* **1984**, *23*, 4173–4184.
(40) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.
(41) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paul, K.; Vestica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolf, A. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
(42) Pettit, G. R.; Meng, Y.; Stevenson, C. A.; Doubek, D. L.; Knight, J. C.; Cichacz, Z.; Pettit, R. K.; Chapuis, J.-C.; Schmidt, J. M. *J. Nat. Prod.* **2003**, *66*, 259–262.

NP070377J