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Design, synthesis, and biological evaluation of combretastatin nitrogen-containing derivatives as inhibitors of tubulin assembly and vascular disrupting agents

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Abstract—A series of analogs with nitro or serinamide substituents at the C-2′-, C-5′-, or C-6′-position of the combretastatin A-4 (CA4) B-ring was synthesized and evaluated for cytotoxic effects against heart endothelioma cells, blood flow reduction to tumors in SCID mice, and as inhibitors of tubulin polymerization. The synthesis of these analogs typically featured a Wittig reaction between a suitably functionalized arylaldehyde and an arylphosphonium salt followed by separation of the resultant *E*- and *Z*-isomers. Several of these nitrogen-modified CA4 derivatives (both amino and nitro) demonstrate significant inhibition of tubulin assembly as well as cytotoxicity and in vivo blood flow reduction. 2′-Aminostilbenoid **7** and 2′-amino-3′-hydroxystilbenoid **29** proved to be the most active in this series. Both compounds, **7** and **29**, have the potential for further pro-drug modification and development as vascular disrupting agents for treatment of solid tumor cancers and certain ophthalmological diseases.

1. Introduction

Tumor vasculature is an important and rapidly emerging target for anticancer therapy because of the requirement of proliferating tumor cells for a functional network of blood vessels and the profound differences between tumor-associated and normal blood vessels.[1–4] Tumor vessels are highly disorganized, have an incomplete underlying basement membrane, and exhibit increased permeability. While the endothelial cells of normal tissues are largely quiescent, those of tumor vessels are activated and are more responsive to angiogenic cell signaling. Two different strategies have been developed for blocking tumor growth and metastasis through targeting the endothelial cells of the tumor-associated microvasculature. Much of the focus of research in this area is on agents that interfere with pro-angiogenic factors or their receptors and a number of antiangiogenic agents that inhibit neovascularization in tumors are undergoing clinical evaluation. Avastin™ (bevacizumab) has recently been approved for treatment of colorectal cancer in combination with chemotherapy.[5] Bevacizumab is a recombinant humanized monoclonal IgG1 antibody that binds to vascular endothelial cell growth factor (VEGF), which is overexpressed in tumors, and blocks VEGF interaction with its corresponding receptors on the surface of endothelial cells. Bevacizumab reduces microvascular growth and inhibits progression of metastatic disease.

In vascular disruption, the aim is to effect a rapid and extensive disruption of the pre-established tumor vasculature.[6–8] Antivascular strategies are characterized by secondary tumor cell death and by extensive tumor necrosis. Vascular targeting can be subdivided into two groups: ligand-directed vascular targeting agents and small-molecule vascular disrupting agents. Both groups aim to exploit the differences between normal

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tissue and tumor vessels. Ligand-directed antivascular agents or biologics include agents that use antibodies and peptides to target toxins, procoagulants, and pro-apoptotic agents to specific cell surface receptors and biomolecules that are localized predominantly in endothelial cells associated with tumors.

Two classes of compounds show promise in pre-clinical and clinical development as small-molecule vascular disrupting agents (VDAs). Although the exact mechanism of action has not been established, the flavenoid, 5,6-dimethylxanthenone-4-acetic acid, DMXAA (AS1404), acts indirectly on tumor cells to induce synthesis and release of tumor nuclear transcription factor (NFκB) resulting in the production of tumor necrosis factor (TNF) and other cytokines which act on tumor endothelial cells to effect vascular shutdown and apoptosis.[9–11] The second class of compounds includes a rapidly growing number of small-molecule, tubulin-binding agents that interfere with microtubule dynamics and acutely disrupt the tumor vasculature. Microtubules are essential cytoskeletal elements formed from the polymerization of subunit dimers composed of the closely related GTP-binding proteins α- and β-tubulin. Microtubules can switch to a fast shrinking state (catastrophe) or transit to a 'rescue' phase characterized by polymerization. This nonequilibrium dynamics is called dynamic instability and is powered by GTP hydrolysis. The dynamics of microtubule assembly are further regulated by a repertoire of microtubule-binding proteins and complexes. Dynamic instability is essential to microtubule function which includes cytoskeletal architecture, intracellular transport, cell migration, wound healing, and mitotic spindle development for chromosome segregation and cell division.[12]

Agents such as colchicine and vinblastine that result in microtubule depolymerization are known to cause vascular disruption, but at concentrations that are too close to their maximum tolerated dose. The colchicine-induced depolymerization of microtubules activates the small guanosine nucleotide triphosphatase, RhoA, which is an intracellular coordinator of the cytoskeletal rearrangement of microtubules and actin.[13] RhoA-GDP is activated by guanosine nucleotide exchange factors (GEFs) that promote the exchange of GTP for GDP. While the exact mechanism by which microtubule depolymerization activates RhoA has not yet been established, binding to microtubules inhibits a number of proteins such as GEF-H1. Upon dissociation from microtubules, GEF-H1 can activate RhoA.[14,15] RhoA-GTP in turn can activate a number of downstream effectors such as RhoA kinase which is able to phosphorylate myosin resulting in increased actinomyosin contractility.[16,17] (Fig. 1)

Combretastatin A-4 (CA4),[18] and combretastatin A-1 (CA1),[19] first isolated from the South African bush willow tree, *Combretum caffrum*, bind to tubulin at the colchicine site and are very effective inhibitors of tubulin polymerization. These compounds and their water-soluble phosphate pro-drugs (Fig. 2) are potent antimitotic agents and are very active VDAs at doses much lower

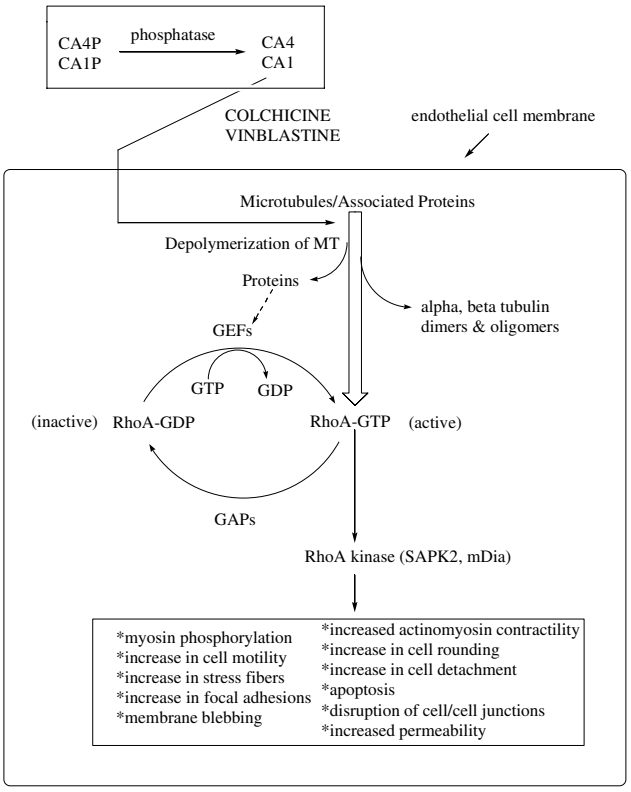


Figure 1. Proposed mechanism of vascular disruption by tubulin-binding agents. Microtubule (MT) depolymerization results in the release of microtubule-associated proteins leading to activation of RhoA and its downstream effectors such as RhoA kinase. The resulting cytoskeletal rearrangements lead to rapid vascular shutdown.

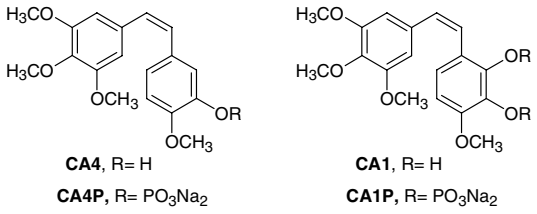


Figure 2. Combretastatin natural products and synthetic phosphate salts.

than their maximum tolerated dose.[20–23] Both compounds are currently in human clinical trials. Upon exposure to cultured endothelial cells, CA4P was converted to CA4 by phosphatases and characteristics of RhoA activation such as phosphorylation of myosin light chain, an increase in stress fibers (bundles of crosslinked actin filaments) and focal adhesions were observed: myosin light chain was phosphorylated, and there was an increase in stress fibers (bundles of crosslinked actin filaments) and focal adhesions. Inhibitors of RhoA-kinase and RhoA abolished these effects. Some cells also exhibited membrane blebbing, a specialized form of membrane protrusion, which was associated with decreased cell viability.[24]

Treatment of SCID mice bearing hemangioendothelioma with a single dose of CA1P (OXi4503) reduced tumor blood flow by 50% in 1 h, and tumor vessel

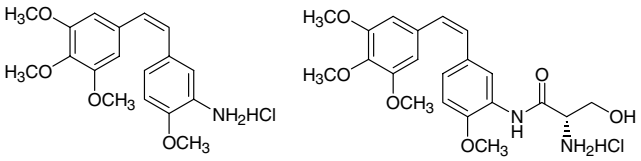


Figure 3. Synthetic aza-CA4 analogs.

permeability increased markedly.[25] Endothelial cells of the tumor vasculature underwent apoptosis resulting in blood flow shutdown and massive tumor necrosis. CA4P is well along in clinical development both as a VDA for cancer chemotherapy as well as a potential treatment agent for certain ocular diseases such as wet, age-related macular degeneration.[23,26,27] OXi4503 has recently entered human clinical trials.[27–30] The robust biological activity as evidenced through clinical success coupled with the structural simplicity of the combretastatins has inspired the synthesis of a growing library of combretastatin analogs.[31–40] These analogs typically include A-ring, bridge-modified, and B-ring alterations. From this library, nitrogen-substituted analogs have emerged as among the most potent biologically. For example, the synthetic analog substituting nitrogen amino for oxygen hydroxyl at C-3′ of the B-ring is extremely active in terms of inhibition of tubulin assembly and in vitro cytotoxicity against selected human cancer cell lines (Fig. 3).[41,42] This compound was originally described in the patent literature by Ajinomoto Inc.[43] and then presented publicly in 1998 by the Ajinomoto group[44] and by Pinney and co-workers[45] independently. A serinamide-based pro-drug (AVE-8062) developed by Ajinomoto has been introduced in clinical development by Aventis Inc.[46,47] Herein, we describe a series of B-ring nitrogen-substituted combretastatin analogs in which the A-ring and ethylene bridge characteristic of CA4 are kept intact.

2. Results and discussion

2.1. Design and synthesis

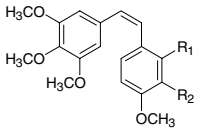
Encouraged by the promising biological activity displayed by certain combretastatins and their synthetic analogs, a series of seventeen new nitrogen-substituted CA4 analogs has been prepared by total synthesis.[48–50] These new analogs contain nitro, amino, amino hydrochloride, or serinamide pro-drug moieties at the C-2′, C-5′, or C-6′ position on the B-ring. Nitrogen substitution at C-3′ of the B-ring has been previously reported[41–45]. In order to incorporate structural functionality deemed significant for enhanced tubulin activity, the molecules maintain the trimethoxyphenyl A-ring and *cis*-ethylene bridge of CA4 as well as oxygen functionality at C-4′ in terms of either a methoxy or hydroxyl group. The compounds are grouped by C-4′-methoxy analogs (Table 1), C-3′-hydroxy-C-4′-methoxy analogs (Table 2), and C-3′-methoxy-C-4′-hydroxy analogs (Table 3). The synthesis of the first series of CA4 analogs and serinamide derivatives is presented in Scheme 1. Bromination at the benzylic positions of 4-methyl-2-nitroanisole and 4-methyl-3-nitroanisole gave compounds **1** and **2**, as previously reported.[41] A considerable amount of dibromide was formed if the reaction was carried out for more than 32 h. After treatment with PPh_3, triphenylphosphonium bromides **3** and **4** were obtained with yields in excess of 90%. A Wittig reaction of 3,4,5-trimethoxybenzaldehyde with phosphonium salts **3** and **4** afforded a mixture of *Z*- and *E*-stilbenes. The *Z*-isomers **5a** and **6a** were separated from their respective *E*-isomers **5b** and **6b** by column chromatography. The *Z*-isomer in a dichloromethane solution and in the presence of UV–vis light has a tendency to isomerize to its respective *E*-isomer as previously reported.[51,52] The stereochemical assignment of the stilbene structures was based on the typical values of the coupling constants for *Z* ($J \approx 12$ Hz) and *E* ($J \approx 16$ Hz). The nitro stilbene derivatives **5a** and **6a** were reduced to the free amines **7** and **8** by refluxing with sodium hydrosulfite in a 3:1 mixture of acetone/water. The serinamide residue was incorporated by reacting **7** and **8** with FMOC-L-Ser (Ac) in the presence of DCC and HOBt to yield compounds **9** and **10**, respectively, which upon further reaction with sodium hydroxide formed serinamide derivatives **11** and **12**. For comparison purposes, AC-7700 **14**, a compound previously reported by Ajinomoto,[46] and its analog **13** were prepared by reacting intermediates **12** and **11** with a dioxane solution of 4 N HCl. Similarly, nitrogen-containing derivatives (C-2, C-5′, and C-6′, **26–32**, Scheme 2) were synthesized from commercially available isovanillin (3-hydroxy-4-methoxybenzaldehyde) to incorporate structural motifs reminiscent of both CA4 and AC-7739.

Accordingly, isovanillin was nitrated using previously reported methods for the preparation of benzaldehydes **17–19** (Scheme 2).[53] Subsequent conversion to the TBS-protected compounds **20–22** was accomplished using TBS-chloride, providing one partner for the requisite Wittig reaction. The 3,4,5-trimethoxybenzylphosphonium bromide, prepared according to methodology reported by Pettit and coworkers,[54] was reacted with sodium hydride to form a brightly colored ylide at 0 °C. The TBS-protected *Z*- and *E*-stilbenes **23–25** were isolated following a Wittig reaction of the ylide with compounds **20–22**. Minor amounts of the TBS-protected 6′-nitrostilbenes **25a** and **25b** were isolated, as a consequence of TBS-deprotection under the reaction conditions to yield the phenolic *Z*-stilbene **28** and its corresponding *E*-isomer. The *Z*-2′-nitroalkene **23a** and *Z*-5′-nitro analog **24a** were converted to the free phenols **26** and **27** by reaction with TBAF. Reduction of the nitro functionality of compounds **26**, **27**, and **28** was accomplished using sodium dithionite as previously discussed.

The *Z*-6′-amino CA4 analog **31** was found to slowly isomerize with prolonged reaction times. The *Z*-2′-amino CA4 analog **29** was converted to the hydrochloride salt **32** using a 4 N HCl-dioxane solution.

Scheme 3 details the analogous synthesis of nitro-substituted combretastatin analogs from commercially

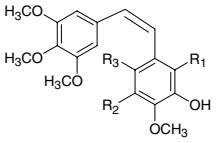
Table 1. Inhibition of tubulin polymerization, cytotoxicity, and blood flow reduction for compounds **8**–**13**



Compound[a]	R_1	R_2	Tubulin inhibition, IC_{50} (μM)	MTT, IC_{50} in vitro cytotoxicity (μM)		In vivo blood flow shutdown (%)	
				1 h	5 days	10 mg/kg	100 mg/kg
CA4	H	OH	1.2 (1.2)[c]	na[b]	na	na	na
CA1	OH	OH	1.9 (2)[d]	na	na	na	na
CA4P	H	OPO_3Na_2	na	0.8	0.0020	10	88
CA1P	OPO_3Na_2	OPO_3Na_2	na	3.2	0.0046	70	99
AC-7739	H	NH_3Cl	1.3 (1)[e]	na	na	na	na
AC-7700	H	NH-SerHCl	11.3	na	na	na	na
8	H	NH_2	2.6 (1.2)[c]	0.8	0.0080	36	100
12	H	NH-Ser	9.6	5.0	0.0140	0	100
7	NH_2	H	1.4	2.0	0.0080	25	50
11	NH-Ser	H	na	10.0	0.050	24	43
13	NH-SerHCl	H	>40	na	na	22.8	na

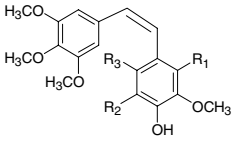
[a] CA4, CA1, CA4P, CA1P, AC-7739, AC-7700, **8**, and **12** have all been previously synthesized and reported in the literature. They are included here for the purpose of comparison (see Refs. 18–21,41,42,46).
[b] na, not analyzed in this study.
[c] Ref. 41.
[d] Ref. 19.
[e] Ref. 57.

Table 2. Inhibition of tubulin polymerization, cytotoxicity, and blood flow reduction for compounds **26**–**31**



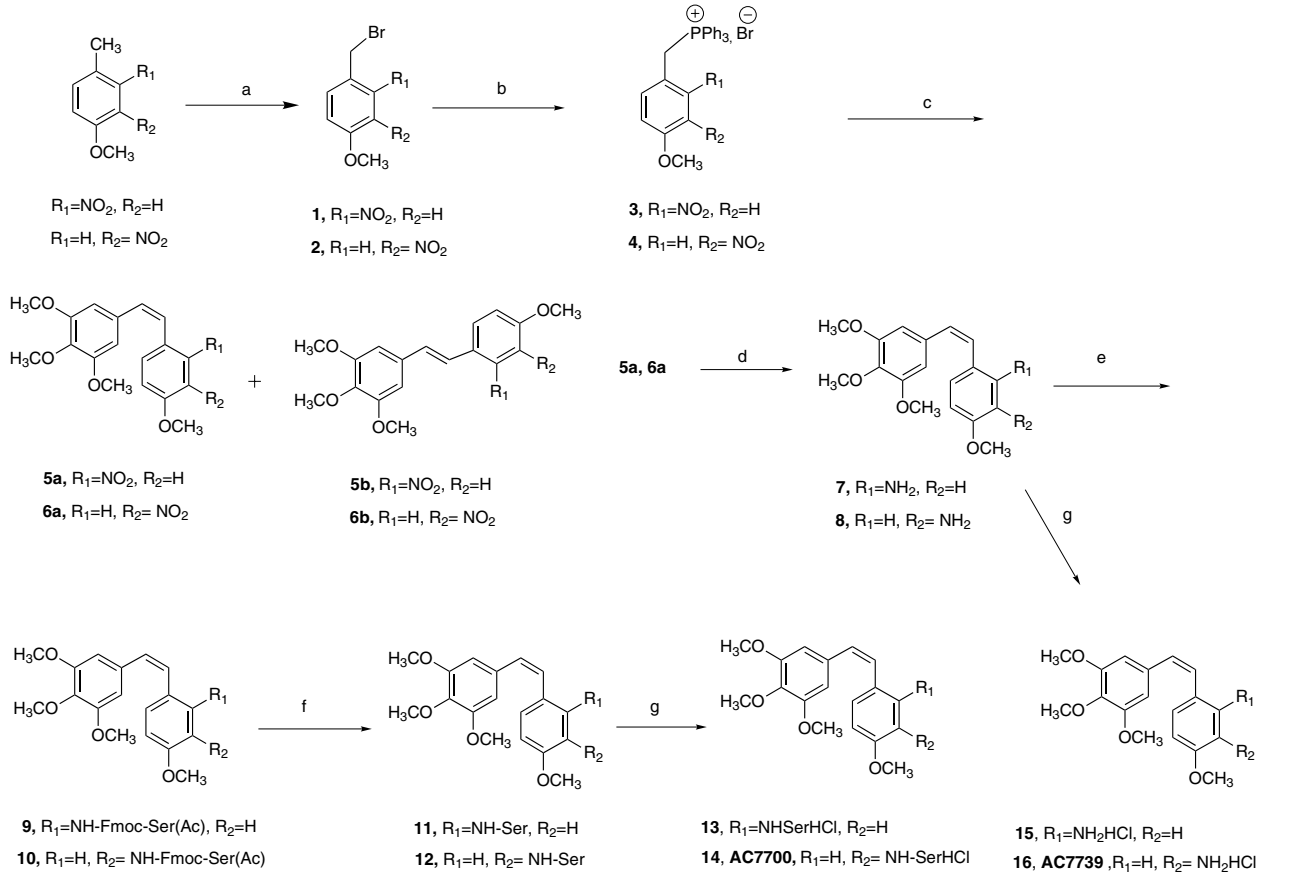
Compound	R_1	R_2	R_3	Tubulin inhibition, IC_{50} (μM)	MTT, IC_{50} in vitro cytotoxicity (μM)		In vivo blood flow shutdown (%)	
					1 h	5 days	10 mg/kg	100 mg/kg
26	NO_2	H	H	1.5	>44.8	0.14	0	4
27	H	NO_2	H	2.0	8	1.3	0	1.5
28	H	H	NO_2	>40	>44.8	>1.4	0	0
29	NH_2	H	H	2.5	1.24	0.017	27.3	59.2
30	H	NH_2	H	>20	44.8	1.35	0	1.5
31	H	H	NH_2	6.2	11.14	1.4	0	0

Table 3. Inhibition of tubulin polymerization, cytotoxicity, and blood flow reduction for compounds **42**–**47**

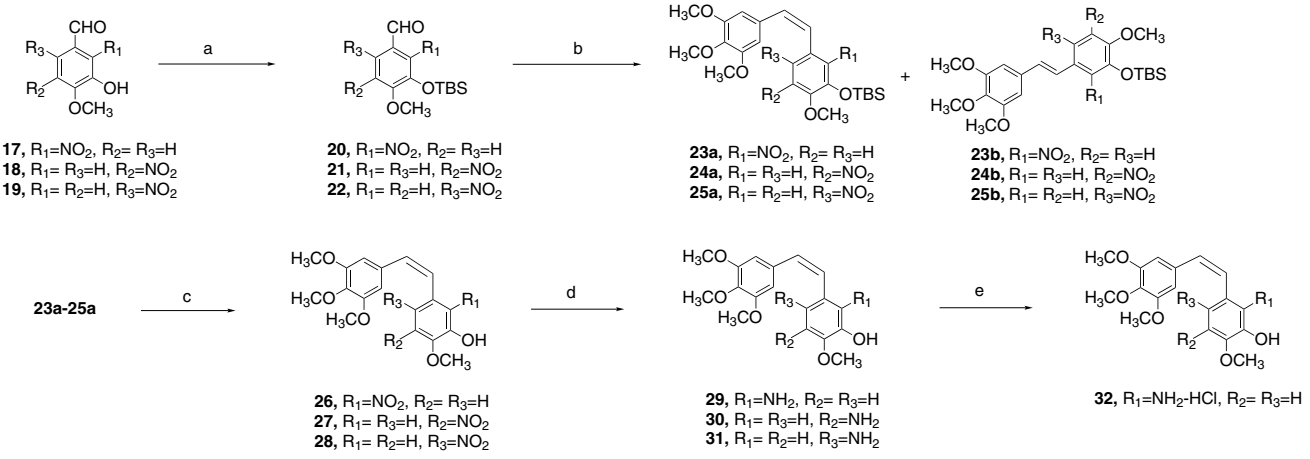


Compound	R_1	R_2	R_3	Tubulin inhibition, IC_{50} (μM)	MTT, IC_{50} in vitro cytotoxicity (μM)		In vivo blood flow shutdown (%)	
					1 h	5 days	10 mg/kg	100 mg/kg
42	NO_2	H	H	>40	na[a]	na	na	na
43	H	NO_2	H	>40	>44.8	>1.4	0	0
44	H	H	NO_2	>40	na	na	na	na
45	NH_2	H	H	>40	na	na	na	na
46	H	NH_2	H	>40	>44.8	>1.4	0	0
47	H	H	NH_2	>40	na	na	na	na

[a] na, not analyzed.

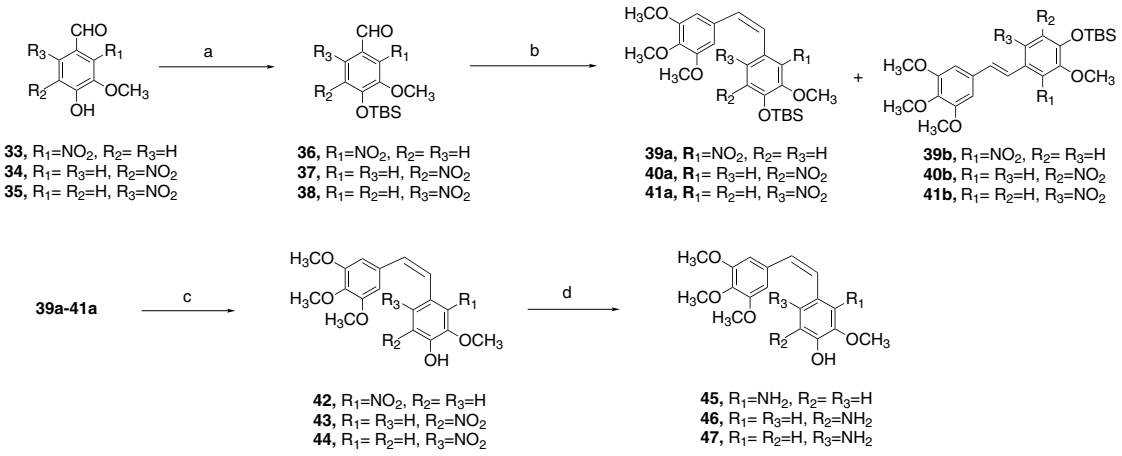


Scheme 1. Synthesis of the first series of CA4 analogs and serinamide derivatives. Reagents and conditions: (a) NBS, AIBN, CCl_4, reflux; (b) PPh_3, dichloromethane, reflux; (c) NaH, 3,4,5-trimethoxybenzaldehyde, dichloromethane, 0 °C to rt; (d) $Na_2S_2O_4$, acetone/water (3:1), 50 °C; (e) DCC, HOBt-H_2O, Fmoc-L-Ser(Ac), DMF (anhydrous), rt; (f) NaOH, dichloromethane/CH_3OH (1:1); (g) 4 N HCl/dioxane, dichloromethane, rt.



Scheme 2. Preparation of the second series of combretastatin analogs from isovanillin. Reagents and conditions: (a) TBS-Cl, DMAP, Et_3N, dichloromethane, 0 °C; (b) NaH, dichloromethane, 3,4,5-trimethoxybenzyltriphenylphosphonium bromide, 0 °C to rt; (c) TBAF, dichloromethane, 0 °C; (d) $Na_2S_2O_4$, acetone/water (3:1), 50 °C; (e) 4 N HCl/dioxane, dichloromethane, rt.

available vanillin (3-methoxy-4-hydroxybenzaldehyde). Reversal of the hydroxyl and methoxy functionalities deemed important at C-3′ and C-4′ in this series of compounds was undertaken to gain additional insight into the relative significance of maintaining oxygen functionality at the 3′- and 4′-positions while incorporating additional nitrogen-containing groups. The synthetic strategy adopted was similar to that used for compounds described in Scheme 2. Nitration of vanillin was accomplished by reported methodologies to yield compounds **33**–**35**.[55] The *Z*-nitro stilbenes **42**–**44** were prepared in moderate to good yield following protection, Wittig reaction, and deprotection. The nitro functionality of compounds **42**–**44** was once again reduced



Scheme 3. Synthesis of the third series of combretastatin analogs from vanillin. Reagents and conditions: (a) TBS-Cl, DMAP, Et_3N, dichloromethane, 0 °C; (b) NaH, dichloromethane, 3,4,5-trimethoxybenzyltriphenylphosphonium bromide, 0 °C to rt; (c) TBAF, dichloromethane, 0 °C; (d) $Na_2S_2O_4$, acetone/water (3:1), 50 °C.

with sodium dithionite to give *Z*-amino combretastatin analogs **45**–**47**.

2.2. Biological evaluation

Biological evaluation of these new nitrogen-modified CA4 analogs features both an in vitro and in vivo component. Initially, compounds are screened for their ability to inhibit tubulin assembly in a cell-free assay using purified tubulin. In addition, selected compounds are evaluated in terms of cytotoxicity (MTT assay) and for their ability to selectively impair blood flow to tumors in SCID mice. The combination of enhanced activity toward tubulin and in vivo blood flow capability has proven to be indicative of pre-clinical efficacy and predictive of potential clinical success. Accordingly, these assays are key components of our drug design and development program as it relates to vascular disrupting agents.

Three compounds, CA4, CA1, and AC-7739, and their water-soluble pro-drug derivatives, CA4P, OXi4503, and AVE-8062 (AC-7700), serve as benchmark reference compounds in order to evaluate new VDA candidates in a pre-clinical sense. CA4, CA1, and AC-7739 all inhibit tubulin assembly with IC_{50} values in the range of 1–2 μM, while CA4P and OXi4503 demonstrate the ability to shutdown tumor blood flow on the order of 80–100% at a dose of 100 mg/kg.

The effects on biological activity of B-ring, nitrogen monosubstitutions in CA4 analogs are summarized in Tables 1–3. Previous studies have shown that amino substitution at the B-ring, 3′-position of CA4 results in analogs (AC-7739, and its serinamide pro-drug, AVE-8062) that are very biologically active. Introduction of an amino group in the B-ring, 2′-position of a combretastatin analog in which the 3′-hydroxyl has been removed, gave a *Z*-stilbenoid compound **7** with an intact A-ring that inhibits tubulin polymerization with an IC_{50} = 1.5 μM which is close to those of CA4 and AC-7739 as shown in Table 1. The *Z*-2′-aminostilbenoid product **7** and its corresponding serinamide pro-drug **11** were also very active in the MTT and in vivo blood flow shutdown assays. The activity of **7** is especially noteworthy in that a substituent at the 3′-position of CA4 analogs is often required for significant cytotoxic activity.[56] Selected biological data for reference compounds, CA4, CA1, CA4P, AC-7739, AC-7700, **8**, and **12**, and compound **13**, the hydrochloride salt of **11**, are also included in Table 1.

Amino and nitro substitutions in the B-ring of CA4 analogs in which the 3′-hydroxyl remains unmodified are summarized in Table 2. CA4 analogs with a nitro moiety at the 2′- or 5′-position of the B-ring (**26** and **27**, respectively) were very effective in their ability to inhibit tubulin assembly with IC_{50} values of 1.5 and 2.0 μM, respectively, but were much less active in the MTT and in vivo blood flow shutdown assays compared to CA4P. A nitro substituent at the 6′-position **28** led to loss of the ability to inhibit tubulin polymerization. Amino substitution at the 2′-position **29** of the B-ring gave an active compound that inhibited tubulin assembly with an IC_{50} value of 2.5 μM and showed significant activity in the MTT and in vivo blood flow shutdown assays (Fig. 4). Substitution of an amino group at the 5′-position gave compound **30** that was much less active. The compound with an amino substituent at the 6′-position **31** demonstrated moderate potency in the inhibition of tubulin assembly (IC_{50} = 6.2 μM) and significant activity in the MTT assay, but no activity in the in vivo blood flow shutdown assay.

Amino and nitro substitutions at the 2′-, 5′-, and 6′-positions of the B-ring of CA4 in which the 3′-hydroxyl and the 4′-methoxy groups have been switched are summarized in Table 3. None of these compounds, **42**–**47**, demonstrated any activity in the inhibition of tubulin polymerization assay. Compounds **43** and **46** were also evaluated in the MTT and in vivo blood flow shutdown assays, but exhibited no activity in either of these assays.

2.3. Conclusions

We were very encouraged to find that the application of existing structure–activity relationships led to the

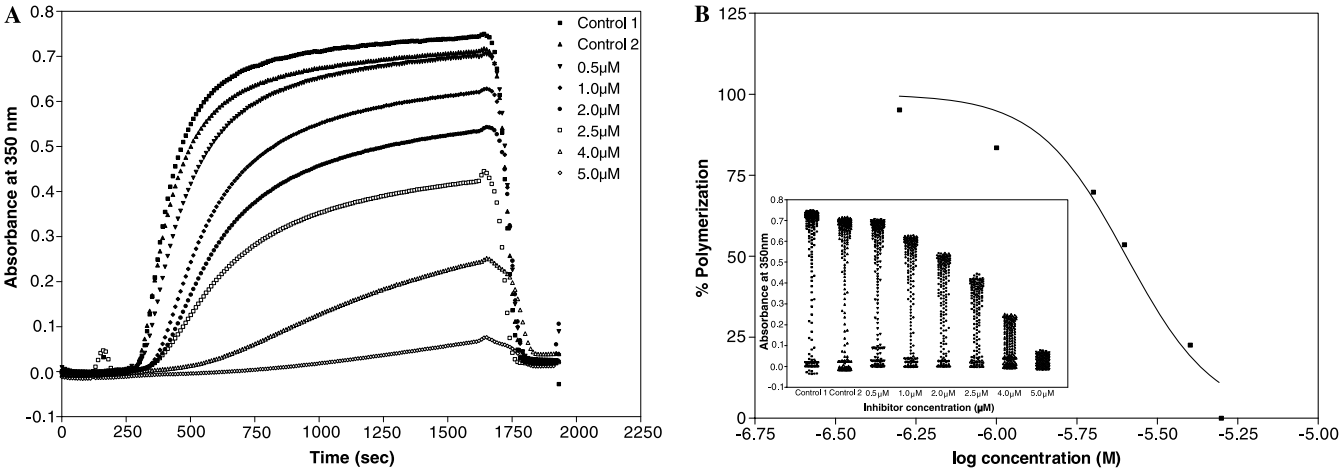


Figure 4. Inhibition of tubulin polymerization by compound **29**. (A) Kinetics of inhibition of microtubule assembly by increasing concentrations of compound **29**. Tubulin (1.0 mg/mL in 1.0 M glutamate buffer, pH 6.6) was incubated with DMSO for controls 1 and 2, or with compound **29** at concentrations of 0.5, 1.0, 2.0, 2.5, 4.0, and 5.0 μM for 15 min at 30 °C and cooled to 0 °C for 15 min on ice. GTP (0.4 mM final concentration) was added to the reaction mixture in a quartz microcuvette. Baseline was established at 350 nm and after 100 s, tubulin polymerization was induced by a temperature jump to 30 °C, and after 1500 s, depolymerization was induced by a drop in the temperature to 0 °C. Turbidity readings were collected every 10 s. (B) The IC_{50} value was obtained from a plot of percent tubulin polymerization against log concentration (M) of compound **29** at 30 °C. Inset: column scatter graph of the absorbance against compound **29** concentration in the reaction mixture. IC_{50} = 2.5 μM, log $IC_{50} \pm SE = -5.596 \pm 0.03$.

development of a small library of new, synthetic, nitrogen-bearing CA4 analogs from which two compounds, **7** and **29**, emerged as potentially viable pre-clinical candidates with respect to their potent inhibition of tubulin assembly and their significant in vivo blood flow shutdown capability. In fact, these compounds are comparable in many ways to AVE-8062 (AC-7700) which is currently in human clinical development. Collectively, this new nitrogen-modified CA4 compound library enhances the overall structure–activity relationship profile that is known for the combretastatins and related analogs. One pro-drug congener **11** is also shown to have significant in vivo activity, and it is anticipated that further pro-drug modification of the analogs may result in improved VDAs with enhanced bioavailability.

3. Experimental

3.1. General procedures

Reactions which involved air or moisture sensitive reagents were performed in oven-dried glassware under an argon atmosphere, unless otherwise stated. Solvents were transferred using cannulas or syringes, which were stored in an oven prior to use. Before use solvents for chromatography and isolation (dichloromethane, ethyl acetate, hexanes, and acetone) were purified by distillation. Anhydrous dichloromethane and diethyl ether were purchased from Aldrich Chemical Company or Fischer Scientific and used as received. All other commercially available reagents were purchased from Aldrich Chemical Company or ACROS Chemical and used as received unless otherwise stated.

All reaction mixtures were magnetically stirred and monitored by TLC on silica plates that were obtained from EM Science and visualized using UV irradiation, PMA (phosphomolybdic acid) staining, or I_2. Gas chromatography (Hewlett Packard 5890 Series II with a SE-54 column) and/or gas chromatography mass spectrometry (Hewlett Packard GCD system with electron impact ionization) were also used to follow the reactions, unless otherwise noted. Flash chromatography was performed with the indicated solvents using silica gel (230–400 mesh) purchased from EM Science.

Characterization of the products was accomplished by NMR spectroscopy using Brüker DPX-300 (300 MHz for ^1H and 75 MHz for ^{13}C) or Brüker AMX-360 (360 MHz for ^1H and 90 MHz for ^{13}C) spectrometers in deuterated chloroform with 0.03% TMS as the internal standard unless otherwise specified. Chemical shifts are expressed in ppm (δ), and peaks are listed as singlets (s), doublets (d), triplets (t), quartets (q), broad singlet (br s), multiplet (m), or combinations of each, with coupling constants (J) expressed in Hertz. All ^{13}C spectra reported are decoupled.

3.2. Synthesis of key intermediates and analogs

3.2.1. 4-Methoxy-2-nitrobenzyl bromide (1). To a well-stirred solution of 4-methyl-3-nitroanisole (7.45 g, 44.1 mmol) in CCl_4 (70 mL), AIBN (0.740 g, 4.41 mmol) and NBS (8.73 g, 48.6 mmol) were added. After the reaction mixture was heated at reflux for 16 h under nitrogen atmosphere, water was added and the product extracted from the aqueous phase with dichloromethane. The resultant organic phase was washed with brine, dried under sodium sulfate, and the solvent evaporated yielding a solid, which was recrystallized (hexanes) to afford 7.90 g of the bromide in a 73% yield. ^1H NMR (300 MHz, $CDCl_3$): δ 7.52 (d, J = 2.7, ArH, 1H), 7.43 (d, J = 8.6, ArH, 1H), 7.11 (dd, J = 8.6, 2.7, ArH, 1H), 4.77

(s, benzylic CH_2, 2H), 3.86 (s, OCH_3, 3H). DEPT 135 NMR (75 MHz, $CDCl_3$): δ 133.6 (*C*H), 120 (*C*H), 110 (*C*H), 56.0 (*C*H_3), 29.1 (*C*H_2). EIMS: *m*/*z* (% rel intensity, ion) 247 (4, M^+ +2), 245 (M^+, 4), 166 (100), 108 (20).

3.2.2. 4-Methoxy-2-nitrobenzyltriphenylphosphonium bromide (3). Bromide **1** (12.0 g, 48.8 mmol) and triphenylphosphine (14.2 g, 53.6 mmol) were dissolved in dichloromethane (150 mL). The reaction mixture was heated at reflux for 16 h at which point water was added and the product extracted by using dichloromethane. The organic phase was washed twice with brine and dried over magnesium sulfate. The resultant solid was washed three times with diethyl ether to afford 23.4 g of the product in a 95% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.95 (dd, J = 8.4, 2.4, Ar*H*, 1H), 7.75 (m, Ar*H*, 3H), 7.61 (m, Ar*H*, 12H), 7.39 (d, J = 2.3, Ar*H*, 1H), 7.09 (dd, J = 8.6, 2.6, Ar*H*, 1H), 5.90 (d, J = 14.3, benzylic CH_2, 2H), 3.78 (s, OCH_3, 3H).

3.2.3. General procedure for the preparation of TBS-protected aldehydes. To a 250 mL round-bottomed flask containing a stir bar and the appropriate nitro-substituted benzaldehyde was added approximately 0.3 equivalents of DMAP, and dichloromethane (~20.0 mL) was transferred to the flask by syringe. The solution was stirred and cooled to 0 °C in an ice-water bath. Triethylamine (1.1 equivalents) was added, followed by 1.2 equivalents of TBS chloride. The reaction was monitored by TLC, and following completion quenched with 20.0 mL of water. The product was extracted with dichloromethane (2× 10 mL), and the organic phases combined. The solvent was removed in vacuo, and the product was purified by flash chromatography.

3.2.4. 3-*tert*-Butyldimethylsilyloxy-4-methoxy-2-nitrobenzaldehyde (20). Yield (86%) of a tan solid. R_f (25% EtOAc/hexanes) = 0.32. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.77 (s, 1H, C*H*O), 7.49 (d, 1H, J = 8.6, Ar*H*), 7.05 (d, 1H, J = 8.6, Ar*H*), 3.96 (s, 3H, OCH_3), 0.94 (s, 9H, *t*-Bu CH_3), 0.21 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 156.1, 186.2, 156.1, 137.8, 125.2, 120.9, 111.7, 100.0, 55.9, 25.5, 18.5, −4.1. EIMS: *m*/*z* (% rel intensity, ion): 311 (M^+), 75 (base peak).

3.2.5. 3-*tert*-Butyldimethylsilyloxy-4-methoxy-5-nitrobenzaldehyde (21). Yield (78%) of a colorless oil. R_f (25% EtOAc/hexanes) = 0.77. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.91 (s, 1H, C*H*O), 7.89 (d, 1H, J = 2.0, Ar*H*), 7.57 (d, 1H, J = 2.0, Ar*H*), 4.03 (s, 3H, OCH_3), 1.05 (s, 9H, *t*-Bu CH_3), 0.28 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 188.9, 151.5, 150.1, 145.5, 131.6, 123.4, 119.6, 62.0, 25.5, 18.2, −4.6. EIMS: *m*/*z* (% rel intensity, ion): 311 (M^+), 254 (base peak).

3.2.6. 3-*tert*-Butyldimethylsilyloxy-4-methoxy-6-nitrobenzaldehyde (22). Yield (61%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.71. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.40 (s, 1H, C*H*O), 7.61 (s, 1H, Ar*H*), 7.38 (s, 1H, Ar*H*), 3.97 (s, 3H, OCH_3), 1.00 (s, 9H, *t*-Bu CH_3), 0.22 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 187.7, 168.7, 154.4, 150.3, 125.7, 119.9, 108.0, 56.3, 25.5, 18.5, −4.5.

3.2.7. 4-*tert*-Butyldimethylsilyloxy-3-methoxy-2-nitrobenzaldehyde (36). Yield (63%) of a yellow solid. R_f (20% EtOAc/hexanes) = 0.60. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.81 (s, 1H, C*H*O), 7.59 (d, 1H, J = 8.4, Ar*H*), 7.07 (d, 1H, J = 8.4, Ar*H*), 3.92 (s, 3H, OCH_3), 1.03 (s, 9H, *t*-Bu CH_3), 0.29 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 185.8, 155.8, 143.4, 127.1, 122.2, 121.2, 62.3, 25.5, 18.3, −4.4. EIMS: *m*/*z* (% rel intensity, ion): 311 (M^+), 254 (base peak).

3.2.8. 4-*tert*-Butyldimethylsilyloxy-3-methoxy-5-nitrobenzaldehyde (37). Yield (64%) of a pale yellow oil. R_f (25% EtOAc/hexanes) = 0.38. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.87 (s, 1H, C*H*O), 7.87 (d, 1H, J = 1.9, Ar*H*), 7.55 (d, 1H, J = 1.9, Ar*H*), 3.94 (s, 3H, OCH_3), 0.98 (s, 9H, *t*-Bu CH_3), 0.25 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 189.1, 152.4, 145.2, 128.7, 121.1, 111.7, 55.9, 25.5, 18.7, −3.9.

3.2.9. 4-*tert*-Butyldimethylsilyloxy-3-methoxy-6-nitrobenzaldehyde (38). Yield (63%) of a yellow solid. R_f (15% EtOAc/hexanes) = 0.51. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 10.43 (s, 1H, C*H*O), 7.58 (s, 1H, Ar*H*), 7.41 (s, 1H, Ar*H*), 3.97 (s, 3H, OCH_3), 1.01 (s, 9H, *t*-Bu CH_3), 0.23 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 187.9, 155.4, 149.3, 143.7, 126.2, 116.9, 110.7, 56.2, 25.5, 18.5, −4.5. EIMS: *m*/*z* (% rel intensity, ion): 311 (M^+), 254 (base peak).

3.2.10. (*Z*)-2-(2′-Nitro-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (5a). 3,4,5-Trimethoxybenzaldehyde (8.27 g, 41.7 mmol) and 4-methoxy-2-nitrobenzyltriphenylphosphonium bromide **3** (19.3 g, 37.9 mmol) were dissolved in 150 mL of anhydrous dichloromethane, and the solution was stirred under nitrogen atmosphere. After the reaction mixture was cooled at 0 °C, NaH (5.05 g, 200 mmol) was added and the mixture was stirred for 16 h at room temperature at which point, water (~10 mL) was added until bubbling stopped. The product was isolated from the aqueous phase with dichloromethane, and the organic phase was washed with brine and dried over sodium sulfate. After evaporation of the solvent, the product was purified by flash chromatography (15% EtOAc/hexanes) to afford 5.29 g of the product in a 41% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.62 (d, J = 2.6, Ar*H*, 1H), 7.27 (d, J = 8.8, Ar*H*, 1H) , 7.04 (dd, J = 8.7, 2.7, Ar*H*, 1H), 6.83 (d, J = 12.1, C=C*H*, 1H), 6.65 (d, J = 12.0, C=C*H*, 1H), 6.31 (s, Ar*H*, 2H), 3.89 (s, OCH_3, 3H), 3.83 (s, OCH_3, 3H), 3.65 (s, OCH_3, 6H). GC–MS *m*/*z* (% rel intensity, ion): 345 (64, M^+), 196 (93), 181 (100), 149 (25), 122 (32).

3.2.11. General Wittig reaction conditions for the preparation of TBS-protected (*Z*)- and (*E*)-stilbenes. To a 100 mL round-bottomed flask containing a stir bar was added approximately 1.1 equivalents of 3,4,5-trimethoxybenzyltriphenylphosphonium bromide and 25 mL of anhydrous dichloromethane. The flask was cooled to 0 °C in an ice-water bath, and 7–8 equivalents of sodium hydride were cautiously transferred to the flask forming the brightly colored ylide. A previously prepared solution of TBS-protected aldehyde (1.0

equivalent in 20 mL of dichloromethane) was transferred to the round-bottomed flask using a cannula. The reaction mixture was allowed to warm from 0 °C to room temperature. The reaction was monitored by TLC and cautiously quenched with 5 mL of water (after approximately 5–8 h). The products were isolated by extraction with dichloromethane and purified with flash chromatography using gradient elution.

3.2.12. (*Z*)-2-(2′-Nitro-3′-*tert*-butyldimethylsilyloxy-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (23a). Yield (47%) of a yellow liquid. R_f (25% EtOAc/hexanes) = 0.41. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.77 (s, 2H, Ar*H*), 6.61 (d, 1H, J = 12.0, vinyl C*H*), 6.41 (s, 2H, Ar*H*), 6.38 (d, 1H, J = 12.0, vinyl C*H*), 3.82 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.67 (s, 6H, OCH_3), 0.94 (s, 9H, *t*-Bu CH_3), 0.19 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.8, 149.9, 144.3, 137.4, 137.2, 133.6, 131.6, 122.8, 122.6, 122.1, 112.4, 106.1, 60.9, 55.8, 55.6, 25.6, 18.5, −4.3.

3.2.13. (*E*)-2-(2′-Nitro-3′-*tert*-butyldimethylsilyloxy-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (23b). Yield (25%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.29. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.23 (d, 1H, J = 8.4, Ar*H*), 6.94 (d, 1H, J = 15.9, vinyl C*H*), 6.93 (d, 1H, J = 8.7, Ar*H*), 6.73 (d, 1H, J = 15.9, vinyl C*H*), 6.66 (s, 2H, Ar*H*), 3.89 (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 0.95 (s, 9H, *t*-Bu CH_3), 0.21 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.4, 150.0, 143.6, 138.4, 137.1, 132.3, 131.7, 122.6, 120.2, 118.1, 112.8, 103.8, 61.0, 56.1, 55.6, 25.6, 18.5, −4.2.

3.2.14. (*Z*)-2-(3′-*tert*-Butyldimethylsilyloxy-4′-methoxy-5′-nitrophenyl)-1- (3,4,5-trimethoxyphenyl)ethene (24a). Yield (33%) of a yellow oil. R_f (25% EtOAc/hexanes) = 0.75. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.31 (d, 1H, J = 2.0, Ar*H*), 6.93 (d, 1H, J = 2.0, Ar*H*), 6.62 (d, 1H, J = 12.0, vinyl C*H*), 6.43 (s, 2H, Ar*H*), 6.43 (d, 1H, J = 12.0, vinyl C*H*), 3.90 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 0.94 (s, 9H, *t*-Bu CH_3), 0.06 (s, 6H, CH_3). ^{13}C ($CDCl_3$, 75 MHz): δ 153.3, 150.2, 145.2, 143.8, 137.7, 133.2, 132.1, 131.8, 127.3, 125.1, 117.6, 105.8, 61.8, 60.9, 56.0, 25.5, 18.1, −4.8.

3.2.15. (*E*)-2-(3′-*tert*-Butyldimethylsilyloxy-4′-methoxy-5′-nitrophenyl)-1 -(3,4,5-trimethoxyphenyl)ethene (24b). Yield (24%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.58. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.54 (d, 1H, J = 2.2, Ar*H*), 7.15 (d, 1H, J = 2.2, Ar*H*), 6.96 (d, 1H, J = 16.2, vinyl C*H*), 6.86 (d, 1H, J = 16.2, vinyl C*H*), 6.73 (s, 2H, Ar*H*), 3.95 (s, 3H, OCH_3), 3.93 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 1.05 (s, 9H, *t*-Bu CH_3), 0.26 (s, 6H, CH_3). ^{13}C ($CDCl_3$, 75 MHz): δ 153.5, 150.8, 145.5, 144.3, 138.5, 133.5, 132.1, 130.6, 125.4, 122.9, 114.9, 103.8, 61.8, 61.0, 56.2, 25.6, 18.3, −4.5.

3.2.16. (*Z*)-2-(3′-Hydroxy-4′-methoxy-6′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (28). Yield (11%) of a yellow solid. R_f (35% EtOAc/hexanes) = 0.21. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.74 (s, 1H, Ar*H*), 6.85 (d, 1H, J = 12.0, vinyl C*H*), 6.83 (s, 1H, Ar*H*), 6.61 (d, 1H, J = 12.0, vinyl C*H*), 6.31 (s, 2H, Ar*H*), 6.08 (s, 1H, Ar*H*), 3.99 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.8, 150.2, 145.4, 140.2, 137.6, 131.4, 130.6, 129.6, 126.6, 117.1, 107.3, 106.5, 60.9, 56.5, 55.9. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found: C, 59.97; H, 5.20; N, 3.91.

3.2.17. (*E*)-2-(3′-Hydroxy-4′-methoxy-6′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (25b)[†]. Yield (33%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.16. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64 (s, 1H, Ar*H*), 7.62 (d, 1H, J = 15.9, vinyl C*H*), 7.22 (s, 1H, Ar*H*), 6.90 (d, 1H, J = 15.9, vinyl C*H*), 6.75 (s, 2H, Ar*H*), 6.15 (s, 1H, Ar*H*), 3.99 (s, 3H, OCH_3), 3.91 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.5, 150.3, 145.6, 140.0, 138.6, 132.8, 132.5, 129.3, 123.8, 113.1, 107.8, 104.1, 61.0, 56.5, 56.2.

3.2.18. (*Z*)-2-(2′-Nitro-3′-methoxy-4′-*tert*-butyldimethylsilyloxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (39a). Yield (21%) of a yellow oil. R_f (25% EtOAc/hexanes) = 0.53. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.88 (d, 1H, J = 8.5, Ar*H*), 6.81 (d, 1H, J = 8.5, Ar*H*), 6.63 (d, 1H, J = 12.0, vinyl C*H*), 6.40 (s, 2H, Ar*H*), 6.37 (d, 1H, J = 12.0, vinyl C*H*), 3.90 (s, 3H, OCH_3), 3.82, (s, 3H, OCH_3), 3.68 (s, 6H, OCH_3), 1.01 (s, 9H, *t*-Bu CH_3), 0.20 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.9, 148.7, 146.4, 143.1, 137.6, 133.9, 131.3, 125.4, 123.8, 122.7, 122.1, 106.1, 61.9, 60.9, 55.8, 25.5, 18.2, −4.6.

3.2.19. (*E*)-2-(2′-Nitro-3′-methoxy-4′-*tert*-butyldimethylsilyloxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (39b). Yield (30%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.45. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.33 (d, 1H, J = 8.7, Ar*H*), 6.96 (d, 1H, J = 16.2, vinyl C*H*), 6.96 (d, 1H, J = 8.7, Ar*H*), 6.75 (d, 1H, J = 16.2, vinyl C*H*), 6.66 (s, 2H, Ar*H*), 3.91 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3), 3.87 (s, 3H, OCH_3), 1.03 (s, 9H, *t*-Bu CH_3), 0.24 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.4, 148.8, 145.7, 143.0, 138.6, 132.3, 132.1, 123.3, 123.0, 121.1, 119.7, 103.9, 61.9, 60.9, 56.1, 25.6, 18.2, −4.5.

3.2.20. (*Z*)-2-(3′-Methoxy-4′-*tert*-butyldimethylsilyloxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (40a). Yield (40%) of a yellow liquid. R_f (25% EtOAc/hexanes) = 0.50. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.27 (d, 1H, J = 1.9, Ar*H*), 6.90 (d, 1H, J = 1.9, Ar*H*), 6.58 (d, 1H, J = 12.1, vinyl C*H*), 6.51 (s, 2H, Ar*H*), 6.43 (d, 1H, J = 12.1, vinyl C*H*), 3.84 (s, 3H, OCH_3), 3.73 (s, 6H, OCH_3), 3.59 (s, 3H, OCH_3), 0.95 (s, 9H, *t*-Bu CH_3), 0.16 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.2, 151.0, 142.6, 138.4, 137.6, 132.0, 131.1, 129.6, 127.7, 117.0, 115.2, 105.9, 60.9, 56.0, 55.4, 25.6, 18.6, −4.2.

3.2.21. (*E*)-2-(3′-Methoxy-4′-*tert*-butyldimethylsilyloxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (40b). Yield (33%) of a yellow solid. R_f (25% EtOAc/

[†] TBS-deprotection occurred in the reaction.

hexanes) = 0.37. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.45 (d, 1H, J = 1.9, Ar*H*), 7.14 (d, 1H, J = 1.9, Ar*H*), 6.96 (d, 1H, J = 16.2, vinyl C*H*), 6.89 (d, 1H, J = 16.2, vinyl C*H*), 6.73 (s, 2H, Ar*H*), 3.92 (s, 9H, OCH_3), 3.88 (s, 3H, OCH_3), 0.98 (s, 9H, *t*-Bu CH_3), 0.21 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.4, 151.8, 142.7, 138.8, 138.2, 132.3, 130.1, 129.3, 125.9, 114.3, 112.1, 103.6, 60.9, 56.1, 55.7, 25.6, 18.6, −4.2.

3.2.22. (*Z*)-2-(3′-Methoxy-4′-tert-butyldimethylsilyloxy-6′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (41a). Yield (64%) of a yellow liquid. R_f (15% EtOAc/hexanes) = 0.33. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67 (s, 1H, Ar*H*), 6.90 (d, 1H, J = 12.0, vinyl C*H*), 6.66 (s, 1H, Ar*H*), 6.65 (d, 1H, J = 12.0, vinyl C*H*), 6.33 (s, 2H, Ar*H*), 3.80 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3), 3.57 (s, 3H, OCH_3), 0.99 (s, 9H, *t*-Bu CH_3), 0.17 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.1, 152.9, 144.3, 140.5, 137.5, 131.7, 130.6, 128.8, 127.2, 117.0, 113.9, 106.2, 60.9, 55.9, 55.7, 25.6, 18.4, −4.7.

3.2.23. (*E*)-2-(3′-Methoxy-4′-*tert*-butyldimethylsilyloxy-6′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (41b). Yield (26%) of a yellow solid. R_f (15% EtOAc/hexanes) = 0.22. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.59 (s, 1H, Ar*H*), 7.05 (s, 1H, Ar*H*), 6.89 (d, 1H, J = 15.9, vinyl C*H*), 6.76 (s, 2H, Ar*H*), 6.63 (d, 1H, J = 15.9, vinyl C*H*), 3.97 (s, 3H, OCH_3), 3.91 (s, 6H, OCH_3), 3.88 (s, 3H, OCH_3), 1.02 (s, 9H, *t*-Bu CH_3), 0.21 (s, 6H, CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 155.4, 153.5, 144.5, 140.3, 138.6, 132.5, 132.2, 128.8, 124.5, 117.4, 109.7, 104.1, 61.0, 56.2, 55.9, 25.6, 18.4, −4.6.

3.2.24. General conditions for TBS-deprotection of (*Z*)-stilbenes. To a 100 mL round-bottomed flask containing a stir bar and the appropriate TBS-protected nitro-substituted (*Z*)-stilbene was added dichloromethane (~20 mL). The flask was cooled to 0 °C in an ice-water bath, and 1.1 equivalents of TBAF (1.0 M in THF) was added. The reaction mixture was stirred and typically complete after 5–10 min as determined by TLC. Water (5 mL) was added and the nitro-substituted combretastatin analog was extracted with dichloromethane. The products were purified with flash chromatography.

3.2.25. (*Z*)-2-(2′-Nitro-3′-hydroxy-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (26). Yield (47%) of a yellow oil. R_f (25% EtOAc/hexanes) = 0.10. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.92 (d, 1H, J = 8.4, Ar*H*), 6.78 (d, 1H, J = 8.4, 0.9, Ar*H*), 6.67 (d, 1H, J = 12.0, vinyl C*H*), 6.58 (d, 1H, J = 12.0, vinyl C*H*), 6.31 (s, 2H, Ar*H*), 3.92 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.8, 148.2, 144.0, 137.4, 135.5, 131.6, 131.1, 126.1, 125.9, 121.9, 115.2, 106.2, 60.9, 56.7, 55.9. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found: C, 59.91; H, 5.30; N, 3.96.

3.2.26. (*Z*)-2-(3′-Hydroxy-4′-methoxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (27). Yield (40%) of a yellow solid. R_f (25% EtOAc/hexanes) = 0.17. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39 (d, 1H, J = 2.0, Ar*H*), 7.16 (d, 1H, J = 2.0, Ar*H*), 6.61 (d, 1H, J = 12.1, vinyl C*H*), 6.47 (s, 2H, Ar*H*), 6.42 (d, 1H, J = 12.1, vinyl C*H*), 5.97 (br s, 1H, O*H*), 3.94 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.72 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.2, 150.1, 142.6, 139.8, 138.0, 133.9, 132.4, 131.3, 126.8, 120.7, 117.3, 106.1, 62.6, 61.0, 56.1. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found: C, 59.80; H, 5.27; N, 3.86.

3.2.27. (*Z*)-2-(2′-Nitro-3′-methoxy-4′-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (42). Yield (73%) of a yellow oil. R_f (50% EtOAc/hexanes) = 0.10. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.94 (s, 2H, Ar*H*), 6.62 (d, 1H, J = 11.9, vinyl C*H*), 6.40 (d, 1H, J = 11.9, vinyl C*H*), 6.37 (s, 2H, Ar*H*), 3.92 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.67 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.9, 148.8, 144.8, 139.1, 137.7, 133.9, 131.3, 126.4, 123.2, 122.2, 118.0, 106.2, 62.7, 60.9, 55.9.

3.2.28. (*Z*)-2-(3′-Methoxy-4′-hydroxy-5′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (43). Yield (62%) of an orange oil. R_f (35% EtOAc/hexanes) = 0.33. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64 (d, 1H, J = 1.9, Ar*H*), 7.03 (d, 1H, J = 1.9, Ar*H*), 6.64 (d, 1H, J = 12.1, vinyl C*H*), 6.49 (s, 2H, Ar*H*), 6.45 (d, 1H, J = 12.1, vinyl C*H*), 3.84 (s, 3H, OCH_3), 3.74 (s, 6H, OCH_3), 3.65 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 153.3, 149.2, 145.6, 137.7, 133.6, 132.1, 131.4, 128.2, 127.5, 118.2, 116.5, 105.9, 61.0, 56.4, 56.1. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found: C, 60.44; H, 5.40; N, 3.78.

3.2.29. (*Z*)-2-(3′-Methoxy-4′-hydroxy-6′-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (44). Yield (73%) of a yellow solid. R_f (50% EtOAc/hexanes) = 0.52. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.75 (s, 1H, Ar*H*), 6.90 (d, 1H, J = 12.0, vinyl C*H*), 6.66 (s, 1H, Ar*H*), 6.64 (d, 1H, J = 12.0, vinyl C*H*), 6.30 (s, 2H, Ar*H*), 5.80 (br s, 1H, O*H*), 3.80 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.9, 150.3, 144.9, 141.3, 137.5, 131.7, 130.7, 127.4, 127.0, 113.0, 111.1, 106.3, 60.9, 56.4, 55.9. Anal. Calcd for $C_{18}H_{19}NO_7$: C, 59.83; H, 5.30; N, 3.88. Found: C, 59.79; H, 5.25; N, 3.89.

3.2.30. General procedure for the reduction of the nitro group for combretastatin analogs. A solution (acetone/water, 3:1 ratio) of a (*Z*)-nitro-substituted combretastatin analog was stirred, and the contents were heated in a water bath at 50 °C for 30 min before adding sodium dithionite (8–10 equivalents). The reaction was held at 50 °C and monitored by TLC. Following completion, the reaction was worked up by adding a 10% sodium bicarbonate solution (~10 mL) and extracting with ethyl acetate (3× 10 mL). The organic phases were combined, dried with sodium sulfate, and concentrated under reduced pressure.

3.2.31. (*Z*)-2-(2′-Amino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (7). Yield (38%). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.03 (d, J = 8.4, Ar*H*, 1H), 6.51 (s, Ar*H*, 2H,), 6.49 (d, J = 11.9, C=C*H*, 1H), 6.42 (d, J = 12.0, C=C*H*, 1H), 6.30 (dd, J = 8.4, 2.5, Ar*H*, 1H), 6.25 (d, J = 2.4, Ar*H*, 1H), 3.80 (s, OCH_3, 3H),

3.75 (s, OCH_3, 3H), 3.64 (s, OCH_3, 6H), 1.56 (br s, NH_2, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 160.1, 152.7, 144.9, 137.2, 132.3, 131.0, 130.6, 125.7, 116.1, 105.8, 104.3, 100.7, 60.9, 55.8, 55.2. DEPT 90 NMR (75 MHz, $CDCl_3$): δ 131.4, 131.0, 126.1, 106.1, 104.7, 101.1. DEPT 135 NMR (75 MHz, $CDCl_3$): δ 131.4 (*C*H), 131.0 (*C*H), 126.1 (*C*H), 106.1 (*C*H), 104.7 (*C*H), 101.1 (*C*H), 61.3 (*C*H_3), 56.2 (*C*H_3), 55.6 (*C*H_3). EIMS: *m/z* (% rel intensity, ion) 315 (100, M^+), 300 (80), 142 (15). Anal. Calcd for $C_{18}H_{21}NO_4$: C, 68.55 H, 6.71 N, 4.44. Found: C, 68.49, H, 6.71, N, 4.25.

3.2.32. (*Z*)-2-(2′-Amino-3′-hydroxy-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (29). Yield (98%) of a yellow oil. R_f (35% EtOAc/hexanes) = 0.26. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.68 (d, 1H, J = 8.4, Ar*H*), 6.52 (s, 2H, Ar*H*), 6.52 (d, 1H, J = 12.0, vinyl C*H*), 6.46 (d, 1H, J = 12.0, vinyl C*H*), 6.32 (d, 1H, J = 8.4, Ar*H*), 3.85 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.7, 145.7, 137.3, 132.6, 132.2, 132.1, 131.0, 125.7, 120.1, 117.6, 105.9, 101.1, 60.9, 56.1, 55.7.

3.2.33. (*Z*)-2-(3′-Hydroxy-4′-methoxy-5′-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (30). Yield (15%) of a brown oil. R_f (50% EtOAc/hexanes) = 0.26. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.54 (s, 2H, Ar*H*), 6.43 (d, 1H, J = 12.1, vinyl C*H*), 6.39 (d, 1H, J = 12.1, vinyl C*H*), 6.35 (d, 1H, J = 1.8, Ar*H*), 6.27 (d, 1H, J = 1.8, Ar*H*), 3.84 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3). Anal. Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 64.55; H, 6.39; N, 4.24.

3.2.34. (*Z*)-2-(3′-Hydroxy-4′-methoxy-6′-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (31). Yield (47%) of a yellow oil. R_f (50% EtOAc/hexanes) = 0.20. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.74 (s, 1H, Ar*H*), 6.53 (s, 2H, Ar*H*), 6.49 (d, 1H, J = 12.0, vinyl C*H*), 6.40 (d, 1H, J = 12.0, vinyl C*H*), 6.28 (s, 1H, Ar*H*), 3.83 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.66 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.7, 146.7, 138.3, 137.3, 136.9, 132.1, 130.9, 125.6, 115.8, 115.1, 105.8, 99.7, 60.9, 55.9, 55.8.

3.2.35. (*Z*)-2-(2′-Amino-3′-methoxy-4′-hydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene (45). Yield (39%) of a yellow oil. R_f (50% EtOAc/hexanes) = 0.17. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.82 (dd, 1H, J = 8.2, 0.9, Ar*H*), 6.51 (d, 1H, J = 11.9, vinyl C*H*), 6.50 (s, 2H, Ar*H*), 6.42 (d, 1H, J = 11.9, vinyl C*H*), 6.36 (d, 1H, J = 8.2, Ar*H*), 3.81 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 3.64 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.8, 148.2, 137.5, 134.3, 132.2, 131.2, 125.5, 125.5, 116.5, 105.8, 105.4, 60.9, 59.7, 55.8.

3.2.36. (*Z*)-2-(3′-Methoxy-4′-hydroxy-5′-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (46). Yield (97%) of a yellow oil. R_f (50% EtOAc/hexanes) = 0.27. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.56 (s, 2H, Ar*H*), 6.42 (d, 1H, J = 12.1, vinyl C*H*), 6.39 (d, 1H, J = 1.8, Ar*H*), 6.38 (d, 1H, J = 12.1, vinyl C*H*), 6.30 (d, 1H, J = 1.8, Ar*H*), 3.83 (s, 3H, OCH_3), 3.72 (s, 6H, OCH_3), 3.67 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.8, 146.2, 137.0, 133.8, 133.0, 132.2, 130.3, 128.7, 128.4, 110.2, 106.0, 102.3, 60.9, 56.0, 55.9.

3.2.37. (*Z*)-2-(3′-Methoxy-4′-hydroxy-6′-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene (47). Yield (68%) of a yellow oil. R_f (50% EtOAc/hexanes) = 0.26. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 6.63 (s, 1H, Ar*H*), 6.53 (s, 2H, Ar*H*), 6.49 (d, 1H, J = 12.0, vinyl C*H*), 6.42 (d, 1H J = 12.0, vinyl C*H*), 6.34 (s, 1H, Ar*H*), 3.81 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.66 (s, 6H, OCH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 152.7, 146.1, 139.7, 138.4, 137.3, 132.2, 130.5, 125.7, 114.3, 112.5, 105.9, 102.8, 60.8, 56.6, 55.8.

3.2.38. General procedure for the preparation of hydrochloride salts of amino combretastatin analogs. To a round-bottomed flask containing a stir bar and a (*Z*)-amino combretastatin analog, anhydrous dichloromethane (~10 mL) was added. The solution was stirred at room temperature and a solution of 4 N HCl/dioxane (3–4 equivalents) was added. The contents were stirred overnight and the solvent was removed to give a brown oil. The product was recrystallized and the salt was collected.

3.2.39. (*Z*)-2-(2′-Methoxy-4′-aminophenyl)-1-(3,4,5-trimethoxyphenyl)ethene hydrochloride (15). Yield (83%) of a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.05 (3H, br s, NH_3^+), 7.18 (1H, d, J = 8.6, Ar*H*), 7.14 (1H, br s, Ar*H*), 6.73 (1H, d, J = 8.2, Ar*H*), 6.71 (1H, d, J = 11.8, C=C*H*), 6.56 (1H, d, J = 11.8, C=C*H*), 6.37 (2H, s, Ar*H*), 3.79 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.60 (6H, s, OCH_3). Anal. Calcd for $C_{18}H_{22}NO_4Cl$: C, 61.45 H, 6.30 N, 3.98. Found: C, 61.16, H, 6.39, N, 3.91.

3.2.40. (*Z*)-2-(2′-Amino-3′-hydroxy-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene hydrochloride (32). Yield (75%) of a tan powder. ^{1}H NMR (D_2O, 300 MHz): δ 6.82 (d, 1H, J = 8.7, Ar*H*), 6.60 (d, 1H, J = 8.7, Ar*H*), 6.57 (d, 1H, J = 12.0, vinyl C*H*), 6.42 (d, 1H, J = 12.0, vinyl C*H*), 6.20 (s, 2H, Ar*H*), 3.68 (s, 3H, OCH_3), 3.51 (s, 3H, OCH_3), 3.39 (s, 6H, OCH_3). ^{13}C NMR (D_2O, 75 MHz): δ 155.2, 150.7, 143.0, 139.3, 136.9, 135.1, 128.0, 126.6, 124.0, 119.2, 115.4, 109.5 63.9, 59.5, 58.7.

3.2.41. (*Z*)-1-(2′-Amino-4′-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene-FMOC-L-serinamide (9). To a well-stirred solution of free amine **7** (0.996 g, 3.07 mmol) in anhydrous DMF (10 mL), DCC (0.767 g, 3.68 mmol), FMOC(Ac) serine (1.37 g, 3.68 mmol), and HOBt·H_2O (0.508 g, 3.68 mmol) were added and the mixture was stirred at room temperature for 16 h at which point the solid formed was filtered and the filtrate was washed 5 times with water and twice with brine. After the organic phase was dried over sodium sulfate, the solvent was evaporated and the crude product of the reaction was purified by flash chromatography (35% EtOAc/hexanes) to afford 1.13 g of the product in a 55% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11 (1H, br s, *NH*), 7.93 (1H, d, J = 2.2, Ar*H*), 7.79 (2H, d, J = 7.4, Ar*H*), 7.59 (2H, d, J = 6.5, Ar*H*), 7.40 (2H, t, J = 7.5, Ar*H*), 7.34 (2H, d,

J = 7.0, Ar*H*), 7.14 (1H, d, J = 8.6, Ar*H*), 6.71 (1H, dd, J = 8.6, 2.5, Ar*H*), 6.35 (1H, d, J = 11.7, C=C*H*), 6.31 (1H, d, J = 11.7, C=C*H*), 6.23 (2H, s, Ar*H*), 5.28 (1H, d, J = 6.6, CH_2), 4.51 (1H, dd, J = 12.9, 6.0, CH_2), 4.40 (2H, br s, CH_2), 4.20 (1H, d, J = 5.5, C*H*), 4.09 (1H, m, C*H*), 3.80 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.48 (6H, s, OCH_3), 1.96 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 166.3, 159.5, 152.9, 143.5, 141.3, 137.8, 135.0, 132.9, 131.1, 129.9, 127.9, 127.8, 127.1, 124.8, 124.7, 123.9, 120.0, 119.9, 111.3, 105.6, 105.5, 63.5, 60.7, 60.4, 55.6, 55.4, 47.1, 20.5, 14.2.

3.2.42. (*Z*)-2-(2′-Amino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethane-L-serinamide (11). FMOC(Ac)-L-serinamide **9** (0.131 g, 0.196 mmol) was dissolved in 6 mL of a mixture of dichloromethane/MeOH (1:1 ratio) and 0.22 mL of an aqueous solution of 2 N sodium hydroxide (0.0176 g, 0.44 mmol) was added. After the reaction mixture was stirred at room temperature for 18 h, dichloromethane (1 mL) was added and the organic phase was washed once with water, twice with brine and dried under sodium sulfate. After the solvent was evaporated, the resulting oil was purified by normal-phase preparative TLC (95% dichloromethane/MeOH) to obtain 0.0529 g of the product in a 67% yield.

^{1}H NMR (300 MHz, CD_2Cl_2): δ 9.66 (1H, br s, N*H*), 7.95 (1H, d, J = 2.6, Ar*H*), 7.15 (1H, d, J = 8.5, Ar*H*), 6.66 (1H, dd, J = 8.5, 2.6, Ar*H*), 6.61 (1H, d, J = 12.0, C=C*H*), 6.49 (1H, d, J = 12.1, C=C*H*), 6.40 (2H, s, Ar*H*), 3.79 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 3.64 (1H, dd, J = 10.7, 5.5, CH_2OH), 3.56 (1H, dd, J = 10.5, 5.6, CH_2OH), 3.57 (6H, s, OCH_3), 3.30 (1H, t, J = 5.4, C*H*NH_2), 2.07 (3H, br s, O*H*, NH_2). ^{13}C NMR (75 MHz, CD_2Cl_2): δ 172.2, 159.8, 153.2, 138.1, 136.3, 132.8, 132.2, 130.4, 124.7, 120.4, 110.5, 106.3, 106.0, 65.2, 60.8, 57.0, 56.1, 55.7. DEPT 45 NMR (75 MHz, CD_2Cl_2): δ 132.5, 130.0, 124.3, 110.1, 105.9, 105.7, 64.9, 60.4, 56.7, 55.7, 55.4. DEPT 90 NMR (75 MHz, $CDCl_3$): δ 132.9, 130.5, 124.7, 111.3, 106.2, 105.7, 56.8. DEPT 135 NMR (75 MHz, CD_2Cl_2): δ 132.5 (*C*H), 130.0 (*C*H), 124.3 (*C*H), 110.1 (*C*H), 105.9 (*C*H), 105.7 (*C*H), 64.9 (*C*H_2), 60.4 (*C*H_3), 56.7 (*C*H), 55.7 (*C*H_3), 55.4 (*C*H_3).

3.2.43. (*Z*)-2-(2′-Amino-4′-methoxyphenyl)-1-(3,4,5-trimethoxyphenyl)ethene-L-serinamide hydrochloride (13). To a well-stirred solution of L-serinamide **11** (0.220 g, 0.546 mmol) in methanol (5 mL), 0.57 mL of a dioxane solution of 4 N hydrochloric acid (0.0832 g, 2.28 mmol) was added dropwise. After the reaction mixture was stirred at room temperature for 22 h, the solvent was removed and the yellow oil formed was triturated with chloroform at 0 °C forming a solid which was filtered and rinsed with chloroform (3 mL). Purification of the salt by reverse-phase preparative TLC (87% dichloromethane/MeOH) afforded 0.0505 g in a 21% yield of the L-serinamide salt. ^{1}H NMR (300 MHz, methanol-d_6): δ 7.37 (1H, d, J = 2.0, Ar*H*), 7.10 (1H, d, J = 8.5, Ar*H*), 6.72 (1H, dd, J = 8.5, 2.3, Ar*H*), 6.57 (1H, d, J = 11.9, C=C*H*), 6.48 (1H, d, J = 12.0, C=C*H*), 6.43 (2H, s, Ar*H*), 4.06 (1H, br s, O*H*), 3.78 (2H, m, CH_2), 3.74 (3H, s, OCH_3), 3.66 (3H, s, OCH_3), 3.55 (6H, s, OCH_3).

3.3. Biological assays

3.3.1. Tubulin polymerization assay. Tubulin was purified from calf brain according to the method of Hamel and Lin.[58] This method is based on six selective polymerization/cold depolymerization cycles. Tubulin purity was determined by SDS–PAGE and by its ability to polymerize. The purified tubulin was flash-frozen and stored in liquid nitrogen. The tubulin concentration was determined by a two-component[59] analysis using the absorption measured at 278 and 255 nm, and the following extinction coefficients: for tubulin, 1.2 L/g-cm at 278 nm (Harrison et al.)[60] and 0.65 L/g-cm at 255 nm; for GTP, 12.17×10^3 and 7.66×10^3 M/cm at 255 and 278 nm, respectively. Tubulin polymerization was induced in the presence of organic acid (sodium glutamate) and GTP by a temperature jump to 30 °C according to the method of Verdier-Pinard et al.[61,62] Polymerization was followed turbidimetrically at 350 nm on an Agilent 8453 spectrophotometer equipped with kinetics program software, a jacketed cell holder, and two circulating water baths. To ice-chilled tubulin at 1.0 mg/mL in 1.0 M glutamate, pH 6.6 (10 μM), pure DMSO (4% v/v final concentration) was added for the control or 2.5–1000 μM of the analog to be analyzed in DMSO, giving a final concentration of 0.1–40.0 μM compound in a total volume of 200 μl of reaction mixture. The mixture was incubated at 30 °C for 15 min and cooled at 0 °C for 15 min. An aliquot of 10 mM GTP (final concentration of 0.4 mM) was added to the reaction mixture on ice, mixed, and immediately transferred into a pre-cooled 250 μl quartz microcuvette. The baseline was established at 350 nm at 0 °C and after 100 s, polymerization was initiated by a temperature jump to 30 °C. After 1500 s, the cell was cooled to 0 °C to induce depolymerization. Turbidity (absorbance) readings were recorded every 10 s. IC_{50} values of the various analogs were determined from the data using nonlinear regression analysis with the Prism software (GraphPad) 3.02 version.

3.3.2. MTT assay. The MTT cell proliferation assay was used to quantify the cell viability, measuring cell survival and proliferation spectrophotometrically.[63] This colorimetric assay measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), reflecting the changes in cell proliferation. The mechanism of this assay involves the cleavage of the tetrazolium ring by active mitochondria in living cells, producing a dark blue formazan product which can be quantified in a rapid colorimetric fashion. The reduction of MTT can only occur with living cells containing active mitochondria. Comparison of the cells treated with the drug to an untreated control group provides the relative cytotoxicity, reflecting the loss of cell viability as MTT reduction decreases. 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 which reduced cell viability by 50% of the control (IC_{50}).

3.3.3. 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.[25] 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, MD) was used to control the picture recording, and image analysis was performed using Image Plus software (Media Cybernetics, MD).

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