Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatins A-4, A-5, and A-6[1a]

George R. Pettit,* Sheo Bux Singh, Michael R. Boyd,[1b] Ernest Hamel,[1c] Robin K. Pettit,[1d] Jean M. Schmidt, and Fiona Hogan

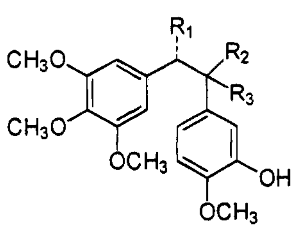
Cancer Research Institute and Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604

Received November 13, 1994®

The antineoplastic constituents of *Combretum caffrum* (Eckl. and Zeyh) Kuntze (Combretaceae family), a species indigenous to South Africa, have been investigated. Subsequently we isolated a series of closely related bibenzyls, stilbenes, and phenanthrenes from *C. caffrum*. Some of the stilbenes proved to be potent antimitotic agents which inhibited both tubulin polymerization and the binding of colchicine to tubulin. Combretastatin A-4 has been shown to be the most potent cancer cell growth inhibitor of the series. Presently this *cis*-stilbene is the most effective inhibitor of colchicine binding to tubulin and the simplest natural product yet described with such potent antitubulin effects. Combretastatin A-4, A-5, and A-6 were also found to inhibit growth of *Neisseria gonorrhoeae*. Details of the isolation and syntheses of combretastatins A-4 (**2a**), A-5 (**2c**), and A-6 (**3a**) have been described.

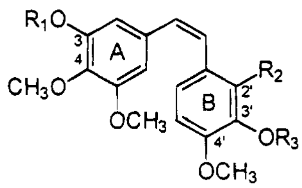
The Combretaceae family of shrubs and trees is well represented in traditional medical practices of, especially, Africa and India.[2-6] Illustrative is a recent study of Combretaceae species used in Somalia.[7] These range from *Combretum hereroense* (young shoots, used for respiratory infection) to *Terminalia brevipes* (root bark employed for hepatitis and malaria) to *Commelina forskoolii* (juice for treatment of uterine cancer). Nine other species including *Anogeissus leiocorpus* (fruit and roots as an anthelmintic treatment), *Guiera senegalensis* (fruit and leaves for leprosy and dysentery), and *Quisqualis indica* (leaves for vermifuge) are more widely used in Africa.[8]

In 1979 we began an in-depth study of cancer cell growth inhibitory constituents of the African willow tree *Combretum caffrum* (Eckl. and Zeyh) Kuntze that resulted in isolation and structural determination of a series of active phenanthrenes, stilbenes, and bibenzyls.[2-4] Initially the bibenzyl combretastatin (**1a**) was



1a, R_1 = OH, R_2 = R_3 = H Combretastatin
1b, R_1 = R_2 = R_3 = H
1c, R_1 = R_2 = H, R_3 = OH Isocombretastatin A
1d, R_1 = H, R_2, R_3 = O

isolated and found to cause substantial astrocyte reversal in the 9ASK system, inhibition of the P388 lymphocytic leukemia cell line (PS cell line), and inhibition of tubulin polymerization. Discovery of the very potent cell growth and tubulin inhibitors combretastatins A-1[2] (**2e**) and A-4[4] (**2a**) was especially important. Both proved to be the strongest presently known inhibitors



2a, R_1 = CH_3, R_2 = R_3 = H Combretastatin A-4
2b, R_1 = CH_3, R_2 = H, R_3 = $Si(CH_3)_2C(CH_3)_3$
2c, R_1 = R_2 = H, R_3 = CH_3 Combretastatin A-5
2d, R_1 = $Si(CH_3)_2C(CH_3)_3$, R_2 = H, R_3 = CH_3
2e, R_1 = CH_3, R_2 = OH, R_3 = H Combretastatin A-1

of colchicine binding to tubulin and to be exceptionally strong inhibitors of tubulin polymerization (IC_{50} values of 2–3 μM).[4] Furthermore, combretastatin A-4 was found to markedly inhibit growth of a selection of colon cancer cell lines. These early results were summarized in a preliminary report.[4] Now follows a detailed description of the isolation, synthesis, and results of expanded biological[9-17] evaluations of combretastatins A-4 to A-6.

The dichloromethane–methanol extract of *C. caffrum* stem wood (77 kg) was fractionated (PS bioassay) as previously described[2-4] using a solvent partition sequence followed by gel filtration of the dichloromethane extract through Sephadex LH-20 (methanol as eluant). Following partition chromatography of the active fraction on Sephadex LH-20 using hexane–toluene–methanol (3:1:1) as the mobile phase, further separation by ambient column and high-performance liquid silica gel chromatography yielded an apparently pure and very active fraction. However, high-resolution ^{1}H-NMR and ^{13}C-NMR spectral data suggested that the fraction was a mixture of at least three substituted stilbenes. Hydrogenation of an aliquot reduced it to a two-component bibenzyl mixture which resisted resolution and suggested that two of the original stilbenes were geometrical isomers while the third was a positional isomer.

The following procedure was found quite effective for resolving the difficultly separable mixture of stilbenes.

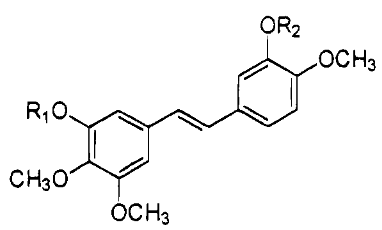
* Author to whom inquiries should be directed.
® Abstract published in *Advance ACS Abstracts*, April 15, 1995.

0022-2623/95/1838-1666$09.00/0

Table 1. ^{1}H-NMR Assignments (at 400 MHz in δ Values) for Combretastatins A-4 (**2a**), A-5 (**2c**), A-6 (**3a**), and Silyl Ether Derivatives in Deuteriochloroform

position	A-4 (**2a**)	*E*-isomer **3c**	A-5 (**2c**)	A-6 (**3a**)	silyl ether **2b**	*E*-isomer **3d**	silyl ether **2d**	silyl ether **3b**
H-2	6.527	6.706	6.569	6.776	6.499	6.712	6.490	6.710
	s	s	d, 2.3	d, 1.88	s	s	d, 1.84	d, 2.0
H-6	6.527	6.706	6.418	6.614	6.499	6.712	6.420	6.639
	s	s	d, 2.3	d, 1.88	s	s	d, 1.84	d, 2.0
H-1a	6.471	6.905	6.478	6.940	6.463	6.896	6.484	6.903
	d, 12.16	d, 16.30	d, 12.24	d, 16.24	d, 12.16	d, 16.28	d, 12.12	d, 16.24
H-1a′	6.412	6.884	6.419	6.850	6.429	6.855	6.428	6.853
	d, 12.16	d, 16.30	d, 12.24	d, 16.24	d, 12.16	d, 16.28	d, 12.12	d, 16.24
H-2′	6.925	7.137	6.841	7.052	6.790	7.035	6.792	7.054
	d, 2.04	d, 2.08	d, 2.0	d, 1.92	d, 2.0	d, 2.0	d, 1.92	d, 2.0
H-5′	6.734	6.824	6.752	6.859	6.735	6.833	6.758	6.859
	d, 8.4	d, 8.32	d, 8.0	d, 8.12	d, 8.28	d, 8.12	d, 8.24	d, 7.96
H-6′	6.799	6.965	6.854	7.039	6.852	7.055	6.843	7.041
	dd, 8.42, 2.04	dd, 8.32, 2.08	dd, 8.0, 2.0	dd, 8.12, 1.92	dd, 8.28, 2.0	dd, 8.12, 2.0	dd, 8.24, 1.92	dd, 7.96, 2.0
–OH	5.509	5.602	5.692	5.772				
3-OCH_3	3.700	3.904			3.702	3.917		
4-OCH_3	3.869	3.861	3.861	3.905	3.832	3.864	3.953	3.799
5-OCH_3	3.700	3.904	3.872	3.915	3.702	3.917	3.757	3.907
3′-OCH_3			3.675	3.949			3.680	3.907
4′-OCH_3	3.844	3.886	3.670	3.919	3.832	3.830	3.674	3.954
$Si(CH_3)_2$					0.059	0.186	0.092	0.210
$SiC(CH_3)_3$					0.929	1.025	0.950	1.030

A portion of the mixture was treated with *tert*-butyldimethylsilyl chloride. The mixture of silyl ethers was separated by preparative thin layer chromatography on silica gel to yield the silyl ether (**2b**, **2d**, and **3b**) derivatives of combretastatins A-4 (**2a**), A-5 (**2c**), and A-6 (**3a**). Structures of the silyl ethers were assigned



3a, R_1 = H, R_2 = CH_3 Combretastatin A-6
3b, R_1 = $Si(CH_3)_2C(CH_3)_3$, R_2 = CH_3
3c, R_1 = CH_3, R_2 = H
3d, R_1 = CH_3, R_2 = $Si(CH_3)_2C(CH_3)_3$

based on results of spectroscopic analyses. High-resolution electron impact mass spectroscopy revealed that all three silyl ethers had the molecular formula $C_{24}H_{34}O_5Si$. High-resolution ^{1}H-NMR spectra (see Table 1) gave evidence of four methoxy groups, one *tert*-butyldimethylsilyl group, and seven protons in the aromatic region. Two of the derivative **2b** methoxy groups were equivalent. Three protons were displayed as an ABC set (J_{AC} = 0) at δ 6.735 (d, J = 8.28 Hz), 6.852 (dd, J = 8.28, 2.0 Hz), and 6.790 (d, J = 2.0 Hz), suggesting ortho–ortho and ortho–meta coupling. The singlet at δ 6.499 integrated for two protons, showing the second aromatic ring to be symmetrically substituted, while two doublets at δ 6.463 and 6.429 (J = 12.16 Hz each) were indicative of a *cis*-stilbene. On this basis structure **2b** was assigned to the combretastatin A-4 silyl ether and subsequently confirmed by synthesis.

The ^{1}H-NMR spectra of silyl ethers **2d** and **3b** each displayed a set of ABC signals resembling that section of the stilbene **2b** spectrum and suggested a similar substitution pattern in one of the aromatic rings. However, none of the methoxy groups were identical,

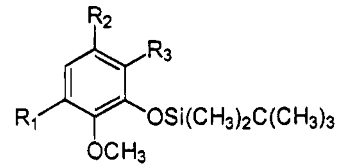
Table 2. Combretastatins A-4, iso-A-4, A-5, and A-6 ^{13}C-NMR (100.6 MHz) Chemical Shift (δ) Assignments Relative to Tetramethylsilane in Deuteriochloroform

carbon position	A-4 (**2a**)	*E*-isomer **3c**	A-5 (**2c**)	A-6 (**3a**)
1	132.67[a]	133.28[a]	133.80	133.79[a]
2	106.07	103.35	108.72	105.96
3	152.82	153.29	149.09[a]	149.42[b]
4	137.14	137.64	134.60	135.15
5	152.82	153.29	151.96	152.44
6	106.07	103.35	104.86	102.39
1a	129.45[b]	127.75[b]	129.77[b]	128.17[c]
1a′	128.98[b]	126.94[b]	128.60[b]	126.55[c]
1′	130.58[a]	130.90[a]	133.80	130.38[a]
2′	115.02	111.73	111.88	108.78
3′	145.77[c]	146.41[c]	148.36[a]	149.15[b]
4′	145.22[c]	145.76[c]	148.25[a]	148.95[b]
5′	110.32	110.65	110.84	111.28
6′	121.06	119.13	121.97	119.85
4-OCH_3	60.85	60.85	60.97	61.06
OCH_3	55.89	56.03	55.83	55.88
OCH_3	55.89	56.03	55.71	55.89
OCH_3	55.89	55.89	55.58	55.95

[a–c] The same superscripts may be interchanged in a vertical column.

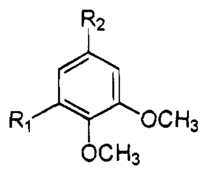
and a set of doublets in each spectrum, both with a coupling constant of 2 Hz indicative of two meta protons, implied the substitution pattern shown in ring A of structures **2d** and **3b**. The spectrum of compound **2d** displayed a set of doublets at δ 6.484 and 6.428 (J = 12.12 Hz) whereas those of ether **3b** appeared at δ 6.903 and 6.853 (J = 16.24 Hz), indicating *cis*- and *trans*-isomers. Furthermore, silyl ether **2d** isomerized to **3b** during the course of NMR experiments. No interconversion of **3b** to **2d** took place under similar conditions. From these observations structures **2d** and **3b** were assigned to the silyl ethers of combretastatins A-5 and A-6, respectively. The above reasoning inferred that combretastatins A-4, A-5, and A-6 corresponded to structures **2a**, **2c**, and **3a**, respectively. Those assignments were confirmed by total syntheses.

A very practical and efficient synthesis of combretastatin A-4 was achieved by a Wittig reaction route analogous to the methods used in our earlier syntheses of combretastatins.[3,10,13] As described previously,[9,13] phosphonium bromide **4a** was prepared by silylation of



4a, $R_1 = R_3 = H$, $R_2 = CH_2PPh_3Br$
4b, $R_1 = OCH_3$, $R_2 = CH_2PPh_3Br$, $R_3 = H$
4c, $R_1 = R_3 = H$, $R_2 = CHO$
4d, $R_1 = H$, $R_2 = CHO$, $R_3 = OSi(CH_3)_2C(CH_3)_3$

isovanillin followed by reduction to the benzyl alcohol, bromination, and reaction with triphenylphosphine. The ylide formed by reaction of bromide **4a** with 1 equiv of butyllithium was treated with 3,4,5-trimethoxybenzaldehyde (**5a**) to afford a mixture of stilbenes **2b** and **3d**



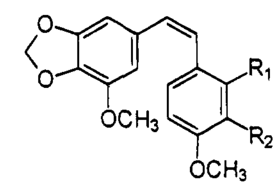
5a, $R_1 = OCH_3$, $R_2 = CHO$
5b, $R_1 = H$, $R_2 = CHO$
5c, $R_1 = OCH_3$, $R_2 = CH_2PPh_3Br$

in 93% yield; ^{1}H-NMR analysis indicated a *Z*/*E* ratio of 1:1.5. The compounds were separated by silica gel column chromatography, and *Z*-isomer **2b** was shown to be identical (by spectroscopic and chromatographic comparisons) to the silyl ether of natural combretastatin A-4. Both *cis*- and *trans*-isomers were desilylated using tetrabutylammonium fluoride to give combretastatin A-4 (**2a**; PS ED_{50} 3.4×10^{-3} μg/mL) and its *trans*-isomer (**3c**; PS ED_{50} 5.0×10^{-2} μg/mL) in 93% and 92% yields, respectively.

Combretastatins A-5 and A-6 were prepared by similar means. Phosphonium bromide **4b** was prepared[3] and treated with butyllithium to form the ylide. Reaction with aldehyde **5b** yielded a mixture of stilbenes **2d** and **3b** in 78% yield with a *Z*/*E* ratio of 1:1. The mixture was separated by silica gel chromatography to afford the *cis*-isomer, identical to the silyl ether of combretastatin A-5 (**2d**), and the *trans*-isomer, identical to the silyl ether of combretastatin A-6 (**3b**). Both products were treated with tetrabutylammonium fluoride to give combretastatins A-5 (PS ED_{50} 0.9 μg/mL) and A-6 (PS ED_{50} 18 μg/mL).

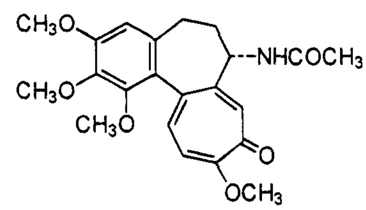
The silyl ether of combretastatin A-4 (**2b**) and its *trans*-isomer **3d** were also synthesized by a Wittig reaction between aldehyde **4c**[9] and the ylide formed from phosphonium bromide **5c**.[2] The yield from this route (65%) was less than in that described above but the *Z*/*E* ratio was higher (3.6:1), thus giving overall a higher yield of the desired *cis*-isomer (**2b**). The different *Z*/*E* ratios obtained in the Wittig reactions leading to the combretastatins have been commented on previously.[2,3] Apparently the presence and position of the bulky *tert*-butyldimethylsilyl ether group are of prime importance in determining both the isomeric ratio and the overall yield. Since large quantities of combretastatin A-4 were needed for biological evaluation, conversion of the silyl ether of the less active *trans*-isomer (**3d**) was effected by irradiating at 254 nm, to afford *cis*-stilbene **2b** in 64% yield.

In order to study structure–activity relationships in the combretastatin series, a number of derivatives have been prepared.[2,18] For example, combretastatin A-4 (**2a**) was hydrogenated over palladium-on-charcoal to give dihydrostilbene **1b**. Stilbene **6a**, a 3,4-methylenedioxy

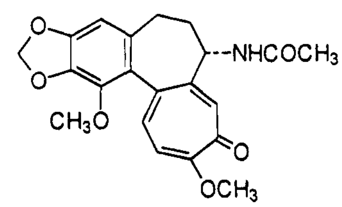


6a, $R_1 = R_2 = OH$
6b, $R_1 = H$, $R_2 = OH$ Combretastatin A-2
6c, $R_1 = R_2 = OSi(CH_3)_2C(CH_3)_3$

(cf. **7** vs **8**) analog of combretastatin A-1 (**2e**),[2] was

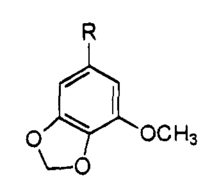


7, Colchicine



8, Cornigerine

synthesized by a Wittig reaction sequence involving protected aldehyde **4d**[2] and phosphonium bromide **9a**, prepared from 3,4-(methylenedioxy)-5-methoxybenzyl alcohol (**9b**).[3] Following separation of the isomeric mixture of ethers, desilylation afforded *cis*-stilbene **6a**. Another synthetic modification is represented by ketone **1d**, prepared by oxidation of isocombretastatin A (**1c**)[10] with DDQ.



9a, $R = CH_2PPh_3Br$
9b, $R = CH_2OH$

Extensive structure–activity studies on effects on tubulin polymerization and colchicine binding have been performed with the combretastatin series of agents[12] and with closely related synthetic compounds.[18] Several generalizations have emerged from these investigations. The *cis*-stilbene configuration is optimal for activity, while the *trans* configuration is probably inactive. Initially[13] it appeared that the *trans*-form (compound **3c**) of combretastatin A-4 (**2a**) had about one-fourth the activity of the *cis*-isomer (i.e., **2a**) as an inhibitor of polymerization, but subsequently we found that freshly prepared stock solutions (in dimethyl sulfoxide) of **3c** were inactive and gained activity with the passage of time.[18] This suggested some conversion of the *trans* (**3c**) to *cis* (**2a**) forms under laboratory storage, and slow conversion could be demonstrated under controlled conditions.[18] However, the bibenzyl or dihydrocombretastatin forms retain significant activity as polymerization inhibitors relative to the cognate *cis*-stilbenes, depending on specific substituents in the two phenyl rings. Ketone **1d** showed greatly reduced activity in all of the tests. In evaluation of ring substituent effects on activity, the bibenzyl configuration is probably more sensitive than the *cis*-stilbene configuration, because of the high activity of the latter. For example, bibenzyl **1b** is only about 50% less active than *cis*-stilbene **2a**, and the reduced form of combretastatin A-1 is similarly about 50% less active than the *cis*-olefin counterpart (**2e**).[12] [Although less potent than stilbene **2a**, bibenzyl **1b** (PS ED_{50} 3.6 × 10^{-2} μg/mL) displayed significant cell growth inhibitory activity.] In contrast, removal of the C-2′ oxygen has a minimal effect on the activity in the *cis*-stilbene configuration but leads to a greater than 2-fold loss of activity in the bibenzyl.[18] Even more striking, the *cis*-stilbenes with a 3,3′-dihydroxy-3,4,5′-trimethoxy and a 3,4,5,3′,4′-pentamethoxy substituent pattern are only 50% less active than combretastatin A-4 (**2a**), but the bibenzyl analogs have negligible effects on tubulin polymerization.[12]

No modification in the substituent pattern in the A ring has yet improved on the cytotoxic, tubulin-inhibiting, or colchicine-binding activity of combretastatin A-4 (**2a**), especially when the cognate bibenzyls are also studied. A 3,4-methylenedioxy bridge, instead of methoxy groups at these positions, yields active compounds combretastatin A-2 (**6b**), compound **6a**, and the bibenzyl equivalent of **6a**.[12] Phenol **6a**, with a 2′-hydroxy group, showed greater tubulin inhibition and colchicine (**7**) binding but less cytotoxicity than the natural combretastatin A-2 (**6b**). However, this stilbene was found to be unstable both as a solid and in solution. Similar high activity but ready oxidation was noted for combretastatin A-1 (**2e**)[2] which is the 2′-hydroxy derivative of combretastatin A-4 (**2a**). Interestingly, biosynthetic processes in *Combretum kraussii* have circumvented this potential problem by elaborating 2′-(β-D-glucopyranosyloxy)combretastatin A-1 along with diphenol **2e**.[19] Neither has any modification in the B ring yet improved on the activity of **2a** and related compounds in all series. An additional hydroxy group at C-2′ seemed to have little effect on the activity of the *cis*-stilbene **2e** or the bibenzyl **1b**, but this addition to the 3,4-methylenedioxy compound **6b** did increase inhibitory effects on tubulin polymerization.[12] The instability of this group of compounds made it difficult to quantify accurately their inhibitory effects. A methyl group at C-4′ instead of a methoxy group (in the series lacking a C-3′ hydroxyl) enhanced activity of the *cis*-stilbene but reduced activity of the bibenzyl.[18]

Table 3. Results of Comparative Antitumor Evaluations of Combretastatins in the NCI *in vitro* Primary Screen[a]

combretastatin nos.	mean panel GI_{50} ($\times 10^{-8}$ M)[b]	*Compare* correlation coefficient[c]
A-1 (**2e**)	1.62	0.80
A-2 (**6b**)	3.16	0.89
A-4 (**2a**)	0.32	1.00
A-5 (**2c**)	165.00	0.84
A-6 (**3a**)	>10 000	<0.5

[a] All compounds were tested in at least quadruplicate at seven different concentrations (10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10}, and 10^{-11} M) against the entire panel of 60 human tumor cell lines composing the NCI screen. [b] Standard errors averaged less than 10−15% of the respective means. [c] Correlation coefficients from the *Compare* pattern-recognition algorithm were calculated using the GI_{50}-centered mean graph profiles of differential cellular sensitivities to the combretastatins. The GI_{50} mean graph profile (see the Experimental Section) of **2a** was used as the benchmark or "seed" for all of the comparisons.[22]

A number of the combretastatins were further evaluated comparatively in the U.S. National Cancer Institute's human tumor cancer cell line panel.[20-22] Consistent with the other testing results, combretastatin A-4 (**2a**) was also the most potent of the series in the NCI screen (Table 3). Combretastatins A-1 (**2e**) and A-2 (**6b**) respectively were about 1/5th and 1/10th as potent as the benchmark A-4 (**2a**). The A-5 (**2c**) was about 1/500th as potent as **2a**, and A-6 (**3a**) was essentially inactive at the concentration tested.

Compare analyses[22,23] of the differential cellular response profile to combretastatin A-4 (**2a**) revealed only a relatively modest correlation (e.g., >0.6−0.7) to known tubulin-interactive, antimitotic standard agents (e.g., vinblastine, vincristine, rhizoxin).[20-23] On the other hand, within the series of combretastatins tested, the screening profiles of A-1, A-2, A-3, and A-5 all were strongly correlated with that of combretastatin A-4 (**2a**; Table 3). The results are consistent with a view that the combretastatins represent a distinct subset among the general class of tubulin-interactive cytotoxins.

Combretastatins A-4, A-5, and A-6 were evaluated for antibacterial and antifungal activity. The minimum inhibitory concentrations of combretastatins A-4 and A-5 for *Neisseria gonorrhoeae* were between 25 and 50 μg/mL. The minimum inhibitory concentration of combretastatin A-6 for *N. gonorrhoeae* was between 50 and 100 μg/mL. These results are important given the increasing prevalence of antibiotic-resistant strains of *N. gonorrhoeae* worldwide. At up to 100 μg/disk, these compounds exhibited no antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans*, or *Cryptococcus neoformans*.

Overall, combretastatins A-1,[2] A-4, and A-2 were found most promising and are being further investigated. The effectiveness of these compounds as antimitotic agents appears to derive primarily from the rapidity of their binding to tubulin and at the colchicine site. A new model for the binding site has been proposed as a result of the combretastatin studies.[14]

Experimental Section

For the general experimental procedures, plant taxonomy, extraction, and solvent partition procedures, see refs 2−4. Ether refers to diethyl ether, and solvent extracts of aqueous solutions were dried over anhydrous sodium sulfate.

Isolation of Combretastatins A-4 (2a), A-5 (2c), and A-6 (3a). The P388 lymphocytic leukemia cell line (PS) active fraction (30.6 g, fraction B[2,3]) obtained by partition chromatography (Sephadex LH-20) of the dichloromethane fraction from the earlier solvent partitioning sequence was further separated on a column of Sephadex LH-20 (eluant: 3:1:1 hexane–toluene–methanol). The greater portion (2.2 g) of the active fraction (2.4 g; PS ED_{50} 0.10 μg/mL) eluted prior to that containing combretastatins A-3 and B-2[3] was subjected to silica gel chromatography (eluant: 3:1 hexane–ethyl acetate) to yield 4′-hydroxy-3,5-dimethoxybibenzyl, 2-hydroxy-3,4,6,7-tetramethoxy-9,10-dihydrophenanthrene, and a very active fraction (0.56 g; PS ED_{50} 4.0 × 10^{-2} μg/mL). The 0.56-g active fraction was rechromatographed on silica gel (eluant: 4:1 hexane–ethyl acetate), and the PS activity was concentrated in two fractions. The more polar (and more active; PS ED_{50} 3.4 × 10^{-2}) fraction, following several PLC and HPLC (Partisil M-9) separations in hexane–2-propanol (9:1), provided an apparently homogeneous fraction (26.4 mg). However, ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) analyses revealed it to be a mixture of at least three closely related stilbenes. A portion (10 mg) was hydrogenated in the presence of 5% Pd/C (10 mg) in methanol for 48 h, but the resultant two-component (^{1}H-NMR) mixture resisted resolution. Successful separation was finally achieved upon silylation of this olefin mixture as described below.

Silylation of Combretastatins A-4, A-5, and A-6. To a solution of the preceding stilbene fraction (7 mg) in dimethylformamide (2 mL) was added diisopropylethylamine (1 mL). Before the addition of *tert*-butyldimethylsilyl chloride (100 mg) the mixture was stirred under argon for 10 min. After 1 h, ice (1 g) was added, followed by a saturated solution of sodium bicarbonate (5 mL). The mixture was extracted with ether (2 × 10 mL), and the ethereal solution was washed with a saturated sodium bicarbonate solution (10 mL) and water (10 mL). Removal of solvent *in vacuo* yielded an oily three-component mixture [R_f 0.37, 0.32, and 0.24; hexane–ethyl acetate (4:1)]. Multiple development PLC (17:3 hexane–ethyl acetate) afforded from the least polar component 3′-[(*tert*-butyldimethylsilyl)oxy]combretastatin A-4 (**2b**; 3.0 mg) as an oil: IR (NaCl) ν_{max}^{neat} 2955, 2931, 1580, 1508, 1464, 1281, 1249, 1236, 1128, 841 cm^{-1}; for the ^{1}H-NMR data see Table 1; HREIMS *m/z* (peak height) 430.2168 (26%, M^+, calcd for $C_{24}H_{34}O_5Si$: 430.2175), 374.1511 (5%), 358.1238 (100%). Removal of the next component [R_f 0.32; hexane–ethyl acetate (4:1)] led to 3′-[(*tert*-butyldimethylsilyl)oxy]combretastatin A-5 (**2d**; 2.0 mg) as an oil: IR (NaCl) ν_{max}^{neat} 2950, 2930, 1574, 1513, 1500, 1468, 1224, 1257, 1251, 1237, 1115, 838 cm^{-1}; the ^{1}H-NMR results appear in Table 1; HREIMS *m/z* (peak height) 430.2164 (14%, M^+, calcd for $C_{24}H_{34}O_5Si$: 430.2175), 373.1472 (8.5%), 358.1221 (100%). The most polar component [R_f 0.24; hexane–ethyl acetate (4:1)] was developed in acetone to afford 3′-[(*tert*-butyldimethylsilyl)oxy]combretastatin A-6 (**3b**; 1.5 mg) as flakes from ethanol: mp 116–117 °C; IR (NaCl) ν_{max}^{neat} 2950, 2932, 1584, 1513, 1502, 1464, 1524, 1264, 1251, 1116, 835 cm^{-1}; refer to Table 1 for the ^{1}H-NMR assignments; HREIMS *m/z* (peak height) 430.2183 (14%, M^+, calcd for $C_{24}H_{34}O_5Si$: 430.2175), 373.1460 (8%), 358.1209 (100%).

3′-[(*tert*-Butyldimethylsilyl)oxy]-3,4,4′,5-tetramethoxy-(*Z*)- and -(*E*)-stilbene: 3′-[(*tert*-Butyldimethylsilyl)oxy]-combretastatin A-4 (2b) and the *trans*-Isomer (3d). Method A. To a cooled (−20 °C) solution of phosphonium bromide **4a** (11.9 g, 20 mmol)[9,13] in tetrahydrofuran (300 mL) was added butyllithium (13.3 mL, 20 mmol) under argon. Before the addition of 3,4,5-trimethoxybenzaldehyde (**5a**; 3.14 g, 16 mmol), the red solution thus formed was stirred at room temperature for 20 min. The red color was discharged and the reaction complete in 10 min. The mixture was distributed between ice–water (100 mL) and ether (300 mL). The organic phase was washed with cold water (2 × 100 mL) and dried. Removal of the solvent *in vacuo* yielded an oil which was subjected to flash column chromatography (eluant: 9:1 hexane–ethyl acetate) to afford the *Z*-isomer (**2b**; 2.2 g) as an oil identical (TLC, IR, ^{1}H-NMR, HREIMS) to the corresponding derivative of the natural product. Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.95; H, 7.96. Found: C, 67.39; H, 8.22.

Continued elution yielded a mixture of *Z*- and *E*-isomers (**2b** and **3d**; 1.23 g) followed by *E*-isomer **3d** (2.98 g, total yield 93%, ratio *Z/E* 1:1.5). The *E*-isomer (**3d**) recrystallized from ethanol as rods: mp 128–130 °C; IR (NaCl) ν_{max}^{neat} 2955, 2931, 2856, 1582, 1509, 1464, 1424, 1273, 1251, 1129 cm^{-1}; see Table 1 for the ^{1}H-NMR data; HREIMS *m/z* (peak height) 430.2168 (22%, M^+, calcd for $C_{24}H_{34}O_5Si$: 430.2175), 373.1469 (20%), 358.1235 (79%). Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.95; H, 7.96. Found: C, 67.17; H, 8.20.

Method B. A homogeneous suspension of phosphonium bromide **5c** (15.1 g)[2] in tetrahydrofuran (900 mL) under argon was cooled to −23 °C and retained at that temperature for 2 h. Butyllithium (18.6 mL) was added dropwise, the resultant red solution was stirred at −23 °C for 60 min, and 3-[(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzaldehyde (**4c**; 7.68 g) was added (dropwise).[15] Stirring was continued at −23 °C for 4 h and at room temperature for 14 h. The red color was discharged as the reaction proceeded. Ice–water (300 mL) was added to the mixture and two phases separated. The aqueous phase was washed with ether (3 × 300 mL), and the ethereal solution was added to the tetrahydrofuran layer from the reaction mixture. The combined organic phase was washed with water (3 × 300 mL) and dried. Repetition (three times) of the reaction under the same conditions and solvent volume was carried out with the following amounts of reagents: **5c**, 20.0, 38.9, and 36.7 g; **4c**, 10.2, 19.7, and 18.7 g; butyllithium, 24.5, 47.8, and 45.2 mL, respectively. The resulting organic phase solutions were combined. Removal of solvent *in vacuo* yielded a crude residue (100 g) which was subjected to flash chromatography in four aliquots [silica gel; eluant: hexane–ethyl acetate (95:5) for each column] to afford isomeric stilbenes **2b** (46.8 g) and **3b** (12.9 g).

Photochemical Isomerization of (*E*)-Stilbene 3d to Combretastatin A-4 Silyl Ether (2b). A solution of silyl ether **3d** (12.9 g) in ethanol (2 L) was irradiated at 254 nm (until TLC showed the appearance of an unwanted product). Removal of solvent followed by flash column chromatography (as above) afforded *cis*-isomer **2b** (8.2 g).

Combretastatin A-4 (2a). A solution of silyl ether **2b** (1.7 g, 3.9 mmol) in tetrahydrofuran (40 mL stirred under argon) was treated with tetrabutylammonium fluoride (4.0 mL, 4.0 mmol). Stirring was continued for 20 min, and then ice (10 g) was added followed by ether (100 mL). The ethereal layer was washed with water (3 × 40 mL) and dried. Removal of solvent *in vacuo* followed by filtration through a short column of silica gel (eluant: 3:2 hexane–ethyl acetate) yielded *cis*-stilbene **2a** as a viscous oil (1.15 g, 93%) which crystallized from ethyl acetate–hexane in fine granules: mp 116 °C; IR (NaCl) ν_{max}^{neat} 3395, 1580, 1508, 1462, 1456, 1420, 1274, 1237, 1221, 773 cm^{-1}. The ^{1}H- and ^{13}C-NMR assignments have been entered in Tables 1 and 2. Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.35; H, 6.37. Found: C, 68.53; H, 6.47.

The reaction sequence was repeated a number of times on smaller (0.63 g of silyl ether → 0.3 g of A-4; 65% yield) and larger (54 g of silyl ether → 33.4 g of A-4; 84% yield) scales.

3′-Hydroxy-3,4,4′,5-tetramethoxy-(*E*)-stilbene (3c). Silyl ether **3d** (2.8 g, 6.6 mmol) was cleaved as above (see **2a**) to give *trans*-phenol **3c** (1.9 g, 92%) as an amorphous solid; mp 103–4 °C; IR (NaCl) ν_{max}^{neat} 3422, 1585, 1509, 1463, 1454, 1418, 1333, 1277, 1261, 1253, 1128 cm^{-1}; refer to Tables 1 and 2 for the NMR data. Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.35; H, 6.37. Found: C, 68.10; H, 6.83.

3-[(*tert*-Butyldimethylsilyl)oxy]-3′,4,4′,5-tetramethoxy-(*Z*)- and -(*E*)-stilbene: 3-[(*tert*-Butyldimethylsilyl)oxy]-combretastatins A-5 (2d) and A-6 (3b). To a cooled (−20 °C) solution of phosphonium bromide **4b** (1.5 g, 2.4 mmol)[3] in tetrahydrofuran (60 mL) was added butyllithium (1.6 mL, 2.4 mmol under argon). Before the addition of 3,4-dimethoxybenzaldehyde (**5b**; 0.33 g, 1.98 mmol), the red solution was stirred at room temperature for 30 min. The red color was discharged and the reaction complete in 10 min. The mixture was distributed between ice–water (15 mL) and ether (100 mL), and the organic phase was washed with cold water (2 × 50 mL). Removal of solvent *in vacuo* yielded an oil which was subjected to flash column chromatography on silica gel [eluant: hexane–ethyl acetate (19:1 → 4:1)] to afford the *Z*-isomer

(**2d**; 0.295 g) as an oil identical (TLC, IR, ^{1}H-NMR, HREIMS) to the corresponding derivative of the natural product (combretastatin A-5, **2c**). Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.95; H, 7.96. Found: C, 67.17; H, 8.20.

Continued elution of the column yielded a mixture of the *Z*- and *E*-isomers (0.10 g) followed by the pure *E*-isomer (**3b**; 0.28 g, total yield 78%, $Z/E \approx 1:1$) identical (TLC, IR, ^{1}H-NMR, HREIMS) to the natural product (combretastatin A-6, **3a**) silyl ether. Anal. Calcd for $C_{24}H_{34}O_5Si$: C, 66.95; H, 7.96. Found: C, 67.34; H, 8.10.

Combretastatin A-5 (2c). Cleavage of silyl ether **2d** (0.17 g, 0.39 mmol) as above (see **2a**) and filtration through silica gel afforded combretastatin A-5 (**2c**) as an oil (0.12 g, 98%): IR (NaCl) ν_{max}^{neat} 3424, 1601, 1583, 1512, 1463, 1429, 1268, 1259, 1237, 1140, 1104 cm^{-1}; for ^{1}H- and ^{13}C-NMR data, seeTables 1 and 2. Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.35; H, 6.37. Found: C, 68.25; H, 6.45.

Combretastatin A-6 (3a). Desilylation of ether **3b** (168 mg, 0.39 mmol) as above (refer to **2a**) afforded combretastatin A-6 (**3a**) which crystallized from acetone–hexane as granules; mp 122–124 °C; IR (NaCl) ν_{max}^{neat} 3419, 1587, 1512, 1464, 1428, 1358, 1264, 1252, 1160, 1139, 1104 cm^{-1}; refer to Tables 1 and 2 for ^{1}H- and ^{13}C-NMR data. Anal. Calcd for $C_{18}H_{20}O_5$: C, 68.3; H, 6.37. Found: C, 68.29; H, 6.33.

2′,3′-Bis[(*tert*-butyldimethylsilyl)oxy]-3,4-(methylenedioxy)-4′,5-dimethoxy-(*Z*)- and -(*E*)-stilbene (6c and *trans*-Isomer). A solution of phosphorus tribromide (1.3 mL, 28 mmol) in dichloromethane (40 mL) was added (dropwise) to a cool (−10 °C) solution of 3,4-methylenedioxy-5-methoxybenzyl alcohol[3] (**9b**; 5.1 g, 28 mmol) under argon. The mixture was stirred for 10 min and the bromination terminated by addition of an ice-cold sodium bicarbonate solution (10%, 20 mL). The organic layer was separated and washed with cold water (2 × 30 mL). Removal (*in vacuo*) of solvent yielded the crude benzyl bromide (6.8 g, 99%) which crystallized from dichloromethane as needles: mp 99–100 °C.

To a solution of the benzyl bromide (6.3 g, 25.7 mmol) in toluene (25 mL) was added a solution of triphenylphosphine (6.73 g, 25.7 mmol) in the same solvent (25 mL), and the mixture was stirred under anhydrous conditions for 48 h at room temperature. The precipitate was collected by filtration and dried to afford phosphonium bromide **9a** as an amorphous powder (11.3 g, 87%): mp 223–226 °C; IR (NaCl) ν_{max}^{neat} 2905, 1636, 1510, 1452, 1438, 1132, 1111, 1093, 748, 690 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.82–7.74 and 7.65–7.60 (15 H, m, aromatic), 6.64 (1 H, dd, *J* = 2.2 Hz), 6.22 (1 H, t, *J* = 2 Hz), 5.87 (2 H, s, $ArCH_2$), 5.43 (2 H, d, *J* = 14 Hz, CH_2), 3.57 (3 H, s, OCH_3).

Butyllithium (7.33 mL, 11.0 mmol) was added to a stirred and cooled (−10 °C) suspension of (3,4-(methylenedioxy)-5-methoxybenzyl)triphenylphosphonium bromide (**9a**; 5.5 g, 11.0 mmol) in tetrahydrofuran (300 mL). The red solution was stirred under argon for 20 min, and 2,3-bis[(*tert*-butyldimethylsilyl)oxy]-4-methoxybenzaldehyde[2] (**4d**; 3.564 g, 9 mmol) was added in one portion. Stirring was continued for 30 min, and the color was discharged. Ice–water (150 mL) was added and the mixture extracted with ether (3 × 150 mL). The ethereal solution was washed with water (150 mL) and concentrated to an oil which was chromatographed on a column of silica gel [200 g; eluant: hexane–ethyl acetate (19:1)] to give *cis*-stilbene **6c** and its *trans*-isomer (*Z*/*E* 3:1, 4.7 g, 96%). *cis*-Silyl ether **6c** crystallized from ethanol: mp 87.5–90.5 °C; ^{1}H-NMR ($CDCl_3$) δ 6.680 (1 H, d, *J* = 8.4 Hz, H-6′), 6.574 (1 H, br s), 6.525 (1 H, d, *J* = 12.2 Hz, *cis*-H), 6.361 (1 H, d, *J* = 8.4 Hz, H-5′), 6.325 (1 H, d, *J* = 12.2 Hz, *cis*-H), 5.923 (2 H, s, OCH_2O), 3.741 (3 H, s, OCH_3), 3.715 (3 H, s, OCH_3), 1.022 (9 H, s, $C(CH_3)_3$), 0.993 (9 H, s, $C(CH_3)_3$), 0.171 (6 H, s, 2 × CH_3), 0.111 (6 H, s, 2 × CH_3). Anal. Calcd for $C_{29}H_{44}O_6Si_2$: C, 63.93; H, 8.14. Found: C, 64.08; H, 8.25.

The higher melting *trans*-isomer **2′,3′-bis[(*tert*-butyldimethylsilyl)oxy]-3,4-(methylenedioxy)-4′,5-dimethoxy-(*E*)-stilbene** also recrystallized easily from ethanol; mp 139–140 °C; IR (NaCl) ν_{max}^{neat} 2954, 2929, 1508, 1495, 1462, 1452, 1307, 1101, 835 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.220 (1 H, d, *J* = 16.4 Hz, *trans*-H), 7.161 (1 H, d, *J* = 8.6 Hz, H-6′), 6.761 (1 H, *J* = 16.4 Hz, *trans*-H), 6.674 (2 H, ABq, *J* = 2.0 Hz, H-2, H-6), 6.543 (1 H, d, *J* = 8.6 Hz, H-5′), 5.974 (2 H, s, OCH_2O), 3.914 (3 H, s, OCH_3), 3.786 (3 H, s, OCH_3), 1.077 (9 H, s, $C(CH_3)_3$), 0.996 (9 H, s, $C(CH_3)_3$), 0.130 (6 H, s, 2 × CH_3), 0.112 (6 H, s, 2 × CH_3). Anal. Calcd for $C_{29}H_{44}O_6Si_2$: C, 63.93; H, 8.14. Found: C, 63.86; H, 8.23.

2′,3′-Dihydroxy-3,4-(methylenedioxy)-4′,5-dimethoxy-(*Z*)-stilbene (6a). To a stirred solution of silyloxy-(*Z*)-stilbene **6c** (1.51 g, 2.77 mmol) in tetrahydrofuran (50 mL) was added a 1 M tetrahydrofuran solution of tetrabutylammonium fluoride (5.54 mL, 5.54 mmol) under argon. The mixture was stirred for 15 min, when the reaction was complete as monitored by TLC (3:2 hexane–ethyl acetate). Ether (100 mL) was added, and the ethereal solution was washed with water (2 × 50 mL) and dried. Removal of solvent *in vacuo* yielded a viscous oil which was passed through silica gel to afford *cis*-stilbene **6a** as a chromatographically homogeneous oil (0.8 g, 91%). Recrystallization from dichloromethane–hexane afforded prisms: mp 124–126 °C; IR (NaCl) ν_{max}^{neat} 3466, 1623, 1508, 1481, 1465, 1449, 1429, 1323, 1290, 1122, 1089 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.730 (1 H, d, *J* = 8.4 Hz, H-6′), 6.535 (1 H, d, *J* = 12.4 Hz, *cis*-H), 6.500 (1 H, d, *J* = 12.4 Hz, *cis*-H), 6.483 (1 H, d, *J* = 1.2 Hz), 6.465 (1 H, d, *J* = 1.2 Hz), 6.381 (1 H, d, *J* = 8.4 Hz, H-5′), 5.925 (2 H, OCH_2O), 5.375 (2 H, br s, 2 × OH), 3.866 (3 H, s, OCH_3), 3.724 (3 H, s, OCH_3). Anal. Calcd for $C_{17}H_{16}O_6$: C, 64.56; H, 5.10. Found: C, 64.14; H, 5.02.

1-(3″-Hydroxy-4″-methoxyphenyl)-2-(3′,4′,5′-tetramethoxyphenyl)ethane (1b). A mixture of combretastatin A-4 (**2a**; 0.20 g) in ethyl acetate–methanol (3:1, 15 mL) and 10% palladium-on-carbon (120 mg) was treated with a positive pressure of hydroen at ambient temperature (overnight). The catalyst was removed by filtration of the solvent through Celite. Purification of the residue by elution through a small silica gel column (eluant: 3:2 hexane–ethyl acetate) yielded dihydrostilbene **1b** as a viscous oil (0.19 g, 94.4%): IR (NaCl) ν_{max}^{neat} 3420, 1590, 1509, 1457, 1421, 1274, 1259, 1239, 1150, 1127, 1009 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 6.80 (1 H, d, *J* = 2 Hz, H-2″), 6.75 (1 H, d, *J* = 8 Hz, H-5″), 6.65 (1 H, dd, *J* = 2, 8 Hz, H-6″), 6.38 (2 H, s, H-2′, H-6′), 5.63 (1 H, br s, OH), 3.86 (3 H, s, OCH_3), 3.82 (9 H, s, 3 × OCH_3), 2.82 (2 H, s, CH_2). Anal. Calcd for $C_{18}H_{22}O_5$: C, 67.91; H, 6.97. Found: C, 67.42; H, 7.08.

1-Oxo-1-(3″-hydroxy-4″-methoxyphenyl)-2-(3′,4′,5′-trimethoxyphenyl)ethane (1d). A solution of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (0.91 g, 4 mmol) in dioxane was added to a solution of isocombretastatin A[10] (**1c**; 1.34 g, 4 mmol) in dioxane (20 mL). The solution was stirred at room temperature for 5.5 h. The hydroquinone side product which precipitated was removed by filtration, and the filtrate was concentrated to dryness. Purification of the residue on a silica gel column (eluant: 7:3 hexane–ethyl acetate) gave ketone **1d** (1.2 g, 88%) which crystallized from acetone–hexane in plates melting at 122–124 °C: IR (NaCl) ν_{max}^{neat} 3400, 1608, 1590, 1509, 1457, 1425, 1317, 1276, 1242, 1124, 1016 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 7.58 (2 H, m, H-2″, H-6″), 6.89 (1 H, d, *J* = 9 Hz, H-5″), 6.47 (2 H, s, H-2′, H-6′), 5.69 (1 H, s, OH), 4.15 (2 H, s, CH_2CO), 3.96 (3 H, s, OCH_3), 3.83 (6 H, s, 2 × OCH_3), 3.82 (3 H, s, OCH_3). Anal. Calcd for $C_{18}H_{20}O_6$: C, 65.11; H, 6.07. Found: C, 65.29, H, 5.95.

Screening Data Summary. Compounds were tested in the NCI screen as described.[20] The negative log GI_{50} values[22] are listed as follows for the benchmark compound (**2a**) with the individual cell line identifiers; the tumor type subpanel identifiers are as follows: I (leukemia); II (non-small cell lung); III (colon); IV (brain); V (melanoma); VI (ovary); VII (kidney); VIII (prostate); IX (breast). [I] CCRF-CEM (9.37), HL60TB (9.74), K-562 (9.64), MOLT-4 (8.80), RPMI-8226 (9.39), SR (9.46); [II] A549/ATCC (7.44), EKVX (6.09), HOP-62 (8.85), HOP-92 (9.31), NCI-H226 (8.51), NCI-H23 (7.85), NCI-H322M (9.23), NCI-H460 (8.96), NCI-H522 (8.85); [III] COLO 205 (6.00), HCC-2998 (6.82), HCT-116 (9.62), HCT-15 (8.64), HT-29 (6.03), KM12 (8.60), SW-620 (9.42); [IV] SF-268 (8.51), SF-295 (8.85), SF-539 (9.33), SNB-19 (8.82), SNB-75 (9.17), U251 (8.72); [V] LOX IMVI (9.23), MALME-3M (8.96), M14 (9.3), SK-MEL-2 (9.32), SK-MEL-28 (9.27), SK-MEL-5 (9.15), UACC-257 (7.66), UACC-62 (9.33); [VI] IGROV1 (8.10), OVCAR-3 (9.15), OVCAR-4 (7.00), OVCAR-5 (6.60), OVCAR-8 (8.80), SK-

OV-3 (7.43); [VII] 786-0 (8.06), A498 (7.89), ACHN (8.62), CAKI-1 (7.62), RXF-393 (7.82), SN12C (8.52), TK-10 (6.52), UO-31 (8.62); [VIII] PC-3 (9.25), DU-145 (8.70); [IX] MCF7 (7.82), MCF7/ADR-RES (9.51), MDA MB-231/ATCC (8.66), HS 578T (8.28), MDA-MB-435 (9.80), MDA-N (9.92), BT-549 (8.49), T-47D (6.04).

Antimicrobial Susceptibility Testing. Antimicrobial disk susceptibility tests were performed according to the method established by the National Committee for Clinical Laboratory Standards.[24] Mueller–Hinton agar was used for susceptibility testing of *Staphylococcus aureus* (ATCC No. 29213), *Enterococcus faecalis* (ATCC No. 29212), and *Escherichia coli* (ATCC No. 25922), Gonococcal Typing agar for *Neisseria gonorrhoeae* (ATCC No. 49226), and YM agar for *Candida albicans* (ATCC No. 90028) and *Cryptococcus neoformans* (ATCC No. 90112). Combretastatins A-4, A-5, and A-6 were reconstructed in sterile DMSO, and 2-fold dilutions, applied to sterile 6 mm disks. Zones of inhibition were recorded after 16 h for bacterial cultures and 42 h for fungal cultures. Combretastatin A-4 and A-5 results are the average of two experiments. Due to a paucity of combretastatin A-6, susceptibility testing was performed a single time.

Acknowledgment. Special thanks and appreciation for support of this research are extended to Outstanding Investigator Grant CA 44344-01A1-06, Grant CA-16049-05-12, and Contract NO1-CM-97262 with the Division of Cancer Treatment, NCI, DHHS, the Arizona Disease Control Research Commission, Mrs. Eleanor W. Libby, The Donald Ware Waddell Foundation, Virginia Piper, Diane Cummings, Gary L. Tooker, and the Robert B. Dalton Endowment. Also, we are pleased to thank for other excellent assistance Drs. Ven Narayanan, Ravi Varma, and Matthew Suffness, Janice M. DeGraziano, Melanie Filiatrault, Pei-ling Cheng, Philip Daschner, the National Science Foundation for equipment Grant CHE-8409644, and the NSF Regional Instrumentation Facility in Nebraska (Grant CHE-8620177).

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JM9407769