Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure−Activity Relationships

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A series of combretastatin A-4 (CA-4) analogues were synthesized, and their cytotoxic effects against murine Colon 26 adenocarcinoma and inhibitory activity on tubulin polymerization were evaluated. Since CA-4 has limited aqueous solubility, the target compounds were designed to improve solubility by introduction of a nitrogen-containing group. Among the compounds synthesized, those with an amino moiety in place of the phenolic OH of CA-4 showed potent antitubulin activity and cytotoxicity against murine Colon 26 adenocarcinoma in vitro. Some of the compounds which were potent in vitro were evaluated in the murine tumor model Colon 26 in vivo. Among these, **13bHCl**, **21aHCl**, and **21bHCl** showed significant antitumor activity in the animal model, while CA-4 was ineffective. **13bHCl** and **21aHCl** were further evaluated in two murine tumor models (Colon 38 and 3LL) and human xenografts HCT-15. These compounds showed potent antitumor activity comparable or superior to that of CDDP. The structure−activity relationships of this series of compounds are also discussed.

Introduction

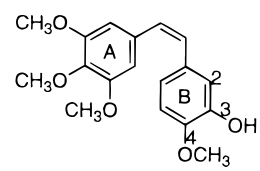
The microtubule system of eukaryotic cells is an important target for the development of anticancer agents. Chemicals which attack microtubules through tubulin disrupt cellular microtubule structure and function resulting in mitotic arrest. Examples of clinically used antimitotic agents are vincristine, which inhibits microtubule polymerization, and paclitaxel, which promotes microtubule assembly and inhibits microtubule disassembly. However, despite their potent antitumor activities, these drugs have undesirable side effects and are subject to multidrug resistance (MDR). Thus, it is essential to develop new anticancer agents with fewer side effects and activity against cancers not effectively treated by existing anticancer drugs.

Combretastatins are mitotic agents isolated from the bark of the South African tree *Combretum caffrum* (Chart 1).[1−3] The most potent of these is combretastatin A-4 (CA-4) (**i**), which has been found to be a potent cytotoxic agent and which strongly inhibits the polymerization of brain tubulin by binding to the colchicine site. CA-4 shows potent cytotoxicity against a wide variety of human cancer cell lines including MDR cancer cell lines.[4,5] Combretastatin A-4 (**i**) is thus attractive as a lead compound for development of anticancer drugs.

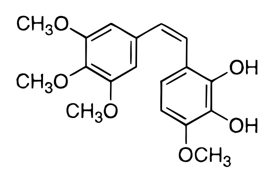
In our laboratory, however, the antitumor activity of combretastatin A-4 against murine Colon 26 adenocarinoma was evaluated in vivo, and it did not show any antineoplastic effects. Since CA-4 is highly lipophilic and has limited aqueous solubility, its lack of efficacy in vivo was considered to be due to its poor pharmacokinetics. A prodrug formulation and derivatization of CA-4 to improve its aqueous solubility have been reported,[6−12] but no studies have confirmed the antitumor activity of CA-4 or its derivatives in vivo.

To obtain compounds with pharmaceutically acceptable properties and improved antitumor activity, we introduced an oxygen or nitrogen-containing group into combretastatin A-4. A number of studies have been reported on structure−activity relationships of CA-4.[13−19] These studies showed that the cis orientation of the two benzene rings is essential and 3,4,5-trimethoxy substituents on the A-ring of CA-4 are indispensable for potent cytotoxicity.[17,18] Thus we mainly examined the substituents on the olefin site and the B-ring.

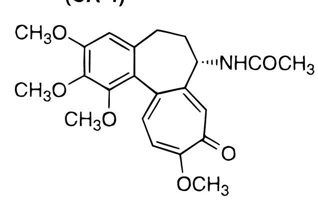
Chart 1



Combretastatin A-4 (i) (CA-4)



Combretastatin A-1 (ii)



Colchicine

In this paper, we describe the syntheses and biological activities of novel derivatives of combretastatin A-4 with improved solubility. The cytotoxic effects against the murine Colon 26 adenocarcinoma, inhibitory activity on tubulin polymerization, and antitumor activity in vivo of these compounds were evaluated.

Chemistry

The syntheses of **3** and **5**−**7** are shown in Scheme 1. Base-catalyzed condensation of 3,4,5-trimethoxybenzaldehyde with 4-methoxyphenylacetic acid (**1a**) in the

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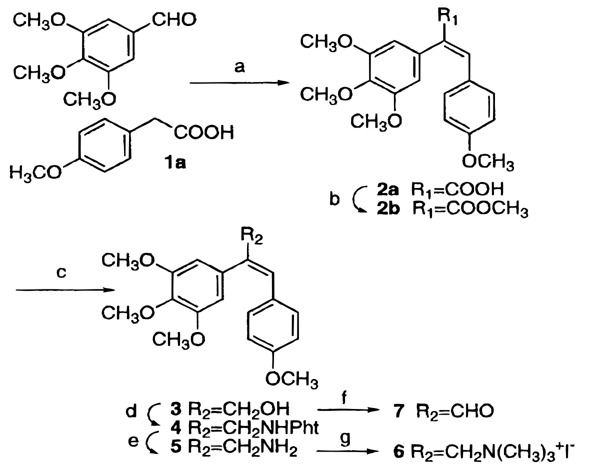
Scheme 1[a]



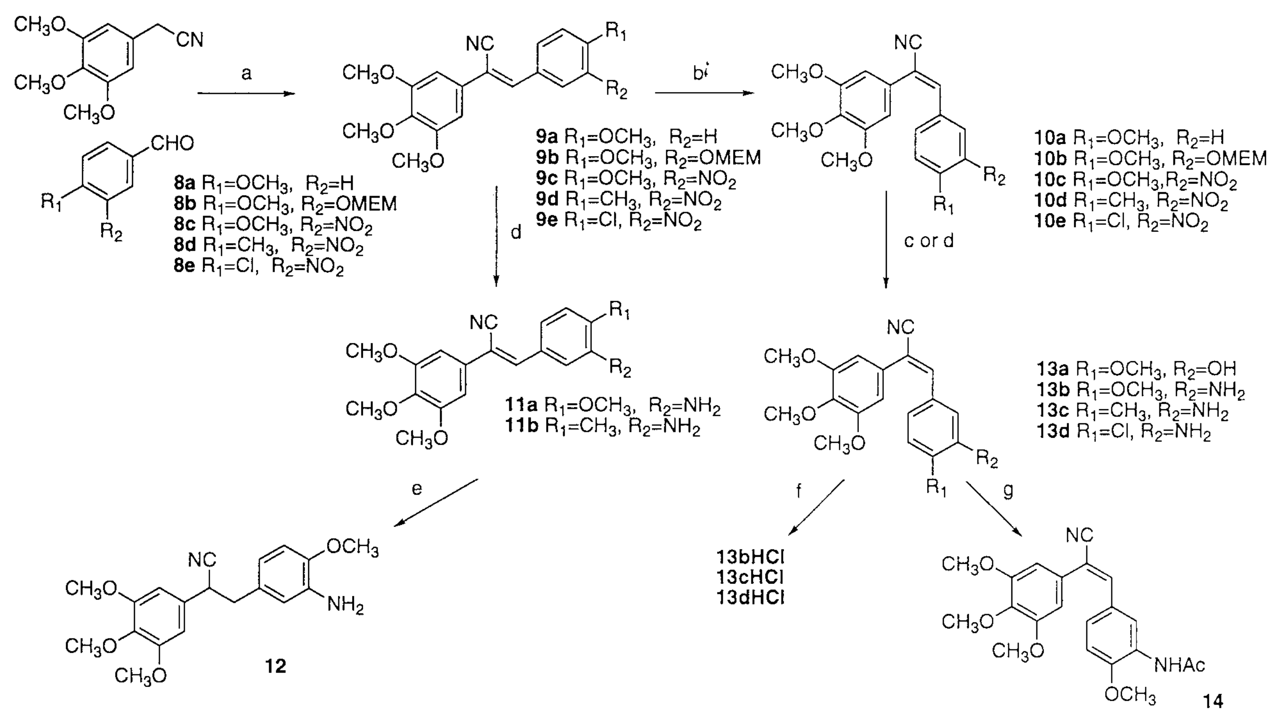
[a] Reagents: (a) (1) Ac_2O, Et_3O, 140 °C, (2) NaOH(aq); (b) MeI, K_2CO_3, DMF, rt; (c) $LiAlH_4$, THF, 0 °C; (d) phthalimide, diethyl azodicaboxylate, PPh_3, rt; (e) hydrazine, EtOH, reflux; (f) MnO_2, CH_2Cl_2, rt; (g) MeI, K_2CO_3, DMF, rt.

presence of triethylamine and acetic anhydride at 140 °C gave the carboxylic acid **2a**. In this reaction, only the *E*-form was obtained.[20] Reaction of **2a** with MeI at room temperature gave methyl ester **2b**. **2b** was then reacted with $LiAlH_4$ to give alcohol **3**. Mitsunobu reaction of **3** with phthalimide gave **4**. **4** was deprotected by hydrazine to give the aminomethyl compound **5**. Reaction of **5** with MeI gave trimethylamino iodide salt **6**. Oxidation of the alcohol **3** with MnO_2 gave ketone **7**.

The syntheses of **9a,c**, **10a**, **11a**,**b**, **12**, **13a−d**, **14**, and **13b−dHCl** are shown in Scheme 2. Base-catalyzed condensation of 3,4,5-trimethoxyphenylacetonitrile with benzaldehyde **8a−e** in the presence of methyltrioctylammonium chloride and NaOH at room temperature gave (*Z*)-acrylonitriles **9a−e** in good yields.[21] Since the active form of combretastatins was reported to be the cis form, we attempted to isomerize the (*Z*)-acrylonitriles to the corresponding (*E*)-acrylonitriles in which two benzene rings were located in the cis orientation. The (*Z*)-acrylonitriles **9a−e** were dissolved in CH_3CN at high dilution (10 mmol/L) and irradiated for 30 min. This reaction gave a 1:1 mixture of *E*- and *Z*-isomers. Prolonged reaction time resulted in a decrease in yield due to formation of byproducts. *E*- and *Z*-isomers were separated easily by crystallization from EtOAc/hexane. *E*- or *Z*-geometries of these compounds were determined by comparison of the chemical shifts of B-ring protons adjacent to the olefin site (protons on the B-ring of *Z*-form derivatives resonate 0.5−1.0 ppm downfield from the corresponding protons of the *E*-form by the anisotropic effect of the nitrile group). **10b** was deprotected with HCl to give phenol **13a**. Nitro compounds (**9c**,**d**, **10c−e**) were reacted with Zn in acetic acid/CH_2Cl_2 to give anilino compounds (**11a**,**b**, **13b−d**) in good yields. The obtained anilino compounds were reacted with 4 N HCl−dioxane to give corresponding hydrochloride salts (**13b−dHCl**). Acetylation of aniline **13b** with Ac_2O gave acetamide **14**. (*Z*)-Acrylonitrile **11a** was hydrogenated on 10% Pd−C to give saturated compound **12**.

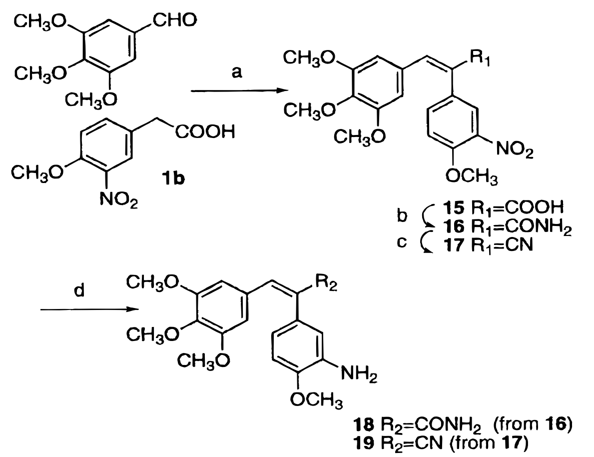
The syntheses of **18** and **19** are shown in Scheme 3. Condensation of 3,4,5-trimethoxybenzaldehyde with 4-methoxy-3-nitrophenylacetic acid (**1b**) in triethylamine and acetic anhydride at 140 °C for 12 h gave acrylic acid **15**. The acid **15** was converted to amide **16** by treatment with $SOCl_2$ followed by aqueous NH_3 treatment. The acrylamide **16** was reacted with $SOCl_2$ to give acrylonitrile **17**. Compounds **16** and **17** were reduced with Zn in acetic acid to give corresponding anilino compounds **18** and **19**, respectively.

Scheme 2[a]



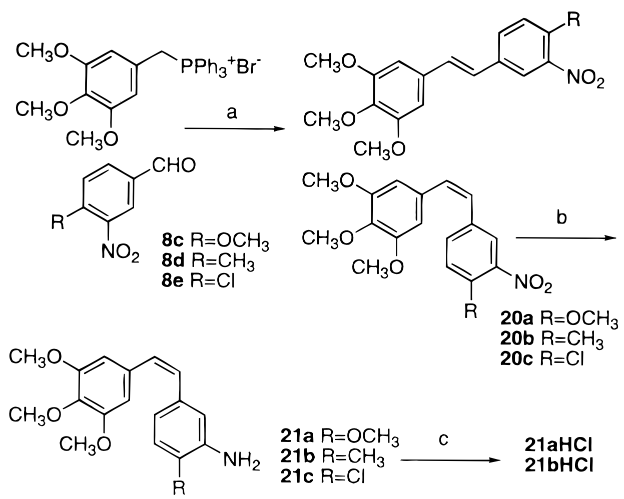
[a] Reagents: (a) NaOH, methyltrioctylammonium chloride, CH_2Cl_2, rt; (b) CH_3CN, irradiation; (c) HCl, AcOH, rt; (d) Zn, AcOH, rt; (e) H2, 10% Pd−C, MeOH, rt; (f) 4 N HCl−dioxane, rt; (g) Ac_2O, Py, rt.

Scheme 3[a]



[a] Reagents: (a) (1) Ac_2O, Et_3N, 140 °C, (2) NaOH(aq); (b) (1) $SOCl_2$, rt, (2) 28% aq NH_3, rt; (c) $SOCl_2$, Py, rt; (d) Zn, AcOH, rt.

Scheme 4[a]

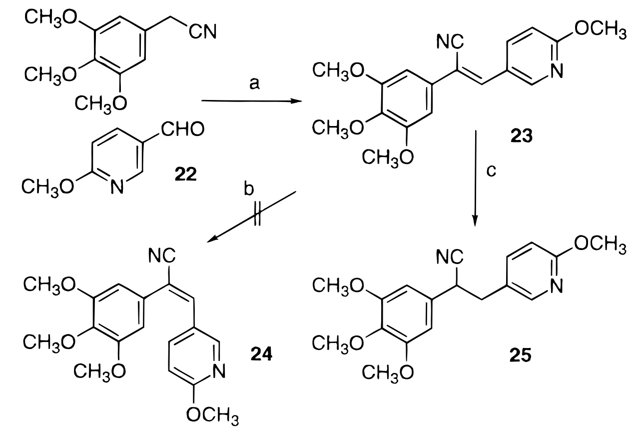


[a] Reagents: (a) NaH, toluene, rt; (b) Zn, AcOH; rt; (c) 4 N HCl–dioxane, CH_2Cl_2, rt.

Syntheses of **21a–c** and **21a,bHCl** are shown in Scheme 4. Wittig reaction of 3,4,5-trimethoxybenzylphosphonium bromide with nitrobenzaldehyde **8c–e** in the presence of sodium hydride in toluene gave a 1:1 mixture of (*E*)- and (*Z*)-stilbenes.[18] **20a** was separated from the mixture by crystallization (from EtOAc, then EtOH). **20b,c** were purified by silica gel column chromatography. The cis and trans geometries of the stilbenes were assigned by the characteristic ^1H NMR coupling constants of the olefinic protons (about 12 Hz for cis and 16 Hz for trans isomers).[22] The obtained (*Z*)-nitro compounds **20a–c** were reacted with Zn in acetic acid–CH_2Cl_2 at room temperature to give anilino compounds **21a–c**. The anilino compounds were reacted with 4 N HCl–dioxane to give hydrochloride salts **21a,bHCl**, respectively.

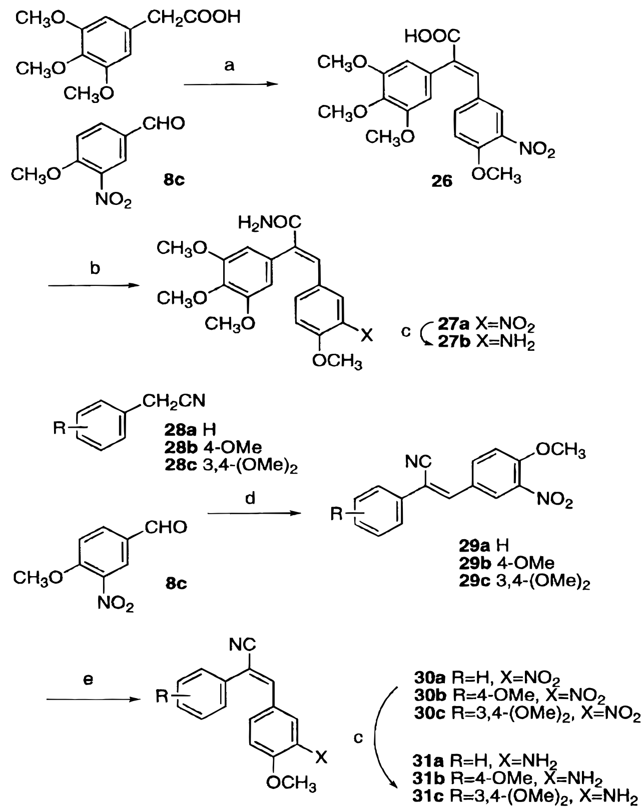
The syntheses of **23** and **25**, in which the B-ring was replaced with 2-methoxypyridine to mimic 3-amino-4-methoxybenzene, are shown in Scheme 5. Base-catalyzed condensation of 3,4,5-trimethoxyphenylacetonitrile and pyridylaldehyde **22** gave (*Z*)-acrylonitrile **23**. To obtain (*E*)-acrylonitrile, **23** was irradiated with visible light; however, this reaction did not give the desired (*E*)-acrylonitrile **24**, and only decomposed product was obtained. Reduction of **23** with 10% Pd–C gave saturated compound **25**.

Scheme 5[a]



[a] Reagents: (a) NaOH, methyltrioctylammonium chloride, CH_2Cl_2, rt; (b) CH_3CN, irradiation; (c) H2, 10% Pd–C, MeOH, rt.

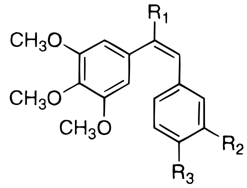
Scheme 6[a]



[a] Reagents: (a) Ac_2O, Et_3N, 140 °C; (b) (1) $SOCl_2$, rt, (2) 28% aq NH_3, rt; (c) Zn, AcOH, rt; (d) NaOH, methyltrioctylammonium chloride, CH_2Cl_2, rt; (e) CH_3CN, irradiation.

The syntheses of **27b** and **31a–c** are shown in Scheme 6. Condensation of 4-methoxy-3-nitrobenzaldehyde (**8c**) with 3,4,5-trimethoxyphenylacetic acid in triethylamine and acetic anhydride at 140 °C for 12 h gave acrylic acid **26**. The acrylic acid **26** was converted to acrylamide **27a** by $SOCl_2$ followed by aqueous NH_3 treatment. **27a** was reacted with Zn in acetic acid to give anilino compound **27b**. Condensation of phenylacetonitriles **28a–c** and 4-methoxy-3-nitrobenzaldehyde (**8c**) in the presence of NaOH gave (*Z*)-acrylonitriles **29a–c**. The acrylonitriles **29a–c** were isomerized by irradiation to give a 1:1 mixture of (*E*)- and (*Z*)-

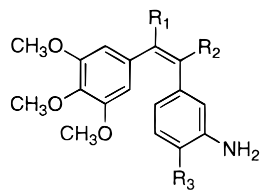
Table 1. Modification of the Olefin and B-Ring Substituents



compd	R_1	R_2	R_3	Colon 26[a] IC_{50} (nM)	antitubulin activity[b] IC_{50} (μM)
3	CH_2OH	H	OCH_3	363	>20
5	CH_2NH_2	H	OCH_3	>3000	>20
6	$CH_2N(CH_3)_4$	H	OCH_3	>3000	>20
7	CHO	H	OCH_3	213	4
10a	CN	H	OCH_3	18.0	6
10c	CN	NO_2	OCH_3	135	5
13a	CN	OH	OCH_3	23.5	5
13b	CN	NH_2	OCH_3	5.9	10
13c	CN	NH_2	CH_3	61.7	10
13d	CN	NH_2	Cl	58.1	6
14	CN	NHAc	OCH_3	18.3	10
combretastatin A-4				18.0	4

[a] Drug concentration required to inhibit the growth of Colon 26 cells by 50%. [b] Tubulin polymerization was determined as described in the text.

Table 2. Modification of the Olefin and B-Ring Substituents



compd	R_1	R_2	R_3	Colon 26[a] IC_{50} (nM)	antitubulin activity[b] IC_{50} (μM)
18	H	$CONH_2$	OCH_3	223	nt
19	H	CN	OCH_3	235	5
27b	$CONH_2$	H	OCH_3	195	3
21a	H	H	OCH_3	5.1	4
21b	H	H	CH_3	43.5	4
21c	H	H	Cl	68.9	3
13b	CN	H	OCH_3	5.9	10
combretastatin A-4				18.0	4

[a] Drug concentration required to inhibit the growth of Colon 26 cells by 50%. [b] Tubulin polymerization was determined as described in the text. nt, not tested.

acrylonitriles. (*E*)-Acrylonitriles **30a**−**c** were purified by silica gel column chromatography and reacted with Zn in acetic acid to give anilino compounds **31a**−**c**.

Results and Discussion

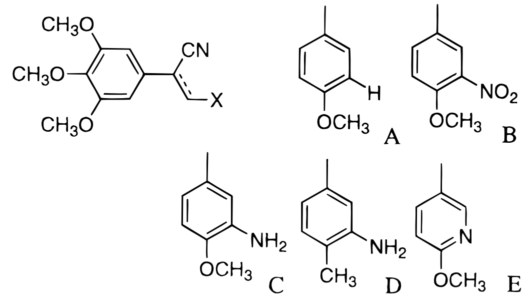
A series of 28 newly synthesized stilbene analogues were evaluated for their cytotoxic effects against murine Colon 26 adenocarcinoma and for tubulin polymerization inhibitory activity (Tables 1−4).

The Purdue group has studied structure−activity relationships of combretastatin A-4 (**i**) extensively.[17,18] They synthesized a large number of A-ring-substituted analogues and found that a 3,4,5-trimethoxy group on the A-ring was essential for strong cytotoxicity and antimitotic activity. They introduced several ester groups on the olefin site, but these alterations resulted in complete loss of antitubulin activity or significant decrease of cytotoxic activity. They examined 4-substituents of the B-ring of CA-4, but other positions of the B-ring have not been examined in detail. Therefore, we examined smaller substituents on the olefin site and substituents on the B-ring other than at the 4-position.

First, substituents were introduced into the olefin site adjacent to the A-ring (Table 1). The Purdue group reported that the 3-hydroxyl group on the B-ring of CA-4 is not necessary for potent activity.[17] Therefore, we used a 4-methoxyphenyl group as the B-ring for ease of preparation (**3**, **5**−**7**, **10a**). Introduction of a hydroxymethyl group (**3**) resulted in loss of antimitotic activity (IC_{50} > 20 μM) and a 20-fold decrease in cytotoxicity compared to that of CA-4. Compounds **5** and **6** diminished antimitotic and cytotoxic activities. These functional groups were bulky, so next we introduced smaller groups into the olefin site (**7**, **10a**). Aldehyde **7** retained antimitotic activity but showed a 10-fold decreased cytotoxicity. Nitrile **10a** showed slightly decreased antimitotic activity (IC_{50} 6 μM) but strong cytotoxicity (IC_{50} 18.0 nM) comparable to CA-4. Insertion of a CN group did not affect the activity of CA-4. These results demonstrate that larger substituents on the olefin site adjacent to the A-ring result in weaker activity and a nitrile group is about the maximum tolerable size.

The presence of a nitrile group is not sufficient to improve solubility or physicochemical property of the

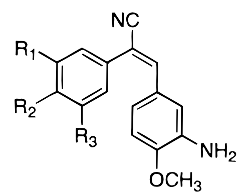
Table 3. Modification of the Aryl Substituents



compd	X	bond type	Colon 26[a] IC_{50} (nM)	antitubulin activity[b] IC_{50} (μM)
9a	A	double	63.7	>20
9c	B	double	>3000	>20
11a	C	double	12.6	>20
11b	D	double	722	>20
12	C	single	22.5	7
23	E	double	2370	>20
25	E	single	>3000	>20

[a] Drug concentration required to inhibit the growth of Colon 26 cells by 50%. [b] Tubulin polymerization was determined as described in the text.

Table 4. Modifications of the Aryl Substituents



compd	R_1	R_2	R_3	Colon 26[a] IC_{50} (nM)	antitubulin activity[b] IC_{50} (μM)
31a	H	H	H	36.0	8
31b	H	OCH_3	H	1430	9
31c	OCH_3	OCH_3	H	8.1	7
13b	OCH_3	OCH_3	OCH_3	5.9	10

[a] Drug concentration required to inhibit the growth of Colon 26 cells by 50%. [b] Tubulin polymerization was determined as described in the text.

mother compound, so we synthesized a series of compounds with a nitrile substituent on the olefin site and substituents on the B-ring were examined (Table 1). A 4-methoxy or 4-methyl group was required in the B-ring for strong activity.[18] Combretastatin A-1 (**ii**) which has an additional 2-OH group on the B-ring of Combretastatin A-4 (**i**) showed significantly decreased cytotoxicity suggesting that a 2-substituent is unacceptable for potent cytotoxicity.[14] So, we examined substituents on the 3-position of the B-ring (**10c**, **13a**,**b**). Introduction of a NO_2 group (**10c**) resulted in a 7-fold decrease in cytotoxicity compared to **10a**. Introduction of OH resulted in a compound (**13a**, IC_{50} 23.5 nM) which was as cytotoxic as the parent compound **10a**. The antimitotic activity of **13a** was also comparable to that of **10a**. The presence of a hydroxyl group on the 3-position of the B-ring did not affect the activity of **10a**. This relationship of **10a** and **13a** was identical to that observed when the 3-hydroxyl group of CA-4 was deleted.[17] These results validate our initial strategy to use a 4-methoxyphenyl group as the B-ring in place of the 3-hydroxyl-4-methoxyphenyl group of CA-4. Introduction of an amino group (**13b**) significantly decreased antimitotic activity (IC_{50} 10 μM), but **13b** showed strong cytotoxicity (IC_{50} 5.9 nM), more potent than those of **10a** and CA-4. Acetamide (**14**) retained cytotoxicity but showed decreased antimitotic activity (IC_{50} 10 μM). By introducing an amino group into the 3-position of the B-ring of **10a**, we obtained the new potent cytotoxic agent **13b**.

Next, 4-substituents of the B-ring of **13b** were examined. Replacement of 4-OMe of the B-ring with a 4-methyl group (**13c**) or 4-chloro group (**13d**) resulted in a 10-fold decrease in cytotoxicity. The antitubulin activity of **13d** (IC_{50} 6 μM) was more potent than that of **13b.** A 3-amino-4-methoxy substituent on the B-ring seemed to be optimal for strong cytotoxicity.

13b showed greater cytotoxicity than CA-4, but its antimitotic activity was significantly decreased (IC_{50} 10 μM). The presence of an amino group on the B-ring in addition to a CN group on the olefin site of *cis*-stilbene seemed to decrease antimitotic activity. To obtain compounds with both potent cytotoxicity and antimitotic activity, we examined substituents on the olefin site again (**18**, **19**, **27b**, **21a**) (Table 2). Compound **19**, a nitrile regioisomer of **13b**, showed a 40-fold decrease in cytotoxicity, but potent antitubulin activity (IC_{50} 5 μM). The amide compounds **18** and **27b**, intermediates of nitrile compounds, showed approximately 30-fold decreases in cytotoxicity. Only **27b** showed strong antitubulin activity (IC_{50} 3 μM). These results indicated that the position of the nitrile group is critical for cytotoxicity and antitubulin activity.[18]

Next, the requirement of the nitrile group on the olefin site for strong cytotoxicity was examined. (*Z*)-Stilbene **21a** which lacked a CN group on the olefin site of **13b** showed strong cytotoxicity (IC_{50} 5.1 nM) and potent antitubulin activity (IC_{50} 4 μM). This result demonstrated that the CN group on the olefin site is not necessary for potent cytotoxicity, and the presence of the CN group weakened the antitubulin activity. The strong cytotoxicity of **21a** seemed to be due to replacement of the phenolic OH of combretastatin A-4 by an NH_2 group.

Next, 4-substituents on the B-ring of **21a** were examined. Replacement of the 4-methoxy group of **21a** with a 4-methyl (**21b**) or 4-chloro (**21c**) group resulted in an order of magnitude decrease in cytotoxicity. On the other hand, **21b**,**c** showed strong antitubulin activities (IC_{50} 3−4 μM). There seemed to be a discrepancy between the potency of cytotoxicity and antitubulin activity.

Table 3 shows the activities of (*Z*)-acrylonitriles, which were obtained as intermediates of (*E*)-acrylonitriles and saturated analogues (**9a**,**c**, **11a**,**b**, **12**, **23**, **25**) All the (*Z*)-acrylonitriles lost their antitubulin activity. Only **9a** and **11a** showed potent cytotoxicity (IC_{50} 63.7 and 12.6 nM, respectively). Compound **12**, a saturated analogue of **11a**, showed strong cytotoxicity (IC_{50} 22.5 nM) and moderate antitubulin activity (IC_{50} 7 μM). The two benzene rings of **12** can adapt a favorable orientation that could mimic (*E*)-acrylonitrile (**13b**) to exert potent activity. (*Z*)-Acrylonitrile **11a** was more cytotoxic than the saturated compound **12** suggesting that **11a** exerts its strong cytotoxicity in the *Z*-form rather than on isomerization to the *E*-form. **11a** may exert its potent cytotoxic effect by a mechanism other than inhibition of tubulin polymerization. Compounds in

Table 5. Antitumor Activities of the Anilino Compounds

compd	doses[a] (mg/kg)	IR (%)[b] Colon 26 (sc-iv)[c]
13bHCl	10	77
13cHCl	5	11
13dHCl	5	−23
21aHCl	40	69
21bHCl	40	62
combretastatin A-4	160	31

[a] Maximum tolerable doses were administered. [b] IR (%) = (1 − T/C) × 100; T, tumor volume (treated); C, tumor volume (untreated). [c] Mice were implanted subcutaneously (sc) with tumor cells, and the drug was administered intravenously (iv).

Table 6. Antitumor Activities of **13bHCl** and **21aHCl**

	IR (%)[a] (sc-iv)[b]		
compd	Colon 38 (dose)[c]	3LL (dose)[c]	HCT-15 (dose)[c]
13bHCl	75 (10)	59 (5)	28 (10)
21aHCl	72 (10)	65 (40)	53 (40)
cisplatin	43 (5)	33 (4)	26 (8)

[a] IR (%) = (1 − T/C) × 100; T, tumor volume (treated); C, tumor volume (untreated). [b] Mice were implanted subcutaneously (sc) with tumor cells, and the drug was administered intravenously (iv). [c] Numbers in parentheses show maximum tolerable doses (mg/kg).

which the B-ring was replaced with 2-methoxypyridine were synthesized to mimic a 3-amino-4-methoxyphenyl group and evaluated. Since we could not synthesize the *E*-form of **23**, we synthesized a saturated analogue to evaluate the activities of the pyridine compounds. However, the pyridine compounds **23** and **25** did not show cytotoxicity or antitubulin activity. Since the saturated compound **25** did not show cytotoxicity, the *E*-form of compound **25** would not show strong cytotoxicity if synthesized.

Finally, we examined the 3,4,5-trimethoxy group in **13b** on the A-ring (**31a**−**c**) The 3,4-dimethoxyphenyl compound **31c** showed strong cytotoxicity (IC_{50} 8.1 nM) comparable to that of **13b** and moderate antimitotic activity (7 μM). 4-Methoxyphenyl **31b** and nonsubstituted compound **31a** showed decreased cytotoxicity. In combretastatin A-4, the 3,4,5-trimethoxy group on the A-ring was thought to be indispensable for its strong cytotoxicity.[18] However, our results indicated that a methoxy group on the 3-position of A-ring is not necessary for potent cytotoxicity of amino acrylonitriles.

The discrepancy between cytotoxicity and antitubulin activity was observed in several compounds. This has been noticed in other classes of antimitotic agents.[23] Since strong cytotoxicity was observed in the amino compounds with weaker antimitotic activity such as **13b**,**c**, there might be another mechanism for exertion of cytotoxicity. The strong cytotoxicity of amino compound **11a**, a *trans*-olefin with complete loss of antitubulin activity, supports this hypothesis. Some other compounds such as **19** and **27b** showed strong antimitotic activity but weak cytotoxicity. Poor permeability into cells is one possibility; however, the decrease in activity other than antitubulin activity is the most possible explanation. The amino group in the 3-position plays no important role in the tubulin binding as the 3-hydroxyl group of combretastatin A-4 does,[17] but it seems to be important for the strong cytotoxicity caused by the other mechanism.

13b−**d** and **21a,b**, which showed potent cytotoxicity against Colon 26 in vitro, were selected for in vivo testing. These compounds have an amino group on the 3-position of the B-ring, and hydrochloride salts were formulated to improve aqueous solubility. The obtained hydrochloride salts of each compound were water-soluble. **13b**−**dHCl** and **21a,bHCl** were evaluated for their antitumor activities against murine Colon 26 adenocarcinoma in vivo (Table 5). The maximum tolerable dose (MTD) of each compound was injected into mice intravenously, and compounds which showed IR (%) > 50 were defined as effective. **13bHCl** and **21a,bHCl** showed marked tumor regression (IR = 77% at 10 mg/kg, 69% at 40 mg/kg, 62% at 40 mg/kg, respectively), while **13c,dHCl** did not show antitumor activity (IR = 11% at 5 mg/kg, −23% at 5 mg/kg, respectively). Combretastatin A-4 also did not show antitumor activity (IR = 31% at 160 mg/kg). **13b**−**dHCl**, each of which had a nitrile group on the olefin site, seemed to be more toxic (MTD 5−10 mg/kg) than **21a,bHCl**, which had no substituent on the olefin site of *cis*-stilbene (MTD 40 mg/kg). The toxic effects of **13c,dHCl** prevented their evaluation at elevated doses.

13bHCl and **21aHCl**, which showed potent activity in the Colon 26 murine tumor model, were further evaluated in the Colon 38 and 3LL murine tumor models and HCT-15 xenografts (Table 6). **13bHCl** showed potent antitumor activity against Colon 38 (IR = 75% at 10 mg/kg) and moderate antitumor activity against 3LL (IR = 59% at 5 mg/kg) but did not show antitumor effects against HCT-15 xenografts (IR = 28% at 10 mg/kg). **21aHCl** showed potent antitumor activity against Colon 38 (IR = 72% at 10 mg/kg), 3LL (IR = 65% at 40 mg/kg), and HCT-15 xenografts (IR = 53% at 40 mg/kg). Cisplatin did not show antitumor activity against Colon 38, 3LL, or HCT-15. **21aHCl** was superior to cisplatin in antitumor activity in these animal models. Thus, we obtained compounds with potent antitumor activity by introducing an amino group in place of the phenolic OH of CA-4. This is the first report of combretastatin A-4 analogues showing potent antitumor activity in vivo.

The marked antitumor activity of **21aHCl** in contrast to combretastatin A-4 may be due to increased hydrophilicity of the compound. **21aHCl** is more soluble (10.0 mg/mL) than CA-4 (0.11 mg/mL) in phosphate-buffered saline solution, and different pharmacokinetics were expected for both drugs. Delivery to the tumor site or duration of contact with the tumor cells of **21aHCl** may be favorable for exerting the antitumor activity in mice.

Conclusion

To improve in vivo antineoplastic activity of combretastatin A-4, we synthesized a series of stilbene compounds with oxygen- or nitrogen-containing groups. By replacing phenolic OH of combretastatin A-4 by amine, compounds with potent cytotoxicity and antitubulin activity were obtained. Among these, **13bHCl** and **21aHCl** showed potent antitumor activity against Colon 26, Colon 38, and 3LL murine tumor models in mice. **21aHCl** also showed antitumor activity against HCT-15 human xenografts in nude mice. **21aHCl** was superior to cisplatin in these tumor models (Colon 38, 3LL, and HCT-15). **21aHCl** showed improved solubility, and further studies are required to clarify the reason for its superior in vivo efficacy over combretastatin A-4. Further experiments to evaluate the potential of **21aHCl** as an anticancer agent for solid tumors are now underway in our laboratory.

Experimental Section

Chemical Procedures. Column chromatography was performed using silica gel (Merck, particle size 0.063−0.200 mm). TLC analyses were performed on silica gel plates (Merck, Art. 5715). All melting points were determined on a Yanaco micromelting point apparatus and are shown uncorrected. NMR spectra were recorded on a Varian EM-390 300-MHz spectrometer with tetramethylsilane as the internal standard; J values are given in hertz (Hz). Mass spectra (MS) were measured on JEOL JMS-DX300 (FD, ESI, and FAB) and JEOL JMS-HX110/HX110 (HRMS) instruments. Analytical results indicated by elemental symbols were within ±0.4% of the theoretical values. For photoisomerization, high-pressure mercury lamp (UVL-400HA, Riko-Kagaku Sangyo Co., Ltd.) was used. Combretastatin A-4 was prepared by the method reported by Pettit.[24] Cisplatin was purchased from Nihon Kayaku Co., Ltd.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-acrylic Acid (2a). A mixture of 3,4,5-trimethoxyphenylacetic acid (30.0 g, 0.13 mol), 4-methoxy benzaldehyde (18.9 g, 0.13 mol), and triethylamine (20 mL) in Ac_2O (100 mL) was heated at 140 °C for 12 h. After cooling, the mixture was evaporated to dryness. The residue was diluted with aqueous NaOH for saponification. Then, the solution was acidified with AcOH and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from EtOAc−hexane to give product **2a** as a white solid (16.5 g, 36%): ^{1}H NMR ($CDCl_3$) δ 7.87 (1H, s), 7.06 (2H, d, J = 8.4), 6.73 (2H, d, J = 8.4), 6.47 (2H, s), 3.90 (3H, s), 3.78 (6H, s), 3.77 (3H, s); MS (FD) 344 (M^+); HRMS found 344.1260, calcd 344.1251.

(*E*)-Methyl 3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylate (2b). To a solution of carboxylic acid **2a** (6.5 g, 18.2 mmol) in DMF (60 mL) were added K_2CO_3 (6.5 g, 47.1 mmol) and methyl iodide (1.35 mL, 21.8 mmol). The reaction mixture was stirred at room temperature for 3 h and filtered, and the filtrate was evaporated to dryness. The residue was dissolved in EtOAc, washed with brine, and then dried over Na_2SO_4. After concentration, the residue was used for the next reaction without further purification.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-prop-2-en-1-ol (3). To a solution of methyl ester **2b** (10.5 g, 29.3 mmol) in anhydrous THF (60 mL) was added 1.0 M $LiAlH_4$−THF (30 mL, 30 mmol). The solution was stirred at 0 °C for 1 h and poured into cold brine. The solution was extracted with CH_2Cl_2 and dried over Na_2SO_4. After concentration, the residue was purified by silica gel column chromatography (33% EtOAc/hexane) to give pure product **3** as an oil (6.0 g, 18.2 mmol, 62%): ^{1}H NMR ($CDCl_3$) δ 6.98 (2H, d, J = 9.0), 6.69 (2H, d, J = 9.0), 6.59 (1H, s), 6.46 (2H, s), 4.43 (2H, s), 3.87 (3H, s), 3.75 (9H, s); MS (FD) 330 (M^+); HRMS found 330.1459, calcd 330.1467. Anal. ($C_{19}H_{22}O_5{\cdot}0.3H_2O$) C, H.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-allylamine (5). To a solution of **3** (384 mg, 1.16 mmol) in THF (4 mL) were added diethyl azodicarboxylate (200 mg, 1.16 mmol), triphenylphosphine (300 mg, 1.16 mmol), and phthalimide (170 mg, 1.16 mmol), and the reaction mixture was stirred at room temperature for 5 h. After concentration, the residue was purified by silica gel column chromatography (33% EtOAc/hexane) to give (*E*)-*N*-3-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-ene phthalimide (**4**) (540 mg, 87%). A mixture of **4** (540 mg, 1.0 mmol) and hydrazine hydrate (71 mg) in ethanol (6 mL) was stirred at 100 °C for 1 h. After concentration, a portion of the residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to give product **5** as an oil (44 mg, 0.13 mmol): ^{1}H NMR ($CDCl_3$) δ 6.94 (2H, d, J = 8.4), 6.65 (2H, d, J = 8.4), 6.53 (1H, s), 6.43 (2H, s), 3.86 (3H, s), 3.74 (9H, s), 3.69 (2H, s); MS (FD) 329 (M^+). Anal. ($C_{19}H_{23}N_1O_4$) C, H, N.

(*E*)-[3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-allyl]trimethylammonium Iodide (6). To a solution of **5** (39 mg, 0.12 mmol) in DMF (2 mL) were added K_2CO_3 (100 mg, 0.72 mmol) and MeI (15μL, 0.36 mmol), and the solution was stirred at room temperature for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with brine, and then dried over Na_2SO_4. After concentration, the residue was purified by alumina column chromatography (5% MeOH/CH_2Cl_2) to give product **6** as an oil (33.6 mg, 56%): ^{1}H NMR ($CDCl_3$) δ 7.27 (1H, d, J = 3.6), 7.00 (2H, d, J = 8.7), 6.66 (2H, d, J = 8.7), 6.61 (2H, s), 5.09 (2H, brs), 3.86 (3H, s), 3.79 (6H, s), 3.75 (3H, s), 3.33 (9H, s); MS (FD) 372 (M^+); formula $(C_{22}H_{30}N_1O_4)^+$ I^-; HRMS calcd 372.2175, found 372.2167.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-propenal (7). To a solution of **3** (2.5 g, 7.6 mmol) in CH_2Cl_2 (25 mL) was added MnO_2 (20 g). The reaction mixture was stirred vigorously at room temperature for 8 h and filtered over Celite, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (33% EtOAc/hexane) to give product **7** (1.71 g, 5.2 mmol, 68%) as white crystals: mp 109−110 °C; ^{1}H NMR ($CDCl_3$) δ 9.71 (1H, s), 7.31 (1H, s), 7.20 (2H, d, J = 7.8), 6.78 (2H, d, J = 7.8), 6.41 (2H, s), 3.91 (3H, s), 3.79 (9H, s); MS (FD) 328 (M^+); HRMS (FAB) calcd 328.1311, found 328.1312. Anal. ($C_{19}H_{20}O_5$) C, H.

General Procedure for the Preparation of 9a−e and 23. A mixture of 3,4,5-trimethoxyphenylacetonitrile (16.6 mmol), benzaldehyde or pyridylaldehyde (16.6 mmol), NaOH (19.9 mmol)−H_2O (15 mL), and methyltrioctylammonium chloride (2.4 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 4 h. The solution was poured into brine, extracted with CH_2Cl_2, and then dried over Na_2SO_4. After concentration, the residue was crystallized from EtOAc to give pure product.

(*Z*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-acrylonitrile (9a): 8.4 g, 75%, yellow crystals, mp 82−83 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (2H, d, J = 8.7), 7.38 (1H, s), 6.98 (2H, d, J = 8.7), 6.85 (2H, s), 3.92 (6H, s), 3.86 (6H, s); MS (FD) 325 (M^+); HRMS calcd 325.1314, found 325.1339. Anal. ($C_{19}H_{19}N_1O_4$) C,H, N.

(*Z*)-3-(3-((Methoxymethyl)oxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (9b): oil, 5.87 g, 95%; ^{1}H NMR ($CDCl_3$) δ 7.71 (1H, d, J = 2.4), 7.37 (1H, s), 7.03 (1H, d, J = 2.4, 8.7), 6.98 (1H, d, J = 8.7), 6.85 (2H, s), 5.29 (2H, s), 3.95 (3H, s), 3.93 (6H, s), 3.89 (3H, s), 3.56 (3H, s); MS (FD) 385 (M^+).

(*Z*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (9c): yellow crystals, 4.42 g, 71%, mp 191−192 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (1H, dd, J = 2.4, 9.0), 8.21 (1H, d, J = 2.4), 7.38 (1H, s), 7.21 (1H, d, J = 9.0), 6.86 (2H, s), 4.05 (3H, s), 3.94 (6H, s), 3.89 (3H, s); MS (FAB) 370 (M^+); HRMS calcd 370.1165 (M^+), found 370.1174. Anal. ($C_{19}H_{18}N_2O_6$) C, H, N.

(*Z*)-3-(4-Methyl-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (9d): yellow crystals, 2.0 g, 31%, mp 162−163 °C; ^{1}H NMR ($CDCl_3$) δ 8.35 (1H, J = 1.5), 8.18 (1H, J = 1.5, 8.1), 7.47 (1H, J = 8.1), 7.44 (1H, s), 6.88 (2H, s), 3.95 (6H, s), 3.90 (3H, s), 2.67 (3H, s); MS (FAB) 354 (M^+); HRMS calcd 354.1216, found 354.1204. Anal. ($C_{19}H_{18}N_2O_5$) C, H, N.

(*Z*)-3-(4-Chloro-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (9e): yellow crystals, 4.9 g, 48%, mp 198−199 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (1H, d, J = 2.1), 8.15 (1H, dd, J = 2.1, 8.4), 7.67 (1H, d, J = 8.4), 7.41 (1H, s), 6.88 (2H, s), 3.94 (6H, s), 3.91 (3H, s); MS (FAB) 374 (M^+). Anal. ($C_{18}H_{15}N_2O_5Cl_1$) C, H, N.

(*Z*)-3-(2-Methoxy-5-pyridyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (23): yellow crystals, 609 mg, 61%, mp 119−120 °C; ^{1}H NMR ($CDCl_3$) δ 8.44 (1H, s), 8.42 (1H, d, J = 9.6), 7.36 (1H, s), 6.86 (2H, s), 6.86 (1H, d, J = 9.6), 4.01 (3H, s), 3.95 (6H, s), 3.89 (3H, s); MS (FAB) 327 (MH^+); HRMS calcd 327.1345 (MH^+), found 327.1344. Anal. ($C_{18}H_{18}N_2O_4$) C, H, N.

General Procedure for the Preparation of 10a−e. A solution of (*Z*)-acrylonitrile **9a** (5.4 mmol) in 500 mL of CH_3CN (CH_3CN was bubbled with N_2 gas to get rid of oxygen before use) was irradiated with a RICO 400-W high-pressure mercury lamp equipped with a Pyrex filter for 30 min. This reaction gave a 1:1 mixture of *E*- and *Z*-isomers. The mixture was concentrated to dryness. The residue was purified by silica gel column chromatography (25% EtOAc/hexane) to give pure product.

(*E*)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)-acrylonitrile (10a): 800 mg (40% from **9a**), yellow crystals, mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 7.23 (1H, s), 7.17 (2H, d, J = 8.4), 6.77 (2H, d, J = 8.4), 6.61 (2H, s), 3.88 (3H, s), 3.79 (3H, s), 3.76 (6H, s); MS (FD) 325 (M^+); HRMS calcd 325.1314, found 325.1340. Anal. ($C_{19}H_{19}N_1O_4$) C, H, N.

(*E*)-3-(3-((Methoxymethyl)oxy)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (10b): *E*- and *Z*-isomers were not separated, and the mixture was used for the next reaction.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (10c): 1.34 g (50% from **9c**), yellow crystals, mp 158−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, d, J = 2.1), 7.35 (1H, dd, J = 2.1, 9.0), 7.19 (1H, s), 6.94 (1H, d, J = 9.0), 6.58 (2H, s), 3.95 (3H, s), 3.89 (3H, s), 3.78 (6H, s); MS (FAB) 370 (M^+); HRMS calcd 370.1165, found 370.1174. Anal. ($C_{19}H_{18}N_2O_6$) C, H, N.

(*E*)-3-(4-Methyl-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (10d): white crystals, 420 mg (42% from **9d**), mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.84 (1H, d, J = 1.8), 7.29 (1H, dd, J = 1.8, 8.1), 7.26 (1H, s), 7.22 (1H, d, J = 8.1), 6.56 (2H, s), 3.89 (3H, s), 3.75 (6H, s), 2.57 (3H, s); MS (FAB) 354 (M^+); HRMS calcd 354.1216, found 354.1216. Anal. ($C_9H_{18}N_2O_5$) C, H, N.

(*E*)-3-(4-Chloro-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (10e): yellow crystals, mp 181−182 °C, 600 mg (40% from **9e**); ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, d, J = 2.1), 7.44 (1H, d, J = 8.7), 7.32 (1H, dd, J = 2.1, 8.7), 7.23 (1H, s), 6.55 (2H, s), 3.89 (3H, s), 3.77 (6H, s); MS (FAB) 374 (M^+).

General Procedure for the Preparation of 11a,b. To a solution of **9c**,**d** (13.6 mmol) in AcOH (200 mL) was added zinc powder (23 g). The reaction mixture was stirred at room temperature for 2 h and filtered over Celite; then the filtrate was evaporated to dryness. After concentration, the residue was purified by silica gel column chromatography (CH_2Cl_2) to give pure product.

(*Z*)-3-(3-Amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (11a): 3.90 g (83% from **9c**), yellow crystals, mp 106−107 °C; ^{1}H NMR ($CDCl_3$) δ 7.40 (1H, d, J = 2.1), 7.30 (1H, s), 7.21 (1H, dd, J = 2.1, 8.4), 6.83 (2H, s), 6.82 (1H, d, J = 8.4), 3.92 (6H, s), 3.92 (3H, s), 3.88 (3H, s); MS (FAB) 340 (M^+); HRMS calcd 340.1423, found 340.1412. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

(*Z*)-3-(3-Amino-4-methylphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (11b): oil, 95.3 mg (95% from **9d**); ^{1}H NMR ($CDCl_3$) δ 7.34 (1H, s), 7.31 (1H, s), 7.13 (2H, s), 6.85 (2H, s), 3.93(6H, s), 3.88 (3H, s), 2.21 (3H, s); MS (FAB) 324 (M^+); HRMS calcd 324.1474, found 324.1479. Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

(*E*)-3-(3-Hydroxy-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (13a): *E*- and *Z*-mixture of **10b** (5.5 g, 14.3 mmol); concentrated HCl (5 mL) in AcOH (55 mL) was stirred at room temperature for 20 min; after concentration, the residue was crystallized from Et_2O to give **13a** (1.95 g, 40%) as white crystals; mp 188−189 °C; ^{1}H NMR ($CDCl_3$) δ 7.18 (1H, s), 6.82 (1H, d, J = 2.1), 6.77 (1H, dd, J = 8.7, 2.1), 6.72 (1H, d, J = 8.7), 5.52 (1H, s), 3.88 (6H, s), 3.77 (6H, s); MS (FD) 341 (M^+); HRMS calcd 341.1263, found 341.1240. Anal. ($C_{19}H_{19}N_1O_5$) C, H, N.

General Procedure for the Preparation of 13b−d. To a solution of nitro compounds **10c**−**e** (8.6 mmol) in AcOH (160 mL) was added zinc powder (32 g). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated to dryness. After concentration, the residue was purified by silica gel column chromatography (25% Et_2O/hexane) to give pure product.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (13b): yellow crystals, 2.2 g (68% from **10c**), mp 144−145 °C; ^{1}H NMR ($CDCl_3$) δ 7.16 (1H, s), 6.65 (4H, s), 6.56 (1H, s), 3.88 (3H, s), 3.84 (3H, s), 3.77 (6H, s); MS (FAB) 340 (M^+); HRMS calcd 340.1423, found 340.1402. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

(*E*)-3-(3-Amino-4-methylphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (13c): yellow crystals, 57.2 mg (60% from **10d**), mp 161−162 °C; ^{1}H NMR ($CDCl_3$) δ 7.20 (1H, s), 6.92 (1H, d, J = 7.5), 6.62 (2H, s), 6.56 (1H, dd, J = 0.9, 7.5), 6.51 (1H, s), 3.87 (3H, s), 3.75 (6H, s), 2.13 (3H, s); MS (FAB) 324 (M^+); HRMS calcd 324.1474, found 324.1481. Anal. ($C_{19}H_{20}N_2O_3$) C, H, N.

(*E*)-3-(3-Amino-4-chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (13d): white crystals, 102 mg (33% from **10e**), mp 150−151 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (1H, s), 7.12 (1H, d, J = 8.1), 6.61 (1H, d, J = 1.8), 6.59 (2H, s), 6.53 (1H, dd, J = 1.8, 8.1), 3.88 (3H, s), 3.75 (6H, s); MS (FAB) 344 (M^+); HRMS calcd 344.0928, found 344.0943. Anal. ($C_{18}H_{17}N_2O_3Cl_1$) C, H, N.

(*E*)-3-(3-(Acetylamino)-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (14). A mixture of **13b** (24 mg, 0.07 mmol) and Ac_2O (1 mL) in pyridine (1 mL) was stirred at room temperature for 1 h. The solution was evaporated to dryness, and the residue was purified by preparative TLC (CH_2Cl_2) to give **14** (26.4 mg, 98%) as white crystals: 230−231 °C; ^{1}H NMR ($CDCl_3$) δ 8.34 (1H, s), 7.62 (1H, brs), 7.25 (1H, s), 6.87 (1H, dd, J = 1.8, 8.7), 6.68 (1H, d, J = 8.7), 3.88 (3H, s), 3.87 (3H, s), 3.76 (6H, s), 2.16 (3H, s); MS (FAB) 382 (M^+); HRMS calcd 382.1529, found 382.1542. Anal. ($C_{21}H_{22}N_2O_5$) C, H, N.

(*E*)-2-(4-Methoxy-3-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)acrylic Acid (15). A mixture of 3,4,5-trimethoxybenzaldehyde (930 mg, 4.7 mmol), 4-methoxy-3-nitrophenylacetic acid (**1b**), and triethylamine (1 mL) in Ac_2O (10 mL) was heated at 140 °C for 12 h. After cooling, the mixture was concentrated to dryness. The residue was diluted with water, and the solution was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by silica gel column chromatography (5% MeOH/CH_2Cl_2) to give **15** as a solid (990 mg, 54%): ^{1}H NMR ($CDCl_3$) δ 7.89 (1H, s), 7.78 (1H, d, J = 1.8), 7.39 (1H, d, J = 7.8), 7.09 (1H, d, J = 7.8), 6.34 (2H, s), 3.95 (3H, s), 3.83 (3H, s), 3.59 (6H, s); MS (FAB) 389 (M^+); HRMS calcd 389.1111, found 389.1091.

(*E*)-2-(4-Methoxy-3-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (16). To a solution of **15** (700 mg, 1.82 mmol) in CH_2Cl_2 (9 mL) was added $SOCl_2$ (0.70 mL) in DMF (0.1 mL). The solution was stirred at room temperature for 1 h; then the mixture was evaporated to dryness. The residue was dissolved with CH_2Cl_2 (5 mL), and the solution was added to well-stirred 28% aqueous NH_3 (30 mL) at room temperature. After 30 min, the reaction mixture was extracted with CH_2Cl_2 and dried over Na_2SO_4. After concentration, the residue was purified by preparative TLC (33% EtOAc/hexane) to give **16** (386 mg, 54%): yellow crystals, mp 193−194 °C; ^{1}H NMR ($CDCl_3$) δ 7.85 (1H, d, J = 2.1), 7.78 (1H, s), 7.49 (1H, dd, J = 2.1), 7.20 (1H, d, J = 2.1, 8.4), 6.28 (2H, s), 3.99 (3H, s), 3.81 (3H, s), 3.60 (6H, s); MS (FAB) 388 (M^+); HRMS calcd 388.1271, found 388.1269.

(*E*)-2-(4-Methoxy-3-nitrophenyl)-3-(3,4,5-trimethoxyphenyl)acrylonitrile (17). To a solution of **16** (116 mg, 0.3 mmol) in pyridine (5 mL) was added $SOCl_2$ (0.10 mL). The reaction mixture was stirred at room temperature for 6 h. The mixture was concentrated to dryness, and the residue was purified by preparative TLC (33% EtOAc/hexane) to give **17** as yellow crystals (48 mg, 43%): ^{1}H NMR ($CDCl_3$) δ 7.92 (1H, d, J = 2.4), 7.60 (1H, dd, J = 2.4, 8.7), 7.31 (1H, s), 7.11 (1H, d. J = 8.7), 6.42 (2H, s), 3.99 (3H, s), 3.86 (3H, s), 3.66 (6H, s); MS (FAB) 370 (M^+); HRMS calcd 370.1165, found 370.1174.

General Procedure for the Preparation of 18 and 19. To a solution of nitro compound **16** (162 mg, 0.42 mmol) in AcOH (2 mL) was added zinc powder (300 mg). The reaction mixture was stirred at room temperature for 1 h. The mixture was filtered over Celite, and the filtrate was concentrated to dryness. The residue was purified by preparative TLC (5% $MeOH/CH_2Cl_2$) to give pure product.

(*E*)-2-(3-Amino-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (18): yellow crystals, 76 mg (50% from **16**), mp 148−149 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (1H, s), 6.87 (1H, s, J = 7.9), 6.64 (1H, d, d, J = 2.0, 7.9), 6.62 (1H, d, J = 2.0), 6.38 (2H, s), 5.73 (2H, br), 5.15 (2H, br), 3.88 (3H, s), 3.80 (3H, s), 3.58 (6H, s); MS (FAB) 358 (M^+); HRMS calcd 358.1529, found 358.1530. Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

(*E*)-2-(3-Amino-4-methoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylonitrile (19): yellow crystals, 21 mg (76% from **17**), mp 128−129 °C; ^{1}H NMR ($CDCl_3$) δ 7.12 (1H, s), 6.77 (3H, brs), 6.49 (2H, s), 3.86 (3H, s), 3.84 (3H, s), 3.64 (6H, s); MS (FAB) 340 (M^+); HRMS calcd 340.1423, found 340.1402. Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

General Procedure for the Preparation of 20a−c. NaH (10.0 mmol, washed with hexane) was added to a stirred suspension of phosphonium bromide (8.4 mmol) and benzaldehyde **8c**−**e** (8.4 mmol) in toluene (60 mL), and the mixture was stirred at room temperature for 12 h. AcOH (5 mL) was added to the reaction mixture, which was then poured onto ice−water and extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from EtOH. The obtained solid was recrystallized from EtOAc/hexane to give the desired *Z*-form product.

(*Z*)-1-Methoxy-2-nitro-4-[2-(3,4,5-trimethoxyphenyl)vinyl]benzene (20a): 1.27 g, 43%, yellow crystals, mp 123−124 °C; ^{1}H NMR ($CDCl_3$) δ 7.79 (1H, d, J = 2.1), 7.42 (1H, dd, J = 2.1, 8.7), 6.93 (1H, d, J = 8.7), 6.58 (1H, d, J = 12.9), 6.47 (2H, s), 6.44 (1H, d, J = 12.9), 3.93 (3H, s), 3.85 (3H, s), 3.71 (6H, s); MS (FAB) 345 (M^+); HRMS calcd 345.1212, found 345.1196. Anal. ($C_{18}H_{19}N_1O_6$) C, H, N.

(*Z*)-1-Methyl-2-nitro-4-[2-(3,4,5-trimethoxyphenyl)vinyl]benzene (20b): 990 mg, 47%, oil; ^{1}H NMR ($CDCl_3$) δ 7.89 (1H, d, J = 1.8), 7.40 (1H, dd, J = 1.8, 7.8), 7.19 (1H, d, J = 7.8), 6.63 (1H, d, J = 12.3), 6.50 (1H, d, J = 12.3), 6.46 (2H, s), 3.85 (3H, s), 3.69 (6H, s), 2.55 (3H, s); MS (FAB) 329 (M^+).

(*Z*)-1-Chloro-2-nitro-4-[2-(3,4,5-trimethoxyphenyl)vinyl]benzene (20c): yellow crystals, mp 73−74 °C, 950 mg, 50%; ^{1}H NMR ($CDCl_3$) δ 7.79 (1H, s), 7.39 (2H, s), 6.70 (1H, d, J = 12.0), 6.47 (1H, d, J = 12.0), 6.44 (2H, s) 3.86 (3H, s), 3.72 (6H, s); MS (FAB) 349 (M^+).

General Procedure for the Preparation of 21a−c. To a solution of nitro compounds **20a**−**c** (2.03 mmol) in AcOH (160 mL) was added zinc powder (32 g). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered over Celite, and the filtrate was evaporated to dryness. After concentration, the residue was purified by silica gel column chromatography (CH_2Cl_2) to give pure product.

(*Z*)-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine (21a): oil, 314 mg (49% from **20a**); ^{1}H NMR ($CDCl_3$) δ 6.69 (1H, s), 6.67 (2H, s), 6.55 (2H, s), 6.45 (1H, d, J = 12.0), 6.36 (1H, d, J = 12.0), 3.84 (3H, s), 3.82 (3H, s), 3.69 (6H, s); MS (FAB) 315 (M^+); HRMS calcd 315.1471, found 315.1462. Anal. ($C_{18}H_{21}N_1O_4$) C, H, N.

(*Z*)-2-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine (21b): oil, 620 mg (82% from **20b**); ^{1}H NMR ($CDCl_3$) δ 6.93 (1H, d, J = 7.5), 6.65 (1H, dd, J = 1.8, 7.5), 6.63 (1H, d, J = 1.8), 6.53 (2H, s), 6.49 (1H, d, J = 12.3), 6.40 (1H, d, J = 12.3), 3.83 (3H, s), 3.68 (6H, s), 2.13 (3H, s); MS (FD) 299 (M^+); HRMS calcd 299.1521, found 299.1523. Anal. ($C_{18}H_{21}N_1O_3 \cdot 0.2H_2O$) C, H, N.

(*Z*)-2-Chloro-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine (21c): oil, 52 mg (66% from **20c**); ^{1}H NMR ($CDCl_3$) δ 7.12 (1H, d, J = 7.8), 6.71 (1H, d, J = 1.8), 6.62 (1H, dd, J = 1.8, 7.8), 6.49 (2H, s), 6.45 (2H, s), 3.84 (3H, s), 3.69 (6H, s); MS (FAB) 319 (M^+); HRMS calcd 319.0975, found 319.0987. Anal. ($C_{17}H_{18}N_1O_3Cl_1 \cdot 0.1H_2O$) C, H, N.

General Procedure of the Preparation of 13b−dHCl and 21a,bHCl. To a solution of anilino compounds **(13b**−**d** and **21a**,**b)** (1.58 mmol) in CH_2Cl_2 (10 mL) was added 4 N HCl−dioxane (1.0 mL). The reaction mixture was stirred at room temperature for 1 h and then concentrated to dryness. The residue was crystallized from $EtOH/Et_2O$ to give the corresponding HCl salt.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile hydrochloride (13bHCl): white crystals (from $EtOH/Et_2O$), mp 154−155 °C, 356 mg (71% from **13b**); ^{1}H NMR (DMSO-d_6), δ 7.51 (1H, s), 7.04−7.14 (2H, m), 6.66 (2H, s), 3.84 (3H, s), 3.71 (3H, s), 3.69 (6H, s). Anal. ($C_{19}H_{20}N_2O_4 \cdot HCl$) C, H, N.

(*E*)-3-(3-Amino-4-methylphenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile hydrochloride (13cHCl): white crystals (from $EtOH/Et_2O$), mp 162−163 °C, 250 mg (72% from **13c**); ^{1}H NMR (DMSO-d_6) δ 7.56 (1H, s), 7.15 (1H, d, J = 7.5), 7.08 (1H, brs), 6,89 (1H, d, J = 7.5), 6.65 (2H, s), 3.71 (3H, s), 3.68 (6H, s), 2.30 (3H, s). Anal. ($C_{19}H_{20}N_2O_3 \cdot HCl$) C, H, N.

(*E*)-3-(3-Amino-4-chlorophenyl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile hydrochloride (13dHCl): white crystals (from $EtOH/Et_2O$), mp 149−150 °C, 364 mg (75% from **13d**); ^{1}H NMR (DMSO-d_6) δ 7.48 (1H, s), 7.12 (1H, d, J = 8.4), 6.75 (1H, d, J = 1.8), 6.65 (2H, s), 6.38 (1H, dd, J = 1.8, 8.4), 3.70 (3H, s), 3.68 (6H, s). Anal. ($C_{18}H_{17}N_2O_3Cl_1 \cdot HCl$) C, H, N.

(*Z*)-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine hydrochloride (21aHCl): white crystals (from $EtOH/Et_2O$), mp 117−118 °C, 8.4 g (82% from **21a**); ^{1}H NMR ($CDCl_3$) δ 7.58 (1H, d, J = 2.1), 7.28 (1H, dd, J = 2.1, 8.7), 6.79 (1H, d, J = 8.7), 6.52 (1H, d, J = 12.0), 6.48 (2H, s), 6.45 (1H, d, J = 12.0), 3.91 (3H, s), 3.83 (3H, s), 3.73 (6H, s). Anal. ($C_{18}H_{21}N_1O_4 \cdot HCl$) C, H, N.

(*Z*)-2-Methyl-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenylamine hydrochloride (21bHCl): oil, 254 mg (72% from **21b**); ^{1}H NMR (DMSO-d_6) δ 7.31 (1H, d, J = 1.5), 7.23 (1H, d, J = 7.8), 7.23 (1H, dd, J = 1.5, 7.8), 6.52 (2H, d, J = 12.3), 3.63 (3H, s), 3.57 (3H, s), 3.56 (3H, s), 2.31 (3H, s). Anal. ($C_{18}H_{21}N_1O_3 \cdot HCl$) C, H, N.

General Procedure of the Preparation of 12 and 25. A mixture of acrylonitrile (11.5 mmol) and 10% Pd−C (3.0 g) in MeOH (120 mL) was stirred at room temperature under hydrogen atmosphere for 3 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness. The residue was purified by silica gel column chromatography (CH_2Cl_2) to give pure product.

3-(3-Amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)propanenitrile (12): white crystals, mp 126−127 °C, 1.81 g (46% from **11a**); ^{1}H NMR ($CDCl_3$) δ 6.69 (1H, d, J = 7.8), 6.51 (1H, s), 6.50 (1H, dd, J = 2.4, 7.8), 6.44 (2H, s), 3.84 (3H, s), 3.83 (3H, s), 3.82 (6H, s), 3.84 (1H, m), 3.06 (1H, dd, J = 8.1, 13.5), 2.97 (1H, dd, J = 8.1, 13.5); MS (FAB) 343 (MH^+); HRMS calcd 343.1658 (MH^+), found 343.1636. Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

3-(2-Methoxy-5-pyridyl)-2-(3,4,5-trimethoxyphenyl)propanenitrile (25): white crystals, mp 67−68 °C, 76.7 mg (76% from **23**); ^{1}H NMR ($CDCl_3$) δ 7.91 (1H, d, J = 2.4), 7.34 (1H, d, J = 2.4, 8.4), 6.68 (1H, d, J = 8.4), 6.41 (2H, s), 3.92 (3H, s), 3.89 (1H, t, J = 7.2), 3.84 (3H, s), 3.82 (6H, s), 3.10 (1H, dd, J = 2.7, 7.2); MS (FAB) 329 (MH^+); HRMS calcd 329.1501, found 329.1483. Anal. ($C_{18}H_{20}N_2O_4$) C, H, N.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylic Acid (26). A mixture of 3,4,5-trimethoxyphenylacetic acid (1.01 g, 4.7 mmol), 4-methoxy-3-nitrobenzaldehyde (850 mg, 4.7 mmol), and triethylamine (1 mL) in Ac_2O (10 mL) was heated at 140 °C for 12 h. After cooling, the mixture was evaporated to dryness. The residue was diluted with water, and the solution was extracted with CH_2Cl_2. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from EtOAc/hexane to give **26** (635 mg, 36%): yellow crystals, mp 217−218 °C; ^{1}H NMR ($CDCl_3$) δ 7.81 (1H, s), 7.65 (1H, d, J = 2.7), 7.21 (1H, dd, J = 2.7, 9.0), 6.89 (1H, d, J = 9.0), 6.45 (2H, s), 3.93 (3H, s), 3.91 (3H, s), 3.79 (6H, s); MS (FAB) 389 (M^+); HRMS calcd 389.1111, found 389.1099. Anal. ($C_{19}H_{19}N_1O_8$) C, H, N.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylamide (27a). To a solution of carboxylic acid **26a** (101 mg, 0.26 mmol) in CH_2Cl_2 (1 mL) were added $SOCl_2$ (38 μL) and pyridine (0.1 mL). The reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated to dryness. The residue was dissolved with CH_2Cl_2 (2 mL), and the solution was added to well-stirred 28% aqueous NH_3 (2 mL). After 1 h, the mixture was diluted with water, extracted with CH_2Cl_2, and dried over Na_2SO_4. After concentration, the residue was purified by preparative TLC (5% MeOH/CH_2Cl_2) to give **27a** (72.8 mg, 73%): yellow crystals, mp 202−203 °C; ^{1}H NMR ($CDCl_3$) δ 7.75 (1H, s), 7.51 (1H, d, J = 2.4), 7.26 (1H, dd, J = 2.4), 6.92 (1H, d, J = 2.4, 9.0), 6.48 (2H, s), 5.59 (2H, br), 3.93 (6H, s), 3.82 (6H, s); MS (FAB) 389 (MH^+); HRMS calcd 388.1271, found 388.1254. Anal. ($C_{19}H_{20}N_2O_7$) C, H, N.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylamide (27b). To a solution of **27a** (204 mg, 0.52 mmol) in AcOH (10 mL) was added zinc powder (2.0 g) at room temperature. The reaction mixture was stirred vigorously for 2 h. The mixture was filtered over Celite, and the filtrate was evaporated to dryness. The residue obtained was purified by preparative TLC (EtOAc) to give **27b** (54 mg, 29%): yellow crystals, mp 182−183 °C; ^{1}H NMR ($CDCl_3$) δ 7.72 (1H, s), 6.61 (1H, d, J = 8.4), 6.51 (2H, s), 6.52 (1H, dd, J = 2.4, 8.4), 6.39 (1H, dd, J = 2.4, 8.4), 5.49 (2H, br), 3.92 (3H, s), 3.82 (9H, s); MS (FAB) 358 (M^+). Anal. ($C_{19}H_{22}N_2O_5$) C, H, N.

General Procedure for the Preparation of 29a−c. A mixture of phenylacetonitriles (15.0 mmol), 4-methoxy-3-nitrobenzaldehyde (15.0 mmol), NaOH (15.0 mmol)−H_2O (15 mL), and methyltrioctylammonium chloride (2.4 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 4 h. The solution was poured onto brine, extracted with CH_2Cl_2, and then dried over Na_2SO_4. After concentration, the residue was crystallized from EtOAc to give pure product.

(*Z*)-3-(4-Methoxy-3-nitrophenyl)-2-phenylacrylonitrile (29a): yellow crystals, mp 151−152 °C; ^{1}H NMR ($CDCl_3$) δ 8.30 (1H, dd, J = 2.4, 8.7), 8.22 (1H, d, J = 2.4), 7.64−7.70 (2H, m), 7.40−7.50 (3H, m), 7.46 (1H, s), 7.21 (1H, d, J = 8.7), 4.05 (3H, s); MS (ESI) 267 (MH^+).

(*Z*)-3-(4-Methoxy-3-nitrophenyl)-2-(4-methoxyphenyl)acrylonitrile (29b): yellow crystals, mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 8.27 (1H, dd, J = 2.1, 8.7), 8.19 (1H, d, J = 2.1), 7.60 (2H, d, J = 9.0), 7.34 (1H, s), 7.19 (1H, d, J = 8.7), 6.97 (2H, d, J = 9.0); MS (ESI) 311 (MH^+).

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4-dimethoxyphenyl)acrylonitrile (29c): yellow crystals, mp 205−206 °C; ^{1}H NMR ($CDCl_3$) δ 8.28 (1H, dd, J = 2.7, 9.0), 8.19 (1H, d, J = 2.7), 7.35 (1H, s), 7.25 (1H, dd, J = 2.1, 8.4), 7.22 (1H, d, J = 9.0), 7.12 (1H, d, J = 2.1), 6.93 (1H, d, J = 8.4); MS (ESI) 341 (MH^+).

General Procedure for the Preparation of 30a−c. A solution of (*Z*)-acrylonitrile **29a−c** (5.0 mmol) in CH_3CN (500 mL) was irradiated with a RICO 400-W high-pressure mercury lamp equipped with a Pyrex filter for 30 min. This reaction gave a 1:1 mixture of *E*- and *Z*-isomers. The mixture was evaporated to dryness, and the residue was purified by silica gel column chromatography (25% EtOAc/hexane) to give pure product.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-phenylacrylonitrile-(30a): yellow crystals, mp 152−153 °C, 405 mg (40% from **29a**); ^{1}H NMR ($CDCl_3$) δ 7.64 (1H, d, J = 2.4), 7.34−7.50 (5H, m), 7.30 (1H, dd, J = 2.4, 9.0), 7.25 (1H, s), 6.92 (1H, d, J = 9.0); MS (FAB) 280 (M^+); HRMS calcd 280.0848, found 280.0861. Anal. ($C_{16}H_{12}N_2O_3$) C, H, N.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(4-methoxyphenyl)acrylonitrile (30b): yellow crystals, mp 95−96 °C, 487 mg (35% from **29b**); ^{1}H NMR ($CDCl_3$) δ 8.01 (1H, d, J = 2.1), 7.66 (1H, dd, J = 2.1, 8.7), 7.61 (2H, d, J = 9.0), 7.48 (1H, s), 7.26 (1H, d, J = 8.7), 7.22 (2H, d, J = 9.0), 4.26 (3H, s), 4.16 (3H, s); MS (FAB) 310 (M^+). Anal. ($C_{17}H_{14}N_2O_4$) C, H, N.

(*E*)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4-dimethoxyphenyl)acrylonitrile (30c): yellow crystals, mp 141−142 °C, 732 mg (41% from **29c**); ^{1}H NMR ($CDCl_3$) δ 7.74 (1H, d, J = 2.4), 7.34 (1H, dd, J = 2.4, 9.0), 7.17 (1H, s), 6.92−6.99 (2H, m), 6.86 (1H, d, J = 9.0), 6.83 (1H, d, J = 2.1); MS (FAB) 340 (M^+). Anal. ($C_{18}H_{16}N_2O_5$) C, H, N.

General Procedure of the Preparation of 31a−c. To a solution of nitro compounds **30a−c** (8.6 mmol) in AcOH (160 mL) was added zinc powder (32 g). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was filtered over Celite, and the filtrate was concentrated to dryness. After concentration, the residue was purified by silica gel column chromatography (25% Et_2O/hexane) to give pure product.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-phenylacrylamide (31a): oil, 820 mg (71% from **30a**); ^{1}H NMR ($CDCl_3$) δ 7.34−7.46 (5H, m), 7.20 (1H, s), 6.63 (1H, d, J = 8.1), 6.58 (1H, dd, J = 2.1, 8.1), 6.46 (1H, d, J = 2.1), 3.83 (3H, s), 3.70 (2H, br); MS (FAB) 250 (M^+); HRMS calcd 250.1106, found 250.1107. Anal. ($C_{16}H_{14}N_2O_1$) C, H, N.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-(4-methoxyphenyl)acrylamide (31b): yellow crystals, mp 121−122 °C, 214 mg (51% from **30b**); ^{1}H NMR ($CDCl_3$) δ 7.33 (2H, d, J = 9.0), 7.13 (1H, s), 6.88 (2H, d, J = 9.0), 6.60−6.66 (2H, m), 6.52 (1H, d, J = 1.8), 3.83 (6H, s), 3.70 (2H, br); MS (FAB) 280 (M^+); HRMS calcd 280.1212, found 280.1217. Anal. ($C_{17}H_{16}N_2O_2$) C,H, N.

(*E*)-3-(3-Amino-4-methoxyphenyl)-2-(3,4-dimethoxyphenyl)acrylamide (31c): yellow crystals, mp 87−88 °C, 588 mg (98% from **30c**); ^{1}H NMR ($CDCl_3$) δ 7.14 (1H, s), 7.01 (1H, dd, J = 2.1, 8.1), 6.90 (1H, d, J = 2.1), 6.84 (1H, d, J = 8.1), 6.63 (2H, s), 6.55 (1H, s), 3.90 (3H, s), 3.83 (3H, s), 3.77 (3H, s); MS (FAB) 310 (M^+); HRMS calcd 310.1301, found 310.1324. Anal. ($C_{18}H_{18}N_2O_3$) C, H, N.

Tubulin Polymerization Inhibition Assay. Electrophoretically homogeneous tubulin was purified from bovine brain.[25] Determination of IC_{50} values for the polymerization of purified tubulin was performed as follows.[26] Tubulin was preincubated at 37 °C with test compounds at various concentrations, and reaction mixtures were chilled on ice. GTP (guanosine 5′-triphosphate; required for the polymerization reaction) was added, and polymerization was followed at 37 °C by turbidimetry measurement at 350 nm in a Gliford recording spectrometer equipped with an electronic temperature controller. The extent of polymerization after 20-min incubation was determined. IC_{50} values were determined graphically. Compounds were examined in three independent assays, and the values shown for these compounds are the averages of three determinations.

Cell Growth Inhibition Assay. Murine Colon 26 adenocarcinoma cells were maintained in plastic dishes in RPMI-1640 supplemented with 10% fetal bovine serum. For in vitro treatment, tumor cells were seeded in 50 μL of culture medium/well in 96-well plates to a final cell density of 1×10^5 cells/ml and incubated in a CO_2 incubator at 37 °C for 24 h. The cells were treated with various concentrations of test compounds and incubated in a CO_2 incubator at 37 °C for 48 h. The number of viable cells was estimated using the tetrazolium dye reduction assay (MTT assay).[27,28] Cytotoxicity of the test compounds was expressed in terms of IC_{50} values. Compounds were examined in three independent assays, and the values shown for these compounds are the averages of three determinations.

In Vivo Antitumor Activity. In transplanted solid tumor models, fragments of Colon 26 tumor (5 mg) were inoculated sc into CD2F1 mice, those of Colon 38 tumor (5 mg) or 3LL (5 mg) were inoculated sc into BD2F1 mice, and fragments of HCT-15 (5 mg) were inoculated sc into ICR nu/nu mice on day 0. Test compounds were given iv on days 7, 11, and 15. On day 21, two diameters of each tumor were measured using calipers. The tumor weights were calculated using the formula: tumor weight (mg) = [length (mm) $\times$ width (mm)^2]/2. The inhibition ratio was evaluated as (1 − T/C) $\times$ 100 (%) (where T is the mean tumor weight of the treated group and C is the mean tumor weight of the control group). Each group consisted of 5 mice.

References

(1) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lohavanijaya, P. Isolation and Structure of Combretastatin. *Can. J. Chem.* **1982**, *60*, 1374−1376.

(2) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. Isolation and Structure of the Strong Cell Growth and Tubulin Inhibitor Combretastatin A-4. *Experientia* **1989**, *45*, 209−211.

(3) Hamel, E.; Lin, C. M. Interactions of Combretastatin, A New Plant-Derived Antimitotic Agent, with Tubulin. *Biochem. Pharmacol.* **1983**, *32*, 3864−3867.

(4) McGown, A. T.; Fox, B. W. Differential Cytotoxicity of Combretastatins A1 and A4 in Two Daunorubicin-Resistant P388 Cell Lines. *Cancer Chemother. Pharmacol.* **1990**, *26*, 79−81.

(5) El-Zayat, A. A. E.; Degen, D.; Drabek, S.; Clark, G. M.; Pettit, G. R.; Von Hoff, D. D. In vitro Evaluation of the Antineoplastic Activity of Combretastatin A-4, A Natural Product from *Combretum caffrum* (arid shrub). *Anti-Cancer Drugs* **1993**, *4*, 19−25.

(6) Bedford, S. B.; Quarteman, C. P.; Rathbone, D. L.; Slack, J. A. Synthesis of Water-Soluble Prodrug of the Cytotoxic Agent Combretastatin A-4. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 157−160.

(7) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Boyd, M. R.; Rener, G. A.; Bansal, N. Antineoplastic Agents 322. Synthesis of Combretastatin A-4 Prodrugs. *Anti-Cancer Drug Des.* **1995**, *10*, 299−309.

(8) Dorr, R. T.; Dvorakova, K.; Snead, K.; Alberts, D. S.; Salmon, S. E.; Pettit, G. R. Antitumor Activity of Combretastatin A-4 Phosphate, A Natural Product Tubulin Inhibitor. *Invest. New Drugs* **1996**, *14*, 131−137.

(9) Shirai, R.; Tokuda, K.; Koiso, Y.; Iwasaki, S. Synthesis and Anti-Tublulin Activity of Aza-Combretastatins. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 699−704.

(10) Cushman, M.; He, H. M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of a Series of Benzylaniline Hydrochlorides as Potential Cytotoxic and Antimitotic Agents Acting by Inhibition of Tubulin Polymerization. *J. Med. Chem.* **1993**, *36*, 2817−2821.

(11) Brown, R. T.; Fox, B. W.; Hadfield, J. A.; McGown, A. T.; Mayalarp, S. P.; Pettit, G. R.; Woods, J. A. Synthesis of Water-Soluble Sugar Derivatives of Combretastatin A-4. *J. Chem. Soc., Perkin Trans. 1* **1995**, *5*, 577−581.

(12) Fulvia, O.; Francesca, P.; Barbara, B.; Giuliana, M. Synthesis of Biologically Active Polyphenolic Glycosides (Combretastatin and Resveratrol series). *Carbohydr. Res.* **1997**, *301*, 95−109.

(13) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. Interactions of Tubulin with Potent Natural and Synthetic Analogues of the Antimitotic Agent Combretastatin: a Structure−Activity Study. *Mol. Pharmacol.* **1988**, *34*, 200−208.

(14) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmit, J. M. Isolation, Structure, and Synthesis of Combretastatins A-1 and B-1, Potent New Inhibitors of Microtubule Assembly, Derived from *Combretum caffrum*. *J. Nat. Prod.* **1987**, *50*, 119−131.

(15) Talvitie, A.; Mannila, E.; Kolehmainen, E. Syntheses of Some Biologically Active Compounds from Stilbenes Isolated from the Bark of *Picea abies*. *Liebigs Ann. Chem.* **1992**, 399−401.

(16) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. Antimitotic Natural Products Combretastatin A-4 and Combretastatin A-2: Studies on the Mechanism of Their Inhibition of the Binding of Colchicine to Tubulin. *Biochemistry* **1989**, *28*, 6984−6991.

(17) Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents That Inhibit Tubulin Polymerization. *J. Med. Chem.* **1991**, *34*, 2579−2588.

(18) Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M.; Lin, C. M.; Hamel, E. Synthesis and Evaluation of Analogues of (*Z*)-1-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl) ethene as Potential Cytotoxic and Antimitotic Agents. *J. Med. Chem.* **1992**, *35*, 2293−2306.

(19) Woods, J. A.; Hadfield, J. A.; Pettit, G. R.; Fox, B. W.; McGown, A. T. The Interaction with Tubulin of a Series of Stilbenes Based on Combretastatin A-4. *Br. J. Cancer* **1995**, *71*, 705−711.

(20) α-Phenylcinnamic Acid. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. IV, pp 777.

(21) Suh, J. T.; Puma, B. M. A Novel Synthesis of 2,3-Disubstituted Indoles, A Study of the Reductive Cyclization of Some 3-Substituted 2-(4,5-dimethoxy-2-nitrophenyl)acrylonitrile. *J. Org. Chem.* **1965**, *30*, 2253.

(22) Silverstein, E. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley and Sons: New York, 1981; pp 264−265.

(23) Goldbrunner, M.; Loidl, G.; Polossek, T.; Mannschreck, A.; Angerer, E. Inhibition of Tubulin Polymerization by 5,6-Dihydroindolo[2,1-a]isoquinoline Derivatives. *J. Med. Chem.* **1997**, *40*, 3524−3533.

(24) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. Antineoplastic Agents. 291. Isolation and Synthesis of Combretastatin A-4, A-5, and A-6. *J. Med. Chem.* **1995**, *38*, 1666−1672.

(25) Hamel, E.; Lin, C. M. Separation of Active Tubulin and Microtubule-Associated Proteins by Ultracentrifugation and Isolation of a Component Causing the Formation of Microtubule Bundles. *Biochemistry* **1984**, *23*, 4173−4184.

(26) Muzaffar, A.; Brossi, A.; Lin, C. M.; Hamel, E. Antitubulin Effects of Derivatives of 3-Demethylthiocolchicine, Methylthio Ethers of Natural Colchicinoids, and Thioketones Derived from Thiocolchicine. Comparison with Colchicinoids. *J. Med. Chem.* **1990**, *33*, 567−571.

(27) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. Feasibility of Drug Screening with Panels of Human Tumor Cell Lines Using a Microculture Tetrazolium Assay. *Cancer Res.* **1988**, *48*, 589−601.

(28) Mossman, T. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

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