Synthesis and Structure−Activity Relationships of 3-Aminobenzophenones as Antimitotic Agents

Jing-Ping Liou,[†,§] Jang-Yang Chang,[‡,§] Chun-Wei Chang, Chi-Yen Chang,[‡] Neeraj Mahindroo,[†] Fu-Ming Kuo,[†] and Hsing-Pang Hsieh*[,†]

Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Taipei, Taiwan, Republic of China and Division of Cancer Research, National Health Research Institutes, Taipei, Taiwan, Republic of China

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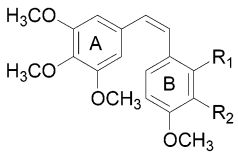
A new series of 3-aminobenzophenone compounds as potent inhibitors of tubulin polymerization was discovered based on the mimic of the aminocombretastatin molecular skeleton. Lead compounds **5** and **11**, with alkoxy groups at the C-4 position of B-ring, were potent cytotoxic agents and inhibitors of tubulin polymerization through the binding to the colchicine-binding site of tubulin. The corresponding antitubulin activities of **5** and **11** were similar to or greater than combretastatin A-4 and AVE-8063. Replacement of the methoxy group with a chloro group in the B ring of aminobenzopheneones (**3**, **8**, and **9**) caused drastic decrease in cytotoxic and antitubulin activity except in compounds **4** and **10**, which could result from a unique alignment between chloro and amino groups located at the para position to each other. SAR information revealed that introduction of an amino group at the C-3 position in B ring of benzophenones, in addition to a methoxy group at the C-4 position, plays an important role for maximal cytotoxicity.

Introduction

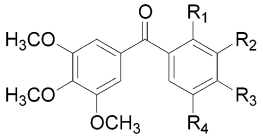
Microtubules are dynamic structures that play a crucial role in cellular division and are recognized as an important target for anticancer therapy.[1] A number of naturally occurring compounds, such as paclitaxel, epothilone A, vinblastine, combretastatin A-4, dolastatin 10, and colchicines, all exhibit their anticancer properties by interfering with the dynamics of tubulin polymerization and depolymerization, resulting in mitotic arrest.[2] Combretastatin A-4 (CA-4), a natural product isolated by Pettit and co-workers in 1982 from the South African bush willow tree *Combretum caffrum*, strongly inhibits the polymerization of tubulin by binding to the colchicine site.[3] CA-4 exerts potent cytotoxicity against a variety of human cancer cells including multidrug resistant (MDR) cancer cell lines.[4] Moreover, CA-4 has been demonstrated to elicit selective and irreversible shutdown of blood flow to neoplastic cells while leaving the blood supply to healthy cells intact.[5]

Despite its very potent cytotoxic and antitubulin activities in vitro, CA-4 as an antimitotic agent showed poor antitumor effects in in vivo models due to, at least in part, its limited water solubility.[1] On the other hand, CA-4P, a disodium phosphate prodrug, displayed potent antivascular and antitumor effects in a wide range of preclinical tumor models[5] and is currently in phase II clinical trials. Due to the dual antivascular/antitumor features of CA-4P, a number of diverse ligands designed to mimic CA-4 have been synthesized[6] and are broadly categorized as CA-4 derivatives and CA-4 analogues. The CA-4 derivatives[6,7] (Chart 1), for example, CA-4P,[7a] CA-1P[7b] (Oxi-4503), AC-7700,[7c] not only demonstrate substantial antivascular activity in tumor blood flow, distinguishing it from the normal tissues,[8,9] but also show antitumor effects in some tumor models,[10] alone or in combination with the other chemotherapeutic drugs, such as cisplatin, carboplatin, 5-FU, and doxorubicin, or radiotherapeutic treatment.[11] The CA-4 analogues[12] (Chart 2), containing a variety of hetero-

Chart 1



Combretastatin A-4 : R_1 = H; R_2 = OH
Combretastatin A-1 : R_1= R_2 = OH
Combretastatin A-1P : R_1 = R_2 = OPO_3Na_2
Combretastatin A-4P : R_1 = H; R_2 = OPO_3Na_2
AVE-8063 : R_1 = H; R_2 = NH_2
AVE-8062 : R_1 = H; R_2 = NH-Serine



Phenstatin : R_1 = R_4 = H; R_2 = OH
R_3 = OCH_3
Hydroxyphenstatin : R_1 = R_4 = H;
R_2 = OH; R_3 = OCH_3
1 : R_1 = NH_2; R_2 = R_4 = H; R_3 = OCH_3
2 : R_1 = NH_2; R_2 = R_3 = H; R_4 = OCH_3

* Corresponding author. Division of Biotechnology and Pharmaceutical Research, National Health Research Institutes, 9F, 161, Sec. 6, Min-Chiuan East Road, Taipei 114, Taiwan, Republic of China. Phone: 886-2-2653-4401 ext. 6577; fax: 886-2-2792-9703; e-mail: hphsieh@nhri.org.tw.

† Medicinal Synthetic Laboratory, Division of Biotechnology and Pharmaceutical Research.

‡ Division of Cancer Research.

§ These authors contributed equally.

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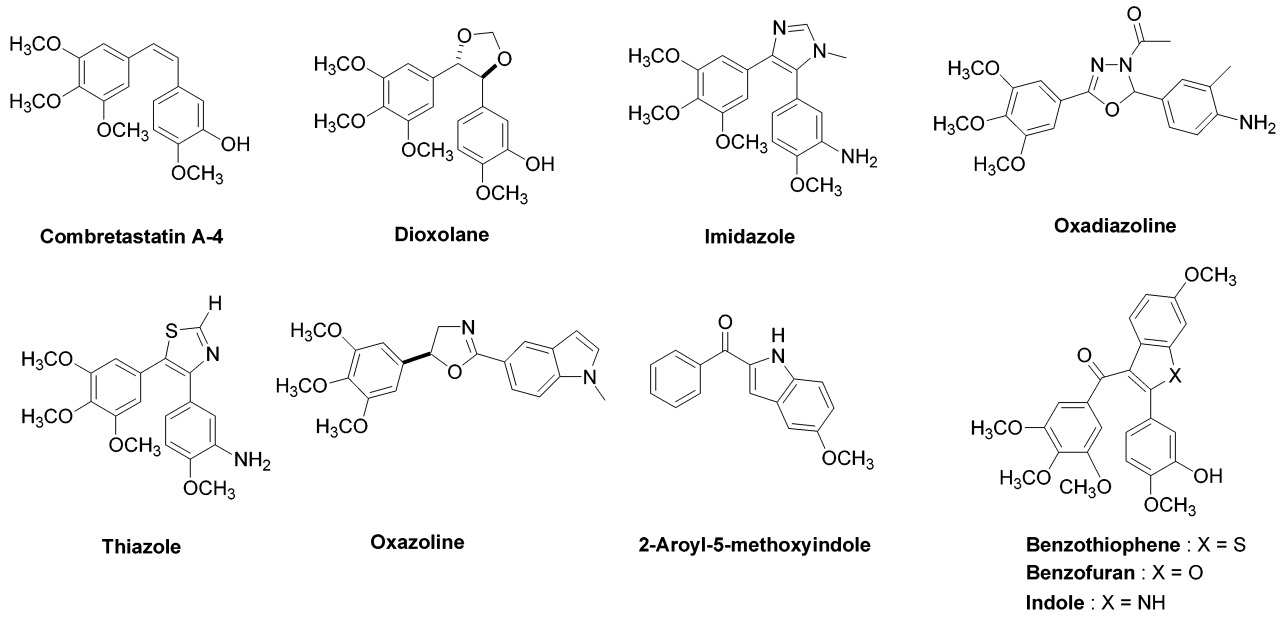
Chart 2



cyclic moieties, such as oxadiazolines, imidazoles, oxazolines, benzothiophenes, benzofurans, indoles, thiazoles and tetrazoles, not only display efficient inhibition of tubulin polymerization but also exert potent cellular growth inhibition in different cancer lines including MDR cancer cells. It is worthy to note that some of CA-4 analogues, such as imidazole-based CA-4[12b] and 2-aroyl-5-methoxyindoles,[13] exhibited oral availability leading to solid tumor regression in in vivo tumor models.

Ohsumi and co-workers reported a series of aminocombretastatins in 1998.[7c] Among these derivatives, AVE-8063, with an amino group replacing the hydroxyl group at the C-3 position of CA-4, showed potent antitubulin activity and cytotoxicity against murine colon 26 adenocarcinoma cells.[9c] Its serine-prodrug AVE-8062 (formally AC-7700) was found to possess more potent antivascular and antitumor activities in comparison with CA-4P.[9c,14] Additionally, Hori and coworkers have demonstrated that AVE-8062 exerts antivascular and antitumor effects both in rapidly proliferating transplanted tumors and in relatively slowly proliferating primary tumors induced by chemical carcinogens.[14] Since early 2002, AVE-8062 is undergoing phase I clinical trials in the Europe and the US.[15] Phenstatin, another CA-4 derivative designed from CA-4 skeleton by replacing the olefin group with a carbonyl group while retaining 3-hydroxy-4-methoxybenzene as B-ring, was discovered as a potent antitubulin agent by Pettit's lab.[7d]

We have been actively engaged in searching for novel antimitotic benzophenone-type molecules that target tubulin[16] and have revealed that the presence of an amino group at the C-2 position of the benzophenone ring, for example 2-aminobenzophenones **1** and **2**, increased cytotoxic activity by 100-fold compared to the corresponding compounds with a hydroxyl group at the C-2 position.[16a] In continuation of our work in this field, we designed a series of 3-aminobenzophenone compounds based on the structures of AVE-8063 and phenstatin (Chart 3) and report herein their synthesis and cytotoxic activities against a variety of human cancer cells including an MDR-positive cancer cell line, antitubulin activity, and the ability of colchicine competition-binding.

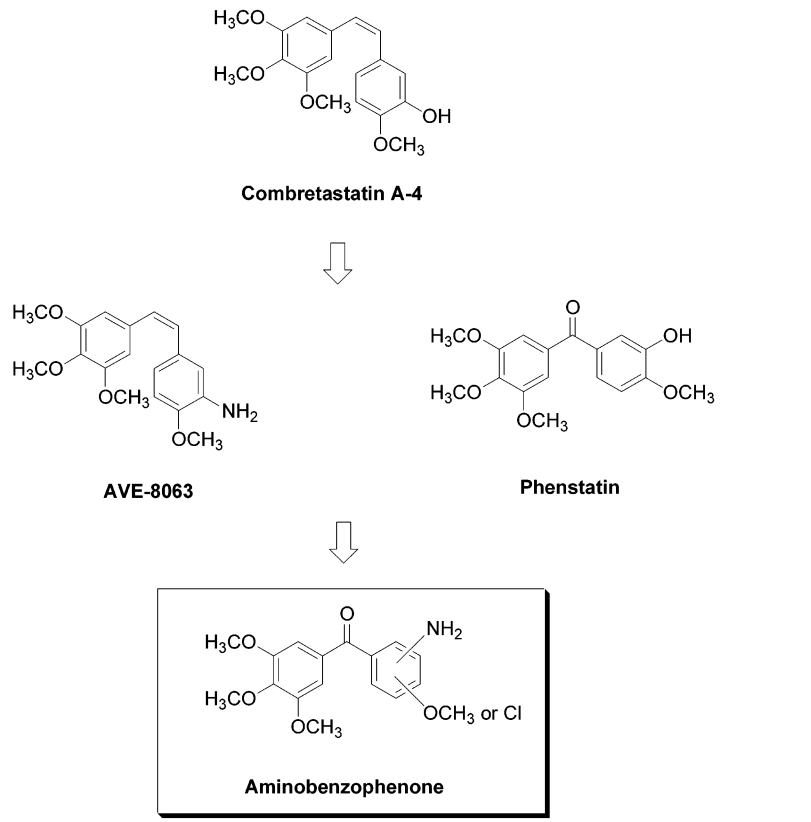
Results and Discussion

Chemistry. The general method for the synthesis of 3-aminobenzophenones is shown in Scheme 1. The preparation involved a straightforward reaction sequence with high yields (overall 54−70% in three steps): (1) Grignard reaction of (3,4,5-trimethoxyphenyl)magnesium bromide with various substituted 3-nitrobenzaldehydes yielded the corresponding secondary benzyl alcohols **A**; (2) pyridinium dichromate (PDC) oxidation of **A** to the corresponding 3-nitrobenzophenones **B**; and (3) reduction of the nitro group in **B** with Fe/AcOH to afford the desired substituted 3-aminobenzophenones **C**.

Most of the methoxy- or chloro-substituted 3-nitrobenzaldehydes were commercially available except for the 3-chloro-5-nitrobenzaldehyde **18** and 3-methoxy-5-nitrobenzaldehyde **22**, which were synthesized as shown in Schemes 2 and 3, respectively. Compound **18** was obtained via a three-step synthesis starting from commercially available 3,5-dinitrobenzyl alcohol (Scheme 2). Selective reduction of 3,5-dinitrobenzyl alcohol with ammonium sulfite in methanol afforded the corresponding 3-amino-5-nitrobenzyl alcohol which was converted to 3-chloro-5-nitrobenzyl alcohol by Sandmeyer reaction in the presence of $NaNO_2$ and $CuCl_2$. Finally, PDC oxidation of which then gave the desired benzaldehyde **18**.[17] Another key building block 3-methoxy-5-nitrobenzaldehyde **22** was obtained from 3,5-dinitrobenzoic acid via sequential reactions: nucleophilic addition by lithium methoxide in hexamethylphosphoric triamide[18] followed by BH_3 reduction and PDC oxidation as shown in Scheme 3.

To elongate the chain length of the alkoxy group at the C-4 position, a series of 4-alkoxy-3-nitrobenzaldehydes **24**−**26** were synthesized (Scheme 4) by base-promoted *O*-alkylation of 4-hydroxy-3-nitrobenzaldehyde with ethyl, propyl, and isopropyl iodide in the

Chart 3

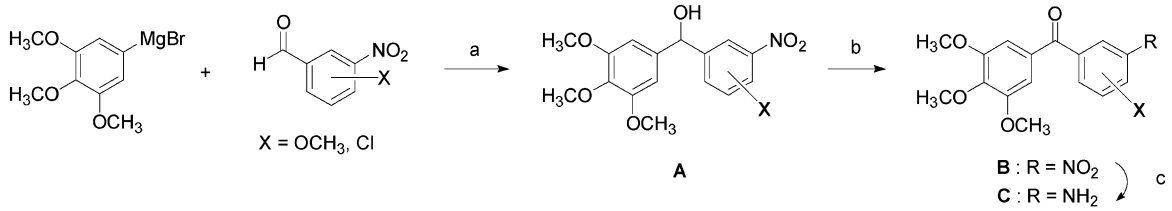


1 : R = 4-OCH_3
2 : R = 5-OCH_3
3 : R = 4-Cl
4 : R = 5-Cl

5 : R = 4-OCH_3; Y = O
6 : R = 5-OCH_3; Y = O
7 : R = 6-OCH_3; Y = O
8 : R = 4-Cl; Y = O
9 : R = 5-Cl; Y = O

10 : R = 6-Cl; Y = O
11 : R = 4-OEt; Y = O
12 : R = 4-OPr; Y = O
13 : R = 4-O^{i}Pr; Y = O
14 : R = 4-OCH_3; Y = H, H

Scheme 1[a]



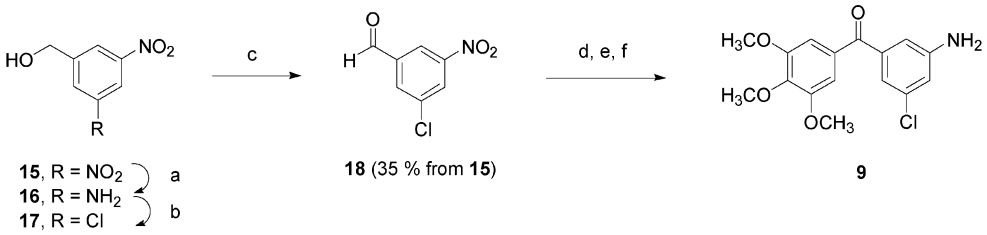
[a] Reagents and conditions: (a) THF, 0−25 °C; (b) PDC, CH_2Cl_2, 25 °C; (c) Fe, AcOH, EtOH, reflux

presence of K_2CO_3 in anhydrous CH_3CN in 75−82% yield. The reduction of the benzylic carbonyl group of compound **5** to the methylene with an efficient protocol utilizing $NaBH_4$/TFA at room temperature afforded **14** in 62% yield (Scheme 5).

Biological Evaluation. (A) In Vitro Cell Growth Inhibitory Activity. The synthesized aminobenzophenones **1**−**14** were evaluated for their cytotoxic activities against three types of human cancer cell lines, oral epidermoid carcinoma KB cells, colorectal carcinoma HT29 cells, and stomach carcinoma TSGH cells, as well as one type of MDR-positive cell line: KB−VIN10 cells, overexpressed P-gp 170/MDR (Table 1).

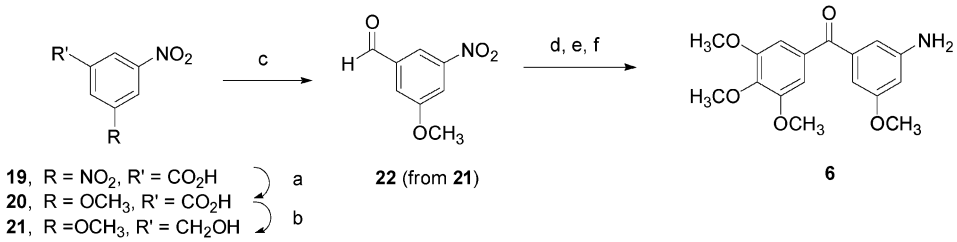
We first evaluated the cytotoxic effect of an amino substitution at the C-3 position. Newly synthesized phenstatin analogue **5**, with an amino group at the C-3 position on the B-ring, exhibited similar or greater cytotoxic activity than phenstatin. Changing the posi-

Scheme 2[a]



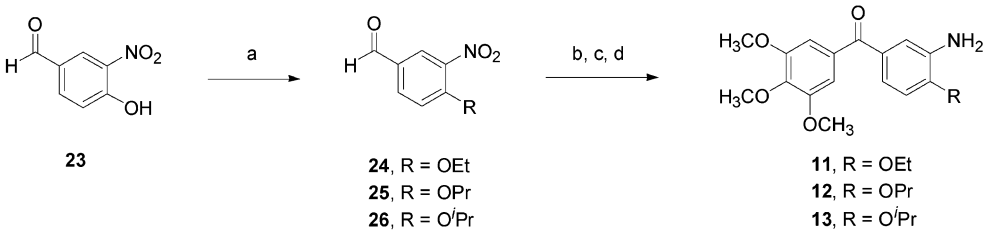
[a] Reagents and conditions: (a) $(NH_4)_2S$, MeOH, reflux; (b) $NaNO_2$, HCl, then $CuCl_2$, 60 °C; (c) MnO_2, CH_2Cl_2, 25 °C; (d) 3,4,5-trimethoxyphenylmagnesium bromide, THF, 0−25 °C; (e) PDC, CH_2Cl_2, 25 °C; (f) Fe, AcOH, EtOH, reflux

Scheme 3[a]



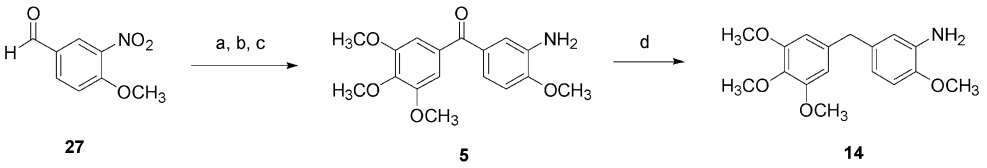
[a] Reagents and conditions: (a)$LiOCH_2$, HMPA, 25 °C; (b) BH_3, THF, 0−25 °C; (c) PDC, CH_2Cl_2, 25 °C; (d) 3,4,5-trimethoxyphenylmagnesium bromide, THF, 0−25 °C; (e) PDC, CH_2Cl_2, 25 °C; (f) Fe, AcOH, EtOH, reflux

Scheme 4[a]



[a] Reagents and conditions: (a) K_2CO_3, EtI, PrI, or *i*-PrI, CH_3CN, reflux; (b) 3,4,5-trimethoxyphenylmagnesium bromide, THF, 0−25 °C; (c) PDC, CH_2Cl_2, 25 °C; (d) Fe, AcOH, EtOH, reflux

Scheme 5[a]



[a] Reagents and conditions: (a) 3,4,5-trimethoxyphenylmagnesium bromide, THF, 0−25 °C; (c) PDC, CH_2Cl_2, 25 °C; (d) Fe, AcOH, EtOH, reflux. (d) $NaBH_4$, TFA, CH_2Cl_2, 25 °C

tion of the methoxy group on the B-ring from C-4 to C-5 and C-6, as in compounds **6** and **7,** respectively, decreased cytotoxicity by 5- to 10-fold as compared to **5**. Similar tendencies were also observed in the 2-aminobenzophenone series (**1** vs phenstatin and **1** vs **2**).

Replacement of a methoxy group (**5**, **6**, and **7**) with a chloro group (**8**, **9**, and **10**) was then examined. It is interesting to note that compound **10** with a chloro group at the C-6 position maintains substantial potency as compared to **5,** while substitution at the C-4 and C-5 positions (**8** and **9**) resulted in significant decrease in cytotoxicity. A comparison of substituent effect by chloro and methoxy groups in 2-aminobenzophenones and 3-aminobenzophenones (**1** vs **3**, **2** vs **4**, **5** vs **8**, **6** vs **9**, and **7** vs **10**) indicates that chloro substitution in 2-aminobenzophenones was more tolerated than in 3-aminobenzophenones. A surprising observation was that a chloro group located at the para position to an amino group (**4** and **10**) retained potency in cytotoxicity assay, although it was previously reported that replacement of a methoxy group with a chloro group resulted in a decrease of potency.[16b] Moreover, chloro substitution at the C-5 position of 2-aminobenzophenones (**4** vs **2**) as well as at the C-6 position of 3-aminobenzophenones (**10** vs **7**) showed maximal effects. Replacing a carbonyl group (**5**) with a methylene group (**14**) resulted in complete loss of activity indicating that the carbonyl moiety between A-ring and B-ring is critical for activity.

To understand the steric effect of alkoxy substituents at the C-4 position, we synthesized ethoxy, propyloxy, and isopropyloxy compounds **11**, **12**, and **13**, respectively. Although compound **11** retained substantial cytotoxicity in comparison with **5** and phenstatin, further increase in the bulkiness of the alkoxy moiety resulted in drastic decrease in potency. This revealed that steric effect of the substitutions at the crucial C-4 position of B-ring in aminobenzophenones significantly influences their cytotoxic activities. This is consistent

Table 1. IC_{50} Values (nM ± SD[a]) of Aminobenzophenone Analogues (**1**−**14**), Phenstatin, AVE-8063, and CA-4

compd	cell type (IC_{50} nM ± SD)[a]			
	KB	KB-Vin10	HT29	TSGH
1[7j]	32 ± 17	24 ± 8	42 ± 7	26 ± 3
2[7j]	127 ± 35	352 ± 87	148 ± 26	100 ± 12
3	710 ± 91	800 ± 73	1000 ± 33	800 ± 63
4[7o]	195 ± 18	162 ± 28	218 ± 42	185 ± 29
5	32 ± 2	31 ± 6	33 ± 9	33 ± 4
6	590 ± 31	307 ± 53	163 ± 14	157 ± 33
7	306 ± 45	324 ± 26	400 ± 78	497 ± 67
8	800 ± 88	3800 ± 278	4000 ± 396	4000 ± 729
9	1400 ± 128	2900 ± 35	1900 ± 77	1500 ± 25
10	180 ± 45	125 ± 18	163 ± 29	157 ± 31
11	59 ± 11	40 ± 9	45 ± 7	42 ± 16
12	3800 ± 260	8600 ± 460	9000 ± 850	4800 ± 244
13	900 ± 84	800 ± 33	1300 ± 169	900 ± 47
14	>10000	>10000	>10000	>10000
Phenstatin[7j]	30 ± 6	30 ± 4	560 ± 28	170 ± 5
AVE-8063[7c]	8.2 ± 1	7.5 ± 2	7.9 ± 8	7.7 ± 9
CA-4[7j]	2.1 ± 3	2.4 ± 5	260 ± 6	45 ± 12

[a] SD: standard deviation, all experiments were independently performed at least three times.

with observations in other literature reports on CA-4 derivatives[19] which suggest that the tolerance of alkoxy substitution in CA-4 family is limited up to ethoxy substitution.

A comparison between cytotoxic activities of **5**, phenstatin, AVE-8063, and CA-4 reveals that the introduction of an amino group at the C-3 position is beneficial for potency especially against HT29 and TSGH cells. This result is in agreement with our observation in the 2-aminobenzophenone series (**1** vs phenstatin). Hence, it may be concluded that an amino group located at the B-ring, either at the C-2 or C-3 position, seems to play an integral role in the inhibition of cellular growth. Moreover, all synthesized 3-aminobenzophenones **6**−**13** overcome MDR-positive resistant cells (KB−Vin10), indicating 3-aminobenzophenones are not substrates for efflux pump.

(B) Inhibition of Tubulin Polymerization. As CA-4, phenstatin, and AVE-8063 have been well documented to show interactions with tubulin, 14 synthesized aminobenzophenones were evaluated for their antitubulin polymerization activities as shown in Table 2. The results demonstrated that the cytotoxicities of the aminobenzophenones correlated well with the inhibitory ability of tubulin polymerization. For instance, six compounds **1**, **2**, **4**, **5**, **10**, and **11** exhibited strong antitubulin activities ($IC_{50} \leq 1.0$ μM) and exhibited higher cytotoxic activities than the other eight aminobenzophenones. It is interesting to note that two chloro-containing compounds **4** and **10** with a chloro group located at the para position to an amino group possess a unique alignment which imparts them with 10-fold more potency than the other two chloro-containing compounds **8** and **9** in the antitubulin activity assay. Another unexpected observation is that compound **4** (IC_{50} = 0.1 μM) displays 3- to 10-fold more potent antitubulin activity than AVE-8063 and CA-4 (0.3 μM and 1.0 μM) but shows only moderate cytotoxic activity.

Taking into account the influence of methoxy substitutent, we found that both 4′-methoxy **5** (IC_{50} = 0.3 μM) and 4′-ethoxy **11** (IC_{50} = 0.7 μM) showed similar or greater antitubulin activities than CA-4, phenstatin, and AVE-8063. Shifting methoxy substitution to different positions (**5** vs **6** and **7**) or changing to a more bulky

Table 2. Inhibition of Tubulin Polymerization by Aminobenzophenone Analogues (**1**−**14**), Phenstatin, AVE-8063, and CA-4

compound	inhibition of tubulin polymerization IC_{50} (μM ± SD)[a]
1	0.4 ± 0.1
2	1.0 ± 0.2
3	2.7 ± 0.5
4	0.1 ± 0.1
5	0.3 ± 0.2
6	2.6 ± 0.6
7	2.9 ± 0.7
8	11 ± 2
9	17 ± 3
10	0.6 ± 0.2
11	0.7 ± 0.3
12	42 ± 8
13	6.4 ± 0.8
14	> 50
Phenstatin	0.4 ± 0.2
AVE-8063	0.3 ± 0.2
CA-4	1.0 ± 0.3

[a] SD: standard deviation, all experiments were independently performed at least three times.

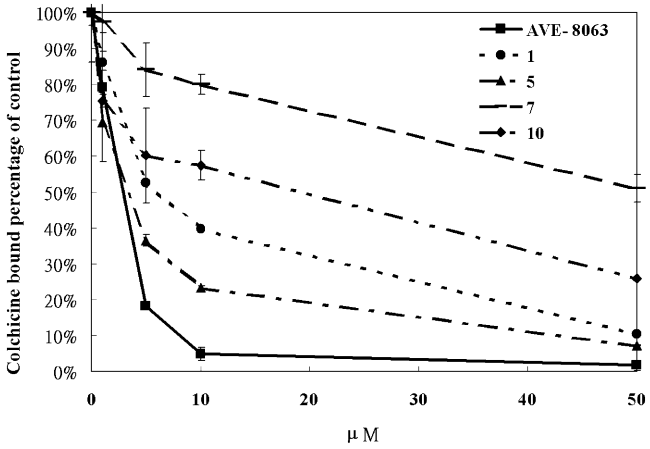


Figure 1. Inhibition of colchicine binding to MAP-rich tubulin for compounds **1**, **5**, **7**, **10**, and AVE-8063 at 1, 5, 10, and 50 μM.

alkoxy substitution (**5** vs **11** and **12**) led to a decrease in activity. The order of inhibition of tubulin polymerization by aminobenzophenones with methoxy groups in the B-ring is **5** ≅ **1** > **2** > **6** ≅ **7,** indicating that a methoxy substitutent at the C-4 position plays an important role to impart both strong antitubulin and cytotoxic activities while the position of amino group is more flexible and can either be at the C-2 or C-3 position.

(C) Inhibition of Colchicine Binding Activity. To examine the binding affinity of aminobenzophenones to the colchicine-binding domain, we selected the compounds **1**, **5**, **7**, and **10** to determine their ability to compete for colchicine-binding sites. It was observed that **1** and **5** showed strong inhibition of colchicine-binding which was correlated with their strong antitubulin and cytotoxic activities whereas compound **7** was less potent in tubulin polymerization due to weak binding affinity to colchicine-binding sites (Figure 1). Additionally, it is suggested that 3-amino-6-chloro-substituted compound **10** exhibited relatively potent cytotoxicity resulting from effective inhibition of tubulin polymerization and colchicine-binding activity.

Conclusions

In our newly synthesized 3-aminobenzophenone series, lead compounds **5** and **11**, with alkoxy groups at the C-4 position of B-ring, are potent cytotoxic agents and inhibitors of tubulin polymerization through the binding with the colchicines-binding site of tubulin. The compounds **5** and **11** exhibit cytotoxic activity with IC_{50} range from 31 to 59 nM in a variety of human cell lines from different organs. They also show similar or greater antitubulin activities than CA-4, phenstatin, and AVE-8063. In addition, the methoxy group should preferably be located at the C-4 position for better activity rather than at the C-5 or C-6 position and the tolerance of alkoxy substitution at the C-4 position could be extended up to an ethoxy group. Furthermore, replacement of the methoxy group with a chloride at the C-4 and C-5 positions (**8** and **9**) results in significant decrease in cytotoxicity. Notably, with a chloro moiety located at the para position to an amino group, compounds **4** and **10** possess an unique alignment to produce both strong cytotoxic and antitubulin activities. The complete loss of activity in **14** indicates a carbonyl linker between A-ring and B-ring is critical for activity. Examination of the SAR in aminobenzophenone analogues revealed that introduction of an amino group in the B ring plays an integral role for maximal cytotoxicity though its position appears to be more flexible as compared to methoxy group and can either be at the C-2 or C-3 position.

Experimental Section

(A) General Methods. Melting points were determined on a Yanaco (MP-500D) melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectra were obtained with a Varian Mercury-300 spectrometer operating at 300 MHz and at 75 MHz, respectively, with chemical shift in parts per million (ppm, δ) downfield from TMS as an internal standard. High-resolution mass spectra (HRMS) were measured with a Finnigan (MAT-95XL) electron impact (EI) mass spectrometer. Elemental analyses were performed on a Heraeus CHN−O Rapid microanalyzer. Flash column chromatography was done using silica gel (Merck Kieselgel 60, No. 9385, 230-400 mesh ASTM). Monitoring of reaction by TLC used Merck 60 F_{254} silica gel glass backed plates (5 × 10 cm); zones were detected visually under ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. All solvents were dried according to standard procedures. All reagents were used as purchased without further treatment unless otherwise stated. All reactions were carried out under an atmosphere of dry nitrogen.

(B) Chemistry. (a) General Procedure for the Preparation of Substituted 3-Aminobenzophenone Derivatives (3, 5−13). Procedure A. 3,4,5-Trimethoxyphenylmagnesium bromide (1.0 M) prepared from 3,4,5-trimethoxyphenyl bromide[16a] (8.08 mmol) and magnesium turnings (8.9 mmol) in anhydrous tetrahydrofuran (8−9 mL) was added slowly to the corresponding substituted 3-nitrobenzoaldehydes in tetrahydrofuran (10 mL) at 0 °C. The reaction mixture was warmed to room temperature, and stirring was continued for another 30 min. A saturated NH_4Cl solution (10 mL) was slowly added to hydrolyze the adduct at 0 °C, and the mixture was extracted with EtOAc (10 mL × 3). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash column to furnish desired benzyl alcohol.

Procedure B. To a stirred solution of benhydrol (4 mmol) and 4 Å molecular sieves (0.60 g) in dichloromethane (20 mL) at room temperature was added pyridinium dichromate (PDC, 6 mmol) in portions. After 16 h, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography to obtain desired substituted 3-nitrobenzophenone.

Procedure C. A stirred suspension of substituted 3-nitrobenzophenone (1 mmol) and iron powder (0.50 g) in ethanol (5 mL), acetic acid (5 mL), water (2 mL), and 37% hydrochloric acid (1 drop) was vigorously stirred and refluxed. After 1 h, the reaction mixture was cooled and filtered through Celite. The filtrate was diluted with sat. $NaHCO_3$ and extracted by CH_2Cl_2 (10 mL × 3). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was further purified by flash chromatography to yield desired substituted 3-aminobenzophenone.

(b) (2-Amino-4-chlorophenyl)(3,4,5-trimethoxyphenyl)-methanone (3). The title compound was obtained in 70% overall yield from 3,4,5-trimethoxyphenyl bromide and 4-chloro-2-nitrobenaldehyde in three steps following procedures A−C; mp 129−130 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 6H, 2 × OCH_3), 3.91 (s, 3H, OCH_3), 6.14 (br, 2H), 6.56 (dd, J = 1.8, 8.4 Hz, 1H), 6.73 (d, J = 1.8 Hz, 1H), 6.85 (s, 2H), 7.42 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 56.2, 60.8, 106.4, 115.6, 116.0, 116.3, 134.5, 135.1, 139.8, 140.5, 151.3, 152.4, 196.7. MS (EI) m/z: 321 (M^+, 100%), 323 (M + 2, 34%). HRMS (EI) for $C_{16}H_{16}ClNO_4$ (M^+): calcd, 321.0774; found, 321.0771. Anal. ($C_{16}H_{16}ClNO_4$) C, H, N.

(c) (3-Amino-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (5). The title compound was obtained in 66% overall yield from 3,4,5-trimethoxyphenyl bromide and 4-methoxy-3-nitrobenaldehyde following procedures A−C; mp 146.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.82 (d, J = 8.4 Hz, 2H), 7.02 (s, 1H), 7.21 (dd, J = 8.1, 2.1 Hz, 1H), 7.26 (s, 1H). ^{13}C NMR ($CDCl_3$): δ 55.7, 56.3, 60.9, 107.2, 108.8, 115.6, 122.1, 130.3, 133.3, 135.9, 141.1, 150.5, 152.4, 194.7. MS (EI) m/z: 317 (M^+, 100%), 274 (43%). HRMS (EI) for $C_{17}H_{19}NO_5$ (M^+): calcd, 317.1271; found, 317.1267. Anal. ($C_{17}H_{19}NO_5$) C, H, N.

(d) (3-Amino-5-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (6). The title compound was obtained in 61% overall yield from 3,4,5-trimethoxyphenyl bromide and 3-methoxy-5-nitrobenaldehyde **16** following procedures A−C; mp 165 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H, OCH_3), 3.88 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 6.43 (t, J = 2.25 Hz, 1H), 6.68−6.69 (m, 2H), 7.08 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 55.4, 56.3, 60.9, 104.6, 104.9, 107.5, 109.2, 132.4, 139.5, 141.6, 147.3, 152.4, 160.1, 195.2. MS (EI) m/z: 317 (M^+, 100%). HRMS (EI) for $C_{17}H_{19}NO_5$ (M^+): calcd, 317.1265; found, 317.1264. Anal. ($C_{17}H_{19}NO_5$) C, H, N.

(e) (3-Amino-6-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (7). The title compound was obtained in 59% overall yield from 3,4,5-trimethoxyphenyl bromide and 2-methoxy-5-nitrobenaldehyde following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.67 (s, 3H, OCH_3), 3.83 (s, 6H, 2 × OCH_3), 3.92 (s, 3H, OCH_3), 6.68 (t, J = 2.4 Hz, 1H), 6.79 (dd, J = 2.1, 8.7 Hz, 1H), 6.83 (d, J = 8.7 Hz, 1H), 7.10 (s, 2H). ^{13}C NMR ($CDCl_3$): δ 56.1, 56.3, 60.8, 107.3, 113.2, 115.9, 118.1, 129.4, 132.6, 139.9, 142.4, 150.0, 152.7, 195.2. MS (EI) m/z: 317 (M^+, 100%). Anal.($C_{17}H_{19}NO_5$) C, H, N.

(f) (3-Amino-4-chlorophenyl)(3,4,5-trimethoxyphenyl)-methanone (8). The title compound was obtained in 69% overall yield from 3,4,5-trimethoxyphenyl bromide and 4-chloro-3-nitrobenaldehyde following procedures A−C; mp 125−126 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 7.02 (s, 2H), 7.04 (dd, J = 8.1, 2.1 Hz, 1H), 7.20 (d, J = 1.8 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 56.6, 60.9, 107.4, 116.3, 120.2, 123.0, 128.8, 132.2, 137.0, 141.8, 142.7, 152.5, 194.5. MS (EI) m/z: 321 (M^+, 100%), 323 (M + 2, 41%). HRMS (EI) for $C_{16}H_{16}ClNO_4$ (M^+): calcd, 321.0764; found, 321.0766. Anal. ($C_{16}H_{16}ClNO_4$) C, H, N.

(g) (3-Amino-4-chlorophenyl)(3,4,5-trimethoxyphenyl)-methanone (9). The title compound was obtained in 58% overall yield from 3,4,5-trimethoxyphenyl bromide and 3-chloro-5-nitrobenaldehyde **15** following procedures A−C; mp 120−121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 6H, 2 × OCH_3),

3.93 (s, 3H, OCH_3), 4.23 (br, 2H), 7.03 (s, 2H), 7.05 (d, J = 2.1, 1H), 7.20 (d, J = 1.8 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H).^{13}C NMR ($CDCl_3$): δ 56.3, 61.0, 107.4, 116.3, 120.2, 123.0, 128.8, 132.2, 137.0, 141.7, 142.8, 152.5, 194.5. MS (EI) *m/z*: 321 (M^+, 100%), 323 (M + 2, 38%). HRMS (EI) for $C_{16}H_{16}ClNO_4$ (M^+): calcd, 321.0770; found, 321.0769. Anal. ($C_{16}H_{16}ClNO_4$) C, H, N.

(h) (5-Amino-2-chlorophenyl)(3,4,5-trimethoxyphenyl)-methanone (10). The title compound was obtained in 64% overall yield from 3,4,5-trimethoxyphenyl bromide and 2-chloro-5-nitrobenaldehyde following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (s, 6H, 2 x OCH_3), 3.91 (s, 3H, OCH_3), 6.63 (d, J = 3 Hz, 1H), 6.70 (dd, J = 2.7, 8.4 Hz. 1H), 7.07 (s, 2H), 7.18 (d, J = 8.4 Hz, 1H). MS (EI) *m/z*: 321 (M^+, 100%), 323 (M + 2, 39%). HRMS (EI) for $C_{16}H_{16}ClNO_4$ (M^+): calcd, 321.0773; found, 321.0770. Anal. ($C_{16}H_{16}ClNO_4$) C, H, N.

(i) (3-Amino-4-ethoxyphenyl)(3,4,5-trimethoxyphenyl)-methanone (11). The title compound was obtained in 62% overall yield from 3,4,5-trimethoxyphenyl bromide and 4-ethoxy-3-nitro- benaldehyde **24** following procedures A−C; mp 128−129 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48 (t, J = 7 Hz, 3H), 3.87 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 4.14 (q, J = 7 Hz, 2H), 6.78 (d, J = 8.4 Hz, 1H), 7.01 (s, 2H), 7.17 (dd, J = 1.95, 8.2 Hz, 1H), 7.24 (d, J = 2.1 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ15.0, 56.3, 60.9, 64.0, 107.2, 109.5, 115.6, 122.1, 130.1, 133.3, 135.9, 141.0, 149.9, 152.4, 194.7. MS (EI) *m/z*: 331 (M^+, 46%), 274 (100%). HRMS (EI) for $C_{18}H_{21}NO_5$ (M^+): calcd, 331.1416; found, 331.1418. Anal. ($C_{18}H_{21}NO_5$) C, H, N.

(j) (3-Amino-4-propoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (12). The title compound was obtained in 70% overall yield from 3,4,5-trimethoxyphenyl bromide and 3-nitro-4-propoxybenaldehyde **25** following procedures A−C; mp 86 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.08 (t, J = 7 0.5 Hz, 3H), 1.88 (h, J = 7.5 Hz, 2H), 3.87 (s, 6H, 2 × OCH_3), 3.92 (s, 3H, OCH_3), 4.04 (q, J = 6.45 Hz, 2H), 6.79 (d, J = 8.1 Hz, 1H), 7.01 (s, 2H), 7.18 (dd, J = 2.1, 8.4 Hz, 1H), 7.25 (d, J = 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ10.7, 22.6, 56.3, 60.9, 69.9, 107.2, 109.5, 115.6, 122.1, 130.0, 133.3, 135.9, 141.0, 150.0, 152.3, 194.7. MS (EI) *m/z*: 345 (M^+, 78%), 303 (100%). HRMS (EI) for $C_{19}H_{23}NO_5$ (M^+): calcd, 345.1582; found, 345.1579. Anal. ($C_{19}H_{23}NO_5$) C, H, N.

(k) (3-Amino-4-isopropoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (13). The title compound was obtained in 54% overall yield from 3,4,5-trimethoxyphenyl bromide and 4-isopropoxy-3-nitrobenaldehyde **26** following procedures A−C; mp 98−99 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (d, J = 5.7 Hz, 6H), 3.87 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 4.66 (p, J = 6.0 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 7.01 (s, 2H), 7.17 (dd, J = 2.1, 8.1 Hz, 1H), 7.24 (d, J = 1.8 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 22.2, 56.2, 60.9, 70.5, 107.2, 110.7, 115.8, 121.9, 129.8, 133.3, 136.5, 140.9, 148.7, 152.3, 194.6. MS (EI) *m/z*: 345 (M^+, 30%), 303 (100%). HRMS (EI) for $C_{19}H_{23}NO_5$ (M^+): calcd, 345.1566; found, 345.1571. Anal. ($C_{19}H_{23}NO_5$) C, H, N.

(l) 2-Methoxy-5-(3,4,5-trimethoxybenzyl)phenylamine (14). To a solution of **5** (0.20 g, 0.63 mmol) in dichloromethane (10 mL) was added sodium borohydride (0.05 g, 1.26 mmol) at room temperature. After 10 min, trifluoroacetic acid (0.23 mL, 3.15 mmol) was added dropwise to the reaction mixture. The reaction was stirred for 16 h at room temperature. The mixture was diluted with cold sat. $NaHCO_3$ and extracted into CH_2Cl_2 (10 mL × 3). The solvents were evaporated, and the concentrate was purified by flash column on silica gel eluting with 15:1 CH_2Cl_2/MeOH to yield the desired product as a yellow solid (0.12 g, 62%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80−3.84 (m, 14H), 6.38 (s, 2H), 6.52−6.56 (m, 2H), 6.71 (d, J = 7.8 Hz, 1H). MS (EI) *m/z*: 303 (M^+, 100). ^{13}C NMR ($CDCl_3$): δ 41.6, 55.4, 55.9, 60.7, 105.5, 110.0, 115.2, 118.2, 133.2, 135.7, 137.0, 145.4, 152.6. MS (EI) *m/z*: 345 (M^+, 30%), 303 (100%). HRMS (EI) for $C_{19}H_{23}NO_5$ (M^+): calcd, 345.1566; found, 345.1571. Anal. ($C_{19}H_{23}NO_5$) C, H, N.Anal. ($C_{17}H_{21}NO_4$) C, H, N.

(m) 3-Methoxy-5-nitrobenzaldehyde (22). To a stirred solution of 3-methoxy-5-nitrobenzoic acid (0.50 g, 2.53 mmol) in tetrahydrofuran at 0 °C was added 1.0 M BH_3/THF (13 mL, 12.65 mmol) dropwise. This solution was allowed to stir at room temperature for 16 h. The reaction was quenched with sat. $NaHCO_3$, extracted with CH_2Cl_2, and then evaporated the organic layer to dryness in vacuo. The residue in the presence of 4 Å molecular sieve (0.38 g) was directly dissolved in dichloromethane (20 mL), followed by addition of pyridinium dichromate (PDC) (1.43 g, 3.8 mmol) and stirred for 5 h at room temperature. The reaction mixture was filtered through a pad of Celite and washed by EtOAc. The filtrate was dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc:*n*-hexane = 1:3) to afford the desired aldehyde **22** as a pale yellow solid (0.33 mg, 71%); mp 96 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.96 (s, 3H, OCH_3), 7.71 (t, J = 1.2 Hz, 1H), 7.97 (t, J = 2.1 Hz, 1H), 8.28 (t, J = 1.2 Hz, 1H), 10.03 (s, 1H).

(n) 4-Ethoxy-3-nitrobenzaldehyde (24). To a solution of 4-hydroxy-3-nitrobenzaldehyde (1.00 g, 5.98 mmol) in acetonitrile (20 mL) were added potassium carbonate (1.65 g, 11.96 mmol) and ethyl iodide (1.00 mL, 11.96 mmol). The reaction mixture was heated under reflux for 15 h and quenched by cold water. The solution was extracted by EtOAc (15 mL × 3), dried over $MgSO_4$, and concentrated in vacuo. The residue was further purified by flash chromatography (EtOAc:*n*-hexane = 1:3) to yield the desired aldehyde **24** as a white solid (0.95 g, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.52 (t, J = 6.9 Hz, 3H), 4.29 (q, J = 7.2 Hz, 2H), 7.19 (d, J = 8.7 Hz, 1H), 8.05 (dd, J = 8.7, 2.1 Hz, 1H), 8.31 (d, J = 2.1 Hz, 1H), 9.91 (s, 1H). MS (EI) *m/z*: 195 (M^+, 100%)

(o) 3-Nitro-4-propoxybenzaldehyde (25). The title compound was obtained as a pale yellow solid in 80% yield in a similar manner for the preparation of **24** by use of propyl iodide instead of ethyl iodide. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.09 (t, J = 7.5 Hz, 3H), 1.91 (m, J = 7.2 Hz, 2H), 4.17 (t, J = 6.3 Hz, 2H), 7.20 (d, J = 8.7 Hz, 1H), 8.05 (dd, J = 8.7, 2.1 Hz, 1H), 8.30 (d, J = 1.8 Hz, 1H), 9.91 (s, 1H). MS (EI) *m/z*: 209 (M^+, 100%).

(p) 4-Isopropoxy-3-nitrobenzaldehyde (26). The title compound was obtained as pale white crystals in 73% yield in a similar manner for the preparation of **24** by use of isopropyl iodide instead of ethyl iodide. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.45 (d, J = 6 Hz, 6H), 4.82 (m, J = 6 Hz, 1H), 7.19 (d, J = 8.7 Hz, 1H), 8.03 (dd, J = 8.4, 1.8 Hz, 1H), 8.27 (d, J = 2.1 Hz, 1H), 9.89 (s, 1H). MS (EI) *m/z*: 209 (M^+, 100%).

(C) Biology. (a) Materials. Regents for cell culture were obtained from Gibco-BRL Life Technologies (Gaitherburg, MD). Microtubule-associated protein (MAP)-rich tubulin was purchased from Cytoskeleton, Inc. (Denver, CO). [^{3}H]Colchicine (specific activity, 60−87 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA).

(b) Cell Growth Inhibitory Assay. Human oral epidermoid carcinoma KB cells, colorectal carcinoma HT29 cells, and stomach carcinoma TSGH were maintained in RPMI-1640 medium supplied with 5% fetal bovine serum. KB-VIN10 cells were maintained in growth medium supplemented with 10 nM vincristine, generated from vincristine-driven selection, and displayed overexpression of P-gp170/MDR (unpublished data). Cell in logarithmic phase were cultured at a density of 5000 cells/mL/well in a 24-well plate. KB-VIN10 cells were cultured in drug-free medium for 3 days prior to use. The cells were exposed to various concentrations of the test drugs for 72 h. The methylene blue dye assay was used to evaluate the effect of the test compounds on cell growth as described previously.[20] The IC_{50} value resulting from 50% inhibition of cell growth was calculated graphically as a comparison with the control. Compounds were examined in at least three independent experiments, and the values shown for these compounds are the mean and standard deviation of these data.

(c) Tubulin Polymerization in Vitro Assay. Turbidimetric assays of microtubules were performed as described by Bollag et al.[21] MAP-rich tubulin (2 mg/mL) in 100 μL buffer containing 100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 1 mM GTP, and 2% (v/v) dimethyl sulfoxide were placed in 96-well microtiter plate in the presence of test compounds. The increase in absorbance was measured at 350 nm in a PowerWave X Microplate Reader (BIO-TEK Instruments, Winooski, VT) at

37 °C and recorded every 30 s for 30 min. The area under the curve (AUC) used to determine the concentration that inhibited tubulin polymerization to 50% (IC_{50}). The AUC of the untreated control and 10μM of colchicine was set to 100% and 0% polymerization, respectively, and the IC_{50} was calculated by nonlinear regression in at least three experiments.[22]

(d) [^{3}H] Colchicine Binding Assay. The assay was basically performed according to the method of Lambeir and Engelborghs.[23] MAP-rich tubulin was incubated with [^{3}H]-colchicine in the presence of various concentrations of test compounds in a buffer containing 0.05 M PIPES (pH 6.9), 1 mM $MgCl_2$, and 1 mM GTP. After incubating at room temperature for 1 h, the samples were centrifuged through Sephadex G-50 columns (Amersham Biosciences, Piscataway, NJ). The eluates in the flow-through were analyzed for radioactivity by scintillation counting.

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Supporting Information Available: Elemental analyses data are available for compounds **3**, **5**−**14**. The material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Li, Q.; Sham, H. L. Discovery and Development of Antimitotic Agents That Inhibit Tubulin Polymerisation for the Treatment of Cancer. *Expert Opin. Ther. Pat.* **2002**, *12*, 1663−1702.

(2) Hamel, E. Antimitotic Natural Products and Their Interactions with Tubulin. *Med. Res. Rev.* **1996**, *16*, 207−231.

(3) 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.

(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) (a) Tozer, G. M.; Kanthou, C.; Parkins, C. S.; Hill, S. A. The Biology of the Combretastatins as Tumour Vascular Targeting Agents. *Int. J. Exp. Pathol.* **2002**, *83*, 21−38. (b) Griggs, J.; Metcalfe, J. C.; Hesketh, R. Targeting Tumour Vasculature: the Development of Combretastatin A4. *Lancet. Oncol.* **2001**, *2*, 82−87.

(6) Nam, N. H. Combretastatin A-4 Analogues as Antimitotic Antitumor Agents. *Curr. Med. Chem.* **2003**, *10*, 1697−1722.

(7) For CA-4 derivatives: (a) Pettit, G. R.; Rhodes, M. R. Antineoplastic Agents 389. New Syntheses of the Combretastatin A-4 Produrg. *Anti-Cancer Drug Design* **1998**, *13*, 183−191. (b) Pettit, G. R.; Lippert, J. W., III. Antineoplastic Agents 429. Syntheses of the Combretastatin A-1 and Combretastatin B-1 Produrgs. *Anti-Cancer Drug Des.* **2000**, *15*, 203−216. (c) Ohsumi, K.; Nakagawa, R.; Fukuda, Y.; Hatanaka, T.; Morinaga, Y.; Nihei, Y.; Ohishi, K.; Suga, Y.; Akiyama, Y.; Tsuji, T. Novel Combretastatin Analogues Effective against Murine Solid Tumors: Design and Structure−Activity Relationships. *J. Med. Chem.* **1998**, *41*, 3022−3032. (d) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. Antineoplastic Agents. 379. Synthesis of Phenstatin Phosphate. *J. Med. Chem.* **1998**, *41*, 1688−1695.

(8) (a) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. Combretastatin A-4, an Agent that Displays Potent and Selective Toxicity toward Tumor Vasculature. *Cancer Res.* **1997**, *57*, 1829−1834. (b) Tozer, G. M.; Prise, V. E.; Wilson, J.; Locke, R. J.; Vojnovic, B.; Stratford, M. R. L.; Dennis, M. F.; Chaplin, D. J. Combretastatin A-4 Phosphate as a Tumor Vascular-Targeting Agent: Early Effects in Tumors and Normal Tissues. *Cancer Res.* **1999**, *59*, 1626−1634. (c) Hill, S. A.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. Preclinical Evaluation of the Antitumor Activity of the Novel Vascular Targeting Agent Oxi 4503. *Anticancer Res.* **2002**, *22*, 1453−1458.

(9) (a) Hori, K.; Saito, S.; Kubota, K. A Novel Combretastatin A-4 Derivative, AC-7700, Strongly Stanches Tumour Blood Flow and Inhibits Growth of Tumors Developing in Various Tissues and Organs. *Br. J. Cancer* **2002**, *86*, 1604−1614. (b) Hori, K.; Saito, S.; Nihei, Y.; Suzuki, M.; Sato, Y. Antitumor Effects Due to Irreversible Stoppage of Tumor Tissue Blood Flow: Evaluation of a Novel Combretastatin A-4 Derivative, AC-7700. *Jpn. J. Cancer Res.* **1999**, *90*, 1026−1038. (c) Nihei, Y.; Suzuki, M.; Okano, A.; Tsuji, T.; Akiyama, Y.; Tsuruo, T.; Saito, S.; Hori, K.; Sato, Y. Evaluation of Antivascular and Antimitotic Effects of Tubulin Binding Agents in Solid Tumor Therapy. *Jpn. J. Cancer Res.* **1999**, *90*, 1387−1395.

(10) (a) Hill, S. A.; Chaplin, D. J.; Lewis, G.; Tozer, G. M. Schedule Dependence of Combretastatin A4 Phosphate in Transplated and Spontaneous Tumor Models. *Int. J. Cancer* **2002**, *102*, 70−74. (b) Boehle, A. S.; Sipos, B.; Kliche, U.; Kalthoff, H.; Dohrmann, P. Combretastatin A-4 Prodrug Inhibits Growth of Human Non-Small Cell Lung Cancer in a Murine Xenotransplant Model. *Ann. Thorac. Surg.* **2001**, *71*, 1657−1665.

(11) (a) Morinaga, Y.; Suga, Y.; Ehara, S.; Harada, K.; Nihei, Y.; Suzuki, M. Combination Effect of AC-7700, a Novel Combretastatin A-4 Derivative, and Cisplatin against Murine and Human Tumors in Vivo. *Cancer Sci.* **2003**, *94*, 200−204. (b) Li, L.; Rojianai, A. M.; Siemann, D. W. Preclinical Evaluations of Therapies Combining the Vascular Targeting Agent Combretastatin A-4 Disodium Phosphate and Conventional Anticancer 41Therapies in the Treatment of Kaposi's Sarcoma. *Acta Oncol.* **2002**, *41*, 91−97. (c) Grosios, K.; Loadman, P. M.; Swaine, D. J.; Pettit, G. R.; Bibby, M. C. Combination Chemotherapy with Combretastatin A-4 Phosphate and 5-Fluorouracil in an Experimental Murine Colon Adenocarcinoma. *Anticancer Res.* **2000**, *20*, 29−234. (d) Nelkin, B. D.; Ball, D. W. Combretastatin A-4 and Doxorubicin Combination Treatment is Effective in a Preclinical Model of Human Medullary Thyroid Carcinoma. *Oncol. Rep.* **2001**, *8*, 157−160. (e) Murata, R.; Overgaard, J.; Horsman. M. R. Combretastatin A-4 Disodium Phosphate: a Vascular Targeting Agent that Improves the Antitumor Effects of Hyperthermia, Radiation, and Mild Thermoradiotherapy. *Int. J. Radiat. Oncol. Biol. Phys.* **2001**, *51*, 1018−1024.

(12) For CA-4 analogues: (a) Shirai, R.; Takayama, H.; Nishikawa, A.; Koiso, Y.; Hashimoto, Y. Asymmetric Synthesis of Antimitotic Combretadioxolane with Potent Antitumor Activity Against Multi-Drug Resistant Cells. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1997−2000. (b) Wang, L.; Woods, K. W.; Li, Q.; Barr, K. J.; McCroskey, R. W.; Hannick, S. M.; Gherke, L.; Credo, R. B.; Hui, Y. H.; Marsh, K.; Warner, R.; Lee, J. Y.; Zielinski-Mozng, N.; Frost, D.; Rosenberg, S. H.; Sham, H. L. Potent, Orally Active Heterocycle-based Combretastatin A-4 Analogues: Synthesis, Structure−Activity Relationship, Pharmacokinetics, and in Vivo Antitumor Activity Evaluation. *J. Med. Chem.* **2002**, *45*, 1697−1711. (b) Szczepankiewicz, B. G.; Liu, G.; Jae, H. S.; Tasker, A. S.; Gunawardana, I. W.; von Geldern, T. W.; Gwaltney, S. L. II; Wu-Wong, J. R.; Gehrke, L.; Chiou, W. J.; Credo, R. B.; Alder, J. D.; Nukkala, M. A.; Zielinski, N. A.; Jarvis, K.; Mollison, K. W.; Frost, D. J.; Bauch, J. L.; Hui, Y. H.; Claiborne, A. K.; Li, Q.; Rosenberg, S. H. New Antimitotic Agents with Activity in Multi-Drug-Resistant Cell Lines and in Vivo Efficacy in Murine Tumor Models. *J. Med. Chem.* **2001**, *44*, 4416−4430. (c) Pinney, K. G.; Bounds A. D.; Dingeman, K. M.; Mocharla, V. P.; Pettit, G. R.; Bai, R.; Hamel, E. A New Anti-Tubulin Agent Containing The Benzo[*b*]thiophene Ring System. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1081−1086. (d) Flynn, B. L.; Hamel, E.; Jung, M. K. One-Pot Synthesis of Benzo[*b*]furan and Indole Inhibitors of Tubulin Polymerization. *J. Med. Chem.* **2002**, *45*, 2670−2673.

(13) (a) Mahboobi; S.; Pongratz; H.; Hufsky; H.; Hockemeyer; J.; Frieser; M.; Lyssenko; A.; Paper; D. H.; Bürgermeister; J.; Böhmer; F. D.; Fiebig; H. H.; Burger; A. M.; Baasner; S.; Beckers; T. Synthetic 2-Aroylindole Derivatives as a New Class of Potent Tubulin-Inhibitory; Antimitotic Agents. *J. Med. Chem.* **2001**, *44*, 4535−4553. (b) Beckers; T.; Reissmann; T.; Schmidt; M.; Burger; A. M.; Fiebig; H. H.; Vanhoefer; U.; Pongratz; H.; Hufsky; H.; Hockemeyer; J.; Frieser; M.; Mahboobi; S. 2-Aroylindoles; a Novel Class of Potent; Orally Active Small Molecule Tubulin Inhibitors. *Cancer Res.* **2002**, *62*, 3113−3119.

(14) (a) Hori, K.; Saito, S. Microvascular Mechanisms by which the Combretastatin A-4 Derivative AC7700 (AVE8062) Induces Tumor Blood Flow Stasis. *Br. J. Cancer* **2003**, *89*, 1334−1344. (b) Hori, K.; Saito, S.; Sato, Y.; Akita, H.; Kawaguchi, T.; Sugiyama, K.; Sato, H. Differential Relationship between Changes in Tumour Size and Microcirculatory Functions Induced by Therapy with an Antivascular Drug and with Cytotoxic Drugs: Implications for the Evaluation of Therapeutic Efficacy of AC7700 (AVE8062). *Eur. J. Cancer* **2003**, *39*, 1957−1966. (c) Nihei, Y.; Suga, Y.; Morinaga, Y.; Ohishi, K.; Okano, A.; Ohsumi, K.; Hatanaka, T.; Nakagawa, R.; Tsuji, T.; Akiyama, Y.; Saito, S.; Hori, K.; Sato, Y.; Tsuruo, T. A Novel Combretastatin A-4 Derivative, AC-7700, Shows Marked Antitumor Activity against Advanced Solid Tumors and Orthotopically Transplanted Tumors. *Jpn. J. Cancer Res.* **1999**, *90*, 1016−1025.

(15) Thorpe, P. E.; Chaplin, D. J.; Blakey, D. C. The First International Conference on Vascular Targeting: Meeting Overview. *Cancer Res.* **2003**, *63*, 1144−1147.

(16) (a) Liou, J. P.; Chang, C. W.; Song, J. S.; Yang, Y. N.; Yeh, C. F.; Tseng, H. Y.; Lo, Y. K.; Chang, Y. L.; Chang, C. M.; Hsieh, H. P. Synthesis and Structure−Activity Relationship of 2-Aminobenzophenone Derivatives as Antimitotic Agents. *J. Med.*

Chem. **2002**, *45*, 2556−2562. (b) Hsieh, H. P.; Liou, J. P.; Lin, Y. T.; Mahindroo, N.; Chang, J. Y.; Yang, Y. N.; Chern, S. S.; Tan, U. K.; Chang, C. W.; Chen, T. W.; Lin, C. H.; Chang, Y. Y.; Wang, C. C. Structure−Activity and Crystallographic Analysis of Benzophenone Derivatives − the Potential Anticancer Agents. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 101−105.

(17) Meindl, W. R.; Angerer, E. V.; Schönenberger, H.; Ruckdeschel, G. Benzylamines: Synthesis and Evaluation of Antimycobacterial Properties. *J. Med. Chem*. **1984**, *27*, 1111−1118.

(18) Herlt, A. J.; Kibby, J. J.; Rickards, R. W. Synthesis of Unlabeled and Carboxyl-Labeled 3-Amino-5-hydroxybenzoic Acid. *Aust. J. Chem*. **1981**, *34*, 1319−1324.

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

(20) Finlay, G. J.; Baguley, B. C.; Wilson, W. R. A Semiautomated Microculture Method for Investigating Growth Inhibitory Effects of Cytotoxic Compounds on Exponentially Growing Carcinoma Cells. *Anal. Biochem*. **1984**, *139*, 272−277.

(21) Bollag, D. M.; McQueney, P. A.; Zhu, J.; Hensens, O.; Koupal, L.; Liesch, J.; Goetz, M.; Lazarides, E.; Woods, C. M. Epothilones, a New Class of Microtubule-stabilizing Agents with a Taxol-like Mechanism of Action. *Cancer Res.* **1995**, *55*, 2325−2333.

(22) Chang, J. Y.; Hsieh, H. P.; Pan, W. Y.; Liou, J. P.; Bey, S. J.; Chen, L. T.; Song, J. S. Dual Inhibition of Topoisomerase I and Tubulin Polymerization by BPR0Y007, a Novel Cytotoxic Agent. *Biochem. Pharmacol.* **2003**, *65*, 2009−2019.

(23) Lambier, A.; Engelborghs, Y. A Quantitative Analysis of Tubulin-colchicine Binding to Microtubules. *Eur. J. Biochem*. **1980**, *109*, 619−624.

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