Synthesis and Structure−Activity Relationship of 2-Aminobenzophenone Derivatives as Antimitotic Agents

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A new type of inhibitor of tubulin polymerization was discovered on the basis of the combretastatin molecular skeleton. The lead compounds in this series, compounds **6** and **7**, strongly inhibited tubulin polymerization in vitro and significantly arrested cells at the G_2/M phase. Compounds **6** and **7** yielded 50- to 100-fold lower IC_{50} values than did combretastatin A-4 against Colo 205, NUGC3, and HA22T human cancer cell lines as well as similar or greater growth inhibitory activities than did combretastain A-4 against DLD-1, HR, MCF-7, DU145, HONE-1, and MES-SA/DX5 human cancer cell lines. Structure−activity relationship information revealed that introduction of an amino group at the ortho position of the benzophenone ring plays an integral role for increased growth inhibition.

Introduction

The microtubule system of eukaryotic cells is an important target for the development of anticancer agents. Consequently, perturbation of normal tubulin polymerization/depolymerization is a popular target for new chemotherapeutic agents.[1] A variety of clinically used compounds such as vinca alkaloids, colchicine, podophyllotoxin, paclitaxel, and epothilone act on microtubules or tubulin to disrupt the cellular microtubule structure and cause mitotic arrest (Chart 1).[2] Combretastatin A-4 (CA-4), isolated by Pettit and co-workers in 1982 from the stem wood of the South African tree *Combretum caffrum*, is one of the most potent antimitotic agents.[3] It exhibits strong growth inhibition against a wide variety of human cancer cells, including multidrug resistant cancer cells.[4,5] Moreover, CA-4 is also structurally similar to colchicine and possesses a higher affinity for the colchicine binding site on tubulin than does colchicine itself.[4b] However, the low water solubility of CA-4 limits its efficacy in vivo. A water-soluble sodium phosphate prodrug (CA-4P, OXIGENE, Boston, MA) is currently in phase I/II clinical trials.[6]

Because CA-4 is currently the simplest structure for a natural product that binds to tubulin, its remarkable anticancer activity has attracted the attention of many medicinal chemists for the rational design of antitubulin agents.[7] A number of structure−activity relationships (SARs) have been reported for the combretastatins.[8] These studies indicate that the cis orientation of the two aromatic rings (*Z* geometry) is the most important factor for the inhibition of cancer cell growth. *Z*-Combretastatin analogues, however, are prone to isomerization (trans-forms/*E*-isomer) during storage and administration. The trans-forms of these compounds display dramatically reduced inhibition of cancer cell growth and tubulin polymerization.[8c] Pettit's group recently synthesized two new benzophenones, phenstatin and hydroxyphenstatin, which display potent anticancer and antimitotic activities comparable to CA-4.[9] On the basis of geometric comparisons, it was suggested that the sp^2-hybridized carbonyl group present in phenstatin and hydroxyphenstatin constrains the two aryl rings in a quasi "cis" orientation that appears to be necessary for significant biological activity.[9b]

Benzophenone-type CA-4 analogues are attractive targets for anti-tubulin agents as the benzophenone backbone not only provides ease of synthesis without the need to control the geometric selectivity (*Z* and *E* geometry) but also increases the pharmacological potential through increased drug stability and water solubility.[7f,g] In the present study, introduction of an amino group at the ortho position of the B-ring was expected to maintain the quasi cis conformation to obtain more potent anti-tubulin agents and also increase water solubility by potential salt formation. Furthermore, we envisioned that variation of the substituents on the B-ring would provide significant SAR information about antitumor activity and the colchicine binding site on β-tubulin.

Chemistry

The general synthetic strategy shown in Scheme 1 was employed for the preparation of the new 2-aminobenzophenone analogues. Grignard reaction of (3,4,5-trimethoxyphenyl)magnesium bromide[10] with several commercially available or synthesized substituted 2-nitrobenzaldehydes yielded benzhydrol derivatives **A** in 80% yield. The substituted 2-nitrobenzophenone derivatives **B** were obtained by pyridinium dichromate (PDC) oxidation of the substituted benzhydrol derivatives. In most cases, the benzhydrol derivatives were used without purification. The desired 2-nitrobenzophenones were obtained in 65% overall yield (two steps). Further

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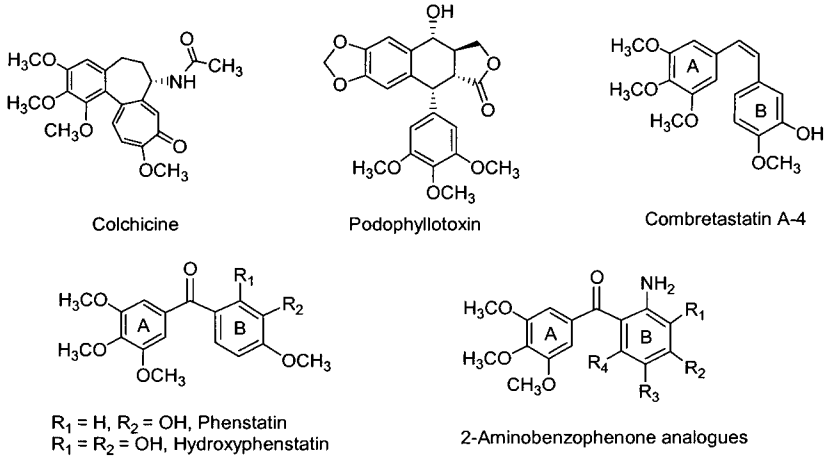
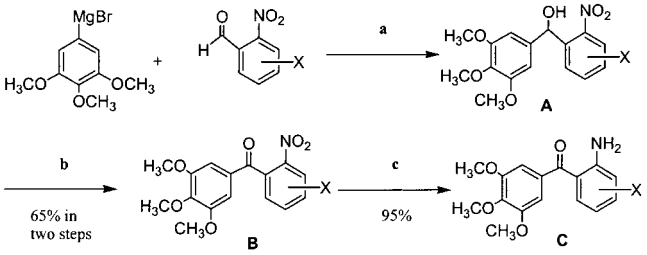
Chart 1

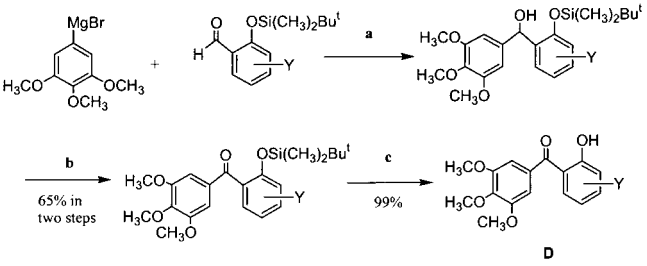


Scheme 1[a]



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

Scheme 2[a]



[a] (a) THF, 0−25 °C; (b) PDC, CH_2Cl_2, 25 °C; (c) TBAF, THF, 25 °C.

reduction of the nitro group of benzophenone **B** with Fe/AcOH yielded the corresponding substituted 2-aminobenzophenones **C** in yields over 95%.

The preparation of 2-hydroxybenzophenone analogues is shown in Scheme 2. The nucleophilic reaction was incomplete and complicated when 2 equiv of (3,4,5-trimethoxyphenyl)magnesium bromide was reacted with 1 equiv of substituted salicylaldehydes. The method was improved by first protecting the hydroxyl group of substituted salicylaldehydes as silyl ethers by *tert*-butyldimethylsilyl chloride (TBSCl) to obtain 2-(*tert*-butyldimethylsilanyloxy)benzhydrol derivatives. Following the approach outlined above, preparation of the 2-hydroxybenzophenone analogues **D** was accomplished in 70−90% yield by Grignard addition, PDC oxidation, and tetrabutylammonium fluoride (TBAF) deprotection.

Biological Results and Discussion

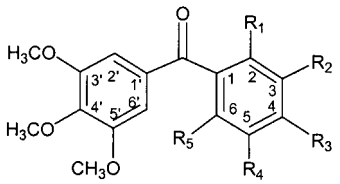
(A) In Vitro Cell Growth Inhibition Assay. A comparison of IC_{50} values for benzophenone analogues (**1**−**14**) is provided in Table 1. All 14 compounds were evaluated for in vitro cell growth inhibition against a panel of human cancer cell lines using the MTS assay.[11] The IC_{50} values represent the drug concentrations producing a 50% decrease in cell growth after 3 days of incubation.

We first evaluated the effect of a hydroxyl substitution at the C-2 position for growth inhibitory activity. A hydroxyl group at the ortho position of the B-ring resulted in less potent activity as compared to phenstatin regardless of whether the methoxy substituent was located at the C-4 or C-5 position (**1** and **2**). Removal of the hydroxyl group at the C-2 position (**4** and **5**) resulted in slightly reduced growth inhibition as compared to phenstatin. Lack of a methoxy group (compound **3**) resulted in significantly decreased growth inhibitory activity. A surprising observation was that placement of the methoxy group at the C-4 position on the B-ring is less important than previously thought,[7f,8d,12b] since analogues **2** and **5** with the methoxy group shifted to the C-5 position have growth inhibitory properties similar to analogues **1** and **4** with the methoxy group at the C-4 position.

Introduction of an amino group at the ortho position produced compounds **6** and **7**, which showed significantly increased growth inhibition against many of the cancer cell lines compared to phenstatin. It is interesting that a methoxy group at the C-3 position (**8**) resulted in complete loss or significant reduction of growth inhibition against the 10 cancer cell lines. The inactivity of the 4,5-dimethoxy analogue **12** and 5,6-dimethoxy analogue **13** indicates that bulky substituents on the B-ring are detrimental to activity. The moderate potency of the 4,5-methylenedioxy bridge analogue **14** suggests that a methylenedioxy substitution on the B-ring may mimic natural anticancer products such as podophyllotoxin and steganacin.[1] Replacement of a 5-methoxy group (**7**) with a bulky *N*,*N*-dimethylamino group (**9**) decreased growth inhibition by 2 orders of magnitude. An order of magnitude increase in potency of the C-2 amino analogue **10** and the C-3 amino analogue **11** as compared to the C-2 hydroxyl analogue **3** indicates that introduction of an amino group at the appropriate position positively contributes to growth inhibition.

To summarize SAR information (**1**−**14**) about growth inhibition, first, the growth inhibitory tendency at the C-2 position was NH_2 > H > OH, on the basis of the IC_{50} values of **1** vs **6**, **2** vs **7**, **3** vs **10**, **4** vs **6**, and **5** vs **7**.

Table 1. IC_{50} Values (nM ± SD[a]) of Benzophenone Analogues (**1**−**14**), Phenstatin, and Combretastatin A-*X*



	compd	IC_{50} for given cell line and cell type									
		Colo 205 colon	NUGC3 stomach	HA22T liver	DLD-1 colon	HR gastric	MCF-7 breast	DU-145 prostate	Hone-1 NPC	MES−SA uterine	MES−SA/ DX5[b] uterine
1	$R_1 = OH, R_2 = R_4 = R_5 = H, R_3 = OCH_3$	3595 ± 808	534 ± 91	3027 ± 384	451 ± **78**	404 ± 30	630 ± 132	2406 ± 114	329 ± 26	666 ± 97	514 ± 42
2	$R_1 = OH, R_2 = R_3 = R_5 = H, R_4 = OCH_3$	2848 ± 664	544 ± 20	7499 ± 774	511 ± 26	829 ± 164	610 ± 103	1903 ± 312	318 ± 35	636 ± 54	365 ± 22
3	$R_1 = OH, R_2 = R_3 = R_4 = R_5 = H$	3652 ± 221	5019 ± 244	5061 ± 554	4544 ± 296	4546 ± 457	6728 ± 569	7103 ± 580	3759 ± 729	5136 ± 680	4256 ± 106
4[c]	$R_1 = H, R_2 = R_4 = R_5 = H, R_3 = OCH_3$	328 ± 33	523 ± 158	506 ± 35	449 ± 84	394 ± 91	586 ± 26	372 ± 117	201 ± 60	394 ± 92	264 ± 28
5	$R_1 = H, R_2 = R_3 = R_5 = H, R_4 = OCH_3$	363 ± 30	537 ± 42	732 ± 162	856 ± 145	669± 83	749 ± 52	790 ± 88	338 ± 6	722 ± 115	426 ± 20
6	$R_1 = NH_2, R_2 = R_4 = R_5 = H, R_3 = OCH_3$	17 ± 3	50 ± 4	48 ± 2	5 ± 1	47± 5	23 ± 2	36 ± 5	3 ± 1	53 ± 1	4 ± 1
7	$R_1 = NH_2, R_2 = R_3 = R_5 = H, R_4 = OCH_3$	20 ± 1	80 ± 16	86 ± 16	94 ± 4	33± 9	61 ± 2	30 ± 4	23 ± 3	46 ± 2	31 ± 2
8	$R_1 = NH_2, R_3 = R_4 = R_5 = H, R_2 = OCH_3$	3622 ± 773	4407 ± 524	>10000	>10000	8535± 1119	7639 ± 720	>10000	4121 ± 956	>10000	>10000
9	$R_1 = NH_2, R_2 = R_3 = R_5 = H, R_4 = N(CH_3)_2$	2945 ± 212	3679 ± 185	5311 ± 603	5174 ± 365	5178± 427	4801 ± 554	4784 ± 387	2512 ± 406	4621 ± 905	3308 ± 54
10	$R_1 = NH_2, R_2 = R_3 = R_4 = R_5 = H$	364 ± 25	485 ± 57	3721 ± 783	1324 ± 547	1535± 552	817 ± 67	3249 ± 244	380 ± 59	2292 ± 750	1535 ± 162
11	$R_2 = NH_2, R_1 = R_3 = R_4 = R_5 = H$	409 ± 14	563 ± 42	5569 ± 1540	4801 ± 1107	2122± 534	702 ± 65	4224 ± 880	405 ± 66	4151 ± 527	2676 ± 820
12	$R_1 = NH_2, R_2 = R_5 = H, R_3 = R_4 = OCH_3$	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
13	$R_1 = NH_2, R_2 = R_3 = H, R_4 = R_5 = OCH_3$	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
14	$R_1 = NH_2, R_2 = R_5 = H, R_3, R_4 = OCH_2O$	338 ± 24	500 ± 58	769 ± 66	503 ± 36	529 ± 54	726 ± 105	639 ± 60	361 ± 37	820 ± 26	377 ± 7
	phenstatin[d]	4860 ± 540	9754 ± 348	3706 ± 130	83 ± 4	35 ± 3	437 ± 67	34 ± 4	87 ± 8	394 ± 11	32 ± 2
	combretastat in A-4[e]	2756 ± 213	8520 ± 771	2708 ± 31	36 ± 7	30 ± 3	25 ± 5	32 ± 2	4 ± 1	5 ± 1	4 ± 1

[a] Standard deviation, all experiments were independently performed at least three times. [b] MES-SA/DX5 is a doxorubicin-resistant cell line. [c] The synthetic method[12a] was modified by starting with 4-methoxybenzaldehyde. [d] The synthetic method was similar to Scheme 2 by starting with 3-(*tert*-butyldimethylsilanyloxy)benzaldehyde. [e] The synthetic methods followed a literature procedure.[13]

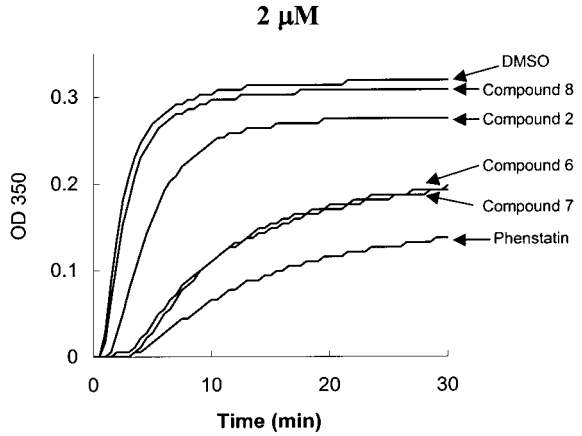


Figure 1. Comparison of inhibition of tubulin polymerization by compounds **2**, **6**, **7**, **8**, phenstatin, and control (DMSO). (All experiments were performed at least twice.)

Second, an amino group at the C-2 or C-3 position (**10** vs **11**) did not greatly affect growth inhibition. Third, a methoxy substituent at the C-4 or C-5 position is important for growth inhibition (**1** or **2** vs **3**, **6** or **7** vs **10** and **8**, **12** or **13** vs **6** or **7**). Finally, a methoxy group located at either the C-4 or C-5 position resulted in compounds with similar potencies (**1** vs **2**, **4** vs **5**, and **6** vs **7**). Thus, introduction of an amino group at the C-2-position with a methoxy group at either the C-4 or C-5 position produced the greatest growth inhibitory activity as seen for compounds **6** and **7**.

Lead compounds **6** and **7** displayed similar or greater growth inhibitory activities than phenstatin against all 10 human cell lines. Compounds **6** and **7** also showed significantly increased growth inhibition against certain cancer cell lines as compared to CA-4. For instance, compounds **6** and **7** were a 50- to 100-fold more potent than CA-4 to Colo 205, NUGC3, and HA22T cancer cells. It is interesting that both phenstatin and CA-4 were relatively inactive against Colo 205, NUGC3, and HA22T cells, whereas compounds **6** and **7** displayed potent growth inhibition against these cells. None of the 16 compounds, including phenstatin and CA-4, exhibited selective resistance to multi-drug resistant MES-SA/DX5 cells, emphasizing the potential use of benzophenone-type CA-4 analogues for multi-drug resistant cancer treatment.[5a]

(B) In Vitro Tubulin Binding Assays. To investigate whether the activities of these compounds were related to interactions with the microtubule system, their in vitro polymerization inhibitory activities were measured (Figure 1). The results demonstrated that drug growth inhibition correlated with inhibition of tubulin polymerization. For instance, lead compounds **6** and **7** displayed similar inhibition of GTP-induced polymerization of Map-rich tubulin and their growth inhibitory activities were strikingly similar. Compound **8**, which was 100-fold less potent than compounds **6** and **7** and 10-fold less potent than compound **2**, also poorly inhibited tubulin polymerization. The order of tubulin polymerization activity was phenstatin > **6**, **7** > **2** ≫ **8**.

All five of the selected compounds competed with colchicine for binding to tubulin (Table 2). The results were consistent with their tubulin polymerization inhibition and growth inhibitory activities; i.e., the two new potent analogues **6** and **7** displayed very strong inhibition of tubulin polymerization, which helps explain their potent growth inhibitory activities.

Table 2. Inhibition of Colchicine Binding to Tubulin for Compounds **2**, **6**−**8**, and Phenstatin at 20 μM

		% Inhibition ± SD[a]
2	$R_1 = OH$, $R_2 = R_3 = R_5 = H$, $R_4 = OCH_3$	50 ± 2
6	$R_1 = NH_2$, $R_2 = R_4 = R_5 = H$, $R_3 = OCH_3$	82 ± 4
7	$R_1 = NH_2$, $R_2 = R_3 = R_5 = H$, $R_4 = OCH_3$	85 ± 2
8	$R_1 = NH_2$, $R_3 = R_4 = R_5 = H$, $R_1 = OCH_3$	8 ± 1
	phenstatin	93 ± 3

[a] All experiments were independently performed three times.

Table 3. Effects[a] of Compounds **2**, **6**−**8**, and Phenstatin on Cell Cycle Progression

compd	G_0/G_1 (%)	S-phase (%)	G_2/M (%)
control (DMSO)	67.7 ± 3.1	13.6 ± 1.8	19.0 ± 1.7
2 (1 μM)	59.7 ± 2.8	13.1 ± 3.1	27.5 ± 3.3
6 (1μM)	6.3 ± 1.8	3.8 ± 0.7	90.1 ± 2.2
7 (1 μM)	5.2 ± 1.1	3.2 ± 1.1	91.6 ± 3.2
8 (1 μM)	67.6 ± 3.0	12.5 ± 0.5	20.2 ± 3.2
phenstatin (1 μM)	64.5 ± 1.6	15.7 ± 0.4	20.1 ± 1.6

[a] All experiments were independently performed three times.

(C) Cell Cycle Analysis. The effects of these compounds on the cell cycle were measured by flow cytometry against NUGC3 human cancer cells after 24 h. The results in Table 3 show that lead compounds **6** and **7** caused significant arrest of the cells at the G_2/M phase relative to the untreated control, consistent with their high antiproliferative potency against NUGC3 cells. On the contrary, the less potent compounds **2**, **8**, and phenstatin produced minimal perturbation of the cell cycle, consistent with the low growth inhibitory activities of these compounds against NUGC3 cells. Thus, the effect of the CA-4 type analogues on cell cycle progression correlated well with their growth inhibitory and antitubulin activities. In summary, lead compounds **6** and **7** have been identified as candidate antineoplastic and antimitotic agents.

Conclusions

We have synthesized a series of benzophenone-type analogues of combretastatin A-4 and have found two of the fourteen compounds to be potent antiproliferative agents, inhibitors of tubulin polymerization, and inhibitors of colchicine binding to tubulin. In addition, those compounds caused G_2/M phase arrest of cells and are considered to be potential new antimitotic agents for clinical use. The two lead compounds **6** and **7** most likely interact with tubulin at the colchicine site and display potent growth inhibitory activity against human cancer cells, including multi-drug resistant cancer cells. Most importantly, compounds **6** and **7** showed a 10- to 100-fold increase in growth inhibition compared to both phenstatin and combretastatin A-4 against several human cancer cell lines. Examination of the SAR in this series of benzophenone-type analogues revealed that introduction of an amino group at the ortho position was important for increased growth inhibition. With an amino group at the C-2 position, a methoxy group at the C-4 or C-5 position produced maximal growth inhibitory activity.

Experimental Section

(A) General Methods. Melting points were determined on a Yanaco (MP-500D) melting point apparatus and are uncor-

rected. EI-MS spectra were recorded using a Hewlett-Packard (1100MSD) spectrometer. High-resolution mass spectra (HRMS) were recorded on a Finnigan (MAT-95XL) spectrometer. Elemental analyses were performed on a Heraeus CHN-O Rapid microanalyzer. ^{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; all values are reported in parts per million (δ) downfield from $(CH_3)_4Si$. Flash-column chromatography was performed on silica gel (Merck Kieselgel 60, No. 9385, 230−400 mesh ASTM). Thin layer chromatography (TLC) was carried out on silica gel plates (E. Merck 60 F_{254}); zones were detected visually by ultraviolet irradiation (254 nm) or by spraying with phosphomolybdic acid reagent (Aldrich Chemical Co., Milwaukee, WI) followed by heating at 100 °C. All reagents were used as purchased unless otherwise stated. All solvents were dried according to standard procedures. All reactions were carried out under an atmosphere of dry nitrogen.

(1) Chemistry. (a) General Procedure for the Preparation of Substituted Benzophenone Analogues (1−14). Procedure A. A Grignard reagent was prepared in an oven-dried three-necked flask outfitted with a reflux condenser, dropping funnel, and magnetic stirrer. Approximately 1/4 of a 10 mmol aliquot of 3,4,5-trimethoxybromobenzene[10] in 5 mL of anhydrous tetrahydrofuran (THF) was added to a mixture of magnesium turnings (10 mmol) in 2.5 mL of anhydrous THF with a small piece of iodine. As soon as the solution became colorless (heating sometimes necessary), the remaining 3,4,5-trimethoxylbromobenzene solution was added dropwise to the solution under mild reflux. Stirring was then continued for 1 h at room temperature. A (trimethoxyphenyl)magnesium bromide solution (10 mmol) was then slowly added to the given substituted 2-nitrobenzoaldehyde (8.35 mmol) in 2.5 mL of anhydrous THF solution at 0 °C. After complete addition, the solution was allowed to stir at room temperature for another 20 min. A saturated NH_4Cl solution (10 mL) was slowly added to hydrolyze the adduct at 0 °C, and the mixture was stirred for 10 min. The phases were separated, and the aqueous layer was extracted with Et_2O (10 mL × 3). The combined organic layers were washed with brine, dried over $MgSO_4$, and filtered. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography to furnish each benzhydrol.

Procedure B. Pyridinium dichromate (PDC, 7.5 mmol) was added to a stirred solution of benzhydrol (5 mmol) and powered 4 Å molecular sieves (0.75 g) in 50 mL of anhydrous CH_2Cl_2 at 0 °C. After complete addition, the mixture was stirred at room temperature for 8 h. The mixture was diluted with anhydrous ether (50 mL) and filtered through a pad of Celite. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography to yield each substituted 2-nitrobenzophenone.

Procedure C. A mixture of substituted 2-nitrobenzophenone (1 mmol) and iron powder (431 mg) was added to a mixture of ethanol (3.5 mL), acetic acid (3.5 mL), water (1.7 mL), and 35% HCl (1 drop). The suspension was refluxed with vigorous stirring for 40 min, cooled, and filtered through Celite. The filtrate was diluted with water (25 mL) and extracted with $CHCl_3$ (3 × 10 mL). The organic layers were combined, sequentially washed with 9% aqueous sodium $NaHCO_3$ (20 mL) and water (2 × 20 mL), dried over $MgSO_4$, and evaporated. The residue was further purified by flash chromatography to yield each substituted 2-aminobenzophenone.

Procedure D. To the substituted 2-(*tert*-butyldimethylsilanyloxy)benzophenone (1 mmol) in anhydrous THF (10 mL) was added Bu_4NF (1.0 M in THF; 3 mmol, 3 mL). After 1 h, the mixture was poured into H_2O (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with brine (50 mL), dried ($MgSO_4$), and evaporated. The residue was chromatographed on silica gel to afford each substituted 2-hydroxybenzophenone.

(b) (2-Hydroxy-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (1). The title compound was obtained in 68% overall yield from 3,4,5-trimethoxybromobenzene and 2-(*tert*-butyldimethylsilanyloxy)-4-methoxybenzaldehyde in three steps following procedures A, B, and D; mp 106−108 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.90 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 6.44 (dd, J = 9.0 Hz, 2.4 Hz, 1H, Ar−H_5), 6.53 (d, J = 2.4 Hz, 1H, Ar−H_3), 6.88 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.58 (d, J = 9.0 Hz, 1H, Ar−H_6), 12.56 (s, 1H, OH). ^{13}C NMR ($CDCl_3$): δ 55.62, 56.30, 60.94, 101.13, 106.58, 107.31, 113.00, 133.42, 134.96, 152.97, 166.19, 166.25, 199.05. MS (EI) m/z: 318 (M^+). HRMS (EI) for $C_{17}H_{18}O_6$ (M^+): calcd, 318.1103; found, 318.1108. Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 63.90; H, 5.90.

(c) (2-Hydroxy-5-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (2). The title compound was obtained in 65% overall yield from 3,4,5-trimethoxybromobenzene and 2-(*tert*-butyldimethylsilanyloxy)-5-methoxybenzaldehyde in three steps following procedures A, B, and D; mp 125−127 °C. IR (cm^{-1}): 2988, 2936, 2831, 1578, 1485, 1411, 1350, 1282, 1218, 1174, 1134. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.73 (s, 3H, OCH_3), 3.91 (s, 6H, 2 × OCH_3), 3.95 (s, 3H, OCH_3), 6.96 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.01−7.05 (m, 1H), 7.13−7.18 (m, 2H), 11.45 (s, 1H, OH). ^{13}C NMR ($CDCl_3$): δ 55.92, 56.33, 60.99, 106.90, 115.79, 118.61, 119.33, 124.13, 132.88, 151.41, 152.97, 157.41, 199.89. MS (EI) m/z: 318 (M^+). HRMS (EI) for $C_{17}H_{18}O_6$ (M^+): calcd, 318.1103; found, 318.1106. Anal. Calcd for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.15; H, 5.81.

(d) (2-Hydroxyphenyl)(3,4,5-trimethoxyphenyl)methanone (3). The title compound was obtained in 68% overall yield from 3,4,5-trimethoxybromobenzene and 2-(*tert*-butyldimethylsilanyloxy)benzaldehyde in three steps following procedures A, B, and D. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 6H, 2 × OCH_3), 3.95 (s, 3H, OCH_3), 6.90 (t, J = 7.5 Hz, 1H, Ar−H_5), 6.94 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.30 (dt, J = 8.4 Hz, 0.6 Hz, 1H, Ar−H_3), 7.50 (m, 1H, Ar−H_4), 7.67 (dd, J = 8.4 Hz, 1.8 Hz, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 56.21, 60.85, 102.55, 106.86, 118.32, 118.49, 118.99, 132.82, 133.16, 136.09, 141.44, 152.85, 162.97, 200.35. MS (ESI): 289 (M + 1).

(e) (4-Methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (4). The title compound[12a] was obtained in 70% overall yield from 3,4,5-trimethoxybromobenzene and 4-methoxybenzaldehyde in two steps following procedures A and B; mp 46−48 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 3H, OCH_3), 3.90 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 6.98 (dd, J = 6.8 Hz, 2.1 Hz, 2H, Ar−H_2, H_6), 7.03 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.83 (dd, J = 6.8 Hz, 2.1 Hz, 2H, Ar−H_3, H_5). ^{13}C NMR ($CDCl_3$): δ 55.43, 56.26, 60.90, 107.45, 113.51, 130.26, 132.33, 133.31, 141.60, 152.80, 163.10, 194.59. MS (EI) m/z: 302 (M^+). HRMS (EI) for $C_{17}H_{18}O_5$ (M^+): calcd, 302.1154; found, 302.1157.

(f) (3-Methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (5). The title compound was obtained in 71% overall yield from 3,4,5-trimethoxybromobenzene and 3-methoxybenzaldehyde in two steps following procedures A and B; mp 70−71 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H, OCH_3), 3.88 (s, 6H, 2 × OCH_3), 3.95 (s, 3H, OCH_3), 7.08 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.12−7.16 (m, 1H), 7.33−7.42 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 55.45, 56.30, 60.94, 107.80, 114.26, 118.67, 122.52, 129.14, 132.59, 139.14, 142.15, 152.85, 159.58. MS (EI) m/z: 302 (M^+). HRMS (EI) for $C_{17}H_{18}O_5$ (M^+): calcd, 302.1154; found, 302.1153.

(g) (2-Amino-4-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (6). The title compound was obtained in 67% overall yield from 3,4,5-trimethoxybromobenzene and 4-methoxy-2-nitrobenzaldehyde in three steps following procedures A−C; mp 88−90 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 3.79 (s, 3H, OCH_3), 3.82 (s, 6H, 2 × OCH_3), 3.84 (s, 3H, OCH_3), 6.13 (dd, J = 8.7 Hz, 2.4 Hz, 1H, Ar−H_5), 6.30 (d, J = 2.4 Hz, 1H, Ar−H_3), 6.82 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.33 (d, J = 8.7 Hz, 1H, Ar−H_6). ^{13}C NMR (CD_3OD): δ 55.87, 56.87, 61.33, 100.00, 105.14, 107.64, 112.75, 137.82, 141.51, 154.30, 156.35, 166.50, 198.57. MS (EI) m/z: 317 (M^+). HRMS (EI) for $C_{17}H_{19}NO_5$ (M^+): calcd, 317.1263; found, 317.1266. Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.20; H, 6.22; N, 4.46.

(h) (2-Amino-5-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (7). The title compound was obtained in 65% overall yield from 3,4,5-trimethoxybromobenzene and

5-methoxy-2-nitrobenzaldehyde in three steps following procedures A−C; mp 127−129 °C. IR (cm^{-1}): 3420, 3307, 3005, 2944, 2832, 1640, 1583, 1411, 1333, 1235, 1212, 1133. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.68 (s, 3H, OCH_3), 3.88 (s, 6H, 2 × OCH_3), 3.95 (s, 3H, OCH_3), 5.62 (br, 2H, NH_2), 6.73 (dd, J = 8.0 Hz, 1.5 Hz, 1H, Ar−H_4), 6.95 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 6.98 (d, J = 8.0 Hz, 1H, Ar−H_3), 7.01 (d, J = 1.5 Hz, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 55.98, 56.27, 60.94, 106.92, 116.67, 118.49, 122.73, 134.82, 141.02, 145.15, 149.87, 152.80, 197.40. MS (EI) m/z: 317 (M^+). HRMS (EI) for $C_{17}H_{19}NO_5$ (M^+): calcd, 317.1263; found, 317.1271. Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.32; H, 6.20; N, 4.18.

(i) (2-Amino-3-methoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (8). The title compound was obtained in 61% overall yield from 3,4,5-trimethoxybromobenzene and 3-methoxy-2-nitrobenzaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 6H, 2 × OCH_3), 3.90 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.56 (t, J = 8.1 Hz, Ar−H_5), 6.89 (dd, J = 8.1 Hz, 0.9 Hz, 1H, Ar−H_3), 6.98 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.14 (dd, J = 8.1, 1.2 Hz, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 55.63, 56.09, 60.76, 106.70, 112.75, 113.73, 117.39, 125.43, 140.57, 141.81, 147.17, 152.57, 197.77. MS (EI) m/z: 317 (M^+). HRMS (EI) for $C_{17}H_{19}NO_5$ (M^+): calcd, 317.1263; found, 317.1266. Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.34; H, 6.03; N, 4.41. Found: C, 64.46; H, 6.17; N, 4.24.

(j) (2-Amino-5-dimethylaminophenyl)(3,4,5-trimethoxyphenyl)methanone (9). The title compound was obtained in 60% overall yield from 3,4,5-trimethoxybromobenzene and 3,5-(dimethylamino)-2-nitrobenzaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.75 (s, 6H, 2 × CH_3), 3.87 (s, 6H, 2 × OCH_3), 3.92 (s, 3H, OCH_3), 6.73 (d, J = 9.0 Hz, 1H, Ar−H_3), 6.92−7.00 (m, 4H, Ar−$H_{2'}$, $H_{6'}$, Ar−H_4, H_6). ^{13}C NMR ($CDCl_3$): δ 29.64, 42.18, 56.21, 60.90, 107.01, 118.32, 118.38, 119.02, 122.88, 134.96, 142.06, 143.26, 152.66, 197.71. MS (ESI): 331 (M + 1).

(k) (2-Aminophenyl)(3,4,5-trimethoxyphenyl)methanone (10). The title compound was obtained in 65% overall yield from 3,4,5-trimethoxybromobenzene and 2-nitrobenzaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 6.00 (br, 2H, NH_2), 6.63 (t, J = 7.2 Hz, 1H, Ar−H_5), 6.66 (d, J = 8.4 Hz, 1H, Ar−H_3), 6.91 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.30 (td, J = 7.2 Hz, 1.5 Hz, 1H, Ar−H_4), 7.50 (dd, J = 8.4 Hz, 1.5 Hz, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 56.26, 60.91, 102.55, 106.86, 11.50, 117.00, 118.24, 134.10, 134.15, 135.13, 150.76, 152.77, 153.18, 198.01. MS (EI) m/z: 287 (M^+). HRMS (EI) for $C_{16}H_{17}NO_4$ (M^+): calcd, 287.1158; found, 287.1153.

(l) (3-Aminophenyl)(3,4,5-trimethoxyphenyl)methanone (11). The title compound was obtained in 64% overall yield from 3,4,5-trimethoxybromobenzene and 3-nitrobenzaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 6H, 2 × OCH_3), 3.94 (s, 3H, OCH_3), 5.30 (br, 2H, NH_2), 6.87−6.91 (m, 1H), 7.08 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.09−7.12 (m, 2H), 7.24 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 56.24, 60.87, 107.68, 115.63, 118.72, 120.21, 128.90, 132.71, 138.80, 141.92, 146.59, 152.72, 195.94. MS (ESI): 288 (M + 1).

(m) (2-Amino-4,5-dimethoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (12). The title compound was obtained in 61% overall yield from 3,4,5-trimethoxybromobenzene and 4,5-dimethoxy-2-nitrobenzaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.70 (s, 3H, OCH_3), 3.88 (s, 6H, 2 × OCH_3), 3.91 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 6.17 (br, 2H, NH_2), 6.22 (s, 1H, Ar−H_3), 6.89 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.00 (s, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 55.84, 56.18, 56.56, 60.90, 99.30, 106.35, 109.79, 116.29, 135.77, 139.66, 140.24, 148.44, 152.76, 155.35, 196.09. MS (EI) m/z: 347 (M^+). HRMS (EI) for $C_{18}H_{21}NO_6$ (M^+): calcd, 347.1369; found, 347.1360.

(n) (6-Amino-2,3-dimethoxyphenyl)(3,4,5-trimethoxyphenyl)methanone (13). The title compound was obtained in 63% overall yield from 3,4,5-trimethoxybromobenzene and 2,3-dimethoxy-6-nitrobenzaldehyde in three steps following procedures A−C; mp 89−91 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.59 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.80 (s, 6H, 2 × OCH_3), 3.88 (s, 3H, OCH_3), 6.42 (d, J = 8.7 Hz, 1H, Ar−H_3), 6.84 (d, J = 8.7 Hz, 1H, Ar−H_4), 7.07 (s, 2H, Ar−$H_{2'}$, $H_{6'}$). ^{13}C NMR ($CDCl_3$): δ 56.07, 56.90, 60.74, 61.23, 106.64, 111.36, 117.10, 120.03, 133.54, 139.55, 142.62, 144.50, 147.75, 152.84, 196.03. MS (EI) m/z: 347 (M^+). HRMS (EI) for $C_{18}H_{21}NO_6$ (M^+): calcd, 347.1369; found, 347.1374.

(o) (6-Aminobenzo[1,3]dioxol-5-yl)(3,4,5-trimethoxyphenyl)methanone (14). The title compound was obtained in 65% overall yield from 3,4,5-trimethoxybromobenzene and 6-nitrobenzo[1,3]dioxole-5-carbaldehyde in three steps following procedures A−C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 6H, 2 × OCH_3), 3.93 (s, 3H, OCH_3), 5.91 (s, 2H, O−CH_2−O), 6.23 (s, 1H, Ar−H_3), 6.35 (br, 2H, NH_2), 6.92 (s, 2H, Ar−$H_{2'}$, $H_{6'}$), 7.00 (s, 1H, Ar−H_6). ^{13}C NMR ($CDCl_3$): δ 56.27, 60.91, 96.81, 101.31, 106.17, 106.78, 110.28, 111.40, 135.98, 138.41, 150.28, 152.85, 153.18, 196.21. MS (EI) m/z: 331 (M^+). HRMS (EI) for $C_{17}H_{17}NO_6$ (M^+): calcd, 331.1056; found, 331.1044.

(2) Biology. (a) Materials. DMEM medium, nonessential amino acids and fetal bovine serum were purchased from GIBCO BRL (Life Technologies, Grand Island, NY). MTS assay kits were obtained from Promega (Madison, WI). Propidium iodide, PIPES, and GTP were purchased from Sigma. [^{3}H]-Colchicine and Sephadex G50 columns were obtained from Amersham Pharmacia (Piscataway, NJ). MAP-rich tubulin was purchased from Cytoskeleton Inc. (Denver, CO).

(b) Cell Growth Inhibitory Assay. Carcinoma cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. For in vitro treatment, 6 × 10^3 cells/well were seeded in 96-well plates and incubated in a CO_2 incubator at 37 °C for 24 h. The cells were treated with at least five different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the tetrazolium dye reduction assay (MTS assay),[11] and the experiment was performed as the manufacturer recommended (Promega, Madison, WI). The absorbance was measured at 490 nm on a Wallac 1420 VICTOR^2 Multilabel counter (Perkin-Elmer, Boston, MA). The results of these assays were used to obtain the dose−response curves from which IC_{50} (nM) values were determined. The IC_{50} value is calculated as follows: % of control = 100% × [($OD490_{compd}$ − $OD490_{blank}$)/($OD490_{DMSO}$ − $OD490_{blank}$)] from a 50% of control inhibitory concentration. An IC_{50} value represents the concentration (nM) of the test compound which produces a 50% cell growth inhibition after 3 days of incubation. The values represent averages of three independent experiments, each with duplicate samples.

(c) Tubulin Polymerization In Vitro Assay. Turbidimetric assays of microtubules were performed as described by Lopes et al.[14] and the manual of Cytoskeleton Inc. (Denver, CO) with some modifications. MAP-rich tubulin (2 mg/mL) was preincubated in polymerization buffer (0.1 M PIPES, pH 6.9, 1 mM $MgCl_2$) with drug at 4 °C for 2 min before the addition of 1 mM GTP. The samples were then rapidly warmed to 37 °C in a 96-well plate thermostatically controlled spectrophotometer (BioCAD SPRINT, PE Biosystems), and the change in absorbance at 350 nm was periodically measured.

(d) Colchicine Binding Assay. Binding of [^{3}H]colchicine was measured by column centrifugation as described by Singer et al.[15] with some modifications. Solutions of 2.5 μM pure tubulin and 2.5 μM [^{3}H]colchicine (0.1 Ci/mmol), with or without drug, were incubated in polymerization buffer at room temperature for 1 h before 50 μL samples were centrifuged through Sephadex G-50 columns. The eluates were analyzed for radioactivity by scintillation counting (Tri-Crab 2100TR, Packard BioScience, Meriden, CT).

(e) Cell Cycle Analysis. Flow cytometric evaluation of the cell cycle status was performed according to Boquest et al.[16] with some modifications.A 5 × 10^5 amount of untreated or drug-treated NUGC3 cells were washed twice with PBS and fixed in 60% methanol for 30 min on ice. The cells were washed once with PBS and then incubated with propidium iodide (25 μg/mL)/RNase A (100 μg/mL) in PBS for 30 min at room temperature in the dark. The samples were analyzed on a FACSCalibur (Becton Dickinson, Heidelberg, Germany) flow

cytometer with excitation at 488 nm and emission measured at 564−606 nm. The number of cells in G_0/G_1, S, and G_2/M phases was calculated using Cell Quest (version 3.1f) cell cycle analysis software.

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Supporting Information Available: ^{1}H NMR, and ^{13}C NMR spectral data are available for compounds **1**−**14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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