Concise Synthesis and Structure−Activity Relationships of Combretastatin A-4 Analogues, 1-Aroylindoles and 3-Aroylindoles, as Novel Classes of Potent Antitubulin Agents

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The synthesis and study of the structure−activity relationships of two new classes of synthetic antitubulin compounds based on 1-aroylindole and 3-aroylindole skeletons are described. Lead compounds **3**, **10**, and **14** displayed potent cytotoxicities with $IC_{50} = 0.9-26$ nM against human NUGC3 stomach, MKN45 stomach, MESSA uterine, A549 lung, and MCF-7 breast carcinoma cell lines. The inhibition of proliferation correlated with in vitro polymerization inhibitory activities. Structure−activity relationships revealed that 6-methoxy substitution of 3-aroylindoles and 5-methoxy substitution of 1-aroylindoles contribute to a significant extent for maximal activity by mimicking the para substitution of the methoxy group to the carbonyl group in the case of aminobenzophenones. Addition of a methyl group at the C-2 position on the indole ring exerts an increased potency. The 3,4,5-trimethoxybenzoyl moiety was necessary for better activity but not essential and can be replaced by 3,5-dimethoxybenzoyl and 3,4,5-trimethoxybenzyl moieties. We conclude that 1- and 3-aroylindoles constitute an interesting new class of antitubulin agents with the potential to be clinically developed for cancer treatment.

Introduction

Microtubules are hollow tubes consisting of α- and *β*-tubulin heterodimers that polymerize parallel to a cylindrical axis. Tubulin-binding molecules interfere with the dynamic instability of microtubules and thereby disrupt microtubules inducing cell cycle arrest in the M-phase, forming abnormal mitotic spindles, and finally leading to apoptotic cell death.[1] A variety of natural compounds, such as paclitaxel, epothilone A, vinblastine, combretastatin A-4 (CA4), dolastatin 10, and colchicine, attack microtubules by interfering with the dynamics of tubulin polymerization and depolymerization, resulting in mitotic arrest.[2] More recently, it has been established that some tubulin binders, CA4, combretastatin A-1, AC-7700, and ZD6126, selectively target the vascular system of tumors (Chart 1).[3] These agents induce morphological changes in the endoethelial cells of the tumor's blood vessels so as to irreversibly shut down the blood flow to neoplastic cells while leaving the blood supply to healthy cells intact.

CA4, a natural product isolated by Pettit and coworkers in 1988 from the South African bush willow tree *Combretum caffrum*, strongly inhibits the polymerization of tubulin by binding to the colchicine-binding site.[4] CA4 exerts a potent cytotoxicity against a variety of human cancer cells including multidrug resistant (MDR) cancer cell lines. CA4P, a disodium phosphate prodrug, is undergoing phase II clinical trials as a tumor vascular targeting agent.[3] It displays potent antitumor effects in a wide variety of preclinical tumor models as well as substantial antivascular activity in tumor blood flow while causing no significant blood flow retention in normal tissues.[5] The encouraging antivascular/anticancer activity of CA4P has stimulated significant interest in a number of diverse ligands designed to mimic CA4 (Chart 2).[6]

We have been actively engaged in searching novel anticancer agents that target tubulin and have earlier reported 2- and 3-aminobenzophenones that show strong growth inhibitory activities against a panel of cancer cell lines including MDR cell lines as compared to CA4 (Chart 3).[7,8] Our work on aminobenzopheneones revealed that the carbonyl moiety located between two aromatic rings should be fixed as a basic motif along with a methoxy group at the para position for maximal cytotoxicity.[7,8] An amino group in the B ring plays an integral role for maximal cytotoxicity although its position appears to be more flexible as compared to the methoxy group and can be at either the C-2 or the C-3 position. Aminobenzophenones **1** and **2** strongly inhibited cancer cellular growth and tubulin polymerization and caused significant arrest of mitosis. In continuation of our earlier work, we have designed two new series of compounds, namely, 1-aroylindoles and 3-aroylindoles, based on bioisosteric replacement of the olefinic linker and the B-ring in the CA4 skeleton. We herein describe the rationale for the design, concise synthesis, and structure−activity relationships (SAR) of 1- and 3-aroylindoles as potent antitubulin agents in continuation of our search for promising anticancer agents.

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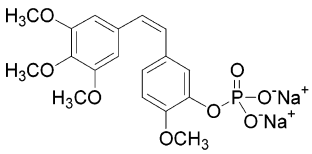
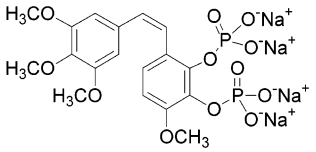
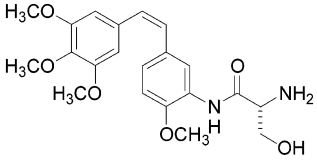
Chart 1



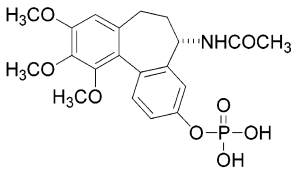
Combretastatin A-4P (Oxigene)



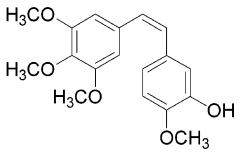
Oxi-4503 (Oxigene)



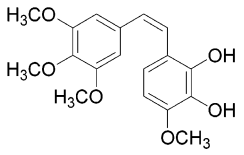
AVE-8062A (Aventis) AC-7700



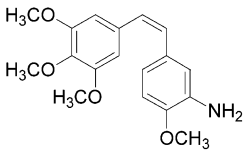
ZD-6126 (AstraZeneca) ANG453



Combretastatin A-4



Combretastatin A-1



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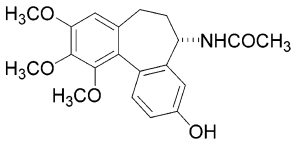
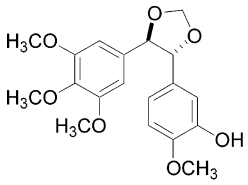
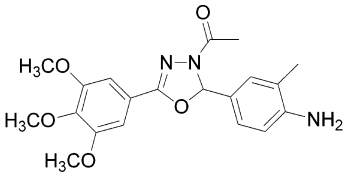


***N*-Acetylcolchinol**

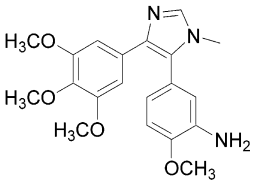
Chart 2



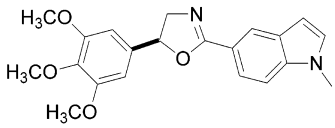
Dioxolane



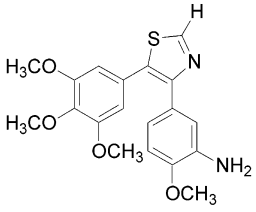
Oxadiazoline



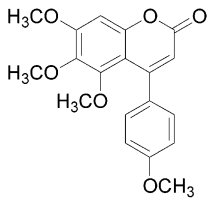
Imidazole



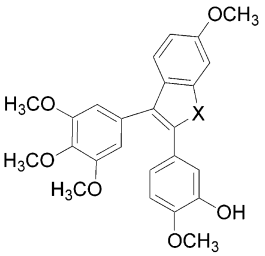
Oxazoline



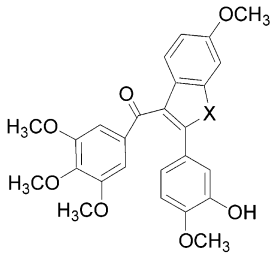
Thiazole



4-Arylcoumarin

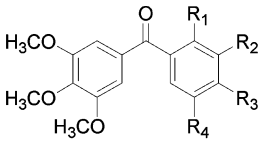


Benzothiophene : X = S
Benzofuran : X = O
Indole : X = NH



Benzothiophene : X = S
Benzofuran : X = O
Indole : X = NH

Chart 3



Phenstatin : $R_1 = R_4 = H$; $R_2 = OH$; $R_3 = OCH_3$

Hydroxyphenstatin : $R_1 = R_2 = R_4 = H$; $R_2 = OH$; $R_3 = OCH_3$

1 : 2-Aminobenzophenone : $R_1 = NH_2$; $R_2 = R_4 = H$; $R_3 = OCH_3$

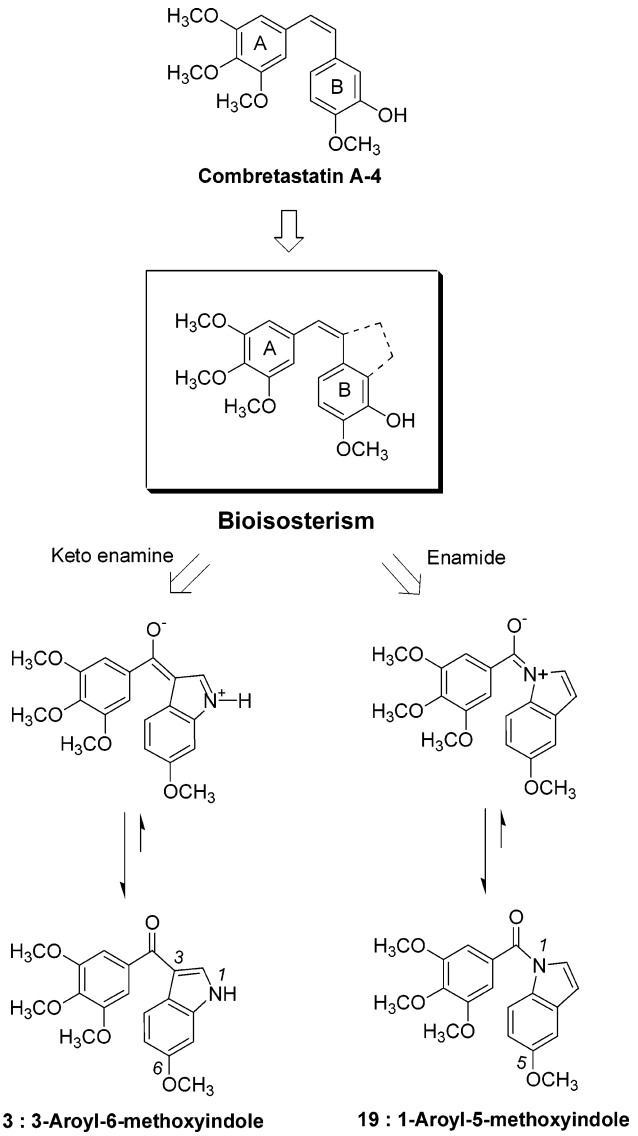
2 : 3-Aminobenzophenone : $R_1 = R_4 = H$; $R_2 = NH_2$, ; $R_3 = OCH_3$

Design

The cis double bond acting as a linker between the A and the B rings of CA4 is the most important factor for the inhibition of cancer cell growth.[6a−c] Many linkers that constrain the two phenyl rings to a similar cis-restricted conformation have been reported to increase the activity and improve the pharmacokinetic profiles of the designed compounds in comparison to CA4.[6b] This intrigued us with an idea to design a series of heterocycle-based CA4 analogues by adding five-membered heterocycles to the B ring of the CA4 structure; i.e., to replace the olefinic moiety and B ring of CA4 with the keto-enamine and enamide groups by introducing 1-aroylindoles and 3-aroylindoles, respectively. On the other hand, we exploited the ketoenamine and enamide groups as bioisosteric replacement in order to examine the effect on cytotoxicity and tubulin polymerization by novel CA4 analogues. Furthermore, utilizing an indole as a model structure, various commercially available scaffolds could be exploited to optimize the activity by manipulating the substituents on the indole ring. Most importantly, we also envisaged that the methoxy group at the C-6 position of 3-aroylindoles and the C-5 position of 1-aroylindoles would mimic the methoxy group present at the para position to the olefinic bond/carbonyl moiety in CA4 and aminobenzophenones, respectively, thus imparting maximum cytotoxicity (Chart 4).

Thirty-two derivatives with 3-aroylindole (**3**−**18**) and 1-aroylindole (**19**−**34**) as basic skeletons were systematically prepared via a concise synthesis from substituted indoles for biological evaluations. The SAR were elucidated with different substitutions at the C-2, C-4, C-5, C-6, or C-7 position on the indole ring, a carbonyl or methylene group at the *N*-1 or C-3 position, and various substitutions on the phenyl ring (Chart 5).

Chart 4



Results and Discussion

Chemistry. The general method for the synthesis of 3-aroylindoles **3**−**8**, **13**, and **15**−**18** is shown in Scheme 1. The preparation involved a one step synthesis with 52−80% yields from di- or trimethoxy-substituted benzoyl chloride and various commercially available substituted indoles. It is well-known that the indoles readily undergo electrophilic reactions particularly at the C-3 position in the presence of Grignard reagents as base and metal chlorides as Lewis acids.[9] The typical experiment included the treatment of substituted indole with EtMgBr, $ZnCl_2$, and $AlCl_3$ followed by stirring with the appropriate benzoyl chloride at room temperature for 5 h to obtain the desired 3-aroylindole. The conversion of a carbonyl group into a methylene was achieved through an efficient synthesis utilizing $NaBH_4$ reduction of **3** at room temperature to afford **18** in 95% yield.

To study the steric effect of the substituent at the C-6 position, a series of 6-alkoxy-3-aroylindoles were prepared as shown in Scheme 2. Demethylation of 6-methoxyindole with BBr_3 followed by silyl ether protection with TBDMSCl gave **36**. Electrophilic substitution of indole with 3,4,5-trimethoxybenzoyl chloride, as described above, gave 3-aroylindole **37**, which was desilylated with the tetrabutylammonium floride (TBAF) to the desired 3-aroyl-6-hydroxyindole **9**. Compound **9** was further reacted with the corresponding alkyl halides in the presence of K_2CO_3 in anhydrous acetone to afford 6-alkoxy-3-aroylindoles (**10**−**12**).

An alkyl group was introduced at the C-2 position of 6-methoxyindole to synthesize compound **14** as outlined in Scheme 3. The *N*-sulfonylation of 6-methoxyindole with benzylsulfonyl chloride and NaOH in the presence of tetra-*n*-butylammonium bromide (TBAB) as a phase transfer catalyst yielded sulfonamide **38**. The lithiated species of **38** with *t*-BuLi in tetrahydrofuran (THF) at −20 °C reacted with CH_3I to give the corresponding 2-methylindole **39**, which on deprotection with aqueous NaOH afforded 6-methoxy-2-methylindole **40**. Once the desired indole **40** was obtained, electrophilic substitution at the C-3 position was performed as described above to afford 6-methoxy-2-methyl-3-aroylindole **14**.

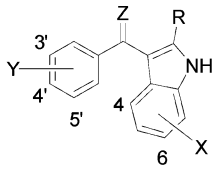
The general method for the synthesis of 1-aroylindoles (**19**−**34**) is depicted in Scheme 4. The preparation involved a one step electrophilic substitution of mono-, di-, or trimethoxybenzoyl chlorides at the *N*-1 position of various commercially available substituted indoles in 58−88% yields. The typical experiment included the treatment of appropriate indole with $NaOBu^t$ in THF followed by stirring with the corresponding benzoyl chloride at room temperature for 2 h to obtain the desired 1-aroylindole. Compound **34**, with a methylene group in place of the carbonyl group, was prepared by treating 5-methoxyindole with 3,4,5-trimethoxybenzyl chloride in the presence of $NaOBu^t$ in 72% yield.

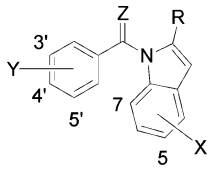
To unequivocally confirm the molecular structures of 1- and 3-aroylindole series, **3** and **19** were subjected to single-crystal X-ray analysis as shown in Figure 1. The dihedral angles between indole and 3,4,5-trimethoxyphenyl rings for **3** and **19** are similar, 53.2 and 52.7°, respectively. It indicates that indole and 3,4,5-trimethoxyphenyl rings are not coplanar as is the case in the dihedral angles between the two phenyl rings of CA1, which is 66.0°.[10]

Biological Evaluation. The two series of synthesized indoles (**3**−**34**) were evaluated for their cytotoxic activities against five human cancer cell lines, two stomach carcinomas (NUGC3 and MKN45), one uterine carcinoma (MESSA), one lung carcinoma (A549), and one breast carcinoma (MCF7). The results are shown in Tables 1 and 2.

3-Aroylindoles. We first evaluated the effect of the methoxy substitution on the indole ring in the 3-aroylindole series for cytotoxic activity. Compounds **3**, **4**, **5**, and **6** with a methoxy group at the C-6, C-4, C-5, and C-7 positions, respectively, on the indole ring were evaluated for cytotoxic activity. The SAR information indicates that in the 3-aroylindole series a methoxy group located at the C-6 position results in the best activity, shifting to the C-5 or C-7 positions results in moderate activity, while shifting to the C-4 position decreases the activity drastically. Notably, compound **3** showed IC_{50} values of 1−23 nM against five different human cancer cell lines, which was 50−500-fold more potent than compounds **4**−**6**. Furthermore, this finding also confirms our rationale that the methoxy group should be related at the C-6 position of the indole ring in 3-aroylindoles for the

Chart 5





3 (BPR0L075) : R = H; X = 6-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

4 : R = H; X = 4-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

5 : R = H; X = 5-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

6 : R = H; X = 7-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

7 : R = H; X = 5,6-di-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

8 : R = H; X = 6-F; Y = 3',4',5'-tri-OCH_3; Z = O

9 : R = H; X = 6-OH; Y = 3',4',5'-tri-OCH_3; Z = O

10 : R = H; X = 6-OEt; Y = 3',4',5'-tri-OCH_3; Z = O

11 : R = H; X = 6-OPr; Y = 3',4',5'-tri-OCH_3; Z = O

12 : R = H; X = 6-O^{i}Pr; Y = 3',4',5'-tri-OCH_3; Z = O

13 : R = CH_3; X = 5-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

14 : R = CH_3; X = 6-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

15 : R = H; X = 6-OCH_3; Y = 3',4'-di-OCH_3; Z = O

16 : R = H; X = 6-OCH_3; Y = 3',5'-di-OCH_3; Z = O

17 : R = H; X = 6-OCH_3; Y = H; Z = O

18 : R = H; X = 6-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = H, H

19 (BPR0L081) : R = H; X = 5-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

20 : R = H; X = 4-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

21 : R = H; X = 6-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

22 : R = H; X = 7-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

23 : R = H; X = 5-CN; Y = 3',4',5'-tri-OCH_3, Z = O

24 : R = H; X = 5-F; Y = 3',4',5'-tri-OCH_3, Z = O

25 : R = H; X = 5-NO_2; Y = 3',4',5'-tri-OCH_3, Z = O

26 : R = CH_3; X = 5-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

27 : R = CH_3; X = 6-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = O

28 : R = H; X = 5-OCH_3; Y = 3',4'-di-OCH_3; Z = O

29 : R = H; X = 5-OCH_3; Y = 3',5'-di-OCH_3; Z = O

30 : R = H; X = 5-OCH_3; Y = 3',4'-OCH_2O; Z = O

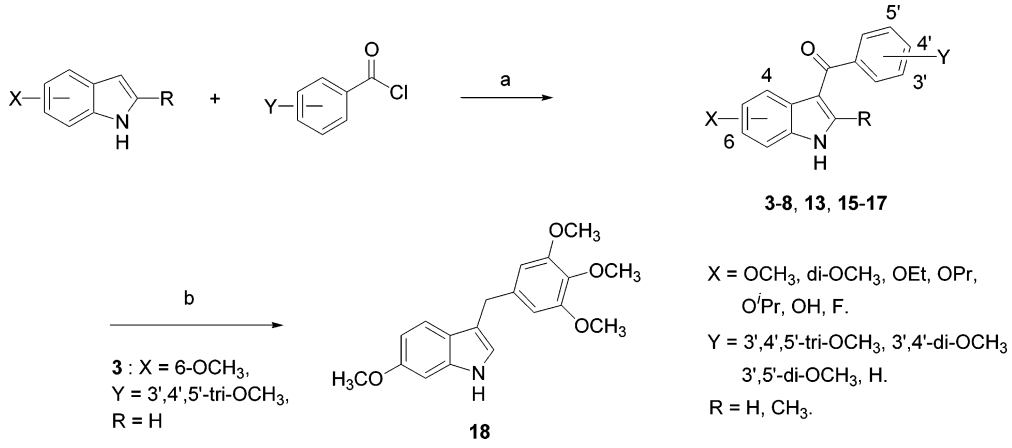
31 : R = H; X = 5-OCH_3; Y = 3'-OCH_3; Z = O

32 : R = H; X = 5-OCH_3; Y = 4'-OCH_3; Z = O

33 : R = H; X = 5-OCH_3; Y = H; Z = O

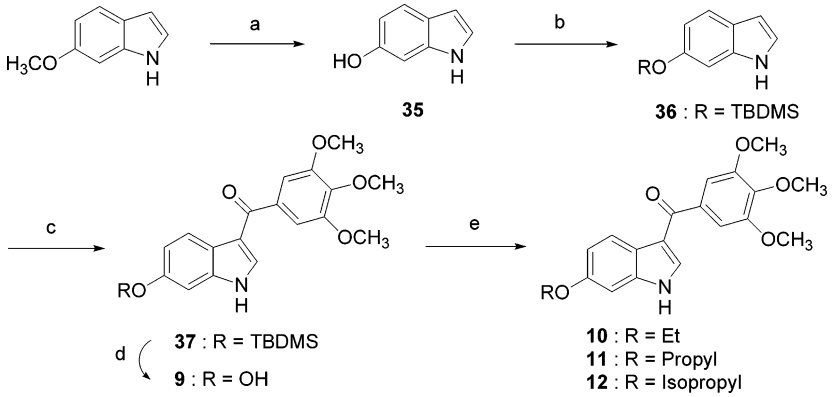
34 : R = H; X = 5-OCH_3; Y = 3',4',5'-tri-OCH_3; Z = H, H

Scheme 1[a]



[a] Reagents and conditions: (a) EtMgBr, $ZnCl_2$, $AlCl_3$, CH_2Cl_2, 52−80%. (b) $NaBH_4$, CH_3OH, 95%.

Scheme 2[a]

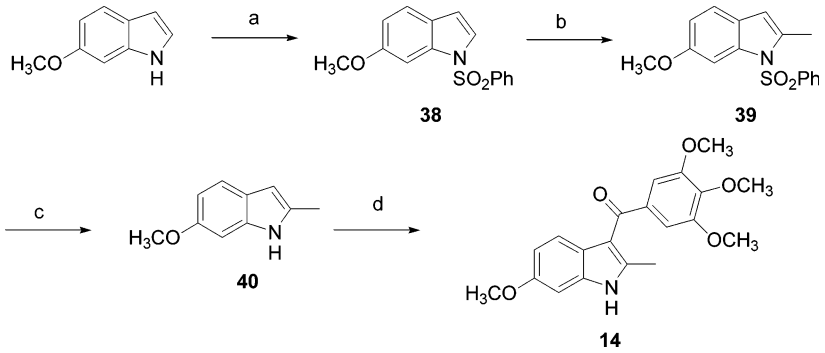


[a] Reagents and conditions: (a) BBr_3, CH_2Cl_2, 0 °C, 72%. (b) Imidazole, TBDMSCl, THF. (c) 3,4,5-Trimethoxybenzoyl chloride, EtMgBr, $ZnCl_2$, $AlCl_3$, CH_2Cl_2. (d) TBAF, THF, 54% from **35**. (e) K_2CO_3, C_2H_5I or C_3H_7I, or *i*-C_3H_7Br, acetone, reflux, 72−82%.

maximal activity to mimic the carbonyl moiety and the methoxy group located at the para position to each other in 2- and 3-aminobenzophenones.[7,8]

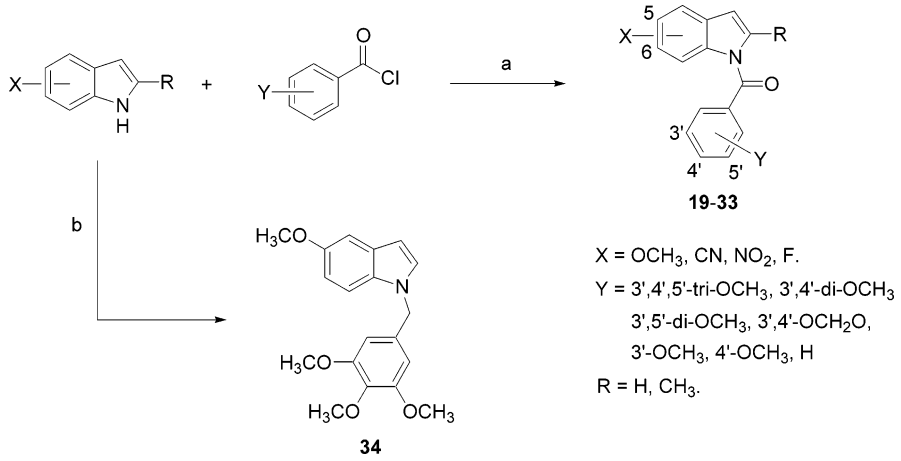
Further investigation of electronic effects on the indole ring revealed that **7** with an additional methoxy group at the C-5 position, **8** with a fluoro substitution

Scheme 3[a]



[a] Reagents and conditions: (a) 50% NaOH, TBAB, $PhSO_2Cl$, THF. (b) *t*-BuLi, CH_3I, THF, −20 °C, 77% in two steps. (c) NaOH, EtOH, reflux, 95%. (d) 3,4,5-Trimethoxybenzoyl chloride, EtMgBr, $ZnCl_2$, $AlCl_3$, CH_2Cl_2, 76%.

Scheme 4[a]



[a] Reagents and conditions: (a) $NaOBu^t$, THF, 58−88%. (b) $NaOBu^t$, 3,4,5-trimethoxybenzyl chloride, CH_3CN, reflux, 72%.

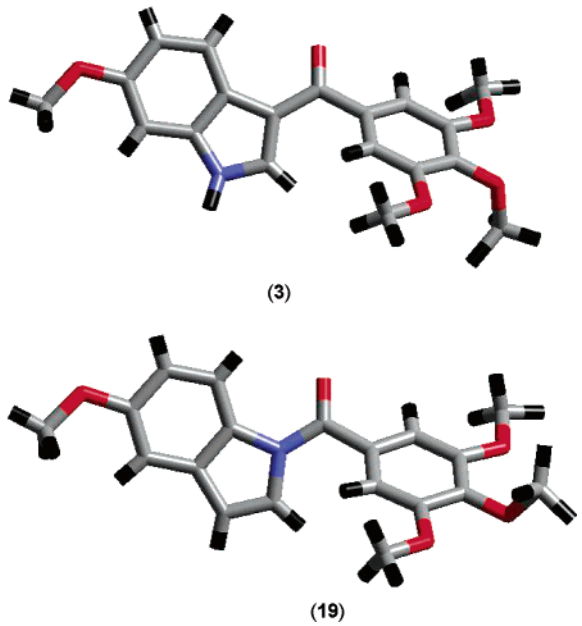


Figure 1. Crystal structures of 3-aroylindole (**3**) and 1-aroylindole (**19**).

at the C-6 position, and **9** with a hydroxyl group at the C-6 position exhibited a drastic loss of activities. It was also noteworthy that the more water soluble 3-aroyl-6-hydroxyindole **9** maintained a moderate cytotoxicity due to the weaker electron-donating ability of the hydroxyl group. In an effort to further understand the steric effect of alkoxy substituents at the C-6 position, ethoxy-, propyloxy-, and *i*-propyloxy-substituted compounds **10**, **11**, and **12**, respectively, were prepared. Compound **10**, with an ethoxy group, retains substantial cytotoxicity in comparison with **3**, but a further increase in the bulkiness of the substituent resulted in a slight decrease in potency thus revealing that the steric effect of the substitutions at the crucial C-6 position of indole ring influences cytotoxic activities.

The introduction of a methyl group at the C-2 position of 3-aroylindoles **5** and **3**, gave 3-aroyl-2-methylindoles **13** and **14**, respectively, with an apparently increased cell growth inhibition as compared to the parent compounds. The moderate activity of **5** was dramatically increased in the case of **13** by an order of magnitude for cytotoxicity against NUGC3, MESSA, and MCF-7 cell lines. A similar phenomenon was also observed in the cases of **3** and **14**, where **the** introduction of a methyl group at the C-2 position resulted in improvement of the IC_{50} values to single digit nanomolar values in all five cell lines.

A report by Cushman et al.[11] that the removal of either the C-4 or the C-5 methoxy group on the A ring causes a substantial loss of cytotoxicity in the CA4 system sparked us to investigate the effect of the 3,4,5-trimethoxybenzoyl group in the 3-aroylindole series. Compound **15**, with a 3,4-dimethoxy-benzoyl group, and compound **17**, with a benzoyl group, exhibited dramatically diminished activities. A surprising observation, however, was that **16**, with a 3,5-dimethoxybenzoyl

Table 1. IC_{50} Values (nM ± SD[a]) of 3-Aroylindoles (**3**−**18**) and CA4

compd	stomach NUGC3	stomach MKN45	uterine MESSA	lung A549	breast MCF-7
3	1 ± 0	4 ± 2	4 ± 1	23 ± 6	7 ± 1
4	3359 ± 1263	487 ± 148	9393 ± 1052	6499 ± 230	9057 ± 1633
5	360 ± 140	326 ± 84	884 ± 246	614 ± 282	639 ± 46
6	474 ± 134	323 ± 28	3524 ± 805	729 ± 157	4772 ± 1697
7	>10000	3708 ± 953	>10000	>10000	9242 ± 1313
8	>10000	669 ± 108	>10000	>10000	>10000
9	585 ± 180	43 ± 7	889 ± 268	815 ± 368	728 ± 74
10	1 ± 0	2 ± 2	3 ± 3	26 ± 21	1 ± 0
11	47 ± 1	11 ± 8	56 ± 15	230 ± 103	52 ± 18
12	41 ± 14	3 ± 2	10 ± 3	121 ± 15	40 ± 1
13	42 ± 5	111 ± 54	160 ± 93	596 ± 294	50 ± 18
14	4 ± 1	3 ± 2	4 ± 1	6 ± 2	0.9 ± 0.1
15	>10000	>10000	>10000	>10000	>10000
16	>10000	1 ± 0	24 ± 9	23 ± 7	20 ± 8
17	>10000	3939 ± 689	8064 ± 2037	8562 ± 2491	>10000
18	2 ± 2	38 ± 4	50 ± 13	52 ± 14	6 ± 0
CA4	>10000	82 ± 8	13 ± 14	117 ± 86	8 ± 1

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

Table 2. IC_{50} Values (nM ± SD[a]) of 1-Aroylindoles (**19**−**34**) and CA4

compd	stomach NUGC3	stomach MKN45	uterine MESSA	lung A549	breast MCF-7
19	368 ± 210	34 ± 11	1687 ± 151	671 ± 326	590 ± 154
20	>10000	3924 ± 1219	>10000	>10000	>10000
21	>10000	3615 ± 859	>10000	>10000	>10000
22	>10000	4074 ± 1100	>10000	>10000	>10000
23	>10000	>10000	>10000	>10000	>10000
24	>10000	5050 ± 1709	>10000	>10000	>10000
25	>10000	>10000	>10000	>10000	>10000
26	75 ± 17	39 ± 40	102 ± 72	62 ± 25	28 ± 28
27	>10000	432 ± 101	>10000	7533 ± 2932	7908 ± 1087
28	9625 ± 867	4488 ± 528	>10000	>10000	>10000
29	>10000	319 ± 73	9279 ± 1248	4486 ± 1043	9429 ± 990
30	>10000	>10000	>10000	>10000	>10000
31	>10000	>10000	>10000	>10000	>10000
32	>10000	>10000	>10000	>10000	>10000
33	>10000	7610 ± 2368	>10000	>10000	>10000
34	2907 ± 185	649 ± 128	9297 ± 1217	9212 ± 1365	8723 ± 2212
CA4	>10000	82 ± 8	13 ± 15	178 ± 140	8 ± 1

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

group, maintained a substantial cytotoxicity against five different cell lines except NUGC3 in comparison with **3**. The replacement of the carbonyl linker (**3**) by a methylene (**18**) resulted in a similar or slightly reduced bioactivity, indicating that sp^2 center of the carbonyl moiety is critical but not essential in the 3-aroylindole series.

In comparison with CA4, five lead compounds (**3**, **10**, **14**, **16**, and **18**) displayed similar or greater growth inhibitory activities against five human cancer cell lines. It is worthy to pinpoint that the cytotoxicities of 3-aroylindoles showed more sensitivity against stomach carcinoma MKN45 cells as compared to other cell lines. For instance, lesser compounds **4**, **7**, **8**, **9**, **11**, **12**, and **17** exhibited 3−15-fold more potent activity against MKN45 cells than the other four human cancers cells.

1-Aroylindoles. As shown in Table 2, 16 1-aroylindoles were also evaluated for their cytotoxic activities against five human cancer cell lines. Similar to the study on 3-aroylindoles, we first systemically evaluated the position of the methoxy substitution on the indole ring in 1-aroylindoles for cytotoxic activity. Compound **19**, with a methoxy group at the C-5 position, exhibited a 10-fold stronger activity than **20**, **21**, and **22** with a methoxy group at the C-4, C-6, and C-7 positions, respectively. It is postulated that a methoxy group at the C-5 position in the 1-aroylindole series is as critical for a strong activity as a methoxy group at the C-6 position in the 3-aroylindole series. Further investigation of the electronic effect revealed that the methoxy group plays a critical role with regard to potency, as **23**, **24**, and **25** with cyano, fluoro, and nitro electron-withdrawing groups, respectively, were completely inactive.

The potency improvement by adding a methyl group at the C-2 position observed in the 3-aroylindole series intrigued us to explore whether 1-aroylindoles also exhibit this effect. A resemblance in the 1-aroylindole series showed that compound **26**, with an additional methyl group as compared to **19**, showed an increased growth inhibition by an order of magnitude in all five cell lines. However, the potency improvement in **27**, with an additional methyl group as compared to **21**, was not apparent, but interestingly, it did show a 10-fold elevation in potency against MKN45 cells.

The unexpected strong potency of the 3,5-dimethoxybenzoyl group (**16**) inspired us to scrutinize whether a similar tendency can be observed in 1-aroylindoles. Replacing the 3,4,5-trimethoxybenzoyl functionality with 3,4-dimethoxybenzoyl, 3,5-dimethoxybenzoyl, 3,4-methylenedioxobenzoyl, 3-methoxybenzoyl, 4-methoxybenzoyl, or benzoyl group (**28**−**33**) yielded inactive

Table 3. Inhibition of Tubulin Polymerization by **3**, **14**, **16**, **19**, **26**, and CA4

compd	inhibition of tubulin polymerization IC_{50} (μM ± SD)[a]
3	2.18 ± 0.16
14	0.90 ± 0.10
16	2.69 ± 0.43
19	3.76 ± 0.51
26	3.31 ± 0.09
CA4	2.03 ± 0.41

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

compounds, except for **29**, with a 3,5-dimethoxybenzoyl group, which still showed a moderate activity against MKN45 cells. Finally, replacing a carbonyl linker (**19**) with methylene (**34**) resulted in a potency decrease by 10−20-fold.

Notably, the consistent tendency of the position effect from both series fully supports our rationale that the methoxy group at the C-6 position of 3-aroylindoles and the C-5 position of 1-aroylindoles would mimic the methoxy group present at the para position to the carbonyl moiety in aminobenzophenones, thus imparting maximum cytotoxicity. The addition of a methyl substituent at the C-2 position resulted in a significant potency increase in both series.

To investigate whether the activities of these indole compounds were related to interactions with the microtubule system, their in vitro polymerization inhibitory activities were measured (Table 3). The results demonstrated that the drug cytotoxicity correlated with the inhibition of tubulin polymerization. For instance, compound **14** clearly displayed a more potent cytotoxicity as well as inhibition of GTP-induced polymerization of MAP-rich tubulin as compared to CA4 and **3**. Compound **19**, which was 100-fold less potent than compounds **3** and **14** and 10-fold less potent than compounds **16** and **26**, weakly inhibited tubulin polymerization. The order of tubulin polymerization activity was **14** > CA4 ∼ **3** > **16** > **26** > **19**.

Conclusions

We have identified two new series of compounds, the 1-aroyl-5-methoxyindoles and 3-aroyl-6-methoxyindoles, as novel classes of highly potent antitubulin agents based on the bioisosterism concept utilizing CA4 as a template. The lead compounds **3**, **10**, **14**, **16**, and **18** showed a 5−10-fold increase in cytotoxicity as compared to CA4 against several human cancer cell lines. The SAR information of the indole substitution pattern revealed that 6-methoxy substitution of 3-aroylindoles and 5-methoxy substitution of 1-aroylindoles contribute to a significant extent for maximal activity by mimicking the para substitution of the methoxy group to the carbonyl group in the case of aminobenzophenones. Adding a methyl group at the C-2 position in both series exerts a potency increase. The 3,4,5-trimethoxybenzoyl functionality can be simplified to the 3,5-dimethoxybenzoyl moiety without a substantial activity loss in the 3-aroylindoles. A slight lowering of the potency by replacing the carbonyl group with the methylene group indicates that the sp^2 center of a carbonyl moiety is critical but not essential. Another noteworthy point is that the preparation of all of the analogues was carried out via a concise and efficient synthesis starting from substituted indoles and that most of the active compounds were prepared by a one step synthesis. Certain 1- and 3-aroylindoles **3**, **10**, **14**, **16**, **18**, and **26**, especially compounds **3** (BPR0L075) and **14**, have the potential as promising anticancer agents for further studies in terms of potent cytotoxic and antitubulin activities.[12]

Experimental Section

Melting points were determined on a Yanaco (MP-500D) melting point apparatus and are uncorrected. NMR (^{1}H NMR and ^{13}C NMR) spectra were obtained with a Varian Mercury-300 spectrometer operating at 300 and 75 MHz, respectively, with chemical shifts in parts per million (ppm, δ) downfield from tetramethylsilane 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). The reactions were monitored by thin-layer chromatography (TLC) using Merck 60 F_{254} silica gel glass-backed plates (5 cm × 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.

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from HyClone, and nonessential amino acids were purchased from Biological Industries. MTS assay kits were obtained from Promega. Propidium iodide, PIPES, and GTP were purchased from Sigma. MAP-rich tubulin was purchased from Cytoskeleton Inc.

6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (3). To a mixture of 6-methoxyindole (0.3 g, 2.03 mmol) and anhydrous zinc chloride (0.56 g, 4.07 mmol) in dry dichloromethane (10 mL), ethylmagnesium bromide (0.9 mL, 3.0 M solution in diethyl ether) was added over 10 min at room temperature. The suspension was stirred for 1 h, and then, the solution of 3,4,5-trimethoxybenzoyl chloride (0.7 g, 3.05 mmol) in dry dichloromethane (10 mL) was added dropwise over 5 min. The reaction mixture was stirred for another 1 h followed by the addition of aluminum chloride (0.27 g, 2.03 mmol). The resultant thick mixture was vigorously stirred for 5 h while monitoring by TLC (EtOAc: *n*-hexane = 1:1). The reaction was quenched with water (10 mL) and extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a brown oil, which was chromatographed over silica gel (EtOAc: *n*-hexane = 1:1) and recrystallized (CH_2Cl_2/EtOAc) to afford compound **3** (0.5 g, 72%) as a white solid; mp 185−187 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H), 3.87 (s, 6H), 3.92 (s, 3H), 6.89 (d, J = 2.1 Hz, 1H), 6.96 (dd, J = 8.9, 2.4 Hz, 1H), 7.09 (s, 2H), 7.59 (d, J = 3 Hz, 1H), 8.23 (d, J = 8.7 Hz, 1H), 9.10 (br, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 56.5, 61.2, 95.1, 106.4, 112.2, 116.9, 120.4, 122.9, 132.7, 135.8, 137.3, 140.8, 152.8, 157.4, 190.3. MS (EI) *m*/*z*: 341 (M^+, 100%), 298 (16%), 174 (21%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1278. Anal. ($C_{19}H_{19}NO_5$) C, H, N.

4-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (4). The title compound was obtained as a pale yellow crystalline solid (recrystallized from CH_2Cl_2/EtOAc) in 70% yield from 3,4,5-trimethoxybenzoyl chloride and 4-methoxyindole in a similar manner as described for the preparation of **3**; mp 232−233 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 3H), 3.84 (s, 6H), 3.94 (s, 3H), 6.65 (d, J = 7.8 Hz, 1H), 7.07 (d, J = 8.1 Hz, 1H), 7.18 (s, 2H), 7.23 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 3.0 Hz, 1H), 8.7 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6, 56.5, 61.2, 100.1, 102.5, 104.6, 107.5, 116.1, 117.8, 124.8, 130.1, 135.5, 137.9, 141.4, 152.4, 154.1, 189.9. MS (EI) *m*/*z*: 341 (M^+, 100%), 311 (17%), 195 (13%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1271.

5-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (5). The title compound was obtained as a pale yellow solid (recrystallized from CH_2Cl_2/EtOAc) in 71% yield from 3,4,5-trimethoxybenzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **3**; mp 194−195 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.86 (s, 9H), 3.92 (s, 3H), 6.94 (dd, J = 9.0, 2.7 Hz, 1H), 7.08 (s, 2H), 7.32 (d, J = 9.0 Hz, 1H), 7.66 (d, J = 3.3 Hz, 1H), 7.91 (d, J = 2.7 Hz, 1H), 9.31 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.9, 56.5, 61.1, 103.7, 106.5, 112.5, 114.7, 116.8, 127.5, 131.5, 134.0, 136.2, 141.0, 153.2, 156.6, 190.8. MS (EI) *m*/*z*: 341 (M^+, 100%), 297 (16%), 173 (21%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1264. Anal. ($C_{19}H_{19}NO_5 \cdot 0.5H_2O$) C, H, N.

7-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (6). The title compound was obtained as a yellow solid in 70% yield from 3,4,5-trimethoxybenzoyl chloride and 7-methoxyindole in a similar manner as described for the preparation of **3**; mp 245−246 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 6H), 3.94 (s, 3H), 3.99 (s, 3H), 6.78 (d, J = 7.8 Hz, 1H), 7.12 (s, 2H), 7.25 (t, J = 8.1 Hz, 1H), 7.71 (d, J = 3.0 Hz, 1H), 7.93 (d, J = 8.1 Hz, 1H), 8.95 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.7, 56.5, 61.2, 100.2, 103.9, 106.5, 114.9, 117.6, 123.4, 127.0, 127.8, 132.3, 136.0, 141.0, 146.0, 152.9, 190.4. MS (EI) *m*/*z*: 341 (M^+, 100%), 298 (12%), 174 (28%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1245.

5,6-Dimethoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (7). The title compound was obtained as a pale white crystalline (recrystallized from CH_2Cl_2/EtOAc) in 75% yield from 3,4,5-trimethoxybenzoyl chloride and 5,6-dimethoxyindole in a similar manner as described for the preparation of **3**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H), 3.92 (s, 6H), 3.98 (s, 3H), 6.93 (s, 1H), 7.11 (s, 2H), 7.59 (d, J = 2.7 Hz, 1H), 7.91 (s, 1H), 8.72 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.3, 56.4, 56.5, 61.1, 94.5, 103.7, 106.5, 117.1, 119.8, 130.7, 131.7, 136.1, 141.1, 147.4, 148.3, 153.1, 190.7. MS (EI) *m*/*z*: 371 (M^+).

6-Fluoro-3-(3′,4′,5′-trimethoxybenzoyl)indole (8). The title compound was obtained as a pale white solid in 52% yield from 3,4,5-trimethoxybenzoyl chloride and 6-fluoroindole in a similar manner as described for the preparation of **3**; mp 214−215 °C. ^{1}H NMR (300 MHz, $CDCl_3$/CD_3OD): δ 3.86 (s, 6H), 3.87 (s, 3H), 7.00 (td, J = 9.6, 2.4 Hz, 1H), 7.04 (s, 2H), 7.11 (dd, J = 9.3, 2.1 Hz, 1H), 7.70 (s, 1H), 8.20 (d, J = 8.7, 5.4 Hz, 1H). MS (EI) *m*/*z*: 329 (M^+, 100%), 287 (20%). HRMS (EI) for $C_{18}H_{16}FNO_4$ (M^+): calcd, 329.1063; found, 329.1053.

6-Hydroxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (9). A solution of **35** (0.5 g, 3.75 mmol) and diisopropylethylamine (1.31 mL, 7.51 mmol) in dry *N*,*N*-dimethylformamide (10 mL) was stirred for 10 min at room temperature. *tert*-Butyldimethylsilyl chloride (0.62 g, 4.13 mmol) was added to the reaction solution in portions with continuous stirring. After 15 h, it was extracted with EtOAc (20 mL × 3), and the combined organic layer was evaporated under reduced pressure. The residual DMF was removed by vacuum to give the crude silyl **36** as a pale yellow oil, which was used for the next step without further purification.

To a mixture of **36** and anhydrous zinc chloride (1.02 g, 7.50 mmol) in dry dichloromethane (30 mL), ethylmagnesium bromide (1.87 mL, 3.0 M solution in diethyl ether) was added over 10 min at room temperature. The suspension was stirred for 1 h, and then, the solution of 3,4,5-trimethoxybenzoyl chloride (1.30 g, 5.63 mmol) in dry dichloromethane (20 mL) was added dropwise over 5 min. The reaction mixture was stirred for another 1 h followed by the addition of aluminum chloride (0.6 g, 4.50 mmol). After the reaction was quenched with water and extracted with CH_2Cl_2, the combined organic layer was evaporated to give a pale yellow residue **37**, which was instantly treated with 1.0 M TBAF (4.5 mL) in THF (15 mL) for 15 h at room temperature. This stirred mixture was evaporated, chromatographed (CH_2Cl_2:CH_3OH = 15:1), and recrystallized (EtOAc/CH_3OH) to afford desired **9** (0.66 g, 54% from **35**) as a white solid; mp 231−232 °C. ^{1}H NMR (300 MHz, CD_3OD): δ 3.83 (s, 3H), 3.85 (s, 6H), 6.79 (dd, J = 8.4, 2.1 Hz, 1H), 6.87 (d, J = 2.1 Hz, 1H), 7.09 (s, 2H), 7.73 (s, 1H), 8.03 (d, 8.4 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 56.8, 61.2, 98.2, 107.3, 113.1, 116.9, 121.0, 123.3, 135.9, 137.3, 139.5, 141.7, 154.0, 155.5, 192.3. MS (EI) *m*/*z*: 327 (M^+, 100%), 284 (16%), 161 (49%). HRMS (EI) for $C_{18}H_{17}NO_5$ (M^+): calcd, 327.1107; found, 327.1087. Anal. ($C_{18}H_{17}NO_5$) C, H, N.

6-Ethoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (10). To a solution of **9** (0.20 g, 0.61 mmol) in acetone (15 mL), potassium carbonate (0.17 g, 1.22 mmol) and iodoethane (0.10 mL, 1.22 mmol) were added. The reaction mixture was heated under reflux for 12 h and quenched with cold water. The solution was extracted with EtOAc (15 mL × 3), dried over anhydrous $MgSO_4$, and concentrated in vacuo. The residue was further purified by flash chromatography (EtOAc:*n*-hexane = 1:1) and recrystallized (CH_2Cl_2/EtOAc) to yield the desired **10** (0.18 g, 82%) as a white solid; mp 180−181 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.44 (t, J = 6.9 Hz, 3H), 3.87 (s, 6H), 3.92 (s, 3H), 4.05 (q, J = 6.9 Hz, 2H), 6.88 (d, J = 2.1 Hz, 1H), 6.95 (dd, J = 8.7, 2.4 Hz, 1H), 7.06 (s, 2H), 7.58 (d, J = 3.0 Hz, 1H), 8.21 (d, J = 9 Hz, 1H), 8.89 (br, 1H, NH). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.2, 56.5, 61.2, 64.0, 95.9, 106.3, 112.7, 116.8, 120.4, 122.8, 132.8, 135.9, 137.4, 140.7, 152.8, 156.6, 190.4. MS (EI) *m*/*z*: 355 (M^+, 100%), 326 (14%). HRMS (EI) for $C_{20}H_{21}NO_5$ (M^+): calcd, 355.1420; found, 355.1404. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

6-Propoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (11). The title compound was obtained as a pale yellow solid in 78% yield in a manner similar for the preparation of **10** by use of propyl iodide; mp 141−143 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.03 (t, J = 7.5 Hz, 3H), 1.81 (m, J = 7.2 Hz, 2H), 3.83 (s, 6H), 3.84 (m, 2H), 3.91 (m, 3H), 6.85 (d, J = 2.1 Hz, 1H), 6.94 (dd, J = 8.7, 2.4 Hz, 1H), 7.07 (s, 2H), 7.57 (d, J = 3.0 Hz, 1H), 8.21 (d, J = 8.7 Hz, 1H), 9.61 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 11.1, 23.1, 56.6, 61.3, 70.2, 96.1, 106.5, 112.9, 116.8, 120.4, 122.9, 133.2, 136.0, 137.6, 140.8, 152.9, 156.9, 190.6. MS (EI) *m*/*z*: 369 (M^+, 100%), 370 (M+1, 21%), 326 (26%). HRMS (EI) for $C_{21}H_{23}NO_5$ (M^+): calcd, 369.1576; found, 369.1591.

6-Isopropoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (12). The title compound was obtained as a pale white crystal in 72% yield in a manner similar to the preparation of **10** by use of isopropyl iodide; mp 164−166 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.28 (d, J = 6 Hz, 6H), 3.80 (s, 6H), 3.93 (s, 3H), 4.48 (m, 1H), 6.87 (d, J = 2.1 Hz, 1H), 6.92 (dd, J = 8.7, 2.1 Hz, 1H), 7.07 (s, 2H), 7.59 (d, J = 3 Hz, 1 H), 8.21 (d, J = 8.7 Hz, 1H), 9.75 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 22.5, 56.6, 61.3, 71.0, 98.4, 106.5, 114.1, 116.7, 120.7, 122.9, 133.4, 136.0, 137.7, 140.8, 152.9, 155.5, 190.6. MS (EI) *m*/*z*: 369 (M^+, 92%), 327 (100%), 284 (17%). HRMS (EI) for $C_{21}H_{23}NO_5$ (M^+): calcd, 369.1576; found, 369.1571.

2-Methyl-5-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (13). The title compound was obtained as a white solid in 73% yield from 3,4,5-trimethoxybenzoyl chloride and 5-methoxy-2-methylindole in a similar manner as described for the preparation of **3**; mp 157−159 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.54 (s, 3H), 3.73 (s, 3H), 3.86 (s, 6H), 3.94 (s, 3H), 6.83 (dd, J = 8.7, 2.7 Hz, 1H), 7.06 (s, 2H), 7.08 (d, J = 2.4, 1H), 7.22 (d, J = 8.7 Hz, 1H), 8.50 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.0, 55.8, 56.4, 61.1, 103.1, 106.2, 107.2, 111.6, 112.2, 128.3, 129.8, 136.4, 140.6, 144.4, 152.7, 155.2, 192.1. MS (EI) *m*/*z*: 355 (M^+, 100%), 340 (35%), 308 (24%). HRMS (EI) for $C_{20}H_{21}NO_5$ (M^+): calcd, 355.1420; found, 355.1442.

2-Methyl-6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (14). The title compound was obtained as a pale white solid in 76% yield from 3,4,5-trimethoxybenzoyl chloride and 6-methoxy-2-methylindole **40** in a similar manner as described for the preparation of **3**; mp 182−184 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.52 (s, 3H), 3.80 (s, 9H), 3.91 (s, 3H), 6.73 (dd, J = 8.7, 2.1 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 7.05 (s, 2H), 7.35 (d, J = 8.7 Hz, 1H), 8.50 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.8, 55.8, 56.5, 61.2, 94.7, 106.6, 110.7, 113.5, 121.5, 135.5, 136.1, 140.9, 142.6, 152.8, 156.3, 191.8. MS (EI) *m*/*z*: 355 (M^+, 100%), 340 (24%), 306 (12%). HRMS (EI) for $C_{20}H_{21}NO_5$ (M^+): calcd, 355.1420; found, 355.1438. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

6-Methoxy-3-(3′,4′-dimethoxybenzoyl)indole (15). The title compound was obtained as a yellow solid in 80% yield

from 3,4-dimethoxybenzoyl chloride and 6-methoxyindole in a similar manner as described for the preparation of **3**; mp 155 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H), 3.96 (s, 3H), 3.97 (s, 3H), 6.81 (dd, J = 8.7, 2.1 Hz, 1H), 6.87 (s, 1H), 6.95 (d, J = 8.4 Hz, 1H), 7.11 (d, J = 1.8 Hz, 1H), 7.54−7.57 (m, 2H), 7.69 (dd, J = 8.4, 1.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 56.4, 56.4, 93.8, 110.2, 111.8, 112.8, 113.0, 122.3, 123.8, 124.0, 131.0, 133.9, 138.9, 149.0, 152.7, 159.5, 185.1. MS (EI) *m*/*z*: 311 (M^+). HRMS (EI) for $C_{18}H_{17}NO_4$ (M^+): calcd, 311.1158; found, 311.1140.

6-Methoxy-3-(3′,5′-dimethoxybenzoyl)indole (16). The title compound was obtained as a white crystalline solid (recrystallized from EtOAc) in 79% yield from 3,5-dimethoxybenzoyl chloride and 6-methoxyindole in a similar manner as described for the preparation of **3**; mp 163−164 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 6H), 3.87 (s, 3H), 6.64 (t, J = 2.4 Hz, 1H), 6.90 (d, J = 2.4 Hz, 1H), 6.97 (d, J = 2.4 Hz, 2H), 7.00 (d, J = 2.4 Hz, 1H), 7.62 (d, J = 2.7 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 8.75 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.6, 55.7, 94.8, 103.3, 106.5, 112.1, 116.8, 120.2, 123.0, 132.8, 137.0, 142.2, 157.2, 160.2, 190.4. MS (EI) *m*/*z*: 311 (M^+, 100%), 296 (11%), 174 (50%), 148 (25%). HRMS (EI) for $C_{18}H_{17}NO_4$ (M^+): calcd, 311.1157; found, 311.1143. Anal. ($C_{18}H_{17}NO_4$) C, H, N.

6-Methoxy-3-benzoylindole (17). The title compound was obtained as a pale gray solid in 53% yield from benzoyl chloride and 6-methoxyindole in a similar manner as described for the preparation of **3**; mp 221−222 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H), 6.90 (d, J = 2.1 Hz, 1H), 6.97 (dd, J = 8.7, 2.4 Hz, 1H), 7.43−7.59 (m, 4H), 7.80−7.82 (m, 2H), 8.28 (d, J = 8.7 Hz, 1H), 8.52 (br, 1H). MS (EI) *m*/*z*: 251 (M^+, 100%), 236 (24%), 175 (61%), 149 (40%). HRMS (EI) for $C_{16}H_{13}NO_2$ (M^+): calcd, 251.0946; found, 251.0943.

6-Methoxy-3-(3′,4′,5′-trimethoxybenzyl)indole (18). A stirred solution of **3** (90 mg, 0.26 mmol) and sodium borohydride (98 mg, 2.6 mmol) in ethanol (10 mL) was heated at reflux for 12 h. The reaction was quenched with water at 0 °C and extracted with CH_2Cl_2 (10 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give a residue that was chromatographed over silica gel (EtOAc:*n*-hexane = 1:2) to afford **18** as a colorless solid in 95% yield; mp 97−98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.79 (s, 9H), 3.83 (s, 3H), 4.02 (s, 2H), 6.52 (s, 2H), 6.77 (dd, J = 8.7, 2.1 Hz, 1H), 6.81 (s, 1H), 6.84 (d, J = 2.1 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 8.03 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 32.2, 55.8, 56.2, 61.0, 94.9, 105.9, 109.5, 115.6, 119.8, 121.4, 122.1, 136.3, 137.2, 137.4, 153.3, 156.7. MS (EI) *m*/*z*: 327 (M^+, 100%), 279 (57%), 167 (78%). HRMS (EI) for $C_{19}H_{21}NO_4$ (M^+): calcd, 355.1471; found, 355.1485. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

5-Methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (19). To a solution of 5-methoxyindole (1 g, 6.79 mmol) in THF (30 mL), sodium *tert*-butoxide (0.98 g, 10.19 mmol) was added and stirred at room temperature for 15 min. 3,4,5-Trimethoxybenzoyl chloride (2.35 g, 10.19 mmol) was added to the reaction mixture in one portion. After 15 h, the solvent was evaporated, and the residue was extracted with CH_2Cl_2 (20 mL × 3). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated to give a yellow oil, which was chromatographed over silica gel (EtOAc:*n*-hexane = 1:3) and recrystallized (EtOAc/*n*-hexane) to afford **19** (2.03 g, 88%) as a pale white solid; mp 98−100 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.91 (s, 9H), 3.94 (s, 3H), 6.56 (d, J = 3.6 Hz, 1H), 6.96 (s, 2H), 7.00 (m, 1H), 7.07 (d, J = 2.4 Hz, 1H), 7.34 (d, J = 3.6 Hz, 1H), 8.27 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.9, 56.5, 61.2, 103.6, 106.7, 108.5, 113.4, 117.1, 128.1, 129.4, 130.6, 131.6, 141.0, 153.0, 156.5, 167.7. MS (EI) *m*/*z*: 341 (M^+, 14%), 195 (100%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1259. Anal. ($C_{19}H_{19}NO_5$) C, H, N.

4-Methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (20). The title compound was obtained as a white solid in 83% yield from 3,4,5-trimethoxybenzoyl chloride and 4-methoxyindole in a similar manner as described for the preparation of **19**; mp 130−131 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.89 (s, 6H), 3.94 (s, 3H), 3.97 (s, 3H), 6.74 (s, 1H), 6.76 (d, J = 3.6 Hz, 1H), 6.97 (s, 2H), 7.27−7.33 (m, 2H), 7.94 (d, J = 8.1 Hz, 1H). MS (EI) *m*/*z*: 341 (M^+, 68%), 195 (100%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1264; found, 341.1245.

6-Methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (21). The title compound was obtained as a white solid in 84% yield from 3,4,5-trimethoxybenzoyl chloride and 6-methoxyindole in a similar manner as described for the preparation of **19**; mp 128−129 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H), 3.85 (s, 6H), 3.91 (s, 3H), 6.49 (d, J = 3.6 Hz, 1H), 6.90 (dd, J = 8.4, 2.1 Hz, 1H), 6.95 (s, 2H), 7.21 (d, J = 3.6 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.97 (d, J = 2.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.0, 56.9, 61.3, 100.5, 106.9, 108.5, 113.4, 121.3, 124.4, 126.4, 129.6, 137.0, 141.1, 153.1, 158.1, 168.3. MS (EI) *m*/*z*: 341 (M^+, 33%), 195 (100%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 341.1248.

7-Methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (22). The title compound was obtained as a pale yellow solid in 83% yield from 3,4,5-trimethoxybenzoyl chloride and 7-methoxyindole in a similar manner as described for the preparation of **19**; mp 91−92 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.67 (s, 3H), 3.73 (s, 6H), 3.86 (s, 3H), 6.51 (d, J = 3.3 Hz, 1H), 6.69 (dd, J = 6.3, 2.4 Hz, 1H), 7.03 (s, 2H), 7.09−7.15 (m, 2H), 7.30 (d, J = 3.6 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 56.5, 61.2, 106.4, 107.4, 107.7, 113.7, 124.3, 125.9, 128.9, 129.3, 133.2, 142.1, 148.2, 152.9, 166.9. MS (EI) *m*/*z*: 341 (M^+, 34%), 195 (100%). HRMS (EI) for $C_{19}H_{19}NO_5$ (M^+): calcd, 341.1263; found, 355.1262.

5-Cyano-1-(3′,4′,5′-trimethoxybenzoyl)indole (23). The title compound was obtained as a pale gray solid in 85% yield from 3,4,5-trimethoxybenzoyl chloride and 5-cyanoindole in a similar manner as described for the preparation of **19**; mp 167−168 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.85 (s, 6H), 3.89 (s, 3H), 6.63 (d, J = 3.6 Hz, 1H), 6.94 (s, 2H), 7.48 (d, J = 3.6 Hz, 1H), 7.52 (d, J = 1.5 Hz, 1H), 7.83 (s, 1H), 8.34 (d, J = 8.4 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.7, 61.3, 107.1, 108.0, 117.1, 119.6, 125.8, 127.8, 128.3, 129.8, 130.7, 137.8, 141.8, 153.2, 167.9. MS (EI) *m*/*z*: 336 (M^+, 100%), 212 (2%). HRMS (EI) for $C_{19}H_{16}N_2O_4$ (M^+): calcd, 336.1111; found, 336.1115.

5-Fluoro-1-(3′,4′,5′-trimethoxybenzoyl)indole (24). The title compound was obtained as a white solid in 62% yield from 3,4,5-trimethoxybenzoyl chloride and 5-fluoroindole in a similar manner as described for the preparation of **19**; mp 106−108 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.88 (s, 6H), 3.93 (s, 3H), 6.57 (d, J = 3.6 Hz, 1H), 6.95 (s, 2H), 7.09 (td, J = 9.3, 2.7 Hz, 1H), 7.22−7.26 (m, 1H), 7.39 (d, J = 3.9 Hz, 1H), 8.32 (dd, J = 9.3, 4.8 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.4, 61.0, 106.1, 106.4, 106.6, 108.0, 112.3, 112.7, 117.1, 117.2, 128.8, 131.4, 131.5, 132.1, 141.0, 152.9, 157.8, 161.0, 167.6. MS (EI) *m*/*z*: 329 (M^+, 27%), 194 (100%). HRMS (EI) for $C_{18}H_{16}FNO_4$ (M^+): calcd, 329.1064; found, 329.1052.

5-Nitro-1-(3′,4′,5′-trimethoxybenzoyl)indole (25). The title compound was obtained as a yellow solid in 58% yield from 3,4,5-trimethoxybenzoyl chloride and 5-nitroindole in a similar manner as described for the preparation of **19**; mp 192−193 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.90 (s, 6H), 3.95 (s, 3H), 6.77 (d, J = 3.6 Hz, 1H), 6.98 (s, 2H), 7.56 (d, J = 3.9 Hz, 1H), 8.26 (dd, J = 9.0, 3.0 Hz, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.53 (d, J = 2.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.5, 61.1, 106.9, 108.5, 116.2, 117.0, 119.9, 127.8, 130.1, 130.3, 138.8, 141.7, 144.1, 153.0, 167.6. MS (EI) *m*/*z*: 356 (M^+, 28%), 195 (100%). HRMS (EI) for $C_{18}H_{16}N_2O_6$ (M^+): calcd, 356.1008; found, 356.1026.

2-Methyl-5-methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (26). The title compound was obtained as a white solid in 81% yield from 3,4,5-trimethoxybenzoyl chloride and 5-methoxy-2-methylindole in a similar manner as described for the preparation of **19**; mp 97−98 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.41 (s, 3H), 3.80 (s, 9H), 3.93 (s, 3H), 6.34 (t, J = 0.9 Hz, 1H), 6.65 (dd, J = 9.0, 2.4 Hz, 1H), 6.92 (d, J = 2.7 Hz, 1H), 6.95 (s, 2H), 6.97 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 15.9, 55.8, 56.5, 61.3, 102.6, 107.4, 108.8, 114.4, 115.3, 130.5, 132.0, 138.9, 142.3, 153.4, 156.0, 169.2. MS (EI) *m*/*z*: 355 (M^+). HRMS (EI) for $C_{20}H_{21}NO_5$ (M^+): calcd, 355.1420; found, 355.1392. Anal. ($C_{20}H_{21}NO_5$) C, H, N.

2-Methyl-6-Methoxy-1-(3′,4′,5′-trimethoxybenzoyl)indole (27). The title compound was obtained as a pale white solid in 80% yield from 3,4,5-trimethoxybenzoyl chloride and 6-methoxy-2-methylindole **40** in a similar manner as described for the preparation of **19**; mp 121−122 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.34 (s, 3H), 3.68 (s, 3H), 3.84 (s, 6H), 3.94 (s, 3H), 6.34 (d, J = 0.9 Hz, 1H), 6.79 (d, J = 2.4 Hz, 1H), 6.82 (s, 1H), 6.96 (s, 2H), 7.33 (dd, J = 7.8, 1.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.2, 55.9, 56.7, 61.4, 99.4, 107.3, 108.5, 111.6, 120.2, 123.4, 130.4, 136.3, 138.1, 142.1, 153.3, 156.6, 169.4. MS (EI) m/z: 355 (M^+, 100%), 226 (10%). HRMS (EI) for $C_{20}H_{21}NO_5$ (M^+): calcd, 355.1420; found, 355.1395.

5-Methoxy-1-(3′,4′-dimethoxybenzoyl)indole (28). The title compound was obtained as a pale white solid in 83% yield from 3,4-dimethoxybenzoyl chloride and 5-methoxyindole in a similar manner as described for the preparatitbon of **19**; mp 153−154 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H), 3.92 (s, 3H), 3.96 (s, 3H), 6.54 (d, J = 3.9 Hz, 1H), 6.94 (d, J = 9.3 Hz, 1H), 6.98 (d, J = 2.7 Hz, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.31−7.35 (m, 2H), 8.23 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.9, 55.4, 103.0, 107.7, 109.9, 111.9, 112.9, 116.6, 122.9, 116.6, 122.9, 126.1, 127.9, 130.3, 131.2, 148.4, 151.7, 156.0, 167.2. MS (EI) m/z: 311 (M^+, 76%), 165 (100%). HRMS (EI) for $C_{18}H_{17}NO_4$ (M^+): calcd, 311.1157; found, 311.1154.

5-Methoxy-1-(3′,5′-dimethoxybenzoyl)indole (29). The title compound was obtained as a white solid in 86% yield from 3,5-dimethoxybenzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **19**; mp 95−97 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.82 (s, 6H), 3.87 (s, 3H), 6.52 (dd, J = 3.6, 0.6 Hz, 1H), 6.64 (t, J = 2.4 Hz, 1H), 6.81 (d, J = 2.4 Hz, 2H), 6.97 (dd, J = 9.0, 2.4 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 7.29 (d, J = 3.6 Hz, 1H), 8.29 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.70, 55.75, 103.4, 103.7, 106.7, 108.4, 113.2, 117.0, 128.0, 130.4, 131.6, 136.1, 156.2, 160.4, 167.8. MS (EI) m/z: 312 (M+1, 48%), 164 (100%). HRMS (EI) for $C_{18}H_{17}NO_4$ (M^+): calcd, 311.1157; found, 311.1126.

5-Methoxy-1-(3′,4′-methylenedioxybenzoyl)indole (30). The title compound was obtained as a pale yellow solid in 73% yield from 3,4-(methylenedioxy)benzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **19**; mp 139−141 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H), 6.04 (s, 2H), 6.51 (d, J = 3.9 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 6.95 (dd, J = 9.0, 2.4 Hz, 1H), 7.02 (d, J = 2.4 Hz, 1H), 7.19−7.26 (m, 2H), 7.29 (d, J = 3.6 Hz, 1H), 8.23 (d, J = 9.0 Hz, 1H). MS (EI) m/z: 295 (M^+, 29%), 149 (100%). HRMS (EI) for $C_{17}H_{13}NO_4$ (M^+): calcd, 295.0845; found, 295.0838.

5-Methoxy-1-(3′-methoxybenzoyl)indole (31). The title compound was obtained as an oil in 84% yield from 3-methoxybenzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **19**. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.84 (s, 3H), 3.86 (s, 3H), 6.53 (d, J = 3.6 Hz, 1H), 6.99 (dd, J = 9.0, 2.7 Hz, 1H), 7.09−7.13 (m, 1H), 7.24−7.28 (m, 3H), 7.40 (t, J = 8.1 Hz, 1H), 8.33 (d, J = 9.0 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.8, 56.0, 103.7, 108.7, 113.5, 114.2, 117.4, 118.0, 121.4, 128.4, 129.7, 130.7, 131.9, 135.8, 156.7, 159.6, 168.1. MS (EI) m/z: 281 (M^+, 38%), 152 (75%), 147 (19%), 135 (100%). HRMS (EI) for $C_{17}H_{15}NO_3$ (M^+): calcd, 281.1052; found, 281.1061.

5-Methoxy-1-(4′-methoxybenzoyl)indole (32). The title compound was obtained as white solid in 81% yield from 4-methoxybenzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **19**; mp 106−108 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.80 (s, 3H), 3.82 (s, 3H), 6.49 (d, J = 3.6 Hz, 1H), 6.92−6.96 (m, 3H), 7.01 (d, J = 2.7 Hz, 1H), 7.27 (d, J = 3.6 Hz, 1H), 7.66 (d, J = 8.7 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.5, 55.7, 103.3, 107.8, 113.1, 113.6, 116.8, 126.3, 128.1, 130.6, 131.3, 156.2, 162.2, 167.5. MS (EI) m/z: 281 (M^+, 22%), 135 (100%). HRMS (EI) for $C_{17}H_{15}NO_3$ (M^+): calcd, 281.1052; found, 281.1066.

5-Methoxy-1-benzoylindole (33). The title compound was obtained as a pale white solid in 75% yield from benzoyl chloride and 5-methoxyindole in a similar manner as described for the preparation of **19**; mp 104−107 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.87 (s, 3H), 6.53 (dd, J = 3.9, 0.9 Hz, 1H), 6.97 (dd, J = 8.7, 2.7 Hz, 1H), 7.04 (d, J = 2.1 Hz, 1H), 7.24 (d, J = 3.6 Hz, 1H), 7.47−7.72 (m, 5H), 8.29 (d, J = 9.0 Hz, 1H). MS (EI) m/z: 251 (M^+, 31%), 122 (61%), 105 (100%). HRMS (EI) for $C_{16}H_{13}NO_2$ (M^+): calcd, 251.0946; found, 251.0925.

5-Methoxy-1-(3′,4′,5′-trimethoxybenzyl)indole (34). To a stirred solution of 5-methoxyindole (0.5 g, 3.39 mmol) and sodium *tert*-butoxide (0.65 g, 6.79 mmol) in anhydrous acetonitrile (15 mL), the solution of 3,4,5-trimethoxybenzyl chloride (0.96 g, 4.42 mmol) in anhydrous acetonitrile (15 mL) was added dropwise at room temperature. The reaction mixture was heated to reflux for 12 h. The solvent was evaporated, and the residue was extracted with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo to give a residue, which was further purified by flash column over silica gel (EtOAc:*n*-hexane = 1:3) to provide pure **34** (0.8 g, 72%) as a white solid; mp 100−102 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.73 (s, 6H), 3.79 (s, 3H), 3.83 (s, 3H), 5.19 (s, 2H), 6.30 (s, 2H), 6.45 (dd, J = 3.0, 0.6 Hz, 1H), 6.82 (dd, J = 8.7, 2.4 Hz, 1H), 7.08 (m, 2H), 7.16 (d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 50.5, 55.8, 56.1, 60.8, 101.1, 102.4, 103.6, 110.2, 111.8, 128.4, 128.8, 131.4, 132.9, 137.0, 153.1, 153.7. MS (EI) m/z: 327 (M^+, 100%), 284 (17%). HRMS (EI) for $C_{19}H_{21}NO_4$ (M^+): calcd, 327.1470; found, 327.1458.

6-Hydroxyindole (35). To a stirred solution of 6-methoxyindole (1 g, 6.79 mmol) in dry dichloromethane (30 mL), boron tribromide (3.4 g, 13.60 mmol) was added dropwise at 0 °C. The reaction mixture was warmed to room temperature. After 15 h, the reaction was quenched with water at 0 °C, followed by extraction with CH_2Cl_2 (20 mL × 3). The combined extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo, and the residue was chromatographed over silica gel (CH_2Cl_2:CH_3OH = 10:1) to provide **35** (0.65 g, 72%) as a pale yellow solid. ^{1}H NMR (300 MHz, CD_3OD): δ 6.30 (d, J = 3.0 Hz, 1H), 6.57 (dd, J = 8.4, 2.1 Hz, 1H), 6.79 (s, 1H), 7.03 (d, J = 3.3 Hz, 1H), 7.31 (d, J = 8.4 Hz, 1H).

1-Benzenesulfonyl-2-methyl-6-methoxylindole (39). A suspension of 6-methoxyindole (3 g, 20.38 mmol), TBAB (0.7 g, 2.03 mmol), and 50% sodium hydroxide (25 mL) in THF (30 mL) and water (10 mL) was stirred vigorously for 20 min at room temperature. The solution of benzenesulfonyl chloride (5.2 mL, 40.76 mmol) in THF (20 mL) was added dropwise to the reaction mixture. After 4 h, the reaction was extracted with EtOAc (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under vacuum to give the residue **38**, which was redissolved in THF (50 mL) at −20 ∼ −30 °C and treated with a solution of *tert*-butyllithium (1.7 M in pentane, 16 mL, 26.5 mmol) dropwise. The resultant mixture was continuously stirred at −30 °C for 30 min and allowed to warm to 0 °C and stirred for another 20 min. The reaction mixture was again cooled to −30 °C before the addition of iodomethane (3.8 mL, 61.1 mmol) dropwise. After continuous stirring at −30 °C for another 1 h, it was allowed to warm to room temperature overnight. The solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (20 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and purified by flash chromatography over silica gel (EtOAc:*n*-hexane = 1:8) to provide **39** (4.6 g, 77% from 6-methoxyindole) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.55 (s, 3H), 3.87 (s, 3H), 6.26 (s, 1H), 6.85 (dd, J = 8.7, 2.4 Hz, 1H), 7.26 (d, J = 8.7 Hz, 1H), 7.41 (m, 2H), 7.52 (m, 1H), 7.77 (m, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.1, 56.0, 99.6, 109.6, 112.4, 120.5, 123.6, 126.3, 129.4, 133.8, 136.1, 138.1, 139.3, 157.4. MS (EI) m/z: 301 (M^+).

2-Methyl-6-methoxyindole (40). A stirred solution of **39** (0.44 g, 1.46 mmol) and 3 M sodium hydroxide (14.6 mL, 43.8 mmol) in ethyl alcohol (30 mL) was heated at reflux for 16 h. The solution was evaporated, and the residue was extracted with CH_2Cl_2 (15 mL × 3). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column over silica gel (EtOAc:*n*-hexane = 1:7) to afford **40** (0.22 g, 95%) as a pale gray solid.

^{1}H NMR (300 MHz, $CDCl_3$): δ 2.36 (s, 3H), 3.82 (s, 3H), 6.15 (s, 1H), 6.70 (d, J = 2.1 Hz, 1H), 6.78 (dd, J = 8.4, 2.1 Hz, 1H), 7.40 (d, J = 8.7 Hz, 1H), 7.78 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 56.0, 94.8, 100.0, 109.2, 120.2, 123.4, 134.2, 136.9, 155.6. MS (EI) m/z: 161 (M^+).

Cell Growth Inhibitory Assay. Carcinoma cells were maintained in DMEM medium supplemented with 10% fetal bovine serum. For in vitro treatment, the carcinoma cells were seeded in 96 well plates and incubated in a CO_2 incubator at 37 °C for 24 h. The seeding numbers were 6000, 3000, 6000, 6500, and 4000 per well for NUGC-3, MKN45, MESSA, MCF7, and A549, respectively. 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), 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. An IC_{50} value represents the concentration (nM) of the tested compound at which a 50% cell growth inhibition after 3 days of incubation is produced. The values represent averages of three or more independent experiments, each with duplicate samples.

Tubulin Polymerization in Vitro Assay. Turbidimetric assays of microtubules were performed as described by Bollag et al.[13] MAP-rich tubulin (2 mg/mL) in 100 μL of buffer containing 100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 1 mM GTP, and 2% (v/v) dimethyl sulfoxide were placed in a 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) was used to determine the concentration that inhibited tubulin polymerization to 50% (IC_{50}). The AUC of the untreated control and 10 μM colchicine was set to 100 and 0% polymerization, respectively, and the IC_{50} was calculated by nonlinear regression in at least three experiments.[14]

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Supporting Information Available: Characterization (^{1}H NMR, ^{13}C NMR) of **3**−**40**. This material is available free of charge via the Internet at http://pubs.acs.org.

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