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# Structure–Activity Relationship Studies of 3-Aroylindoles as Potent Antimitotic Agents

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*The concise synthesis and structure–activity relationship (SAR) studies of 3-aroylindoles were carried out in an effort to improve the potency and solubility of anticancer drug candidate BPR0L075 (**8**) by exploring structure modifications through three regimens: substitution of the B ring, at the N1 position, and of the 3-carbonyl linker. The SAR information revealed that the methoxy group of the B ring could be replaced with an electron-donating group such as methyl (in compound **9**) or* N,N*-dimethylamino (in compound **13**) while retaining both strong cytotoxic and antitubulin activities. The introduction of amide (compounds **30**–**33**) and carbamate (compounds **34**–**37**) functionalities at the N1 position of **8** gave analogues with potent antiproliferative activities. The cytotoxic potency of **8** was improved by replacing the carbonyl group with sulfide (compound **41**) or oxygen (compound **43**), indicating that the carbonyl moiety is important but not essential. The* N,N*-dimethylamino derivative **13** not only displayed potent cytotoxicity and antitubulin activity, but also showed a markedly improved physicochemical profile relative to the parent compound.*

## Introduction

Microtubule-targeting agents such as taxanes and vinca alkaloids have played a central role in the treatment of a variety of human cancers over the past decade. There is a tremendous interest in inhibitors of tubulin polymerization that are not substrates for multidrug-resistance (MDR) mechanisms and which interact at sites that are near to, overlapping with, or different from those of taxanes or vinca alkaloids.[1,2] However, many clinically promising compounds face substantial limitations such as high systemic toxicity, poor water solubility and bioavailability, as well as complex syntheses and isolation procedures. Recently a number of low-molecular-weight tubulin polymerization inhibitors have been reported that have improved oral bioavailability and activity against MDR-positive phenotypes.[3]

Many anticancer drugs have poor and highly variable oral bioavailability. As pharmacokinetics plays a very important role in determining drug dose, exposure, and drug activity, an improved pharmacokinetic profile would result in enhanced antitumor activity and decreased systemic toxicity. Current anticancer drugs require chronic administration to elicit the desired effects.[4,5] Thus, the development of orally administrable anticancer drugs is receiving increased attention from medicinal chemists with conscious efforts to improve the physicochemical and pharmacokinetic profiles of newly designed molecules.

The tubulin binding agents combretastatin A4 disodium phosphate (CA4P, **2**), combretastatin A1 disodium phosphate (CA1P, OXI4503, **4**), and compounds **5**, **6**, and **7** are currently under clinical development.[2,6,7] They were also reported to have profound effects on tumor vasculature, an attractive target for anticancer therapy, as its disruption would block the supply of nutrients and oxygen to the tumor cells in addition to blocking the main route of metastasis.[7,8] As single agents, these compounds have shown efficacy and tumor selectivity in clinical trials.[7]

We have been actively exploring antimitotic agents based on combretastatins[9–12] and recently reported the discovery of a potent antitubulin agent, the 3-aroylindole **8**, which was designed by bioisosteric replacement of the olefinic linker and

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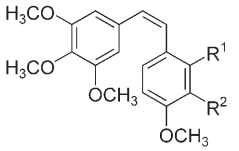
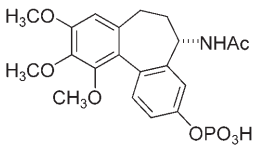
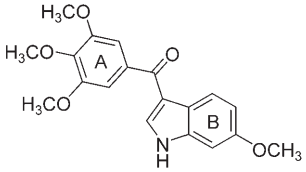


**1** combretastatin A-4: $R^1$ = H, $R^2$ = OH
**2** combretastatin A-4P: $R^1$ = H, $R^2$ = $OPO_3Na_2$
**3** combretastatin A-1: $R^1$ = $R^2$ = OH
**4** combretastatin A-1P: $R^1$ = $R^2$ = $OPO_3Na_2$
**5** AVE-8063 : $R^1$ = H, $R^2$ = $NH_2$
**6** AVE-8062 : $R^1$ = H, $R^2$ = NH-serine



**7**: ZD-6126



**8**: BPR0L075

the B ring of the CA4 (**1**) skeleton.[12,13] Compound **8** exerts strong inhibition of tubulin polymerization by binding to the colchicine binding site of tubulin and causes $G_2$/M phase arrest in a concentration- and time-dependent manner, leading to cell death through an apoptotic pathway. It was equally effective in vitro against three KB-derived MDR-positive cell lines through inhibition of tubulin polymerization, regardless of P-glycoprotein 170/MDR or MRP status (MRP = multidrug-resistance-associated protein). It showed a dose-dependent decrease in tumor volume toward human tumor xenografts in mice.[13] We report herein an extensive structure–activity relationship (SAR) study for analogues of **8** which was done in an effort to further improve the potency and solubility profile of this compound class.

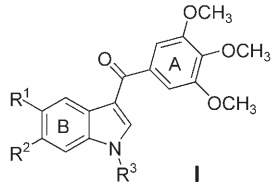
Based on our previous studies, we had reported that the 3,4,5-trimethoxybenzoyl moiety is necessary for improved activity, and the 6-methoxy substitution on the 3-aroylindole group contributes significantly to maximize activity.[12] Building upon these observations, we further studied the SAR of 3-aroylindoles by three regimens: 1) modifications of the B ring of **8** with electron-donating groups; 2) substitution of **8** at the N1 position with various functional groups such as carbonyl, alkyl, and sulfonyl; 3) modifications at the carbonyl bridgehead. Herein we report the cytotoxicity profiles of the synthesized compounds against a variety of human cancer cells including MDR-positive cells, and antitubulin activities of the potent compounds. A lead compound with improved water solubility was also subject to pharmacokinetic studies.

## Results and Discussion

### Chemistry

The synthesis of compounds **9**, **15**, and **16**, which represent modifications on the B ring of **8**, is shown in Scheme 1. The direct electrophilic substitution of commercially available 6-methylindole (**45**) and 5-benzyloxy-6-methoxyindole (**46**) with 3,4,5-trimethoxybenzoyl chloride in the presence of EtMgBr, $ZnCl_2$, and $AlCl_3$ gave the desired 3-aroylindoles **9** and **16** in 78 and 42% yield, respectively. The Pd/C-catalyzed cleavage of the 5-benzyloxy group of **16** afforded 5-hydroxy-6-methoxy-3-aroylindole **15** in 83% yield.

The synthesis of the 6-amino-3-aroylindoles **11**, **12**, and **13** was carried out as shown in Scheme 2 to study the effect of replacement of the 6-methoxy group with an amino or substituted amino group. The conventional electrophilic reaction of indoles with 3,4,5-trimethoxybenzoyl chloride at the C3 position



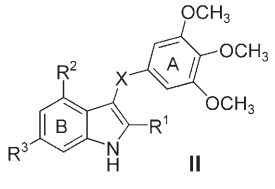
I

regimen 1: (scaffold I)

**8**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = H
**9**: $R^1$ = H, $R^2$ = $CH_3$, $R^3$ = H
**10**: $R^1$ = H, $R^2$ = OH, $R^3$ = H
**11**: $R^1$ = H, $R^2$ = $NH_2$, $R^3$ = H
**12**: $R^1$ = H, $R^2$ = $NH(CH_3)$, $R^3$ = H
**13**: $R^1$ = H, $R^2$ = $N(CH_3)_2$, $R^3$ = H
**14**: $R^1$ = $NH_2$, $R^2$ = $OCH_3$, $R^3$ = H
**15**: $R^1$ = OH, $R^2$ = $OCH_3$, $R^3$ = H
**16**: $R^1$ = $OCH_2C_6H_5$, $R^2$ = $OCH_3$, $R^3$ = H

regimen 2: (scaffold I)

**17**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $CH_3$
**18**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $C_2H_5$
**19**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = *n*-$C_3H_7$
**20**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = *i*-$C_3H_7$
**21**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = *n*-$C_4H_9$
**22**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $C_2H_5N(CH_3)_2$
**23**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $CH_2CO_2H$
**24**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $CH_2CH_2CO_2H$
**25**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = benzyl
**26**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 4-nitrobenzyl
**27**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 4-cyanobenzyl
**28**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 4-methoxybenzyl
**29**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 4-pyridylmethyl
**30**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $(CO)C_6H_5$
**31**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 2-furoyl
**32**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 2-thiophenecarbonyl
**33**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = (CO)*t*-$C_4H_9$
**34**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $(CO)OC_6H_5$
**35**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = (CO)O*t*-$C_4H_9$
**36**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $(CO)OCH_3$
**37**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $(CO)OC_2H_5$
**38**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $(CO)N(CH_3)_2$
**39**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = 4-morpholinecarbonyl
**40**: $R^1$ = H, $R^2$ = $OCH_3$, $R^3$ = $SO_2C_6H_5$

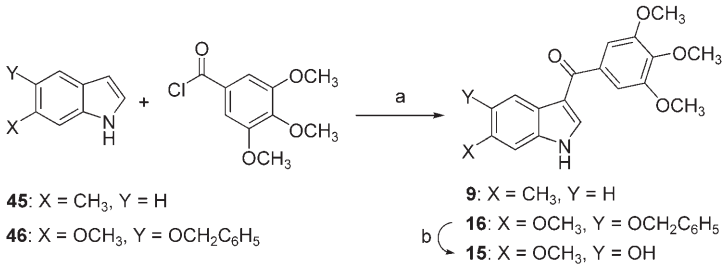


II

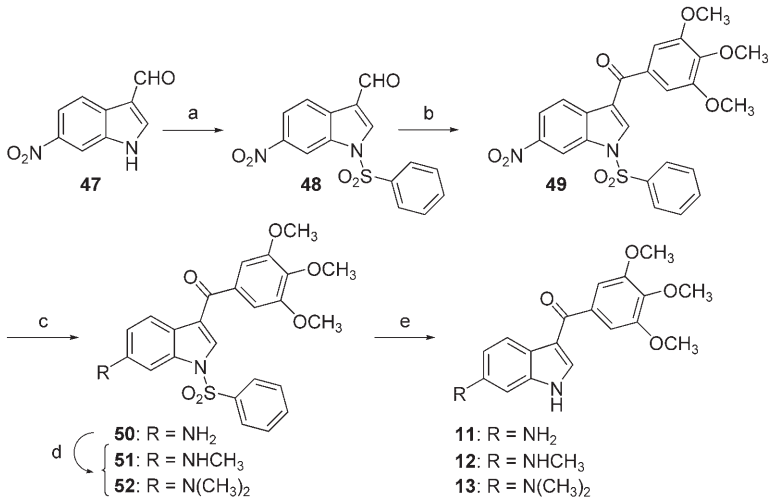
regimen 3: (scaffold II)

**41**: $R^1$ = H, $R^2$ = H, $R^3$ = $OCH_3$, X = S
**42**: $R^1$ = H, $R^2$ = H, $R^3$ = $OCH_3$, X = $SO_2$
**43**: $R^1$ = $CH_3$, $R^2$ = H, $R^3$ = $OCH_3$, X = O
**44**: $R^1$ = $CH_3$, $R^2$ = $OCH_3$, $R^3$ = H, X = O




**Scheme 1.** Reagents and conditions: a) 3.0 M EtMgBr, $ZnCl_2$, $AlCl_3$, $CH_2Cl_2$, RT; b) $H_2$, Pd/C, EtOAc, AcOH.

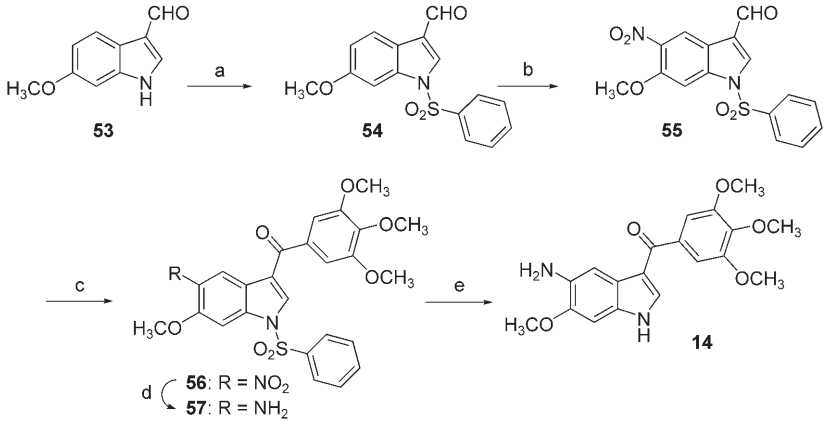



**Scheme 2.** Reagents and conditions: a) $Bu_4NHSO_4$, NaOH, $PhSO_2Cl$, $CH_2Cl_2$, RT; b) 1. (3,4,5-trimethoxyphenyl)magnesium bromide, THF, 0 °C; 2. pyridinium dichromate (PDC), $CH_2Cl_2$, molecular sieves, RT; c) Fe/AcOH, HCl; d) $K_2CO_3$, $CH_3I$, *N*,*N*-dimethylformamide (DMF), RT; e) 3 N NaOH, EtOH, reflux.

did not work in case of the 6-nitroindole owing to its low reactivity. The alternative approach by Grignard reaction of (3,4,5-trimethoxyphenyl)magnesium bromide[9] with the N1-benzenesulfonyl-protected 6-nitro-1*H*-indole-3-carbaldehyde **48**[14] followed by PDC oxidation gave the 1-benzenesulfonyl-6-nitro-3-aroylindole **49**. The Fe/AcOH-mediated reduction of **49** afforded 6-amino-1-benzenesulfonyl-3-aroylindole **50**. Alkylation of **50** with $CH_3I/Et_3N$ gave the methylamino form **51** and dimethylamino form **52**. Deprotection of **50**, **51**, and **52** at reflux with a solution of 3 N NaOH/EtOH afforded the desired compounds **11**, **12**, and **13**, respectively.

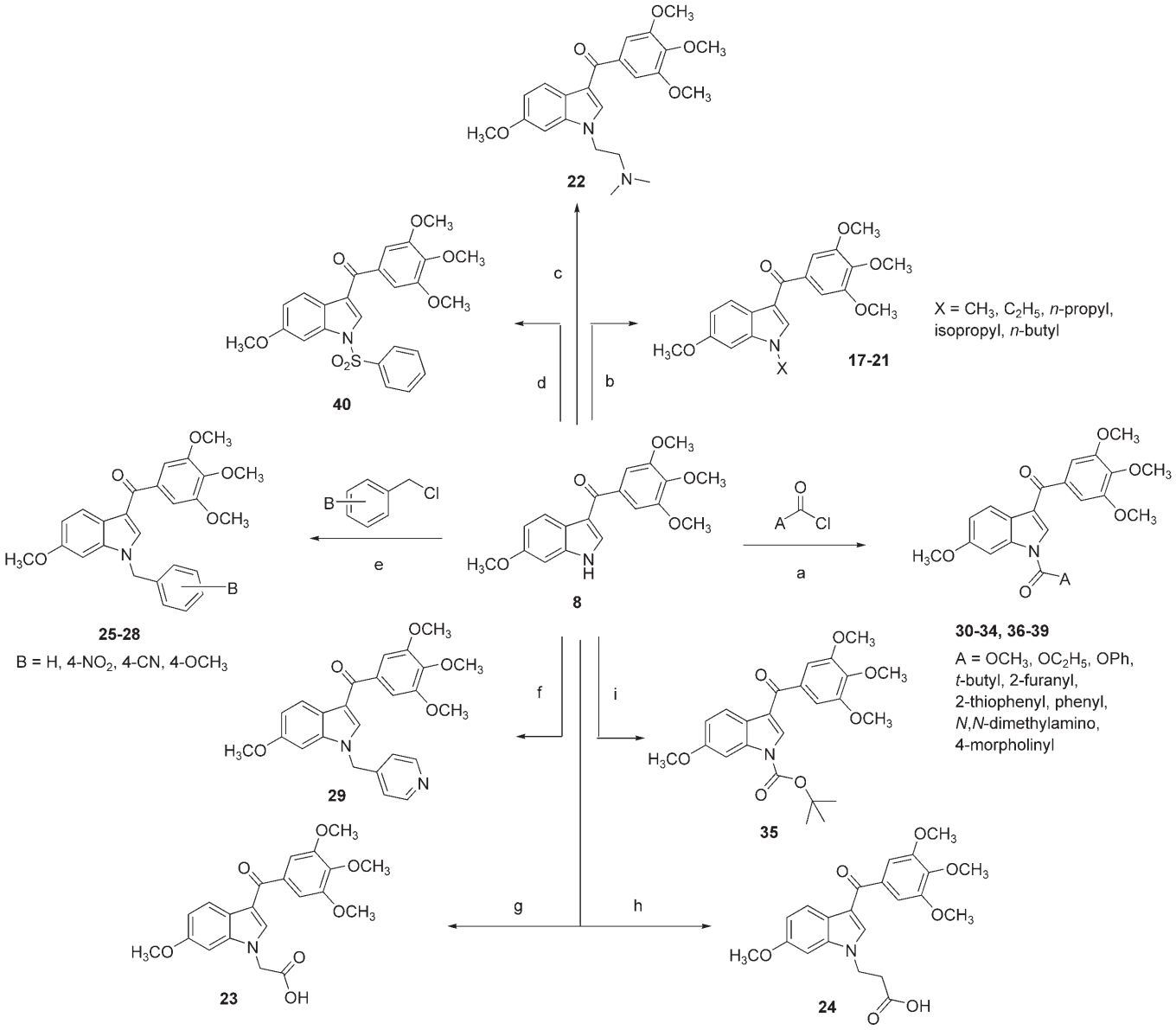
Compound **14**, with an additional amino group at the 5 position of indole in **8**, was prepared by starting from 6-methoxy-1*H*-indole-3-carbaldehyde **53**[15] in five steps, as shown in Scheme 3. N1-benzenesulfonyl protection of **53** followed by nitration with $HNO_3/H_2SO_4$ gave the 5-nitro-substituted indole **55**. Compound **14** was synthesized from **55** in a manner similar to the synthesis of compound **11** from **48**, as described in Scheme 3.

The SAR at the N1 position of **8** was further studied by synthesizing a series of alkyl- (**17**–**21**), alkylamino- (**22**), alkylcarboxylic acid (**23**–**24**), and benzyl- (**25**–**29**) substituted analogues as shown in Scheme 4. The amide (**30**–**33**), carbamate (**34**–**37**), urea (**38**–**39**), and sulfonamide (**40**) functionalities were also introduced at the N1 position of the 3-aroylindole skeleton of **8**. The general method for synthesis of compounds **30**–**34** and **36**–**39** included treatment of compound **8** with KO*t*Bu in THF followed by stirring with the corresponding acyl chloride at room temperature for 1–3 h to obtain the desired N1-substituted 3-aroylindole in 70–95% yields. Compound **35**, with a 1-*tert*-butoxy group, was prepared in 76% yield by treating **8** with di-*tert*-butyl dicarbonate in the presence of KO*t*Bu. The N1-alkyl-substituted compounds **17**–**21** were synthesized in 68–83% yields from **8** by allowing it to react with the corresponding alkyl halide at room temperature using KO*t*Bu as a base. Treatment of **8** with $Cs_2CO_3$, KI, and 2-dimethylaminoethyl chloride hydrochloride in $CH_3CN$ at reflux for 5 h gave the 1-dimethylethylamino analogue **22** in 53% yield. N1-benzylation of **8** with the corresponding 4-substituted benzyl chlorides in the presence of KO*t*Bu and THF gave **25**–**28** in 61–74% yields. Compound **29**, with an *N*-4-pyridylmethyl group, was prepared by treating **8** with 4-picolyl




**Scheme 3.** Reagents and conditions: a) $Bu_4NHSO_4$, NaOH, $PhSO_2Cl$, $CH_2Cl_2$, RT; b) $HNO_3$, $H_2SO_4$, RT; c) 1. (3,4,5-trimethoxyphenyl)magnesium bromide, THF, 0 °C; 2. PDC, $CH_2Cl_2$, molecular sieves, RT; d) Fe/AcOH, HCl; e) 3 N NaOH, EtOH, reflux.



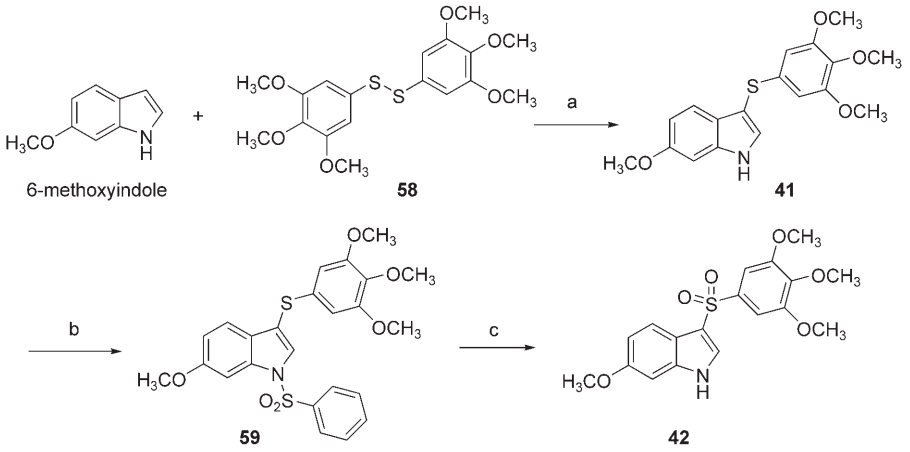

**Scheme 4.** Reagents and conditions: a) KO*t*Bu, THF, 0 °C→RT; b) KO*t*Bu, THF, RT; c) $Cs_2CO_3$, KI, 2-dimethylaminoethyl chloride hydrochloride, $CH_3CN$, reflux; d) $Bu_4NHSO_4$, NaOH, $PhSO_2Cl$, $CH_2Cl_2$, RT; e) KO*t*Bu, THF, reflux; f) $Cs_2CO_3$, KI, 4-picolyl chloride hydrochloride, $CH_3CN$, reflux; g) 1. $Cs_2CO_3$, ethyl bromoacetate, $CH_3CN$, reflux; 2. LiOH, MeOH; h) 1. $Cs_2CO_3$, methyl acrylate, $CH_3CN$, RT; 2. LiOH, MeOH; i) KO*t*Bu, di-*tert*-butyl dicarbonate, THF, RT.

chloride hydrochloride in $CH_3CN$ at reflux for 6 h using $Cs_2CO_3$ as a base and KI as a catalyst to give 53% yield. 1-Alkylcarboxylic acids **23** and **24** were obtained through a two-step synthesis. Treatment of compound **8** with $Cs_2CO_3$ and ethyl bromoacetate in $CH_3CN$ at reflux followed by hydrolysis with LiOH afforded the desired **23** in 67% yield (two steps). Michael addition of methyl acrylate to **8** at room temperature in the presence of $Cs_2CO_3$ followed by hydrolysis with LiOH/MeOH gave the 1-(3-propanoic acid)-3-aroylindole **24** in 75% yield (two steps).

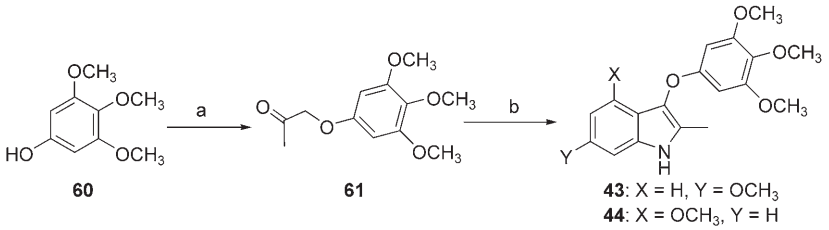
Diarylthioether **41** and diarylsulfone **42** were synthesized from the key reagent bis(3,4,5-trimethoxyphenyl)disulfide (**58**), which was prepared following published methodology.[16] Treatment of 6-methoxyindole with EtMgBr and $ZnCl_2$ followed by stirring with **58** at room temperature for 5–6 h gave the sulfide **41** in 49% yield. The conversion of sulfide **41** into sulfone **42** was carried out in three steps through protection with a benzenesulfonyl group, *m*-CPBA-mediated oxidation, and deprotection with NaOH to give **42** in 49% yield (three steps, Scheme 5).

Compound **43**, the ether analogue of **41**, was synthesized starting from 3,4,5-trimethoxyphenol (**60**), which was allowed to react with bromoacetone in the presence of $Cs_2CO_3$ in DMF to afford **61** in 92% yield. Compound **61** was converted into the corresponding phenylhydrazone by reaction with 3-methoxyphenylhydrazine hydrochloride in the presence of triethylamine. Finally, stirring phenylhydrazone with $PCl_3$ gave the desired compound **43** and the positional isomer **44** in 48 and 19% yields, respectively (Scheme 6).




**Scheme 5.** Reagents and conditions: a) 3.0 M EtMgBr, $ZnCl_2$, $CH_2Cl_2$, RT; b) $Bu_4NHSO_4$, NaOH, $PhSO_2Cl$, $CH_2Cl_2$, RT; c) 1. MCPBA, $CH_2Cl_2$, RT; 2. 3 N NaOH, EtOH, reflux.




**Scheme 6.** Reagents and conditions: a) $CH_3COCH_2Br$, $Cs_2CO_3$, DMF, 25 °C; b) 1. (3-methoxyphenyl)hydrazine·HCl, $Et_3N$, MeOH, 25 °C; 2. $PCl_3$, $CH_2Cl_2$, 25 °C.

**Biological evaluation: A) in vitro cell growth inhibition**

The synthesized compounds **9**–**43** were evaluated for cytotoxic activities against three types of human cancer cell lines: oral epidermoid carcinoma KB cells, stomach carcinoma MKN-45 cells, and lung carcinoma H460 cells (Table 1). These cell lines were selected for screening, as compound **8** has shown in vivo efficacy against KB and MKN-45 xenografts in nude mice.[13] Potent compounds were further examined for antiproliferative activity against two types of MDR-positive human cancer cell lines, including KB-vin10 and KB-7D. (Table 2)

***Ring B modification of 8***

We previously reported that the electron-donating C6 methoxy group is important for maximal cytotoxicity, as it mimics the 4-methoxy group in the B ring of CA4 and aminobenzophenones. Replacing it with the weaker electron-donating and more water-soluble hydroxy group (compound **10**) resulted in a moderate loss of activity, while an electron-withdrawing group caused drastic loss of activity.[12] In further exploration of the structure–activity relationships at the C6 position of indole, we evaluated the effect of replacement of the C6 methoxy group in ring B of **8** with electron-donating methyl (in **9**), amino (in **11**), *N*-methylamino (in **12**), and *N,N*-dimethylamino (in **13**) groups. The 4′-methoxy group in CA4 and its analogues is important for potent bioactivity, although some analogues with a 4′-dimethylamino group have shown similar potency.[17,18] Along similar lines, we wanted to study the effect of replacement of C6 methoxy in **8** with alkylamino groups, with an aim to improve solubility while retaining cytotoxicity. Surprisingly, the moderately electron-donating methyl analogue **9** displayed substantially better cytotoxic activity than the strong electron-donating group analogues **11** (amino) and **12** (*N*-methylamino). The *N,N*-dimethylamino analogue **13** displayed antiproliferative potency similar to that of the parent compound **8**, with $IC_{50}$ values of 3–7 nM against KB, MKN-45, and H460 cell lines. The methylamino-substituted **12** displayed moderate cytotoxicity ($IC_{50}$: 36–65 nM) against all three cancer cell lines. The hydrochloride salt of compound **13** also displayed a significant improvement in solubility in water (268.5 $\mu g\,mL^{-1}$) and phosphate-buffered saline (PBS, 11.6 $\mu g\,mL^{-1}$) relative to that of **8** (0.9 $\mu g\,mL^{-1}$ in water and 0.6 $\mu g\,mL^{-1}$ in PBS). Compound **13**, with an *N,N*-dimethylamino group, appears to be a better candidate than the methyl group-bearing **9** for further studies in the consideration of potency and water solubility.

In an effort to design potential prodrugs with higher water solubility in a manner similar to CA4P (**2**) and **6**, we decided to introduce an additional hydroxy (in **15**) or amino (in **14**) group at the C5 position of the indole (ortho to the methoxy group), keeping the C6 methoxy group intact. To our surprise, this led to a drastic decrease in potency in contrast to the activity of CA4 and many of its analogues that have hydroxy or amino groups ortho to the 4′-methoxy function. Compound **16**, the 5-benzyloxy analogue of **8**, showed some restoration of activity especially against the KB and MKN-45 cell lines. In summary, the electron-donating methyl or the more water-soluble electron-donating dimethylamino groups can replace the methoxy group at the position 6 of the indole moiety of **8**, playing an integral role in antiproliferative activity. An additional hydroxy or amino group at the C5 position of indole is detrimental to cytotoxic activity.

***N1 substitution of 8***

The N1 position of 3-aroylindoles is a facile site for modification with a variety of functional groups to allow an extensive SAR study at this site. Functionalities such as alkyl, alkylamino, alkylcarboxylic acid, aroyl, heteroaroyl, carbamoyl, arylsulfonyl, alkyloxycarbonyl, aryloxycarbonyl, and benzyl were introduced

**Table 1.** $IC_{50}$ values ([nM] ± SD[a]) of 3-aroylindole derivatives **8**–**44**.

| Compd | Cell Type | | |
|---|---|---|---|
| | KB | MKN-45 | H460 |
| **8** | 4 ± 0.5 | 3 ± 0.9 | 7 ± 0.4 |
| **9** | 8 ± 0.8 | 8 ± 0.1 | 4 ± 0.2 |
| **10** | 88 ± 8 | 70 ± 36 | 380 ± 37 |
| **11** | 360 ± 20 | 482 ± 68 | 1360 ± 89 |
| **12** | 36 ± 6 | 65 ± 9 | 37 ± 3 |
| **13** | 6 ± 0.1 | 5 ± 2.1 | 4 ± 0.5 |
| **14** | 5223 ± 1047 | 3202 ± 1658 | 4130 ± 78 |
| **15** | 1685 ± 1125 | 1100 ± 520 | 2070 ± 153 |
| **16** | 318 ± 122 | 255 ± 35 | 880 ± 45 |
| **17** | 34 ± 0.9 | 23 ± 3 | 36 ± 2 |
| **18** | 64 ± 3 | 56 ± 21 | 69 ± 5 |
| **19** | 177 ± 13 | 83 ± 19 | 285 ± 47 |
| **20** | 287 ± 16 | 175 ± 14 | 368 ± 77 |
| **21** | 203 ± 11 | 104 ± 27 | 310 ± 65 |
| **22** | 880 ± 26 | 500 ± 27 | 910 ± 15 |
| **23** | 1825 ± 32 | 1200 ± 800 | 1980 ± 69 |
| **24** | 3746 ± 321 | 3100 ± 30 | 3546 ± 402 |
| **25** | 424 ± 33 | 318 ± 43 | 604 ± 17 |
| **26** | 293 ± 39 | 128 ± 21 | 350 ± 23 |
| **27** | 243 ± 29 | 93 ± 37 | 293 ± 18 |
| **28** | 207 ± 35 | 95 ± 5 | 266 ± 33 |
| **29** | 140 ± 5 | 77 ± 12 | 161 ± 7 |
| **30** | 6 ± 2 | 5 ± 1 | 8 ± 0.9 |
| **31** | 4 ± 0.5 | 6 ± 3 | 7 ± 0.5 |
| **32** | 6 ± 1 | 6 ± 2 | 7 ± 1 |
| **33** | 20 ± 2 | 13 ± 8 | 10 ± 3 |
| **34** | 7 ± 3 | 3 ± 0.9 | 7 ± 1 |
| **35** | 12 ± 3 | 6 ± 1 | 21.7 ± 0.9 |
| **36** | 13 ± 0.9 | 14 ± 6 | 16 ± 3 |
| **37** | 73 ± 5 | 56 ± 26 | 88 ± 9 |
| **38** | 285 ± 6 | 143 ± 44 | 335 ± 23 |
| **39** | 237 ± 47 | 193 ± 48 | 615 ± 34 |
| **40** | 198 ± 10 | 73 ± 19 | 103 ± 18 |
| **41** | 2 ± 0.5 | 1 ± 0.4 | 7 ± 0.8 |
| **42** | 214 ± 119 | 75 ± 7 | 170 ± 15 |
| **43** | 1.2 ± 0.5 | 1.1 ± 0.1 | 2.0 ± 0.8 |
| **44** | > 10000 | > 10000 | > 10000 |
| **CA4**[10,12] | 6.2 ± 0.5 | 82 ± 8 | 17 ± 6 |

[a] All experiments were independently performed at least three times.

**Table 2.** Comparison of cytotoxicities ($IC_{50}$ [nM] ± SD)[a] against KB and KB drug-resistant cell lines.

| Compd | Cell Type | | |
|---|---|---|---|
| | KB | KB-7D | KB-vin10 |
| **8** | 4 ± 1 | 9 ± 2 | 8 ± 2 |
| **9** | 8 ± 0.8 | 3 ± 1 | 7.7 ± 1.2 |
| **13** | 6 ± 0.1 | 9.7 ± 1.1 | 14.9 ± 1.3 |
| **17** | 34 ± 0.9 | 16 ± 1 | 21.3 ± 3.5 |
| **30** | 6 ± 2 | 3 ± 1 | 5 ± 2 |
| **31** | 4 ± 0.5 | 1 ± 0.3 | 1 ± 0.3 |
| **33** | 20 ± 2 | 22 ± 3 | 26 ± 4 |
| **34** | 7 ± 3 | 3.3 ± 0.7 | 2 ± 0.5 |
| **35** | 12 ± 3 | 18 ± 2 | 19 ± 1 |
| **41** | 2 ± 0.5 | 2 ± 0.4 | 2 ± 0.5 |
| **42** | 214 ± 11 | 81 ± 7 | 151 ± 10 |
| **CA4**[10] | 6.2 ± 0.5 | 7.9 ± 0.5 | 9.5 ± 0.6 |

[a] All experiments were independently performed at least three times.

at the N1 position of **8** in an effort to study the effect of size, hydrogen bonding capability, and hydrophobicity on activity and water solubility.

The N1-alkyl-substituted compounds **17**–**21**, modified with methyl, ethyl, propyl, isopropyl, and butyl groups, exhibited decreased cytotoxicity relative to **8**. The N1-methyl-substituted **17** was 3–4 fold less potent than **8**, while the ethyl-substituted **18** showed a further decrease in activity, being 8–10-fold less potent than the parent compound. Any further increase in the size of the alkyl substituent resulted in a considerable loss of activity, with $IC_{50}$ values for compounds **19**–**21** ranging between 100–300 nM for three cell lines. In an effort to improve water solubility, dimethylamino and carboxylic acid groups were introduced on the alkyl substituent at the N1 position in **17** and **18**. Although 2-(*N,N*-dimethylamino)ethyl-substituted **22** showed a significant improvement in solubility (941.1 $\mu g\,mL^{-1}$ in water, 626.8 $\mu g\,mL^{-1}$ in PBS), it showed a substantial decrease in cytotoxic activity. The ethanoic and propanoic acid analogues **23** and **24** showed a dramatic decrease in activity to micromolar $IC_{50}$ values. In summary, alkylation at the N1 position of **8** with methyl or ethyl groups caused a 10-fold decrease in cytotoxicity relative to the parent compound; a further increase in the bulkiness of the substituents resulted in a 50-fold decrease in potency. The 2-(*N,N*-dimethylamino)ethyl substitution at the N1 position of **8** improved water solubility but resulted in decreased activity; the alkanoic acid substitutions drastically decreased the activity.

Benzyl groups were introduced at the N1 position to determine whether an aryl group with various substituents could retain activity and also create a new site for modification to improve drugability. Compounds **25**–**29**, with benzyl, 4-nitrobenzyl, 4-cyanobenzyl, 4-methoxybenzyl, and 4-pyridylmethyl groups, exhibited moderate cytotoxicity against KB and H460 cell lines and comparatively greater activity against the MKN-45 cell line. The 4-pyridylmethyl-substituted **29** showed better activity than other benzyl analogues. The potency of benzyl-substituted compounds was similar to that of compounds **19**–**21** with bulkier alkyl groups, thus indicating that an increase in size of the N1 substituent may result in decreased activity. Furthermore, various functionalities such as amides (in **30**–**33**), carbamates (in **34**–**37**), and ureas (in **38** and **39**) were introduced at the N1 position. Compounds **30**–**33**, with benzoyl, 2-furoyl, 2-thienoyl, and pivaloyl substituents at N1, exhibited $IC_{50}$ values in the range 4–20 nM against all three cell lines, indicating that compounds with an amide group at the N1 position of **8** retain activity equivalent to that of the parent compound.

The introduction of a carbamate instead of an amide in compounds **30** and **33** gave the phenyl carbamate **34** and the *tert*-butyl carbamate **35**, respectively, which retained a potent capacity to inhibit cell growth. The methyl and ethyl carbamates **36** and **37** also displayed good activity, although the ethyl carbamate analogue **37** was 4–5-fold less potent.

The flexibility of the N1 position to accommodate different functionalities further prompted us to synthesize urea analogues **38** and **39** by introducing dimethylamino carbonyl and morpholine-4-carbonyl groups at the N1 position, respectively.

In contrast to the amides and carbamates, both compounds showed a significant decrease in activity relative to **8**.

As amide **30** and carbamate **34** exhibited potent activity, phenylsulfonamide **40** was also synthesized and evaluated for cytotoxicity. However, it showed a substantial decrease in cytotoxicity in comparison with the amide and carbamate analogues.

In summary, the substitution of alkyl or benzyl groups at the N1 position of **8** generally led to a decrease in activity. Amide and carbamate analogues of **8** displayed excellent antiproliferative activities, but the urea analogues were 50- to 100-fold less active.

***Modifications at the carbonyl bridgehead***

We previously reported that the replacement of the 3-carbonyl group of **8** with a methylene group could maintain good cytotoxicity.[12] On a similar note, we continued our efforts to explore the replacement of the carbonyl group with sulfide (in **41**), sulfone (in **42**), and oxy (in **43** and **44**) groups. The idea, which employs the sulfide and sulfone as a replacements for the 3-carbonyl group of 3-aroylindoles, was originally based on the expectation that the sulfide **41** and sulfone **42** possibly form a sulfoxide through metabolic oxidative or reductive reactions in vivo. De Martino et al.[19] recently reported arylthioindoles as potent inhibitors of tubulin polymerization. Methyl 3-[(3,4,5-trimethoxyphenyl)thio]-5- methoxy-1*H*-indole-2-carboxylate was the most potent compound, with an $IC_{50}$ value of 13 nM against MCF-7 human breast carcinoma cells, while the sulfones were much less potent. Along similar lines, compound **41** displayed potent cytotoxicity, with $IC_{50}$ values of 1–8 nM against KB, MKN-45, and H460 human cancer cell lines, while the sulfone **42** was 50- to 100-fold less potent. The oxygen analogue **43** showed similar activity to the sulfide analogue **41**. It is worth noting that **41** and **43** showed similar or greater activities of growth inhibition relative to **8** against all the three cell lines. Shifting of the methoxy group to the 4 position of indole (in **44**) resulted in a loss of activity.

Selected compounds with potent cytotoxicity against KB, MKN-45, and H460 were further evaluated for their antiproliferative activity against the MDR-positive human cancer cell lines KB-7D and KB-vin10, as shown in Table 2. All evaluated 3-aroylindoles **9**, **13**, **17**, **30**, **31**, **33**, **34**, **35**, **41**, and **42** were active against MDR-positive cells, indicating that they are not substrates for the efflux pump.

### Biological evaluation: B) inhibition of tubulin polymerization

Compound **8** was shown to have strong interactions with tubulin which correlates well with its cytotoxicity.[13] The newly synthesized 3-aroylindoles were also evaluated for their antitubulin polymerization activities (Table 3). The B-ring-modified compounds **9** and **13**, with an electron-donating methyl and dimethylamino group at the C6 position of indole, respectively, were potent inhibitors of tubulin polymerization. Compound **15**, the 5-hydroxy analogue of **8**, showed weak antitubulin polymerization activity, which correlates to its weak cytotoxicity. The N1-substituted analogues are generally weak inhibitors of tubulin polymerization. The 1-benzoyl analogue **30** and 1-phenoxy-carbonyl analogue **34**, though potent cell growth inhibitors against various cell lines including MDR-positive cell lines, showed weak antitubulin activities. The sulfide bridgehead analogue **41** also exhibited potent antitubulin activity, which correlates well with its cytotoxicity.

**Table 3.** Inhibition of tubulin polymerization by 3-aroylindoles.

| Compd | $IC_{50}$ [μM] ± SD[a] |
|---|---|
| **8** | 2.8 ± 0.3 |
| **9** | 2.3 ± 0.5 |
| **13** | 1.0 ± 0.1 |
| **15** | 30 ± 4 |
| **30** | 9 ± 3 |
| **34** | 6.6 ± 0.6 |
| **41** | 1.9 ± 0.1 |
| **42** | 11.9 ± 2.0 |
| **CA-4**[12] | 2.0 ± 0.4 |

[a] All experiments were independently performed at least three times.

***Pharmacokinetic studies***

The pharmacokinetic profile of the hydrochloride salt of **13**, which showed improved physicochemical properties, particularly aqueous solubility, was determined in male Sprague–Dawley rats. It showed 9% oral bioavailability and a short half-life (0.4 h) which were similar to the parent compound **8** (Table 4).

**Table 4.** Pharmacokinetic parameters of the hydrochloride salts of **8** and **13** in male Sprague–Dawley rats.[a]

| Parameter | **8**[b] | | **13**[c] | |
|---|---|---|---|---|
| | po | iv | po | iv |
| Dose [mg kg$^{-1}$] | 25.1 | 4.6 | 19.7 | 1.6 |
| AUC [ng h mL$^{-1}$][d] | 1288 ± 357 | 2734 ± 763 | 480 ± 472 | 454 ± 25 |
| $C_{max}$ [ng mL$^{-1}$][e] | 187 ± 34 | | 425 ± 32 | |
| $T_{max}$ [h][f] | 3.5 ± 1.0 | | 0.6 ± 0.4 | |
| $t_{1/2}$ [h][g] | 2.8 ± 1.47 | 5.8 ± 1.7 | | 0.4 ± 0.1 |
| $V_{ss}$ [L kg$^{-1}$][h] | | 1.7 ± 0.9 | | 1.0 ± 0.2 |
| $CL$ [mL min$^{-1}$ kg$^{-1}$][i] | | 29.8 ± 9.1 | | 60 ± 3.3 |
| $F$ [%][j] | 8.8 | | 9 | |

[a] All values represent the mean ± SD, $n = 3$. [b] 0.5% CMC suspension. [c] EtOH/Tween 80/$H_2O$ 5:15:80. [d] Estimated area under the curve (plasma concentration vs. time) after intravenous and oral dosing, $t =$ 24 h. [e] Maximum plasma concentration after oral dosing. [f] Time taken to reach maximum concentration. [g] Half-life. [h] Volume of distribution during steady state. [i] Clearance. [j] Oral bioavailability.

## Conclusions

Encouraged by the preliminary preclinical studies of the anticancer drug candidate **8**, further SAR studies of the 3-aroylindoles were carried out to improve the potency the solubility of

this compound class. The structure modifications were focused on three regimens: substitution at the B ring, at the N1 position, and of the 3-carbonyl linker. The SAR information revealed that the methoxy group in the B ring could be replaced with an electron-donating group such as methyl (in **9**) or *N,N*-dimethylamino (in **13**), which retain both strong cytotoxic and antitubulin activities. An additional OH or $NH_2$ group at the 5 position of indole resulted in a dramatic loss of potency. The hydrochloride salt of compound **13** displayed much better solubility in both water and PBS and improved bioavailability relative to the parent compound. Alkyl or benzyl substitution at the N1 position of **8** led to a substantial decrease in activity, although the hydrochloride salt of the *N,N*-dimethylaminoethyl-substituted compound **22** showed a significantly improved physicochemical profile over that of **8**. The introduction of amide (**30**–**33**) and carbamate (**34**–**37**) functionalities at the N1 position of **8** gave analogues with excellent antiproliferative activities, but the urea moiety (in **38**–**39**) at the same position led to a decrease in activity. The cytotoxic potency of **8** was improved on replacing the carbonyl group with sulfur (in **41**) or oxygen (in **43**), indicating that the carbonyl moiety is important but not essential for activity. The *N,N*-dimethylamino analogue **13**, which has potent cytotoxicity and antitubulin activity and significantly improved physicochemical profile in comparison with the parent compound has potential for further development as an anticancer agent, and is being developed as a backup candidate for **8**.

## Experimental Section

All reagents were used as purchased without further treatment unless otherwise stated. All reactions were carried out under an atmosphere of dry nitrogen. All solvents were dried according to standard procedures. Melting points were determined on a Yanaco (MP-500D) melting point apparatus and are reported uncorrected. Flash column chromatography was performed with silica gel (Merck Kieselgel 60, 230–400 mesh). The reactions were monitored by thin-layer chromatography (TLC) using Merck 60 $F_{254}$ silica gel glass-backed plates ($5\times10$ cm$^2$); zones were detected visually under UV irradiation ($\lambda=254$ nm) or by spraying with phosphomolybdic acid reagent (Aldrich) followed by heating at 80 °C. NMR spectra ($^1$H and $^{13}$C) were recorded in deuterated solvents specified on a Varian Mercury-300 spectrometer operating at 300 and 75 MHz, respectively, with chemical shifts reported in ppm ($\delta$) downfield from tetramethylsilane as an internal standard. High-resolution mass spectrometry (HRMS) was carried out on a Finnigan (MAT-95XL) fast atom bombardment (FAB) mass spectrometer. Elemental analyses were performed on a Heraeus CHN-O Rapid microanalyzer. RPMI 1640 medium and fetal bovine serum were purchased from HyClone, and nonessential amino acids were purchased from Biological Industries (Place). MTS assay kits were obtained from Promega (Place). Propidium iodide, PIPES, and GTP were purchased from Sigma. MAP-rich tubulin was purchased from Cytoskeleton Inc. (Denver, USA). The animal use protocols were reviewed and approved by the Institutional Animal Care and Use Committee of National Health Research Institutes, Taiwan.

**6-Methyl-3-(3′,4′,5′-trimethoxybenzoyl)indole (9)**: Ethylmagnesium bromide (3.3 mL, 3.0 M in diethyl ether) was added to a mixture of 6-methyindole (1.00 g, 7.62 mmol) and anhydrous zinc chloride (2.07 g, 15.10 mmol) in dry dichloromethane (50 mL), over 10 min at room temperature. The suspension was stirred for 1 h, and then the solution of 3,4,5-trimethoxybenzoyl chloride (2.10 g, 9.10 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 (1.01 g, 7.57 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 (50 mL) and extracted with $CH_2Cl_2$ ($3\times$ 50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a brown oil, which was subjected to silica gel chromatography (EtOAc/*n*-hexane 1:1) and recrystallized ($CH_2Cl_2$/EtOAc) to afford compound **9** (78% yield); mp: 201.5–202.2 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=2.48$ (s, 3H), 3.88 (s, 6H), 3.93 (s, 3H), 7.10 (s, 2H), 7.16 (d, $J=8.1$ Hz, 1H), 7.23 (d, $J=0.6$ Hz, 1H), 7.65 (d, $J=3$ Hz, 1H), 8.23 (d, $J=8.1$ Hz, 1H), 8.81 ppm (br, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=21.7$, 56.2, 60.9, 106.3, 111.2, 116.9, 121.9, 124.1, 124.4, 132.7, 134.0, 135.9, 136.8, 140.8, 152.9, 190.4 ppm; MS (FAB, NBA *m/z*) 326 [*M*+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{19}H_{20}NO_4$ [*M*+H$^+$] 326.1392, found 326.1398; anal. ($C_{19}H_{19}NO_4$) C, H, N.

**6-Amino-3-(3′,4′,5′-trimethoxybenzoyl)indole (11)**: A stirred solution of **50** (0.046 g, 0.10 mmol) and 3 N sodium hydroxide (10 mL, 30 mmol) in ethyl alcohol (30 mL) was heated at reflux for 16 h. The solution was evaporated, and the residue was extracted with $CH_2Cl_2$ ($3\times30$ mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel ($CH_2Cl_2$/MeOH 30:1) to afford **11** (90% yield) as dark brown solid; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=3.83$ (s, 6H), 3.89 (s, 3H), 6.68–6.74 (m, 2H), 7.04 (s, 2H), 7.47 (d, $J=3.0$ Hz, 1H), 8.09 (d, $J=8.4$ Hz, 1H), 9.10 ppm (br, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=56.3$, 60.9, 97.0, 106.1, 113.1, 116.1, 119.5, 122.7, 132.2, 135.7, 137.5, 140.4, 142.4, 152.5, 190.0 ppm; MS (EI *m/z*) 326 [*M*$^+$]; HRMS (FAB *m/z*) calcd for $C_{18}H_{18}N_2O_4$ [*M*$^+$] 326.1267, found 326.1262.

**6-Methylamino-3-(3′,4′,5′-trimethoxybenzoyl)indole (12) and 6-dimethylamino-3-(3′,4′,5′-trimethoxybenzoyl)indole (13)**: Iodomethane (0.19 mL, 3.03 mmol) was added to a stirred solution of **50** (0.475 g, 1.01 mmol) in DMF (10 mL), and the mixture was heated at 50 °C overnight. The reaction was quenched with water, and extracted with EtOAc ($3\times20$ mL). The combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, evaporated in vacuo, and purified by silica gel chromatography (EtOAc/*n*-hexane 1:3) to give an inseparable mixture of **51** and **52**. The mixture was hydrolyzed in a manner similar to that described for the preparation of **11** to give **12** and **13. 12**: $^1$H NMR (300 MHz, $CDCl_3$): $\delta=2.85$ (s, 3H), 3.85 (s, 6H), 3.91 (s, 3H), 6.55 (d, $J=1.8$ Hz, 1H), 6.66 (dd, $J=1.9$, 8.5 Hz, 1H), 7.07 (s, 2H), 7.48 (d, $J=2.7$ Hz, 1H), 8.10 (d, $J=8.4$ Hz, 1H), 8.91 ppm (br, 1H); MS (EI *m/z*) 341 [*M*$^+$]; HRMS (FAB *m/z*) calcd for $C_{19}H_{20}N_2O_4$ [*M*$^+$] 340.1423, found 340.1402. **13**: mp: 162.5–164.0 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=$ 2.92 (s, 6H), 3.82 (s, 6H), 3.90 (s, 3H), 6.64 (d, $J=2.1$ Hz, 1H), 6.86 (dd, $J=2.2$, 8.8 Hz, 1H), 7.07 (s, 2H), 7.50 (d, $J=2.7$ Hz, 1H), 8.17 (d, $J=8.7$ Hz, 1H), 9.46 ppm (br, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta=$ 29.8, 41.5, 56.2, 60.9, 94.5, 106.1, 111.2, 116.4, 117.7, 122.2, 132.3, 135.8, 138.0, 140.3, 148.4, 152.4, 190.1 ppm; MS (EI *m/z*) 355 [*M*+H$^+$]; HRMS (FAB *m/z*) calcd for $C_{20}H_{22}N_2O_4$ [*M*$^+$] 354.1580, found 354.1578.

**5-Amino-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (14)**: The title compound was obtained as gray solid in 94% yield from **57** in a manner similar to that described for the preparation of **11**; mp: 193.1–194.3 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta=3.71$ (br), 3.89

(m, 9H), 3.91 (s, 3H), 6.87 (s, 1H), 7.06 (s, 2H), 7.54 (s, 1H), 7.72 ppm (s, 1H); MS (FAB, NBA *m/z*) 356 [$M^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{19}H_{20}N_2O_5$ [$M^+$] 356.1372, found 356.1377.

**5-Hydroxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (15)**: 30% palladium on charcoal (0.024 g) was added to a solution of **16** (0.15 g, 0.33 mmol) in ethyl acetate (10 mL) and ethanol (10 mL). The mixture was hydrogenated at 310 kPa for 12 h and filtered through a pad of celite, which was washed with ethyl acetate (3×20 mL). The filtrate was evaporated to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 2:1) to afford **15** (83% yield) as brown solid; $^1$H NMR (300 MHz, $CD_3OD/CDCl_3$): $\delta$ = 3.87 (m, 9H), 3.90 (s, 3H), 6.93 (s, 1H), 7.03 (s, 2H), 7.56 (s, 1H), 7.71 ppm (s, 1H); MS (FAB *m/z*) 359 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{19}H_{20}NO_6$ [$M$+$H^+$] 358.1291, found 358.1285.

**5-Benzyloxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (16)**: The title compound was obtained as a brown solid in 42% yield from 5-benzyloxy-6-methoxyindole and 3,4,5-trimethoxybenzoyl chloride in a manner similar to that described for the preparation of **8**; mp: 191.6–192.2 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.82 (s, 3H), 3.85 (s, 6H), 3.92 (s, 3H), 5.18 (s, 2H), 6.85 (s, 1H), 7.06 (s, 2H), 7.30 (m, 3H), 7.88 (m, 3H), 7.99 (s, 1H), 9.33 ppm (s, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 56.5, 56.6, 61.2, 71.6, 95.1, 106.4, 106.6, 117.3, 119.8, 127.8, 128.0, 128.7, 131.1, 131.8, 136.2, 137.5, 141.2, 146.2, 149.1, 153.2, 190.6 ppm; MS (FAB *m/z*) 448 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{26}H_{26}NO_6$ [$M$+$H^+$] 448.1760, found 448.1756.

**1-Methyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (17)**: Potassium *tert*-butoxide (0.050 g, 0.44 mmol) was added to a solution of **8** (0.100 g, 0.29 mmol) in THF (5 mL) under vigorous stirring at room temperature. Stirring was continued for 15 min followed by the addition of iodomethane (64 μL, 1.02 mmol) in one portion. After 5 h the solvent was evaporated, then water was added and the mixture was extracted with EtOAc (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:3) to afford **17** (83% yield) as a dark yellow solid; mp: 121.2–122.0 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.77 (s, 3H), 3.87 (s, 3H), 3.88 (s, 6H), 3.91 (s, 3H), 6.77 (d, $J$ = 1.8 Hz, 1H), 6.95 (dd, $J$ = 2.2, 8.8 Hz, 1H), 7.05 (s, 2H), 7.46 (s, 1H), 8.22 ppm (d, $J$ = 7.8 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 33.5, 55.5, 56.1, 60.8, 93.0, 105.8, 111.6, 115.0, 120.8, 122.9, 135.7, 136.2, 138.0, 140.2, 152.4, 156.9, 189.2 ppm; MS (FAB *m/z*) 356 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{20}H_{22}NO_5$ [$M$+$H^+$] 356.1498, found 356.1497; anal. ($C_{20}H_{21}NO_5$·0.5 $CH_2Cl_2$) C, H, N.

**1-Ethyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (18)**: The title compound was obtained as a pale yellow solid in 77% yield from **8** and iodoethane in a manner similar to that described for the preparation of **17**; mp: 102.5–103.6 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.50 (t, $J$ = 7.3 Hz, 3H), 3.88 (s, 9H), 3.92 (s, 3H), 4.16 (q, $J$ = 7.3 Hz, 2H), 6.82 (d, $J$ = 2.1 Hz, 1H), 6.96 (dd, $J$ = 2.4, 8.7 Hz, 1H), 7.07 (s, 2H), 7.54 (s, 1H), 8.23 ppm (d, $J$ = 9 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 15.1, 41.6, 55.6, 56.2, 60.8, 93.3, 105.9, 111.5, 115.2, 121.0, 123.1, 134.6, 135.8, 137.2, 140.2, 152.4, 156.8, 189.2 ppm; MS (FAB *m/z*) 370 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{21}H_{24}NO_5$ [$M$+$H^+$] 370.1654, found 370.1646; anal. ($C_{21}H_{23}NO_5$) C, H, N.

**1-Propyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (19)**: The title compound was obtained as a pale yellow solid in 78% yield from **8** and 1-iodopropane in a manner similar to that described for the preparation of **17**; mp: 120.4–121.1 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 0.96 (t, $J$ = 7.35 Hz, 3H), 1.89 (heptet, $J$ = 7.2 Hz, 2H), 3.87 (s, 3H), 3.88 (s, 6H), 3.92 (s, 3H), 4.06 (t, $J$ = 6.9 Hz, 2H), 6.81 (d, $J$ = 2.1 Hz, 1H), 6.95 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.07 (s, 2H), 7.52 (s, 1H), 8.24 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 11.4, 22.9, 48.5, 55.5, 56.0, 60.7, 93.4, 105.8, 111.3, 114.8, 121.0, 122.8, 135.5, 135.7, 137.2, 140.1, 152.3, 156.7, 189.1 ppm; MS (FAB *m/z*) 384 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{22}H_{26}NO_5$ [$M$+$H^+$] 384.1811, found 384.1812.

**1-Isopropyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (20)**: The title compound was obtained as a pale yellow solid in 68% yield from **8** and 2-bromopropane in a manner similar to that described for the preparation of **17**; mp: 140.5–141.5 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 1.55 (s, 3H), 1.57 (s, 3H), 3.89 (s, 9H), 3.93 (s, 3H), 4.64 (septet, $J$ = 6.6 Hz, 1H), 6.87 (d, $J$ = 2.1 Hz, 1H), 6.97 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.08 (s, 2H), 7.63 (s, 1H), 8.22 ppm (d, $J$ = 9 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 22.5, 29.6, 47.6, 55.7, 56.1, 60.9, 93.8, 106.2, 111.6, 115.6, 121.3, 123.3, 131.9, 136.1, 137.4, 140.6, 152.8, 157.1, 189.7 ppm; MS (EI *m/z*) 383 [$M^+$]; HRMS (EI *m/z*) calcd for $C_{22}H_{25}NO_5$ [$M^+$] 383.1733, found 383.1736.

**1-Butyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (21)**: The title compound was obtained as a white solid in 72% yield from **8** and 1-bromobutane in a manner similar to that described for the preparation of **17**; mp: 112.8–113.9 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 0.94 (t, $J$ = 7.3 Hz, 3H), 1.36 (heptet, $J$ = 7.5 Hz, 2H), 1.84 (pentet, $J$ = 7.2 Hz, 2H), 3.87 (s, 3H), 3.88 (s, 6H), 3.92 (s, 3H), 4.10 (t, $J$ = 6.9 Hz, 2H), 6.82 (d, $J$ = 2.1 Hz, 1H), 6.95 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.07 (s, 2H), 7.51 (s, 1H), 8.24 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 13.6, 20.0, 31.6, 46.6, 55.5, 56.0, 60.7, 93.4, 105.9, 111.3, 114.9, 121.0, 122.9, 135.5, 135.7, 137.3, 140.2, 152.4, 156.7, 189.1 ppm; MS (FAB *m/z*) 398 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{23}H_{28}NO_5$ [$M$+$H^+$] 398.1967, found 398.1974.

**1-(*N*,*N*-Dimethylaminoethyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (22)**: A stirred solution of **8** (0.010 g, 0.29 mmol), cesium carbonate (0.286 g, 0.87 mmol), potassium iodide (0.024 g, 0.14 mmol) and 2-dimethylaminoethyl chloride hydrochloride (0.063 g, 0.44 mmol) in dried acetonitrile (10 mL) was heated at reflux overnight. The reaction was quenched with water and extracted with EtOAc (3×20 mL). The organic layers were dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude product, which was purified by silica gel chromatography ($CH_2Cl_2$/MeOH 4:1) to afford **22** (53% yield) as brown solid; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.30 (s, 6H), 2.74 (t, $J$ = 6.4 Hz, 2H), 3.90 (s, 9H), 3.93 (s, 3H), 4.21 (t, $J$ = 6.6 Hz, 2H), 6.86 (d, $J$ = 2.4 Hz, 1H), 6.98 (dd, $J$ = 2.2, 8.8 Hz, 1H), 7.11 (s, 2H), 7.62 (s, 1H), 8.26 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 44.7, 45.4, 55.6, 56.1, 58.1, 60.8, 93.5, 106.2, 111.6, 115.4, 121.2, 123.3, 135.9, 136.2, 137.6, 140.6, 152.8, 157.3, 189.7 ppm; MS (FAB *m/z*) 413 [$M$+$H^+$]; HRMS (FAB *m/z*) calcd for $C_{23}H_{29}N_2O_5$ [$M$+$H^+$] 413.2076, found 413.2068.

**[6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indol-1-yl]acetic acid (23)**: A stirred solution of **8** (0.30 g, 0.88 mmol), potassium *tert*-butoxide (0.19 g, 1.75 mmol), and ethyl bromoacetate (0.15 mL, 1.32 mmol) in dried acetonitrile (30 mL) was heated at reflux for 4 h. The reaction was quenched with water and extracted with EtOAc (3×30 mL). The combined organic layers were dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:1) to afford ethyl ester product (76% yield). The ethyl ester compound was then dissolved in methanol (10 mL) and water (2 mL), and lithium hydroxide (0.032 g, 1.32 mmol) was added to the reaction mixture and heated at reflux under vigorous stirring overnight. The solution was evaporated and extracted with

EtOAc (3×20 mL). The organic layers were collected and dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude product, which was purified by silica gel chromatography ($CH_2Cl_2$/MeOH 12:1) to afford **23** (88% yield) as a pale yellow solid; mp: 233.1–234.4 °C; $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ = 2.29 (br), 3.89 (s, 3H), 3.91 (s, 6H), 3.92 (s, 3H), 3.99 (br), 4.87 (s, 2H), 6.82 (d, $J$ = 2.1 Hz, 1H), 6.98 (dd, $J$ = 2.4, 8.7 Hz, 1H), 7.11 (s, 2H), 7.63 (s, 1H), 8.21 ppm (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ = 48.0, 55.8, 56.4, 61.1, 93.6, 106.6, 112.3, 116.3, 121.1, 123.4, 136.0, 137.7, 138.5, 141.0, 153.0, 157.8, 169.8, 191.0 ppm; MS (EI *m/z*) 399 [$M^+$]; HRMS (FAB *m/z*) calcd for $C_{21}H_{21}NO_7$ [$M^+$] 399.1318, found 399.1315.

**[6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indol-1-yl]propionic acid (24)**: Ethyl acrylate (0.19 g, 1.75 mmol) was added to a stirred mixture of **8** (0.30 g, 0.88 mmol) and cesium carbonate (0.573 g, 1.75 mmol) in dried acetonitrile (30 mL) at room temperature, and stirring was continued overnight. The reaction was quenched with water and extracted with EtOAc (3×30 mL). The organic layers were dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:1) to afford ethyl ester compound (83% yield). The ethyl ester was hydrolyzed to give **24** (90% yield) as a pale yellow solid in a manner similar to that described for the preparation of **23**; mp: 167.6–168.3 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 2.86 (t, $J$ = 6.3 Hz, 2H), 3.912 (s, 3H), 3.916 (s, 6H), 3.93 (s, 3H), 4.43 (t, $J$ = 6.3 Hz, 2H), 6.85 (d, $J$ = 2.1 Hz, 1H), 6.99 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.10 (s, 2H), 7.68 (s, 1H), 8.24 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 33.7, 42.2, 55.7, 56.2, 60.8, 93.5, 106.3, 111.7, 115.4, 121.3, 123.4, 135.8, 136.8, 137.3, 140.7, 152.7, 157.3, 172.6, 190.1 ppm; MS (EI *m/z*) 413 [$M^+$]; HRMS (FAB *m/z*) calcd for $C_{22}H_{23}NO_7$ [$M^+$] 413.1475, found 413.1473.

**1-Benzyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (25)**: A stirred solution of **8** (0.10 g, 0.29 mmol), potassium carbonate (0.050 g, 0.44 mmol), and benzyl bromide (52 μL, 0.44 mmol) in anhydrous acetonitrile (10 mL) was heated at reflux overnight. The reaction was quenched with water and extracted with EtOAc (3×20 mL). The organic layers were combined and dried over anhydrous $MgSO_4$ and then evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 2:1) to afford **25** (61% yield) as a white solid; mp: 129.9–130.4 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.82 (s, 9H), 3.90 (s, 3H), 5.28 (s, 2H), 6.79 (d, $J$ = 2.1 Hz, 1H), 6.97 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.04 (s, 2H), 7.15–7.33 (m, 5H), 7.49 (s, 1H), 8.26 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 50.6, 55.6, 56.1, 60.9, 93.8, 106.0, 111.6, 115.6, 121.1, 123.1, 126.9, 128.0, 128.7, 135.3, 135.5, 135.6, 137.8, 140.4, 152.5, 157.0, 189.2 ppm; MS (FAB, NBA *m/z*) 432 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{26}H_{26}NO_5$ [$M$+H$^+$] 432.1811, found 432.1806.

**1-(4-Nitrobenzyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (26)**: The title compound was obtained as a yellow solid in 74% yield from **8** and 4-nitrobenzyl chloride in a manner similar to that described for the preparation of **25**; mp: 228.3–230.8 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.80 (s, 3H), 3.86 (s, 6H), 3.91 (s, 3H), 5.43 (s, 2H), 6.65 (d, $J$ = 2.1 Hz, 1H), 6.99 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.07 (s, 2H), 7.25 (d, $J$ = 8.7 Hz, 2H), 7.55 (s, 1H), 8.16 (dd, $J$ = 1.9, 6.7 Hz, 2H), 8.26 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 50.0, 55.7, 56.3, 61.0, 93.7, 106.2, 111.8, 116.5, 121.2, 123.5, 124.1, 127.1, 135.2, 135.4, 137.5, 140.8, 142.8, 147.4, 152.6, 157.4, 189.4 ppm; MS (FAB, NBA *m/z*) 477 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{26}H_{25}N_2O_7$ [$M$+H$^+$] 477.1662, found 477.1659.

**1-(4-Cyanobenzyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (27)**: The title compound was obtained as a pale yellow solid in 70% yield from **8** and 4-cyanobenzyl chloride in a manner similar to that described for the preparation of **25**; mp: 193.1–192.4 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.79 (s, 3H), 3.84 (s, 6H), 3.90 (s, 3H), 5.38 (s, 2H), 6.65 (d, $J$ = 2.1 Hz, 1H), 6.96 (dd, $J$ = 2.2, 8.8 Hz, 1H), 7.06 (s, 2H), 7.19 (d, $J$ = 8.4 Hz, 2H), 7.55 (s, 1H), 7.57 (d, $J$ = 8.4 Hz, 2H), 8.24 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 50.0, 55.6, 56.1, 60.8, 93.6, 106.0, 111.7, 111.8, 1116.2, 117.9, 121.0, 123.3, 126.9, 132.4, 135.2, 135.3, 137.4, 140.6, 140.9, 152.5, 157.2, 189.2 ppm; MS (FAB, NBA *m/z*) 457 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{27}H_{25}N_2O_5$ [$M$+H$^+$] 457.1763, found 457.1763.

**1-(4-Methoxybenzyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (28)**: The title compound was obtained as a pale yellow oil in 71% yield from **8** and 4-methoxybenzyl chloride in a manner similar to that described for the preparation of **25**; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.74 (s, 3H), 3.79 (s, 9H), 3.89 (s, 3H), 5.16 (s, 2H), 6.80–6.83 (m, 3H), 6.93 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.02 (s, 2H), 7.10 (d, $J$ = 8.7 Hz, 2H), 7.46 (s, 1H), 8.25 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 49.8, 55.0, 55.4, 55.9, 60.6, 93.6, 105.8, 111.4, 113.9, 115.1, 121.0, 122.8, 127.0, 128.4, 135.2, 135.4, 137.5, 140.1, 152.2, 156.7, 158.9, 188.9 ppm; MS (FAB, NBA *m/z*) 462 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{27}H_{28}NO_6$ [$M$+H$^+$] 462.1917, found 462.1918.

**1-(4-Pyridylmethyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (29)**: The title compound was obtained as a pale yellow solid in 53% yield from **8** and picolyl chloride hydrochloride in a manner similar to that described for the preparation of **22**; mp: 188.5–189.9 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.82 (s, 3H), 3.86 (s, 6H), 3.92 (s, 3H), 5.34 (s, 2H), 6.67 (d, $J$ = 1.8 Hz, 1H), 6.99–7.02 (m, 3H), 7.09 (s, 2H), 7.55 (s, 1H), 8.28 (d, $J$ = 8.7 Hz, 1H), 8.56 ppm (d, $J$ = 6 Hz, 2H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 49.4, 55.6, 56.2, 60.9, 93.7, 106.3, 112.0, 116.6, 121.30, 121.34, 123.7, 135.5, 135.7, 137.8, 141.0, 144.9, 150.4, 152.9, 157.7, 189.8 ppm; MS (FAB, NBA *m/z*) 433 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{25}H_{25}N_2O_5$ [$M$+H$^+$] 433.1763, found 433.1759; anal. ($C_{25}H_{24}N_2O_5 \cdot 0.25\,H_2O$): C, H, N.

**1-Benzoyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (30)**: Potassium *tert*-butoxide (0.05 g, 0.44 mmol) was added to a solution of **8** (0.10 g, 0.29 mmol) in THF (10 mL) under vigorous stirring at 0 °C, and stirring was continued for 15 min. Benzoyl chloride (51 μL, 0.44 mmol) was added slowly to the reaction mixture and stirred for 12 h. The solvent was evaporated, then water was added and the mixture was extracted with EtOAc (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:3) to afford **30** (84% yield) as a white solid; mp: 136.3–138.1 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.85 (s, 6H), 3.88 (s, 3H), 3.90 (s, 3H), 7.05 (dd, $J$ = 2.4, 8.7 Hz, 1H), 7.09 (s, 2H), 7.49–7.65 (m, 4H), 7.74 (s, 1H), 7.75 (d, $J$ = 8.7 Hz, 1H), 7.90 (d, $J$ = 2.1 Hz, 1H), 8.11 ppm (d, $J$ = 8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 55.6, 56.1, 60.9, 99.6, 106.1, 114.3, 119.9, 121.7, 122.6, 128.6, 128.8, 132.4, 132.8, 133.2, 133.9, 137.2, 141.3, 152.6, 158.5, 168.4, 189.2 ppm; MS (FAB, NBA *m/z*) 446 [$M$+H$^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{26}H_{24}NO_6$ [$M$+H$^+$] 446.1604, found 446.1603; anal. ($C_{26}H_{23}NO_6$) C, H, N.

**1-(Furan-2-carbonyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (31)**: The title compound was obtained as a white solid in 70% yield from **8** and 2-furoyl chloride in a manner similar to that described for the preparation of **30**; mp: 157.4–157.8 °C; $^1$H NMR (300 MHz, $CDCl_3$): 3.92 (s, 9H), 3.95 (s, 3H), 6.68 (dd, $J$ = 1.8, 3.6 Hz, 1H), 7.07 (dd, $J$ = 2.4, 8.7 Hz, 1H), 7.20 (s, 2H), 7.52 (dd, $J$ = 0.6,

3.6 Hz, 1H), 7.67 (dd, $J$=0.6, 1.8 Hz, 1H), 8.05 (d, $J$=2.4 Hz, 1H), 8.12 (d, $J$=8.4 Hz, 1H), 8.45 ppm (s, 1H); MS (FAB, NBA $m/z$) 436 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{24}H_{22}NO_7$ [$M$+H$^+$] 436.1396, found 436.1390; anal. ($C_{24}H_{21}NO_7$) C, H, N.

**1-(Thiophene-2-carbonyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (32)**: The title compound was obtained as dark yellow solid in 70% yield from **8** and thiophene-2-carbonyl chloride in a manner similar to that described for the preparation of **30**; mp: 165.9–166.8 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.91 (s, 9H), 3.94 (s, 3H), 7.08 (dd, $J$=1.8, 6.9 Hz, 1H), 7.15 (s, 2H), 7.21 (t, $J$=4.2 Hz, 1H), 7.75 (d, $J$=3.6 Hz, 1H), 7.78 (d, $J$=5.1 Hz, 1H), 7.91 (d, $J$=1.8 Hz, 1H), 8.04(s, 1H), 8.13 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 452 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{24}H_{22}NO_6S$ [$M$+H$^+$] 452.1168, found 452.1158; anal. ($C_{24}H_{21}NO_6S$) C., H, N.

**1-(2,2-Dimethylpropionyl)-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (33)**: The title compound was obtained as a pale yellow solid in 70% yield from **8** and pivaloyl chloride in a manner similar to that described for the preparation of **30**; mp: 131.6–132.4 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=1.54(s, 9H), 3.91 (s, 9H), 3.97 (s, 3H), 7.05 (dd, $J$=2.4, 8.7 Hz, 1H), 7.16 (s, 2H), 8.08–8.11 ppm (m, 5H); MS (FAB, NBA $m/z$) 426 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{24}H_{28}NO_6$ [$M$+H$^+$] 426.1917, found 426.1907; anal. ($C_{24}H_{27}NO_6$) C, H, N.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole-1-carboxylic acid phenyl ester (34)**: The title compound was obtained as a white solid in 74% yield from **8** and phenyl chloroformate in a manner similar to that described for the preparation of **30**; mp: 153.8–154.6 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.85 (s, 3H), 3.90 (s, 6H), 3.94 (s, 3H), 7.01 (dd, $J$=2.4, 9 Hz, 1H), 7.16 (s, 2H), 7.28–7.49 (m, 5H), 7.77 (d, $J$=2.1 Hz, 1H), 8.10 (s, 1H), 8.12 ppm (d, $J$=9 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$=55.5, 56.2, 60.8, 98.9, 106.2, 113.5, 120.3, 120.9, 121.5, 122.8, 126.5, 129.4, 130.9, 133.9, 136.5, 141.3, 148.6, 149.5, 152.6, 158.4, 189.3 ppm; MS (FAB, NBA $m/z$) 462 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{26}H_{24}NO_7$ [$M$+H$^+$] 462.1553, found 462.1555; anal. ($C_{26}H_{23}NO_7$) C, H, N.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole-1-carboxylic acid *tert*-butyl ester (35)**: The title compound was obtained as a white solid in 76% yield from **8** and di-*tert*-butyldicarbonate in a manner similar to that described for the preparation of **10**; mp: 95.1–95.7 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=1.69 (s, 9H), 3.92 (s, 9H), 3.96 (s, 3H), 7.02 (dd, $J$=2.4, 8.7 Hz, 1H), 7.16 (s, 2H), 7.74 (d, $J$=1.8 Hz, 1H), 8.01 (s, 1H), 8.15 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 442 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{24}H_{28}NO_7$ [$M$+H$^+$] 442.1866, found 442.1865; anal. ($C_{24}H_{27}NO_7$) C, H, N.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole-1-carboxylic acid methyl ester (36)**: The title compound was obtained as a white solid in 94% yield from **8** and methyl chloroformate in a manner similar to that described for the preparation of **30**; mp: 178.1–178.5 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.92 (s, 9H), 3.96 (s, 3H), 4.09 (s, 3H), 7.04 (dd, $J$=2.4, 8.7 Hz, 1H), 7.14 (s, 2H), 7.78 (d, $J$=2.1 Hz, 1H), 7.98 (s, 1H), 8.16 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 400 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{21}H_{22}NO_7$ [$M$+H$^+$] 400.1396, found 400.1403.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole-1-carboxylic acid ethyl ester (37)**: The title compound was obtained as a pale yellow solid in 95% yield from **8** and ethyl chloroformate in a manner similar to that described for the preparation of **30**; mp: 176.8–177.6 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=1.26 (t, $J$=7.2 Hz, 3H), 3.91 (s, 9H), 3.96 (s, 3H), 4.54 (q, $J$=7.2 Hz, 2H), 7.03 (dd, $J$=2.4, 9.0 Hz, 1H), 7.15 (s, 2H), 7.78 (d, $J$=2.1 Hz, 1H), 8.01 (s, 1H), 8.15 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 414 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{22}H_{24}NO_7$ [$M$+H$^+$] 414.1553, found 414.1546.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole-1-carboxylic acid dimethylamide (38)**: The title compound was obtained as a pale yellow solid in 72% yield from **8** and dimethylcarbamoyl chloride in a manner similar to that described for the preparation of **30**; mp: 133.4–134.1 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.11 (s, 6H), 3.89 (s, 9H), 3.93 (s, 3H), 7.01 (d, $J$=8.7 Hz, 1H), 7.11 (s, 3H), 7.67 (s, 1H), 8.17 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 413 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{22}H_{25}N_2O_6$ [$M$+H$^+$] 413.1713, found 413.1716.

**6-Methoxy-1-(morpholin-4-carbonyl)-3-(3′,4′,5′-trimethoxybenzoyl)indole (39)**: The title compound was obtained as a white solid in 90% yield from **8** and 4-morpholinecarbonyl chloride in a manner similar to that described for the preparation of **30**; mp: 170.2–170.9 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.63 (d, $J$=4.5 Hz, 4H), 3.77 (d, $J$=4.5 Hz, 4H), 3.90 (s, 9H), 3.94 (s, 3H), 7.01 (d, $J$=8.7 Hz, 1H), 7.11 (s, 2H), 7.12 (s, 1H), 7.69 (s, 1H), 8.15 ppm (d, $J$=8.7 Hz, 1H); MS (FAB, NBA $m/z$) 455 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{24}H_{27}N_2O_7$ [$M$+H$^+$] 455.1818, found 455.1820.

**1-Benzenesulfonyl-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)indole (40)**: Sodium hydroxide (0.047 g, 1.17 mmol) and tetra-*n*-butylammonium hydrogen sulfate (0.005 g, 0.01 mmol) were added to a solution of **8** (0.10 g, 0.29 mmol) in dichloromethane (10 mL) under vigorous stirring at 0 °C, and stirring was continued for 30 min. Benzenesulfonyl chloride (56 μL, 44 mmol) was added slowly to the reaction mixture. After 12 h the mixture was quenched with water and extracted with EtOAc (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:4) to afford **33** (89% yield) as a pale yellow solid; mp: 165.1–166.3 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.90 (s, 3H), 3.91 (s, 6H), 3.96 (s, 3H), 6.99 (dd, $J$=2.2, 8.8 Hz, 1H), 7.11 (s, 2H), 7.44–7.62 (m, 4H), 7.78–7.93 (m, 3H), 8.10 ppm (d, $J$=8.7 Hz, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$=55.8, 56.3, 61.0, 97.3, 106.3, 113.5, 120.3, 121.9, 123.2, 126.6, 129.3, 131.5, 133.8, 134.3, 135.8, 137.2, 141.6, 152.7, 158.4, 189.1 ppm; MS (FAB, NBA $m/z$) 482 [$M$+H$^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{25}H_{24}NO_7S$ [$M$+H$^+$] 482.1273, found 482.1266.

**6-Methoxy-3-(3′,4′,5′-trimethoxyphenylsulfanyl)indole (41)**: Ethylmagnesium bromide (0.58 mL, 3.0 M in diethyl ether) was added dropwise over 10 min at 0 °C to a stirred solution of 6-methoxyindole (0.20 g, 1.35 mmol) and anhydrous zinc chloride (0.37 g, 2.71 mmol) in dichloromethane (10 mL); stirring was continued for 1 h. A solution of bis(3,4,5-trimethoxyphenyl)disulfide **58**[15] (0.54 g, 1.35 mmol) in dichloromethane (5 mL) was added to the reaction mixture and vigorously stirred for 5 h. The reaction was quenched with water and extracted with EtOAc (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:3) to afford compound **41** (49% yield) as a pale red solid; mp: 118.5–119.6 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$=3.66 (s, 6H), 3.76 (s, 3H), 3.85 (s, 3H), 6.37 (s, 2H), 6.82 (dd, $J$=2.1, 8.7 Hz, 1H), 6.89 (d, $J$=2.1 Hz, 1H), 7.37 (d, $J$=2.4 Hz, 1H), 7.47 (d, $J$=8.7 Hz, 1H), 8.28 ppm (br, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$=55.6, 56.0, 60.8, 94.9, 102.5, 103.2, 110.5, 119.9, 122.9, 129.2, 134.1, 135.2, 136.9, 153.0, 156.7 ppm; MS (FAB, NBA $m/z$) $m/z$: 345 [$M^+$]; HRMS (FAB, NBA $m/z$) calcd for $C_{18}H_{19}NO_4S$ [$M^+$] 345.1035, found 345.1033; anal. ($C_{18}H_{19}NO_4S$) C, H, N.

**6-Methoxy-3-(3′,4′,5′-trimethoxybenzenesulfonyl)indole (42)**: *m*-Chloroperoxybenzoic acid (0.07 g, 0.42 mmol) was added slowly over 10 min at 0 °C to a stirred solution of compound **59** (0.10 g, 0.21 mmol) in dichloromethane (10 mL) which was then allowed to warm to room temperature. After 1 h, the solution was quenched with water and extracted with EtOAc (3×20 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:2) to afford the desired sulfoxide compound (63% yield) as a white solid. The sulfoxide compound was hydrolyzed by using the same procedure as described for the preparation of **11** to afford compound **42** (88% yield) as a white solid; mp: 185.2–186.5 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.73 (s, 3H), 3.83 (s, 3H), 3.85 (s, 6H), 6.84 (d, $J$ = 2.1 Hz, 1H), 6.88 (dd, $J$ = 2.1, 8.7 Hz, 1H), 7.23 (s, 2H), 7.71 (d, $J$ = 2.7 Hz, 1H), 7.75 (d, $J$ = 8.7 Hz, 1H), 9.45 ppm (br, 1H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 55.5, 56.4, 60.8, 95.2, 103.8, 112.5, 116.1, 117.2, 119.6, 128.6, 137.1, 137.6, 141.1, 152.9, 157.2 ppm; MS (FAB, NBA *m/z*) 377[$M^+$]; HRMS (FAB, NBA *m/z*) calcd for $C_{18}H_{19}NO_6S$ [$M^+$] 377.0933, found 377.0931; anal. ($C_{18}H_{19}NO_6S$·0.5 $(CH_3CH_2O)_2$) C, H, N.

**6-Methoxy-2-methyl-3-(3′,4′,5′-trimethoxyphenoxy)indole (43) and 4-Methoxy-2-methyl-3-(3′,4′,5′-trimethoxyphenoxy)indole (44)**: $Et_3N$ (0.1 mL, 0.98 mmol) was added to a solution of **61** (170 mg, 0.98 mmol) in MeOH. After 30 min, 3-methoxyphenyl hydrazine hydrochloride (200 mg, 382 mmol) was introduced by syringe. The reaction mixture was stirred for 45 min while monitoring by TLC (EtOAc/*n*-hexane 1:1), dried over anhydrous $MgSO_4$ and evaporated to give the desired phenylhydrazone, which was used immediately without further purification.

$PCl_3$ (0.26 mL) was added to a solution of the above-obtained phenylhydrazone in $CH_2Cl_2$ (20 mL) at room temperature. The reaction was stirred at room temperature for 12 h before water (5 mL) was introduced and the reaction was vigorously stirred for another 10 min. The reaction was neutralized to pH 7 by adding 2 N aqueous NaOH and extracted with $CH_2Cl_2$ (3×10 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a pale yellow oil, which was subjected to silica gel chromatography (EtOAc/*n*-hexane 1:5) to afford **43** (48% yield) and **44** (19% yield). **43**: IR (neat): $\tilde{\nu}$ = 3385, 2944, 1598, 1500, 1220, 989 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.28 (s, 3H), 3.70 (s, 6H), 3.77 (s, 3H), 3.81 (s, 3H), 6.21 (s, 2H), 6.66–6.69 (m, 1H), 6.76 (s, 1H), 7.14 (d, $J$ = 4 MHz, 1H), 7.50 ppm (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 10.1, 55.5, 55.8, 55.9, 60.9, 92.8, 92.9, 94.6, 109.0, 116.0, 117.9, 122.4, 130.0, 132.4, 133.8, 153.5, 155.6, 156 ppm; MS (EI *m/z*) 343 [$M^+$]; HRMS (EI *m/z*) calcd for $C_{19}H_{21}NO_5$ [$M^+$] 343.1419, found 343.1426. **44**: IR (neat): $\tilde{\nu}$ = 3401, 1600, 1500, 1462, 1264, 1127, 989 cm$^{-1}$; $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ = 2.26 (s, 3H), 3.69 (s, 3H), 3.70 (s, 6H), 3.77 (s, 3H), 6.22 (s, 2H), 6.43 (d, $J$ = 3.8 Hz, 1H), 6.87 (d, $J$ = 8 Hz, 1H), 7.68 ppm (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 10.0, 55.5, 56.0, 60.9, 92.9, 100.3, 104.1, 112.3, 122.3, 122.7, 129.8, 132.2, 134.5, 152.5, 153.4, 156.8 ppm; MS (EI *m/z*) 343 [$M^+$]; HRMS (EI *m/z*) calcd for $C_{19}H_{21}NO_5$ [$M^+$] 343.1419, found 343.1412.

**1-Benzenesulfonyl-6-nitroindole-3-carbaldehyde (48)**: The title compound was obtained as a white solid in 65% yield from **47**[14] in a similar manner to that described for the preparation of **40**; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 7.55–7.68 (m, 3H), 8.01–8.04 (m, 2H), 8.25 (dd, $J$ = 1.6, 9.1 Hz, 1H), 8.39 (d, $J$ = 9 Hz, 1H), 8.44 (s, 1H), 8.86 (d, $J$ = 1.8 Hz, 1H), 10.11 ppm (s, 1H); MS (EI *m/z*) 330 [$M^+$].

**6-Amino-1-benzenesulfonyl-3-(3′,4′,5′-trimethoxybenzoyl)indole (50)**: A stirred solution of **48** (0.369 g, 1.11 mmol) in THF (10 mL) was cooled to 0 °C. A solution of 3,4,5-trimethoxyphenylmagnesium bromide (1.68 mL, 1.0 M in THF, prepared in advance) was added dropwise to the reaction over 10 min with vigorous stirring for 1 h. The mixture was quenched with water, neutralized with saturated $NH_4Cl$, and extracted with EtOAc (3×20 mL). The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo to give crude product, which was dissolved in dichloromethane (20 mL) and cooled to 0 °C with stirring. Powdered molecular sieves (4 Å, 0.84 g) and pyridinium dichromate (0.84 g, 2.22 mmol) were added with stirring at room temperature overnight. The mixture was diluted with anhydrous diethyl ether (20 mL) and stirred further for 1 h, and then filtered through a pad of celite and washed three times with solution of dichloromethane/*n*-hexane (1:1). The filtrate was concentrated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:2) to afford nitroindole compound **49**.

Compound **49** (0.10 g, 0.20 mmol) and iron powder (0.078 g, 1.39 mmol) were added to a mixture of ethanol (4 mL), acetic acid (4 mL), water (2 mL), and 35% HCl (1 drop). The suspension was heated at reflux with vigorous stirring for 1 h. The mixture was cooled to room temperature and filtered through celite. The filtrate was dilute with water (10 mL), carefully neutralized with aqueous sodium hydrogen carbonate, and extracted with EtOAc (3×20 mL). The organic layer was combined, dried over $MgSO_4$, and evaporated to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:1) to afford **50** (93% yield) as a yellow solid; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.90 (s, 6H), 3.95 (s, 3H), 6.75 (dd, $J$ = 1.9, 8.5 Hz, 1H), 7.10 (s, 2H), 7.31 (d, $J$ = 2.1 Hz, 1H), 7.43–7.58 (m, 3H), 7.82 (s, 1H), 7.86–7.89 (m, 2H), 7.97 ppm (d, $J$ = 8.4 Hz, 1H); MS (EI *m/z*) 466[$M^+$].

**1-Benzenesulfonyl-6-methoxyindole-3-carbaldehyde (54)**: The title compound was obtained as a yellow solid in 83% yield from **53**[15] in a manner similar to that described for the preparation of **40**; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.87 (s, 3H), 6.95 (dd, $J$ = 2.2, 8.5 Hz, 1H), 7.44 (d, $J$ = 2.1 Hz, 1H), 7.47–7.52 (m, 3H), 7.57–7.62 (m, 2H), 7.92–7.95 (m, 2H), 8.08 (d, $J$ = 8.7 Hz, 1H), 8.11 (s, 1H), 10.01 ppm (s, 1H).

**1-Benzenesulfonyl-6-methoxy-5-amino-3-(3′,4′,5′-trimethoxybenzoyl)indole (57)**: Nitric acid (34 μL, 0.80 mmol) was added to a vigorously stirred solution of **54** (0.20 g, 0.63 mmol) in sulfuric acid (5 mL) at room temperature. After 2 h, the mixture was poured into crushed ice, followed by extraction with EtOAc (3×10 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated in vacuo to give crude product, which was purified by silica gel chromatography (EtOAc/*n*-hexane 1:2) to afford the desired nitration product **55**. Compound **55** was alkylated with 3,4,5-trimethoxyphenylmagnesium bromide[12] followed by oxidation in the same manner as described for the preparation of **49** to give **56** as a pale yellow solid, which was reduced immediately to give the desired product **57** in a similar manner as described for the preparation of **50**; $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.90 (s, 6H), 3.95 (s, 3H), 3.97 (s, 3H), 7.08 (s, 2H), 7.42–7.47 (m, 3H), 7.55–7.60 (m, 2H), 7.82–7.86 ppm (m, 3H); MS (EI *m/z*) 496 [$M^+$].

**1-Benzenesulfonyl-6-methoxy-3-(3′,4′,5′-trimethoxyphenylsulfanyl)indole (59)**: The title compound was obtained as a white solid in an 89% yield from **41** in a similar manner as described for the preparation of **40**. $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ = 3.61 (s, 6H), 3.72 (s, 3H), 3.77 (s, 3H), 6.34 (s, 2H), 6.85 (d, $J$ = 2.4 Hz, 1H), 6.95 (dd, $J$ = 2.5, 9.1 Hz, 1H), 7.40–7.54 (m, 3H), 7.74 (s, 1H), 7.86–7.92 ppm (m, 3H); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ = 55.6, 56.0, 60.8, 102.2, 104.8, 112.4, 114.5, 114.7, 126.5, 129.1, 129.5, 130.2, 130.7, 131.8, 133.7, 136.3, 137.6, 153.1, 156.5 ppm; MS (EI *m/z*) 485 [$M^+$].

**1-(3,4,5-Trimethoxyphenoxy)acetone (61)**: $Cs_2CO_3$ (0.300 g, 2.4 mmol) was added to a stirred solution of 3,4,5-trimethoxyphenol **60** (0.300 g, 1.6 mmol) in DMF (15 mL) at room temperature. After 30 min, bromoacetone (0.435 g, 3.2 mmol) was introduced by syringe. The reaction mixture was stirred for 2 h, quenched with water (20 mL), and extracted with EtOAc (3×10 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and evaporated to give a pale yellow oil, which was subjected to silica gel chromatography (EtOAc/*n*-hexane 1:5) to afford compound **61** (92% yield); IR (neat): $\tilde{\nu}=3081$, 1736, 1596, 1465 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$): $\delta=2.25$ (s, 3H), 3.75 (s, 3H), 3.80 (s, 6H), 4.47 (s, 2H), 6.09 ppm (s, 2H); $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta=26.3$, 55.8, 60.6, 73.0, 92.1, 132.6, 153.5, 154.1, 205.0 ppm; MS (EI *m/z*) 240 [$M^+$]; HRMS (EI *m/z*) calcd for $C_{12}H_{16}O_5$ [$M^+$] 240.0998, found 240.0997.

**Cell culture**: Human cervical carcinoma KB cells and gastric MKN-45 carcinoma cells were maintained in RPMI 1640 medium supplied with 5% fetal bovine serum. Human lung carcinoma H460 cells were maintained in RPMI 1640 medium supplied with 10% fetal bovine serum and 1.0 mM sodium pyruvate. KB-derived MDR-positive cell lines KB-vin10, and KB-7D were maintained in growth medium supplemented with 10 nM vincristine and 7 μM VP-16, respectively.

**Growth inhibition assay**: KB and H-460 cells in logarithmic growth phase were cultured at a density of 7000 cells $mL^{-1}$ per well in a 24-well plate, whereas MKN-45 cells were at a density of 20000 cells $mL^{-1}$ per well. Drug-resistant cells were cultured in drug-free medium for 3 days before use. The cells were exposed to various concentrations of the test compounds and incubated at 37 °C in a humid environment for 3 days. The effect of test compounds on cell growth was evaluated by the methylene blue assay. The $IC_{50}$ value (obtained from 50% inhibition of cell growth) relative to control growth was calculated.[13]

**In vitro tubulin polymerization assay**: Microtubule-associated protein-rich tubulin (0.001 g per 250 μL buffer) in 100 μL buffer containing 100 mM PIPES (pH 6.9), 2 mM $MgCl_2$, 1 mM GTP, and 2% (*v/v*) DMSO was transferred to 96-well microtiter plates in the presence of test compounds. The increase in absorbance was measured at $\lambda=350$ nm in a PowerWave X microplate reader (BIO-TEK Instruments, Winooski, USA) at 37 °C and read at the time interval of 30 s for 30 min. The concentration that inhibited tubulin polymerization by 50% ($IC_{50}$) was obtained by setting the area under the curve of the untreated control to 100% polymerization and 10 μM colchicine to 0% polymerization. The $IC_{50}$ value was then determined by nonlinear regression in at least three experiments.[13]

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