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# Synthesis and Structure–Activity Relationships of *N*-Methyl-5,6,7-trimethoxylindoles as Novel Antimitotic and Vascular Disrupting Agents

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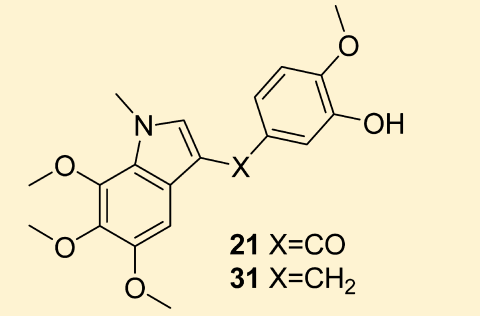
Supporting Information

**ABSTRACT:** Several new series of 5,6,7-trimethoxyindole derivatives were synthesized and their structure–activity relationships (SARs) were studied. Some of these compounds exhibited strong antiproliferative activities in the submicromolar range. *N*-Methyl-5,6,7-trimethoxylindoles **21** and **31** displayed the highest antiproliferative activities, with $IC_{50}$ values ranging from 22 to 125 nM in four human cancer cell lines and activated human umbilical vein endothelial cells (HUVECs). In addition to vascular disrupting activity verified by in vitro assays, compounds **21** and **31** displayed much higher selectivity for activated HUVECs versus quiescent HUVECs than those of colchicine and combretastatinA-4. The polymerization of cancer cell tubulin was inhibited and the cell cycle was arrested in the G2/M phase after treatment with **21** and **31**. It was showed that **21** disrupted tumor vasculature by use of in vivo assay. Our results suggest that these two new compounds we synthesized may become the promising leads for the development of vascular disrupting agents.




## INTRODUCTION

The vasculature has a pivotal role in growth and survival of solid tumors.[1] Given its inherent differences with normal vasculature, the tumor vasculature has become an attractive target for anticancer drug discovery.[2] Vascular-disrupting agents (VDAs) selectively target established tumor vasculature to cause a significant shutdown in blood flow to solid tumors, resulting in extensive tumor-cell necrosis while the blood flow in normal tissues remains relatively intact.[3] VDAs have many advantages over other cancer therapies.[4] Currently, two major groups of VDAs, small-molecule VDAs and ligand-directed VDAs, are constructed for cancer treatment.

A number of antimitotic agents, such as colchicine **3** (Figure 1), vincristine, and vinblastine, show potent vascular disrupting properties.[5] More recently, many agents binding tubulin at or near the colchicine binding site, including combretastatinA-4 (CA-4) **1**,[6] **2** (Oxi4503),[7] **4** (ZD6126),[8] and **5** (BNC105),[9] among others, are currently in clinical trials or undergoing preclinical testing.

It is well-known that the damage of antimitotic agents on tumor vasculature has been ascribed to a direct effect on endothelial cells.[3,10] To discover new antimitotic agents which selectively disrupt tumor vasculature, it is important to evaluate these analogues not only regarding their cytotoxicity and antitubulin activity but also regarding their ability to selective

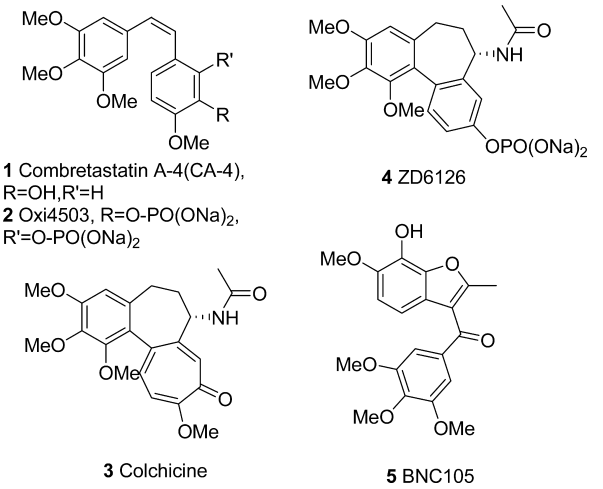



**Figure 1.** Some anitmitotic agents with vascular-disrupting activities.

cytotoxicity against tumor endothelial cells.[10,11] Tumor endothelial cells are constantly exposed to proangiogenic growth factors and, as a consequence, are in a constant state

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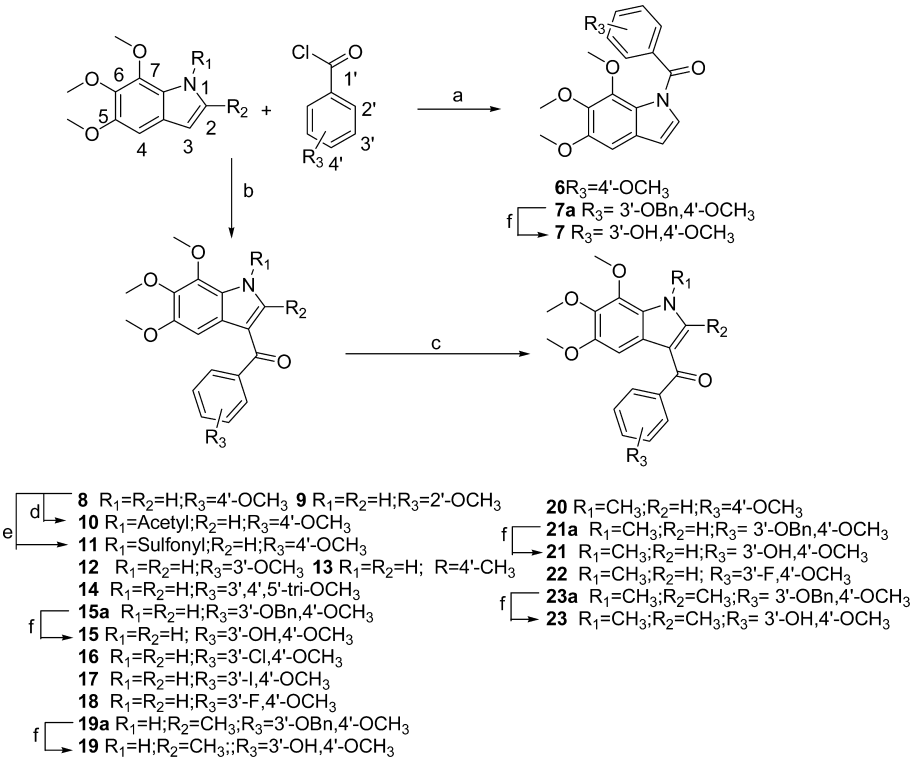
Scheme 1[a]




[a]Reagents and conditions: (a) THF, *t*-BuOK, 61−65%; (b) EtMgBr, $ZnCl_2$, $AlCl_3$, $CH_2Cl_2$, 37−62%; (c) $CH_3I$, THF, *t*-BuOK, 93−98%; (d) acetyl chloride, THF, *t*-BuOK, 52%; (e) benzenesulfonyl chloride, THF, *t*-BuOK, 46%; (f) 10% Pd/C, $NH_4COOH$, $CH_3OH$, reflux, 90−99%.

of activation and angiogenesis.[12] Therefore, human umbilical vein endothelial cells (HUVECs) grown in the presence (activated) and absence (quiescent) of growth factors may model the endothelial cells of tumor and normal vasculature, respectively.[7,8,11,13] Evaluating the selectivity of tested compounds for activated HUVECs compared with quiescent HUVECs may be useful for identifying their selectivity toward tumor vasculature. Indeed, this approach has been applied to the discover process of **5**, which selectively disrupts tumor vasculature and displays a wider therapeutic margin.[9,11] Herein, we also applied this approach to discover new antimitotic agents that display potent and selective toxicity toward tumor vasculature.

Compounds **1** and **4** are thought to be the lead compounds among the class both as antimitotic agents and VDAs.[4] Hundreds of compounds, mimicking **1** and **4**, have been synthesized.[14] Through structure−activity relationships (SARs) studies, it has been established that these compounds bear two polyoxygenated aromatic rings, and the trimethoxybenzene moiety is crucial to obtain antiproliferative and antimitotic activities.[14] However, it should be noted that most efforts have been so far concentrated on increasing their cytotoxic and capacity to inhibit tubulin, while little efforts were paid in the antivascular effects.[10,14] With respect to antivascular activity, it is difficult to derive meaningful structure−activity relationships from the data obtained, and more extensive structural modifications need to be exploited.[10]

The replacement of the trimethoxybenzene ring by benzoheterocyclic structures has received little attention so far.[14] Recently, some trimethoxyindole derivatives have been reported as potent antimitotic agents with antivascular activity.[15] To discover potential antitumor agents with a high degree of antivascular selectivity, following the SAR-guided discovery process, we replaced the trimethoxybenzene moiety with a more hindered trimethoxyindole skeleton, and synthesizing series of 5,6,7-trimethoxyindole derivatives: (1) 1-aroylindoles and 1-benzylindoles, (2) 3-aroylindoles and 3-benzylindoles, and (3) N1-substituted-3-aroylindoles and N1-substituted-3-benzylindoles. The screening results of our series biological experiments demonstrated that both **21** and **31** among our synthesized compounds had high degree of selectivity for activated HUVECs and are promising leads for the development of vascular-disrupting agents.

## RESCULT AND DISCUSSION

**Chemistry.** The synthetic route followed for the synthesis of the desired aroylindoles **6**−**23** is outlined in Scheme 1. The 5,6,7-trimethoxylindole and 5,6,7-trimethoxy-2-methylindole was prepared according to the procedure previously reported.[16] The direct electrophilic substitution of 5,6,7-trimethoxylindole and with corresponding benzoyl chloride in the presence of EtMgBr, $ZnCl_2$, and $AlCl_3$ gave the desired 3-aroylindoles **8**, **12**−**14**, **15a**, **16**−**18**, and **19a** in 37−62% yields.[17] The N1-aroylindoles **6** and **7a** were synthesized in 61−65% yields from corresponding benzoyl chloride by allowing it to react with the 5,6,7-trimethoxylindole at room temperature in the presence of *t*-BuOk. In a similar manner, compounds **10** and **11** were prepared from **8** using acetyl chloride and benzenesulfonyl chloride, respectively. When 3-aroylindoles **8**, **15a**, and **19a** reacted with iodomethane, compounds **20**, **21a**, and **23a** were obtained in excellent yields, respectively. Transfer hydrogenation of **7a**, **15a**, **19a**, **21a**, and **23a** with ammonium formate and palladium on charcoal yielded the desired **7**, **15**, **19**, **21**, and **23**.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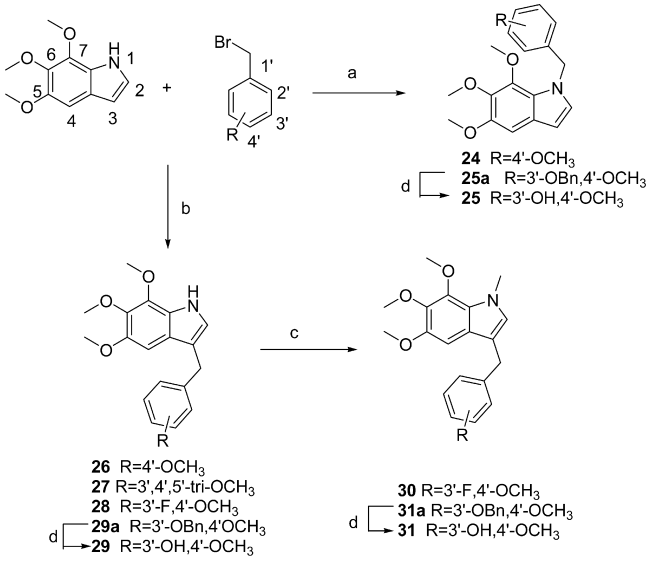
The benzylindoles **24**–**31** were synthesized as shown in Scheme 2. The N1-benzylindoles **24** and **25a** were synthesized

**Scheme 2**[a]




[a]Reagents and conditions: (a) DMF, KOH, KI, 39–47%; (b) $NH_4HCO_3$, 80% aqueous acetone (v/v), 44–55%; (c) $CH_3I$, THF, *t*-BuOK, 93–98%; (d) 10% Pd/C, $NH_4COOH$, $CH_3OH$, reflux, 90–99%.

in 39–47% yields from corresponding benzyl bromide by allowing it to react with the 5,6,7-trimethoxylindole at room temperature in the presence of KI and KOH.[18] Because our attempts to convert 3-aroylindoles into 3-benzylindoles have failed, the synthesis of 3-benzylindoles was accomplished via Friedel–Crafts acylation.[19] The 3-benzylindoles **26**–**29a** and its isomer 2-benzylindoles were obtained by a one-step procedure applied to 5,6,7-trimethoxylindole and the appropriate benzyl bromide with ammonium hydrogencarbonate as base in 80% aqueous acetone (v/v). The 5:1 mixture of the 3-benzylindoles and its isomer were finally separated by reversed-phase silica gel column chromatography. When 3-benzylindoles **28** and **29a** reacted with iodomethane, compounds **30** and **31a** were obtained in excellent yields, respectively. In the manner described above, transfer hydrogenation of **25a**, **29a**, and **31a** yielded the desired **25**, **29**, and **31**, respectively.

**Antiproliferative Activity.** All the synthesized trimethoxyindole derivatives and reference compounds colchicine and **1** were initially screened for their antiproliferative activity against the human lung arcinoma cell line A549 (Table 1). Cell proliferation was determined by CCK-8 assay after a treatment period of 72 h. Compounds **21** and **31** displayed potent antiproliferative activities in A549 cells, with $IC_{50}$ values of 28 and 72 nM, respectively. Compounds **23** and **29** also showed considerable antiproliferative activities, with $IC_{50}$ values of 108 and 118 nM, respectively. A preliminary comparison between antiproliferative activities of synthesized trimethoxyindole derivatives suggested that 3-aroyltrimethoxyindole and 3-benzyltrimethoxyindole derivatives had a higher antiproliferative activity than 1-aroyltrimethoxyindole (**6**, **7**) and 1-benzyltrimethoxyindole derivatives (**24**, **25**). The antiproliferative activities of 3-benzyltrimethoxyindole derivatives were comparable to those of 3-aroyltrimethoxyindole derivatives. The comparison among the 3-aroyltrimethoxyindole derivatives

**Table 1. Antiproliferative Activity[a] of the Synthesized Compounds against A-549 Human Lung Adenocarcinoma**

| compd | $IC_{50}$ = (nM ± SDs)[b] | compd | $IC_{50}$ = (nM ± SDs)[b] |
|---|---|---|---|
| **6** | >10000 | **20** | 481.7 ± 7.47 |
| **7** | >10000 | **21** | 28.30 ± 6.36 |
| **8** | 8289 ± 397 | **22** | 146.15 ± 6.00 |
| **9** | >10000 | **23** | 107.8 ± 18.9 |
| **10** | 5274 ± 267 | **24** | 3088 ± 426 |
| **11** | >10000 | **25** | 3192.4 ± 185.5 |
| **12** | >10000 | **26** | 2987 ± 448 |
| **13** | >10000 | **27** | >10000 |
| **14** | >10000 | **28** | 387.4 ± 28.5 |
| **15** | 166.08 ± 8.68 | **29** | 117.99 ± 10.88 |
| **16** | >10000 | **30** | 144.3 ± 23.42 |
| **17** | >10000 | **31** | 71.72 ± 7.22 |
| **18** | >10000 | colchicine | 20.00 ± 2.04 |
| **19** | 2623 ± 153 | **1** | 3.64 ± 0.54 |

[a]Antiproliferative activity was determined by CCK-8 assay. [b]$IC_{50}$ = compound concentration required to inhibit tumor cell proliferation by 50% after cells were treated with compounds for 72 h. Data are expressed as means ± SDs (standard deviations) from at least three independent experiments.

**8** and **12**–**18** and 3-benzyltrimethoxyindole derivatives **26**–**29** demonstrated that the 3′-hydroxy-4′-methoxy substitution (**15**, **29**) resulted in best activity.

The introduction of a methyl group at the N-1 position of 3-aroylindoles (**20**–**22**) and 3-benzylindoles (**30**, **31**) resulted in apparently increased antiproliferative activities as compared to its parent compounds, but, in contrast, the introduction of an acetyl (**10**) or a sulfonyl group (**11**) at the N-1 position and the introduction of a methyl group at the C-2 position of 3-aroylindoles (**23**) resulted in reduced activities. In these series, the replacement of 3′-OH group with a halogen atom (**16**–**18**, **22**, **28**, **30**) caused loss of antiproliferative activities.

The antiproliferative property of the trimethoxyindole derivatives **21**, **23**, **29**, and **31** were further confirmed in other three human cancer cell lines (Table 2). The

**Table 2. Antiproliferative Activity[a] of Selected Compounds against Different Human Cancer Cell Lines**

| | $IC_{50}$ = (nM ± SDs)[b] | | |
|---|---|---|---|
| compd | HeLa | HepG2 | HT1080 |
| colchicine | 37.38 ± 9.58 | 46.74 ± 2.63 | 15.38 ± 0.18 |
| **21** | 125.37 ± 4.66 | 44.45 ± 1.88 | 26.32 ± 3.98 |
| **23** | 632.2 ± 68.5 | 91.63 ± 12.17 | 573.94 ± 86.0 |
| **29** | 148.51 ± 1.81 | 72.17 ± 14.20 | 117.86 ± 11.65 |
| **31** | 40.68 ± 8.20 | 45.49 ± 0.48 | 42.18 ± 10.39 |

[a]Antiproliferative activity was determined by CCK-8 assay. [b]$IC_{50}$ = compound concentration required to inhibit tumor cell proliferation by 50% after cells were treated with compounds for 72 h. Data are expressed as means ± SDs (standard deviations) from at least three independent experiments.

antiproliferative activities of compounds **21** and **31** were higher than the **23** and **29**, with $IC_{50}$ values ranging from 26 to 125 nM. These result were quiet coincident with SRAs described above. Although less potent than **1**, the antiproliferative activities of **21** and **31** are comparable to those of colchicine.

**Endothelial Cell Proliferation Assay.** According to the method of Flynn et al.,[11] key compounds were evaluated for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antiproliferative activity in activated HUVECs and quiescent HUVECs. As shown in Table 3, compounds **21** and **31**

**Table 3. Antiproliferative Activity[a] of Selected Compounds against HUVECs**

| compd | actived HUVEC[b] $IC_{50}$ = (nM ± SDs) | quesicent HUVEC[c] $IC_{50}$ = (nM ± SDs) | selective ratio[d] |
|---|---|---|---|
| colchicine | 8.91 ± 0.52 | 7.60 ± 0.44 | 0.85 |
| **1** | 3.34 ± 0.45 | 8.90 ± 0.62 | 2.66 |
| **15** | 327 ± 17.35 | 2975 ± 218 | 9.10 |
| **21** | 25.3 ± 3.13 | 5240 ± 280 | 207 |
| **22** | 96.4 ± 11.8 | 150.7 ± 8.62 | 1.56 |
| **23** | 43.7 ± 5.03 | 160.7 ± 22.1 | 3.68 |
| **29** | 228 ± 21.3 | 2453 ± 225 | 10.8 |
| **31** | 27 ± 4.58 | 6296 ± 790 | 233 |

[a]These procedures were modified from previously described protocols. Antiproliferative activity was determined by CCK-8 assay. $IC_{50}$ = compound concentration required to inhibit tumor cell proliferation by 50% after cells were treated with compounds for 48 h. Data are expressed as means ± SDs (standard deviations) from at least three independent experiments [b]For activated growth conditions, HUVEC cells were cultured in endothelial cell medium (M&C Gene Technology, China) containing 0.03 mg/mL endothelial cell growth supplement. [c]For quiescent growth conditions, HUVEC cells were seeded at 15000 cells/well in endothelial cell basal medium (M&C Gene Technology, China) containing 0.5% fetal calf serum. [d]Selectivity ratio = ($IC_{50}$ quiescent)/($IC_{50}$ activated).

exhibited 207- and 233-fold selectivity (ratio) for activated HUVECs verse quiescent HUVECs, respectively. In contrast, **1** and colchicine only exhibited 2.66- and 0.85-fold selectivity, respectively. In addition, **15**, **22**, **23**, and **29** showed relatively low selectivity (9.10-, 1.56-, 3.68-, and 10.8-fold, respectively).

**Capillary Formation and Disruption Assays.** To further verify the selectivity observed in **21** and **31** and their vascular-disrupting capability, their activities on formation of capillary-like assay (activated HUVECs engaged in) as well as capillary-like structure disruption assay (quiescent HUVECs engaged in) were carried out as described.[11] HUVECs were plated on Matrigel and allowed to form capillary tubes in the presence or absence of compound from the beginning. We found that **21**, **31**, and reference compound colchicine inhibited the formation of capillary tubes at 0.1 $\mu$M (Figure 2). It suggested that compounds **21** and **31** were highly potent against activated endothelial cells that are engaged in capillary-like structures formation.

Subsequently, tested compounds and control was added to capillary tubes formed by endothelial cells on Matrigel 16 h after seeding (Figure 3).[20] Cultures were photographed immediately following compound addition and 4 h after exposure to test compounds. Total capillary length was determined by measuring with ImageJ software. Compared to the total capillary length at the 0 h, the length at the 4 h time point decreased, by 11% in the DMSO treatment control group, 13% in the treatment of 0.1 $\mu$M **21**, 10% in the treatment of 0.1 $\mu$M **31**, and 56% in the treatment of 0.1 $\mu$M colchicine group, respectively. It showed that both **21** and **31** did not disrupt preformed capillaries at 0.1 $\mu$M, while they were able to inhibit the formation of capillary tubes. In contrast, colchicine not only inhibited the formation of capillary tubes but also disrupted formed capillaries at the concentration of 0.1 $\mu$M. These results consisted with the endothelial cell proliferation assay results, which further demonstrated that

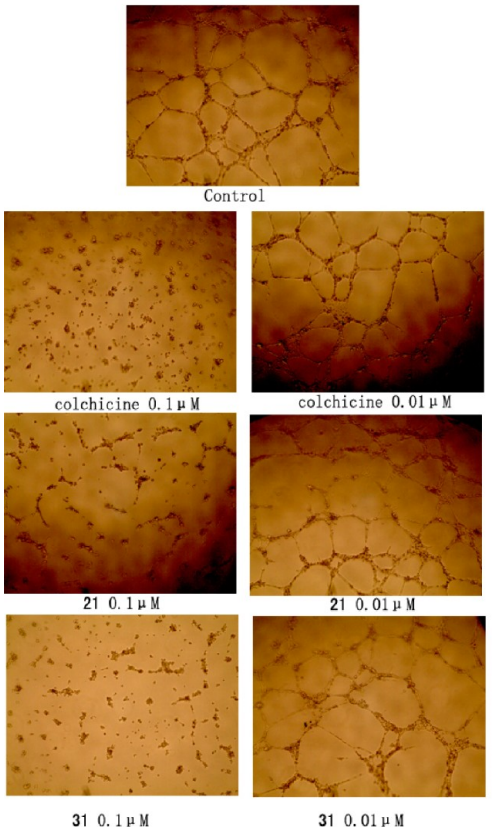


**Figure 2.** Effects of tested compounds on the formation of capillary-like structures by endothelial cells. Human umbilical vein endothelial cells (HUVECs) were plated onto a thick layer of Matrigel in the presence of vehicle (control) or tested compounds at the indicated concentrations. Images were taken 22 h after plating. Magnification ×40.

compounds **21** and **31** have higher selectivity for activated endothelial cells.

Moreover, after the addition of 1 $\mu$M of **21** and **31**, there were 49% and 51% decreases at the 4 h time point, respectively. These results showed that both **21** and **31** have vascular-disrupting properties.

**Immunocytochemistry, Tubulin Polymerization Assay and Cell Cycle Analysis.** To confirm the mechanism of action of these compounds, we performed immunocytochemistry, tubulin polymerization assay, and flow cytometry analysis. First, A549 cells were treated with two potent compounds (**21** and **31**) and reference compound **1**, and microtubule structure was detected using immunocytochemistry staining. As shown in Figure 4A, the microtubule structures in control cells were slim and fibrous. After treatment with the compounds, microtubules became short and wrapped around the nucleus.

Subsequently, we performed a tubulin polymerization assay as described previously.[21,22] A549 cells were exposed to **1**, **21**, or **31** for 6 h and then harvested in lysis buffer containing paclitaxel, which prevents further tubulin rearrangements. The lysates were then centrifuged to separate the polymerized (pellet) and soluble (supernatant) tubulin. Both the pellet and the supernatant were collected and Western blotting was conducted to determine levels of the polymerized (pellet) tubulin and soluble form (supernatant). As shown in parts B and C of Figure 4, while in control cells the amount of polymerized tubulin (46% of total tubulin) was almost equal to


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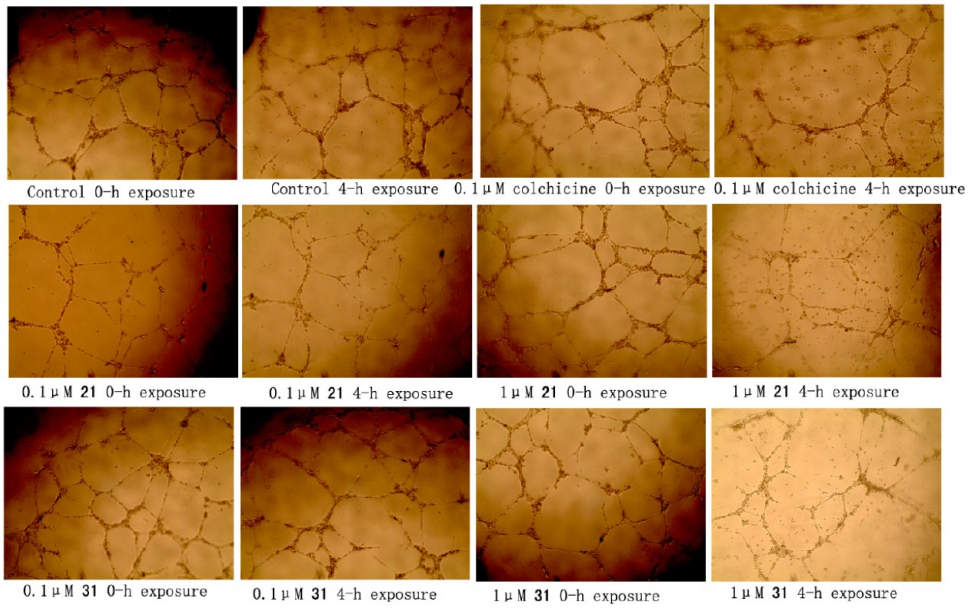


**Figure 3.** Effects of tested compounds on performed capillary-like structures. Control (0.1% DMSO) or tested compounds at the indicated concentrations were added to cords formed by endothelial cells on Matrigel 16 h after seeding. Images were taken 0 and 4 h after tested compounds addition. Magnification ×40.

the soluble form (54% of total tubulin), the cellular polymerized tubulin decreased dramatically when cells were treated with the tested compounds. Total tubulin values of 21%, 27% and 31% in the polymerized state were found after cells were treated with **1**, **21**, and **31**, respectively. These data showed that **21** and **31** inhibited tubulin polymerization which were similar to **1**.

Last, we evaluated effects of **21** and **31** on cell cycle. A549 cells were treated with **21**, **31**, and **1** at indicated concentrations for 24 h. After staining with propidium iodide, cells then were analyzed by flow cytometry. As shown in Figure 5 and Table 4, cells treated with **21** and **31** resulted in severe G2/M arrest (exhibited 79% and 80% of cells arrest at the G2/M-phase, respectively), while control cells were mainly in G1 phase. These results demonstrate that these compounds act as antimitotic agents.

**Perfused Vascular Volume Assay in Vivo.** The efficacy of the representative compound **21** in disrupting tumor vasculature was determined using perfused vascular volume assay in vivo, as reported previously.[6,22] The nude mice bearing size-matched A549-derived tumors were treated with a single administration of tested compounds (100 mg/kg) or vehicle. After 16 h of administration, functional vascular volume of tumors was assessed using Hoechst 33342, which acts as a marker of blood perfusion. Perfused vessels were identified by their fluorescent outlines, and the results for treated tumors were expressed as a percentage of control values. Quantitative analysis of the tumor sections showed that following treatment with **21** and **1**, functioning vascular volumes were reduced by 68% and 72%, respectively (Figure 6A,B). These data demonstrate that **21** is a potential efficient vascular disrupting agent in vivo.

## CONCLUSION

We have synthesized several series of 5,6,7-trimethoxyindole derivatives that mimick colchcine and **1**. SARs observed for members of these series revealed that the synthesized 3-aroyindoles and 3-benzylindoles had higher antiproliferative activities than other series; adding a methyl group at the N-1 position in both series gave compounds **15**, **21**, **22**, **29**, and **31** and exerts a potency increase. The N1-methyl 5,6,7-trimethoxyindole derivatives **21** and **31** showed potent antiproliferative activities against several tumor cell lines. Furthermore, two independent in vitro assay paradigms confirmed that compounds **21** and **31** are far more potent against activated HUVECs. This degree of selectivity was not observed with colchicine and **1**. In addition, **21** and **31** disrupted the network of capillary-like structures, indicating potential vascular-disrupting activity. Immunocytochemistry staining assay demonstrated that **21** and **31** disrupted the microtubule structure. Tubulin polymerization assay showed that **21** and **31** act as tubulin polymerization inhibitors. Similar to other antitubulin agents, **21** and **31** caused arrest at G2/M phase of the cell cycle. Finally, the representatively compound **21** significantly reduced vascular volume of A549 tumors in vivo, which demonstrated the in vivo efficacy of **21** as a vascular disrupting agent.

Taken together, these in vitro and in vivo data suggest that compounds **21** and **31** may become promising leads for further studies in terms of potential vascular-disrupting activity and high degree of selectivity for activated endothelial cells. The replacement of the trimethoxybenzene by *N*-methyl-5,6,7-trimethoxyindole leads to a increase in the selectivity against activated HUVECs. These results demonstrate that *N*-methyl-5,6,7-trimethoxyindole could be considered to replace the 3,4,5-trimethoxybenzene moiety of colchcine and **1**, revealing another avenue for the development of novel vascular disrupt agents.

## EXPERIMENTAL SECTION

**Chemistry.** All commercial chemicals and solvents are reagent grade and were used without further treatment unless otherwise noted. All reactions were carried out under an atmosphere of dry argon. $^1$H NMR spectra were obtained with a Varian Mercury-300BB NMR (300 MHz) and Varian Mercury Plus-400 (400 MHz) spectrometers with


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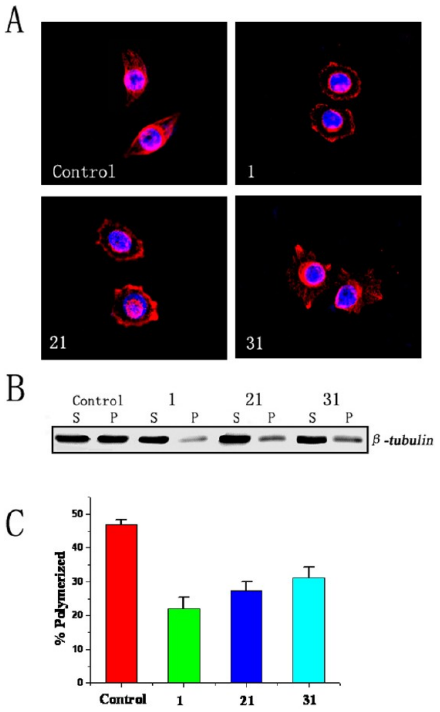


**Figure 4.** Effect of tested compounds on microtubules. (A) Control cells (0.1% DMSO) or cells were treated for 16 h with **21** (0.05 $\mu$M), **31** (0.1 $\mu$M), or **1** (0.01 $\mu$M), then fixed and immunofluorescent staining with an anti-$\beta$-tubulin antibody (red) and DIPA (blue, for nuclear staining). Images were captured with Zeiss microscopy (63× oil immersion objective). Results are representative of three separate experiments which yielded comparable results. (B) Effect of tested compounds on cellular tubulin polymerization. Control cells (0.1% DMSO) or cells were treated for 6 h with indicated compounds (all at 0.1 $\mu$M), then lysed in the presence of paclitaxel and centrifuged to separate polymerized fraction (P) from soluble fraction (S), then both fractions were analyzed by Western blot. (C) Statistical chart of (B) quantified by ImageJ software. The % polymerized was calculated by dividing the $\beta$-tubulin in the polymerized fraction by the sum of the $\beta$-tubulin in the polymerized and soluble fractions; bars, the mean ± SE of at least three independent experiments.

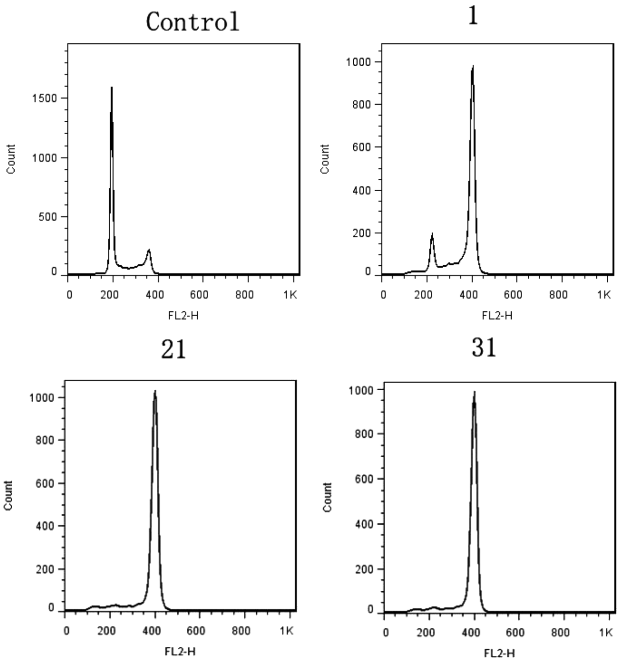


**Figure 5.** Effects of **21**, **31**, and **1** on cell cycle. A549 cells were harvested after treatment with 0.1 $\mu$M **21**, **31**, and 0.01 $\mu$M **1** for 24 h. DMSO-treated (0.1%) cells served as controls. The percentage of cells in each phase of cell cycle was analyzed by the standard propidium iodide procedure as described in the Experimental Section.

**Table 4. Cell Cycle Distribution of A549 Cell Line Treated with 21, 31, and 1**[a]

| compd | % of cells in G1 phase | % of cells in S phase | % of cells in G2/M phase |
|---|---|---|---|
| control | 53.37 | 32.56 | 8.52 |
| **1** | 9.11 | 26.40 | 58.61 |
| **21** | 5.07 | 14.34 | 78.85 |
| **31** | 3.90 | 14.70 | 80.48 |

[a]Cell cycle analysis was performed after treatment of cells with **21**, **31**, and **1** using a FACSCalibur (BD Biosciences, Mountain View, CA). A549 cells were treated with 0.1 $\mu$M **21** and **31**, and 0.01 $\mu$M **1** for 24 h. Cell cycle distribution was analyzed by the standard propidium iodide procedure as mentioned under the Experimental Section.

TMS as internal standard. HRESIMS data were measured on a Bruker Daltonics APEX II 47e spectrometer. Column chromatography (CC): YMC gel (ODS-A, 12 nm, S-50 $\mu$m, YMC Co., Kyoto, Japan); silica gel (200−300 mesh, Qingdao Marine Chemical Factory, Qingdao, PR China). TLC: silica gel GF 254 (Qingdao Marine Chemical Factory, Qingdao, PR China). Purity of the final compounds were determined using an Waters 1525−2998 HPLC (Waters Co., USA) system using C-18 column (Sun Fire, 5 $\mu$m, 4.6 mm × 150 mm) and were found to be ≥95%, unless otherwise stated.

*(5,6,7-Trimethoxy-1H-indol-1-yl)(4-methoxyphenyl)methanone (**6**).* To a solution of 5,6,7-trimethoxy-1*H*-indole (0.35 g, 1.69 mmol) in THF (10 mL), potassium *tert*-butoxide (0.284 g, 2.54 mmol) was added at room temperature. At this temperature, the reaction mixture was stirred for 15 min before the addition of 4-methoxybenzoyl chloride (2.54 mmol) in dry THF (5 mL). After stirring for another 6 h at room temperature, the reaction mixture was evaporated and then water was added and the mixture was extracted with diethyl ether. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 5:1) to afford compound **6** (350 mg, 61%) as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.82 (d, $J$ = 8.8 Hz, 2H), 7.23 (d, $J$ = 3.6 Hz, 1H), 6.97 (d, $J$ = 8.8 Hz, 2H), 6.84 (s, 1H), 6.48 (d, $J$ = 3.2 Hz, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.88 (s, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.9, 163.4, 151.3, 142.3, 140.7, 132.4, 132.4, 129.0, 127.5, 126.3, 124.0, 113.8, 113.8, 106.9, 97.9, 61.1, 60.4, 56.2, 55.5. HRMS calcd for $C_{19}H_{19}NO_5$ $[M + H]^+$, 342.1336; found, 342.1339.

*(3-Hydroxy-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-1-yl)-methanone (**7**).* The crude intermediate **7a** was prepared as described for **6** using 5,6,7-trimethoxy-1*H*-indole and 3-(benzyloxy)-4-methoxybenzoyl chloride. **7** was prepared as described for **21** using **7a**. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 3:1) to afford compound **7** as an off-white solid (58% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.44 (d, $J$ = 2.4 Hz, 1H), 7.42 (dd, $J$ = 8.4 Hz, 2 Hz, 1H), 7.24 (d, $J$ = 3.6 Hz, 1H), 6.92 (d, $J$ = 8.4 Hz, 1H), 6.80 (s, 1H), 6.47 (d, $J$ = 3.6 Hz, 1H), 3.97 (s, 3H), 3.92 (s, 3H), 3.91 (s, 6H), 3.89 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 166.8, 151.4, 150.5, 145.5, 142.3, 140.7, 129.1, 127.6, 127.1, 123.9, 123.6, 116.3, 110.0, 106.9, 97.9, 61.2, 60.4, 56.2, 56.1. HRMS calcd for $C_{19}H_{19}NO_6$[M + H]$^+$, 358.1285; found, 358.1287.

*(5,6,7-Trimethoxy-1H-indol-3-yl)(4-methoxyphenyl)methanone (**8**).* To a mixture of 5,6,7-trimethoxy-1*H*-indole (0.249 g, 1.2 mmol) and anhydrous zinc chloride (0.327 g, 2.4 mmol) in dry dichloromethane (10 mL), ethylmagnesium bromide (0.54 mL, 3.0 M solution in diethyl ether) was added at room temperature. At this temperature, the reaction mixture was stirred for 1 h before the addition of 4-methoxybenzoyl chloride (1.8 mmol) in dry dichloromethane (10


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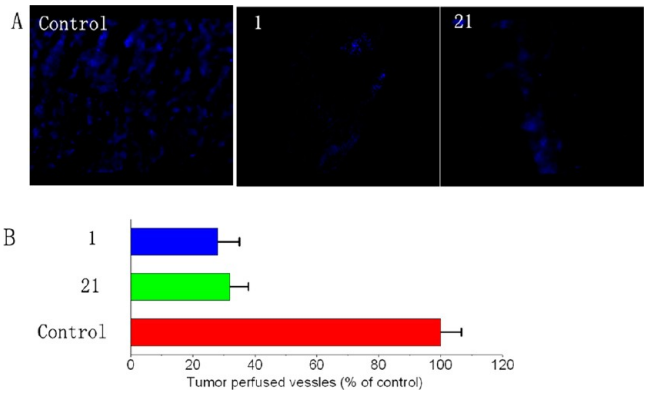


**Figure 6.** Effects of tested compounds on perfused vascular volume assay. (A) Representative images of perfused A549 tumors vascular (blue staining) following the administration of **21** or **1**. After intraperitoneal (ip) administration of **21** and **1** (100 mg/kg) for 16 h, the fluorescent dye Hoechst 33342 was injected iv at a dose of 10 mg/kg, then tumors were excised and frozen 1 min later, and sections were imaged on an inverted Zeiss Imager Z2 microscope. Magnification ×40. (B) Statistical chart of (A). Data are expressed as a percentage of the perfusion measured in untreated tumors; bars, the mean ± SE of six tumors.

mL). The reaction mixture was stirred for another 1 h, followed by the addition of aluminum chloride (0.27 g, 2.03 mmol). After stirring for another 5 h at room temperature, the reaction mixture quenched with water (10 mL) and extracted with $CH_2Cl_2$. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 2:1) to afford compound **8** (180 mg, 44%) as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.3 (brs, 1H), 7.82 (d, $J$ = 8 Hz, 2H), 7.67 (s, 1H), 7.54 (d, $J$ = 2 Hz, 1H), 6.95 (d, $J$ = 8 Hz, 2H), 4.05 (s, 3H), 3.91 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 190.4, 162.2, 151.3, 138.7, 138.7, 133.1, 132.4, 130.8, 124.7, 122.5, 113.5, 113.5, 117.1, 98.6, 61.4, 61.1, 56.2, 55.3. HRMS calcd for $C_{19}H_{19}NO_5$ $[M + H]^+$, 342.1336; found, 342.1331.

*(5,6,7-Trimethoxy-1H-indol-3-yl)(2-methoxyphenyl)methanone (**9**).* **9** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 2-methoxybenzoyl chloride; white solid (41% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.99 (brs, 1H), 7.67 (s, 1H), 7.39 (m, 3H), 6.98 (m, 2H), 4.10 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.75 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.8, 156.7, 151.4, 138.7, 130.9, 130.7, 128.7, 124.7, 120.1, 118.8, 111.5, 98.8, 61.4, 61.2, 56.2, 55.7. HRMS calcd for $C_{19}H_{19}NO_5$ $[M + H]^+$, 342.1336; found, 342.1328.

*(1-Acetyl-5,6,7-trimethoxy-1H-indol-3-yl)(4-methoxyphenyl)-methanone (**10**).* To a solution of **8** (0.1 g, 0.29 mmol) in THF (5 mL), potassium *tert*-butoxide (0.049 g, 0.44 mmol) was added at room temperature. At this temperature, the reaction mixture was stirred for 15 min before the addition of acetyl chloride (0.6 mmol). After stirring for another 6 h at room temperature, the reaction mixture was evaporated, and then water was added and the mixture was extracted with diethyl ether. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 5:1) to afford compound **10** (58 mg, 52%) as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.89 (s, 1H), 7.85 (d, $J$ = 8.0 Hz, 2H), 7.63 (s, 1H), 6.97 (d, $J$ = 8.0 Hz, 2H), 3.98 (s, 3H), 3.94 (s, 3H), 3.92 (s, 3H), 3.87 (s, 3H), 2.69 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 189.9, 169.5, 163.0, 152.4, 141.6, 141.4, 134.0, 131.9, 131.1, 131.1, 126.2, 123.0, 119.4, 113.7, 113.7, 99.6, 61.2, 61.2, 56.2, 55.4, 25.4. HRMS calcd for $C_{21}H_{21}NO_6$ $[M + H]^+$, 384.1442; found, 384.1439.

*(1-Benzenesulfonyl-5,6,7-trimethoxy-1H-indol-3-yl)(4-methoxyphenyl)methanone (**11**).* To a solution of **8** (0.1 g, 0.29 mmol) in THF (5 mL), potassium *tert*-butoxide (0.049 g, 0.44 mmol) was added at room temperature. At this temperature, the reaction mixture was stirred for 15 min before the addition of benzenesulfonyl chloride (0.7 mmol). After stirring for another 6 h at room temperature, the reaction mixture was evaporated, and then water was added and the mixture was extracted with diethyl ether. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 5:1) to afford compound **11** (64 mg, 46%) as a light-yellow solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.20 (s, 1H), 7.92 (d, $J$ = 8.8 Hz, 2H), 7.87 (d, $J$ = 8.4 Hz, 2H), 7.63 (s, 1H), 7.58 (m, 1H), 7.49 (m, 2H), 7.03 (d, $J$ = 2 Hz, 2H), 3.91 (s, 6H), 3.81 (s, 3H), 3.79 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 189.7, 163.2, 152.5, 141.4, 140.9, 138.8, 135.1, 133.8, 131.8, 131.3, 131.3, 129.1, 129.1, 127.4, 127.4, 125.9, 123.0, 118.9, 113.9, 113.9, 99.3, 61.1, 61.0, 56.2, 55.5. HRMS calcd for $C_{25}H_{23}NO_7S$ $[M + H]^+$, 482.1268; found, 482.1272.

*(5,6,7-Trimethoxy-1H-indol-3-yl)(3-methoxyphenyl)methanone (**12**).* **12** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3-methoxybenzoyl chloride; off-white solid (49% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.65 (brs, 1H), 7.70 (s, 1H), 7.61 (d, $J$ = 3.2 Hz, 1H), 7.39 (m, 3H), 7.10 (m, 1H), 4.10 (s, 3H), 3.97 (s, 3H), 3.92 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.0, 159.5, 151.6, 141.9, 138.8, 138.6, 132.6, 129.2, 124.6, 122.2, 121.1, 117.4, 117.3, 113.4, 98.7, 61.4, 61.2, 56.3, 55.4. HRMS calcd for $C_{19}H_{19}NO_5$ $[M + H]^+$, 342.1336; found, 342.1327.

*(5,6,7-Trimethoxy-1H-indol-3-yl)(p-tolyl)methanone (**13**).* **13** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 4-methybenzoyl chloride; off-white solid (55% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.93 (brs, 1H), 7.73 (d, $J$ = 8 Hz, 2H), 7.70 (s, 1H), 7.56 (d, $J$ = 2 Hz, 1H), 7.27 (d, $J$ = 8 Hz, 2H), 4.08 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H), 2.43 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.2, 151.4, 141.7, 138.8, 138.6, 137.9, 132.5, 128.9, 128.8, 128.8, 124.6, 122.3, 117.3, 98.7, 61.4, 61.2, 56.3, 21.5. HRMS calcd for $C_{19}H_{19}NO_4$ $[M + H]^+$, 326.1387; found, 326.1377.

*(5,6,7-Trimethoxy-1H-indol-3-yl)(3,4,5-trimethoxyphenyl)-methanone (**14**).* **14** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3,4,5-trimethoxybenzoyl chloride; off-white solid (62% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.07 (brs, 1H), 7.67 (s, 1H), 7.63 (d, $J$ = 2.8 Hz, 1H), 7.11 (s, 2H), 4.09 (s, 3H), 3.96 (s, 3H), 3.93 (s, 3H), 3.93 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 190.4, 152.9, 152.9, 151.5, 140.9, 138.9, 138.7, 135.9, 132.4, 124.7, 122.4, 117.0, 106.3, 106.3, 98.6, 61.4, 61.2, 61.0, 56.3, 56.2. HRMS calcd for $C_{21}H_{23}NO_7$ $[M + H]^+$, 402.1557; found, 402.1554.

*(3-(Benzyloxy)-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-3-yl)methanone (**15a**).* **15a** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3-(benzyloxy)-4-methoxybenzoyl chloride; yellow solid (57% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 8.47 (brs, 1H), 7.65 (s, 1H), 7.5−7.3 (m, 7H), 7.21 (d, $J$ = 2.0 Hz), 6.97 (d, $J$ = 8.1 Hz, 1H), 5.23 (s, 2H), 4.10 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H) 3.92 (s, 3H).

*(3-Hydroxy-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-3-yl)-methanone (**15**).* **15** was prepared as described for **21** using **7a**; off-white solid (93% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.42 (brs, 1H), 7.67 (s, 1H), 7.54 (t, $J$ = 2 Hz), 7.44 (d, $J$ = 2 Hz, 1H), 7.38 (d, $J$ = 8 Hz, 1H), 6.88 (s, 1H), 4.04 (s, 3H), 3.91 (s, 3H), 3.90 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 190.4, 151.3, 149.5, 145.2, 138.7, 133.8, 133.0, 132.9, 124.7, 122.7, 121.9, 116.9, 115.3, 110.0, 98.7, 61.4, 61.2, 56.3, 56.0. HRMS calcd for $C_{19}H_{19}NO_6$ $[M + H]^+$, 358.1285; found, 358.1277.

*(3-Chloro-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-3-yl)-methanone (**16**).* **16** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3-chloro-4-methoxy benzoyl chloride; off-white solid (45% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.94 (brs, 1H), 7.91 (s, 1H), 7.76 (dd, $J$ = 8.8 Hz, $J$ = 2.8 Hz, 1H), 7.63 (s, 1H), 7.56 (d, $J$ = 2.8 Hz, 1H), 7.00 (d, $J$ = 8.8 Hz, 1H), 4.09 (s, 3H), 3.98 (s, 3H), 3.95 (s, 3H), 3.92 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 188.9, 157.5, 151.6, 138.9, 138.7, 133.8, 132.2, 131.0, 129.0, 124.7, 122.4, 122.3, 117.0, 111.4, 98.5, 61.4, 61.3, 56.3, 56.3. HRMS calcd for $C_{19}H_{18}ClNO_5$ $[M + H]^+$, 376.0946; found, 376.0944.


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*(3-Iodo-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-3-yl)-methanone (**17**).* **17** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3-iodo-4-methoxy benzoyl chloride; off-white solid (37% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.11 (brs, 1H), 8.29 (d, $J$ = 2.0 Hz, 1H), 7.83 (dd, $J$ = 8.4 Hz, $J$ = 2.0 Hz, 1H), 7.62 (s, 1H), 7.56 (d, $J$ = 3.2 Hz, 1H), 6.88 (d, $J$ = 8.4 Hz, 1H), 4.07 (s, 3H), 3.95 (s, 3H), 3.94 (s, 3H), 3.91 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 188.7, 160.5, 151.5, 140.3, 138.9, 138.7, 134.9, 132.3, 130.8, 124.7, 122.4, 117.0, 110.2, 98.6, 85.5, 61.5, 61.3, 56.6, 56.4. HRMS calcd for $C_{19}H_{18}INO_5$ $[M + H]^+$, 468.0302; found, 468.0304.

*(3-Fluoro-4-methoxyphenyl)(5,6,7-trimethoxy-1H-indol-3-yl)-methanone (**18**).* **18** was prepared as described for **8** using 5,6,7-trimethoxy-1*H*-indole and 3-fluoro-4-methoxy benzoyl chloride; off-white solid (41% yield). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 12.12 (d, $J$ = 2.1 Hz 1H), 7.80 (d, $J$ = 3.2 Hz, 1H), 7.65 (dd, $J$ = 8.4 Hz, $J$ = 1.2 Hz, 1H), 7.60 (dd, $J$ = 12 Hz, $J$ = 2.0 Hz, 1H), 7.30 (t, $J$ = 8.4 Hz, 1H), 3.97 (s, 3H), 3.93 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 187.4, 152.2, 150.5, 149.7, 139.0, 138.4, 134.2, 133.1, 125.7, 124.6, 122.5, 115.8, 115.1, 113.2, 98.5, 61.2, 60.9, 56.2, 55.9. HRMS calcd for $C_{19}H_{18}FNO_5$ $[M + H]^+$, 360.1242; found, 360.1239.

*(3-Hydroxy-4-methoxyphenyl)(5,6,7-trimethoxy-2-methyl-1H-indol-3-yl)methanone (**19**).* **19** was prepared as described for **21** using **19a**. The crude intermediate **19a** was prepared as described for **8** using 5,6,7-trimethoxy-2-methyl-1*H*-indole and 3-(benzyloxy)-4-methoxy-benzoyl chloride. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 3:1) to afford compound **19** as an off-white solid (52% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.40 (brs, 1H), 7.38 (d, $J$ = 2 Hz, 1H), 7.34 (dd, $J$ = 8.4 Hz, 2 Hz, 1H), 6.90 (d, $J$ = 8.4 Hz, 1H), 6.89 (s, 1H), 5.68 (brs, 1H), 4.07 (s, 3H), 3.97 (s, 3H), 3.89 (s, 6H), 3.77 (s, 3H), 2.45 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.8, 150.3, 149.5, 145.3, 141.4, 138.0, 137.8, 134.5, 123.6, 122.8, 122.3, 115.4, 114.6, 109.8, 98.2, 61.4, 61.2, 56.3, 56.1, 14.6. HRMS calcd for $C_{20}H_{21}NO_6$ $[M + H]^+$, 372.1442; found, 372.1445.

*(5,6,7-Trimethoxy-1-methyl-1H-indol-3-yl)(4-methoxyphenyl)-methanone (**20**).* To a solution of **8** (0.05 g, 0.145 mmol) in THF (5 mL), potassium *tert*-butoxide (0.025 g, 0.22 mmol) was added at room temperature. At this temperature, the reaction mixture was stirred for 15 min before the addition of iodomethane (0.6 mmol). After stirring for another 6 h at room temperature, the reaction mixture was evaporated, and then water was added and the mixture was extracted with diethyl ether. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel to afford compound **20** (50 mg, 97%) as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.81 (d, $J$ = 8.4 Hz, 2H), 7.73 (s, 1H), 7.35 (s, 1H), 6.97 (d, $J$ = 8.4 Hz, 2H), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.92 (s, 3H), 3.92 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 189.7, 162.1, 151.2, 140.4, 139.7, 137.9, 133.6, 130.8, 130.8, 124.9, 124.4, 115.1, 113.5, 113.5, 99.1, 62.0, 61.3, 56.2, 55.4, 36.5. HRMS calcd for $C_{20}H_{21}NO_5$ $[M + H]^+$, 356.1492; found, 356.1496.

*(3-(Benzyloxy)-4-methoxyphenyl)(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)methanone (**21a**).* **21a** was prepared from **19a** as described for **20**; off-white solid (98% yield). $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 7.73 (s, 1H), 7.47−7.31 (m, 7H), 7.11 (s, 1H), 6.98 (d, $J$ = 8.8 Hz, 1H), 5.23 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 3.95 (s, 6H), 3.91 (s, 3H).

*(3-Hydroxy-4-methoxyphenyl)(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)methanone (**21**).* A mixture of **21a** (0.28 g, 0.63 mmol), ammonium formate (0.400 g, 6.3 mmol), 10% palladium on carbon (0.1 g), and 30 mL of methanol was heated under reflux for 1 h. After it was cooled to room temperature, the solution was filtered and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (eluent, petroleum/ethyl acetate 3:1) to give 0.202 g (90% yield) of **21** as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.74 (s, 1H), 7.43 (d, $J$ = 1.6 Hz, 1H), 7.39 (d, $J$ = 8.8 Hz, 1H), 7.38 (s, 1H), 6.92 (dd, $J$ = 1.6 Hz, 8.4 Hz, 1H), 4.03 (s, 3H), 3.99 (s, 3H), 3.95 (s, 6H), 3.91(s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 189.6, 151.2, 149.2, 145.2, 140.4, 139.7, 138.1, 134.3, 124.9, 124.4, 121.7, 115.2, 115.0, 110.0, 99.2, 62.0, 61.3, 56.2, 56.1, 36.5. HRMS calcd for $C_{20}H_{21}NO_6$ $[M + H]^+$, 372.1442; found, 372.1443.

*(3-Fluoro-4-methoxyphenyl)(5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)methanone (**22**).* **22** was prepared from **18** as described for **20**; off-white solid (93% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.73 (s, 1H), 7.57−7.62 (m, 2H), 7.35 (s, 1H), 7.03 (t, $J$ = 8.0 Hz, 1H), 4.04 (s, 3H), 4.02 (s, 3H), 3.96 (s, 6H), 3.95 (s, 3H), 3.92 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 188.3, 153.1, 151.4, 150.3, 140.5, 139.9, 137.8, 133.8, 125.5, 124.9, 124.3, 116.6, 114.8, 112.5, 99.1, 62.0, 61.3, 56.3, 56.2, 36.6. HRMS calcd for $C_{20}H_{20}FNO_5$ $[M + H]^+$, 374.1398; found, 374.1395.

*(3-Hydroxy-4-methoxyphenyl)(5,6,7-trimethoxy-1,2-dimethyl-1H-indol-3-yl)methanone (**23**).* **23** was prepared as described for **21** using **23a**. The crude intermediate **23a** was prepared from **19a** as described for **20**. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 3:1) to afford compound **23** as an off-white solid (82% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.40 (brs, 1H), 7.38 (d, $J$ = 2 Hz, 1H), 7.34 (dd, $J$ = 8.4 Hz, 2 Hz, 1H), 6.90 (d, $J$ = 8.4 Hz, 1H), 6.89 (s, 1H), 5.68 (brs, 1H), 4.07 (s, 3H), 3.97 (s, 3H), 3.89 (s, 6H), 3.77 (s, 3H), 2.45 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 191.8, 150.3, 149.5, 145.3, 141.4, 138.0, 137.8, 134.5, 123.6, 122.8, 122.3, 115.4, 114.6, 109.8, 98.2, 61.4, 61.2, 56.3, 56.1, 14.6. HRMS calcd for $C_{20}H_{23}NO_6$ $[M + H]^+$, 386.1598; found, 386.1598.

*1-(4-Methoxybenzyl)-5,6,7-trimethoxy-1H-indole (**24**).* To a solution of 5,6,7-trimethoxy-1*H*-indole (0.03 g, 0.14 mmol) in DMF (5 mL), KOH (0.012 g, 0.214 mmol) and KI (0.024 g, 0.14 mmol) were added at room temperature. At this temperature, the reaction mixture was stirred for 1 h. The reaction mixture was then cooled to 0 °C and treated with 4-methoxybenzyl bromide (0.028 g, 0.14 mmol) with continuous stirring at 0 °C for 1 h. The reaction mixture was then quenched with water and extracted with diethyl ether. The combined organic extracts was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 12:1) to afford compound **24** (21 mg, 47%) as an off-white solid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.00 (d, $J$ = 8.8 Hz, 2H), 6.96 (d, $J$ = 3.2 Hz, 1H), 6.83 (d, $J$ = 2.4 Hz, 1H), 6.82 (d, $J$ = 8.4 Hz, 2H), 6.39 (d, $J$ = 2.8 Hz, 1H), 5.48 (s, 2H), 3.89 (s, 3H), 3.88 (s, 3H), 3.76 (s, 3H), 3.74 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 158.8, 148.9, 140.5, 138.8, 131.4, 129.3, 127.7, 127.7, 125.4, 123.7, 113.9, 113.9, 101.5, 97.5, 61.3, 61.2, 56.2, 55.2, 51.2. HRMS calcd for $C_{19}H_{21}NO_4$ $[M + H]^+$, 328.1543; found, 328.1550.

*2-Methoxy-5-(5,6,7-trimethoxy-1H-indol-1-yl)methyl)phenol (**25**).* The crude intermediate **25a** was prepared as described for **24** using 5,6,7-trimethoxy-1*H*-indole and 3-(benzyloxy)-4-methoxybenzyl bromide. **25** was prepared as described for **21** using **25a**. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 5:1) to afford compound **25** as an off-white solid (35% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.95 (d, $J$ = 3.2 Hz, 1H), 6.82 (s, 1H), 6.71 (d, $J$ = 8.4 Hz, 1H), 6.68 (d, $J$ = 1.6 Hz, 1H), 5.68 (s, 1H), 5.43 (s, 2H), 3.87 (s, 3H), 3.87 (s, 3H), 3.79 (s, 3H), 3.74 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 148.7, 145.6, 145.6, 140.3, 138.6, 132.4, 129.2, 125.2, 123.5, 117.7, 112.8, 110.5, 101.4, 97.4, 61.2, 61.1, 56.1, 55.7, 51.1. HRMS calcd for $C_{19}H_{21}NO_5$ $[M + H]^+$, 344.1492; found, 344.1497.

*3-(4-Methoxybenzyl)-5,6,7-trimethoxy-1H-indole (**26**).* To a mixture of 5,6,7-trimethoxy-1*H*-indole (0.65 g, 3.14 mmol) and $NH_4HCO_3$ (0.1 g, 1.29 mmol) in 20 mL 80% aqueous acetone (v/v), 4-methoxybenzyl bromide (0.13 g, 0.65 mmol) was added at room temperature. After stirring for 2 h at room temperature, water was added and the mixture was extracted with diethyl ether. The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude residue purified by column chromatography on reversed-phase silica gel (eluent, methanol/water 1:1) to afford compound **26** (94 mg, 44%) as a colorless liquid. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.0q (brs, 1H), 7.21 (d, $J$ = 8.4 Hz, 2H), 6.84 (d, $J$ = 8.8 Hz, 2H), 6.82 (d, $J$ = 0.8 Hz, 1H), 6.68 (s, 1H), 4.06 (s, 3H), 4.00 (s, 2H), 3.90 (s, 3H), 3.85 (s, 3H), 3.80 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 157.8, 148.9, 138.7, 138.1, 133.2, 129.5, 129.5, 124.8, 123.1, 121.9,


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116.5, 113.7, 113.7, 96.0, 61.4, 61.0, 56.4, 55.2, 30.8. HRMS calcd for $C_{19}H_{21}NO_4$ $[M + H]^+$, 328.1543; found, 328.1550.

*3-(3,4,5-Trimethoxybenzyl)-5,6,7-trimethoxy-1H-indole (**27**).* **27** was prepared as described for **26** using 5,6,7-trimethoxy-1*H*-indole and 5,6,7-trimethoxybenzyl bromide. The crude residue purified by column chromatography on reversed-phase silica gel (eluent, methanol/water 1:1) to afford compound **27** as an colorless liquid (55% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.07 (brs, 1H), 6.84 (d, $J$ = 2.4 Hz, 1H), 6.71 (s, 1H), 6.53 (s, 2H), 4.06 (s, 3H), 3.99 (s, 2H), 3.96 (s, 3H), 3.90 (s, 3H), 3.83 (s, 3H), 3.80 (s, 6H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 153.1, 153.1, 149.0, 138.8, 138.1, 136.7, 136.2, 124.9, 123.1, 122.0, 115.8, 105.8, 105.8, 95.9, 61.4, 61.0, 60.8, 56.5, 56.1, 56.1, 32.1. HRMS calcd for $C_{21}H_{25}NO_6$ $[M + H]^+$, 388.1755; found, 388.1759.

*3-(3-Fluoro-4-methoxybenzyl)-5,6,7-trimethoxy-1H-indole (**28**).* **28** was prepared as described for **26** using 5,6,7-trimethoxy-1*H*-indole and 3-fluoro-4-methoxybenzyl bromide. The crude residue purified by column chromatography on reversed-phase silica gel (eluent, methanol/water 6:4) to afford compound **28** as an colorless liquid (52% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 8.02 (brs, 1H), 7.00 (d, $J$ = 2.0 Hz, 1H), 6.98 (d, $J$ = 7.2 Hz, 1H), 6.89 (d, $J$ = 8.4 Hz, 1H), 6.85 (d, $J$ = 2.0 Hz, 1H), 6.34 (s, 1H), 4.06 (s, 3H), 3.97 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 152.3 (d, $J$ = 244 Hz), 149.1, 145.7, 138.8, 138.1, 134.3, 124.9, 123.9, 123.0, 122.0, 116.3, 115.6, 113.3, 95.8, 61.4, 61.0, 56.5, 56.4, 30.7. HRMS calcd for $C_{19}H_{20}FNO_4$ $[M + H]^+$, 346.1449; found, 346.1445.

*2-Methoxy-5-((5,6,7-trimethoxy-1H-indol-3-yl)methyl)phenol (**29**).* **29** was prepared as described for **21** using **29a**. The crude intermediate **29a** was prepared as described for **26** using 5,6,7-trimethoxy-1*H*-indole and 3-(benzyloxy)-4-methoxybenzyl bromide. The crude intermediate **29a** purified by column chromatography on reversed-phase silica gel (eluent, methanol/water 6:4). Transfer hydrogenation of **29a** afforded compound **29** as an colorless liquid (51% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.98 (brs, 1H), 6.87 (d, $J$ = 2.4 Hz, 2H), 6.78 (s, 2H), 6.70 (s, 1H), 5.56 (s, 1H), 4.06 (s, 3H), 3.97 (s, 2H), 3.90 (s, 3H), 3.87 (s, 3H), 3.86 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 148.9, 145.5, 144.8, 138.7, 138.1, 134.5, 124.9, 123.2, 121.9, 119.9, 116.2, 114.9, 110.6, 96.0, 61.4, 61.0, 56.5, 56.0, 31.0. HRMS calcd for $C_{19}H_{21}NO_5$ $[M + H]^+$, 344.1492; found, 344.1495.

*3-(3-Fluoro-4-methoxybenzyl)-5,6,7-trimethoxy-1-methyl-1H-indole (**30**).* **30** was prepared as described for **20** using **29**; colorless liquid (95% yield). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.99 (m, 1H), 6.97 (s, 1H), 6.87 (t, $J$ = 8.8 Hz, 1H), 6.63 (s, 1H), 6.59 (s, 1H), 4.00 (s, 3H), 3.93 (s, 2H), 3.91 (s, 3H), 3.90 (s, 3H), 3.86 (s, 3H), 3.84 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 152.3 (d, $J$ = 243 Hz), 148.5, 145.7 (d, $J$ = 11 Hz), 140.4, 139.0, 134.5 (d, $J$ = 6 Hz), 128.2, 124.8, 124.4, 123.9 (d, $J$ = 3 Hz), 116.3 (d, $J$ = 18 Hz), 113.5, 113.4 (d, $J$ = 4 Hz), 96.0, 61.9, 61.3, 56.4, 56.4, 35.2, 30.5. HRMS calcd for $C_{20}H_{22}FNO_4$ $[M + H]^+$, 360.1606; found, 360.1602.

*2-Methoxy-5-((5,6,7-trimethoxy-1-methyl-1H-indol-3-yl)methyl)-phenol (**31**).* **31** was prepared as described for **21** using **31a**. The crude intermediate **31a** was prepared as described for **20** using **29a**. The crude residue purified by column chromatography on silica gel (eluent, petroleum/ethyl acetate 3:1) to afford compound **31** as an colorless liquid (84% yield, two steps). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 6.85 (d, $J$ = 1.2 Hz, 1H), 6.77 (s, 1H), 6.76 (s, 1H), 6.67 (s, 1H), 6.59 (s, 1H), 4.00 (s, 3H), 3.91 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 148.4, 145.4, 144.8, 140.3, 138.8, 134.6, 128.2, 124.7, 124.5, 119.9, 114.9, 114.1, 110.5, 96.0, 61.9, 61.3, 56.3, 56.0, 35.1, 30.8. HRMS calcd for $C_{20}H_{23}NO_5$ $[M + H]^+$, 358.1649; found, 358.1652.

**Cell Culture.** Human cervix epitheloid carcinoma HeLa, and nonsmall cell lung cancer A549 were cultured in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Gibco) and penicillium/streptomycin at 37 °C in humidified 5% $CO_2$ incubator. Hepatocellular carcinoma HepG2 were cultured in Dulbecco's Modified Essential Medium (Gibco) with the same supplements as above. Human fibrosarcoma HT1080 were cultured in Alpha-Minimum Essential Medium with the same supplements as above. HUVEC were purchased from PriCells (Wuhan, China) and routinely cultured in endothelial cell medium (M&C Gene Technology, China) supplemented with 0.03 mg/mL endothelial cell growth supplement (M&C Gene Technology, China), 10% fetal bovine serum, 0.1 mg/mL heparin (M&C Gene Technology, China), and penicillium/streptomycin. HUVECs at passages 2−6 were used from for all experiments.

**Cell Proliferation Assay.** Cell proliferation was determined using CCK-8 dye (Beyotime Inst Biotech, China) according to manufacture's instructions. Briefly, A549 ($3 \times 10^3$ cells/well), HT1080 ($1.5 \times 10^3$ cells/well), HeLa ($1.5 \times 10^3$ cells/well), or HepG2 ($3 \times 10^3$ cells/well) were plated in 96-well plate and allowed to adhere overnight. Activated and quiescent HUVECs proliferation assay were carried out in 96-well plates. To assess the effects of compounds on activated endothelial cells, HUVECs plated at 2500 cells per well and cultured in endothelial cell medium (M&C Gene Technology, China) supplemented with 0.03 mg/mL endothelial cell growth supplement (M&C Gene Technology, China) and 10% fetal bovine serum.[11] To assess the effects of compounds on quiescent endothelial cells, HUVECs were plated at 15000 cells per well in basal medium supplemented with 0.5% fetal calf serum. After incubation with tested compounds for 48 or 72 h, 10 $\mu$L of CCK-8 dye then was add to each well, and cells were incubated at 37 °C for 2 h. The absorbance was finally determined at 450 nm using a microplate reader (TECAN Infinite M200).

**Capillary Formation and Disruption Assays.** Capillary formation assays were conducted according to the modified protocols previously described.[11] HUVEC cells were plated on a thicker Matrigel layer (BD Biosciences, 75 $\mu$Lwell$^{-1}$) in a density of 25000 cells per well in 96-well plates and incubated for 22 h. Capillary disruption assays were carried out when capillaries were allowed to form over a 16 h period and then added the test compound or control. Images were captured by light microscopy (40× magnification) immediately following compound addition and 4 h after exposure to test compound. Tube formation was quantified by measuring the total length of capillary structures using the software WCIF ImageJ. Two representative fields were counted in each experiment.

**Immunocytochemistry.** A549 cells were plate on glass coverslips and grown in the presence or absence of tested compounds for 24 h. Cell cultures were then rinsed twice with PBS, fixed in 4% paraformaldehyde for 20 min at room temperature, and permeabilized with 0.5% Triton X-100 in PBS for 10 min on ice. Fixed cells were rinsed in PBS and blocked with 5% normal goat serum (NGS) in PBS for at least 60 min at room temperature. After a brief wash, the cells were incubated with the anti-$\beta$-tubulin antibodies (Sigma-Aldrich, St Louis, Mo) (1:2000) for 2 h at room temperature, followed by incubated with the a goat antimouse secondary Texas Red-conjugated antibody (ZSGB−Bio, China) for 1 h at room temperature. Finally, the cells were incubated with DAPI (ZSGB−Bio, China) prior to mounting. Images were captured with Zeiss microscopy under the 63× oil immersion objective.

**Tubulin Polymerization Assay.** Tubulin polymerization assay was carried out essentially as described previously with some modifications. Briefly, A549 cells were treated with tested compounds or vehicle (0.1% DMSO) for 6 h, then trypsinized and washed once with PBS. Cells were then resuspended in 75 $\mu$L of lysis buffer (20 mM Tris-HCl, pH 6.8, 1 mM $MgCl_2$, 2 mM EGTA, protease inhibitors, 0.5% Nonidet P-40) containing 1 $\mu$g/mL paclitaxel and incubated for 15 min at 4 °C. Lysates were then centrifuged at 13000 rpm for 15 min at 4 °C. The pellet was then resuspended in 75 $\mu$L of lysis buffer, and both the pellet and the supernatant were added 15 $\mu$L of 5× SDS-loading buffer. Equal aliquots of pellet and supernatant were loaded on SDS-PAGE, and tubulin was identified with an anti-$\beta$-tubulin primary antibody (1:1000, Sigma-Aldrich). Western blots were quantified using the WCIF Image J software, and the error bars correspond to standard deviations ($n \geq 3$).The percentage of polymerized was determined by dividing the $\beta$-tubulin in the polymerized fraction by the sum of the $\beta$-tubulin in the polymerized and soluble fractions. Given the polymerized and soluble fractions are equalized for each pair, the proportion of the polymerized to the soluble tubulin fraction is irrelevant to the amount of total protein loaded for each sample.[22]

**Cell Cycle Analysis.** A549 cells treated with tested compounds for 24 h were washed once in PBS and resuspended in 1 mL of 70% ice cold ethanol and stored at −20 °C. Fixed cells were washed twice in PBS and then treated with 1 mL of 0.1 mg/mL of RNase A solution at 37 °C for 1 h. DNA was then stained with a PBS solution containing 0.1 mg/mL propidium iodide for 30 min at room temperature in the dark. Cell cycle analysis was determined with FACSCalibur (BD Biosciences, Mountain View, CA).

**Perfused Vascular Volume Assay.** The use of animals was approved by Institutional Animal Care and Use Committee of Lanzhou University, with confirmed adherence to the ethical guide lines for the care and use of animals. Female BALB/c nu/nu mice at 6−8 weeks (Beijing HFK Bioscience, Beijing, China) were sc implanted with $1 \times 10^7$ A549 cells mixed with equal volume of Matrigel (Becton Dickinson) in 0.2 mL at one flank per mouse. When their tumors reached about 100 $mm^3$, the xenograft tumor-bearing nude mice were given a single ip injection of **21** and **1** (dissolved in a mixture of DMSO/cremophor/saline (1:4:15)) at a dose of 100 mg/kg. The control group was treated with vehicle mixture only. Sixteen hours after administration, animals were iv injected with 10 mg/kg of Hoechst 33342 (Invitrogen). After 1 min, tumors were excised and cryostat sections were processed. Images of histologic sections were captured with an inverted Zeiss Imager Z2 microscope. Quantification of H33342 Hoechst staining was done using the software WCIF ImageJ. Twenty representative fields were counted in each tumor.

## ■ ASSOCIATED CONTENT

### Supporting Information

The HPLC results and $^1H$ NMR and $^{13}C$ NMR spectra of target compounds **8**−**31**. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

CA4, combretastatin A-4; HUVECs, human umbilical vein endothelial cells; SAR, structure−activity relationship; VDAs, vascular-disrupting agents; THF, tetrahydrofuran; DMF, *N*,*N*-dimethylformamide; $IC_{50}$, half-maximum inhibitory concentration; DMSO, dimethylsulfoxide; DAPI, 4′,6-diamidino-2-phenylindole; TMS, tetramethylsilane; HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography; PBS, phosphate buffered saline; Tris, tris(hydroxymethyl)-aminomethane; EGTA, ethylene glycol tetraacetic acid; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis

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