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Synthesis and biological evaluation of new disubstituted analogues of 6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (BPR0L075), as potential antivascular agents

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ABSTRACT

6-Methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (BPR0L075) (**1**) is a potent inhibitor of tubulin polymerization which exhibits both in vitro and in vivo activities against a broad spectrum of solid tumors. This compound was designed as a heterocyclic analogue of combretastatin A4 (CA-4), a natural stilbene derivative that disrupts the tumor vasculature and causes tumor regression. In the present work, we describe the design and synthesis of several new disubstituted analogues of **1**, along with their biological evaluation as potential antivascular agents. Compound **13**, bearing a hydroxyl group at the 7-position of the indole nucleus that mimics the hydroxyl group at the 3-position of the B-ring of CA-4, was identified as a potent inhibitor of tubulin polymerization and also as a cytotoxic agent against B16 melanoma cells at sub-micromolar concentration. In addition, compound **13** displayed marked morphological activity (rounding up) at nanomolar concentrations on endothelial cells (EA.hy 926 cells), which is indicative of potential antivascular activity. Interestingly, the corresponding 7-*O*-mesylate derivative **28** (an intermediate in the synthesis of **13**), was also found active in cellular assays, although it was moderately active in the tubulin polymerization inhibition test. Finally, in order to better understand the SAR of disubstituted analogues of **1**, two other position isomers (**10** and **14**), were synthesized and evaluated for their biological activities. It was noted that the 7-hydroxysubstituted analogue **13** was more potent than the 5-hydroxysubstituted analogue **10**. In conclusion, this work has allowed the identification of biologically potent CA-4 analogues (**13** and **28**) and also contributes to a better understanding of the SAR of **1**.

1. Introduction

Microtubules are key components of the cytoskeleton and play an essential role in all eukaryotic cells in the development and maintenance of cell shape, in the transport of vesicles, mitochondria and other components throughout cells, in cell signaling, and in cell division and mitosis. Microtubules are composed of α-tubulin and β-tubulin heterodimers that form highly dynamic polymers. The central role of microtubules in the process of separating duplicated chromosomes before cell division makes them a particularly important target for anticancer drugs. Indeed, many compounds which interfere with the dynamic of microtubules are active anticancer drugs. Microtubule active drugs generally bind to one of the three main classes of sites on tubulin, that is, the colchicine domain, the vinca alkaloid site, and the paclitaxel site.[1]

Tumour vasculature is an important and highly interesting target for anticancer therapy because of the requirement of the tumor cells for a functional network of blood vessels and also because tumor vasculature is profoundly different compared to normal blood vessels.[2] Two main strategies have been developed to target tumor vasculature, the antiangiogenesis approach that prevents the formation of new vessels in tumors, and the antivascular approach that aims at disrupting the existing tumor vasculature. Compounds that selectively target the pre-existing tumor vasculature and cause damage to the endothelial cell layer are able to stop the tumor blood supply and waste removal leading to tumor necrosis. These compounds are called 'vascular disrupting agents' (VDA) and several of them are currently undergoing phase I–III clinical trials, most often in combination therapies with cytotoxic agents.[3]

In the vascular disrupting agents, combretastatin A-4 (CA-4), first isolated from the bark of the South African bush willow tree

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Combretum caffrum Kuntze (Combretaceae), emerged as lead compound which displayed strong inhibitory activity against tubulin polymerization.[4,5] The corresponding water-soluble disodium phosphate prodrug of CA-4 (CA-4P, brand name: Zybrestat™) represents the lead compound in the group of tubulin binding small molecules possessing tumor vascular disrupting activity. Developed by the Oxigene company, CA-4P recently entered phase II/III studies in the US in anaplastic thyroid cancer.[3e,6,7]

Recently, the 6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole **1** (BPR0L075), was designed as a heterocyclic analogue of CA-4 (Fig. 1). Compound **1** was shown to be a potent inhibitor of tubulin polymerization and binds tubulin at the colchicine site.[8,9] It exhibits a strong cytotoxic activity (in the nanomolar range) against a panel of human cancer cell lines, including multidrug-resistant cells.[10] This compound was also found to exert significant antitumor activity in several human xenografted tumors and has recently been selected for further preclinical development as an antimitotic anticancer drug.[10,11]

We recently reported the in vitro activity of **1** on the morphology of endothelial cells (rounding up at nanomolar concentrations), suggesting its potential in vivo antivascular activity.[9] Our previous studies also disclosed a good correlation between the rounding up effect of **1** on endothelial cells and its inhibition of tubulin polymerization.[9]

Compound **1** and CA-4 share several common structural features. Both possess two aryl rings linked by a two-carbon chain, including an identical 3,4,5-trimethoxyphenyl ring, and a second aromatic ring bearing a methoxy group (Fig. 1). Structure–activity relationships (SAR) in the 3-aroylindole series have been extensively studied, particularly modifications at the N1-position and at the carbonyl bridge.[8,9,12] An unsubstituted N1-position and a sp^2 center (carbonyl or thiocarbonyl[9]) are structural requirements for potent cytotoxic and tubulin polymerization inhibitory activities,[8,9] but some modifications are tolerated in these positions without loss of potency (for example, replacement of the carbonyl bridge by a methylene[8,9] or a sulfur[12,13]; substitution at the N1-position by a phenyloxycarbonyl[9,12] or a benzoyl[12] group). Specific examples include compounds **2**–**6** (Fig. 2).

Introduction of a methyl group at the C2-position, exemplified by compound **7**, led to a substantial improvement in cytotoxic and antitubulin activities.[8] In addition, in 2001, the Pinney[14] and the Flynn[15] groups reported concomitantly the synthesis of 2-phenylindole **8** that displayed strong tubulin polymerization inhibitory activities. However, this 3-aroylindole derivative was designed (and is still considered) as a *cis*-restricted analogue of CA-4, with an ethene bridge as part of a heterocycle (Fig. 2). Concerning the substitution of the benzene ring of the indole nucleus, monosubstituted indoles, mainly in positions 5 and 6 were investigated.[8,9,12] These SAR studies underlined the critical role of the methoxy group linked at the position 6 of the indole nucleus. Furthermore, it mimics the *p*-methoxy group of CA-4, which is also known to play a crucial role in the activities of **1**, particularly on the ability to inhibit tubulin polymerization.[8,9,12] SAR studies carried out by Liou and coworkers also emphasize that the 6-methoxy group can be replaced by another electron donating group (i.e., *p*-dimethylamino and methyl groups) without unfavorable effects on biological activities.[12] In contrast, SAR of 3-aroylindoles containing disubstituted indoles have not been studied in detail, probably due to the difficulties encountered in the synthesis of the corresponding indole precursors. The synthesis and biological evaluation of a short series of 5,6-disubstituted-3-aroylindoles **9**–**11** (Fig. 3) were reported by J.-P. Liou et al.[8,12] The three compounds possess a methoxy substituent in the 6-position and **10**, particularly, an additional hydroxyl group at the C5 position which could mimic the 3-hydroxyl group on ring B of CA-4. Surprisingly, these analogues were found to exhibit weak antimitotic and cytotoxic activities.[10,14] Moreover, Duan et al. showed that substitution at the 7-position of indole with a bulky acetylene group (compound **12**, Fig. 3) led to a dramatic reduction in potency.[16]

Taking into account the above-mentioned SARs in the 3-aroylindole series, we decided to explore the possibility of introducing a hydroxyl group at the 7-position of the indole nucleus and to evaluate the influence of this modification on cytotoxic and antitubulin

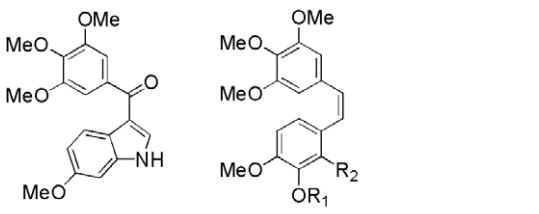


Figure 1. BPR0L075, CA-4, CA-4P, and CA-1.

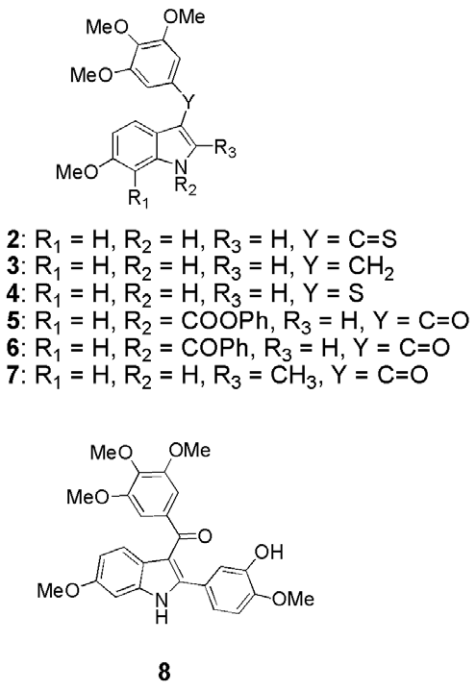


Figure 2. Selected analogues of BPR0L075.

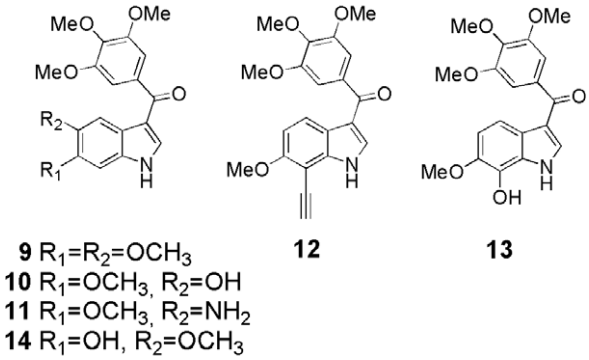


Figure 3. Structures of 5,6- and 6,7-disubstituted 3-aroylindoles.

activities. Thus, we now report our studies on the synthesis and the biological evaluation of the 7-hydroxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (**13**).

Compound **13** was found to inhibit tubulin polymerization at low concentrations. In order to compare properly our results with those already reported for 5,6-disubstituted analogues of **1**, compound **10**, previously synthesized and reported by J.-P. Liou et al.[12] was resynthesized, using a new and efficient short-step method for the preparation of the indole intermediate precursor of **10**. Moreover, the new isomeric precursor 6-hydroxy-5-methoxydisubstituted indole was concomitantly obtained in the same step. Both precursors were further coupled with the 3,4,5-trimethoxybenzoyl fragment and submitted to partial or total deprotection to afford five new 5,6-disubstituted 3-aroylindoles, particularly the new analogue **14** and the desired compound **13** (Fig. 3).

Finally, seven new disubstituted 3-aroylindoles were synthesized (five in the 5,6-disubstituted series and two in the 6,7-series) and evaluated for their antiproliferative activities against murine B16 melanoma cells, their capacity to inhibit tubulin polymerization, and their ability to induce morphological changes on endothelial cells (EA.hy 926).[17] Our results provide new interesting and important SAR in the series of disubstituted 3-aroylindoles, and have allowed the identification of new biologically potent CA-4 analogues.

2. Chemistry

2.1. Synthesis of 5,6-disubstituted 3-aroylindoles

A previous publication of the synthesis of the 3-aroylindole **10** involved an acylation of 5-benzyloxy-6-methoxy-1*H*-indole with 3,4,5-trimethoxybenzoylchloride in the presence of EtMgBr, $ZnCl_2$, and $AlCl_3$ and subsequent removal of the benzyl protecting group under catalytic hydrogenation conditions.[12] The aforementioned indole could be prepared from isovanillin in four steps, including a reductive cyclization of the corresponding 2-nitro-β-nitrostyrene, with a moderate overall yield of 28%. Moreover, the 6-benzyloxy-5-methoxy-1*H*-indole isomer was similarly obtained from vanillin in an overall yield of 27%.[18] We have developed a new efficient and simple method for the direct synthesis of the two 5,6-disubstituted indoles, as convenient precursors of the 3-aroylindoles **10** and **14**.

The starting material of this synthesis is the non-commercial 5,6-dimethoxy-*N*-benzenesulfonyl-1*H*-indole **18**. This compound was prepared on a gram-scale in a 73% overall yield from the aniline **15**, according to a modified Bischler strategy previously developed in our laboratory (Scheme 1).[9]

3,4-Dimethoxyaniline **15** was quantitatively transformed into its *N*-phenylsulfonyl derivative **16** by treatment with phenylsulfonyl chloride, then alkylated by bromoacetaldehyde diacetal to give **17** (Scheme 1). Finally, the indole cyclization was performed in the presence of boron trifluoride etherate and afforded **18** in 80% yield. The mono-*O*-demethylation of **18** was achieved by treatment of this compound with one equivalent of BBr_3 in CH_2Cl_2 at −15 °C. The reaction did not proceed with significant regioselectivity and led to a mixture of isomeric indoles **19a** and **19b** (a ratio of 55:45 was estimated by ^1H NMR) which proved difficult to resolve. Each of the two isomers was unambiguously identified on the basis of HMBC and NOESY experiments.[19] Particularly, the NOESY spectrum of **19a** showed a strong correlation signal between the 5-methoxy protons at δ 3.88 ppm and the H-4 at δ 6.91 ppm, whereas for **19b**, a NOESY coupling was observed between the 6-methoxy signal at δ 3.99 ppm and H-7 at δ 7.54 ppm.

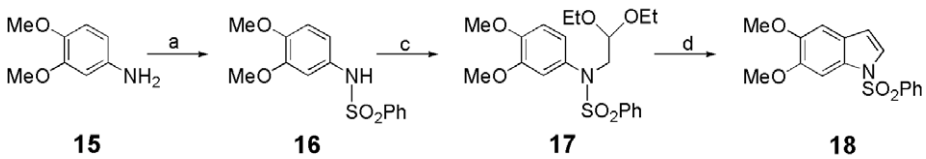
In order to facilitate the chromatographic separation of both indoles, the latter mixture was directly treated with an excess of methanesulfonylchloride in presence of Et_3N and the corresponding *O*-mesylates **20a** and **20b** were thus easily obtained pure in 47% and 38% isolated yield, respectively, from the indole **18**.

Both indole mesylates were further selectively acylated at the 3-position under Friedel-Crafts conditions, using the 3,4,5-trimethoxybenzoylchloride as electrophilic reactant (Scheme 2). The corresponding coupling products were obtained in moderate to good yields, depending on the substitution pattern of the starting indole. Thus, the coupling step proceeded in 76% yield for the indole **20a**, which bears a methoxy group at the 5-position, and in only 35% yield for the indole with a methoxy at the 6-position (**20b**). The different protecting groups of the 3-aroylindoles **21a**,**b** were cleaved in two successive steps. First, the *N*-phenylsulfonyl group was selectively removed by treatment with LiOH in methanol at room temperature, to afford the free NH 3-aroylindoles **22a** or **22b** in 73% and 76% yield, respectively. Thereafter, various conditions of deprotection of the *O*-mesylate esters were tested. Under classical basic conditions (KOH or K_2CO_3) only degradation products were formed. Finally, this step was cleanly achieved under transesterification conditions using a large excess of magnesium methoxide[20] in refluxing methanol, to give the expected deprotected compounds **14** and **10** in 67% and 65% yield, respectively.

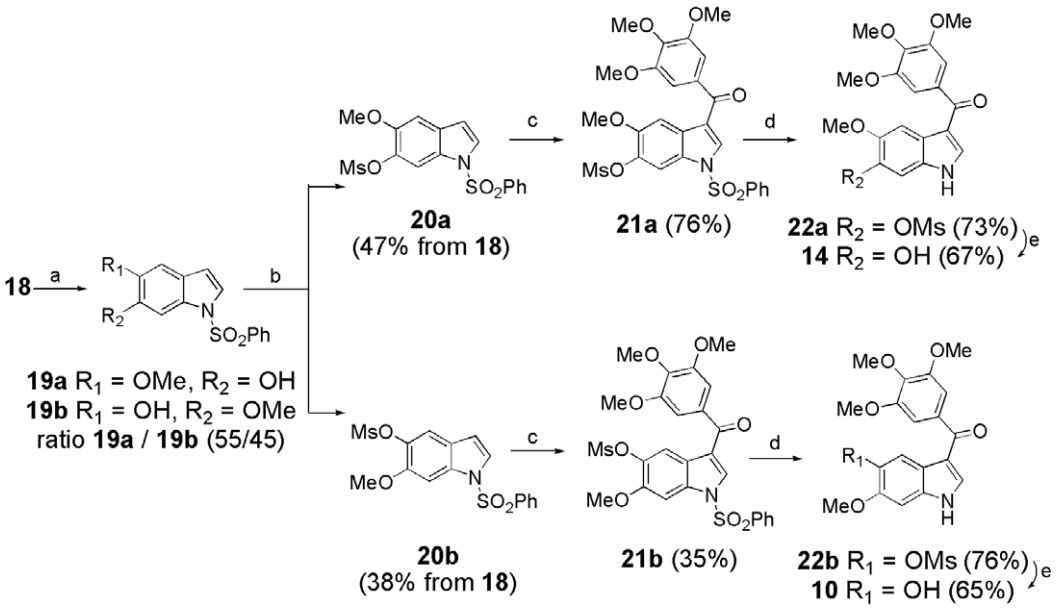
2.2. Synthesis of 6,7-disubstituted 3-aroylindoles

Synthesis of the 3-aroylindole **13** involves preparation of the 7-hydroxy-6-methoxy-1*H*-indole with suitable protecting groups. Indole **27** was first synthesized according to a modified Fukuyama et al.[21] procedure. Particularly, *O*-mesylate ester instead of *O*-benzyl ether was used to protect the phenol function, in order to allow some comparison of the biological activities between this series and the 5,6-disubstituted series (Scheme 3).

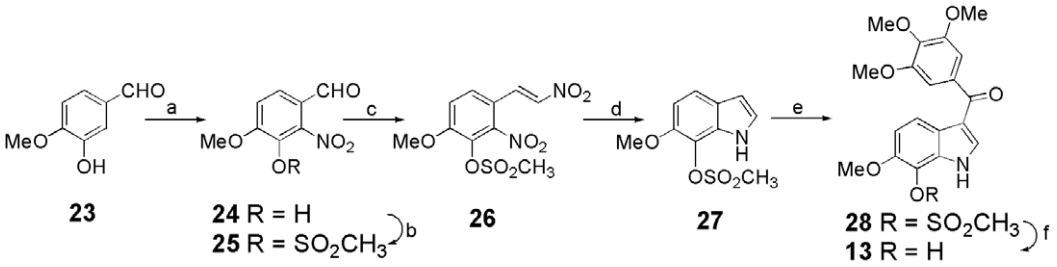
Thus, nitration of isovanillin **23** with fuming nitric acid[22] in acetone at −40 °C afforded the expected 2-nitro isomer **24**[21,22] as the minor product (18% yield), while the 6-nitro isomer was identified as the major product (31% yield). The use of NO_2BF_4 as nitrating agent[21] did not improve the yield, because both reactions suffered from difficulties in purifying the reaction products. Mesylation of **24** with methanesulfonylchloride in the presence of triethylamine in CH_2Cl_2 at 0 °C gave the 3-*O*-mesylate ester **25** in 83% yield. This compound was treated under modified Henry's conditions[21] (CH_3NO_2, LHMDS in THF) to afford the 2-nitro-β-nitrostyrene **26** in 74% yield. The formation of the indolic ring was achieved in 54% yield, via a reductive cyclization under catalytic hydrogenation conditions. Further acylation of the indole **27** with 3,4,5-trimethoxybenzoylchloride in the presence of EtMgBr, $ZnCl_2$, and



Scheme 1. Synthesis of *N*-phenylsulfonyl-5,6-dimethoxy-1*H*-indole (**18**). Reagents and conditions: (a) $PhSO_2Cl$, pyridine, CH_2Cl_2, rt, 100%; (b) $BrCH_2CH(OEt)_2$, NaH, anhyd DMF, reflux, 94%; (c) $BF_3{\cdot}Et_2O$, CH_2Cl_2, rt, 80%.



Scheme 2. Synthesis of 5,6-disubstituted 3-aroylindoles (**10** and **14**). Reagents and conditions: (a) BBr_3, CH_2Cl_2, −15 °C; (b) MsCl, Et_3N, rt, 15 min; (c) $(OCH_3)_3PhCOCl$, $AlCl_3$, CH_2Cl_2, rt, 2–6 h; (d) LiOH (2–3 equiv), MeOH, rt, 15 min–2 h; (e) $Mg(OCH_3)_2$ (1.4 M), MeOH, reflux, 12–24 h.



Scheme 3. Synthesis of 6,7-disubstituted 3-aroylindole (**13**). Reagents and conditions: (a) fuming HNO_3, acetone, −40 °C, 1 h, 18%; (b) CH_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C, 30 min, 83%; (c) i—CH_3NO_2, LHMDS in toluene, THF, −78 °C, 2.5 h; ii—AcONa, Ac_2O, 60 °C, 3.5 h, 74%; (d) H_2, 10% Pd/C, DMF/MeOH (1:3), rt, 16.5 h, 54%; (e) EtMgBr, $ZnCl_2$, $(OCH_3)_3PhCOCl$, $AlCl_3$, CH_2Cl_2, rt, 7 h, 19%; (f) LDA, THF, −78 to 0 °C, 30 min, 100%.

$AlCl_3$[23] afforded the 3-aroylindole **28** in low yield (19%). Finally, the mesylate protecting group was cleanly removed using LDA at −78 °C in THF to afford **13** in quantitative yield.

3. Biological evaluation

The biological activities of the compounds are summarized in Table 1 and were evaluated using three complementary assays, that is, the inhibition of tubulin polymerization, cytotoxicity against murine B16 melanoma cells, and the induction of a rapid morphological change of EA.hy 926 endothelial cells (rounding up), which is considered a useful model of potential in vivo antivascular effects.[24] BPR0L075 (**1**) was used as the reference standard in the present study, thus allowing a direct comparison with its disubstituted analogues.

Within the series of newly synthesized compounds, only compounds **13** and **28** presented significant biological activities. Compound **13** was the best inhibitor of tubulin polymerization reported to date within the series of disubstituted 3-aroylindoles and was nearly as potent as the reference **1** with an ITP value of 1.3.[25] In terms of cytotoxicity against B16 melanoma cells, the IC_{50} value was about 18-fold higher than that of the reference compound **1**, but was still in the submicromolar range (IC_{50} = 0.24 μM). Both activities were well correlated with a potent morphological effect on EA.hy 926 endothelial cells, and the lowest concentration that induced a rounding-up of the cells was 0.047 μM for a 2 h exposure time. For the mesylic ester **28**, a slight decrease in tubulin inhibition was observed (ITP = 2.7), in comparison with that of **13**. However, **28** was slightly more cytotoxic than **13**, with an IC_{50} value of 0.17 μM. The most surprising and interesting result was the observation that **28** could cause the rounding up of endothelial cells in the nanomolar concentration range (0.013 μM).

Figure 4 depicts representative changes in the morphology of endothelial cells (EA.hy 926) after a brief 2 h exposure time to reference compounds and to newly synthesized combretastatin analogues **13** and **28**. Control cells exposed to the solvent alone (1% DMSO) did not cause a rounding up of the endothelial cells. As expected, for the reference compounds CA-4 and compound **1** (BPR0L075), a rounding up of the cells was clearly observed at concentrations as low as 0.008 and 0.007 μM, respectively. Compounds **13** and **28** presented clear rounding up activity (Fig. 4).

Other compounds **14**, **21a,b**, and **22a,b** were either weak inhibitors of tubulin polymerization (**14** and **21a,b**) or inactive (**22a,b**). However, compounds **14** and **21a,b** exhibited inhibitory effects on the proliferation of B16 melanoma cells, but these effects were observed only in the micromolar range (5.07, 3.14, and 3.29 μM, respectively). Furthermore, a morphological rounding up effect was noted for **21a** at a relatively low concentration (1.30 μM), as depicted in Figure 4. It is interesting to note that the good morpho-

Table 1
Inhibition of tubulin polymerization, cytotoxicity on B16 melanoma cells and morphological effects on EA.hy 926 endothelial cells of disubstituted 3-aroylindoles

Compound	ITP[a,b] (IC_{50} compound IC_{50} colchicine)	Cytotoxicity IC_{50}[c] (μM)	Morphological activity on endothelial cells[d] (μM)
BPR0L075 (**1**)	0.4[e]	0.013 ± 0.004	0.007
Colchicine	1.0[f]	0.031 ± 0.003	0.003
CA-4	0.7[g]	0.003 ± 0.001	0.008
10[12]	31[e]	12.18 ± 5.06	17.49
13	1.3	0.24 ± 0.14	0.047
14	12	5.07 ± 0.91	8.74
21a	18	3.14 ± 2.17	1.30
21b	15	3.29 ± 1.15	10.86
22a	Inactive[b]	32.42 ± 2.24	28.71
22b	Inactive[b]	13.67 ± 1.40	14.35
28	2.7	0.17 ± 0.05	0.013

[a] Inhibition of tubulin polymerization (ITP) is the ratio of the IC_{50} value of a synthesized compound over the IC_{50} value of colchicine, used as the reference.
[b] Compounds were designated as 'inactive' when the measured ratio was greater than 40.
[c] IC_{50} ± SEM of 3–7 independent triplicate experiments (B16 melanoma cells line).
[d] Morphological activity (rounding up) on modified HUVEC cells (EA.hy 926) is expressed as the lowest concentration (μM) at which cell rounding up was observed after a 2 h incubation period with the test compound. Experiments done in triplicate.
[e] Experimental data. Values of IC_{50} from Ref. 12: 2.8 ± 0.3 μM for BPR0L075 and 30 ± 4 μM for compound **10**.
[f] IC_{50} (colchicine) varies from 1.8 to 6.7 μM for the different experiments according to the tubulin concentration.
[g] Value of IC_{50} from Ref. 32: 2.2 ± 0.2 μM.

logical activity of compound **21a**, is not correlated with a strong antitubulin activity.

Finally, the 5-hydroxy-6-methoxy-1*H*-indole **10** showed very similar biological activities to those previously described in the literature.[12] This molecule also exhibited low micromolar cytotoxicity against B16 melanoma cells (IC_{50} = 12 μM vs 0.013 μM for **1**), as well as no significant effects on the morphology of endothelial cells.

This study provides new SAR information, particularly regarding the tolerance for substitution at the 7-position. As mentioned in Section 1, the previously reported analogues of BPR0L075, substituted at either position 5 or 7 of the indole nucleus, were found to loose the antitubulin and cytotoxic activities of the parent compound. Particularly, substitution at the 7-position with a bulky acetylene group was not tolerated. Results obtained with compound **13** indicate that position 7 of **1** can accommodate a hydroxyl without a dramatic loss in biological activities. However, it should be mentioned that compound **13** was slightly less potent than the parent compound **1**. Apart from quite obvious structural differences between both the 3-aroylindole and the stilbene skeletons, we hypothesize that the indolic nitrogen atom may also play a role in the SAR. Indeed, we found that **13** was sensitive to air oxidation and quickly turned brown on standing without precautions. This finding may suggests that a *O*-quinoneimine type derivative was formed upon oxidation of the 2-aminophenol structure. Similar observations were noted in the case of combretastatin A1 (CA-1), a 2-hydroxylated analogue of CA-4 (Fig. 1). Although CA-1 was approximately 200- to 300-fold less cytotoxic than CA-4,[4b] this compound (precisely its disodium phosphate diester prodrug, designated as OXi4503), demonstrated a greater in vivo antitumor activity than CA-4P[26] and is currently undergoing phase II clinical trials.[7]

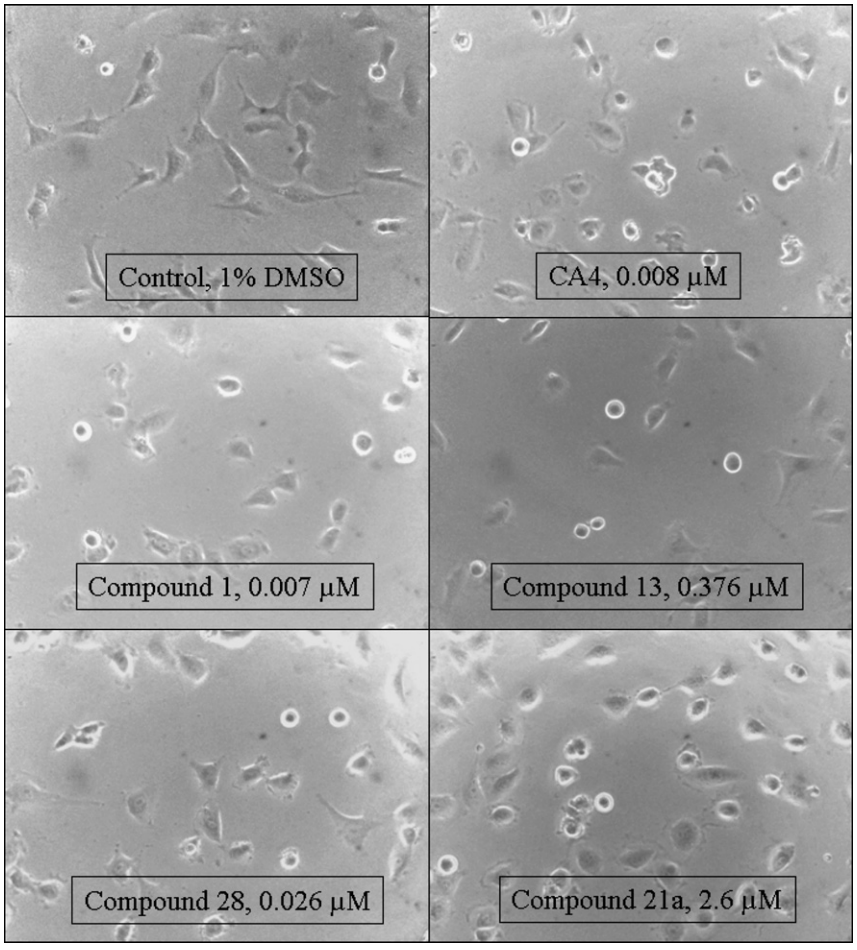


Figure 4. Morphological effects of combretastatin A4 (CA-4) analogues. Endothelial cells (EAhy 926 cells) were exposed to the test compounds and incubated for 2 h at the indicated concentrations. Representative photographs were taken at a magnification of 360×.

The relationship between the chemical structure and the increased morphological activity of the 7-*O*-mesylate **28** deserves further investigation. Indeed, **28** displayed a noteworthy effect on endothelial cell shape, at a similar concentration to that of the reference compounds CA-4 and **1**. Moreover, among all the new disubstituted 3-aroylindoles synthesized in this study, **28** was the most cytotoxic against B16 melanoma cells. However, these effects were not well correlated with inhibition of tubulin polymerization, that was somewhat weaker compared to **1** and **13**. Contrary to **13**, compound **28** could be isolated as a stable crystalline form and it would be therefore interesting to determine whether **28** could act as a prodrug of **13**.

Introduction of a hydroxyl or a mesylic ester group at the 5-position of **1** (compounds **10** and **22b**, respectively) is deleterious for both antitubulin and cytotoxic activities, which confirms previous observations.[8,12] Shifting the methoxy group from position 6 to 5 of the indole ring (compounds **14** and **22a**), also affect biological activities, but, surprisingly, **14** was about twofold more active in all the three tests than its isomer **10**.

Finally, for analogues containing a *N*-phenylsulfonyl protecting group (i.e., **21a**,**b**), the SARs remains somewhat unclear, particularly since the biological activities were not obviously correlated (e.g., poor antitubulin activity and good morphological effect for **21a** or cytotoxicity not correlated with antitubulin activity for both compounds). However, these findings suggest that other mechanisms of action are involved, particularly with regard to the morphological activity on endothelial cells (e.g., actin polymerization, signaling pathways, or interaction with microtubule associated proteins).

4. Conclusion

In the present study, 7 new disubstituted 3-aroylindoles were synthesized and evaluated in terms of inhibition of tubulin polymerization, cytotoxicity against the B16 melanoma cancer cell line, and morphological effects on endothelial cells. This work contributes to a better understanding of the SARs of 6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (BPR0L075, **1**). Particularly, we demonstrated that, contrary to the 5-position, the 7-position of the indole nucleus can accommodate a hydroxyl group without dramatic loss of cytotoxic and inhibition of polymerization activities. The 7-hydroxy-substituted analogue of **1** (compound **13**) was shown to be more potent than the 5-hydroxy-substituted analogue **10**. Furthermore, the corresponding 6-*O*-mesylate **28** displayed a strong activity in the endothelial cell morphology assay. Although **28** was not a strong inhibitor of tubulin polymerization, morphological effect was observed in the low nanomolar range, similar to those of BPR0L075 and CA-4. Compound **28** therefore deserves further in vivo investigations to fully assess its potential antitumor antivascular activity and also to elucidate its molecular mechanism of action.

5. Experimental

Melting points were determined on a LEICA VM microscope equipped with a heating stage and are uncorrected. Infrared spectra were obtained with a Nicolet 510 FT-IR apparatus. NMR spectra were recorded at 300 or 400 MHz (^{1}H NMR) and at 75 or 100 MHz (^{13}C NMR) with a AC 300 and a Avance 400 BRUKER spectrometers; chemical shifts are given in parts per million (ppm, δ) relative to solvent peaks as internal standards (δ: $CDCl_3$: 7.27 ppm (^{1}H), 77 ppm. (^{13}C); DMSO-d_6: 2.50 ppm (^{1}H), 40.6 ppm (^{13}C); acetone-d_6: 2.05 ppm (^{1}H), 29.8 and 206.0 ppm (^{13}C)); ^{1}H and ^{13}C signals were unambiguously attributed by 2D NMR experiments; coupling constants are given in Hertz (Hz, *J*). Mass spectra (MS) were measured with a Nermag R 10-10C mass spectrometer (CI/NH_3) or with a ZQ2000 Waters mass spectrometer (ESI); HRMS spectra were performed at the Laboratoire de Spectrométrie de Masse (I.C.S.N./C.N.R.S., Gif sur Yvette, France). Elemental analyses were performed at the Laboratoire de microanalyses (Université Paris 7 - Denis Diderot, Paris, France). Flash column chromatography was done using silica gel (SDS 60 ACC 35–70 μM). The reactions were monitored by thin-layer chromatography (TLC) using Merck Kieselgel 60 F_{254} silica gel; zones were detected visually under ultraviolet irradiation (254 and 366 nm) and read after spraying with sulfuric vanillin followed by heating. All solvents were dried according to standard procedures. All reagents were used as purchased without further treatment unless otherwise stated.

5.1. *N*-Benzenesulfonamide-3,4-dimethoxyphenylaniline (16)

To a solution of 4-aminoveratrole **15** (8.14 g, 53.2 mmol) in a 2/8 mixture of pyridine and anhydrous CH_2Cl_2 (120 mL), benzenesulfonyl chloride (8.18 mL, 63.8 mmol) was slowly added at 0 °C. After 5 min of stirring at room temperature, the reaction mixture was evaporated to dryness in vacuo and the residue was taken up with CH_2Cl_2 (50 mL). The organic solution was washed with water, 10% aqueous HCl, water and brine, dried over $MgSO_4$, and concentrated in vacuo to give 15.6 g of analytically pure **16** as transparent crystals (quantitative yield). Recrystallization from a 9:1 mixture of CH_2Cl_2 and MeOH gave prismatic transparent crystals. Mp 127–130 °C. ^{1}H NMR ($CDCl_3$) δ 3.73 (s, 3H, $^3OCH_3$); 3.79 (s, 3H, $^4OCH_3$); 6.56 (dd, J = 8.5 Hz, J = 2.4 Hz, 1H, H_6); 6.66 (s, J = 8.5 Hz, 1H, H_5); 6.68 (s, J = 2.4 Hz, 1H, H_2); 7.16 (br s, D_2O exch., 1H, NH); 7.41 (m, 2H, $H_{3'-5'}$); 7.52 (m, 1H, $H_{4'}$); 7.74 (m, 2H, $H_{2'-6'}$); ^{13}C NMR ($CDCl_3$) δ 55.9 ($^3OCH_3$); 56.0 ($^4OCH_3$); 108.0 (C_2); 111.3 (C_5); 115.8 (C_6); 127.3 ($C_{2'-6'}$); 128.9 ($C_{3'-5'}$); 129.2 (C_1); 132.9 ($C_{4'}$); 138.8 ($C_{1'}$); 147.4 (C_4); 149.2 (C_3); MS (ZQ2000/ES+) m/z 316 $[M+Na]^+$; HRMS (ES+) ($C_{14}H_{16}NO_4S$, $[M+H]^+$): found: 294.0802; calcd: 294.0800.

5.2. *N*-(2,2-Diethoxyethyl)-*N*-(3,4-dimethoxyphenyl) benzenesulfonamide (17)

To a solution of **16** (1.1 g, 3.75 mmol) in anhydrous DMF (10 mL), NaH (216 mg, 4.50 mmol) was added at room temperature. The suspension was stirred for a few minutes, and then, bromoacetaldehyde diethyl acetal (846 μL, 5.60 mmol) was added and the mixture was warmed at 110 °C. After 24 h, another 1.87 mmol of NaH (90 mg) and bromoacetaldehyde diethyl acetal (282 μL) were added and heating was continued for an additional 24 h. The reaction mixture was quenched with MeOH and DMF was removed under reduced pressure. The residue was taken up with CH_2Cl_2 and the organic solution was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to yield 1.4 g of analytically pure **17** (91%) as an amber oil. ^{1}H NMR ($CDCl_3$) δ 1.11–1.14 (m, 6H, 2× CH_3); 3.48 (m, 2H, CH_2CH_3); 3.61 (d, J = 5.5 Hz, 2H, –CH_2–); 3.62 (m, 2H, CH_2CH_3); 3.69 (s, 3H, $^3OCH_3$); 3.84 (s, 3H, $^4OCH_3$); 4.60 (t, J = 5.5 Hz, 1H, CH); 6.51 (d, J = 2.0 Hz, 1H, H_2); 6.55 (dd, J = 8.5 Hz, J = 2.0 Hz, 1H, H_6); 6.72 (d, J = 8.5 Hz, 1H, H_5); 7.43 (m, 2H, $H_{3'-5'}$); 7.55 (m, 1H, $H_{4'}$); 7.60 (m, 2H, $H_{2'-6'}$); ^{13}C NMR ($CDCl_3$) δ 15.2 (2× CH_3); 53.5 (–CH_2–); 55.8 ($^4OCH_3$); 55.9 ($^3OCH_3$); 62.1 (2× CH_2CH_3); 100.8 (CH); 110.7 (C_5); 112.5 (C_2); 121.2 (C_6); 127.8 ($C_{2'-6'}$); 128.7 ($C_{3'-5'}$); 132.6 ($C_{4'}$); 132.8 (C_1); 138.3 ($C_{1'}$); 148.7 (C_3+C_4); MS (ZQ2000/ES+) m/z 448 $[M+K]^+$, 432 $[M+Na]^+$. Anal. ($C_{20}H_{27}NO_6S$). Found: C, 58.90%; H, 6.93%; N, 3.53%; calcd: C, 58.66%; H, 6.65%; N, 3.42%.

5.3. *N*-Phenylsulfonyl-5,6-dimethoxy-1*H*-indole (18)

To a magnetically stirred solution of **17** (1.14 g, 2.78 mmol) in anhydrous CH_2Cl_2 (10 mL), BF_3–Et_20 (526 μL, 4.17 mmol) was

added dropwise under argon at 0 °C. The reaction mixture was quenched after 15 min with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$ and concentrated in vacuo. Flash chromatography using cyclohexane/EtOAc (7:3) gave 705 mg of **18** (80%) as a white solid. Recrystallization from a 9:1 mixture of $CHCl_3$ and *n*-hexane gave transparent crystals. Mp 130–133 °C. ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H, $^6OCH_3$); 3.96 (s, 3H, $^5OCH_3$); 6.55 (d, J = 3.6 Hz, 1H, H_3); 6.94 (s, 1H, H_4); 7.39–7.43 (m, 3H, $H_2 + H_{3'\text{-}5'}$); 7.51 (m, 1H, $H_{4'}$); 7.56 (s, 1H, H_7); 7.82 (m, 2H, $H_{2'\text{-}6'}$); ^{13}C NMR ($CDCl_3$) δ 56.1 ($^6OCH_3$); 56.3 ($^5OCH_3$); 97.3 (C_7); 102.7 (C_4); 109.6 (C_3); 123.6 (C_{3a}); 125.0 (C_2); 126.5 ($C_{2'\text{-}6'}$); 129.2 ($C_{3'\text{-}5'}$); 129.3 (C_{7a}); 133.7 ($C_{4'}$); 138.2 ($C_{1'}$); 147.2 (C_6); 148.2 (C_5); MS (CI/NH_3) m/z 318 $[M+H]^+$, 178. Anal. ($C_{16}H_{15}NO_4S$, 0.6$H_2$0). Found: C, 58.41%; H, 4.90%; N, 4.32%; calcd: C, 58.55%; H, 4. 99%; N, 4.27%.

5.4. *N*-Phenylsulfonyl-6-hydroxy-5-methoxy-1*H*-indole (19a) and *N*-phenylsulfonyl-5-hydroxy-6-methoxy-1*H*-indole (19b)

To a solution of **18** (88 mg, 0.28 mmol) in anhydrous CH_2Cl_2 (5 mL), a molar solution of BBr_3 in CH_2Cl_2 (332 μL, 0.33 mmol) was added dropwise at −15 °C. The reaction mixture was immediately quenched by addition of chilled MeOH (1 mL) and then, a saturated solution of aqueous $NaHCO_3$ was added. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, and concentrated in vacuo to afford **19a** and **19b** in a hardly separable mixture (79 mg, 94%). A ratio of 55:45 was estimated by ^{1}H NMR. Pure analytical samples of each isomer were obtained by a slow chromatography of the mixture on 20–45 μm silica gel (eluent: cyclohexane/EtOAc (8:2)).

5.5. *N*-Phenylsulfonyl-6-hydroxy-5-methoxy-1*H*-indole (19a)

Mp 154–155 °C (CH_2Cl_2/*n*-hexane (9:1), colorless crystals); ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3H, OCH_3); 5.83 (br s, D_2O exch., 1H, OH); 6.54 (d, J = 3.5 Hz, 1H, H_3); 6.91 (s, 1H, H_4); 7.39–7.44 (m, 3H, $H_{3'\text{-}5'} + H_2$); 7.52 (m, 1H, $H_{4'}$); 7.60 (s, 1H, H_7); 7.85 (m, 2H, $H_{2'\text{-}6'}$); ^{13}C NMR ($CDCl_3$) δ 56.2 (OCH_3); 99.8 (C_7); 102.0 (C_4); 109.2 (C_3); 123.3 (C_{3a}); 124.9 (C_2); 126.7 ($C_{2'\text{-}6'}$); 129.2 ($C_{7a} + C_{3'\text{-}5'}$); 133.6 ($C_{4'}$); 138.3 ($C_{1'}$); 144.5 ($C_6$); 144.7 ($C_5$); MS (ZQ2000/ES+) m/z 342 $[M+K]^+$, 326 $[M+Na]^+$; HRMS (ES+) ($C_{15}H_{14}NO_4S$, $[M+H]^+$): found: 304.0640; calcd: 304.0644.

5.6. *N*-Phenylsulfonyl-5-hydroxy-6-methoxy-1*H*-indole (19b)

Colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.99 (s, 3H, OCH_3); 5.60 (br s, D_2O exch., 1H, OH); 6.53 (d, J = 3.6 Hz, 1H, H_3); 7.01 (s, 1H, H_4); 7.41 (d, J = 3.6 Hz, 1H, H_2); 7.43 (m, 2H, $H_{3'\text{-}5'}$); 7.54 (m, 2H, $H_{4'} + H_7$); 7.82 (m, 2H, $H_{2'\text{-}6'}$); ^{13}C NMR ($CDCl_3$) δ 56.4 (OCH_3); 96.6 (C_7); 105.3 (C_4); 109.6 (C_3); 124.3 (C_{3a}); 125.3 (C_2); 126.5 ($C_{2'\text{-}6'}$); 129.2 ($C_{3'\text{-}5'} + C_{7a}$); 133.7 ($C_{4'}$); 138.4 ($C_{1'}$); 143.4 ($C_5$); 145.6 ($C_6$); MS (ZQ2000/ES+) m/z 326 $[M+Na]^+$.

5.7. *N*-Phenylsulfonyl-6-methanesulfonyloxy-5-methoxy-1*H*-indole (20a) and *N*-phenylsulfonyl-5-methanesulfonyloxy-6-methoxy-1*H*-indole (20b)

To a solution of the mixture of **19a** and **19b** (65 mg, 0.99 mmol) in CH_2Cl_2 (5 mL), Et_3N (280 μL, 2.01 mmol), and methanesulfonyl chloride (160 μL, 2.03 mmol) were successively added at room temperature. The reaction mixture was quenched after 15 min by addition of few drops of aq 10% HCl, and then, was evaporated in vacuo and purified by chromatography on 20–45 μM silica gel, using cyclohexane/EtOAc (6.5:3.5) in order to obtain 168 mg of **20b** (38% from **18**) and 165 mg of **20a** (47% from **18**). Both compounds were recrystallized as colorless crystals from a 9:1 mixture of CH_2Cl_2 and MeOH.

5.8. *N*-Phenylsulfonyl-6-methanesulfonyloxy-5-methoxy-1*H*-indole (20a)

Mp 174–177 °C; IR (NaCl film) ν' (cm^{-1}) 3385, 1474, 1423, 1365, 1221, 1182, 1139, 1108, 1085, 1019, 964, 871, 785, 723; ^{1}H NMR ($CDCl_3$) δ 3.19 (s, 3H, CH_3); 3.88 (s, 3H, $^5OCH_3$); 6.60 (d, J = 3.4 Hz, 1H, H_3); 7.07 (s, 1H, H_4); 7.45 (m, 2H, $H_{3'\text{-}5'}$); 7.50–7.58 (m, 3H, $H_2 + H_{4'}$); 7.90 (m, 2H, $H_{2'\text{-}6'}$); 8.01 (s, 1H, H_7); ^{13}C NMR ($CDCl_3$) δ 38.2 (CH_3); 56.3 ($^5OCH_3$); 104.1 (C_4); 109.4 (C_3); 110.3 (C_7); 126.8 ($C_{2'\text{-}6'}$); (C_2); 148.7 (C_5); MS (ZQ2000/ES+) m/z 404 $[M+Na]^+$; HRMS (ES+) ($C_{16}H_{16}NO_6S_2$, $[M+H]^+$): found: 382.0421; calcd: 382.0419.

5.9. *N*-Phenylsulfonyl-5-methanesulfonyloxy-6-methoxy-1*H*-indole (20b)

Mp 143–145 °C; IR (NaCl film) ν' (cm^{-1}) 3144, 3120, 2941, 2836, 1618, 1583, 1529, 1482, 1447, 1365, 1322, 1217, 1178, 1128, 1085, 1015, 972, 867, 816, 754, 727, 684; ^{1}H NMR ($CDCl_3$) δ 3.17 (s, 3H, CH_3); 3.97 (s, 3H, $^6OCH_3$); 6.60 (d, J = 3.0 Hz, 1H, H_3); 7.45–7.51 (m, 4H, $H_4 + H_{3'\text{-}5'} + H_2$); 7.58 (m, 1H, $H_{4'}$); 7.64 (s, 1H, H_7); 7.86 (m, 2H, $H_{2'\text{-}6'}$); ^{13}C NMR ($CDCl_3$) δ 38.0 (CH_3); 56.3 ($^6OCH_3$); 97.8 (C_7); 109.0 (C_3); 116.6 (C_4); 123.5 (C_{3a}); 126.1 (C_2); 126.5 ($C_{2'\text{-}6'}$); 129.3 ($C_{3'\text{-}5'}$); 133.5 (C_{7a}); 134.1 ($C_{4'}$); 135.8 (C_5); 137.8 ($C_{1'}$); 149.5 (C_6); MS (ZQ2000/ES+) m/z 404 $[M+Na]^+$. Anal. ($C_{16}H_{15}NO_6S_2$). Found: C, 50.66%; H, 4.21%; N, 3.77%; calcd: C, 50.38%; H, 3.96%; N, 3.67%.

5.10. 6-Methanesulfonyloxy-5-methoxy-*N*-phenylsulfonyl-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (21a)

To a magnetically stirred suspension of $AlCl_3$ (171 mg, 1.28 mmol) in anhydrous CH_2Cl_2, 3,4,5-trimethoxybenzoyl chloride (222 mg, 0.96 mmol) was added at 25 °C and the mixture was stirred for 15 min to afford a red suspension. A solution of indole **20a** (245 mg, 0.64 mmol) in anhydrous CH_2Cl_2 was added dropwise and the mixture was stirred at 25 °C for 2 h and poured onto crushed ice. The aqueous layer was separated and extracted with CH_2Cl_2, and the combined organic extracts were washed with saturated aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated in vacuo. Chromatography on 20–45 μm silica gel, using cyclohexane/EtOAc (6:4) gave 276 mg of **21a** (76%) as a yellowish powder. Recrystallization from a 9:1 mixture of CH_2Cl_2 and MeOH gave orange crystals. Mp 216–220 °C; IR (NaCl film) ν' (cm^{-1}) 3113, 3000, 2934, 2832, 1638, 1583, 1532, 1501, 1470, 1408, 1365, 1326, 1233, 1217, 1186, 1155, 1124, 1015, 898, 867, 762, 723; ^{1}H NMR ($CDCl_3$) δ 3.20 (s, 3H, SO_2CH_3); 3.94 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.96 (s, 3H, $^5OCH_3$); 3.98 (s, 3H, $^{4'}OCH_3$); 7.20 (s, 2H, $H_{2'\text{-}6'}$); 7.50 (m, 2H, $H_{3''\text{-}5''}$); 7.62 (m, 1H, $H_{4''}$); 7.89 (s, 1H, H_7); 7.96 (m, 2H, $H_{2''\text{-}6''}$); 8.06 (s, 2H, $H_4 + H_2$); ^{13}C NMR ($CDCl_3$) δ 38.4 (SO_2CH_3); 56.3 ($^{3'\text{-}5'}OCH_3$); 56.4 ($^5OCH_3$); 61.1 ($^{4'}OCH_3$); 105.3 (C_4); 106.6 ($C_{2'\text{-}6'}$); 110.3 (C_7); 119.8 (C_3); 127.2 ($C_{2''\text{-}6''}$); 128.8 (C_{7a}); 129.8 ($C_{3''\text{-}5''}$); 133.7 (C_2); 133.8 ($C_{1'}$); 134.9 ($C_{4''}$); 137.0 ($C_{1''}$); 137.3 (C_6); 142.3 ($C_{4'}$); 149.9 (C_5); 153.2 ($C_{3'\text{-}5'}$); 189.6 (CO); MS (ZQ2000/ES+) m/z 614 $[M+K]^+$, 598 $[M+Na]^+$. Anal. ($C_{26}H_{25}NO_{10}S_2$, 0.5$H_2$0). Found: C, 53.32%; H, 4.53%; N, 2.43%; calcd: C, 53.25%, H, 4.51%; N, 2.39%.

5.11. 5-Methanesulfonyloxy-6-methoxy-*N*-phenylsulfonyl-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (21b)

The same procedure was performed with **20b** (226 mg, 0.59 mmol), 237 mg (1.78 mmol) of $AlCl_3$ and 205 mg (0.89 mmol) of 3,4,5-trimethoxybenzoyl chloride. The mixture was in this case,

stirred for 6 h. Chromatography on 20–45 μm silica gel, using cyclohexane/EtOAc (6:4) gave 122 mg of **21b** (35%) as a yellowish powder. Recrystallization from a 9:1 mixture of CH_2Cl_2 and MeOH gave yellow transparent crystals. Mp 234–236 °C; IR (NaCl film) ν' (cm^{-1}) 3132, 3004, 2934, 2844, 1630, 1587, 1536, 1505, 1482, 1447, 1412, 1369, 1330, 1241, 1182, 1128, 1089, 1019, 999, 968, 906, 867, 805, 762, 731, 684; ^{1}H NMR ($CDCl_3$) δ 3.24 (s, 3H, SO_2CH_3); 3.92 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.97 (s, 3H, $^{4'}OCH_3$); 4.01 (s, 3H, $^{6}OCH_3$); 7.10 (s, 2H, $H_{2'\text{-}6'}$); 7.53 (m, 2H, $H_{3''\text{-}5''}$); 7.64–7.68 (m, 2H, $H_7 + H_{4''}$); 7.92 (m, 2H, $H_{2''\text{-}6''}$); 7.99 (s, 1H, H_2); 8.16 (s, 1H, H_4); ^{13}C NMR ($CDCl_3$) δ 56.3 ($^{3'\text{-}5'}OCH_3$); 56.5 ($^{6}OCH_3$); 61.0 ($^{4'}OCH_3$); 97.4 (C_7); 106.6 ($C_{2'\text{-}6'}$); 117.9 (C_4); 120.5 (C_3); 121.5 (C_{3a}); 126.9 ($C_{2''\text{-}6''}$); 129.8 ($C_{3''\text{-}5''}$); 132.1 (C_2); 133.7 ($C_{1'}$); 133.9 (C_{7a}); 134.9 ($C_{4''}$); 137.1 (C_5); 137.4 ($C_{1''}$); 142.2 ($C_{4'}$); 151.0 (C_6); 153.1 ($C_{3'\text{-}5'}$); 188.9 (CO); MS (ZQ2000/ES+) m/z 598 $[M+Na]^+$. Anal. ($C_{26}H_{25}NO_{10}S_2$). Found: C, 54.69%; H, 4.87%; N, 2.63%; calcd: C, 54.25%; H, 4.38%; N, 2.43%.

5.12. 6-Methanesulfonyloxy-5-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (22a)

To a magnetically stirred solution of **21a** (190 mg, 0.35 mmol) in a 3:4 mixture of MeOH and THF (35 mL), LiOH (17 mg, 0.69 mmol) was added at room temperature. The reaction mixture was quenched after 15 min by addition of few drops of 10% aqueous HCl. The reaction mixture was evaporated in vacuo and the residue was taken up with CH_2Cl_2. The organic solution was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Flash chromatography using EtOAc/cyclohexane (7:3) gave 110 mg of **22a** (73%). Recrystallization from a 9:1 mixture of CH_2Cl_2/MeOH gave yellow crystals. Mp 148–151 °C; IR (NaCl film) ν' (cm^{-1}) 3327, 3000, 2934, 2832, 1614, 1575, 1497, 1478, 1412, 1361, 1326, 1237, 1178, 1124, 1104, 1073, 999, 968, 871, 832, 766, 735, 672; ^{1}H NMR ($CDCl_3$) δ 3.22 (s, 3H, SO_2CH_3); 3.92 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.95 (s, 3H, $^{4'}OCH_3$); 4.00 (s, 3H, $^{5}OCH_3$); 7.11 (s, 2H, $H_{2'\text{-}6'}$); 7.47 (s, 1H, H_7); 7.76 (s, 1H, H_2); 8.08 (s, 1H, H_4); 8.90 (s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 38.1 (SO_2CH_3); 56.3 ($^{3'\text{-}5'}OCH_3$); 56.4 ($^{5}OCH_3$); 61.0 ($^{4'}OCH_3$); 104.9 (C_4); 106.3 ($C_{2'\text{-}6'}$); 108.0 (C_7); 116.5 (C_3); 125.7 (C_{3a}); 129.7 (C_{7a}); 134.2 (C_2); 135.6 ($C_{1'}$); 136.4 (C_6); 141.1 ($C_{4'}$); 148.3 (C_5); 153.1 ($C_{3'\text{-}5'}$); 190.3 (CO); MS (ZQ2000/ES+) m/z 458 $[M+Na]^+$; HRMS (ES−) ($C_{10}H_{20}NO_8S$, $[M-H]^+$): found: 434.0906; calcd: 434.0910.

5.13. 5-Methanesulfonyloxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (22b)

To a magnetically stirred solution of **21b** (93 mg, 0.16 mmol) in a 1:2 mixture of MeOH and THF (25 mL), LiOH (8 mg, 0.32 mmol) was added at room temperature. The reaction mixture was quenched after 2 h by addition of few drops of 10% aqueous HCl. The reaction mixture was evaporated in vacuo and the residue was taken up with CH_2Cl_2. The organic solution was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Chromatography on 20–45 μm silica gel using EtOAc gave 53 mg of **22b** (76%). Recrystallization from a 9:1 mixture of CH_2Cl_2 and MeOH gave yellowish needles. Mp 189–193 °C; IR (NaCl film) ν' (cm^{-1}) 3350, 3124, 3004, 2937, 2836, 1571, 1525, 1501, 1459, 1412, 1357, 1322, 1272, 1233, 1159, 1124, 1065, 999, 968, 875, 832, 809, 770, 731; ^{1}H NMR ($CDCl_3$) δ 3.21 (s, 3H, SO_2CH_3); 3.79 (s, 3H, $^{6}OCH_3$); 3.85 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.90 (s, 3H, $^{4'}OCH_3$); 6.91 (s, 1H, H_7); 7.05 (s, 2H, $H_{2'\text{-}6'}$); 7.64 (s, 1H, H_2); 8.22 (s, 1H, H_4); 9.74 (br s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 38.2 (SO_2CH_3); 56.1 ($^{6}OCH_3$); 56.2 ($^{3'\text{-}5'}OCH_3$); 60.9 ($^{4'}OCH_3$); 95.6 (C_7); 106.3 ($C_{2'\text{-}6'}$); 116.5 (C_3); 116.9 (C_4); 119.3 (C_{3a}); 133.8 (C_2); 135.2 ($C_{1'}$); 135.3 (C_{7a}); 135.5 (C_5); 141.0 ($C_{4'}$); 149.3 (C_6); 152.9 ($C_{3'\text{-}5'}$); 190.2 (CO); MS (ZQ2000/ES+) m/z 458 $[M+Na]^+$. Anal. ($C_{20}H_{21}NO_8S$, $0.5H_20$). Found: C 54.11%; H, 4.94%; N, 3.13%; calcd: C, 54.04%; H, 5.00%; N, 3.15%.

5.14. 6-Hydroxy-5-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (14)

To a freshly prepared 1.4 M solution of $Mg(OCH_3)_2$ in dry MeOH (3 mL), **22a** (40 mg, 0,09 mmol) was added at room temperature and the reaction mixture was refluxed for 12 h. Solvent was evaporated under reduced pressure and the residue was taken up with EtOAc. The organic solution was washed with saturated aqueous NH_4Cl, brine and dried over $MgSO_4$, and then, concentrated in vacuo. Chromatography on 20–45 μm silica gel, using EtOAc gave 22 mg of **14** as a colorless amorphous powder (67%). ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 6H, $^{3'-5'}OCH_3$); 3.94 (s, 3H, $^{4'}OCH_3$); 4.02 (s, 3H, $^{5}OCH_3$); 5.88 (br s, D_2O exch., 1H, OH); 7.00 (s, 1H, H_7); 7.12 (s, 2H, $H_{2'\text{-}6'}$); 7.59 (d, J = 2.7 Hz, 1H, H_2); 7.93 (s, 1H, H_4); 8.55 (br s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 56.3 ($^{3'-5'}OCH_3$); 56.4 ($^{5}OCH_3$); 61.0 ($^{4'}OCH_3$); 96.5 (C_7); 103.0 (C_4); 106.3 ($C_{2'\text{-}6'}$); 116.9 (C_3); 119.3 (C_{3a}); 130.9 (C_{7a}); 131.8 (C_2); 136.0 ($C_{1'}$); 140.9 ($C_{4'}$); 144.5 (C_5); 144.8 (C_6); 153.0 ($C_{3'-5'}$); 190.5 (CO); MS (ZQ2000/ES+) m/z 380 $[M+Na]^+$. Anal. ($C_{19}H_{19}NO_6$). Found: C, 64.45%; H, 5.40%; N, 3.88%; calcd: C, 63.86%; H, 5.36%; N, 3.92%.

5.15. 5-Hydroxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (10)[12]

The same procedure was performed with **22b** (66 mg, 0.15 mmol), though the reaction mixture was refluxed for 24 h. Flash chromatography using EtOAc gave 35 mg of **10** as a colorless amorphous powder (65%). ^{1}H NMR (DMSO-d_6) δ 3.73 (s, 3H, $^{4'}OCH_3$); 3.79 (s, 3H, $^{6}OCH_3$); 3.82 (s, 6H, $^{3'-5'}OCH_3$); 6.95 (s, 1H, H_7); 7.02 (s, 2H, $H_{2'\text{-}6'}$); 7.63 (s, 1H, H_4); 7.80 (d, J = 2.8 Hz, 1H, H_2); 8.68 (br s, D_2O exch., 1H, OH); 11.62 (br s, D_2O exch., 1H, NH); ^{13}C NMR (DMSO-d_6) δ 56.1 ($^{6}OCH_3$); 56.4 ($^{3'-5'}OCH_3$); 60.5 ($^{4'}OCH_3$); 95.7 (C_7); 106.4 ($C_{2'-6'}$); 106.8 (C_4); 115.0 (C_3); 120.2 (C_{3a}); 130.9 (C_{7a}); 134.1 (C_2); 136.5 ($C_{1'}$); 140.2 ($C_{4'}$); 144.1 (C_5); 146.8 (C_6); 153.0 ($C_{3'-5'}$); 189.2 (CO); MS (ZQ2000/ES+) m/z 380 $[M+Na]^+$.

5.16. 3-Hydroxy-4-methoxy-2-nitrobenzaldehyde (24)[21,22]

Compound **24** was synthesized according a previously described procedure.[22] Isovanilline **23** (3 g, 19.7 mmol) was nitrated in presence of 1.2 equiv of fuming nitric acid (2 mL) to afford 1.26 g of 3-hydroxy-4-methoxy-6-nitrobenzaldehyde (31%) and 700 mg of **24** (18%), after purification by flash chromatography using cyclohexane/acetone (7:3). Mp 142–144 °C (EtOH) (lit. 143–145 °C)[22]; IR (NaCl film) ν' (cm^{-1}) 3216, 2848, 1673, 1285; ^{1}H NMR ($CDCl_3$) δ 4.14 (s, 3H, OCH_3); 7.14 (d, J = 8.4 Hz, 1H, H_6); 7.50 (d, J = 8.4 Hz, 1H, H_5); 10.10 (s, 1H, CHO); 10.50 (s, 1H, OH); MS (ZQ2000/ES+) m/z 236 $[M+K]^+$, 220 $[M+Na]^+$.

5.17. 4-Methoxy-3-methanesulfonyloxy-2-nitrobenzaldehyde (25)

To a solution of **24** (256 mg, 1.29 mmol) in anhydrous CH_2Cl_2 (7 mL), Et_3N (260 μL, 2.62 mmol) was added at 0 °C, and then, methanesulfonyl chloride (205 μL, 2.64 mmol) was added 10 min later. The reaction was followed by TLC (cyclohexane/acetone (1:1)) and quenched after 30 min by adding 30 mL of distillated water. The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography using cyclohexane/EtOAc (3:2) to give 295 mg of **25** (83%) which was recrystallized

from EtOH. Mp 133–135 °C; IR (NaCl film) ν' (cm^{-1}) 2848, 1697, 1371, 1290; ^1H NMR (acetone-d_6) δ 3.55 (s, 3H, CH_3), 4.19 (s, 3H, OCH_3); 7.68 (d, J = 8.8 Hz, 1H, H_6); 8.14 (d, J = 8.8 Hz, 1H, H_5); 9.92 (s, 1H, CHO); MS (ZQ2000/ES+) m/z 298 $[M+Na]^+$.

5.18. 6-Methoxy-2-nitro-3-(2-nitrovinyl)phenyl methanesulfonate (26)

To a stirred solution of dry CH_3NO_2 (720 μL, 13.3 mmol) in dry THF (10 mL) cooled at −78 °C were successively added a 1 M solution of LHMDS in toluene (6.65 mL) and, 10 min later, a solution of **25** (915 mg, 3.33 mmol) in dry THF (25 mL). After stirring for 2.5 h at the same temperature, the reaction mixture was quenched with saturated aqueous NH_4Cl (100 mL) and extracted with EtOAc. The combined organic layers were successively washed with water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was dissolved in Ac_2O (6 mL), and then, AcONa (90 mg, 1.10 mmol) was added. The reaction mixture was heated at 60 °C for 3.5 h, then diluted with toluene and concentrated in vacuo. The crude product was taken up with CH_2Cl_2 and the organic solution was washed with water, brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure to afford 787 mg of **26** as an amorphous solid used without further purification (74%). ^1H NMR ($CDCl_3$) δ 3.38 (s, 3H, CH_3), 4.07 (s, 3H, OCH_3), 7.24 (d, J = 8.9 Hz, 1H, H_5), 7.50 (d, J = 13.5 Hz, 1H, Ha), 7.60 (dd, J = 8.9 Hz,J = 0.4 Hz, 1H, H_6), 7.86 (d, J = 13.5 Hz, 1H, Hb).

5.19. 6-Methoxy-7-methanesulfonyloxy-1*H*-indole (27)

To a solution of **26** (787 mg, 2.47 mmol) in a 4:1 mixture of MeOH/DMF (28 mL), 10% Pd/C (263 mg, 0.25 mmol) was added and the reaction mixture was stirred at room temperature under hydrogen for 16.5 h. The suspension was filtered on a Celite layer and concentrated under pressure. The residue was purified by flash chromatography using cyclohexane/EtOAc (4:1) to afford **27** as a pale pink solid, recrystallized from a mixture of CH_2Cl_2 and *n*-hexane. Mp 130–131 °C; IR (NaCl film) ν' (cm^{-1}) 3408, 2938, 2834, 1636, 1452, 1353, 1325, 1254, 1173, 1087; ^1H NMR ($CDCl_3$) δ 3.25 (s, 3H, CH_3); 3.93 (s, 3H, OCH_3); 6.49 (dd, J = 3.1 Hz, J = 2.1 Hz, 1H, H_3); 6.87 (d, J = 8.7 Hz, 1H, H_5); 7.16 (dd, J = 3.1 Hz, J = 2.4 Hz, 1H, H_2); 7.48 (d, J = 8.7 Hz, 1H, H_4); 8.64 (br s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 37.4 (CH_3), 57.2 (OCH_3), 102.7 (C_3), 107.0 (C_5), 119.8 (C_4), 123.9 (C_7), 125.2 (C_2), 125.4 (C_{3a}), 130.5 (C_{7a}), 146.9 (C_6); MS (ZQ2000/ES+) m/z 280 $[M+K]^+$, 264 $[M+Na]^+$, 242 $[M+H]^+$; HRMS (ES+) ($C_{10}H_{11}NO_4NaS$, $[M+Na]^+$): found: 264.0308; calcd: 264.0306.

5.20. 7-Methanesulfonyloxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (28)

To a suspension of **27** (120 mg, 0.50 mmol) and anhydrous $ZnCl_2$ (135.6 mg, 1.0 mmol) in dry CH_2Cl_2 (1.5 mL), a 3 M solution of EtMgBr in Et_2O (215 μL, 0.65 mmol) was added dropwise. The suspension was stirred for 1 h, then a solution of 3,4,5-trimethoxybenzoyl chloride (230 mg, 1.0 mmol) in dry CH_2Cl_2 (2.0 mL) was added dropwise. The reaction mixture was stirred for another 1 h followed by the addition of $AlCl_3$ (67 mg, 0.5 mmol). The resultant suspension was vigorously stirred for 7 h and was quenched with water and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography using cyclohexane/EtOAc (1:1) and 41.4 mg of pure **28** (19%) was obtained as brown crystals after crystallization in a mixture of CH_2Cl_2/MeOH. Mp 209–210 °C; IR (NaCl film) ν' (cm^{-1}) 3372, 2935, 2844, 1580, 1415, 1365, 1125; ^1H NMR ($CDCl_3$) δ 3.32 (s, 3H, CH_3); 3.93 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.96 (s, 3H, $^6OCH_3$); 4.01 ($^{4'}OCH_3$); 7.10 (d, J = 8.8 Hz, 1H, H_5); 7.13 (s, 2H, $H_{2'\text{-}6'}$); 7.71 (d, J = 2.9 Hz, 1H, H_2); 8.30 (d, J = 8.8 Hz, J = 0.4 Hz, 1H, H_4); 9.18 (br s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 37,6 (CH_3); 56.3 ($^{3'\text{-}5'}OCH_3$); 57.1 ($^6OCH_3$); 61.0 ($^{4'}OCH_3$); 106.3 ($C_{2'\text{-}6'}$); 109.3 (C_5); 117.2 (C_3); 122.1 (C_4); 123.2 (C_7); 123.4 (C_{3a}); 131.2 (C_{7a}); 133.6 (C_2); 135.5 ($C_{1'}$); 141.2 ($C_{4'}$); 148.3 (C_6); 153.1 ($C_{3'\text{-}5'}$); 190.1 (CO); MS (ZQ2000/ES+) m/z 476 $[M+K]^+$, 458 $[M+Na]^+$; HRMS (ES−) ($C_{10}H_{20}NO_8S$, $[M-H]^+$): found: 434.0906; calcd: 434.0910.

5.21. 7-Hydroxy-6-methoxy-3-(3′,4′,5′-trimethoxybenzoyl)-1*H*-indole (13)

To a solution of **28** (20 mg, 0.046 mmol) in anhydrous THF (3 mL) cooled at −78 °C, a 2 M solution of LDA in THF (115 μL, 0.23 mmol) was slowly added and the reaction mixture was stirred for 5 min at the same temperature, then further 30 min at 0 °C. The reaction mixture was quenched with saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography using cyclohexane/EtOAc (3:7) to give pure **13** as a pink oil (16.4 mg, 100%). IR (NaCl film) ν' (cm^{-1}) 3332, 2939, 2836, 1575, 1515, 1416, 1360, 1125, 1026; ^1H NMR ($CDCl_3$) δ 3.90 (s, 6H, $^{3'\text{-}5'}OCH_3$); 3.94 (s, 3H, $^6OCH_3$); 3.98 (s, 3H, $^{4'}OCH_3$); 5.97 (br s, D_2O exch., 1H, OH); 7.02 (d, J = 8.7 Hz, 1H, H_5); 7.12 (s, 2H, $H_{2'\text{-}6'}$); 7.68 (br s, 1H, H_2); 7.87 (d, J = 8.6 Hz, 1H, H_4); 9.09 (br s, D_2O exch., 1H, NH); ^{13}C NMR ($CDCl_3$) δ 56.3 ($^{3'\text{-}5'}OCH_3$); 57.4 ($^6OCH_3$); 61.0 ($^{4'}OCH_3$); 106.3 ($C_{2'\text{-}6'}$); 109.0 (C_5); 113.4 (C_4); 117.0 (C_3); 122.7 (C_{3a}); 126.1 (C_{7a}); 131.2 (C_7); 133.5 (C_2); 136.0 ($C_{1'}$); 140.9 ($C_{4'}$); 142.5 (C_6); 152.9 ($C_{3'\text{-}5'}$); 190.5 (CO); MS (ZQ2000/ES+) m/z 396 $[M+K]^+$, 380 $[M+Na]^+$, 358 $[M+H]^+$; HRMS (ES+) ($C_{19}H_{20}NO_6$, $[M+H]^+$): found: 358.1287; calcd: 358.1291.

5.22. Tubulin-binding assay

Calf brain tubulin was purified according to the method of Shelanski et al.[27] by three cycles of assembly–disassembly and then dissolved at a final concentration of 2–3 mg/mL in the assembly buffer (pH 6.6) containing 0.1 M MES, 0.5 mM $MgCl_2$, 2 mM EGTA, and 1 mM GTP. Tubulin assembly was monitored and recorded continuously by turbidimetry at 400 nm in a UV spectrophotometer, equipped with a thermostated cell at 37 °C.[28] The IC_{50} value, defined as the concentration of inhibitor which decreased by 50% the maximum assembly rate of tubulin in control cell without test compound, was determined for each newly synthesized compound. The IC_{50} of all compounds were compared to the IC_{50} of colchicine (Sigma–Aldrich), measured the same day under the same conditions. The results are presented as inhibition of tubulin polymerization (or ITP), which is the ratio of the IC_{50} value of a given synthesized compound to the IC_{50} value of colchicine (ITP = IC_{50} test compound/IC_{50} colchicine).

5.23. Evaluation of cytotoxicity in murine B16 melanoma cells

Murine B16 melanoma cells were grown in DMEM containing 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (37 °C, 5% CO_2). All compounds were initially dissolved in DMSO at a stock concentration of 2.5 mg/mL and were further diluted in cell culture medium. For comparative purposes, CA-4 (synthesized according to Ref. 29) and colchicine (Sigma–Aldrich) were routinely included in the experiments as reference control compounds. Exponentially growing B16 cells were plated onto 96-well plates at 5000 cells per well in 100 μL of culture medium. Twenty-four hours after plating, 100 μL of medium containing the compound of interest (final concentrations ranging

from 0.25 ng/mL to 25 μg/mL, in 10-fold dilutions) was added to the wells (in triplicate) containing the cells, and incubated for 48 h at 37 °C and 5% CO_2. After the 48 h exposure period to the test compounds, cell viability was assayed using the MTT test[30] and absorbance was read at 562 nm in a microplate reader (BioKinetics Reader, EL340). Appropriate controls with DMEM only and MTT were run to subtract background absorbance. Results are presented as percent of controls containing 1% DMSO, which was not cytotoxic at this concentration. The concentration of compound that inhibited cell viability by 50% (inhibitory concentration for 50% of cells, or IC_{50}) was determined using the GraphPad Prism software. Results are presented as means ± SEM of 3–7 independent experiments each run in triplicate.

5.24. Effect on the morphology of transformed HUVEC cells (EA.hy 926 cells)

To assess the effects of the compounds on the morphology of endothelial cells, we used the EA.hy 926 cell line which is derived from the fusion of human umbilical vein endothelial cells (HUVEC) with the permanent human cell line A549.[17] The EA.hy 926 cell line is considered as one of the best immortalized HUVEC cell lines because these cells express most of the biochemical markers of parental HUVEC.[31] EA.hy 926 cells, originally obtained from Dr. Cora-Jean S. Edgell (Pathology Department, University of North Carolina, Chapel Hill, NC 27599-7525, USA) were used with her permission, and were grown in DMEM containing 2 mM L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (37 °C, 5% CO_2). Exponentially growing EA.hy 926 cells were plated onto 96-well plates at 5000 cells/100 μL/well. Twenty-four h after plating, the medium was aspirated, and 100 μL of medium containing the test compound was added to the well containing the cells (in triplicate) in 10-fold dilutions, and incubated for 2 h at 37 °C and 5% CO_2. After the 2 h-incubation period, digital photographs were taken of srepresentative center areas of each well at a magnification of 360×. Combretastatin A4 (CA-4) and colchicine were routinely included in the experiments as internal standards.

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Supplementary data

NMR data. ^1H and ^{13}C spectra for compounds **10**, **13**, **14**, **19a,b**, **21a,b**, **22a,b**, and **28** are available, as well as HMQC, HMBC, and NOESY spectra for compounds **19a** and **19b**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.06.002.

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