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SCIENCE DIRECT
Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 14 (2006) 4410–4426

# Synthesis and biological evaluation of (3,4,5-trimethoxyphenyl)indol-3-ylmethane derivatives as potential antivascular agents

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Received 26 October 2005; revised 16 February 2006; accepted 21 February 2006
Available online 10 March 2006

**Abstract**—Combretastatin A-4 (CSA-4), a stilbene derivative, is a potent vascular disrupting agent (VDA) with the structural requirement of a *cis*-configuration to maintain a molecular geometry and a correct orientation of both phenyl groups. A series of indolic analogues of CSA-4 was synthesized by means of an efficient strategy. Six compounds (**20b**, **25b**–**27b**, **32b**, and **35b**) were identified as potent inhibitors of tubulin polymerization and also displayed cytotoxic activities on B16 melanoma cells at a nanomolar level. Both activities were well correlated with the ability to induce morphological changes of EA·hy 926 endothelial cells. In conclusion, the *cis*-stilbene skeleton of CSA-4 could conveniently be replaced by the 3-aroylindolic moiety, thus avoiding any isomerization leading to inactive *trans* compounds.


## 1. Introduction

The mitotic spindle, whose formation and activity are required for chromosome segregation and cell division, is constituted by microtubules generated by polymerization of tubulin α,β-dimers.[1] Many drugs which interfere with the dynamic behavior of the microtubules are potential anticancer drugs.[2] Three main sites are involved in the ligand binding on tubulin: the colchicine **1** site[3,4] which is involved in binding of various natural and synthetic compounds suggesting a high plasticity of the target protein at this place,[5] the vinca alkaloid site,[6] and the paclitaxel site.[7,8]

Microtubules of the cytoskeleton also play a major role in maintaining cell shape. The elongated endothelial cells of the neovasculature are particularly sensitive to drugs that cause depolymerization of the microtubules like combretastatin A-4 (CSA-4) **2** and combretastatin A-1 (CA-1) **3** (Fig. 1), which are both natural compounds isolated from the bark of the South African bush willow tree *Combretum caffrum* Kuntze (Combretaceae).[9]

Tumor metastasis, growth, and survival are highly dependent on the development of new blood vessels.[10] Therefore, several approaches have been explored to selectively prevent the development of new blood vessels in tumors, or to attack selectively the existing tumor vasculature with tubulin binding agents, of which CSA-4 emerged as the prototype. In contrast to drugs that target tumor angiogenesis, CSA-4 targets the newly formed tumor vasculature.[11–15]

After administration of relatively non-toxic doses, CSA-4 causes a rapid tubulin depolymerization of endothelial

*Keywords*: Combretastatin A-4; Tubulin; 3-Aroylindole; EA·hy 926 endothelial cells.
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doi:10.1016/j.bmc.2006.02.037

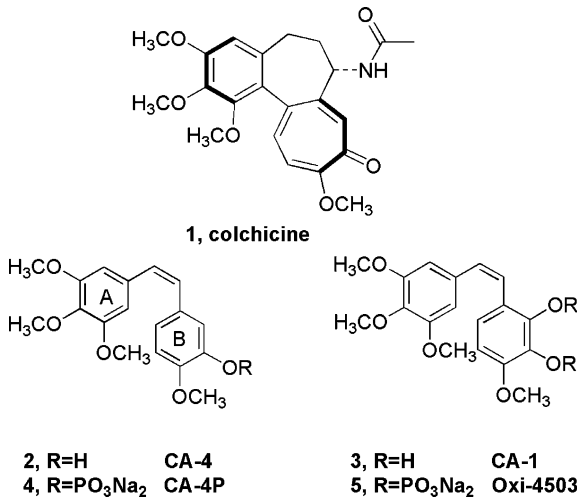



**Figure 1.** Structures of colchicines and combretastatin derivatives.

cells which induces morphological changes including rounding up and membrane blebbing. This rapid collapse in tumor blood flow in vivo is most likely due to morphological and functional changes associated with endothelial cytoskeleton, similar to those that occur in endothelial cells in vitro after short drug exposure to CSA-4.[16] This cascade of events leads to extensive shut down of tumor vasculature and consequently to tumor cell necrosis.[17,18]

Combretastatins have received a great deal of attention[19] due to their relatively simple structures,[20] high potency as cytotoxic agents,[20] and antivascular activity.[11,21] Major problems are their poor water solubility and the structural instability of the *cis*-configuration which is necessary for tubulin activity. To allow easier administration in vivo, water-soluble derivatives have been prepared, for example, the combretastatin A-4 sodium phosphate prodrug (CSA-4P) **4** and the combretastatin A-1 sodium diphosphate prodrug (CA-1P or Oxi4503) **5**, which are both developed by Oxigene as vascular-disrupting agents (VDAs).[12]

As a result of the SAR studies in the CSA-4 series, it was deduced that a 3,4,5-trimethoxyphenyl and a 4-methoxy-3-X-substituted-phenyl system (with X = OH and $NH_2$), linked by a two-atom bridge and a *cis*-configuration allowing a dihedral angle of about 66° between the two rings,[22,23] are the common structural characteristics most important for the activity of these compounds. These structural requirements have been achieved in derivatives and analogues of diverse types, either conserving the *cis* olefin, bearing an olefin stabilized in the *cis* configuration by added substituents[24] or bearing an olefin involved in a more or less unsaturated cycle.[19,25,26] Formal replacements of A- or B-ring by heteroaromatic groups have been synthesized. Only a few results concerning compounds bearing an indolic part have been previously published. The indole nucleus has been used to obtain *cis*-restricted analogues of CSA-4 and some 2,3-diarylindoles have been synthesized by Medarde (**6**),[27] Flynn and Pinney (**7** and **8**).[28,29] The B-ring can also be replaced by an indolyl part like the 5-indolyl derivative **9**,[30] the A-289099 (**10**)[31] and compound **11**[32] developed by Abbott, the heterocombretastatin **12**,[33] and the arylthioindole **13**[34] prepared, respectively, by Medarde and De Martino (Fig. 2).

Other indolic agents, less obviously comparable to the CSA-4 pattern, display powerful inhibitory activities of tubulin polymerization: the indolyl-3-glyoxamide D-24851 **14**,[35] the 2-aroylindoles **15a**–**c**[36] discovered by Baxter oncology, and the 3-formyl-2-phenylindole **16**[37] designed by von Angerer.

The aim of the present study was to synthesize 3-aroylindole analogues of CSA-4 in order to avoid the problematic isomerization of the *cis*-configuration. The two-carbon linker of our parent compound was maintained between the 3′,4′,5′-trimethoxyphenyl ring and the phenyl part of the 3-indolyl moiety (Fig. 3). This latter is thus considered as a mimic of CSA-4 B-ring. To determine the optimal distance and angle between the two rings, we chose to prepare indolylmethane derivatives with various substituted bridges (X = O; S; H, OH; H, $OCH_3$; H, $OCOCH_3$; and H, H). The influence of the substitution of the indolic nitrogen was also studied. In order to mimic the *p*-methoxy group, which is considered essential for CSA-4 activity, derivatives bearing a 6-methoxy group on the indole nucleus were prepared.

The biological activities of the synthesized compounds were assessed by the tubulin polymerization inhibition assay, by their cytotoxicity against the B16 melanoma cells, and also by their effect on the morphology of EA·hy 926 endothelial cells,[38] which are immortalized human umbilical vein endothelial cells (HUVEC). We report herein both the synthesis and biological activity of 23 compounds. Six of them (**20b**, **25b**–**27b**, **32b**, and **35b**) were identified as promising compounds on the basis of their potent activities.

## 2. Chemistry

The key step of our synthesis was a Friedel–Crafts acylation of the corresponding *N*-benzenesulfonyl-1*H*-indole (i.e., **19a**,**b**) with the 3,4,5-trimethoxybenzoyl chloride (Scheme 1). This reaction was quickly performed in methylene chloride in a low excess of aluminum chloride at room temperature to afford **20a**,**b** with a very good yield (80% and 86% respectively) due to the phenylsulfonyl group which enhances the selectivity of the substitution on the 3-position of the indole nucleus.

The non-commercial 6-methoxy-*N*-benzenesulfonyl-1*H*-indole **19b** was prepared by means of a modified Bischler strategy,[39,40] optimized in order to obtain large amount of **19b** with an improved overall yield (Scheme 2). Benzenesulfonylation of *m*-anisidine gave **17b** in a quantitative yield. Sulfonamide **17b** was alkylated by the bromoacetaldehyde diacetal in the presence of NaH to afford **18b**. Finally, a boron trifluoride etherate promoted cyclization affords **19b** in a quantitative yield.

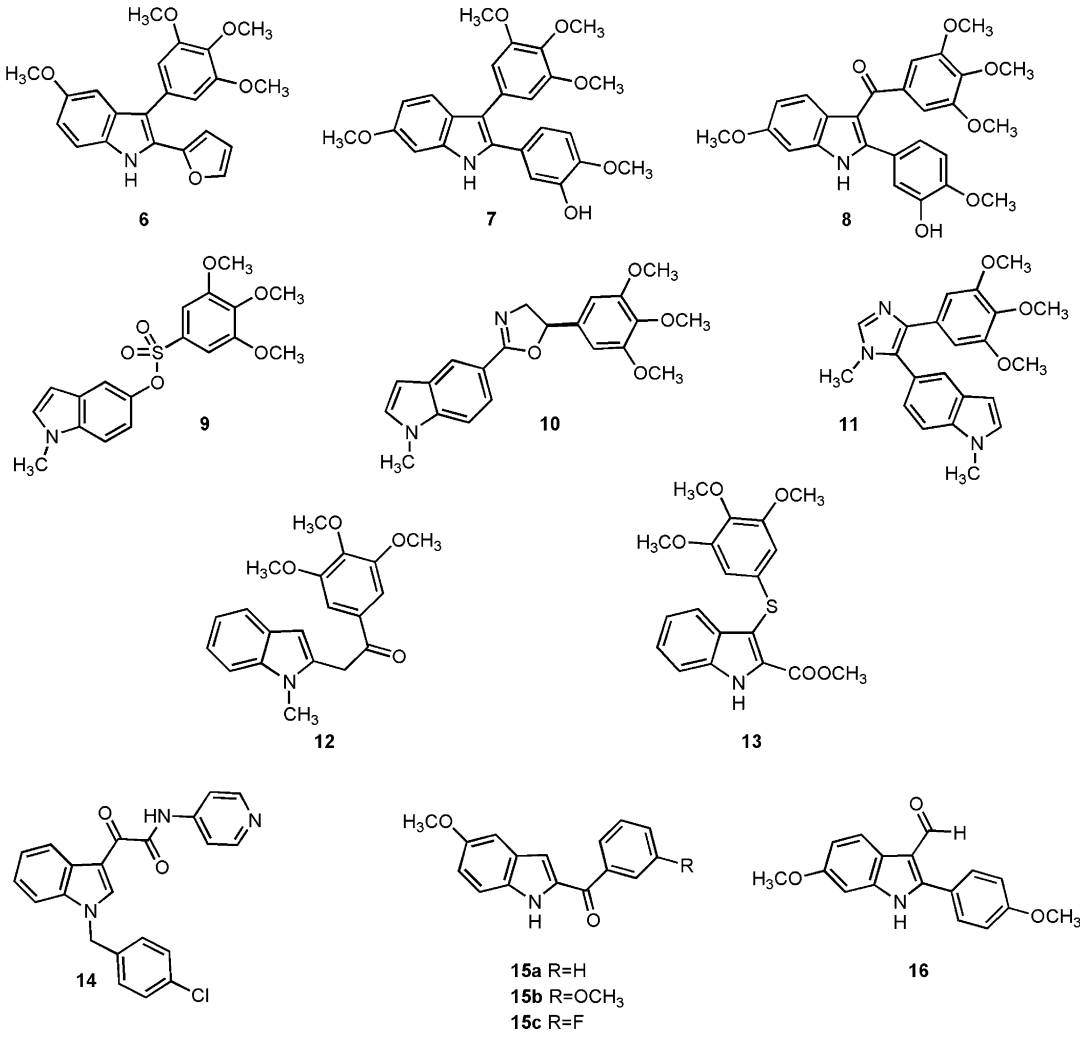



**Figure 2.** Structures of *cis*-restricted analogues of CSA-4.

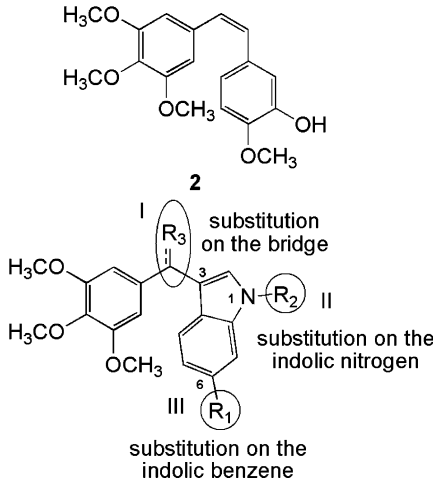


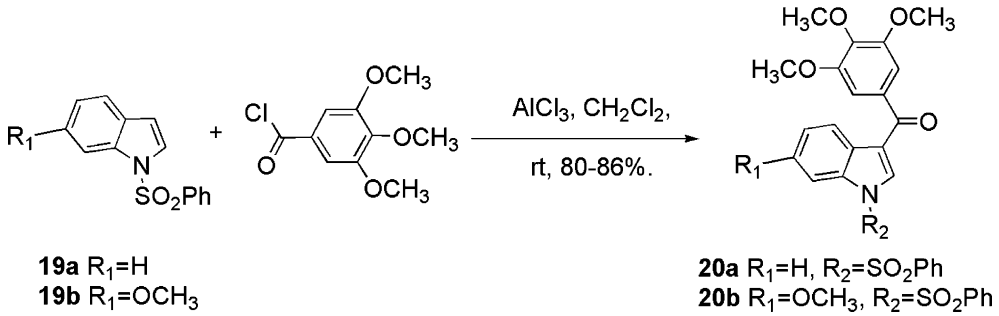

**Figure 3.** Modifications of the 3-indolyl methane skeleton.

## 2.1. Modification of the bridge

Carbinols **21a**,**b** were obtained in a quantitative yield by reduction of the carbonyl group with sodium borohydride in methanol at room temperature. Methylenes **24a**,**b** were obtained from the corresponding carbinol by reduction with triethylsilane in trifluoroacetic acid, conditions previously published for the reduction of 3-aroylindoles.[41] Two other modifications were obtained from the carbinol **21a** due to the extreme lability of its benzylic hydroxyl group: the substitution by a methoxy group (**22a**) occurred in methanol in the presence of *p*-toluene sulfonic acid and the substitution by an acetoxyl group (**23a**) occurred in a mixture of acetic acid and acetic anhydride in the presence of 4-DMAP (these conditions also probably lead to a direct acetylation of carbinol). Nevertheless, these two latter types of substitution were not attempted on the 6-substituted series due to the relative lack of stability of these products.

## 2.2. Deprotection of the 1-position of the indole nucleus

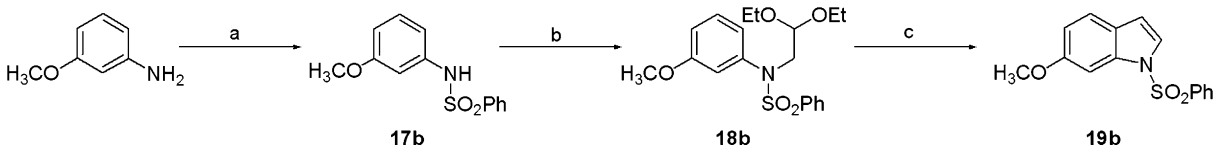
The nitrogen substituent was also modified (Scheme 3). Carbonyls **20a**,**b** were easily deprotected in refluxing aqueous methanol in the presence of $K_2CO_3$ to give **25a**,**b**. Deprotection of the carbinol **21a** in these conditions led to the isolation of 1*H*-indole as major product. The instable carbinol function probably underwent a




**19a** $R_1$=H
**19b** $R_1$=$OCH_3$

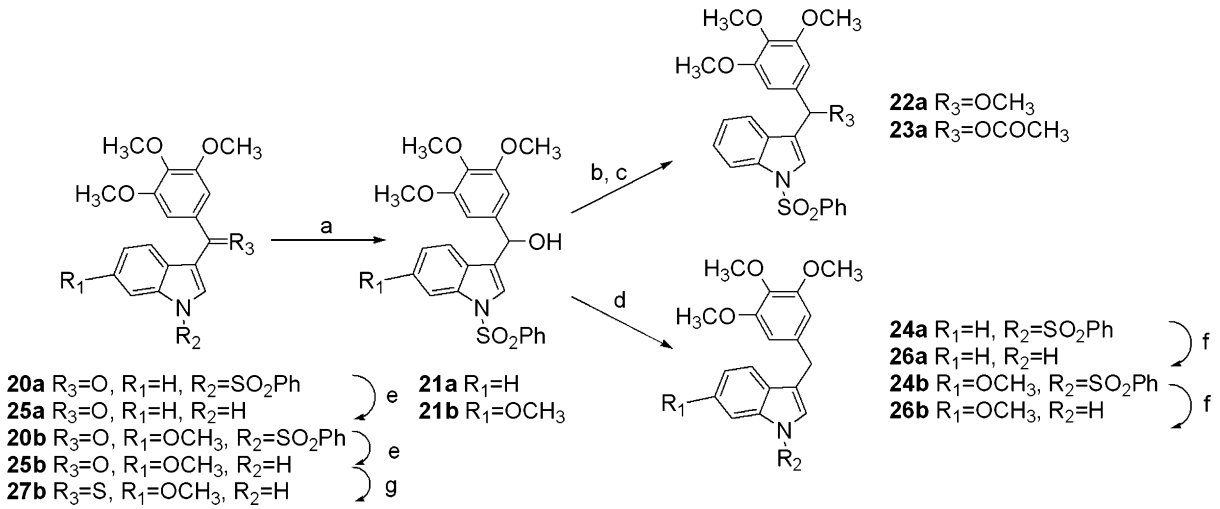
**20a** $R_1$=H, $R_2$=$SO_2Ph$
**20b** $R_1$=$OCH_3$, $R_2$=$SO_2Ph$

**Scheme 1.** Synthesis of 3-aroyl indoles **20a** and **20b**.



**17b** **18b** **19b**

**Scheme 2.** Synthesis of 6-methoxyindole **19b**. Reagents and conditions: (a) $PhSO_2Cl$, pyridine, $CH_2Cl_2$, rt, 98%; (b) $BrCH_2CH(OEt)_2$, NaH, anhyd DMF, reflux, 82%; (c) $BF_3{\cdot}Et_2O$, $CH_2Cl_2$, rt, 97%.



**22a** $R_3$=$OCH_3$
**23a** $R_3$=$OCOCH_3$

**20a** $R_3$=O, $R_1$=H, $R_2$=$SO_2Ph$
**25a** $R_3$=O, $R_1$=H, $R_2$=H
**20b** $R_3$=O, $R_1$=$OCH_3$, $R_2$=$SO_2Ph$
**25b** $R_3$=O, $R_1$=$OCH_3$, $R_2$=H
**27b** $R_3$=S, $R_1$=$OCH_3$, $R_2$=H

**21a** $R_1$=H
**21b** $R_1$=$OCH_3$

**24a** $R_1$=H, $R_2$=$SO_2Ph$
**26a** $R_1$=H, $R_2$=H
**24b** $R_1$=$OCH_3$, $R_2$=$SO_2Ph$
**26b** $R_1$=$OCH_3$, $R_2$=H

**Scheme 3.** Modifications of the bridge and deprotection of the indole nucleus. Reagents and conditions: (a) $NaBH_4$, MeOH/THF, rt, 92–95%; (b) PTSA, MeOH, rt, 85%; (c) $Ac_2O$, AcOH, 4-DMAP, rt, 95%; (d) $Et_3SiH$, TFA, $CH_2Cl_2$, 75–90%; (e) $K_2CO_3$, MeOH/$H_2O$, reflux, 94–99%; (f) KOH, MeOH/$H_2O$, reflux, 88% or MeONa (0.1 M), MeOH, reflux, 81%; (g) Lawesson's reagent, THF, rt, 91%.

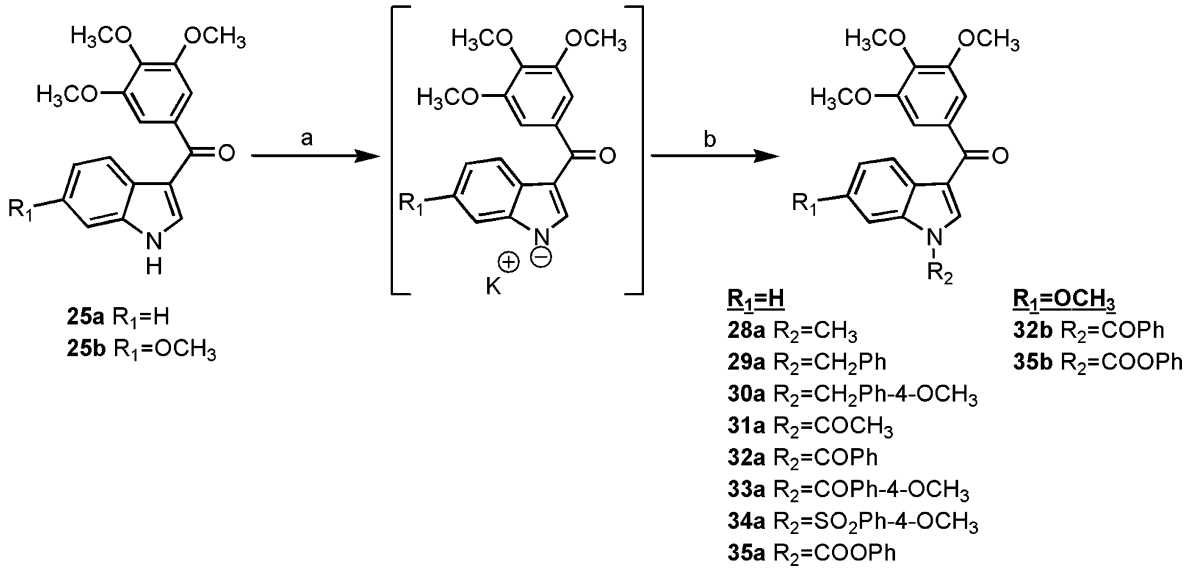
retro-addition reaction in this basic medium. Consequently, deprotections of neither the carbinol **21b** in the 6-methoxy series nor derivatives **22a** and **23a** were not investigated anymore. Deprotection of the methylenes **24a**,**b** was only achieved with a stronger base (KOH) to afford **26a**,**b**. This contrasts with the easier deprotection of carbonyls **20a**,**b**, where the carbonyl group contributes to the formation of a more stable indolyl anion by charge delocalization. The thioketone **27b** was synthesized from the deprotected carbonyl **25b** using Lawesson's reagent in THF at room temperature in a quantitative yield.

### 2.3. Modification of the 1-position of the indole nucleus (N-substitution)

Diverse groups were introduced on the 1-position of the indole ring: (a) electron-donating groups: $-CH_3$ (**28a**), $-CH_2Ph$ (**29a**), and $-CH_2Ph$–*p*-$OCH_3$ (**30a**); (b) electron-withdrawing groups: $-COCH_3$ (**31a**), –COPh (**32a**,**b**), –COPh–*p*-$OCH_3$ (**33a**), $-SO_2Ph$–*p*-$OCH_3$ (**34a**), and –COOPh (**35a**,**b**). All compounds were synthesized according to the same general method (Scheme 4). Carbonyls **25a**,**b** were deprotonated by KOH in EtOH. After evaporation of the solvent, the corresponding salts were taken up with acetone or DMF and alkyl or acyl halides were added.

## 3. Biological activity

Twenty-three CSA-4 analogues were synthesized and evaluated for their biological activities, including the inhibition of tubulin polymerization (ITP), the cytotoxicity against murine B16 melanoma cells, and their effects on the morphology of EA·hy 926 endothelial cells




**Scheme 4.** Modifications of the N-position of the indole nucleus. Reagents and conditions: (a) KOH, EtOH, rt; (b) $R_1X$ or $R_1COX$, acetone or DMF, rt, 65–96% in one-pot.

(modified HUVEC), which are considered as a good predictor of potential in vivo antivascular effects.[42,43]

### 3.1. Modifications of the methane bridge of the 1*H*-indol-3-yl-(3,4,5-trimethoxyphenyl) methane

Compound **20a** ($R_1$ = $SO_2Ph$ and R = O) was our starting scaffold and therefore all the modifications were aimed at improving the activity of that scaffold (Table 1). Compound **20a** presented a medium inhibition of tubulin polymerization activity with an ITP value of 11 (results expressed in terms of the relative $IC_{50}/IC_{50colchicine}$ are summarized in Table 1). Analogue **20a** was moderately cytotoxic in the micromolar range (8.4 μM) against B16 melanoma cells and could alter the morphology (rounding up) of EA·hy 926 endothelial cells at a rather high concentration of 25 μg/mL for a 2 h exposure time.

**Table 1.** Biological consequences of modifications of the methane bridge for (1*H*-indol-3-yl)-3,4,5-trimethoxyphenyl-methane compounds **20a**–**24b**

| Compound | ITP ($IC_{50}/IC_{50colchicine}$)[a,b] | Cytotoxicity $IC_{50}$[c] (μM) | Morphological activity on EA·hy 926[d] |
|---|---|---|---|
| **20a** | 11 | 8.4 ± 0.9 | 1+ |
| **21a** | 9 | 16.2 ± 0.8 | 1+ |
| **22a** | Inactive | 20.9 ± 6.7 | — |
| **23a** | Inactive | 7.5 ± 1.2 | 2+ |
| **24a** | Inactive | 6.1 ± 0.7 | — |

[a] Inhibition of tubulin polymerization (ITP).
[b] Compounds were designated as 'inactive' when the measured ratio was greater than 57.
[c] $IC_{50}$ ± SEM of 3–7 independent triplicate experiments (B16 melanoma cell line).
[d] Morphological activity (rounding up) on modified HUVEC (EA·hy 926) is expressed as the lowest concentration at which cell rounding up was observed after a 2 h incubation period with the test compound. The compounds were tested at concentrations ranging from 0.00025 to 25 μg/mL. The number of + sign indicates rounding up more than 10% of cells at the following concentrations: 1+ at 25; 2+ at 2.5; 3+ at 0.25; 4+ at 0.025; and 5+ at 0.0025 μg/mL. A minus sign (−) indicates no rounding up.

The influence of a modification of the bridge was first evaluated. Among the compounds tested, only the carbinol **21a** had a close ITP value of 9 compared to **20a**, but this modification was accompanied by a 2-fold decrease in cytotoxic activity. Effects on the morphology of EA·hy 926 endothelial cells were similar to **20a**, with rounding up activity noted at a high concentration of 25 μg/mL.

Other derivatives, the methoxy **22a**, the acetoxy **23a**, and the methylene **24a**, were rather ineffective on tubulin inhibition (Table 1). However, compounds **23a** and **24a** presented cytotoxic activity similar to **20a**. Interestingly, the acetoxy derivative **23a** exhibited a slightly better activity on EA·hy 926 cell rounding up (at 2.5 μg/mL) than the starting compound **20a**.

These results show the difficulty in trying to establish structure–activity relationships for these types of modifications since good ITP and cytotoxicity values are not always correlated. No apparent correlation was also noted with regard to morphological effects on EA·hy 926 endothelial cells. Therefore, the global activity of our synthon was not markedly enhanced by replacing the carbonyl group. It can be hypothesized that this carbonyl group interacts at its binding site or that it allows a better positioning between the two rings. It was therefore decided to maintain the carbonyl group on the bridge and to modify the nature of the indolic nitrogen substituent.

### 3.2. Modifications of the N-position of the 1*H*-indol-3-yl-(3,4,5-trimethoxyphenyl) methane

Various nitrogen substitutions of the N-position were studied (Scheme 4). As previously observed for the

modifications of the methane bridge, correlations between the three biological tests were not clear, except for compounds **29a** and **30a** which are devoid of significant activities.

In terms of ITP, the benzenesulfonyl **20a** and the phenyl carbamate **35a** gave the best results (11 and 5, respectively) (Table 2). In terms of cytotoxicity the deprotected indole **25a** (5.0 μM) and the indoles bearing a carbonyl group (**32a**: COPh, **33a**: *p*-OMe–COPh, and **35a**: COOPh) yielded the best results (3.5, 5.3, and 5.2 μM, respectively). Only the phenyl carbamate **35a** exhibits a rather good correlation between both cytotoxic and antitubulin activities ($IC_{50}$ of 5.2 μM and ITP of 5). Substitution of the benzene sulfonyl by a methoxy group in *para* position decreases ITP and cytotoxic activities (compare **20a** and **34a**), indicating that the arylsulfonyl group does not mimic the B-ring of CSA-4.

Derivatives **25a**, **31a**, and **32a** were found to be the more efficient on cell shape rounding up of EA·hy 926 endothelial cells since they were still active at the concentration of 2.5 μg/mL (or 2+).

### 3.3. 6-Methoxy-1*H*-indol-3-yl-(3,4,5-trimethoxyphenyl) methane derivatives

The above SAR showed that the best biologically active molecules bear a carbonyl group on the bridge, and a carbonyl, a sulfonyl, or no substituent on the nitrogen. On the basis of these previous results, a new series of compounds bearing a methoxy group on the 6 position of the indole nucleus was prepared in an attempt to mimic the *p*-$OCH_3$ of the CSA-4. Indeed, this substituent is well known to play a major role on the activities.[20] To facilitate comparisons, the results of the two series are described in Table 3. The biological activity of colchicine and CSA-4 was included as reference compounds.

**Table 2.** Biological consequences of modifications of the N-position for (1*H*-indol-3-yl)-3,4,5-trimethoxyphenyl-methane compounds (**20a**, **25a**, and **28a**–**35a**)

| Compound | ITP ($IC_{50}/IC_{50colchicine}$)[a,b] | Cytotoxicity $IC_{50}$[c] (μM) | Morphological activity on EA·hy 926[d] |
|---|---|---|---|
| **20a** | 11 | 8.4 ± 0.9 | 1+ |
| **25a** | 57 | 5.0 ± 1.0 | 2+ |
| **28a** | 40 | 7.0 ± 1.6 | 1+ |
| **29a** | Inactive | 22.7 ± 1.9 | — |
| **30a** | 41 | 36.0 ± 5.2 | — |
| **31a** | 35 | 7.3 ± 3.2 | 2+ |
| **32a** | 31 | 3.5 ± 0.7 | 2+ |
| **33a** | 24 | 5.3 ± 1.1 | 1+ |
| **34a** | 24 | 23.9 ± 4.7 | 1+ |
| **35a** | 5 | 5.2 ± 2.2 | 1+ |

[a] Inhibition of tubulin polymerization (ITP).
[b] Compounds were designated as 'inactive' when the measured ratio was greater than 57.
[c] $IC_{50}$ ± SEM of 3–7 independent triplicate experiments (B16 melanoma cell line).
[d] Morphological activity (rounding up) on modified HUVEC (EA·hy 926) is expressed as the lowest concentration at which cell rounding up was observed after a 2 h incubation period with the test compound. The compounds were tested at concentrations ranging from 0.00025 to 25 μg/mL. The number of + sign indicates rounding up more than 10% of cells at the following concentrations: 1+ at 25; 2+ at 2.5; 3+ at 0.25; 4+ at 0.025; and 5+ at 0.0025 μg/mL. A minus sign (−) indicates no rounding up.

For most compounds, the presence of a 6-methoxy group markedly increased the ITP activity compared to the corresponding compound with no substituent (Table 3). Several of the 6-methoxylated compounds presented lower ITP values compared to the reference colchicine, for example, compounds **25b**, **26b**, **27b**, and **35b**, which were 2.5 (ITP = 0.4) to 5 (ITP = 0.2) times better than colchicine at inhibiting tubulin polymerization. These values are very close to that measured for CSA-4 (ITP = 0.7).

In parallel to their potent tubulin polymerization inhibition activity, the 6-methoxylated compounds **25b**–**27b** and **35b** also presented nanomolar $IC_{50}$ values against the B16 melanoma cell line of 13, 45, 24, and 23 nM, respectively (Table 3). These $IC_{50}$ values were close to that of colchicine (31 nM) or of reference compound CSA-4 (3 nM). Compounds **20b** and **32b** present despite their moderate ITP value a potent cytotoxicity with respective values of 211 nM and 35 nM. The intermediate **19b**, also evaluated under the same conditions, was devoid of activity (ITP value of 32 μM, $IC_{50}$ (B16) of 67 μM).

In addition, compounds which were most cytotoxic were also the ones that presented the best activity on the morphology of EA·hy 926 endothelial cells, with rounding up activity still observed at concentrations as low as 25 ng/mL. For the best compounds, the calculated concentrations for the rounding up of 50% of EA·hy 926 cells were as follows: CSA-4 = 1 ng/mL (3.0 nM); compound **25b** = 2 ng/mL (6.2 nM); compound **27b** = 19 ng/mL (52.6 nM); compound **35b** = 2 ng/mL (4.6 nM).

Figure 4 depicts representative changes in the morphology of EA·hy 926 cells after exposure to CSA-4 and to compounds **27b** and **35b**. Compared to control DMSO-treated cells (panel 1), it can be observed that within 2 h, the cells presented a characteristic rounding up morphology for CSA-4 (panel 2) used as a positive control. Similarly, the synthesized compounds **27b** and **35b** (panels 3 and 4, respectively) presented a rounding up aspect resembling that of CSA-4 (panel 2). The dual staining used for tubulin and actin allowed us to visualize the change in the distribution of these cytoskeleton components: in control cells, tubulin was relatively uniformly distributed within the entire cell area, whereas actin was diffusely distributed in the cell periphery; in cells treated with CSA-4, or compounds **27b** and **35b**, the rounded cell area was markedly reduced compared to controls, and the tubulin was localized close to the nuclear margin, whereas actin was localized on the cell periphery with frequent blebbing observed.

**Table 3.** Biological consequences of modifications on the N- and the C-6 position of (1*H*-indol-3-yl)-3,4,5-trimethoxyphenyl-methane

| Substitution | | $R_1$ = H (**a**) | | | | $R_1$ = $OCH_3$ (**b**) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Compound | ITP[a,b] | Cytotoxicity $IC_{50}$ (μM)[c] | EA·hy 926[d] | Compound | ITP[a] | Cytotoxicity $IC_{50}$ (μM)[c] | EA·hy 926[d] |
| $R_1$ = H | R = O | **25a** | 57 | 5.0 ± 1.0 | 2+ | **25b** | 0.4 | 0.013 ± 0.004 | 5+ |
| | R = S | | | | | **27b** | 0.2 | 0.024 ± 0.007 | 5+ |
| | R = H, H | **26a** | Inactive | 6.1 ± 1.0 | 2+ | **26b** | 0.2 | 0.045 ± 0.006 | 4+ |
| $R_1$ = $SO_2Ph$ | R = O | **20a** | 11 | 8.4 ± 0.9 | 1+ | **20b** | 12 | 0.211 ± 0.040 | 4+ |
| | R = H, OH | **21a** | 9 | 16.2 ± 0.8 | 1+ | **21b** | 13 | >51.70 | 1+ |
| | R = H, H | **24a** | Inactive | 6.1 ± 0.7 | — | **24b** | 4 | 7.0 ± 0.4 | 2+ |
| $R_1$ = COPh | R = O | **32a** | 31 | 3.5 ± 0.7 | 2+ | **32b** | 11 | 0.035 ± 0.022 | 3+ |
| $R_1$ = COOPh | R = O | **35a** | 5 | 5.2 ± 2.2 | 1+ | **35b** | 0.2 | 0.023 ± 0.010 | 5+ |

| Reference compounds | ITP[a] | Cytotoxicity $IC_{50}$[c] (μM) | Morphological activity on EA·hy 926[d] |
|---|---|---|---|
| Colchicine | 1.0[e] | 0.031 ± 0.003 | 3+ |
| CSA-4 | 0.7[f] | 0.003 ± 0.001 | 5+ |

[a] Inhibition of tubulin polymerization (ITP).

[b] Compounds were designated as 'inactive' when the measured ratio was greater than 57.

[c] $IC_{50}$ ± SEM of 3–7 independent triplicate experiments (B16 melanoma cell line).

[d] Morphological activity (rounding up) on modified HUVEC (EA·hy 926) is expressed as the lowest concentration at which cell rounding up was observed after a 2 h incubation period with the test compound. The compounds were tested at concentrations ranging from 0.00025 to 25 μg/mL. The number of + sign indicates rounding up of more than 10% of cells at the following concentrations: 1+ at 25; 2+ at 2.5; 3+ at 0.25; 4+ at 0.025; and 5+ at 0.0025 μg/mL. A minus sign (−) indicates no rounding up.

[e] $IC_{50colchicine}$ varies from 1.8 to 6.7 μM for the different experiments according to the tubulin concentration.

[f] Lit., $IC_{50}$ (CSA-4) = 2.2 ± 0.2 μM and $IC_{50}$ (colchicine) = 3.2 ± 0.4 μM.[34]

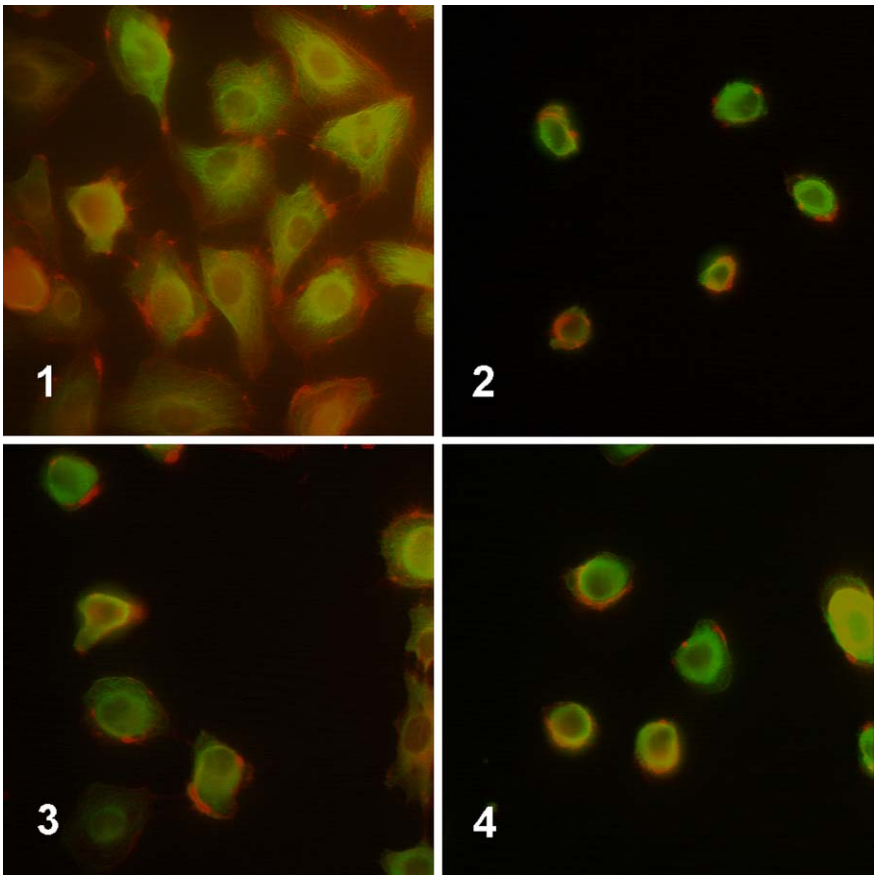



**Figure 4.** Representative photographs depicting the morphological aspects of EA·hy 926 endothelial cells after treatment with analogues of combretastatin A-4 (CSA-4). (1) Control cells exposed to 1% DMSO; (2) CSA-4 at 25 ng/mL; (3) compound **27b** at 25 ng/mL; and (4) compound **35b** at 25 ng/mL. Cells were exposed to the compounds for 2 h and fixed in ethanol. Tubulin (green) was visualized by employing a primary antibody to tubulin and a secondary antibody coupled to fluorescein. Actin (red) was visualized with phalloidin coupled to rhodamine. Photographs were taken at a magnification of 360× under fluorescent illumination.

## 4. Discussion

Among the synthesized compounds, the most biologically active were the ones bearing a $sp^2$ center between both aromatic groups (except for **26b**) and furthermore a 6-methoxy group, which markedly increased tubulin polymerization inhibition, cytotoxic activities, and morphological effects on EA·hy 926 cells. Compounds **20b** and **32b** and particularly **25b**, **27b**, and **35b** were found to display the stronger activities. The SAR observed in this study thus confirms further the interest of substituting the indole nucleus with a 6-methoxy group to increase its biological potency. In the course of the biological evaluation of our molecules, Liou et al., in the continuation of their previous work on phenstatin analogues,[44] have also recovered the potent activity of compounds **25b** and **26b**. The compound **25b** was submitted to further in vivo development as an antimitotic antitumoral agent under the reference number BPR0L075.[45] For both compounds **25b** and **26b**, good cytotoxic and ITP activities were also recovered. Similar potent activities were observed with the thioketone **27b** and the carbamate **35b**, showing that the bridge and the N-position accept some structural modifications. Results obtained with **27b** underline the importance for activities of a $sp^2$ center as linker between the two aryl parts of the molecule. Interestingly, the phenyl carbamate **35b** exhibits potent effects in both tests, whereas the benzamide **32b** displays a good cytotoxic activity and a moderate inhibition of tubulin polymerization. However, possibility that these protecting groups were cleaved in the conditions of experiments, leading to unsubstituted indoles, could not be discarded. Indeed, the *N*-1 bond is known to be very liable in the presence of a carbonyl group at the 3-position of the indole nucleus.[46]

Nevertheless, for the most active compounds, a good correlation was observed between both their cytotoxic and antitubulin activities.

The study of the morphological changes on EA·hy 926 cells allowed us to identify six potent compounds presenting a score greater than 3+ (colchicine value): **20b**, **25b**–**27b**, **32b**, and **35b**. Three of them, **25b**, **27b**, and **35b**, were as efficient as CSA-4 with a score of 5+. These results are in good agreement with the aforementioned data. It is the first report on the morphological effects of 3-aroylindole derivatives on endothelial cells, suggesting that these compounds may act in vivo as antivascular antitumoral agents.

## 5. Conclusion

Of twenty-three CSA-4 analogues synthesized and evaluated for their biological activities, four new compounds (**20b**, **27b**, **32b**, and **35b**) emerged as leads that presented activities as good as CSA-4, in terms of inhibition of tubulin polymerization and cytotoxicity on B16 melanoma cells. In addition, these compounds were as active as CSA-4 at altering the morphology of EA·hy 926 endothelial cells and therefore merit further testing in vivo to ascertain their potential antivascular antitumor activity.

## 6. 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. UV spectra were obtained with a BECKMAN DU 640 spectrophotometer apparatus. NMR spectra were recorded at 300 or 400 MHz ($^1$H NMR) and at 75 or 100 MHz ($^{13}$C NMR) with an AC 300 and an Avance 400 BRUKER spectrometers; chemical shifts are given in parts per million (ppm, $\delta$) relative to solvent peaks as internal standards ($\delta$: $CDCl_3$: 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 (DIC/$NH_3$) or with a ZQ2000 Waters mass spectrometer (ESI). Elemental analyses were performed at the Laboratoire de microanalyses (Université Paris 7—Denis Diderot, Paris, France). Flash chromatography was performed 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.

### 6.1. *N*-Benzenesulfonamide-3-methoxyaniline (17b)

To a mixture of anhydrous pyridine (6 mL) and dry $CH_2Cl_2$ (25 mL), *m*-anisidine (1.82 mL, 16.0 mmol) was added at room temperature. After a few minutes of stirring, benzenesulfonyl chloride (2.5 mL, 19.5 mmol) was added dropwise. The reaction monitored by TLC (toluene/EtOAc (5:5)) was stopped after 5 min. The solvents were evaporated to dryness and the residue was taken up with $CH_2Cl_2$. The organic solution was washed with water, HCl 10%, water again, and brine dried ($MgSO_4$), and concentrated in vacuo to yield 4.13 g of analytically pure **17b** (98%). Recrystallization from a 1:1 mixture of $CH_2Cl_2$ and MeOH gave large prismatic crystals. Mp 75–77 °C; IR (NaCl film) $\nu'$ ($cm^{-1}$) 3250, 3009, 2963, 2937, 1591, 1448, 1407, 1324, 1283, 1139, 1083, 1047, 939, 893, 754, 688; $^1$H NMR ($CDCl_3$) $\delta$ 3.68 (s, 3H, $C_3$–$OCH_3$), 6.61 (m, $J_{4-5}$ = 8.1 Hz, 1H, $H_4$), 6.73 (d, $J_{6-5}$ = 8.1 Hz, $H_6$), 6.77 (br s, 1H, $H_2$), 7.08 (t, $J_{5-6}$ = 8.1 Hz, 1H, $H_5$), 7.39 (m, 2H, $H_{3'}$ and $H_{5'}$), 7.48 (m, 1H, $H_{4'}$), 7.87 (d, $J_{2'-3'}$ = 7.40 Hz, 2H, $H_{2'}$ and $H_{6'}$), 8.01 (s, 1H, NH, $D_2O$ exch.); $^{13}$C NMR ($CDCl_3$) $\delta$ 55.4 ($C_3$–O*C*$H_3$), 106.9 ($C_2$), 111.0 ($C_4$), 113.4 ($C_6$), 127.4 ($C_{2'}$ and $C_{6'}$), 129.2 ($C_{3'}$ and $C_{5'}$), 130.1 ($C_5$), 133.1 ($C_{4'}$), 138.0 ($C_1$), 139.0 ($C_{1'}$), 160.3 ($C_3$); MS (ESI) *m*/*z* 308 $[M+2Na]^+$, 286 $[M+Na]^+$; Anal. ($C_{13}H_{13}NO_3S$) found: C, 59.05; H, 4.89; N, 5.21; calcd: C, 59.30; H, 4.98; N, 5.32.

### 6.2. *N*-(2,2-Diethoxyethyl)-*N*-(3-methoxyphenyl)benzenesulfonamide (18b)[40]

To a stirred solution of **17b** (427 mg, 1.62 mmol) in anhydrous DMF (20 mL), $NaHCO_3$ (269 mg, 1.94 mmol) was added at room temperature. The suspension was stirred for a few minutes, and then, a solution of bromoacetaldehyde diethyl acetal (584 μL, 3.88 mmol) was added dropwise, and the mixture was kept at 110 °C. After 20 h, 1.62 mmol (244 μL) of the acetal was added and the mixture was kept at 110 °C for 20 h. Another 1.62 mol (244 μL) of acetal was added and heating was continued for an additional 8 h. Then, DMF was removed under reduced pressure and the residue was taken up with $CH_2Cl_2$. The organic solution was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo to yield 565 mg of analytically pure **18b** (82%) as an amber oil. IR (NaCl film) $\nu'$ ($cm^{-1}$) 1605, 1485, 1450, 1350, 1285, 1260, 1205, 1170, 1060, 955, 705, 690; $^1$H NMR ($CDCl_3$) $\delta$ 1.38 (t, $J$ = 7.0 Hz, 6H, 2× $OCH_2CH_3$), 3.50 (ddd, $^2J$ = 14.1 Hz, $J$ = 5.5 Hz, $J$ = 5.5 Hz, 2H, $CH_2CH$), 3.64 (m, 4H, 2× $OCH_2CH_3$), 3.73 (s, 3H, $C_3$–$OCH_3$), 4.63 (t, $J$ = 5.5 Hz, 1H, $CH_2$ *CH*), 6.55–6.65 (m, 2H, $H_2$ and $H_4$), 6.83 (m, $J_{6-5}$ = 8.0 Hz, 1H, $H_6$), 7.18 (t, $J_{5-6}$ = 8.0 Hz, 1H, $H_5$), 7.46 (m, 2H, $H_{3'}$ and $H_{5'}$), 7.57 (m, 1H, $H_{4'}$), 7.63 (br d, $J$ = 8.5 Hz, 2H, $H_{2'}$ and $H_{6'}$); $^{13}$C NMR ($CDCl_3$) $\delta$ 15.2 ($OCH_2CH_3$), 53.3 ($CH_2CH$), 55.3 ($C_3$–$OCH_3$), 62.2 ($OCH_2CH_3$), 100.9 ($CH_2CH$), 113.9 ($C_2$), 114.5 ($C_4$), 120.7 ($C_6$), 127.7 ($C_{2'}$ and $C_{6'}$), 128.7 ($C_{3'}$ and $C_{5'}$), 129.4 ($C_5$), 132.7 ($C_{4'}$), 138.2 ($C_{1'}$), 141.2 ($C_1$), 159.8 ($C_3$); MS (ESI) *m*/*z* 402 $[M+Na]^+$.

### 6.3. *N*-Benzenesulfonyl-6-methoxy-1*H*-indole (19b)[40]

To a stirred mixture of **18b** (188 mg, 0.49 mmol) in $CH_2Cl_2$ (7 mL), boron trifluoride etherate (93.5 μL, 0.74 mmol) was added dropwise at 0 °C under argon. The reaction monitored by TLC (cyclohexane/EtOAc, (7:3)) was quenched after 15 min with a solution of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with $CH_2Cl_2$ and then washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give without further purification 138 mg of **19b** (97%) as a crystalline transparent solid. Recrystallization from $CH_2Cl_2$ and MeOH gave large prismatic crystals. Mp 132–135 °C (lit.[47] mp 140–142 °C ($CH_2Cl_2$/*n*-hexane); lit.,[40] mp 137–139 °C (ether)); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3138, 3111, 2975, 2847, 1618, 1580, 1446, 1363, 1281, 1216, 1180, 1118, 997, 858, 810, 725; $^1$H NMR ($CDCl_3$) $\delta$ 3.89 (s, 3H, $C_6$–$OCH_3$), 6.59 (dd, $J_{3,2}$ = 3.67 Hz,

$J_{3,7}$ = 0.65 Hz, 1H, $H_3$), 6.87 (dd, $J_{5,4}$ = 8.61 Hz, $J_{5,7}$ = 2.29 Hz, 1H, $H_5$), 7.39 (br d, $J_{4,5}$ = 8.61 Hz, 1H, $H_4$), 7.42–7.47 (m, 3H, $H_{3'}$, $H_{5'}$ and $H_2$), 7.52–7.55 (m, 2H, $H_{4'}$ and $H_7$), 7.85–7.89 (m, 2H, $H_{2'}$ and $H_{6'}$); $^{13}$C NMR ($CDCl_3$): $\delta$ 55.9 ($C_6$–$OCH_3$), 98.0 ($C_7$), 109.3 ($C_3$), 112.7 ($C_5$), 121.9 ($C_4$), 124.5 ($C_{3a}$), 125.2 ($C_2$), 126.8 ($C_{2'}$ and $C_{6'}$), 129.3 ($C_{3'}$ and $C_{5'}$), 133.9 ($C_{4'}$), 137.7 ($C_{7a}$), 138.4 ($C_{1'}$), 159.7 ($C_6$); MS (ESI) *m/z* 310 $[M+Na]^+$; Anal. ($C_{15}H_{13}NO_3S$) found: C, 63.05; H, 4.66; N, 4.98; calcd: C, 62.70; H, 4.56; N, 4.87.

### 6.4. (*N*-Benzenesulfonyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (20a)

To a magnetically stirred suspension of $AlCl_3$ (517 mg, 3.88 mmol) in $CH_2Cl_2$ at 25 °C was added 3,4,5-trimethoxybenzoyl chloride (538 mg, 2.33 mmol), and the mixture was stirred for 15 min to a red suspension. A solution of 1-(phenylsulfonyl)indole **19a** (500 mg, 1.94 mmol) in $CH_2Cl_2$ was added dropwise; the mixture was stirred at 25 °C for 30 min and poured onto crushed ice. The aqueous layer was extracted with $CH_2Cl_2$, and the organic extract was washed with brine, saturated aqueous $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography using cyclohexane/EtOAc (8:2) gave 707 mg of **20a** (80%) as a yellow powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave transparent crystals. Mp 142–143 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 227.0 ($\log\varepsilon$ = 4.48), 299.0 ($\log\varepsilon$ = 4.23) nm; IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2934, 2834, 1639, 1579, 1533, 1503, 1448, 1375, 1323, 1171, 1129; $^1$H NMR ($CDCl_3$) $\delta$ 3.93 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.98 (s, 3H, $C_{4'}$–$OCH_3$), 7.15 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.41 (t, $J_{5,4}$ = 7.2 Hz, $J_{5,6}$ = 7.2 Hz, 1H, $H_5$), 7.45 (dd, $J_{6,5}$ = 7.2 Hz, $J_{6,7}$ = 8.2 Hz, 1H, $H_6$), 7.50 (m, 2H, $H_{5''}$ and $H_{3''}$), 7.62 (m, 1H, $H_{4''}$), 7.95 (br d, $J$ = 7.7 Hz, 2H, $H_{2''}$ and $H_{6''}$), 8.04 (d, $J_{7,6}$ = 8.2 Hz, 1H, $H_7$), 8.07 (s, 1H, $H_2$), 8.26 (d, $J_{4,5}$ = 7.2 Hz, 1H, $H_4$); $^{13}$C NMR ($CDCl_3$) $\delta$ 53.3 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 106.7 ($C_{2'}$ and $C_{6'}$), 113.2 ($C_7$), 120.5 ($C_3$), 122.9 ($C_4$), 124.8 ($C_5$), 126.0 ($C_6$), 127.0 ($C_{2''}$ and $C_{6''}$), 128.5 ($C_{3a}$), 129.6 ($C_{3''}$ and $C_{5''}$), 132.8 ($C_2$), 134.1 ($C_{1'}$), 134.5 ($C_{4''}$), 135.1 ($C_{7a}$), 137.7 ($C_{1''}$), 142.2 ($C_{4'}$), 153.1 ($C_{3'}$ and $C_{5'}$), 189.5 (CO); MS (DIC/$NH_3$) *m/z* 452 $[M+H]^+$, 312 $[M+H-SO_2Ph]^+$; Anal. ($C_{24}H_{21}NO_6S$) found: C, 64.42; H, 4.59; N, 2.88; calcd: C, 63.8; H, 4.69; N, 3.10.

### 6.5. (*N*-Benzenesulfonyl-6-methoxy-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (20b)

The same procedure as described above was performed with **19b** (340 mg, 1.18 mmol) and gave after flash chromatography using cyclohexane/EtOAc (7:3) 490 mg of analytically pure **20b** (86%) as a yellowish crystal. Recrystallization from $CH_2Cl_2$ and MeOH gave large yellowish crystals. Mp 162–165 °C; IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2939, 2830, 1581, 1492, 1378, 1220, 1127, 1003, 726; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228 ($\log\varepsilon$ = 4.49), 246.0 ($\log\varepsilon$ = 4.32), 268.0 ($\log\varepsilon$ = 4.27), 295.0 ($\log\varepsilon$ = 4.22); $^1$H NMR ($CDCl_3$) $\delta$ 3.92 (s, 3H, $C_6$–$OCH_3$), 3.93 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.98 (s, 3H, $C_{4'}$–$OCH_3$), 7.02 (dd, $J_{5,4}$ = 8.8 Hz, $J_{5,7}$ = 2.3 Hz, 1H, $H_5$), 7.13 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.50 (m, 2H, $H_{5''}$and $H_{3''}$), 7.54 (d, $J_{7,5}$ = 2.3 Hz, 1H, $H_7$),7.62 (m, 1H, $H_{4''}$), 7.92 (m, 2H, $H_{2''}$ and $H_{6''}$), 7.95 (s, 1H, $H_2$), 8.12 (d, $J_{4,5}$ = 8.8 Hz, 1H, $H_4$); $^{13}$C NMR ($CDCl_3$) $\delta$ 55.8 ($C_6$–$OCH_3$), 56.3 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 97.5 ($C_7$), 106.7 ($C_{2'}$ and $C_{6'}$), 113.7 ($C_5$), 120.6 ($C_3$), 122.2 ($C_{3a}$), 123.4 ($C_4$), 126.9 ($C_{2''}$ and $C_{6''}$), 129.6 ($C_{3''}$ and $C_{5''}$), 131.7 ($C_2$), 134.1 ($C_{1'}$), 134.5 ($C_{4''}$), 136.2 ($C_{7a}$), 137.7 ($C_{1''}$), 142.0 ($C_{4'}$), 153.1 ($C_{3'}$ and $C_{5'}$), 158.8 ($C_6$), 189.6 (CO); MS (ESI) *m/z* 504 $[M+Na]^+$; Anal. ($C_{25}H_{23}NO_7S$) found: C, 62.43; H, 4.71; N, 3.02; calcd: C, 62.36; H, 4.81; N, 2.91.

### 6.6. (*N*-Benzenesulfonyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanol (21a)

To a magnetically stirred suspension of **20a** (500 mg, 1.11 mmol) in a 20/1 mixture of MeOH and THF, $NaBH_4$ (84 mg, 2.22 mmol) was added portionwise at room temperature. The reaction was monitored by TLC (cyclohexane/EtOAc (5:5)) and stopped after 5 min. The solvents were evaporated in vacuo, and the residue was taken up in $CH_2Cl_2$. The organic extract was washed with water, then with brine, dried ($MgSO_4$), and concentrated in vacuo to give 477 mg of analytically pure **21a** (95%). Recrystallization from THF and *n*-hexane gave large prismatic crystals. Mp 140 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 227.0 ($\log\varepsilon$ = 4.32); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3371, 2994, 2963, 2940, 2831, 1595, 1503, 1446, 1360, 1322, 1176, 1120; $^1$H NMR (DMSO-$d_6$) $\delta$ 3.58 (s, 3H, $C_{4'}$–$OCH_3$), 3.68 (s, 6H, $C_{3'-5'}$–$OCH_3$), 5.84 (d, $J_{CH,OH}$ = 4.0 Hz, 1H, CH), 5.97 (d, $J_{OH,CH}$ = 4.0 Hz, 1H, $D_2O$ exch., OH), 6.73 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.17 (dd, $J_{5,6}$ = 7.7 Hz, $J_{5,4}$ = 7.6 Hz,, 1H, $H_5$), 7.29 (dd, $J_{6,7}$ = 8.3 Hz, $J_{6,5}$ = 7.7 Hz, 1H, $H_6$), 7.53–7.56 (m, 3H, $H_4$, $H_{3''}$ and $H_{5''}$), 7.66 (m, 1H, $H_{4''}$), 7.68 (s, 1H, $H_2$), 7.91 (br d, $J_{7,6}$ = 8.3 Hz, 1H, $H_7$), 7.97 (br d, $J$ = 7.7 Hz, 2H, $H_{2''}$ and $H_{6''}$); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 56.9 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 69.4 (CHOH), 104.7 ($C_{2'}$ and $C_{6'}$), 114.3 ($C_7$), 122.3 ($C_4$), 124.1 ($C_2$), 124.3 ($C_5$), 125.8 ($C_6$), 127.7 ($C_{2''}$ and $C_{6''}$), 128.6 ($C_3$), 129.8 ($C_{3a}$), 130.8 ($C_{3''}$ and $C_{5''}$), 135.6 ($C_{4''}$), 135.9 ($C_{7a}$), 137.5 ($C_{4'}$), 138.1 ($C_{1''}$), 140.5 ($C_{1'}$), 153.6 ($C_{3'}$ and $C_{5'}$); MS (DIC/$NH_3$) *m/z* 471 $[M+NH_4]^+$; Anal. ($C_{24}H_{23}NO_6S$) found: C, 63.71; H, 5.09; N, 2.96; calcd: C, 63.56; H, 5.11; N, 3.09.

### 6.7. (*N*-Benzenesulfonyl-6-methoxy-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanol (21b)

The same procedure as described above was performed with **20b** (400 mg, 0,83 mmol) and gave 370 mg of analytically pure **21b** (92%). IR (NaCl film) $\nu'$ ($cm^{-1}$) 3371, 2994, 2963, 2940, 2831, 1595, 1503, 1446, 1360, 1322, 1176, 1120; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.19 (d, $J_{OH,CH}$ = 4.1 Hz, 1H, $D_2O$ exch., OH), 3.79 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.85 (s, 3H, $C_{4'}$–$OCH_3$), 3.87 (s, 3H, $C_6$–$OCH_3$), 5.91 (d, $J_{CH,OH}$ = 4.1 Hz, 1H, CH), 6.62 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.82 (dd, $J_{5,4}$ = 8.7 Hz, $J_{5,7}$ = 2.3 Hz, 1H, $H_5$), 7.30 (d, $J_{4,5}$ = 8.7 Hz, 1H, $H_4$), 7.36 (d, $J_{2,CH}$ = 0.9 Hz, 1H, $H_2$), 7.45 (m, 2H, $H_{5''}$ and $H_{3''}$), 7.54–7.56 (m, 2H, $H_7$ and $H_{4''}$), 7.88 (m, 2H, $H_{2''}$ and $H_{6''}$); $^{13}$C NMR ($CDCl_3$) $\delta$ 55.8 ($C_6$–$OCH_3$), 56.1 ($C_{3'-5'}$–$OCH_3$), 60.8 ($C_{4'}$–$OCH_3$), 70.4 (CHOH), 98.2

($C_7$), 103.8 ($C_{2'}$ and $C_{6'}$), 112.5 ($C_5$), 121.1 ($C_4$), 122.5 ($C_2$ and $C_{3a}$), 125.6 ($C_3$), 126.7 ($C_{2''}$ and $C_{6''}$), 129.2 ($C_{3''}$ and $C_{5''}$), 133.8 ($C_{4''}$), 136.8 ($C_{7a}$), 137.6 ($C_{1'}$ and $C_{4'}$), 138.3 ($C_{1''}$), 153.4 ($C_{3'}$ and $C_{5'}$), 158.2 ($C_6$); MS (ESI) *m/z* 506 $[M+Na]^+$; Anal. ($C_{25}H_{25}NO_7S$) found: C, 61.82; H, 5.11; N, 2.71; calcd: C, 62.10; H, 5.21; N, 2.90.

### 6.8. *N*-Benzenesulfonyl-3-[methoxy-(3,4,5-trimethoxyphenyl) methyl]-1*H*-indole (22a)

To a solution of **21a** (200 mg, 0.44 mmol) in a 20/1 mixture of MeOH and THF, pyridinium *p*-toluenesulfonate (223 mg, 0.89 mmol) was added and the reaction mixture was magnetically stirred at room temperature during 15 h. Solvents were evaporated under reduced pressure. The residue was dissolved in $CH_2Cl_2$, washed with brine, dried, and evaporated in vacuo. Flash chromatography using cyclohexane/EtOAc (6:4) gave 174 mg of **22a** (85%) as a colorless oil. UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 227.0 ($\log\varepsilon = 4.36$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2990, 2938, 2828, 1592, 1505, 1448, 1372, 1234, 1177, 1127, 1006, 986, 749, 728, 686; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.27 (s, 3H, –$OCH_3$), 3.59 (s, 3H, $C_{4'}$–$OCH_3$), 3.69 (s, 6H, $C_{3'-5'}$–$OCH_3$), 5.50 (s, 1H, CH), 6.71 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.19 (dd, $J_{5,4} = 7.9$ Hz, $J_{5,6} = 7.7$ Hz, 1H, $H_5$), 7.31 (dd, $J_{6,7} = 8.3$ Hz, $J_{6,5} = 7.7$ Hz, 1H, $H_6$), 7.51 (br d, $J_{4,5} = 7.9$ Hz, 1H, $H_4$), 7.55 (m, 2H, $H_{5''}$ and $H_{3''}$), 7.66 (m, 1H, $H_{4''}$), 7.75 (s, 1H, $H_2$), 7.91 (br d, $J_{7,6} = 8.3$ Hz, 1H, $H_7$), 7.98 (br d, $J = 7.7$ Hz, 2H, $H_{2''}$ and $H_{6''}$); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 56.9 ($C_{3'-5'}$–$OCH_3$), 57.3 ($OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 78.9 (CH), 105.0 ($C_{2'}$ and $C_{6'}$), 114.3 ($C_7$), 122.0 ($C_4$), 124.5 ($C_5$), 125.0 ($C_2$), 125.3 ($C_3$), 126.0 ($C_6$), 127.7 ($C_{2''}$ and $C_{6''}$), 129.5 ($C_{3a}$), 130.8 ($C_{3''}$ and $C_{5''}$), 135.6 ($C_{4''}$), 135.9 ($C_{7a}$), 137.1 ($C_{1'}$), 137.9 ($C_{4'}$), 138.0 ($C_{1''}$), 153.8 ($C_{3'}$ and $C_{5'}$); MS (DIC/$NH_3$) *m/z* 485 $[M+NH_4]^+$; Anal. ($C_{25}H_{25}NO_6S$) found: C, 64.05; H, 5.92; N, 2.68; calcd: C, 64.22; H, 5.39; N, 3.00.

### 6.9. Acetic acid (*N*-benzenesulfonyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methyl ester (23a)

To a solution of **21a** (200 mg, 0.44 mmol) in a mixture of acetic anhydride (2.5 mL) and acetic acid (2.5 mL) was added 4-DMAP (5 mg, 0.04 mmol) and the reaction mixture was magnetically stirred at room temperature. The reaction was monitored by TLC (cyclohexane/EtOAc (5:5)) and was quenched after 48 h. Water (2 mL) was added to hydrolyze the residual anhydride and solvents were evaporated in vacuo after addition of toluene (5 mL). The residue was dissolved in $CH_2Cl_2$, washed with brine, dried, and evaporated in vacuo. Compound **23a** was obtained without further purification as a colorless oil (207 mg, 95%). Mp 176–177 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 223.0 ($\log\varepsilon = 4.91$), 229.0 ($\log\varepsilon = 4.89$), 258.0 ($\log\varepsilon = 4.57$), 301.0 ($\log\varepsilon = 4.49$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3002, 2959, 2939, 2835, 1723, 1581, 1542, 1449, 1383, 1324, 1219, 1197, 1127; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.16 (s, 3H, $COCH_3$), 3.60 (s, 3H, $C_{4'}$–$OCH_3$), 3.70 (s, 6H, $C_{3'-5'}$–$OCH_3$), 6.76 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.96 (s, 1H, CH), 7.23 (dd, $J_{5,6} = 7.8$ Hz, $J_{5,4} = 7.6$ Hz, 1H, $H_5$), 7.34 (dd, $J_{6,7} = 8.3$ Hz, $J_{6,5} = 7.8$ Hz, 1H, $H_6$), 7.56–7.57 (m, 3H, $H_4$, $H_{5''}$ and $H_{3''}$), 7.68 (m, 1H, $H_{4''}$), 7.83 (s, 1H, $H_2$), 7.93 (br d, $J_{7,6} = 8.3$ Hz, 1H, $H_7$), 8.01 (br d, $J = 8.2$ Hz, 2H, $H_{2''}$ and $H_{6''}$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 21.9 ($COCH_3$), 57.0 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 71.4 (*CH*$OCOCH_3$), 105.2 ($C_{2'}$ and $C_{6'}$), 114.4 ($C_7$), 121.7 ($C_4$), 123.6 ($C_3$), 124.7 ($C_5$), 125.2 ($C_2$), 126.2 ($C_6$), 127.8 ($C_{2''}$ and $C_{6''}$), 129.1 ($C_{3a}$), 130.9 ($C_{3''}$ and $C_{5''}$), 135.4 ($C_{1'}$), 135.6 ($C_{7a}$), 135.8 ($C_{4''}$), 137.9 ($C_{1''}$), 138.2 ($C_{4'}$), 153.8 ($C_{3'}$ and $C_{5'}$), 170.6 (*CO*$CH_3$); MS (DIC/$NH_3$) *m/z* 436 $[M+H-CH_3COOH]^+$; Anal. ($C_{26}H_{25}NO_7S$) found: C, 66.59; H, 5.50; N, 3.92; calcd: C, 63.02; H, 5.09; N, 2.83.

### 6.10. *N*-Benzenesulfonyl-3-(3,4,5-trimethoxybenzyl)-1*H*-indole (24a)

To a magnetically stirred suspension of **21a** (50 mg, 0.11 mmol) in $CH_2Cl_2$ (5 mL) at 25 °C was added trifluoroacetic acid (82 μL, 1.1 mmol) and then triethylsilane (178 μL, 1.1 mmol). The reaction was followed by TLC (cyclohexane/EtOAc (5:5)) and was stopped after 15 min. The solvent was evaporated in vacuo, and the residue was dissolved in $CH_2Cl_2$. The organic extract was washed with an aqueous saturated solution of $NaHCO_3$, brine, dried ($MgSO_4$), and concentrated in vacuo. Recrystallization from THF and *n*-hexane gave analytically pure **24a** (43 mg, 90%) as large prismatic crystals. Mp 127–129 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.51$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3056, 2994, 2937, 2837, 1590, 1506, 1448, 1371, 1238, 1175, 1126, 725; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.57 (s, 3H, $C_{4'}$–$OCH_3$), 3.66 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.93 (s, 2H, $CH_2$), 6.58 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.21 (dd, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 7.5$ Hz, 1H, $H_5$), 7.31 (dd, $J_{6,7} = 8.4$ Hz, $J_{6,5} = 7.5$ Hz, 1H, $H_6$), 7.53–7.55 (m, 3H, $H_4$, $H_{5''}$ and $H_{3''}$), 7.66 (m, 1H, $H_{4''}$), 7.71 (s, 1H, $H_2$), 7.92 (br d, $J_{7,6} = 8.4$ Hz, 1H, $H_7$), 7.95 (br d, $J = 7.5$ Hz, 2H, $H_{2''}$ and $H_{6''}$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 31.6 ($CH_2$), 56.8 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 106.9 ($C_{2'}$ and $C_{6'}$), 114.3 ($C_7$), 121.2 ($C_4$), 123.8 ($C_3$), 124.4 ($C_5$), 125.0 ($C_2$), 125.9 ($C_6$), 127.5 ($C_{2''}$ and $C_{6''}$), 130.7 ($C_{3''}$ and $C_{5''}$), 131.4 ($C_{3a}$), 135.5 ($C_{4''}$), 135.7 ($C_{7a}$), 136.1 ($C_{1'}$), 136.8 ($C_{4'}$), 138.0 ($C_{1''}$), 153.7 ($C_{3'}$ and $C_{5'}$); MS (DIC/$NH_3$) *m/z* 455 $[M+NH_4]^+$, 438 $[M+H]^+$; Anal. ($C_{24}H_{23}NO_5S$) found: C, 66.03; H, 5.27; N, 3.13; calcd: C, 65.89; H, 5.30; N, 3.20.

### 6.11. *N*-Benzenesulfonyl-6-methoxy-3-(3,4,5-trimethoxybenzyl)-1*H*-indole (24b)

The same procedure as described above was performed with **21b** (73 mg, 0.15 mmol) and gave after recrystallization from $CH_2Cl_2$ and *n*-hexane 53.4 mg of **24b** (75%) as a greenish crystal. Mp 130–133 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 209.0 ($\log\varepsilon = 4.62$), 219.0 ($\log\varepsilon = 4.67$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2959, 2938, 2831, 1590, 1506, 1448, 1364, 1238, 1174, 1126, 725; $^1H$ NMR ($CDCl_3$) $\delta$ 3.75 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.83 (s, 3H, $C_{4'}$–$OCH_3$), 3.88 (s, 3H, $C_6$–$OCH_3$), 3.90 (s, 2H, $CH_2$), 6.39 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.83 (dd, $J_{5,4} = 8.6$ Hz, $J_{5,7} = 2.3$ Hz, 1H, $H_5$), 7.19 (s, 1H, $H_2$), 7.24 (d, $J_{4,5} = 8.6$ Hz, 1H, $H_4$), 7.43 (m, 2H, $H_{3''}$ and $H_{5''}$), 7.54 (m, 1H, $H_{4''}$), 7.55 (d, $J_{7,5} = 2.3$ Hz, 1H,

$H_7$), 7.85 (m, 2H, $H_{2''}$ and $H_{6''}$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 31.7 ($CH_2$), 55.7 ($C_6$–$OCH_3$), 56.0 ($C_{3'-5'}$–$OCH_3$), 60.8 ($C_{4'}$–$OCH_3$), 98.1 ($C_7$), 105.4 ($C_{2'}$ and $C_{6'}$), 112.3 ($C_5$), 120.3 ($C_4$), 122.5 ($C_2$ and $C_3$), 124.5 ($C_{3a}$), 126.6 ($C_{2''}$ and $C_{6''}$), 129.1 ($C_{3''}$ and $C_{5''}$), 133.7 ($C_{4''}$), 134.7 ($C_{1'}$), 136.3 ($C_{4'}$), 136.6 ($C_{7a}$), 138.2 ($C_{1''}$), 153.1 ($C_{3'}$ and $C_{5'}$), 158.1 ($C_6$); MS (ESI) *m/z* 490 $[M+Na]^+$; Anal. ($C_{25}H_{25}NO_6S$) found: C, 62.45; H, 5.56; N, 2.95; calcd: C, 64.22; H, 5.39; N, 3.00.

### 6.12. (1*H*-Indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (25a)

To a magnetically stirred solution of **20a** (300 mg, 0.66 mmol) in a 4/1 mixture of MeOH and $H_2O$, $K_2CO_3$ was added (230 mg, 1.65 mmol) and the reaction mixture was refluxed under $N_2$. The reaction was monitored by TLC (cyclohexane/EtOAc (5:5)) and stopped after 1 h by evaporation of MeOH. The aqueous residue was thoroughly extracted with $CH_2Cl_2$. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo to give 208 mg of **25a** (99%) as a white powder. Recrystallization from $CH_2Cl_2$ and MeOH gave transparent crystals. Mp 174–176 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.28$), 250.0 ($\log\varepsilon = 4.15$), 272.0 ($\log\varepsilon = 4.17$), 306.0 ($\log\varepsilon = 4.22$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3189, 2927, 2828, 1574, 1503, 1414, 1232, 1127, 744; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.75 (s, 3H, $C_{4'}$–$OCH_3$), 3.84 (s, 6H, $C_{3'-5'}$–$OCH_3$), 7.08 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.22-7.25 (m, 2H, $H_5$ and $H_6$), 7.50 (br d, $J_{7,6} = 7.8$ Hz, 1H, $H_7$), 8.09 (s, 1H, $H_2$), 8.23 (br d, $J_{4,5} = 7.2$ Hz, 1H, $H_4$), 12.02 (br s, 1H, $D_2O$ exch., NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 57.0 ($C_{3'-5'}$–$OCH_3$), 61.1 ($C_{4'}$–$OCH_3$), 107.1 ($C_{2'}$ and $C_{6'}$), 113.9 ($C_7$), 115.9 ($C_3$), 122.4 ($C_4$), 122.8 ($C_5$), 124.0 ($C_6$), 127.4 ($C_{3a}$), 136.5 ($C_2$), 136.8 ($C_{1'}$), 137.7 ($C_{7a}$), 141.1 ($C_{4'}$), 153.6 ($C_{3'}$ and $C_{5'}$), 189.9 (CO); MS (DIC/$NH_3$) *m/z* 312 $[M+H]^+$; Anal. ($C_{18}H_{17}NO_4$) found: C, 69.38; H, 5.35; N, 4.32; calcd: C, 69.44; H, 5.50; N, 4.50.

### 6.13. (6-Methoxy-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (25b)[44]

The same procedure as described above was performed with **20b** (60 mg, 0.12 mmol). Flash chromatography using EtOAc/cyclohexane (6:4) gave 40 mg of analytically pure **25b** (94%) as a white powder. Recrystallization from $CH_2Cl_2$ and MeOH gave transparent crystals. Mp 166–168 °C (lit.[44] mp 185–187 °C ($CH_2Cl_2$/EtOAc)); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3418, 3005, 2951, 2928, 2835, 1633, 1578, 1502, 1414, 1323, 1236, 1156, 1126, 770; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.88 (s, 3H, $C_6$–$OCH_3$), 3.90 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.94 (s, 3H, $C_{4'}$–$OCH_3$), 6.93 (d, $J_{7,5} = 2.1$ Hz, 1H, $H_7$), 6.99 (dd, $J_{5,4} = 8.7$ Hz, $J_{5,7} = 2.1$ Hz, 1H, $H_5$), 7.12 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.63 (d, $J_{2,NH} = 2.9$ Hz, 1H, $H_2$), 8.26 (d, $J_{4,5} = 8.7$ Hz, 1H, $H_4$), 8.61 (br s, 1H, $D_2O$ exch., NH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 55.5 ($C_6$–$OCH_3$), 56.2 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 95.0 ($C_7$), 106.2 ($C_{2'}$ and $C_{6'}$), 112.2 ($C_5$), 116.7 ($C_3$), 120.4 ($C_{3a}$), 122.8 ($C_4$), 133.0 ($C_2$), 135.9 ($C_{1'}$), 137.4 ($C_{7a}$), 140.7 ($C_{4'}$), 152.8 ($C_{3'}$ and $C_{5'}$), 157.4 ($C_6$), 190.6 (CO); MS (ESI) *m/z* 364 $[M+Na]^+$, 342 $[M+H]^+$; Anal. ($C_{19}H_{19}NO_5$) found: C, 65.81; H, 6.05; N, 3.69; calcd: C, 66.85; H, 5.61; N, 4.10.

### 6.14. 3-(3,4,5-Trimethoxybenzyl)-1*H*-indole (26a)

To a magnetically stirred solution of **24a** (60 mg, 0.20 mmol) in a 10/1 mixture of MeOH and $H_2O$, KOH was added (112 mg, 2 mmol) and the reaction mixture was refluxed for 48 h. MeOH was evaporated in vacuo, and the aqueous residue was thoroughly extracted with $CH_2Cl_2$. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography using $CH_2Cl_2$ gave 36 mg of **26a** (88%) as a white powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave a white solid. Mp 125–128 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.45$), 279.0 ($\log\varepsilon = 3.77$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2990, 2936, 2835, 1590, 1505, 1420, 1122, 1126, 1091, 994, 737; $^1H$ NMR ($CDCl_3$) $\delta$ 3.82 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.85 (s, 3H, $C_{4'}$–$OCH_3$), 4.08 (s, 2H, $CH_2$), 6.58 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.12 (m, 1H, $H_5$), 7.20 (m, 1H, $H_6$), 6.95 (br s, 1H, $H_2$), 7.38 (br d, $J_{7,6} = 8$ Hz, 1H, $H_7$), 7.58 (br d, $J_{4,5} = 7.5$ Hz, 1H, $H_4$), 8.05 (br s, 1H, $D_2O$ exch., NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 32.1 ($CH_2$), 56.1 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 105.8 ($C_{2'}$ and $C_{6'}$), 111.3 ($C_7$), 115.6 ($C_3$), 119.1 ($C_5$), 119.4 ($C_4$), 122.1 ($C_6$), 122.5 ($C_2$), 127.5 ($C_{3a}$), 136.2 ($C_{4'}$), 136.6 ($C_{7a}$), 137.1 ($C_{1'}$), 153.2 ($C_{3'}$ and $C_{5'}$); MS (DIC/$NH_3$) *m/z* 315 $[M+NH_4]^+$, 298 $[M+H]^+$; Anal. ($C_{18}H_{19}NO_3$) found: C, 72.33; H, 6.98; N, 4.39; calcd: C, 72.60; H, 6.40; N, 4.70.

### 6.15. 6-Methoxy-3-(3,4,5-trimethoxybenzyl)-1*H*-indole (26b)[44]

To a magnetically stirred solution of **24b** (150 mg, 0.32 mmol) in MeOH, a 0.1 M methanolic solution of MeONa (10 mL) was added and the reaction mixture was refluxed for 72 h. MeOH was evaporated in vacuo, and the residue was taken up with $CH_2Cl_2$. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography using cyclohexane/EtOAc (7:3) gave 85 mg of **26a** (81%) as a greenish powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave a white/greenish solid. Mp 95–97 °C (lit.[44] mp 97–98 °C); UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.60$), 272.0 ($\log\varepsilon = 3.77$), 293.0 ($\log\varepsilon = 3.66$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3372, 2994, 2938, 2901, 2834, 1629, 1591, 1504, 1457, 1237, 1125, 806; $^1H$ NMR ($CDCl_3$) $\delta$ 3.80 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.83 (s, 3H, $C_6$–$OCH_3$), 3.85 (s, 3H, $C_{4'}$–$OCH_3$), 4.03 (s, 2H, $CH_2$), 6.53 (s, 2H, $H_{2'}$ and $H_{6'}$), 6.77 (dd, $J_{5,4} = 8.6$ Hz, $J_{5,7} = 2.2$ Hz, 1H, $H_5$), 6.83 (br s, 1H, $H_2$), 7.41 (d, $J_{4,5} = 8.6$ Hz, 1H, $H_4$), 7.89 (br s, 1H, $D_2O$ exch., NH); $^{13}C$ NMR ($CDCl_3$) $\delta$ 32.1 ($CH_2$), 55.7 ($C_6$–$OCH_3$), 56.0 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 94.7 ($C_7$), 105.6 ($C_{2'}$ and $C_{6'}$), 109.3 ($C_5$), 115.4 ($C_3$), 119.7 ($C_4$), 121.2 ($C_2$), 121.7 ($C_{3a}$), 136.0 ($C_{4'}$), 137.1 ($C_{1'}$), 137.3 ($C_{7a}$), 153.1 ($C_{3'}$ and $C_{5'}$), 156.5 ($C_6$); MS (ESI) *m/z* 366 $[M+K]^+$, 350 $[M+Na]^+$; Anal. ($C_{19}H_{21}NO_4$) found: C, 69.62; H, 6.09; N, 4.27; calcd: C, 69.71; H, 6.47; N, 4.28.

### 6.16. (6-Methoxy-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanethione (27b)

To a magnetically stirred solution of **25a** (65 mg, 0.19 mmol) in anhydrous THF at 25 °C was added Lawesson's reagent (89 mg, 0.23 mmol). The mixture monitored by TLC (cyclohexane/EtOAc (5:5)) was stirred for 1h. THF was evaporated in vacuo, and the residue was dissolved in $CH_2Cl_2$. The organic extract was washed with water, dried ($MgSO_4$), and concentrated in vacuo. Flash chromatography using cyclohexane/EtOAc (5:5) gave analytically pure 62 mg of **27b** (91%) as a reddish oil. UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 227.0 ($\log\varepsilon = 4.40$), 291 ($\log\varepsilon = 3.96$), 392 ($\log\varepsilon = 4.04$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3311, 2934, 2835, 1625, 1579, 1487, 1448, 1411, 1324, 1232, 1161, 1125, 1028, 1001, 822; $^1H$ NMR ($CDCl_3$) $\delta$ 3.87 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.89 (s, 3H, $C_6$–$OCH_3$), 3.92 (s, 3H, $C_{4'}$–$OCH_3$), 6.93 (d, $J_{7,5} = 2.2$ Hz, 1H, $H_7$), 6.96 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.01 (dd, $J_{5,4} = 8.9$ Hz, $J_{5,7} = 2.2$ Hz, 1H, $H_5$), 7.59 (d, $J_{2,NH} = 3.1$ Hz, 1H, $H_2$), 8.58 (d, $J_{4,5} = 8.9$ Hz, 1H, $H_4$), 8.64 (br s, 1H, $D_2O$ exch., NH); $^{13}C$ NMR ($CDCl_3$): $\delta$ 55.6 ($C_6$–$OCH_3$), 56.2 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 95.4 ($C_7$), 106.4 ($C_{2'}$ and $C_{6'}$), 112.7 ($C_5$), 120.9 ($C_3$), 123.7 ($C_4$), 129.3 ($C_{3a}$), 132.2 ($C_2$), 138.4 ($C_{7a}$), 139.7 ($C_{4'}$), 145.1 ($C_{1'}$), 152.3 ($C_{3'}$ and $C_{5'}$), 157.8 ($C_6$), 222.6 (CS); MS (ESI) *m/z* 356 $[M-H]^-$; Anal. ($C_{19}H_{19}NO_4S$) found: C, 64.42; H, 5.90; N, 3.76; calcd: C, 63.85; H, 5.36; N, 3.92.

### 6.17. General procedure for the preparation of the N-substituted methanones 28a–35a, 32b, and 35b

A solution of **25a** or **25b** (50 mg) in 5 mL of EtOH was magnetically stirred during 15 min at room temperature in the presence of KOH (1.2 equiv). EtOH was evaporated under reduced pressure. The residue was taken up in 5 mL of dry acetone. Anhydrous sodium sulfate (2 equiv) and a slight excess (1.2–1.5 equiv) of alkyl or acyl halides were successively added. The reaction mixture was monitored by TLC and stopped by filtration and solvent evaporation. The residue was taken up in $CH_2Cl_2$ and washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude residue was then purified by flash chromatography.

**6.17.1. (*N*-Methyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (28a).** The general procedure was performed with **25a** (207 mg, 0.66 mmol) and dimethylsulfate (63 μL, 0.79 mmol). The reaction was monitored by TLC (cyclohexane/EtOAc (5:5)). The residue was purified by flash chromatography using cyclohexane/EtOAc (6:4) to give 195 mg of analytically pure **28a** (90%) as an oil. UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.42$), 260.0 ($\log\varepsilon = 4.11$), 314.0 ($\log\varepsilon = 4.12$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2932, 2831, 1633, 1581, 1464, 1367, 1323, 1218, 1125; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.75 (s, 3H, $C_{4'}$–$OCH_3$), 3.86 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.88 (s, 3H, $NCH_3$), 7.07 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.28 (dd, $J_{5,4} = 7.6$ Hz, $J_{5,6} = 7.3$ Hz, 1H, $H_5$), 7.32 (dd, $J_{6,7} = 7.6$ Hz, $J_{6,5} = 7.3$ Hz, 1H, $H_6$), 7.57 (br d, $J_{7,6} = 7.6$ Hz, 1H, $H_7$), 8.13 (s, 1H, $H_2$), 8.26 (br d, $J_{4,5} = 7.6$ Hz, 1H, $H_4$); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 34.2 ($NCH_3$), 57.1 ($C_{3'-5'}$–$OCH_3$), 61.2 ($C_{4'}$–$OCH_3$), 107.1 ($C_{2'}$ and $C_{6'}$), 111.7 ($C_7$), 114.7 ($C_3$), 122.6 ($C_4$), 123.2 ($C_5$), 124.2 ($C_6$), 127.8 ($C_{3a}$), 136.8 ($C_{1'}$), 138.4 ($C_{7a}$), 140.3 ($C_2$), 141.1 ($C_{4'}$), 153.7 ($C_{3'}$ and $C_{5'}$), 189.6 (CO); MS (DIC/$NH_3$) *m/z* 326 $[M+H]^+$; Anal. ($C_{19}H_{19}NO_4$) found: C, 64.2; H, 5.97; N, 3.83; calcd: C, 70.14; H, 5.89; N, 4.31.

**6.17.2. (*N*-Benzyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (29a).** The general procedure was performed with **25a** (50 mg, 0.16 mmol) and benzyl bromide (24 μL, 0.19 mmol) and gave after flash chromatography using cyclohexane and EtOAc (6:4), 62 mg of **29a** (96%) as a colorless oil. UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 226.0 ($\log\varepsilon = 4.35$), 260.0 ($\log\varepsilon = 4.10$), 274.0 ($\log\varepsilon = 4.06$), 314.0 ($\log\varepsilon = 4.18$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3055, 3001, 2937, 2836, 1621, 1580, 1521, 1503, 1463, 1412, 1382, 1323, 1234, 1169, 1126, 813, 750; $^1H$ NMR ($CDCl_3$) $\delta$ 3.85 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.92 (s, 3H, $C_{4'}$–$OCH_3$), 5.37 (s, 2H, $CH_2$), 7.08 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.21 (m, 2H, $H_{2''}$ and $H_{6''}$), 7.32–7.44 (m, 6H, $H_{3''}$, $H_{5''}$, $H_{4''}$, $H_5$, $H_6$ and $H_7$), 7.63 (s, 1H, $H_2$), 8.41 (m, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 50.6 ($CH_2$), 56.2 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 106.4 ($C_{2'}$ and $C_{6'}$), 110.0 ($C_7$), 115.9 ($C_3$), 122.7 ($C_4$ and $C_5$), 123.8 ($C_6$), 127.2 ($C_{3a}$, $C_{2''}$ and $C_{6''}$), 128.3 ($C_{4''}$), 129.0 ($C_{3''}$ and $C_{5''}$), 135.7 ($C_{1''}$), 135.9 ($C_{1'}$), 136.3 ($C_2$), 137.0 ($C_{7a}$), 141.0 ($C_{4'}$), 152.9 ($C_{3'}$ and $C_{5'}$), 192.2 (CO); MS (ESI) *m/z* 424 $[M+Na]^+$, 402 $[M+H]^+$; Anal. ($C_{25}H_{23}NO_4$) found: C, 70.6; H, 6.17; N, 3.12; calcd: C, 74.79; H, 5.77; N, 3.49.

**6.17.3. [*N*-(4-Methoxybenzyl)-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (30a).** The general procedure was performed with **25a** (50 mg, 0.16 mmol) and *p*-methoxybenzyl chloride (27 μL, 0.19 mmol) and gave after flash chromatography using cyclohexane/EtOAc (6:4), 63 mg of **30a** (91%) as a colorless oil. UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 227.0 ($\log\varepsilon = 4.53$), 268.0 ($\log\varepsilon = 4.17$), 315.0 ($\log\varepsilon = 4.21$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3045, 3000, 2937, 2836, 1613, 1580, 1515, 1463, 1412, 1382, 1324, 1249, 1175, 1127, 750; $^1H$ NMR ($CDCl_3$): $\delta$ 3.79 (s, 3H, $C_{4''}$–$OCH_3$), 3.85 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.92 (s, 3H, $C_{4'}$–$OCH_3$), 5.29 (s, 2H, $CH_2$), 6.87 (m, 2H, $H_{3''}$ and $H_{5''}$), 7.07 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.16 (m, 2H, $H_{2''}$ and $H_{6''}$), 7.32–7.37 (m, 2H, $H_5$ and $H_6$), 7.42 (m, 1H, $H_7$), 7.59 (m, 1H, $H_2$), 8.40 (m, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 50.2 ($CH_2$), 55.3 ($C_{4''}$–$OCH_3$), 56.2 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 106.4 ($C_{2'}$ and $C_{6'}$), 110.1 ($C_7$), 114.4 ($C_{3''}$ and $C_{5''}$), 115.7 ($C_3$), 122.7 ($C_4$ and $C_5$), 123.7 ($C_6$), 127.5 ($C_{1''}$ and $C_{3a}$), 128.9 ($C_{2''}$ and $C_{6''}$), 136.0 ($C_{1''}$), 136.3 ($C_2$), 137.1 ($C_{7a}$), 140.9 ($C_{4'}$), 152.9 ($C_{3'}$ and $C_{5'}$), 159.6 ($C_{4''}$), 189.8 (CO); MS (ESI) *m/z* 454 $[M+Na]^+$; Anal. ($C_{26}H_{25}NO_5$) found: C, 72.01; H, 6.52; N, 2.82; calcd: C, 72.37; H, 5.84; N, 3.25.

**6.17.4. [*N*-Acetyl-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (31a).** The general procedure was performed with **25a** (50 mg, 0.16 mmol) and acetyl chloride (14 μL, 0.20 mmol) and gave after flash chromatography using cyclohexane/EtOAc (6:4), 48 mg of **31a** (85%) as a crystalline solid. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave large prismatic crystals.

Mp 180–182 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 229.0 ($\log\varepsilon = 4.49$), 256.0 ($\log\varepsilon = 4.22$), 306.0 ($\log\varepsilon = 4.25$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3002, 2967, 2939, 2835, 1722, 1634, 1581, 1449, 1383, 1324, 1219, 1197, 1126; $^1H$ NMR (DMSO-$d_6$) $\delta$ 2.74 (s, 3H, $COCH_3$), 3.77 (s, 3H, $C_{4'}$–$OCH_3$), 3.87 (s, 6H, $C_{3'-5'}$–$OCH_3$), 7.23 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.42 (dd, $J_{5,4} = 7.3$ Hz, $J_{5,6} = 7.1$ Hz, 1H, $H_5$), 7.45 (dd, $J_{6,5} = 7.1$ Hz, $J_{6,7} = 7.0$ Hz, 1H, $H_6$), 8.20 (br d, $J_{4,5} = 7.3$ Hz, 1H, $H_4$), 8.40–8.41 (m, 2H, $H_7$ and $H_2$); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 25.0 ($COCH_3$), 57.2 ($C_{3'-5'}$–$OCH_3$), 61.2 ($C_{4'}$–$OCH_3$), 107.7 ($C_{2'}$ and $C_{6'}$), 116.9 ($C_7$), 119.3 ($C_3$), 122.6 ($C_4$), 125.7 ($C_5$), 126.9 ($C_6$), 129.0 ($C_{3a}$), 135.0 ($C_{1'}$), 136.5 ($C_2$ and $C_{7a}$), 141.8 ($C_{4'}$), 153.9 ($C_{3'}$ and $C_{5'}$), 171.7 ($COCH_3$), 190.3 (CO); MS (DIC/$NH_3$) $m/z$ 354 $[M+H]^+$, 312 $[M+H-COCH_3]^+$; Anal. ($C_{20}H_{19}NO_5$) found: C, 67.99; H, 5.41; N, 4.00; calcd: C, 67.98; H, 5.42; N, 3.96.

**6.17.5. (*N*-Benzoyl-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (32a).** The general procedure was performed with **25a** (50 mg, 0.16 mmol) and benzoyl chloride (23 μL, 0.19 mmol) and gave after flash chromatography using cyclohexane/EtOAc (6:4), 56 mg of **32a** (84%) as a white powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave colorless needles. Mp 138–140 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.55$), 310.0 ($\log\varepsilon = 4.23$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2940, 2837, 1698, 1637, 1581, 1541, 1504, 1450, 1377, 1327, 1128; $^1H$ NMR ($CDCl_3$) $\delta$ 3.88 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.93 (s, 3H, $C_{4'}$–$OCH_3$), 7.13 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.45–7.50 (m, 2H, $H_5$ and $H_6$), 7.55 (m, 2H, $H_{3''}$ and $H_{5''}$), 7.67 (m, 1H, $H_{4''}$), 7.79 (br d, $J = 7.2$ Hz, 2H, $H_{2''}$ and $H_{6''}$), 7.82 (s, 1H, $H_2$), 8.29 (m, 1H, $H_4$), 8.35 (m, 1H, $H_7$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 56.3 ($C_{3'-5'}$–OMe), 61.0 ($C_{4'}$–OMe), 106.6 ($C_{2'}$ and $C_{6'}$), 116.0 ($C_7$), 120.3 ($C_3$), 122.5 ($C_4$), 125.3 ($C_5$), 126.2 ($C_6$), 128.6 ($C_{3a}$), 128.9 ($C_{3''}$ and $C_{5''}$), 129.2 ($C_{2''}$ and $C_{6''}$), 132.9 ($C_{4''}$), 133.5 ($C_{1''}$), 134.1 ($C_2$), 134.3 ($C_{1'}$), 136.6 ($C_{7a}$), 141.7 ($C_{4'}$), 153.1 ($C_{3'}$ and $C_{5'}$), 168.6 (NCO), 191.2 (CO); MS (ESI) $m/z$ 438 $[M+Na]^+$; Anal. ($C_{25}H_{21}NO_5$) found: C, 72.51; H, 5.28; N, 2.75; calcd: C, 72.28; H, 5.10; N, 3.37.

**6.17.6. (*N*-Benzoyl-6-methoxy-1*H*-indol-3-yl)-(3,4,5-trimethoxyphenyl) methanone (32b).** The general procedure was performed with **25b** (52 mg, 0.15 mmol) and benzoyl chloride (22 μL, 0.18 mmol) and gave after flash chromatography using cyclohexane/EtOAc (7:3), 50 mg of **32b** (74%) as a yellowish powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave yellowish crystals. Mp 118–121 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 229.0 ($\log\varepsilon = 4.54$), 273.0 ($\log\varepsilon = 4.42$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2940, 2835, 1698, 1634, 1581, 1492, 1324, 1217, 1127; $^1H$ NMR ($CDCl_3$): 3.87 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.92 (s, 6H, $C_6$–$OCH_3$ and $C_{4'}$–$OCH_3$), 7.10 (dd, $J_{5,4} = 8.8$ Hz, $J_{5,7} = 2.3$ Hz, 1H, $H_5$), 7.11 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.55 (m, 2H, $H_{3''}$ and $H_{5''}$), 7.69 (s, 1H, $H_2$), 7.78 (m, 2H, $H_{2''}$ and $H_{6''}$), 7.95 (d, $J_{7,5} = 2.3$ Hz, 1H, $H_7$), 8.12 (m, 1H, $H_{4''}$), 8.15 (d, $J_{4,5} = 8.8$ Hz, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) 55.7 ($C_6$–$OCH_3$), 56.3 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 99.9 ($C_7$), 106.6 ($C_{2'}$ and $C_{6'}$), 114.6 ($C_5$), 120.3 ($C_3$), 122.1 ($C_{3a}$), 123.0 ($C_4$), 128.9 ($C_{3''}$ and $C_{5''}$), 129.2 ($C_{2''}$ and $C_{6''}$), 130.2 ($C_{4''}$), 132.8 ($C_{1''}$), 133.1 ($C_2$), 134.3 ($C_{1'}$), 137.6 ($C_{7a}$), 141.9 ($C_{4'}$), 153.1 ($C_{3'}$ and $C_{5'}$), 159.0 ($C_6$), 168.9 (CO), 189.8 (CO); MS (ESI) $m/z$ 468 $[M+Na]^+$, 446 $[M+H]^+$; Anal. ($C_{26}H_{23}NO_6$) found: C, 70.14; H, 5.53; N, 3.22; calcd: C, 70.10; H, 5.20; N, 3.14.

**6.17.7. [*N*-(4-Methoxybenzoyl)-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (33a).** The general procedure was performed with **25a** (24 mg, 0.08 mmol) and *p*-methoxybenzoyl chloride (16 mg, 1.00 mmol) and gave after recrystallization from $CH_2Cl_2$ and *n*-hexane 23 mg of **33a** (67%) as a white solid. Mp 137–139 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 225.0 ($\log\varepsilon = 4.49$), 260.0 ($\log\varepsilon = 4.37$), 309.0 ($\log\varepsilon = 4.27$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3000, 2963, 2929, 2829, 1691, 1635, 1602, 1575, 1445, 1322, 1258, 1165, 1130; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.89 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.92 (s, 3H, $C_{4''}$–$OCH_3$), 3.93 (s, 3H, $C_{4'}$–$OCH_3$), 7.02 (br d, $J = 8.7$ Hz, 2H, $H_{3''}$ and $H_{5''}$), 7.14 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.45–7.47 (m, 2H, $H_5$ and $H_6$), 7.79 (br d, $J = 8.7$ Hz, 2H, $H_{2''}$ and $H_{6''}$), 7.87 (s, 1H, $H_2$), 8.25 (m, 1H, $H_7$), 8.29 (m, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 55.6 ($C_{4''}$–$OCH_3$), 56.3 ($C_{3'-5'}$–$OCH_3$), 60.9 ($C_{4'}$–$OCH_3$), 106.6 ($C_{2'}$ and $C_{6'}$), 114.3 ($C_{3''}$ and $C_{5''}$), 115.7 ($C_7$), 119.9 ($C_3$), 122.4 ($C_4$), 125.0 ($C_5$), 125.3 ($C_{1''}$), 126.0 ($C_6$), 128.5 ($C_{3a}$), 131.9 ($C_{2''}$ and $C_{6''}$), 134.4 ($C_2$ and $C_{1'}$), 136.8 ($C_{7a}$), 141.8 ($C_{4'}$), 153.1 ($C_{3'}$ and $C_{5'}$), 163.7 ($C_{4''}$), 168.3 (NCO), 190.4 (CO); MS (ESI) $m/z$ 468 $[M+Na]^+$; Anal. ($C_{26}H_{23}NO_6$) found: C, 70.93; H, 5.47; N, 3.18; calcd: C, 70.10; H, 5.20; N, 3.14.

**6.17.8. [*N*-(4-Methoxybenzenesulfonyl)-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (34a).** The general procedure was performed with **25a** (40 mg, 0.13 mmol) and *p*-methoxybenzenesulfonyl chloride (32 mg, 0.16 mmol) and gave after flash chromatography using cyclohexane/EtOAc (6:4), 54 mg of **34a** (87%) as a yellow powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave yellow crystals. Mp 161–162 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.38$), 249.0 ($\log\varepsilon = 4.42$), 299.0 ($\log\varepsilon = 4.24$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3001, 2938, 2834, 1635, 1577, 1496, 1414, 1374, 1168, 1130; $^1H$ NMR (DMSO-$d_6$) $\delta$ 3.82 (s, 3H, $C_{4''}$–$OCH_3$), 3.93 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.98 (s, 3H, $C_{4'}$–$OCH_3$), 6.92 (br d, $J = 9.0$ Hz, 2H, $H_{3''}$ and $H_{5''}$), 7.14 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.39 (ddd, $J_{5,4} = 7.5$ Hz, $J_{5,6} = 7.3$ Hz, $J_{5,7} = 1.0$ Hz, 1H, $H_5$), 7.43 (ddd, $J_{6,7} = 7.5$ Hz, $J_{6,5} = 7.3$ Hz, $J_{6,4} = 1.0$ Hz, 1H, $H_6$), 7.88 (br d, $J = 9.0$ Hz, 2H, $H_{2''}$ and $H_{6''}$), 8.01 (br d, $J_{7,6} = 7.5$ Hz, 1H, $H_7$), 8.06 (s, 1H, $H_2$), 8.26 (br d, $J_{4,5} = 7.5$ Hz, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) $\delta$ 55.8 ($C_{4''}$–$OCH_3$), 56.3 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 106.7 ($C_{2'}$ and $C_{6'}$), 113.2 ($C_7$), 114.8 ($C_{3''}$ and $C_{5''}$), 120.2 ($C_3$), 122.8 ($C_4$), 124.7 ($C_5$), 125.9 ($C_6$), 128.5 ($C_{3a}$), 128.9 ($C_{1''}$), 129.4 ($C_{2''}$ and $C_{6''}$), 133.0 ($C_2$), 134.2 ($C_{1'}$), 135.0 ($C_{7a}$), 142.1 ($C_{4'}$), 153.2 ($C_{3'}$ and $C_{5'}$), 164.4 ($C_{4''}$), 189.6 (CO); MS (ESI) $m/z$ 504 $[M+Na]^+$, 482 $[M+H]^+$; Anal. ($C_{25}H_{23}NO_7S$) found: C, 61.06; H, 4.90; N, 3.03; calcd: C, 62.36; H, 4.81; N, 2.91.

**6.17.9. [*N*-Phenyloxycarbonyl-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (35a).** The general procedure was performed with **25a** (60 mg, 0.19 mmol) and phenylchloroformate (30 μL, 0.23 mmol) and gave after flash

chromatography using cyclohexane/EtOAc (8:2), 40 mg of **35a** (67%) as a white powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave prismatic colorless crystals. Mp 155–156 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228 ($\log\varepsilon = 4.51$), 249 ($\log\varepsilon = 4.24$), 299 ($\log\varepsilon = 4.24$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 3002, 2936, 2835, 1755, 1639, 1581, 1452, 1225, 1127, 1004; $^1H$ NMR (acetone-$d_6$) $\delta$ 3.83 (s, 3H, $C_{4'}$–$OCH_3$), 3.93 (s, 6H, $C_{3'-5'}$–$OCH_3$), 7.29 (s, 2H, $H_{2'}$ and $H_{6'}$), 7.39 (m, 1H, $H_{4''}$), 7.44–7.55 (m, 6H, $H_{2''}$, $H_{6''}$, $H_{3''}$, $H_{5''}$, $H_5$ and $H_6$), 8.28 (br d, $J_{7,6} = 7.8$ Hz, 1H, $H_7$), 8.32 (br d, $J_{4,5} = 7.8$ Hz, 1H, $H_4$), 8.47 (s, 1H, $H_2$); $^{13}C$ NMR (acetone-$d_6$) 57.2 ($C_{3'-5'}$–$OCH_3$), 61.3 ($C_{4'}$–$OCH_3$), 108.2 ($C_{2'}$ and $C_{6'}$), 116.4 ($C_7$), 121.2 ($C_3$), 123.1 ($C_{2''}$ and $C_{6''}$), 123.8 ($C_4$), 126.0 ($C_5$), 127.3 ($C_6$), 128.1 ($C_{4''}$), 130.0 ($C_{3a}$), 131.1 ($C_{3''}$ and $C_{5''}$), 135.1 ($C_2$ and $C_{1'}$), 135.9 ($C_{7a}$), 141.9 ($C_{4'}$), 148.9 (NCOO), 150.5 ($C_{1''}$), 154.9 ($C_{3'}$ and $C_{5'}$), 190.7 (CO); MS (ESI) *m/z* 454 $[M+Na]^+$; Anal. ($C_{25}H_{21}NO_6$) found: C, 69.49; H, 4.75; N, 3.21; calcd: C, 69.60; H, 4.91; N, 3.25.

**6.17.10. [*N*-Phenyloxycarbonyl-6-methoxy-1*H*-indol-3-yl]-(3,4,5-trimethoxyphenyl) methanone (35b).** The general procedure was performed with **25b** (57 mg, 0.17 mmol) and phenylchloroformate (26 μL, 0.20 mmol) and gave after flash chromatography using cyclohexane/EtOAc (8:2), 50 mg of **35b** (65%) as a white powder. Recrystallization from $CH_2Cl_2$ and *n*-hexane gave colorless needle crystals. Mp 90–93 °C; UV ($CH_2Cl_2$) $\lambda_{max}$ (nm) 228.0 ($\log\varepsilon = 4.55$), 264.5 ($\log\varepsilon = 4.36$); 299.5 ($\log\varepsilon = 4.17$); IR (NaCl film) $\nu'$ ($cm^{-1}$) 2998, 2940, 2837, 1760, 1637, 1581, 1493, 1391, 1326, 1214, 1127, 1003; $^1H$ NMR ($CDCl_3$) 3.90 (s, 3H, $C_6$–$OCH_3$), 3.93 (s, 6H, $C_{3'-5'}$–$OCH_3$), 3.96 (s, 3H, $C_{4'}$–$OCH_3$), 7.08 (dd, $J_{5,4} = 8.8$ Hz, $J_{5,7} = 2.3$ Hz, 1H, $H_5$), 7.30 (m, 2H, $H_{2''}$ and $H_{6''}$), 7.37 (m, 1H, $H_{4''}$), 7.50 (m, 2H, $H_{3''}$ and $H_{5''}$), 7.85 (d, $J_{7,5} = 2.3$ Hz, 1H, $H_7$), 8.16 (s, 1H, $H_2$), 8.17 (d, $J_{4,5} = 8.8$ Hz, 1H, $H_4$); $^{13}C$ NMR ($CDCl_3$) 55.7 ($C_6$–$OCH_3$), 56.4 ($C_{3'-5'}$–$OCH_3$), 61.0 ($C_{4'}$–$OCH_3$), 99.4 ($C_7$), 106.7 ($C_{2'}$ and $C_{6'}$), 114.1 ($C_5$), 121.0 ($C_3$), 121.3 ($C_{2''}$ and $C_{6''}$), 121.5 ($C_{3a}$), 123.3 ($C_4$), 126.9 ($C_{4''}$), 129.9 ($C_{3''}$ and $C_{5''}$), 131.2 ($C_2$), 134.4 ($C_{1'}$), 137.0 ($C_{7a}$), 142.1 ($C_{4'}$), 149.1 (NCOO), 150.0 ($C_{1''}$), 153.2 ($C_{3'}$ and $C_{5'}$), 159.1 ($C_6$), 190.0 (CO); MS (ESI) *m/z* 484 $[M+Na]^+$, 462 $[M+H]^+$; Anal. ($C_{26}H_{23}NO_7$) found: C, 65.36; H, 5.25; N, 2.71; calcd: C, 67.67; H, 5.02; N, 3.04.

## 7. Tubulin binding assay

Calf brain tubulin was purified according to the method of Shelanski et al.,[48] 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.[49] 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, 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/$IC_{50colchicine}$). Compounds with a ratio value greater than 57C are designated as 'inactive' in Tables 1–3.

## 8. 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, combretastatin A-4 phosphate (CSA-4) and colchicine were routinely included in the experiments as internal standards. 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[50] and absorbance was read at 562 nm in a microplate reader (BioKinetics Reader, EL340). Appropriate controls with DMEM only and MTT were run to substract 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.

## 9. Effect on the morphology of transformed HUVEC (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.[38] The EA·hy 926 cell line is considered as one of the best immortalized HUVEC lines because these cells express most of the biochemical markers of parental HUVEC.[51] 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 hours 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) at final concentrations ranging from 0.25 ng/mL to 25 μg/mL, 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 representative center areas of each well at a magnification of 360×. The presence of rounded cells was scored for each concentration, and the results are presented as the presence of rounded cells for more than 10% of cells in a given field, because the DMSO-treated cells presented about 10% of rounded cells. The number of + sign indicates rounding up at the following concentrations: 1+ at 25; 2+ at 2.5; 3+ at 0.25; 4+ at 0.025; and 5+ at 0.0025 μg/mL. A minus sign (−) indicates no rounding up. Combretastatin A-4 (CSA-4) and colchicine were routinely included in the experiments as internal standards. For the best compounds, the concentrations for the rounding up of 50% of EA·hy 926 cells were calculated from the percent plot of rounded cells as a function of the logarithm of the concentrations of compounds by using the GraphPad Prism software.

## Acknowledgments

We thank Dr Julia Kaffy of the Institut Curie, Paris, for providing us with synthetic CSA-4. G.D. was supported by a studentship from the Ministère de l'Education et de la Recherche.

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