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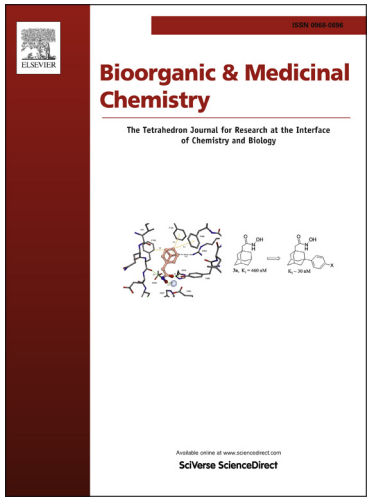

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Synthesis and biological evaluation of indole-based, anti-cancer agents inspired by the vascular disrupting agent 2-(3 -hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-methoxyindole (OXi8006)

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ABSTRACT.

The discovery of a 2-aryl-3-aroyl indole-based small-molecule inhibitor of tubulin assembly (referred to as **OXi8006**) inspired the design, synthesis, and biological evaluation of a series of diversely functionalized analogues. In the majority of examples, the pendant 2-aryl ring contained a 3-hydroxy-4-methoxy substitution pattern, and the fused aryl ring featured a 6-methoxy group. Most of the variability was in the 3-aroyl moiety, which was modified to incorporate methoxy (**33-36**), nitro (**25-27**), halogen (**28-29**), trifluoromethyl (**30**), or trifluoromethoxy (**31-32**) functionalities. In two analogues (**34** and **36**), the methoxy substitution pattern in the fused aryl ring varied, while in another derivative (**35**) the phenolic moiety was translocated from the pendant 2-aryl ring to position-7 of the fused aryl ring. Each of the compounds were evaluated for their cytotoxicity (*in vitro*) against the SK-OV-3 (ovarian), NCI-H460 (lung), and DU-145 (prostate) human cancer cell lines and for their ability to inhibit tubulin assembly. Four of the compounds (**30**, **31**, **35**, **36**) proved to be potent inhibitors of tubulin assembly (IC_{50} < 5 M), and three of these compounds (**31**, **35**, **36**) were strongly cytotoxic against the three cancer cell lines. The most active compound (**36**) in this series, which incorporated a methoxy group at position-7, was comparable in terms of inhibition of tubulin assembly and cytotoxicity to the lead compound **OXi8006**.

Keywords: Vascular disrupting agent (VDA), inhibitor of tubulin assembly, functionalized indole, combretastatin.

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1. Introduction

The exploration and assessment of the tumor microenvironment and its physiology have revealed a number of prospective molecular targets for selective therapeutic intervention by small-molecule anti-cancer agents. A well-established target is the dynamic tubulin-microtubule protein system. Microtubules are structurally characterized as biopolymers composed of -tubulin heterodimers.[1-5] The dynamic assembly and disassembly of microtubules is linked to a variety of cellular functions, including cell shape, intracellular motility, cellular division, and apoptosis.[1-5] More recently, certain small-molecule inhibitors of tubulin assembly have been identified as vascular disrupting agents (VDAs).[6] These compounds selectively disrupt tumor vasculature by interfering with the tubulin-microtubule protein system of the endothelial cells lining tumor microvessels, which sets in motion a cascade of cell signaling events leading to morphology changes (rounding up) of these endothelial cells. This results in the occlusion of the vessels, which limits tumor blood flow. This in turn restricts the oxygen and nutrients vital for tumor survival. The vascular network feeding tumors is distinct from normal tissue vasculature and incorporates branching that is often unsystematic and convoluted.[7-9] In addition, increased rates of tumor cell proliferation coupled with underdeveloped endothelium, in contrast to normal tissue vasculature, has established tumor vasculature as a selective therapeutic target for anti-cancer agents.[9] This approach has led to the development of a class of therapeutics referred to as vascular targeting agents (VTAs). This class is further subdivided into two discrete sub-classes centered upon distinct mechanism(s) of action: vascular disrupting agents (VDAs) and angiogenesis inhibiting agents (AIAs).[10] VDAs damage existing tumor vasculature while AIAs impede new tumor vessel formation.[10-12] VDAs can be further divided into two

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distinct groups: biologics and small-molecules. One strategy focuses on the development of indole-based small-molecule VDAs that bind at the colchicine site, named after the natural product originally described as binding at the site (Figure 1)[13] and whose interaction with tubulin led to the original isolation of the protein.[14] Synthetic and biological studies with indole-based, colchicine site VDAs were originally prompted by the discovery of the potent natural products combretastatin A-4 (**CA4**) and combretastatin A-1 (**CA1**) that were isolated from the African bush willow tree, *Combretum caffrum*, by Pettit and co-workers (Figure 1).[15-16] **CA4** emerged as a benchmark VDA, and its corresponding prodrug salt **CA4P** (Zybrestat™) was the first small-molecule tubulin binding VDA to enter clinical trials.[17-19] Although no VDA is yet in routine clinical use, several small-molecule VDAs interacting at the colchicine site are in clinical trials.[17-21]

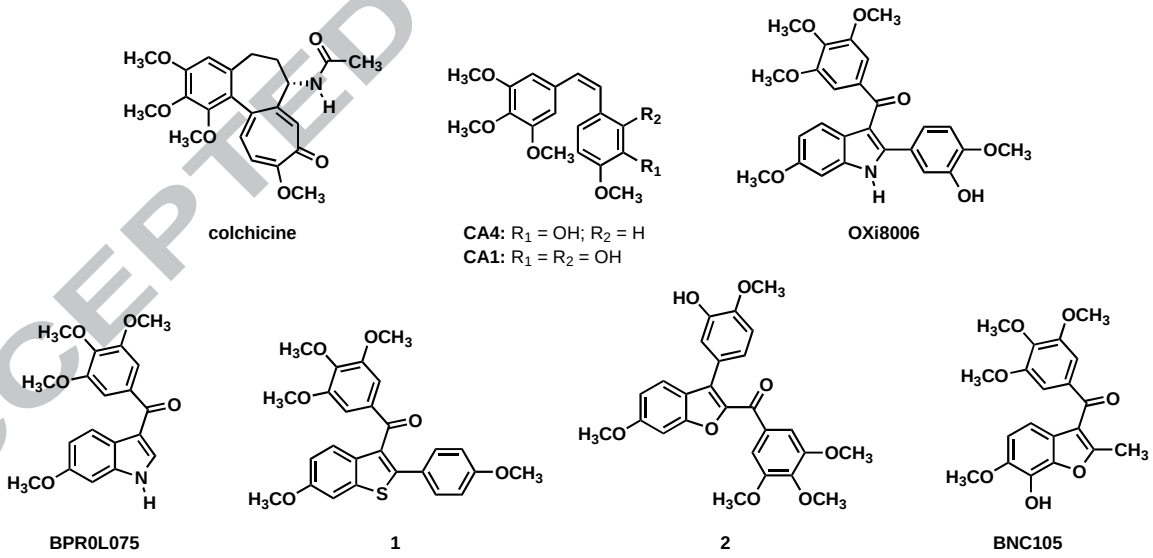

Figure 1. Selected colchicine site tubulin binding agents.

VDAs derived from the combretastatin family demonstrate potent antiproliferative activity in various human cancer cell lines *in vitro* through the inhibition

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of tubulin polymerization.[22-31] These findings led us and others to explore indole-based compounds for potential VDA and antitubulin activities by incorporating into their design structural similarities to the combretastatin series. Our work led to the potent compound 2-(3 -hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-methoxyindole (referred to as **OXi8006**),[32-35] and Flynn[36] has subsequently pursued this compound (through a separate synthetic route) and structurally similar, highly active compounds. Because **OXi8006** potently inhibits tubulin assembly (IC_{50} = 1.1 M) and cell growth (for example, GI_{50} = 3.45 nM against SK-OV-3 cells), we initiated further structural studies. As an initial finding, a water-soluble, disodium phosphate prodrug salt, **OXi8007**, demonstrated distinct *in vivo* VDA activity in a study employing a SCID mouse model bearing an orthotopic PC-3 (prostate) tumor as imaged by color Doppler ultrasound.[37]

Herein, we report the synthesis and biological evaluation of a series of functionalized analogues of **OXi8006** in an effort to further explore the molecular space inherent to 2-aryl-3-aroyl indole-based anti-cancer agents. Our finding[32-35] that **OXi8006** is a potent tubulin binding agent combined with the work of Hseih[38] with **BPR0L075** (Figure 1) provided preliminary structural parallels defining distinct associations between the stilbene aryl rings of **CA4** and the aryl and aroyl rings of **OXi8006** and **BPR0L075**. These correlations were further expanded by our previous identification of benzo[*b*]thiophene **1** and benzo[*b*]furan **2** as tubulin interacting compounds[39-42] and the subsequent studies by Flynn leading to the benzofuran-based **BNC105** (Figure 1), a VDA currently undergoing clinical trials.[43-45] A narrow but focused literature survey of inhibitors of tubulin assembly that incorporate the indole molecular template confirms the importance of the 3-(3 -trimethoxybenzoyl)indole functionality while allowing for

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structural diversity within the indole core (Figure 2). This is exemplified by structures that include variation in alkoxy substitution (structure **I**, Fig. 2),[38] halogen incorporation (structures **I** and **III**),[38,46] heterocyclic substitution at the 2-position (structure **IV**),[47] and derivatives of **BRP0L075** (such as compound **II**).[48]

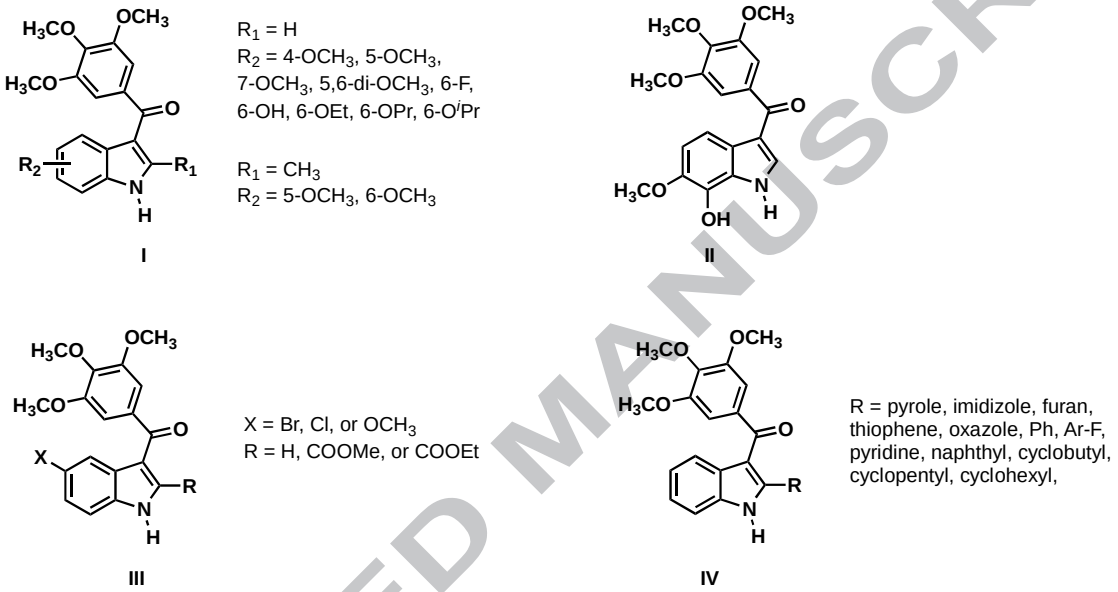

Figure 2. Structural diversity within the 3-(3 -trimethoxybenzoyl)indole molecular space

The potent inhibition of tubulin assembly and cytotoxicity of **OXi8006** and **BPR0L075**, in addition to the previous studies with benzo[*b*]thiophene and benzo[*b*]furan derivatives, led to the present study, which investigates a small collection of diversely modified 2-aryl-3-aroyl indole-based analogues to gain further insight into the structural features of **OXi8006** that are most important for biological activity (inhibition of tubulin assembly and cytotoxicity).

2. Results and discussion

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2.1 Chemistry

The synthetic route to derivatized **OXi8006** analogues **25**-**36** involved the previously described bromoacetophenone **3**[37] and commercially available bromoacetophenone **4** as key intermediates. 2-Aryl substituted indoles **5**-**11** were prepared by condensation of bromoacetophenone **3** or **4** with suitable anilines under Bischler-Mohlau conditions[49-50] (Scheme 1). Further modification of 2-aryl indole **8** by selective demethoxylation in the presence of ionic liquid $[TMAH][Al_2Cl_7]$[51] (generated from $AlCl_3$ and trimethylamine hydrochloride (TMAH)) and microwave irradiation yielded the phenolic 2-aryl indole **9**, which was subsequently protected as its corresponding TBS derivative **10** (Scheme 2). The regioselectivity of the demethylation reaction was confirmed by X-ray crystallographic analysis of TBS indole **10** (see Supplementary data). Treatment of 2-aryl indoles **5**-**11** with appropriate functionalized benzoyl chloride derivatives resulted in 2-aryl-3-aroyl indole analogues **12**-**24** through a benzoylation reaction. Final desilylation with TBAF provided the parent 2-aryl-3-aroyl free phenol indole analogues **25**-**36**.

Scheme 1. Synthetic route to OXi8006 analogues from bromoacetophenones 3 and 4.

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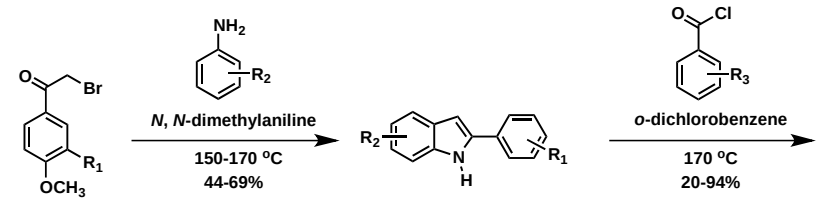
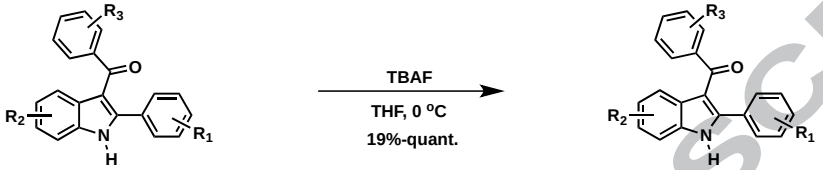


3: R_1 = OTBS
4: R_1 = H

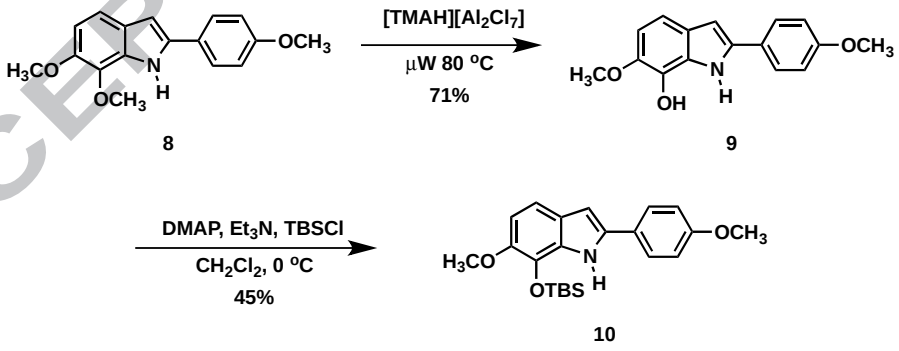
5: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3
6: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OTBS
7: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 4,5,6-tri-OCH_3
8: R_1 = 4'-OCH_3, R_2 = 6,7-di-OCH_3
10: R_1 = 4'-OCH_3, R_2 = 6-OCH_3, 7-OTBS
11: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6,7-di-OCH_3



12: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",4",5"-tri-OCH_3
13: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",5"-di-NO_2
14: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-NO_2
15: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3"-NO_2
16: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",4",5"-tri-F
17: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-F
18: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",5"-di-CF_3
19: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3"-OCF_3
20: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-OCF_3
21: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6-OTBS, R_3 = 3",4",5"-tri-OCH_3
22: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 4,5,6-tri-OCH_3, R_3 = 3",4",5"-tri-OCH_3
23: R_1 = 4'-OCH_3, R_2 = 6-OCH_3, 7-OTBS, R_3 = 3",4",5"-tri-OCH_3
24: R_1 = 3'-OTBS, 4'-OCH_3, R_2 = 6,7-di-OCH_3, R_3 = 3",4",5"-tri-OCH_3

OXi8006: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",4",5"-tri-OCH_3
25: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",5"-di-NO_2
26: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-NO_2
27: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3"-NO_2
28: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",4",5"-tri-F
29: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-F
30: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3",5"-di-CF_3
31: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 3"-OCF_3
32: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OCH_3, R_3 = 4"-OCF_3
33: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6-OH, R_3 = 3",4",5"-tri-OCH_3
34: R_1 = 3'-OH, 4'-OCH_3, R_2 = 4,5,6-tri-OCH_3, R_3 = 3",4",5"-tri-OCH_3
35: R_1 = 4'-OCH_3, R_2 = 6-OCH_3, 7-OH, R_3 = 3",4",5"-tri-OCH_3
36: R_1 = 3'-OH, 4'-OCH_3, R_2 = 6,7-di-OCH_3, R_3 = 3",4",5"-tri-OCH_3

Scheme 2. Synthetic route to modified indole 10.



2.2 Biological Evaluation

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The series of 2-aryl-3-aroyl indole analogues (Fig. 3) were evaluated for their cytotoxicity against the SK-OV-3, NCI-H460, and DU-145 human cancer cell lines (Table 1) and for their ability to inhibit tubulin assembly (Table 2). The two most active compounds (**35** and **36**) in the series featured substitution at position-7 in the fused aryl ring. Compound **36** (7-methoxy) was comparable to **OXi8006** in terms of both cytotoxicity (sub-micromolar) and inhibition of tubulin assembly (IC_{50} = 1.1 M), and analogue **35**, in which the hydroxyl group was transposed from the pendant 2-aryl ring to position-7 of the fused aryl ring, was nearly equipotent. Replacement of the 6-methoxy group with a 6-hydroxy moiety (analogue **33**) resulted in a loss of antitubulin activity (> 20 M) and a significant decrease in cytotoxicity. All structural modifications in the 3-aroyl moiety that replaced the 3,4,5-trimethoxy motif (inherent to **OXi8006**) with a different functionality resulted in a decrease in cytotoxicity (compared to **OXi8006** and the reference stilbene compound, **CA4**). However, the 3,5-*bis*-trifluoromethyl analogue (**30**) and the 3-trifluoromethoxy derivative (**31**) remained relatively good inhibitors of tubulin assembly (3.1 and 3.7 M, respectively). Although selective fluorine substitution in **CA4** analogues has been generally well-tolerated,[52] this trend did not carry forward to this indole series of compounds. A 3,4,5-trifluoro analogue (**28**) demonstrated only modest inhibition of tubulin assembly (7.5 M), while a 3-fluoro derivative (**29**) was inactive (> 20 M) in this assay. Nitro-bearing analogues (**25**-**27**) were similarly inactive. These results suggest the importance of the 3,4,5-trimethoxy substitution pattern in the 3-aroyl moiety and appropriate substitution at positions 6 and 7 of the fused aryl ring for maintaining potent cytotoxicity and inhibition of tubulin assembly in this series of compounds.

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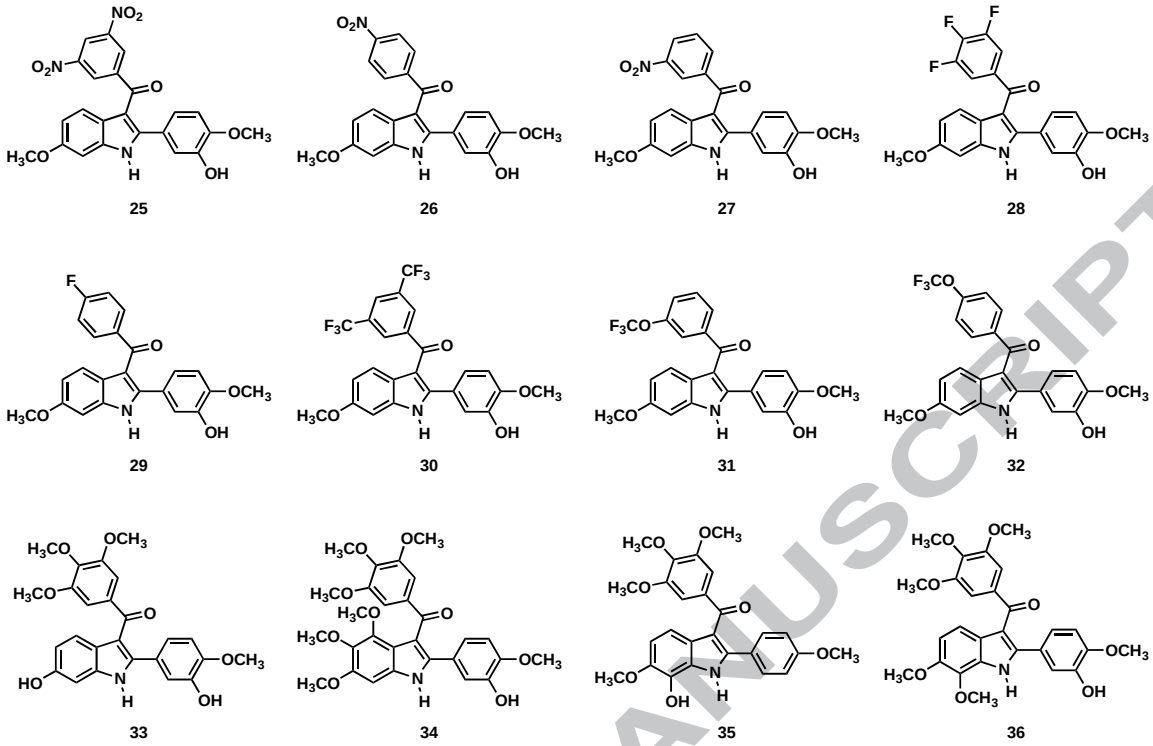

Figure 3. Molecular structures of synthesized 2-aryl-3-aroylindole analogues **25-36**.

Table 1. Cytotoxicity against human cancer cell lines SK-OV-3, NCI-H460, and DU-145

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	GI_{50} (M) ± SD Sulforhodamine B assay[a]		
Compound	SK-OV-3	NCI-H460	DU-145
CA4	0.00533 ± 0.00180	0.00449 ± 0.0000648[b]	0.00484 ± 0.000848[b]
OXi8006	0.00345 ± 0.000409	0.0379 ± 0.00182	0.0356 ± 0.00107
25	4.35 ± 0.290	4.08 ± 0.119	5.52 ± 0.106
26	21.1 ± 2.45	16.8 ± 2.13	8.16 ± 4.41
27	2.65 ± 1.94	15.7 ± 1.64	7.11 ± 2.16
28	19.8 ± 3.95	5.54 ± 0.505	10.0 ± 9.83
29	15.0 ± 9.60	49.6 ± 3.20	10.0 ± 4.21
30	1.45 ± 0.365	2.86 ± 0.104	3.01 ± 0.0829
31	0.283 ± 0.0395	3.57 ± 0.508	2.99 ± 0.235
32	3.05 ± 0.895	2.35 ± 0.159	3.21 ± 0.294
33	2.167 ± 0.3657	2.910 ± 0.4833	3.401 ± 1.471
34	25.1 ± 0.262	41.8 ± 2.52	28.3 ± 18.0
35	0.264 ± 0.0418	0.177 ± 0.0245	0.309 ± 0.0143
36	0.0119 ± 0.00422	0.992 ± 0.320	0.0181 ± 0.000772

[a] Average of n ≥ 3 independent determinations. [b] For additional data see refs. 15-16.

Table 2. Inhibition of tubulin polymerization and colchicine binding

	Inhibition of colchicine binding (%) ± SD

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Compound	Inhibition of tubulin polymerization IC_{50} (M) ± SD	1 M	5 M
CA4	1.3 ± 0.07	88 ± 2	98 ± 0.5
OXi8006	1.1 ± 0.04	40 ± 0.2	75 ± 0.2
25	> 20	nd[a]	nd
26	> 20	nd	nd
27	19 ± 0.8	nd	21 ± 1
28	7.5 ± 2	nd	26 ± 2
29	> 20	nd	nd
30	3.1 ± 0.2	nd	26 ± 2
31	3.7 ± 0.4	nd	19 ± 4
32	> 20	nd	nd
33	> 20	nd	nd
34	> 20	nd	nd
35	1.0 ± 0.1	51 ± 0.4	85 ± 0.7
36	1.1 ± 0.4	31 ± 4	67 ± 3

[a] nd = not determined in this study.

3. Conclusion

In summary, the results of this study have significantly extended our knowledge of functional group tolerability for 2-aryl-3-aroyl indole analogues. The most promising new analogues (**35**, **36**) demonstrated inhibition of tubulin assembly comparable to the reference compounds **OXi8006** and **CA4**, and future studies will evaluate these compounds (as their corresponding water-soluble phosphate prodrug salts) for their potential to function as VDAs.

4. Experimental

4.1 Chemistry

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4.1.1 Materials and instrumentation

CH_2Cl_2 and tetrahydrofuran (THF) were used in their anhydrous forms, as obtained from the chemical suppliers. Reactions were performed under an inert atmosphere using nitrogen gas, unless specified otherwise. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system (Biotage Isolera 1 or 4) using silica gel (200-400 mesh, 60 Å) prepacked columns. Reactions carried out under microwave irradiation were performed with a Biotage Initiator Microwave Synthesizer. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), and ^{19}F NMR (470 MHz) spectroscopic data using a Varian VNMRS 500 MHz instrument. Spectra were recorded in $CDCl_3$, $(CD_3)_2SO$, or $(CD_3)_2CO$. All of the chemical shifts are expressed in ppm ( ), coupling constants (*J*) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), double doublet (dd), double triplet (dt), triplet of triplets (tt), doublet of doublets of doublets (ddd), and multiplet (m). Purity of the final compounds was further analyzed at 25 °C using an Agilent 1200 HPLC system with a diode-array detector (= 190-400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm - 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; solvent A: acetonitrile, solvent B: H_2O; gradient: 10%A / 90%B to 100%A / 0%B over 0 to 40 min; post-time 10 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 254, 230, 280, and 360 nm. Mass spectrometry was carried out under positive ESI (electrospray ionization) using a Thermo scientific LTQ Orbitrap Discovery instrument.

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4.1.2. 2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-6-methoxyindole (5)[37]

To a solution of *m*-anisidine (2.05 mL, 18.4 mmol) dissolved in *N*,*N*-dimethylaniline (20 mL) at 170 °C was added dropwise bromoacetophenone **3** (2.0 g, 5.6 mmol) in EtOAc (5 mL). The reaction mixture was stirred at 170 °C for 12 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2.6 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulted in the desired 2-phenylindole derivative **5** (1.49 g, 3.88 mmol, 69%, R_f = 0.48 (50:50 hexanes:EtOAc)) as light tan crystals. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.11 (br s, 1H, NH), 7.47 (d, *J* = 8.5 Hz, 1H, ArH), 7.16 (dd, *J* = 2.0 Hz, 8.5 Hz 1H, ArH), 7.13 (d, *J* = 2.5 Hz, 1H, ArH), 6.90 (d, *J* = 8.5 Hz, 1H, ArH), 6.89 (d, *J* = 2.5 Hz, 1H, ArH), 6.79 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, ArH), 6.64 (dd, *J* = 1.0 Hz, 2.0 Hz 1H, ArH), 3.86 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 1.04 (s, 9H, $C(CH_3)_3$), 0.21 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 156.3, 150.5, 145.4, 137.4, 136.9, 125.8, 123.7, 120.9, 118.2, 117.8, 112.4, 109.9, 98.6, 94.5, 55.6, 55.4, 25.7, 18.5, -4.6.

4.1.3. 2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-6-*tert*-butyldimethylsilyloxyindole (6)

To a solution of 3-(*tert*-butyldimethylsilyloxy)aniline (2.06 g, 9.21 mmol) in *N*,*N*-dimethylaniline (20 mL) at 170 °C was added compound **3** (1.00 g, 2.79 mmol) dropwise

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in EtOAc (5 mL). The reaction mixture was stirred at 170 °C for 12 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2%A / 98%B (4 CV), 2%A / 98%B → 20%A / 80%B (10 CV), 20%A / 80%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulted in the desired 2-phenylindole **6** (0.59 g, 1.23 mmol, 44%, R_f = 0.39 (90:10 hexanes:EtOAc)) as a brown solid. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.09 (br s, 1H, NH), 7.37 (d, *J* = 8.5 Hz, 1H, ArH), 7.10 (d, *J* = 1.5 Hz, 1H, ArH), 7.01 (dd, *J* = 1.5 Hz, 8.5 Hz, 1H, ArH), 6.82 (s, 1H, ArH), 6.72 (d, *J* = 8.5 Hz, 1H, ArH), 6.67 (dd, *J* = 1.5 Hz, 8.5 Hz, 1H, ArH), 6.54 (s, 1H, ArH), 3.70 (s, 3H, OCH_3), 1.02 (s, 9H, $C(CH_3)_3$), 1.01 (s, 9H, $C(CH_3)_3$), 0.20 (s, 6H, $Si(CH_3)_2$), 0.18 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 150.7, 150.6, 145.4, 137.7, 137.3, 126.0, 124.5, 120.6, 118.5, 118.0, 114.6, 112.4, 101.8, 98.7, 55.4, 25.93, 25.89, 18.6, 18.4, -4.3, -4.5. **HPLC**: 25.45 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{27}H_{42}NO_3Si_2$ $[M+H]^+$ 484.2698, found 484.2698.

4.1.4. 2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-4,5,6-trimethoxyindole (7)

To a solution of 3,4,5-trimethoxyaniline (0.336 g, 1.84 mmol) in *N*,*N*-dimethylaniline (20 mL) at 170 °C was added compound **3** (0.20 g, 0.56 mmol) dropwise in EtOAc (5 mL). The reaction mixture was stirred at 170 °C for 12 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a

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prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulted in the desired 2-phenylindole **7** (0.14 g, 0.32 mmol, 58%, R_f = 0.31 (70:30 hexanes:EtOAc)) as colorless crystals. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.06 (br s, 1H, NH), 7.15 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 7.10 (d, *J* = 2.0 Hz, 1H, ArH), 6.90 (d, *J* = 8.5 Hz, 1H, ArH), 6.70 (dd, *J* = 1.0 Hz, 2.0 Hz, 1H, ArH), 6.66 (d, *J* = 0.5 Hz, 1H, ArH), 4.13 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 1.03 (s, 9H, $C(CH_3)_3$), 0.19 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 151.0, 150.6, 145.6, 145.5, 136.4, 135.8, 133.9, 125.8, 118.3, 117.9, 116.6, 112.5, 96.3, 89.8, 61.6, 60.9, 56.2, 55.2, 25.9, 18.5, -4.6. **HPLC**: 20.17 min., purity at 254 nm 94.2%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{24}H_{34}NO_5Si$ $[M+H]^+$ 444.2201, found 443.2200.

4.1.5. 2-(4 -Methoxyphenyl)-6,7-dimethoxyindole (8)

To a solution of 2,3-dimethoxyaniline (0.92 mL, 6.85 mmol) dissolved in *N*,*N*-dimethylaniline (10 mL) was added 4-methoxybromoacetophenone **4** (0.79 g, 3.43 mmol). The solution was heated to reflux and stirred at 150 °C for 12 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (4 CV), 5%A / 95%B → 40%A / 60%B (10 CV), 40%A / 60%B (4 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulted in the desired 6,7-dimethoxy-2-phenylindole **8** (0.50 g, 1.76 mmol, 51%, R_f = 0.35 (80:20 hexanes:EtOAc)) as a tan

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solid. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.61 (br s, 1H, NH), 7.61 (d, *J* = 8.7 Hz, 2H, ArH), 7.28 (d, *J* = 8.5 Hz, 1H, ArH), 6.97 (d, *J* = 8.7 Hz, 2H, ArH), 6.87 (d, *J* = 8.6 Hz, 1H, ArH), 6.66 (d, *J* = 2.1 Hz, 1H, ArH), 4.09 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3). **^{13}C NMR** ($CDCl_3$, 125 MHz): 159.2, 147.1, 138.0, 134.2, 131.3, 126.4, 126.0, 125.3, 115.3, 114.5, 108.5, 98.8, 61.1, 57.4, 55.4. **HPLC**: 15.30 min., purity at 254 nm 90.6%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{17}H_{18}NO_3$ $[M+H]^+$ 284.1281, found 284.1282.

4.1.6. 2-(4 -Methoxyphenyl)-6-methoxy-7-hydroxyindole (9)

Trimethoxyindole **8** (0.61 g, 2.16 mmol) was dissolved in a solution of $[Al_2Cl_7][TMAH]$ (6.3 mL, 3.13 mmol, 0.496 M in CH_2Cl_2). The reaction mixture was sealed and subjected to microwave irradiation at 80 °C for 1 h. Upon completion of the reaction, the reaction mixture was diluted with $NaHCO_3$ and extracted with CH_2Cl_2 (3 x 20 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulted in the desired 6-methoxy-7-hydroxy-2-phenylindole **9** (0.42 g, 1.55 mmol, 71%, R_f = 0.36 (70:30 hexanes:EtOAc)) as a tan solid. **^{1}H NMR** ($(CD_3)_2CO$, 500 MHz): 10.11 (br s, 1H, NH), 7.85 (d, *J* = 8.7 Hz, 2H, ArH), 7.66 (s, 1H, OH), 6.98 (m, 3H, ArH), 6.81 (d, *J* = 8.5 Hz, 1H, ArH), 6.66 (d, *J* = 2.2 Hz, 1H, ArH), 3.83 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2CO$, 125 MHz): 159.9, 142.5, 138.8, 133.1, 128.4, 127.2, 127.1, 126.5, 115.0, 111.3, 108.9, 99.0, 58.3, 55.7. **HPLC**: 13.47 min., purity at 254 nm 85.8%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{16}H_{16}NO_3$ $[M+H]^+$ 270.1125, found 270.1129.

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4.1.7. 2-(4 -Methoxyphenyl)-6-methoxy-7-*tert*-butyldimethylsilyloxyindole (10)

To a solution of free phenol indole **9** (0.08 g, 0.28 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added Et_3N (0.04 mL, 0.31 mmol) and DMAP (0.01 g, 0.11 mmol). The reaction mixture was stirred for 10 min, and TBSCl (0.05 g, 0.31 mmol) was added gradually. The solution was allowed to warm to room temperature over 12 h. Upon completion of the reaction, water (10 mL) was added, and the reaction mixture was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2%A / 98%B (4 CV), 2%A / 98%B → 20%A / 80%B (10 CV), 20%A / 80%B (5.2 CV); flow rate: 35 mL/min; monitored at 254 and 280 nm] resulted in the TBS indole product **10** (0.05 g, 0.02 mmol, 45%, R_f = 0.64 (70:30 hexanes:EtOAc))) as a light tan solid. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.03 (br s, 1H, N<u>H</u>), 7.53 (d, *J* = 8.7, 2H, Ar<u>H</u>), 7.13 (d, *J* = 8.5 Hz, 1H, Ar<u>H</u>), 6.98 (d, *J* = 8.7 Hz, 2H, Ar<u>H</u>), 6.80 (d, *J* = 8.5 Hz, 1H, Ar<u>H</u>), 6.61 (d, *J* = 2.2 Hz, 1H, Ar<u>H</u>), 3.86 (s, 6H, OC<u>H</u>$_3$), 1.11 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.24 (s, 6H, Si(C<u>H</u>$_3$)$_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 159.3, 145.2, 137.5, 131.2, 130.2, 126.2, 125.9, 125.6, 114.6, 112.9, 108.5, 99.0, 57.0, 55.5, 26.3, 18.8, -4.2. **HPLC**: 21.73 min., purity at 254 nm 93.7%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{22}H_{30}NO_3Si$ $[M+H]^+$ 384.1989, found 384.1990.

4.1.8. 2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-6,7-dimethoxyindole (11)

To a solution of 2,3-dimethoxyaniline (2.19 mL, 16.3 mmol) dissolved in *N*,*N*-dimethylaniline (20 mL) was added bromoacetophenone **3** (2.93 g, 8.16 mmol). The

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solution was heated to reflux and stirred at 150 °C for 12 h. Upon completion of the reaction, the reaction mixture was cooled to room temperature and extracted with EtOAc (3 x 50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5%A / 95%B (4 CV), 5%A / 95%B → 40%A / 60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] resulted in the desired 6,7-dimethoxy-2-phenylindole **11** (1.43 g, 3.47 mmol, 43%, R_f = 0.40 (80:20 hexanes:EtOAc)) as a tan solid. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.63 (br s, 1H, NH), 7.33 (d, *J* = 8.5 Hz, 1H, ArH), 7.28 (d, *J* = 2.2 Hz, 1H, ArH), 7.26 (dd, *J* = 8.5 Hz, 2.2 Hz 1H, ArH), 6.92 (dd, *J* = 8.4 Hz, 1.4 Hz, 2H, ArH), 6.71 (d, *J* = 2.2 Hz, 1H, ArH), 4.15 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 1.15 (s, 9H, $C(CH_3)_3$), 0.31 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 150.7, 147.1, 145.4, 137.9, 134.2, 131.2, 126.0, 125.7, 118.4, 118.1, 115.2, 112.3, 108.7, 99.0, 60.9, 57.3, 55.4, 25.8, 18.5, -4.5. **HPLC**: 21.28 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{32}NO_4Si$ $[M+H]^+$ 414.2095, found 414.2095.

4.1.9. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,5 dinitrobenzoyl)-6-methoxyindole (13)

To a solution of compound **5** (0.50 g, 1.30 mmol) in *o*-dichlorobenzene (20 mL) was added 3,5-dinitrobenzoylchloride (0.45 g, 1.90 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (11 CV), 60%A / 40%B (2 CV);

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flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **13** as a pale yellow powder (0.40 g, 0.69 mmol, 53%, R_f = 0.59 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.85 (t, *J* = 2.0 Hz, 1H, ArH), 8.62 (d, *J* = 2.0 Hz, 2H, ArH), 8.60 (br s, 1H, NH) 8.15 (d, *J* = 8.5 Hz, 1H, ArH), 7.00 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.95 (d, 2.0 Hz, 1H, ArH), 6.87 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.61 (d, *J* = 8.5 Hz, 1H, ArH), 6.56 (d, *J* = 2.5 Hz, 1H, ArH), 3.90 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 0.89 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 187.3, 158.1, 152.1, 147.8, 145.3, 145.1, 143.1, 136.5, 129.1, 123.5, 123.4, 122.7, 122.4, 122.3, 120.1, 112.8, 112.5, 111.8, 95.0, 55.9, 55.5, 25.6, 18.4, -4.8. **HPLC**: 20.28 min., purity at 254 nm 93.1%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{29}H_{32}N_3O_8Si$ $[M+H]^+$ 578.1953, found 578.1950.

4.1.10. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(4 -nitrobenzoyl)-6-methoxyindole (14)

To a solution of compound **5** (0.20 g, 0.52 mmol) in *o*-dichlorobenzene (10 mL) was added 3-nitrobenzoylchloride (0.15 g, 0.78 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (8.8 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **14** as a yellow powder (0.14 g, 0.28 mmol, 51%, R_f = 0.36 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.37 (br s, 1H, NH), 8.06 (d, *J* = 8.5 Hz, 1H, ArH), 7.97 (d, *J* = 8.5 Hz, 2H, ArH) 7.69 (d, *J* = 8.5 Hz, 2H, ArH), 6.97 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.93 (d, *J* =

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2.5 Hz, 1H, ArH), 6.75 (m, 2H, ArH), 6.58 (d, *J* = 8.0 Hz, 1H, ArH), 3.89 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.07 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 190.9, 157.8, 152.1, 148.9, 145.6, 145.2, 144.4, 136.5, 130.3, 124.1, 123.7, 123.0, 122.69, 122.67, 121.7, 113.0, 112.3, 111.7, 94.9, 55.9, 55.6, 25.8, 18.6, -4.6. **HPLC**: 20.23 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{29}H_{33}N_2O_6Si$ $[M+H]^+$ 533.2102, found 533.2100.

4.1.11. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 -nitrobenzoyl)-6-methoxyindole (15)

To a solution of compound **5** (0.10 g, 0.26 mmol) in *o*-dichlorobenzene (10 mL) was added 3-nitrobenzoylchloride (0.07 g, 0.39 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2.8 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **15** as pale yellow crystals (0.13 g, 0.25 mmol, 94%, R_f = 0.63 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 9.15 (br s, 1H, NH), 8.30 (t, *J* = 2.0 Hz, 1H, ArH), 8.06 (ddd, *J* = 1.0 Hz, 2.0 Hz, 8.0 Hz, 1H, ArH) 8.03 (d *J* = 9.5 Hz, 1H, ArH), 7.88 (dt, *J* = 1.0 Hz, 8.0 Hz, 1H, ArH), 7.29 (t, *J* = 8.0 Hz, 1H, ArH), 6.92 (dd, *J* = 2.0 Hz, 7.0 Hz, 1H, ArH), 6.91 (d, *J* = 2.0 Hz, 1H, ArH), 6.78 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.66 (d, *J* = 2.5 Hz, 1H, ArH), 6.50 (d, *J* = 8.5 Hz, 1H, ArH), 3.82 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 0.91 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 190.4, 157.6, 151.7, 147.4, 144.9, 144.6, 141.3, 136.6, 135.0, 128.9, 125.4, 124.7, 124.1, 123.4, 122.7, 122.3,

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122.0, 112.4, 112.3, 111.6, 94.9, 55.7, 55.4, 25.7, 18.4, -4.7. **HPLC**: 20.13 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{29}H_{33}N_2O_6Si$ $[M+H]^+$ 533.2102, found 533.2100.

4.1.12. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trifluorobenzoyl)-6-methoxyindole (16)

To a solution of compound **5** (0.61 g, 1.59 mmol) in *o*-dichlorobenzene (10 mL) was added 3,4,5-trifluorobenzoylchloride (3.12 mL, 2.38 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 97%B → 60%A / 40%B (10 CV), 60%A / 40%B (5.5 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **16** as a white powder (0.70 g, 1.29 mmol, 81%, R_f = 0.48 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.44 (br s, 1H, NH), 7.95 (d, *J* = 9.0 Hz, 1H, ArH), 7.25 (m, 2H, ArH) 6.94 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, ArH), 6.91 (d, *J* = 2.0 Hz, 1H, ArH), 6.83 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.79 (d, *J* = 2.0 Hz, 1H, ArH), 6.70 (d, *J* = 8.5 Hz, 1H, ArH), 3.87 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.09 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 189.1, 157.7, 152.1, 150.6 (ddd, J_{C-F} = 3.1 Hz, 10.0 Hz, 250.0 Hz), 145.4, 143.5, 141.8 (dt, J_{C-F} = 15.5 Hz, 255.9 Hz), 136.5, 135.6 (d, J_{C-F} = 3.9 Hz), 124.4, 123.2, 122.8, 121.4, 121.6, 114.1 (dd, J_{C-F} = 5.1 Hz, 16.9 Hz), 112.3, 112.2, 112.0, 94.8, 55.9, 55.6, 25.7, 18.5, -4.8. **^{19}F NMR** ($CDCl_3$, 470 MHz): -134.0 (dd, *J* = 7.5 Hz, 21.2 Hz, 2F, ArF), -155.6 (tt, *J* = 6.6 Hz, 20.2 Hz, 1F, ArF). **HPLC**: 21.32 min.,

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purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{29}H_{30}F_3NNaO_4Si$ $[M+Na]^+$ 564.1788, found 564.1786.

4.1.13. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(4 -fluorobenzoyl)-6-methoxyindole (17)

To a solution of compound **5** (0.10 g, 0.26 mmol) in *o*-dichlorobenzene (10 mL) was added 4-fluorobenzoylchloride (0.05 mL, 0.39 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **17** as pale yellow crystals (0.09 g, 0.18 mmol, 69%, R_f = 0.73 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.59 (br s, 1H, NH), 7.88 (d, *J* = 9.5 Hz, 1H, ArH), 7.65 (dt, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH) 7.64 (dt, *J* = 2.0 Hz, 9.0 Hz, 1H, ArH), 6.89 (m, 2H, ArH), 6.82 (m, 4H, ArH), 6.60 (d, *J* = 8.0 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 0.96 (s, 9H, $C(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 191.7, 164.8 (d, J_{C-F} = 251 Hz), 157.4, 151.7, 145.1, 142.8, 136.4, 136.09, 136.06, 132.2 (d, J_{C-F} = 9 Hz), 124.6, 123.4, 123.1, 122.4, 121.6, 114.9 (d, J_{C-F} = 22 Hz), 113.0, 111.8, 94.7, 55.8, 55.5, 25.8, 18.6, -4.6. **^{19}F NMR** ($CDCl_3$, 470 MHz): -108.1 (m, 1F, ArF). **HPLC**: 20.59 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{29}H_{33}FNO_4Si$ $[M+H]^+$ 506.2157, found 506.2155.

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4.1.14. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,5 -*bis*-trifluoromethylbenzoyl)-6-methoxyindole (18)

To a solution of compound **5** (0.20 g, 0.52 mmol) in *o*-dichlorobenzene (15 mL) was added 3,5-*bis*-trifluoromethylbenzoyl chloride (0.14 mL, 0.78 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **18** as pale orange crystals (0.26 g, 0.42 mmol, 81%, R_f = 0.47 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.70 (br s, 1H, NH), 8.07 (d, *J* = 9.0 Hz, 1H, ArH), 8.03 (s, 2H, ArH) 7.74 (s, 1H, ArH), 6.96 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.92 (d, *J* = 2.0 Hz, 1H, ArH), 6.85 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.65 (d, *J* = 8.5 Hz, 1H, ArH), 6.62 (d, *J* = 2.5 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 0.91 (s, 9H, $C(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 189.5, 157.8, 152.0, 145.3, 144.5, 141.5, 136.6, 131.3 (q, J_{C-F} = 33 Hz), 129.7 (m), 124.47 (q, J_{C-F} = 7 Hz), 124.46, 124.0, 123.1 (q, J_{C-F} = 275 Hz), 122.9, 122.7, 122.5, 122.2, 112.4, 112.1, 94.9, 55.8, 55.5, 25.6, 18.4, -4.8. **^{19}F NMR** ($CDCl_3$, 470 MHz): -62.8 (s, 6F, CF_3). **HPLC**: 22.22 min., purity at 254 nm 96.3%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{31}H_{32}F_6NO_4Si$ $[M+H]^+$ 624.1999, found 624.1997.

4.1.15. (2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-3-(3 -trifluoromethoxybenzoyl)-6-methoxyindole (19)

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To a solution of compound **5** (1.14 g, 2.97 mmol) in *o*-dichlorobenzene (15 mL) was added 3-trifluoromethoxybenzoylchloride (0.70 mL, 4.45 mmol). The reaction mixture was heated to reflux at 170 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark green colored solid was subjected to flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **19** as a yellow powder (1.21 g, 2.11 mmol, 71%, R_f = 0.43 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.31 (br s, 1H, NH), 7.96 (d, *J* = 8.4 Hz, 1H, ArH), 7.52 (dt, *J* = 1.3 Hz, 7.5 Hz, 1H, ArH) 7.48 (s, 1H, ArH), 7.17 (t, *J* = 7.8 Hz, 1H, ArH), 7.13 (d, *J* = 8.2 Hz, 1H, ArH), 6.93 (m, 2H, ArH), 6.81 (m, 2H, ArH), 6.62 (d, *J* = 9.0 Hz, 1H, ArH), 3.88 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.08 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 191.8, 157.4, 151.6, 148.8, 144.9, 144.1, 142.1, 136.6, 129.2, 128.0, 124.3, 123.6, 123.5, 122.9, 122.2, 122.0, 121.4, 120.4 (q, J_{C-F} = 256 Hz), 112.6, 112.0, 111.6, 94.8, 55.7, 55.3, 25.7, 18.4, -4.7. **^{19}F NMR** ($CDCl_3$, 470 MHz): -57.8 (s, 3F, OCF_3). **HPLC**: 21.53 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{30}H_{33}F_3NO_5Si$ $[M+H]^+$ 572.2075, found 572.2071.

4.1.16. (2-(3 -*tert*-Butyldimethylsilyloxy-4 -methoxyphenyl)-3-(4 -trifluoromethoxybenzoyl)-6-methoxyindole (20)

To a solution of compound **5** (1.14 g, 2.97 mmol) in *o*-dichlorobenzene (15 mL) was added 3-trifluoromethylbenzoylchloride (0.70 mL, 4.45 mmol). The reaction mixture was heated to reflux at 170 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark green colored solid was subjected to flash

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chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (1.1 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **20** as a yellow powder (0.92 g, 1.61 mmol, 54%, R_f = 0.43 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.29 (br s, 1H, NH), 8.00 (d, *J* = 8.6 Hz, 1H, ArH), 7.64 (d, *J* = 8.7 Hz, 2H, ArH) 6.97 (d, *J* = 8.0 Hz, 2H, ArH), 6.93 (m, 2H, ArH), 6.83 (d, *J* = 2.2 Hz, 1H, ArH), 6.73 (dd, *J* = 2.2 Hz, 8.3 Hz, 1H, ArH), 6.58 (d, *J* = 8.4 Hz, 1H, ArH), 3.88 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 0.98 (s, 9H, $C(CH_3)_3$), 0.10 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 191.9, 157.3, 151.6, 151.0, 144.8, 144.1, 138.4, 136.6, 131.3, 124.2, 123.8, 122.8, 122.2, 121.4, 120.3 (q, J_{C-F} = 256 Hz), 119.7, 112.6, 111.9, 111.3, 94.8, 55.6, 55.1, 25.6, 18.4, -4.8. **^{19}F NMR** ($CDCl_3$, 470 MHz): -57.7 (s, 3F, OCF_3). **HPLC**: 21.61 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{30}H_{33}F_3NO_5Si$ $[M+H]^+$ 572.2075, found 572.2075.

4.1.17. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-*tert*-butyldimethylsiloxyindole (21)

To a solution of compound **6** (0.19 g, 0.39 mmol) in *o*-dichlorobenzene (20 mL) was added 3,4,5-trimethoxybenzoylchloride (0.13 g, 0.58 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark colored solid was subjected to flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in di-TBS-indole **21** as a pale yellow powder (0.04 g, 0.59 mmol, 20%, R_f = 0.33 (70:30 hexanes:EtOAc)). **^{1}H**

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NMR ($CDCl_3$, 500 MHz): 8.31 (br s, 1H, NH), 7.90 (d, *J* = 8.5 Hz, 1H, ArH), 6.99 (s, 2H, ArH) 6.94 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.89 (d, *J* = 2.0 Hz, 1H, ArH), 6.82 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.76 (d, *J* = 2.5 Hz, 1H, ArH), 6.70 (d, *J* = 8.0 Hz, 1H, ArH), 3.79 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.69 (s, 6H, OCH_3), 1.01 (s, 9H, $C(CH_3)_3$), 0.94 (s, 9H, $C(CH_3)_3$), 0.22 (s, 6H, $Si(CH_3)_2$), 0.04 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 191.8, 153.0, 152.6, 151.7, 145.2, 142.3, 141.3, 136.5, 134.6, 125.2, 123.9, 122.3, 121.9, 116.7, 112.9, 111.8, 107.4, 105.1, 101.6, 60.9, 56.1, 55.5, 25.9, 25.8, 18.46, 18.45, -4.2, -4.8. **HPLC**: 23.31 min., purity at 254 nm 90.6%. **HRMS (ESI^+)**: *m/z* calculated for $C_{37}H_{52}NO_7Si_2$ $[M+H]^+$ 678.3277, found 678.3279.

4.1.18. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-4,5,6-trimethoxyindole (22)

To a solution of compound **7** (0.05 g, 0.11 mmol) in *o*-dichlorobenzene (10 mL) was added 3,4,5-trimethoxybenzoylchloride (0.04 g, 0.17 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark green colored solid was subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **22** as a pale yellow powder (0.03 g, 0.05 mmol, 46%, R_f = 0.50 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.25 (br s, 1H, NH), 7.19 (s, 2H, ArH), 6.99 (dd, *J* = 2.5 Hz, 8.5 Hz, 1H, ArH) 6.88 (d, *J* = 2.5 Hz, 1H, ArH), 6.75 (d, *J* = 8.0 Hz, 1H, ArH), 6.69 (s, 1H, ArH), 3.90 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.74 (s, 6H, OCH_3), 3.72 (s, 3H, OCH_3), 0.93 (s, 9H,

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C(C<u>H</u>$_3$)$_3$), 0.04 (s, 6H, Si(C<u>H</u>$_3$)$_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 193.4, 152.9, 152.1, 151.4, 146.4, 145.3, 142.2, 137.8, 136.6, 134.2, 132.4, 124.7, 121.1, 120.5, 116.7, 114.4, 112.3, 107.6, 89.8, 61.4, 61.0, 60.8, 56.4, 56.3, 55.6, 25.8, 18.5, -4.7. **HPLC**: 18.60 min., purity at 254 nm 90.7%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{34}H_{44}NO_9Si$ $[M+H]^+$ 638.2780, found 638.2780.

4.1.19. 2-(4 -Methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-methoxy-7-*tert*-butyldimethylsilyloxyindole (23)

To a solution of compound **10** (0.05 g, 0.13 mmol) in *o*-dichlorobenzene (10 mL) was added 3,4,5-trimethoxybenzoylchloride (0.05 g, 0.20 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark green colored solid was subjected to flash chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10%A / 90%B (4 CV), 10%A / 90%B → 80%A / 20%B (10 CV), 80%A / 20%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **23** as a yellow powder (0.06 g, 0.10 mmol, 76%, R_f = 0.36 (60:40 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.20 (br s, 1H, N<u>H</u>), 7.56 (d, *J* = 8.7 Hz, 1H, Ar<u>H</u>), 7.26 (d, *J* = 9.0 Hz, 2H, Ar<u>H</u>) 6.96 (s, 2H, Ar<u>H</u>), 6.94 (d, *J* = 8.7 Hz, 1H, Ar<u>H</u>), 6.78 (d, *J* = 8.6 Hz, 2H, Ar<u>H</u>), 3.88 (s, 3H, OC<u>H</u>$_3$), 3.80 (s, 3H, OC<u>H</u>$_3$), 3.76 (s, 3H, OC<u>H</u>$_3$), 3.70 (s, 6H, OC<u>H</u>$_3$), 1.09 (s, 9H, C(C<u>H</u>$_3$)$_3$), 0.26 (s, 6H, Si(C<u>H</u>$_3$)$_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 192.0, 160.2, 152.6, 146.0, 142.8, 141.3, 134.9, 130.3, 129.99, 129.95, 125.0, 124.8, 114.4, 114.2, 113.4, 109.9, 107.5, 61.0, 56.7, 56.2, 55.5, 26.3, 18.8, -4.1. **HPLC**: 20.21 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{32}H_{39}NNaO_7Si$ $[M+Na]^+$ 600.2388, found 600.2383.

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4.1.20. 2-(3 -*tert*-Butyldimethylsiloxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6,7-dimethoxyindole (24)

To a solution of compound **11** (0.25 g, 0.60 mmol) in *o*-dichlorobenzene (10 mL) was added 3,4,5-trimethoxybenzoylchloride (0.15 g, 0.66 mmol). The reaction mixture was heated to reflux at 160 °C for 12 h. The *o*-dichlorobenzene was removed by simple distillation, and the resulting dark green colored solid was subjected to flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (5.2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole **24** as a yellow powder (0.08 g, 0.14 mmol, 23%, R_f = 0.17 (70:30 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.53 (br s, 1H, NH), 7.71 (d, *J* = 8.5 Hz, 1H, ArH), 6.98 (m, 4H, ArH) 6.77 (d, *J* = 2.0 Hz, 1H, ArH), 6.73 (d, *J* = 8.5 Hz, 1H, ArH), 4.06 (s, 3H, OCH_3) 3.96 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.69 (s, 6H, OCH_3), 0.94 (s, 9H, $C(CH_3)_3$), 0.03 (s, 6H, $Si(CH_3)_2$). **^{13}C NMR** ($CDCl_3$, 125 MHz): 191.8, 152.6, 151.8, 148.0, 145.2, 142.8, 141.3, 134.6, 134.0, 130.2, 125.2, 125.1, 122.3, 122.2, 116.8, 113.1, 111.8, 110.4, 107.4, 61.3, 60.9, 57.3, 56.1, 55.5, 25.8, 18.5, -4.7. **HPLC**: 19.24 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{33}H_{41}NNaO_8Si$ $[M+Na]^+$ 630.2494, found 630.2491.

4.1.21. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,5 -dinitrobenzoyl)-6-methoxyindole (25)

To a well-stirred solution of compound **13** (0.40 g, 0.69 mmol) in THF (10 mL) at 0 °C was added tetrabutylammonium fluoride (TBAF) (1.03 mL, 1.03 mmol, 1 M in

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THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **25** (0.15 g, 0.33 mmol, 47%, R_f = 0.12 (70:30 hexanes:EtOAc)) as a yellow powder. **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz): 12.21 (br s, 1H, NH), 9.06 (br s, 1H, OH), 8.68 (t, *J* = 2.0 Hz, 1H, ArH), 8.41 (d, *J* = 2.0 Hz, 2H, ArH) 8.05 (d, *J* = 8.5 Hz, 1H, ArH), 6.97 (dd, *J* = 2.0 Hz, 1H, ArH), 6.92 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.71 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.68 (d, *J* = 8.5 Hz, 1H, ArH), 6.54 (d, *J* = 2.0 Hz, 1H, ArH), 3.83 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz): 186.7, 156.8, 148.2, 147.1, 146.7, 146.1, 142.8, 136.7, 128.5, 123.3, 121.77, 121.75, 121.7, 119.3, 117.2, 112.0, 111.7, 111.1, 95.0, 55.7, 55.3. **HPLC**: 13.87 min., purity at 254 nm 93.4%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{18}N_3O_8$ $[M+H]^+$ 464.1088, found 464.1087.

4.1.22. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(4 -nitrobenzoyl)-6-methoxyindole (26)

To a well-stirred solution of compound **14** (0.14 g, 0.27 mmol) in THF (10 mL) at 0 °C was added TBAF (0.40 mL, 0.40 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica

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column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (3 CV), 12%A / 88%B → 100%A / 0%B (11 CV), 100%A / 0%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole phenol ligand **26** (0.02 g, 0.05 mmol, 19%, R_f 0.23 (50:50 hexanes:EtOAc)) as a yellow powder. **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz): 12.04 (br s, 1H, NH), 9.05 (s, 1H, OH), 7.98 (d, *J* = 8.0 Hz, 2H, ArH), 7.83 (d, *J* = 8.5 Hz, 1H, ArH), 7.61 (d, *J* = 8.5 Hz, 2H, ArH), 6.95 (d, *J* = 1.0 Hz, 1H, ArH), 6.85 (dd, *J* = 1.5 Hz, 8.5 Hz, 1H, ArH), 6.71 (s, 1H, ArH), 6.68 (d, *J* = 8.5 Hz, 1H, ArH), 6.63 (d, *J* = 8.5 Hz, 1H, ArH), 3.81 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz): 190.1, 156.6, 148.3, 148.0, 146.1, 146.0, 145.6, 136.6, 129.8, 123.9, 122.6, 122.0, 121.47, 121.45, 116.8, 111.62, 111.55, 111.4, 94.9, 55.7, 55.3. **HPLC**: 13.19 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{19}N_2O_6$ $[M+H]^+$ 419.1238, found 419.1237.

4.1.23. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 -nitrobenzoyl)-6-methoxyindole (27)

To a well-stirred solution of compound **15** (0.13 g, 0.25 mmol) in THF (10 mL) at 0 °C was added TBAF (0.38 mL, 0.38 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 15%A / 85%B (4.5 CV), 40%A / 60%B (16 CV), 40%A / 60%B → 100%A / 0%B (2 CV), 100%A / 0%B (10.5 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole phenol ligand **27** (0.10 g, 0.25 mmol, R_f = 0.28 (50:50 hexanes:EtOAc)) quantitatively as a

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yellow powder. **^{1}H NMR** ($(CD_3)_2CO$, 500 MHz): 10.95 (br s, 1H, NH), 8.25 (t, *J* = 2.0 Hz, 1H, ArH), 8.13 (ddd, *J* = 1.0 Hz, 2.0 Hz, 8.0 Hz, 1H, ArH), 8.02 (d, *J* = 8.5 Hz, 1H, ArH), 7.93 (dt, *J* = 1.0 Hz, 7.5 Hz, 1H, ArH), 7.69 (s, 1H, OH), 7.49 (t, *J* = 8.0 Hz, 1H, ArH), 7.06 (d, *J* = 2.0 Hz, 1H, ArH), 6.91 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.81 (d, *J* = 2.0 Hz, 1H, ArH), 6.78 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H, ArH), 6.73 (d, *J* = 8.0 Hz, 1H, ArH), 3.86 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2CO$, 125 MHz): 190.5, 158.5, 149.2, 148.5, 147.4, 146.0, 143.1, 138.0, 135.9, 130.1, 125.8, 125.7, 124.8, 123.7, 123.0, 122.9, 117.5, 113.0, 112.7, 112.3, 95.8, 56.5, 56.0. **HPLC**: 12.97 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{19}N_2O_6$ $[M+H]^+$ 419.1238, found 419.1236.

4.1.24. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trifluorobenzoyl)-6-methoxyindole (28)

To a well-stirred solution of compound **16** (0.70 g, 1.29 mmol) in THF (10 mL) at 0 °C was added TBAF (1.94 mL, 1.94 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **28** (0.35 g, 0.41 mmol, 64%, R_f = 0.18 (50:50 hexanes:EtOAc)) as a tan powder. **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz): 12.02 (br s, 1H, NH), 9.12 (br s, 1H, OH), 7.85 (d, *J* = 8.5 Hz,

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1H, ArH), 7.28 (t, *J* = 7.5 Hz, 2H, ArH) 6.94 (d, *J* = 2.0 Hz, 1H, ArH), 6.86 (dd, *J* = 2.0 Hz, 9.0 Hz, 1H, ArH), 6.80 (d, *J* = 8.0 Hz, 1H, ArH), 6.71 (d, *J* = 2.0 Hz, 1H, ArH), 6.69 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 3.81 (s, 3H, OCH$_3$), 3.73 (s, 3H, OCH$_3$). **^{13}C NMR** ((CD$_3$)$_2$SO, 125 MHz): 188.0, 156.6, 149.5 (ddd, J_{C-F} = 3.0 Hz, 10.2 Hz, 247.6 Hz) 148.2, 146.1, 145.5, 140.0 (dt, J_{C-F} = 15.5 Hz, 251.4 Hz), 136.71 (d, J_{C-F} = 5.6 Hz), 136.65, 124.2, 122.0, 121.4, 121.2, 116.8, 113.5 (dd, J_{C-F} = 4.8 Hz, 16.6 Hz), 111.8, 111.5, 110.8, 94.9, 55.8, 55.3. **^{19}F NMR** ((CD$_3$)$_2$SO, 470 MHz): -135.5 (dd, *J* = 8.5 Hz, 21.6 Hz, 2F, ArF), -158.6 (tt, *J* = 6.6 Hz, 21.2 Hz, 1F, ArF). **HPLC**: 14.33 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{17}F_3NO_4$ $[M+H]^+$ 428.1104, found 428.1104.

4.1.25. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(4 -fluorobenzoyl)-6-methoxyindole (29)

To a well-stirred solution of compound **17** (0.09 g, 0.18 mmol) in THF (10 mL) at 0 °C was added TBAF (0.30 mL, 0.38 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 35%A / 65%B (5 CV), 35%A / 65%B → 50%A / 50%B (17.5 CV), 100%A / 0%B (7 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole phenol ligand **29** (0.05 g, 0.12 mmol, 64%, R_f = 0.31 (50:50 hexanes:EtOAc)) as a tan powder. **^{1}H NMR** ((CD$_3$)$_2$CO, 500 MHz): 10.78 (br s, 1H, NH), 7.77 (d, *J* = 8.5 Hz, 1H, ArH), 7.66 (m, 3H, ArH) 7.03

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(d, *J* = 2.5 Hz, 1H, ArH), 6.96 (tt, *J* = 2.5 Hz, 9.5 Hz, 2H, ArH), 6.92 (d, *J* = 2.0 Hz, 1H, ArH), 6.84 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.81 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.78 (d, *J* = 8.5 Hz, 1H, ArH), 3.84 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3. **^{13}C NMR** ($(CD_3)_2CO$, 125 MHz): 190.6, 164.3 (d, J_{C-F} = 248 Hz), 157.2, 147.9, 146.3, 143.0, 137.00, 136.98, 136.9, 131.8 (d, J_{C-F} = 9 Hz), 125.1, 123.0, 121.7, 121.5, 116.0, 114.4 (d, J_{C-F} = 22 Hz), 111.17, 111.15, 94.5, 55.4, 55.9. **^{19}F NMR** ($(CD_3)_2CO$, 470 MHz): -110.8 (m, 1F, ArF). **HPLC**: 12.91 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{23}H_{19}FNO_4$ $[M+H]^+$ 392.1293, found 392.1291.

4.1.26. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,5 -*bis*-trifluoromethylbenzoyl)-6-methoxyindole (30)

To a well-stirred solution of compound **18** (0.26 g, 0.42 mmol) in THF (10 mL) at 0 °C was added TBAF (0.63 mL, 0.63 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (5.2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **30** (0.22 g, 0.42 mmol, R_f = 0.21 (70:30 hexanes:EtOAc)) quantitatively as an orange powder. **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz): 12.10 (br s, 1H, NH), 9.00 (br s, 1H, OH), 7.99 (m, 2H, ArH), 7.90 (s, 2H, ArH) 6.96 (d, *J* = 2.0 Hz, 1H, ArH), 6.89 (dd, *J* = 2.5 Hz, 9.0 Hz, 1H, ArH), 6.64 (m, 3H, ArH), 3.82 (s, 3H, OCH_3), 3.66 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2SO$,

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125 MHz): 188.4, 156.7, 148.2, 146.3, 146.2, 142.2, 136.7, 129.7 (q, J_{C-F} = 33 Hz), 128.9 (m), 123.7 (m), 123.5, 123.0 (q, J_{C-F} = 272 Hz), 122.0, 121.6, 121.4, 116.9, 111.7, 111.6, 111.0, 94.9, 55.6, 55.3. **^{19}F NMR** ($(CD_3)_2SO$, 470 MHz): -61.4 (s, 6F, C$\underline{F}_3$). **HPLC**: 16.17 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{25}H_{18}F_6NO_4$ $[M+H]^+$ 510.1135, found 510.1134.

4.1.27. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 -trifluoromethoxybenzoyl)-6-methoxyindole (31)

To a well-stirred solution of compound **19** (1.21 g, 2.11 mmol) in THF (10 mL) at 0 °C was added TBAF (3.20 mL, 3.17 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (4 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **31** (0.67 g, 1.51 mmol, 71%, R_f = 0.33 (50:50 hexanes:EtOAc)) as an orange powder. **^{1}H NMR** ($(CD_3)_2CO$, 500 MHz): 10.89 (br s, 1H, N$\underline{H}$), 7.89 (d, *J* = 8.7 Hz, 1H, Ar$\underline{H}$), 7.72 (s, 1H, O$\underline{H}$), 7.55 (d, *J* = 7.4 Hz, 1H, Ar$\underline{H}$), 7.47 (s, 1H, Ar$\underline{H}$), 7.30 (t, *J* = 7.8 Hz, 1H, Ar$\underline{H}$), 7.26 (d, *J* = 8.3 Hz, 1H, Ar$\underline{H}$), 7.04 (d, *J* = 2.2 Hz, 1H, Ar$\underline{H}$), 6.93 (d, *J* = 2.0 Hz, 1H, Ar$\underline{H}$), 6.88 (dd, *J* = 8.7, 2.3 Hz, 1H, Ar$\underline{H}$), 6.78 (dd, *J* = 8.3, 2.0 Hz, 1H, Ar$\underline{H}$), 6.72 (d, *J* = 8.3 Hz, 1H, Ar$\underline{H}$), 3.84 (s, 3H, OC$\underline{H}_3$), 3.76 (s, 3H, OC$\underline{H}_3$). **^{13}C NMR** ($(CD_3)_2CO$, 125 MHz): 191.4, 158.1, 149.4 (q, J_{C-F} = 2 Hz), 148.9, 147.2, 145.2, 143.8, 137.8, 130.3,

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128.9, 125.6, 123.9, 123.7, 122.63, 122.59, 122.2, 121.3 (q, J_{C-F} = 254.75 Hz), 116.9, 112.8, 112.3, 112.0, 95.6, 56.2, 55.8. **^{19}F NMR** ($(CD_3)_2CO$, 470 MHz): -58.5 (s, 6F, OC<u>F</u>$_3$). **HPLC**: 14.71 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{24}H_{19}F_3NO_5$ $[M+H]^+$ 458.1210, found 458.1210.

4.1.28. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(4 -trifluoromethoxybenzoyl)-6-methoxyindole (32)

To a well-stirred solution of compound **20** (0.92 g, 1.60 mmol) in THF (5 mL) at 0 °C was added TBAF (2.5 mL, 2.40 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (4 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **32** (0.43 g, 0.96 mmol, 60%, R_f = 0.33 (50:50 hexanes:EtOAc)) as a yellow powder. **^{1}H NMR** ($(CD_3)_2CO$, 500 MHz): 10.86 (br s, 1H, N<u>H</u>), 7.91 (d, *J* = 8.8 Hz, 1H, Ar<u>H</u>), 7.69 (s, 1H, O<u>H</u>), 7.65 (d, *J* = 8.5 Hz, 2H, Ar<u>H</u>), 7.10 (d, *J* = 8.5 Hz, 2H, Ar<u>H</u>), 7.03 (d, *J* = 2.2 Hz, 1H, Ar<u>H</u>), 6.90 (s, 1H, Ar<u>H</u>), 6.87 (dd, *J* = 2.2 Hz, 8.8 Hz, 1H, Ar<u>H</u>), 6.71 (m, 2H, Ar<u>H</u>), 3.84 (s, 3H, OC<u>H</u>$_3$), 3.77 (s, 3H, OC<u>H</u>$_3$). **^{13}C NMR** ($(CD_3)_2CO$, 125 MHz): 191.6, 158.1, 151.3 (q, J_{C-F} = 2 Hz), 148.9, 147.2, 145.1, 140.5, 137.8, 132.0, 125.7, 123.7, 122.72, 122.67, 121.7 (q, J_{C-F} = 254.75 Hz), 120.7, 116.9, 113.0, 112.3, 111.8, 95.5, 55.1, 55.8. **^{19}F NMR** ($(CD_3)_2CO$, 470 MHz): -58.5 (s, 6F, OC<u>F</u>$_3$). **HPLC**: 14.80 min., purity at 254 nm

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>99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{24}H_{19}F_3NO_5$ $[M+H]^+$ 458.1210, found 458.1210.

4.1.29. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-hydroxyindole (33)

To a well-stirred solution of compound **21** (0.40 g, 0.59 mmol) in THF (10 mL) at 0 °C was added TBAF (1.00 mL, 0.89 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted using EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (1.4 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole free phenol ligand **33** (0.11 g, 0.25 mmol, 42%, R_f = 0.03 (70:30 hexanes:EtOAc)) as a yellow powder. **^{1}H NMR** ($(CD_3)_2SO$, 500 MHz): 11.62 (br s, 1H, NH), 9.19 (br s, 1H, OH), 9.00 (br s, 1H, OH), 7.66 (d, *J* = 8.5 Hz, 1H, ArH), 6.82 (d, *J* = 2.0 Hz, 1H, ArH) 6.78 (s, 2H, ArH), 6.74 (d, *J* = 9.0 Hz, 2H, ArH), 6.67 (t, *J* = 2.5 Hz, 1H, ArH), 6.65 (t, *J* = 3.0 Hz, 1H, ArH), 3.69 (s, 3H, OCH_3), 3.61 (s, 6H, OCH_3), 3.59 (s, 3H, OCH_3). **^{13}C NMR** ($(CD_3)_2SO$, 125 MHz): 192.0, 155.2, 153.4, 148.5, 147.0, 143.7, 141.8, 138.0, 136.4, 126.5, 123.4, 122.7, 122.0, 116.9, 113.1, 112.3, 111.8, 106.0, 97.4, 60.4, 56.18, 56.17. **HPLC**: 8.95 min., purity at 254 nm 90.7%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{25}H_{24}NO_7$ $[M+H]^+$ 450.1547, found 450.1547.

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4.1.30. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-4,5,6-methoxyindole (34)

To a well-stirred solution of compound **22** (0.03 g, 0.05 mmol) in THF (10 mL) at 0 °C was added TBAF (0.1 mL, 0.08 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired indole phenol ligand **34** (0.02 g, 0.03 mmol, 60%, R_f = 0.10 (50:50 hexanes:EtOAc)) as a yellow powder. **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.30 (br s, 1H, NH), 7.15 (s, 2H, ArH), 6.99 (d, *J* = 2.0 Hz, 1H, ArH) 6.89 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H, ArH), 6.71 (d, *J* = 8.5 Hz, 1H, ArH), 6.68 (s, 1H, ArH), 5.62 (s, 1H, OH), 3.90 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3) 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.74 (s, 6H, OCH_3), 3.69 (s, 3H, OCH_3). **^{13}C NMR** ($CDCl_3$, 125 MHz): 193.4, 152.8, 152.2, 146.8, 146.5, 145.8, 142.1, 137.8, 137.0, 134.6, 132.4, 125.1, 120.5, 116.1, 113.7, 112.6, 110.9, 107.5, 89.7, 61.4, 61.0, 60.8, 56.4, 56.3, 56.1. **HPLC**: 11.13 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{28}H_{30}NO_9$ $[M+H]^+$ 524.1915, found 524.1912.

4.1.31. 2-(4 -Methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6-methoxy-7-hydroxyindole (35)

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To a well-stirred solution of compound **23** (0.008 g, 0.014 mmol) in THF (10 mL) at 0 °C was added TBAF (0.01 mL, 0.01 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%A / 93%B (4 CV), 7%A / 93%B → 60%A / 40%B (10 CV), 60%A / 40%B (5 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] resulting in free phenol indole **35** as a dark brown powder (0.006 g, 0.013 mmol, 90%, R_f = 0.22 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.57 (br s, 1H, NH), 7.52 (d, *J* = 8.4 Hz, 1H, ArH), 7.29 (d, *J* = 8.1 Hz, 2H, ArH) 6.95 (br s, 3H, ArH), 6.74 (d, *J* = 8.1 Hz, 2H, ArH), 5.76 (br s, 1H, OH), 3.97 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 3.69 (s, 6H, OCH_3). **^{13}C NMR** ($CDCl_3$, 125 MHz): 192.2, 160.1, 152.6, 143.7, 142.3, 141.3, 134.8, 131.0, 130.4, 125.5, 125.3, 124.5, 114.0, 113.0, 112.8, 108.7, 107.5, 61.0, 57.6, 56.2, 55.4. **HPLC**: 11.67 min., purity at 254 nm 96.9%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{26}H_{26}NO_7$ $[M+H]^+$ 464.1704, found 464.1704.

4.1.32. 2-(3 -Hydroxy-4 -methoxyphenyl)-3-(3 ,4 ,5 -trimethoxybenzoyl)-6,7-dimethoxyindole (36)

To a well-stirred solution of compound **24** (0.08 g, 0.14 mmol) in THF (10 mL) at 0 °C was added TBAF (0.21 mL, 0.21 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min while warming to room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 x 10 mL). The

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combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by flash column chromatography using a prepacked 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12%A / 88%B (4 CV), 12%A / 88%B → 100%A / 0%B (10 CV), 100%A / 0%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulting in free phenol indole **36** as a dark brown powder (0.02 g, 0.04 mmol, 27%, R_f = 0.14 (50:50 hexanes:EtOAc)). **^{1}H NMR** ($CDCl_3$, 500 MHz): 8.57 (br s, 1H, NH), 7.71 (d, *J* = 9.0 Hz, 1H, ArH), 6.972 (d, *J* = 9.0 Hz, 1H, ArH) 6.971 (d, *J* = 2.0 Hz, 1H, ArH), 6.94 (s, 2H, ArH), 6.79 (dd, *J* = 8.3 Hz, 2.0 Hz, 1H, ArH), 6.64 (d, *J* = 8.3 Hz, 1H, ArH), 5.63 (br s, 1H, OH), 4.06 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3). **^{13}C NMR** ($CDCl_3$, 125 MHz): 192.0, 152.6, 148.1, 147.2, 145.7, 143.2, 141.1, 135.0, 134.0, 130.2, 125.4, 125.0, 122.0, 116.8, 114.8, 113.4, 110.52, 110.45, 107.3, 61.3, 60.9, 57.4, 56.17, 56.15. **HPLC**: 11.45 min., purity at 254 nm >99%. **HRMS (ESI$^+$)**: *m/z* calculated for $C_{27}H_{27}NNaO_8$ $[M+Na]^+$ 516.1629, found 516.1626.

4.2 Biological evaluation

4.2.1. SRB Assay[53-54]

We assessed inhibition of human cancer cell growth using the National Cancer Institute's standard sulforhodamine B assay, as previously described.[53] Briefly, cancer cell lines in a 5% fetal bovine serum/RPMI1640 medium, 1% gentamicin solution were plated in 96-well plates and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the cells were fixed with trichloroacetic acid, stained with sulforhodamine B, and read with an automated Biotek plate reader. A growth inhibition

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of 50% (GI_{50} or the drug concentration causing a 50% reduction in the net protein increase) was calculated from optical density data.

4.2.2. Colchicine Binding Assay

Inhibition of [^{3}H]colchicine binding was determined using 100 L reaction mixtures containing 1.0 M tubulin, 5.0 M [^{3}H]colchicine (from Perkin-Elmer), 5% (v/v) dimethyl sulfoxide, and potential inhibitors at 1.0 or 5.0 M. Reaction mixtures also contained components shown to potently stabilize the colchicine binding activity of tubulin:[55] 1.0 M monosodium glutamate (pH 6.6 as above), 0.5 mg/mL bovine serum albumin, 0.1 M glucose-1-phosphate, 1.0 mM $MgCl_2$, and 1.0 mM GTP. Incubation was for 10 min at 37 °C, a time point at which the reaction in the control is 40-60% complete. Reactions were stopped by adding 2.0 mL of ice-cold water and placing the samples on ice prior to filtration. Each sample was poured onto a stack of two DEAE-cellulose filters, followed immediately by 6 mL of ice-cold water, and the water was aspirated under reduced vacuum. The filters were washed with additional water and placed into vials containing 5 mL of Biosafe II scintillation cocktail. The samples were counted the next day in a Beckman scintillation counter. Samples with potential inhibitors were compared to controls with no inhibitor to determine percent inhibition.

4.2.3. Inhibition of Tubulin Polymerization

Tubulin assembly experiments were performed with 0.25 mL reaction mixtures (final volume). The mixtures contained 1 mg/mL (10 M) purified bovine brain tubulin, 0.8 M tubulin monosodium glutamate (adjusted to pH 6.6 with HCl in 2.0 M stock solution), 4% (v/v) dimethyl sulfoxide, 0.4 mM GTP, and varying concentrations of

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compound. Initially, all components except GTP were preincubated for 15 min at 30 °C in a 0.24 mL volume. After chilling the mixtures on ice, 10 L of 10 mM GTP was added to each sample. The reaction mixtures were then transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was jumped to 30 °C over about 30 s, and polymerization was followed turbidimetrically at 350 nM for 30 min. Each reaction set included a reaction mixture without compound, and the IC_{50} was defined as the concentration of compound that inhibited the extent of assembly versus the control after 20 min at 30 °C. These values were obtained by interpolation between the actual experimental values.

Supplementary Data.

Characterization data (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, HPLC, and HRMS) for final compounds and X-ray crystallography for compound **10** are available free of charge via the internet at http://pubs.acs.org.

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