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Synthesis of a 2-Aryl-3-Aroyl-Indole Salt (OXi8007) Resembling Combretastatin A-4 with Application as a Vascular Disrupting Agent

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Abstract

The natural products colchicine and combretastatin A-4 (CA4) are potent inhibitors of tubulin assembly, and they have inspired the design and synthesis of a large number of small-molecule, potential anticancer agents. The indole-based molecular scaffold is prominent among these SAR modifications, leading to a rapidly increasing number of agents. The water-soluble phosphate prodrug **33** (OXi8007) of a 2-aryl-3-aroilindole-based phenol **8** (OXi8006) was prepared by chemical synthesis and found to be strongly cytotoxic against selected human cancer cell lines (GI₅₀ = 36 nM against DU-145 cells, for example). The free phenol, **8** (OXi8006), was a strong inhibitor (IC₅₀ = 1.1 μM) of tubulin assembly. The corresponding phosphate prodrug **33** (OXi8007) also demonstrated pronounced interference with tumor vasculature in a preliminary in vivo study utilizing a SCID mouse model bearing an orthotopic PC-3 (prostate) tumor as imaged by color Doppler ultrasound. The combination of these results provides evidence that the indole-based phosphate prodrug **33** (OXi8007) functions as a vascular disrupting agent (VDA) that may prove useful for the treatment of cancer.

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[∇]Dedicated to the memory of Dr. Anjan Ghatak, who passed away on July 22, 2003

ASSOCIATED CONTENT.

Supporting Information. Characterization data for selected intermediates and the final compounds are available free of charge via the Internet at <http://pubs.acs.org>.

One of the co-authors (K.G.P.) is a paid consultant (and stock shareholder) with a corporate sponsor of this research (Oxigene Inc.) and another co-author (D.J.C.) is the former Chief Scientific Officer, and a current Board member and stock shareholder, with Oxigene Inc.

The vast majority of natural products and synthetic compounds that bind to the tubulin-microtubule protein system and subsequently interfere with the dynamic assembly/disassembly inherent to the α,β -tubulin/microtubule system do so by many variations of this overall mechanism of action. The most thoroughly studied are interactions at the colchicine, vinca alkaloid, and taxoid sites.^{1,2} A significant number of antiproliferative, anticancer agents function through this basic mechanism of action. The combretastatin family of natural products consists of a variety of *cis*-stilbenoid compounds isolated from the South African bush willow tree, *Combretum caffrum* Kuntze (Combretaceae).¹ Combretastatin A-4 (CA4) is among the most potent antimitotic agents from this family of compounds and binds to the colchicine site on tubulin (Figure 1, compound **2**).^{3–5} As with many natural products, the challenge of water solubility led to the development of a disodium phosphate prodrug **3**, combretastatin A-4P (CA4P).⁶ The discovery of CA4 has led to a diverse library of anti-tubulin agents designed to mimic the simple stilbenoid structure.^{1,7}

The indole structure is prevalent as a core molecular component in a variety of inhibitors of tubulin assembly.⁸ As an early example, the vinca alkaloid natural products vinblastine and vincristine, originally isolated from the madagascar periwinkle plant *Catharanthus roseus* (L.) G. Don (Apocynaceae), both incorporate two key indole ring systems.^{9,10} A significant number of compounds that bind to the colchicine site and inhibit microtubule formation are also indole-based (see Figure 2 for representative molecules). To the best of our knowledge, the first examples of such colchicine site interactive, indole-based compounds were reported by von Angerer et al.¹¹ (Figure 2, compound **6**) and separately by Pinney et al.^{12,13} (Figure 2, compounds **7–8**) in the middle-to-late 1990s. Our (Pinney and co-workers) 2-aryl-3-aryl-indole analogues^{12–14} were originally inspired, in part, by the combretastatin collection of natural products (pioneered by George R. Pettit)^{1,15} and the non-steroidal, selective estrogen receptor modulator (SERM) work of Eli Lilly, Inc., featuring benzo[*b*]thiophene templates.¹⁶ A judicious combination of key structural features inherent to both of these molecules led to the preparation of benzo[*b*]thiophene-based analogues (Figure 1, compounds **4–5**) that proved to be potent inhibitors of tubulin assembly through a binding interaction at the colchicine site.^{17,18} A logical course of structure-activity relationship (SAR) considerations led to the idea of incorporating a nitrogen atom in the form of an indole-based analogue.

Herein, we report the synthesis of this indole-based compound **8** (OXi8006), along with a water-soluble phosphate salt **33** (OXi8007) and the corresponding *N*-methyl derivatives (**30** and **34**). Cytotoxicity of these compounds against selected human cancer cell lines and their ability to inhibit tubulin polymerization are described. In addition, a preliminary color Doppler ultrasound experiment with a PC-3 tumor in a SCID mouse model is presented, demonstrating a time-progressive disruption of tumor blood flow, thus aiding in the establishment of phosphate prodrug **33** (OXi8007) as among the first indole-based, vascular disrupting agents (VDAs). A structurally similar 3-arylindole (Figure 2, compound **11**, also referred to as BPR0L075)^{21,28–29} was recently described by Mason et al. as a VDA.³⁰ In addition, *N*-methyl-5,6,7-trimethoxy indole VDAs have been recently reported by Hu et al.³¹

A previous study by Dalal and Burchill in a TC-32 tumor model found minimal necrosis and vessel occlusion with indole salt **33** (OXi8007) and CA4P as compared with OXi4503.³² Compounds delineated as VDAs function by disrupting existing tumor vasculature, ultimately leading to oxygen and nutrient deprivation to the tumor. VDAs are a separate class of anticancer agents that are mechanistically distinct from the angiogenesis inhibiting agents (AIAs).^{33,34} VDAs cause morphology changes (rounding up) of the endothelial cells lining the microvessels feeding tumors leading to hypoxia, and ultimately to tumor necrosis.³⁵ No VDA has been approved by the FDA to date, although several are currently

progressing in clinical trials. Our original patent publications inspired Flynn and co-workers to apply their elegant heteroatom ring formation synthetic strategy towards the synthesis of indole **8** (OXi8006).³⁶ Their important contributions in this area ultimately led to their design and synthesis of BNC105, a benzofuran-based VDA that is currently undergoing clinical trials.^{37,38}

RESULTS AND DISCUSSION

The synthesis of indole analogues **8** and **30** involved substituted bromoacetophenone **24** as a key intermediate, which was prepared from commercially available isovanillin **19** (Scheme 1). After protection of the phenol as its corresponding silyl ether **20**, addition of methylolithium afforded the secondary alcohol **21**, which upon oxidation with PCC furnished the expected acetophenone derivative **22**. Treatment with LDA followed by TMSCl resulted in silyl enol ether **23**, which was converted to its corresponding bromide **24**. The 2-aryl substituted indole derivative **25** was prepared following the Bischler-Mohrlau method, by condensation of the substituted bromoacetophenone **24** with 3-methoxyaniline in the high boiling solvent *N,N*-dimethyl aniline.³⁹ Subsequent reaction of indole **25** with 3,4,5-trimethoxybenzoyl chloride yielded the 2-aryl-3-trimethoxybenzoyl substituted indole derivative **27** through a Friedel-Crafts benzoylation reaction. Desilylation of compound **27** with TBAF afforded the desired parent indole-based phenolic ligand **8** (OXi8006). Preparation of the corresponding *N*-methyl indole derivative **30** followed a similar synthetic pathway (Scheme 1) except that the TBS protecting group on intermediate **25** was replaced with an isopropyl group (intermediate **26b**) prior to the benzoylation reaction. In general, the benzoylation reaction proceeded with higher yields with the isopropyl protecting group rather than with the TBS group. However, the isopropyl group was not used throughout the synthetic sequence since other reactions tended to have higher yields with the TBS group in place, and product purification following deprotection of the isopropyl group was more involved, hence the preference for the TBS group.

Reaction of either phenol (**8** or **30**) with in situ-generated dibenzyl chlorophosphite resulted in the expected indole-based dibenzyl phosphate ester derivative (**31** or **32**). Catalytic hydrogenolysis of the benzylic carbon-oxygen bonds with hydrogen gas and palladium on activated carbon (10%), followed by an acid-base reaction between the resulting phosphoric acid and sodium methoxide in methanol resulted in the desired disodium phosphate indole prodrug **33** (OXi8007) or the *N*-methyl derivative **34**.⁶ Initial debenzoylation attempts involved reaction of the dibenzyl ester with in situ-generated iodotrimethylsilane, from chlorotrimethylsilane and sodium iodide. The hydrogenolysis reaction offered the advantages of ease of work up and isolation of the product and better yields compared with the iodotrimethylsilane reaction.⁶

The two indole free phenolic analogues **8** (OXi8006) and **30** and their corresponding water-soluble prodrug salts **33** (OXi8007) and **34** were evaluated for their ability to inhibit tubulin polymerization (in a cell-free, pure protein assay). Indole **8** was found to be a potent inhibitor of tubulin assembly ($IC_{50} = 1.1 \mu M$) comparable with CA4 (Table 1). While CA4P (containing the bulky phosphate prodrug moiety) is inactive ($IC_{50} > 40 \mu M$) as an inhibitor of tubulin assembly,⁴⁰ the indole-based phosphate prodrug **33** (OXi8007) was found to be strongly inhibitory ($IC_{50} = 4.2 \mu M$). The addition of the *N*-methyl group resulted in a loss of the ability to inhibit tubulin polymerization ($IC_{50} > 20 \mu M$) for both the free phenol **30** and its corresponding phosphate prodrug salt **34**. In a binding assay against tritium-labeled colchicine, indole **8** (OXi8006) demonstrated modest inhibition at a concentration of $1 \mu M$ (40%) that increased at $5 \mu M$ (75%). CA4, as a control, strongly inhibited colchicine binding even at $1 \mu M$ (91%). The phosphate prodrug salt, **33** (OXi8007), was only minimally inhibitory (26%) at a concentration of $5 \mu M$.

Both of the *N*-methyl analogues **30** and **34** showed only modest inhibition (34% and 29%, respectively) at a high concentration (50 μ M). Two possible conclusions can be inferred from these data. First, it seems likely that the non-methylated, free phenol **8** (OXi8006) interacts with the colchicine binding site in an orientation that places the free phenolic moiety outside of the binding pocket. This seems plausible since the corresponding prodrug **33** (OXi8007), containing the bulky phosphate salt moiety, also demonstrated strong inhibition of tubulin assembly, which is typically not the case for small-molecule phosphate prodrugs of active tubulin binding agents (such as CA4P). Secondly, incorporation of a methyl group on the indole-nitrogen atom severely limited the ability of these analogues to interact with tubulin.

The phenolic indole **8** (OXi8006) and its corresponding prodrug salt **33** (OXi8007) demonstrated consistently strong cytotoxicity (GI_{50} values ranging from 3.5 to 38 nM) against the NCI-H460 (lung), DU-145 (prostate), and SK-OV-3 (ovarian) human cancer cell lines (Table 2). Cytotoxicity was greatly diminished (IC_{50} approximately 3,000 nM) with the closely related *N*-methyl phenol **30** and prodrug **34**. These data track with the inhibition of tubulin assembly data. Thus, it is instructive to briefly address the question (and subsequent confusion) that occasionally arises in regard to why compounds that are in the nM concentration range in terms of GI_{50} values for cytotoxicity against selected human cancer cell lines, rarely show lower than micromolar activity in terms of their measured IC_{50} value in the inhibition of tubulin assembly assay. This is commonly noted for many compounds including CA4, for example, as well as indole **8** (OXi8006).

Although the reason is not known with certainty, several factors may contribute. For example, the stoichiometry that exists between the concentration of compound and the concentration of tubulin needed to achieve an IC_{50} value in the micromolar range in the pure protein (cell-free) assay may be drastically different from the requisite stoichiometry that exists when inhibiting tubulin in a cell-based assay leading to a measured GI_{50} value for cytotoxicity in the nanomolar range. It should be emphasized that the pure protein assay incorporates no cellular components other than tubulin. Importantly, tubulin disassembly (in cells) may release factors that are involved in intramolecular signal transduction leading to a major amplification. In addition, the amount of tubulin needed in the pure protein assay in order to accurately measure inhibition of microtubule formation (by monitoring absorbance at 350 nm spectrophotometrically) is large enough that the practical lower limit for this assay is around 0.5 to 1 micromolar (IC_{50} value) for even the most active inhibitors of tubulin polymerization.

As a preliminary test of vascular disrupting activity *in vivo*, color Doppler ultrasound was applied to a PC-3 human tumor xenograft growing in a SCID mouse. Images were acquired over a period of 80 min starting immediately after administration of indole prodrug **33** (OXi8007). The tumor was clearly visible (Figure 3) with extensive bidirectional blood flow displayed in red and blue. Blood flow was also observed in vessels outside the tumor. Blood flow decreased progressively and by 80 min had essentially ceased in the core of the tumor. The extratumoral vessels remained undisrupted throughout. The differential activity in the tumor and animal tissue provides preliminary evidence of selective vascular disruption *in vivo*. Ultrasound has emerged as a valuable imaging technique for VDAs⁴² and has recently been validated as a corollary imaging strategy to bioluminescence imaging (BLI)³³ for the assessment of VDAs.⁴³

In summary, an indole-based, water-soluble phosphate prodrug salt **33** (OXi8007) was prepared by chemical synthesis and found to be strongly cytotoxic against a selection of human cancer cell lines. Its parent phenolic analogue **8** (OXi8006) was a potent inhibitor of tubulin assembly. A preliminary Doppler ultrasound experiment demonstrated that indole

prodrug **33** (OXi8007) has VDA characteristics that are potentially suitable for the treatment of cancer. Additional studies are underway with indole prodrug **33** (OXi8007) and related compounds to further understand the biological mechanism of action and to investigate structure-activity relationships within the 2-aryl-3-arylindole molecular framework.

EXPERIMENTAL SECTION

General Experimental Methods

Dichloromethane, acetonitrile, dimethylformamide (DMF), methanol, ethanol, 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. 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 Biotage Isolera flash purification system using silica gel (200–400 mesh, 60 Å) or RP-18 prepacked columns or manually in glass columns.

Intermediates and products synthesized were characterized on the basis of their ¹H NMR (500 or 300 MHz), ¹³C NMR (125 or 75 MHz), and ³¹P NMR (200 or 120 MHz) spectroscopic data using a Varian VNMRs 500 MHz or a Bruker DPX 300 MHz instrument. Spectra were recorded in CDCl₃, D₂O, or CD₃OD. All chemical shifts are expressed in ppm (δ), coupling constants (*J*) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), double doublet (dd), 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 (λ = 190–400 nm), a Zorbax XDB-C₁₈ HPLC column (4.6 mm – 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; method A: solvent A, acetonitrile, solvent B, 0.1% TFA in H₂O; or method B: solvent A, acetonitrile, solvent B, H₂O; 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.

3-(*tert*-Butyldimethylsilyloxy)-4-methoxybenzaldehyde (20).⁴⁴—To a solution of 3-hydroxy-4-methoxybenzaldehyde **19** (25.00 g, 164.4 mmol) dissolved in CH₂Cl₂ (250 mL) at 0 °C was added triethylamine (Et₃N) (25.2 mL, 180.9 mmol) followed by *N,N*-dimethylaminopyridine (DMAP) (2.01 g, 16.4 mmol). The reaction mixture was stirred for 10 min, and *tert*-butyldimethylsilyl chloride (TBSCl) (27.26 g, 180.9 mmol) was then added gradually. The solution was allowed to warm to room temperature over 12 h. Upon completion of the reaction, the reaction mixture was quenched with water (150 mL) and extracted with CH₂Cl₂. The extracted layers were combined, dried over Na₂SO₄, and concentrated under reduced pressure. The TBS benzaldehyde product **20** [47.09 g, 176.9 mmol, *R*_f = 0.50 (hexanes-EtOAc 70:30)] was isolated quantitatively as a yellow oil and was taken to the next step without further purification. ¹H NMR (CDCl₃, 500 MHz) δ 9.80 (1H, s, CHO), 7.45 (1H, dd, *J* = 8.5 Hz, *J* = 2.0 Hz, ArH), 7.35 (1H, d, *J* = 2.0 Hz, ArH), 6.93 (1H, d, *J* = 8.5 Hz, ArH), 3.87 (3H, s, OCH₃), 0.99 (9H, s, C(CH₃)₃), 0.16 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 190.2, 156.2, 145.2, 130.0, 126.0, 119.4, 110.9, 55.1, 25.3, 18.0, –5.0.

3-*tert*-Butyldimethylsilyloxy-1-(1'-hydroxyethyl)-4-methoxybenzene (21).^{12,44}—Crude TBS benzaldehyde **20** (47.09 g, 176.9 mmol) dissolved in tetrahydrofuran (THF, 500 mL) at 0 °C was treated with CH₃Li (144 mL, 230 mmol) dropwise. The solution was allowed to reach room temperature over 12 h. Upon completion of the reaction, the reaction

mixture was slowly quenched with water (200 mL) and extracted with EtOAc. The organic extract was dried over Na₂SO₄ and concentrated under reduced pressure, resulting in secondary alcohol **21** [48.97 g, 173.4 mmol, 98%, *R_f* = 0.40 (hexanes-EtOAc 70:30)] as a yellow oil, which was taken to the next step without further purification. ¹H NMR (CDCl₃, 500 MHz) δ 6.88 (2H, m, ArH), 6.83 (1H, d, *J* = 8.1 Hz, ArH), 4.81 (1H, q, *J* = 6.3 Hz, CH), 3.79 (3H, s, OCH₃), 1.82 (1H, s, OH), 1.45 (3H, d, *J* = 6.3, CH₃), 0.99 (9H, s, (CH₃)₃), 0.15 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 149.7, 144.5, 138.9, 118.4, 118.0, 111.7, 69.1, 55.1, 25.5, 24.9, 18.2, -4.8.

3-*tert*-Butyldimethylsilyloxy-4-methoxyacetophenone (22).^{17,44}—To a solution of crude alcohol **21** (48.97 g, 173.4 mmol) and Celite® (35 g) in CH₂Cl₂ (500 mL) at 0 °C was added pyridinium chlorochromate (PCC, 41.12 g, 190.7 mmol) in small increments (1–3 g), allowing 10 min of stirring between each addition. The reaction mixture was allowed to warm to room temperature over 12 h. Upon completion of the reaction, the reaction mixture was filtered through a 50:50 plug of silica gel-Celite®, and the plug was rinsed well with CH₂Cl₂. The filtrate was concentrated under reduced pressure providing the desired acetophenone derivative **22** [37.94 g, 135.3 mmol, 78%, *R_f* = 0.50 (hexanes-EtOAc 70:30)] as a pale yellow solid. ¹H NMR (CDCl₃, 500 MHz) δ 7.57 (1H, dd, *J* = 8.5 Hz, 2.0 Hz, ArH), 7.46 (1H, d, *J* = 2.0 Hz, ArH), 6.86 (1H, d, *J* = 8.5 Hz, ArH), 3.86 (3H, s, OCH₃), 2.52 (3H, s, CH₃), 1.00 (9H, s, C(CH₃)₃), 0.16 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 196.7, 155.3, 144.8, 130.5, 123.5, 120.4, 110.7, 55.4, 26.2, 25.6, 18.4, -4.7.

1-(3-*tert*-Butyldimethylsilyloxy-4-methoxyphenyl)-1-trimethylsilylethene (23).¹⁷—To a solution of diisopropylamine (16.6 mL, 117 mmol) in THF (300 mL) at 0 °C was added *n*-butyllithium (47.04 mL, 117.4 mmol) dropwise. The LDA solution was allowed to stir for 15 min, and then a solution of TBS-acetophenone **22** (21.95 g, 78.27 mmol) in THF (100 mL) was added dropwise. The solution was stirred for 10 min, and trimethylsilyl chloride (TMSCl) (14.9 mL, 117.4 mmol) was also added dropwise. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion of the reaction, the solution was diluted with NaHCO₃ (10%, 200 mL). The reaction mixture was extracted with diethyl ether. Next, the extract was dried over Na₂SO₄, and the organic phase was concentrated under reduced pressure to provide crude TMS-enol ether **23** [30.45 g, 86.12 mmol] as a dark yellow oil, which was taken to the next step without purification. ¹H NMR (CDCl₃, 500 MHz) δ 7.18 (1H, dd, *J* = 8.5 Hz, 2.5 Hz, ArH), 7.12 (1H, d, *J* = 2.5 Hz, ArH), 6.80 (1H, d, *J* = 8.5 Hz, ArH), 4.78 (1H, d, *J* = 1.5 Hz, CH₂), 4.34 (1H, d, *J* = 1.5 Hz, CH₂), 3.81 (3H, s, OCH₃), 1.03 (9H, s, C(CH₃)₃), 0.27 (9H, s, Si(CH₃)₃), 0.18 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 155.3, 151.1, 144.4, 130.6, 118.8, 118.1, 111.2, 89.5, 55.4, 25.7, 18.4, 0.03, -4.7.

3'-(*tert*-Butyldimethylsilyloxy)-4'-methoxy-2-bromoacetophenone (24).¹⁷—To a solution of crude **23** (30.45 g, 86.12 mmol) and anhydrous K₂CO₃ (0.51 g, 3.7 mmol) in CH₂Cl₂ (250 mL) at 0 °C, was added bromine (2.66 mL, 51.7 mmol) dropwise. The solution was allowed to stir for 30 min, diluted with sodium thiosulfate (10%), and extracted with CH₂Cl₂. The extract was dried over Na₂SO₄ 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 (1.2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded bromoacetophenone analogue **24** [18.10 g, 50.38 mmol, 58%, *R_f* = 0.37 (hexanes-EtOAc 90:10)] as a tan red solid. ¹H NMR (CDCl₃, 500 MHz) δ 7.61 (1H, dd, *J* = 8.5 Hz, 2.5 Hz, ArH), 7.48 (1H, d, *J* = 2.5 Hz, ArH), 6.88 (1H, d, *J* = 8.5 Hz, ArH), 4.37 (2H, s, CH₂), 3.88 (3H, s, OCH₃), 1.00 (9H, s, C(CH₃)₃), 0.17 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 189.8, 156.1, 145.1, 127.1, 124.2, 121.0, 111.0, 55.5, 30.7, 25.6, 18.4,

–4.6; HPLC: method B, t_R 9.10 min; (+) HRESIMS m/z found 381.0496 $[M+Na]^+$ (calcd for $C_{15}H_{23}BrNaO_3Si$, 381.0492).

2-(3'-*tert*-Butyldimethylsilyloxy-4'-methoxyphenyl)-6-methoxyindole (25).⁴⁵

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 **24** (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 × 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 gel 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 **25** [1.49 g, 3.88 mmol, 69%, R_f = 0.48 (hexanes-EtOAc 50:50)] as light tan crystals. 1H NMR ($CDCl_3$, 500 MHz) δ 8.11 (1H, br s, NH), 7.47 (1H, d, J = 8.5 Hz, ArH), 7.16 (1H, dd, J = 8.5 Hz, 2.0 Hz, ArH), 7.13 (1H, d, J = 2.5 Hz, ArH), 6.90 (1H, d, J = 8.5 Hz, ArH), 6.89 (1H, d, J = 2.5 Hz, ArH), 6.79 (1H, dd, J = 8.5 Hz, 2.5 Hz, ArH), 6.64 (1H, dd, J = 2.0 Hz, 1.0 Hz, ArH), 3.86 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 1.04 (9H, s, $C(CH_3)_3$), 0.21 (6H, s, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 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; HPLC: method B, t_R 21.09 min; (+) HRESIMS m/z found 384.1989 $[M+H]^+$ (calcd for $C_{22}H_{30}NO_3Si$, 384.1989).

2-(3'-Hydroxy-4'-methoxyphenyl)-6-methoxyindole (26a).⁴⁶—To a solution of TBS-protected phenol **25** (0.10 g, 0.26 mmol) in THF (10 mL) at 0 °C was added tetrabutylammonium fluoride (TBAF, 0.4 mL, 0.4 mmol, 1 M in THF) dropwise. The reaction mixture was stirred for 30 min and then allowed to reach room temperature. The reaction mixture was quenched with water (10 mL) and extracted with EtOAc (3 × 10 mL). The combined organic layers were 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: 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: 25 mL/min; monitored at 254 and 280 nm] afforded the desired free phenol indole **26a** [0.72 g, 0.27 mmol, R_f = 0.22 (hexanes-EtOAc 70:30)] as a yellow powder. 1H NMR ($CDCl_3$, 500 MHz) δ 8.12 (1H, br s, NH), 7.47 (1H, d, J = 8.6 Hz, ArH), 7.20 (1H, d, J = 2.1 Hz, ArH), 7.11 (1H, dd, J = 8.3 Hz, 2.1 Hz, ArH), 6.90 (1H, m, ArH), 6.78 (1H, dd, J = 8.6 Hz, 2.2 Hz, ArH), 6.64 (1H, d, J = 1.6 Hz, ArH), 5.67 (1H, br s, OH), 3.93 (3H, s, OCH_3), 3.86 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.6, 146.20, 146.15, 137.6, 136.9, 126.5, 123.8, 121.2, 116.8, 111.4, 111.2, 110.1, 99.1, 94.6, 56.2, 55.9; HPLC: method B, t_R 12.33 min; (+) HRESIMS m/z found 270.1128 $[M+H]^+$ (calcd for $C_{16}H_{16}NO_3$, 270.1125).

2-(3'-Isopropoxy-4'-methoxyphenyl)-6-methoxyindole (26b).⁴⁵—To a well-stirred solution of free phenol **26a** (0.07 g, 0.27 mmol) and K_2CO_3 (0.12 g, 0.89 mmol) in DMF (10 mL) at 100 °C was added 2-bromopropane (0.05 mL, 0.54 mmol) dropwise. The reaction mixture was stirred for 12 h at 100 °C. The mixture was cooled to room temperature and extracted with EtOAc (3×10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure to afford the desired isopropyl protected indole **26b** [0.05 g, 0.27 mmol, 60%, R_f = 0.50 (hexanes-EtOAc 60:40)] as a tan solid, which was carried to the next step without further purification. 1H NMR ($CDCl_3$, 500 MHz) δ 8.43 (1H, br s, NH), 7.47 (1H, d, J = 8.6 Hz, ArH), 7.21 (1H, d, J = 2.1 Hz, ArH), 7.16 (1H, dd, J = 8.3 Hz, 2.1 Hz, ArH), 6.90 (1H, d, J = 8.4 Hz, ArH), 6.86 (1H, d, J = 2.1 Hz, ArH), 6.79 (1H, dd, J = 8.6 Hz, 2.3 Hz, ArH), 6.67 (1H, m, ArH), 4.61 (1H, sept, J = 6.0 Hz, CH), 3.87 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 1.39 (6H, d, J = 6.1 Hz, $(CH_3)_2$); ^{13}C NMR ($CDCl_3$,

125 MHz) δ 156.5, 150.2, 147.7, 137.6, 137.2, 125.9, 123.8, 121.0, 117.9, 113.4, 112.5, 110.0, 98.7, 94.7, 71.9, 56.1, 55.8, 22.3; (+) HRESIMS m/z found 312.1596 $[M+H]^+$ (calcd for $C_{19}H_{22}NO_3$, 312.1594).

2-(3'-tert-Butyldimethylsiloxy-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-6-methoxyindole (27).¹³

—To a solution of compound **25** (3.12 g, 8.14 mmol) in *o*-dichlorobenzene (30 mL) was added 3,4,5-trimethoxybenzoylchloride (2.82 g, 12.2 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-colored crude oil was subjected to flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A/90% B (4 CV), 10% A/90% B $\rightarrow$ 80% A/20% B (10 CV), 80% A/20% B (2.8 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] resulting in TBS-indole analogue **27** [1.60 g, 2.77 mmol, 34%, R_f = 0.38 (hexanes-EtOAc 60:40)] as a yellow powder. ¹H NMR (CDCl₃, 500 MHz) δ 8.42 (1H, br s, NH), 7.93 (1H, d, J = 9.5 Hz, ArH), 6.99 (2H, s, ArH), 6.94 (1H, dd, J = 8.0 Hz, 2.0 Hz, ArH), 6.91 (1H, dd, J = 9.0 Hz, 2.0 Hz, ArH), 6.91 (1H, d, J = 2.0 Hz, ArH), 6.77 (1H, d, J = 2.0 Hz, ArH), 6.70 (1H, d, J = 8.5 Hz, ArH), 3.87 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 3.74 (3H, s, OCH₃), 3.69 (6H, s, OCH₃), 0.94 (9H, s, C(CH₃)₃), 0.04 (6H, s, Si(CH₃)₂); ¹³C NMR (CDCl₃, 125 MHz) δ 191.9, 157.4, 152.6, 151.6, 145.2, 142.1, 141.3, 136.5, 134.6, 125.2, 123.4, 122.6, 122.3, 121.9, 112.9, 111.8, 111.7, 107.4, 94.6, 60.9, 56.1, 55.9, 55.5, 25.8, 18.5, -4.7.

2-(3'-Isopropoxy-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-6-methoxyindole (28).³⁶

—To a well-stirred solution of compound **26b** (1.07 g, 3.45 mmol) in *o*-dichlorobenzene (20 mL) was added 3,4,5-trimethoxybenzoylchloride (1.39 g, 6.04 mmol). The reaction mixture was heated to reflux at 160 °C for 16 h. The excess *o*-dichlorobenzene was removed by simple distillation, and the resulting dark-colored solid was subjected to glass column flash chromatography (silica gel, hexanes-EtOAc 50:50), yielding the desired isopropyl-indole analogue **28** [0.96 g, 1.90 mmol, 55%, R_f = 0.29 (hexanes-EtOAc 50:50)] as a yellow solid. ¹H NMR (CDCl₃, 300 MHz) δ 8.79 (1H, br s, NH), 7.94 (1H, d, J = 9.4 Hz, ArH), 6.99 (1H, dd, J = 8.2 Hz, J = 2.1 Hz, ArH), 6.98 (2H, s, ArH), 6.92 (1H, dd, J = 6.1 Hz, 2.3 Hz, ArH), 6.90 (1H, d, J = 2.3 Hz, ArH), 6.74 (1H, d, J = 2.1 Hz, ArH), 6.73 (1H, d, J = 8.4 Hz, ArH), 4.16 (1H, m, CH), 3.85 (3H, s, OCH₃), 3.79 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 3.66 (6H, s, OCH₃), 1.21 (3H, d, J = 5.6 Hz, CH₃), 1.19 (3H, d, J = 5.6 Hz, CH₃); ¹³C NMR (CDCl₃, 75 MHz) δ 191.8, 157.3, 152.5, 150.9, 147.1, 142.3, 141.2, 136.4, 134.5, 124.8, 123.2, 122.4, 121.0, 117.3, 112.6, 111.6, 111.5, 107.2, 94.5, 71.7, 64.4, 60.8, 55.9, 55.6, 25.3, 21.9.

2-(3'-Isopropoxy-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-N-methyl-6-methoxyindole (29).⁴⁵

—To a solution of compound **28** (0.52 g, 1.03 mmol) in CH₂Cl₂ (20 mL) at 0 °C was added sodium hydride (0.04 g, 1.75 mmol) slowly and carefully. The solution was stirred for 5 min, and methyl iodide (0.13 mL, 2.16 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature over 19 h. Upon completion of the reaction, the solution was diluted with water (15 mL) and extracted with CH₂Cl₂ (2 $\times$ 15 mL). The combined organic phases were dried over Na₂SO₄ and concentrated under reduced pressure. Purification by column flash column chromatography (silica gel, hexanes-EtOAc 70:30) afforded methyl indole analogue **29** [0.43 g, 0.82 mmol, 80%, R_f = 0.18 (hexanes-EtOAc 60:40)] as a white solid. ¹H NMR (CDCl₃, 300 MHz) δ 8.00 (1H, d, J = 8.7 Hz, ArH), 6.97 (1H, dd, J = 8.7 Hz, 2.3 Hz, ArH), 6.88 (1H, dd, J = 7.9 Hz, 1.9 Hz, ArH), 6.87 (1H, d, J = 2.4 Hz, ArH), 6.84 (2H, s, ArH), 6.81 (1H, d, J = 8.3 Hz, ArH), 6.66 (1H, d, J = 1.9 Hz, ArH), 4.28 (1H, m, CH), 3.93 (3H, s, CH₃), 3.82 (3H, s, OCH₃), 3.78 (3H, s, OCH₃), 3.73 (6H, s, OCH₃), 3.66 (3H, s, OCH₃), 1.27 (6H, d, J = 6.1 Hz, (CH₃)₂); ¹³C NMR (CDCl₃, 75 MHz) δ 191.8, 157.2, 152.2, 150.6, 146.7, 145.2, 140.5,

138.1, 135.3, 123.5, 123.3, 122.5, 121.8, 118.4, 114.0, 111.5, 111.2, 106.7, 93.6, 71.4, 60.7, 55.90, 55.86, 55.7, 31.4, 21.9; HPLC: method B, t_R 15.14 min; (+) HRESIMS m/z found 542.2150 $[M+Na]^+$ (calcd for $C_{30}H_{33}NNaO_7$, 542.2149).

2-(3'-Hydroxy-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-6-methoxyindole (8) (OXi8006).¹²—To a well-stirred solution of compound **27** (4.45 g, 7.70 mmol) in THF (15 mL) at 0 °C was added tetrabutylammonium fluoride (TBAF, 11.55 mL, 11.55 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 × 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 (1 CV), 12% A/88% B → 100% A/0% B (10 CV), 100% A/0% B (5 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] afforded the desired phenolic indole **8** (OXi8006) [2.49 g, 5.37 mmol, 70%, R_f = 0.28 (hexanes-EtOAc 50:50)] as a yellow powder; 1H NMR ($CDCl_3$, 500 MHz) δ 8.30 (1H, br s, NH), 7.93 (1H, d, J = 9.5 Hz, ArH), 6.96 (2H, s, ArH), 6.95 (1H, d, J = 2.0 Hz, ArH), 6.93 (1H, dd, J = 9.5 Hz, 2.5 Hz, ArH), 6.92 (1H, d, J = 2.5 Hz, ArH), 6.78 (1H, dd, J = 8.0 Hz, 2.0 Hz, ArH), 6.65 (1H, d, J = 8.5 Hz, ArH), 5.55 (1H, s, OH), 3.89 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.80 (3H, s, OCH_3), 3.71 (6H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.7, 157.1, 152.5, 147.0, 145.3, 143.3, 141.0, 136.6, 135.0, 125.1, 123.0, 122.1, 121.5, 115.1, 112.6, 111.6, 110.3, 107.4, 94.8, 60.8, 56.0, 55.8, 55.6; HPLC: method B, t_R 11.43 min; (+) HRESIMS m/z found 464.1706 $[M+H]^+$ (calcd for $C_{26}H_{26}NO_7$, 464.1704).

2-(3'-Hydroxy-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-N-methyl-6-methoxyindole (30).⁴⁵—To a solution of isopropyl ether **29** (0.426 g, 0.819 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added $AlCl_3$ (0.33 g, 2.46 mmol). The reaction mixture was allowed to warm to room temperature and stirring at this temperature continued for 2 h. Examination by TLC indicated the presence of starting material so an additional 3 molar equivalents (0.33 g, 2.46 mmol) of $AlCl_3$ was added, and the reaction mixture was stirred for an additional 1.5 h. Upon completion of the reaction, the reaction mixture was quenched with water (15 mL) and extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column flash column chromatography (silica gel, hexanes-EtOAc 60:40) afforded the free phenolic N-methyl indole analogue **30** [0.286 g, 0.599 mmol, 73%, R_f = 0.17 (hexanes-EtOAc 50:50)] as a colorless solid. 1H NMR ($CDCl_3$, 500 MHz) δ 8.02 (1H, d, J = 8.7 Hz, ArH), 6.96 (1H, dd, J = 8.7 Hz, 2.2 Hz, ArH), 6.86 (1H, d, J = 2.1 Hz, ArH), 6.79 (1H, d, J = 8.0 Hz, ArH), 6.78 (2H, s, ArH), 6.65 (2H, m, ArH), 5.77 (1H, s, OH), 3.92 (3H, s, CH_3), 3.82 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 3.74 (6H, s, OCH_3), 3.65 (3H, s, OCH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.2, 157.3, 152.3, 147.0, 145.4, 145.2, 140.2, 138.2, 135.8, 124.0, 123.6, 122.7, 121.9, 117.0, 114.4, 111.7, 110.2, 106.6, 93.7, 60.8, 56.1, 56.0, 55.9, 31.5; HPLC: method B, t_R 12.38 min; (+) HRESIMS m/z found 478.1857 $[M+H]^+$ (calcd for $C_{27}H_{28}NO_7$, 478.1860).

2-(3'-Dibenzyl phosphate-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-6-methoxyindole (31).⁴⁵—To a solution of compound **8** (1.90 g, 4.09 mmol) in acetonitrile (70 mL) at -25 °C was added CCl_4 (3.50 mL, 35.98 mmol). The solution was stirred for 10 min, and ethyldiisopropylamine (1.50 mL, 8.63 mmol) and DMAP (0.05 g, 0.41 mmol) were added. After 5 min of stirring, dibenzyl phosphite (1.36 mL, 6.17 mmol) was added, and the reaction mixture was stirred for 2 h while allowing the solution to reach room temperature. Upon completion of the reaction, the reaction was terminated by adding a solution of KH_2PO_4 (15 mL, 0.5 M) and extracted with EtOAc (3 × 50 mL). The 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 $\rightarrow$ 100% A/0% B (10 CV), 100% A/0% B (5.2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] afforded the desired phosphate ester **31** [2.71 g 3.75 mmol, 91 %, R_f = 0.57 (hexanes-EtOAc 50:50)] as a yellow powder. ^1H NMR (CDCl_3 , 500 MHz) δ 9.20 (1H, br s, NH), 7.78 (1H, d, J = 8.5 Hz, ArH), 7.35 (10H, m, ArH), 7.25 (1H, m, ArH), 6.96 (1H, dd, J = 9.0 Hz, 2.5 Hz, ArH), 6.93 (2H, s, ArH), 6.91 (1H, d, J = 2.0 Hz, ArH), 6.86 (1H, dd, J = 9.0 Hz, 2.5 Hz, ArH), 6.51 (1H, d, J = 8.5 Hz, ArH), 5.17 (4H, d, J = 8.0 Hz CH_2), 3.82 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 3.65 (6H, s, OCH_3), 3.54 (3H, s, OCH_3); ^{13}C NMR (CDCl_3 , 125 MHz) δ 192.1, 157.3, 152.7, 150.73, 150.69, 141.3, 141.2, 136.7, 135.6 (d, J = 7.3 Hz), 135.0, 128.84, 128.78, 128.1, 127.8, 124.6, 123.1, 122.3, 121.4, 112.8, 112.0, 111.7, 107.4, 94.9, 70.3 (d, J = 6.0 Hz), 60.9, 56.2, 55.8, 55.7; ^{31}P NMR (CDCl_3 , 200 MHz) δ 6.1; HPLC: method B, t_R 16.40 min; (+) HRESIMS m/z found 746.2126 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{40}\text{H}_{38}\text{NNaO}_{10}\text{P}$, 746.2126).

2-(3'-Dibenzyl phosphate-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-N-methyl-6-methoxyindole (32).⁴⁵—To a well-stirred solution of compound **30** (0.312 g, 0.653 mmol) dissolved in acetonitrile (15 mL) at -25°C was added CCl_4 (0.56 mL, 5.75 mmol). The reaction mixture was stirred for 10 min and ethyl diisopropylamine (0.24 mL, 1.37 mmol) and DMAP (0.01 g, 0.06 mmol) were added. After 5 min, dibenzylphosphite (0.22 mL, 0.99 mmol) was added, and the reaction mixture was allowed to reach room temperature over 1.5 h. A solution of KH_2PO_4 (20 mL, 0.5 M) was added, and the reaction mixture was extracted with EtOAc (3×50 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column flash column chromatography (silica gel, hexanes-EtOAc 50:50) yielded the desired phosphate ester **32** [0.35 g, 0.47 mmol, 73%, R_f = 0.52 (hexanes-EtOAc 30:70)] as a colorless solid. ^1H NMR (CDCl_3 , 300 MHz) δ 7.88 (1H, d, J = 8.7 Hz, ArH), 7.31 (10H, m, ArH), 7.17 (1H, m, ArH), 7.03 (1H, m, ArH), 6.93 (1H, dd, J = 8.8 Hz, 2.2 Hz, ArH), 6.84 (1H, d, J = 2.2 Hz, ArH), 6.83 (2H, s, ArH), 6.78 (1H, d, J = 8.5 Hz, ArH), 5.14 (4H, d, J = 8.1 Hz, CH_2), 3.91 (3H, s, CH_3), 3.79 (3H, s, OCH_3), 3.76 (3H, s, OCH_3), 3.70 (6H, s, OCH_3), 3.54 (3H, s, OCH_3); ^{13}C NMR (CDCl_3 , 125 MHz) δ 191.7, 157.4, 152.5, 150.99, 150.95, 144.1, 140.7, 138.2, 135.7 (d, J = 6.8 Hz), 135.6, 129.0, 128.74, 128.70, 128.1, 123.84, 123.82, 122.8, 121.7, 114.5, 112.2, 111.7, 106.9, 93.7, 70.1 (d, J = 5.8 Hz), 60.9, 56.2, 56.1, 55.9, 31.4; ^{31}P NMR (CDCl_3 , 120 MHz) δ 5.95; HPLC: method B, t_R 16.84 min; (+) HRESIMS m/z found 760.2280 $[\text{M}+\text{Na}]^+$ (calcd for $\text{C}_{41}\text{H}_{40}\text{NNaO}_{10}\text{P}$, 760.2282).

2-(3'-Disodium phosphate-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-6-methoxyindole (33) (OXi8007).¹²—To a solution of dibenzyl ester **31** (2.71 g, 3.74 mmol) in methanol (70 mL) was added 10% palladium-carbon (1.2 g). The flask was evacuated under vacuum, and H_2 gas was introduced via a balloon. The reaction proceeded for 30 min, and the solution was filtered using Celite® with EtOAc. The filtrate was concentrated under reduced pressure to give the crude phosphoric acid derivative as a greenish-yellow oil (2.29 g, 4.22 mmol). The oil was dissolved in methanol (20 mL), and a solution of sodium methoxide (2.0 mL, 25% w/v in methanol) was added. The reaction mixture was stirred at room temperature for 12 h, and the methanol was removed under reduced pressure. Purification by flash chromatography using a prepacked 25 g reversed-phase silica column [solvent A: water; solvent B: acetonitrile; gradient: 100% A/0% B (3 CV), 100% A/0% B $\rightarrow$ 20% A/80% B (10 CV), 0% A/100% B (7.3 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] resulted in the desired disodium phosphate salt **33** (OXi8007) [1.49 g, 2.54 mmol, 60%] as a yellow powder. ^1H NMR (D_2O , 500 MHz) δ 8.03 (1H, d, J = 9.0 Hz, ArH), 7.71 (1H, m, ArH), 7.21 (1H, d, J = 2.0 Hz, ArH), 7.03 (1H,

dd, $J = 9.0$ Hz, 2.5 Hz, ArH), 6.93 (2H, s, ArH), 6.67 (1H, d, $J = 8.5$ Hz, ArH), 6.63 (1H, dd, $J = 9.0$ Hz, 2.0 Hz, ArH), 3.95 (3H, s, OCH₃), 3.77 (3H, s, OCH₃), 3.75 (6H, s, OCH₃), 3.72 (3H, s, OCH₃); ¹³C NMR (D₂O, 125 MHz) δ 195.1, 156.3, 151.7, 150.5, 150.4, 147.7, 142.8 (d, $J = 5.6$ Hz), 139.7, 136.6, 135.4, 125.8, 123.8, 122.2, 121.5, 120.4, 111.7, 111.2, 107.8, 94.5, 60.8, 56.1, 55.8, 55.7; ³¹P NMR (D₂O, 200 MHz) δ 0.57; HPLC: method A, t_R 8.32 min; (+) HRESIMS m/z found 588.1008 [M+H]⁺ (calcd for C₂₆H₂₅NNa₂O₁₀P, 588.1006).

2-(3'-Disodium phosphate-4'-methoxyphenyl)-3-(3'',4'',5''-trimethoxybenzoyl)-N-methyl-6-methoxyindole (34).⁴⁵—To a solution of dibenzyl ester **32** (0.32 g, 0.43 mmol) in ethanol (10 mL) was added 5% palladium-carbon (0.10 g). The flask was evacuated by aspirator vacuum, and H₂ gas was introduced into the solution via balloon. The reaction proceeded for 30 min, and the solution was filtered through Celite® in EtOAc. The filtrate was concentrated under reduced pressure to afford the crude phosphoric acid derivative as a yellow foam (0.223 g, 0.400 mmol). The foam product was dissolved in methanol (15 mL), and a solution of sodium methoxide (0.18 mL, 0.80 mmol, 4.37 M solution in methanol) was added. The reaction mixture was stirred at room temperature for 12 h. Next, the methanol was removed under reduced pressure. Purification by recrystallization from water-acetone afforded the desired salt **34** (0.168 g, 0.279 mmol, 64%) as a yellow solid. ¹H NMR (CD₃OD, 500 MHz) δ 7.89 (1H, d, $J = 8.8$ Hz, ArH), 7.79 (1H, m, ArH), 7.02 (1H, d, $J = 2.0$ Hz, ArH), 6.88 (1H, dd, $J = 8.8$ Hz, 2.2 Hz, ArH), 6.76 (2H, s, ArH), 6.62 (1H, d, $J = 8.3$ Hz, ArH), 6.58 (1H, dd, $J = 8.4$ Hz, 2.1 Hz, ArH), 3.90 (3H, s, CH₃), 3.77 (3H, s, OCH₃), 3.76 (3H, s, OCH₃), 3.74 (6H, s, OCH₃), 3.69 (3H, s, OCH₃); ¹³C NMR (CD₃OD, 75 MHz) δ 194.9, 158.8, 153.7, 152.2, 149.1, 148.9, 145.6 (d, $J = 5.5$ Hz), 141.5, 139.9, 137.4, 126.5, 124.3, 124.0, 123.2, 114.6, 112.9, 112.6, 108.0, 94.8, 61.1, 56.8, 56.6, 56.2, 32.4; ³¹P NMR (CD₃OD, 120 MHz) δ 1.84; HPLC: method B, t_R 8.74 min; (+) HRESIMS m/z found 602.1180 [M+H]⁺ (calcd for C₂₇H₂₇NNa₂O₁₀P, 602.1162).

Effects on Tubulin Polymerization

Bovine brain tubulin was purified using methods previously described.⁴⁷ The effect of compounds on tubulin assembly in vitro was determined by using a series of concentrations that were preincubated with 10 μ M tubulin (1.0 mg/mL) in glutamate buffer at 30 °C, followed by cooling to 0 °C. After GTP was added, the samples were mixed and transferred to cuvettes at 0 °C in a recording spectrophotometer and warmed to 30 °C to initiate polymerization. Tubulin assembly was observed turbidimetrically at 350 nm.⁴⁸ Polymer disassembly was confirmed by cooling to 0 °C. The calculated compound concentration that inhibited the extent of tubulin assembly by 50% after a 20 min incubation was defined as the IC₅₀ value.

Colchicine Binding Assay

Colchicine binding was measured as previously described.^{49,50} Solutions containing 1 μ M tubulin, 5.0 μ M [³H]colchicine, and inhibitor at either 50 μ M, 5 μ M, and/or 1 μ M (as specified) were used.

Cell Lines and Sulforhodamine B (SRB) Assay

Cancer cell lines were obtained from ATCC (DU-145 (prostate), SK-OV-3 (ovarian), and NCI-H460 (lung)) and maintained as recommended. Media included gentamicin and amphotericin B. The National Cancer Institute's standard SRB assay assessed cancer cell line growth inhibition, as previously described, with the GI₅₀ being the drug concentrations calculated to cause a 50% reduction in net protein increase relative to untreated cells.^{51–53} Results reported are averages of at least three separate experiments, each of which was carried out in triplicate.

Color Doppler Ultrasound Imaging with indole 33 (OXi8007)

PC-3-luc cells were grown in culture and harvested, as described previously.⁴³ Cells (2×10^6) were implanted orthotopically in the prostate of a SCID mice (NIH, Frederick, MD), and the tumor was allowed to grow to approximately 5–7 mm in diameter. At this stage NAIR[®] Lotion (Church and Dwight, Princeton, NJ) was applied to ensure complete local hair removal. The mouse was anesthetized (2% isoflurane in oxygen), and gel was applied to ensure effective ultrasound coupling to the solid-state transducers: MS400 (24 MHz). Data were acquired using a Vevo 2100 (Visual Sonics, Inc. Toronto, Ontario, Canada) in both B and color Doppler modes. Compound **33** (OXi8007) (350 mg/kg in saline) was injected ip, and ultrasound images were acquired over the next 80 min.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1: Colchicine

2: Combretastatin A-4: R = OH

3: Combretastatin A-4P: R = OP(O)O₂[⊖] Na₂[⊕]

4: benzo[b]thiophene: R = H

5: benzo[b]thiophene: R = OH

Figure 1.
Representative small-molecule inhibitors of tubulin assembly.

Figure 2.

Compilation of indole-based inhibitors of tubulin assembly (6: von Angerer, 1998¹¹ 7–8: Pinney, 2001¹² 9: Mahboobi and Beckers, 2001¹⁹ 10: Silvestri, 2004²⁰ 11: Lee and Hsieh, 2004²¹ 12: Chang, 2007²² 13: Cachet, 2008²³ 14–15: Chang, 2008²⁴ 16: Romagnoli, 2008²⁵ 17: Silvestri, 2011²⁶ 18: Liou, 2011²⁷).

Figure 3.

Dynamics of vascular disruption caused by **33** (OXi8007) in human prostate tumor PC-3 xenograft visualized by doppler ultrasound. Sequential transaxial images were acquired over a period of 80 min following injection of indole prodrug **33** (OXi8007) (350 mg/kg ip) in a SCID mouse. (A) Immediately post injection, (B) 20 min, (C) 40 min, (D) 60 min, (E) 80 min. Each image was acquired in color Doppler mode with extensive vasculature initially observed both within the tumor (outline in yellow) and in surrounding tissue. Within 20 min, the tumor vascular perfusion had decreased with further progressive decline, so that by 80 min no vascular flow was observed within the tumor. Flow was, however, still apparent in surrounding normal tissues (white arrow). White scale bar 2 mm. Heat scale bar representing flow ranging from ± 64.2 mm/s.

Scheme 1.
Synthesis of indole analogues **8** and **30**.

Scheme 2. Preparation of indole-based disodium phosphate prodrug salts **33** and **34**.

Table 1

Inhibition of Tubulin Polymerization and Colchicine Binding.

compound	inhibition of tubulin polymerization IC ₅₀ (μM)	inhibition of colchicine binding (%)		
		1 μM	5 μM	50 μM
CA4	0.96 ± 0.07	91 ± 1	99 ± 0.8	nd ^a
8 (OXi8006)	1.1 ± 0.04	40 ± 0.2	75 ± 0.2	nd
33 (OXi8007)	4.2 ± 0.1	nd	26 ± 4	nd
30	> 20	nd	nd	34 ± 2
34	> 20	nd	nd	29 ± 2

^a nd = not determined in this study.

Table 2

Cytotoxicity Against Human Cancer Cell Lines NCI-H460, DU-145, and SK-OV-3.

compound	GI ₅₀ (μM) SRB assay ^a		
	NCI-H460	DU-145	SK-OV-3
CA4	0.0028 ^b	0.00054 ^b	0.00042
CA4P	0.066 ^c	0.016 ^c	nd ^d
8 (OXi8006)	0.0379	0.0356	0.00345
33 (OXi8007)	0.0311	0.0297	0.0223
30	4.14	2.68	2.05
34	3.56	4.67	8.64

^a Average of *n* = 3 independent determinations.^b For additional data, see Ref.⁴⁰^c See Ref.⁴¹^d nd = not determined in this study.