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Structure Guided Design, Synthesis and Biological Evaluation of Novel Benzosuberene Analogues as Inhibitors of Tubulin Polymerization

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Abstract

A promising design paradigm for small-molecule inhibitors of tubulin polymerization that bind to the colchicine site draws structural inspiration from the natural products colchicine and combretastatin A-4 (**CA4**). Our previous studies with benzocycloalkenyl and heteroaromatic ring systems yielded promising inhibitors with dihydronaphthalene and benzosuberene analogues featuring phenolic (**KGP03** and **KGP18**) and aniline (**KGP05** and **KGP156**) congeners emerging as lead agents. These molecules demonstrated dual mechanism of action, functioning as both potent vascular disrupting agents (VDAs) and as highly cytotoxic anticancer agents. A further series of analogues was designed to extend functional group diversity and investigate regioisomeric tolerance. Ten new molecules were effective inhibitors of tubulin polymerization ($IC_{50} < 5 \mu M$) with seven of these exhibiting highly potent activity comparable to **CA4**, **KGP18**, and **KGP03**. For one of the most effective agents, dose-dependent vascular shutdown was

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Supporting Information:

Supporting Information: ¹H NMR, ¹³C NMR, ³¹P NMR, HRMS, HPLC for final target compounds and intermediates (¹H NMR, ¹³C NMR only) associated with this article, a table of biological data including standard deviations, and a separate table of biological data including molecular formula strings (as SMILES) can be found, in the online version, at <http://10.1021/acs.jmedchem.9b00551>.

demonstrated using dynamic bioluminescence imaging in a human prostate tumor xenograft growing in a rat.

Keywords

inhibitors of tubulin polymerization; benzosuberene analogues; vascular disrupting agents; small-molecule synthesis

Introduction

Solid tumors require a functional vasculature to supply oxygen and nutrients to their cells when they exceed 1 mm³ in size.^{1,2} Unlike normal vasculature, the tumor-associated vascular network tends to expand irregularly, incorporating fragile and chaotic bulges and blind ends.^{3–5} The primitive character and inherent fragility of tumor-associated vasculature, along with the seminal observations by Denekamp and co-workers that blocking established tumor-associated blood flow leads to tumor regression in mice, positioned tumor-associated vasculature as a promising target for cancer therapy.^{6–8} Two categories of small-molecule, vascular-targeted therapies have been developed: angiogenesis inhibiting agents (AIAs) that inhibit neovascularization in developing tumors; and, separately, vascular disrupting agents (VDAs) that irreversibly damage established tumor-associated vasculature.^{9–13} The two major sub-divisions of VDAs include biologics and small-molecule anticancer agents. The majority of small-molecule VDAs function as inhibitors of tubulin polymerization, which destabilize the tubulin-microtubule protein system by binding to the colchicine site on β -tubulin in close proximity to the α,β -tubulin heterodimer interface. The endothelial cells lining microvessels undergo rapid cytoskeletal disruption, manifested by morphological changes (flat to round) in response to inhibition of their tubulin-microtubule protein system cytoskeleton triggered by VDA binding to the colchicine site. This rapid endothelial cell cytoskeletal rearrangement leads to irreversible damage to the tumor-associated vasculature, culminating in tumor necrosis.^{4,14–17}

The natural products colchicine, combretastatin A-4 (CA4), and combretastatin A-1 (CA1), along with the synthetic analogue phenstatin (Fig. 1), are potent colchicine site inhibitors of tubulin polymerization that function as promising VDAs.^{18–24} These molecules have provided structural inspiration and guidance for the design, synthesis, and biological evaluation of many second-generation (and beyond) molecules in a world-wide effort to identify a small-molecule colchicine site agent with the necessary efficacy coupled with safety to be utilized as a cancer therapeutic in humans. To date, no small-molecule therapeutic agent that interacts with the colchicine site and functions as either an antiproliferative agent or a VDA (or demonstrates a dual mechanism of action) has reached FDA approval. Structural similarities between these natural products include a trimethoxy phenyl ring, a separate hydroxylated *p*-methoxy aryl moiety, and a bridging functionality connecting the two rings with a comparable centroid-to-centroid distance. Our long-standing program focused on molecular recognition for the colchicine site has led to the discovery of promising synthetic analogues and derivatives,^{25–28} including stilbenoid,^{14,29–31} benzo[*b*]thiophene,^{32–34} benzofuran,³⁴ dihydronaphthalene,^{8,35,36} benzosuberene,^{8,25,35–38}

and indole-based³⁹ molecules (Fig. 1). Numerous other studies have investigated a myriad of structural and functional group modifications to the A-ring, the B-ring, and the ethylene bridge of combretastatin A-4; the results of which are conveyed in extensive publications (too numerous to cite herein) and summarized in various review articles, yet none are comprehensive.^{40–49}

Two benzosuberene-based analogues (referred to as **KGP18**^{8,25,37} and its amino congener **KGP156**^{35,38}) emerged from our previous studies as molecules with high relevance as potential preclinical candidates due, in part, to their potent inhibition of tubulin polymerization, pronounced cytotoxicity against human cancer cell lines, and promising activity as VDAs.^{8,25,38}

Studies by us and others have investigated a variety of functional group modifications on both the fused and pendant aryl rings of tubulin-binding benzosuberene and dihydronaphthalene molecular frameworks (Fig. 2).^{8,25,35,36,54,55} In particular, Maderna and co-workers contributed efficient methodology towards the benzosuberene structural motif and presented a number of important B-ring modified benzosuberene analogues (Fig. 2) along with an evaluation of their biological potency and their drugability characteristics.^{56,57} Interestingly, a benzosuberene B-ring diene analogue was identified as one of the most potent cytotoxic agents amongst their synthesized series of eleven members,⁵⁷ and this same molecule (Maderna compound, listed as **68** herein) was obtained during our synthetic campaign as an unexpected product. It is also noteworthy that a new class of benzodiazepines has been reported as inhibitors of tubulin polymerization.⁵⁸

Herein we designed, synthesized and biologically evaluated a series of structurally varied analogues using **KGP18** and **KGP05** as lead compounds and further adopted our methodology to investigate functional group modifications on the A-ring (C-4 position) and the B-ring (C-6, 7, 8, 9 positions), along with regiochemical translocation of the pendant aryl ring (C-ring) in regard to their influence on inhibition of tubulin polymerization and cytotoxicity against several human cancer cell lines.

Results and Discussion

Molecular Design and Synthesis

Eighteen benzosuberene and dihydronaphthalene analogues (Fig. 3) were prepared by chemical synthesis and evaluated for their cytotoxicity against selected human cancer cell lines and their ability to inhibit tubulin polymerization. Structural modifications included: 1) functional group (R) modifications on the fused aryl ring including the installation of alcohol, aldehyde, nitrile, and ester groups along with ether linkages to facilitate extension of the polar alcohol moiety away from the fused six-seven ring system; 2) R₂ and R₃ incorporation at the olefinic and allylic positions of the seven-membered ring introduced -Br, -OH, and -NHAc groups; 3) modification (R₄ position) of the fused aliphatic ring adjacent to the tertiary alcohol site; 4) olefination and pendant trimethoxy phenyl ring regiochemistry on the fused non-aromatic ring. The synthesis of analogues **3**, **6**, **8**, **11**, **13**, **15** was initiated from a common intermediate ketone **1** that was readily available utilizing our previous methodology.²⁵ Treatment of benzosuberone **1** with trimethoxyphenyllithium (prepared from

the corresponding bromide) generated tertiary alcohol **2**, which was subsequently converted to diol **3** upon removal of the phenolic TBS protecting group. Separately, tertiary alcohol **2** was converted to its corresponding benzosuberene **4**, which underwent treatment with a series of oxidants (*m*-CPBA, NBS, OsO₄) to facilitate epoxidation followed by ring opening and oxidation, bromination, and Upjohn dihydroxylation.^{59,60} Following the removal of protecting groups, target compounds **6**, **13**, and **15** were obtained. Similarly, reaction of 4-methylbenzosuberene **12** (prepared by us previously²⁵) with NBS/AIBN afforded vinylbromide **13**. Lead compound **7** (referred to as **KGP18**), also available through this methodology, was directly converted to its corresponding ether analogues **8** and **11** (Scheme 1).^{25,61}

Lead compounds benzosuberene **16** (referred to as **KGP156**) and its corresponding dihydronaphthalene analogue **17** (referred to as **KGP05**), which were readily available from our previous synthetic studies, were subjected to a Sandmeyer radical-nucleophilic aromatic substitution protocol to generate nitrile analogues **18** and **19** (Scheme 2).^{35,38,62,63} The benzosuberene aldehyde analogue **20** was obtained after subsequent reduction of the nitrile analogue (Scheme 2).

As part of a larger program focused on the use of potentially cytotoxic benzosuberene and dihydronaphthalene analogues as payloads in antibody-drug conjugates (ADCs) and as prodrugs targeted for selective release in regions of profound tumor hypoxia, we were intrigued by the possibility of replacing the heteroatom [oxygen (phenol) or nitrogen (aniline)] at the 4-position with a short carbon chain terminating with a primary alcohol (or amino) moiety, thus maintaining its hydrogen-bond donor nature and serving as a viable position for future attachment of various linkers. Accordingly, methylation of phenolic bromo-aldehyde **21** (Scheme 3), followed by Wittig olefination and subsequent hydrogenation (under Ph₂S mediation to maintain the aryl bromine group) afforded methyl ester **24**.⁶⁴ Benzosuberone **25** was obtained through an intramolecular Friedel-Crafts annulation facilitated by Eaton's reagent (7.7% weight percent P₂O₅ in CH₃SO₃H), and subsequent treatment of compounds **25** with trimethoxyphenyllithium, followed by reaction work-up under acidic conditions, generated benzosuberene intermediate **26**.^{65,66} We have previously evaluated corresponding fluorine and chlorine benzosuberene analogues.²⁵ Halogen-lithium exchange, followed by reaction with ethyl chloroformate, afforded ethyl ester **27**, which was reduced (LiAlH₄) to generate benzylic alcohol **28**. Notably, while it proved possible to obtain analogue **28** directly from intermediate **26**, the isolated yield was quite low (~8%) under these conditions, which was likely due, in part, to the low solubility of paraformaldehyde at low temperature in THF and its low reactivity as a polymer (Scheme 3).

Secondary allylic alcohol **37** and its corresponding *N*-acetyl congener **49** (Scheme 4) were prepared to investigate structure-activity relationship correlations associated with heteroatom incorporation on the conformationally flexible fused seven-membered ring. Appropriate aldehyde chain elongations were facilitated by Meldrum's acid (towards **37**) and Wittig-ylide methodology (towards **49**), and subsequent functional group transformations (including installation of carboxylic acid moieties obtained under saponification conditions) afforded protected alcohol **34** and *N*-acetamide **45**, separately. Lewis acid mediated

cyclization to obtain the benzosuberone molecular core was achieved by treatment of the requisite acyl chloride with either tin tetrachloride (to obtain ketone **35**) or Eaton's reagent (to obtain ketone **46**). In each case, the pendant ring was installed through reaction with 3,4,5-trimethoxyphenyllithium. The secondary alcohol moiety (target compound **37**) was revealed (by deprotection) prior to the organolithium step, while the phenolic moiety (target compound **49**) was revealed after the organolithium reaction (Scheme 4).^{8,67-69} Both compounds **36** and **37** undergo elimination first (allylic alcohol), then demethylation if treated with a Lewis acid such as AlCl₃ or BCl₃.

Translocation of the trimethoxyphenyl group (Scheme 5) was achieved by initial cyclization (Eaton's reagent) of carboxylic acid **54** with concomitant elimination to afford α,β -unsaturated ketone **55**. With the α,β -unsaturated ketone in hand, 1,2- and 1,4- addition reactions were conducted using the appropriate aryl-lithium and Gilman reagents to provide tertiary alcohol analogue **56** (with unsaturated 7-membered ring to maintain rigidity), and separately the Michael adduct, trimethoxy pendant phenyl ring shifted analogue **57** (Scheme 5).⁷⁰

During the course of an investigation centered on variability in phenolic moiety protecting groups for a subset of benzosuberone analogues, we were surprised by the formation of diene **67**, obtained upon 1,2-addition of trimethoxyphenyllithium to ketone **66**, followed by reaction work-up. In this case, the secondary alcohol demonstrated a propensity to undergo elimination even under mild acidic or basic conditions such as TBAF deprotection or BCl₃ cleavage at lowered temperature. We attempted various combinations of 4-position (phenolic moiety on the fused aryl ring) and allylic alcohol protecting group strategies, which eventually led to the unanticipated formation of diene **68**. It is important to note that this diene (**68**) was previously obtained by Maderna and co-workers.⁵⁷ Having this compound in hand, and noting its exceptional biological activity (inhibition of tubulin polymerization and cytotoxicity against human cancer cell lines, Table 1),⁵⁷ motivated us to prepare the corresponding water-soluble phosphate prodrug disodium salt **69** to facilitate *in vivo* studies in a mouse model of prostate cancer to evaluate the efficacy of this compound as a VDA, as evidenced by bioluminescence imaging (BLI) (Scheme 6).^{8,25,57,68,69}

Biological Evaluation

Each of the eighteen compounds (Fig. 3) was evaluated for its cytotoxicity against human cancer cell lines [SK-OV-3 (ovarian), NCI-H460 (lung), DU-145 (prostate)] and for the ability to inhibit tubulin polymerization (Table 1). Ten of the evaluated molecules were identified as strong inhibitors of tubulin assembly (IC₅₀ < 5 μ M, cell-free assay), while seven of the ten were highly active (IC₅₀ = 1.2 μ M). CA4 (IC₅₀ \approx 1 μ M) and **KGP18** (compound **7**, IC₅₀ \approx 0.85 μ M) were utilized as comparative compounds. Three of the benzosuberone and dihydronaphthalene analogues (compounds **13**, **19**), along with the Maderna compound (**68**), were more potent against tubulin in comparison to our dihydronaphthalene lead compound **KGP03** (IC₅₀ = 0.5 μ M). Excellent IC₅₀ values for inhibition of tubulin polymerization were retained upon alteration of the 4-position phenolic moiety into nitrile, ethyl ester, and CH₂OH groups (**18**, **19**, **27**, **28**), along with modifications to the double bond on the seven-member ring that included replacement with ketone and

tertiary alcohol groups, substitution with a bromine group, and increased unsaturation (**6**, **13**, **68**). Extension of the alkyl chain (at position 4) through an ether linkage that terminated with a polar alcohol group (compound **11**) and separately a methoxy moiety (compound **8**) resulted in loss of inhibitory activity. Incorporation of a hydrogen bond donor at the allylic position on the seven-member ring (compounds **37** and **49**) and substituted saturation of the double bond (compounds **3** and **15**) both reduced inhibition of tubulin polymerization. The lack of tubulin activity observed with compound **56** was unanticipated, since semi-rigidity of the seven-membered ring was maintained through a double bond one carbon removed from the stereogenic center, and its parent benzosuberene analogue (dimethoxy group on the fused aryl ring) demonstrated a moderate degree of inhibition of tubulin polymerization ($IC_{50} = 3.1 \mu M$) reported in our previous study.²⁵ The lack of activity of compound **57** in regard to inhibition of tubulin polymerization suggested that the trimethoxy pendant aryl ring situated at the benzylic position on the fused-ring system was closely correlated to biological efficacy (at least in regard to inhibition of tubulin polymerization). While we have previously described similar pendant ring shifts in related benzosuberene analogues, this β -position substitution with a trimethoxy aryl ring has not been previously investigated.⁸

Among the eighteen benzosuberene and dihydronaphthalene analogues investigated in this study (Fig. 3), the most cytotoxic agents were compounds **6**, **13**, **18**, **19**, **28**, **68** ($GI_{50} = 0.0314, 0.0221, 0.0648, 0.0384, 0.0403 \mu M$, and $0.00690 \mu M$, respectively, against the SK-OV-3 ovarian cancer cell line, for example). Judiciously selected structural modifications to the 4, 8, and 9-positions in the parent benzosuberene scaffold accounted for the majority of the highly potent analogues evaluated in this study. While the strong cytotoxicity of this subset of molecules is encouraging, it is noteworthy that all molecules proved less cytotoxic than the lead benzosuberene **KGP18** and less cytotoxic (with the partial exception of the Maderna compound **68**) than the natural product CA4, despite demonstrating similar inhibition of tubulin polymerization (cell free assay). These observations provided an important extension to the known SAR considerations regarding structural modifications to **KGP18**. As anticipated (and similarly observed for combretastatin A-4 phosphate),^{15,71} the benzosuberene phosphate prodrug salt **69** was inactive as an inhibitor of tubulin polymerization in this cell-free assay, presumably due to the lack of phosphatase enzymes necessary to cleave the prodrug to its parent phenolic (biologically active) agent. Prodrug **69** was an active cytotoxic agent since non-specific phosphatase activity is present in these cancer cell-based cytotoxicity assays.^{72,73}

Assessment of Vascular Damage

Ultimately, VDAs will be used *in vivo*, and thus it is crucial to understand both efficacy of vascular disruption and potential off target toxicity *in vivo*. As a preliminary investigation, the extent of vascular damage was assessed in a human prostate tumor line in rats treated with the water-soluble prodrug salt **69** compared to CA4P as control. Many imaging methods have been developed to assess vascular disruption non-invasively *in vivo*.¹¹ We favor dynamic bioluminescence imaging (BLI) for initial validation of VDA activity since it provides a fast, non-invasive and easy method and allows comparison of repeat or sequential investigations. BLI does require the use of cells transfected to express luciferase (*Luc*), but

these are commonly available and we have used this approach extensively^{11,25,39,53,75} as have others.⁷⁶ We used the human prostate cancer PC3 line, in which the tumor suppressor protein DAB2IP had been knocked down and luciferase introduced.⁷⁷ BLI requires administration of luciferin substrate, which readily crosses membranes and is carried throughout the vasculature. The measurement of light emission dynamics is related to vascular delivery of the luciferin substrate, and thus it provides a measure of vascular patency.⁷⁸ Disruption of tumor vasculature blocks delivery of the substrate and consequently results in a quantifiable decrease in bioluminescent signal. The extent of vascular shutdown was evaluated using IP doses of 10 and 40 or 80 mg/kg of **69** and compared to a dose of 30 mg/kg of CA4P, which had been shown previously to cause extensive vascular shutdown in rats at this dose.^{79–81} It should also be noted that the lead benzosuberene KGP18 and the dihydronaphthalene KGP03 (both as their corresponding water-soluble phosphate prodrug salts (KGP265 and KGP04, respectively), along with other structurally modified benzosuberene analogues, demonstrated vascular shutdown (as evidenced by similar BLI imaging studies or color Doppler ultrasound).^{25,37,75}

Prodrug **69** administered at 10 mg/kg resulted in minimal change in light emission. A subsequent dose of 40 mg/kg **69** resulted in substantially diminished light emission (signal reduction 95%; Figs. 4 and 5), but with substantial recovery by 24 h. When CA4P (30 mg/kg) was administered 4 days later it caused a very similar effect in terms of extent and longevity of diminished BLI signal as a surrogate for vascular shutdown. Similar activity was observed when a treatment naïve rat was given 40 mg/kg **69** (Fig. 5) with substantial recovery at 24 h. At 48 h the rats appeared healthy and the signal increased marginally. A dose of 80 mg/kg was also well tolerated, but this higher dose did not show additional vascular disruption.

Conclusions:

These studies have expanded our SAR knowledge regarding the impact of structural modifications to lead benzosuberene and dihydronaphthalene analogues on inhibition of tubulin polymerization and cytotoxicity against human cancer cell lines. Amongst this group of seventeen new molecules [along with the Maderna compound (**68**), accessed (in this study) through separate synthesis], emerged several promising analogues (compounds **6**, **13**, **18**, **19**, **28**, **68**) that elicited inhibition (IC₅₀) of tubulin assembly (cell free assay) greater than or comparable to that of the lead natural product **CA4** and our lead benzosuberene analogues **KGP18** and **KGP156**. These compounds demonstrated potent cytotoxicity (GI₅₀) against SK-OV-3 (ovarian), NCI-H460 (lung), and DU-145 (prostate) cells typically in the low to mid nM range. Preliminary investigation of water-soluble benzosuberene phosphate prodrug salt **69** at 40 mg/kg *in vivo* revealed vascular disruption in a PC3-DAB2IP-*luc* human prostate tumor xenograft based on BLI (Figs. 4 and 5), which was similar to that obtained with CA4P.

Experimental Section

Chemistry

General Materials and Methods—Tetrahydrofuran (THF), carbon tetrachloride, dichloromethane, methanol, dimethylformamide (DMF), and acetonitrile were used in their anhydrous forms. Reactions were performed under nitrogen gas. 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 pre-packed columns or manually in glass columns. Intermediates and products synthesized were characterized on the basis of their ^1H NMR (500 or 600 MHz), ^{13}C NMR (125 or 150 MHz) spectroscopic data using a Varian VNMRS 500 MHz or Bruker DPX 600 MHz instrument. Spectra were recorded in CDCl_3 , D_2O , $(\text{CD}_3)_2\text{CO}$, or CD_3OD . All chemical shifts are expressed in ppm (δ), and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), sextet (sext), septet (sept), double doublet (dd), double double doublet (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 ($\lambda = 190\text{--}400$ nm), a Zorbax XDB-C18 HPLC column (4.6 mm \times 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; Method: 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 1.0mL/min; injection volume 20 μL ; monitored at wavelengths of 210, 230, 254, 280, and 320 nm. Purity of target molecules (with reported biological data) was 95% (as determined by HPLC at one or more scanned wavelengths) with the exception of compound 27 (94.3% at 254 nm). Mass spectrometry was carried out under positive or negative ESI (electrospray ionization) using a Thermo Scientific LTQ Orbitrap Discovery instrument.

1-((*Tert*-butyldimethylsilyloxy)-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-ol (2)²⁵: To an oven dried flask, THF (10 mL) and 3, 4, 5-trimethoxyphenyl bromide (0.89 g, 3.6 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (1.44 mL, 3.60 mmol) was added dropwise to the reaction mixture, which was then stirred at -78 °C for 1 h. TBS-protected ketone (**1**) (0.77 g, 2.4 mmol) in THF (5 mL) was then added slowly to the flask, and the reaction mixture was stirred while warming from -78 °C to room temperature over 12 h. The reaction mixture was quenched with water and extracted with EtOAc (3 \times 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A / 98% B (1 CV), 2% A / 98% B \rightarrow 20% A / 80% B (10 CV), 20% A / 80% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford tertiary alcohol **2** (1.05 g, 2.15 mmol, 89%) as a clear oil. ^1H NMR (500 MHz, CDCl_3) δ 7.15 (1H, d, $J = 9$ Hz), 6.69 (1H, d, $J = 9$ Hz), 6.50 (2H, s), 3.84 (3H, s), 3.80 (3H, s), 3.75 (6H, s), 3.29 (1H, m), 2.56 (1H, m), 2.26 (1H, m), 2.12 (2H, m), 1.90 (1H, m), 1.75 (2H, m), 0.99 (9H, s), 0.17 (3H, s), 0.15 (3H, s). ^{13}C NMR (125 MHz, CDCl_3) δ 153.1, 149.4, 142.0,

141.9, 138.7, 137.3, 132.9, 119.8, 108.0, 104.4, 80.2, 61.0, 56.2, 54.8, 41.4, 27.1, 26.4, 26.2, 25.5, 19.1, -3.8, -4.0.

2-Methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulene-1,5-diol (3)²⁵: TBS-protected tertiary alcohol **2** (0.41 g, 0.84 mmol) was dissolved in THF (6 mL), and TBAF (1.01 mL, 1 M in THF, 1.01 mmol) was added, and the reaction mixture was stirred at room temperature for 4 h. The solution was washed with water and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 3% A / 97% B (1 CV), 3% A / 97% B → 30% A / 70% B (10 CV), 30% A / 70% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford phenol (0.11 g, 0.29 mmol, 35%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 7.04 (1H, d, *J* = 9 Hz), 6.70 (1H, d, *J* = 9 Hz), 6.52 (2H, s), 5.79 (1H, s), 3.91 (3H, s), 3.84 (3H, s), 3.76 (6H, s), 3.23 (1H, m), 2.56 (1H, m), 2.35 (1H, m), 2.11 (1H, m), 1.92 (1H, m), 1.75 (2H, m), 1.47 (1H, m). ¹³C NMR (125 MHz, CDCl₃) δ 153.1, 145.6, 142.7, 141.9, 139.4, 137.3, 127.2, 118.2, 107.3, 104.4, 80.2, 61.0, 56.3, 56.0, 41.5, 26.8, 26.3, 24.7. HRMS: Obsvd 397.1623 [M + Na⁺], Calcd for C₂₁H₂₆O₆Na: 397.1622. HPLC: 16.33 min.

Tert-butyl((3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)dimethylsilane (4)³⁷: TBS-protected tertiary alcohol **2** (0.64 g, 1.3 mmol) was dissolved in acetic acid (10 mL), and the reaction mixture was stirred at room temperature for 6 h. The unreacted acetic acid was removed under reduced pressure. The resulting reaction mixture was washed with water and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, evaporated under reduced pressure and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc, solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 50% A / 50% B (10 CV), 50% A / 50% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford a clear oil that solidified as a colorless solid of TBS-protected benzosuberene analogue **4** (0.41 g, 0.87 mmol, 66%). ¹H NMR (CDCl₃, 500 MHz) δ 6.68 (1H, d, *J* = 8.5 Hz), 6.61 (1H, d, *J* = 8.5 Hz), 6.48 (2H, s), 6.32 (1H, t, *J* = 7 Hz), 3.85 (3H, s), 3.81 (3H, s), 3.79 (6H, s), 2.76 (2H, t, *J* = 7 Hz), 2.10 (2H, m), 1.95 (2H, m), 1.04 (9H, s), 0.23 (6H, s). ¹³C NMR (CDCl₃, 125 MHz) δ 152.9, 148.8, 143.2, 141.6, 138.8, 137.3, 133.9, 133.4, 127.0, 122.5, 108.5, 105.3, 61.0, 56.2, 54.8, 34.1, 26.3, 25.7, 24.4, 19.2, -3.7.

1-(Tert-butyl(dimethylsilyl)oxy)-5-hydroxy-2-methoxy-5-(3,4,5-trimethoxyphenyl)-5,7,8,9-tetrahydro-6H-benzo[7]annulen-6-one (5): To a solution of TBS-protected benzosuberene **4** (0.51 g, 1.1 mmol) dissolved in CH₂Cl₂ (20 mL) was added *m*-CPBA (0.36 g, 2.1 mmol) at -5 °C, and the reaction mixture was stirred for 2 h and then at room temperature for 12 h. The solution was washed with saturated Na₂S₂O₃ and saturated NaHCO₃ and extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to

afford tertiary alcohol **5** (0.256 g, 0.51 mmol, 47%) as a yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 7.26 (1H, d, $J = 9$ Hz), 6.76 (1H, d, $J = 9$ Hz), 6.42 (2H, s), 5.01 (1H, s), 3.83 (3H, s), 3.81 (3H, s), 3.75 (6H, s), 3.10 (2H, m), 2.82 (1H, m), 2.70 (1H, m), 1.98 (1H, m), 1.76 (1H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 211.1, 153.4, 150.2, 142.1, 138.0, 137.5, 131.4, 131.1, 127.8, 109.1, 105.2, 85.6, 61.0, 56.3, 54.8, 39.7, 26.2, 25.8, 24.2, 19.1, -3.7, -3.9.

1,5-Dihydroxy-2-methoxy-5-(3,4,5-trimethoxyphenyl)-5,7,8,9-tetrahydro-6H-

benzo[7]annulen-6-one (6): TBS-protected benzosuberane **5** (0.17 g, 0.33 mmol) was dissolved in THF (10 mL). TBAF (0.33 mL, 1 M, 0.33 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h at 0 °C. A brine (30 mL) solution was added, and the reaction mixture was extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate, filtered, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford phenol **6** (123 mg, 0.320 mmol, 96%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.17 (1H, d, $J = 8.4$ Hz), 6.77 (1H, d, $J = 9$ Hz), 6.43 (2H, s), 5.88 (1H, s), 5.00 (1H, s), 3.90 (3H, s), 3.82 (3H, s), 3.74 (6H, s), 3.08 (2H, m), 2.84 (1H, m), 2.68 (1H, m), 1.99 (1H, m), 1.83 (1H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 211.0, 153.3, 146.4, 142.8, 138.0, 137.2, 131.6, 125.7, 120.2, 108.4, 105.2, 85.5, 60.9, 56.3, 56.0, 39.4, 25.4, 23.3. HRMS: Obsvd 411.1414 [M + Na⁺], Calcd for $\text{C}_{21}\text{H}_{24}\text{O}_7\text{Na}$: 411.1414. HPLC: 15.75 min.

3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-ol (7):

TBS-protected benzosuberene **4** (0.41 g, 0.87 mmol) was dissolved in THF (10 mL). TBAF (1.13 mL, 1.13 mmol) was added, and the reaction mixture was stirred at room temperature for 1 h. The solution was washed with water and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 3% A / 97% B (1 CV), 3% A / 97% B → 30% A / 70% B (10 CV), 30% A / 70% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford phenol **7** (0.25 g, 0.70 mmol, 81%) as a white solid. ^1H NMR (CDCl_3 , 500 MHz) δ 6.71 (1H, d, $J = 9$ Hz), 6.56 (1H, d, $J = 9$ Hz), 6.50 (2H, s), 6.34 (1H, t, $J = 7.5$ Hz), 5.74 (1H, s), 3.91 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.76 (2H, t, $J = 7$ Hz), 2.14 (2H, m), 1.97 (2H, m). ^{13}C NMR (CDCl_3 , 125 MHz) δ 152.9, 145.2, 142.9, 142.4, 138.6, 134.4, 127.9, 127.4, 121.0, 110.1, 107.8, 105.4, 61.1, 56.3, 56.1, 33.7, 25.9, 23.7.

3-Methoxy-4-(2-(2-methoxyethoxy)ethoxy)-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-

benzo[7]annulene (8): Phenol **7** (0.11 g, 0.31 mmol) was dissolved in DMF (6 mL), and K_2CO_3 (0.12 g, 0.86 mmol) was added. The solution was stirred at room temperature for 20 min. 1-Bromo-2-(2-methoxyethoxy) ethane in 90% purity (0.07 mL, 0.5 mmol) was added, the reaction mixture was stirred at room temperature for 15 h, followed by an additional 3 h of stirring at 150 °C. The solution was washed with water and extracted with EtOAc (3 × 40 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a

pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 3% A / 97% B (1 CV), 3% A / 97% B → 30% A / 70% B (20 CV), 30% A / 70% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford ether **8** (25 mg, 0.06 mmol, 18%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃) δ 6.74 (2H, m), 6.48 (2H, s), 6.32 (1H, t, *J* = 7.5 Hz), 4.18 (2H, t, *J* = 5 Hz), 3.87 (2H, t, *J* = 5.5 Hz), 3.85 (6H, s), 3.79 (6H, s), 3.76 (2H, m), 3.60 (2H, m), 3.40 (3H, s), 2.78 (2H, t, *J* = 6.5 Hz), 2.13 (2H, m), 1.93 (2H, m). ¹³C NMR (125 MHz, CDCl₃) δ 152.9, 151.5, 145.1, 142.9, 138.5, 137.4, 136.1, 133.8, 127.3, 125.3, 109.3, 105.3, 72.8, 72.2, 70.78, 70.77, 61.0, 59.2, 56.2, 55.7, 34.5, 25.7, 24.2. HRMS: Obsvd 481.2198 [M + Na⁺], Calcd for C₂₆H₃₄O₇Na: 481.2197. HPLC: 21.65 min.

(8-Bromo-3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)(tert-butyl)dimethylsilane (9): To a solution of TBS-protected benzosuberene (102 mg, 0.22 mmol) in CCl₄ (30 mL) was added NBS (46 mg, 0.26 mmol) and AIBN (3.6 mg, 0.02 mmol). The solution was heated at reflux for 2 h, followed by the addition of water (20 mL) and extraction with CH₂Cl₂ (3 × 30 mL). The combined organic phase was dried over sodium sulfate, filtered, and the solvent was removed under reduced pressure. The crude product was obtained as a yellow oil and taken to the next step directly without any further purification.

1-((Tert-butyl)dimethylsilyloxy)-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulene-5,6-diol (10): To a solution of TBS-protected benzosuberene **4** (1.00 g, 2.12 mmol) in acetone/ water (35 mL/ 15 mL) were added OsO₄ (270 mg, 1.06 mmol) and *N*-methylmorpholine-*N*-oxide (0.66 mL, 4.8 M, 3.2 mmol) at room temperature, and the reaction mixture was stirred for 12 h. A saturated sodium hydrosulfite (20 mL) solution was added, and the reaction mixture was extracted with EtOAc (5 × 20 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, filtered, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford diol **10** (0.35 mg, 0.69 mmol, 33%) as an orange oil. ¹H NMR (600 MHz, CDCl₃) δ 7.30 (1H, d, *J* = 8.4 Hz), 6.74 (1H, d, *J* = 9 Hz), 6.47 (2H, s), 4.51 (1H, s, b), 3.81 (3H, s), 3.80 (3H, s), 3.72 (6H, s), 3.42 (1H, m), 3.33 (1H, s), 2.15 (1H, m), 1.96 (2H, m), 1.83 (1H, m), 1.62 (1H, m), 1.51 (1H, m), 0.98 (9H, s), 0.15 (6H, d, *J* = 3.6 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 153.1, 149.8, 142.0, 138.8, 137.7, 133.1, 132.7, 122.3, 108.5, 105.0, 83.1, 76.5, 60.9, 56.2, 54.7, 32.7, 26.2, 25.9, 21.3, 19.0, -3.9, -4.0.

2-((3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-yl)oxy)ethan-1-ol (11): Phenol **7** (0.14 g, 0.39 mmol) was dissolved in DMF (3 mL), then ethylene carbonate (70 mg, 0.79 mmol) and tetrabutyl ammonium bromide (0.13 g, 0.39 mmol) were added together. The solution was stirred and heated at reflux for 24 h. The reaction mixture was diluted with brine, extracted with EtOAc (3 × 10 mL), and the combined organic layers were dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A /

93%B → 60%A / 40%B (10 CV), 60%A / 40%B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm) to afford alcohol **11** (0.11 g, 0.27 mmol, 68%). ¹H NMR (500 MHz, CDCl₃) δ 6.76 (2H, m), 6.47 (2H, s), 6.33 (1H, t, *J* = 7.5 Hz), 4.12 (2H, m), 3.89 (2H, m), 3.88 (3H, s), 3.84 (3H, s), 3.79 (6H, s), 2.75 (2H, t, *J* = 7 Hz), 2.15 (2H, m), 1.95 (2H, m). ¹³C NMR (125 MHz, CDCl₃) δ 152.9, 151.1, 145.0, 142.8, 138.3, 137.4, 136.1, 134.2, 127.3, 125.7, 109.2, 105.3, 76.1, 62.2, 61.0, 56.2, 55.8, 34.6, 25.6, 24.6. HRMS: Obsvd 423.1780 [M + Na⁺], Calcd for C₂₃H₂₈O₆Na: 423.1778. HPLC: 13.77 min.

8-Bromo-3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-4-ol (13): Brominated benzosuberene **9** (0.12 g, 0.22 mmol, crude) was dissolved in THF (20 mL), and TBAF (0.22 mL, 1 M, 0.22 mmol) was added to the solution at 0 °C. The reaction mixture was stirred for 1 h, washed with brine (20 mL), and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The resulting material was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm) to afford brominated phenol **13** (97 mg, 0.22 mmol, 100% over two steps) as a white crystalline solid. ¹H NMR (600 MHz, CDCl₃) δ 6.63 (1H, d, *J* = 8.4 Hz), 6.45 (2H, s), 6.41 (1H, d, *J* = 8.4 Hz), 5.74 (1H, s), 3.88 (3H, s), 3.87 (3H, s), 3.80 (6H, s), 2.88 (2H, t, *J* = 7.2 Hz), 2.58 (2H, t, *J* = 7.2 Hz), 2.26 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 152.7, 145.5, 142.5, 140.8, 137.9, 137.3, 135.3, 126.4, 121.5, 121.1, 108.0, 107.5, 61.0, 56.3, 56.1, 38.5, 32.5, 23.2. HRMS: Obsvd 457.0621 [M + Na⁺], Calcd for C₂₁H₂₃BrO₅Na: 457.0621. HPLC: 17.54 min.

8-Bromo-3-methoxy-4-methyl-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene (14): KGP391 **12** (68 mg, 0.19 mmol) was dissolved in CCl₄ (20 mL), and NBS (37 mg, 0.21 mmol) and AIBN (3.1 mg, 0.02 mmol) were added carefully avoiding shaking or metal spatula since AIBN can be explosive. The reaction mixture was refluxed and stirred for 2 h. The solution was washed with water and extracted by CH₂Cl₂, the organic phase was further washed by brine and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 6% A / 94% B (1 CV), 6% A / 94% B → 70% A / 30% B (10 CV), 70% A / 30% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm) to afford brominated benzosuberene analogue **14** (66 mg, 0.15 mmol, 80%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 6.70 (1H, d, *J* = 7 Hz), 6.62 (1H, d, *J* = 7 Hz), 6.47 (2H, s), 3.88 (3H, s), 3.81 (9H, s), 2.81 (2H, m), 2.53 (2H, m), 2.26 (3H, s), 2.24 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 156.8, 152.7, 141.4, 140.2, 138.0, 137.2, 134.2, 127.6, 123.5, 120.5, 107.7, 107.4, 61.0, 56.3, 55.6, 38.3, 33.0, 27.5, 11.9. HRMS: Obsvd 457.0808 [M + Na⁺], Calcd for C₂₂H₂₅BrO₄Na: 455.0828. HPLC: 25.38 min.

2-Methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulene-1,5,6-triol (15): To a solution of TBS-protected phenol **10** (0.35 g, 0.69 mmol) in THF (20 mL) was added TBAF (0.76 mL, 1 M in THF, 0.76 mmol) at 0 °C. The reaction mixture was stirred for 1 h, subsequently washed with brine (30 mL), and extracted with EtOAc (3 × 30

mL). The resultant organic phase was dried over sodium sulfate, filtered, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 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 (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford diol phenol **15** (138 mg, 0.35 mmol, 51%) as a yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.24 (1H, d, *J* = 8.4 Hz), 6.78 (1H, d, *J* = 8.4 Hz), 6.51 (2H, s), 5.82 (1H, s), 4.56 (1H, m), 3.93 (3H, s), 3.83 (3H, s), 3.75 (6H, s), 3.37 (1H, m), 3.21 (1H, s), 2.24 (1H, m), 2.05 (1H, m), 1.96 (1H, m), 1.69 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 153.3, 146.1, 142.8, 138.6, 137.9, 133.8, 127.0, 120.6, 107.9, 105.2, 83.3, 76.8, 61.0, 56.3, 56.0, 32.7, 25.1, 21.2. HRMS: Obsvd 413.1571 [M + Na⁺], Calcd for C₂₁H₂₆O₇Na: 413.1571. HPLC: 13.71 min.

3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene-4-carbonitrile (18)³⁸: To **KGP156** (0.10 g, 0.28 mmol) in a 2 M HCl/ CH₃OH solution (5 mL/ 5 mL) was added NaNO₂ (77.7 mg, 1.12 mmol) at 0 °C, and the mixture was stirred for 1 h. CuCN was added (50.4 mg, 0.56 mmol), and the reaction mixture was heated at 60 °C for 2 h. Na₂CO₃ and NaCN were added (50 mg of each), and the reaction mixture was stirred for 12 h at room temperature. A saturated FeCl₃ solution (50 mL) was added to quench the reaction, and the reaction mixture was extracted with EtOAc (3 × 30 mL). The combined organic phase was washed with brine and a saturated NaHCO₃ solution, then dried over sodium sulfate and evaporated under reduced pressure. The crude reaction was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford nitrile **18** (41 mg, 0.11 mmol, 40%) as a yellow solid. ¹H NMR (600 MHz, CDCl₃) δ 7.20 (1H, d, *J* = 8.4 Hz), 6.80 (1H, d, *J* = 8.4 Hz), 6.43 (1H, t, *J* = 7.8 Hz), 6.42 (2H, s), 3.94 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.90 (2H, t, *J* = 7.2 Hz), 2.26 (2H, m), 1.94 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 160.7, 153.1, 147.5, 141.6, 137.6, 137.5, 134.9, 133.4, 128.7, 116.1, 108.5, 105.0, 101.7, 61.1, 56.3, 56.2, 34.8, 25.7, 25.4. HRMS: Obsvd 388.1521 [M + Na⁺], Calcd for C₂₂H₂₃NO₄Na: 388.1519. HPLC: 20.75 min.

2-Methoxy-5-(3,4,5-trimethoxyphenyl)-7,8-dihydronaphthalene-1-carbonitrile (19)³⁵: **KGP05** (48.6 mg, 0.14 mmol) was dissolved in 2 M HCl/ CH₃OH (2 mL/ 2 mL). The solution was cooled to 0 °C, NaNO₂ (39.2 mg, 0.56 mmol) was added, and the resultant reaction mixture was stirred at 0 °C for 1 h. The reaction mixture was heated at 60 °C for 2 h before the addition of CuCN (25.5 mg, 0.28 mmol). After the reaction mixture was cooled to room temperature, Na₂CO₃ and NaCN were added to adjust the pH to 10 and provide more nitrile ions for improving the yield, followed by an additional 12 h of stirring. FeCl₃ was added to quench the reaction, followed by extraction with EtOAc (3 × 20 mL). The combined organic phase was washed with brine and a saturated NaHCO₃ solution, dried over sodium sulfate, and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford nitrile **19** (17 mg, 0.046 mmol, 32%) as a white foam. ¹H NMR (600 MHz, CDCl₃) δ 7.20

(1H, d, $J=8.4$ Hz), 6.69 (1H, d, $J=9$ Hz), 6.50 (2H, s), 6.03 (1H, t, $J=4.8$ Hz), 3.91 (3H, s), 3.88 (3H, s), 3.84 (6H, s), 3.06 (2H, t, $J=7.8$ Hz), 2.43 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 160.3, 153.3, 142.9, 138.5, 137.6, 135.8, 130.8, 128.8, 126.1, 115.6, 108.3, 105.8, 101.6, 61.1, 56.3, 56.2, 26.8, 22.7. HRMS: Obsvd 374.1363 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{21}\text{H}_{21}\text{NO}_4\text{Na}$: 374.1363. HPLC: 20.92 min.

3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene-4-carbaldehyde (20):

To a solution of nitrile **18** (48 mg, 0.13 mmol) in toluene (15 mL) was added DIBAL-H (0.16 mL, 1 M, 0.16 mmol) at 0 °C, and the resultant solution was stirred for 12 h while warming to room temperature. 1 M HCl (100 mL) was added to the reaction mixture, which was stirred for 30 min at room temperature while the solution color turned to yellow. EtOAc (3 \times 50 mL) was used to extract the organic compound. The combined organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B \rightarrow 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford aldehyde **20** (37.8 mg, 0.10 mmol, 78%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 10.68 (1H, s), 7.18 (1H, d, $J=7.2$ Hz), 6.82 (1H, d, $J=8.4$ Hz), 6.47 (2H, s), 6.43 (1H, t, $J=7.8$ Hz), 3.92 (3H, s), 3.86 (3H, s), 3.81 (6H, s), 2.99 (2H, m), 2.27 (2H, m), 1.91 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 193.3, 161.8, 153.2, 145.2, 141.9, 138.2, 137.6, 135.5, 134.7, 128.8, 123.7, 108.7, 105.3, 61.1, 56.3, 55.9, 31.7, 25.7, 22.8. HRMS: Obsvd 391.1519 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{22}\text{H}_{24}\text{O}_5\text{Na}$: 391.1516. HPLC: 22.39 min.

2-Bromo-3-methoxybenzaldehyde (22): To a solution of 2-bromo-3-hydroxybenzaldehyde **21** (2.50 g, 12.4 mmol) in DMF (50 mL) was added CH_3I (1.01 mL, 16.2 mmol) and K_2CO_3 (1.35 g, 13.7 mmol). The reaction mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the residue was washed with water (50 mL) and extracted with EtOAc (3 \times 50 mL). The organic phases were combined and concentrated without further purification to afford 2-bromo-3-methoxybenzaldehyde **22** (2.67 g, 12.4 mmol, 100%) as a brown solid. ^1H NMR (600 MHz, CDCl_3) δ 10.44 (1H, s), 7.52 (1H, d, $J=7.8$ Hz), 7.38 (1H, t, $J=7.8$ Hz), 7.13 (1H, d, $J=8.4$ Hz), 3.96 (3H, s). ^{13}C NMR (150 MHz, CDCl_3) δ 192.4, 156.4, 134.9, 128.5, 121.6, 117.3, 117.1, 56.8.

5-(2-Bromo-3-methoxyphenyl)pent-4-enoic acid (23): To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (5.33 g, 12.4 mmol) in THF (250 mL) was added potassium *tert*-butoxide (3.08 g, 27.3 mmol), and the reaction mixture was stirred at room temperature for 1 h. 2-Bromo-3-methoxybenzaldehyde **22** (2.67 g, 16.2 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The THF was removed under reduced pressure, and the resulting material was quenched and acidified with 2 M HCl (30 mL) and extracted with EtOAc (3 \times 50 mL). The combined organic phase was dried over sodium sulfate, filtered, and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B \rightarrow 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min;

monitored at 254 and 280 nm] to afford carboxylic acid **23** (1.74 g, 6.10 mmol, 49%) as a white solid. NMR characterization was performed after the next step.

Methyl 5-(2-bromo-3-methoxyphenyl)pentanoate (24): To dissolved carboxylic acid **23** (0.69 g, 2.42 mmol) in CH₃OH (30 mL) was added 10% palladium on carbon (0.26 g), Ph₂S (40 μL, 0.24 mmol), and two balloons with hydrogen gas. After stirring for 24 h, the mixture was filtered through Celite[®], and the Celite[®] was washed with EtOAc (3 × 50 mL). The combined organic phase (CH₃OH and EtOAc) was evaporated under reduced pressure. The residue was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford saturated ester **24** (0.48 g, 1.6 mmol, 66%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.54 (1H, t, *J* = 7.8 Hz), 7.19 (1H, d, *J* = 8.4 Hz), 7.10 (1H, d, *J* = 7.8 Hz), 4.24 (3H, s), 4.02 (3H, s), 3.14 (2H, m), 2.72 (2H, m), 2.07 (2H, m), 2.02 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 174.2, 156.1, 143.3, 127.8, 122.5, 113.9, 109.5, 56.4, 51.6, 36.1, 34.0, 29.4, 24.8.

1-Bromo-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (25): To ester **24** (0.48 g, 1.6 mmol) was added Eaton's reagent (8.5 mL), and the mixture was stirred at room temperature for 12 h. The reaction mixture was then poured over ice and neutralized with sodium carbonate. The aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic phase was dried over sodium sulfate, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **25** (0.20 g, 0.74 mmol, 47%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.63 (1H, d, *J* = 8.4 Hz), 6.82 (1H, d, *J* = 8.4 Hz), 3.94 (3H, s), 3.17 (2H, m), 2.69 (2H, m), 1.85 (2H, m), 1.75 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 205.2, 159.0, 142.1, 133.7, 129.1, 114.1, 109.4, 56.6, 40.5, 31.2, 23.9, 20.7.

4-Bromo-3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene (26): To an oven dried flask, THF (20 mL) and 3, 4, 5-trimethoxyphenyl bromide (1.12 g, 4.53 mmol) were added, and the solution was cooled to -78 °C. *n*-Buli (1.81 mL, 2.5 M, 4.52 mmol) was slowly added to the reaction mixture, which was then stirred at -78 °C for 45 min. Benzosuberone **25** (0.61 g, 2.3 mmol) was then added to the flask dropwise, and the reaction mixture was stirred while warming from -78 °C to room temperature over 12 h. The reaction was quenched with excess HCl (2 M, 50 mL), and stirred for 30 min at room temperature. The reaction mixture was washed with water and extracted with EtOAc (3 × 40 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 20% A / 80% B (10 CV), 20% A / 80% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford brominated benzosuberene **26** (0.46 g, 1.1 mmol, 49%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 6.97 (1H, d, *J* = 8.4 Hz), 6.75 (1H, d, *J* = 8.4 Hz), 6.48 (2H, s), 6.37 (1H, t, *J* = 7.8 Hz), 3.92 (3H, s), 3.86 (3H, s),

3.81 (6H, s), 2.95 (2H, t, $J = 7.2$ Hz), 2.17 (2H, m), 1.92 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 154.9, 153.1, 143.0, 142.8, 138.0, 137.6, 134.4, 129.1, 127.8, 113.4, 109.1, 105.3, 61.0, 56.4, 56.3, 33.8, 31.9, 25.4.

Ethyl 3-methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene-4-carboxylate (27): To a solution of brominated benzosuberene **26** (0.15 g, 0.36 mmol) in THF (20 mL) was added *n*-Buli (0.34 mL, 1.6 M, 0.54 mmol) dropwise at -78 °C. The reaction mixture was stirred at -78 °C for 30 min, followed by the addition of ethyl chlorofomate (61.5 μL , 0.64 mmol). The reaction mixture was stirred while warming from -78 °C to room temperature over 12 h. The reaction mixture was washed with water and extracted with EtOAc (3×30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93%B (1 CV), 7% A / 93%B \rightarrow 40% A / 60%B (10 CV), 40% A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford benzosuberene ester **27** (51.8 mg, 0.13 mmol, 35%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 7.03 (1H, d, $J = 8.4$ Hz), 6.76 (1H, d, $J = 9$ Hz), 6.47 (2H, s), 6.37 (1H, t, $J = 7.8$ Hz), 4.43 (2H, q, $J = 7.2$ Hz), 3.85 (3H, s), 3.84 (3H, s), 3.79 (6H, s), 2.56 (2H, t, $J = 6.6$ Hz), 2.17 (2H, m), 1.96 (2H, m), 1.41 (3H, t, $J = 7.2$ Hz). ^{13}C NMR (150 MHz, CDCl_3) δ 168.8, 155.2, 153.0, 142.3, 140.2, 138.1, 137.5, 133.2, 131.6, 127.7, 123.4, 108.6, 105.2, 61.4, 61.0, 56.3, 55.9, 34.9, 29.6, 25.3, 14.4. HRMS: Obsvd 435.1778 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{24}\text{H}_{28}\text{O}_6\text{Na}$: 435.1778. HPLC: 22.42 min.

(3-Methoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulene-4-yl)methanol (28): Ester **27** (0.12 g, 0.28 mmol) was dissolved in THF (10 mL), and the solution was cooled to 0 °C. LiAlH_4 (77 μL , 4 M in ether, 0.31 mmol) was added to the solution dropwise, and the reaction mixture was stirred while warming to room temperature for 1 h. The reaction mixture was washed with water and extracted with EtOAc (3×20 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88%B (1 CV), 12% A / 88%B \rightarrow 60% A / 40%B (10 CV), 60% A / 40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford benzyl alcohol **28** (54.3 mg, 0.15 mmol, 52%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 6.97 (1H, d, $J = 9$ Hz), 6.75 (1H, d, $J = 9$ Hz), 6.50 (2H, s), 6.35 (1H, t, $J = 7.8$ Hz), 4.87 (2H, s), 3.89 (3H, s), 3.86 (3H, s), 3.81 (6H, s), 2.79 (2H, t, $J = 7.2$ Hz), 2.16 (2H, p, $J = 7.2$ Hz), 1.92 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 157.1, 153.1, 143.3, 142.4, 138.5, 137.5, 133.8, 130.2, 127.1, 126.0, 107.9, 105.4, 61.1, 57.7, 56.3, 55.7, 35.3, 27.6, 25.4. HRMS: Obsvd 393.1672 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_5\text{Na}$: 393.1672. HPLC: 19.09 min.

3-(2,3-Dimethoxyphenyl)propanoic acid (30): To cinnamic acid **29** (5.0 g, 24 mmol) was added methanol (50 mL) and 10% Pd/C (0.8 g). Two hydrogen balloons were installed through the rubber septum, and the reaction mixture was stirred at room temperature for 4 h. The reaction mixture was filtered through Celite®, and the Celite® was washed with EtOAc (3×50 mL). The organic solvents (CH_3OH and EtOAc) were evaporated under reduced

pressure to afford carboxylic acid **30** (5.0 g, 24 mmol, quantitative) as a white solid. No further purification was needed. ^1H NMR (600 MHz, CDCl_3) δ 6.98 (1H, t, $J=7.8$ Hz), 6.78 (2H, m), 3.86 (3H, s), 3.84 (3H, s), 2.95 (2H, t, $J=7.8$ Hz), 2.66 (2H, t, $J=7.8$ Hz). ^{13}C NMR (150 MHz, CDCl_3) δ 178.9, 152.8, 147.2, 134.1, 124.1, 121.8, 110.9, 60.7, 55.8, 34.8, 25.4.

Methyl 5-(2,3-dimethoxyphenyl)-3-oxopentanoate (31): To dissolved carboxylic acid **30** (5.05 g, 24.0 mmol) in dichloromethane (96 mL) were added oxalyl chloride (4.12 mL, 47.2 mmol) and a catalytic amount of DMF (0.15 mL). The reaction mixture was stirred at room temperature for 1 h, at which time an additional catalytic amount of DMF (0.15 mL) was added, and the reaction solution stirred for 1 h at room temperature. The solvent and unreacted oxalyl chloride were removed under reduced pressure to afford acyl chloride as a yellow crystalline solid, which was re-dissolved in dichloromethane (50 mL) and cooled to 0°C . Meldrum's acid (3.47 g, 24.1 mmol) and pyridine (4.33 mL, 53.8 mmol) were added, and the reaction mixture was stirred for 30 min at 0°C , then 1 h at room temperature. The mixture was diluted with dichloromethane (50 mL), and washed with 2 M HCl (20 mL), followed by brine (30 mL). The organic layer was dried over sodium sulfate and concentrated in vacuo. The residue was dissolved in CH_3OH (50 mL) and heated at reflux for 3 h. The solvent was removed under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B \rightarrow 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford the ester **31** (3.57 g, 13.4 mmol, 56%) as a pale-yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 6.96 (1H, t, $J=7.8$ Hz), 6.77 (1H, d, $J=9$ Hz), 6.74 (1H, d, $J=7.8$ Hz), 3.84 (3H, s), 3.81 (3H, s), 3.71 (3H, s), 3.44 (2H, s), 2.89 (2H, m), 2.83 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 202.1, 167.6, 152.7, 147.0, 134.2, 124.0, 121.8, 110.6, 60.5, 55.6, 52.3, 49.0, 43.6, 24.2.

Methyl 5-(2,3-dimethoxyphenyl)-3-hydroxypentanoate (32): To a well-stirred solution of ketone **31** (0.50 g, 1.9 mmol) in CH_3OH (8 mL) at 0°C , sodium borohydride (24 mg, 0.63 mmol) was added in one aliquot. The reaction mixture was initially stirred at 0°C for 1 h, and then stirred at room temperature for another 1 h. The solvent was removed under reduced pressure. The residue was washed with water (10 mL) and extracted with diethyl ether (3×10 mL). The combined organic phase was dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography using a prepacked 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (3 CV), 7% A / 93% B \rightarrow 60% A / 40% B (10 CV), 60% A / 40% B (1 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford alcohol **32** (0.40 g, 1.5 mmol, 79%). ^1H NMR (600 MHz, CDCl_3) δ 6.98 (1H, t, 7.8 Hz), 6.78 (2H, m), 3.97 (1H, m), 3.85 (3H, s), 3.82 (3H, s), 3.69 (3H, s), 2.76 (2H, t, $J=7.8$ Hz), 2.48 (2H, m), 1.78 (2H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 173.3, 152.8, 147.2, 135.4, 124.2, 122.1, 110.5, 67.2, 60.8, 55.8, 51.8, 41.4, 37.6, 25.9.

Methyl 3-((tert-butyl) diphenylsilyloxy)-5-(2,3-dimethoxyphenyl)pentanoate (33): To a solution of alcohol **32** (0.38 g, 0.14 mmol) and imidazole (0.16 g, 2.3 mmol) in DMF (2.6 mL) at room temperature was added TBDPSCl (0.55 mL, 2.1 mmol) in one aliquot. The

reaction mixture was stirred for 12 h, diluted with brine (10 mL), and extracted with Et₂O (3 × 10 mL). The organic extracts were combined and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 30% A / 70% B (10 CV), 30% A / 70% B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to afford ester **33** (0.35 g, 0.69 mmol, 49%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.72 (3H, m), 7.67 (1H, m), 7.38 (6H, m), 6.91 (1H, t, *J* = 7.8 Hz), 6.73 (1H, d, *J* = 9.6 Hz), 6.55 (1H, d, *J* = 9 Hz), 4.29 (1H, m), 3.83 (3H, s), 3.71 (3H, s), 3.54 (3H, s), 2.58 (4H, m), 1.76 (2H, m), 1.06 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 172.0, 152.8, 147.1, 136.1, 136.0, 135.9, 135.3, 134.9, 134.2, 134.1, 129.8, 129.7, 127.9, 127.7, 127.6, 123.9, 121.8, 110.2, 70.5, 60.7, 55.8, 51.5, 41.9, 38.2, 27.1, 26.7, 25.4.

3-((*Tert*-butyldiphenylsilyloxy)-5-(2,3-dimethoxyphenyl)pentanoic acid (34): To a solution of ester **33** (0.67 g, 1.3 mmol) in CH₃OH/THF (2.2 mL/ 1.1 mL) at 0 °C was added 2.5 M NaOH (1.76 mL). The reaction mixture was stirred for 1 h at 0 °C, and then 13 h at room temperature, acidified by 2 M HCl (10 mL), and extracted with Et₂O (3 × 10 mL). The organic extracts were combined and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 30% A / 70% B (10 CV), 30% A / 70% B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **34** (0.26 g, 0.53 mmol, 40%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.67 (4H, m), 7.41 (6H, m), 6.89 (1H, t, *J* = 8.4 Hz), 6.72 (1H, d, *J* = 8.4 Hz), 6.52 (1H, m), 4.20 (1H, m), 3.83 (3H, s), 3.69 (3H, s), 2.50 (4H, m), 1.80 (2H, m), 1.06 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 152.6, 146.94, 146.93, 135.9, 135.8, 129.9, 129.8, 129.77, 127.7, 127.6, 123.81, 123.80, 121.6, 110.2, 70.2, 60.5, 55.6, 40.8, 37.6, 26.9, 25.3, 19.3.

7-((*Tert*-butyldiphenylsilyloxy)-1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (35): To a solution of carboxylic acid **34** (5.37g, 10.9 mmol) in dichloromethane (40 mL) was added oxalyl chloride (4.5 mL, 52 mmol) and 3 drops of DMF as catalyst at room temperature. The resultant reaction mixture was stirred for 2 h at 0 °C. The solvent and unreacted oxalyl chloride were removed under reduced pressure. The residue acyl chloride was dissolved in dichloromethane (50 mL). The solution was cooled to -10 °C, at which point SnCl₄ (3.63 mL, 1 M in CH₂Cl₂, 3.63 mmol) was added, followed by stirring at -10 °C for 30 min. The reaction was quenched with cold water and extracted with EtOAc (3 × 50 mL). The organic extracts were combined and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to afford cyclized ketone **35** (2.80 g, 5.90 mmol, 54%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.63 (4H, m), 7.56 (1H, d, *J* = 8.4 Hz), 7.39 (6H, m), 6.81 (1H, d, *J* = 9 Hz), 6.29 (1H, m), 3.90 (3H, s), 3.78 (3H, s), 3.17 (1H, m), 3.03 (2H, m), 2.88 (1H, m), 1.98 (1H, m), 1.84 (1H, m), 1.03 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 199.5, 155.8, 146.2, 137.9, 136.0, 135.9, 134.1, 133.9, 133.1, 129.9, 129.8, 127.81, 127.77, 125.7, 109.6, 68.3, 60.9, 55.9, 50.4, 36.2, 27.0, 21.3, 19.3.

7-Hydroxy-1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (36): To a solution of ketone **35** (0.73 g, 1.5 mmol) in THF (10 mL) was added TBAF (3.1 mL 1 M in THF, 3.1 mmol), and the reaction mixture was stirred for 30 min at 0 °C and 16 h at room temperature. The reaction was quenched with brine (10 mL) and extracted with EtOAc (3 × 20 mL). The organic extracts were combined and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B → 20% A / 80% B (10 CV), 20% A / 80% B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to afford alcohol **36** (0.16 g, 0.66 mmol, 49%) as a yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.60 (1H, d, *J* = 8.4 Hz), 6.84 (1H, d, *J* = 8.4 Hz), 4.33 (1H, m), 3.91 (3H, s), 3.80 (3H, s), 3.08 (3H, m), 2.99 (1H, m), 1.89 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 199.3, 156.0, 146.3, 137.7, 132.6, 125.8, 109.8, 67.3, 60.9, 56.0, 50.3, 35.8, 21.4.

3,4-Dimethoxy-9-(3,4,5-trimethoxyphenyl)-6,7-dihydro-5H-benzo[7]annulen-7-ol (37): To a solution of 3, 4, 5-trimethoxyphenyl bromide (0.49 g, 2.0 mmol) in THF (20 mL) at -78 °C was added *n*-BuLi (1.85 mL, 1.6 M in hexanes, 2.98 mmol), and the reaction mixture was stirred for 1 h. Benzosuberone **36** (0.16 g, 0.66 mmol) in THF (5 mL) was added slowly. The reaction mixture was stirred at 0 °C for 20 h. 2 M HCl (20 mL) was added, and the mixture was extracted with EtOAc (4 × 20 mL). The combined organic phase was further washed by brine and dried over sodium sulfate, filtered, and concentrated under reduced pressure and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B → 80% A / 20% B (10 CV), 80% A / 20% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford alcohol **37** (0.10 g, 0.26 mmol, 39%) as a brown solid. ¹H NMR (600 MHz, CDCl₃) δ 6.75 (2H, m), 6.51 (2H, s), 6.28 (1H, d, *J* 4.8 Hz), 4.18 (1H, m), 3.877 (3H, s), 3.875 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 3.16 (1H, m), 2.53 (1H, m), 2.43 (1H, m), 2.15 (1H, m). ¹³C NMR (150 MHz, CDCl₃) δ 153.1, 152.0, 146.1, 139.4, 137.8, 137.4, 135.7, 132.9, 131.5, 125.5, 109.6, 105.5, 70.0, 61.4, 61.1, 56.3, 55.8, 43.2, 22.4. HRMS: Obsvd 409.1621 [M + Na⁺], Calcd for C₂₂H₂₆O₆Na: 409.1622. HPLC: 16.79 min.

2-((Tert-butyl dimethylsilyl)oxy)-3-methoxybenzaldehyde (39): To a well-stirred solution of 2-hydroxy-3-methoxybenzaldehyde **38** (0.50 g, 3.3 mmol) in dichloromethane (30 mL) was added TBSCl (0.74 g, 4.9 mmol), DMAP (0.12 g, 0.99 mmol), and Et₃N (0.69 mL, 4.9 mmol). The reaction mixture was stirred for 12 h at room temperature, at which point brine (50 mL) was added, and the reaction mixture was extracted with dichloromethane (3 × 40 mL). The organic extracts were combined and dried over sodium sulfate, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 75 mL/min; monitored at 254 and 280 nm] to afford protected aldehyde **39** (0.50 g, 1.86 mmol, 57%) as a pale yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 10.51 (1H, s), 7.36 (1H, d, *J* = 7.8 Hz), 7.03 (1H, d, *J* = 7.8 Hz), 6.94 (1H, t, *J* = 8.4 Hz), 3.81 (3H, s), 0.99 (9H, s), 0.20 (6H,

s). ^{13}C NMR (150 MHz, CDCl_3) δ 190.4, 150.8, 149.2, 127.9, 121.2, 119.1, 117.0, 55.2, 26.0, 19.0, 4.1.

Ethyl 5-(2-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-3-oxopent-4-enoate (40): To dissolved ethyl 3-oxo-4-(triphenylphosphoranylidene) butanoate (3.22 g, 8.26 mmol) in THF (20 mL) was added protected aldehyde **39** (2.2 g, 8.3 mmol), and the reaction mixture was heated at reflux and stirred for 17 h. The solvent was removed under reduced pressure, and the residue was taken up as a slurry and purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B \rightarrow 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford ester **40** (2.50 g, 6.59 mmol, 80%) as an off white solid. NMR characterization was conducted after the next step.

Ethyl 5-(2-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-3-oxopentanoate (41): To dissolved ester **40** (2.50 g, 6.59 mmol) in methanol (60 mL) was added 10% palladium on carbon (0.54 g), and hydrogen gas was introduced with a balloon. The reaction mixture was stirred at room temperature for 12 h and filtered through Celite®, and the Celite® was washed with EtOAc (3 \times 40 mL). The combined organic phase (CH_3OH and EtOAc) was evaporated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B \rightarrow 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford saturated ester **41** (1.15 g, 3.02 mmol, 46%) as a light-yellow oil. ^1H NMR (600 MHz, CDCl_3) δ 6.81(1H, m), 6.71 (1H, d, $J = 7.8$ Hz), 4.16 (2H, q, $J = 7.2$ Hz), 3.76 (3H, s), 3.39 (2H, s), 2.90 (2H, m), 2.83 (2H, m), 1.25 (3H, t, $J = 7.2$ Hz), 0.98 (9H, s), 0.18 (6H, s). ^{13}C NMR (150 MHz, CDCl_3) δ 202.3, 167.2, 150.0, 142.8, 131.8, 121.9, 121.0, 109.7, 61.4, 54.8, 49.4, 43.2, 26.2, 24.8, 18.9, 14.2, -3.7.

Ethyl (Z)-3-amino-5-(2-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)pent-2-enoate (42): To keto-ester **41** (1.10 g, 2.89 mmol) dissolved in methanol (15 mL) was added dry ammonium acetate (1.11 g, 14.5 mmol). The reaction mixture was stirred at 35 °C for 16 h. The methanol was removed under vacuum, and the residue was suspended in EtOAc (30 mL) and filtered. The filtrate was washed with EtOAc (4 \times 20 mL). The combined organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford amine **42** (1.02 g, 2.69 mmol, 93%) as pale yellow crystals. No further purification was performed. ^1H NMR (600 MHz, CDCl_3) δ 6.82 (1H, m), 6.72 (2H, m), 4.58 (1H, s), 4.11 (2H, m), 3.78 (3H, s), 2.87 (2H, m), 2.40 (2H, m), 1.26 (3H, t, $J = 7.2$ Hz), 1.00 (9H, s), 0.19 (6H, s). ^{13}C NMR (150 MHz, CDCl_3) δ 170.7, 163.6, 150.0, 142.8, 131.8, 122.0, 121.1, 109.8, 83.5, 58.7, 54.8, 36.7, 29.3, 26.3, 19.0, 14.7, -3.6.

Ethyl (Z)-3-acetamido-5-(2-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)pent-2-enoate (43): To amine **42** (4.02 g, 10.6 mmol) dissolved in THF (50 mL) was added pyridine (1.71 mL, 21.2 mmol) and acetic anhydride (6.00 mL, 63.6 mmol). The reaction mixture was stirred for 48 h under reflux. The THF was removed under vacuum, and the residue was dissolved in EtOAc (50 mL) and washed with water (50 mL), 2 M HCl (20 mL),

saturated NaHCO₃ (50 mL) and brine (50 mL). The combined organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford *N*-acetamide **43** (2.17 g, 5.15 mmol, 49%) as a yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 6.80 (2H, m), 6.70 (1H, m), 4.90 (1H, s), 4.14 (2H, m), 3.76 (3H, s), 3.00 (2H, m), 2.86 (2H, m), 2.15 (3H, s), 1.26 (3H, m), 0.98 (9H, s), 0.17 (6H, s). ¹³C NMR (150 MHz, CDCl₃) δ 169.3, 168.3, 158.2, 149.8, 142.7, 132.0, 122.2, 120.9, 109.5, 96.2, 59.8, 54.7, 34.5, 28.7, 26.2, 25.3, 18.9, 14.3, -3.8.

Ethyl 3-acetamido-5-(2-((*tert*-butyldimethylsilyloxy)-3-methoxyphenyl)pentanoate

(44): Unsaturated *N*-acetamide **43** (2.17 g, 5.15 mmol) was dissolved in CH₃OH (30 mL). Palladium (10%) on carbon (0.53 g) and a hydrogen gas balloon were introduced, and the solution was stirred at room temperature for 60 h and filtered through Celite®. The Celite® was washed with EtOAc (3 × 50 mL). The combined organic phase (CH₃OH and EtOAc) was evaporated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 50% A / 50% B (10 CV), 50% A / 50% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford saturated *N*-acetamide **44** (0.96 g, 2.3 mmol, 44%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.83 (1H, t, *J* = 7.8 Hz), 6.71 (2H, m), 6.03 (1H, d, *J* = 9 Hz), 4.29 (1H, m), 4.11 (2H, q, *J* = 7.2 Hz), 3.77 (3H, s), 2.74 (1H, m), 2.62 (1H, m), 2.59 (1H, m), 2.51 (1H, m), 1.96 (3H, s), 1.82 (2H, m), 1.24 (3H, t, *J* = 7.2 Hz), 1.00 (9H, s), 0.17 (6H, d, *J* = 10.8 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 172.1, 169.6, 150.0, 142.7, 132.7, 121.9, 121.0, 109.4, 60.7, 54.8, 46.3, 38.8, 34.3, 27.6, 26.3, 23.7, 19.0, 14.3, -3.6, -3.7.

3-Acetamido-5-(2-hydroxy-3-methoxyphenyl)pentanoic acid (45): To dissolved unsaturated ester **44** (0.96 g, 2.3 mmol) in methanol (5 mL) was added 1 M KOH (7.48 mL). The reaction was stirred from 0 °C to room temperature over 3 h. The methanol was removed under vacuum, and 2 M HCl (5 mL) was added to the residue, which was then extracted with EtOAc (3 × 20 mL). The combined organic phase was evaporated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B → 100% A / 0% B (35 CV), 100% A / 0% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **45** (0.38 g, 1.8 mmol, 58%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.74 (3H, m), 6.34 (1H, d, *J* = 9 Hz), 4.25 (1H, m), 3.85 (3H, s), 2.64 (4H, m), 1.97 (3H, s), 1.91 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 175.6, 171.0, 146.6, 143.5, 127.3, 122.5, 119.8, 108.9, 56.2, 46.7, 38.9, 34.1, 26.8, 23.5.

***N*-(1-hydroxy-2-methoxy-5-oxo-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-7-yl)acetamide**

(46): Carboxylic acid **45** (0.70 g, 2.5 mmol) was dissolved in Eaton's reagent (14 mL), and the reaction mixture was stirred at room temperature for 12 h. Ice was added to the reaction mixture, which generated a significant amount of heat. A saturated sodium carbonate

solution was added until neutral pH was achieved. The mixture was extracted with dichloromethane (4 × 30 mL). The organic phase was further washed by brine and dried over sodium sulfate, filtered, and concentrated under reduced pressure and purified by flash chromatography using a pre-packed 50 g silica column [solvent A: CH₃OH; solvent B: CH₂Cl₂; gradient: 1% A / 99%B (1 CV), 1% A / 99%B → 10% A / 90%B (10 CV), 10% A / 90%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford cyclized ketone **46** (0.37 g, 1.4 mmol, 57%) as a yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.34 (1H, d, *J* = 8.4 Hz), 6.79 (1H, d, *J* = 9 Hz), 5.91 (1H, s), 4.48 (1H, m), 3.94 (3H, s), 3.23 (1H, m), 3.13 (1H, m), 2.83 (2H, m), 2.73 (1H, m), 2.44 (1H, m), 1.96 (3H, s). ¹³C NMR (150 MHz, CDCl₃) δ 201.1, 169.6, 149.3, 142.9, 133.1, 129.0, 121.1, 108.2, 56.3, 47.0, 45.7, 32.9, 23.6, 22.6.

***N*-(1-((*tert*-butyldimethylsilyloxy)-2-methoxy-5-oxo-6,7,8,9-tetrahydro-5H-benzo[7]annulen-7-yl)acetamide (47):** To a solution of cyclized ketone **46** (0.37 g, 1.4 mmol) in dichloromethane (20 mL) at room temperature was added TBSCl (0.32 g, 2.1 mmol), DMAP (52 mg, 0.42 mmol) and trimethylamine (0.30 mL, 2.1 mmol), and the resultant reaction mixture was stirred for 12 h. The reaction mixture was subsequently washed with brine (30 mL), and extracted with dichloromethane (3 × 40 mL). The combined organic layer was dried over sodium sulfate and concentrated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: CH₃OH; solvent B: CH₂Cl₂; gradient: 0% A / 100%B (1 CV), 0% A / 100%B → 5% A / 95%B (10 CV), 5% A / 95%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford protected ketone **47** (0.36 g, 0.95 mmol, 68%) as an off-white solid. ¹H NMR (600 MHz, CDCl₃) δ 7.41 (1H, d, *J* = 8.4 Hz), 6.78 (1H, d, *J* = 9 Hz), 5.61 (1H, br), 4.46 (1H, m), 3.84 (3H, s), 3.26 (1H, m), 3.16 (1H, m), 2.80 (2H, m), 2.71 (1H, m), 2.45 (1H, m), 1.96 (3H, s), 1.00 (9H, s), 0.17 (6H, d, *J* = 14.4 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 201.1, 169.6, 153.4, 142.4, 134.7, 129.0, 122.6, 109.1, 55.1, 46.9, 45.8, 40.0, 33.2, 26.2, 23.6, 19.1, -3.7, -3.8.

***N*-(1-((*tert*-butyldimethylsilyloxy)-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-7-yl)acetamide (48):** To an oven-dried flask, THF (20 mL) and 3, 4, 5-trimethoxyphenyl bromide (0.16 g, 0.64 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (1.6 M, 0.59 mL, 0.94 mmol) was added to the reaction mixture slowly, which was then stirred at -78 °C for 45 min. Benzosuberone **47** (80 mg, 0.21 mmol) was then added dropwise to the flask, and the reaction mixture was stirred while warming from -78 °C to room temperature over 12 h. 2 M HCl (20 mL) was added, and the reaction mixture was stirred for 10 min, then extracted with EtOAc (3 × 50 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 20 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 (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford cross-coupling product benzosuberone **48** (63.6 mg, 0.120 mmol, 57%) as a crystalline white solid. ¹H NMR (600 MHz, CDCl₃) δ 6.68 (1H, d, *J* = 9 Hz), 6.58 (1H, d, *J* = 8.4 Hz), 6.47 (2H, s), 5.98 (1H, d, *J* = 6 Hz), 5.54 (1H, d, *J* = 8.4 Hz, br), 4.39 (1H, m), 3.85 (3H, s), 3.80 (3H, s), 3.79 (9H, s), 3.20–2.46 (4H,

m), 1.95 (3H, s), 1.03 (9H, s), 0.25 (3H, s), 0.22 (3H). ^{13}C NMR (150 MHz, CDCl_3) δ 169.0, 152.8, 149.1, 141.7, 141.5, 137.9, 137.6, 132.7, 132.3, 128.4, 122.9, 108.9, 105.4, 60.9, 56.2, 54.7, 47.8, 41.0, 26.2, 23.6, 22.9, 19.0, -3.6, -3.9.

***N*-(1-hydroxy-2-methoxy-5-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-**

benzo[7]annulen-7-yl)acetamide (49): Protected benzosuberene *N*-acetamide **48** (63.6 mg, 0.12 mmol) was dissolved in THF (2 mL) and cooled to 0 °C. TBAF (0.24 mL, 0.24 mmol) was added, and the reaction mixture was stirred at 0 °C for 2 h. The solution was washed with water and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over sodium sulfate, filtered, and concentrated under reduced pressure and purified by flash chromatography using a pre-packed 25 g silica column [solvent A: CH_3OH ; solvent B: CH_2Cl_2 ; gradient: 5% A / 95% B (1 CV), 10% A / 90% B → 10% A / 90% B (10 CV), 10% A / 90% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford benzosuberene *N*-acetamide **49** (22 mg, 0.05 mmol, 44%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 6.71 (1H, d, $J = 9$ Hz), 6.54 (1H, d, $J = 8.4$ Hz), 6.48 (2H, s), 5.98 (1H, d, $J = 6$ Hz), 5.76 (1H, s), 5.56 (1H, d, $J = 9$ Hz, br), 4.38 (1H, m), 3.91 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 3.17–2.43 (4H, m), 1.96 (3H, s). ^{13}C NMR (150 MHz, CDCl_3) δ 169.2, 153.0, 145.7, 142.5, 141.4, 137.83, 137.75, 133.3, 128.8, 126.9, 121.5, 108.3, 105.6, 61.1, 56.4, 56.2, 48.1, 40.8, 23.8, 22.3. HRMS: Obsvd 436.1730 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{23}\text{H}_{29}\text{NO}_6\text{Na}$: 436.1731. HPLC: 9.92 min.

Ethyl 5-(2,3-dimethoxyphenyl)-3-oxopentanoate (52): To a solution of 2, 3-dimethoxybenzaldehyde **50** (1.06 g, 6.38 mmol) in THF (50 mL) were added ethyl 3-oxo-4-(triphenylphosphoranylidene) butyrate (3.00 g, 7.65 mmol) and potassium *t*-butoxide (1.73 g, 15.3 mmol) at room temperature, and the reaction mixture was stirred for 12 h. The THF was removed under reduced pressure, and the resulting material was quenched with 2 M HCl (20 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were evaporated under reduced pressure, and the crude product (**51**) was dissolved in CH_3OH (40 mL). To this solution was added 10% palladium on carbon (0.24 g) and balloons with hydrogen gas. The reaction mixture was stirred at room temperature for 12 h and filtered through Celite®, and the Celite® was washed with EtOAc (3 × 30 mL). The combined organic phase (CH_3OH and EtOAc) was evaporated under reduced pressure. The resulting organic material was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford saturated ester **52** (1.45 g, 5.17 mmol, 81%) as a colorless oil. ^1H NMR (600 MHz, CDCl_3) δ 6.97 (1H, t, $J = 7.8$ Hz), 6.78 (1H, d, $J = 9$ Hz), 6.75 (1H, d, $J = 7.8$ Hz), 4.17, (2H, q, $J = 7.2$ Hz), 3.85 (3H, s), 3.82 (3H, s), 3.43 (2H, s), 2.90 (2H, m), 2.84 (2H, m), 1.26 (3H, t, $J = 6.6$ Hz). ^{13}C NMR (150 MHz, CDCl_3) δ 202.4, 167.3, 152.9, 147.2, 134.5, 124.1, 122.0, 110.8, 61.5, 60.7, 55.8, 49.5, 43.8, 24.5, 14.2.

Ethyl 5-(2,3-dimethoxyphenyl)-3-hydroxypentanoate (53): To a solution of ketone **52** (1.45 g, 5.17 mmol) in CH_3OH (20 mL) at 0 °C was added NaBH_4 (110 mg, 2.91 mmol), and the reaction mixture was stirred for 1 h at 0 °C, then stirred for 3 h at ambient temperature. The CH_3OH was removed under reduced pressure, and the resulting material

was washed with water and extracted with EtOAc (3 × 30 mL). The combined organic phase was concentrated under reduced pressure to afford crude alcohol product **53** (1.20 g, 4.25 mmol, 82%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.98 (1H, t, *J* = 7.8 Hz), 6.78 (2H, m), 4.15 (2H, m), 3.97 (1H, m), 3.85 (3H, s), 3.83 (3H, s), 2.76 (2H, m), 2.47 (2H, m), 1.80 (1H, m), 1.72 (1H, m), 1.25 (3H, t, *J* = 7.2 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 173.0, 152.8, 147.2, 135.5, 124.2, 122.1, 110.4, 67.3, 60.8, 55.8, 41.5, 37.6, 25.9, 14.3.

5-(2,3-Dimethoxyphenyl)-3-hydroxypentanoic acid (54): To a solution of ester **53** in CH₃OH/ THF (8 mL/ 4mL) was added a 2.5 M NaOH aqueous solution (6 mL) at 0 °C, and the reaction mixture was stirred for 1 h at 0 °C, then warmed to room temperature over 12 h. The CH₃OH and THF were removed under reduced pressure, and the resulting material was washed with water and extracted with EtOAc (3 × 30 mL). The combined organic extract was dried over Na₂SO₄, filtered and concentrated under pressure to afford carboxylic acid **54** (0.88 g, 3.3 mmol, 77%) as an orange oil (no further purification was needed). ¹H NMR (600 MHz, CDCl₃) δ 7.00 (1H, t, *J* = 8.4 Hz), 6.78 (2H, dd, *J* = 6.6 Hz, 6.6 Hz), 3.92 (1H, m), 3.86 (3H, s), 3.84 (3H, s), 2.76 (2H, m), 2.51 (2H, m), 1.82 (1H, m), 1.73 (1H, m). ¹³C NMR (150 MHz, CDCl₃) δ 176.8, 152.7, 146.9, 134.9, 124.5, 122.2, 110.6, 66.9, 61.0, 55.8, 41.2, 37.6, 25.6.

1,2-Dimethoxy-8,9-dihydro-5H-benzo[7]annulen-5-one (55): To carboxylic acid **54** (0.88 g, 3.3 mmol) was added Eaton's reagent (15.7 mL), and the mixture was stirred at room temperature for 14 h. The mixture was then poured over ice and neutralized with sodium carbonate. The aqueous layer was extracted with EtOAc (3 × 40 mL). The combined organic phase was dried over sodium sulfate, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A / 93% B (1 CV), 7% A / 93% B → 30% A / 70% B (10 CV), 30% A / 70% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford unsaturated cyclized ketone **55** (0.360 g, 1.65 mmol, 50%) as a yellow crystalline solid. ¹H NMR (600 MHz, CDCl₃) δ 7.55 (1H, d, *J* = 9 Hz), 6.83 (1H, d, *J* = 9 Hz), 6.71 (1H, td, *J* = 4.8 Hz, 12 Hz), 6.23 (1H, td, *J* = 1.8 Hz, 12 Hz), 3.91 (3H, s), 3.78 (3H, s), 3.16 (2H, m), 2.55 (2H, m). ¹³C NMR (150 MHz, CDCl₃) δ 193.9, 156.2, 147.0, 145.2, 134.4, 134.3, 132.7, 126.7, 110.0, 61.2, 55.9, 29.6, 25.1.

1,2-Dimethoxy-5-(3,4,5-trimethoxyphenyl)-8,9-dihydro-5H-benzo[7]annulen-5-ol (56): To an oven-dried flask, THF (30 mL) and 3,4,5-trimethoxyphenyl bromide (0.87 g, 3.5 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (1.41 mL, 2.5 M, 3.52 mmol) was slowly added to the reaction mixture, which was then stirred at -78 °C for 30 min. Unsaturated cyclized ketone **55** (0.35 g, 1.6 mmol) in THF (5 mL) was then added dropwise to the flask, and the reaction mixture was stirred while warming from -78 °C to room temperature over 12 h. The reaction mixture was washed with 2 M HCl (10 mL) and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 12% A / 88% B (1 CV), 12% A / 88% B → 60% A / 40% B (10 CV), 60% A / 40% B (5 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford

tertiary alcohol **56** (0.457 g, 1.18 mmol, 74%) as a white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.54 (1H, d, $J=9$ Hz), 6.82 (1H, d, $J=8.4$ Hz), 6.59 (2H, s), 6.07 (1H, d, $J=12$ Hz), 5.77 (1H, td, $J=1.2$ Hz, 12 Hz), 3.90 (3H, s), 3.82 (3H, s), 3.78 (6H, s), 3.75 (3H, s), 3.05 (1H, m), 2.48 (1H, m), 2.43 (1H, m), 2.15 (1H, m). ^{13}C NMR (150 MHz, CD_3OD) δ 153.9, 153.2, 147.5, 145.1, 141.7, 138.3, 137.2, 134.1, 130.8, 121.6, 109.9, 106.0, 78.6, 61.4, 61.1, 56.5, 56.1, 30.0, 23.4. HRMS: Obsvd 409.1622 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_6\text{Na}$: 409.1622. HPLC: 17.36 min.

1,2-Dimethoxy-7-(3,4,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benz[7]annulen-5-one (57): To an oven-dried flask, THF (30 mL) and 3,4,5-trimethoxyphenyl bromide (0.47 g, 1.9 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (0.76 mL, 2.5 M, 1.9 mmol) was slowly added to the reaction mixture, which was stirred at -78 °C for 45 min then moved to a -10 °C bath. CuI (0.181 g, 0.95 mmol) was added in one aliquot to the flask, and the reaction mixture was stirred at -10 °C for 1 h. Unsaturated ketone **55** (0.104 g, 0.47 mmol) in THF (10 mL) was then added dropwise to the flask, and the reaction mixture was stirred while warming from -78 °C to room temperature over 7 h. A saturated NH_4Cl solution and ammonium hydroxide (20 mL/ 20mL), were added followed by stirring for 30 min at room temperature and subsequent extraction with EtOAc (3×50 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B \rightarrow 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 110 mL/min; monitored at 254 and 280 nm] to afford Michael addition ketone **57** (81.7 mg, 0.21 mmol, 44%) as a light white solid. ^1H NMR (600 MHz, CDCl_3) δ 7.56 (1H, d, $J=8.4$ Hz), 6.88 (1H, d, $J=9$ Hz), 6.41 (2H, s), 3.93 (3H, s), 3.83 (6H, s), 3.824 (3H, s), 3.819 (3H, s), 3.34 (1H, td, $J=4.8$ Hz, 15 Hz), 3.09 (1H, m), 3.04 (1H, m), 2.94 (2H, m), 2.16 (1H, m), 1.97 (1H, m). ^{13}C NMR (150 MHz, CDCl_3) δ 203.0, 156.4, 153.4, 146.1, 141.4, 136.6, 135.5, 132.5, 125.7, 110.1, 104.0, 61.3, 61.0, 56.2, 56.0, 47.9, 39.9, 34.6, 23.4. HRMS: Obsvd 409.1624 [$\text{M} + \text{Na}^+$], Calcd for $\text{C}_{22}\text{H}_{26}\text{O}_6\text{Na}$: 409.1622. HPLC: 18.77 min.

2-Isopropoxy-3-methoxybenzaldehyde (58): To a solution of 2-hydroxy-3-methoxybenzaldehyde **38** (5.00 g, 32.9 mmol) in DMF (100 mL) were added K_2CO_3 (14.97 g, 98.58 mmol) and 2-iodopropane (6.54 mL, 65.7 mmol), and the reaction mixture was stirred at 50 °C for 20 h. The DMF was removed under reduced pressure, and the resulting material was washed with water (100 mL) to remove the excess salt and extracted with EtOAc (3×100 mL). The combined organic phase was dried over sodium sulfate and concentrated to afford protected aldehyde **58** (6.12 g, 31.6 mmol, 96%) as a colorless oil without further purification. ^1H NMR (600 MHz, CDCl_3) δ 10.44 (1H, s), 7.41 (1H, d, $J=7.8$ Hz), 7.10 (2H, m), 4.62 (1H, m), 3.86 (3H, s), 1.31 (6H, d, $J=6$ Hz). ^{13}C NMR (150 MHz, CDCl_3) δ 191.0, 153.4, 150.7, 131.0, 123.7, 119.0, 118.0, 76.3, 56.1, 22.4.

Ethyl 5-(2-isopropoxy-3-methoxyphenyl)-3-oxopent-4-enoate (59): To dissolved ethyl 3-oxo-4-(triphenylphosphoranylidene) butanoate (0.85 g, 2.2 mmol) in THF (50 mL) were added potassium *tert*-butoxide (0.50 g, 4.4 mmol) and aldehyde **58** (0.35 g, 1.8 mmol), and the resultant reaction mixture was stirred at room temperature for 12 h. The THF was

removed under reduced pressure, and the resulting material was neutralized with 2 M HCl (10 mL) and extracted with EtOAc (3 × 20 mL). The combined organic layers were evaporated under reduced pressure, and the crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90% B (1 CV), 10% A / 90% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford unsaturated ester **59** (0.40 g, 1.3 mmol, 72%) as a yellow oil. NMR characterization was conducted after the next step.

Ethyl 5-(2-isopropoxy-3-methoxyphenyl)-3-oxopentanoate (60): To dissolved unsaturated ester **59** (0.39 g, 1.3 mmol) in CH₃OH (20 mL) was added 10% palladium on carbon (0.2 g) and balloons with hydrogen gas. The reaction mixture was stirred at room temperature for 12 h, followed by filtration through Celite®, and the Celite® was washed with EtOAc (3 × 20 mL). The combined organic phase (CH₃OH and EtOAc) was evaporated under reduced pressure to afford saturated ester **60** (0.23 g, 0.73 mmol, 57%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.93 (1H, t, *J* = 7.8 Hz), 6.75 (2H, m), 4.51 (1H, sept, *J* = 6 Hz), 4.17 (2H, q, *J* = 7.2 Hz), 3.81 (3H, s), 3.41 (2H, s), 2.92 (2H, t, *J* = 7.8 Hz), 2.83 (2H, t, *J* = 8.4 Hz), 1.25 (6H, d, *J* = 6 Hz), 1.25 (3H, t, 7.2 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 202.5, 167.2, 152.9, 144.9, 135.0, 123.5, 121.9, 110.7, 74.6, 61.4, 55.7, 49.4, 43.6, 25.0, 22.7, 14.2.

Ethyl 3-hydroxy-5-(2-isopropoxy-3-methoxyphenyl)pentanoate (61): To dissolved ketone **60** (2.32 g, 7.52 mmol) in CH₃OH (30 mL) was added NaBH₄ (96 mg, 2.5 mmol) at 0 °C. The reaction was stirred for 1 h and then returned to room temperature for an additional 1 h. The CH₃OH was removed under reduced pressure, and the residue was washed with water and extracted with EtOAc (3 × 30 mL). The combined organic phase was evaporated under reduced pressure, and the crude reaction product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90% B (1 CV), 10% A / 90% B → 40% A / 60% B (10 CV), 40% A / 60% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford alcohol **61** (1.80 g, 5.80 mmol, 77%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.96 (1H, t, *J* = 8.4 Hz), 6.77 (1H, d, *J* = 6.6 Hz), 6.75 (1H, d, *J* = 7.8 Hz), 4.52 (1H, sept, *J* = 6.6 Hz), 4.13 (2H, q, *J* = 7.2 Hz), 3.91 (1H, m), 3.82 (3H, s), 2.82 (1H, m), 2.74 (1H, m), 2.45 (1H, m), 2.44 (1H, m), 1.78 (1H, m), 1.71 (1H, m), 1.28 (3H, t, *J* = 6.6 Hz), 1.26 (3H, t, *J* = 6.6 Hz), 1.25 (3H, t, *J* = 7.2 Hz). ¹³C NMR (150 MHz, CDCl₃) δ 172.8, 152.9, 144.8, 136.0, 123.7, 122.1, 110.3, 74.9, 67.1, 60.7, 55.7, 41.6, 37.5, 41.6, 37.5, 26.3, 22.9, 22.6, 14.3.

Ethyl 3-((*tert*-butyldiphenylsilyl)oxy)-5-(2-isopropoxy-3-methoxyphenyl)pentanoate (62): To dissolved alcohol **61** (0.77 g, 2.5 mmol) in DMF (10 mL) were added *tert*-butyl(chloro)diphenylsilane (TBDPSCl) (0.96 mL, 3.7 mmol) and imidazole (0.280 g, 3.98 mmol) at room temperature, and the solution was stirred for 12 h at room temperature. The DMF was removed under reduced pressure, and the resulting material was washed with brine (50 mL) and extracted with diethyl ether (3 × 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90% B (1 CV), 10% A / 90% B → 40% A /

60%B (10 CV), 40%A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford protected alcohol **62** (0.960 g, 1.75 mmol, 71%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.70 (4H, m), 7.39 (6H, m), 6.87 (1H, t, *J* = 7.8 Hz), 6.70 (1H, d, *J* = 8.4 Hz), 6.52 (1H, d, *J* = 7.8 Hz), 4.41 (1H, m), 4.29 (1H, m), 4.00 (2H, m), 3.80 (3H, s), 2.55 (4H, m), 1.76 (2H, m), 1.19 (6H, m), 1.18 (3H, t, *J* = 7.2 Hz), 1.08 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 171.6, 152.9, 144.9, 136.5, 136.1, 135.3, 135.0, 134.3, 129.8, 129.7, 127.9, 127.6, 123.3, 121.6, 110.1, 74.4, 70.6, 60.4, 55.8, 42.1, 37.8, 26.7, 25.6, 22.7, 19.5, 19.2, 14.2.

3-((Tert-butyl)diphenylsilyloxy)-5-(2-isopropoxy-3-methoxyphenyl)pentanoic acid

(63): To dissolved protected alcohol **62** (7.80 g, 14.2 mmol) in CH₃OH/ THF (60 mL/ 30 mL) was added 2.5 M NaOH (20 mL) at 0 °C, and the solution was stirred for 1 h and then at room temperature for 12 h. The organic solvents (CH₃OH and THF) were removed under reduced pressure, and water (30 mL) was added to the resulting suspension, followed by extraction with diethyl ether (3 × 50 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90%B (1 CV), 10% A / 90%B → 60%A / 40%B (15 CV), 60% A / 40%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **63** (3.49 g, 6.70 mmol, 46%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 7.67 (4H, m), 7.39 (6H, m), 6.86 (1H, t, *J* = 7.8 Hz), 6.70 (1H, d, *J* = 8.4 Hz), 6.50 (1H, d, *J* = 7.2 Hz), 4.41 (1H, m), 4.20 (1H, m), 3.79 (3H, s), 2.56 (4H, m), 1.82 (2H, m), 1.19 (6H, t, *J* = 6 Hz), 1.06 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 175.5, 152.9, 144.8, 136.0, 133.7, 133.6, 129.9, 127.8, 123.4, 121.6, 110.2, 74.5, 70.5, 55.8, 41.2, 37.5, 27.1, 25.7, 22.7, 19.4.

7-((Tert-butyl)diphenylsilyloxy)-1-isopropoxy-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (**64**):

To dissolved carboxylic acid **63** (3.49 g, 6.70 mmol) in dichloromethane (30 mL) were added oxalyl chloride (2.77 mL, 31.8 mmol) and DMF (0.1 mL) as catalyst at room temperature, and the solution was stirred for 2 h at room temperature. The solvent and unreacted oxalyl chloride were removed under reduced pressure. The yellow acyl chloride was then dissolved in dichloromethane (40 mL) and cooled to -10 °C. To this solution, 1 M SnCl₄ in a dichloromethane solution (7.4 mL, 7.4 mmol) was added, and the reaction mixture was stirred for 30 min at -10 °C. The reaction was quenched by the addition of water, followed by extraction with dichloromethane (3 × 40 mL). The combined organic phase was dried over sodium sulfate and concentrated. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90%B (1 CV), 10% A / 90%B → 40% A / 60%B (10 CV), 40% A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford ketone **64** (2.19 g, 4.50 mmol, 67%) as a pale-yellow gel. ¹H NMR (600 MHz, CDCl₃) δ 7.66 (4H, m), 7.54 (1H, d, *J* = 9.6 Hz), 7.38 (6H, m), 6.79 (1H, d, *J* = 9 Hz), 4.36 (1H, m), 4.28 (1H, m), 3.86 (3H, s), 3.14 (1H, m), 3.03 (2H, m), 2.88 (1H, m), 1.94 (2H, m), 1.27 (6H, m), 1.03 (9H, s). ¹³C NMR (150 MHz, CDCl₃) δ 199.7, 156.0, 144.1, 138.5, 136.1, 136.0, 135.9, 134.2, 133.9, 133.1, 129.9, 129.8, 127.9, 127.8, 127.7, 125.3, 109.5, 75.2, 68.4, 55.9, 50.4, 36.1, 27.0, 22.7, 22.6, 22.0, 19.3.

7-Hydroxy-1-isopropoxy-2-methoxy-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (65): Ketone **64** (2.19 g, 4.50 mmol) was dissolved in THF (20 mL), TBAF (9.00 mL, 9.00 mmol) was added at 0 °C, and the reaction mixture was stirred for 30 min, followed by additional stirring at room temperature for 6 h. Brine (30 mL) was added, and the resultant solution was extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90%B (1 CV), 10% A / 90%B → 40% A / 60%B (10 CV), 40% A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford alcohol **65** (0.41 g, 1.7 mmol, 37%) as a yellow oil. ¹H NMR (600 MHz, CDCl₃) δ 7.57 (1H, d, *J* = 8.4 Hz), 6.8 (1H, d, *J* = 8.4 Hz), 4.41 (1H, m), 4.31 (1H, m), 3.88 (3H, s), 3.11 (2H, m), 3.06 (1H, m), 2.99 (1H, m), 2.15 (1H, m), 1.87 (1H, m), 1.29 (6H, m). ¹³C NMR (150 MHz, CDCl₃) δ 199.6, 156.3, 144.2, 138.4, 132.6, 125.4, 109.7, 75.3, 67.3, 55.9, 50.3, 35.8, 22.7, 22.6, 22.1.

1-Isopropoxy-2-methoxy-7-((trimethylsilyl)oxy)-6,7,8,9-tetrahydro-5H-benzo[7]annulen-5-one (66): To a solution of alcohol **65** (0.35 g, 1.33 mmol) in DMF (20 mL) were added imidazole (0.27 g, 6.4 mmol) and TMSCl (4.26 mmol) at room temperature, and the reaction mixture was stirred for 12 h. The solvent was removed under reduced pressure, and brine (20 mL) was added, followed by extraction with EtOAc (3 × 30 mL). The organic extract was dried over sodium sulfate, filtered, and concentrated under reduced pressure, and the residue was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90%B (1 CV), 10% A / 90%B → 40% A / 60%B (10 CV), 40% A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford TMS protected ketone **66** (0.19 g, 0.56 mmol, 43%) as a yellow oil. ¹H NMR (500 MHz, CDCl₃) δ 7.54 (1H, d, *J* = 10.2 Hz), 6.79 (1H, d, *J* = 10.8 Hz), 4.39 (1H, m), 4.19 (1H, m), 3.86 (3H, s), 3.22 (1H, m), 2.99 (2H, m), 2.90 (1H, m), 1.91 (2H, m), 1.27 (6H, d, *J* = 9 Hz), 0.11 (9H, s). ¹³C NMR (125 MHz, CDCl₃) δ 199.7, 156.1, 144.0, 138.6, 132.6, 125.3, 109.5, 75.1, 67.3, 55.8, 51.0, 36.4, 22.6, 22.5, 21.7.

4-Isopropoxy-3-methoxy-9-(3,4,5-trimethoxyphenyl)-5H-benzo[7]annulene (67): To an oven-dried flask, THF (20 mL) and 3,4,5-trimethoxyphenyl bromide (0.21 g, 0.85 mmol) were added, and the solution was cooled to -78 °C. *n*-BuLi (0.34 mL, 2.5 M, 0.85 mmol) was slowly added to the reaction mixture, which was then stirred at -78 °C for 45 min. Ketone **66** (0.19 g, 0.56 mmol) was then added dropwise to the flask, and the reaction mixture was stirred (-78 °C to 0 °C) over 4 h, and at 0 °C for an additional 1 h. 2 M HCl (20 mL) was added at 0 °C followed by stirring for 10 min and extraction with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A / 90%B (1 CV), 10% A / 90%B → 40% A / 60%B (10 CV), 40% A / 60%B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford diene **67** (60 mg, 0.15 mmol, 27%) as a colorless oil. ¹H NMR (600 MHz, CDCl₃) δ 6.81 (1H, d, *J* = 8.4 Hz), 6.71 (1H, d, *J* = 9 Hz), 6.61 (2H, s), 6.60 (1H, d, *J* = 5.4 Hz), 6.17 (1H, m), 5.87 (1H, m), 4.48 (1H, p, *J* = 6.6 Hz), 3.91 (3H, s), 3.86 (6H, s), 3.85 (3H, s), 3.26 (2H, m, b), 1.38 (6H, d, *J* = 6 Hz). ¹³C NMR

(150 MHz, CDCl₃) δ 153.8, 153.0, 145.6, 142.0, 140.4, 137.5, 134.5, 131.7, 128.5, 126.9, 125.9, 124.8, 109.1, 106.7, 75.0, 61.0, 56.3, 55.8, 26.7, 22.8.

3-Methoxy-9-(3,4,5-trimethoxyphenyl)-5H-benzo[7]annulen-4-ol (68): Isopropyl protected phenol **67** (60 mg, 0.15 mmol) was dissolved in CH₂Cl₂ (10 mL), to which BCl₃ (0.17 mL, 1 M, 0.17 mmol) was added, and the reaction mixture was stirred at 0 °C for 1 h. The solution was washed with water and 2 M HCl and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction was purified by flash chromatography using a pre-packed 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A / 95% B (1 CV), 5% A / 95% B → 60% A / 40% B (10 CV), 60% A / 40% B (2 CV); flow rate: 100 mL/min; monitored at 254 and 280 nm] to afford phenol **68** (40 mg, 0.11 mmol, 75%) as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 6.67 (1H, d, *J* = 8.4 Hz), 6.62 (1H, d, *J* = 9 Hz), 6.60 (2H, s), 6.59 (1H, d, *J* = 5.4 Hz), 6.15 (1H, m), 5.94 (1H, m), 3.89 (6H, s), 3.84 (6H, s), 3.23 (2H, m, b). ¹³C NMR (150 MHz, CDCl₃) δ 153.0, 147.1, 145.5, 140.6, 140.3, 137.5, 132.2, 128.3, 127.0, 126.2, 126.0, 120.1, 107.6, 106.7, 61.1, 56.3, 56.2, 25.7. HRMS: Obsvd 377.1361 [M + Na⁺], Calcd for C₂₁H₂₂O₅Na: 377.1359. HPLC: 19.52 min.

Sodium 3-methoxy-9-(3,4,5-trimethoxyphenyl)-5H-benzo[7]annulen-4-yl phosphate (69): To phenol **68** (95 mg, 0.27 mmol) dissolved in CH₂Cl₂ (25 mL) was added POCl₃ (0.11 mL, 1.1 mmol) and pyridine (0.078 mL, 0.97 mmol), and the reaction mixture was stirred from 0 °C to room temperature for 8 h. The CH₂Cl₂ was removed under reduced pressure, the residue was dissolved in H₂O/ THF (10 mL/ 5 mL) at room temperature, and the solution was stirred for 1 h. A NaOH solution (0.1 M) was added to the reaction mixture to adjust to pH = 10 at 0 °C, and the solution was stirred at 0 °C for 30 min. Water was removed under reduced pressure. The crude product was purified by flash chromatography using a pre-packed 12 g C-18 column [solvent A: acetonitrile; solvent B: water; gradient: 0% A / 100% B (1 CV), 0% A / 100% B → 10% A / 90% B (10 CV), 10% A / 90% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford phosphate salt **69** (65.8 mg, 0.14 mmol, 51%) as a yellow solid. ¹H NMR (500 MHz, D₂O) δ 6.65 (2H, s), 6.60 (2H, m), 6.53 (1H, d, *J* = 6 Hz), 6.10 (1H, m), 5.98 (1H, m), 3.78 (3H, s), 3.72 (3H, s), 3.70 (6H, s), 3.29 (2H, b). ¹³C NMR (125 MHz, D₂O) δ 153.0, 152.1, 144.5, 140.8, 138.4, 136.0, 134.5, 131.1, 129.8, 126.2, 126.1, 124.2, 109.1, 106.7, 60.9, 55.9, 55.7, 26.9. ³¹P NMR (200 MHz, D₂O) δ 0.81. HRMS: Obsvd 479.0841 [M + H⁺], Calcd for C₂₁H₂₂O₈Na₂P⁺: 479.0842. HPLC: 14.22 min

Confirmation that target molecules are not PAINS molecules: Each of the target molecules and several common motifs were screened using the zinc15 docking algorithm (<http://zinc15.docking.org/patterns/home>), which provided confirmation that these benzosuberene-based molecules are not Pan Assay Interference compoundS (PAINS). A molecule previously identified as a PAIN molecule (Capuzzi et al., *J. Chem. Inf. Model.* 2017, 57, 417–427) was screened to provide positive confirmation that the algorithm was functioning accurately.

Biology

Cell lines and sulforhodamine B (SRB) assay—The sulforhodamine B (SRB) assay was used to assess growth inhibition of human cancer cells, as previously described.^{29,82,83} DU-145, SK-OV-3, and NCI-H460 cancer cell lines (obtained from ATCC) were maintained in T75 flasks (Corning) using high glucose DMEM supplemented with 10% fetal bovine serum (Gibco)/ 1% gentamicin sulfate for a maximum of 15 passages. For these experiments, cells were trypsinized, counted, and plated at 7000–8000 cells/well into 96-well plates (Corning) and incubated for 24 h at 37 °C in a humidified incubator in a 5% CO₂ atmosphere. Compounds to be tested were dissolved in DMSO to generate a 10 mg/mL stock solution, and serial dilutions added in media to the plates. Doxorubicin (Sigma-Aldrich) and paclitaxel (Tokyo Chemical) were used as positive controls. After a 48 h treatment, the cells were fixed with trichloroacetic acid (10% final concentration), washed, dried, stained with SRB dye, washed to remove excess dye, and dried. SRB dye was solubilized, and absorbances were measured at wavelength 540 nm and normalized to values at wavelength 630 nm using an automated Biotek plate reader. A growth inhibition of 50% (GI₅₀ or the drug concentration causing 50% reduction in the net protein increase) was calculated from the absorbance data.

84 Colchicine binding assay.⁸⁴—Inhibition of [³H]colchicine binding to tubulin was measured in 0.1 mL reaction mixtures, each containing 1.0 μM tubulin, 5.0 μM [³H]colchicine (Perkin-Elmer), 5% (v/v) dimethyl sulfoxide, compounds at 1.0 or 5.0 μM, as indicated, and components that stabilize the colchicine binding activity of tubulin (1.0 M monosodium glutamate [adjusted to pH 6.6 with HCl in a 2.0 M stock solution], 0.5 mg/mL bovine serum albumin, 0.1 M glucose-1-phosphate, 1.0 mM MgCl₂, and 1.0 mM GTP). Incubation was for 10 min at 37 °C, when in control reaction mixtures colchicine binding is 40–60% complete. Reactions were stopped with 2.0 mL of ice-cold water, with the reaction mixtures being placed on ice. Each diluted sample was poured onto a stack of two DEAE-cellulose filters (GE Biomedical), followed by 3 successive 2 mL aliquots of ice-cold water. A reduced vacuum was used to remove excess water from the filters, which were washed three times with 2 mL water and placed into vials containing 5 mL of Biosafe II scintillation cocktail. Samples were counted 18 h later in a Beckman scintillation counter. Samples with inhibitors were compared to samples with no inhibitor, and percent inhibition was determined, correcting all values for the amount of radiolabel bound to the filters in the absence of tubulin.

85 Inhibition of tubulin polymerization.⁸⁵—Tubulin polymerization was evaluated in 0.25 mL reaction mixtures (final volume) containing 1 mg/mL (10 μM) purified bovine brain tubulin, 0.8 M monosodium glutamate (pH 6.6), 4% (v/v) dimethyl sulfoxide, 0.4 mM GTP, and different compound concentrations. All components except GTP were preincubated for 15 min at 30 °C in 0.24 mL. The assay mixtures were cooled to 0 °C, and 10 μL of 0.01 M GTP was added to each sample. Reaction mixtures were transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was increased to 30 °C, over about 30 s, and polymerization was followed turbidimetrically at 350 nm for 20 min. The IC₅₀ was defined as the compound concentration inhibiting extent of polymerization by 50% after 20 min.

In vivo vascular disruption—The human prostate cancer cell line (PC3) was modified by knock down of a tumor suppressor protein PC3-DAB2IP and further modified by transfection with a firefly luciferase reporter, as described in detail previously.⁷⁷ Male Copenhagen rats (originally from Charles River and bred at UT Southwestern) were inoculated subcutaneously with 5×10^5 PC3-DAB2IP-luc cells mixed with 30% Matrigel® in the right thigh. These rats were approximately 6 weeks old and weighed between 100–120 g on date of implantation. Tumors were allowed to grow to a diameter of at least 1 cm before treatment commenced. Three rats were treated with VDA prodrug **69** (25 mg/mL in saline) administered IP. Rat 1 received 10 mg/kg followed by 40 mg/kg 24 h later and CA4P (30 mg/kg at 20 mg/mL in saline) was administered IP as a control 4 days later. Rat 2 received a single dose of 40 mg/kg of VDA prodrug **69**, and rat 3 received 80 mg/kg of **69**. Bioluminescence imaging (BLI) was performed before treatment (baseline), and at 4 and 24 h after treatment using an IVIS Spectrum® (Perkin-Elmer). Briefly, anesthetized rats (breathing oxygen with 3% isoflurane, Henry Schein Inc.) were injected subcutaneously in the foreback neck region with 120 mg/kg *D*-luciferin sodium salt (Gold Biotechnology, St. Louis, MO) and imaged over a time course of about 30 min. Fresh luciferin was administered at each time point. The resulting light intensity time curves were analyzed using Living Image® Software and light emission measured for a region of interest encompassing the tumor. All animal procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health as well as the Institutional Animal Care and Use Committee approved protocols (APN 2017–102168) at the University of Texas Southwestern Medical Center.

Supplementary Material

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Abbreviations Used:

VDA	(vascular disrupting agent)
AiAs	(angiogenesis inhibiting agents)
TBDPSCI	(<i>tert</i> -butyldiphenylsilyl chloride)
GI₅₀	(half maximal growth inhibition)

BLI	(bioluminescence imaging)
ND	(not determined)
NR	(not reported)
DEAE	(diethylaminoethyl)

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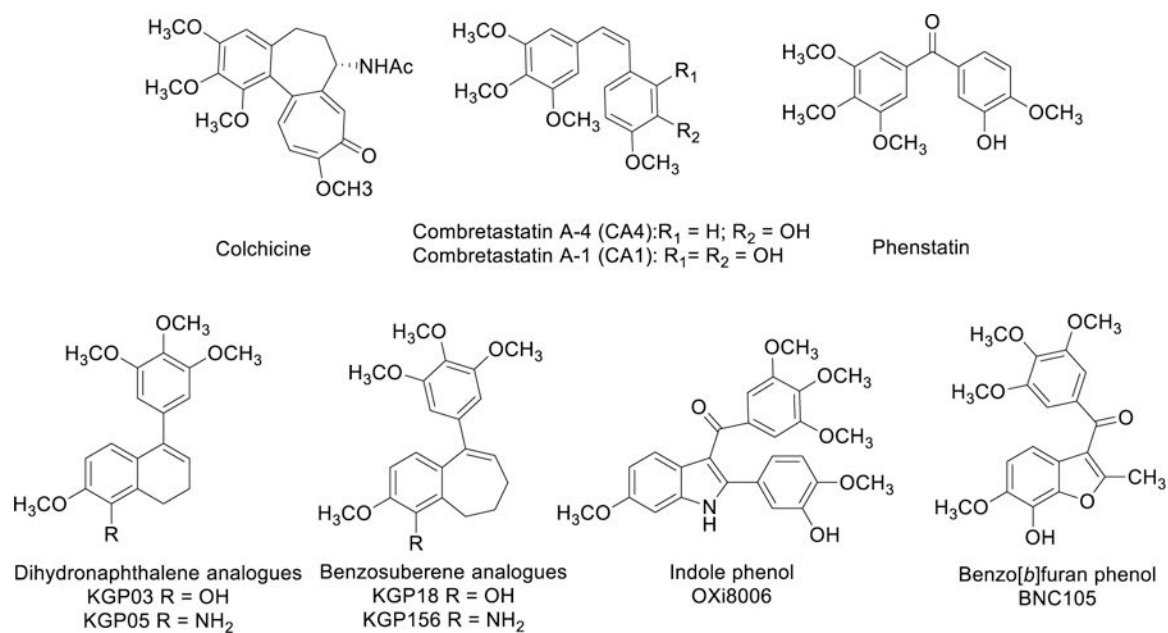
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**Figure 1.**

Representative small-molecule inhibitors of tubulin polymerization: **colchicine**,²¹ combretastatins (**CA4**, **CA1**),^{15,23} **phenstatin**, dihydronaphthalene analogues (**KGP03**, **KGP05**),^{35,36} benzosuberene analogues (**KGP18**, **KGP156**),^{8,25,37,38} indole analogue (**OXi8006**),^{39,50} and benzo[*b*]furan analogue (**BNC105**).^{51–53}

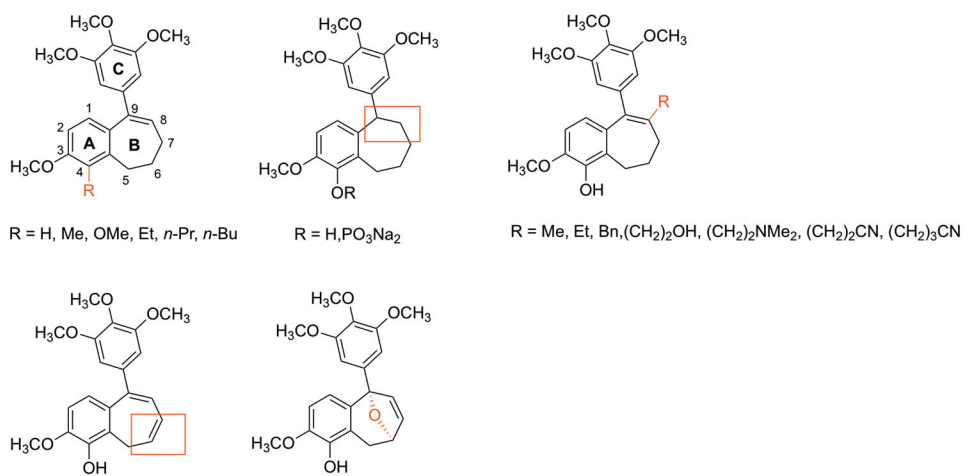
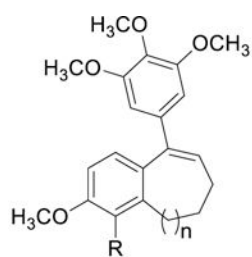
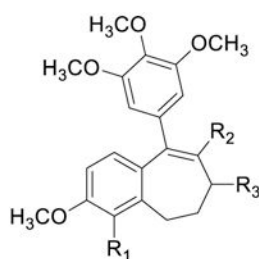


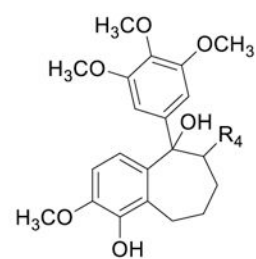
Figure 2. Selected **KGP18** derivatives as inhibitors of tubulin polymerization with modifications at the C-4 position of the A ring and the C-6, 7, 8 positions of the B ring.^{25,57}



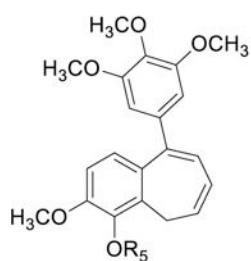
- 8** R = O(CH₂)₂O(CH₂)₂OCH₃ n = 1
11 R = O(CH₂)₂OH n = 1
27 R = COOEt n = 1
28 R = CH₂OH n = 1
18 R = CN n = 1
20 R = CHO n = 1
19 R = CN n = 0



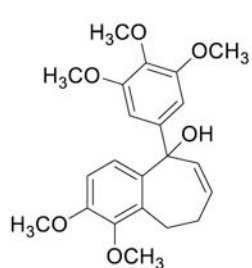
- 14** R₁ = CH₃, R₂ = Br, R₃ = H
13 R₁ = OH, R₂ = Br, R₃ = H
37 R₁ = OCH₃, R₂ = H, R₃ = OH
49 R₁ = OH, R₂ = H, R₃ = NHAc



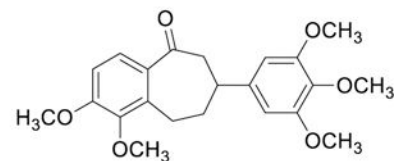
- 3** R₄ = H
15 R₄ = OH
6 R₄ = (=O)



- 68** R₅ = H
69 R₅ = PO(ONa)₂



56



57

Figure 3.

Compilation of synthesized benzosuberene, benzosuberane, benzosuberone, and dihydronaphthalene analogues

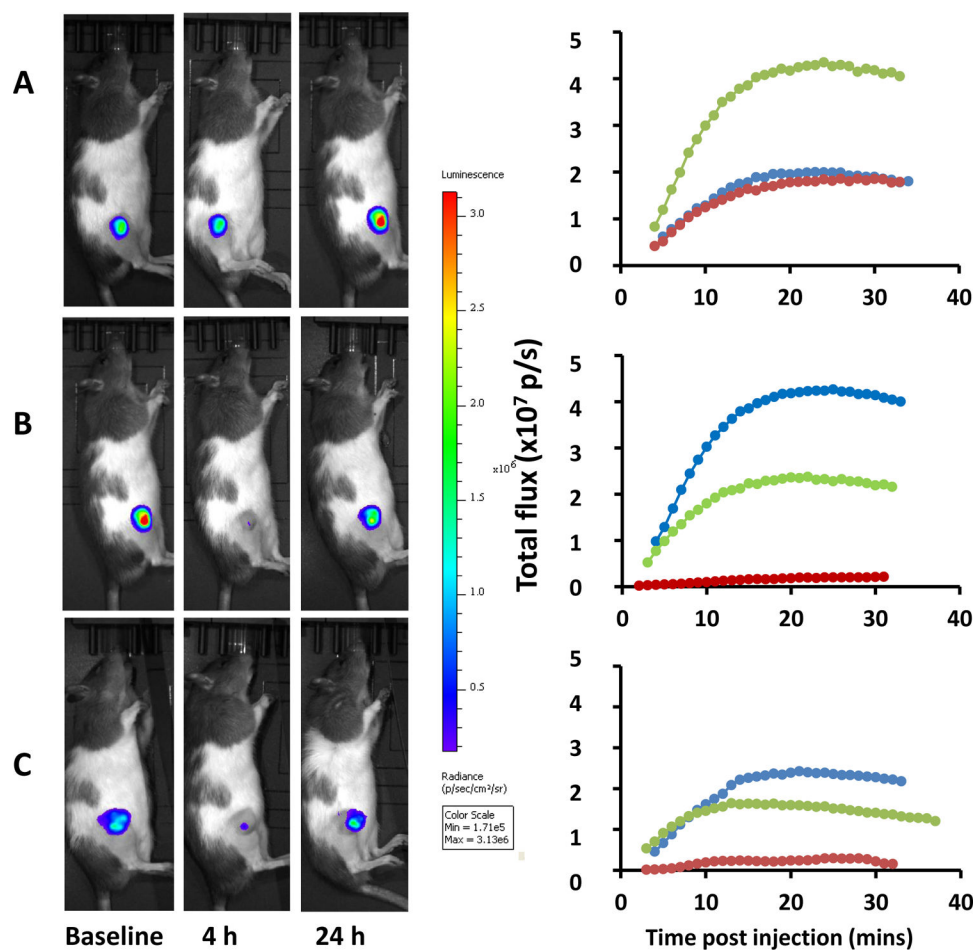


Figure 4.

BLI assessment of vascular response to VDA. Left) Heat maps overlaid on photographs of male Copenhagen rat with subcutaneous PC3-DAB2IP-luc human prostate tumor xenograft showing light emission about 20 min after administration of *D*-luciferin (120 mg/kg) at various times with respect to VDA administration IP. Right) Corresponding dynamic light emission curves acquired over about 30 min following luciferin administration. Blue shows baseline; red about 4 h after VDA and green at 24 h. A) 10 mg/kg compound **69** indicating no vascular perturbation, but increased signal at 24 h consistent with rapid tumor growth. B) 6 h later 40 mg/kg compound **69** was administered to the same rat generating about 95% reduced signal at 4 h, consistent with substantial vascular shutdown and showing substantial recovery by 24 h. C) Four days later 30 mg/kg CA4P was administered to this rat eliciting BLI response similar to the BLI response shown in B.

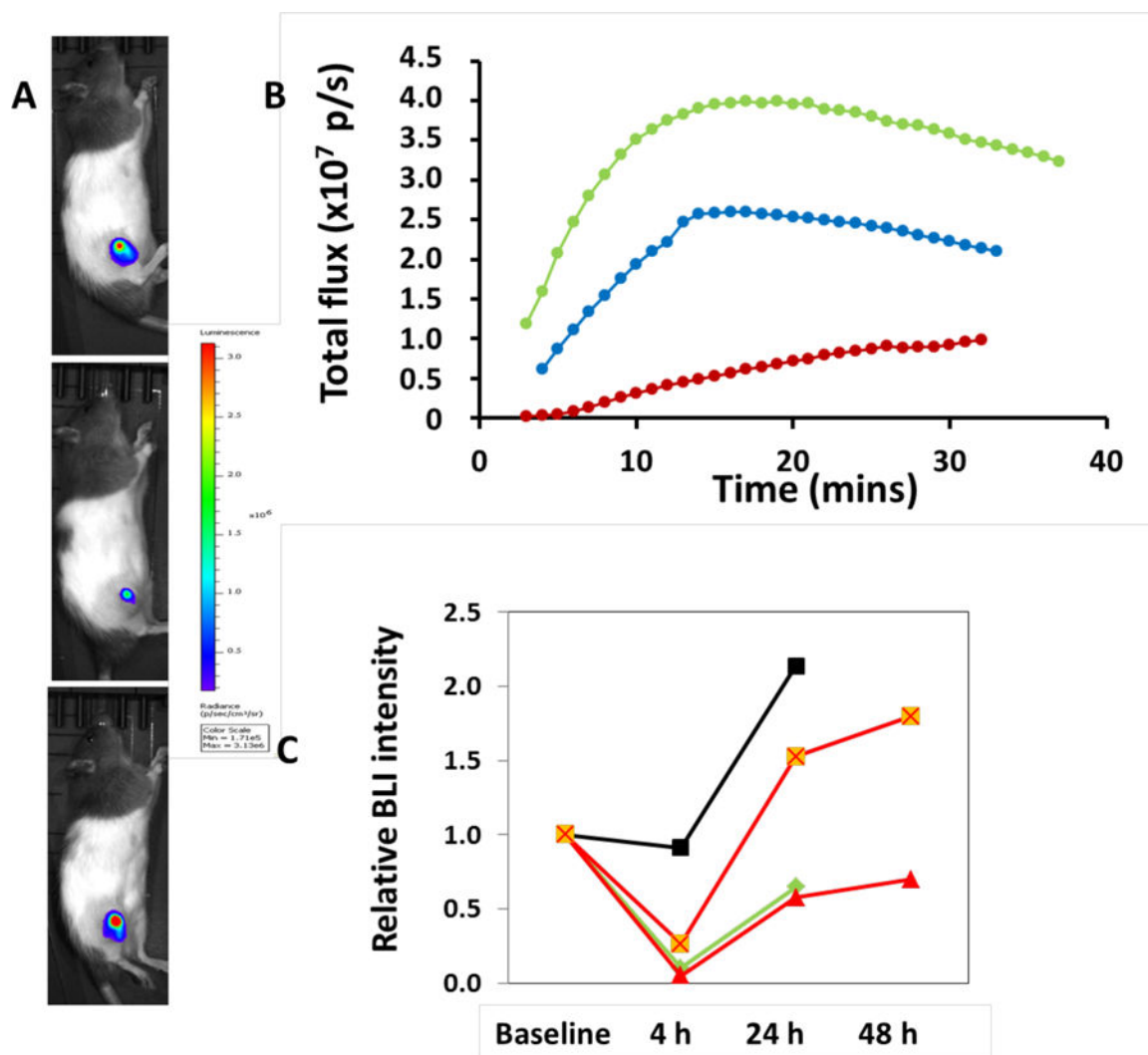
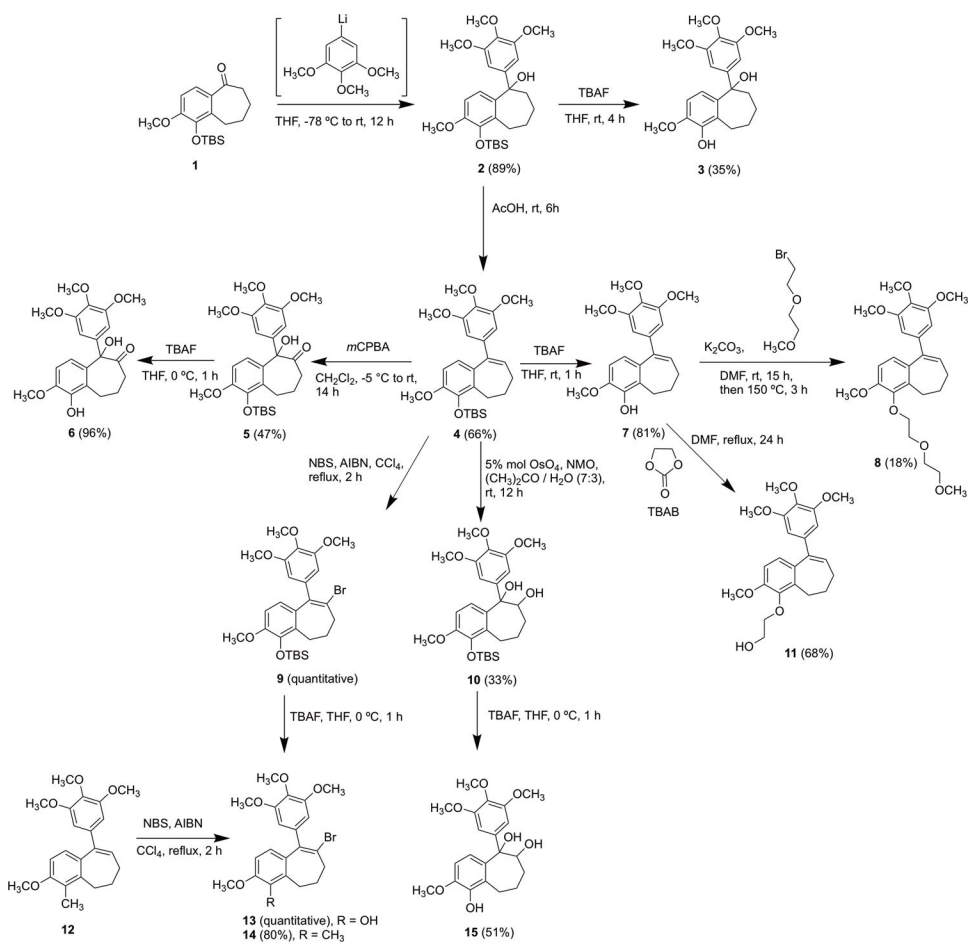
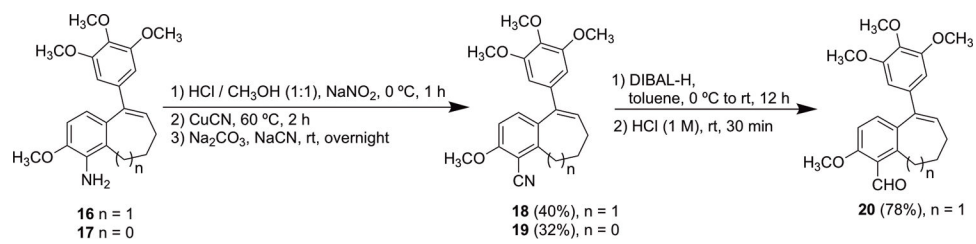
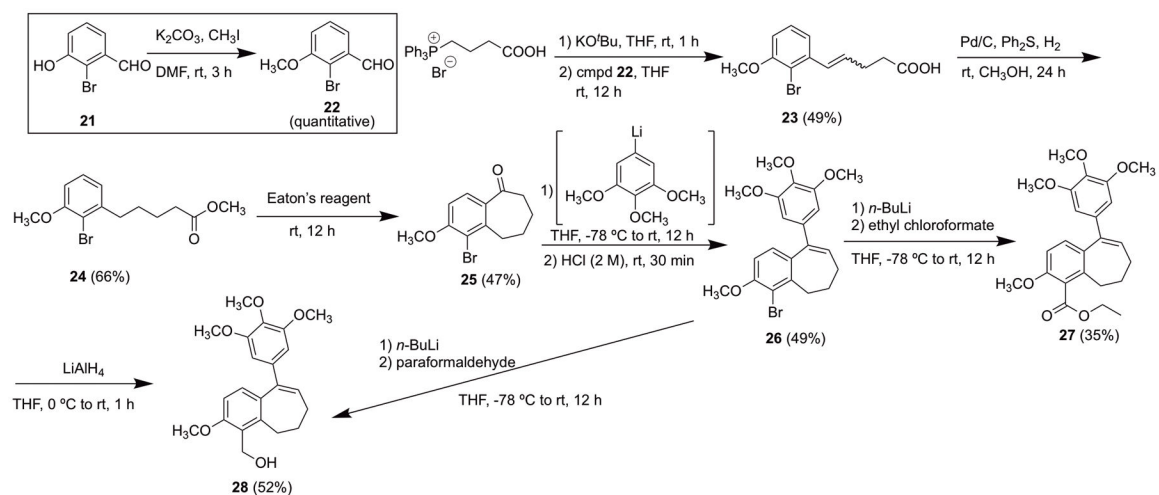


Figure 5. Relative light emission following administration of VDAs. A) Relative signal intensity is shown about 20 min after administration of *D*-luciferin subcutaneously in the foreback neck region of a rat with a subcutaneous PC3-DAB2IP-*luc* prostate tumor xenograft in the thigh. Top) baseline (no prior drug), center) 4 h after 40 mg/kg **69**, and bottom) 24 h after **69**. B) Corresponding light emission dynamic curve at baseline (blue), 4 h after **69** (red) and 24 h after **69** (green). C) Normalized BLI signal at various times for the rat in Fig 4 receiving **69** sequentially at 10 mg/kg (black), 40 mg/kg (red) and 30 mg/kg CA4P (green), together with the treatment naive rat in Fig 5 A, B receiving 40 mg/kg **69** (orange).

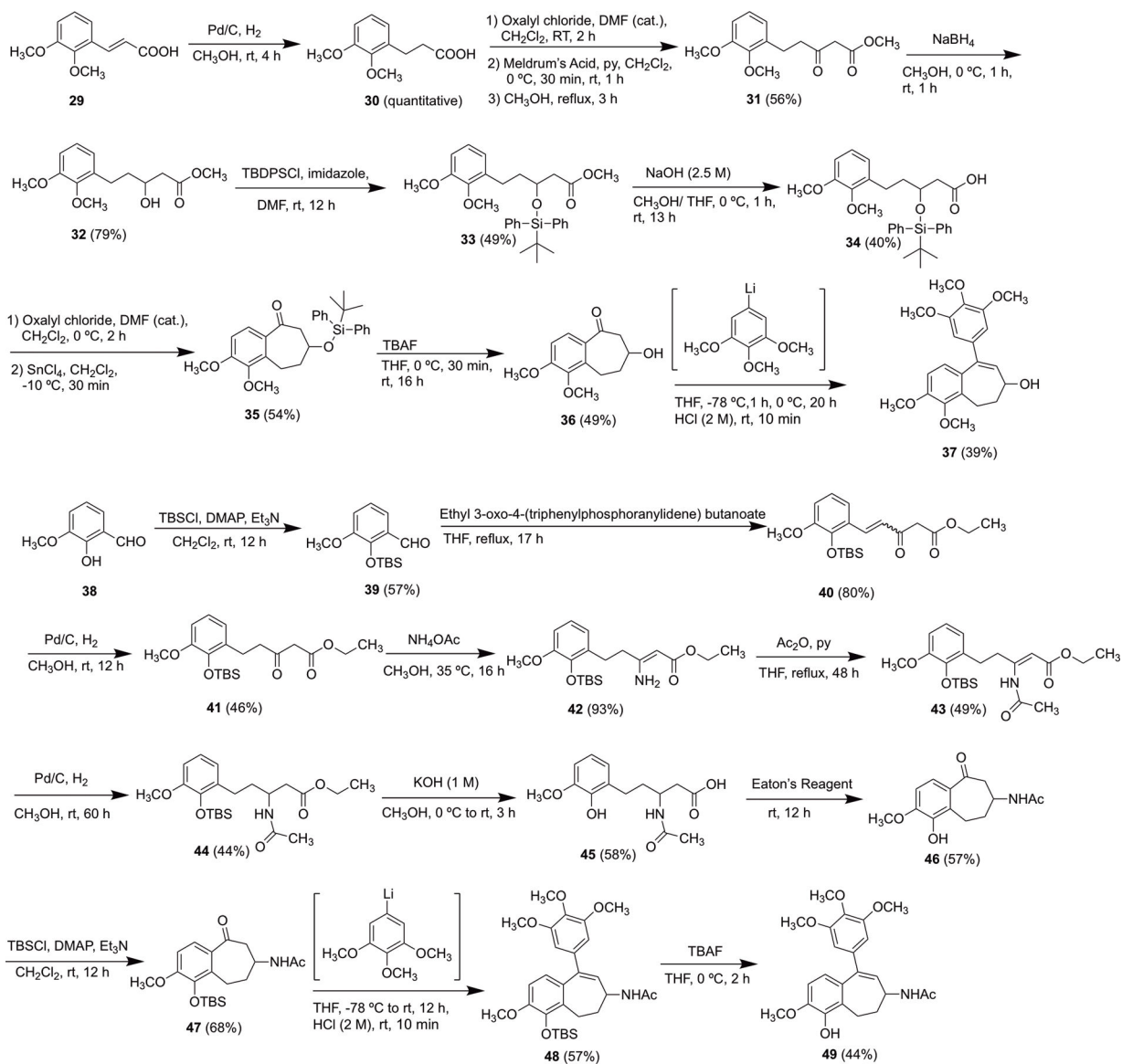


Scheme 1.
Synthesis of benzosuberene and benzosuberane intermediates and analogues.

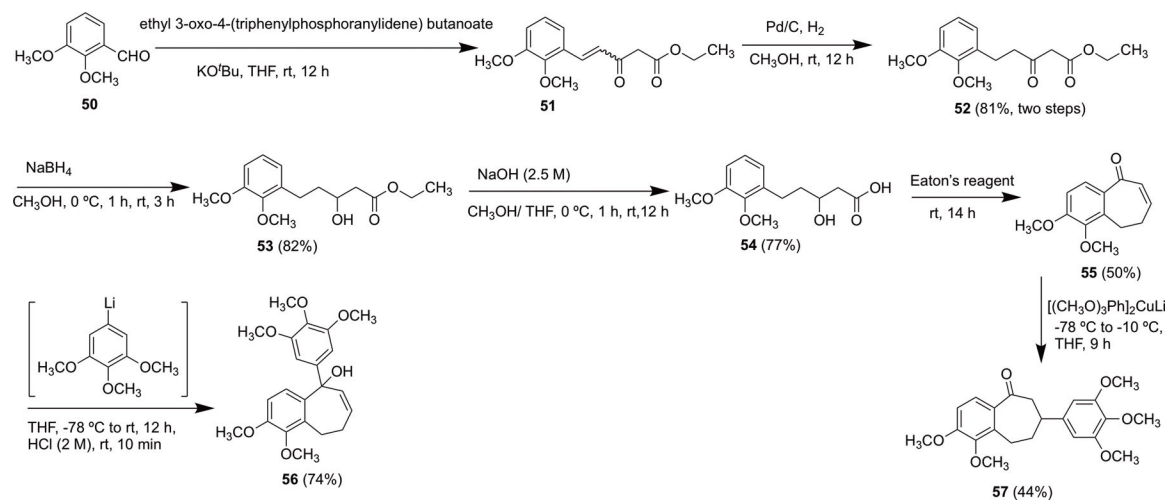
**Scheme 2.**Synthesis of nitrile- and aldehyde- substituted R position analogues **18–20**.

**Scheme 3.**

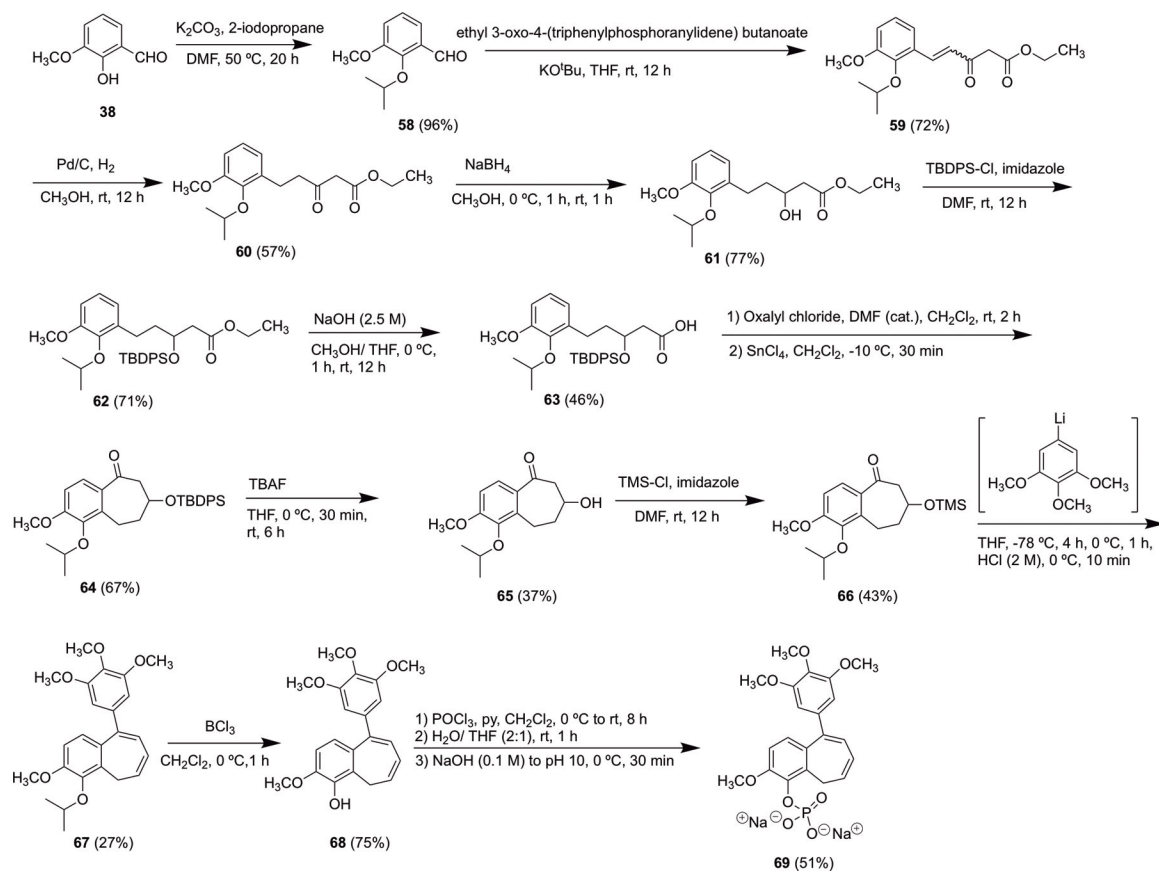
Synthesis of ester analogue **27** and primary alcohol analogue **28**.



Scheme 4.
Synthesis of allylic alcohol analogue **37** and allylic *N*-acetamide analogue **49**.

**Scheme 5.**

Synthesis of α -unsaturated tertiary alcohol analogue **56** and pendant ring shifted analogue **57**.



Scheme 6.
Synthesis of benzosuberdiene **68** and phosphate salt **69**.

Table 1.

Inhibition of tubulin polymerization, percent inhibition of colchicine binding, and cytotoxicity of the benzosuberene and dihydronaphthalene analogues.

Compound	Inhibition of tubulin polymerization IC ₅₀ (μM) ± SD	% Inhibition of colchicine binding ± SD	GI ₅₀ (μM) SRB assay ^a		
			SK-OV-3	NCI-H460	DU-145
CA4	1.0 ^b	84 ± 3 (1 μM), 98 ± 0.007 (5 μM)	0.00455	0.00223 ^c	0.00327 ^c
CA4P	>40 ^b	ND	0.00119	0.00194 ^c	0.00323 ^c
KGP18	0.85 ± 0.02 ^d	73 ± 5 (1 μM), 95 ± 0.5 (5 μM)	0.0000543 ^e	0.0000418 ^e	0.0000249 ^e
KGP03	0.5 ^f	90 ± 2 (1 μM), 98 ± 0.3 (5 μM)	0.0029 ^f	0.0032 ^f	0.00040 ^f
Doxorubicin	ND	ND	0.0789	0.123	0.134
Paclitaxel	NR	NR	0.00134	0.00176	0.00147
3	>20	ND	0.394	0.173	0.0330
6	1.2 ± 0.1	72 ± 2 (5 μM)	0.0314	0.0476	0.141
8	>20	ND	6.52	1.90	5.66
11	>20	ND	3.22	0.855	3.76
13	0.39 ± 0.06	88 ± 1 (5 μM)	0.0221	0.0353	0.0362
14	4.9 ± 0.1	35 ± 5 (1 μM)	0.312	0.449	0.423
15	>20	0 (5 μM)	3.03	3.86	4.04
18	1.1 ± 0.1	62 ± 0.7 (5 μM)	0.0648	0.434	0.860
19	0.37 ± 0.08	73 ± 0.6 (5 μM)	0.0384	0.0605	0.0252
20	3.2 ± 0.08	61 ± 3 (5 μM)	0.0572	0.0847	0.0402
27	1.0 ± 0.07	47 ± 1 (5 μM)	0.299	0.353	0.631
28	0.63 ± 0.03	76 ± 2 (5 μM)	0.0403	0.0628	ND
37	3.8 ± 0.5	28 ± 4 (5 μM)	0.263	0.432	0.439
49	>20	0.4 ± 0.6 (5 μM)	ND	0.334	1.20 ^g
56	>20	0 (5 μM)	27.2	70.5	26.0
57	>20	0 (5 μM)	5.77	2.00	4.45
68	0.48 ± 0.08	68 ± 1 (0.5 μM), 95 ± 0.8 (5 μM)	0.00690	0.0581	0.0976
69	16 ± 0.7	45 ± 2 (5 μM)	0.0153	0.0291	0.0239

^a Average of n = 3 independent determinations (unless otherwise noted)

^b Data from ref. 24²⁴ and ref. 74⁷⁴

^c For additional data, see ref. 24²⁴

^d For additional data, see ref. 37³⁷

^e For additional data, see ref. 8⁸

^f For additional data, see ref. 36³⁶ and ref. 75⁷⁵

^g Average of n = 2 independent determinations (of duplicates)

ND = Not Determined

NR = Not Relevant (paclitaxel enhances microtubule assembly)

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