Bioorganic & Medicinal Chemistry 16 (2008) 8161–8171

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and biological evaluation of dihydronaphthalene and benzosuberene analogs of the combretastatins as inhibitors of tubulin polymerization in cancer chemotherapy

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ARTICLE INFO

Article history:
Received 11 June 2008
Revised 17 July 2008
Accepted 17 July 2008
Available online 24 July 2008

Keywords:
Benzosuberenes
Dihydronaphthalenes
Combretastatins
Tubulin
Vascular disrupting agents
Cancer Chemotherapy

ABSTRACT

A novel series of dihydronaphthalene and benzosuberene analogs bearing structural similarity to the combretastatins in terms of 1,2-diarylethene, trimethoxyphenyl, and biaryl functionality has been synthesized. The compounds have been evaluated in regard to their ability to inhibit tubulin assembly and for their cytotoxicity against selected human cancer cell lines. From this series of compounds, benzosuberene analogs **2** and **4** inhibited tubulin assembly at concentrations comparable to that of combretastatin A-4 (CA4) and combretastatin A-1 (CA1). Furthermore, analog **4** demonstrated remarkable cytotoxicity against the three human cancer cell lines evaluated (for example GI_{50} = 0.0000032 μM against DU-145 prostate carcinoma).

1. Introduction

Solid tumors larger than 1–3 mm in diameter depend extensively on their own vasculature for the supply of nutrients and oxygen.[1,2] Since the discovery of the concept that blocking blood supply to solid tumors led to tumor regression in mice by Denekamp,[3,4] compounds that target well-established tumor vasculature have gained considerable significance over the past two decades. Tumor blood vessels, unlike the normal arteries and veins, lack the hierarchical branching patterns and are often chaotic, dilated, and tortuous, and have poorly developed endothelial monolayers.[5–7] In addition, the increased rate of proliferation renders the tumor vascular endothelial cells as a specific target for anti-cancer agents.[6] Molecules that specifically interact with the tumor vasculature are known as vascular targeting agents (VTAs). VTAs are further sub-divided into two classes:[8–10] (a) anti-angiogenic drugs that prevent neovascularization in tumors and (b) vascular disrupting agents (VDAs) that target already established tumor vessels.[10] VDAs that are currently in clinical development are either ligand-directed biological agents or small molecule compounds that include flavonoids such as DMXAA[11] and the microtubule depolymerizing agents represented by combretastatin A-4 phosphate (CA4P also known as Zybrestat™), and combretastatin A-1 phosphate (CA1P also known as Oxi4503).[12–14] In addition, AVE8062,[15,16] a serinamide prodrug is currently in phase I/II human clinical development as a VDA, while ZD6126,[12,17,18] a colchicine analog, has recently been halted in human clinical trials (Fig. 1). The combretastatins (CSAs), discovered by Pettit and coworkers in the late 1970s, were originally isolated from the African Cape Bushwillow tree *Combretum caffrum* Kuntze.[10,19,20]

VDAs, such as CA4P, undergo de-phosphorylation and subsequently function by binding to an internal system of cross-linked proteins called the tubulin cytoskeleton (microtubules; polymers of α,β-tubulin heterodimers) in the endothelial cells lining the inner layer of tumor vasculature.[21,22] This results in microtubule depolymerization, which is accompanied by cell signaling pathways, followed by cytoskeletal rearrangement and ultimately irreversible microvessel damage selectively in the tumor microenvironment.[5,22] Colchicine was the first drug reported to function through a tubulin binding mechanism in the late 1930s.[23,24] However, colchicine was eventually withdrawn from clinical use in cancer treatment due to significant in vivo toxicity.[24,25] Nonetheless, colchicine has been used in the treatment of gout at very low doses since the late 1950s.[26]

Numerous structure–activity relationship (SAR) studies have focused on compounds that bind to tubulin at the colchicine site. Collectively, these studies suggest important pharmacophore components that include the trimethoxyphenyl unit, *p*-methoxyphenyl unit, presence of the aromatic rings in a *cis*-configuration, and a

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doi:10.1016/j.bmc.2008.07.050

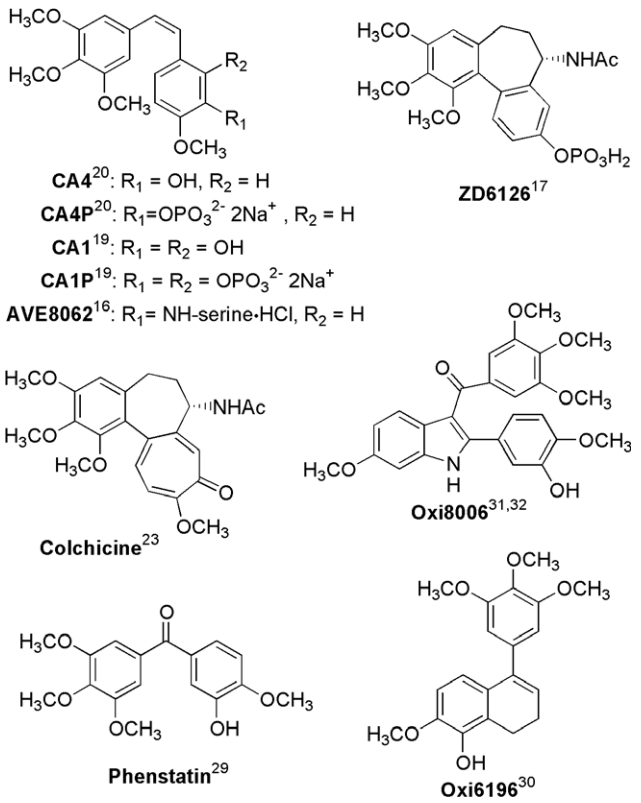


Figure 1. Representative inhibitors of tubulin assembly and vascular disrupting agents (VDAs).

distance of approximately 4–5 Å between the two aryl rings.[10,27,28] A variety of combretastatin analogs have been synthesized in an effort to optimize the distance between the two aryl rings while maintaining them in a *cis* or quasi *cis*-configuration. Representative analogs include the phenstatins synthesized by Pettit and co-workers,[29] and the dihydronaphthalenes (ex. Oxi6196)[30] and indole analogs synthesized by both Pinney and co-workers (Fig. 1),[31,32] and later by Flynn and co-workers (in the case of indole analogs).[33]

In this paper, the design and synthesis of a series of molecules containing the dihydronaphthalene and benzosuberene scaffolds structurally comparable to CA4, CA1, and colchicine are reported. Preliminary biochemical evaluation of the compounds in terms of inhibition of tubulin polymerization along with cytotoxicity studies against NCI-H460 (non-small cell lung carcinoma), DU-145 (prostate carcinoma), and SK-OV-3 (ovarian adenocarcinoma) human cancer cell lines is presented.

2. Results and discussions

2.1. Chemistry

The designed molecules fall into two classes[34] (Fig. 2): class I includes compounds **1** through **4** that are structurally analogous to the dihydronaphthalene Oxi6196,[30] (Fig. 1); while class II includes compounds **5** through **8** in which the aryl rings are connected via a carbonyl bridge. Compounds **9** and **10** were prepared serendipitously.

Key intermediates **17** and **19** were prepared from 5-hydroxy-6-methoxytetralin **13** (Scheme 1).[30,35] Following introduction of an isopropyl protecting group, compound **14** was oxidized selectively at the benzylic position with DDQ to afford tetralone **15** in good yield.[35] A Wittig reaction followed by a cyanogen azide ring expansion reaction[36,37] gave ketone **17**, which was converted to isomeric

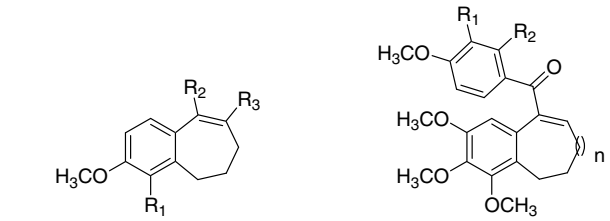


Figure 2. Class I and class II compounds.

ketone **19** through a Clemmensen reduction followed by chromium trioxide oxidation.[34–37] Some interesting observations were made during the synthesis of the compounds described in scheme 1. Initial attempts to protect the hydroxy functionality in tetralin **13a** with an isopropyl group in the presence of potassium carbonate[38] or triethylamine as base were unsuccessful. However, use of cesium carbonate led successfully to compound **14**.[39] To avoid partial deprotection of the isopropyl group observed during the Clemmensen reduction of ketone **17**, a Wolff-Kishner reduction[40] was attempted; however, these conditions did not yield the desired product **18**. Finally, an effort to oxidize **18** at the benzylic position using DDQ (following a protocol similar to that used for the benzylic oxidation of compound **14**)[35] was also unsuccessful.

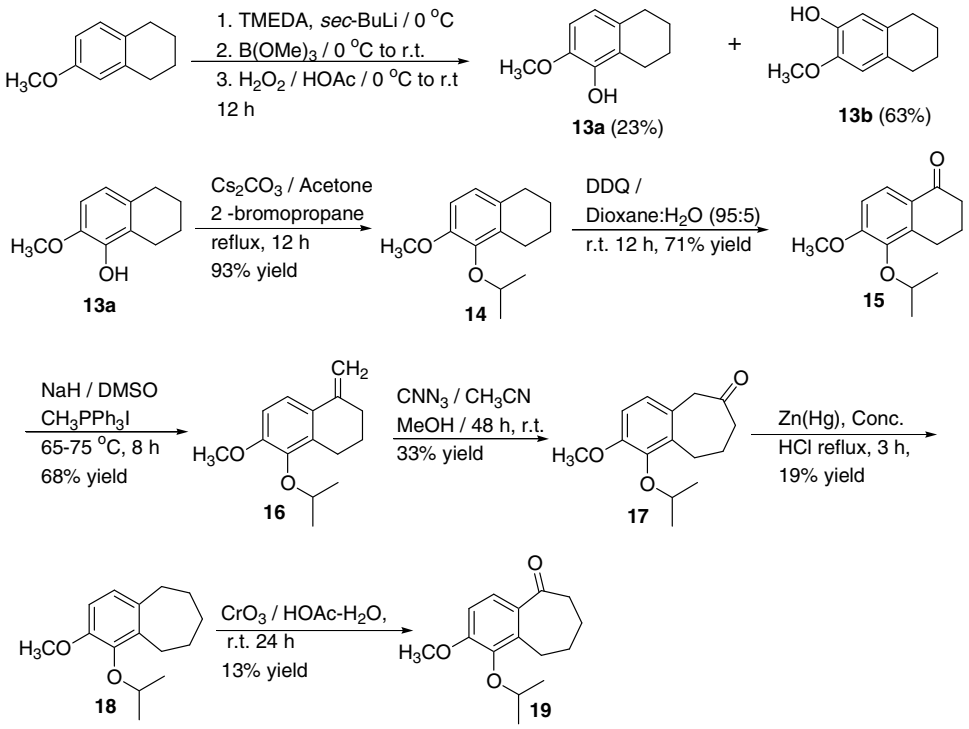
The benzosuberones (**11**,[37] **12**,[37] **17**, and **19**) were then coupled to lithiated 3,4,5-trimethoxybenzene (Schemes 2 and 3) to obtain tertiary alcohols **20**, **23**, **21**, and **24**, respectively.

The alcohols were subjected to acid catalyzed elimination of water followed by deprotection (in the case of compounds **22** and **25**) to afford benzosuberenes **1**–**4** in moderate yields.

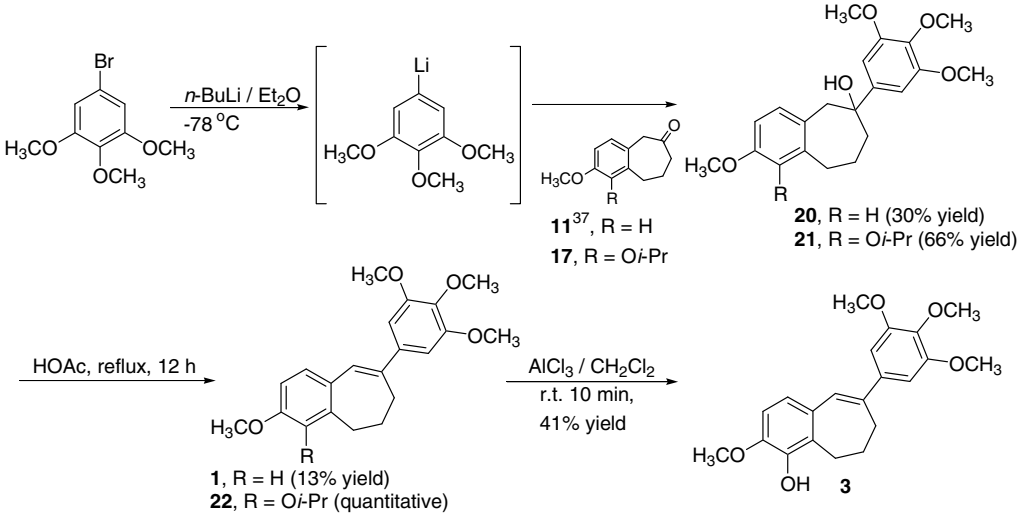
The synthesis of compounds **5** through **8** was achieved via a modified Shapiro coupling reaction (Scheme 4).[41] 5,6,7-Trimethoxytetralone **26** and 6,7,8-trimethoxybenzosuberone **27** were prepared according to the reported procedures by Gardner[42] and Khanolkar and co-workers.[43] The ketones were then converted to their respective hydrazones **28** and **29**.[41] Formation of the corresponding vinyl lithium adducts followed by treatment with aldehydes **38**[44] and **39**[45] gave secondary alcohols **30** through **33**. The alcohols were converted to the requisite ketones **34** through **37**, respectively, via Dess–Martin oxidation.[46] Ketones **34** and **35** were selectively debenzylated without affecting the α,β-unsaturated ketone moiety using 1,4-cyclohexadiene in the presence of Pd–C,[47] to obtain the desired dihydronaphthalene **5** and benzosuberene **6** in 46% and 63% yields, respectively. The isopropyl groups in ketones **36** and **37** were removed using anhydrous $AlCl_3$ at reduced temperature to yield the final products **7** and **8** in yields of 44% and 66%, respectively.[33]

On one occasion involving the deprotection of the benzyl groups from ketone **35**, H_2 gas was unintentionally used as a source of hydrogen instead of 1,4-cyclohexadiene (Scheme 5). This resulted in not only removal of the benzyl groups, but also in reduction of the double bond and the ketone to afford benzocyclic analog **9** in 23% yield. The structure of compound **9** was confirmed by X-ray crystallographic analysis (Fig. 3).[48]

Another interesting observation was made when the attempted deprotection of the isopropyl group in ketone **36** (with anhydrous $AlCl_3$ at room temperature rather than −78 °C as in Scheme 4) resulted in an unexpected cyclization to afford the isopropyl protected benzo[*a*]fluorene **40** in a 51% yield (Scheme 6). X-ray crystallographic analysis confirmed the structure of compound



Scheme 1. Synthesis of benzosuberones **17** and **19**.



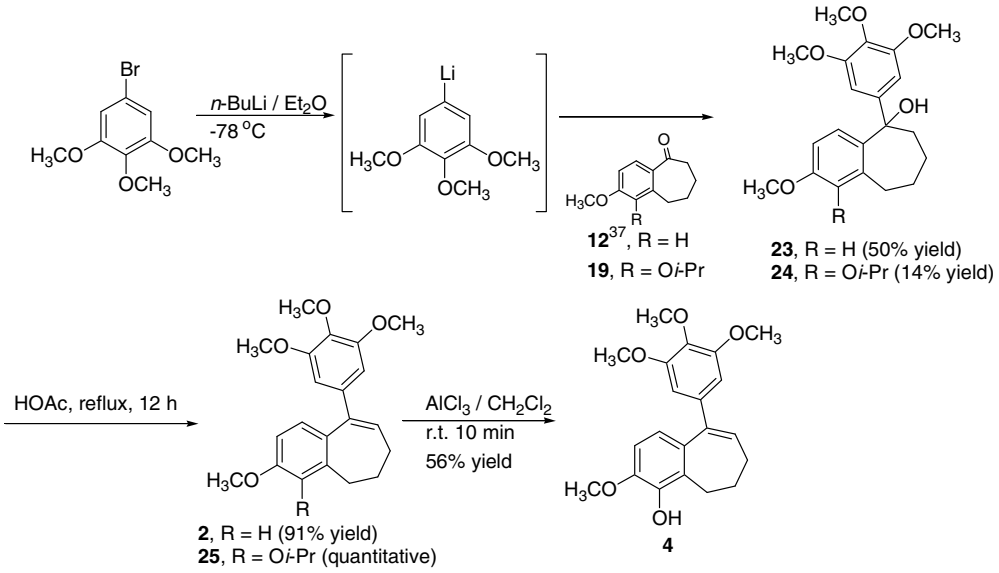
Scheme 2. Synthesis of benzosuberene analogs **1** and **3**.

40[48] (Fig. 4), the formation of which may have taken place through a Nazarov type of cyclization.[49] Deprotection of the isopropyl group at reduced temperature in the benzo[*a*]fluorene **40** afforded phenol **10**.

2.2. Biology

Vascular disrupting agents such as CA4P are, in general, potent inhibitors of tubulin assembly in parent compound form (CA4, IC_{50} = 1.2 μM). Therefore, the benzocyclic compounds in this series were evaluated for their ability to inhibit tubulin assembly. Compounds **2** and **4** inhibited tubulin polymerization with IC_{50} values of 1.4 μM and 1.7 μM, respectively (Table 1). These results are comparable with those of CA4 and CA1, which are between 1.2 and 1.9 μM.[50] It is intriguing to note that while benzosuberene **2** (with a hydrogen at C-6) and benzosuberene **4** (with a hydroxy at C-6) were both strong inhibitors of tubulin assembly, compound **4** demonstrated remarkable cytotoxicity against NCI-H460, DU-145, and SK-OV-3 cancer cell lines compared to compound **2**. This suggests the possibility that benzosuberene **4** may demonstrate an additional mechanism of inhibition of cancer cell growth beyond that attributable to the tubulin-based mechanism alone.

Compounds **1** and **3** in which the trimethoxy aryl ring is located at C-2 are not inhibitors of tubulin polymerization (IC_{50} > 40 μM); however, these compounds do maintain considerable cytotoxicity against the three human cancer cell lines studied. Introduction of a carbonyl moiety along with positioning the trimethoxy motif on the A-ring of the fused ring system resulted in compounds **6** and **8** (a di-phenol and mono-phenol, respectively) that were inactive both as inhibitors of tubulin assembly and in regard to



Scheme 3. Synthesis of benzosuberene analogs **2** and **4**.

cytotoxicity toward the cancer cell lines. Compound **9**, the chemically reduced analog of **6**, was similarly inactive against human cancer cell lines; however, it demonstrated moderate activity in regard to inhibition of tubulin polymerization.

The analogous tetralin compounds (**5** and **7**) were also inactive or minimally active as inhibitors of tubulin assembly but maintained limited cytotoxicity. The tetracyclic analog **10**, which is essentially a ring-closed variant of compound **7**, was inactive as an inhibitor of tubulin assembly; however, the two compounds (**10** and **7**) showed disparate cytotoxicity.

3. Conclusions

A novel series of ten benzocyclic compounds bearing structural similarity to the combretastatins, colchicine, and Oxi6196 have been synthesized and assessed for their preliminary biochemical and biological activity. Benzosuberenes **2** and **4** are potent inhibitors of tubulin polymerization, and compound **4** exhibited outstanding cytotoxicity against all three cancer cell lines evaluated. While compound **1**, the regioisomer of benzosuberene **2**, was not active as an inhibitor of tubulin assembly, it was cytotoxic against the cancer cell lines. The remarkable activity of benzosuberene **4** suggests that a suitable prodrug formulation of this compound may lead to a new vascular disrupting agent for the potential treatment of certain solid tumor cancers.

4. Experimental[51]

All reactions were performed under inert atmosphere using either nitrogen or argon gas unless specified differently. Chemical reagents used in the synthetic procedures were obtained from various chemical suppliers (Sigma–Aldrich Chem. Co., Acros Chem. Co., Alfa Aesar, Fisher Scientific, EMD Chemicals, and VWR). Silica gel (200–400 mesh, 60 Å) used for column chromatography was obtained from either Silicycle Inc. or VWR. TLC plates (pre-coated glass plates with silica gel 60 F254, 0.25 mm thickness, EMD chemicals, VWR) were used to monitor reactions. Intermediates and products synthesized were characterized based on ^{1}H NMR (Bruker DPX operating at 300 MHz or Varian operating at 500 MHz), and ^{13}C NMR (Bruker DPX operating at 75 MHz or Varian operating at 125 MHz). Deuterated $CDCl_3$ (with 0.03% TMS as internal standard) was used as common solvent for recording the NMR. All the chemical shifts are expressed in ppm (δ), coupling constants (J, Hz), and peak patterns are reported as broad (b), singlets (s), doublets (d), triplets (t), quartets (q), pentets (p), septets (sep), and multiplets (m). Elemental analysis was performed by Atlantic Microlab, Norcross, GA. High resolution mass spectra (HRMS) were obtained in the Baylor University Mass Spectrometry Core Facility on a VG Prospec Micromass spectrometer using EI.

4.1. Chemistry

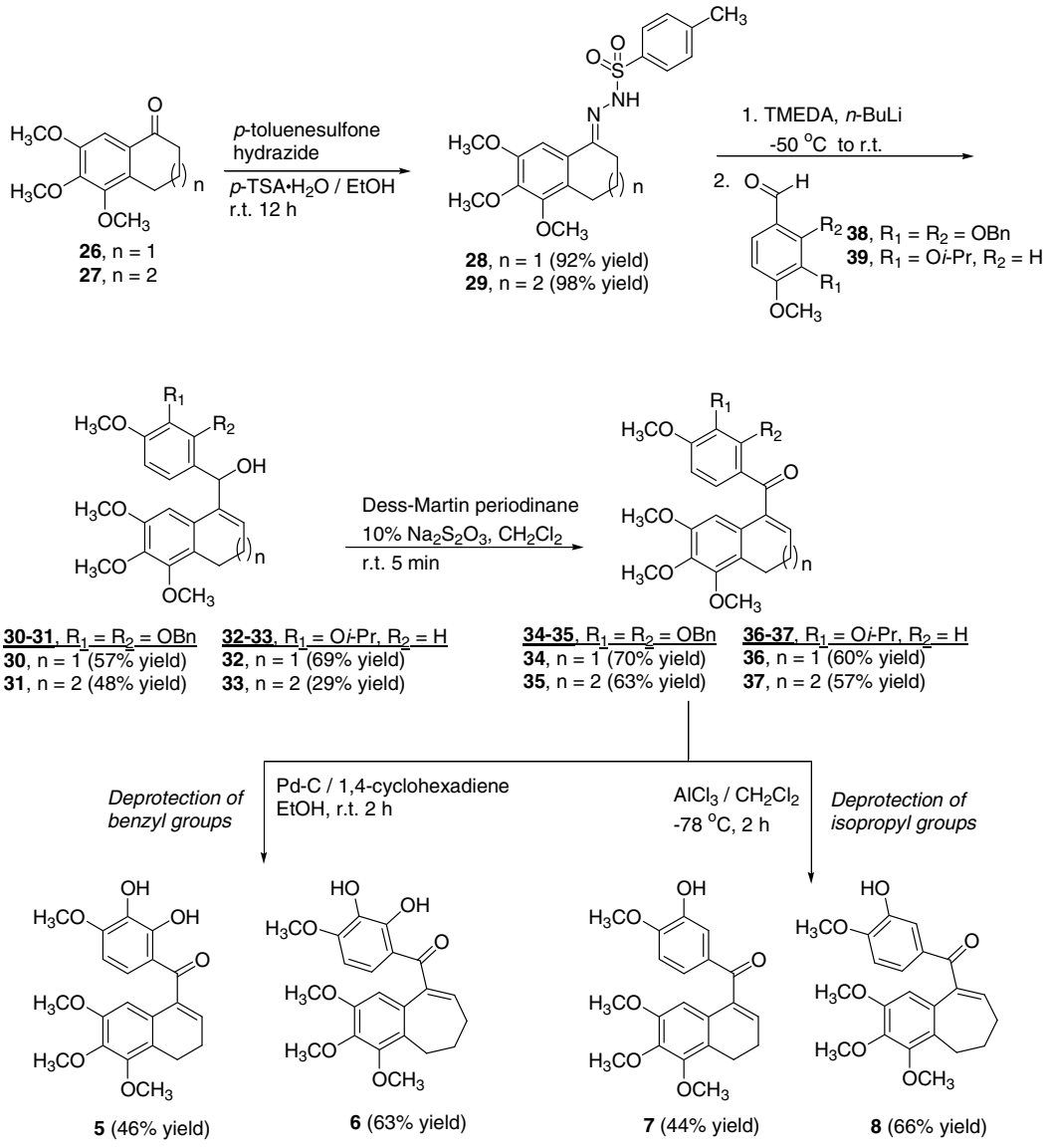
4.1.1. 5-Hydroxy-6-methoxy-1,2,3,4-tetrahydronaphthalene (13a)[35]

To a well-stirred solution of 6-methoxy-1,2,3,4-tetrahydronaphthalene (14.15 g, 86.75 mmol) in *sec*-BuLi (100 mL, 110 mmol) at 0 °C was added freshly distilled TMEDA (13.6 mL) dropwise. After the addition was complete, the reaction mixture was stirred at rt for 1 h. The reaction mixture was cooled again to 0 °C and $B(OMe)_3$ (12.5 mL, 110 mmol) was added dropwise. Then the reaction mixture was stirred for 1 h at rt. The reaction mixture was cooled back to 0 °C and glacial HOAc (7 mL) was added dropwise, followed by the dropwise addition of 35% wt. H_2O_2 (15 mL). Finally, the reaction mixture was allowed to stir at rt for 12 h. Saturated aqueous NH_4Cl (100 mL) was added and product was extracted with Et_2O (3× 400 mL). The combined organic phases were washed with brine and dried over Na_2SO_4, filtered, and concentrated in vacuo. Purification by flash column chromatography (silica gel, EtOAc/hexanes, gradient 2:98 to 5:95) yielded 5-hydroxy-6-methoxy-1,2,3,4-tetrahydronaphthalene **13a** (3.50 g, 19.6 mmol, 23% yield) as an off-white solid and 7-hydroxy-6-methoxy-1,2,3,4-tetrahydronaphthalene **13b** (9.70 g, 54.4 mmol, 63%) as colorless crystals, R_{f13a} = 0.48, R_{f13b} = 0.37 (15:85, EtOAc/hexanes).

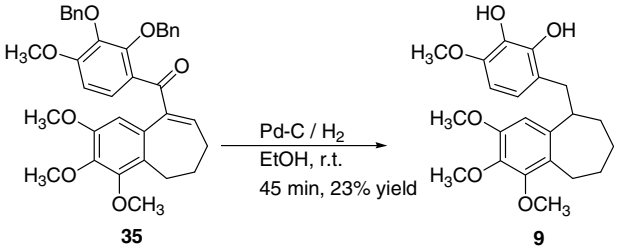
^{1}H NMR for **13a** ($CDCl_3$, 500 MHz) δ 6.67 (d, J = 8.5 Hz, 1H), 6.23 (d, J = 8.9 Hz, 1H), 5.63 (s, 1H), 3.86 (s, 3H), 2.70 (t, J = 6.0 Hz, 4H), 1.71 (m, 4H); ^{13}C NMR for **13a** ($CDCl_3$, 125 MHz) δ 143.8, 143.0, 130.8, 123.7, 119.4, 108.1, 56.2, 29.1, 23.1, 23.0, 22.6.

4.1.2. 5-Isopropoxy-6-methoxy-1,2,3,4-tetrahydronaphthalene (14)

Anhydrous Cs_2CO_3 (66.55 g, 204.3 mmol) was added to a well-stirred solution of **13a**[35] (4.55 g, 25.5 mmol) in anhydrous acetone



Scheme 4. Synthesis of dihydronaphthalene and benzosuberene compounds **5**–**8**.



Scheme 5. Preparation of compound **9**.

(50 mL). 2-Bromopropane (23.9 mL, 255 mmol) was added and the reaction mixture was heated at reflux for 12 h at which point the reaction mixture was filtered and the solvent evaporated in vacuo. Purification by flash column chromatography (silica gel, 1:99, EtOAc/hexanes) afforded 5-isopropoxy-6-methoxytetralin **14** (5.23 g, 23.7 mmol, 93% yield) as a pale yellow oil, R_f = 0.61 (15:85, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.77 (d, J = 8.3 Hz, 1H), 6.71 (d, J = 8.3 Hz, 1H), 4.46 (sep, J = 6.2 Hz, 1H), 3.80 (s, 3H), 2.71 (m, 4H), 1.72 (m, 4H), 1.28 (d, J = 6.2 Hz, 6H).

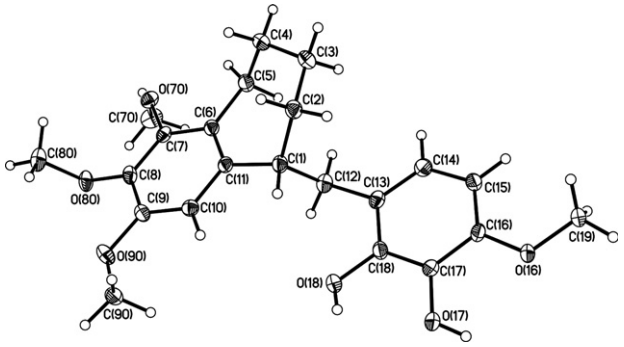
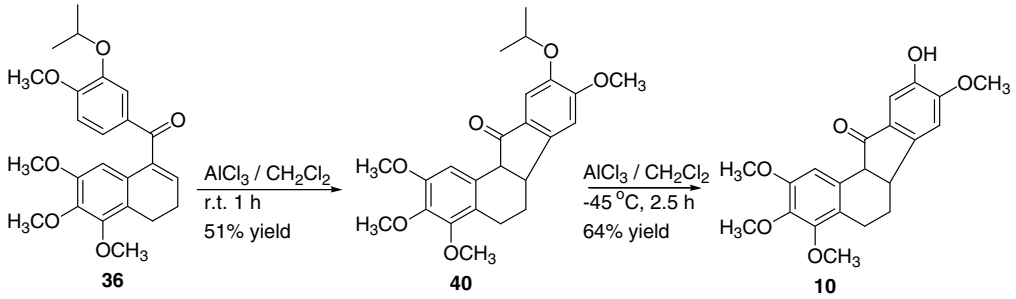


Figure 3. Thermal ellipsoid plot at 50% probability for compound **9**.

4.1.3. 5-Isopropoxy-6-methoxy-3,4-dihydro-2H-naphthalen-1-one (15)

To a well-stirred solution of tetralin **14** (0.12 g, 0.55 mmol) in H_2O/dioxane (5:95, 5 mL) was added a solution of DDQ (0.25 g, 1.09 mmol) in dioxane (5 mL) dropwise. The reaction mixture was stirred for 12 h at rt. The precipitate was filtered and washed



Scheme 6. Preparation of compounds **40** and **10**.

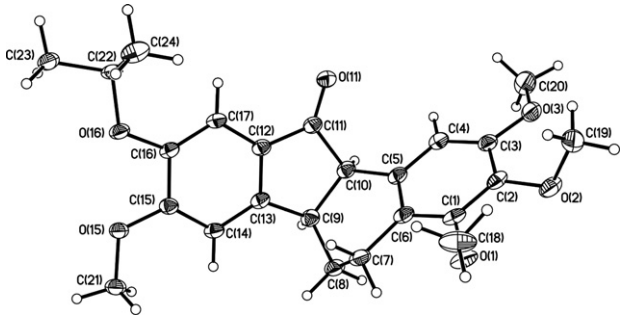


Figure 4. Thermal ellipsoid plot at 50% probability for compound **40**.

Table 1
Inhibition of tubulin polymerization and cytotoxicity studies against human cancer cell lines NCI-H460, DU-145, and SK-OV-3

Compound	Inhibition of tubulin polymerization IC_{50} (μM)	GI_{50} (μM) SRB assay		
		NCI-H460	DU-145	SK-OV-3
CA4	1.2[a]	0.002[b]	0.002[b]	0.00013
CA1	1.9[a]	2.2[b]	0.51[b]	nd[c]
1	>40[d]	0.47	0.55	0.44
2	1.4[d]	0.051	0.027	0.052
3	>40	1.2	0.44	0.27
4	1.7	0.000028	0.0000032	<0.00003
5	>40	2.8	3.4	1.4
6	>40	>10	>10	nd
7	24	6.0	3.1	nd
8	>40	>10	>10	nd
9	7.4	>10	>10	nd
10	>40	>10	>10	nd

[a] See Ref. 50.
[b] See Ref. 29.
[c] nd, not determined in this study.
[d] See Ref. 55

with EtOAc. The filtrate was concentrated under reduced pressure, saturated aqueous $NaHCO_3$ solution (10 mL) was added, and the resulting solution was extracted with Et_2O (3× 25 mL). The combined organic phases were dried over Na_2SO_4, filtered, and the solvent evaporated in vacuo. Purification by flash column chromatography (silica gel, 30:70, EtOAc/hexanes) afforded tetralone **15** (0.09 g, 0.38 mmol, 71% yield) as a colorless oil, R_f = 0.458 (40:60, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 4.45 (sep, J = 6.0 Hz, 1H), 3.89 (s, 3H), 2.95 (t, J = 6.0 Hz, 2H), 2.59 (t, J = 6.5 Hz, 2H), 2.06 (p, J = 6.5 Hz, 2H), 1.29 (d, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 197.7, 157.0, 143.2, 139.4, 126.7, 124.0, 109.9, 74.7, 55.7, 38.8, 24.2, 23.0, 22.6.

4.1.4. 5-Isopropoxy-6-methoxy-1-methylene-1,2,3,4-tetrahydronaphthalene (16)

Anhydrous DMSO (35 mL) was added to NaH (1.14 g, 47.4 mmol) and the reaction mixture was heated to 70–75 °C for 1–2 h, until the evolution of hydrogen ceased. The reaction mixture was cooled to room temperature and additional DMSO (20 mL) was added. Methyltriphenylphosphonium iodide (27.02 g, 66.84 mmol) was added in portions over a period of 30 min and the reaction mixture was subsequently stirred for 20 min at room temperature. A solution of tetralone **15** (7.83 g, 33.4 mmol) in anhydrous DMSO (8 mL) was added and the reaction mixture was stirred at 60–65 °C for 8 h. The reaction mixture was poured into a 250 mL Erlenmeyer flask containing ice/hexanes (50:50, 100 mL), stirred vigorously for 15 min, and then extracted with hexanes (2× 100 mL). The combined organic layers were washed with DMSO/H_2O (1:1), dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Purification by flash column chromatography (silica gel, 5:95, EtOAc/hexanes) yielded benzosuberone **16** (5.31 g, 22.9 mmol, 68% yield) as a colorless oil, R_f = 0.63 (30:70, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 (d, J = 8.5 Hz, 1H), 6.75 (d, J = 8.5 Hz, 1H), 5.35 (s, 1H), 4.84 (s, 1H), 4.46 (sep, J = 6.5 Hz, 1H), 3.83 (s, 3H), 2.82 (t, J = 6.5 Hz, 2H), 2.48 (t, J = 6.0 Hz, 2H), 1.82 (p, J = 6.0 Hz, 2H), 1.27 (d, J = 6.5 Hz, 6H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.1, 144.1, 143.5, 132.6, 128.6, 119.4, 110.1, 106.1, 74.2, 55.7, 33.1, 25.1, 23.5, 22.6.

4.1.5. 1-Isopropoxy-2-methoxy-5,7,8,9-tetrahydrobenzocyclohepten-6-one (17)

Preparation of CNN_3: finely powdered NaN_3 (0.510 g, 7.85 mmol) was added rapidly to a solution of CNBr (0.830 g, 7.85 mmol) in CH_3CN (anhydrous, 5 mL) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C, after which the clear supernatant solution containing CNN_3 was drawn into a syringe to be used for the ring expansion reaction.

Ring expansion reaction: to a stirred solution of exocyclic olefin **16** (0.32 g, 1.3 mmol) in MeOH/CH_3CN (1:1, 5 mL) at room temperature was added freshly prepared CNN_3, and the reaction mixture was stirred for 48 h. HCl (6 M, 5 mL) was added and the reaction mixture was heated at 50 °C for 4 h. The reaction mixture was then cooled to room temperature, and extracted with Et_2O (2× 25 mL). The ethereal extracts were washed with H_2O until neutral and then dried over Na_2SO_4. The organic phase was then percolated through a column of basic alumina capped with a layer of Celite™ to remove the potentially explosive azides. Evaporation of the solvent and subsequent purification by flash column chromatography (silica gel, 8:92, EtOAc/hexanes) yielded benzosuberone **17** (110 mg, 0.44 mmol, 33% yield) as an off-white solid, R_f = 0.48 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.84 (d, J = 8.3 Hz, 1H), 6.72 (d, J = 8.2 Hz, 1H), 4.43 (sep, J = 6.1 Hz, 1H), 3.81 (s, 3H), 3.64 (s, 2H),

3.04 (t, J = 6.9 Hz, 2H), 2.51 (t, J = 6.9 Hz, 2H), 1.94 (p, J = 6.9 Hz, 2H), 1.28 (d, J = 6.1 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) 209.5, 152.2, 144.4, 134.6, 126.9, 124.2, 109.9, 74.8, 55.6, 49.7, 43.2, 25.3, 24.4, 22.5.

4.1.6. 1-Isopropoxy-2-methoxy-6,7,8,9-tetrahydro-5H-benzocycloheptane (18)

Amalgamated zinc was prepared by shaking Zn dust (16 g), $HgCl_2$ (1.6 g), H_2O (16 mL), and HCl (concd, 1 mL) in a 100 mL round-bottomed flask for 10 min. The supernatant liquid was decanted and benzosuberone **17** (0.830 g, 1.13 mmol) was added, followed by HCl (concd, 60 mL). After heating at reflux for 3 h, the reaction mixture was cooled and extracted with Et_2O (3× 30 mL) and the combined ethereal extracts were washed with H_2O until neutral, then dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Separation by flash column chromatography (neutral alumina, 4:96, EtOAc/hexanes) afforded benzosuberan **18** (150 mg, 0.64 mmol, 19% yield) as a colorless oil, R_f = 0.55 (15:85, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.77 (d, J = 8.2 Hz, 1H), 6.60 (d, J = 8.2 Hz, 1H), 4.31 (sep, J = 6.2 Hz, 1H), 3.78 (s, 3H), 2.88 (m, 2H), 2.73 (m, 2H), 1.76 (m, 2H), 1.61 (m, 4H), 1.27 (d, J = 6.2 Hz, 6H).

4.1.7. 1-Isopropoxy-2-methoxy-6,7,8,9-tetrahydrobenzocyclohepten-5-one (19)

CrO_3 (0.47 g, 4.7 mmol) dissolved in HOAc (2 mL) and H_2O (0.5 mL) was added dropwise to a well-stirred solution of benzosuberan **18** (0.37 g, 1.6 mmol) in glacial HOAc (5 mL). The resulting reaction mixture was then stirred at room temperature for 28 h. H_2O (10 mL) was added and the product was extracted in Et_2O (3× 25 mL). The combined ethereal extracts were washed with a 5% aqueous NaOH solution until the aqueous phases tested basic with pH paper. The combined organic phases were first washed with H_2O until neutral, followed by brine, and then dried over Na_2SO_4. The solution was filtered and the solvent was removed under reduced pressure to obtain the crude product which was then subjected to flash column chromatography (neutral alumina, 4:96, EtOAc/hexanes) to afford benzosuberone **19** (0.05 g, 0.2 mmol, 13% yield) as colorless crystals, R_f = 0.60 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.51 (d, J = 8.6 Hz, 1H), 6.83 (d, J = 8.6 Hz, 1H), 4.41 (sep, J = 6.2 Hz, 1H), 3.88 (s, 3H), 3.03 (m, 2H), 2.69 (m, 2H), 1.79 (m, 4H), 1.29 (d, J = 6.2 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 205.1, 156.2, 143.7, 136.2, 124.9, 109.5, 74.9, 55.6, 40.6, 24.7, 23.8, 22.4, 21.5, 20.9.

4.1.8. 1-Isopropoxy-2-methoxy-6-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzocyclohepten-6-ol (21)

To a well-stirred solution of 3,4,5-trimethoxybromobenzene (1.50 g, 6.05 mmol) in dry Et_2O (200 mL) at −78 °C was added n-BuLi (3.20 mL, 8.06 mmol) dropwise. The reaction mixture was stirred until the temperature rose to −30 °C. Ketone **17** (1.05 g, 4.03 mmol) dissolved in dry Et_2O (25 mL) was added dropwise. The reaction mixture was then gradually allowed to warm to room temperature. At this point, H_2O (30 mL) was added and the organic phase was separated. The aqueous layer was extracted with Et_2O (2× 100 mL). The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was evaporated under reduced pressure. Purification of the crude product by flash column chromatography (silica gel, 30:70, EtOAc/hexanes) yielded alcohol **21** (1.15 g, 2.76 mmol, 66% yield) as a white crystalline solid, R_f = 0.15 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (d, J = 8.1 Hz, 1H), 6.78 (s, 2H), 6.58 (d, J = 8.1 Hz, 1H), 4.39 (m, 1H), 3.87 (s, 6H), 3.85 (s, 3H), 3.79 (s, 3H), 3.60 (m, 2H), 2.90 (d, J = 14.1 Hz, 1H), 2.49 (m, 1H), 2.16 (m, 2H), 1.55 (s, 2H), 1.29 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.7, 152.2, 146.0, 143.9, 137.8, 136.3, 128.2, 126.8, 109.1, 101.3, 74.8, 72.2, 60.6, 55.5, 48.2, 45.7, 26.4, 22.9, 22.5, 22.3.

4.1.9. 2-Hydroxy-7-methoxy-2-(3′,4′,5′-trimethoxyphenyl)-benzosuberan (20)

Following the general procedure for **21**, compound **20** (0.52 g, 1.4 mmol, 30% yield) was obtained from ketone **11** as a pale yellow oil, R_f = 0.25 (35:65, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.03 (d, J = 8.1 Hz, 1H), 6.79 (s, 2H), 6.75 (d, J = 2.6 Hz, 1H), 6.68 (dd, J = 8.1 Hz, 2.6 Hz, 1H), 3.89 (s, 6H), 3.86 (s, 3H), 3.80 (s, 3H), 3.59 (d, J = 15.2 Hz, 1H), 2.88 (m, 3H), 1.98 (m, 4H).

4.1.10. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydrobenzocyclohepten-5-ol (23)

Following the general procedure for **21**, compound **23** (0.81 g, 2.3 mmol, 50% yield) was obtained from ketone **12** as a pale yellow oil, R_f = 0.16 (30:70, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 2.6 Hz, 1H), 6.81 (m, 1H), 6.49 (d, J = 2.6 Hz, 2H), 3.84 (s, 3H), 3.82 (s, 3H), 3.76 (s, 6H), 2.64 (m, 2H), 2.07 (m, 6H).

4.1.11. 1-Isopropoxy-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol (24)

Following the general procedure for **21**, compound **24** (0.012 g, 0.029 mmol, 14% yield) was obtained from ketone **19** as a colorless oil, R_f = 0.14 (30:70, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, J = 8.7 Hz, 1H), 6.76 (d, J = 8.7 Hz, 1H), 6.47 (s, 2H), 4.38 (sep, J = 6.2 Hz, 1H), 3.87 (s, 3H), 3.85 (s, 3H), 3.73 (s, 6H), 3.33 (m, 1H), 2.56 (m, 1H), 2.12 (m, 6H), 1.29 (d, J = 6.2 Hz, 3H), 1.25 (d, J = 6.2 Hz, 3H).

4.1.12. 4-Isopropoxy-3-methoxy-8-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5H-benzocycloheptene (22)

A mixture of alcohol **21** (1.15 g, 2.64 mmol) in HOAc (30 mL) was heated at reflux for 12 h. The reaction mixture was cooled, extracted with CH_2Cl_2 (3× 50 mL), and the combined organic phases were dried over Na_2SO_4. The solvent was evaporated and the crude product was purified by flash column chromatography (silica gel, 10:90, EtOAc/hexanes) to afford trimethoxyphenylbenzocycloheptene **22** (1.04 g, 2.61 mmol, 95% yield) as a white crystalline solid, R_f = 0.56 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.95 (d, J = 8.4 Hz, 1H), 6.76 (d, J = 8.4 Hz, 1H), 6.71 (s, 1H), 6.69 (s, 2H), 4.44 (sep, J = 6.1 Hz, 1H), 3.90 (s, 6H), 3.87 (s, 3H), 3.84 (s, 3H), 2.93 (t, J = 6.0 Hz, 2H), 2.62 (t, J = 6.7 Hz, 2H), 2.19 (p, J = 6.5 Hz, 2H), 1.32 (d, J = 6.1 Hz, 6H).

4.1.13. 3-Methoxy-8-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5H-benzocycloheptene (1)

Following the general procedure for compound **22**, tertiary alcohol **20** yielded benzosuberene **1** (0.06 g, 0.18 mmol, 13% yield) as a colorless oil, R_f = 0.37 (35:65, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (d, J = 7.9 Hz, 1H), 6.73 (m, 5H), 3.90 (s, 6H), 3.87 (s, 3H), 3.82 (s, 3H), 2.80 (t, J = 6.2 Hz, 2H), 2.63 (t, J = 6.2 Hz, 2H), 2.21 (p, J = 6.2 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 158.3, 152.9, 142.8, 140.8, 140.4, 137.3, 131.8, 129.8, 127.9, 114.7, 111.0, 103.6, 60.9, 56.1, 55.2, 34.8, 33.1, 29.9; HRMS (EI) Calcd for 340.1675, found 340.1681.

4.1.14. 3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5H-benzocycloheptene (2)

Following the general procedure for compound **22**, tertiary alcohol **23** yielded benzosuberene **2** (0.690 g, 2.03 mmol, 91% yield) as a white solid, R_f = 0.43 (30:70, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.99 (d, J = 8.5 Hz, 1H), 6.83 (d, J = 2.6 Hz, 1H), 6.75 (dd, J = 8.5 Hz, 2.6 Hz, 1H), 6.49 (s, 2H), 6.35

(t, J = 7.3 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.80 (s, 6H), 2.64 (t, J = 7.0 Hz, 2H), 2.18 (p, J = 7.0 Hz, 2H), 1.98 (m, 2H); Anal. Calcd for $C_{21}H_{24}O_4$: C, 74.09; H, 7.09. Found: C, 73.98; H, 7.09; HRMS (EI) Calcd for 340.1675, found 340.1672.

4.1.15. 1-Isopropoxy-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzocycloheptene (25)

Following the general procedure for compound **22**, tertiary alcohol **24** yielded benzosuberene **25** (0.01 g, 0.02 mmol, quantitative yield) as a colorless oil, R_f = 0.46 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.73 (s, 2H), 6.47 (s, 2H), 6.33 (t, J = 7.3 Hz, 1H), 4.50 (sep, J = 6.2 Hz, 1H), 3.85 (s, 6H), 3.79 (s, 6H), 2.77 (t, J = 6.9 Hz, 2H), 2.21 (m, 2H), 1.97 (m, 2H), 1.33 (d, J = 6.2 Hz, 6H).

4.1.16. 2-Methoxy-6-(3′,4′,5′-trimethoxyphenyl)-8,9-dihydro-7H-benzocyclohepten-1-ol (3)

To a stirred solution of compound **22** (0.22 g, 0.55 mmol) in anhydrous CH_2Cl_2 (15 mL) at room temperature was added $AlCl_3$ (0.150 g, 1.10 mmol). After 10 min, H_2O (10 mL) was added and the product was extracted in CH_2Cl_2 (2× 20 mL). The combined organic phases were dried over Na_2SO_4, evaporated under reduced pressure, and purified by flash column chromatography (silica gel, 90:10, hexanes/EtOAc). Phenol **3** (0.08 g, 0.2 mmol, 41% yield) was obtained as a colorless oil, R_f = 0.54 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.78 (d, J = 8.4 Hz, 1H), 6.72 (d, J = 8.4 Hz, 1H), 6.70 (s, 3H), 5.77 (s, 1H), 3.90 (s, 6H), 3.89 (s, 3H), 3.86 (s, 3H), 2.93 (t, J = 6.1 Hz, 2H), 2.63 (t, J = 6.1 Hz, 2H), 2.23 (p, J = 6.1 Hz, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8, 145.0, 142.4, 141.2, 140.2, 137.2, 131.6, 128.1, 126.8, 121.7, 107.6, 103.5, 60.8, 56.1, 55.9, 32.8, 29.8, 24.8; HRMS (EI) Calcd for 356.1624, found 356.1620.

4.1.17. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5H-benzocyclohepten-1-ol (4)

Following the general procedure for compound **3**, benzosuberene **25** yielded phenol **4** (0.005 g, 0.012 mmol, 56% yield) as a colorless oil, R_f = 0.35 (70:30, EtOAc/hexanes).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.71 (d, J = 8.4 Hz, 1H), 6.57 (d, J = 8.4 Hz, 1H), 6.50 (s, 2H), 6.34 (t, J = 7.3 Hz, 1H), 5.74 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H), 3.80 (s, 6H), 2.76 (t, J = 7.3 Hz, 2H), 2.14 (p, J = 7.1 Hz, 2H), 1.97 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 152.8, 145.0, 142.7, 142.3, 138.4, 137.3, 134.2, 127.7, 127.2, 120.8, 107.6, 105.3, 60.8, 56.1, 55.9, 33.5, 25.6, 23.5; HRMS (EI) Calcd for 356.1624, found 356.1624.

4.1.18. Synthesis of tosylhydrazones (28 and 29)—General procedure

4.1.18.1. (5,6,7-Trimethoxy-3,4-dihydro-2H-naphthalen)-1-*p*-toluenesulfonylhydrazone (28). To a well-stirred solution of cyclic ketone **26** (2.96 g, 12.5 mmol) in anhydrous EtOH (60 mL) was added *p*-toluenesulfonylhydrazide (2.34 g, 12.5 mmol). Solution was achieved within 5 min at which point *p*-TSA monohydrate (0.11 g, 0.63 mmol) was added and the reaction mixture was stirred for 12 h at room temperature. Hydrazone **28** precipitated out as a white solid, which was then filtered, washed with ice-cold EtOH, and dried under reduced pressure to afford **28** (4.65 g, 11.5 mmol, 92% yield) as a white solid, R_f = 0.31 (60:40, hexanes/EtOAc).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.30 (s, 1H), 7.83 (d, J = 8.2 Hz, 2H), 7.41 (d, J = 8.2 Hz, 2H), 7.14 (s, 1H), 3.77 (s, 3H), 3.74 (s, 3H), 3.70 (s, 3H), 2.57 (t, J = 5.7 Hz, 2H), 2.45 (t, J = 6.3 Hz, 2H), 2.37 (s, 3H), 1.71 (m, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 152.6, 151.1, 150.0, 143.3, 143.1, 136.0, 129.2, 127.8, 126.9, 126.6, 102.6, 60.4, 55.4, 25.4, 21.6, 21.0, 20.9.

4.1.18.2. (6,7,8-Trimethoxy-2,3,4,5-tetrahydrobenzocycloheptene)-1-*p*-toluenesulfonylhydrazone (29). Reaction of **27** (8.21 g, 32.8 mmol) with *p*-toluenesulfonylhydrazide (6.11 g, 32.8 mmol) under similar reaction conditions (as described for compound **28**) yielded hydrazone **29** (13.50 g, 32.26 mmol, 98% yield) as a white solid, R_f = 0.32 (60:40, hexanes/EtOAc).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.33 (s, 1H), 7.83 (s-broad, 2H), 7.51 (s-broad, 2H), 6.38 (s, 1H), 3.73 (s, 3H), 3.69 (s, 6H), 2.73 (m, 7H), 1.60 (s, 2H), 1.47 (s, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 160.4, 151.3, 150.7, 143.8, 143.1, 136.5, 133.6, 129.8, 128.1, 124.8, 107.6, 61.7, 60.8, 55.9, 27.8, 25.2, 22.7, 21.4, 21.2.

4.1.19. Modified Shapiro coupling reactions to prepare 30–33—General synthetic procedure

4.1.19.1. (2″,3″-Bis-benzyloxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanol (30). *n*-BuLi (7.42 mL, 14.8 mmol) was added to freshly distilled TMEDA (20 mL), and the mixture was cooled to −50 °C. At this point, hydrazone **28** (1.49 g, 3.71 mmol) was added, and the reaction mixture was stirred until the temperature reached 25 °C. Aldehyde **38** (5.18 g, 14.8 mmol) was added, and the reaction mixture was stirred for 1 h. H_2O (25 mL) was added and the product was extracted in ethyl acetate (2× 100 mL). The combined organic phases were washed with 10% aqueous $CuSO_4$ solution (100 mL), followed by brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Purification of the crude product by flash column chromatography (silica gel, 16:84, EtOAc/hexanes) yielded alcohol **30** (1.20 g, 2.11 mmol, 57% yield) as a pale yellow oil, R_f = 0.40 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (m, 10H), 7.03 (d, J = 8.6 Hz, 1H), 6.66 (m, 2H), 6.16 (t, J = 4.6 Hz, 1H), 5.93 (d, J = 3.0 Hz, 1H), 5.19 (m, 2H), 5.03 (m, 2H), 3.84 (s, 6H), 3.80 (s, 3H), 3.65 (s, 3H), 2.87 (m, 2H), 2.68 (m, 2H), 1.77 (d, J = 3.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 153.6, 151.1, 150.2, 150.0, 141.2, 141.0, 129.2, 129.0, 128.7, 128.5, 128.4, 128.34, 128.31, 128.0, 125.0, 122.5, 122.3, 107.9, 104.0, 75.8, 75.4, 67.4, 60.8, 60.7, 56.0, 55.9, 22.5, 20.1.

4.1.19.2. (2″,3″-Bis-benzyloxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro-7′H-benzocyclohepten-5′-yl)-methanol (31). Coupling between hydrazone **29** (1.54 g, 3.68 mmol) and aldehyde **38** (5.14 g, 14.7 mmol) under similar conditions (as described for compound **30**) afforded alcohol **31** (1.02 g, 1.75 mmol, 48% yield) as a pale yellow oil, R_f = 0.56 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (m, 10H), 7.13 (d, J = 8.6 Hz, 1H), 6.69 (d, J = 8.6 Hz, 1H), 6.63 (s, 1H), 6.09 (t, J = 7.1 Hz, 1H), 5.86 (d, J = 4.2 Hz, 1H), 5.06 (m, 4H), 3.85 (s, 3H), 3.82 (s, 6H), 3.63 (s, 3H), 3.01 (t, J = 7.0, 2H), 2.06 (t, J = 7.0, 2H), 1.86 (m, 2H).

4.1.19.3. (3″-Isopropoxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanol (32). Coupling of hydrazone **28** (0.998 g, 2.47 mmol) with aldehyde **39** (1.93 g, 9.89 mmol) under similar conditions (as described for compound **30**) yielded alcohol **32** (0.70 g, 1.7 mmol, 69% yield) as a pale yellow oil, R_f = 0.31 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (m, 3H), 6.66 (s, 1H), 6.17 (t, J = 4.5 Hz, 1H), 5.65 (s, 1H), 4.45 (sep, J = 6.0 Hz, 1H), 3.85 (s, 6H), 3.81 (s, 3H), 3.66 (s, 3H), 2.75 (t, J = 7.8 Hz, 2H), 2.34 (m, 2H), 1.32 (m, 6H).

4.1.19.4. (3″-Isopropoxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro7′H-benzocyclohepten-5′-yl)-methanol (33). Hydrazone **29** (1.05 g, 2.40 mmol), when coupled with aldehyde **39** (1.86 g, 9.56 mmol) under similar Shapiro conditions (as described for compound **30**) afforded alcohol **33** (0.30 g, 0.70 mmol, 29% yield) as a pale yellow oil, R_f = 0.29 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.95 (m, 2H), 6.80 (d, J = 8.2 Hz, 1H), 6.65 (s, 1H), 6.30 (t, J = 8.0 Hz, 1H), 5.53 (s, 1H), 4.45 (sep, J = 6.0 Hz, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H), 2.53 (m, 2H), 2.05 (m, 2H), 1.91 (m, 2H), 1.29 (d, J = 6.0 Hz, 6H).

4.1.20. Synthesis of compounds 34–37—Dess–Martin oxidation—General methodology

4.1.20.1. (2″,3″-Bis-benzyloxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanone (34). To a well-stirred solution of Dess–Martin periodinane (1.30 g, 2.24 mmol) in dry CH_2Cl_2 (20 mL) at room temperature was added a solution of alcohol **30** (1.16 g, 2.04 mmol) in anhydrous CH_2Cl_2 (20 mL) followed by 10% aqueous $Na_2S_2O_3$ (0.05 mL). After stirring for 5 min, a solution of 10% aqueous $Na_2S_2O_3$ and saturated aqueous $NaHCO_3$ (30 mL, 1:1 ratio) was added, and the reaction mixture was stirred for 5 min. The product was extracted in CH_2Cl_2 (3× 50 mL). The combined organic phases were washed with brine and dried over Na_2SO_4. The organic phases were filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 10:90, EtOAc/hexanes) to afford ketone **34** (0.80 g, 1.4 mmol, 70% yield) as a pale yellow oil, R_f = 0.41 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (m, 11H), 7.05 (s, 1H), 6.72 (d, J = 8.5 Hz, 1H), 6.34 (t, J = 4.5 Hz, 1H), 5.07 (s, 2H), 5.02 (s, 2H), 3.90 (s, 6H), 3.82 (s, 3H), 3.76 (s, 3H), 2.66 (t, J = 7.9 Hz, 2H), 2.28 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.9, 156.9, 152.2, 151.5, 150.5, 142.1, 141.5, 139.8, 139.6, 137.3, 137.2, 128.8, 128.6, 128.5, 128.4, 128.3, 128.0, 127.5, 126.2, 122.4, 107.1, 106.5, 76.1, 75.6, 61.1, 56.3, 56.2, 23.4, 19.6.

4.1.20.2. (2″,3″-Bis-benzyloxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro-7′H-benzocyclohepten-5′-yl)-methanone (35). Oxidation of alcohol **31** (1.60 g, 2.75 mmol) under similar Dess–Martin reaction conditions (as described for compound **34**) afforded ketone **35** (1.05 g, 1.80 mmol, 63% yield) as a yellow oil, R_f = 0.43 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.40 (m, 10H), 7.13 (d, J = 8.4 Hz, 1H), 6.80 (t, J = 7.3 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 6.61 (s, 1H), 5.09 (s, 2H), 5.02 (s, 2H), 3.90 (s, 3H), 3.89 (s, 3H), 3.85 (s, 3H), 3.70 (s, 3H), 2.54 (t, J = 6.5 Hz, 2H), 2.10 (m, 4H).

4.1.20.3. (3″-Isopropoxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanone (36). Secon dary- alcohol **32** (0.71 g, 1.7 mmol) was oxidized under similar Dess–Martin reaction conditions (as described for compound **34**) to yield ketone **36** (0.42 g, 1.0 mmol, 60% yield) as a colorless oil, R_f = 0.51 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (m, 2H), 6.87 (d, J = 8.3 Hz, 1H), 6.69 (s, 1H), 6.40 (t, J = 4.7 Hz, 1H), 4.61 (sep, J = 6.0 Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.73 (s, 3H), 2.84 (t, J = 7.8 Hz, 2H), 2.44 (m, 2H), 1.38 (d, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.9, 154.6, 151.5, 150.4, 147.1, 142.0, 138.2, 134.8, 130.7, 127.7, 125.1, 121.8, 115.7, 110.4, 105.9, 71.4, 60.9, 56.0, 22.8, 21.9, 19.8.

4.1.20.4. (3″-Isopropoxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro-7′H-benzocyclohepten-5′-yl)-methanone (37). Oxidation of alcohol **31** (0.30 g, 0.70 mmol) under similar Dess–Martin reaction conditions (as described for compound **34**) afforded ketone **37** (0.17 g, 0.39 mmol, 57% yield) as a pale yellow oil, R_f = 0.62 (40:60, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.43 (dd, J = 8.4 Hz, 2.03 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 6.80 (m, 2H), 6.47 (s, 1H), 4.49 (sep, J = 6.0 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 6H), 3.69 (s, 3H), 2.77 (t, J = 6.6 Hz, 2H), 2.22 (m, 4H), 1.33 (d, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.4, 154.0, 151.3, 146.7, 142.6, 141.6, 140.4, 132.8, 130.5, 127.0, 124.5, 116.1, 110.5, 108.4, 71.2, 61.5, 60.8, 56.0, 55.9, 33.9, 26.0, 23.8, 21.9.

4.1.21. Synthesis of compounds 5 and 6—deprotection of benzyl groups—General methodology

4.1.21.1. (2″,3″-Dihydroxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanone (5). To a well-stirred solution of ketone **34** (0.796 g, 1.41 mmol) in EtOH (20 mL) was added Pd–C (10% wt., 0.5 g) followed by 1,4-cyclohexadiene (3.37 mL, 35.3 mmol). The reaction mixture was stirred for 1.75 h. The solution was filtered through Celite™, and the residue was washed with EtOAc. The combined filtrates were evaporated under reduced pressure and purified by flash column chromatography (silica gel, 20:80, EtOAc/hexanes) to obtain diol **5** (0.25 g, 0.65 mmol, 46% yield) as a yellow solid, R_f = 0.37 (50:50, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.44 (s, 1H), 7.27 (d, J = 8.0 Hz, 1H), 6.52 (s, 2H), 6.45 (d, J = 8.0 Hz, 1H), 6.29 (t, J = 5.0 Hz, 1H), 5.53 (s, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.87 (s, 3H), 3.72 (s, 3H), 2.86 (t, J = 8.5 Hz, 2H), 2.45 (m, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 201.8, 152.6, 151.9, 151.2, 150.8, 142.5, 137.4, 133.6, 127.5, 125.7, 121.9, 114.9, 105.6, 102.7, 61.2, 61.1, 56.5, 56.4, 22.9, 20.0; HRMS (EI) Calcd for 386.1366, found 386.1364.

4.1.21.2. (2″,3″-Dihydroxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro-7′H-benzocyclohepten-5′-yl)-methanone (6). Debenzylation of ketone **35** (0.995 g, 1.72 mmol) under similar reaction conditions (as described for compound **5**) afforded benzosuberene diol **6** (0.55 g, 1.4 mmol, 80% yield) as a yellow solid, R_f = 0.40 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 500 MHz) δ 12.49 (s, 1H), 7.07 (d, J = 9.0 Hz, 1H), 6.68 (t, J = 6.8 Hz, 1H), 6.41 (s, 1H), 6.39 (d, J = 9.0 Hz, 1H), 5.50 (s, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.88 (s, 3H), 3.69 (s, 3H), 2.75 (t, J = 6.5 Hz, 2H), 2.18 (m, 4H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 200.7, 151.9, 151.3, 151.0, 141.8, 141.4, 139.1, 133.3, 132.3, 127.2, 125.1, 114.3, 107.8, 102.5, 61.5, 60.7, 56.1, 55.9, 33.8, 26.0, 23.8; Anal. Calcd for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04. Found: C, 66.02; H, 6.05; HRMS (EI) Calcd for 400.1522, found 400.1509.

4.1.22. Synthesis of compounds 7 and 8—deprotection of isopropyl groups—General methodology

4.1.22.1. (3″-Hydroxy-4″-methoxyphenyl)-(5′,6′,7′-trimethoxy-3′,4′-dihydronaphthalen-1′-yl)-methanone (7). To a solution of ketone **36** (0.11 g, 0.24 mmol) in anhydrous CH_2Cl_2 (10 mL) at −78 °C was added $AlCl_3$ (anhydrous, 0.08 g, 0.6 mmol). The reaction mixture was stirred for 3 h. NH_4Cl (saturated, 5 mL) was added and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic phases were dried over Na_2SO_4, filtered, and the solvent evaporated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 30:70, EtOAc/hexanes) to yield phenol **7** (0.04 g, 0.11 mmol, 44% yield) as a pale yellow oil, R_f = 0.22 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (m, 2H), 6.88 (d, J = 8.0 Hz, 1H), 6.70 (s, 1H), 6.39 (t, J = 4.6 Hz, 1H), 5.78 (s, 1H), 3.95 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.72 (s, 3H), 2.82 (t, J = 7.8 Hz, 2H), 2.42 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.9, 151.4, 150.6, 150.4, 145.2, 142.0, 137.94, 135.2, 131.5, 127.5, 123.7, 121.8, 109.7, 105.8, 60.8, 56.1, 56.0, 22.8, 19.7; Anal. Calcd for $C_{21}H_{22}O_6{\cdot}H_2O$: C, 64.94; H, 6.23. Found: C, 64.96; H, 5.94; HRMS (EI) Calcd for 370.1416, found 370.1416.

4.1.22.2. (3″-Hydroxy-4″-methoxyphenyl)-(1′,2′,3′-trimethoxy-8′,9′-dihydro-7′H-benzocyclohepten-5′-yl)-methanone (8). Deprotection of the isopropyl group in ketone **37** (69 mg, 0.16 mmol) under similar reaction conditions (as described for

compound **7**) afforded **8** (35 mg, 0.09 mmol, 56% yield) as a colorless oil, R_f = 0.23 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.41 (d, J = 2.0 Hz, 1H), 7.34 (dd, J = 8.3 Hz, 2.0 Hz, 1H), 6.85 (d, J = 8.4 Hz, 1H), 6.79 (t, J = 6.9 Hz, 1H), 6.5 (s, 1H), 3.96 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.72 (s, 3H), 2.72 (t, J = 6.6 Hz, 2H), 2.14 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 195.7, 151.1, 150.1, 145.1, 142.2, 141.6, 141.2, 132.5, 131.6, 127.3, 132.4, 115.9, 109.6, 108.4, 61.5, 60.8, 56.0, 55.9, 33.8, 26.0, 23.7; Anal. Calcd for $C_{22}H_{24}O_6{\cdot}H_2O$: C, 65.66; H, 6.51. Found: C, 65.59; H, 6.23; HRMS (EI) Calcd for 384.1573, found 384.1570.

4.1.23. 2,3-Bis-benzyloxy-4-methoxybenzaldehyde (38)

Anhydrous K_2CO_3 (14.39 g, 104.1 mmol) was added to a well-stirred solution of 2,3-dihydroxy-4-methoxybenzaldehyde[35] (5.31 g, 29.8 mmol) in MeOH (anhydrous, 50 mL). Benzyl bromide (10.7 mL, 89.2 mmol) was added and the reaction mixture was heated at reflux for 1.5 h. The reaction mixture was filtered and the solvent was evaporated under reduced pressure. H_2O (100 mL) and CH_2Cl_2 (100 mL) were added to the crude reaction mixture. The organic phases were separated and the aqueous phase was extracted with additional CH_2Cl_2 (2× 150 mL). The combined organic phases were washed with H_2O (2× 200 mL), followed by brine, and dried over anhydrous Na_2SO_4. The organic phases were filtered and the solvent was evaporated under reduced pressure. The crude product obtained was purified by flash column chromatography (5:95, EtOAc/hexanes) to afford bis-*o*-benzyl-aldehyde **38** (10.2 g, 29.3 mmol, 98% yield) as a yellow solid, R_f = 0.51 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.10 (s, 1H), 7.60 (d, J = 8.8 Hz, 1H), 7.41 (m, 10H), 6.77 (d, J = 8.8 Hz, 1H), 5.20 (s, 2H), 5.07 (s, 2H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 188.8, 159.4, 155.8, 140.7, 137.0, 136.2, 128.7, 128.5, 128.3, 128.2, 127.6, 126.9, 124.1, 123.9, 107.7, 75.5, 65.3, 56.1.

4.1.24. 3-Isopropoxy-4-methoxybenzaldehyde (39)[45]

Anhydrous K_2CO_3 (36.35 g, 262.9 mmol) was added to a solution of isovanillin (20.34 g, 131.5 mmol) in anhydrous DMF (250 mL) at 50 °C. The reaction mixture was then stirred for 15 min, and 2-bromopropane (24.6 mL, 262 mmol) was added. The reaction mixture was then stirred for 8 h at 90 °C. H_2O (200 mL) was added and the product was extracted with CH_2Cl_2 (3× 300 mL). The combined organic phases were washed with H_2O (2× 300 mL), followed by brine, and then dried over Na_2SO_4. The organic phases were filtered and concentrated to dryness in vacuo. The crude product was subjected to flash column chromatography (silica gel, 5:95, hexanes/EtOAc) to obtain product **39** (22.71 g, 116.9 mmol, 89% yield) as pale yellow crystals, R_f = 0.40 (70:30, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.84 (s, 1H), 7.43 (m, 2H), 6.84 (d, J = 8.0 Hz, 1H), 4.65 (sep, J = 6.0 Hz, 1H), 3.93 (s, 3H), 1.40 (d, J = 6.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 190.8, 155.5, 147.7, 129.9, 126.3, 112.6, 110.9, 71.2, 56.0, 21.8.

4.1.25. 3-Methoxy-6-((1′,2′,3′-trimethoxy-6′,7′,8′,9′-tetrahydro-5′H-benzo[7]annulen-5′-yl)benzene-1,2-diol (9)

To a solution of ketone **35** (0.98 g, 1.7 mmol) in anhydrous EtOH (100 mL) was added Pd–C (10% wt., 1.0 g). The reaction mixture was de-gassed under vacuum and then stirred for 1 h under H_2 (using a balloon filled with H_2 gas). The reaction mixture was filtered through Celite™. The solvent was evaporated under reduced pressure. The crude product was recrystallized with EtOAc/hexanes to obtain diol **9** (0.15 g, 0.38 mmol, 23% yield) as white crystals, R_f = 0.31 (50:50, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.55 (m, 2H), 6.37 (d, J = 8.4 Hz, 1H), 5.38 (s, 1H), 5.37 (s, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.79 (s, 6H), 3.05 (m, 5H), 1.68 (m, 6H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 150.8, 150.4, 145.0, 142.2, 141.7, 140.1, 132.1, 128.5, 120.7, 102.3, 61.3, 60.8, 56.1, 56.0, 33.1, 32.2, 29.0, 27.8, 24.9; Anal. Calcd for $C_{22}H_{28}O_6$: C, 68.02; H, 7.27. Found: C, 68.04; H, 7.26; HRMS (EI) Calcd for 388.1886, found 388.1887.

4.1.26. 9-Isopropoxy-2,3,4,8-tetramethoxy-5,6-dihydro-benzo[*a*]fluoren-11-one (40)

Anhydrous $AlCl_3$ (0.05 g, 0.4 mmol) was added to a well-stirred solution of ketone **36** (0.14 g, 0.34 mmol) in anhydrous CH_2Cl_2 (10 mL) at 5 °C under N_2↑. The reaction mixture was stirred for 1 h. H_2O (5 mL) was added and the product was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude product obtained was crystallized using EtOAc/hexanes (15:85) to afford benzo[*a*]fluorene **40** (0.07 g, 1.7 mmol, 51% yield) as white crystals, R_f = 0.32 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (s, 1H), 7.07 (s, 1H), 6.99 (s, 1H), 4.57 (sep, J = 6.1 Hz, 1H), 3.99 (s, 3H), 3.95 (s, 3H), 3.85 (s, 3H), 3.77 (m, 5H), 2.74 (m, 1H), 2.16 (m, 1H), 2.01 (m, 2H), 1.39 (d, J = 6.1 Hz, 3H), 1.37 (d, J = 6.1 Hz, 3H).

4.1.27. 9-Hydroxy-2,3,4,8-tetramethoxy-5,6-dihydro-benzo[*a*]fluoren-11-one (10)

To a solution of benzo[*a*]fluorene **40** (73 mg, 0.17 mmol) in anhydrous CH_2Cl_2 (10 mL) at −45 °C was added anhydrous $AlCl_3$ (64 mg, 0.43 mmol). The reaction mixture was stirred for 2.5 h at which point H_2O (5 mL) was added and the organic phases were separated. The aqueous layer was extracted with CH_2Cl_2 and the combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was purified by flash column chromatography (silica gel, 35:65, EtOAc/hexanes) to yield alcohol **10** (0.04 g, 0.1 mmol, 64% yield) as a colorless solid, R_f = 0.14 (60:40, hexanes/EtOAc).

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24 (s, 1H), 7.06 (s, 1H), 6.99 (s, 1H), 5.70 (s, 1H), 4.04 (s, 3H), 3.94 (s, 3H), 3.85 (s, 3H), 3.77 (m, 5H), 2.70 (m, 1H), 2.11 (m, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 202.7, 153.1, 151.7, 151.2, 150.2, 146.1, 140.8, 130.1, 128.0, 123.6, 109.0, 108.4, 105.7, 60.8, 56.3, 56.0, 51.1, 38.2, 28.6, 19.3; Anal. Calcd for $C_{21}H_{22}O_6$: C, 68.10; H, 5.99. Found: C, 67.63; H, 6.04; HRMS (EI) Calcd for 370.1416, found 370.1414.

4.2. Biology

4.2.1. Tubulin polymerization assay[22,52]

Tubulin was purified from calf brain following a method reported by Hamel and Lin.[52] Polymerization was followed turbidimetrically at 350 nm. IC_{50} values of the various analogs were determined from the data using nonlinear regression analysis with Prism software (GraphPad) 3.02 version.

4.2.2. SRB assay[50,53,54]

Inhibition of human cancer growth was assessed using the National Cancer Institute's standard sulforhodamine B assay, as previously described.[54] Briefly, cells were distributed into 96-well plates, followed by treatment with study compounds and doxorubicin as a control at concentrations between 0.000005 and 50.0 μg/mL at 37 °C for 48 h. Due to its extreme cytotoxicity, compound **4** was screened at concentrations between 0.5×10^{-13} μg/mL and 50.0 μg/mL at 37 °C for 48 h. A growth inhibition of 50% in comparison to untreated controls (GI_{50} or the drug concentrations causing a 50% reduction in net protein increase) was calculated by nonlinear regression analysis.[50,53,54]

Acknowledgments

The authors are grateful to Oxigene Inc. (Waltham, MA, grants to K.G.P. and M.L.T.), the Welch Foundation (Grant No. AA-1278 to

K.G.P.), and the National Science Foundation for funding both the Varian 500 MHz NMR spectrometer (Award CHE-0420802) and the Bruker X8 APEX diffractometer (Grant CHE-0321214). The authors are thankful to Dr. Benon E. Mugabe (former Baylor Graduate student) for performing the tubulin assay for compounds **1** and **2**. The authors would like to thank Dr. Alejandro Ramirez (Mass Spectrometry Core Facility, Baylor University) for HRMS analysis. The authors also thank H&B Packing (Waco, TX) for providing calf brain, and are grateful to Ms. Beverly Herrington (Undergraduate student, Baylor University, 2003) and Mr. Ankur Gupta [Baylor high school summer science research program (HSSSRP), 2003] for their valuable assistance with chemical synthesis.

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