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Structural interrogation of benzosuberene-based inhibitors of tubulin polymerization

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ABSTRACT

The discovery of 3-methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (a benzosuberene-based analogue referred to as **KGP18**) was originally inspired by the natural products colchicine and combretastatin A-4 (**CA4**). The relative structural simplicity and ease of synthesis of **KGP18**, coupled with its potent biological activity as an inhibitor of tubulin polymerization and its cytotoxicity (in vitro) against human cancer cell lines, has resulted in studies focused on new analogue design and synthesis. Our goal was to probe the relationship of structure to function in this class of anticancer agents. A series of twenty-two new benzosuberene-based analogues of **KGP18** was designed and synthesized. These compounds vary in their methoxylation pattern and separately incorporate trifluoromethyl groups around the pendant aryl ring for the evaluation of the effect of functional group modifications on the fused six-membered aromatic ring. In addition, the 8,9-saturated congener of **KGP18** has been synthesized to assess the necessity of unsaturation at the carbon atom bearing the pendant aryl ring. Six of the molecules from this benzosuberene-series of compounds were active (IC_{50} < 5 μM) as inhibitors of tubulin polymerization while four analogues were comparable (IC_{50} approximately 1 μM) in their tubulin inhibitory activity to **CA4** and **KGP18**. The potency of a bis-trifluoromethyl analogue **74** and the unsaturated **KGP18** derivative **73** as inhibitors of tubulin assembly along with their moderate cytotoxicity suggested the potential utility of these compounds as vascular disrupting agents (VDAs) to selectively target microvessels feeding tumors. Accordingly, water-soluble and DMSO-soluble phosphate prodrug salts of each were synthesized for preliminary in vivo studies to assess their potential efficacy as VDAs.
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1. Introduction

As solid tumors grow beyond approximately 1 mm^3 in size, they require an ever larger vascular network to supply oxygen and nutrients to the cells and remove cellular waste products.[1] Since the vasculature feeding tumors tends to grow rapidly to keep up with tumor expansion, it has a tendency to vary in diameter and incorporate bulges and blind ends, rendering it somewhat fragile and chaotic in character.[2–4] The primitive nature of the vascular network in tumors makes it a promising target for cancer therapy.

There are two types of antivascular therapies: angiogenesis inhibiting agents (AIAs) and vascular disrupting agents (VDAs).[5–7] AIAs inhibit the formation of new vasculature in developing tumors, while VDAs damage the already existing tumor vasculature.[6,8,9] VDAs are further subdivided into biologics and small-molecule anticancer agents. Inhibitors of tubulin polymerization represent one class of small-molecule VDAs. These compounds disrupt the tubulin-microtubule protein system and cause structural changes to the endothelial cells lining the vasculature, in response to cell signaling events. These morphological changes eventually lead to irreversible damage to the tumor vasculature, thus starving

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the tumor of nutrients and oxygen, ultimately leading to tumor necrosis.[10–19]

One class of VDAs interact with the colchicine site on β-tubulin near the α,β-tubulin heterodimer interface. Two of the most potent colchicine site binding VDAs are the natural products combretastatin A-4 (**CA4**)[20] and combretastatin A-1 (**CA1**)[21] originally isolated from *Combretum caffrum*, the South African bushwillow tree (Fig. 1). The corresponding phosphate prodrug salts combretastatin A-4P (**CA4P**)[22,23] and combretastatin A-1P (**CA1P**)[15] have improved water solubility and have been extensively evaluated in both preclinical experiments and clinical studies in humans.[24–29]

Significant structural similarities exist among the natural products colchicine, **CA4**, and **CA1** (Fig. 1), including a trimethoxy phenyl ring, a separate *p*-methoxy phenyl moiety, and bridging functionality connecting the two rings at a comparable centroid to centroid distance. The relative structural simplicity of **CA4** has inspired the synthesis of a vast array of synthetic analogues and derivatives in which both aryl rings and the ethylene bridge have been structurally modified. An early initial molecular design paradigm led us to utilize clinically relevant non-steroidal, selective estrogen receptor modulators (SERMs) and related compounds as molecular templates modified to mimic colchicine and **CA4**.[37–39] This led us to the discovery and establishment of benzo[*b*]thiophene,[40–42] benzofuran,[42,43] dihydronaphthalene,[30–32] benzosuberene,[30–34] and indole-based[35] analogues as potent inhibitors of tubulin polymerization (Fig. 1). Two benzosuberene analogues (referred to as **KGP18**[30,33] and its amino congener **KGP156**[31,34]) are especially promising anticancer agents based, in part, on their pronounced cytotoxicity against human cancer cell lines and their efficacy as inhibitors of tubulin polymerization. Our previous studies in this area[30,33,34] resulted in two separate synthetic strategies towards the pendant 9-aryl, fused six-seven ring system present in the benzosuberene analogues **KGP18** and **KGP156**. These studies included a variety of functional group modifications designed to probe structural diversity as it relates to biological function. Inspired by our original work with these and related benzosuberene analogues, Maderna and co-workers at Pfizer developed a separate synthetic approach utilizing a ring-closing metathesis (RCM) reaction to form the benzosuberene molecular core and a Suzuki coupling to install the pendant aryl ring.[44] They prepared and evaluated a series of structurally diverse benzosuberene analogues.[45] Using **KGP18** and **KGP156** as models, we developed a series of analogues to analyze further functional group modifications for their effects on cytotoxicity and inhibition of tubulin polymerization.

2. Results and discussion

2.1. Synthesis

Twenty-two benzosuberene analogues (Fig. 2) were synthesized and evaluated for both their ability to inhibit tubulin polymerization and for their in vitro cytotoxicity against selected human cancer cell lines. Structural modifications to the R_1 and R_2 positions of the fused aryl ring as well as the pendant aryl ring were explored in order to evaluate their impact on tubulin dynamics and cytotoxicity. The synthesis of each benzosuberene analogue involved a Wittig olefination reaction followed by hydrogenation to afford carboxylic acid derivatives **7**–**12**. An intramolecular Friedel–Crafts annulation facilitated by Eaton's reagent (7.7 weight percent P_2O_5 in CH_3SO_3H)[46,47] yielded benzosuberone analogues **13**–**18**, which were subsequently treated with requisite aryl-lithium reagents to generate tertiary alcohols **19**–**27**, which upon dehydration afforded the final benzosuberene analogues **28**–**36** (Scheme 1).

Concomitant elimination accompanied the addition of 4-methoxyphenyl lithium, which resulted in benzosuberene analogue **37** (Scheme 2).

Benzosuberone analogues **13** and **14** were also subjected to a demethylation reaction using the ionic liquid [TMAH][Al_2Cl_7][48] to afford phenolic derivatives **38**–**40**. We previously demonstrated the regioselective demethylation of compound **13**.[33,48] Protection of the phenolic moieties as their corresponding *tert*-butyldimethylsilyl (TBS) ethers followed by nucleophilic addition with an appropriately substituted lithiated aryl ring produced tertiary alcohols **44**–**50**, which were subsequently dehydrated to yield TBS protected benzosuberene analogues **51**–**57** (Scheme 3). Removal of the TBS protecting groups upon treatment with TBAF resulted in benzosuberene analogues **64**–**70** (Scheme 4).

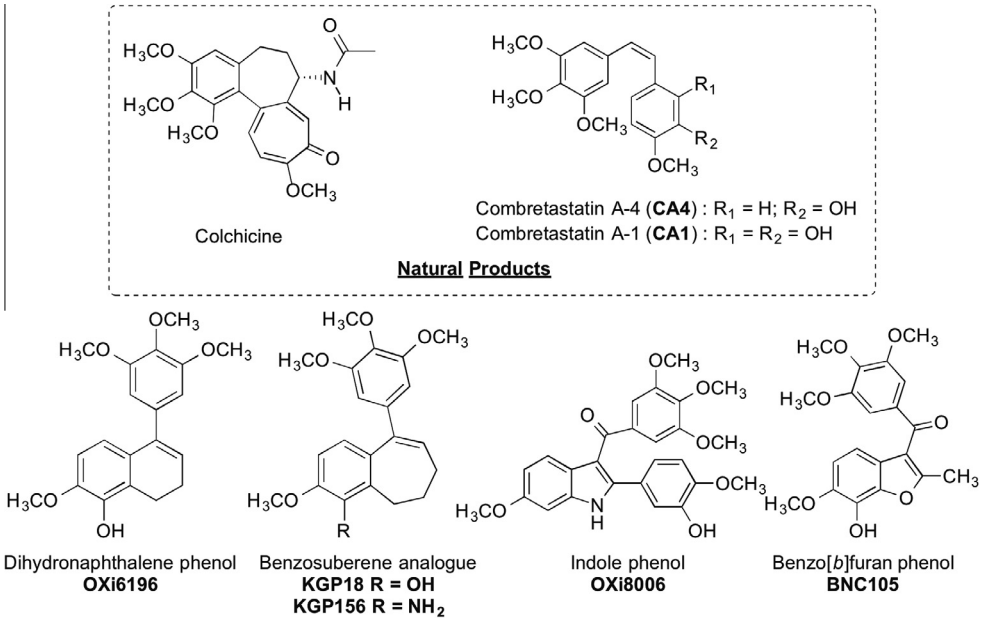


Figure 1. Representative small-molecule inhibitors of tubulin polymerization: colchicine, combretastatins (**CA4**, **CA1**),[20,21] dihydronaphthalene analogue (**OXi6196**),[30–32] benzosuberene analogues (**KGP18** and **KGP156**),[30,33,34] indole analogue (**OXi8006**)[35] and benzo[*b*]furan analogue (**BNC105**).[36]

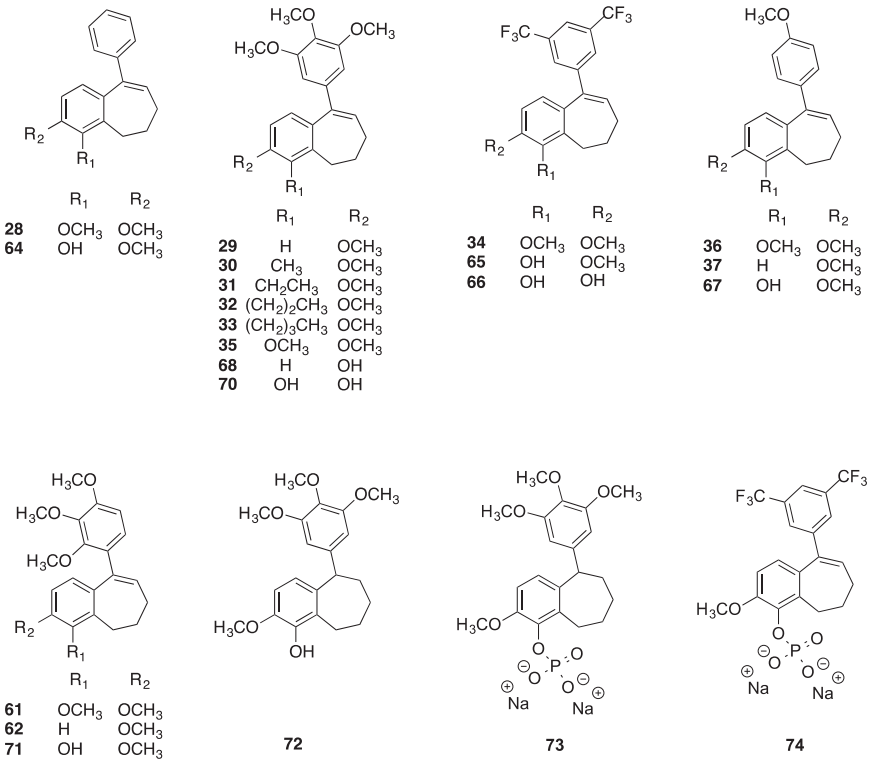


Figure 2. Compilation of synthesized benzosuberene analogues.

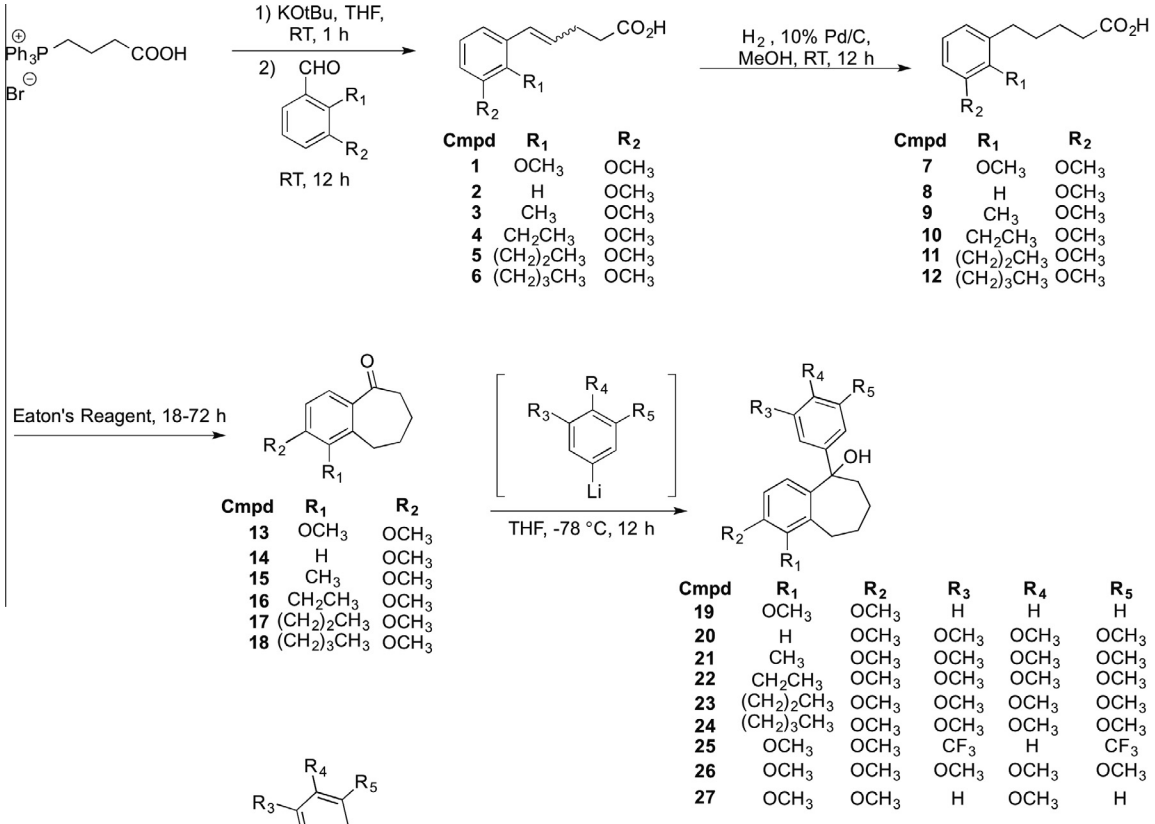
In our hands, desired benzosuberene analogues **61**, **62**, and **71** were not accessible by the methodology involving aryl lithium addition, instead yielding recovered starting material. It is possible that competing enolate formation was faster than 1,2-carbonyl addition in these cases. Alternatively, the synthesis of analogues **61**, **62**, and **71** was accomplished using a Suzuki coupling to attach the pendant aryl ring, similar to the methodology of Maderna and co-workers.[44] Vinyl triflates **58**, **59**, and **60** were reacted with the corresponding boronic acids to generate target benzosuberene analogues **61** and **62** and TBS protected compound **63**, which, after deprotection with TBAF, afforded benzosuberene analogue **71** (Scheme 4).

The double bond of benzosuberene **69** (**KGP18**) was reduced to afford benzosuberane analogue **72**, which was subsequently converted to its corresponding phosphate salt **73**. Benzosuberene analogue **65** was also converted to its phosphate prodrug salt **74** under analogous reaction conditions (Scheme 5).

2.2. Biological evaluation

Each of the twenty-two analogues was evaluated biologically for its ability to inhibit tubulin polymerization (cell free assay), as well as its cytotoxicity against human cancer cell lines [SK-OV-3 (ovarian), NCI-H460 (lung), and DU-145 (prostate)]. It is important to note that these two assays, while providing complementary structure activity relationship (SAR) information, represent very different approaches to analyzing the biological activity of the target benzosuberene analogues. It is common for biologically active, small-molecule inhibitors of tubulin polymerization to demonstrate IC_{50} values in the low micromolar range (in this type of cell free assay) while demonstrating sub-micromolar to nanomolar GI_{50} values in terms of cytotoxicity against human cancer cell lines. This activity differential may be due, in part, to requisite stoichiometry differences in regard to the number of molecules of inhibitor bound to tubulin in a cell-based assay versus a pure protein assay, as well as practical assay limits (in the low micromolar range) in the pure protein assay (no cells and no additional microtubule-associated proteins). Inhibitor generated interference within the dynamic that is inherent to the tubulin-microtubule protein system in cells may influence signal transduction leading to an amplification of activity in cell-based assays.[35,49] An initial structure–activity analysis for the 22 compounds for which we obtained biological data (Table 1), is perhaps best accomplished by focusing on the studies on the presumptive intracellular target, tubulin. The most extensive data were obtained for effects on tubulin assembly, in part because we have rarely observed substantial inhibition of colchicine binding (>50%, with 5 μM inhibitor) if the assembly IC_{50} is >3 μM.

Ten of the evaluated compounds were active inhibitors of tubulin polymerization (IC_{50} values <20 μM). Structural variability was tolerated (in terms of retained tubulin inhibitory activity) by the incorporation of several groups (H, OCH_3, CH_3) at R_1 while the pendant 3,4,5-trimethoxyaryl motif reminiscent of **CA4** and colchicine was maintained. This mirrored our previous observations with other functional groups [OH (parent **KGP18**), NH_2 (parent **KGP156**), Br, Cl] situated at R_1 in this same molecular template. Replacement of the R_2 methoxy group with a hydroxyl group, while either maintaining R_1 = OH or modifying R_1 to be a hydrogen atom, led to analogues (**70** and **68**, respectively) that were also active inhibitors of tubulin polymerization. Variation of the methoxylation pattern (2,3,4-trimethoxy) within the pendant aryl ring also led to active inhibitors of tubulin polymerization when R_1 was H or OH (**62** and **71**, respectively). Replacement of the trimethoxyaryl ring with either a 3,5-bis-trifluoromethyl aryl ring or a 4-methoxyaryl ring with maintenance of R_1 = OH (compounds **65** and **67**, respectively) resulted in benzosuberene analogues that were still inhibitory against tubulin polymerization. Intriguingly, the double-bond reduced analogue **72** was the most active

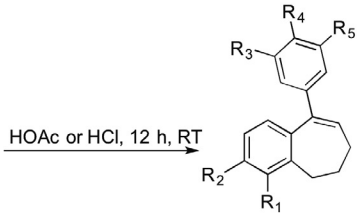


Cmpd	R_1	R_2
1	OCH_3	OCH_3
2	H	OCH_3
3	CH_3	OCH_3
4	CH_2CH_3	OCH_3
5	$(CH_2)_2CH_3$	OCH_3
6	$(CH_2)_3CH_3$	OCH_3

Cmpd	R_1	R_2
7	OCH_3	OCH_3
8	H	OCH_3
9	CH_3	OCH_3
10	CH_2CH_3	OCH_3
11	$(CH_2)_2CH_3$	OCH_3
12	$(CH_2)_3CH_3$	OCH_3

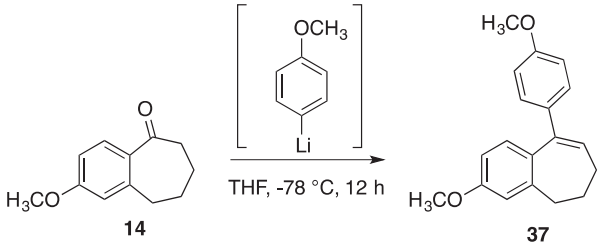
Cmpd	R_1	R_2
13	OCH_3	OCH_3
14	H	OCH_3
15	CH_3	OCH_3
16	CH_2CH_3	OCH_3
17	$(CH_2)_2CH_3$	OCH_3
18	$(CH_2)_3CH_3$	OCH_3

Cmpd	R_1	R_2	R_3	R_4	R_5
19	OCH_3	OCH_3	H	H	H
20	H	OCH_3	OCH_3	OCH_3	OCH_3
21	CH_3	OCH_3	OCH_3	OCH_3	OCH_3
22	CH_2CH_3	OCH_3	OCH_3	OCH_3	OCH_3
23	$(CH_2)_2CH_3$	OCH_3	OCH_3	OCH_3	OCH_3
24	$(CH_2)_3CH_3$	OCH_3	OCH_3	OCH_3	OCH_3
25	OCH_3	OCH_3	CF_3	H	CF_3
26	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
27	OCH_3	OCH_3	H	OCH_3	H



Cmpd	R_1	R_2	R_3	R_4	R_5
28	OCH_3	OCH_3	H	H	H
29	H	OCH_3	OCH_3	OCH_3	OCH_3
30	CH_3	OCH_3	OCH_3	OCH_3	OCH_3
31	CH_2CH_3	OCH_3	OCH_3	OCH_3	OCH_3
32	$(CH_2)_2CH_3$	OCH_3	OCH_3	OCH_3	OCH_3
33	$(CH_2)_3CH_3$	OCH_3	OCH_3	OCH_3	OCH_3
34	OCH_3	OCH_3	CF_3	H	CF_3
35	OCH_3	OCH_3	OCH_3	OCH_3	OCH_3
36	OCH_3	OCH_3	H	OCH_3	H

Scheme 1. Synthesis of benzosuberene analogues **28**–**36**.

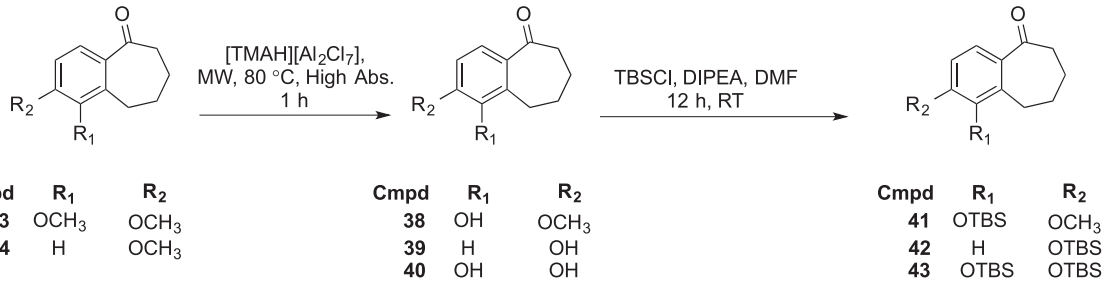


Scheme 2. Synthesis of benzosuberene analogue **37**.

inhibitor of tubulin assembly within the entire series of benzosuberene analogues analyzed. Loss of inhibition of tubulin polymerization was observed when the trimethoxy aryl substituent was replaced with an unsubstituted phenyl ring (**28** and **64**). While the R_1 methyl analogue **30** functioned as an inhibitor of tubulin polymerization, extension of the alkyl chain to ethyl, propyl, and butyl resulted in the loss of inhibitory activity (as observed with compounds **31**, **32**, and **33**). While ten of the analogues inhibited tubulin assembly, only **29**, **30**, **62**, and **72** demonstrated inhibition values (1.0 μM, 1.6 μM, 1.2 μM, and 0.70 μM, respectively) comparable to those observed with lead compounds **KGP18** and **CA4**.

Molecular docking studies were carried out on several analogues that were active inhibitors of tubulin polymerization (compounds **29**, **62** and **72**), and compared to compound **33** with an IC_{50} >20 μM (inactive) in this assay. Docking placed the trimethoxyphenyl ring of all three active analogues in a similar position to that of the trimethoxyphenyl moiety of *N*-deacetyl-*N*-(2-mercaptoacetyl)-colchicine in the structure co-crystallized with tubulin. In contrast, modeling placed multiple top conformations of compound **33** with its trimethoxyphenyl ring outside of this pocket (see Supplementary data).

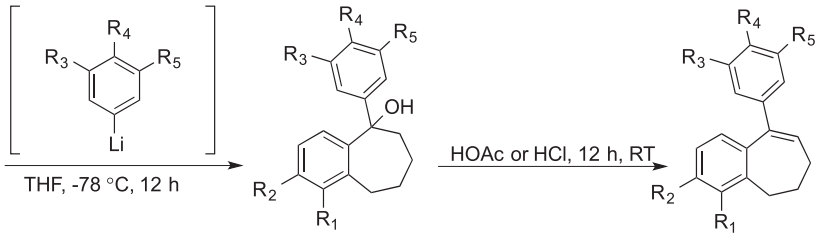
Among the twenty-two benzosuberene analogues investigated in this study, the most cytotoxic agents were compounds **29** and **62** (for example, GI_{50} = 0.0516 μM and 0.0432 μM, respectively,



Cmpd	R_1	R_2
13	OCH_3	OCH_3
14	H	OCH_3

Cmpd	R_1	R_2
38	OH	OCH_3
39	H	OH
40	OH	OH

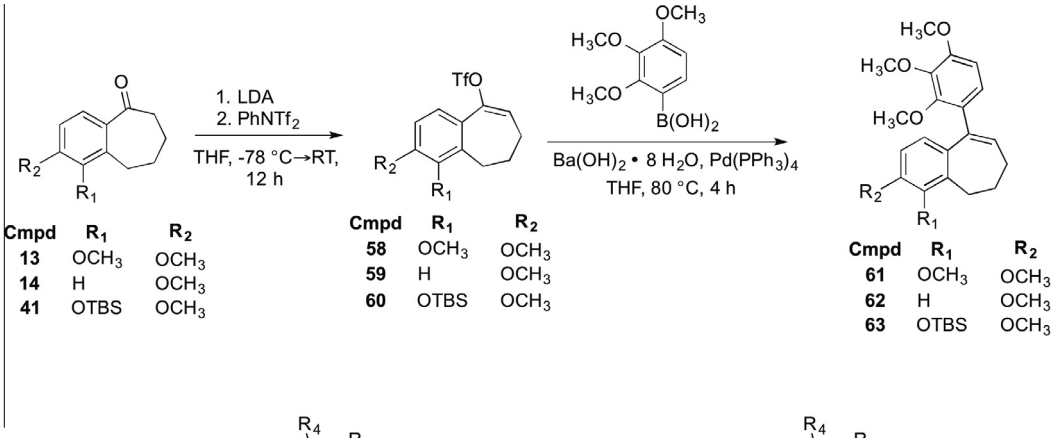
Cmpd	R_1	R_2
41	OTBS	OCH_3
42	H	OTBS
43	OTBS	OTBS



Cmpd	R_1	R_2	R_3	R_4	R_5
44	OTBS	OCH_3	H	H	H
45	OTBS	OCH_3	CF_3	H	CF_3
46	OTBS	OTBS	CF_3	H	CF_3
47	OTBS	OCH_3	H	OCH_3	H
48	H	OTBS	OCH_3	OCH_3	OCH_3
49	OTBS	OCH_3	OCH_3	OCH_3	OCH_3
50	OTBS	OTBS	OCH_3	OCH_3	OCH_3

Cmpd	R_1	R_2	R_3	R_4	R_5
51	OTBS	OCH_3	H	H	H
52	OTBS	OCH_3	CF_3	H	CF_3
53	OTBS	OTBS	CF_3	H	CF_3
54	OTBS	OCH_3	H	OCH_3	H
55	H	OTBS	OCH_3	OCH_3	OCH_3
56	OTBS	OCH_3	OCH_3	OCH_3	OCH_3
57	OTBS	OTBS	OCH_3	OCH_3	OCH_3

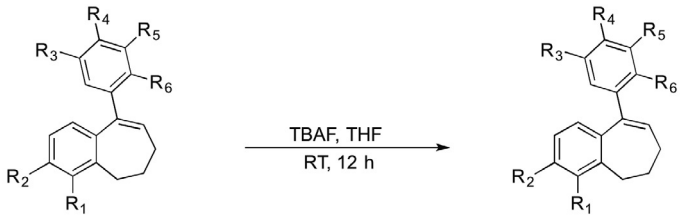
Scheme 3. Synthesis of TBS protected analogues **51**–**57**.



Cmpd	R_1	R_2
13	OCH_3	OCH_3
14	H	OCH_3
41	OTBS	OCH_3

Cmpd	R_1	R_2
58	OCH_3	OCH_3
59	H	OCH_3
60	OTBS	OCH_3

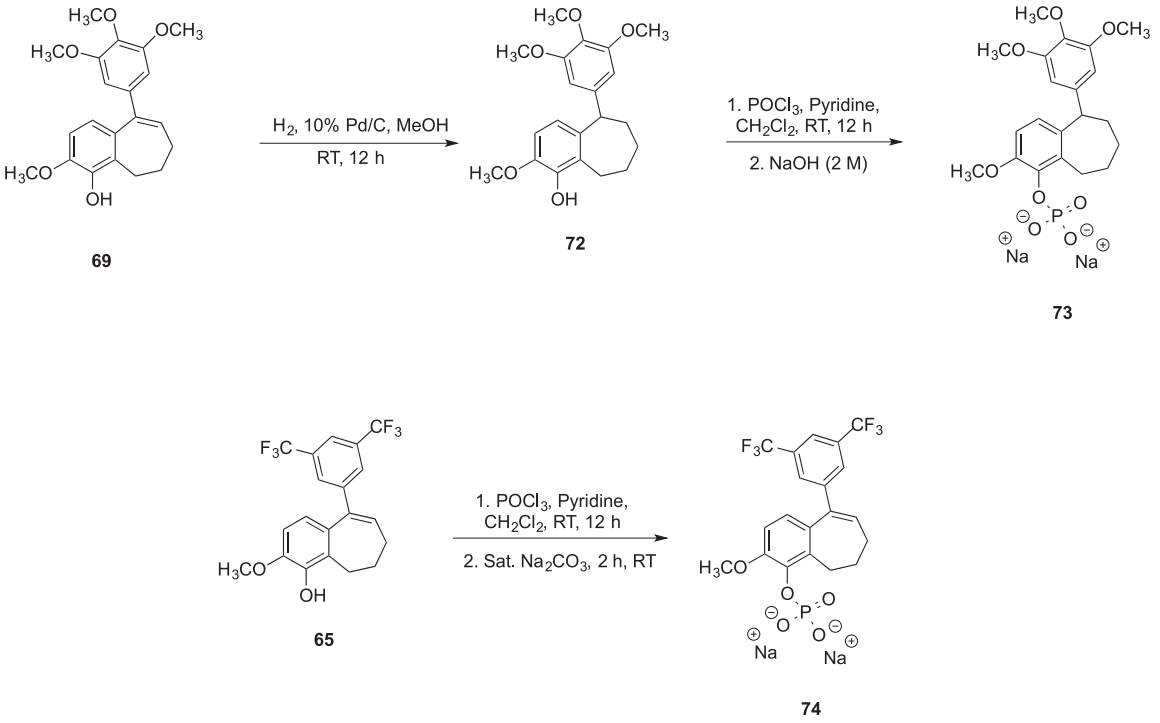
Cmpd	R_1	R_2
61	OCH_3	OCH_3
62	H	OCH_3
63	OTBS	OCH_3



Cmpd	R_1	R_2	R_3	R_4	R_5	R_6
51	OTBS	OCH_3	H	H	H	H
52	OTBS	OCH_3	CF_3	H	CF_3	H
53	OTBS	OTBS	CF_3	H	CF_3	H
54	OTBS	OCH_3	H	OCH_3	H	H
55	H	OTBS	OCH_3	OCH_3	OCH_3	H
56	OTBS	OCH_3	OCH_3	OCH_3	OCH_3	H
57	OTBS	OTBS	OCH_3	OCH_3	OCH_3	H
63	OTBS	OCH_3	H	OCH_3	OCH_3	OCH_3

Cmpd	R_1	R_2	R_3	R_4	R_5	R_6
64	OH	OCH_3	H	H	H	H
65	OH	OCH_3	CF_3	H	CF_3	H
66	OH	OH	CF_3	H	CF_3	H
67	OH	OCH_3	H	OCH_3	H	H
68	H	OH	OCH_3	OCH_3	OCH_3	H
69	OH	OCH_3	OCH_3	OCH_3	OCH_3	H
70	OH	OH	OCH3	OCH_3	OCH_3	H
71	OH	OCH_3	H	OCH_3	OCH_3	OCH_3

Scheme 4. Synthesis of benzosuberene analogues **61**, **62**, and **64**–**71**.



Scheme 5. Synthesis of reduced benzosuberane analogue **72** and phosphate salts **73** and **74**.

Table 1
Inhibition of tubulin polymerization, percent inhibition of colchicine binding, and cytotoxicity of the target benzosuberene 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]	nr	0.00119	0.00194[c]	0.00323[c]
KGP18	1.4[d]	nr	0.0000543[e]	0.0000418[e]	0.0000249[e]
28	>20	nr	32.7	37.5	89.3
29	1.0 ± 0.02	37 ± 5 (1 μM), 72 ± 0.8 (5 μM)	0.0516	0.0527	0.0619
30	1.6 ± 0.2	65 ± 0.6 (5 μM)	0.330	0.422	0.644
31	>20	nr	0.568	0.763	1.51
32	>20	nr	2.96	3.32	6.03
33	>20	nr	11.5	16.1	12.2
34	>20	nr	31.1	25.5	52.1
35	3.1 ± 0.03	30 ± 4 (5 μM), 56 ± 4 (50 μM)	0.277	0.593	0.708
36	>20	nr	20.5	33.4	48.3
37	>20	nr	40.7	57.7	68.7
61	>20	nr	6.96	10.5	26.2
62	1.2 ± 0.007	36 ± 5 (1 μM), 69 ± 3 (5 μM)	0.0432	0.120	0.0562
64	>20	nr	0.557	0.652	4.40
65	3.8 ± 0.3	8.5 ± 4 (5 μM), 37 ± 5 (50 μM)	4.81	4.39	4.92
66	>20	nr	16.8	25.0	21.8
67	7.4 ± 0.06	nr	18.4	10.6	8.59
68	2.7 ± 0.1	27 ± 5 (5 μM)	0.527	0.647	1.02
70	7.7 ± 0.2	nr	0.346	0.691	1.53
71	11 ± 0.4	nr	3.53	4.24	7.54
72	0.70 ± 0.1	21 ± 0.9 (1 μM), 67 ± 0.6 (5 μM)	0.408	0.141	0.570
73	>20	nr	0.357	0.145	0.753
74	>20	nr	17.2	16.3	17.5

nr = not reported.
[a] Average of $n \geqslant 3$ independent determinations.
[b] Data from Ref. 50.
[c] For additional data, see Ref. 50.
[d] Data from Ref. 33.
[e] For additional data, see Ref. 30.

against the SK-OV-3 cell line). Both of these compounds bear trimethoxy aryl groups, but, unlike **KGP18**, they each contain a hydrogen atom at the R_1 position, rather than a hydroxyl group. The cytotoxicity, inhibition of colchicine binding, and the inhibition of tubulin polymerization correlate well for these compounds. Compounds **31** and **64** were not inhibitors of tubulin polymerization (IC_{50} >20 μM), but they were found to be cytotoxic (GI_{50} <1 μM) against two of the three cell lines utilized in this study. Although

these compounds are structurally similar to others in this library, they may have an alternate mechanism of inhibiting cell growth. We note the strong antitubulin activity of compound **72**, in which the double bond in the seven-membered fused ring was reduced, although this modification appears to be associated with reduced cytotoxicity. This reduction in cytotoxicity (for compound **72**) correlates with a decrease in the percent inhibition of colchicine binding (at 1 μM) relative to compounds **29** and **62**, although all three compounds (**29**, **62**, and **72**) are comparable in the colchicine binding assay at 5 μM.

Two compounds, **65** and the strong tubulin inhibitor **72**, were selected for conversion to prodrugs (**74** and **73**, respectively) by phosphorylation, in order to improve water-solubility and potentially bioavailability. As with **CA4** (phosphorylated to **CA4P**), this synthetic transformation eliminated (IC_{50} >20 μM) the ability to inhibit tubulin polymerization (cell-free assay), while the cytotoxicity was maintained presumably due to phosphatase activity present in the cell-based assay.

We previously demonstrated that dynamic bioluminescence imaging (BLI) provides a facile indication of vascular disruption in luciferase transfected tumors.[7,51,52] Analysis is noninvasive, and each tumor acts as its own control. Specifically, the substrate luciferin normally diffuses into the blood stream following subcutaneous injection, and, when it reaches a tumor, light emission occurs. Vascular disruption impairs delivery, and reduced light emission is observed. Analogue **73** showed no obvious acute toxicity over 24 h to breast tumor bearing SCID mice following IP administration of saline solutions delivering doses up to 40 mg/kg. Doses of 20 or 30 mg/kg showed a similar modest reduction of BLI signal 4 h after administration. At 40 mg/kg there was approximately a 50% reduction of the emitted signal. In each case, the signal generally returned to its original level within 24 h. By comparison, saline controls showed a highly reproducible signal, and **CA4P** (a well-established VDA in clinical development) showed greater than 90% reduction at 4 h, which remained depressed (>75%, after 24 h). These data are presented in Figures 3 and 4. Patterns of light emission are presented in Figure 5. The signal intensity for saline emphasizes reproducibility of signal (hence assessment of vasculature) for repeat measurements. As reported previously, **CA4P**[7,51,52] caused significant vascular impairment at 4 h. Compound **73** had little effect at 20 or 30 mg/kg, but caused about 50% reduction in light emission at 40 mg/kg. While these initial, preliminary studies at 20, 30, and 40 mg/kg suggested a potential VDA mechanism for analogue **73**, future dose escalation studies will be necessary to establish a maximum tolerated dose (MTD) in this mouse model and to confirm the extent to which analogue **73** is capable of disrupting tumor-associated vasculature.

3. Conclusion

In summary, the results of these experiments have expanded our SAR knowledge regarding effects that modifications of the benzosuberene skeleton play in relationship to cytotoxicity and antitubulin activity. The most promising analogues evaluated in this study demonstrated inhibition of tubulin assembly comparable to **CA4** and **KGP18**, but these compounds had reduced inhibitory effects on colchicine binding and on cell growth. Preliminary in vivo BLI evaluation of the VDA capability of benzosuberene analogue **73** against an MDA-MB-231-luc xenograft (in a SCID mouse model) showed efficacy, but, at the doses examined, the effect of **73** was less pronounced than that obtained with an established VDA (in this case, **CA4P**) currently in clinical development. Future studies with compound **73** and related benzosuberene analogues involving dose escalation and other tumor models to assess vascular damage appear warranted.

4. Experimental section

4.1. Chemistry

4.1.1. General materials and methods

Tetrahydrofuran (THF), dichloromethane, ethanol, methanol, dimethylformamide (DMF), and acetonitrile were used in their anhydrous forms. Reactions were performed under nitrogen gas, unless otherwise specified. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F254, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a Biotage Isolera or Teledyne Combiflash 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 ^{1}H NMR (500 or 300 MHz), ^{13}C NMR (125 or 75 MHz), ^{19}F (470 MHz) and ^{31}P NMR (200 or 120 MHz) spectroscopic data using a Varian VNMRS 500 MHz or a Bruker DPX 300 MHz instrument. Spectra were recorded in $CDCl_3$, D_2O, $(CD_3)_2CO$, 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 (λ = 190–400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm Å~150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; Method A: solvent A, acetonitrile, solvent B, 0.1% TFA in H_2O; or Method B: solvent A, acetonitrile, solvent B, H_2O; gradient, 10% A/90% B to 100% A/0% B over 0–40 min; post-time 10 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 210, 230, 254, 280, and 320 nm. Mass spectrometry was carried out under positive or negative ESI (electrospray ionization) using a Thermo Scientific LTQ OrbitrapDiscovery instrument.

4.1.1.1. 5-(2′,3′-Dimethoxyphenyl)pent-4-enoic acid (1).[31,33] To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (13.04 g, 30.39 mmol) in THF (500 mL) was added potassium *tert*-butoxide (7.43 g, 66.2 mmol), and the reaction mixture was stirred at room temperature for 1 h. 2,3-Dimethoxybenzaldehyde (5.02 g, 30.1 mmol) dissolved in THF (100 mL) was added, and the mixture was stirred at room temperature for 12 h. The THF was removed under reduced pressure, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were evaporated under reduced pressure, and the crude reaction 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 → 50% A/50% B (10 CV), 50% A/50% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford compound **1** (5.39 g, 21.5 mmol, 72%) as a yellow oil. NMR characterization was conducted after the next step.

4.1.1.2. 5-(2′,3′-Dimethoxyphenyl)pentanoic acid (7).[31,33,53] To dissolved carboxylic acid **1** (5.39 g, 21.5 mmol) in methanol (100 mL) was added 10% palladium on carbon (0.43 g) and 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 × 50 mL). The combined organic phase (MeOH 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: 40 mL/min;

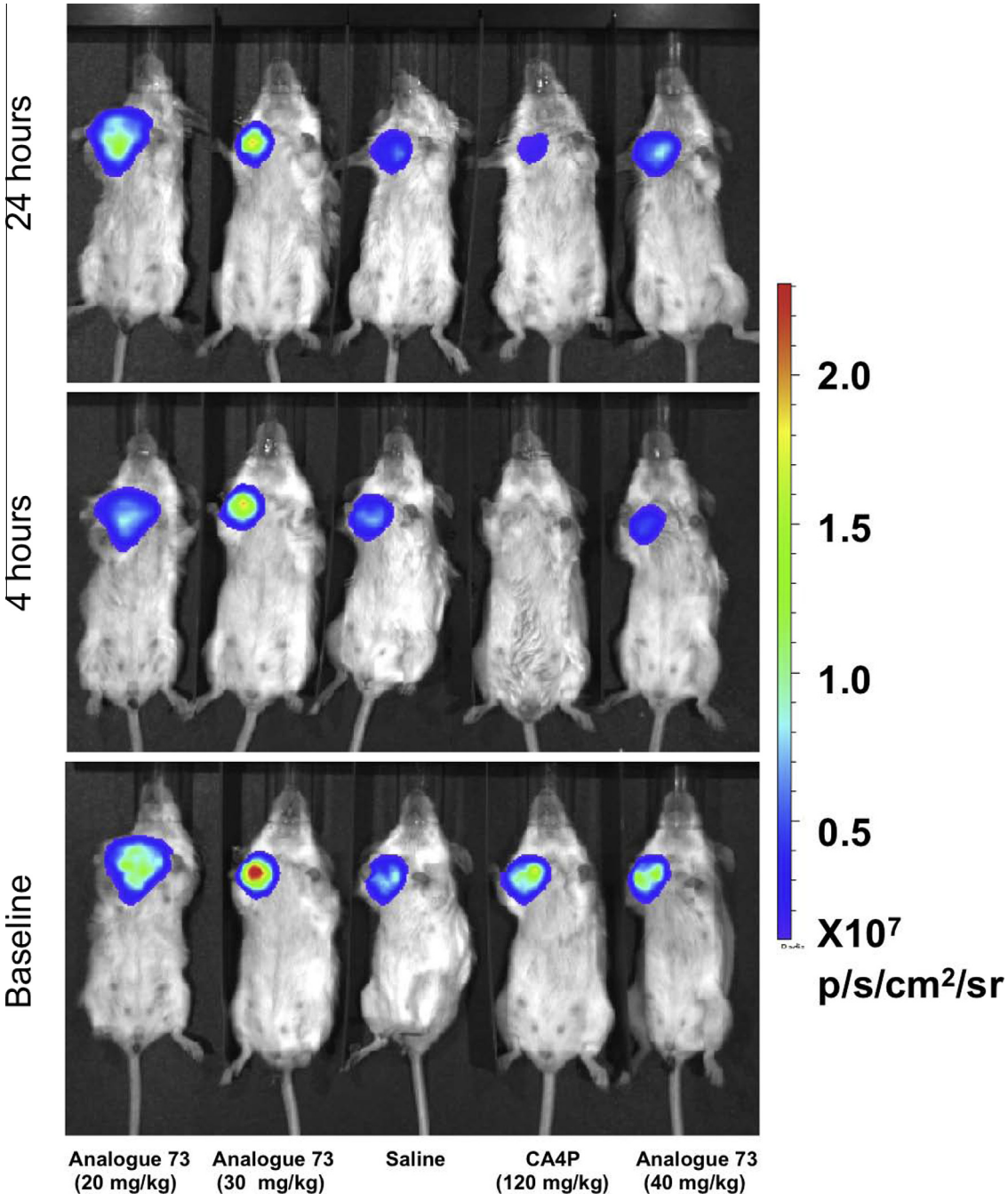


Figure 3. BLI assessment of vascular disruption caused in MDA-MB-231-luc orthotopic human tumor xenografts by analogue **73**. Dynamic BLI was performed at baseline (bottom row), 4 h after VDA administration (middle), and after 24 h (top), and images are shown for representative mice 17 min after administering fresh luciferin substrate on each occasion to each animal. Images show bioluminescent signal intensity overlaid on photographs of the mice. Analogue **73** was administered at 20, 30 or 40 mg/kg ip in saline, and additional mice received saline control or **CA4P** (120 mg/kg) for comparison. Analogue **73** caused a reduced signal at all doses at 4 h with substantial recovery by 24 h.

monitored at 254 and 280 nm] to afford carboxylic acid **7** (4.45 g, 18.7 mmol, 82%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.67 (1H, s), 6.99 (1H, t, J = 8 Hz), 6.78 (2H, m), 3.86 (3H, s), 3.84 (3H, s), 2.68 (2H, t, J = 8 Hz), 2.41 (2H, t, J = 7.5 Hz), 1.70 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.2, 152.7, 147.1, 135.8, 123.8, 121.9, 110.2, 60.6, 55.6, 34.0, 30.8, 29.4, 24.5.

4.1.1.3. 1,2-Dimethoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (13).[31,33,54] To carboxylic acid **7** (3.55 g, 14.9 mmol) was added Eaton's reagent (29 mL, 3 g per mmol of compound **7**), and the reaction mixture was stirred at room temperature for 12 h. It was then poured over ice and neutralized with sodium bicarbonate. The reaction mixture was extracted with EtOAc (3 × 50 mL), and the combined organic phase was dried over sodium sulfate and evaporated under reduced pressure. The reaction product was purified by flash chromatography using a prepacked 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: 40 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **13** (2.43 g, 11.0 mmol, 74%) as a light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (1H, d, J = 8.5 Hz), 6.67 (1H, d, J = 9 Hz), 3.72 (3H, s), 3.62 (3H, s), 2.83

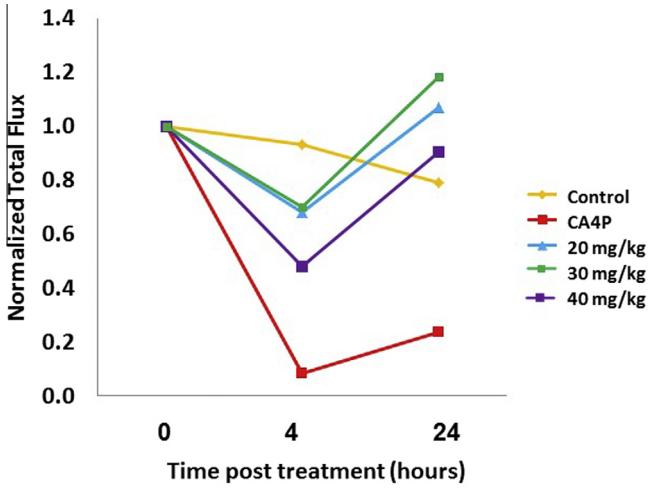


Figure 4. Vascular disruption caused by analogue **73**. Relative signal intensity is plotted for the mice shown in Figure 3.

(2H, t, J = 6 Hz), 2.50 (2H, t, J = 6 Hz), 1.66 (2H, p, J = 6.5 Hz), 1.59 (2H, p, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.2, 155.9, 145.8, 135.5, 132.6, 125.2, 109.6, 60.8, 55.6, 40.4, 24.7, 23.0, 20.7.

4.1.1.4. [TMAH][Al_2Cl_7].[48] To dry dichloromethane (150 mL) was added $AlCl_3$ (19.84 g, 149.1 mmol), and the mixture was stirred and cooled to 0 °C. Trimethylamine hydrochloride (7.11 g, 74.5 mmol) was added, and the reaction mixture was allowed to stir for 2 h at room temperature. The resulting liquid was stored at room temperature under nitrogen.

4.1.1.5. 1-Hydroxy-2-dimethoxy-6,7,8,9-tetrahydro-5*H*-benzo [7]annulen-5-one (38).[31,33,55] To benzosuberone **13** (1.01 g, 4.54 mmol) in a 20 mL microwave vial was added [TMAH][Al_2Cl_7] (18.3 mL, 9.08 mmol), and the mixture was subjected to microwave irradiation for 1 h at 80 °C on high absorbance. The solution was then poured into water (50 mL) and extracted with EtOAc (3 × 25 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 → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **38** (0.61 g, 3.0 mmol, 65%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (1H, d, J = 8.5 Hz), 6.67 (1H, d, J = 9 Hz), 6.26 (1H, s), 3.78 (3H, s), 2.93 (2H, t, J = 5.5 Hz), 2.61 (2H, t, J = 6.5 Hz), 1.71 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.0, 149.5, 142.5, 133.0, 127.8, 120.7, 107.9, 55.9, 40.6, 24.4, 23.0, 21.2.

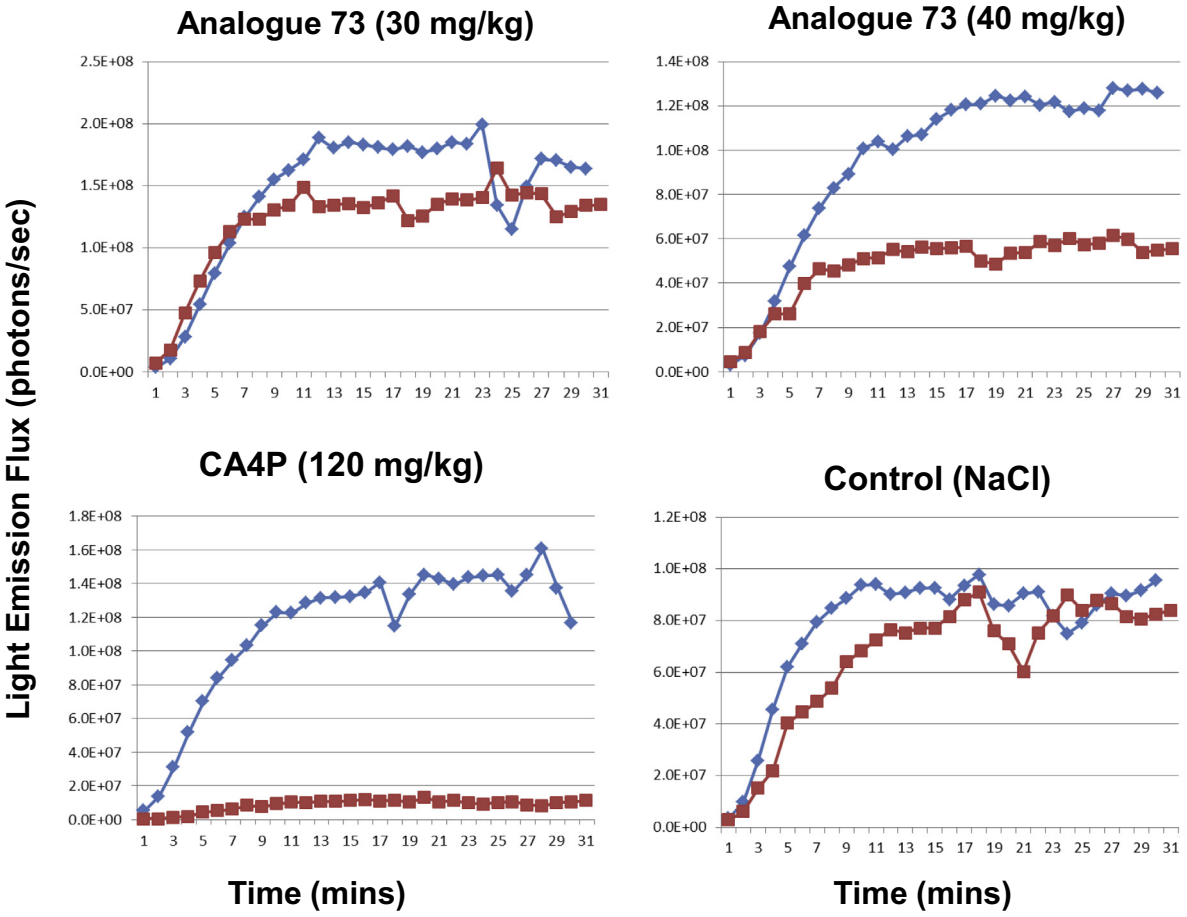


Figure 5. Dynamic bioluminescence with respect to vascular disruption. Graphs show evolution of light emission from individual MDA-MB-231-luc tumors following administration of luciferin substrate subcutaneously in the fore-back region of each mouse at baseline (blue) and 4 h after administration of agent (red). Analogue **73** had a modest effect at 30 mg/kg and a greater effect at 40 mg/kg. By comparison control saline showed a high degree of reproducibility and **CA4P** showed >90% reduction in light emission.

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4.1.1.6. 1-((*tert*-Butyldimethylsilyl)oxy)-2-dimethoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (41).[31,33] Benzosuberone **38** (2.16 g, 10.5 mmol) was dissolved in dimethylformamide (50 mL). TBSCl (3.16 g, 21.0 mmol) and DIPEA (5.50 mL, 31.6 mmol) were added, and the solution was stirred for 12 h at room temperature. The reaction mixture was washed with water and extracted with EtOAc (5 × 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 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 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 TBS protected analogue **41** (2.37 g, 7.38 mmol, 71%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (1H, d, J = 8.5 Hz), 6.62 (1H, d, J = 9 Hz), 3.67 (3H, s), 2.88 (2H, t, J = 5.5 Hz), 2.53 (2H, t, J = 5.5 Hz), 1.64 (4H, m), 0.89 (9H, s), 0.06 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.3, 152.9, 141.6, 132.9, 132.7, 122.2, 108.6, 54.6, 40.5, 26.0, 24.6, 23.8, 21.1, 18.8, −4.0.

4.1.1.7. 4-((*tert*-Butyldimethylsilyl)oxy)-3-methoxy-6,7-dihydro-5*H*-benzo[7]annulen-9-yl trifluoromethanesulfonate (60). To an oven-dried flask, diisopropylamine (0.18 mL, 1.3 mmol) dissolved in THF (50 mL) was added and cooled to −78 °C, and *n*-BuLi (0.51 mL, 1.3 mmol) was added. The reaction mixture was stirred for 15 min. TBS protected **41** (0.37 g, 1.2 mmol) dissolved in THF (10 mL) was added dropwise, and the reaction mixture was stirred for 2 h at −78 °C. *N*-Phenyl-bis(trifluoromethanesulfonimide) (0.45 g, 1.3 mmol) dissolved in THF (10 mL) was then added dropwise, and the reaction mixture was stirred for 12 h while warming from −78 °C to room temperature. After 12 h, the THF was evaporated under reduced pressure, and the resulting solid was washed with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 20% A/80% B (10 CV), 20% A/80% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford triflate **60** (0.17 g, 0.38 mmol, 33%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 (1H, d, J = 8.5 Hz), 6.79 (1H, d, J = 8.5 Hz), 6.09 (1H, t, J = 6.5 Hz), 3.82 (3H, s), 2.88 (2H, t, J = 6 Hz), 2.15 (2H, q, J = 6.5 Hz), 2.03 (2H, p, J = 6.5 Hz), 1.03 (9H, s), 0.20 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.8, 146.5, 132.9, 130.9, 129.9, 126.0, 120.9, 119.7, 108.8, 54.7. 30.5, 26.0, 25.0, 24.5, 18.9, 4.0.

4.1.1.8. *tert*-Butyl((3-methoxy-9-(2′,3′,4′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)oxy)dimethylsilane (63). Triflate **60** (0.17 g, 0.38 mmol) was dissolved in THF (25 mL) and 2,3,4-trimethoxyphenyl boronic acid (0.09 g, 0.41 mmol), barium hydroxide octahydrate (0.18 g, 0.57 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.013 g, 0.011 mmol) were added to the solution and refluxed at 80 °C for 2 h. The solution was then filtered through Celite®, and the Celite® was washed with dichloromethane. The organic solution (dichloromethane and THF) was evaporated under reduced pressure. The crude reaction product was purified using flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 20% A/80% B (10 CV), 20% A/80% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **63** (0.07 g, 0.15 mmol, 41%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, d, J = 8.5 Hz), 6.64 (1H, d, J = 8.5 Hz), 6.60 (1H, d, J = 8 Hz), 6.44 (1H, d, J = 8 Hz), 6.10 (1H, t, J = 7 Hz), 3.87 (3H, s), 3.83 (3H, s), 3.76 (3H, s), 3.38 (3H, s), 2.89 (2H, t, J = 6.5 Hz), 2.12 (2H, p, J = 7 Hz), 1.95 (2H, q, J = 7 Hz), 1.05 (9H, s), 0.22 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.0, 151.7, 148.5, 142.4, 140.3, 135.8, 132.5, 131.1, 128.2, 124.9, 120.7, 108.0, 106.6, 105.2, 60.6, 60.4, 55.9, 54.6, 33.8, 26.2, 25.5, 24.2, 19.0, −3.9.

4.1.1.9. 3-Methoxy-9-(2′,3,′4′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (71). TBS-protected benzosuberene **63** (0.068 g, 0.146 mmol) was dissolved in THF (5 mL), and tetrabutylammonium fluoride (0.18 mL, 0.18 mmol) was added. The reaction was stirred at room temperature for 12 h, washed with water, extracted with EtOAc (3 × 25 mL), 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: 7% A/93% B (1 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene analogue **71** (0.053 g, 0.15 mmol, 96%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, d, J = 8.5 Hz), 6.63 (1H, d, J = 8.5 Hz), 6.61 (1H, d, J = 8.5 Hz), 6.37 (1H, d, J = 8.5 Hz), 6.10 (1H, t, J = 7 Hz), 5.71 (1H, s), 3.87 (3H, s), 3.85 (3H, s), 3.82 (3H, s), 3.42 (3H, s), 2.87 (2H, t, J = 7 Hz), 2.15 (2H, p, J = 7 Hz), 1.96 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.0, 151.8, 144.7, 142.39, 142.36, 140.0, 136.2, 131.0, 128.6, 126.8, 125.0, 119.0, 107.3, 106.6, 60.7, 60.5, 55.94, 55.90, 33.6, 25.6, 23.4. HRMS: Obsd 379.1516 [M+Na$^+$], Calcd for $C_{21}H_{24}O_5Na$: 379.1516. HPLC (Method B): 16.18 min.

4.1.1.10. 3,4-Dimethoxy-6,7-dihydro-5*H*-benzo[7]annulen-9-yl trifluoromethanesulfonate (58). Diisopropylamine (0.84 mL, 6.0 mmol) was dissolved in THF (20 mL) and cooled to −78 °C. *n*-BuLi (2.4 mL, 6.0 mmol) was added dropwise, and the solution was stirred for 15 min. Benzosuberone **13** (1.2 g, 5.4 mmol) dissolved in THF (10 mL) was added dropwise, and the reaction was stirred for 2 h at −78 °C. *N*-Phenyl-bis(trifluoromethanesulfonimide) (2.14 g, 5.99 mmol) dissolved in THF (10 mL) was then added dropwise, and the reaction mixture was stirred for 12 h while warming from −78 °C to room temperature. After 12 h, the THF was evaporated under reduced pressure, and the resulting solid was washed with water (50 mL) and extracted with EtOAc (3 × 50 mL). The organic layer 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: 5% A/95% B (1 CV), 5% A/95% B → 40% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford triflate **58** (1.01 g, 2.87 mmol, 50%) as a yellow oil. NMR characterization was performed after the next step.

4.1.1.11. 3,4-Dimethoxy-9-(2′,3′,4′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (61). Triflate **58** (0.46 g, 1.3 mmol) was dissolved in THF (25 mL) and 2,3,4-trimethoxyphenyl boronic acid (0.31 g, 1.4 mmol), barium hydroxide octahydrate (0.62 g, 2.0 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.05 g, 0.04 mmol) were added to the solution, which was refluxed at 80 °C for 2 h. The solution was then filtered through Celite®, and the Celite® was washed with dichloromethane. The organic solution (dichloromethane and THF) was evaporated under reduced pressure. The crude reaction product was purified using 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 → 45% A/55% B (10 CV), 45% A/55% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **61** (0.07 g, 0.19 mmol, 15%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.91 (1H, d, J = 8.5 Hz), 6.64 (2H, overlapping d, J = 8.5 Hz), 6.58 (1H, d, J = 8.5 Hz), 6.11 (1H, t, J = 7 Hz), 3.86 (3H, s), 3.84 (3H, s), 3.82 (3H, s), 3.81 (3H, s), 3.38 (3H, s), 2.86

(2H, t, J = 7 Hz), 2.15 (2H, p, J = 6 Hz), 1.95 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.1, 151.7, 151.0, 146.2, 142.4, 140.1, 135.9, 134.9, 130.8, 128.4, 124.9, 123.5, 108.9, 106.7, 61.2, 60.6, 60.3, 55.9, 55.6, 34.4, 25.4, 23.9. HRMS: Obsd 393.1740 [M+Na$^+$], Calcd for $C_{22}H_{26}O_5Na$: 393.1672. HPLC (Method B): 18.25 min.

4.1.1.12. 1-((*tert*-Butyldimethylsilyl)oxy)-2-methoxy-5-phenyl-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (44). To an oven dried flask, THF (50 mL) and phenyl bromide (0.69 mL, 6.5 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (2.74 mL, 6.86 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. TBS-protected **41** (1.55 g, 4.83 mmol) in THF (25 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 water and 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 50 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 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 **44** (0.80 g, 2.01 mmol, 42%) as a clear oil. NMR characterization was performed after the next step.

4.1.1.13. *tert*-Butyl((3-methoxy-9-phenyl-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)oxy)dimethylsilane (51). Acetic acid (10 mL) was added to alcohol **44** (0.80 g, 2.0 mmol), and the reaction mixture was stirred for 12 h at room temperature. The mixture was washed with water and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried over sodium sulfate. The organic phase was evaporated under reduced pressure, and the crude reaction product was purified by flash chromatography using a pre-packed 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 20% A/80% B (10 CV), 20% A/80% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **51** (0.38 g, 1.0 mmol, 49%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (5H, m), 6.70 (1H, d, J = 8 Hz), 6.60 (1H, d, J = 8.5 Hz), 6.37 (1H, t, J = 7.5 Hz), 3.81 (3H, s), 2.79 (2H, t, J = 7 Hz), 2.13 (2H, p, J = 7 Hz), 1.98 (2H, q, J = 7.5 Hz), 1.07 (9H, s), 0.26 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 148.6, 143.0, 142.8, 141.6, 134.1, 133.3, 128.0, 127.3, 126.8, 122.1, 108.8, 108.4, 54.7, 33.8, 26.2, 25.6, 24.2, 19.0, −3.80.

4.1.1.14. 3-Methoxy-9-phenyl-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (64). TBS-protected benzosuberene **51** (0.38 g, 1.0 mmol) was dissolved in THF (25 mL), TBAF (1.20 mL, 1.20 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution 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 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 benzosuberene analogue **64** (0.12 g, 0.45 mmol, 44%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (5H, m), 6.71 (1H, d, J = 8.5 Hz), 6.54 (1H, d, J = 8.5 Hz), 6.38 (1H, t, J = 7.5 Hz), 5.77 (1H, s), 3.91 (3H, s), 2.79 (2H, t, J = 7 Hz), 2.16 (2H, p, J = 7 Hz), 1.99 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.0, 142.8, 142.7, 142.4, 134.6, 128.02, 128.00, 127.8, 127.6, 126.9, 120.6, 107.7, 55.9, 33.5, 25.7, 23.5. HRMS: Obsd 267.1385 [M+H$^+$], Calcd for $C_{18}H_{19}O_2$: 267.1380. HPLC (Method B): 17.89 min.

4.1.1.15. ((5-(3′,5′-Bis(trifluoromethyl)phenyl)-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-1-yl)oxy)(*tert*-butyl)dimethylsilane (45). 1-Bromo-3,5-bis(trifluoromethyl)benzene (0.36 g, 1.2 mmol) was dissolved in THF (13 mL), and the reaction flask was cooled to −78 °C. *n*-BuLi (0.55 mL, 2.5 M) was added to the reaction mixture, which was stirred for 1 h. Ketone **41** (0.29 g, 0.91 mmol) was dissolved in THF (5 mL) and slowly added to the reaction mixture over a period of 15 min. The reaction mixture was stirred for 20 h while warming from −78 °C to room temperature. The reaction mixture was diluted with H_2O (25 mL) and extracted with EtOAc (2 × 25 mL), and the organic extract was dried over Na_2SO_4, 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 **45** (0.40 g, 0.75 mmol, 82%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, s), 7.74 (2H, s), 6.95 (1H, d, J = 8.7 Hz), 6.70 (1H, d, J = 8.7 Hz), 3.80 (3H, s), 3.32 (1H, ddd, J = 14.8, 7.6, 2.0 Hz), 2.57 (1H, ddd, J = 14.2, 7.6, 3.0 Hz), 2.41 (1H, s), 2.34–2.21 (1H, m), 2.16 (1H, ddd, J = 13.8, 10.3, 3.1 Hz), 1.97–1.90 (1H, m), 1.75–1.68 (1H, m), 1.67–1.57 (1H, m), 1.56–1.48 (1H, m), 0.99 (9H, s), 0.18 (3H, s), 0.17 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.9, 149.7, 142.5, 137.1, 132.7, 131.6 (q, J = 33.2 Hz), 127.3 (q, J = 3 Hz), 123.5 (q, J = 272.8 Hz), 121.2 (hept, J = 3.8 Hz), 120.3, 108.6, 79.8, 54.8, 41.6, 26.7, 26.2, 25.3, 25.3, 19.1, −3.94, −3.95.

4.1.1.16. ((9-(3′,5′-Bis(trifluoromethyl)phenyl)-3-methoxy-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)oxy)(*tert*-butyl)dimethylsilane (52). 2 M HCl (8 mL, 16 mmol) was added to a well-stirred solution of alcohol **45** (0.68 g, 1.3 mmol) in EtOH (5 mL), and the reaction mixture was stirred for 12 h at ambient temperature. The reaction mixture was then extracted with EtOAc (4 × 15 mL), and the organic extract was dried over Na_2SO_4, 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: 2% A/98% B (1 CV), 2% A/98% B → 15% A/85% B (10 CV), 15% A/85% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **52** (0.43 g, 0.84 mmol, 66%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, s), 7.74 (2H, s), 6.73 (1H, d, J = 8.5 Hz), 6.50 (1H, t, J = 7.1 Hz), 6.49 (1H, d, J = 8.5 Hz), 3.84 (3H, s), 2.80 (2H, t, J = 6.9 Hz), 2.16 (2H, p, J = 7.0 Hz), 2.04 (2H, q, J = 7.1 Hz), 1.07 (9H, s), 0.27 (6H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.3, 145.0, 142.1, 141.1, 133.5, 132.5, 131.5 (q, J = 33.3 Hz), 130.8, 128.0 (q, J = 3.4 Hz), 123.7 (q, J = 272.7 Hz), 121.9, 120.6 (hept, J = 3.7 Hz), 109.0, 54.8, 33.8, 26.3, 26.0, 24.4, 19.2, −3.6.

4.1.1.17. 9-(3′,5′-Bis(trifluoromethyl)phenyl)-3-methoxy-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (65). To a solution of TBS-protected analogue **52** (0.43 g, 0.84 mmol) in THF (5 mL) was added TBAF (1.0 mL, 1 M in THF), and the reaction mixture was stirred for 18 h at ambient temperature and then concentrated under reduced pressure. The reaction mixture was washed with water (5 mL) and extracted with EtOAc (3 × 10 mL). The organic extract was dried over Na_2SO_4, 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 → 50% A/50% B (10 CV), 50% A/50% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford phenolic benzosuberene analogue **65** (0.26 g, 0.66 mmol, 78%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, s), 7.74 (2H, s), 6.74 (1H, d, J = 8.3 Hz), 6.51 (1H, t, J = 7.3 Hz), 6.45 (1H, d, J = 8.4 Hz), 5.83 (1H, s), 3.93 (3H,

s), 2.79 (2H, t, J = 6.9 Hz), 2.19 (2H, p, J = 7.1 Hz), 2.05 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 145.8, 144.9, 142.9, 140.8, 133.0, 131.5 (q, J = 33.4 Hz), 131.2, 128.03, 127.99, 123.6 (q, J = 272.8 Hz), 120.6 (hept, J = 3.9 Hz), 120.4, 108.2, 56.1, 33.4, 26.1, 23.7. ^{19}F NMR ($CDCl_3$, 470 MHz) δ −62.80. HRMS: Obsd 401.0963 [M–H]$^-$, Calcd for $C_{20}H_{15}F_6O_2$: 401.0976. HPLC (Method B): 20.39 min.

4.1.1.18. 9-(3′,5′-Bis(trifluoromethyl)phenyl)-3-methoxy-6,7-dihydro-5*H*-benzo[7]annulen-4-yl disodium phosphate (74). To a well-stirred solution of phenol **65** (0.10 g, 0.25 mmol) in CH_2Cl_2 (10 mL), $POCl_3$ (153.3 mg, 1.00 mmol) and pyridine (70.8 mg, 0.9 mmol) were added to the reaction flask. After the reaction mixture was stirred for 15 h at ambient temperature, the solvent was evaporated under reduced pressure. Saturated aqueous Na_2CO_3 (20 mL) was added to the flask, and the reaction mixture was stirred for another 2 h. The reaction mixture was concentrated to dryness with a stream of N_2 gas and purified by flash chromatography using a prepacked C-18 30 g reversed phase column [solvent A: acetonitrile; solvent B: water; gradient: 30% A/70% B (1 CV), 30% A/70% B → 100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford phosphate salt **74** (0.043 g, 0.082 mmol, 33%) as a white solid. ^{1}H NMR (D_2O, 500 MHz) δ 7.96 (1H, s), 7.87 (2H, s), 6.91 (1H, d, J = 8.6 Hz), 6.72 (1H, d, J = 8.4 Hz), 6.62 (1H, t, J = 7.4 Hz), 3.84 (3H, s), 2.76 (2H, t, J = 7.0 Hz), 2.17 (2H, p, J = 7.1 Hz), 1.97 (2H, q, J = 7.2 Hz). ^{13}C NMR (CD_3OD, 125 MHz) δ 153.1, 146.6, 142.0, 141.9, 137.2, 133.3, 132.6 (q, J = 33.0 Hz), 132.4, 129.0 (q, J = 2.5 Hz), 124.9 (q, J = 271.3 Hz), 125.2 (hept, J = 3.8 Hz), 121.2, 111.1, 56.4, 34.6, 26.9, 25.9. ^{19}F NMR (D_2O, 470 MHz) δ −62.8. ^{31}P NMR (D_2O, 200 MHz) δ −3.5. HRMS: Obsd 527.0429 [M+H]$^+$, Calcd for $C_{19}H_{16}F_6O_2Na_2O_5P$: 527.0429. HPLC (Method A): 13.79 min.

4.1.1.19. 1-((*tert*-Butyldimethylsilyl)oxy)-5-(4-hydroxyphenyl)-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (47). To a solution of 4-methoxyphenyl bromide (0.52 g, 2.8 mmol) in THF (40 mL) at −78 °C was added *n*-BuLi (0.34 mL, 2.5 M in hexanes), and the reaction mixture was stirred for 30 min. Benzosuberone **41** (0.61 g, 1.9 mmol) in THF (20 mL) was added dropwise over a period of 15 min. The reaction mixture was allowed to reach room temperature over 12 h. Upon completion, water was added, and the mixture was extracted with EtOAc (3 × 20 mL). The organic extract was washed with brine, dried over Na_2SO_4, 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 → 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 alcohol **47** (0.32 g, 0.75 mmol, 39%) as a clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (1H, d, J = 8.6 Hz), 7.15 (2H, d, J = 8.8 Hz), 6.81 (2H, d, J = 8.8 Hz), 6.73 (1H, d, J = 8.7 Hz), 3.81 (3H, s), 3.79 (3H, s), 3.30 (1H, dd, J = 14.6, 7.5 Hz), 2.67–2.59 (1H, m), 2.14–2.04 (2H, m), 1.77–1.65 (2H, m), 1.35–1.25 (2H, m), 0.99 (9H, s), 0.20 (3H, s), 0.14 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.7, 149.2, 141.8, 139.0, 137.6, 132.7, 128.3, 119.1, 113.6, 107.8, 79.4, 55.2, 54.6, 41.2, 27.1, 26.7, 26.1, 25.5, −3.86, −4.22.

4.1.1.20. *tert*-Butyl((3-methoxy-9-(4′-methoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)oxy)dimethylsilane (54). Tertiary alcohol **47** (0.53 g, 1.2 mmol) was dissolved in acetic acid (5 mL) and refluxed for 5 h. No apparent change in TLC was observed, so water (15 mL) was added to the reaction mixture, which was refluxed for 2 h. The solvents were evaporated, and the resulting product was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with satd $NaHCO_3$, brine, dried over Na_2SO_4, filtered, 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 **54** (0.43 g, 1.1 mmol, 85%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20 (2H, d, J = 8.7 Hz), 6.83 (2H, d, J = 8.8 Hz), 6.68 (1H, d, J = 8.4 Hz), 6.58 (1H, d, J = 8.4 Hz), 6.26 (1H, t, J = 7.3 Hz), 3.81 (3H, s), 3.79 (3H, s), 2.75 (2H, t, J = 6.9 Hz), 2.09 (2H, p, J = 7.1 Hz), 1.93 (2H, q, J = 7.2 Hz), 1.04 (9H, s), 0.23 (6H, s). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.7, 148.5, 142.4, 141.5, 135.5, 134.3, 133.3, 129.0, 125.7, 122.0, 113.4, 108.3, 55.3, 54.7, 33.94, 26.2, 25.5, 24.2, 19.0, −3.9.

4.1.1.21. 3-Methoxy-9-(4′-methoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (67). The TBS-protected analogue **54** (0.33 g, 0.8 mmol) was dissolved in THF (5 mL). To the solution, TBAF–$3H_2O$ (0.96 mmol) was added, and the mixture was stirred for 3 h at room temperature. The reaction was quenched with water (15 mL), followed by the evaporation of organic solvent under reduced pressure. The resultant aqueous phase was then extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine solution, dried over Na_2SO_4, filtered, evaporated under reduced pressure, and purified by flash chromatography using a pre-packed 40 g silica column [solvent A, EtOAc, solvent B, hexanes; gradient 0% A/100% B →100% A/0% B over 9.0 min; flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford the benzosuberene analogue **67** (0.21 g, 0.71 mmol, 89%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.21 (2H, d, J = 8.7 Hz), 6.83 (2H, d, J = 8.7 Hz), 6.70 (1H, d, J = 8.4 Hz), 6.54 (1H, d, J = 8.4 Hz), 6.28 (1H, t, J = 7.4 Hz), 5.72 (1H, s), 3.90 (3H, s), 3.81 (3H, s), 2.75 (2H, t, J = 7.0 Hz), 2.13 (2H, p, J = 7.0 Hz), 1.95 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 144.9, 142.4, 142.2, 135.3, 134.8, 129.0, 127.8, 126.1, 120.6, 113.4, 107.6, 55.9, 55.3, 33.7, 25.6, 23.5. HRMS: Obsd 297.1492 [M+H$^+$], Calcd for $C_{19}H_{21}O_3$: 297.1485. HPLC (Method B): 17.42 min.

4.1.1.22. 1-((*tert*-Butyldimethylsilyl)oxy)-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (49).[31,33] To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (2.46 g, 9.97 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (4.2 mL, 10 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **41** (2.37 g, 7.38 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. The reaction mixture was washed with water and 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 100 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/80% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford compound **49** (1.80 g, 3.70 mmol, 50%) as a clear oil. NMR characterization was performed after the next step.

4.1.1.23. *tert*-Butyl((3-methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-yl)oxy)dimethylsilane (56). Acetic acid (20 mL) was added to tertiary alcohol **49** (1.80 g, 3.70 mmol), and the reaction mixture was stirred for 12 h at room temperature. The 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 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 → 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 TBS-protected benzosuberene **56** (1.43 g, 8.38 mmol, 83%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.67 (1H, d, J = 8.5 Hz), 6.61 (1H, d, J = 8.5 Hz), 6.50 (2H, s), 6.32 (1H, t, J = 7.5 Hz), 3.85 (3H, s), 3.78 (3H, s), 3.77 (6H, s), 2.78 (2H, t, J = 7 Hz), 2.11 (2H, p, J = 7 Hz), 1.95 (2H, q, J = 7.5 Hz), 1.06 (9H s), 0.25 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.8, 148.6, 143.1, 141.5, 138.6, 137.3, 133.8, 133.2, 126.7, 122.4, 108.4, 105.3, 60.7, 56.0, 54.5, 34.0, 26.2, 25.6, 24.2, 19.0, −3.8.

4.1.1.24. 3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-ol (69).[30,31,33,44,56] Benzosuberene **56** (1.43 g, 3.04 mmol) was dissolved in THF (10 mL), TBAF (3.65 mL, 3.6 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution 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 affording benzosuberene **69** (0.66 g, 1.9 mmol, 61%) as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.72 (1H, d, J = 8.5 Hz), 6.58 (1H, d, J = 8 Hz), 6.51 (2H, s), 6.35 (1H, t, J = 7 Hz), 5.74 (1H, s), 3.93 (3H, s), 3.87 (3H, s), 3.81 (6H, s), 2.77 (2H, t, J = 7 Hz), 2.15 (2H, p, J = 7 Hz), 1.97 (2H, q, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 152.8, 145.0, 142.8, 142.3, 138.5, 137.3, 134.2, 127.7, 127.2, 120.8, 107.6, 105.3, 60.9, 56.1, 55.9, 33.6, 25.7, 23.5.

4.1.1.25. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-1-ol (72). To benzosuberene **69** (0.66 g, 1.9 mmol) was added methanol (50 mL) and 10% Pd/C (0.42 g). Hydrogen gas was added, and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through Celite®, and the Celite® was washed with EtOAc (3 × 30 mL). The organic phase (MeOH and EtOAc) was evaporated under reduced pressure. The crude reaction product was 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: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberane analogue **72** (0.16 g, 0.45 mmol, 24%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.54 (1H, d, J = 8.5 Hz), 6.47 (2H, s), 6.17 (1H, d, J = 8 Hz), 5.84 (1H, s), 4.17 (1H, d, J = 9.5 Hz), 3.89 (3H, s), 3.84 (9H, s), 3.28 (1H, q, J = 7.5 Hz), 2.73 (1H, t, J = 12 Hz), 2.17 (1H, m), 2.02 (2H, m), 1.86 (2H, m), 1.46 (1H, q, J = 12 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.1, 144.7, 142.6, 141.2, 139.6, 136.2, 128.4, 118.6, 108.1, 105.7, 60.9, 56.1, 55.9, 49.5, 34.7, 30.6, 27.2, 25.4. HRMS: Obsd 381.1764 [M+Na$^+$], Calcd for $C_{21}H_{26}O_5Na^+$: 381.1672. HPLC (Method B): 15.30 min.

4.1.1.26. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-1-yl disodium phosphate (73). To benzosuberane analogue **72** dissolved in CH_2Cl_2 (25 mL) was added $POCl_3$ (0.11 mL, 1.1 mmol) and pyridine (0.1 mL, 1 mmol), and the reaction mixture was stirred at room temperature for 12 h. 2 M NaOH (1.69 mL, 3.38 mmol) was added, and the reaction was stirred for 5 min and extracted with CH_2Cl_2, and the organic layer was evaporated under reduced pressure. NaOH (2 mL, 2 M) was added to the resulting oil, and the solution was refluxed at 60 °C for 15 min. Water was removed under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 12 g C-18 column [solvent A: water; solvent B: acetonitrile; gradient: 0% A/100% B (1 CV), 0% A/100% B → 100% A/0% B (10 CV), 0% A/100% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford the benzosuberane phosphate salt **73** (0.024 g, 0.050 mmol, 17%) as a white solid. ^{1}H NMR (500 MHz, D_2O) δ 6.43 (2H, s), 6.36 (1H, d, J = 8.5 Hz), 6.07 (1H, d, J = 8.5 Hz), 4.04 (1H, d, J = 9.5 Hz), 3.61 (6H, s) 3.60 (3H, s), 3.56 (3H, s), 3.26 (1H, m), 2.55 (1H, t, J = 12 Hz), 1.90 (1H, m), 1.65 (4H, m), 1.16 (1H, q, J = 10.5 Hz). ^{13}C NMR (125 MHz, D_2O) δ 152.2, 150.3, 142.8, 140.5, 140.4, 139.0, 137.3, 134.6, 121.8, 108.6, 105.9, 60.8, 55.4, 48.6, 33.7, 30.0, 33.7, 29.7, 26.6. ^{31}P NMR (200 MHz, D_2O, 85% phosphoric acid reference) δ 0.97. HRMS: Obsd 483.1190 [M+H$^+$], Calcd for $C_{21}H_{26}O_5Na_2P^+$: 483.1155. HPLC (Method A): 14.23 min.

4.1.1.27. 1,2-Dimethoxy-5-(4′-methoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (27). To a solution of 4-methoxyphenyl bromide (0.719 g, 3.84 mmol) in THF (50 mL) at −78 °C was added *n*-BuLi (2.6 mL, 2.5 M in hexanes), and the reaction mixture was stirred for 30 min. Benzosuberone **13** (0.58 g, 2.6 mmol) in THF (15 mL) was added dropwise over 15 min. The reaction mixture was warmed to room temperature over 12 h. Upon completion, water was added, and the mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and purified by flash chromatography using a pre-packed 80 g silica column [solvent A, EtOAc, solvent B, hexanes; gradient 0% A/100% B → 100% A/0% B over 20.3 min; flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford alcohol **27** (0.45 g, 1.4 mmol, 54%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39 (1H, d, J = 8.7 Hz), 7.15 (2H, d, J = 8.8 Hz), 6.83 (2H, d, J = 8.8 Hz), 6.79 (1H, d, J = 8.7 Hz), 3.89 (3H, s), 3.79 (3H, s), 3.75 (3H, s), 3.25–3.18 (1H, m), 2.67–2.59 (1H, m), 2.15–2.08 (2H, m), 1.93–1.86 (1H, m), 1.80–1.69 (2H, m), 1.43–1.35 (1H, m). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 151.7, 146.3, 139.1, 137.5, 135.4, 128.2, 122.31, 113.7, 108.8, 79.4, 61.0, 55.6, 55.2, 41.2, 27.3, 26.6, 25.2.

4.1.1.28. 3,4-Dimethoxy-9-(4′-methoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (36). Tertiary alcohol analogue **27** (0.401 g, 1.22 mmol) was dissolved in acetic acid (10 mL), and the mixture was stirred for 12 h at ambient temperature. Water (30 mL) was added, and the reaction mixture was stirred for 2 h. The reaction mixture was extracted with EtOAc (3 × 20 mL). The organic solvent was evaporated under reduced pressure and the residue extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, 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: 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 benzosuberene analogue **36** (0.31 g, 1.0 mmol, 82%) as a clear oil. ^{1}H NMR (CD_3OD, 500 MHz) δ 7.17 (2H, d, J = 8.8 Hz), 6.87 (1H, d, J = 8.5 Hz), 6.86 (2H, d, J = 8.8 Hz), 6.71 (1H, d, J = 8.5 Hz), 6.30 (1H, t, J = 7.4 Hz), 3.89 (3H, s), 3.85 (3H, s), 3.81 (3H, s), 2.74 (2H, t, J = 7.0 Hz), 2.15 (2H, p, J = 7.1 Hz), 1.94 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 158.8, 151.3, 146.1, 142.2, 135.9, 135.2, 134.3, 129.0, 125.9, 125.0, 113.5, 109.2, 61.2, 55.6, 55.3, 34.6, 25.5, 24.0. HRMS: Obsd 281.1597 [M+H$^+$], Calcd for $C_{19}H_{23}O_2$: 281.1536. HPLC (Method B): 19.08 min.

4.1.1.29. 1,2-Dimethoxy-5-phenyl-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (19). To an oven dried flask, THF (50 mL) and phenylbromide (0.25 mL, 2.4 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (1.0 mL, 2.5 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **13** (0.39 g, 1.8 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. The reaction mixture was washed with water and 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 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 tertiary alcohol **19** (0.29 g, 0.97 mmol, 55%) as a pale yellow oil. NMR characterization was performed after the next step.

4.1.1.30. 3,4-Dimethoxy-9-phenyl-6,7-dihydro-5*H*-benzo[7]annulene (28). Acetic acid (15 mL) was added to tertiary alcohol **19** (0.29 g, 0.97 mmol), and the reaction mixture was stirred for 12 h at room temperature. The mixture was washed with water (50 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 10 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A/95% B (1 CV), 5% A/95% B → 50% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene analogue **28** (0.10 g, 0.36 mmol, 37%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (5H, m), 6.78 (2H, s), 6.40 (1H, t, J = 7.5 Hz), 3.91 (3H, s), 3.90 (3H, s), 2.81 (2H, t, J = 7 Hz), 2.19 (2H, p, J = 7 Hz), 2.01 (2H, q, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.5, 146.1, 142.9, 142.6, 135.9, 134.2, 128.1, 128.0, 127.5, 127.0, 125.1, 109.4, 61.2, 55.6, 34.5, 25.6, 24.1.HRMS: Obsd 303.1363 [M+Na$^+$], Calcd for $C_{19}H_{20}O_2Na$: 303.1356. HPLC (Method B): 19.93 min.

4.1.1.31. 1,2-Dimethoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (26).[31] To a solution of 3,4,5-trimethoxyphenyl bromide (0.67 g, 2.7 mmol) in THF (100 mL) at −78 °C was added *n*-BuLi (1.1 mL, 2.5 M in hexanes), and the reaction mixture was stirred for 30 min. Benzosuberone **13** (0.60 g, 2.7 mmol) in THF (15 mL) was added dropwise over 15 min. The reaction mixture was warmed to room temperature over 12 h. Water was added, and the mixture was extracted with EtOAc (100 mL). The organic extract was washed with brine, dried over Na_2SO_4, 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 → 56% A/44% B (9.2 CV), 56% A/44% B (1 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford alcohol **26** (0.39 g, 0.99 mmol, 34%) as a light yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (1H, d, J = 8.7 Hz), 6.75 (1H, d, J = 8.8 Hz), 6.49 (2H, s), 3.87 (3H, s), 3.83 (3H, s), 3.74 (3H, s), 3.73 (6H, s), 3.26–3.20 (1H, m), 2.56 (1H, ddd, J = 14.1, 6.9, 3.0 Hz), 2.38–2.30 (1H, m), 2.21–2.21 (1H, m), 2.11 (1H, ddd, J = 14.0, 10.7, 3.1 Hz), 1.93 (1H, ddd, J = 15.3, 7.4, 3.8 Hz), 1.81–1.71 (2H, m), 1.50–1.42 (1H, m). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 153.0, 151.8, 146.2, 141.6, 138.6, 137.2, 135.5, 123.0, 108.8, 104.2, 79.9, 61.0, 60.8, 56.1, 55.5, 41.3, 27.1, 26.3, 25.1.

4.1.1.32. 3,4-Dimethoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (35).[31] Tertiary alcohol analogue **26** (0.5 g, 1 mmol) was dissolved in acetic acid (10 mL) and refluxed for 2 h. Water (30 mL) was added, and the reaction was refluxed for 2 h. The white precipitate thus obtained was filtered and washed with hexanes. On drying, it afforded benzosuberene analogue **35** (0.444 g, 1.2 mmol, 93%) as a colorless solid, which was not further purified. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.79 (1H, d, J = 8.5 Hz), 6.77 (1H, d, J = 8.5 Hz), 6.51 (2H, s), 6.35 (1H, t, J = 7.3 Hz), 3.90 (3H, s), 3.89 (3H, s), 3.88 (3H, s), 3.82 (6H, s), 2.77 (2H, t, J = 7.0 Hz), 2.17 (2H, p, J = 7.1 Hz), 1.98 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 152.9, 151.5, 146.1, 142.9, 138.4, 137.4 135.9, 133.8, 127.1, 125.3, 109.3, 105.3, 61.3, 60.9, 56.17, 55.6, 34.6, 25.6, 24.2. HRMS: Obsd 393.1682 [M+Na$^+$], Calcd for $C_{22}H_{26}O_5Na$: 393.1672. HPLC (Method B): 17.52 min.

4.1.1.33. 5-(3′,5′-Bis(trifluoromethyl)phenyl)-1,2-dimethoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (25). To a solution of 1-bromo-3,5-bis(trifluoromethyl)benzene (0.36 g, 1.2 mmol) in THF (13 mL) at −78 °C was added *n*-BuLi (0.55 mL, 2.5 M), and the reaction mixture was stirred for 1 h. Benzosuberone **13** (0.20 g, 0.91 mmol) in THF (5 mL) was added dropwise over 15 min. The reaction mixture was stirred for 20 h and was warmed to room temperature. The reaction mixture was diluted with H_2O (25 mL) and extracted with EtOAc (2 × 25 mL). The organic extract was dried over Na_2SO_4, 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 (15 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford alcohol **25** (0.45 g, 1.0 mmol, 84%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.70 (1H, s), 7.67 (2H, s), 6.92 (1H, d, J = 8.7 Hz), 6.63 (1H, d, J = 8.8 Hz), 3.76 (3H, s), 3.67 (3H, s), 3.16 (1H, ddd, J = 14.6, 7.8, 1.9 Hz), 2.50 (1H, s), 2.46 (1H, ddd, J = 14.3, 8.2, 2.8 Hz), 2.37–2.24 (1H, m), 2.06 (1H, ddd, J = 14.2, 9.6, 3.0 Hz), 1.91–1.85 (1H, m), 1.73–1.61 (1H, m), 1.59–1.50 (2H, m). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.4, 149.8, 146.6, 137.2, 135.6, 131.6 (q, J = 33.1 Hz), 127.1 (q, J = 3.8 Hz), 123.5 (q, J = 272.7 Hz), 123.7, 121.3 (hept, J = 4.0 Hz), 109.5, 79.8, 61.1, 55.7, 41.6, 27.0, 25.2, 24.8.

4.1.1.34. 9-(3′,5′-Bis(trifluoromethyl)phenyl)-3,4-dimethoxy-6,7-dihydro-5*H*-benzo[7]annulene (34). To a solution of tertiary alcohol **25** (0.45 g, 1.0 mmol) in EtOH (10 mL) was added 2 M HCl (10 mL, 20 mmol), and the reaction mixture was stirred overnight. The reaction mixture was then extracted using EtOAc (4 × 15 mL). The organic extract was dried over Na_2SO_4, 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: 5% A/95% B (1 CV), 5% A/95% B → 40% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **34** (0.22 g, 0.54 mmol, 54%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (1H, s), 7.71 (2H, s), 6.77 (1H, d, J = 8.5 Hz), 6.63 (1H, d, J = 8.5 Hz), 6.49 (1H, t, J = 7.3 Hz), 3.90 (3H, s), 3.88 (3H, s), 2.76 (2H, t, J = 6.9 Hz), 2.18 (2H, p, J = 7.1 Hz), 2.03 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.2, 146.6, 144.7, 140.8, 136.1, 132.4, 131.6 (q, J = 33 Hz), 131.1, 128.0 (q, J = 3.8 Hz), 124.8, 123.6 (q, J = 272.7 Hz), 120.7 (hept, J = 4.0 Hz), 109.9, 61.4, 55.8, 34.3, 26.0, 24.3. ^{19}F NMR ($CDCl_3$, 470 MHz) δ −62.81. HRMS: Obsd 401.0965 [M–CH_3]$^-$, Calcd for $C_{20}H_{15}F_6O_2$: 401.0976. HPLC (Method B): 21.57 min.

4.1.1.35. 1,2-Dihydroxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (40).[31,57] Ketone **13** (0.88 g, 4.0 mmol) was added to the ionic liquid [TMAH][Al_2Cl_7](20.0 mL, 0.497 M). The reaction mixture was subjected to microwave irradiation at 80 °C and 1 atm for 1 h. H_2O (20 mL) was added to the mixture, and the resulting brown liquid was extracted with dichloromethane (3 × 20 mL). The organic extracts were dried over Na_2SO_4, filtered, 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 → 60% A/40% B (13 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **40** (0.50 g, 2.6 mmol, 65%) as a brown solid. ^{1}H NMR ((CD_3)$_2$CO, 500 MHz) δ 7.14 (1H, d, J = 8.3 Hz), 6.78 (1H, d, J = 8.8 Hz), 3.07–2.99 (2H, m), 2.67–2.59 (2H, m), 1.84–1.77 (2H, m), 1.77–1.71 (2H, m). ^{13}C

NMR ($(CD_3)_2CO$, 126 MHz) δ 205.4, 148.1, 142.0, 132.3, 128.9, 120.6, 112.3, 40.3, 24.4, 22.8, 21.0.

4.1.1.36. 1,2-Bis((*tert*-butyldimethylsilyl)oxy)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (43).[31] To a solution of catechol **40** (0.68 g, 3.5 mmol) and DIPEA (2.7 mL, 16 mmol) in DMF (5 mL) at 0 °C was added TBSCl (1.60 g, 10.6 mmol) in portions. The reaction mixture was stirred for 18 h, diluted with H_2O (5 mL), and extracted with Et_2O (2 × 20 mL). The organic extract was dried over Na_2SO_4, 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: 0% A/100% B (1 CV), 0% A/100% B → 30% A/70% B (10 CV), 30% A/70% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford ketone **43** (1.51 g, 3.59 mmol, 99%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.29 (1H, d, J = 8.5 Hz), 6.75 (1H, d, J = 8.5 Hz), 2.97–2.94 (2H, m), 2.70 (2H, t, J = 6.2 Hz), 1.89–1.70 (4H, m), 1.02 (9H, s), 0.96 (9H, s), 0.24 (6H, s), 0.15 (6H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 205.3, 151.2, 143.9, 135.5, 134.0, 122.6, 118.6, 41.0, 26.5, 26.5, 25.3, 25.1, 21.9, 19.2, 18.9, −3.15, −3.19.

4.1.1.37. 1,2-Bis((*tert*-butyldimethylsilyl)oxy)-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (50).[31] To a solution of 3,4,5-trimethoxyphenyl bromide (0.458 g, 1.85 mmol) in THF (20 mL) at −78 °C was added *n*-BuLi (0.96 mL, 2.5 M in hexanes), and the reaction mixture was stirred for 1 h. Benzosuberone **43** (0.639 g, 1.51 mmol) in THF (20 mL) was added dropwise over 15 min. The reaction mixture was stirred while warming to room temperature over 12 h. Water was added, and the mixture was extracted with EtOAc (4 × 20 mL). The organic extract was washed with brine, dried over Na_2SO_4, 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: 10% A/90% B (1 CV), 10% A/90% B → 42% A/58% B (6 CV), 42% A/58% B→70% A/30% B (1 CV), 70% A/30% B → 100% A/0% B, 100% A/0% B (1.1 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford alcohol **50** (0.480 g, 0.81 mmol, 54%) as a clear oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.10 (1H, d, J = 8.7 Hz), 6.72 (1H, d, J = 8.6 Hz), 6.46 (2H, s), 3.83 (3H, s), 3.74 (6H, s), 3.23–3.15 (1H, m), 2.57 (1H, ddd, J = 14.0, 6.2, 3.0 Hz), 2.23–2.07 (3H, m), 1.94–1.84 (1H, m), 1.81–1.66 (2H, m), 1.46–1.33 (1H, m), 1.00 (9H, s), 0.95 (9H, s), 0.24 (3H, s), 0.23 (3H, s), 0.15 (3H, s), 0.10 (3H, s). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 152.9, 146.4, 143.7, 141.8, 139.1, 137.1, 133.9, 120.0, 117.5, 104.1, 80.0, 60.8, 56.0, 40.9, 26.8, 26.3, 26.2, 26.1, 25.9, 18.9, 18.6, −3.4, −3.6.

4.1.1.38. ((9-(3′,4′,5′-Trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene-3,4-diyl)bis(oxy))bis(*tert*-butyldimethylsilane) (57).[31] Tertiary alcohol **50** (0.44 g, 0.75 mmol) was dissolved in acetic acid (5 mL) and stirred for 12 h at room temperature. The reaction was quenched with water (10 mL) and extracted with Et_2O (3 × 10 mL). The combined organic extract was washed with satd $NaHCO_3$, brine, dried over Na_2SO_4, filtered, and evaporated under reduced pressure to afford the clear oil of TBS-protected benzosuberene analogue **57** (0.38 g, 0.66 mmol, 89%), which was used without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.69 (1H, d, J = 8.4 Hz), 6.52 (1H, d, J = 8.4 Hz), 6.45 (2H, s), 6.33 (1H, t, J = 7.3 Hz), 3.85 (3H, s), 3.78 (6H, s), 2.70 (2H, t, J = 6.9 Hz), 2.10 (2H, q, J = 7.0 Hz), 1.95 (2H, q, J = 7.0 Hz), 1.04 (9H, s), 0.95 (9H, s), 0.24 (6H, s), 0.20 (6H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.7, 145.9, 143.4, 143.0, 138.6, 137.1, 134.4, 134.2, 126.8, 122.7, 117.9, 105.0, 60.9, 56.0, 33.9, 26.27, 26.25, 25.7, 24.5, 18.9, 18.7, −3.3, −3.4.

4.1.1.39. 9-(3′,4′,5′-Trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene-3,4-diol (70). The di-TBS-protected analogue **57** (0.32 g, 0.56 mmol) was dissolved in THF (5 mL). To the solution, TBAF–$3H_2O$ (1.4 mmol) was added and stirred for 3 h at room temperature. The reaction was quenched with water (15 mL), and the organic solvent was evaporated under reduced pressure. The resulting aqueous phase was then extracted with EtOAc (3 × 20 mL). The combined organic extract was washed with brine, dried over Na_2SO_4, filtered, evaporated under reduced pressure and purified by flash chromatography using a pre-packed 25 g silica gel 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 (5.2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford catechol analogue **70** (0.17 g, 0.49 mmol, 88%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.68 (1H, d, J = 8.2 Hz), 6.52 (1H, d, J = 8.2 Hz), 6.49 (2H, s), 6.33 (1H, t, J = 7.4 Hz), 5.29 (1H, s), 5.28 (1H, s), 3.86 (3H, s), 3.79 (6H, s), 2.71 (2H, t, J = 7.0 Hz), 2.15 (2H, p, J = 7.1 Hz), 1.96 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 152.8, 142.9, 141.8, 140.8, 138.3, 137.3, 134.1, 128.5, 127.0, 121.7, 112.3, 105.3, 60.9, 56.1, 33.8, 25.6, 23.8. HRMS: Obsd 365.1444 [M+H$^+$], Calcd for $C_{12}H_{23}O_5$: 365.1359. HPLC (Method B): 16.18 min.

4.1.1.40. 5-(3′,5′-Bis(trifluoromethyl)phenyl)-1,2-bis((*tert*-butyldimethylsilyl)oxy)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (46). 1-Bromo-3,5-bis(trifluoromethyl)benzene (0.36 g, 1.2 mmol) was dissolved in THF (13 mL) at −78 °C and *n*-BuLi (0.55 mL, 2.5 M) was then added. The reaction mixture was stirred for 1 h, and then ketone **43** (0.38 g, 0.91 mmol) in THF (5 mL) was added dropwise over 15 min. The reaction mixture was stirred for 20 h, warming from −78 °C to room temperature, and then diluted with H_2O (25 mL) and extracted with EtOAc (3 × 25 mL). The organic extract was dried over Na_2SO_4, 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 → 100% A/0% B (10 CV), 100% A/0% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford alcohol **46** (0.43 g, 0.68 mmol, 74%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (1H, s), 7.72 (2H, s), 6.92 (1H, d, J = 8.6 Hz), 6.73 (1H, d, J = 8.7 Hz), 3.25 (1H, ddd, J = 14.7, 7.6, 1.9 Hz), 2.57 (1H, ddd, J = 14.2, 7.3, 3.1 Hz), 2.31 (1H, s), 2.22–2.14 (2H, m), 1.96–190 (1H, m), 1.74–170 (1H, m), 1.64–1.58 (1H, m), 1.54–148 (1H, m), 1.01 (9H, s), 0.97 (9H, s), 0.26 (3H, s), 0.23 (3H, s), 0.19 (3H, s), 0.09 (3H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.7, 147.2, 144.3, 137.6, 134.0, 131.6 (q, J = 33.1 Hz), 127.2 (q, J = 3.2 Hz), 123.5 (q, J = 273.0 Hz), 121.3 (hept, J = 4.0 Hz), 120.6, 118.3, 79.8, 41.2, 26.8, 26.39, 26.35, 25.9, 25.2, 19.0, 18.7, −3.1, −3.2, −3.5, −3.9.

4.1.1.41. ((9-(3′,5′-Bis(trifluoromethyl)phenyl)-6,7-dihydro-5*H*-benzo[7]annulene-3,4-diyl)bis(oxy))bis(*tert*-butyldimethylsilane) (53). Tertiary alcohol **46** (0.43 g, 0.67 mmol) was dissolved in EtOH (5 mL) and 2 M HCl (10 mL, 20 mmol) was then added. The reaction mixture was stirred overnight and then extracted using EtOAc (4 × 15 mL). The organic extract was dried over Na_2SO_4, 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: 2% A/98% B (1 CV), 2% A/98% B → 15% A/85% B (10 CV), 15% A/85% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **53** (0.22 g, 0.35 mmol, 53%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (1H, s), 7.69 (2H, s), 6.72 (1H, d, J = 8.4 Hz), 6.49 (1H, t, J = 7.2 Hz), 6.40 (1H, d, J = 8.4 Hz), 2.73 (2H, t, J = 6.9 Hz), 2.15 (2H, p, J = 7.0 Hz), 2.03 (2H, q, J = 7.1 Hz), 1.07 (9H, s), 0.98 (9H, s), 0.26 (6H, s), 0.23 (6H, s). ^{13}C

NMR ($CDCl_3$, 125 MHz) δ 146.8, 145.0, 144.0, 141.1, 134.7, 133.0, 131.5 (q, J = 33.0 Hz), 130.8, 127.9 (q, J = 3.1 Hz), 123.6 (q, J = 272.7 Hz), 122.3, 120.6 (hept, J = 3.9 Hz), 118.5, 33.8, 26.43, 26.39, 26.1, 24.8, 19.1, 18.9, −3.1, −3.2.

4.1.1.42. 9-(3′,5′-Bis(trifluoromethyl)phenyl)-6,7-dihydro-5*H*-benzo[7]annulene-3,4-diol (66). TBS-protected analogue **53** (0.43 g, 0.84 mmol) was dissolved in THF (5 mL), and TBAF (1.00 mL, 1 M in THF) was added to the reaction flask. The reaction mixture was stirred for 18 h at ambient temperature, concentrated under reduced pressure, and H_2O (5 mL) was then added. The reaction mixture was extracted with EtOAc (3 × 10 mL). The organic extract was dried over Na_2SO_4, 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 catechol **66** (0.077 g, 0.20 mmol, 57%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (1H, s), 7.71 (2H, s), 6.71 (1H, d, J = 8.2 Hz), 6.50 (1H, t, J = 7.3 Hz), 6.38 (1H, d, J = 8.2 Hz), 5.36 (1H, s), 5.25 (1H, s), 2.73 (2H, t, J = 6.9 Hz), 2.19 (2H, p, J = 7.0 Hz), 2.04 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 144.7, 142.4, 141.43, 140.9, 132.9, 131.6 (q, J = 32.9 Hz), 131.1, 128.8, 128.0 (q, J = 2.9 Hz), 123.6 (q, J = 272.7 Hz), 121.2, 120.7 (hept, J = 3.9 Hz), 112.9, 33.7, 26.0, 23.9. ^{19}F NMR ($CDCl_3$, 470 MHz) δ −62.83. HRMS: Obsd 387.0805 $[M-H]^-$, Calcd for $C_{19}H_{13}F_6O_2$: 387.0820. HPLC (Method B): 18.11 min.

4.1.1.43. 5-(3′-Methoxyphenyl)pent-4-enoic acid (2).[31,33] To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (15.92 g, 37.09 mmol) in THF (500 mL) was added potassium *tert*-butoxide (8.20 g, 73.4 mmol), and the reaction mixture was stirred at room temperature for 1 h. 3-Methoxybenzaldehyde (4.5 mL, 37 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The THF was evaporated, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 × 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 → 70% A/30% B (10 CV), 70% A/30% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **2** (5.63 g, 27.3 mmol, 74%) as a yellow solid. NMR characterization was performed after the next step.

4.1.1.44. 5-(3′-Methoxyphenyl)pentanoic acid (8).[31,33,53] To dissolved carboxylic acid **2** (5.63 g, 27.3 mmol) in MeOH (100 mL) was added 10% palladium on carbon (0.44 g) and hydrogen gas. The reaction was stirred at room temperature for 12 h. The reaction mixture was then filtered through Celite®, and the Celite® was washed with EtOAc (3 × 50 mL). The organic phase (MeOH and EtOAc) was evaporated under reduced pressure to afford carboxylic acid **8** (4.27 g, 20.5 mmol, 75%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.2 (1H, s), 7.03 (1H, d, J = 8 Hz), 6.62 (2H, d, J = 5 Hz), 6.59 (1H, d, J = 8.5 Hz), 3.59 (3H, s), 2.44 (2H, t, J = 7.5 Hz), 2.21 (2H, d, J = 7.5 Hz), 1.52 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.2, 159.7, 143.7, 129.4, 120.9, 114.3, 111.1, 55.0, 35.6, 34.1, 30.7, 24.5.

4.1.1.45. 2-Methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (14). To carboxylic acid **8** (4.43 g, 21.3 mmol) was added Eaton's reagent (43 mL, 3 g per mmol of compound **8**), and the mixture was stirred at room temperature for 12 h. The mixture was then poured over ice and neutralized with sodium bicarbonate. The aqueous layer was extracted with EtOAc (3 × 50 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 benzosuberone **14** (2.80 g, 14.7 mmol, 70%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (1H, d, J = 8.5 Hz), 6.61 (1H, dd, J = 8.5, 2.5 Hz), 6.51 (1H, d, J = 2.5 Hz), 3.63 (3H, s), 2.70 (2H, t, J = 6 Hz), 2.51 (2H, t, J = 6 Hz), 1.67 (2H, p, J = 7.5 Hz), 1.59 (2H, p, J = 5.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.5, 162.5, 144.1, 131.3, 131.0, 114.7, 111.6, 55.1, 40.5, 32.6, 24.9, 20.5.

4.1.1.46. 2-Hydroxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (39).[31,55] To benzosuberone **14** (0.89 g, 4.7 mmol) in a 20 mL microwave vial was added $[TMAH][Al_2Cl_7]$ (22 mL, 0.53 M), and the reaction mixture was subjected to microwave irradiation for 1 h at 80 °C. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3 × 25 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 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7%/93% B (1 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford alcohol **39** (0.66 g, 3.8 mmol, 80%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (1H, d, J = 8.5 Hz), 6.75 (1H, dd, J = 8.5, 2.5 Hz), 6.67 (1H, d, J = 2 Hz), 3.84 (1H, s), 2.87 (2H, t, J = 6 Hz), 2.71 (2H, t, J = 6 Hz), 1.82 (4H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 204.9, 159.9, 144.8, 131.6, 131.3, 116.3, 113.7, 40.7, 32.7, 25.0, 20.7.

4.1.1.47. 2-((*tert*-Butyldimethylsilyl)oxy)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (42).[31] Phenol **39** (0.42 g, 2.4 mmol) was dissolved in dimethylformamide (50 mL). TBSCl (0.72 g, 4.8 mmol) and DIPEA (1.24 mL, 7.14 mmol) were added, and the solution was stirred for 12 h at room temperature. The reaction mixture was washed with water (50 mL) and extracted with EtOAc (5 × 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: 7% A/93% B (1 CV), 7% A/93% B → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford TBS-protected **42** (0.53 g, 1.82 mmol, 77%) as a clear oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (1H, d, J = 8.5 Hz), 6.64 (1H, dd, J = 8.5, 2 Hz), 6.56 (1H, d, J = 2 Hz), 2.77 (2H, t, J = 6 Hz), 2.59 (2H, t, J = 6 Hz), 1.75 (2H, p, J = 6.5 Hz), 1.68 (2H, p, J = 6 Hz), 0.90 (9H, s), 0.14 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 203.7, 159.0, 143.9, 132.0, 130.9, 120.8, 117.8, 40.5, 32.5, 25.5, 25.0, 20.6, 18.0, −4.5.

4.1.1.48. 2-((*tert*-Butyldimethylsilyl)oxy)-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (48).[31] To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (1.01 g, 4.04 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (1.71 mL, 4.27 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **42** (0.53 g, 3.0 mmol) in THF (25 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 water and 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 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: 50 mL/min; monitored at 254 and 280 nm] to afford tertiary alcohol **48** (0.470 g, 1.02 mmol, 34%) as a clear oil. NMR characterization was performed after the next step.

4.1.1.49. *tert*-Butyldimethyl((9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-3-yl)oxy)silane (55).[31] Acetic acid (10 mL) was added to tertiary alcohol **48** (0.47 g, 1.0 mmol), and the reaction mixture was stirred for 12 h at room temperature. The 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 to afford protected benzosuberene **55** (0.39 g, 0.89 mmol, 87%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.90 (1H, d, J = 8 Hz), 6.77 (1H, d, J = 2.5 Hz), 6.67 (1H, dd, J = 8.5, 2.5 Hz), 6.49 (2H, s), 6.34 (1H, t, J = 7 Hz), 3.86 (3H, s), 3.79 (6H, s), 2.60 (2H, t, J = 7 Hz), 2.15 (2H, p, J = 7.5 Hz), 1.96 (2H, q, J = 7 Hz), 1.00 (9H, s), 0.23 (6H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.4, 152.8, 143.7, 142.8, 138.4, 137.3, 133.0, 130.5, 127.0, 120.0, 117.3, 105.2, 60.9, 56.1, 35.0, 32.6, 25.7, 25.5, 18.2, −4.3.

4.1.1.50. 9-(3′,4′,5′-Trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-3-ol (68).[31] TBS-protected **55** (0.39 g, 0.89 mmol) was dissolved in THF (25 mL), TBAF (1.06 mL, 1.06 mmol) was added, and the reaction mixture was stirred at room temperature for 12 h. The solution 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 reaction product was 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 benzosuberene **68** (0.13 g, 0.40 mmol, 45%) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.89 (1H, d, J = 8 Hz), 6.78 (1H, d, J = 3 Hz), 6.67 (1H, dd, J = 8.5 Hz, 2.5 Hz), 6.50 (2H, s), 6.33 (1H, t, J = 7.5 Hz), 6.21 (1H, s), 3.88 (3H, s), 3.79 (6H, s), 2.59 (2H, t, J = 7 Hz), 2.14 (2H, p, J = 7 Hz), 1.95 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 154.9, 152.8, 144.0, 142.7, 138.7, 137.0, 132.1, 130.7, 127.1, 115.4, 112.9, 105.3, 61.0, 56.1, 35.0, 32.6, 25.5. HRMS: Obsd 349.1417 [M+Na$^+$], Calcd for $C_{20}H_{22}O_4Na$: 349.1410. HPLC (Method B): 14.30 min.

4.1.1.51. 2-Methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (20).[30,31,56] To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (2.82 g, 11.4 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (4.9 mL,12 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **14** (1.60 g, 8.41 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. The reaction mixture was washed with water and 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 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 (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford tertiary alcohol **20** (2.29 g, 6.48 mmol, 76%) as a light yellow oil. NMR characterization was performed after the next step.

4.1.1.52. 3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (29).[30,31,56] Acetic acid (15 mL) was added to tertiary alcohol **20** (2.29 g, 6.38 mmol), and the reaction mixture was stirred for 12 h at room temperature. The 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 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 → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **29** (0.362 g, 1.06 mmol, 17%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (1H, d, J = 8.5 Hz), 6.84 (1H, d, J = 2.5 Hz), 6.74 (1H, dd, J = 8.5, 2.5 Hz), 6.53 (2H, s), 6.37 (1H, t, J = 7.5 Hz), 3.78 (3H, s), 3.81 (3H, s), 3.79 (6H, s) 2.65 (2H, t, J = 7 Hz), 2.18 (2H, p, J = 7 Hz), 1.98 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 152.8, 143.7, 142.7, 138.5, 137.3, 132.3, 130.5, 126.8, 113.8, 111.1, 105.1, 60.7, 55.9, 54.9, 35.0, 32.7, 25.4. HRMS: Obsd 363.1574 [M+Na$^+$], Calcd for $C_{21}H_{24}O_4Na$: 363.1567. HPLC (Method B): 18.33 min.

4.1.1.53. 3-Methoxy-6,7-dihydro-5*H*-benzo[7]annulen-9-yl trifluoromethanesulfonate (59). To an oven dried flask, diisopropylamine (1.74 mL, 12.4 mmol) dissolved in THF (50 mL) was added and cooled to −78 °C. Then *n*-BuLi (5.0 mL, 12 mmol) was added, and the reaction was stirred for 15 min. Benzosuberone **14** (2.14 g, 11.3 mmol) dissolved in THF was added dropwise and stirred for 2 h at −78 °C. *N*-Phenyl-bis(trifluoromethanesulfonimide) (4.42 g, 12.4 mmol) dissolved in THF was then added dropwise, and the reaction mixture was stirred for 12 h while warming from −78 °C to room temperature. After 12 h, the THF was evaporated under reduced pressure, and the resulting solid was washed with water and extracted with EtOAc (3 × 50 mL). The combined organic layer was dried over sodium sulfate and evaporated under reduced pressure. The crude reaction product was purified by flash chromatography using a pre-packed 25 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 20% A/80% B (10 CV), 20% A/80% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford triflate **59** (2.28 g, 7.07 mmol, 68%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) 7.51 (1H, d, J = 8.5 Hz), 6.86 (1H, dd, J = 8.5, 2.5 Hz), 6.81 (1H, d, J = 3 Hz), 6.15 (1H, t, J = 6 Hz), 3.79 (3H, s), 2.77 (2H, t, J = 6.5 Hz), 2.20 (2H, q, J = 7 Hz), 2.04 (2H, q, J = 7 Hz).

4.1.1.54. 3-Methoxy-9-(2′,3′,4′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (62). Triflate **59** (2.28 g, 7.07 mmol) was dissolved in THF, and 2,3,4-trimethoxyphenyl boronic acid (1.65 g, 7.78 mmol), barium hydroxide octahydrate (3.35 g, 10.6 mmol), and tetrakis(triphenylphosphine)palladium(0) (0.24 g, 0.21 mmol) were added to the solution and refluxed at 80 °C for 2 h. The solution was then filtered through Celite®, and the Celite® was washed with dichloromethane (3 × 30 mL). The combined organic solution (THF and dichloromethane) was evaporated under reduced pressure. The crude reaction mixture 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 → 45% A/55% B (10 CV), 45% A/55% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **62** (1.05 g, 3.08 mmol, 44%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.02 (1H, d, J = 8.5 Hz), 6.88 (1H, d, J = 2.5 Hz), 6.77 (1H, dd, J = 8.5, 2.5 Hz), 6.58 (2H, s), 6.41 (1H, t, J = 7.5 Hz), 3.91 (3H, s), 3.83 (3H s), 3.82 (6H, s), 2.69 (2H, t, J = 7 Hz), 2.21 (2H, p, J = 7 Hz), 2.00 (2H, q, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 153.4, 152.8, 143.6, 142.7, 138.3, 137.4, 132.3, 130.4, 126.7, 123.5, 113.8, 111.1, 105.2, 60.6, 60.1, 55.8, 54.8, 35.0, 32.7, 25.4. HRMS: Obsd 363.1573 [M+Na$^+$], Calcd for $C_{21}H_{24}O_4Na$: 363.1567. HPLC (Method B): 18.23 min.

4.1.1.55. 3-Methoxy-9-(4′-methoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (37). To a solution of 4-methoxyphenyl bromide (0.886 g, 4.73 mmol) in THF (50 mL) at −78 °C was added

n-BuLi (3.4 mL, 2.5 M in hexanes), and the reaction mixture was stirred for 30 min. Benzosuberone **14** (0.601 g, 3.15 mmol) in THF (25 mL) was added dropwise over a period of 15 min. The reaction mixture was stirred while warming to room temperature over 12 h. Water was added, and the mixture was extracted with EtOAc (100 mL). The organic extract was washed with brine, dried over Na_2SO_4, filtered, concentrated under reduced pressure, and subjected to flash chromatography using a pre-packed 40 g silica column [solvent A, EtOAc, solvent B, hexanes; gradient 0% A/100% B → 100% A/0% B over 22.9 min; flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberene analogue **37** (0.32 g, 1.1 mmol, 36%) as a white solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20 (2H, d, J = 8.7 Hz), 6.94 (1H, d, J = 8.4 Hz), 6.83 (2H, d, J = 8.5 Hz), 6.82 (1H, d, J = 2.5 Hz), 6.73 (1H, dd, J = 8.5, 2.7 Hz), 6.29 (1H, t, J = 7.5 Hz) 3.83 (3H, s), 3.81 (3H, s), 2.63 (2H, t, J = 7.0 Hz), 2.16 (2H, p, J = 7.1 Hz), 1.96 (2H, q, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 158.3, 143.8, 142.1, 135.3, 133.0, 130.4, 125.9, 113.9, 113.50, 113.49, 111.1, 55.3, 55.2, 35.2, 32.8, 25.4. HRMS: Obsd 311.1713 [M+H$^+$], Calcd for $C_{20}H_{21}O_3$: 311.1642. HPLC (Method B): 17.65 min.

4.1.1.56. 3-Methoxy-2-methylbenzaldehyde.[58] *N*′,*N*′,*N*′-Trimethylethylene diamine (1.84 mL, 14.3 mmol) was dissolved in benzene (25 mL) and cooled to 0 °C. *n*-BuLi (5.5 mL, 13 mmol) was added dropwise, and the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was again cooled to 0 °C, and *m*-anisaldehyde (1.54 mL, 13.3 mmol) was added. The reaction mixture was stirred for 15 min at room temperature and then cooled to 0 °C. Phenyllithium (22.0 mL, 40.0 mmol) was added dropwise. The reaction mixture was stirred for 12 h at room temperature. THF (20 mL) was then added, and the reaction mixture was cooled to −78 °C. Methyl iodide (5.0 mL, 80 mmol) was added dropwise, and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was poured into cold 10% HCl (50 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 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 → 20% A/80% B (10 CV), 20% A/80% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford 3-methoxy-2-methylbenzaldehyde (2.01 g, 14.3 mmol, 98%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.97 (1H, s), 7.10 (1H, d, J = 8 Hz), 6.98 (1H, t, J = 8 Hz), 6.74 (1H, d, J = 8 Hz), 3.54 (3H, s), 2.23 (3H, s). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.0, 157.8, 134.9, 128.9, 126.3, 122.7, 114.8, 55.3, 10.0.

4.1.1.57. 5-(3′-Methoxy-2′-methylphenyl)pent-4-enoic acid (3). To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (6.55 g, 15.3 mmol) in THF was added potassium *tert*-butoxide (3.30 g, 29.0 mmol), and the reaction mixture was stirred at room temperature for 1 h. 3-Methoxy-2-methylbenzaldehyde (2.01 g, 13.4 mmol) dissolved in THF was added to the original reaction mixture, and the reaction mixture was stirred at room temperature for 12 h. The THF was evaporated, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phase was evaporated under reduced pressure 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 → 75% A/25% B (10 CV), 75% A/25% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **3** (2.12 g, 9.62 mmol, 69%) as a yellow oil. NMR characterization was performed after the next step.

4.1.1.58. 5-(3′-Methoxy-2′-methylphenyl)pentanoic acid (9). To dissolved carboxylic acid **3** (2.12 g, 9.62 mmol) in MeOH (100 mL) was added 10% palladium on carbon (0.44 g) and hydrogen gas. The reaction mixture was stirred at room temperature for 12 h. The mixture was then filtered through Celite®, and the Celite® was washed with EtOAc (3 × 50 mL). The combined organic phase (MeOH and EtOAc) was evaporated under reduced pressure. The residue 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: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **9** (1.20 g, 5.40 mmol, 59%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.94 (1H, s), 7.23 (1H, t, J = 8 Hz), 6.91 (1H, d, J = 7.5 Hz), 6.84 (1H, d, J = 8.5 Hz), 3.93 (3H, s), 2.77 (2H, t, J = 8 Hz), 2.51 (2H, t, J = 7 Hz), 2.34 (3H, s), 1.86 (2H, p, J = 7.5 Hz), 1.75 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.4, 157.9, 141.6, 126.1, 124.5, 121.6, 108.0, 55.5, 34.1, 33.4, 30.0, 24.7, 11.3.

4.1.1.59. 2-Methoxy-1-methyl-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (15). To carboxylic acid **9** (1.20 g, 5.40 mmol) was added Eaton's reagent (10.8 mL, 3 g per mmol of compound **9**), and the mixture was stirred at room temperature for 12 h. It was then poured over ice and neutralized with sodium bicarbonate. The aqueous layer was extracted with EtOAc (3 × 50 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: 5% A/95% B (1 CV), 5% A/95% B → 40% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **15** (2.43 g, 11.0 mmol, 74%) as a light yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.42 (1H, d, J = 8.5 Hz), 6.64 (1H, d, J = 8.5 Hz), 3.72 (3H, s), 2.76 (2H, t, J = 6 Hz), 2.53 (2H, t, J = 6 Hz), 2.09 (3H, s), 1.69 (2H, p, J = 7.5 Hz), 1.61 (2H, p, J = 6.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 205.9, 160.4, 140.4, 132.6, 127.4, 123.7, 107.6, 55.4, 40.4, 27.0, 24.1, 20.6, 11.0.

4.1.1.60. 2-Methoxy-1-methyl-5-(3,′4′,5-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (21). To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (0.85 g, 3.4 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (1.5 mL, 3.6 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **15** (0.52 g, 2.6 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. The reaction mixture was washed with water and 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 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 10% A/90% B (1 CV), 10% A/90% B → 60% A/50% B (10 CV), 60% A/50% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford tertiary alcohol **21** (2.29 g, 6.39 mmol, 76%) as a light yellow oil. NMR characterization was performed after the next step.

4.1.1.61. 3-Methoxy-4-methyl-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (30). Acetic acid (15 mL) was added to tertiary alcohol **21** (0.48 g, 1.3 mmol), and the reaction mixture was stirred for 12 h at room temperature. The 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 reaction

product was purified by flash chromatography using a pre-packed 10 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: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **30** (0.16. g, 0.45 mmol, 36%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (1H, d, J = 8.5 Hz), 6.69 (1H, d, J = 8.5 Hz), 6.53 (2H, s), 6.33 (1H, t, J = 7 Hz), 3.87 (3H, s), 3.82 (3H s,) 3.80 (6H, s), 2.69 (2H, t, J = 6.5 Hz), 2.29 (3H, s), 2.12 (2H, p, J = 7 Hz), 1.91 (2H, q, J = 7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 152.8, 143.5, 141.6, 138.5, 137.3, 132.9, 127.4, 126.4, 123.1, 107.4, 105.3, 60.8, 56.1, 55.4, 34.0, 27.7, 25.5, 11.8. HRMS: Obsd 377.1731 [M+Na$^+$], Calcd for $C_{22}H_{26}O_5Na$: 377.1723. HPLC (Method B): 19.56 min.

4.1.1.62. 2-Ethyl-3-methoxybenzaldehyde.[59] *N*′,*N*′*N*′-Trimethylethlene diamine (1.36 mL, 10.5 mmol) was dissolved in benzene (25 mL) and cooled to 0 °C. *n*-BuLi (4.05 mL, 10.1 mmol) was added dropwise, and the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was again cooled to 0 °C, *m*-anisaldehyde (1.34 mL, 9.84 mmol) was added, and the reaction mixture was stirred for 15 min at room temperature. The reaction mixture was then cooled to 0 °C, and phenyllithium (16.4 mL, 29.5 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature. THF (20 mL) was then added, and the reaction mixture was cooled to −78 °C. Ethyl iodide (3.7 mL, 59 mmol) was added dropwise, and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was poured into cold 10% HCl (50 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 using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 5% A/95% B (1 CV), 5% A/95% B → 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 2-ethyl-3-methoxybenzaldehyde (0.91 g, 5.6 mmol, 57%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.24 (1H, s), 7.37 (1H, dd, J = 7.5, 1 Hz), 7.23 (1H, t, J = 8 Hz), 7.01 (1H, dd, J = 8.5, 1 Hz), 3.79 (3H, s), 3.01 (2H, q, J = 7.5 Hz), 1.14 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.1, 157.6, 135.9, 134.4., 126.7, 122.6, 115.5, 55.7, 17.5, 15.3.

4.1.1.63. 5-(2′-Ethyl-3′-methoxyphenyl)pent-4-enoic acid (4). To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (3.56 g, 8.30 mmol) in THF (500 mL) was added potassium *tert*-butoxide (2.03 g, 18.1 mmol), and the reaction mixture was stirred at room temperature for 1 h. 2-Ethyl-3-methoxybenzaldehyde (1.35 g, 8.22 mmol) dissolved in THF (100 mL) was added to the original reaction mixture and stirred at room temperature for 12 h. The THF was evaporated, and the resulting material was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layer was 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 → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **4** (1.78 g, 7.60 mmol, 92%) as an orange-yellow oil. NMR characterization was performed after the next step.

4.1.1.64. 5-(2′-Ethyl-3′-methoxyphenyl)pentanoic acid (10). To dissolved carboxylic acid **4** (1.78 g, 7.60 mmol) in MeOH (50 mL) was added 10% palladium on carbon (0.58 g) and hydrogen gas. The reaction was stirred at room temperature for 12 h. The reaction mixture was then filtered through Celite®, and the Celite® was washed with EtOAc (3 × 50 mL). The combined organic phase (MeOH and EtOAc) was 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: 7% A/93% B (1 CV), 7% A/93% B → 40% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **10** (0.79 g, 3.3 mmol, 44%) as a clear oil. It is likely in this case that the carboxylic acid became methylated. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (1H, t, J = 8 Hz), 6.82 (1H, d, J = 7.5 Hz), 6.75 (1H, d, J = 8.5 Hz), 3.83 (3H, s), 3.70 (3H, s), 2.76 (2H, q, J = 7.5 Hz), 2.70 (2H, t, J = 8 Hz), 2.39 (2H, J = 7.5 Hz), 1.79 (2H, p, J = 7 Hz), 1.68 (2H, p, J = 8 Hz), 1.22, (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.7, 157.6, 140.9, 130.6, 126.2, 121.7, 108.1, 55.2, 21.2, 33.9, 32.6, 21.1, 25.1, 19.2, 14.5.

4.1.1.65. 1-Ethyl-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (16). To carboxylic acid **10** (0.79 g, 3.3 mmol) was added Eaton's reagent (6.7 mL, 3 g per mmol of compound **10**), and the reaction mixture was stirred at room temperature for 12 h. The mixture was then poured over ice and neutralized with sodium bicarbonate. The aqueous layer was extracted with EtOAc (3 × 50 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 → 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 benzosuberone **16** (0.32 g, 1.6 mmol, 52%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (1H, d, J = 8.5 Hz), 6.70 (1H, d, J = 8.5 Hz), 3.77 (3H, s), 2.82 (2H, t, J = 6 Hz), 2.65 (2H, q, J = 7.5 Hz), 2.57 (2H, t, J = 6 Hz), 1.75 (2H, p, J = 7 Hz), 1.66 (2H, p, J = 6.5 Hz), 1.03 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.3, 160.3, 139.5, 132.9, 130.0, 127.7, 108.0, 55.4, 40.4, 25.3, 24.9, 20.4, 18.9, 14.5.

4.1.1.66. 1-Ethyl-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (22). To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (0.52 g, 2.1 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (0.88 mL, 2.2 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **16** (0.32 g, 1.54 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. The reaction mixture was washed with water and 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 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 tertiary alcohol **22** (0.20 g, 0.52 mmol, 33%) as a light yellow oil. NMR characterization was performed after the next step.

4.1.1.67. 4-Ethyl-3-methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (31). Acetic acid (15 mL) was added to tertiary alcohol **22** (0.20 g, 0.52 mmol), and the reaction mixture was stirred for 12 h at room temperature. 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 reaction product was 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/50% B (10 CV), 60% A/40% B (2 CV); flow rate: 25 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **31** (0.085 g, 0.32 mmol, 45%) as a yellow solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.85 (1H, d, J = 8.5 Hz), 6.69

(1H, d, J = 8.5 Hz), 6.52 (2H, s), 6.32 (1H, t, J = 7.5 Hz), 3.86 (3H, s), 3.83 (3H, s), 3.81 (6H, s), 2.78 (2H, q, J = 7.5 Hz), 2.69 (2H, t, J = 6.5 Hz), 2.14 (2H, p, J = 7 Hz), 1.92 (2H, q, J = 7 Hz), 1.18 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.5, 153.1, 143.8, 141.3, 139.0, 137.6, 133.4, 129.9, 127.9, 126.7, 107.8, 105.6, 61.2, 56.4, 55.7, 35.2, 27.5, 25.7, 20.0, 15.2. HRMS: Obsd 391.1891 [M +Na$^+$], Calcd for $C_{23}H_{28}O_4Na$: 391.1880. HPLC (Method B): 20.66 min.

4.1.1.68. 3-Methoxy-2-propylbenzaldehyde.[60] *N*′,*N*′,*N*′-Trimethylethlene diamine (1.55 mL, 12.0 mmol) was dissolved in benzene (25 mL) and cooled to 0 °C. *n*-BuLi (4.6 mL, 11 mmol) was added dropwise, and the reaction mixture was stirred for 10 min at room temperature. The reaction mixture was again cooled to 0 °C, and *m*-anisaldehyde (1.30 mL, 11.2 mmol) was added. The reaction mixture was stirred for 15 min at room temperature and cooled to 0 °C. Phenyllithium (18.7 mL, 33.7 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature. THF (20 mL) was then added, and the reaction mixture was cooled to −78 °C. Propyl iodide (6.7 mL, 67.32 mmol) was added dropwise, and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was poured into cold 10% HCl (50 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 using a pre-packed 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 2% A/98% B (1 CV), 2% A/98% B → 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 3-methoxy-2-propylbenzaldehyde (1.82 g, 10.2 mmol, 91%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.19 (1H, s), 7.32 (1H, d, J = 8 Hz), 7.16 (1H, t, J = 7.5 Hz), 6.95 (1H, d, J = 8 Hz), 3.71 (3H, s), 2.94 (2H, t, J = 7 Hz), 1.48 (2H, sext, J = 7.5 Hz), 0.88 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.9, 157.8, 134.8, 134.3, 126.7, 122.3, 115.3, 55.5, 25.8, 24.2, 14.0.

4.1.1.69. 5-(3′-Methoxy-2′-propylphenyl)pent-4-enoic acid (5). To dissolved 3-(carboxypropyl)triphenyl phosphonium bromide (4.43 g, 10.3 mmol) in THF (500 mL) was added potassium *tert*-butoxide (2.52 g, 22.5 mmol), and the reaction mixture was stirred at room temperature for 1 h. 3-Methoxy-2-propylbenzaldehyde (1.82 g, 10.2 mmol) dissolved in THF (100 mL) was added to the original reaction mixture and stirred at room temperature for 12 h. The THF was evaporated, and the residue was quenched with 2 M HCl (75 mL) and extracted with EtOAc (3 × 100 mL). The combined organic phase was 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 → 60% A/40% B (10 CV), 60% A/40% B (2 CV); flow rate: 50 mL/min; monitored at 254 and 280 nm] to afford compound **5** (2.25 g, 9.06 mmol, 89%) as a yellow oil. NMR characterization was performed after the next step.

4.1.1.70. 5-(3′-Methoxy-2′-propylphenyl)pentanoic acid (11). To dissolved compound **5** (2.25 g, 9.06 mmol) in MeOH (50 mL) was added 10% palladium on carbon (0.47 g) and hydrogen gas. The reaction mixture was stirred at room temperature for 12 h. The mixture was then filtered through Celite®, and the Celite® was washed with EtOAc (3 × 50 mL). The combined organic phase (MeOH and EtOAc) was 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: 7% A/93% B (1 CV), 7% A/93% B → 40% A/60% B (10 CV), 40% A/60% B (2 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford compound **11** (0.87 g, 3.5 mmol, 38%) as a clear oil. It is likely that the carboxylic acid was methylated during this reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (1H, t, J = 8 Hz), 6.83 (1H, d, J = 7.5 Hz), 6.75 (1H, d, J = 8 Hz), 3.82 (3H, s), 3.71 (3H, s), 2.71 (4H, m), 2.40 (2H, t, J = 7.5 Hz), 1.79 (2H, p, J = 7.5 Hz), 1.66 (4H, m), 1.09 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.4, 157.5, 140.9, 128.8, 125.9, 121.2, 107.6, 54.8, 50.9, 33.5, 32.3, 30.7, 27.8, 24.7, 23.1, 14.2.

4.1.1.71. 2-Methoxy-1-propyl-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (17). To carboxylic acid **11** (0.87 g, 3.5 mmol) was added Eaton's reagent (7.0 mL, 3 g per mmol of compound **11**), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was then poured over ice and neutralized with sodium bicarbonate. The aqueous layer was 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 50 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: 50 mL/min; monitored at 254 and 280 nm] to afford benzosuberone **17** (0.56 g, 2.41 mmol, 69%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (1H, d, J = 8.5 Hz), 6.68 (1H, d, J = 8.5 Hz), 3.75 (3H, s), 2.81 (2H, t, J = 6.5 Hz), 2.59 (4H, m), 1.74 (2H, p, J = 7 Hz), 1.65 (2H, p, J = 6 Hz), 1.42 (2H, sext, J = 7.5 Hz), 0.91 (3H, t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.2, 160.4, 139.7, 132.9, 128.5, 127.6, 107.8, 55.2, 40.3, 27.6, 25.3, 24.8, 23.2, 20.3, 14.2.

4.1.1.72. 2-Methoxy-1-propyl-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (23). To an oven dried flask, THF (50 mL) and 3,4,5-trimethoxyphenyl bromide (0.80 g, 3.3 mmol) were added, and the solution was cooled to −78 °C. *n*-BuLi (1.4 mL, 3.4 mmol) was slowly added to the reaction mixture, which was then stirred at −78 °C for 1 h. Benzosuberone **17** (0.56 g, 2.4 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. The reaction mixture was washed with water and 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 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 tertiary alcohol **23** (0.53 g, 1.3 mmol, 52%) as a colorless oil. NMR characterization was performed after the next step.

4.1.1.73. 3-Methoxy-4-propyl-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (32). Acetic acid (10 mL) was added to tertiary alcohol **23** (0.53 g, 1.3 mmol), and the reaction mixture was stirred for 12 h at room temperature. 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 reaction mixture was purified by flash chromatography using a pre-packed 10 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: 12 mL/min; monitored at 254 and 280 nm] to afford benzosuberene **32** (0.13 g, 0.34 mmol, 25%) as a cream-colored solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.86 (1H, d, J = 8.5 Hz), 6.70 (1H, d, J = 8.5 Hz), 6.53 (2H, s), 6.33 (1H, t, J = 7.5 Hz), 3.88 (3H, s), 3.84 (3H, s), 3.82 (6H, s), 2.72 (4H, m), 2.15 (2H, p, J = 7 Hz), 1.93 (2H, q, J = 7 Hz), 1.59 (2H, sext, J = 7 Hz), 1.04 (3H,

t, J = 7.5 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.4, 152.8, 143.5, 141.3, 138.7, 137.3, 133.1, 128.3, 127.6, 126.4, 107.5, 105.3, 60.9, 56.2, 55.4, 35.0, 28.6, 27.3, 25.5, 23.8, 14.5. HRMS: Obsd 405.2043 [M+Na$^+$], Calcd for $C_{24}H_{30}O_4Na^+$: 405.2036. HPLC (Method B): 21.53 min.

4.1.1.74. 2-(2′,3′-Dimethoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole.[61,62] A mixture of 2,3-dimethoxybenzoic acid (5.00 g, 27.5 mmol) in $SOCl_2$ (10 mL) was stirred at room temperature for 24 h. The excess of $SOCl_2$ was evaporated under reduced pressure, and the residue was diluted with CH_2Cl_2 (80 mL). This solution was added dropwise to a solution of 2-amino-2-methylpropan-1-ol (4.89 g, 55 mmol) in CH_2Cl_2 (150 mL) at −10 °C and stirred for 20 h while warming to room temperature. The resulting suspension was filtered, and the filtrate was evaporated to give the crystalline amide. The latter was treated dropwise with $SOCl_2$ (10 mL) and stirred at room temperature for another 7 h. The mixture was then poured into diethyl ether (400 mL) to form a suspension, which was then cooled to 0 °C, and aqueous NaOH solution (20%, 100 mL) was added. The aqueous layer was extracted with diethyl ether (3 × 100 mL). The combined organic phase was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 2-(2,3-dimethoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole (5.22 g, 22.2 mmol, 81%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.31–7.00 (3H, m), 4.11 (2H, s), 3.86 (6H, m), 1.39 (6H, s). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 161.3, 153.3, 148.7, 123.8, 123.4, 122.6, 115.0, 79.2, 67.3, 61.4, 56.1, 28.3.

4.1.1.75. 2-(2′-Butyl-3′-methoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole.[63] A solution of 2-(2,3-dimethoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole (5.02 g, 21.4 mmol) in THF (80 mL) was stirred and cooled to −40 °C in a cyclohexanone/dry ice bath. *n*-BuLi (13 mL, 2.5 M) was added dropwise to the reaction flask. The reaction mixture was stirred for 5 h while warming to 0 °C. The reaction was quenched with saturated aqueous NH_4Cl and extracted with diethyl ether (3 × 30 mL). The combined organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 2-(2-butyl-3-methoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole as yellow liquid (4.99 g, 19.1 mmol, 89%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23 (1H, dd, J = 7.5 Hz, 1.2 Hz), 7.15 (1H, t, J = 8.0 Hz), 6.91 (1H, dd, J = 8.1, 1.1 Hz), 4.07 (2H, s), 3.82 (3H, s), 1.53–1.47 (2H, m), 1.38 (6H, s), 1.41–1.33 (4H, m), 0.93 (3H, t, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 163.0, 157.7, 132.0, 129.2, 126.1, 121.9, 112.3, 78.8, 67.7, 55.7, 32.4, 28.4, 26.5, 23.1, 14.0.

4.1.1.76. 2-Butyl-3-methoxybenzoic acid.[63,64] A solution of 2-(2-butyl-3-methoxyphenyl)-4,4-dimethyl-4,5-dihydrooxazole (4.69 g, 17.95 mmol) in 4.5 M HCl (100 mL) was refluxed for 21 h. After cooling to room temperature, the reaction mixture was extracted with diethyl ether (3 × 30 mL), and the combined organic phase was washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 2-butyl-3-methoxybenzoic acid as a colorless liquid (2.45 g, 11.8 mmol, 65%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.55 (1H, dd, J = 7.9 Hz, 1.1 Hz), 7.24 (1H, t, J = 8.0 Hz), 7.05 (1H, dd, J = 8.3 Hz, 0.9 Hz), 3.87 (3H, s), 3.01 (2H, m), 1.54 (2H, m), 1.43 (2H, m), 0.95 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.1, 158.0, 134.5, 129.9, 126.2, 122.9, 114.4, 55.8, 32.4, 26.3, 23.1, 13.9.

4.1.1.77. (2-Butyl-3-methoxyphenyl)methanol. 2-butyl-3-methoxybenzoic acid (2.45 g, 11.8 mmol) was dissolved in THF (40 mL) and stirred for 10 min. $LiAlH_4$ (7.6 mL, 2.0 M) was then added dropwise, and the reaction mixture was stirred for 10 h while warming to room temperature. The reaction was carefully quenched with a H_2O/THF (1:4) solution, followed by aqueous NaOH (15%, 20 mL), and a precipitate formed. The unwanted precipitate was removed by filtration through Celite®. The Celite® and unwanted precipitate were washed with CH_2Cl_2. The H_2O/THF/CH_2Cl_2 filtrate was extracted with EtOAc (3 × 30 mL), and the organic layer was rinsed with brine and dried with Na_2SO_4. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 (2-butyl-3-methoxyphenyl) methanol as a colorless liquid (1.94 g, 9.97 mmol, 85%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.19 (1H, t, J = 8.5 Hz), 7.01 (1H, d, J = 7.6 Hz), 6.83 (1H, d, J = 8.3 Hz), 4.72 (2H, d, J = 5.3 Hz), 3.83 (3H, s), 2.69 (2H, t, J = 7.4 Hz), 1.49 (4H, m), 0.95 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 157.7,139.6, 129.8, 126.6, 120.4, 110.0, 63.2, 55.5, 32.4, 25.5, 23.1, 14.0.

4.1.1.78. 2-Butyl-3-methoxybenzaldehyde. To a well stirred solution of pyridinium chlorochromate (2.586 g, 12 mmol) in CH_2Cl_2 (20 mL) at room temperature, (2-butyl-3-methoxyphenyl) methanol (1.936 g, 9.97 mmol) dissolved in CH_2Cl_2 (15 mL) was slowly added via syringe. The reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered through Celite®, and the Celite® was washed thoroughly with CH_2Cl_2. The filtrate was concentrated under reduced pressure and further purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 2-butyl-3-methoxybenzaldehyde as a dark yellow liquid (1.91 g, 9.93 mmol, 99%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.34 (1H, s), 7.46 (1H, dd, J = 7.8 Hz, 1.1 Hz), 7.30 (1H, t, J = 8.0 Hz), 7.09 (1H, dd, J = 8.1 Hz, 0.75 Hz), 3.87 (3H, s), 3.06 (2H, m), 1.53 (2H, m), 1.42 (2H, m), 0.95 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.3, 157.8, 135.0, 134.7, 126.7, 122.1, 115.5, 55.8, 33.5, 23.8, 22.8, 13.9.

4.1.1.79. 5-(2′-Butyl-3′-methoxyphenyl)pent-4-enoic acid (6). A mixture of 3-(carboxypropyl) triphenylphosphonium bromide (4.52 g, 10.6 mmol) and potassium *tert*-butoxide (2.62 g, 23.2 mmol) in THF (100 mL) was stirred for 1 h at room temperature. 2-Butyl-3-methoxybenzaldehyde (2.03 g, 10.6 mmol) in THF (20 mL) was added dropwise to the reaction mixture and stirred for 12 h at room temperature. The reaction was quenched with 2 M HCl (15 mL), then extracted with EtOAc (3 × 50 mL). The combined organic phase was washed with brine, dried with Na_2SO_4, and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/3% B → 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 carboxylic acid **6** (2.38 g, 9.07 mmol, 86%). NMR characterization was performed after the next step.

4.1.1.80. 5-(2′-Butyl-3′-methoxyphenyl)pentanoic acid (12). Carboxylic acid **6** (2.38 g, 9.07 mmol) was mixed with 10% Pd/C (0.15 g). Methanol (25 mL) was slowly added, and the reaction mixture was stirred for 12 h at room temperature under a H_2 atmosphere. The suspension was filtered through Celite®, and the Celite® was rinsed with EtOAc. The filtrate (MeOH and EtOAc) was concentrated under reduced pressure, and the crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 20% A/80% B (3 CV), 20% A/80% B → 100% A/0% B (10 CV), 100% A/0% B (1 CV); flow rate: 40 mL/min; monitored at 254 and 280 nm] to afford carboxylic acid **12** (1.48 g, 56.0 mmol, 61%) as a yellow-brown liquid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.09 (1H, t, J = 7.9 Hz), 6.77 (1H, d, J = 7.6 Hz), 6.72 (1H, d, J = 8.1 Hz), 3.81 (3H, s), 2.63 (4H, m), 2.40 (2H, t, J = 7.3 Hz), 1.75 (2H, m), 1.64 (2H, m), 1.44 (4H, m), 0.96 (3H, t, J = 7.2 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 179.4, 157.7, 141.1, 129.5, 126.1, 121.5, 108.1, 55.4, 34.0, 32.5, 32.3, 30.8, 25.7, 24.8, 23.2, 14.0.

4.1.1.81. 1-Butyl-2-methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one (18). Eaton's reagent (28 mL) was added to carboxylic acid **12** (1.48 g, 5.6 mmol) and sonicated until the **12** dissolved. The reaction mixture was then stirred at room temperature for 12 h. Ice was poured into the reaction flask, and then the reaction mixture was neutralized with a satd $NaHCO_3$ solution and extracted with EtOAc (3 × 50 mL). The combined organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 ketone **18** (1.13 g, 4.59 mmol, 82%) as a yellow liquid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.53 (1H, d, J = 8.6 Hz), 6.77 (1H, d, J = 8.6 Hz), 3.86 (3H, s), 2.89 (2H, m), 2.67 (4H, m), 1.83 (2H, p, J = 6.5 Hz), 1.75 (2H, m), 1.42 (4H, m), 0.96 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.8, 160.6, 139.8, 133.0, 128.9, 127.7, 108.0, 55.5, 40.5, 32.5, 26.5, 25.5, 25.5, 23.0, 20.0, 20.5, 14.0.

4.1.1.82. 1-Butyl-2-methoxy-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol (24). 5-Bromo-1,2,3-trimethoxybenzene (1.70 g, 6.9 mmol) was dissolved in THF (20 mL) and cooled to −78 °C. *n*-BuLi (4.06 mL, 2.5 M) was added dropwise, and the reaction mixture was stirred at −78 °C. After 1 h, ketone **18** (1.13 g, 4.6 mmol) in THF (15 mL) was added dropwise to the reaction flask. The reaction mixture was stirred for 12 h while warming to room temperature. The reaction was quenched with water (40 mL) and extracted with EtOAc (3 × 30 mL). The combined organic phase was dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction product was purified by flash chromatography using a prepacked 100 g silica column [solvent A: EtOAc; solvent B: hexanes; gradient: 7% A/93% B (3 CV), 7% A/93% B → 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 tertiary alcohol **24** (0.76 g, 1.8 mmol, 40%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39 (1H, d, J = 8.7 Hz), 6.72 (1H, d, J = 8.8 Hz), 6.51 (2H, s), 3.84 (3H, s), 3.82 (3H, s), 3.74 (6H, s), 2.96 (2H, m), 2.67 (4H, m), 1.82 (4H, m), 1.39 (4H, m), 0.92 (3H, t, J = 6.9 Hz).

4.1.1.83. 4-Butyl-3-methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene (33). The tertiary alcohol **24** (0.76 g, 1.8 mmol) was dissolved in acetic acid (10 mL), and the reaction mixture was stirred for 6 h. The reaction was quenched with water (50 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine, dried with Na_2SO_4 and concentrated under reduced pressure. The crude reaction 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 → 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 benzosuberene **33** (0.76 g, 1.8 mmol, quantitative) as a yellowish oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.84 (1H, d, J = 8.5 Hz), 6.69 (1H, d, J = 8.5 Hz), 6.51 (2H, s), 6.32 (1H, t, J = 7.4 Hz), 3.86 (3H, s), 3.83 (3H, s), 3.81 (6H, s), 2.74 (2H, m), 2.68 (2H, t, J = 6.9 Hz), 2.13 (2H, p, J = 7.0 Hz), 1.91 (2H, q, J = 7.3 Hz), 1.53 (2H, m), 1.46 (2H, m), 0.98 (3H, t, J = 7.3 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4, 152.8, 143.5, 141.2, 138.7, 137.3, 133.1, 128.5, 127.5, 126.4, 107.5, 105.3, 60.9, 56.2, 55.4, 34.9, 32.9, 27.3, 26.3, 25.5, 23.2, 14.1. HRMS: Obsd 397.2374 $[M+H]^+$, calcd for $C_{25}H_{33}O_4$: 397.2373. HPLC (Method B): 22.30 min.

4.2. Biological evaluations

4.2.1. SRB assay[65,66]

Inhibition of human cancer cell growth was assessed using the sulforhodamine B assay, as previously described.[65] Cancer cell lines were plated at 9000 cells/well into 96-well plates using DMEM supplemented with 5% fetal bovine serum/1% gentamicin sulfate and incubated for 24 h. Serial dilutions of the compounds were then added. After 48 h, the cells were fixed with trichloroacetic acid, washed, dried, stained with sulforhodamine B dye (Acid red 52), solubilized, and read at 540 nm and normalized to 630 nm with an automated Biotek plate reader. A growth inhibition of 50% (GI_{50} or the drug concentration causing 50% reduction in the net protein increase) was calculated from the absorbance data.

4.2.2. Colchicine binding assay

Inhibition of [^{3}H]colchicine binding to tubulin was determined using 0.1 mL reaction mixtures. Each reaction mixture contained 1.0 μM tubulin, 5.0 μM [^{3}H]colchicine (from Perkin–Elmer), 5% (v/v) dimethyl sulfoxide, potential inhibitors at 1.0, 5.0 or 50 μM and components that were previously demonstrated to stabilize the colchicine binding activity of tubulin[67] (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 $MgCl_2$, and 1.0 mM GTP). Incubation was for 10 min at 37 °C, a time point at which the binding reaction in the control is 40–60% complete. Reactions were stopped by adding 2.0 mL of ice-cold water and placing the samples on ice. Each sample was poured onto a stack of two DEAE-cellulose filters, followed immediately by 6 mL of ice-cold water, and the water was aspirated under reduced vacuum. The filters were washed with 2 mL water × 3 and, following removal of excess water under a strong vacuum, placed into vials containing 5 mL of Biosafe II scintillation cocktail. Samples were counted the next day in a Beckman scintillation counter. Samples with potential inhibitors were compared to controls with no inhibitor to determine percent inhibition. All samples were corrected for the amount of colchicine that bound to the filters in the absence of tubulin.

4.2.3. Inhibition of tubulin polymerization

Tubulin assembly experiments were performed using 0.25 mL reaction mixtures (final volume).[68] The mixtures contained 1 mg/mL (10 μM) purified bovine brain tubulin, 0.8 M monosodium glutamate (pH 6.6, as above), 4% (v/v) dimethyl sulfoxide, 0.4 mM GTP, and varying compound concentrations. Initially, all components except GTP were preincubated for 15 min at 30 °C in 0.24 mL. After chilling the mixtures on ice, 10 μL of 10 mM GTP was added. The reaction mixtures were then transferred to cuvettes held at 0 °C in Beckman DU-7400 and DU-7500 spectrophotometers equipped with electronic temperature controllers. The temperature was

jumped to 30 °C over about 30 s, and polymerization was followed turbidimetrically at 350 nm for 20 min. Each reaction set included a reaction mixture without compound, and the IC_{50} was defined as compound concentration that inhibited extent of assembly by 50% after 20 min at 30 °C.

4.2.4. In vivo tumor model

Human breast cancer cells, MDA-MB-231 (ATCC), were transfected with a lentivirus containing a firefly luciferase reporter. Highly expressing stable clones were isolated to create the cell line, MDA-MB-231-luc, which was kindly provided by Dr. Edward Graves, Stanford University.[51] Induction of tumors was carried out by injecting 10^6 cells mixed with 30% Matrigel™ (BD Biosciences, San Jose, CA) into the mammary fat pads of female SCID mice (UTSW breeding colony). Tumors were allowed to grow to a size of approximately 5 mm in diameter, determined by calipers, before selection for BLI or histological analysis. All animal procedures were approved by the University of Texas Southwestern Medical Center Institutional Animal Care and Use Committee.

4.2.5. In vivo bioluminescence imaging (BLI)

Bioluminescence imaging was carried out as described previously.[69] Briefly, anesthetized, tumor bearing mice (O_2, 2% isoflurane, Henry Schein Inc., Melville, NY) were injected subcutaneously in the fore-back neck region with 80 μL of a solution of luciferase substrate, *D*-luciferin (sodium salt, 120 mg/kg, in saline, Gold Biotechnology, St. Louis, MO). Mice were maintained under anesthesia (2% isoflurane in oxygen, 1 dm^3/min), while baseline bioluminescence imaging was performed using a Xenogen IVIS® Spectrum (Perkin–Elmer, Alameda, CA). A series of BLI images was collected over 35 min using the following settings: auto exposure time, f-stop = 2, Field of view = D, binning = 4 (medium). Light intensity–time curves obtained from these images were analyzed using Living Image® software. Mice were injected intraperitoneally with either 120 μL of saline (vehicle), **CA4P** (provided by OXiGENE 120 mg/kg in saline as used previously[7] or analogue **73** (20, 30 or 40 mg/kg) in saline immediately after baseline BLI. Bioluminescence imaging was repeated, with new luciferin injections 4 and 24 h later. Dosing with 20 and 30 mg/kg was repeated in a separate cohort of mice.

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Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, ^{31}P NMR, HPLC, HRMS for final target compounds and intermediates (^{1}H NMR, ^{13}C NMR, ^{19}F NMR only), X-ray crystallography for compound **72**, an alternative synthetic procedure for compound **30**, and molecular docking for compounds **29**, **62**, and **72**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2015.10.012.

References and notes

1. Siemann, D. W.; Chaplin, D. J.; Horsman, M. R. *Cancer* **2004**, *10*, 2491.
2. Siemann, D. W. *Cancer Treat. Rev.* **2011**, *37*, 63.
3. Tozer, G. M.; Kanthou, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423.
4. Kanthou, C.; Tozer, G. M. *Expert Opin. Ther. Targets* **2007**, *11*, 1443.
5. Horsman, M. R.; Bohn, A. B.; Busk, M. *Exp. Oncol.* **2010**, *32*, 143.
6. Siemann, D. W.; Bibby, M. C.; Dark, G. G.; Dicker, A. P.; Eskens, F. A.; Horsman, M. R.; Marme, D.; Lorusso, P. M. *Clin. Cancer Res.* **2005**, *11*, 416.
7. Mason, R. P.; Zhao, D.; Liu, L.; Trawick, M. L.; Pinney, K. G. *Integrat. Biol.* **2011**, *3*, 375.
8. Dougherty, G. J.; Chaplin, D. J. In *Vascular Disruptive Agents for the Treatment of Cancer*;; Meyer, T., Ed.; Springer: New York, 2010; pp 1–27. Chapter 1.
9. Siemann, D. W. In *Vascular-Targeted Therapies in Oncology*; Siemann, D., Ed.; John Wiley & Sons: London, UK, 2006; pp 1–8.
10. Pettit, G. R.; Singh, S. B.; Boyd, M. R. *J. Med. Chem.* **1995**, *38*, 1666.
11. Oshumi, K.; Nakagawa, R.; Fukuda, Y. *J. Med. Chem.* **1998**, *41*, 3022.
12. McGowan, A. T.; Fox, B. W. *Cancer Chemother. Pharmacol.* **1990**, *26*, 79.
13. El-Zayat, A. A. E.; Degan, D.; Drabek, S. *Anticancer Drug Des.* **1993**, *4*, 19.
14. Pettit, G. R.; Minardi, M. D.; Boyd, M. R.; Pettit, R. K. *Anticancer Drugs Des.* **2000**, *15*, 397.
15. Pettit, G. R.; Lippert, J. W. III *Anticancer Drug Des.* **2000**, *15*, 203.
16. Boehle, A. S.; Sipos, B.; Kliche, U.; Kalthoff, H.; Dohrmann, P. *Ann. Thorac. Surg.* **2001**, *71*, 1657.
17. Pettit, G. R.; Moser, B. R.; Boyd, M. R. *Anticancer Drug Des.* **2001**, *16*, 185.
18. Ohno, T.; Kawano, K.; Tahara, K. *Gastroenterology* **2001**, *120*, 2831.
19. Oshumi, K.; Hatanaka, R.; Nakagawa, R. *Anticancer Drugs* **1999**, *14*, 539.
20. Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experentia* **1989**, *45*, 209.
21. Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119.
22. Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829.
23. Pettit, G. R.; Rhodes, M. R. *Anticancer Drug Des.* **1998**, *13*, 183.
24. Cooney, M. M.; Ortiz, J.; Bukowski, R. M.; Remick, S. C. *Curr. Oncol. Rep.* **2005**, *7*, 90.
25. Kirwan, I. G.; Liadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W. III; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446.
26. Kretzschmann, V. K.; Fürst, R. *Phytochem. Rev.* **2014**, *13*, 191.
27. Baguley, B. C.; McKeage, M. J. C. *J. Clin. Invest.* **2012**, *2*, 985.
28. Liu, P.; Qin, Y.; Wu, L.; Yang, S.; Li, N.; Wang, H.; Xu, H.; Sun, K.; Zhang, S.; Han, X.; Sun, Y.; Shi, Y. *Anticancer Drugs* **2014**, *25*, 462.
29. Chaplin, D. J.; Horsman, M. R.; Siemann, D. W. *Curr. Opin. Investig. Drugs* **2006**, *7*, 522.
30. Sriram, M.; Hall, J. J.; Grohmann, N. C.; Strecker, T. E.; Wootton, T.; Franken, A.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2008**, *16*, 8161.
31. Pinney, K. G.; Sriram, M.; George, C.; Tanpure, R. P. PCT Int. Appl. WO 2,012,068,284 A2 20120524, 2012.
32. Pinney, K. G.; Mocharla, V. P.; Chen, Z.; Garner, C. M.; Ghatak, A.; Hadimani, M.; Kessler, J.; Dorsey, J. M.; Edvardsen, K.; Chaplin, D. J.; Prezioso, J.; Ghatak, U. R. U.S. Patent Appl. Publ. 20,040,043,969 A1, 2004.
33. Tanpure, R. P.; George, C. S.; Strecker, T. E.; Devkota, L.; Tidmore, J. K.; Lin, C. M.; Herdman, C. A.; MacDonough, M. T.; Sriram, M.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2013**, *21*, 8019.
34. Tanpure, R. P.; George, C. S.; Sriram, M.; Strecker, T. E.; Tidmore, J. T.; Hamel, E.; Charlton-Sevcik, A. K.; Chaplin, D. J.; Trawick, M. L.; Pinney, K. G. *MedChemComm* **2012**, *3*, 720.
35. Hadimani, M. B.; MacDonough, M. T.; Ghatak, A.; Strecker, T. E.; Lopez, R.; Sriram, M.; Nguyen, B. L.; Hall, J. J.; Kissler, R. J.; Shiral, A. R.; Liu, L.; Garner, C. M.; Pettit, G. R.; Hamel, E.; Chaplin, D. J.; Mason, R. P.; Trawick, M. L.; Pinney, K. G. *J. Nat. Prod.* **2013**, *76*, 1668.
36. Flynn, B. L.; Gill, G. S.; Grobelny, D. W.; Chaplin, J. H.; Paul, D.; Leske, A. F.; Lavranos, T. C.; Chalmers, D. K.; Charman, S. A.; Kostewicz, E.; Shackleford, D. M.; Morizzi, J.; Hamel, E.; Jung, M. K.; Kremmidiotis, G. *J. Med. Chem.* **2011**, *54*, 6014.

37. Tanpure, R. P.; Harkrider, A. R.; Strecker, T. E.; Hamel, E.; Trawick, M. L.; Pinney, K. G. *Bioorg. Med. Chem.* **2009**, *17*, 6993.
38. Pinney, K. G.; Pettit, G. R.; Trawick, M. L.; Jelinek, C.; Chaplin, D. J. In *Antitumor Agents from Natural Products*; Kingston, D., New man, D., Cragg, G., Eds., 2nd ed.; CRC Press, Taylor and Francis Group: Boca Raton, FL, 2012; pp 27–63.
39. Pinney, K. G. In *Vascular-Targeted Therapies in Oncology*; Siemann, D. W., Ed.; John Wiley & Sons: London, UK, 2006; pp 95–121.
40. Chen, Z.; Mocharla, V. P.; Farmer, J. M.; Pettit, G. R.; Hamel, E.; Pinney, K. G. *J. Org. Chem.* **2000**, *65*, 8811.
41. Pinney, K. G.; Bounds, A. D.; Dingeman, K. M.; Mocharla, V. P.; Pettit, G. R.; Bai, R.; Hamel, E. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1081.
42. Pinney, K. G.; Pettit, G. R.; Mocharla, V. P.; Del, P. M. M.; Shirali, A. PCT Int. Appl. WO 9839323 A1 19980911, 1998.
43. Kessler, R. J. Synthesis and Evaluation of New Inhibitors of Tubulin Polymerization and Their Corresponding Prodrugs as Potential Vascular Targeting Agents. Masters Thesis. Baylor University, Waco, TX, December 2002.
44. Chen, Z.; O'Donnell, C. J.; Maderna, A. *Tetrahedron Lett.* **2012**, *53*, 64.
45. Chen, Z.; Maderna, A.; Sukuru, S. C. K.; Wagenaar, M.; O'Donnell, C. J.; Lam, M. H.; Musto, S.; Loganzo, F. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 6688.
46. Eaton, P. E.; Carlson, G. R.; Lee, J. T. *J. Org. Chem.* **1973**, *38*, 4071.
47. Tanis, V. M.; Moya, C.; Jacobs, R. S.; Little, R. D. *Tetrahedron* **2008**, *64*, 10649.
48. Kemperman, G.; Roeters, T.; Hilberink, P. *Eur. J. Org. Chem.* **2003**, 1681.
49. Pascal, V. P.; Kepler, J. A.; Pettit, G. R.; Hamel, E. *Mol. Pharm.* **2000**, *57*, 180.
50. Pettit, G. R.; Grealish, M. P.; Hearld, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2000**, *43*, 2731.
51. Zhao, D.; Richer, E.; Antich, P. P.; Mason, R. P. *FASEB J.* **2008**, *22*, 2445.
52. Alhasan, M. K.; Liu, L.; Lewis, M. A.; Magnusson, J.; Mason, R. P. *PLoS One* **2012**, *7*, e46106.
53. Gardner, P. D.; Horton, W. J.; Thompson, G.; Twelves, R. R. *J. Am. Chem. Soc.* **1952**, *74*, 5527.
54. Caunt, D.; Crow, W. D.; Haworth, R. D.; Vodoz, C. A. *J. Chem. Soc.* **1950**, 1631.
55. Horton, W. J.; Pitchforth, L. L. *J. Org. Chem.* **1960**, *25*, 131.
56. Pinney, K. G., Sriram, M. PCT Int. Appl. WO 2,006,138,427 A2 20061228, 2006.
57. Carpenter, P. D.; Humphreys, D. J.; Proctor, G. R.; Rees, L. G. *J. Chem. Soc., Perkin Trans. 1* **1974**, *13*, 1527.
58. Comins, D. L.; Brown, J. D. *J. Org. Chem.* **1989**, *54*, 3730.
59. Richtzenhain, H.; Meyer-Delius, M. *Chem. Ber.* **1948**, *81*, 81.
60. Gapinksi, D. M.; Roman, C. R.; Rinkema, L. E. *J. Med. Chem.* **1988**, *31*, 172.
61. Meyers, A. I.; Mihelich, E. D. *J. Am. Chem. Soc.* **1975**, *97*, 7383.
62. Meyers, A. I.; Gabel, R.; Mihelich, E. D. *J. Org. Chem.* **1978**, *43*, 1372.
63. Flippin, L. A.; Berger, J.; Parnes, J. S.; Gudiksen, M. S. *J. Org. Chem.* **1996**, *61*, 4812.
64. Flippin, L. A.; Carter, D. S.; Dubree, N. J. P. *Tetrahedron Lett.* **1993**, *34*, 3255.
65. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, M.; Mayo, J.; Boyd, M. *J. Natl Cancer Inst.* **1991**, *83*, 767.
66. Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, *1*, 1112.
67. Hamel, E.; Lin, C. M. *Biochim. Biophys. Acta* **1981**, *675*, 226.
68. Hamel, E. *Cell Biochem. Biophys.* **2003**, *38*, 1.
69. Liu, L.; Beck, H.; Wang, X.; Hsieh, H.; Mason, R. P.; Liu, X. *PLoS One* **2012**, *7*, e43314.

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