**Supporting Information**

# An Amino-Benzosuberene Analogue That Inhibits Tubulin Assembly and Demonstrates Remarkable Cytotoxicity

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**General Experimental Procedures.** Anhydrous methanol (MeOH) was obtained from commercial sources, and tetrahydrofuran (THF) was dried using a solvent purification system. Thin-layer chromatography (TLC) plates (pre-coated glass plates with silica gel 60 $F_{254}$, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system using silica gel (200-400 mesh, 60 Å) prepacked columns. Intermediates and products synthesized were characterized on the basis of their $^{1}$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) spectroscopic data. TMS was used as an internal standard for spectra recorded in $CDCl_3$. All of the chemical shifts are expressed in ppm ($\delta$), coupling constants ($J$) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), and multiplet (m). HRMS were obtained using (+ve or –ve) electrospray ionization (ESI) techniques. Purity of the final compounds was further analyzed at 25 °C using a Agilent 1200 HPLC system with a diode-array detector ($\lambda$ = 190-400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm × 150 mm, 5 $\mu$m), and a Zorbax reliance cartridge guard-column; eluents, solvent A, water, solvent B, acetonitrile; gradient, 90% A/10% B →0% A/100% B over 0 to 20 min; 0% A/100% B over 20 to 22 min; 0% A/100% B →90% A/10% B over 22 to 26 min; stop time 30 min; post-time 5 min; flow rate 1.5 mL/min; injection volume 20 $\mu$L; monitored at wavelengths ($\lambda$ 254, 264, 280 and 300 nm).

[Scheme 2, Route 1]

**5-(3′-Methoxy-2′-nitrophenyl)pent-4-enoic acid** (**8**): To a solution of anhydrous THF (60 mL) under $N_2$ was added 3-(carboxypropyl)triphenylphosphonium bromide (2.66 g,

6.21 mmol) and potassium *tert*-butoxide (1.68 g, 14.9 mmol). The solution was allowed to stir for 1 h at ambient temperature. 3-Methoxy-2-nitrobenzaldehyde (1.07 g, 5.91 mmol) was added dropwise to the reaction mixture in anhydrous THF (5 mL), which was allowed to stir for 12 h at ambient temperature. The solution was quenched with 2 M HCl (20 mL), and the organic solvent was evaporated under reduced pressure. The aqueous phase was extracted with EtOAc (4 x 30 mL), washed with brine, dried over $Na_2SO_4$, filtered, evaporated under reduced pressure, and purified by flash chromatography with a prepacked 100 g silica gel column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 10% A/90% B ⇀ 12% A/88% B (1 CV), 12% A/88% B ⇀ 100% A/0% B (13 CV), 100% A/0% B (1.5 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm]. Pentenoic acid analogue **8** (0.879 g, 3.46 mmol, 58% yield) was obtained as a mixture of *E* and *Z* isomers that was a red solid, $R_f$ = 0.16 (50:50 hexanes:EtOAc).

**$^1$H NMR** ($CDCl_3$, 500 MHz) Reported as *E* & *Z* mixture: $\delta$ 7.38 (1H, t, *J* = 8.1 Hz), 7.34 (1H, t, *J* = 8.2 Hz), 7.12 (1H, dd, *J* = 8.0 Hz, 0.8 Hz), 6.96 (1H, *J* = 8.4 Hz), 6.89 (2H, d, *J* = 8.4 Hz), 6.37 (1H, d, *J* = 11.4 Hz), 6.31 (2H, m), 5.84 (1H, dt, *J* = 11.4 Hz, 7.3 Hz), 3.90 (3H, s), 3.88 (3H, s), 2.54 (4H, m), 2.45 (4H, m).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz) Reported as *E* & *Z* mixture: $\delta$ 178.1, 178.0, 150.76, 150.74, 134.8, 134.3, 130.62, 130.60, 123.6, 123.5, 121.6, 118.0, 111.2, 110.9, 56.41, 56.39, 33.4, 33.1, 28.0, 23.8.

**HRMS**, *m/z*: observed 252.0868 $[M + H]^+$, (calcd for $C_{12}H_{14}NO_5^+$, 252.0866).

**5-(3′-Methoxy-2′-nitrophenyl)pentanoic acid** (**9**): Pentenoic acid analogue **8** (2.06 g, 8.21 mmol) under $N_2$ was added to anhydrous MeOH (100 mL) under $N_2$. To this solution was added 10% Pd/C (2.00 g) and stirred for 10 min at ambient temperature. 1,4-Cyclohexadiene (16.4 g, 205 mmol) was added to the mixture, which was stirred for 4 h. The reaction mixture was filtered through celite, washed with EtOAc (4 x 25 mL), evaporated under reduced pressure, and purified by flash chromatography using 20% EtOAc/80% hexanes as eluent. Pentanoic acid analogue **9** was obtained as a tan solid (1.54 g, 6.10 mmol, 74% yield), $R_f$ = 0.22 (50:50, hexanes:EtOAc)

**$^1$H NMR** ($CDCl_3$, 500 MHz): $\delta$ 7.33 (1H, t, $J$ = 8.1 Hz), 6.87 (2H, dd, $J$ = 8.1 Hz, 3.7 Hz), 3.87 (3H, s), 2.58 (2H, m), 2.36 (2H, m), 1.67 (4H, m).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz): $\delta$ 179.4, 150.7, 141.8, 134.7, 130.7, 121.6, 110.1, 56.3, 33.6, 30.6, 29.7, 24.2.

**HRMS**, *m/z*: observed 276.0845 $[M + Na]^+$, (calcd for $C_{12}H_{14}NNaO_5^+$, 276.0842).

**2-Methoxy-1-nitro-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one** (**10**):[S2A] To a flask containing pentanoic acid analogue **9** (0.281 g, 1.11 mmol) was added 5.5 mL of Eaton's reagent (7.7% $P_2O_5$ in $CH_3SO_3H$). The solid slowly dissolved with vigorous stirring and was allowed to stir for 12 h at ambient temperature. The solution was poured over ice, which was allowed to melt, then slowly neutralized with $NaHCO_3$ (aq.). The aqueous phase was extracted with EtOAc (4 x 25 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, filtered, evaporated under reduced pressure, and purified using flash chromatography with a prepacked 25 g silica column [eluents;

solvent A, EtOAc, solvent B, hexanes; gradient, 5% A/95% B → 7% A/93% B (1 CV), 7% A/93% B → 60% A/40% B (13 CV), 60% A/40% B (1 CV); flow rate, 25 mL/min; monitored at λ's 254 and 280 nm]. Ketone **10** (0.222 g, 0.945 mmol, 85% yield) was obtained as a white solid, $R_f$ = 0.34 (70:30 hexanes:EtOAc).

**$^1$H NMR** ($CDCl_3$, 500 MHz): $\delta$ 7.84 (1H, d, $J$ = 8.8 Hz), 6.97 (1H, d, $J$ = 8.8 Hz), 3.94 (3H, s), 2.79 (2H, m), 2.71 (2H, m), 1.90 (2H, m), 1.81 (2H, m).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz): $\delta$ 203.1, 153.3, 141.4, 134.0, 132.2, 131.9, 110.3, 56.6, 40.3, 26.2, 24.4, 20.2.

**HRMS**, *m/z*: observed 236.0917 $[M + H]^+$, (calcd for $C_{12}H_{14}NO_4^+$, 236.0917).

[Scheme 2, continued from Route 1]

**2-Methoxy-1-nitro-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol** (**11**): A solution of 3,4,5-trimethoxyphenylbromide (0.389 g, 1.57 mmol) in anhydrous THF (15 mL) under $N_2$ was cooled to —78 °C, then *n*-BuLi (0.57 mL, 2.5 M in hexanes) was added and stirred for 1 h. Ketone **10** (0.222 g, 0.945 mmol) was slowly added to the reaction mixture in anhydrous THF (3 mL) and allowed to warm to ambient temperature overnight with continuous stirring. On completion, the reaction mixture was quenched with $H_2O$ (5 mL), and the solvent was evaporated under reduced pressure. The aqueous phase was extracted using EtOAc (4 x 15 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, filtered, evaporated under reduced pressure, and purified by flash chromatography using a prepacked 25 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 5% A/95% B (1 CV),

5% A/95% B → 12% A/88% B (1 CV), 12% A/88% B → 100% A/0% B (13 CV), 100% A/0% B (2 CV); flow rate, 40 mL/min; monitored at λ's 254 and 280 nm]. Tertiary alcohol **11** (0.320 g, 0.794 mmol, 84% yield) was obtained as a white solid, $R_f$ 0.20 (50:50, hexanes:EtOAc).

**$^1$H NMR** ($CDCl_3$, 500 MHz): $\delta$ 7.68 (1H, d, $J$ = 8.8 Hz), 6.88 (1H, d, $J$ = 8.9 Hz), 6.46 (2H, s), 3.90 (3H, s), 3.85 (3H, s), 3.77 (6H, s), 3.10 (2H, m), 2.40 (1H, m), 2.19 (1H, s), 2.12 (1H, m), 1.94 (1H, m), 1.79 (2H, m), 1.54 (1H, m).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz): $\delta$ 153.3, 149.4, 142.4, 140.4, 138.5, 137.7, 133.5, 129.3, 109.2, 104.0, 79.7, 60.9, 56.2, 56.2, 40.9, 28.6, 26.3, 25.9.

**HRMS**, *m/z*: observed 426.1525 $[M + Na]^+$, (calcd for $C_{21}H_{25}NNaO_7^+$, 246.1523).

**3-Methoxy-4-nitro-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene** (**4**): Tertiary alcohol **11** (0.266 g, 0.660 mmol) was dissolved in AcOH (10 mL) and refluxed at 140 °C for 3 h. The reaction was then cooled to ambient temperature and slowly neutralized with $NaHCO_3$ (aq.). The aqueous phase was then extracted with EtOAc (4 x 50 mL). The combined organic extracts were washed with brine, dried over $Na_2SO_4$, filtered, evaporated under reduced pressure, and purified by flash chromatography using a prepacked 25 g silica gel column [eluent; solvent A, EtOAc, solvent B, hexanes; gradient 5% A/95% B → 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 λ's 254 and 280 nm]. Nitro analogue **4** (0.189 g, 0.491 mmol, 74% yield) was obtained as a white solid, $R_f$ = 0.29 (70:30, hexanes:EtOAc).

**$^{1}$H NMR** ($CDCl_3$, 500 MHz): $\delta$ 7.12 (1H, d, $J$ = 8.7 Hz), 6.87 (1H, d, $J$ = 8.5 Hz), 6.45 (2H, s), 6.43 (1H, t, $J$ = 7.4 Hz), 3.91 (3H, s), 3.87 (3H, s), 3.82 (6H, s), 2.56 (2H, t, $J$ = 6.9 Hz), 2.22 (2H, p, $J$ = 7.0 Hz), 2.00 (2H, q, $J$ = 7.3 Hz).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz): $\delta$ 153.1, 149.3, 141.5, 141.5, 137.7, 137.3, 134.9, 133.7, 131.8, 128.6, 109.7, 105.1, 60.9, 56.3, 56.2, 34.7, 27.3, 25.2.

**HRMS**, *m/z*: observed 386.1599 $[M + H]^+$, (calcd for $C_{21}H_{24}O_6^+$, 386.1598).

**3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-amine** (**3**): Nitro analogue **4** (0.139 g, 0.361 mmol) was dissolved in AcOH (8 mL). Zinc dust (0.550 g, 8.42 mmol) was added to the solution, and the reaction mixture was stirred for 7 h at ambient temperature. The reaction was quenched with $NaHCO_3$ (aq.) and monitored by pH paper until a neutral pH was reached. The aqueous reaction mixture was extracted with EtOAc (4 x 25 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the crude product was purified by flash chromatography using a prepacked 25 g silica gel column [eluent; solvent A, EtOAc, solvent B, hexanes; gradient 5% A/95% B ⇀ 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 λ's 254 and 280 nm]. The amino analogue **3** (0.103 g, 0.290 mmol, 80% yield) was obtained as a yellow solid, $R_f$ = 0.47 (70:30, hexanes:EtOAc).

**$^{1}$H NMR** ($CDCl_3$, 500 MHz): $\delta$ 6.67 (1H, d, $J$ = 8.4 Hz), 6.52 (2H, s), 6.49 (1H, d, $J$ = 8.4 Hz), 6.30 (1H, t, $J$ = 7.4 Hz), 3.88 (3H, s), 3.86 (3H, s), 3.80 (6H, s), 2.59 (2H, t, $J$ = 6.9 Hz), 2.12 (2H, p, $J$ = 7.0 Hz), 1.95 (2H, q, $J$ = 7.2 Hz).

**$^{13}$C NMR** ($CDCl_3$, 125 MHz): $\delta$ 152.8, 146.3, 143.5, 138.6, 137.2, 133.6, 132.4, 126.8, 126.3, 119.8, 107.6, 105.3, 60.9, 56.1, 55.6, 33.2, 25.6, 25.3.

**HRMS**, *m/z*: observed 356.1857 $[M + H]^+$, (calcd for $C_{21}H_{26}O_4^+$, 356.1856).

[Scheme 2, Route 2]

**(*Z*)/(*E*)- 5-(3′-Methoxyphenyl)pent-4-enoic acid** (**13**): K-O*t*Bu (25.5 g, 227 mmol) was added to a well-stirred solution of 3-(carboxypropyl)triphenylphosphonium bromide (46.7 g, 109 mmol) in THF (500 mL, anhyd) at rt. The reaction mixture was then stirred for 15 mins. A solution of 3-methoxyaldehyde (14.2 g, 104 mmol) in THF (50 mL, anhyd) was added dropwise to the reaction mixture, which was stirred at rt for 8h. The reaction was quenched by careful addition of $H_2O$ (100 mL) and extracted with $Et_2O$ (2 × 500 mL). The aqueous phase was acidified with 2M HCl until the product precipitated making the solution cloudy, which then eventually turned clear again. This acidified aqueous phase was extracted with EtOAc (3 × 200 mL). The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered and evaporated under reduced pressure. Flash chromatography of the crude using a prepacked 160 g silica column, Eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 30% A/70% B over 4.0 min (1 CV), 30% A/70% B →70% A/30% B over 40.0 min (10 CV), 70% A/30% B over 8.0 min (2 CV); flow rate 50.0 mL/min; monitored at λ's 254 and 280 nm afforded the mixture of *E*/*Z*-isomers **13** (16.9 g, 82.2 mmol, 79 % yield), as a pale yellow liquid.

$^1$H NMR (**mixture of *E*/*Z*-isomers**) (500 MHz, $CDCl_3$) δ 7.21 (1H, t, *J* = 7.9 Hz), 6.94 (1H, dt, *J* = 7.6 Hz), 6.88 (1H, dd, *J* = 2.3, 1.7 Hz), 6.77 (1H, ddd, *J* = 8.25, 2.6, 0.8 Hz), 6.42 (1H, d, *J* = 15.8 Hz), 6.21 (1H, d, *J* = 15.8 Hz), 3.80 (3H, s, $OCH_3$), 2.55 (2H, m), 2.54 (2H, m). $^1$H NMR (500 MHz, $CDCl_3$) *δ* 7.69 (1H, dt, *J* = 7.7, 1.1 Hz), 7.60 (1H, dd, *J* = 2.6, 1.5 Hz), 7.25 (1H, t, *J* = 7.8 Hz), 6.46 (1H, dt, *J* = 11.6 Hz), 5.63 (1H, dt, *J* = 11.6 Hz), 3.85 (3H, s, $OCH_3$), 2.67 (2H, m), 2.49 (2H, m); $^{13}$C NMR (**mixture of *E*/*Z*-isomers**) (126 MHz, $CDCl_3$) δ 179.38, 179.35, 172.01, 159.74, 159.56, 159.42, 138.72, 138.46, 131.07, 130.53, 130.31, 130.16, 129.51, 129.48, 129.23, 128.35, 122.64, 121.20, 120.48, 118.76, 114.30, 114.23, 112.84, 112.38, 111.44, 77.29, 77.03, 76.78, 55.44, 55.19, 34.13, 33.75, 27.86, 23.80.

**HRMS**, *m*/*z*: observed 205.0868 $[M-1]^-$, (calcd for $C_{12}H_{13}O_3^-$, 205.0870).

**5-(3′-Methoxyphenyl)pentanoic acid** (**14**): A suspension of 10% Pd/C (0.154 g) and **13** (1.50 g, 7.27 mmol) in MeOH (20 mL) was stirred under $H_2$ gas for 24 h. The reaction was checked for completion by filtering a little amount of the reaction mixture through celite and evaporating the solvent to record NMR data. On completion the reaction mixture was filtered through celite and the solvent was evaporated under reduced pressure to obtain a pale yellow liquid. Flash chromatography of the crude mixture using a prepacked 25 g silica column, Eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 30% A/70% B over 1.19 min (1 CV), 30% A/70% B →80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 25.0 mL/min; monitored at λ's 254 and 280 nm afforded **14** (1.38 g, 6.63 mmol, 91 % yield), as a pale yellow liquid.

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.19 (1H, t, $J$ = 7.5 Hz), 6.76 (1H, d, $J$ = 7.5 Hz), 6.73 (1H, d, $J$ = 7.5 Hz), 6.72 (1H, s), 3.79 (3H, s, $OCH_3$), 2.61 (2H, m), 2.38 (2H, m), 1.68 (2H, m); $^{13}$C NMR (126 MHz, $CDCl_3$) δ 179.89, 159.60, 143.65, 129.29, 120.82, 114.16, 111.06, 77.28, 77.03, 76.77, 55.14, 35.56, 30.64, 24.28.

**2-Methoxy-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-one** (**15**)[S1]: Eaton's reagent (60.0 g) was added to compound **14** (3.01 g, 14.5 mmol) and the solution was stirred for 12 h under $N_2$. The reaction mixture was poured over ice and the ice was allowed to melt. The aqueous phase was extracted with $CH_2Cl_2$ (2 × 100 mL) and the combined organic phase was washed with $NaHCO_3$ (Satd. soln.) (2 × 100 mL). The organic phase was dried over $Na_2SO_4$ and the solvent was evaporated under reduced pressure to obtain a pale yellow liquid. Flash chromatography of the crude using a prepacked 25 g silica column, Eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 10% A/90% B over 1.19 min (1 CV), 10% A/90% B →50% A/50% B over 13.12 min (10 CV), 50% A/50% B over 2.38 min (2 CV); flow rate 25.0 mL/min; monitored at λ's 254 and 280 nm afforded benzosuberone **15** (2.10 g, 11.0 mmol, 76 % yield), as a white solid.

$^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.78 (1H, d, $J$ = 8.7 Hz, H-4), 6.81 (1H, dd, $J$ = 8.7, 2.5 Hz, H-3), 6.70 (1H, d, $J$ = 2.5 Hz, H-1), 3.85 (3H, s, $OCH_3$-2), 2.91 (2H, dd, $J$ = 6.5, 6.2 Hz, $CH_2$-9), 2.71 (2H, dd, $J$ = 6.0 Hz, $CH_2$-6), 1.88 (2H, m, $J$ = 6.5 Hz, $CH_2$-8), 1.80 (2H, dd, $J$ = 6.2 Hz, $CH_2$-7); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 204.3 (C, C-5), 162.7 (C, C-2), 144.2 (C, C-1a), 131.6 (C, C-4a), 131.3 (C, C-4), 114.9 (C, C-1), 111.7 (C, C-3), 55.4 ($CH_3$, $OCH_3$-2), 40.7 (C, C-6), 32.8 (C, C-9), 25.1 (C, C-8), 20.7 (C, C-7).

**HRMS**, *m*/*z*: observed 191.1071 $[M+1]^+$, (calcd for $C_{12}H_{15}O_2^+$, 190.1067).

**2-Methoxy-1-nitro-6,7,8,9-tetrahydro-5*H*- benzo[7]annulen-5-one** (**10**) [S2B]: To a solution of benzosuberone **15** (7.0 g, 36.8 mmol) in acetic anhydride (75 mL) cooled to -10 ºC, a solution (1:1) of $HNO_3$ and acetic acid (16 mL) was added dropwise with stirring. After stirring for 3h, ice cold water was added to quench the reaction. The mixture was stirred vigorously for 2 min and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 x 100 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and the solvents evaporated under reduced pressure. Flash chromatography of the crude mixture using a prepacked 50 g silica column, Eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 20% A/80% B over 4.57 min (1 CV), 20% A/80% B →80% A/20% B over 16.30 min (10 CV), 80% A/20% B over 3.18 min (2 CV); flow rate 40.0 mL/min; monitored at λ's 254 and 280 nm; afforded 1-nitrobenzosuberone **10** (3.24 g, 13.8 mmol, 37%) and 3-nitrobenzosuberone **12** (3.54 g, 15.1 mmol, 41 %).

$^1$H NMR (**10**) (500 MHz, $CDCl_3$) $\delta$ 7.84 (1H, d, $J$ = 8.8 Hz, H-4), 6.97 (1H, d, $J$ = 8.8 Hz, H-3), 3.94 (3H, s, $OCH_3$-2), 2.79 (2H, dd, $J$ = 6.5 Hz, H-9), 2.72 (2H, dd, $J$ = 6.0 Hz, H-6), 1.92 (2H, m, $J$ = 6.5 Hz, H-8), 1.82 (2H, m, $J$ = 6.0 Hz, H-7); $^{13}$C NMR (**10**) (126 MHz, $CDCl_3$) $\delta$ 203.1 (C, C-5), 153.3 (C, C-2), 141.4 (C, C-1), 134.0 (C, C-1a), 132.2 (C, C-4a), 131.9 (C, C-4), 110.3 (C, C-3), 56.6 ($CH_3$, $OCH_3$-2), 40.3 (C, C-6), 26.2 (C, C-9), 24.4 (C, C-8), 20.2 (C, C-7);

**Anal**., Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.82. Found: C, 61.17; H, 5.55; N, 5.82.

**HRMS**, *m*/*z*: observed 236.0919 $[M+1]^+$, (calcd for $C_{12}H_{14}NO_4^+$, 236.0917).

$^1$H NMR (**12**) (500 MHz, $CDCl_3$) $\delta$ 8.32 (1H, s, H-4), 6.88 (1H, s, H-1), 4.01 (3H, s, $OCH_3$-2), 2.99 (2H, dd, *J* = 6.6, 6.3 Hz, H-9), 2.76 (2H, dd, *J* = 6.1, 4.1 Hz, H-6), 1.94 (2H, m, *J* = 6.6, 6.3 Hz, H-8), 1.84 (2H, m, *J* = 6.1, 4.1 Hz, H-7); $^{13}$C NMR (**12**) (126 MHz, $CDCl_3$) $\delta$ 202.0 (C, C-5), 155.1 (C, C-2), 148.8 (C, C-1a), 138.2 (C, C-3), 131.0 (C, C-4a), 127.3 (C, C-4), 114.1 (C, C-1), 56.7 ($CH_3$, $OCH_3$-2), 40.4 (C, C-6), 33.1 (C, C-9), 24.7 (C, C-8), 20.3 (C, C-7);

**HRMS**, *m*/*z*: observed 236.0921 $[M+1]^+$, (calcd for $C_{12}H_{14}NO_4^+$, 236.0917).

[Scheme 2, continued from Route 2]

**2-Methoxy-1-nitro-5-(3′,4′,5′-trimethoxyphenyl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-5-ol: (11):** To a solution of 3,4,5-trimethoxyphenylbromide (5.01 g, 20.3 mmol) in anhydrous THF (200 mL) at -78 ºC, *n*-BuLi (8 mL, 2.5 M) was added and the reaction stirred for 30 min. Benzosuberone **10** (2.35 g, 10 mmol) in THF (25 mL) was added using a dropping funnel over a period of 15 min. The reaction mixture was stirred overnight and allowed to warm to ambient temperature. The reaction mixture was quenched with $H_2O$ (100 mL), and extracted with EtOAc (2 × 100 mL). The combined organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. Flash chromatography of the crude product using a prepacked 50 g

silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 20% A/80% B over 1.39 min (1 CV), 20% A/80% B →80% A/20% B over 16.3 min (10 CV), 80% A/20% B over 3.18 min (2 CV); flow rate 25.0 mL/min; monitored at λ 254 and 280 nm] afforded alcohol **11** (3.57 g, 8.8 mmol, 88% yield) as a white solid:

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.68 (1H, d, $J$ = 8.9 Hz, H-4), 6.88 (1H, d, $J$ = 8.9 Hz, H-3), 6.46 (2H, S, H-2′, -6′), 3.89 (3H, s, $OCH_3$-2), 3.84 (3H, s, $OCH_3$-4′), 3.76 (3H, s, $OCH_3$-3′, -5′), 2.62-2.58 (1H, m, H-9), 2.61-2.56 (1H, m, H-6), 2.42-2.36 (1H, m, H-9), 2.15-2.09 (1H, m, H-6), 1.96-1.93 (1H, m, H-8), 1.85-1.80 (1H, m, H-8), 1.80-1.72 (1H, m, H-7), 1.55-1.48 (1H, m, H-7); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 153.3 (C, C-3′, -5′), 149.3 (C, C-2), 142.4 (C, C-1), 140.4 (C, C-1′), 138.5 (C, C-4a), 137.6(C, C-4′), 133.5 (C, C-1a), 129.3 (CH, C-4), 109.2 (CH, C-3), 104.0 (CH, C-2′, -6′), 79.7 (C, C-5), 60.9 ($CH_3$, $OCH_3$-4′), 56.23 ($CH_3$, $OCH_3$-2), 56.18 ($CH_3$, $OCH_3$-3′, -5′), 40.8 ($CH_2$, $CH_2$-6), 28.6 ($CH_2$, $CH_2$-9), 26.3 ($CH_2$, $CH_2$-7), 25.9 ($CH_2$, $CH_2$-8).

**HRMS**, *m*/*z*: observed 426.1523 $[M+Na]^+$, (calcd for $C_{21}H_{25}NO_7Na^+$, 426.1523).

**3-Methoxy-4-nitro-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulene** (**4**): A solution of **11** (3.5 g, 8.7 mmol) in AcOH (50 mL) and $H_2O$ (50 mL) was heated to reflux at 160 °C for 12 h. The reaction mixture was cooled and the aqueous solvents evaporated under reduced pressure to obtain a crude product. Flash chromatography of the crude product using a prepacked 25 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 20% A/80% B over 1.19 min (1 CV), 20% A/80% B →80% A/20% B over 13.12 min (10 CV), 80% A/20% B over 2.38 min (2 CV); flow rate 25.0

mL/min; monitored at λ 254 and 280 nm] afforded benzosuberene **4** (2.96 g, 7.68 mmol, 88% yield) as a white solid:

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 7.12 (1H, d, $J$ = 9.0 Hz, H-4), 6.87 (1H, d, $J$ = 9.0 Hz, H-3), 6.45 (2H, S, H-2′, -6′), 6.43 (2H, t, $J$ = 7.5 Hz, H-6), 3.91 (3H, s, $OCH_3$-3), 3.87 (3H, s, $OCH_3$-4′), 3.82 (3H, s, $OCH_3$-3′, -5′), 2.56 (2H, t, $J$ = 7.0 Hz, H-9), 2.22 (2H, p, $J$ = 7.0 Hz, H-8), 2.00 (2H, dt, $J$ = 7.0, 7.5 Hz, H-7); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 153.1 (C, C-3′, -5′), 149.3 (C, C-3), 141.5 (C, C-4), 141.5 (C, C-5), 137.7 (C, C-4′), 137.3 (C, C-1′), 134.9 (C, C-4a), 133.7 (C, C-1a), 131.8 (CH, C-1), 128.6 (CH, C-8), 109.6 (CH, C-2), 105.1 (CH, C-2′, -6′), 60.9 ($CH_3$, $OCH_3$-4′), 56.3 ($CH_3$, $OCH_3$-3′, -5′), 56.2 ($CH_3$, $OCH_3$-3), 34.7 ($CH_2$, $CH_2$-6), 27.3 ($CH_2$, $CH_2$-5), 25.2 ($CH_2$, $CH_2$-7).

**Anal**., Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.41; H, 6.19; N, 3.58.

**HRMS**, *m*/*z*: observed 386.1600 $[M+1]^+$, (calcd for $C_{21}H_{24}NO_6^+$, 386.1598).

**3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-amine** (**3**): A suspension of **4** (0.049 g, 0.127 mmol) and zinc (0.43 g) in AcOH (10 mL) was stirred for 2h. The reaction mixture was filtered and the aqueous solvents evaporated under reduced pressure to obtain a crude product. Flash chromatography of the crude product using a prepacked 10 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 30% A/70% B over 1.15 min (1 CV), 30% A/70% B →30% A/70% B over 13.07 min (10.5 CV), 80% A/20% B over 2.3 min (2 CV); flow rate 12.0 mL/min;

monitored at λ 254 and 280 nm] afforded benzosuberene **3** (0.040 g, 0.113 mmol, 88% yield) as a white solid:

$^{1}$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.67 (1H, d, $J$ = 8.4 Hz, H-3), 6.52 (2H, S, H-2′, -6′), 6.49 (1H, d, $J$ = 8.4 Hz, H-4), 6.30 (2H, t, $J$ = 7.3 Hz, H-6), 3.88 (3H, s, $OCH_3$-2), 3.86 (3H, s, $OCH_3$-4′), 3.80 (3H, s, $OCH_3$-3′, -5′), 2.59 (2H, t, $J$ = 7.0 Hz, H-9), 2.12 (2H, p, $J$ = 7.0 Hz, H-8), 1.95 (2H, dt, $J$ = 7.0, 7.3 Hz, H-7); $^{13}$C NMR (126 MHz, $CDCl_3$) $\delta$ 152.8 (C, C-3′, -5′), 146.3 (C, C-2), 143.5 (C, C-5), 138.5 (C, C-1′), 137.2 (C, C-4′), 133.5 (C, C-4a), 132.4 (C, C-1), 126.8 (C, C-1a), 126.3 (CH, C-6), 119.8 (CH, C-4), 107.5 (CH, C-3), 105.2 (CH, C-2′, -6′), 60.9 ($CH_3$, $OCH_3$-4′), 56.1 ($CH_3$, $OCH_3$-3′, -5′), 55.5 ($CH_3$, $OCH_3$-2), 33.2 ($CH_2$, $CH_2$-8), 25.6 ($CH_2$, $CH_2$-7), 25.3 ($CH_2$, $CH_2$-9).

**HRMS**, *m*/*z*: observed 356.1862 $[M+1]^+$, (calcd for $C_{21}H_{26}NO_4^+$, 356.1856).

Purity by HPLC: $R_f$ = 10.65 min, 95% at 254, and 280 nM.

[Scheme 3]

**3-Methoxy-9-(3′,4′,5′-trimethoxyphenyl)-6,7-dihydro-5*H*-benzo[7]annulen-4-amine hydrochloride** (**16**): Amine analogue **3** (0.049 g, 0.137 mmol) was dissolved in $Et_2O$ (4 mL). To this solution was added 1M HCl in $Et_2O$ (4 mL) and the solution was stirred for 2 h at room temperature. The reaction mixture was then filtered through a nylon membrane and washed with $Et_2O$ (3 x 3 mL). The amine salt **16** (0.028 g, 0.072 mmol, 52% yield) was obtained as a white solid.

**$^1$H NMR** ($D_2O$, 500 MHz): $\delta$ 6.97 (1H, d, $J$ = 8.6 Hz), 6.95 (1H, d, $J$ = 8.6 Hz), 6.54 (2H, s), 6.45 (1H, t, $J$ = 7.3 Hz), 3.84 (3H, s), 3.682 (6H, s), 3.676 (3H, s), 2.57 (2H, t, $J$ = 7.0 Hz), 2.09 (2H, p, $J$ = 7.1 Hz), 1.82 (2H, q, $J$ = 7.1).

**$^1$H NMR** ($CD_3OD$, 500 MHz): $\delta$ 7.10 (1 H, d, $J$ = 8.8 Hz), 7.08 (1 H, d, $J$ = 8.8 Hz), 6.494 (2 H, s), 6.491 (1 H, t, $J$ = 7.4 Hz), 4.00 (3 H, s), 3.77 (3 H, s), 3.75 (6 H, s), 2.74 (2 H, t, $J$ = 7.0 Hz), 2.25 (2 H, p, $J$ = 7.2 Hz), 1.95 (2 H, q, $J$ = 7.3 Hz).

**$^{13}$C NMR** ($CD_3OD$, 125 MHz): $\delta$ 154.4, 152.9, 143.4, 139.2, 138.9, 137.8, 135.8, 132.0, 129.0, 118.1, 110.7, 106.6, 61.2, 57.0, 56.6, 34.9, 26.8, 26.1.

**HRMS**, *m*/*z*: observed 356.1865 $[M+1]^+$, (calcd for $C_{21}H_{26}NO_4^+$, 356.1856). [Note: Calculated based on protonated free amine].

Purity by HPLC: $R_f$ = 8.12 min, 98% at 254, and 280 nM.

**Biology:**

**Effects on Tubulin Polymerization.** Bovine brain tubulin was purified using methods previously described.[S3] The effect of compounds on tubulin assembly in vitro was determined by using a series of concentrations that were preincubated with 10 $\mu$M tubulin (1.0 mg/mL) in glutamate buffer at 30 °C, followed by cooling to 0 °C. After GTP was added, the samples were mixed and transferred to cuvettes at 0 °C in a recording spectrophotometer and warmed to 30 °C to initiate polymerization. Tubulin assembly was observed turbidimetrically at 350 nm.[S4] Tubulin disassembly was confirmed by cooling to 0 °C. The calculated compound concentration that inhibited tubulin assembly by 50% after a 20 min incubation was defined as the $IC_{50}$ value.

**Colchicine Binding Assay.** Colchicine binding was measured as previously described.[S4,S5] Solutions containing 1 μM tubulin, 5.0 μM [$^3$H]colchicine, and inhibitor at either 5 μM or 1 μM (as specified) was used for all assay conditions. The values presented herein were attained from greater than 3 independent determinations.

**Cell lines, and Sulforhodamine B (SRB) assay.** Cancer cell lines were obtained from ATCC (DU-145 (prostate), SK-OV-3 (ovarian), and NCI-H460 (lung)) and maintained according to recommended conditions. Media was enriched with the recommended concentration of fetal bovine serum, as well as gentamicin and amphotericin B. The National Cancer Institute's standard SRB assay assessed cancer cell line growth inhibition, as previously described as the $GI_{50,}$ or the drug concentrations calculated to cause a 50% reduction in net protein increase relative to untreated cells.[S6-S8] Results reported are averages of at least three separate experiments, each of which was carried out in triplicate.

**Endothelial Tube Disruption Assay Experimental**

Matrigel™ basement membrane matrix (BD Biosciences Inc., Bedford, MA) was thawed on ice at 4 $^oC$ and then swirled to homogeneity prior to use. To a pre-cooled tissue culture treated 24-well culture plate (Corning Inc., Corning, NY), 300 μL of Matrigel™ (9.5 mg/mL) was dispensed into each well. The plates were then incubated at 37 $^oC$ for 1 hour prior to seeding with HUVEC cells. HUVEC cells, cultured in a 75 $cm^2$ tissue culture treated cell culture flask (Corning Inc., Corning, NY), were allowed to grow to 90% confluency in Medium 200 (Gibco®, Grand Island, NY) supplemented with

Gentamycin Sulfate (100 µg/mL, Teknova Inc., Hollister, CA), Amphotericin B (0.25 µg/mL, Mediatech Inc., Manassas, VA) and a low serum growth supplement (50x, 10 mL, Gibco®, Grand Island, NY) containing fetal bovine serum (2%), hydrocortisone (1 µg⁄ml), human epidermal growth factor (10 ng⁄ml), basic fibroblast growth factor (3 ng⁄ml), and heparin (10 µg⁄ml). Cells were removed from the culture flask by treatment with a trypsin/ EDTA solution (1x, 3 mL, Gibco®, Grand Island, NY) followed by incubation in a 37 $^{o}$C incubator for 4 minutes. The trypsin was then neutralized by treatment with a trypsin neutralizer solution (1x, 4 mL, Gibco®, Grand Island, NY). Cells were counted on a Beckman Z1 Coulter Particle Counter (Beckman Coulter™, USA ) and the cells were then centrifuged at 650 rpm for 6 minutes using an Eppendorf 5810 R centrifuge with a swing-bucket A-4-81 rotor (Eppendorf Inc., Hamburg, Germany). Cells were then resuspended in Medium 200 (Gibco®, Grand Island, NY) growth media containing gentamycin sulfate (100 µg/mL, Teknova Inc., Hollister, CA), Amphotericin B (0.25 µg/mL, Mediatech Inc., Manassas, VA), and an endothelial cell growth kit (ATCC Primary Cell Solutions™, Manassas, VA) containing rh VEGF (5 ng/mL), rh EGF (5 ng/mL), Rh FGF basic (5 ng/mL), Rh IGF-1 (15 ng/mL), L-glutamine (10 mM), heparin sulfate (0.75 units/mL), hydrocortisone hemisuccinate (1 µg/mL), fetal bovine serum (2%), and ascorbic acid (50 µg/mL). HUVEC cells (300 µL - 413333 cells/mL) were added to each well of the 24-well culture plate and the plate was allowed to incubate for 16 hours at 37 $^{o}$C in a NAPCO Series 8000 WJ $CO_2$ incubator (Thermo Electron Corporation, USA) containing a 5% $CO_2$ atmosphere to allow the HUVEC cells to grow tube-like structures. After the 16 hour incubation period, the cells were treated with varying concentrations of compounds dissolved in Medium 200 growth media and

1% DMSO and then the cells were allowed to further incubate at 37 $^{o}$C for 2 hours in the incubator containing a 5% $CO_2$ atmosphere. After the incubation period, the media was removed from the cells followed by 2 washes using 400 µL of the Medium 200 growth media. The effect of the compounds on the disruption of endothelial tubes was evaluated by light microscopy. Photos were taken (9-fields/well) on a Zeiss Axiovert 40 CFL confocal microscope (Carl Zeiss Inc., Thornwood, NY) with a x40 objective lens. Three independent experiments were conducted for each tested compound. This procedure was modified from the BD BioCoat™ Angiogenesis System-Endothelial Cell Tube Formation's Guidelines for Use (Catalog No. 354149, 354150) which can be found at the following web address:

http://www4.bdj.co.jp/external_files/dl/doc/manuals/live/web_enabled/354149_354150_pug.pdf

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