Supporting Information

Pyrrole-Based Antitubulin Agents: Two Distinct Binding Modalities Are Predicted for C-2 Analogues in the Colchicine Site

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Experimental Procedures

Chemistry

General

All chemicals were used as received from the manufacturer (Aldrich Chemicals and Fisher Scientific) and all reactions were carried out under a nitrogen or argon atmosphere. All solvents were dried over 4 angstrom molecular sieves prior to their use. NMR spectra were obtained on either a Bruker 300 MHz spectrometer or a Bruker 500 MHz spectrometer in either $CDCl_3$, d_6-DMSO or d_6-acetone solutions. IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrometer with an HATR attachment. High-resolution mass spectra were provided on a Biotof Q electrospray mass spectrometer at the University of Richmond. Low-resolution GC-MS spectra were obtained on a Shimadzu QP 5050 instrument. Melting points and boiling points are uncorrected. Flash chromatographic separations were carried out on a Biotage SP-1 instrument, which was equipped with a silica cartridge, and ethyl acetate/hexane was used as the eluant. Microwave accelerated reactions were carried out in a Biotage Liberator system. Microwave reactions were controlled at a constant temperature whereby the microwave power was allowed to fluctuate so as to maintain a constant temperature and safe pressure limits. TLC analyses were conducted on silica plates with hexane/ethyl acetate as the eluant. Vinamidinium salts utilized for pyrrole formation were prepared according to standard procedures.[S1] All purified reaction products gave TLC results, GC-MS spectra, flash chromatograms and ^{13}C NMR spectra consistent with a sample purity of >95%.

Assessment of purity

All samples listed in Table 1, with the exception of **7j** and **7k**, were purified by preparative chromatography. A typical chromatogram, for **7e**, is shown in Figure

S1. Compounds **7a-7d** and **7f-7i** produced very similar chromatograms. Compounds **7j** and **7k** were obtained by simply making the hydrochloride salts of **7h** and **7i**, which were purified by flash chromatography. All compounds listed in Table 1 gave ^{1}H and ^{13}C NMR spectra completely analogous to those shown for **7e** in Figures S2 and S3, respectively.

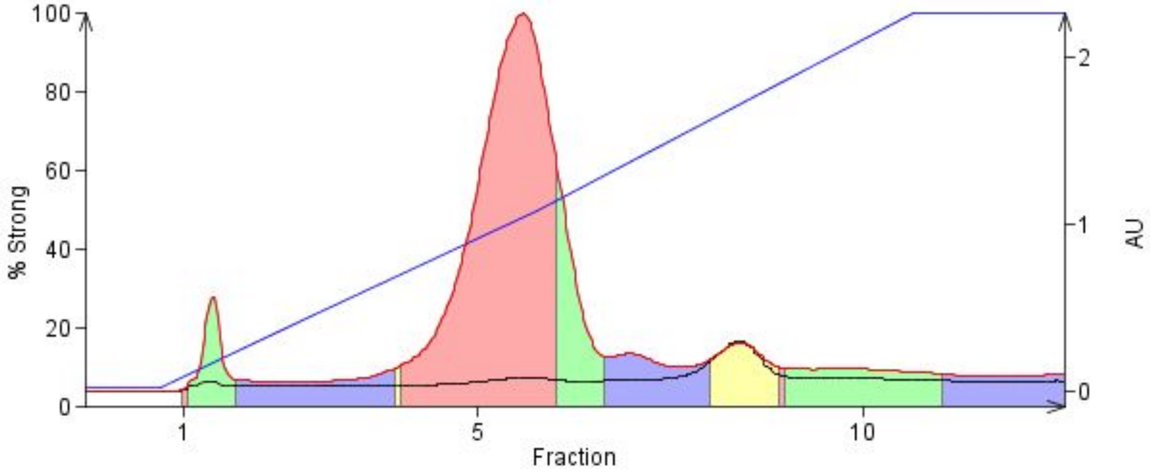

Figure S1. This material (**7e**) was purified on a Biotage SP-1 preparative chromatograph using a silica column with a hexane/ethyl acetate gradient. Fraction 5 represents the pyrrole fraction collected and characterized as compound **7e**.

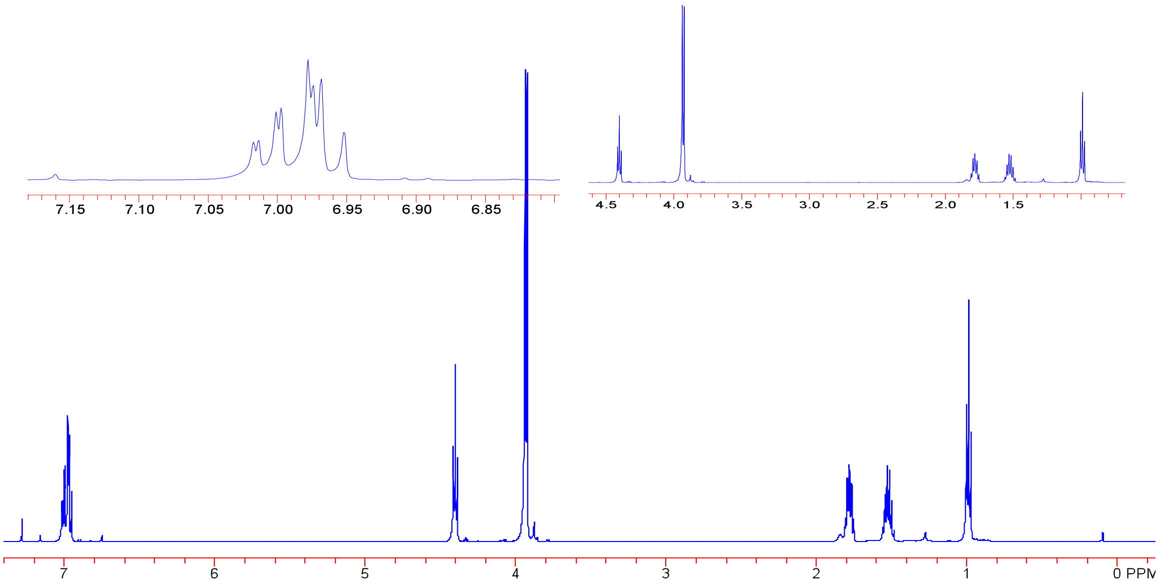

Figure S2. ^{1}H NMR spectrum for **7e**.

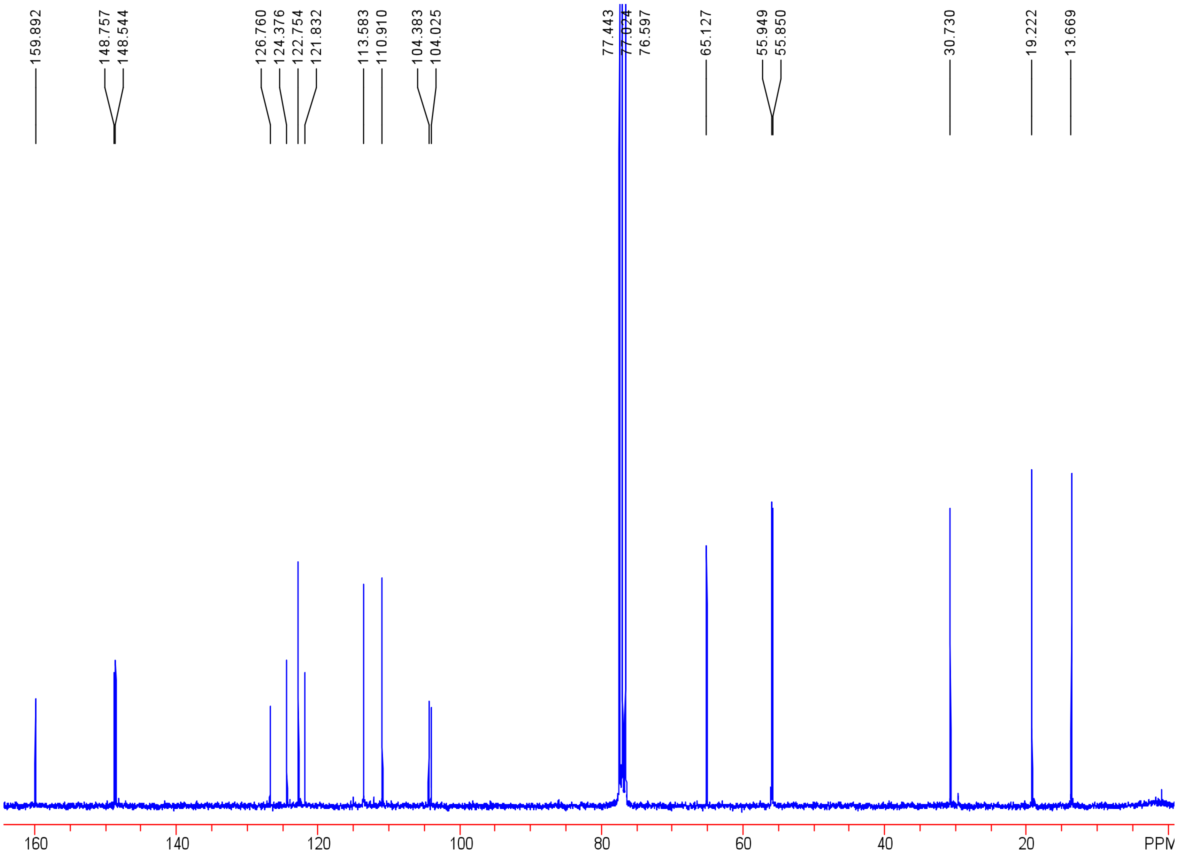

Figure S3. ^{13}C NMR spectrum for **7e**.

<u>Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester **4a.**</u> The preparation of this material has been previously reported.[S2]

<u>Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid t-butyl ester **4b.**</u> Into a 100 mL round bottom flask, equipped with a magnetic stir bar, was placed the 2-(3,4-dimethoxyphenyl) vinamidinium salt (**3**)[S1] (1.00 g, 2.49 mmol), glycine t-butyl ester (1.23 g, 7.35 mmol) along with 10 mL of DMF and the mixture was stirred for 30 mins. To the resulting reaction mixture was slowly added a solution of sodium t-butoxide, prepared previously by adding t-butanol (1.4 mL, 14.9 mmol) to sodium hydride (0.240 g, 9.96 mmol) in 10 mL of DMF in a round bottom flask equipped with a stir bar and reflux condensor. The resulting reaction mixture was refluxed overnight under a nitrogen atmosphere. After cooling the reaction mixture to room temperature, it was diluted with 50 mL of water and extracted with ethyl acetate (3 x 50 mL). The combined organic extract was washed with brine (2 x 15 mL), dried over anhydrous sodium sulfate, filtered and concentrated *in vacuo*. The crude product was taken up in ethyl acetate and passed through a short plug of silica gel on a fritted glass filtering funnel. The silica plug was washed with additional ethyl acetate and the combined filtrate was concentrated *in vacuo* to yield a brown solid (0.680 g, 92%). This material exhibited the following properties: mp 126 – 129°C; ^{1}H NMR ($CDCl_3$) δ 7.09 (m, 4H), 6.87 (d, J = 8.1 Hz, 1H), 3.93 (s, 3H) and

3.89 (s, 3H) and 1.61 (s, 9H) ; ^{13}C NMR ($CDCl_3$) δ 160.09, 149.3, 147.8, 128.1, 126.6, 125.0, 118.6, 117.7, 111.9, 111.8, 109.2, 81.1, 56.0, 55.9 and 28.4 ; IR (neat) 3328 and 1685 cm^{-1}; HRMS (ES) m/z calcd for $C_{17}H_{22}NO_4$ 304.1543, found 304.1533.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid, **5.** Into a 100 mL round bottom flask, equipped with a magnetic stir bar and a reflux condenser, was placed potassium hydroxide (0.061 g, 1.08 mmol) and a 50:50 mixture of ethanol and water (20 mL). The mixture was stirred until all the solid material was dissolved. The 4-(3, 4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester[S2] (**4a**, 0.100 g, 0.360 mmol) was added to the flask and the reaction mixture was refluxed for 3 h. The reaction mixture was subsequently allowed to cool in an ice-bath and the pH of the mixture was adjusted to 2 by the slow addition of 6 M hydrochloric acid. The resulting aqueous solution was extracted with 3 x 30 mL of ethyl acetate. The combined organic phases were washed with brine (1 x 30 mL) and dried over anhydrous sodium sulfate. The solution was filtered, evaporated in *vacuo* and dried using a Kugelrohr apparatus to give a dark solid (0.084 g, 94% yield). This material exhibited the following physical properties: mp 185 - 187°C; ^{1}H NMR (Acetone - d_6) δ 7.44 (m, 1H), 7.23 (d, *J* = 2.5Hz, 1H), 7.20 (m, 1H), 7.16 (dd, *J* = 2.5 Hz, *J* = 8.0 Hz, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 3H) and 3.81 (s, 3H); ^{13}C NMR (Acetone - d_6) δ 161.5, 149.9, 148.1, 128.4, 126.3, 123.5, 119.9, 117.2, 112.6, 112.2, 109.6, 55.4 and 55.3; IR (neat) 3347 and 1679 cm^{-1}; HRMS (ES) m$^-$/z calcd for $C_{13}H_{12}NO_4$ 246.0761, found 246.0753.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid n-butyl ester, **6a.** Into a 100 mL round bottom flask, equipped with a magnetic stir bar and reflux condenser, was placed carbonyl diimidazole (CDI) (0.146 g, 1.01 mmol), 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid (**5**, 0.250 g, 1.01 mmol) in 2 mL of DMF under a nitrogen atmosphere. The resulting mixture was stirred for 1 h at 40°C and n-butanol (0.185 mL, 2.02 mmol) and DBU (0.152 g, 1.01 mmol) were then added to the reaction mixture and the resulting solution was stirred for 24 h at 40°C. After cooling to room temperature, the reaction mixture was diluted with ethyl acetate (20 mL) and the resulting solution was extracted with 10% hydrochloric acid (1 x 20 mL), water (1 x 20 mL) and aqueous sodium bicarbonate (1 x 20 mL). The combined organic extract was dried over anhydrous sodium sulfate and evaporated *in vacuo* to give a dark brown solid. The crude product was purified using a Biotage Flash Purification SP-1 system with a silica column and a hexane/ethyl acetate gradient to give a light yellow solid (0.275 g, 90% yield). This material exhibited the following physical properties: mp 79 – 81 °C; ^{1}H NMR ($CDCl_3$) δ 7.17 (m, 2H), 7.07 (dd, *J* = 2.1 Hz, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 2.1 Hz, 1H), 6.88 (d, *J* = 8.4 Hz, 1H), 4.32 (t, *J* = 6.9 Hz, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 1.75 (pent, *J* = 7.5 Hz, 2H), 1.48 (hex, *J* = 7.5 Hz, 2H) and 0.99 (t, *J* = 7.5 Hz, 3H) ; ^{13}C NMR ($CDCl_3$) δ 161.4, 149.3, 147.9, 127.90, 126.7, 123.6, 119.2, 117.7, 112.3, 111.8, 109.2, 64.4, 56.0, 55.9, 30.9, 19.2 and 13.7 ; IR (neat) 3289 and 1674 cm^{-1}; HRMS (ES) m/z calcd for $C_{17}H_{22}NO_4$ 304.1543, found 304.1534.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid methyl ester, **6b.** This compound was prepared according to the previous procedure with the exception that methanol was used in the reaction instead of n-butanol in which case a light yellow solid (86% yield) was obtained. This material exhibited the following physical properties: mp 150 – 152 °C; ^{1}H NMR (CDCl$_3$) δ 7.19 (m, 1H), 7.17 (m, 1H), 7.09 (dd, *J* = 2.0 Hz, *J* = 8.5 Hz, 1H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 3.96 (s, 3H), 3.92 (s, 3H) and 3.91 (s, 3H) ; ^{13}C NMR (CDCl$_3$) δ 161.7, 149.3, 147.9, 127.8, 126.8, 123.21, 119.3, 117.7, 112.5, 111.8, 109.1, 56.0, 55.9 and 51.6 ; IR (neat) 3304 and 1681 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{16}NO_4$ 262.1074, found 262.1078.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid n-propyl ester, **6c.** This compound was prepared according to the previous procedure with the exception that n-propanol was used in the reaction instead of n-butanol in which case a light tan solid (96% yield) was obtained. This material exhibited the following physical properties: mp 85 – 87 °C; ^{1}H NMR (CDCl$_3$) δ 7.17 (m, 1H), 7.15 (m, 1H), 7.09 (dd, *J* = 2.0 Hz, J = 8.4 Hz, 1H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.89 (d, *J* = 8.4 Hz, 1H), 4.28 (t, *J* = 7.2 Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 1.79 (hex, *J* = 7.2 Hz, 2H) and 1.04 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (CDCl$_3$) δ 161.5, 149.3, 147.9, 127.9, 126.7, 123.65, 119.2, 117.7, 112.3, 111.8, 109.2, 66.1, 56.0, 55.9, 22.2 and 10.4 ; IR (neat) 3303 and 1677 cm^{-1}; HRMS (ES) m/z calcd for $C_{16}H_{20}NO_4$ 290.1387, found 290.1401.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid i-propyl ester, **6d.** This compound was prepared according to the previous procedure with the exception that i-propanol was used in the reaction instead of n-butanol in which case an orange solid (65% yield) was obtained. This material exhibited the following physical properties: mp 143 – 145 °C; ^{1}H NMR (CDCl$_3$) δ 7.16 (m, 2H), 7.08 (m, 2H), 6.87 (d, *J* = 8.1 Hz, 1H), 5.26 (hept, *J* = 6.3 Hz, 1H), 3.93 (s, 3H), 3.89 (s, 3H) and 1.37 (d, *J* = 6.3 Hz, 6H) ; ^{13}C NMR (CDCl$_3$) δ 160.1, 149.3, 147.8, 128. 0, 126.6, 124.0, 119.1, 117.7, 112.2, 111.8, 109.2, 68.0, 56.0, 55.9 and 22.0; IR (neat) 3266 and 1676 cm^{-1}; HRMS (ES) m/z calcd for $C_{16}H_{20}NO_4$ 290.1387, found 290.1403.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid n-hexyl ester, **6e.** This compound was prepared according to the previous procedure with the exception that n-hexanol was used in the reaction instead of n-butanol in which case a brown solid (95% yield) was obtained. This material exhibited the following physical properties: mp 74 – 76 °C; ^{1}H NMR (CDCl$_3$) δ 7.18 (m, 1H), 7.14 (m, 1H), 7.09 (dd, *J* = 1.5 Hz, J = 8.1 Hz, 1H), 7.07 (broad s, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 4.31 (t, *J* = 6.9 Hz, 2H), 3.95 (s, 3H), 3.91 (s, 3H), 1.77 (hext, J = 6.9 Hz, 2H), 1.37 (m, 6H) and 0.93 (t, J = 6.9 Hz, 3H) ; ^{13}C NMR (CDCl$_3$) δ 161.5, 149.3, 147.8, 127.90, 126.7, 123.6, 119.3, 117.7, 112.3, 111.8, 109.2, 64.7, 56.0, 55.9, 31.5, 28.8, 25.6, 25.5 and 14.0; IR (neat) 3296 and 1683 cm^{-1}; HRMS (ES) m/z calcd for $C_{19}H_{26}NO_4$ 332.1856, found 332.1861.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid benzyl ester, **6f.** This compound was prepared according to the previous procedure with the exception that benzyl alcohol was used in the reaction instead of n-butanol in which case a brown solid (92% yield) was obtained. This material exhibited the following physical properties: mp 79 – 81 °C; ^{1}H NMR (CDCl$_3$) δ 7.47 (d, *J* = 6.5 Hz, 2H), 7.41 (t, *J* = 6.5 Hz, 2H), 7.38 (t, *J* = 6.5 Hz, 1H), 7.21 (m, 1H), 7.18 (m, 1H), 7.09 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.89 (d, *J* = 8.0 Hz, 1H), 5.37 (s, 2H), 3.95 (s, 3H) and 3.92 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 160.9, 149.3, 147.9, 136.1, 128.6, 128.3, 128.2, 127.8, 126.8, 123.2, 119.4, 117.6, 112.7, 111.8, 109.1, 66.1, 56.0 and 55.9; IR (neat) 3314 and 1675 cm^{-1}; HRMS (ES) m/z calcd for $C_{20}H_{20}NO_4$ 338.1387, found 338.1388.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminopropyl ester, **6g.** This compound was prepared according to the previous procedure with the exception that N,N-3-dimethylaminopropanol was used in the reaction instead of n-butanol in which case a brown liquid (97% yield) was obtained. This material exhibited the following physical properties: bp 56-57°C at 0.12 torr; ^{1}H NMR (CDCl$_3$) δ 7.19 (m, 1H), 7.16 (m, 1H), 7.09 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 7.05 (d, *J* = 2.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 4.37 (t, *J* = 6.5 Hz, 2H), 3.96 (s, 3H), 3.92 (s, 3H), 2.52 (quintet, *J* = 6.5 Hz, 2H), 2.48 (t, *J* = 6.5 Hz, 2H) and 2.30 (s, 6H); ^{13}C NMR (CDCl$_3$) δ 161.3, 149.2 , 147.7, 128.0, 126.4, 123.4, 119.7, 117.6, 112.4, 111.8, 109.1, 62.7, 56.1, 55.9, 55.8, 45.3 and 27.0; IR (neat) 1696 cm^{-1}; HRMS (ES) m/z calcd for $C_{18}H_{25}N_2O_4$ 333.1809, found 333.1892.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminoethyl ester, **6h.** This compound was prepared according to the previous procedure with the exception that N,N-2-dimethylaminoethanol was used in the reaction instead of n-butanol in which case a brown liquid (91% yield) was obtained. This material exhibited the following physical properties: bp 45-46°C at 0.31 torr; bp 45°C at 0.31 torr; ^{1}H NMR (CDCl$_3$) δ 7.19 (m, 1H), 7.16 (m, 1H), 7.08 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 7.04 (d, *J* = 2.0 Hz, 1H), 6.88 (d, *J* = 8.0 Hz, 1H), 4.41 (t, *J* = 5.5 Hz, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 2.72 (t, *J* = 5.5 Hz, 2H) and 2.36 (s, 6H); ^{13}C NMR (CDCl$_3$) δ 161.0, 149.1, 147.6, 128.0, 126.3, 123.2, 119.8, 117.6, 112.7, 111.8, 109.0, 61.4, 57.8, 55.9, 55.8 and 45.3 ; IR (neat) 1697 cm^{-1}; HRMS (ES) m/z calcd for $C_{17}H_{23}N_2O_4$ 319.1652, found 319.1674.

Synthesis of 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid 4-methoxyphenyl ester, **6i.** This compound was prepared according to the previous procedure with the exception that 4-methoxyphenol was used in the reaction instead of n-butanol in which case a yellow solid (84% yield) was obtained. This material exhibited the following physical properties: mp 161 – 163°C; ^{1}H NMR (CDCl$_3$) δ 7.36 (broad s, 1H), 7.13 (m, 5H), 6.92 (m, 3H), 3.95 (s, 3H), 3.91 (s, 3H) and 3.82 (s, 3H); ^{13}C NMR (CDCl$_3$) δ 160.0, 157.4, 149.3, 148.0, 144.0, 127.6, 127.1, 122.6, 120.4, 117.7, 114.6, 113.7, 111.8, 109.2, 56.0, 55.9 and 55.6; IR (neat) 1707 cm^{-1}; HRMS (ES) m/z calcd for $C_{20}H_{20}NO_5$ 354.1336, found 354.1360.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester, **1**. The synthesis of this compound has been previously reported.[S2]

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *n*-butyl ester, **7e.** Into a 100 mL round bottom flask equipped with a magnetic stir bar and condensor, was placed 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *n*-butyl ester (0.15 g, 0.495 mmol) in 20 mL of dry chloroform along with dibromo-dimethylhydantoin (0.142 g, 0.495 mmol). The reaction mixture was refluxed for 4 hours, cooled to room temperature and washed with aqueous sodium thiosulfate (2 x 25 mL). The organic layer was subsequently washed with brine and dried over anhydrous sodium sulfate and concentrated in *vacuo* to give a brown solid (0.235 g). The crude product was purified using a Biotage Flash Purification SP-1 system with a silica column and an ethyl acetate/hexane gradient to yield a light yellow solid (0.194g, 85%). This material exhibited the following physical properties: mp 165 - 167°C; ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.40 (t, *J* =7.0 Hz, 2H), 3.94 (s, 3H), 3.92 (s, 3H), 1.78 (pent, *J* = 7.0 Hz, 2H), 1.52 (hext, *J* =7.0 Hz, 2H) and 0.99 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.9, 148.8, 148.5, 126.8, 124.4, 122.8, 121.8, 113.6, 110.9, 104.4, 104.0, 65.1, 55.9, 55.8, 30.7, 19.2 and 13.7; IR (neat) 3243 and 1671 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{17}H_{20}Br_2NO_4$ 459.9754, found 459.9702.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid methyl ester, **7a.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid methyl ester was used as the starting material. The product was obtained in 95% yield and exhibited the following physical properties: mp 158 - 160°C; ^{1}H NMR ($CDCl_3$) δ 6.95 (m, 3H) and 3.94 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 160.3, 148.8, 148.6, 126.7, 124.3, 122.7, 121.5, 113.6, 110.9, 104.8, 104.2, 56.0, 55.9 and 52.1; IR (neat) 3238 and 1678 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{14}H_{14}Br_2NO_4$ 417.9284, found 417.9769.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *n*-propyl ester, **7b.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid n-propyl ester was used as the starting material. The product was obtained in 90% yield and exhibited the following physical properties: mp 110 - 112°C; ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, *J* = 2.5 Hz, *J* = 8.0 Hz, 1H), 6.98 (broad s, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.34 (t, *J* =6.5 Hz, 2H), 3.95 (s, 3H), 3.93 (s, 3H), 1.82 (hext, *J* = 6.5 Hz, 2H) and 1.07 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.7, 148.8, 148.6, 126.8, 124.3, 122.7, 121.8, 113.5, 110.9, 104.2, 104.0, 66.9, 55.9, 55.8, 22.1 and 10.6; IR (neat) 3231 and 1670 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{16}H_{17}Br_2NO_4Na$ 467.9417, found 467.9400.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *i*-propyl ester, **7c.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *i*-propyl ester was used as the starting material. The product was obtained in 90% yield and exhibited the following physical properties: mp 138 - 140°C; ^{1}H NMR

(CDCl$_3$) δ 6.70 (dd, *J* = 1.8 Hz, *J* = 8.4 Hz, 1H), 6.97 (broad s, 1H), 6.95 (d, *J* = 8.4 Hz, 1H), 5.30 (hept, *J* = 6.0 Hz, 1H), 3.94 (s, 3H), 3.93 (s, 3H) and 1.41 (d, *J* = 6.0 Hz, 6H); ^{13}C NMR (CDCl$_3$) δ 159.6, 148.7, 148.5, 126.6, 124.5, 122.8, 122.0, 113.6, 110.9, 104.5, 104.0, 69.4, 55.9, 55.8 and 22.0; IR (neat) 3248 and 1705 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{16}H_{18}Br_2NO_4$ 445.9597, found 445.9595.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *t*-butyl ester, **7d.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *t*-butyl ester was used as the starting material. The product was obtained in 92% yield and exhibited the following physical properties: mp 125 - 127°C; ^{1}H NMR (CDCl$_3$) δ 6.99 (m, 3H), 3.95 (s, 3H), 3.93 (s, 3H) and 1.61 (s, , 9H); ^{13}C NMR (CDCl$_3$) δ 159.2, 148.7, 148.5, 126.5, 124.6, 122.8, 122.7, 113.6, 110.9, 103.9, 103.8, 82.8, 55.9, 55.8 and 28.4; IR (neat) 3225 and 1659 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{17}H_{19}Br_2NO_4Na$ 481.9573, found 481.9539.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *n*-hexyl ester, **7f.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid *n*-hexyl ester was used as the starting material. The product was obtained in 97% yield and exhibited the following physical properties: mp 163 - 164°C; ^{1}H NMR (CDCl$_3$) δ 7.01 (dd, *J* = 2.0 Hz, *J* = 8.5 Hz, 1H), 6.97 (broad s, 1H), 6.96 (d, *J* = 8.5 Hz, 1H), 4.36 (t, *J* =6.5 Hz, 2H), 3.95 (s, 3H), 3.94 (s, 3H), 1.79 (pent, *J* = 7.5 Hz, 2H), 1.49 (m, 2H), 1.37 (m, 4H) and 0.92 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (CDCl$_3$) δ 159.7, 148.8, 148.5, 126.8, 124.3, 122.7, 121.8, 113.5, 110.9, 104.2, 104.0, 65.4, 55.9, 55.8, 31.4, 28.7, 25.6, 22.5 and 14.0; IR (neat) 3246 and 1673 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{19}H_{23}Br_2NO_4Na$ 509.9886, found 509.9876.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid benzyl ester, **7g.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid benzyl ester was used as the starting material. The product was obtained in 90% yield and exhibited the following physical properties: mp 150 - 152°C; ^{1}H NMR (CDCl$_3$) δ 7.50 (d, *J* = 7.0 Hz, 2H), 7.40 (t, *J* = 7.0 Hz 2H). 7.37 (t, *J* = 7.0 Hz 1H), 7.0 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 5.43 (s, 2H), 3.94 (s, 3H) and 3.93 (s, 3H) ; ^{13}C NMR (CDCl$_3$) δ 154.3, 143.6, 143.4, 130.4, 123.4, 123.2, 123.0, 121.7, 119.1, 117.6, 116.2, 108.4, 105.7, 99.7, 99.5, 61.6, , 50.8, and 50.6; IR (neat) 3234 and 1690 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{20}H_{18}Br_2NO_4$ 493.9597, found 493.9556.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminopropyl ester, **7h.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminopropyl ester was used as the starting material. The product was obtained in 90% yield and exhibited the following physical properties: mp 169 - 171°C; ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.96 (d, *J* = 8.0 Hz, 1H), 4.41 (t, *J* =6.0 Hz, 2H), 3.95 (s, 3H), 3.94 (s, 3H), 2.76 (m, 2H), 2.49 (s, 6H) and 2.10 (t, *J* =6.0 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 159.9, 148.6, 148.4, 126.3, 124.6, 122.8, 121.6, 113.6, 110.9, 105.9, 104.1, 63.0, 56.3, 55.9, 55.8, 44.9 and 26.1; IR (neat) 2960 and 1709 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{18}H_{23}Br_2N_2O_4$ 489.0019, found 488.9987.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminoethyl ester, **7i.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminoethyl ester was used as the starting material. The product was obtained in 89% yield and exhibited the following physical properties: mp 144 - 146°C; ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, *J* = 2.0 Hz, *J* = 8.5 Hz, 1H), 6.97 (d, *J* = 2.0 Hz, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 4.45 (t, *J* =5.5 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 2.92 (broad m, 2H) and 2.54 (broad s, 6H) ; ^{13}C NMR ($CDCl_3$) δ 158.6, 148.5, 148.3, 125.9, 124.6, 122.8, 120.8, 113.8, 110.8, 105.3, 104.3, 59.7, 57.3, 55.9, 55.8 and 44.6; IR (neat) 2935 and 1709 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{17}H_{21}Br_2N_2O_4$ 474.9863, found 474.9849.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid 4-methoxyphenyl ester, **7l.** This material was prepared according to the previous procedure with the exception that 4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid 4-methoxyphenyl ester was used as the starting material. The product was obtained in 96% yield and exhibited the following physical properties: mp 154 - 156°C; ^{1}H NMR ($CDCl_3$) δ 10.77 (d, *J* = 6.9 Hz, 2H), 10.63 (dd, *J* = 2.1 Hz, *J* = 8.1 Hz, 1H), 10.55 (m, 4H), 3.95 (s, 3H), 3.94 (s, 3H) and 3.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.1, 157.5, 148.9, 148.6, 143.6, 127.3, 124.1, 122.8, 122.4, 120.9, 114.6, 113.5, 110.9, 105.7, 105.5, 56.0, 55.9 and 55.7; IR (neat) 3324 and 1702 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{20}H_{18}Br_2NO_5$ 509.9546, found 509.9529.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminpropyl ester Hydrochloride, **7j.** Into a round bottom flask equipped with a magnetic stir bar, was placed 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminopropyl ester (**7h**, 0.200 g, 0.410 mmol). In addition, 4.0 M HCl in dioxane (0.015 g, 0.41 mmol) along with 5 mL of dioxane was added to the reaction vessel and the reaction mixture was stirred overnight. The reaction mixture was subsequently evaporated *in vacuo* to give a residue, which was triturated with a 1:1 mixture of hexane:THF, and after drying *in vacuo,* yielded a brown solid (0.201 g, 93% yield). This brown solid exhibited the following physical properties; mp 205 - 208°C; ^{1}H NMR ($CDCl_3$) δ 7.01 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 4.34 (t, *J* =5.0 Hz, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.29 (m, 2H), 2.90 (d , *J* = 5.0 Hz, 6H) and 2.40 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 158.5, 148.7, 148.5, 126.6, 124.4, 122.8, 113.6, 110.9, 105.7, 105.2, 61.0, 56.0, 55.9, 55.6, 43.4 and 24.4; IR (neat) 3001 and 1709 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{18}H_{23}Br_2N_2O_4$ 489.0019, found 488.9987.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminoethyl ester Hydrochloride, **7k**. Into a round bottom flask equipped with a magnetic stir bar, was placed 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid N,N-dimethylaminoethyl ester (**7i**, 0.200 g, 0.420 mmol). In addition, 4.0 M HCl in dioxane (0.015 g, 0.41 mmol) along with 5 mL of dioxane was added to the reaction vessel and the reaction mixture was stirred overnight. The reaction mixture was subsequently evaporated *in vacuo* to give a residue, which was triturated with a 1:1 mixture of hexane:THF, and after drying *in vacuo,* yielded a brown solid (0.206 g, 96% yield). This solid exhibited the following physical properties; mp 178 - 180°C; ^{1}H NMR ($CDCl_3$) δ 7.02 (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 6.98 (d, *J* = 2.0 Hz, 1H), 6.95 (d, *J* = 8.0 Hz, 1H), 4.57 (broad m, 2H), 3.94 (s, 3H), 3.93 (s, 3H), 3.45 (broad m, 2H) and 2.98 (d, *J* = 4.5 Hz, 6H) ; ^{13}C NMR ($CDCl_3$) δ 157.2, 148.6, 148.5, 126.6, 124.4, 122.8, 118.7, 113.7, 110.9, 106.8, 106.4, 56.9, 56.0, 55.9, 55.8 and 43.2; IR (neat) 2906 and 1714 cm^{-1}; HRMS (ES) *m/z* calcd for $C_{17}H_{21}Br_2N_2O_4$ 474.9863, found 474.9871.

Bioassays

Cell Culture. The MDA-MB-435 human melanoma cancer cell line was obtained from the Lombardi Cancer Center (Georgetown University; Washington, DC) and grown in Richter's IMEM medium (Invitrogen; Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone; Logan, UT) and 25 μg/mL gentamicin sulfate (Invitrogen). The A-10 embryonic rat aortic smooth muscle cell line was purchased from American Type Culture Collection (Manassas, VA) and cultured in Basal Medium Eagle medium (Sigma; St. Louis, MO) with 10% fetal bovine serum and 50 μg/ml gentamicin sulfate.

Inhibition of Cellular Proliferation. Antiproliferative effects were evaluated using the SRB assay[S3] as previously described.[S4] The concentration of drug that caused a 50% inhibition of cellular proliferation (IC_{50}) was calculated from the linear portion of the log of the dose response curve. Each IC_{50} represents the mean and standard deviation from three independent experiments, each performed in triplicate. Colchicine is included as a reference compound.

Immunofluorescence. Cellular microtubules in interphase were visualized using indirect immunofluorescence techniques as previously described.[S4] Cells were treated for 18 h with vehicle or drug, fixed with methanol and microtubules visualized with a β-tubulin antibody using a Nikon Eclipse 80i fluorescence microscope. Percent microtubule depolymerization as compared to vehicle treated controls was determined visually.

Molecular Modeling

The X-ray crystal structure of αβ-tubulin complexed with DAMA-colchicine (pdbid: 1SA0) was prepared with Sybyl 8.1.[S5] The stathmin-like domain and the C and D subunits were deleted. Hydrogen atoms were added and their orientations were optimized by the Tripos force field to a gradient of 0.005 kcal mol^{-1} $Å^{-1}$. The docking studies were performed using GOLD 5.0.[S6] The ligands were docked in the active site, which was defined by the space in a 6 Å radius around DAMA-colchicine. Docking conformations generated with GOLD and filtered initially by GoldScore were further analyzed with HINT.[S7] The most active ligand JG-03-14 (**1**) was docked first with GOLD without constraints. The resulting conformations were rescored with HINT and the best docking pose of **1** was defined as its binding mode. This pose was then used to define a similarity constraint in GOLD such that other ligands (**2**-**13**) were docked in the way that best matches this shape. For these ligands, the conformations/binding modes were chosen based on the highest calculated HINT score.

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