Supplementary Information

Developing novel C-4 analogues of pyrrole-based antitubulin agents: weak but critical hydrogen bonding in the colchicine site

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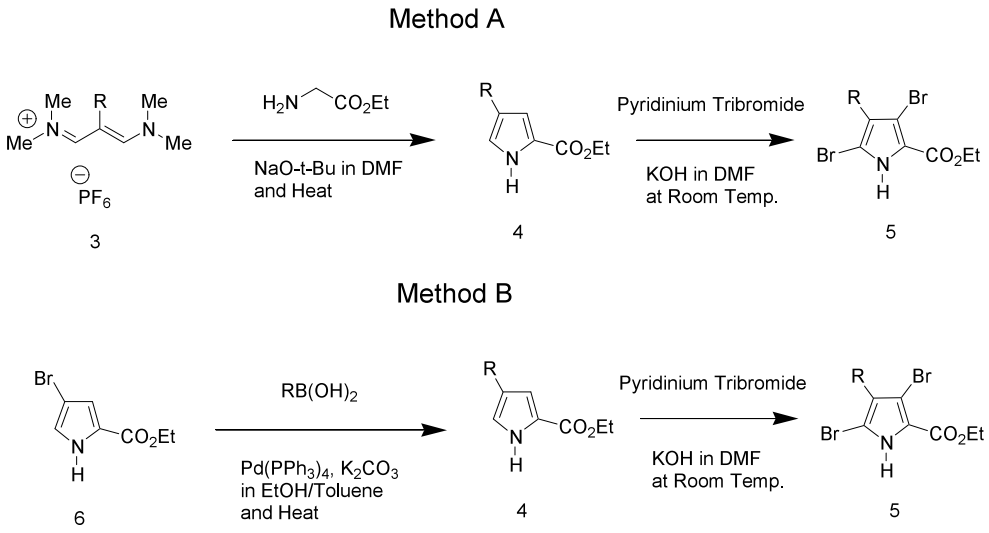
Experimental Procedures

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 Å molecular sieves prior to their use. NMR spectra were obtained on either a Bruker 300 MHz spectrometer or a Bruker 500 MHz spectrometer in $CDCl_3$, 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 or an AxION 2 time of flight mass spectrometer at Virginia Commonwealth University. Low-resolution 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 used as the eluant. Microwave accelerated reactions were carried out in a Biotage Initiator 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, MS spectra, flash chromatograms and ^{13}C NMR spectra consistent with a sample purity of >95%. Scheme 1 depicts Method A and Method B, which were used for the preparation of the pyrrole analogs with one exception. Compound **5q** was prepared by bromination of compound **2** with dibromdimethylhydantioin as indicated in the experimental procedures that follow.



Scheme S1. Preparation of Pyrrole Analogs.

Assessment of purity. All new compounds (including their precursors) listed in Table 1 were purified by preparative chromatography. A typical chromatogram, as illustrated for compound 4g is shown in Figure S1 and subsequent HPLC analysis of the purified material is presented in Figure S2.

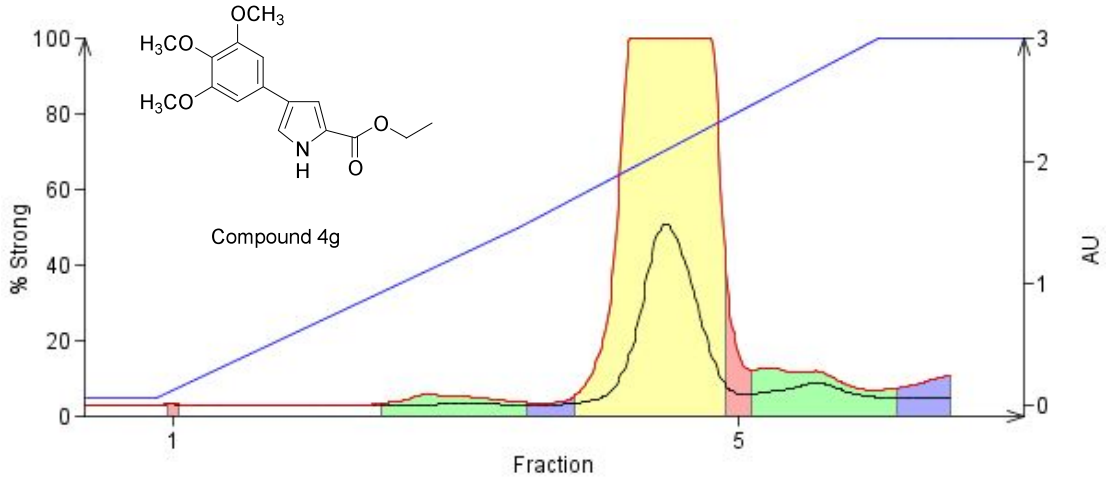
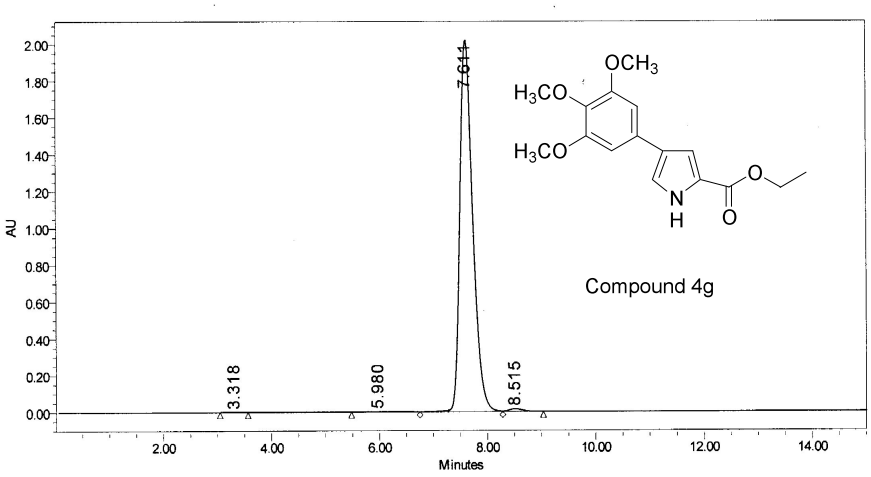


Figure S1. This material (**4g**) was purified on a Biotage SP-1 preparative chromatograph using a silica column with a hexane/ethyl acetate gradient. The fraction represented in yellow was collected and characterized as compound **4g**.



	RT	Area	% Area	Height
1	3.318	13451	0.04	1043
2	5.980	94278	0.28	1851
3	7.611	33397826	98.89	2024419
4	8.515	267518	0.79	15409

Figure S2. This chromatogram represents the HPLC analysis of purified compound **4g** on an Inertsil ODS-II column in an isocratic mode with a 1:1 mixture of methanol:acetonitrile.

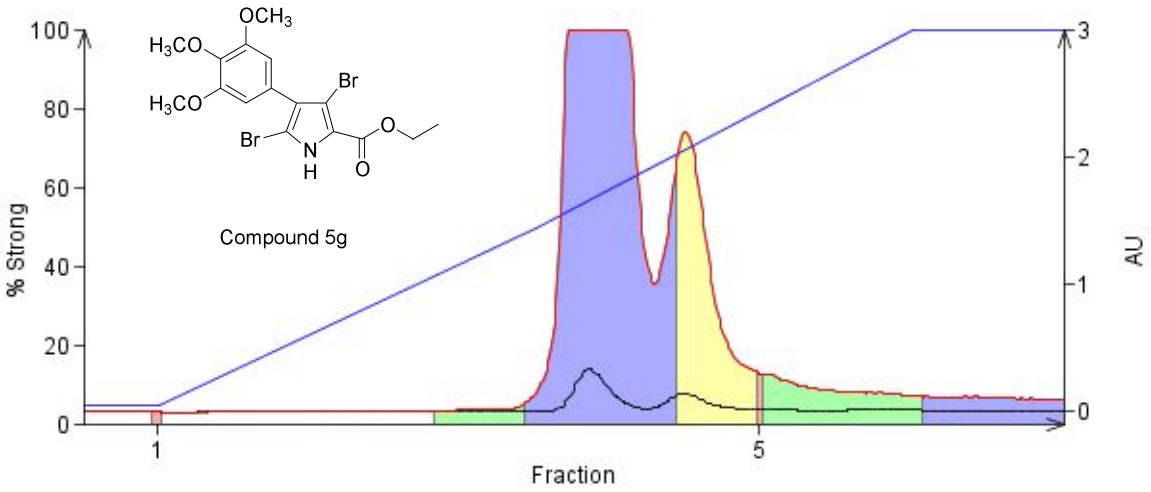
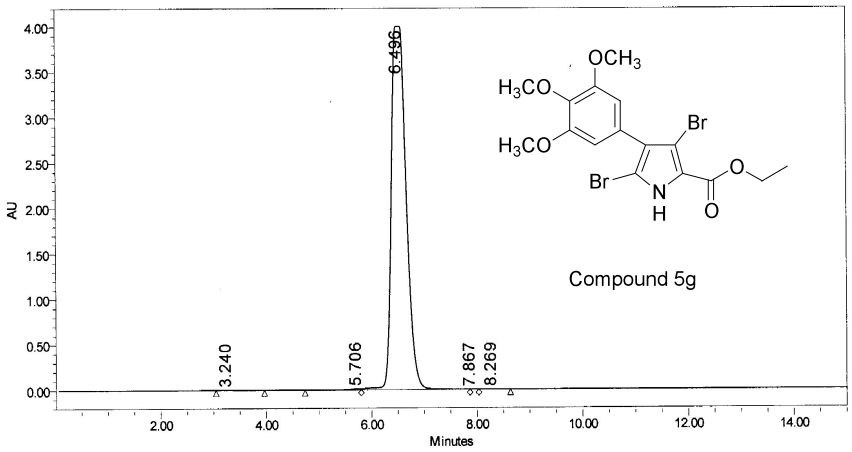


Figure S3. This material (**5g**) was purified on a Biotage SP-1 preparative chromatograph using a silica column with a hexane/ethyl acetate gradient. The fraction represented in blue was collected and characterized as compound **5g**.



	RT	Area	% Area	Height
1	3.240	7422	0.01	500
2	5.706	128352	0.16	11702
3	6.496	77675795	99.77	4032436
4	7.867	4659	0.01	572
5	8.269	40835	0.05	2481

Figure S4. This chromatogram represents the HPLC analysis of purified compound **5g** on an Inertsil ODS-II column in an isocratic mode with a 1:1 mixture of methanol:acetonitrile.

Synthesis of 4-phenyl-1H-pyrrole-2-carboxylic acid ethyl ester ***4a.*** The preparation of this material has been previously reported.[S1]

Synthesis of 4-(4-methylphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4b.*** The preparation of this material has been previously reported.[S1]

Synthesis of 4-(4-chlorophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4c.*** The preparation of this material has been previously reported.[S1]

Synthesis of 4-(4-bromophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4d.*** The preparation of this material has been previously reported.[S1]

Synthesis of 4-(4-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4e.*** The preparation of this material has been previously reported.[S1]

Synthesis of 4-(3-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4f.*** **Method A.** Into a 100 mL, three-necked round bottom flask was placed sodium hydride [0.127 g (60% by weight), 5.28 mmol], t-butanol (0.75 mL, 7.9 mmol) and 30 mL of dry DMF. The sodium hydride was allowed to react until there was no evolution of hydrogen gas. The reaction mixture was then cooled in an ice bath and glycine ethyl ester hydrochloride (0.368g, 2.64 mmol) was added, and the resulting mixture was stirred for 30 mins at room temperature. Subsequently, 3-methoxyphenylvinamidinium hexafluorophosphate[S1] (0.500 g, 1.32 mmol) was added to the reaction flask and the resulting contents were refluxed for 24 hrs. After cooling to room temperature, the reaction mixture was diluted with 100 mL of water and extracted with ethyl

acetate (2 x 50 mL). The combined organic extracts were washed with water (2 x 50 mL), brine (2 x 50 mL) and dried over anhydrous sodium sulfate. The organic phase was filtered and concentrated *in vacuo* to give a dark solid (0.317 g). This material was purified by flash chromatography using a hexane/ethyl acetate gradient to yield a light yellow solid (0.264 g, 77% yield), which exhibited the following physical properties: mp 100 – 105 °C; ^{1}H NMR ($CDCl_3$) δ 7.26-7.32 (m, 2H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.11 (broad s, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 4.40 (q, *J* = 7.2 Hz, 2H), 2.53 (s, 3H) and 1.44 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.7, 160.1, 136.2, 129.8, 126.5, 123.6, 120.3, 117.9, 112.7, 111.6, 111.0, 60.7, 55.2 and 29.8; IR (neat) 3248 and 1665 cm^{-1}; MS (EI) m/z calcd for $C_{14}H_{15}NO_3$ 245, found 245.

Synthesis of 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4g.*** The preparation of this material has been previously reported.[S2]

Synthesis of 4-(1-naphthyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4h.*** This compound was prepared by Method A with the exception that the 1-naphthylvinamidinium hexafluorophosphate was used in the pyrrole forming reaction in place of the 3-methoxyphenylvinamidinium hexafluorophosphate, in which case a 14% yield of a yellow solid was obtained, which exhibited the following physical properties: mp 126 – 129 °C; ^{1}H NMR ($CDCl_3$) δ 8.31 (m, 1H), 7.95 (m, 2H), 7.86 (t, *J* = 4.8 Hz, 1H), 7.56 (m, 3H), 7.30 (m, 1H), 7.23 (m, 1H), 4.46 (q, *J* = 7.2 Hz, 2H) and 1.46 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.7, 134.0, 133.4, 131.9, 128.4, 127.2, 126.9, 126.1, 125.9, 125.8, 125.6, 125.2, 123.2, 122.6, 116.4, 60.6 and 14.5; IR (neat) 3296 and 1677 cm^{-1}; HRMS (ES) m/z calcd for $C_{17}H_{16}NO_2$, 266.1176 found 266.1177.

Synthesis of 4-(3-indolyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4i.*** This compound was prepared by Method A with the exception that the 3-indolylvinamidinium hexafluorophosphate was used in the pyrrole forming reaction in place of the 3-methoxyphenylvinamidinium hexafluorophosphate, in which case a 31% yield of a brown solid was obtained, which exhibited the following physical properties: mp 135 – 136 °C; ^{1}H NMR ($CDCl_3$) δ 7.87 (d, *J* = 8.0 Hz, 1H), 7.55 (d, *J* = 2.5 Hz, 1H),7.46 (d, *J* = 8.0 Hz, 1H), 7.44 (m, 1H), 7.21 (m, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.12 (t, *J* = 8.0 Hz, 1H), 4.32 (q, *J* = 7.0 Hz, 2H) and 1.36 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.7, 137.1, 126.0, 123.0, 121.4, 121.3, 120.4, 119.7, 119.4, 119.3, 112.8, 111.5, 110.9, 59.5 and 13.9; IR (neat) 3386, 3310 and 1682 cm^{-1}; HRMS (ES) m/z calcd for $C_{15}H_{15}N_2O_2$, 255.1128 found 255.1127.

Synthesis of 4-(4-trifluoromethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4j.*** **Method B**. Into a 20 mL microwave vial equipped with a stirring bar was placed 4-bromo-1H-pyrrole-2-carboxylic acid ethyl ester (**6**)[S3] (0.250 g, 1.15 mmol), 4-trifluoromethoxyphenylboronic acid (0.708 g, 3.43 mmol), tetrakis(triphenylphosphine)palladium (0) (0.013 g, 0.012 mmol) and anhydrous potassium carbonate (0.540 g, 3.91 mmol). To this reaction vessel was added 10 mL of a 3:1 mixture of toluene:ethanol. The reaction vessel was capped with a crimping tool and heated in a Biotage Initiator microwave reactor for 2 hrs at 110 °C. After cooling to room temperature, the reaction mixture was filtered through a short plug of silica gel and the filtrate was concentrated *in vacuo* to give a dark brown solid, which was purified by flash

chromatography using a hexane/ethyl acetate gradient to yield a light yellow solid (0.287 g, 84% yield). This material exhibited the following physical properties: mp 95 – 98 °C; ^{1}H NMR ($CDCl_3$) δ 7.54 (d, *J* = 9.0 Hz, 2H), 7.23 (m, 3H), 7.19 (m, 1H), 4.39 (q, *J* = 7.0 Hz, 2H) and 1.42 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.6, 147.6, 133.6, 126.4, 125.4, 123.9, 121.3, 120.6 (q, *J* = 256.5 Hz), 119.9, 112.5, 60.7 and 14.3; IR (neat) 3296 and 1687 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{13}F_3NO_3$ 300.0842, found 300.0827.

Synthesis of 4-(4-thiomethylphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4k.*** This compound was prepared according to Method B with the exception that 4-thiomethylphenylboronic acid was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case an 80% yield of a light yellow solid was obtained. This material exhibited the following physical properties: mp 136 – 138 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, *J* = 8.4 Hz, 2H), 7.29 (d, *J* = 8.4 Hz, 2H), 7.22 (m, 1H), 7.18 (m, 1H), 4.37 (q, *J* = 6.9 Hz, 2H), 2.52 (s, 3H) and 1.40 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.2, 136.0, 131.8, 127.5, 126.3, 125.7, 123.8, 119.3, 112.2, 60.5, 16.3 and 14.5; IR (neat) 3295 and 1677 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{16}NO_2S$, 262.0896, found 262.0921.

Synthesis of 4-(3,4-dichlorophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4l.*** This compound was prepared according to Method B with the exception that 3,4-dichlorophenylboronic acid was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case an 87% yield of a light tan solid was obtained. This material exhibited the following physical properties: mp 138 – 140 °C; ^{1}H NMR ($CDCl_3$) δ 7.61 (d, *J* = 2.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), (dd, *J* = 2.0 Hz, *J* = 8.0 Hz, 1H), 7.24 (m, 1H), 7.17 (m, 1H), 4.37 (q, *J* = 7.0 Hz, 2H) and 1.42 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.1, 134.8, 132.8, 130.6, 129.8, 127.0, 124.5, 124.1, 119.8, 112.3, 60.8 and 14.4; IR (neat) 3239 and 1673 cm^{-1}; MS (ES) m/z calcd for $C_{13}H_{12}Cl_2NO_2$, 284 found 284.

Synthesis of 4-(3-fluoro-4-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4m.*** This compound was prepared according to Method B with the exception that 3-fluoro-4-methoxyphenylboronic acid was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case a 91% yield of a light yellow solid was obtained. This material exhibited the following physical properties: mp 105 – 107 °C; ^{1}H NMR ($CDCl_3$) δ 7.24 (m, 2H), 7.17 (m, 1H), 7.13 (m, 1H), 6.98 (t, *J* = 9.0 Hz, 1H), 4.38 (q, *J* = 7.0 Hz, 2H), 3.93 (s, 3H) and 1.41 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.5, 152.7 (d, *J* = 245.0 Hz), 146.0 (d, *J* = 10.3 Hz), 128.3 (d, *J* = 6.9 Hz), 125.5, 123.7, 120.8, 119.5, 114.0, 113.1 (d, *J* = 19.0 Hz), 112.3, 60.6, 56.4 and 14.4; IR (neat) 3288 and 1681 cm^{-1}; MS (ES) m/z calcd for $C_{14}H_{15}FNO_3$, 264 found 264.

Synthesis of 4-(6-ethoxynaphthalen-2-yl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4n.*** This compound was prepared according to Method B with the exception that (6-ethoxynaphthalen-2-yl)boronic was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case an 14% yield of a yellow solid was obtained. This material exhibited the following physical properties: mp 180 – 181 °C; ^{1}H NMR ($CDCl_3$) δ 7.91 (d, *J* = 2.0 Hz, 1H), 7.74 (d, *J* = 8.5 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.64 (dd, *J* = 2.0 Hz, *J* = 8.5 Hz, 1H), 7.34 (s, 1H), 7.32 (s,

1H), , 7.16 (dd, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H), 7.13 (d, *J* = 2.5 Hz, 1H), 4.38 (q, *J* = 7.0 Hz, 2H), 4.19 (q, *J* = 7.0 Hz, 2H), 1.51 (t, *J* = 7.0 Hz, 3H); and 1.42 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.1, 156.7, 133.4, 129.7, 129.3, 129.2, 127.2, 127.1, 124.8, 123.9, 123.1, 119.4, 119.3, 112.4, 106.6, 63.5, 60.5, 14.8 and 14.5; IR (neat) 1670 cm^{-1}; MS (ES) m/z calcd for $C_{19}H_{20}NO_3$, 310 found 310.

Synthesis of 4-benzo[1,3]dioxol-5-yl-1H-pyrrole-2-carboxylic acid ethyl ester ***4o.*** This compound was prepared according to Method B with the exception that 4-benzo[1,3]dioxol-5-ylboronic was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case an 82% yield of a tan solid was obtained. This material exhibited the following physical properties: mp 161 – 164 °C; ^{1}H NMR ($CDCl_3$) δ 7.13 (m, 2H), 7.02 (m, 2H), 6.73 (d, *J* = 8.4 Hz, 1H), 5.98 (s, 2H), 4.36 (q, *J* = 7.2 Hz, 2H) and 1.40 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 161.3, 148.1, 146.1, 128.9, 126.7, 123.6, 119.1, 118.6, 112.3, 108.6, 106.2, 101.0, 60.6 and 14.5; IR (neat) 1678 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{14}NO_4$, 260.0917 found 260.0925.

4-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-1H-pyrrole-2-carboxylic acid ethyl ester ***4p****.* This compound was prepared according to Method B with the exception that 4-(2,3-dihydro-benzo[1,4]dioxin-6-ylboronic acid was used instead of 4-trifluoromethoxyphenylboronic acid for the cross-coupling reaction in which case an 85% yield of a tan solid was obtained. This material exhibited the following physical properties: mp 93-95 °C; ^{1}H NMR ($CDCl_3$) δ 7.04 (m, 2H), 6.95 (m, 1H), 6.92 (m, 2H), 4.32 (q, *J* = 6.9 Hz, 2H), 4.30 (s, 4H) and 1.37 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.9, 147.5, 143.3, 129.4, 124.5, 123.2, 123.0, 117.1, 116.9, 97.6, 64.7, 64.2, 60.9 and 14.4; IR (neat) 1666 cm^{-1}; MS (ES) m/z calcd for $C_{15}H_{16}NO_4$, 274 found 274.

Synthesis of 3,5-dibromo-4-(3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***2.*** The preparation of this material has been previously reported.[S4]

Synthesis of 3,5-dibromo-4-phenyl-1H-pyrrole-2-carboxylic acid ethyl ester ***5a.*** The preparation of this material has been previously reported.[S4]

Synthesis of 3,5-dibromo-4-(4-methylphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5b.*** This compound was prepared according to the procedure used for compound **5g** with the exception that compound **4b** was used as the starting material. Subsequently, a 74% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 145 – 147 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (d, *J* = 8.1 Hz, 2H), 7.27 (d, *J* = 8.1 Hz, 2H), 4.44 (q, *J* = 7.2 Hz, 2H), 2.43 (s, 3H) and 1.44 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.7, 137.7, 130.1, 129.0, 128.8, 126.9, 121.7, 104.4, 104.2, 61.3, 21.4 and 14.4;IR (neat) 3211 and 1664 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{13}Br_2NaNO_2$, 407.9205 found 407.9203.

Synthesis of 3,5-dibromo-4-(4-chlorophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5c.*** The preparation of this material has been previously reported.[S4]

Synthesis of 3,5-dibromo-4-(4-bromophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5d.*** The preparation of this material has been previously reported.[S4]

Synthesis of 3,5-dibromo-4-(4-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5e.*** The preparation of this material has been previously reported.[S4]

Synthesis of 3,5-dibromo-4-(3-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5f.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4f** was used as the starting material. Subsequently, a 39% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 75 – 77 °C; ^{1}H NMR ($CDCl_3$) δ 7.34 (t, *J* = 7.8 Hz, 1H), 7.03 (m, 2H), 6.93 (d, *J* = 8.1 Hz, 1H), 4.43 (q, *J* = 6.9 Hz, 2H) and 1.41 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.2, 159.2, 133.3, 129.2, 126.5, 122.7, 121.9, 115.9, 113.4, 105.1, 103.9, 61.4, 55.3 and 14.4; IR (neat) 1670 cm^{-1}; MS (ES) m/z calcd for $C_{14}H_{14}Br_2NO_3$, 404 found 404.

Synthesis of 3,5-dibromo-4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5g.*** Into a 100 mL round bottom flask equipped with a magnetic stir bar, was placed 4-(3,4,5-trimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester **4g** (0.250 g, 0.819 mmol) and potassium hydroxide (0.184 g, 3.27 mmol) in 20 mL of dry DMF. The reaction mixture was stirred for 15 mins at room temperature after which pyridinium tribromide (0.524 g, 1.64 mmol) was added and the reaction mixture was allowed to stir overnight at room temperature. The reaction mixture was subsequently diluted with 30 mL of water, extracted with ethyl acetate (3 x 25 mL), washed with brine (1 x 15 mL) and dried over anhydrous sodium sulfate. After filtering off the drying agent the organic phase was concentrated in *vacuo* to give a light yellow solid. The crude product was purified using a Biotage Flash Purification SP-1 system to give a light yellow solid (0.274 g, 73% yield). This material exhibited the following physical properties: mp 108 – 110 °C; ^{1}H NMR ($CDCl_3$) δ 6.67 (s, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.93 (s, 3H), 3.90 (s, 6H) and 1.43 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.4, 152.9, 137.6, 127.1, 126.8, 121.8, 107.4, 104.2, 103.9, 61.3, 60.9, 56.2 and 14.4; IR (neat) 3239 and 1666 cm^{-1}; MS (ES) m/z calcd for $C_{16}H_{18}Br_2NO_5$, 464 found 464.

Synthesis of 3,5-dibromo-4-(1-naphthyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5h.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4h** was used as the starting material. Subsequently, a 62% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 144 – 146 °C; ^{1}H NMR ($CDCl_3$) δ 7.94 (m, 2H), 7.67 (d, *J = 7.8 Hz,* 1H), 7.51 (m, 4H), 4.48 (q, *J* = 6.9 Hz, 2H) and 1.46 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.3, 133.7, 132.2, 129.9, 129.3, 129.0, 128.4, 126.3, 126.1, 126.0, 125.9, 125.3, 122.0, 106.3, 106.0, 61.5 and 14.4; IR (neat) 3239 and 1662 cm^{-1}; MS (ES) m/z calcd for $C_{17}H_{14}Br_2NO_2$, 421 found 421.

Synthesis of 3,5-dibromo-4-(3-indolyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5i.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4i** was used as the starting material. Subsequently, a 68% yield of a dark solid was obtained and this material exhibited the following physical properties: mp 118 – 121 °C; ^{1}H NMR ($CDCl_3$) δ 7.42 (d, *J* = 7.5 Hz, 2H), 7.18 (t, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 6.99 (s, 1H), 4.31 (q, *J* = 7.2 Hz, 2H) and 1.34 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 158.6, 137.1, 126.9, 123.3, 122.1, 120.6, 118.7,

115.2, 113.8, 111.9, 108.1, 106.7, 105.7, 60.4 and 13.8; IR (neat) 3282 and 1688 cm^{-1}; HRMS (ES) m/z calcd for $C_{15}H_{13}Br_2N_2O_2$, 410.9383 found 410.9344.

Synthesis of 3,5-dibromo-4-(4-trifluoromethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5j.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4j** was used as the starting material. Subsequently, a 71% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 132 – 135 °C; ^{1}H NMR ($CDCl_3$) δ 7.47 (d, *J* = 8.7 Hz, 2H), 7.29 (d, *J* = 8.7 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H) and 1.41 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.2, 148.8, 131.7, 130.6, 125.6, 122.1, 120.5, 105.0, 103.9, 61.6 and 14.3; IR (neat) 3223 and 1670 cm^{-1}; MS (ES) m/z calcd for $C_{14}H_{11}Br_2F_3NO_3$, 458 found 458.

Synthesis of 3,5-dibromo-4-(4-thiomethylphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5k.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4k** was used as the starting material. Subsequently, a 65 % yield of a tan solid was obtained and this material exhibited the following physical properties: mp 153-156 °C; ^{1}H NMR ($CDCl_3$) δ 7.37 (d, *J* = 8.7 Hz, 2H), 7.32 (d, *J* = 8.7 Hz, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 2.54 (s, 3H) and 1.43 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.5, 138.4, 130.5, 128.3, 126.3, 126.0, 121.8, 104.3, 104.0, 61.3, 15.5 and 14.4: IR (neat) 3232 and 1664 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{14}Br_2NO_2S$, 417.9107 found 417.9119.

Synthesis of 3,5-dibromo-4-(3,4-dichlorophenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5l.***This compound was prepared in a manner identical to compound **5g** with the exception that compound **4l** was used as the starting material. Subsequently, a 56% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 180 – 183 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, *J* = 8.5 Hz, 1H), 7.63 (d, *J* = 2.5 Hz, 1H), 7.43 (dd, *J* = 2.5 Hz, *J* = 8.5 Hz, 1H), 4.33 (q, *J* = 7.0 Hz, 2H) and 1.35 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.6, 135.5, 132.9, 131.6, 129.6, 123.1, 116.5, 115.4,109.2, 96.7, 60.3 and 13.6; IR (neat) 3223 and 1671 cm^{-1}; MS (ES) m/z calcd for $C_{13}H_9Br_2Cl_2NO_2$, 439 found 439.

Synthesis of 3,5-dibromo-4-(3-fluoro-4-methoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5m.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4m** was used as the starting material. Subsequently, a 55% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 169 –171 °C; ^{1}H NMR ($CDCl_3$) δ 7.14-7.21 (m, 2H), 7.05 (t, *J* = 8.7 Hz, 1H), 4.41 (q, *J* = 7.2 Hz, 2H) and 1.43 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.6, 151.9 (d, *J* = 244.3 Hz),150.2, 149.6 (d, *J* = 10.3 Hz), 131.0 (d, *J* = 3.5 Hz), 123.3, 120.7 (d, *J* = 16.1 Hz), 114.8, 112.7, 96.5, 60.1, 55.4 and 13.8; IR (neat) 3227 and 1668 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{13}Br_2FNO_3$, 419.9221 found 419.9263.

Synthesis of 3,5-dibromo-4-(6-ethoxynaphthalen-2-yl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5n.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4n** was used as the starting material. Subsequently, a 71% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 200 – 203 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (m, 3H), 7.50 (dd, *J* = 1.8 Hz, *J* = 8.1 Hz, 1H), 7.19 (m, 2H), 4.42 (q, *J* = 7.2 Hz, 2H),

4.19 (q, *J* = 6.9 Hz, 2H), 1.52 (t, *J* = 6.9 Hz, 3H) and 1.45 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.5, 157.4, 134.0, 129.6, 129.3, 128.6, 128.3, 127.1, 126.9, 126.5, 121.8, 119.4, 106.5, 104.4, 104.3, 63.6, 61.3, 14.8 and 14.4; IR (neat) 3246 and 1665 cm^{-1}; HRMS (ES) m/z calcd for $C_{19}H_{18}Br_2NO_3$, 465.9653 found 465.9658.

Synthesis of 3,5-dibromo-4-benzo[1,3]dioxol-5-yl-1H-pyrrole-2-carboxylic acid ethyl ester ***5o.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4o** was used as the starting material. Subsequently, a 68% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 125 – 127 °C; ^{1}H NMR ($CDCl_3$) δ 6.90 (broad s, 3H), 6.03 (s, 2H), 4.41 (q, *J* = 7.2 Hz, 2H) and 1.42 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 159.7, 147.4, 147.3, 126.7, 125.4, 124.1, 121.7, 110.7, 108.2, 104.5, 104.2, 101.2, 61.3 and 14.4; IR (neat) 3283 and 1678 cm^{-1}; HRMS (ES) m/z calcd for $C_{14}H_{12}Br_2NO_4$, 415.9128 found 415.9117.

Synthesis of 3,5-dibromo-4-(2,3-dihydro-benzo[1,4]dioxin-6-yl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5p.*** This compound was prepared in a manner identical to compound **5g** with the exception that compound **4p** was used as the starting material. Subsequently, a 93% yield of a tan solid was obtained and this material exhibited the following physical properties: mp 153 – 155 °C; ^{1}H NMR ($CDCl_3$) δ 6.94 (m, 3H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.32 (s, 4H) and 1.43 (t, *J* = 7. Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.1, 143.4, 143.2, 126.3, 125.1, 123.5, 121.7, 119.1, 117.1, 104.8, 104.2, 64.4, 64.3, 61.4 and 14.4; IR (neat) 3250 and 1673 cm^{-1}; HRMS (ES) m/z calcd for $C_{15}H_{14}Br_2NO_4$, 432 found 432.

Synthesis of 3,5-dibromo-4-(2-bromo-3,4-dimethoxyphenyl)-1H-pyrrole-2-carboxylic acid ethyl ester ***5q.*** Into a 100 mL round bottom flask equipped with a magnetic stir bar, was placed compound **2** (0.125 g, 0.290 mmol), dibromodimethylhydantoin (0.083 g, 0.290 mmol) and 15 mL of chloroform. The resulting reaction mixture was refluxed for 4 hours and cooled to room temperature. The reaction mixture was then diluted with 20 mL of chloroform and washed with sodium thiosulfate solution (2 x 30 mL), brine (2 x 30mL), dried with anhydrous sodium sulfate, filtered and concentrated *in vacuo* to give a light yellow solid (0.12 g, 76% yield), which exhibited the following physical properties: mp 165 – 167 °C; ^{1}H NMR ($CDCl_3$) δ 6.97 (m, 2H), 4.42 (q, *J* = 7.2 Hz, 2H), 3.89 (s, 6H) and 1.39 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 160.3, 148.6, 148.4, 126.5, 124.5, 122.8, 121.7, 113.5, 110.8, 105.1, 104.1, 61.5, 55.9, 55.8 and 14.4; IR (neat) 1701 cm^{-1}; MS (ES) m/z calcd for $C_{15}H_{15}Br_3NO_4$, 509.9 found 509.9.

Biological Activity

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[S5] as previously described.[S6] 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.[S6] 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

Sybyl 8.1[S7] was used to prepare the X-ray crystal structure models of αβ-tubulin complexed with different ligands (pdbid: 1sa0, 1sa1, 3hkc, 3hkd and 3hke).[S8,S9] For each structure, the procedure was the same as previously reported.[S10] The stathmin-like domain, 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} $Å^{-1}$. GOLD 5.1[S11] was used for docking studies. The ligands were docked in the active site, which was defined by the space in a 6 Å radius around the complexed small molecule. One hundred GA runs generated one hundred docking conformations for each ligand with GOLD and were filtered initially by GoldScore. They were further analyzed with HINT.[S12] First, the ligands were docked to all five tubulin structures with GOLD without constraints. The resulting conformations were rescored with HINT and the best docking poses were indicated by the highest HINT score. Next, the differences between ligand binding to the five receptors for each ligand were checked and it was found that binding with 3hkc generally gave higher scores. The resulting 3hkc-ligand complexes were further minimized with the Tripos force field and rescored again. The minimized conformation and the new HINT score were defined as the docking conformation/binding mode and binding score of the ligand.

It is important to note that although it binds in a similar fashion, the complexed ligand in 3hkc, N-{2-[(4-hydroxylphenyl)amino]pyridin-3-yl}-4-methoxybenzenesulfonamide (Figure S5) is structurally different from the pyrrole-based compounds reported here or colchicine.

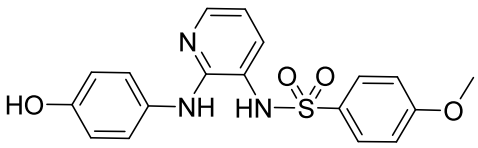


Figure S5. N-{2-[(4-hydroxylphenyl)amino]pyridin-3-yl}-4-methoxybenzenesulfonamide

The total HINT scores of C-4 analogues fail to show a tight relationship with pEC_{50} (Figure S6).

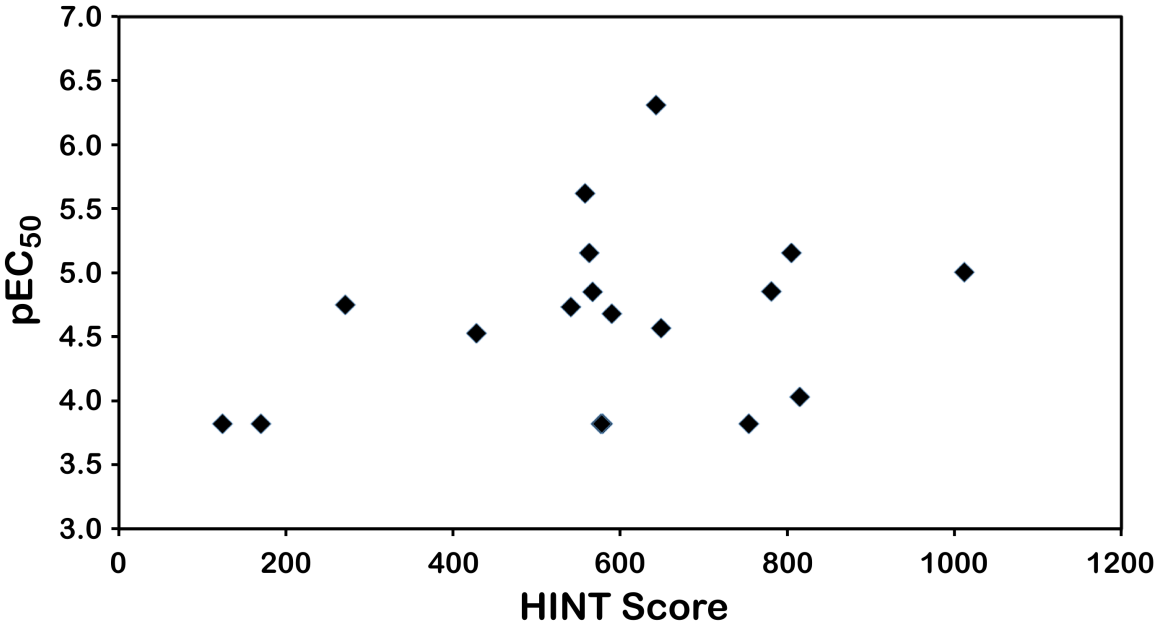


Figure S6. Plot of pEC_{50} vs. total HINT score

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