

# An Investigation into the Cytotoxicity and Mode of Action of Some Novel *N*-alkyl Substituted Isatins

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## Spectroscopic (NMR and MS) Data for Compounds 3-5, 7-20 and 22-25.

Compounds 3-5, 7-20 and 22-25 were synthesized using the general alkylation method given in the main paper. Petroleum spirit (pet. spirit) had a bp range of 40-60 °C.

*5,7-Dibromo-N-(2'-methoxyethyl)isatin (3)*: The crude product was purified using flash chromatography eluting with CHCl<sub>3</sub>. The product was a dark red oil that solidified on standing (84 mg, 35%), mp 112 - 114 °C, R<sub>f</sub> 0.38 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 3.34 (s, 3H, CH<sub>3</sub>), 3.68 (t, *J* = 6 Hz, 2H, H2'), 4.40 (t, *J* = 6 Hz, 2H, H1'), 7.68 (d, *J* = 2 Hz, 1H, H4), 7.86 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 127 MHz) δ 40.6 (C1'), 59.0 (OCH<sub>3</sub>), 69.8 (C2'), 105.0, 116.9, 121.5, 127.5 (C4), 145.1 (C6), 146.8, 158.3 (C2), 181.3 (C3). LREI-MS *m/z* 361/363/365 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>); HREI-MS *m/z* calcd for [M+2]<sup>+</sup> C<sub>11</sub>H<sub>9</sub><sup>79</sup>Br<sup>81</sup>BrNO<sub>3</sub>: 362.8929, found: 362.8926.

*5,7-Dibromo-N-(3'-methylbutyl)isatin (4)*: The product was a bright red oil (167 mg, 67%), R<sub>f</sub> 0.57 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 0.90 (d, *J* = 6 Hz, 6H, 2 × CH<sub>3</sub>), 1.57 (m, 1H, H3'), 1.66 (m, 2H, H3'), 4.09 (t, *J* = 8 Hz, 2H, H1'), 7.60 (d, *J* = 2 Hz, 1H, isatin ArH), 7.80 (d, *J* = 2 Hz, 1H, isatin ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 22.4, 26.1, 37.9, 40.3, 104.7, 116.7, 121.4, 127.4, 145.1, 146.9, 157.9, 181.7. LREI-MS *m/z* 373/375/377 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>); HREI-MS *m/z* calcd for [M]<sup>+</sup> C<sub>13</sub>H<sub>13</sub><sup>79</sup>Br<sub>2</sub>NO<sub>2</sub>: 372.9313, found: 372.9311.

*N-Benzyl-5,7-dibromoisatin (5)*: The product was a bright red solid (209 mg, 80%) mp 150 - 152 °C (lit.<sup>1</sup> 149 – 150 °C), R<sub>f</sub> 0.47 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 5.40 (s, 2H, H1'), 7.18-7.36 (overlapping m, 5H, phenyl ArH), 7.69 (d, *J* = 2 Hz, 1H, H4), 7.79 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75MHz) δ 44.6, 105.2, 117.1, 121.3, 126.3, 127.4, 127.7, 128.8, 135.6, 145.2, 146.6, 158.2, 181.2. LREI-MS *m/z* 393/395/397 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>).

*5-Bromo-N-(p-methoxybenzyl)isatin (9)*: This compound was made from technical grade 5-bromoisatin which contained 10% isatin and gave a mixture of two *N*-alkylated products. The major product was the bright orange-red 5-bromoisatin derivative (138 mg, 20% based on the amount of 5-bromoisatin in the starting material), mp 144 - 146 °C, R<sub>f</sub> 0.43 (CH<sub>2</sub>Cl<sub>2</sub>, silica) <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 3.77 (s, 3H, OCH<sub>3</sub>), 4.84 (s, 2H, H1'), 6.69 (d, *J* = 8 Hz, 1H, H7), 6.86 (d, *J* = 8 Hz, 2H, phenyl ArH), 7.23 (d, *J* = 8

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<sup>1</sup> Pummerer, R.; Meininger, F. Indigo Dyes. IV. A new method for the preparation of *N,N'*-alkylated indigo dyes from commercial indigosols. *Ann.* **1954**, 590, 173-194.

Hz, 2H, phenyl ArH), 7.57 (dd,  $J = 2, 8$  Hz, 1H, H6), 7.68 (d,  $J = 2$  Hz, 1H, H4).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  43.6, 55.3, 112.7, 114.3, 116.6, 118.8, 125.9, 128.1, 128.8, 140.4, 149.4, 157.4, 159.5, 182.2. LREI-MS  $m/z$  345/347 ( $[\text{M}]^+ / [\text{M}+2]^+$ ); HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{16}\text{H}_{12}^{79}\text{BrNO}_3$ : 345.0001, found: 344.9998. *N*-(*p*-methoxybenzyl)isatin (**7**): The minor product from isatin was a bright orange solid (25 mg, 27% based on the amount of isatin in the starting material), mp 169 - 171 °C (lit.<sup>2</sup> 171 – 172 °C),  $R_f$  0.31 ( $\text{CH}_2\text{Cl}_2$ , silica).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.78 (s, 3H,  $\text{OCH}_3$ ), 4.86 (s, 2H, H1'), 6.80 (d,  $J = 8$  Hz, 1H, H7), 6.86 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.08 (t,  $J = 8$  Hz, 1H, H5), 7.27 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.48 (dt,  $J = 1, 8$  Hz, 1H, H6), 7.59 (d,  $J = 7$  Hz, 1H, H4).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  43.5, 55.3, 110.0, 114.4, 117.7, 123.7, 125.3, 126.4, 128.9, 138.2, 150.7, 158.2, 159.4, 183.3. LREI- MS  $m/z$  267 ( $[\text{M}]^+$ ).

*4-Bromo-N*-(*p*-methoxybenzyl)isatin (**8**): The product was the bright orange solid (142 mg, 65%), mp 173 - 175 °C,  $R_f$  0.42 ( $\text{CH}_2\text{Cl}_2$ , silica)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.77 (s, 3H,  $\text{OCH}_3$ ), 4.85 (s, 2H, H1'), 6.76 (d,  $J = 8$  Hz, 1H, isatin ArH), 6.85 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.19 (d,  $J = 8$  Hz, 1H, isatin ArH), 7.24 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.29 (t,  $J = 8$  Hz, 1H, H6).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  43.5, 55.2, 109.8, 114.4, 116.4, 121.5, 126.0, 128.4, 128.8, 138.2, 152.2, 157.2, 159.5, 180.6. LREI-MS  $m/z$  345/347 ( $[\text{M}]^+ / [\text{M}+2]^+$ ); HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{16}\text{H}_{12}^{79}\text{BrNO}_3$ : 345.0001, found: 344.9998.

*6-Bromo-N*-(*p*-methoxybenzyl)isatin (**10**): The product was the bright orange solid (122 mg, 42%), mp 177 - 179 °C,  $R_f$  0.45 ( $\text{CH}_2\text{Cl}_2$ , silica)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.80 (s, 3H,  $\text{OCH}_3$ ), 4.84 (s, 2H, H1'), 6.89 (d,  $J = 9$  Hz, 2H, H4'), 6.98 (d,  $J = 8$  Hz, 1H, H7), 7.24 (dd,  $J = 8, 1$  Hz, 1H, H5), 7.26 (d,  $J = 9$  Hz, 2H, H3'), 7.45 (d,  $J = 8$  Hz, 1H, H4).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  43.7, 55.3, 114.47, 114.51, 116.3, 125.9, 126.3, 127.0, 128.9, 133.4, 151.5, 158.0, 159.6, 182.0. LREI-MS  $m/z$  345/347 ( $[\text{M}]^+ / [\text{M}+2]^+$ ); HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{16}\text{H}_{12}^{79}\text{BrNO}_3$ : 345.0001, found: 344.9997.

*7-Bromo-N*-(*p*-methoxybenzyl)isatin (**11**): The product was the bright orange-red solid (145 mg, 65%), mp 159 - 161 °C,  $R_f$  0.53 ( $\text{CH}_2\text{Cl}_2$ , silica)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.78 (s, 3H,  $\text{OCH}_3$ ), 5.37 (s, 2H, H1'), 6.85 (d,  $J = 9$  Hz, 2H, phenyl ArH), 6.99 (t,  $J = 8$  Hz, 1H, H5), 7.23 (d,  $J = 9$  Hz, 2H, phenyl ArH), 7.61 (d,  $J = 8$  Hz, 1H, H4 or H6), 7.66 (d,  $J = 8$  Hz, 1H, H4 or H6).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)

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<sup>2</sup> Tacconi, G.; Gamba, A.; Marinone, F.; Desimoni, G. Heterodiene synthesis. V. 1,2- versus 1,4-cycloaddition reactions of enamines to *N*-substituted 3-oxindolideneacetophenones. *Tetrahedron*, **1971**, 27, 561-579.

$\delta$  44.0, 55.2, 104.4, 114.1, 120.9, 124.7, 125.2, 127.9(6), 128.0(5), 144.1, 147.8, 159.0, 159.1, 182.4. LREI-MS  $m/z$  345/347 ( $[M]^+/[M+2]^+$ ); HREI-MS  $m/z$  calcd for  $[M]^+ C_{16}H_{12}^{79}BrNO_3$ : 345.0001, found: 344.9992.

*5,7-Dibromo-N-(p-methoxybenzyl)isatin (12)* (CAS No. 620932-73-2): The product was a bright red-orange solid (174 mg, 62%), mp 166 – 168 °C,  $R_f$  0.49 ( $CH_2Cl_2$ , silica).  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  3.76 (s, 3H,  $OCH_3$ ), 5.33 (s, 2H,  $H1'$ ), 6.83 (d,  $J$  = 8 Hz, 2H, phenyl ArH), 7.18 (d,  $J$  = 8 Hz, 2H, phenyl ArH), 7.68 (d,  $J$  = 2 Hz, 1H,  $H4$ ), 7.79 (d,  $J$  = 2 Hz, 1H,  $H6$ ).  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  44.1, 55.2, 105.2, 114.2, 117.0, 121.4, 127.4, 127.5, 128.0, 145.2, 146.7, 158.3, 159.1, 181.3. LREI-MS  $m/z$  423/425/427 ( $[M]^+/[M+2]^+/[M+4]^+$ ); HREI-MS  $m/z$  calcd for  $[M+2]^+ C_{19}H_{17}^{79}Br^{81}BrNO_2$ : 424.9085, found: 424.9077.

*5,7-Dibromo-N-(m-methoxybenzyl)isatin (13)*: The product was a bright orange-red solid (237 mg, 85%), mp 110 - 112 °C,  $R_f$  0.32 ( $CH_2Cl_2$ , silica)  $^1H$  NMR ( $CDCl_3$ , 500 MHz)  $\delta$  3.77 (s, 3H,  $OCH_3$ ), 5.37 (s, 2H,  $H1'$ ), 6.77 (s, 1H, phenyl ArH), 6.80-6.82 (overlapping m, 2H, phenyl ArH), 7.24 (d,  $J$  = 8 Hz, 1H, phenyl ArH), 7.71 (d,  $J$  = 2 Hz, 1H, isatin ArH) 7.81(d,  $J$  = 2 Hz, 1H, isatin ArH).  $^{13}C$  NMR ( $CDCl_3$ , 126 MHz)  $\delta$  44.6, 55.2, 105.3, 112.55, 112.58, 117.2, 118.5, 121.4, 127.5, 129.9, 137.3, 145.3, 146.7, 158.2, 159.9, 181.2. LREI-MS  $m/z$  423/425/427 ( $[M]^+/[M+2]^+/[M+4]^+$ ); HREI-MS  $m/z$  calcd for  $[M+2]^+ C_{19}H_{17}^{79}Br^{81}BrNO_2$ :424.9085, found: 424.9079.

*5,7-Dibromo-N-(p-nitrobenzyl)isatin (14)*: The product was a bright orange solid (216 mg, 74%), mp 185 - 187 °C,  $R_f$  0.34 ( $CH_2Cl_2$ , silica).  $^1H$  NMR ( $CDCl_3$ , 300 MHz)  $\delta$  5.49 (s, 2H,  $H1'$ ), 7.42 (d,  $J$  = 9 Hz, 2H, phenyl ArH), 7.76 (d,  $J$  = 2 Hz, 1H, isatin ArH), 7.84 (d,  $J$  = 2 Hz, 1H, isatin ArH), 8.21 (d,  $J$  = 8 Hz, 2H, phenyl ArH).  $^{13}C$  NMR ( $CDCl_3$ , 75 MHz)  $\delta$  44.4, 104.9, 117.8, 121.4, 124.2, 127.2, 127.9, 143.2, 145.3, 146.0, 147.6, 158.2, 180.1. LREI-MS  $m/z$  438/440/42 ( $[M]^+/[M+2]^+/[M+4]^+$ ); HREI-MS  $m/z$  calcd for  $[M]^+ C_{15}H_8Br_2N_2O_4$ : 437.8851 , found: 437.8846.

*5,7-Dibromo-N-(o-nitrobenzyl)isatin (15)*: The product was a bright orange solid (278 mg, 96%), mp 198 – 200 °C,  $R_f$  0.56 ( $CH_2Cl_2$ , silica).  $^1H$  NMR ( $CDCl_3$ , 500 MHz)  $\delta$  5.75 (s, 2H,  $H1'$ ), 7.22 (d,  $J$  = 8 Hz, 1H, phenyl ArH), 7.51 (t,  $J$  = 8 Hz, 1H, phenyl ArH), 7.61 (t,  $J$  = 8 Hz, 1H, phenyl ArH), 7.77 (d,  $J$  = 2 Hz, 1H, isatin ArH), 7.81 (d,  $J$  = 2 Hz, 1H, isatin ArH), 8.21 (d,  $J$  = 8 Hz, 1H, phenyl ArH).  $^{13}C$  NMR ( $CDCl_3$ , 126 MHz),  $\delta$  43.5, 105.1, 117.7, 121.3, 125.9, 126.7, 127.8, 128.6, 132.2, 134.3, 145.4, 146.1, 147.2, 158.2, 180.7. LREI-MS  $m/z$  438/440/442 ( $[M]^+/[M+2]^+/[M+4]^+$ ); HREI-MS  $m/z$  calcd for  $[M]^+ C_{15}H_8^{79}Br_2N_2O_4$ : 437.8851, found: 437.8872.

*5,7-Dibromo-N-(p-chlorobenzyl)isatin (16)*: The crude product was purified using flash chromatography on silica eluting with 7:3 CH<sub>2</sub>Cl<sub>2</sub>/pet. spirit. The product was a bright orange solid (253 mg, 89%), mp 159 - 161 °C, R<sub>f</sub> 0.66 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.36 (s, 2H, H1'), 7.18 (d, *J* = 8 Hz, 2H, H3'), 7.30 (d, *J* = 8 Hz, 2H, H4'), 7.72 (d, *J* = 2 Hz, 1H, H4), 7.78 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 44.1 (C1'), 105.0, 117.3 (C5), 121.4, 127.6 (C4), 127.9, 129.0, 133.6, 134.2, 145.3 (C6), 146.4 (C7a), 158.2 (C2), 181.0 (C3). LREI-MS *m/z* 427/429/431 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>), HREI-MS *m/z* calcd for [M+2]<sup>+</sup> C<sub>15</sub>H<sub>8</sub><sup>79</sup>Br<sup>81</sup>BrClNO<sub>2</sub>: 428.8590, found: 428.8593.

*5,7-Dibromo-N-(p-bromobenzyl)isatin (17)*: The product was a bright orange solid (221 mg, 71%), mp 160-161 °C, R<sub>f</sub> 0.50 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz) δ 5.31 (s, 2H, H1'), 7.11 (d, *J* = 8 Hz, 2H, phenyl ArH), 7.41 (d, *J* = 8 Hz, 2H, phenyl ArH), 7.66 (d, *J* = 2 Hz, 1H, isatin ArH), 7.77 (d, *J* = 2 Hz, 1H, isatin ArH). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 75 MHz) δ 44.1, 105.0, 117.2, 121.3, 121.6, 127.5, 128.1, 131.8, 134.7, 145.1, 146.2, 158.1, 180.9. LREI-MS *m/z* 471/473/475/477 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>/[M+6]<sup>+</sup>), HREI-MS *m/z* calcd for [M]<sup>+</sup> C<sub>15</sub>H<sub>8</sub><sup>79</sup>Br<sub>3</sub>NO<sub>2</sub>: 470.8105, found: 470.8113.

*5,7-Dibromo-N-(p-iodobenzyl)isatin (18)*: The product was a bright orange solid (257 mg, 75%), mp 141 - 142 °C, R<sub>f</sub> 0.45 (CH<sub>2</sub>Cl<sub>2</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.32 (s, 2H, H1'), 6.99 (d, *J* = 8 Hz, 2H, H3'), 7.63 (d, *J* = 8 Hz, 2H, H4'), 7.69 (d, *J* = 2 Hz, 1H, H4), 7.79 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 44.2 (C1'), 93.2, 105.1, 117.3, 121.3, 127.6 (C4), 128.3 (C3'), 135.4 (C5'), 137.8 (C4'), 145.2 (C6), 146.3, 158.2 (C2), 180.9 (C3). LREI-MS *m/z* 519/521/523 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>); HREI-MS *m/z* calcd for [M+2]<sup>+</sup> C<sub>15</sub>H<sub>8</sub><sup>79</sup>Br<sup>81</sup>BrINO<sub>2</sub>: 520.7946, found: 520.7959.

*5,7-Dibromo-N-(p-trifluoromethylbenzyl)isatin (19)*: The crude product was purified using flash chromatography with 7:3 CH<sub>2</sub>Cl<sub>2</sub>/pet. spirit. The product was a bright yellow-orange solid (137 mg, 45%), mp 131 - 132 °C, R<sub>f</sub> 0.64 (CHCl<sub>3</sub>, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.45 (s, 2H, H1'), 7.36 (d, *J* = 8 Hz, 2H, H3'), 7.59 (d, *J* = 8 Hz, 2H, H4'), 7.72 (d, *J* = 2 Hz, 1H, H4), 7.81 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 44.4 (C1'), 105.0, 117.5, 121.4, 123.9 (d, <sup>1</sup>*J*<sub>CF</sub> = 269 Hz, CF<sub>3</sub>), 125.8 (q, <sup>3</sup>*J*<sub>CF</sub> = 4 Hz, C4'), 126.7, 128.0, 129.7 (d, <sup>2</sup>*J*<sub>CF</sub> = 33 Hz, C5'), 139.8, 146.2, 158.2 (C2), 180.8 (C3). LREI-MS *m/z* 461/463/465 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>), HREI-MS *m/z* calcd for [M]<sup>+</sup> C<sub>16</sub>H<sub>8</sub><sup>79</sup>Br<sub>2</sub>F<sub>3</sub>NO<sub>2</sub>: 460.8874, found: 460.8866, [M+2]<sup>+</sup> C<sub>16</sub>H<sub>8</sub><sup>79</sup>Br<sup>81</sup>BrF<sub>3</sub>NO<sub>2</sub>: 462.8853, found: 462.8842, [M+4]<sup>+</sup> C<sub>16</sub>H<sub>8</sub><sup>81</sup>Br<sub>2</sub>F<sub>3</sub>NO<sub>2</sub>: 464.8833, found: 464.8823.

*6-Bromo-N-(p-trifluoromethylbenzyl)isatin (20)*: The crude product was purified using flash chromatography with 7:3 pet. spirit/EtOAc. The product was a bright orange solid (41 mg, 24 %), mp 134 - 136 °C,  $R_f$  0.50 (7:3 pet. spirit/EtOAc, silica).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.96 (s, 2H, H1'), 6.92 (d,  $J = 2$  Hz, 1H, H7), 7.28 (dd,  $J = 2, 8$  Hz, 1H, H5), 7.45 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.48 (d,  $J = 8$  Hz, 1H, H4), 7.63 (d,  $J = 8$  Hz, 2H, phenyl ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  43.6 (C1'), 114.1, 116.3, 124.0 (d,  $^1J_{\text{CF}} = 251$  Hz,  $\text{CF}_3$ ), 126.2 (q,  $^3J_{\text{CF}} = 4$  Hz, C4'), 125.6, 127.4, 127.6, 130.7 (d,  $^2J_{\text{CF}} = 33$  Hz, C5'), 133.7, 138.1, 151.0, 158.1 (C2), 181.5 (C3). LREI-MS  $m/z$  383/385 ( $[\text{M}]^+ / [\text{M}+2]^+$ ), HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{16}\text{H}_9^{79}\text{BrF}_3\text{NO}_2$ : 382.9769, found: 382.9771.

*4-[(5,7-Dibromo-2,3-dihydro-2,3-dioxo-1H-indol-1-yl)methyl]benzoic acid methyl ester (22)*: The product was a bright red solid (193 mg, 65%), mp 165 - 166 °C,  $R_f$  0.36 ( $\text{CH}_2\text{Cl}_2$ , silica).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  3.89 (s, 3H,  $\text{OCH}_3$ ), 5.43 (s, 2H, H1'), 7.29 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.72 (d,  $J = 2$  Hz, 1H, isatin ArH), 7.79 (d,  $J = 2$  Hz, 1H, isatin ArH), 7.98 (d,  $J = 8$  Hz, 2H, phenyl ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  44.6, 52.1, 105.1, 117.4, 121.4, 126.2, 127.6, 129.7, 130.1, 140.9, 145.2, 146.4, 158.2, 166.5, 180.9. LREI-MS  $m/z$  451/453/455 ( $[\text{M}]^+ / [\text{M}+2]^+ / [\text{M}+4]^+$ ); HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{17}\text{H}_{11}^{79}\text{Br}_2\text{NO}_4$ : 450.9055, found: 450.9071.

*5,7-Dibromo-N-(p-tertbutylbenzyl)isatin (23)*: The product was a bright red-orange solid (224 mg, 75%), mp 159 - 161 °C,  $R_f$  0.62 ( $\text{CH}_2\text{Cl}_2$ , silica).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  1.29 (s, 9H,  $\text{CH}_3$ ), 5.38 (s, 2H, H1'), 7.18 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.33 (d,  $J = 8$  Hz, 2H, phenyl ArH), 7.70 (d,  $J = 2$  Hz, 1H, isatin ArH), 7.81 (d,  $J = 2$  Hz, 1H, isatin ArH).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz),  $\delta$  31.3, 34.5, 44.4, 105.3, 117.1, 121.4, 125.7, 126.3, 127.4, 132.5, 145.4, 146.9, 150.8, 158.3, 181.4. LREI-MS  $m/z$  449/451/453 ( $[\text{M}]^+ / [\text{M}+2]^+ / [\text{M}+4]^+$ ); HREI-MS  $m/z$  calcd for  $[\text{M}]^+ \text{C}_{19}\text{H}_{17}^{79}\text{Br}_2\text{NO}_2$ : 448.9626, found: 448.9626.

*5,7-Dibromo-N-(cinnamyl)isatin (24)*: The product was a bright red solid (133 mg, 48%), mp 115 - 117 °C,  $R_f$  0.53 ( $\text{CH}_2\text{Cl}_2$ , silica).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 500 MHz)  $\delta$  4.92 (dd,  $J = 1, 6$  Hz, 2H, H1'), 6.29 (dt,  $J = 6, 16$  Hz, 1H, H2'), 6.63 (d,  $J = 16$  Hz, 1H, H3'), 7.23 (t,  $J = 7$  Hz, 1H, H7'), 7.29 (d,  $J = 7$  Hz, 2H, H6'), 7.33 (d,  $J = 7$  Hz, 2H, H5'), 7.67 (d,  $J = 2$  Hz, 1H, H4), 7.84 (d,  $J = 2$  Hz, 1H, H6).  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 126 MHz)  $\delta$  42.8 (C1'), 104.9, 117.0, 121.3, 122.5 (C2'), 126.4 (C5'), 127.5 (C4), 128.1 (C7'), 128.6 (C6'), 133.7, 135.9, 145.1 (C6), 146.6, 157.8 (C2), 181.3 (C3). LREI-MS  $m/z$  419/421/423 ( $[\text{M}]^+ / [\text{M}+2]^+ / [\text{M}+4]^+$ ), HREI-MS  $m/z$  calcd for  $[\text{M}+2]^+ \text{C}_{17}\text{H}_{11}^{79}\text{Br}^{81}\text{BrNO}_2$ : 420.9136, found: 420.9130.

*5,7-Dibromo-N-(p-phenylbenzyl)isatin (25)*: The crude product was purified using flash chromatography using gradient elution of 1:1 to 7:3 CH<sub>2</sub>Cl<sub>2</sub>/pet. spirit. The product was a bright red solid (118 mg, 38%) mp 159 - 160 °C, R<sub>f</sub> 0.47 (7:3 CH<sub>2</sub>Cl<sub>2</sub>/pet. spirit, silica). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ 5.45 (s, 2H, H1'), 7.32 (d, *J* = 8 Hz, 2H, H3'), overlapping 7.34 – 7.35 (m, 1H, H8'), 7.42 (t, *J* = 8 Hz, 2H, H7'), 7.55 (d, *J* = 8 Hz, 4H, H2' and 6'), 7.72 (d, *J* = 2 Hz, 1H, H4), 7.82 (d, *J* = 2 Hz, 1H, H6). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 126 MHz) δ 44.6 (CH<sub>2</sub>), 105.2, 117.2, 121.4, 126.9 (CH), 127.0 (CH), 127.4 (CH), 127.5 (CH), 127.6 (CH), 128.8 (CH), 134.6, 140.4, 140.7, 145.3 (CH), 146.7, 158.3 (C2), 181.2 (C3). LREI-MS *m/z* 469/471/473 ([M]<sup>+</sup>/[M+2]<sup>+</sup>/[M+4]<sup>+</sup>), HREI-MS *m/z* calcd for [M+2]<sup>+</sup> C<sub>21</sub>H<sub>13</sub><sup>79</sup>Br<sup>81</sup>BrNO<sub>2</sub>: 470.9293, found: 470.9293.

**Table S1:** HPLC Purity Data for Compounds **2–25**.

<b>Compound</b>	<b>t<sub>R</sub> (min)<sup>a</sup></b>	<b>Purity (%)</b>	<b>Compound</b>	<b>t<sub>R</sub> (min)</b>	<b>Purity (%)</b>
<b>2</b>	5.27	> 95	<b>14</b>	5.45	> 95
<b>3</b>	5.05	> 95	<b>15</b>	5.44	> 95
<b>4</b>	6.44	> 98	<b>16</b>	6.18	> 95
<b>5</b>	5.66	> 95	<b>17</b>	6.20	> 95
<b>6</b>	6.12	> 95	<b>18</b>	6.48	> 98
<b>7</b>	4.71	> 98	<b>19</b>	5.80	> 98
<b>8</b>	5.07	> 98	<b>20</b>	5.47	> 95
<b>9</b>	5.10	> 98	<b>21</b>	2.30	> 95
<b>10</b>	5.27	> 98	<b>22</b>	5.48	> 95
<b>11</b>	5.20	> 98	<b>23</b>	6.68	> 95
<b>12</b>	5.71	> 95	<b>24</b>	6.21	> 98
<b>13</b>	5.64	> 95	<b>25</b>	6.62	> 95

<sup>a</sup> HPLC was performed using a Phenomenex® Luna C18 5µm column (150 × 4.6 mm) with a flow rate of 1.00 mL min<sup>-1</sup> and UV detection at 254 nm. The compounds were dissolved in DMF for injection and subject to a three minute gradient from 60% water: 40% acetonitrile to 100% acetonitrile and then isocratic conditions were maintained for 25 min.

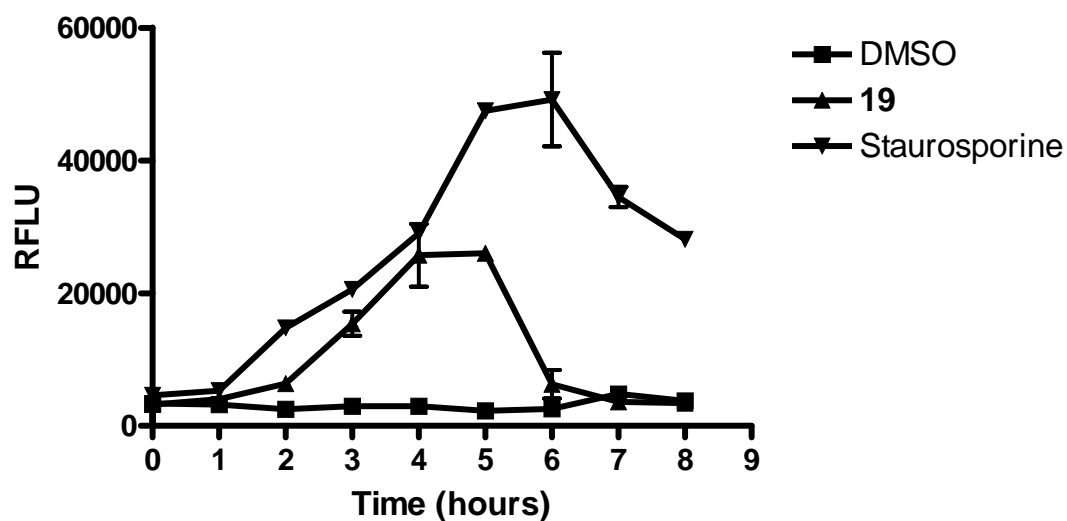


**Table S2:** Physiochemical properties<sup>a</sup> of selected *N*-alkylisatins.

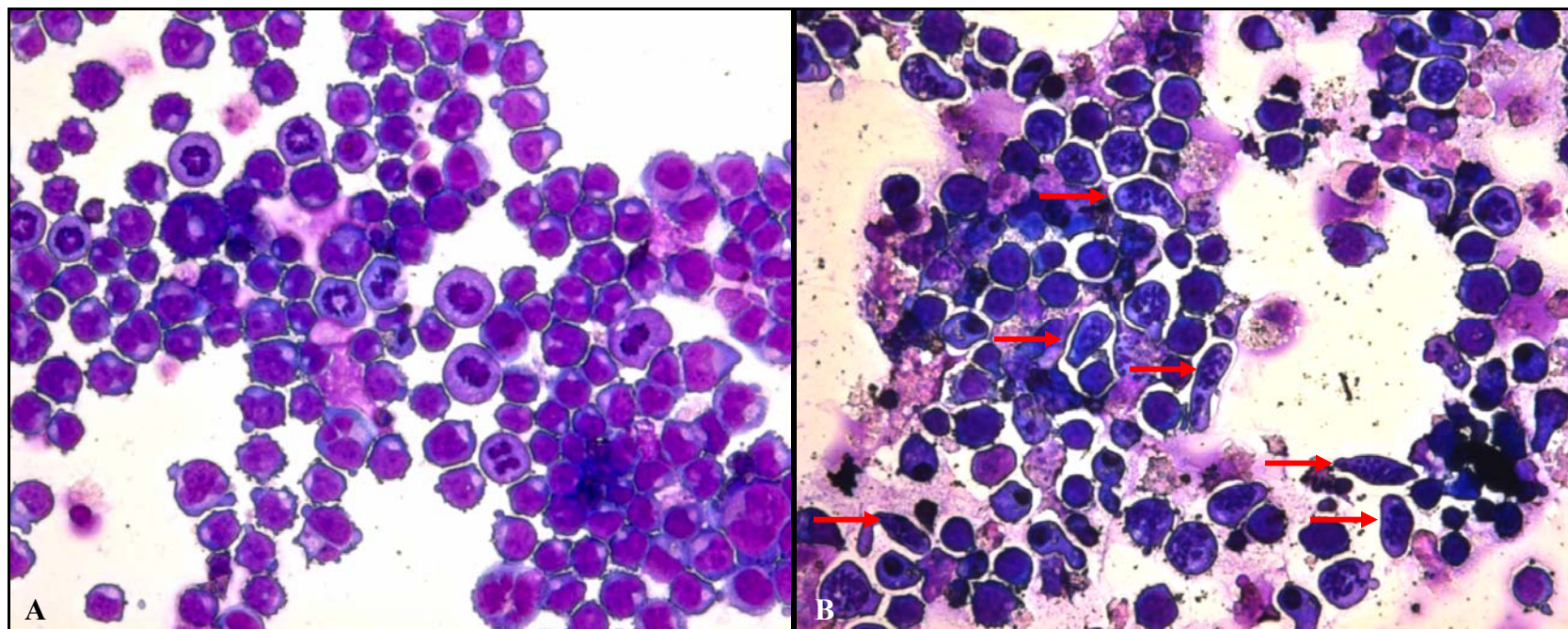
Compound	Substituent	<i>c</i> Log <i>P</i>	$\pi$	$\sigma_p$	<i>E</i> <sub>s</sub>	IC <sub>50</sub> (μM) Jurkat
<b>5</b>	-H	4.3	0.00	0.00	0	1.1
<b>6</b>	-CH <sub>3</sub>	4.8	0.56	-0.17	-1.2	0.49
<b>14</b>	-NO <sub>2</sub>	4.0	-0.80	0.78	-0.5	0.89
<b>16</b>	-Cl	4.2	1.1	0.23	-0.5	0.50
<b>17</b>	-Br	5.2	0.86	0.23	-2.5	0.63
<b>18</b>	-I	5.4	1.1	0.18	-0.9	0.58
<b>19</b>	-CF <sub>3</sub>	5.2	0.88	0.54	-1.1	0.69
<b>21</b>	-COOH	4.0	-0.16	0.45	-1.4	>14
	-COO <sup>-</sup>	1.8				>14
<b>23</b>	-C(CH <sub>3</sub> ) <sub>3</sub>	6.1			-1.7	0.66
<b>25</b>	-Ph	6.2	2.0	-0.01	-3.8	0.74
<b>Correlation Coefficient (R)<sup>b</sup></b>		<b>0.09</b> ( <i>P</i> = 0.82)	<b>0.45</b> ( <i>P</i> = 0.27)	<b>-0.31</b> ( <i>P</i> = 0.46)	<b>-0.09</b> ( <i>P</i> = 0.83)	

<sup>a</sup> *c* log*P* values were calculated using ChemDraw Ultra V. 8.0 (CambridgeSoft Corporation). Other physiochemical constants were obtained from Hansch, C. and Leo, A., Substituent Constants for Correlation Analysis in Chemistry and Biology, Wiley, New York, 1979.

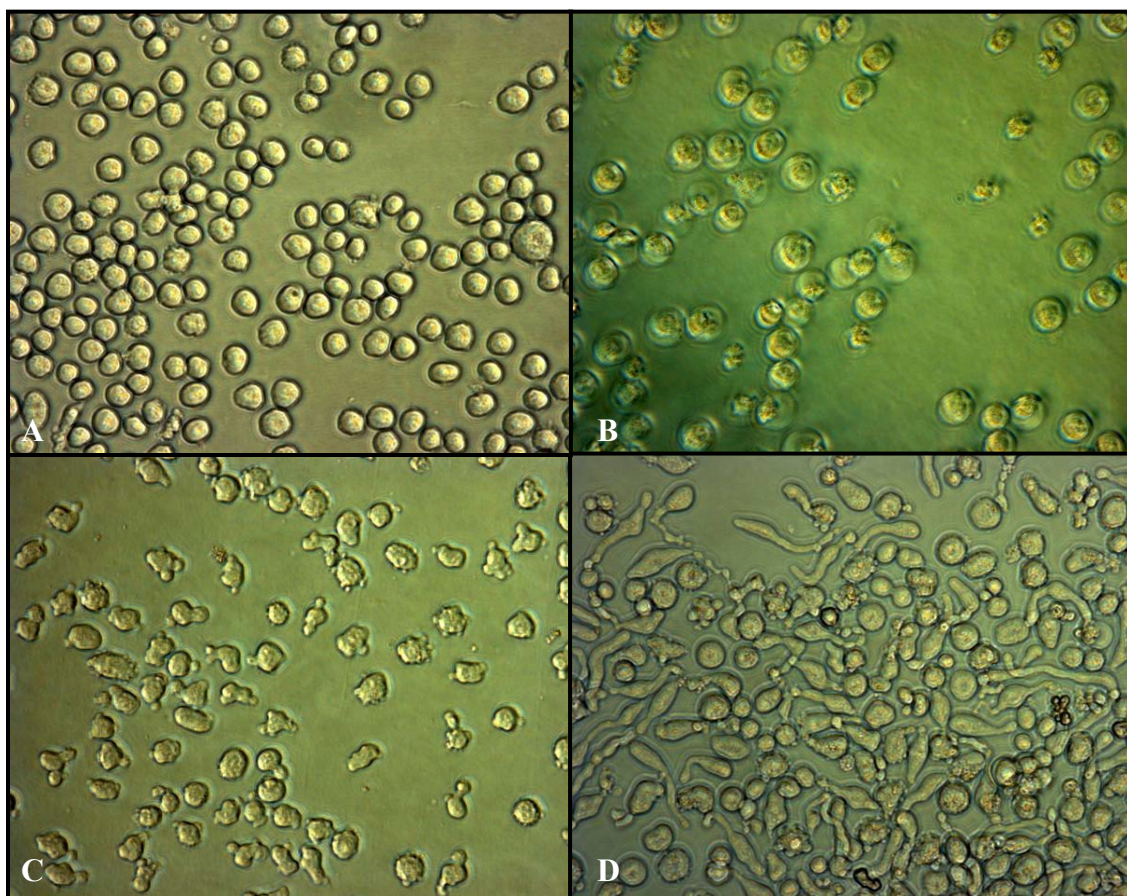
<sup>b</sup> The correlation coefficient (*R*) was calculated using the Pearson correlation test (GraphPad Prism V 4.0).



**Figure S1** Activation of the effector Caspases 3 and 7 in Jurkat cells. Cells ( $2.0 \times 10^4$ ) were exposed to either ■ 2.5% DMSO vehicle control, ▲ 6.8  $\mu$ M 5,7-dibromo-*N*-(*p*-trifluoromethylbenzyl)isatin (**19**) or ▼ 2  $\mu$ M staurosporine for up to 8 h at 37°C. Cells were then incubated with the Caspase-3/7 reagent for 1 h at room temperature and fluorescence measured at an excitation wavelength of 485 nm and 520 nm emission. Data are means  $\pm$  SE of one representative experiment performed in triplicate.

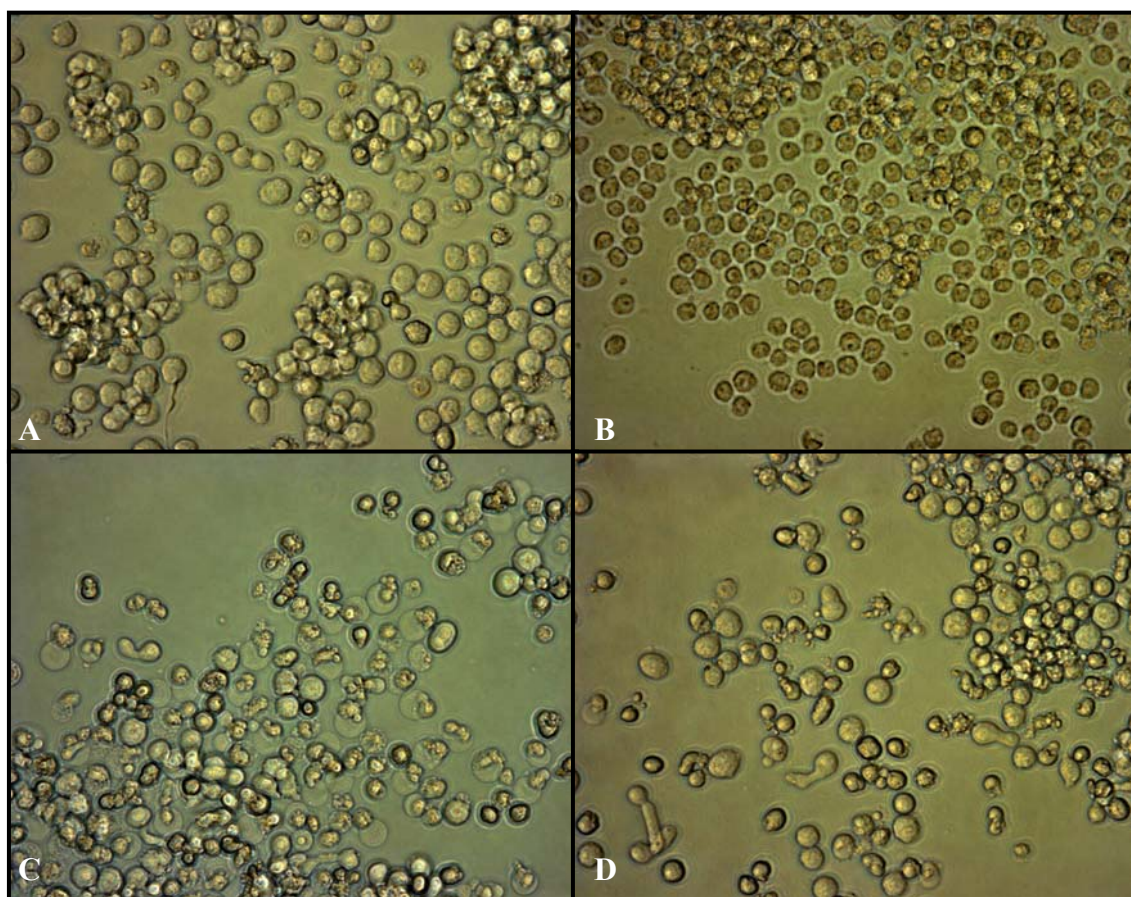


**Figure S2:** Morphological evaluation of nuclei stained with Diff Quik™ after 24 h of treatment with compound **18**. Briefly, U937 cells were treated with either A) DMSO vehicle control or B) 5,7-dibromo-*N*-(*p*-iodobenzyl)isatin (**18**) at 0.39  $\mu\text{g/mL}$  (0.75  $\mu\text{M}$ ). Treatment dose was based on the concentration that induced the greatest amount of morphological change in U937 cells at 24 h. Red arrows indicate morphologically altered cells containing fragmented nuclei. Magnification  $\times 1000$ .

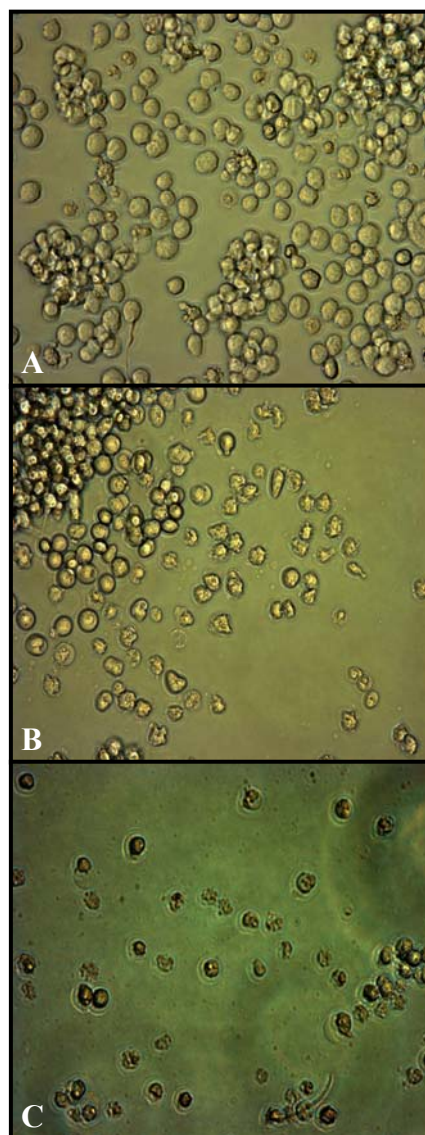


**Figure S3:** Morphological effects of compound 5,7-dibromo-*N*-(cinnamyl)isatin (**24**) on human monocyte-like, histiocytic lymphoma (U937) cells. Briefly cells ( $1.0 \times 10^4$ ) were incubated with either A) DMSO vehicle control for 24 h, B) compound **24** at 12.5 µg/mL (30 µM) for 5 h, C) compound **24** at 0.39 µg/mL (0.9 µM) for 5 h or D) compound **24** at 0.39 µg/mL (0.9 µM) for 24 h. Images were obtained by brightfield microscopy on an inverted light microscope using a Leica DC500 12-megapixel high-performance FireWire camera system. Images were viewed at 1000 × magnification.

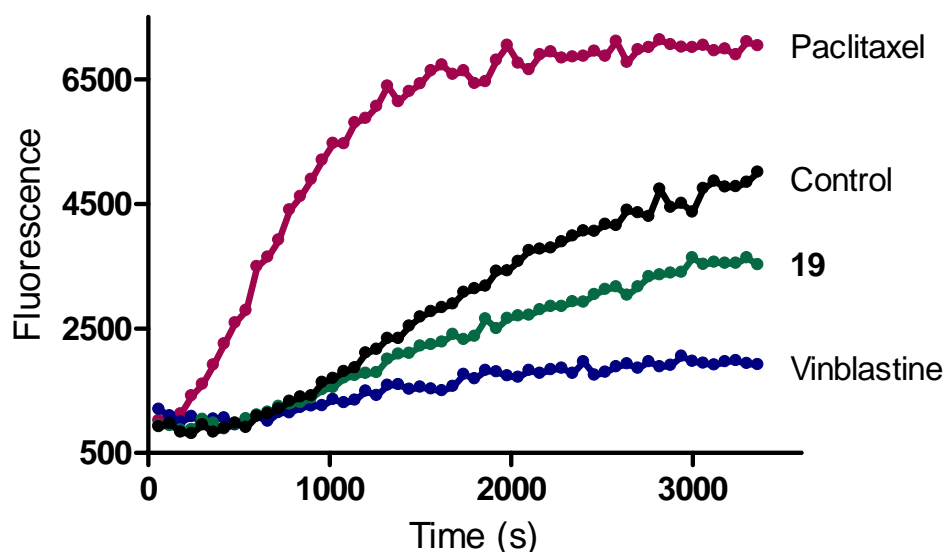




**Figure S4:** Morphological effects of compound 5,7-dibromo-*N*-(*p*-iodobenzyl)isatin (**18**) on human leukemic (Jurkat) T-cells. Briefly cells ( $1.0 \times 10^4$ ) were incubated with either A) DMSO vehicle control for 24 h, B) compound **18** at 12.5 µg/mL (24 µM) for 5 h, C) compound **18** at 0.39 µg/mL (0.7 µM) for 5 h or D) compound **18** at 0.39 µg/mL (0.7 µM) for 24 h. Images were obtained by brightfield microscopy on an inverted light microscope using a Leica DC500 12-megapixel high-performance FireWire camera system. Images were viewed at 1000 × magnification.



**Figure S5:** Morphological effects of staurosporine on human leukemic (Jurkat) T-cells. Briefly cells ( $1.0 \times 10^4$ ) were incubated with either A) DMSO vehicle control for 24 h, B) staurosporine at  $2 \mu\text{M}$  for 5 h or C) staurosporine at  $2 \mu\text{M}$  for 24 h. Images were obtained by brightfield microscopy on an inverted light microscope using a Leica DC500 12-megapixel high-performance FireWire camera system. Images were viewed at  $1000 \times$  magnification.



**Figure S6** The effect of compound **19** and commercial anticancer agents on tubulin polymerization as determined in the *in vitro* microtubule polymerization assay. Purified bovine neuronal tubulin was used to assay for microtubule formation in the presence of either: ●) vehicle control, ●) paclitaxel 3  $\mu$ M, ●) vinblastine sulfate 3  $\mu$ M or ●) 5,7-dibromo-*N*-(*p*-trifluoromethylbenzyl) isatin (**19**) 3  $\mu$ M at 37°C. A shift of the curve to the left or right of the control is indicative of either an increase or decrease, respectively, in the rate of tubulin polymerization. Changes in fluorescence were measured at an excitation wavelength of  $360 \pm 10$  nm and the fluorescence was collected at  $440 \pm 10$  nm. Data points are the means of duplicate experiments.