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## SYNTHESIS OF $\beta$ -DIHYDROPYRROLYL AND $\beta$ -PYRROLYL ACRYLATES AND THEIR ANTIPROLIFERATIVE ACTIVITY AGAINST HCT-116 AND HL-60 CELLS

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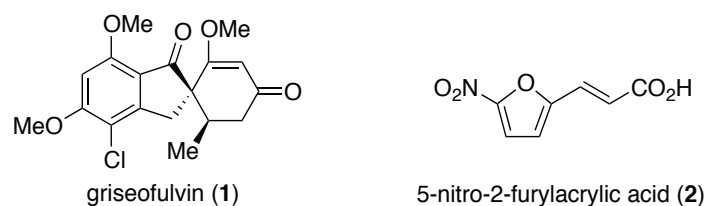
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*This paper is dedicated to Professor Dr. Masakatsu Shibasaki, The University of Tokyo, on the celebration of his 70th birthday.*

**Abstract** – The  $\alpha,\beta$ -unsaturated carbonyl derivatives have been reported as bioactive substances, in particular acting as antiproliferative and antifungal agents. In this study,  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates were synthesized by copper-mediated 5-endo cyclization of isolable Blaise-type  $\beta$ -enamino esters and were assessed for antiproliferative activity against HCT-116 and HL-60 cells. Some  $\beta$ -dihydropyrroles and  $\beta$ -pyrroles showed such activities, which were found to be due to different mechanisms: the  $\beta$ -dihydropyrroles act as cytotoxic agents, whereas the  $\beta$ -pyrroles act as cell cycle inhibitors.

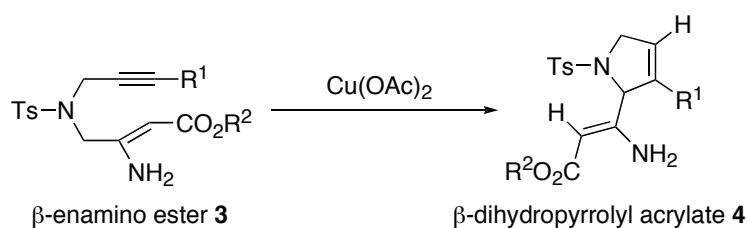
Cancer is defined as a disease occurring due to the malignant growth of the cells. According to WHO data for 2011, cancer causes more deaths than stroke or any other coronary heart disease.<sup>1</sup> While cancer can be effectively treated by chemotherapy performed using cytotoxic anticancer drugs and targeted drugs, the use of most chemotherapeutic drugs requires careful attention because of their narrow therapeutic window<sup>2,3</sup> and the problem of acquired resistance to current drugs.<sup>4</sup> Therefore, developing novel anticancer agents having more potent activities and better safety profiles is necessary.<sup>5</sup>

Enzymes in most human organs<sup>6-8</sup> and fungi<sup>9</sup> have been reported to be influenced when exposed to conjugated  $\alpha,\beta$ -unsaturated carbonyl derivatives that can be linked with a soft nucleophile<sup>10</sup> such as sulfur atoms in enzymes.<sup>11</sup> Griseofulvin (**1**),<sup>9</sup> which is an antifungal agent including with the  $\alpha,\beta$ -unsaturated ketone, is an example of an agent that can link with a soft nucleophile<sup>11</sup> such as enzyme's sulfur atoms in biological systems and has been hypothesized to act by nucleic acid binding and by inhibition of tubulin polymerization.<sup>12-14</sup> Electronic and lipophilic characteristics have been shown to play an important role in the antifungal activity of griseofulvins.<sup>15</sup> Among antitumor agents, the use of 3-(2-furyl)acrylic acid **2** led to a prolonged lifespan in mice bearing Ehrlich ascites tumor.<sup>16</sup> The National Cancer Institute has performed a comprehensive computer-assisted evaluation of structure-activity correlations of antitumoral  $\beta$ -aryl acrylic acid derivatives,<sup>17</sup> finding that the best activity against P388 mouse leukemia cells was observed when the  $\beta$  position was introduced on a ring other than phenyl. These reports suggested that  $\alpha,\beta$ -unsaturated carbonyl derivatives with a ring except phenyl at the  $\beta$  position are bioactive compounds, in particular, acting as antiproliferative agents.



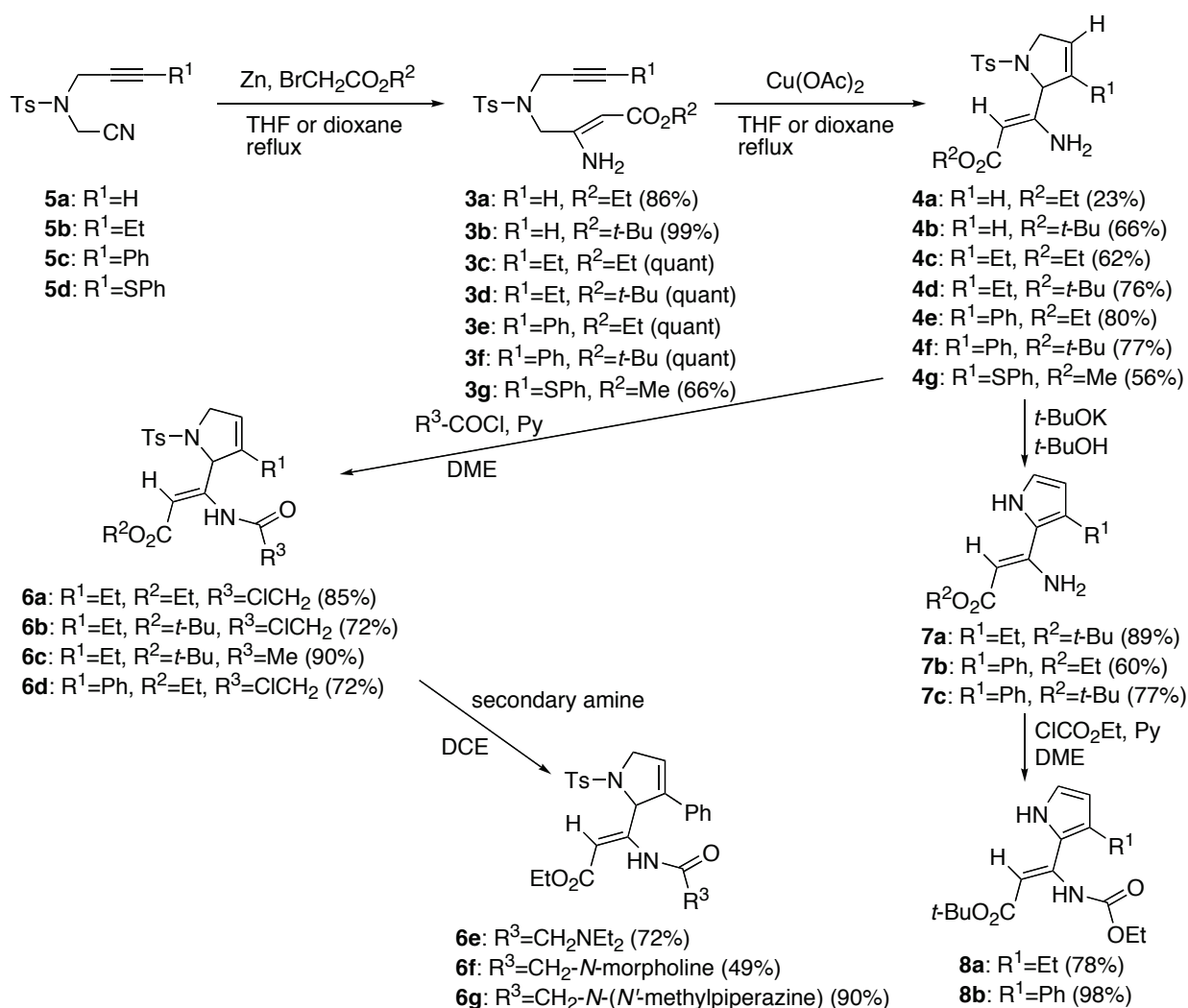
**Figure 1.** Structures of bioactive  $\alpha,\beta$ -unsaturated carbonyl derivatives

Most recently, we reported that isolable Blaise-type  $\beta$ -enamino esters **3** uniquely underwent copper-mediated 5-endo cyclization and afforded  $\beta$ -dihydropyrrolyl acrylates **4** (Scheme 1).<sup>18</sup> Using this method, in the current study, we synthesized the acrylates with  $\beta$ -dihydropyrrolyl **6-7** or  $\beta$ -pyrrolyl **8-9** and assessed their antiproliferative activity against HCT-116 human colon tumor and HL-60 human leukemia cells.



**Scheme 1.** Copper-mediated 5-endo cyclization of isolable Blaise intermediate

The isolable Blaise intermediates **3a-3g**, obtained by the reaction of ynenitriles **5a-5d** with the Reformatsky reagent in good or excellent yields, were subjected to the copper-mediated cyclization to afford the  $\beta$ -dihydropyrrolyl acrylates **4a-4g** (Scheme 2).<sup>18</sup> The cyclization of  $\beta$ -enamino esters **3a-3f** was used for conversion into only the dihydropyrroles **4a-4f**, even though the cyclization of  $\beta$ -enamino esters **3g** obtained both desired dihydropyrrole **4g** and undesired [6 + 1]annulated azepine in nearly the same yield, 56% and 44%, respectively. Treatment of acrylates **4c-4e** with acyl chlorides afforded the corresponding amides **6a-6d** in good yields. Subsequent amination of amide **6d** with secondary amines afforded the desired amines **6e-6g** in moderate or good yields. On the other hand, the  $\beta$ -pyrrolyl acrylates **7a-7c** were obtained in good yield by the treatment of  $\beta$ -dihydropyrrolyl esters **4a-4g** with *t*-BuOK. Treatment of *t*-butyl esters **7a** and **7c** with ethyl chloroformate afforded the corresponding amides **8a** and **8b** in good yields.



**Scheme 2.** Synthesis of  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates

The synthesized acrylates were assessed for their antiproliferative activity against HCT-116 human colon tumor and HL-60 human leukemia cells, with the obtained data shown in Table 1. With the exception of **6a** and **6g**, treatments by 100  $\mu\text{M}$  for most  $\beta$ -dihydropyrrole derivatives **4a-4g** and **6b-6f** did not inhibit the cell growth of both tumors. On the contrary, excluding **8a**,  $\beta$ -pyrrole derivatives **7-8**, exhibited good antiproliferative potential. Five derived compounds, **6a**, **6g**, **7b**, **7c**, and **8b**, completely inhibited the growth of tumor cells by treatment at a concentration of 100  $\mu\text{M}$ , and their concentrations for 50% of cell growth inhibition ( $\text{IC}_{50}$ ) were found to be less than 50  $\mu\text{M}$ . The  $\beta$ -dihydropyrrole derivative **6a** showed the best antiproliferative activity of all derivatives, with  $\text{IC}_{50}$  values of 9.56 and 6.75  $\mu\text{M}$  against HCT-116 and HL-60 cells, respectively. The  $\text{IC}_{50}$  values for  $\beta$ -dihydropyrrole derivative **6g** and  $\beta$ -pyrrole derivative **7c** and **8b** were almost identical.

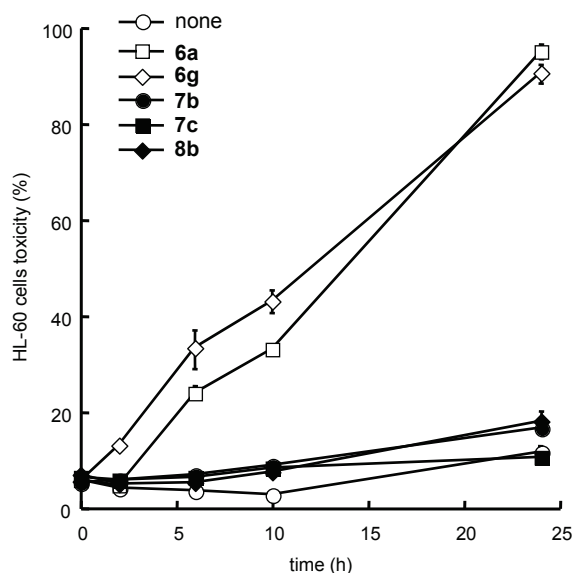
**Table 1.** Effects of  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates on antiproliferative activities against HCT-116 and HL-60 cells

Compd				$\text{IC}_{50}$ values ( $\mu\text{M}$ )		Compd				$\text{IC}_{50}$ values ( $\mu\text{M}$ )	
	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	HCT-116 cells	HL-60 cells		R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>	HCT-116 cells	HL-60 cells
<b>4a</b>	—H	—Et	—H	> 100	> 100	<b>7b</b>	—Ph	—Et	—H	42.92	49.75
<b>4c</b>	—Et	—Et	—H	> 100	> 100	<b>7c</b>	—Ph	— <i>t</i> -Bu	—H	19.55	27.14
<b>4e</b>	—Ph	—Et	—H	> 100	> 100	<b>8a</b>	—Et	— <i>t</i> -Bu		> 100	> 100
<b>4g</b>	—SPh	—Me	—H	> 100	> 100	<b>8b</b>	—Et	— <i>t</i> -Bu		20.70	22.93
<b>6a</b>	—Et	—Et		9.56	6.75						
<b>6b</b>	—Et	— <i>t</i> -Bu		> 100	> 100						
<b>6c</b>	—Et	— <i>t</i> -Bu		> 100	> 100						
<b>6d</b>	—Ph	—Et		> 100	> 100						
<b>6e</b>	—Ph	—Et		> 100	> 100						
<b>6f</b>	—Ph	—Et		> 100	> 100						
<b>6g</b>	—Ph	—Et		23.09	28.51						

Data are shown as mean  $\pm$  sem values ( $n = 3$ ).

The decrease of cell viability was caused by various mechanisms, including arresting of the cell cycle, necrosis, and apoptosis. The arresting of the cell cycle simply attenuates the cell growth, while necrosis and apoptosis conduct cytotoxic processes resulting in cell death. Cytotoxicities for HL-60 cells by five antiproliferative derivatives **6a**, **6g**, **7b**, **7c**, and **8b** were assessed using a CellTox™ Green Dye that stained the DNA of dead cells.<sup>19</sup> It was found that HL-60 cells toxicity of the  $\beta$ -dihydropyrrole derivatives **6a** and **6g** increased with time, while the cytotoxicities of  $\beta$ -pyrrole derivatives **7b**, **7c**, and **8b** were

almost the same as that of the untreated control (Figure 2). The data indicated that the  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates could exhibit antiproliferative activity via different mechanisms;  $\beta$ -dihydropyrroles function as cytotoxic reagents, while the  $\beta$ -pyrroles function as cell cycle inhibitors.



**Figure 2.** Cytotoxicity of  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates against HL-60 cells. HL-60 cells were treated with  $\beta$ -dihydropyrroles **6a** (open square), **6g** (open diamond), and  $\beta$ -pyrroles **7b** (filled circle), **7c** (filled square), **8b** (filled diamond), and their cytotoxicities were analyzed using the CellTox<sup>TM</sup> Green Dye. Data are shown as mean  $\pm$  sem values ( $n = 3$ ).

In the present study,  $\beta$ -dihydropyrrolyl and  $\beta$ -pyrrolyl acrylates were synthesized and their antiproliferative activities were assessed. Some of these compounds, in particular, almost all  $\beta$ -pyrroles, exhibited antiproliferative activity against HCT-116 and HL-60 cells. Furthermore, it was shown that the  $\beta$ -dihydropyrroles function as cytotoxic agents, whereas the  $\beta$ -pyrroles function as cell cycle inhibitors.

## EXPERIMENTAL

### General method

All new compounds were fully identified. Melting points were obtained with a J-Science Lab. micro melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FT-IR 460-PLUS infrared spectrometer and are expressed in reciprocal centimeter ( $\text{cm}^{-1}$ ).  $^1\text{H}$  NMR spectra were recorded on a JEOL ECA600 (600 MHz) spectrometer with respect to tetramethylsilane as an internal standard.  $^{13}\text{C}$  NMR spectra were obtained on a JEOL ECA600 spectrometer with respect to  $\text{CDCl}_3$  as an internal standard ( $\delta = 77.0$ ). Chemical shifts are expressed in parts per million (ppm) and splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet. EI mass spectra (MS) were obtained

using JEOL MS-700 spectrometer with direct-insertion probe at 70 eV. All high resolution mass determinations were obtained on the JMSD300 JMS 2000 on line system. Elemental analyses of new compounds were performed at the Center of Instrumentation of Gifu University.

**(Z)-*t*-Butyl (2-chloroacetyl)amino-3-(2,5-dihydro-3-ethyl-1-tosylpyrrol-2-yl)acrylate (6b).** To a dioxane (5.0 mL) solution of *t*-butyl 3-amino-4-(3-ethyl-*N*-tosylprop-2-ynylamino)but-2-enoate (**3d**)<sup>18</sup> (500 mg, 1.27 mmol) was added copper acetate (0.31 g, 1.53 mmol) at room temperature. The reaction mixture was heated at 100 °C for 40 min and then the cooled mixture was poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel eluting with AcOEt-*n*-hexane (1:5). (*Z*)-*t*-Butyl 3-amino-3-(3-ethyl-2,5-dihydro-1-tosyl-1*H*-pyrrol-2-yl)acrylate (**4d**) (307 mg, 60%) was obtained as yellow powders: mp 136-137 °C; IR (KBr, cm<sup>-1</sup>)  $\nu$  3457, 3335, 2977, 2928, 2876, 1667, 1625, 1558, 1457, 1393, 1365, 1352, 1287, 1252, 1164, 1094, 1056, 1016, 854, 817, 794, 671, 587, 550; MS *m/z* 392 (M<sup>+</sup>). Anal. Calcd for C<sub>20</sub>H<sub>28</sub>N<sub>2</sub>O<sub>4</sub>S: C, 61.20; H, 7.19; N, 7.14. Found: C, 60.97; H, 7.18; N, 7.13.

To a DME (0.50 mL) solution of *t*-butyl 3-amino-3-(2,5-dihydro-3-ethyl-1-tosylpyrrol-2-yl)acrylate (30 mg, 0.08 mmol) and pyridine (**4d**) (30.1 mg, 0.38 mmol) and chloroacetyl chloride (26.0 mg, 0.23 mmol) at 0 °C. The reaction mixture was stirred for 10 min at 0 °C and then poured into water (50 mL). The workup procedure and purification by preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:5) gave (*Z*)-*t*-butyl (2-chloroacetyl)amino-3-(2,5-dihydro-3-ethyl-1-tosylpyrrol-2-yl)acrylate (**6b**) (26.0 mg, 72%) as pale yellow powders: mp 46-47 °C (from CH<sub>2</sub>Cl<sub>2</sub>-*n*-hexane), IR (KBr, cm<sup>-1</sup>)  $\nu$  2975, 2932, 2872, 1704, 1673, 1596, 1478, 1459, 1369, 1339, 1287, 1273, 1250, 1241, 1159, 1094, 997, 923, 902, 812, 761, 753, 671, 548; <sup>1</sup>HNMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  0.95 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 1.53 (9H, t, *J* = 10.3 Hz, *t*-Bu), 2.43 (3H, s, CH<sub>3</sub>), 4.01 (1H, dt, *J* = 14.4 Hz, CH), 4.12 (1H, d, *J* = 14.4 Hz, CHCl), 4.21 (1H, d, *J* = 15.0 Hz, CHCl), 4.25 (1H, dq, *J* = 14.5 Hz, CH), 5.27 (1H, d, *J* = 2.1 Hz, CH), 5.49 (1H, s, CH), 6.69 (1H, brs, CH), 7.33 (2H, d, *J* = 8.3 Hz, ArH), 7.76 (2H, d, *J* = 8.2 Hz, ArH), 11.93 (1H, s, NH); <sup>13</sup>CNMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  11.2 (q), 20.5 (q), 21.5 (q), 28.5 (q), 43.3 (t), 54.9 (t), 64.9 (d), 81.3 (s), 101.8 (d), 117.4 (d), 127.9 (d x 2), 129.8 (d x 2), 133.5 (s), 143.7 (s), 144.7 (s), 156.7 (s), 165.7 (s), 168.5 (s); MS *m/z* 468 (M<sup>+</sup>), 367 (M<sup>+</sup>-CO<sub>2</sub>*t*-Bu). Anal. Calcd for C<sub>22</sub>H<sub>29</sub>N<sub>2</sub>O<sub>5</sub>SCl: C, 56.34; H, 6.23; N, 5.97. Found: C, 56.22; H, 6.19; N, 5.85.

**(Z)-*t*-Butyl 3-acetylamino-3-(2,5-dihydro-3-ethyl-1-tosylpyrrol-2-yl)acrylate (6c).** To a DME (1.0 mL) solution of *t*-butyl 3-amino-3-(2,5-dihydro-3-phenyl-1-tosylpyrrol-2-yl)acrylate (**4d**)<sup>18</sup> (60 mg, 0.15 mmol) was added pyridine (120 mg, 1.53 mmol) and acetyl chloride (120 mg, 1.53 mmol) at 0 °C. The reaction mixture was stirred for 16 h and poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The

solvent was removed under reduced pressure. The residue was purified by preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:5). (*Z*)-*t*-Butyl 3-acetylamino-3-(2,5-dihydro-3-enyl-1-tosylpyrrol-2-yl)acrylate (**6c**) (60 mg, 90%) was obtained as white powders: mp 42-44 °C, IR (KBr, cm<sup>-1</sup>)  $\nu$  2975, 2360, 1711, 1667, 1634, 1493, 1458, 1367, 1350, 1300, 1252, 1238, 1163, 1148, 1095, 1056, 845, 817, 668, 589, 547; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  0.94 (3H, t, *J* = 7.6 Hz, CH<sub>3</sub>), 1.49 (9H, s, *t*-Bu), 1.88-1.96 (2H, m, CH<sub>2</sub>), 2.22 (3H, s, CH<sub>3</sub>), 2.42, (3H, s, CH<sub>3</sub>), 3.98-4.01 (1H, dt, *J* = 2.1 and 14.4 Hz, CH), 4.23-4.26 (1H, dq, *J* = 2.1 and 14.4 Hz, CH), 5.24 (1H, d, *J* = 2.1 Hz, CH), 5.36 (1H, s, CH), 6.78 (1H, brs, CH), 7.33 (2H, d, *J* = 8.3 Hz, ArH), 7.76 (2H, d, *J* = 8.2 Hz, CH<sub>2</sub>), 11.26 (1H, s, NH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  11.2 (q), 20.5 (t), 21.5 (q), 25.6 (q), 28.2 (q x 3), 54.9 (t), 64.8 (d), 80.9 (s), 98.9 (d), 117.0 (d), 127.9 (d x 2), 129.8 (d x 2), 133.4 (s), 143.6 (s), 145.0 (s), 158.5 (s), 169.2 (s), 169.5 (s); MS *m/z* 435 (M<sup>+</sup>+1), 361 (M<sup>+</sup>-*Ot*-Bu), 279 (M<sup>+</sup>-Tos).

**(Z)-Ethyl 3-(diethylaminoacetylamino)-3-(2,5-dihydro-3-phenyl-1-tosylpyrrol-2-yl)acrylate (6e).** Diethylamine (44.9 mg, 0.61 mmol) was added to a DCE (0.50 mL) solution of (*Z*)-ethyl 3-[(2-chloroacetylamino)-3-(2,5-dihydro-3-phenyl-1-tosylpyrrol-2-yl)acrylate (**4e**)<sup>18</sup> (30 mg, 0.06 mmol) at room temperature. The reaction mixture was stirred for 12 h and then poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure. The residue was purified by preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:5). (*Z*)-Ethyl-3-[(2-diethylaminoacetyl)amino]-3-(2,5-dihydro-3-phenyl-1-tosylpyrrol-2-yl)acrylate (**6e**) (26.3 mg, 81%) was obtained as a pale yellow oil: IR (KBr, cm<sup>-1</sup>)  $\nu$  974, 1683, 1624, 1598, 1494, 1402, 1350, 1308, 1286, 1250, 1228, 1198, 1164, 1095, 1050, 1018, 846, 821, 756, 698, 668, 594, 550; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  1.05 (6H, t, *J* = 7.3 Hz, CH<sub>3</sub> x 3), 1.25 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 2.39 (3H, s, CH<sub>3</sub>), 2.62 (4H, m, CH<sub>2</sub> x 2), 3.12 (1H, d, *J* = 17.4 Hz, CH), 3.32 (1H, d, *J* = 17.4 Hz, CH), 4.02-4.24 (3H, m, CH<sub>3</sub>), 4.42 (1H, dd, *J* = 5.5 and 16.0 Hz, CH), 5.31 (1H, s, CH), 5.96 (1H, d, *J* = 1.8 Hz, CH), 7.22-7.27 (2H, m, ArH), 7.32 (2H, d, *J* = 8.2 Hz, ArH), 7.36-7.38 (3H, m, ArH), 7.58 (1H, d, *J* = 5.5 Hz, CH), 7.88 (2H, d, *J* = 8.3 Hz, ArH), 12.00 (1H, s, NH); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>)  $\delta$  11.9 (q x 2), 14.2 (q), 21.53 (q), 48.3 (t x 2), 55.4 (t), 58.8 (t), 59.8 (t), 62.0 (d), 99.4 (d), 121.0 (d), 126.8 (d x 2), 127.8 (d x 2), 128.3 (d), 128.5 (d x 2), 129.8 (d x 2), 132.1 (s), 133.6 (s), 141.7 (s), 143.6 (s), 156.3 (s), 167.5 (s), 173.2 (s); MS *m/z* 525 (M<sup>+</sup>), 370 (M<sup>+</sup>-Tos); high-resolution mass calcd for C<sub>28</sub>H<sub>35</sub>N<sub>3</sub>O<sub>5</sub>S: 525.2297, found *m/z* 525.2256.

***t*-Butyl (Z)-3-[(ethoxycarbonyl)amino]-3-(3-ethyl-1H-pyrrol-2-yl)acrylate (8a).** To a *t*-BuOH (9.1 mL) solution of *t*-butyl (*Z*)-3-amino-3-(3-ethyl-1-tosyl-2,5-dihydro-1H-pyrrol-2-yl)acrylate (**4d**)<sup>18</sup> (205 mg, 0.52 mmol) was added *t*-BuOK (293 mg, 2.61 mmol) at room temperature. The reaction mixture was stirred for 10 min and then poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The solvent was

removed under reduced pressure. The residue was separated by the preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:5). *t*-Butyl (*Z*)-3-amino-3-(3-ethyl-1*H*-pyrrol-2-yl)acrylate (**7a**) (110 mg, 89%) was obtained as a yellow oil: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.23 (3H, t, *J* = 7.6 Hz, CH<sub>3</sub>), 1.50 (9H, s, CH<sub>3</sub> x 3), 2.69 (2H, q, *J* = 7.6 Hz, CH<sub>2</sub>), 4.70 (1H, s, olefinicH), 6.15 (1H, t, *J* = 2.7 Hz, ArH), 6.31 (2H, brs, NH<sub>2</sub>), 6.74 (1H, d, *J* = 2.7 Hz, ArH), 8.37 (1H, brs, NH). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 15.2 (q), 19.8 (t), 28.6 (q x 3), 78.5 (s), 84.6 (d), 110.5 (d), 119.1 (d), 123.7 (s), 127.0 (s), 152.9 (s), 170.6 (s).

To a DME (1.3 mL) solution of *t*-butyl (*Z*)-3-amino-3-(3-ethyl-1*H*-pyrrol-2-yl)acrylate (**7a**) (47 mg, 0.20 mmol) was added pyridine (79 mg, 1.0 mmol) and ethyl chloroformate (65 mg, 0.60 mmol) at 0 °C. The reaction mixture was stirred for 20 min and then poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure. The residue was purified by preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:10). *t*-Butyl (*Z*)-3-[(ethoxycarbonyl)amino]-3-(3-ethyl-1*H*-pyrrol-2-yl)acrylate (**8a**) (48 mg, 78%) was obtained as white powders: mp 97-98 °C. IR (KBr, cm<sup>-1</sup>) ν 3380, 2979, 2935, 1733, 1665, 1619, 1553, 1492, 1426, 1393, 1369, 1311, 1221, 1155, 1135, 1057, 1023, 871, 827, 760; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.21 (3H, t, *J* = 7.6 Hz, CH<sub>3</sub>), 1.26 (3H, t, *J* = 7.6 Hz, CH<sub>3</sub>), 1.50 (9H, s, CH<sub>3</sub> x 3), 2.60 (2H, q, *J* = 7.6 Hz, CH<sub>2</sub>), 4.13 (2H, q, *J* = 7.6 Hz, CH<sub>2</sub>), 5.09 (1H, s, olefinicH), 6.16 (1H, brs, ArH), 6.78 (1H, brs, ArH), 8.83 (1H, brs, NH), 10.37 (1H, brs, NH); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 14.3 (q), 14.9 (q), 20.0 (t), 28.3 (q x 3), 61.7 (t), 80.4 (d), 99.1 (d), 109.8 (d), 119.5 (d), 121.4 (s), 128.5 (s), 144.8 (s), 153.4 (s), 168.8 (s); MS *m/z* 308 (M<sup>+</sup>), 251 (M<sup>+</sup>-*t*-Bu), 235 (M<sup>+</sup>-CO<sub>2</sub>Et), 207 (M<sup>+</sup>-CO<sub>2</sub>*t*-Bu). Anal. Calcd for C<sub>16</sub>H<sub>24</sub>N<sub>2</sub>O<sub>4</sub>: C, 62.32; H, 7.84; N, 9.08. Found: C, 62.13; H, 7.63; N, 8.82.

***t*-Butyl (*Z*)-3-[(ethoxycarbonyl)amino]-3-(3-phenyl-1*H*-pyrrol-2-yl)acrylate (**8b**).** To a DME (3.0 mL) solution of *t*-butyl (*Z*)-3-amino-3-(3-phenyl-1*H*-pyrrol-2-yl)acrylate (**7c**)<sup>18</sup> (94 mg, 0.33 mmol) was added pyridine (131 mg, 1.65 mmol) and ethyl chloroformate (108 mg, 0.99 mmol) at 0 °C. The reaction mixture was stirred for 15 min and then poured into water (50 mL). The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic layer was dried over MgSO<sub>4</sub>. The solvent was removed under reduced pressure. The residue was purified by preparative TLC on silica gel eluting with AcOEt-*n*-hexane (1:5) to give *t*-butyl (*Z*)-3-[(ethoxycarbonyl)amino]-3-(3-phenyl-1*H*-pyrrol-2-yl)acrylate (**8b**) (115 mg, 98%) as white powders: mp 127-131 °C; IR (KBr, cm<sup>-1</sup>) ν 3262, 2980, 1728, 1666, 1619, 1552, 1497, 1449, 1391, 1368, 1296, 1223, 1156, 1140, 1093, 1057, 1037, 985, 942, 903, 876, 844, 825, 816, 755, 736, 711, 699, 684, 660, 612, 604; <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 1.06 (3H, t, *J* = 6.9 Hz, CH<sub>3</sub>), 1.46 (9H, s, CH<sub>3</sub> x 3), 3.92 (2H, q, *J* = 6.9 Hz, CH<sub>2</sub>), 5.08 (1H, s, olefinic H), 6.34 (1H, brs, ArH), 6.81 (1H, brs, ArH), 7.18-7.21 (1H, m, ArH), 7.29-7.32 (2H, m, ArH), 7.40-7.41 (2H, m, ArH), 8.85 (1H, brs, NH), 10.10 (1H, brs, NH); <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 14.1 (q), 28.3 (q x 3), 61.6 (t),

80.5 (d), 100.2 (d), 110.5 (d), 119.7 (s), 121.2 (s), 126.0 (d), 126.8 (s), 128.1 (d x 2), 128.2 (d x 2), 135.8 (s), 145.0 (s), 152.7 (s), 168.4 (s); MS  $m/z$  356 ( $M^+$ ), 299 ( $M^+-t\text{-Bu}$ ), 283 ( $M^+-\text{CO}_2\text{Et}$ ), 255 ( $M^+-\text{CO}_2t\text{-Bu}$ ), 182 ( $M^+-(\text{CO}_2\text{Et}+\text{CO}_2t\text{-Bu})$ ). Anal. Calcd for  $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_4$ : C, 67.40; H, 6.79; N, 7.86. Found: C, 67.32; H, 6.75; N, 7.83.

#### *Cell lines and cell cultures*

Two types of cancer cell lines derived from human species were used to assess the antiproliferative and cytotoxic activity of the synthesized compounds. HCT-116 cells (derived from human colon cancer) and HL-60 cells (derived from human promyelocytic leukemia) were purchased from American Type Culture Collection (VA, USA), were maintained in McCoy 5A medium supplemented with L-glutamine and 10% heat inactivated (55 °C for 30 min) fetal bovine serum (FBS) at 37 °C in a 5%  $\text{CO}_2$  atmosphere. The HL-60 cells were cultured in RPMI-1640 medium supplemented with L-glutamine and 10% heat inactivated FBS at 37 °C in a 5%  $\text{CO}_2$  atmosphere.

#### *Cell viability assays*

The HCT-116 cells viability assay was carried out by the MTT method following the method described by Mosmann.<sup>20</sup> Briefly, the cells were placed in 96-well flat-bottomed tissue culture plates with  $2.0 \times 10^3$  cells per well in 100  $\mu\text{L}$  culture medium. This was followed by incubation at 37 °C in an atmosphere of 5%  $\text{CO}_2$  for 48 h to allow the cells' attachment onto the wells. The cells were treated with the indicated concentrations of test agents in the culture medium without FBS. Following a further 24 h of incubation, 10  $\mu\text{L}$  of MTT (5 mg/mL in PBS buffer) was added per well and the plate was incubated for 4 h to allow metabolism of MTT by cellular mitochondrial dehydrogenases. The excess MTT was aspirated, and the formazan crystals were dissolved by the addition of 100  $\mu\text{L}$  of DMSO. The absorbance of purple formazan was read at 570 nm using a microplate reader. The results following the exposure of the test agents were calculated as a percentage relative to untreated controls.

The HL-60 cells viability assay was carried out by the WST-1 method adopting the procedure described by Ishiyama.<sup>21</sup> The cells were seeded in 96-well flat-bottomed tissue culture plates with  $2.0 \times 10^4$  cells per well in 100  $\mu\text{L}$  of the FBS-containing culture medium with the indicated concentrations of test agents. Following a further 48 h incubation, 10  $\mu\text{L}$  of a mixture of WST-1/1-methoxy PMS solution containing 5 mM WST-1 and 0.2 mM 1-methoxy PMS in 40 mM HEPES-NaOH (pH 7.4) was added per well and the plate was incubated for 3 h to allow metabolism of WST-1 by cellular mitochondrial dehydrogenases. The absorbance of yellow formazan was recorded at 415 nm using a microplate reader. The results following the exposure of the test agents were calculated as a percentage relative to untreated controls.

### *Cytotoxicity assay*

HL-60 cells were seeded in 96-well flat-bottomed culture plates at a density of  $1.0 \times 10^4$  cells per well in 100  $\mu$ L of the FBS-containing culture medium with test agents and CellTox™ Green Dye obtained from Promega (WI, USA).<sup>22</sup> After the cells had been incubated for the indicated number of hours, the fluorescence of the wells was measured at an excitation wavelength of 480 nm and emission wavelength of 530 nm using a microplate reader. The results following the exposure of the test agents were calculated as a percentage relative to cytotoxicity controls.

### *Statistical calculation*

Concentration-cell viability relations were fitted to a four-parameter logistic equation using a nonlinear curve-fitting program that then derived the IC<sub>50</sub> values (Kaleida-graph; Synergy Software, Reading, PA). Where appropriate, the results were expressed as means  $\pm$  sem, with n = 3 or higher in one of at least three similar experiments.

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