

# Synthesis and Biological Evaluation of Urea Derivatives of Combretastatins as Anticancer Agents

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**Abstract:** A series of new combretastatin urea derivatives (**12a-j**) were synthesized by reacting of substituted aromatic isocyanates (**11a-j**) to the combretastatin pyrazole-amine. The synthesized derivatives **11a-j** were evaluated for their cytotoxicity against four human cancer cell lines (breast, lung and ovarian). Among them, compounds **12a**, **12c**, **12d**, **12f**, **12g** and **12i** were showed potent anticancer activity.

**Keywords:** Combretastatin A-4, Sorafenib, Linifanib, Anticancer activity

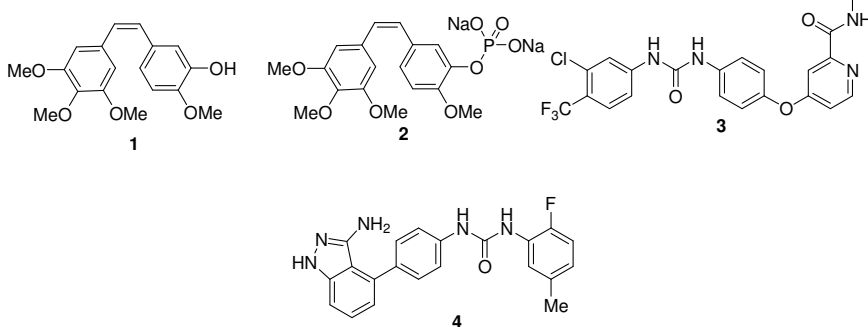
## Introduction

Combretastatin A-4 (CA-4, **1**, Figure 1), which is an alkaloid extracted from the South African tree *Combretum caffrum*<sup>1,2</sup>. It affects microtubule dynamics by binding to the colchicine site and shows potent cytotoxicity<sup>3-5</sup>. CA-4 also acts as a vascular disrupting agent (VDA) and induces blood flow reduction and subsequent tumor cell death<sup>6</sup>. However, its poor aqueous solubility, low bioavailability and dose-dependent toxicity restrict its clinical application<sup>7-9</sup>. Therefore to enhance these drawback the water-soluble sodium phosphate prodrug of CA-4, fosbretabulin (CA-4P, **2**), was designed and is currently in phase II/III clinical trials<sup>10,11</sup>.

Nitrogen containing heterocycles are part of organic chemistry and plays a lead role in the biological activities. Urea was the first synthesized organic compound by Wohler in 1828 and played important roles in animal kingdom. Aryl urea moiety was a important functional group in numerous natural products and drug intermediates<sup>12</sup> and building blocks for various heterocyclic compounds<sup>13,14</sup>. Some of the aryl urea derivatives showed a numerous biological activities such as antitumor<sup>15</sup>, inhibit the polymerization of tubulin<sup>16</sup> anti-inflammatory<sup>17</sup>, antimalarial<sup>18</sup> and antidiabetic<sup>19</sup>, antituberculosis, antibacterial and

anticonvulsant<sup>20-22</sup> antibiotic<sup>23</sup> and hypoglycemic activities<sup>24</sup>. Sorafenib (**3**) was a bi-aryl urea (Figure 1) developed by Bayer, is currently used for the treatment of hepatocellular carcinoma (HCC)<sup>25</sup>, renal cell carcinoma (RCC)<sup>26</sup>. It was also showed anti-inflammatory activity<sup>27</sup>. Linifanib (**4**) was developed by Abbott, is used for the treatment of colorectal cancer<sup>28</sup>.

In view of above biological importance of combretastatin-A-4 and urea derivatives and continuous of efforts, we have synthesized a novel series of urea fused combretastatin derivatives and evaluated for their anticancer activity against human cancer cell lines.



**Figure 1.** Structure of compounds **1-4**

## Experimental

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich, St. Louis, MO, USA), Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA) and were used without further purification. Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254 and visualization on TLC was achieved by UV light or iodine indicator. <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on Bruker, Bruker UXNMR/XWIN-NMR (400 MHz, 300 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from internal TMS standard. ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector. Melting points were determined with an electrothermal melting point apparatus and are uncorrected.

### *(E)-3-(4-Methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylic acid (7)*

The compound 3,4,5-trimethoxyphenyl acetic acid (**5**) (7 g, 30.9 mmol) was dissolved in 20 mL of AC<sub>2</sub>O, followed by addition of substituted 4-methoxy-3-nitrobenzaldehyde (**6**) (5.6 g, 30.9 mmol) and Et<sub>3</sub>N (3 mL). The reaction mixture was heated at 140 °C for 12 hours. After cooling, the reaction mixture was acidified with 35% aq HCl. The reaction mixture was left overnight and the precipitated product was filtered. The precipitate was recrystallized from absolute ethanol afford the pure compound **7**, 6.2 g in 52% yield. M.p: 217-218 °C; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>): δ 3.80 (s, 6H), 3.89 (s, 3H), 3.94 (s, 3H), 6.40 (s, 2H), 6.66 (d, 1H, *J* = 7.3 Hz), 6.84-6.90 (m, 1H), 7.16 (d, 1H, *J* = 7.3 Hz), 7.77 (s, 1H); MS (ESI): 390 [M+H]<sup>+</sup>.

### *(E)-Methyl 3-(4-methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylate (8)*

Concentrated H<sub>2</sub>SO<sub>4</sub> (5 mL) was added to a stirred solution of carboxylic acid **7** (5 g, 12.85 mmol) in absolute methanol (40 mL) and the mixture was heated under reflux for 6 h. About 90% of the excess methanol was removed by evaporation, and the residue was poured into ice water (300 mL). The product was extracted with ether (2×40 mL) and the combined extracts were washed with 2% aqueous NaOH solution (2×50 mL) followed by water (200 mL). Evaporation of ether from the dried (Na<sub>2</sub>SO<sub>4</sub>) solution gave the desired

products **8**, 4.9 g in 95% yield.  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.71 (s, 3H), 3.80 (s, 6H), 3.86 (s, 3H), 3.96 (s, 3H), 6.43 (s, 2H), 6.67 (d, 1H,  $J = 7.25$  Hz), 7.77 (s, 1H), 7.83-7.86 (m, 1H), 7.89-7.92 (m, 1H); MS (ESI): 404  $[\text{M}+\text{H}]^+$ .

*(E)-5-(4-Methoxy-3-nitrophenyl)-4-(3,4,5-trimethoxyphenyl)-3-oxopent-4-enenitrile (9)*

Mixed (E)-methyl 3-(4-methoxy-3-nitrophenyl)-2-(3,4,5-trimethoxyphenyl)acrylate (**8**) (4 g, 9.92 mmol) with NaH (476 mg, 19.8 mmol) in boiling tetrahydrofuran (15 mL) and then followed by drop wise addition of solution of acetonitrile (5.1 mL, 9.92 mmol) in tetrahydrofuran (10 mL). The resulting mixture was refluxed for 4 hours and then cooling down to room temperature, after which the solution was diluted with diethylether (15 mL) and left to stand at room temperature for 48 hours. The precipitated sodium salt was filtered and washed with diethylether. The dry compound was dissolved in water (5 mL) and acidified with HCl (1 mol/L) to pH 2. The collected extracts were crystallized from toluene, filtered and dried with  $\text{Na}_2\text{SO}_4$  to afford pure compound **9**, 3.86 g in 96% yield. M.p.: 220-222  $^\circ\text{C}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.81 (s, 6H), 3.86 (s, 3H), 3.97 (s, 3H), 4.16 (s, 2H), 6.46 (s, 2H), 6.68 (d, 1H,  $J = 7.24$  Hz), 7.78 (s, 1H), 7.86-7.89 (m, 1H), 7.93-7.95 (m, 1H); MS (ESI): 413  $[\text{M}+\text{H}]^+$ .

*5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-amine (10)*

To a stirred mixture of compound **9** (3 g, 7.28 mmol) in ethanol (25 mL) were added  $\text{NH}_2\text{NH}_2 \cdot \text{H}_2\text{O}$  (2.7 mL, 8.73 mmol) and  $\text{CH}_3\text{SO}_3\text{H}$  (0.9 mL, 1.45 mmol) at room temperature and the mixture was stirred under reflux for 1 hour. The precipitate was purified by column chromatography over silica gel with ethylacetate/hexane (4:6) to afford pure compound **10**, 2.7 g in 87% yield. M.p: 177-179  $^\circ\text{C}$ ;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.81 (s, 6H), 3.86 (s, 3H), 3.97 (s, 3H), 4.78 (s, 2H), 5.77 (s, 1H), 6.46 (s, 2H), 6.69 (d, 1H,  $J = 7.28$  Hz), 7.78 (s, 1H), 7.86-7.88 (m, 1H), 7.93-7.96 (m, 1H), 11.68 (s, 1H); MS (ESI): 427  $[\text{M}+\text{H}]^+$ .

*Procedure for synthesis of novel combretastatin urea derivatives (12a-j)*

The amine (**10**) (0.46 mmol) was dissolved in 10 mL of dry  $\text{CH}_2\text{Cl}_2$ , followed by addition of 1-isocyanatobenzene (**11a-j**) (0.46 mmol). The reaction mixture was stirred at room temperature for 12 hours. After completion of reaction product was precipitated. The precipitated product was filtered and recrystallized with ethanol to afford the pure compounds **12a-j**.

*1-(5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)-3-phenylurea (12a)*

81% Yield; 210-212  $^\circ\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.86 (s, 3H), 3.90 (s, 6H), 3.94 (s, 3H), 5.76 (s, 1H), 6.23 (s, 2H), 6.78-6.84 (m, 2H), 7.76-7.87 (m, 3H), 7.93 (d, 2H,  $J = 8.19$  Hz), 8.05 (d, 1H,  $J = 7.68$  Hz), 8.19 (s, 1H), 9.04 (s, 1H), 10.56 (s, 1H), 11.67 (s, 1H),  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.5, 59.3, 61.5, 94.3, 115.6, 116.7, 120.7, 123.6, 127.8, 129.6, 130.7, 131.8, 133.4, 138.5, 140.6, 140.8, 141.5, 142.3, 143.7, 150.5, 153.5, 154.6, 155.7; MS (ESI): 546  $[\text{M}+\text{H}]^+$ .

*1-(5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)-3-(3,4,5-trimethoxyphenyl)urea (12b)*

70% Yield; Mp: 215-217  $^\circ\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.86 (s, 3H), 3.89 (s, 6H), 3.92 (s, 3H), 3.95 (s, 6H), 3.97 (s, 3H), 5.77 (s, 1H), 6.24 (s, 2H), 6.84 (s, 2H), 7.76 (s, 1H),

7.88 (d, 1H,  $J = 8.24$  Hz), 7.92 (d, 1H,  $J = 8.17$  Hz), 8.19 (s, 1H), 9.05 (s, 1H), 10.57 (s, 1H), 11.66 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.5, 58.5, 59.4, 61.4, 61.9, 94.6, 112.6, 115.6, 116.8, 127.5, 129.6, 130.5, 131.4, 132.5, 133.7, 138.6, 140.6, 141.5, 141.7, 143.6, 147.8, 150.5, 153.5, 153.9, 155.8; MS (ESI): 636  $[\text{M}+\text{H}]^+$ .

*1-(5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)-3-(4-methoxyphenyl)urea (12c)*

78% Yield.; Mp: 201-203  $^{\circ}\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.85 (s, 3H), 3.88 (s, 6H), 3.91 (s, 3H), 3.94 (s, 3H), 5.77 (s, 1H), 6.23 (s, 2H), 6.80 (d, 2H,  $J = 7.65$  Hz), 7.75 (s, 1H), 7.86 (d, 1H,  $J = 8.28$  Hz), 7.89 (d, 2H,  $J = 7.65$  Hz), 7.91 (d, 1H,  $J = 8.20$  Hz), 8.19 (s, 1H), 9.06 (s, 1H), 10.56 (s, 1H), 11.66 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  56.4, 57.8, 59.6, 61.5, 94.6, 115.8, 116.7, 119.7, 122.4, 127.8, 130.4, 131.2, 133.4, 134.7, 138.5, 141.4, 141.8, 143.5, 150.4, 153.4, 153.7, 154.6, 155.9; MS (ESI): 576  $[\text{M}+\text{H}]^+$ .

*1-(4-Chlorophenyl)-3-(5-((E)-2-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)urea (12d)*

81% Yield.; Mp: 220-222  $^{\circ}\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.86 (s, 3H), 3.90 (s, 6H), 3.94 (s, 3H), 5.78 (s, 1H), 6.25 (s, 2H), 6.85 (d, 2H,  $J = 8.23$  Hz), 7.76 (s, 1H), 7.87 (d, 1H,  $J = 8.29$  Hz), 7.90 (d, 2H,  $J = 8.23$  Hz), 7.93 (d, 1H,  $J = 8.25$  Hz), 8.20 (s, 1H), 9.07 (s, 1H), 10.57 (s, 1H), 11.67 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.6, 59.3, 61.4, 94.6, 115.6, 116.7, 117.9, 125.7, 127.7, 130.5, 130.7, 131.3, 133.5, 137.6, 138.4, 140.5, 141.4, 141.7, 143.6, 150.5, 153.4, 153.6, 155.8; MS (ESI): 580  $[\text{M}+\text{H}]^+$ .

*1-(4-Bromophenyl)-3-(5-((E)-2-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)urea (12e)*

79% Yield.; Mp: 225-227  $^{\circ}\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.87 (s, 3H), 3.90 (s, 6H), 3.96 (s, 3H), 5.78 (s, 1H), 6.26 (s, 2H), 7.76 (s, 1H), 7.86 (d, 1H,  $J = 8.30$  Hz), 7.90 (d, 2H,  $J = 8.36$  Hz), 7.94-8.04 (m, 3H), 8.20 (s, 1H), 9.08 (s, 1H), 10.57 (s, 1H), 11.67 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.6, 59.7, 61.4, 94.7, 110.6, 115.6, 116.7, 122.5, 127.6, 130.5, 131.7, 132.5, 133.4, 138.5, 138.8, 140.4, 141.3, 141.7, 143.5, 150.5, 153.4, 153.7, 155.9; MS (ESI): 625  $[\text{M}+\text{H}]^+$ .

*1-(4-Fluorophenyl)-3-(5-((E)-2-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)urea (12f)*

86% Yield.; Mp: 218-220  $^{\circ}\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.86 (s, 3H), 3.90 (s, 6H), 3.94 (s, 3H), 5.77 (s, 1H), 6.25 (s, 2H), 7.75 (s, 1H), 7.85-7.90 (m, 3H), 7.94 (d, 2H,  $J = 8.35$  Hz), 7.97 (d, 1H,  $J = 8.14$  Hz), 8.17 (s, 1H), 9.06 (s, 1H), 10.56 (s, 1H), 11.66 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.5, 59.5, 61.9, 94.7, 115.6, 116.7, 117.8, 121.6, 127.7, 130.5, 131.5, 133.4, 135.3, 136.5, 138.6, 140.5, 141.3, 141.7, 143.6, 150.6, 153.4, 153.7, 155.9; MS (ESI): 564  $[\text{M}+\text{H}]^+$ .

*1-(5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)-3-(4-nitrophenyl)urea (12g)*

83% Yield.; Mp: 229-231  $^{\circ}\text{C}$ ,  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  3.87 (s, 3H), 3.91 (s, 6H), 3.95 (s, 3H), 5.78 (s, 1H), 6.26 (s, 2H), 7.76 (s, 1H), 7.86 (d, 1H,  $J = 8.36$  Hz), 8.10-8.17 (m, 3H), 8.20-8.25 (m, 3H), 9.07 (s, 1H), 10.57 (s, 1H), 11.67 (s, 1H);  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  57.5, 59.9, 61.9, 94.6, 115.6, 116.7, 120.7, 122.6, 127.8, 130.6, 131.6, 133.5, 138.6, 140.5, 141.4, 141.7, 142.3, 143.5, 144.5, 150.6, 153.4, 153.8, 155.9; MS (ESI): 591  $[\text{M}+\text{H}]^+$ .

*1-(5-((E)-2-(4-Methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)-3-(4-nitrophenyl)urea (12h)*

77% Yield.; Mp: 198-200 °C, <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 2.67 (s, 3H), 3.85 (s, 3H), 3.90 (s, 6H), 3.93 (s, 3H), 5.76 (s, 1H), 6.24 (s, 2H), 6.85 (d, 2H, *J* = 8.32 Hz), 7.75 (s, 1H), 7.82 (d, 1H, *J* = 8.13 Hz), 7.87 (d, 2H, *J* = 8.32 Hz), 7.89 (d, 1H, *J* = 8.15 Hz), 8.18 (s, 1H), 9.05 (s, 1H), 10.55 (s, 1H), 11.65 (s, 1H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 27.4, 57.6, 59.8, 61.5, 94.5, 115.6, 116.7, 121.7, 127.4, 127.9, 130.5, 131.2, 131.7, 133.5, 138.5, 139.6, 140.5, 141.3, 141.8, 143.6, 150.7, 153.4, 153.8, 155.9; MS (ESI): 560 [M+H]<sup>+</sup>.

*1-(4-Cyanophenyl)-3-(5-((E)-2-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)urea (12i)*

68% Yield.; Mp: 230-232 °C, <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 3.87 (s, 3H), 3.92 (s, 6H), 3.94 (s, 3H), 5.78 (s, 1H), 6.26 (s, 2H), 7.76 (s, 1H), 7.82 (d, 1H, *J* = 8.24 Hz), 7.87 (d, 2H, *J* = 8.40 Hz), 7.90-7.96 (m, 3H), 8.20 (s, 1H), 9.08 (s, 1H), 10.57 (s, 1H), 11.67 (s, 1H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 57.6, 59.6, 61.9, 94.6, 103.5, 115.7, 116.5, 120.7, 121.4, 127.8, 130.2, 131.4, 133.6, 135.6, 138.5, 140.5, 141.2, 141.5, 142.5, 143.7, 150.6, 153.7, 155.7; MS (ESI): 571 [M+H]<sup>+</sup>.

*1-(4-Tert-butylphenyl)-3-(5-((E)-2-(4-methoxy-3-nitrophenyl)-1-(3,4,5-trimethoxyphenyl)vinyl)-1H-pyrazol-3-yl)urea (12j)*

70% Yield. Mp: 190-192 °C, <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 1.45 (s, 9H), 3.85 (s, 3H), 3.90 (s, 6H), 3.93 (s, 3H), 5.76 (s, 1H), 6.24 (s, 2H), 7.75 (s, 1H), 7.81-7.89 (m, 3H), 7.92 (d, 2H, *J* = 7.89 Hz), 7.94 (d, 1H, *J* = 8.20 Hz), 8.19 (s, 1H), 9.05 (s, 1H), 10.56 (s, 1H), 11.65 (s, 1H); <sup>13</sup>C NMR (100 MHz, DMSO-d<sub>6</sub>): δ 33.5, 36.7, 57.7, 59.5, 61.5, 94.6, 115.6, 116.7, 121.6, 127.8, 128.7, 130.6, 131.4, 133.6, 138.6, 138.9, 140.6, 141.3, 141.7, 143.6, 144.6, 150.6, 153.5, 153.7, 155.7; MS (ESI): 602 [M+H]<sup>+</sup>.

## Results and Discussion

### Chemistry

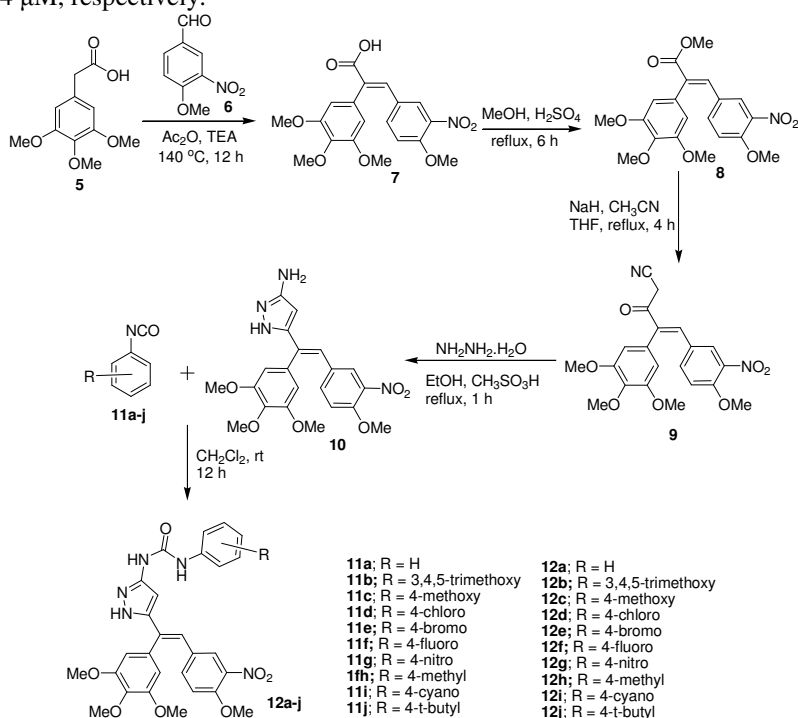
The general route to prepare compounds (**12a-j**) is described in Scheme 1. The appropriate 3,4,5-trimethoxy phenylacetic acid (**5**) was reacted with 4-methoxy-3-nitrobenzaldehyde (**6**) in acetic anhydride, triethyl amine at 140 °C for 12 hours to afford pure acrylic acid (**7**) in good yield. This intermediate **7** was undergoes to esterification with methanol and catalytic amount of H<sub>2</sub>SO<sub>4</sub> at reflux for 6 hours to afford ester compound **8**, it was reacted with CH<sub>3</sub>CN in THF, NaH at reflux for 4 hours to afford pure nitrile intermediate (**9**), this intermediate was cyclization with hydrazine hydrate in ethanol, methanesulphonic acid at reflux for 1 hours to afford pure pyrazole-amine (**10**) in a high yield. Finally amine intermediate **10** was reacted with different substituted readily available aromatic isocyanates (**11a-j**) in CH<sub>2</sub>Cl<sub>2</sub> at room temperature for 12 hours to afford pure compounds (**12a-j**).

### Biological evaluation

#### *In vitro* cytotoxicity

The antitumor activity of these (**12a-j**) compounds was screened in four cancer cell lines, namely Zr-75-1, MCF7 (human Breast cancer), A549 (human lung cancer) and A2780 (human Ovarian cancer), using sulforhodamine B (SRB) method. Adriamycin was used as a positive control. The biological data, expressed as GI<sub>50</sub> values, are listed in Table 1.

Interestingly, the results of this screening revealed dramatic differences in the activities of these compounds. Among them, compounds **12a**, **12c**, **12d**, **12f**, **12g** and **12i** were showed potent anticancer activity in all the tested cancer cell lines, with GI<sub>50</sub> values between 0.12-89.4  $\mu$ M, respectively.



**Table 1.** Cytotoxic activity (GI<sub>50</sub>  $\mu$ M) of compounds **12a-j**

Compound	Breast cancer		Lung cancer	Ovarian cancer
	Zr-75-1	MCF7	A549	A2780
<b>12a</b>	0.23	0.12	2.34	1.34
<b>12b</b>	-	2.11	1.67	4.78
<b>12c</b>	45.2	1.11	0.34	34.6
<b>12d</b>	1.56	1.89	3.45	8.23
<b>12e</b>	3.67	12.3	10.7	25.7
<b>12f</b>	7.80	1.20	56.2	9.30
<b>12g</b>	2.55	1.55	3.40	-
<b>12h</b>	56.2	89.4	77.4	-
<b>12i</b>	44.78	1.22	1.66	15.4
<b>12j</b>	-	-	17.4	18.9
Adriamycin	1.79	0.17	7.25	0.16

#### Procedure of the SRB-assay

The synthesized compounds (**12a-j**) have been evaluated for their *in vitro* cytotoxicity in human cancer cell lines. A protocol of 48 hours continuous drug exposure has been used and a sulforhodamine B (SRB) protein assay has been used to estimate cell viability or growth.

The cell lines were grown in DMEM medium containing 10% fetal bovine serum and 2 mM L-glutamine and were inoculated into 96 well microtiter plates in 90 mL at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO<sub>2</sub>, 95% air and 100% relative humidity for 24 hours prior to addition of experimental drugs. Aliquots of 10 mL of the drug dilutions were added to the appropriate microtiter wells already containing 90 mL of cells, resulting in the required final drug concentrations. For each compound four concentrations (0.1, 1, 10 and 100 µM) were evaluated and each was done in triplicate wells. Plates were incubated further for 48 hours and assay was terminated by the addition of 50 mL of cold trichloroacetic acid (TCA) (final concentration, 10% TCA) and incubated for 60 minutes at 4 °C. The plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (50 mL) at 0.4% (w/v) in 1% acetic acid was added to each of the cells and plates were incubated for 20 minutes at room temperature. The residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm reference wavelengths. Percent growth was calculated on a plate by plate basis for test wells relative to control wells. The above determinations were repeated three times. Percentage growth was expressed as the (ratio of average absorbance of the test well to the average absorbance of the control wells) \*100. Growth inhibition of 50% (GI<sub>50</sub>) was calculated from  $[(Ti-Tz)/(C-Tz)]*100$  1/4 50, which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Where, Tz1/4 Optical density at time zero, OD of control 1/4 C, and OD of test growth in the presence of drug 1/4 Ti.

## Conclusion

We have synthesized a novel series of urea derivatives of combretastatins and evaluated for their *in vitro* anticancer activity against four human cancer cell lines Zr-75-1, MCF7 (human Breast cancer), A549 (human lung cancer) and A2780 (human Ovarian cancer). The compounds that exhibit GI<sub>50</sub> ≤ 10<sup>-5</sup> M are considered to be active on the respective cell lines. Among them, compounds **12a**, **12c**, **12d**, **12f**, **12g** and **12i** were showed potent anticancer activity in all the tested cancer cell lines, with GI<sub>50</sub> values between 0.12-89.4 µM, respectively.

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