

# **Synthesis and biological evaluation of amidinourea derivatives against Herpes Simplex Viruses.**

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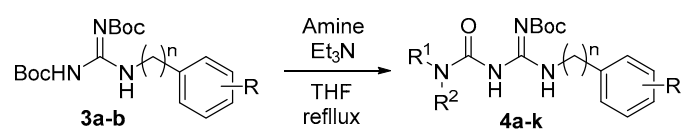
## **SUPPORTING INFORMATION**

## General Procedure for the synthesis of guanidines **3a-b**

Guanidines **3a-b** have been synthesised as previously described in literature.<sup>1</sup>

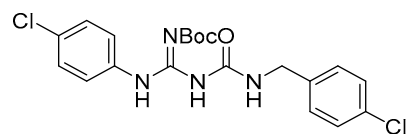
Into a round bottom flask, the 1,3-di-Boc-2-trifluoromethylsulfonyl guanidine (1 mmol) was dissolved in DCM (5 mL). The appropriate aniline (1 mmol) and Et<sub>3</sub>N (2 mmol) were added and the resulting mixture was stirred at room temperature for 1 h and then refluxed for 24-48 h. The reaction was monitored by TLC and stopped upon consumption of the starting material. mixture was diluted with DCM and washed once with HCl 0.1 N and once with brine. The organic extract was dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure to yield guanidines **3a-b** which proved to be pure enough to be used in the next steps without any further purification. <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data are identical with those described in the literature.<sup>1</sup>

## General procedure for the synthesis of Boc-amidinoureas **4a-k**



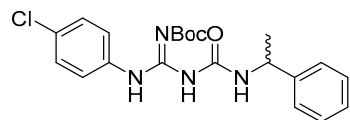
The appropriate guanidine **3a-b** (0.5 mmol) was dissolved in 5 mL of THF. The appropriate amine (1 mmol) and Et<sub>3</sub>N (2 mmol) were added to the solution and the resulting mixture was refluxed for 24-48h. The solvent was then removed under reduced pressure and the residue was purified by flash chromatography (Hexane/AcOEt 9:1) affording the desired amidinoureas **4a-k**.

### Boc-amidinourea **4a**



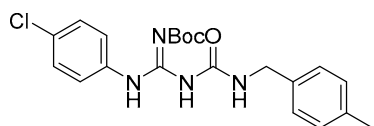
Yield: 51% (111 mg). Pale oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 12.21 (br. s., 1H, NH), 10.17 (br. s., 1H, NH), 7.46 - 7.51 (m, 2H, *Phenyl*), 7.20 - 7.29 (m, 6H, *Phenyl*), 5.64 (br. s., 1H, NH), 4.35 (s, 2H, CH<sub>2</sub>), 1.49 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ 153.4 (C=O), 151.9 (C=N), 137.4, 133.0, 128.8, 128.7, 128.6, 128.5, 128.1, 123.5, 123.4, 83.4 (C(CH<sub>3</sub>)<sub>3</sub>), 43.4 (CH<sub>2</sub>), 28.0 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. LRMS *m/z* (ES<sup>+</sup>) *m/z*: 459 [M+Na]<sup>+</sup>.

### Boc-amidinourea **4b**



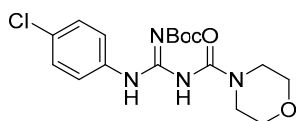
Yield: 38% (79 mg). oil. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 12.19 (br. s., 1H, NH), 10.09 (br. s., 1H, NH), 7.42 - 7.46 (m, 2H, *Phenyl*), 7.27 (s, 3H, *Phenyl*), 7.16 - 7.22 (m, 4H, *Phenyl*), 4.87 (m, 1H, CH), 1.40 - 1.43 (m, 12H, CH<sub>3</sub> + C(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>): δ 163.4 (C=O), 153.4 (C=O), 151.8 (C=N), 143.9, 135.8, 129.4, 128.7, 128.6, 127.1, 125.9, 125.5, 123.3, 83.2 (C(CH<sub>3</sub>)<sub>3</sub>), 49.7 (CH), 28.0 (C(CH<sub>3</sub>)<sub>3</sub>), 22.5 (CH<sub>3</sub>) ppm. LRMS *m/z* (ES<sup>+</sup>) *m/z*: 439 [M+Na]<sup>+</sup>.

### Boc-amidinourea 4c



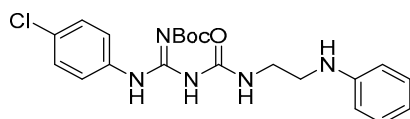
Yield: 44% (91.5 mg). Pale oil.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.30 (br. s., 1H, NH), 10.17 (br. s., 1H, NH), 7.51 (d,  $J = 8.80$  Hz, 2 H, *Cl-Phenyl*), 7.24 (s, 2H, *Phenyl*), 7.18 - 7.22 (m, 2H, *Phenyl*), 7.12 - 7.16 (m, 2H, *Phenyl*), 5.59 (br. s., 1H), 4.37 (s, 2H,  $\text{CH}_2$ ), 2.33 (s, 3H,  $\text{CH}_3$ ), 1.51 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  153.4 (C=O), 151.7 (C=N), 137.0, 135.6, 129.3, 128.7, 127.6, 126.8, 123.2, 83.3 ( $\text{C}(\text{CH}_3)_3$ ), 43.9 ( $\text{CH}_2$ ), 28.0 ( $\text{C}(\text{CH}_3)_3$ ), 21.1 ( $\text{CH}_3$ ) ppm. LRMS  $m/z$  (ES+)  $m/z$ : 439  $[\text{M}+\text{Na}]^+$ .

### Boc-amidinourea 4d



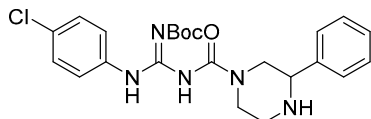
Yield: 44% (84 mg). Pale oil.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.33 (br. s., 1H, NH), 10.14 (br. s., 1H, NH), 7.42 (d,  $J = 8.80$  Hz, 2H, *Cl-Phenyl*), 7.22 - 7.27 (d,  $J = 8.80$  Hz, 2H, *Cl-Phenyl*), 3.50 - 3.68 (m, 8H,  $4x\text{CH}_2$  morpholine), 1.45 - 1.49 (m, 9H,  $\text{C}(\text{CH}_3)_3$ ) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  162.7 (C=O), 153.5 (C=O), 152.0 (C=N), 135.8, 129.6, 128.7, 123.5, 83.3 ( $\text{C}(\text{CH}_3)_3$ ), 66.9 ( $\text{CH}_2$  morpholine), 66.8 ( $\text{CH}_2$  morpholine), 45.1 ( $\text{CH}_2$  morpholine), 42.7 ( $\text{CH}_2$  morpholine), 28.0 ( $\text{C}(\text{CH}_3)_3$ ) ppm. LRMS  $m/z$  (ES+)  $m/z$ : 405  $[\text{M}+\text{Na}]^+$ .

### Boc-amidinourea 4e



Yield: 51% (110 mg). Dark oil.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.15 (br. s., 1H, NH), 10.17 (br. s., 1H, NH), 7.49 (d,  $J = 7.98$  Hz, 2H, *Cl-Phenyl*), 7.28 (s, 2H, *Phenyl*), 7.14 - 7.22 (m, 2H, *Phenyl*), 6.72 (t,  $J = 7.34$  Hz, 1H, *Phenyl*), 6.58 - 6.66 (m, 2H, *Phenyl*), 5.59 (br. s., 1H, NH), 3.46 (m, 2H,  $\text{CH}_2\text{NHC=O}$ ), 3.27 - 3.33 (m, 2H,  $\text{CH}_2\text{NHPh}$ ), 1.53 (s, 9H,  $\text{C}(\text{CH}_3)_3$ ) ppm.  $^{13}\text{C NMR}$  (101 MHz,  $\text{CDCl}_3$ ):  $\delta$  153.4 (C=O), 151.8 (C=O), 147.8 (C=N), 135.6, 129.7, 129.3, 128.8, 123.7, 123.5, 117.7, 112.8, 83.5 ( $\text{C}(\text{CH}_3)_3$ ), 44.1 ( $\text{CH}_2\text{NHC=O}$ ), 39.6 ( $\text{CH}_2\text{NHPh}$ ), 28.0 ( $\text{C}(\text{CH}_3)_3$ ) ppm. LRMS  $m/z$  (ES+)  $m/z$ : 454  $[\text{M}+\text{Na}]^+$ .

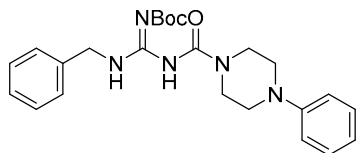
### Boc-amidinourea 4f



Yield: 65% (148.5 mg). Pale oil.  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ ):  $\delta$  12.47 (d,  $J = 18.43$  Hz, 1H, NH), 10.16 (d,  $J = 18.43$  Hz, 1H, NH), 7.41 - 7.52 (m, 3H, *Phenyl*), 7.27 - 7.40 (m, 5H, *Phenyl*), 7.19 (m, 1H, *Phenyl*), 4.62 (br. s., 1H, NH), 4.35 - 4.52 (m, 1H, *CH-Ph*), 3.71 (dt,  $J = 10.43, 3.54$  Hz, 1H, *CH-piperazine*), 3.14 (d,  $J = 9.63$  Hz, 1H, *CH-piperazine*), 2.99 - 3.10 (m, 1H, *CH-piperazine*), 2.83 - 2.96 (m, 2H,  $\text{CH}_2$ -piperazine), 2.68 - 2.83 (m, 1H, *CH-piperazine*), 1.51 (d,  $J = 1.74$  Hz, 9H,  $\text{C}(\text{CH}_3)_3$ ) ppm.

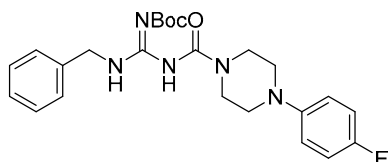
**<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>): δ 162.5 (C=O), 162.4 (C=O), 153.5 (C=N), 151.9, 141.4, 135.9, 129.5, 128.7, 128.6, 127.7, 126.5, 123.6, 123.4, 83.2 (C(CH<sub>3</sub>)<sub>3</sub>), 60.4 (CH-Ph), 60.3 (CH<sub>2</sub>), 46.3 (CH<sub>2</sub>), 42.6 (CH<sub>2</sub>), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 480 [M+Na]<sup>+</sup>.

**Boc-amidinourea 4g<sup>1</sup>**



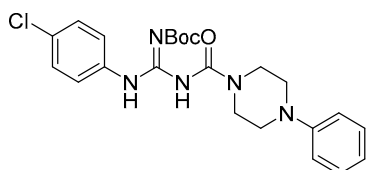
Yield 67% (146 mg). Oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 12.31 (br. s., 1H, NH), 8.48 (br. s., 1H, NH), 7.34-7.25 (m, 7H, *Phenyl*), 6.93-6.86 (m, 3H, *Phenyl*), 4.54 (m, 2H, CH<sub>2</sub>Ph), 3.86 (m, 2H, CH<sub>2</sub>), 3.72 (m, 2H, CH<sub>2</sub>), 3.14 (m, 2H, CH<sub>2</sub>), 3.05 (m, 2H, CH<sub>2</sub>), 1.47 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 162.8 (C=O), 154.5 (C=O), 153.1 (C=N), 151.4, 138.3, 129.2, 128.7, 127.5, 127.4, 120.1, 116.5, 82.5 (C(CH<sub>3</sub>)<sub>3</sub>), 49.6 (2xCH<sub>2</sub>), 49.5 (2xCH<sub>2</sub>), 44.8 (CH<sub>2</sub>Ph), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 460 [M+Na]<sup>+</sup>.

**Boc-amidinourea 4h<sup>1</sup>**



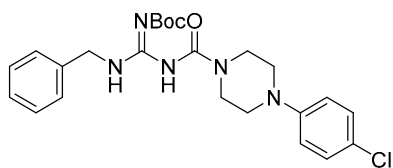
Yield 62% (141 mg). Oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 8.46 (br, 1H, NH), 7.33-7.25 (m, 5H, *Phenyl*), 6.97-6.93 (m, 2H, *Phenyl*), 6.87-6.84 (m, 2H, *Phenyl*), 4.55 (d, 2H, *J* = 5.50 Hz, CH<sub>2</sub>Ph), 3.84 (m, 2H, CH<sub>2</sub>-piperazine), 3.70 (m, 2H, CH<sub>2</sub>-piperazine), 3.03 (m, 2H, CH<sub>2</sub>-piperazine), 2.95 (m, 2H, CH<sub>2</sub>-piperazine), 1.45 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 162.8 (C=O), 154.5 (C=O), 153.4 (C=N), 148.1, 138.3, 128.7, 127.5, 127.4, 118.5, 118.4, 115.7, 115.4, 82.6 (C(CH<sub>3</sub>)<sub>3</sub>), 50.6 (2xCH<sub>2</sub>), 44.8 (CH<sub>2</sub>), 44.7 (CH<sub>2</sub>), 42.1 (CH<sub>2</sub>Ph), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 478 [M+Na]<sup>+</sup>.

**Boc-amidinourea 4i<sup>1</sup>**



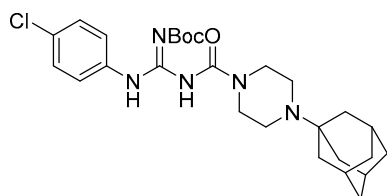
Yield 65% (148 mg). Oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 7.52-7.45 (m, 3H, *Phenyl*), 7.29-7.23 (m, 4H, *Phenyl*), 6.92-6.90 (m, 2H, *Phenyl*), 3.85 (m, 2H, CH<sub>2</sub>), 3.73 (m, 2H, CH<sub>2</sub>), 3.14 (m, 2H, CH<sub>2</sub>), 3.10 (m, 2H, CH<sub>2</sub>), 1.49 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 162.6 (C=O), 153.6 (C=O), 152.0 (C=N), 151.2, 135.9, 129.6, 129.2, 128.7, 123.6, 120.2, 116.6, 83.3 (C(CH<sub>3</sub>)<sub>3</sub>), 49.7 (CH<sub>2</sub>), 49.5 (CH<sub>2</sub>), 44.7 (CH<sub>2</sub>), 42.3 (CH<sub>2</sub>), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 480 [M+Na]<sup>+</sup>.

### Boc-amidinourea **4j**<sup>1</sup>



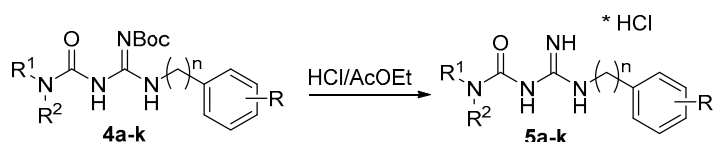
Yield 68% (160 mg). Oil. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>) δ 8.48 (br, 1H, NH), 7.34-7.25 (m, 5H, *Phenyl*), 7.19 (d, 2H, *J* = 8.0 Hz, *Phenyl*), 6.81 (d, 2H, *J* = 8.0 Hz, *Phenyl*), 4.55 (d, 2H, *J* = 5.5 Hz, CH<sub>2</sub>Ph), 3.83 (m, 2H, CH<sub>2</sub>-piperazine), 3.69 (m, 2H, CH<sub>2</sub>-piperazine), 3.08 (m, 2H, CH<sub>2</sub>-piperazine), 2.99 (m, 2H, CH<sub>2</sub>-piperazine), 1.45 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (100 MHz CDCl<sub>3</sub>) δ 162.6 (C=O), 153.5 (C=O), 152.5 (C=N), 152.2, 135.6, 129.1, 128.8, 128.3, 123.7, 119.2, 114.1, 110.5, 83.4 (C(CH<sub>3</sub>)<sub>3</sub>), 48.6 (CH<sub>2</sub>), 48.4 (CH<sub>2</sub>), 44.2 (CH<sub>2</sub>), 41.8 (CH<sub>2</sub>), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. LRMS *m/z* (ES+) *m/z*: 494 [M+Na]<sup>+</sup>.

### Boc-amidinourea **4k**<sup>1</sup>



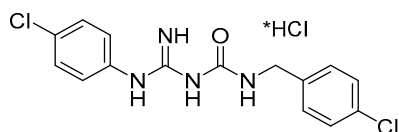
Yield 58% (149 mg). Oil. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>) δ 7.46 (d, 2H, *J* = 8.0 Hz, *Phenyl*), 7.26 (d, 2H, *J* = 8.0 Hz, *Phenyl*), 3.67 (m, 2H, CH<sub>2</sub>), 3.55 (m, 2H, CH<sub>2</sub>), 2.60-2.55 (m, 4H, 2xCH<sub>2</sub>), 2.08 (m, 2H, Adamantyl), 1.66-1.55 (m, 13H, Adamantyl), 1.47 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>) ppm. <sup>13</sup>C NMR (100 MHz CDCl<sub>3</sub>) δ 162.4 (C=O), 153.6 (C=O), 151.7 (C=N), 136.0, 129.3, 128.6, 123.4, 83.1 (C(CH<sub>3</sub>)<sub>3</sub>), 54.2 (CH<sub>2</sub>), 45.5 (CH<sub>2</sub>), 44.5 (Adamantyl), 44.2 (Adamantyl), 43.1 (Adamantyl), 38.5 (Adamantyl), 36.9 (Adamantyl), 29.6 (Adamantyl), 28.1 (C(CH<sub>3</sub>)<sub>3</sub>) ppm. LRMS *m/z* (ES+) *m/z*: 539 [M+Na]<sup>+</sup>.

### General procedure for the synthesis of amidinoureas **5a-k**



The appropriate amidinourea **4a-k** (0.2 mmol) was placed in a vial. 3 mL of a freshly prepared HCl/AcOEt solution was then added. The mixture was stirred for 48 h. The solvent was then removed under reduced pressure and the product washed several times with cold Et<sub>2</sub>O. The desired amidinoureas **5a-k** were obtained as oil HCl salts in quantitative yields.

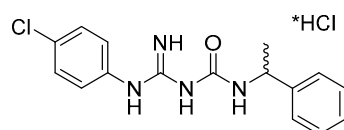
### Amidinourea **5a**



Obtained 73 mg. Oil. <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>): δ 10.87 (br. s., 1H, NH), 10.55 (br. s., 1H, NH), 8.97 (br. s., 1H, NH), 7.50 – 7.56 (m, 2H, *Phenyl*), 7.29 – 7.44 (m, 6H, *Phenyl*), 4.32 (s, 2H, CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-d<sub>6</sub>): δ 156.5 (C=N), 154.0 (C=O), 138.1, 133.2, 132.5, 132.1, 130.2,

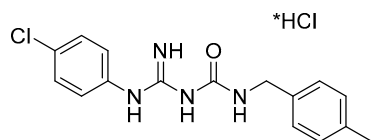
129.6, 128.8, 128.3, 42.6 (CH<sub>2</sub>) ppm. **LRMS** *m/z* (ES+) *m/z*: 337 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>15</sub>H<sub>15</sub>N<sub>4</sub>Cl<sub>2</sub>O<sup>+</sup> (M + H<sup>+</sup>) 337.0617, found 337.0614.

#### Amidinourea 5b



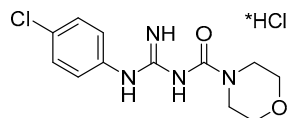
The reaction was carried out on 70 mg (0.17 mmol) of **4b**. Obtained 58 mg. Pale oil. **<sup>1</sup>H NMR** (400 MHz, CD<sub>3</sub>OD): δ 7.52 (m, 2H, *Phenyl*), 7.33 – 7.37 (m, 7H, *Phenyl*), 4.92 (m, 1H, *CH*), 1.50 (d, 3H, *CH*<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, CD<sub>3</sub>OD): δ 154.3 (C=N), 152.6 (C=O), 143.0, 134.2, 131.7, 130.0, 128.3, 127.9, 125.6, 49.8 (*CH*), 21.5 (*CH*<sub>3</sub>) ppm. **LRMS** *m/z* (ES+) *m/z*: 317 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 317.1164, found 317.1168.

#### Amidinourea 5c



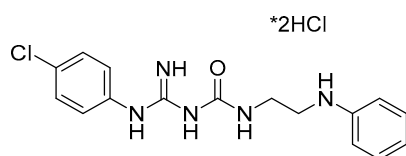
Obtained 69 mg. Oil. **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 7.51 – 7.56 (m, 2H, *Phenyl*), 7.35 – 7.39 (m, 2H, *Phenyl*), 7.12 – 7.21 (m, 4H, *Phenyl*), 4.27 (s, 2H, *CH*<sub>2</sub>), 2.28 (s, 3H, *CH*<sub>3</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 154.1 (C=N), 152.3 (C=O), 136.7, 135.8, 130.2, 129.4, 128.2, 127.8, 43.0 (*CH*<sub>2</sub>), 21.1 (*CH*<sub>3</sub>) ppm. **LRMS** *m/z* (ES+) *m/z*: 317 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>16</sub>H<sub>18</sub>N<sub>4</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 317.1164, found 317.1165.

#### Amidinourea 5d



Obtained 62 mg. Oil. **<sup>1</sup>H NMR** (400 MHz, CDCl<sub>3</sub>): δ 11.74 (br. s., 1H, *NH*), 9.90 (br. s., 1H, *NH*), 7.47 (m, 2H, *Phenyl*), 7.24 (m, 2H, *Phenyl*), 3.98-3.58 (m, 8H, *Morpholine 4xCH*<sub>2</sub>) ppm. **<sup>13</sup>C NMR** (101 MHz, CDCl<sub>3</sub>): δ 155.8 (C=N), 153.1 (C=O), 130.9, 130.4, 127.4, 66.4 (2x*CH*<sub>2</sub>), 31.5 (2x*CH*<sub>2</sub>) ppm. **LRMS** *m/z* (ES+) *m/z*: 283 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>12</sub>H<sub>16</sub>N<sub>4</sub>ClO<sub>2</sub><sup>+</sup> (M + H<sup>+</sup>) 283.0956, found 283.0958.

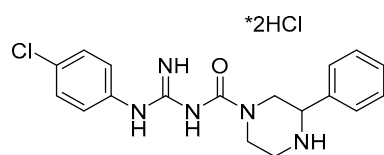
#### Amidinourea 5e



Obtained 79 mg. Dark oil. **<sup>1</sup>H NMR** (400 MHz, DMSO-*d*<sub>6</sub>): δ 10.94 (br. s., 1H, *NH*), 10.51 (br. s., 1H, *NH*), 8.08 (br. s., 1H, *NH*), 7.54 (d, 2H, *J* = 8.5 Hz, *Phenyl-Cl*), 7.29 – 7.39 (m, 5H, *Phenyl*), 7.11 – 7.18 (m, 2H, *Phenyl*), 4.14 (br. s., 1H, *NH-Ph*), 3.42 (m, 2H, (C=O)*NHCH*<sub>2</sub>), 3.29 (m, 2H, *CH*<sub>2</sub>*NHPh*) ppm. **<sup>13</sup>C NMR** (101 MHz, DMSO-*d*<sub>6</sub>): δ 154.0 (C=N), 153.3 (C=O), 133.1, 132.6, 130.2, 129.9, 128.2,

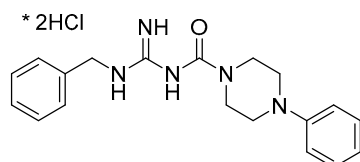
120.8, 113.2, 43.6 (CH<sub>2</sub>), 38.4 (CH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 332 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>16</sub>H<sub>19</sub>N<sub>5</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 332.1273, found 332.1270.

#### Amidinourea 5f



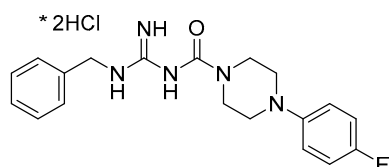
Obtained 84 mg. Oil. **<sup>1</sup>H NMR** (400 MHz, DMSO-d<sub>6</sub>): δ 9.92 (br. s., 1H, NH), 7.70 (d, 2H, *J* = 6.5 Hz, *Phenyl-Cl*), 7.44 – 7.56 (m, 5H, *Phenyl*), 7.37 (m, 2H, *Phenyl*), 4.50 (m, 1H, *CH*-piperazine), 3.49 – 3.60 (m, 6H, 3*x*CH<sub>2</sub>-piperazine) ppm. **<sup>13</sup>C NMR** (101 MHz, DMSO-d<sub>6</sub>): δ 155.2 (C=N), 154.6 (C=O), 144.5, 137.6, 134.4, 130.1, 129.3, 128.3, 126.6, 123.5, 123.0, 58.4 (CH), 58.1 (CH<sub>2</sub>), 43.5 (CH<sub>2</sub>), 40.1 (CH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 358 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>18</sub>H<sub>21</sub>N<sub>5</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 358.1429, found 358.1427.

#### Amidinourea 5g<sup>1</sup>



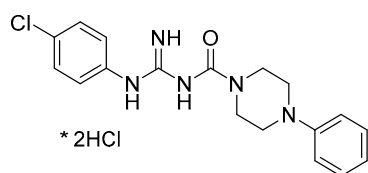
Obtained 80 mg. Yellow oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 10.23 45 (br, 1H, NH), 9.98 (br, 1H, NH), 8.06 (br, 1H, NH), 7.30-7.24 (m, 10H, *Phenyls*), 4.53 (m, 2H, CH<sub>2</sub>Ph), 3.52 (m, 4H, 2*x*CH<sub>2</sub> piperazine), 3.31 (m, 4H, 2*x*CH<sub>2</sub> piperazine) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 155.0 (C=N), 153.0 (C=O), 141.4, 134.5, 130.6, 129.1, 128.4, 127.6, 121.5, 54.8 (2*x*CH<sub>2</sub>), 45.8 (CH<sub>2</sub>), 45.7 (CH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 338. **HRMS (ESI)**: calcd for C<sub>19</sub>H<sub>24</sub>N<sub>5</sub>O<sup>+</sup> (M + H<sup>+</sup>) 338.1978, found 338.1974.

#### Amidinourea 5h<sup>1</sup>



Obtained 83 mg. Yellow oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 7.93 (br, 1H, NH), 7.34 (m, 6H, *Phenyl*), 7.15 (m, 3H, *Phenyl*), 4.61 (m, 2H, CH<sub>2</sub>Ph), 4.35 (m, 4H, 2*x*CH<sub>2</sub> piperazine), 3.60 (m, 4H, 2*x*CH<sub>2</sub> piperazine) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 155.6 (C=N), 152.9 (C=O), 140.9, 133.9, 129.4, 128.2, 127.5, 123.9, 117.8, 117.6, 55.2 (CH<sub>2</sub>), 53.5 (CH<sub>2</sub>), 45.9 (2*x*CH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 356 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>19</sub>H<sub>23</sub>FN<sub>5</sub>O<sup>+</sup> (M + H<sup>+</sup>) 356.1881, found 356.1883.

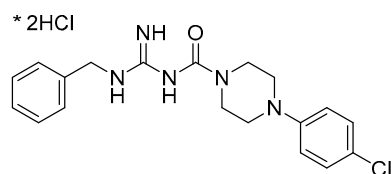
#### Amidinourea 5i<sup>1</sup>



Obtained 85 mg. Yellow oil. **<sup>1</sup>H NMR** (400 MHz CD<sub>3</sub>OD) δ 7.77-7.75 (m, 2H, *Phenyl-Cl*), 7.56-7.48 (m, 5H, *Phenyl*), 7.36-7.34 (m, 2H, *Phenyl-Cl*), 3.78-3.68 (m, 4H, 2*x*CH<sub>2</sub> piperazine), 3.25 (m, 4H, 2*x*CH<sub>2</sub> piperazine) ppm. **<sup>13</sup>C NMR** (100 MHz CD<sub>3</sub>OD) δ 155.6 (C=N), 153.2 (C=O), 142.6, 134.8,

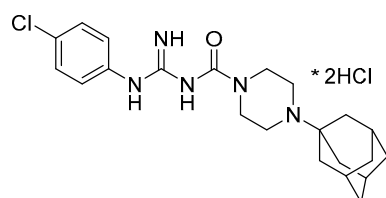
132.5, 131.0, 130.7, 130.5, 128.1, 121.7, 121.5, 54.9 (2xCH<sub>2</sub>), 49.1 (2xCH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 358 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>18</sub>H<sub>21</sub>N<sub>5</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 358.1429, found 358.1431.

### Amidinourea 5j<sup>1</sup>



Obtained 85 mg. Yellow oil. **<sup>1</sup>H NMR** (400 MHz CDCl<sub>3</sub>) δ 7.66 (br, 1H, NH), 7.34-7.25 (m, 9H, *Phenyls*), 4.57 (m, 2H, CH<sub>2</sub>Ph), 3.61-3.38 (m, 8H, 4xCH<sub>2</sub> piperazine) ppm. **<sup>13</sup>C NMR** (100 MHz CDCl<sub>3</sub>) δ 155.2 (C=N), 153.0 (C=O), 143.0, 134.3, 130.5, 129.2, 128.5, 127.5, 122.4, 53.9 (CH<sub>2</sub>), 50.6 (CH<sub>2</sub>), 45.8 (2xCH<sub>2</sub>) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 372 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>19</sub>H<sub>23</sub>N<sub>5</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 372.1586, found 372.1588.

### Amidinourea 5k<sup>1</sup>



Obtained 95 mg. Yellow oil. **<sup>1</sup>H NMR** (400 MHz DMSO-d<sub>6</sub>) δ 7.52-7.50 (m, 2H, 2xCH *Phenyl*), 7.38-7.32 (m, 2H, 2xCH *Phenyl*), 3.70-3.46 (m, 8H, 4xCH<sub>2</sub> piperazine), 2.12 (m, 3H, *Adamantyl*), 1.94 (m, 6H, *Adamantyl*), 1.59 (m, 6H, *Adamantyl*) ppm. **<sup>13</sup>C NMR** (100 MHz DMSO-d<sub>6</sub>) δ 155.0 (C=N), 151.9 (C=O), 136.4, 130.3, 129.0, 128.8, 124.8, 64.2 (2xCH<sub>2</sub> piperazine), 63.9 (2xCH<sub>2</sub> piperazine), 44.1 (C-N-adamantyl), 35.8 (3xCH<sub>2</sub>-adamantyl), 35.6 (3xCH<sub>2</sub>-adamantyl), 29.4 (3xC-adamantyl) ppm. **LRMS** *m/z* (ES<sup>+</sup>) *m/z*: 416 [M+H]<sup>+</sup>. **HRMS (ESI)**: calcd for C<sub>22</sub>H<sub>31</sub>N<sub>5</sub>ClO<sup>+</sup> (M + H<sup>+</sup>) 416.2212, found 416.2210.

### Purity check

All the compounds were analysed by LC-MS prior submission for the biological assays and proved to be pure at >95%. The LC-MS analysis was carried out using the following conditions: flow 1.3 mL/min; eluent H<sub>2</sub>O/CH<sub>3</sub>CN 70:30; column, Thermo Scientific Acclaim 120 C18 Columns, 100mm L x 4.6mm ID, 5.0μm.

Compound	Retention time	Compound	Retention time
<b>5a</b>	7.29 min <sup>a</sup>	<b>5g</b>	15.7 min
<b>5b</b>	20.5 min	<b>5h</b>	18.3 min
<b>5c</b>	24.2 min	<b>5i</b>	8.4 min <sup>a</sup>
<b>5d</b>	3.8 min	<b>5j</b>	9.9 min <sup>a</sup>
<b>5e</b>	15.3 min	<b>5k</b>	21.6 min
<b>5f</b>	6.1 min		

<sup>a</sup>Eluent used is H<sub>2</sub>O/CH<sub>3</sub>CN 60:40



## References

1. A. Magri, R. Reilly, N. Scalacci, M. Radi, M. Hunter, M. Ripoll, A. H. Patel, D. Castagnolo, *Bioorg. Med. Chem. Lett.* **2015**, 25, 5372-5376.