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Citation: Cellier, Marie, Gignoux, Amandine, James, Arthur, Orenga, Sylvain, Perry, John, Robinson, Shaun, Stanforth, Stephen and Turnbull, Graeme (2015) 2-(Nitroaryl)benzothiazole and benzoxazole derivatives as fluorogenic substrates for the detection of nitroreductase activity in clinically important microorganisms. *Bioorganic & Medicinal Chemistry Letters*, 25 (24). pp. 5694-5698. ISSN 0960-894X

Published by: Elsevier

URL: <http://dx.doi.org/10.1016/j.bmcl.2015.10.099>  
<<http://dx.doi.org/10.1016/j.bmcl.2015.10.099>>

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2-(Nitroaryl)benzothiazole and benzoxazole derivatives as fluorogenic substrates for the detection of nitroreductase activity in microorganisms

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### Supplementary information

#### Synthetic work

<sup>1</sup>H-NMR spectra (400 MHz) and <sup>13</sup>C-NMR spectra (101 MHz) were recorded on a Jeol ECS400 instrument. High resolution mass spectrometry (HRMS) was performed by the EPSRC mass spectrometry service. Infrared spectra were obtained *via* a diamond anvil sample cell using a Perkin Elmer 1000 spectrometer. Melting points are reported uncorrected as determined on a Stuart SMP 1 melting point apparatus. Thin layer chromatography was performed on Merck plastic foil plates pre-coated with silica gel 60 F<sub>254</sub>. Merck silica gel 60 was used for column chromatography. Compound **6a** was prepared following a literature procedure (ref. 13 in text). The synthesis of compounds **3c** and **4c** have previously been described by us (ref. 11 in text).

#### General method for the synthesis of substrates **7a-d**.

A mixture of 3-amino-4-hydroxybenzoic acid or 4-amino-3-hydroxybenzoic acid (1.0 equiv) and an appropriate 2-nitrobenzaldehyde derivative (1.0 equiv) was heated in EtOH at reflux for 1 h. The reaction mixture was allowed to cool to room temperature and the resulting precipitate (compound **12**) was collected, washed with water, dried under vacuum overnight and used directly in the next step. The precipitate and DDQ (1.0 equiv) in anhydrous 1,4-dioxane was stirred at room temperature. The reaction mixture was then filtered and the filtrate was evaporated giving the substrate **7**.

#### *2-(2-Nitrophenyl)benzoxazole-6-carboxylic acid (7a).*

4-Amino-3-hydroxybenzoic acid (0.30 g, 1.96 mmol) and 2-nitrobenzaldehyde (0.30 g, 1.96 mmol) in ethanol (30 mL) at reflux for 16 hours gave compound **12a** which was reacted with DDQ (0.44 g, 1.96 mmol) in 1,4-dioxane (50 mL) for 16 h. Compound **7a** was obtained as a brown solid (0.53 g, 1.86 mmol, 95%), m.p. 252-254 °C; HRMS found M+H: 285.0510. Calcd. for C<sub>14</sub>H<sub>9</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup>, M+H: 285.0506; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 3000 (OH), 1688 (C=O), 1530, 1417, 1294, 1276; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.32 (1H, d, *J* = 1.6 Hz, C7-*H*), 8.25 (1H, m, Ar-*H*), 8.18 (1H, m, Ar-*H*), 8.07 (1H, dd, *J* = 8.2 and 1.6 Hz, C5-*H*), 7.96 (3H, m, 3 x Ar-*H*); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  167.2 (C=O), 161.1 (C), 150.6 (C), 149.2 (C), 145.0 (C), 134.0 (CH), 133.9 (CH), 132.0 (CH), 129.4 (C), 127.0 (CH), 125.1 (CH), 120.7 (CH), 120.0 (C), 112.9 (CH).

*2-(2-Nitrophenyl)benzoxazole-5- carboxylic acid (7b).*

3-Amino-4-hydroxybenzoic acid (1.00 g, 6.53 mmol) and 2-nitrobenzaldehyde (1.09 g, 7.18 mmol) in ethanol (50 mL) at reflux for 16 hours gave compound **12b** which was reacted with DDQ (0.95 g, 4.19 mmol) in 1,4-dioxane (50 mL) for 62 h. Compound **7b** was obtained as a brown solid (0.53 g, 1.86 mmol, 53%), m.p. 240-243 °C; HRMS found M+H: 285.0509.

Calcd. for C<sub>14</sub>H<sub>9</sub>N<sub>2</sub>O<sub>5</sub><sup>+</sup>, M+H: 285.0506; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 3108 (OH), 1672 (C=O), 1534, 1554, 1268, 1172; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.37 (1H, broad s, C4-H), 8.24 (1H, d, *J* = 7.3 Hz, Ar-H), 8.18 (1H, d, *J* = 7.3 Hz, Ar-H), 8.11 (1H, d, *J* = 8.7 Hz, Ar-H), 7.95 (3H, m, 3 x Ar-H); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  167.3 (C=O), 160.3 (C), 153.5 (C), 149.1 (C), 141.6 (C), 133.9 (2 x 2CH), 132.0 (CH), 128.8 (C), 128.3 (CH), 125.2 (CH), 122.1 (CH), 120.1 (C), 111.9 (CH).

*2-(5-Fluoro-2-nitrophenyl)benzoxazole-6- carboxylic acid (7c).*

4-Amino-3-hydroxybenzoic acid (0.20 g, 1.31 mmol) and 5-fluoro-2-nitrobenzaldehyde (0.22 g, 1.31 mmol) in ethanol (30 mL) at reflux for 16 hours gave compound **12c** which was reacted with DDQ (0.30 g, 1.31 mmol) in 1,4-dioxane (50 mL) for 16 hours. Compound **7c** was obtained as a brown solid (0.04 g, 0.15 mmol, 11%), m.p. 236-238 °C after purification by column chromatography over silica gel (eluent: ethyl acetate); HRMS found M+H:

303.0407. Calcd. for C<sub>14</sub>H<sub>8</sub>FN<sub>2</sub>O<sub>5</sub><sup>+</sup>, M+H: 303.0412; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 3000 (OH), 1679 (C=O), 1541, 1496, 1268, 1216; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.31 (2H, m, 2 x Ar-H), 8.11 (1H, dd, *J* = 8.2 Hz and 2.8 Hz, Ar-H), 8.07 (1H, dd, *J* = 8.2 Hz and 1.4 Hz, Ar-H), 7.98 (1H, d, *J* = 8.2 Hz, Ar-H), 7.83 (1H, m, Ar-H); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  167.1 (C=O), 165.1 (d, *J* = 254.0 Hz, CF), 160.3 (C), 150.6 (C), 145.6 (C), 144.8 (C), 129.7 (C), 128.5 (d, *J* = 9.6 Hz, CH), 127.1 (CH), 123.2 (d, *J* = 10.5 Hz, C), 120.9 (CH), 120.7 (CH), 119.4 (d, *J* = 26.8 Hz, CH), 112.9 (CH).

*2-(5-Fluoro-2-nitrophenyl)benzoxazole-5- carboxylic acid (7d).*

Compound **7d** (95%) was prepared in a similar manner to compound **7c** and was obtained as a yellow solid m.p. 217-220 °C. HRMS found M+H: 303.0416. Calcd. for C<sub>14</sub>H<sub>8</sub>FN<sub>2</sub>O<sub>5</sub><sup>+</sup>, M+H: 303.0412; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 1683 (C=O), 1537, 1292; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.32 (1H, d, *J* = 1.6 Hz, C4-H), 8.26 (1H, dd, *J* = 8.7 and 4.6 Hz, Ar-H), 8.07 (1H, dd, *J* = 8.7 and 1.6 Hz, C6-H), 8.04 (1H, dd, *J* = 8.7 and 2.9 Hz, Ar-H), 7.89 (1H, d, *J* = 8.7 Hz, C7-H), 7.76 (1H, qd, *J* = 7.8 and 2.9 Hz, Ar-H); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  167.2 (C=O), 163.8 (d, *J* = 254.0 Hz, CF), 159.2 (C), 153.5 (C), 145.5 (d, *J* = 2.9 Hz, C), 141.4 (C), 128.9 (C), 128.5 (CH), 128.4 (CH), 123.1 (d, *J* = 10.5 Hz, C) 122.2 (CH), 120.7 (d, *J* = 23.0 Hz, CH), 119.3 (d, *J* = 26.8 Hz, CH), 112.0 (CH).

**Potassium salt of carboxylic acid 7a.**

*Potassium 2-(2-nitrophenyl)benzoxazole-6-carboxylate*

To compound **7a** (98 mg, 0.35 mmol) was added a solution of methanolic KOH solution (34 mM, 10.3 mL, 0.35 mmol) at room temperature with stirring. The solvent was evaporated to yield the potassium salt (106 mg, 94%) as a dark brown solid;  $\delta_{\text{H}}$  (400 MHz, D<sub>2</sub>O) 7.61-7.57 (2H, m, 2 x Ar-H), 7.55-7.48 (2H, m, 2 x Ar-H), 7.37 (2H, m, 2 x Ar-H), 7.28 (1H, dd,  $J = 8.2$  and 1.8 Hz, C7-H).

### General procedure for the preparation of the benzothiazole derivatives **9a-9c**.

Compound **3c** (1 equiv), an appropriate amine (1.1 equiv) and NaHCO<sub>3</sub> (2.5 equiv) were added to THF and H<sub>2</sub>O (50 mL, 1:1) and the mixture was heated under reflux for 16 h. The reaction was allowed to cool and the THF was evaporated. The remaining solution was then acidified to pH 1-2 with 2M aqueous HCl. The resulting precipitate was collected giving the desired compound.

#### *(2R)-1-[3-(1,3-Benzothiazol-2-yl)-4-nitrophenyl]pyrrolidine-2-carboxylic acid (9a).*

Compound **9a** was prepared from compound **3c** (0.10 g, 0.37 mmol), L-proline (0.05 g, 0.40 mmol) and NaHCO<sub>3</sub> (0.08 g, 0.91 mmol). Compound **9a** was obtained as a yellow solid (0.13 g, 0.35 mmol, 95%), m.p. decomposes from 132 °C. HRMS found M+H: 370.0857. Calcd for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>4</sub>S<sup>+</sup>, M+H: 370.0856; IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3000 (OH), 1728 (C=O), 1594, 1504, 1309; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.15 (1H, d,  $J = 7.3$  Hz, Ar-H), 8.07 (1H, d,  $J = 9.2$  Hz, Ar-H), 8.01 (1H, d,  $J = 7.8$  Hz, Ar-H), 7.54 (1H, td,  $J = 6.9$  and 1.4 Hz, Ar-H), 7.48 (1H, td,  $J = 6.9$  and 1.4 Hz, Ar-H), 6.67 (2H, broad s, 2 x Ar-H), 4.49 (1H, dd,  $J = 8.7$  and 2.3 Hz, N-CH), 3.54 (1H, m, N-CH), 3.43 (1H, m, N-CH), 2.26 (1H, m, CH), 2.11 (1H, m, CH), 1.97 (2H, m, 2 x CH); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  173.9 (C=O), 165.2 (C), 153.3 (C), 150.5 (C), 136.2 (C), 136.0 (C), 131.5 (C), 128.3 (CH), 127.1 (CH), 126.2 (CH), 123.6 (CH), 122.8 (CH), 114.6 (CH), 113.1 (CH), 60.9 (N-CH), 49.0 (N-CH<sub>2</sub>), 30.7 (CH<sub>2</sub>), 23.9 (CH<sub>2</sub>).

#### *(2S)-1-[3-(1,3-Benzothiazol-2-yl)-4-nitrophenyl]pyrrolidine-2-carboxylic acid (9b).*

Compound **9b** was prepared from compound **3c** (0.10 g, 0.37 mmol), D-proline (0.05 g, 0.40 mmol) and NaHCO<sub>3</sub> (0.08 g, 0.91 mmol). Compound **9b** was obtained as a yellow solid (0.11 g, 0.30 mmol, 80%), m.p. decomposes from 132 °C. HRMS found M+H: 370.0850. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>4</sub>S<sup>+</sup>, M+H: 370.0856; IR  $\nu_{\text{max}}$  (cm<sup>-1</sup>): 3338 (OH), 1730 (C=O), 1596, 1500, 1309; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.19 (1H, d,  $J = 7.3$  Hz, Ar-H), 8.11 (1H, d,  $J = 9.6$  Hz, Ar-H), 8.05 (1H, d,  $J = 7.8$  Hz, Ar-H), 7.58 (1H, td,  $J = 7.8$  Hz and 1.4 Hz, Ar-H), 7.52 (1H, td,  $J = 7.8$  Hz and 1.4 Hz, Ar-H), 6.71 (2H, broad s, 2 x Ar-H), 4.53 (1H, dd,  $J = 8.7$  and 2.3 Hz, N-CH), 3.60 (2H, m, 2 x N-CH), 2.30 (1H, m, CH), 2.15 (1H, m, CH), 2.00 (2H, m, 2 x CH); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  173.9 (C=O), 165.2 (C), 153.3 (C), 150.5 (C), 136.2 (C), 136.0 (C), 131.5 (C), 128.3 (CH), 127.1 (CH), 126.2 (CH), 123.6 (CH), 122.8 (CH), 114.6 (CH), 113.1 (CH), 60.9 (N-CH), 49.0 (N-CH<sub>2</sub>), 30.7 (CH<sub>2</sub>), 23.8 (CH<sub>2</sub>).

*4-[[3-(1,3-Benzothiazol-2-yl)-4-nitrophenyl](methyl)amino]butanoic acid (9c).*

Compound **9c** was prepared from compound **3c** (0.10 g, 0.37 mmol), *N*-methylaminobutyric acid hydrochloride (0.05 g, 0.40 mmol) and NaHCO<sub>3</sub> (0.08 g, 0.91 mmol). Compound **9c** was obtained as a yellow solid (0.11 g, 0.29 mmol, 79%), m.p. 163-166 °C. HRMS found M+H: 372.1014. Calcd for C<sub>18</sub>H<sub>18</sub>N<sub>3</sub>O<sub>4</sub>S<sup>+</sup>, M+H: 372.1013; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 2919 (OH), 1718 (C=O), 1597, 1493, 1302; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.19 (1H, d, *J* = 7.7 Hz, Ar-*H*), 8.09 (1H, d, *J* = 9.2 Hz, Ar-*H*), 8.06 (1H, d, *J* = 7.7 Hz, Ar-*H*), 7.58 (1H, td, *J* = 7.7 and 1.4 Hz, Ar-*H*), 7.52 (1H, td, *J* = 7.7 and 1.4 Hz, Ar-*H*), 6.97 (1H, dd, *J* = 9.2 and 2.8 Hz, Ar-*H*), 6.92 (1H, d, *J* = 2.8 Hz, C2'-*H*), 3.51 (2H, t, *J* = 7.3 Hz, N-CH<sub>2</sub>), 3.09 (3H, s, N-CH<sub>3</sub>), 2.29 (2H, t, *J* = 7.3 Hz, CH<sub>2</sub>-CO<sub>2</sub>H), 1.77 (2H, quintet, *J* = 7.3 Hz, CH<sub>2</sub>); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  174.7 (C=O), 165.6 (C), 153.3 (C), 152.5 (C), 136.1 (C), 135.4 (C), 131.8 (C), 128.4 (CH), 127.0 (CH), 126.1 (CH), 123.6 (CH), 122.8 (CH), 113.8 (CH), 112.2 (CH), 51.5 (N-CH<sub>2</sub>), 38.9 (N-CH<sub>3</sub>), 31.0 (CH<sub>2</sub>), 22.0 (CH<sub>2</sub>).

**General procedure for the preparation of the benzoxazole derivatives 10a-c.**

Compound **4c** (1 equiv), an appropriate amine (1.1 equiv) and NaHCO<sub>3</sub> (2.5 equiv) were added to EtOH and H<sub>2</sub>O (50 mL, 1:1) and the mixture was heated under reflux for 16 h. The reaction was allowed to cool and the EtOH was evaporated. The remaining solution was then acidified to pH 1-2 with 2M aqueous HCl. EtOAc was then added and the organic layer was separated. The organic layer was washed sequentially with H<sub>2</sub>O and then brine, dried (MgSO<sub>4</sub>) and evaporated yielding the product.

*(2R)-1-[3-(1,3-Benzoxazol-2-yl)-4-nitrophenyl]pyrrolidine-2-carboxylic acid (10a).*

Compound **10a** was prepared from compound **4c** (0.50 g, 1.93 mmol), L-proline (0.25 g, 2.13 mmol) and NaHCO<sub>3</sub> (0.41 g, 4.83 mmol). Compound **10a** was obtained as a yellow solid (0.49 g, 1.38 mmol, 71%), 207-209 °C. HRMS found M+H: 354.1084. Calcd. for C<sub>18</sub>H<sub>16</sub>N<sub>3</sub>O<sub>5</sub><sup>+</sup>, M+H: 354.1084; IR  $\nu_{\max}$  (cm<sup>-1</sup>): 2858 (OH), 1728 (C=O), 1586, 1287; <sup>1</sup>H-NMR (400 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{H}}$  8.17 (1H, d, *J* = 9.2 Hz, Ar-*H*), 7.86 (1H, m, Ar-*H*), 7.78 (1H, m, Ar-*H*), 7.47 (2H, m, 2 x Ar-*H*), 6.93 (1H, broad s, Ar-*H*), 6.81 (1H, broad s, Ar-*H*), 4.55 (1H, dd, *J* = 8.2 Hz and 1.8 Hz, N-CH), 3.59 (1H, m, N-CH), 3.49 (1H, m, N-CH), 2.31 (1H, m, CH), 2.17 (1H, m, CH), 2.02 (2H, m, 2 x CH); <sup>13</sup>C-NMR (101 MHz, d<sub>6</sub>-DMSO)  $\delta_{\text{C}}$  173.7 (C=O), 161.2 (C), 151.0 (C), 150.8 (C), 141.5 (C), 136.0 (C), 128.3 (CH), 126.3 (CH), 125.6 (C), 125.4 (CH), 120.6 (CH), 115.0 (CH), 113.8 (CH), 111.6 (CH), 60.9 (N-CH), 49.1 (N-CH<sub>2</sub>), 30.7 (CH<sub>2</sub>), 23.8 (CH<sub>2</sub>).

*(2S)-1-[3-(1,3-Benzoxazol-2-yl)-4-nitrophenyl]pyrrolidine-2-carboxylic acid (10b).*

Compound **10b** was prepared from compound **4c** (0.50 g, 1.93 mmol), D-proline (0.25 g, 2.13 mmol) and NaHCO<sub>3</sub> (0.41 g, 4.83 mmol). Compound **10b** was obtained as a yellow solid (0.59 g, 1.68 mmol, 87%), m.p. 207-209 °C. HRMS found M+H: 354.1082. Calcd. for

$C_{18}H_{16}N_3O_5^+$ , M+H: 354.1084; IR  $\nu_{max}$  ( $cm^{-1}$ ): 1727 (C=O), 1586, 1287;  $^1H$ -NMR (400 MHz,  $d_6$ -DMSO)  $\delta_H$  8.18 (1H, d,  $J = 9.2$  Hz, Ar-H), 7.87 (2H, m, 2 x Ar-H), 7.79 (1H, m, Ar-H), 7.48 (2H, m, 2 x Ar-H), 6.94 (1H, broad s, Ar-H), 6.80 (1H, broad s, Ar-H), 4.57 (1H, dd,  $J = 8.7$  and  $1.8$  Hz, N-CH), 3.60 (1H, m, N-CH), 3.50 (1H, m, N-CH), 2.32 (1H, m, CH), 2.18 (1H, m, CH), 2.02 (2H, m, 2 x CH);  $^{13}C$ -NMR (101 MHz,  $d_6$ -DMSO)  $\delta_C$  173.7 (C=O), 161.2 (C), 151.0 (C), 150.8 (C), 141.5 (C), 136.0 (C), 128.3 (CH), 126.3 (CH), 125.6 (C), 125.4 (CH), 120.6 (CH), 115.0 (CH), 113.8 (CH), 111.6 (CH), 60.9 (N-CH), 49.1 (N-CH<sub>2</sub>), 30.7 (CH<sub>2</sub>), 23.8 (CH<sub>2</sub>).

*4-[[3-(1,3-Benzoxazol-2-yl)-4-nitrophenyl](methyl)amino]butanoic acid (10c).*

Compound **10c** was prepared from compound **4c** (0.50 g, 1.93 mmol), *N*-methylaminobutyric acid hydrochloride (0.33 g, 2.13 mmol) and NaHCO<sub>3</sub> (0.41 g, 4.83 mmol). Compound **10c** was obtained as a yellow solid (0.58 g, 1.64 mmol, 85%), m.p. 168-170 °C. HRMS found M+H: 356.1240. Calcd. for  $C_{18}H_{18}N_3O_5^+$ , M+H: 356.1241; IR  $\nu_{max}$  ( $cm^{-1}$ ): 2940 (OH), 1727 (C=O), 1587, 1308;  $^1H$ -NMR (400 MHz,  $d_6$ -DMSO)  $\delta_H$  8.14 (1H, d,  $J = 9.2$  Hz, Ar-H), 7.85 (1H, m, Ar-H), 7.78 (1H, m, Ar-H), 7.46 (2H, m, Ar-H), 7.12 (1H, d,  $J = 2.8$  Hz, C2'-H), 7.02 (1H, dd,  $J = 9.6$  and  $2.8$  Hz, Ar-H), 3.53 (2H, t,  $J = 7.3$  Hz, NCH<sub>2</sub>), 3.10 (3H, s, CH<sub>3</sub>), 2.30 (2H, t,  $J = 7.3$  Hz, CH<sub>2</sub>-CO<sub>2</sub>H), 1.78 (2H, quintet,  $J = 7.3$  Hz, CH<sub>2</sub>);  $^{13}C$ -NMR (101 MHz,  $d_6$ -DMSO)  $\delta_C$  174.7 (C=O), 161.5 (C), 152.9 (C), 151.0 (C), 141.6 (C), 135.1 (C), 128.3 (CH), 126.2 (CH), 126.0 (CH), 125.3 (CH), 120.6 (CH), 114.2 (CH), 112.9 (CH), 111.6 (CH), 51.5 (N-CH), 39.0 (N-CH<sub>2</sub>), 31.0 (CH<sub>2</sub>), 22.1 (CH<sub>2</sub>).

## Microbiological work

### Agar plate preparation

Each substrate (10 mg) was dissolved in a minimal volume of 1-methyl-2-pyrrolidone (200-400  $\mu$ L) and added to molten Columbia agar (100 mL) (Oxoid, Basingstoke) at 50 °C to a final concentration of 100 mg L<sup>-1</sup>. Agar plates were then prepared and dried to remove excess moisture. Bacterial strains and yeasts obtained from various national culture collections (see Tables) were sub-cultured onto Columbia agar. Colonies of each strain were sampled using a loop and suspended in 0.85 % sterile saline to generate a suspension equivalent to 10<sup>8</sup> colony forming units (cfu) per mL using a densitometer. Each agar plate was then inoculated with 1  $\mu$ L of this suspension using a multipoint inoculator that delivered suspensions of 20 strains per plate. Plates were incubated at 37 °C in air for 24 h. Control plates without the substrate were prepared and inoculated concomitantly.

### Fluorescence response at varying concentrations

Compound **7a** (10 mg) was dissolved in 0.95 mL sterile deionised water and 50  $\mu$ L of 1M sodium hydroxide solution was added to produce a solution of 10000 mg/L. The following volumes were added to brain heart infusion broth (Oxoid) to give a final volume of 20 mL: 200  $\mu$ L, 100  $\mu$ L, 50  $\mu$ L, 25  $\mu$ L and 12.5  $\mu$ L. The final concentration range was 0.33 – 0.02

mmol/L. A Gram-negative isolate (*Enterobacter cloacae*) and a Gram-positive isolate (*Staphylococcus aureus*) were inoculated at a final inoculum of  $10^5$  CFU/mL and the broths were incubated overnight for 18 h at 37°C. Substrate-free and bacteria-free controls were included and tests were performed in duplicate. Fluorescence was read before and after incubation on a Synergy HT microplate reader using 365 nm excitation and 440 emission wavelengths.