

Chromogenic enzyme substrates based on [2-(nitroaryl)ethenyl]pyridinium and quinolinium derivatives for the detection of nitroreductase activity in clinically important microorganisms.

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Supplementary information

General experimental directions

NMR spectra were recorded on a Jeol spectrometer at either 270 or 400 MHz for ^1H spectra and either 68 or 100 MHz for ^{13}C spectra. All chemical shifts are quoted in ppm relative to tetramethylsilane. High resolution mass spectra (HRMS) were obtained from the EPSRC Mass Spectrometry Facility (Swansea).

Several of the compounds described in this paper have previously been reported in the literature without supporting NMR spectral data. NMR spectral data is therefore reported for all compounds.

The preparation of agar plates was conducted as described previously.¹

4-[(*E*)-2-(4-Nitrophenyl)ethenyl]pyridine **12a**^{2,3}

To a stirred suspension of 4-nitrobenzaldehyde (3.0 g, 19.9 mmol) in acetic anhydride (10 mL) at room temperature was added 4-methylpyridine (1.86 g, 20 mmol) in one portion. The mixture was heated at reflux (5 hours) during which time a solid started to form. The mixture was allowed to cool to room temperature and allowed to stand overnight. The light brown coloured solid was collected, washed with a little acetic anhydride and allowed to dry giving the acetate salt of compound **12a** (3.44 g). $^1\text{H-NMR}$ (d_6 -DMSO): δ 8.55 (2H, d, $J = 5$ Hz), 8.22 (2H, d, $J = 8$ Hz), 7.87 (2H, d, $J = 8$ Hz), 7.64 (1H, $J = 16$ Hz), 7.58 (2H, $J = 5$ Hz), 7.46 (1H, d, $J = 16$ Hz), 3.33 (1H, broad s), 1.87 (3H, s). The acetate salt (2.4 g) was partitioned between CH_2Cl_2 and 2N aqueous NaOH solution. The organic layer was separated, washed with H_2O , dried (MgSO_4) and evaporated yielding compound **12a** (1.82 g, 42%). $^1\text{H-NMR}$ (d_6 -DMSO): δ 8.56 (2H, d, $J = 4$ Hz), 8.22 (2H, d, $J = 7$ Hz), 7.87 (2H, d, $J = 7$ Hz), 7.65 (1H, d, $J = 16$ Hz), 7.57 (2H, d, $J = 4$ Hz), 7.47 (1H, d, $J = 16$ Hz). $^{13}\text{C-NMR}$ (d_6 -DMSO): 150.7, 144.0, 143.4, 131.4, 131.1, 128.5, 124.6, 121.8.

4-[(*E*)-2-(2-Methoxy-4-nitrophenyl)ethenyl]pyridine **12b**

A stirred mixture of 2-methoxy-4-nitrobenzaldehyde (0.18 g, 1.0 mmol), 4-methylpyridine (0.10 g, 1.1 mmol) and acetic anhydride (5 mL) was heated (5 h) at reflux, allowed to cool to room temperature and then poured onto ice. The mixture was basified by the addition of 3M aqueous NaOH solution and then extracted twice with CH_2Cl_2 . The combined organic extracts were washed with H_2O , dried (MgSO_4) and evaporated giving the crude product as a dark semi-solid which was contaminated by unreacted 4-methylpyridine. Purification by column chromatography over silica (eluent: CH_2Cl_2 : ethyl acetate, 95:5) gave compound **12b** (0.25 g, 85%). $^1\text{H-NMR}$ (CDCl_3): δ 8.61 (2H, d, $J = 5$ Hz), 7.77 - 7.62 (4H, m), 7.40 (2H, d, $J = 5$ Hz), 7.22 (1H, d, $J = 16$ Hz), 3.95 (3H, s). $^{13}\text{C-NMR}$ (CDCl_3): δ 157.2, 150.5, 148.3, 143.8, 131.6, 130.5, 127.1, 126.1, 121.2, 116.2, 106.2, 56.3.

HRMS: $\text{C}_{14}\text{H}_{13}\text{N}_2\text{O}_3$ [$\text{M}+\text{H}$] $^+$. Calculated mass of molecular ion 257.0926. Measured mass: 257.0927.

4-[(E)-2-(5-Nitrothienyl)ethenyl]pyridine **12c**⁴

To a stirred suspension of 2-nitrothiophene-5-carboxaldehyde (3.1 g, 20 mmol) in acetic anhydride (15 mL) at room temperature was added 4-methylpyridine (1.86 g, 20 mmol). The mixture was heated at reflux (3 hours), allowed to cool to room temperature and H₂O (25 mL) was added. After standing for 4 days, the dark mixture was poured into more H₂O and CH₂Cl₂ (ca 20 mL) was added. The mixture was agitated and sufficient 2M aqueous NaOH solution was added to neutralise the aqueous layer. The dark mixture was filtered through celite and the celite was rinsed through with CH₂Cl₂. The organic portion of the filtrate was separated, washed with H₂O, dried (MgSO₄) and evaporated yielding the crude product as a dark coloured solid (4.2 g). Purification by column chromatography over silica (eluent: CH₂Cl₂: EtOH, 95:5) gave compound **12c** (1.83 g, 40%) as a yellow-brown solid. ¹H-NMR (d₆-DMSO): δ 8.55 (2H, d, J = 5 Hz), 8.08 (1H, d, 4 Hz), 7.76 (1H, d, 16 Hz), 7.54 (2H, d, J = 5 Hz), 7.39 (1H, d, J = 4 Hz), 7.35 (1H, d, J = 16 Hz). ¹³C-NMR (d₆-DMSO): 150.8, 150.0, 149.7, 143.3, 131.5, 131.5, 128.0, 125.4, 121.7.

4-[(E)-2-(4-Nitrophenyl)ethenyl]quinoline **13a**^{5,6}

A mixture of 4-nitrobenzaldehyde (7.56 g, 0.05 mol), 4-methylquinoline (7.15 g, 0.05 mol) and acetic anhydride (15 mL) were heated under reflux for 9 h. The mixture was allowed to cool to room temperature, the resulting solid was collected and then suspended in H₂O. After stirring (2 h) the solid was collected and recrystallised from EtOH giving compound **13a** as a yellow solid (12.97 g, 94%). ¹H-NMR (d₆-DMSO): δ 8.91 (1H, d, J = 5 Hz), 8.56 (1H, d, J = 7 Hz), 8.34 (1H, d, J = 16 Hz), 8.28 (2H, d, J = 9 Hz), 8.11 (2H, d, J = 9 Hz), 8.04 (1H, dd, J = 8 and 1 Hz), 7.89 (1H, d, J = 5 Hz), 7.80 (1H, t, J = 7 Hz), 7.73 (1H, d, J = 16 Hz), 7.66 (1H, t, J = 7 Hz). ¹³C-NMR (d₆-DMSO): δ 150.8, 148.8, 147.4, 143.6, 141.9, 133.4, 130.1, 130.1, 129.0, 127.6, 127.3, 126.3, 124.7, 124.6, 117.7.

4-[(E)-2-(2-Methoxy-4-nitrophenyl)ethenyl]quinoline **13b**

Following a similar procedure to that described above for the preparation of compound **13a**, 2-methoxy-4-nitrobenzaldehyde (0.8 g, 4.4 mmol) and 4-methylquinoline (0.7 g, 4.9 mmol) in acetic anhydride (6 mL) afforded the acetate salt of compound **13b** (1.2 g) as a yellow solid. ¹H-NMR (d₆-DMSO): δ 11.96 (1H, broad s), 8.86 (1H, d, J = 4 Hz), 8.44 (1H, d, J = 8 Hz), 8.24 - 8.19 (2H, m), 8.00 (1H, d, J = Hz), 7.85 - 7.68 (6H, m), 4.00 (3H, s), 1.87 (3H, s). The mother liquor from the acetate salt was poured into water and, after standing for 2 days, the resulting solid was collected and identified as unreacted 2-methoxy-4-nitrobenzaldehyde (0.43 g) by ¹H-NMR spectroscopy. The acetate salt was partitioned between CH₂Cl₂ and 1M aqueous NaOH solution followed by separation, drying (MgSO₄) and evaporation of the organic layer affording compound **13b** (0.42 g, 31%) as a yellow solid. ¹H-NMR (d₆-DMSO): δ 8.86 (1H, d, J = 5 Hz), 8.44 (1H, d, J = 8 Hz), 8.26 (1H, d, J = 16 Hz), 8.20 (1H, d, J = 8 Hz), 8.01 (1H, d, J = 8 Hz), 7.86 (1H, d, J = 9 Hz), 7.79-7.62 (m, 5H), 3.99 (3H, s). ¹³C-NMR (d₆-DMSO): δ 157.5, 150.9, 148.8, 148.4, 142.2, 132.1, 130.1, 128.7, 127.8, 127.3, 126.2, 124.6, 117.6, 116.3, 106.8, 57.0.

HRMS: C₁₈H₁₅N₂O₃ [M+H]⁺. Calculated mass of molecular ion 307.1077. Measured mass: 307.1082.

4-[(E)-2-(5-Nitrothienyl)ethenyl]quinoline **13c**⁷

Following a similar procedure to that described above for the preparation of compound **13a**, 2-nitrothiophene-5-carboxaldehyde (1.5 g, 9.55 mmol) and 4-methylquinoline (1.4 g, 9.8 mmol) in acetic anhydride (10 mL) afforded the acetate salt of compound **13c** (1.2 g) as a yellow solid. ¹H-NMR (CDCl₃): δ 8.93 (1H, d, J = 4 Hz), 8.25 - 8.15 (2H, m), 7.90-7.55 (5H, m), 7.36 (1H, d, J = 16 Hz), 7.14 (1H, d, J = 4 Hz), 2.10 (3H, s). Partitioning this salt between CH₂Cl₂ and aqueous NaHCO₃ solution followed by separation, drying (MgSO₄) and evaporation of the organic layer afforded compound **13c** (1.02 g, 37%) as a yellow solid. ¹H-NMR (CDCl₃): δ 8.92 (1H, d, J = 4 Hz), 8.14 (2H, m), 7.88-7.60 (5H, m), 7.56 (1H, d, J = 4 Hz), 7.36 (1H, d, J = 16 Hz), 7.14 (1H, J = 4 Hz). ¹³C-NMR (CDCl₃): δ 150.7, 150.2, 148.8, 148.4, 140.6, 130.5, 129.8, 129.5, 127.7, 127.3, 126.5, 126.4, 126.0, 123.1, 117.4.

HRMS: C₁₅H₁₁N₂O₂S [M+H]⁺. Calculated mass of molecular ion 283.0536. Measured mass: 283.0544.

4-[(E)-2-(4-Nitrophenyl)ethenyl]-1-methylpyridinium iodide **14a**^{3,8}

To compound **12a** (0.23 g, 1.0 mmol) in a mixture of CH₃CN (10 mL) and CH₂Cl₂ (2 mL) at room temperature was added methyl iodide (1.5 mL, 24 mmol) in one portion with stirring. The mixture was heated at reflux (3 hours) during which time it attained an orange colour and a precipitate started to form. The mixture was allowed to cool to room temperature and the precipitate was collected and washed with a little CH₃CN giving compound **14a** (0.28 g, 76%) as an orange solid. ¹H-NMR (d₆-DMSO): δ 8.91 (2H, d, J = 6 Hz), 8.31 - 8.26 (4H, m), 8.10 (1H, d, J = 17 Hz), 17.97 (2H, d, J = 8 Hz), 7.72 (1H, d, J = 17 Hz), 4.26 (3H, s). ¹³C-NMR (d₆-DMSO): δ 152.0, 148.2, 146.0, 141.9, 138.0, 129.5, 124.8, 124.7, 47.8.

HRMS: C₁₄H₁₃N₂O₂ [M]⁺. Calculated mass of molecular ion 241.0972. Measured mass: 241.0975.

4-[(E)-2-(2-Methoxy-4-nitrophenyl)ethenyl]-1-methylpyridinium iodide **14b**⁹

Following a similar procedure to that described above for the preparation of compound **14a**, compound **12b** (0.23 g, 0.09 mmol) afforded compound **14b** (0.21 g, 78%) as a brown coloured solid. ¹H-NMR (d₆-DMSO): δ 8.86 (2H, d, J = 6 Hz), 8.27 (2H, d, J = 6 Hz), 8.02 - 7.85 (m, 3H), 7.72 (1H, d, J = 16 Hz), 4.24 (s, 3H), 4.02 (s, 3H). ¹³C-NMR (d₆-DMSO): δ 158.4, 152.3, 149.4, 145.9, 133.5, 130.6, 129.8, 128.5, 124.7, 116.4, 107.3, 57.1, 47.7.

HRMS: C₁₅H₁₅N₂O₃ [M]⁺. Calculated mass of molecular ion 271.1077. Measured mass: 271.1075.

4-[(E)-2-(5-Nitro-2-thienyl)ethenyl]-1-methylpyridinium iodide **14c**

Following a similar procedure as described above for the preparation of compound **14a**, compound **12c** (0.23 g, 1.0 mmol) and iodomethane (1 mL, 16 mmol) afforded compound **14c** (0.28 g, 76%) as a rust-coloured solid. ¹H-NMR (d₆-DMSO): δ 8.88 (2H, d, J = 7 Hz), 8.21 - 8.14 (4H, m), 7.57 (1H, d, J = 17 Hz), 7.54 (1H, d, J = 4 Hz), 4.24 (3H, s). ¹³C-NMR (d₆-DMSO): δ 151.8, 151.3, 147.7, 146.0, 132.1, 131.5, 130.3, 127.9, 124.6, 47.8.

HRMS: C₁₂H₁₁N₂O₂S [M]⁺. Calculated mass of molecular ion 247.0536. Measured mass: 247.0533

4-[(E)-2-(5-Nitro-2-thienyl)ethenyl]-1-hexylpyridinium iodide **14d**

To a stirred mixture of compound **12c** (0.10 g, 0.43 mmol) in DMF (2 mL) was added 1-iodohexane (0.15 mL, 0.9 mmol). The mixture was stirred (55 °C) for 4 hours during which time a reddish colour developed. The solution was allowed to cool to room temperature and Et₂O (*ca* 3 volumes) was added dropwise with stirring. The Et₂O was decanted from the red-coloured oil and a further portion of Et₂O was added. The oil was agitated with a glass rod and the Et₂O was decanted. This process was repeated resulting in the formation of compound **14d** (0.10 g, 53%) as a rust-coloured solid. ¹H-NMR (d₆-DMSO): δ 9.00 (2H, d, J = 6 Hz), 8.24 - 8.14 (4H, m), 7.59 (1H, d, J = 16 Hz), 7.54 (1H, d, J = 4 Hz), 4.48 (2H, t, J = 8 Hz), 2.51 (2H, overlapping DMSO signal), 1.87 (2H, broad s), 1.24 (3 x 2H, broad s), 0.83 (3H, t, J = 7 Hz). ¹³C-NMR (d₆-DMSO): δ 151.8, 151.8, 147.7, 145.2, 132.3, 131.4, 130.3, 127.9, 125.0, 60.6, 34.9, 31.1, 25.6, 22.4, 14.4.

HRMS: C₁₇H₂₁N₂O₂S [M]⁺. Calculated mass of molecular ion 317.1318. Measured mass: 317.1314.

4-[(E)-2-(4-Nitrophenyl)ethenyl]-1-methylquinolinium iodide **15a**¹⁰

Following a similar procedure as described above for the preparation of compound **14a**, compound **13a** (0.27 g, 0.98 mmol) and iodomethane (1 mL, 16 mmol) afforded compound **15a** (0.40 g, 98%) as a red solid. ¹H-NMR: (d₆-DMSO) δ 9.43 (1H, d, J = 6 Hz), 9.04 (1H, d, J = 8 Hz), 8.53 - 8.46 (3H, m), 8.35 - 8.22 (6H, m), 8.09 (1H, t, J = 8 Hz), 4.56 (3H, s). ¹³C-NMR: (d₆-DMSO) 152.1, 149.1, 148.3, 142.2, 140.3, 139.2, 135.7, 130.2, 130.1, 127.1, 124.8, 124.6, 120.0, 117.8, 45.6.

HRMS: C₁₈H₁₅N₂O₂ [M]⁺. Calculated mass of molecular ion 291.1128. Measured mass: 291.1127.

4-[(E)-2-(2-Methoxy-4-nitrophenyl)ethenyl]-1-methylquinolinium iodide **15b**⁹

Following a similar procedure as described above for the preparation of compound **14a**, compound **13b** (0.17 g, 0.56 mmol) and iodomethane (1.5 mL, 24 mmol) afforded compound **15b** (0.22 g, 88%) as a red solid. ¹H-NMR: (d₆-DMSO) δ 9.36 (1H, d, J = 6 Hz), 8.94 (1H, d, J = 8 Hz), 8.44 - 8.52 (3H, m), 8.35 (1H, d, J = 8 Hz), 8.26 (1H, t, J = 7 Hz), 8.15 (1H, d, J = 15 Hz), 8.05 (1H, t, J = 7 Hz), 7.93 (1H, dd, J = 8 and 2 Hz), 7.86 (1H, d, J = 2 Hz), 4.55 (3H, s), 4.01 (3H, s). ¹³C-NMR: (d₆-DMSO) δ 158.4, 152.4, 149.6, 149.2, 139.2, 135.7, 134.9, 130.9, 130.2, 130.0, 127.1, 127.0, 125.2, 120.1, 118.0, 116.3, 107.3, 57.1, 45.8.

HRMS: C₁₉H₁₇N₂O₃ [M]⁺. Calculated mass of molecular ion 321.1234. Measured mass: 321.1228.

4-[(E)-2-(5-Nitro-2-thienyl)ethenyl]-1-methylquinolinium iodide **15c**

Following a similar procedure as described above for the preparation of compound **14a**, compound **13c** (0.14 g, 0.50 mmol) and iodomethane (1 mL, 16 mmol) afforded compound **15c** (0.19 g, 90%) as a purple solid. ¹H-NMR (CF₃CO₂D): δ 9.00 (1H, d, J = 6 Hz), 8.68 (1H, d, J = 8 Hz), 8.40 - 8.30 (3H, m), 8.22 (1H, d, J = 6 Hz), 8.15-8.05 (3H, m), 7.92 (1H, d, J = 16 Hz), 7.46 (1H, d, J = 4 Hz), 4.67 (3H, s). This compound was insufficiently soluble in d₆-DMSO to obtain a ¹³C-NMR spectrum.

HRMS: C₁₆H₁₃N₂O₂S [M]⁺. Calculated mass of molecular ion 297.0692. Measured mass: 297.0695.

4-(4-Nitrocinnamylidene)-*N*-methyl quinolinium iodide **16a**

To a stirred solution of 1,4-dimethylquinolinium iodide (0.26 g, 0.9 mmol) and 4-nitrocinnamaldehyde (0.16 g, 0.9 mmol) in ethanol (15 mL) at reflux was added a solution of ammonium acetate (0.1 g, 1.3 mmol) in ethanol (5 mL), drop-wise over 2 minutes. The mixture gradually developed a brown colour. The mixture was heated at reflux (1 hour), allowed to cool to room temperature and filtered. The brown solid was washed with ethanol and allowed to dry in air giving compound **16a** (0.27 g, 66%). ¹H-NMR: (d₆-DMSO) δ 4.50 (3H, s), 7.26 (1H, d, J = 16 Hz), 7.63 (1H, dd, J = 8 and 16 Hz), 7.87 (2H, d, J = 8 Hz), 7.98 - 8.07 (3H, m), 8.20 - 8.28 (3H, m), 8.42 (2H, m), 8.73 (1H, d, J = 8 Hz), 9.32 (1H, d, J = 6 Hz); ¹³C-NMR: (d₆-DMSO) δ 45.4, 117.1, 120.1, 124.8, 126.2, 126.8, 126.9, 128.7, 130.2, 133.5, 135.6, 138.1, 139.3, 142.8, 143.1, 147.6, 148.8, 152.0.

HRMS: C₂₀H₁₇N₂O₂ [M]⁺. Calculated mass of molecular ion 317.1285. Measured mass 317.1287.

4-[(*Z*)-1-Ethoxycarbonyl-2-(5-nitro-2-thienyl)ethenyl]pyridine **17**

A stirred mixture of ethyl 4-pyridylacetate (0.80 g, 4.85 mmol), 2-nitrothiophene-5-carboxaldehyde (0.80 g, 5.1 mmol) and triethylamine (0.70 mL, 5.06 mmol) in acetic anhydride was heated (3 hours) at 80 °C. The reaction mixture was allowed to cool to room temperature and H₂O (50 mL) was added. Stirring was continued overnight and the resulting dull, green solid was collected and washed with H₂O. This solid was allowed to dry giving compound **17** (1.19 g, 81%) which did not require any further purification. ¹H-NMR (CDCl₃): δ 8.77 (2H, d, J = 6 Hz), 7.90 (1H, s), 7.72 (1H, d, J = 4 Hz), 7.20 (2H, d, J = 6 Hz), 7.04 (1H, d, J = 4 Hz), 4.25 (2H, q, J = 7 Hz), 1.27 (3H, t, J = 7 Hz). ¹³C-NMR (CDCl₃): δ 165.7, 154.0, 151.2, 143.0, 142.6, 132.5, 132.1, 127.8, 124.1, 62.2, 14.2.

HRMS: C₁₄H₁₃N₂O₄S [M+H]⁺. Calculated mass of molecular ion 305.0591. Measured mass: 305.0596.

4-[(*Z*)-1-Ethoxycarbonyl-2-(5-nitro-2-furyl)ethenyl]pyridine **18**

To ethyl 4-pyridyl acetate (0.33 g, 2.0 mmol) was added acetic anhydride resulting in the formation of a yellow solid. 2-Nitrofuran-5-carboxaldehyde (0.28 g, 2.0 mmol) was then added to the mixture followed by triethylamine (0.4 mL, 2.9 mmol). The mixture was then stirred (3 hours) at 70 °C, allowed to cool to room temperature and then kept for 3 days. The resulting green-yellow solid was collected, washed with water and allowed to dry giving the crude product (0.20 g). A portion (0.15 g) of this material was purified by column chromatography over silica (eluent: CH₂Cl₂:EtOH, 100:5) affording compound **18** (0.13 g, 49%) as a dull yellow solid. ¹H-NMR (CDCl₃): δ 8.66 (2H, d, J = 6 Hz), 7.34 (3H, m), 6.88 (1H, s), 6.80 (1H, d, J = 4 Hz), 4.53 (2H, q, J = 8 Hz), 1.38 (3H, t, J = 8 Hz). ¹³C-NMR (CDCl₃): δ 166.7, 151.7, 150.7, 142.0, 135.8, 123.8, 120.4, 117.4, 115.5, 113.1, 62.9, 14.1.

HRMS: C₁₄H₁₃N₂O₅ [M+H]⁺. Calculated mass of molecular ion 289.0819. Measured mass: 289.0827.

4-[(*Z/E*)-1-Ethoxycarbonyl-2-(5-nitro-2-thienyl)ethenyl]-1-methylpyridinium iodide **19**

A mixture of compound **17** (0.630 g, 2.07 mmol) and iodomethane (0.6 mL, 10 mmol) in CH₃CN (20 mL) was stirred at room temperature overnight. The resulting solid was collected giving compound **19** (0.542 g, 59%) as a mixture (*ca* 5:2) of *Z* and *E* geometrical isomers. ¹H-NMR (d₆-DMSO): δ 9.11 (1.4H, d, J = 6 Hz), 8.99 (0.6H, d, J = 6 Hz), 8.27 - 8.04 (4H, m), 7.70 (0.3H, d, J = 4 Hz), 7.55 (0.7H, d, J

= 4 Hz), 4.40 (3H, s), 4.30 (0.6H, q, J = 7 Hz), 4.18 (1.4H, q, J = 7 Hz), 1.26 (0.9H, t, J = 7 Hz), 1.78 (2.1H, t, J = 7 Hz).

HRMS: C₁₅H₁₅N₂O₄S [M]⁺. Calculated mass of molecular ion 319.0747. Measured mass: 319.0744.

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