Electronic supporting information

The antibacterial activity of a photoactivatable diarylacetylene against Gram-positive bacteria

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1. Candidate structures for screening experiments



Figure S1: Chemical structures of the six candidate screening compounds.

2. Compound Synthesis

2.1 General chemical information

Reagents were purchased from Sigma-Aldrich, Acros Organics, Alfa-Aesar and Fluorochem. They were purified, if required, by recrystallisation or distillation/sublimation under vacuum. Solvents were used as supplied from Fisher Scientific or Sigma Aldrich, and dried before use if required with appropriate drying agents. Thin-layer chromatography (TLC) was conducted using Merck Millipore silica gel 60G F254 25 glass plates and/or TLC-PET foils of aluminium oxide with fluorescent indicator 254 nm (40 × 80 mm) with visualisation by UV lamp or appropriate staining agents. Flash column chromatography was performed using SiO₂ from Sigma-Aldrich (230-400 mesh, 40-63 μ M, 60 Å), and monitored using TLC. NMR spectra were recorded using Bruker Avance Neo-700, Bruker Avance-600 or Bruker Avance-400 spectrometers operating at ambient probe temperature. NMR peaks are reported as singlet (s), doublet (d), triplet (t), quartet (q), broad (br), septet (sept), combinations thereof, or as a multiplet (m), with reference to the following deuterated solvent signals: CDCl₃ (¹H = 7.26 ppm, ¹³C = 77.0 ppm), (CD₃)₂SO (¹H = 2.50 ppm, ¹³C = 39.5 ppm). ESMS was performed using a TQD (Waters Ltd., UK) mass spectrometer with an Acquity UPLC (Waters Ltd., UK), and accurate mass measurements were obtained using a QtoF Premier mass spectrometer with an Acquity UPLC (Waters Ltd., UK). IR spectra were recorded using a Perkin Elmer FTIR spectrometer.

2.2 5 - lodothiophene - 2 - carbaldehyde, 7

To a solution of 2-thiophenecarboxaldehyde (37.4 mL, 400.0 mmol) in EtOH (300 mL) at 50 °C was added *N*-iodosuccinimide (99.0 g, 440.0 mmol) and *p*-toluenesulfonic acid monohydrate (7.60 g, 40.0 mmol), whereupon the resultant solution was stirred at 50 °C for 50 min. The solution was cooled to 35 °C whereupon 1M HCl (500 mL) was added, and the mixture was then extracted with EtOAc, washed with sat. Na₂S₂O₃, sat. NaHCO₃, H₂O and brine, dried (MgSO₄) and evaporated to give compound **7** as a brown oil that slowly crystallised (85.2 g, 90%): ¹H NMR (400 MHz, CDCl₃) δ 7.39 (s, 2H), 9.77 (s, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 87.8, 137.0, 138.2, 149.6, 181.1. All other data matched the literature.¹ Note: the material contained residual succinimide: ¹H NMR (400 MHz, CDCl₃) δ 2.77 (s, 4H); along with trace EtOAc and EtOH.

2.3 5 - [2 - (Trimethylsilyl)ethynyl]thiophene - 2-carbaldehyde, 8

Compound **7** (78.3 g, 329 mmol) was dissolved in Et₃N (1000 mL) and the resulting solution was degassed by sonication under vacuum, replacing the atmosphere with Ar (10x). Trimethylsilylacetylene (50.1 mL, 362 mmol), Pd(PPh₃)₂Cl₂ (2.31 g, 3.29 mmol) and Cul (0.64 g, 3.29 mmol) were then added under Ar and the resultant suspension was stirred at 40 °C for 18 h. The mixture was diluted with Et₂O and passed through Celite/SiO₂ to give a crude brown oil (72 g). This was purified by SiO₂ chromatography (cyclohexane/EtOAc, 9:1) to give compound **8** as a light brown oil that slowly crystallised (64.22 g, 94%): ¹H NMR (400 MHz, CDCl₃) δ 0.26 (s, 9H), 7.25 (d, *J* = 3.9 Hz, 1H), 7.61 (d, *J* = 3.9 Hz, 1H), 9.84 (s, 1H); ¹³C NMR (101 MHz, CDCl₃) δ -0.5, 26.9, 96.3, 104.6, 132.5, 133.1, 135.7, 143.8, 182.4; IR (ATR) *v*_{max}/cm⁻¹ 2960w, 2899w, 2833w, 2148m, 1666s, 1438s, 1249s, 1223s, 1207s, 838s; MS (ES) *m*/*z* = 209.0 [M+H]⁺; HRMS (ES) calcd. for C₁₀H₁₃SOSi [M+H]⁺: 209.0451, found 209.0454.

2.4 Tert - butyl (2E) - 3 - {5 - [2 - (trimethylsilyl)ethynyl]thiophen - 2 - yl}prop - 2 - enoate, 9

Tert-butyl diethylphosphonoacetate (40.6 mL, 173 mmol) and LiCl (7.33 g, 173 mmol) were added to anhydrous THF (700 mL) at 0 °C and the resultant solution was stirred for 15 min, whereupon compound **8** (30.0 g, 144 mmol) was added. To this solution was slowly added DBU (25.9 mL, 173 mmol), and the resultant slurry was stirred at RT for 16 h. This was poured into crushed ice and extracted with EtOAc. The organics were washed with H₂O and brine, dried (MgSO₄) and evaporated to give a crude brown oil (49.6 g). This was purified by SiO₂ chromatography to give compound **9** as an orange oil, containing approximately 25% of the TMS-deprotection product, compound **10** (37.47 g, 85%): ¹H NMR (400 MHz, CDCl₃) δ 0.25 (s, 8H), 1.51 (s, 9H), 6.13 (dd, *J* = 15.7, 0.4 Hz, 1H), 7.05 (d, *J* = 3.9 Hz, 1H), 7.12 (d, *J* = 3.9 Hz, 1H), 7.57 (dd, *J* = 15.7, 0.4 Hz, 1H). Note: the percentage yield of this step was calculated on the basis of compound **9**; TMS deprotection was observed during purification on SiO₂.

2.5 Tert - butyl (2E) - 3 - (5 - ethynylthiophen - 2 - yl)prop - 2 - enoate, 10

Compound **9** (37.47 g, 122 mmol) was dissolved in a mixture of DCM (300 mL) and MeOH (30 mL), whereupon K₂CO₃ (33.7 g, 244 mmol) was added. The resultant suspension was stirred at RT for 18 h before being diluted with DCM and H₂O. The organics were washed with sat. NH₄Cl and H₂O, dried (MgSO₄) and evaporated to give a crude orange oil (28 g). This was purified by SiO₂ chromatography (95:5, cyclohexane/EtOAc) to give compound **10** as an orange oil that slowly darkens (26.33 g, 83%): ¹H NMR (300 MHz, CDCl₃) δ 1.51 (s, 9H), 3.44 (s, 1H), 6.15 (d, *J* = 15.7 Hz, 1H), 7.07 (d, *J* = 3.8 Hz, 1H), 7.17 (d, *J* = 3.8 Hz, 1H), 7.58 (dd, *J* = 15.7, 0.6 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 26.9, 28.1, 80.8, 83.4, 120.2, 124.0, 130.0, 133.8, 135.1, 141.2, 165.7; IR (ATR) *v*_{max}/cm⁻¹ 2978w, 2933w, 1702m, 1623m, 1447m, 1392w, 1368m, 1148s, 753m; MS (ES) *m/z* = 235.1 [M+H]⁺; HRMS (ES) calcd. for C₁₃H₁₅SO₂ [M+H]⁺: 235.0787, found 235.0786. Note: Compound 10 was generated and reacted in the next step as soon as possible. Slow polymerisation and darkening occurred if left to stand at RT after 24 h under Ar.

2.6 *Tert* - butyl (2*E*) - 3 - (5 - {2 - [4 - (piperazin - 1 - yl)phenyl]ethynyl}thiophen - 2 - yl)prop - 2 - enoate, 2

Compound **10** (26.33 g, 112.4 mmol) was dissolved in Et₃N (750 mL) and the solution was degassed by sparging with Ar for 1 h. 1-(4-lodophenyl)piperazine,² compound **11** (29.45 g, 102.2 mmol), Pd(PPh₃)₂Cl₂ (3.59 g, 5.11 mmol) and Cul (0.97 g, 5.11 mmol) were then added under Ar and the resultant suspension was stirred at 60 °C for 72 h. The resultant suspension was diluted with DCM and washed with sat. NaHCO₃ and water, dried (MgSO₄) and evaporated to give a crude orange solid (57 g). This was purified by SiO₂ chromatography (95:5, DCM/MeOH, 1% Et₃N) followed by recrystallisation from MeCN to give compound **2** as a yellow/orange solid (27.01 g, 67%): ¹H NMR (400 MHz, CDCl₃) δ 1.51 (s, 9H), 2.98 – 3.05 (m, 4H), 3.17 – 3.25 (m, 4H), 6.12 (d, *J* = 15.7 Hz, 1H), 6.80 – 6.88 (m, 2H), 7.08 (d, *J* = 3.9 Hz, 1H), 7.10 (d, *J* = 3.8 Hz, 1H), 7.36 – 7.43 (m, 2H), 7.59 (dd, *J* = 15.7, 0.6 Hz, 1H); ¹³C NMR (101 MHz, CDCl₃) δ 28.1, 45.9, 49.1, 80.6, 81.1, 96.4, 112.0, 114.8, 119.2, 126.4, 130.6, 131.8, 132.6, 135.5, 140.1, 151.6, 165.9; IR (ATR) v_{max}/cm^{-1} 2977w, 2929w, 2820w, 2194w, 1698s, 1617m, 1602m, 1526w, 1323m, 1141s, 812w; MS(ES): *m/z* = 395.3 [M+H]⁺; HRMS (ES) calcd. for C₂₃H₂₇N₂O₂S [M+H]⁺: 395.1793, found 395.1792.

3. ¹H and ¹³C NMR Spectra

5-lodothiophene-2-carbaldehyde, 7



5-[2-(Trimethylsilyl)ethynyl]thiophene-2carbaldehyde, 8









Tert-butyl (2E)-3-(5-ethynylthiophen-2-yl)prop-2-enoate, 10





Tert-butyl (2E)-3-(5-{2-[4-(piperazin-1-yl)phenyl]ethynyl}thiophen-2-yl)prop-2-enoate, 2

Solvent	λ _{abs} (max)/nm, (ε/M ⁻¹ cm ⁻¹)	λ _{em} (max)/nm	φ	т/ns
Toluene	389	491	0.58	1.64
CHCl₃	384 (35700 +/- 1200)	531	0.50	2.33
DMSO	396 (28400 +/- 500)	588	0.0013	-

4. Photophysical characterisation of compound 2

Table S1: Photophysical properties of compound 2 in toluene, chloroform and DMSO.



5. Non-irradiated and DMSO controls of compound screening

Figure S2. Controls for screening lead compounds for antibacterial activity against Gram-positive and Gramnegative bacteria. Two-fold dilutions of six lead compounds were applied in 6 µl volumes to the surface of a soft agar overlay inoculated with *B. subtilis*, *S. epidermidis*, *E. coli* or *P. fluorescens*. With a control of 0.1% DMSO (C) spotted onto the bottom row. The LB agar plates were incubated for 24 hours at 37°C prior to imaging. Minimal zones of inhibition were seen relative to the photoactivated plates. All spots appear to leave a slight zone of growth inhibition regardless of compound concentration, suggesting the simple application of liquid to the surface of the overlay affects the normal growth of the bacterial lawn beneath.

6. Ethanol controls of Propidium lodide assays



Figure S3. Effect of compound 2 and 50% ethanol on bacterial membrane integrity. Bacteria were grown to mid-log phase in the presence or absence of 2 μ M compound 2 as described in the Material and methods. When samples containing compound 2 were photoactivated 50 μ I of 100% ethanol was added to 50 μ I of bacteria suspended in PBS to give a final concentration of 50% ethanol. The relative fluorescence units (RFU) at an emission of 645 nm were normalised against controls containing appropriate control concentrations of DMSO.

7. Time lapse of membrane integrity following photoactivation



Supplementary Video 1. Real-time monitoring of *S. epidermidis* membrane integrity. The BacLight assay of membrane integrity following photoactivation of compound 2. Bacteria in the mid-log phase of growth were stained with PI (magenta) and SYTO 9 (yellow) and imaged by confocal microscopy using a time series function, with images taken every 6 seconds for 10 mins. Showing the gradual colour change as the PI displaces the SYTO9 within the cell. Scale bar is at 3μ M. The laser was applied at 30% power for 1 minute.

8. References

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