Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry
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Structure-function analysis of the C-3 position in analogues of *Pseudomonas aeruginosa* behavioural modulators HHQ and PQS

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Supplementary Material

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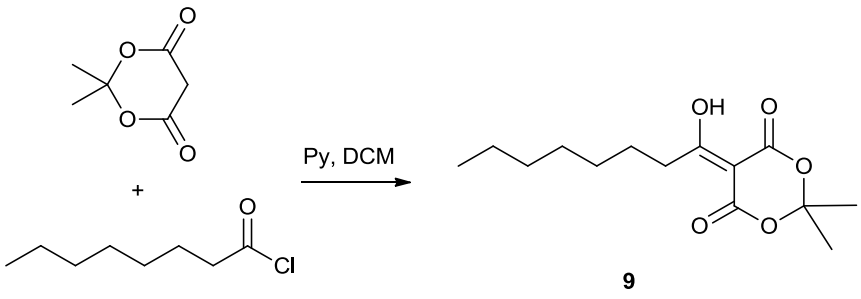
1. Chemical Synthesis

Solvents and reagents were used as obtained from commercial sources and without purification with exception of dichloromethane which was distilled from CaH_2 and acetone which was distilled from K_2CO_3 and stored under nitrogen. ^{1}H NMR (400 MHz) spectra and ^{1}H NMR (300 MHz) spectra were recorded on Bruker Avance 400 and Bruker Avance 300 NMR spectrometers respectively in proton coupled mode. ^{13}C NMR (125 MHz) spectra and ^{13}C NMR (75.5 MHz) spectra were recorded on Bruker Avance 500 and Bruker Avance 300 NMR spectrometers respectively in proton decoupled mode at 20°C in deuterated chloroform, dimethylsulfoxide or methanol using tetramethysilane as internal standard. The Microanalysis Laboratory, National University of Ireland, Cork, performed elemental analysis using a Perkin-Elmer 240 and Exeter Analytical CE440 elemental analysers. Low-resolution mass spectra were recorded on a Waters Quattro Micro triple quadropole instrument in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. High resolution precise mass spectra (HRMS) were recorded on a Waters LCT Premier Tof LC-MS instrument in electrospray ionisation (ESI) mode using 50% acetonitrile-water containing 0.1% formic acid as eluent; samples were made up in acetonitrile or methanol. Infrared spectra were measured as pressed potassium bromide (KBr) for solids or thin films on sodium chloride plates for liquids on a Perkin-Elmer FT-IR spectrometer. Melting points were carried out on a uni-melt Thomas Hoover Capillary melting point apparatus and are uncorrected. Column chromatography was performed with Aldrich silica gel (pore size 60 Å, 220-440 mesh, 35-75 µm for flash chromatography). Analytical thin layer chromatography was performed employing 0.2 mm coated commercial silica gel plates (Merck KGaA TLC Silica gel 60 F_{254}).

Preparation of 9[1]

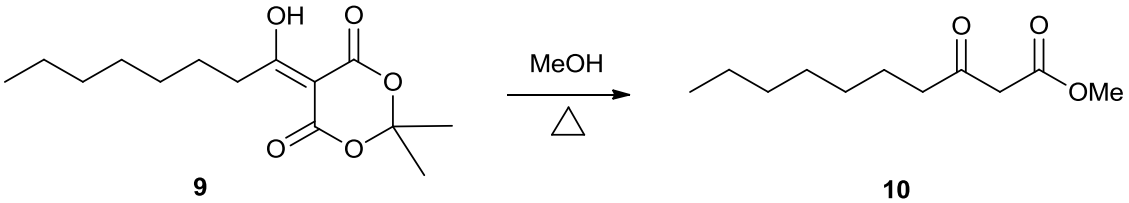


9

2,2-dimethyl-1,3-dioxane-4,6-dione (Meldrum's Acid) (15.0 g, 0.13 mol) was dissolved in distilled dicholormethane (190 mL) and cooled to 0°C over a N_2 atmosphere. To this solution was added pyridine (20.57 g, 0.26 mol) followed by dropwise addition of octanoyl chloride (23.89 mL, 0.14 mol). The resulting orange solution was allowed to stir at 0 °C for 1 h then at room temperature for 1 h. Reaction progress was monitored by TLC analysis. On completion, the reaction mixture was washed with 5% HCl solution (3 × 75 mL). The organic layer was then washed with distilled water (75 mL) before being dried over anhydrous $MgSO_4$, filtered and concentrated *in vacuo* to yield **9** as a brown oil (35.24 g, 99%) which was used in the subsequent step without further purification. δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, J = 6.9 Hz, CH_3), 1.29–1.41 (8H, m, 4 × CH_2), 1.61–1.70 (2H, m, CH_2), 1.74, (6H, s, 2 × CH_3), 3.04–3.09 (2H, m, CH_2), 15.31 (1H, bs, OH). δ_C ($CDCl_3$, 75.5 MHz) 14.0, 22.5, 26.1 (3 × CH_3),

26.7, 28.8, 29.3, 31.6, 35.2, 35.6 (6 × CH_2), 91.2, 104.7(C=*C*-C=O), 160.1 (C=C-OH), 170.5, 198.2 (2 × C=O). *m/z* (ES-) 269 [$(M - H)^+$, 10%].

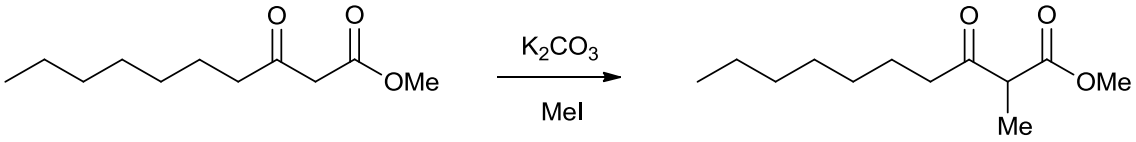
Preparation of 10[2]



9 **10**

Compound **9** (35.24 g, 0.13 mol) was dissolved in methanol (180 mL) and heated at reflux for 5 h. The reaction was allowed to cool and the solvent was removed *in vacuo* yielding the crude product as an orange oil. Purification was achieved using fractional distillation affording β-ketoester **10** as a pale orange oil (9.13 g, 35%). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, *J* = 6.5 Hz, CH_3), 1.27–1.31 (8H, m, 4 × CH_2), 1.54–1.63 (2H, m, C*H*$_2$$CH_2$CO), 2.53 (2H, t, *J* = 7.5 Hz, CH_2C*H*$_2$CO), 3.46 (2H, s, C*H*$_2$$CO_2$Me), 3.74 (3H, s, OCH_3). δ_C ($CDCl_3$, 75.5 MHz) 14.0 (CH_3), 22.6, 23.4, 28.9, 29.0, 31.6, 43.1(6 × CH_2), 49.0 (CO-C*H*$_2$-CO), 52.3 (OCH_3), 167.7 (C=O ester), 202.9 (C=O ketone). *m/z* (ES+) 201 [$(M + H)^+$, 8%].

Preparation of 11



10 **11**

To β-ketoester **10** (2.74 g, 13.7 mmol) was added dry acetone (35 mL). This solution was added to a flask containing dry potassium carbonate (1.76 g, 12.7 mmol) over a N_2 atmosphere. The reaction mixture was allowed to stir for 20 min before the addition of methyliodide (1.02 mL, 16.4 mmol). Stirring was continued at room temperature overnight before being heated at reflux for 6 h. The mixture was allowed to cool and the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification was carried out using silica column chromatography to yield methylated β-ketoester **11** as a pale yellow oil (1.17 g, 40%). (Found: C, 67.15; H, 10.2. $C_{12}H_{22}O_3$ requires C, 67.3; H, 10.35%). ν_{max} (film)/cm^{-1} 2930 (CH stretch), 2857 (CH stretch), 1749 (C=O ketone), 1717 (C=O ester), 1456 (CH scissor, bending), 1204 (C-O ester). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, *J* = 6.7 Hz, CH_3), 1.23–1.27 (8H, m, 4 × CH_2), 1.33 (3H, d, *J* = 7.2 Hz, CO-CH(C*H*$_3$)-CO), 1.53–1.66 (2H, m, C*H*$_2$$CH_2$CO), 2.52 (2H, qt, *J* = 7.4, 17.2, 27.4, 44.6 Hz, C*H*$_2$CO), 3.53 (1H, q, *J* = 7.1 Hz, CO-C*H*(CH_3)-CO), 3.73 (3H, s, OCH_3). δ_C ($CDCl_3$, 75.5 MHz) 12.8, 14.0 (2 × CH_3), 22.6, 23.5, 28.98, 29.00, 31.6, 41.4 (6 × CH_2), 52.3 (CO-*C*H-CO), 52.7 (OCH_3), 171.1 (C=O ester), 205.9 (C=O ketone). Exact mass calculated for $C_{12}H_{23}O_3$ [$(M+H)^+$], 215.1647. Found 215.1642, *m/z* (ES+) 215 [$(M+H)^+$, 30%].

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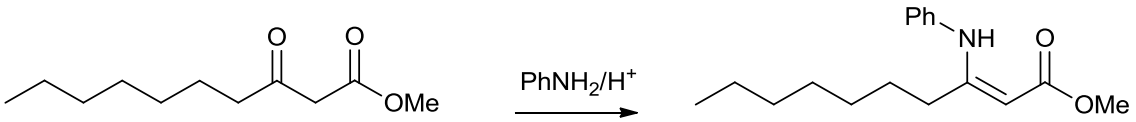
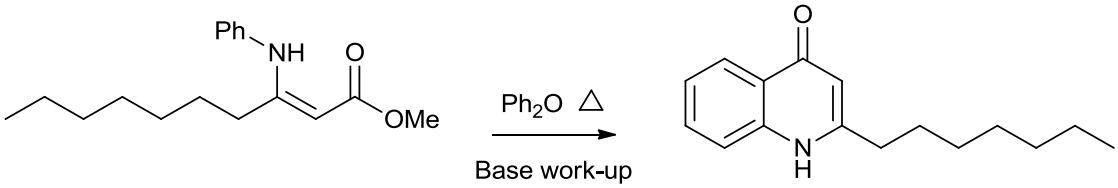
Preparation of 12



10 **12**

To a solution of **10** (1.27 g, 5.94 mmol) in dry hexane (30 mL) was added aniline (0.57 mL, 6.24 mmol) and *p*-toluene sulfonic acid (0.023 g, 0.12 mmol). The reaction mixture was heated at reflux under a N_2 atmosphere for 16 h. The reaction was allowed to cool and the solvent was removed *in vacuo* yielding **12** as an orange oil (1.35 g, 79%). (Found: C, 74.3; H, 9.2; N, 5.2. $C_{18}H_{27}NO_2$ requires C, 74.7; H, 9.4; N, 4.8%). ν_{max} (film)/cm^{-1} 3216 (NH stretch), 2952 (CH stretch), 2928 (CH stretch), 2856 (CH stretch), 1744 (C=O), 1657 (C=C), 1612, 1594 (NH bend), 1252 (C-O), 1229 (C-O), 1164(C-O). δ_H ($CDCl_3$, 400 MHz) 0.84 (3H, t, *J* = 7.0 Hz, CH_3), 1.17–1.29 (8H, m, 4 × CH_2), 1.37–1.46 (2H, m, CH_2), 1.59 (1H, s, CH_3), 1.86 (1H, s, CH_3), 2.26–2.30 (1H, m, CH_2), 2.34–2.38 (1H, m, CH_2), 3.52–3.75 (4H, m, CH_3), 7.03–7.10 (2H, m, 2 × ArH), 7.13–7.19 (1H, m, ArH), 7.26–7.35 (2H, m, 2 × ArH), 10.81 (1H, bs, NH). δ_C ($CDCl_3$, 75.5 MHz) 12.5, 14.0 (2 × CH_3), 22.6, 27.7, 28.7, 28.8, 29.4, 31.6 (6 × CH_2), 50.7 (CH_3), 84.5 (CH), 124.8 (quaternary C), 125.1, 125.6, 128.9, 129.1, 129.3 (5 × ArC), 160.8 (ArC-N), 163.8 (C-N), 171.7 (C=O). Exact mass calculated for $C_{18}H_{28}NO_2$ [(M+H)$^+$], 290.2120 . Found 290.2116, *m/z* (ES+) 290 [(M+H)$^+$, 56%].

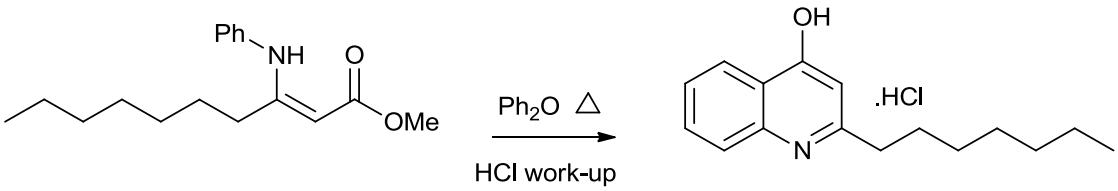
Formation of 2-heptylquinolin-4(1H)-one, HHQ, 1[3]



12 **HHQ, 1**

Diphenyl ether (35 mL) was heated at reflux (270°C) and **12** (40.6 g, 0.18 mol) was added dropwise over 90 min ensuring reflux was maintained and the mixture was heated for an additional 30 min. The mixture was then allowed cool to room temperature and ether (90 ml) and 2M aq. HCl (120) were added. After allowing to settle overnight the aqueous layer was removed. To the aqueous layer was added ethyl acetate (50 ml) and 12 M ammonium hydroxide solution until a solid crashed out in the organic layer. The basic aqueous layer was removed and the precipitate in the organic layer filtered under vacuum to yield the crude product as a brown solid which was purified by recrystallisation in ethanol to yield **1** as a cream solid (14.44g, 33%). δ_H ($CDCl_3$, 300 MHz) 0.79–0.84 (3H, m, CH_3), 1.18–1.33 (8H, m, 4 × CH_2), 1.66–1.78 (2H, m, CH_2), 2.65–2.70 (2H, m, CH_2), 6.23 (1H, s, CH) 7.30–7.36 (1H, m, ArH), 7.56–7.62 (1H, m, ArH), 7.71–7.74 (1H, m, ArH), 8.36 (1H, dd, *J* = 1.2, 8.2 Hz, ArH), 11.78 (1H, bs, NH). δ_C ($CDCl_3$, 75 MHz) 14.0 (CH_3), 22.6, 29.0, 29.1, 29.2, 31.7, 34.4 (6 × CH_2), 108.2 (CH), 118.5, 123.6 (2 × Ar-CH), 125.0 (quaternary C), 125.3, 131.8 (2 × Ar-CH), 140.6, 155.1 (2 × quaternary C), 178.9 (C=O). *m/z* (ES+) 243 [(M + H)$^+$, 100%].

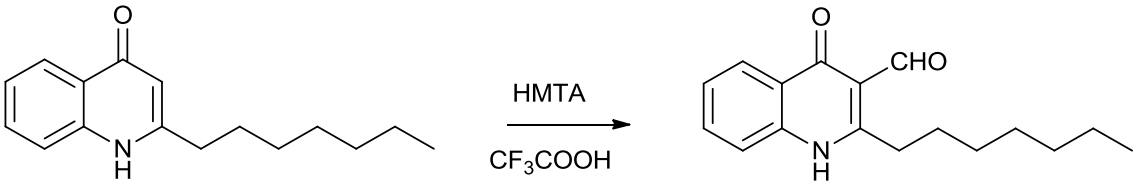
Preparation of HHQ.HCl, 1.HCl



12 **HHQ.HCl 1.HCl**

Diphenyl ether (45 mL) was heated at reflux (270°C) and **12** (51.36 g, 0.18 mol) was added dropwise over 90 min ensuring reflux was maintained and the mixture was heated for an additional 1 h. The mixture was then allowed cool to room temperature and the formed methanol was removed *in vacuo*. To the isolated residue was added diethyl ether (120 ml) and 2M HCl solution (160 ml). The mixture was then allowed stand at room temperature for 18 h. The precipitate which formed was filtered and washed with diethyl ether to afford a yellow solid which was triturated in warm ethyl acetate yielding **1.HCl** as a cream solid (19.91 g, 46 %). Mp 110-113°C. (Found: C, 69.0; H, 7.9; N, 5.0 $C_{16}H_{22}ClNO$ requires C, 68.7; H, 7.9; N, 5.0%). ν_{max} (KBr)/cm^{-1} 3103, 3060, 2930, 1772, 1702, 1639, 1594, 1488, 1182. δ_H ($CDCl_3$, 300 MHz) 0.77 (3H, t, *J* = 6.6 Hz, CH_3), 1.05–1.25 (6H, m, 3 × CH_2), 1.25–1.38 (2H, m, CH_2), 1.45–1.73 (1H, bs, OH), 1.72–1.88 (2H, m, CH_2), 3.12 (2H, t, *J* = 7.8 Hz, CH_2), 7.63 (1H, m, ArH), 7.84 (1H, t, *J* = 7.2 Hz, ArH), 8.34 (1H, d, *J* = 7.5 Hz, ArH), 8.53 (1H, d, *J* = 8.4 Hz, ArH). δ_C ($CDCl_3$, 75 MHz) 14.0 (CH_3), 22.5, 28.9, 29.3, 29.8, 31.6, 34.3 (6 × CH_2), 105.4 (CH), 119.8 (C), 119.9 (CH), 123.8 (C-H), 127.2 (CH), 134.0 (CH), 139.7 (C-N), 160.9 (C=N), 169.7 (C-OH). *m/z* (ES-) 278 [(M-H)$^-$, 38%].

Preparation of 2[3]



HHQ, 1 **2**

HHQ **1** (2.07 g, 8.52 mmol) and hexamethylenetetramine (0.6 g, 4.28 mmol) were stirred under a nitrogen atmosphere for 15 min. Trifluoroacetic acid (3.3 mL) was added and reaction vessel flushed with nitrogen. The reaction was heated to reflux overnight, with further additions of trifluoroacetic acid (2 × 5 mL) after 2 and 4 h. Distilled water (20 mL) and methanol (20 mL) were added and the reaction mixture allowed to reflux for 2 h 30 min. 2M HCl (12 mL) was added and reflux maintained for a further 1.5 h. The reaction mixture was allowed to cool and the precipitate was filtered and washed with acetone (30 mL). The crude product was then triturated with acetone (10 mL) and filtered, yielding **2** as a white powder (1.30g, 56%). δ_H ($[CD_3]_2SO$, 300 MHz) 0.82–0.88 (3H, m, CH_3), 1.27–1.38 (8H, m, 4 × CH_2), 1.56–1.72 (2H, m CH_2), 3.03–3.08 (2H, m, CH_2), 7.43 (1H, t, *J* = 7.4 Hz, ArH), 7.60–7.62 (1H, m, ArH), 7.71–7.80 (1H, m, ArH), 8.14–8.19 (1H, m, ArH), 10.39 (1H, s, CHO), 12.17 (1H, bs, OH). δ_C ($[CD_3]_2SO$, 75.5 MHz) 13.9 (CH_3), 22.0, 28.3, 28.8, 29.0, 31.1, 31.5 (6 × CH_2), 113.3 (quaternary C), 118.7 (Ar-CH), 124.90, 124.94, 126.2, (3 × Ar-

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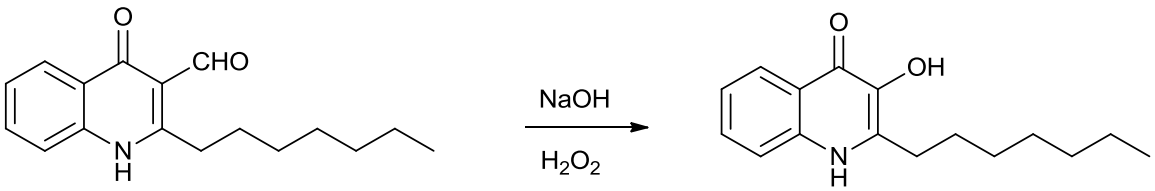
CH), 133.3, 139.1 (2 × quaternary C), 160.0 (quaternary C), 178.0 (C=O ketone), 190.8 (C=O aldehyde). *m/z* (ES+) 272 [$(M+H)^+$, 100%].

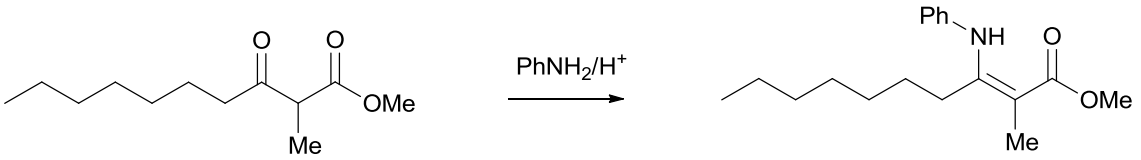
Preparation of PQS, 3[3]



2 **3, PQS**

Aqueous hydrogen peroxide (1.05 M, 1.49 ml, 1.56 mmol) was added to a solution of 3-formyl-2-heptylquinolone (0.41 g, 1.49 mmol) in ethanol (4.5 ml) and aqueous sodium hydroxide (1.08 M, 1.49 ml, 1.6 mmol) under argon, and the mixture was stirred at room temperature for 6 h. The precipitate was removed by filtration, was air dried, and was crystallized from ethyl acetate to give PQS, **3** (0.114 g, 29%) as off-white needles. δ_H ($[CD_3]_2SO$, 400 MHz) 0.83–0.87 (3H, m, CH_3), 1.16–1.33 (8H, m, 4 × CH_2), 1.65–1.68 (2H, m, CH_2), 2.67–2.75 (2H, m, CH_2), 7.18–7.24 (1H, m, ArH), 7.51–7.53 (2H, m, 2 × ArH), 8.09 (1H, d, *J* = 8.2Hz, ArH). δ_C ($[CD_3]_2SO$, 75 MHz) 13.9 (CH_3), 22.0, 27.8, 28.1, 28.4, 28.7, 31.2 (6 × CH_2), 117.8, 121.4, 122.1, 124.4, 129.8, 135.6, 137.4, 168.7 *m/z* (ES+) 260 [$(M+H)^+$, 100%].

Preparation of 13



11 **13**

To a solution of methyl 2-methyl-3-oxodecanoate **11** (1.27 g, 5.94 mmol) in dry hexane (30 mL) was added aniline (0.57 mL, 6.24 mmol) and *p*-toluene sulfonic acid (0.023 g, 0.12 mmol). The reaction mixture was heated at reflux under a N_2 atmosphere for 16 h. The reaction was allowed to cool and the solvent was removed *in vacuo* yielding **13** as an orange oil (1.35 g, 79%). (Found: C, 74.3; H, 9.2; N, 5.2. $C_{18}H_{27}NO_2$ requires C, 74.7; H, 9.4; N, 4.8%). ν_{max} (film)/cm^{-1} 3216 (NH stretch), 2952 (CH stretch), 2928 (CH stretch), 2856 (CH stretch), 1744 (C=O), 1657 (C=C), 1612, 1594 (NH bend), 1252 (C-O), 1229 (C-O), 1164(C-O). δ_H ($CDCl_3$, 400 MHz) 0.84 (3H, t, *J* = 7.0 Hz, CH_3), 1.17–1.29 (8H, m, 4 × CH_2), 1.37–1.46 (2H, m, CH_2), 1.59 (1H, s, CH_3), 1.86 (1H, s, CH_3), 2.26–2.30 (1H, m, CH_2), 2.34–2.38 (1H, m, CH_2), 3.52–3.75 (4H, m, CH_3), 7.03–7.10 (2H, m, 2 × ArH), 7.13–7.19 (1H, m, ArH), 7.26–7.35 (2H, m, 2 × ArH), 10.81 (1H, bs, OH). δ_C ($CDCl_3$, 75.5 MHz) 12.5, 14.0 (2 × CH_3), 22.6, 27.7, 28.7, 28.8, 29.4, 31.6 (6 × CH_2), 50.7 (CH_3), 124.8 (quaternary C), 125.1, 125.6, 128.9, 129.1, 129.3 (5 × ArC), 160.8 (ArC-N), 163.8 (C-N), 171.7 (C=O). Exact mass calculated for $C_{18}H_{28}NO_2$ [$(M+H)^+$], 290.2120 . Found 290.2116, *m/z* (ES+) 290 [$(M+H)^+$, 56%].

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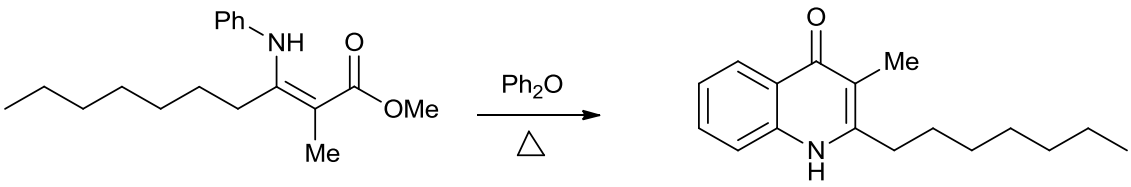
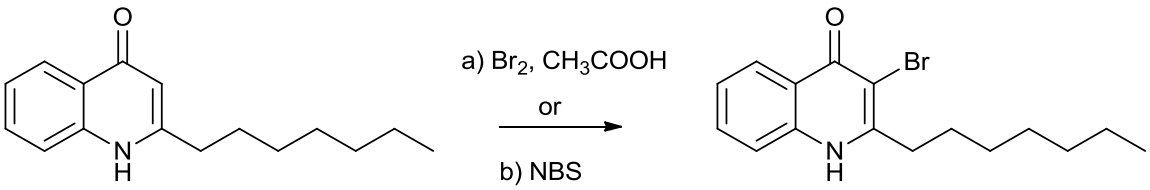
Preparation of 4[4]



13 **4**

Diphenyl ether (45 mL) was heated at reflux (270°C) and **13** (1.35g, 4.68 mmol) was added dropwise over 90 min ensuring reflux was maintained and the mixture was heated for an additional 1 h. The mixture was then allowed cool to room temperature and the formed methanol was removed *in vacuo*. 4M HCl (6 mL) was then added and the organic layer extracted with ethyl acetate (2 × 8 mL) dried over anhydrous $MgSO_4$, filtered and the solvent removed *in vacuo* to yield crude product as a brown oil. Purification was achieved using silica column chromatography to yield product as a dark brown solid followed by two recrystallisations from methanol to yield **4** as a white crystalline solid (10.6 mg, 10%). δ_H (CD_3OD, 400 MHz) 0.88 (3H, t, J = 6.8 Hz, CH_3), 1.30–1.46 (8H, m, 4 × CH_2), 1.68–1.75 (2H, m, CH_2), 2.15 (3H, s, CH_3), 2.81 (2H, t, J = 7.9 Hz, CH_2), 7.33 (1H, t, J = 8.1 Hz, ArH), 7.53–7.55 (1H, m, ArH), 7.60–7.64 (1H, m, ArH), 8.22 (1H, d, J = 7.5 Hz, ArH). δ_C (CD_3OD, 75.5 MHz) 10.8, 14.4 (2 × CH_3), 23.7, 30.0, 30.2, 30.5, 32.9, 33.5 (6 × CH_2), 116.2 (quaternary C), 118.7 (ArC), 124.4 (quaternary C), 124.5, 126.2, 132.7 (3 × ArC), 140.6, 153.4 (2 × quaternary C), 179.5 (C=O). *m/z* (ES+) 258 [$(M + H)^+$, 100%].

Preparation of 5



HHQ, 1 **5**

Method a. To a stirred solution of **1** (0.5 g, 2.05 mmol) in dichloromethane (10 mL) and methanol (2.5 mL) was added portionwise *N*-bromosuccinimide (0.73 g, 4.1 mmol) and the reaction was stirred at room temperature for 24 h. The solvent was removed *in vacuo* and the crude product was purified by recrystallisation in ethanol yielding **5** as a white solid (0.245g, 37%).

Method b. To a stirred solution of **1** (0.389 g, 1.6 mmol) in acetic acid (4 mL) was added dropwise over 30 min, a solution of bromine (0.1 mL, 1.8 mmol) in acetic acid (1 mL). Reaction progress was monitored by TLC analysis. After 1 h, the reaction mixture was poured into 1 % aqueous sodium sulfite (100 mL). The precipitate was filtered and washed with water yielding the product **5** as a white solid (0.245g, 47%). Mp 245-248°C (EtOH). ν_{max}(KBr)/cm^{-1} 3432 (OH stretch), 2926 (CH stretch), 2855 (CH stretch), 1631 (C=N), 1607 (aromatic), 1559 (C=N conjugated), 1475 (C=C stretch aromatic), 572 (C-Br). δ_H ($[CD_3]_2SO$, 300MHz) 0.85 (3H, s, CH_3), 1.26–1.34 (8H, m, 4 × CH_2), 1.70 (2H, m, CH_2), 2.84–2.89 (2H,

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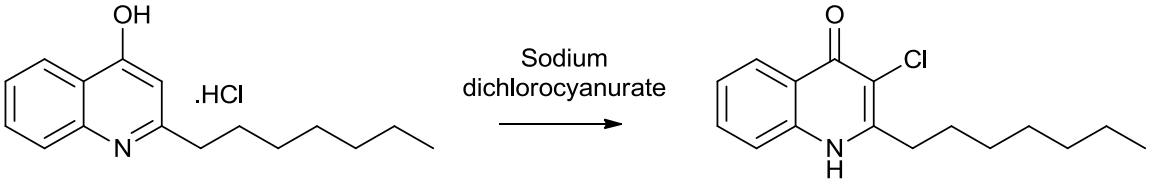
m, CH_2), 7.33–7.38 (1H, m, ArH), 7.57–7.70 (2H, m, 2 × ArH), 8.09 (1H, d, *J* = 7.9 Hz, ArH), 12.03 (1H, bs, OH). δ_C ($[CD_3]_2SO$, 75 MHz) 13.9 (CH_3), 22.0, 27.6, 28.3, 28.6, 31.1, 34.5 (6 × CH_2), 105.5 (C-Br), 117.8 (ArC), 122.7 (quaternary C), 123.6, 125.2 131.9 (3 × ArC), 138.7 (quaternary C), 152.0 (C=N), 171.24 (C-OH). Exact mass calculated for $C_{16}H_{21}NOBr$ [$(M+H)^+$], 322.0807. Found 322.0792, *m/z* ES^+ 322.3 [$(M+H)^+$, 100%].

Preparation of 6

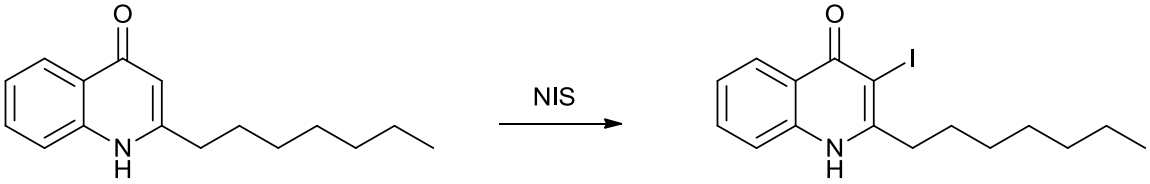


HHQ.HCl
1.HCl

6

HHQ.HCl (**1.HCl)** (0.839 g, 3.0 mmol) was dissolved in methanol (50 mL) before addition of 2M NaOH until neutral followed by water (10 mL). Sodium dichloroisocyanurate (0.363 g, 1.65 mmol) was then added to the reaction mixture. The reaction was allowed stir at room temperature overnight. The precipitate was filtered and washed with methanol. The filtrate was then acidified to pH 4 and placed in the fridge overnight. The precipitate was filtered to give an off-white solid. Purification by recrystallisation in ethanol yielded **6** as a white crystalline solid (0.167 g, 46%). Mp 269–272°C (EtOH). (Found: C, 68.7; H, 7.1; N, 5.1; Cl, 12.5. $C_{16}H_{20}ONCl$ requires C, 69.2; H, 7.3; N, 5.0; Cl, 12.8%). ν_{max}(KBr)/cm^{-1} 3454 (OH stretch), 2927 (CH stretch), 2857 (CH stretch), 1634 (C=C stretch, conjugated), 1563 (C=N conjugated), 1504 (C-C stretch, in ring, aromatic), 1477 (C=C stretch, aromatic), 1356 (CN stretch), 584 (C-Cl). δ_H ($[CD_3]_2SO$, 300 MHz) 0.88 (3H, t, *J* = 6.7 Hz, CH_3), 1.26–1.34 (8H, m, 4 × CH_2), 1.65–1.75 (2H, m, CH_2), 2.84 (2H, t, *J* = 7.8 Hz, CH_2), 7.32–7.37 (1H, m, ArH), 7.57–7.70 (2H, m, 2 × ArH), 8.1 (1H, d, *J* = 8.1 Hz, ArH), 12.03 (1H, bs, OH). δ_C ($[CD_3]_2SO$, 150 MHz) 13.9 (CH_3), 22.0, 27.5, 28.4, 28.6, 31.1, 32.1 (6 × CH_2), 113.3 (C-Cl), 118.0 (Ar-CH), 123.4(quaternary C), 123.5, 125.1, 131.8 (3 × Ar-CH), 138.6 (quaternary C), 150.7 (ArC), 170.9 (C=O). Exact mass calculated for $C_{16}H_{21}NOCl$ [$(M+H)^+$], 278.1312. Found 278.1317, *m/z* (ES+) 278 [$(M+H)^+$, 100%].

Preparation of 3-iodo-2-heptylquinolin-4(1*H*)-one, 7



HHQ, 1

7

To a stirred solution of **1** (0.333g, 1.37 mmol) in glacial acetic acid (10 mL) was added portionwise *N*-iodosuccinimide (0.315g, 1.40 mmol). Reaction progress was monitored by TLC analysis and after 2 h, the precipitate was filtered, washed with acetic acid and

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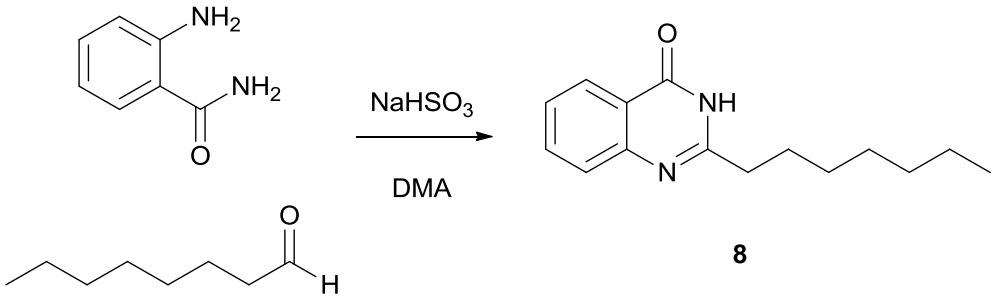
acetonitrile. Purification was achieved by silica column chromatography (80/20 ethyl acetate/ hexane, ramping to 100% ethyl acetate) to yield **7** as a white crystalline solid (0.22g, 48%). Mp 221-225°C (EtOAc). (Found: C, 52.4; H, 5.4; N, 3.9. $C_{16}H_{20}INO$ requires C, 52.0; H, 5.5; N, 3.8%). ν_{max}(film)/cm^{-1} 3419 (OH stretch), 2921 (CH stretch), 1627 (C=N), 1557 (C=N conjugated), 1474 (C=C stretch aromatic), 1134 (C-O alcohol), 571 (C-I). δ_H ([CD_3]$_2$SO, 300MHz) 0.84–0.88 (3H, m, CH_3). 1.27–1.37 (8H, m, 4 × CH_2), 1.63–1.71 (2H, m, CH_2), 2.88–2.93 (2H, m, CH_2), 7.30–7.35 (1H, m, ArH), 7.57–7.68 (2H, m, 2 × ArH), 8.05–8.08 (1H, d, *J* = 8.1 Hz, ArH). δ_C ([CD_3]$_2$SO, 75MHz) 13.9 (CH_3), 22.0, 27.9, 28.3, 28.7, 31.1, 38.7 (6 × CH_2), 85.8 (C-I), 117.8 (ArC), 120.6 (quaternary C), 123.8, 125.4, 131.9 (3 × ArC), 139.0 (quaternary C), 154.5 (C=N), 173.1 (C-OH). Exact mass calculated for $C_{16}H_{21}NOI$ [(M+H)$^+$], 370.0668. Found 370.0664, *m/z* ES$^+$ 370.3 [(M+H)$^+$, 100%].

Preparation of 2-heptylquinazolin-4-one, 8



8

A mixture of anthranilamide (20.5 mmol, 2.791 g), *n*-octanal (20.5 mmol, 3.2 mL) and sodium bisulfite (30.75 mmol, 3.2 g) in dimethylacetamide (30 mL) was stirred at 150°C for 2h. Reaction progress was monitored by TLC analysis. The reaction mixture was poured into water (500 mL) and the precipitate filtered. The precipitate was recrystallised from ethanol to give product **8** as an off-white crystalline solid (3.82 g, 76%). Mp 124-127°C (EtOH). (Found: C, 73.4; H, 8.2; N, 11.4. $C_{15}H_{20}N_2O$ requires C, 73.7; H, 8.25; N, 11.5%). ν_{max}(KBr)/cm^{-1} 3448 (OH stretch), 3034 (C-H stretch aromatic), 2919 (CH stretch), 2855 (CH stretch) 1674 (C=C), 1616 (C=N), 1470 (CH_2 bend), 1341 (C-N stretch), 1149 (C-O alcohol). δ_H ($CDCl_3$, 300 MHz) 0.88 (3H, t, *J* = 6.8 Hz, CH_3), 1.25–1.51 (8H, m, 4 × CH_2), 1.82–1.92 (2H, m, CH_2), 2.74 – 2.79 (2H, m, CH_2), 7.44–7.49 (1H, m, ArH), 7.68–7.80 (2H, m, 2 × ArH), 8.29 (1H, dd, *J* = 1.1, 8.0 Hz, ArH), 11.10 (1H, bs, OH). δ_C ($CDCl_3$, 75MHz) 14.1 (CH_3), 22.6, 27.6, 29.0, 29.2, 31.7, 36.1 (6 × CH_2), 120.5 (quaternary C), 126.3, 126.4, 127.2, 134.8 (4 × ArC), 149.4 (quaternary C), 156.8 (C=N), 164.0 (C-OH). Exact mass calculated for $C_{15}H_{20}N_2O$ [(M+H)$^+$], 245.1654. Found 245.1654, *m/z* ES$^+$ 245 [(M+H)$^+$, 80%].

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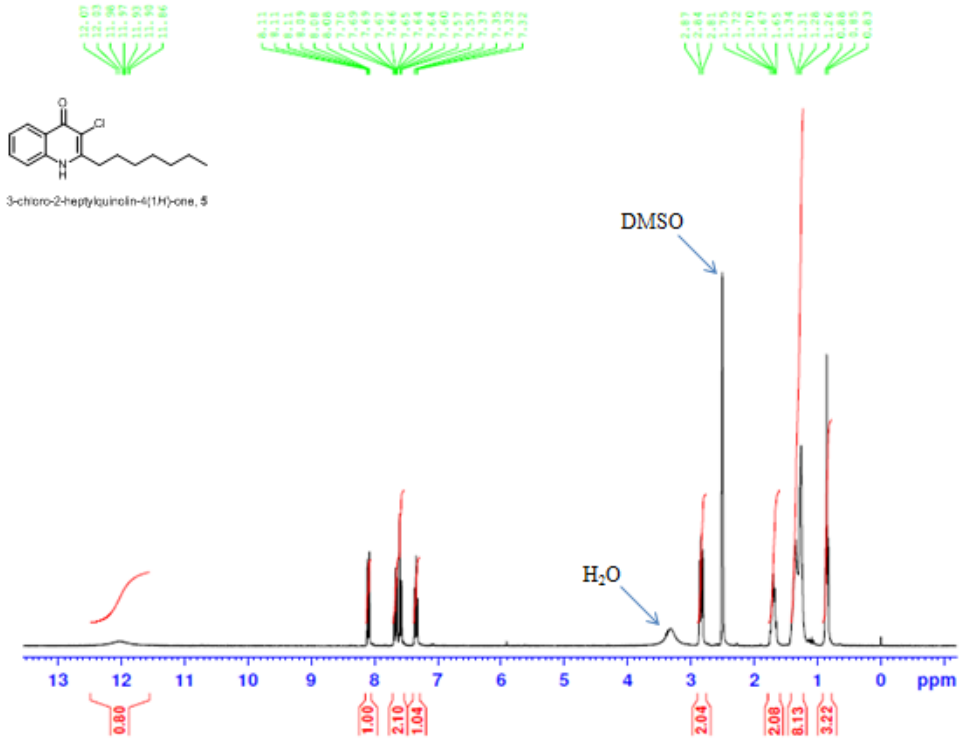
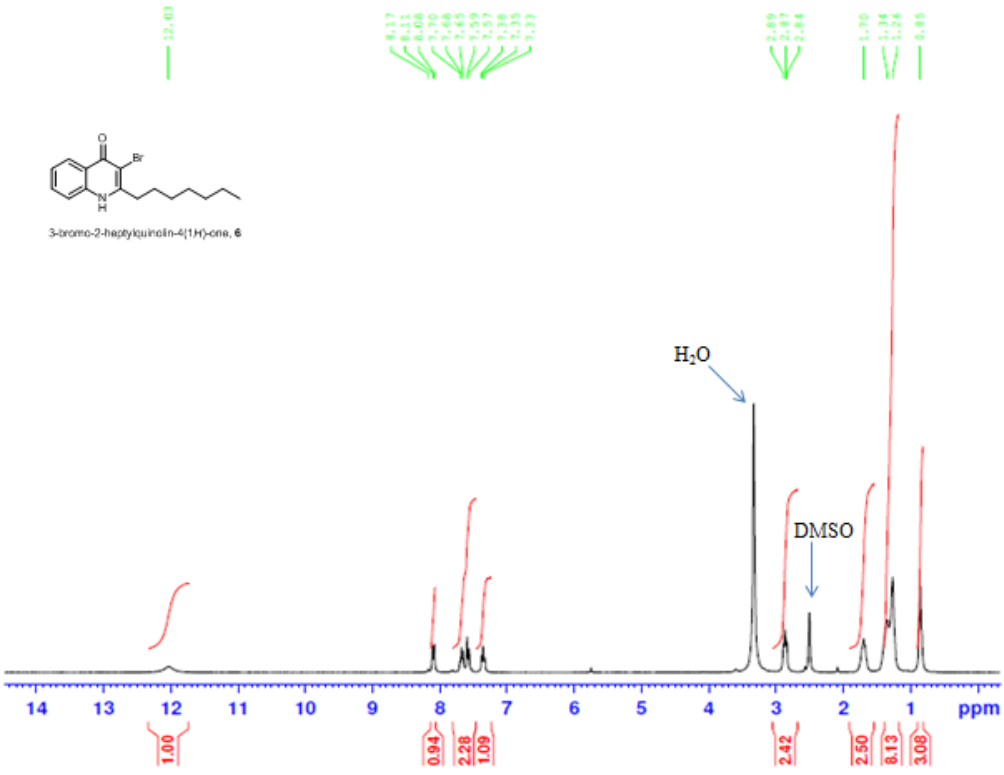
2. ^{1}H NMR spectra

Four novel PQS analogues were synthesised for biological testing, the ^{1}H NMR spectra of which are provided below.





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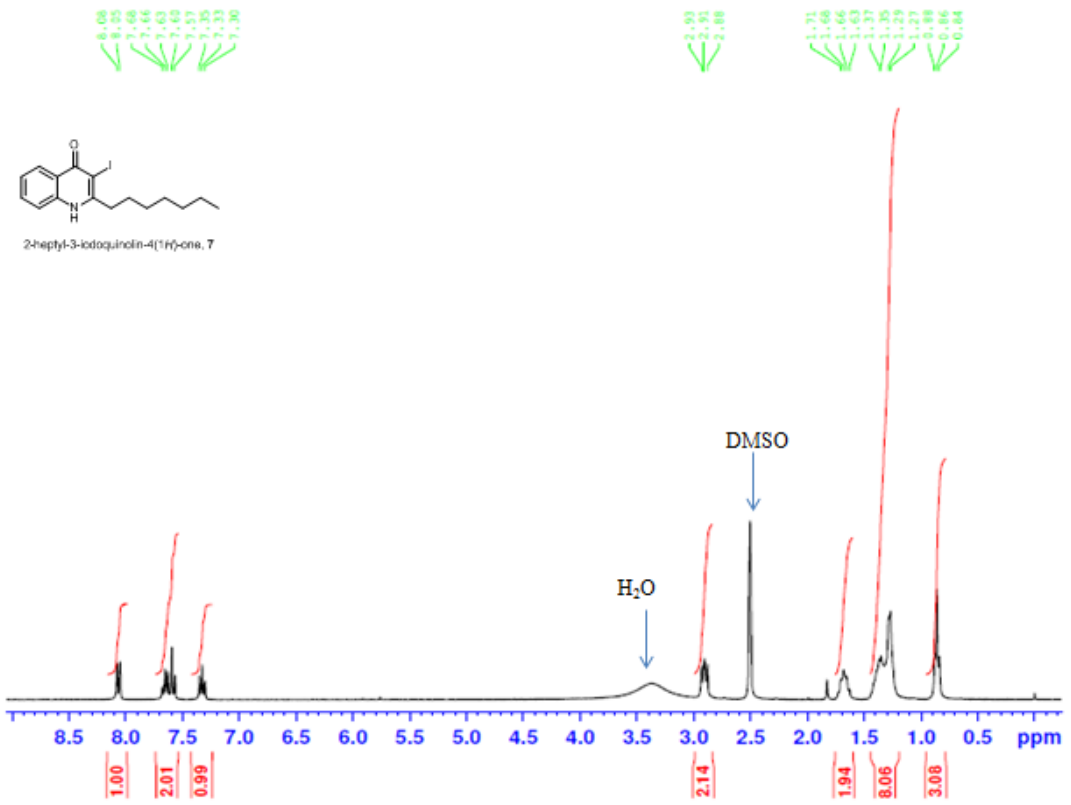

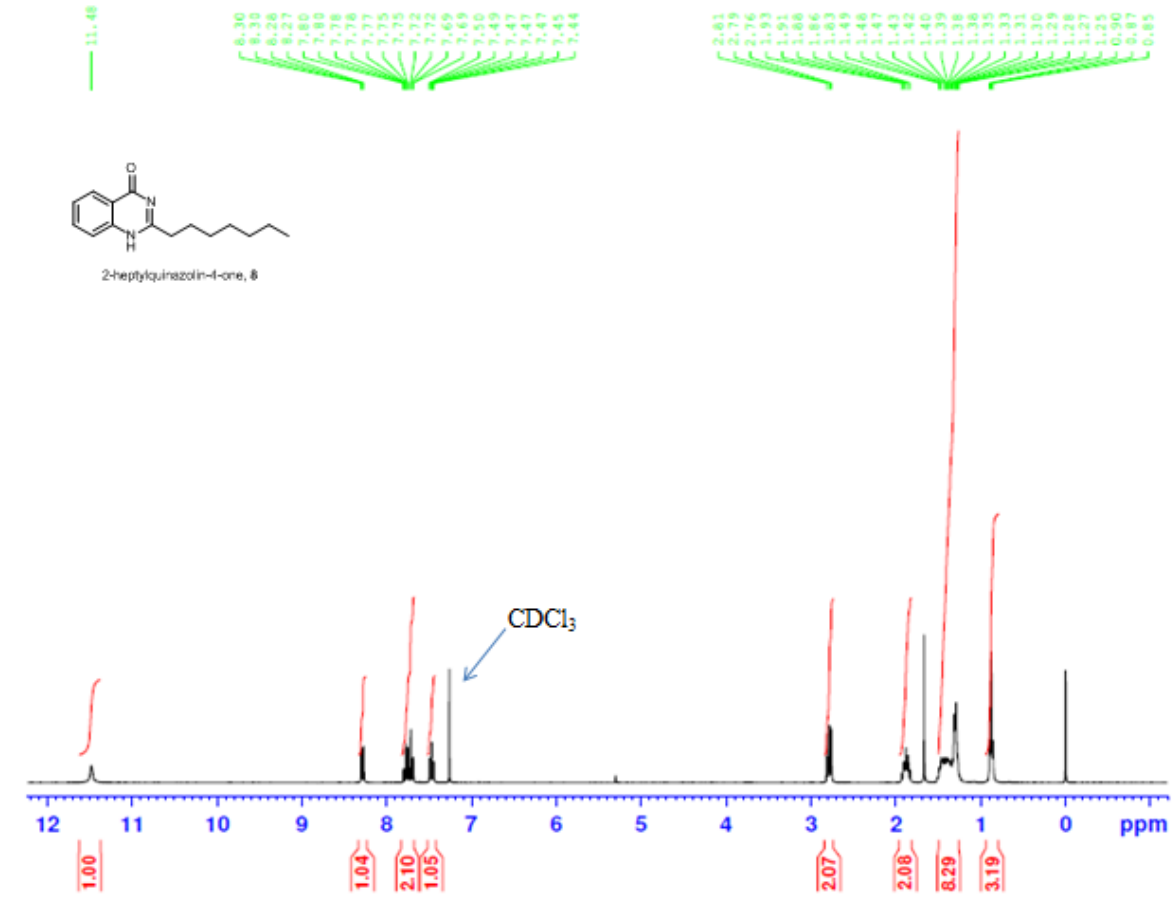

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3. Biological methods

Microbial behavioural analysis in response to AQ analogues.

1. *Phenazine extraction*
Cells of *P. aeruginosa* PAO1 and its isogenic *pqsA* mutant were grown overnight in Luria Bertani (LB) media at 37°C and shaking at 180 rpm. Following removal of supernatant, cells were inoculated into fresh LB media, starting $OD600_{nm}$ 0.05, with and without 10 µM concentrations of HHQ, PQS, or AQ analogue. Corresponding concentrations of methanol or ethanol were added as controls. Cultures were grown 37°C with shaking (at 180 rpm) for 16 hrs and cell-free supernatants (5 mL) were obtained. Chloroform (3 mL) was added to the supernatant and mixed by vortex. Subsequent to centrifugation, the lower organic phase was removed and transferred into fresh tubes into which 0.2 N HCl (2 mL) was added. Samples were mixed by vortex and the top phase was analysed by spectrophotometry (570 nm). Phenazine production in each sample was calculated using the following algorithm: Abs_{570nm} x 2 x 17.072.

2. *Growth profiling*
Antibacterial activity of AQ analogues was investigated using agar plate assays. A HHQ sensitive marine isolate *Algoriphagus spp*., isolated from marine sponges taken from Lough Lyne, off the Southwest coast of Ireland, was streaked onto Starch Yeast Peptone (SYP) marine agar supplemented with 10 µM AQs, and incubated at room temperature. Growth was scored as present or absent on the presence of colonies after 2-4 days. All experiments were performed in triplicate and data presented is representative of at least three independent biological replicates.

3. Motility analysis
Motility of *B. subtilis* NCTC10073 was analysed on Trypticase Soy Agar (TSA) 0.3% (w/v) agar. AQ derivatives were added to the molten medium at a final concentration of 10 µM immediately before pouring plates. Aliquots of overnight *B. subtilis* cultures (3 µL) were spotted into the centre of the plate and the zone of motility was measured against control plates containing methanol or ethanol. All experiments were performed in triplicate and data presented is the result of at least three independent biological replicates.

4. Bacterial biofilm formation
P. aeruginosa wild-type and *pqsA* mutant overnight cultures were transferred into fresh LB media, starting OD_{600nm} 0.05. *B. subtilis* NCTC10073 cultures were incubated overnight and transferred into fresh Tryptone Soy Broth (TSB) to an OD_{600nm} of 0.05. Aliquots (1 mL) were transferred into 24-well plates and AQs or controls added at a final concentration of 10 µM. Plates were incubated overnight at 37°C and attachment/biofilm was evaluated visually and by crystal violet [0.1% (w/v)] staining after washing to quantify attached cells. The degree of crystal violet staining was evaluated spectrophotometrically at Abs_{595nm}. Unpaired Student's t-test was performed by comparison of *B. subtilis* cells treated with AQ analogues with cells treated with methanol or ethanol (***, p-value $\leq$ 0.001).

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Cytotoxicity towards airway epithelial cells (LDH release & Cell morphology).

1. Cell culture

IB3-1 (ATCC CRL-2777) is a bronchial epithelial cell line derived from a CF patient with CFTR ΔF508/W1282X alleles (CF-affected cells). IB3-1 cell line was purchased from the American type Culture Collection (ATCC, LGC Standards). IB3-1 cells were cultured on bovine serum albumin-collagen-fibronectin-coated flask using LHC-8 medium (Invitrogen) supplemented with 5% foetal bovine serum (FBS) (Sigma), 100 units/ml penicillin, 100 µg/ml streptomycin (Invitrogen) under a 5% CO2 humidified atmosphere at 37 °C.

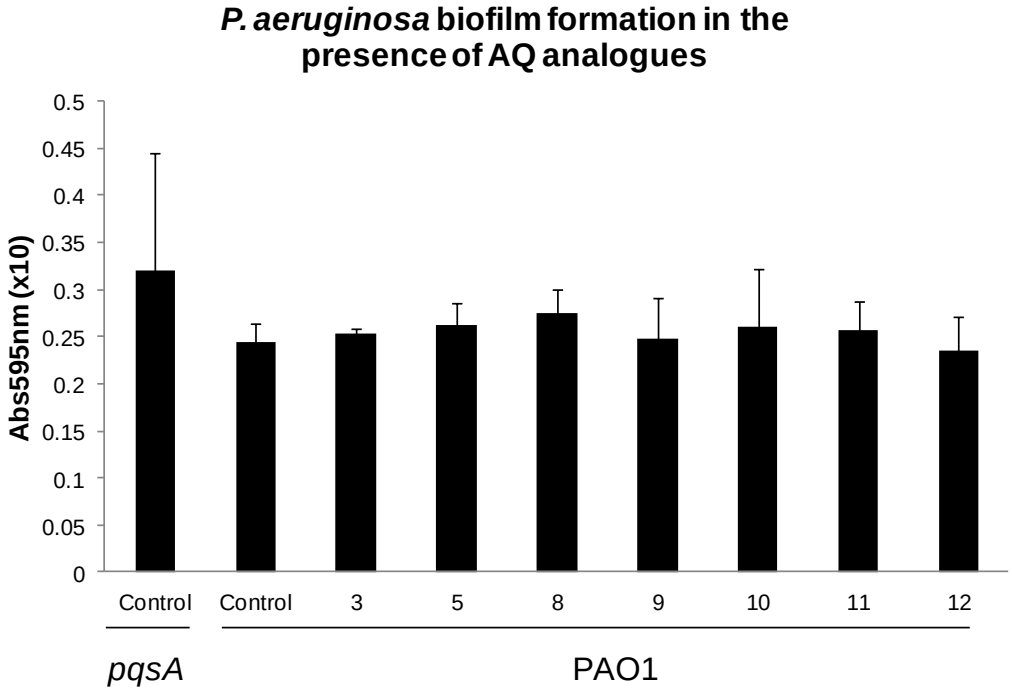
2. AQ analogues treatment

At 80% of confluence, IB3-1 cells were treated by AQ analogues at concentration of 100 µM for 16 hours and with the corresponding methanol and ethanol dilution, used as control, in LHC-8 medium without FBS.

3. LDH release

The release of lactate dehydrogenase (LDH) into culture supernatants was measured using an LDH cytotoxicity detection kit (Roche) according to the manufacturer's instructions. Cytotoxicity was expressed as a percentage of the total amount of LDH released from cells treated with methanol or ethanol, or with 100 µM of the AQ analogues in comparaison with cells treated with 0.1% Triton X-100, given the percentage of 100. Data (means ± SD) are representative of three independent biological experiments. Two-tailed unpaired Student's t-test was performed by comparison of IB3-1 cells treated with AQ analogues with IB3-1 cells treated with methanol or ethanol (**, p-value ≤ 0.01; ***, p-value ≤ 0.001).

4. Additional biological results and summary table



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Supplementary Figure. AQ analogues do not interfere with the initial stages of biofilm formation and attachment in *P. aeruginosa*. A *pqsA* mutant was not deficient in attachment to multiwell plates and addition of AQ analogues (10 µM) did not prevent attachment of the wild-type strain as seen in crystal violet staining assays. Experiments were performed in quadruplicate and the same trend was observed in three independent experiments.

	P. aeruginosa		*Interkingdom*			
Compound	**Phenazine production**	**Biofilm /Attachment**	**Biofilm repression**	**Motility repression**	**Antibacterial activity**	**Cytotoxicity**
3	+	-	+[a]	+	+	+
3.HCl	nt[b]	nt	+	+	+	+
5 PQS	+	-	-	+	-	-
8	-	-	-	-	+	-
9	-	-	-	-	+	-
10	-	-	-	-	+	-
11	-	-	-	-	+	-
12	-	-	-	-	-	+

[a] Biological activity presented as + Active or – Inactive
[b] Not tested

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