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Synthesis of 3-halo-analogues of HHQ, subsequent cross-coupling and first crystal structure of *Pseudomonas* quinolone signal (PQS)

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ABSTRACT

2-Aryl- and 2-alkyl-quinolin-4-ones and their N-substituted derivatives have several important biological functions such as the *Pseudomonas* quinolone signal (PQS) molecule participation in quorum sensing. Herein, we report the synthesis of its biological precursor, 2-heptyl-4-hydroxy-quinoline (HHQ) and possible isosteres of PQS; the C-3 Cl, Br and I analogues. N-Methylation of the iodide was also feasible and the usefulness of this compound showcased in Pd-catalysed cross-coupling reactions, thus allowing access to a diverse set of biologically important molecules. The first crystal structure of PQS is also included.

Quinolones are best known as broad-spectrum antibacterial agents,[1] for example, fluoroquinolone sales accounted for 18% of the antibacterial market in 2006.[2] An attractive feature of these molecules is their ability to kill bacteria very rapidly; an ability not widely attributable to other antibacterial agents. The related 2-aryl and 2-alkylquinolin-4-ones have recently received considerable attention due to their more wide ranging pharmacological applications. For example, 2-arylquinolin-4-one derivatives also exhibit anti-bacterial[3] and anti-tumour properties.[4] N-Substituted 2-arylquinoline derivatives can act as anti-malarial agents, immunostimulants and non-nucleoside HIV-1 inhibitors.[5] 2-Heptyl-4-hydroxyquinoline N-oxide (HHQNO) is effective against *Staphylococcus aureus*.[6] 2-Heptyl-3-hydroxy-4-quinolone,[7] otherwise known as the *Pseudomonas* quinolone signal (PQS, Fig. 1), has emerged as a key regulator of bacterial cooperative behaviour known as quorum sensing in the antibiotic resistant human pathogen *Pseudomonas aeruginosa*.[8] Derived from its biological precursor, 2-heptyl-4-quinolone (HHQ), PQS has a vast and varied array of biological functions[9] influencing iron homeostasis,[10] vesicle formation,[11] secondary metabolite production and biofilm formation.[12] *P. aeruginosa* PQS signaling is highly responsive to environmental and host-specific cues, including Mg^{2+} and the CF therapeutic colistin.[13] Recent evidence has revealed that PQS is capable of modulating immune responses and human T-cell proliferation.[14]

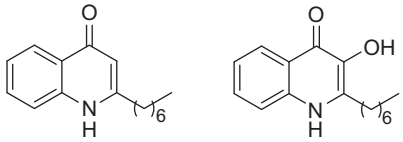

Figure 1. HHQ and PQS structures.

Our interest is twofold; firstly, in the synthesis of 3-haloquinolin-4-ones as analogues of PQS. These substrates will facilitate mechanistic studies into PQS signaling in virulent *Pseudomonas* populations with important clinical applications. Secondly, to explore if a new *N*-methyl version can be used in palladium cross-coupling reactions, thus providing access to an array of new biologically important quinolones. Importantly, the 2-heptyl chain is essential for certain biological functions such as the stimulation of outer vesicle formation in *P. aeruginosa*[11] and thus synthetic procedures on compounds bearing this bulky and hydrophobic substituent are important. There are no reports of halogenation or subsequent cross-coupling of HHQ. From a synthetic viewpoint, the presence of the long hydrophobic chain represents a challenge due to low solubility and the obvious steric hindrance.

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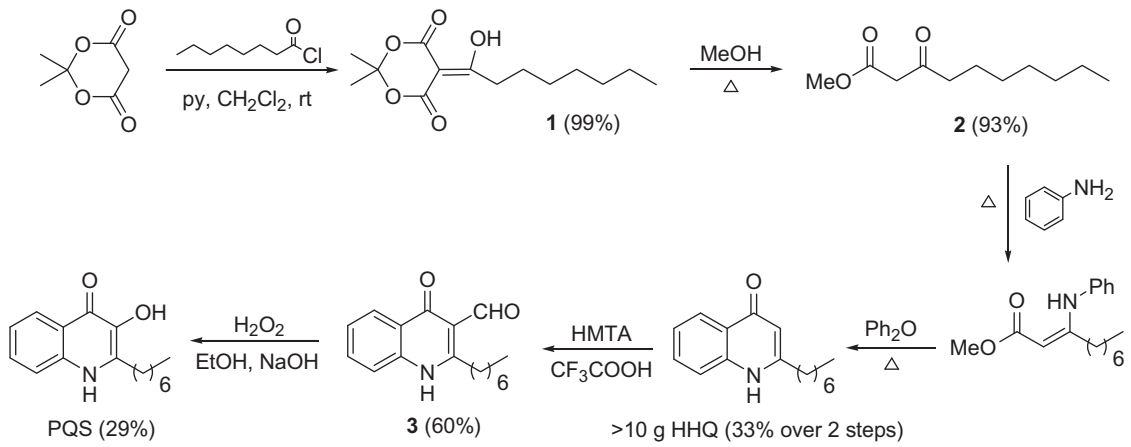


Scheme 1. Synthesis of HHQ and PQS.

Our initial goal was the synthesis of multi-gram quantities of the useful precursor HHQ and to investigate if HHQ could be halogenated at the 3-position. A modified route was designed. Initially Meldrum's acid (0.14 mol) was reacted with octanoyl chloride giving compound **1** followed by boiling in MeOH affording β-ketoester **2** in excellent yield (Scheme 1).[15] Formation of the enamine by reaction with aniline using Dean–Stark apparatus occurred with >98% conversion (^{1}H NMR) over 16 h.[16] Conrad–Limpach cyclisation occurred best using a method described by Bangdiwala and Desai.[17] An alternative cyclisation method reported by Woschek et al. failed to give any product in our hands.[18] As quantities of PQS were also required for biological testing, we carried out our synthesis based on conditions described by Pesci et al.[7]

The Duff formylation reaction proved problematic. In fact no isolable aldehyde could be obtained using the experimental conditions reported. We found using two equivalents of hexamine (HMTA) crucial to obtain a decent yield of aldehyde **3**. Oxidation of precursor **3** proceeded with moderate yield to give PQS as described.[7] For the first time X-ray crystallographic data were obtained for PQS (Fig. 2).[19] Interesting dimeric H-bonding indicates the potential for similar interactions in biological systems.

Chlorination of HHQ occurred smoothly using sodium dichloroisocyanurate.[20] Bromination also proceeded in reasonable yield with either pyridinium tribromide (PTB) or Br_2. Iodination with I_2 in basic THF afforded **6**.[21] The anticipated low reactivity associated with the sterically demanding neighbouring alkyl chain never materialised in these reactions. Furthermore, iodide **6** could be easily methylated under standard conditions affording **7** in 67% yield (Scheme 2).[22] To our delight, Pd-cross-coupling reactions could be carried out on **7**. Using $Pd(PPh_3)_4$ as catalyst a phenyl group could be introduced at the 3-position.[23]

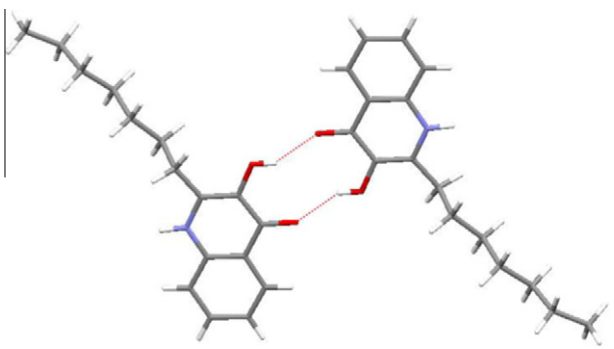
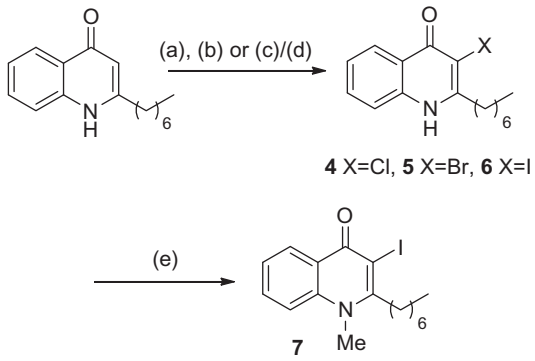


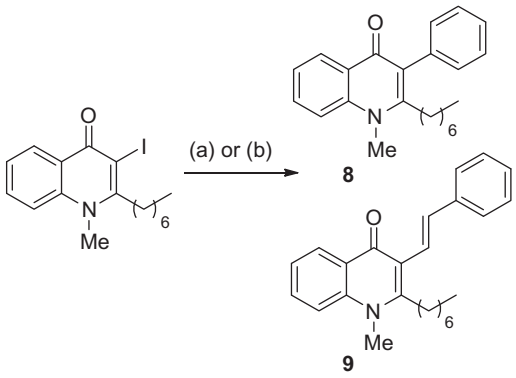
Figure 2. X-ray crystal structure of PQS depicting dimeric H-bonding.[19]



Scheme 2. Reagents and conditions: (a) $C_3Cl_2N_3NaO_3$, 2 M NaOH, MeOH, H_2O, 59% (b) PTB, AcOH, 68% (c) Br_2, 1 I_2 crystal, AcOH, 44% (d) I_2, Na_2CO_3, THF, 48% (e) NaH, DMF, MeI.

After heating at 130 °C for 30 min, palladium black was seen to precipitate and the reaction was stopped. The coupled product **8** was isolated in 50% yield.[24] An alkenyl group was also introduced using the Mizoroki–Heck reaction, iodide **7** and styrene giving alkene **9**. Two catalytic systems were tried over 16 h at 100 °C using $Pd_2(dba)_3$.dba and $Pd(PPh_3)_4$ with NMR analysis indicating conversions of ca. 15% and 30%, respectively. Using $Pd(PPh_3)_4$ at 120 °C only improved the conversion to 60% with an isolated yield of 52% (Scheme 3). No further optimisation was carried out.

In conclusion, we have described the synthesis of >10 g quantities of HHQ, its halogenation at the 3-position, subsequent



Scheme 3. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, DMF, 2 M Na_2CO_3, 50% (b) styrene, $Pd(PPh_3)_4$, NMP, Et_3N, 52%.

N-methylation and finally Pd-cross coupling of 3-iodo-HHQ. The crystal structure of the prominent biological agent PQS is also described. These compounds and analogues are currently undergoing full biological evaluation, which will be reported in due course.

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19. Notable features include a dimeric structure with two moderate strength hydroxy-carbonyl intermolecular H-bonds with a discrete amino carbonyl H-bond capping the dimer. A second crystallographically different dimer was also observed (omitted in diagram for clarity). The data has been deposited at the CCDC 780780.
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21. Example of halogenation: 3-Iodo-2-heptylquinolin-4(1*H*)-one: A mixture of HHQ (0.2 g, 0.823 mmol), iodine (0.418 g, 1.646 mmol) and Na_2CO_3 (0.131 g, 1.235 mmol) in THF (4 mL) was stirred at rt for 18 h. The mixture was quenched with $Na_2S_2O_3$ (0.613 g, 3 equiv) and the precipitate was collected by filtration before washing with ice-cold H_2O (50 mL). Recrystallisation was carried out (EtOH) affording **6** (146 mg) in 48% yield. Mp: 241–243 °C. IR ν_{max} (KBr): 3210, 3060, 2923, 2851, 2360 1628, 1578, 1555, 1497, 1473, 1435 cm^{-1}; ^1H NMR (400 MHz CD_3SOCD_3) δ: 0.86 (3H, t, *J* 8.5), 1.27–1.42 (8H, m), 1.68 (2H, m), 2.91 (2H, t, *J* 9.9), 7.33–7.38 (1H, m), 7.58 (1H, d, *J* 10.1), 7.65–7.7 (1H, m), 8.07 (1H, d, *J* 8.7), 12.08 (1H, br s); ^{13}C NMR (400 MHz CD_3SOCD_3) δ: 13.9, 22.0, 27.9, 28.3, 28.6, 31.1, 38.7, 85.7, 117.8, 120.6, 123.8, 125.5, 131.9, 139.0, 154.6, 173.2. Exact mass calcd for $C_{16}H_{21}INO$ $(M+H)^+$, 370.0668. Found 370.0656.
22. N-Methylation: 2-Heptyl-3-iodo-1-methylquinolin-4(1*H*)-one: A stirred suspension of **6** (120 mg, 0.446 mmol) in dry DMF (3 mL) was treated with NaH (60% dispersion, 1.5 equiv) at room temperature under a nitrogen atmosphere then stirred at 40 °C for 5 h. The mixture was treated with iodomethane (1.5 equiv, 69 mg) and stirred for 12 h at 40 °C. The mixture was quenched with cold H_2O. The product was extracted with $CHCl_3$, washed with brine and dried ($MgSO_4$). The solvent was evaporated and the product was purified using column chromatography (1:1 hexane/EtOAc) affording **7** (82 mg) in 66% yield. Mp: 67–69 °C. IR ν_{max} (KBr): 3374, 2926, 2854, 2361, 1617, 1592.8, 1519, 1462 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ: 0.91 (3H, t, *J* 6.9), 1.34 (6H, m), 1.53 (2H, m), 1.68 (2H, m), 3.22 (2H, t, *J* 7.9), 3.89 (3H, s), 7.36 (1H, t, *J* 7.1), 7.52 (1H, d, *J* 8.6), 7.62–7.65 (1H, m), 8.44 (1H, d, *J* 6.6); ^{13}C NMR (400 MHz $CDCl_3$) δ: 14.1, 22.6, 27.6, 28.9, 29.6, 31.7, 36.7, 40.1, 90.4, 115.3, 122.6, 124.2, 127.9, 132.4, 140.9, 155.0, 173.8. Exact mass calcd for $C_{17}H_{23}INO$ $(M+H)^+$, 384.0824. Found 384.0806.
23. For a similar reaction, see: Mphahele, M. J.; Nwamadi, M. S.; Mabeta, P. *J. Heterocycl. Chem.* **2006**, *43*, 255.
24. Example of Pd-coupling: 2-heptyl-1-methyl-3-phenylquinolin-4-one: A stirred mixture of **7** (55 mg, 0.143 mmol), phenylboronic acid (2 equiv, 35 mg) and $Pd(PPh_3)_4$ (5 mol %) in DMF (2.5 ml) and aqueous 2 M Na_2CO_3 (1.5 mL) was heated at 130 °C for 2 h and then cooled to room temperature. The mixture was poured into ice-cold H_2O and the precipitate was taken-up into $CHCl_3$, washed with brine and dried. The solvent was evaporated and the product was purified using column chromatography (1:1 hexane/EtOAc) affording **8** (24 mg) in 50% yield. Mp: 211–215 °C. IR ν_{max} (KBr): 2926, 2854, 1618, 1592, 1538, 1498 cm^{-1}; ^1H NMR (400 MHz $CDCl_3$) δ: 0.85 (3H, t, *J* 7.2), 1.19 (8H, m), 1.55 (2H, m), 2.63 (2H, t, *J* 8.2), 3.83 (3H, s), 7.20–7.45 (6H, m), 7.55 (1H, d, *J* 8.6), 7.65–7.75 (1H, m), 8.5 (1H, dd, *J* 1.4, 8); ^{13}C NMR (400 MHz $CDCl_3$) δ: 14.0, 22.6, 28.5, 28.9, 29.4, 31.5, 31.8, 35.0, 115.2, 123.3, 124.3, 126.2, 127.0, 127.3, 128.4, 130.7, 132.0, 137.2, 141.6, 152.4, 176.4. Exact mass calcd for $C_{23}H_{28}NO$ $(M+H)^+$, 334.2171. Found 334.2164.