Electronic Supplementary Material (ESI) for Chemical Communications

Detection of Pseudomonas Quinolone Signal (PQS) by cyclic voltammetry and amperometry using a boron doped diamond electrode

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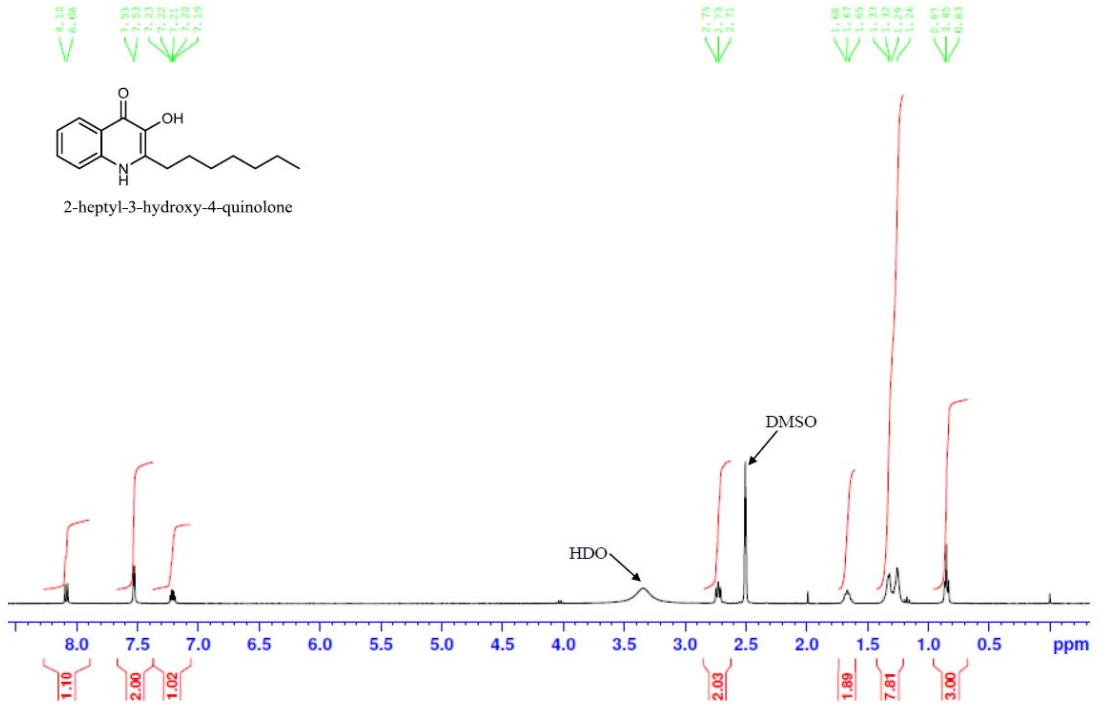
Supplementary Material

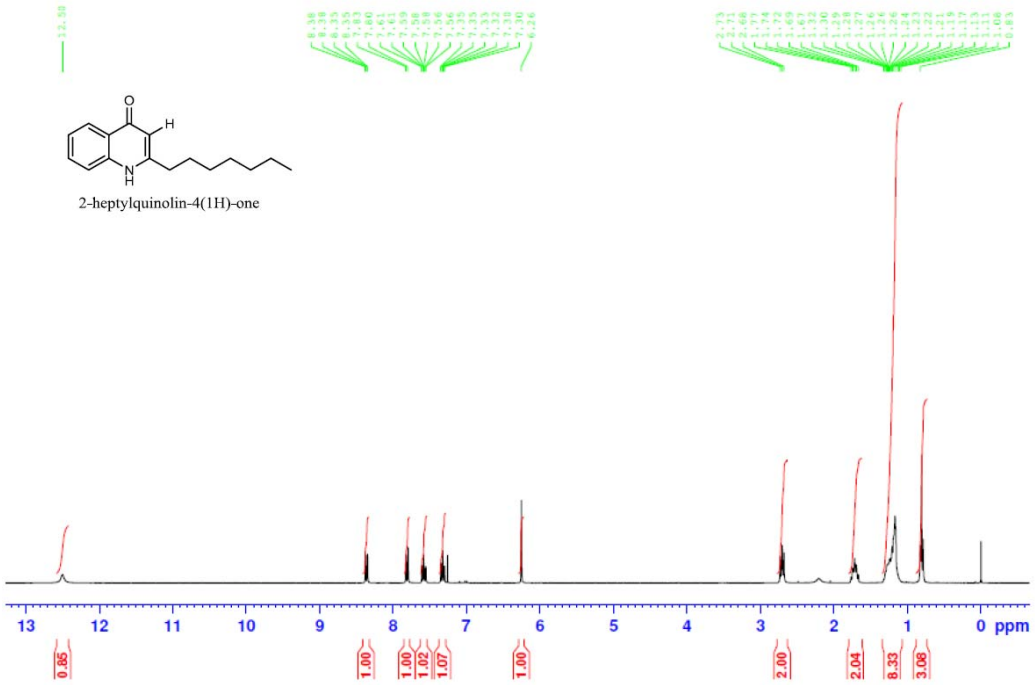
1. Chemical synthesis:

The compounds in this study are known. PQS and HHQ were synthesised by published methods. [1] as were the C-2 methyl analogues quinolone 1[2] and 2.[3] All analysis was in full agreement with that previously reported. ^{1}H spectra (300 MHz, in DMSO-δ6) shown below.

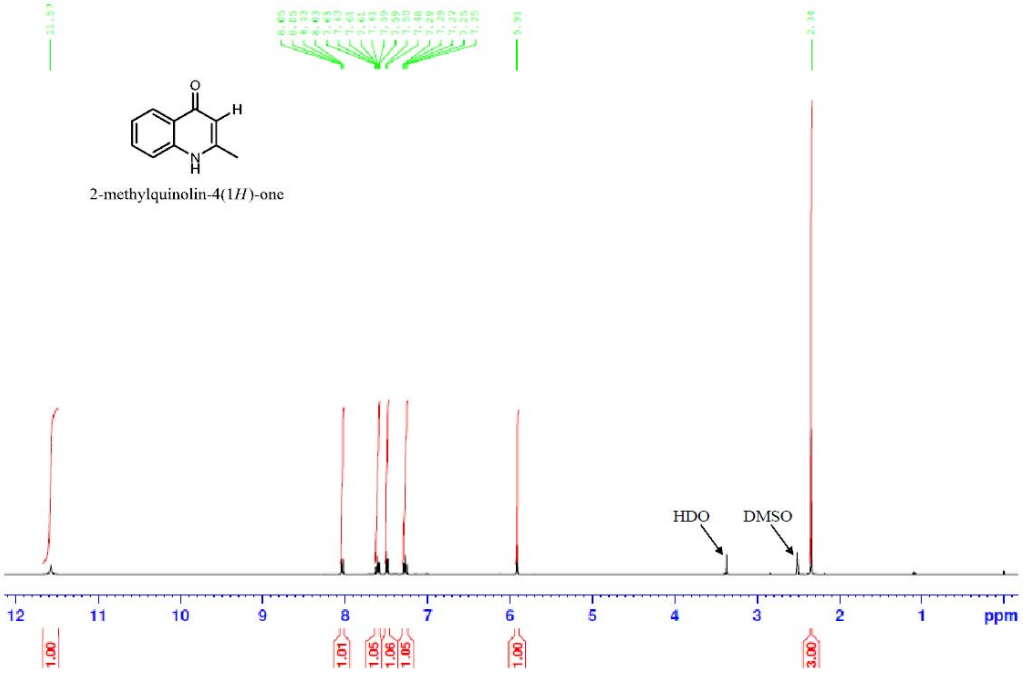
PQS



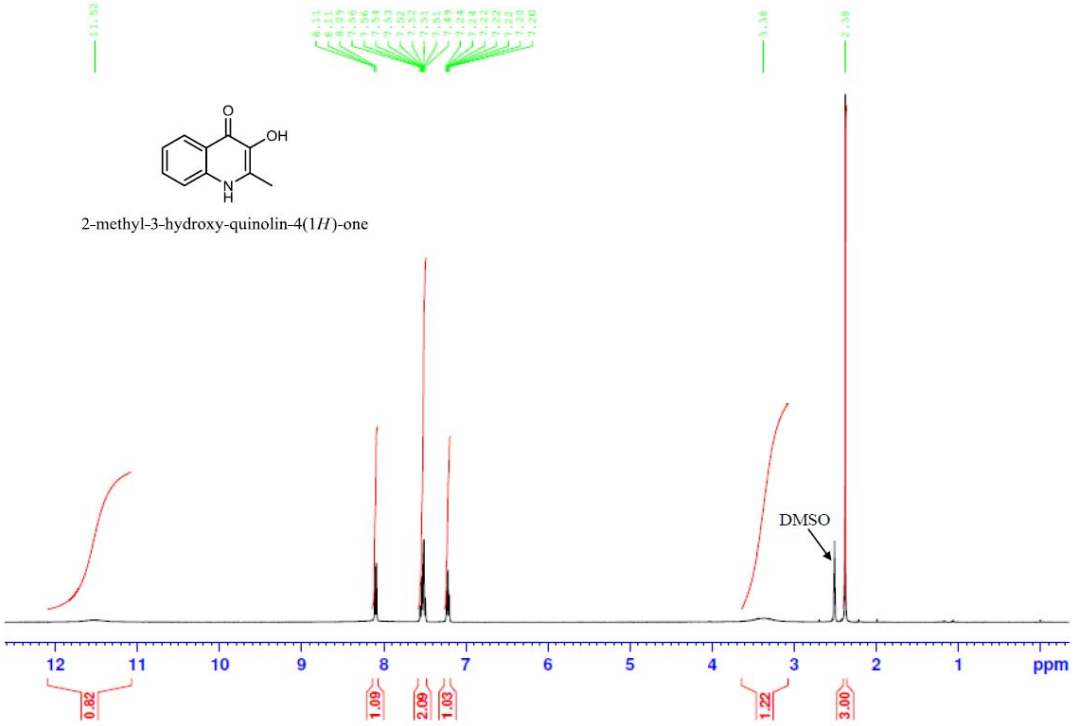
HHQ



Quinolone 1



Quinolone 2



2. Methods of analysis

Phosphoric acid (H_3PO_4), sodium phosphate monobasic (NaH_2PO_4), sodium phosphate dibasic (Na_2HPO_4), sodium hydroxide (NaOH) and acetonitrile (ACN) were purchased from Sigma-Aldrich (Dublin, Ireland). Deionized water (18.2 MΩ·cm) was obtained from a Milli-Q (Millipore, Ireland) water purification system. All reagents were of analytical grade with highest purity. Unless otherwise stated, a 15 mM $H_3PO_4$was adjusted to pH 2with 1M HCl and mixed with ACN by 50% supporting electrolyte. The other pH of buffer is prepared by adjusting 15 mM H_3PO_4 with either 1M HCl or 1M NaOH. The standard stock solution (5.0 mM) of the HHQ and PQS were prepared daily in supporting electrolyte.

Electrode preparation

BDD, 3 mm diameter, 0.1% doped boron (Windsor Scientific, Slough, Berkshire, U.K.) was polished with polishing paper (grid 2000, Hand American Made Hardwood Products, South Plainfield, NJ) and subsequently with alumina (Buehler, UK) until a mirror finish was obtained. After thorough rinsing with deionized water, the electrode was sonicated in 2-propanol and deionized water for 5 and 10 min, respectively. The electrode was transferred to an electrochemical cell for cleaning by cyclic voltammetry between -0.5 and +2.0V versus Ag/AgCl (3M NaCl, BAS, West Layette, IN) at 0.1 V s^{-1} in 50 mM phosphate buffer, pH 7 until a stable CV profile was obtained.

Instrumentation

Amperometric measurement (I/t) and cyclic voltammerty (CV) were performed using a CHI 1040 A electrochemical workstation (CH Instruments, Austin, TX) at room temperature. The three-electrode system consists of a boron doped diamond electrode (Windsor Scientific, Slough, Berkshire, U.K., an Ag/AgCl (3M NaCl) reference electrode (BAS, West Layette, IN) and a Pt wire counter electrode (Sigma, Dublin, Ireland). The convective transport during the amperometric determination was performed with magnetic stirring at 800 rpm.

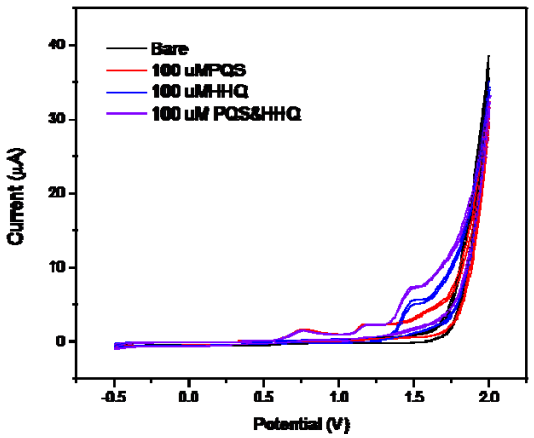


Figure 1: Cyclic voltamogrammagrams of a mixture of PQS and HHQ

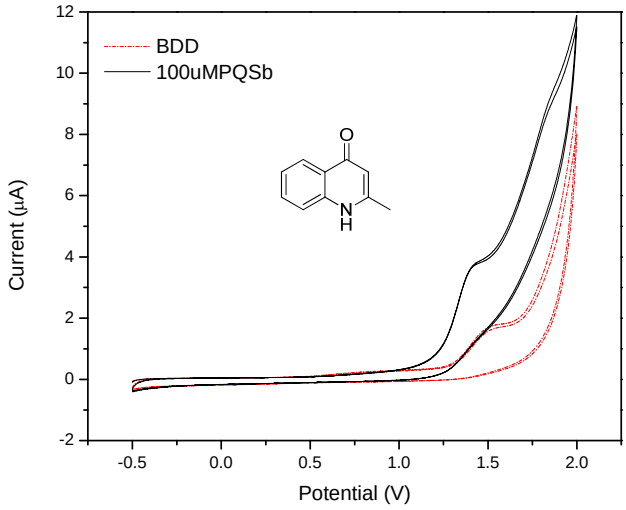


Figure 2: Cyclic voltamogrammagrams of quinolone 1

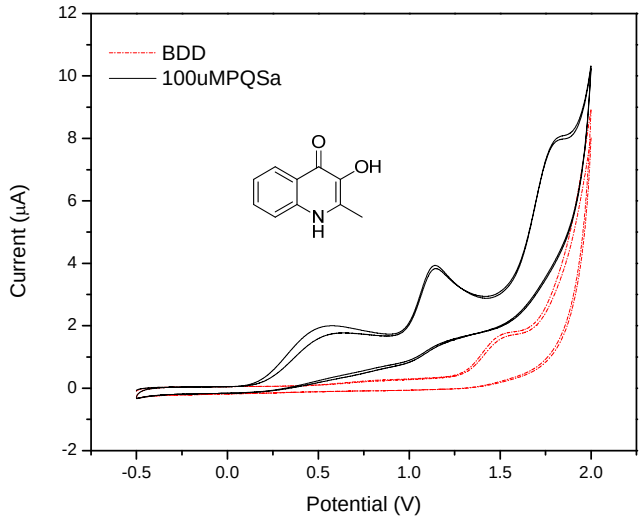


Figure 3: Cyclic voltamogrammagrams of quinolone 2

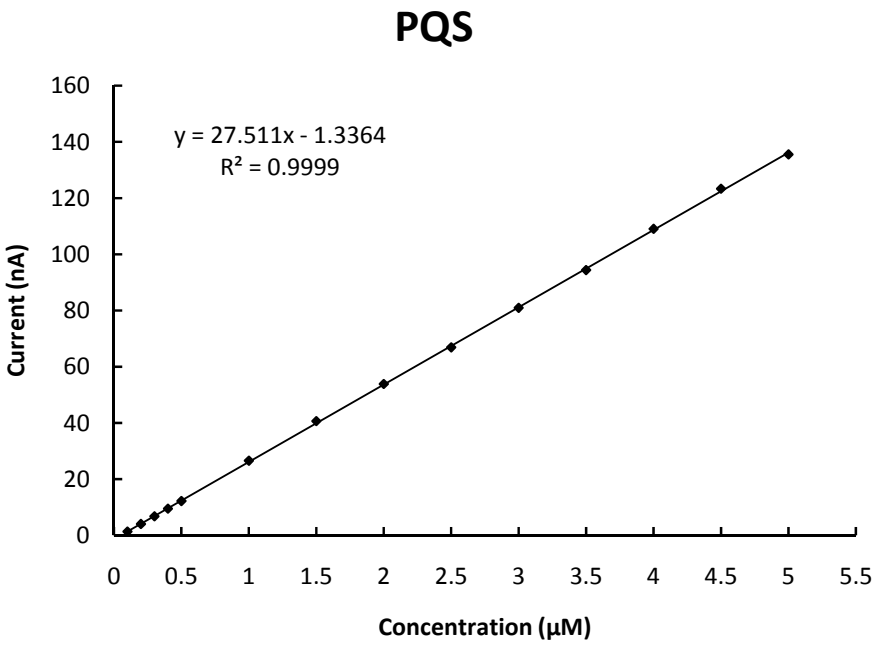


Figure 4: Calibration curve for PQS

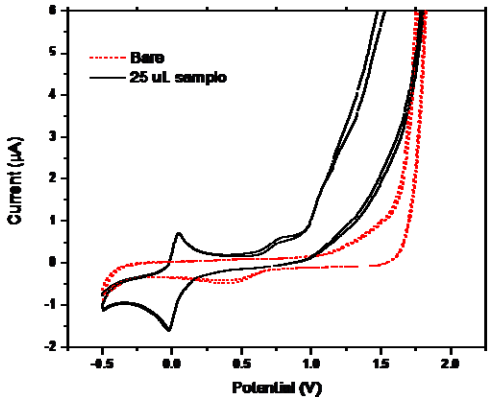


Figure 5: Cyclic voltamogrammagrams of supernatant from *P. aeruginosa pqsL*$^-$

3. Preparation of biological samples

Supernatant extracts for PQS analysis were obtained using a modified version of the Fletcher protocol.[4] Briefly, cultures of Pseudomonas aeruginosa PA14 pqsL$^-$ mutant strain were incubated overnight in Luria Bertani broth at 37°C (Total 40 mL). Culture supernatants were obtained by centrifugation (5000 rpm for 10 mins) and subsequently filter sterilised using Minisart (Sartorius) 0.2 μM filters into clean 50 ml centrifuge tubes. An equal volume of acidified ethyl acetate [0.01% (v/v) glacial acetic acid] was added to the cell-free supernatant and vortexed for 30 s, after which samples were separated into two phases by centrifugation (5000 rpm for 5 mins). The top organic phase was removed and the process repeated a further two times to maximise the extraction (Total 40 mL). The samples could be used directly in EtOAc or evaporated to dryness.

References

(1) (a) McGlacken, G. P.; McSweeney, C. M.; O'Brien, T.; Lawrence, S. E.; Elcoate, C. J.; Reen, F. J.; O'Gara, F. *Tetrahedron Lett.* **2010**, *51*, 5919(b) Pesci, E. C.; Milbank, J. B. J.; Pearson, J. P.; McKnight, S.; Kende, A. S.; P., G. E.; Iglewski, E. H. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 11229.

(2) Cross, M. R.; Manetsch, R. *J. Org. Chem.* **2010**, *75*, 8654.

(3) Hradil, P.; Hlaváč, J.; Lemr, K. *J. Heterocyclic Chem.* **1999**, *36*, 141.

(4) Fletcher, M. P.; Diggle, S. P.; Cámara, M.; Williams, P. *Nat. Prot.* **2007**, *2*, 1254.