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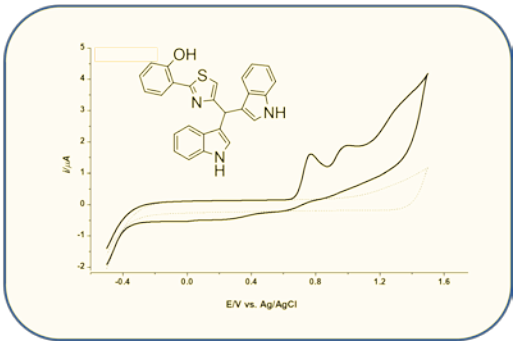
Graphical Abstract

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Synthesis and sensitive electrochemical detection of a thiazolyl-indole natural product isolated from the nosocomial pathogen *Pseudomonas aeruginosa*

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Synthesis and sensitive electrochemical detection of a thiazolyl-indole natural product isolated from the nosocomial pathogen *Pseudomonas aeruginosa*

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ABSTRACT

Pseudomonas aeruginosa, a gram-negative opportunistic pathogen, capable of surviving in a broad range of natural environments, is an antibiotic-resistant human pathogen associated with hospital-acquired infections. Simple and sensitive methods for the detection of biomolecules as indicators of *P. aeruginosa* infection would be of great clinical importance. Here we report the synthesis of the *P. aeruginosa* natural product, barakacin, which was recently isolated from the strain ZIO. A simple and sensitive electrochemical method was used for barakacin detection using boron-doped diamond, and glassy carbon electrodes, based on cyclic voltammetry and differential pulse voltammetry.

1. Introduction

The versatile and ubiquitous *Pseudomonas aeruginosa*, a gram-negative, multidrug resistant bacterium, is the third most common cause of nosocomial infections in Europe after *Escherichia coli* and *Klebsiella pneumoniae*, respectively.[1] It is also the primary cause of morbidity and mortality in cystic fibrosis patients.[2-4] *P. aeruginosa* expresses a large number of extracellular virulence factors, such as lipases, proteases and toxins that cause tissue damage, delay wound healing, and produce an innate immune response.[5] Quorum sensing (QS) is a chemical cell-to-cell communication process used by bacteria regulated by small extracellular signalling molecules, which allows populations of bacteria to collectively control gene expression and synchronize group behaviour in a cell density-dependent manner. QS signals are small diffusible molecules, which act as a means of intercellular communication to co-ordinate bacterial behaviour such as secondary metabolite production, virulence, biofilm formation and swimming and swarming motility.[6, 7] A significant feature of *P. aeruginosa* infection is the formation of biofilms (microcolonies surrounded by an exopolysaccharide alginate), which is partially controlled by QS molecules.[8] Biofilm acts as a direct barrier to phagocytic cells and offers innate resistance to antibiotics and disinfectants.[9] An important class of small molecules which are involved in bacterial communication systems are the 2-alkyl-4(1H)-quinolones (AHQs), primarily the Pseudomonas Quinolone Signal, (PQS), **1**, and its biosynthetic precursor, HHQ, **2** as illustrated in **Fig. 1**.[10, 11] More recently, Lee *et al.* reported the identification of a new potential QS signal, 2(2-hydroxyphenyl)-thiazole-4-carbaldehyde **3**, which they gave the name IQS.[12]

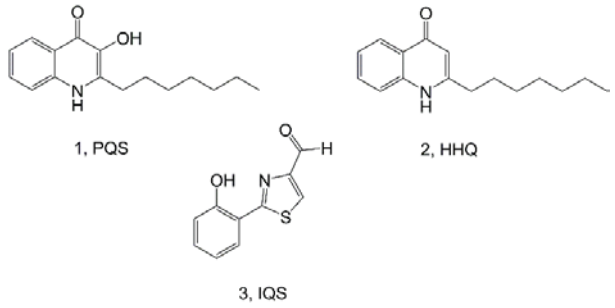


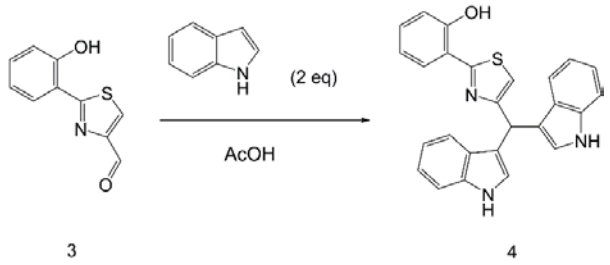
Figure 1. The structure of *P. aeruginosa* AHQ signals.

As QS molecules, PQS, HHQ and IQS could act as potential biomarkers for the bacteria *P. aeruginosa* and allow for early medical intervention, perhaps prior to the onset of biofilm formation.

Traditional methods for the positive determination of *P. aeruginosa* include the expensive and time-consuming culture growth method. Alternatively, the use of standard laboratory markers have been implemented.[13, 14] Although offering a number

of advantages over culture growth, the routine employment of molecular (PCR) detection of *P. aeruginosa* directly from the sputum of CF patients represents a time-intensive and very costly procedure. A recent biosensor-based detection of PQS and HHQ has recently been reported by Williams.[15] Their complex bioreporter can identify HHQ and PQS with a detection limit of 12 nM. We have previously reported the detection of HHQ and PQS using a boron doped diamond electrode.[16] We have also synthesised and investigated the electrochemical profile of IQS.[17] Ultimately, these methods of analysis allow for a quantitative description of the bacterial profile, facilitating a more informed clinical assessment.

Recently, Zendah *et al.* reported the isolation of a new thiazolyl-indole alkaloid from a ruminal *P. aeruginosa* strain.[18, 19] The molecule was identified as 2-{4-[bis-(1H-indol-3-yl)-methyl]-thiazol-2-yl}-phenol **4** (**Fig. 2**), and was given the name barakacin. Barakacin showed negligible antibacterial activity against selected gram-positive bacteria compared to structurally similar thiazole alkaloids, while it exhibited a weak, unselective cytotoxic activity against a range of human cancer cell lines. However to us, it possessed a chemical framework likely to display strong electrochemical properties. We identified a rational synthesis from IQS **3** via the condensation of the heteroaryl aldehyde with indole in the presence of acid (**Fig. 2**).[20] Thus IQS **3** was reacted with 2 equivalents of indole at room temperature in acetic acid giving barakacin **4** in 42% yield after purification (see SI for details).



3 4

Figure 2. Synthesis of barakacin.

With barakacin in hand, we then focused on its electrochemical properties using 1) boron-doped diamond (BDD) and 2) glassy carbon (GC) electrodes. The BDD electrode has superior features such as high current density, wide potential range, low background current, extreme electrochemical stability and high resistance to fouling. [21, 22] The GC electrode displays good electrical conductivity and positive potential range, low porosity and permeability to gases, high biocompatibility and hardness.[23]

The redox behaviour of barakacin was characterised using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The CV presented two well-defined oxidation peaks at +0.76 V, +0.97 V and a third broad peak in the potential range of +1.2 V to +1.4 V at pH 2.0 on the BDD electrode (**Fig. 3A**). Similarly, the CV of barakacin on the GC electrode showed two distinct anodic peaks at +0.70 and +0.96 V (**Fig. 3B**). The BDD electrode also detected three small reduction peaks (-0.019 V, -0.38 V and -0.62 V), compared to one small reduction peak on the GC electrode (-0.58 V). Other pH values were tested (**SI, Fig. S1**), but at higher pHs only smaller or broader peaks were observed.

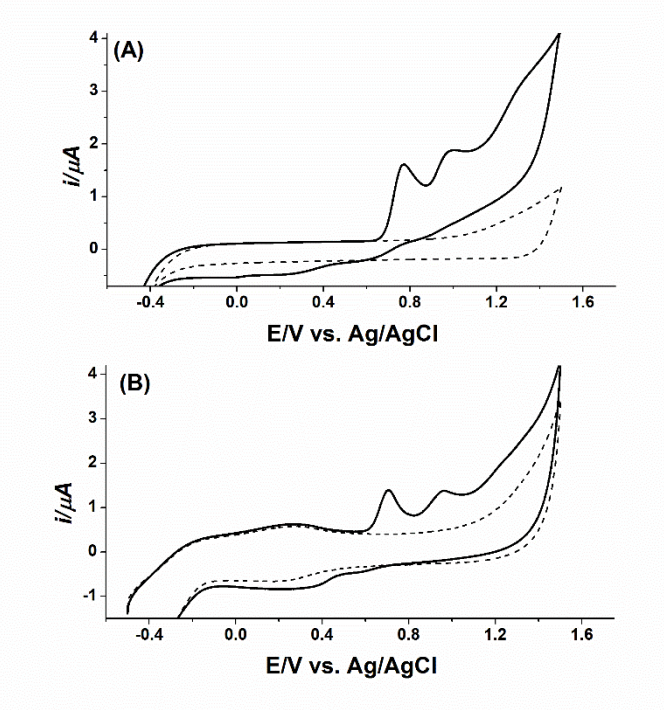


Figure 3. CV of 100 µM barakacin at the (A) BDD and (B) GC electrodes vs. Ag/AgCl in 50 mM phosphate buffer (pH 2.0) containing 20% ACN.

The pH effect on the peak potential (Ep) and peak current [23] was also investigated on the BDD and GC electrodes. It has been noticed that with increasing the pH, the peak potential (Ep) shifted to less positive values on the BDD and GC electrodes, indicating that the electrooxidation of barakacin is pH-dependent. Moreover, the peak current[23] intensity was decreased by increasing the pH. Overall, pH 2.0 was selected as the optimum buffer pH as it resulted in higher current intensity (**Fig. 4A and 4B**).

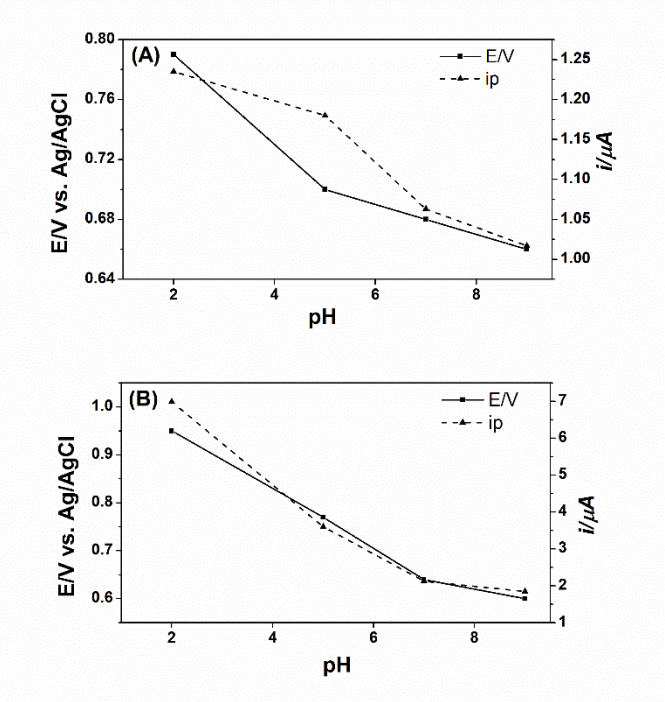


Figure 4. CV response the effect of pH on the peak potential (Ep) and peak current[23] towards 100 µM barakacin on the (A) BDD electrode vs. Ag/AgCl and (B) GC electrode vs. Ag/AgCl.

To improve sensitivity, DPV was selected for further experiments. The use of DPV indicated barakacin oxidation on the BDD electrode in the linear range 1-10 µM at pH 2.0 (**Fig. 5**), 5.0, 7.0 and 9.0 (**SI, Fig. S2**). The calibration curves were obtained with excellent linearity at different pHs. The analytical performance of barakacin oxidation on BDD electrode is shown in the SI (**Table S1)**. To obtain a calibration curve using the BDD electrode, the oxidation peaks of +0.68 V and +0.63 V were used at pH 2.0 and 5.0, respectively. The detection limits were 5, 100, 125 and 10 nM (S/N = 3) for the BDD electrode at pH 2.0, 5.0, 7.0 and 9.0, respectively. The relative standard deviation (RSD) values of the anodic peak current were 2 % to 6 %, indicating good precision for the barakacin detection by the BDD, while RSD values of the potential were 0.5 % to 1 %, showing very good repeatability for the method.

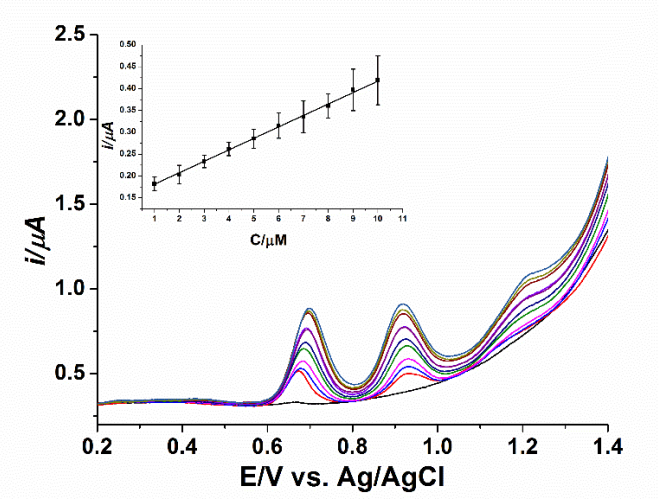


Figure 5. DPV of various barakacin concentration (1-10 µM) at the BDD electrode vs. Ag/AgCl in 50 mM phosphate buffer (pH 2.0) containing 20 % ACN.

The DPV exhibited the response of barakacin oxidation on the GC electrode at different pHs (**SI, Fig. S3**). An example, at pH 2.0 is shown in **Fig. 6**. The calibration plots were achieved with good linearity.

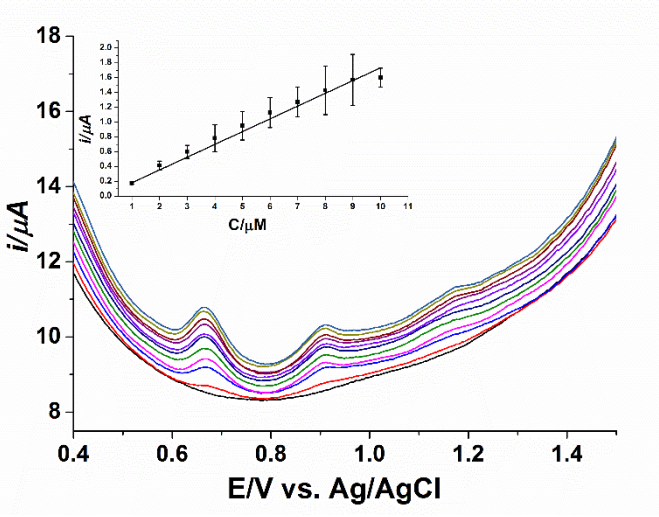


Figure 6. DPV of various barakacin concentrations at the GC electrode vs. Ag/AgCl in 50 mM phosphate buffer (pH 2.0) containing 20 % ACN.

The analytical parameters of barakacin determination on the GC electrode are presented in the SI (**SI, Table S2**). For calibration curves on the GC electrode, an oxidation peak at +0.67 V, +0.52 V and +0.57 V was chosen for pH 2.0, 5.0 and 7.0, respectively. The detection limits were 500, 600, 800 and 500 nM (S/N = 3) for the GC electrode at pH 2.0, 5.0, 7.0 and 9.0, respectively. The RSD

values obtained from the anodic peak current were 2 % to 34 %, demonstrating that the GC electrode is subject to surface fouling by oxidation products. However, the RSD values of the potential were 0.4 % to 0.6 %, indicating good repeatability of the method. In contrast, the BDD electrode, in addition to exhibiting remarkable sensitivity at a wide pH range, also displayed low background current and excellent repeatability compared to the GC electrode.

In conclusion, the synthesis of a bioactive compound from *P. aeruginosa* ZIO, barakacin, has been completed. A simple electrochemical analytical method was employed for its detection using the BDD and GC electrodes. The BDD electrode shows remarkable sensitivity for barakacin. Studies on the detection of barakacin from incubated *P. aeruginosa* isolates and from both industrial and clinical samples are underway and will be reported in due course.

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