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# Quorum sensing inhibitory activities of surface immobilized antibacterial dihydropyrrolones *via* click chemistry

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ABSTRACT

Device-related infection remains a major barrier to the use of biomaterial implants as life-saving devices. This study aims to examine the effectiveness and mechanism of action of surface attached dihydropyrrolones (DHPs), a quorum sensing (QS) inhibitor, against bacterial colonization. DHPs were covalently attached on glass surfaces *via* copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) click reaction. The covalent attachment of DHP surfaces was confirmed by X-ray photoelectron spectroscopy (XPS) and contact angle measurements, and the antimicrobial efficacy of the DHP coatings was assessed by confocal laser scanning microscopy (CLSM) and image analysis. The results demonstrated that covalently bound DHP compounds are effective in reducing the adhesion by up to 97% ($p < 0.05$) for both *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Furthermore, using the green fluorescent protein (Gfp)-based reporter technology, it is demonstrated that surface attached DHPs were able to repress the expression of a *lasB-gfp* reporter fusion of *P. aeruginosa* by 72% ($p < 0.001$) without affecting cell viability. This demonstrates the ability of the covalently bound QS inhibitor to inhibit QS and suggests the existence of a membrane-based pathway(s) for QS inhibition. Hence, strategies based on incorporation of QS inhibitors such as DHPs represent a potential approach for prevention of device-related infections.



## 1. Introduction

Bacterial infections associated with the use of biomedical devices present a serious ongoing problem [1,2]. For example, the emergence of multi-drug resistant bacteria results in reduced efficacy of antibiotic prophylaxis and/or treatments [3]. Consequently, there is a clear need to develop new strategies to reduce infection associated with the use of in-dwelling biomaterials. One strategy involves impairing the bacterial communication system of gene regulation termed quorum sensing (QS). It has been found that bacterial QS controls fundamental bacterial processes including bacterial physiology, adhesion, biofilm formation and virulence [4,5]. There is increasing evidence that QS plays an integral role in bacterial pathogenesis and is a major cause of persistent infections [6–8]. Several studies have also demonstrated QS-deficient bacteria produce less virulence factors and result in milder infections as compared to their wild-type counterparts [9–12]. For example in *Pseudomonas aeruginosa*, the *las* QS system is responsible for the production of a number of virulence factors [13] and the ability of the bacterium to form biofilms [14]. More importantly, disruption of QS does not affect the viability of bacteria [15,16], therefore it is less likely to induce selective pressures for the development of resistance. Hence, coatings designed to disrupt QS and inhibit biofilm formation of bacterial pathogens represent new approaches for the prevention of device-related infections.

Dihydropyrrolones (DHPs) derived from natural QS inhibitors, fimbrolides produced from the marine red alga *Delisea pulchra*, have been shown to interfere with QS in various pathogens while exhibiting low cytotoxicity towards mammalian cells [17]. Fimbrolides have been shown to displace the native bacterial signal, *N*-acyl homoserine lactone (AHL), from its binding site in the signal receptor protein LasR [18] and also to interfere with other Lux-type QS systems as well as the AI-2 system [18–22]. Thus, it is believed that DHP with structural similarity to fimbrolides may act in a

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similar manner inhibiting not only the *las* system but other AHL-mediated QS systems in other Gram-negative bacteria.

Our recent studies demonstrated that DHPs were able to reduce bacterial adhesion and prevent biofilm formation of *P. aeruginosa* and *Staphylococcus aureus in vitro* and *in vivo* when covalently attached to substrates [15,16]. DHP-coated substrates were shown to reduce the pathogenic potential of staphylococcal infection in a subcutaneous infection mouse model [16]. However, the exact mechanism of action for surface immobilized DHPs remains largely unexplored.

In this study, DHPs were covalently attached onto glass substrates by a copper-catalyzed azide-alkyne 1,3-dipolar cycloaddition (CuAAC) click reaction. The CuAAC click reaction is regioselective, chemoselective and can be performed in aqueous medium at room or physiological temperature [23]. This reaction's versatility is demonstrated by its wide range of applications in polymer science [24,25], biomaterials [26] and biochemistry [27,28]. In order to identify the best methodology for attachment of DHPs, a series of alkyne- and azido-derivatives of DHPs were synthesized (Fig. 1) and covalently attached to substrates by CuAAC click reaction *via* two complimentary approaches. The ability of the surface to prevent the expression of a *Pseudomonas las* QS system was assessed. The antimicrobial activity, cytotoxicity and surface property of the resulting substrates were also examined and compared with previously developed DHP coatings.

## 2. Materials and methods

### 2.1. Synthesis of DHP derivatives

#### 2.1.1. 1-(4-Azidophenyl)-3-butyl-5-(dibromomethylene)-1H-pyrrol-2(5H)-one (azide-F8-phenyl-DHP)

A mixture of 3-butyl-5-(dibromomethylene)furan-2(5*H*)-one (furanone 8; 0.43 g; 1.39 mmol) and 4-azidoaniline (0.46 g; 2.22 mmol) were heated together at 120 °C. The residue was redissolved in $CH_2Cl_2$ and successively washed with water. The organic phase was separated and dried with sodium sulfate and the solvent evaporated *in vacuo*. The reactant residual solid was purified by chromatography on silica gel using ethylacetate/*n*-hexane to yield 1-(4-Azidophenyl)-3-butyl-5-(dibromomethyl)-5-hydroxy-1*H*-pyrrol-2(5*H*)-one as a brown solid. The lactam intermediate was then mixed with $P_2O_5$ (0.56 g; 3.96 mmol) in $CH_2Cl_2$ (10 mL) and refluxed for 2 h. The solvent was evaporated under vacuum and the residue was purified by silica gel chromatography (ethylacetate/$CH_2Cl_2$) to yield the title compound as a white solid (0.26 g; 44%).

#### 2.1.2. 3-Butyl-5-(dibromomethylene)-1-(4-(prop-2-yn-1-yloxy)phenyl)-1H-pyrrol-2(5H)-One (alkyne-F8-phenyl-DHP)

3-Butyl-5-(dibromomethylene)furan-2(5*H*)-one (furanone 8; 0.34 g; 1.10 mmol) and 1,4-bis((trimethylsilyl)oxy)benzene (1.5 g; 5.90 mmol) were heated together with stirring at 120 °C for 24 h. The mixture was cooled to room temperature, washed with HCl (2 M) and extracted into $CH_2Cl_2$. The solution was then dried with sodium sulfate and passed through a flash column with $CH_2Cl_2$ to remove any unreacted furanone 8. Finally, the product was eluted with 50% $CH_2Cl_2$/ethylacetate, and solvent removed by vacuum to yield the intermediate product 3-butyl-5-(dibromomethyl)-5-hydroxy-1-(4-((trimethylsilyl)oxy)phenyl)-1*H*-pyrrol-2(5*H*)-one as white solid (0.40 g; 74%).

The intermediate product (0.40 g; 0.95 mmol) was dehydrated with borontrifluoride diethyl etherate ($BF_3.O(C_2H_5)_2$; 300 μL; 1.12 mmol) in dry $CH_2Cl_2$ (10 mL), with stirring and refluxed for 2 h. Purification of the residue by silica gel chromatography (20% ethylacetate/$CH_2Cl_2$) yielded 3-butyl-5-(dibromomethylene)-1-(4-hydroxyphenyl)-1*H*-pyrrol-2(5*H*)-one as colorless solid needles (0.32 g; 83%). The compound (0.19 g; 0.47 mmol) was then dissolved in dry acetone (5 mL), followed by the addition of $K_2CO_3$ (0.15 g; 1.09 mmol) and propargyl bromide (80 %wt in toluene; 200 μL; 1.80 mmol). The solution was stirred and refluxed for 4 h, and the product was purified by column chromatography (50% $CH_2Cl_2$/*n*-hexane) to yield the desired product as white solid (0.21 g; 99%). M.p. 86–87 °C; $^1$H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 1.37–1.44 (m, 2H, $CH_2$), 1.58–1.63 (m, 2H, $CH_2$), 2.34–2.39 (m, 2H, $CH_2$), 2.53 (t, $J$ = 4.8 Hz, 1H, ≡CH), 4.70 (d, $J$ = 2.4 Hz, 2H, OCH2), 6.99–7.02 (m, 2H, 2 x CH aryl), 7.12–7.15 (m, 3H, 2 x CH acryl, CH). $^{13}$C NMR: δ 13.8 ($CH_3$), 22.4 ($CH_2$), 27.3 ($CH_2$), 31.4 ($CH_2$), 56.1 ($OCH_2$), 74.2 ($CBr_2$), 76.2 (≡CH), 77.5 (CCH), 119.4 (2 x CH aryl), 128.4 (C), 130.6 (2 x CH aryl), 131.8 (C), 138.9 (C), 140.2 (C), 157.5 (C), 171.9 (C=O). IR (KBr, $\nu_{max}$, $cm^{-1}$): 3256, 2958, 2921, 2848, 2126, 1895, 1701, 1606, 1588, 1509, 1463, 1449, 1380, 1293, 1241, 1193, 1175, 1122, 1031, 844, 825, 723, 682, 661, 533; HRMS (ESI) m/z Calcd. for $C_{18}H_{17}Br_2NO_2Na$ $(M + Na)^+$ 459.9529. Found 459.9527.

#### 2.1.3. 3-Butyl-5-(dibromomethylene)-1-(prop-2-yn-1-yl)-1H-pyrrol-2(5H)-One (alkyne-F8-DHP)

A solution of 3-butyl-5-(dibromomethylene)furan-2(5*H*)-one (0.41 g; 1.21 mmol) in $CH_2Cl_2$ (5 mL) was stirred in an ice bath at 0 °C followed by dropwise addition of propargylamine (0.38 mL; 5.93 mmol) in $CH_2Cl_2$ (10 mL). The solution was then heated to 40 °C and allowed to react for 2 h. The resulting brown mixture was concentrated under reduced pressure. The lactam intermediate was then mixed with $P_2O_5$ (0.72 g; 5.07 mmol) in $CH_2Cl_2$ (15 mL) and refluxed for 2 h. The solvent was evaporated under vacuum and the residue was purified by silica gel chromatography (10% ethylacetate/$CH_2Cl_2$) to yield the title compound as light brown solid (0.32 g; 70%). M.p. 36 °C; $^1$H NMR ($CDCl_3$): δ 0.95 (t, $J$ = 7.2 Hz, 3H, $CH_3$), 1.37–1.44 (m, 2H, $CH_2$), 1.58–1.63 (m, 2H, $CH_2$), 2.24 (t, $J$ = 4.8 Hz, 1H, ≡CH), 2.34–2.39 (m, 2H, $CH_2$), 4.78 (d, 2H, $-CH_2-$), 7.04 (s, 1H, H4); $^{13}$C NMR: δ 13.8 ($CH_3$), 22.4 ($CH_2$), 25.3

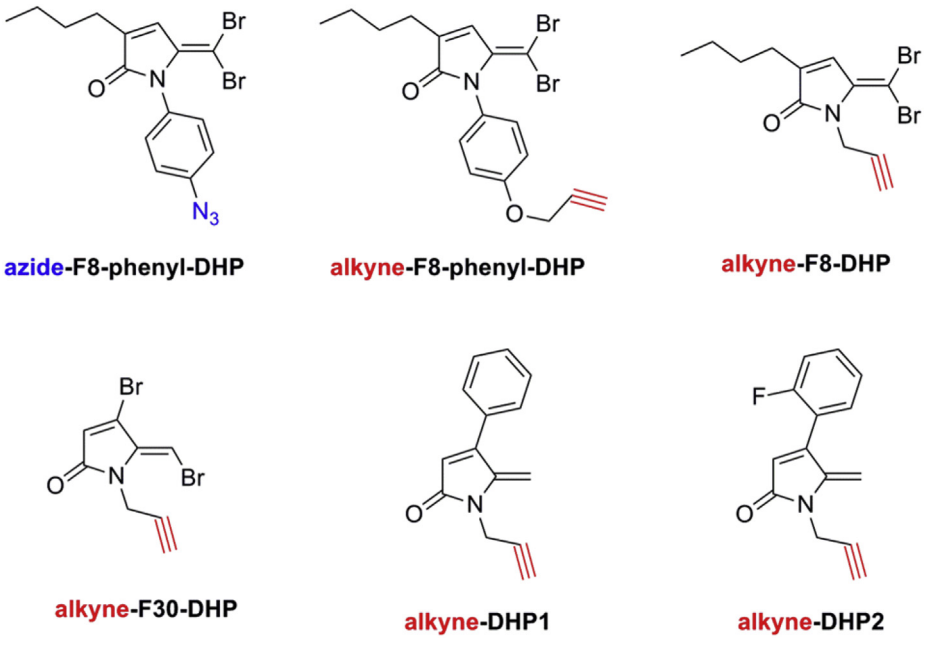


**Fig. 1.** Chemical structure of the azide-/alkyne-DHP derivatives.