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Alkyne-Substituted Dihydropyrolones as Bacterial Quorum Sensing Inhibitors

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Abstract: Bacteria regulate their virulence factor production and biofilm formation through an intercellular communication system mediated by the binding of signaling molecules to QS receptors such as LasR. A range of natural and synthetic brominated furanones known as fimbrolides and their dihydropyrolones counterparts have been found to act as inhibitors of QS-dependent bacterial phenotypes. In this study, a range of dihydropyrrolone (DHP) analogues were synthesized *via* the lactone-lactam conversion of lactone intermediates followed by the formation of novel acetylene analogues of dihydropyrrolones. New novel alkyne analogues of DHPs with various substitution patterns and aliphatic chain lengths were successfully synthesised via lactamisation and Sonogashira coupling reactions in moderate to high yields. The Sonogashira reaction was carried out with DHPs and alkynes in the presence of CuI, palladium catalyst $PdCl_2(PPh_3)_2$ and TEA. Biological testing demonstrated that several compounds showed low to moderate activity against the *P. aeruginosa* MH602 reporter strain with little bactericidal effect. The present study represents the first application of the Sonogashira reaction to DHP scaffolds for the synthesis of novel bacterial QS inhibitors.

Keywords: Dihydropyrrolone (DHP), Alkyne-containing dihydropyrrolone **;** Sonogashira coupling reactions**;** Quorum sensing**;** Quorum sensing inhibition**;** Pseudomonas aeruginosa

1. Introduction

Fimbrolides such as **1** and **2** have been shown to act as QS inhibitors of AHL mediated QS phenotypes[1-2] but are limited by their instability, ease of hydrolysis and toxicity (Figure 1). [3] Fimbrolides can be useful as a starting template suitable for chemical manipulation into 5-dihydropyrrol-2-ones (DHPs) such as compound **3**. Examples of reported dihydropyrolones with QSI activity are compounds 4a-c (Figure 1).[4] A number of synthetic analogues of DHPs were synthesised and have demonstrated good QS inhibition against *P. aeruginosa*[4] and *E. coli*[5] and antibiofilm activities with minimal effect on bacterial growth.[5] Moreover, fimbrolides frequently contain brominated exocyclic alkene at the 5-position, which can be exploited for derivatisation, such as the introduction of an acetylene group *via* the Sonogashira coupling reaction.

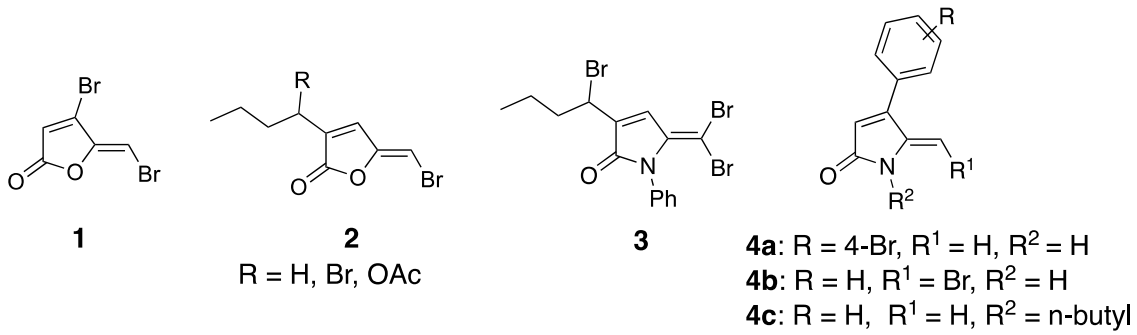


Figure 1. Furanones and DHP examples.

The rationale of the design was to study the effect of an acetylene group at the 5-position of the DHP on the biological or the QS activity. This was based on a previous study in our group where a lactone derivative or furanone 30 containing an acetylene group was found to have enhanced biological activity. The acetylene functionality is frequently encountered in many bioactive natural and synthetic products. Acetylene groups are commonly used in medicinal chemistry for their electronic effects, equivalence to aromatic rings and structural rigidity[6]. In a recent paper by our group, acetylene-containing furanones were found to exhibit low to moderate quorum sensing (QS) inhibitory activity.[7] We also previously reported the QS inhibitory activity of dihydropyrrolone compounds (e.g compound **4**, Figure 1).[4] Prompted by these previous findings[4, 7], in this study, we investigated novel alkyne-substituted analogues of dihydropyrrolones.

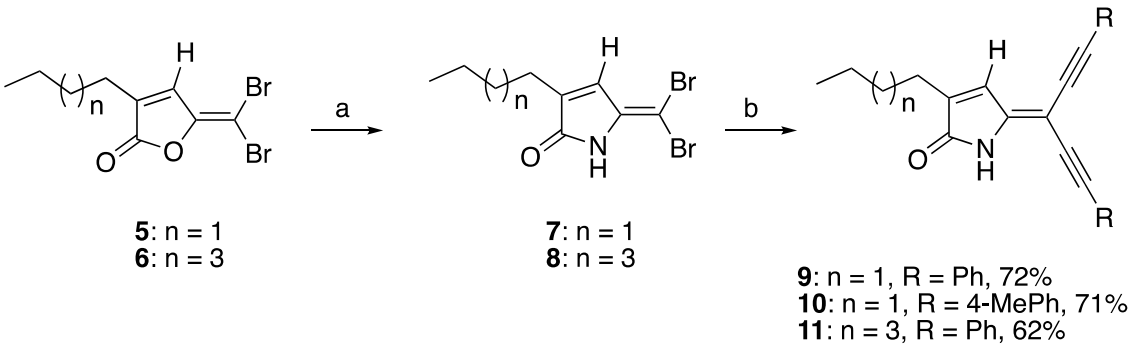
2. Results and Discussion

2.1. Synthesis

5-Dibrominated furanones, first synthesised in 1997 by Manny et al.[8], are known to possess antimicrobial and quorum sensing inhibitory (QSI) activities.[1-2, 9] Furthermore, we have previously developed a method to convert brominated furanones into DHPs via a ring-opening/ring-closing lactamisation reaction with amine nucleophiles. [10] Furanones and/or their DHP analogues were envisaged to allow the introduction of acetylene substituents via the Sonogashira cross-coupling reaction. Therefore, in this study, we investigated the Sonogashira coupling reaction of brominated DHPs with alkynes.

To synthesise the alkynylated DHPs, we envisaged that the 5-(dibromomethylene)-2(5H)-furanones **5**-**6** could undergo lactamisation with ammonia gas, followed by their dehydration to give the 5-(bromomethylene)-2(5H)-dihydropyrrolones **7**-**8** (Scheme 1). Lactamisation of **5**-**6** with aqueous ammonia was not successful unlike our previous report for synthesis of DHP derivatives.[4] This could be attributed to the presence of the bromine on the exocyclic double bond, which hindered the ring opening of the lactone to lactam.

In addition, our first attempt of lactamisation of the Sonogashira furanone products was not successful. This could be attributed to the reduced reactivity of the acetylene containing furanones towards lactamisation. Therefore, to synthesise compounds **9**-**11**, the Sonogashira reaction was carried out between brominated DHPs **7**-**8** and phenylacetylene (2.5 eq), at reflux in the presence of CuI (0.1 eq), bistriphenylphosphine)palladium(II) dichloride ($PdCl_2(PPh_3)_2$ (0.1 eq), triethylamine (TEA, 1 eq), and degassed THF by purging with argon or nitrogen gas. We found that heating was crucial for the success of the reaction and to minimize the formation of the acetylene homo-adduct. While THF was the best solvent for the synthesis of Sonogashira products, the reaction was also completed successfully in the presence of triethylamine as a solvent and a base. The NH DHPs **9**-**11** bearing two alkyne groups were obtained in 62-72 % yields (Scheme 1).



Scheme 1. Lactamisation of furanone and Sonogashira coupling reaction of compounds **9**-**11.** Reaction condition: a) ammonia gas, DCM, rt; b) $PdCl_2(PPh_3)_2$ CuI, TEA, argon, alkyne.

2.2. QS inhibition

A QS inhibition assay was performed on the DHPs to evaluate the efficacy of the newly synthesised acetylene compounds and to study the structure activity relationships (SAR). The compounds were tested against *P. aeruginosa* MH602 following the method reported by Hentzer et al.[1]

The assay uses a reporter strain that measures the level of green fluorescent protein (GFP). An inhibitor is expected to reduce the expression and the production of GFP. In this study, the liquid cultures of the reporter strain MH602 were incubated with various amounts of the synthesised compounds. The acetylene compounds reduced QS at concentrations of 250 μM, 125 μM and 62.5 μM. Moreover, the majority of the alkyne derivatives did not have a substantial effect on the viability of bacterial cells.

3. Conclusions

Fifteen novel alkyne analogues of DHPs with various substitution patterns and aliphatic chain lengths were successfully synthesised via lactamisation and Sonogashira coupling reactions in moderate to high yields and the full results will be puplished soon at another communications. The Sonogashira reaction was carried out with DHPs and alkynes in the presence of CuI, palladium catalyst $PdCl_2(PPh_3)_2$ and TEA. Biological testing demonstrated that the newly synthesised alkyne DHP showed low to moderate activity against the *P. aeruginosa* MH602 reporter strain with little bactericidal effect. The present study represents the first application of the Sonogashira reaction to DHP scaffolds for the synthesis of novel bacterial QS inhibitors.

4. Methods and Experimental

Quorum Sensing inhibition assay for PAMH602

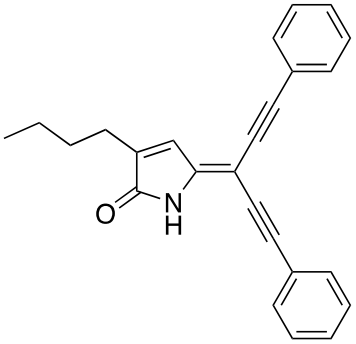
The *P. aeruginosa* MH602 PlasB::gfp (ASV) reporter strain was used. An overnight culture was prepared in Luria-Bertani (LB10) media supplemented with gentamycin (40 μM). This bacterial culture solution was diluted (1 in 100) with LB10 supplemented with gentamycin (15 μM). Stock solutions of the synthesized compounds were prepared at 20 mM in DMSO. Compounds were pipetted into each well with final concentrations of 250 μM, 125 μM and 62.5 μM (in triplicate) with a final volume of 200 μL with the prepared bacterial culture. The negative control was prepared containing 200 μL of the bacterial culture without the tested compounds. The plates were incubated at 37 °C for 15 h. The plates were measured for GFP expression (fluorescence: excitation 485 nm, emission 535 nm) using a microplate reader (Wallac Victor, Perkin-Elmer), and the cell growth was also assessed by recording the OD at 600 nm.

Sonogashira Coupling reaction procedure

A mixture of acetylene (2.5 eq), and TEA (1 eq) in THF was purged with argon or nitrogen for 20 minutes before addition of the 5-bromo DHPs (1 mmol), CuI (0.1 eq) and $PdCl_2(PPh_3)_2$ (0.1 eq) and the mixture was heated at 60 °C for 18 hours. The THF in the reaction mixture was evaporated, redissolved in DCM and was washed with 2M HCL (5 ml x 2) and the organic layer was dried over sodium sulphate. The crude mixtures were purified by flash chromatography using gradient solvent mixture of dichloromethane and hexane (10 to 50% gradient).

DHP acetylene analogues

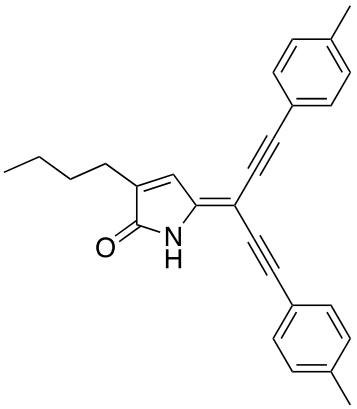
3. -Butyl-5-(1,5-diphenylpenta-1,4-diyn-3-ylidene)-1,5-dihydro-2H-pyrrol-2-one (9)



Yellow solid (72%); m.p. 215 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.93 (t, *J* = 8 Hz, 3H, CH_3), 1.38-1.48 (m, 2H, CH_3), 1.58-1.61 (m, 2H, CH_2), 2.40-2.42 (q, *J* = 8 Hz, 2H, CH_2), 7.11 (s, 1H, C4-H), 7.26-7.38 (m, 6H, ArH), 7.53-7.57 (m, 4H, ArH), 8.06 (s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.9 (CH_3),

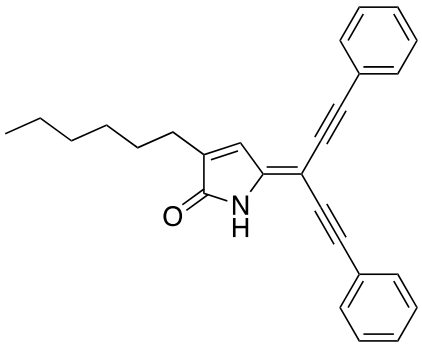
22.5 (CH_2), 25.4 (CH_2), 29.9 (CH_2), 65.86 (=C-Br_2), 83.8 (C), 86.9 (C), 93.5 (C), 97.5 (C), 122.1 (C), 122.5, 128.0 (C), 128.4 (CH), 128.5 (CH), 128.8 (C), 129.2 (C), 131.6 (CH), 131.7 (CH), 141.1 (C), 148.6 (C), 170.6 (C=O); IR (ATR): ν_{max} 752, 848, 1124, 1486, 1685, 3155; UV-VIS (MeOH): λ_{max} 390 nm (ε 12962 $cm^{-1}M^{-1}$) 295 (10741); HRMS ($C_{25}H_{21}NO$) calcd m/z 352.1696 $[M+H]^+$, obsd m/z 352.1695 $[M+H]^+$

3. -butyl-5-(1,5-di-p-tolylpenta-1,4-diyn-3-ylidene)-1,5-dihydro-2H-pyrrol-2-one (10)



Yellow semi-solid (71%); m.p. 148 °C;^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.97 (t, *J* = 8 Hz, 3H, CH_3), 1.41-1.46 (m, 2H, CH_2), 1.59-1.65 (m, 4H, CH_2), 2.42-2.46 (m, 2H, CH_2), 7.13 (s, 1H, C4-H), 7.19-7.21 (m, 4H, ArH), 7.28-7.46 (m, 4H, ArH), 7.87 (s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.8 (CH_3), 21.6 (CH_2), 22.5 (CH_2), 25.4 (CH_2), 30.0 (CH_2), 83.5 (=C-Br_2), 87.4 (C), 87.4 (C), 93.7 (C), 97.8 (C), 119.0 (C), 128.0 (C), 129.2 (CH), 131.5 (CH), 139.1 (C), 139.5 (C), 141.8 (C), 148.0 (C), 170.5 (C=O); IR (ATR): ν_{max} 753, 812, 1090, 1508, 1685, 2959; UV-VIS (MeOH): λ_{max} 390 nm (ε 5560 $cm^{-1}M^{-1}$) 300 (5370); HRMS ($C_{27}H_{25}NO$) calcd m/z 380.2009 $[M+H]^+$, obsd m/z 380.2009 $[M+H]^+$

5. -(1,5-Diphenylpenta-1,4-diyn-3-ylidene)-3-hexyl-1,5-dihydro-2H-pyrrol-2-one (11)



Yellow semi-solid (62%); m.p. 156 °C;^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.92 (t, *J* = 8 Hz, 3H, CH_3), 1.28-1.40 (m, 6H, CH_2), 1.42-1.66 (m, 2H, CH_2), 2.42-2.46 (m, 2H, CH_2), 7.14 (s, 1H, C4-H), 7.28-7.41 (m, 6H, ArH), 7.56-7.60 (m, 4H, ArH), 8.11 (s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1 (CH_3), 22.5 (CH_2), 25.7 (CH_2), 27.9 (CH_2), 29.1 (CH_2), 31.6 (CH_2), 83.8 (C), 86.9 (C), 93.5 (C), 97.5 (C), 122.1 (C), 128.0 (C), 128.5 (CH), 128.9 (C), 129.2 (C), 131.6 (CH), 141.1 (C), 148.6 (C), 170.6 (C=O); IR (ATR): ν_{max} 752, 845, 1096, 1370, 1488, 1684, 2918; UV-VIS (MeOH): λ_{max} 390 nm (ε 5655 $cm^{-1}M^{-1}$), 295 (4934); HRMS ($C_{27}H_{25}NO$) calcd m/z 380.2009 $[M+H]^+$, obsd m/z 380.2010 $[M+H]^+$

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