Quinolone Natural Products

The Synthesis of Quinolone Natural Products from Pseudonocardia sp.

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Abstract: The synthesis of four quinolone natural products from the actinomycete Pseudonocardia sp. is reported. The key step involved a sp2–sp3 Suzuki–Miyaura reaction between a common boronic ester lateral chain and various functionalised quinolone cores. The quinolones slowed growth of E. coli and S. aureus by inducing extended lag phases.

Introduction

Quinolone natural products have been noted for their biological activities since the 1950s, and in the 1980s the introduction of synthetic fluoroquinolones provided potent new antibiotics.[1] More recently, quinolones have been found to play a role in bacterial cell-cell signalling, known as quorum sensing (QS), in the pathogen Pseudomonas aeruginosa[2] which produces over 50 quinolones.[3] Since this discovery it has become apparent that other bacterial species including Burkholderia and Altermonas spp. also produce quinolones.[4] Such findings have led to the hypothesis that in the bacterial world quinolones may play an underlying role in gene regulation and inter-species modulation in complex multi-bacterial communities.[5,6] Intriguingly, both Burkholderia sp. and P. aeruginosa which can be found in the natural environment, including soil and water sources, are major pathogens in cystic fibrosis patients and form mixed biofilms.[7] Quinolones produced by P. aeruginosa have also been found to influence a number of phenotypes in other bacterial species.[6]

Considering this role, and our research interests involving quinolone signalling and bacterial signaling,[8] we became interested in the natural quinolones produced by the actinomycete Pseudonocardia sp. CL38489, isolated from a soil sample in India by Dekker et al.[9] (Figure 1). A number of these quinolone natural products were originally noted for their potent antibacterial activity against the Gram-negative bacteria Helicobacter pylori, which is implicated in pathogenesis of chronic gastritis, peptic ulcers and gastric cancers.[9] Quinolones 1–4 also bear structural resemblance to a number of natural alkyl-substituted quinolones produced by P. aeruginosa and Burkholderia spp.[3,4]

We envisaged a divergent total synthesis[10] that will be efficient for the production of quinolone natural products 1–4 for further biological evaluation of their effects on QS, inter-species signaling, and antibacterial activities. The first total synthesis of 1 was recently reported by Abe et al.[11] when synthetic efforts within our group was also aiming at these natural products.

Scheme 1. Retrosynthetic analysis of quinolones 1–4. Disconnection b proved successful.
Our retrosynthetic analysis of quinolones 1–4, similarly to Abe et al., incorporated three main disconnection strategies (a, b and c, Scheme 1). Disconnection (a) involved a final intramolecular cyclisation step with 5 to set up the quinoline core. Disconnection (b) would require the preparation of halogen functionalised quinoline building blocks 6 and a common boronic ester lateral chain 7 that would be combined via palladium sp²–sp³ coupling chemistry. Cross-couplings of this sort are notoriously challenging; however, this would enable a very expedient and modular route towards the quinoline natural products that should prove highly amenable to analogue preparation. Disconnection (c) required quinoline building blocks of the type 9 and the commercially available ketone 8, which could be converted into the final quinolone natural products utilizing Wittig chemistry.

Results and Discussion

Initial studies focused upon the exploration of disconnection strategies (a) and (c) (Scheme 1). Unfortunately, these both proved to be unsatisfactory. Under various cyclisation conditions with intermediate 5 only the undesired pyridone regiosomer could be detected and isolated. Preparation of the quinoline intermediate 10 was very low yielding as upon formation of 10 elimination of the halide readily occurred to give the undesired alkene substituted quinoline.

Attempts to perform Wittig chemistry between 8 and 9 resulted in complex mixtures, with no detected desired product. Attention thus shifted towards disconnection strategy (b), which was initially regarded as being the more challenging of the three proposed options. 5-Bromo-2-methyl-pent-2-ene 11 which could be purchased, or readily prepared by literature procedure, served as the starting material for the synthesis of the lateral chain 7 (Scheme 2). Compound 11 was subjected to an S$_2$2 reaction with lithium acetylide to afford the alkylene 12 $^{[13]}$ Zr-catalysed carboalumination followed by reaction with iodine generated compound 13 as a single stereoisomer in modest yield. $^{[14]}$ The last step was the formation of the pinacolboronate 7 via the Miyaura borylation reaction, a single stereoisomer was isolated and the desired (E) geometry was confirmed by NOESY analysis. For the synthesis of the quinoline building blocks for natural products 1–3, esters 17–19 were synthesized in a two-step sequence, utilizing the Conrad–Limpach method in good to moderate yields (Scheme 3).$^{[15]}$ The subsequent reduction of esters 17–19 in the presence of lithium aluminium hydride or sodium borohydride afforded the alcohols 20–22 in good yields. Lithium aluminium hydride proved to be a non-chemo selective reducing agent in the case of N-methyl-substituted quinoline 19, with reduction of both the quinoline core and the ester group observed. Substitution of the hydroxy group of 20–22 by chlorination with thionyl chloride led to the formation of the halide substituted quinoline cores 23–25 in good yields. Unfortunately, the dimethyl-substituted quinoline core 29, required for quinoline natural product 4, could only be prepared in poor yields using the Conrad–Limpach reaction that was utilized for the formation of esters 17–19.

Thus, an alternative strategy was employed for the synthesis of 4, based upon the use of compound 20. The hydroxy group of 20 was protected with TBDMS-Cl to afford the desired silyl ether in good yield (Scheme 4). Methylation of the nitrogen resulted in the formation of 27 in moderate yield; the O-methyl-ated regiosomer of 27 was also detected as a side product. Deprotection of 27 using TBAF and chlorination using thionyl chloride afforded 29 in good yield.

With the halide substituted quinolones 23, 24, 25, 29 and pinacolborate 7 in hand, we were ready to attempt the key Suzuki–Miyaura coupling reaction (Scheme 5). Pleasingly, microwave irradiation of a suspension of 7, quinolone 23, 24, 25 or 29, sodium carbonate and tetrakis(triphenylphosphine)palladium(0) in 1,4-dioxane led to the formation of desired products 1–4 respectively in good to moderate yields, aside from 2 (final

![Scheme 2. Preparation of common boronic ester lateral chain.](Image 77x70 to 262x216)

![Scheme 3. Preparation of quinoline cores for natural products 1–3.](Image 48x740 to 71x765)
purification of 2 for biological testing proved particularly challenging), however significant quantities were obtained for biological studies. The four natural products 1–4 were obtained with high levels of purity and stereoselectivity (≥ 99 % E-selectivity; the E-geometry of the double bond was confirmed by NOESY analysis). The bromine analogues of quinolones 23, 24, 25 and 29 were also prepared however there was no significant improvement in yield of the final coupling step compared to the chlorine substituted quinolones.

With the natural products in hand we next sought to investigate their effects on quinolone signaling on other bacterial species. Our initial assay involved focusing on PqsR (also known as MvfR), the receptor protein utilized by P. aeruginosa for quinolone mediated signalling, in a heterologous E. coli system.[16,8b] The natural products 1–4 failed to modulate PqsR dependent transcriptional activity in any agonistic or antagonistic fashion (data not shown). However, it cannot be ruled out that other phenotypes independent of PqsR could be modulated by analogues 1–4, as Burkhodera and Altermonas spp produce quinolones but no PqsR homologue has been identified to date. Inspired by the antibacterial activities of compounds 1–4 against Helicobacter pylori, we decided to screen the natural products to see if they had any noticeable bioactivity in a range of bacterial species. We initially screened four bacterial species; Pseudomonas aeruginosa wild-type strain PA01, Staphylococcus aureus 25923, Escherichia coli ESS (Figure 2) and Serratia marcescens 274 (data not shown). The growth of P. aeruginosa and S. marcescens were not affected by the presence of the natural products. Intriguingly, there was a large effect on the growth of the E. coli and S. aureus strains;[17] however this effect was notably different to the gentamicin control (which prevented growth of all the species tested).

In S. aureus cultures, all four natural products caused a marked delay in the onset of growth. A similar effect was observed in E. coli, although natural product 3 was inactive. This observed delay in the onset of growth resembles an extended lag phase in the growth profile of the bacteria. Thus, quinolones 1–4 are slowing growth (decreased OD$_{600}$ values for the first 6 hours of growth compared to the DMSO control) rather than directly inhibiting growth (bacteriostatic antibiotic) or killing the bacteria (bacteriolytic antibiotic). In the case of a bacteriolytic or bacteriostatic antibiotic, similar to the gentamicin control, growth would be completely inhibited over the 8 hour period. Toyofuku and co-workers have reported that the structurally related signalling molecule utilized by P. aeruginosa [2-heptyl-3-hydroxy-4(1H)-quinolone] also known as the Pseudomonas Quinolone Signal (PQS), also effects the growth of several Gram-negative and Gram-positive bacterial species by slowing growth and inducing an extended lag phase.[18] Although the underlying biochemical basis for this induced extended lag phase is not known, it is tempting to speculate that the quinolones may be interfering with the electron transport chain and affecting cellular respiration, as has previously been reported for other quinolone natural products.[19] Previous
studies have also shown that the uptake of hydrophobic quinolone antibiotics by \(S.\, aureus\) and \(E.\, coli\) occurs by passive diffusion, whereas uptake by \(P.\, aeruginosa\) was found to be more complex. An inadequate uptake may explain why \(P.\, aeruginosa\) showed to be insensitive to the tested natural products. One hypothesis for the observed growth defect by the natural product quinolones such as \(1-4\), PQS and possibly other natural quinolones produced by bacteria, is that such quinolone proteins could be functioning in multi-bacterial communities in the natural environment to perturb the growth of competing bacterial species.

**Conclusions**

In conclusion we have developed an efficient and direct synthetic route to four quinolone natural products \(1-4\) produced by the actinomycete \textit{Pseudonocardia} sp. The route involved a traditionally challenging sp\(^2\)-sp\(^3\) Suzuki step between various functionalised quinolone cores and a common lateral chain, which proceeded in high stereoselectivity. The quinolone core building blocks and lateral chain could be readily prepared from easily accessible starting materials (longest linear sequence of four steps). It was established that the natural products \(1-4\) do not interfere with signalling by the quinolone receptor protein PqsR of \(P.\, aeruginosa\). However, similar to PQS the natural products were found to slow the growth of other bacterial species, including \(E.\, coli\) and \(S.\, aureus\). Further studies of such quinolones and their biological activities in bacterial species should lead to a greater understanding of inter-species signaling in the natural environment and potential novel antibacterial agents.

**Experimental Section**

**Supporting Information** (see footnote on the first page of this article): Full experimental protocols, characterisation data, and \(^1\)H and \(^13\)C NMR spectra.

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