2-Methoxycyclopentyl analogues of a *Pseudomonas aeruginosa* quorum sensing modulator[†][‡]

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Received 29th January 2008, Accepted 29th February 2008 First published as an Advance Article on the web 18th March 2008 DOI: 10.1039/b801563e

Diastereomeric 2-methoxycyclopentyl analogues of a natural quorum sensing signaling molecule from *Pseudomonas aeruginosa* were synthesized and screened in pigment production assays with *P. aeruginosa* and *Serratia* strain ATCC39006.

Many bacteria co-ordinate their genome expression patterns in step with their population density via the intercellular signalling mechanism: quorum sensing.¹ Several pathogens use quorum sensing to regulate a diverse range of processes such as virulence factor production, secondary metabolite biosynthesis and biofilm formation.² Disruption of this pathway represents an attractive way to modulate bacterial pathogenicity without the inherent difficulties associated with growth inhibition.³ Many Gram-negative bacteria utilize quorum sensing systems comprising LuxI synthase homologues that synthesize N-acyl-L-homoserine lactone (AHL) signal molecules, which then bind to their cognate LuxR-type transcriptional regulators.⁴ The most extensively studied quorum sensing system is that of Pseudomonas aeruginosa which uses N-butyryl-L-homoserine lactone (BHL)⁵ and N-(3-oxododecanyl)-L-homoserine lactone (OdDHL)⁶ as the signalling molecules (Fig. 1). This bacterium is a leading pathogen in a range of life threatening nosocomial infections, and is the primary cause of mortality in cystic fibrosis sufferers.⁷ Structural information of the LasR-OdDHL complex from P. aeruginosa has been reported recently,8 which has aided the design of AHL analogues (Fig. 2). Cell-permeable modulators of LuxRtype receptors are valuable for dissecting the role of quorum sensing in vivo, and may have chemotherapeutic potential.³ Therefore, identification of small molecules which disrupt quorum sensing pathways have been investigated actively.¹⁰

Non-enzymatic lactone hydrolysis is significant for AHL degradation *in vivo*, for example BHL has a half-life of *ca*. 1 day at pH 7.3 and 37 °C.¹¹ Previously, we and others have described the synthesis of the non-hydrolyzable BHL analogues 1 and 2.^{11,12} Compound (*S*,*S*)-2 was a weak agonist,



Fig. 1 Quorum sensing agonists of *Pseudomonas aeruginosa* (BHL and OdDHL), previous derivatives and the synthetic targets of this report.

whereas, (S)-1 was more potent than BHL in a *P. aeruginosa* pigmentation assay (BHL-RhlR signaling system). Epimerization under physiological conditions, albeit slow,§ was a disadvantage of aminoketone 1;¹¹ therefore, configurationally stable methoxy analogues of BHL were investigated. The methoxy substituent should be a hydrogen bond acceptor for the conserved tryptophan residue in LuxR-type proteins (*e.g.* OdDHL-LasR, Fig. 2), whilst not being a hydrogen bond donor. The weak activity of **2** was hypothesized to be due to either, an unfavourable hydrogen bond donation, or, an unfavourable spatial preference of the *trans*-amino alcohol.



Fig. 2 The OdDHL binding site in LasR (code 2UV0),⁸ illustrating the conserved hydrogen bond between Trp60 and the lactone carbonyl of OdDHL (2.97 Å). Note that the endocyclic lactone oxygen is not involved directly in LasR receptor binding, a feature consistent with AHL binding with TraR.⁹

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[†] This article is part of a *Molecular BioSystems* 'Emerging Investigators' issue highlighting the work of outstanding young scientists at the chemical- and systems-biology interfaces.

[‡] Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/b801563e



Scheme 1 Synthesis of compounds 3 and 4.

These hypotheses could be addressed by testing the activity of *trans*- and *cis*-methoxy analogues of BHL (3 and 4, respectively). Herein, we describe the synthesis of these BHL analogues (3 and 4), and report their biological activity in phenotypic assays with *P. aeruginosa* and *Serratia*.

BHL analogues 3 and 4 were prepared as shown in Scheme 1. Analogue 3 was obtained in four steps from aminoalcohol 5. The protected aminoalcohol 6 (obtained by heating 5 with phthalic anhydride) was O-alkylated cleanly by Meerwein's salt and proton sponge[®]. Phthalimide removal with hydrazine and N-acylation with butryryl chloride under Schotten-Baumen conditions gave the amide 3. Analogue 4 was obtained in seven steps from 5. Treatment of 5 with Boc₂O, followed by mesylation of the hydroxyl group resulted in concomitant in situ cyclization to afford oxazolidinone 8. Hydrolysis of the oxazolidinone to the cis-aminoalcohol, followed by N-Boc protection, gave 9. O-Methylation of 9 by a variety of reagents was very slow, but could be achieved Meerwein's salt and proton sponge with over of 10 and N-acylation with butryryl chloride afforded 4 (Scheme 1).

A pigment production phenotypic $assay^{11}$ with a *P. aeruginosa* mutant was used to test the two BHL analogues (3 and 4). The green pigmentation of *P. aeruginosa* cultures, which forms



Fig. 3 *P. aeruginosa* strain PAO-JP2 pigmentation assay.¹¹ All wells contain PAO-JP2 in an alanine–glucose salts medium, $10 \,\mu$ M OdDHL (unless indicated otherwise), and the indicated concentration of **3**, **4** or BHL. The intensity of green colouration in each well reflects the level of pigment biosynthesis.



Fig. 4 Serratia strain ATCC39006 smal mutant prodigiosin assay.¹¹ All wells contain Serratia 39006 smal mutant in a peptone–glycerol medium, and the indicated decreasing concentration of **3**, **4** or BHL. The intensity of red colouration of the spots reflects the level of prodigiosin biosynthesis.

the visual colourimetric output of the assay, requires both BHL and OdDHL (or suitable agonists) to be present at miduM concentrations. The P. aeruginosa mutant PAO-JP2 can synthesize neither OdDHL nor BHL;13 however, addition of both autoinducers and incubation for 18 hours at 37 °C restores the wild-type phenotype. Substitution of BHL with either 3 or 4 revealed a difference in the production of pigmentation as shown in Fig. 3. The intensity of green colouration at the end of the test reflects the level of pigment biosynthesis and thus the potency of the analogues. As expected, no pigment was produced in wells that lack the presence of OdDHL. In this test, the *trans*-diastereoisomer 3 produces a stronger colouration than the cis-diastereoisomer 4. In order to confirm and extend the results to another bacterial quorum sensing system a more sensitive bioassay involving Serratia strain ATCC39006 was conducted.

The Serratia 39006 strain is a Gram-negative motile rod that contains the LuxR homologs CarR_{sma} and SmaR.¹⁴ These proteins respond to BHL, which is produced by SmaI. Prodigiosin is a bright red tripyrrole secondary metabolite pigment produced in Serratia 39006 and its biosynthesis is controlled by quorum sensing.¹⁴ A Serratia 39006 smaI mutant,¹⁵ which cannot produce BHL, was used to test the ability of **3** and **4** to induce pigment production (Fig. 4). The intensity of red colouration of the spots reflects the level of prodigiosin biosynthesis. The natural ligand BHL was the most potent compound activating pigment synthesis at around 100 nM. The BHL analogues both activated pigment synthesis at a concentration between 3.7–11 μ M, with the trans-diastereo-isomer (**3**) being a slightly better agonist.

In both phenotypic assays it is notable that the BHL analogues **3** and **4** are significantly less potent than cyclic ketone (S)-1, which has activity similar to BHL. Additional assays showed that the compounds did not display any significant inhibitory activity. We conclude that the weak activity of **2–4** appears not to be simply due to either an unfavourable hydrogen bond donation, or an unfavourable spatial preference of the oxygen lone pair. The acidities of protonated ethers *vs.* ketones (p K_a values -3.5 vs. -7, respectively)¹⁶ indicates that the methoxy-ethers (**3** and **4**) should be

better hydrogen bond acceptors to the conserved tryptophan in the AHL binding pockets, compared to the ketone (S)-1. However, it is possible that there is an unfavourable steric interaction due to the methyl group, or that the ketone may be hydrated in the binding pocket. Additional potent quorum sensing modulators are being discovered and will be reported in due course.

In summary, we have synthesised *cis*- and *trans*-2-methoxycyclopentyl analogues of BHL (**3** and **4**) and screened them in *P. aeruginosa* and *Serratia* 39006 phenotypic assays. The diastereomeric compounds were agonists, but were significantly less active relative to a 2-oxocyclopentyl analogue (S)-**1** and BHL.

Acknowledgements

We thank EPSRC, BBSRC, MRC and the Augustus and Harry Newman Foundation for financial support. Barbara Iglewski (U. Rochester, USA) is thanked for generously supplying the *P. aeruginosa* strains PAO1 and PAO-JP2.

References

 $\$ The racemization of the six-membered cyclic aminoketone analogue of BHL was shown to have a half-life of 36 h at pH 7.3 and 37 °C. At 30 °C, α -deprotonation was less than 20% after 7 days. 11

¶ Methylation of the phthalamide protected *cis*-aminoalcohol could not be achieved using a wide range of reagents (MeI, Me₃OBF₄, Me₂SO₄).

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