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Inhibition of the production of the *Pseudomonas aeruginosa* virulence factor pyocyanin in wild-type cells by quorum sensing autoinducer-mimics[†]

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Pseudomonas aeruginosa is a notorious human pathogen associated with a range of life-threatening nosocomial infections. There is an increasing problem of antibiotic resistance in P. aeruginosa, highlighted by the emergence of multi-drug resistant strains. Thus the exploration of new strategies for the treatment of *P. aeruginosa* infections is clearly warranted. *P. aeruginosa* is known to produce a range of virulence factors that enhance its ability to damage the host tissue and cause disease. One of the most important virulence factors is pyocyanin. P. aeruginosa regulates pyocyanin production using an intercellular communication mechanism called quorum sensing, which is mediated by small signalling molecules termed autoinducers. One native autoinducer is N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL). Herein we report the synthesis of a collection of abiotic OdDHL-mimics. A number of novel compounds capable of competing with the endogenous OdDHL and consequently, inhibiting the production of pyocyanin in cultures of wild type *P. aeruginosa* were identified. We present evidence suggesting that compounds of this general structural type act as direct antagonists of quorum sensing in P. aeruginosa and as such may find value as molecular tools for the study and manipulation of this signalling pathway. A direct quantitative comparison of the pyocyanin suppressive activities of the most active OdDHL-mimics with some previously-reported inhibitors (based around different general structural frameworks) of quorum sensing from the literature, was also made.

Introduction

Pseudomonas aeruginosa is a major opportunistic pathogen. This Gram-negative bacterium causes a variety of nosocomial infections and life-threatening diseases in immunocompromised and debilitated patients¹ and is the most predominant cause of chronic pulmonary infections in cystic fibrosis sufferers.² Infections of this bacterium are notoriously difficult to eradicate due to high levels of intrinsic antibiotic resistance¹ and the propensity of *P. aeruginosa cells* to form antibiotic-resistant biofilms.³ Indeed, *P. aeruginosa* represents one of the most challenging pathogenic bacteria to treat¹ and multi-drug resistant *P. aeruginosa* nosocomial infections are increasingly being recognized worldwide.⁴ Thus the exploration of innovative new therapeutic strategies for tackling *P. aeruginosa* infections is urgently required.^{1,2}

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The ability of pathogenic bacteria such as P. aeruginosa to cause disease is dependent upon the production of agents termed 'virulence factors', such as toxins and adhesion molecules, that actively cause damage to host tissues.⁵⁻⁷ The targeting of virulence factors (e.g. inhibition of their production, delivery or function) has gained increasing attention as a potential new antibacterial strategy; in principle this would 'disarm' pathogens and allow the host immune system a better chance of clearing the infection before the bacteria cause too much tissue damage.⁶ This approach has a number of theoretical advantages over standard antibiotic treatment.⁷ For example, many virulence factors are organism-specific and therefore virulence-targeting drugs should have a minimum impact upon the host's commensal flora.^{7,8} In addition, provided the targeted virulence factor is not essential for bacterial survival in vivo then there should be a weaker selective pressure for the development of resistant mutants relative to traditional antibiotic treatments.^{7,8} Recent years have witnessed considerable interest in the targeting of virulence factors as a novel therapeutic strategy and there is a growing body of *in vivo* data to support this approach.^{7,8}

Amongst the arsenal of virulence factors produced by *P. aeru*ginosa is pyocyanin (1-hydroxy-5-methyl-phenazine), a low molecular weight redox-active phenazine dye.⁹ *In vitro* studies

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Fig. 1 BHL, OdDHL and PQS are natural autoinducers employed by *P. aeruginosa* in quorum sensing. 1-5 are synthetic OdDHL-based small molecule inhibitors of LasR-dependent quorum sensing. 1-3 were discovered by our group in a previous study;¹⁷ 4 and 5 were reported by Blackwell and co-workers³² and Yoon and co-workers³⁶ respectively.

have shown pyocyanin to have multiple deleterious effects upon mammalian cells.¹⁰ Pyocyanin has been found to be critical for P. aeruginosa lung infections in mice and it is regarded to play an important role in colonizing the airways of cystic fibrosis patients.^{10,11} Overall there is clear evidence that pyocyanin is important to the pathogenesis of P. aeruginosa infections.^{2,11} Unsurprisingly therefore, the targeting of pyocyanin has been suggested as a therapeutic strategy for treating infections by this organism.^{2,8,11} Some of pyocyanin's toxic effects appear to result from its ability to redox cycle and place cellular systems under increased oxidative stress through the production of reactive oxygen species.9,12 Accordingly several antioxidant therapies have proved valuable in cystic fibrosis.^{2,13–15} The inhibition of pyocyanin production has also been identified as a possible approach for attenuating the pathogenicity of P. aeruginosa infections.^{2,11,16} Indeed, recent years have witnessed considerable interest in the discovery of synthetic and non-native naturally occurring compounds which have the ability to inhibit pyocyanin biosynthesis.17-21

An intercellular signalling process known as quorum sensing regulates pyocyanin production by P. aeruginosa.^{22,23} This communication mechanism is mediated by small molecules termed autoinducers which are synthesized intracellularly by bacterial cells throughout their growth and are continually released into the surroundings.²⁴⁻²⁶ Therefore the extracellular concentration of the autoinducer (typically) increases in concert with the bacterial population cell density; once a threshold concentration is reached (at which point the population is said to be "quorate") a signal transduction cascade is initiated leading to populationwide changes in gene expression.^{24,27–32} Quorum sensing systems can be found in many species of Gram-negative bacteria, most of which employ *N*-acylated-L-homoserine lactones (AHLs) as autoinducers.^{21,24} The majority of natural AHLs are based around the same general structural framework, namely a homoserine lactone ring (the 'head group') unsubstituted at the β and γ positions, which is *N*-acylated at the α position with an acyl group (the 'tail group').^{17,21,24} These small molecules are generated by LuxI-type synthase enzymes and bind to cognate cytoplasmic LuxR-type receptors to initiate the expression of genes associated with bacterial group processes.^{21,24,26,33} Typically, each bacterial species responds specifically to its own unique AHL(s), with different LuxI-type synthase and LuxRtype receptors employed.^{17,24,32} P. aeruginosa uses two AHLbased quorum sensing systems. One system employs N-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) as the autoinducer,

which is generated by LasI with LasR as the cognate receptor (Fig. 1). The second involves *N*-butanoyl-L-homoserine lactone (BHL) as the signalling molecule, which is generated by RhII and detected by RhIR. These AHL-based systems are interlinked with a third system employing a chemically distinct autoinducer termed the *Pseudomonas* quinolone signal, PQS. The result is an intricate hierarchical quorum sensing network.^{17,24,34,35}

Pvocvanin production is regulated by RhlR. Transcription of the *rhlR* gene is itself regulated by LasR.²² Thus, *lasR* mutants no longer produce pyocyanin, and inhibitors of LasR would be expected to attenuate pyocyanin biosynthesis.²³ Indeed, abiotic small molecules with this activity profile are known.^{21,24,37-39} The structure of the natural LasR agonist OdDHL has often served as a template for the design and synthesis of such agents.^{17,24,21,40} For example, we have recently reported the discovery of potent OdDHL-based inhibitors of pyocyanin production, in wild-type P. aeruginosa cells in which the native 3-oxo-dodecanoyl tail group is present but the natural homoserine lactone moiety has been substituted with a non-native aromatic head group (compounds 1-3 Fig. 1).¹⁷ These data clearly suggest that the OdDHL framework can serve as a promising lead scaffold in developing further novel small molecule inhibitors of pyocyanin production.

Inspired by our previous study,¹⁷ we sought to examine the pyocyanin inhibitory activity of additional OdDHL analogues containing non-native head groups in order to: (i) identify new inhibitors of this phenotype and (ii) to gain new insights into the molecular basis of the activity of compounds of this structural class. Herein we report the results of this study. A small library of compounds of this general structural type was synthesized, a number of which were found to be capable of suppressing the production of pyocyanin in a wild-type strain of *P. aeruginosa*. Such compounds could potentially be exploited in a therapeutic context for the treatment of human bacterial infections. A comparison with the data obtained from our previous study yielded some new structure-activity information with respect to pyocyanin inhibition. Given the strong dependence of pyocyanin production upon LasR-mediated quorum sensing (vide supra) we were also interested in benchmarking the anti-pyocyanin activity of our OdDHL-mimics against some other compounds previously reported to have inhibitory activity against LuxR-type receptors by other researchers. However, a significant issue encountered when examining the use of small molecules to modulate phenotypes regulated by quorum sensing pathways is the lack of standardization between the assays used in different reports to assess the biological effects of such agents.^{21,24,41} Also, it is generally accepted that direct quantitative comparison of the activities of such compounds obtained from different studies by different research groups can be misleading and is not appropriate in many cases.^{21,24,41,42} Therefore, and to circumvent these issues, we synthesized several compounds that had previously been reported by other researchers to have inhibitory activity against LuxR-type quorum sensing systems. These included compounds of the same structural class as our library members (*i.e.* OdDHL-mimics with non-native head groups). All compounds were evaluated for their ability to inhibit pyocyanin production using the same assay.

Results and discussion

OdDHL mimics

Synthesis of OdDHL mimics. A range of OdDHL-mimics incorporating aromatic head groups differing in both steric and electronic properties was readily prepared *via* a sequence which utilized 2-(2-ethyl-1,3-dioxolan-2-yl)acetic acid (7) as a common intermediate (Scheme 1).^{17,43} EDC-mediated coupling of 7 with a range of aromatic amines 8 furnished ketone-protected amide derivatives 9. Acetal deprotection using TFA yielded the desired final compounds 4, 5 and 10–18. Compounds 4 (Blackwell and co-workers³²) and 5 (Yoon and co-workers⁴⁴) have previously been reported to be LasR antagonists.

Biological screening of OdDHL-mimics

Assay protocol. Compounds 4, 5 and 10–18 were evaluated for their effects upon pyocyanin production by the wild type *P. aeru*ginosa strain PAO1. Cells were grown for 13 hours in the presence of the indicated synthetic AHL mimic (at a concentration of 200 μ M) added at the start of the growth period. The amount of pyocyanin present was measured and compared with that obtained when the cells were grown for 13 hours in the absence of any synthetic AHL mimic (Fig. 2). All compounds were found to decrease pyocyanin production without affecting growth. These data could be directly compared with those obtained for compounds 1-3 from our previous study as an identical assay protocol was employed (Table 1).

Mode of action considerations. Compounds that affect pyocyanin production in P. aeruginosa may be inhibitors of LasR-based quorum sensing (vide supra). OdDHL, the natural binding partner of LasR, is very closely related in structure to compounds 1-5, 10-18. Thus it reasonable to propose that the inhibitory effects of 1-5, 10-18 upon pyocyanin production result from an inhibition of quorum sensing, with the compounds possibly acting at the level of the LasR receptor (i.e. they are LasR antagonists). Compound 3 has previously been reported to inhibit the production of elastase, another P. aeruginosa virulence factor which is regulated by the las branch of the quorum sensing system (and to a lesser extent, also by the subordinate rhl branch); a result that is also consistent with direct inhibition of LasR-based quorum sensing.¹⁷ It is worth noting however, that *lasR* mutants can still, under certain conditions, express multiple virulence factors, including pyocyanin, by utilising the Rhl and PQS systems and there are additional studies which indicate that the quorum sensing hierarchy in this organism is more complex than the simple las/rhl hierarchy reported previously.^{22,46} Thus it is possible that inhibitors of pyocyanin production in wild-type P. aeruginosa may not affect the LasR-receptor directly, but instead have an alternative mode of action.

Structure–activity observations. Compound **5**, previously identified as a LasR antagonist by Yoon and co-workers,⁴⁴ was found to be less active than six of the novel analogues disclosed



Scheme 1 Synthesis of OdDHL mimics with non-native head groups. The synthesis of 7 from Meldrum's acid (6) was achieved by following a previously reported route.⁴³ rt = room temperature. TFA = trifluoroacetic acid.



Fig. 2 Inhibitory effects of OdDHL-mimics **4**, **5** and **10–18** on pyocyanin production in PAO1. Cultures of PAO1 were grown in Luria broth medium in the presence of OdDHL-mimics (200 μ M) with good aeration at 37 °C for 13 hours (initial OD₆₀₀ of 0.05 *t* = 0). After growth pyocyanin production was quantified as previously described.⁴⁵ No effect on growth was observed for any of the analogues (data not shown). DMSO (–) was added as a control. The data represents the averages and standard deviations from the results of three independent biological repeats.

Table 1	Summary of the	activities of t	the OdDHL	-mimics	examined	in
our two st	udies					

Compound	% Inhibition ^a	
1	67 ± 2^b	
2	73 ± 4^b	
3	93 ± 2^b	
4	80 ± 4^b	
5	45 ± 6	
10	70 ± 4	
11	38 ± 6	
12	49 ± 4	
13	62 ± 4	
14	47 ± 17	
15	12 ± 8	
16	39 ± 21	
17	64 ± 5	
18	68 ± 3	

 a % Inhibition of pyocyanin production as determined from the data illustrated in Fig. 2. b Data obtained from previous study. 17 Compound concentration of 200 μM in all cases.

in this report and also less active than compounds 1-3 identified in our previous study.¹⁷ The most active of the AHL-mimic screened in the current study was 4, a compound that had previously been reported by the Blackwell group to be a potent LasR antagonist.³² The activity of 4 compares favourably with the three strongest inhibitors of pyocyanin production that we identified previously,¹⁷ with only 3 being noticeably more active (Table 1). 3 bears an electron-donating *meta*-methoxy group; the aryl ring would therefore be expected to be more electron rich than that of 4. Compound 12 also contains an electron donating substituent in the *meta*-position; however, this derivative was found to be considerably less active than 3 (and indeed, slightly less active than 13 which has an electron withdrawing *meta*-nitro group). These data suggest that steric, as well as electronic factors, play an important role in determining activity. Consistent with this, moving a methoxy group from the *meta* to *ortho* position (compounds **3** *versus* **5**, respectively) resulted in a considerable drop in activity. Assuming these OdDHL-mimics exert their anti-pyocyanin effects *via* interaction with the LasR receptor (*vide supra*) this may be because *ortho* groups may inhibit efficient binding in the LasR pocket, possibly due to steric factors. Furthermore, the introduction of a second methoxy group at the *ortho* position of **3** (to form **15**) severely weakened the pyocyanin antagonistic activity of the ligand. The halide-functionalized derivatives (**2** and **10**) had similar inhibitory activities, suggesting that there is some flexibility in terms of the steric bulk at the *meta* position that the LasR binding pocket can accommodate, again assuming the effects of the compounds are mediated *via* binding to the LasR receptor.

Of the OdDHL-mimics examined in this study, **4** and **10** are the only examples which do not contain a heteroatom on the aromatic head group which would be expected to be capable of forming hydrogen bonding interactions. If one supposes that these two OdDHL-mimics act as competitive LasR antagonists, then the relatively high inhibitory activities of these compounds corroborates recent observations by our own group (and that of Blackwell and co-workers) which question the proposed critical nature of a hydrogen bonding interaction between the head group of AHLs or AHL-mimics and the LasR receptor for strong binding.³²

Overall, from the available data it is difficult to draw any firm SAR conclusions regarding the effects of the aromatic ring head group on the ability of members of the OdDHL-mimic compound class to inhibit pyocyanin production. There appears to be a subtle interplay between the structural and electronic properties of the aromatic moiety governing compound activity, which is which is consistent with previous observations by the Blackwell and co-workers.³² Consequently the rational modification of compounds of this sort in order to improve properties (*e.g.* activity) is challenging. This is a well-known problem associated with AHL-based compounds.^{24,41}

Comparative assessment of OdDHL-mimics and some structurally distinct literature inhibitors of LuxR-type proteins

Synthesis of some literature inhibitors of LuxR-type proteins. Of the OdDHL-mimics with non-native head groups examined, the two most potent inhibitors of pyocyanin production (and by inference LasR-mediated quorum sensing) were found to be 3 and 4. We were interested in comparing the activity of these compounds with some examples of literature inhibitors of LuxRtype quorum sensing which were based around different general structural frameworks in an attempt to gain further insights into the molecular features required for inhibition of pyocyanin production. Specifically we focused upon: (i) 19-20, AHL-mimics with non-native tail groups reported by Geske *et al.*,⁴⁷ (ii) 21-22, AHL mimics in which both the natural homoserine lactone head group and acyl chain had been replaced with nonnative structural moieties (identified by Teasdale et al.⁴⁸) and (iii) PD-12, an example of a quorum sensing inhibitor with a non-AHL core scaffold identified by Müh et al.⁴⁹ (Fig. 3).

19 and **20** were readily accessed *via* EDC-mediated coupling of (S)-(-)- (α) -amino- γ -butyrolactone hydrobromide (**23**) with indole-3-butyric acid and 4-bromophenyl acetic acid respectively (Scheme 2). In a similar fashion, amide bond formation between 2-phenylethylamine (**24**) and isobutyric acid furnished **21** whereas the use of isovaleric acid generated target compound **22** (Scheme 3). **PD-12** was synthesized *via* a three-step sequence from ethyl cyanoacetate (**25**) (Scheme 4).^{50,51} Reaction with sodium azide furnished tetrazole **26**. Alkylation with



Fig. 3 OdDHL-mimics 3 and 4 together with the structures of known literature quorum sensing modulators examined in this report. 19–20 are potent antagonists against LasR (Geske *et al.*⁴⁷), 21–22 have been reported to inhibit quorum sensing-controlled phenotypes in Gram negative bacteria, presumably by competing with native AHLs for binding to the LuxR-type receptors⁴⁸ and PD-12 is an inhibitor of LasR-dependent gene expression which is also believed to interact directly with the native AHL-binding site on LasR.⁴⁹



Scheme 2 Synthesis of AHL-mimics 19 and 20 with non-native tail groups. rt = room temperature.



Scheme 3 Synthesis of AHL-mimics 21 and 22. rt = room temperature.



Scheme 4 Synthesis of PD-12. rt = room temperature.

1-bromododecane yielded **27** and subsequent ester hydrolysis furnished the desired target compound.

Biological screening. Compounds 19–22 and PD-12 were evaluated for their effects upon pyocyanin production by the wild type *P. aeruginosa* strain PAO1 using the method described above at a compound concentration of 200 μ M. However, all these compounds were found to have very similar levels of inhibitory activities at this concentration, therefore experiments were repeated at a lower ligand concentration (50 μ M) to increase resolution. (Fig. 4 and Table 2). The OdDHL-mimics **3** and **4** were found to be significantly stronger inhibitors of pyocyanin synthesis. This was not necessarily unexpected in the case of compounds **21–22**, which had not previously been tested





Fig. 4 Inhibitory effects of various compounds on pyocyanin production in PAO1. Cultures of PAO1 were grown in Luria broth medium in the presence of compounds (50 μ M) with good aeration at 37 °C for 13 hours (initial OD₆₀₀ of 0.05 t = 0). After growth pyocyanin production was quantified as previously described.⁴⁵ No effect on growth was observed for any of the analogues (data not shown). DMSO (–) was added as a control. The data represents the averages and standard deviations from the results of three independent biological repeats.

 Table 2
 Summary of the comparative assessment

Compound	% Inhibition ^a		
3	74 ± 3		
4	77 ± 4		
19	22 ± 5		
20	13 ± 7		
21	30 ± 2		
22	25 ± 4		
PD-12	9 ± 11		

 a % Inhibition of pyocyanin production as determined from the data illustrated in Fig. 2. Compound concentration of 50 μM in all cases.

against *P. aeruginosa*. In addition, our earlier study suggested that the incorporation of both non-native head and tail regions into an AHL-mimic (as is the case in 21-22) is generally associated with decreased inhibitory activity (relative to compounds which retain the native acyl chain). The data for 19-20 implies that non-native tail groups are not well tolerated with respect to pyocyanin inhibition by AHL-mimics, though additional analogues which retain the natural homoserine lactone head group but which incorporate other abiotic tail moieties need to be examined in order to explore this trend further. Overall the data for the AHL-mimics 3-4 and 19-22 suggests that the natural 3-oxo-dodecanoyl tail group of OdDHL is important for the inhibition of pyocyanin production by compounds which mimic the structure of AHLs. Assuming that these compounds affect

pyocyanin production by targeting the LasR receptor (vide supra) these data corroborate our earlier study, and observations from other researchers, which suggest that the natural OdDHL acyl tail group is important for the LasR modulatory activity of compounds which are based around the AHL scaffold.^{17,21,24,32,52} The large difference in activity between the two OdDHL-mimics 3-4 and PD-12 was surprising. Though PD-12 it is based upon a structural framework that is clearly distinct from that of AHLs, it does retain the natural 3-oxo-dodecanoyl tail group of OdDHL. PD-12 has been identified as a potent LasR antagonist in a bacterial reporter strain.⁴⁹ PD-12 has also previously been reported to inhibit pyocyanin production by approximately 40% in a wild-type PAO1 strain (derived from an alternative source to that used in our study) at a compound concentration of 10 µM (in an assay that also employed slightly different conditions to those used in our study).⁴⁹ This provides a clear illustration of how misleading it can be to directly compare, in a quantitative fashion, the activities of compounds affecting quorum-sensing regulated phenotypes obtained from different studies by different research groups (even in studies investigating the modulation of the same protein).^{21,24,42}

Probing the binding of OdDHL-mimcs to LasR. Bottomley et al.⁵³ and Schuster et al.⁵⁴ have reported that the LasR protein is soluble and stable only if it is expressed in the presence of its cognate ligand, OdDHL.41 Presumably correct binding of OdDHL to LasR causes the protein to adopt a soluble conformation.⁴¹ This observation provided a convenient (albeit crude) assay to determine whether our most active antagonist (compound 3) might bind directly to LasR and thereby inhibit pyocyanin production. The expression of full length LasR yields only insoluble inclusion bodies, even in the presence of the native ligand OdDHL.^{41,53,54} However, when the DNA-binding domain is removed (leaving just the ligand-binding domain and dimerization determinants) the protein was fully soluble when expressed in trans in Escherichia coli in the presence of OdDHL (Fig. 5). In the absence of OdDHL, the LasR-LBD exclusively partitioned into insoluble inclusion bodies (Fig. 5). In the presence of compound 3 the protein was roughly equally distributed between the soluble and insoluble fractions, indicating that this compound probably binds directly to the LasR-LBD and elicits a conformational change similar to that caused by OdDHL Bottomley et al. previously showed that weak binding of non-cognate AHLs to LasR elicits a similar result.⁵³ This lends credence to the hypothesis that the inhibitory effect of 3 (and presumably, also the other OdDHL-mimics examined,) upon pyocyanin production results - at least in part - from modulation of LasR-based quorum sensing as a consequence of direct binding to the LasR receptor. Compounds of this general form may therefore represent antagonists of quorum sensing in P. aeruginosa.

Conclusions

We have reported the design, synthesis and biological evaluation of a collection of abiotic compounds based around the structure of the natural *P. aeruginosa* signalling molecule OdDHL in which the native homoserine lactone head group has been replaced with a non-native aromatic moiety. The majority of



Fig. 5 A Coomassie blue-stained 12% SDS-polyacrylamide gel showing the partitioning of LasR-LBD in the insoluble pellet (P) and soluble (S) fractions of E. *coli* cell lysates obtained from cultures grown in the presence or absence of OdDHL and compound **3**. The far left lane is a molecular weight ladder. From bottom to top of the molecular weight ladder: the bands below the red rectangle are molecular weights of 7 kDa and 17 kDA; the bands above the rectangle are 23 kDa and 30 kDa.

these OdDHL-mimics, including several structurally novel derivatives, were found to inhibit the production of pyocyanin, a molecule that enhances the virulence of *P. aeruginosa*, in a wild-type strain of the organism. The fact that this class of compounds effectively compete with endogenously-produced OdDHL to antagonize pyocyanin production means that they have the potential to be exploited in a therapeutic context for the development of novel anti-pseudomonal agents. Of more general significance it has been argued that the identification of such small molecule modulators is needed in order to better evaluate the therapeutic potential of targeting virulence factors.⁸

Overall it proved difficult to delineate SARs for pyocyanin inhibition by this class of compounds. There appears to be a subtle interplay between the structural and electronic properties of the aromatic head group governing compound activity, which is difficult to rationalize. Thus the deliberate modification of compounds of this sort in order to improve properties (*e.g.* activity) would be expected to be challenging, which may hinder their suitability as lead-compounds for further rational development.

The OdDHL-based derivatives 1–5, 10–18 are closely related in structure to the natural autoinducer OdDHL, which is known to interact with the LasR receptor, and pyocyanin production is regarded as being regulated by LasR-based quorum sensing. These observations are consistent with the notion that 1–5, 10–18 reduce the level of pyocyanin production by disrupting OdDHL-dependent activation of LasR. Furthermore we have presented direct experimental evidence that OdDHL-mimic 3, one of the most potent inhibitors of pyocyanin production explored in our two studies, is capable of causing LasR to adopt a soluble conformation. This result is indicative of direct binding of 3 to LasR and provides further support that 3 (and, by inference, possibly the other OdDHL-mimics examined) is an antagonist of the LasR receptor and an inhibitor of LasR-based quorum sensing in P. aeruginosa (note again that the las system is generally regarded as standing at the apex of the quorum sensing hierarchy in *P. aeruginosa*). The possibility that the OdDHL-mimics examined may inhibit quorum sensing in this organism has some interesting implications. In addition to pyocyanin production, quorum sensing also regulates additional elements associated with *P. aeruginosa* pathogenicity.^{8,24,55–57} These include biofilm formation (in many growth conditions)³ and the production of the virulence factors elastase, exotoxin A and alkaline protease (specifically regulated by the *las* system).⁵⁸ In addition, the *rhl* system regulates the virulence factor hydrogen cyanide and is also required for optimal production of alkaline protease and elastase.57,58 Thus there is tremendous interest in disrupting AHL-mediated quorum sensing in *P. aeruginosa*;²¹ it is possible that, as putative quorum sensing inhibitors, the OdDHL-mimics studied in this report may have broad-spectrum inhibitory activity against several aspects of P. aeruginosa virulence. Furthermore such compounds could have value as molecular tools to study and manipulate quorum sensing pathways in P. aeruginosa. Potentially these compounds could affect the quorum sensing systems of other species of Gram-negative bacteria and may therefore may prove to be of value in a wide range of other applications where the inhibition of quorum sensing is desirable.^{24,49,59} However, there is evidence that the length of the AHL acyl chain, even in non-lactone derivatives, can give high levels of receptor selectivity.³² Thus the OdDHL-mimics may only have a significant impact upon quorum sensing in P. aeruginosa. This could be advantageous in the context of possible therapeutic applications of such agents; in contrast to broadspectrum quorum sensing inhibitors, narrow-spectrum inhibitors of this sort would be expected to interfere less with the commensal (and beneficial) microbial flora of the host.⁶⁰ Investigations exploring both species selectivity of the OdDHL-mimics and their ability to affect other quorum sensing-regulated phenotypes in P. aeruginosas are on going and results will be reported in due course.

Of the OdDHL-mimics with non-native aromatic head groups examined, the two most potent inhibitors of pyocyanin production in wild-type P. aeruginosa cells (strain PAO1) were found to be 3, discovered by our research group 17 and 4, initially reported to be a LasR antagonist by the Blackwell group.³² A comparison of the anti-pyocyanin activities of these compounds with some known literature inhibitors of LuxR-type quorum sensing, which were based around different general structural frameworks, was then made. Importantly all compounds were synthesized in house and biologically evaluated using the same assay system to allow a direct quantitative comparison of activities. In general the data suggested that the natural 3-oxo-dodecanoyl tail group of OdDHL is important for the inhibition of pyocyanin production by compounds which mimic the structure of AHLs. This adds to the growing body of evidence that this structural feature is important for the LasR modulatory activity of compounds that are based around the AHL scaffold.^{17,21,24,52} Compounds 3 and 4 were found to be significantly stronger inhibitors of pyocyanin production than the other compounds examined. Significantly this included the compound PD-12, a molecule based around a non-AHL core structure, which had previously been reported to be a potent inhibitor of this phenotype in a wild-type strain of this organism.⁴⁹ In our hands, the

potency of **PD-12** in inhibiting pyocyanin production was significantly lower than that reported in the literature. This provides a clear illustration of the importance of adopting standardized assay systems to enable truly comparative analyses to be made. In this context the establishment of a small 'test' compound library of known quorum sensing inhibitors may be valuable. Such a library could be made available to all quorum sensing researchers that publish new inhibitors, thus allowing the activities of new agents to be directly benchmarked against know inhibitors.

It is worth noting that small molecules that modulate AHLbased quorum sensing are typically discovered through culturebased screening assays that employ bacterial reporter strains (so-called biosensor strains) where the expression of an easily assayable phenotypic output is under the control of a LuxR-(type)-regulated promoter.^{17,21,60} Functional AHL synthases are not present in such strains; thus transcription of the reporter gene is, in principle, entirely dependent upon the addition of the exogenous AHL (or a functional equivalent).^{17,21,60} Antagonist screening trials using biosensor strains involve the addition of the cognate AHL at a fixed concentration (usually, just enough to stimulate expression of the reporter gene); antagonists are identified by their ability to compete with the native autoinducer and reduce reporter gene expression.^{17,21,60} The advantage of such a system is that it is well-defined 17,60 However, it does not accurately mimic the situation in wild-type cells, where the endogenous AHL is continually produced and can therefore more effectively outcompete any added antagonist.^{17,21,60} In this study we therefore chose to work with wild-type cells and screen for the inhibition of a quorum sensing-dependent phenotype (pyocyanin production). Studies such as this, which use wild-type bacteria, may be of more direct relevance to native situations than those employing biosensor strains, and may therefore better facilitate the discovery of small molecule modulators of quorum sensing with useful real-world applications.⁶⁰

Experimental

General information

Reactions were performed using oven-dried glassware under an atmosphere of nitrogen with anhydrous, freshly distilled solvents unless otherwise stated. Dichloromethane, ethyl acetate, methanol, n-hexane, acetonitrile and toluene were distilled from calcium hydride. Diethyl ether was distilled over a mixture of lithium aluminium hydride and calcium hydride. Petroleum ether was distilled before use and refers to the fraction between 30-40 °C. All other reagents were used as obtained from commercial sources. Room temperature refers to ambient temperature. Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. All flash chromatography was carried out using slurry-packed Merck 9325 Keiselgel 60 silica gel. Were possible, reactions were monitored by thin layer chromatography (TLC) performed on commercially prepared glass plates pre-coated with Merck silica gel 60 F254 or aluminium oxide 60 F254. Visualisation was by the quenching of UV fluorescence ($v_{max} = 254$ nm) or by staining with ceric ammonium molybdate, potassium permanganate or Dragendorff 's reagent (0.08% w/v bismuth subnitrate and 2% w/v

KI in 3 M aq. AcOH). Infrared spectra were recorded neat or as a solution in the designated solvent on a Perkin-Elmer Spectrum One spectrometer with internal referencing. Selected absorption maxima (v_{max}) are reported in wavenumbers (cm^{-1}) . The abbreviation "br" indicates a broad peak. Melting points were obtained using a Büchi® melting point apparatus (model B-545) and are uncorrected. Proton magnetic resonance spectra were recorded using an internal deuterium lock at ambient probe temperatures (unless otherwise stated) on the following instruments: Bruker DPX-400 (400 MHz), Bruker Avance 400 QNP (400 MHz) Bruker Avance 500 BB ATM (500 MHz) and Bruker Avance 500 Cryo Ultrashield (500 MHz). Chemical shifts ($\delta_{\rm H}$) are quoted in ppm, to the nearest 0.01 ppm, and are referenced to the residual non-deuterated solvent peak. Coupling constants (J) are reported in Hertz to the nearest 0.5 Hz. Data are reported as follows: chemical shift, integration, multiplicity [br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; sextet; sept, septet; m, multiplet; or as a combination of these (e.g. dd, dt, etc.)], coupling constant(s) and assignment. Proton assignments were determined either on the basis of unambiguous chemical shift or coupling pattern, by patterns observed in 2D experiments (¹H-¹H COSY, HMBC and HMQC) or by analogy to fully interpreted spectra for related compounds. Carbon magnetic resonance spectra were recorded by broadband proton spin decoupling at ambient probe temperatures (unless otherwise stated) using an internal deuterium lock on the following instruments: Bruker DPX-400 (100 MHz), Bruker Avance 400 QNP (100 MHz) and Bruker Avance 500 BB ATM (125 MHz) and Bruker Avance 500 Cryo Ultrashield (125 MHz). Chemical shifts ($\delta_{\rm C}$) are quoted in ppm, to the nearest 0.1 ppm, and are referenced to the residual non-deuterated solvent peak. Where appropriate, coupling constants are reported in Hertz to the nearest 0.5 Hz and data are reported as for proton magnetic resonance spectra without integration. Assignments were supported by DEPT editing and determined either on the basis of unambiguous chemical shift or coupling pattern, by patterns observed in 2D experiments (HMBC and HMQC) or by analogy to fully interpreted spectra for related compounds. LCMS spectra were recorded on an HP/Agilent LCMS APCI 120-1000 full gradient machine. The ionisation technique used was electron ionisation (EI). High resolution mass spectroscopy measurements were recorded in-house using a Waters LCT Premier Mass Spectrometer or a Micromass Quadrapole-Time of Flight (Q-ToF) spectrometer. Mass values are reported within the error limits of ± 5 ppm mass units. The ionisation technique used was electrospray ionization (ESI).

General procedure 1: synthesis of β -keto amides from 2-(2nonyl-1,3-dioxolan-2-yl)acetic acid (7). EDC (1.2 equiv.), DMAP (1.7 equiv.) and the appropriate aromatic amine (1.3 equiv.) were added to a solution of 2-(2-nonyl-1,3-dioxolan-2yl)acetic acid (7, 1.0 equiv.) in CH₂Cl₂ (~7 mL per mmol 7) at room temperature. The reaction mixture was stirred at room temperature until TLC analysis indicated complete consumption of the starting acid (~18 hours). Aqueous HCl (10% v/v solution, ~7 mL per mmol 7) was added to the reaction mixture with stirring. The aqueous and organic layers were separated and the aqueous layer was extracted with CH₂Cl₂ (×3). The combined organic extracts were dried (MgSO₄) and the solvent removed under reduced pressure to yield the ketone-protected amide (the crude product material could be purified by column chromatography if desired or used directly in the next step). TFA (~10 equiv.) and H₂O (~0.2 mL per mmol ketone-protected amide substrate) were added dropwise to the ketone-protected amide with stirring at room temperature. The reaction mixture was stirred at room temperature until TLC analysis indicated complete consumption of starting material (~18 hours). Sat. aqueous NaHCO₃ (~15 mL per mmol substrate) was added dropwise at room temperature with stirring. CH₂Cl₂ was added and the aqueous and organic layers separated. The aqueous layer was extracted with CH₂Cl₂ (×3). The combined organic extracts were dried (MgSO₄) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography.

3-Oxo-N-phenvldodecanamide (4). Prepared by general procedure 1 using aniline (0.92 g, 9.8 mmol). The crude ketoneprotected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO_2 , 8 : 2 petroleum ether : EtOAc) to yield the title compound as a white solid (44% overall). R_f 0.3 (8:2 petroleum ether: EtOAc); δ_H (400 MHz; CDCl₃) 9.16 (1H, s, NH) 7.56-7.53 (2H, m, aryl H), 7.35-7.31 (2H, m, aryl H), 7.14-7.10 (1H, m, aryl H), 3.56 (2H, s, $COCH_2CO$), 2.58 (2H, t, J = 7.5 Hz, $COCH_2(CH_2)_7CH_3$), 1.66–1.58 (2H, m, CH₂), 1.32–1.26 (12H, m, 6 × CH₂), 0.89–0.86 (3H, m, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 208.0 (C=O ketone) 163.2 (C=O amide), 137.5 (C), 128.9 (CH), 124.5 (CH), 120.1 (CH), 48.8 (COCH₂CO₂), 44.3 (COCH₂-(CH₂)₇CH₃), 31.8 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.0 (CH₂), 23.4 (CH₂), 22.6 (CH₂), 14.1 (CH₃); v_{max} (neat)/ cm⁻¹ 2918 (NH), 1729 (C=O ketone), 1709 (C=O amide); HRMS (ESI+) m/z found $[M + H]^+$ 290.2119, $C_{18}H_{28}NO_2^$ requires 290.2120; m.p. 80-85 °C (8:2 petroleum ether: EtOAc). This data is consistent with that previously reported.³²

N-(2-Methoxyphenyl)-3-oxododecanamide (5). Prepared by general procedure 1 using o-anisidine (0.12 mL, 1.05 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 7:3petroleum ether: EtOAc) to yield the title compound as a pale brown solid (36% overall). $R_{\rm f}$ 0.8 (8:2 petroleum ether: EtOAc); δ_H (400 MHz; CDCl₃) 9.30 (1H, s, NH), 8.32 (1H, dd, J = 8.0 Hz, 2.0 Hz, aryl H), 7.05 (1H, td, J = 8.0 Hz, 2.0 Hz, aryl H), 6.96 (1H, dd, J = 8.0, 1.5 Hz, aryl H), 6.90–6.87 (1H, m, aryl H), 3.92 (3H, s, OCH₃), 3.57 (2H, s, COCH₂CO), 2.58 $(2H, t, J = 7.5 \text{ Hz}, \text{COC}H_2(\text{CH}_2)_7\text{CH}_3), 1.65-1.59 (2H, m, m)$ CH₂), 1.31–1.22 (12H, m, $6 \times$ CH₂), 0.89–0.86 (3H, m, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 207.0 (C=O ketone) 163.3 (C=O amide), 148.3 (COCH₃), 127.4 (C), 124.0 (CH), 120.9 (CH), 120.1 (CH), 110.0 (CH), 55.8 (COCH₃), 50.0 (COCH₂CO₂), 44.1 (COCH₂(CH₂)₇CH₃), 35.4 (CH₂), 31.8 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.0 (CH₂), 23.4 (CH₂), 22.6 (CH₂), 14.1 (CH₃); v_{max} (neat)/cm⁻¹ 2921 (NH), 1711 (C=O ketone), 1669 (C=O amide); HRMS (ESI+) m/z found $[M + Na]^+$ 342.2059, C19H29NO3Na⁺ requires 342.2040; m.p. 36-38 °C (7:3 petroleum ether : EtOAc).

N-(3-Bromophenvl)3-oxododecanammide (10). Prepared by general procedure 1 using 3-bromoaniline (0.11 mL, 1.01 mmol). The crude ketone-protected amide was purified by column chromatography (SiO₂, 7:3 petroleum ether: EtOAc) to yield the ketone-protected amide as a red oil (0.154 g, 48%) which was used in the next step of the reaction. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 5:5 petroleum ether: Et₂O) to yield the title compound as a pale pink solid (0.094 g, 69%). $R_{\rm f}$ 0.4 (5:5 petroleum ether: Et₂O); v_{max} (neat)/cm⁻¹ 3283, 3253, 2916 (NH), 1715 (C=O ketone), 1660 (C=O amide), 1587, 1547, 1479; δ_H (500 MHz; CDCl₃) 9.34 (1H, s, NH), 7.81 (1H, t, J = 2.0 Hz, NHCCHBr), 7.43 (1H, dt, J = 8.0 Hz, 1.5 Hz, aryl CH), 7.23 (1H, dt, J = 8.0 Hz, 1.5 Hz, aryl CH), 7.16 (1H, t, J = 8.0 Hz, aryl CH), 3.55 (2H, s, COCH₂CO), 2.56 (2H, t, J = 7.5 Hz, $COCH_2(CH_2)_7CH_3$), 1.60 (2H, t, J = 7.5 Hz, CH_2CH_2CO), 1.36–1.16 (12H, br m, 6 × CH_2), 0.87 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (125 MHz; CDCl₃) 207.9 (C=O ketone), 163.7 (C=O amide), 138.8 (NHC), 130.2 (CH), 127.4 (CH), 123.0 (CH), 122.6 (C), 118.5 (CH), 48.7 (COCH₂CO), 44.2 (CH₂), 31.8 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.9 (CH₂), 23.3 (CH₂), 22.6 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z found $[M + H]^+$ 368.1233, $C_{18}H_{27}NO_2Br^{79+}$ requires 368.1220; m.p. 55–58 °C (5 : 5 petroleum ether : Et_2O).

3-Oxo-*N*-((3-trifluoromethoxy)phenyl)dodecanamide (11). Prepared by general procedure 1 using 3-(trifluoromethoxy) aniline (0.93 g, 5.88 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 8:2 petroleum ether: EtOAc) to yield the title compound (0.1113 g, 37% overall). $R_{\rm f}$ 0.63 (8:2 petroleum ether : EtOAc); v_{max} (neat)/cm⁻¹ 2912 (N–H), 1713 (C=O ketone), 1665 (C=O amide); δ_H (500 MHz; CDCl₃) 9.40 (1H, s, NH), 7.59 (1H, s, aryl CH), 7.40 (1H, m, aryl CH), 7.31 (1H, t, J = 8.0 Hz, aryl CH), 7.00-6.94 (1H, m, aryl CH), 3.56 $(2H, s, COCH_2CO), 2.56 (2H, t, J = 7.5 Hz,$ COCH₂(CH₂)₇CH₃), 1.31–1.23 (14H, m, 7 × CH₂), 0.86 (3H, t, J = 6.5 Hz, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 208.1 (C=O ketone), 163.5 (C=O amide), 149.5 (C), 138.8 (C), 129.9 (CH), 118.0 (CH), 116.5 (CH), 112.8 (CH), 48.3 (CH₂), 44.3 (CH₂), 31.8 (CH₂), 29.3 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.5 (CH₂), 23.3 (CH₂), 22.6 (CH₂), 14.0 (CH₃); $\delta_{\rm F}$ (376 MHz; CDCl₃) -57.99 (CF₃); m.p. 50-55 °C (8:2 petroleum ether: EtOAc).

N-(3-Ethoxyphenyl)-3-oxododecanamide (12). Prepared by general procedure 1 using *m*-phenetidine (0.14 mL, 1.07 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, gradient 8:2 petroleum ether: EtOAc to 7:3 petroleum ether: EtOAc) to yield the title compound (0.032 g, 12% overall). $R_{\rm f}$ 0.6 (7:3 petroleum ether: EtOAc); $v_{\rm max}$ (neat)/cm⁻¹ 3299 (C–H), 2916 (N–H), 1718 (C=O ketone), 1661 (C=O amide), 1606, 1535, 1496; $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.15 (1H, s, NH), 7.26–7.25 (1H, m, aryl CH), 7.20 (1H, t, J = 8.0 Hz, aryl CH), 7.03 (1H, ddd, J = 8.0 Hz, 2.0 Hz, 1.0 Hz, aryl CH), 6.69–6.61 (1H, m, aryl CH), 4.02 (2H, q, J = 7.0 Hz, OCH₂CH₃), 3.55

(2H, s, COCH₂CO), 2.57 (2H, t, J = 7.5 Hz, COCH₂(CH₂)₇CH₃), 1.66–1.58 (2H, m, CH₂CH₂CO), 1.40 (3H, t, J = 7.0 Hz, OCH₂CH₃), 1.33–1.19 (12H, br m, 6 × CH₂), 0.88 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (125 MHz; CDCl₃) 208.0 (C=O ketone), 163.4 (C=O amide), 159.5 (C), 138.7 (C), 129.6 (CH), 112.1 (CH), 111.0 (CH), 106.3 (CH), 63.5 (CH₂), 48.8 (CH₂), 44.1 (CH₂), 31.8 (CH₂), 29.35 (CH₂), 29.35 (CH₂), 29.2 (CH₂), 29.0 (CH₂), 23.3 (CH₂), 22.6 (CH₂), 14.8 (CH₃), 14.1 (CH₃); HRMS (ESI+) *m/z* found [M + Na]⁺ 356.2217, C₂₀H₃₁NO₃Na⁺ requires 356.2196; m.p. 41–44 °C (7:3 petroleum ether : EtOAc).

N-(3-Nitrophenyl)-3-oxododecanamide (13). Prepared by general procedure 1 using 3-nitroaniline (0.18 g, 1.37 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 6:4petroleum ether: EtOAc) to yield the title compound (0.0512 g, 18% overall). $R_{\rm f}$ 0.77 (6:4 petroleum ether: EtOAc); $v_{\rm max}$ (neat)/cm⁻¹ 2919 (N-H), 1716 (C=O ketone), 1678 (C=O amide); $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.65 (1H, s, NH), 8.44 (1H, s, aryl CH), 7.92 (1H, d, J = 8.5 Hz, aryl CH), 7.89 (1H, d, J =8.0 Hz aryl CH), 7.49-7.44 (1H, m, aryl CH), 3.60 (2H, s, COCH₂CO), 2.57 (2H, t, J = 7.5 Hz, COCH₂(CH₂)₇CH₃), 1.31–1.18 (14H, m, 7 × CH₂), 0.85 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 207.8 (C=O ketone), 164.1 (C=O amide), 148.5 (C), 138.6 (C), 129.7 (CH), 125.6 (CH), 119.0 (CH), 114.8 (CH), 48.4 (CH₂), 44.1 (CH₂), 31.7 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.9 (CH₂), 23.2 (CH₂), 22.6 (CH₂), 14.0 (CH₃); HRMS (ESI+) m/z found $[M + H]^+$ 335.1996, $C_{18}H_{27}N_2O_4^+$ requires 335.1971; m.p. 68–93 °C (6 : 4 petroleum ether : EtOAc).

3-Oxo-N-(3-phenoxyphenyl)oxododecanamide (14). Prepared by general procedure 1 using 3-phenoxyaniline (0.22 g, 1.23 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography $(SiO_2, 8:2 \text{ petroleum ether}: EtOAc)$ to yield the title compound (0.1086 g, 41% overall). $R_{\rm f}$ 0.36 (8 : 2 petroleum ether : EtOAc); v_{max} (neat)/cm⁻¹ 2916 (N–H), 1717 (C=O ketone), 1660 (C=O amide); $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.25 (1H, s, NH), 7.34–7.25 (3H, m, aryl CH), 7.25-7.20 (2H, m, aryl CH), 7.12-7.06 (1H, m, aryl CH), 7.01-6.97 (2H, m, aryl CH), 6.75-6.71 (1H, m, aryl CH), 3.49 (2H, s, COCH₂CO), 2.52 (2H, t, J = 7.5 Hz, $COCH_2(CH_2)_7CH_3$, 1.26–1.24 (14H, m, 7 × CH₂), 0.87 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 207.4 (C=O ketone), 163.7 (C=O amide), 157.7 (C), 156.7 (C), 138.8 (C), 129.8 (CH), 129.6 (CH), 123.3 (CH), 118.9 (CH), 114.6 (CH), 114.5 (CH), 110.5 (CH), 49.0 (CH₂), 43.8 (CH₂), 31.7 (CH₂), 29.2 (CH₂), 29.2 (CH₂), 29.1 (CH₂), 28.8 (CH₂), 23.2 (CH₂), 22.5 (CH₂), 14.0 (CH₃); HRMS (ESI+) m/z found [M + H]⁺ 382.2375, $C_{24}H_{32}NO_3^+$ requires 382.2382; m.p. 40–45 °C (8 : 2 petroleum ether : EtOAc).

N-(2,5-Dimethoxyphenyl)-3-oxododecanamide (15). Prepared by general procedure 1 using 2,5-dimethoxyaniline (0.16 g, 1.06 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography $(SiO_2, 8:2 petroleum ether: EtOAc)$ to yield the title compound (0.0109 g, 44% overall). $R_f 0.28 (8:2 \text{ petroleum ether}: EtOAc);$ v_{max} (neat)/cm⁻¹ 2914 (N–H), 1703 (C=O ketone), 1677 (C=O amide); $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.29 (1H, s, NH), 8.02 (1H, d, J = 3.0 Hz, aryl CH), 6.75 (1H, dd, J = 9.0 Hz and 3.0 Hz, aryl CH), 6.54 (1H, dd, J = 9.0 Hz and 3.0 Hz, aryl CH), 3.83 (3H, s, OCH₃), 3.73 (3H, s, OCH₃), 3.52 (2H, s, COCH₂CO), 2.56 (2H, t, J = 7.5 Hz, COCH₂(CH₂)₇CH₃), 1.31–1.28 (14H, m, 7 × CH₂), 0.84 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (100 MHz; CDCl₃) 206.8 (C=O ketone), 163.5 (C=O amide), 153.7 (C), 142.6 (C), 128.1 (C), 110.9 (CH), 108.6 (CH), 106.4 (CH), 56.4 (CH₃), 55.7 (CH₃), 50.0 (CH₂), 44.0 (CH₂), 31.8 (CH₂), 29.3 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.9 (CH₂), 23.3 (CH₂), 22.7 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z found $[M + H]^+$ 350.2327, C₂₀H₃₂NO₄⁺ requires 350.2331; m.p. 48–55 °C (8:2 petroleum ether : EtOAc).

N-(3,4-Dimethoxyphenyl)-3-oxododecanamide (16). Prepared by general procedure 1 using 3,4-dimethoxyaniline (0.16 g, 1.08 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography $(SiO_2, 8:2 \text{ petroleum ether}: EtOAc)$ to yield the title compound (0.173 g, 56% overall). R_f 0.83 (8 : 2 Petroleum Ether : EtOAc); v_{max} (neat)/cm⁻¹ 2914 (N–H), 1703 (C=O ketone), 1677 (C=O amide); $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.09 (1H, s, NH), 7.23 (1H, d, J = 2.5 Hz, aryl CH), 6.95 (1H, dd, J = 8.5 Hz and 2.5 Hz, aryl CH), 6.77 (1H, d, J = 8.5 Hz aryl CH), 3.82 (3H, s, OCH₃), 3.81 (3H, s, OCH₃), 3.50 (2H, s, COCH₂CO), 2.53 (2H, t, J = 7.5 Hz, COCH₂(CH₂)₇CH₃), 1.31–1.18 (14H, m, 7 × CH₂), 0.84 (3H, t, J = 7.0 Hz, CH₃); δ_{C} (100 MHz; CDCl₃) 207.9 (C=O ketone), 163.5 (C=O amide,) 148.9 (C), 145.9 (C), 131.2 (C), 112.1 (CH), 111.2 (CH), 104.9 (CH), 56.0 (CH₃), 55.8 (CH₃), 48.9 (CH₂), 44.1 (CH₂), 31.8 (CH₂), 29.3 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 28.9 (CH₂), 23.3 (CH₂), 22.7 (CH₂), 14.0 (CH₃); HRMS (ESI+) m/z found $[M + H]^+$ 350.2338, $C_{20}H_{32}NO_4$ 350.2331; m.p. 69–74 °C (8:2 requires petroleum ether : EtOAc).

N-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-3-oxododecanamide (17). Prepared by general procedure 1 using 6-amino-1,4-benzodioxan (0.13 mL, 1.06 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 7:3 petroleum ether: EtOAc) to yield the title compound (0.07 g, 26% overall). $R_{\rm f}$ 0.7 (7:3 petroleum ether : EtOAc); v_{max} (neat)/cm⁻¹ 3298 (C-H), 2918 (N-H), 1711 (C=O ketone), 1655 (C=O amide), 1547, 1507, 1466, 1417; $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.00 (1H, s, NH), 7.18 (1H, d, J = 2.5 Hz, aryl CH), 6.91 (1H, dd, J = 9.0 Hz, 2.5 Hz, aryl CH), 6.79 (1H, d, J = 9.0 Hz, aryl CH), 4.28–4.17 (4H, m, OCH₂CH₂O), 3.52 (2H, s, COCH₂CO), 2.55 (2H, t, J = 7.5 Hz, $COCH_2(CH_2)_7CH_3$), 1.60 (2H, quint, J = 7.5 Hz, CH_2CH_2CO), 1.36–1.14 (12H, br m, $6 \times CH_2$), 0.87 (3H, t, J = 7.0 Hz, CH_3); $\delta_{\rm C}$ (125 MHz; CDCl₃) 207.9 (C=O ketone), 163.3 (C=O amide), 143.4 (C), 140.5 (C), 131.2 (C), 117.1 (CH), 113.7 (CH), 109.9 (CH), 64.4 (CH₂), 64.2 (CH₂), 48.8 (CH₂), 44.1 (CH₂), 31.8 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.0 (CH₂), 23.3 (CH₂), 22.6 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z found $[M + Na]^+$ 370.2000, $C_{20}H_{29}NO_4Na^+$ requires 370.1994; m.p. 53–55 °C (7 : 3 petroleum ether : EtOAc).

N-(Benzo[d][1,3]dioxol-5-yl)-3-oxododecanamide (18). Prepared by general procedure 1 using 3.4-(methylenedioxy)aniline (0.145 g, 1.06 mmol). The crude ketone-protected amide was used directly without further purification. The crude material obtained after treatment with TFA was purified by column chromatography (SiO₂, 7:3 petroleum ether: EtOAc) to yield the title compound as a light brown solid (0.0346 g, 13% overall). $R_{\rm f}$ 0.6 (7 : 3 petroleum ether : EtOAc); $v_{\rm max}$ (neat)/cm⁻¹ 3294 (CH), 2916 (N-H), 1710 (C=O ketone), 1654 (C=O amide), 1507, 1495, 1414; $\delta_{\rm H}$ (500 MHz; CDCl₃) 9.09 (1H, s, NH), 7.24 (1H, d, J = 2.0 Hz, aryl CH), 6.84 (1H, dd, J = 8.5 Hz, 2.0 Hz, aryl CH), 6.74 (1H, d, J = 8.5 Hz, aryl CH), 5.94 (2H, s, OCH₂O), 3.53 (2H, s, COCH₂CO), 2.56 (2H, t, J = 7.5 Hz, COCH₂(CH₂)₇CH₃), 1.65–1.59 (2H, m, CH₂CH₂CO), 1.32–1.21 (12H, br m, $6 \times CH_2$), 0.87 (3H, t, J = 7.0 Hz, CH_3); $\delta_{\rm C}$ (125 MHz; CDCl₃) 208.0 (C=O ketone), 163.3 (C=O amide), 147.7 (C), 144.3 (C), 131.8 (C), 113.7 (CH), 108.0 (CH), 102.9 (CH), 101.2 (CH₂), 48.6 (CH₂), 44.2 (CH₂), 31.8 (CH₂), 29.4 (CH₂), 29.3 (CH₂), 29.2 (CH₂), 29.0 (CH₂), 23.3 (CH₂), 22.6 (CH₂), 14.1 (CH₃); HRMS (ESI+) m/z found $[M + H]^+$ 334.2044, $C_{19}H_{28}NO_4^+$ requires 334.2013; m.p. 81-85 °C (7:3 petroleum ether: EtOAc).

(S)-4-(1H-Indol-2-yl)-N-(2-oxotetrahydrofuran-3-yl)butanamide (19). EDC (282 mg, 1.5 mol equiv.) and DMAP (72 mg, 0.6 mol equiv.) were added to a stirred solution of (S)-(-)- (α) amino- γ -butyrolactone hydrobromide (23, 178 mg, 0.98 mmol) and indole-3-butyric acid (200 mg, 0.98 mmol) in anhydrous CH₂Cl₂ (10 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 18 hours and then diluted with CH₂Cl₂, washed with HCl (10% v/v solution), dried $(MgSO_4)$ and the solvent removed under reduced pressure. The resulting orange oil was purified by column chromatography (SiO₂, gradient 6:1 CH₂Cl₂: EtOAc to 1:1 CH₂Cl₂: EtOAc) to give the title compound as a slightly opaque oil (180 mg, 64%). $R_{\rm f}$ 0.21 (1:1 EtOAc: CH₂Cl₂); $v_{\rm max}$ (neat)/cm⁻¹ 3377 (NH indole), 3301 (NH amide), 2916 (CH), 1765 (C=O ester), 1649 (C=O amide), 1522 (C=C aromatic); $\delta_{\rm H}$ (500 MHz; CDCl₃) 8.14 (1H, br s, NH indole), 7.58 (1H, d, J = 8.0 Hz, aryl CH), 7.34 (1H, dt, J = 8.0 Hz and 1.0 Hz, aryl CH), 7.18 (1H, ddd, J = 8.0 Hz, 7.0 Hz, 1.0 Hz, aryl CH), 7.10 (1H, ddd, J = 8.0 Hz, 7.0 Hz and 1.0 Hz, aryl CH), 6.95 (1H, d, J = 2.0 Hz, aryl CH), 6.14 (1H, d, J = 6.0 Hz, NH amide), 4.50 (1H, ddd, J = 11.5 Hz, 9.0 Hz and 6.5 Hz, NHCHC(=O)), 4.39 (1H, td, J = 9.0 Hz and 1.5 Hz, CHH'OC(=O)), 4.20 (1H, ddd, J = 11.0 Hz, 9.0 Hz and 6.0 Hz, CHH'OC(=0)), 2.85-2.74 (2H, m, C(=0)CH₂), 2.69 (1H, dddd, J = 12.5 Hz, 9.0 Hz, 6.0 Hz and 1.5 Hz, CHH' CHNH), 2.25 (2H, dd, J = 8.5 Hz and 7.0 Hz, CH₂), 2.09–1.98 (3H, m, CHH'CHNH and CH₂); $\delta_{\rm C}$ (100 MHz; CDCl₃) 175.7 (C=O ester), 173.7 (C=O amide), 136.3 (C), 127.4 (C), 121.9 (CH), 121.8 (CH), 119.2 (CH), 118.8 (CH), 115.4 (C), 115.2 (C), 111.2 (CH), 66.1 (CH₂), 49.0 (CH), 35.4 (CH₂), 25.6 (CH₂), 24.3 (CH₂); LCMS (ES-) (MeCN) 285 (M - H)⁻. This data is in accordance with that previously reported.⁴⁷

(S)-2-(4-Bromophenyl)-N-(2-oxotetrahydrofuran-3-yl)acetamide (20). EDC (267 mg, 1.5 mol equiv.) and DMAP (68 mg, 0.6 mol equiv.) were added to a stirred solution of (S)-(-)- (α) amino-y-butyrolactone hydrobromide (23, 169 mg, 0.93 mmol) and 4-bromophenyl acetic acid (200 mg, 0.93 mmol) in anhydrous CH₂Cl₂ (10 mL). The reaction mixture was stirred at room temperature for 18 hours and then diluted with CH₂Cl₂, washed with HCl (10% v/v solution), dried (MgSO₄), and the solvent removed under reduced pressure. The resulting yellow-white solid was purified by column chromatography (SiO₂, 5:1 $EtOAc: CH_2Cl_2$) to yield the title compound as a white solid (233 mg, 84%). $R_{\rm f}$ 0.26 (5:1 EtOAc:CH₂Cl₂); $v_{\rm max}$ (neat)/ cm⁻¹ 3291 (NH), 1778 (C=O ester), 1649 (C=O amide), 1542 (C=C aromatic); $\delta_{\rm H}$ (500 MHz; CDCl₃) 7.49–7.46 (2H, m, aryl CH), 7.17–7.14 (2H, m, aryl CH), 6.09 (1H, d, J = 6.5 Hz, NH), 4.56-4.46 (1H, m, NHCH), 4.44 (1H, td, J = 9.0 Hz and 1.0 Hz, CHH'OC(=O)), 4.26 (1H, ddd, J = 11.5 Hz, 9.5 Hz and 6.0 Hz, CHH'OC(=O)), 3.56 (2H, s, C(=O)CH₂), 2.81-2.75 (1H, m, CHH'CHNH), 2.17–2.04 (1H, m, CHH'CHNH₂); $\delta_{\rm C}$ (100 MHz; CDCl₃) 175.0 (C=O ester), 170.8 (C=O amide), 133.0 (C), 132.2 (CH), 1331.0 (CH), 121.6 (C), 66.0 (CH₂), 49.4 (CH), 42.5 (CH₂), 30.3 (CH₂); LCMS (ES+) (MeCN) 299 (M + H)⁺; m.p. 154-156 °C (5 : 1 EtOAc : CH₂Cl₂). This data is in accordance with that previously reported.47

N-Phenethylisobutyramide (21). A solution of isobutyric acid (0.21 mL, 2.26 mmol), EDC (0.525 g, 2.73 mmol), DMAP (0.03 g, 0.24 mmol) and 2-phenylethylamine (24, 0.29 mL, 2.3 mmol) in anhydrous CH₂Cl₂ (6 mL) was stirred at room temperature under nitrogen for 12 hours. The solution was concentrated under high vacuum and aqueous HCl (5 mL, 10% v/v solution) was added dropwise with stirring. The aqueous and organic layers were separated and the aqueous layer extracted with CH₂Cl₂. The organic extracts were combined and washed with aqueous HCl (10% v/v solution). The organic extracts were washed with sat. aqueous NaHCO3, dried (MgSO4) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography (SiO₂, 5:5petroleum ether: EtOAc) to yield the title compound as a light brown solid (44%). $R_{\rm f}$ 0.5 (5:5 petroleum ether: EtOAc); $v_{\rm max}$ (neat)/cm⁻¹ 3295 (C-H), 2968 (N-H), 1639 (C=O), 1587, 1545, 1455; $\delta_{\rm H}$ (500 MHz; CDCl₃) 7.36–7.07 (5H, m, aryl CH), 5.86 (1H, br s, NH), 3.47 (2H, dt, J = 7.0 Hz, 6.5 Hz, NHCH₂), 2.79 (2H, t, J = 7.0 Hz, NHCH₂CH₂), 2.29 (1H, hept, J = 7.0Hz, $C(O)CH((CH_3)_2)$), 1.09 (6H, d, J = 7.0 Hz, C(O)CH $((CH_3)_2)$; δ_C (125 MHz; CDCl₃) 177.1 (C=O), 139.0 (C), 128.8 (CH), 128.6 (CH), 126.4 (CH), 40.5 (CH₂), 35.7 (CH₂), 35.5 (CH), 19.6 (CH₃); HRMS (ESI+) m/z found [M + H]⁺ 192.1391, C₁₂H₁₈NO⁺ requires 192.1383; m.p. 88–91 °C (5:5 petroleum ether : EtOAc).

3-Methyl-N-phenethylbutanamide (22). A solution of isovaleric acid (0.22 mL, 2.0 mmol), EDC (0.462 g, 2.41 mmol), DMAP (0.03 g, 0.27 mmol) and 2-phenylethylamine (**24**, 0.25 mL, 2.0 mmol) in anhydrous CH_2Cl_2 (6 mL) was stirred at room temperature under nitrogen for 12 hours. The semi-solid mixture was re-dissolved in CH_2Cl_2 and aqueous HCl (5 mL, 10% v/v solution) was added dropwise with stirring. The aqueous and organic layers were separated and the aqueous layer extracted with CH_2Cl_2 . The organic extracts were combined and washed with aqueous HCl (10% v/v solution). The organic extracts were washed with sat. aqueous NaHCO₃, H₂O dried (MgSO₄) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography (SiO₂, 3 : 7 petroleum ether : EtOAc) to yield the title compound as a yellow crystalline solid (44%). R_f 0.5 (3 : 7 petroleum ether : EtOAc); v_{max} (neat)/cm⁻¹ 3301 (C–H), 2962 (N–H), 1637 (C=O), 1541, 1496, 1454; δ_H (500 MHz; CDCl₃) 7.28–7.08 (5H, m, aryl CH), 6.20 (1H, br s, NH), 3.46 (2H, td, J = 7.0 Hz, 6.0 Hz, NHCH₂), 2.78 (2H, t, J = 7.0 Hz, NCH₂CH₂), 2.10–1.95 (3H, m, C(O)CH₂ and CH((CH₃)₂)), 0.88 (6H, d, J = 7.0 Hz, CH((CH₃)₂)); δ_C (125 MHz; CDCl₃) 173.0 (C=O), 138.9 (C), 128.7 (CH), 128.6 (CH), 126.4 (CH), 45.9 (CH₂), 40.6 (CH₂), 35.7 (CH₂), 26.1 (CH), 22.4 (CH₃); HRMS (ESI+) m/z found [M + H]⁺ 206.1543, C₁₃H₂₀NO⁺ requires 206.1539; m.p. 61–65 °C (3 : 7 petroleum ether : EtOAc).

Ethyl 2-(2H-tetrazol-5-yl)acetate (26). Sodium azide (0.500 g, 7.69 mmol), ammonium chloride (0.411 g, 7.69 mmol) and ethyl cyanoacetate (25, 0.790 g, 6.66 mmol) were dissolved in 7 mL of DMF and stirred for 5 min. The temperature was then raised to 100 °C and the solution was stirred at this temperature for 16 hours. The reaction mixture was allowed to cool to room temperature and a small amount of methanol was added. The solvent was removed under reduced pressure and the residue redissolved in a minimum amount of H₂O. Aqueous HCl (1 N) was then added until pH 2, and the resulting white solid precipitate collected by vacuum filtration. This was washed with small portions of cold H₂O and the solvent removed under reduced pressure to yield the title compound as an off white solid (0.501 g, 42%). v_{max} (neat)/cm⁻¹ 2910 (N–H), 1741 (C=O), 1563, 1454; δ_H (500 MHz, d₆-DMSO) 4.13 (2H, s, C(O)CH₂), 4.13 (2H, q, J = 7.0 Hz, OCH₂CH₃, 1.20 (3H, t, J = 7.0 Hz, OCH₂CH₃); δ_C (125 MHz, d₆-DMSO) 167.7 (C=O), 150.5 (N-C=N), 61.2 (CH_2CH_3), 29.5 ($COCH_2C$), 14.0 (CH_2CH_3); LCMS (EI+) (MeCN) 157 $(M + H)^+$.

Ethyl 2-(2-dodecyl-2H-tetrazol-5-yl)acetate (27). Ethyl 2-(2Htetrazol-5-yl)acetate (26, 0.230 g, 1.49 mmol) and triethylamine (0.180 g, 1.78 mmol) were dissolved in 3 mL of MeCN and stirred to 90 °C over 15 min. 1-bromododecane (0.406 g, 1.63 mmol) was then added dropwise and the solution stirred at reflux for 19 hours. The solution was cooled to room temperature and filtered, and the solvent removed under reduced pressure to give an oil. This was partitioned between H₂O and EtOAc, with the organic phase collected, and the aqueous layer washed a further 2 times with EtOAC. The combined EtOAc washings were dried (MgSO₄) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography (SiO₂, gradient 9.5:0.5 hexane: EtOAc to 8: 2 hexane : EtOAc) to yield the title compound as a colourless oil (0.105 g, 22%). v_{max} (neat)/cm⁻¹ 2918, 1736 (C=O), 1469, 1342; $\delta_{\rm H}$ (500 MHz, d₆-DMSO) 4.64 (2H, t, J = 7.0 Hz, $NCH_2(CH_2)_{10}$, 4.11 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.03 (2H, s, C(O)CH₂C), 1.92–1.83 (2H, m, NCH₂CH₂) 1.33–1.12 (21H, m, 9 × CH₂ and OCH₂CH₃) 0.88–0.83 (3H, m, (CH₂)₁₁CH₃); $\delta_{\rm C}$ (125 MHz, d₆-DMSO): 168.4 (C=O), 159.9 (NC=N), 60.9 (CO₂CH₂CH₃), 52.5 (CH₂CO₂CH₂), 31.3 (CH₂), 31.2 (CH₂), 31.0 (CH₂), 30.8 ((CH₂)₁₁CH₃) 29.0 (CH₂), 28.9 (CH₂), 28.8 (CH₂), 28.7 (CH₂), 28.6 (CH₂), 28.2 (CH₂), 25.6 (CH₂),

22.1 (CH₂), 14.0 (CO₂CH₂CH₃); LCMS (EI+) (MeCN) 325 $(M + H)^+$.

PD-12. Ethyl 2-(2-dodecyl-2*H*-tetrazol-5-yl)acetate (27, 0.140 g, 0.43 mmol) was dissolved in 1.5 mL of EtOH and stirred at room temperature for 5 min. To this was added powdered KOH (0.029 g, 0.52 mmol in 1.5 mL H₂O) was added and the solution stirred for 4.5 hours. EtOAc was then added and the aqueous layer extracted, this was acidified with 1 N HCl until between pH 1-2 and EtOAc added. The organic phase was extracted and dried (MgSO₄) and the solvent removed under reduced pressure to give the title compounds as a white solid (0.069 g, 59%). v_{max} (neat)/cm⁻¹ 2917 (NH), 1740 (C=O), 1520, 1467; $\delta_{\rm H}$ (500 MHz; CDCl₃) 4.59 (2H, t, J = 7.0 Hz, NCH_2CH_2), 4.04 (2H, s, CH_2CO_2H), 2.00 (2H, dd, J = 10.0 Hz, 5.0 Hz, NCH₂CH₂), 1.38–1.18 (18H, m, 9 × CH₂), 0.88 (3H, t, J = 7.0 Hz, CH₃); $\delta_{\rm C}$ (125 MHz; CDCl₃) 172.5 (C=O), 159.3 (N-C=N), 53.3 (NCH₂CH₂), 31.9 (CH₂), 31.4 (CH₂), 29.57 (CH₂), 29.56 (CH₂), 29.5 (CH₂), 29.31 (CH₂), 29.30 (CH₂), 29.2 (CH₂), 28.8 (CH₂), 26.2 (CH₂), 22.7 (CH₂), 14.1 (CH₃); LCMS (ES+) (MeCN) 297 (M + H)⁺; m.p. 67-69 °C.

Analysis of ligand binding to LasR in vivo. The ligand binding domain encoded by lasR was PCR amplified from PAO1 genomic DNA using primers NlasRFxSph (5' GTTGGTACCATCGAGGGAAGGGCCTTGGTTGACGGTTT-CTTG 3') and ClasRHDIIIN3 (5' GTTAAGCTTATTTGCT-GACCGGATGTTCG 3'). The NlasRFxSph primer anneals to the 5' end of the lasR open reading frame (ORF) directly after the ATG start codon and incorporates a SphI cleavage site into the DNA amplicon. This primer also generates a Factor Xa proteolytic cleavage site near to the N-terminus of the encoded protein. The ClasRHDIIIN3 primer anneals to the sequence region just 5' of nucleotide 519 in the lasR ORF and incorporates a HindIII restriction enzyme site. The PCR amplicon (encoding residues 2-173 of the lasR ORF) was end-polished with SphI and HindIII and sub-cloned into SphI/HindII-digested dephosphorylated pQE80. The pQE80 vector incorporates an in-frame hexa-histidine tag onto the N-terminal end of the encoded protein. The N-terminal His-tag and Factor Xa sites were incorporated in the eventuality that the LasR-LBD might need to be purified by column chromatography, although this did not prove necessary. The pQE80(lasR-LBD) vector was introduced into E. coli strain DH5a by electroporation. The recombinant plasmid insert was confirmed by sequencing. The resulting E. coli strain was grown up overnight in LB medium (containing 50 μ g mL⁻¹ carbenicillin). The overnight culture was used to inoculate 50 mL cultures of LB/carbenicillin. After the cultures reached an optical density (600 nm) of 0.5, IPTG (1 mM) was added (to induce expression of *lasR* from the lac promoter on the pQE80 plasmid) to all of the flasks. At the same time, some flasks also received OdDHL (10 µM), compound 3 (50 µM) or just DMSO (solvent) as a control. After a further 2 hours of growth, the cultures were harvested by centrifugation (5000g, 10 min, 4 °C) and the cell pellets were resuspended in 5 mL of lysis buffer (0.1 M Tris-HCl, 0.2 M NaCl, 10 mM DTT (pH 7.5)) and sonicated on ice. The sonicated samples were clarified by centrifugation (12000g, 5 min, 4 °C) and the soluble/insoluble (pelleted) material was analysed by SDS-PAGE.

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