PAPER

Design, synthesis and biological evaluation of non-natural modulators of quorum sensing in *Pseudomonas aeruginosa*†‡

James T. Hodgkinson,[a] **Warren R. J. D. Galloway,**[a] **Megan Wright,**[a] **Ioulia K. Mati,**[a] **Rebecca L. Nicholson,**[a] **Martin Welch**[b] **and David R. Spring***[a]

Many species of bacteria employ a mechanism of intercellular communication known as quorum sensing which is mediated by small diffusible signalling molecules termed autoinducers. The most common class of autoinducer used by Gram-negative bacteria are *N*-acylated-L-homoserine lactones (AHLs). *Pseudomonas aeruginosa* is a clinically important bacterium which is known to use AHL-mediated quorum sensing systems to regulate a variety of processes associated with virulence. Thus the selective disruption of AHL-based quorum sensing represents a strategy to attenuate the pathogenicity of this bacterium. Herein we describe the design, synthesis and biological evaluation of a collection of structurally novel AHL mimics. A number of new compounds capable of modulating the LasR-dependent quorum sensing system of *P. aeruginosa* were identified, which could have value as molecular tools to study and manipulate this signalling pathway. Worthy of particular note, this research has delivered novel potent quorum sensing antagonists, which strongly inhibit the production of virulence factors in a wild type strain of this pathogenic bacterium.

Introduction

Quorum sensing is a mechanism of intercellular communication employed by numerous species of bacteria.[1–3] This process allows the cells comprising a bacterial colony to coordinate their genome expression in a cell-density dependent manner, thus facilitating population-dependent adaptive activity.[1,4–7] The behaviours regulated by quorum sensing are extremely diverse in nature, with many playing critical roles in the mediation of both pathogenic and symbiotic bacteria–host interactions.[1,8,9] For example, root nodulation is a significant quorum sensing-dependent phenotype in symbiots and several clinically relevant pathogens use quorum sensing to control processes associated with virulence.[1,10–14] Given the large variety of behaviours regulated by quorum sensing and the widespread impact of these upon healthcare, the environment and agriculture, it is unsurprising that there has been significant interest in further understanding this form of intercellular communication.[1,15,16]

Quorum sensing is mediated by small diffusible signalling molecules termed autoinducers that are synthesized intracellularly are released into the surrounding milieu.[1] The reliance of quorum sensing upon this 'language' of small molecules provides chemical opportunity to investigate these signalling pathways at a molecular level.[1,15,17] Indeed, recent years have witnessed significant efforts directed towards the discovery of non-native compounds that can modulate quorum sensing systems, with either agonist or antagonist activity.[1,15]

The most common class of autoinducer used by Gram-negative bacteria are *N*-acylated-L-homoserine lactones (AHLs).[1–3,15,18] Most natural AHLs share conserved structural characteristics, 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').[1,19] In the majority of Gram-negative bacterial species, AHL-based quorum sensing typically involves the same series of events. The AHL is generated by an enzyme (a LuxI-type synthase) whereupon it freely diffuses out of the cell. Above a certain threshold concentration, the AHL binds to its cognate cytoplasmic receptor (a LuxR-type transcriptional regulator). The resulting AHL–LuxR-type protein complex then modulates the expression of genes associated with bacterial group processes.[1,3,15,20] Homologues of LuxI and LuxR have been identified in a large number of bacterial genomes; in general each bacterial species responds specifically to its own unique AHL(s), with different LuxI-type synthase and LuxR-type receptors employed.[1,9]

[a]*Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK. E-mail: spring@ch.cam.ac.uk; Fax: +44 (0) 1223-336362; Tel: +44 (0) 1223-336498*
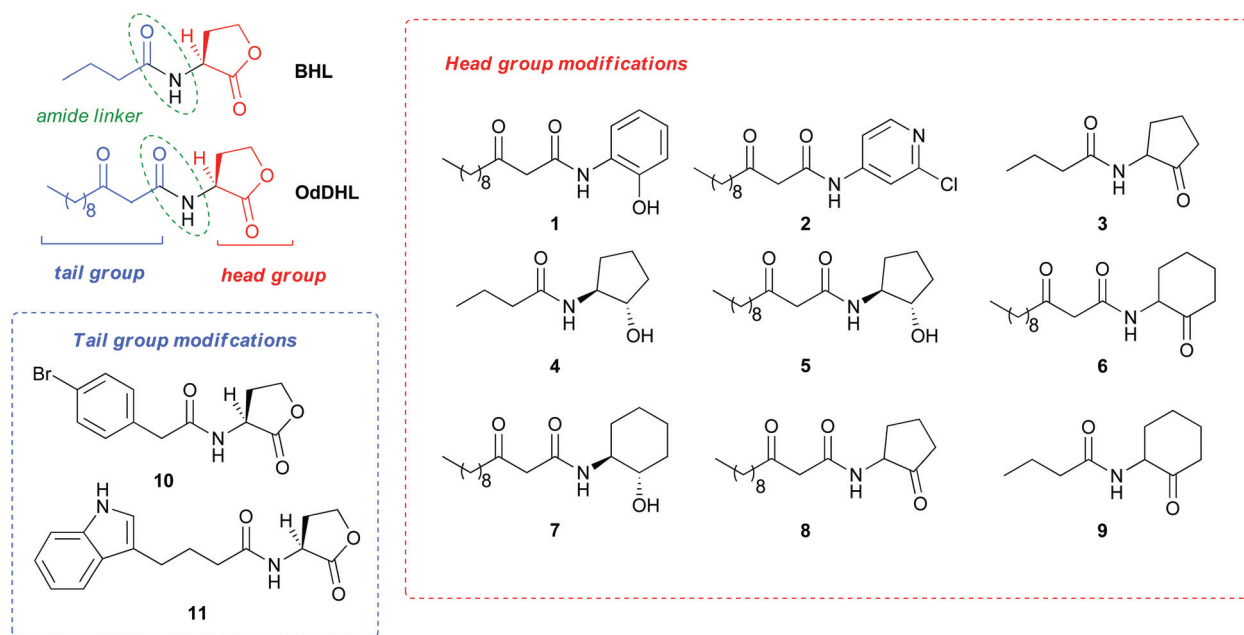[b]*Department of Biochemistry, University of Cambridge, Cambridge, UK*

Fig. 1 (A) Natural AHL molecules employed by *P. aeruginosa* in quorum sensing. (B) Some examples of AHL-mimics capable of modulating quorum sensing in *P. aeruginosa* which contain modifications in the head and tail groups. 1 (Smith *et al.*[42]) and 2 (Marsden *et al.*[43]) are inhibitors of LasR mediated quorum sensing. 3 and 4 are agonists of RhlR-mediated signalling (Lee *et al.*[44] and Glansdorp *et al.*[45]). 5–9 modulate the activity of LuxR homologue mediated quorum sensing in *P. aeruginosa*: 5 and 6 are LasR antagonists, 7 and 8 are LasR agonists and 9 is a RhlR agonist (Smith *et al.*[27,42]). 10 and 11 are potent antagonists against LasR (Geske *et al.*[46]).

The Gram-negative bacterium *Pseudomonas aeruginosa* is a clinically important pathogen, associated with a range of life-threatening hospital-acquired infections and a common cause of mortality in cystic fibrosis patients.[21–26] *P. aeruginosa* uses (at least) three different types of quorum sensing systems, two of which are AHL-based.[1,27–29] Each AHL system involves a distinct AHL molecule, LuxI-type synthase LuxR-type receptor. *N*-(3-Oxododecanoyl)-L-homoserine lactone (OdDHL) is generated by LasI with LasR being the cognate receptor. *N*-Butanoyl-L-homoserine lactone (BHL) is generated by RhlI and is detected by the RhlR protein.[1,27–29] These AHL-dependent systems are interlinked with a third system employing a chemically distinct autoinducer (termed the *Pseudomonas* quinolone signal, PQS[23,24]), forming an intricate hierarchical signaling network.[1,13,29] The Las system is considered to stand at the top, with LasR-OdDHL positively regulating the Rhl and PQS signalling systems;[1,30–35]

P. aeruginosa is known to use quorum sensing to control a variety of processes associated with virulence.[1,21,29,36] For example the Las system regulates the production of the virulence factors elastase, alkaline protease and exotoxin A[37] and the Rhl system regulates rhamnolipid production (a rhamnose-based bio-surfactant) and the virulence factors hydrogen cyanide and pyocyanin. The Rhl system is also required for optimal production of elastase and alkaline protease.[38] Thus the selective disruption of AHL-based quorum sensing using non-native small molecules represents a strategy to attenuate the virulence of the bacterium, allowing the host immune system a better chance of clearing the infection before the bacteria cause too much tissue damage.[1,15,23,24,29] Indeed, there is proof-of-concept from animal studies that the virulence of *P. aeruginosa* can be partially

attenuated *in vivo* by the inhibition of quorum sensing.[1,39,40] *P. aeruginosa* is well known for its low susceptibility to antibiotics and antibiotic resistant strains of *P. aeruginosa* are widely reported; thus novel methods to inhibit its virulence would be of significant clinical value.[41]

Most work on the small molecule modulation of AHL-based quorum sensing in Gram negative bacteria has focused upon the identification of compounds that can interact with the LuxR-type receptor proteins.[1] Given the fact that the Las system is generally considered to be located at the top of the quorum sensing hierarchy in *P. aeruginosa* (*vide supra*), it is unsurprising that the LasR receptor is usually the main target for activator or inhibitor development in this bacterium.[1,17] Indeed, in recent years a range of non-natural compounds capable of modulating LasR-mediated quorum sensing *via* interaction with this receptor protein have been reported.[1,15] Synthetic agents targeting the BHL-RhlR signalling system are also known.[1,15] Many of these Las- and Rhl-system modulators are based upon the structure of the natural AHL signalling molecules, OdDHL and BHL, with modifications in either the 'head' or 'tail' portions[1,15,29] (Fig. 1). For example, the Suga group has identified several non-natural agonists and antagonists of natural AHL binding to LasR in which the head group of OdDHL had been replaced with an aromatic group (*e.g.* 1, Fig. 1).[42] Recently, our own group has reported the discovery of a novel chloro-pyridine pharmacophore that can be used in the place of the native homoserine lactone moiety.[43] For example, compound 2 was found to be an inhibitor of a LasR mediated quorum sensing phenotype in *P. aeruginosa* (Fig. 1). OdDHL and BHL analogues bearing cyclic carbocycles (*e.g.* cyclopentanone, cyclohexanol) as head group replacements have also been reported to be capable of modulating LasR and

This journal is © The Royal Society of Chemistry 2012

Org. Biomol. Chem., 2012, **10**, 6032–6044 | 6033

RhlR mediated quorum sensing in this bacterium (**3–9**, Fig. 1).[27,28,42,44] The Blackwell group have identified a range of non-native AHL analogues containing modifications in the tail region which were capable of modulating the activity of the LasR receptor in *P. aeruginosa*. Of particular interest, the incorporation of aromatic functionalities in the tail section (*e.g.* 4-bromo benzene in **10** and indole in **11**, Fig. 1) generally resulted in analogues with inhibitor activity.[16,46] It has also been shown that the central amide connective function of AHLs (the 'amide linker', Fig. 1) represents a suitable target for chemical modification.[3] For example, Doutheau and co-workers have reported a range of synthetic modulators of LuxR-based quorum sensing in *Vibrio fischeri* in which the amide function has been replaced with various non-native moieties.[47–49]

Inspired by our results in this field,[43–45] and those obtained by the research groups of Blackwell[46] and Suga,[27,42] we sought to design new AHL-mimics incorporating non-native head and tail structural moieties which were capable of modulating quorum sensing in *P. aeruginosa*. This would expand the set of chemical tools available for the study and manipulation of this form of intercellular communication in this bacterium. In particular, structurally novel antagonists would be of interest from a therapeutic perspective. Herein we describe the design, synthesis and biological evaluation of novel AHL mimics based upon two general structural frameworks (Fig. 2). Guided by previous results from Suga and our own research group, one set of compounds (of the general form **12**) was based around the structures of OdDHL and BHL in which the natural acyl chains were retained but the homoserine lactone head group was substituted with non-natural aromatic and heteroaromatic moieties.

Most studies on the small molecule modulation of AHL-based quorum sensing have employed compounds which retained either the natural head or tail portions of the native AHL autoinducer. We were therefore interested in carrying out exploratory studies into the quorum sensing modulatory activity of a small set of 'chimeric' compounds in which *both* the natural head and tail sections had been replaced with non-native structural moieties of proven biological relevance (that is non-native head or tail sections from molecules which are known to modulate quorum sensing).[50] Specifically, the exploratory studies described in this report focused upon a small collection of compounds of the general form **13** that contained a (hetero)aromatic or carbocyclic head group together with a bromo benzene or indole tail. Through this work a series of structurally novel modulators of LasR-mediated quorum sensing in *P. aeruginosa* were identified. In particular, new potent antagonists which were capable of strongly inhibiting the formation of virulence factors were discovered. Overall, the data obtained from these studies provide further insights into the molecular features required for small molecule modulation of LasR-mediated quorum sensing in *P. aeruginosa* which should provide valuable information for the rational design of next-generation chemical tools to study and manipulate this system.[1,3,51]

Results and discussion

Synthesis of AHL-mimics with (hetero)aromatic head groups

Mimics of BHL (**14–17**) were readily prepared by the direct coupling of butyryl chloride (**18**) with a range of (hetero)aromatic amines **19** (Scheme 1). OdDHL-mimics which incorporated an (hetero)aromatic head group (compounds **20–23**) were generated by modification of or previously reported synthetic route towards natural beta-ketoamide AHLs (Scheme 2).[52] Reaction of Meldrum's acid (**24**) with decanoyl chloride (**25**) generated adduct **26**. Treatment of this crude material with methanol furnished methyl 3-oxododecanoate (**27**). The ketone group was then protected as an acetal to form **28** and subsequent ester hydrolysis yielded acid derivative **29**. DCC-mediated coupling of this key intermediate with a range of aromatic amines (**30**) furnished ketone-protected amide derivatives **31**. Subsequent
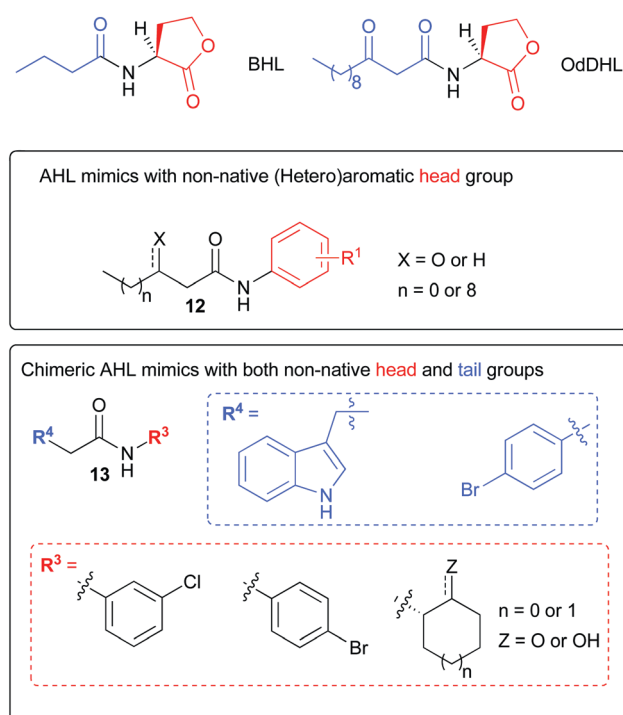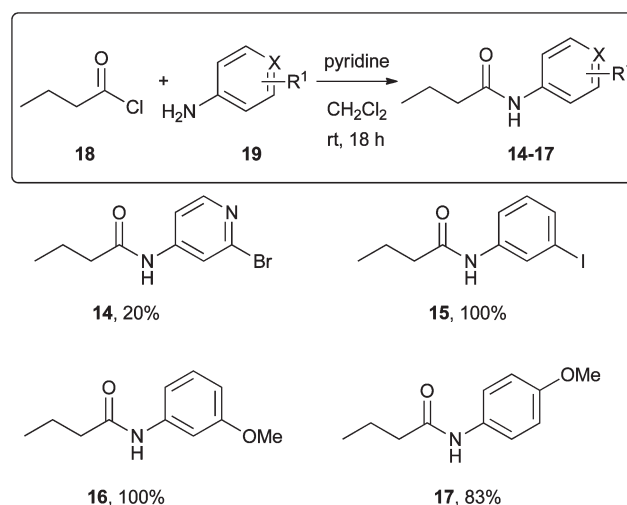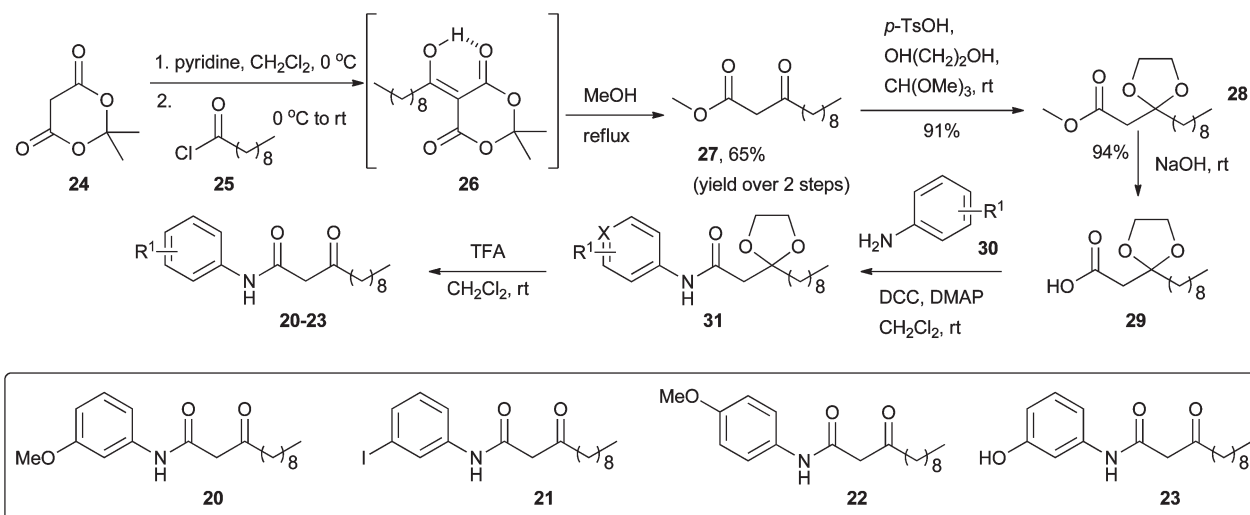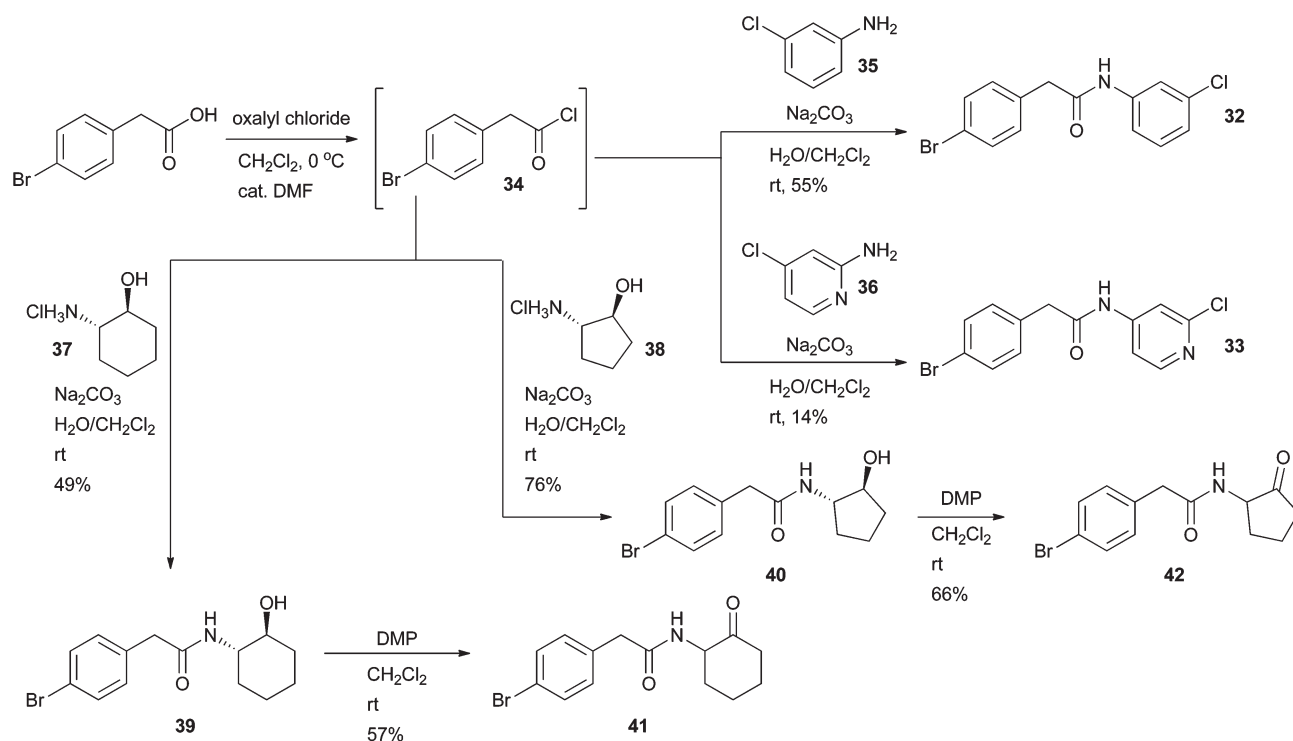

Fig. 2 General structures of target AHL mimics with head and tail modifications.

Scheme 1 Synthesis of BHL mimics with a (hetero)aromatic head group.

Scheme 2 Synthesis of OdDHL mimics with an aromatic head group. Note that 23 has previously been reported by the Suga group.[40]

Scheme 3 Synthesis of chimeric AHL mimics. DMP = Dess–Martin periodinane.

TFA-mediated deprotection yielded the final OdDHL-mimics 20–23.

Synthesis of 'chimeric' AHL mimics with head and tail modifications

We were interested in carrying out some preliminary exploratory studies on the quorum sensory modulatory activity of a small set of 'chimeric' AHL mimics which incorporated a (hetero)aromatic or carbocyclic head group together with a bromo benzene or indole tail. Compounds 32–33 containing a bromo phenylacetyl tail group and a (hetero)aromatic head group, were prepared in two steps by the coupling of readily synthesised 4-bromophenylacetyl chloride (34) with the appropriate (hetero)-aromatic amine (35 and 36 respectively, Scheme 3) under Schotten–Baumann conditions. Alternatively, reaction of chloride 34 with the amino alcohols 37 or 38 furnished compounds 39 and 40 respectively which contained a bromophenylacetyl tail group with a carbocyclic head group. Oxidation using Dess–Martin periodinane then generated the corresponding keto-derivatives 41 and 42. Compound 43, which incorporates an indole-functionalised tail group and an aromatic head group, could be readily

accessed by the DCC-mediated coupling of indole-3-butyric acid (**44**) with the aromatic amine **45** (Scheme 4).

Biological screening

Evaluation of compounds for LasR agonistic activity. Compounds were evaluated for LasR agonism by measuring their effect upon the production of the virulence factor pyocyanin by a *lasI* mutant of *P. aeruginosa* (PAO-JP1).[53] This strain does not contain a functional LasI synthase and thus cannot produce endogenous OdDHL. It has been shown that *lasI* mutants are compromised with regards to pyocyanin production.[54,55] The *lasI* mutant retains the LasR receptor and therefore pyocyanin production would be expected to be stimulated by the addition of exogenous OdDHL.

PAO-JP1 cells were grown for 13 h in the presence or absence of exogenously-supplied OdDHL (10 μM) added at the start of the growth period (positive and negative controls respectively) and the amount of pyocyanin produced measured (Fig. 3). As expected, a much larger quantity of pyocyanin was present in cells grown in the presence of OdDHL, the natural agonist of LasR. These values were compared with those obtained when
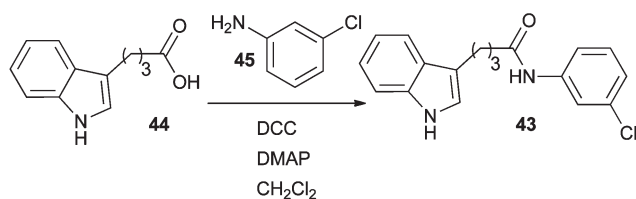
PAO-JP1 was grown for 12 h in the presence of one of the synthetic AHL mimics (10 μM concentration) added at the start of the growth curve in the place of OdDHL. Agonists of the LasR receptor would be expected to increase the amount of pyocyanin present at the end of the growth period relative to that observed in the negative control where no OdDHL was present. None of the compounds examined were found to have significant agonistic activity, though there was evidence that some (**23**, **16**, **14** and **32**) could be partial agonists (partial efficacy relative to the OdDHL, the full agonist).

Evaluation of compounds for LasR antagonistic activity. Small molecules that antagonize quorum sensing are commonly screened using tailored bacterial reporter strains. Typically in such systems, expression of an easily-assayable output (*e.g.*, *lacZ*-encoded β-galactosidase) is driven from a LuxR-dependent promoter. Usually, elsewhere on the same construct, *luxR* is engineered to be expressed constitutively. When such a reporter construct is introduced into a *luxI*-genetic background, transcription of the reporter gene (*lacZ*) becomes entirely dependent on the addition of exogenous AHLs. In antagonist screening trials, the cognate AHL is added at a fixed concentration (usually, just enough to stimulate robust expression of the reporter gene) and antagonist molecules are identified by their ability to compete with the cognate AHL and reduce reporter gene expression. Such a system offers the clear advantage of being defined. However, the disadvantage is that it does not faithfully mimic the situation in wild-type cells, where the endogenous AHL is continually produced and can therefore more effectively "outcompete" any added antagonist. This is why many antagonists that appear extremely potent when identified using engineered reporter systems often fail to elicit the anticipated response when

Scheme 4 Synthesis of AHL mimic **43** with an indole head group.
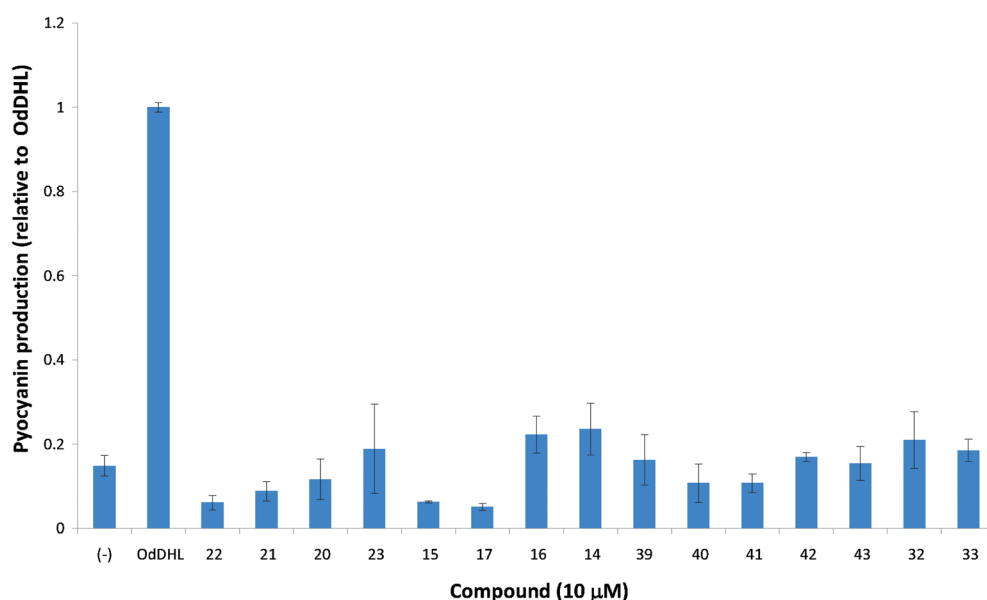
Fig. 3 Stimulation of pyocyanin activity by AHL analogues. Cultures of the *P. aeruginosa* strain PAO-JP1 were grown in Luria broth (LB) medium in the presence of OdDHL (10 μM) or the indicated AHL analogues (10 μM) with good aeration at 37 °C for 13 h (initial OD_{600} of 0.05 $t = 0$). After growth pyocyanin production was quantified as previously described.[56] The amount of pyocyanin produced by each culture in the presence of the AHL analogues was compared directly to the pyocyanin produced in the presence of OdDHL. No effect on growth was observed for any of the analogues (see ESI†). DMSO (−) was added as a control. The data represent the averages and standard deviations from the results of 3 independent biological repeats.

tested in wild-type cells. In the current work, we therefore chose to screen our small molecules by looking for inhibition of quorum sensing-dependent phenotypes in wild-type cells. Specifically, the compounds were initially evaluated for LasR antagonism by measuring their effects upon pyocyanin production by the wild type *P. aeruginosa* strain PAO1. Cells were grown for 13 h in the presence of a synthetic AHL mimic (at a concentration of 200 μM) 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 h in the absence of any synthetic AHL mimic; antagonists of the LasR receptor would be expected to reduce the quantity of pyocyanin produced compared (Fig. 4).

The 'chimeric' compounds (which contained both non-native head and tail regions) were found to be largely inactive (**32**, **39–43**) or only moderately active (**33**). Compounds which retained the acyl chain (tail) of the native signalling molecule of the Rhl-system, BHL, were typically found to display only slight antagonistic effects (**14–17**). In contrast, the majority of compounds which contained the tail region found in OdDHL (specifically compounds **20**, **21** and **22**) were found to have strong antagonistic activities without affecting cell growth. In particular, compounds **22** and **20** inhibited the production of pyocyanin by 73 and 93% respectively. These findings corroborate those of the Greenberg,[57] Suga[27,42] and Blackwell[9] groups in which structurally related OdDHL analogues bearing aromatic head groups were reported to be LasR antagonists.[58] Taken together, our data strongly suggest that the natural 3-oxo-dodecanoyl tail group of OdDHL is important for the LasR modulatory activity of compounds which are based around the AHL scaffold

which is in agreement with other studies.[1,9] A comparison with literature data suggests that the presence of the native OdDHL acyl chain may be especially important for inducing strong LasR antagonism when non-native head groups are present.[59] For example, compound **10**, which has a non-native tail region and native head group has been reported to be a potent LasR antagonist.[46] On the other hand the chimeric compounds examined in this study which contained the same tail group (**32–33**, **39–42**) displayed only moderate levels of activity.[60] Indeed, the relatively poor results obtained with such compounds containing *both* abiotic head and tail moieties imply that one cannot generate potent antagonists simply by making chimeras of previously identified active agents without consideration of other factors.

There are some interesting additional observations. Compound **23** was found to be much less active than **20** despite being very similar in molecular structure. This is in agreement with previous work by the Suga group, in which **23** was reported to have no antagonist activity upon *P. aeruginosa* (in a quorum sensing controlled reporter gene assay).[42] The authors postulated that, in the case of OdDHL mimics containing the native acyl chain and aromatic head groups, a hydrogen bond acceptor is required in either the *meta* or *para* position for antagonistic activity, with the exact position depending on the nature of the substituent; in the case of a hydroxyl group, *ortho* placement is required. Suga and co-workers have suggested that this substituent is involved in a key hydrogen bonding interaction within the binding pocket of the LasR protein, a role which is presumably fulfilled by the carbonyl group of the homoserine lactone moiety in the native autoinducer OdDHL.[42] The high antagonistic activities of methoxy analogues **22** and **20** are broadly consistent with this model;
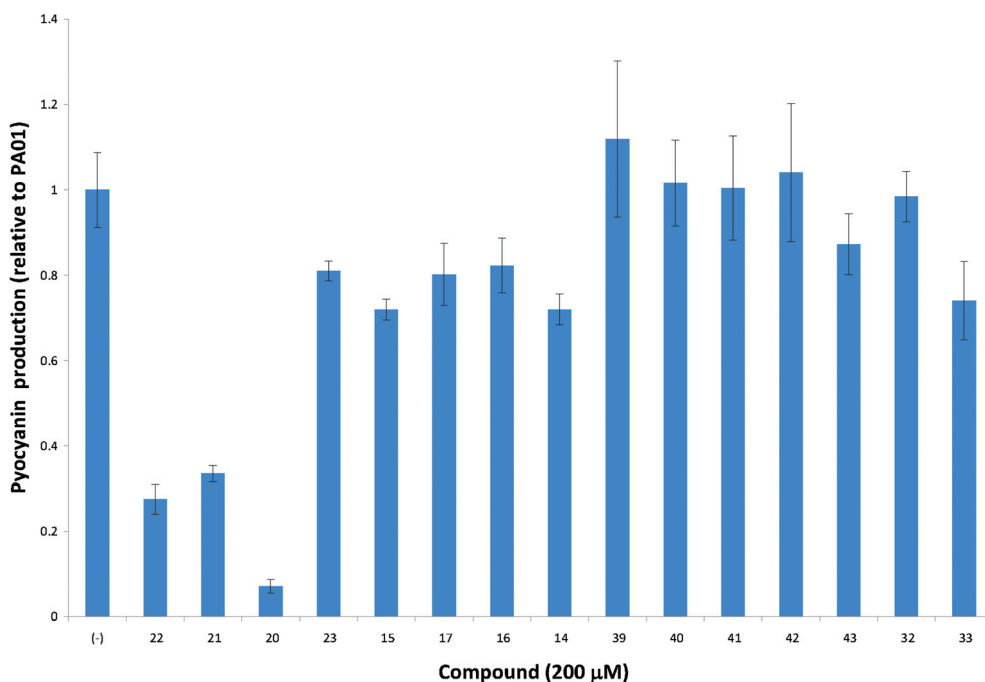
Fig. 4 Antagonist activity of AHL analogues on pyocyanin production in PA01. Cultures of PA01 were grown in Luria broth (LB) medium in the presence of AHL analogues (200 μM) with good aeration at 37 °C for 13 h (initial OD$_{600}$ of 0.05 $t = 0$). After growth pyocyanin production was quantified as previously described.[56] No effect on growth was observed for any of the analogues (see ESI†). DMSO (−) was added as a control. The data represent the averages and standard deviations from the results of 3 independent biological repeats.

methoxy groups are capable of acting as hydrogen bond acceptors and the higher antagonism activity of **22** could possibly be attributed to a more favourable positioning of the methoxy group in the LasR binding pocket for formation of the hydrogen bonding interaction. However, the relatively high antagonistic activity of compound **21** would not be predicted on the basis of these binding requirements; **21** contains an iodine atom in the *meta* position of the aromatic ring which would not be expected to be capable of forming hydrogen bonding interactions. If one assumes that **20–22** act as antagonists by targeting the same site of the LasR receptor (and that the binding mode of these compounds is the same as the native ligand OdDHL), than the antagonistic activity of **21** corroborates recent observations by the Blackwell group which question the proposed critical nature of a hydrogen bonding interaction between the head group of AHLs and AHL mimics and the LasR receptor protein for binding.[9] In addition, it has been suggested that π–π stacking or hydrophobic interactions could play key roles in the binding of aromatic-type AHL mimics to the LasR receptor.[9]

Cells grown in the presence of the two most active antagonists (**20** and **22**) were also assayed for the amount of elastase present (Fig. 5). Elastase production is regulated by the las system (and to a lesser extent, also by the rhl system); antagonists of LasR-mediated quorum sensing would therefore be expected to decrease the amount of elastase produced in a given time period. Both **20** and **22** were found to be antagonists by this assay, with **20** identified as the most active. These results mirror the relative LasR-antagonistic activities identified by the pyocyanin assay.

Conclusions

Herein we have described the design and synthesis of a collection of structurally novel AHL mimics incorporating various non-native head and tail moieties. Biological evaluation of these compounds led to the identification of a number of analogues capable of modulating LasR-based quorum sensing in the pathogenic bacterium *P. aeruginosa*. Thus, through this work the set of chemical tools available for the study and manipulation of this method of intercellular communication has been expanded. In terms of agonism, only weakly active compounds were identified; arguably of more interest are the results obtained in the context of antagonism. The inhibition of quorum sensing in *P. aeruginosa* using non-natural small molecules has been identified as an attractive alternative approach to the use of traditional antibiotics for the treatment of infections caused by this organism.[1] Three compounds with significant LasR-antagonistic activity in *P. aeruginosa* were discovered (**20–22**), none of which were found to be toxic to the host bacterium (Table 1). More specifically, these compounds were able to strongly inhibit the production of virulence factors that contribute to the pathogenicity of this clinically-relevant pathogen in a wild type strain of the organism.

In addition, some general SAR trends could be delineated in the context of LasR antagonism. Compounds containing both non-native head and tail regions were found to be largely inactive and the highest levels of antagonist activity were observed in compounds which contained the tail region found in OdDHL, the native signalling molecule of the LasR-system. In addition,
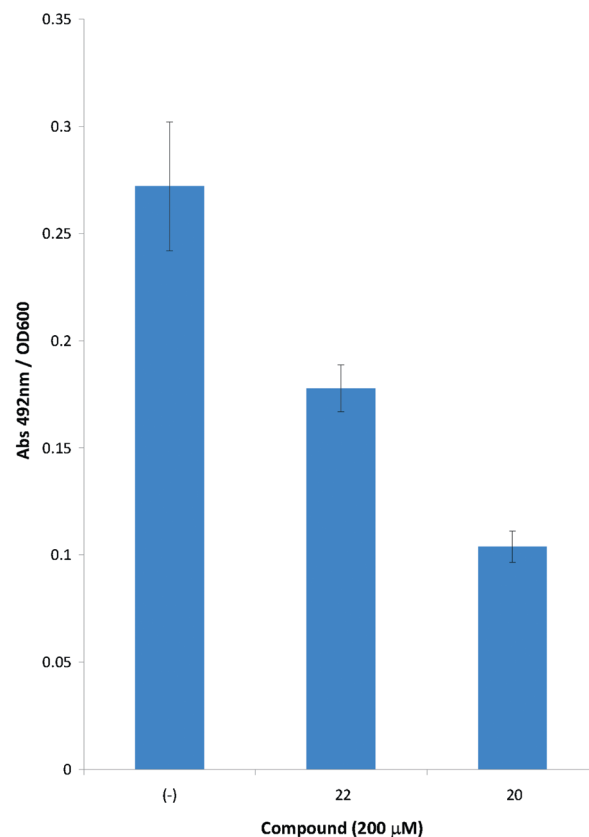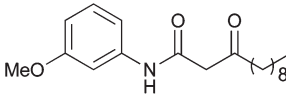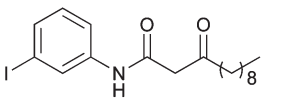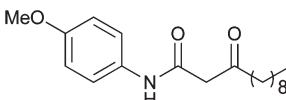
Fig. 5 Antagonist activity of AHL analogues **20** and **22** on elastase production in PA01. Cultures of PA01 were grown in the presence of the AHL analogues (200 μM) in LB medium with good aeration at 37 °C for 7 h (initial OD_{600} of 0.05 $t = 0$). No effect on growth was observed for any of the analogues (see ESI†). DMSO (−) was added as a control. The amount of secreted elastase present in the culture supernatant was quantified as previously described[61] with minor modifications (see ESI†). DMSO (−) was added as a control. The data represent the averages and standard deviations from the results of 3 independent biological repeats.

Table 1 Summary of the activities of three most active antagonists

Compound	% Inhibition[a]	
	Pyocyanin	Elastase
20	93 ± 2	63 ± 3
21	67 ± 2	ND
22	73 ± 4	34 ± 4

[a] % Inhibition of virulence factor production as determined from the data illustrated in Fig. 4 and 5. ND = not determined.

our data corroborate recent observations which question the critical requirement of a hydrogen bonding interaction to the head group of AHLs and AHL-mimics head group for strong LasR binding.[9] SAR data of this sort may facilitate the rational design of *de novo* design of next-generation agents with improved activities.[1,62] Further analogue syntheses and SAR studies which build on the results presented in this report are ongoing and the results of this work will be reported in due course.

Experimental

General information

Reactions were performed using oven-dried glassware apparatus 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 40–60 °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 Kieselgel 60 silica gel. Where possible, reactions were monitored by thin layer chromatography (TLC) performed on commercially prepared glass plates precoated 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 (δ_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 (^{1}H–^{1}H 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 (δ_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.

General procedure 1: coupling of acid chlorides with (hetero)aromatic amines

Pyridine (1.1 equiv.) and butyryl chloride (1 equiv.) were added to a solution of the appropriate (hetero)aromatic amine (1 equiv.) in CH_2Cl_2 (~1 mL per mmol amine) at room temperature. The reaction mixture was stirred until TLC analysis indicated complete consumption of the amine (~18 h). The mixture was diluted with CH_2Cl_2, washed with aqueous HCl (~3 N solution), dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography if required.

N-(2-Bromopyridin-4-yl)butyramide (14). Prepared by general procedure 1 using 4-amino-2-bromopyridine (200 mg, 1.16 mmol), pyridine (0.10 mL, 1.24 mmol) and butyryl chloride (0.12 mL, 1.16 mmol). The crude product material was purified by column chromatography (SiO$_2$, Et$_2$O) to yield the title compound as an orange oil (55 mg, 20%). R_f 0.3 (SiO$_2$; diethyl ether); v_{max} (neat)/cm^{-1} 1468, 1504, 1575, 1687 (amide), 2874, 2963, 3048, 3142, 3240; δ_H (400 MHz, CDCl$_3$) 8.47 (1H, s, N*H*), 8.12 (1H, d, *J* = 5.5 Hz, C*H*NCBr), 7.82 (1H, d, *J* = 1.5 Hz, NHCC*H*CBr), 7.42 (1H, dd, *J* = 5.5 Hz, 1.5 Hz, NHCC*H*CHN), 2.33 (2H, t, *J* = 7.5 Hz, C*H*$_2$CO), 1.67 (2H, sextet, *J* = 7.5 Hz, C*H*$_2$CH$_2$CO), 0.91 (3H, t, *J* = 7.5 Hz, C*H*$_3$); δ_C (100 MHz; CDCl$_3$) 172.2 (*C*=O (amide)), 150.3 (*C*HNCBr), 147.0 (NH*C*), 142.7 (*C*Br), 117.1 (*C*HCBr), 112.8 (*C*HCHN), 39.5 (*C*H$_2$CO), 18.6 (*C*H$_2$CH$_2$CO), 13.6 (*C*H$_3$); LCMS (ES+) (MeCN) 243 (M$^+$).

N-(3-Methoxyphenyl)butyramide (16). Prepared by general procedure 1 using *meta*-anisidine (0.13 mL, 1.16 mol), pyridine (0.10 mL, 1.24 mmol) and butyryl chloride (0.12 mL, 1.16 mmol). The crude product material was purified by column chromatography (SiO$_2$, 2 : 1 Et$_2$O–pet. ether) to yield the title compound as a brown oil (224 mg, 100%). R_f 0.3 (SiO$_2$; 2 : 1 Et$_2$O–pet. ether); v_{max} (neat)/cm^{-1} 1427, 1454, 1492, 1543, 1598, 1661 (amide), 2963, 3301; δ_H (400 MHz, CDCl$_3$) 7.13 (1H, s, N*H*), 7.36 (1H, s, NHCC*H*COMe), 7.22 (1H, t, *J* = 8.0 Hz, NHCCHC*H*), 6.97 (1H, d, *J* = 8.0 Hz, NHCC*H*CH), 6.67 (1H, dd, *J* = 8.0 Hz, 2.0 Hz, NHCCHCHC*H*), 3.83 (3H, s, OC*H*$_3$), 2.35 (2H, t, *J* = 7.5 Hz, C*H*$_2$CO), 1.79 (2H, sextet, *J* = 7.5 Hz, C*H*$_2$CH$_2$CO), 1.03 (3H, t, *J* = 7.5 Hz, C*H*$_3$); δ_C (100 MHz; CDCl$_3$) 171.5 (*C*=O (amide)), 160.1 (*C*OMe), 139.2 (NH*C*), 129.6 (NHCC*H*CH), 111.9 (NHCCHCHC*H*),

This journal is © The Royal Society of Chemistry 2012

Org. Biomol. Chem., 2012, **10**, 6032–6044 | 6039

110.1 (NHC*C*HCOMe), 105.5 (NHC*C*HCH), 55.3 (O*C*H$_3$), 39.7 (*C*H$_2$CO), 19.0 (*C*H$_2$CH$_2$CO), 13.7 (*C*H$_3$); LCMS (ES+) (MeCN) 194 (M + H)$^+$.

N-(3-Iodophenyl)butyramide (15). Prepared by general procedure 1 using 3-iodoaniline (0.14 mL, 1.16 mol), pyridine (0.10 mL, 1.24 mmol) and butyryl chloride (0.12 mL, 1.16 mmol). The crude product material was purified by column chromatography (SiO$_2$, 1 : 1 Et$_2$O–pet. ether) to yield the title compound as a brown oil (335 mg, 100%). R_f 0.25 (SiO$_2$; 1 : 1 Et$_2$O–pet. ether); v_{max} (neat)/cm^{-1} 1414, 1474, 1529, 1580, 1662 (amide), 2872, 2931, 2962, 3071, 3106, 3175, 3291; δ_H (400 MHz, CDCl$_3$) 7.14 (1H, s, N*H*), 7.73 (1H, s, NHCC*H*Cl), 7.28 (1H, d, J = 7.5 Hz, NHCCHCHC*H*), 7.22 (1H, d, J = 7.5 Hz, NHCC*H*CH), 6.82 (1H, t, J = 8.0 Hz, NHCCHC*H*), 2.12 (2H, t, J = 7.0 Hz, C*H*$_2$CO), 1.54 (2H, sextet, J = 7.5 Hz, C*H*$_2$CH$_2$CO), 0.8 (3H, t, J = 7.5 Hz, C*H*$_3$); δ_C (100 MHz; CDCl$_3$) 171.4 (C=O (amide)), 139.1 (NH*C*), 133.2 (NHC*C*HCH*C*H), 130.4 (NHC*C*HCH), 128.5 (NHC*C*HCl), 119.0 (NHC*C*HCH), 94.1 (*C*I), 39.6 (*C*H$_2$CO), 18.9 (*C*H$_2$CH$_2$CO), 13.7 (*C*H$_3$); LCMS (ES+) (MeCN) 290 (M + H)$^+$; m.p. 62–64 °C (1 : 1 Et$_2$O–pet. ether).

N-(4-Methoxyphenyl)butyramide (17). Prepared by general procedure 1 using *para*-anisidine (143 mg, 1.16 mol), pyridine (0.10 mL, 1.24 mmol) and butyryl chloride (0.12 mL, 1.16 mmol). The crude product material was purified by column chromatography (SiO$_2$, 2 : 1 Et$_2$O–pet. ether) to yield the title compound as a brown oil (185 mg, 83%).

R_f 0.3 (SiO$_2$; 2 : 1 Et$_2$O–pet. ether); v_{max} (neat)/cm^{-1} 1411, 1466, 1510, 1530, 1609, 1648 (amide), 2838, 2872, 2967, 3280; δ_H (400 MHz, CDCl$_3$) 7.19 (1H, s, N*H*), 7.42 (2H, dd, J = 6.5 Hz, 2.0 Hz, NHCC*H*), 6.87 (2H, dd, J = 6.5 Hz, 2.0 Hz, NHCCHC*H*), 3.8 (3H, s, OC*H*$_3$), 2.33 (2H, t, J = 7.5 Hz, C*H*$_2$CO), 1.77 (2H, sextet, J = 7.5 Hz, C*H*$_2$CH$_2$CO), 1.02 (3H, t, J = 7.5 Hz, C*H*$_3$); δ_C (100 MHz; CDCl$_3$) 171.2 (C=O (amide)), 156.3 (*C*OMe), 131.1 (NH*C*), 121.8 (NHC*C*HCH), 114.1 (NHCC*H*CH), 55.5 (O*C*H$_3$), 39.4 (*C*H$_2$CO), 19.1 (*C*H$_2$CH$_2$CO), 13.7 (*C*H$_3$); LCMS (ES+) (MeCN) 194 (M + H)$^+$; m.p. 87–89 °C (2 : 1 Et$_2$O–pet. ether).

General procedure 2: coupling of 2-(2-nonyl-1,3-dioxolan-2-yl)-acetic acid with (hetero)aromatic amines

DCC (1.5 equiv.) and DMAP (0.6 equiv.) were added to a solution of 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (**29**, 1.0 equiv.) in CH$_2$Cl$_2$ (~5 mL per mmol **29**) at room temperature. The reaction mixture was stirred at room temperature for 20 min. The appropriate (hetero)aromatic amine (1 equiv.) was added and the reaction mixture stirred at room temperature until TLC analysis indicated complete consumption of the starting acid (~18 h). The reaction mixture was filtered through a pad of Celite® washing with CH$_2$Cl$_2$, the filtrate washed with aqueous HCl (~3 N solution), dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography if required.

General procedure 3: acetal group removal

TFA (~20 equiv.) was added to a solution of the protected dicarbonyl (1 equiv.) in CH$_2$Cl$_2$ (~20 mL mmol^{-1}) at room temperature. The reaction mixture was stirred at room temperature until TLC analysis indicated complete consumption of starting material (~18 h). The solvent was removed under reduced pressure and the crude product material purified by column chromatography.

N-(3-Methoxyphenyl)-3-oxododecanamide (20). The protected analogue of the final product was prepared by general method 2 using 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (**29**, 200 mg, 0.78 mmol), CH$_2$Cl$_2$ (5 mL), DCC (240 mg, 1.16 mmol), DMAP (57 mg, 0.47 mmol) and *meta*-anisidine (0.09 mL, 0.78 mmol). The crude material was purified by column chromatography (SiO$_2$, 1 : 2 EtOAc–pet. ether) to yield a brown oil (155 mg, 55%). A sample of this material (133 mg) was deprotected by general method 3 using 0.5 mL TFA and 5 mL CH$_2$Cl$_2$. The crude material was purified by column chromatography (SiO$_2$, 1 : 1 EtOAc–pet. ether) to give the title compound as a white solid (63 mg, 54%). R_f 0.6 (SiO$_2$; 1 : 1 EtOAc–pet. ether); v_{max} (neat)/cm^{-1} 1429, 1456, 1494, 1548, 1596, 1659 (amide), 1717 (ketone), 2854, 2924, 3293; δ_H (400 MHz; CDCl$_3$) 9.16 (1H, s, N*H*), 7.27 (1H, t, J = 2.0 Hz, NHCC*H*COMe), 7.21 (1H, t, J = 8.0 Hz, NHCCHC*H*), 7.03 (1H, dd, J = 8.0 Hz, 1.0 Hz, NHCC*H*CH), 6.66 (1H, dd, J = 8.0 Hz, 2.0 Hz, NHCCHCHC*H*), 3.80 (3H, s, OC*H*$_3$), 3.54 (2H, s, C(O)C*H*$_2$CO), 2.56 (2H, t, J = 7.5 Hz, C*H*$_2$CO), 1.69–1.53 (2H, m, J = 7.0, C*H*$_2$CH$_2$CO), 1.31–1.25 (12H, br m, alkyl C*H*$_2$), 0.87 (3H, t, J 7.0, C*H*$_3$); δ_C (100 MHz; CDCl$_3$) 208.0 (C=O (ketone)), 163.4 (C=O (amide)), 160.1 (*C*OMe), 138.7 (NH*C*), 129.6 (NHC*C*H), 112.2 (NHCC*H*CH*C*H), 110.4 (NHC*C*HCOMe), 105.7 (NHC*C*HCH), 55.3 (O*C*H$_3$), 48.8 (C(O)*C*H$_2$CO), 44.2 (*C*H$_2$C(O)CH$_2$CO), 31.8 (*C*H$_2$), 29.34 (*C*H$_2$), 29.30 (*C*H$_2$), 29.20 (*C*H$_2$), 28.9 (*C*H$_2$), 23.3 (*C*H$_2$), 22.6 (*C*H$_2$), 14.1 (*C*H$_3$); LCMS (ES+) (MeCN) 320 (M + H)$^+$.

N-(3-Iodophenyl)-3-oxododecanamide (21). The protected analogue of the final product was prepared by general method 2 using 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (**29**, 200 mg, 0.78 mmol), CH$_2$Cl$_2$ (5 mL), DCC (240 mg, 1.16 mmol), DMAP (57 mg, 0.47 mmol) and 3-iodoaniline (0.09 mL, 0.78 mmol). The crude material was purified by column chromatography (SiO$_2$, 2 : 1 Et$_2$O–pet. ether) to yield an orange oil (257 mg, 72%). A sample of this material (237 mg) was deprotected by general method 3 using 0.7 mL TFA and 5 mL CH$_2$Cl$_2$. The crude material was purified by column chromatography (SiO$_2$, 1 : 2 Et$_2$O–pet. ether) to give the title compound as a white solid (168 mg, 78%). R_f 0.17 (SiO$_2$; 1 : 2 Et$_2$O–pet. ether (30–40)); v_{max} (neat)/cm^{-1} 1474, 1542, 1586, 1654 (amide), 1715 (ketone), 2850, 2916, 3284; δ_H (500 MHz, CDCl$_3$) 9.27 (1H, s, N*H*), 7.97 (1H, t, J = 2.0 Hz, NHCC*H*Cl), 7.53–7.50 (1H, m, NHCC*H*CH), 7.45 (1H, ddd, J = 8.0 Hz, 1.5 Hz, 1.0 Hz, NHCCHCHC*H*), 7.04 (1H, t, J = 8.0 Hz, NHCC*H*CH), 3.55 (2H, s, C(O)C*H*$_2$CO), 2.57 (2H, t, J = 7.5 Hz, C*H*$_2$CO), 1.65–1.59 (2H, m, C*H*$_2$CH$_2$CO), 1.29–1.21 (12H, br m, alkyl C*H*$_2$), 0.88 (3H, t, J = 7.0 Hz, C*H*$_3$); δ_C (125 MHz; CDCl$_3$) 208.1 (C=O (ketone)), 163.5 (C=O (amide)), 138.6 (NH*C*), 133.5 (NHC*C*HCH<u>C</u>H), 130.4 (NHC*C*HCH), 128.7

(NHCC*H*CHCl), 119.2 (NHCCH*C*HCl), 94.1 (NHCCH*C*HCl), 48.4 (C(O)*C*H$_2$CO), 44.3 (CH$_2$C(O)*C*H$_2$CO), 31.8 (*C*H$_2$), 29.4 (*C*H$_2$), 29.3 (*C*H$_2$), 29.2 (*C*H$_2$), 29.0 (*C*H$_2$), 23.3 (*C*H$_2$), 22.7 (*C*H$_2$), 14.1 (*C*H$_3$); LCMS (ES+) (MeCN) 416 (M + H)$^+$; m.p. 73–75 °C (1 : 2 Et$_2$O–pet. ether).

N-(4-Methoxyphenyl)-3-oxododecanamide (22). The protected analogue of the final product was prepared by general method 2 using 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (**29**, 200 mg, 0.78 mmol), CH$_2$Cl$_2$ (5 mL), DCC (240 mg, 1.16 mmol), DMAP (57 mg, 0.47 mmol) and *para*-anisidine (96 mg, 0.78 mmol). The crude material was purified by column chromatography (SiO$_2$, 2 : 1 Et$_2$O–pet. ether) to yield a white solid (236 mg, 83%). A sample of this material (214 mg) was deprotected by general method 3 using 0.7 mL TFA and 5 mL CH$_2$Cl$_2$. The crude material was purified by column chromatography (SiO$_2$, 1 : 1 Et$_2$O–pet. ether) to give the title compound as a white solid (126 mg, 67%). R_f 0.18 (SiO$_2$; 1 : 1 Et$_2$O–pet. ether); v_{max} (neat)/cm^{-1} 1418, 1468, 1517, 1552, 1654 (amide), 1711 (ketone), 1727, 2849, 2918, 3301; δ_H (500 MHz, CDCl$_3$) 9.00 (1H, s, N*H*), 7.46–7.43 (2H, m, NHCC*H*), 6.88–6.85 (2H, m, NHCCHC*H*), 3.79 (3H, s, OC*H$_3$*), 3.55 (2H, s, C(O)C*H$_2$*CO), 2.57 (2H, t, J = 7.5 Hz, C*H$_2$*CO), 1.65–1.59 (2H, m, C*H$_2$*CH$_2$CO), 1.30–1.26 (12H, br m, alkyl C*H$_2$*), 0.88 (3H, t, J = 7.0 Hz, C*H$_3$*); δ_C (125 MHz; CDCl$_3$) 208.1 (*C*=O (ketone)), 163.2 (*C*=O (amide)), 156.5 (*C*OMe), 130.7 (NH*C*), 121.9 (NHC*C*), 114.1 (NHCCH*C*H), 55.5 (O*C*H$_3$), 48.7 (*C*(O)CH$_2$CO), 44.2 (*C*H$_2$C(O)CH$_2$CO), 31.8 (*C*H$_2$), 29.4 (*C*H$_2$), 29.3 (*C*H$_2$), 29.2 (*C*H$_2$), 29.0 (*C*H$_2$), 23.4 (*C*H$_2$), 22.7 (*C*H$_2$), 14.1 (*C*H$_3$); LCMS (ES+) (MeCN) 320 (M + H)$^+$; m.p. 91–94 °C (1 : 1 Et$_2$O–pet. ether).

N-(3-Hydroxyphenyl)-3-oxododecanamide (23). The protected analogue of the final product was prepared by general method 2 using 2-(2-nonyl-1,3-dioxolan-2-yl)acetic acid (**29**, 200 mg, 0.78 mmol), CH$_2$Cl$_2$ (5 mL), DCC (240 mg, 1.16 mmol), DMAP (57 mg, 0.47 mmol) and 3-aminophenol (85 mg, 0.78 mmol). The crude material was purified by column chromatography (SiO$_2$, 1 : 1 EtOAc–pet. ether) to yield a yellow solid (68 mg, 25%). A sample of this material (58 mg) was deprotected by general method 3 using 0.25 mL TFA and 5 mL CH$_2$Cl$_2$. The crude material was purified by column chromatography (SiO$_2$, 4 : 1 Et$_2$O–pet. ether) to give the title compound as a white solid (11 mg, 23%). R_f 0.3 (SiO$_2$; 4 : 1 Et$_2$O–pet. ether); v_{max} (neat)/cm^{-1} 1446, 1491, 1506, 1601, 1641 (amide), 1698 (ketone), 2384, 2851, 2915, 3201; δ_H (400 MHz; CDCl$_3$) 9.43 (1H, N*H*), 7.79 (1H, s, NHCC*H*COH), 7.18 (1H, t, J = 8.0 Hz, NHCCHC*H*), 6.74 (1H, d, J = 8.0 Hz, NHCC*H*CH), 6.66 (1H, dd, J = 8.0 Hz, 2.0 Hz, NHCCHCHC*H*), 3.61 (2H, s, C(O)C*H$_2$*CO), 2.59 (2H, t, J = 7.0 Hz, C*H$_2$*CO), 1.6–1.66 (2H, m, C*H$_2$*CH$_2$CO), 1.23–1.3 (12H, br m, alkyl C*H$_2$*), 0.9 (3H, t, J = 7.0 Hz, C*H$_3$*); δ_C (100 MHz; MeOD) 207.8 (*C*=O (ketone)), 168.5 (*C*=O (amide)), 160.0 (*C*OH), 141.6 (NH*C*), 131.6 (NHCC*H*), 113.5, 113.3 (NHC*C*HCH and NHCCHC*H*CH), 109.4 (NHC*C*HCOH), 44.9 (*C*(O)CH$_2$CO), 34.1 (*C*H$_2$C(O)CH$_2$CO), 31.17–31.59, 25.5, 24.7 (seven signals, *C*H$_2$), 15.4 (*C*H$_3$); LCMS (ES+) (MeCN) 306 (M + H)$^+$; m.p. 105–110 °C (4 : 1 Et$_2$O–pet. ether). This compound has been reported previously but spectroscopic data is not available.

N-(3-Chlorophenyl)-4-(1*H*-indol-3-yl)butanamide (43). DCC (303 mg) and DMAP (72 mg) were added to a solution of indole-3-butyric acid (200 mg, 0.98 mmol) in anhydrous CH$_2$Cl$_2$ (5 ml) under nitrogen at room temperature. The mixture was stirred for 20 min at room temperature. 3-Chloroaniline (0.1 ml, 0.98 mmol) was added and the reaction mixture stirred for 12 h at rt. The reaction mixture was filtered through a pad of Celite® washing with CH$_2$Cl$_2$, the filtrate washed with aqueous HCl (~3 N solution), dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography (SiO$_2$, Et$_2$O) to yield the title compound as a yellow oil (225 mg, 0.72 mmol, 73%). R_f 0.46 (SiO$_2$, Et$_2$O); v_{max} (CDCl$_3$)/cm^{-1} 3403 (NH indole), 3301 (NH amide), 2927 (CH), 1666 (C=O amide), 1593 (C=C aromatic), 1530 (C=C aromatic); δ_H (400 MHz, CDCl$_3$) 8.04 (1H, br s. indole N*H*), 7.58 (1H, d, J = 7.0 Hz, aryl C*H*), 7.53 (1H, s, aryl C*H*), 7.33 (1H, d, J = 8.0 Hz, aryl C*H*), 7.27 (1H, br s, N*H* amide), 7.25 (1H, partially obscured by solvent, aryl C*H*), 7.21–7.16 (2H, m, aryl C*H*), 7.11 (1H, ddd, J = 8.0 Hz, 8.0 Hz, 1.0 Hz, aryl C*H*), 7.05 (1H, ddd, J = 8.0 Hz, 2.0 Hz, 1.0 Hz, aryl C*H*), 6.94 (1H, d, J = 2.0 Hz, aryl C*H*), 2.86–2.81 (2H, m, C*H$_2$*C(=O)NH), 2.37–2.33 (2H, m, NHC(=O)CH$_2$CH$_2$C*H$_2$*), 2.16–2.11 (2H, m, NHC(=O)CH$_2$C*H$_2$*CH$_2$); δ_C (100 MHz; CDCl$_3$) 171.6 (*C*=O amide), 139.0 (*C*), 136.4 (*C*), 134.5 (*C*), 129.9 (*C*H), 127.4 (*C*), 124.1 (*C*H), 122.0 (*C*H), 121.7 (*C*H), 119.9 (*C*H), 119.3 (*C*H), 118.8 (*C*H), 117.7 (*C*H), 115.3 (*C*) 111.2 (*C*H), 36.9 (*C*H$_2$), 25.7 (*C*H$_2$), 24.4 (*C*H$_2$); LCMS (ES−) (MeCN) 313.2 (M − H)$^+$ for ^{37}Cl, 311.2 (M − H)$^+$ for ^{36}Cl.

4-Bromophenylacetyl chloride (34). Oxalyl chloride (0.30 ml, 3.5 mmol, 1.5 equiv.) was added to a stirred solution of 4-bromophenyl acetic acid (500 mg, 2.33 mmol, 1.0 equiv.) in anhydrous CH$_2$Cl$_2$ (20 ml) at 0 °C under a nitrogen atmosphere. One drop of DMF was then added and the reaction mixture stirred for 30 min at 0 °C. The flask was warmed to room temperature and stirred for at least 80 min. The solvent was removed under reduced pressure to yield the title compound as a crude yellow oil which was used immediately and without purification in the next steps.

General procedure 4: coupling of 4-bromophenylacetyl chloride (34) with (hetero)aromatic amines

A solution of 4-bromophenylacetyl chloride (1 equiv.) in CH$_2$Cl$_2$ (~7 mL per mmol susbtrate) was added dropwise to a vigorously stirred mixture of the appropriate (hetero)aromatic amine (1 equiv.) and Na$_2$CO$_3$ (2 equiv.) in water (~7 mL per mmol substrate) at room temperature. The reaction was stirred vigorously at room temperature open to the air for 12 h. The reaction mixture was diluted with CH$_2$Cl$_2$ and the aqueous and organic layers separated. The aqueous layer was washed with CH$_2$Cl$_2$ and the combined organic layers dried (MgSO$_4$) and the solvent removed under reduced pressure. The crude product material was purified by column chromatography if required.

2-(4-Bromophenyl)-N-(3-chlorophenyl)acetamide (32). Prepared by general procedure 4 using 3-chloroaniline (0.1 mL, 0.86 mmol), Na$_2$CO$_3$ (273 mg), water (6 mL) and 4-bromophenylacetyl chloride (200 mg, 0.86 mmol) dissolved in CH$_2$Cl$_2$

This journal is © The Royal Society of Chemistry 2012

Org. Biomol. Chem., 2012, **10**, 6032–6044 | 6041

(6 mL). The crude product material (white solid) was purified by column chromatography (SiO$_2$, 1 : 1 Et$_2$O–pet. ether) to yield the title compound as an off-white solid (154 mg, 55%). R_f 0.29 (SiO$_2$, 1 : 1 Et$_2$O–pet. ether); ν_{max} (MeOD)/cm^{-1} 3332 br (OH solvent), 3276 (NH amide), 2395, 1654 (C=O amide), 1590 (C=C aromatic), 1482 (C=C aromatic); δ_H (400 MHz, MeOD) 7.74 (1H, dd, J = 2.0 Hz, NHCC*H*CCl), 7.50 (2H, d, J = 8.5 Hz, CBrC*H*), 7.43 (1H, ddd, J = 8.0 Hz, 1.0 Hz, 1.0 Hz, NHCC*H*CH), 7.29 (2H, d, J = 8.5 Hz, CBrCHC*H*), 7.29 (1H, dd, J = 8.0 Hz, 8.0 Hz, NHCCHC*H*), 7.11 (1H, ddd, J = 8.0 Hz, 2.0 Hz, 1.0 Hz, NHCCHCHC*H*), 3.68 (2H, s, C*H$_2$*); δ_C (100 MHz; MeOD) 171.7 (*C*=O), 141.2 (NH*C*), 135.7 (*C*), 135.4 (*C*), 132.6 (*C*H), 132.1 (*C*H), 131.0 (NHCC*H*CH), 125.0 (C*C*lCHCH), 121.8 (*C*Br), 120.9 (NHCC*H*CCl), 119.1 (NHCC*H*CH), 43.8 (*C*H$_2$); LCMS (ES+) (MeCN) 325.8 (M + H)$^+$ for ^{35}Cl and ^{81}Br, 327.8 (M + H)$^+$ for ^{37}Cl and ^{81}Br; m.p. 136–138 °C (1 : 1 Et$_2$O–pet. ether).

2-(4-Bromophenyl)-*N*-(2-chloropyridin-4-yl)acetamide (33). Prepared by general procedure 4 using 4-amino-2-chloropyridine (111 mg, 0.86 mmol), Na$_2$CO$_3$ (273 mg), water (6 mL) and 4-bromophenylacetyl chloride (200 mg, 0.86 mmol) dissolved in CH$_2$Cl$_2$ (6 mL). The crude product material (yellow oil) was purified by column chromatography (SiO$_2$, 20 : 1 Et$_2$O–pet. ether) followed by washing with HCl (to remove unreacted starting amine running with the same R_f) to yield the title compound as an amorphous white solid (39 mg, 14%). R_f 0.22 (SiO$_2$, 20 : 1 Et$_2$O–pet. ether); ν_{max} (CDCl$_3$)/cm^{-1} 3246 (amide NH), 3053 (CH aromatic), 2927 (CH), 1681 (amide C=O), 1578 (C=N), 1504 (C=C aromatic); δ_H (400 MHz, CDCl$_3$) 8.17 (1H, br d, J = 5.0 Hz, NHCCHC*H*N), 7.55 (1H, s, C*H*CCl), 7.47 (2H, d J = 8.5 Hz, CBrC*H*), 7.31 (1H, br d, J = 4.5 Hz, NHCC*H*CHN), 7.14 (2H, d, J = 8.0 Hz, CBrCHC*H*), 3.67 (2H, s, C*H$_2$*); δ_C (100 MHz; CDCl$_3$) 169.4 (*C*=O), 151.7 (*C*), 149.4 (*C*H), 147.6 (*C*), 132.3 (*C*H), 132.3 (*C*), 131.0 (*C*H), 122.0 (*C*), 113.6 (*C*H), 112.6 (*C*H), 44.0 (*C*H$_2$); LCMS (ES+) (MeCN) 324.9 (M + H)$^+$ for ^{35}Cl and ^{79}Br, 326.9 (M + H)$^+$ for ^{35}Cl and ^{81}Br and/or ^{37}Cl and ^{79}Br, 328.8 (M + H)$^+$ for ^{37}Cl and ^{81}Br.

2-(4-Bromophenyl)-*N*-((1*S**,2*S**)-2-hydroxycyclohexyl)acetamide (39). Prepared by general procedure 4 using *trans*-2-aminocyclohexanol hydrochloride (177 mg, 1.17 mmol), Na$_2$CO$_3$ (372 mg), water (6 mL) and 4-bromophenylacetyl chloride (273 mg, 1.17 mmol) dissolved in CH$_2$Cl$_2$ (6 mL). The crude product material (white solid) was purified by column chromatography (SiO$_2$, 20 : 1 EtOAc–CH$_2$Cl$_2$) to yield the title compound as a white solid (179 mg, 49%). R_f 0.20 (SiO$_2$, 4 : 1 EtOAc–CH$_2$Cl$_2$); ν_{max} (CDCl$_3$)/cm^{-1} 3300 br (OH), 3286 (NH amide), 2932 (CH), 2851 (CH), 1644 (C=O amide), 1618, 1550 (C=C aromatic); δ_H (400 MHz, CDCl$_3$) 7.47 (2H, d, J = 8.5 Hz, CBrC*H*), 7.14 (2H, d, J = 8.5 Hz, CBrCHC*H*), 5.34 (1H, br s, N*H*), 3.65–3.57 (1H, m, NHCHC*H*OH), 3.54 (2H, s, C*H$_2$*C(=O)), 2.05–1.99 (1H, m), 1.89–1.82 (1H, m), 1.73–1.62 (2H, m), 1.35–1.14 (1H, m), 1.12–1.02 (1H, m); δ_C (100 MHz; CDCl$_3$) 172.1 (*C*=O), 133.6 (CBrCHCHC*H*), 132.1 (CBrC*H*), 131.0 (CBrCHC*H*), 121.5 (*C*Br), 75.4 (*C*HOH), 56.0 (NH*C*H), 43.0 (*C*H$_2$), 34.5 (*C*H$_2$), 31.3 (*C*H$_2$), 24.5 (*C*H$_2$), 23.9 (*C*H$_2$); LCMS (ES+) (MeCN) 313.9 (M + H)$^+$ for ^{81}Br; m.p. 110–114 °C (4 : 1 EtOAc–CH$_2$Cl$_2$).

2-(4-Bromophenyl)-*N*-((1*S**,2*S**)-2-hydroxycyclopentyl)acetamide (40). Prepared by general procedure 4 using *trans*-2-aminocyclopentanol hydrochloride (161 mg, 1.17 mmol), Na$_2$CO$_3$ (372 mg), water (6 mL) and 4-bromophenylacetyl chloride (273 mg, 1.17 mmol) dissolved in CH$_2$Cl$_2$ (6 mL). The crude product material (pale yellow solid) was purified by column chromatography (SiO$_2$, 4 : 1 EtOAc–CH$_2$Cl$_2$) to yield the title compound as a white solid (266 mg, 76%).

R_f 0.20 (SiO$_2$, 4 : 1 EtOAc–CH$_2$Cl$_2$); ν_{max} (CDCl$_3$)/cm^{-1} 3350 br (OH), 3281 (NH amide), 2952 (CH), 1631 (C=O amide), 1553 (C=C amide); δ_H (400 MHz, CDCl$_3$) 7.52 (2H, d, J = 8.5 Hz, CBrC*H*), 7.16 (2H, d, J = 8.5 Hz, CBrCHC*H*), 5.52 (1H, br s, O*H*), 4.27 (1H, br d, J = 1.5 Hz, N*H*), 3.95 (1H, dq, J = 6.0 Hz, 1.5 Hz, C*H*OH), 3.86–3.79 (1H, m, NHC*H*), 3.55 (2H, s, C*H$_2$*C(=O)), 2.14–1.98 (2H, m), 1.85–1.75 (1H, m), 1.72–1.62 (2H, m), 1.37–1.29 (1H, m); δ_C (100 MHz; CDCl$_3$) 172.4 (*C*=O), 133.4 (CBrCHCHC*H*), 132.1 (CBrC*H*), 131.0 (CBrCHC*H*), 121.5 (*C*Br), 79.6 (*C*HOH), 61.1 (NH*C*H), 42.7 (*C*H$_2$C(=O)), 32.6 (COH*C*H$_2$), 30.5 (NHCH*C*H$_2$), 21.3 (*C*H$_2$CH$_2$CH$_2$CHOH); LCMS (ES+) (MeCN) 299.9 (M + H)$^+$ for ^{81}Br; m.p. 110–114 °C (4 : 1 EtOAc–CH$_2$Cl$_2$).

General procedure 5: oxidation using Dess–Martin periodinane

Dess–Martin periodinane (1.1 equiv.) was added to a solution of the amide (1 equiv.) in anhydrous CH$_2$Cl$_2$ under nitrogen at room temperature. The reaction mixture was stirred at room temperature for 12 h and the solvent removed under reduced pressure. The crude product material was purified by column chromatography if required.

2-(4-Bromophenyl)-*N*-(2-oxocyclopentyl)acetamide (42). Prepared by general procedure 5 using amide **40** (266 mg, 0.89 mmol), anhydrous CH$_2$Cl$_2$ (10 mL). The crude product material (white solid) was purified by column chromatography (SiO$_2$, 1 : 1 CH$_2$Cl$_2$–EtOAc) to yield the title compound as a white solid (175 mg, 66%). R_f 0.35 (SiO$_2$, 1 : 1 EtOAc–CH$_2$Cl$_2$); ν_{max} (CDCl$_3$)/cm^{-1} 3251 (NH amide), 3063 (CH aromatic), 2962 (CH), 2906 (CH), 1745 (C=O ketone), 1641 (C=O amide), 1547 (C=C aromatic); δ_H (400 MHz, CDCl$_3$) 7.40 (2H, d, J = 8.5 Hz, CBrC*H*), 7.09 (2H, d, J = 8.5 Hz, CBrCHC*H*), 5.76 (1H, br s, N*H*), 4.03 (1H, ddd, J = 13.5 Hz, 7.5 Hz, 7.5 Hz, NHC*H*), 3.47 (2H, s, C*H$_2$*C(=O)), 2.57–2.51 (1H, m, C(=O)C*H*), 2.33 (1H, dd, J = 9.0 Hz, 1.5 Hz, C(=O)C*H*′), 2.14–2.05 (1H, m), 2.02–1.94 (1H, m), 1.84–1.71 (1H, m), 1.54–1.43 (1H, dddd, J = 12.5 Hz, 12.5 Hz, 12.5 Hz, 7.0 Hz); δ_C (100 MHz; CDCl$_3$) 215.2 (*C*=O ketone), 171.0 (*C*=O amide), 134.4 (CBrCHCHC*H*), 132.5 (CBrC*H*), 131.5 (CBrCHC*H*), 121.8 (*C*Br), 58.6 (NH*C*H), 43.1 (*C*H$_2$C(=O)), 35.3 (C(=O)*C*H$_2$), 30.3 (*C*H$_2$), 18.4 (*C*H$_2$); LCMS (ES+) (MeCN) 297.9 (M + H)$^+$ for ^{81}Br; m.p. 124–127 °C (1 : 1 EtOAc–CH$_2$Cl$_2$).

2-(4-Bromophenyl)-*N*-(2-oxocyclohexyl)acetamide (41). Prepared by general procedure 5 using amide **39** (179 mg, 0.58 mmol), anhydrous CH$_2$Cl$_2$ (10 mL). The crude product material (white solid) was purified by column chromatography (SiO$_2$, 3 : 1 CH$_2$Cl$_2$–EtOAc) to yield the title compound as a white solid (102 mg, 57%). R_f 0.38 (SiO$_2$, 1 : 3 EtOAc–

CH$_2$Cl$_2$); ν_{max} (CDCl$_3$)/cm^{-1} 3256 (NH amide), 3068 (CH aromatic), 2942 (CH), 2861 (CH), 1719 (C=O ketone), 1638 (C=O amide), 1553 (C=C aromatic); δ_H (400 MHz, CDCl$_3$) 7.40 (2H, d, J = 8.0 Hz, CBrCH), 7.09 (2H, d, J = 8.5 Hz, CBrCHCH), 6.35 (1H, br s, NH amide), 4.38 (1H, ddd, J = 12.5 Hz, 6.5 Hz, 6.5 Hz, NHCH), 3.45 (2H, s, CH_2C(=O)), 2.55 (1H, ddddd, J = 6.0 Hz, 6.0 Hz, 3.0 Hz, 3.0 Hz, 3.0 Hz), 2.46–2.40 (1H, m), 2.34–2.25 (1H, m), 2.10–2.02 (1H, m), 1.83–1.75 (1H, m), 1.73–1.65 (1H, m), 1.54 (1H, qt, J = 13.5 Hz, 4,5 Hz), 1.23 (1H, qd, J = 12.5 Hz, 4.5 Hz); δ_C (100 MHz; CDCl$_3$) 208.0 (C=O ketone), 170.0 (C=O amide), 134.0 (CBrCHCHC), 132.4 (CBrC), 131.4 (CBrCHCH), 121 (CBr), 58.6 (NHCH), 43.4 (CH$_2$C(=O)), 41.5 (NHCHC(=O)CH$_2$), 35.7 (CH$_2$), 28.4 (CH$_2$), 24.4 (CH$_2$); LCMS (ES+) (MeCN) 312.0 (M + H)$^+$ for ^{81}Br, 313.03; m.p. 116–118 °C (1 : 3 EtOAc–CH$_2$Cl$_2$).

Acknowledgements

Notes and references

1 W. R. J. D. Galloway, J. T. Hodgkinson, S. D. Bowden, M. Welch and D. R. Spring, *Chem. Rev.*, 2011, **111**, 28–67.
2 M. E. Churchill and L. Chen, *Chem. Rev.*, 2011, **111**, 68–85.
3 A. M. Stevens, Y. Queneau, L. Soulere, S. von Bodman and A. Doutheau, *Chem. Rev.*, 2011, **111**, 4–27.
4 S. Atkinson and P. Williams, *J. R. Soc. Interface*, 2009, **6**, 959–978.
5 W. C. Fuqua, S. C. Winans and E. P. Greenberg, *J. Bacteriol.*, 1994, **176**, 269–275.
6 C. Fuqua, M. R. Parsek and E. P. Greenberg, *Annu. Rev. Genet.*, 2001, **35**, 439–468.
7 B. L. Bassler and R. Losick, *Cell*, 2006, **125**, 237–246.
8 M. Boyer and F. Wisniewski-Dye, *FEMS Microbiol. Ecol.*, 2009, **70**, 1–19.
9 C. E. McInnis and H. E. Blackwell, *Bioorg. Med. Chem.*, 2011, **19**, 4812–4819.
10 T. Praneenararat, G. D. Geske and H. E. Blackwell, *Org. Lett.*, 2009, **11**, 4600–4603.
11 J. E. Gonzalez and N. D. Keshavan, *Microbiol. Mol. Biol. Rev.*, 2006, **70**, 859–875.
12 P. Williams, *Microbiology*, 2007, **153**, 3923–3938.
13 P. Williams, K. Winzer, W. C. Chan and M. Camara, *Philos. Trans. R. Soc. London, Ser. B*, 2007, **362**, 1119–1134.
14 M. Sanchez-Contreras, W. D. Bauer, M. S. Gao, J. B. Robinson and J. A. Downie, *Philos. Trans. R. Soc. London, Ser. B*, 2007, **362**, 1149–1163.
15 G. D. Geske, J. C. O'Neill and H. E. Blackwell, *Chem. Soc. Rev.*, 2008, **37**, 1432–1447.
16 G. D. Geske, J. C. O'Neill, D. M. Miller, M. E. Mattmann and H. E. Blackwell, *J. Am. Chem. Soc.*, 2007, **129**, 13613–13625.
17 J. T. Hodgkinson, M. Welch and D. R. Spring, *ACS Chem. Biol.*, 2007, **2**, 715–717.
18 D. Smith, J. H. Wang, J. E. Swatton, P. Davenport, B. Price, H. Mikkelsen, H. Stickland, K. Nishikawa, N. Gardiol, D. R. Spring and M. Welch, *Sci. Prog.*, 2006, **89**, 167–211.
19 S. R. Chhabra, B. Philipp, L. Eberl, M. Givskov, P. Williams and M. Camara, Extracellular communication in bacteria, in *Chemistry of Pheromones and Other Semiochemicals II*, ed. S. Schultz, Springer Berlin, Heidelberg, Germany, 2005, vol. 240, pp. 279–315.
20 W. L. Ng and B. L. Bassler, *Annu. Rev. Genet.*, 2009, **43**, 197–222.
21 R. Popat, S. A. Crusz and S. P. Diggle, *Br. Med. Bull.*, 2008, **87**, 63–75.
22 S. P. Diggle, K. Winzer, S. R. Chhabra, S. R. Chhabra, K. E. Worrall, M. Camara and P. Williams, *Mol. Microbiol.*, 2003, **50**, 29–43.
23 J. Hodgkinson, S. D. Bowden, W. R. J. D. Galloway, D. R. Spring and M. Welch, *J. Bacteriol.*, 2010, **192**, 3833–3837.
24 J. T. Hodgkinson, W. R. J. D. Galloway, S. Saraf, I. R. Baxendale, S. V. Ley, M. Ladlow, M. Welch and D. R. Spring, *Org. Biomol. Chem.*, 2011, **9**, 57–61.
25 P. Grossi and D. Dalla Gasperina, *Expert Rev. Anticancer Ther.*, 2006, **4**, 639–662.
26 S. Lanini, S. D'Arezzo, V. Puro, L. Martini, F. Imperi, P. Piselli, M. Montanaro, S. Paoletti, P. Visca and G. Ippolito, *PLoS One*, 2011, 6.
27 K. M. Smith, Y. G. Bu and H. Suga, *Chem. Biol.*, 2003, **10**, 81–89.
28 G. J. Jog, J. Igarashi and H. Suga, *Chem. Biol.*, 2006, **13**, 123–128.
29 N. A. Whitehead, A. M. L. Barnard, H. Slater, N. J. L. Simpson and G. P. C. Salmond, *FEMS Microbiol. Rev.*, 2001, **25**, 365–404.
30 C. Attila, A. Ueda and T. K. Wood, *Appl. Microbiol. Biotechnol.*, 2008, **78**, 293–307.
31 S. P. Diggle, P. Cornelis, P. Williams and M. Camara, *Int. J. Med. Microbiol.*, 2006, **296**, 83–91.
32 C. Kim, J. Kim, H. Y. Park, J. H. Lee, H. J. Park, C. K. Kim and J. Yoon, *Appl. Microbiol. Biotechnol.*, 2009, **83**, 1095–1103.
33 M. E. Skindersoe, M. Alhede, R. Phipps, L. Yang, P. O. Jensen, T. B. Rasmussen, T. Bjarnsholt, T. Tolker-Nielsen, N. Hoiby and M. Givskov, *Antimicrob. Agents Chemother.*, 2008, **52**, 3648–3663.
34 M. Hentzer, K. Riedel, T. B. Rasmussen, A. Heydorn, J. B. Andersen, M. R. Parsek, S. A. Rice, L. Eberl, S. Molin, N. Hoiby, S. Kjelleberg and M. Givskov, *Microbiology*, 2002, **148**, 87–102.
35 Recent evidence indicates that the quorum sensing hierarchy is more complex than this model presents. See: V. Dekimpe and E. Deziel, *Microbiology*, 2009, **155**, 712–723.
36 T. B. Rasmussen and M. Givskov, *Microbiology*, 2006, **152**, 895–904.
37 M. J. Gambello, S. Kaye and B. H. Iglewski, *Infect. Immun.*, 1993, **61**, 1180–1184.
38 J. M. Brint and D. E. Ohman, *J. Bacteriol.*, 1995, **177**, 7155–7163.
39 M. Hentzer, H. Wu, J. B. Andersen, K. Riedel, T. B. Rasmussen, N. Bagge, N. Kumar, M. A. Schembri, Z. J. Song, P. Kristoffersen, M. Manefield, J. W. Costerton, S. Molin, L. Eberl, P. Steinberg, S. Kjelleberg, N. Hoiby and M. Givskov, *EMBO J.*, 2003, **22**, 3803–3815.
40 H. Wu, Z. Song, M. Hentzer, J. B. Andersen, S. Molin, M. Givskov and N. Hoiby, *J. Antimicrob. Chemother.*, 2004, **53**, 1054–1061.
41 H. Giamarellou, *Int. J. Antimicrob. Agents*, 2010, **36**, S50–S54.
42 K. M. Smith, Y. G. Bu and H. Suga, *Chem. Biol.*, 2003, **10**, 563–571.
43 D. M. Marsden, R. L. Nicholson, M. E. Skindersoe, W. R. J. D. Galloway, H. F. Sore, M. Givskov, G. P. C. Salmond, M. Ladlow, M. Welchd and D. R. Spring, *Org. Biomol. Chem.*, 2010, **8**, 5313–5323.
44 L. Y. W. Lee, T. Hupfield, R. L. Nicholson, J. T. Hodgkinson, X. B. Su, G. L. Thomas, G. P. C. Salmond, M. Welch and D. R. Spring, *Mol. BioSyst.*, 2008, **4**, 505–507.
45 F. G. Glansdorp, G. L. Thomas, J. J. K. Lee, J. M. Dutton, G. P. C. Salmond, M. Welch and D. R. Spring, *Org. Biomol. Chem.*, 2004, **2**, 3329–3336.
46 G. D. Geske, R. J. Wezeman, A. P. Siegel and H. E. Blackwell, *J. Am. Chem. Soc.*, 2005, **127**, 12762–12763.
47 M. Boukraa, M. Sabbah, L. Soulere, M. L. El Efrit, Y. Queneau and A. Doutheau, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 6876–6879.
48 M. Frezza, L. Soulere, S. Reverchon, N. Guiliani, C. Jerez, Y. Queneau and A. Doutheau, *Bioorg. Med. Chem.*, 2008, **16**, 3550–3556.
49 M. Frezza, S. Castang, J. Estephane, L. Soulere, C. Deshayes, B. Chantegrel, W. Nasser, Y. Queneau, S. Reverchon and A. Doutheau, *Bioorg. Med. Chem.*, 2006, **14**, 4781–4791.
50 A notable example of a similar study was recently reported by McInnis and Blackwell[9]. In this elegant piece of work, the effects of various AHL mimics containing different head and tail groups on LuxR-type quorum sensing was examined. These authors first coined the phrase 'chimeric compounds' to refer to such AHL-type analogues. However, all compounds tested the report of McInnis and Blackwell[9] were structurally different to the compounds revealed in this work.
51 Non-native AHLs capable of modulating quorum sensing at the LuxR-type receptor level can generally be thought of as being 'semi-rationally designed' molecules. Such agents are not typically designed *de novo* by researchers, instead being based upon the structure of a known autoinducer. Typically AHL-based inhibitors are presumed to act in a competitive fashion (that is, they target the binding site occupied by the natural AHL

This journal is © The Royal Society of Chemistry 2012

Org. Biomol. Chem., 2012, **10**, 6032–6044 | 6043

ligand). There is a relative lack of work pertaining to the *de novo* rational design of LuxR-modulators that act *via* by an alternative mode of action or are structurally distinct from AHLs (see ref. 1). Worthy of note in this context is a recent report by Amara *et al*. These researchers used the crystal structure of the ligand binding domain of LasR to rationally design a range of compounds which could covalently modify this receptor protein and consequently attenuate quorum sensing in *P. aeruginosa*. See: N. Amara, R. Mashiach, D. Amar, P. Kreif, S. A. H. Spiser, M. J. Bottomley, A. Aharoni and M. M. Meijler, *J. Am. Chem. Soc.*, 2009, **131**, 10610–10619.

52 J. T. Hodgkinson, W. R. J. D. Galloway, M. Casoli, H. Keane, X. B. Su, G. P. C. Salmond, M. Welch and D. R. Spring, *Tetrahedron Lett.*, 2011, **52**, 3291–3294.

53 J. P. Pearson, E. C. Pesci and B. H. Iglewski, *J. Bacteriol.*, 1997, **179**, 5756–5767.

54 K. Evans, L. Passador, R. Srikumar, E. Tsang, J. Nezezon and K. Poole, *J. Bacteriol.*, 1998, **180**, 5443–5447.

55 G. W. Lau, D. J. Hassett, H. Ran and F. Kong, *Trends Mol. Med.*, 2004, **10**, 599–606.

56 D. W. Essar, L. Eberly, A. Hadero and I. P. Crawford, *J. Bacteriol.*, 1990, **172**, 884–900.

57 U. Muh, M. Schuster, R. Heim, A. Singh, E. R. Olson and E. P. Greenberg, *Antimicrob. Agents Chemother.*, 2006, **50**, 3674–3679.

58 Despite the structural similarities between the antagonists of this sort disclosed in this report and those found in previous studies by other research groups, a direct quantitative comparison of the levels of activities is not appropriate. There is a lack of standardisation between the experimental techniques employed in different reports to assess the biological effects of such agents. Even in studies investigating the same target protein (*e.g.* LasR) there is often variation in the bacterial strains, growth media and assays used which can have a large impact on the observed biological effects of a small molecule agent. For further discussion see Galloway *et al.*[1] and references therein.

59 McInnis and Blackwell[9] have noted a similar observation with regards to the chimeric compounds they examined (see ref. 50).

60 It is worth noting that the head groups in all these chimeric compounds were selected from molecules which are known to modulate LuxR-type quorum sensing.

61 D. E. Ohman, S. J. Cryz and B. H. Iglewski, *J. Bacteriol.*, 1980, **142**, 836–842.

62 W. R. J. D. Galloway, J. T. Hodgkinson, M. Welch and D. R. Spring, *Chem. Biol.*, 2009, **16**, 913–914.