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Synthesis and biological evaluation of homoserine lactone derived ureas as antagonists of bacterial quorum sensing

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Abstract—A series of 15 racemic alkyl- and aryl-N-substituted ureas, derived from homoserine lactone, were synthesized and tested for their ability to competitively inhibit the action of 3-oxohexanoyl-L-homoserine lactone, the natural inducer of bioluminescence in the bacterium *Vibrio fischeri*. *N*-alkyl ureas with an alkyl chain of at least 4 carbon atoms, as well as certain ureas bearing a phenyl group at the extremity of the alkyl chain, were found to be significant antagonists. In the case of *N*-butyl urea, it has been shown that the antagonist activity was related to the inhibition of the dimerisation of the N-terminal domain of ExpR, a protein of the receptor LuxR family. Molecular modelling suggested that this would result from the formation of an additional hydrogen bond in the protein acylhomoserine lactone binding cavity.

1. Introduction

In many bacteria, the expression of some sets of genes is regulated in response to population density. This process is based on the production, by bacteria, of small signalling molecules, called autoinducers (AI), which are released in the extracellular medium and sensed by other individual bacteria through specific receptors.[1] This cell-to-cell communication system is termed quorum sensing (QS) and has been detected in a growing number of pathogenic bacteria. In these bacteria, QS prevents premature virulence factor production, and the subsequent activation of host defence responses, and allows them to invade an infected host once their numbers are sufficient enough to carry out a full-scale attack. Thus, QS has emerged as a possible target pathway for the design of a novel antimicrobial therapy since QS antagonists, that is, molecules that can bind to quorum sensing receptors but block them in an inactive conformation, would constitute a new class of potential antibacterial agents.[2] QS disrupting agents were also shown to be active against biofilm formation, whereas traditional antibiotics were ineffective for killing bacteria which adopt a biofilm life-style.[3]

More than 20 years ago Eberhard et al.[4] demonstrated that the marine bacteria *Vibrio fischeri* use acylhomoserine lactones (AHLs), particularly *N*-hexanoyl-L-homoserine lactone (C_6-HSL) **1** and *N*-3-oxohexanoyl-L-homoserine lactone (3-oxo-C_6-HSL) **2** (Scheme 1), to regulate bioluminescence in response to cell density. The LuxI protein is responsible for AHL biosynthesis, while the LuxR quorum sensing receptor is a transcriptional regulator that activates bioluminescence genes. The current model for quorum sensing in *V. fischeri* maintains that the association of 3-oxo-C_6-HSL with LuxR is a key step enabling the activator to bind to *lux* box DNA at the *luxICDABEG* operon, thereby facilitating the binding and activation of RNA polymerase at the *luxI* promoter.[5] Since these initial observations, more than 40 Gram negative bacterial species have been shown to possess QS systems using AHLs as autoinducers and containing proteins of the LuxI–LuxR family.[6]

Keywords: Quorum sensing; *Vibrio fischeri*; AHLs; Ureas; Antagonists.
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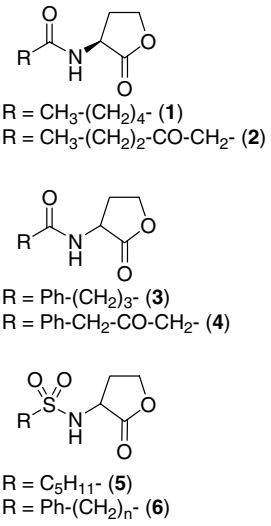


Scheme 1. Structure of compounds **1–6**.

Different types of AHL analogs have already been synthesized and evaluated as potential inhibitors of QS by several research groups.[7,8] As part of an ongoing program aimed at obtaining such compounds, we have previously identified two families of antagonists of QS in *V. fischeri* bacteria: AHL analogs bearing an aromatic ring at the extremity of the alkyl side chain[8a] and sulfonylamides,[8b] the most active compounds being, respectively, **3** and **4** in the first series and **5** and **6a** ($n = 2$) in the second.

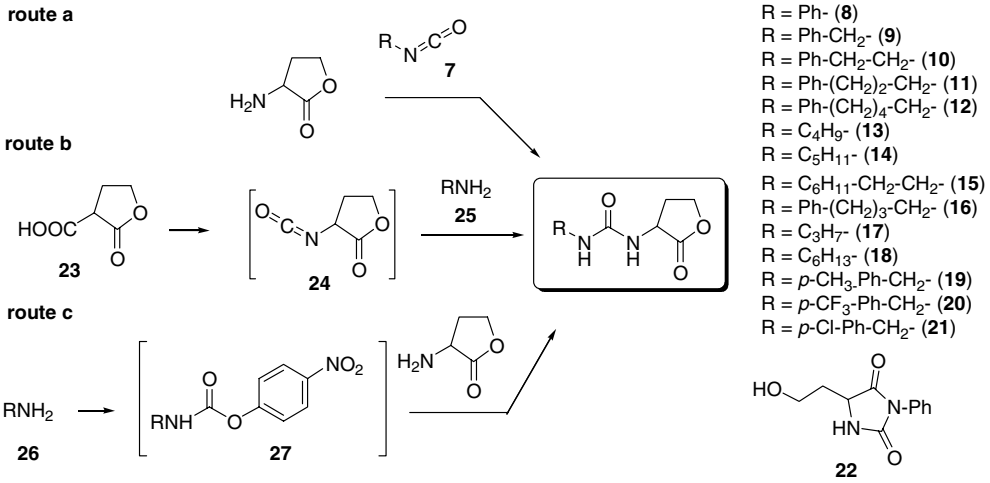
In keeping with our research into new antagonists of QS based on the replacement of the amide function of AHLs by other linkages,[8b] we considered it to be of interest to evaluate urea analogs. Indeed, acyclic and cyclic ureas are often biologically active, nontoxic compounds, probably due to the fact that the ureido (–NH-CO-NH–) unit is a pseudodipeptide motif.[9] We report here the synthesis and the results of the biological assays of a series of these new AHL analogs.

2. Chemistry

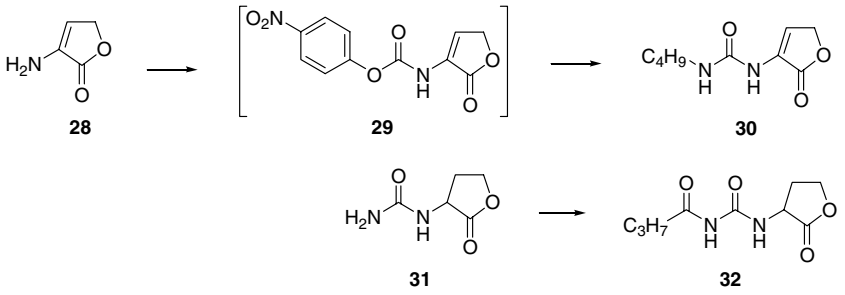
In order to examine the effect of the substitution on the activity, a series of 15 racemic[10] alkyl- and aryl-N-substituted ureas derived from homoserine lactone, as well as 3 related compounds, were synthesized and tested. Ureas **8–21** were easily prepared following three routes (Scheme 2). Ureas **8–15** were obtained (route a) by reacting homoserine lactone with isocyanates **7**, either commercially available (synthesis of **8–10**, **13** and **14**), or obtained in situ by the Curtius rearrangement of the corresponding acyl azide. The latter compounds were obtained either (**11**,**15**) by reacting sodium azide with an acid chloride or (**12**) from the reaction of diphenylphosphoryl azide with 6-phenylhexanoic acid. The reaction of homoserine lactone with isocyanates was achieved in the presence of dimethylaminopyridine (DMAP) except for urea **8**, for which these experimental conditions led only to hydantoin **22** resulting from the opening of the lactone ring by the urea moiety.[11] This particular behaviour was probably due to the acidity of the urea group in **8** which gave rise, in the basic reaction mixture, to a nucleophilic amidure type species. By replacing DMAP with the less basic Et_3N, we obtained a mixture of urea **8** and hydantoin **22** from which pure **8** was obtained by two consecutive separations, using silica gel column chromatography.

Ureas **16–18** resulted (route b) from the reaction between amines **25** with α-isocyanato-γ-butyrolactone **24** prepared in situ by adding diphenylphosphoryl azide to known[12]α-carboxy-γ-butyrolactone **23**. Ureas **19–21** were prepared (route c) by reacting 4-nitro-phenylchloroformate with benzylamines **26** to generate the corresponding intermediate carbamate **27**, followed by the addition of homoserine lactone.

Ureas **30** and **32** were prepared according to the synthetic sequences depicted in Scheme 3. Enamino-urea **30** was obtained by first reacting the known[13] enamino lactone **28** with 4-nitro-phenylchloroformate to produce the intermediate carbamate **29,** which was then submitted



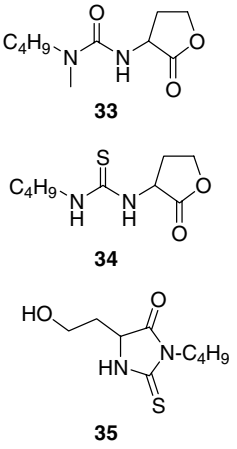
Scheme 2. Synthesis of ureas **8–21**. Structure of hydantoin **22**.



Scheme 3. Synthesis of ureas **30** and **32**.

to the action of butylamine. Acylurea **32** was prepared by reacting known[14] urea **31** with butanoyl chloride.

The *N*-methyl urea **33** (Scheme 4) was obtained according to route b, using *N*-methylbutylamine. The thiourea **34** was prepared according to route a: the action of homoserine lactone on commercially available butyl isothiocyanate gave rise to a mixture of expected **34** and thiohydantoin **35**.[15]



Scheme 4. Structures of compounds **33**, **34** and **35.**

3. Biological results and molecular modelling

3.1. Inhibitory activity

The above ureas **8–21**, **30** and **32–34** were evaluated for their ability to interfere with the induction of luminescence by *N*-3-oxohexanoyl-L-homoserine lactone in the *V. fischeri* bacteria QS system (see Section 6.1).

3.1.1. Aryl ureas 8–12, and 16. Since AHL analogs, bearing a phenyl group at the extremity of the alkyl chain, were often shown to display antagonist activities,[7d,i,j,8] we first prepared, and evaluated, the biological activities of phenyl substituted ureas **8-12** and **16** with 4–9 σ-bonds between the aromatic and the lactone rings. With the exception of compound **8**, all these ureas proved to be antagonists (Fig. 1). Compounds **9**, **11**, **12** and **16,** in which the phenyl group is distant from the lactone ring of 5, 7, 8 and 9 bonds, respectively, displayed inhibitory activity of about the same magnitude ($IC_{50} \approx 4$–7 μM). Urea **10,** in which this distance is of 6 bonds, is significantly less active. It is interesting to note that a similar effect was observed in the case of phenylalkylsulfonyl HSLs **6** when the alkyl spacer length was varied from $n = 1$ to $n = 3$, corresponding, respectively, to 4- to 6-bond distances between the phenyl and lactone rings.[8b] The strongest inhibitory activity was for a 5-bond spacing (compound **6a**, $n = 2$), whereas 6 (**6b**, $n = 3$) or 4-bond (**6c**, $n = 1$) spacing led to a lower, or nearly absent, activity, respectively.

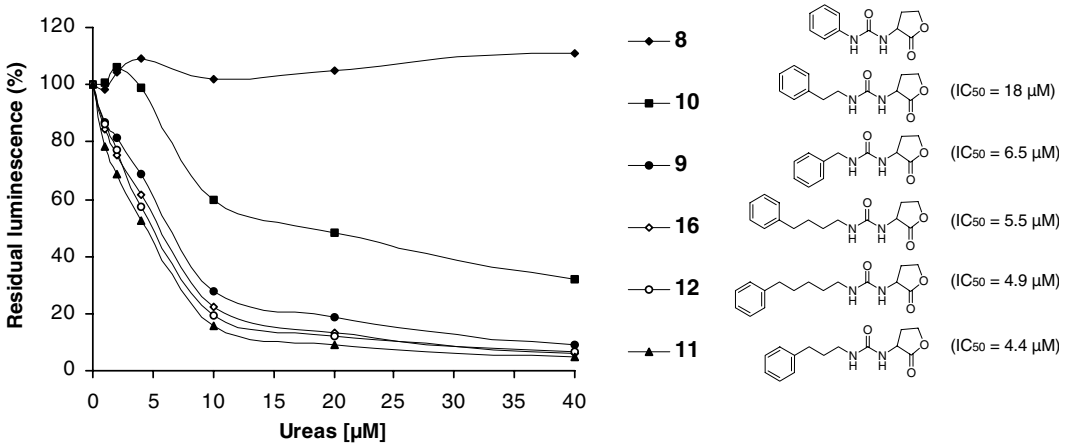


Figure 1. Inhibitory activity of ureas **8–12** and **16.** Effect of the distance between the lactone and the phenyl groups on antagonist activity.

3.1.2. 4-Substituted phenyl ureas 19–21. The effect of the aromatic ring substitution on the antagonist activity was then briefly investigated with the 4-substituted analogs **19**–**21** of **9**, easily prepared from commercially available amines. These new analogs displayed inhibitory activity of about the same significant magnitude as the parent compound **9** (Fig. 2). However, a slight enhancement of the inhibitory activity was observed when the phenyl group was substituted with a methyl group or a chlorine atom as electrodonating substituents (**19** and **21** $IC_{50} \approx 3$ μM), whereas substitution by the strongly electron-withdrawing trifluoromethyl group was without any significant effect (**20** and **9**, $IC_{50} \approx 7$ μM).

3.1.3. Alkyl ureas and related compounds. We then tested the alkyl ureas **13–15**, **17**, **18** and the bis-alkyl urea **33**, as well as the enamino-urea **30**, the acylurea **32** and the thiourea **34**. Among these compounds, the *n*-alkyl ureas **13–15** and **18** displayed good antagonist activity (Fig. 3). If the propyl urea **17** is essentially inactive, the inhibitory activity of **13**, **14**, and **18** increased with alkyl chain length. However, beyond 5 carbon atoms the activity reached a maximum and **14** (pentyl) and **18** (hexyl) both displayed about the same activity ($IC_{50} \approx 1$ μM). Interestingly **15**, bearing a cyclohexyl substituent, exhibited a fairly similar inhibitory effect as *n*-hexyl urea **18.** Alkylation of the urea nitrogen with a methyl group (urea **33**) resulted in a drastic decrease in antagonist activity. Finally, we observed that compounds **30**, **32** and **34** were deprived of any inhibitory activity (data not shown).

3.2. Dimerisation of the N-terminal domain of a protein of the LuxR family

To analyse conformational transition induced by 3-oxo-C_6-HSL in a protein of the LuxR family, we tried to purify the N-terminal domain of ExpR, an *Erwinia chrysanthemi* quorum sensing regulator which is responsive to 3-oxo-C_6-HSL as LuxR. Attempts to overproduce LuxR in the absence of ligand were unsuccessful, suggesting that LuxR was unstable under such conditions.[5] By contrast, we obtained large amounts of the ExpR N-terminal domain$(His)_6$, henceforth designated as ExpR-N. Addition of 3-oxo-C_6-HSL to this purified domain modified its electrophoretic mobility in a concentration dependent manner (Fig. 4A). This finding suggested that 3-oxo-C_6-HSL induced a conformational transition in ExpR-N. In size-exclusion chromatography, purified ExpR-N was eluted with a single peak corresponding to a

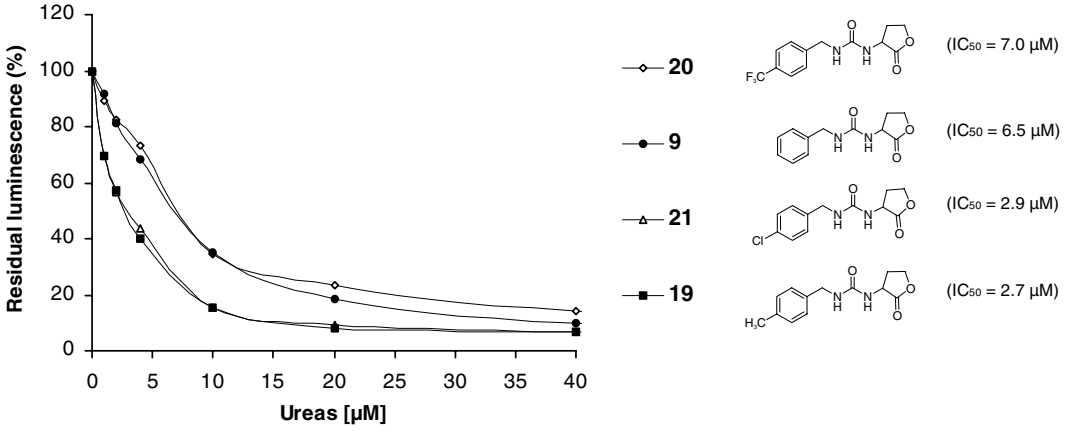


Figure 2. Inhibitory activity of ureas **9** and **19–21**. Effect of the phenyl group substitution on antagonist activity.

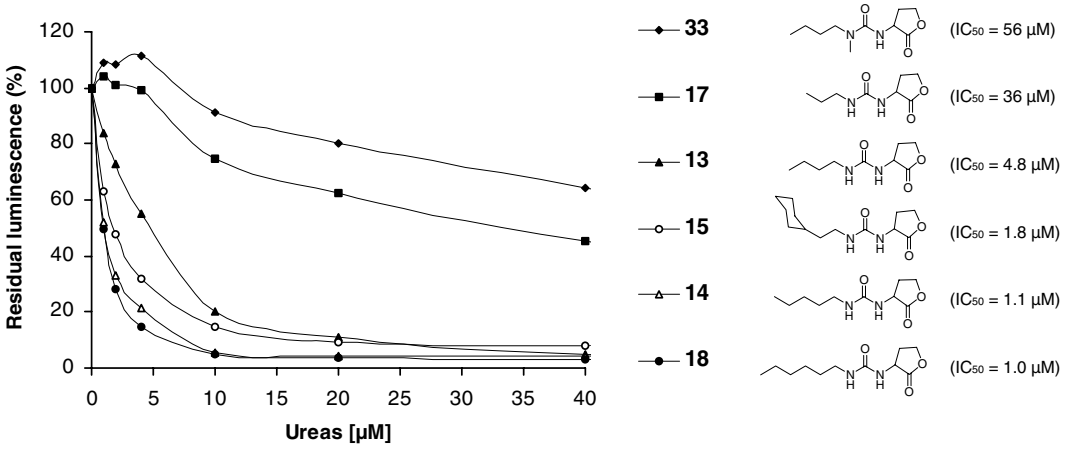


Figure 3. Inhibitory activity of ureas **13–15**, **17**, **18** and **33.** Effect of the alkyl chain on antagonist activity.

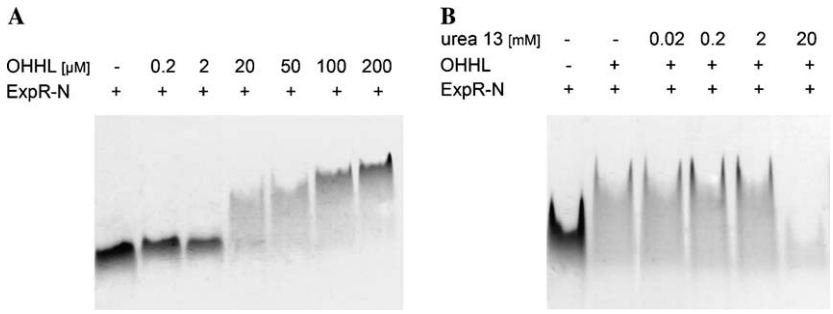


Figure 4. Electrophoretic mobility of the ExpR-N terminal domain$(His)_6$. (A) Influence of 3-oxo-C_6-HSL (OHHL) on electrophoretic mobility of the ExpR-N terminal domain$(His)_6$. (B) Electrophoretic mobility of the ExpR-N terminal domain$(His)_6$ in the presence of 20 μM OHHL and an increasing concentration of urea **13**.

molecular mass of 25.5 kDa, a value analogous with the calculated size of 20 kDa for one monomer of this protein. On the other hand, ExpR-N complexed with 3-oxo-C_6-HSL eluted with a single peak corresponding to a molecular mass of 40.2 kDa, a value in agreement with the calculated size of one homodimer of this protein (data not shown). These results demonstrated that 3-oxo-C_6-HSL induces the dimerisation of ExpR-N.

3.3. Urea-HSL analogs inhibit dimerisation of the ExpR N-terminal domain

To gain insights into the molecular effects of urea-HSL analogs on quorum sensing inhibition, we analysed the effect of urea **13** on the electrophoretic mobility of ExpR-N complexed with 3-oxo-C_6-HSL (Fig. 4B). In the presence of 20 μM of 3-oxo-C_6-HSL, approximately 50% of the ExpR-N was in a dimer state (Fig. 4A). Increased amounts (0–20 mM) of **13** were added to ExpR-N in the presence of 20 μM 3-oxo-C_6-HSL (Fig. 4B). At the highest concentration of urea **13** the dimer was no longer present. This observation suggested that **13** competes with 3-oxo-C_6-HSL to occupy the ligand-binding pocket of ExpR and inhibits dimerisation of the N-terminal domain.

3.4. Molecular modelling

To investigate the structure–activity relationships of AHL derived ureas, correlated with their possible interactions with the transcriptional regulator LuxR, the compound **13** was modelled in the active site of the LuxR model,[8b] whose construction was based on the folding of TraR.[16] Preferential conformations of **13** were first calculated by varying the key torsion angles and they were compared to the natural ligand of TraR.[16] The resulting conformation was then docked as a ligand in the active site of LuxR. The analysis of the docking result (Fig. 5A) suggests that (i) the urea function permits the formation of an additional hydrogen bond with the residue Asp79 (3.0 Å) in addition to the one already present with the amide function of the AHLs (see Fig. 5B for the hydrogen bond network); (ii) the planar urea functional group allows a favourable orientation of the alkyl chain in the hydrophobic pocket (Fig. 5C); and (iii) the urea moiety fits well in the hydrophilic domain of the active site (Fig. 5D).

4. Discussion

Molecular modelling suggests that the geometry of the urea function fits well in the AHL-binding cavity of LuxR. Moreover, this functional group is capable of forming two hydrogen bonds with the residue Asp79 (3.0 Å). This residue was shown to be essential for AHL-binding in different proteins of the LuxR family since alteration of the corresponding residues of TraR (Asp70)[17], LasR (Asp73)[18] and of LuxR (Asp79)[19] completely abolishes the AHL-binding capacity of these proteins, which failed to dimerise and lacked any detectable transcriptional activity. Why the enforced hydrogen bonding might be a determining factor in conferring an antagonist activity to the AHL derived ureas is not clear, but the importance of the hydrogen atom of the external NH of the urea function was confirmed by the inactivity of the *N*-methylated urea **33**.

With the aryl substituted ureas **8–12** and **16**, it seems that the antagonist activity depends on the distance between the phenyl group and the lactone ring. This could result from the relative positions of the phenyl group and the aromatic residues, Tyr62 and Tyr70, located in the hydrophobic pocket of the active site (Fig. 5A). Depending on the distance between the lactone and the aromatic rings, urea **9** (5 bond spacing) and **11** (7 bond spacing) would develop an attractive interaction with either Tyr62 or Tyr 70. On the other hand, such a favourable interaction would not be permitted for ureas equipped with an inappropriate spacer, either too short in **8** (4-bond spacing) or intermediate in **10** (6-bond spacing). The strong activity of **12** and **16**, similar to that of **11**, is probably due to the increased hydrophobic character of a longer alkyl chain. The slight enhancement of the activity of ureas **19** and **21**, compared to **9**, appears to be consistent with the effect of electrodonating substituents. The biological results obtained with the alkyl substituted derived ureas **13–15**, **17** and **18** showed that the antagonist activity increases with the hydrophobic character of the ligand to reach a maximum value for the hexyl substituent (IC_{50} = 1 μM), probably correlated with the enhanced affinity for the hydrophobic protein pocket. Moreover, alkyl substituted ureas seem to show stronger activity compared to more sterically hindered aryl substituted ureas. In enaminourea **30** the flattening of the lactone ring, resulting from the introduction of a carbon–carbon

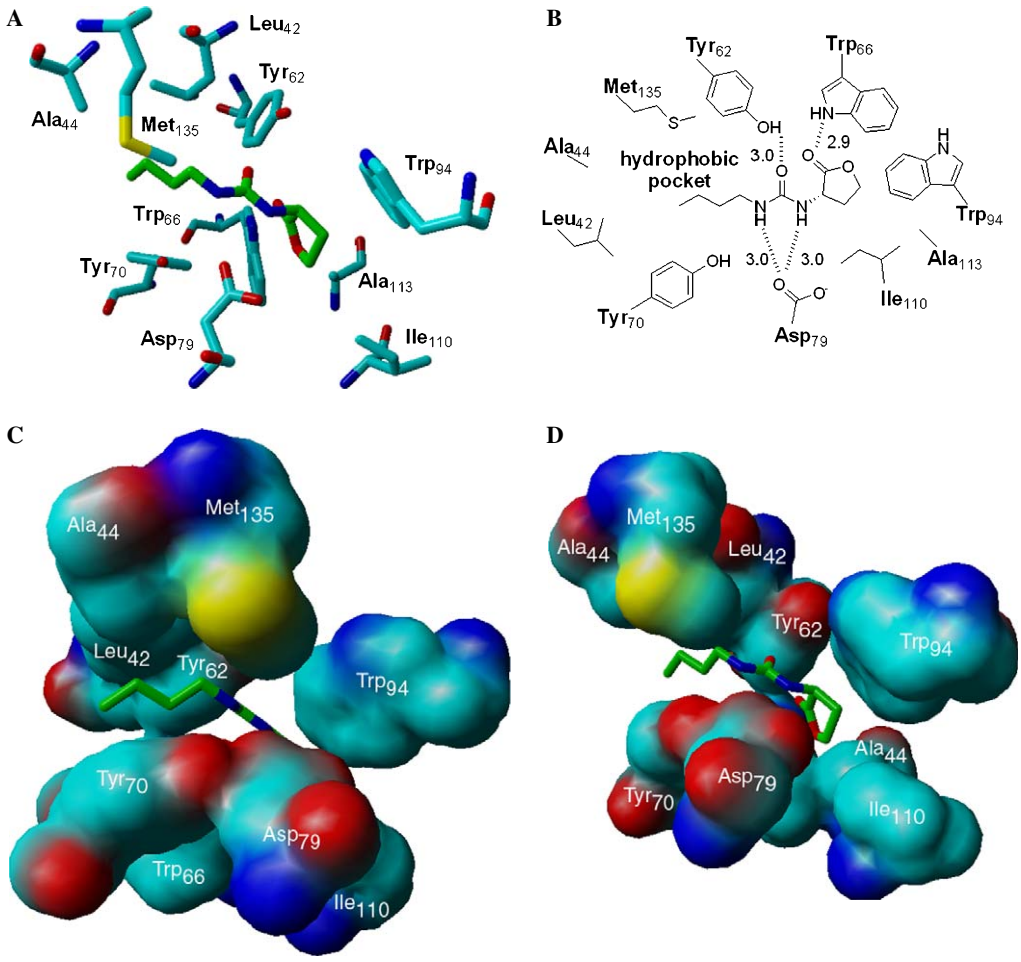


Figure 5. Docking result of urea **13** in the active site of the LuxR model. (A) Visualisation of the simplified active site with urea **13**. (B) Schematic overview of the active site displaying the hydrogen bond network with urea **13**. (C) The interactions between the alkyl chain and the hydrophobic pocket and (D) shows the interactions of the urea functional group with polar residues (residues are represented with van der Waals surfaces).

double bond, would be the cause of its inactivity. As in the case of sulfonylamides[8b], we observed that the presence of an additional carbonyl function in urea **32** resulted in a nearly total loss of activity. The data obtained from molecular modelling (not shown) indicated that the introduction of a sp^2 carbon resulted in a conformation of the acyl chain which is not tolerated in the binding site. The loss of activity when changing the carbonyl function of the urea to a thiocarbonyl one (compound **34**) could result either from the bigger size of the sulfur atom or from the weaker capacity of sulfur, compared with oxygen, to act as a hydrogen bonding acceptor.

5. Conclusion

In this report, we have prepared 15 homoserine lactone derived ureas and three related compounds, and evaluated their ability to inhibit the QS system in *V. fischeri* bacteria. We observed that two classes of these new AHL analogs displayed significant antagonist activity: (1) certain ureas bearing a phenyl group at the extremity of the alkyl chain, (2) *N*-alkyl substituted ureas with an alkyl chain of at least 4 carbon atoms long. We observed, in the case of *N*-butyl urea, that the antagonist activity was related to the dimerisation of the N-terminal domain of ExpR, a protein of the LuxR family. Molecular modelling suggested that this would result from the formation of an additional hydrogen bond in the protein AHL-binding cavity.

6. Experimental

6.1. Antagonist activity measurement

Compounds **8–21**, **30** and **32–34** were evaluated for their ability to interfere with the induction of luminescence by *N*-3-oxohexanoyl-L-homoserine lactone (3-oxo-C6-HSL, **2**) in the *V. fischeri* QS system For that purpose, we used the recombinant *Escherichia coli* biosensor strain NM522 containing the plasmid pSB401.[20] In this plasmid, the *luxR* and the *luxI* promoter from *V. fischeri* have been coupled to the entire *lux* structural operon *(luxCDABE)* from *Photorhabdus luminescens*. This biosensor strain produces luminescence in response to exogenously provided AHL. AHL activity was measured in a microtitre plate format, with bioluminescence quantified using a luminescence reader (Luminoskan Ascent, Labsystems). Competition assays were performed in LB

growth medium in the presence of 200 nM of 3-oxo-C6-HSL and with simultaneously added analogs, in concentrations ranging from 20 nM to 40 μM. After inoculation by the biosensor strain, the plate was incubated at 30 °C. The amount of light produced by the bacteria was detected after 4–5 h and was expressed in relative light units (RLU). The experiments were carried out in triplicate and the standard deviation (data not shown) did not exceed 10% of the mean value. A negative control was performed in the absence of 3-oxo-C6-HSL and this gave the basal level of bioluminescence in the absence of an inducer. A positive control was performed in the presence of 3-oxo-C6-HSL only. We also checked that the analogs did not inhibit bacterial growth by measuring the turbidity of the bacterial culture at OD 600 nm for the highest analog concentration used. Similarly, to be sure that the analogs inhibited induction of *luxCDABE* genes and did not inhibit Lux enzyme activity, an additional control was performed in which the highest analog concentration was added to glowing bacteria after the 4–5 h growth period and we verified that bioluminescence was not suppressed.

6.2. Inhibition of dimerisation

6.2.1. Overproduction and purification of the N-terminal domain of ExpR. The ExpR N-terminal domain-coding region (amino acids 1–163) was amplified from *Erwinia chrysanthemi* strain 3937 chromosome by PCR, using the two primers ExpR deb (5′-GGAATCCATATGTC TATATCATTCTCTAACG-3′) and ExpR-Nterm fin (5′-CCGCTCGAGTCGGTACAGAGAGGTGAG-3′). Artificial *Nde*I and *Xho*I restriction sites were incorporated in the primers (restriction sites are underlined). The resulting PCR product was digested with *Nde*I and *Xho*I, and then cloned into pET20(b+) (Novagen) to form pSC2833. In the resulting pSC2833 plasmid, the ExpR N-terminal domain is fused with a His-tag at the C-terminus. The integrity of the N- terminal *expR* coding region was confirmed by sequencing. For ExpR N-terminal domain$(His)_6$ overproduction, colonies of *E. coli* BL21(DE3) pLysS (Novagen) carrying pSC2833 were grown overnight on LB-ampicillin–chloramphenicol plates and were used to inoculate 200 ml of pre-warmed LB containing 100 μg/ml ampicillin and 20 μg/ ml chloramphenicol. The culture was incubated at 25 °C until reaching an optical density of 0.6 at 600 nm. Bacterial cells were then induced with isopropyl-β-D-thiogalactoside (0.2 mM final concentration), and incubated at 25 °C for an additional 2.5 h. Cells were harvested by centrifugation at 5000*g* for 10 min. All subsequent steps were carried out at 4 °C. The cell pellets from 200 ml culture were washed and resuspended in 25 ml of extraction buffer (10 mM Tris–HCl, pH 7.9, 300 mM KCl, 2 mM β-mercaptoethanol, 10% glycerol and 1 mM phenylmethylsulfonyl fluoride (PMSF)). Crude protein extract was obtained by disrupting bacteria at 138,000 kPa in a French pressure cell (AMINCO). Insoluble material was removed by 2 steps of centrifugation (20,000*g*, for 10 min). The cellular extract obtained was then submitted to a 0–20% ammonium sulfate precipitation. After centrifugation, the supernatant was dialysed against 2× 2 L of extraction buffer to eliminate the residual ammonium sulfate. After dilution with one volume of extraction buffer without KCl and filtration on a 0.45 μm filter, the protein solution was applied to a PAK DEAE 15HR column (50 mm × 100 mm; Waters). The column was washed with extraction buffer and eluted using a linear KCl gradient (0.075–0.6 M) at a flow rate of 1.4 ml.min^{-1}. After SDS–PAGE analysis, the ExpR N-terminal protein$(His)_6$ was then observed in the fraction corresponding to the injection peak. This fraction was dialysed against phosphate buffer (50 mM NaH_2PO_4, pH 8.0, 300 mM NaCl, 10 mM imidazole, 2 mM β-mercaptoethanol, 10% glycerol, 1 mM PMSF) and then purified on Ni–NTA resin (QIAGEN) with empty columns (QIAGEN), according to the manufacturer's instructions. Fractions were analysed by SDS–PAGE and those containing the ExpR N-terminal domain$(His)_6$ were pooled and dialyzed against 3× 1.5 L of buffer (10 mM Tris–HCl, pH 7.9, 200 mM NaCl, 2 mM β-mercaptoethanol, 1 mM EDTA and 1 mM PMSF). The resulting ExpR N-terminal domain(His)6 preparation was >95% pure, as determined by SDS–PAGE, and was stored at 4 °C. Protein concentration was measured using the Bradford assay.

6.2.2. 3-oxo-C_6-HSL effect on electrophoretic mobility of the ExpR N-terminal domain. Purified ExpR N-terminal domain$(His)_6$ (20 μM) was incubated with increasing amounts of 3-oxo-C_6-HSL (from 0 to 200 μM) at 30 °C for 30 min. After the addition of an adequate volume of Laemmli sample buffer, without SDS and β-mercaptoethanol, the reaction mixtures were electrophoresed on native 12% Tris–glycine polyacrylamide gels (1:30 bis-acrylamide to acrylamide ratio) at 10 V/cm and at room temperature. Gels were then stained with Coomassie blue and dried.

6.2.3. Analysis of the oligomeric state of the ExpR N-terminal domain. For gel filtration analysis, 0.2 ml of the ExpR N-terminal domain$(His)_6$ (25 μM) was chromatographed on a Superdex 75 HR 10/30 column (Amersham), at 15 °C, using a Bio-Rad Biologic fast performance liquid chromatography apparatus. This experiment was performed either in the absence or presence of 250 μM 3-oxo-C_6-HSL. Samples were incubated at 30 °C for 30 min before filtration and injection. The column was equilibrated and eluted with gel filtration buffer (50 mM Tris–HCl, pH 7.9, 150 mM NaCl and 1 mM EDTA) at a flow rate of 0.5 ml/min and fractions were collected at 0.25 ml/min. 3-Oxo-C6-HSL was not incorporated in the gel filtration buffer. However, we observed that when dimerisation is achieved, the dimer is quite stable and is not dissociated during elution. Similar results were obtained with two other quorum sensing regulators of the LuxR family, namely TraR and LasR, which tightly bind their cognate AHL and did not dissociate even after prolonged dialysis.[21,22] The column was calibrated with protein standards from a molecular weight gel filtration kit (SERVA). Fractions were then analysed by dot blot using anti-ExpR polyclonal antibodies.

6.3. Molecular modelling

A conformational study was carried out on the butylurea using the Tripos Force-field method with Sybyl

7.0 for Linux. Stable conformations were superimposed on the natural ligand and the closest was then manually docked into the active site of the LuxR model[8b] to analyse intermolecular interactions. Hydrogen bonds were assigned based on the distance measured between the donor and the acceptor. Figure 5 was created using YASARA.[23]

6.4. Chemistry

6.4.1. General remarks. All chemicals were purchased from Aldrich. Organic solutions were dried over anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen and were monitored by thin-layer chromatography on Silica Gel 60 F_{254} (Merck); detection was carried out by charring with a 5% phosphomolybdic acid solution in ethanol containing 10% of H_2SO_4. Silica gel (Kieselgel 60, 70–230 mesh ASTM, Merck) was used for flash chromatography. Melting points were determined on a Kofler block apparatus. The ^{1}H (200 MHz or 300 MHz) and ^{13}C NMR (50 MHz or 75 MHz) spectra were recorded with a Bruker AC200, ALS300 or DRX300 spectrometer. The signal of the residual protonated solvent was taken as reference. Chemical shift (δ) and coupling constants (J) are reported in ppm and Hz, respectively. Elemental analyses were performed by 'Service Central de Microanalyses du CNRS' 69360 Solaize (France).

6.4.2. General procedure for the synthesis of ureas 8–10, 13–14 and 34. To a solution of D,L-α-amino-γ-butyrolactone hydrobromide (0.50 g, 2.75 mmol) and triethylamine (0.28 g, 2.75 mmol) (**8** and **34**) or 4-dimethylaminopyridine (0.33 g, 2.75 mmol) (**9**, **10**, **13** and **14**) in dry THF (30 mL) was added at 0 °C the appropriate commercially available isocyanate or isothiocyanate (2.75 mmol). The reaction mixture was allowed to warm at rt and stirred for 8 to 48 h. The solution was filtered and the solvent was evaporated. The residue was purified by flash chromatography to give a solid (except for **34** and **35**), which was recrystallised.

6.4.2.1. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-phenyl-urea (8) and 5-(2-hydroxy-ethyl)-3-phenyl-imidazolidine-2,4-dione (22). Reaction time: 8 h. Chromatography: EtOAc/pentane, 8:2. Compound **8** was first eluted (R_f: 0.48) and then **22** (R_f: 0.18). The urea **8** was further purified by a second chromatography (EtOAc/pentane, 9:1). Compound **8**. Recrystallisation: EtOAc/pentane. White solid (14%). Mp: 161–162 °C. ^{1}H NMR (acetone-d_6, 200 MHz): δ 2.21–2.42 (m, 1H), 2.58–2.73 (m, 1H), 4.24–4.47 (m, 2H), 4.53–4.67 (m, 1H), 6.24 (d, J = 6 Hz, 1H), 6.90–6.99 (t, J = 7.3 and J = 1.2 Hz, 1H), 7.20–7.28 (t, J = 7.5 Hz, 2H), 7.47–7.52 (dd, J = 7.5 and J = 1.2 Hz, 2H), 8.19 (s, 1H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 28.8, 48.8, 65.2, 117.9, 121.4, 128.7, 140.0, 154.7, 176.0. Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.24; H, 5.66; N, 12.70. Compound **22**. Recrystallisation: EtOAc/pentane. White solid. Mp: 119–120 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ 1.80–2.04 (m, 2H), 3.53–3.69 (m, 2H), 4.29 (t, J = 5.7 Hz, 1H), 4.70 (t, J = 4.7 Hz, 1H), 7.33–7.52 (m, 5H), 8.50 (s, 1H). In agreement with reported data.[10] ^{13}C NMR (DMSO-d_6, 50 MHz): δ 34.5, 53.8, 56.7, 126.7, 127.6, 128.6, 132.4, 155.8, 173.8.

6.4.2.2. 1-Benzyl-3-(2-oxo-tetrahydro-furan-3-yl)-urea (9). Reaction time: 36 h. Chromatography: EtOAc/pentane, 9/1. Recrystallisation: EtOAc/pentane. White solid (22%). Mp: 143–144 °C. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 2.05–2.27 (m, 1H), 2.34–2.44 (m, 1H), 4.13–4.54 (m, 3H), 4.24 (d, J = 6 Hz, 2H), 6.51 (d, J = 7 Hz, 1H), 6.69 (t, J = 5.9 Hz, 1H), 7.20–7.37 (m, 5H). ^{13}C NMR (DMSO-d6, 50 MHz): δ 29.1, 42.9, 48.9, 65.1, 126.6, 127.0, 128.2, 140.6, 157.4, 176.3. Anal. Calcd for $C_{12}H_{14}N_2O_3$: C, 61.53; H, 6.02; N, 11.96. Found: C, 60.54; H, 6.13; N, 11.77.

6.4.2.3. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-phenethyl-urea (10). Reaction time: 24 h. Chromatography: EtOAc. Recrystallisation: EtOAc/pentane. White solid (84%). Mp: 113–114 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.05–2.27 (m, 1H), 2.56–2.69 (m, 1H), 2.77 (t, J = 7.2 Hz, 2H), 3.33–3.43 (q, J = 6.7 Hz, 2H), 4.16–4.29 (m, 1H), 4.34–4.49 (m, 2H), 5.47 (t, J = 5.6 Hz, 1H), 5.79 (d, J = 6.6 Hz, 1H), 7.15–7.32 (m, 5H).^{13}C NMR ($CDCl_3$, 50 MHz): δ 30.5, 36.6, 41.7, 49.9, 66.2, 126.4, 128.6, 128.8, 139.2, 158.3, 177.3. Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.92; H, 6.68; N, 11.35.

6.4.2.4. 1-Butyl-3-(2-oxo-tetrahydro-furan-3-yl)-urea (13). Reaction time: 48 h. Chromatography: EtOAc. Recrystallisation: EtOAc/pentane. White solid (83%). Mp: 100–101 °C. ^{1}H NMR (acetone-d_6, 200 MHz): δ 0.90 (t, J = 7.3 Hz, 3H), 1.24–1.51 (m, 4H), 2.11–2.33 (m, 1H), 2.50–2.64 (m, 1H), 3.09–3.18 (q, J = 6.5 Hz, 2H), 4.19–4.57 (m, 3H), 5.78 (s, 1H), 5.90 (s, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.8, 20.1, 30.4, 32.3, 40.1, 49.9, 66.2, 158.4, 177.4. Anal. Calcd for $C_9H_{16}N_2O_3$: C, 53.98; H, 8.05; N, 13.99. Found: C, 54.18; H, 8.42; N, 14.12.

6.4.2.5. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-pentyl-urea (14). Reaction time: 24 h. Chromatography: EtOAc/pentane, 9/1. Recrystallisation: EtOAc/pentane. White solid (75%). Mp: 106–107 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.85 (t, J = 6.6 Hz, 3H), 1.22–1.33 (m, 4H), 1.35–1.47 (m, 2H), 2.17–2.33 (m, 1H), 2.53–2.66 (m, 1H), 3.05–3.14 (q, J = 6.5 Hz, 2H), 4.17–4.55 (m, 3H), 5.76 (t, J = 5.3 Hz, 1H), 6.06 (d, J = 7.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 14.0, 22.4, 29.1, 29.9, 30.1, 40.4, 49.8, 66.1, 158.4, 177.4. Anal. Calcd for $C_{10}H_{18}N_2O_3$: C, 56.06; H, 8.47; N, 13.07. Found: C, 56.40; H, 8.39; N, 13.03.

6.4.2.6. 1-Butyl-3-(2-oxo-tetrahydro-furan-3-yl)-thio-urea (34) and 3-butyl-5-(2-hydroxy-ethyl)-2-thioxo-imidazolidine-4-one (35). Reaction time: 24 h. Chromatography: EtOAc/pentane, 55:45. Compound **34** was first eluted (R_f: 0.38) and then **35** (R_f: 0.22). Compound **34**: colourless oil (21%). ^{1}H NMR (acetone-d_6, 200 MHz): δ 0.92 (t, J = 7.2 Hz, 3H), 1.28–1.46 (m, 2H), 1.51–1.66 (m, 2H), 2.11–2.28 (m, 1H), 2.75–2.88

(m, 1H), 3.50–3.53 (m, 2H), 4.28–4.46 (m, 2H), 5.32–5.46 (m, 1H), 7.04 (s, 1H), 7.27 (s, 1H). ^{13}C NMR (acetone-d_6, 50 MHz): δ 14.7, 21.3, 31.6, 32.5, 45.5, 54.5, 66.9, 176.8, 185.3. HR-MS (EI): M^+. Calcd for $C_9H_{16}N_2O_2S$: 216.0932. Found: 216.0934. Compound **35**: colourless oil. ^{1}H NMR ($CDCl_3$, 200 MHZ): δ 0.94 (t, J = 7.2 Hz, 3H), 1.24–1.44 (m, 2H), 1.57–1.72 (m, 2H), 1.82–2.05 (m, 1H), 2.12–2.26 (m, 1H), 2.65 (s, 1H), 3.79 (t, J = 7.5 Hz, 2H), 3.88–3.93 (m, 2H), 4.23 (dd, J = 3.9 Hz and 9.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.7, 20.0, 29.7, 33.6, 41.2, 58.1, 59.6, 175.1, 183.8.

6.4.3. General procedure for the synthesis of ureas 11 and 15. To a solution of sodium azide (1.04 g, 16.0 mmol) in water (4 mL) was added dropwise a solution of acyl chloride (5.3 mmol) in acetone (4 mL). After 1 h of stirring at rt, the solution was extracted with dichoromethane (2× 20 mL). The organic phase was washed with water and dried. The solvent was evaporated to give the corresponding carboxylic azide as an oil, which was refluxed in dry THF (50 mL) for 1 h. The reaction mixture was then cooled to rt and D,L-α-amino-γ-butyrolactone hydrobromide (0.92 g, 5.0 mmol) followed by 4-dimethylaminopyridine (0.68 g, 5.6 mmol) were added. After 24 h, the reaction mixture was filtered and the solvent was evaporated. The residue was purified by flash chromatography to give a solid, which was recrystallised.

6.4.3.1. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-(3-phenyl-propyl)-urea (11). Chromatography: EtOAc. Recrystallisation: EtOAc/cyclohexane. White solid (82%). Mp: 80–81 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 1.71–1.86 (m, 2H), 2.09–2.31 (m, 1H), 2.58–2.71 (m, 3H), 3.13–3.23 (q, J = 6.6 Hz, 2H), 4.16–4.29 (m, 1H), 4.34–4.56 (m, 2H), 5.63 (t, J = 5.6 Hz, 1H), 5.90 (d, J = 6.6 Hz, 1H), 7.13–7.30 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 30.6, 31.8, 33.1, 40.0, 50.0, 66.3, 126.0, 128.4, 128.5, 141.6, 158.2, 177.5. Anal. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68. Found: C, 64.44; H, 7.17; N, 10.64.

6.4.3.2. 1-(2-Cyclohexyl-ethyl)-3-(2-oxo-tetrahydro-furan-3-yl)-urea (15). Chromatography: EtOAc. Recrystallisation: EtOAc/cyclohexane. White solid (61%). Mp: 117–118 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.87–0.97 (m, 2H), 1.15–1.44 (m, 6H), 1.67–1.72 (m, 5H), 2.05-2.31 (m, 1H), 2.69–2.83 (m, 1H), 3.19 (t, J = 7.2 Hz, 2H), 4.22–4.35 (m, 1H), 4.39–4.57 (m, 2H), 5.26 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 26.3, 26.6, 30.6, 33.2, 35.8, 37.6, 38.3, 49.9, 66.2, 158.3, 177.4. Anal. Calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.01. Found: C, 61.57; H, 8.62; N, 11.18.

6.4.4. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-(5-phenyl-pentyl)-urea (12). To a solution of 6-phenyl-hexanoic acid (0.38 g, 2.0 mmol) and triethylamine (0.20 g, 2.0 mmol) in dry THF (10 mL) was added diphenylphosphorylazide (0.55 g, 2.0 mmol). The solution was refluxed for 2 h and then cooled to rt D,L-α-amino-γ-butyrolactone hydrobromide (0.37 g, 2.0 mmol) and triethylamine (0.20 g, 2 mmol) were then added. After 16 h of stirring at rt, the solvent was evaporated. The residue was purified by flash chromatography (EtOAc/pentane, 9:1) to give a solid, which was recrystallised (EtOAc/pentane). White solid (54%). Mp: 78–79 °C.^{1}H NMR (acetone-d_6, 300 MHz): δ 1.32–1.42 (m, 2H), 1.47–1.57 (m, 2H), 1.59–1.69 (m, 2H), 2.16–2.30 (m, 1H), 2.52–2.60 (m, 1H), 2.61 (t, J = 7.8 Hz, 2H), 3.15 (q, J = 6.6 Hz, 2H), 4.21–4.30 (m, 1H), 4.37 (dt, J = 1.8 and J = 9 Hz, 1H), 4.48–4.57 (m, 1H), 5.86 (t, J = 4.8 Hz, 1H), 5.99 (d, J = 7.2 Hz, 1H), 7.14–7.30 (m, 5H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 27.2, 30.8, 31.0, 32.1, 36.4, 40.6, 50.1, 66.0, 126.4, 129.0, 129.2, 143.5, 158.5, 176.8. Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65. Found: C, 66.34; H, 7.89; N, 9.64.

6.4.5. General procedure for the synthesis of ureas 16–18 and 33. To a solution of α-carboxy-γ-butyrolactone[11] (0.26 g, 2.0 mmol) in dry THF (30 mL) were added diphenylphosphorylazide (0.55 g, 2.0 mmol) and triethylamine (0.20 g, 2.0 mmol). The solution was refluxed for 3.5 h and then cooled to rt. The appropriate amine (2.0 mmol) was then added. After 18 h of stirring at rt, the solvent was evaporated. The residue was purified by flash chromatography to give a solid (except **33**), which was recrystallised.

6.4.5.1. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-(4-phenyl-butyl)-urea (16). Chromatography: EtOAc/pentane, 85:15. Recrystallisation (EtOAc/pentane). White solid (21%). Mp: 81–82 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.43–1.67 (m, 4H), 2.05–2.28 (m, 1H), 2.57–2.71 (m, 3H), 3.11–3.21 (q, J = 6.4 Hz, 2H), 4.15–4.55 (m, 3H), 5.49 (s, 1H), 5.79 (s, 1H), 7.13–7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 28.7, 29.8, 30.6, 35.6, 40.2, 50.0, 66.2, 125.8, 128.3, 128.4, 142.3, 158.2, 177.4. Anal. Calcd for $C_{15}H_{20}N_2O_3$: C, 65.20; H, 7.30; N, 10.14. Found: C, 65.27; H, 7.40; N, 9.92.

6.4.5.2. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-propyl-urea (17). Chromatography: EtOAc. Recrystallisation: EtOAc/pentane. White solid (24%). Mp: 109–110 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, J = 7.2 Hz, 3H), 1.42–1.52 (m, 2H), 2.16–2.28 (m, 1H), 2.64–2.73 (m, 1H), 3.10 (q, J = 7.0 Hz, 2H), 4.25–4.31 (m, 1H), 4.42 (dt, J = 1.5 and J = 9 Hz, 1H), 4.47–4.56 (m, 1H), 5.49 (t, J = 5.3 Hz, 1H), 5.80 (d, J = 6.8 Hz, 1H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 12.6, 25.2, 31.6, 43.4, 51.1, 67.0, 159.6, 177.9. Anal. Calcd for $C_8H_{14}N_2O_3$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.33; H, 7.72; N, 14.90.

6.4.5.3. 1-Hexyl-3-(2-oxo-tetrahydro-furan-3-yl)-urea (18). Chromatography: EtOAc/pentane, 9:1. Recrystallisation: EtOAc/pentane. White solid (16%). Mp: 96–97 °C. ^{1}H NMR (acetone-d_6, 300 MHz): δ 0.88 (t, J = 6.6 Hz, 3H), 1.23–1.35 (m, 6H), 1.42–1.48 (m, 2H), 2.15–2.29 (m, 1H), 2.51–2.61 (m, 1H), 3.12 (q, J = 6.6 Hz, 2H), 4.21–4.29 (m, 1H), 4.33–4.39 (dt, J = 1.5 Hz and J = 8.8 Hz, 1H), 4.46–4.55 (m, 1H), 5.85 (s, 1H), 5.98 (d, J = 6.6 Hz, 1H).^{13}C NMR (acetone-d_6, 75 MHz): δ 15.1, 24.0, 28.1, 31.6, 31.8, 33.1, 41.5, 50.9, 66.7, 159.3, 177.6. Anal. Calcd for $C_{11}H_{20}N_2O_3$: C, 57.87; H, 8.83; N, 12.27. Found: C, 57.33; H, 8.87; N, 12.12.

6.4.5.4. 1-Butyl-1-methyl-3-(2-oxo-tetrahydro-furan-3-yl)-urea (33). Chromatography: EtOAc. Yellow oil (13%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.89 (t, J = 7.5 Hz, 3H), 1.22–1.34 (m, 2H), 1.43–1.53 (m, 2H), 2.09–2.23 (m, 1H), 2.68–2.77 (m, 1H), 2.86 (s, 3H), 3.13–3.30 (m, 2H), 4.19–4.28 (m, 1H), 4.38–4.50 (m, 2H), 5.19 (d, J = 5.1 Hz, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.1, 20.2, 30.3, 31.1, 34.5, 48.9, 50.7, 66.4, 157.7, 177.2. HR-MS (EI): M^+. Calcd for $C_{10}H_{18}N_2O_3$: 214.1317. Found: 214.1318.

6.4.6. General procedure for the synthesis of ureas 19–21. To a solution of 4-nitrophenyl chloroformate (0.55 g, 2.7 mmol) and diisopropylethylamine (0.36 g, 2.7 mmol) in dry THF (10 mL) was added dropwise a solution of the appropriate amine (2.7 mmol) in THF (5 mL). After 4 h of stirring at rt, D,L-α-amino-γ-butyrolactone hydrobromide (0.5 g, 2.7 mmol) followed by diisopropylethylamine (0.355 g, 2.7 mmol) were added. After 18 h, the solution was filtered and the solvent was evaporated. The residue was purified by flash chromatography to give a solid which was recrystallised.

6.4.6.1. 1-(4-Methyl-benzyl)-3-(2-oxo-tetrahydro-furan-3-yl)-urea (19). Chromatography: EtOAc/pentane, 9:1. Recrystallisation: EtOAc/pentane. White solid (70%). Mp: 161–162 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.08–2.22 (m, 1H), 2.27 (s, 3H), 2.35–2.44 (m, 1H), 4.14–4.28 (m, 1H), 4.15 (d, J = 5.7 Hz, 2H), 4.31 (dt, J = 1.9 and 9 Hz, 1H), 4.40–4.49 (m, 1H), 6.46 (d, J = 7.8 Hz, 1H), 6.60 (t, J = 6.0 Hz, 1H), 7.10–7.16 (m, 4H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 20.7, 29.2, 42.7, 48.9, 65.1, 127.1, 128.8, 135.7, 137.4, 157.4, 176.4. Anal. Calcd for $C_{13}H_{16}N_2O_3$: C, 62.89; H, 6.50; N, 11.28. Found: C, 62.61; H, 6.63; N, 11.20.

6.4.6.2. 1-(2-Oxo-tetrahydro-furan-3-yl)-3-(4-trifluoromethyl-benzyl)-urea (20). Chromatography: EtOAc. Recrystallisation: EtOAc/pentane. White solid (60%). Mp: 132–133 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.09–2.24 (m, 1H), 2.35–2.45 (m, 1H), 4.14–4.23 (m, 1H), 4.28–4.34 (m, 3H), 4.41–4.51 (m, 1H), 6.61 (d, J = 7.5 Hz, 1H), 6.81 (t, J = 6.0 Hz, 1H), 7.46 (d, J = 8.1 Hz, 2H), 7.67 (d, J = 8.1 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 29.1, 42.6, 48.9, 65.1, 124.4 (q, J = 270 Hz), 125.0 (q, J = 3.5 Hz), 127.3 (q, J = 31.5 Hz), 127.6, 145.7, 157.4, 176.3. Anal. Calcd for $C_{13}H_{13}F_3N_2O_3$: C, 51.66; H, 4.34; N, 9.27. Found: C, 51.52; H, 4.48; N, 9.25.

6.4.6.3. 1-(4-Chloro-benzyl)-3-(2-oxo-tetrahydro-furan-3-yl)-urea (21). Chromatography: EtOAc/pentane, 9:1. Recrystallisation: EtOAc/pentane. White solid (46%). Mp: 149–150 °C. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.07–2.21 (m, 1H), 2.34–2.43 (m, 1H), 4.13–4.20 (m, 3H), 4.30 (t, J = 7.8 Hz, 1H), 4.39–4.48 (m, 1H), 6.52 (d, J = 7.8 Hz, 1H), 6.70 (t, J = 6.0 Hz, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 29.1, 42.3, 48.9, 65.1, 128.2, 128.9, 131.1, 139.8, 157.4, 176.4. Anal. Calcd for $C_{12}H_{13}ClN_2O_3$: C, 53.64; H, 4.88; N, 10.43. Found: C, 53.39; H, 4.85; N, 10.13.

6.4.7. 1-Butyl-3-(2-oxo-2,5-dihydro-furan-3-yl)-urea (30). To a solution of 4-nitrophenyl chloroformate (0.48 g, 2.4 mmol) and triethylamine (0.24 g, 2.4 mmol) in dry THF (15 mL) was added dropwise a solution of 3-amino-5*H*-furan-2-one **28**[12] (0.20 g, 2.0 mmol) in THF (5 mL). After 4 h of stirring at rt, butylamine (0.15 g, 2.0 mmol) was added. After 48 h, the solution was filtered and the solvent was evaporated. The residue was purified by flash chromatography (EtOAc/pentane: 7:3). White solid (26%). Mp: 101–103 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.93 (t, J = 7.2 Hz, 3H), 1.30–1.44 (m, 2H), 1.48–1.57 (m, 2H), 3.26 (t, J = 6.8 Hz, 2H), 4.90 (d, J = 1.8 Hz, 2H), 5.92 (s, 1H), 7.27 (t, J = 1.8 Hz, 1H), 7.83 (s, 1H). ^{13}C NMR (acetone-d_6, 75 MHz): δ 14.8, 21.4, 33.7, 40.9, 71.8, 122.1, 128.3, 156.1, 172.0. HR-MS (EI): M^+. Calcd for $C_9H_{14}N_2O_3$: 198.1004. Found: 198.1005.

6.4.8. 1-Butyryl-3-(2-oxo-tetrahydro-furan-3-yl)-urea (32). To a solution of (2-oxo-tetrahydro-furan-3-yl)-urea (**31**)[13] (0.45 g, 3.1 mmol) in dry pyridine was added dropwise at 0 °C butyryl chloride (0.33 g, 3.1 mmol). After 18 h of stirring at room temperature, the solvent was evaporated. The residue was purified by flash chromatography (EtOAc/pentane: 5:5) to give **32** (0.44 g, 66%) as a white solid. Mp: 168–169 °C. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 0.99 (t, J = 7.3 Hz, 3H), 1.61–1.79 (m, 2H), 2.32 (t, J = 7.3 Hz, 2H), 2.29–2.45 (m, 1H), 2.64–2.74 (m, 1H), 4.23–4.36 (m, 1H), 4.45–4.60 (m, 2H), 8.98 (s, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 13.6, 18.3, 29.3, 38.8, 49.3, 65.7, 154.5, 174.3, 175.2. Anal. Calcd for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.83; H, 6.87; N, 13.04.

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References and notes

1. For recent reviews, see: (a) Miller, M. B.; Bassler, B. L. *Annu. Rev. Microbiol.* **2001**, *55*, 165–199; (b) Whitead, N. A.; Barnard, A. M. L.; Slater, H.; Simpson, N. J. L.; Salmond, G. P. C. *FEMS Microbiol. Rev.* **2001**, *5*, 365–404; (c) Lyon, G. J.; Muir, T. W. *Chem. Biol.* **2003**, *10*, 1007; (d) Waters, C. M.; Bassler, B. L. *Annu. Rev. Cell. Dev. Biol.* **2005**, *21*, 319–346.
2. (a) Suga, H.; Smith, K. M. *Curr. Opin. Chem. Biol.* **2003**, *7*, 586–591; (b) Persson, T.; Givskov, M.; Nielsen, J. *Curr. Med. Chem.* **2005**, *12*, 3103–3115; (c) Raffa, R. B.; Iannuzzo, J. R.; Levine, D. R.; Saeid, K. K.; Schwartz, R. C.; Sucic, N. T.; Terleckyj, O. D.; Young, J. M. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 417–423.
3. (a) Kjelleberg, S.; Molin, S. *Curr. Opin. Microbiol.* **2002**, *5*, 254–258; (b) Hentzer, M.; Eberl, L.; Nielsen, J.; Givskov, M. *BioDrugs* **2003**, *17*, 241–250; (c) Rice, S. A.; McDougald, D.; Kumar, N.; Kjelleberg, S. *Curr. Opin. Investig. Drugs* **2005**, *6*, 168–184.

4. Eberhard, A.; Burlingame, A. L.; Eberhard, C.; Kenyon, G. L.; Nealson, K. H.; Oppenheimer, N. J. *Biochemistry* **1981**, *20*, 2444–2449.
5. Urbanowski, M. L.; Lostroh, C. P.; Greenberg, E. P. *J. Bacteriol.* **2004**, *186*, 631–637.
6. (a) Lerat, E.; Moran, N. A. *Mol. Biol. Evol.* **2004**, *21*, 903–913; (b) Parsek, M. R.; Greenberg, E. P. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8789–8793.
7. (a) Eberhard, A.; Widrig, C. A.; McBath, P.; Schineller, J. B. *Arch. Microbiol.* **1986**, *146*, 35–40; (b) Chaabra, S. R.; Stead, P.; Bainton, N. J.; Salmond, G. P. C.; Stewart, G. S. A. B.; Williams, P.; Bycroft, B. W. *J. Antibiot.* **1993**, *46*, 441–454; (c) Passador, L.; Tucker, K. D.; Guertin, K. R.; Journet, M. P.; Kende, A. S.; Iglewski, B. H. *J. Bacteriol.* **1996**, 5995–6000; (d) Schaefer, A. L.; Hanzelka, B. L.; Eberhard, A.; Greenberg, E. P. *J. Bacteriol.* **1996**, 2897–2901; (e) Kline, T.; Bowman, J.; Iglewski, B. H.; de Kievit, T.; Kakai, Y.; Passador, L. *Biorg. Med. Chem. Lett.* **1999**, *9*, 3447–3452; (f) Smith, K. M.; Bu, Y.; Suga, H. *Chem. Biol* **2003**, *10*, 81–89; (g) Smith, K. M.; Bu, Y.; Suga, H. *Chem. Biol.* **2003**, *10*, 563–571; (h) Welch, M.; Dutton, J. M.; Glansdorp, F. G.; Thomas, G. L.; Smith, D. S.; Coulthurst, S. J.; Barnard, A. M. L.; Salmond, G. P. C.; Spring, D. R. *Biorg. Med. Chem. Lett.* **2005**, *15*, 4235–4238; (i) Persson, T.; Hansen, T. H.; Rasmussen, T. B.; Skinderso, M. E.; Givskov, M.; Nielsen, J. *Org. Biomol. Chem.* **2005**, *3*, 253–262; (j) Geske, G. D.; Wezeman, R. J.; Siegel, A. P.; Blackwell, H. E. *J. Am. Chem. Soc.* **2005**, *127*, 12762–12763, and references cited therein.
8. (a) Reverchon, S.; Chantegrel, B.; Deshayes, C.; Doutheau, A.; Cotte-Pattat, N. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1153–1157; (b) Castang, S.; Chantegrel, B.; Deshayes, C.; Dolmazon, R.; Gouet, P.; Haser, R.; Reverchon, S.; Nasser, W.; Hugouvieux-Cotte-Pattat, N.; Doutheau, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5145–5149.
9. For recent reviews, see: (a) Chrusciel, R. A.; Strohbach, J. W. *Curr. Top. Med. Chem.* **2004**, *4*, 1097–1114; (b) Dumas, J. *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 718–727.
10. When both L- and D-isomers of natural AHLs were tested on *P. aeruginosa*, bacteria with an AHL-dependent QS system, only the L-isomers displayed autoinducing activity, whereas the D-isomers were inactive. Moreover, the activity of the L-isomers was not inhibited by the presence of the D-isomer: Ikeda, T.; Kajiyama, K.; Kita, T.; Takiguchi, N.; Kuroda, A.; Kato, J.; Ohtake, H. *Chem. Lett.* **2001**, 314–315.
11. Kraatz, U.; Wamhoff, H.; Korte, F. *Liebigs Ann. Chem.* **1971**, *744*, 33–41.
12. Rao, V. S. *Synth. Commun.* **1989**, *19*, 1389–1393.
13. Kraatz, U.; Hasenbrink, W.; Wamhoff, H.; Korte, F. *Chem. Ber.* **1971**, *104*, 2458–2466.
14. Knobler, Y.; Bonni, E.; Frankel, M. *Tetrahedron* **1967**, *23*, 1557–1563.
15. Silica gel chromatography of the reaction mixture provided **34** still contaminated by a small amount (≈10%) of **35**.
16. Zhang, R. G.; Pappas, T.; Brace, J. L.; Miller, P. C.; Oulmassov, T.; Molyneaux, J. M.; Anderson, J. C.; Bashkin, J. K.; Winans, S. C.; Joachimiak, A. *Nature* **2002**, *417*, 971–974.
17. Luo, Z. Q.; Smyth, A. J.; Gao, P.; Qin, Y.; Farrand, S. K. *J. Biol. Chem.* **2003**, *278*, 13173–13182.
18. Kiratisin, P.; Tucker, K. D.; Passador, L. *J. Bacteriol.* **2002**, *184*, 4912–4919.
19. Shadel, G. S.; Young, R.; Baldwin, T. O. *J. Bacteriol.* **1990**, *172*, 3980–3987.
20. Winson, M. K.; Swift, S.; Fish, L.; Throup, J. P.; Jorgensen, F.; Chhabra, S. R.; Bycroft, B. W.; Williams, P.; Stewardt, M. K. *FEMS Microbiol. Lett.* **1998**, *163*, 185–192.
21. Schuster, M.; Urbanowski, M. L.; Greenberg, E. P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15833–15839.
22. Zhu, J.; Winans, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4832–4837.
23. Krieger, E.; Koraimann, G.; Vriend, G. *Proteins* **2002**, *47*, 393–402.