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Synthesis and biological evaluation of new *N*-acyl-homoserine-lactone analogues, based on triazole and tetrazole scaffolds, acting as LuxR-dependent quorum sensing modulators

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ABSTRACT

New analogues of *N*-acyl-homoserine-lactone (AHL), in which the amide was replaced by a triazole or tetrazole ring, were prepared and tested for their activity as LuxR-dependent QS modulators. Several compounds showed a level of antagonistic or agonistic activity, notably some 1,4-triazolic and 1,5-tetrazolic derivatives, whereas the 2,5-tetrazolic compounds were inactive. In 1,5-tetrazoles, substituted with butyrolactone and an alkyl chain, the activity was reversed, depending on the connection between the lactone and the tetrazole. The C–N connected compounds were agonists whereas the C–C connected ones were antagonists.

1. Introduction

Bacterial quorum sensing (QS) refers to a cell-to-cell communication system which allows bacteria to synchronize the expression of specific genes, encoding for phenotype expression, as a function of their population density.[1–5] This communication is based on the synthesis and release of small diffusible signal molecules, known as autoinducers, which bind to their cognate transcriptional regulatory proteins. QS regulates diverse phenotypes, such as bioluminescence, antibiotic production, symbiosis, biofilm formation or virulence factors, in a variety of microorganisms found in plants, animals and humans. Thus, QS modulation is considered as a promising new strategy for controlling both pathogenesis and symbiosis, which have important impacts on human health and the environment,[6–11] either by stimulation or inhibition of communication. Therefore, the development of synthetic ligands, which are able to behave as antagonists of autoinducers, constitutes an important research strategy for the design of potential antibacterial drugs.

In Gram negative bacteria, *N*-acyl-homoserine lactones (AHLs) (Fig. 1) are the main autoinducers and LuxR-type proteins are the transcriptional factors.[12–14] In recent years we have reported several classes[15–22] of QS inhibitors in *Vibrio fischeri* bacteria. Among those showing close structural analogy with AHLs, we have investigated, in particular, the effect of replacing the amide function with amide bioisosteres, such as sulfonamide,[19] urea,[20] sulfonylurea[21] or reverse-amides.[22]

Heterocycles, containing several nitrogen atoms, are scaffolds that are frequently considered when designing bioactive compounds and some of them behave as biomimetics. However, within the field of QS modulation, very few AHL analogues built on a central multi-nitrogen heterocycle have been reported to date, with the exception of one series of tetrazolic compounds described by Greenberg's group[23] and one family of imidazolium compounds

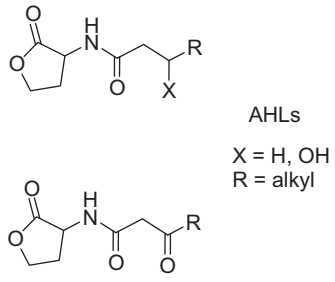

Figure 1. Structures of AHLs.

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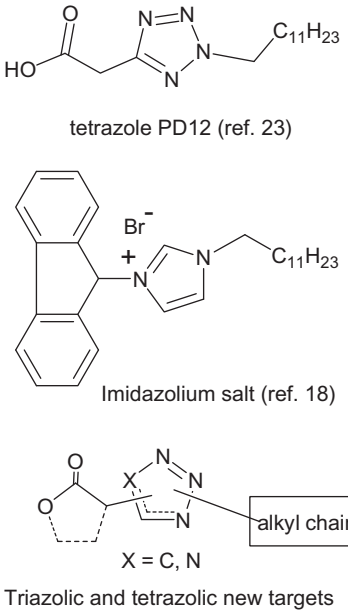
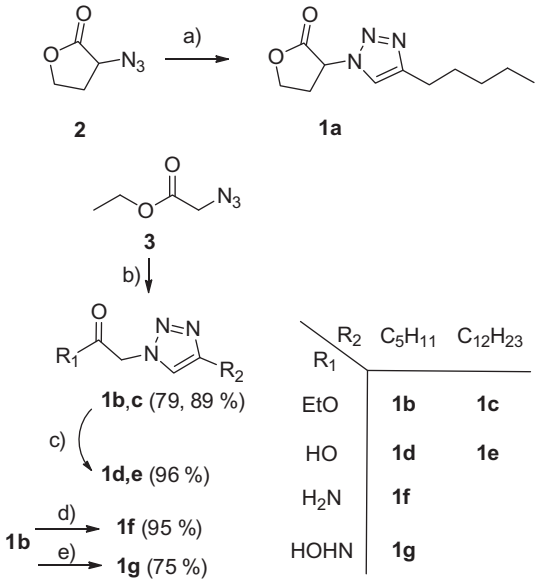


Figure 2. Known and targeted QS modulators constructed on a central multi-nitrogen heterocyclic scaffold.



Scheme 1. Preparation of triazole **1a–1g**. Reagents and conditions: (a) hept-1-yne, CuI, H_2O/CH_3CN (1:1), rt, 72 h; (b) CuIsupp, CH_2Cl_2, rt, 12 h; (c) KOH, Ethanol, rt, 4 h; (d) NH_3, methanol, −30 °C to rt, 16 h; (e) NH_2OHaq, KCN, THF/MeOH (1:1), rt, 24 h.

described by some of us (Fig. 2).[18] Pursuing our efforts to identify new QS inhibitors by replacing the amide of AHLs with various functions, we report here the synthesis and the biological evaluation of new AHL analogues, based on the 1,2,3-triazole or 1,2,3,4-tetrazole scaffolds (Fig. 2), as LuxR-dependent QS agonists or antagonists.

2. Triazoles

The triazole ring displays some similarity with amide bonds, in terms of distance and planarity, and has been known for a long time as an amide bond mimic.[24–26] This motif can be easily obtained using Huisgen's 1,3-dipolar cycloaddition between an azide and an alkyne.[27] Using improved experimental conditions, this reaction can result in good to excellent yields and complete regioselectivity,[28–30] making this strategy attractive for the design of bioactive compounds.[31] In this context, we decided to prepare an AHL analogue of type **1**, in which a 1,2,3-triazole ring is introduced in place of the amide function of AHLs (Fig. 3). The first targeted triazole derivative in the series was **1a**, in which the lactone ring is retained and equipped with an alkyl chain of 5 carbon atoms, an appropriate length according to our previous studies.[19–21] Since this compound was active (vide infra), and inspired by Greenberg's results[23] showing that a carboxylic acid was a possible substitution in tetrazolic compounds bearing a long alkyl chain (although the activity was demonstrated in another biological assay related to *Pseudomonas aeruginosa*), triazoles **1b–g**, lacking the lactone ring and bearing longer alkyl chains, were then prepared. Finally, inverted triazoles of type **2**, isomers of type **1** compounds, were synthesized in order to evaluate any effects on the biological activity due to the position of the nitrogen atoms with respect to the substituents on the triazole ring.

2.1. Synthesis

Racemic 1,2,3-triazole **1a** was prepared from a 1,3-dipolar cycloaddition reaction between the known[32] racemic azido-lactone **2** and hept-1-yne, using copper iodide as a catalyst (Scheme 1).[28] Triazoles **1b–g** were obtained from known[33] ethyl-2-azidoacetate **3**. Huisgen's 1,3 dipolar cycloaddition reaction of **3** with hept-1-yne or tetradec-1-yne, in the presence of a polymer-supported copper catalyst,[34] led to the desired triazoles **1b**,**c** in 79–89% of isolated yields. Triazoles with an acid function, **1d** and **1e**, were obtained by saponification of the corresponding ethyl esters, **1b** and **1c**. The reaction of **1b** with ammonia in methanol[35] or in aqueous hydroxylamine, using potassium cyanide as a catalyst,[36] produced the triazole-amide **1f** and triazole-hydroxamate **1g**, respectively (Scheme 1).

Type **2** triazoles were prepared in three or four steps (Scheme 2). The reaction of pentyl bromide, **4a**, and dodecyl bromide, **4b**, with sodium azide resulted in azidoalkanes **5a** and **5b** in 36% and 80% isolated yields, respectively.[33] Also, a good yield of triazoles **6** was obtained by reacting azidoalkanes **5** with 3-butyn-1-ol, under the same cycloaddition conditions as stated above. Oxidation of compounds **6** with Jones reagent led to the desired triazoles **2c** and **2d** in 66% and 69% yields, respectively, which could then be transformed into the corresponding esters **2a**,**b** (Scheme 2).

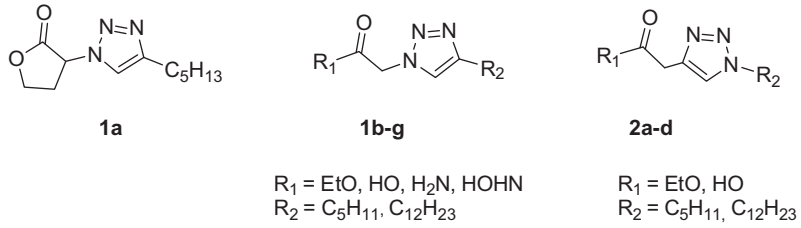
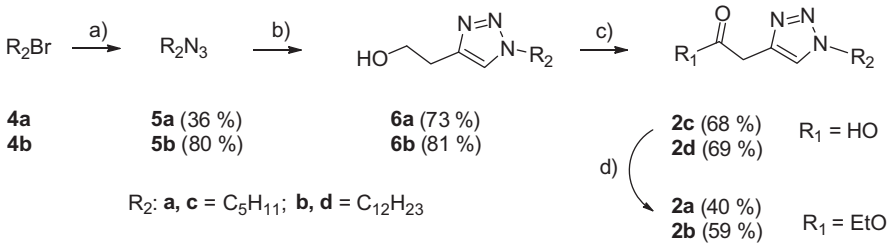


Figure 3. Structure of triazoles of type **1** and **2**.



Scheme 2. Preparation of triazole **2a–d**. Reagents and conditions: (a) NaN_3, acetone/H_2O (3:1), rt, 1 h; (b) 3-butyn-1-ol, CuIsupp, CH_2Cl_2, rt, 12 h; (c) chromic acid, sulfuric acid, acetone, 0 °C, 12 h; (d) sulfuric acid, ethanol, 0 °C to rt, 4 h.

2.2. Biological evaluation of triazoles

Compounds **1** and **2** were first tested for their ability to induce luminescence in an *Escherichia coli* biosensor strain containing a plasmid that couples the *luxR* and *luxICDABE* promoter region of *Vibrio fischeri* to the *luxCDABE* operon of *Photorhabdus luminescens*.[15] Neither of them displayed any agonistic activity at concentrations up to 200 μM. QS inhibition was then evaluated by measuring the decrease in bioluminescence induced by 3-oxo-C6-HSL (200 nM), the main QS autoinducer in *Vibrio fischeri*. The lactone **1a** proved to be active, with significant antagonist activity (IC_{50} = 51 ± 2 μM). Among the non-lactonic analogues, ethyl ester **1b,** equipped with a short alkyl chain, displayed activity (IC_{50} = 94 ± 4 μM) whereas neither the corresponding acid **1d,** nor the amide **1f** and hydroxamate **1g** were active. The longer chain compounds **1c** and **1e**, as well as all type **2** triazoles, were found to be inactive.

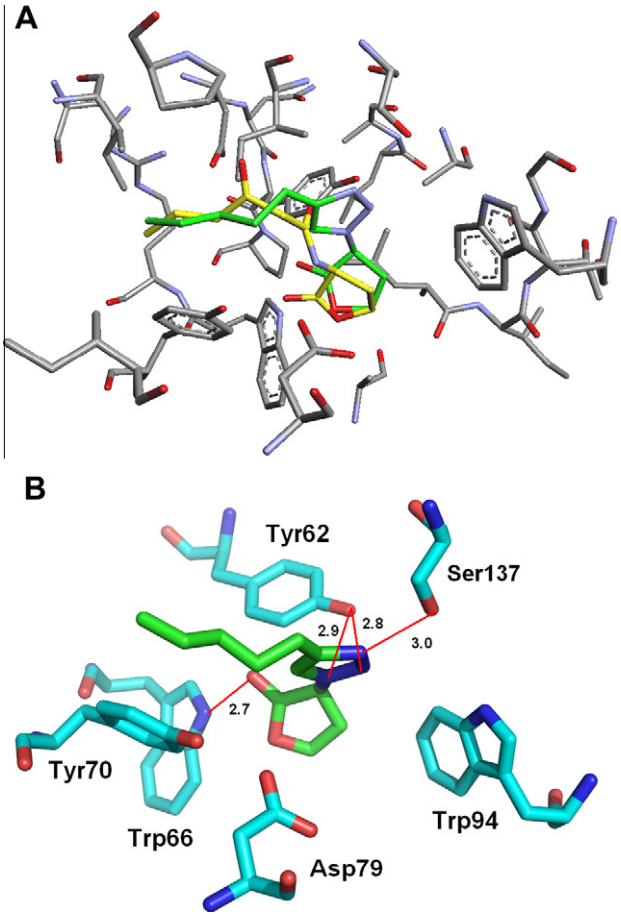


Figure 4. Proposed binding mode of compound **1a** in the ligand binding site of the LuxR model: (A) Overview of compound **1a** and 3-oxo-hexanoyl homoserine lactone (OHHL) within the binding site; (B) magnification and simplification of the binding mode showing interactions with conserved residues.

2.3. Molecular modeling of triazole 1a

The proposed binding mode of the triazole derived compound, **1a**, was obtained as a result of flexible docking in the ligand binding site of the LuxR model[37] (Fig. 4), constructed on the basis of the X-ray structure of TraR.[38]

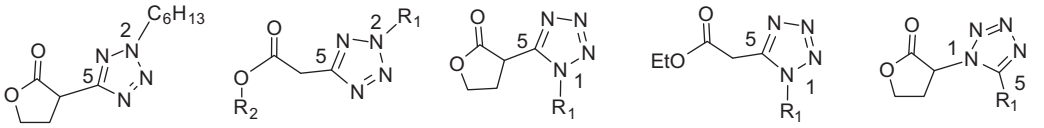
This binding mode suggests that compound **1a** has a similar position in the active site to the natural ligand 3-oxo-hexanoyl homoserine lactone (OHHL),[16,38] with the triazole ring located in the same area of the active site as the amide function in the autoinducer (Fig. 4A). Three hydrogen bonds are shown in the model, two of which are similar to those observed in the case of the natural ligand,[16] involving Trp66 and the carbonyl function of the lactone ring and Tyr62 and the nitrogen atoms of the triazolyl moiety, respectively. The third H-bond indicated in this model, which involves the triazole ring and Ser137, might explain the antagonist activity of triazole **1a** (Fig. 4B).

3. Tetrazoles

Tetrazoles are often important in biology and medicine.[39] In particular, 5-substituted tetrazoles are nonclassical isosteres of the carboxyl group and 1,5-disubstituted tetrazoles can be used as isosteres of the cis-amide bond of peptides.[40–42] Although they have been studied less, examples of biologically active 2,5-disubstituted tetrazoles have also been reported.[43–45] Notably, among the tetrazoles reported by Greenberg and colleagues as being QS inhibitors in *Pseudomonas aeruginosa*, the most potent was the 2,5-disubstituted derivative PD12 (Fig. 2). In the present study, we have focused our attention on new butyrolactone–tetrazole hybrids in which the lactone moiety is connected either to the carbon atom of the tetrazole ring (**7a** and **8a,d,e**) or to the nitrogen atom (**9a–d**). All the compounds in this series are racemic. Analogues with an ethyl ester (**7b,c** and **8b,c**) were also prepared in view of our previous results in the triazole series, as were the carboxylic acids (**7d,e**) for comparison purposes (Fig. 5).

3.1. Synthesis

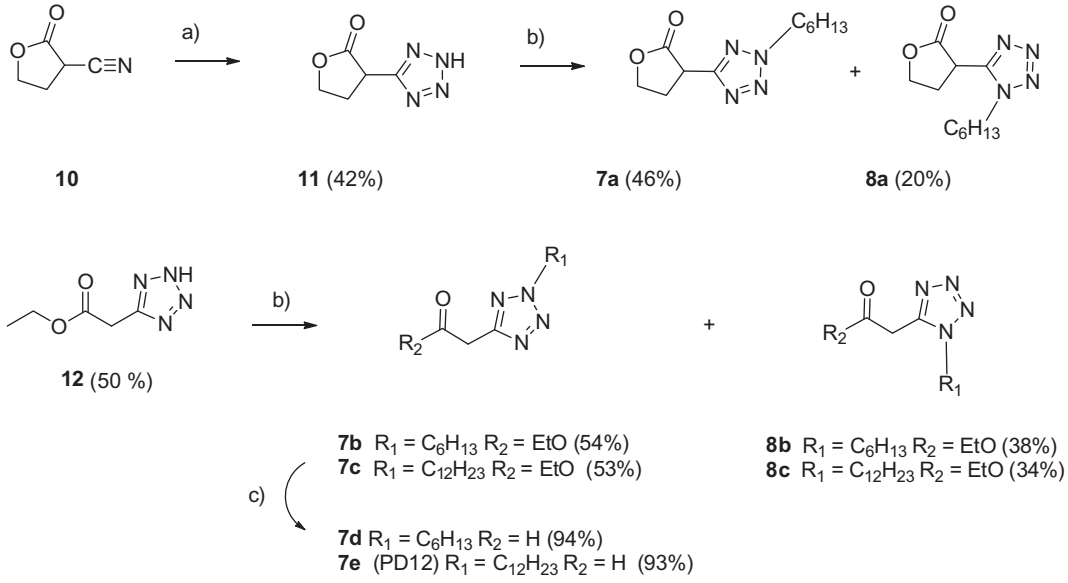
The reaction of the known[46] racemic lactone **10** with sodium azide, in the presence of ammonium chloride,[47] gave a moderate yield of the tetrazole **11**. Subsequent alkylation with hexyl bromide led to a mixture of the racemic tetrazoles **7a** and **8a** in 66% yield (Scheme 3), which were then separated by silica gel column chromatography. Compounds **7b,c** and **8b,c**, were obtained in good yields by the same method, from the known[48] tetrazole **12** using hexyl or dodecyl chloride. The corresponding acids, **7d** and **7e**, were obtained by saponification of the ester-tetrazoles **7b** and **7c**, in almost quantitative yields.



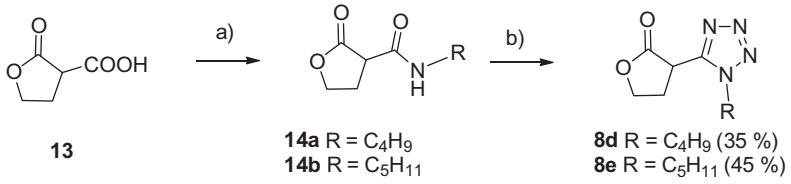
7a **7b-e** **8a,d,e** **8b,c** **9a-d**

R_1 = alkyle R_2 = H, Et

Figure 5. Structure of tetrazoles **7**, **8** and **9**.

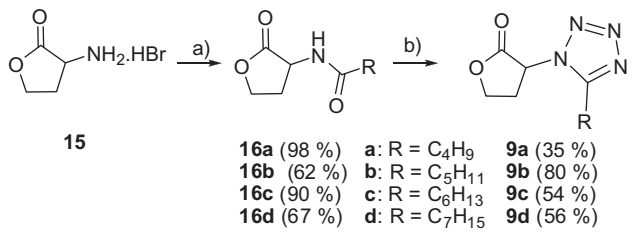


Scheme 3. Simultaneous preparation of tetrazoles **7a–e** and **8a–c**. Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, 105 °C, 6 h, then HCl_{conc} (b) alkyl bromide, Et_3N, acetonitrile, reflux, 18 h; (c) KOH, ethanol, rt, 4 h.



Scheme 4. Preparation of tetrazole **8d** and **8e**. Reagents and conditions: (a) butylamine/pentylamine, DCC, DMAP, CH_2Cl_2, 0 °C to rt, 14 h; (b) NaN_3, Tf_2O, CH_3CN, rt, 10 h.

Tetrazoles **8d** and **8e** were obtained from a two step reaction (Scheme 4). Reaction of the known[49] racemic butyrolactone **13** with butylamine and hexylamine led to amides **14a** and **14b** (reverse amide AHL analogues previously described by our group).[22]



Scheme 5. Preparation of tetrazole **9a** to **9d**. Reagents and conditions: (a) acyl chloride, pyridine, $CHCl_3$, 0 °C to rt, 16 h; (b) NaN_3, Tf_2O, CH_3CN, rt, 10 h.

A subsequent reaction with sodium azide, in the presence of triflic anhydride,[50] led to the corresponding tetrazoles **8d** and **8e**.

Tetrazoles **9** were prepared from reactions of the racemic aminolactone **15** with diverse acyl chlorides, under basic conditions, resulting in the intermediate amides **16**. A further reaction with sodium azide[50] (Scheme 5) led to the racemic tetrazoles **9a–d** in yields ranging from 35% to 80%.

3.2. Biological evaluation

The series of 14 new tetrazoles was evaluated using the same biological assay, as described above, for triazoles. All 2,5-disubstituted compounds of type **7** proved to be inactive in this test, which relates to the QS system of *Vibrio fischeri*. It is interesting to note that compound **7e** was reported, by Greenberg, to be active in another QS system, that of *Pseudomonas aeruginosa* which involves a different type of receptor protein.[23] This is another example amongst many

Table 1
Inhibition or induction of bioluminescence obtained with compounds **8a**, **b**, **d**, **e** and **9b**, **c**

Compounds	IC_{50}[a,b] (μM)	Compounds	EC_{50}[c,d] (μM)	*Biolum.* $_{max}$ (RLU)
8a	74 (±1)	**9b**	10 (±1)	*59 (±2)*
8b	>200	**9c**	35 (±1)	*102 (±3)*
8d	>200			
8e	80 (±2)			

[a] Concentration (μM) required to reduce the bioluminescence, induced by 200 nM of 3-oxo-C_6-HSL, to a level of 50% intensity (IC_{50}).
[b] Values are the means of three experiments; standard deviation is given in brackets.
[c] Concentration (μM) required to induce 50% intensity of bioluminescence.
[d] Values are the means of two experiments; standard deviation is given in brackets.

compounds which exhibit a distinct activity, sometimes even reverse activity, when applied to one bacterium species or another.[12–14,51]

Among the 1,5-disubstitued tetrazoles, only compounds substituted with a short alkyl chain displayed significant activity, either antagonistic (**8a** and **8e**) or agonistic (**9b** and **9c**) (Table 1). Thus, just a slight modification in the structure, namely the position of the nitrogen atoms in the tetrazole ring, is enough to convert the antagonist tetrazoles **8a** and **8e** into the agonist isomeric tetrazoles **9c** and **9b**, respectively. It should be noted that the tetrazole **9c**, which displays a much higher EC_{50} than the autoinducer (3-oxo-C_6-HSL, EC_{50} = 0.2 μM),[15] is nevertheless quite active since at high concentrations (⩾100 μM) these two compounds induce the same bioluminescence intensity, that is 99 (±3) and 102 (±3) RLU, respectively.

3.3. Molecular modeling of tetrazoles 8a and 9b

The proposed binding modes of tetrazole **8a** and **9b**, obtained with the protein LuxR, are depicted in Figure 6.

Compounds **9b** and **8a** are shown to occupy the same general area within the active site as the autoinducer. According to this model, compound **9b**, which exhibits agonistic activity, would display similar interactions as the natural ligand, with hydrogen bonds involving Trp 66 and Tyr 62.[16] For compound **8a**, which exhibits antagonistic activity, the same two hydrogen bonds with Trp66 would appear to be possible, whereas the heterocycle would now interact tightly with Tyr70 instead of Tyr 62. It is suggested that the difference of activity between the two compounds is mainly driven by these interactions, either with Tyr62 or with Tyr70.

4. Conclusion

Several new compounds with a triazolic or tetrazolic core, to which a lactone is attached, are shown to behave as LuxR-dependent QS modulators, with activity levels within the moderate 10–100 μM range. Modeling of the most active compounds in the study suggests that the triazolic or tetrazolic rings are located where the amide function of the autoinducer normally sits. Apart from one triazolic compound, which bears an ethyl ester motif instead of the lactone, all active compounds in this study retain the lactone moiety. Among the tetrazolic compounds, only the 1,5-systems have shown any activity whereas the 2,5-ones proved to be inactive, possibly due to the relative ability, or inability, of such linkages to serve as amide mimics. In the case of the active 1,5-tetrazoles, the way in which the lactone is connected to the tetrazole appears to

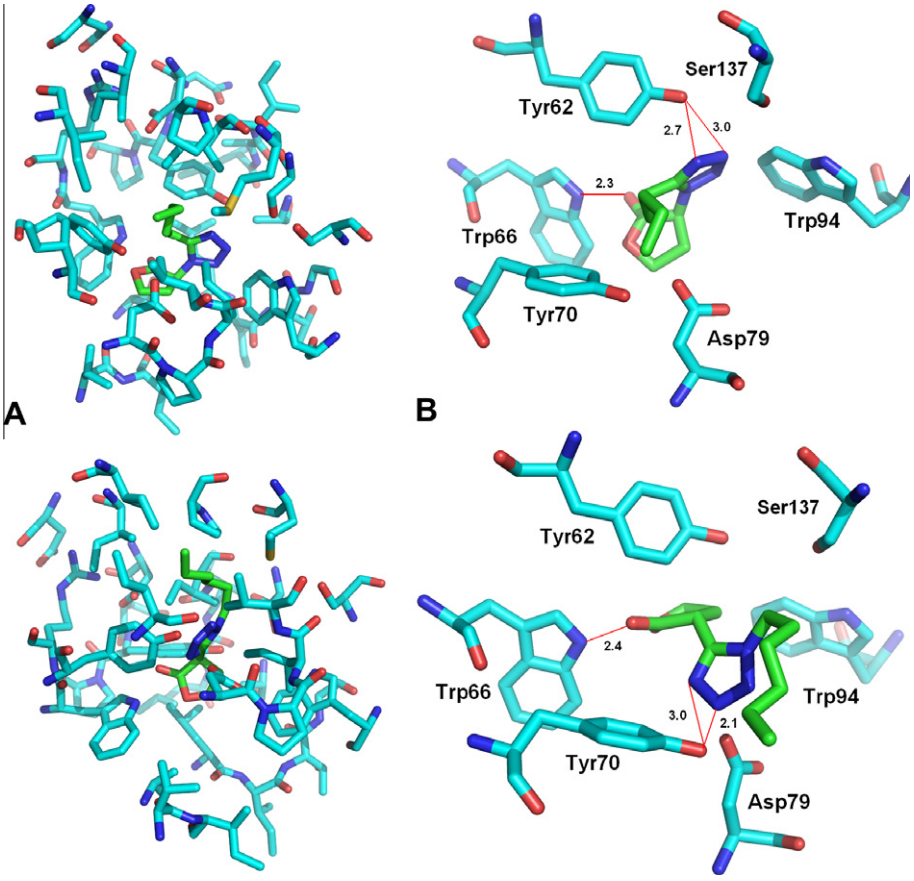


Figure 6. Proposed binding mode of agonist **9b** (top) and antagonist **8a** (bottom) in the ligand binding site of the LuxR model: (A) Overview of compounds **9b** and **8a** with all the residues of the binding site; (B) magnification and simplification of the binding mode showing interactions with conserved residues.

be critical, as the C–N connected compounds are agonists whereas the C–C connected ones are antagonists. Overall, this study confirms that multi-nitrogen heterocycles can serve as scaffolds for designing new QS modulators.

5. Experimental section

5.1. Synthesis

The solvents were distilled and dried prior to use: dichloromethane from calcium hydride and tetrahydrofuran from sodium benzophenone ketyl. Organic solutions were dried over anhydrous sodium sulfate. The reactions were performed under a constant flow of nitrogen. The reactions were monitored, by t.l.c., on Silica Gel 60 F254 (Merck) and detection was carried out by UV light (254 nm) and/or charring with a 5% phosphomolybdic acid solution in ethanol containing 10% of H_2SO_4, or a 1% potassium permanganate solution in water. Silica gel (Kieselgel 60, 70–230 mesh ASTM, Merck) was used for column chromatography. Melting points were determined on a Kofler block apparatus.

The ^{1}H NMR (300 MHz or 400 MHz) and ^{13}C NMR (75 MHz or 100 MHz) spectra were recorded with Brucker ALS300, DRX300, and DRX400 spectrometers. Chemical shifts are given in ppm. Coupling constants are expressed in Hertz and splitting pattern abbreviations are: s, singlet; d, doublet; t, triplet; q, quartet; quint, quintuplet; m, multiplet; M, massif, p, pseudo; br, broad. Multiplicity (^{13}C NMR) was determined by DEPT sequences. High resolution mass spectra were obtained using an electro spray technique, in positive mode with a ThermoFinnigan MAT 95 XL spectrometer. Amides **14a**,**b**,[22] and amides **16**[52] were obtained following the procedures reported in the literature.

5.1.1. 3-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)dihydrofuran-2(3*H*)-one (1a)

Copper(I) iodide (30 mg, 0.15 mmol) and hept-1-yne (394 μL, 3 mmol) were added to a stirred solution of 3-azidodihydrofuran-2(3*H*)-one[32] **2** (381 mg, 3 mmol) in 12 mL of acetonitrile/water (1:1). The mixture was stirred at room temperature for 72 h, washed with water (50 mL) and then extracted with ethyl acetate (2 × 100 mL). The organic phase was dried, filtered, and concentrated, under reduced pressure, to provide the compound **1a** (288 mg, 43%), after purification by flash chromatography using pentane/ethyl acetate (4/6) as the eluent. White solid mp = 75–76 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, J = 7.2 Hz, 3H); 1.35 (m, 4H); 1.69 (m, 2H); 2.73 (t, J = 7.5 Hz, 2H); 2.94–3.15 (m, 2H); 4.53–4.57 (m, 1H); 4.65–4.72 (dt, J = 3.6 Hz et 8.1 Hz, 1H); 5.29 (t, J = 9.6 Hz, 1H); 7.54 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1; 22.5; 25.7; 29.0; 29.4; 31.5; 57.6; 66.4; 120.9; 149.1; 171.4. HRMS ESI+ calculated for $C_{11}H_{17}N_3O_2$ H$^+$: 224.1394; found: MH$^+$, 224.1391.

5.1.2. General procedure A for the preparation of triazoles (1b, 1c, 6a, 6b), using a polymer supported catalyst[34]

Dry Amberlyst A-21 (1.0 g, 4.8 mmol amine) was added to a solution of copper(I) iodide (381 mg, 2.00 mmol) in acetonitrile (15 mL) and gently shaken on an orbital stirrer for 17 h. The solvent was filtered, the resin was washed with CH_3CN (2 × 15 mL) and CH_2Cl_2 (2 × 15 mL), and then dried in vacuum (0.01 mm Hg) at 40 °C. The increase in weight was 0.307 g (1.61 mmol CuI), which gave the polymer a loading of 1.23 mmol CuI g^{-1}.

The polymer supported catalyst (Amberlyst A-21·CuI, 0.076 equiv) was added to a solution of azides (1 equiv) and alkynes (1.2 equiv) in dichloromethane. The suspension was orbitally stirred for 12 h at room temperature. The solution was filtered and the reactors and polymers were washed with CH_2Cl_2. The extracts were evaporated under reduced pressure and the products dried in vacuum.

5.1.2.1. Ethyl 2-(4-pentyl-1*H*-1,2,3-triazol-1-yl)acetate (1b).

Following the general procedure A, compound **1b** (481 mg, 79%) was obtained as a green oil from ethyl azidoacetate **3** (349 mg, 2.7 mmol) and hept-1-yne (354 μL, 3.24 mmol). Eluent: pentane/ dichloromethane (8/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (m, 3H); 1.30 (m, 7H); 1.69 (m, 2H); 2.74 (t, J = 7.7 Hz, 2H); 4.26 (q, J = 7.2 Hz, 2H); 5.11 (s, 2H); 7.41 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0; 14.0; 22.4; 25.6; 29.0; 31.4; 50.8; 62.3; 122.0; 148.9; 166.6. HRMS ESI+ calculated for $C_{11}H_{19}N_3O_2$ H$^+$: 226.1550; found: MH$^+$, 226.1547.

5.1.2.2. Ethyl 2-(4-dodecyl-1*H*-1,2,3-triazol-1-yl)acetate (1c).

Following the general procedure A, compound **1c** (753 mg, 89%) was obtained as a white solid from ethyl azidoacetate **3** (338 mg, 2.6 mmol) and tetradec-1-yne (966 μL, 3.9 mmol). Mp = 75–76 °C, Eluent: pentane/dichloromethane (8/2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.7 Hz, 3H); 1.30 (m, 21H); 1.68 (m, 2H); 2.73 (t, J = 7.8 Hz, 2H); 4.26 (q, J = 7.1 Hz, 2H); 5.12 (s, 2H); 7.41 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1; 14.2; 22.7; 25.7; 29.3; 29.4 (2C); 29.4; 29.6; 29.7 (2C); 29.7; 32.0; 50.8; 62.3; 122.0; 149.0; 166.6. HRMS ESI+ calculated for $C_{18}H_{33}N_3O_2$ H$^+$: 324.2646; found: MH$^+$, 324.2647.

5.1.2.3. 2-(1-Pentyl-1*H*-1,2,3-triazol-4-yl)ethanol (6a).

Following the general procedure A, compound **6a** (670 mg, 73%) was obtained as a green oil from 1-azidopentane **5a** (500 mg, 4.42 mmol) and 3-butyn-1-ol (402 μL, 5.3 mmol). Eluent: methanol/ethyl acetate (5/95). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.9 Hz, 3H); 1.32 (m, 4H); 1.89 (quint, J = 7.5 Hz, 2H); 2.31 (M, 2H); 2.93 (t, J = 5.9 Hz, 2H); 3.99 (M, 1H, OH); 4.31 (t, J = 7.2 Hz, 2H); 7.43 (m, 1H). HRMS ESI+ calculated for $C_9H_{17}N_3O$ Na$^+$: 206.1264; found: MNa$^+$, 206.1259.

5.1.2.4. 2-(1-Dodecyl-1*H*-1,2,3-triazol-4-yl)ethanol (6b).

Following the general procedure A, compound **6b** (920 mg, 83%) was obtained as a white solid from 1-azidododecane **5b** (853 mg, 4.03 mmol) and 3-butyn-1-ol (366 μL, 4.84 mmol). Mp = 67–68 °C, eluent: methanol/ethyl acetate (1/9). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.7 Hz, 3H); 1.28 (m, 18H); 1.61 (s, 1H); 1.89 (quint, J = 6.8 Hz, 2H); 2.61 (t, J = 5.9 Hz, 1H); 2.95 (t, J = 5.6 Hz, 2H); 3.96 (m, 2H); 4.32 (t, J = 7.2 Hz, 2H); 7.36 (s, 1H). HRMS ESI+ calculated for $C_{16}H_{32}N_3O$ H$^+$: 282.2540; found: MH$^+$, 282.2539.

5.1.3. 2-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)acetamide (1f)

Triazole **1b** (200 mg, 0.89 mmol) was added to a saturated solution of NH_3 in MeOH (10 mL) at −30 °C. The mixture was stirred for 16 h at room temperature. After evaporation, the residue was purified by recrystallisation (MeOH/pentane) to produce pure **1f** (166 mg, 95%) as a white solid. Mp = 164–165 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, J = 6.6 Hz, 3H); 1.29 (m, 4H); 1.59 (m, 2H); 2.60 (t, J = 7.5 Hz, 2H); 4.98 (s, 2H); 7.34 (s, 1H, NH); 7.67 (s, 1H, NH); 7.76 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9; 21.9; 24.9; 28.7; 30.8; 51.3; 123.3; 146.6; 167.5. HRMS ESI+ calculated for $C_9H_{16}N_4O$ Na$^+$: 219.1216; found: MNa$^+$, 219.1217.

5.1.4. *N*-Hydroxy-2-(4-pentyl-1*H*-1,2,3-triazol-1-yl)acetamide (1g)

Aqueous hydroxylamine (1.1 mL, 17.7 mmol) and KCN (4 mg, 0.062 mmol) were added to a solution of triazole **1b** (400 mg, 1.77 mmol) in a THF/methanol mixture (3 mL, 1:1). The mixture was stirred at room temperature for 24 h. The reaction was diluted with EtOAc (50 mL) and washed with saturated $NaHCO_3$ (2 × 30 mL) and brine (30 mL). The organic layer was then dried and concentrated in vacuum. The product **1g** (282 mg, 75%) was

obtained as a white solid after purification by silica gel chromatography, using ethyl acetate/methanol (95:5) as the eluent. Mp = 159–160 °C, ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.87 (t, J = 6.6 Hz, 3H); 1.30 (m, 4H); 1.59 (m, 2H); 2.60 (s, J = 7.5 Hz, 2H); 4.90 (s, 2H); 7.79 (s, 1H); 9.10 (s, 1H); 11.00 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 13.9; 21.8; 24.9; 28.6; 30.8; 49.2; 123.1; 146.7; 171.5. HRMS ESI+ calculated for $C_9H_{16}N_4O_2$ Na^+: 235.1165; found: MNa^+, 235.1165.

5.1.5. General procedure B for saponification

A solution of potassium hydroxide (2 equiv) was added to a solution of the corresponding ester (**1b**, **1c**) (1 equiv) in ethanol. The reaction mixture was stirred at room temperature for 4 h. The solvent was removed under pressure, the residue was dissolved in water and the pH of this solution was adjusted to 2, by adding 1 N hydrochloric acid, and extracted with ethyl acetate. The combined ethyl acetate extract was washed with saturated NaCl solution, dried and concentrated.

5.1.5.1. 2-(4-Pentyl-1*H*-1,2,3-triazol-1-yl)acetic acid (1d). Following the general procedure B, compound **1d** (300 mg, 96%) was obtained as a white solid from triazole **1b** (357 mg, 1.58 mmol). Mp = 124–125 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.86 (t, J = 6.9 Hz, 3H); 1.30 (m, 4H); 1.59 (q, J = 7.3 Hz, 2H); 2.61 (t, J = 7.5 Hz, 2H); 5.19 (s, 2H); 7.81 (s, 1H); 13.31 (br, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9; 21.9; 24.9; 28.7; 30.9; 50.4; 123.2; 147.1; 168.8. HRMS ESI+ calculated for $C_9H_{15}N_3O_2$ H^+: 198.1237; found: MH^+, 198.1232.

5.1.5.2. 2-(4-Dodecyl-1*H*-1,2,3-triazol-1-yl)acetic acid (1e). Following the general procedure B, compound **1e** (300 mg, 96%) was obtained as a white solid from triazole **1c** (342 mg, 1.02 mmol). Mp = 135–136 °C. ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.85 (t, J = 6.7 Hz, 3H); 1.24 (M, 18H); 1.58 (m, 2H); 2.60 (t, J = 7.5 Hz, 2H); 5.19 (s, 2H); 7.80 (s, 1H); 13.32 (br, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.0; 22.2; 25.1; 28.8; 28.9; 29.0; 29.1 (2C); 29.2; 29.3 (2C); 31.5; 49.2; 123.2; 147.1; 168.9. HRMS ESI+ calculated for $C_{16}H_{29}N_3O_2$ H^+: 296.2333; found: MH^+, 296.2331.

5.1.6. General procedure C for Jones oxidation (2c, 2d)

A solution of the corresponding alcohol **6** (1 equiv), in acetone, was added dropwise to a stirred solution of chromium trioxide (2 equiv) in H_2SO_4 (5 M, 3 mL/1 mmol of alcohol), at 0 °C. The reaction mixture was allowed to warm to room temperature and stirring was continued for 12 h. The mixture was diluted with water and extracted with ethyl acetate. The organic phase was washed with brine, dried over Na_2SO_4, filtered and then concentrated under reduced pressure.

5.1.6.1. 2-(1-Pentyl-1*H*-1,2,3-triazol-4-yl)acetic acid (2c). Following the general procedure C, compound **2c** (800 mg, 68%) was obtained as a white solid from triazole **6a** (1.1 g, 6 mmol) and chromium trioxide (1.19 g, 10 mmol). Mp = 75–76 °C, eluent: ethyl acetate/methanol (95/5). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (m, 3H); 1.33 (m, 4H); 1.19 (m, 2H); 4.35 (t, J = 7.6 Hz, 2H); 5.32 (s, 2H); 7.63 (s, 1H); 9.46 (br, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 14.1; 26.2; 30.8; 31.4; 32.5; 51.6; 123.2; 139.2; 170.9. HRMS ESI+ calculated for $C_9H_{15}N_3O_2$ H^+: 198,1237; found: MH^+, 198,1239.

5.1.6.2. 2-(1-Dodecyl-1*H*-1,2,3-triazol-4-yl)acetic acid (2d). Following the general procedure C, compound **2d** (635 mg, 69%) was obtained as a white solid from triazole **6b** (875 mg, 3.1 mmol) and chromium trioxide (622 mg, 6.2 mmol). Mp = 94–95 °C, eluent: ethyl acetate/pentane/CH_3COOH (60/40/0.2). ^{1}H NMR (300 MHz, acetone-d_6): δ 0.87 (t, J = 6.7 Hz, 3H); 1.30 (m, 18H); 1.91 (p, J = 7.2 Hz, 2H); 3.76 (s, 2H); 4.39 (t, J = 7.2 Hz, 2H); 7.87 (s, 1H); 9.49 (br, 1H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 13.9; 22.1; 25.9; 28.5; 28.8; 28.9; 29.0; 29.1 (2C); 29.8; 31.3; 31.5; 49.2; 123.3; 140.6; 171.5. HRMS ESI+ calculated for $C_{16}H_{29}N_3O_2$ H^+: 296.2333; found: MH^+, 296.2340.

5.1.7. General procedure D for the esterification of triazoles with a carboxylic acid function (2a, 2b)

A few drops of sulfuric acid (18 M) were added to a solution of triazole (**2c** and **2d**) in ethanol (5 mL/1 mmol of triazole). The reaction mixture was stirred at 80 °C for 4 h. The solvent was removed under pressure and the residue was dissolved in water, then extracted with ether. The organic solution was washed with a saturated solution of $NaHCO_3$, dried and evaporated under reduced pressure.

5.1.7.1. Ethyl 2-(1-pentyl-1*H*-1,2,3-triazol-4-yl)acetate (2a). Following the general procedure D, compound **2a** (441 mg, 40%) was obtained as a green oil from triazole-acid **2c** (1 g, 5.02 mmol). Eluent: ethyl acetate/pentane (3/7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, J = 6.6 Hz, 3H); 1.24–1.38 (t, J = 7.2 Hz, 3H)-(m, 4H); 1.9 (quint, J = 7.2 Hz, 2H); 3.82 (s, 2H); 4.18 (q, J = 7.2 Hz, 2H); 4.33 (t, J = 7.2 Hz, 2H); 7.58 (s, 1H). RMN ^{13}C (75 MHz, $CDCl_3$): δ 13.8; 14.1; 22.1; 28.1; 29.9; 31.9; 50.3; 61.1; 122.3; 140.5; 170.3. HRMS ESI+ calculated for $C_{11}H_{19}N_3O_2$ H^+: 226.155; found: MH^+, 226.1547.

5.1.7.2. Ethyl 2-(1-dodecyl-1*H*-1,2,3-triazol-4-yl)acetate (2b). Following the general procedure D, compound **2b** (197 mg, 59%) was obtained as a colorless oil from triazole-acid **2d** (308 mg, 1.04 mmol). Eluent: ethyl acetate/pentane (3/7). ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (t, J = 6.7 Hz, 3H); 1.27 (m, 21H); 1.89 (quint, J = 7.0 Hz, 2H); 3.81 (s, 2H); 4.18 (q, J = 7.2 Hz, 2H); 4.32 (t, J = 7.2 Hz, 2H); 7.58 (s, 1H). RMN ^{13}C (75 MHz, $CDCl_3$): δ 14.1; 14.2; 22.7; 25.7; 29.3; 29.4 (2C); 29.4; 29.6; 29.7 (2C); 29.7; 32.0; 50.8; 62.3; 122.0; 149.2; 166.6. HRMS ESI+ calculated for $C_{18}H_{33}N_3O_2$ H^+: 324.2646; found: MH^+, 324.2643.

5.1.8. 3-(2*H*-Tetrazol-5-yl)dihydrofuran-2(3*H*)-one (11)

A mixture of cyanolactone **10** (800 mg, 7.2 mmol), sodium azide (586 mg, 9 mmol), and ammonium chloride (485 mg, 9 mmol) in dimethylformamide (7 mL) was maintained at 100 °C for 7 hrs. After cooling to room temperature, the solution was concentrated and was co-evaporated with water. Finally, it was dissolved in water (10 mL) and the pH of this solution was adjusted to 1.8, by adding hydrochloric acid, and extracted with ethyl acetate (3 × 20 mL). The combined ethyl acetate extract was washed with saturated NaCl solution, dried over Na_2SO_4 and concentrated. The crude product was purified by flash chromatography on silica gel, with ethyl acetate-methanol (9/1) as the eluent, producing compound **11** (252 mg, 23%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.90 (m, 2H); 4.28 (t, J = 9.4 Hz, 1H); 4.46 (m, 1H); 4.63 (m, 1H); 5.87 (br, 1H). HRMS ESI+ calculated for $C_5H_6N_4O_2$ H^+: 155.0569; found: MH^+, 155.0571.

5.1.9. General procedure E for the alkylation of tetrazoles 11, 12

Triethylamine (1.5 equiv) was added to different solutions of alkyl bromide (1.5 equiv) with tetrazole **11** or **12** (l equiv) in acetonitrile. The reaction mixture was refluxed for 16 h and the solvent was then removed under vacuum after filtration. Flash chromatography on the crude product allowed us to purify and separate the two regioisomers.

5.1.9.1. 3-(2-Hexyl-2*H*-tetrazol-5-yl)dihydrofuran-2(3*H*)-one 7a and 3-(1-hexyl-1*H*-tetrazol-5-yl)dihydrofuran-2(3*H*)-one 8a. Following the general procedure E, the reaction between tetrazole **11** (140 mg, 0.91 mmol) and bromohexane (192 μL, 1.37 mmol) gave the compounds **7a** (99 mg) and **8a** (42 mg) as yellow oils, in a 65% total yield. Eluent: ethyl acetate–dichloromethane (2/8). ^{1}H NMR of **7a** (300 MHz, $CDCl_3$): δ 0.81 (m, 3H), 1.26 (m, 6H), 1.19 (m, 2H), 2.75 (m, 2H), 4.18 (t, J = 9.33 Hz, 1H), 4.38 (m, 1H), 4.51 (m, 3H). ^{13}C NMR of **7a** (75 MHz, $CDCl_3$): δ 13.9, 22.3, 25.9, 28.7, 29.1, 30.9, 37.7, 53.4, 67.2, 162.2, 174.1. ^{1}H NMR of **8a** (300 MHz, $CDCl_3$): δ 0.86 (m, 3H), 1.32 (m, 6H), 1.96 (m, 2H), 2.78 (m, 1H), 3.17 (m, 1H), 4.06 (t, J = 8.8 Hz, 1H), 4.4–4.5 (m, 3H), 4.7 (m, 1H). ^{13}C NMR of **8a** (75 MHz, $CDCl_3$): δ 14, 22.5, 26.3, 27.4, 29.6, 31.2, 36.1, 48.1, 68, 152.4, 172.3. HRMS CI + calculated for $C_{11}H_{18}N_4O_2$ H^+: 239.1508; found: MH^+, 239.1507.

5.1.9.2. Ethyl 2-(2-hexyl-2*H*-tetrazol-5-yl)acetate (7b) and ethyl 2-(1-hexyl-1*H*-tetrazol-5-yl)acetate (8b). Following the general procedure E, the reaction between tetrazole **12** (1 g, 6.49 mmol) and bromohexane (1.2 mL, 8.5 mmol) gave the compounds **7b** (811 mg) and **8b** (525 mg) as colorless oils, in an 86% total yield. Eluent: pentane/ether (6:4). ^{1}H NMR of **7b** (300 MHz, DMSO-d_6): δ 0.83 (m, 3H), 1.17 (t, J = 7.1 Hz, 3H), 1.24 (m, 6H), 1.18 (m, 2H), 4.04 (s, 2H), 4.10 (q, J = 7.1 Hz, 2H), 4.65 (t, J = 6.9 Hz, 2H). ^{13}C NMR of **7b** (75 MHz, $CDCl_3$): δ 14.0, 14.1, 22.4, 26.0, 29.3, 31.1, 32.0, 53.3, 61.6, 160.1, 168.4. ^{1}H NMR of **8b** (300 MHz, DMSO-d_6): δ 0.85 (m, 3H), 1.19 (t, J = 7.1 Hz, 3H), 1.27 (m, 6H), 1.79 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 4.26 (s, 2H), 4.34 (t, J = 7.2 Hz, 2H). ^{13}C NMR of **8b** (75 MHz, $CDCl_3$): δ 14.0, 14.1, 22.5, 26.2, 29.4, 29.9, 31.2, 47.9, 62.4, 148.9, 166.6. HRMS ESI+ calculated for $C_{11}H_{20}N_4O_2Na^+$: 263.1478; found: MNa^+, 263.1479.

5.1.9.3. Ethyl 2-(2-dodecyl-2*H*-tetrazol-5-yl)acetate (7c) and ethyl 2-(1-dodecyl-1*H*-tetrazol-5-yl)acetate (8c). Following the general procedure E, the reaction between tetrazole **12** (997 mg, 6.43 mmol) and bromododecane (2 mL, 8 mmol) gave the compounds **7c** (1.12 g), as a colorless oil, and **8c** (780 mg) as a white solid, in a 92% total yield. Eluent: pentane/ether (9:1). ^{1}H NMR of **7c** (300 MHz, DMSO-d_6): δ 0.85 (m, 3H), 1.17 (t, J = 7.1 Hz, 3H), 1.22 (m, 18H), 1.88 (m, 2H), 4.03 (s, 2H), 4.10 (q, J = 7.1 Hz, 2H), 4.64 (t, J = 6.9 Hz, 2H). ^{13}C NMR of **7c** (75 MHz, $CDCl_3$): δ 14.2, 14.2, 22.8, 26.4, 28.9, 29.3, 29.4 (2C), 29.6, 29.7 (2C), 31.9, 32.0, 53.3, 61.6, 160.1, 168.4. Mp of **7c** = 77 °C. ^{1}H NMR of **8c** (300 MHz, DMSO-d_6): δ 0.85 (m, 3H), 1.11–1.40 (m, 21H), 1.79 (m, 2H), 4.12 (q, J = 7.15 Hz, 2H), 4.25 (s, 2H),, 4.33 (t, J = 7.2 Hz, 2H). ^{13}C NMR of **8c** (75 MHz, $CDCl_3$): δ 14.1, 14.1, 22.7, 26.5, 29.0, 29.3, 29.4 (2C), 29.5, 29.6 (2C), 29.8, 31.9, 47.8, 62.3, 148.8, 166.6. HRMS ESI+ calculated for $C_{17}H_{32}N_4O_2Na^+$: 347.2417; found: MNa^+, 347.2427.

5.1.10. 2-(2-Hexyl-2*H*-tetrazol-5-yl)acetic acid (7d)

Following the general procedure B, compound **7d** (348 mg, 98%) was obtained as a white solid from tetrazole **7b** (404 mg, 1.69 mmol). Mp = 67 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.80 (m, 3H), 1.25 (m, 6H), 1.93 (m, 2H), 3.99 (s, 2H), 4.53 (t, J = 7.1 Hz, 2H), 10.92 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.8, 22.3, 25.9, 29.1, 30.9, 31.4, 53.3, 159.5, 172.9. HRMS ESI+ calculated for $C_9H_{16}N_4O_2Na^+$: 235.1165; found: MNa^+, 235.1168.

5.1.11. 2-(2-Dodecyl-2*H*-tetrazol-5-yl)acetic acid (7e)

Following the general procedure B, compound **7e** (392 mg, 87%) was obtained as a white solid from tetrazole **7c** (495 mg, 1.53 mmol). Mp = 72 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.87 (m, 3H), 1.24 (m, 18H), 1.20 (m, 2H), 4.03 (s, 2H), 4.59 (t, J = 7.1 Hz, 2H); 11.1 (br, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.2, 22.8, 26.4, 28.9, 29.3, 29.4 (2C), 29.6, 29.7 (2C), 31.6, 32.0, 53.5, 159.6, 173.6. HRMS ESI+ calculated for $C_{15}H_{28}N_4O_2Na^+$: 319.2104; found: MNa^+, 319.2103.

5.1.12. General procedure F for the synthesis of tetrazole (8d, 8e and 9)

Triflic anhydride (4 equiv) was added to a stirred suspension of amide (1 equiv) and sodium azide (3 equiv) in acetonitrile, under a nitrogen atmosphere. The mixture rapidly resulted in a homogenous solution. After 20 h, the mixture was poured into a 5% $NaHCO_3$ solution and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was then purified, by silica gel chromatography, using AcOEt/ether (1:1) to yield the desired tetrazole.

5.1.12.1. 3-(1-Butyl-1*H*-tetrazol-5-yl)dihydrofuran-2(3*H*)-one (8d). Following the general procedure F, compound **8d** (118 mg, 35%) was obtained from amide **14a** (prepared as in Ref. 22) (300 mg, 1.61 mmol) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, J = 7.4, 3H), 1.36 (m, 2H), 1.93 (m, 2H), 2.77 (m, 1H), 3.13 (m, 1H), 4.13 (t, J = 9 Hz, 1H), 4.46 (m, 3H), 4.66 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.5, 19.8, 27.4, 31.5, 36.0, 47.7, 68.0, 150.8, 172.5 HRMS CI + calculated for $C_9H_{14}N_4O_2$ H^+: 211.1195; found: MH^+, 211.1197.

5.1.12.2. 3-(1-Pentyl-1*H*-tetrazol-5-yl)dihydrofuran-2(3*H*)-one (8e). Following the general procedure F, compound **8e** (151 mg, 45%) was obtained from amide **14b** (prepared as in Ref. 22) (300 mg, 1.5 mmol) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (t, J = 6.8 Hz, 3H), 1.34 (m, 4H), 1.99 (m, 2H), 2.78 (m, 1H), 3.14 (m, 1H), 4.10 (t, J = 8.9 Hz, 1H), 4.47 (m, 3H), 4.66 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.9, 22.4, 27.4, 28.6, 29.3, 36.0, 48.0, 68.0, 150.7, 172.4. HRMS CI + calculated for $C_{10}H_{16}N_4O_2$ H^+: 225.1352; found: MH^+, 225.1355.

5.1.12.3. 3-(5-Butyl-1*H*-tetrazol-1-yl)dihydrofuran-2(3*H*)-one (9a). Following the general procedure F, compound **9a** (193 mg, 68%) was obtained from amide **16a** (prepared as in Ref. 52) (250 mg, 1.35 mmol) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (t, J = 7.3 Hz, 3H), 1.45 (m, 2H), 1.86 (m, 2H), 2.90 (m, 3H), 3.18 (m, 1H), 4.55 (m, 1H), 4.76 (m, 1H), 5.18 (t, J = 9.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.6, 22.2, 22.8, 28.7, 29.1, 54.9, 66.5, 156.5, 170.5. HRMS CI + calculated for $C_9H_{14}N_4O_2$ H^+: 211.1195; found: MH^+, 211.1194.

5.1.12.4. 3-(5-Pentyl-1*H*-tetrazol-1-yl)dihydrofuran-2(3*H*)-one (9b). Following the general procedure F, compound **9b** (179 mg, 80%) was obtained from amide **16b** (prepared as in Ref. 52) (200 mg, 1 mmol) as an orange solid. Mp = 63 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, J = 7.2 Hz, 3H), 1.37 (m, 4H), 1.86 (m, 2H), 2.90 (m, 3H), 3.16 (m, 1H), 4.52 (m, 1H), 4.76 (m, 1H), 5.22 (t, J = 9.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 22.3, 23.3, 26.9, 28.6, 31.3, 54.9, 66.6, 156.3, 170.1. HRMS CI +: calculated for $C_{10}H_{16}N_4O_2$ H^+: 225.1352; found: MH^+, 225.1356.

5.1.12.5. 3-(5-Hexyl-1*H*-tetrazol-1-yl)dihydrofuran-2(3*H*)-one (9c). Following the general procedure F, compound **9c** (258 mg, 53%) was obtained from amide **16c** (prepared as in Ref. 52) (400 mg, 1.88 mmol) as an orange solid. Mp = 76 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.89 (t, 3H), 1.38 (m, 6H), 1.87 (m, 2H), 2.92 (m, 3H), 3.16 (m, 1H), 4.54 (m, 1H), 4.78 (m, 1H), 5.17 (t, J = 9.1 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.0, 22.4, 23.1, 27.0,

28.7, 28.75, 31.3, 54.9, 66.5, 156.5, 170.4. HRMS CI + calculated for $C_{11}H_{18}N_4O_2$ H^+: 225.1352; found: MH^+, 225.1357.

5.1.12.6. 3-(5-Heptyl-1*H*-tetrazol-1-yl)dihydrofuran-2(3*H*)-one 9d. Following the general procedure F, compound **9d** (187 mg, 56%) was obtained from amide **16d** (prepared as in Ref. 52) (300 mg, 1.32 mmol) as an orange solid. Mp = 58–60 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (t, J = 7.2 Hz, 3H), 1.35 (m, 8H), 1.85 (m, 2H), 2.91 (m, 3H), 3.13 (m, 1H), 4.53 (m, 1H), 4.74 (m, 1H), 5.24 (t, J = 9.2 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.1, 22.7, 23.3, 27.2, 28.6, 28.9, 29.2, 31.7, 54.9, 66.6, 156.3, 170.1. HRMS CI + calculated for $C_{12}H_{20}N_4O_2$ H^+: 253.1664; found: MH^+, 253.1664.

5.2. Biological evaluation

The recombinant *Escherichia coli* strain NM522, containing the sensor plasmid pSB401, was used. In pSB401, the *LuxR* and the *LuxI* promoter from *V. fischeri* have been coupled to the entire *Lux* structural operon (*LuxCDABE*) from *Photorhabdus luminescens*. Bacterial cultures were grown to exponential phase in Luria broth, in the presence of tetracycline (20 μg mL^{-1}), at 30 °C.

5.2.1. Agonistic activity

The inducing activity of the various acyl-HSL analogues was monitored using the *E. coli* biosensor strain. Acyl-HSL activity was measured in a microtitre plate format, using a Luminoskan luminometer to quantify the bioluminescence. Concentrations of analogues, ranging from 0.1 to 200 μM, were made up to 0.1 mL volumes with growth medium. The amount of light produced by the bacteria was measured after 4–5 h and it was expressed in relative light units (RLU).

5.2.2. Antagonistic activity

The influence of acyl-HSL analogues on the induction of bioluminescence by 3-oxo-C_6-HSL was determined at concentrations ranging from 1 to 200 μM as described above, except that 3-oxo-C_6-HSL was included at a final concentration of 200 nM together with the analogue. 3-Oxo-C_6-HSL, at 200 nM, induced a 124 ± 8 RLU after 4–5 h, when the ratio of induced to background light was at its maximum.

5.3. Molecular modeling

All calculations were performed, using ArgusLab[53] as the software, on a Dell OPTIPLEX GX620 PC. Docking experiments were carried out with the docking module of ArgusLab. The protein model of LuxR,[37] based on the X-ray structure of TraR,[38] was created using the SWISS-MODEL[54] with ClustalW.[55]

Docking studies were performed using the ligand binding site of the LuxR model after the docking of 3-oxo-C_6-HSL.[16] Docking experiments were carried out with the following parameters: Docking box centred on 3-oxo-C_6-HSL: $X = Y = Z$ = 15 Å, ligand option: flexible; calculation type: Dock; Docking engine: GADock (Genetic Algorithm).[56] Genetic algorithm dock engine settings: default advanced parameters; hydrogen bonds were assigned within a distance of 3 Å.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.06.007.

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