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N-Acyl-3-amino-5*H*-furanone derivatives as new inhibitors of LuxR-dependent quorum sensing: Synthesis, biological evaluation and binding mode study

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ABSTRACT

New *N*-acyl homoserine lactone analogues, *N*-acyl-3-amino-5*H*-furanone derivatives and some 4-halogeno counterparts, were synthesised and tested for their ability to modulate LuxR-dependent bacterial quorum sensing. Both types of analogues proved to be inhibitors, the halogenated compounds being significantly more active. Molecular modelling suggested that the conjugated enamide group induces two preferential conformations leading to specific binding modes. In addition, the presence of the halogen atom could enhance the fitting of the lactone ring through specific interactions with strictly conserved or conservatively replaceable residues in the LuxR protein family, namely Asp79, Trp94 and Ile81.

Bacteria are able to communicate through small diffusible molecules called autoinducers. The concentration of these chemical messengers in a specific environment reflects the bacterial density and, when a critical concentration is reached, bacteria change their biological activities. This cell to cell communication system, named 'quorum sensing', allows bacteria to coordinate their behaviour for a specific activity, such as biofilm formation or virulence,[1–4] rendering it an attractive target for the conception of new antibacterial agents.[5–12] In Gram-negative bacteria, the quorum sensing is orchestrated by *N*-acyl-L-homoserine lactones (AHLs) acting as autoinducers, AHL synthase, an AHL-dependent transcriptional regulator and specific genes encoding different phenotypes.[13] Previously we have reported the antagonist activity and molecular modelling studies of AHL analogues with structural modifications on the acyl moiety, either with a terminal aromatic group[14] or resulting from the replacement of the amide function by sulfonamide or urea functions.[15–18] We present here the synthesis, the biological evaluation and the binding mode study of new AHL analogues, namely 3-amino-5*H*-furanone derivatives **5**–**7** and some 4-halogenated counterparts **8**–**10** (Scheme 1). Compounds **5**–**7** were designed to evaluate the modulation of the antagonist activity induced by conformational and H-bonding modifications resulting from the replacement of the amide by an enamide function. Because of their analogy with natural brominated furanones, known to be quorum sensing inhibitors which display antimicrobial activities,[19–21] the halogenated analogues **8**–**10** were also prepared.

The 3-amino-5*H*-furanone derivatives **5**–**10** were obtained from known[22] 3-amino-5*H*-furan-2-one (**2**) prepared by reacting sodium azide with α-bromo-γ-butyrolactone (**1**) in dimethylformamide (Scheme 1).[23] This compound **2** was further halogenated with *N*-bromo or *N*-chloro-succinimide to afford known[24] 3-amino-4-bromo-5*H*-furan-2-one (**3**) and 3-amino-4-chloro-5*H*-furan-2-one (**4**), respectively. The furanones **2**–**4** were then acylated with acyl chlorides under standard conditions to furnish the compounds **5** and **7**–**10**. Compound **6** was prepared by acylation of Meldrum's acid with butyryl chloride and then by further reaction with **2**. Pure **6** was obtained in low yield because crude product was a mixture of **6** and of *N*-butanoyl-3-amino-5*H*-furan-2-one, arising from reaction between **2** and butyryl chloride, two compounds that proved to be difficult to separate by column chromatography.

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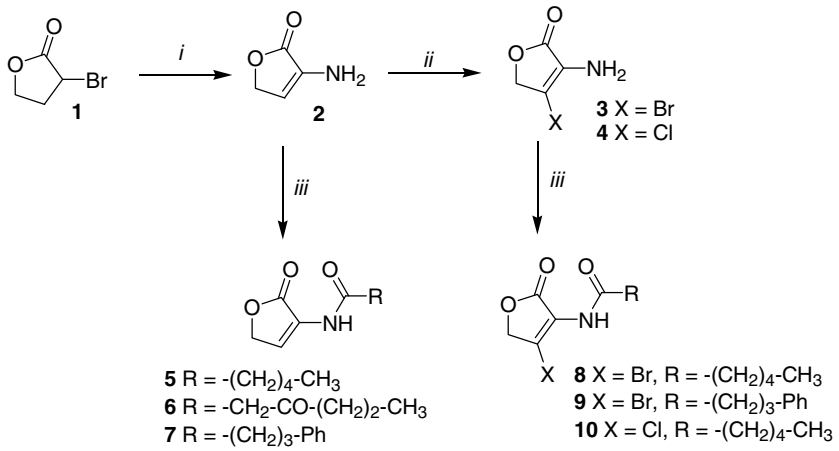

Scheme 1. Synthesis of 3-amino-5*H*-furanone derivatives **5**–**10**. Reagents and conditions: (i) NaN_3, Et_3N, DMF, 80 °C, 5 h, 60%; (ii) *N*-bromosuccinimide or *N*-chlorosuccinimide, CH_2Cl_2, 50 °C, 2 h, 82% (**3**), 66% (**4**); (iii) RCOCl, diisopropylethylamine or triethylamine, THF, 0 °C then rt, 5–18 h, 95% (**5**), 95% (**7**), 85% (**8**), 70% (**9**), 69% (**10**); Meldrum's acid, butyryl chloride, pyridine, CH_2Cl_2, 0 °C then rt, 2 h, **2**, dichloroethane, 80 °C, 24 h, 12% (**6**).

Compounds **5–10** were evaluated for their ability to inhibit the induction of luminescence obtained with 3-oxo-hexanoylhomoserine lactone (OHHL), the natural ligand of LuxR in the *V. fischeri* QS system (Table 1).[16] The structurally closest analogues **5** and **6** of OHHL displayed an IC_{50} of 85 and 55 μM, respectively, whereas compound **7**, bearing a phenyl group on the terminal alkyl chain, was found to be inactive, in contrast with previous results obtained with phenyl-substituted OHHL analogues.[6,14,25] Interestingly, the brominated analogues in position 4, **8** and **9**, exerted a stronger inhibitory activity compared with their hydrogenated counterparts, which are either inactive (**7**) or about 10 times less active (**5**). In order to confirm the effect of the halogen atom on antagonist activity, we prepared the chlorinated enamide **10**. This compound proved to be as active as **8** with about the same IC_{50} value of 6.9 μM.

A molecular modelling study was performed to understand the structure–activity relationship observed. Amino acid sequence alignments revealed a high homology between the two proteins, LuxR and TraR, especially for the strictly conserved residues located in the ligand binding site (Tyr62, Trp66, Tyr70, Asp72, Trp94, Ala113 and Gly121 for LuxR). A LuxR homology model was developed[17] based on a TraR structure (PDB code: 1L3L).[26] To perform docking experiments with 3-amino-5*H*-furanone derivatives, we defined the ligand binding site as a docking box with the natural ligand 3-oxohexanoylhomoserine lactone (OHHL). The docking result (Fig. 1A) showed a very similar binding mode of OHHL as compared to the binding mode of OOHL co-crystallised in the active site of TraR, with the same hydrogen bond network and only slight differences in terms of inter-atomic distances (Fig. 1B).[26]

Table 1
Inhibition of bioluminescence obtained with compounds **5**–**10**

Compound	R	X	IC_{50}[a,b] (μM)
5	$-(CH_2)_4-CH_3$	H	85 (±2)
6	$-CH_2-CO-(CH_2)_2-CH_3$	H	55 (±2)
7	$-(CH_2)_3$-Ph	H	na[c]
8	$-(CH_2)_4-CH_3$	Br	6.5 (±0.1)
9	$-(CH_2)_3$-Ph	Br	84 (±1)
10	$-(CH_2)_4-CH_3$	Cl	6.9 (±0.4)

[a] Concentration (μM) required to reduce to 50% intensity (IC_{50}) the bioluminescence induced by 200 nM of 3-oxo-hexanoylhomoserine lactone.
[b] Values are the means of three experiments; standard deviation is given in brackets.
[c] Not active at concentrations up to 100 μM.

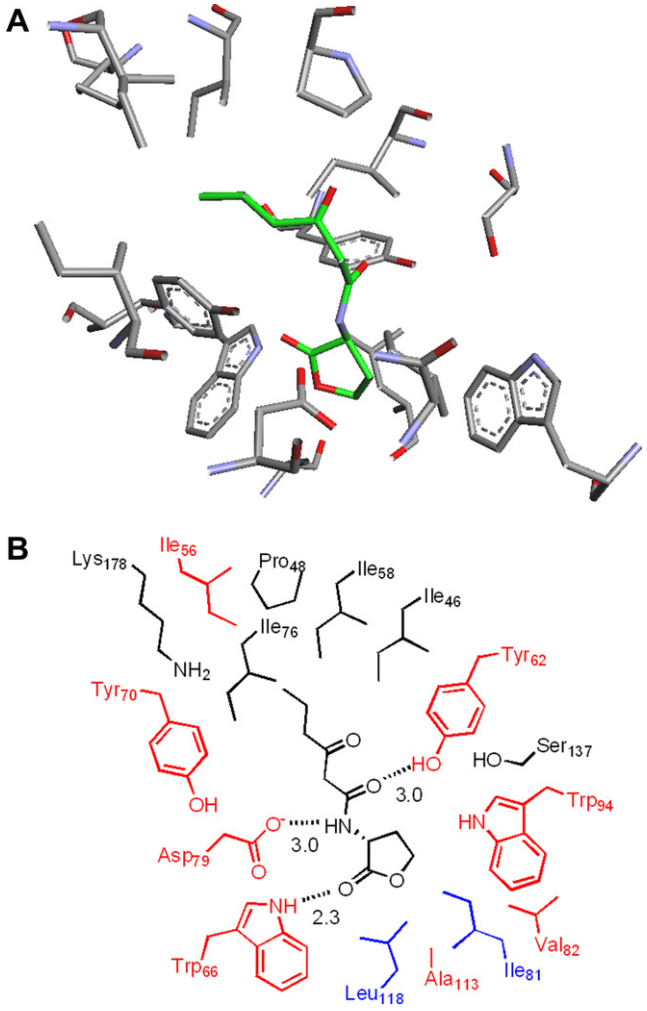

Figure 1. (A) Docking result of OHHL in the ligand binding site of LuxR; (B) schematic overview displaying the hydrogen bond network with distances; strictly conserved residues and conservatively replaceable residues are coloured, respectively, in red and in blue.

Docking experiments were then conducted on compounds **5** and **8**. The binding modes **I** and **II** were obtained as a result of the flexible docking experiments with these compounds (Fig. 2).