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AHL-dependent quorum sensing inhibition: Synthesis and biological evaluation of α-(*N*-alkyl-carboxamide)-γ-butyrolactones and α-(*N*-alkyl-sulfonamide)-γ-butyrolactones

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ABSTRACT

New *N*-acylhomoserine lactone (AHL) analogues in which the amide function is replaced by a reverse-amide one have been studied as AHL QS modulators. The series of compounds consists of α-(*N*-alkyl-carboxamide)-γ-butyrolactones, α-(*N*-alkyl-sulfonamide)-γ-butyrolactones, and 2-(*N*-alkyl-carboxamide)-cyclopentanones and cyclopentanols. Most active compounds exhibited antagonist activities against LuxR reaching the 30 μM range.

The communication system referred to as quorum sensing (QS) is used by bacteria for adapting themselves to their environment. QS is based on the regulation of the expression of genes encoding for phenotype expression via small molecules called autoinducers which interact with their cognate receptor proteins.[1–6] Important phenotypes such as bioluminescence, virulence expression, biofilm formation are regulated by QS. Therefore modulation of QS can have significant impact on human health and environment. QS modulation can be achieved by several approaches, one of them being the design of compounds able to act as agonist or antagonists of autoinducers.[7–13] New QS modulators can bring insights into bacterial signaling from the fundamental and applied points of view, including the possible development of new antibacterial strategies.

N-Acyl-homoserine lactones (AHLs) are an important family of QS autoinducers, and the main one in Gram negative bacteria.[14–17] AHLs are rather simple molecules for which one can delimit three areas ideal for chemical modification: (1) the acyl chain including the characteristic carbonyl or hydroxyl substitution or lack thereof at the C-3 position, (2) the central amide connective function, and (3) the lactone moiety (Fig. 1). In the context of AHL-dependent QS modulation, the strategies used in the design of active compounds have included the synthesis of close structural analogues of AHLs in which the three areas have been modified.[8,9,17,18] With respect to structural variations of the central amide zone, our group has reported the synthesis and the evaluation of several families of active analogues, including sulfonamides,[19] ureas,[20] and sulfonylureas,[21] all this functions being considered as amide bioisosteres.

In keeping with our work focusing on the amide function of AHLs, we were interested in testing new analogues having a 'reverse-amide' linkage. This approach is a common one for altering amide containing compounds, notably for the design of peptide analogues.[22,23] Surprisingly, despite the known antagonist activity

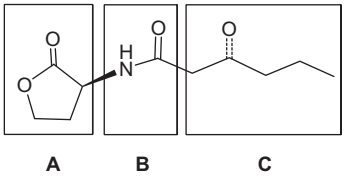

Figure 1. The three areas of AHLs: the lactone moiety (A), the central amide function (B), and the side acyl chain (C).

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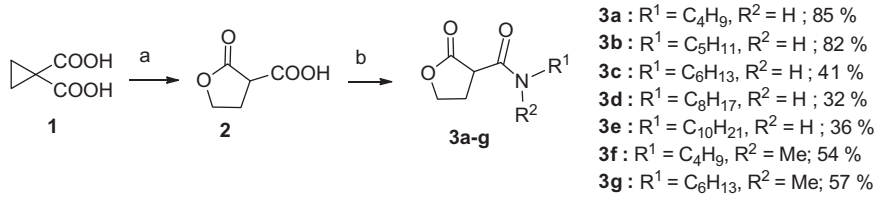
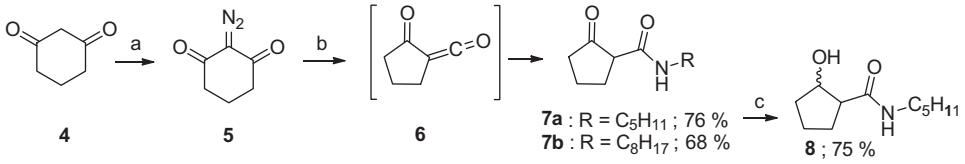
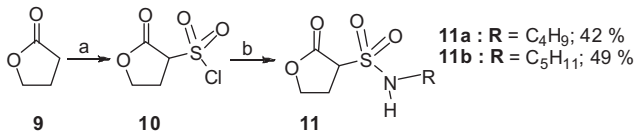


Scheme 1. Preparation of reverse amides **3a**–**g**. Reagents and conditions: (a) NBu_4Br, CH_3CN, 75 °C, 16 h; (b) R^1R^2NH, DCC, DMAP, CH_2Cl_2, 0 °C, rt, 14 h.



Scheme 2. Preparation of cyclopentyl reverse amides **7** and **8**. Reagents and conditions: (a) TsN_3, Et_3N, CH_2Cl_2, 0 °C, 1 h, 77%; (b) RNH_2, xylene, reflux 1 h; (c) $NaBH_4$, MeOH, rt, 2 h.



Scheme 3. Preparation of reverse-sulfonamides **11**. Reagents and conditions: (a) $SOCl_2$, $ClSO_3H$, 120 °C, 2 h; (b) RNH_2, CH_2Cl_2, −10 °C, rt, 1.5 h.

of some homoserine lactone amides,[13] and sulfonamides,[19] to our knowledge, only the reverse-amides analogues of oroidin, a marine alkaloid possessing anti-biofilm properties, have been designed and tested as QS inhibitors.[24,25] We report hereafter the synthesis and the biological evaluation of some various AHL analogues having a reverse-amide or a reverse-sulfonamide functions, as well compounds in which the lactone ring is also modified as a cyclopentanone or cyclopentanol one.

Reverse-amide AHLs analogues, namely α-(*N*-alkyl-carboxamide)-γ-butyrolactones **3**, were obtained in two steps (Scheme 1). Reaction of butyrolactone **2**[26] prepared from commercially available cyclopropane dicarboxylic acid (**1**) with primary alkylamines (C_4, C_5, C_6, C_8, C_{10}) led to the desired amides **3a–e** in 36–85% isolated yields. Reaction with secondary amines *N*-methyl-*N*-butyl and *N*-methyl-*N*-hexyl led to the corresponding N-methylated amides **3f–g**.

Cyclopentanones analogues **7** were prepared in three steps from commercially available cyclohexane-1,3-dione (**4**) (Scheme 2). Reaction of **4** with tosylazide in the presence of triethylamine[27] led to the 2-diazo **5** which underwent a Wolff rearrangement to give the intermediate ketene **6**, which reacted with pentylamine or octylamine to give 2-(*N*-alkyl-carboxamide)-cyclopentanones **7a** and **7b** in good yields. Reduction of compound **7a** with sodium borohydride in methanol led to alcool **8a** obtained in 75% yield, as a mixture of two diasteroiomers, from which one of them could be isolated as a pure sample.

Reverse-sulfonamides were prepared in two steps (Scheme 3) from γ-butyrolactone (**9**) via its α-chlorosulfonyl **10** derivative obtained by reaction with thionyl chloride in presence of chlorosulfonic acid.[28] Subsequent reaction of **10** with butylamine or pentylamine led to the corresponding α-(*N*-alkyl-sulfonamide)-γ-butyrolactones **11a–b** in moderate yields.

Compounds **3a–g**, **7a,b**, **8,** and **11a,b** 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*.[29] None of them proved to display any agonistic activity at any concentration (0.1–200 μM). QS inhibition was then evaluated by measuring the decrease of the bioluminescence induced by 3-oxo-C_6-HSL, the main autoinducer in *Vibrio fischeri* (Table 1). Most compounds displayed antagonist activity in the whole concentration range (1–200 μM). The reverse amides **3a–e** were first tested, all of them exhibiting antagonist activity, with IC_{50} ranging from 34 to 96 μM, the most active one being the *N*-pentyl derivative. Surprisingly, a slightly shorter *N*-butyl chain resulted in a significantly lower activity (96 μM), whereas compounds having chain length elongated to six, eight or 10 carbon atoms proved to be only moderately less efficient antagonists. Since native AHLs with various chain lengths have been reported to be either agonists or antagonists with activities reaching the micromolar range,[8,9,17] this suggests that the α-amidolactone motif in AHLs is an important structural signature for efficient binding to the transcriptional factors LuxR type proteins. *N*-methylamides **3f–g** were inactive, showing the importance of the NH system for binding within the receptor active site. The cyclopentanones **7a** and **7b** also exhibited antagonistic activity, in the same range of activity measured for the corresponding lactones **3a** and **3d**, respectively. Alteration of the lactone ring would be of limited effect once the α-amidolactone linkage is replaced by a 1,3-dicarboxy system. By contrast, the presence of an hydroxyl group instead of the keto one in cyclopentanols **8** having the pentyl chain resulted in loss of activity either for

Table 1
Inhibition of bioluminescence obtained with compounds **3**, **7**, **8**, and **11**

Compounds	IC_{50}[a,b] (μM)
3a	96 (±4)
3b	34 (±5)
3c	54 (±3)
3d	52 (±3)
3e	75 (±1)
7a	40 (±2)
7b	77 (±1)
8	200 (±8)
11a	>200
11b	70 (±5)

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

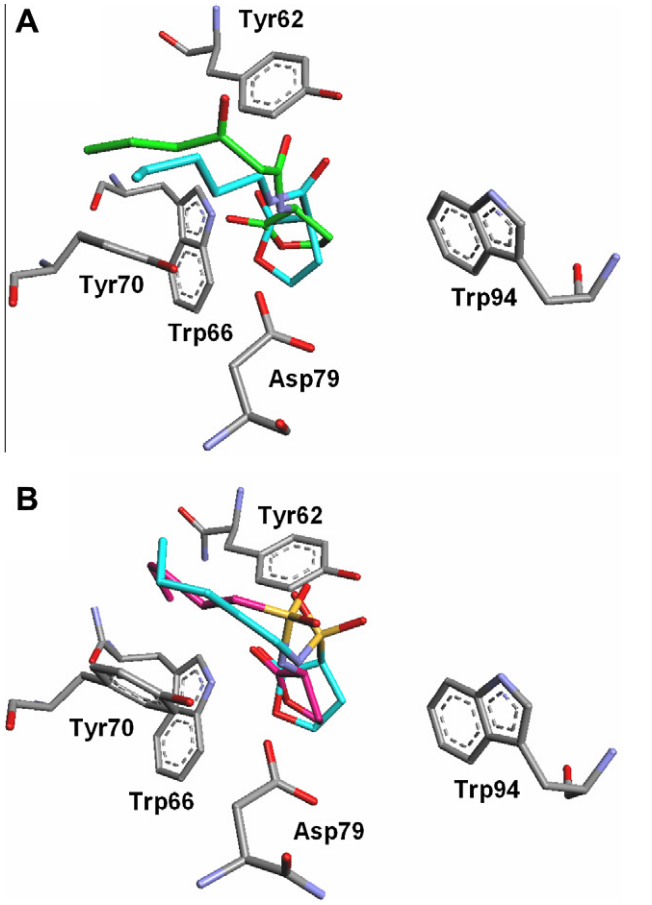


Figure 2. (A) Proposed binding modes of reverse amide **3b** (in blue) and 3-oxo-C_6-HSL (in green). (B) Proposed binding modes of reverse-sulfonamide **10b** (in blue) and C5 sulfonamide (in pink).

Table 2
Hydrogen bonds network with distances in Å between residues Trp66, Tyr62 and Asp79 and different functions of 3-oxo-C_6-HSL, reverse-amide **3b**, C5-sulfonamide[19] and reverse-sulfonamide **11b**

	Trp66	Tyr62	Asp79
3-oxo-C_6-HSL	2.3 (C=O lactone)	3.0 (C=O amide)	3.0 (NH)
Reverse amide **3b**	2.3 (C=O lactone)	2.8 (C=O amide)	3.0 (NH)
(C5) Sulfonamide	2.6 (C=O lactone)	3.1 (S=O)	3.0 (NH)
Reverse sulfonamide **11b**	2.3 (C=O lactone)	2.9 and 2.9 (O=S=O)	2.9 (NH)

the mixture of the two diastereoisomers (IC_{50} = 200 μM) or for the single one (IC_{50} = 92 μM), as compared to cyclopentanone **7a** (IC_{50} = 40 μM).

The two sulfonamides **11a** and **11b** possessing side chains of similar length as those in the most active QS inhibitors AHL analogues sulfonamides[19] were found to decrease bioluminescence. However, the shorter butyl derivative **11a** exhibited a rather low activity (IC_{50} >200 μM), whereas the *N*-pentylsulfonamide **11b** was significantly more active (IC_{50} = 70 μM). Consistently with amides versus reverse-amides, the reverse-sulfonamides exhibit lower activities compared to the sulfonamides, clearly confirming the key role of the α-amidolactone system in AHL binding.

A molecular modeling study was investigated with the LuxR model[30] and compounds **3b**, **11b** as well as with the previously reported C5 sulfonamide[19] and 3-oxo-C_6-HSL.[31] All compounds showed similar binding modes interacting with conserved residues Trp66, Tyr62, Asp79 (Figs. 2A and B). Distances between key atoms involved in hydrogen bonds were not significantly different for C=O or S=O groups and basically identical for the NH group (Table 2). Modeling of compound **3b** compared to the natural ligand 3-oxo-C_6-HSL,[31] (Fig. 2A) showed a different orientation of the lactone and the alkyl chain. For compound **11b** (Fig. 2B) versus C5 sulfonamide, a different orientation of the sulfonamide function is observed with two hydrogen bonds with Tyr62 for **11b** and only one for C5 sulfonamide. In both cases, it is seen that the NH function locations are nearly superimposable. Antagonist activity of reverse analogues can be explained by the necessary adaptation of the location of the molecule when forced to match as much as possible the H-bond network imposed by the binding site.

In conclusion, new AHL analogues, designed by replacing the amide function by a reverse-amide or a reverse-sulfonamide one, have been prepared and their activity as QS modulators has been evaluated. Some reverse-amides [α-(*N*-alkyl-carboxamide)-γ-butyrolactones] and reverse-sulfonamides [α-(*N*-alkyl-sulfonamide)-γ-butyrolactones] having short alkyl side chains exhibited significant QS antagonist activity, the most active having a 30 μM IC_{50}.

Altering the lactone to a cyclopentanone in the reverse carboxamides did not result in significant change in the activity. Finally, *N*-methylated reveres-amides proved to be inactive. Besides the interest of enlarging the structural scope of AHL antagonists, these results also point out the key role of the NH group in the positioning of the AHL analogue in the binding site.

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Supplementary data

Supplementary data (experimental section for synthesis, biological evaluation and modeling) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.010.

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