Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 1153–1157

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

New Synthetic Analogues of *N*-Acyl Homoserine Lactones as Agonists or Antagonists of Transcriptional Regulators Involved in Bacterial Quorum Sensing

Sylvie Reverchon,[a,*] Bernard Chantegrel,[b] Christian Deshayes,[b] Alain Doutheau[b] and Nicole Cotte-Pattat[a]

[a]*Unité de Microbiologie et Génétique CNRS-INSA-UCB UMR 5122, INSA, Batiment Louis Pasteur, 11 Avenue Jean Capelle, 69621 Villeurbanne, France*

[b]*Laboratoire de Chimie Organique, INSA, Batiment Jules Verne, 17 Avenue Jean Capelle, 69621 Villeurbanne, France*

Received 17 October 2001; revised 24 January 2002; accepted 18 February 2002

Abstract—A series of 22 novel synthetic *N*-acyl-homoserine lactone analogues has been evaluated for both their inducing activity and their ability to competitively inhibit the action of 3-oxo-hexanoyl-L-homoserine lactone, the natural inducer of bioluminescence in the bacterium *Vibrio fischeri*. In the newly synthesized analogues, the extremity of the acyl chain was modified by introducing ramified alkyl, cycloalkyl or aryl substituents at the C-4 position. Most of the analogues bearing either acyclic or cyclic alkyl substituents showed inducing activity. In contrast, the phenyl substituted analogues displayed significant antagonist activity. We hypothesized that the antagonist activity of the phenyl compounds may result from the interaction between the aryl group and aromatic amino acids of the LuxR receptor, preventing it from adopting the active dimeric form. © 2002 Elsevier Science Ltd. All rights reserved.

Introduction

N-Acyl-L-homoserine lactones (acyl-HSLs) are produced by a variety of Gram-negative bacteria which use them as extracellular quorum sensing signals.[1] Depending on the bacterial species, these molecules differ in the length of their acyl chain (4–14 carbons) and in the eventual presence of double bonds or functional groups (keto, hydroxyl). By activating proteins belonging to the LuxR family of transcriptional regulators, these signals allow for population density-dependent expression of multiple genes. Many pathogens, such as *Pseudomonas aeruginosa*,[2] *Erwinia carotovora*[3] or *Erwinia chrysanthemi*,[4] use acyl-HSLs to regulate the production of virulence factors. Thus, an attractive way of controlling virulence could be to interfere with quorum sensing signals by designing acyl-HSL analogues that bind to, but fail to activate, the LuxR-type transcriptional regulators. The design of *N*-acyl-HSL analogues has been previously performed in four different systems: the 3-oxo-C6-HSL and LuxR of *Vibrio fischeri*,[5,6] the 3-oxo-C6-HSL and CarR of *E. carotovora*,[7] the 3-oxo-C8-HSL and TraR of *Agrobacterium tumefaciens*[8] and the 3-oxo-C12-HSL and LasR of *P. aeruginosa*.[9] From these different studies, the following general features emerge: the homoserine lactone ring is very important for biological activity while the nature of the acyl chain is not as critical. Previous attempts to obtain acyl-HSL antagonists by modifying the acyl chain have focused mainly on its length, on the modification of the 3-oxo functionality and on the introduction of unsaturations. In contrast, compounds with ramified substituents have received less attention. As part of a program aimed at obtaining compounds with antagonist activity, we synthesized a range of analogues of 3-oxo-C6-HSL and C6-HSL bearing either ramified, cycloalkyl or aryl substituents at the C-4 position of the acyl chain. We then studied their ability to competitively inhibit the activity of natural inducers in the *V. fischeri* LuxR model system.

Experimental Procedures

Synthesis of acyl-HSL analogues[10]

Racemic mixtures of 3-oxo-C6-HSL analogues **1**–**5**, **7**–**19** were obtained through a two step synthesis:[11] the reaction of the requisite acid chloride with Meldrum's acid, in the presence of pyridine in dichloromethane, at room temperature and the subsequent work up yielded crude intermediate acyl Meldrum's acid, which was then

*Corresponding author. Tel.: +33-4-7243-8088; fax: +33-4-7243-8714; e-mail: revercho@insa-lyon.fr

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00124-5

dissolved in 1,2-dichloroethane and refluxed in the presence of D,L-homoserine lactone hydrobromide and pyridine. Racemic mixtures of C6-HSL analogues **6**, **20**–**22** were prepared by reacting, at room temperature, D,L-homoserine lactone hydrobromide, pyridine and the corresponding acid chlorides in dichloromethane. After the usual workup, crude products were purified by silica gel chromatography and obtained in yields ranging from 20 to 70%.

Bacterial strain and culture conditions

We used the recombinant *Escherichia coli* strain NM522 containing the sensor plasmid pSB401 to measure the induction of luminescence by various acyl-HSL analogues. In pSB401, the *LuxR* and the *LuxI* promoter from *V. fischeri* have been coupled to the entire *Lux* structural operon (*LuxCDABE*) from *Photorhabdus luminescens*. This construct, present in the biosensor strain (NM522/pSB401), responds to a wide range of acyl-HSLs by producing bioluminescence.[12] Bacterial cultures were grown in Luria broth, in the presence of tetracycline (20 μg mL^{-1}), at 30 °C.

Detection of biological activity using the biosensor strain

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, with bioluminescence quantified using a Luminoskan luminometer. Concentrations of analogues, ranging from 20 to 40 μM, were made up to 0.1 mL volumes with growth medium. The same volume (0.1 mL) of a 1:10 dilution of an overnight culture of the biosensor strain was then added and the plate was incubated at 30 °C. The amount of light produced by the bacteria was detected after 4–5 h, when the ratio of induced to background light was at its maximum. The amount of light measured was expressed in relative light units (RLU).

Competition assays using the biosensor strain

The influence of acyl-HSL analogues on the induction of bioluminescence by 3-oxo-C6-HSL was determined as described above, except that 3-oxo-C6-HSL was included at a final concentration of 200 nM together with the analogue. This concentration of 3-oxo-C6-HSL was required for 1/2 maximal induction of luminescence under our conditions. This value is 2- to 4-fold higher than that previously reported.[5,6] This is probably due to the fact that we used a racemic mixture of 3-oxo-C6-HSL.[13] In addition, bioassays were performed under different cultural conditions.

Results

Structures of synthetic analogues of the natural LuxR inducers

We prepared a series of analogues of the two natural inducers of the LuxR regulator which are produced by the acyl-HSL synthase LuxI of *V. fischeri*: 3-oxo-C6-HSL, the most efficient inducer and C6-HSL, a secondary inducer. We synthesized a range of derivatives bearing a ramified alkyl (**1**, **2**), cycloalkyl (**3**–**6**), aryl (**7**–**17**) or heteroaromatic (**18**, **19**) substituent at the C-4 position of the 3-oxo-acyl chain. Three analogues of the C6-HSL, bearing either a phenyl group at C-4 (**20**[6]) or a phenyl group at C-4 together with either an oxygen or a sulphur atom instead of the C-3 methylene (**21**, **22**), were also prepared.

Effect of acyl-HSL analogues on the induction of bioluminescence

The ability of each analogue to activate the LuxR protein was determined by following the luminescence of the biosensor strain. The natural inducers 3-oxo-C6-HSL and C6-HSL were used as controls (Fig. 1). Four compounds (**1**, **3**, **4**, **6**) with alkyl or cycloalkyl substitution activated luminescence whereas the other compounds showed no inducing activity. Compound **3** (cyclopentyl) is as active as 3-oxo-C6-HSL whereas **1** (isopropyl) and **4** (cyclohexyl) displayed a lower activity. Compound **6** (cyclohexyl with a C-3 methylene) appears to be as active as the corresponding C6-HSL natural inducer. These results indicate that the inducing activity is more or less retained if one branching is introduced at the C-5 position of the acyl chain, either in acyclic or cyclic form. The introduction of two branchings at C-5 resulted in the absence of any inducing activity, as shown with compounds **2** (*tert*-butyl) and **5** (adamentyl). Interestingly, all of the aryl compounds (**7**–**17**) were also deprived of inducing activity, indicating that the flattening of the chain extremity due to the hybridization modification of the C-5 atom, from sp^3 to sp^2, resulted in a dramatic effect.

Inhibition of 3-oxo-C6-HSL activity by analogues

The analogues devoid of inducing activity were tested for their ability to interfere with the induction of luminescence by 3-oxo-C6-HSL. The alkyl derivative **2**, bearing a *tert*-butyl group at C-4, proved to be less active that all the 4-aryl-3-oxo-derivatives (**7**–**15**) (Figs. 2 and 3). Among the latter compounds, the antagonist activity appeared to be of the same significant magnitude with the phenyl compound **7** and the *p*-substituted phenyl compounds **8**, **9** and **10** bearing, respectively, a chloro, methoxy or bromo group. The activity was slightly lower with the *p*-fluorophenyl and tolyl derivatives **11** and **12** and significantly decreased in the case of the *p*-trifluoromethylphenyl derivative **13** (Fig. 2). The influence of the position of the substituent on the aromatic ring was examined with chloro derivatives (Fig. 3). A decrease in inhibitory activity was observed following the sequence *para* (**8**), *meta* (**15**) and *ortho* (**14**). Thus the best antagonist activity was obtained with a phenyl group or a phenyl group bearing a heteroatom in position *para*.

Naphthyl (**16**) and biphenyl (**17**) compounds (Fig. 4) showed no inhibitory activity, presumably because of steric hindrance limitation; this kind of limitation was also observed with the alkyl derivative **5** bearing a bulky adamentyl group at C-4 which displayed no antagonist

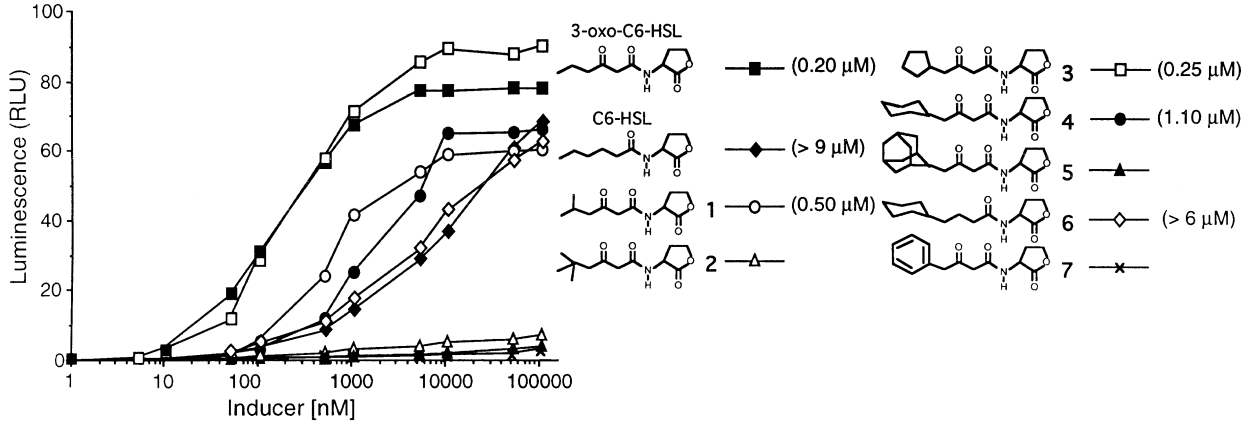

Figure 1. Agonist effects of *N*-acyl-HSL analogues **1**, **2**, **3**, **4**, **5**, **6** and **7**. The concentrations (μM) required for half-maximal activation are given in parentheses.

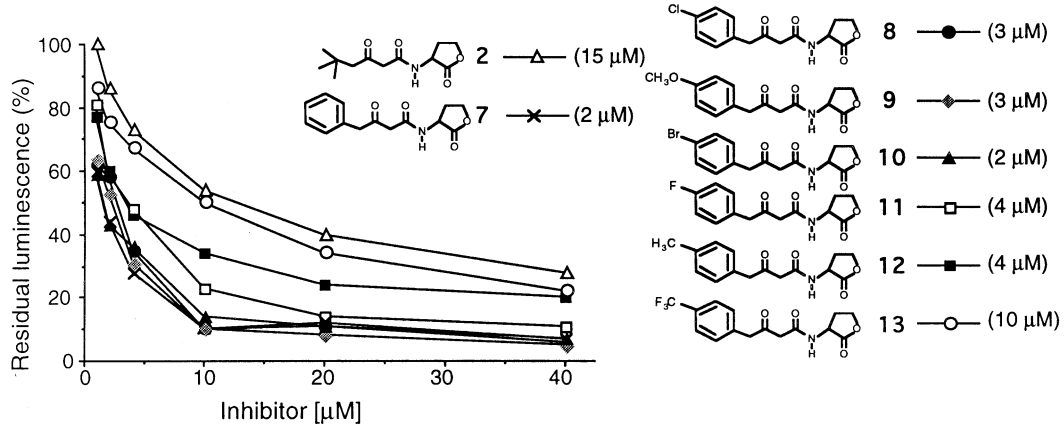

Figure 2. Antagonist effects of *N*-acyl-HSL analogues **2**, **7**, **8**, **9**, **10**, **11**, **12** and **13**. The concentrations (μM) required for 50% inhibition are given in parentheses.

activity. Surprisingly, when the phenyl group in compound **7** was replaced by an isosteric thiophenyl group[14] (**18** and **19**, Fig. 4), no antagonist activity was observed either.

To evaluate the influence of the 3-oxo moiety for antagonist activity, we tested the C6-HSL analogues **20**, **21** and **22** bearing a phenyl substituent at C-4. These compounds were inhibitors but they appeared to be slightly less efficient than the corresponding 3-oxo-C6-HSL analogue **7** (Fig. 5). Thus the 3-oxo moiety, which is important for the inducing capacity of natural inducers, also favours the antagonist activity of phenyl derivatives. This statement is in agreement with previous results from the literature.[6]

Discussion

Considering their biological activity, secondary alkyl or aryl acyl-HSLs clearly delimit two classes of compounds: secondary alkyl derivatives (**1**, **3**, **4**, **6**) show agonist activity while the aryl derivatives (**7**–**15**, **20**–**22**) and, to a lesser extent, the tertiary alkyl derivative **2** show antagonist activity. Compounds with larger alkyl (**5**) or aryl (**16**, **17**) moieties are deprived of any activity, indicating that the presence of an excessively bulky group prevents interaction with LuxR.

To our knowledge, such clear-cut differences of behaviour among HSL analogues bearing alkyl and aryl substituents have not previously been reported. It is very

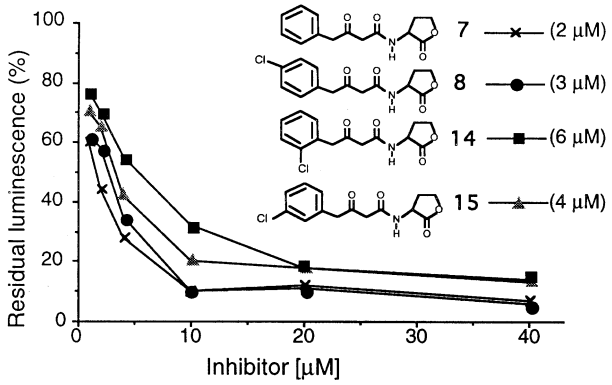

Figure 3. Antagonist effects of *N*-acyl-HSL analogues **7**, **8**, **14** and **15**. The concentrations (μM) required for 50% inhibition are given in parentheses.

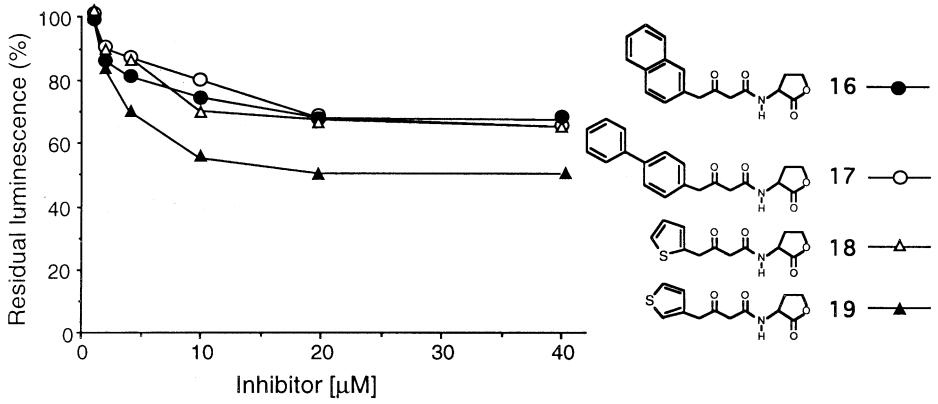

Figure 4. Antagonist effects of *N*-acyl-HSL analogues **16**, **17**, **18** and **19**.

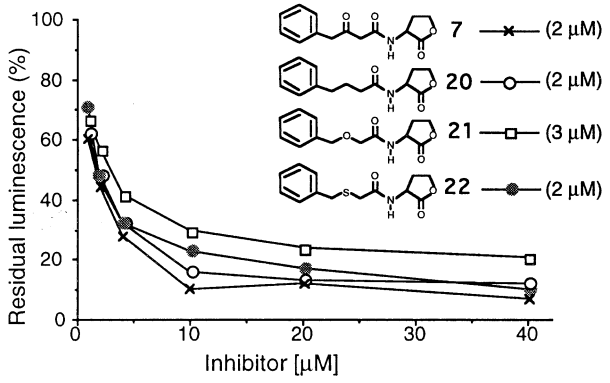

Figure 5. Antagonist effects of *N*-acyl-HSL analogues **7**, **20**, **21** and **22**. The concentrations (μM) required for 50% inhibition are given in parentheses.

unlikely that these differences can be explained on the basis of steric effects. More probably, the presence of an aryl group allows for a very specific interaction to take place with the LuxR protein. The specific character of this interaction is also illustrated by the surprising absence of inhibitory activity observed with the isosteric thiophenyl derivatives **18** and **19**.

It is well recognized that LuxR-type transcriptional regulators activate gene transcription only after dimerization. For the LuxR protein, the accepted model is that dimerization requires the preliminary binding of acyl-HSL.[15] One can speculate that the antagonist activity of 4-aryl acyl-HSLs may result from their interaction with one or several aromatic amino acid(s) of the LuxR protein, in the region containing the HSL-binding site or in the dimerization domain or both. Previous work indicates[16,17] that the HSL-binding site of LuxR contains three aromatic residues and is followed by the hydrophobic dimerization domain which contains six aromatic residues.

We plan to use a genetic system, based on the cI repressor of phage λ,[18] to determine whether the mechanism of inhibition is really due to the prevention of LuxR dimerization in the presence of 4-aryl-3-oxo-butanoyl-HSLs.

Acknowledgements

We thank G. Condemine for his critical opinions and V. James for reading the manuscript. We are much indebted to Professor Loic Blum for generously making his Luminometer available to us and to Agnes Degiuli for guiding us in its use. This work was supported by grants from the CNRS, from the Ministère de l'Education Nationale et de la Recherche and by specific grants from INSA (Bonus Qualite Recherche) and from the Fonds National pour la Science 2000 (Programme Microbiologie).

References and Notes

1. Fuqua, C.; Winans, S. C.; Greenberg, E. P. *Annu. Rev. Microbiol.* **1996**, *50*, 727.
2. Latifi, A.; Winson, M. K.; Foglino, M.; Bycroft, B. W.; Stewart, G. S.; Lazdunski, A.; Williams, P. *Mol. Microbiol.* **1995**, *17*, 333.
3. Jones, S.; Yu, B.; Bainton, N. J.; Birdsall, M.; Bycroft, B. W.; Chhabra, S. R.; Cox, A. J. R.; Golby, P.; Reeves, P. J.; Stephens, S.; Winson, M. K.; Salmond, G. P. C.; Stewart, G. S. A. B.; Williams, P. *EMBO J.* **1993**, *12*, 2477.
4. Reverchon, S.; Bouillant, M.; Salmond, G.; Nasser, W. *Mol. Microbiol.* **1998**, *29*, 1407.
5. Eberhard, A.; Widrig, C. A.; McBath, P.; Schineller, J. B. *Arch. Microbiol.* **1986**, *146*, 35.
6. Schaefer, A. L.; Hanzelka, B. L.; Eberhard, A.; Greenberg, E. P. *J. Bacteriol.* **1996**, *178*, 2897.
7. Chhabra, S. R.; Stead, P.; Bainton, N. J.; Salmond, G. P.; Stewart, G. S.; Williams, P.; Bycroft, B. W. *J. Antibiot. (Tokyo)* **1993**, *46*, 441.
8. Zhu, J.; Beaber, J. W.; More, M. I.; Fuqua, C.; Eberhard, A.; Winans, S. C. *J. Bacteriol.* **1998**, *180*, 5398.
9. Passador, L.; Tucker, K. D.; Guertin, K. R.; Journet, M. P.; Kende, A. S.; Iglewski, B. H. *J. Bacteriol.* **1996**, *178*, 5995.
10. For a recent review on chemical synthesis of acyl-HSLs and analogues, see: Eberhard, A.; Schineller, J. B. *Methods Enzymol.* **2000**, *305*, 301.

11. Moya, P.; Cantin, A.; Castillo, M. A.; Primo, J.; Miranda, M. A.; Primo-Yufera, E. *J. Org. Chem.* **1998**, *63*, 8530.
12. Winson, M. K.; Swift, S.; Fish, L.; Throup, J. P.; Jorgensen, F.; Chhabra, S. R.; Bycroft, B. W.; Williams, P.; Stewart, G. S. A. B. *FEMS Microbiol. Lett.* **1998**, *163*, 185.
13. Ikeda, T.; Kajiyama, K.; Kita, T.; Takiguchi, N.; Kuroda, A.; Kato, J.; Ohtake, H. *Chem. Lett.* **2001**, 314.
14. Patani, G. A.; LaVoie, E. *J. Chem. Rev.* **1996**, *96*, 3147.
15. Fuqua, C.; Greenberg, E. P. *Curr. Opin. Microbiol.* **1998**, *1*, 183.
16. Shadel, G. S.; Young, R.; Baldwin, T. O. *J. Bacteriol.* **1990**, *172*, 3980.
17. Slock, J.; VanRiet, D.; Kolibachuk, D.; Greenberg, E. P. *J. Bacteriol.* **1990**, *172*, 3974.
18. Hu, J. C. *Structure* **1995**, *3*, 431.