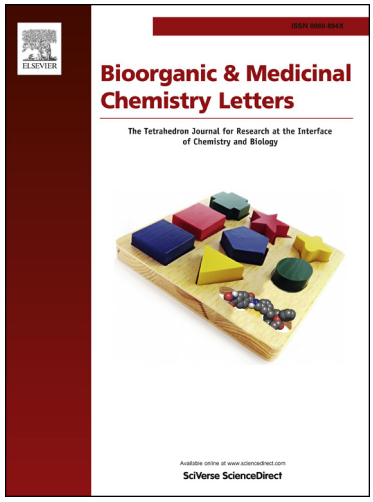


Design, synthesis and antibacterial evaluation of novel AHL analogues

Jing-Li Ren, En Zhang, Xian-Wei Ye, Meng-Meng Wang, Bin Yu, Wen-Hua Wang, Ya-Zhuo Guo, Hong-Min Liu

PII: S0960-894X(13)00630-6
DOI: http://dx.doi.org/10.1016/j.bmcl.2013.05.035
Reference: BMCL 20496

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 11 April 2013
Revised Date: 8 May 2013
Accepted Date: 10 May 2013

Please cite this article as: Ren, J-L., Zhang, E., Ye, X-W., Wang, M-M., Yu, B., Wang, W-H., Guo, Y-Z., Liu, H-M., Design, synthesis and antibacterial evaluation of novel AHL analogues, *Bioorganic & Medicinal Chemistry Letters* (2013), doi: http://dx.doi.org/10.1016/j.bmcl.2013.05.035

Design, synthesis and antibacterial evaluation of novel AHL analogues

Jing-Li Ren, En Zhang, Xian-Wei Ye, Meng-Meng Wang, Bin Yu, Wen-Hua Wang, Ya-Zhuo Guo, Hong-Min Liu*.

School of Pharmaceutical Sciences and New Drug Research & Development Center, Zhengzhou University, Zhengzhou 450001, P. R. China

Abstract: Two series of novel AHL analogues were designed, synthesized and evaluated for antibacterial activity under cell membrane conditions in vitro. Analogues **4a-c** and **4g-m** presented potent activity against Gram-positive bacteria. Especially the analogue **4l** exerted the most potent inhibition against *Bacillus subtilis* with MIC_{50} value of 1.443 μg/ml. To our surprise, analogues **6a-c** and **6g** showed weak inhibition against Gram-negative bacteria with MIC_{50} values ranging from 17.589 to 67.840 μg/ml. This was the first report about synthesis and antibacterial evaluation in vitro of AHL analogues containing dithioester linkage.

Key words: antibacterial; quorum sensing; dithiocarbamates; AHLs.

Bacterial resistance to the existing drugs is still a global problem, which develops at increasingly fast speed in hospitals and community settings during these years. Thus research of more effective antibacterial agents is of considerable significance to public health especially in developing countries.

Bacterial quorum sensing (QS) refers to a chemical communication system, in which bacteria use diffusible, small molecular signals namely autoinducers to monitor their local population densities. QS regulates diverse phenotypes, such as bioluminescence, biofilm formation, virulence expression and antibiotic production, in various bacteria survived in plants and mammals.[1,2] Different kinds of compounds are employed by bacteria as QS signals.[3] To Gram-negative bacteria, *N*-acylhomoserine lactones (AHLs) (Figure 1, Ⅰand Ⅱ) are the most studied class of autoinducers. Numerous researches on synthetic analogues (Figure 1, Ⅲ-Ⅴ) and their signaling action mechanisms have been carried out during these years.[4,5,6,7] The AHL, 3-oxo-dodecanoyl-*L*-homoserine lactone (OdDHL, figure 1, Ⅰ) produced by the prevalent pathogen *Pseudomonas aeruginosa*, has been shown to induce apoptosis of macrophages and human breast cancer cell lines but not common ones and even to enhance sensitivity of H630 colon cancer cells to 5-Fluorouracil.[8,9,10] Except for the action to eukaryotic cells, OdDHL and some other synthetic 3-oxo derivatives have been reported to inhibit the growth of Gram-positive bacteria (*Bacillus cereus*, *Bacillus subtilis* and *Staphylococcus aureus*) selectively in high concentration range.[3] So far, strategies to intercept QS have been considered as potential anti-infective therapies.

* Corresponding author. Tel.: +86 371 67781739; fax: +86 371 67781739. E-mail address: liuhm@zzu.edu.cn (H.-M. Liu).

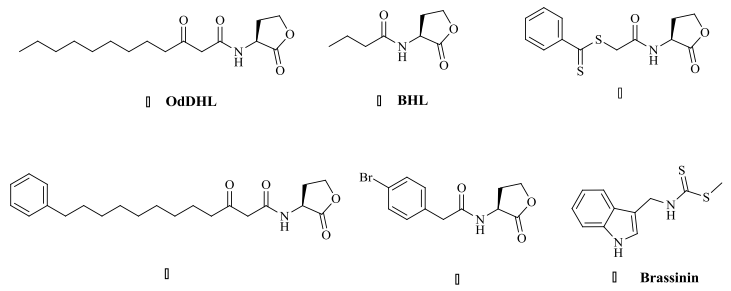
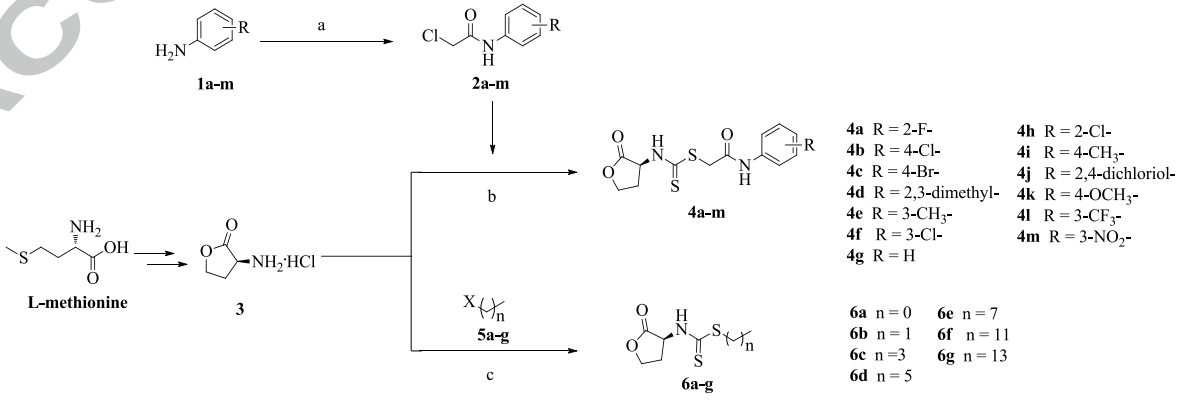


Figure 1. Some natural occurring AHLs I-II and synthetic quorum sensing molecules III-IVand the natural dithiocarbamate Brassinin VI.

Dithiocarbamates have received considerable attention for their excellent biological activities during these years.[11,12,13] The natural product brassinin (Figure 1, VI) was reported benign antifungal activity and as a moderate inhibitor of a novel cancer immunosuppression target (Indoleamine 2,3-dioxygenase, IDO).[11] Lots of trials have been done in order to obtain more novel dithocarbamates with good biological activities. Dithiocarbamates were efficiently prepared by multicomponient one-pot reaction, in which the primary or secondary amines reacted with carbon disulfide and halides in the presence of weak bases.[14,15]

The intrinsic liposolubility of natural AHLs enables QS signals to cross cell membranes in unregulated diffusion way. In considering this key element, we designed to prepare two series of AHL analogues containing dithioester linkage by introduction of *N*-phenyl acetamide a benign antimicrobial pharmacophore[16,17] and hydrophobic alkyl chains as side chains respectively. Herein, in this letter we first reported the synthesis and antibacterial evaluation of AHL analogues containing dithoester lingkage.

The synthetic route towards target AHL Analogues (**4a-m** and **6a-g**) was shown in Scheme 1. The starting material *L*-homoserine lactone hydrochloride **3** and the key intermediates **2a-m** were prepared according to the literature reported methods.[16,18] The target analogues were easily obtained in high yields with the mature reaction conditions developed by our group[14,15]. Specifically, **2a-m** reacted with compound **3** and carbon disulfide to afford **4a-m.** Compound **3** reacted with commercially available halids **5a-g** to synthesize **6a-g**. Finally, all the structures of **4a-m** and **6a-g** were fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS.



Scheme 1. Synthesis of AHL analogues **4a-m** and **6a-g**. Reagents and conditions: (a) $ClCH_2COCl$, Anhyd.K_2CO_3, Acetone, rt. (b) **2a-m**, CS_2, $Na_3PO_4{\cdot}12H_2O$, Acetone, rt. (c) **5a-g**, CS_2, $Na_3PO_4{\cdot}12H_2O$, Acetone, rt.

Analogues **4a-m** and **6a-g** were screened against Gram-positive bacteria (*Staphylococcus*

aureus, *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli*) in vitro by microdilution broth method with levofloxacin as the positive control. MIC_{50} values of the tested compounds were listed in Table 1. It was observed that **4a-m** displayed benign inhibitory action against Gram-positive bacteria and relatively weak inhibition against Gram-negative bacteria, while **6a-g** showed weak inhibition against most of the tested bacteria. Specifically, compounds **4a-c** and **4g-m** could potently inhibit the growth of *S. aureus* with the MIC_{50} values ranging from 3.498 to 9.515 μg/ml. Analogues **4h** (2-Cl), **4j** (4-OCH_3), **4l** (3-CF_3) and **4m** (3-NO_2) showed potent inhibition against *B. subtilis* with the MIC_{50} of 9.626, 5.600, 1.443 and 6.289 μg/ml, respectively. It is worth mentioning that compound **4l** presented the most excellent inhibition against *B. subtilis* with the MIC_{50} of 1.443 μg/ml. To our surprise, the analogues **6a-c** showed weak inhibitory effect against Gram-negative bacteria with the MIC_{50} range of 17.589-44.631 μg/ml.

Table 1

Antibacterial activity (MIC_{50}, μg/ml) of compouds **4a-m**, **6a-g**.

compound	$Gram^+$ bacteria		$Gram^-$ bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
4a	6.534	>128	>128	40.488
4b	3.498	>128	>128	>128
4c	3.691	30.233	54.525	24.859
4d	>128	>128	>128	>128
4e	>128	>128	37.192	>128
4f	>128	>128	>128	>128
4g	7.722	47.907	48.467	>128
4h	5.621	9.629	>128	>128
4i	3.489	>128	63.765	30.589
4j	4.898	5.600	>128	20.995
4k	9.515	>128	>128	54.211
4l	5.807	1.443	>128	>128
4m	8.674	6.289	59.935	12.808
6a	>128	>128	31.934	17.589
6b	>128	>128	34.557	25.224
6c	>128	>128	44.631	>128
6d	>128	>128	>128	>128
6e	>128	>128	>128	>128
6f	50.569	>128	>128	>128
6g	>128	>128	>128	67.840
Levofloxacin[a]	<0.25	<0.25	<0.25	<0.25

[a] the control drug for antibacterial assays.

The synthesized compounds showed varying degrees of antibacterial activity against Gram-positive and Gram-negative bacteria. The analogues with hydrophobic alkyl chains stimulated the growth of Gram-positive bacteria except for **6f**, while against Gram-negative bacteria they exerted weak inhibitory activity and the activity reduced with the length of

hydrophobic alkyl chains increased. The introduction of substituted N-phenyl acetamides as side chains contributed to antibacterial activities immensely when compared with hydrophobic alkyl chains. Analogues with different substituents on phenyl ring exemplified varying degrees of inhibition against Gram-positive bacteria. Analogues **4l** and **4m** with strong electron-withdrawing groups -CF_3 and -NO_2 as meta-substitutions showed benign antibacterial activities, while no activity was exerted by **4e** with the electron-donating group -CH_3 at meta-position. Analogues **4b** (4-Cl), **4c** (4-Br), **4i** (4-CH_3), **4j** (2, 4-dichloride), **4h** (2-Cl) and **4l** (3-CF_3) presented potent inhibition against *S. aureus*. The antibacterial inhibition of **4b** (4-Cl) and **4i** (4-CH_3) with the substituents at para-position were better than **4h** (2-Cl), **4f** (3-Cl) and **4e** (3-CH_3) respectively. No activity improvement was exhibited by **4j** (2, 4-dichloride) compared to **4b** (4-Cl). Compound **4l** (3-CF_3) presented the best activity against *B. subtilis*, and the activity disappeared when the aryl hydrogen atom was replaced by the electron-donating groups. Compound **4j** (2, 4-dichloride) had better antibacterial activity than compound **4h** (2-Cl).

In conclusion, we synthesized two series of novel AHL analogues and evaluated their antibacterial activity in vitro against Gram-positive and Gram-negative bacteria. The nature of the side chains and the substitutions on the phenyl ring influenced the antibacterial activities remarkably. Analogues **6a-g** with hydrophobic alkyl chains showed weak inhibition against most of the tested bacteria. The analogues **4a-m** obtained via introduction of different substituted *N*-phenyl amides as side chains presented benign antibacterial activity to the tested bacterial strains. Amongst them, compound **4l** presented excellent inbititory effect against *B. subtilis* (MIC_{50} = 1.443 μg/ml)**,** which is under further investigation as lead structure**.** Further modifications and SAR study are undergoing and will be reported elsewhere.

Acknowledgments

This work was supported by the National Natural Sciences Foundations of China (No. 8112937, U1204206), China Postdoctoral Science Foundation (No. 20100480857, 201104402) and New Teachers' Fund for Doctor Stations, Ministry of Education (No. 20114101120013).

References

1. Margrith, E. M.; Helen, E. B. *J. Org. Chem.* **2010**, *75*, 6737.
2. Grant, D. G.; Rachel, J. W.; Adam, P. S.; Helen, E. B. *J. Am. Chem. Soc*. **2005**, *127*, 12762.
3. Armando, M. P.; Anita, J. M. *J. Nat. Prod*. **2008**, *71*, 1032.
4. Grant, D. G.; Jennifer, C. O.; David, M. M.; Margrith, E. M.; Helen, E. B. *J. Am. Chem. Soc*. **2007**, *129*, 13613.
5. Andrew, G. P.; Evan, S.; Helen, E. B. *ACS Chem. Biol*. **2011**, *6*, 1348.
6. Thanit, P.; Teresa, M. J. B.; Anthony, S. B.; Helen, E. B. *Bioorg. Med. Chem. Lett*. **2011**, *21*, 5054.
7. Danielle, M. S.; Michael, A. W.; Philip, N. R.; Helen, E. B. *ACS Chem. Biol*. **2012**, *7*, 1719.
8. Manabu, H.; Kazuhiro, T.; Etsu, T.; Yoshikazu, I.; Chihiro, U.; Tohru, T.; Shinichi, M.; Keizou, Y.; Masaji, I. *Bioorg. Med. Chem. Lett*. **2006**, *16*, 2130.
9. Li, L.; Doreen, H.; Siri, R. C.; David, P.; Peter, E. S. *Oncogene*. **2004**, *23*, 4894.
10. Ree, D.; Qi, W.; Norman, J. A.; Lawrence, V. S.; Kuan, C. C.; Janice, R. S.; Bruce, J. D. *Cancer Res*. **2005**, *13*, 5917.
11. Paul, G.; Tinku, B.; William, P. M.; Alexander, J. M.; George, C. P.; James, D.; Shauna, B.; Ashley, M. D. *J. Med. Chem.* **2006,** *49*, 684.
12. Ghali, B.; Fathima, R. K.; Annalisa, F.; Daniela, B.; Jitka, S.; Andrea, B.; Angelika, M. B.;

Andrew, D. W. *J. Med. Chem.* **2010,** *53*, 2757.

13. Fabrizio, C.; Mayank, A.; Alfonso, M.; Andrea, S.; Robert, M.; Emanuela, M.; Claudiu, T. S. *J. Med. Chem.* **2012,** *55*, 1721.
14. Wang, X. J.; Xu, H. W.; Guo, L. L.; Zheng, J. X.; Xu, B.; Guo, X.; Zheng, C. X.; Liu, H. M. *Bioorg. Med. Chem. Lett*. 2011, *21*, 3074.
15. Wang, X. J.; Xu, H. W.; Guo, L. L.; Zhang, E.; Chen, G.. W.; Guo, X.; Liu, H. M. *Heterocycles*. **2011**, *83*, 1005.
16. Rahul, V. P.; Paresh, K. P.; Premlata, K.; Dhanji, P. R.; Kishor, H. C. *Eur. J. Med. Chem*. **2012**, *53*, 41.
17. Signh, D. C. P.; Hashim, S. R.; Singhal, R. G.. *E-Journal of Chemistry*. **2011**, *8*, 635.
18. Peter, H. B.; Anthony, P. D.; Kevin, J. D.; Gladys, D. H. *Tetrahedron: Asymmetry*. **1995**, *6*, 2819.