Synthesis and Biological Activities of Some 1,3-Benzoxazol-2(3*H*)-One Derivatives as Anti-Quorum Sensing Agents

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Abstract

Antibiotics are commonly used to treat microbial infections. Due to misuse or large-scale use of antibiotics, many pathogens have gained resistance which makes antibiotic treatments ineffective. The discovery that many bacteria use quorum sensing (QS) to regulate their virulence factor and pathogenicity production makes the QS system an attractive target for antimicrobial therapy. A series of 1,3-benzoxazol-2(3*H*)-one derivatives were designed and synthesized as QS inhibitors (QSIs) and tested for their QS inhibitory activities. In vitro quorum sensing inhibitor screen (QSIS) assay indicated that the 1,3-benzoxazol-2(3*H*)-one (compound 1), 5-chloro-1,3-benzoxazol-2(3*H*)-one (compound 6), 6-methyl-1,3-benzoxazol-2(3*H*)-one (compound 11), and 5-methyl-1,3-benzoxazol-2(3*H*)-one (compound 16), inhibit QS system in quorum sensing selector (QSIS)1 strain. These 4 QSIs also significantly reduced elastase production, biofilm formation and swarming motility of *Pseudomonas aeruginosa* PA01 strain. These results suggest that compound 1, 6, 11 and 16 may provide a starting point for the design and development of new anti-pathogenic drugs that restrict virulence of *P. aeruginosa* and possibly other clinically important human pathogens. In addition, these QSI molecules could potentially be used in combination with conventional antibiotics to increase the efficiency of disease control and to extend the life span of established antimicrobials.

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Introduction

Bacterial cell to cell communication can regulate community-wide behaviors including biofilm formation, conjugation, swarming, motility, the production of virulence factors, bioluminescence and antibiotic biosynthesis through a process called quorum sensing (QS) [1–3]. QS depends on the production of diffusible signal molecules termed auto-inducers that are synthesized intracellular. Different bacteria may use different auto-inducers to communicate intercellular. *N*-acylated-L-homoserine lactones (AHLs) are the most common signaling molecules found in Gram-negative bacteria while cyclic peptides are the major class of auto-inducers used by Gram-positive bacteria [4]. *P. aeruginosa* is an important human pathogen which is responsible for opportunistic infections in cancer, AIDS and cystic fibrosis (CF) patients [5–7]. A wide variety of extracellular enzymes including elastase, protease, hemolysins, exotoxin A, rhamnolipid biosurfactants and phospholipase contribute to the virulence of *P. aeruginosa*. These extracellular factors are collectively capable of causing extensive tissue damage in humans and other mammals [8, 9]. *P. aeruginosa* utilizes QS to coordinate the expression of virulence genes. In *P. aeruginosa*, QS is mediated by 3-oxo-C12-HSL(OdDHL) synthesized by LasI, C4-HSL (BHL) synthesized by RhlI and by 2-alkyl-4-quinolones (*Pseudomonas* quinolone signal PQS) [10–12] (**Fig. 1**).

P. aeruginosa also employs QS to control the formation of biofilms [13]. Biofilms are dense extracellular polymeric matrices in which the bacteria embed themselves. Biofilm growth also occurs in otitis media, chronic rhinosinusitis, chronic osteomyelitis and prosthetic joint infection and it is the handicap for the medical devices such as catheters, stents, prostheses and artificial heart valves [14–17]. Biofilm formation is thought to protect the microorganisms from host defenses

and provide increased resistance to antibiotics [14–19]. With the widespread appearance of antibiotic-resistant bacteria, there is an increasing demand for new strategies to control infectious diseases. Consequently, inhibitors and antagonists of bacterial quorum sensing are important research tools and potential therapeutic agents. Up to date, many acylhomoserine lactone analogs, furanone derivatives and different heterocycles have been reported to possess QS inhibitory activity [14–16,19,20], (**Fig. 2**). However, most of these QS inhibitors are unsuitable for human use. Toxicity and in vivo efficacy tests significantly narrow down the potential candidates.

In the present study, we describe the synthesis and preliminary biological evaluation of 2(3H)-benzoxazolone, 5-chloro-1,3-benzoxazol-2(3*H*)-one, 6-methyl-1,3-benzoxazol-2(3*H*)-one and 5-methyl-1,3-benzoxazol-2(3*H*)-one and their *N*-long chain acyl derivatives (**Fig. 2**). These compounds were screened for their QS inhibitory activities. 4 compounds, 1,3-benzoxazol-2(3*H*)-one (compound **1**), 5-chloro-1,3-benzoxazol-2(3*H*)-one (compound **6**), 6-methyl-1,3-benzoxazol-2(3*H*)-one (compound **11**) and 5-methyl-1,3-benzoxazol-2(3*H*)-one (compound **16**), showed significant inhibition of QS regulated phenotypes in human pathogen *P. aeruginosa*.

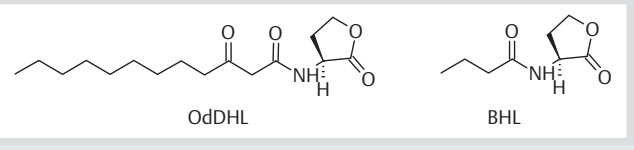


Fig. 1 Acylhomoserine lactone molecules in *P. aeruginosa*.

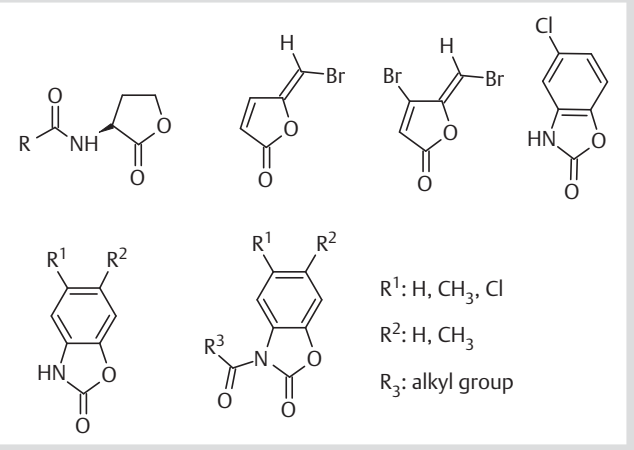


Fig. 2 General structure of AHL, synthetic furanone derivatives, chlorzoxazon and synthesized compounds.

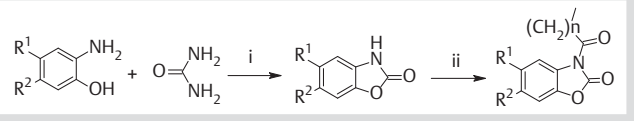


Fig. 3 Synthesis of 1,3-benzoxazol-2(3*H*)-one derivatives, compounds **1–18**. Reagents: (i) MWI, 10 min; (ii) Acylhalide, triethylamine, tetrahydrofurane, reflux under MWI, 20 min. R^1: H, CH_3, Cl. R^2: H, CH_3. n: 5–8.

Materials and Methods

Experimental procedures, spectral characterization data were given in Supporting Information Part.

Results and Discussion

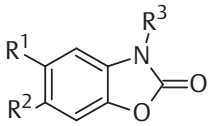
The synthetic routes for the synthesized compounds are outlined in **Fig. 3**. The starting compound, 1,3-benzoxazol-2(3H)-one **(1)** was readily prepared by the reaction of urea and o-aminophenol under microwave irradiation (MWI) and the procedures is in accordance with a previously published method [21]. Other starting compounds, 6-methyl-1,3-benzoxazol-2(3H)-one **(11)** and 5-methyl-1,3-benzoxazol-2(3H)-one **(16),** were prepared by the reaction of urea and 5-methyl-2-aminophenol/4-methyl-2-aminophenol under MWI. Acylation of core rings with acyl chloride derivatives was carried out in tetrahydrofurane (THF) by using microwave assisted method. The 1,3-benzoxazol-2(3*H*)-one [21], 5-methyl-1,3-benzoxazol-2(3*H*)-one [22,23], 6-methyl-1,3-benzoxazol-2(3*H*)-one [22,24,25] and 3-decanoyl-1,3-benzoxazol-2(3*H*)-one [26] was previously reported.

All the synthesized 1,3-benzoxazol-2(3*H*)-one derivatives were first tested for their antibacterial activity [27] against reference strains, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* RSKK 574 and clinical isolates of these microorganisms. The minimum inhibitory concentration (MIC) values were determined by microdilution method. Ampicillin, gentamicin sulfate, ofloxacin, tetracycline, ceftriaxone, meropenem and amoxicilin-clavulanic acid were used as the reference. Antibacterial activity results of the synthesized compounds are summarized in **Table 1**. The results indicated that among the synthesized compounds, compound **5** displayed antibacterial activity against various tested bacterial strains when compared to the other synthesized compounds. All of the test compounds did not show significant activity against *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* isolates while they exhibited moderate inhibitory effect with MIC values between 64 and 32 µg/ml against *P. aeruginosa* ATCC 27853 and *P. aeruginosa* isolate.

In order to determine the QS inhibitory potential of the synthesized compounds, they were initially screened by QSIS1 assay based on recombinant bacteria background giving rise to a blue circle of growth on X-Gal supplemented medium at concentration (sub-MIC) as described by Rasmussen et al. [28]. Patulin used as a positive control. Of the 18 compounds screened, significant QS inhibitory activity was detected in Compounds **1, 6, 11** and **16** (**Fig. 4**). *N*-acyl derivatives of these compounds did not show any inhibition. Compound **6** (chlorzoxazon) was discovered previously to be an inhibitor of QS in *P. aeruginosa*[20]. However, there is no published data on anti QS activities of compounds **1, 11** and **16**. In agreement with Yang's study [20], our findings have further verified Compound **6** (chlorzoxazon) as a QS inhibitor. Interestingly, Compounds **1** and **16** displayed a higher level of inhibition in QSIS1 assay than known QSI compound **6** (chlorzoxazon) (**Fig. 4**).

We tested the effects of compounds **1, 6, 11** and **16** on the production of 3 QS-regulated virulence factors such as elastase, biofilm formation and swarming motility of human pathogen *P. aeruginosa* PA01 strain. The production of elastase by the *P. aeruginosa* PA01 strain was examined by the Elastin Congo Red assay [29]. *P. aeruginosa* PA01 was grown in the presence and

Table 1 Antibacterial activities of the synthesized compounds.



Comp.	R^1	R^2	R^3	A	B	C	D	E	F	G
1	H	H	H	128	128	64	64	32	64	128
2	H	H	Heptanoyl	128	128	128	64	64	64	128
3	H	H	Octanoyl	128	128	128	64	64	64	128
4	H	H	Nonanoyl	128	128	128	64	32	128	128
5	H	H	Decanoyl	64	64	64	64	32	32	64
6	Cl	H	H	128	128	64	64	32	128	128
7	Cl	H	Heptanoyl	128	128	128	64	64	64	128
8	Cl	H	Octanoyl	128	128	128	64	64	64	128
9	Cl	H	Nonanoyl	128	128	128	64	32	128	128
10	Cl	H	Decanoyl	128	128	128	64	64	64	128
11	H	CH_3	H	128	128	128	64	32	128	128
12	H	CH_3	Heptanoyl	128	128	128	64	64	32	128
13	H	CH_3	Octanoyl	128	128	128	64	64	64	128
14	H	CH_3	Nonanoyl	128	128	128	64	32	128	128
15	H	CH_3	Decanoyl	128	128	128	64	64	64	128
16	CH_3	H	H	128	128	128	64	32	128	128
17	CH_3	H	Heptanoyl	128	128	128	64	64	128	128
18	CH_3	H	Nonanoyl	128	128	128	64	32	128	128
Ampicillin				4	64	64	–	–	2	64
Gentamicin				0.25	0.25	256	0.25	2	<0,0125	<0,0125
Ofloxacin				0.015	–	32	1	2	0.25	0.5
Tetracycline				0.5	–	256	8	8	0.25	4
Ceftriaxone				0.12	–	512	64	64	2	<0.25*
Meropenem				<0,0125	<0,0125	<0,0125	0.25	4	<0,0125	<0,0125
Amoxicillin/clavulanic acid				4	8	64	–	–	1	64

A: *E. coli* ATCC 25922, **B:** *E. coli* ATCC 35218, **C:** *E. coli* isolate, **D:** *P. aeruginosa* ATCC 27853, **E:** *P.aeruginosa* isolate, **F:** *K. pneumoniae* RSKK 574, **G:** *K. pneumoniae* isolate, **E.coli* and *K. pneumoniae* isolates have ESBL and *P. aeruginosa* isolate is resistant to ceftriaxone

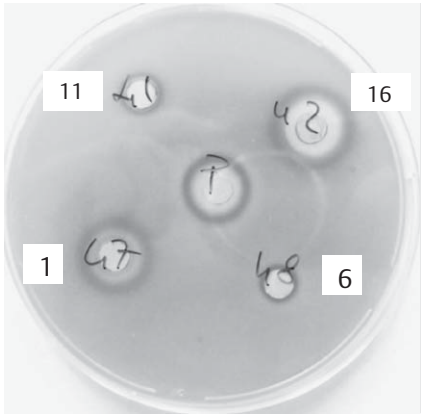


Fig. 4 Samples screened for QSI activity using QSIS1 strain P; Patulin (positive control), compounds **1, 6, 11 and 16**.

absence of compounds **1, 6, 11** and **16**. As shown in ◉ **Fig. 5**, all compounds, showed significant reductions in the elastase production. Among them, compound **6** had the greatest inhibitory activity (83.1%). This was followed by compounds **11, 16** and **1** which decreased elastase activity by 42.2%, 34.1% and 20.4%, respectively. QS has been reported to play an important role in the maturation of *P. aeruginosa* biofilms and biofilm related antibiotic tolerance [30–32]. We, therefore, examined the effect of compounds **1, 6, 11** and **16** on *P. aeruginosa* PA01 strain biofilm formation. Biofilms were grown on LB medium in 96-well polystyrene plates in the presence or absence of sub-MIC concentrations of the compounds **1, 6, 11** and **16.** Treatment of *P. aeruginosa* PA01 strain with these 4 compounds resulted in significant reduction in the biofilm formation capacity as follows: PA01 100% production, compound **1** 60%; compound **6**, 53.8%; compound **11**, 47.7%; compound **16**, 46.5% (◉ **Fig. 6**). The compounds did not inhibit growth at the concentration used.

The ability of the compounds to reduce the swarming motility of PA01 was also tested [33]. The results showed that swarming motility was significantly reduced by these four compounds compared to untreated *P. aeruginosa* PA01 (◉ **Fig. 7**). We confirmed that the compounds do not affect the growth of *P. aeruginosa* PA01 at concentrations used in the swarming assay (data not shown). Yang et al. [20] have reported that chlorzoxazon (Compound **6**) is capable of decreasing the production of a range of QS-regulated virulence factors such as exogenous proteases, pyoverdin, rhamnolipid and biofilm formation in *P. aeruginosa.* In this study, we showed that elastase production and swarming motility in *P. aeruginosa* were also inhibited by chlorzoxazon. In addition, Yang et al. [20] also discovered that salicylic acid and nifuroxazide were capable of interfering with QS in *P. aeruginosa.* Up to now, a large number of synthetic QS inhibitors have been discovered. A range of recognized drugs have been shown to have quorum sensing inhibitory activities. For example, some macrolide and nonmacrolide antibiotics have been shown to have effects upon AHL-mediated quorum sensing in Gram-negative bacteria. Skindersoe et al. [34] identified that ceftazidime and iprofloxacin reduced QS-regulated gene expression in *P. aeruginosa.* The sub-inhibitory concentration of antibiotic

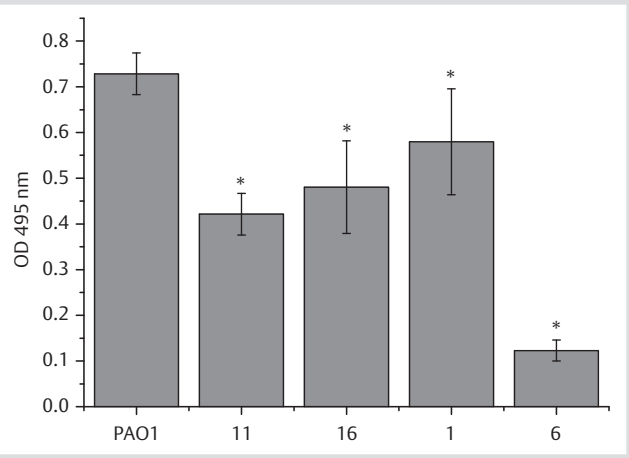


Fig. 5 Inhibition of elastase production in *P. aeruginosa*. PA01, untreated; 11, compound **11**, treated PA01; 16, compound **16** treated PA01; 1, compound **1** treated PA01; 6, compound **6** treated PA01. Values represent the mean of three independent experiments ± SD.

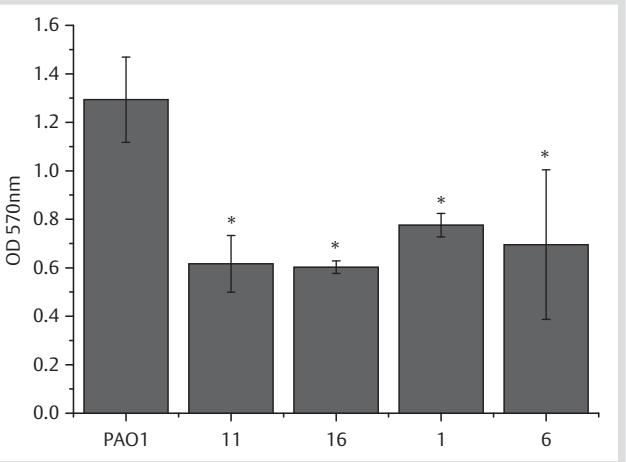


Fig. 6 Results of biofilm formation capacity. PA01, untreated; 11, compound **11**, treated PA01; 16, compound **16** treated PA01; 1, compound **1** treated PA01; 6, compound **6** treated PA01. Values represent the mean of 3 independent experiments ± SD.

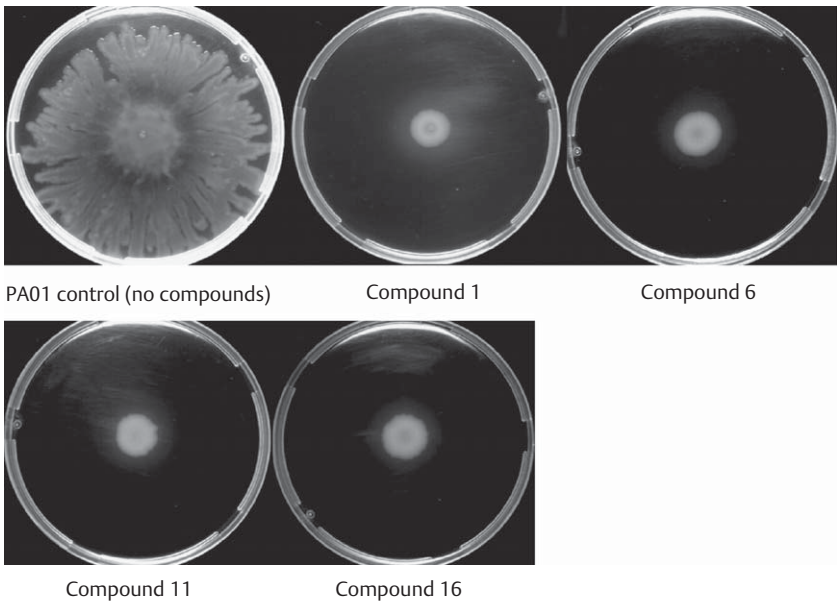


Fig. 7 Effect of compounds **1**, **6**, **11** and **16** on swarming motility of *P. aeruginosa* PA01.

tobramycin has also been reported to reduce elastase production in *P. aeruginosa*[35]. Although compounds **1, 11** and **16** synthesized in this study are known molecules, anti QS activities of these compounds have not been reported earlier.

Conclusions

In this study, the effects of compounds **1**, **6**, **11**, **16** and their *N*-long chain acyl derivatives on the production of extracellular virulence factors of *P. aeruginosa* were investigated. Our results showed that four compounds, compounds **1**, **6**, **11**, and **16**, but not their *N*-long chain acyl derivatives, inhibited quorum-sensing regulated phenotypes such as elastase production, swarming motility, and biofilm formation in *P. aeruginosa*. Among these compounds, compound **6** (chlorzoxazone) was identified previously to be a QS inhibitor. Our screening identified 3 new QSIs, compounds **1, 11** and **16**. The capability of these compounds to inhibit bacterial QS has not been reported earlier. In conclusion, these compounds appear to provide a starting point for the design and development of the novel and more active QS inhibitors that restrict pathogenesis of *P. aeruginosa* and other clinically significant pathogens. The interruption of QS system can render pathogenic bacteria non-virulent.

Conflict of Interest

The authors declare that they have no conflict of interest.

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