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REVIEW

Quorum Quenching: A Potential Target for Antipseudomonal Therapy

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Fatemeh Hemmati[1,2]
Roya Salehi[3]
Reza Ghotaslou[4]
Hossein Samadi Kafil[3,4]
Alka Hasani[4]
Pourya Gholizadeh[1,2]
Roghayeh Nouri[1,2]
Mohammad Ahangarzadeh Rezaee[1,4]

[1]Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; [2]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran; [3]Drug Applied Research Center, Tabriz University of Medical Science, Tabriz, Iran; [4]Department of Medical Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract: There has been excessive rate of use of antibiotics to fight *Pseudomonas aeruginosa* (*P. aeruginosa*) infections worldwide, which has consequently caused the increased resistance to multiple antibiotics in this pathogen. Due to the widespread resistance and the current poor effect of antibiotics consumed to treat *P. aeruginosa* infections, finding some novel alternative therapeutic methods are necessary for the treatment of infections. The *P. aeruginosa* biofilms can cause severe infections leading to the increased antibiotic resistance and mortality rate among the patients. In this regard, there are no approaches that can efficiently manage these infections; therefore, novel and effective antimicrobial and antibiofilm agents are needed to control and treat these bacterial infections. Quorum sensing inhibitors (QSIs) or quorum quenchings (QQs) are now considered as potential therapeutic alternatives and/or adjuvants to the current failing antibiotics, which can control the virulence traits of the pathogens, so as a result, the host immune system can quickly eliminate bacteria. Thus, the aims of this review article were presenting a brief explanation of the research reports on the natural and synthetic QSIs of *P. aeruginosa*, and the assessment of the current understanding on the QS mechanisms and various QQ strategies in *P. aeruginosa*.

Keywords: *Pseudomonas aeruginosa*, quorum quenchings, quorum sensing, nanoparticle, natural compounds, synthetic compounds

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is a Gram-negative pathogen[1,2] that causes acute and chronic infections in immunocompromised patients such as cystic fibrosis and burn patients.[3] The treatment of *P. aeruginosa* by conventional antibiotics has become very difficult, due to the rise of multi-drug resistance strains. Therefore, there is an urgent need to find some novel antimicrobial agents and recognize novel approaches to treat or prevent bacterial infections.[4–7]

The quorum sensing (QS) plays a critical role in multi-drug resistance of *P. aeruginosa*, which can upregulate both biofilms-associated matrix and efflux pump genes to improve resistance of bacteria against antibacterial agents.[8] A new promising approach to treat *P. aeruginosa* infections is its QS blocking without killing any of the target bacteria.[3] Efforts to disturb bacterial biofilms and reduce the expression of efflux pump genes have provided the recognition of molecules produced by prokaryotes and eukaryotes with the capability of inhibiting the QS signals, named as quorum quenchings (QQ) or quorum sensing inhibitor (QSI). Since QQs do not effect the growth of bacterial, they do not inflict potent selective pressures on the increased resistance compared to antibiotics. Therefore, they have been considered as an ideal target for novel anti-virulence drugs.[9–11] The QQs could significantly affect the

Correspondence: Mohammad Ahangarzadeh Rezaee
Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
Tel + 98-4133364661
Email rezaee.mohammad@gmail.com

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treatment of a broad range of pathogenic bacterial infections.[12] Moreover, they can aggressively control the QS signals as well as providing a chance to improve novel agents against QS signals to fight pathogens.[13]

The aims of this review article were presenting a brief explanation of the research reports on the natural and synthetic QSIs of *P. aeruginosa*, and the assessment of the current understanding on the QS mechanisms and various QQ strategies in *P. aeruginosa*.

Quorum Sensing

Bacteria at low cell densities behave like single cellular organisms; however, when their population density reaches the concentration threshold, they may change their behavior to the "multicellular" type via sensing. In this step, they communicate via autoinducers (AIs), which enable them to express genes for various phenotypes, particularly those that are responsible for their virulent behavior.[14] This system, known as the bacterial QS, can be divided into several steps. In the first step, signaling molecules, also called AIs, are produced by the bacterial cell that are then released either actively or passively into the surrounding environment. After reaching the concentration threshold, signal molecules can be recognized by specific receptors and the signal molecules can lead to some changes in the gene's expression and regulation.[15] Opportunistic bacteria such as *P. aeruginosa* select to lie "dormant" and postponement their virulent factors until their population density has sufficiently increased to overcome the host's defense systems.[13]

The QS systems operate via a broad range of signals as follows: (a) Oligopeptides,[16] (b) N-acyl homoserine lactones (AHLs, AI-1),[16] (c) Furanosyl borate (AI-2),[13,14] (d) Hydroxyl-palmitic acid methyl ester,[13,14] (e) Methyl dodecanoic acid,[14] and (f) Diffusible signal factor (DSF); cis-11-methyl-2-dodecenoic acid.[17] In addition, the two most broadly studied signaling molecules are as follows: (1) Peptide based QS system or oligopeptides, containing between five and 34 amino acid residue, which are generally involved in intercellular communication in Gram-positive bacteria, and (2) AHLs, which differ in the length and oxidation state of their acyl side chains and are produced by Gram-negative bacteria to screen their population density in QS control of gene expression (Figure 1).[4,13,18–20] Also, many other signaling molecules have been known that can act as QS signals. Among these signaling molecules, the cis-2-unsaturated fatty acids are included, often referred to as DSF family signals. The first molecule of the DSF family, cis-11-methyl-2-dodecenoic acid, was discovered in phytopathogen *Xanthomonas campestris* pv. *campestris*. Subsequently, other DSF family signals were also described in *Burkholderia cenocepacia* and *P. aeruginosa*, which can synthesize cis-2-dodecenoic acid (BDSF) and cis-2-decenoic acid (PDSF), respectively.[17,21]

There is a significant correlation among QS and the pathogenic factors production, motility, plasmid transfer, antibiotic production, and biofilm formation. Accordingly, the QS system facilitates the population to live and multiply in a better environment with effective intercellular communication, since it can contribute to several behaviors that enable bacteria to resist antibacterial compounds or antibiotics like biofilm development.[22] Due to the significance of bacterial communication in the expression of pathogenic factors, QQ can be considered as a potential target to prevent bacterial infection.[23]

Quorum Sensing Systems in *P. aeruginosa*

Of Gram-negative bacteria, *P. aeruginosa* is the common pathogen in the AHL AIs studying and has been extensively used for performing studies on QS.[4] The *P. aeruginosa* has four QS systems as follows: LasI/LasR, RhlI/RhlR systems, which both are two AHL-based signaling systems, as Pseudomonas quinolone signal (PQS), which is a non-AHL-based signaling system, as well as the newly identified QS named integrated QS signal (IQS) (Figure 2).[24–26]

In *P. aeruginosa*, Las and Rhl are two critical QS systems represented by LasI/LasR and RhlI/RhlR system. N-3-oxo-dodecanoyl homoserine lactone (3OC12-HSL) is synthesized by LasI synthase in las system, which forms a LasR-3OC12HSL complex by binding to LasR and can initiate the transcription of many virulence genes such as *lasB, apr*, and *toxA*.[18,24] This complex indues the expressions of LasI and RhlR; therefore, both AHL-based QS systems can be positively regulated.[18] The RhlR is one of the QS transcription factors that is able of autoinduction, as well as replying to N-butanoyl-homoserine lactone (C4-HSL) produced by RhlI. The RhlR-C4HSL complex indues the expression of pathogenesis factors such as pyocyanin, rhamnolipid, and elastase. In addition, RhlR has no direct effect on the LasR system.[18,24,27,28]

The PQS system can be mediated by 2-alkyl-quinolones.[27] In this regard, the pqsABCDE and pqsH systems are two members of quinolone-dependent QS system, which directly synthesize PQS and HHQ (4-hydroxy-2-heptylquinoline)

signals, respectively. Although pseudomonas quinolone signal positively regulates the Rhl QS system and indues RhlR expression, it has no direct effect on the LasR system. Also, HHQ and PQS signals link to the transcriptional regulator pqsR, and besides, positively modulate the expression of pathogenic factors such as production of biofilm and swarming and twitching motilities.[18,29–31]

The 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde is a novel class of QS signal molecules, which belongs to IQS that has been recognized in *P. aeruginosa*, and is able to integrate the environmental stress cues with the QS system. The ambBCDE cluster encodes the enzymes for L-2-amino-4-methoxy-trans-3-butenoic acid (AMB) biosynthesis occurring through a non-ribosomal peptide synthase (NRPS) pathway that directly synthesize the IQS. When IQS biosynthesis is disturbed, it can disable the Pqs and Rhl systems, which attenuate the pathogenicity of bacteria.[25,31,32] In addition, the IQS contributes to the greater pathogenicity of *P. aeruginosa* in several animal models such as mice,[14] zebrafish,[14] fruitfly and nematode.[32] In addition, IQS is able to sense the phosphate-reduction stress by networking with the QS system. Therefore, in the phosphate reduction stress situation, it can partly take over the purposes of the Las system, which provides some important clues in comprehending the confusing phenomenon.[25,32]

Overall, the QS signals are hierarchically organized as follows: Las system positively regulates Rhl, Pqs, and Iqs systems.[32] Moreover, the RhlI/RhlR and the PqsABCDE/PqsR systems regulate each other, and besides, the ambBCDE/IqsR system regulates the PqsABCDE/PqsR system.[26] In addition, each one of these systems is modified by a collection of extra regulators in both transcriptional and post-transcriptional steps.[26]

Quorum Quenching

Due to the increased antibiotic resistance among the human bacterial pathogens and the current poor effects of antibiotics to treat bacterial infections, finding novel alternative antimicrobial approaches is urgently needed.[5–7,33] Correspondingly, one of these approaches is targeting the expression of the QS-regulated pathogenic factors using the analogs of the QS molecules. In this regard, they have been developed as

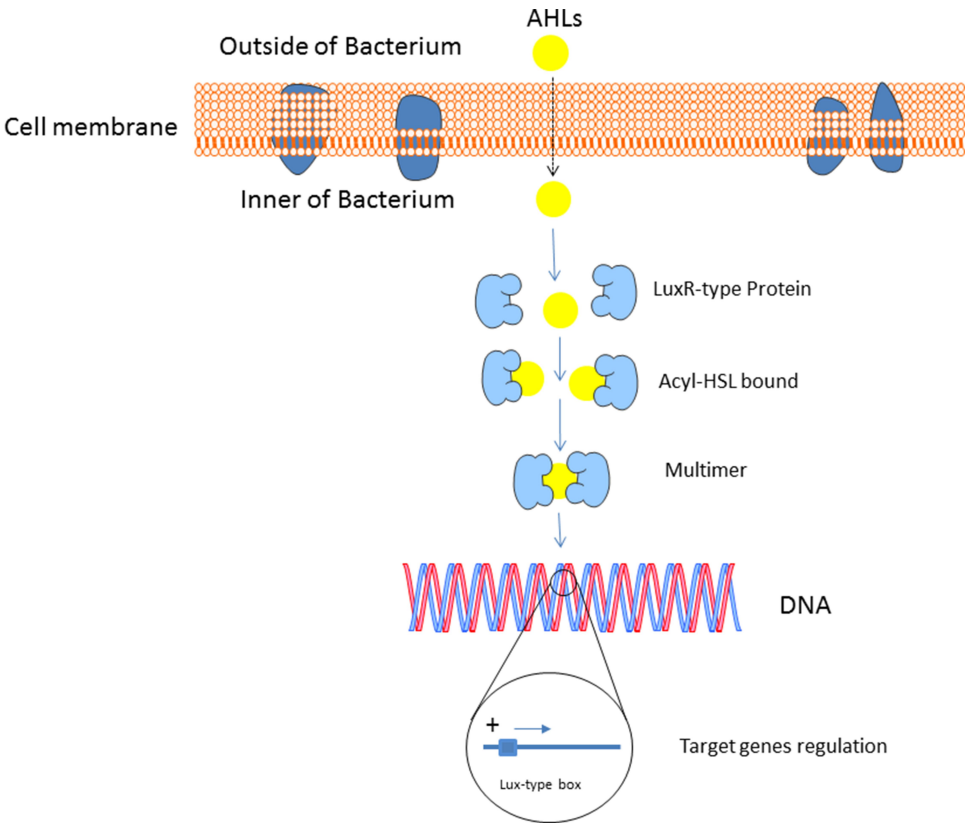

Figure 1 The LuxR/AHL-mediated quorum sensing the regulation of the target genes' expressions in *P. aeruginosa*. At a threshold level of AHL, a positive feedback loop is formed causing more AHL to be synthesized. Afterward, the reaction takes place between the AHLs and the LuxR receptor in the cytoplasm of the cell, leading to target genes' expression of quorum sensing.

different methods to produce novel antimicrobial agents. This aim can be attained by the control of the synthesis of AIs and their contact with the receptors, as well as the raise of their disintegration.[26] In this case, several natural and synthetic compounds have been characterized, which can degrade and also inactivate the QS molecules (Figure 3).

The system of QS can be disturbed via various methods including: (a) decreasing in the activity of the AHL synthase, (b) inhibition of the production of AHLs, (c) degradation of the AHLs, and (d) the use of various compounds as the antagonists of the signaling molecules.[13,34,35]

Natural Compounds

Natural-originated compounds are always taken into consideration in medical fields, because they are biodegradable and usually very useful, so they serve as a convenient compound for the inhibition of biological infection. Several studies have shown that the use of natural eukaryotic or prokaryotic derived compounds can reduce the bacterial virulence and modulates QS.[4,36-40] In this regard, natural compounds are assumed to be better than other QSIs, so for this reason, they can be used more confidently for a prolonged time and can reach the situation of GRAS (generally recognized as safe).[41]

Prokaryotic QSIs

In recent years, several QSIs have been reported in bacteria. Singh et al[42] in their study showed that *Delftia tsuruhatensis* SJ01 isolated from the rhizosphere has the AHL degrading activity as well as an anti-biofilm potential. Secondary metabolites of *Vibrio alginolyticus* inhibit the *P. aeruginosa* PAO1 virulence factors by downregulating the motility ability, elastase activity, and rhamnolipid production. In addition, the *Vibrio alginolyticus* extract inhibits the production of biofilm in *P. aeruginosa* PAO1.[43]

Moreover, some species of bacteria are capable of producing QQ enzymes that are as follows: (I) Firmicutes: *Arthrobacter, Bacillus*, and *Oceanobacillus*; (II) Actinobacteria: *Rhodococcus* and *Streptomyces*; (III) Proteobacteria: *Acinetobacter, Agrobacterium tumefaciens, Alteromonas, Comomonas, Halomonas, Hyphomonas, Klebsiella pneumoniae, P. aeruginosa, Ralstonia, Stappia*, and *Variovorax paradoxus*; (IV) Bacteroidetes: *Tenacibaculum*; and (V) Cyanobacteria: *Anabaena*.[14,44-49]

Prokaryotes have three types of enzymes that target the AHLs and play an essential role in QQ including AHL lactonases, AHL acylases, and AHL oxidoreductases.[40,41]

AHL-Lactonase Enzymes

One of the metallo-β-lactamase (MBL) family members is AHL-lactonases, which can hydrolyze the lactone ring.[50] Owing to conservation of targeted homoserine lactone ring among all the AHLs and nonspecific interactions within the active-site cavity of the enzymes that are created by variable acyl chains, AHL lactonases have a very broad AHL substrate specificity.[44–47] Autoinducer inactivation gene (*aiiA*), as the first described AHL lactonase, was

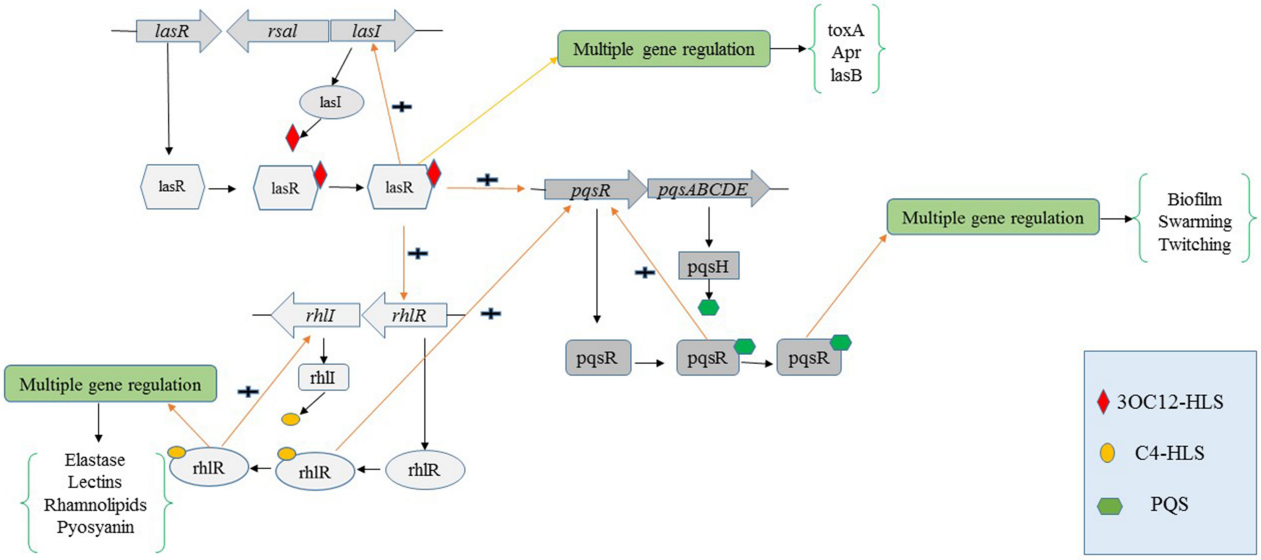

Figure 2 Graphical plot of the quorum-sensing system of *P. aeruginosa*. The AIs 3OC12-HSL, C4-HSL, and PQS/HHQ are synthesized by the AIs synthases, LasI, RhlI, and PqsABCDE, respectively. AIs are also identified by the receptors in the cell cytoplasm LasR, RhlR, and PqsR. Protein receptors in the cell cytoplasm regulate the expression of its corresponding AIs synthase as well as new targets, as demonstrated by the arrows. Arrows labeled (+) demonstrate a positive feedback.

identified in *Bacillus* spp. Correspondingly, the expression of *aiiA* alleles in some pathogenic bacteria such as *P. aeruginosa* and *Burkholderia thailandensis*, declined AHL accumulation and also changed the QS-dependent behaviors.[51–53]

Other AHL-lactonase enzymes could alter the production of virulence factors in *P. aeruginosa* PAO114 like Mom1 in Muricauda olearia, interfere with swarming motility of *P. aeruginosa* like HqiA in *Pectobacterium carotovorum*,[18,54] or could decrease the production of pathogenic factors like proteases and pyocyanin, as well as decreasing the biofilm production of *P. aeruginosa* like Ssopox in *Sulfolobus solfactaricus*.[10]

AHL-Acylase Enzymes

The AHL acylases belongs to the novel family of N-terminal nucleophile (NTN) that cleave the acyl side chains in the homoserine lactone, resulting in disabling the AHLs. The acylase enzymes are also recognized as amidase enzymes, which hydrolyze the amide bond between the acyl chain and the homoserine lactone ring.[55] Moreover, there are many different types of acylases in terms of the various acyl chain exchanges on AHLs. Also, for the first time, the deacylation activity of AHL was detected in *Variovorax paradoxus* VAI-C. Accordingly, this strain can use AHLs as a source of nitrogen and energy.[56–58]

Acyl homoserine lactone acylases have been found in numerous bacteria, such as AhlM in *Streptomyces* sp. strain M664,[59] PvdQ and QuiP in *P. aeruginosa* PAO1,[58,60] AiiC in *Anabaena* sp. strain PCC7120[61] and AiiD in *Ralstonia* sp strain XJ12B.[62] Investigating AiiD enzyme of *P. aeruginosa* PAO1 showed that it is very diverse and shared only 39% similarity at the amino-acid level.[13,57] AiiD homologs have also been identified in numerous of *Pseudomonas* spp., which can have AHL-acylase activity.[58,62,63] Notably, the AHL acylases could potentially modulate bacterial behavior by interfering with the production of virulence factors. Also, motility phenotypes in *P. aeruginosa* PAO1 can be changed by the expression of AHL acylases *aiiD*.[62,64]

The *P. aeruginosa* PAO1 contains four acylases homologues, which belong to NTN hydrolase enzymes including PvdQ (PA2385), QuiP (PA1032), HacB (PA0305), and PA1893. Accordingly, PA1893 is the known member of QS-regulons, while the other homologues have acylase activities.[18,57] Also, NTN hydrolase enzymes can decrease the secretion level of pathogenic factors in *P. aeruginosa*.[18,55,65]

AHL-Oxidoreductase Enzymes

The AHL oxidoreductase modifies the chemical structure of the AHLs by oxidizing or decreasing the acyl side chain at the third carbon position without damaging the AHLs.[57] In general, to the best of our knowledge, there are few studies performed on the disabling of AHLs through the oxidation of the acyl side chain compared to the AHL degradation by lactonases and acylases. Accordingly,

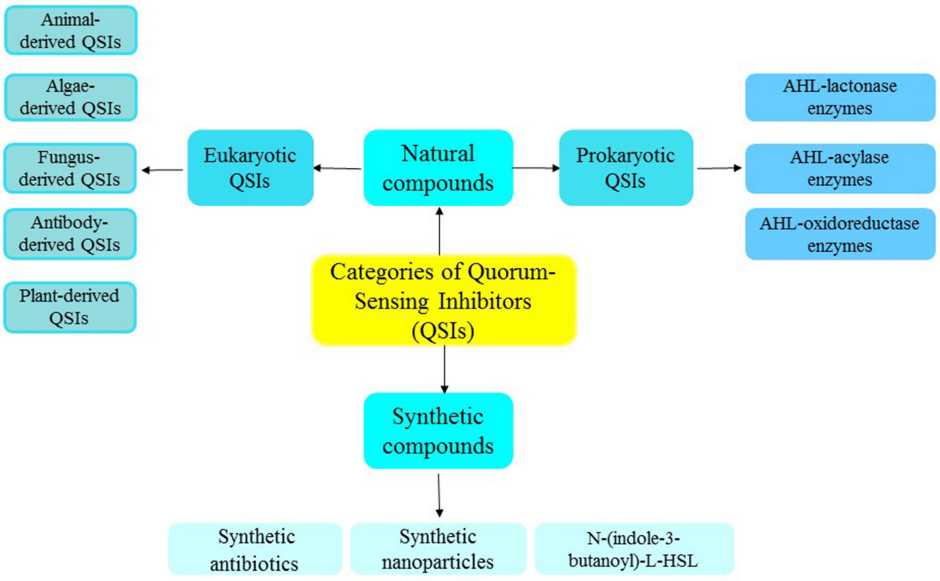

Figure 3 Schematic diagram demonstrating quorum sensing inhibitors compounds of *P. aeruginosa.*

these enzymes were firstly observed in *Rhodococcus erythropolis* that is capable of using a range of AHLs as nitrogen and carbon sources.[18,55,66] In recent years, a novel oxidoreductase, known as BpiB09, has been identified that can inactivate 3OC12-HSL. In addition, oxidoreductase BpiB09 in *P. aeruginosa* PAO1 reduces the accumulation of AHLs, followed by reducing motility phenotypes, pyocyanin secretion, and biofilm production.[18,55]

Eukaryotic QSIs

The eukaryotic-derived compounds including animals, antibodies, plants, fungus, and algae derived compounds are capable of interfere in bacteria cell-to-cell signalling molecules.[4] In this regard, they are usually used in medical field, since they are bio-compatible, usually very efficient, and known as excellent candidates for biological anti-infectious approaches. So, studies have suggested the use of the eukaryotic-derived compounds to decrease bacteria pathogenicity and QS modification.[36,67]

It has been recognized that animals have evolved multiple defence strategies including anti-microbial peptides, lysozymes, and antibodies to protect themselves against bacterial pathogens. Additionally, interactions between animal hosts and pathogenic bacteria provoke a broad range of reactions, particularly in the presence of QS molecules.[13,68] Enzymes of QQ have been discovered in several animals such as mice, rats, and zebrafish. Notably, the enzymes of QQ have been discovered in several animals such as mice, rats, and zebrafish. Acylase I enzyme of porcine kidney could disable QS signals of N-Hexanoyl-L-homoserine lactone (C6HSL) and 3OC12HSL; however, it was shown that it has no effect on C4HSL.[14] Acylase I can have effect on diminishing biofilm production by *Aeromonas hydrophila* and *Pseudomonas putida*.[69] A group of mammalian enzymes known as paraoxonases 1, 2, and 3 (PON) has been found to have hydrolytic activities on esters and lactones, which are relevant to drug metabolism and detoxification of the nerve agents. PON-lactonases vary from prokaryotic lactonases due to the loss of the "HCDH~H~D" motif, and besides, they need calcium ion for their functions.[13,70,71] The human epithelial cells and mammalian sera have PON enzymes, which are able to disable and destroy AHLs.[18] Several studies have shown that the PON by an active site, can hydrolyze many various substrates such as lactones, esters, and phosphotriesters.[18,72,73]

Antibody based QSI as one of the methods for anti-infective therapy have been proposed to inhibit QS signals.[35] The anti-QS activity of antibodies was firstly reported by Sandra De Lamo Marin's group.[74] They revealed that some of the anti-AHL antibodies could inhibit the 3OC12-AHL-based QS system. In this regard, XYD-11G2 is one of the most efficient antibodies that have capability of inhibiting 3OC12HSL in *P. aeruginosa*.[74] It has been shown that the generation of the monoclonal antibody RS2-IG9 against the 3OC12HSL analog RS2 could be efficient on destroying 3OC12HSL of *P. aeruginosa*.[75] Also, some studies have shown that antibody catalysis could create a novel approach for the inhibition QS in bacteria.[35,74]

Plant-derived compounds are mostly secondary metabolites that are used for their antibacterial properties, since many years ago. Moreover, they have been used for decreasing bacterial virulence and production of biofilm.[4] Notably, many natural compounds have been described as QSIs, and some of the most promising QSI molecules have been found in various plants. These compounds can serve as both autoinducer agonists and antagonists.[18,76,77] Also, herbal extracts can act as QSIs, which are structural analogs of AHLs and disrupt the QS system by binding to LuxR/LasR-receptors.[78,79] Some of these herbal extracts that act as QSIs as well as their effects on *P. aeruginosa* are shown in Table 1.

The QSI has been also reported from algae. Some studies have shown that several algal species have natural defense strategies to inhibit microbial accumulation.[4,23] Regarding this, halogenated furanones created by the red marine macroalga *Delisea pulchra* were firstly recognized as anti-QS compounds.[80,81] *D. pulchra* can produce some of the nontoxic and halogenated metabolites, especially brominated furanones that can act as mimics of the bacterial AHL QS signals[23] and also inhibit the QS-regulated behaviors by competitively linking to the LuxR-receptors.[80,81] Moreover, these compounds are known as structural analogs of AHLs, which control the QS regulation in various bacteria to prevent biofilm formation and subsequent accumulation effectively.[23,82] Most of the algal QS modulators investigations have been conducted on the furanones and their derivatives.[4] Furanone-mediated inhibition of QS signaling indicated some significant effects on the disruption of expression of *P. aeruginosa* virulence gene. It is noteworthy that, treatment of bacteria with low concentrations of furanones decrease the secretion of exoprotease enzymes, pyoverdin production, and biofilm

formation. The AHL mimic compounds, as halogenated furanones from *D. pulchra*, serve in the inhibition of the QS-regulated responses.[82] The disruption of QS by furanones can result in the improved animal survival after being exposed to the lethal dose of *P. aeruginosa* inoculations.[18]

Fungal secondary metabolites can also act as QSI. Penicillic acid and patulin are the secondary metabolites produced by of *Penicillium* spp., which are able to inhibit the QS system.[83] Equisetin is a secondary metabolite of marine-derived fungi that could inhibit the biofilm production, motility phenotypes, and other pathogenic factors in *P. aeruginosa*. It could also downregulate the expression of *lasB, lasI, lasR, pqsA, pqsR, rhlA, rhlI*, and *rhlR* genes.[84]

Synthetic Compounds

QSIs production is naturally occurred in a large number of organisms; however, their main limitation is the low levels, in which they are generated as well as the related toxicity in some cases.[13] In recent years, control of bacterial virulence using chemical compounds has considerably received many attention. Therefore, these studies aimed to extend targeted synthetic QS regulators.[4,13] The process of the inhibition of QS by synthetic compounds can be followed by various mechanisms in *P. aeruginosa* as follows (Table 2): I) synthetic signal analogs II) modifications in the AHL side-chain, III) modifications in the AHL ring moiety, and IV) antagonists of the receptor-ligand interactions.[13,18]

In recent years, nanoparticles (NPs) have received many consideration, due to their antimicrobial activities. Moreover, the use of NPs is one of the most promising strategies to fight against microbial drug resistance.[85] Suitable therapeutic compounds are not only required to display a low toxicity index, but also to have appropriate pharmacokinetic features for clinical usages.[86] Nanoparticles are also recognized as synthetic QSIs. However, more studies are required to explore these nanoparticles additionally. Researchers have recently utilized nanotechnology for the extension of innovative nanomaterials targeting QS-regulated pathogenic factors, which create new insights on the expansion of some alternative antibacterial treatments.[87,88]

Chitosan is a cationic polysaccharide formed by N-acetylglucosamine and glucosamine. Correspondingly, it is linked by β-(1, 4) glycosidic linkages,[89] showing many unique properties, such as bio-compatibility,[89] biological activity,[90] nontoxicity,[91] bioadhesion,[92] anti-hypercholesterolemia,[92] antioxidant[93] and antimicrobial activity.[94] Moreover, chitosan and its derivatives have an anti-biofilm activity.[95,96] Also, the antimicrobial properties of chitosan, due to the presence of its positive charge amino groups, can react with negatively charged lipopolysaccharides of *P. aeruginosa*, which can consequently inhibit the bacterial proliferation.[85,97] Chitosan derivatives such as chitosan NPs have also shown biological activities against microorganisms. Recent studies have shown that the enhanced antimicrobial activity of chitosan NPs is related to the increase of surface area to volume ratio as the particle size decreases. Therefore, the chitosan NPs with vast surface areas modifies the bacterial membrane penetrability via the membrane incorporation and also leads to the death of bacteria.[33,97-99] Furthermore, chitosan NPs have shown anti-QS properties by disrupting biofilm production, decreasing the expression of *lasR* and *rhlR* genes, and reducing the secretion of pyocyanin and proteases in *P. aeruginosa*.[100] In addition, chitosan NPs have a negative effect on the pathogenic factors produced by *P. aeruginosa* PAO1.[101] In this regard, Muslim et al,[100] in their study demonstrated that chitosan significantly decrease the biofilm formation, pyocyanin, protease secretion, as well as the expressions of *lasR* and *rhlR* genes in *P. aeruginosa*. In addition, Ilka et al reported that the loaded kaempferol into chitosan NPs have QSI activity and suggested that a combination of materials with chitosan NPs can inhibit the QS-related encoding genes, which can serve as a new approach for antibacterial treatment acting as the QS-based antibiofilm agents.[95]

In recent years, zinc oxide (ZnO) NPs have been recognized as efficacious QSIs for *P. aeruginosa* PAO1, by reducing the generation of different pathogenic factors with no growth-inhibitory efficacy.[102] Lee et al[102] demonstrated that Zn^{2+} and ZnO NPs have no bactericidal activities against *P. aeruginosa* at the concentration levels of <3 mM; however, they have antivirulence activities. In addition, generation of reactive oxygen species (ROS) on the surface of ZnO induce some serious damages to bacteria cell. Also, the attachment of the ZnO NPs on the bacterial surface or cumulating of NPs in the cytoplasm area induces disturbance of cellular action as well as the disorder of the bacterial membrane.[103,104] Lara et al reported that ZnO NPs could significantly reduce the secretion of elastase, pyocyanin, and the production of biofilm in *P. aeruginosa*, which demonstrate that ZnO NPs have

Table 1 Herbal Extracts as Quorum Sensing Inhibitors and Their Effects on the *P. aeruginosa* QS System

Quorum Sensing Inhibitor Compounds	Quorum Sensing Efficacy	Ref.
Coumarin	Production of biofilm and pathogenic factors production	[76]
Pistacia atlantica crude extract	Production of biofilm and pyocyanin secretion	[11]
Curcumin	Motility phenotypes and production of biofilm	[131]
Ginseng	Production of LasA and LasB and synthesis of the AHL molecules	[141]
Baicalin	Production of biofilm and pathogenic factors (protease, elastase, pyocyanin, rhamnolipid, motilities and exotoxin A)	[142]
Flavonoids (naturally-produced plant Metabolites)	LasR and RhlR receptors	[143]
Ajoene, a Sulfur-Rich Molecule from Garlic	Pathogenic factors production	[144]
Berberine	Production of biofilm, secretion of protease, pyoverdin, pyocyanin and the expressions of *lasI, lasR, rhlI, rhlR* genes	[133]
Trans-cinnamaldehyde	Secretion of protease, elastase, pyocyanin and production of biofilm and the expressions of *lasI, lasR, rhlI, rhlR* genes	[145]
Salicylic acid	Secretion of protease, elastase, pyocyanin and production of biofilm and the expressions of *lasI, lasR, rhlI, rhlR* genes	[145]
Phillyrin	Pyocyanin, rhamnolipid, elastase, swimming and twitching motility and biofilm formation	[146]
Trans-anethole	Swarming motility, secretion of protease, elastase, pyocyanin and the expressions of *lasB* gene	[147]
Cinnamic acid	Secretion of pyocyanin, proteases, elastase and production biofilm	[148]
Hordenine	Swarming motility, secretion of pyocyanin, elastase, rhamnolipid, production of biofilm and the expressions of *lasI, lasR, rhlI, rhlR* genes	[149]
Eugenol from clove extract	Pathogenic factors and production of biofilm	[150]
Ocimum sanctum	Secretion of pyocyanin, protease, elastase and production of biofilm	[151]
Musa paradisiaca	Pyocyanin, protease, elastase and biofilm formation	[151]
Caffeine	Motility phenotypes	[151]
Methanolic extract of Phyllanthus amarus	Motility phenotypes, pyocyanin secretion	[152]
Zingerone	Motility phenotypes, production of biofilm and pathogenic factors production	[153]
Combretum albiflorum	Elastase secretion and production of biofilm	[154]
Allium sativum (garlic) extract	Production of biofilm, elastase secretion	[155,156]
FL fraction of Psidium guajava L	Virulence factors production and production of biofilm	[157]
Clove oil	Protease, chitinase and pyocyanin secretion, swimming motility and production of biofilm	[158]
The methanol extract of fenugreek	Protease, LasB elastase, pyocyanin and chitinase production, swarming motility and production of biofilm	[159]

(*Continued*)

Table 1 (Continued).

Quorum Sensing Inhibitor Compounds	Quorum Sensing Efficacy	Ref.
The dichloromethane extract of Cordiagilletii	Pyocyanin secretion, the expression of genes *lasB, rhlA, lasI, lasR, rhlI*, and *rhlR* genes and production of biofilm	[160]
Manilkara zapota	Pyocyanin, protease and elastase secretion and production of biofilm	[151]
Diarylheptanoids from the barks of Alnus viridis and Alnus glutinosa	Pyocyanin secretion, twitching motility and production of biofilm	[161]
Gingerol	Exoprotease, rhamnolipid and pyocyanin secretion and biofilm formation	[162]
Diterpene phytol	Pyocyanin secretion, twitching motility and production of biofilm	[163]

a wide spectrum, so it can be considered as an alternative for the treatment of *P. aeruginosa* infections.[105]

Other attractive compounds are Engineered nanoparticles (ENPs), which are used as QSIs. Several recent researchers have described different mechanisms for the toxicity of ENPs as follows: (a) metal ions toxicity of ENPs,[106] (b) enhancement in the generation of reactive oxygen species,[107] and (c) the damage to DNA and proteins.[108] Also, Li et al[109] reported that different ENPs with various physicochemical traits have dissimilar effects on QS systems of *P. aeruginosa* PAO1. Although upper silver (Ag) ENPs concentrations (mg/L) induce the anti-QS activity in *P. aeruginosa* PAO1, its lower concentrations (μg/L) have shown to increase its QS activity. In contrast to Ag ENPs, ferrous (Fe) ENPs enhance the concentration level of 3OC12-HSL, but it has no effect on the other QS activities. Mohanty et al[110] also showed that Ag ENPs can decrease the release of C4-HSL, C6-HSL, 3OC6-HSL, C8-HSL, and 3OC8-HSL in *Pseudomonas syringae*. Moreover, Singh et al[111] reported that mfAg (mycofabricated Ag NPs) ENPs can decrease C4-HSL and 3OC12-HSL release in *P. aeruginosa*. In addition, mfAgNPs reduce the biofilm production and QS activities by reducing the expressions of *lasIR* and *rhlIR* genes. Furthermore, Wagh et al[112] showed that silver nanowires (SNWs) had some hopeful properties of the QS-mediated controlling of biofilm in *P. aeruginosa*. Notably, when *P. aeruginosa* was treated in various concentrations of SNWs, it was detected that the most reduction was at 4 mg/mL in the production of biofilm, without inhibiting bacterial growth. Nevertheless, the concentration enhancement (≥5mg/mL) declines the number of viable cells. Therefore, 4 mg/mL of SNWs could dramatically inhibit the production of biofilm without affecting viability, while higher concentrations could inhibit bacterial growth. Gholamrezazadeh et al[113] demonstrated that Ag NPs and benzalkonium chloride efficiently induced the *rhlR* gene expression. Prateeksha et al[114] have reported that the selenium NPs harbor a superior QSI property, antibiofilm activity, and antivirulence potential in *P. aeruginosa*. Moreover, Nafee et al[115] reported that ultra-small solid lipid nanoparticles (US-SLNs) inhibited the QS-dependent phenotype like the secretion of pyocyanin in *P. aeruginosa*.

Antibiotics as QSI

Antibiotics, besides having therapeutic efficacy in killing or inhibiting bacterial proliferation, can also act as signaling molecules, which are capable of reducing the expressions of virulence factors in bacterial populations.[116–119]

Some studies have demonstrated that several antibiotics are capable of inhibiting virulence factors in *P. aeruginosa*.[13,18,120] In this study, the reply of *P. aeruginosa* to azithromycin antibiotic was analyzed by the use of the microarray method. Also, its phenotype investigation showed that there is a link between genes regulated by QS and by azithromycin.[120] Several studies have revealed that azithromycin significantly has anti-QS activity, and the subinhibitory concentrations (SICs) of azithromycin are capable of blocking many genes regulated by QS.[120–122]

Sofer et al[123] in their study indicated that erythromycin therapy decrease the AHL production in bacteria. Another study has also shown that the treatment of *P. aeruginosa* with azithromycin reduce the C4HSL and 3OC12HSL production.[124] The SICs of macrolides and β-lactam

Table 2 Synthetic Quorum Sensing Inhibitors, Their Targets, and Effects on the *P. aeruginosa* QS system

Quorum Sensing Inhibitor Compounds	Target	Quorum Sensing Efficacy	Ref.
N-decanoyl-L-homoserine benzyl ester	AHL-mediated	Protease, elastase, rhamnolipid secretion and motility phenotypes	[164]
Synthetic triazole containing analogs of AHL	LuxR-LasR analogs	LasR receptor	[165]
N-(indole-3-butanoyl)-L-HSL	AHL-mediated	Pathogenic factors production, production of biofilm	[166]
2-heptyl-6-nitro-4-oxo-1,4-dihydroquinoline-3-	PqsR analog	Virulence factor production	[115]
N-(heptyl-sulfanyl acetyl)-L-HSL (HepS-AHL)	AHL-mediated	transcriptional regulator - LasR	[167]
N-arylglyoxamide Derivatives	LasR analog	Pyocyanin secretion	[168]
Diarylheptanoids	AHL-mediated	Production of biofilm and pyocyanin secretion	[161]
Aspirin	AHL-mediated	Virulence factor production, expressions of *lasI, lasR, rhlI, rhlR, pqsA* and *pqsR* genes and expression of *Pseudomonas* toxins *exoS* and *exoY*	[128]
Meloxicam and Piroxicam	AHL-mediated	LasR and PqsE protein	[129]
N-decanoyl-L-homoserine benzyl ester	AHL-mediated	Virulence factors production	[164]
3-nitro phenylacetanoyl HL	AHL-mediated	LasR reporter	[169]
1,3-benzoxazol-2(3 *H*)-one, 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	Unknown	Elastase secretion, production of biofilm and motility phenotypes	[170]
(*S,E*)-2-hydroxy-*N*-(3-hydroxy-5-(hydroxymethyl)-2-27 methylpyridin-4-yl)propane hydrazide (pyridoxal lactohydrazone)	Unknown	Motility phenotypes, production of biofilm and pyocyanin secretion	[171]
Diastereomeric 2-methoxycyclopentyl	AHL-mediated	Pigmentation production	[172]
benzamide-benzimidazole	Transcriptional regulator	Reduced acute and persistent pathogenicity	[12]
(S,E)-2-hydroxy-N-(2-hydroxy-5-nitrobenzylidene) propane hydrazide (lacto hydrazone)	Unknown	Motility phenotypes, production of biofilm and pyocyanin secretion	[173]
Meta-bromo-thiolactone	LasR/RhlR analogs	Secretion of pyocyanin and production of biofilm	[174]

antibiotics can reduce the pathogenic factors' expression of *P. aeruginosa* such as the diminished of exotoxin A secretion, pyocyanin, protease, DNase, and phospholipase C, as well as significantly removing the QS activity of *P. aeruginosa*.[125] In addition, SICs of tobramycin could block the expressions of *rhlI* and *rhlR* genes by decreasing C4-HSL generation. Previous studies have also confirmed the impact of tobramycin, as a signaling molecule, on virulence genes expression in the transcriptional step.[126,127]

Skindersoe et al[120] investigated several antibiotics for their capabilities in intervening with the bacterial signaling systems. Of the antibiotics used, azithromycin displayed high levels of QSI activity, followed by ciprofloxacin and ceftazidime that had potent QSI activities. Whereas aminoglycoside antibiotics, piperacillin, spectinomycin, and streptomycin had either low levels or no QSI activity. In their study, the protease secretion has been reduced by azithromycin, ciprofloxacin, and ceftazidime, as well as ciprofloxacin and ceftazidime that diminished the elastase activity.[120]

Furthermore, the nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin, piroxicam, and meloxicam are chemical compounds that can be used as the potential

inhibitors for controlling the P. aeruginosa QS signaling system as well as biofilm formation. The NSAID drugs can reduce the level of AHL-mediated quorum sensing in *P. aeruginosa* such as Las, Rhl, and Pqs.[77,128,129] Generally, some antibiotics and drugs have QSI potentials that can reduce the levels of AHL synthesis in *P. aeruginosa*. Therefore, diminishing in the bacterial population by antibiotics can result in the decreased levels of pathogenic factors.

Synergism Between QSIs and Antibiotics

A single QSI can not be very effective on bacteria, and could also lead to resistance against QSI. Therefore, a combination therapy of QSIs and antibiotics are suggested. Accordingly, this combination therapy prevents the resistance to a single QSI and can also increase the effectiveness of therapy without enhancing the toxicity of the antibiotics.[41,130]

Vadekeetil et al[41] also reported the synergistic interaction between proanthocyanidin active fraction and ciprofloxacin against *P. aeruginosa* QS. Moreover, there was a considerable decrease in the amount of the expressions of several pathogenic factors such as motility phenotypes and biofilm formation; however, it did not affect the secretion of elastase and protease. The significant suppressing effect of ciprofloxacin has been also observed on the twitching motility of *P. aeruginosa*. In addition, their study demonstrated that ciprofloxacin combined with proanthocyanidin active fraction have a higher anti-motility property on all three forms of motilities (ie, swimming, swarming, and twitching).

The inhibitory effects of ciprofloxacin on biofilm formation were also observed at SICs. However, it was found that, in the presence of proanthocyanidin active fraction, ciprofloxacin decreases biofilm production up to fivefold compared to the control sample.[41]

The synergistic efficacy of curcumin with ceftazidime and ciprofloxacin on signaling system in *P. aeruginosa* PAO1, was investigated by Roudashti et al.[131] Their findings indicated that SICs of curcumin, ceftazidime, and ciprofloxacin both alone and in combination can dramatically decrease motility phenotypes and the production of biofilm. Furthermore, these compounds, alone and in combination, can also reduce the expression of the genes regulated by QS.[131] Bahari et al[132] evaluated the synergistic efficacy of curcumin combined with azithromycin and gentamicin on signaling system in *P. aeruginosa* PAO1, and reported that the curcumin in combination with antibiotics drastically decline 3OC12-HSL and C4-HSL signals. Moreover, the above-mentioned compounds, alone and in combination, can considerably decrease motility phenotypes and biofilm production of *P. aeruginosa* PAO1.[132]

Li et al[133] also found that azithromycin and berberine could significantly reduce the production of several pathogenic factors such as biofilm production, as well as secretion of pyocyanin and elastase, and remarkably inhibition of the QS system and the expressions of the genes regulated by QS. In their study, it was also demonstrated that LasA activity was drastically decreased after the administration of azithromycin and berberine, separately and in combination.

Chanda et al[134] showed that linolenic acid and tobramycin (LNA+TOB) had significant impacts on downregulating the QS-mediated genes. Also, they have revealed that LNA+TOB therapy could inhibit motility phenotypes and reduce the development of infection. Therefore, it can be deduced that LNA+TOB is more effective on the inhibition of the pathogenic secretion and biofilm production compared to alone LNA or TOB in targeting the QS system of P. aeruginosa. Similar to these QSIs, it was observed that, Aminoglycosides in combination with resveratrol dramatically decrease the production of biofilm in comparison to each one of the agents alone, besides, it could significantly inhibit the expression of the QS regulatory genes.[135]

Bacterial Resistance to QSIs

The proposal reporting that bacteria may develop resistance to the QSIs compounds was offered for the first time in 2010.[136] The base for this assumption came from some studies demonstrating that the expression of the central QS genes was extremely diverse among various strains of the bacteria such as *Vibrio* spp. and *P. aeruginosa*.[137] Lately, resistance mechanisms to the best-QSIs have been observed in the in vitro, and also in clinical isolates indicating that the increased resistance to these types of compounds is the facility. Brominated furanone C-30 is one of the best QSIs that is effluxed by the MexAB-OpmR pump. Bacterial species that have mutations in their efflux pump-encoding genes *mexR* and *nalC* are also resistant to C-30, which was observed in *P. aeruginosa* for the first time.[138] 5-fluorouracil also is another QSI that some of the clinical isolates of *P. aeruginosa* are resistant to it.[137,139,140] It has been proposed that the probabilities of QSIs resistance are lower compared to those for conventional antibiotics. In this regard, the combination of QSIs and antibiotics to hamper biofilms formation and reduce the pathogenic factors in bacteria, can be considered as an alternative approach.

Conclusion

The QS inhibition is a broadly accepted anti-virulence and non-bactericidal mechanism. The development of diverse QS suppressing agents and the inhibition of QS mediators might be known as an evolutionary alteration that can decrease the resistance of the fouling bacteria. In addition, QS inhibition alone cannot affect the antibiotic susceptibility of bacteria. Therefore, more studies are required to demonstrate their mechanisms of action and the optimal amounts of the QS inhibitory compounds that are safe and applicable.

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