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Synthetically Modified Quorum Sensing (QS) Inhibitor: A Review

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Review Article

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ABSTRACT

The growing threat of antimicrobial resistance of human and animal origin has become a major global public health challenge in the treatment of microbial infections. The discovery and development of novel drugs such as Quorum Sensing (QS) inhibitors are urgently needed to mitigate this problem, which has greatly undermined the clinical effectiveness of conventional antibiotics. QS Systems, which are cell-to-cell communications among bacteria, allow bacteria to adapt rapidly to unfavourable conditions in their environment, promoting the formation of antibiotic-tolerant biofilm communities. It is well known that QS enhances the recalcitrant mode of growth in biofilm formation and also increases bacterial resistance and virulence to conventional antibiotics. QS inhibitors can eliminate the QS signals and hinder biofilm formation, reduce bacterial virulence and prevent antimicrobial resistance by the pathogens. Natural, semi-synthetic and synthetic inhibitors have been identified as potential QS inhibitors in which synthetically modified QS inhibitors with lactone moieties can be designed as an alternative to conventional antibiotics. This review will expand drug design researchers and pharmaceutical companies knowledge base on the development of synthetically modified QS inhibitors and their applicability in treatment.

INTRODUCTION

Bacteria have developed resistance to the various classes of antibiotics, from natural to semi-synthetic and synthetic antimicrobials, since the discovery of antibiotics (e.g. penicillin, streptomycin etc.) in the 18th century. Infectious diseases have been reported as the second-leading cause of death worldwide despite the numerous clinical antibiotics that are available, causing chronic infections. This has posed a great challenge to researchers developing natural and synthetic alternative therapeutic agents against infectious diseases without inducing antimicrobial

resistance. With the introduction of only one new class of antibiotic for over two decades, the emergence of multidrug-resistant pathogens has been reported. It is imperative to develop new antimicrobials with novel mechanisms of action to combat evolving bacterial infections [1-3]. Many pathogenic bacteria implicated in chronic infections are known to be characterized by the presence of biofilms, virulence factors and other biological behaviours *via* a chemical signalling process called QS [3].

LITERATURE REVIEW

Microorganisms in the microbial community show social cohesion by producing chemical signalling molecules or hormones that they use to communicate with one another. The mediation of this signalling mechanism is done by a process called QS *via* the secretion and perception of a quorum hormone. QS is regarded as cell-to-cell communication of various cellular or physiological processes in bacteria, comprising extracellular signalling molecules or metabolites from contributing planktonic bacteria that are detected and responded to by other planktonic members of the association. The extracellular signalling molecules or metabolites are called Autoinducers (AI). They coordinate interaction, transfer of conjugative plasmids, conjugation, the expression of specific genes, virulence factors, susceptibility to antibiotics, antibiotic resistance, antimicrobial peptide synthesis, motility, swarming, sporulation, bacterial cell adhesion, bioluminescence and the formation of biofilms, which enable bacteria to survive any stressed environment. AI concentration is determined by the cell density of bacteria, which act as individuals when cell density is low but increase the AI concentration when cell density increases, thus enhancing the attachment of AI to specific receptors and stimulating regulatory genes responsible for the collective interaction among the population [1-8].

Biofilm formation, which involves the production of extracellular polymer and an adhesion matrix is a critical requirement for bacterial adhesion and growth. This further leads to fundamental changes in bacterial growth and gene expression. The biofilm acts as a defence against antibacterial agents and radiation, thus reducing the sensitivity of bacteria. The significant challenge to the effectiveness of conventional antibiotics is the biofilm lifestyle, which is considered a suitable environment for antibiotic resistance. Virulence factors produced by bacteria during the evasion of the host's immune system are a major contributory factor to the pathogenesis of infections. QS alteration is considered an attractive therapeutic strategy against the production of virulence factors [2,4].

Biofilm is often referred to as microbial cities. The bacterial communities within the microbial city are fused into an Extracellular Polymeric Substance (EPS) of structural scaffold matrix, consisting of biomolecules, exopolysaccharides, polypeptides, a hydrated polar mixture and extracellular DNA (eDNA), which comprise 85% of the total biofilm mass. The EPS cements together and protects the microbial city against physical factors as well as antimicrobial agents. The planktonic bacteria are first attached reversibly to a suitable surface for growth during biofilm formation and remain momentary, waiting for a prompt from an environmental signal to form a less transitory relationship followed by irreversible bacterial attachment to form microcolonies in the EPS matrix (biofilm formation is initiated by a specific set of signals from bacterial species within the environment). With time, the confluences formed by the microcolonies develop more structured phenotypes with noncolonized spaces, which are then filled with bacteria covering the entire surface and the bacteria eventually re-enter a planktonic state by dispersing from the sessile structure, spreading and colonizing other surfaces [8-10].

QS molecules coordinate the behaviour of the bacteria in a population density-dependent manner, which is related to Cyclic-di-GMP second-messenger signalling pathways, small RNA regulation, two-component systems, flagella regulation and protein secretion systems. During bacterial growth, signal molecules are secreted and accumulate as bacterial population density increases in the surrounding environment, until a critical threshold concentration is reached following the activation of certain sets of genes. QS only establishes the fact that a good number of bacteria or quorum should be present either for the induction or repression of expressed target genes. Most bacteria produce QS signals as well as QS inhibitors. The basic QS circuit machinery contains three essential elements, including the signal synthase, signal receptor and signal molecules [5,8,9,11]. Three main QS systems identified among bacteria are as follows: (i) Gram-positive bacteria use small oligopeptides (Autoinducing Peptide; AIP) QS; (ii) Gram-negative bacteria use Acyl Homoserine Lactones (AHL); and (iii) Autoinducer-2 (AI-2) is used both in gram-positive and gram-negative bacteria [9,11].

Two kinds of AHL receptors have been reported: (i) LuxR-type receptors are cytoplasmic AHL-binding transcription factors possessing variable Ligand-Binding Domains (LBD) and well-conserved helix-turn-helix DNA-Binding Domains (DBD). (ii) LuxN-type receptors are two-component membrane-spanning signalling proteins that bind AHLs in their periplasmic regions, transducing information regarding ligand occupancy internally by phosphorylation and dephosphorylation cascades [7]. For example, the QS system in *Vibrio fischeri*, a gram-negative bacteria, is dependent on homologues of the LuxI and LuxR regulatory proteins. The quorum signals are synthesized by the LuxI-like proteins, and the autoinducer compounds are AHLs, which are also known as Autoinducer 1 (AI-1). The AHLs consist of a homoserine lactone ring with a variable-length acyl side chain (Figure 3). The concentration of AHLs increases as the bacterial population increases. The AHL is synthesized within the cell and is either diffused or secreted outside its external environment. Thus, when the AHLs reach a critical threshold level, they re-enter the bacterial cell to bind to the LuxR-like protein receptors. The LuxR-AHL complexes formed activate or repress target gene transcription [8].

In recent times, increasing attention has been paid to discovering new and novel therapeutic strategies, specifically targeting QS signalling systems and the biofilm mode of growth, which will provide the foundation upon which the future of next-generation antimicrobial agents will be developed [2]. Natural AHLs mostly share conserved structural motifs, and their biosynthesis derived from fatty acids occurs *via* a sequentially ordered reaction, *viz.*, an unsubstituted homoserine lactone ring at the end positions and the addition of an acyl group at the R-position (N-acylated). The acyl side chain of AHL is synthesized during the fatty acid biosynthesis pathway; the homoserine lactone moiety is synthesized by S-adenosyl methionine (an amino donor for the formation of the homoserine lactone ring), which coupled with the acyl side chain forms AHL. Proteins such as acyl carrier protein, Enoyl-ACP reductase, Fab I and AHL synthase are involved in this pathway. LuxR-type transcription factor is another important protein involved in AHL biosynthesis that is involved in activating the expression and production of AHL. The AHL synthases are highly conserved enzymes in QS-regulating bacteria. Studies showed that Fab I reduce Enoyl-ACP to produce acyl-ACP, which reacts with S-adenosyl methionine to produce AHL in the presence of the AHL synthase enzyme [9].

Through an interspecies QS system involving furanosyl diesters, which in some cases contain boron and an Autoinducer 2 (AI-2), many bacteria are also able to detect and respond to the presence of unrelated species. The LuxS gene involved in AI-2 synthesis has been identified in more than 50 different bacteria. QS regulating the expression of pathogenicity-related phenotypes (biofilm formation and virulence) in many bacteria, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* has become a potential target for anti-

pathogen treatment. Many signal interference mechanisms referred to as anti-QS mechanisms exist and are distinguished as QS inhibition for those that interfere with the QS gene regulation system and quorum quenching for those that degrade the AI molecules. Because QS regulation is so complex, new anti-QS targets are being discovered and studied [11].

DISCUSSION

Naturally occuring QS inhibitors

Natural QS inhibitors are regarded as anti-QS bioactive compounds and are obtained from fungi, marine organisms, and plants. Sourcing for plants secondary metabolites has since been explored with bioprospecting of natural ecosystems. Several naturally occurring bioactive compounds with a broad range of antimicrobial activities have been reported and could serve as novel QS inhibitory agents owing to their low toxicity, bioavailability and therapeutic applications against clinical and food pathogens. Medicinal plants containing secondary metabolites (terpenoids, phenolics, coumarins, tannins, flavonoids, saponins, quinones, alkaloids and polyacetylenes) have been found to act against the QS system [1,2]. These anti-QS inhibitory molecules from plants include: cinnamic acids (cinnamon), flavanones (i.e. naringenin, eriodictyol and taxifolin), curcumin (turmeric), resveratrol (grape fruit), thymoquinone (Nigella sativa seeds), flavan-3-ol catechin, salicylic acid (willow bark or aspirin), limon ellagic acid, quercetin, cinnamaldehyde (cinnamon), kaempferol and apigenin (fruits and veggies) [12-17].

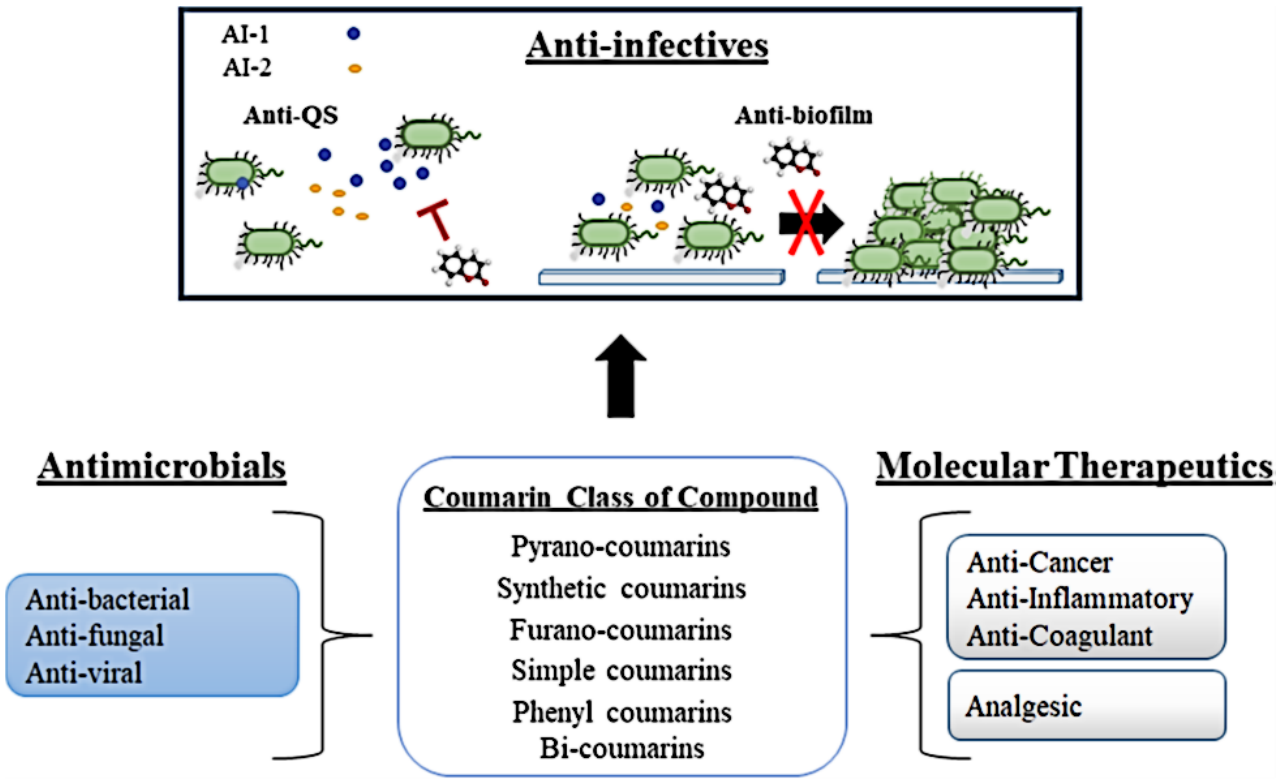
Lower plant species (Bryophytes and Pteridophytes) have no QS inhibitors [5]. Findings showed that lichen produces usnic acid, a metabolite possessing inhibitory activity against bacterial and fungal biofilms formed *via* QS. QS inhibitors naturally increase the susceptibility of biofilms to antibiotics and are generally regarded as safe in humans. Hamamelitannin obtained from the extract of willow bark has been found to inhibit QS [8]. Garlic inhibits the expression of several genes that control bacterial QS. The major QS inhibitor in garlic is ajoene, a sulfur-containing compound in pulverized garlic that inhibits the production of rhamnolipids that prevent the activity of white blood cells against biofilms. When exposed to oxygen, ajoene loses its bioactivity quickly [8]. Studies revealed that over 90% of biofilm bacteria were killed with a combination of ajoene and the antibiotic tobramycin [18].

The rate of inhibition by QS inhibitors depends on the structure and chemical composition of the compound. For example, curcumin produced from *Curcuma longa*, inhibits *Pseudomonas aeruginosa* biofilm formation at early stages. Bacteria are also known to possess several enzymes that are QS inhibitors, e.g. AHL lactonase (*Agrobacterium tumefaciens*) and lactonase (*Bacillus thuringiensis*) have the ability to degrade AHL, while AHL acylase (*Pseudomonas aeruginosa*) can degrade long-chain AHLs etc. Paraoxonases in mammalian serum and human cell lines have the ability to hydrolyze esters and lactones. The antibody XYD-11G2 is capable of degrading *Pseudomonas aeruginosa* 3OC12HSL and acting as a QS inhibitor. Acylases from animal models have been reported to reduce biofilm formation in *Pseudomonas aeruginosa* [9]. The role of biofilm in disrupting the function of flavonoids appears to be unknown. Flavonoids appear to suppress the formation of biofilms *via* a non-specific QS inhibition. The flavonoid phloretin inhibited biofilm formation in *Escherichia coli* O157:H7, without harming beneficial biofilms and enhancing colon inflammation in rats. The red pigments in cranberries are capable of preventing bacteria from attaching to surfaces and host tissues, thus inhibiting biofilm formation in the mouth and urinary tract [8].

Coumarins are a large family of naturally derived fused benzene and pyrone rings found primarily in different plant sources. Coumarins are regarded as phytoalexins and synthesized by plant tissues in response to pathogenic

infection as plant resistance compounds [19]. Members of the coumarin class of compound have also been identified in bacteria such as *Streptomyces* species as novobiocin, coumermycin and in fungi, they are aflatoxins from different *Aspergillus* species [20]. Bergamottin and dihydroxybergamottin are furocoumarins (Figure 1) isolated from grapefruit juice and are able to suppress QS biofilm formation in *Escherichia coli* O157:H7 by about 72% and 58.3% respectively. The anti-biofilm activity of these compounds was also observed (15.5% and 46.5% respectively) against *Salmonella enterica* serovar Typhimurium and *Pseudomonas aeruginosa* (18.1% and 27.3% respectively). In another study, the authors demonstrated how both furocoumarins were able to inhibit AI-1 (N-acyl homoserine lactones, AHLs) and AI-2 (furanosyl borate diester) signalling using Tn5 mutants of *Vibrio harveyi* as reporter strains [2].

Figure 1. The coumarin class of plant phenolic compound have been shown to possess several important pharmacological properties. More recently, a role in the modulation of microbial behaviour has emerged, with several reports describing interference with cell-cell communication (QS) and the formation of multicellular microbial structures (biofilms). Particular emphasis has been placed on the ability of coumarins to disrupt AI-1 and AI-2 signalling in a range of important microbial pathogens.



The anti-biofilm activity of coumarin and umbelliferone against the Escherichia coli O157:H7 strain showed a high level of biofilm inhibition, reaching values of 80% and 90%, respectively, while the qRT-PCR analysis revealed that none of these compounds had any inhibitory effect on AI-2 signalling, thus revealing the expression of the LuxS gene encoding the synthase responsible for AI-2 production. Nonetheless, the expression of QS controlled IsrA gene was

decreased in response to coumarin and umbelliferone [2]. Table 1 revealed various natural compounds possessing anti-biofilm properties.

Table 1. Natural compounds and extracts as biofilm disrupting agents and QS inhibitors.

Natural agent	Bacterial species	Further information	Bibliographic reference
Chitosan	*Pseudomonas aeruginosa* PA01, *Streptococcus mutans* ATCC 35668, *Staphylococcus aureus* MW-2, *Acinetobacter baumannii* ATCC 19606, *Klebsiella pneumoniae* ATCC13883, *Enterococcus faecalis* ATCC51299	Chitosan derivatized with arginine and lactobionic acid could disaggregate a two-day old biofilm	[21]
Vitamin E	*Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii, Pseudomonas aeruginosa, Pseudomonas putida*	An oily topical formulation to limit bacterial and fungal biofilm production was tested in a clinical trial involving 20 wounded patients	[21]
Abscissic acid	*Propionibacterium acnes, Staphylococcus aureus, Pseudomonas aeruginosa*	Abscissic acid, alone or in association with plant extracts (*Morinda citrifolia L.*, *Olea europaea var. sylvestris Brot.*, *Curcuma longa L.*) was assayed for: The inhibitory activity against *Propionibacterium acnes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Malassezia furfur*; the inhibition of the synthesis of QS molecules; and the reduction of	[21]

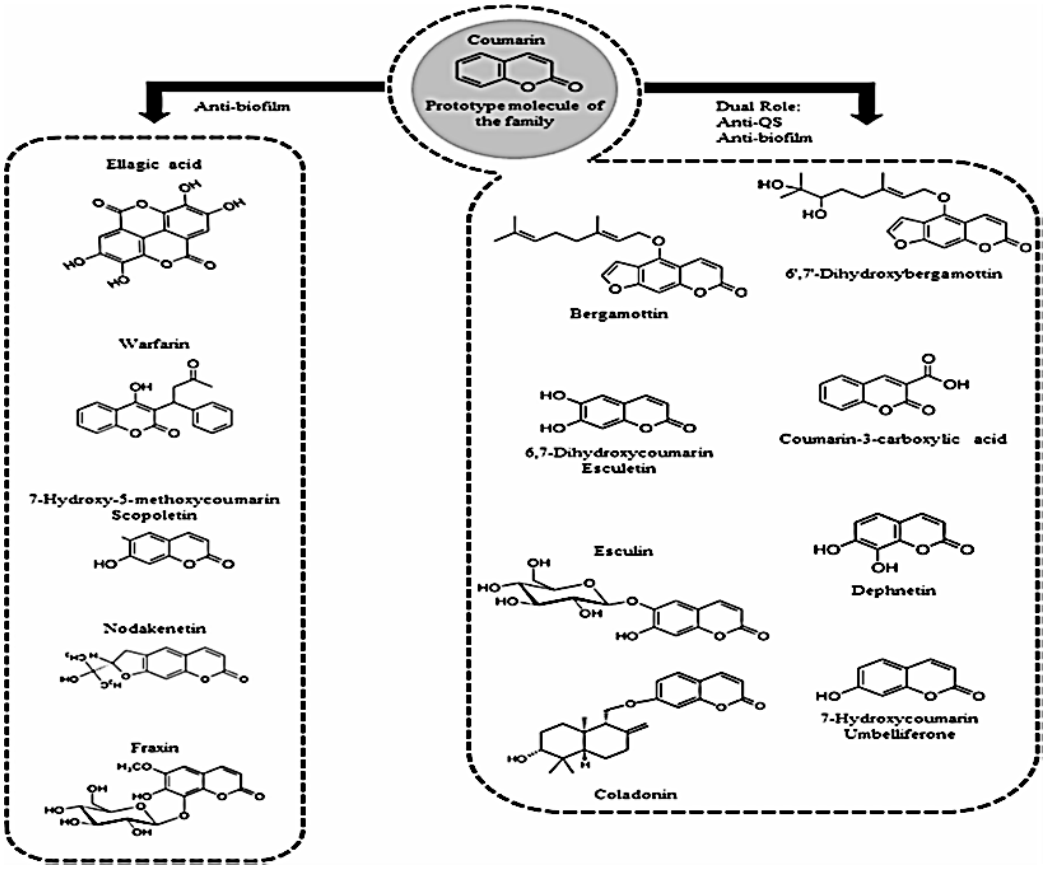
		inflammation markers in keratinocytes infected with bacteria. In addition, a synergistic effect between abscissic acid (30 µg/mL-60 µg/mL) and plant extracts (2%-15% by weight) against *Staphylococcus aureus* and *Propionibacterium acnes* biofilm was assessed	
Aqueous and ethanolic extracts of *Rhamnus prinoides* L'Hér. stem and leaves and their main constituents	*Streptococcus mutans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus subtilis*	Extracts (3 mg/mL) and single bioactives (4-hydroxy-4-methyl-2-pentanone, ethyl 4-ethoxybenzoate and benzoic compounds) were tested against polymicrobial biofilms in-vitro of *Staphylococcus aureus* and *Pseudomonas aeruginosa*	[21]
Mannose, Methyl α -Dmannopyranoside, 2-Deoxy-Dglucose, Methyl α -D-glucopyranoside	*Desulfovibrio vulgaris*ATCC 29579, *Desulfovibrio desulfuricans* DSM 12129	High concentrations (1 mM-500 mM) of each compound gave a consistent eradication of a mature biofilm if applied for 2 h-14 h. Mannose was the best biofilm dispersing agent for both species	[21]

Lipopolysaccharide	*Vibrio vulnificus, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria monocytogenes*	Detoxified lipopolysaccharide from Bacteroides vulgatus MGM001 was effective in association with lipoteichoic acid to reduce biofilm formation on various materials, except acryl matrices	[21]
Amorfrutin B	*Pseudomonas aeruginosa*	showed promising activity with inhibition	[1]
Coumarin and hydroxylated derivatives	*Chromobacterium violaceum* CV026	All the different coumarins tested with the exception of 4-hydroxycoumarin and dihydrocoumarin, inhibited the violacein production.	[22]

Structure activity relationships of anti-quorum sensing compounds

The reason for the loss or low activity of simple coumarin structures in the absence of long hydrocarbon chains has not been understood in spite of the broad range of activity of coumarin compounds (Figure 2). Coumarins having methoxy side chains function at C-7 and if O-H groups are present at either the C-6 or the C-8 position, they are always effective. The presence of aromatic dimethoxy side chains was reported to be favourable against bacteria that require special growth factors (beta-haemolytic *Streptococcus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*). Nonetheless, rather than coumarin to limit the growth of microbial pathogens, it targets a key microbial cell-cell communication system enhancing the ability to inhibit antibiotic tolerant colonisation structures called biofilms [2].

Figure 2. A schematic overview of coumarin structures and their anti-infective properties. Compounds for which anti-biofilm properties have been established are presented on the left, while those with dual activity are presented on the right [2].



D'Almeida, et al. compared the bioassay of seven structurally related coumarins (coumarin and different hydroxylated derivatives) using the QS biosensor *Chromobacterium violaceum* CV026 with the addition of exogenous Hexanoyl Homoserine Lactone (HHL). It was observed that, with the exception of 4-hydroxycoumarin and dihydrocoumarin, all the different coumarins tested inhibited violacein production. The different coumarins were tested against the biofilm formation of *Pseudomonas aeruginosa*, and it was noted that esculetin, umbelliferone, and coumarin had the highest rate of biofilm inhibition, while 4-hydroxycoumarin and dihydrocoumarin showed the lowest activity. This was in contrast to the relatively low antibiofilm activity of esculetin tested against Escherichia coli, as reported by Lee, et al. which suggests that species or strain heterogeneity may play some role in this response.

Lee, et al. also reported the bioactivity of hydroxylated coumarins in Escherichia coli, revealing that at low activity with the hydroxylation at position 4 or position 8 (dephnetin) of the coumarin structure, there is a drastic reduction in anti-biofilm activity, while an enhanced activity of the same modification at position 7 was observed. A study found that di-hydroxylation of coumarin at position 6 and position 7 (esculetin) reduced biofilm activities when compared to simple coumarin [23]. However, noted that the replacement of the 6-hydroxy group with a 6-methoxy group (scopoletin) did not affect antibiofilm activity, whereas the addition of a carboxy group at position 3 reduced the activity of the parent compound. Reen, et al. observed that the introduction of a sesquiterpene in position 7 (coladonin) of coumarin did not increase or reduce biofilm activity.

Bacterial enzymes as QS inhibitors

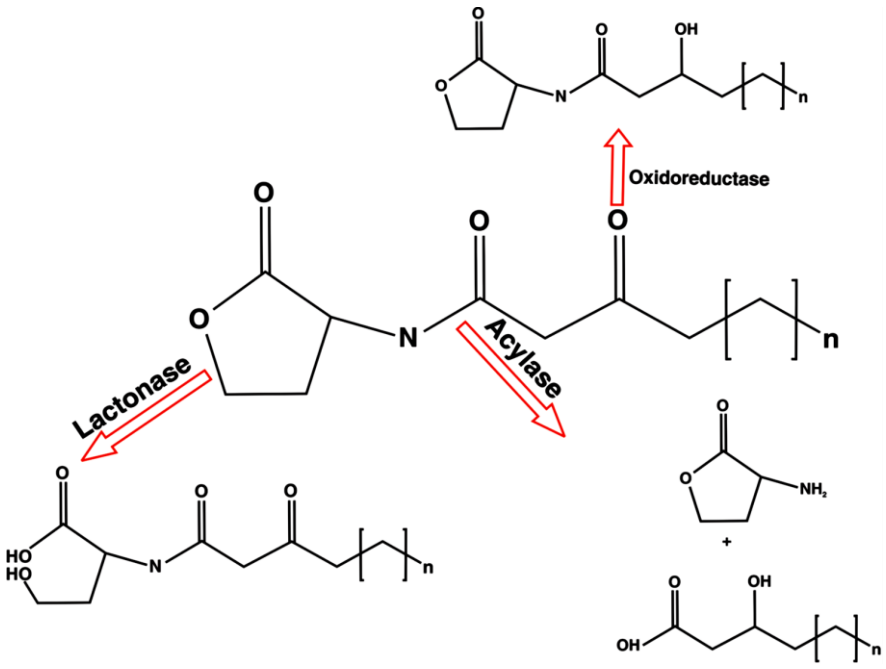
According to Gupta, et al. depending on the site of action, some gram-negative bacteria contain three different types of QS inhibitors: AHL lactonase, AHL acylase and oxido-reductase (Figure 3).

AHL lactonases: The biological activity of the homoserine lactone ring of AHLs is decreased when reversibly hydrolyzed by AHL lactonase (Figure 3). Based on the amino acid sequence and structure of the enzyme, four families have been identified: (1) metallo–lactamase (Aiia, AhlD), (2) phosphotriesterase (Ssopox, Sislac), (3) paraoxanases and (4) hydrolase fold lactonases (AidH). Mammals have been found to develop Paraoxanases, which possess lactonase activity, as a natural response to the bacterial virulence. This lactonase family is made up of three enzymes: Paraoxanases 1 (PON1), PON2 and PON3. While PON1 and PON3 are mainly expressed in the liver and kidney cells, PON2 is known to be expressed in different tissues.

AHL acylases: The expression of a potent enzyme, AHL acylase in *Pseudomonas aeruginosa* growing in *Caenorhabditis elegans* has a deleterious impact on AHL-based quorum sensing (biofilm formation and virulence factors).

Oxido-reductase: Oxidoreductase is known to target the acyl side chain of AHL and catalyse a modification of the chemical structure of the signal by either oxidation or reduction but not degradation (Figure 3). This affects the specificity of the AHL signal, obstructing the activation of QS-mediated genes regulating a particular AHL. The expression of PAO1 in *Pseudomonas aeruginosa* significantly reduced pyocyanin production, decreased motility, and inhibited biofilm formation.

Figure 3. Chemical structure of AHL showing the target site for different quorum-quenching enzymes on this molecule. The figure shows the cleavage of ester bond inside the lactone ring by AHL lactonase, cleavage of the acyl chain of AHL at the site of amine linkage by AHL acylase, and reduction of the carbonyl group present in the acyl chain to the hydroxyl group by AHL oxido-reductase.



QS inhibitors with synthetic modification

Much of the problem exists and has limited the biological activities of natural sources of QS inhibitors owing to the low level of exploration or extraction for therapeutic use, which has now been overcome by chemical synthesis or modification of the natural product molecules [1]. Quorum quenching is a current approach explored where bacterial communication is dependent upon the efficient production, secretion and binding of the signalling molecule to its receptor and is impeded in different ways to render the bacteria avirulent. QS has been targeted mostly at three different levels in preventing the activation of selective effector functions in bacteria, either by interrupting the signal generator, signal receptor or signal molecule [5].

Signal generator: In the signal generator, regulation of the production of AHL, Pseudomonas Quinolone Signalling (PQS), Methyl Thioadenosine deaminase (MTAN) and AI-2 molecules in gram-negative bacteria has been achieved *via* chemical signalling. In gram-positive bacteria, QS inhibition has been achieved by inhibiting either AI-2 or peptide autoinducer production.

Signal receptors: This is the most studied strategy for QS inhibition to block the activation of LuxR homologues, involving the use of small molecules. It has also been hypothesized that changes in the natural signals can be maintained *via* the signal receptor interaction by producing a non-productive signal receptor complex while disrupting the downstream signalling, which would competitively block binding by the natural signal.

According to Roychoudhury, synthetic quorum quenchers (inhibitors) can be designed using different reactions: (i) substitution and alternate introduction into the lactone ring of an acyl side chain (ii) substitution and alternate introduction of another side chain in the lactone ring leaving the acyl side chain unchanged (iii) modifications of both the acyl side chain and the lactone ring (iv) by introducing an acyl side chain containing sulphur instead of a C3 atom, LuxR-controlled and LasR-controlled.

The derivatives of cinnamic acid (such as allyl cinnamate, cinnamyl alcohol and methyltrans-cinnamate) are synthetic quorum inhibitors that inhibit violacein, the virulence factor produced by *Chromobacterium violaceum*. The use of AHL analogues can be used to prevent biofilm formation and QS communication in *Acinetobacter baumanii* [9].

Synthesized quorum sensing inhibitors from synthetic or natural compounds are described below, with the summary displayed in Table 2.

Table 2. Summary of biological activity studies of synthesized QS inhibitors.

Compound	Microbial biofilm (target)	Reference
Cyclopenta(hepta)thiophene derivative	QS inhibitors against bacteria	[24]
N-sulfonyl Homoserine Lactone	QS inhibitors against *Chromobacterium violaceum* CV026	[25]
Llimaquinone derivatives	QS inhibitory activity against the bioluminescent bacterium *Vibrio harveyi* and the minimal delay time for the onset of bioluminescence	[26]

Substituted Pyridine/Pyrimidine/Pyrazole derivatives	QS inhibitors possessing LsrK inhibition	[27]
Homoserine lactone derivatives	QS inhibition of the green fluorescent protein (GFP) fluorescence in *Escherichia coli*	[28]
Alkyno/Azido-alkyno/Phenyl glyoxamide derivatives	QS and biofilm inhibition against *Pseudomonas aeruginosa* MH602 and *E. coli* MT102	[29]
Gingerol structural analogues	Biofilm inhibitor against *Pseudomonas aeruginosa* by competing LasR	[30]
Allyl cinnamate, cinnamyl alcohol and methyltrans-cinnamate	QS inhibition of violacein (virulence factor produced by *Chromobacterium Violaceum*) and prevention of biofilm formation in *Acinetobacter baumanii*	[9]
Norspermidine (a type of polyamine)	QS inhibition of expressed genes in *Pseudomonas aeruginosa* and biofilm formation	[9]
Galactose-Modified Di-block Co-polymer	QS inhibition of Pseudomonas aeruginosa PAO1 Biofilm Formation and lectin binding (LecA)	[6]

Synthesis of 4-thioketoanalogs: QS and biofilm inhibition have gained strategic preference as potential future treatments for infectious diseases. A QS system using 2-alkyl-4-quinolone (AQ) signalling molecules has been identified in *Pseudomonas aeruginosa*, the PQS system. Different AQs are known, and the most abundant and well-studied are 3-hydroxy-2-heptyl-4-quinolone (PQS) and its biosynthetic precursor, 2-heptyl-4-quinolone (HHQ), which may both have distinct roles in cell-to-cell communication. In the generation of a structurally modified library of HHQ and PQS derivatives, heteroatom substitutions were performed at positions 1, 3 and 4 of the 2-alkyl-4-quinolone scaffolds [29]. The thionation of the 4-keto-compounds HHQ and PQS using P4S10 in pyridine under reflux conditions yielded 4-thioketo analogues 7 and 8. This reaction was extremely reliable as it yielded the desired molecules without interfering with hydroxyl or amine functionalities at the same time, whereas the Lawessons reagent did not produce successful thionation of the ketones [29].

Cyclopenta(Hepta)thiophene derivative synthesis: Twenty-one new analogues of cyclopenta(hepta)[b]thiophene bearing pyrazole, pyridazine and pyrazole were synthesized. Pyridine and pyrimidine moieties were reported for their effective antimicrobial activities; however, only two compounds are designated as QS inhibitors [28].

Synthesis of N-Sulfonyl homoserine lactone: N-sulfonyl homoserine lactone was synthesized from the main AHL QS inhibitor having a 4-aminobenzenesulfonyl moiety as a side chain by incorporating L-methionine and bromoacetic acid into the reaction with 4-acetylaminobenzenesulfonyl chloride. One of the N-sulfonyl homoserine lactone

derivatives was reported to have very high QS inhibitory activity with an IC50 value of 6.19 M against *Chromobacterium violaceum* CV026 [29].

Llimaquinone derivatives: Llimaquinone is a phytochemical compound obtained from *Dactylospongia metachromia*, a sponge. Its derivatives were studied using ring-distortion studies involving reorganization of the quinone ring and rearrangement of the sesquiterpene moiety. Some of the derivatives obtained were found to have QS inhibitory activity against the bioluminescent bacterium *Vibrio harveyi*, and the delay time for the onset of bioluminescence was minimal [30].

Substituted pyridine, pyrimidine and pyrazole derivatives: A number of heterocyclic derivatives, as described by Stotani, et al. were prepared and evaluated for QS interference through LsrK inhibition in both gram-positive and gram-negative bacteria. Derivatives such as substituted pyridine, pyrimidines, and pyrazoles were synthesized from (S)-4,5-Dihydroxy-2-pentanedione (DPD) using different reactions. The derivatives were found to be QS inhibitors possessing LsrK inhibition with an IC50 in the range of 119 M–475 M.

Homoserine lactone derivatives: Four homoserine lactone-derived TGK series compounds were synthesized by refluxing an ester with an amine having an equivalent ratio of 1:2.5 in methanol for 9 h. All the compounds formed showed significant inhibition (at 50 M concentration) of the Green Fluorescent Protein (GFP) fluorescence induced by N-(3-oxohexanoyl)-homoserine lactone (OC6HSL) in the *Escherichia coli* QS biosensor strain [30].

Derivatives of alkyno/azido-alkyno/phenyl glyoxamide: New acyclic and cyclic glyoxamide-based derivatives were synthesized and evaluated by Nizalapur, et al. against bacterial QS and biofilm inhibition activities. Some of the synthesized compounds (at 250 M concentration) revealed high QS and biofilm inhibition activity when evaluated on *Pseudomonas aeruginosa* MH602 and *Escherichia coli* MT102. The *in-vitro* toxicity test performed on the compounds against MRC-5 lung fibroblast cells found them to be non-toxic to human cells.

Gingerol structural analogues: The natural product (S)-6-gingerol isolated from ginger has been reported as a biofilm inhibitor against Pseudomonas aeruginosa by competing LasR. It is of note that a higher activity against biofilm formation and LasR antagonism has been reported on the structural analogues of gingerol (8-gingerol) [31].

CONCLUSION

QS allows biofilm initiation, expression and transfer of virulence genes, as well as antibiotic resistance among microbial pathogens. However, biological compounds of plant or animal origin have been discovered to hinder or reduce the QS system in bacteria. Therefore, for an adequate response to the global threat from antibiotic resistance, synthetically modified QS inhibitors with little or no side effects and a faster mode of action are highly needed to combat the scourge plaguing human and animal health. *In-vivo* studies on various animal models should be encouraged as a future challenge in order to investigate the therapeutic applications of QS inhibitors. New classes of QS inhibitors should be investigated to expand the drug development base. Drug combination studies of these QS inhibitors with conventional antibiotics could be undertaken to improve the effectiveness of therapeutic drugs.

AUTHORS' CONTRIBUTIONS

Ojo Stephen Kayode Simpa conceptualise the title, participated in review writing and editing; Okunade Stephen Oyeoluwa, Aliu Omotayo Rachael and Adeyemo Michael Bolaji participated in the journal search and review writing.

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None

REFERENCES

1. Majik MS, et al. Next generation quorum sensing inhibitors: Accounts on structure activity relationship studies and biological activities. Bioorg Med Chem. 2020;28:115728.
2. Reen FJ, et al. Coumarin: A novel player in microbial quorum sensing and biofilm formation inhibition. Appl Microbiol Biotechnol. 2018;102:2063-2073.
3. Teasdale ME, et al. Gram-positive marine bacteria as a potential resource for the discovery of quorum sensing inhibitors. Mar Biotechnol. 2011;13:722-732.
4. Haque M, et al. Quorum sensing: A new prospect for the management of antimicrobial-resistant infectious diseases. Expert Rev Anti Infect Ther. 2021;19:571-586.
5. Gupta K, et al. Parallels among natural and synthetically modified quorum-quenching strategies as convoy to future therapy. J Microbiol. 2019;165:1265-1281.
6. Flockton TR, et al. Inhibition of *Pseudomonas aeruginosa* biofilm formation with surface modified polymeric nanoparticles. Pathog. 2019;55:1-13.
7. Amelia RM, et al. Structural determinants driving homoserine lactone ligand selection in the *Pseudomonas aeruginosa* LasR quorum-sensing receptor. Biol Sci. 2019;116:245-254.
8. Usman NA, et al. Natural and synthetic antibiofilm compounds: A Review. Bayero J Pure Appl Sci. 2019;12:619-627.
9. Roychoudhury A. Bioactive quorum quenchers antagonizing *Pseudomonas aeruginosa* biofilm. Drug Dev Ind Pharm. 2020;4:29-45.
10. Stowe SD, et al. Anti-biofilm compounds derived from marine sponges. Mar Drugs. 2011;9:2010-2035.
11. Schipper C, et al. Metagenome-derived clones encoding two novel lactonase family proteins involved in biofilm inhibition in *Pseudomonas aeruginosa.* Appl Environ Microbiol. 2009;75:224-233.
12. Chang CY, et al. Non-antibiotic quorum sensing inhibitors acting against N-acyl homoserine lactone synthase as druggable target. Sci Rep. 2014;28:7245.
13. Singh BN, et al. Quercetin sensitizes fluconazole-resistant *Candida albicans* to induce apoptotic cell death by modulating quorum sensing. Antimicrob Agents Chemother. 2015;59:2153-2168.
14. Truchado MP, et al. Plant food extracts and phytochemicals: Their role as quorum sensing inhibitors. Trends Food Sci Tech. 2015;43:189-204.
15. Sarabhai S, et al. Ellagic acid derivatives from *Terminalia chebula* Retz. downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 virulence. PLoS One. 2013;8:e53441.

16. Vandeputte OM, et al. The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. Microbiol. 2011;157:2120-2132.
17. Vikram A, et al. Suppression of bacterial cell-cell signalling, biofilm formation and type III secretion system by citrus flavonoids. J Appl Microbiol. 2010;109:515-527.
18. Jakobsen TH, et al. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. Antimicrob Agents Chemother. 2012;56:2314-2325.
19. Yang L, et al. Exposure to umbelliferone reduces *Ralstonia solanacearum* biofilm formation, transcription of type III secretion system regulators and effectors and virulence on tobacco. Front Microbiol. 2017;8:1234.
20. Kumar P, et al. Aflatoxins: A global concern for food safety, human health and their management. Front Microbiol. 2016;7:2170.
21. Guglielmi P, et al. Natural compounds and extracts as novel antimicrobial agents. Expert Opin Ther Pat. 2020;30:949-962.
22. Dalmeida RE, et al. Comparison of seven structurally related coumarins on the inhibition of quorum sensing of *Pseudomonas aeruginosa* and *Chromobacterium violaceum*. Bioorg Chem. 2017;73:37-42.
23. Lee JH, et al. Coumarins reduce biofilm formation and the virulence of *Escherichia coli* O157:H7. Phytomed. 2014;21:1037-1042.
24. Abdel RSA, et al. Synthesis, antimicrobial, antiquorum-sensing, antitumor and cytotoxic activities of new series of cyclopenta(hepta)[b]thiophene and fused cyclohepta[b]thiophene analogs. Eur J Med Chem. 2017;140:200-211.
25. Sun Q, et al. Design, synthesis and activity evaluation study of novel substituted N-sulfonyl homoserine lactone derivatives as bacterial quorum sensing inhibitors. Med Chem Res. 2017;26:3345-3353.
26. Evanno L, et al. A ring-distortion strategy from marine natural product ilimaquinone leads to quorum sensing modulators. Eur J Org Chem. 2018;20:2486-2497.
27. Stotani S, et al. DPD-Inspired discovery of novel LsrK kinase inhibitors: An opportunity to fight antimicrobial resistance. J Med Chem. 2019;62:2720-2737.
28. Qin X, et al. Synthetic homoserine lactone analogues as antagonists of bacterial quorum sensing. Bioorg Chem. 2020;98:103698.
29. Nizalapur S, et al. Synthesis and biological evaluation of novel acyclic and cyclic glyoxamide based derivatives as bacterial quorum sensing and biofilm inhibitors. Org Biomol Chem. 2017;15:5743-5755.
30. Choi H, et al. Structure-activity relationships of 6- and 8-gingerol analogs as anti-biofilm agents. J Med Chem. 2017;60:9821-9837.
31. Szamosvari D, et al. Synthetic quinolone signal analogues inhibiting the virulence factor elastase of *Pseudomonas aeruginosa*. Chem Commun. 2016;52:13440-13443.