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Perspective

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# *Pseudomonas aeruginosa* Quorum Sensing Systems as Drug Discovery Targets: Current Position and Future Perspectives

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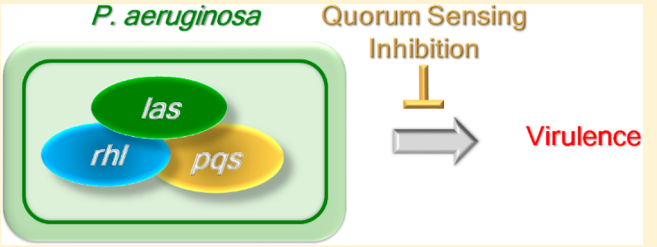
**ABSTRACT:** Antimicrobial resistance (AMR) is a serious threat to public health globally, manifested by the frequent emergence of multidrug resistant pathogens that render current chemotherapy inadequate. Health organizations worldwide have recognized the severity of this crisis and implemented action plans to contain its adverse consequences and prolong the utility of conventional antibiotics. Hence, there is a pressing need for new classes of antibacterial agents with novel modes of action. Quorum sensing (QS), a communication system employed by bacterial populations to coordinate virulence gene expression, is a potential target that has been intensively investigated over the past decade. This Perspective will focus on recent advances in targeting the three main quorum sensing systems (*las*, *rhl*, and *pqs*) of a major opportunistic human pathogen, *Pseudomonas aeruginosa*, and will specifically evaluate the medicinal chemistry strategies devised to develop QS inhibitors from a drug discovery perspective.




## INTRODUCTION

Antimicrobial resistance is a global threat that is imposing an ever increasing burden on public health because of the rapid selection of antibiotic resistance associated with the over- and misuse of antibacterial reagents.[1,2] The withdrawal of most major pharmaceutical companies from antibiotic discovery and their alternative focus on chronic, noncommunicable diseases reflects the difficulties in developing novel antibacterial agents and the enormous cost of bringing new therapeutics to the clinic. In addition, the increasing complexity of the legislation imposed by regulatory bodies and risks associated with antibacterial drug discovery research has restricted further advances in this field.[3,4] Over the past 17 years, only four new classes of antibiotics have been discovered with the majority of FDA-approved drugs being based on alterations to existing structures (Figure 1).[4−6]

The antibiotic crisis is associated with the appearance of multidrug resistant pathogens, also known as "superbugs" that are capable of surviving antibiotic treatment as in the case of the so-called "ESKAPE" panel pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).[7] According to the World Health Organization (WHO), *Pseudomonas aeruginosa* represents one of the "critical priority pathogens" that requires urgent attention because of its multidrug resistance (MDR) to a broad spectrum of antibiotics including carbapenems and third generation cephalosporins.[8,9] *P. aeruginosa* is commonly responsible for lung, skin, eye, wound, blood-borne, and urinary tract infections occurring in both hospitals and the community.[10,11] This Gram-negative bacterium is a common cause of nosocomial infections and a major pathogen in both cystic fibrosis (CF) and immunocompromised patients and those with burns, open fractures, or implanted medical devices such as catheters.[12,13]

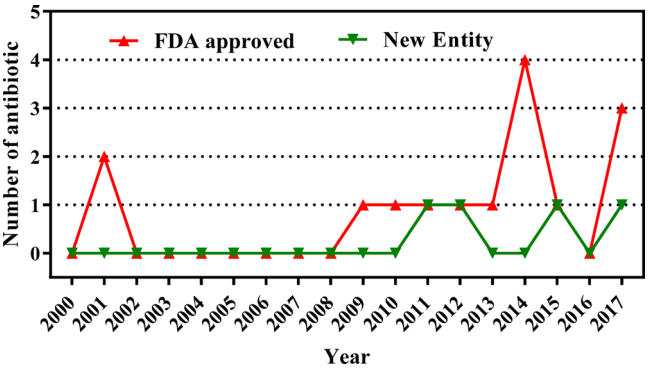



**Figure 1.** FDA approved antibiotics for the period 2000−2017 (red) and novel approved antibiotic classes with unprecedented chemical structures and modes of action (green). Data are collected from U.S. Food and Drug Administration (FDA) (www.fda.gov as of March 15, 2018).

## VIRULENCE OF *P. aeruginosa*

The clinical significance of *P. aeruginosa* arises from its ability to express a plethora of virulence factors that aid invasion of, and cause damage to, host tissues.[14] Among these, flagella and pili contribute to tissue surface adhesion as well as to tissue migration via swarming and twitching motility.[15,16] *P. aeruginosa* also secretes multiple tissue degrading exoenzymes, exotoxins, and host defense-inactivating effector proteins which play key

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roles in virulence and survival.[14,17] Furthermore, *P. aeruginosa* produces multiple secondary metabolites including hydrogen cyanide (HCN) and is able to interfere with host oxidative stress responses via the redox reactive pigment pyocyanin.[18] It also scavenges essential nutrients such as iron from the host proteins transferrin and lactoferrin via the siderophores pyoverdine and pyochelin.[14,19] During chronic infections, *P. aeruginosa* forms biofilms, communities of bacteria usually attached to a surface and surrounded by an extracellular matrix composed of exopolysaccharides, proteins, nucleic acids, and lipids. Biofilms are highly tolerant to antibiotics and the immune system.[20] Consequently, this extensive secreted macromolecular and secondary metabolite "toolbox" of virulence factors makes *P. aeruginosa* a formidable opportunistic pathogen.

## *P. aeruginosa* AS A "SUPERBUG"

*P. aeruginosa* is highly resistant to antimicrobials due to intrinsic, acquired, and evolved mechanisms. *P. aeruginosa* exhibits intrinsic resistance to antibiotics because of the low permeability of its outer membrane and the presence of at least 12 efflux pumps which are able to expel various antibiotics including cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides.[21] In addition, $\beta$-lactamase genes are frequently chromosomally encoded making *P. aeruginosa* resistant to penicillins and cephalosporins.[22] Acquired resistance in *P. aeruginosa* is mainly driven by horizontal gene transfer whereby genes coding for specific resistance traits are transferred from one bacterium to another. Acquired resistance can also be induced through a mutational change, for example, in DNA gyrase, resulting in lower affinity for fluoroquinolones.[23] A third mechanism for developing resistance is known as evolved resistance, whereby *P. aeruginosa* responds to numerous stimuli, for instance, subinhibitory concentrations of antibiotics, nutrient deprivation, pH, and temperature dependence and through the expression of genes which enhance specific activities such as efflux pump mechanisms and/or those that modify cell envelope composition.[23] For these reasons, the effectiveness of molecules targeting *P. aeruginosa* infections can be significantly compromised by these bacterial defense mechanisms. Therefore, it is important that knowledge of existing resistance mechanisms is considered when introducing new molecular scaffolds into the rational design of inhibitors of bacterial QS regulatory pathways.

## QUORUM SENSING AS A DRUG DISCOVERY TARGET

QS is a mechanism for cell to cell communication between bacteria that relies on the production and sensing of diffusible quorum sensing signal molecules (QSSMs) that are sometimes referred to as autoinducers (AIs). Once a bacterial population reaches a certain threshold that is reflected by the concentration of QSSMs in the surrounding environment, the transcription of multiple genes is synchronized enabling the population to behave collectively. This diffusible signal-mediated regulation controls a wide range of activities from swarming and swimming motility, biofilm maturation, virulence factor, and secondary metabolite production as well as antibiotic resistance.[24] In recent years, attempts to develop new classes of antimicrobial agents have included the targeting of specific virulence factors or virulence regulatory mechanisms rather than cell viability with a view to minimize the selective pressures that lead to the emergence of resistance.[25−27] One of these strategies is directed toward interference with QS-mediated signaling to disrupt bacterial communication in order to attenuate virulence such that the infecting bacteria can be cleared by the host defenses. Hence, the use of QS inhibitors (QSIs) that do not directly compromise bacterial viability should impose less selective pressure with respect to resistance than conventional antibiotics.[28] QS inhibitors (QSIs) alone may not be sufficient to eradicate infections especially in immunocompromised individuals but are likely to act synergistically in combination with growth inhibitory antibiotics. QSIs may however be very effective as prophylactics. Since 2000, the number of QS publications has shown a significant upward trajectory mostly with respect the underlying molecular biology with medicinal chemistry related papers and published patent applications representing only a small percentage of the total (Figure 2).

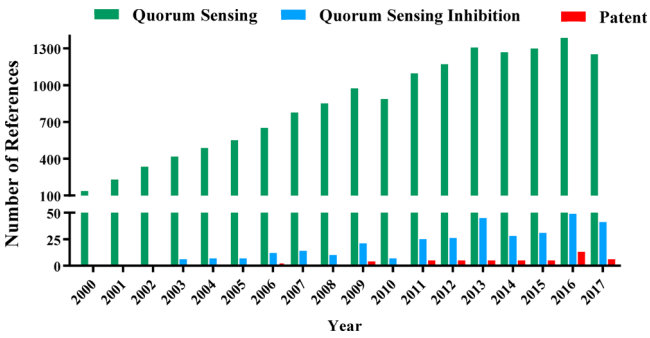


**Figure 2.** Representation of the number of publications related to QS for the period 2000−2017 as analyzed using the Scifinder Scholar search tool (https://scifinder.cas.org, as of March 15, 2018).

## QUORUM SENSING SYSTEMS IN *Pseudomonas aeruginosa*

*P. aeruginosa* possesses three major QS systems, *las*, *rhl*, and *pqs* that are interconnected and highly integrated, with each system being autoregulatory while also modulating the activities of the others (Figure 3). The *las* system for example positively controls both *rhl* and *pqs* system genes that code for QSSM receptors (*rhlR* and *pqsR*) and synthase genes (*rhlI* and *pqsH*). However, while some target genes are specifically regulated by *las* and others by *rhl*, some require both of these QS systems for full activation.[29] The *las* and *rhl* systems rely on two different *N*-acyl-L-homoserine lactone (AHL) type signal molecules (Figure 3, **1**, **2**). The third QS circuit, *pqs*, employs 2-alkyl-4-quinolones (Figure 3, **3**, 2-heptyl-4-hydroxyquinoline (HHQ), or **4**, 2-heptyl-3-hydroxy-4(1*H*)-quinolone (PQS)) as QSSMs. The pathogenicity of *P. aeruginosa* strains with mutations in the key QS genes from the *las*, *rhl*, or *pqs* systems is highly attenuated in experimental infection models making QS a putative target for novel antibacterial agents.[30]

For each QS system, activation of the receptor protein (LasR, RhlR, and PqsR) by the cognate QS signal molecule activates expression of the biosynthetic genes setting up an autoinduction loop to generate more signal molecules while also being responsible for the up-regulation of diverse genes associated with virulence, secondary metabolism, and biofilm development. From a drug discovery point of view, the QS systems can be targeted at four main levels: signal biosynthesis, signal reception, signal sequestration, and signal degradation.[28] This review will focus mainly on antagonism of QSSM biosynthesis and response in *P. aeruginosa*.


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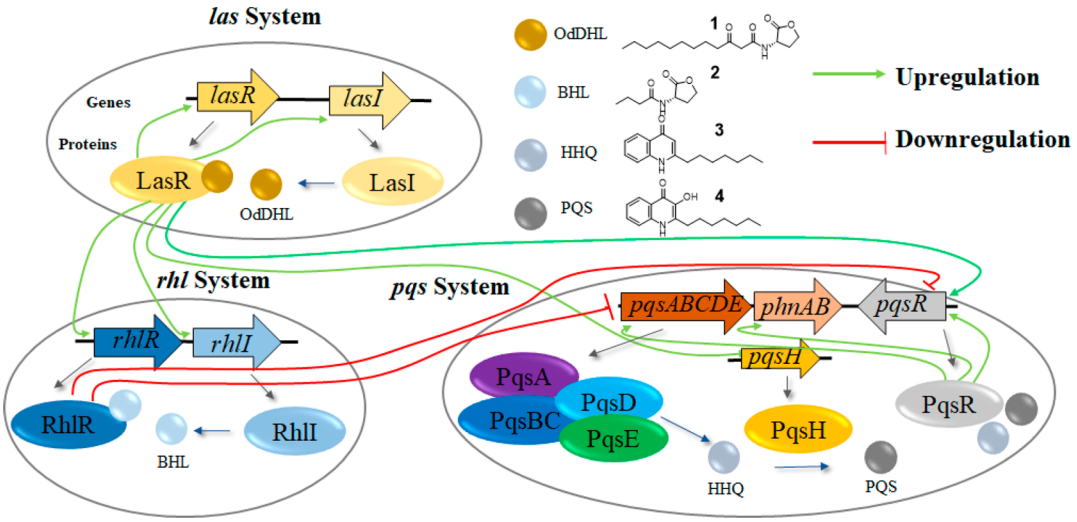


**Figure 3.** Schematic diagram of the interconnected *las*, *rhl*, and *pqs* quorum sensing systems in *P. aeruginosa*. Green arrows and red blocked lines indicate up- or down-regulation, respectively. Oval shapes represent various proteins, color coded circle shapes represent QSSMs, and large colored arrows represent genes. Thin gray arrows represent protein expression, and thin blue arrows indicate QSSMs biosynthesis.

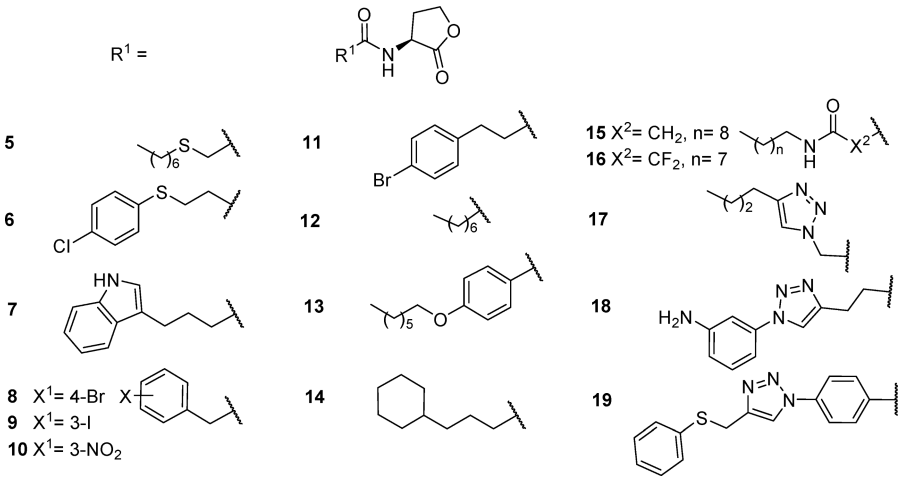


**Figure 4.** LasR inhibitors: AHL analogs with modified tail structures.

## INHIBITION OF THE *las* QUORUM SENSING SYSTEM

The *las* system in *P. aeruginosa* employs *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL) as the cognate QSSM that, upon binding the LuxR type transcriptional regulator LasR, activates the expression of multiple genes. These include *lasI* which codes for the 3OC12-HSL synthase as well as numerous virulence factor genes (e.g., the elastases LasA and LasB, alkaline protease, and exotoxin A) required for biofilm development and the *rhl* and *pqs* systems.[20] Recent work has confirmed that pharmacological antagonism of the LasR receptor induces and stabilizes conformational changes that prevent the complex (LasR−antagonist) from binding to DNA so preventing transcription of the target genes.[31]

## LasR INHIBITORS

LasR has attracted attention as a drug discovery target driven by its position in the *P. aeruginosa* QS hierarchy. There is a considerable amount of literature describing inhibitors for LasR from the past 15 years, and these can be classified into four categories: AHL-like antagonists, non-AHL-like antagonists, covalent binders, and natural-product-based inhibitors. It is also noteworthy that several assays for evaluating LasR inhibition have been described. These are mostly bacterial cell-based employing transcriptional fusions to LasR target gene promoters coupled to reporter genes providing bioluminescent or fluorescent readouts.[32,33] However, these assays have not been standardized and employ different homologous (*P. aeruginosa*) or heterologous (*E. coli*) host strains making direct comparisons of inhibitor potencies between studies challenging.

## AHL-LIKE INHIBITORS

**Inhibitors with Modified Tail Structures.** One of the earliest attempts to modify 3OC12-HSL was through the introduction of a sulfur containing tail which had variable effects on LasR antagonism depending on chain length and the oxidation state of the sulfur, with the best inhibitor **5** (Figure 4) displaying 50% LasR inhibition at 6 $\mu$M using an *E. coli* reporter strain.[34] A recent patent described a sulfur-based tail **6** with $IC_{50}$ of 5.2 $\mu$M in a *P. aeruginosa* reporter strain.[35] Another tail group modification was introduced by Geske et al., who shortened the aliphatic chain and incorporated an aromatic end group (**7** and **8**). Both compounds showed inhibition in a *P. aeruginosa*


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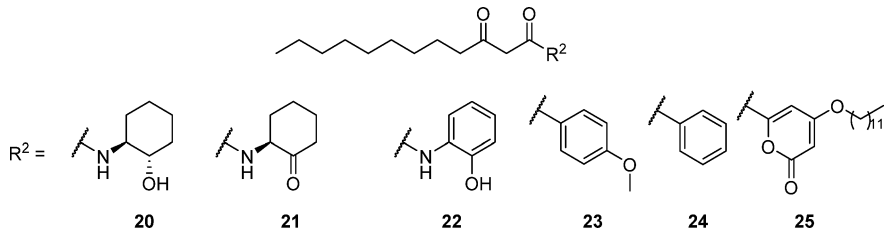


**Figure 5.** Structures of LasR inhibitors: AHL analogs with modified head structures.

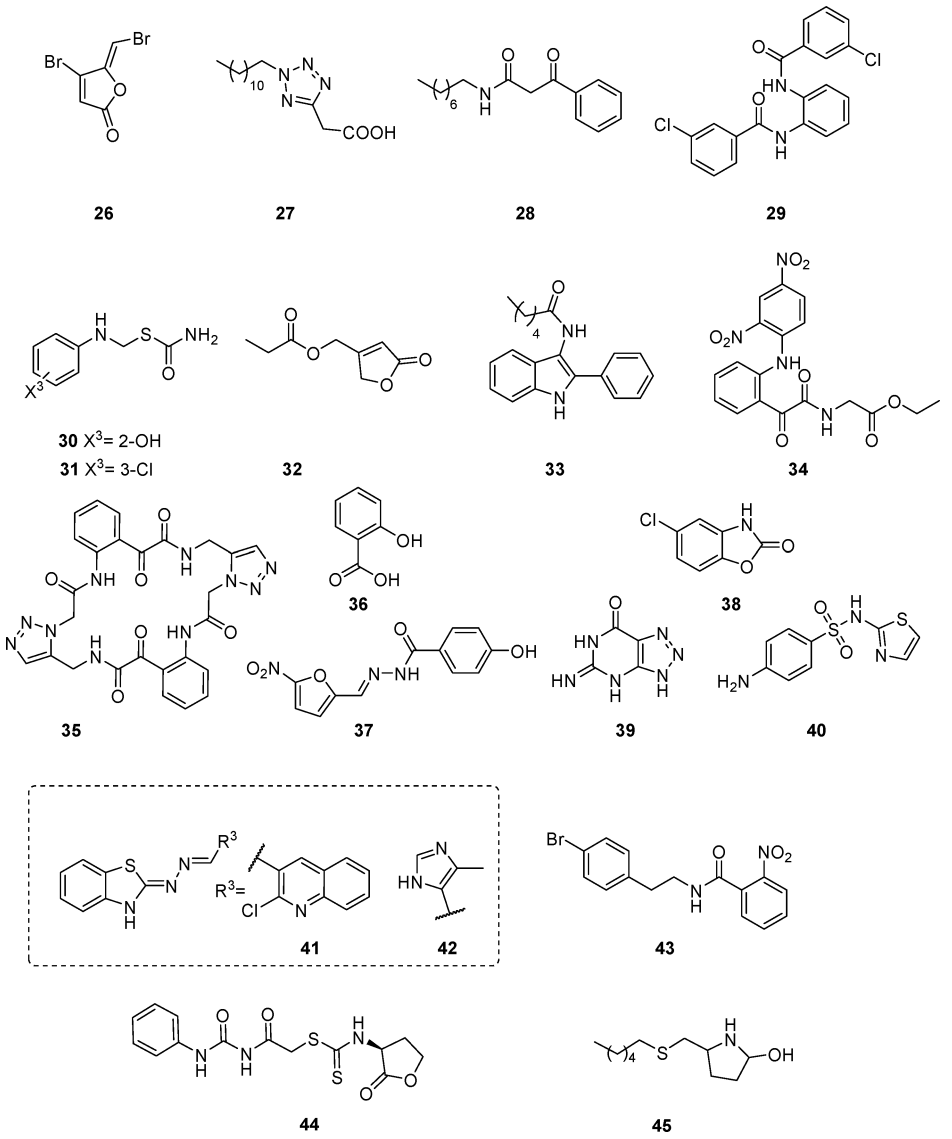


**Figure 6.** Structures of non-native LasR inhibitors with non-AHL-like cores.

reporter strain with $IC_{50}$ values of 16.1 and 14.8 $\mu$M, respectively.[36] In a later study, the same group of researchers expanded the SAR of these inhibitors and submicromolar antagonists were identified using an *E. coli* reporter strain (**9**, 1.72 $\mu$M; **10**, 610 nM; **11**, 250 nM; **12**, 340 nM).[37] Similar structures have also been published as LasR antagonists with $IC_{50}$ values of 1 and 10 $\mu$M for **13** and **14**, respectively.[38] Although Jadhav et al. focused on the structure−activity relationship of 3OC12-HSL analogs as immune modulators, a series of LasR antagonists was found bearing modifications of the hydrophobic tail region. The compounds were screened using a native *P. aeruginosa* reporter assay at 100 $\mu$M ligand concentration to reveal that **15** and **16** had reduced the activity of LasR to less than 6%.[39] Triazole-derived tail structures were designed by Stacy et al. with various linker lengths from the headgroup, and the compounds were assessed using an *E. coli* LasR reporter assay to identify three LasR inhibitors with low micromolar $IC_{50}$ (**17**, 3.27 $\mu$M; **18**, 4.03 $\mu$M; **19**, 2.64 $\mu$M).[40]

**Modified Head Structures.** Suga et al. published a series of compounds with modifications to the headgroup with various saturated and aromatic ring replacements preserving the tail structure which resulted in a number of agonists and antagonists


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with little structural diversity. One of the highlighted agonists in their work was compound **20** (Figure 5) which is based on 2-aminocyclohexanol. Interestingly, the keto derivative **21** was shown to be a weak antagonist at ligand concentrations of 50 $\mu$M. Replacing the saturated ring with an aromatic ring provided antagonist **22** that exhibited reduced activity of LasR in PAO-JP2 *lasI-gfp*, a *lasI rhlI* double mutant transformed with a plasmid containing the *lasI* promoter fused to green fluorescent protein gene (*gfp*). Possible RhlR antagonism in the reporter screens was also noted at concentrations of 10 $\mu$M or greater. However, these results did not translate into virulence factor attenuation with respect to pyocyanin or biofilm reduction.[41,42] The latter observation could be interpreted by the recent finding of Moore et al. that **20** is actually a partial agonist and had no antagonistic activity.[43] Compound **23**, containing a *p*-methoxyphenyl group, exhibited inhibitory activity on pyocyanin and elastase production with no evidence of reporter strain inhibition.[44] Modifications of the headgroup by Mclnnis et al. were shown to be detrimental for activity with a phenyl group **24** being the best replacement among the published compounds. Nevertheless, it had relatively weak activity when tested in a *P. aeruginosa* reporter compared with *E. coli*.[45] Park et al. designed a series of compounds with a pyrone headgroup and aliphatic tail that were validated using a biofilm assay to conclude that **25** had the strongest effect particularly at 100 $\mu$M ligand concentration. Even though the study presented some molecular docking data on **25** binding to LasR, no experiments were performed to validate this in silico modeling.[46]

## NON-AHL-LIKE STRUCTURES

The design of LasR inhibitors has also focused on addressing the chemical and enzymatic stabilities associated with the original lactone-based structure. The AHL lactone ring under alkaline conditions undergoes a ring opening reaction to the corresponding $\gamma$-hydroxycarboxylate.[47] Further, the AHL structure is also prone to enzymatic degradation by lactonases and amino acylases which render these QSSMs inactive.[48]

In a search for non-AHL-like LasR inhibitors, Hentzer et al. disclosed a halogenated furanone **26** (Figure 6), which is a synthetic analog of a furanone-derived natural product isolated from the marine alga *Delisia pulchra*.[49] Compound **26** showed a dose-dependent inhibition of virulence and the development of antibiotic resistant biofilms. Transcriptomic profiling after treatment of *P. aeruginosa* with **26** resulted in the repression of diverse genes controlled by AHL-dependent QS. Most importantly, **26** at a dose of 0.7 mg/kg had significant efficacy in treating *P. aeruginosa* lung infections in a mouse infection model.[50,51] The mechanism of action of **26** however has not been elucidated. The compound is toxic at concentrations of $\geq$100 $\mu$M, and although surface enhanced Raman scattering showed signal-specific structural changes in LasR upon ligand binding, Moore et al. were unable to demonstrate inhibition of LasR activity at subgrowth inhibitory concentrations in the *lasR* bioreporter PAO-JP2 *lasI-gfp*.[43]

An ultrahigh throughput screen (UHTS) was performed on a library of 200 000 compounds by Müh et al. using a *P. aeruginosa* fluorescently tagged reporter strain which gave two LasR antagonists **27** and **28** with $IC_{50}$ values of 30 nM and 10 $\mu$M, respectively. The inhibition of LasR correlated with reduced elastase and pyocyanin production. It is noteworthy that the hydrophobic tail is still preserved to a certain extent in these two molecules.[52] Moreover, the screen also identified a LasR inhibitor **29** with a low potency ($IC_{50}$ = 50 $\mu$M). Similar compounds to **29** have been shown to be activators of the receptor, and cocrystal structures with LasR have been obtained.[52,53] Borlee et al. screened a synthetic compound library of 16 000 compounds using a recombinant *lasR* expressing *Pseudomonas putida* for both agonists and antagonists. LasR inhibitors with a thiocarbamate functionality (**30** and **31**) were their most active hits showing antagonism of 50−60% of 3OC12-HSL (50 nM) at a concentration of 20 $\mu$M.[54] Yoon et al. described a furanone-based series that was tested in an *E. coli*-based LasR reporter strain to demonstrate that compound **32** was the most potent analog which also impacted on biofilm formation. However, concentration and dose response curves were lacking.[55] Biswas et al. proposed an indole derivative **33** as a replacement for the lactone, but their data suggest weak inhibition (65%) at concentration of 250 $\mu$M,[56] while Nizalapur et al. designed a new compound containing a glycine ethyl ester branch **34** which inhibited 3OC12-HSL-dependent activation of LasR in the *P. aeruginosa* MH602 reporter moderately by 48% at 250 $\mu$M although there was minimal effect on pyocyanin production.[57] However, **34** was identified as a pan-assay interfering compound (PAIN) and hence may have given a false positive result.[58] The glyxoamide-based macrocycle **35** exhibited inhibitory activity of the bioreporter strain *P. aeruginosa* PAO1 MH64 and biofilm formation at 250 $\mu$M.[59]

Nielsen et al. performed a structure-based virtual screening on known LasR agonists on QS in *P. aeruginosa* using a library of approved drugs and natural products followed by in vitro assessment of the effects of three candidates: salicylic acid **36**, nifuroxazide **37**, and chlorzoxazone **38**. The results indicated that these three drugs can variably inhibit the three quorum sensing systems (*pqs*, *las*, *rhl*) and reduce biofilm biomass at submillimolar concentrations.[60] Another example of the virtual screening of a compound library was accomplished by Yang-Yi Tan et al., who concluded that **39** was a LasR antagonist ($IC_{50}$ = 0.64 $\mu$M) and significantly reduced elastase production and biofilm formation. However, **39** demonstrated multiple effects on both the *rhl* and *pqs* QS systems.[61] Utilization of computer-aided virtual screening to assist the identification of new compounds was carried out by Skovstrup et al. and led to the discovery of a novel LasR inhibitor scaffold. The hits were evaluated using a *P. aeruginosa* bioreporter, and **40** was shown to have an $IC_{50}$ of 9 $\mu$M.[62] In another report, compounds containing a nonsymmetrical azine core were found to have an inhibitory activity on LasR in the reporter assay. Specifically, **41** and **42** showed a dose-dependent response and biofilm disruption at concentrations lower than 50 $\mu$M.[63] Reilly et al. designed a hybrid compound **43** with an $IC_{50}$ (4.8 $\mu$M) in an *E. coli lasR* reporter.[64]

A recent patent described the *N*-thioacyl homoserine lactone **44** as a *las* quorum sensing inhibitor with extended effects on *pqs* and *rhl* at subinhibitory concentrations; however, the concentration used was not stated.[65] Another patent reported that the pyrrolidin-2-ol derivative **45** inhibited both LasR and RhlR at concentrations around 400 $\mu$M without affecting growth.[66]

## COVALENT BINDERS AS INHIBITORS FOR LasR

Amara et al. developed a series of covalent antagonists with an electrophilic warhead, such as isothiocyanate, that is able to form a covalent bond with cysteine (Cys79) within the LasR ligand binding site. The study focused on **46** (Figure 7) as it is a covalent binder with an $IC_{50}$ of 134 $\mu$M in the *P. aeruginosa* reporter strain PAO-JP2 *lasI-gfp*. Biofilm and pyocyanin production were also reduced at a 50 $\mu$M concentration.[67]


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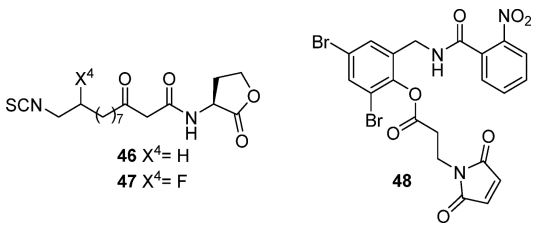


**Figure 7.** Structures of LasR covalent binders.

In a follow-on study, **46** was refined by the introduction of an electronegative halogen at the $\beta$-position to increase the electrophilicity of the isothiocyanate. Compound **47** was thoroughly investigated for its binding to LasR Cys79, used in an ex vivo human wound infection model and in a *Caenorhabditis elegans* survival model where it protected the worms from *P. aeruginosa* infections.[68] Nevertheless, these results have been recently contradicted by Moore et al., who reported that **46** is a LasR agonist that induces LasB elastase production.[43] O'Brien et al. developed the irreversible binder **48** based on **29** with substitution of 2-chlorobenzoate by a maleimide linker that was proposed to form a covalent bond with Cys79. In an *E. coli* based reporter assay, **48** showed a concentration-dependent inhibition with an $IC_{50}$ of 4.8 $\mu$M and was able to reduce pyocyanin production and biofilm formation in *P. aeruginosa*.[69]

## LasI INHIBITORS

LasI, the 3OC12-HSL synthase, has received less attention as a target for disrupting the *las*-dependent QS. There appears to be only one report describing **49** (Figure 8) as a LasI inhibitor

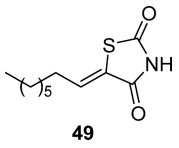


**Figure 8.** Structure of a LasI inhibitor.

(~40% inhibition at 20 $\mu$M) in a heterologous *E. coli*-based reporter strain causing a significant reduction in biofilm and swarming motility. A microarray-based transcriptomic assay revealed that *lasI* along with the *pqsABCDE* genes were down-regulated after treatment with **49** compared to the untreated cells, a result that would be expected given that LasR/3OC12-HSL is involved in regulating both AHL and AQ biosynthesis in *P. aeruginosa* (Figure 3).[70]

## INHIBITION OF THE *rhl* QUORUM SENSING SYSTEM

The *P. aeruginosa rhl* system employs **2** *N*-butanoyl-L-homoserine lactone (C4-HSL) and is responsible for the expression of multiple virulence factors including rhamnolipids, HCN, swarming motility and contributes to biofilm maturation. The expression of the *rhl* system is controlled by the *las* system as the LasR/3OC12-HSL complex activates the transcription of both *rhlR* and *rhlI* leading to more C4-HSL and the activation of a wide range of RhlR/C4-HSL controlled genes. The *rhl* QS system has received less attention compared with the *las* system as most published work has focused on the latter probably due to its location at the top of the *P. aeruginosa* QS hierarchy. Hence it remains unclear as to whether RhlI and RhlR both represent a valid drug discovery target especially since a *rhlI* mutant in contrast to a *rhlR* mutant retains full virulence in a mouse infection model.[71]

The RhlR modulator **50** (Figure 9) was reported to act as an antagonist in the presence of C4-HSL and an agonist in its

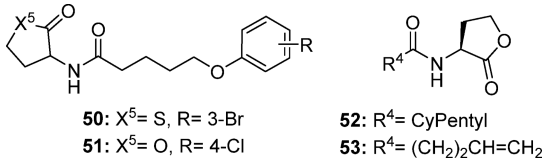


**Figure 9.** Structures of RhlR modulators.

absence. **50** reduced pyocyanin production ($IC_{50}$ = 8 $\mu$M), inhibited biofilm formation, and enhanced *C. elegans* survival during a *P. aeruginosa* PA14 infection at 50 $\mu$M ligand concentration.[72,73] However, a subsequent study, described **50** as an agonist for RhlR, inhibiting pyocyanin production through down-regulation of the *pqs* system.[74] Furthermore, **52** and **53** were identified as RhlR agonists with $EC_{50}$ values of 4.5 and 7.2 $\mu$M in *P. aeruginosa* PAO-JP2 *lasI-gfp* reporter, respectively, significantly reducing pyocyanin but not rhamnolipid production. It is noteworthy that these compounds appeared in a recent patent along with other analogs with the thiolactone headgroup.[75] The study concluded that for an RhlR modulator to function as an antivirulence agent, it is required to be an agonist rather than antagonist since it reduces pyocyanin production through *pqs* down-regulation.[74,76] However, in such cases RhlR activation would lead to overproduction of rhamnolipids, a virulence factor involved in swarming, biofilm maturation and detachment, and early infiltration of *P. aeruginosa* into human airway epithelia.[77−79] Hence, RhlR requires further investigation and evaluation as antiquorum sensing target before considering it for any further medicinal chemistry optimization.

## NATURAL PRODUCTS

There is considerable body of literature describing various natural products that interfere with *P. aeruginosa* QS particularly the *las* and *rhl* systems. These efforts are summarized in Table 1.

## PERSPECTIVE VIEW

The *las* QS system has been the most intensively investigated as an antivirulence drug target. Despite the numerous attempts to target signal reception (LasR) or signal synthesis (LasI), most inhibitors lack the lead-like properties required and failed to proceed to preclinical development due to one or more of the following drawbacks: (i) presence of hydrolytically and/or metabolically labile groups such as lactone/thiolactone or reactive species (i.e., isothiocyanate); (ii) unsurmountable physiochemical properties (e.g., high lipophilicity and/or molecular weight); (iii) weak potency and ambiguity of antivirulence profiles and efficacy toward *P. aeruginosa* clinical isolates; (iv) lack of uniformity and standardization of methodology governing the assessment of the compounds, e.g., use of heterologous *E. coli* reporters. It should also be noted that *lasR* mutants frequently arise in chronic human *P. aeruginosa* infections.[92] Nevertheless, the knowledge gained, along with the availability of crystal structures for LasR and LasI, should facilitate the future discovery and evaluation of more drug-like molecules. In addition, the administration route and indications for such inhibitors plays a major role in


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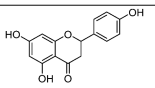
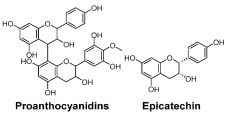
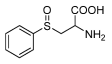
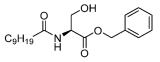
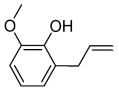
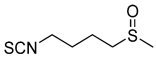
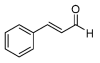
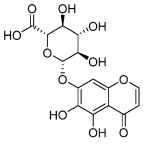
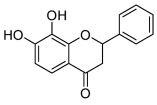
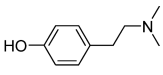
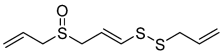
**Table 1. Summary of Natural Product with *P. aeruginosa* QS Quenching Activities**

| Natural Product | Structure | Effect |
|---|---|---|
| Naringenin (derived from *Cobretum albiflorum*) |  **54** | Down regulation of *lasI*, *lasR*, *rhlI and rhlR* genes and reduced C4-HSL and 3OC12-HSL at 4 mM. [80] |
| Proanthocyanidins |  Proanthocyanidins Epicatechin **55** **56** | Reduced the expression of *lasR, lasI, rhlR* and *rhlI* genes and antagonized LasR and RhlR promoting survival of *Drosophila melanogaster* at doses of 200 μg/mL.[81] |
| (phenylsulfinyl)alanine |  **57** | Inhibited biofilm development at 1 mM through the inhibition of *las* and *rhl* systems.[82] |
| benzyl decanoyl-L-serinate |  **58** | Reduced production of elastase and rhamnolipids and potentiated antibiotic activities at 100 μM through partial down regulation of *lasR, lasI, rhlR* and *rhlI*.[83] |
| Coumarin |  **59** | At 1.36 mM, coumarin slightly inhibited *pqsA* and *rhlI* expression, biofilm formation and swarming.[84] |
| Eugenol |  **60** | At 400 μM, eugenol inhibited *pqs* and *las* in heterologous *E. coli* transcriptional reporter assays, biofilm formation and swarming motility.[85] |
| Sulforaphane from broccoli |  **61** | ~100 μM antagonized LasR in both PAO1 and heterologous *E. coli* reporters, inhibited pyocyanin and biofilm.[86] |
| Trans-cinnam aldehyde |  **62** | At 2.27 mM, inhibited C4-HSL and pyocyanin production (42.06 %) likely to be mediated *via* RhlI rather than LasI inhibition.[87] |
| Baicalin from Scutellaria baicalensis extract. |  **63** | Baicalin at a sub-minimum growth inhibitory concentration (MIC) of 256 μg/ml (equal to 573 μM) inhibited AHL synthesis and biofilm development, enhanced antibiotic efficacy in a mouse foreign body infection model, disrupted motility and promoted survival in *C. elegans*. Baicalin downregulated the *P. aeruginosa* QS network genes (*lasI*, *lasR*, *rhlI*, *rhlR*, *pqsA* and *pqsR*).[88] **63** was identified as a PAINS filter hit. |
| 7,8-dihydroxy flavone |  **64** | Non-competitive inhibition (>70%) of LasR and RhlR in heterologous *E.coli* reporters, reduced pyocyanin and swarming.[89] **64** identified as a PAINS filter hit. |
| Hordenine |  **65** | At 6 mM, **65** inhibited C4-HSL by ~70% and 3OC12-HSL by ~30%, and reduced rhamnolipid, elastase, protease, alginate, pyocyanin and biofilm. Hordenine also downregulated *lasI, lasR, rhlI* and *rhlR* expression.[90] |
| Ajoene derived from garlic extract |  **66** | LasR ($IC_{50}$ 15 μM) and RhlR (50 μM) using *P. aeruginosa* reporter strains.[91] |

physiochemical property criteria definition; for instance, transdermal treatment (i.e., for wound infections) will require different compound properties to those desirable for oral administration.[93] With respect to *rhl* inhibition, it is not yet clear whether antagonizing this system alone would yield therapeutic benefits. Moreover, the lack of structural information for RhlR and RhlI makes compound design more difficult. Therefore, *rhl* requires further validation as a target.

## THE *PSEUDOMONAS* QUINOLONE SYSTEM (pqs)

The *P. aeruginosa pqs* QS system relies on 2-alkyl-4-quinolone signal molecules rather than AHLs that interact with their



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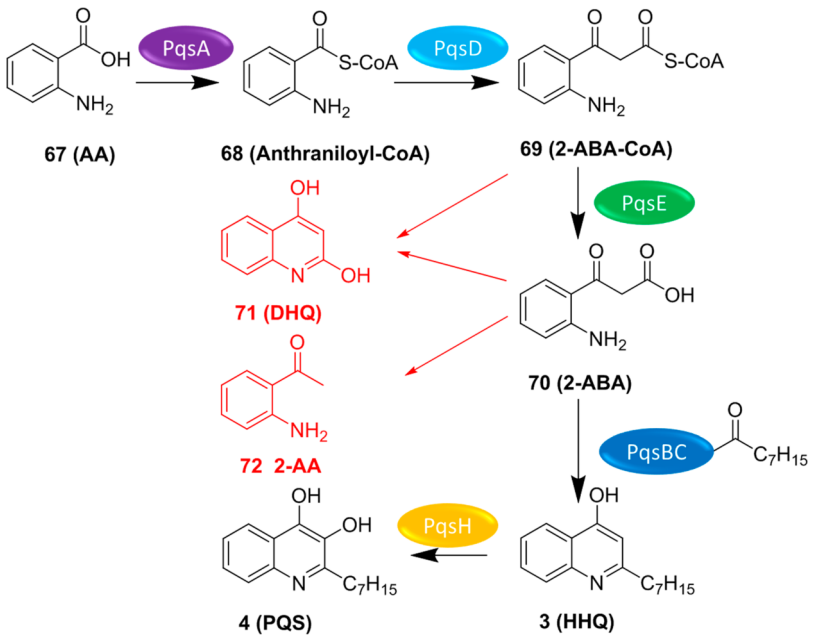


**Figure 10.** Biosynthesis of alkylquinolones starting from activated anthranilic acid and mediated via PqsA, PqsBC, PqsD, and PqsE to generate HHQ which is converted to PQS via PqsH. Non-AQ side products of this route are highlighted in red.

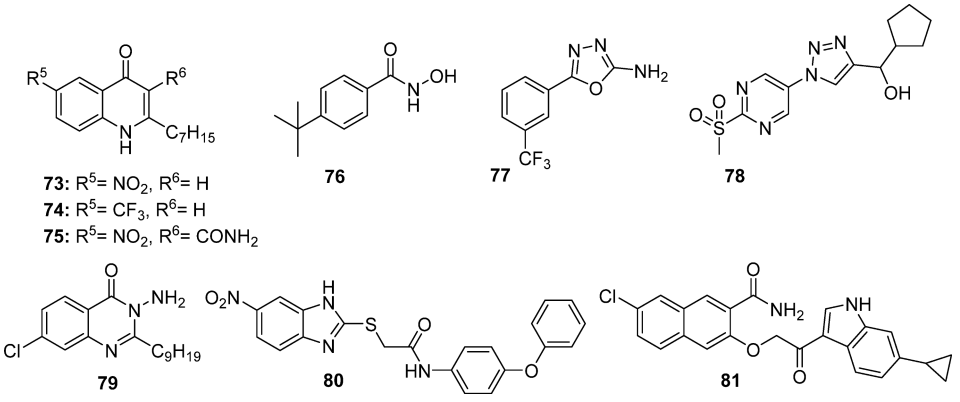


**Figure 11.** Structures of various PqsR antagonists.

cognate receptor PqsR (also known as MvfR), a LysR family transcriptional regulator that characteristically consists of an N-terminal DNA-binding domain and a C-terminal ligand binding domain (Figure 3 and Figure 10). *P. aeruginosa* produces a diverse range of over 50 AQ molecules of three main classes, 2-alkyl-4-hydroxyquinolines, 2-alkyl-3-hydroxy-4-quinolones, and 2-alkyl-4-hydroxyquinoline-*N*-oxides including HHQ **3** (2-heptyl-4-hydroxyquinolone), and the *Pseudomonas* quinolone signal **4** (PQS) (2-heptyl-3-hydroxy-4(1*H*)-quinolone). PQS/HHQ and their C-9 congeners are all able to activate PqsR. In contrast to HHQ, PQS is an iron chelator and regulates the expression of genes involved in the iron-starvation response and virulence factor production via both PqsR-dependent and PqsR-independent pathways.[94,95] AQ biosynthesis is achieved via the condensation of **70** and *β*-keto fatty acids mediated by the heterodimeric enzyme PqsBC to afford HHQ which can be hydroxylated at the 3-position via PqsH to yield PQS.[96,97] The Co-A derivative of **70** is a product of the PqsA and PqsD reactions starting from anthranilic acid **67**. PQS and HHQ are both able to interact with PqsR to form a PqsR/AQ protein complex which in turn binds to the *pqsA* promoter leading to further activation of the *pqsABCDEphnAB* operon hence triggering the autoinduction response characteristic of most QS systems.[24,95] Recent studies have revealed that although PqsE is a thioesterase that contributes to AQ biosynthesis, the mechanism by which PqsE controls a subset of virulence factors including pyocyanin is still not understood.[98,99] Interestingly, the pathogenicity of a *P. aeruginosa pqsE* mutant in contrast to a *pqsA* mutant was not attenuated in a mouse wound infection model. However, *pqsE* alone in the absence of AQ production can restore the virulence of a *pqsA* mutant.[99,100]

The AQ system is linked with *las* and *rhl* QS as LasR (positively) and RhlR (negatively) regulate PqsR through binding to its promoter.[101] Moreover, *pqsH* is positively regulated by LasR/3OC12-HSL which increases the abundance of PQS.[102] The *pqs* system plays a major role in the virulence of *P. aeruginosa* as demonstrated by the attenuation of *pqsA* and *pqsR* mutants in murine infection models.[99,103] In contrast to LasR/3OC12-HSL and RhlR/C4-HSL that bind to the promoters of multiple target genes, transcriptome experiments suggest that PqsR has only a single target promoter, that of *pqsA*.[95] However, using ChIPseq, Maura et al. suggested that PqsR bound to 35 locations on the *P. aeruginosa* chromosome, although only 22% of these were to promoter regions.[104] Most of


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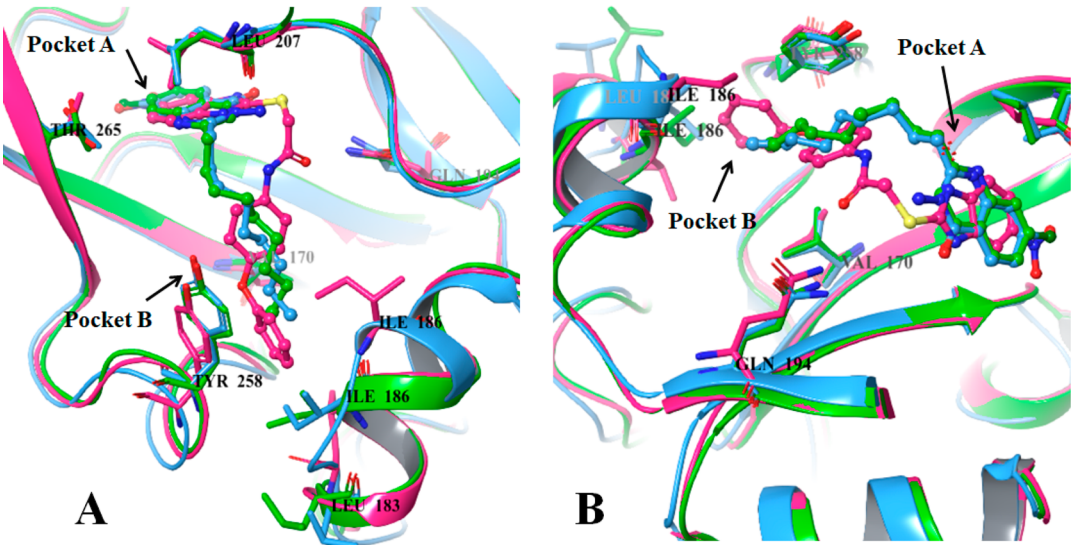


**Figure 12.** Overlay poses of the cocrystal structures of PqsR ligand binding domain with a natural agonist NHQ (PDB code 4JVD, green), quinazolinone based antagonist **79** (PDB code 4JVI, blue), and thioacetamide based antagonist **80** (PDB code 6B8A, pink). Figure 12A highlights the hydrophobic residues surrounding pocket B. Figure 12B shows the binding pose of heterocyclic headgroup residing in pocket A. The poses were generated using Maestro, Schrödinger, LLC, New York, NY (2018).

the binding sites were located inside a gene or overlapping several genes, and hence their functionality as PqsR binding sites remains to be validated.[104]

## INHIBITION OF *pqs* QUORUM SENSING IN *P. aeruginosa*

**Inhibition of Signal Reception by PqsR.** Lu et al. discovered a PqsR antagonist based on HHQ by introducing modifications to the benzene moiety of the quinolone ring and the aliphatic side chain. The highest potencies, as demonstrated using a recombinant *E. coli* transcriptional reporter, were achieved upon the introduction of a strong electron withdrawing group (Figure 11; **73**, $IC_{50}$ = 51 nM; **74**, $IC_{50}$ = 54 nM) at the 6-position of the HHQ quinoline ring. Despite the fact that these compounds showed good potency using the *E. coli* reporter, in *P. aeruginosa*, only a modest reduction in pyocyanin was noted and they failed to reduce elastase, rhamnolipids, or AQ levels.[105,106] When **73** and **74** were further tested in *P. aeruginosa*, it became clear that PqsH-mediated the oxidation at the 3-position of the quinoline ring of these inhibitors converting them to potent agonists. This effect was not observed for compound **75** ($IC_{50}$ = 35 nM, *E. coli* reporter, $IC_{50}$ = 4 $\mu$M, *P. aeruginosa* reporter) where a blocking group ($CONH_2$) was introduced at the 3-position to preserve the antagonistic activity in *P. aeruginosa*.[107−109] Klein et al. reported another series of weak PqsR inhibitors derived from *N*-hydroxybenzamides with modification at the *para*-position. Only modest activity against PqsR was attained with **76** ($IC_{50}$ in *E. coli* of 12.5 $\mu$M vs $IC_{50}$ in *P. aeruginosa* of 23.6 $\mu$M) which weakly affected pyocyanin at $IC_{50}$ concentrations.[110] In a follow-up study, the carboxamide group was replaced by a 1,3,4-oxadiazole moiety to provide **77** with similar antagonist activity to **76** along with marginal activity on pyocyanin and AQ production.[111]

In a similar effort, the same group published a series of weak PqsR antagonists based on a triazole scaffold (**78**, $IC_{50}$ = 26 $\mu$M) in a pursuit of dual PqsR/PqsD inhibitors. Compound **79**, the first PqsR inhibitor with low micromolar potency in *P. aeruginosa*, was discovered by Ilangovan et al. based on a quinazolinone headgroup with a hydrazide at the 3-position. **79** displayed a clear effect on pyocyanin production, AQ synthesis, and biofilm formation.[112] Shortly after this study, Starkey et al. reported a PqsR antagonist **80** with submicromolar potency and a substantial effect on virulence factors at concentrations of >1 $\mu$M. In addition, **80** enhanced the effect of tobramycin in clearing *P. aeruginosa* PA14 biofilms. Moreover, this inhibitor potentiated the effect of ciprofloxacin, reduced persistence, and increased postinfection survival rates in burn and lung infection models in mice.[113,114] Spero Therapeutics published a patent for aryloxyacetoindoles as PqsR inhibitors detailing an extensive SAR. One of the optimal compounds **81** demonstrated submicromolar potency (50−250 nM) and around 50% reduction of AQs in an in vivo acute thigh infection model in mice after oral administration with 4 doses of 200 mg/kg postinfection.[115] No further development of these inhibitors has been reported.

## STRUCTURAL INSIGHTS OF PqsR LIGAND BINDING DOMAIN

To date, there is no report describing the crystal structure for the full length PqsR protein; however, the truncated co-inducer binding domain of PqsR ($PqsR^{cbd}$) has been described in literature.[112,116−118] Ilangovan et al. reported the first crystal structure of $PqsR^{cbd}$ bound to a natural agonist (2-nonylquinolin-4(1*H*)-one, NHQ; PDB code 4JVD) to show that the PqsR ligand binding site consists of two subdomains: pocket A which accommodates the co-inducer quinolone ring and pocket B where the aliphatic chain resides supported by hydrophobic interactions with Tyr 258, Ile189, Ile186, and Val170 (Figure 12). The same group also described the cocrystal structure of $PqsR^{CBD}$ with the quinazolinone-derived inhibitor **79** (PDB code 4JVI) which induced a subtle conformational change at the level of Thr265 in pocket A to accommodate the chlorine substituent at the 7-position. The binding of **79** also featured a hydrogen bond between the hydrazide group and the side chain of Leu207.[112] Kitao et al. recently reported the crystal structure of **80** (PDB code 6B8A, Figure 12) which adopted a similar binding mode to **79** with the benzimidazole ring residing in place of quinazolinone and the thioacetamide linker making a


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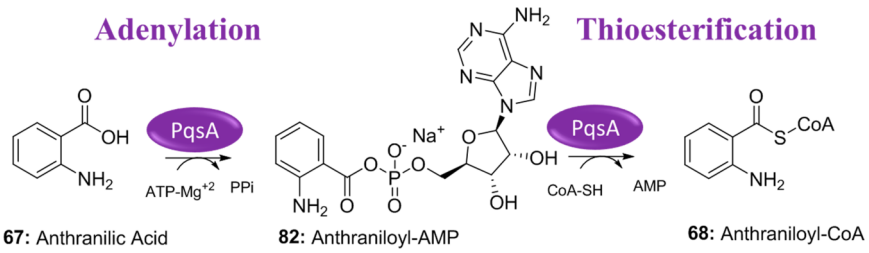


**Figure 13.** Formation of anthraniloyl-CoA mediated by PqsA through two consecutive half-reactions, first is adenylation to form **82** followed by thioesterification to provide **68**.

hydrogen bond to Gln194. It is noteworthy that the bulky phenoxyphenyl group occupied the cigar shaped hydrophobic pocket B and induced the reorientation of Leu183 and Ile186 residues.[118]

## INHIBITION OF AQ BIOSYNTHESIS

**Inhibitors of PqsA.** PqsA, a CoA-ligase enzyme, is the first enzyme in the AQ biosynthetic pathway responsible for the conversion of anthranilic acid **67** into anthraniloyl-CoA **68** mediated via adenylation to give **82** followed by a thioesterification (Figure 13).[119] PqsA represents a valid antivirulence target due its essential role in AQ biosynthesis. *P. aeruginosa pqsA* mutants exhibit reduced biofilm formation compared with the isogenic wild type strains.[100,120,121]

Initial attempts to inhibit this enzyme were based on substrate analogs such as halogenated anthranilate derivatives (Figure 14, **83**, **84**, **85**) which showed weak inhibition with millimolar concentrations of the ligands being required to demonstrate an effect.[119,122] Nevertheless, these PqsA inhibitors increased survival rates in mice infected with *P. aeruginosa* and reduced bacterial systemic dissemination.[122] Ji et al. recently reported the design of anthraniloyl-AMP analogs as PqsA inhibitors through the replacement of the phosphate bridge by a sulfonamide based linker. Despite the high affinity of **86** and **87** in the enzymatic assay (**86**, $K_i$ = 88 nM; **87**, $K_i$ = 109 nM), the inhibitors only weakly reduced AQ and pyocyanin levels in *P. aeruginosa* most likely due to their limited bacterial cell permeability.[123] The resolution of the PqsA ligand binding domain crystal structure by Witzgall et al. should aid the design of future enzyme inhibitors with improved potency and permeability.[124]

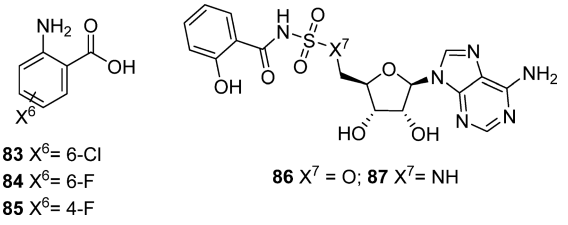


**Figure 14.** Structures of reported PqsA inhibitors.

**Inhibitors of PqsD.** PqsD is the second enzyme in the HHQ biosynthetic pathway and is responsible for the condensation of anthraniloyl-CoA **68** (Figure 10) with malonyl-CoA to produce 2-aminobenzoylacetate-CoA **69**.[125] The first weak PqsD antagonists (Figure 15, **88**, $IC_{50}$ = 65 $\mu$M; **89**, $IC_{50}$ = 35 $\mu$M using in vitro enzymatic assays) were derived from inhibitors of FabH a structural and functional homolog of PqsD.[126] These compounds were further optimized to low micromolar potencies (**90**, $IC_{50}$ = 1.1 $\mu$M; **91**, $IC_{50}$ = 1.6 $\mu$M following the same assay) and were able to compete with anthraniloyl-CoA for the substrate pocket.[127,128] Strikingly, this benzamide−benzoic acid scaffold was employed by the same group for the search of bacterial polymerase inhibitors (RNAP) as antibacterial agents.[129] Although a follow-up study highlighted the areas of the molecules that contribute to selectivity against PqsD, this was only improved by 50-fold.[128] Moreover, there was a lack of evidence for the effect of these PqsD inhibitors on *P. aeruginosa* growth particularly given that RNAP inhibition was solely assessed in an *E. coli* based assay. Following a ligand-based approach, inhibitor **92** was identified with $IC_{50}$ of 3.2 $\mu$M in PqsD functional assay (ITC: $K_d$ = 13 $\mu$M). However, the in vitro effect of **92** on biofilm and AQ production was only achieved using high concentrations (250−500 $\mu$M).[130] A follow-on paper described the SAR of **93** with little improvement in cellular activity.[131] Urea-based PqsD inhibitors were also described by Sahner et al. with $IC_{50}$ values of 0.5 $\mu$M and 0.14 $\mu$M for compounds **93** and **94**, respectively.[132,133] Once again, a similar

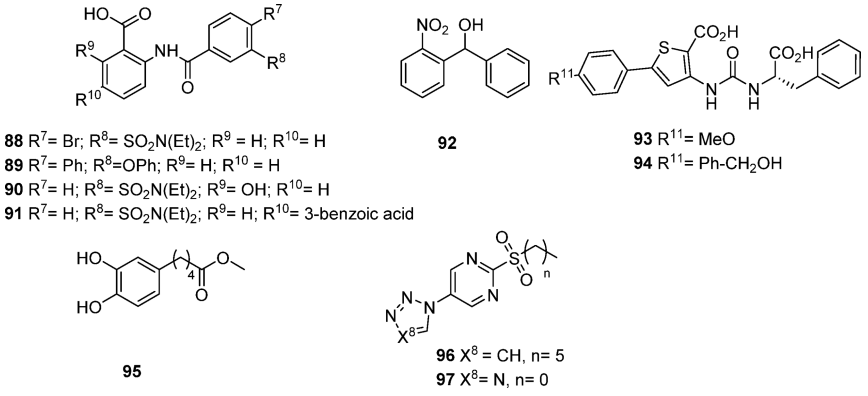


**Figure 15.** Structures of reported PqsD inhibitors.



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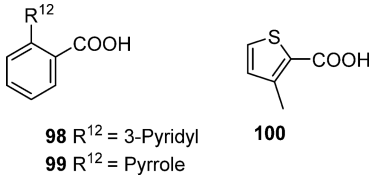
scaffold was used by the same group to identify RNAP inhibitors in *E. coli* which questions the selectivity of **93** and **94** against PqsD and their effect on bacterial growth.[132] Catechol-derived scaffold **95** was reported as a PqsD inhibitor based on the structural similarity between PqsD and CHS2, a chalcone synthase from *Medicago sativa*. Despite the micromolar potency in the enzymatic assay for **95** ($IC_{50}$ = 7.9 $\mu$M), only a slight effect was obtained in cells at concentration of 0.25 mM.[134] Moreover, **95** was identified as PAINS structure and therefore could act as a false positive in the enzymatic assay. A sulfonyl pyrimidine scaffold was employed in the discovery of dual PqsD/PqsR inhibitors by Thomann et al., who reported compound **96** with selectivity against PqsD ($IC_{50}$ = 1.7 $\mu$M) that caused a 60% reduction in PA14 biofilm at concentration of 100 $\mu$M.[135] Interestingly, compound **97** which shares the same scaffold with **96** showed weak dual inhibition against both targets (PqsD, $IC_{50}$ = 21 $\mu$M; PqsR $IC_{50}$ = 15 $\mu$M) with effects on pyocyanin and biofilm at high micromolar concentrations.[135,136]

**Inhibitors of PqsE.** PqsE is a thioesterase enzyme capable of transforming the PqsD reaction product **69** (2-ABA-CoA) into 2-aminobenzoylacetate **70**, the HHQ precursor. Although *PqsE* mutants produce similar levels of HHQ to the wild type strain they generate more DHQ arising from the intramolecular cyclization of the PqsD product, 2-ABA-CoA.[98,100] 2-Aminoacetophenone **72** is another metabolite obtained from this pathway through the decarboxylation of 2-ABA-CoA **69**. PqsE therefore plays a central role in AQ biosynthesis and balances the formation of AQs, **71** and **72** from 2-ABA-CoA with the dead-end product, **71** and **72**. However, the functions of PqsE are not fully understood since the thioesterase activity does not account for the AQ-independent activities of PqsE in regulating virulence factors including pyocyanin, HCN, and rhamnopliids in the absence of AQ production.[95,100] The only attempt to inhibit PqsE was achieved through fragment based drug discovery, and three fragments (Figure 16, **98**, **99**, **100**) were




**98** $R^{12}$ = 3-Pyridyl
**99** $R^{12}$ = Pyrrole
**100**

**Figure 16.** Structures of reported PqsE inhibitors.

identified as inhibitors with submillimolar potencies with their binding confirmed by cocrystallization experiments and isothermal calorimetry. Although these compounds attenuated the thioesterase activity of PqsE, as demonstrated by the accumulation of DHQ and 2-ABA, they failed to modulate pyocyanin production.[137] Therefore, the validity of PqsE as a drug target requires further investigation particularly as another broad spectrum thioesterase (TesB) in *P. aeruginosa* may be able take over its biological function with respect to AQ biosynthesis.[98]

**Inhibitors of PqsBC.** PqsBC is a heterodimeric $\beta$-keto acyl synthase III enzyme responsible for the condensation of 2-ABA **70** and octanoyl CoA to form HHQ.[97] It was reported that 2-aminoacetophenone (2-AA) **72**, a secondary metabolite from the AQ biosynthesis pathway, was able to inhibit PqsBC with an $IC_{50}$ of 46 $\mu$M in a PqsBC-based biochemical assay[97] and reduces virulence in an acute infection model in mice.[138] Maura et al. reported the first synthetic inhibitors for PqsBC (Figure 17, **101**) which are based on a benzimidazole scaffold, previously described for PqsR inhibitor **80**. Through using LCMS/MS to quantify AQ synthesis intermediates, they found that some analogs were able to inhibit PqsBC as evidenced by a reduction in HHQ production concomitant with increased levels of 2-AA and DHQ. The $EC_{50}$ values for **102** (dual PqsR and PqsBC) and **103** (PqsBC) were determined in a PqsBC enzymatic assay to be 13.4 $\mu$M and 12.5 $\mu$M, respectively. It is intriguing that minor structural changes to **94** (higher activity toward PqsR) enhanced the activity toward PqsBC. It has been shown that selective PqsBC inhibitors induced less tolerance in *P. aeruginosa* cells toward the $\beta$-lactam antibiotic meropenem compared to dual or selective PqsR inhibitors.[139] Allegretta et al. re-evaluated previously published *pqs* inhibitors and their effect on PqsBC inhibition and showed that compounds **104** and **105** are able to significantly induce an increase in 2-AA and DHQ levels at concentrations of 10 $\mu$M and 250 $\mu$M.[140] It is noteworthy that compound **104** was reported as a weak PqsD inhibitor in a previous study.[131] However, the validity of PqsBC as antivirulence drug target remains doubtful as even though PqsBC inhibition leads to a reduction of AQ signal synthesis, it induces accumulation of 2-AA and DHQ, molecules that enhance the persistence of *P. aeruginosa* and promote chronic infections.[138,140,141] Hence, PqsBC inhibitor combination therapy would be advisible with other *pqs* pathway inhibitors.

## GENERAL AND MULTITARGET *pqs* INHIBITORS

In addition to the compounds listed above, there are other reports of *pqs* inhibitors with no specific, defined targets. For instance, recent work described 4-aminoquinoline derived molecules as inhibitors for *pqs* signaling with a potency of ~2 $\mu$M for compound **106** (Figure 18) against *P. aeruginosa* PA14. The study demonstrated the effect of this class of inhibitors in a series of phenotypic assays including biofilm formation in two different laboratory strains of *P. aeruginosa* (PAO1-L, PA14). Molecular docking studies implicated PqsR as the plausible target, but this was not confirmed experimentally.[142] The 7-chloroquinoline scaffold was also presented in another study showing that **107** was able to disrupt biofilm formation and pyocyanin production at a concentration of 138 $\mu$M through inhibition of PQS signaling (81%).[143] Pyrrol derivative **108** was reported in a patent as a *pqs* inhibitor with $IC_{50}$ values of 22 $\mu$M and 17 $\mu$M in strains PAO1-L and PA14, respectively. **108** reduced pyocyanin and AQ biosynthesis without affecting bacterial growth up to a concentration of 100 $\mu$M.[144] Fong et al. reported a "pan" QS inhibitor **109** for *P. aeruginosa* with low-micromolar activity ($IC_{50}$ of 0.56 $\mu$M for *las*, 3.49 $\mu$M for *rhl*, and 5.63 $\mu$M for *pqs* using *P. aeruginosa* reporters) leading to the down-regulation of multiple virulence factors (pyocyanin, rhamnolipids, elastase). **109** also exhibited high clearance rate of bacteria post foreign body infections in mice.[145]

## PERSPECTIVE VIEW

The *pqs* system in *P. aeruginosa* is crucial for the full virulence and persistence of this human pathogen as well as some of its immune modulatory effects. Reports describing the occurrence of *lasR* mutations in clinical *P. aeruginosa* isolates from chronic infections such as those encountered in cystic fibrosis lend further significance to *pqs* signaling as a target for antipseudomonal drugs. Now that the mechanistic biochemical basis for *pqs* biosynthesis and signal transduction have been elucidated and complemented with an understanding of the structural basis for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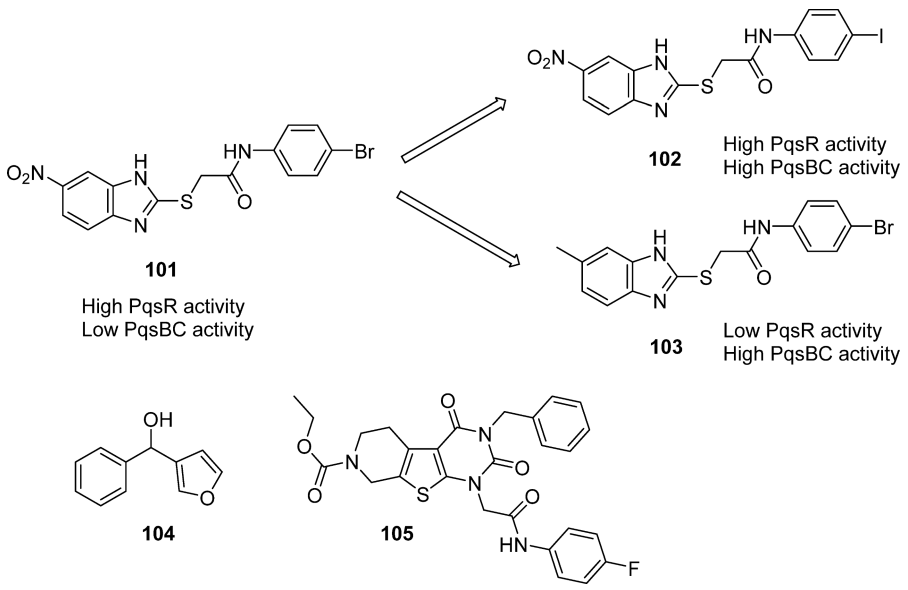



**Figure 17.** Structures of published PqsBC inhibitors.

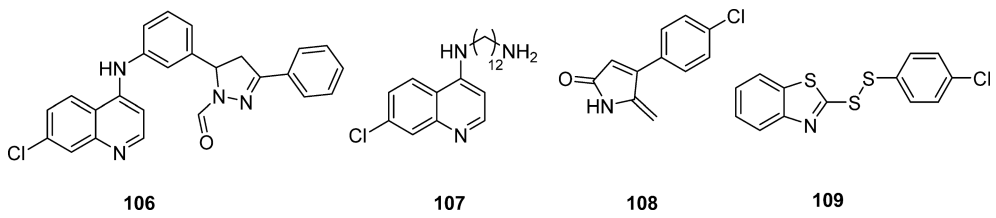
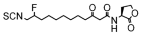
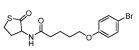
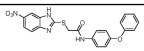
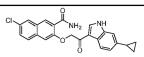
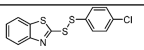



**Figure 18.** Structures of reported general and multitarget *pqs* inhibitors.

**Table 2. Summary of Preclinical QS Inhibitors in *P. aeruginosa* with Their Predicted Physicochemical Properties**[a]

| ID | Structure | Target | *In vivo* | LogP* | HBA* | HBD* | TPSA* | Lipinski rule* | Lead likeness* |
|---|---|---|---|---|---|---|---|---|---|
| **47** |  | LasR | *C. elegans* | 2.9 | 4 | 1 | 84.8 | √ | X |
| **50** |  | RhlR | *C. elegans* | 2.8 | 3 | 1 | 55.4 | √ | √ |
| **80** |  | PqsR | mice | 4.6 | 4 | 2 | 110.2 | √ | X |
| **81** |  | PqsR | mice | 4.4 | 3 | 2 | 85.2 | √ | X |
| **109** |  | mixed | mice | 6.0 | 1 | 0 | 12.9 | X | X |

[a]The asterisk (∗) indicates that these parameters were predicted using Instant JChem 18.8.0, 2018, ChemAxon (http://www.chemaxon.com).

its essential components including crystal structures for PqsR, PqsA, PqsD, PqsBC, and PqsE, it is clear that certain elements of the *pqs* system represent attractive drug discovery targets. Indeed, inhibitors **80** and **81** have advanced in preclinical stages and the available data provide a robust proof of concept for targeting PqsR. PqsA, PqsD are emerging as additional new targets that have yet to be fully explored. Despite the fact that the PqsBC heterodimer is critical for AQ biosynthesis, its validity as antivirulence targets remains to be elucidated. The lack of attenuation of *pqsE* mutants in mouse infection models indicates that PqsE is unlikely to be a good target.

## RESISTANCE TO ANTIQUORUM SENSING AGENTS

There is some evidence that QS mutants arise in bacterial populations specifically under conditions where QS is essential for bacterial growth but only where they continue to benefit to from the metabolic activities of QS competent cells.[146] QSI resistance could fall into one of the following categories: (i) overexpression of QS signal receptor genes or a receptor homologue to overcome inhibition, (ii) point mutations in the receptor such that it becomes signal independent, and (iii) preference of one particular QS system over others.[147]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There are few reports describing the development of resistance to QSIs in *P. aeruginosa*. For the furanone-derived compound **27**, the underlying resistance mechanism was solely reasoned to the up-regulation of an efflux pump due to a mutation in *mexR* and *nalC* regulatory genes.[148,149] However, it is noteworthy that the QSI concentration used for this study was at least 25-fold higher than that previously reported for QS inhibition by **26** in the original literature. At such elevated concentrations, **26** is cytotoxic and growth inhibitory and so will exert selective pressures on the bacteria to drive the evolution of mutations that confer resistance.

Currently, the literature relating to QSI resistance is scarce and limited to certain cases and specific growth conditions. Further investigations of selection for QSI resistance in conditions that mimic in vivo infections will be vital to establish a sound platform for the future design and development of QSIs with nanomolar potencies.

## CONCLUSION

Through reviewing the medicinal chemistry related QS literature, it is clear that more effort needs to be directed toward the design of drug-like molecules with favorable physiochemical properties. It becomes evident that the majority of the inhibitors identified to date function as useful probes for mechanistic studies rather than lead-like compounds for further drug development as summarized in Table 2.

In addition, the lack of methodological standardization in assessing QSI candidates including the use of single laboratory-adapted *P. aeruginosa* strains limits the broad validity of any findings such that these may be distant from relevant clinical infections and so constitute a major pitfall in this field. Within the *P. aeruginosa* QS circuitry, the *pqs* system holds promise for prospective therapeutics particularly at the level of PqsR where inhibitors with nanomolar potencies and lead-like properties have already been developed. However, it is important to note that QS inhibitors are most likely to be beneficial as adjuvants for conventional antibiotics rather than as standalone therapeutic agents, although they may prove useful for prophylaxis. Undoubtedly, polypharmacology through the concurrent use of inhibitors for various targets/QS systems could also prove highly beneficial in combating multiantibiotic bacterial resistance.

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### Notes

The authors declare no competing financial interest.

### Biographies

**Fadi Soukarieh** obtained his Ph.D. in Medicinal Chemistry from University of Nottingham (U.K.) in 2013 under supervision of Professor Peter M. Fischer. He was then appointed as a Postdoctoral Research Fellow working on anticancer project targeting CDK9. He then joined Prof. Cámara, Dr. Stocks, and Prof. Williams groups to work on multinational project (SENBIOTAR) for the discovery of new PqsR antagonist as novel antipseudomonal agents. Along his work, he contributed to teaching and supervision of a number of M.Sc. and Ph.D. research students.

**Paul Williams** is currently Professor of Molecular Microbiology in the School of Life Sciences, Faculty of Medicine and Health Sciences, University of Nottingham, U.K. From 1996 to 2008 he was Director of the Institute of Infections & Immunity after which he became Head of the School of Molecular Medical Sciences, University of Nottingham. His research interests focus primarily on the regulation of gene expression in bacteria through cell−cell communication (quorum sensing) and the development of novel antibacterial agents and bacterial biofilm resistant polymers. He has published over 300 research papers and reviews, has served on the Medical Research Council UK MRC Infection and Immunity board and the scientific advisory board of the EU Joint Programming Initiative in Antimicrobial Resistance and is a Wellcome Trust Senior Investigator.

**Michael J. Stocks** was appointed as an Associate Professor in Medicinal Chemistry in 2012 within the School of Pharmacy at The University of Nottingham. He has over 20 years of industrial experience in drug discovery within AstraZeneca, and during his industrial career, he was both the lead scientist and project leader on multiple preclinical research projects as well as the synthetic medicinal chemistry lead of the AstraZeneca compound enhancement initiative. Since joining the School of Pharmacy in 2012, Michael has grown his research group and his research has focused on the medicinal chemistry design of compounds to study and modulate the function of biological targets.

**Miguel Cámara** is a Professor of Molecular Microbiology in the School of Life Sciences, University of Nottingham since 2009. He has 24 years of expertise studying molecular mechanisms of quorum sensing-based control of gene expression in bacteria with emphasis on biofilms, the influence on the behavior of polymicrobial communities, and the interaction with mammalian hosts and eukaryotic organisms. He has also been working on the identification of novel antimicrobial targets to treat detrimental biofilms and coordinated several international antimicrobial programs mainly focused on quorum sensing inhibition as the main target. He has >120 research papers in peer reviewed journals and is co-director of the National Biofilms Innovation Centre and a member of the UK Cystic Fibrosis Trust Strategic Implementation Board.

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## ABBREVIATIONS USED

2-ABA, 2-aminobenzoylacetic acid; 3OC12-HSL, *N*-(3-oxodo-decanoyl)-L-homoserine lactone; AHL, acylhomoserine lactone; AI, autoinducer; AMR, antimicrobial resistance; AQ, alkyl quinolone; C4-HSL, *N*-butanoyl-L-homoserine lactone; FDA, U.S. Food and Drug Administration; HCN, hydrogen cyanide; HHQ, 2-heptyl-4-hydroxyquinoline; $K_d$, dissociation constant; $K_i$, association constant; mAb, monoclonal antibody; MDR, multidrug resistance; PQS, *Pseudomonas* quinolone signal; QS, quorum sensing; QSI, quorum sensing inhibitor; QSSM, quorum sensing signal molecule; QZN, quinazolinone; WHO, World Health Organisation.


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