Next Generation Biofilm Inhibitors for *Pseudomonas aeruginosa*: Synthesis and Rational Design Approaches

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Abstract:
The bacterial biofilms and the emergence of multiple drug resistance have become a major threat for current medical treatment of nosocomial infections. It has been estimated that about 65-80% of microbial infections in the developed countries are associated with biofilms. Given the prominence of biofilms in infectious diseases, increasing efforts toward the development of small molecules that will modulate bacterial biofilm development and maintenance is on the rise. Till date, marine natural products have shown a tremendous potential as pharma leads and also given new skeletons which would be used as biofilm/QS inhibitors. Medically relevant biofilm forming bacteria such as Pseudomonas aeruginosa which is most frequently isolated bacteria in nosocomial infection is believed to be a model organism for biofilm studies. Hence, in this review, we have highlighted the development of small molecules that inhibit and/or disperse bacterial biofilms of P. aeruginosa in particular. Additionally, the rational design approaches as well as synthetic methodologies along with biological studies has been accounted in this article.

Keywords. Marine natural products, Biofilms, Quorum sensing inhibitors, Antifouling, Chemical signaling
INTRODUCTION

Since the discovery of Quorum sensing (QS) (cell-cell signaling system) in 1996, it has been identified in numerous pathogens. During cell growth, small signaling molecules were synthesized and secreted into the surrounding environment. As a result, the local QS signal concentration increases with cell density [1]. When cell density is above a certain threshold, a significant amount of signal molecules bind to membrane receptors and trigger the expression of series of genes to control a wide spectrum of phenotypes such as bioluminescence in *Vibrio fischeri*, bacterial biofilm formation, and many others (Fig. (1)). Biofilm formation has tremendous detrimental effects and has brought huge amounts of problems to our everyday life (Fig. (2)). In addition, biofilms show resistance to a wide range of antibiotics such as ampicillin, streptomycin, tetracyclines, gentamicin *etc.* Also, the conventional methods such as antibiotics, and disinfectants to control the infections caused by biofilm formation are often ineffective due to their special physiology and physical matrix barrier [2]. Consequently, the development of novel strategies based on understanding of the biofilm formation mechanism are urgently in demand for the benefit of mankind.

![Biofilms Impact](http://www.erc.montana.edu/)

**Fig. (1). Problems due to bacterial biofilms in everyday life** (copied from http://www.erc.montana.edu/)
The formation of biofilms is dynamic and complicated process which involves many physiological activities and is highly dependent on the environmental factors (e.g. oxygen level, shear force, nutrition). Medically relevant bacteria that are responsible for formation of biofilms and their detrimental effect on human being are depicted in Table 1. Natural products, in particular, marine natural products have been proven useful in providing new chemical entities to target biofilm inhibition and serve as a rich source of privileged skeletons for studying chemical as well as biological applications [4].
<table>
<thead>
<tr>
<th>Organism</th>
<th>Classification</th>
<th>Biofilm associated disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Gram (-) γ-proteobacterium</td>
<td>Cystic Fibrosis lung infection</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>Gram (-) β-proteobacterium</td>
<td>Cystic Fibrosis lung infection</td>
</tr>
<tr>
<td><em>Acinetobacter baumannii</em></td>
<td>Gram (-) γ-proteobacterium</td>
<td>Burn wound, trauma infection</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Gram (-) γ-proteobacterium</td>
<td>Urinary and gastrointestinal infection</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>Gram (-) β-proteobacterium</td>
<td>Respiratory infection</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Gram (+)</td>
<td>Burn wound, catheter, lung infection</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Gram (+)</td>
<td>Nosocomial sepsis, catheter infection</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em></td>
<td>Gram (+)</td>
<td>Dental caries</td>
</tr>
</tbody>
</table>

**HISTORIAL BACKGROUND OF BIOFILMS/QUORUM SENSING: ROLE OF SMALL MOLECULES**

Bacteria have evolved chemical-mediated cell-to-cell communication systems to coordinate gene expression and group activities within communities, which was defined as ‘quorum sensing’ by Dr. Peter Greenberg in 1994 [5]. Within a community, individual bacterial cells produce and respond to their specific secreted small molecules which were termed as autoinducers. Thus, signaling molecules co-ordinate the gene expression of individual cells within the bacterial communication. The bacterial quorum sensing systems are generally classified into three major classes based on the type of autoinducer signals and the receptors used for its detection. First, Gram negative bacteria typically use *LuxI/R* quorum sensing systems [5]. *LuxI* is an enzyme that synthesizes an acylated homoserine lactone
(AHL) based autoinducer which interacts with the transcriptional regulator *LuxR* and then controls transcription of quorum-sensing target genes. *LuxI/R* systems exist in more than 70 species of Gram negative bacteria [6]. QS system based on homoserine lactones (acyl-HSLs or AHLs) are commonly found in Gram negative bacteria. In general, each AHL signal is generated by a synthase (called I protein) and is detected by a specific autoinducer receptor (called R protein). Particularly, *P. aeruginosa* produces and responds to two AHLs signals namely *N*-((3-oxododecanoyl)-HSL (synthesized by *LasI* and detected by *LasR*) [7], and *N*-butyryl-HSL (synthesized by *RhII* and detected by *RhIR*) [8]. The specific binding of a QS signal to its receptor is critical for the regulation of gene expression. AHLs have been found during bacterial infections in human and also been detected in animal models such as, in lung tissues of mice and ischemic wound of rat infected with *P. aeruginosa* [9]. These facts together provide evidence that the AHLs are potential targets for both diagnosis and drug development. 4-Quinolones is another class of autoinducer in Gram negative bacteria wherein 2-heptyl-3-hydroxy-4-quinolone in *Pseudomonas spp.* is widely studied and called as Psedomonas Quinolone Signals (PQS). The expression of virulence determinants in *P. aeruginosa* is controlled by PQS [10]. Autoinducing peptides are the key signals for QS in many Gram-negative bacteria.

The second class of quorum sensing systems exist in Gram positive bacteria, wherein modified oligopeptides are used as autoinducers. The extracellular peptide autoinducer is recognized by a two-component signal transduction protein called sensor histidine kinase [11]. The sensor histidine kinase then transmits sensory information via phosphorylation of a two-component response regulator protein and modifies its DNA binding activity and thus enables it to control transcription of quorum sensing target genes [11]. A group of γ-butyrolactone having structural similarity with AHLs is another class of autoinducer in Gram +ve bacteria found in *Streptomyces* species [12]. These signals have been shown to bind to a repressor-type receptor protein (ArpA) and induce the production of antibiotics such as streptomycin in *Streptomyces griseus*. The third class of quorum sensing systems is a combination of the Gram negative and Gram positive systems. Small molecule autoinducer (e.g. AHL) is synthesized and released into the environment like the first quorum sensing systems among Gram negative bacteria. However, the sensing of the autoinducer and control of transcription of quorum-sensing target genes is through a two-component signal transduction system like the second class of quorum sensing system among Gram positive bacteria [13]. There are also other signal systems developed for both intra and
inter-species QS, in addition to the AHLs in Gram -ve bacteria and peptides in Gram +ve bacteria i.e. AI-II derived from (S)-4,5-dihydroxy-2,3-pentanedione (DPD) with the activity of LuxS as QS signal in bacteria [14]. Additionally, AI-II play a role in biofilm formation as that of AHLs and has been demonstrated as interspecies signal for communication among both Gram –ve and +ve species. Cis-11-methyl-2-dodecenoic acid signal was found to function in multiple species. It helps to control flagellum synthesis, aerobic respiration and resistance to toxins in Xanthomonas campestris [15]. In a nutshell, QS has been found in Gram –ve, Gram +ve bacteria and fungi whose signals are mainly categorized as AHLs, quinolones, autoinducing peptides (AI-II, AI-III) and farnesoic acid [16]. Besides the above three quorum sensing systems, there are many minor quorum sensing systems which only exist in certain organisms. Quorum sensing systems are used to regulate important behaviors that are most effective when coordinated within a large population, including production of virulence factors, motilities, production of iron chelators, exoenzymes, antibiotics, biofilm formation etc. [17].

WORKING OF BIOFILM/QS-INHIBITORS AND METHODS TO CONTROL BIOFILM FORMATION

Quorum sensor is microbial cell-to-cell signaling systems that correlate gene expression with cell population density and hence for the QS system to work requires following criteria: a) the cell must be able to synthesize the signaling molecules; b) detect the signals through specific molecular interactions, and c) activate or inactivate the target genes by responding to specific signal receptor binding events. Breakpoint of any of these steps could result in repression of virulence and failure for the microbes to infect the host. The general mechanistic approach for QS inhibitor is given in Fig. (3).
The treatment of bacterial infectious diseases caused due to detrimental effects of biofilms and the emergence of antibiotic resistance have become a major threat for current medical cure [18]. Due to the extensive use of antibiotics, clinically significant antibiotic resistance has arisen against virtually every antibiotic developed [19]. It is a big challenge for researchers to develop novel classes of antibiotics to catch up our growing need for such drugs. Also, novel strategies to inhibit bacterial biofilm formation and disrupt mature biofilms are obviously in need. Considering the serious health related problems caused by biofilm formation, various approaches for preventing and controlling biofilm formation have been developed for last several decades. These includes: a) prevention of bacterial cell adhesion, b) reduction of polysaccharide production, c) disruption of cell-to-cell communications in bacteria. In the following text, the concept of anti virulence strategy and anti biofilm strategy will be introduced briefly.

**Anti virulence strategy development**

Since the discovery of penicillin in 1928, regular antibiotics have routinely been identified based on their ability to kill bacteria (bacteriocidal) or inhibit bacterial growth (bacteriostatic). These regular antibiotics could inhibit a range of bacterial functions that are essential for *in vitro* growth such as cell wall synthesis (β-lactams), DNA replication (Fluoroquinolones), and protein synthesis
(Aminoglycosides). However, the high mutation rate of bacterial genome has made it possible for bacteria to evolve resistant towards almost all classes of regular antibiotics [20]. Pathogenic bacteria usually possess a wide range of virulence factors to enable themselves to successfully invade host tissues and cause disease. These virulence factors include adhesion, extracellular toxins, iron chelating siderophores and so on. For example, \textit{P. aeruginosa} mutants who lack type IV pili or flagellum were seriously deficient in causing infections based on animal models [21]. Therefore, development of anti virulence therapies endeavors at inhibiting the invasion abilities of the pathogenic bacteria.

\textbf{Anti biofilm strategy development}

Once the biofilms developed to a mature stage, it is extremely difficult to eradicate them with traditional antimicrobial agents [22]. Over the years, different anti biofilm strategies have been tried that were targeting different biofilm development mechanisms such as non bactericidal and bactericidal approaches. In general, these approaches are similar to the antibiotic based approaches as far as their development is concerned. Digestion of EPS matrix materials may be another way to reduce biofilm formation. Without these ‘house of biofilm cells’, bacteria will only loosely attach to each other and will be sensitive towards the treatment of antimicrobial agents and host immune attack. It was reported that alginate lyase could enhance antibiotic killing of mucoid \textit{P. aeruginosa} in biofilms [23]. Azithromycin was reported to be able to block EPS material alginate formation and quorum sensing [24] and it was further shown to improve lung function of CF patients, especially in the subgroup colonized with \textit{Pseudomonas}. Targeting the iron up take pathways may also be a good choice to battle biofilm infections. Singh and coworkers has used an innate immunity component, lactoferrin, to prevent \textit{P. aeruginosa} biofilm formation [25]. By chelating iron, lactoferrin could stimulate the type IV pili mediated twitching motility of \textit{P. aeruginosa} thus block it to settle down and form biofilms [25]. Furthermore, Kaneko and colleagues have investigated a “Trojan horse” strategy by using the transition metal gallium to disrupt \textit{P. aeruginosa} iron metabolism and exploit the iron stress of \textit{in vivo} environments [26]. Due to its chemical similarity to iron, gallium can substitute for iron in many biological systems and inhibit Fe-dependent processes. They have found that gallium could inhibit \textit{P. aeruginosa} growth and biofilm formation [26]. Iron salts such as ferric ammonium citrate is utilized by Musk \textit{et al.} to treat fully developed \textit{P. aeruginosa} biofilms and found that iron salts not only perturb biofilm formation but also disrupt existing biofilms [27]. Even though biofilm cells are very resistant
toward conventional antimicrobial agents, there are still approaches to kill biofilm cells. One way is to
 treat biofilms with different combinations of antimicrobial agents. Natural product extracts has been also
 used in antibiofilm development process. It was showed that garlic extract containing quorum sensing
 inhibitors could repress genes involved in oxidative stress response [28] and in combination with
 hydrogen peroxide could efficiently eradicate *P. aeruginosa* biofilms.

**BIOFILM/ QS-INHIBITORS FOR *PSEUDOMONAS AERUGINOSA*: CHEMICAL SYNTHESIS
 & RATIONAL DESIGN APPROACHES**

The interruption in the steps of QS sequence of events such as signal production/detections, gene
 activation/inactivation could stop the QS to cause detrimental effects and hence cause struggle for
 survival/pathogenesis of bacteria and fungi. The natural products or its synthetically modified analogues
 could lead to be better methods to control infections [29]. A number of natural and synthetic remedies
 have been reported in literature to control QS. In this section, we had described the utility of various
 agents as biofilm inhibitors, their rational design approaches inspired from natural products in particular,
 as well as synthetic modifications to increase their potency. *P. aeruginosa* is a Gram -ve bacterium
 capable of infecting insects, plants, animals, and even humans. It is one of the most frequently isolated
 bacteria in nosocomial infection and hence we have stressed more on the potent QS inhibitors of
 bacterial strains in particular to *P. aeruginosa* strains. Also for few instance, we had highlighted the
 effects of inhibitors on other strains responsible for nosocomial infections. Chemical classifications of
 biofilm/ QS inhibitors are categorized in following order: a) Halogenated furanones inspired by marine
 natural products, b) AHL-based compounds, c) Amino imidazoles/ Indole derivatives, d) Organosulfur
 compounds, e) Miscellaneous

(a) **Halogenated furanones inspired from marine natural products.**

The involvement of QS in biofilm formation was originally reported for *P. aeruginosa*. Biofilms
 produced by *P. aeruginosa* are much more tolerant to treatment with antibiotics than planktonic cells
 and hence, infections by bacteria in the biofilm mode are difficult to eradicate. However, blocking the
 QS receptor with brominated ylidenebutanolide (halogenated furanone 1-6) has shown better biofilm
 inhibitory sensitivity compared to the treatment with tobramycine Fig. (4). Brominated
 ylidenebutanolide is considered as a biomimetic of AHLs [*N-(3-oxododecanoyl)-L-homoserine lactone*],
 a signal molecule produced by *P. aeruginosa*. This butanolide is synthetic analogue of marine natural
product fimbrolide which was isolated from red seaweed *Delisea pulchra* along with many other related metabolites. Additionally, some of the marine organisms are known to produce compounds that can inhibit bacterial QS in particular those based on AHL-signalling especially, brominated furanone from seaweeds. The binding of the furanone to the AHL-receptors induces a conformational change in LuxR, which subsequently results in a non-compatible complex for subsequent gene express. Thus, these molecules were well documented as Quorum sensing inhibitors or antibiofilm agents.

![Image](natural_product_fimbrolide.png)

**Fig. (4).** Halogenated furanone derivatives as potent biofilm inhibitors

Estephane *et al.* [30] described the synthesis of AHL analogues, 3-amino-5H-furanone derivatives 10a-c and 4-halogenated counterpart 11a-c Fig. (5) and evaluated their ability to modulate LuxR-dependent bacterial quorum sensing.

![Image](reagents_condition.png)

**Fig. (5).** *Reagents and condition:* a) NaN₃, Et₃N, DMF, 80 °C, 5 h; b) NBS or NCS, CH₂Cl₂, 50 °C, 2 h; c) RCOCl, i-Pr₂EtN or Et₃N, THF, 0 °C then rt, 5-18 h.
Reaction of α-bromo-γ-butyrolactone 7 with sodium azide resulted in the formation of 3-amino-5H-furan-2-one 8 which on treatment with NBS or NCS afforded the corresponding bromo or chloro compounds 9. N-acylation of the 3-amino-5H-furan-2-one and their halogenated counterparts were achieved using standard procedure to give the target compounds 10a-c and 11a-c, except compound 10b. Compound 10b was prepared by acylation of Meldrum’s acid with butyryl chloride and then by reaction with 3-amino-5H-furan-2-one in DCE at 80°C. Inhibition of bioluminescence obtained with compounds 10a, 10c and 11a-c are shown in table 2 below.

Table 2. Inhibition data of halogenated furanones

<table>
<thead>
<tr>
<th>Compound</th>
<th>10a</th>
<th>10b</th>
<th>10c</th>
<th>11a</th>
<th>11b</th>
<th>11c</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 (µM)</td>
<td>85 (±2)</td>
<td>55 (±2)</td>
<td>not active</td>
<td>6.5 (±0.1)</td>
<td>84 (±1)</td>
<td>6.9 (±0.4)</td>
</tr>
</tbody>
</table>

In general, halogenated compounds exerted a stronger inhibitory activity compared with their hydrogenated counterparts probably due to particular interactions between halogen atom and specific residues of LuxR. Compounds 11a and 11c proved to be more active with IC50 values of 6.5 and 6.9 µM respectively.

Sabbah and others [31] demonstrated a new synthetic route for the preparation of analogues of fimbrolides Fig. (6) and identified one of the most active furanone-type QS inhibitors. Synthesis begins with lactone 12 which was either alkylated or subjected to an aldol condensation to give the desired compound 13. Compound 13 was then subjected to oxidation-elimination reaction which led to compound 14. Reaction of 14 with LDA and CH2=N+Me2I- resulted in the formation of compound 15 with exocyclic double bond that were converted to bromolactones 16 via dibromination-elimination sequence.
Fig. (6). Reagents and condition: (a) LDA, RCHO or LDA, HMPA, RCH₂I, THF, -78 °C, 5 h; (b) 1) m-CPBA, CH₂Cl₂, -10 °C, 10 min, 2) toluene, reflux, 1 h; (c) LDA, CH₂=N+Me₂I-, THF, -78 °C, 5 h; (d) 1) Br₂, CCl₄, 0 °C, 5 min, 2) Et₃N, DBU cat., CHCl₃, 0 °C, 7 h.

The synthesized furanones 12-16 were tested for their ability to interfere with the QS dependent bioluminescence production in an E. coli biosensor strain (Table 3).

**Table 3. Inhibition data of synthesised furanones 14-16**

<table>
<thead>
<tr>
<th>IC₅₀ (µM)</th>
<th>14</th>
<th>15</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>0.6 (±0.05)</td>
</tr>
<tr>
<td>b</td>
<td>&gt;200</td>
<td>&gt;200</td>
<td>3 (±0.1)</td>
</tr>
<tr>
<td>c</td>
<td>Inactive</td>
<td>52 (±2.3)</td>
<td>(Z)14 (±1.1); (E)18 (±1.7)</td>
</tr>
<tr>
<td>d</td>
<td>Inactive</td>
<td>8 (±1.2)</td>
<td>6 (±0.8)</td>
</tr>
<tr>
<td>e</td>
<td>Inactive</td>
<td>15 (±1.5)</td>
<td>25 (±2.1)</td>
</tr>
</tbody>
</table>

The furanones 14 displayed either very low activity or are inactive. The furanones 15 without the hydroxyl group showed low activity whereas with the hydroxyl group displayed significantly good
activity. Bromo-substituted furanones 16, the analogues of naturally occurring fimbrolides shows a very
good activity with most active being the 3-butyl-5-bromomethylene-2(5H)-furanone 16a (IC$_{50}$ = 0.6
µM).

Shetye et al. [32] reported the synthesis of brominated furanones (BFs) Fig. (7) and screened them for
biofilm inhibition activity against *E. coli* and *P. aeruginosa*. The brominated furanones 1 [33-34], 3
[35], and 17 [36] were prepared using previously reported procedure while 18 was synthesized by
bromination of 4-oxohexanoic acid 19 followed by dehydration with P$_4$O$_{10}$ and HBr elimination with
triethyl amine.

![Chemical structures](image)

**Fig. (7). Reagents and condition:** (a) Br$_2$, CH$_2$Cl$_2$, rt, 1 h; (b) 1) P$_4$O$_{10}$, CH$_2$Cl$_2$, 0 °C to reflux, 1 h, 2)
Et$_3$N, CH$_2$Cl$_2$, 0 °C to reflux, 1 h.

The structure-activity relationships of these brominated furanones indicated that the methyl substituent
on the furanone ring and on the exocyclic vinyl double bond is important for relieving the inhibitory
effect on the growth of *E. coli* and *P. aeruginosa*. Lack of methyl group at C2 position or on the
exocyclic vinyl group impaired bacterial growth while methyl substitution introduces nonmicrobial
inhibition of biofilm formation. Furthermore, brominated furanones exhibited antagonistic activities to
LasR protein in the las system, but agonistic to the rhlR protein in the rhl system of *P. aeruginosa* which
is contrary to the other known anti-biofilm agents that exhibit antagonistic effect on both the las and rhl
pathways [33-36].
(b) Acylated Homoserine lactones as QS inhibitors:

From the vast collections of QS inhibitors reported in literature, HSL-moiety ranked first and hence great majority of this class of compounds have been explored.

\[
\text{O} \quad \text{O} \\
\text{N} \\
\text{O} \\
\text{R}_1 = \text{H}, \text{OH}, =\text{O} \\
\text{R}_2 = \text{C}_n\text{H}_{2n+2}, \text{C}_n\text{H}_{2n}
\]

**Fig. (8).** The molecular structure of \(N\)-acyl homoserine lactones (AHLs)

The structure of AHLs determines not only their signaling function but also their mode of interactions with environmental factors during cell-to-cell communications. AHLs 21 consist of five membered lactone ring with varied amide linked side chains as depicted in Fig. (8). The structural diversity of AHL-related compounds 22-25 result in large difference in genetic responses and activity Fig. (9). AHLs diffuse through the cell and hence enhance communication between cell and leads to biofilm formation. Nevertheless, the chemical structures of AHLs can be modified in number of ways to form different analogues which still remains some degree of induction activity that can be either positive or negative. The majority of published protocol utilized the similar procedures i.e. the coupling of carboxylic acid with HSL.

**Fig. (9).** Some selected \(N\)-acyl homoserine lactones (AHLs) scaffolds as biofilm inhibitors.
Hjelmgaard *et al.* [37] reported the synthesis of furanone-based product analogues, 5- & 3-(1'-hydroxyalkyl)-substituted 5H-furan-2-ones 29 & 34 (Fig. (10) & Fig. (11)) and their structure-activity relationship (SAR) towards two different quorum sensing systems (Table 3).

![Synthesis diagram](image)

**Fig. (10).** *Reagents and condition:* (a) HCOOH, H2O2, Na2SO4, K2CO3, H2O, CH2Cl2, reflux to rt; then Et3N, toluene, rt; (b) TMSCl, Et3N, 0 °C to rt; (c) CH3(CH2)n-CHO, TESOTf, CH2Cl2, -78 °C; then 1M HCl, rt; (d) Ac2O, Et3N, DMAP, CH2Cl2, rt.

5-(1'-Hydroxyalkyl)-substituted 5H-furan-2-ones 29 were synthesized starting from furfural. Oxidation of furfural 26 with H2O2 in presence of formic acid and then isomerization with Et3N gave 5H-furan-2-one 27 which was reacted with TMSCl to furnish 2-(tri-methylsilyloxy)furan 28. Vinylogous Mukaiyama aldol condensation between 2-(tri-methylsilyloxy)furan 28 and aliphatic aldehyde in presence of TESOTf afforded the 5-(1'-hydroxyalkyl)-5H-furan-2-ones 29 (erythro and threo) with threo as the major isomer (determined by NMR). Acetylation of 29 resulted in elimination giving 5-[(E)/(Z)-alkylidene]-5H-furan-2-ones 30.

![Synthesis diagram](image)

**Fig. (11).** *Reagents and condition:* (a) i-Pr2NH, n-BuLi, THF, -78 °C; then PhSSPh, THF, -78 °C; (b) i-Pr2NH, n-BuLi, THF, -78 °C; then CH3(CH2)n-CHO, THF, -50 °C; (c) m-CPBA, CH2Cl2, 0 °C; then toluene, reflux.

Reaction of lithium enolate of γ-valerolactone 31 with diphenyldisulfide gave phenylsulfanyl-dihydrofuran-2-one 32. Lithiation of phenylsulfanyl-dihydrofuran-2-one 32 with aliphatic aldehydes and
subsequent oxidation of the resultant 3-(1’-hydroxyalkyl)-5-methyl-3-phenylsulfanyl-dihydro-furan-2-ones 33 with \(m\)-CPBA and elimination by refluxing in toluene yielded 3-(1’-hydroxyalkyl)-5-methyl-5\(H\)-furan-2-ones 34.

All the synthesized compounds were subjected to two assays showing the antagonists effect on different quorum sensing systems. In general, the compounds with shorter alkyl chains showed more active quorum sensing antagonists than the longer alkyl chain counterparts. The synthesized compounds were less efficient as quorum sensing antagonists compared to naturally occurring halogenated furanone 35 and a synthetic analogue 36 Fig. (12).

<table>
<thead>
<tr>
<th>Compound</th>
<th>29, 30, 34</th>
<th>35</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>(IC_{50}) (mol antagonist)</td>
<td>95-231</td>
<td>11.9</td>
<td>8.2</td>
</tr>
</tbody>
</table>

Fig. (12). Selected halogenated furanone and their biological data

Horikawa and coworkers [38] demonstrated the synthesis of the analogues of \(N\)-3-oxododecanoyl-L-homoserine lactone (3-oxo-C\(_{12}\)-HSL) and studied their SAR for the apoptotic induction in macrophages, P388D1.
Fig. (13). Reagents and condition: (a) 1) NaH, $n$-BuLi, 41, 2) TFA, CH$_2$Cl$_2$, 3) 42, EDCI, HOAt, CH$_3$CN; (b) CH$_2$N$_2$, PdCl$_2$(CH$_3$CN)$_2$, Et$_2$O, rt; (c) 1) NaH, $n$-BuLi, 44, 2) TFA, CH$_2$Cl$_2$, 3) 42, EDCI, HOAt, CH$_3$CN; (d) 45, 46, CH$_2$Cl$_2$, 40 °C; (e) H$_2$, 10% Pd/C, EtOAc, rt.

The acyl-HSL 37-39 (Fig. (13)), each possessing either a cyclopropane ring or a phenyl group at the end of acyl side chain was prepared using tert-butyl acetoacetate 40 as the starting material. Generation of dianion of tert-butyl acetoacetate with NaH, $n$-BuLi and treatment with bromo-compounds afforded the corresponding β-ketoesters. Removal of tert-butyl group with TFA and subsequent coupling with L-HSL 42 using EDCI, HOAt provided 7,8-dehydro-acyl-HSL 43 which on cyclopropanation with diazomethane in presence of PdCl$_2$(CH$_3$CN)$_2$ yielded the corresponding cyclopropyl-acyl-HLS 37. In a similar manner, phenylacyl-HSL 38 was synthesized from 1-bromo-4-phenylbutane 44 while phenylacyl-HSL 39 were prepared by olefin cross-metathesis reaction of the 7,8-dehydro-acyl-HSL 43 with 4-phenyl-1-butene 45 and subsequent hydrogenation of the resultant endo-olefins 47.

Fig. (14). Reagents and condition: (a) EtOH, H$_2$SO$_4$; (b) CrO$_3$, acetone; (c) KOH, MeOH; d) 42, EDCI, dioxane.

Next modification involves the introduction of long chain on N-acylated HSL containing keto functionality in chain. Ethanolysis of γ- or δ-dodecanolactone 48 in presence of H$_2$SO$_4$ followed by oxidation of the resultant alcohol 49 with CrO$_3$ gave keto ester. Hydrolysis of the keto ester and subsequent coupling with L-HSL 42 using EDCI, HOAt furnished the γ- or δ-oxoacyl-HSL 51 (Fig. (14)).
Another variation in this class of compounds involves introduction of COOH functionality at the end of side chain. Hence, the requisite methyl ketone 54 was prepared from 10-undecen-1-ol in five steps as shown in Fig. (15). Generation of anion of methyl ketone using LiHMDS followed by addition of crushed ice and subsequent coupling of the resultant acid with L-HSL 42 using EDCI and removal of THP group provided 12-hydroxy-3-oxo-C12-HSL 55. Treatment of 55 with succinic anhydride in presence of pyridine gave 12-succinyl-3-oxo-C12-HSL 56.

The apoptosis-inducing activities of all the compounds 37-39, 51, 55-56 along with previously synthesized compounds were measured on the macrophage P388D1 cell line and compared with that of natural counterpart, N-3-oxododecanoyl-L-homoserine lactone 57 Fig. (16). The present SAR study revealed that 3-oxo group in the acyl side chain and the homoserine lactone moiety is important for the apoptosis-inducing activity in macrophages.
Mattmann and others [39] discovered a series of synthetic AHLs that are capable of activating or inhibiting the LuxR-type receptor, QscR from P. aeruginosa. They screened the libraries of AHL for agonistic and antagonistic activity against QscR using a previously reported [40], recombinant E. coli strain that reports QscR activity via the production of β-galactosidase from a promoter fusion and identified the compounds 58-66 (Fig. (17)) as the most active QscR agonists and compounds 67-75 (Fig. (18)) as the QscR antagonists.

Fig. (17). Structures of the synthetic QscR agonists

Fig. (18). Structures of the synthetic QscR antagonists

The dose-response agonism assays on the most active AHLs revealed that the compound 59 exhibited the lowest EC_{50} values (EC_{50} = 6 nM), with compound 61 having the next lowest value (EC_{50} = 11 nM). The antagonism assays on the selected AHLs revealed that the compound 69 as the most active QscR antagonist with IC_{50} = 30 nM. None of the other QscR antagonists displayed appreciable inhibitory activity.
Boukraa et al. [41] reported the synthesis and biological evaluation of various AHL analogues having a reverse-amide or a reverse-sulfonamide functionality as well as cyclopentyl reverse amides.

\[
\begin{align*}
\text{COOH} & \quad \text{COOH} \\
\text{R1} & \quad \text{R2} \\
\text{R1} = & \text{C}_4\text{H}_9, \text{C}_5\text{H}_{11}, \text{C}_6\text{H}_{13}, \text{C}_8\text{H}_{17}, \text{C}_{10}\text{H}_{21} \\
\text{R2} = & \text{H}, \text{CH}_3
\end{align*}
\]

**Fig. (19).** Reagents and condition: (a) NBu_4Br, CH_3CN, 75 °C, 16 h; (b) R_1R_2NH, DCC, DMAP, CH_2Cl_2, 0 °C, rt, 14 h.

Reverse-amide AHL-analogues, \( \alpha \)-(N-alkyl-carboxamide)-\( \gamma \)-butyrolactones 78 were synthesized by reacting butyrolactone 77 (prepared from cyclopropane dicarboxylic acid 76) with corresponding primary or secondary amines (Fig. (19)). Furthermore, the lactol derivatives of this series are prepared to check their effects on inhibitory activity.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{R} & \quad \text{N} \\
\text{R} = & \text{C}_4\text{H}_9, \text{C}_6\text{H}_{11}
\end{align*}
\]

**Fig. (20).** Reagents and condition: (a) TsN_3, Et_3N, CH_2Cl_2, 0 °C, 1 h; (b) RNH_2, xylene, reflux, 1 h; (c) NaBH_4, MeOH, rt, 2 h.

Hence, cyclohexane-1,3-dione 79 was treated with tosylazide to give 2-diazoo derivative 80, which subsequently underwent Wolff rearrangement to form intermediate ketene and then undergoes in situ reaction with pentylamine or octylamine to furnish 81 (Fig. (20)). Cyclopentanone was reduced using NaBH_4 to give corresponding alcohol 82.

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{H} & \quad \text{N} \\
\text{R} = & \text{C}_4\text{H}_9, \text{C}_6\text{H}_{11}
\end{align*}
\]

**Fig. (21).** Reagents and condition: (a) SOCl_2, ClSO_3H, 120 °C, 2 h; (b) RNH_2, CH_2Cl_2, -10 °C, rt, 1.5 h.
The next variation in AHL-class involves the replacement of reverse sulfonamide function in place of reverse amide moiety. Synthesis of reverse-sulfonamide i.e. $\alpha$-(N-alkyl-sulfonamide)-$\gamma$-butyrolactones 85 were achieved by reacting $\gamma$-butyrolactone 83 with thionyl chloride in presence of chlorosulfonic acid and subsequent reaction of the thus formed $\alpha$-chlorosulfonyl derivative 84 with butylamine or pentyamine (Fig. (21)).

Activities of the modified AHLs 78, 82, and 85 as QS modulators have been evaluated. Reverse-amide exhibits antagonist activity with IC$_{50}$ ranging from 34 to 96 µM with most active one being the N-pentyl derivative. N-Methyl amides were inactive which indicates the importance of NH system for binding within the receptor active site. Cyclopentalactone shows moderate to good antagonistic activity with IC$_{50}$ 40 and 77 µM respectively whereas compound 82 with hydroxyl group (lactol) resulted in loss of activity. Among the two sulfonamides 85, N-pentylsulfonamide was significantly more active (IC$_{50}$ = 70 µM) than the shorter butyl derivative (IC$_{50}$ = >200 µM).

Mattmann et al. [42] identified a set of potent non-native AHLs that are capable of strongly modulating QscR in $P$. aeruginosa. In preliminary studies, library of approximately 100 non-native AHL derivatives were screened for QscR inhibition and activation in an $E$. coli reporter strain [43]. This analysis framed the design of 29 new synthetic AHLs that comprise the libraries Q, R and S as shown in Fig. (22).

![Libraries of modified AHLs for biofilm inhibition.](image)

**Fig. (22).** Libraries of modified AHLs for biofilm inhibition.
Over two-thirds of the AHLs in library Q inhibited QscR by at least 50%. The inhibitory activities of bromo substituted BnHLs Q1 and Q2 increased as the substituents moved further from the amide bond (ortho < meta < para). Among the para-substituted halo BnHLs, the inhibitory activity increases with the size of halogen (Q3 < Q4 < Q5). BnHL bearing the EWG (4-NO2) decreases QscR activity to the same level as the EDG (4-CH3), providing support that steric bulk, as opposed to electronic nature, on the aryl group para to the amide group is favorable for QscR antagonism. The bulkier Q10 and Q11 were less potent antagonists (<45%) while Q9 and Q13 appears to be beneficial for QscR antagonism. The most active inhibitor from this family of antagonists was Q9 with IC50 = 11 nM while only Q10 showed appreciable agonistic activity (30%).

Library R which contains Q9 analogues with acyl tails of varying lengths and branching were subjected to the analogues QscR antagonism and agonism screening as for library Q. In general, it was found that library R provided weaker QscR antagonists as compared to Q9 with most potent antagonists being AHLs R1 and R6 (IC50 = 1.9 µM & 57 nM respectively). Library S which was designed for QscR agonistic activity was screened in the E. coli QscR reporter strain. The most active QscR agonists were the branched-alkyl HLs S3 and S7 (both with EC50 = 80 nM). Agonistic activity decreased as the lengths of the branched alkyl tails decreased.

Brackman and co-workers [44] demonstrated the synthesis of series of AHL analogues, triazolyldihydrofuranones (Fig. (23)) in which the amide function was replaced by triazole group and evaluated their effect on QS and biofilm formation in B. cenocepacia and P. aeruginosa.

![Fig. (23). Reagents and condition: (a) NaN3, acetone/H2O, overnight; (b) 88, CuSO4/Na-ascorbate/t-BuOH/H2O, 18-24 h, rt.](image)

3-(1,2,3-Triazol-1-yl)dihydrofuran-2(3H)-one derivatives 89 were prepared in two steps. Reaction of α-bromo-γ-butyrolactone 86 with sodium azide provided 3-azidodihydrofuran-2(3H)-one 87. Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction between 3-azidodihydrofuran-2(3H)-one and the
appropriate alkyne 88 yielded the corresponding triazolyldihydrofuranones 89. The compounds were tested for their ability to inhibit LasR-dependent QS in a *P. aeruginosa* QSIS2 assay. Compounds with R = C₁₀H₂₂, C₁₂H₂₅, CH₂Ph, (CH₂)₂ were moderately potent inhibitors while the remaining compounds did not display antagonistic activity at the tested concentrations. IC₅₀ value of the most active QS inhibitor in this study (R = C₈H₁₇, IC₅₀ = 307.7 µM) was found to be higher than IC₅₀ values obtained for most of the previously described AHL analogues (IC₅₀ = 4 –100 µM). The compounds were also screened for their ability to induce QS in *E. coli* JB523 and *P. aeruginosa* QSIS2 biosensor. Compounds with longer acyl-chain were strongly activators whereas those with shorter chain were either moderate activators or incapable of activating QS in the *P. aeruginosa* QSIS2 biosensor. Most of the compounds also displayed agonistic activities in at least one concentration in the *E. coli* JB523 biosensor.

Stacy and colleagues [45] developed a methodology for the synthesis of triazole-containing *N*-acyl homoserine lactones using Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reactions and evaluated their ability to agonize or antagonize two QS receptors, LasR and AbaR in *P. aeruginosa* and *A. baumannii* respectively. Combination of azide and alkyne building blocks yielded four sets of triazole-containing AHL analogues (Type I-IV) and varied combinations of building blocks yielded multivalent AHL analogues (Type V) in a minimal number of synthetic steps. Representative synthesis of Type I triazole HLs was shown in Fig. (24). The azide and alkyne building blocks were either commercially available or generated from simple precursors via Sonogashira cross-coupling and Cu-catalyzed aromatic azidation of aromatic halides.

![Fig. (24). Representative synthesis of Type I triazole HLs. Reagents and condition: (a) NaHCO₃, BrCH₂COBr, NaN₃, CH₂Cl₂:H₂O (1:1), rt, 16h; (b) 91, Cul (10 mol%), DIPEA, CH₃CN, rt, 16 h.](image-url)
Fig. (25). Triazole-AHLs Type II – V.

Several triazole derivatives were identified, which were capable of modulating the LasR and AbaR are depicted in Fig. (25) and Fig. (26).
Fig. (26). General SAR trends for LasR and AbaR modulators identified in triazol-HL scaffolds.

Among the five triazole types, in general the Type I and II triazole HLs were most active. The strongest LasR antagonists contained short alkyl chains. Cycloalkyl and heterocyclic Type I and II triazole HLs were active inhibitors of both receptors. Of the Type III and IV triazole HLs, the Type IV with variable substituents was more active. The Type V triazole HLs displayed modest LasR agonistic and antagonistic activities.

(c) Aminoimidazole/ Indole derivatives as QS inhibitors:

Marine organisms are well known for their chemical defenses by producing diverse skeletons of chemical compounds. The marine sponges of family Agelasidae produces oroidin class of alkaloids which possess nitrogen-rich architectures characterized by the presence of at least one 2-aminoimidazol subunit (Fig. (27)). In 1990, oroidin and bromoageliferin are documented to display anti-biofouling activity against the Gram-negative marine Rhodospirillum salexigens bacterium and leading as first
report of oroidin family members to inhibit formation of biofilms [46]. Based upon this finding, it was hypothesized that, 2-aminoimidazole motif in these molecules could be a key pharmacophore responsible for observed biological activity. This motivated medicinal chemists to undertake the synthesis of 2-aminoimidazole containing compounds and show their potential as antibiofilm agents or QS inhibitors which could be further use in treatment of various diseases including nosocomial infections (Fig. (27)).

Fig. (27). Aminoimidazole alkaloids from marine sponge and their modifications

Initial studies by Melander and coworkers [47] resulted in identification of two simplified analogues of bromoageliferin, TAGE (trans-bromoageliferin 100) and CAGE (cis-bromoageliferin 101) as inhibitors of bacterial biofilms of *P. aeruginosa*. The rational approach for this investigation inspired from marine natural product along with the simplified bromoageliferin scaffolds are depicted in Fig. (28).
Fig. (28). Rational approach inspired from marine natural product along with the simplified bromoageliferin scaffolds.

The approach commenced with known diol 103 [48] which was converted to dimesylate and then to diazide 104 in 90% yield (Fig. (29)). Epoxidation of double bond, followed by the treatment with sodium azide furnished triazido alcohol 105 which on hydrogenation in the presence of (Boc)$_2$O gave tri-Boc protected alcohol. Next, the requisite tri-Boc protected α-aminoketone 106 was prepared from alcohol using PDC. The important 2-aminoimidazole skeleton was constructed employing Boc-deprotection using TFA followed by condensation with cyanamide to give TAGE 100 in 24% overall yield with eight step sequence. The another diastereomer of TAGE i.e CAGE 101 was prepared using synthetic protocol, analogous to that utilized for preparation of TAGE from known cis diol 107 [49] instead of trans diol (18% overall yield).

**Fig. (29).** Reagents and condition: (a) 1) MsCl, Et$_3$N, CH$_2$Cl$_2$, -78 °C to -25 °C, 2) NaN$_3$, DMF, 100 °C; (b) 1) $m$-CPBA, CH$_2$Cl$_2$, 2) NaN$_3$, DMF, 100 °C; (c) 1) H$_2$, 10% Pd/C, (Boc)$_2$O, DMF, 2) PDC, DMF; (d) 1) TFA, CH$_2$Cl$_2$, then HCl, MeOH, 2) NH$_2$CN, H$_2$O/ pH = 4.3, 95 °C.
In this study, bacterial strains PA01 or PA14 of *P. aeruginosa* were allowed to form biofilms in 96-well plate in the presence and the absence of TAGE and CAGE. Both these compounds showed inhibition in formation of *P. aeruginosa* biofilms. The IC$_{50}$ value of biofilm inhibition for TAGE 100 against PA01 is 100 µM & PA14 I is 190 µM whereas IC$_{50}$ value for CAGE 101 against PA01 and PA14 I is 100 µM & 180 µM respectively. Additionally, 4-(3-aminopropyl)-2-aminoimidazole 102 displayed only marginal activity at 500 µM and did not inhibit the biofilm development at 100-400 µM. Interestingly, at the higher conc., CAGE 101 (400 & 500 µM) showed significant antimicrobial activity.

**Fig. (30).** Rational approach to diacylated TAGE and TAGE-triazoles

Further modification in this series involves the installation of di-brominated acylpyrrole moieties (similar to parent bromoageliferin) on to the TAGE scaffold to give compound 108 (Fig. (30)), which showed enhance antibiofilm activity with IC$_{50}$ value 1.77 µM, 12.0 µM against PA01 and PA14 strains of *P. aeruginosa*. The synthetic approach involves the preparation of TAGE using previously reported protocol by Melander and coworkers [48]. Finally, the synthesized TAGE was coupled with different substituted pyrrole trichloromethyl ketone [50] to generate corresponding TAGE derivatives as shown in Fig. (31).

**Fig. (31).** Synthesis of Acylated TAGE derivatives
Pyrrole TAGE 110a is inactive against *P. aeruginosa* at concentration up to 400 µM whereas bromo-TAGE 110b showed 3-fold increase in activity compared to parent TAGE with IC<sub>50</sub> value 32.5 µM against PA01. But unfortunately, these acylated derivatives were not able to disperse pre-formed biofilms with the same efficiency as that of TAGE 100 [51].

Melander research group [52] has further introduced diverse library of TAGE-triazole conjugate and screened for their antibiofilm activity against *P. aeruginosa, S. aureus, A. baumannii* bacterial strains. The synthetic strategy utilized the known procedure for preparation of azido TAGE as described in Fig. (29). Towards this end, click chemistry was carried out in the presence of the HCl salt of azido TAGE, substituted alkyne, copper sulfate and sodium ascorbate to give TAGE-triazole derivatives (Fig. (32))

**Fig. (32). Reagents and condition:** (a) 1) NH<sub>4</sub>OH, H<sub>2</sub>O, MeOH (NH₃), reflux, 2) Boc₂O, acetone, H<sub>2</sub>O, Na₂CO₃, reflux, 3) PDC, DMF, rt; (b) 1) TFA, CH₂Cl₂, 0 °C to rt, 2) NH₂CN, H₂O, pH 4; (c) substituted terminal alkyne, CuSO₄, Na ascorbate, EtOH: H₂O: CH₂Cl₂.

The TAGE-triazole analogues were initially screened for antibiofilm activity against *A. baumannii* at 400 µM, wherein decyl side chain (alkyne used in click chemistry is 1-dodecyne) containing triazole derivative showed very weak inhibition where as branching of the butyl side chain (5-methyl-1-hexyne coupling with triazole) increases the inhibition activity. Amongst this TAGE-triazole series, 3 members containing hexyl, cyclohexyl, and phenyl (R group) showed maximum inhibitory activity against *P. aeruginosa* at 500 µM i.e. >95% inhibition against PA01. Additionally, the lead TAGE-triazole 109
containing hexyl side chain (R = hexyl) demonstrated inhibition potency at PA01 and PA14 higher than that of parent TAGE. It is also worth to note that the several of these TAGE-triazoles were capable of inhibiting biofilm formation against different strain of *A. baumannii, S. aureus*, and *P. aeruginosa* biofilms.

To investigate whether simpler derivatives bearing similar core as that of ageliferin skeleton would show any effect on antibiofilm properties, Melander and co-workers continued their effort towards synthesis of library of oroidin family. Initially, the libraries of oroidin core scaffolds were constructed by varying three regions within the oroidin template to understand the essential structural feature of molecules for biological activity (Fig. (33)).

![Rational design of marine metabolite oroidin template for SAR study](image)

Fig. (33). Rational design of marine metabolite oroidin template for SAR study

Initially, oroidin analogues were synthesized employing modifications at tail end region i.e. tail group was modified as: removed from skeleton, NH pyrrole derivative or *N*-methyl pyrrole derivative. The necessary intermediate halogenated (trichloroacetyl) pyrroles were synthesized employing bromination or chlorination of (trichloroacetyl) pyrrole using reported protocol [54]. Similarly, dibromopyrrole carboxylic acid and *N*-methyl dibromocarboxylic acid was prepared using bromination of pyrrole as depicted in Fig. (34). Finally, (trichloroacetyl) pyrroles were coupled with unprotected 2-aminoimidazoles in the presence of base to give tail group analogues of oroidin class which has been screened at 500 µM against *P. aeruginosa* strain for its biofilm activity.
Fig. (34). Reagents and condition: (a) Br₂, CHCl₃, 0 °C; (b) SO₂Cl₂, CHCl₃, reflux; (c) Br₂, HOAc, CHCl₃, 50 °C; (d) 1) K₂CO₃, MeOH, 2) LiOH, MeOH/THF/H₂O; (e) 1) pyrrole derivative, Na₂CO₃, DMF, rt, 2) HCl in MeOH.

In this class of oroidin family (tail modified derivatives), the non-brominated derivatives showed a slight increase in activity relative to dihydroclathrodin (DHC) 120a. In comparison with NH pyrrole derivative, N-methyl dihydrohymenidin 120b showed substantial increase in activity i.e. 88% for PA01 and 83% for PA14. Overall, the dihydrosventrin (DHS) 120c was the most active with an IC₅₀ of 51±9 µM against PA01 and 111±8 µM against PA14 strain of *P. aeruginosa*. Hence the degree of bromination on pyrrole ring increases antibiofilm activity.

Next, the linker modified oroidin analogues have been evaluated for their biofilm inhibitory activity. The synthetic approach is depicted in Fig. (35) which involves the construction of 2-aminomidazole skeleton (122 & 125) in the first step followed by coupling with trichloroacetyl pyrrole derivatives.
The IC$_{50}$ values for both compounds indicate the appearance of minor effects of alkyl linker length on inhibitory activity wherein, both modification results in decreasing activity in relation to DHS (dihydrosventrin). Also, the optimum chain length between the 2-aminoimidazole head and pyrrole tail was three carbon units. Authors have also demonstrated the importance of 2-AI moiety of oroidin alkaloids for its observed antibiofilm activity. Hence, different head group modified analogues have been synthesized. These two modifications involve the replacement of 2-AI group with a tryptophan residue or removal of amino functionality of imidazole moiety. Tryptamine.HCl or histamine.HCl was coupled with different (trichloroacetyl)pyrrole to give tryptophan analogues (Fig. (36)).

Fig. (36). Modified oroidin skeleton containing indole moiety.

However, replacement of 2-AI moiety with a tryptophan abolished all activity at 500 µM against PA01 and PA14 of *P. aeruginosa*. Furthermore removal of amino functionality decreases the antibiofilm
activity. Another variation of this class of compound involve introduction of sulfur atom (synthesis of thioimidazolone/2-aminothioazole (2-AT) scaffold) for SAR studies, which has been discussed in next section (class of sulfur compounds) of this review.

The marine natural oroidin 128 containing 2-AI skeleton had been further modified as reverse amide (RA) to explore their potential as biofilm inhibitors by Melander group [55]. The rational to this study is depicted in Fig. (37).

![Fig. (37). Oroidin and reverse amide (RA) scaffold for antibiofilm inhibitory activity and structural variation is highlighted](image)

**Fig. (37).** Oroidin and reverse amide (RA) scaffold for antibiofilm inhibitory activity and structural variation is highlighted

**Fig. (38).** Reagents and condition: (a) 1) CH$_2$N$_2$, Et$_2$O-CH$_2$Cl$_2$, 0°C, 2) conc. HCl or conc. HBr, 3) Boc-guanidine, NaI, DMF; (b) 1) AlCl$_3$, NH$_2$R, DCE, 0°C to 60°C, 2) TFA, CH$_2$Cl$_2$, 3) 2M HCl in Et$_2$O; (c) 1) (COCl)$_2$, DMF, CH$_2$Cl$_2$, 2) CH$_2$N$_2$, Et$_2$O-CH$_2$Cl$_2$, 0°C, 3) conc HBr, 4) Boc-guanidine, DMF, 5) H$_2$ (1 atm), 10% Pd/C, THF; (d) 1) EDC, HOBt, NH$_2$R, DMF, 2) TFA, CH$_2$Cl$_2$, 3) 2M HCl in Et$_2$O.

The synthetic approach started from commercially available acid chloride 130 which on treatment with diazomethane followed by quenching with conc HCl or HBr yielded corresponding α-halo ketone. This on condensation with Boc-quinidine in DMF gave N-Boc protected 2-aminoimidazole derivative 131. Unfortunately the authors were unable to proceed forward with path A due to the problem encountered in direct aminolysis for preparation of RA scaffold. This prompted the authors to give their second generation approach which utilizes change in protecting group *i.e.* benzyl ester instead of ethyl ester.
(advantages in preparation of corresponding acid by hydrolysis). The known monobenzyl ester acid 132 [56] is converted to benzyl protected $\alpha$-bromoketone via acid chloride followed by diazomethane homologation and concomitant quenching with conc HBr. Boc-protected 2-aminoimidazole was prepared by condensation of benzyl protected $\alpha$-bromoketone with Boc-guanidine in 66% yield. Finally, debenzylolation using hydrogen atmosphere gave pure Boc-protected acid 133 in quantitative yield. The preparation of libraries of final RA scaffold involve the coupling of N-Boc protected acids with different amines wherein, EDC-HOBt combination was found to be better option amongst the number of activating agents evaluated (DCC, EDC, HCTU, CDI, cyanuric chloride). The final step of the synthesis involves the N-Boc deprotection using TFA followed by HCl exchange of corresponding TFA salt (Fig. (38)).

![Chemical Structure](image)

**Table:**

<table>
<thead>
<tr>
<th>Amine</th>
<th>PA01 IC$_{50}$ in µM</th>
<th>PA14 IC$_{50}$ in µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexylamine</td>
<td>32.7 ± 6.5</td>
<td>39.9 ± 13.0</td>
</tr>
<tr>
<td>Octylamine</td>
<td>18.4 ± 2.3</td>
<td>13.3 ± 1.8</td>
</tr>
<tr>
<td>Decylamine</td>
<td>8 ± 1</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Dododecylamine</td>
<td>2.84 ± 0.93</td>
<td>2.26 ± 0.83</td>
</tr>
</tbody>
</table>

**Fig. (39).** Reagents and condition: (a) Al(CH$_3$)$_3$, ester 131, DCE, 0 °C to 60 °C, or EDC, HOBt, above acid 133, DMF; (b) 1) TFA, CH$_2$Cl$_2$, 2) 2M HCl in Et$_2$O.

The series of synthesized reverse amide (RA) along with parent oroidin were screened at 500 µM in a 96-well format using crystal violet reporter assay to evaluate their capacity to inhibit the formation of PA01 and PA14 biofilms. These results reveal that the aliphatic chain derivative and oroidin were very potent inhibitor in *P. aeruginosa* biofilms (> 95% inhibition) and were selected for IC$_{50}$ value determination against PA01 and PA14 (Fig. (39)). SAR analyses indicate that with increasing chain length from six to twelve carbons led to increase in their inhibitory activity over both the strains. Also all linear carbon chain analogues were found to be significantly more potent than parent oroidin (IC$_{50}$ value 190±9 µM and 166±23 µM vs PA01 and PA14 resp). Overall, oroidin was found to have similar activity as that of TAGE and CAGE, confirming that the simpler skeleton based upon 2-aminoimidazole scaffold was viable as an antibiofilm agent.
The further modification of oroidin core includes the incorporation of triazole moiety keeping 2-aminoimidazole part intact, which resulted in a number of highly active compounds as shown in Fig. (40).

![Figure 40](image)

**Fig. (40).** Selected oroidin analogues containing triazole moiety as biofilm inhibitors

Again, Melander group [57] has introduced new series containing 2-aminoimidazole triazole (2-AIT) conjugates with the most active compound possessing a substituted unsaturated aryl group. This study has been initiated from previously disclosed dihydrosventrin (DHS) (Fig. (34)), as inhibitor of *P. aeruginosa, A. baumannii* biofilms. The modification involves the replacement of the pyrrole core of DHS with the triazole subunit to access 2-aminoimidazole/triazole (2-AIT) conjugate. The synthetic protocol for the preparation of libraries of 2-AITs started with amino acid ester 136, which on Akabori reduction followed by condensation with cyanamide delivered alkyne 137 (Fig. (41)).

![Figure 41](image)

**Fig. (41).** *Reagents and condition:* (a) 1) 5% Na/Hg, H₂O, pH 1.5-2.0, 0-5 °C, 2) cyanamide, H₂O, pH 4.3, 95 °C; (b) CuSO₄, sodium ascorbate, t-BuOH: H₂O: CH₂Cl₂ (1:1:1), rt.

The next crucial step involves [3+2] alkyne/azide cycloaddition (click reaction) to generate 2-AIT derivatives. The click reaction of alkyne 137 with azide 138 in the presence of CuSO₄ and sodium ascorbate in 1:1:1 mixture of solvents t-BuOH: H₂O: CH₂Cl₂ gave 93% 2-AIT 139 (attempted different
conditions for standardization of click reaction protocol). The libraries of 2-AIT derivatives were prepared using click chemistry approach as shown in Fig. (42)

![Chemical structures of 2-AIT derivatives](image)

**Fig. (42).** Synthesis of libraries of 2-AITs and selected most active inhibitors

In this series, compound **142a** was active against Gram +ve and Gram –ve bacteria with IC$_{50}$ values of 5.6 µM, 530 nM against PA01, PA14 resp. and 980 nM and 810 nM against *A. baumannii*, and *S. aureus* respectively. In addition, this compound also exhibited synergistic effect with different antibiotics in the dispersion of pre-established biofilms of bacteria strains. The inhibitory activity against *A. baumannii* increases with the increase in the number of methylene units between 2-AI and triazole core. Hence, another series of compounds of this class has been prepared by increasing the methylene linker of 2-AIT lead. The biological data is shown in Fig. (43).

![Table of IC$_{50}$ values](image)

**Fig. (43).** Effect of chain length of 2-AITs on biofilm inhibitory activity
Furthermore, the same research group [58] extended their work by the replacement of aryl appendage of 142a with different linkers containing alkyl amide which resulted in 2-aminoimidazole/triazole scaffolds with different N-alkyl substituent at amide functionality (Fig. (44)). Amongst this series of 2-AIT, compound 143a and 143b showed the promising activity and inhibited biofilm formations in E. coli by 90.8% and 95% at 400 µM with IC$_{50}$ values 36.9±1 µM and 13.0±1 µM respectively. Also, this study highlighted the importance of precise placement of triazole moiety and amide functionality within the structure of the 2-AIT conjugate and their effects on inhibitory activity.

![Fig. (44). Structure and activity comparison of biofilm inhibitor of 2-aminoimidazole/triazole scaffolds](image)

Recently, Melander and colleagues [59] discovered another series of 1,4-disubstituted 2-aminoimidazoles to explore their antibiofilm activity against multidrug resistance bacteria (Fig. (45)). The synthesis of 1,4-disubstituted 2-aminoimidazole/triazole conjugate 145 was achieved via one-pot N-alkylation/cyanamide cyclization protocol. These compounds were found to be active biofilm inhibitors of Staphylococcus aureus (MRSA) with the lead compound being n-pentyl derivative (MIC/MRSA 12.5 µM).

![Fig. (45). Reagents and conditions:](image)

**Reagents and conditions:** (a) 1) RNH$_2$, EtOH, 30 min, 2) NH$_2$CN, pH adjusted to 4.3 using 0.1 N HCl (aq.) followed by reflux for 3 h.
Melander and coworkers [60] disclosed a new concept of hybrid 2-aminoimidazole/menthyl carbamate antibiotic film agents and demonstrated their applications to inhibit biofilm formation and dispersion of mature pre-formed biofilms of *P. aeruginosa*, *S. aureus*, *A. baumannii* (Fig. (46)).

![Chemical structure](image)

**Fig. (46).** Reagents and condition: (a) NaH, DMF, -(CH₂)₅CH₂I; (b) 1) CuSO₄, Na-ascorbate, EtOH, CH₂Cl₂, H₂O, 2) TFA/CH₂Cl₂ then HCl/MeOH; (c) 1) H₂, 10% Pd/C, MeOH, 2) (-)-menthyl chloroformate, Et₃N, CH₂Cl₂, 3) TFA/CH₂Cl₂ then HCl/MeOH; (d) 1) (-)-menthyl chloroformate, Et₃N, CH₂Cl₂, 2) CuSO₄, Na-ascorbate, t-BuOH, CH₂Cl₂, H₂O, 3) TFA/CH₂Cl₂ then HCl/MeOH.

The rationale to this work was inspired from the structurally simple marine natural product ethyl *N*-(*2*-phenethyl) carbamate [61] in combination with antibiotic agent (-)-menthol as well as 2-aminoimidazole, oroidin skeleton. The synthesis involves the alkylation of (-)-menthyl carbamate 146.
with 8-iodo-1-octyne to deliver carbamate derivative 147. 2-Aminoimidazole containing azide 148 (n = 1, 2, 3) were reacted with carbamate alkyne derivative 147 in the presence of copper sulfate and sodium ascorbate followed by Boc-deprotection and subsequent HCl exchange to give hybride derivatives 149a-c containing triazole, 2-AI and menthyl moiety. Also hybrid 2-AI and menthyl derivative were synthesized from 2-aminoimidazole azide 150 via azide reduction, carbamate formation and Boc-deprotection/HCl exchange. Next series of 2-aminoimidazole-triazole derivatives 154a-b were prepared by coupling of amino azide 152 with (-)-menthyl carbamate in the presence of Et3N. Subsequently, coupling of this azido carbamate with known 2-AI alkyne 153 via Cu(I) mediated click chemistry approach gave Boc-protected 2-AI analogues. Finally, removal of Boc group followed by HCl exchange afforded 2-AI triazole 154a-b.

Table 4. Biofilm inhibition and IC50 in μM against various bacterial strains

<table>
<thead>
<tr>
<th>Compounds</th>
<th>P. aeruginosa PA 14</th>
<th>A. baumannii</th>
</tr>
</thead>
<tbody>
<tr>
<td>149a</td>
<td>&gt;200</td>
<td>19.2</td>
</tr>
<tr>
<td>149b</td>
<td>&gt;200</td>
<td>18.4</td>
</tr>
<tr>
<td>149c</td>
<td>&gt;200</td>
<td>16.7</td>
</tr>
<tr>
<td>151a</td>
<td>18.0</td>
<td>19.2</td>
</tr>
<tr>
<td>151b</td>
<td>18.0</td>
<td>-</td>
</tr>
<tr>
<td>151c</td>
<td>-</td>
<td>16.7</td>
</tr>
<tr>
<td>154a</td>
<td>58.7</td>
<td>94.9</td>
</tr>
<tr>
<td>154b</td>
<td>40.3</td>
<td>-</td>
</tr>
</tbody>
</table>

These synthesized compounds were initially screened against P. aeruginosa and A. baumannii to explore their biofilm inhibitory potentials as depicted in table 4. Compounds 151a-b and 154a-b inhibited PA14 biofilm formation whereas compound 149a and 151c exhibited the lowest MIC values against the S. aereus and A. baumannii strains. Overall, most of the 2-AI/carbamate hybride demonstrated their anti-biofilm activity via underlying microbicidal means and this study also resulted in a new class of nontoxic inhibitors for P. aeruginosa strain PA14 (151a-b, 154a-b).
The natural product inspired motifs of 2-aminoimidazole (roidin core) have shown great success as inhibitors of the Gram +ve and Gram –ve biofilms, but 2-aminobenzimidazole (2-ABI) is rarely studied. Melander and co-workers [62] designed and synthesized scaffolds related to aromatized analogue of TAGE i.e benzimidazole skeleton to provide improved antibiofilm activity in comparison with 2-aminoimidazole Fig. (47).

![Chemical Structures](image)

**Fig. (47). Reagents and condition:** (a) 1) acyclic anhydride, CH$_2$Cl$_2$; 2) HCl, H$_2$O, THF; (b) 1) cyclic anhydride, toluene, 110 °C; 2) HCl, H$_2$O, THF.

The synthesis started from tri-Boc-protected-5-amino-2-ABI 155 which was acylated with acyclic anhydride and then subjected to Boc-deprotection to furnish 2-ABI derivatives 156. Also, related derivatives such as 2-ABI-imides 157 are prepared by reaction of tri-Boc-protected-5-amino-2-ABI 155 with cyclic anhydride under refluxing toluene, followed by Boc-deprotection under acidic condition.

Unlike the 2-AI class of antibiofilm agents, none of the above 2-aminobenzimidazole (2-ABI) derivatives were able to inhibit biofilm formation of γ–proteobacteria (P. aeruginosa PA01, A. baumannii) at 100 μM. Amongst the series of compounds tested against Gram positive bacteria, 2-ABI derivative 156b showed best activity profile with IC$_{50}$ 890 nM, 1.4 μM and 570 nM against MRSA, E. faecium and S. epidermidis respectively. The investigation of mechanistic basis for 156b as inhibitor and disperser of Gram +ve biofilm confirm the Zn(II)-dependent mode of action of this compound.
Another modification by Melander group [63] in 2-aminobenzimidazole (2-ABI) class of compounds involves preparation of \( N \)-1-substituted-2-aminobenzimidazole as zinc-dependent \( S. \) aureus biofilm inhibitors. The acylation of 4-fluoro-3-nitroaniline 158 with 4-pentylbenzoyl chloride 159 gave corresponding \( N \)-substituted derivative 160. The various \( N \)-substituted 2-ABI derivatives 161 were prepared from nitro-derivative 160 in 3-steps via nucleophilic aromatic substitution, followed by nitro reduction using hydrogenation and finally condensation with cyanogen bromide (Fig. (48)).

![Chemical Structure](image)

**Fig. (48). Reagents and condition:** (a) 4-pentyl benzoyl chloride, DMAP, CH\(_2\)Cl\(_2\); (b) 1) RNH\(_2\), EtOH, 2) HCOONH\(_4\), Pd/C, EtOH, 3) BrCN, EtOH/CHCl\(_3\), 4) MeOH/HCl

All these 2-ABI 161 were initially tested for their antibacterial properties wherein, the \( N \)-substituted 2-ABI 161a-b adversely affect their potential to act on the Gram –ve \( A. \) baumannii (BAA-1605) and \( P. \) aeruginosa (PA14). Overall, \( N \)-1-substituted-2-ABI retained their anti-biofilm properties when substituted with various heterocycles and their indole derivative (\( R \) = indolyl) was the most potent with IC\(_{50} \) values 3.7, 4.4 µM against MRSA 43300 and BAA 44 respectively.

Next work by Melander group [64] involves the construction of 30-membered library based upon a aryl 2-AI skeleton (rational approach involves modification of 2-ABI) and evaluated for biofilm inhibitory activity against three Gram –ve bacteria \( E. \) coli, \( P. \) aeruginosa, and \( A. \) baumannii. Initially, para-acylated 2-amino imidazole derivative 165 has been prepared employing previously reported methodology described for accessing aryl halide 2-AI via palladium catalysed Sonogashira cross coupling [65]. The synthesis commenced with commercially available \( p \)-azidobenzoic acid 162 which on treatment with oxalyl chloride and then with diazomethane followed by quenching with conc. HBr gave \( \alpha \)-bromoketone (Fig. (49)). The condensation of \( \alpha \)-bromoketone with Boc-guanidine delivered Boc-protected 2-AI 163. Boc protection of exocyclic amino functionality followed by reduction of azide
and subsequently acylation of aniline with various acid chloride gave Boc-protected compounds. Boc-deprotection using TFA followed by exchange with HCl gave para-substituted aryl 2-AI analogues 165.

**Fig. (49). Reagents and condition:** (a) 1) (COCl)$_2$, DMF, CH$_2$Cl$_2$, rt, 2) CH$_2$N$_2$, Et$_2$O, 0 °C, 3) conc. HBr, 4) Boc-guanidine, DMF, rt; (b) (Boc)$_2$O, Et$_3$N, THF, rt; (c) 1) H$_2$ (1 atm), 10% Pd/C, THF, rt, 2) RCOCl, Et$_3$N, CH$_2$Cl$_2$, THF, -78 °C-rt, 3) TFA, CH$_2$Cl$_2$, 0 °C, 4) 2 M HCl in Et$_2$O.

The ortho- and meta-substituted acylated 2-aminoimidazoles were prepared using corresponding amino acetophenone (Fig. (50)). $N$-protection of aniline 166 & 169 with Cbz group and mono bromination of methyl ketone followed by condensation with Boc-protected guanidine gave 2-AI aniline products 167 & 170. Finally, $N$-acylation using various acid chloride followed by Boc-deprotection using TFA and counterion exchange with chloride gave corresponding ortho- and meta-acylated 2-AI aniline analogues 168 & 171.

**Fig. (50). Reagents and condition:** (a) 1) CbzCl, NaHCO$_3$, H$_2$O, acetone, 0 °C-rt, 2) Br$_2$, AlCl$_3$ (cat.), 0 °C, 3) Boc-guanidine, DMF, rt, 4) H$_2$ (35 psi), 10% Pd/C, EtOH, rt; (b) 1) RCOCl, Et$_3$N, CH$_2$Cl$_2$, THF, -78 °C-rt, 2) TFA, CH$_2$Cl$_2$, 0 °C, 3) 2M HCl in Et$_2$O.
Amongst the series of aryl-2-AI, fluoro compound 165a was found to be most active against inhibition of biofilm formation in E. coli and P. aeruginosa as depicted in Fig. (51). However, numbers of these derivatives have been found to show a toxic effect to growth of planktonic E. coli, P. aeruginosa and A. baumannii. Furthermore with intention of improving the activity of aryl 2-AI skeleton, triazole isoster was incorporated in aryl-2-AI scaffold (Fig. (52)).

![Fig. (51). Biofilm inhibitory activity of aryl-2-aminoimidazole](image)

2-AI azide 172 was subjected to N-protection using Boc-anhydride, followed by copper mediated [3+2] cycloaddition with 1-ethynyl-3,5-difluorobenzene and finally Boc-deprotection gave aryl-2-AI triazole derivative 174. Unfortunately, the evaluation of this triazole for inhibition of biofilm at 100 µM against bacterial strains revealed very weak activity against cell strains (35% inhibition against E. coli, <5% inhibition against P. aeruginosa, 21% inhibition against A. baumannii).

![Fig. (52). Reagents and condition](image)

Recently, an elegant study by Blackwell and coworkers [66] have demonstrated the importance of hybrid compounds i.e 2-aminobenzimidazole (2-ABI) derived from the structures of three natural products (bromoageliferin, 3-indolyl acetonitrile, resveratrol) with known QS inhibitory activities and also their simplified derivatives were synthesized to understand SAR. Initially, the 2-ABI stilbene was prepared from 2-nitro-4-iodoaniline 175 by Heck reaction with styrene followed by reduction of nitro
functionality to furnish diamine intermediate. The condensation of diamine with cyanogen bromide gave 2-ABI stilbene derivative 176. Furthermore, new series of 2-AI stilbene derivative 178 were prepared by palladium-catalyzed regioselective arylation of imidazo[1,2-a]pyrimidine and then pyrimidine ring cleavage by treatment with hydrazine hydrate (Fig. (53)).

![Chemical structure](image)

**Fig. (53).** Reagents and condition: (a) Styrene, Pd(OAc)$_2$, CH$_3$CN, DIPEA, 80 °C, 2) SnCl$_2$.2H$_2$O, EtOAc, 80 °C, 3) CNBr, MeOH/H$_2$O (1:1), 80 °C; (b) 1) imidazo[1,2-a] pyrimidine.HBr, Pd(OAc)$_2$, PPh$_3$, Cs$_2$CO$_3$, 1,4-dioxane, 100 °C, 2) 20% N$_2$H$_4$/EtOH, 105 °C.

Furthermore, 2-ABI stilbene, 2-AI stilbene, as well as some indole stilbenes were tested to inhibit biofilm formation against *P. aeruginosa* strain (PA01) at 500 µM employing static biofilm growth assays, wherein 2-ABI and 2-AI derivatives showed good potential to inhibit biofilm growth by 56 and 48% respectively at 100 µM. However, indole stilbene did not show appreciable antibiofilm activity. Next, in order to identify the potential structural features within the lead compound 2-ABI stilbene that were responsible for its biological activity, the molecule is further dissected in 5 more simple structural analogues as depicted in Fig. (54).
Accordingly, these compounds were synthesized and evaluated for their antibiofilm activity. Removal of amino group of 2-AI unit (179, 180) led to complete loss of biofilm inhibitory activity, whereas removal of styrene moiety furnished 2-ABI 181 which enhances the inhibitory activity compared to parent 2-ABI stilbene. This observation motivated others to prepare small library of 2-ABI derivative for SAR analyses. The condensation of various functionalized o-diaminobenzenes with cyanogen bromide gave 2-ABI derivatives as shown in Fig. (55). Most of the synthesized 2-ABI derivatives were capable of inhibiting biofilm growth with improved potencies (i.e. lower IC$_{50}$ values) relative to parent 2-ABI.

**Fig. (55).** Reagents and condition: (a) CNBr, MeOH/H$_2$O (1:1), 50 °C, 1h
Most of the synthesized 2-ABI derivatives 183 were capable of inhibiting biofilm growth with improved potencies (i.e. lower IC₅₀ values) relative to parent 2-ABI 181. The halide or methyl substitution on 2-ABI aryl ring exhibited greater activity. The most potent *P. aeruginosa* inhibitor identified was 5,6-dimethyl 2-ABI with IC₅₀ 4.0 µM i.e. approximately 10-fold more active than potent 2-ABI. The aromatic ring containing hydrogen-bond donors or acceptors led to total loss or reduction in inhibitory activity. It is worth to note that this study discovered several 2-ABI derivatives as most active *P. aeruginosa* biofilm modulator. However, it is contrary to previous data on related 2-ABI derivatives which indicated they were inactive in *P. aeruginosa*.

**Organosulfur compounds:**

Few sulfur containing compounds have also been used as biofilm inhibitors. The garlic (genus *Allium sativum* L.) is well known for its antibiotic activity which is mainly attributed to the sulfur compounds present in it [67]. Neilsen and co-workers [68] has described rational studies toward the synthesis of QS inhibitors inspired from acylated homoserine lactones (AHLs) and natural products of garlic (Fig. (56)). The extract of garlic were shown to inhibit LuxR and LasR based QS systems wherein compound 184 and 185 possessed QS activity in particular.

![Fig. (56). Rational design study for sulfated AHLs scaffold](image)

Neilsen group has highlighted the one-pot or sequential approach employing parallel solution phase synthetic protocol to construct sulfated AHL analogues (Fig. (57)). Towards this, the reaction of alkylamines 187 with bromoacetyl bromide gave corresponding N-bromoacetyl derivatives 188, which on treatment with alkyl thiol (RSH) gave sulfanyl acetamide 189 (R is alkyl or aryl).
**Fig. (57). Reagents and condition:** (a) Bromoacetyl bromide, Et$_3$N, CH$_2$Cl$_2$, -78 °C to rt; (b) R$_2$SH, Et$_3$N, EtOH, rt; (c) Bromoacetyl bromide, Et$_3$N, CH$_2$Cl$_2$, -20 °C to rt then R$_2$SH, Et$_3$N; (d) (COCl)$_2$, DMSO, CH$_2$Cl$_2$, -20 °C.

This series of compound were screened in two QS system designed from the LuxR (*V. fischeri*) and LasR (*P. aeruginosa*) system. The highest activities were found for sulfated AHL containing heptyl linkage i.e. the optimal chain length was two carbon atoms shorter than for the natural ligand. Further, in the search of more potent pharmacophore, the garlic component was combined with AHL, which furnished another class of sulfated AHLs (Fig. (58)). Although, the garlic component possesses an inhibitory activity, the garlic derived synthesized-dithianes 191 were found to possess antagonism against LuxR but not LasR. The reason for decrease in potency for these derivatives compared to parent compounds could be attributed for the presence of wrong linkers between this skeleton, or due to the absence of double bond in modified analogues of dithiane.

**Fig. (58). Reagents and condition:** (a) isobutyl chloroformate, Et$_3$N, CH$_2$Cl$_2$, 0 °C.

The sulfide AHL analogues of the first series indicated the presence of alkyl *i.e.* heptyl in particular enhances the activity. The further modification in this series involves replacement of sulfur by sulfoxide
or sulfone functionality. Hence, sulfide 192 was oxidized to sulfoxide 193 and sulfone derivative 194 by \( m \)-CPBA as oxidizing agent as depicted in Fig. (59).

![Chemical structure](image)

**Fig. (59).** *Reagents and condition:* (a) \( m \)-CPBA, CH\(_2\)Cl\(_2\), -10 \(^\circ\)C; (b) \( m \)-CPBA, CH\(_2\)Cl\(_2\), rt.

In this series of sulfoxide, AHL derivative 193 containing heptyl side chain is possess highest activity as similar to that of parent sulfide AHL derivative 192. Thus the efficiency of the diffusion of molecules with a long carbon chain through membranes is to a greater extent determined by its length. Overall, the cyclic dithianes 184, 185 obtained from garlic extract was found to inhibit the formation of biofilm and also \( N \)-(heptylsulfanylacetyl)-L-homoserine lactone was identified as potent QS inhibitor in this class of sulfur compounds.

Musah and coworkers [69] has demonstrated the effects of several \( S \)-substituted cysteine sulfoxide and their corresponding disulfide derivatives on *P. aeruginosa* PA01 biofilm formation (Fig. (60)). As discussed earlier, the garlic derived natural products 184 & 185 found to inhibit \( lasL/lasR \)-based QS system that are found in *P. aeruginosa* species as well as \( luxI/luxR \) systems of other bacteria. Bioassay guided fraction of garlic extract has revealed that the derivatives responsible for the QSI activity includes ajoene 198a, sulfide 198b, polysulfide 198c-e and vinyl dithianes 184 and 185. All these seven compounds are derivatives of the cysteine sulfoxide alliin 195. These sulfur containing derivatives are biosynthetically produced in the reaction cascade, that begins in macerated garlic tissue with alliinase-catalysed breakdown of the precursor \( S \)-alk(en)yl cysteine sulfoxide alliin.
Fig. (60). C-S lyase (alliinase) mediated cleavage of cystein sulfoxides.

The reaction with cysteine sulfoxide derivative 195 yields a sulfinic acid 196, where in two molecules condense to give thiosulfinates 197. These thiosulfinates further react to yield a variety of organosulfur compounds 198a-e, 184, 185 from garlic (Fig. (60)).

Fig. (61). Alliinase mediated cleavage of cystein sulfoxides

The P. alliacea alliinase is shown to have broad substrate specificity and degrade a variety of cysteine sulfoxide derivative 199 (Fig. (61)). Amongst the series of sulfur derivatives, S-phenyl-L-cysteine sulfoxide 199 and diphenyl disulfide 201a demonstrated significant biofilm inhibitory activity (Dunnett’s test, P<0.01), each at concentration of 1 µM. Also diphenyl disulfide 201b retained significant inhibitory activity at 0.1 µM.

Melander and coworkers introduced detail SAR studies of oroidin alkaloid containing biofilm inhibitors and assayed this oroidin library against P. aeruginosa biofilms [55]. One such modification involves the replacement of 2-AI of oroidin with 2-thioimidazone and 2-aminothiazole (2-AT). The synthesis commenced with ornithine methyl ester 202 which on Akabori reduction, followed by cyclization with
KSCN under pH controlled condition gave 2-thioimidazone 203. Subsequently, acylation of terminal amine with 4,5-substituted(trichloroacetyl) pyrrole (NH & NCH₃ pyrrole) 204 gave 2-thioimidazolone derivatives 205. All these synthesized compounds were able to inhibit biofilm formation in range of 27-61% for PA01 strain and 14-57% for PA14 at 500 µM as shown in (Fig. (62)).

![Chemical structure (1)](image1)

**Fig. (62). Reagents and condition:** (a) 1) Na/Hg, H₂O, 5 °C, pH 1.5, 2) KSCN, H₂O, 95 °C, pH 4.3; (b) Na₂CO₃, DMF, rt, pyrrole 204.

Also, 2-aminothiazoles (2-AT) was synthesized in this study as shown in Fig. (63). The synthetic approach involves the chlorination of 4-phthalimidobutanoic acid 206 followed by diazomethane homologation and concomitant quenching with HBr giving α–bromoketone. Treatment of α–bromoketone with thiourea under neutral condition gave 2-aminothioazole (2-AT) 207. Finally, removal of phthlamide using hydrazine followed by acylation with (trichloroacetyl)pyrrole 204 gave 2-AT analogues 208. However, this 2-AT sublibrary of oroidin family was found to be completely inactive against PA01 and PA14 strains. Overall from this study, it has been concluded that the presence of 2-AI moiety is important feature for the observed inhibitory activity of oroidin class of compounds.

![Chemical structure (2)](image2)
Fig. (63). **Reagents and condition:** (a) 1) (COCl)$_2$, DMF (cat.), CH$_2$Cl$_2$, 2) CH$_2$N$_2$, Et$_2$O/CH$_2$Cl$_2$, 0 °C, 3) conc. HBr, 4) Thiourea, DMF, 0-25 °C; (b) 1) N$_2$H$_4$, MeOH, 25-55 °C, 2) K$_2$CO$_3$, DMF, rt, dibromo(trichloroacetyl)pyrrole 204, 3) HCl in MeOH.

Brominated furanones isolated from macroalgae *Delisea pulchra* and other five membered heterocycles such as thiophene [70] and pyrrolone [71] have been identified as the effective QS inhibitors. Recently, Benneche and coworkers [72] reported new methodology for the synthesis of five membered heterocyclic QS inhibitors 210 by cleavage of the corresponding 2-acyl-5-methoxy heterocycles 209 using oxalyl bromide (Fig. (64)).

![Fig. (64). Reagents and condition: (a) oxalyl bromide, CH$_2$Cl$_2$, rt; (b) oxalyl chloride, CH$_2$Cl$_2$, 0 °C; (c) CH$_2$Cl$_2$, rt.](image)

Treatment of 2-acyl-5-methoxy heterocycle with oxalyl bromide in CH$_2$Cl$_2$ at ambient temperature gave corresponding heterocyclic derivatives. This represents the simple and short method towards this class of QS inhibitors.

**Miscellaneous:**

The development of non native (AHL) ligands has emerged as a promising strategy to inhibit biofilm formation of the different bacterial strains. Recently, our group [73] introduced pyrrolidine based skeleton as a new class of biofilm inhibitor inspired from natural product (R)-bgugaine (Fig. (65)). The first step involves preparation of *N*-carbobenzyloxy polinal from proline. Phosphorane 212 of tridecyl bromide 211 was prepared *in situ* and reacted with *N*-protected prolinal to give olefin 213. Finally, the reduction of double bond and deprotection gave norbgugaine 214.
The synthesized norbgugaine was evaluated against biofilms of *P. aeruginosa*. The norbgugaine was found to inhibit various motilities, pyocyanin pigmentation, *lasA* protease rhamnolipid production and biofilm formation in *P. aeruginosa*, wherein swarming motility is reduced by 95% and biofilm formation by 83%.

Wnuk and coworkers [74] introduced new class of optically pure γ-lactams and their reduced cyclic azahemiacetal analogues and evaluated their effect on the AHL-dependent *P. aeruginosa las* and *rhl* QS pathways. The lactam ring was chosen to explore effect of non-native AHL scaffold on QS because it is more stable isoster of the lactone ring present in AHL inhibitors (Fig. (66)). The (S)-5-(bromomethyl)pyrrolidin-2-one prepared from L-pyroglutamic acid, was subjected to bromide displacement with sodium propanethiolate to deliver (S)-5-(propylthiomethyl)pyrrolidin-2-one 216a. Previously, it has been demonstrated that the length of side chain is important for biological activity, hence lactam containing C6, C9, & C12 alkylthio chain length 216 were prepared. Also, cyclic azahemiacetals 217 (N, O-acetals or hemiaminals) with a hydroxyl group instead of carbonyl oxygen at C2 was explored in this study.
Fig. (66). Reagents and condition: (a) R1SH, NaH, DMF; (b) (Boc)_2O, DMAP, CH2Cl2; (c) 1) LiEt3BH, THF, CH2Cl2, -78 °C, 2) TFA, rt; (d) MsCl, Et3N, CH2Cl2, rt; (e) NaIO4, hydrated RuO2, EtOAc, H2O, rt; (f) RSH, NaH, DMF; (g) TFA, H2O; (h) LiEt3BH, THF, CH2Cl2, -78 °C.

The reduction of N-Boc protected lactam afforded azahemicetals which on subsequent deprotection with TFA gave (5S)-(alkylthiomethyl)pyrrolidin-2-one 217. With the intention of increasing the polarity/solubility of previously prepared lactam and azahemiacetal analogues, authors prepared another series of these compounds with hydroxyl group at C-3 and C-4 position (aza sugars). The N-Boc-protected 1,4-dideoxy-1,4-imino-D-ribitol 218a (prepared from D-gulonic-γ-lactone) on mesylation followed by selective oxidation gave mesylate 219. The displacement of mesylate with sodium hexanethiolate and sodium nonanethiolate produced corresponding 5-alkylthiomethyl lactam 220a-b which on deprotection with TFA gave (5S)-(hexyl or nonyl thiomethyl)-3,4-dihydroxypyrrolidin-2-one 221a-b. Next modification involves the reduction of 5-alkylthiomethyl lactams with LiBEt3H to give cyclic azahemiactals 222. Finally, deprotection with TFA provided (5S)-(alkyl thiomethyl)-3,4-dihydroxypyrrolidin-2-ols 223. Among this lactam analogues tested, hexyl thiolactam inhibit las activity against *P. aeruginosa*. Also the inhibitory potential increased with increase in alkylthio chain length. In particular, nonylthiolactam and dodecyl thiolactam was found to possess greatest inhibition in this series.
of compounds. The lactams \textbf{216a-c} does not modulate \textit{rhl} activity while hexylthio lactam \textbf{216b} significantly modulate \textit{rhl} QS activities at higher concentration. Overall, the cyclic azahemiacetal (217, 223) with shorter propylthio or hexylthio substituent was found to strongly inhibit both \textit{las} and \textit{rhl} signaling at higher concentration, while the propylthio analogues strongly stimulated the \textit{las} QS system at lower concentration.

Melander and coworkers \cite{75} introduced a new class of antibiofilm inhibitors inspired from the marine bacterial metabolite ethyl \textit{N}-(2-phenethyl)carbamate \textbf{225}. The synthetic method for preparation of ethyl \textit{N}-(2-phenethyl)carbamate involves the utilization of well known acylation method using phenethylamine \textbf{224} and ethylchloroformate (Fig. (67)). Ethyl \textit{N}-(2-phenethyl)carbamate \textbf{225} displayed antibiofilm activity against \textit{R. salexigens} giving 60\% inhibition at 200 \textmu M concentration. Additionally, this compound also inhibit biofilm formation for \textit{S. epidermidis}, \textit{S. aureus} (MRSA), \textit{E. faecium} (VRE), \textit{A. baumannii} (MDRAB) and \textit{E.coli} in 63\%, 68\%, 80\%, 52\% and 41\% respectively.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig67.png}
\caption{Fig. (67). \textit{Reagents and condition}: (a) EtCOOCl, Et\textsubscript{3}N, CH\textsubscript{2}Cl\textsubscript{2}, 0 °C to rt,}
\end{figure}

Other natural product derivatives of this class of compound have been synthesized using the above route. Three modifications in this series involves: aryl head group, cabamate linker, and tail group. The requisite aryl-head groups are introduced by selecting particular amine as depicted in Fig. (67). The carbamate linker core was varied through substitution with thiocarbamate, urea and thiourea linkage. The tail modification was made through the introduction of the (\textit{-})-menthyl, benzyl, \textit{t}-butyl, and
cholesteryl group. The most potent biofilm formation inhibitors (226 & 227) of *S. aureus* are shown in Fig (67).

Kato group [76] has introduced *N*-acyl cyclopentylamide 229 as a moderate inhibitor of quorum sensing in *P. aeruginosa* PA01. The rational approach suggested that the lactone ring of AHLs 228 is replaced by cyclopentane ring to deliver new class of biofilm inhibitor (Fig. (68)).

![Rational approach for *N*-acyl cyclopentylamide from AHLs](image)

The libraries of *N*-acyl-pentylamide analogues 229 are prepared by varying chain length of acyl moiety to understand the effect of side chain on their inhibitory activity. The *N*-acylated compounds with varying chain length ranging from C4 to C12 abbreviated as C4-CPA to C12-CPA were prepared by reaction of acyl chloride 230 with cycloamines 231.

![Synthesis of *N*-acyl cyclopentylamide analogues](image)

Initially synthesized *N*-octanoyl cyclopentyl amide (C8-CPA) moderately inhibited the expression of transcriptional fusion gene of *P. aeruginosa* PA01, which behave as a lead compound for the synthesis of series of structural analogues of C8-CPA to obtain stronger QS inhibitor. Overall, C10-CPA was found to have strong inhibitory effects on expression of QS-controlled genes and virulence factors in *P. aeruginosa*. Also, the length of the acyl side chain and the ring structure are crucial for the inhibitory activity of C10-CPA.
CONCLUSIONS:
Infectious diseases like nosocomial infections are still a major scourge of human life, and recent emergence of multidrug-resistance to antibiotics has left physicians with little option for the medical treatment. A number of novel strategies have been developed to solve such problems, and one such novel development in this field is “quorum sensing inhibition (QSI)”. In recent years, a hunt for a novel scaffold for treatment of infectious diseases is in demand. Moreover, most of the active biofilm inhibitors discovered till date has been based upon the motivation and inspiration from the molecular scaffolds of marine natural products. Halogenated furanones and AHLs are widely used class of compounds for development of biofilm inhibitors. Also, oroidin based 2-aminoimidazole and 2-aminobenzimidazole are used as privileged scaffolds for biofilm inhibition of P. aeruginosa and other medically relevant strains. These inhibitors play important roles to interrupt QS by repressing signal generation, blocking signal receptors or disrupting QS signal and also provide an opportunity to control infections with alternative mechanisms. However, some issues such as toxicity, carcinogenic property, instability under aqueous conditions still needs to be addressed to allow the use of these compounds for human treatment therapies. In the absence of new antibiotic agents, new modalities of treatment should be developed in future drug discovery. Hence, the development of alternative hydrolytically stable molecular scaffold for QS or biofilm infection represents an extremely important goal and will have tremendous potential for treatment of many infectious diseases.

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