

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

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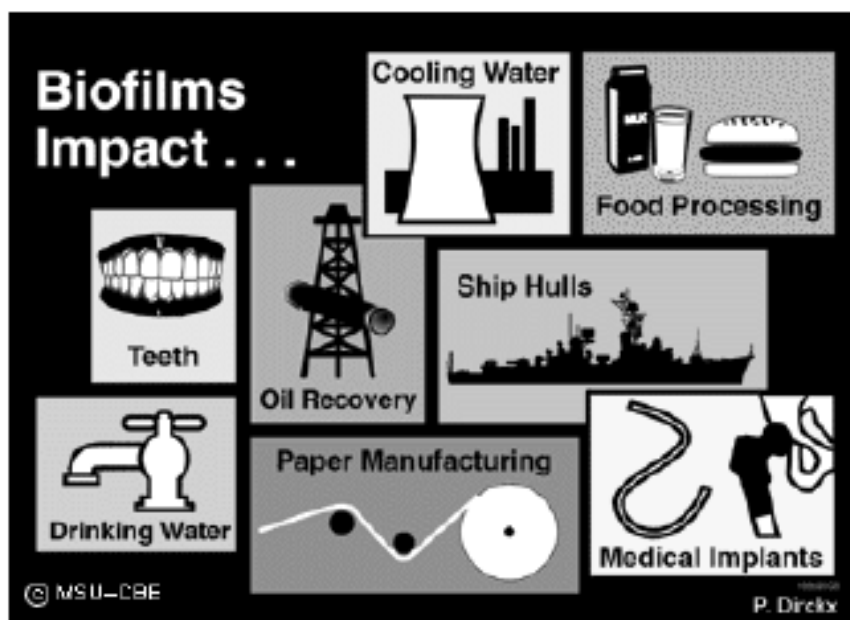
### **Abstract:**

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.

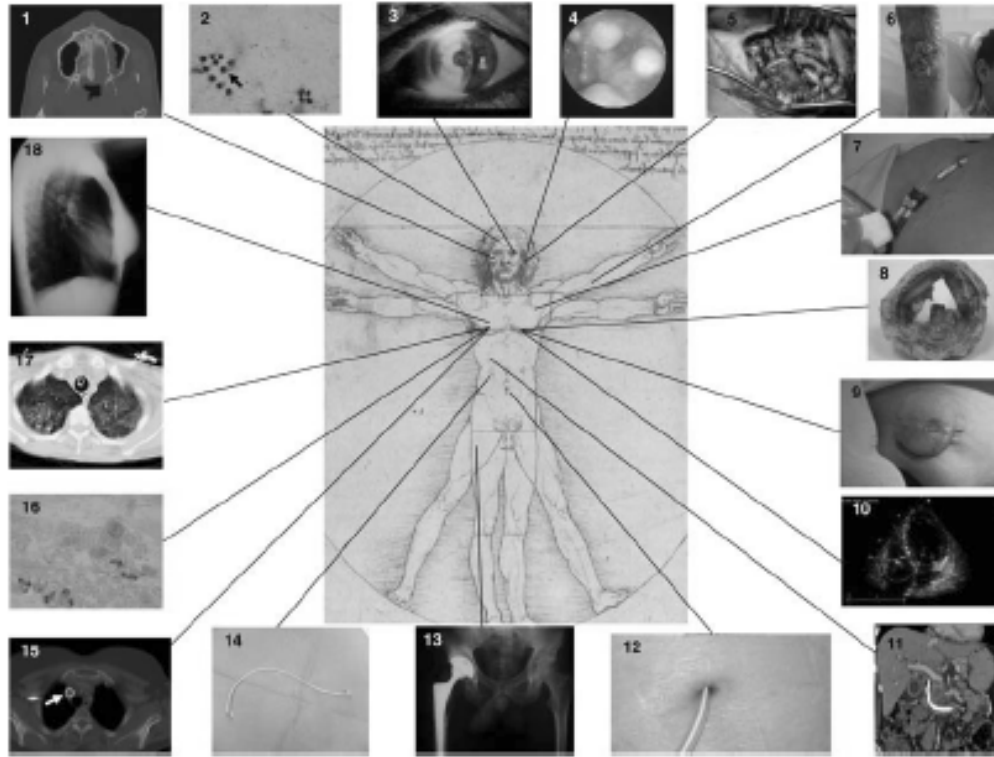
**Keywords.** Marine natural products, Biofilms, Quorum sensing inhibitors, Antifouling, Chemical signaling

## 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.



**Fig. (1).** Problems due to bacterial biofilms in everyday life (copied from <http://www.erc.montana.edu/>)



**Fig. (2). Human infectious diseases caused by bacterial biofilms** (del Pozo & Patel, 2007) [3]. (1) Chronic sinusitis. (2) Central nervous system shunt infection. (3) Contact lens-associated keratitis. (4) Chronic otitis media. (5) Cochlear implant infection. (6) Burn-related infection. (7) Intravascular catheter infection. (8) Prosthetic valve endocarditis. (9) Pacemaker infection. (10) Electrophysiological wire endocarditis. (11) Biliary stent infection. (12) Peritoneal dialysis catheter infection. (13) Prosthetic joint infection. (14) Urinary stent infection. (15) Intravascular stent infection. (16) Pulmonary infection in cystic fibrosis patient. (17) Ventilator associated pneumonia. (18) Breast implant infection.

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 1.** Medically relevant biofilm forming bacteria and the diseases caused in human

<b>Organism</b>	<b>Classification</b>	<b>Biofilm associated disease</b>
<i>Pseudomonas aeruginosa</i>	Gram (-) $\gamma$ -proteobacterium	Cystic Fibrosis lung infection
<i>Burkholderia cepacia</i>	Gram (-) $\beta$ -proteobacterium	Cystic Fibrosis lung infection
<i>Acinetobacter baumannii</i>	Gram (-) $\gamma$ -proteobacterium	Burn wound, trauma infection
<i>Escherichia coli</i>	Gram (-) $\gamma$ -proteobacterium	Urinary and gastrointestinal infection
<i>Bordetella pertussis</i>	Gram (-) $\beta$ -proteobacterium	Respiratory infection
<i>Staphylococcus aureus</i>	Gram (+)	Burn wound, catheter, lung infection
<i>Staphylococcus epidermidis</i>	Gram (+)	Nosocomial sepsis, catheter infection
<i>Streptococcus mutans</i>	Gram (+)	Dental caries

## **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

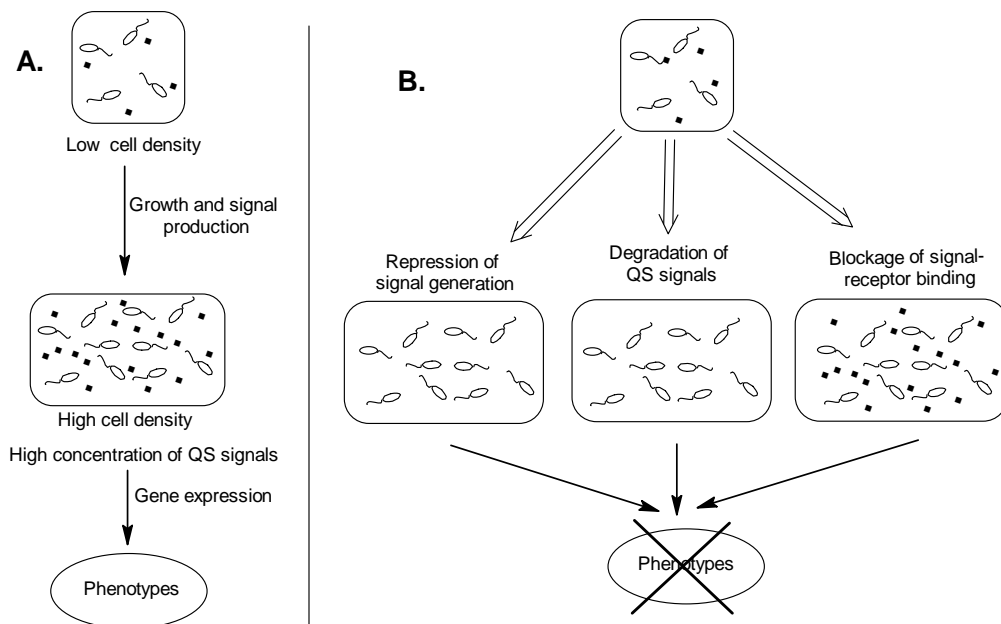
(AHL) based autoinducer which interacts with the transcriptional regulator *LuxR* and then controls transcription of quorum-sensing target genes. *LuxI/R* systems exist in more than 70 species of Gram negative bacteria [6]. QS system based on homoserine lactones (acyl-HSLs or AHLs) are commonly found 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 *Pseudomonas* 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  $\gamma$ -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).



**Fig. (3). General mechanism of QS.** (A) Significant binding of QS signals with receptors leading activation of genes; (B) QS-regulated phenotypes inhibition.

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 ( $\beta$ -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, *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.

### **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 *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 *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 *P. aeruginosa* biofilm formation [25]. By chelating iron, lactoferrin could stimulate the type IV pili mediated twitching motility of *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 *P. aeruginosa* iron metabolism and exploit the iron stress of *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 *P. aeruginosa* growth and biofilm formation [26]. Iron salts such as ferric ammonium citrate is utilized by Musk *et al.* to treat fully developed *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.

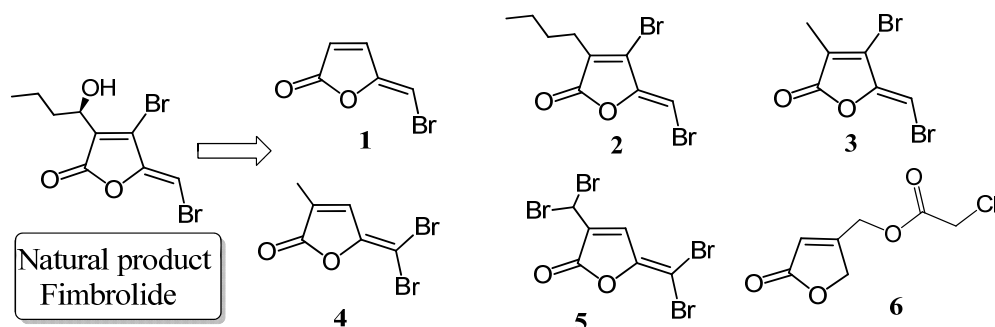
## **BIOFILM/ QS-INHIBITORS FOR *PSEUDOMONAS AERUGINOSA*: CHEMICAL SYNTHESIS & RATIONAL DESIGN APPROACHES**

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.**

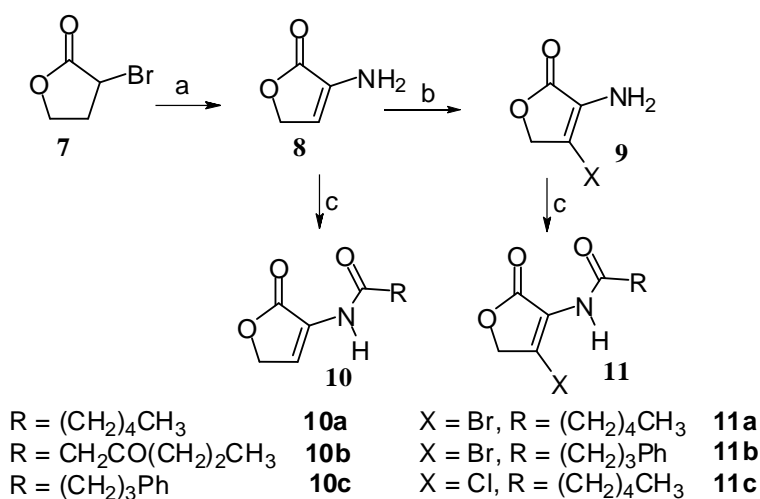
The involvement of QS in biofilm formation was originally reported for *P. aeruginosa*. Biofilms produced by *P. aeruginosa* are much more tolerant to treatment with antibiotics than planktonic cells and hence, infections by bacteria in the biofilm mode are difficult to eradicate. However, blocking the 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.



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

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



**Fig. (5).** Reagents and condition: a) NaN<sub>3</sub>, Et<sub>3</sub>N, DMF, 80 °C, 5 h; b) NBS or NCS, CH<sub>2</sub>Cl<sub>2</sub>, 50 °C, 2 h; c) RCOCl, *i*-Pr<sub>2</sub>EtN or Et<sub>3</sub>N, THF, 0 °C then rt, 5-18 h.

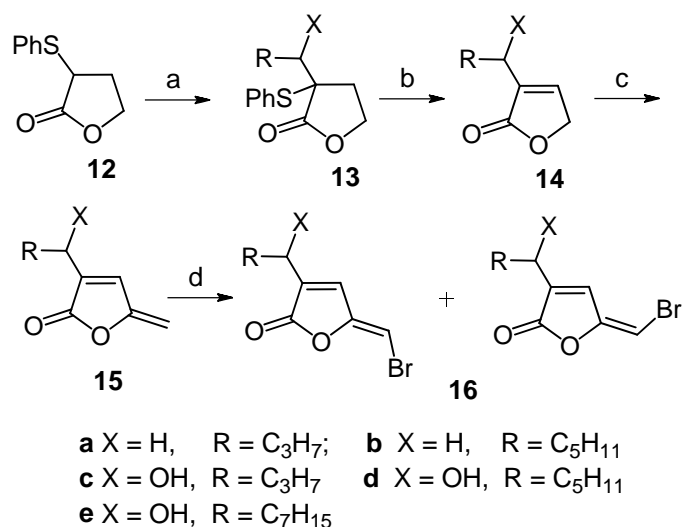
Reaction of  $\alpha$ -bromo- $\gamma$ -butyrolactone **7** with sodium azide resulted in the formation of 3-amino-5*H*-furan-2-one **8** which on treatment with NBS or NCS afforded the corresponding bromo or chloro compounds **9**. *N*-acylation of the 3-amino-5*H*-furan-2-one and their halogenated 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-5*H*-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

Compound	<b>10a</b>	<b>10b</b>	<b>10c</b>	<b>11a</b>	<b>11b</b>	<b>11c</b>
IC <sub>50</sub> ( $\mu$ M)	85 ( $\pm$ 2)	55 ( $\pm$ 2)	not active	6.5 ( $\pm$ 0.1)	84 ( $\pm$ 1)	6.9 ( $\pm$ 0.4)

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 IC<sub>50</sub> values of 6.5 and 6.9  $\mu$ M 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 CH<sub>2</sub>=N<sup>+</sup>Me<sub>2</sub>I<sup>-</sup> 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, RCH<sub>2</sub>I, THF, -78 °C, 5 h; (b) 1) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, -10 °C, 10 min, 2) toluene, reflux, 1 h; (c) LDA, CH<sub>2</sub>=N<sup>+</sup>Me<sub>2</sub>I<sup>-</sup>, THF, -78 °C, 5 h; (d) 1) Br<sub>2</sub>, CCl<sub>4</sub>, 0 °C, 5 min, 2) Et<sub>3</sub>N, DBU cat., CHCl<sub>3</sub>, 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).

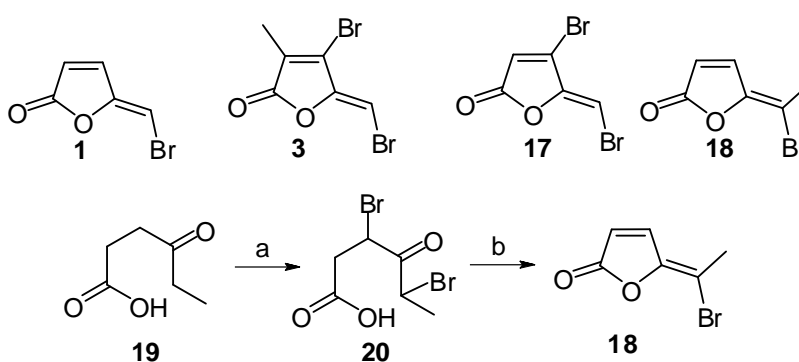
**Table 3.** Inhibition data of synthesised furanones **14-16**

IC <sub>50</sub> (μM)	<b>14</b>	<b>15</b>	<b>16</b>
<b>a</b>	>200	>200	0.6 (±0.05)
<b>b</b>	>200	>200	3 (±0.1)
<b>c</b>	Inactive	52(±2.3)	( <i>Z</i> )14 (±1.1); ( <i>E</i> )18 (±1.7)
<b>d</b>	Inactive	8(±1.2)	6 (±0.8)
<b>e</b>	Inactive	15(±1.5)	25 (±2.1)

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(5*H*)-furanone **16a** ( $IC_{50} = 0.6 \mu\text{M}$ ).

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  $P_4O_{10}$  and HBr elimination with triethyl amine.

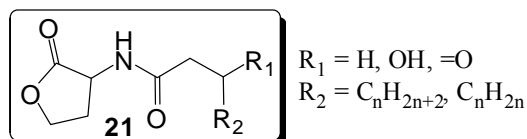


**Fig. (7).** Reagents and condition: (a)  $Br_2$ ,  $CH_2Cl_2$ , rt, 1 h; (b) 1)  $P_4O_{10}$ ,  $CH_2Cl_2$ ,  $0^\circ\text{C}$  to reflux, 1 h, 2)  $Et_3N$ ,  $CH_2Cl_2$ ,  $0^\circ\text{C}$  to reflux, 1 h.

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].

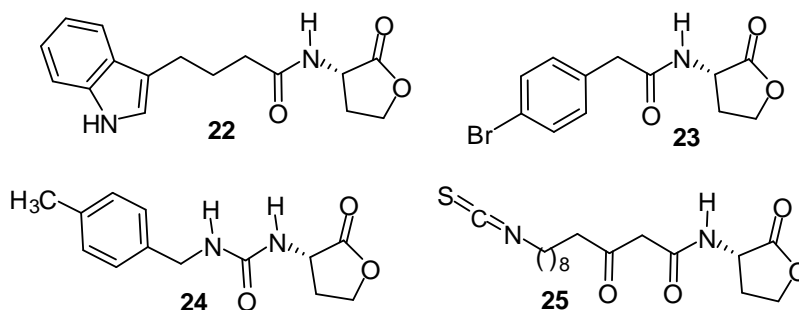
### (b) Acylated Homoserine lactones as QS inhibitors:

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.



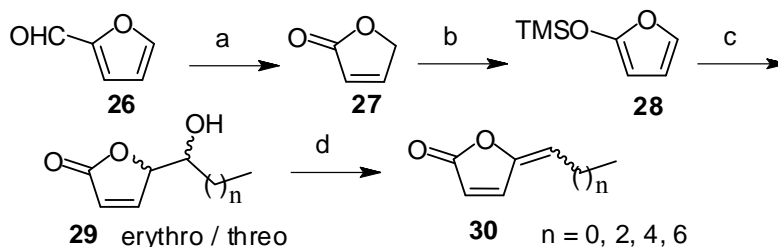
**Fig. (8).** The molecular structure of *N*-acyl homoserine lactones (AHLs)

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.



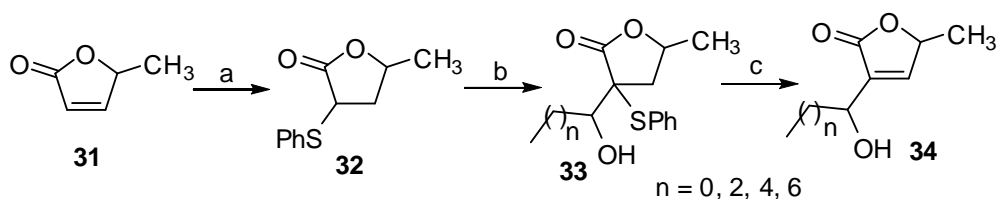
**Fig. (9).** Some selected *N*-acyl homoserine lactones (AHLs) scaffolds as biofilm inhibitors.

Hjelmgaard *et al.* [37] reported the synthesis of furanone-based product analogues, 5- & 3-(1'-hydroxyalkyl)-substituted 5*H*-furan-2-ones **29** & **34** (Fig. (10) & Fig. (11)) and their structure-activity relationship (SAR) towards two different quorum sensing systems (Table 3).



**Fig. (10).** *Reagents and condition:* (a) HCOOH, H<sub>2</sub>O<sub>2</sub>, Na<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>CO<sub>3</sub>, H<sub>2</sub>O, CH<sub>2</sub>Cl<sub>2</sub>, reflux to rt; then Et<sub>3</sub>N, toluene, rt; (b) TMSCl, Et<sub>3</sub>N, 0 °C to rt; (c) CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub>-CHO, TESOTf, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C; then 1M HCl, rt; (d) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>, rt.

5-(1'-Hydroxyalkyl)-substituted 5*H*-furan-2-ones **29** were synthesized starting from furfural. Oxidation of furfural **26** with H<sub>2</sub>O<sub>2</sub> in presence of formic acid and then isomerization with Et<sub>3</sub>N gave 5*H*-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)-5*H*-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]-5*H*-furan-2-ones **30**.

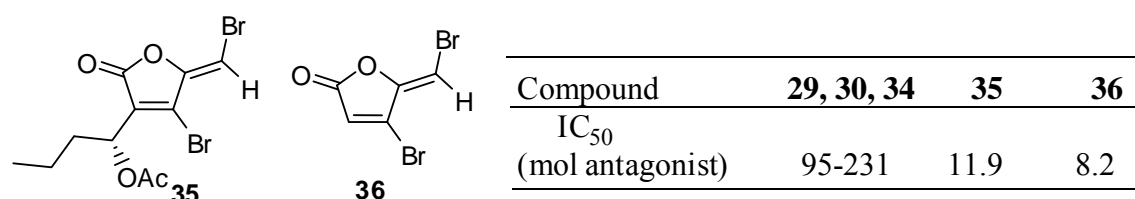


**Fig. (11).** *Reagents and condition:* (a) *i*-Pr<sub>2</sub>NH, *n*-BuLi, THF, -78 °C; then PhSSPh, THF, -78 °C; (b) *i*-Pr<sub>2</sub>NH, *n*-BuLi, THF, -78 °C; then CH<sub>3</sub>(CH<sub>2</sub>)<sub>n</sub>-CHO, THF, -50 °C; (c) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; then toluene, reflux.

Reaction of lithium enolate of  $\gamma$ -valerolactone **31** with diphenyldisulfide gave phenylsulfanyl-dihydrofuran-2-one **32**. Lithiation of phenylsulfanyl-dihydrofuran-2-one **32** with aliphatic aldehydes and

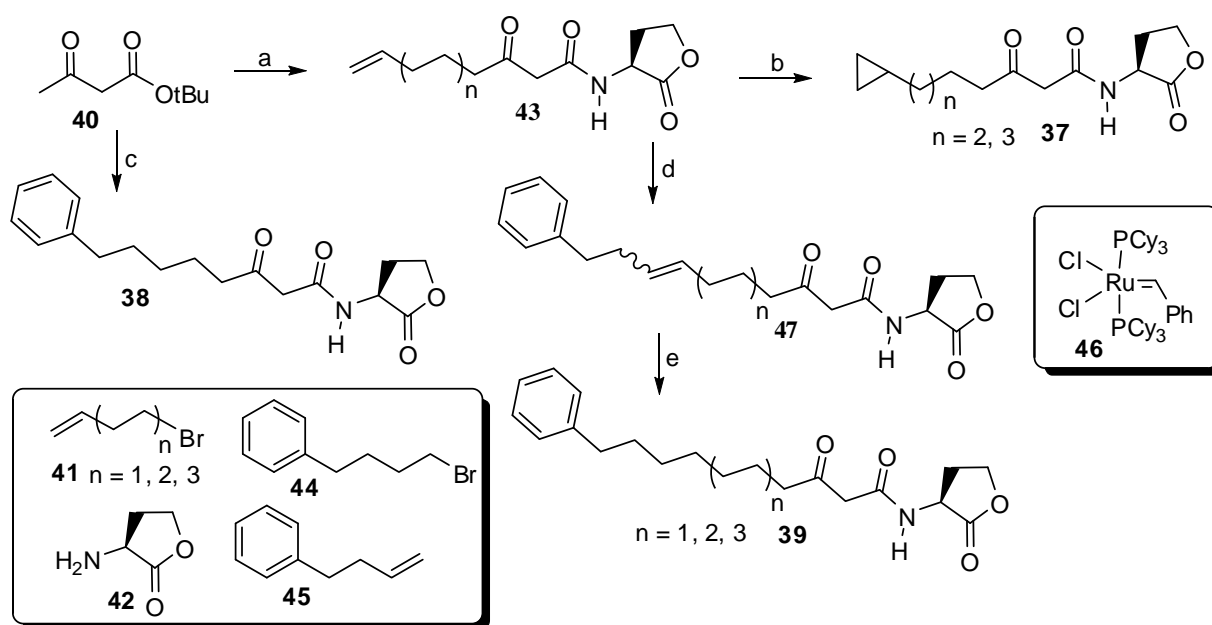
subsequent oxidation of the resultant 3-(1'-hydroxyalkyl)-5-methyl-3-phenylsulfanyl-dihydro-furan-2-ones **33** with *m*-CPBA and elimination by refluxing in toluene yielded 3-(1'-hydroxyalkyl)-5-methyl-5*H*-furan-2-ones **34**.

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).



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

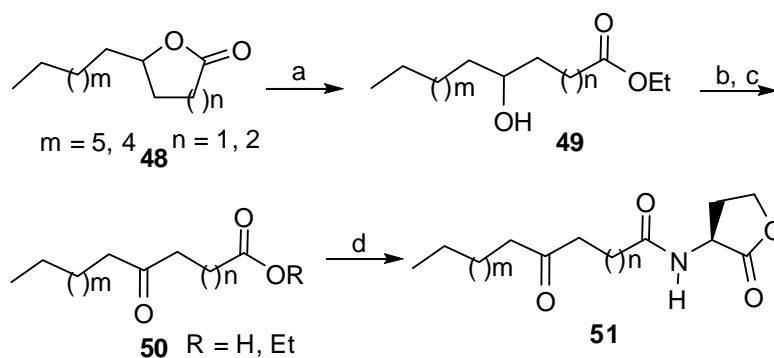
Horikawa and coworkers [38] demonstrated the synthesis of the analogues of *N*-3-oxododecanoyl-L-homoserine lactone (3-oxo-C<sub>12</sub>-HSL) and studied their SAR for the apoptotic induction in macrophages, P388D1.





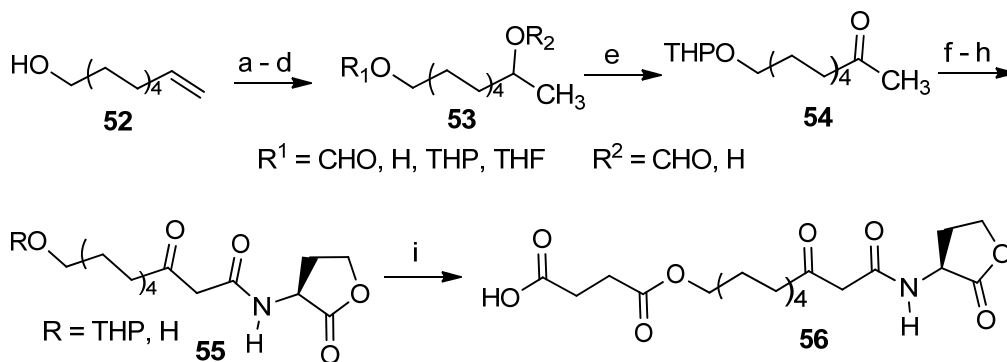
**Fig. (13).** *Reagents and condition:* (a) 1) NaH, *n*-BuLi, **41**, 2) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 3) **42**, EDCI, HOAt, CH<sub>3</sub>CN; (b) CH<sub>2</sub>N<sub>2</sub>, PdCl<sub>2</sub>(CH<sub>3</sub>CN)<sub>2</sub>, Et<sub>2</sub>O, rt; (c) 1) NaH, *n*-BuLi, **44**, 2) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 3) **42**, EDCI, HOAt, CH<sub>3</sub>CN; (d) **45**, **46**, CH<sub>2</sub>Cl<sub>2</sub>, 40 °C; (e) H<sub>2</sub>, 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  $\beta$ -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<sub>2</sub>(CH<sub>3</sub>CN)<sub>2</sub> 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<sub>2</sub>SO<sub>4</sub>; (b) CrO<sub>3</sub>, acetone; (c) KOH, MeOH; d) **42**, EDCI, dioxane.

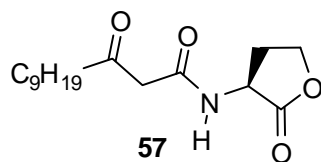
Next modification involves the introduction of long chain on *N*-acylated HSL containing ketone functionality in chain. Ethanolysis of  $\gamma$ - or  $\delta$ -dodecanolactone **48** in presence of H<sub>2</sub>SO<sub>4</sub> followed by oxidation of the resultant alcohol **49** with CrO<sub>3</sub> gave keto ester. Hydrolysis of the keto ester and subsequent coupling with L-HSL **42** using EDCI, HOAt furnished the  $\gamma$ - or  $\delta$ -oxoacyl-HSL **51** (Fig. (14)).



**Fig. (15).** Reagents and condition: (a) HCOOH, HClO<sub>4</sub>; (b) K<sub>2</sub>CO<sub>3</sub>, MeOH; (c) DHP, PPTS, CH<sub>2</sub>Cl<sub>2</sub>; (d) NaOH, MeOH; (e) CrO<sub>3</sub>, acetone; (f) LiHMDS, THF, then dry ice; (g) **42**, EDCI, dioxane; (h) H<sub>2</sub>O, AcOH; (i) succinic anhydride, pyridine.

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-C<sub>12</sub>-HSL **55**. Treatment of **55** with succinic anhydride in presence of pyridine gave 12-succinyl-3-oxo-C<sub>12</sub>-HSL **56**.

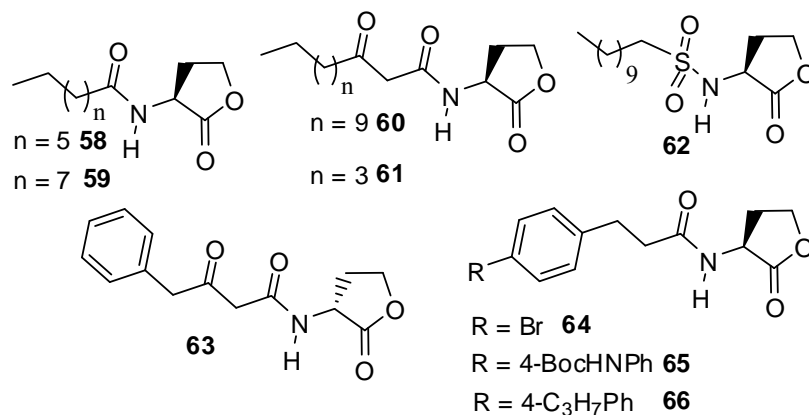
The apoptosis-inducing activities of all the compounds **37-39**, **51**, **55-56** along with previously synthesized compounds were measured on the macrophage P388D1 cell line and compared with that of natural counterpart, *N*-3-oxododecanoyl-L-homoserine lactone **57** Fig. (16). The present SAR study revealed that 3-oxo group in the acyl side chain and the homoserine lactone moiety is important for the apoptosis-inducing activity in macrophages.



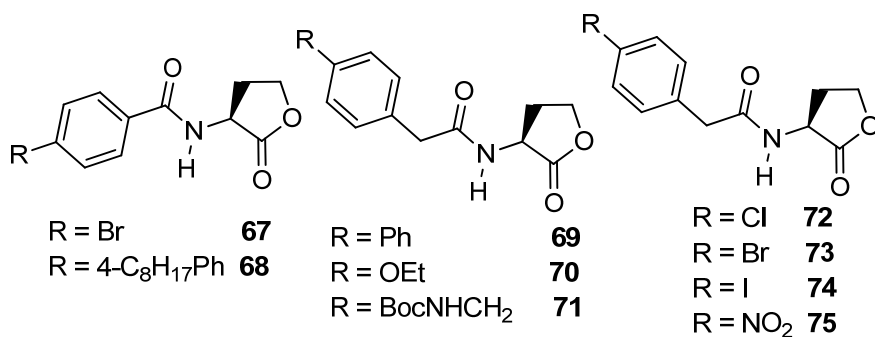
**Fig. (16).** Structure of natural *N*-3-oxododecanoyl-L-homoserine lactone **57**

The longer and bulkier hydrophobic acyl side chains than that of **57** kept the activity while the shorter ones lost the activity. Also, the polar group at the end of the acyl side chains eliminates the activity.

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  $\beta$ -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.



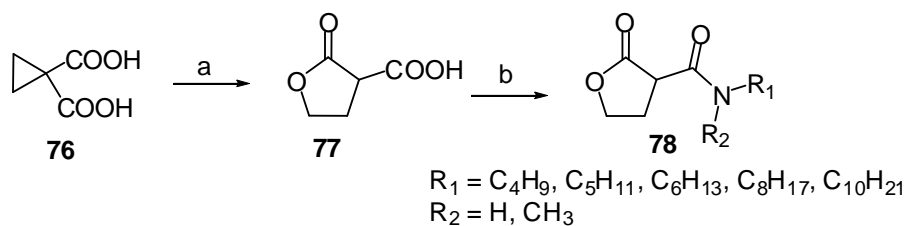
**Fig. (17).** Structures of the synthetic *QscR* agonists



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

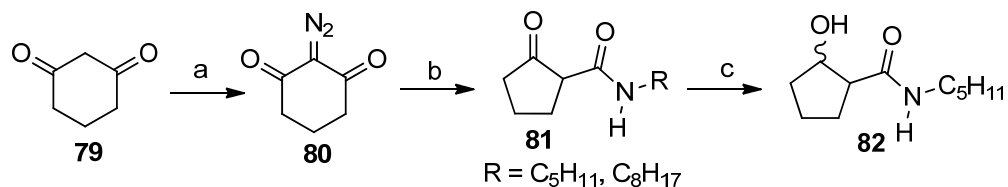
The dose-response agonism assays on the most active AHLs revealed that the compound **59** exhibited the lowest EC<sub>50</sub> values (EC<sub>50</sub> = 6 nM), with compound **61** having the next lowest value (EC<sub>50</sub> = 11 nM). The antagonism assays on the selected AHLs revealed that the compound **69** as the most active *QscR* antagonist with IC<sub>50</sub> = 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.



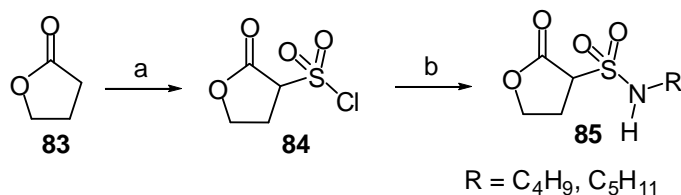
**Fig. (19).** *Reagents and condition:* (a)  $NBu_4Br$ ,  $CH_3CN$ ,  $75\text{ }^\circ C$ , 16 h; (b)  $R_1R_2NH$ , DCC, DMAP,  $CH_2Cl_2$ ,  $0\text{ }^\circ C$ , rt, 14 h.

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.



**Fig. (20).** *Reagents and condition:* (a)  $TsN_3$ ,  $Et_3N$ ,  $CH_2Cl_2$ ,  $0\text{ }^\circ C$ , 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-diazo 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**.

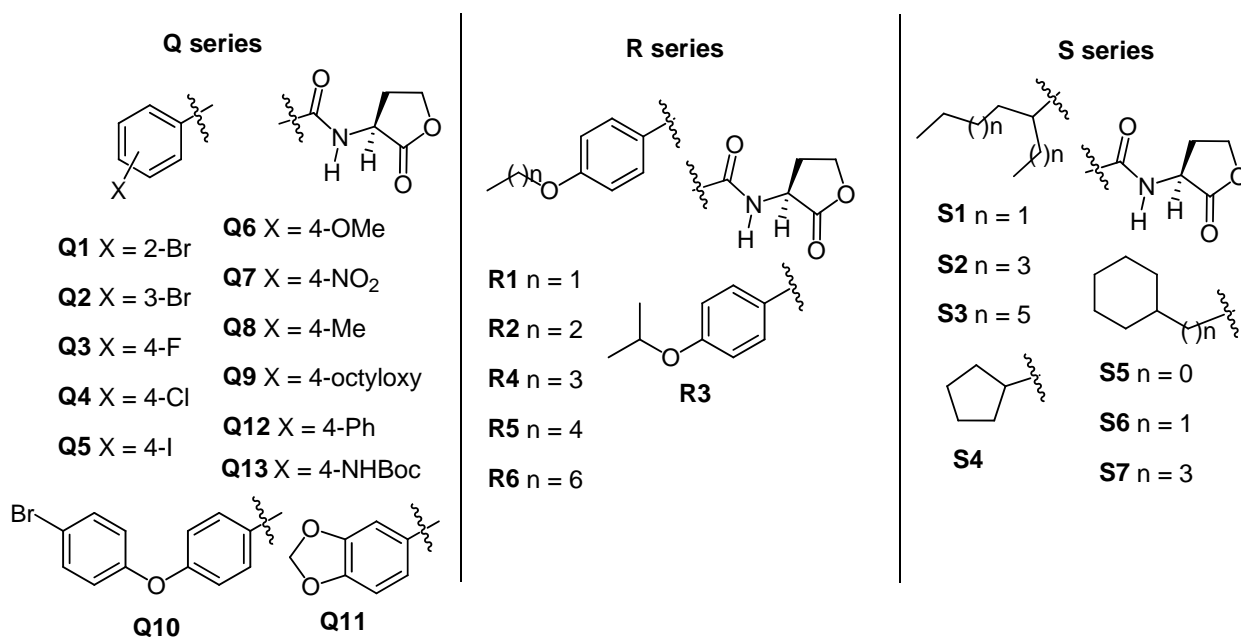


**Fig. (21).** *Reagents and condition:* (a)  $SOCl_2$ ,  $ClSO_3H$ ,  $120\text{ }^\circ C$ , 2 h; (b)  $RNH_2$ ,  $CH_2Cl_2$ ,  $-10\text{ }^\circ C$ , rt, 1.5 h.

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 pentylamine (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  $\mu$ M 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  $\mu$ M 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  $\mu$ M) than the shorter butyl derivative ( $IC_{50}$  = >200  $\mu$ M).

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).

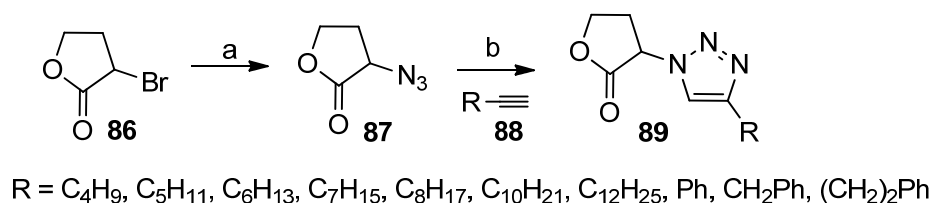


**Fig. (22).** Libraries of modified AHLs for biofilm inhibition.

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-NO<sub>2</sub>) decreases *QscR* activity to the same level as the EDG (4-CH<sub>3</sub>), 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 IC<sub>50</sub> = 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** (IC<sub>50</sub> = 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 EC<sub>50</sub> = 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, triazolylidihydrofuranones (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*.

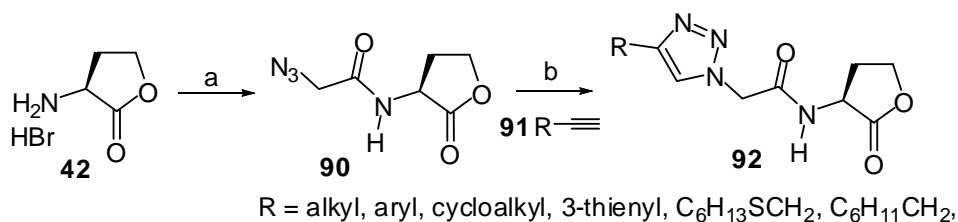


**Fig. (23).** Reagents and condition: (a) NaN<sub>3</sub>, acetone/H<sub>2</sub>O, overnight; (b) **88**, CuSO<sub>4</sub>/Na-ascorbate/*t*-BuOH/H<sub>2</sub>O, 18-24 h, rt.

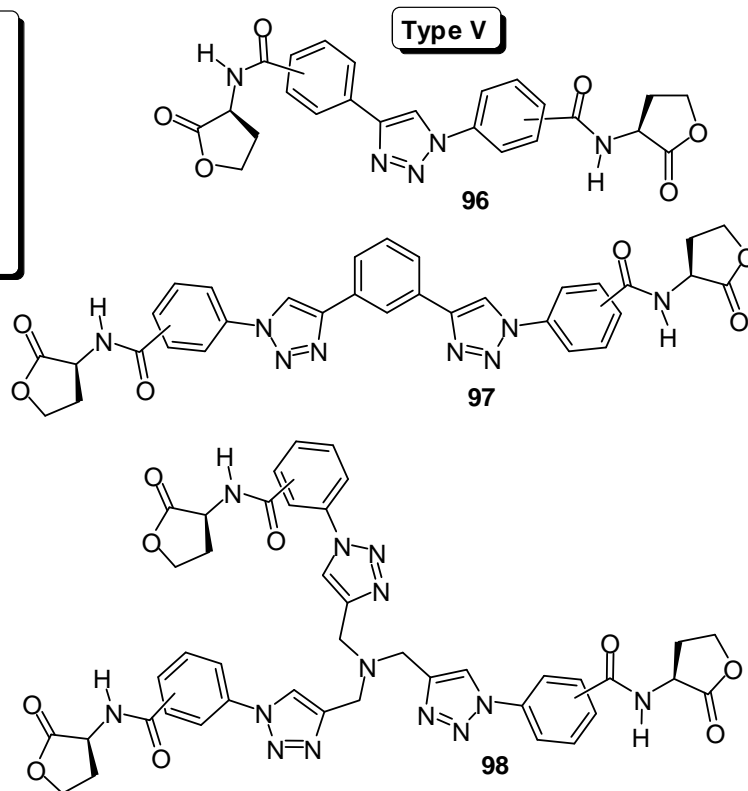
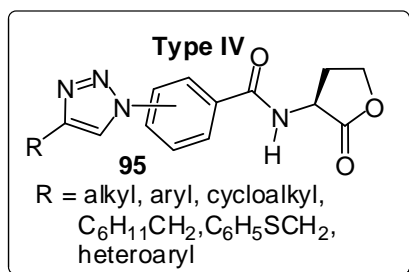
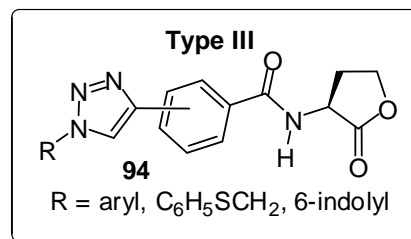
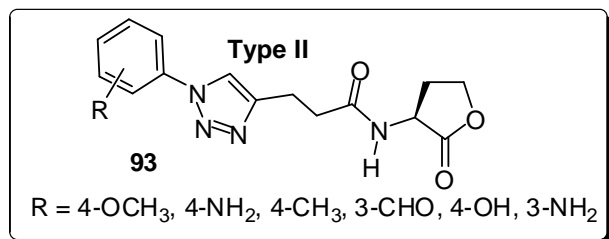
3-(1,2,3-Triazol-1-yl)dihydrofuran-2(3*H*)-one derivatives **89** were prepared in two steps. Reaction of α-bromo-γ-butyrolactone **86** with sodium azide provided 3-azidodihydrofuran-2(3*H*)-one **87**. Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction between 3-azidodihydrofuran-2(3*H*)-one and the

appropriate alkyne **88** yielded the corresponding triazolyldihydrofuranones **89**. The compounds were tested for their ability to inhibit *LasR*-dependent QS in a *P. aeruginosa* Q<sub>S</sub>SIS2 assay. Compounds with R = C<sub>10</sub>H<sub>22</sub>, C<sub>12</sub>H<sub>25</sub>, CH<sub>2</sub>Ph, (CH<sub>2</sub>)<sub>2</sub> were moderately potent inhibitors while the remaining compounds did not display antagonistic activity at the tested concentrations. IC<sub>50</sub> value of the most active QS inhibitor in this study (R = C<sub>8</sub>H<sub>17</sub>, IC<sub>50</sub> = 307.7 μM) was found to be higher than IC<sub>50</sub> values obtained for most of the previously described AHL analogues (IC<sub>50</sub> = 4 –100 μM). The compounds were also screened for their ability to induce QS in *E. coli* JB523 and *P. aeruginosa* Q<sub>S</sub>SIS2 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* Q<sub>S</sub>SIS2 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.



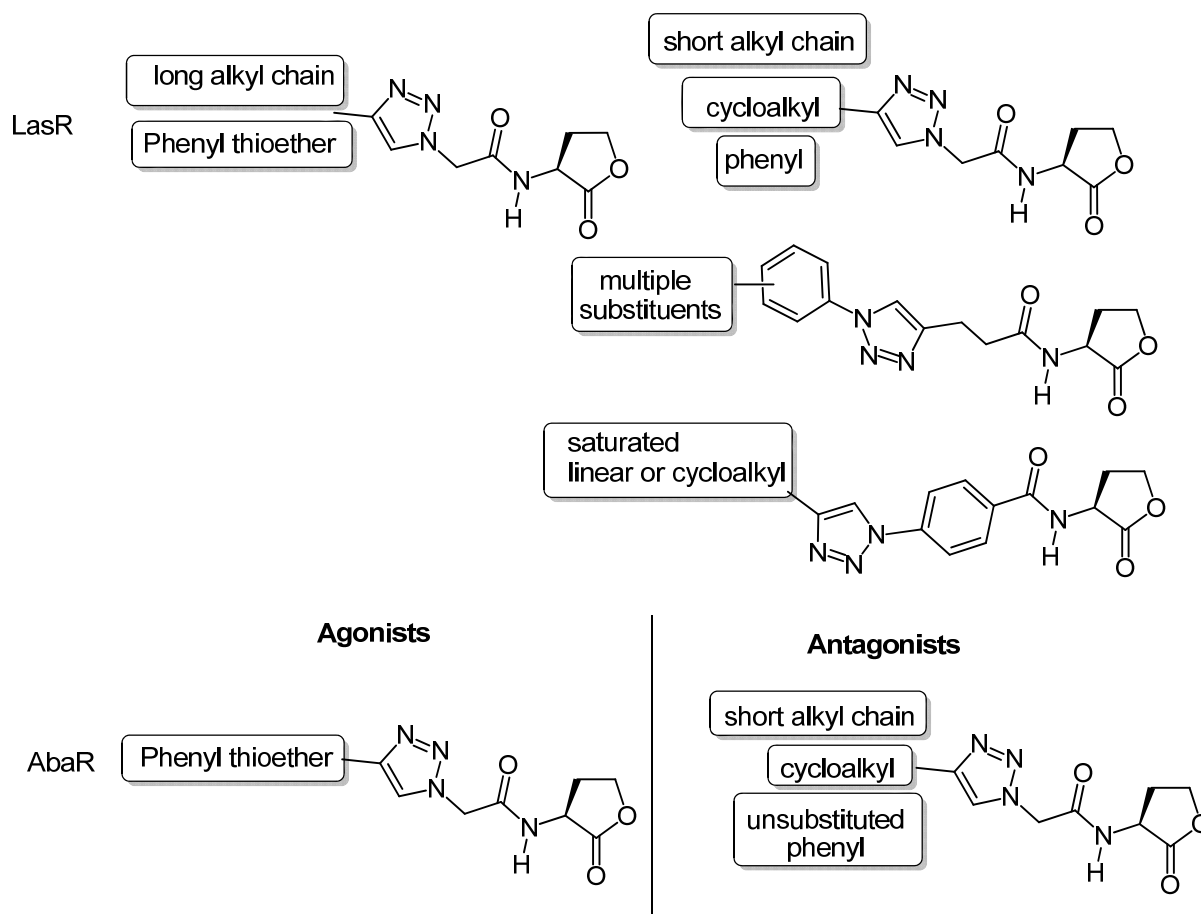
**Fig. (24).** Representative synthesis of Type **I** triazole HLs. *Reagents and condition:* (a) NaHCO<sub>3</sub>, BrCH<sub>2</sub>COBr, NaN<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>:H<sub>2</sub>O (1:1), rt, 16h; (b) **91**, CuI (10 mol%), DIPEA, CH<sub>3</sub>CN, rt, 16 h.



**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).





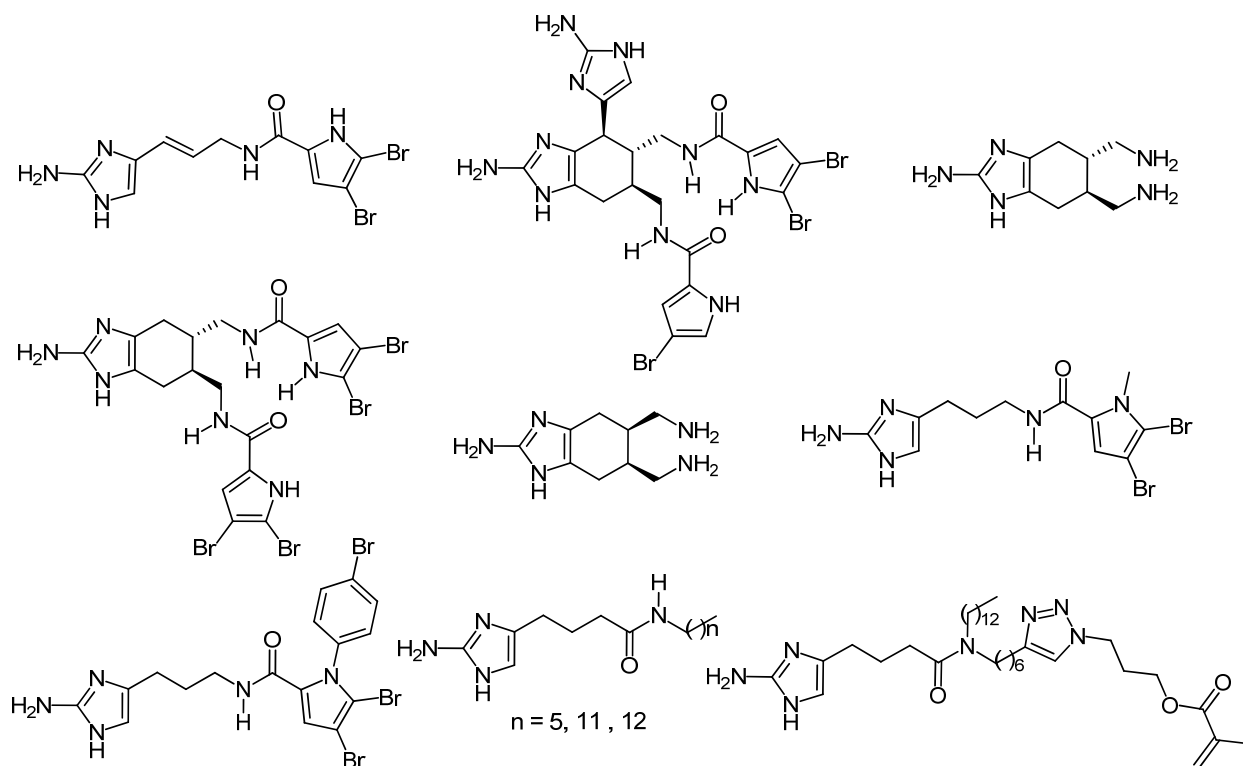
**Fig. (26).** General SAR trends for *LasR* and *AbaR* modulators identified in triazol-HL scaffolds.

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:

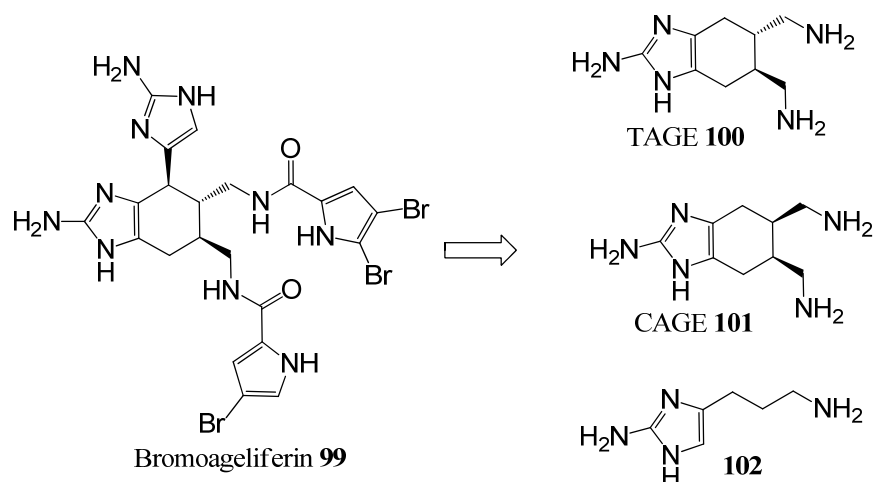
Marine organisms are well known for their chemical defenses by producing diverse skeletons of chemical compounds. The marine sponges of family *Agelasidae* produces oroidin class of alkaloids which possess nitrogen-rich architectures characterized by the presence of at least one 2-aminoimidazol subunit (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)).



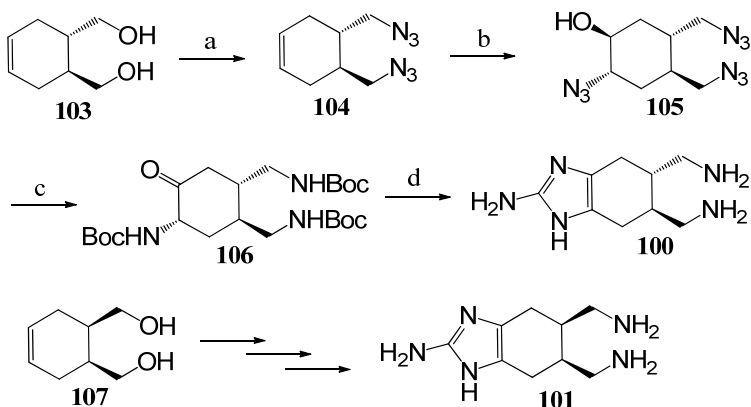
**Fig. (27).** Aminoimidazole alkaloids from marine sponge and their modifications

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).



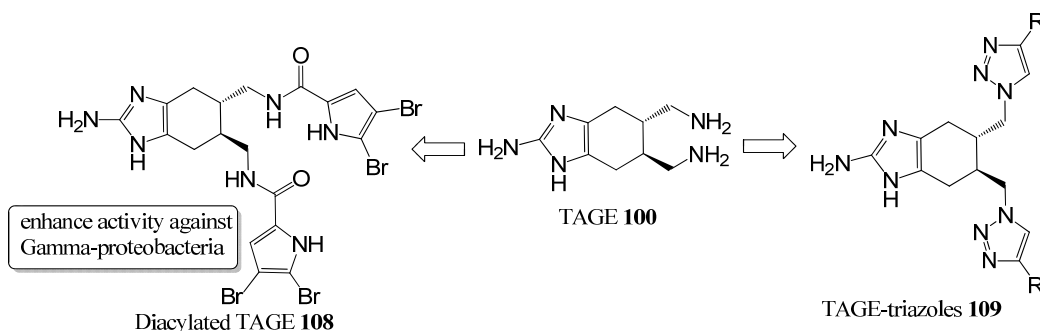
**Fig. (28).** Rational approach inspired from marine natural product along with the simplified bromoageliferin scaffolds.

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)<sub>2</sub>O gave tri-Boc protected alcohol. Next, the requisite tri-Boc protected  $\alpha$ -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).



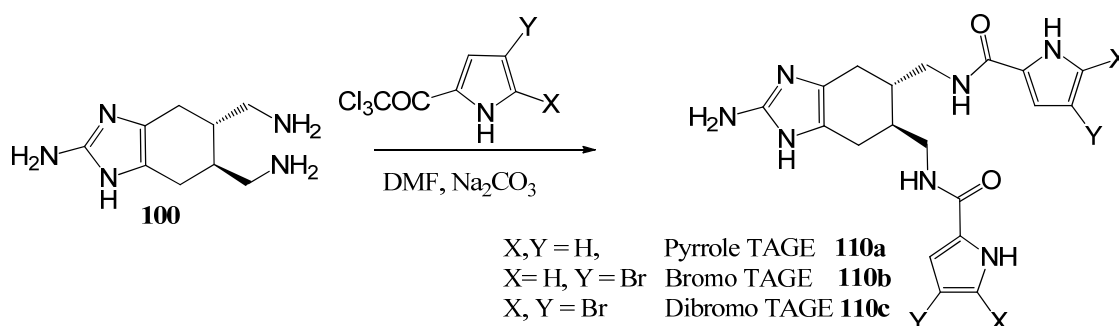
**Fig. (29).** Reagents and condition: (a) 1) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C to -25 °C, 2) NaN<sub>3</sub>, DMF, 100 °C; (b) 1) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, 2) NaN<sub>3</sub>, DMF, 100 °C; (c) 1) H<sub>2</sub>, 10% Pd/C, (Boc)<sub>2</sub>O, DMF, 2) PDC, DMF; (d) 1) TFA, CH<sub>2</sub>Cl<sub>2</sub>, then HCl, MeOH, 2) NH<sub>2</sub>CN, H<sub>2</sub>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<sub>50</sub> value of biofilm inhibition for TAGE **100** against PA01 is 100 μM & PA14 I is 190 μM whereas IC<sub>50</sub> 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

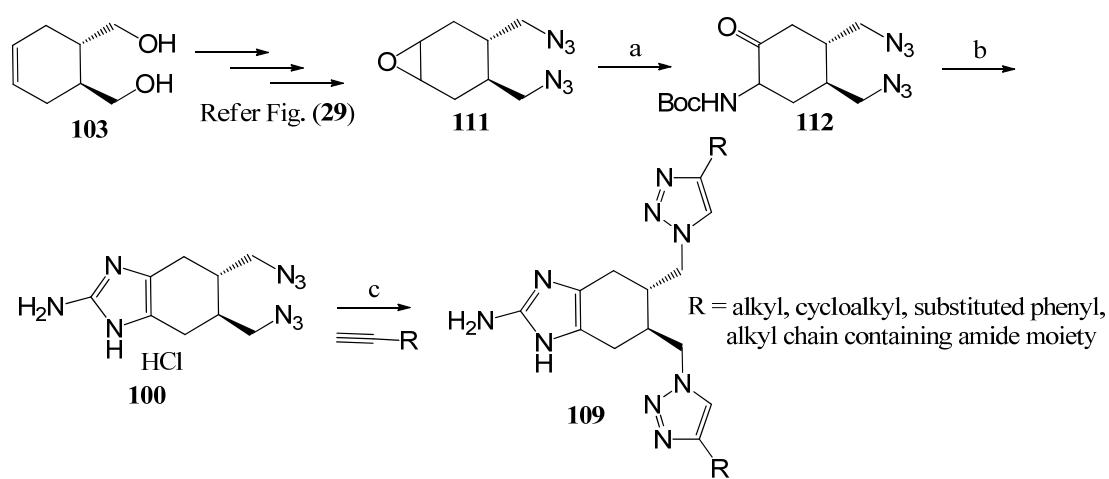
Further modification in this series involves the installation of di-brominated acylpyrrole moieties (similar to parent bromoageliferin) on to the TAGE scaffold to give compound **108** (Fig. (30)), which showed enhance antibiofilm activity with IC<sub>50</sub> value 1.77 μM, 12.0 μM against PA01 and 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 upto 400  $\mu\text{M}$  whereas bromo-TAGE **110b** showed 3-fold increase in activity compared to parent TAGE with  $\text{IC}_{50}$  value 32.5  $\mu\text{M}$  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))

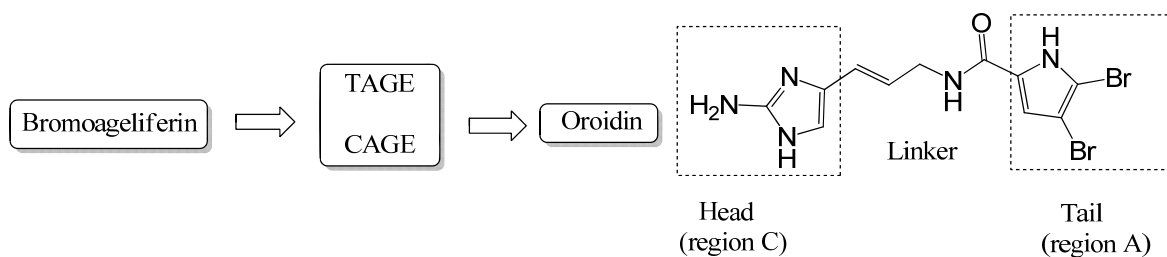


**Fig. (32).** Reagents and condition: (a) 1)  $\text{NH}_4\text{OH}$ ,  $\text{H}_2\text{O}$ ,  $\text{MeOH}$  ( $\text{NH}_3$ ), reflux, 2)  $\text{Boc}_2\text{O}$ , acetone,  $\text{H}_2\text{O}$ ,  $\text{Na}_2\text{CO}_3$ , reflux, 3) PDC, DMF, rt; (b) 1) TFA,  $\text{CH}_2\text{Cl}_2$ ,  $0^\circ\text{C}$  to rt, 2)  $\text{NH}_2\text{CN}$ ,  $\text{H}_2\text{O}$ , pH 4; (c) substituted terminal alkyne,  $\text{CuSO}_4$ , Na ascorbate,  $\text{EtOH}:\text{H}_2\text{O}:\text{CH}_2\text{Cl}_2$ .

The TAGE-triazole analogues were initially screened for antibiofilm activity against *A. baumannii* at 400  $\mu\text{M}$ , 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  $\mu\text{M}$  i.e. >95% inhibition against PA01. Additionally, the lead TAGE-triazole **109**

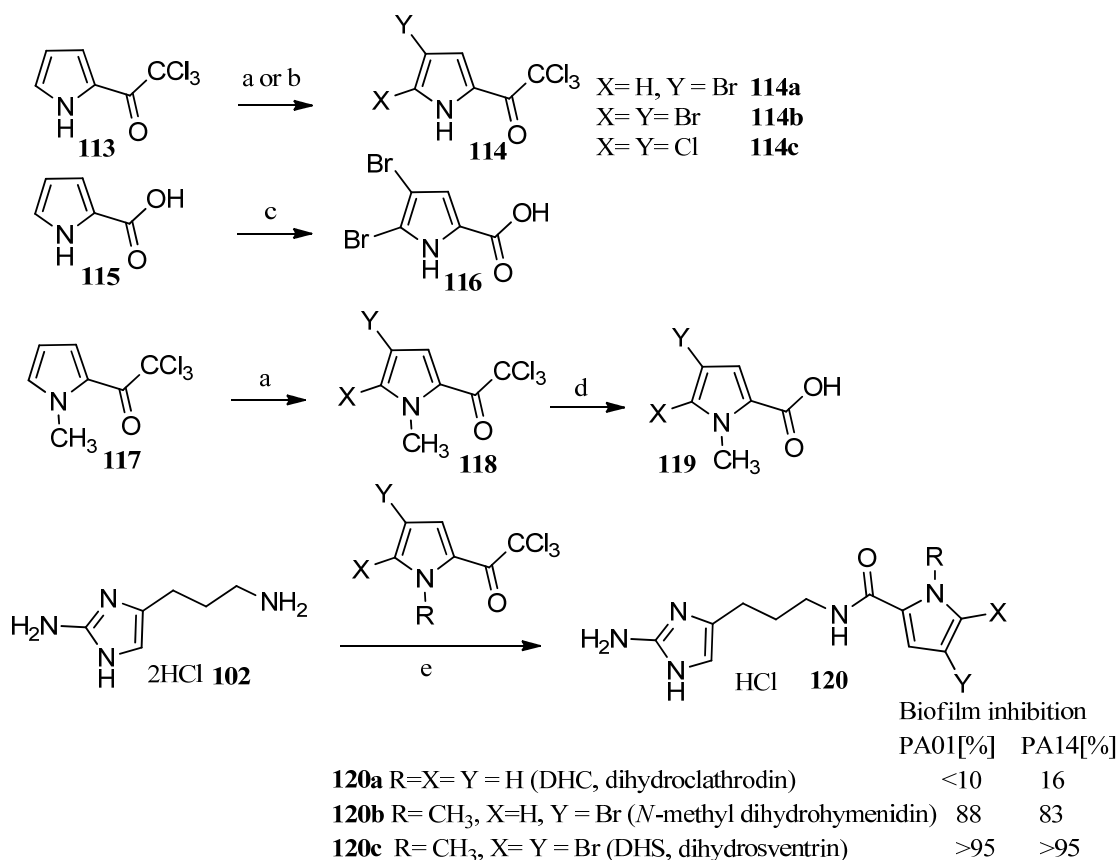
containing hexyl side chain (R = hexyl) demonstrated inhibition potency at PA01 and PA14 higher than that of parent TAGE. It is also worth to note that the several of these TAGE-triazoles were capable of inhibiting biofilm formation against different strain of *A. baumannii*, *S. aureus*, and *P. aeruginosa* 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)).



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

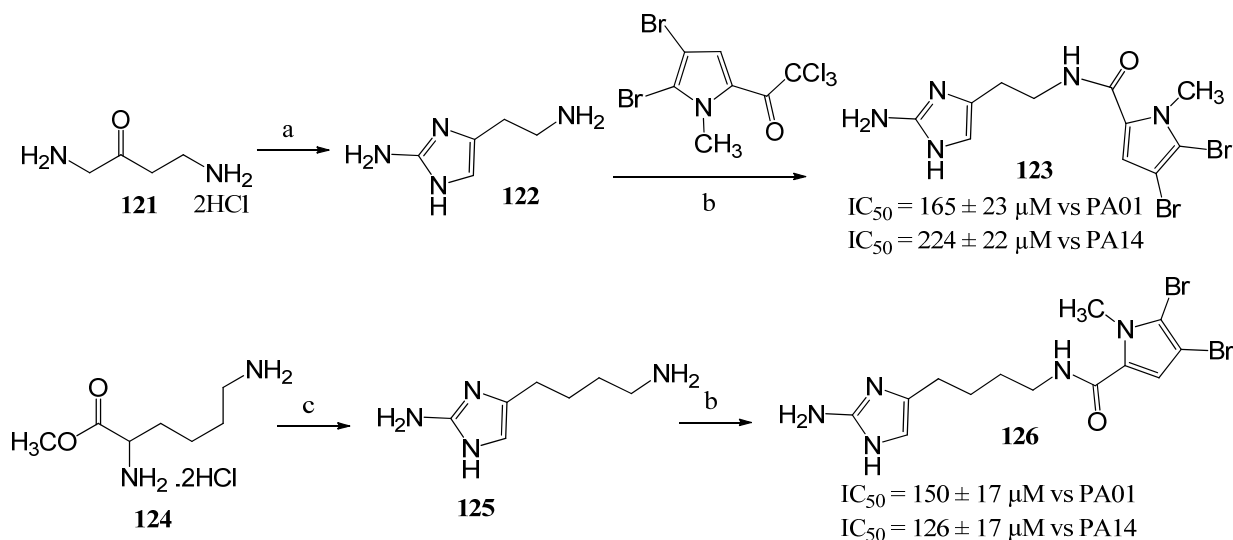
Initially, oroidin analogues were synthesized employing modifications at tail end region i.e. tail group was modified as: removed from skeleton, NH pyrrole derivative or *N*-methyl pyrrole derivative. The necessary intermediate halogenated (trichloroacetyl) pyrroles were synthesized employing bromination 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  $\mu$ M against *P. aeruginosa* strain for its biofilm activity.



**Fig. (34).** Reagents and condition: (a) Br<sub>2</sub>, CHCl<sub>3</sub>, 0 °C; (b) SO<sub>2</sub>Cl<sub>2</sub>, CHCl<sub>3</sub>, reflux; (c) Br<sub>2</sub>, HOAc, CHCl<sub>3</sub>, 50 °C; (d) 1) K<sub>2</sub>CO<sub>3</sub>, MeOH, 2) LiOH, MeOH/THF/H<sub>2</sub>O; (e) 1) pyrrole derivative, Na<sub>2</sub>CO<sub>3</sub>, DMF, rt, 2) HCl in MeOH.

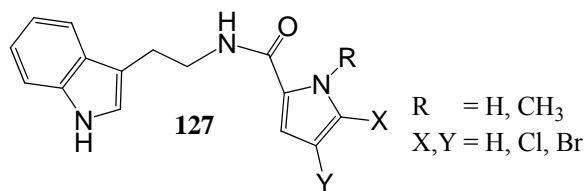
In this class of oroidin family (tail modified derivatives), the non-brominated derivatives showed a slight increase in activity relative to dihydroclathrocin (DHC) **120a**. In comparison with NH pyrrole derivative, *N*-methyl dihydrohymenidin **120b** showed substantial increase in activity *i.e.* 88% for PA01 and 83% for PA14. Overall, the dihydrosventrin (DHS) **120c** was the most active with an IC<sub>50</sub> 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-aminoimidazole skeleton (**122** & **125**) in the first step followed by coupling with trichloroacetyl pyrrole derivatives.



**Fig. (35).** Reagents and condition. (a)  $\text{NH}_2\text{CN}$ ,  $\text{H}_2\text{O}$ ,  $95^\circ\text{C}$ , pH 4.3; (b) 1) *N*-methyl-4,5-dibromo-2-(trichloroacetyl)pyrrole,  $\text{Na}_2\text{CO}_3$ , DMF, rt; 2) HCl in MeOH; (c) Na/Hg,  $\text{H}_2\text{O}$ ,  $5^\circ\text{C}$ , pH 1.5 then  $\text{NH}_2\text{CN}$ ,  $\text{H}_2\text{O}$ ,  $95^\circ\text{C}$ , pH 4.3.

The  $\text{IC}_{50}$  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 (dihydrostreptin). 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)).



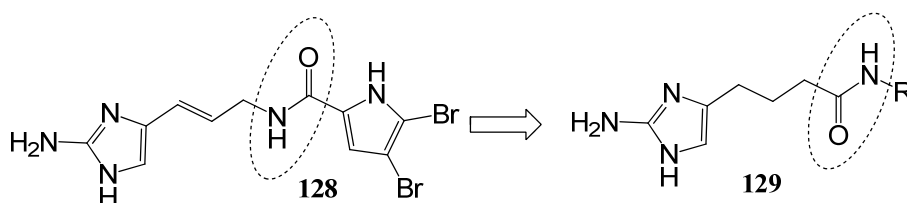
**Fig. (36).** Modified oroidin skeleton containing indole moiety.

However, replacement of 2-AI moiety with a tryptophan abolished all activity at  $500 \mu\text{M}$  against PA01 and PA14 of *P. aeruginosa*. Furthermore removal of amino functionality decreases the antibiofilm

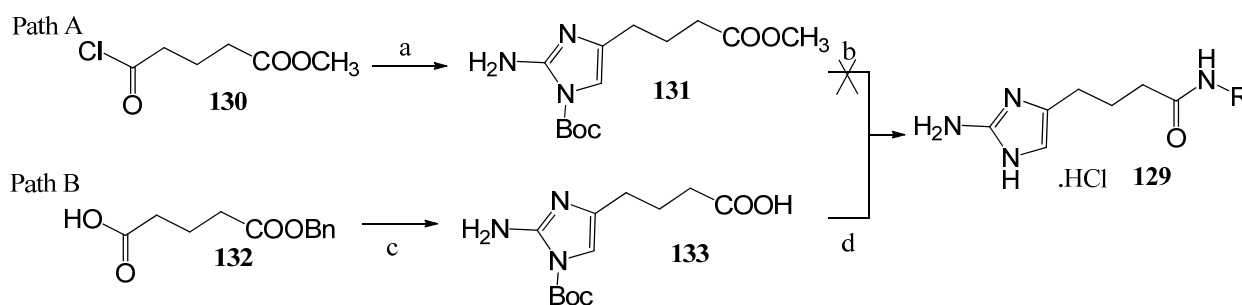


activity. Another variation of this class of compound involve introduction of sulfur atom (synthesis of thioimidazolone/2-aminothioazole (2-AT) scaffold) for SAR studies, which has been discussed in next section (class of sulfur compounds) of this review.

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 rationale to this study is depicted in Fig. (37).



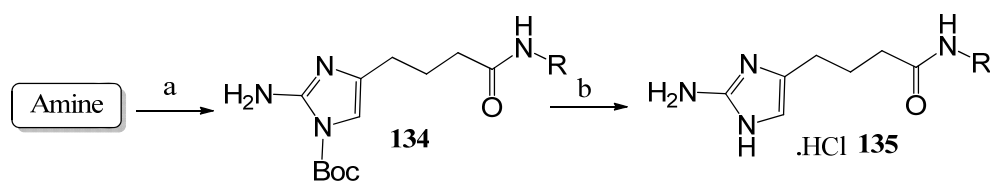
**Fig. (37).** Oroidin and reverse amide (RA) scaffold for antibiofilm inhibitory activity and structural variation is highlighted



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

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  $\alpha$ -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, debenzylation 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)).

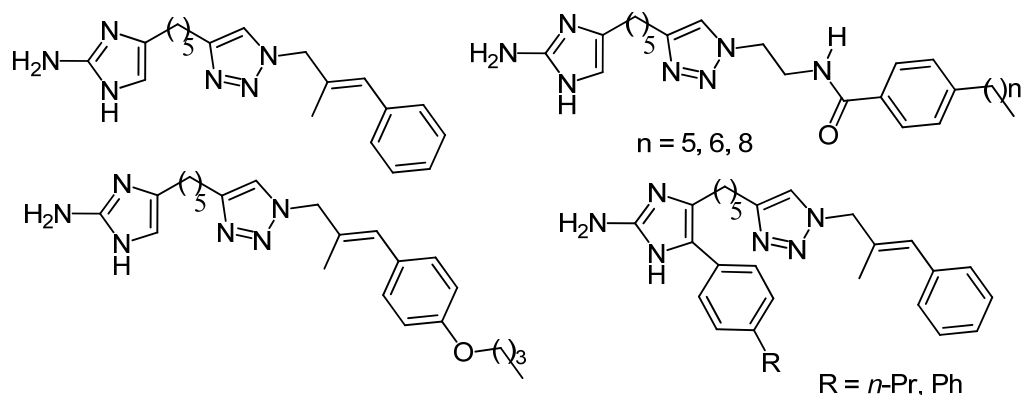


Amine	PA01 IC <sub>50</sub> in $\mu$ M	PA14 IC <sub>50</sub> in $\mu$ M
Hexylamine	32.7 $\pm$ 6.5	39.9 $\pm$ 13.0
Octylamine	18.4 $\pm$ 2.3	13.3 $\pm$ 1.8
Decylamine	8 $\pm$ 1	6 $\pm$ 1
Dododecylamine	2.84 $\pm$ 0.93	2.26 $\pm$ 0.83

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

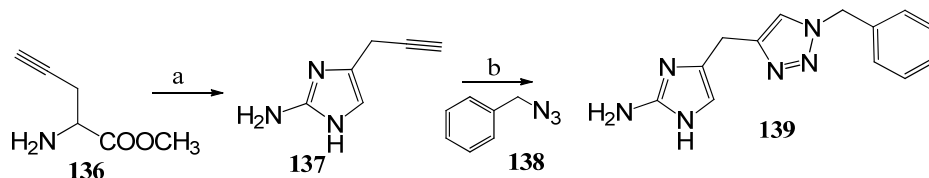
The series of synthesized reverse amide (RA) along with parent oroidin were screened at 500  $\mu$ M in a 96-well format using crystal violet reporter assay to evaluate their capacity to inhibit the formation of PA01 and PA14 biofilms. These results reveal that the aliphatic chain derivative and oroidin were very potent inhibitor in *P. aeruginosa* biofilms (> 95% inhibition) and were selected for IC<sub>50</sub> 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<sub>50</sub> value 190 $\pm$ 9  $\mu$ M and 166 $\pm$ 23  $\mu$ M 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).



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

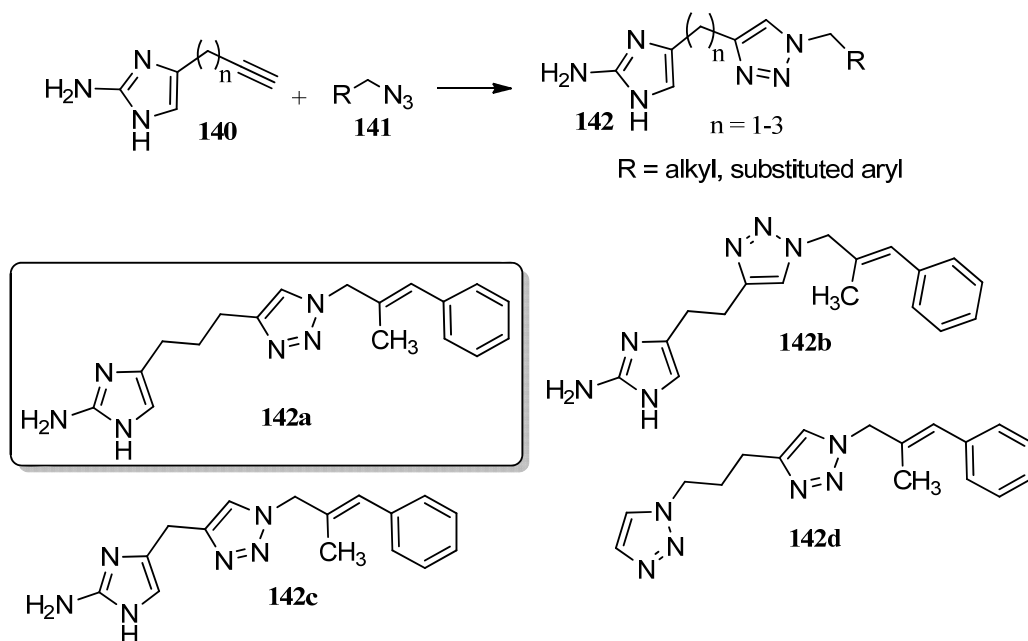
Again, Melander group [57] has introduced new series containing 2-aminoimidazole triazole (2-AIT) conjugates with the most active compound possessing a substituted unsaturated aryl group. This study has been initiated from previously disclosed dihydrosventrin (DHS) (Fig. (34)), as inhibitor of *P. aeruginosa*, *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)).



**Fig. (41).** Reagents and condition: (a) 1) 5% Na/Hg, H<sub>2</sub>O, pH 1.5-2.0, 0-5 °C, 2) cyanamide, H<sub>2</sub>O, pH 4.3, 95 °C; (b) CuSO<sub>4</sub>, sodium ascorbate, *t*-BuOH: H<sub>2</sub>O: CH<sub>2</sub>Cl<sub>2</sub> (1:1:1), rt.

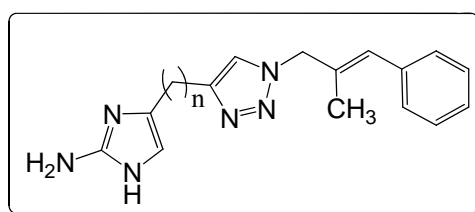
The next crucial step involves [3+2] alkyne/azide cycloaddition (click reaction) to generate 2-AIT derivatives. The click reaction of alkyne **137** with azide **138** in the presence of CuSO<sub>4</sub> and sodium ascorbate in 1:1:1 mixture of solvents *t*-BuOH: H<sub>2</sub>O: CH<sub>2</sub>Cl<sub>2</sub> 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)



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

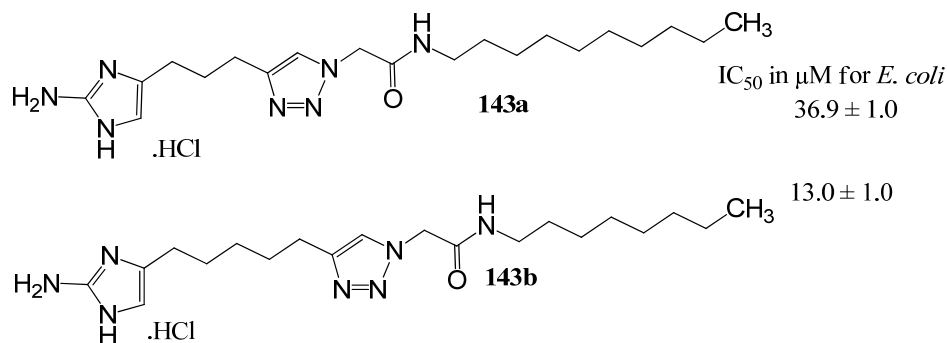
In this series, compound **142a** was active against Gram +ve and Gram –ve bacteria with  $\text{IC}_{50}$  values of 5.6  $\mu\text{M}$ , 530 nM against PA01, PA14 resp. and 980 nM and 810 nM against *A. baumannii*, and *S. aureus* respectively. In addition, this compound also exhibited synergistic 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).



$\text{IC}_{50}$ values in mM			
n	PA01	PA14	<i>S. aureus</i>
4	15	4	7
5	5.6	0.53	0.81
6	2.7	22	4.6

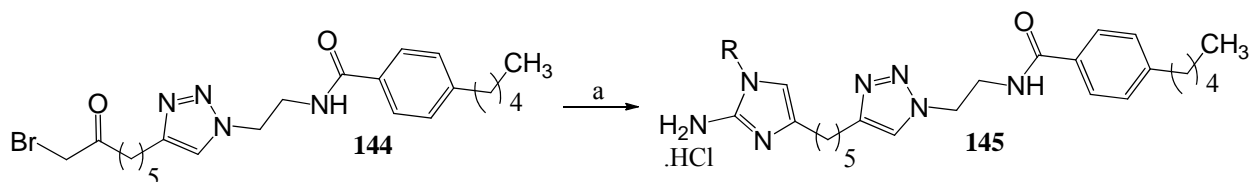
**Fig. (43).** Effect of chain length of 2-AITs on biofilm inhibitory activity

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  $\mu\text{M}$  with  $\text{IC}_{50}$  values  $36.9 \pm 1 \mu\text{M}$  and  $13.0 \pm 1 \mu\text{M}$  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

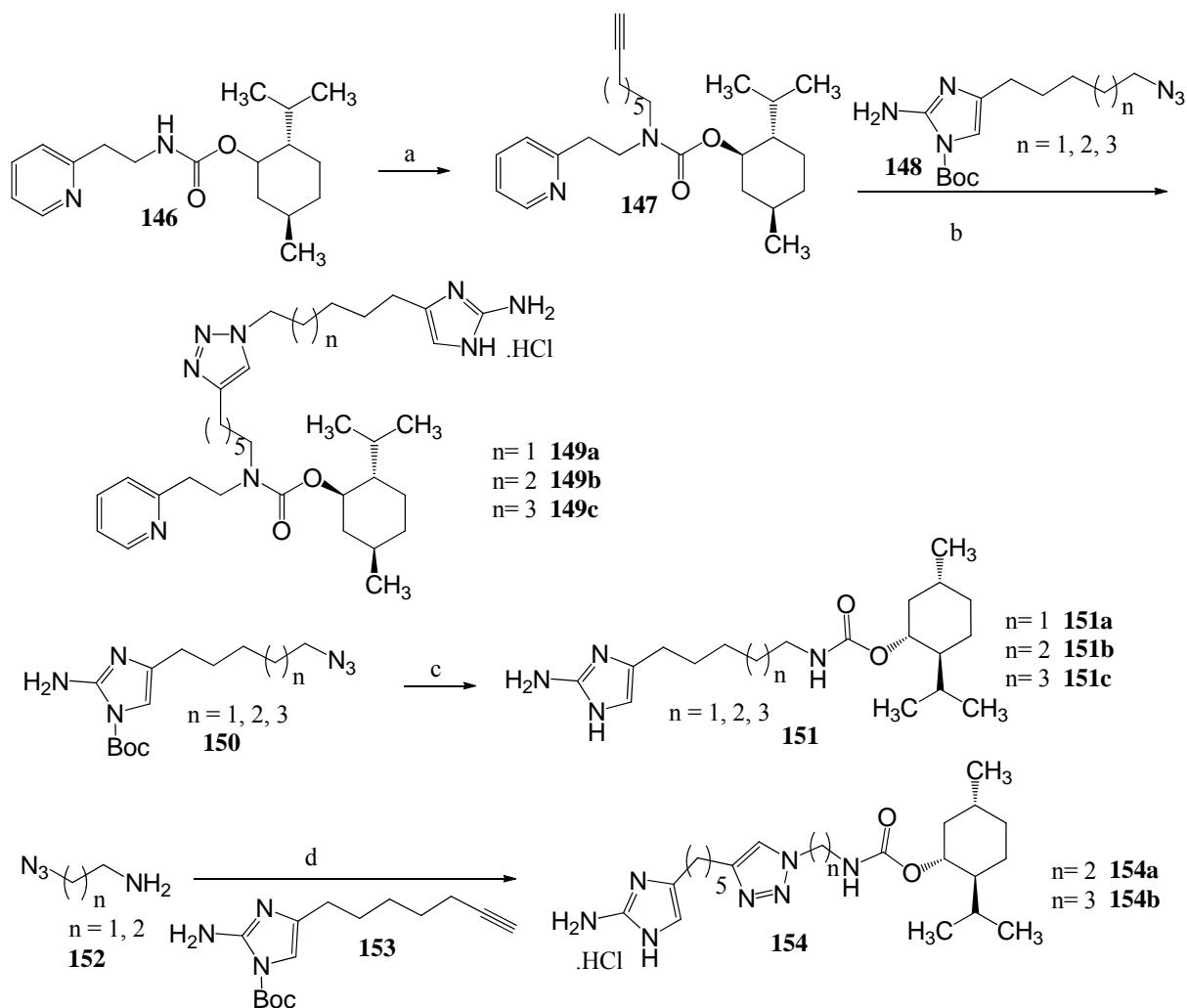
Recently, Melander and colleagues [59] discovered another series of 1,4-disubstituted 2-aminoimidazoles to explore their antibiofilm activity against multidrug resistance bacteria (Fig. (45)). The synthesis of 1,4-disubstituted 2-aminoimidazole/triazole conjugate **145** was achieved via one-pot *N*-alkylation/cyanamide 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  $\mu\text{M}$ ).



R = cyclopropyl, cyclopentyl, cyclohexyl, isopropyl, isobutyl, allyl, butyl, pentyl, hexyl, phenyl, benzyl

**Fig. (45).** Reagents and conditions: (a) 1)  $\text{RNH}_2$ , EtOH, 30 min, 2)  $\text{NH}_2\text{CN}$ , 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 antibiofilm 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)).



**Fig. (46).** Reagents and condition: (a) NaH, DMF,  $\equiv\text{-(CH}_2\text{)}_5\text{CH}_2\text{I}$ ; (b) 1)  $\text{CuSO}_4$ , Na-ascorbate, **148**, EtOH,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ , 2) TFA/  $\text{CH}_2\text{Cl}_2$  then HCl/MeOH; (c) 1)  $\text{H}_2$ , 10% Pd/C, MeOH, 2) (-)-menthyl chloroformate,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 3) TFA/  $\text{CH}_2\text{Cl}_2$  then HCl/MeOH; (d) 1) (-)-menthyl chloroformate,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 2)  $\text{CuSO}_4$ , Na-ascorbate, **153**, *t*-BuOH,  $\text{CH}_2\text{Cl}_2$ ,  $\text{H}_2\text{O}$ , 3) TFA/  $\text{CH}_2\text{Cl}_2$  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 antibiofilm agent (-)-menthol as well as 2-aminoimidazole, oroidin skeleton. The synthesis involves the alkylation of (-)-menthyl carbamate **146**

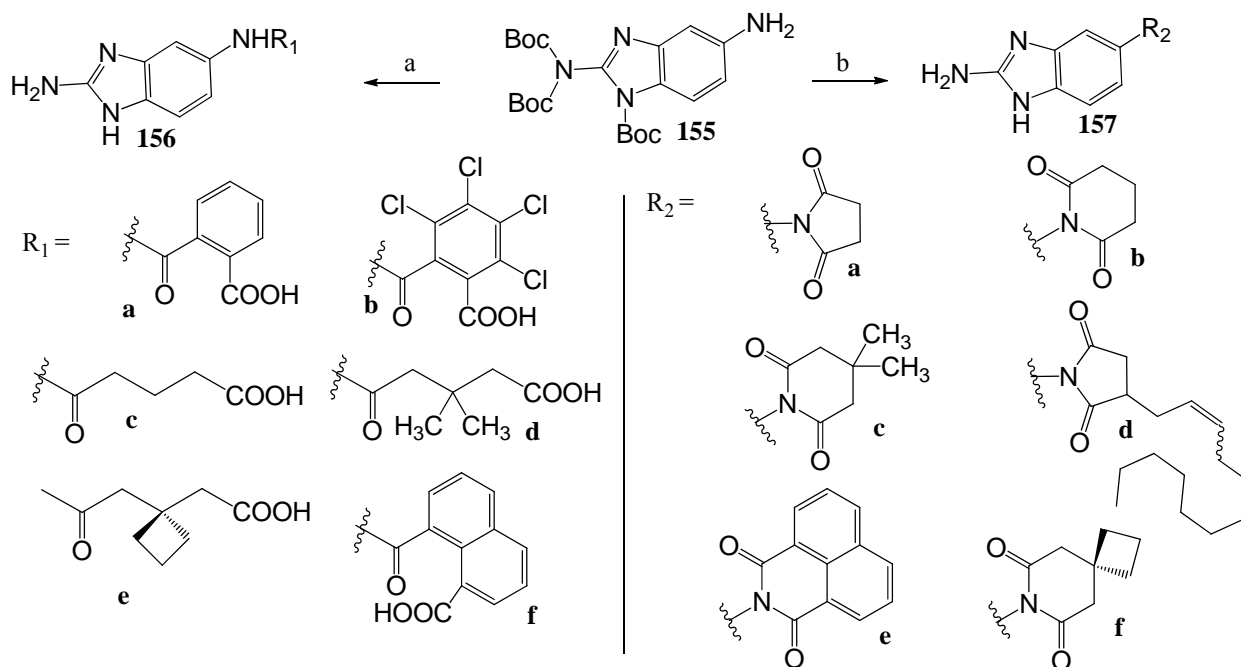
with 8-iodo-1-octyne to deliver carbamate derivative **147**. 2-Aminoimidazole containing azide **148** (n = 1, 2, 3) were reacted with carbamate alkyne derivative **147** in the presence of copper sulfate and sodium ascorbate followed by Boc-deprotection and subsequent HCl exchange to give hybride derivatives **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 Et<sub>3</sub>N. 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 IC<sub>50</sub> in μM against various bacterial strains

Compounds	<i>P. aeruginosa</i> PA 14	<i>A. baumannii</i>
<b>149a</b>	>200	19.2
<b>149b</b>	>200	18.4
<b>149c</b>	>200	16.7
<b>151a</b>	18.0	19.2
<b>151b</b>	18.0	-
<b>151c</b>	-	16.7
<b>154a</b>	58.7	94.9
<b>154b</b>	40.3	-

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. aureus* 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 (oroidin 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).

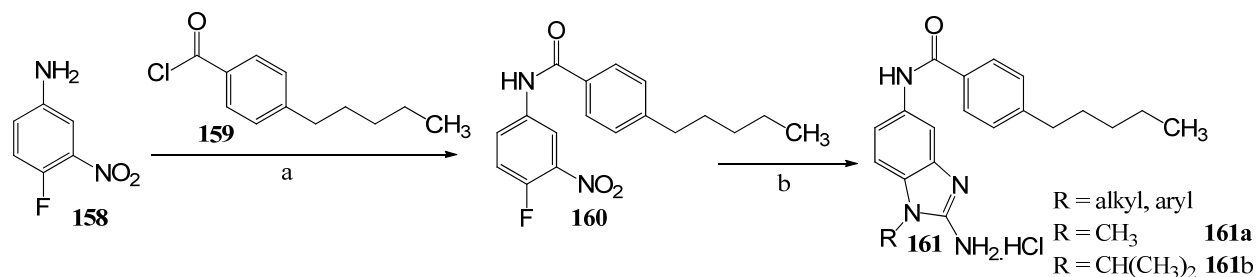


**Fig. (47).** Reagents and condition: (a) 1) acyclic anhydride,  $\text{CH}_2\text{Cl}_2$ ; 2) HCl,  $\text{H}_2\text{O}$ , THF; (b) 1) cyclic anhydride, toluene,  $110^\circ\text{C}$ ; 2) HCl,  $\text{H}_2\text{O}$ , THF.

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  $\gamma$ -proteobacteria (*P. aeruginosa* PA01, *A. baumannii*) at  $100\ \mu\text{M}$ . Amongst the series of compounds tested against Gram positive bacteria, 2-ABI derivative **156b** showed best activity profile with  $\text{IC}_{50}$  890 nM,  $1.4\ \mu\text{M}$  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)).

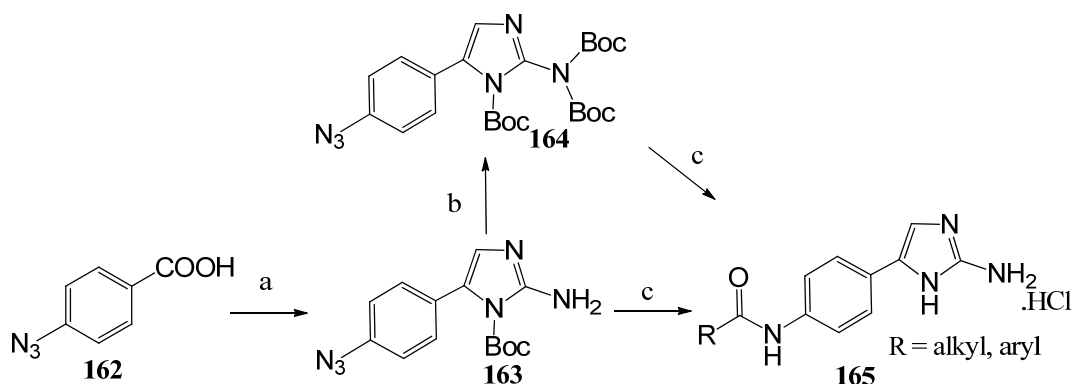


**Fig. (48).** Reagents and condition: (a) 4-pentyl benzoyl chloride, DMAP, CH<sub>2</sub>Cl<sub>2</sub>; (b) 1) RNH<sub>2</sub>, EtOH, 2) HCOONH<sub>4</sub>, Pd/C, EtOH, 3) BrCN, EtOH/CHCl<sub>3</sub>, 4) MeOH/HCl

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<sub>50</sub> values 3.7, 4.4 μM against MRSA 43300 and BAA 44 respectively.

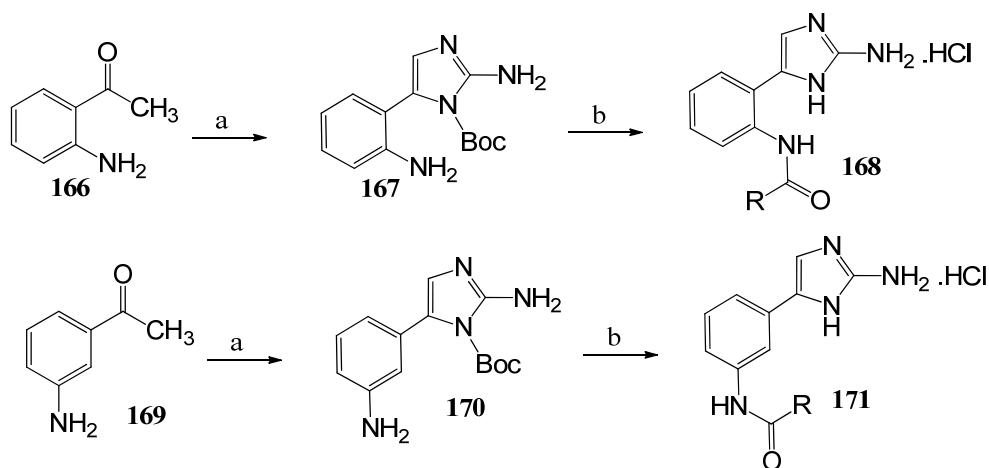
Next work by Melander group [64] involves the construction of 30-membered library based upon a aryl 2-AI skeleton (rational approach involves modification of 2-ABI) and evaluated for biofilm inhibitory activity against three Gram –ve bacteria *E. coli*, *P. aeruginosa*, and *A. baumannii*. Initially, *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 α-bromoketone (Fig. (49)). The condensation of α-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**.



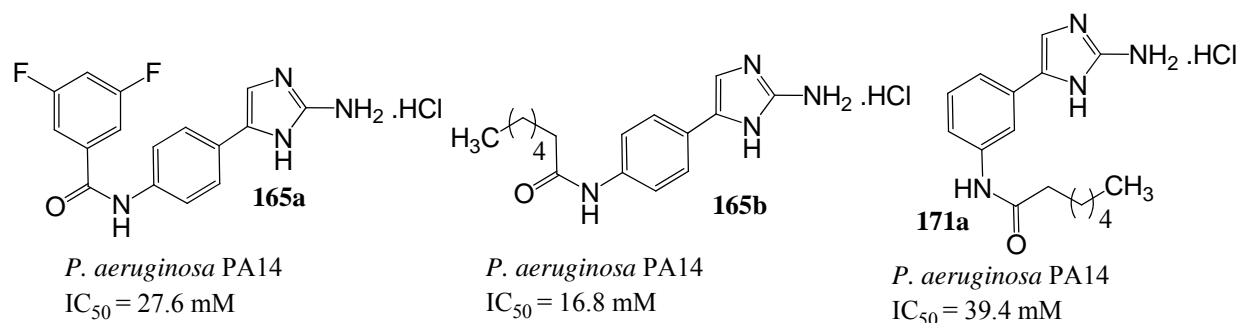
**Fig. (49).** Reagents and condition: (a) 1)  $(\text{COCl})_2$ , DMF,  $\text{CH}_2\text{Cl}_2$ , rt, 2)  $\text{CH}_2\text{N}_2$ ,  $\text{Et}_2\text{O}$ ,  $0\text{ }^\circ\text{C}$ , 3) conc. HBr, 4) Boc-guanidine, DMF, rt; (b)  $(\text{Boc})_2\text{O}$ ,  $\text{Et}_3\text{N}$ , THF, rt; (c) 1)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

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**.



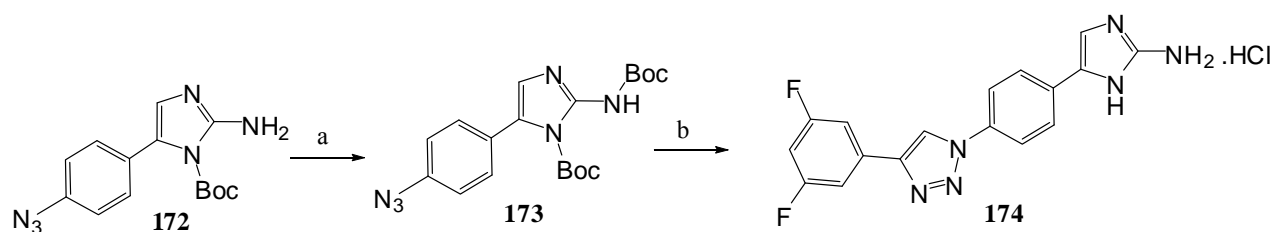
**Fig. (50).** Reagents and condition: (a) 1) CbzCl,  $\text{NaHCO}_3$ ,  $\text{H}_2\text{O}$ , acetone,  $0\text{ }^\circ\text{C}$ -rt, 2)  $\text{Br}_2$ ,  $\text{AlCl}_3$  (cat.),  $0\text{ }^\circ\text{C}$ , 3) Boc-guanidine, DMF, rt, 4)  $\text{H}_2$  (35 psi), 10% Pd/C,  $\text{EtOH}$ , rt; (b) 1)  $\text{RCOCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , THF,  $-78\text{ }^\circ\text{C}$ -rt, 2) TFA,  $\text{CH}_2\text{Cl}_2$ ,  $0\text{ }^\circ\text{C}$ , 3) 2M HCl in  $\text{Et}_2\text{O}$ .

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 intension 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

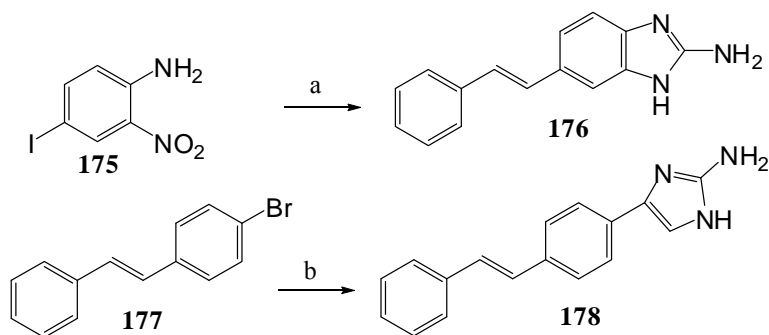
2-AI azide **172** was subjected to *N*-protection using Boc-anhydride, followed by copper mediated [3+2] cycloaddition with 1-ethynyl-3,5-difluorobenzene and finally Boc-deprotection gave aryl-2-AI triazole derivative **174**. Unfortunately, the evaluation of this triazole for inhibition of biofilm at 100  $\mu$ M 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: (a) LiHMDS, (Boc)<sub>2</sub>O, THF, 0 °C; (b) 1) CuSO<sub>4</sub>.5H<sub>2</sub>O, Na ascorbate, 1-ethynyl-3,5-difluorobenzene, CH<sub>2</sub>Cl<sub>2</sub>, EtOH, H<sub>2</sub>O, rt, 2) TFA, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 3) 2M HCl in Et<sub>2</sub>O.

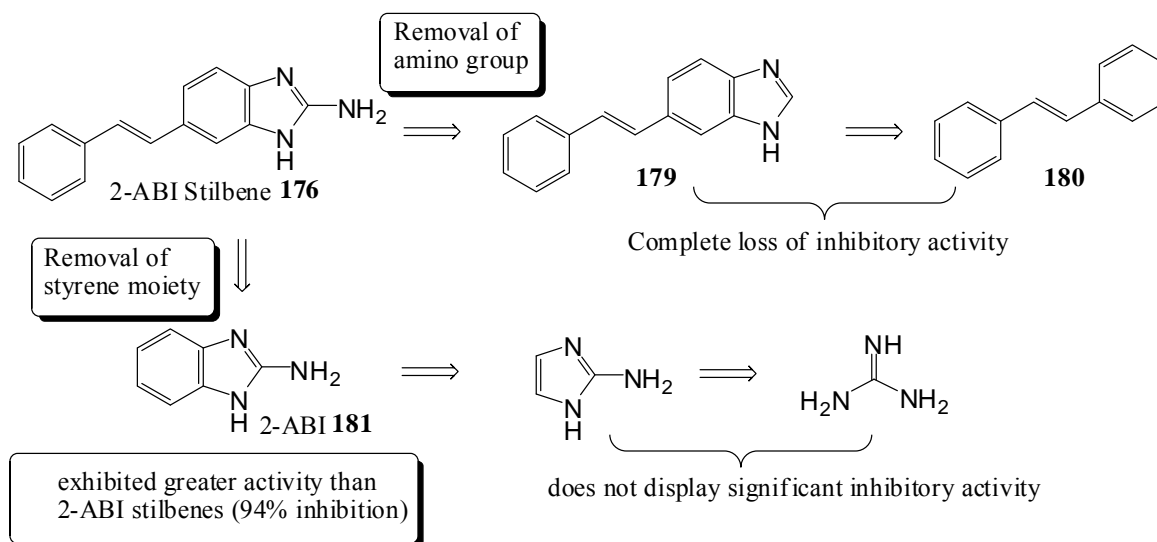
Recently, an elegant study by Blackwell and coworkers [66] have demonstrated the importance of hybrid compounds *i.e* 2-aminobenzimidazole (2-ABI) derived from the structures of three natural products (bromoageliferin, 3-indolylacetonitrile, resveratrol) with known QS inhibitory activities and also their 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)).



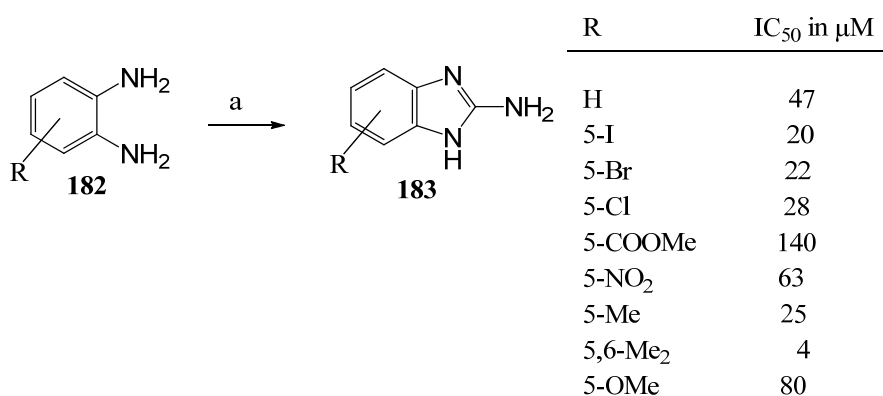
**Fig. (53).** Reagents and condition: (a) Styrene, Pd(OAc)<sub>2</sub>, CH<sub>3</sub>CN, DIPEA, 80 °C, 2) SnCl<sub>2</sub>·2H<sub>2</sub>O, EtOAc, 80 °C, 3) CNBr, MeOH/H<sub>2</sub>O (1:1), 80 °C; (b) 1) imidazo[1,2-*a*] pyrimidine.HBr, Pd(OAc)<sub>2</sub>, PPh<sub>3</sub>, Cs<sub>2</sub>CO<sub>3</sub>,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



**Fig. (54).** Rational design approach starting from 2-ABI stilbene as lead compound and essential molecular scaffold for improved biological activity.

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  $\text{IC}_{50}$  values) relative to parent 2-ABI.

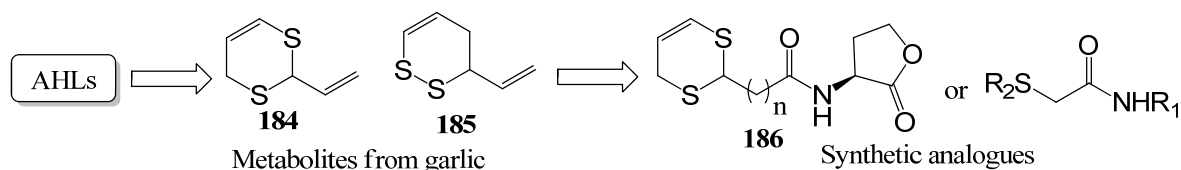


**Fig. (55).** Reagents and condition: (a) CNBr, MeOH/H<sub>2</sub>O (1:1), 50 °C, 1h

Most of the synthesized 2-ABI derivatives **183** were capable of inhibiting biofilm growth with improved potencies (*i.e.* lower IC<sub>50</sub> 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 IC<sub>50</sub> 4.0 μM *i.e.* approximately 10-fold more active than parent 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*.

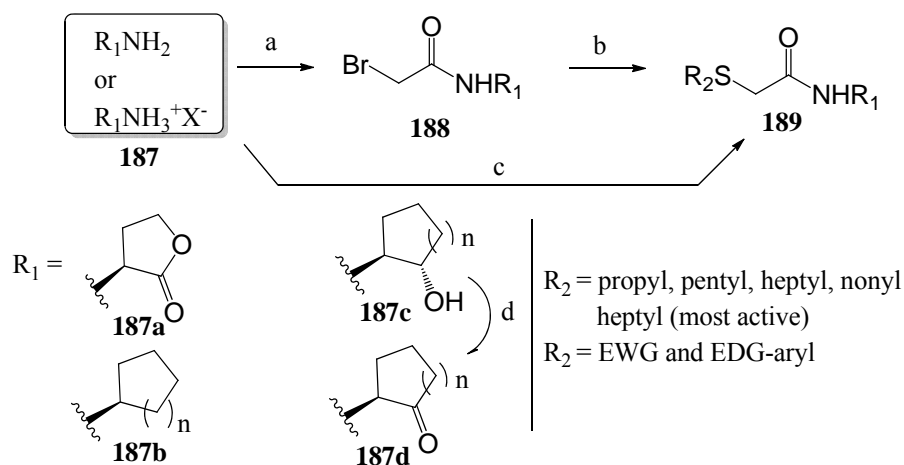
### Organosulfur compounds:

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.



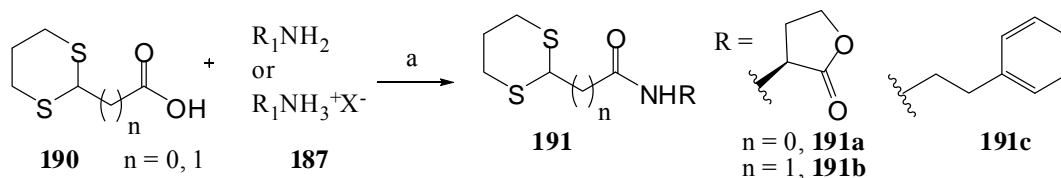
**Fig. (56).** Rational design study for sulfated AHLs scaffold

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<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C to rt; (b) R<sub>2</sub>SH, Et<sub>3</sub>N, EtOH, rt; (c) Bromoacetyl bromide, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, -20 °C to rt then R<sub>2</sub>SH, Et<sub>3</sub>N; (d) (COCl)<sub>2</sub>, DMSO, CH<sub>2</sub>Cl<sub>2</sub>, -20 °C.

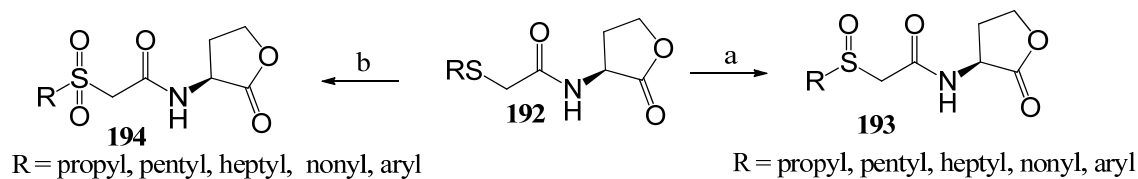
This series of compounds were screened in two QS systems designed from the *LuxR* (*V. fischeri*) and *LasR* (*P. aeruginosa*) systems. The highest activities were found for sulfated AHLs containing heptyl linkage, i.e. the optimal chain length was two carbon atoms shorter than for the natural ligand. Further, in the search for more potent pharmacophores, 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 the decrease in potency for these derivatives compared to parent compounds could be attributed to the presence of wrong linkers between this skeleton, or due to the absence of a double bond in modified analogues of dithiane.



**Fig. (58).** Reagents and condition: (a) isobutyl chloroformate, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 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).

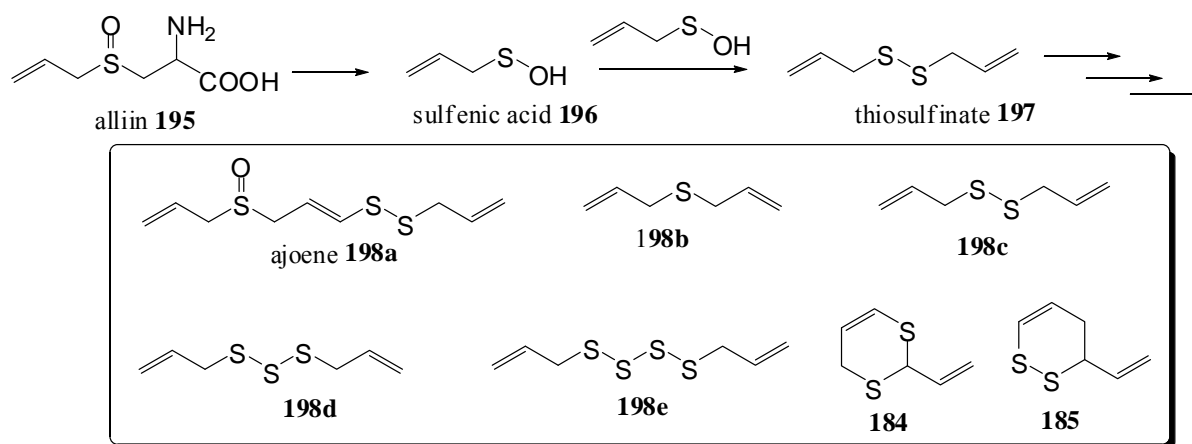


**Fig. (59).** Reagents and condition: (a) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, -10 °C; (b) *m*-CPBA, CH<sub>2</sub>Cl<sub>2</sub>, rt.

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*-(heptylsufanylacetyl)-L-homoserine lactone was identified as potent QS inhibitor in this class of sulfur compounds.

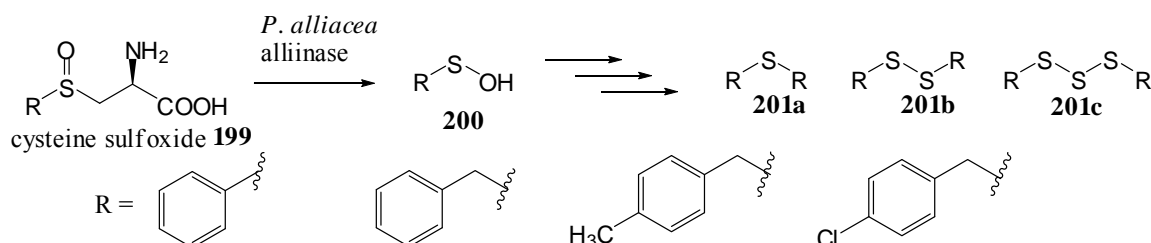
Musah and coworkers [69] has demonstrated the effects of several *S*-substituted cysteine sulfoxide and their corresponding disulfide derivatives on *P. aeruginosa* PA01 biofilm formation (Fig. (60)). As discussed earlier, the garlic derived natural products **184** & **185** found to inhibit *lasL/lasR*-based 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 sulfenic acid **196**, where in two molecules condense to give thiosulfinate **197**. These thiosulfinate further react to yield a variety of organosulfur compounds **198a-e**, **184**, **185** from garlic (Fig. (60)).

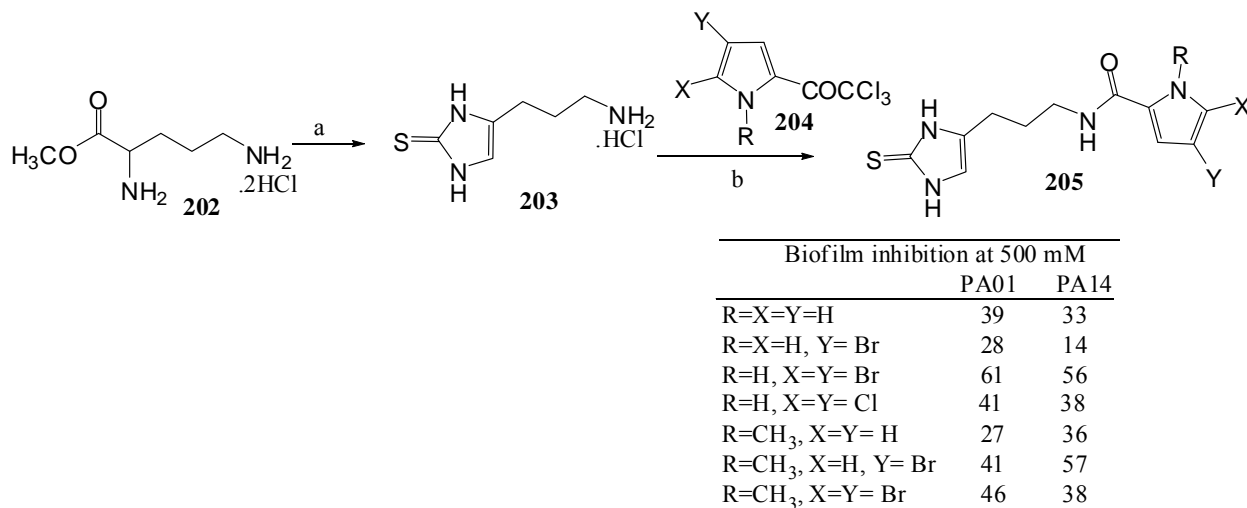


**Fig. (61).** Alliinase mediated cleavage of cystein sulfoxides

The *P. 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  $\mu\text{M}$ . Also diphenyl disulfide **201b** retained significant inhibitory activity at 0.1  $\mu\text{M}$ .

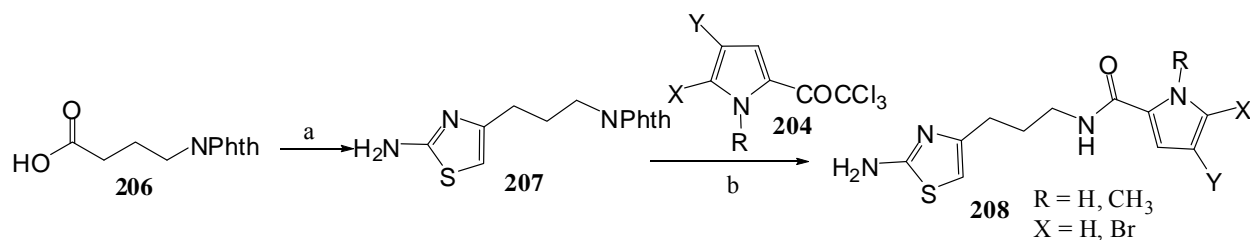
Melander and coworkers introduced detail SAR studies of oroidin alkaloid containing biofilm inhibitors and assayed this oroidin library against *P. aeruginosa* biofilms [55]. One such modification involves the replacement of 2-AI of oroidin with 2-thioimidazole and 2-aminothiazole (2-AT). The synthesis commenced with ornithine methyl ester **202** which on Akabori reduction, followed by cyclization with

KSCN under pH controlled condition gave 2-thioimidazole **203**. Subsequently, acylation of terminal amine with 4,5-substituted(trichloroacetyl) pyrrole (NH & NCH<sub>3</sub> 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  $\mu$ M as shown in (Fig. (62)).



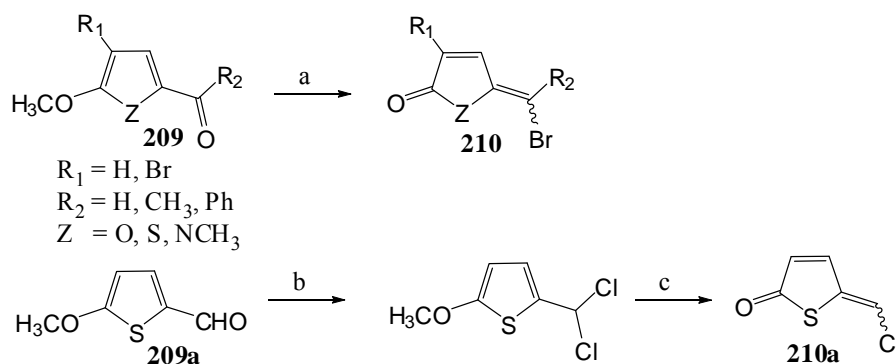
**Fig. (62).** Reagents and condition: (a) 1) Na/Hg, H<sub>2</sub>O, 5 °C, pH 1.5, 2) KSCN, H<sub>2</sub>O, 95 °C, pH 4.3; (b) Na<sub>2</sub>CO<sub>3</sub>, DMF, rt, pyrrole **204**.

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  $\alpha$ -bromoketone. Treatment of  $\alpha$ -bromoketone with thiourea under neutral condition gave 2-aminothiazole (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.



**Fig. (63).** *Reagents and condition:* (a) 1) (COCl)<sub>2</sub>, DMF (cat.), CH<sub>2</sub>Cl<sub>2</sub>, 2) CH<sub>2</sub>N<sub>2</sub>, Et<sub>2</sub>O/CH<sub>2</sub>Cl<sub>2</sub>, 0 °C, 3) conc. HBr, 4) Thiourea, DMF, 0-25 °C; (b) 1) N<sub>2</sub>H<sub>4</sub>, MeOH, 25-55 °C, 2) K<sub>2</sub>CO<sub>3</sub>, 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)).

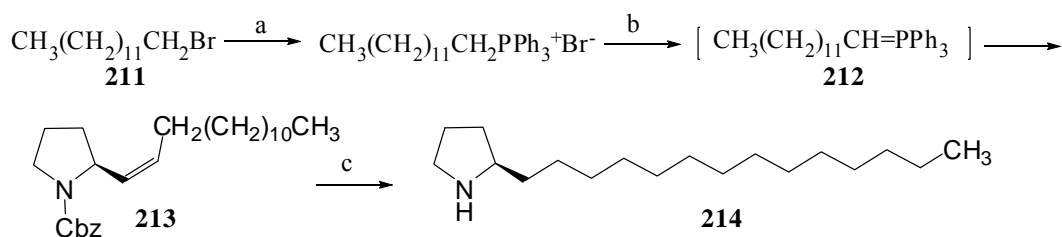


**Fig. (64).** *Reagents and condition:* (a) oxalyl bromide, CH<sub>2</sub>Cl<sub>2</sub>, rt; (b) oxalyl chloride, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C; (c) CH<sub>2</sub>Cl<sub>2</sub>, rt.

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

### Miscellaneous:

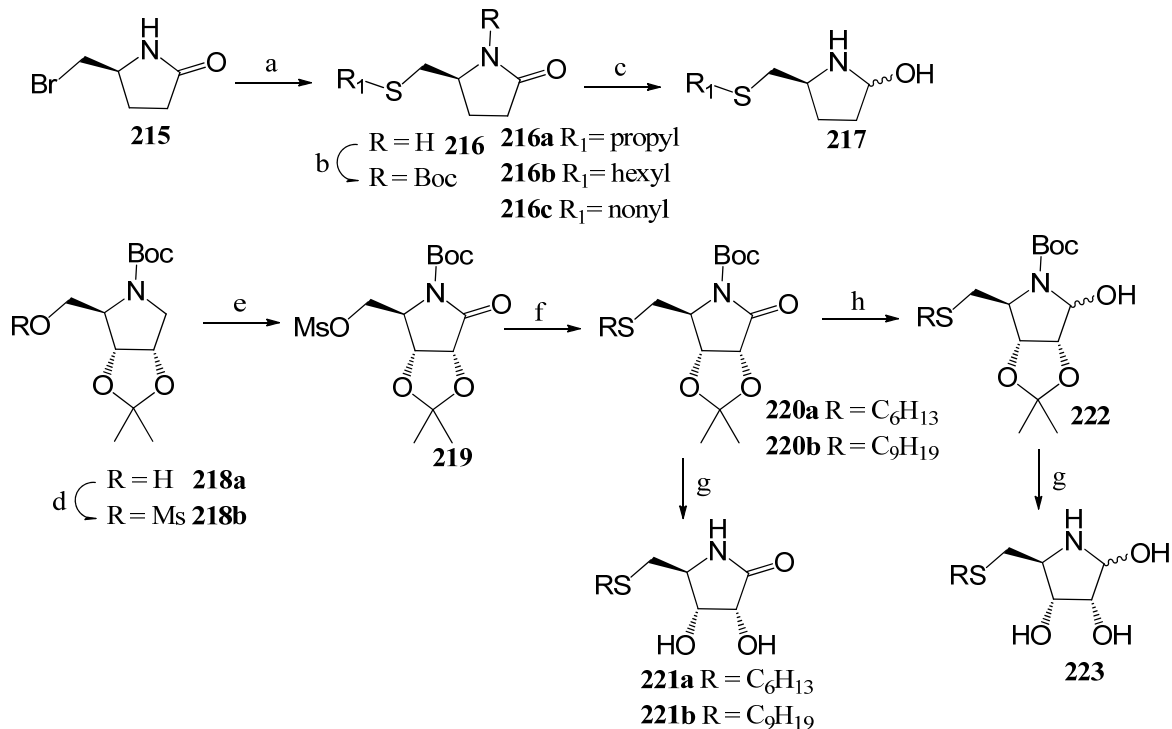
The development of non native (AHL) ligands has emerged as a promising strategy to inhibit biofilm formation of the different bacterial strains. Recently, our group [73] introduced pyrrolidine based skeleton as a new class of biofilm inhibitor inspired from natural product (*R*)-bgugaine (Fig. (65)). The first step involves preparation of *N*-carbobenzyloxy prolinal from proline. Phosphorane **212** of tridecyl bromide **211** was prepared *insitu* and reacted with *N*-protected prolinal to give olefin **213**. Finally, the reduction of double bond and deprotection gave norbgugaine **214**.



**Fig. (65).** Reagents and condition: (a) PPh<sub>3</sub>, toluene, reflux, 24 h; (b) *n*-BuLi, Et<sub>2</sub>O, *N*-carbobenzyloxy prolinal; (c) H<sub>2</sub>, Pd/C, EtOH, rt.

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  $\gamma$ -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-pyrroglutamic 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.

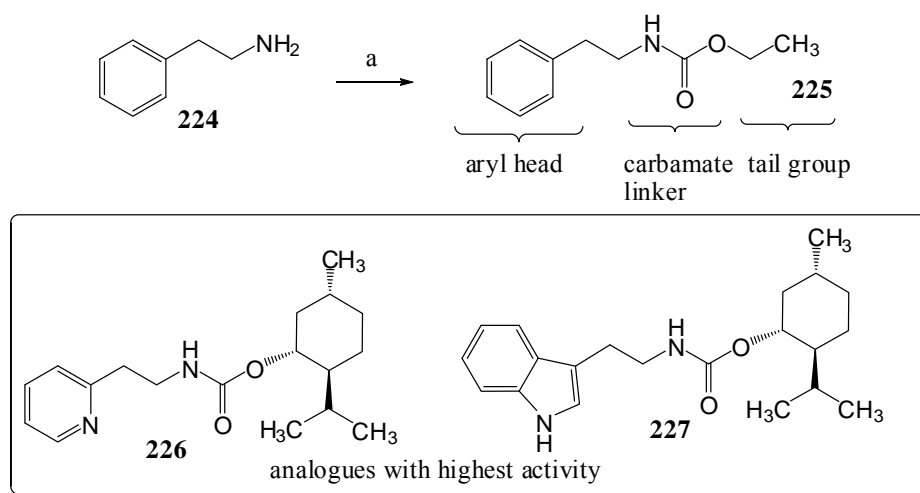


**Fig. (66).** Reagents and condition: (a)  $R_1\text{SH}$ , NaH, DMF; (b)  $(\text{Boc})_2\text{O}$ , DMAP,  $\text{CH}_2\text{Cl}_2$ ; (c) 1)  $\text{LiEt}_3\text{BH}$ , THF,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ , 2) TFA, rt; (d)  $\text{MsCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , rt; (e)  $\text{NaIO}_4$ , hydrated  $\text{RuO}_2$ , EtOAc,  $\text{H}_2\text{O}$ , rt; (f)  $\text{RSH}$ , NaH, DMF; (g) TFA,  $\text{H}_2\text{O}$ ; (h)  $\text{LiEt}_3\text{BH}$ , THF,  $\text{CH}_2\text{Cl}_2$ ,  $-78^\circ\text{C}$ .

The reduction of *N*-Boc protected lactam afforded azahemiacetals which on subsequent deprotection with TFA gave (5*S*)-(alkylthiomethyl)pyrrolidin-2-one **217**. With the intension 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- $\gamma$ -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 (5*S*)-(hexyl or nonyl thiomethyl)-3,4-dihydroxypyrrolidin-2-one **221a-b**. Next modification involves the reduction of 5-alkylthiomethyl lactams with  $\text{LiEt}_3\text{BH}$  to give cyclic azahemiacetals **222**. Finally, deprotection with TFA provided (5*S*)-(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 **216a-c** does not modulate *rhl* activity while hexylthio lactam **216b** significantly modulate *rhl* QS activities at higher concentration. Overall, the cyclic azahemiacetal (**217**, **223**) with shorter propylthio or hexylthio substituent was found to strongly inhibit both *las* and *rhl* signaling at higher concentration, while the propylthio analogues strongly stimulated the *las* QS system at lower concentration.

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

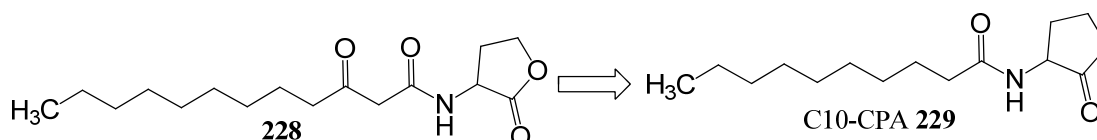


**Fig. (67).** Reagents and condition: (a) EtCOOCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 0 °C to rt,

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 (-)-menthyl, benzyl, *t*-butyl, and

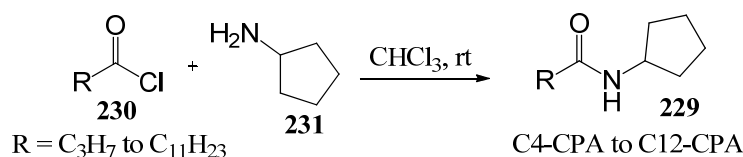
cholesteryl group. The most potent biofilm formation inhibitors (**226** & **227**) of *S. aureus* are shown in Fig (67).

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)).



**Fig. (68).** Rational approach for *N*-acyl cyclopentylamide from AHLs.

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**.



**Fig. (69).** Synthesis of *N*-acyl cyclopentylamide analogues

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.

## Acknowledgments

The authors thank the Director, CSIR-National Institute of Oceanography for constant encouragement. Financial assistance provided by the OCEAN FINDER and EU-FP7-KBBE-2009-3-245137 MAREX is highly acknowledged. Authors are grateful to CSIR-NIO for the award of Scientist Fellow-QHS.

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