AHL bioisosteres as a strategy for minimizing or eliminating the virulence of gram-negative bacteria

Alicia Reyes-Arellano1,*, Alejandro Bucio-Cano1, Héctor Salgado-Zamora1 and Everardo Curiel-Quesada2

1 Departamento de Química Orgánica, 2 Departamento de Bioquímica, Escuela Nacional de Ciencias Biológicas, IPN. Carpio y Plan de Ayala S/N, Colonia Santo Tomás, 11340, México. D. F. México.
*Corresponding autor: e-mail: areyesarellano@yahoo.com.mx

All biological phenomena can be observed, understood and treated as chemical processes. Since the fundamental constitution of living beings is molecular, chemical and biological phenomena are closely related. A biological phenomenon that has been the focus of recent research is the bacterial cell-to-cell communication known as quorum sensing. This communication is carried out through small easily diffusible molecules, such as acyl homoserine lactones (AHLs), in Gram-negative bacteria. These small molecules are capable of sensing the density of the bacterial population. Apparently as a result of evolution and in an attempt to avoid alerting the host immune system of their presence, pathogenic microorganisms delay the production of virulence factors until the density of the bacterial population is sufficiently high to assure a successful infection.

Research into quorum sensing has gained importance due to the increasing resistance of bacteria to antibiotics. Through the process of natural selection and evolution, bacteria have developed resistance strategies against the majority of antibiotics used today. These drugs are natural products or derivatives of them, therefore bacteria have interacted for a long time with molecules of similar structures; this prolonged contact as well as the indiscriminate use of antibiotics have had the tendency to select for resistant bacterial strains, leading to a serious public health problem that is especially grave for immunosuppressed and hospitalized patients.

Hence, quorum sensing inhibitors hold promise as therapeutic strategies to minimize or eradicating the virulence of pathogenic microorganisms. One of the research methods used to find agents capable of disrupting this chemical communication (quorum sensing) in pathogens is based on bioisosterism. Accordingly, small organic molecules have been designed based on the structure of AHLs. The current contribution is a mini-review of the main bioisosteres synthesized to date, and their capacity of attenuating or eliminating the virulence of Gram-negative bacteria.

Keywords AHL, bioisosteres, quorum sensing, Gram-negative bacteria

1. General remarks

Quorum sensing (QS), the focus of intense research around the world, is a form of cell-to-cell communication by bacteria that senses the population density of these microorganisms in a given microenvironment mediated by acyl homoserine lactone in Gram-negative bacteria (AHL) [1]. This communication mediates the expression by bacteria of genes that are related to distinct phenomena, including virulence factors. Apparently as a result of evolution and in an attempt to avoid alerting the host immune system of their presence, pathogenic microorganisms delay the production of virulence factors until the density of the bacterial population is sufficiently high to assure a successful infection.

The general thrust of recent research on quorum sensing is to identify natural and synthetic molecules that can act as antagonists, which may contribute to overcome the bacterial resistance or agonists. The dependence of bacteria on quorum sensing has made these signaling systems an attractive target for the design of new therapeutic agents. Thus, the identification of different classes of autoinducers is important, as it is an understanding of their interaction with different receptors of QS systems. Within this context, related topics include affinity to bacterial protein receptors, the structural requirements of synthetic structures, and the mechanisms of action of inducer molecules (semiochemicals) [2] in Gram-negative bacteria.

Some methods for rapid screening for potential quorum sensing inhibitors (QSIs) compounds have been reported [3-4]. In addition several strategies do exist to synthesize novel compounds with optimized biological activity such as the bioisosterism [5, 6] which is a rational approach in medicinal chemistry for drug design that begins with a lead compound [7] and searches among its derivatives for the maximum positive pharmacological effect with the minimum side effects. Several excellent reviews on the subject of QS are available, but they treat it from a biochemical viewpoint. In the current contribution we focus on the bioisosteric approach used to create or synthesize alternative molecules useful as inhibitors of QSIs.

Great interest is now being given to the development of quorum sensing inhibitors because of the increasing resistance of bacteria to antibiotics. Even newly developed antibiotics often meet successful bacterial resistance strategies within a relatively short time after their introduction to clinical practice. The resistance strategies of bacteria are various, and have been classified [8] as: a) the overexpression of enzymes, b) the mutation of the bacterial target site, c) the export of antibiotic drugs to the extracellular media, or d) the loss of the porin channel. Among these
bacterial strategies, the first modifies the drug and therefore inactivates it, while the second avoid effective drug-receptor interaction. The third and fourth strategies minimize or avoid drug bioavailability.

The development of bacterial resistance to antibiotics has become a major health issue with multifactorial causes, including the extensive and indiscriminate use of antibiotics, auto-medication, and an increased use of indwelling medical devices [9]. The latter, affecting mainly hospitalized and immunosuppressed patients, provide a propitious environment for the growth of biofilms [10, 11], which are both tenacious and highly resistant to antimicrobial treatment.

Hence, it is necessary to employ new strategies to combat bacterial proliferation, and one option consists of interrupting the pathway of bacterial communication [8, 12, 13]. It is known that this communication might be interrupted in three ways: a) blockading the AHL synthesis, b) destroying AHL and c) avoiding the interaction AHL-receptor in order to attenuate or eliminate the virulence of Gram-negative bacteria [14,15]. Compounds aimed at interrupting quorum sensing through the last way, have been synthesized and could be classified on the basis of bioisosterism. [5, 6]

2. Quorum sensing and acyl homoserine lactones

Some bacteria utilize AHLs as a device to communicate, a chemical compound which contain a chiral center in the \( \alpha \) C of a lactone ring with an S configuration. The number of C atoms, vary from 4 to 18 in the side chain of the acyl group, depending on the bacterial strain. This chain is found to be substituted at the \( \beta \) position by OH or C=O. [16, 17, 18]

Since AHLs are of low polarity, they flow freely across both internal and external membranes. At pH 5-8, these molecules are highly stable. [19]

![Fig. 1 General structure of acyl homoserine lactones](image)

In the search for new QSIs to eliminate or minimize the pathogenicity of Gram-negative bacteria, AHLs have provided the lead compound in the studies involving punctual or diverse modifications to AHLs, or the design of completely new molecules. Most of the compounds synthesized so far can be considered bioisosteric replacements. Table 1 summarizes the substitutions carried out on AHLs in accordance with the following classification:

1) Classical bioisosteres
   a) Monovalent bioisosteres.
   b) Divalent bioisosteres: \( \text{CH}_2 \text{vs} -\text{S}- \), involved in the acyl chain
   c) Divalent bioisosteres : \( \text{C} = \text{O} \text{vs} \text{S} = \text{O} \), involved in the acyl chain.
   d) Ring equivalent bioisosteres:
      i) Lactone vs thiolactone replacements.
      ii) Lactone vs cyclopentanone replacements.
      iii) Lactone vs cyclopentanol, cyclopentanone and methoxy-cyclopentane replacements.
      iv) Lactone vs pyrrolidine replacements.

2) Non-classical bioisosteres
   a) Non-cyclic vs cyclic bioisosteric replacements.
      i) Replacement of \( \text{CH}_3 \) or some part of the chain with aryl group.
      ii) Replacement of the whole chain with aryl group.
   b) Non-classical bioisosteric replacement of functional groups.
      i) Replacement of \( \text{C} = \text{O} \text{vs} \text{O} = \text{S} = \text{O} \), involved in the acyl chain.
      ii) Replacement of the amide group with -O-, involved the acyl chain.
      iii) Replacement lactone vs other heterocycles: imidazoline and tetrazole.
   c) Retroisosteres
### Table 1 Bioisosteric replacements carried out on AHL.

<table>
<thead>
<tr>
<th>Bioisoster type</th>
<th>Bioisoster type</th>
<th>Bioisoster groups exchange</th>
<th>Compound/ Evaluated on</th>
<th>Moiety involved</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classic bioisosteres</strong></td>
<td><strong>Monovalent</strong></td>
<td>H vs Br</td>
<td>---</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H vs OH</td>
<td>---</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H vs COOH</td>
<td>---</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H vs COOEt</td>
<td>---</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H vs F</td>
<td>---</td>
<td>---</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><strong>Divalent</strong></td>
<td>CH$_2$ vs S</td>
<td>8 / V. fischeri; 8-10 / V. ruckeri</td>
<td>Acyl chain</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C=O vs S=O</td>
<td>11 / LuxR and LasR</td>
<td>Lactone ring</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td><strong>Equivalent rings</strong></td>
<td>Lactone vs thiolactone (CH$_2$ vs S)</td>
<td>12-14 / C. violaceum; 15-16, 18-26 / V. fischeri; 15, 17, 20, 21, 23 / E. coli LasR</td>
<td>Lactone ring</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactone vs cyclopentane</td>
<td>27 / P. aeruginosa; 28 / S. marcescens; 29-31 / V. fischeri</td>
<td>Lactone ring</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactone vs cyclopentanone, cyclopentanol, methoxycyclopentane</td>
<td>32, 33 / P. aeruginosa; 34, 35 / P. aeruginosa y S. marcescens</td>
<td>Lactone ring</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lactone vs pyrrolidine</td>
<td>36, 37 / P. gingivalis</td>
<td>Lactone ring</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td><strong>Non-cyclic vs cyclic</strong></td>
<td>CH$_3$ or part of the chain vs aryl group</td>
<td>38, 39 / P. aeruginosa and A. tumefaciens; 40-44 / A. baumannii; 45, 46 / P. aeruginosa; 47 / C. violaceum</td>
<td>Acyl chain</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole chain with aryl group</td>
<td>48-51 / A. tumefaciens and P. aeruginosa; 52-54 / P. aeruginosa</td>
<td>Acyl chain</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td><strong>Non-classical bioisosteres</strong></td>
<td>C=O vs O=S=O</td>
<td>55-57 / V. fischeri; 58-60 / P. aeruginosa</td>
<td>Acyl chain</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^3$C=ONH vs –O- Replacement of functional groups</td>
<td>61-64 / C. violaceum and S. marcescens</td>
<td>Acyl chain</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$^4$Lactone vs imidazoline</td>
<td>65, 66 / C. violaceum and S. marcescens</td>
<td>Acyl chain</td>
<td>37, 38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tetrazole vs C=ONH</td>
<td>67, 68 / V. fischeri</td>
<td>Acyl chain</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C=ONH vs NHSO$_2$</td>
<td>69, 70 / V. fischeri</td>
<td>Acyl chain</td>
<td>40</td>
</tr>
</tbody>
</table>

*a The phenyl ring was placed as a connector between the imidazoline and the acyl chain.

*b The phenyl ring was placed as a connector between the imidazoline and the alkoxy chain.
3. Selected bioisosteres replacements and impact as QS inhibitors

3.1. Classical bioisosteres

3.1.1. Monovalent bioisosteres

Monovalent AHL bioisosteres do exist, nevertheless most of them have not been used as antiquorum sensing molecules, but for other purposes. Thus, SAR studies were conducted to compare the immune modulatory activity for a range of bioisosteres of *Pseudomonas aeruginosa* AHL, N-(3-oxododecanoyl)-L-homoserine lactone (3O, C12-HSL, 1). The structural segments modified in these studies were the acyl chain, the oxoacyl and the hetero ring. From the IC_{50} values obtained with a murine splenocyte proliferation assay, it is apparent that acylated L-homoserine lactones with an 11-13 C side chain, either containing a 3-oxo, or a 3-hydroxy group, 6, are optimal structures for immune suppressive activity. The monovalent bioisosteric replacement in the acyl chain of H (with Br, 2 or OH, 3) at terminal CH\text{3} gave good inhibitory activity. The best inhibitor in the series was bioisoster bromo derivative 2 (at 3 \(\mu\)M). The replacement of H in the \(\omega\) position with –COOH, 4, led to a loss of activity, while replacement with COOEt, 5, gave moderate activity. In this study, structures lacking the homoserine lactone ring or the L-configuration at the chiral center were essentially devoid of activity [20].

Fluor has been an excellent bioisoster of H, such as in the extraordinary anticancer drug fluorouracil. It is therefore worth mentioning that replacement of H with F at the \(\alpha\)-position of the carbonyl in the oxo dodecyl acyl homoserine lactone gave 3-oxo-4,4-difluoro-C12-HSL, 7, which turned out to be an excellent biofilm sensitizer of *Pseudomonas aeruginosa*. Originally compound 7 was considered as a high agonist of LasR [21] however Davies and Costerton recently filed a patent on biofilm regulators that includes this compound [22].

3.1.2. Divalent bioisosteres: CH\(_2\) vs -S-, involved in the acyl chain.

Compound 8 is a typical divalent bioisoster, where replacement of a methylene group by a sulfur atom was carried out. N-(heptylsulfanylacetyl)-L-homoserine lactone 8 was used against *Vibrio fischeri* QS bacteria, a blocking of the expression of LuxR and LasR QS reporters was obtained. Sulfur appears at the \(\beta\) position, which was previously occupied by either OH or C=O (i.e., it is in this position that changes were made with respect to the natural AHLs) [23].

In contrast this compound (8), N-pentylsulfanylacetyl (9) and N-propyl sulfanylacetyl (10) did not inhibit protease production on *Yersinia ruckeri*, the authors argued that the protease production may not be QS regulated in *Y. ruckeri* [24].
3.1.3. Divalent bioisosteres: C=O vs S=O, involved in the acyl chain.

_Divalent replacement involving a double bond._ Compound 11 is a typical divalent bioisoster using a double bond in substitution of the β CO of β-oxo DHL. A library of compounds has been synthesized by Persson _et al_, [23] which were inspired on sulphur compounds isolated from garlic and known to be active QS inhibitors. Derivatives containing a sulphone group in the chain, for instance 11, were active against LuxR and LasR.

![Image](image1)

3.1.4. Ring equivalent bioisosteres

3.1.4.1. Divalent bioisosteres involving the lactone ring. Lactone vs thiolactone replacements.

Interestingly, the replacement of -O- by -S- in the lactone ring did not have any effect on QS inhibition in *Chromobacterium violaceum* CV026. Thus, _N_-butanoyl-L-homocysteine thiolactone, 12, _N_-hexanoyl-L-homocysteine thiolactone, 13 and _N_-(3-oxohexanoyl)-homocysteine thiolactone, 14 were unable to either induce or antagonize _N_-(hexanoy1)-L-homoserine lactone, HHL [25].

![Image](image2)

In another study, a library of AHL thiolactone bioisosteres was synthesized and evaluated for both antagonistic and agonistic activity in three relevant LuxR-type receptors (LasR, LuxR and TraR) using cell-based reporter gene assays in *Pseudomonas aeruginosa*, *Vibrio fischeri* and *Agrobacterium tumefaciens*. Although the screening data provided a complex set of activity profiles for these compounds (most notably in LasR), several new and highly active QS modulators were discovered, many with multireceptor activity. Compounds 15-16 and 18-26 all had nanomolar IC₅₀ values in *V. fischeri*, and are among the most potent LuxR antagonists reported to date. OOHHL thiolactone bioisoster 16 is also a strong LuxR antagonist, and at the same time is the strongest synthetic TraR agonist yet reported in *V. fischeri*. In terms of LasR, thiolactones 20, 21 and 23 displayed nanomolar IC₅₀ values in the *E. coli* LasR reporter. The thiolactone bioisosteres of both OdDHL and its non-3-oxo HL bioisoster (15 and 22) are strong LasR agonists in the *E. coli* system. However, the trend does not always hold true, as was found that the OHHL thiolactone analog with a six-carbon shorter acyl tail, 17 had similar LasR agonistic activity as 15. [26] In previous studies authors used compounds with the stereochemistry of the native AHL.

![Image](image3)

3.1.4.2. Lactone with cyclopentane

Ishida _et al_ [27], studied the replacement of the lactone ring with cyclopentane. It was found that _N_-cyclopentyldecanamide, 27 was the most effective inhibitor of the LasR and RhlR _quorum sensing_ systems in *P. aeruginosa*. Derivative 28 was the most effective inhibitor in *Serratia marcescens*, and compounds 29-31 were most effective in *Vibrio fischeri*. 

![Image](image4)
3.1.4.3. Lactone with cyclopentanone, cyclopentanol and methoxycyclopentane.

Derivatives 32-35 represent the bioisosteric replacement of equivalent rings. Several compounds with no sensibility to hydrolysis were synthesized by Suga and his coworkers and were tested on *P. aeruginosa*. It was found, compounds 32-33 showed agonist activity. [28] In another study cis and trans-2-methoxycyclopentyl butyramides 34-35 were prepared and evaluated on *P. aeruginosa* and *Serratia marcescens*, both compounds showed agonist activity. [29]

![Chemical structures](image)

3.1.4.4. Lactone with pyrrolidine

An interesting bioisosteric replacement was made by Noiri and his coworkers, they replaced the lactone ring with pyrrolidine and compounds were tested as inhibitors on *Porphyromonas gingivalis*. It was observed that compound 36 did not have inhibitory activity, while derivative 37 significantly reduced the number of biofilm-forming cells during *P. gingivalis* biofilm formation. [30]

![Chemical structures](image)

3.2. Non-classical bioisosteres

3.2.1. Bioisosteric replacement non-cyclic vs cyclic.

3.2.1.1. Replacement of CH₃ or some part of the chain with aryl group.

Several examples may be grouped in this type of replacement, for instance a library of both natural and synthetic AHLs have been obtained in which the main structural alterations consisted of replacing the methyl group or part of the chain with an aryl group. These compounds were screened in *P. aeruginosa* and *A. tumefaciens*, two well-characterized bacterial reporter strains for antagonism of quorum sensing. Among the various synthetic AHL derivatives, 38 and 39 were found to be inhibitors of bacterial quorum sensing, it is notable that the latter displayed 50% inhibition at 100 nM. These two ligands were identified as antagonists in *P. aeruginosa* and strongly inhibit *P. aeruginosa* biofilm formation. [31]

Other molecular structures with similar bioisosteric replacement, were assayed by Blackwell *et al*. [32] Thus, a series of compounds was evaluated on *A. baumanii* and it was found that compounds 40-44 reduced the biofilm expression in this bacteria moreover they inhibited film formation and are the most active inhibitors of the series. It is convenient to mention that the 4-bromophenyl 39 was one of the most active inhibitors in *P. aeruginosa*, however derivative 3-iodophenyl 42 gave remarkable inhibition in *A. baumanii*. [32]

Compounds 45 and 46 showed inhibitory activity in *P. aeruginosa* and both compounds were tested as inhibitors cancer cell growth. [33] Comparative SAR analysis demonstrates that both anticancer and QS signaling systems require long acyl side chains with a 3-oxo substitution for maximum activity. In addition 2-(4’-chlorophenoxy)-N-butanoyl homoserine lactone, 47 has been reported as an excellent *C. violacein* inhibitor. [34] The terminal methyl replacement by a phenyl substituent offers a pool for more isosteric variations that could enhance both antineoplastic as well as anti-QS activities.
Replacement of the whole chain with aryl group

Replacement of the alkyl moiety by an aryl group leaving the amido functionality, can take place to deliver non classical bioisosteres. Compounds in this group include aroyl HL, where aryl may be pyrazinyl, pyridyl and phenyl. Compounds 48-51 showed inhibitory activities on quorum sensing in A. tumefaciens, and inhibit biofilm formation on these bacteria as well as on P. aeruginosa [35]. At this point it is convenient to recall that the semiochemical compounds of A. tumefaciens and P. aeruginosa differ only in the number of C atoms present in the chain, eight in the autoinducer of A. tumefaciens and twelve in the autoinducer of P. aeruginosa. This fact has been taken as remarkable and we considered that it might be explained in terms of the whole supramolecular interaction between the protein and the ligand molecule. Docking studies have shown that these types of interactions of AHLs, or synthetic inhibitors with the protein are determinant. Likewise, other molecules with a completely different structure compared to the native AHL and having inhibitory activity may be explained on similar grounds.

In contrast, compounds 52-54, with bioisosteric replacement by other aryl groups, did not show activity against P. aeruginosa. [21] The reason appears to be steric impediment, due to the ortho substitution on all compounds that prevents a proper interaction with the receptor site.

3.2.2. Non-classical bioisosteric replacement of functional groups.

3.2.2.1. Replacement of C=O vs O=S=O, involved in the acyl chain.

Sulphone (O=S=O) has been used as a replacement for the carbonyl functional group, for instance, Castang and coworkers synthesized a series of 11 N-sulphonyl homoserine lactones, some of which (55-57) were found to competitively inhibit the action of 3-oxohexanoyl-L-homoserine lactone, the AHL inducer of bioluminescence in the bacterium Vibrio fischeri. Molecular modeling suggests a possible explanation based on the prevention of structural rearrangements necessary for the formation of the active dimer of LuxR. [36]
In another study the carbonyl group has been replaced with a sulphonyl group to give compounds 58-60, the lead compound being 3-oxo-C8-HSL. These three compounds showed inhibitory activity in A. tumefaciens and P. aeruginosa. [35]. The results confirm that the interaction is not atom-by-atom, but instead a total supramolecular interaction.

![Chemical structures](image)

3.2.2.2. Replacement of the amido group with $-\text{O}-$, involved the acyl chain.

A non-classical bioisoster with two replacements: a) amide with ether group and simultaneously b) lactone with imidazoline was made. Furthermore a connector between both moieties was placed to give molecules 61-64, which were tested on C. violaceum. Molecules 61-62 resulted violacein inhibitors. It is important to notice the electronic effect of the alkoxy group at the para position resulted in biological activity, whereas at the meta position (no conjugated) the activity was suppressed, derivatives 63 and 64 were inactive. [37]

![Chemical structures](image)

3.2.2.3. Replacement lactone vs imidazoline

Another non-classical bioisoster containing the imidazoline ring but keeping the N-acyl moiety was made. Thus, 2-substituted imidazolines 65-66 were synthesized; the side chains were hexanoyl and nonanoyl, moreover a connector was placed between the moieties. Compounds were evaluated as violacein production inhibitors in Chromobacterium violaceum, employing dose-response experiments. It was found that imidazolines 65 and 66 inhibited violacein production at different concentrations. Remarkably 66 was active at 1 nM concentration, suggesting an anti-quorum sensing profile against Gram-negative bacteria.[37] The docking analysis confirmed anti quorum sensing behaviors of the tested molecules. [38]

![Chemical structures](image)

3.2.2.4. Tetrazole as bioisosteric replacements for $-\text{C=ONH}$-

The tetrazole ring can mimic the amido group, as some researchers have found. Soulère et al have recently shown that the tetrazole ring is a good bioisoster of the AHL amido group. Tetrazoles 67 and 68 were evaluated in an E. coli biosensor strain containing a plasmid of Vibrio fischeri, whose main inductor is 3-oxo- C6-HSL. Derivative 67 was antagonist whereas derivative 68 was inactive. Bioluminescent inhibition was found at 200 nM. [39]
3.2.3. Retroisosteres

Sulphoxide has been used by Soulère et al. as a retrobioisoster in some sulfonamides. Sulfonamide 69 and 70 showed QS inhibition in *Vibrio fischeri* with IC$_{50}$ >200 and 70 μM, respectively. Docking studies of 69 with a LuxR model showed the positive interaction between sulfonamides and residues Trp66, Try62, Asp79. Hydrogen bond distances in the key interactions involving S=O are not significantly different from those in the interactions with C=O [40].

Once again, it is clear that the recognition between natural or synthetic AHLs and the receptor is based on the supramolecular rather than atom-by-atom interaction [2, 41]. That is, in the total interaction at the binding site, a very important factor is whether the host and guest (protein-molecule) are complementary, based on topology, size, shape, conformation, and electronic properties. The latter include H donor and H acceptor properties at the active site of the protein where natural AHL or AHL bioisosteres bind.

\[
\begin{array}{c}
R = \text{C}_4\text{H}_{13} \\
R = \text{C}_5\text{H}_{11}
\end{array}
\]

4. Docking studies in the search for bioisosteric inhibitors of AHL

Molecular modeling, by simulating the binding of a ligand with the receptor site of a target macromolecule, allows for the identification of possibly relevant amino acid residues in the binding site, as well as the conformations of the receptors that favor the union. [42]

In the search for QS inhibitors, the docking analysis is basically used in two ways to explore the binding mode of ligands with proven QS inhibitory activity, and to screen new and yet untested compounds. Olson et al. conducted an experimental screening of 200,000 compounds in *Pseudomonas aeruginosa*, where active compounds were found that apparently do not bear any relevant structural resemblance to AHL.[4] However the docking analysis of such compounds and protein LasR of *Pseudomonas aeruginosa* suggested that the activity of ligands such as TP-1 is due to a conformational similarity with the octanoyl 3-oxo homoserine lactone. [43] Similarly, Vivas-Reyes et al. docked a series of QS inhibitors, analogues of AHL, in bacteria *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*. [44] The docking analyses on proteins LasR and TraR showed that ligands with affinity share the same binding site. In addition, the amino acids that make up the binding site in the R isof orm are preserved in TraR.[45] Inhibition of QS activity in *Chromobacterium violaceum* by a series of 2-imidazolines has been reported.[37] The corresponding docking analysis of these molecules and of the N-hexanoyl lactone performed on the CviR protein [38] showed that the phenyl and imidazole rings are essential for the anti QS activity. Figure 2 shows π-π interactions (among others) of amino acids residues Trp111 and Tyr88 with the phenyl group of 61.

![Fig. 2 Interactions of 61 with protein CviR.](image-url)
A docking analysis of a series of N-acyl-3-amino-5H-furanones was performed to screen these compounds as possible inhibitors of the QS of *Vibrio Fischeri*. The LuxR protein from this bacterium was used and the results were compared with a docking carried out between the oxo homoserin lactone and the LuxR protein. Tolker-Nielsen et al conducted a virtual screening of a library of 147 compounds containing known drugs and natural products. The docking was carried out between the compounds and the LasR protein complexes of *Pseudomonas aeruginosa*, and compared with the complex formed between AHL-LasR. In subsequent experimental studies, a correlation was observed with the docking studies. [45]

5. May QS inhibitors become clinical drugs?

Today there is still skepticism about the clinical use of quorum sensing inhibitors, although their potential use has already been recognized [12]. In vitro studies exploring the effect of QSIs on bacterial pathogenicity have been encouraging, as it is known that QS controls the majority of toxin production by bacteria [46]. Thus, Müh et al showed that two bioisosteres of AHL, with the lactone ring substituted by a thiazol ring or a phenyl ring, inhibited production of two quorum-controlled extracellular virulence factors, pyocyanin and elastase, in wild-type *P. aeruginosa*. Inhibition of pyocyanin production was about 90% by the bioisoster with the phenyl ring, and about 40% by the bioisoster with the thiazol ring. On the other hand, inhibition of elastase production in this same bacterium was about 60% by the bioisoster with the phenyl ring, and about 20% by the bioisoster with the tetrazol ring. [4]

Another strategy to overcome the resistance of bacteria is the use of QSIs as adjuncts to therapy with antibiotics, giving a synergic effect with good results. Several *in vitro* studies have corroborated this use of QSIs as adjuncts [47, 48, 49]. For instance, Rogers et al reported that the 2-aminoimidazole/triazole conjugate resensitizes multidrug-resistant *Acinetobacter baumanii* [50].

Acknowledgment AR. gratefully acknowledges the financial support provided for this work by SECITI (Mexico D. F.) through Research Grant No. 325/2011, and by IPN through Research Grant: SIP 20131523.

References


© FORMATEX 2013


[34] Manuscript in preparation.


