N-Heterocycles Scaffolds as Quorum Sensing Inhibitors. Design, Synthesis, Biological and Docking Studies

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Abstract: Quorum sensing is a communication system among bacteria to sense the proper time to express their virulence factors. Quorum sensing inhibition is a therapeutic strategy to block bacterial mechanisms of virulence. The aim of this study was to synthesize and evaluate new bioisosteres of N-acyl homoserine lactones as Quorum sensing inhibitors in Chromobacterium violaceum CV026 by quantifying the specific production of violacein. Five series of compounds with different heterocyclic scaffolds were synthesized in good yields: thiazoles, 16a–c, thiazolines 17a–c, benzimidazoles 18a–c, pyridines 19a–c and imidazolines 32a–c. All 15 compounds showed activity as Quorum sensing inhibitors except 16a. Compounds 16b, 17a–c, 18a, 18c, 19c and 32b exhibited activity at concentrations of 10 μM and 100 μM, highlighting the activity of benzimidazole 18a (IC50 = 36.67 μM) and 32b (IC50 = 85.03 μM). Pyridine 19c displayed the best quorum sensing inhibition activity (IC50 = 9.66 μM). Molecular docking simulations were conducted for all test compounds on the Chromobacterium violaceum CviR protein to gain insight into the process of quorum sensing inhibition. The in-silico data reveal that all 15 the compounds have higher affinity for the protein than the native AHL ligand (1). A strong correlation was found between the theoretical and experimental results.

Keywords: heterocycles synthesis; new AHL bioisosteres; π–π interactions; quorum sensing

1. Introduction

Quorum sensing (QS), a mechanism of cell-to-cell communication in bacteria and fungi, involves self-produced chemical signals called autoinducers that function as semiochemicals [1]. Bacteria use this mechanism to communicate among themselves through the recognition and measurement of extracellular autoinducers, which accumulate in the local environment until reaching a certain level. At such a point, the bacterial population is sufficient to allow for group actions and therefore signaling pathways are activated and specific genes (some related to virulence factors) are transcribed [2–5].

Since the disruption of QS could plausibly attenuate or halt bacterial virulence and overcome bacterial resistance, it is an attractive target for drug discovery [6,7]. In Gram-negative bacteria the autoinducers for QS are N-acyl homoserine lactones (AHLs 1). They are synthesized and released to the extracellular medium, diffuse freely through the bacterial membrane, and bind to specific cytoplasmic receptors. Once reaching a certain level, AHLs 1 promote specific gene expression
An excellent model for this purpose is Chromobacterium violaceum to provide insights into the process of QS inhibition.

In hospital-acquired infections and their increasing resistance to numerous drugs [6,11]. Finally, molecular docking simulations were conducted for all 15 test compounds on C. violaceum benzimidazoles (each containing a distinct heterocyclic scaffold in its structure: thiazoles (16a–c), thiazolines (17a–c), benzimidazoles (18a–c), pyridines (19a–c) and imidazolines (32a–c) to be assayed experimentally. Finally, molecular docking simulations were conducted for all 15 test compounds on C. violaceum CviR protein to provide insights into the process of QS inhibition.

One of the most common strategies used to interfere with QS is the antagonism of AHL signaling by organic molecules [7]. Accordingly, researchers have developed bioisosteres of AHL (although they are not always designated as such), most of which are monovalent bioisosteres and act as antagonists [12,13]. Thus, the use of AHL as a lead compound has led to the development of a variety of QS inhibitors (QSIs) (Figure 1). The inhibition of QS has been achieved in Chromobacterium violaceum by a set of N-substituted homocysteine thiolactones (2–5) [14], in Pseudomonas aeruginosa by AHL bioisosteres based on indole (6–7) [15], in Vibrio fischeri by a N-(2-nitrophenyl)-amide scaffold (8–9) as an AHL bioisosteres [16], in C. violaceum and P. aeruginosa by a set of AHL bioisosteres (10–13) by modifying the acyl side chain [17], and in P. aeruginosa by a set of AHL bioisosteres based on the itaconimide scaffold (14–15) [18].

After synthesizing AHL bioisosteres, it is important to demonstrate their capacity to inhibit QS. An excellent model for this purpose is Chromobacterium violaceum due to it is a QS biosensor [19]. The aim of the present study was to synthesize new bioisosteres of AHL and evaluate them in C. violaceum CV026 as QSIs by quantifying the specific production of violacein. Five series of compounds were synthesized, each containing a distinct heterocyclic scaffold in its structure: thiazoles (16a–c), thiazolines (17a–c), benzimidazoles (18a–c), pyridines (19a–c) and imidazolines (32a–c) to be assayed experimentally. Finally, molecular docking simulations were conducted for all 15 test compounds on C. violaceum CviR protein to provide insights into the process of QS inhibition.

![Figure 1. AHL and some known bioisosteres.](image-url)

After synthesizing AHL bioisosteres, it is important to demonstrate their capacity to inhibit QS.
2. Results and Discussion

2.1. Bioisosteres Design

New non-classical bioisosteres [12] of AHL were designed by modifying hexanoyl homoserine lactone 1 (i.e., \( R_1 = H, R_2 = C_3H_7 \)), an autoinducer of Chromobacterium violaceum. Specifically, the lactone ring 1 was bioisostERICALLY replaced by heterocyclic rings of thiazoles (16a–c), thiazolines (17a–c), benzimidazoles (18a–c), pyridines (19a–c) and imidazolines (32a–c) (Figure 2). The lactone ring has been previously substituted by other heterocycles and even phenyl (Figure 1) [14–18]. Furthermore, there are reports of its replacement with cyclopentane, cyclopentanol, cyclopentanone, oxazolidinone, benzothiazol and thiadiazol [13]. Our group reported a substitution with imidazoline and oxazoline in prior studies [20,21]. In the design of the present bioisosteres, the amido group and the lateral chain were conserved but the length of the latter varied. Additionally, an aromatic ring was included in all the molecules to promote \( \pi-\pi \) interactions with the receptor. Thus, five series of compounds emerged (Figure 2).

**Figure 2.** AHL bioisosteres design presently.

Thiazoles 16a–c. Although compounds 16 contain two structural elements (an amido group and a lateral chain) similar to hexanoyl homoserine lactone 1, they are non-classical bioisosteres [12], formed by exchanging one functional group with another (i.e., lactone with pyridine). The ester group could have been removed, but it was left in place to observe the effect.

Thiazolines 17a–c. The phenyl ring in these compounds serves both as a linker and the aromatic factor required in the design.

Benzimidazoles 18a–c. The aim with this series was to combine a fragment-based QSI design [22] with a bioisosteric substitution of the lactone ring of AHL by a benzimidazole ring. Thioacetamide is a fragment in a compound reported to have good activity as a QSI [23], furthermore the thioacetamide group could be considered a retroisoster [12] of the amido group of AHL. In the case of 18a‒b, the lateral chain was replaced by a phenyl ring, which was 3-Cl-phenyl for 18a and 4-Cl-benzyl for 18b. The amido
group of 18c was substituted by a sulfur, and the nonyl chain was left in place since it has worked well in other bioisosteres [24].

Pyridines 19a–c. Although the pyridines contain two elements of AHL, they are regarded as non-classical bioisosteres because the pyridine ring is completely different from the lactone ring. Consequently, they are based on an exchange of one functional group for another [12].

2.2. Synthesis

2.2.1. Synthesis of 2-Acylamino-thiazole-4-carboxylic Acid Ethyl Esters (16a–c)

The synthesis of 16a–c began with the formation of amino thiazole 22, utilizing thiourea and ethyl bromopyruvate as raw materials (Scheme 1).

\[
\begin{align*}
\text{Br} & \quad \text{O} \\
\text{O} & \quad \text{N} \\
\text{H} & \quad \text{S} \\
\text{N} & \quad \text{NH}_2
\end{align*}
\]

Scheme 1. Synthetic route to obtain the N-(thiazol-2-yl)-amine (22). Reagents and conditions: a: EtOH, rt, 16 h.

To obtain 2-amino thiazol 22, Hantzsch synthesis was carried out with an α-halo carbonyl compound, α-bromo ethyl pyruvate (20) and thiourea as raw materials and EtOH as solvent according to the methodology published by Kaushik [25]. The reaction mixture was stirred for 16 h at room temperature (rt), furnishing the desired product in good yields. (Scheme 1.) Subsequently, intermediary 22 was acylated with the corresponding carboxylic acid, a reaction described by various authors [26–29]. Such a reaction occurs in 2-amine thiazole with electron with drawing groups in position 4, for which heterogeneous yields have been documented in the literature. For instance, low yields (17–47%) were provided when Parish performed the acylation reaction with acyl chlorides in the presence of bases [26]. The acylation reaction has also been carried out with carboxylic acids and coupling reagents CDI or EDCI, affording moderate to good yields (54–69%) [27]. Considering, this background, distinct conditions were herein investigated but two procedures were followed: MW energy, as reported by You [28], and DCC as a coupling reagent with conventional heating as the energy source [29] (Table 1).

| Experiment | R       | Thiazole | Procedure | Temperature (°C) | Yield (%) |
|------------|---------|----------|-----------|------------------|-----------|
| 1          | -C₃H₇  | 16a      | A         | 150              | 82        |
| 2          | -C₃H₇  | 16a      | B         | rt               | 12        |
| 3          | -C₅H₁₁ | 16b      | A         | 150              | 85        |
| 4          | -C₅H₁₁ | 16b      | B         | rt               | 20        |
| 5          | -C₇H₁₅ | 16c      | A         | 180              | 80        |

Conditions: (A) 2-amino thiazole (1 eq), carboxylic acid (2 mL), 90 min. (B) 2-amino thiazole (1 eq), DCC (1 eq), carboxylic acid (1 eq), CH₂Cl₂ (20 mL), N₂ atmosphere, 16 h.
Data in the literature [27] indicate that the acylation reaction of compounds like 22 depends on the coupling reagents, the solvent and the mixture of these two factors. Additionally, it is necessary to consider the low nucleophilicity of the amine group conjugated to an electron withdrawing group. When Toyoshima [30] carried out the synthesis of compounds 16, the yields were lower than those found presently. In the acylation reaction of an amine, the excess concentration of the acid is important due to autocatalysis [31]. Compounds 16 have been tested as antivirals and anti-tuberculosis drugs [30] but never as QSIs.

2.2.2. Synthesis of N-[4-(Thiazolin-2-yl)-phenyl]-amides (17a–c)

The synthetic pathway began with the reaction between 4-amino benzonitrile and cysteamine hydrochloride to prepare intermediate 25.

To obtain 25, 4-amine benzonitrile and cysteamine hydrochloride were reacted by using EtOH: H$_2$O 1:1 as solvent (Table 2).

![Table 2. Results obtained to achieve 4-(4′5′-dihydrothiazol-2-yl) aniline 25.](image)

| Experiment | Base (eq) | Time (h) | Temperature (°C) | Yield (%) |
|------------|-----------|----------|------------------|-----------|
| 1          | NaOH (0.2)| 2        | 80               | 38        |
| 2          | NaOH (0.2)| 16       | 80               | 40        |
| 3          | NaOH (1.5)| 16       | 100              | 17        |
| 4          | K$_2$CO$_3$ (5) | 4   | 110              | 61        |
| 5          | K$_2$CO$_3$ (5) | 16  | 110              | 82        |

Conditions: 4-amine benzonitrile, (1 eq), cysteamine hydrochloride (1.5 eq), EtOH/H$_2$O 1:1 (5 mL).

Cazin conditions [32] provided a low yield for 25 (Table 2, experiment 1), observing an incomplete reaction. Lengthening the reaction time (experiment 2) did not improve the outcome, nor did a greater amount of base (experiment 3), which caused the yield to drop sharply due to the hydrolysis of benzonitrile to carboxylic acid. The combination of a weaker base (K$_2$CO$_3$ instead of NaOH) and a higher reaction temperature, as described by Hintermann [33], gave better results (experiment 4). Since the raw material was detected in TLC, the reaction time was increased (experiment 5), leading to a high yield with the complete reaction of the raw material.

It is known that the transformation of benzonitrile to thiazoline was established in accordance with the computational studies by Mor and Cavalli [34], (Figure 3).

![Figure 3. Transformation of benzonitriles to 2-thiazolines.](image)
dichloride [36]. The methodology chosen, based on the acylation of thiazole 22 (Table 3), furnished the novel thiazolines 17a–c in good yields.

2.2.3. Synthesis of 6-Chloro-2-alkylsulfanyl-1H-benzimidazoles (18a–c)

The two-step synthetic procedure to obtain benzimidazoles 18a–c (Scheme 2) began with the reaction between carbon disulfide and 4-chloro-1,2-phenylenediamine 26 to afford 5-chloro-benzimidazole-2-thione 27 [37] (Table 4).

| Experiment | R         | Compound | Temperature (°C) | Yield (%) |
|------------|-----------|----------|------------------|-----------|
| 1          | -C3H7     | 17a      | 150              | 82        |
| 2          | -C5H11    | 17b      | 150              | 85        |
| 3          | -C7H15    | 17c      | 180              | 80        |

Conditions: 4-(thiazolin-2-yl)aniline (1 eq), carboxylic acid (2 mL), 90 min.

Scheme 2. Synthetic procedure to obtain the 6-chloro-2-alkylsulfanyl-1H-benzimidazoles (18a–c). Reagents and conditions: a: potassium hydroxide, EtOH/H2O (7:3), 80 °C, 5 h; b: EtOH, 60 °C, 16 h.

To prepare intermediary 27, variations in the conditions were based on previous reports [37]. (Table 4).
were synthesized with 2-bromo acetyl bromide and 3-Cl aniline in very good yields at 3 h of reaction. In our hands, however, the outcome was different, detecting the presence of benzimidazoles 18 after 3 h (experiment 1). A change to MW energy (experiment 2) was also unsuccessful. By using conventional energy and increasing the reaction time from 3 to 5 h (experiment 3), the total consumption of raw material was observed and thione 27 was produced in a higher yield. In accordance with the work of Sartori [38], the proposed reaction sequence is the formation of ethyl(2-amino-5-chlorophenyl)carbamothioate 29 followed by its conversion into isothiocyanonitrile 30 and finally into thione 27 (Scheme 3).

Experiment 1 was carried out under the conditions described by Micheva [37], who obtained very good yields at 3 h of reaction. In our hands, however, the outcome was different, detecting the presence of phenylenediamine after 3 h (experiment 1). A change to MW energy (experiment 2) was also unsuccessful. By using conventional energy and increasing the reaction time from 3 to 5 h (experiment 3), the total consumption of raw material was observed and thione 27 was produced in a higher yield. In accordance with the work of Sartori [38], the proposed reaction sequence is the formation of ethyl(2-amino-5-chlorophenyl)carbamothioate 29 followed by its conversion into isothiocyanonitrile 30 and finally into thione 27 (Scheme 3).

This intermediate 27 was then subjected to an acylation reaction [25] with the bromoacetamides 28a, 28b or alkylation with n-bromononane 28c, to obtain 18a–c, Scheme 3. Bromoacetamides 28a–b were synthesized with 2-bromo acetyl bromide and 3-Cl aniline 31 or 4-Cl benzylamine 32 (Scheme 4).

Finally 28 were reacted with 27 to achieve 18a–c. Scheme 2. There are reports of benzimidazole acetamides as antidiabetic drugs [39], antimicrobial agents [40], and QSI in P. aeruginosa [41]. However, benzimidazoles 18 are new compounds that open a broad range of opportunities for research into QSIs.

| Assay | Energy | Time (h) | Yield (%) |
|-------|--------|---------|-----------|
| 1     | Conventional | 3     | 52        |
| 2     | MW     | 3      | 24        |
| 3     | Conventional | 5     | 88        |

Reactions conditions: Phenylenediamine (1 eq), CS₂ (1.3 eq), KOH (3 eq), EtOH/H₂O 7:3 (5 mL), 80 °C.

**Table 4.** Synthesis of 5-chloro-1,3-dihydro-2H-benzo[d]imidazole-2-thione 27.
2.2.4. Synthesis of \( N\)-(5-Chloro-pyridin-2-yl)-amides (19a–c)

Compounds 19a–c were synthesized by the acylation of 5-chloro-2-amino pyridine 33 under the same reaction conditions as for 17a–c and 18a–c. The \( N\)-(5-Chloro-pyridin-2-yl)-amides 19 were generated in excellent yields (87–91%) (Table 5).

Table 5. Synthesis of 5-chloropyridin-2-ylalkylamides 19.

| Experiment | R          | Compound | Temperature (°C) | Yield (%) |
|------------|------------|----------|------------------|-----------|
| 1          | -C\(_3\)H\(_7\) | 19a      | 150              | 91        |
| 2          | -C\(_5\)H\(_{11}\) | 19b      | 150              | 87        |
| 3          | -C\(_3\)H\(_{17}\) | 19c      | 180              | 90        |

Conditions: 5-chloropyridine-2-amine (1 eq), carboxylic acid (2 mL), 90 min.

Although compounds 19 are known and their use has been reported [42,43], their possible application as QSIs is unexplored. Characterization of pyridines 19 was not found.

2.2.5. Synthesis of \( N\)-Substituted-2-[4-(imidazolin-2-yl)-phenoxy]-acetamides (32a–c)

In addition to designing bioisosters 16–19, three other novel imidazoline acetamides were synthesized (32a–c) because of the similarity of their structure to that of imidazoline, the QS inhibitory activity of which is well recognized [20]. Moreover, their structural elements coincide with those considered presently. In a previous theoretical study, the \( \Delta G \) of the complexes formed by 32a–c and the CviR protein were calculated, finding much lower values than for the complex of AHL with the same protein [44].

Based on the method described by our group [20], imidazolines 32 were synthesized with different substituents on the amide group. Obtaining compounds 32 began with the synthesis of the aldehydes 35, for this it was necessary to obtain acetamide 34 (Scheme 5).

Scheme 5. Procedure to synthesize \( \alpha\)-bromo-\( N\)-propylacetamide 34. Conditions: a. Amine (1 eq), bromoacetyl bromide (1.2 eq), CH\(_2\)Cl\(_2\) (15 mL), rt, 20 min.

To prepare aldehydes 35, a previous report was considered [20] (Table 6).
Table 6. Results from the synthesis of aldehydes 35.

| Experiment | R          | Compound | Time (h) | Yield (%) |
|------------|------------|----------|----------|-----------|
| 1          | -C₆H₄-3-Cl | 35a      | 18°C     | 63        |
| 2          | -C₆H₄-3-Cl | 35a      | 6        | 65        |
| 3          | -CH₂-C₆H₄-4-Cl | 35b | 6        | 73        |
| 4          | -C₃H₇      | 35c      | 6        | 79        |

Conditions: amide (1 eq), 4-hydroxybenzaldehyde (1.1 eq), DBU (1 eq), acetonitrile (20 mL).

Compared to the report on the preparation of aldehyde 35a, a shorter time was herein achieved (Table 6) by using 2-bromoacetyl bromide rather than the previously employed α-bromoacetic acid and DCC [20]. Compounds 35 were subjected to cyclization with ethylenediamine and subsequent oxidation with NBS under ultrasound irradiation, as reported by Torres [29]. The corresponding imidazolines 32a–c were provided in good yields (85–90%) (Scheme 6).

Scheme 6. Synthetic procedure to obtain the imidazoline acetamides (32a–c). Reagents and conditions: (a) CH₂Cl₂, rt, 20 min; (b) DBU, ACN, 70 °C, 8 h; (c) ethylenediamine, ACN, 15 min; then NBS, ACN, 10 min.

2.3. Characterization of Compounds

All compounds were characterized by spectroscopic methods utilizing IR, NMR, and DIP-MS. Spectra were assigned with the help of heteronuclear correlation (gHSQC, gHMBC) and in some cases with homonuclear correlation (gCOSY). Mass spectra were recorded with positive and negative ions. Spectra for all compounds are attached in the Supplementary Materials (Figures S1–S104).

3. Evaluation of QS Inhibition on Chromobacterium violaceum CV026

The synthesized compounds were added (at 10, 100 and 1000 µM) to the bacteria (OD600 of 0.12) in thioglycolate broth supplemented with C6-AHL, followed by incubation for 16 h. The determination of the specific production of violacein, calculated as the ratio of detectable pigment (OD577) per amount of bacteria (OD720), demonstrated the existence of QS inhibitory activity for almost all test compounds. (Figures 4–8). There is a clear difference between quorum sensing and antibacterial activity. In this
research, growth inhibition by the evaluated heterocycles results in a decrease in OD720. While this
decrease was statistically significant, taking cultures grown in the absence of inhibitors as a basis of
comparison, this is denoted with a letter ‘a’ on the top of the corresponding bar and stated as ‘a’ the
footnote of the figure.

Figure 4. Specific production of violacein by Chromobacterium violaceum CV026 in the presence of the
thiazoles (16a–c) at 10, 100 and 1000 µM. Data are expressed as a percentage of the specific production of
violacein (mean ± SEM) and normalized by considering the value without the addition of a compound
as 100%. Significance was confirmed by the Student’s t-test with an accuracy of * p < 0.05 at the marked
bars. a Denotes an antimicrobial effect of the compound. Experiments were performed with n = 6.

Figure 5. Violacein specific production by Chromobacterium violaceum CV026 in the presence of the
synthesized thiazolines at 10, 100 and 1000 µM concentrations. Data are expressed as the percentage of
the specific production of violacein (mean ± SEM) and normalized on the violacein production without
compound addition and this was considered as 100%. Significance was confirmed by the Student’s t-test with an accuracy of * p < 0.05 at the marked
bars; a represents antimicrobial effect of the tested
compound. Experiments were performed with n = 6.
lactone (IC50 = 377 nM) [7].

### 3.3. Benzimidazoles

respectively, presented a value of IC50 of 36.67 µM, and behaved as an antimicrobial at 1000 µM. For synthesized inhibitors like 4-nitro-pyridine-1′-(pentyloxy)phenyl]-4,5-dihydro-1H-imidazole (IC50 = 56.38 µM) but is not better than 4-[(4′-chlorophenoxy)-N-(2-oxotetrahydrofuran-3-yl)]butanamide (IC50 = 377 nM) [7] nor better than CV026 in the presence of the tested compound. Experiments were performed with n = 6.

**Figure 6.** Violacein specific production by *Chromobacterium violaceum* CV026 in the presence of the synthesized benzimidazoles at 10, 100 and 1000 µM concentrations. Data are presented as percentages of violacein specific production (mean ± SEM) and normalized on the violacein production without compound addition and this was considered as 100%. Significance was confirmed by the Student’s t-test with an accuracy of * p < 0.05 at the marked bars. a Indicates an antimicrobial effect of the tested compound. Experiments were performed with n = 6.

### 3.5. Imidazolines

In the case of synthesized thiazolines at 10, 100 and 1000 µM concentrations. Data are expressed as the percentage of violacein specific production (mean ± SEM) and normalized on the violacein production without compound addition and this was considered as 100%. Significance was confirmed by the Student’s t-test with an accuracy of * p < 0.05 at the labelled bars; a Indicates an antimicrobial effect of the compound. Experiments were performed with n = 6.

**Figure 7.** The specific production of violacein by *Chromobacterium violaceum* CV026 in the presence of the synthesized compounds (19a–c) at 10, 100 and 1000 µM. Data are expressed as a percentage of specific production of violacein (mean ± SEM), normalized on the violacein production without compound addition and this considered as 100%. Significance was confirmed by the Student’s t-test with an accuracy of * p < 0.05 at the marked bars. a Indicates an antimicrobial effect of the tested compound. Experiments were performed with n = 6.
3.1. Thiabanes

The QS inhibitory activity of the series of thiabanes is illustrated in Figure 4. No such effect was found for 16a at any concentration. Thiabane 16b at 10, 100 and 1000 µM showed low-moderate QS inhibitory activity (32%, 39% and 51%, respectively) with an IC50 = 925.0 µM. The thiabane 16c was QSI only at 10 µM, causing limited inhibition (22%).

3.2. Thiabesines

The results for thiabesine series are shown in Figure 5. Thiabesine 17a displayed a moderate QS inhibition at 10 and 100 µM being of 44% and 47% respectively, while at 1000 µM it behaved as antimicrobial. Thiabesine 17b exhibited a limited to good inhibition at 10, 100 and 1000 µM being of 23%, 37% and 65% respectively, in addition 17b gave an IC50 value of 517.86 µM. Compound 17c reduced violacein production 23%, 24% and 48% at 10, 100 and 1000 µM respectively.

3.3. Benzimidazoles

For the benzimidazole series (Figure 6), 18a gave 27% and 85% QS inhibition at 10 and 100 µM respectively, presented a value of IC50 of 36.67 µM, and behaved as an antimicrobial at 1000 µM. For 18b at 100 and 1000 µM, good and excellent activity as a QSI was found (31% and 95%, respectively). The IC50 value of 18b is 376.92 µM. For 18c the inhibitory effect was 17%, 24% and 23% at 10, 100 and 1000 µM respectively.

Activity as QSI of benzimidazole 18a (85% QS inhibition at 100 µM, IC50 of 36.67 µM) is comparable than 2-[4-(hexyloxy)phenyl]-4,5-dihydro-1H-imidazole (65% QS inhibition at 90 µM, IC50 = 56.38 µM) [21], more active than 4-nitro-pyridine-N-oxide (active at 100 µM), the best synthetic quorum sensing inhibitor (QSI) in a screening of 27 synthetic compounds and 27 natural extracts [11,45]. However benzimidazole 18a is not QSI like 2-(4′-chlorophenoxy)-N-butanoyl homoserine lactone (IC50 = 377 nM) [7].
3.4. Pyridines

Pyridine 19a exhibited QS inhibition activity (17%) at 100 µM, while at 1000 µM it behaved as an antimicrobial. Compound 19b was active against QS only at 1000 µM, eliciting a 21% inhibition. In the case of 19c, all three concentrations (10, 100 and 1000 µM) showed an QS inhibitory effect (52%, 55% and 58%, respectively). (Figure 7). According to the experimental data, the IC50 was calculated to be 9.66 µM. Pyridine 19c is the best compound of all those tested in this work, its activity as QSI is comparable to 2 and 3 (IC50 = 1.1 µM and 2.2 µM respectively) [7]. It is more active than other synthesized inhibitors like 4-nitro-pyridine-N-oxide (active at 100 µM) [11,45] or 2-[4’-(pentyloxy)phenyl]-4,5-dihydro-1H-imidazole (IC50 = 56.38 µM) but is not better than 4-[(4’-chorophenoxy)-N-(2-oxotetrahydrofuran-3-yl)butanamide (IC50 = 377 nM) [7] nor better than N-[4’-(4,5-dihydro-1H-imidazol-2-yl)phenyl]nonamide (activity as QSI from 1 nM) [24].

3.5. Imidazolines

For the series of imidazolines (Figure 8), 32a and 32c at 100 µM exhibited 24% and 34% activity as QSI, respectively. Imidazoline 32b was QSI at all concentrations and displayed IC50 = 65.09 µM. The QS inhibition activity of 32b is comparable to other synthesized bioisosteres [21]. Imidazoline 32c at 1000 µM afforded 46% activity and compound 32a behaved as an antimicrobial at the latter concentration. Compound 32b is one of the top three in the series of compounds synthesized in this work, its QS inhibition activity is lower than that of ethyl (4-fluorobenzoyl) acetate (IC50 = 23 µM) the best QSI of a library with 26 compounds [46], but it is similar to 2-[4’-(pentyloxy)phenyl]-4,5-dihydro-1H-imidazole (IC50 = 56.38 µM) and more active than QSI 4-nitro-pyridine-N-oxide (active at 100 µM) [11,45].

4. Docking Results

Molecular docking was carried out to provide insight into the non-bonding interactions of the test compounds with the CviR protein (PDB code: 3QP6). The binding modes were observed and the affinity energy of each compound was calculated and expressed as ΔG (kcal/mol). The Docking simulations were performed with AutoDock 4.2, utilizing the blind docking method. The grid box size was adjusted to 126 Å³ with a grid spacing of 0.375 Å. The Lamarckian genetic algorithm was employed with a randomized initial population of 100 individuals and a maximum number of energy evaluations of 1 × 10⁶. [47] The docking results were analyzed and visualized on AutoDockTools and PyMOL [48], represented as 3D plot conformations. The affinity energies of the evaluated compounds are shown in Table 7.

Molecular Docking of compounds 32a-c were performed by us in a previous study [44] obtaining the following results: 32a, ΔG = −11.39 kcal/mol; 32b, ΔG = −8.52 kcal/mol y 32c, ΔG = −10.42 and AHL (1), ΔG = −7.26 kcal/mol.

The theoretical calculations reveal that all tested compounds had greater affinity for the protein than the native ligand C6-AHL (1, R1 = H, R2 = C₃H₇). In addition, a strong correlation exists between the in silico and experimental results. For example, 17b-c, 18a-c and 19c displayed good experimental inhibition and had the greatest affinity energies in silico.

The amino acids residues of the protein involved in the interaction with the C6-AHL, the native ligand, also participate in the binding of the most test compounds. There are hydrogen bond interactions of the polar groups of the compounds with the TRP84, ASP97 and SER155 residues. Additionally, there are π-π interactions of the phenyl ring on the thiazolines 17a and 17b with the phenyl group of TYR88, of the benzimidazoles 18a-b with TYR80, and of the pyridine ring of compound 19c with TRP111. Moreover, hydrophobic interactions took place with most of the residues at the binding site, including ILE57, VAL59, MET72, LEU85, ILE99, PHE126, ALA130 and MET135. (Figure 9).
### Table 7. Affinity energies (ΔG) and amino acid residues of the binding site for the ligands evaluated.

| Ligand  | ΔG (kcal/mol) | Amino Acid Residues of the Binding Site                                      |
|---------|---------------|-----------------------------------------------------------------------------|
| 16a     | −7.13         | ILE57, VAL59, TYR80, TRP84 *, LEU85, TYR88, ASP97 *, ILE99, TRP111, PHE115, PHE126, MET135, SER155 |
| 16b     | −7.5          | VAL59, MET72, TYR80 *, TRP84, LEU85, TYR88, ASP97 *, ILE99, TRP111, PHE115, PHE126, ALA130, MET135, ILE153, SER155 * |
| 16c     | −7.19         | MET72, TYR80 *, TRP84, LEU85, TYR88, SER89, MET100, ILE153, SER155 *          |
| 17a     | −7.7          | VAL75, TYR80 *, LEU85, TYR88 *, ASP97 *, ILE99, TRP111, SER155 *              |
| 17b     | −8.51         | MET72, VAL75, TYR80, TRP84, LEU85, TYR88 *, ASP97 *, ILE99, TRP111, PHE126, SER155 * |
| 17c     | −8.77         | MET72, VAL75, TYR80, TRP84, LEU85, TYR88, ASP97, ILE99, TRP111, PHE115, PHE126, ALA130, MET135, ILE153, SER155 |
| 18a     | −9.58         | ILE57, VAL59, MET72, TYR80 *, TRP84, LEU85, TYR88, SER89, ILE99, TRP111, PHE126, ALA130, MET135, ILE153, SER155 * |
| 18b     | −9.51         | ILE57, VAL59, MET72, VAL75, TYR80 *, TRP84, LEU85, TYR88, SER89, ILE99, TRP111, PHE126, ALA130, MET135, ILE153, SER155 * |
| 18c     | −8.52         | ILE57, VAL59, TYR80, LEU85, TYR88, SER89, ILE99, TRP111, PHE126, ALA130, MET135, SER155 * |
| 19a     | −7.15         | ILE57, VAL59, TYR80, TRP84 *, TYR88, ASP97 *, ILE99, TRP111, PHE115, PHE126, MET135 |
| 19b     | −7.96         | TYR80, TRP84, TYR85 *, ASP97 *, ILE99, TRP111, PHE115, PHE126, MET135, SER155 * |
| 19c     | −8.09         | ILE57, VAL59, MET72, TRP84 *, LEU85, TYR88, ASP97, ILE99, TRP111, PHE115, PHE126, ALA130, MET135, SER155 * |
| 1 (R₁ = H, R₂ = C₃H₇) | −6.63         | ILE57, TYR80, TRP84 *, LEU85, TYR88, ASP97 *, TRP111, PHE126, SER155          |

Hydrogen bond interaction (*), π–π interaction (*).
Additionally, there are $\pi$–$\pi$ interactions of the phenyl ring on the thiazolines 17a and 17b with the phenyl group of TYR88, of the benzimidazoles 18a–b with TYR80, and of the pyridine ring of compound 19c with TRP111. Moreover, hydrophobic interactions took place with most of the residues at the binding site, including ILE57, VAL59, MET72, LEU85, ILE99, PHE126, ALA130 and MET135. (Figure 9).

The non-covalent interactions of the most active ligands evaluated (17b, 18a and 19c) are observed in the Figures 10–12. The $\pi$–$\pi$ interactions in the protein-compound complexes highlight the relevance of the phenyl moiety in the chemical structure of the test compounds. Other non-covalent interactions are involved in these complexes, such as hydrophobic and hydrogen bond interactions.
5. Structure-Activity Relationship

The elements present in the bioisosteres of this work were a heterocycle, the amide group and the side chain. In one of the 5 bioisosteres, a phenyl ring was introduced as a linker between the amide and the heterocycle (17).
The chain length is important for the activity, not because of the interactions that it establishes, since they are all hydrophobic, but rather because of the size that permits other interactions; thus the compound 16a that has an aromatic ring, the thiazol, and the amide group but a chain of only 3C, did not display activity at any concentration. According to the Docking analysis this compound does not satisfy the size required to give all interactions like its homologous 16b and 16c. The latter compounds showed hydrogen bonding between the C=O of the amido group and TYR 80 and SER 155.

Thiazolines 17 contain a thiazoline ring, a phenyl as a connector, the amido group, and propyl, pentyland heptyl chains. They exhibited some degree of QS inhibition, except for 17a at 1000 µM, showing antimicrobial activity. In an earlier work, our group synthesized thiazolines, 33a–c, (Figure 13) like the thiazolines 17 but without the phenyl connector; all those thiazolines were inactive as QSI [29,49]. Thiazoline 17b afforded a low activity as QSI, 23% and 37% at 10 and 100 µM, respectively, as well as 65% at 1000 µM. The phenyl linker enables a greater number of interactions than the aliphatic chains alone, and increases the length of the compound. The π–π interactions of the complex formed by compound 17b and tyrosine 88 at the active site the CviR protein is illustrated in Figure 12.

![Inactive thiazolines as QSI.](image)

All benzimidazoles (18) with a thiol functionality display good QSI activity, 18a and 18b, so the compound 18a exhibited 85% QSI at 100 µM and 18b 95% at 1000 µM. Benzimidazol 18c displayed only 17, 24 and 23% inhibitory activity at 10, 100 and 1000 µM, respectively. According to the Docking simulations, the benzimidazole ring occupies the space of the lactone and the amide of AHL, while thioacetamide engages the space of the aliphatic chain of AHL. The interactions of the protein with the benzimidazole rings were similar to those found with the lactone ring and the amido group of AHL. Moreover, the phenyl ring of 18a y 18b presents π–π interactions with the receptor. Conversely, no π–π interactions were observed for 18c because there is no phenyl ring in its structure, and the benzimidazol ring, which could give these interactions, establishes the interactions mentioned above. Benzimidazoles 18a–b do not contain an aliphatic chain; but have a thioacetamide group, giving them greater length and flexibility.

Since 18a is QSI at 100 µM and antimicrobial at 1000 µM, it is possible to find a concentration that is able to restore sensitivity to an antibiotic. It is known that the QSI addition to an antibiotic can restore the sensitivity to the drug and increase its potency [11,50], therefore we consider that benzimidazol 18a that behaves as very good QSI at one concentration and antimicrobial at another is very promising.

Pyridine 19a with a propyl chain exhibited little QSI activity, 17% at 100µM and behaved as an antimicrobial at 1000 µM suggesting that chain was too short to properly couple to the protein. Among the pyridines, the best activity as a QSI was produced by 19c, the compound with a nonyl chain. Its inhibitory activity was surprisingly similar at all three concentrations (52, 55 and 58% at 10, 100 and 1000 µM, respectively), which has no apparent explanation. Pyridines 19a and 19b generate the same hydrogen bond as AHL between NH and Asp 97. With 19b, there is in an additional hydrogen bond of C=O of the amido group with SER 155 and TYR 80. It was observed that 19c is rotated 180° in relation to AHL and forms two hydrogen bonds: one between the compound 19c carbonyl and SER 155, and the other between N and TRP 84. All imidazolines displayed activity as QSI at 100 µM and 1000 µM, although only 32b (at 1000 µM) showed good activity. Compound 32c, containing a propyl lateral chain, gave rise to 34 and 46% QSI at 100 and 1000 µM respectively.
6. Materials and Methods

6.1. General

All reagents and solvents were purchased from Sigma Aldrich (Toluca, Mexico) and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) on Merck F253 silica gel aluminum sheets, and spots were revealed with ultraviolet (UV) light (254 nm). NMR experiments were carried out in Varian NMR System (500 MHz and 125 MHz), Varian Mercury (300 MHz and 75 MHz) and Bruker ASCEND (600 MHz and 150 MHz). $^1$H NMR and $^{13}$C spectra were assigned with the help of 2-D experiments (gHSQC and gHMBC). The chemical shifts ($\delta$) are given in ppm. Mass spectra (MS) were recorded on a Bruker Amazon Speed (ESI). Infrared (IR) spectra were obtained on a Perkin Elmer FT-IR Spectrum 2000 spectrometer from the ENCB-IPN spectroscopy instrumentation center. Melting points were determined on an Electrothermal MEL-TEMP apparatus and are uncorrected. Microwave reactions were accomplished on a CEM Discovery SP apparatus.

6.2. Procedure for the Synthesis of Ethyl 2-Aminothiazol-4-carboxylate, 22

Ethyl bromine pyruvate (7.69 mmol, 1 eq), thiourea (7.69 mmol, 1 eq) and 15 mL of EtOH were added to a flask equipped with a magnetic stirring bar and left to reaction mixture at rt for 16 h. At the end of this period, the solvent was evaporated under reduced pressure, followed by the addition of 40 mL of 20% aqueous potassium carbonate to the residue. The suspension formed was maintained under constant stirring for 30 min before subjecting it to vacuum filtration. The solid retained on the filter paper was washed with 40 mL of distilled water in two 20 mL portions and oven-dried at 40 °C.

Ethyl 2-Aminothiazol-4-Carboxylate, 22

Yield: 85%. White solid. $^1$H NMR (300 MHz, DMSO-d$_6$) $\delta$: 7.44 (s, 1H, H-5), 7.24 (s, 2H, NH), 4.17 (q, $J$ = 7.10 Hz, 2H, H-8), 1.22 (t, $J$ = 7.10 Hz, 3H, H-9).

$^{13}$C NMR (75 MHz, DMSO-d$_6$) $\delta$: 168.6 (C-2), 161.5 (C-6), 142.6 (C-4), 117.4 (C-5), 60.6 (C-8), 14.6 (C-9). DIP-MS (ESI; M + Na$^+$) $m/z$ (calculated): 195.01, $m/z$ (measured): 195.00.

6.3. Synthesis of Ethyl 2-Acylamidothiazol-4-carboxylate, 16

2-Amino thiazole (1.74 mmol, 1 eq) and 2 mL of carboxylic acid (butanoic acid at 150 °C, hexanoic acid at 150 °C, octanoic acid at 180 °C) were added to a reaction vial equipped with magnetic stirring, the reaction mixture was warmed for 90 min. It was then transferred to an aqueous solution of 20% potassium carbonate (40 mL) and allowed the mixture to stir for 30 min. Subsequently, the suspension was vacuum filtered and the solid obtained washed with distilled water (2 x 20 mL) and oven-dried at 40 °C.

Ethyl 2-Butyramidothiazol-4-Carboxylate, 16a

Yield: 82%. Brownish solid; m.p. 145–148 °C. Lit. [30] 150–151 °C R$_f$ = 0.6 (AcOEt). IR (CH$_2$Cl$_2$) $\tilde{\nu}$ = 3256.8, 1720.1, 1690.4, 1546.0, 1215.1 cm$^{-1}$. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.81 (s, 1H, H-5’), 4.35 (q, $J$ = 7.12 Hz, 2H, H-3’’), 2.43 (t, $J$ = 7.47 Hz, 2H, H-2), 1.70 (m, 2H, H-3), 1.35 (t, $J$ = 7.12 Hz, 3H, H-4’’), 0.94 (t, $J$ = 7.47 Hz, 3H, H-4). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 171.9 (C-1), 161.4 (C-1’’), 159.0 (C-2’), 141.1 (C-4’), 122.2 (C-5’), 61.4 (C-3’’), 37.9 (C-2), 18.3 (C-3), 14.3 (C-4’’), 13.6 (C-4). DIP-MS (ESI; M + Na$^+$) $m/z$ (calculated): 265.06, $m/z$ (measured): 265.10.

Ethyl 2-Hexanamidothiazol-4-Carboxylate, 16b

Yield: 85%. Brownish solid; m.p. 129–132 °C. Lit. [30] 135–136 °C R$_f$ = 0.6 (AcOEt). IR (CH$_2$Cl$_2$) $\tilde{\nu}$ = 3256.8, 1723.4, 1680.7, 1547.6, 1211.1 cm$^{-1}$. $^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.82 (s, 1H, H-5’), 4.35 (q, $J$ = 7.13 Hz, 2H, H-3’’), 2.44 (t, $J$ = 7.59 Hz, 2H, H-2), 1.68 (m, 2H, H-3), 1.36 (t, $J$ = 7.13 Hz, 3H, H-4’’), 1.28 (m, 4H, H-4,5), 0.86 (m, 3H, H-6). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$: 171.9 (C-1), 161.4 (C-1’’), 159.0 (C-2’), 141.1 (C-4’), 122.2 (C-5’), 61.4 (C-3’’), 37.9 (C-2), 18.3 (C-3), 14.3 (C-4’’), 13.6 (C-4). DIP-MS (ESI; M + Na$^+$) $m/z$ (calculated): 265.06, $m/z$ (measured): 265.10.
Ethyl 2-Octanamidothiazol-4-Carboxylate, 16c

Yield: 80%. Brownish solid; m.p. 102–104 °C. Lit. [30] 109–111 °C. Rf = 0.68 (AcOEt). IR (CH2Cl2) \( \bar{\nu} = 3245.9, 1725.1, 1689.5, 1544.2, 1214.2 \) cm\(^{-1}\). 1H NMR (300 MHz, CDCl3) \( \delta \): 7.64 (d, \( J = 8.57 \) Hz, 2H, H-3), 6.63 (d, \( J = 8.57 \) Hz, 2H, H-2), 4.38 (t, \( J = 8.2 \) Hz, 2H, H-4\(^{\prime}\)), 3.96 (s, 2H, NH), 3.34 (t, \( J = 8.2 \) Hz, 2H, H-5\(^{\prime}\)). 13C NMR (75 MHz, CDCl3) \( \delta \): 167.9 (C-2\(^{\prime}\)), 149.3 (C-1), 130.0 (C-3), 123.5 (C-4), 114.2 (C-2), 64.9 (C-4\(^{\prime}\)), 33.5 (C-5). DIP-MS (ESI; M + H) \( m/z \) (calculated): 284.29, 177.19. IR (CH2Cl2) \( \bar{\nu} = 2931.0, 1667.6, 1598.3, 1527.5, 845.5 \) cm\(^{-1}\).

6.4. Procedure for the Synthesis of 4-(Thiazolin-2-yl)-aniline, 25

4-aminobenzonitrile (4.23 mmol, 1 eq), cysteamine hydrochloride (6.34 mmol, 1.5 eq), potassium carbonate (21.16 mmol, 5 eq) and 5 mL of a EtOH/H2O (1:1) mixture were added to an ACE pressure tube equipped with a magnetic stirring bar. The reaction mixture was subjected to heating in a sand bath at 110 °C for 16 h. At the end of this period, the reaction mixture was transferred to an aqueous solution of 20% potassium carbonate (40 mL) and left to stand under constant stirring for 90 min. The suspension was subjected to vacuum filtration and the solid retained on the filter paper dried with anhydrous Na2SO4 and evaporated in a ball flask under reduced pressure. The residue was purified by chromatographic column, using silica gel as the stationary phase and an 8:2 hexane/AcOEt mixture as the mobile phase.

4-(Thiazolin-2-yl) Aniline, 25

Yield: 82%. Pink solid. 1H NMR (300 MHz, CDCl3) \( \delta \): 7.64 (d, \( J = 8.57 \) Hz, 2H, H-3), 6.63 (d, \( J = 8.57 \) Hz, 2H, H-2), 4.38 (t, \( J = 8.2 \) Hz, 2H, H-4\(^{\prime}\)), 3.96 (s, 2H, NH), 3.34 (t, \( J = 8.2 \) Hz, 2H, H-5\(^{\prime}\)). 13C NMR (75 MHz, CDCl3) \( \delta \): 167.9 (C-2\(^{\prime}\)), 149.3 (C-1), 130.0 (C-3), 123.5 (C-4), 114.2 (C-2), 64.9 (C-4\(^{\prime}\)), 33.5 (C-5). DIP-MS (ESI; M + H) \( m/z \) (calculated): 179.06, 177.19. IR (CH2Cl2) \( \bar{\nu} = 2931.0, 1667.6, 1598.3, 1527.5, 845.5 \) cm\(^{-1}\).

6.5. Synthesis of N-[4-(Thiazolin-2-yl)-phenyl]-carboxamides, 17

4-(Thiazolin-2-yl)-Aniline (1.96 mmol, 1 eq) and 2.5 mL of carboxylic acid (butanoic, hexanoic, octanoic acids) were added to a reaction vial equipped with a magnetic stirring bar. The vial was closed and the reaction mixture warmed (butanoic acid at 150 °C, hexanoic acid at 150 °C, octanoic acid at 180 °C) for 90 min. At the end of that period, the reaction mixture was transferred to an aqueous solution of 20% potassium carbonate (40 mL) and left to stand under constant stirring for 30 min. The suspension was subjected to vacuum filtration and the solid retained on the filter paper was washed with 40 mL of distilled water in two 20 mL portions, then oven-dried at 40 °C.

N-(4-(Thiazolin-2-yl) Phenyl) Butiramide, 17a

Yield: 85%. Yellow solid; m.p. 138–140 °C. Rf = 0.3 (AcOEt). IR (CH2Cl2) \( \bar{\nu} = 3296.6, 1667.6, 1598.3, 1527.5, 845.5 \) cm\(^{-1}\). 1H NMR (300 MHz, CDCl3) \( \delta \): 7.80 (s, 1H, NH), 7.76 (d, \( J = 8.58 \) Hz, 2H, H-3\(^{\prime}\)), 7.58 (d, \( J = 8.58 \) Hz, 2H, H-2\(^{\prime}\)), 4.42 (t, \( J = 8.28 \) Hz, 2H, H-4\(^{\prime\prime}\)), 3.39 (t, \( J = 8.28 \) Hz, 2H, H-5\(^{\prime\prime}\)), 2.32 (t, \( J = 7.42 \) Hz, 2H, H-2), 1.73 (m, 2H, H-3), 0.97 (t, \( J = 7.35 \) Hz, 3H, H-4). 13C NMR (75 MHz, CDCl3) \( \delta \): 171.8 (C-1), 168.0 (C-2\(^{\prime}\)), 140.7 (C-1\(^{\prime}\)), 129.3 (C-3\(^{\prime}\)), 128.7 (C-4\(^{\prime}\)), 119.1 (C-2\(^{\prime}\)), 65.0 (C-4\(^{\prime\prime}\)), 39.6 (C-2), 33.7 (C-5\(^{\prime\prime}\)), 19.0 (C-3), 13.7 (C-4). DIP-MS (ESI; M + H) \( m/z \) (calculated): 249.10, 249.15. IR (CH2Cl2) \( \bar{\nu} = 3301.0, 1669.4, 1598.7, 1529.8, 843.9 \) cm\(^{-1}\).
7.65 (s, 1H, NH), 7.58 (d, J = 8.59 Hz, 2H, H-2′), 4.42 (t, J = 8.3 Hz, 2H, H-4′′)), 3.39 (t, J = 8.3 Hz, 2H, H-5′′), 2.34 (t, J = 7.55 Hz, 2H, H-2), 1.70 (m, 2H, H-3), 1.33 (m, 4H, H-4,5), 0.88 (t, J = 6.44 Hz, 3H, H-6). 13C NMR (75 MHz, CDCl3) δ 171.7 (C-1), 167.9 (C-2′′), 140.6 (C-1′′), 129.3 (C-3′′), 128.7 (C-4′′), 119.0 (C-2′′), 65.0 (C-4′′), 37.8 (C-2), 33.677 (C-5′′), 31.4 (C-4), 25.2 (C-3), 22.4 (C-5), 13.9 (C-6). DIP-MS (ESI; M + Na) m/z (calculated): 299.11, m/z (measured): 299.20.

N-(4-Thiazolin-2-yl) Phenyl Octanamide, 17c

Yield: 79%. Brownish solid; m.p. 68–70 °C. Rf = 0.51 (AcOEt). IR (CH2Cl2) ν = 3301.9, 1667.5, 1597.6, 1530.8, 844.1 cm−1. 1H NMR (300 MHz, CDCl3) δ: 7.90 (1H, NH), 7.76 (2H, H-3′′), 7.59 (2H, H-2′′), 4.42 (2H, H-4′′), 3.39 (2H, H-5′′), 2.35 (2H, H-2), 1.69 (2H, H-3), 1.26 (8H, H-4,5,6,7), 0.86 (3H, H-8). 13C NMR (75 MHz, CDCl3) δ 171.7 (C-1), 167.9 (C-2′′), 140.7 (C-1′′), 129.3 (C-3′′), 118.9 (C-4′′), 64.9 (C-4′′), 33.7 (C-2), 31.6 (C-5′′), 29.2, 29.0 28.9, 25.5, 22.6, 14.1 (C-8). DIP-MS (ESI; M + H) m/z (calculated): 305.16, m/z (measured): 305.27.

6.6. Procedure for the Synthesis of 5-Chloro-benzimidazole-2-thione, 27

4-Chloro-phenylenediamine (5.61 mmol, 1 Eq), carbon disulfide (7.23 mmol, 1.3 Eq), potassium hydroxide (16.83 mmol, 3 eq) and 5 mL of an ethanol/water mixture (7:3) were placed in an ACE pressure tube charged with magnetic stirring. The reaction mixture was heated at 80 °C for 5 h, then the solvent was evaporated under reduced pressure and 15 mL of a 5% aqueous HCl solution were added to the residue. The suspension was left with stirring for 15 min and subsequently it was vacuum filtered, the solid obtained was washed with 2 × 20 mL of distilled water, the solid was dried at 40 °C in an oven.

5-chloro-1,3-dihydro-2H-Benzimidazol-2-thione, 27

Yield: 88%. Brownish solid. 1H NMR (300 MHz, Acetone-d6) δ: 7.17 (dd, J = 1.86, 0.86 Hz, 1H, H-6), 7.15 (d, J = 0.86 Hz, 1H, H-4), 7.13 (d, J = 1.86 Hz, 1H, H-7). 13C NMR (75 MHz, Acetone-d6) δ 170.4 (C-2), 133.768 (C-4a), 131.7 (C-7a), 127.1 (C-5), 122.1 (C-6), 110.5 (C-7), 109.4 (C-4). DIP-MS (ESI; M + H) m/z (calculated): 182.97, m/z (measured): 182.88.

6.7. Alkylation of 5-Chloro-benzimidazole-2-thione, 18

Benzimidazol-2-thione (1.35 mmol, 1 eq), alkyl halide (1.35 mmol, 1 eq) and 15 mL of ethanol were placed in a 100 mL ball flask equipped with a magnetic stirring bar and the reaction mixture was left to stand for 16 h at 60 °C before evaporating the solvent. The solid was suspended in an aqueous solution of 20% potassium carbonate (30 mL) and vacuum filtered. This solid was washed with 2 × 20 mL of distilled water and oven-dried at 40 °C.

2-[(6-chloro-1H-Benzimidazol-2-yl thio)-N-(3-Chlorophenyl)] Acetamide, 18a

Yield: 80%. Purple solid; m.p. 228–231 °C. Rf = 0.28 (hexane/AcOEt 7:3). IR (CH2Cl2) ν = 1652.2, 1586.4, 1423.7 cm−1. 1H NMR (300 MHz, DMSO-d6) δ: 10.77 (s, 1H, NH), 7.77 (s, 1H, H-2′′), 7.64 (d, J = 1.64 Hz, 1H, H-7′′), 7.57 (d, J = 8.63 Hz, 1H, H-4′′), 7.43 (d, J = 8.36 Hz, 1H, H-6′′), 7.32 (m, 2H, H-5′′,5′′″), 7.10 (d, J = 7.84 Hz, 1H, H-4′), 4.41 (s, 2H, H-2). 13C NMR (75 MHz, DMSO-d6) δ 166.1 (C-1), 152.3 (C-2′′), 140.5 (C-1′′), 137.2 (C-7a″), 134.9 (C-4a″), 133.5 (C-3′′), 131.0 (C-5′′), 128.3 (C-6′′), 124.1 (C-5′′″), 123.8 (C-4′), 119.0 (C-2′′), 118.0 (C-6′), 115.1 (C-4′′), 113.8 (C-7″), 36.9 (C-2). DIP-MS (ESI; M-H) m/z (calculated): 349.99, m/z (measured): 349.93.

2-[(6-chloro-1H-Benzimidazol-2-yl thio)-N-(4-chlorobenzyl] Acetamide, 18b

Yield: 83%. Blue solid; m.p. = 115–118 °C. Rf = 0.15 (hexane/AcOEt 7:3); IR (CH2Cl2) ν = 3735.7, 3649.3, 3283.2, 1640.0, 1557.9, 1393.9 cm−1. 1H NMR (300 MHz, DMSO-d6) δ: 8.87 (t, J = 5.95 Hz, 1H, NH), 7.54 (d, J = 1.98 Hz, 1H, H-7″), 7.48 (d, J = 8.64 Hz, 1H, H-4′″), 7.23 (m, 5H, H-3′′,4′′,5′′″), 4.25 (d, J = 5.95 Hz, 2H, H-1″), 4.11 (s, 2H, H-2). 13C NMR (75 MHz, DMSO-d6) δ 167.5
(C-1), 152.1 (C-2”), 139.4 (C-7a”), 138.4 (C-2’), 136.9 (C-4a”), 131.7 (C-5’), 129.4 (C-3’), 128.5 (C-4’), 127.2 (C-6”), 123.0 (C-5’), 115.2 (C-4’), 114.0 (C-7’’), 42.3 (C-1’), 35.5 (C-2’). DIP-MS (ESI; M-H) m/z (calculated): 364.00, m/z (measured): 363.95.

6-chloro-2-(Nonylthio)-1H-Benzimidazole, 18c

Yield: 70%. Pink solid; m.p. 73–76 °C. Rf = 0.66 (hexane/AcOEt 7:3). IR (CH2Cl2) ν = 3032.6, 1389.6 cm⁻¹. 1H NMR (300 MHz, CDCl3): δ: 7.72 (s, 1H, NH), 7.58 (d, J = 1.97 Hz, 1H, H-7), 7.42 (d, J = 8.56 Hz, 1H, H-4), 7.16 (dd, J = 8.56, 1.97 Hz, 1H, H-5), 3.28 (m, 2H, H-1’), 1.72 (m, 2H, H-2’), 1.28 (m, 12H, H-3’,4’,5’,6’,7’,8’), 0.85 (t, J = 6.83 Hz, 3H, H-9”). 13C NMR (75 MHz, CDCl3) δ: 152.6 (C-2), 139.9 (C-7a), 137.9 (C-4a), 127.2 (C-6”), 123.0 (C-5’), 114.6 (C-4), 113.9 (C-7), 32.7 (C-1), 35.5 (C-2’). DIP-MS (ESI; M + Na) m/z (calculated): 309.11, m/z (measured): 309.05.

6.8. Procedure for the Synthesis of Compounds, 19

In a vial for microwave reaction equipped with a magnetic stirring bar, a mixture was made (0.15 mmol, 1 eq) and bromine acetyl bromide (0.47 mmol, 1.2 eq) were added. The reaction mixture was stirred at rt for 20 min. Subsequently, 40 mL of a 20% aqueous sodium carbonate solution was added and separated by liquid-liquid extraction with CH2Cl2 (2 × 10 mL). The combined organic phases were

N-(5-Chloropyridin-2-yl) Butamide, 19a

Yield: 91%. White solid; m.p. 102–104 °C. Rf = 0.64 (hexane/AcOEt 7:3). IR (CH2Cl2) ν = 3300.9, 1697.8, 1582.4 cm⁻¹. 1H NMR (300 MHz, CDCl3): δ: 8.41 (s, 1H, NH), 8.22 (d, J = 8.95 Hz, 1H, H-3’), 8.19 (d, J = 2.54 Hz, 1H, H-6’), 7.65 (dd, J = 8.95, 2.54 Hz, 1H, H-4’), 2.36 (t, J = 7.43 Hz, 2H, H-2), 1.74 (m, 2H, H-3), 0.98 (t, J = 7.38 Hz, 3H, H-4). DIP-MS (ESI; M + Na) m/z (calculated): 221.04, m/z (measured): 221.07.

N-(5-Chloropyridin-2-yl) Hexanamide, 19b

Yield: 87%. White solid; m.p. 103–106 °C. Rf = 0.73 (hexane/AcOEt 7:3). IR (CH2Cl2) ν = 3275.3, 1690.5, 1582.3 cm⁻¹. NMR 1H (600 MHz, CDCl3): δ: 8.37 (s, 1H, NH), 8.23 (d, J = 8.9 Hz, 1H, H-3’), 8.21 (d, J = 2.5 Hz, 1H, H-6’), 7.66 (dd, J = 8.9, 2.5 Hz, 1H, H-4’), 2.49 (t, J = 7.58 Hz, 2H, H-2), 1.73 (m, 2H, H-3), 1.35 (m, 4H, H-4,5), 0.90 (t, J = 7.13 Hz, 3H, H-6). NMR 13C (125 MHz, CDCl3) δ: 171.9 (C-1), 149.9 (C-2’), 146.3 (C-6’), 138.0 (C-4’), 126.5 (C-5’), 114.8 (C-3’), 37.7 (C-2), 31.3 (C-4’), 25.0 (C-3’), 22.4 (C-5’), 13.9 (C-6). DIP-MS (ESI; M+Na) m/z (calculated): 249.07, m/z (measured): 249.11.

N-(5-Chloropyridin-2-yl) Nonamide, 19c

Yield: 90%. White solid; m.p. 64–66 °C. Rf = 0.8 (hexane/AcOEt 7:3). IR (CH2Cl2) ν = 3271.7, 1687.1, 1582.7 cm⁻¹. 1H NMR (600 MHz, CDCl3) δ: 8.874 (s, 1H, NH), 8.25 (d, J = 8.88 Hz, 1H, H-3’), 8.19 (d, J = 2Hz, 1H, H-6’), 7.64 (dd, J = 8.88, 2 Hz, 1H, H-4’), 2.38 (t, J = 7.59 Hz, 2H, H-2), 1.70 (m, 2H, H-3), 1.28 (m, 10H, H-4,5,6,7,8), 0.85 (t, J = 6.88 Hz, 3H, H-9’). 13C NMR (125 MHz, CDCl3) δ: 172.1 (C-1), 150.1 (C-2’), 146.1 (C-6’), 138.7 (C-4’), 126.5 (C-5’), 115.0 (C-3’), 37.6 (C-2), 31.8, 29.3, 29.2, 29.1, 25.3 (C-3’), 22.6, 14.0 (C-9). DIP-MS (ESI; M+Na) m/z (calculated): 291.12, m/z (measured): 291.19.

6.9. Procedure for the Synthesis of N-Substituted 2-(4-formylphenoxy) Acetamides, 35

In a 50 mL ball flask loaded with magnetic stirring bar, 15 mL of CH2Cl2, the corresponding amine (3.91 mmol, 1 eq) and bromine acetyl bromide (4.70 mmol, 1.2 eq) were added. The reaction mixture was stirred at rt for 20 min. Subsequently, 40 mL of a 20% aqueous sodium carbonate solution was added and separated by liquid-liquid extraction with CH2Cl2 (2 × 10 mL). The combined organic phases were
dried with anhydrous Na$_2$SO$_4$ and filtered, then evaporated under vacuum. Once the residue was dry, it was transferred to a 100 mL ball flask equipped with a magnetic stirring bar and reacted with 4-hydroxybenzaldehyde (4.31 mmol, 1.1 eq) and DBU (3.91 mmol, 1 eq) at 70 °C for 6 h, employing acetonitrile (20 mL) as solvent. Upon completion of that period, the solvent was evaporated under reduced pressure, and 30 mL of a 5% HCl solution was added to the residue. Afterwards liquid-liquid extractions were carried out with AcOEt (3 × 10 mL) and the organic phases were dried with anhydrous Na$_2$SO$_4$ and filtered. The filtrate was evaporated under reduced pressure and the residue was purified by column chromatography, with silica gel as a stationary phase and a hexane/AcOEt mixture 7: 3 as the mobile phase.

N-(3-Chlorophenyl)-2-(4-Formylphenoxy) Acetamide, 35a

Yield: 65%. White solid. $^1$H NMR (600 MHz, DMSO-d$_6$) δ: 10.364 (s, 1H, NH), 9.89 (s, 1H, CHO), 7.90 (d, $J = 8.77$ Hz, 2H, H-3′′), 7.85 (t, $J = 1.81$ Hz, 1H, H-2′), 7.55 (d, $J = 8.11$ Hz, 1H, H-6′), 7.35 (t, $J = 8.11$ Hz, 1H, H-5′), 7.20 (d, $J = 8.77$ Hz, 2H, H-2′′), 7.14 (d, $J = 8.11$ Hz, 1H, H-4′), 4.89 (s, 2H, H-2), 13C NMR (150 MHz, DMSO-d$_6$) δ 191.7 (CHO), 166.8 (C-1), 163.1 (C-1′), 140.2 (C-1′′), 133.6 (C-3′), 132.2 (C-3′′), 130.9 (C-5′), 130.6 (C-4′′), 123.9 (C-4′), 119.7 (C-2′), 118.5 (C-6′), 115.6 (C-2′′), 67.5 (C-2). DIP-MS (ESI; M-H) $m/z$ (calculated): 288.04, $m/z$ (measured): 288.05.

N-(4-Chlorobenzyl)-2-(4-Formylphenoxy) Acetamide, 35b

Yield: 73%. White solid. $^1$H NMR (600 MHz, DMSO-d$_6$) δ: 9.98 (s, 1H, CHO), 8.77 (t, $J = 6$ Hz, 1H, NH), 7.89 (d, $J = 8.72$ Hz, 2H, H-3′), 7.37 (d, $J = 8.4$ Hz, 2H, H-4′), 7.29 (d, $J = 8.4$ Hz, 2H, H-3′′), 7.16 (d, $J = 8.7$ Hz, 2H, H-2′′), 4.71 (s, 2H, H-2), 4.34 (d, $J = 6$ Hz, 2H, H-1′). 13C NMR (150 MHz, DMSO-d$_6$) δ 191.3 (CHO), 167.2 (C-1), 162.5 (C-1′′), 138.2 (C-2′), 131.6 (C-3′′), 131.3 (C-5′), 130.1 (C-4′′), 129.0 (C-3′), 128.1 (C-4′), 115.2 (C-2′′), 66.9 (C-2), 41.1 (C-1′). DIP-MS (ESI; M-H) $m/z$ (calculated): 302.05, $m/z$ (measured): 302.09.

2-(4-Formylphenoxy)-N-propyl Acetamide, 35c

Yield: 79%. White solid. $^1$H NMR (500 MHz, DMSO-d$_6$) δ: 9.87 (s, 1H, CHO), 8.15 (s, 1H, NH), 7.87 (d, $J = 8.7$ Hz, 2H, H-3′), 7.13 (d, $J = 8.7$ Hz, 2H, H-2′), 4.60 (s, 2H, H-2), 3.08 (dd, $J = 13.15$, 6.84, 2H, H-2′), 0.82 (t, $J = 7.42$ Hz, 3H, H-3′). 13C NMR (125 MHz, DMSO-d$_6$) δ 191.786 (CHO), 167.3 (C-1), 163.1 (C-1′), 132.1 (C-3′′), 130.5 (C-4′′), 115.6 (C-2′′), 67.5 (C-2), 40.6 (C-1′), 22.8 (C-2′), 11.7 (C-3′). DIP-MS (ESI; M + Na) $m/z$ (calculated): 244.09, $m/z$ (measured): 244.10.

6.10. **Synthesis of N-Substituted 2-(4-(imidazolin-2-yl)-phenoxy) Acetamides**, 32

Once the aldehydes (1.20 mmol, 1 eq) were obtained, they were placed in a 100 mL ball flask containing 50 mL of acetonitrile. Afterwards, they were subjected to a reaction with ethylenediamine (1.20 mmol, 1 eq) with ultrasound for 15 min. Upon completion of this time, NBS (1.45 mmol, 1.2 Eq) was added to the reaction mixture and it was reacted by ultrasound for 10 min. Then the solvent was evaporated under reduced pressure, followed by the addition of 30 mL of an aqueous solution of 20% NaOH. The resulting suspension was stirred for 20 min and vacuum filtered. The solid obtained was washed with 40 mL of distilled water (2 × 20 mL) and oven-dried at 40 °C.

N-(3-Chlorophenyl)-2-(4-(Imidazolin-2-yl) Phenoxy) Acetamide, 32a

Yield: 88%. Orange solid; m.p. 192–195 °C. R$_f$ = 0.17 (MeOH/NH$_4$OH 20:1). IR (CH$_2$Cl$_2$) $\nu$ = 3584.6, 3203, 1655.9, 1532.8 cm$^{-1}$. $^1$H NMR (500 MHz, DMSO-d$_6$) δ: 10.47 (s, 1H, NH), 7.84 (t, $J = 1.99$ Hz, 1H, H-2′), 7.77 (d, $J = 8.7$ Hz, 2H, H-3′), 7.54 (m, 1H, H-6′), 7.33 (t, $J = 8.12$ Hz, 1H, H-5′), 7.13 (m, 1H, H-4′), 7.03 (d, $J = 8.7$ Hz, 2H, H-2′′), 4.77 (s, 2H, H-2), 3.56 (s, 4H, H-7′′). 13C NMR (125 MHz, DMSO-d$_6$) δ 167.2 (C-1), 163.6 (C-5′′), 159.7 (C-1′′), 140.3 (C-1′′′), 133.5 (C-3′), 130.9 (C-5′), 129.1 (C-3′′), 124.3 (C-4′′), 123.8 (C-4′), 119.6 (C-2′), 118.5 (C-6′), 114.7 (C-2′′′), 67.4 (C-2), 49.9 (C-7′′). DIP-MS (ESI; M-H$_2$O + MeOH) $m/z$ (calculated): 344.11, $m/z$ (measured): 344.20.
N-(4-Chlorobenzyl)-2-(4-(Imidazolin-2-yl) Phenoxy) Acetamide, 32b

Yield: 90%. Yellow solid; m.p. 149–155 °C. R₁ = 0.2 (MeOH/NH₄OH 20:1). IR (CH₂Cl₂) ν = 3250.1, 1594.5, 1545.1, 1516.9, 839.1 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆) δ: 8.74 (t, J = 6.14 Hz, 1H, NH), 7.79 (d, J = 8.78 Hz, 2H, H-3′′), 7.38 (d, J = 8.4 Hz, 2H, H-4′′), 7.29 (d, J = 8.4 Hz, 2H, H-3′), 7.02 (d, J = 8.78 Hz, 2H, H-2′′), 4.62 (s, 2H, H-2), 4.35 (d, J = 6.14 Hz, 2H, H-1′′), 3.59 (s, 4H, H-7′′). ¹³C NMR (125 MHz, DMSO-d₆) δ 168.1 (C-1), 163.6 (C-5′′), 159.6 (C-1′′), 159.6 (C-1′′′), 154.5 (C-1′′), 155.1 (C-1′′), 131.6 (C-5′), 129.6 (C-3′), 129.1 (C-3′′), 128.6 (C-4′), 124.3 (C-4′′), 114.8 (C-2′′), 67.4 (C-2), 49.8 (C-7′′), 41.7 (C-1′). DIP-MS (ESI; M+H) m/z (calculated): 344.11, m/z (measured): 344.02.

2-(4-(Imidazolin-2-yl) Phenoxy)-N-Propyl Acetamide, 32c

Yield: 85%. White solid; m.p. 175–178 °C. R₁ = 0.15 (MeOH/NH₄OH 20:1). IR (CH₂Cl₂) ν = 3376.1, 3202.8, 1659.4, 1615.4, 1537.3, 1519.5 cm⁻¹. ¹H NMR (500 MHz, DMSO-d₆) δ: 8.10 (t, J = 5.36 Hz, 1H, NH), 7.75 (m, 2H, H-3′′), 6.91 (m, 2H, H-2′′′), 4.50 (s, 2H, H-2), 3.56 (s, 4H, H-7′′), 3.08 (m, 2H, H-1′), 1.43 (m, 2H, H-2′), 0.81 (t, J = 7.42 Hz, 3H, H-3′). ¹³C NMR (125 MHz, DMSO-d₆) δ 167.7 (C-1), 163.6 (C-5′′), 159.6 (C-1′′), 159.6 (C-1′′′), 129.0 (C-3′′), 124.1 (C-4′′), 114.7 (C-2′′), 67.4 (C-2), 49.7 (C-7′′), 40.5 (C-1′), 22.8 (C-2′), 11.7 (C-3′). DIP-MS (ESI; M+H) m/z (calculated): 262.15, m/z (measured): 262.04.

6.11. Preparation of Culture Media, Test Compounds, and Inoculum

The Luria Bertani (LB) broth was prepared in one liter of distilled water by adding 10 g peptone, 5 g yeast extract, and 5 g NaCl and then sterilized in an autoclave at 15 psi and 121 °C for 15 min. For the LB solid medium, 15 g of bacteriological agar was added and the solution was made up to a liter with distilled water. Thioglycolate medium was prepared according to the fabricant specifications. For the LB solid medium, 15 g of bacteriological agar was added and the solution was made up to a liter with distilled water. Thioglycolate medium BBL was prepared according to the fabricant specifications. The amount of each test compound required for a concentration of 100 mM Pleas was weighed. With the resulting solution, serial 1:10 dilutions were prepared to obtain concentrations 1000 µM, 100 µM and 10 µM.

6.12. Preparation of CV026 Suspensions

C. violaceum CV026 from a cryovial was streaked onto L-agar plates containing 30 µg/mL kanamycin. Bacteria were incubated at 29 °C for 24 h. The next day an isolated colony was inoculated into 5 mL LB medium with 30 µg/mL kanamycin, followed by incubation at 29 °C and 200 rpm for 15 h.

6.13. Evaluation of Compounds as Quorum Sensing Inhibitors in Chromobacterium violaceum CV026

C. violaceum CV026 was cultured in 50 mL of thioglycolate medium until reaching an optical density of 0.12 to 0.60 nm, then 430 µL of an 80 µM C6-AHL solution were added (680 nM final concentration) to the broth. Subsequently 1980 µL of the suspension was transferred to a 5 mL tube and 20 µL of the dilutions of the test compounds were added until reaching the final concentration of 1000 µM, 100 µM, 10 µM. Tubes were incubated at 29 °C and 800 rpm for 16 h. Upon completion of the incubation time, cell density was determined by absorbance at 720 nm by using the thioglycolate medium as the blank. Finally, the absorbance of violacein was measured. 500 µL of the bacterial culture was placed in a 2 mL tube and then 500 µL of acetone were added. The tubes were vortexed and centrifuged at 15,000 rpm for 1 min. The specific production of violacein was calculated by dividing the value of the reading at 577 nm by that at 720 nm. Experiments with additional concentrations were performed in order to determine the IC₅₀ values. Each experiment was performed 6 times/compound, and the results were graphed. Statistical significance was analyzed by Student’s t-test.

6.14. Modeling and Optimization of Ligands.

Docking studies of the target compounds were carried out on the CviR protein (PDB code: 3QP6).
Prior to docking, the ligands were drawn with the ACD/ChemSketch program [51]. A geometric pre-optimization was created in 3D maintaining the stereochemistry. The 3D structures were submitted to a geometric and energetic optimization at the AM1 (Austin Model 1) semiempirical level using the Gaussian 09 program [52]. The output files were saved as (PBD).

6.15. Molecular Docking

The docking studies were performed on the AutoDock 4.2 program, which maintains the macromolecule rigid while allowing flexibility in the ligand. Polar hydrogens were added to the ligand, and protein atoms and partial charges were assigned to the receptor (Kollman) and ligand (Gasteiger). Blind docking was achieved using a grid box of $126 \text{ Å}^3$ with a grid spacing of $0.375 \text{ Å}^3$ using the Hybrid Genetic Algorithm of Lamarckian with an initial population of 100 randomized individuals and a maximum number of energy evaluations of $10^7$. The results of these docking simulations experiments were examined on AutoDockTools version 1.5.2 and PyMol visualizer to describe the non-bond interaction from the ligand-protein complex.

7. Conclusions

Compounds 16a–c, 17a–c, 18a–c, 19a–c and 32a–c were synthesized in moderate to very good global yields, finding MW energy and ultrasound to be extremely useful for the corresponding reactions. The synthesis of the ethyl 2-acylamino thiazole-4-carboxylate 16a–c, was improved using MW in the acylation of the electronically deactivated amino, obtaining higher yields than those reported previously. A methodology was established in the current contribution for the synthesis of N-[4-(thiazolin-2-yl) -phenyl]-carboxamides using MW as an energy source and obtaining 3 new compounds. Three novel benzimidazoles 18a–c were synthesized. A new methodology was developed for the synthesis of N-(5-chloropyridin-2-yl) amides using MW, obtaining higher yields than those in reported procedures and in shorter times. A new methodology was established for the synthesis of α-phenoxy-imidazolin-2-yl acetamides, involving better yields and shorter times at all reaction stages and achieving 3 new compounds, 32a–c.

Docking and biological data were in good agreement, which confirms that 16b–c, 17a–c, 18a–c, 19a–c, and 32a–c conduct as bioisosteres of AHL. Such behavior is not attributed to the atom-by-atom or group by group interaction of these compounds with the CviR protein, but rather to the total supramolecular interplay of the compounds with the receptor. Nevertheless, the importance of the $\pi-\pi$ interactions emphasizes the fundamental role of the aromatic ring in the inhibitory activity.

The inhibitory activity of quorum sensing shown by the active compounds ranges from low to good, highlighting the activities of benzimidazole 18a and pyridine 19c.

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Abbreviations, Acronyms and Symbols

QS  quorum sensing  
QSI quorum sensing inhibitor  
QSIs quorum sensing inhibitors  
AHL microwave irradiation  
MW microwave irradiation  
ΔG affinity energy  
UV ultraviolet  
IR infrared  
IR infrared  
NMR Nuclear Magnetic Resonance  
DIP-MS Direct Insertion Probe-Mass Spectrometry  
ESI Electrospray Ionization

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