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Theoretical and structural analysis of the active site of the transcriptional regulators LasR and TraR, using molecular docking methodology for identifying potential analogues of acyl homoserine lactones (AHLs) with anti-quorum sensing activity

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ABSTRACT

In the present study the homology of transcriptional receptors LuxR type were evaluated using as point of reference the receptors TraR and LasR of the bacterial types *Agrobacterium tumefaciens* and *Pseudomonas aureginosa* respectively.

A series of alignments were performed in order to demonstrate that the active site of the protein is conserved in wide range of gram negative bacteria. Moreover, some docking calculations were carried out for analogs of the acyl homoserin lactones (AHLs) and regulatory proteins LasR and TraR, to understand the complex microenvironment in which the ligands are exposed. The molecular alignments show clearly that there are preserved motifs in the residues (Y53, Y61, W57, D70, W85 to TraR, Y56, Y64, W60, D73, W88 to LasR) analyzed, which may serve as site-specific targets for the development of potential antagonists. In this study was found that the anti-quorum sensing activity of the AHLs molecular analogs appears to depend on; the structure of the lactone ring and on appropriate combination of absolute and relative stereochemistry of the carbonyl (C═O) and amide (NH_2) groups of the side chain of these AHLs molecular analogs, in combination with the interactions with the conserved amino acids (D73, W60, Y56, S129 to LasR and D70, W57, Y53 to TraR) of the LuxR type protein family.

1. Introduction

Since was discovered more than three decades ago that bacterium may communicate via release and detection of diffusible chemical signals [1–6] the approach to study and analyze bacterium life have changed. These chemicals signals can stimulate diverse kinds of behaviors, including bioluminescence, horizontal transfer of DNA, biofilm formation, production of pathogenic factors, antibiotics resistance, and other secondary metabolites, among others [7–10]. This type of behavior in bacterium is now known as quorum sensing (QS). The term "quorum sensing" was coined to describe this kind of behavior, because the population must reach a "quorum level" before some of these mentioned fact can be produced [11–13]. Even though the phenomenon of QS was first recognized more than 30 years ago in *Vibrio fischeri* bacteria the significance of the observations have taken some time to become widely recognized as a scientific fact [5,6].

In Gram-negative bacterium, QS often involves the production of acyl-homoserine lactones (AHLs) [14]. In such system, binding of an acyl-homoserine lactone signal to its cognate transcriptional regulator (R-protein) frequently induces stabilization and subsequent dimerization of the R-protein, which results in the regulation of downstream gene expression. Accumulation of the AHL molecules to a threshold concentration, which corresponds to bacteria reaching a critical density via reproduction, results in the full activation of the AHL-binding transcription factor, and it is thereby rendered capable of inducing gene expression for functional regulation [14–20].

In this study was chosen the mechanism of QS in *Pseudomonas aeruginosa* (*PA*) and *Agrobacterium tumefaciens* (*AT*), due to clinical relevance of *PA* [21,22] and besides for the vast amount of information of structural and biochemical mechanisms of *AT* [14,23,24]. Transcriptional regulators of *PA* and *AT* are LasR and TraR respectively. Different types of analogues have been synthesized and evaluated as potential inhibitors of QS by several research groups [14,25–28]. In order to shed some light onto understanding of the molecular interaction between molecules analogs and regulators of the QS, were carried out a series of studies of molecular docking

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                    70        80        90       100       110       120
                     |         |         |         |         |         |
CepRxx3      YGIRVPLPVS---KPAVAIFDTYPDGWMAHYQAQNYIEIDSTVRDGALNTNMIVWPDVDR
SolRxx8      YGARMPLPVS---KPAVAIFDTYPAGWMQHYQANGFLDIDPTVRAGASSGDMIVWPVSIR
SdiAxx7      LCVRHPVPFT---RPKVAFYTNYPEAWVSYYQAKNFLAIDPVLNPENFSQGHLMWNDDLF
ExpRxx5      LNKKNPS---------EMLIISSYPDEWVNLYKENKYQHIDPVVLASFNKISPFSWEKSLV
ExpRxx6      MNKKKPT---------DVVIISNYPSEWVEIYRSNNYQHIDPVILTAINKISPFSWDDNLV
yenRxx11     MNKKTPL---------HPTIISNYPLDWVKKYKKNSYHLIDPVILTAKDKVAPFAWDDNSV
LasRxx0      FGLLPKDSQD---YENAFIVGNYPAAWREHYDRAGYARVDPTVSHCTQSVLPIFWEPSIY
LuxRxx2      LAIIYPHSMV---KSDISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIFEN
CerRxx10     YATTSPMTG------AVQGYANYPDSWKMHYMRRNLHRVDPTIHKSALSIAPVDWSRFER
RaiRxx9      IAYGSPCDLAGATDSNPLLMLTYPPEWVKQYRDRDYFSIDPVVRLGRRGFLPVEWSASGW
TraRxx1      YLHIQHK---------HTIAVTNYHRDWRSAYFENNFDKLDPVVKRAKSRKHVFAWSGEQE
CviRxx4      LALGRLNNQNQIQRLERVLNVSYPSDWLDQYMKENYAQHDPILR-IHLGQGPVMWEERFN
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Fig. 1. Multiple alignment of amino acid sequences of 12 members of the family of LuxR-type transcriptional receptors. In Table 1 shows each regulator and the bacterium to which it belongs. Here is showed just one segment of the alignment, which is demonstrated experimentally, join the autoinducers. The identical residues are shown in a red row, they are involved in the reception of the ligand. The alignment was generated using Clustal-W.

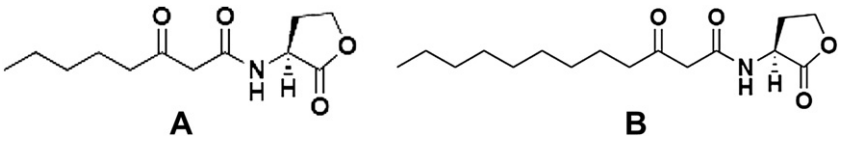

Fig. 2. Structure of the 2D native autoinductores for both proteins LasR y TraR. A *N*-(3-oxo-octanoil)-L-homoseryn lactone and B. *N*-(3-oxo-dodecanoil)-L-homoserine lactone.

among several similar AHLs and regulatory proteins LasR and TraR in order to get inside [29,30], and understand microenvironment in which they are exposed to these analogues, with the purpose to obtain information which may assist the « *in silico* » design of antagonists quorum sensing [31,32].

2. Materials and methods

All calculations were performed through a set of computer programs such as SYBYL 7.0 [33], Pymol [34], Gaussian 03 [35], GOLD version 2.1 [36], y FlexX [37,38]. The molecular analogues of AHLs that experimentally showed inhibitory activity against the quorum sensing of *AT* and *PA* were optimized using the program Gaussian 03, with the semiempirical method PM3 [39].

The amino acids (AA) sequences of transcriptional regulators LasR and TraR were obtained from the NCBI [40] and subjected to a bioinformatic analysis on the ExPASy web server [41]. A multiple alignments of the AA Sequences of transcriptional regulators in several Gram-negative bacteria were made using the web server Clustal-W [42] to find regions conserved between these proteins. The 3D structures of the transcriptional regulators LasR and TraR were obtained from the Protein Data Bank (PDB) with codes (1L3L to TraR and 2UV0 for LasR) [43,44]. The 3D structures of LasR and TraR were analized and optimized using the force field of molecular mechanics MMFF94s with the Gasteiger-Marsili charges, using the SYBYL 7.0 program. The docking studies were performed using the programs FlexX and Gold. The site on which the docking was chosen to be carried out was a radius of 3.5 Å of the ligand in the case of FlexX and 10 Å in the case of Gold. The results of the docking calculations were analyzed using the module Cscore (Consensus Score) module in SYBYL, allowing the most robust and accurate possible evaluation of the conformations for ligand–receptor interactions [45], found about 30 conformations for each ligand. The interactions between the ligand–protein complexes obtained were displayed and analyzed using the Pymol program [46].

3. Results and discussion

The results of the multiple alignments (Figs. 1 and 2), showed that the protein LasR and TraR have a remote homology with the proteins belonging to the family transcriptional regulators type

Table 1
Some organisms possessing LuxI/LuxR homologues: the regulatory proteins and the HSL autoinducers.

Organism	Transcriptional regulating mechanism	Autoinducer
Pseudomonas aeruginosa	LasR	*N*-(3-oxododecanoyl)-HSL
Agrobacterium tumefaciens	TraR	*N*-(3-oxooctanoyl)- HSL
Escherichia coli	SdiA	*n*-octanoyl-L-homoserine lactone
Aeromonas salmonicida	AsaR	*N*-butanoyl-HSL
Erwinia chrysanthemi	ExpR	*N*-(3-oxohexanoyl)- HSL
Yersinia enterocolitica	YenR	*N*-hexanoyl-HSL, *N*-(3-oxohexanoyl)-HSL
Vibrio fischeri	LuxR	*N*-(3-oxohexanoyl)- HSL
Rhizobium etli	RaiR	Multiple, unconfirmed
Chromobacterium violaceum	CviR	*N*-hexanoyl-HSL
Rhodobacter sphaeroides	CerR	7,8-*cis*-*N*- (tetradecanoyl)-HSL

LuxR [47], this remote homology may be related to the divergent evolution of each one. Such differences exist among the likely transcriptional regulators since each system has been optimized to provide survival in the specialized niche in which a particular species of bacteria reside [10,48].

In Table 1 is depicted some gram-negative bacteria with their respective regulatory proteins and their specific autoinducers (AI) [19,49].

Based on the results of multiple alignments it can be deducted that amino acids sequences are preserved and play a similar role in the function of this protein family. In Fig. 1 it is shown that some amino acids are kept identical in all proteins, these are the ones which are involved in forming interactions such as hydrogen bridges with autoinducers (AI) AHLs, This suggests that the active site of the protein in this family is kept, in addition to believe that all the protein-type LuxR can have a very similar 3D structure on

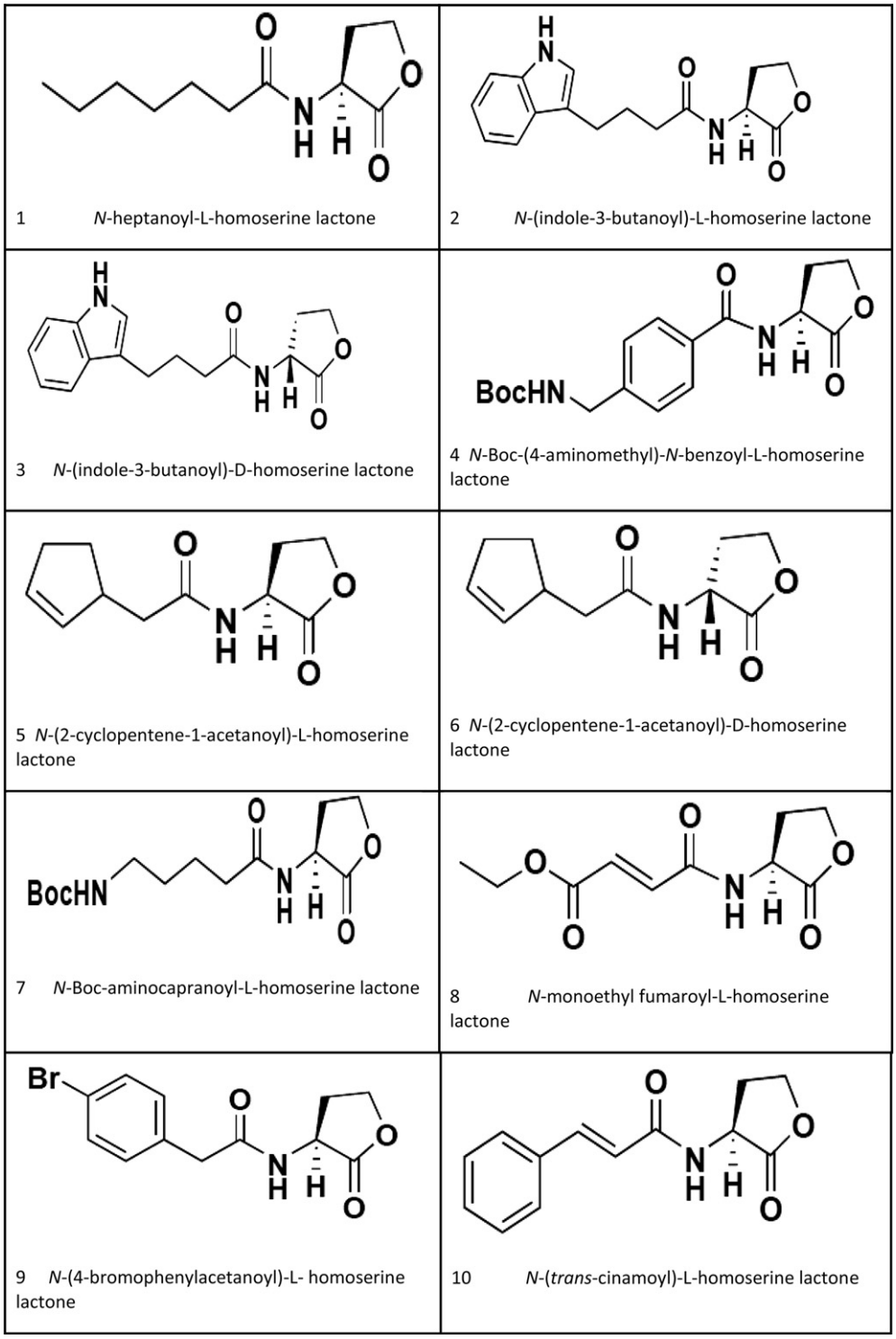

Fig. 3. Structure of molecular analogues of AHL.

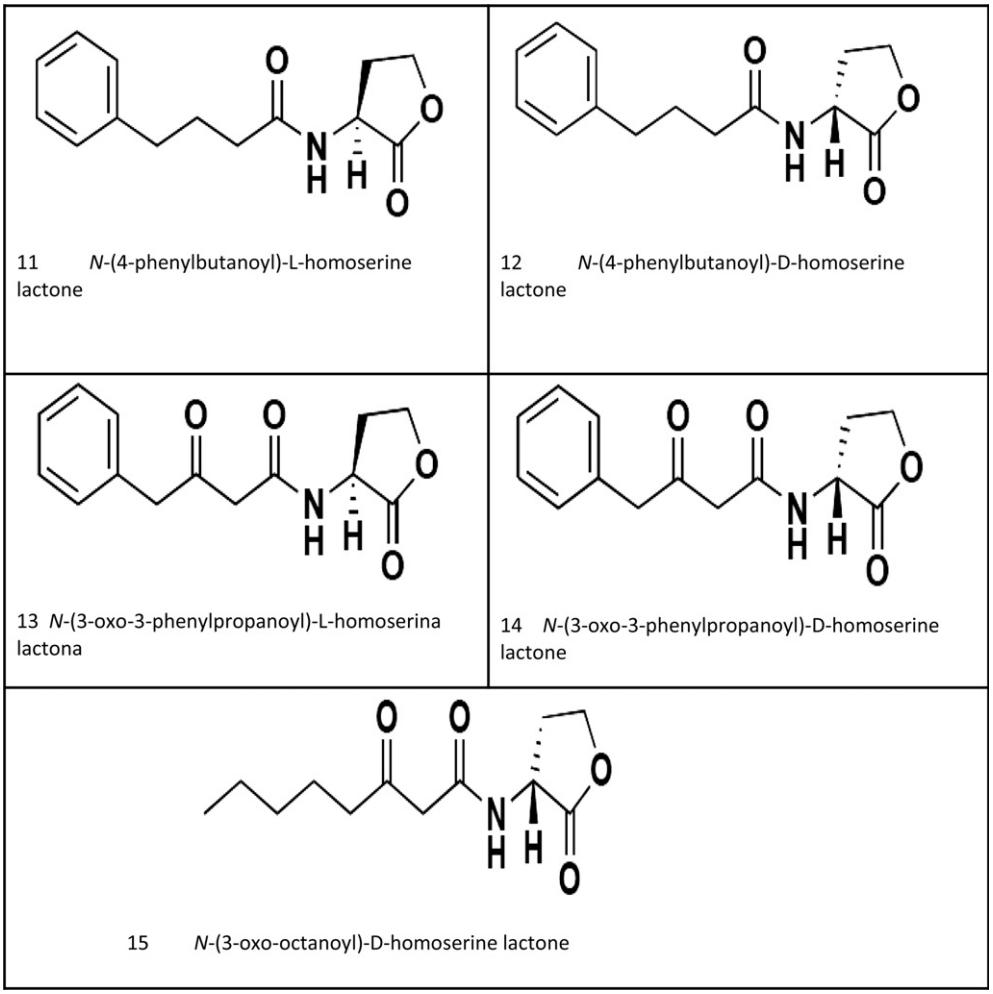

Fig. 3. (*continued*).

the site of the autoinducers receiver and also have the same interactions with the AHLs characteristics. This also allows us to establish that all the protein LuxR type can show a very similar 3D on the receptor site of the auto-inducing and also have the same characteristic interactions with the AHLs.

Some studies have shown [50,51] that the protein family LuxR-type regulates the transcription of key genes, which control the bacterial virulence by interaction with auto-inducing AHLs, directing the dimerization and subsequent formation of DNA-AHLs-transcriptional regulators [52]. It is known that complexes regulators LasR [28], TraR [53] and SdiA [54], the first two solved by the technique of X-ray diffraction and the last one through NMR technique. The analysis of these three proteins in the region where it bind to the AI reveals a high structural homology and also show that keys residues interact with the native ligand, are preserved and located in the same position in all three active sites (Y53, Y61, W57, D70, W85 to TraR, Y56, Y64, W60, D73, W88 to LasR and Y63, Y71, W67, D80, W95 for SdiA).

Table 2
Comparison of the Residues involved in the Active Site of TraR and LasR. The residues marked in red are identical, green very similar in characteristics; blue slightly similar and unmarked refer to non-conserved residues.

TraR	LasR	TraR	LasR	TraR	LasR
Y37	I35	Q58	R61	F101	F101
A38	L36	S59	E62	Y102	F102
Y39	F37	Y61	Y64	A105	A105
L40	G38	D70	D73	S106	S106
A49	I52	P71	P74	I110	L110
Y53	Y56	V72	T75	T115	T115
H54	P57	V73	V76	F128	L128
W57	W60	W85	W88	T129	S129

3.1. Conformational analysis and studies of docking

The docking analysis between proteins and the analogues (Fig. 3) was performed and some minor modification on LasR and

Table 3
IC_{50} inhibition values for AHL derivatives in *Agrobacterium tumefaciens* and *Pseudomonas aeruginosa*.

Ligands	*A. tumefaciens* IC50 (μM)	*P. aeruginosa* IC50 (μM)
1	0.13	51.50
2	1.10	14.80
5	1.00	33.10
9	0.25	16.10
13	0.50	53.60

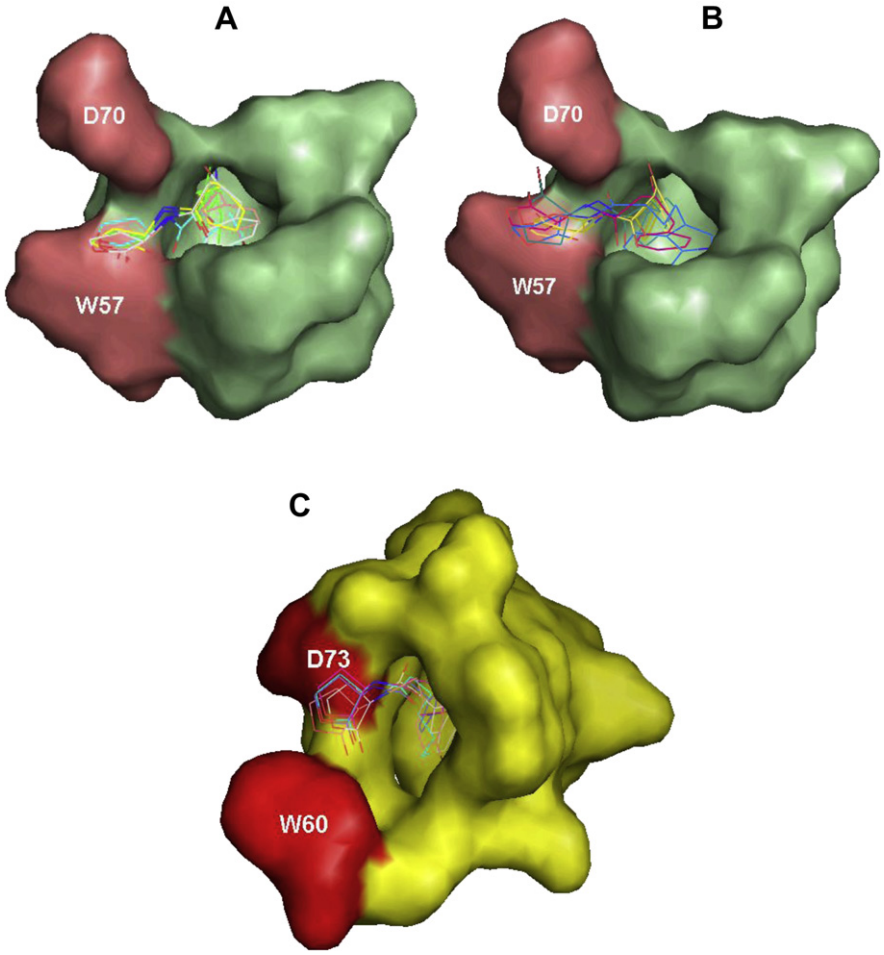

Fig. 4. Active site of transcriptional regulators LasR and TraR are shown. A) Shows the superposition of analogues 1, 2, 5, 9 and 13 in the active site of the receptor TraR, it can be seen the proper orientation of the amino and carboxyl groups of ligands to the amino acids, D70 and W57. B) Shows the superposition of different analogues in the active site of regulator TraR. It can be seen a misdirection in groups of ligands that form hydrogen bridges. C) Shows the overlap of analogues 1, 2, 5, 9 and 13 in the active site of the receptor LasR. It can be seen the proper orientation of the amino and carboxyl groups of ligands to the amino acids D73 and W60.

TraR molecules were made, such as adding hydrogen atoms, eliminating water molecules, eliminating LP (pairs free of heavy atoms) after that, both proteins were optimized (1L3L to TraR and 2UV0 for LasR) (minimizing geometric). Based on the studies [55,56] and papers, on LasR and TraR, it was selected the amino acids involved in the binding site, in order to carry out studies of molecular docking on this site, were taken the amino acids that were found in a radius of about 3.5 Å around of the native ligand of TraR. In the same way was done to LasR. In Table 2 are depicted the amino acids to each case.

The geometries of molecular analogues of AHLs were optimized using the semiempirical method PM3, in the same way was done with the IAs native, LasR and TraR, the structure of the 2D native autoinductores for both proteins are shown below.

The structures of 2D molecular analogues of AHLs with inhibitory activity against quorum sensing in *AT* and *PA* are shown in Fig. 3. These molecular analogues are similar in nature, and have in common that keep the lactone ring. This is in line with the experimental studies that have shown that these molecular analogues retaining the lactone ring [57], is recognized by several amino acids involved in the active site of this family of proteins, and taking into account that this family of proteins is not when folded completely bind with their AHL native this is crucial to complete the folding of the protein, it is logical to think that this protein alone interact with ligands that have very similar basic structure of the AHL native, designed in this way with similar structure and to prevent the proper functioning of the protein, we would be talking about a kind of alosteric inhibition [58].

Different side chains of these molecular analogues of AHLs could be responsible for allowing or not the proper folding of protein in question and thus, controlling the activity of the protein based on the fact that the structure and function of proteins is closely related. Among these analogues only the ligands 1, 2, 5, 9 and 13 show activity (Table 3) of anti-quorum sensing and amongst these ligands 1 and 13 are already known as inhibitors. In the results part the molecular docking will analyze the microenvironment of different analogues, the structure of each analogue and its position in the active site.

3.2. *Docking between regulators TraR and LasR ligands analogues AHL*

As can been seen in Fig. 4 the active site of transcriptional regulators LasR and TraR have been divided into two regions, the first one is a polar region (red) and the second one is a hydrophobic region (green) in the case of TraR and yellow to LasR. The docking with the 15 AHL analogues showed that only the ligands 1, 2, 5, 9 and 13 have very similar poses to those taken by native

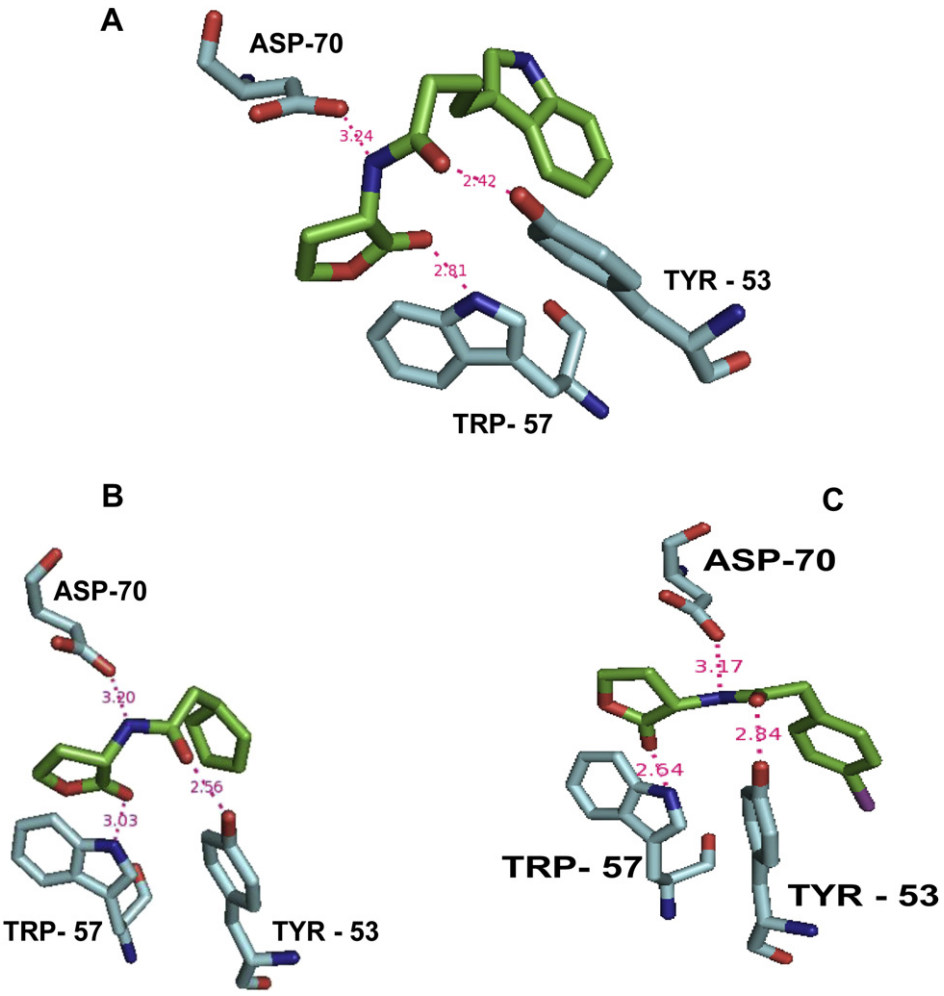

Fig. 5. Docking results of analogues 2, 5, and 9 in transcriptional active receptor site of TraR.

ligands in active site of transcriptional regulators LasR and TraR, with its amino group (NH_2) with orientation to aspartic acid (D), where they form an interaction with hydrogen atoms and carboxyl group (C═O) oriented to tryptophan (W) with the interaction forming another kind of bridge hydrogen (Fig. 4a and c), while in Fig. 4b it is shown the wrong orientation of the other ligands, the particular case of TraR, for LasR is the same, these results are consistent with the experimental data where only these 5 ligands show high inhibitory activity, based on these results alone will analyze the interactions of these ligands in particular ligands 2, 5 and 9, since these inhibitors have been recently found out [14].

The docking analysis to ligands 2, 5 and 9 and the protein TraR show that in all cases the hydrogen bridges are present in the structure co-crystallized protein–ligand in the native protein code 1L3L Data Bank, also an interaction with the residue Tyr-53 is formed as shown in Fig. 5, (a residue conserved in this family of proteins see Fig. 1), this interaction with Tyr-53 is due to the good location of the side chains the ligands in the hydrophobic pocket of the active site of the transcriptional regulator TraR, allowing not only the interactions of hydrogen bridge, but also a large number of hydrophobic interactions, since the side chains of these ligands are very hydrophobic. These interactions combined with the type of Hydrogen Bridge provide stability to protein–ligand complex formed in all cases.

In the analysis of docking results for ligands 2, 5 and 9 and the protein LasR, was found the interactions type hydrogen bridge that are present in the co-crystallized structure protein–ligand under native code 1L3L in PDB. Besides also show two types of attractive interactions between ligands and residues Ser129 and Tyr-56 as is shown in Fig. 6. Residue Tyr-56 as mentioned previously is a conserved residue of the family of LuxR type regulators transcriptionals, as well as show the appropriate location of the side chains of the ligands in the hydrophobic pocket of the receptor is crucial for transcriptional LasR to have an extra attractive interactions but in this case not only with Tyr-56 but also with Ser-129, these extra interactions are responsible for the stability of protein–ligand complex produced by our docking results.

Attractive interactions with the residue Tyr-53, Ser-56 and 129 in the case of LasR are coupled with the interactions occurring between hydrophobic side chains of aromatic and bulky ligands. These ligands are possibly responsible for the antagonist activity of such analogs as ligands that interact with conserved residues of this protein family and is known by experimental data that the interaction between the ligand and the native residues are crucial for the proper folding protein, therefore, we can affirm that if similar to the native ligand interacting with the conserved residues of this protein family protein folding is incorrect and will generate a conformational change which affects the biological function of the protein, keeping in mind that protein dimerizes and then binds to DNA.

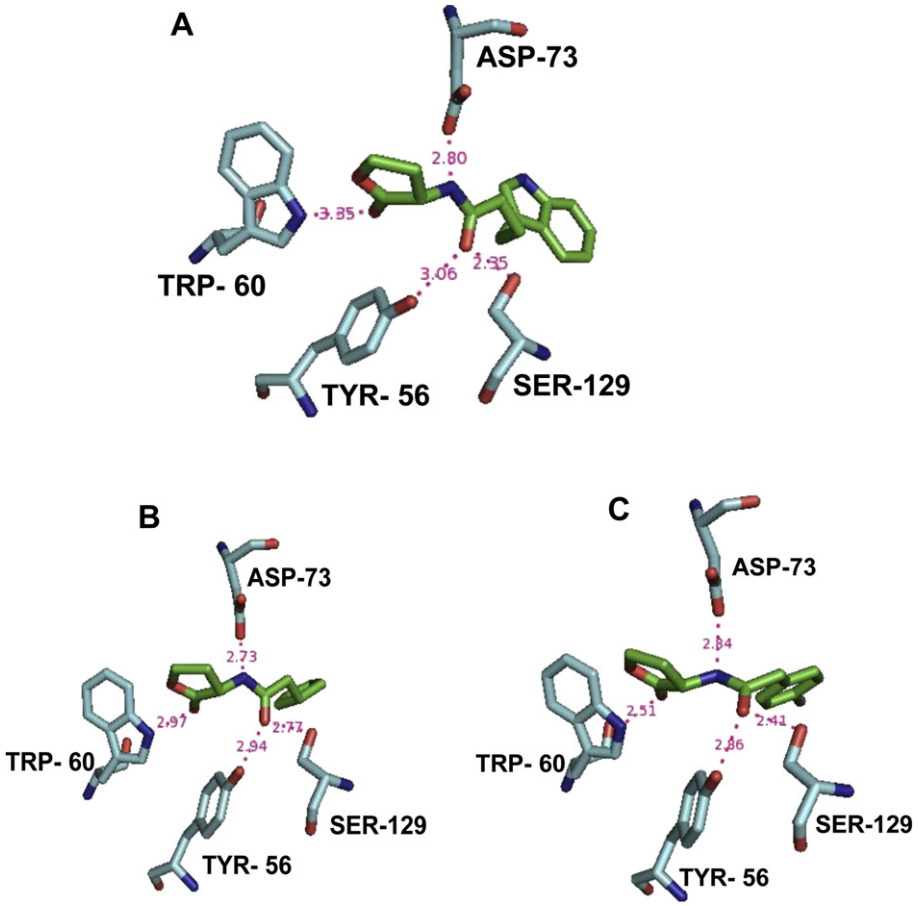

Fig. 6. Docking results of analogues 2, 5, and 9 in transcriptional LasR active site.

4. Conclusions

The docking studies were possible due to structural homology of the family of LuxR-type protein, allowing the use of proteins TraR and LasR as molecular reference to carried out studies of docking molecular with derivatives of AHLs. Our studies shows the importance of conserved residues in the interaction with the possible inhibitors, therefore these residues can be used as specific targets for the development of potential antagonists.

In this study was found that the activity anti-quorum sensing of the AHLs molecular analogs may depend on the structure of the lactone ring, on appropriate combination of absolute and relative stereochemistry of the carboxy (C═O) and amide (NH_2) groups of the side chain of these molecular analogs of AHLs in combination with the interactions with the conserved amino acids (D73, W60, Y56, S129 to LasR and D70, W57, Y53 to TraR) of the LuxR type protein family.

This study also opens perspectives for the rational design and computational screening of new potential modulators of QS using the transcriptional regulators TraR and LasR as speciphics targets. These result may be used to study some mechanism aspects of QS on different kind of pathogen bacteria, this can be done based on fact that transcriptional regulators of Gram bacteria have a high level of homology between them and also they are very conserved.

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