

# VIRTUAL SCREENING: IDENTIFICATION OF COMPOUNDS WITH POSSIBLE QUORUM SENSING AGONISTIC ACTIVITY IN *Pseudomonas aeruginosa*

CRIBADO VIRTUAL: IDENTIFICACIÓN DE POSIBLES COMPUESTOS CON ACTIVIDAD MODULADORA DEL QUORUM SENSING EN *Pseudomonas aeruginosa*

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## ABSTRACT

**Background:** *Quorum sensing* (QS) is a cell density dependent mechanism that allows bacteria to regulate the expression of specific genes in response to changes in their population density, thus controlling their activities in order to produce a response as a unit multicellular. These responses include production of virulence factors, formation of biofilm, bioluminescence, sporulation, among other behavior. **Objectives:** The objective of this work was to obtain pharmacophore models able to filter and identify molecules with possible agonist activity of uorum sensing and to find possible candidates based on calculations of molecular docking. **Methods:** The structure of the receptor was taken from the Protein Data Bank (PDB). The program *AutoDock 4.2* was used to perform docking calculations. The 3D structure of the ligand *TP1* was extracted from the complex co-crystallized identified with the code PDB 3IX3. The geometries of ligands were optimized using the PM3 semiempirical method. **Results:** Two pharmacophoric models were designed, the first one was made using the most active compound (*TP-1*), highlighting the most important chemical characteristics for molecular recognition. The second model was based on the alignment of three of the most active ligands (*TP-1*, *TP-3* and *TP-4*). These models were used as a filter in a screening on a database of conformations of several compounds with possible agonist activity of the main circuit of QS present in *Pseudomonas aeruginosa*. The pharmacophoric model based on alignment of the most active compounds showed greater capacity to select or identify compounds exhibiting significant structural and chemical characteristics to be considered possible *hit*. With this model, 22 compounds were identified. These compounds were subjected to a series of calculations of docking. The outcomes of the *docking* were used to identify interactions making a SAR analysis and were used as support to understand how chemically distinct compounds can be accommodated by a highly selective receptor, and provide the framework for the development of novel quorum-sensing regulators, utilizing the 2-benzamido(methyl) phenyl benzoate scaffold and to assess the possibility of synthetic routes, considering the structural similarity presenting between these compounds, generating in this way an alternative to find new compounds with modulating activity QS. These two strategies were used to select a list of potential modulators of quorum sensing or new pharmacophoric candidates.

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**Conclusions:** The two pharmacophoric models designed in this study, the number 2 (model based on the alignment of the most active compounds) showed greater ability to select or identify compounds that had important structural and chemical characteristics to be considered possible hits. With this model, 22 compounds were identified, which were subsequently subjected to docking calculations. In general, the docking protocol used is adequate, since in validating the conformation of the co-crystallized ligand.

**Keywords:** Quorum sensing, virtual screening, *Pseudomonas aeruginosa*, Docking.

## RESUMEN

**Antecedentes:** *Quorum sensing* (QS) es un mecanismo dependiente de la densidad celular que le permite a las bacterias regular la expresión de genes específicos en respuesta a cambios en su densidad poblacional, controlando de esta manera sus actividades, con el fin de producir una respuesta como una unidad multicelular. Estas repuestas incluyen, producción de factores de virulencia, formación de bio-películas, bioluminiscencia, esporulación, entre otros comportamientos. **Objetivos:** Obtener modelos farmacofóricos capaces de filtrar e identificar moléculas con posible actividad agonista del QS y buscar posibles candidatos basados en cálculos de docking molecular. **Métodos:** La estructura del receptor *LasR* fue obtenida del *Protein Data Bank* (PDB). El programa utilizado para el acoplamiento fue *AutoDock 4.2*. Las estructuras de los cuatro compuestos identificados como líderes en el estudio realizado por Muh *et al.*, (2006), fueron utilizadas con el fin de diseñar modelos farmacofóricos capaces de identificar moléculas con posible actividad agonista del QS. La estructura 3D del ligando *TP-1* fue extraída del complejo co-cristalizado identificado con el código PDB 3IX3. Las geometrías de los ligandos obtenidos como resultado de la búsqueda farmacofórica fueron optimizadas usando el método semiempírico *PM3*, para posteriormente realizar los cálculos de *docking molecular*. **Resultados:** Se diseñaron dos modelos farmacofóricos, el primero se creó utilizando el compuesto más activo identificado como *TP-1*, resaltando las características químicas más importantes para el reconocimiento molecular. El segundo modelo se basó en el alineamiento de tres de los ligandos más activos (*TP-1*, *TP-3* y *TP-4*). Estos modelos se utilizaron como filtro en un cribado sobre una base de datos de conformaciones de varios compuestos con posible actividad agonista del circuito principal de QS presente en *Pseudomonas aeruginosa*. El modelo farmacofórico basado en el alineamiento de los compuestos más activos se utilizó para seleccionar o identificar los compuestos que presentaban características estructurales y químicas importantes para ser considerados posibles *hits*. Con este modelo se identificaron 22 compuestos. A estos 22 compuestos se les realizó una serie de cálculos de docking molecular. Los resultados del docking sirvieron para identificar las interacciones haciendo un análisis SAR (structure–activity relationship) y fueron usados como soporte para entender como compuestos distintos químicamente se pueden acoplar selectivamente al receptor *LasR* y apoyo para evaluar la posibilidad de rutas de síntesis, teniendo en cuenta la similitud estructural que presentan estos compuestos con el núcleo base 2-(benzamidometil)fenil benzoato, generando una alternativa para encontrar nuevos compuestos con actividad agonista del QS. Estas dos estrategias fueron usadas para seleccionar una lista de posibles moduladores del QS e identificar un nuevo farmacoforo. **Conclusiones:** Los dos modelos farmacofóricos diseñados en este estudio, el número 2 (modelo basado en la alineación de los compuestos más activos) mostró mayor habilidad para seleccionar o identificar compuestos que tenían las características estructurales y químicas importantes para ser considerados como posibles fármacos. Con este modelo, se identificaron 22 compuestos, los cuales fueron posteriormente sometidos a cálculos de acoplamiento. En general, el protocolo de acoplamiento utilizado fue el adecuado, ya que se validó la conformación del ligando co-cristalizado

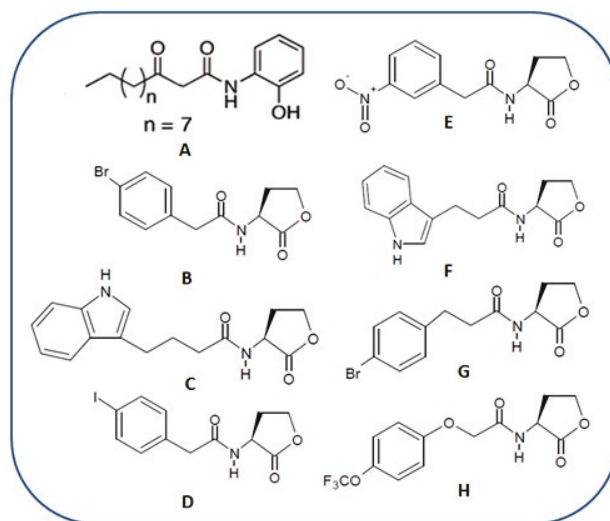
**Palabras clave:** *Quorum sensing*, cribado virtual, *Pseudomonas aeruginosa*, *acople molecular*.

## INTRODUCTION

*Quorum sensing* (QS) is a mechanism allows to bacteria regulating the specific genes expression in response to changes in their population density, coordinating their activities as a multicellular entity (1,2). This mechanism is based on the production, by the bacteria, of small molecules signals called autoinducers (AIs) (3), which are released into the extracellular environment and detected by other bacteria through specific receptors (3). In Gram negative bacteria, at low bacterial density, small amounts of the autoinducer (AI) are produced by the presence of a *LuxI*-type enzyme, the *AIs* passively diffuse into the extracellular medium in favor of a concentration gradient and immediately diluted on the surroundings of the environment. An increase in bacterial population density results in a gradual accumulation of AIs into and surrounding the cells (4-6). The *AIs* specifically activate a transcriptional receptor (proteins type *LuxR*) by union to this one. The activated receptors then interact with white DNA sequences improving or locking the transcription of the genes regulated by QS, resulting in the synchronized activation of some phenotypes in the bacterial population such as the production of virulence factors, formation of biofilm, bioluminescence, sporulation and other behaviors, which have a significant impact on health, agriculture and the environment (7-9).

Knowing the nature of this phenomenon has generated great interest in the development of methods to control this signaling mechanism. In the last 25 years, significant advances have been achieved through both theoretical and experimental studies, focusing the efforts on design and synthesis of ligands able of modulating the different circuits from QS present at the negative Gram bacteria *Pseudomonas aeruginosa*, due to the potential fatal effects of their infections, causing death to miles of immune-engaged persons each year (10-12). *Pseudomonas aeruginosa* presents at least three well characterized QS circuits, being *LasI-AI-LasR* the most studied of all (13). Until present days, the QS synthetic agonist and antagonist are very rare and mainly structurally analogous to native N-acyl homoserina lactonas (*AHL*), as can be observed at Figure 1. Recently, a set of 200000 compounds was tasted against a *P. aeruginosa* strain, unable of producing *AHLs*, being identifies powerful *LasR*

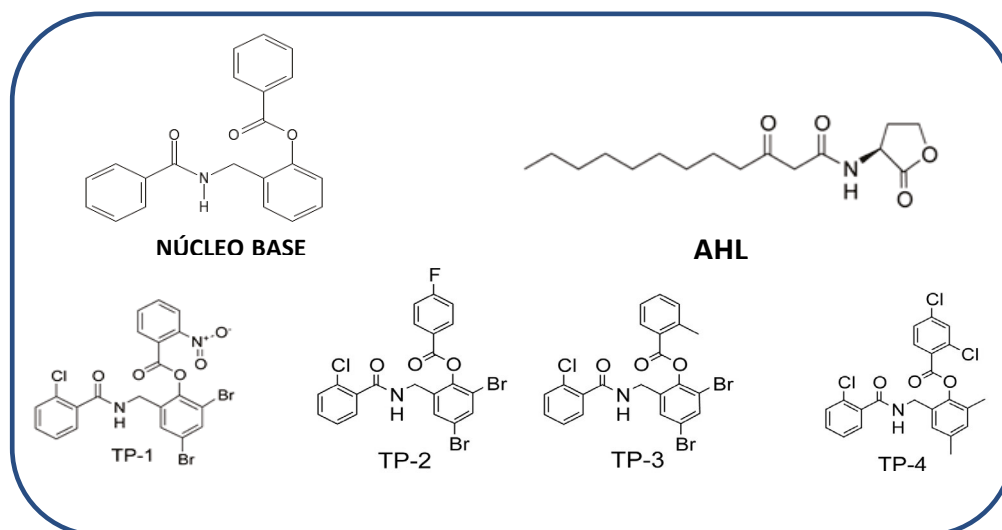
(14, 15) activators composed by nucleus with base 2-(benzamido methyl)phenyl benzoate, which possess poor or null chemical similitude with *AIs*, *AHLs* of *P. aeruginosa* (Figure 2). These compounds, designed as *TP-1* and its derivative *TP-2*, *TP-3*,  $\gamma$  *TP-4*, have probed union affinities by *LasR* receptor, which are several orders of magnitude lower than any other type of activator and they don't show crossed reactivity with the related signal receptors *QScR*, *LuxR*, and *RhlR* (15).



**Figure 1.** *LasR* antagonist synthetic reported until present days. Compound A, reported by Smith et al., 2003. Compounds B-H reported by Blackwell et al., 2007.

More than 50 species of Gram negative bacteria use the QS communication systems and more specifically use the *AHLs* as signal molecules, and therefore to conduct research in this area of microbiology is of vital importance due to the resistance that bacteria have developed to the antibiotics.

Nowadays, computational chemistry tools are being used to support the development of new antibiotics with restriction each time major allow performing tests with specific receptors and later inferring about the rest of the receptors of this proteins family. The application of the virtual screening has become in a procedure much more simple of understanding and also faster of developing. It consists of computational analysis of databases of compounds, focused to identifying and selecting a limited number of candidates possess the wish biological activity over a specific therapeutic goal (16).



**Figure 2.** Structure of the molecules with nucleus with base 2-(benzamidomethyl) phenyl benzoate, *TP-1*, *TP-2*, *TP-3* y *TP-4*. The structure of the native ligand (signal molecule) for *LasR* independent highlighting process, *AHL* is included as reference.

There are two approaches: virtual screening based on ligand and virtual screening based on the receptor structure. Among the ligand-based methods, pharmacophore search is one of the methods used for this propose, when a compound or a set of active compounds is available (which have in common certain steric and electronic features necessary to guarantee an optimal supramolecular interaction with a biological structure and for activating or locking its biological answer) a pharmacophoric model can be generated, which can be used as a filter to suppress those compounds do not comply with the conditions of the necessary activities (17).

By the other hand, the molecular docking is a computational strategy based on the receptor structure, which has as last goal predicting (or usually reproducing in retrospective) the best way for one compound can couple into the binding site of one macromolecular object, evaluating the intermolecular interactions present in each explored shaping (18). Therefore, for a study of molecular docking requires two essential components: the search algorithm and the evaluation function.

In the present research, there were applied two virtual screening strategies in a sequential way, first the generation of pharmacophoric models later used as filters to screen a database of compounds for finally obtain a list of possible QS agonist, which were categorized by evaluation functions of molecular couple programs (docking). After developing and analyzing the outcomes of docking, those

ligands with the best evaluation performance are possible candidates to be synthesized or they can be compared for being used during the experimental researches.

## MATERIALS AND METHODS

For the identification of the possible compounds with agonist activity of the QS the following methodology was followed.

### Analysis and comparison of interactions

Given the availability of crystalline structures from *LasR-AHL*, *LasR-TP-1*, *LasR-TP-2*, *LasR-TP-3* and *LasR-TP-4* (19), there was performed an analysis of different interactions of each one of these molecules, obtaining biochemical and structural information allowed understanding the microenvironment the ligands are exposed into the active receptor (19).

### Database

The base structure 2-(benzamidomethyl) phenyl benzoate present in the four compounds reported as agonists (14) *LasR-LasI* and *RhlR-RhII*. *LasI* catalyzes the synthesis of N-3-oxododecanoyl homoserine lactone (3OC12-HSL (Figure 2), was used to develop a search based on the substructure (only 2D structure or topology of the molecule) over the *PubChem database* (20), the search result generated 32 compounds with structures similar to the nucleus base.

There was obtained information related with the molecular shape, ramification degree, molecular size and structural flexibility of the compounds resulting from the search. These compounds were used to generate a new focused database includes the 32 compounds obtained from the search over *PubChem*, the four compounds reported as agonist (14, 21) and 5 compound belonging to a group of possible active reported (22). On this database, there is a totality of 9 real active and the 32 compound with possible activity, those last one based on the supposing of compounds with similar structure also possess similar activity; summarizing 41 compounds. Once created the database, a multi-conformational database was generated using the program *Moe* (23).

### Generation of pharmacophoric models

Taking into account the structural and chemical features of the compounds *TP-1*, *TP-2*, *TP-3* y *TP-4*, two pharmacophoric models were developed using *Moe program* (23). There were manually generated due to the common features into the active ligands set can be easily identified and also there exists experimental evidence of the functional groups must be present in the ligand to get a good activity (24).

#### *Pharmacophoric model I*

To the bioactive conformation of the most active compound (*TP-1*), hydrogens were added and optimized using the MMFF94 force field. This pharmacophoric model was manually generated setting the position, rates and other set of restrictions related to location and pharmacophoric features of the ligand all in an interactive way. For getting that goal there was used *Pharmacophore Query Editor* de *Moe*, which automatically detects the pharmacophoric features generating the pharmacophoric hypothesis (23). Pharmacophoric features used were, donor and acceptor capacity of hydrogen, hydrophobic and aromatic hydrophobic bridges.

#### *Pharmacophoric model II*

The generation of the second pharmacophoric model was performed based on the alignment of the most active compounds *TP-1*, *TP-3*, *TP-4*, using *Pharmacophore Consensus* de *Moe* (23), there was applied the same procedure that at model I except by the hydrophobic features.

### Obtaining and hits addition

Once the pharmacophoric models were generated, the search was performed over a multi-conformational database (database of conformations of each of the considered compounds) previously calculated and not generated during the search because each one of the database entries is straight superposed over the generated pharmacophoric hypothesis (using *Pharmacophore Search* de *Moe*) generating a list of compounds, which represent possible hits present in the screened database. The result obtained is a list with the compounds considered as active (Hit list), which is ordered taking into account the average squared distances, expressed as *RMSD* among each pharmacophoric feature from the model and its corresponding point in the conformation evaluated.

To evaluate the hits list and the precision of the applied methods the following metrics were used, typically applied in searches with pharmacophoric models (25).

$$falsos- = A - Ha$$

$$falsos + Ht - Ha$$

$$Y = \frac{Ha}{Ht} \times 100$$

$$A = \frac{Ha}{A} \times 100$$

For a database D of n compounds, A represents the number of active compounds in database D, Ht is the number of compounds in the list of hits, and Ha is the number of active in the list of hits.

### Molecular Docking

The receptor structure was obtained from *Protein Data Bank* (26). The program used for molecular docking was *AutoDock 4.2* (27). Validation of the modeling system was previously performed. The 3D structure of the *TP-1* ligand was extracted from the co-crystallized complex with *PDB code 3IX3* (19), using the program *Sybyl 7.3* (28) and was subjected to a geometric optimization using the *PM3* semiempiric method (29) implemented on *Gaussian 03* (30). The structure protein was visualized and modified (all water molecules removing) with *Sybyl 7.3*. *TP-1* ligand was prepared with *AutoDock tools* (31) to check the polar hydrogens, *Gasteiger* charges

were added (32) and routable bonds were specified. On the other hand, only the polar hydrogens were added to the protein and the Gasteiger type loads were calculated. For the grid preparation a box was taken centered at the ligand binding site with a size  $50 \times 50 \times 50$  with a spacing between the points of  $0.375 \text{ \AA}$ . For the generation of the poses was selected the genetic algorithm lamarckian (33), 100 runs were calculated. The other parameters were selected by default.

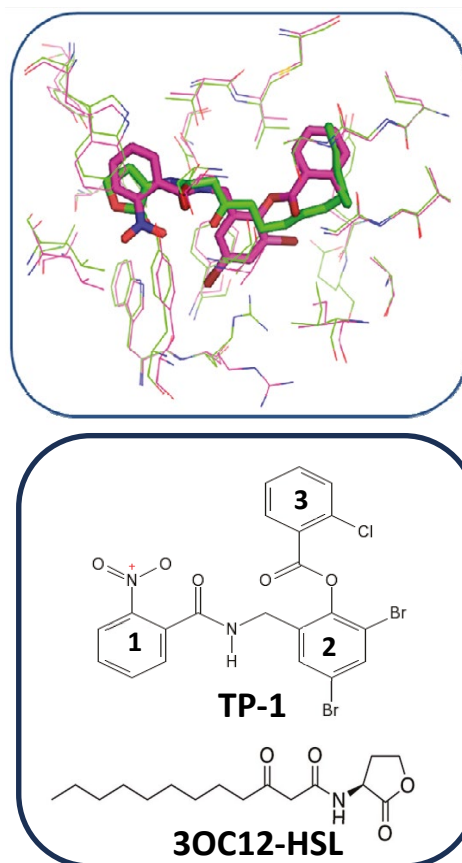
After coupling, the co-crystallized structure of the *TP-1* ligand was compared versus its coupled structure into the active site of *LasR* receptor, using the standard mean square deviation (RMSD) criterion. The 22 ligands obtained from the pharmacophoric search were also optimized and coupled following the same protocol previously describe during the validation. The best conformation of each ligand was selected taking into account the docking free energy and its position inside the binding site.

## RESULTS

### Interactions analysis

In order to identify the pharmacophore and structural characteristics of the *LasR* agonists, previous docking calculations between the active site of *LasR* and its native ligand and between the active site of *LasR* and the ligand *TP-1* (Figure 3) were performed. A great similarity in the orientation was taken by each ligand in the active site. The hydrogen bonding interactions present between *LasR* and its native ligand are also present between this receptor and *TP-1* ligand. In addition, the latter ligand has three aromatic rings in its structure that intervene in other similar interactions with the receptor (Figure 3). Ring 1 is stacked on the indole group of *Trp-88* by a “face-to-face” interaction and interacts with the aromatic ring of *Phe-101* as reported by *Zou et al* (19).

Ring 2, on the other hand, has two bromine atoms, which participate in halogen bridges with the carbonyl oxygen of *Tyr-47* ( $3.52 \text{ \AA}$ ) and the  $\text{N}\epsilon 1$  of *Trp-60* ( $3.69 \text{ \AA}$ ) and participates in a “Face to face” ( $\pi$ - $\pi$  interaction) with the aromatic ring of *Tyr-64*. Finally, ring 3 mimics the orientation taken by the hydrocarbon chain of the native ligand in the hydrophobic *LasR* pocket, establishing Van der Waals interactions with the residues present in this pocket.

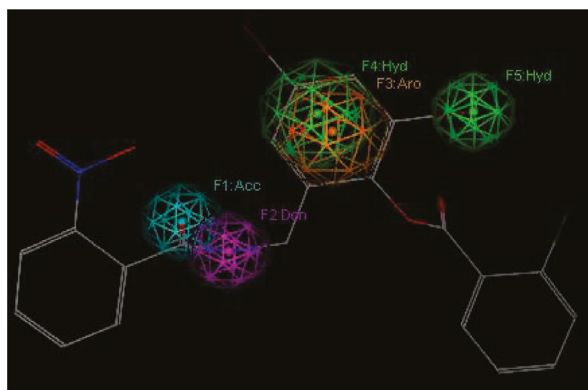


**Figure 3** Alignment between the active site of *LasR* co-crystallized with its native ligand (in green, 3OC12-HSL) and the active site of *LasR* co-crystallized with the *TP-1* ligand (in pink pocket).

### Pharmacophoric model I: Based on the bioactive conformation of *TP-1*

The database created is composed of 41 molecules, 9 real active and 32 obtained from the *PubChem* database (possible active). The pharmacophoric model I (Figure 4) generated a hit list of 31 molecules, 25 of them are possible actives and 6 are real actives. This model presents an actives return of 19.35% and an actives ratio of 66.66% (Table 1). In general, this model recognizes as hits to the four real agonists (14) and also two of the compounds with agonistic activity reported by *Blackwell et al*, (22), which is an evidence of its selectivity, only classifies 3 real active molecules as false negatives, as can be seen in Table 1. By the other hand, it identifies 25 compounds (false positives) that comply with the pharmacophoric characteristics, such as a hydrophobic region and a region with donor and electron acceptor groups.





**Figure 4.** Representation of the pharmacophoric model I. features are represented with colored spheres on the *TP-1* compounds (the most active): hydrophobic (Green), Donor (lilac), acceptor (cyan).

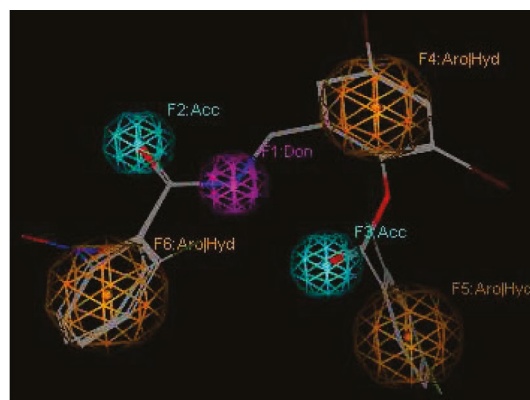
**Table 1.** Retrospective analysis of the pharmacophoric model I.

	Ha	Ht	False +	False -	ef	%Y	%A
Model I	6	31	25	3	0.88	19.35	66.66

*Pharmacophoric Model II: based on the alignment*

The *pharmacophoric model II* (Figure 5) generated a hits list of 26 molecules, 22 of them are possible actives and 4 are real actives. This model presents an asset yield of 15.3% and an actives ratio of 44.4% (Table 2). In general, this model recognizes four real agonists, the compounds with nucleus base 2-(benzamidomethyl)phenyl benzoate (13), being an indication of its selectivity, classifies five real active molecules as false negatives, as can be seen in Table 2. By tradition, the model with major actives performance value (%Y) is the best, and

more selective will be its pharmacophore over the database domain, therefore, the first model is more selective, being its more logical selection to follow with the research.

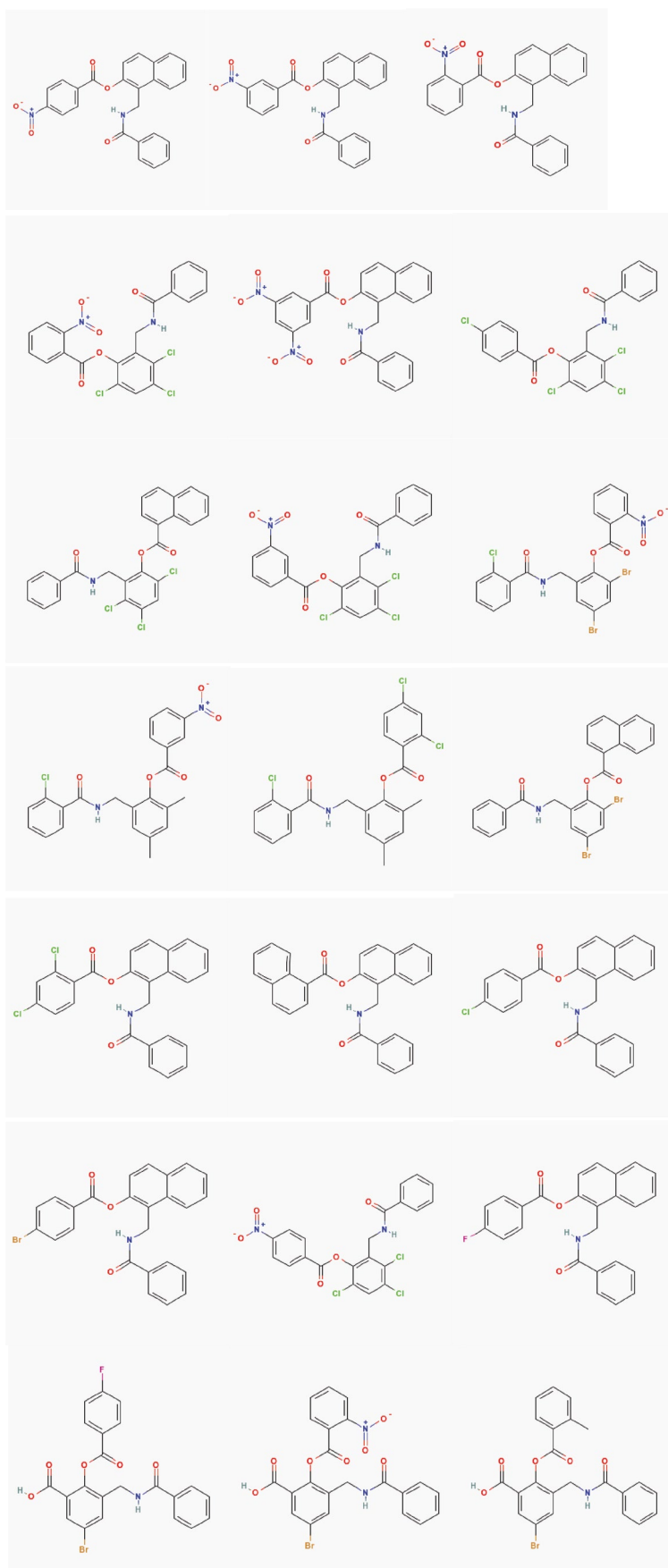


**Figure 5.** Representation of the pharmacophoric model II. Features are represented with colored spheres over the alignment compounds 2: Aro (orange), Donnor (lilac), acceptor (cyan).

**Table 2.** Retrospective analysis of the pharmacophoric model II.

	Ha	Ht	False +	False -	ef	%Y	%A
Model II	4	26	22	5	0.70	15.3	44.4

Analyzing the pharmacophoric features of model II and comparing with the interaction type presents into the co-crystallized complex from *TP-1*, *TP-2*, *TP-3*, *TP-4*, there was found a high relation among them. Therefore, the 22 false positives identified by this model (Figure 6), were submitted to a molecular docking calculation with the studied receptor (*LasR*).



**Figure 6.** Compounds obtained from the pharmacophoric search using model II.



## Molecular Docking

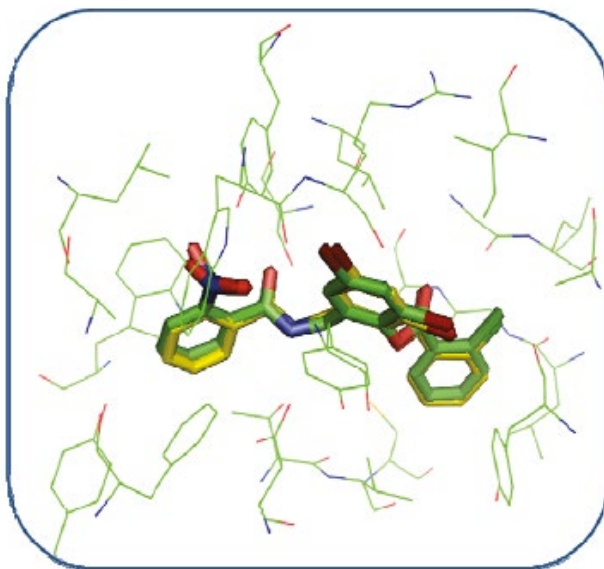
In the validation of the docking methodology applied in this work the co-crystallized structure of the *TP-1* ligand was compared to its structure coupled to the active site of the LasR receptor, using in addition to the visual criterion, the criterion of mean square deviation (RMSD). Figure 7 shows the alignment between the conformation of the *TP-1* ligand corresponding to the co-crystallized complex in green color and the conformation result of the docking is shown in yellow color. As can be observed, the coupled structure is located at the LasR binding site taking a very similar conformation to that presented by the *TP-1* ligand in its crystallized structure, superimposing the three aromatic rings almost identically to the ligand Presented in the co-crystallized structure. The only difference is found in the nitro substituent in ring number 1 of *TP-1*.

The qualitative analysis described above was confirmed by the RMSD value, calculated for the conformation coupled to the conformation of the *TP-1* ligand present in the co-crystallized complex, for this calculation a value of 0.62 Å was obtained, this value is an indication that our Docking program (*AutoDock 4.2*) correctly reproduces the binding form and the interactions present between the *LasR* receptor and the *TP-1* ligand. This value is an indication of our *Docking (AutoDock 4.2)* program effectively reproduces the union shape and the interactions present between the *LasR* receptor and the *TP-1* ligand.

An analysis starting with the hydrogen bonding interactions between *TP-1* and the active site of *LasR* (Figure 7) was observed that the ligand regions involved in this type of interactions are likely to recognize and need the nascent polypeptide to begin their folding, for such reason, these polar groups present in the ligands are given great importance at the time of synthesizing compounds capable of modulating the receptor-ligand circuit present in the QS, in particular the adjacent carbonyl and amine groups.

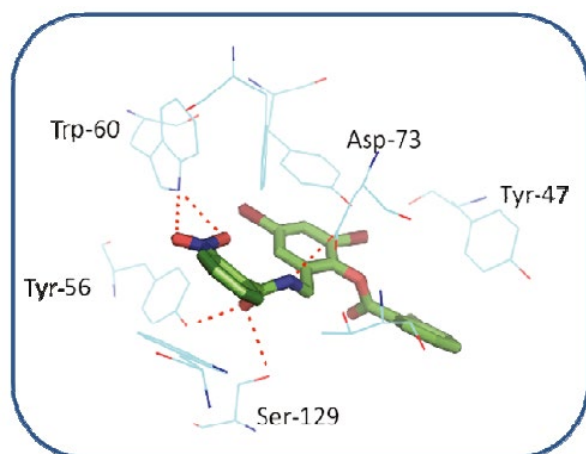
There have been demonstrated by multiple alignment between different transcriptional receptors type *LuxRs* (34) that the aminoacids implicated into hydrogen bridge interactions are conserved in a wide variety of these proteins(*LuxR type*) from

Gram negative bacteria (34-36) some docking calculations were carried out for analogs of the acyl homoserin lactones (AHLs). It is thus that the chemical characteristics of the functional groups present in the residues that participate in this type of interactions are considered as key points for the search and rational design of modulators of the QS circuits present in Gram negative bacteria.



**Figure 7.** Alignment between the *TP-1* ligand present in the co-crystallized complex (green) and the coupled ligand (yellow) using the *AutoDock 4.2* program.

On the other hand, when analyzing possible interactions between the aromatic rings of the *TP-1* ligand and the aromatic side chains of some aminoacids at the active site of the *LasR* receptor (Figure 8) between the ring number 2 of the *TP-1* ligand and the aromatic ring of *Tyr-64* there is a “face to face” arrangement allowing, in this way, a favorable interaction between the rings. Ring number 1 again has a face-to-face arrangement with *Trp-88* and an edge-to-face arrangement with *Phe-101*. The presence of the nitro group allows a favorable interaction with the indol ring of *Trp-88*, as well as the aromatic ring from *Phe-101*. Ring number 3, presents a T-shaped arrangement with *Tyr-47*, as can be observed in Figure 9. The interactions present in this type of stacks are of type CH- $\pi$ , very similar to the interactions that are present between a weak base and a weak acid.



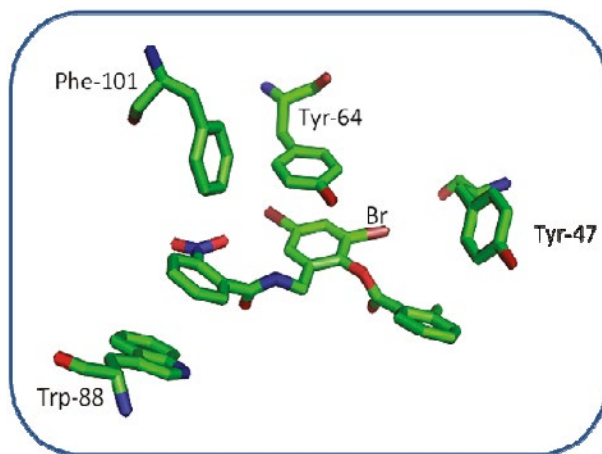
**Figure 8.** Interactions between some aminoacids of the active site of *LasR* and *TP-1*. The amino acid Tyr-47, has a small interaction with the bromine atom in the meta position of ring number 2.

The interactions mentioned above are those that allow nucleoside 2-(benzamido(methyl) phenyl benzoate compounds to stabilize the folding of the transcriptional receptor *LasR*. For this reason, when analyzing the results of our docking calculations, we will take into account the compounds having the polar groups mentioned above and the aromatic groups capable of reproducing all the  $\pi$ - $\pi$  type interactions.

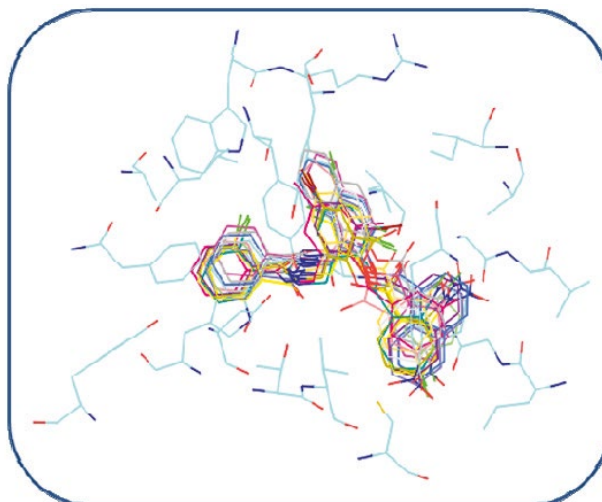
### Docking between *LasR* and the possible agonists

In general, the coupled compounds took a conformation very similar to that taken by the *TP-1* ligand; in some cases it is evident that the conformation allows the ligands to form all the existing interactions in *TP-1*, the aromatic rings of all coupled compounds oriented correctly towards the amino acids with which they form the  $\pi$ - $\pi$  interactions (Figure 10). The compounds shown in Figures 11a and 11c, having a base similar to 2-(benzamido(methyl) phenyl benzoate, are considered potential candidates for synthesis and in vitro agonist activity tests.

However, for other cases, marked differences between the conformation of the ligands under study and the conformation of the *TP-1* ligand (Figure 11b and 11d) were observed. In Figure 11b it is observed that the chemical structure of the coupled ligand differs from the base structure of the *TP-1* ligand by the presence of a bicyclic at the position of ring number 2 of these compounds.

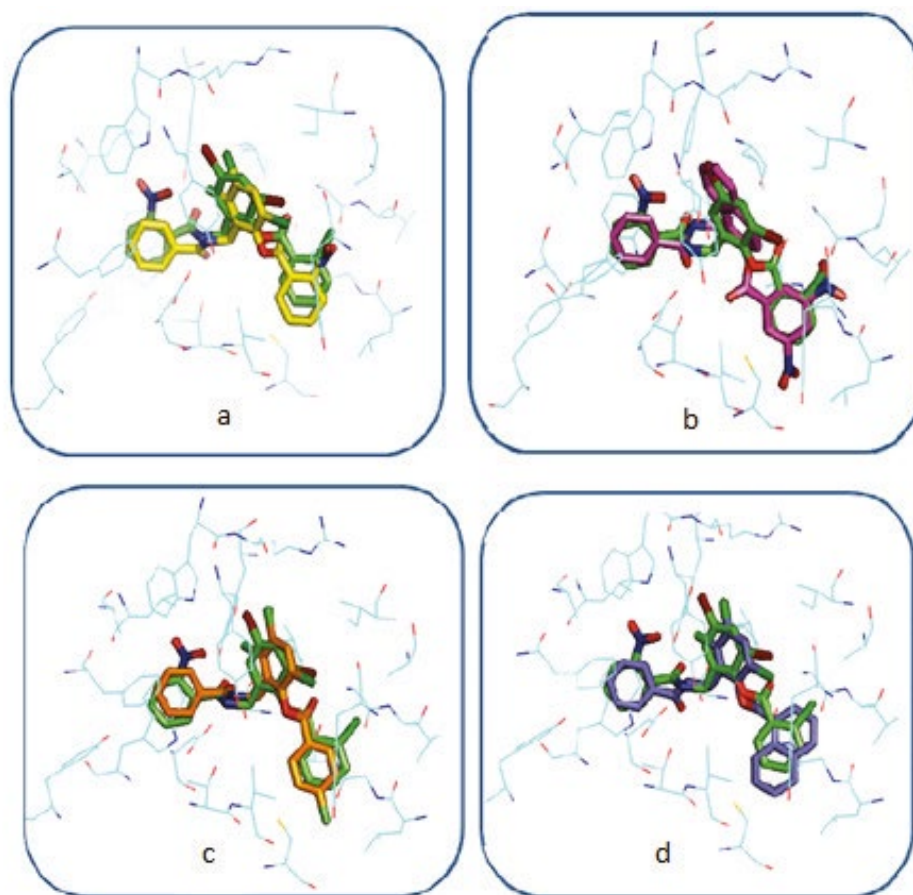


**Figure 9.** Interactions between the aromatic rings of the residues Tyr-47, TYR-64, Phe-101, Trp-88 and the aromatic rings of the *TP-1* ligand.



**Figure 10.** Alignment of the best conformations of all compounds identified as hit in our pharmacophoric model within the *LasR* binding site.

This fact can have consequences from the steric point of view, for which this characteristic must be taken into account in a possible synthesis. In the case of Figure 11d also a structural difference was observed with the base structure of 2-(benzamido(methyl) phenyl benzoate. There is a bicyclic at the position of ring number 3, unlike the steric effect that would possibly cause the bicyclic of Figure 11b to the complex. The effect in this case is different because the bicyclic is located in a hydrophobic pocket, a much larger volume so it would be ideal to establish interactions of the Van der Waals type, which would possibly increase the stability of the receiver.



**Figure 11.** The best conformation of four of the compounds identified as possible agonists, superimposed on the co-crystallized conformation of *TP-1*.

## DISCUSSION

The alignment shown at Figure 3 evidences the tridimensional orientation taken by *TP-1* ligand into the active site from *LasR*, which allows knowing the structural features and the interactions between this compound and *LasR*, establishing conditions to attend a focused search about new possible QS antagonists, using pharmacophoric models in our case.

The Figure 5 shows the pharmacophoric features present into the model II, which are more than those proposed in the model I, for that reason the compounds pass thru this filter should be chemical and structurally very similar to the compounds used for performing the alignment, being the possibly identified as *Hit*. The final goal to develop these pharmacophoric model was to use them filters to identify significant features in the molecular acknowledge. This research that procedure has a binary character type because answer to requests about the pharmacophoric features or not.

In the validation of the docking protocol the calculated RMSD value was 0.62 Å, an RMSD value less than 2 Å is considered a reliable result (37) and in addition the structural similarity between the hits and the four core compound 2-(benzamidomethyl) phenyl benzoate can give some certainty about the results that *AutoDock 4.2* shows. The interactions between each ligand and the conserved residues of this family of proteins are those that allow molecular recognition, whereas other interactions, such as  $\pi$ - $\pi$  type interactions, are those that allow the folding of the transcriptional receptor type *LasR*. For this reason, when analyzing the results of our docking, we took into account the compounds that have the polar groups mentioned above and the aromatic groups capable of reproducing all the interactions type  $\pi$ - $\pi$ . These interactions are reproduced in each of the 22 compounds shown in Figure 6, this may be an indication of the possibilities possessed by this group of compounds to be considered for a rational synthesis.

In general the compounds found can be classified into three groups which possess the bicyclo at the position corresponding to the ring number two of the 2-(benzamido(methyl) phenyl benzoate derivatives, which have the bicyclo at the position corresponding to ring number three of The 2-(benzamido(methyl) phenyl benzoate derivatives and those having the same base structure as the 2-(benzamidomethyl) phenyl benzoate derivatives. Finally, it is important to mention that for further studies it is of vital importance to use compound with biological activities, to be able to relate to the data obtained from the theoretical calculations, in order to move from classification models to predictive models.

## CONCLUSIONS

Among the two pharmacophoric models designed in this study, the number 2 (model based on the alignment of the most active compounds) showed greater ability to select or identify compounds that had important structural and chemical characteristics to be considered possible hits. With this model, 22 compounds were identified, which were subsequently subjected to docking calculations.

In general, the docking protocol used is adequate, since in validating the conformation of the co-crystallized ligand, the program reproduces it adequately, in addition to the visual criterion an RMSD value of 0.62 Å was obtained in validation processes A computed conformation is considered correct if its RMSD value is less than 2 Å. The above is a valid reason to use *AutoDock 4.2* as a program to work the existing links between this type of ligand and the transcriptional receptors belonging to the *LuxR* family of proteins.

The antagonist capacity of *TP-1*, *TP-2*, *TP-3*, *TP-4* compounds is mainly due to their chemical structure and the pharmacophoric groups present, which allow them to be recognized by the *LasR* receptor to start their folding while supply structural requirements and interactions necessary for the correct *LasR* folding

The results of the docking were supported to affirm that the identified compounds can be considered possible candidates for computational analysis more expensive and also to evaluate the possibility of routes of synthesis, taking into account the structural similarity that these compounds present with the derivatives of 2-benzamido(methyl) phenyl

benzoate, thus providing an alternative to find new compounds with QS agonist activity.

## CONFLICT OF INTEREST

There is no conflict of interest of the authors, with the results of the investigation.

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## AUTHORS' CONTRIBUTIONS

All authors contributed extensively to the work presented in this paper. M.A, M.V and R, V-R assembled input data, ran the model, analyzed output data and wrote the manuscript.

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