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Exploring the active site of acyl homoserine lactones-dependent transcriptional regulators with bacterial quorum sensing modulators using molecular mechanics and docking studies

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Abstract

A comparative molecular modelling study of acyl homoserine lactones-dependent transcriptional regulators (TraR, SdiA, LuxR and LasR) involved in bacterial quorum sensing (QS) revealed a high structural homology of their active site. Docking studies within the active site of TraR of fixed conformations obtained using molecular mechanics calculations showed that TraR, for which the crystalline structure is known, is a relevant model for the study of other protein–ligand interactions in the same protein family. Structure–activity relationships of AHLs derived QS modulators including carboxamides, sulfonamides and ureas were thus investigated. The results show that Tyr61, a residue conserved in the LuxR-proteins family, is involved in attractive interactions with aromatic carboxamide antagonists. Tyr53, Tyr61 and Asp70, conserved residues, are implicated in both the development of additional hydrogen bonds and attractive interactions with the *N*-sulfonyl homoserine lactones and AHLs derived ureas antagonists.

Keywords: Quorum sensing; AHLs; TraR; Modulators; Conformational analysis; Docking

1. Introduction

Bacteria communicate using chemical messengers known as auto-inducers (AIs) able to bind regulatory proteins of gene expression encoding for specific phenotypes such as the regulation of virulence factors production, biofilm formation, luminescence [1,2]. This process, named quorum sensing (QS) [3,4], allows bacteria to adapt their behaviour in response to their population density. This cell to cell communication is extensively studied for the understanding of molecular mechanisms involved in transcriptional regulation. QS is also targeted to develop new therapies of bacterial infections which often require antibiotic treatment leading to the emergence of resistant bacteria [5]. Important classes of auto-inducers have been identified with the corresponding transcriptional regulators, i.e. AI-2 with LuxP-proteins family (in Gram positive and negative bacteria) [6], cyclic peptides which induce a phosphorylation cascade activating Agr-proteins family [7] and the QS system with acyl homoserine lactones (AHLs) associated to LuxR-proteins family [8,9] (in Gram negative bacteria). The latter has aroused a great interest in order to design AHL analogues which could act as transcriptional regulator antagonists [10–14]. As a part of this aim, we recently described the synthesis and the biological evaluation of several series of AHL derived compounds that showed agonist or antagonist activities towards the QS system based on the AHLs-dependant transcriptional regulators LuxR in *Vibrio fischeri* [15–17]. Among them, compounds **2** and **3** (Fig. 1) are agonist and activate the luminescence with IC_{50} values of 0.25 and 0.5 μM respectively comparable to the activity of the natural ligand of LuxR (compound **1**). Other compounds (**4–8**)

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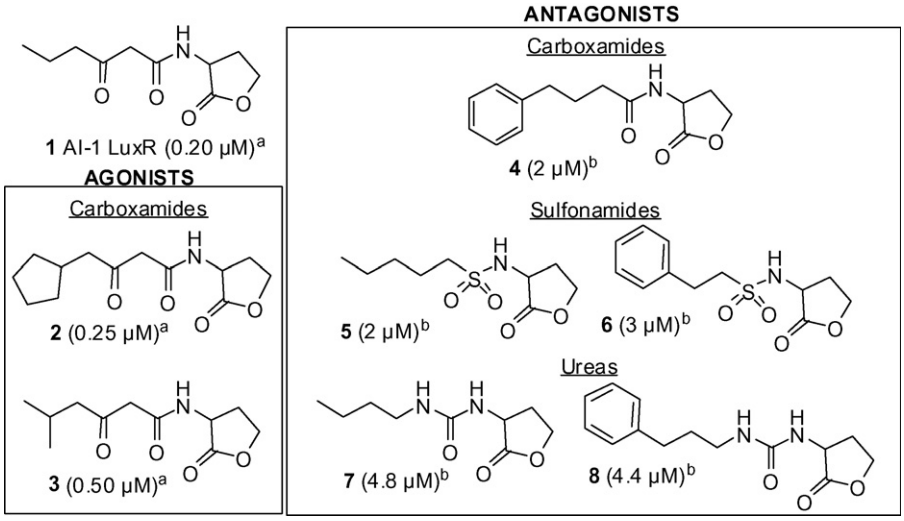

Fig. 1. Structures of QS modulators: natural ligand of LuxR (**1**), agonists (**2** and **3**) and antagonists (**4**–**8**). [a]The concentrations required for half-maximal activation are given in parentheses. [b]The concentrations required for 50% inhibition are given in parentheses.

displayed an antagonist activity and inhibit the bioluminescence with EC_{50} ranging from 2 to 4.8 μM.

Here, we describe a comparative molecular modelling study of the LuxR-proteins family. We also report a study of the structure–activity relationship of the QS modulators presented above using conformation analysis and docking using the TraR protein as reference model [18,19] to explore the active site of LuxR-proteins family.

2. Materials and methods

All calculations were performed on a Dell OPTIPLEX GW 620 PC equipped with a double processor and with the Sybyl 7.2 package for Linux [20], ArgusLab [21] and YASARA [22] as software. Conformation analyses were carried out using the grid search module of Sybyl. Docking experiments were performed with both the docking module of Sybyl and of ArgusLab. Fig. 2A showing the complex of the TraR with DNA and Fig. 2B showing the secondary structure of the protein receiver domain with the auto-inducer were generated from the crystal structure of TraR *Agrobacterium tumefaciens* (pdb code 1L3L) [23]. Fig. 3 showing the protein domain receiver with secondary structure of TraR and SdiA and their active site was created using the RX-structure of TraR (pdb code 1L3L) and the NMR-solved structure of SdiA1-171 from *Escherichia coli* (pdb code 2AVX) [24]. Multiple sequence alignment (Fig. 4) was generated with T-COFFEE [25] with the protein sequences of TraR *A. tumefaciens* (NCBI protein code 1L3LA), SdiA from *E. coli* (NCBI protein code 2AVXA), LuxR from *V. fischeri* (NCBI protein code CAA68561) and LasR from *Pseudomonas aeruginosa* (NCBI protein code AAA25874). The proteins models of LuxR and LasR (Fig. 5) were created using SWISS-MODEL [26] with Clustal W [27]. The crystal structure of TraR [23] (pdb code 1L3L) was used as initial structure and three of the four monomers together with the DNA double strand were deleted. The docking box (Fig. 6) was generated by selecting residues within a distance of 3.5 Å from the ligand. Residues which were not included in the selection

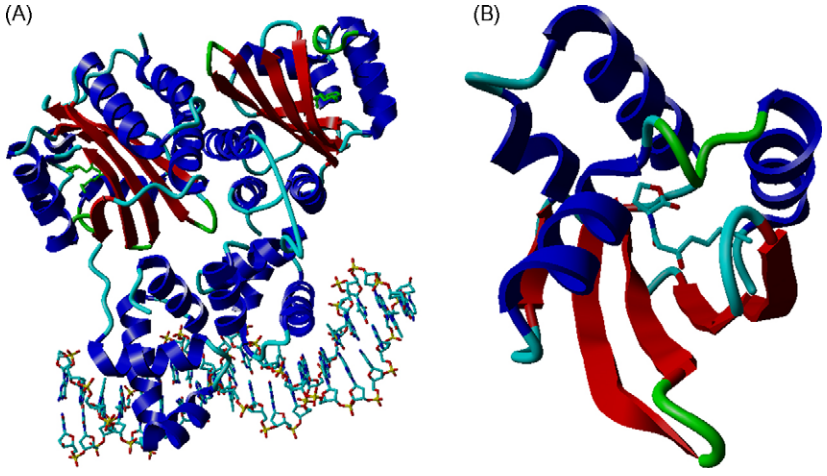

Fig. 2. (**A**) Structure of the complex between DNA and the homodimeric TraR protein bound with the auto-inducer 3-oxo-octanoyl-L-homoserine lactone (**AI-1**). (**B**) Representation with secondary structure of the protein receiver domain with the auto-inducer (**AI-1**).

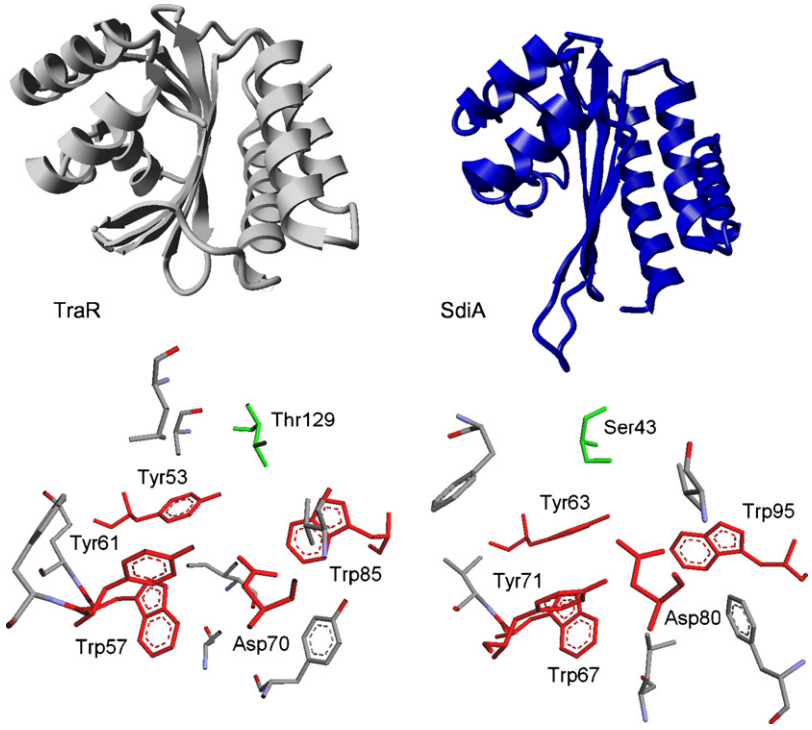

Fig. 3. Representation of the overall folding with secondary structure of the domain receiver and of the active site for the proteins TraR and SdiA (conserved and homologous residues are indicated in red and green, respectively).

```
TraR ----------XQHWLDKLTDLAAIEGDECILKTGLADIADHFGFT----GYAYLH-IQHR
SdiA MSDKDFFSWRRTMLLRFQRMETAEE---VYHE--IELQAQQLEYDYYSLCVRHPVPFTRP
LuxR --MKNINADDTYRIINKIKACRAYDINQCLSD--MTKMVHCEYY--LTLAIIYPHSMVKS
LasR ----------MALVDGFLELERSSGKLEWSAI--LQKMASDLGFSKILFGLLPKDSQDYE
                              :           :   .    :.................

TraR HITAVTNYHRQWQSTYFDKKFEALDPVVKRARSRKHIFTWSGEHERPTLSKDERAFYDHA
SdiA KVAFYTNYPEAWVSYYQAKNFLAIDPVLNPENFSQGHLMWN---DDL--FSEAQPLWEAA
LuxR DISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWN-IFENNAVNKKSPNVIKEA
LasR NAFIVGNYPAAWREHYDRAGYARVDPTVSHCTQSVLPIFWE---PSIYQTRKQHEFFEEA
     .     **   * . *         ** :.        : *.          .   . . *

TraR SDFGIRSGITIPIKTANGFXSXFTXASD-KPVIDLDREIDAVAAAATIGQIHARISF--L
SdiA RAHGLRRGVTQYLMLPERALGFLSFSR--------------------------------
LuxR KTSGLITGFSFPIHTANNGFGMLSFAHSEKDNYIDSLFLHACM-NIPLIVPSLVDNYRKI
LasR SAAGLVYGLTMPLHGARGELGALSLSVEAENRAEANRFMESVLPTLWMLKDYALQSGAGL
        *:  *.:  :  ..   . :: :.....................................

TraR RTTPTAEDAAWLDPKEATYLRWIAVGKTXEEIADVEGVKYNSVRVKLREAXKRFDVRSKA
SdiA ----------------------------------CSAREIPILSDELQLKMQLLVRE
LuxR NIANNKSNND-LTKREKECLAWACEGKSSWDISKILGCSERTVTFHLTNAQMKLNTTNRC
LasR AFEHPVSKPVVLTSREKEVLQWCAIGKTSWEISVICNCSEANVNFHMGNIRRKFGVTSRR
                                                .   : .   :   ::    :.

TraR -HLTALAIRRKLI-------
SdiA SLMALMRLNDE---------
LuxR -QSISKAILTGAIDCPYFKN
LasR -VAAIMAVNLGLITL-----
           :............
```

Fig. 4. Multiple sequence alignment of TraR homologues (TraR from *Agrobacterium tumefaciens*, SdiA from *Escherichia coli*, LuxR from *Vibrio fischeri* and LasR from *Pseudomonas aeruginosa*). Conserved residues are indicated with stars, conserved residues located in the active sites and interacting with the ligand are boxed in red. The multiple sequence alignment was generated using T-COFFEE [25].

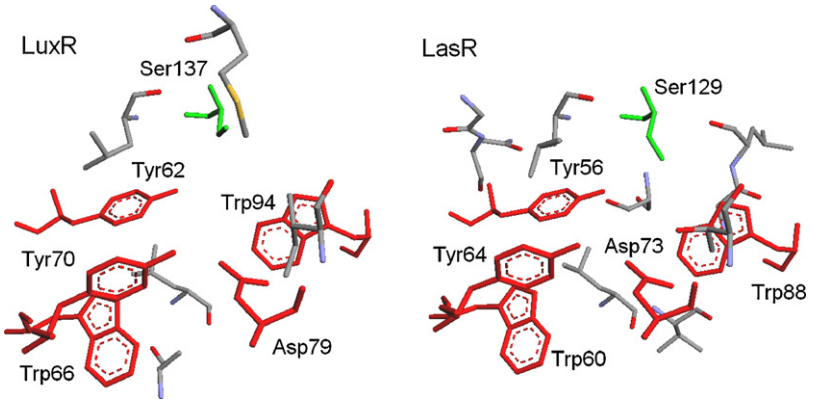

Fig. 5. Representation of the active sites with conserved residues of the LuxR and LasR models.

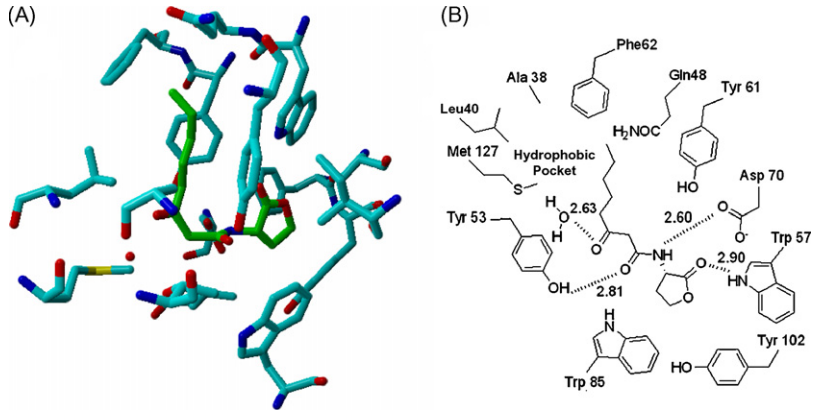

Fig. 6. (A) Structure of the active site used as docking box with residues selected within a distance of 3.5 Å from the auto-inducers. (B) Schematic overview of the active site with **AI-1** displaying the hydrogen bonds network.

were then deleted. Conformation analyses and minimization calculations were performed using the TRIPOS force field with the conjugate Gradient method and Gasteiger–Hückel charges. The systematic search was carried out on each compound by varying key torsions angles ω_1 to ω_5 from 0° to 360° with a 60° increment for compound **1–3** (7776 conformers were generated); ω_1 to ω_4 from 0° to 360° and ω_5 from 0° to 180° with a 60° increment for compound **4** (3888 conformers were generated); ω_a to ω_c from 0° to 360° and ω_d from 0° to 180° with a 30° increment for compound **6** (10368 conformers were generated); ω_I to ω_{IV} from 0° to 360° and ω_V from 0° to 180° with a 60° increment for compound **8** (3888 conformers were generated) (Fig. 7). Resulting conformers were then classified by increasing order of energy to analyse conformations of low energy. Representative preferential conformers (20–30 fixed conformers) obtained as a result of the conformational analysis were then docked as rigid ligand in the docking box. Docking results were analysed for the conformers with the best ligand pose to describe interactions between the ligand and the active site of TraR, hydrogen bonds were assigned within a distance of 3 Å. All important residues conserved in LuxR-proteins family were then selected and others were deleted to generate a TIFF picture as docking results. Tyr53 and Tyr61 were selected as important residue located at the entrance of the hydrophobic pocket. Trp57, Asp70 with or without the water molecule were selected as polar residues able to form hydrogen bonds.

3. Results and discussion

3.1. Structure of LuxR-proteins family

AHLs-dependant transcriptional regulators have been well studied to get insights in molecular mechanism involved in QS system of Gram negative bacteria [12]. These studies showed

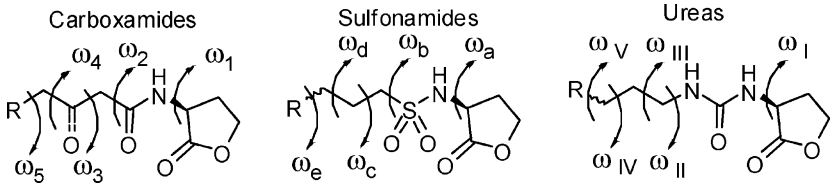

Fig. 7. Conformational analysis using a systematic grid search by varying key torsion angles.

that LuxR-proteins family regulates the transcription of key genes which control the bacterial virulence by interacting with AHLs derived auto-inducers leading to dimerisation and subsequent AHLs-transcriptional regulators-DNA complex formation. The structure of this complex has been recently crystallised and resolved in the case of the TraR protein bounded with the auto-inducer 3-oxo-octanoyl-L-homoserine lactone (**AI-1**) (Fig. 2A) [23]. The analysis of the structure shows that the active site is located between four α-helix and five β-sheets, constituents of the protein receiver domain (Fig. 2B).

The structure of receiver domain of the protein SdiA from *E. coli*, a protein of the LuxR family has been recently elucidated using NMR data [24]. The comparison of the overall folding and of the structure of active site between this protein and the protein TraR reveals a high structural homology (Fig. 3). Key residues interacting with the ligand are conserved and they are located at the same position in the two active sites (Tyr 53, Tyr61, Trp57, Asp70, Trp85 for TraR and Tyr63, Tyr71, Trp67, Asp80, Trp95 for SdiA).

Analysis of the multiple sequence alignment shows that residues located in the active site and interacting with the ligand are strictly conserved in TraR, SdiA, LuxR and LasR (Fig. 4). This observation strongly suggests that the folding of LuxR and LasR should be the same as the proteins TraR, SdiA, to preserve similar interactions with the ligand.

Based on these observations and using the protein sequence alignment of the four proteins with clustal W [27], we then constructed the models of the protein LuxR and LasR derived from the structure of TraR. The structure of their active site with conserved residue is shown in Fig. 5.

To summarize, the comparative study of the LuxR-proteins family with the resolved tri-dimensional structures of TraR, SdiA and the analysis of the multiple alignment sequence of these two proteins with LuxR and LasR showed that (1) the proteins TraR and SdiA adopt the same overall folding and they have a similar active site with conserved residues and (2) the multiple alignment sequence of the four proteins proved that residues implicated in the active site are strictly conserved in the protein family. This comparative study showing a high homology for the active site suggests that the TraR protein should be a relevant model for the docking of potential quorum sensing modulators.

3.2. Conformational analysis using molecular mechanics and docking studies

Based on the comparative study of the LuxR-proteins family, we built a docking box for the TraR protein by selecting keys residues, i.e. residues within a distance of 3.5 Å of the ligand (Fig. 6A). The active site of TraR embodies two hydrophobic pockets, one interacting with the alkyl chain of **AI-1** and a smaller interacting with the lactone. The hydrogen bonds network between the ligand and polar residues is depicted in Fig. 6B and shows the participation of a water molecule forming a hydrogen bond with the ketone function of **AI-1**. With this docking box we then turned out to the study of the possible interactions of QS modulators.

In order to get QSAR for each AHL derived analogues **1**–**8**, we first examined their preferential conformations employing a systematic grid search by varying key torsion angles (Fig. 7) [18]. All resulting conformations were classified by increasing

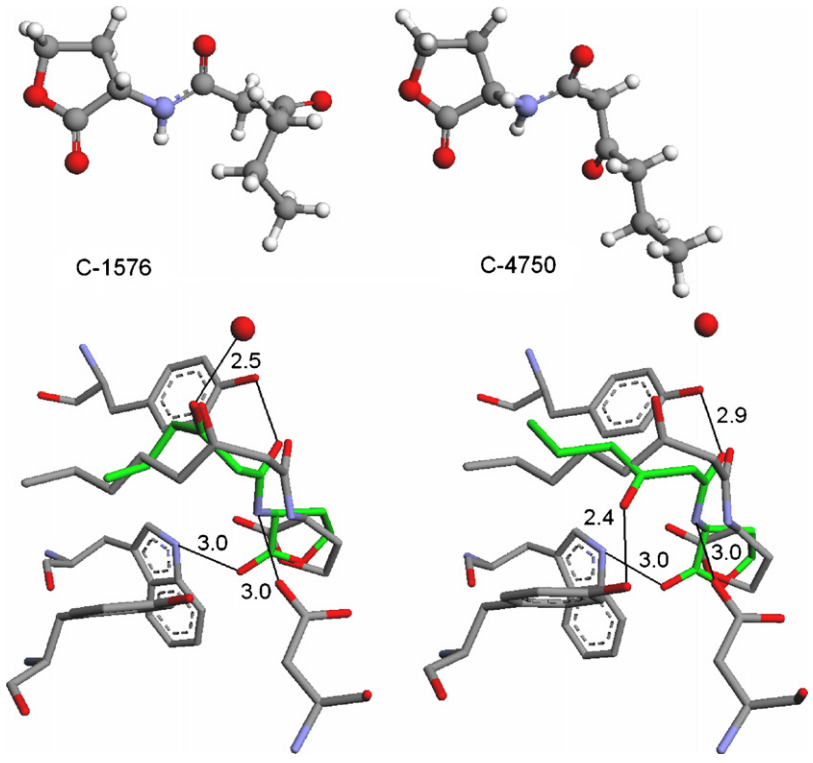

Fig. 8. Docking results of the two conformations **C-C1576** and **C-C4750** in the active site of TraR.

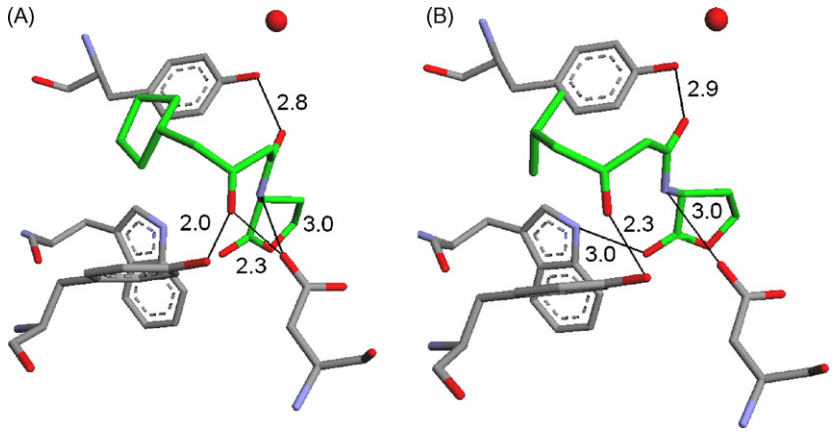

Fig. 9. Best ligand poses obtained as a result of the docking in the active site of TraR for compounds **2**(A) and **3** (B).

order of energy to study the preferential conformations with the lowest energies. Preferential conformers were then used as rigid ligands for docking studies with the protein TraR.

3.2.1. Study of compound 1 (natural ligand of LuxR)

The conformational analysis of the natural ligand of LuxR (compound **1**) was first performed. The comparative analysis of all preferential conformations revealed that the alkyl chain adopts many orientations suggesting important flexibility of the hydrophobic moiety. Among all conformations, two conformers described in Fig. 8 led to a best ligand pose as a result of the docking. Other conformations were also studied but did not lead to satisfactory results. It should be noted that the preferential conformation **C-C1576** was found to be very similar to the conformation adopted by the 3-oxo-octanoyl-L-homoserine lactone co-crystallized with TraR. Fig. 8 describes the binding mode of the 3-oxo-hexanoyl-L-homoserine lactone (compound **1**), natural ligand of LuxR. As expected considering the similarity between the conformer **C-C1576** and the natural ligand of TraR, the docking result showed that the binding mode of this conformer is also very close and preserves notably the same hydrogen bonds (Fig. 6). A similar result was obtained with the conformer **C-C4750** with a difference for the hydrogen bond of the 3-oxo function which takes place with the Tyr53 instead of the molecule of water. It is reasonable to hypothesize that this binding mode exists between the compound **1** and the protein LuxR. Moreover these results are in agreement with the NMR study of the complex between the 3-oxo-octanoyl-L-homoserine lactone and SdiA showing the flexibility of the ligand in the active site [24].

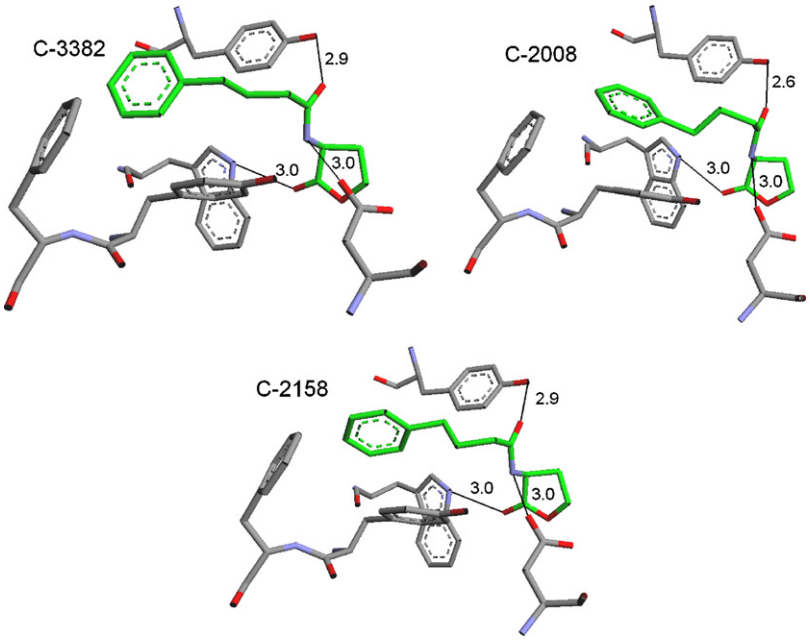

Fig. 10. Docking results in the active site of TraR obtained for compound **4** with the preferential conformations **C-3382**, **C-2008** and **C-2158** obtained as a result of the conformation analysis.

*3.2.2. Study of compounds **2** and **3***

Conformation analyses and docking studies were then applied to compounds **2** and **3** which were found to exert an agonist activity with protein LuxR [15]. Similar results to those observed with **1** were obtained for the binding mode of these compounds, i.e. a binding mode homolog to the one obtained for the conformation **C-C1576** and a best ligand pose obtained for the conformation homologue to **C-C4750** with a hydrogen bond between the 3-oxo function and the Tyr53 (Fig. 9). The similarity of the binding mode of compound **2** and **3** compared to compound **1** may explain their agonist activity for LuxR with IC_{50} of respectively 0.25 and 0.5 μM (IC_{50} = 0.2 μM for **1**).

3.2.3. Study of antagonists

3.2.3.1. Compound 4. The conformation analysis of compound **4** was carried by generating conformers by varying the key torsion angles ω_1 to ω_5. Among them, only preferential conformers were selected to be used as rigid ligand for docking studies. The conformations which led to the best ligand poses are depicted in Fig. 10. The conformation analysis shows that the value of about 180° for the torsion angle ω_1 is conserved in all conformers suggesting the importance of this angle for the binding to preserve the hydrogen bond with Trp57. In contrast, the orientation of the hydrophobic moiety (the alkyl chain and the terminal aromatic group) is variable showing its flexibility. The analysis of the docking for those three conformations showed that the hydrogen bond network observed for the natural ligand is preserved (Fig. 8). However, the flexibility of the hydrophobic moiety allows in all cases favourable interactions in the hydrophobic pocket especially attractive interactions with the aromatic residue Tyr61, residue conserved in LuxR protein family. These additional aromatic interactions with this specific residue may explain the antagonist activity of this compound.

*3.2.3.2. Study of N-sulfonyl homoserine lactones **5** and **6** as antagonists.* We have reported the antagonist activity of various *N*-sulfonyl homoserine lactones [16]. Among them, compounds **5** and **6** were found to be the most active. In this previous study, we delineated the general binding mode of compound **5** in the active site of the LuxR model. The binding mode of this compound can be described using TraR. As a consequence, we docked in the active site of TraR the preferential conformation of compound **5** which was used in the preceding study as rigid ligand for the docking with the LuxR model (Fig. 11). The docking result of compound **5** in the active site of TraR is quite similar to the one obtained for the docking in active site of the LuxR model [16]. Some small differences are notable for the hydrogen bond network with the hydrogen bond of the sulfonamide with Thr129 which corresponds to Ser137 in LuxR and an additional hydrogen bond between this function and the molecule of water. However, all interactions of compound **5** in active site of LuxR can be described using TraR.

We then studied the binding mode of the compound **6**. The conformational analysis led to stable conformations which led to a best fit in the active site of TraR (Fig. 12). The values of the torsion angle ω_a are about 177° for the conformers **C-4447** and

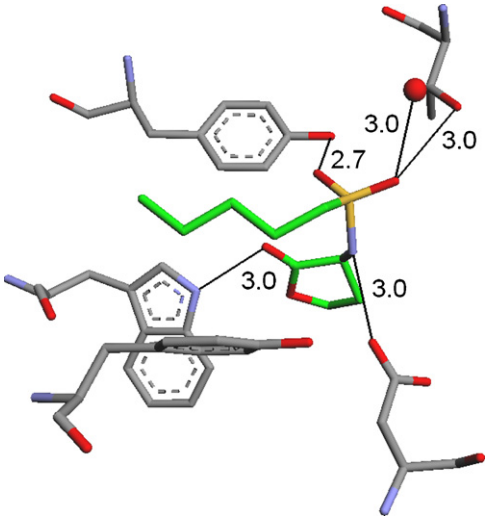

Fig. 11. Docking result of compound **5** in the active site of TraR.

C-4400, 250° for **C-6129** and 235° for **C-4351** suggesting that for the binding of this compound, the degree of freedom obtained by rotation around the sigma bond between the lactone ring and the sulfonamide function is higher than the one for the carboxamide function (see compound **4**). The tetrahedral geometry of the sulfonamide function and the presence of an additional hydrogen bond donor may explain this result by increasing the possibility of interactions with polar residues.

Docking experiments of those conformers (Fig. 12) indeed showed that hydrogen bonds are formed between the sulfonamide function and either Tyr61 (**C-4447**) or Tyr53 (**C-4351**) or with both Tyr61 and Tyr53 for **C-6129** and **C-4400**. Moreover, the flexibility of the hydrophobic moiety observed for conformers induced attractive interactions with the residue Tyr61. The conformation analysis followed by docking experiments of stable conformers of compound **6** showed that this compound interacts in the hydrophilic part of the active site of TraR with Asp70 (NH group), with Trp57 (C=O group of the lactone) and also with Tyr61 and Tyr53 (SO_2 group) by probably switching from conformers **C-4447** to **C-4351**, **C-4400** and **C-6129**. This particularity might explain the high antagonist activity exerts by this compound (IC_{50} = 3 μM) whereas the hydrophobic moiety is less preponderant than the one of compound **4** which displays about the same activity (IC_{50} = 2 μM).

*3.2.3.3. Study of acyl homoserine lactone derived ureas **7** and **8**.* Acyl homoserine lactone derived ureas compounds **7** and **8** were found to exert an antagonist activity with IC_{50} of 4.4 and 4.8 μM [17]. In this previous study, we described the binding mode of the compound **7** in the active site of the LuxR model. Docking of the same conformer in the active site of TraR led to a similar result since all interactions in LuxR can be describe with TraR (Fig. 13). The general binding mode of acyl homoserine lactone derived ureas is induced by the planar geometry of the urea function that allows the formation of an additional hydrogen bond between the supplementary NH group with Asp70 (Asp79 in LuxR). As for *N*-sulfonyl

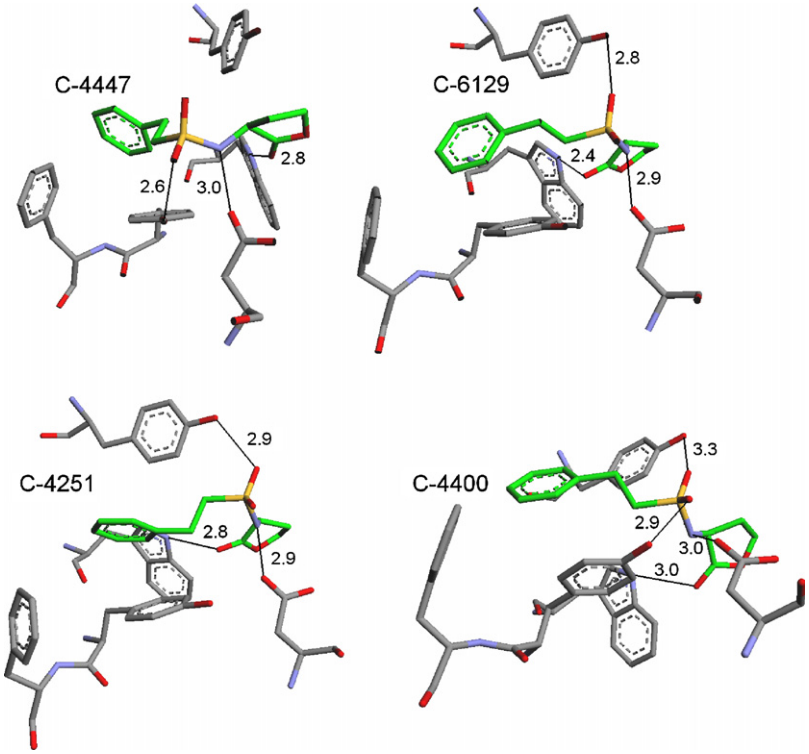

Fig. 12. Docking result of compound **6** in the active site of TraR (conformers **C-4447**, **C-4351**, **C-6129**, and **C-4400** were obtained as a result of the conformation analysis).

homoserine lactone with the sulfonamide function, the urea function develops favourable interactions with the hydrophilic part of the active site and also allows a favourable orientation of the hydrophobic moiety into the hydrophobic pocket.

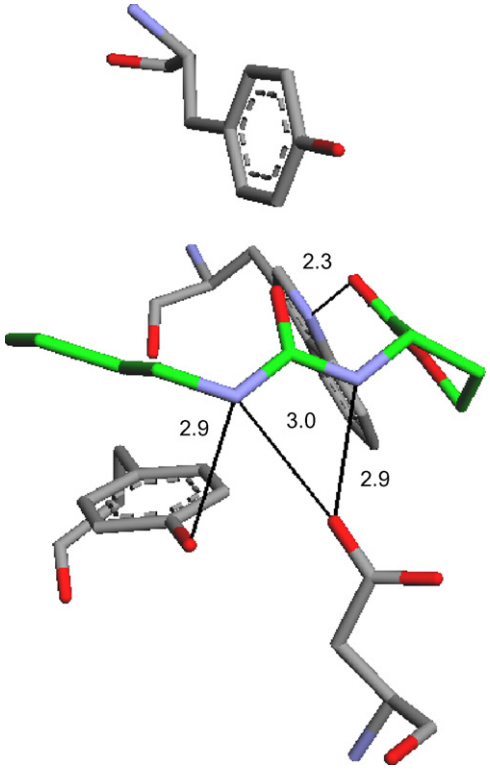

Fig. 13. Docking result of compound **7** in the active site of TraR.

The conformational analysis of compound **8** was then performed. Among all preferential conformations analysed as rigid ligand for this compound, the conformer **C-2140** led to the best ligand pose (Fig. 14). Analysis of the docking result showed that the general binding mode observed for compound **7** is preserved with the same hydrogen bond network. However, the compound **8** develops attractive interactions with Tyr61, residue conserved in LuxR-proteins family. Thus, the hydrophobic moiety fits well in the hydrophobic pocket. As for the urea derivative **7**, the urea function of **8** develops polar

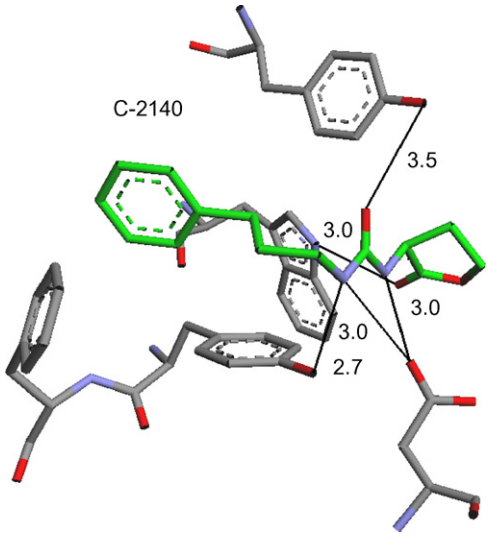

Fig. 14. Best ligand pose for compound **8** as a result of the docking of the conformer **C-2140**.

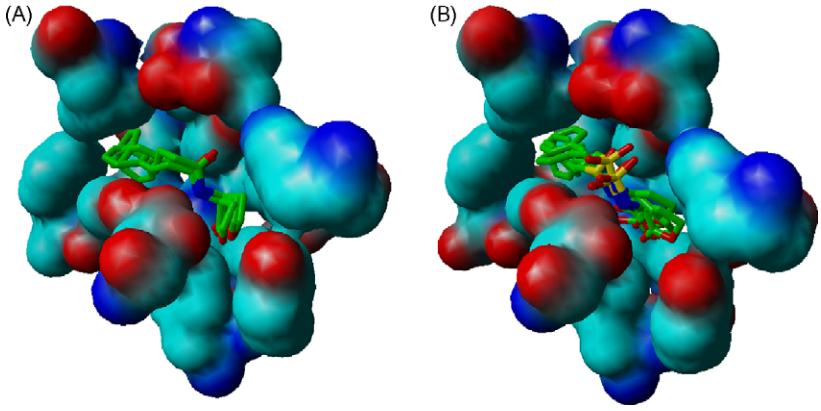

Fig. 15. Representation of the general binding mode for compound **4** (superposition obtained by consecutive docking of conformers **C-3382**, **C-2008** and **C-2158**) and compound **6** (superposition obtained by consecutive docking of conformers **C-4447**, **C-6129**, **C-4251** and **C-4400**). Residues are represented with Van der Waals surface.

interactions especially with Asp70 by forming two hydrogen bonds.

4. Structure–activity relationship

4.1. Interactions in the hydrophobic pocket

For carboxamides derivatives (compounds **1**–**4**), the structural difference between agonists and antagonists is the presence of the aromatic group. Alkyl substituents induce an agonist activity whereas aromatic substituents an antagonist one. The docking studies of compound **4** bearing a terminal aromatic group suggest that this compound develops attractive interactions with the residue Tyr61, residue conserved in LuxR-proteins family. The antagonist activity of this compound may be attributed to these interactions together with the sterical hindrance of the aromatic group in the hydrophobic pocket which could perturb the folding of the receiver domain and could thus disable downstream effect, i.e. the dimerisation with the other subunit or the interaction of the complex with DNA.

4.2. Interactions in the polar area of the active site

The polar area of the active site of LuxR-proteins family is constituted of residues strictly conserved in the family with Asp70 located in the middle of this area, Tyr53, Trp57 and Tyr61 located at the junction between the polar area and the hydrophobic pocket. These residues permit the anchoring of the ligand via polar interactions especially hydrogen bonds (Fig. 6B). Ligands with function analog to the carboxamide, i.e. sulfonamide and urea bearing additional polar group develop new interactions with the polar area by creating new hydrogen bonds. These new interactions might affect the hydrogen network between the residues of the protein, and thus, the overall folding of the receiver domain. This effect may inhibit the dimerisation of subunits. Examination of the whole protein conformation after energy minimization of the two complexes protein–antagonist or protein–natural ligand did not reveal high differences. However, the comparison of the complexes showed only additional interactions with the protein in the case of antagonists. These differences should be determinant to affect the dimerisation process [17].

4.3. General remarks

This present study suggests that carboxamide AHL analogues displaying an agonist activity adopt a similar binding mode as natural ligands whereas carboxamide AHL analogues with a terminal aryl substituent displaying an antagonist activity develop attractive interactions with Tyr61 (Fig. 15A). Importantly, this residue is conserved in the LuxR protein family. AHL derived ureas with alkyl or aryl substituents displaying an antagonist activity develop an additional hydrogen bond with Asp70 and attractive interactions with Tyr61. However, there is no synergy for the antagonist activity between attractive interactions with Tyr61 and the additional hydrogen bond with Asp70 (IC_{50} = 4.4 and 4.8 μM for compounds **7** and **8**, respectively). Fig. 15B shows that the sulfonamide function of *N*-sulfonyl homoserine lactones displaying an antagonist activity may adopt different orientation by conformational switches. This function could thus develop new hydrogen bonds with conserved residues Tyr61, Tyr53 and Asp70. The attractive interactions of the aromatic substituent of compound **6** with Tyr61 do not induce a synergy as for AHL derived ureas.

5. Conclusion

The structural homology of the LuxR-proteins family allows the use of the TraR protein as reference for docking studies of AHLs derived compounds. Here we have applied docking studies to AHL derived carboxamides, sulfonamides and ureas to get insight to their structure–activity relationship. This study shows that important conserved residues, in particular Asp70 and Tyr61, could specifically be targeted to develop potential antagonists. This study also opens perspectives for the rational design or computational screening of new potential QS modulators using the TraR protein.

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