Biotechnological Journal of Environmental Microorganisms (BJEM)

BJEM

Biotechnological Journal of Environmental Microorganisms(BJEM) 1(4) 2022 201-206

In silico Analysis of Chemical Interaction Space Governed by Diclofenac Sodium and Las Quorum Sensing Receptors in *Pseudomonas aeruginosa*

Seyyedeh Shirin Shahangian[1]*

[1]Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

Received: 16 November 2022/ Revised: 03 December 2022 /Accepted: 16 December 2022

Abstract

Most of pathogenic characteristics of *Pseudomonas aeruginosa*, a very well-known opportunist gram-negative bacteria, are modulated by its quorum sensing systems. Therefore, blocking quorum sensing pathways can be used as a strategy to confront *P. aeruginosa*. Non-steroidal anti-inflammatory drugs are among the most popular chemicals used as therapeutics against microbial infections. Chemical interaction space of diclofenac sodium, a well-known non-steroidal anti-inflammatory drug, has been investigated herein against two major receptors (LasI and LasR) involved in quorum sensing system of *P. aeruginosa*. Optimized structures of ligands and receptors were subjected to molecular docking simulations, applying AutoDock Vina plugin available in PyRx software. Results obtained from docking and non-covalent interaction space analyses revealed suitable binding energies against both LasI and LasR receptors. However, binding energy of diclofenac sodium was more negative for LasR, showing its higher affinity for LasR receptor. Finally, based on our results, it is suggested that diclofenac sodium has a good potential to bind both LasI and LasR receptors. This, in turn, can followed by downregulation of some virulence factors genes. Therefore, diclofenac sodium can be considered as a potent inhibitor of quorum sensing in *P. aeruginosa*.

Key words: LasI; LasR; Non-steroidal anti-inflammatory drugs; Molecular docking simulation

*Corresponding Author: E-mail: shahangian@guilan.ac.ir

Introduction

Pseudomonas aeruginosa is recognized as an opportunistic nosocomial pathogen associated with various acute and chronic infections. These infections include respiratory tract infections, bloodstream infection, urinary tract infections, wound infections, and skin and soft tissue infections, particularly in immunocompromised patients (Lorenz et al. 2016). Quorum sensing (QS) is a bacterial communication mechanism in which bacterial cells initiate a series of coordinated behaviors based on the density of signaling molecules (Rutherford and Bassler, 2012). Numerous aspects of *P. aeruginosa* pathogenesis, including the secretion of toxins and degrading enzymes, iron acquisition, biofilm formation, and motility, are controlled by quorum sensing (Bjarnsholt et al. 2010). *P. aeruginosa* possesses a complex network of quorum sensing pathways, including Las, Rhl, PQS, and IQS, which form an intricate and interdependent system. These pathways are intricately connected, featuring autoregulation and control mechanisms that influence the overall activity of one another (Papenfort and Bassler, 2016). IQS utilizes a novel signaling molecule, 2-(2-Hydroxyphenyl)-4-thiazolecarbaldehyde. Besides assessing bacterial population density, IQS also responds to phosphate scarcity, a frequent stress during infections, to control the production of virulence factors. Additionally, IQS hinders host cell growth, induces apoptosis, and impairs host DNA damage repair (Wang et al. 2019). The Las and Rhl circuits utilize N-acyl-L-homoserine lactones (AHLs), whereas the PQS system use quinolones as signal molecules. In the Las and Rhl networks, following an increase in bacterial population density, LasI and RhlI synthases generate the N-3-oxo-dodecanoyl-L-homoserine lactone (3O-C12-HSL) and N-butanoyl-L-homoserine lactone (C4-HSL) signals molecules, respectively. At a critical concentration, AHL molecules bind to their receptors and initiate the expression of various genes.

3O-C12-HSL binds to the LasR regulator, and C4-HSL binds to the RhlR regulator (Miranda et al. 2022). Discovering substances capable of inhibiting the quorum sensing system is a promising strategy to address bacterial infections and drug resistance (Marshall, 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) have recently gained attention as promising chemicals in microbial infection chemotherapy (She et al. 2018; Askoura et al. 2019; Khodaparast et al. 2022). Applying molecular docking simulations, herein, chemical interaction space governed by diclofenac sodium and LasI/LasR receptors has been investigated in *P. aeruginosa*.

Materials and methods

Preparation of chemical structures

Diclofenac sodium

The 3-D SDfile structure of diclofenac sodium was retrieved from PubChem chemical library (Kim et al. 2023). Obtained structure was geometrically optimized in SYBYL7.3. Tripos force field with distance-dependent dielectric and Powell conjugate gradient algorithm with convergence criterion of 0.01 kcal/mol Å were applied for optimization. Partial atomic charges were calculated by Gasteiger method (Gasteiger and Marsili 1980).

LasI/LasR receptors

The crystallized structures of LasI Synthase (PDB ID: 1RO5) and transcriptional activator protein LasR (PDB ID: 3IX3) were obtained from Protein Data Bank (PDB). Co-crystallized ligands and water molecules were removed from PDB structures before molecular docking simulations. Then, polar hydrogens were added, and the Gasteiger method was used to assign partial atomic charges (Gasteiger and Marsili 1980), followed by energy minimization utilizing the 1000 steepest descent gradient algorithm (Pettersen et al. 2004; Meza 2010).

Docking simulation analysis

Molecular docking simulation was performed to detect the chemical interaction space governed by of diclofenac sodium and quorum sensing receptors (LasI and LasR) of *P. aeruginosa*. Prior to docking, structures of diclofenac sodium and receptors were prepared according to methods stated earlier above. Afterwards, the SDF file of diclofenac sodium was imported into OpenBabel software and converted to PDBQT format,

followed by energy minimization via Universal Force Field (UFF) algorithm (Dallakyan and Olson, 2015). The molecular docking simulations were carried out using the AutoDock Vina (Eberhardt et al. 2015) plugin integrated into the PyRx software (Dallakyan and Olson, 2015). The simulation runs were conducted on a desktop equipped with an Intel® Core™ i7-8700K 3.60 GHz processor and 24 GB DDR4 memory. Ultimately, conformations with the lowest binding energy were selected and their chemical binding patterns were analyzed using Discovery Studio Visualizer and Chimera software.

Results

Docking simulation analysis

The lowest binding energy values observed in docking analysis results were -6 kcal/mol and -8.6 kcal/mol, respectively, for diclofenac sodium/LasI and diclofenac sodium/LasR complexes (Table 1). This suggests a higher affinity of diclofenac sodium for LasR compared to that for LasI. Two- and three-dimensional views of diclofenac sodium/receptors complexes are shown in Figures 1 and 2, while important non-covalent interactions which govern diclofenac sodium/receptors chemical spaces are listed in Table 1.

Table 1. Binding affinities and interacting residues in diclofenac sodium/LasI and diclofenac sodium/LasR complexes

Receptor name	Binding energy (kcal/mol)	No. of H-bonds	Residues forming H-bonds	No. of hydrophobic interactions	Residues forming hydrophobic interactions
LasI	-6	2	Phe105, Thr144	4	Val26, Arg30, Ile107, Val148
LasR	-8.6	2	Asp73, Ser129	11	Leu36, Leu40, Tyr47, Ala50, Val76, Leu125, Gly126, Ala127

Based on docking simulation analyses, diclofenac sodium makes two conventional hydrogen bonds with LasI trough Phe105 and Thr144 (Fig. 1, Table 1). Similarly, the same number of hydrogen bonds has been made between diclofenac sodium and LasR trough Asp73, Ser129 (Fig. 2, Table 1). Differential chemical binding pattern relates to hydrophobic interactions, where diclofenac sodium/LasR complex shows more than double interactions compared to that of diclofenac sodium/LasI complex (Fig. 1 and Fig. 2). This suggests that the higher affinity of diclofenac sodium for LasR is likely related to the hydrophobic chemical space governed by diclofenac sodium and LasR receptor.

A closer insight into the hydrophobic chemical space available between diclofenac sodium and LasI shows one pi-cation interaction and one alkyl interaction built through Arg30 and Val26 residues, respectively (Fig. 1). Moreover, Ile107 and Val148 residues of LasI receptor are involved in making pi-alkyl interactions with diclofenac sodium (Fig. 1). Regarding diclofenac sodium/LasR complex, on the other hand, there are one pi-pi T-shaped interaction and one amide-pi stacked interaction, made respectively by Tyr47 and Gly126, in addition to five pi-alkyl interactions made by Leu36, Val76, Leu125, and Ala127 residues (Fig. 2). Moreover, other four alkyl interactions are also available between diclofenac sodium and LasR which are built through Leu40, Ala50, Val76 and Ala127 residues (Fig. 2). The higher number of hydrophobic interactions in diclofenac sodium/LasR complex compared to that in diclofenac sodium/LasI complex is highly compatible with calculated binding energies (Table 1), suggesting higher affinity of diclofenac sodium for LasR.

Discussion

Pseudomonas aeruginosa is associated with different acute and chronic infections such as respiratory tract, bloodstream, urinary tract, wound, skin and soft tissue infections. Many pathogenesis features of *P. aeruginosa* such as toxins/degrading enzymes secretion, biofilm formation and motility are engaged by its quorum sensing systems. Therefore, discovery of chemical compounds which are able to block quorum sensing systems of P.aeruginosa, can be a promising strategy to use against bacterial infections

and bacterial drug resistance. Since NSAIDs have been recently in the center of attention for microbial infection therapies, chemical interaction space governed by diclofenac sodium as a well-known NASID and major quorum sensing receptors (LasI and LasR) of *P. aeruginosa* has been investigated herein using *in silico* methods. Applying docking simulations, binding energies and non-covalent interactions patterns have been extracted and compered between diclofenac sodium/LasI and diclofenac sodium/LasR complexes. The results showed acceptable binding energies for diclofenac sodium against both receptors. However, binding energy of diclofenac sodium was more negative for LasR, suggesting its higher affinity towards this receptor. Esnaashari et al. have recently conducted a docking study to investigate interaction patterns between naproxen (another well-known NASID) and Las receptors in *P. aeruginosa*. Similar to the present study, they have also observed higher affinity of naproxen for LasR compared to that for LasI. They reported binding energies of -6.2 kcal/mol and -9.2 kcal/mol for naproxen/LasI and naproxen/LasR complexes, respectively (Esnaashari et al. 2023). Moreover, another study performed by Zahmatkesh et al. showed similar results with regard to a set of NASIDs. Chemical interaction space of Ibuprofen, Ketoprofen, Mefenamic acid, Meloxicam and Tenoxicam was separately studied against Las receptors in *P. aeruginosa*. In case of all drugs, NASID/LasR complex showed lower binding energy than NASID/LasI, suggesting higher affinities of NASIDs for LasR receptor (Zahmatkesh and Rasti, 2022). These findings are in good agreement with outcomes observed here for diclofenac sodium and Las receptors. This was further strengthened by analysis of non-covalent interactions patterns, where diclofenac sodium/LasR complex was shown to contain more than twice as much non-covalent interaction as diclofenac sodium/LasI complex. Finally, it is suggested that good capacity of diclofenac sodium to bind to LasI synthase can lead to inhibition of production of the signaling C12-HSL molecule. This, in turn, can interfere with the activation of the LasR system and followed by downregulation of some virulence factors genes. Accordingly, diclofenac sodium can be considered as a potent candidate with ability to inhibit quorum sensing in *P. aeruginosa*.

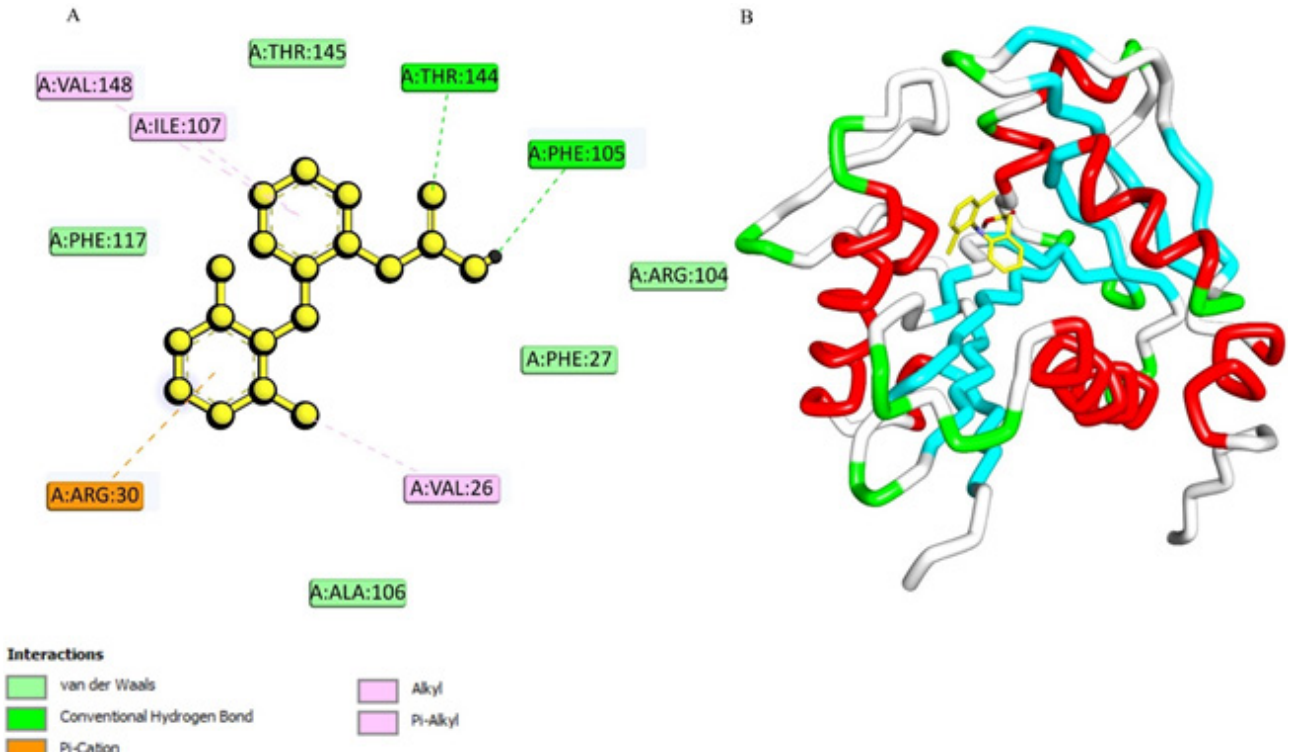

Fig. 1. Chemical interaction space governed by diclofenac sodium and LasI. (A) Two-dimensional and (B) Three-dimensional views

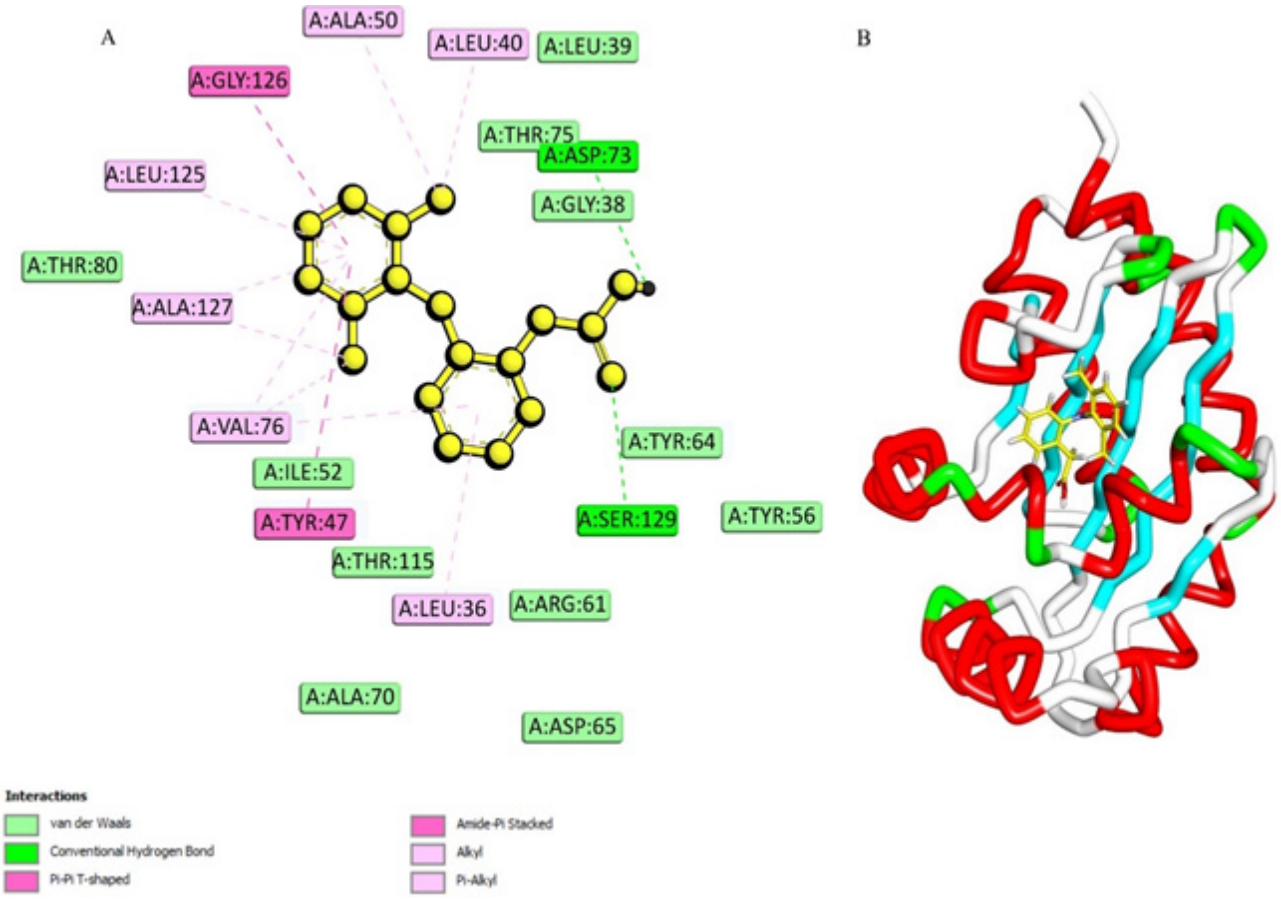

Fig. 2. Chemical interaction space governed by diclofenac sodium and LasR. (A) Two-dimensional and (B) Three-dimensional views

Conclusion

Pseudomonas aeruginosa is responsible for many acute/chronic infectious diseases such as wound infections, respiratory tract infections, urinary tract infections, etc. Since quorum sensing systems are significantly engaged with pathogenic characteristics of *P. aeruginosa*, blocking QS pathways can be considered as an efficient strategy to confront pathogenicity of *P. aeruginosa*. Non-steroidal anti-inflammatory drugs are among the most popular chemicals used as therapeutics against microbial infections. Herein, *in silico* docking simulations have been carried out to investigate chemical interaction space governed by diclofenac sodium and Las QS receptors. Non-covalent interactions analysis revealed good potential of diclofenac sodium to interact with both LasI and LasR, suggesting diclofenac sodium as a good inhibitor for quorum sensing mechanism in *P. aeruginosa*.

Acknowledgments

I would like to thank University of Guilan for providing facilities to carry out this study.

Conflict of Interest

No conflict of interest was declared.

References

Askoura M, Saleh M, Abbas H (2020) An innovative role for tenoxicam as a quorum sensing inhibitor in *Pseudomonas aeruginosa*. Arch Microbiol. https://doi.org/10.1007/s00203-019-01771-4

Bjarnsholt T, Jensen PØ, Jakobsen TH, Phipps R, Nielsen AK, Rybtke MT (2010) Quorum sensing and virulence of *Pseudomonas aeruginosa* during lung infection of cystic fibrosis patients. PLoS One. https://doi.org/10.1371/journal.pone.0010115

Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods Mol Biol. https://doi.org/10.1007/978-1-4939-2269-7_19

Eberhardt J, Santos-Martins D, Tillack AF, Forli S (2021) AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. J Chem Inf Model. https://doi.org/10.1021/acs.jcim.1c00203

Esnaashari F, Rostamnejad D, Zahmatkesh H, Zamani H (2023) In vitro and *in silico* assessment

of anti-quorum sensing activity of Naproxen against *Pseudomonas aeruginosa*. World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-023-03690-5

Gasteiger J, Marsili M (1980) Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges.Tetrahedron. https://doi.org/10.1016/0040-4020(80)80168-2

Khodaparast S, Ghanbari F, Zamani H (2022) Evaluation of the effect of ibuprofen in combination with ciprofloxacin on the virulence-associated traits, and efflux pump genes of *Pseudomonas aeruginosa*. World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-022-03316-2

Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA, Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE (2023) PubChem 2023 update. Nucleic Acids Res. https://doi.org/10.1093/nar/gkac956

Lorenz A, Pawar V, Haussler S, Weiss S (2016) Insights into host-pathogen interactions from state-of-the-art animal models of respiratory *Pseudomonas aeruginosa* infections. FEBS Lett. https://doi.org/10.1002/1873-3468.12454

Marshall J (2013) Quorum sensing. PANS. https://doi.org/10.1073/pnas.13014 32110

Meza JC (2010) Steepest Descent. WIREs Comp Stat. https://doi.org/10.1002/wics.117

Miranda SW, Asfahl KL, Dandekar AA, Greenberg EP (2022) *Pseudomonas aeruginosa* quorum sensing. Adv Exp Med Biol. https://doi.org/10.1007/978-3-031-08491-1_4

Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. https://doi.org/10.1038/nrmicro.2016.89

Pettersen EF, Goddard TD, Huang CC et al (2004) UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem. https://doi.org/10.1002/jcc.20084

Rutherford ST, Bassler BL (2012) Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harb. Perspect. Med. https://doi.org/10.1101/cshperspect.a012427

She P, Wang Y, Luo Z, Chen L, Tan R, Wang Y, et al. (2018) Meloxicam inhibits biofilm formation and enhances antimicrobial agents efficacy by *Pseudomonas aeruginosa*. Microbiologyopen. https://doi.org/10.1002/mbo3.545

Wang J, Wang C, Yu HB, Ahator SD, Wu X, Lv S, Zhang LH (2019) Bacterial quorum-sensing signal IQS induces host cell apoptosis by targeting POT1-p53 signalling pathway. Cell Microbiol. https://doi.org/10.1111/cmi.13076

Zahmatkesh H, Rasti B, (2022) *In silico* Analysis of Inhibitory Potential of Major Non-steroidal Anti-inflammatory Drugs against Las-quorum Sensing Circuit in *Pseudomonas aeruginosa*. Biotechnological Journal of Environmental Microorganisms.https://doi.org/10.30495/bioem.2022.704264