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In silico Analysis of Inhibitory Potential of Major Non-steroidal Anti-inflammatory Drugs against Las-quorum Sensing Circuit in *Pseudomonas aeruginosa*

Hossein Zahmatkesh[1]*, Behnam Rasti[2]

[1]Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University (IAU), Lahijan, Guilan, Iran

[2]Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University (IAU), Lahijan, Guilan, Iran

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Abstract

The emergence of drug resistance, therapeutic failure, and the development of *Pseudomonas aeruginosa* infections are primarily attributed to biofilm formation and quorum sensing (QS) dependent virulence factors. The antimicrobial potential of some non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, has been determined in laboratory studies. Herein, a docking analysis was conducted to examine the interaction between seven NSAIDs and the proteins of the Las system. Initially, the three-dimensional structure of selected NSAIDs (Diclofenac sodium, Ibuprofen, Ketoprofen, Mefenamic acid, Meloxicam, Naproxen, and Tenoxicam), and natural ligand of LasR (3-oxo-C12-HSL) were retrieved from PubChem database. Also, crystal structures of LasI Synthase and transcriptional activator protein LasR were obtained from Protein Data Bank. Subsequently, the molecular docking analysis utilizing AutoDock Vina software was employed to investigate the capability of the selected NSAIDs to inhibit the LasI/LasR receptor. Based our findings, the majority of the selected NSAIDs exhibited favorable interactions with LasI/R proteins. Moreover, ketoprofen exhibited the strongest interactions with both proteins. In summary, this work suggested that NSAIDs, especially ketoprofen and naproxen, have promising potential as candidates for further in vitro and in vivo investigations to inhibit the QS circuits of P. aeruginosa.

Key words: *Pseudomonas aeruginosa*; Quorum sensing; NSAIDs; LasI; LasR; Molecular Docking

*Corresponding Author E-mail: h_zahmatkesh@liau.ac.ir

Introduction

Pseudomonas aeruginosa is an opportunistic human pathogen associated with various diseases, such as urinary tract, pulmonary, wound, and bloodstream infections (Khodaparast et al., 2022). This bacterium can produce a wide array of virulence factors, including proteases, elastase, rhamnolipids, and pyocyanin (Thees et al., 2021; Zahmatkesh et al., 2022). These factors play critical roles in initial surface colonization, host tissue adhesion, biofilm formation, and the progression of infections (Kazmierczak et al., 2015).

Cell-to-cell signaling known as quorum sensing (QS) is an essential mechanism involved in the production of virulence factors, biofilm formation, and the evasion of the immune system (Lee and Zhang, 2015). The Las system is composed of the LasI synthase and LasR transcriptional regulator. The LasI synthase is responsible for the production of the signaling molecule N-(3-oxododecanoyl)-l-homoserine lactone (3-oxo-C12-HSL). After binding to the 3O-C12-HSL molecule, the LasR regulator undergoes multimerization and initiates the transcription of various genes associated with virulence factors (Wang et al., 2018).

Identifying compounds that can inhibit the QS system is a promising approach to combat bacterial infections and drug resistance (Marshall, 2013). Non-steroidal anti-inflammatory drugs (NSAIDs) have emerged as a novel strategy in the chemotherapy of microbial infections. Previous studies have demonstrated the antibacterial activity of some NSAIDs, including ibuprofen, meloxicam, diclofenac, and tenoxicam, against P. aeruginosa (Khodaparast et al. 2022; She et al. 2018; Abbas 2015; Askoura et al. 2019). In this study, we conducted an in-silico investigation to explore the potential of seven NSAIDs to interact with the Las QS proteins (LasI, LasR) of P. aeruginosa.

Materials and methods

Protein Preparation

X-ray structures of LasI Synthase (PDB ID: 1RO5; resolution: 2.30 Å) and transcriptional activator protein LasR (PDB ID: 3IX3; resolution: 1.40 Å) were downloaded from Protein Data Bank (Bernstein et al., 1978). Prior to molecular docking, the protein structures were prepared using the DockPrep tool in UCSF Chimera by removing co-crystallized ligands and water molecules, followed by adding polar hydrogens and partial atomic charges by the Gasteiger method (Gasteiger and Marsili, 1980). Next, energy minimization of proteins was accomplished using Chimera software via the 1000 steepest descent gradient algorithm (Pettersen et al., 2004).

Ligand Preparation

the 3D structures of NSAIDs (Diclofenac sodium, Ibuprofen, Ketoprofen, Mefenamic acid, Meloxicam, Naproxen, and Tenoxicam), and natural ligand of LasR (3-oxo-C12-HSL) were obtained from PubChem (Bolton et al., 2011). 3D ligand structures (.sdf format) were imported into the OpenBabel plugin embedded in PyRx and energy minimization was conducted using Universal Force Field (UFF) followed by a conjugate gradient optimization algorithm. Next, the minimized ligands were converted to the PDBQT format and utilized for docking (Dallakyan and Olson, 2015).

Molecular Docking Using AutoDock VINA

All docking experiments were run on a desktop with Intel®CoreTM i7-8700K 3.60 GHz processor and 24 GB DDR4 memory. AutoDock vina Plugin implanted in PyRx software was employed to execute molecular docking simulations (Trott and Olson, 2009). The binding patterns of protein-ligand complexes were subsequently explored utilizing LigPlot, and Chimera programs.

Results and Discussion

Docking between NSAIDs and LasI

The 2D docking pattern and the molecular interactions of selected NSAIDs with LasI are represented in Fig. 1. According to docking results, the binding energies of Ibuprofen and Ketoprofen were -6.5 and -6.4 kcal/mol, respectively. Ketoprofen formed four H-bonds with Phe105, Ile107, and Thr144, whereas Ibuprofen interacts with the cavity of the LasI through two H-bonds (Phe105 and Thr144). Moreover, Meloxicam formed four hydrogen bonds with Arg30, Phe105, Thr144, and Thr145. Altogether, Phe105 and Thr144 resi-

Table 1 Binding affinities and interacting residues of NSAIDs with LasI of *P. aeruginosa*

Compound name	Binding affinity (kcal/mol)	No. of H-bond	Residues forming H-bonds	Residues forming hydrophobic
Diclofenac sodium	-6	2	Phe105, Thr144	Phe27, Arg30, Ile107, Phe117, Val148
Ibuprofen	-6.5	2	Phe105, Thr144	Phe27, Arg30, Trp33, Arg104, Ala106, Ile107, Phe117, Thr145, Val148
Ketoprofen	-6.4	4	Phe105, Ile107, Thr144	Val26, Phe27, Arg30, Ala106, Phe117, Thr145, Val148
Mefenamic acid	-6	3	Arg30, Phe105	Val26, Phe27, Ile107, Ser109, Phe117, Val148
Meloxicam	-5.8	4	Arg30, Phe105, Thr144, Thr145	Phe27, Trp33, Phe117, Val143, Gly147, Val148, Glu171
Naproxen	-6.2	3	Ile107, Thr144	Val26, Phe27, Arg30, Phe105, Phe117, Val143, Val148
Tenoxicam	-6	3	Arg30, Ile107	Gln25, Val26, Phe105, Ser109, Val148

Table 2 Binding affinities and interacting residues of NSAIDs with LasR of *P. aeruginosa*

Compound name	Binding affinity (kcal/mol)	No. of H-bond	Residues forming H-bonds	Residues forming hydrophobic
Diclofenac sodium	-8.6	4	Tyr64, Asp73, Thr75, Ser129	Leu36, Leu40, Tyr47, Ile52, Arg61, Val76, Cys79, Leu125, Ala127
Ibuprofen	-7.8	2	Trp60, Asp73	Leu36, Tyr47, Tyr56, Arg61, Tyr64, Ala70, Thr75, Val76, Trp88, Phe101, Ser129
Ketoprofen	-10	4	Tyr64, Asp73, Thr75, Ser129	Leu36, Leu40, Tyr47, Ile52, Arg61, Val76, Cys79, Leu125, Ala127
Mefenamic acid	-9.1	3	Thr115, Ser129	Leu36, Tyr56, Trp60, Arg61, Tyr64, Thr75, Val76, Trp88, Tyr93, Phe101, Leu110, Ala127
Meloxicam	-6.2	5	Ser77, Ile92, Gln98	Arg71, pro74, His78, Gln81
Naproxen	-9.3	3	Arg61, Asp65, Ser129	Leu36, Tyr47, Ala50, Ile52, Tyr56, Tyr64, Ala70, Asp73, Thr75, Val76, Trp88, Ala127
Tenoxicam	-8.6	5	Arg61, Tyr64, Asp73, Thr75, Ser129	Leu36, Gly38, Tyr47, Ala50, Ile52, Tyr56, Asp65, Val76, Trp88, Leu110, Ala127

Table 3 Binding affinity and interacting residues of the natural ligand with LasR of *P. aeruginosa*

Compound name	Binding affinity (kcal/mol)	No. of H-bond	Residues forming H-bonds	Residues forming hydrophobic
C12-HSL (Natural ligand for LasR)	-7.9	3	Trp60, Asp73, Ser129	Leu36, Leu40, Tyr47, Ala50, Tyr56, Tyr64, Ala70, Thr75, Val76, Trp88, Phe101, Leu110, Gly126, Ala127

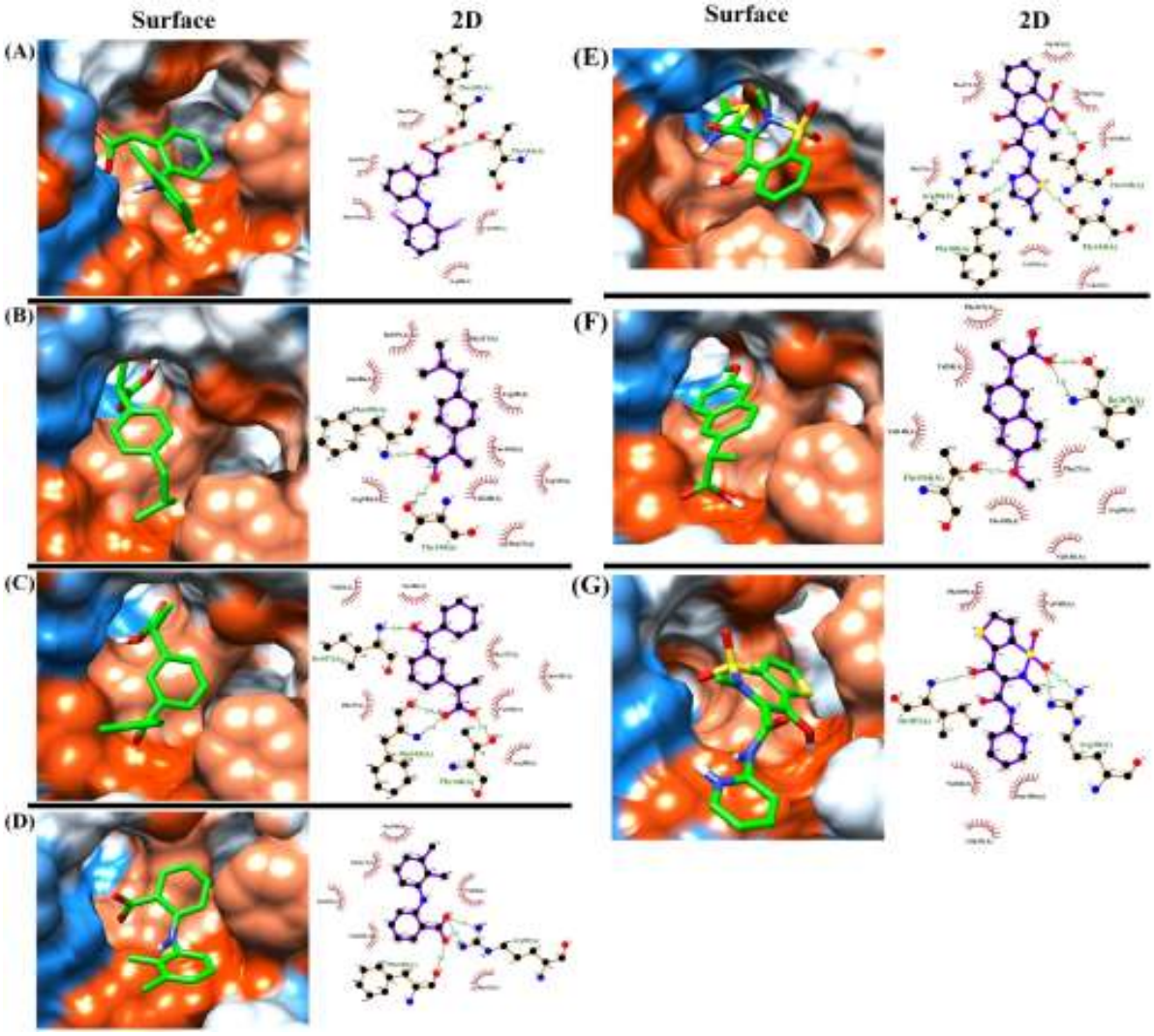

Fig. 1 Molecular docking analyses of NSAIDs with LasI receptor: the images are in surface mode and 2D view. Images represent the interactions between LasI and (A) Diclofenac sodium; (B) Ibuprofen, (C) Ketoprofen, (D) Mefenamic acid, (E) Meloxicam, (F) Naproxen, and (G) Tenoxicam.

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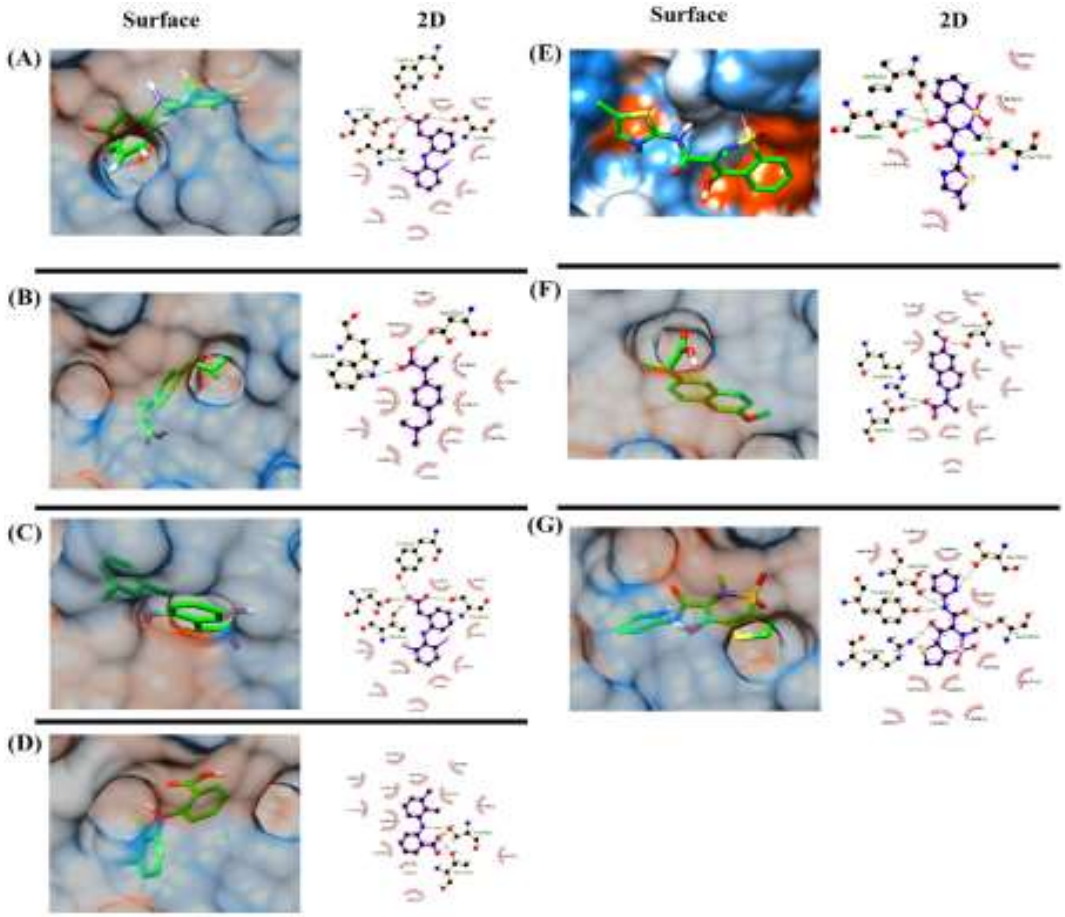

Fig. 2 Molecular docking analyses of NSAIDs with LasR receptor: the images are in surface mode and 2D view. Images represent the interactions between LasR and (A) Diclofenac sodium; (B) Ibuprofen, (C) Ketoprofen, (D) Mefenamic acid, (E) Meloxicam, (F) Naproxen, and (G) Tenoxicam.

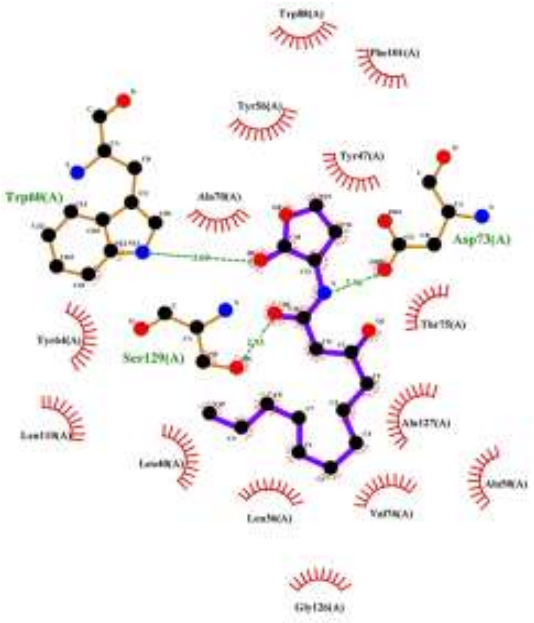

Fig. 3 2D docking pattern of 3-oxo-C12-HSL in the active site of LasR

dues were more involved in hydrogen bonds than other residues (Table 1).

Investigated drugs showed a good potential to bind to the LasI. Based on the binding energies, Ibuprofen and Ketoprofen had the strongest interaction with LasI. As well, 2D interaction patterns showed that Meloxicam, Mefenamic acid, and Ketoprofen formed 3 hydrogen bonds with key residues of LasI: (Arg30, Phe105, Thr144), (Arg30, Phe105), and (Phe105, Thr144) respectively.Inhibition of the LasI system reduces the production of C12-HSL signaling molecule. In consequence, reducing the production of signaling molecules can interfere with the activation of the LasR system, which subsequently causes a downregulation in the expression of virulence factors.

Docking between NSAIDs and LasR

The 2D docking pattern and the molecular interactions of NSAIDs with LasR are depicted in Fig. 2. As indicated in Table 2, all seven compounds exhibited hydrogen bond interactions, with active site residues in the LasR. According to docking simulation, binding affinities of Ketoprofen, Naproxen, and Mefenamic acid were -10, -9.3, and -91 kcal/mol, respectively. Tenoxicam and Meloxicam interact with the cavity of the LasR through five H-bonds (Arg61, Tyr64, Asp73, Thr75, and Ser129) and (Ser77, Ile92, and Gln98) respectively. Also, Diclofenac sodium with -8.6 Kcal/mol binding energy and Ketoprofen interact with LasR through the similar four H-bonds (Tyr64, Asp73, Thr75, and Ser129). Generally, Ser129 residue was more involved in hydrogen bonds than other residues.

Based on the 2D interaction patterns, Tenoxicam, Ketoprofen, and Diclofenac sodium formed 4 hydrogen bonds with key residues of LasR: Tyr64, Asp73, Thr75, and Ser129. Thus, these compounds can disrupt the binding of natural ligands to the LasR transcriptional regulator and deactivate the QS system.

Docking studies of natural ligand

Molecular docking of natural ligand revealed that 3-oxo-C12-HSL with -7.9 kcal/mol binding affinities bind to LasR (Table 3). Apart from Ibuprofen and meloxicam with -7.8 and -6.2 kcal/mol binding energies, respectively, other studied drugs showed a higher affinity to bind to LasR than its natural ligand. Also, the 2D docking pattern showed that 3-oxo-C12-HSL interacts with the LasR through three H-bonds (Trp60, Asp73, Ser129) (Fig. 3). Overall, Apart from Ibuprofen and meloxicam, the comparison of binding energies of LasR-NSAIDs complex with those of LasR-Natural ligand complex revealed that the investigated NSAIDs interacted with the transcriptional regulator more strongly, proposing them as selective inhibitors of LasR.

According to docking simulation, Ketoprofen, Naproxen, and Mefenamic acid are the superior three ligands whose docking energies extremely exceeded C12-HSL (-10, -9.3, and -9.1 kcal/mol, respectively).

Conclusion

In summary, this in-silico study investigated the inhibitory potential of major NSAIDs against the P. aeruginosa Las-QS system. The findings suggest that NSAIDs, specifically ketoprofen and naproxen, exhibit a high potential for controlling the LasI/LasR system.

CONFLICT OF INTEREST: No conflict of interest declared.

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