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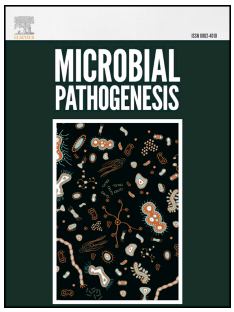

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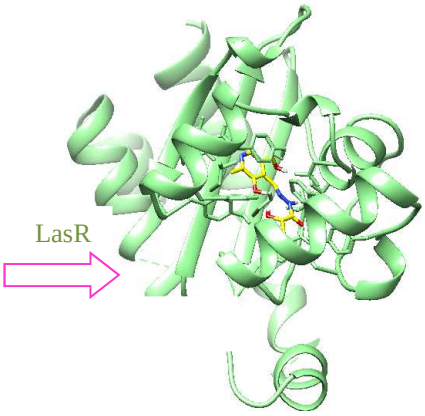

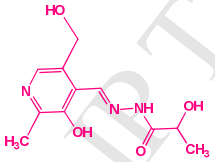

LasR

Inhibition of quorum sensing related virulence factors of *Pseudomonas aeruginosa* by Pyridoxal lactohydrazone

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Short Title: Virulence factors inhibition by pyridoxal lactohydrazone

Abstract

Pseudomonas aeruginosa quorum sensing (QS) system is a cell to cell signaling mechanism that regulates virulence factors and pathogenicity. Therefore, the QS system in *P. aeruginosa* may be an important target for pharmacological intervention. The present study aimed to investigate the effects of sub-MIC concentrations of (*S,E*)-2-hydroxy-*N*-(3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)propane hydrazide (pyridoxal lactohydrazone) against *P. aeruginosa* QS related virulence factors. We investigated the effect of sub-MIC concentrations of chiral pyridoxal lactohydrazone, which formed by the reaction of chiral lactic acid hydrazide and pyridoxal (one form of Vitamin B6) as bioactive reagents, on virulence factors. Treated PAO1 cultures in the presence of tested compound at 1/4 and 1/16 MIC (32 and 8 µg/mL respectively) showed significant inhibition of virulence factors including motility, alginate and pyocyanin production and susceptibility to H_2O_2 ($P< 0.001$). Also, the pyridoxal lactohydrazone showed anti-QS activity in *Chromobacterium violaceum* CV026 biosensor bioassay. Because of quorum sensing is a promising target for anti-virulence therapy and also important role of LasR regulatory protein in the initiation of *P. aeruginosa* QS system, we carried out molecular docking for understanding the interactions of pyridoxal lactohydrazone with the LasR receptor. The results of docking study suggested that the pyridoxal lactohydrazone has potential to inhibit the LasR protein. The results indicated that sub-MIC concentrations of this compound exhibited inhibitory effect on *P. aeruginosa* QS related virulence factors.

Keywords: *Pseudomonas aeruginosa*, Pyridoxal lactohydrazone, Quorum sensing, Virulence factors, Molecular docking.

1. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen that causes acute and chronic infections and is resistant to many antimicrobial agents [1]. The pathogenicity of *P. aeruginosa* is controlled by quorum sensing regulatory system. Quorum sensing (QS) is a cell to cell signaling mechanism that controls the gene expression in response to cell density using chemical hormone-like molecules called autoinducers. This regulatory system plays an important role in controlling of virulence factors, antibiotic resistance and biofilm formation [2,3,4]. *P. aeruginosa* has three distinct QS systems including LasI/LasR, RhlI/RhlR, and PQS/MvfR which utilize small chemical signaling molecules called autoinducers (Fig. 1). The lasI/lasR system regulates the production of *N*-3-oxododecanoyl-homoserinelactone (3-oxo-C_{12}-HSL) and rhlI/rhlR system regulates the production of *N*-butyryl-homoserine lactone (C_4-HSL) [4,5]. The pqs produce 2-heptyl-3-hydroxy-4-quinolone (PQS) molecule that have interconnecting role between Las and Rhl in quorum sensing of *P. aeruginosa* [6]. These autoinducer molecules (3-oxo-C_{12}-HSL, C_4-HSL and PQS) interact with intracellular receptors and trigger transcription of QS-controlled genes in the bacteria including virulence factors such as bacterial adhesion, biofilm formation, motility, and swarming [4].

In recent years, synthesis of *N*-acyl-homoserine lactone analogues is considered as a promising strategy for the design of potential quorum sensing inhibitors (QSI) and anti-bacterial drugs [7,8,9]. Furthermore, natural QSIs have been recognized from bio-resources such as zingerone [10, 11] furanone [12], ascorbic acid (vitamin C) [13], alginate derived oligosaccharides [14], and curcumin [15,16] derivatives. Chemical modification of biologically active compounds from natural sources is promising as the efficient approaches in drug development [17].

In our previous study, in order to synthesis of new compounds from naturally sources and the evaluation of biological activity, we synthesized new hydrazone compounds such as pyridoxal lactohydrazone from chiral lactic acid and pyridoxal (scheme 1) which showed anti-bacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pneumonia* [18]. Pyridoxal is one of natural forms of vitamin B6 and its biologically active form is pyridoxal 5-phosphate (PLP). It is main component in metabolism process of amino acids in living organisms. It is reported that biological potentialities of PLP is related to presence of pyridine nitrogen atom, aldehyde and also phenolic hydroxyl groups. As well as, hydroxyl methyl moiety of pyridoxal play a main physiologically role in attachment of phosphate in pyridoxal phosphate [19,20,21]. The presence of multiple functional groups, hydrogen bond acceptance and donation of hydrazones [20,21,22], were lead to continuous interest about their biological activity such as anti-cancer [23,24,25], anti-convulsant [26], anti-hepatitis C [24,27], analgesic [28], anti-inflammatory [29], anti-depressant [30], anti-platelet [31], anti-leishmanial [32], anti-malarial [33], anti-microbial [34,35], anti-mycobacterial [36,37,38], and insecticidal [39] activities. Furthermore, hydrazone or acyl-hydrazide ($R-C(=O)-NH-NH_2$) moiety has anti-virulence effects in *P. aeruginosa*. Inhibitory activity of salicylidene acyl-hydrazone was found on biofilm formation and virulence of *P. aeruginosa* [40]. The aim of present study was to investigate the activity of (*S*,*E*)-2-hydroxy-*N*-(3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)propane hydrazide (or pyridoxal lactohydrazone) against *P. aeruginosa* QS related virulence factors. To further understand the QSI activity of this compound at molecular level, we carried out molecular docking to investigate the possible interactions of this compound with the QS regulatory protein of LasR.

2. Materials and Methods

2.1. Bacterial strains, growth media and conditions

P. aeruginosa PAO1, as the wild type strain, and *Chromobacterium violaceum* CV026 were used for the assay of QSI activity of pyridoxal lactohydrazone. *N*-acyl-homoserine lactone (C_6-HSL) was purchased from Sigma (Switzerland), aliquot and used at 10 µM. Cultivation and enrichment of bacterial strains were performed with Luria Bertani broth/agar (Merck, Germany) at 37 °C for PAO1 and 30 °C for CV026 during 16-18 h. Bacterial strains were preserved in Tripticase soy broth (Merck, Germany) containing 20% (v/v) glycerolat –70 °C.

2.2. Measurement of cell growth

In order to measurement of pyridoxal lactohydrazone effect on cell growth inhibition, the broth dilution method was used according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [41]. Briefly, overnight inoculums of PAO1 and CV026 were exposed to different concentrations (8-512µg/mL) of tested compound during 18 h at 37 and 30 °C, respectively. After the incubation time, the absorbance was read at 620 nm and percentage of cell growth was calculated by deviation of A_{620} of the strain with pyridoxal lactohydrazone (treated strain) to A_{620} of the strain without tested compound (untreated strain) according to equation (1). Also, tested compound without bacteria strain was considered as negative control.

$$\text{Cell growth\%} = \frac{A_{620}(\text{treated strain})}{A_{620}(\text{untreated strain})} \times 100 \qquad (1)$$

2.3. Biosensor bioassay for detection of anti-quorum sensing activity of pyridoxal lactohydrazone

Well diffusion assay was performed using the reporter strain *C. violaceum* CV026 for detection of anti QS activity of tested compound as described previously [42]. 50 µL of different

concentrations of pyridoxal lactohydrazone (256, 128, 64, 32, 16 and 8 µg/mL) were loaded onto wells of 5 mm on the LB agar plates seeded with 100 µL of *C. violaceum* CV026 and supplemented with 100 µL of 10 µg/mL of C_6-HSL. Inhibition of QS was detected after 24 h incubation at 30 °C by the presence of a zone of pigmentless but viable cells around the wells. DMSO and Kanamycin (100 µg/mL) were used as controls.

2.4. MIC Determination of pyridoxal lactohydrazone

Minimum Inhibitory Concentration (MIC) of pyridoxal lactohydrazone for PAO1was determined in our previous study [18] and MIC of tested compound for *C. violaceum* CV026 was determined using the broth macrodilution method. For quorum sensing assays, concentrations below MIC were considered as sub-MIC concentrations. Stock solution of tested compound was prepared in dimethyl sulfoxide (DMSO) with the final concentration of 1000 µg/mL.

2.5. Effects on biofilm formation

The effect of pyridoxal lactohydrazone on biofilm formation was determined using a microtiter plate assay in our previous study [18].

2.6. Effects on Motility

Bacterial twitching and swarming motilities were assayed on agar plates containing specialized medium with or without a sub-MIC concentrations of pyridoxal lactohydrazone as described previously [43].

Twitching: Luria–Bertani agar plates (1% Bacto agar) with and without sub-MIC concentrations (1/4 and 1/16 MIC respectively) of the pyridoxal lactohydrazone were prepared. Overnight cultures were stabbed with a sterile toothpick through the agar layer to the bottom of the Petri dish. The plates were then incubated at 37 ºC for 48 h. The ability of bacterium to stick on the

polystyrene surface was tested by removing the agar and staining the attached cells with crystal violet (0.1 %, w/v). The plates were washed gently with tap water to get rid of any unattached cells before staining. The diameter of the stained zone was recorded to assess the twitching motility.

Swarming: Nutrient broth (8 g/L) with 5 % glucose was prepared and supplemented with sub-MIC (1/4 and 1/16 MIC) concentrations of pyridoxal lactohydrazone (32 and 8 µg/mL respectively). The medium was solidified by the addition of 0.5% (w/v) Bacto agar. Treated and untreated plates were inoculated with 2 µL of the diluted PAO1 culture and incubated at 37 °C for 16 h.

2.7. Effects on alginate production

The effect of sub-MIC concentrations of the pyridoxal lactohydrazone on alginate production was assayed as described previously [44- 46]. Briefly, 1 mL of bacterial suspension (1×10^8 CFU mL^{-1}) was cultured with and without sub-MIC concentrations (1/4 and 1/16 MIC) of tested compound in a final volume of 100 mL, at 37 °C for 48 h. After 48 h of incubation, pellets were removed and 1 mL of culture was centrifuged for 30 min at 12,000 rpm. The supernatant was retained, and maintained at 80 °C in a water bath for 30 min and then centrifuged at 12,000 rpm for 30 min. The refined supernatant was precipitated with 3 mL of ice-chilled ethanol 99% (v/v) at 4 °C for 2 h. Then, 1 mL of sterile saline (0.9%) was mixed with the precipitate. The carbazole–borate assay was then carried out to determine the amount of uronic acid production. In brief, one mL of borate–sulfuric acid reagent (100 mM H_3BO_3 in concentrated H_2SO_4) was added to 118 µL of culture on ice, and 34 µL of carbazole reagent (0.1% in ethanol) was then added. The mixture allowed to warm up 55 °C for 30 min, and the absorbance (OD_{530}) was measured.

2.8. Effects on pyocyanin production

Pyocyanin production was determined with and without sub-MIC concentrations (1/4 and 1/16 MIC) of pyridoxal lactohydrazone, as described previously [14,45,46]. 500 µL of bacteria (1×10^8 CFU ml^{-1}) were cultured with tested compound at 37 °C for 24 h, in a final volume of 10 mL. In order to extraction of pyocyanin, 3 mL of chloroform mixed with 5 mL of supernatant. After mixing, when two distinct phases were formed, the chloroform extracted phase transferred to another test tube and 1 mL of hydrochloric acid (0.2 M in distilled water) was added. After centrifugation at 12,000 rpm at 4 °C for 10 min, the absorbance (520 nm) was recorded. Pyocyanin concentration was calculated as $OD_{520} \times 17.07$.

2.9. Effects on hydrogen peroxide susceptibility

The susceptibility of hydrogen peroxide was determined according to He et al [14]. Briefly, 100 µL of bacteria (1×10^8 CFU mL^{-1}) were cultured with the pyridoxal lactohydrazone at 37 °C for 48 h, in a final volume of 2 mL. After pellet removal, 100 µL of the supernatant were poured onto nutrient agar plates and a filter paper (pore size 0.45 µm) saturated with 20 µL of H_2O_2 (10%, v/v) was placed in the center of the plates. The plates were incubated at 37 °C for 48 h and diameter of the inhibition zones was expressed in mm.

2.10. Molecular Docking

High resolution X-ray crystal structure of *P. aeruginosa* LasR with the agonist 3-oxo-C_{12}-HSL was obtained from the Protein Data Bank website (PDB ID: 2UV0) and molecular docking studies was carried out using Auto Dock Tools (ADT, version 4.2.6) [47]. The 3D crystal structure of protein was prepared for docking by removing the co-crystallized ligand and waters. Also, the ligand was prepared for docking by energy minimization using the molecular mechanics force field of MM+ and the semi-emperical method of AM1 with Hyperchem

Professional 8.0 program. Partial charges were calculated by Gasteiger method and the grid box was settled to include all amino acid residues in the active site. The best docked conformation of the pyridoxal lactohydrazone was identified based on docking score and also by analyzing the active site hydrogen bonds and π-interactions. The graphical representations were shown using UCSF Chimera 1.10.2 program.

2.11. Statistical analysis

The data were analyzed by SPSS version 17.0 software (SPSS, Inc., Chicago, IL). The one way ANOVA (Tukey) test was used to compare the statistical significance of the data. *P*- value< 0.05 was considered significant.

3. Results

3.1. Cell growth inhibition

The cell growth curves of PAO1 and *C. violaceum* CV026 in the presence of different concentrations of pyridoxal lactohydrazonewere shown in Fig 2. It was observed that the presence of pyridoxal lactohydrazonewith concentration of 128 µg/mL decreased the cell growth to 92.8% and 62.4% in PAO1 and CV026 in comparison with control, respectively ($P<0.001$).

3.2. Inhibition of quorum sensing activity by pyridoxal lactohydrazone

The pyridoxal lactohydrazone showed anti-QS activity in *C. violaceum* CV026 biosensor bioassay and inhibition of violacein production observed in well diffusion assay. The biomarker strain *C. violaceum* CV026 is a mini-Tn5mutant derived from ATCC 31532. It is unable to synthesize the autoinducer C_6-HSL, but can respond to an exogenous short chain AHL by producing the purple pigment of violacein [48]. After 24 h incubation, the presence of a pigmentless zone but viable cells around the wells with 16 mm and <10 mm diameter was

observed at the concentrations of 128 µg/ml and 64 and 32 µg/ml of pyridoxal lactohydrazone, respectively.

3.3. Determination of sub-inhibitory concentrations of pyridoxal lactohydrazone

According to our previous study [18], MIC of pyridoxal lactohydrazone for *P. aeruginosa* PAO1 was determined as 128 µg/mL. Sub-inhibitory concentrations of 1/4 and 1/16 MIC (32 and 8 µg/mL, respectively) were used to assess inhibitory activity of pyridoxal lactohydrazone on virulence factors.

3.4. Effects on biofilm formation

As reported in our previous study [18], treated PAO1 cultures with concentrations of 1/4 and 1/16 MIC of pyridoxal lactohydrazone showed a significant decrease (P<0.001) in biofilm formation when compared to untreated PAO1 (Fig. 3).

3.5. Effect of pyridoxal lactohydrazoneon motility

The effect of pyridoxal lactohydrazone on twitching and swarming motilities of *P. aeruginosa* was determined by inoculating overnight cultures of PAO1 onto motility plates. It was observed that the presence of tested compound with concentrations of 1/4 and 1/16 MIC decreased swarming and twitching motilities with significant value (P<0.05). The inhibitory effect was concentration dependent manner and 1/4 MIC of pyridoxal lactohydrazone demonstrated greater effects on motility inhibition (Fig. 4).

3.6. Effects of pyridoxal lactohydrazone virulence factors

The effect of pyridoxal lactohydrazone on alginate and pyocyanin production and hydrogen peroxide susceptibility was shown in Fig. 5. The results of treatment with 1/4 and 1/16 MIC

concentrations of pyridoxal lactohydrazone showed significant decrease on alginate production to 64% and 40% respectively in comparison with untreated PAO1(P<0.001). Furthermore, 1/4 and 1/16 MIC concentrations of tested compound was significantly effective in decreasing the production of pyocyanin to 79% and 67% respectively (P<0.001). An agar overlay assay was used to measure the H_2O_2 susceptibility of PAO1 strain. Also, the growth of *P. aeruginosa* was affected significantly by compound. Furthermore, concentrations of 1/4 and 1/16 MIC of this compound increased the sensitivity of PAO1 to H_2O_2 in comparison to untreated PAO1 (P< 0.001).

3.7. Molecular docking

In order to understand the interactions of pyridoxal lactohydrazone with LasR, it was necessary to study the binding site of this compound in the QS regulatory protein. The synthesized molecule was docked to LasR to determine the preferred binding sites. The *in silico* results of molecular docking were illustrated that this compound in the best docked pose showed binding energy of -6.30 kcal/mol in the active site of LasR.

Also, in order to assess the accuracy of docking method for the LasR receptor system, the root mean square distance (RMSD) value between the co-crystallized inhibitor (3-oxo-C_{12}-HSL) conformation and the best docked pose of native ligand was calculated. The RMSD value of the docked conformer relative to its crystal orientation was found within the range of 2 Å that suggesting good docking reliability [49].

Figure 6 shows the pyridoxal lactohydrazone made hydrogen bonding interactions with Arg 61, Ser 129, Trp 60, and Thr 75 in the active site of LasR. In addition, figure 7 shows the overlay of the native crystal orientation of 3-oxo-C_{12}-HSL with pyridoxal lactohydrazone in the active site

of LasR. The pyridine ring and lactic moiety of pyridoxal lactohydrazone overlapped with the side chain and homoserine ring respectively in LasR.

4. Discussion

Inhibition of QS is a potential strategy for decrease the virulence genes expression and control of microbial infections. The results of this study demonstrated that pyridoxal lactohydrazone has the capacity to inhibit the QS system based on biosensor strain *C. violaceum* CV026. Results showed that the tested compound inhibits QS-regulated violacein pigment production in CV026.

In literatures, the effect of sub-inhibitory concentrations of various antibiotics such as azithromycin, ceftazidime and ciprofloxacin as a QS inhibitors [43,1] has been studied on the expression of resistance related genes [50], morphology and biochemical properties [51], biofilm formation [52], motility and flagella formation in *P. aeruginosa* [51,53]. We investigated the effects of sub-inhibitory concentrations of pyridoxal lactohydrazone as a naturally and bioactive compound on QS related virulence factors in *P. aeruginosa*. According to the previous results, MIC of pyridoxal lactohydrazone for PAO1 was determined as 128 µg/mL [18]. Treated PAO1 cultures in the presence of pyridoxal lactohydrazone at 1/4 and 1/16 MIC showed significant inhibition of virulence factors including motility, biofilm formation, alginate and pyocyanin production and susceptibility to H_2O_2 (P<0.001).

Our results showed that tested compound significantly inhibited the cell growth in PAO1 as 92.8% in concentration of 128 µg/mL and decreased the biofilm formation (44-71%) and pyocyanin production (67-79%) in sub-MIC concentrations in comparison with untreated strain. Pyocyanin as an important virulence factor of *P. aeruginosa* is a blue–green phenazine pigment. It is an intracellular redox buffer that inhibits the oxidative burst of phagocytic cells by apoptosis in host cells and effect to bacterial pathogenesis [54]. Inhibition of pyocyanin has the bactericidal

activity and increase the susceptibility to oxygen free radicals. Also, it is reported that pyocyanin is a potential biomarker with diagnostic property for identification of *P. aeruginosa* infections [55, 56]. Previous studies showed that QS participates in H_2O_2 resistance of *P. aeruginosa* using production of superoxide dismutase and antioxidants catalase enzymes, resulting in the resistance to the toxic free oxygen radicals [57]. In this study we confirmed that this compound significantly made bacterial strains more susceptible to H_2O_2. Similar results were reported by He et al. that demonstrated the feasibility of attenuating the tolerance of *P. aeruginosa* to azithromycin by using marine oligosaccharides [14]. These preliminary results suggest that the pyridoxal lactohydrazone compound exhibit inhibitory effect on cell growth and quorum sensing related virulence factors in *P. aeruginosa*. Also, tested compound showed quorum sensing inhibitory activity on CV026 and exhibited inhibitory activity of violacein synthesis in tested concentrations.

Additionally, the docking results suggested that H-bonding and hydrophobic interactions to amino acid residues play an important role in the interpretation of molecular mechanism of QSI activity of pyridoxal lactohydrazone in *P. aeruginosa*. The LasR make many H-bonds to the HSL of autoinducer 3-oxo-C_{12}-HSL such as Arg 61, Tyr 56, Trp 60, Ser 129, Asp 73, and Thr 75 [58], which is similar to that observed for pyridoxal lactohydrazone. Pyridine ring in pyridoxal moiety had formed hydrogen bonding through nitrogen atom with Arg 61 (2.08, 2.69 Å). Also, oxygen atoms of hydroxyl methyl and hydroxyl substitutes of pyridine ring interact with Ser 129 and Trp 60 within distance of 2.56 and 2.26 Å respectively. In addition, the NH group in lactic moiety had formed hydrogen bond with Thr 75 (2.33 Å) in the active site of LasR (Fig. 6). Also, π-π interaction between pyridine ring and the phenyl ring of Tyr 64 enhanced hydrazone-LasR complex stability which leads to more inhibitory activity of this compound in LasR.

It was found in literatures that presence of -NH- group in the side chain of synthesized QSI compounds enhances its inhibitory activity [59,60]. As well as, in present study, NH group in side chain of pyridoxal lactohydrazone play important role in inhibitory activity through interaction with Thr 75 in LasR.

These results indicated that the QSI activity of tested compound may be depends on the presence of nitrogen atoms and hydroxyl groups which interacted with the amino acid residues in LasR.

5. Conclusion

To our knowledge, this is the first study to show anti-quorum sensing activity of pyridoxal lactohydrazone against *P. aeroginosa*. Therefore, this compound could lead to the development of new antimicrobial agent against *P. aeruginosa*.

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484

485 **Figure Legends**

486 **Scheme 1.** The synthetic pathway of (*S*,*E*)-2-hydroxy-*N*-((3-hydroxy-5-(hydroxymethyl)-2-
487 methylpyridin-4-yl)propane hydrazide (pyridoxal lactohydrazone)

488 **Fig. 1.** Chemical structure ofautoinducers:PQS, QZN, 3-oxo-C_{12}-HSL, C_4-HSL and inhibitor in
489 this study (pyridoxal lactohydrazone)

490 **Fig. 2.** Effect of different concentrations of pyridoxal lactohydrazone on cell growth inhibition in
491 PAO1 and CV026**.**

492 **Fig. 3.** Effect of sub-MIC concentrations of pyridoxal lactohydrazone on biofilm formation**.** The
493 results are presented as means ±SD, * P< 0.001.

494 **Fig. 4.** Effect of sub-MIC concentrations of pyridoxal lactohydrazone on the motility of
495 *Pseudomonas aeruginosa* PAO1.**a)** The results are presented as means ±SD, * P< 0.001; **
496 P<0.05. **b)** Swarming motility of PAO1 in the absence and presence of tested compound.

497 **Fig. 5.** Effect of sub-MIC concentrations of pyridoxal lactohydrazoneon **a)** alginate production,
498 **b)** pyocyanin production, and **c)** hydrogen peroxide susceptibility. The results are presented as
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500 **Fig. 6.** Ribbon view of docked conformer of pyridoxal lactohydrazone (yellow colour stick
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503 oxo-C_{12}-HSL (blue colour stick model) in the active site of LasR.

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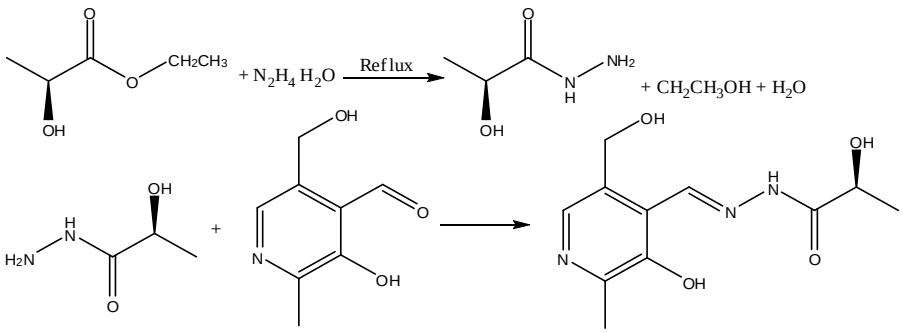

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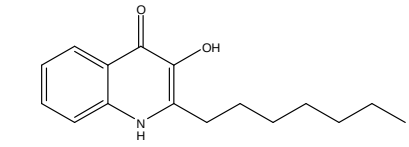
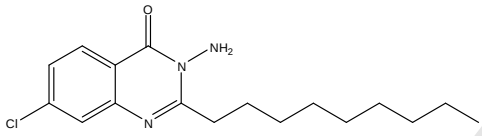


PQS



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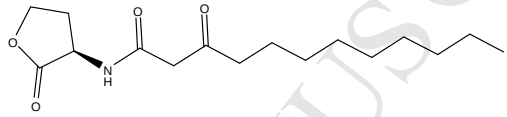
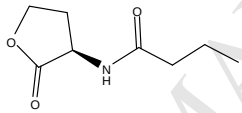


3-oxo-C12-HSL



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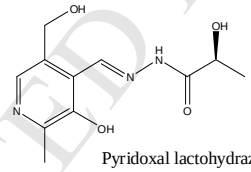

512 Pyridoxal lactohydrazone

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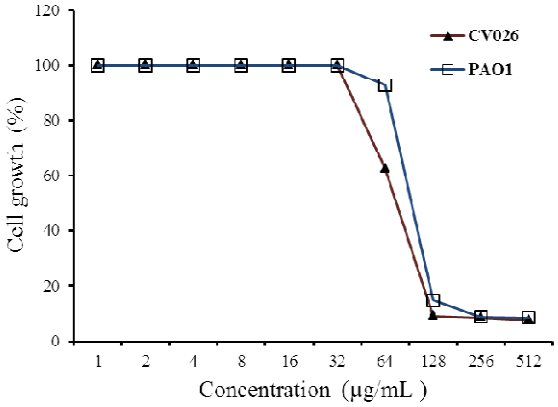

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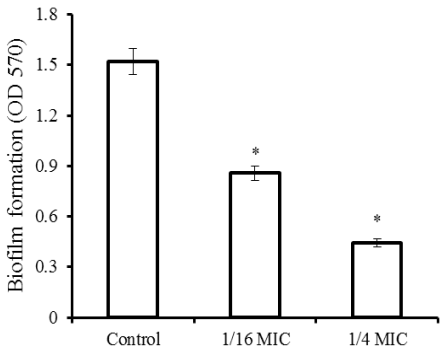
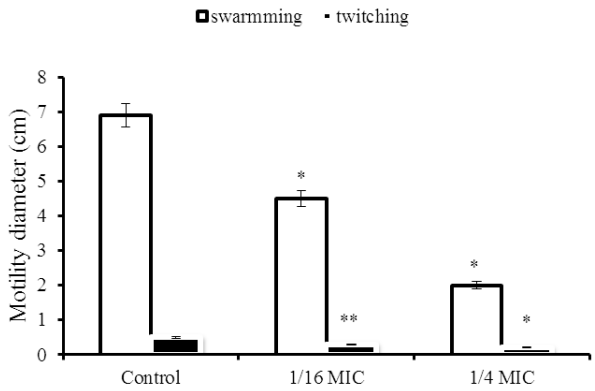
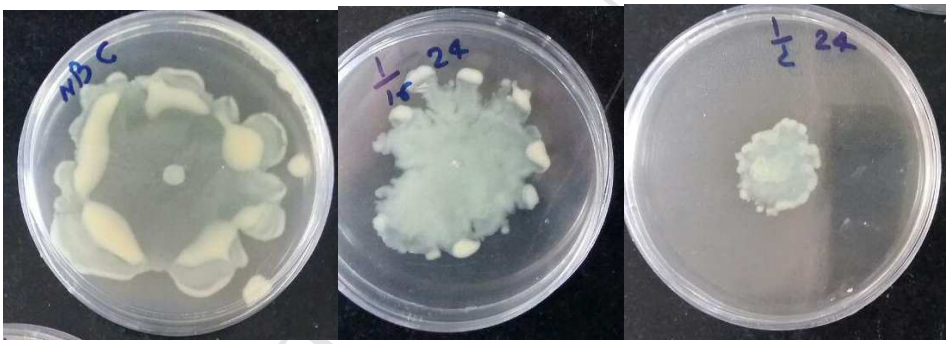


Fig. 3. Effect of sub-MIC concentrations of pyridoxal lactohydrazone on biofilm formation**.** The results are presented as means ±SD, * P< 0.001.

a)



b)

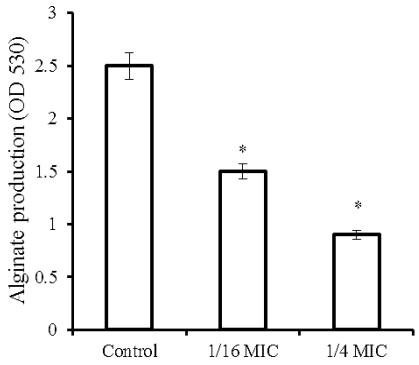


Control 1/16 MIC 1/4 MIC

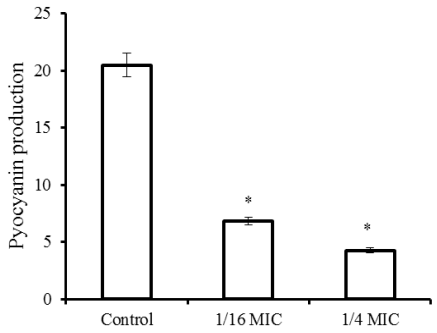
Fig. 4. Effect of sub-MIC concentrations of pyridoxal lactohydrazoneon the motility of *Pseudomonas aeruginosa* PAO1. **a)** The results are presented as means ±SD, * P< 0.001; ** P<0.05. **b)** Swarming motility of PAO1 in the absence and presence of tested compound.

545

546 **a)**



547 **b)**



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549 **c)**

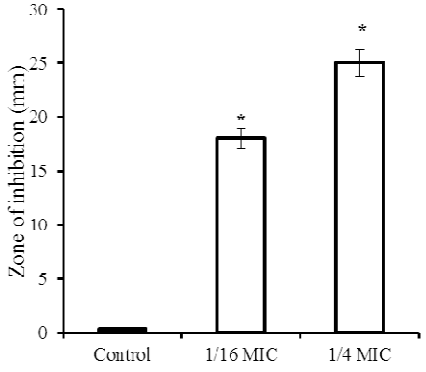

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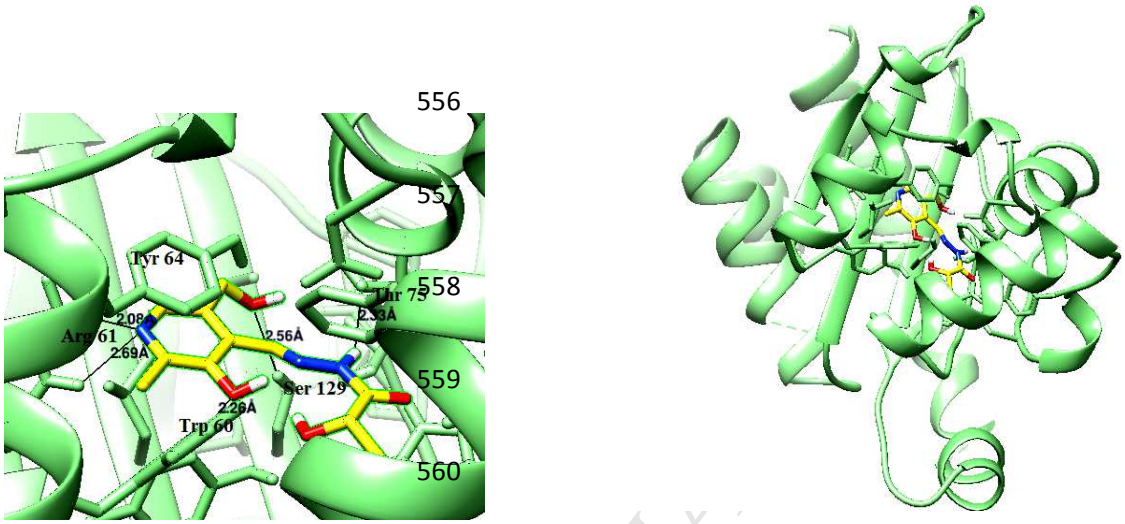
Fig. 5. Effect of sub-MIC concentrations of pyridoxal lactohydrazone on **a)** alginate production, **b)** pyocyanin production, and **c)** hydrogen peroxide susceptibility. The results are presented as means ±SD, * P< 0.001.



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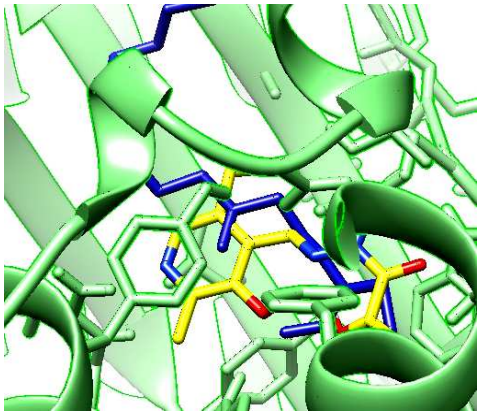

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Highlights

- Pyridoxal lacto hydrazone showed significant inhibition of virulence factors including motility, biofilm formation, alginate and pyocyanin production and susceptibility to H_2O_2 against *Pseudomonas aeruginosa.*
- Molecular docking results suggested that pyridoxal lacto hydrazone have potent to inhibit the QS regulatory protein of LasR.