



Revitalizing common drugs for antibacterial, quorum quenching, and antivirulence potential against *Pseudomonas aeruginosa*: in vitro and in silico insights

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Abstract

In the post-antibiotic era, antivirulence therapies are becoming refractory to the clinical application of existing antimicrobial regimens. Moreover, in an attempt to explore alternate intervention strategies, drug repurposing is gaining attention over development of novel drugs/antimicrobials. With the prevalence of multidrug resistance and high medical burden associated with *Pseudomonas aeruginosa*, there is an urgent need to devise novel therapeutics to combat this bacterial pathogen. In this context, the present study was undertaken to scrutinize the anti-quorum sensing (QS) and antivirulence potential of commonly consumed drugs such as fexofenadine (FeX), ivermectin (IvM), nitrofurantoin (NiT), levocetirizine (LvC), atorvastatin (AtS), and aceclofenac (AcF), against *P. aeruginosa*. The methodology involved assessment of antibacterial activity against *P. aeruginosa* PAO1 and quorum quenching (QQ) potential using *Agrobacterium tumefaciens* NTL4 biosensor strain. The antivirulence prospects were investigated by estimating the production of hallmark virulence factors in *P. aeruginosa* accompanied by molecular docking to predict drug associations with the QS receptors. Interestingly, all the drugs harbored antibacterial, anti-QS, and antivirulence potential in vitro, which consequently disrupted QS circuits and attenuated pseudomonal virulence phenotypically by significantly lowering the production of pyocyanin, hemolysin, pyochelin, and total bacterial protease in vitro. Moreover, the findings were validated by computational studies that predicted strong molecular interactions between the test drugs and QS receptors of *P. aeruginosa*. Hence, this study is the first to suggest the prospect of repurposing FeX, IvM, NiT, LvC, AtS, and AcF against *P. aeruginosa*.

Keywords Quorum quenching and antivirulence therapy · *Pseudomonas aeruginosa* · Atorvastatin and aceclofenac · Cetirizine and levocetirizine · Ivermectin and nitrofurantoin

Abbreviations

AcF	Aceclofenac
AHL	Acyl-homoserine lactone
AtS	Atorvastatin
FDA	US Food and Drug Administration
FeX	Fexofenadine
IvM	Ivermectin
LcV	Levofloxacin
MIC	Minimum inhibitory concentration
NiT	Nitrofurantoin

QQ	Quorum quenching
QS	Quorum sensing

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Pseudomonas aeruginosa is a Gram-negative opportunistic bacterial pathogen recognized as ‘Priority 1’ or critical superbug by the World Health Organization (Tacconelli et al. 2018). Due to extensive multidrug resistance profiles, it causes hard-to-treat nosocomial infections, including urinary tract infections (UTIs), burn wound infections, chronic bronchitis in cystic fibrosis patients, ventilator-associated pneumonia, keratitis, bone and joint infections, and bloodstream-associated infections in immunocompromised individuals (Chadha et al. 2021b). The pathogen harbors a genome of ~6.3 Mbp which encodes a multitude of virulence factors that contribute to bacterial pathogenesis, disease progression, and persistence (Moradali et al. 2017). Interestingly, the virulence of *P. aeruginosa* is stringently controlled

Fig. 1 Evaluation of antibacterial potential of the test drugs at various concentrations against *P. aeruginosa* PAO1 (test concentrations; 1: 2 mg/mL, 2: 5 mg/mL, 3: 10 mg/mL 4: solvent control 10% DMSO/DMF)

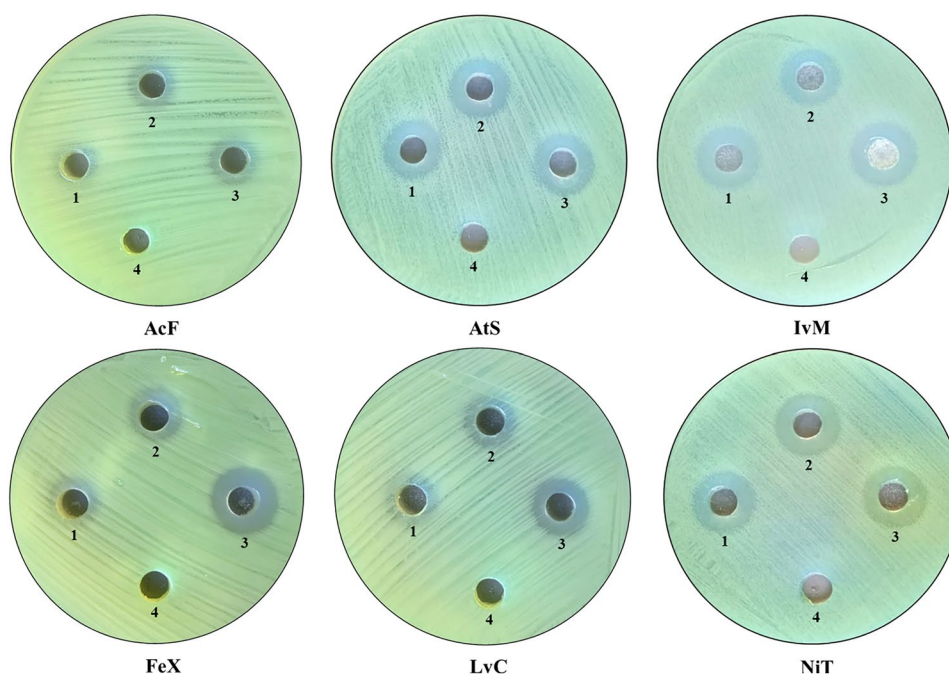


Table 1 Growth inhibition zones obtained with the test drugs against *P. aeruginosa* PAO1

Test drugs	Diameter of inhibition zones (in mm) obtained against <i>P. aeruginosa</i> PAO1 at a test concentration of		
	2 mg/mL	5 mg/mL	10 mg/mL
IvM	17.8 ± 0.76	16.8 ± 0.28	19.0 ± 0
FeX	6.66 ± 0.57	8.0 ± 1.0	13.0 ± 1.0
NiT	17.8 ± 0.28	18.8 ± 0.28	18.0 ± 1.0
LcV	5.66 ± 0.57	7.0 ± 1.0	10.6 ± 1.15
AcF	3.66 ± 1.52	6.33 ± 0.57	7.66 ± 0.57
AtS	17.0 ± 0	19.0 ± 0.5	18 ± 1.0

by quorum sensing (QS), a cell density-dependent phenomenon, which directly influences bacterial social behavior and synchronizes communal responses (Mudgil et al. 2024). The acyl-homoserine lactone (AHL)-dependent Las and Rhl systems along with non-AHL-based Pqs system constitute the intricate QS circuitry of *P. aeruginosa*, which functions in a hierarchical manner (Chadha et al. 2021a). As an innovative approach to combat *P. aeruginosa*, targeting the QS mechanisms has been regarded as a viable alternative to existing antimicrobial therapies (Chadha et al. 2021a). Furthermore, QS inhibitor-based antivirulence drugs are known to reduce the emergence of antimicrobial resistance in bacterial pathogens as they interfere only with virulence pathways (at sub-lethal/inhibitory concentrations), rather than inducing bacterial death (Chadha et al. 2021b). Such antivirulence responses can be induced via (i) hydrolytic enzymes like

lactonase and acylase that degrade AHL molecules, (ii) targeted disruption of QS signaling pathways through competitive inhibition by drug molecule, and (iii) abrogation of AHL biosynthesis (Chadha et al. 2024a).

In recent times, scientific advances have been made to develop novel antivirulence approaches based on the principle of QQ to tackle the notorious pathogen. Various natural products such as essential oils, plant extracts, and phytochemicals have been shown to extend antivirulence potential against *P. aeruginosa* (Chadha et al. 2021a, 2024c). Apart from these, antibiotics, chemically synthesized compounds, and US FDA-approved drugs are also being exploited for the same (Baldelli et al. 2020). Since traditional drug development procedures are extensively lengthy, laborious, and cost-intensive, drug repurposing proves to be a considerable option since it bypasses all these limitations associated with the early stages of development (Krishnamurthy et al. 2022). In this context, the antivirulence and/or antifouling potential of FDA-approved drugs such as niclosamide, azathioprine, doxorubicin, raloxefine, azithromycin, pentetic acid, and flucytosine against *P. aeruginosa* has been well documented (Walker et al. 2017). Fascinated by the potentiality of pre-existing drugs in targeting the QS circuits and consequently attenuating the phenotypic virulence of *P. aeruginosa*, this study was carried out to scrutinize the anti-QS prospects of some commonly consumed FDA-approved drugs, including fexofenadine (FeX), ivermectin (IvM), nitrofurantoin (NiT), levocetirizine (LvC), atorvastatin (AtS), and aceclofenac (AcF) for effectively disarming bacterial virulence in *P. aeruginosa*. Both FeX and LvC belong to the class of anti-histamines and find their application in the treatment of

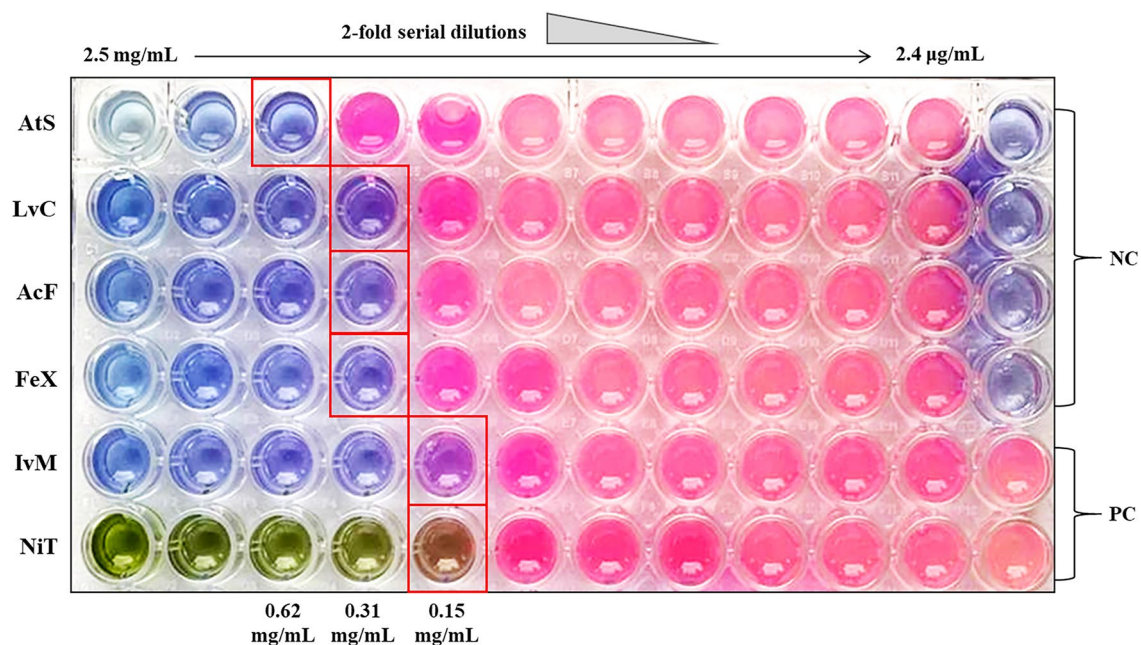
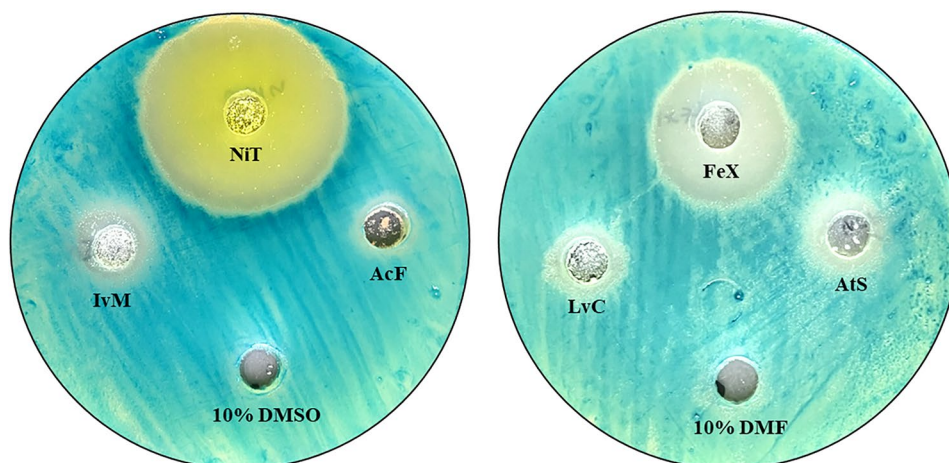


Fig. 2 Qualitative assessment of the anti-QS potential of test drugs (at MIC levels) using the biosensor strain *Agrobacterium tumefaciens* NTL4

Fig. 3 Minimum inhibitory concentrations (MICs) indicating the antimicrobial potency of the test drugs against *P. aeruginosa* PAO1 using microbroth dilution method coupled with resazurin dye reduction



allergic rhinitis (Bachert 2009), while AtS is a commonly used anti-cholesterol drug (McIver and Siddique 2024). Contrarily, NiT (antibiotic) is employed for treating UTI (Huttner et al. 2015), whereas IvM is administered as an anti-parasitic medication (Laing et al. 2017). Moreover, AcF is a non-steroidal anti-inflammatory drug (NSAID) indicated for relieving chronic inflammation and pain in bones/joints (Legrand 2005).

The study was initiated by procuring all the drugs from nearby pharmacies (GlaxoSmithKline Pharmaceuticals) and examining the antibacterial potential of the six (test) drugs against the standard strain of *P. aeruginosa* PAO1. Previously established protocols following Clinical Laboratory Standards Institute (CLSI) guidelines were employed for

performing agar well diffusion assays (Negi et al. 2024). Subsequently, microbroth dilution method coupled with resazurin dye reduction was used to determine the minimum inhibitory concentrations (MICs) of the test drugs (Chadha et al. 2022). Further, the QQ potential of the drugs was qualitatively tested using a biosensor strain, *Agrobacterium tumefaciens* NTL4 (pZLR4), which exhibits active QS in the presence of AHLs (QS molecules). This genetically engineered strain shows AHL-induced expression of β -galactosidase (*lacZ*) from the *traI* promoter on pZLR4 plasmid, resulting in enzymatic degradation of X-Gal (chromogenic substrate), yielding a blue-colored bacterial growth (Chadha et al. 2024b). The growth profile of *P. aeruginosa* PAO1 was then studied spectrophotometrically (A_{600}) in the

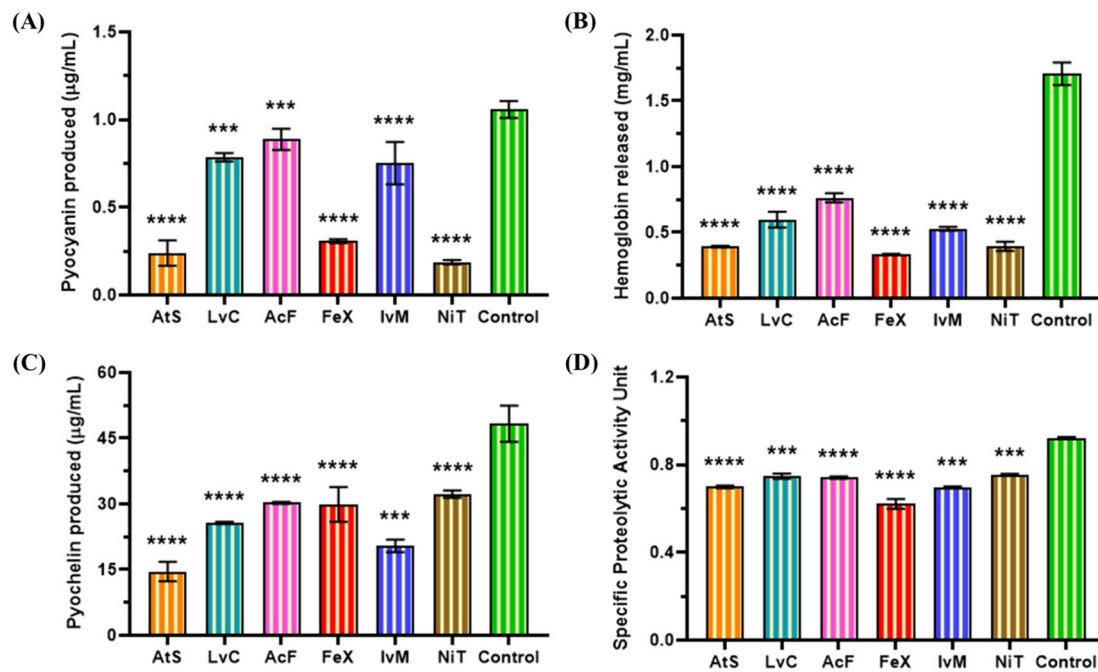


Fig. 4 Antivirulence potential of test drugs in terms of quantitative production of different QS-regulated virulence factors in *P. aeruginosa* PAO1. **A** Pyocyanin production, **B** hemolysin production, **C**

pyochelin production, and **(D)** total bacterial protease (** $p \leq 0.001$, $p^{****} \leq 0.0001$)

presence of different sub-MICs (1/2, 1/4, 1/8, and 1/16) of the test drugs to identify drug concentrations that do not inhibit bacterial growth (Chadha 2021; Chadha and Khullar 2021). The antivirulence prospects were then tested by culturing *P. aeruginosa* PAO1 in the presence of the drugs (at individual sub-MICs) and quantitatively assessing the levels of pyocyanin, hemolysin, total protease activity, and pyochelin production in cell-free supernatants using standard laboratory protocols (Chadha et al. 2023a, 2024a). The experimental findings for each experiment were compared with those of its drug-free control (untreated). Since high-affinity interactions with QS receptors is an effective mechanism of QQ (QS signal inhibition), computational studies and molecular docking were performed using AutoDock Vina (version 1.5.4) to predict the molecular interactions/associations between the test drugs and QS receptors (LasR, RhIR, and PqsR) of *P. aeruginosa* (Chadha et al. 2022, 2023b).

The preliminary findings from the agar well diffusion assay indicated the antibacterial potential of all the test drugs at varying concentrations against *P. aeruginosa* PAO1 (Fig. 1). The diameters of growth inhibition increased in a dose-dependent manner with maximum bacterial inhibition being observed with IvM, NiT, and AtS and least with AcF (Table 1). Subsequently, the antibacterial potency of the

drugs was quantitatively determined in terms of MIC using the microbroth dilution method. The MIC values of AtS, LvC, AcF, FeX, IvM, and NiT against *P. aeruginosa* PAO1 were found to be 0.62, 0.31, 0.31, 0.31, 0.15, and 0.15 mg/mL, respectively (Fig. 2), suggesting that the latter two drugs exhibit comparatively higher antibacterial effectiveness. These results draw parallels with recent studies that document repurposing of FDA-approved drugs such as metformin (Chadha et al. 2023a), paracetamol (Seleem et al. 2021), and albendazole (Chadha et al. 2024a) for their antibacterial properties against *P. aeruginosa* with MIC values reported to be 100 mg/mL, 256 μg/mL, and 625 μg/mL, respectively. Considering the high MIC values of the test drugs (in mg/mL), their potential as antibacterial agents against *P. aeruginosa* is deemed inferior to existing antibiotics that effectively eliminate bacterial pathogens at extremely low concentrations (in μg/mL). Hence, the anti-QS potential of the six drugs (at MIC levels) was qualitatively tested using the *A. tumefaciens* NTL4 biosensor strain. Interestingly, NiT, FeX, and IvM resulted in the development of two distinct zones, i.e., a larger antibacterial zone with no growth (clear) of 31.0 ± 1.15 , 21.3 ± 1.15 , and 6.6 ± 0.57 mm, respectively, and a smaller white-colored anti-QS zone, against a blue-colored bacterial lawn (Fig. 3). These results are in accordance with existing literature which highlights the antibacterial prospects of repurposed compounds at higher concentrations and their anti-QS potential at lower concentrations that do not induce bacterial killing (Kumar et al.

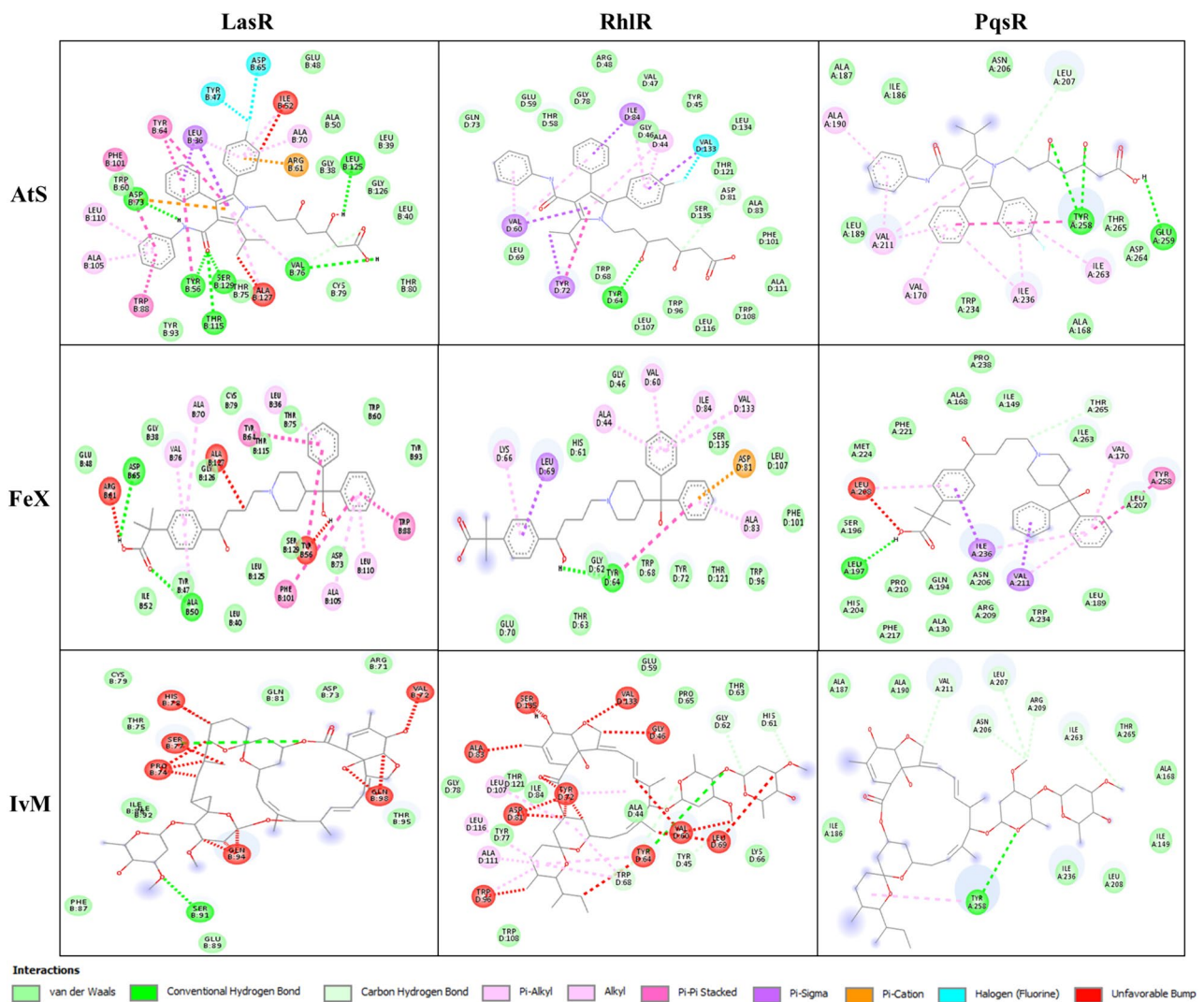


Fig. 5 Schematic representation of the molecular interactions predicted between AtS/FeX/IvM and QS receptors of *P. aeruginosa* (LasR, RhlR, and PqsR) using AutoDock Vina (version 1.5.4). Molecular interactions were visualized using BIOVIA Discovery Studio (version 21.1)

2021; Chadha et al. 2022). On the other hand, AcF, LvC, and AtS yielded only white-colored zones around the wells measuring 3.0 ± 1.0 , 6.6 ± 0.57 , and 7.0 ± 1.0 mm, respectively, indicating their anti-QS potential (Fig. 3). Since the biosensor strain does not exhibit intrinsic QS mechanisms, but rather induced expression of β -galactosidase resulting from externally supplemented AHLs, the QQ potential of the drugs can be attributed to their interference via competitive inhibition (Chadha et al. 2021b). These findings provided fertile grounds to consider the possible role of selected drugs in lowering phenotypic virulence in *P. aeruginosa*.

To investigate the antivirulence prospects, *P. aeruginosa* PAO1 was cultured in the presence of the drugs at various sub-MICs and changes in A_{600} were measured spectrophotometrically. Interestingly, higher drug concentrations (1/2, 1/4, 1/8 MIC) altered bacterial growth kinetics, while all the

test drugs at their respective 1/16 MIC values did not affect bacterial growth, resembling growth profiles similar to that of the drug-free control (data not shown). Hence, PAO1 cultures were raised in the presence of drugs at their 1/16 MIC values and the production of hallmark virulence factors in drug-treated cell-free supernatants was quantitatively estimated. As anticipated, the phenotypic expression of all four virulence factors was significantly lowered upon drug treatment (Fig. 4). The highest degree of virulence inhibition was observed with AtS, followed by NiT, FeX, IvM, LvC, and AcF in decreasing order. With AtS treatment, the production of pyocyanin, hemolysin, pyochelin, and total bacterial protease in PAO1 was diminished by 77.24%, 77.1%, 69.84%, and 24.1%, while LvC lowered the same by 25.81%, 65.02%, 46.88%, and 18.78%, respectively (Fig. 4). Exposure to AcF also reduced the phenotypic virulence by 16.1%, 55.31%,

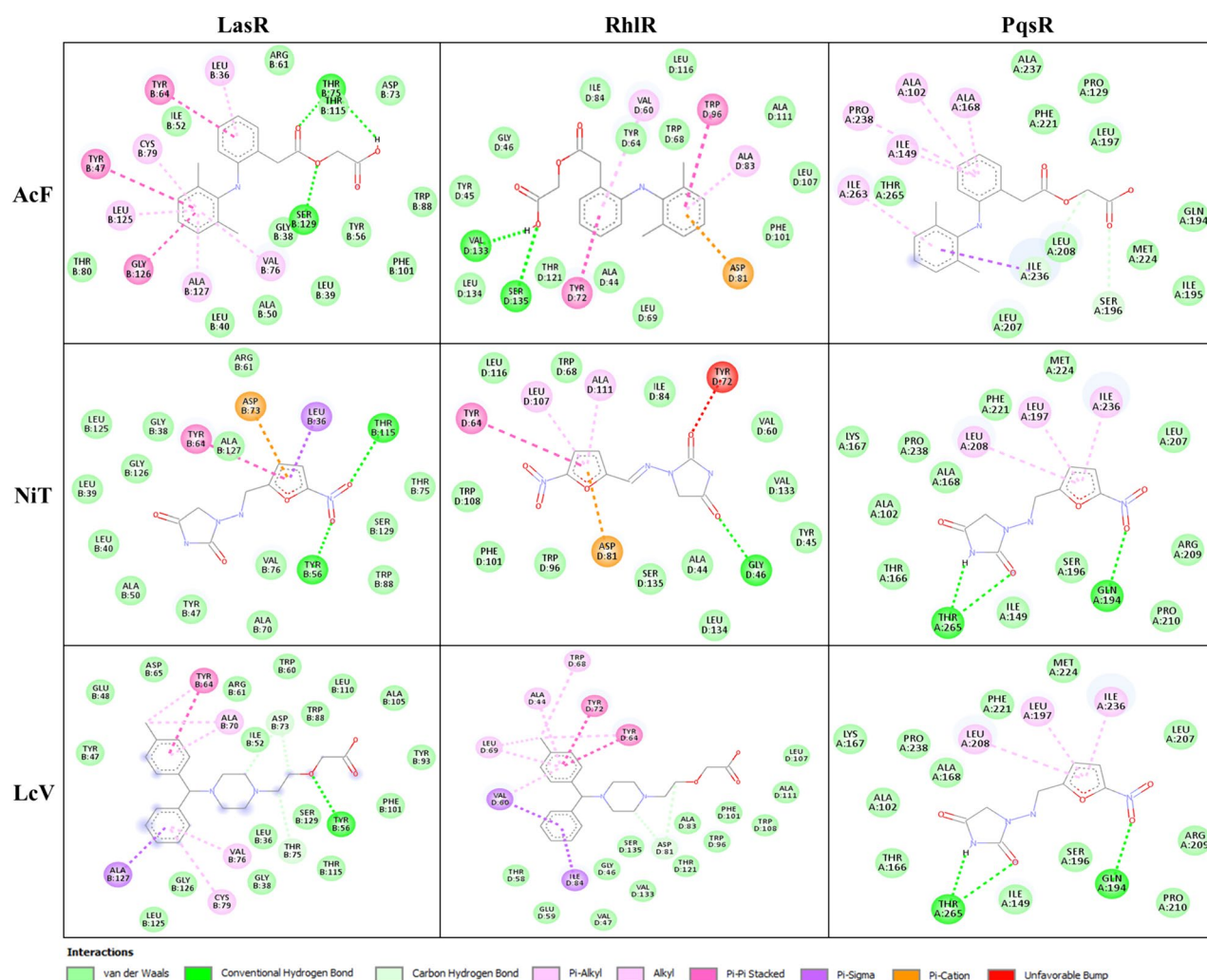


Fig. 6 Schematic representation of the molecular interactions predicted between AcF/NiT/LcV and QS receptors of *P. aeruginosa* (LasR, RhlR, and PqsR) using AutoDock Vina (version 1.5.4). Molecular interactions were visualized using BIOVIA Discovery Studio (version 21.1)

37.38%, and 19.54%, whereas FeX extended comparatively higher inhibitory effects on pseudomonal virulence factors by 70.98%, 80.54%, 38.21%, and 32.57%, respectively. Similarly, the production of the above-mentioned virulence determinants was impeded by 29.1%, 69.07%, 57.67%, and 24.34% with IvM, while treatment with NiT extended inhibition by 82.3%, 76.92%, 33.25%, and 18.1%, respectively, in PAO1 as compared to the untreated control (Fig. 4). Overall, it was evident that all the selected drugs at sub-MICs were capable of lowering the production of hallmark pseudomonal virulence factors phenotypically, which can be accredited to their potential in disrupting QS circuits in the pathogen. Similar findings have been widely reported with other drugs such as albendazole (Chadha et al. 2024a) and sitagliptin (Abbas et al. 2020), where anti-QS properties of the compounds consequently resulted in lowering pseudomonal virulence, thereby negatively impacting bacterial

pathogenesis and disease progression. The experimental findings were then computationally validated using molecular docking to study possible interactions between the test drugs and QS receptors of *P. aeruginosa*. Exploring this aspect becomes virtually critical for successfully delineating the possible mechanism of QQ and identifying drug targets, which in this case were probably the LasR, RhlR, and/or PqsR QS receptors. The natural ligands (3-oxo-C12-HSL, C4-HSL, and PQS) were used as control molecules for the respective QS receptor alongside furanone C-30, a known QS inhibitor. In silico analysis strongly correlated with the in vitro experimentation and indicated strong molecular interactions between the drugs and QS receptors (Figs. 5, 6). The drugs formed multiple hydrogen bonds (H-bonds), van der Waal (vdW) interactions (hydrophobic bonds), π -cation bonds (electrostatic interactions), and non-covalent bonds (π -alkyl and π -sigma) with all three QS receptors (Figs. 5,

Table 2 Binding energies and molecular interactions predicted between the six test drugs and LasR/RhlR/PqsR QS receptors of *P. aeruginosa* along with their natural ligands and furanone C-30

QS receptor	Ligands	Binding energy (in kcal/mol)	Interacting amino acid residues
LasR	3-oxo-C12-HSL	−8.2	Leu36, Gly38, Tyr47, Ile52, Tyr56, Trp60, Arg61, Tyr64, Ala70, Asp73, Thr75, Val76, Trp88, Tyr93, Phe101, Ala105, Thr115, Gly126, Ala127, Ser129
	AtS	−4.7	Leu36, Gly38 , Leu39, Leu40, Tyr47 , Glu48, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64 , Asp65, Ala70, Asp73, Thr75, Val76 , Cys79, Thr80, Trp88, Tyr93, Phe101, Ala105 , Leu110, Thr115 , Leu125, Gly126, Ala127, Ser129
	FeX	−5.3	Leu36, Gly38 , Leu40, Tyr47 , Glu48, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64 , Asp65, Ala70, Asp73, Thr75, Val76 , Cys79, Trp88, Tyr93, Phe101, Ala105 , Leu110, Thr115 , Leu125, Gly126, Ala127, Ser129
	IvM	−2.1	Arg71, Val72, Asp73 , Pro74, Thr75 , Ser77, His78, Cys79, Gln81, Ile86, Phe87, Glu89, Ser91, Ile92, Gln94, Thr95, Gln98
	AcF	−8.8	Leu36, Gly38 , Leu39, Leu40, Tyr47, Ala50, Ile52, Tyr56, Arg61, Tyr64, Asp73, Thr75, Val76 , Cys79, Thr80, Trp88, Phe101, Thr115 , Leu125, Gly126, Ala127, Ser129
	NT	−8.5	Leu36, Gly38 , Leu39, Leu40, Tyr47, Ala50, Tyr56, Arg61, Tyr64, Ala70, Asp73, Thr75, Val76, Trp88, Thr115 , Leu125, Gly126, Ala127, Ser129
	LcV	−7.5	Leu36, Gly38, Tyr47 , Glu48, Ile52, Tyr56, Trp60, Arg61, Tyr64 , Asp65, Ala70, Asp73, Thr75, Val76 , Cys79, Trp88, Tyr93, Phe101, Ala105 , Leu110, Thr115 , Leu125, Gly126, Ala127, Ser129
	Furanone C-30	−6.6	Leu36, Tyr47, Ala50, Ile52, Tyr56, Trp60, Arg61, Tyr64, Thr75, Val76, Ala127, Ser129
RhlR	C4-HSL	−6.6	Ala44, Val60, Tyr64, Trp68, Tyr72, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111, Thr121, Ser135
	AtS	−2.6	Ala44 , Tyr45, Gly46, Val47, Arg48, Thr58, Gly59, Val60, Tyr64, Trp68 , Leu69, Tyr72 , Gly78, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111 , Leu116, Thr121, Val133 , Leu134, Ser135
	FeX	−5.6	Ala44, Val60 , His61, Gly62, Thr63, Tyr64 , Leu65, Lys66, Trp68 , Glu70, Tyr72, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Thr121, Val133, Ser135
	IvM	−3.0	Ala44 , Tyr45, Gly46, Glu59, Val60 , His61, Gly62, Thr63, Tyr64 , Pro65, Lys66, Trp68 , Leu69, Tyr72 , Tyr77, Gly78, Asp81, Ala83, Ile84, Trp96, Leu107, Trp108, Ala111 , Leu116, Thr121, Val133, Ser135
	AcF	−8.5	Ala44, Tyr45 , Gly46, Val60, Trp68 , Leu69, Tyr72, Asp81, Ala83, Ile84, Phe101, Leu107, Ala111, Thr121, Val133 , Leu134, Ser135
	NT	−7.6	Ala44 , Tyr45, Gly46, Val60, Tyr64, Trp68 , Tyr72, Asp81, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111 , Leu116, Val133, Leu134, Ser135
	LcV	−6.8	Ala44 , Gly46, Val47, Thr58, Gly59, Val60, Tyr64, Trp68 , Leu69, Tyr72, Asp81, Ala83, Ile84, Trp96, Phe101, Leu107, Trp108, Ala111, Thr121, Val133, Ser135
	Furanone C-30	−5.7	Ala44, Val60, Trp68, Tyr72, Asp81, Ala83, Trp96, Leu107, Val133, Ser135
PqsR	PQS	−7.0	Ile149, Ala168, Val170, Ser196, Leu197, Leu207, Leu208, Phe221, Met224, Ile236, Tyr258, Ile263, Thr265
	AtS	−7.1	Ala168, Val170 , Ile186, Ala187, Leu189, Ala190, Asn206, Leu207 , Val211, Trp234, Ile236, Tyr258 , Glu259, Ile263, Asp264, Thr265
	FeX	−9.5	Ala130, Ile149, Ala168, Val170 , Leu189, Gln194, Ser196, Leu197 , His204, Asn206, Leu207, Leu208 , Arg209, Val211, Phe217, Phe221, Met224, Trp234, Ile236, Pro238, Tyr258, Ile263, Thr265
	IvM	−11.6	Ile149, Ala168 , Ile186, Ala187, Ala190, Asn206, Leu207, Leu208 , Arg209, Val211, Ile236, Tyr258, Ile263, Thr265
	AcF	−7.7	Ala102, Pro129, Ile149, Ala168, Gln194 , Ile195, Ser196, Leu197, Leu207, Leu208, Phe221, Met224, Ile236, Ala237, Pro238, Ile263, Thr265
	NT	−6.7	Ala102, Ile149 , Thr166, Lys167, Ala168, Gln194, Ser196, Leu197, Leu207, Leu208 , Arg209, Pro210, Phe221, Met224, Ile236, Pro238, Thr265
	LcV	−6.6	Pro129, Ile149, Ala168, Val170 , Leu189, Leu197, Asn206, Leu207, Leu208 , Val211, Phe221, Met224, Trp234, Ile236, Ala237, Pro238, Tyr258, Ile263, Thr265
	Furanone C-30	−5.9	Ala102, Ser128, Pro129, Ala130, Ile149, Gln194, Leu197, Leu208, Phe221, Ile236, Ala237, Pro238

Table 2 (continued)

Boldface amino acid residues interacting with the test drugs were found to have overlapping interactions with that of either the natural ligand of each QS receptor or furanone C-30

6), which were found to be overlapping with either the natural ligands and/or furanone C-30 (boldface residues: Table 2). The binding energies for all control and test ligands toward the QS receptors have been collated along with their interacting amino acid residues in Table 2. From the binding energies predicted, AcF showed the overall highest affinity toward all the QS receptors, while IvM displayed the lowest affinity (except for PqsR). Overall, it was evident that all the drugs were capable of strongly associating with the QS receptors, which may be responsible for disrupting QS circuits by competitively inhibiting the binding of natural ligands to their respective ligand-binding domain within the QS receptors, thereby achieving targeted inhibition over QS signal reception (Chadha et al. 2023b).

Collectively, the current study validates the antibacterial, anti-QS, and antivirulence potential of the six FDA-approved drugs, AtS, LvC, AcF, FeX, IvM, and NiT, against *P. aeruginosa* PAO1. By combining standard in vitro experimentation and in silico methods, the study elucidated that these drugs can be repurposed beyond their existing medical applications to effectively attenuate QS circuits and ultimately disarm phenotypic virulence of *P. aeruginosa*. Due to the prevalence of widespread drug resistance and limited options available to treat pseudomonal infections, these drugs can prove to be vital in clinical settings. The biomedical application/repurposing of these drugs can be extended for curbing *P. aeruginosa*, provided comprehensive preclinical studies combining in vitro experimentation and in vivo investigations are carried out in animal infections models to prove their therapeutic efficacy. Moreover, if not alone, such QQ drugs may be given in combination with existing antimicrobial regimens for improving the therapeutic outcome, quality of patient life, and lowering medical burden in healthcare facilities, thereby lowering the dependence on antibiotic-based treatment strategies.

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Data availability statement The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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