Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - XXXIX

Edaravone derivatives as potential Quorum Sensing inhibitors in *Pseudomonas aeruginosa*

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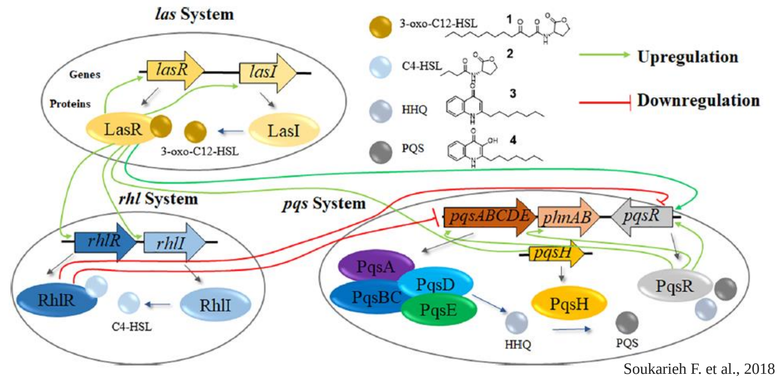
Background

Pseudomonas aeruginosa is an opportunistic human pathogen causing nosocomial infections and is one of the major bacteria involved in chronic infections in patients with Cystic Fibrosis. Its ability to form biofilms, along with its multidrug resistance, underscores the urgent need for the development of novel antimicrobial strategies [1, 2].

In *P. aeruginosa*, three main quorum sensing (QS) systems regulate the expression of virulence genes and biofilm production: Las, Rhl, and PQS, which are organized in a hierarchical manner [1]. At the top of this signaling cascade is the Las system, which is activated when LasR binds its signal molecule, the autoinducer N-3-oxo-dodecanoyl-L-homoserine lactone (3OC12-HSL). The LasR–HSL complex activates the transcription of its own regulon, as well as those of the Rhl and PQS systems, along with several virulence-associated genes (**Figure 1**).

Disrupting the QS system through the use of quorum sensing inhibitors (QSIs) represents a promising therapeutic alternative to traditional antibiotics. This approach can reduce bacterial virulence, which is essential for the establishment of infection, and increase the susceptibility of microbial biofilms to antibiotic treatment [3].

Figure 1. Quorum sensing systems in P. aeruginosa

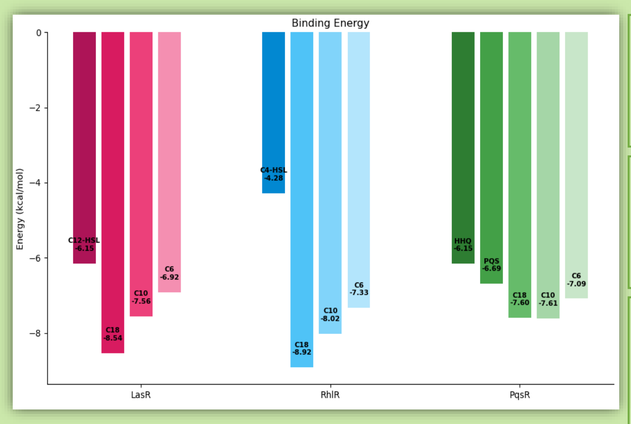


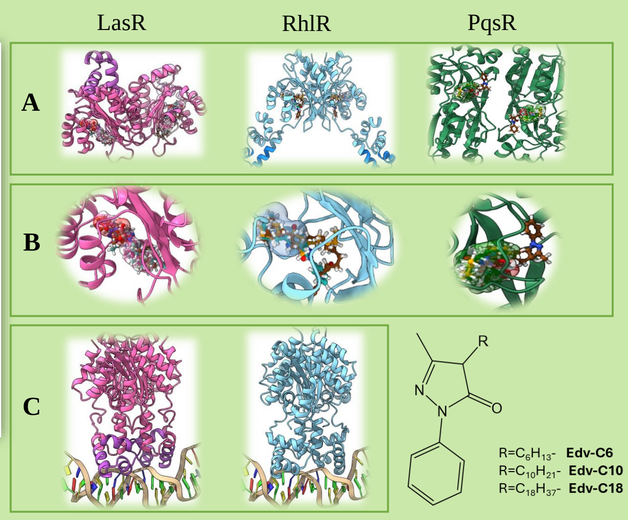
Soukarieh F. et al., 2018

Aim

In this study, we investigated Edaravone and its lipophilic derivatives (Edv) as potential QSIs, based on the *in silico* structural similarity of Edaravone to *P. aeruginosa* autoinducers. Derivatives were obtained by introducing a fully saturated lipophilic chain (C6, C10, or C18) at the C4 position. The effects of Edv-C6, Edv-C10, and Edv-C18 on biofilm formation and production of the virulence factor **pyocyanin** were evaluated in the *P. aeruginosa* PAO1 strain. Furthermore, transcriptional interference by Edv compounds was assessed by quantifying the expression levels of key QS-regulatory genes: *lasR*, *rhlR*, and *pqsR*.

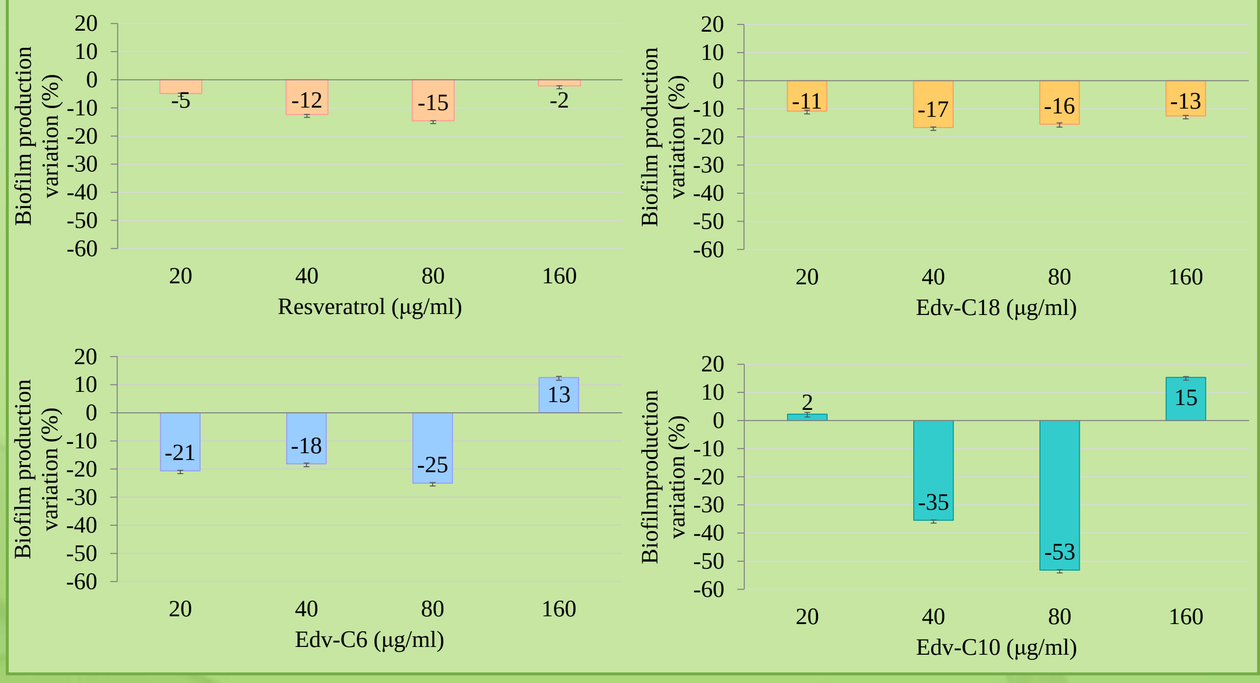
Molecular docking and dynamics





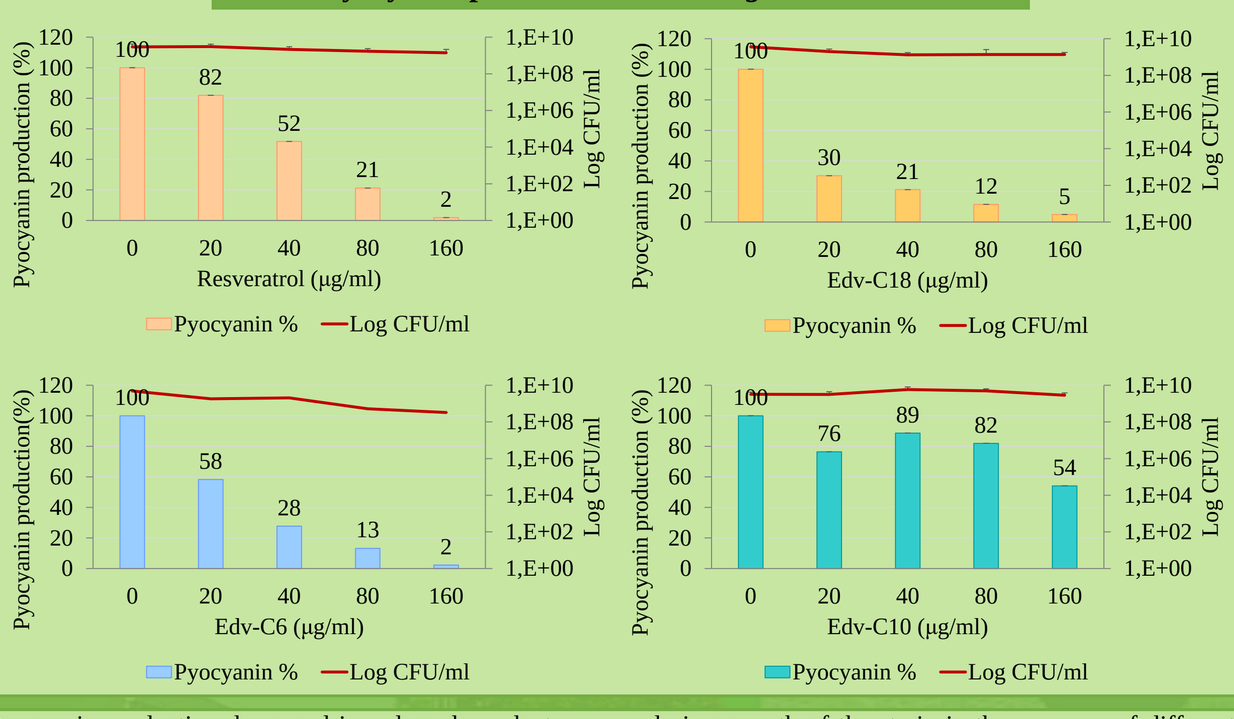
Binding energy of Edv-derivatives compared to native autoinducers (AIs). As the aliphatic chain length increases from Edv-C6 to Edv-C10 and Edv-C18, binding energy becomes more negative, indicating stronger interactions with the receptor. Edv-C18 consistently exhibits the most favorable binding energies. **Molecular docking and dynamic simulation results.** "down" conformation of QS-receptors LasR, RhlR and PqsR (**A**) and Edv-derivatives (R,S enantiomers) ability to bind the ligand-binding pocket as natural AIs (**B**) preventing the active "up" state (**C**).

Biofilm production in *P. aeruginosa* PAO1



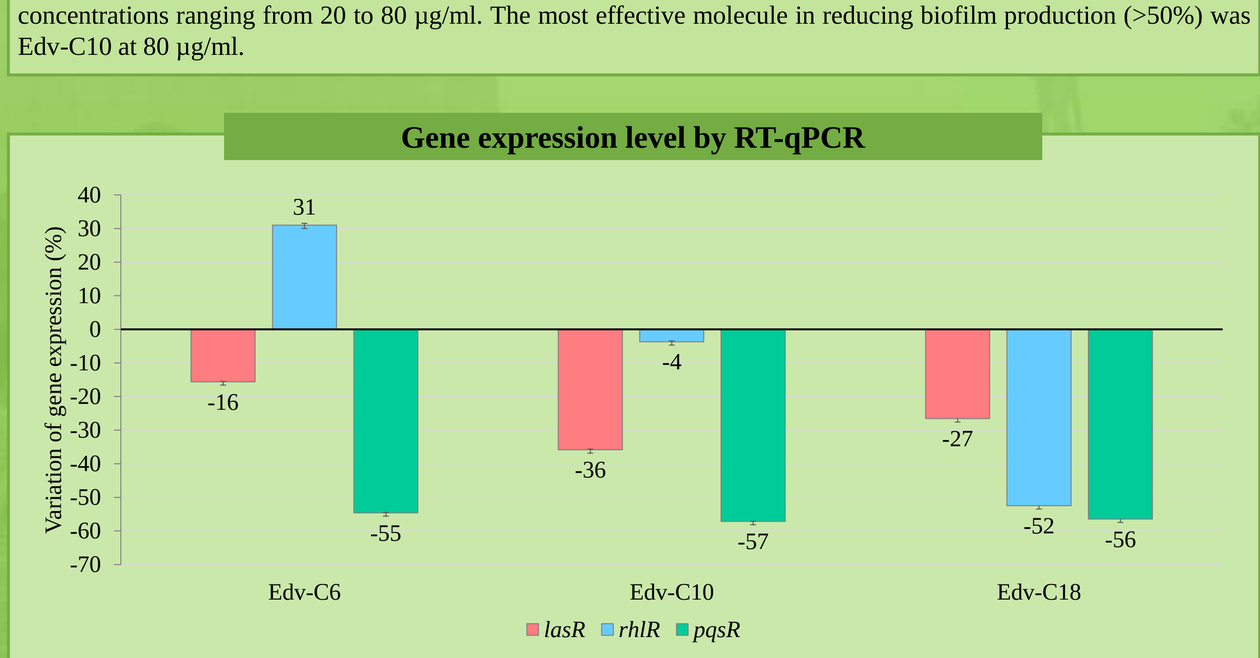
Biofilm formation in the presence and absence of compounds at sub-inhibitory concentrations was evaluated. The graphs report the % variation in biofilm production in the presence of the three Edv compounds and Resveratrol (used as a known QSI) at different concentrations. Overall, biofilm formation was reduced by all compounds at concentrations ranging from 20 to 80 µg/ml. The most effective molecule in reducing biofilm production (>50%) was Edv-C10 at 80 µg/ml.

Pyocyanin production in *P. aeruginosa* PAO1



Pyocyanin production decreased in a dose-dependent manner during growth of the strain in the presence of different Edv concentrations, particularly with Edv-C6 and Edv-C18. At 160 µg/mL, these compounds reduced pigment production to just 2–5% of the amount secreted by the strain in the absence of the compounds. This behavior was comparable to that of Resveratrol (a natural plant-derived compound), described as a QSI with high binding affinity for the LasR receptor (5). The reduction of pyocyanin production was not determined by the decreased viability of cells as showed by the CFU counts.

Gene expression level by RT-qPCR



Relative expression (%) of the *lasR*, *rhlR*, and *pqsR* genes compared to their basal expression levels (indicated by the black line). Both *lasR* and *pqsR* were downregulated when PAO1 was grown in the presence of 40 µg/ml of the derivatives, with Edv-C18 causing the greatest reduction. Notably, Edv-C18 was also the only compound to reduce *rhlR* expression by 50%.

Material and methods

In silico assays: by a drug repurposing (DR)/3D-QSAR approach and molecular docking/dynamics analysis, we selected synthetic Edaravone derivatives (Edv) based on the binding core structure between the regulatory proteins LasR/RhlR and their signal molecules (N-3-oxododecanoyl-L-homoserine lactone and N-butyryl-L-homoserine lactone respectively). The same computational protocol has been extended to PqsR, Autodock 4.2 and GROMACS 2024.3 have been used for docking and MD simulation; MM/PBSA methods has been used for free energy calculations: the obtained values confirms the docking scores trend.

Synthesis of derivatives: Edaravone was functionalized at the C4 position by introducing a fully saturated lipophilic chain (C6, C10 and C18) to increase its binding affinity to QS proteins and to act as powerful QSI [6,7]. *In silico* analysis and derivatives synthesis were conducted by chemistry group of Prof. Galeazzi.

In vitro microbiological assays: antibacterial activity was tested by MIC determination using the broth microdilution method. Biofilm formation was measured using a crystal violet staining microplate assay (4). Pyocyanin production was evaluated by a spectrophotometric assay in presence or not of sub-inhibitory concentrations of the compounds (3). The expression of *lasR*, *rhlR* and *pqsR* genes, encoding QS regulatory proteins, was analysed by RT-qPCR after exposure to the QSIs.

References

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