In vitro characterization of novel *P. aeruginosa* QS Inhibitors identified by *In silico* screening

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Introduction

The Multiple Virulence Factor Regulator (MvfR) is a *Pseudomonas aeruginosa* (Pa) Quorum Sensing (QS) transcriptional factor that regulates virulence functions critical for both acute and chronic infections in Cystic Fibrosis patients, making it an interesting drug target.

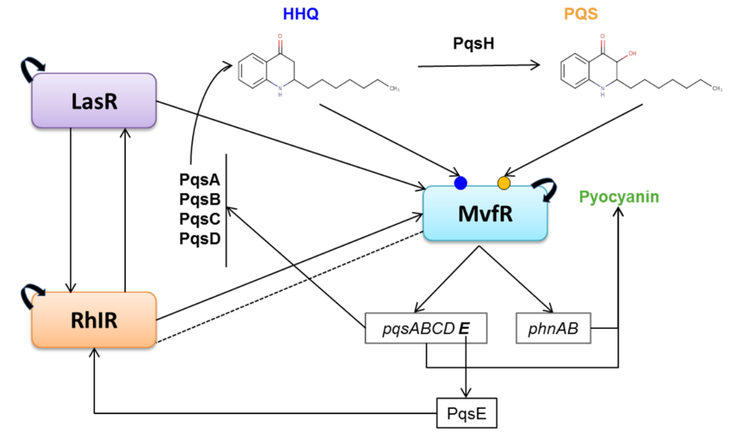

Figure 1. Role of MvfR direct regulation and the systems that feedback to it either positively (☐) or negatively (----).

Objective

In vitro characterization of novel Pa Quorum Sensing Inhibitors selected from a structure-based *in silico* screening approach based on docking studies on M64, a compound showed to inhibit MvfR regulon.

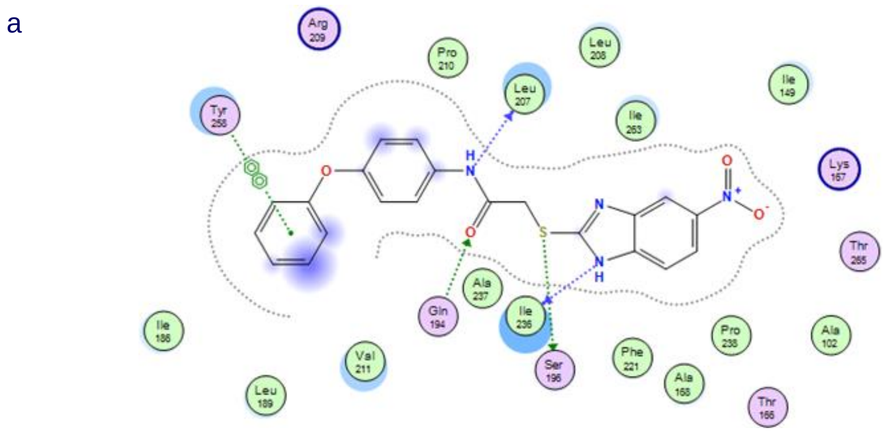

Figure 2. M64 into the MvfR pocket and ligand interactions with residues (residues within ~4.5A from the ligand)

Methods

- Pyocyanin was measured using a 96-well-MTP colorimetric assay after centrifugation
- PQS and HHQ levels quantification was carried out using the isotope-dilution method, a well-established LC-MS/MS technique for quantifying those HAQs
- Binding to the MvfR protein was determined by an optimized biophysical assay based on surface plasmon resonance (SPR) using a Biacore T200 system. MvfR protein including the binding site was immobilized on CM7 sensor chip by amine coupling.

Results

In silico screening results and pyocyanin screening

The computational chemistry approach allowed the initial identification of ~2K hits.
141 of them were selected by applying drug-like physic-chemical structural filters.
The most effective inhibitor, Compound 21, was selected by functional assay for reduced levels of pyocyanin. Differences between PA14 and the sample containing Compound 21 were statistical significant.

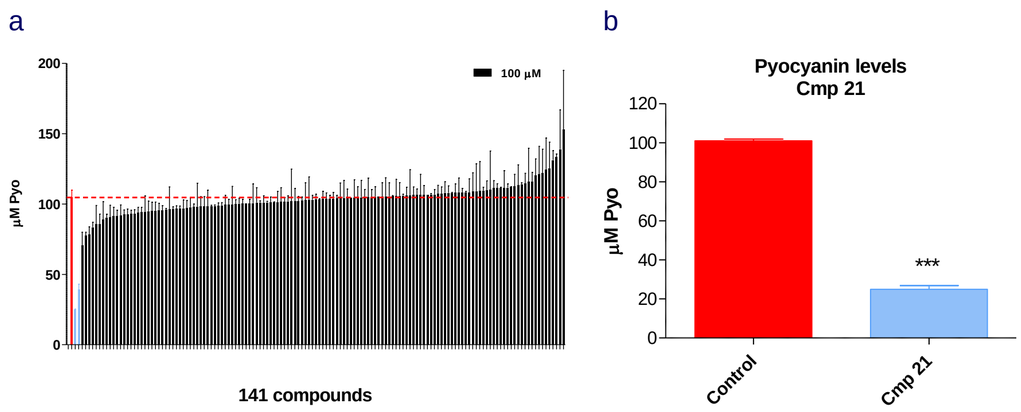

Figure 3. a) Pyocyanin production of PA14 in presence of 141 possible new MvfR inhibitors. Pyocyanin levels were quantified in response to 100 µM compounds (black bars). Untreated PA14 cells was the control (red bar)
b) Statistical significance (t test, ***, P <0.001) of Compound 21 (blue bar). Error bars show SD of at least 2 replicates.

Results

In vitro Characterization of Compound 21

Compound 21 was able to inhibit HAQs molecules at both 6 and 24h. 50% of inhibition was recorded at 6h using both HHQ and PQS molecules whereas Compound 21 induced higher HHQ inhibition at 24h compared to PQS. Affinity analysis allowed calculation of the equilibrium constant KD = 66.85 µM ± 7 (n=2).

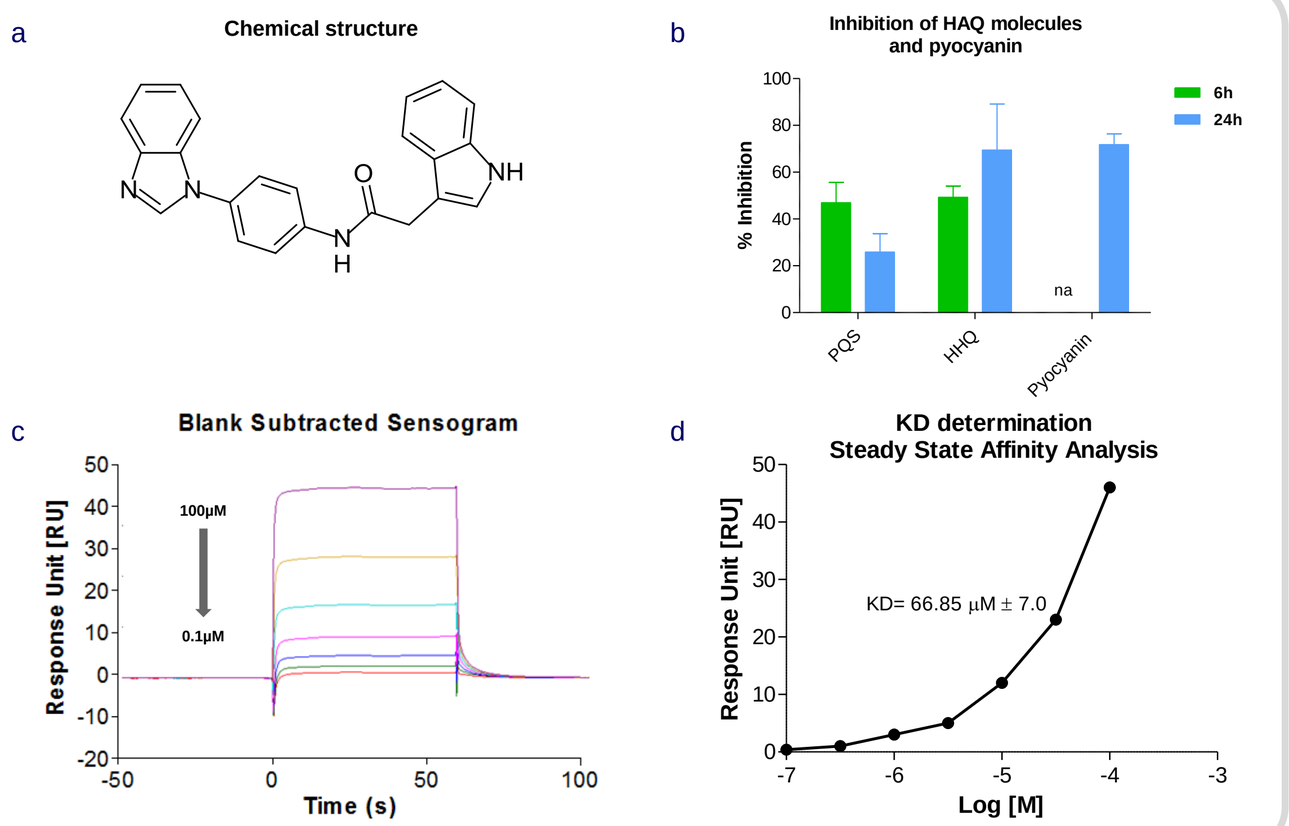

Figure 4. a) Structure and biological activity of Compound 21 for inhibition of HAQs and pyocyanin production. HHQ, PQS and pyocyanin levels were quantified in response to 100 µM at both 6 (green bars) and 24h (blue bars).b) Sensogram of Compound 21 signals on MvfR – immobilized surface. c) Dependence of RU change on the concentration of Compound 21. KD was determined by Steady State Affinity Analysis.

Chemical structure modifications and *in vitro* pattern

Initial medicinal chemistry exploration on Compound 21 (KD= 66.8 µM ± 7.0) led to a better interaction with the protein binding site (KD = 28.7 µM ± 1.1) Compound 21C, which translated into a higher inhibitory pattern. Compound 21C show higher pyocyanin and PQS inhibition than Compound 21. Compound 21C generated similar inhibition to Compound 21.

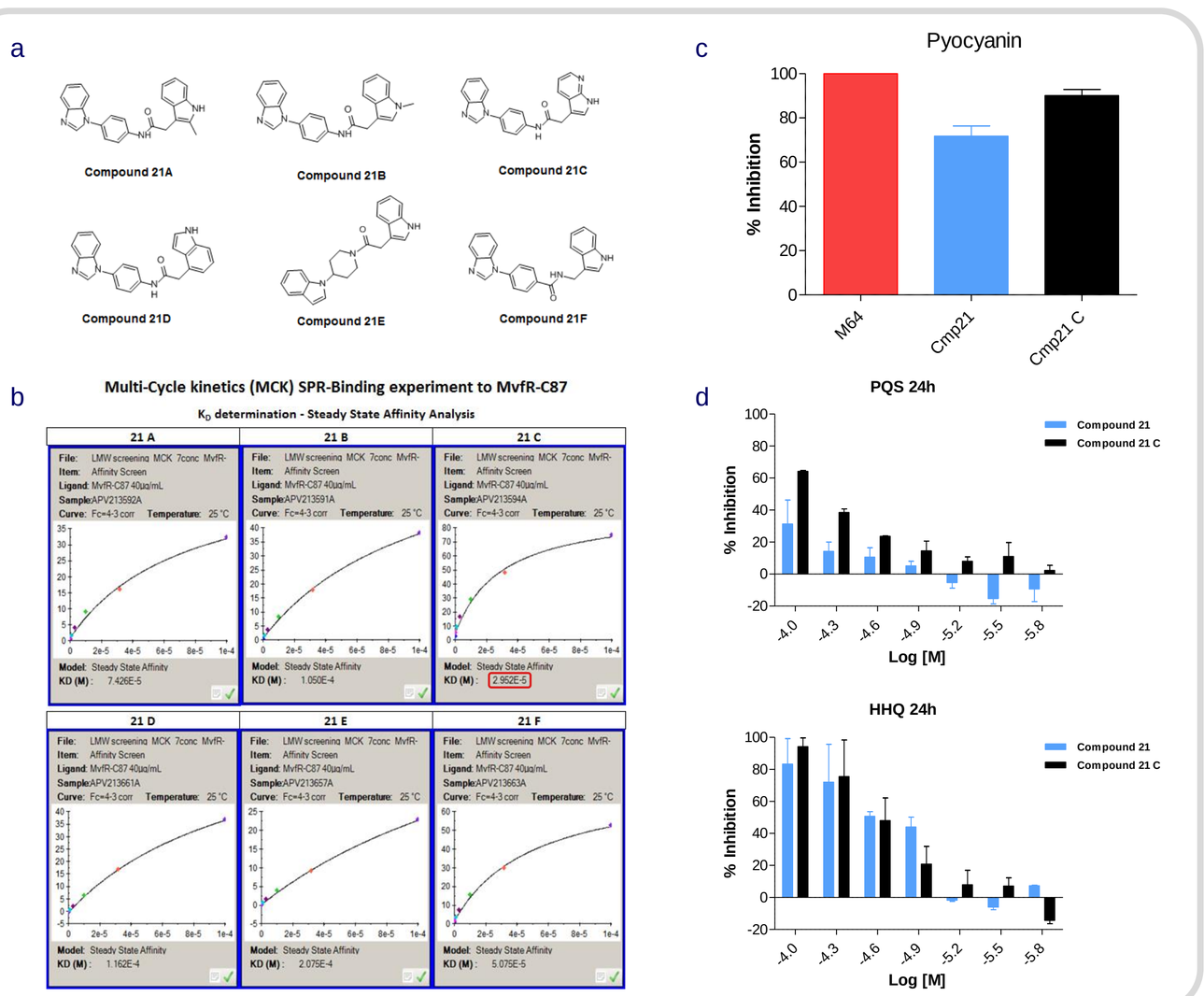

Figure 5. a) Chemical structure of Compound 21 derivatives. b) SPR binding experiment to MvfR-C87. KD determination by Steady State Affinity Analysis. c) Pyocyanin % inhibition in presence of compound 21 (blue bars) and Compound 21 C (black bars). M64 (red bars) has been used as reference MvfR inhibition d) % Inhibition of HAQs molecules in presence of different concentration of Compound 21 and 21C. Errors bars show SD of at least 2 replicates.

Conclusion

In silico screening enabled the identification of ~2K MvfR hits. 141 compounds were selected by applying drug-like physiochemical filters. Compound 21 showed a promising *in vitro* profile compatible with an anti-MvfR inhibitor. Profile improvement was obtained by minor chemical modifications of the compound scaffold leading to an improvement in the overall profile. Additional effort is required to design improved molecules for evaluation in pharmacodynamics models of infections of clinical relevance.

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