**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.

**Objective**

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

**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 physio-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.

**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.