Fall 2023 OVPR Seed Grant Program

Title: Structure-based design of anti-bacterial agents targeting LpxC

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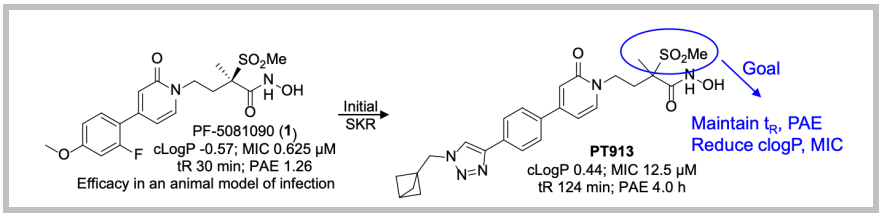

Figure 1. Long term goal

OVERVIEW / ABSTRACT

New drugs are needed to treat Multi Drug Resistant *Pseudomonas aeruginosa* (MDR-PA) which is a serious threat to human health.[1,2] The zinc deacetylase *pa*LpxC catalyzes the first committed step in Lipid A biosynthesis and is an unexploited MDR-PA drug target. Initial efforts to develop antibiotics that target LpxC were hindered by safety concerns. Several studies now indicate that the alkyne group in many LpxC inhibitors is a metabolic liability, and Pfizer developed a series of pyridone sulfones such as PF5081090 (**1**) (**Figure 1**) which lack the alkyne and are thought to be safer. Compound **1** has a residence time of 30 min on *pa*LpxC, causes a PAE of 1.26 h on the growth of *P. aeruginosa* PAO1, and has efficacy in an animal model of infection. In order to further improve safety, our goal is to develop analogs of **1** that result in an increase in PAE, which we hypothesize will enable dosing frequency to be reduced thereby widening the therapeutic window and improving safety. A strong positive correlation exists between inhibitor residence time and PAE, and consequently we performed a structure kinetic relationship (SKR) study to identify analogs of **1** with increased residence time on *pa*LpxC. This resulted in the discovery of inhibitors such as **PT913** that has a residence time of 2 h and causes a PAE of 4 h (**Figure 1**). However, **PT913** has an MIC of only 12.5 µM compared to **1** (0.625 µM) likely due to the increase in cLogP of **PT913** compared to **1** (0.44 vs -0.57). Consequently, our goal is to develop analogs of **1** with increased residence time and PAE, on *pa*LpxC *and* with physicochemical and microbiological properties suitable for *in vivo* analysis. We will accomplish this goal by modifying the sulfone headgroup of **1** which is oriented toward a pocket adjacent to the active site zinc, and our *approach* is to modify a primary inhibitor scaffold which has a chemically tractable head group. The rationale is that by computationally screening for modifications to the head group we can prioritize a more optimal subset for experimental validation. The expected outcomes are: (1) computational assembly and screening of reaction-compatible head groups conjoined to a core inhibitor scaffold, (2) prioritization and purchase of precursors for the most favorable head groups, (3) chemical synthesis to attach head groups to the primary scaffolds, (4) experimental testing to identify inhibitors with the most favorable antibacterial activity and drug-like properties.

Aim #1: Identify molecules that mimic native interactions of the endogenous substrate. Computationally identify head group precursors which recreate biologically relevant interactions. Create docking libraries by attaching headgroups to scaffolds using amine alkylation, amide condensation, or triazole-based click reactions which mimic experimental protocols. Dock libraries to a high-resolution *pa*LpxC structure. Prioritize compounds for synthesis and evaluation using protein-ligand energy, molecular similarity, and cheminformatic descriptors derived from Gram-negative antibiotics.[3] Develop analogs around experimentally-verified hits.

Aim #2: Synthesize and characterize compounds for *pa*LpxC inhibition and microbiological activity. Top scoring compounds will be synthesized and characterized. K_i (inhibition) and t_R (residence times) will be determined using fluorescence competition assays with *pa*LpxC. The microbiological activity of the inhibitors will be evaluated using a wild-type strain (PAO1) and efflux-pump mutant (MexABCDXY) of *P. aeruginosa* to determine the minimum inhibitory concentration (MIC) and post-antibiotic effect (PAE).