Decoupling Proton Motive Force to Overcome Antibacterial Resistance

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Bacterial resistance to antibiotics is a continuing concern for clinicians around the globe. In order to overcome this problem, many researchers have attempted to identify new chemical entities with novel mechanisms of action in an effort to combat pre-existing resistance. However, a common mechanism of resistance involves the utilization of multi-drug resistance efflux pumps, some of which are responsible for drug resistance of chemotypes ranging from β-lactams to tetracyclines. Many of these pumps are the cause clinical resistance to one or multiple classes of drugs, complicating treatment regiments and extending the course of treatment. These efflux pumps either utilize ATP or proton motive force (PMF) directly as a power source to remove problematic entities. Overcoming the resistance imposed by these efflux pumps would restore the action of many drugs for many pathogens. In order to overcome these multi-drug resistance efflux pumps, we've envisioned decoupling PMF to block bacterial metabolism as well as efflux.

We are looking into multiple metabolic poisons that disrupt PMF to recover the action of a variety of drugs in multiple pathogens including gram-negative infectious agents and *M. tuberculosis*, the cause of tuberculosis. PMF poisons, such as 2,4-dinitrophenol (DNP), can unselectively disrupt the gradient of protons over a membrane, disrupting cellular metabolism and efflux mechanisms. To overcome this limitation, we have chosen to study the effects of PMF poisons with a chemical handle which can be used to attach a targeting moiety do reduce the effects of the poisons to affect one cell type. Herein we describe a limited set of PMF poisons which show synergy with FDA-approved drugs in pathogenic bacteria such as the gram negative agent *P. aeruginosa* and the acid-fast bacillus *M. tuberculosis*. We will also show that β-lactam conjugates of these poisons will offer selectivity for bacteria, with β-lactamase activity, over mammalian cells using a targeted release strategy we and others have already used for targeted drug release. Combining our PMF poison with known drugs has shown significant synergy with heavily resisted chemotherapeutics. *In-vitro* testing of our PMF poison in combination with tetracycline in checkerboard assays has shown reduced fractional inhibitory concentrations (FIC) of each compound by 32-fold compared to monotherapies for *P. aeruginosa* (MICs). We will present detailed characterization of the nature of this extensive synergy in *P. aeruginosa* and investigate other pathogens with similar efflux machinery.

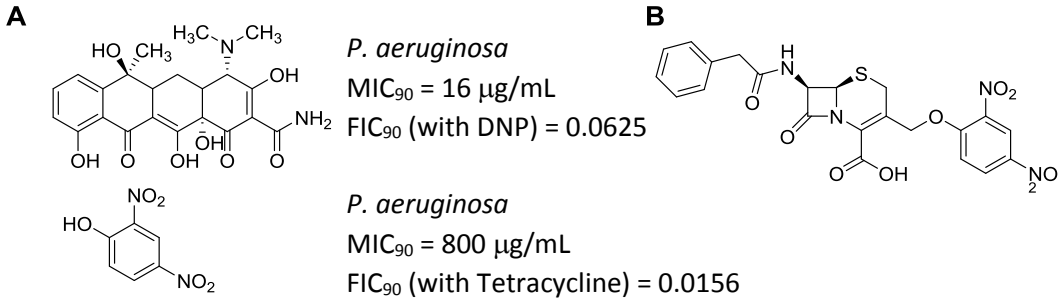

Figure 1. A) Minimum inhibitory concentrations (MIC) and fractional inhibitory concentrations (FIC) of tetracycline and 2,4-dinitrophenol in *P. aeruginosa*; B) 2,4-Dinitrophenol conjugated to a cephalosporin targeting moiety.