Synthesis and Antibiotic Evaluation of Bedaquiline Analogs in Complex with E. coli’s Gram-Negative F-ATP Synthase

Faculty Advisor:

Abstract:

The rising epidemic due to lack of treatments for drug-resistant gram-negative bacteria call for new advances and approaches to antibiotic discovery. The novel drug, Bedaquiline (BDQ), bypasses regular antibiotic mechanisms of cellular membrane penetration and targets the F-ATP synthase (F-ATPase) of Mycobacteria tuberculosis (M. tb); however, BDQ is specific to M. tb. Analogs of BDQ will be synthesized to determine factors influencing binding affinity to E. coli’s F-ATPase; however, due to the differences in amino acid sequence between it and M. tb at positions 32, 65, and 67, research will be primarily focused there. Synthesis of the base analog structure begins with a methoxy addition, a dimethylamine addition, and a Grignard synthesis. Targeted analogs are those with strong hydrogen bonding at position 65. Position 32 facilitates steric alignment and, therefore, long carbon chains will be added to the α carbon to mimic BDQ but reduce steric hindrance. Antibiotic potential will be assessed through liquid bacterial inhibition assay against a variety of Gram-negative pathogens.

Proposal:
Tuberculosis (TB) is contracted by the airborne-dormant bacteria Mycobacterium tuberculosis (M. tb), affecting mainly the lung, if not treated, can lead to death in the most severe cases. Current treatments consist of several rounds of antibiotics due to gram-negative M. tb. Gram-positive bacteria have three layers of outer membranes; however, Gram-negative bacteria have an additional two layers, which makes penetration and ultimate destruction of gram-negative bacteria especially difficult. Antibiotics are widely used generating strains of multi-drug resistant (MDR) bacteria. New drugs are being researched to combat MDR bacteria; however, discovery of molecules with high efficacy rates have been few and far between. A new drug, Bedaquiline (BDQ), bypasses cell-membrane penetration that most antibiotics are based upon and targets F-ATP synthase on the cellular membrane of M. tb stopping production of ATP, increasing the acidity of the cell, and ceasing cellular function. Therefore, the synthesis of BDQ analogs shall be conducted to target F-ATP synthase in other gram-negative mycobacteria.
F-ATP synthase (F-ATPase) is responsible for the production and hydrolysis of adenosine triphosphate (ATP) in cells. ATP is an energy carrier used in various metabolic pathways that power cells. F-ATPase’s structure consists of two domains, F₀ and F₁. The lower F₀ domain functions as a H⁺ pump and rotates the c-ring generating torque to the upper F₁ region allowing ADP and Pᵢ/ATP to combine/disassociate. BDQ binds to the F₀ c-ring subunit stopping rotation and production/hydrolysis of ATP through its bulky sterics. As of yet, there are only two known drugs, oligomycin and dicyclohexylcarbodiimide, that bind to the F₀ c-rings as well. The sterics of BDQ build a hydrophobic platform with other amino acids (AA) that allow strong H-bonding that is facilitated by the OH- and amino groups in complex with Glutamate (Glu, E) at position 65 (E65).
Figure 2. General structure of F-ATP synthase divided into its sub-regions and units. ADP and P_i bind to the α and β subunits where reformation/hydroxylation of ATP is completed. F-ATP synthase sits in the cellular membrane of bacteria, the thylakoid membrane in chloroplasts, and the inner membranes of eukaryotic mitochondria.\(^7\)

The F-ATPase in \textit{M. Tb}, as well as \textit{Escherichia coli} (\textit{E. coli}) is located on the plasma membrane where it pumps H\(^+\) from the outside to the inside of the cell, and vice versa. However, the amino acid sequence of \textit{E. coli}'s F-ATPase differs from \textit{M. tb}'s, reducing overall BDQ binding affinity. AA positions important to binding are 32, 65, and 67. Changes between the two cause the target F-ATPase to be less polar and more hydrophobic.\(^{1,2}\) Extensive research has shed light on the mechanism of bactericide that BDQ uses; however, researchers primarily focused on \textit{M. tb} and synthesizing BDQ analogs to hindering \textit{M. tb} F-ATPase.\(^3\)

Analogs of the substituents of BDQ show promise with low minimum inhibitory concentrations (MICs) in \textit{M. Tb}. In conjunction with the quinoline and hydroxyl groups, the amino group seems to show antibiotic potential. In previous studies, compounds with the best MICs (0.43-6.8 \(\mu\)g/mL) had a 3(4-(\(N,N\)-dimethylamino)methyl)phenyl group, or some variation of amine functional groups.\(^5\) This is likely due to the strong H-bonding of E65 with the dimethylamino and alcohol groups. Therefore, in order to establish 4-(2-methoxyquinolin-3-yl)-\(N,N\)-dimethylbutanamine's antibiotic potential in \textit{E. coli}, synthesis and microbial assays will be conducted.

Synthesis will follow the scheme shown below (Figure 4). F-ATPase assays will be completed using \textit{E. coli} inside-out (ISO) vesicles and tested for fluorescence using the ACMA, a fluorescent compound. As F-ATPase pumps H\(^+\) ions, ACMA is pulled into the vesicle, reducing fluorescence in solution. If the F-ATPase is inhibited by the compound, as the assay runs, there will be more fluorescence in solution.

Figure 3. Synthetic pathway for analog 1 and base structure for future synthesis of analogs (5).

References:


**Presentation/Publication Outlet:**

Research is being presented at SERMACS 2017, the UNC-Asheville Fall Research Symposium, and eventual publication in the UNC-Asheville Journal of Undergraduate Research.

**Funding:**

1) 10 mg Bedaquiline (BDQ) $387.00
   Used as a control for testing the analogous compounds against.

2) 1 L Dimethyl Sulfoxide (DMSO) BioReagent (124.77 each) $124.77
   A reagent to test BDQ and the other compounds for ATP Synthase assays

**Total:** $511.77