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

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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*.[1] 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.[1] Therefore, the synthesis of BDQ analogs shall be conducted to target F-ATP synthase in other gram-negative mycobacteria.

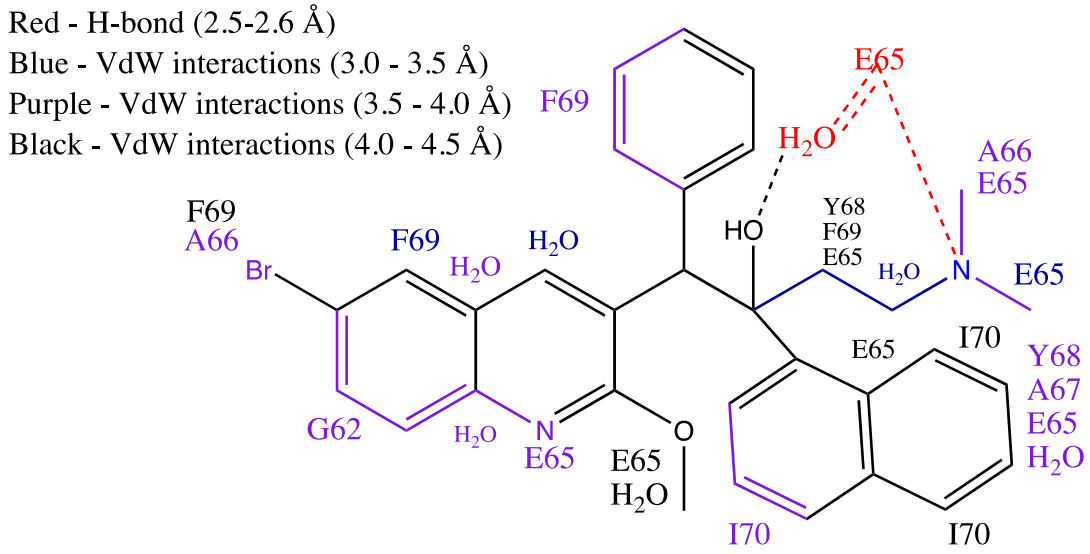

Figure 1. Structure of BDQ with interaction areas highlights and their respective amino acids. through H-bonding and Van der Waals. Strongest binding is at position 65. Not shown is I32, however, it acts as a hydrophobic platform that strongly facilitates binding.[2]

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, F_0 and F_1. The lower F_0 domain functions as a H^+ pump and rotates the c-ring generating torque to the upper F_1 region allowing ADP and P_i/ATP to combine/disassociate.[2] BDQ binds to the F_0 c-ring subunit stopping rotation and production/hydrolysis of ATP through its bulky sterics.[2] As of yet, there are only two known drugs, oligomycin and dicyclohexylcarbodiimide, that bind to the F_0 c-rings as well.[4] 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).[2]

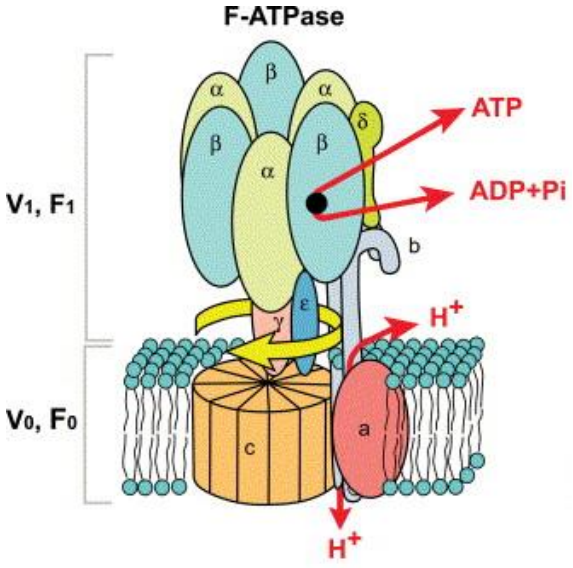

Figure 2. General structure of F-ATP synthase divided into its sub-regions and units. ADP and P_i bind to the α and β subunits where reformation/hydroxylation of ATP is completed. F-ATP synthase sits in the cellular membrane of bacteria, the thylakoid membrane in chloroplasts, and the inner membranes of eukaryotic mitochondria.[7]

The F-ATPase in *M. Tb.* as well as *Escherichia coli* (*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 *E. coli's* F-ATPase differs from *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 *M. tb* and synthesizing BDQ analogs to hindering *M. tb* F-ATPase.[3]

Analogs of the substituents of BDQ show promise with low minimum inhibitory concentrations (MICs) in *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 μg/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 *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 *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.

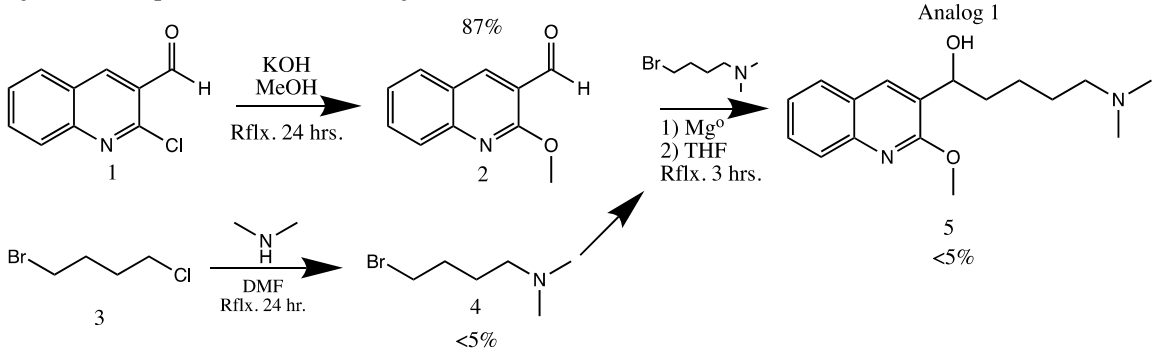

Figure 3. Synthetic pathway for analog 1 and base structure for future synthesis of analogs (5).

References:

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Presentation/Publication Outlet:

Research is being presented at SERMACS 2017, the UNC-Asheville Fall Research Symposium, and eventual publishment 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.
   http://www.cellagentech.com/tmc207-bedaquiline/
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
   https://www.fishersci.com/shop/products/dimethyl-sulfoxide-fisher-bioreagents-3/bp2311?searchHijack=true&searchTerm=BP2311&searchType=RAPID&matchedCatNo=BP2311

Total: $511.77