University of North Carolina Asheville
Journal of Undergraduate Research
Asheville, North Carolina
May 2022

Synthesis of diarylquinoline thiols for use as selective Pseudomonas aeruginosa ATP synthase inhibitors

Dietrich O. Jackson
Department of Chemistry & Biochemistry
The University of North Carolina Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Amanda Wolfe

Abstract

Antibiotic resistant infections affect millions of people each year, killing tens of thousands of people in the United States alone. The rate at which bacteria are becoming resistant is surpassing the rate that novel antibiotics are able to be produced, and thus it is becoming clear that new targets are necessary. One possible target of inhibition is ATP synthase, which has the potential to kill both Gram-negative and Gram-positive bacteria. Bedaquiline (BDQ), the first new FDA approved drug to treat *Mycobacterium tuberculosis* in decades, is an ATP synthase inhibitor, the discovery of which opens the door to a large quantity of potential antibiotics. Herein, the synthesis of diarylquinoline benzyl thiol with amino acid substituents, analogues based off of BDQ, was developed to selectively inhibit ATP synthase of *Pseudomonas aeruginosa*, a highly infectious Gram-negative pathogen.

1. Introduction

1.1 General Background

More than 2.8 million people are infected by antibiotic-resistant bacteria each year in the United States alone, and 35,000 people die as a result.[1] If the current trend in infections continue, it is projected that by 2050 the world could have more than 10 million people dying each year from these infections.[2] Conversely antibiotic discoveries have been on the decline since the 1980s due to a combination of lack of funding creating a discovery void.[3]

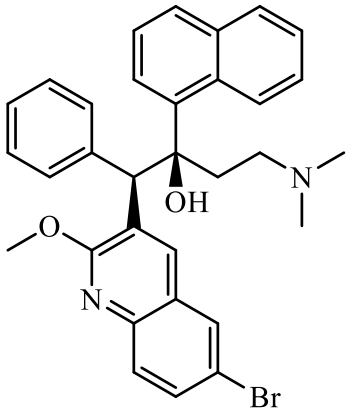


Figure 1: Bedaquiline Structure

Antibiotic resistance has developed through improper usage of antibiotics, either through not following a prescription all the way through or by over prescription, causing selective pressure and opportunity for mutations. On the molecular level, bacteria have the ability to modify their antibiotic active sites by producing enzymes that degrade drug candidates, reducing small molecule penetration of the outer membrane, or by modifying what the efflux pumps are able to eject from the cell. Due to the fact that Gram-negative bacteria have an outer membrane that reduces permeability on top of ever-increasing antibiotic resistance, they are becoming increasingly difficult to treat. The CDC has identified multiple Gram-negative bacteria that are in critical need for functioning antibiotics for these reasons.[1] Specifically, *Pseudomonas aeruginosa* (PA) is one of the most difficult to treat due to a more densely packed outer membrane. Up until this point all antibiotics have targeted DNA replication, protein synthesis, and cell wall synthesis. It has become clear that these methods cannot keep up with the evolution of bacteria as they become more and more resistant to our antibiotics, and new types of antibiotics should be pursued to combat this.

Most antibiotics used today target only a few essential functions in the bacterial cell, all of which have resistance mechanisms. One new antibiotic that has been developed in the past decade with a novel mechanism of action is bedaquiline (BDQ), which has been FDA approved to treat tuberculosis (TB). TB is the second deadliest infectious disease in the world, with a mortality rate of 2 to 3 million deaths each year.[4] The bacteria that causes TB is known as *Mycobacterium tuberculosis* (M. tb). BDQ, also known as TMC207 and R207910, as shown in Figure 1, is a molecule originally synthesized in 2004 and was approved in 2013 by the FDA to treat M. tb.[4,5] BDQ has a unique mechanism of action, targeting the proton pump of M. tb.'s ATP synthase by binding to subunit c, as seen in Figure 2.

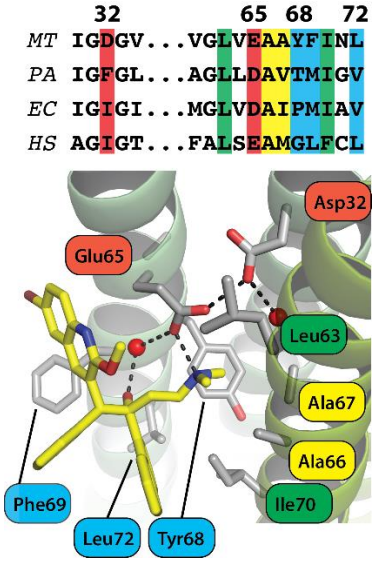


Figure 2: BDQ's binding to *M. tb.* ATP synthase. *Provided by Dr. Steed*

This binding halts ATP production and thus eventually kills the cell.[4] This method has the potential to be very effective at being an antibiotic if it is possible to modify BDQ to target specific ATP synthases in a desired target bacterium. Alongside this, since ATP inhibition is such a new concept in the world of antibiotics, there is a very low probability of antibiotic resistant bacteria to be resistant to this method of attack.

ATP synthase is an essential protein complex that works to produce adenosine triphosphate (ATP). This occurs through the conversion of adenosine diphosphate (ADP) and inorganic phosphate (P_i) with the help of a proton motor force. This force comes from an electrochemical gradient of protons (H^+), ultimately promoting the rotary movement of ATP synthase to generate ATP. This synthase is the primary source of ATP production, being crucial for cellular bioenergetics. This makes ATP synthase an appealing target, since ATP is required for many biological processes. On top of that, ATP synthases are unique for each organism, as seen in Figure 2, which allows us to specifically bind to different ATP synthases. By taking the differences in amino acid sequences into consideration, we can design molecules to inhibit normal function.

1.2 Previous Works

In 2012, Guillemot and coworkers evaluated BDQ analogues for use as broad-spectrum antibiotics against all types of bacteria. They investigated around 700 molecules with lateral chains for antibacterial properties. Of those tested, some had significant inhibition with a minimum inhibitory concentration (MIC) of 0.25 μg/mL against *Streptococcus pneumoniae*, and some only inhibited *Staphylococcus aureus* at high concentrations. Among the analogues, there was some inhibition of other Gram-negative bacteria such as *Escherichia coli* with a half maximal inhibitory concentration (IC_{50}) value of 8 μg/mL, but with less pronounced inhibition. It is theorized that this less pronounced inhibition is likely due to the bacteria potentially having less of a dependency on ATP synthase.[6]

In 2017, Surase and coworkers delved into the concept of finding another ATP synthase inhibitor, because BDQ had some unwanted adverse effects on patients. Out of 10,000 chemically diverse molecules screened, and extensive structure-activity relationship (SAR) studies, they found a hit on a molecule they called compound 1 as shown in Figure 3.

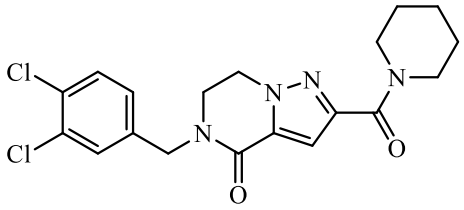


Figure 3: Compound 1

This compound had a MIC value of 16 μg/mL in a biochemical assay against M. tb. From a glance, this molecule seems to be structurally unique compared to BDQ, but it does include a bi-cyclic structure similar to the diarylquinoline of BDQ. That structure became the central core of their analogues due to their original search region having too narrow of an SAR. Of the analogues of compound 1 that were tested, they some showed potent ATP synthase inhibition activity, but had only modest whole cell activity. It should also be noted that in this process of testing, Surase et. al did not specify the region of ATP synthase being targeted by their compounds. This is most likely due to the ambiguous nature of the exact structure of ATP synthase at the time of publication.[7]

In 2019, Wang and coworkers discovered a new potential for an ATP inhibitor called optochin as seen in Figure 4.

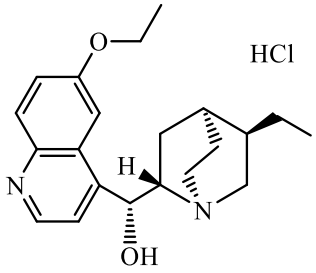


Figure 4: Optochin

Optochin has a similar structure to that of BDQ, both having a diarylquinoline core structure. There is a difference in their attachments, most notably is the lack of a terminal basic nitrogen group off of the diarylquinoline structure, something that has been proven to be necessary for antitubercular activity[8]. Due to this fact, it was expected that optochin and its analogues would not have any activity towards M. tb. in regards to ATP synthase inhibition. These analogues happen to bind very closely to the ion binding site for subunit c in ATP synthase. However, these compounds are quite cytotoxic with IC_{50} values between 20 and 30 μM for *Homo sapiens*.[9]

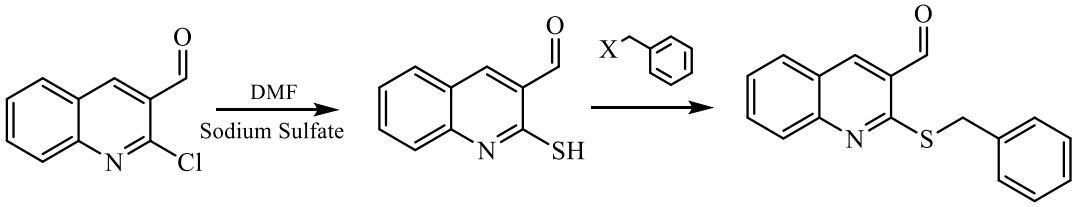
The goal of this research is to modify the core of BDQ to specifically target PA ATP synthase by making selective modifications to the base diarylquinoline structure. These attachments will be various amino acids at the C-1 position

to establish effectiveness against bacteria utilizing general cell death assays alongside ATP inhibition assays. Based on preliminary data, successful analogues will be modified further based on other literature to test the effectiveness of any additions. Docking energies will also be computed to establish binding affinity of analogues within subunit c of ATP synthase utilizing Autodock vina and Pymol.

2. Methods and Experimental

2.1 Chemistry

All reagents and solvents were purchased from commercially available sources and were used without further purification. The following solvent abbreviations are used: (DMF) dimethylformamide, (Hex) hexane, (EA) ethyl acetate, and (DCM) dichloromethane. All reactions were monitored by TLC, and flash column chromatography was performed with silica gel (mesh size 250). Purity was determined by NMR spectroscopy. ^{1}H NMR spectral data was obtained using $CDCl_3$ as the solvent on a Varian Oxford Instruments 400 Hz superconducting magnet using TMS as internal standard. ^{1}H NMR data reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constants (Hz), integration, and proton identifier.

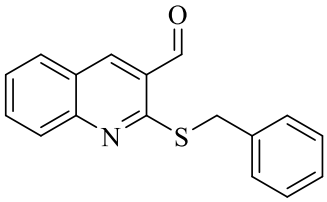


2.2 General Procedure for Addition of Thioethers[10]

A heat-dried, 1 neck 100mL round bottom flask (RBF) was prepared, where a stir bar, sodium sulfide (1.5 mmol), and 2-chloroquinoline-3-carbaldehyde (1 mmol), were added. The mixture of solids was then placed under an inert Ar atmosphere. Once under Ar, 13mL of DMF (64 mmol) was added via syringe while stirring to make a darkening yellow solution. This mixture was allowed to react for 12 hours, followed by the addition of the desired aryl compound, which was left to react for 30 additional minutes before being put in an ice bath. TLC was utilized as verification of synthesis, using 2:1 Hex:EA.

Once verified, the reaction mixture was poured into ice-cooled water, where a precipitate was formed. The precipitate was then filtered out via vacuum filtration. DCM was added to attempt to retrieve solids that ended up in the filter, which caused complications with DMF. Liquid-liquid extraction with deionized water and DCM then occurred over multiple days to attempt to get as much product out of solution. H-NMR was then taken for further identification, and a mixture of desired product and minor starting material was identified.

Purification of the product was conducted using flash column chromatography (SiO_2, 80:20 Hex:EA, increasing to 70:30 Hex:EA after fraction 30) was conducted.



Compound 1a
(90%)

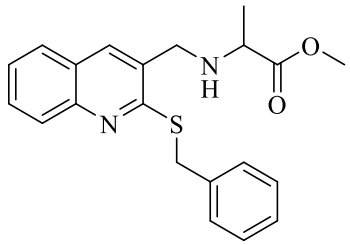
2-(benzylthio)quinoline-3-carbaldehyde (1a): The general procedure for adding thiols was repeated using 2-hydroxyquinoline-3-carbaldehyde (0.5 g, 1.0 mmol), sodium sulfide (0.3 g, 1.5 mmol), and benzyl bromide as the aryl compound (0.3 mL, 1 mmol). Crude product was purified using flash chromatography (SiO_2, 2 x 10 cm, 70:30-80:20

Hex:EA gradient dilution) resulting in 1a as a mustard colored oil (89.5%). ^{1}H NMR: ($CDCl_3$, 400 Hz) δ 10.2 (s, 1H), 8.40 (s, 1H), 7.98 (s, 1H), 7.80 (m, 2H), 7.50 (s, 3H), 7.20 (m, 3H), 4.60 (s, 2H).

2.3 General Procedure for Addition of Amino Acids[11]

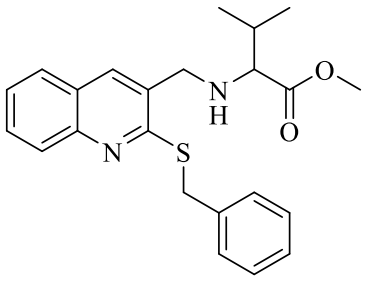
A 1 neck 100 mL RBF was placed in an ice bath with the respective amino acid (20 mmol), diisopropylethylamine (20 mmol), and methanol (until just dissolved). While this mixture was stirring, compound 1a was added (24 mmol) and subsequently taken off ice to stir under argon overnight. If solid was still present during this time, methanol was added slowly until the solid was mostly dissolved to prevent solidification on the bottom of flask. Upon TLC verification of product (80:20 Hex:EA), $NaBH_4$ (40 mmol) was added and the reaction was put on ice for 3 hours. After 3 hours, the ice bath was removed and the reaction was left to react until TLC showed no starting material.

Quenching of these reactions involved acidification of the flask using $KHSO_4$ (10% m/v), removing the aqueous layer containing diisopropylethylamine and aqueous soluble molecules. The organic layer was then neutralized using sodium bicarbonate, and was separated using liquid-liquid extraction using EA, water, and brine as needed to assist in the separation of layers. All compounds were then purified using Flash chromatography (SiO_2, 2 x 10 cm, 70:30-80:20 Hex:EA gradient dilution), unless specified.



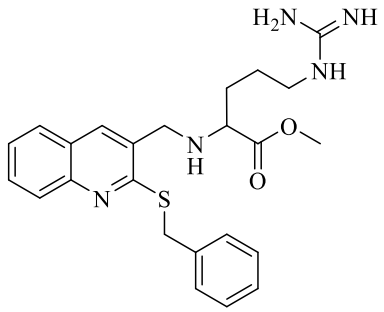
Compound 2a
(89%)

((2-(benzylthio)quinolin-3-yl)methyl)alanine (2a): The general procedure was followed using compound 1a (0.207 g, 20 mmol), alanine methyl ester hydrochloride (0.078g, 20 mmol), methanol (1.9 mL), triethylamine in place of diisopropylethylamine (0.1 mL, 20 mmol) and $NaBH_4$ (0.057g, 40 mmol). Product was a brownish-yellow solid (89%). ^{1}H NMR: ($CDCl_3$, 400 Hz) δ 8.05 (m, 3H), 7.50 (m, 3H), 7.80 (m, 2H), 7.50 (m, 5H), 4.80 (s, 2H), 4.70 (m, 3H), 3.9 (m, 1H), 6.68 (s, 2H), 1.36 (m, 3H).



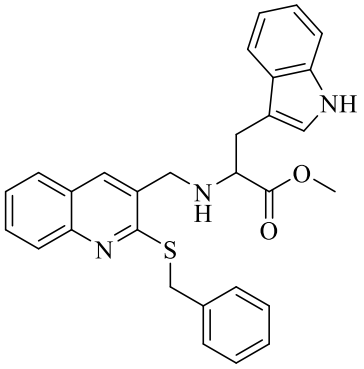
Compound 2b
(55%)

((2-(benzylthio)quinolin-3-yl)methyl)valine (2b): The general procedure was followed using compound 1a (0.195 g, 20 mmol), valine methyl ester hydrochloride (0.08g, 20 mmol), methanol (0.9 mL), triethylamine in place of diisopropylethylamine (0.8 mL, xs) and $NaBH_4$ (0.057g, 40 mmol). This resulted in a brown oil (55%). ^{1}H NMR: ($CDCl_3$, 400 Hz) δ 8.06 (m, 2H), 7.78 (m, 1H), 7.70 (m, 1H), 7.50 (m, 1H), 7.30 (m, 4H), 4.70 (m, 2H), 3.70 (m, 4H), 3.15 (m, 1H).



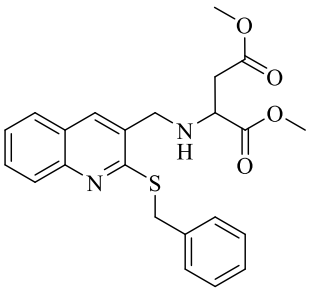
Compound 2c
(66%)

methyl ((3-(benzylthio)naphthalen-2-yl)methyl)argininate (2c): The general procedure was followed using compound 1a (0.2022 g, 20 mmol), arginine methyl ester hydrochloride (0.114g, 20 mmol), methanol (0.9 mL), triethylamine in place of diisopropylethylamine (0.1 mL, 20 mmol) and $NaBH_4$ (0.056g, 40 mmol). This produced a yellowish-brown product (66%). ^1H NMR: ($CDCl_3$, 400 Hz) δ 7.62 (m, 1H), 7.50 (m, 1H), 4.66 (m, 2H), 3.7 (m, 4H).



Compound 2d
(71%)

methyl ((2-(benzylthio)quinolin-3-yl)methyl)tryptophanate (2d): The general procedure was followed using compound 1a (0.202 g, 20 mmol), tryptophan methyl ester hydrochloride (0.151g, 20 mmol), methanol (10.5 mL), diisopropylethylamine (0.12 mL, 20 mmol) and $NaBH_4$ (0.047g, 40 mmol). This yielded an orange-brown solid (71%). ^1H NMR: ($CDCl_3$, 400 Hz) δ 7.97 (d, 1H), 7.88 (s, 1H), 7.73 (s, 1H), 7.60 (m, 2H), 7.47 (m, 4H), 7.39 (m, 1H), 7.26 (m, 7H), 7.18 (m, 1H), 7.13 (m, 2H), 4.60 (m, 2H), 3.93 (d, 1H), 3.66 (m, 5H), 3.26 (q, 1H), 3.17 (q, 1H).



Compound 2e
(crude 59%)

dimethyl ((3-(benzylthio)naphthalen-2-yl)methyl)aspartate (2e): The general procedure was followed using compound 1a (0.213 g, 20 mmol), aspartic acid methylesterhydrochloride (0.138g, 20 mmol), methanol (5.0 mL), diisopropylethylamine (0.15 mL, 20 mmol) and $NaBH_4$ (0.046g, 40 mmol). Product was a dark orange/brown oily

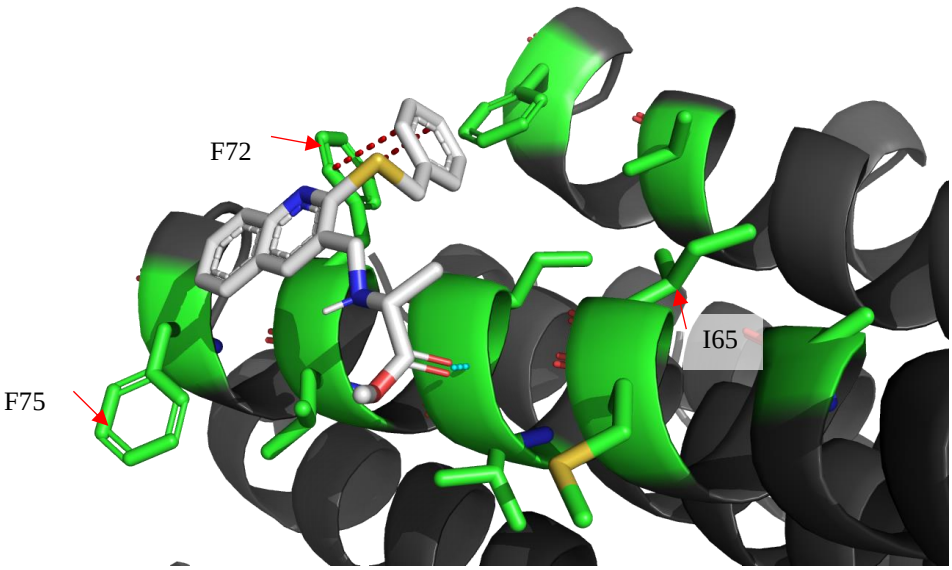
solid (crude 59%). Flash chromatography was not conducted on this compound. ^{1}H NMR: ($CDCl_3$, 400 Hz) δ 10.25 (s, 1H), 8.00 (m, 2H), 7.77 (m, 1H), 7.50 (m, 4H), 4.80 (s, 1H), 4.70 (d, 2H), 4.10 (d, 1H), 3.65 (m, 3H), 2.7 (m, 1H), 0.90 (q, 1H).

2.4 Computational Docking

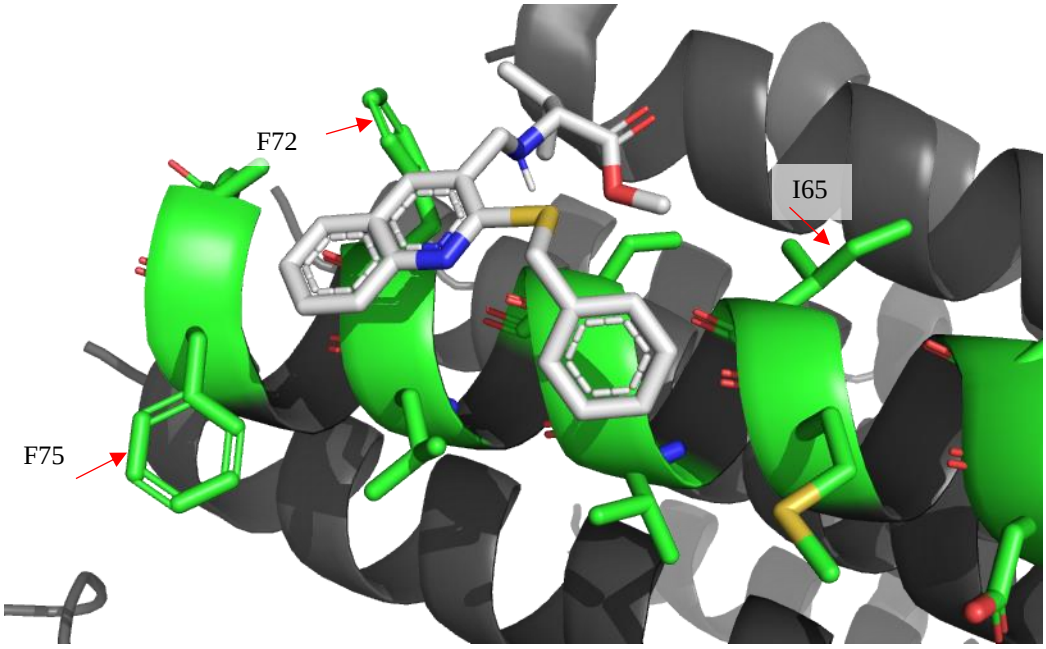
Likely binding poses and energies were predicted computationally using Autodock Vina, a genetic search algorithm that estimates binding energies based on experimentally determined values, for compounds 2a-2e. Proposed analogues and ATP synthase were prepped in Autodock Tools. This includes defining torsion roots for the analogues, and the addition of hydrogens for the analogous ATP synthase model. An analogous model for PA ATP synthase was used since no crystal structure for PA ATP synthase exists yet. This process involves overlaying the amino acid sequence over another known crystal structure to get a predicted model. Once in the proper format, the search grid was set around the 60th amino acid in the c-subunit, with a radius of 70, spanning an entire section of the c-subunit. This position was based on the binding site of BDQ in m. tb, as it was hypothesized that similar compounds would bind similarly in other ATP synthases. Vina was used to computationally dock each proposed molecule to the binding site.[12]

Analogue	Compound 2a	Compound 2b	Compound 2c	Compound 2d	Compound 2e
$\Delta G_{binding}$ (Kcal/mol)	-6.0	-6.0	-6.2	-5.5	-5.6

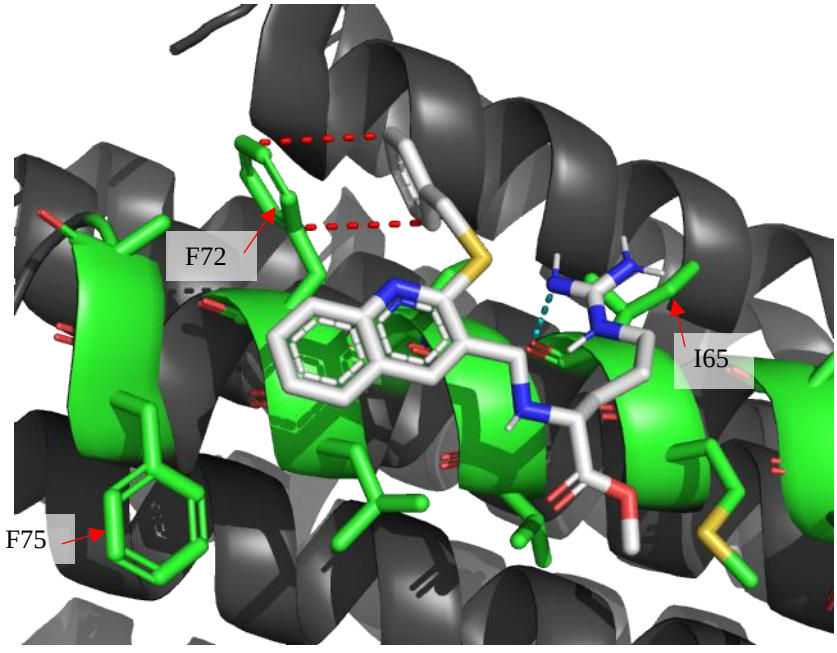
A

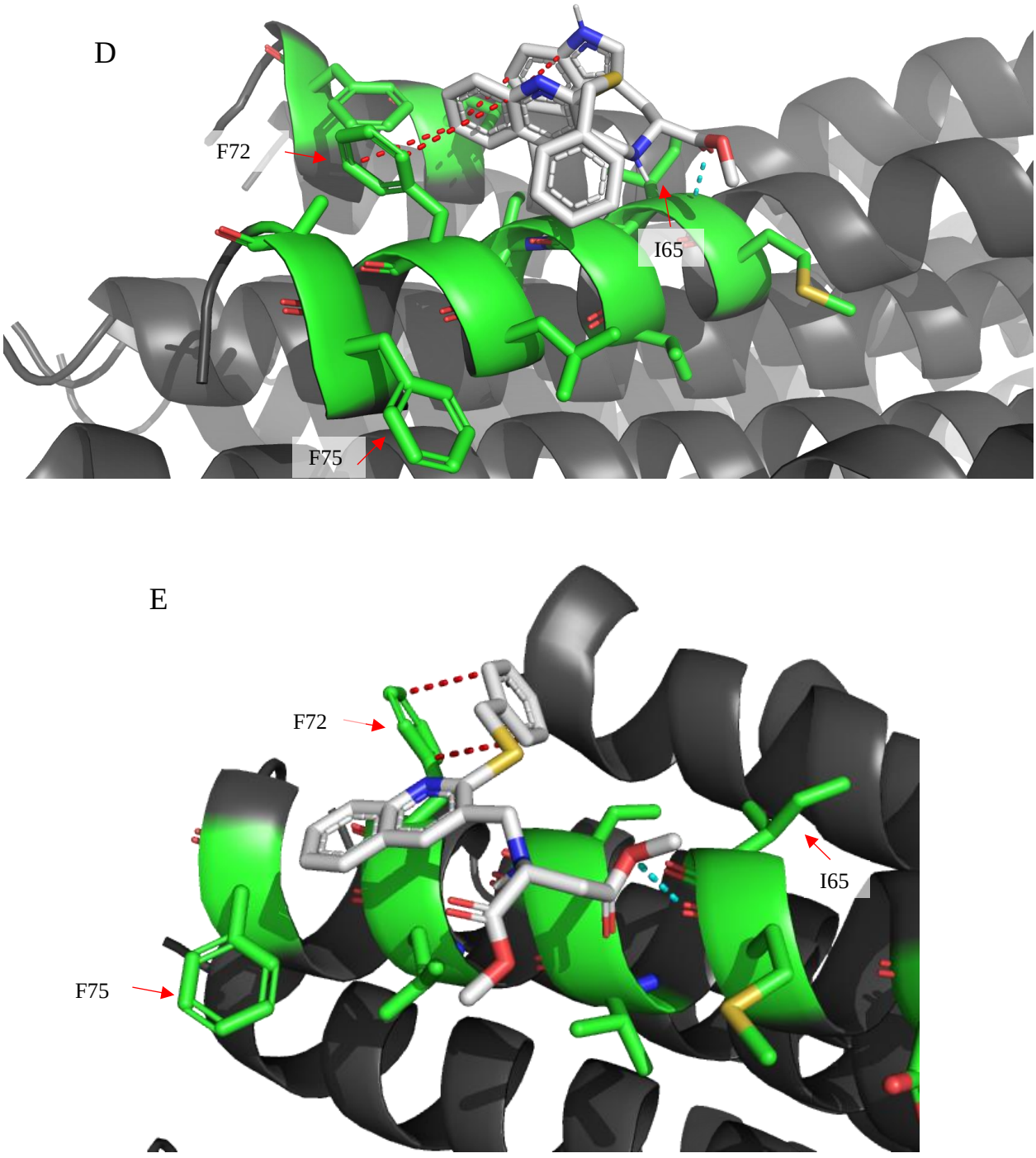


B



C





Figures 5: Docking images of proposed analogues A) Compound 2a, B) Compound 2b, C) Compound 2c, D) Compound 2d, and E) Compound 2e in an analogous model of *Pseudomonas aeruginosa* ATP synthase.

3. Results and Discussion

Currently several compounds have been synthesized and purified, as shown in Figure 6. Progress thus far has been focused on obtaining better yields, improving docking imaging and other various troubleshooting.

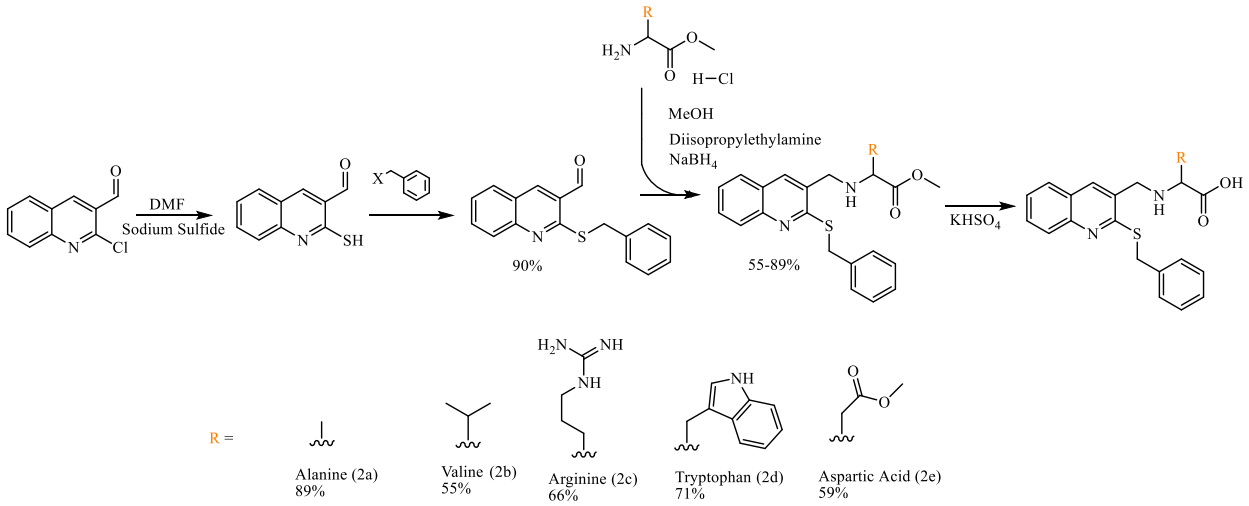


Figure 6: Overall reaction scheme for synthesis. All percent yields available are shown, any reaction step not labeled is such because: 1) all of the compound is immediately used for the next reaction or 2) has not been successfully synthesized.

Looking at the docking results for each of the compounds, it is seen that each compound interacts with the same residues for the most part. Our computational models prefer binding to the edge of the c-subunit, namely phenylalanine 72 (or 75 in some cases when looking at secondary docking positions). These results were not initially expected, as it was theorized that molecules would bind similarly to BDQ in M. tb.

4. Conclusions

Significant synthesis optimization has been performed over the course of a year and a half. Percent yields to produce compound 1a have increased by ~200%, and percent yields to produce the variety of compound 2s have also increased by ~200% from their initial runs respectively. Additionally, five novel compounds have been synthesized in decent to great yield.

From the docking process, we can make the prediction that all five compounds will interact with ATP synthase similarly. Regardless of the fact that the computational data does not line up with initial predictions, these compounds should still inhibit rotation of the c-subunit, providing the same effect. It should also be noted that each compound has a different binding energy; however, the energies provided are in Kcal/mol, meaning that the differences shown are not as significant as one may believe upon first glance. This is demonstrated when the $\Delta G_{binding}$ is converted to its dissociation constant counterpart, K_D, where all compounds previously mentioned are within 6.0×10^{-5} M of each other. K_D is the concentration at which half of the ligand binding sites are occupied, so with such a low ΔK_D these values are practically identical.

For the future of this project, more analogues will be synthesized, and compounds will be tested in ATP synthase assays along with general cell death assays. These new compounds doubled with proper assay testing should provide great insight into the effectiveness of these compounds as novel antibiotics.

5. Acknowledgements

I would like to acknowledge Dr. Amanda Wolfe, the Wolfe Research Team, Dr. Steed, the Chemistry Scholars Program, the National Institutes of Health for funding, UNCA Chemistry and Biochemistry Department, and the Undergraduate Research Department at University of North Carolina Asheville.

6. References

(1) Services, U. . D. of H. and H. Antibiotic Resistance Threats in the United States. *Centers Dis. Control Prev.* **2019**, 1–113.

(2) Neill, J. O. '. Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations The Review on Antimicrobial Resistance Chaired. **2014**, No. December.

(3) Payne, D. J.; Gwynn, M. N.; Holmes, D. J.; Pompliano, D. L. Drugs for Bad Bugs: Confronting the Challenges of Antibacterial Discovery. *Nat. Rev. Drug Discov.* **2007**, *6* (1), 29–40. https://doi.org/10.1038/nrd2201.

(4) Andries, K.; Verhasselt, P.; Guillemont, J.; Göhlmann, H. W. H.; Neefs, J. M.; Winkler, H.; Van Gestel, J.; Timmerman, P.; Zhu, M.; Lee, E.; Williams, P.; De Chaffoy, D.; Huitric, E.; Hoffner, S.; Cambau, E.; Truffot-Pernot, C.; Lounis, N.; Jarlier, V. A Diarylquinoline Drug Active on the ATP Synthase of Mycobacterium Tuberculosis. *Science (80-.).* **2005**, *307* (5707), 223–227. https://doi.org/10.1126/science.1106753.

(5) Fox, G. J.; Menzies, D. A Review of the Evidence for Using Bedaquiline (TMC207) to Treat Multi-Drug Resistant Tuberculosis. *Infectious Diseases and Therapy*. **2013**. https://doi.org/10.1007/s40121-013-0009-3.

(6) Balemans, W.; Vranckx, L.; Lounis, N.; Pop, O.; Guillemont, J.; Vergauwen, K.; Mol, S.; Gilissen, R.; Motte, M.; Lançois, D.; De Bolle, M.; Bonroy, K.; Lill, H.; Andries, K.; Bald, D.; Koul, A. Novel Antibiotics Targeting Respiratory ATP Synthesis in Gram-Positive Pathogenic Bacteria. *Antimicrob. Agents Chemother.* **2012**, *56* (8), 4131–4139. https://doi.org/10.1128/AAC.00273-12.

(7) Surase, Y. B.; Samby, K.; Amale, S. R.; Sood, R.; Purnapatre, K. P.; Pareek, P. K.; Das, B.; Nanda, K.; Kumar, S.; Verma, A. K. Identification and Synthesis of Novel Inhibitors of Mycobacterium ATP Synthase. *Bioorganic Med. Chem. Lett.* **2017**, *27* (15), 3454–3459. https://doi.org/10.1016/j.bmcl.2017.05.081.

(8) He, C.; Preiss, L.; Wang, B.; Fu, L.; Wen, H.; Zhang, X.; Cui, H.; Meier, T.; Yin, D. Structural Simplification of Bedaquiline: The Discovery of 3-(4-(N,N-Dimethylaminomethyl)Phenyl)Quinoline-Derived Antitubercular Lead Compounds. *ChemMedChem* **2017**, *12* (2), 106–119. https://doi.org/10.1002/cmdc.201600441.

(9) Wang, X.; Zeng, Y.; Sheng, L.; Larson, P.; Liu, X.; Zou, X.; Wang, S.; Guo, K.; Ma, C.; Zhang, G.; Cui, H.; Ferguson, D. M.; Li, Y.; Zhang, J.; Aldrich, C. C. A Cinchona Alkaloid Antibiotic That Appears to Target ATP Synthase in Streptococcus Pneumoniae. *J. Med. Chem.* **2019**, *62* (5), 2305–2332. https://doi.org/10.1021/acs.jmedchem.8b01353.

(10) Srivastava, A.; Singh, R. M. Vilsmeier-Haack Reagent: A Facile Synthesis of 2-Chloro-3-Formylquinolines from N-Arylacetamides and Transformation into Different Functionalities. *Indian J. Chem. - Sect. B Org. Med. Chem.* **2005**, *44* (9), 1868–1875. https://doi.org/10.1002/chin.200602144.

(11) Chamoin, S., Roth, H. J., Borek, C., Jary, A., & Maillot, H. (2003). Automated parallel synthesis of N-alkylated-α-amino methyl esters in gram quantities. *Chimia*, *57*(5), 241–247. https://doi.org/10.2533/000942903777679370

(12) O. Trott, A. J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, Journal of Computational Chemistry 31 (2010) 455-461. https://doi.org/10.1002/jcc.21334