

Combating Antibiotic Resistance with Novel Fluoroquinolone-Based Compounds
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According to the CDC, at least 2 million people in the United States alone acquire infections from resistant bacteria and at least 23,000 die from such infections. As a result, it is no surprise that bacterial resistance to currently available antimicrobial agents is a growing threat to public health. The most commonly prescribed class of antibiotic drugs are the Fluoroquinolones (FQ). This class of drugs show greater penetration into both Gram-positive and Gram-negative bacteria than any other drug class in previous use. However due to growing resistance to even FQs, novel FQ based compounds are required to be effective in resistant strains.

Ideally, these novel FQ-based compounds will interact with the target enzymes in a different manner than FQs like Ciprofloxacin or Moxifloxacin. These FQs take part in DNA cleavage and ligation reactions involving bacterial type II topoisomerases; DNA Gyrase and Topoisomerase IV. In cells, DNA gyrase introduces negative supercoils and removes torsional strain ahead of the replication fork to facilitate DNA strand separation. Topoisomerase IV decatenates daughter chromosomes after replication. Once either of these enzymes bind to the DNA, they form enzyme-DNA cleavage complexes, a characteristic which FQs exploit. FQs increase the concentration of these complexes, trigger DNA repair pathways and eventually overwhelm the cell and cause cell death. The main mechanism of action of these potent FQ drugs is through a Mg^{2+} -H₂O ion bridge interaction via active site residues Ser84 and Glu88. These two residues are most commonly mutated in FQ-resistant strains.

As the basis of my research this summer, I set out to better understand if and how the novel FQ-based compounds synthesized by the Barbachyn lab were effective in penetrating the double cell wall of Gram-negative bacteria and binding to the DNA-enzyme complex. Furthermore, I wanted to recognize any differences in structural interactions and orientations between FQs and the new compounds, and if these differences would suggest effectiveness in resistant strains.

Out of the 13 novel compounds I screened, 3 showed promising results when tested against strains of 4 Gram-negative and 1 Gram-positive bacteria. All 3 compounds bound effectively to the enzyme when tested using assay kits and all 3 presented a respectable EC₅₀ value. This EC₅₀ value tells us the concentration of drug required to give half the maximal response in a cell. When grown at various concentrations in bacterial cultures, two of the compounds showed promising results of having penetrated the cell wall to interact with the enzymes. The third compound displayed interesting activity, not having penetrated the cell wall of the bacteria, despite interacting well with the purified enzymes.

When comparing the new compounds to common FQs in terms of molecular modelling and orientation in the active site, the new compounds appeared to orient themselves very differently towards the Mg^{2+} ion and the two key residues. Some conformations showed clashes with the Mg^{2+} , suggesting that it may fit better in the active site without this divalent metal ion. The importance of the ion in the active site is one possible area of further study on this project. I am currently in the process of developing a *K. pneumoniae* strain that is resistant to Moxifloxacin for further tests with the new compounds. Sequencing this resistant strain will tell us more about the

residues mutated and if these mutations affect the role of the Mg^{2+} ion in the bridge. Eventually I would hope to test these new compounds with Human Topoisomerase II to assess its selectivity for the bacterial enzyme over the human enzyme and once this is done, it will be possible to talk of these novel compounds as potentially being a viable option for treating FQ-resistant strains.