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Structural and Functional Characterization of S06017, a Potential Novel Inhibitor of ADC-7

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β-lactams, such as penicillin, are the most widely prescribed class of antibiotics. In response to their extensive use and misuse, bacteria have become resistant to a growing number of these drugs. Many antibiotic-resistant bacteria express the enzyme β-lactamase, the most widespread resistance mechanism to β-lactams. These enzymes cleave the defining lactam ring, rendering the antibiotic inactive against its original cellular target, thereby allowing the bacteria to survive. As a result, antibiotic resistance has become a critical concern to human health. An example of an antibiotic resistant bacterial species that has shown pathogenic properties is Acinetobacter baumannii. A significant portion of the resistance of this organism is due to its expression of the β-lactamase, Acinetobacter-derived cephalosporinase-7 (ADC-7). In order to combat the antibiotic resistance in Acinetobacter, molecules were designed that could potentially bind ADC-7 and inhibit its ability to break down antibiotics. However, to facilitate the characterization and optimization of inhibitor design, the molecular structure of ADC-7 needs to be determined. In collaboration with Dr. Rachel Powers, we have recently determined the structure of ADC-7 with and without a bound inhibitory molecule. Currently, we are attempting to identify a specific molecule that can bind ADC-7 with high affinity and sufficiently inhibit ADC-7 to be considered an effective drug.

The goal of this study is to characterize a promising new inhibitory compound of ADC-7 (S06017) and determine the efficacy with which this drug can inactivate the β-lactamase enzyme. In order to determine whether the inhibitor substantially decreases the enzyme activity, competition kinetics were performed with S06017 against a diagnostic substrate of ADC-7 (nitrocefin). Competition kinetics assays yield a Ki value, which is a measurement of the ADC-7/inhibitor binding affinity, with lower Ki values indicating higher binding affinities. While the resulting Ki of 6.108 µM demonstrates that S06017 does bind and inhibit ADC-7’s ability to break down nitrocefin, the ADC-7/S06017 binding affinity is weaker than other related compounds. For example, the inhibitory compound S02030 binds ADC-7 with a Ki of 0.0373 µM, and competition kinetics performed with CR192 resulted in an average Ki of 0.00045 µM. Even though S06017 binds ADC-7 with lower affinity than related molecules, the question remains: what specific parts of these molecules are responsible for binding tightly to ADC-7?

By determining the X-ray crystal structure of ADC-7 with S06017 bound in its active site, it is possible to compare the specific orientation and interactions involved in inhibitor binding. In addition, by comparing the ADC-7 structures in a complex with different compounds, it is possible to optimize the molecular structure of compound that will serve as the most efficient inhibitor of ADC-7. Comparing all of the X-ray crystal structures, as well as the Ki values from competition kinetics, will hopefully lead to the discovery of an inhibitory drug that will significantly inhibit ADC-7, which could aid in combating antibiotic resistance in Acinetobacter baumannii.

*This scholar and faculty mentor have requested that only an abstract be published.