Evaluation of Various Analogs of High-Throughput Screening Target Molecules as DapE Inhibitors Using the DapE Ninhydrin Assay for Novel Antibiotic Discovery

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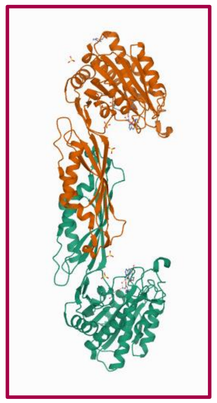
In our medicinal organic chemistry research lab, I am performing a ninhydrin-based enzyme inhibition assay, previously developed in our lab, to discover novel antibiotics to combat antibiotic resistance that poses an imminent public health crisis. These drugs target the bacterial enzyme *N*-succinyl-L,L-diaminopimelic acid desuccinylase (DapE), a crucial enzyme to bacterial survival due to its involvement in the biosynthetic pathway of lysine, an amino acid essential to protein synthesis and mDAP, which is essential for bacterial cell wall construction. This pathway is exclusive to bacteria and is not found in mammalian cells, thus inhibition of this enzyme should be selectively toxic to bacteria. The assay enables testing of potential new inhibitors of DapE, done by spectrophotometric analysis of the products of the in vitro enzyme assay, quantitatively analyzing the amount of enzyme substrate converted to product. The goal is to evaluate several newly discovered inhibitor series involving indoline sulfonamides, tetrazoles, and N-aryl sulfonamides. Studying analogs of these compounds will greatly aid our understanding of the enzyme dynamics involved in the inhibition process. This information will broaden our understanding of the enzyme's catalytic machinery to significantly aid in our future endeavors of drug synthesis and drug discovery for the optimization of new inhibitors.

Background

Although the world currently faces a major battle against the global COVID-19 pandemic, a longer-term ongoing fight continues against an increase in antibiotic resistant bacteria, underscoring the urgent need to discover antibiotics with a new mechanism of action.[1] The Becker research group is addressing this increase in mortality and morbidity in order to advance our fight against antibiotic resistance. Our medicinal chemistry research has revealed the possibility of an entirely new class of antibiotics, which targets a previously-unexplored biochemical pathway,

Utilizing inhibition of the enzymatic activity of *N*-succinyl-L,L-diaminopimelic acid desuccinylase, or DapE (inset).[2] This enzyme is part of a crucial biosynthetic pathway that produces the amino acid lysine and mDAP, both essential to protein synthesis and bacterial peptidoglycan cell wall construction, respectively. Therefore, inhibition of DapE enzymatic activity is very attractive because its inhibition should be selectively lethal to bacteria, while safe for humans, as we receive lysine from our diet, and we do not express the DapE enzyme.



Objectives

To work in collaboration with graduate students as well as undergraduate students within Dr. Becker's research group who are synthesizing compounds that require testing in the assay to obtain their inhibitory potency against DapE.

Through the utilization of the computational suite, the Molecular Operating Environment (MOE), and a High Throughput Screen (HiTS), we discovered several potential new inhibitor series for DapE, which include indoline sulfonamides[4], tetrazoles, and N-aryl sulfonamides. Our group has also designed cyclobutanones as DapE inhibitors.[5]

After hypothesizing that these molecules would inhibit DapE, analogs of the original hits were either previously synthesized or continue to be synthesized, and then, each of these compounds must be assessed in the assay to obtain inhibition data.

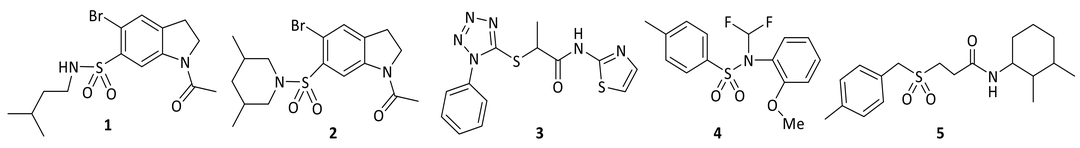
Once the inhibition data is obtained, the molecular interactions between DapE's structure and the potential inhibitors may be analyzed to provide greater understanding of the enzyme dynamics involved in the inhibition process. This greater understanding will significantly aid in future endeavors of drug synthesis and drug discovery for the optimization of new inhibitors.

Methodology

In order to demonstrate inhibition of DapE to test potential antibiotic efficacy, the biochemical assay developed in our research group[3] is utilized. This consists of the incubation of the active DapE enzyme, a modified substrate, *N*-methylsuccinyl diaminopimelic acid (N-Me-SDAP), HEPES buffer, and the inhibitor. In addition, the potential inhibitors are tested at various concentrations at 30°C for 10 minutes, then the reaction is quenched by heating for 1 minute to deactivate the enzyme. Then, ninhydrin is added, which reacts with the primary amine of the now-cleaved product, converting the solution to a shade of purple. The absorbance at 570 nm is then measured in a spectrophotometer through the utilization of a well-plate reader, and different absorbance values are obtained, which represent the amount of substrate converted to product within the assay. After subtracting background absorbance, the inhibition for each concentration of inhibitor is calculated. After running the assay in triplicate, using 100% enzymatic activity, 0% enzymatic activity, and glutamic acid controls, the IC_{50} of each potential inhibitor can be accurately obtained.[3] Within medicinal chemistry and drug discovery, the IC_{50} refers to half the maximum inhibitory concentration, and it is utilized to compare the effect that potential drugs have on specific biological pathways or processes. It is one of the most common ways to report inhibitory effects of small molecule drug candidates. Now, with this inhibition data, the synthesized compounds may be compared with previously identified DapE inhibitors, such as the thiol-containing ACE inhibitor captopril, to determine their efficacy as potential new antibiotics. In addition, these data facilitate an understanding of the structure-activity relationships (SAR) between our synthesized compounds and the enzyme and enable us to iteratively improve our analogs.

Lead Molecules as DapE Inhibitors

Figure 1 contains the lead structure from each inhibitor series to demonstrate the wide variety of inhibitors being tested in our critical assay. Some lead molecules are in their beginning stages of synthesis, while the Becker group has just published advanced inhibitors based on the indolines **1** and **2**.[4]



1

IC50 = 42% @ 200 uM
MW = 389
cLogP = 3.29
PSA = 66.5 Å^2

2

IC50 = 133 uM (nor-Me)
MW = 415
cLogP = 3.5
PSA = 57.6 Å^2

3

IC50 = 89 uM
MW = 332
cLogP = 1.89
PSA = 81.8 Å^2

4

IC50 = 72.9 uM
MW = 327
cLogP = 3.69
PSA = 46.6 Å^2

5

IC50 30% @ 20 uM (insol)
MW = 351
cLogP: 3.53
PSA: 63.2 Å^2

Figure 1. Lead molecules as DapE inhibitors

Results and Conclusions

Inhibition data for a series of indolines has recently published by our group.[4] We have obtained preliminary inhibition data for series **3** and **4** inhibitors, as well as for cyclobutanones, and this data will be published in due course. Furthermore, after performing these reactions and obtaining DapE inhibition data, we will co-crystallize the drug candidates in collaboration with Argonne National Laboratories and obtain high-resolution co-crystal structures of the inhibitors with the DapE enzyme to elucidate the binding mode of the inhibitors as well as the distinct enzyme conformations[6] with the different compound moieties bound to the active sites. With this information, we will be able to advance our understanding of the enzyme-drug interactions through an improved knowledge of the structure activity relationship (SAR) between the different classes of inhibitors with the enzyme, which will in turn enhance our effort toward the discovery of new antibiotics.

Continued Work

In addition, updates and improvements have been made for streamlining our assay to enable faster throughput and more accurate results. We are now utilizing a thermal cycler, an instrument that automatically regulates the temperature of the sample and the incubation time for each of those temperatures, all possible through programming. With the reduction of error, every IC_{50} value calculated utilizing the thermal cycler will be that much more accurate. This will greatly impact every single project in our research lab.

Additionally, it is imperative to note our ongoing work to broaden the scope of our research to combat growing antibiotic resistance. Therefore, in the future, we will be moving to set up the assay with the DapE enzyme from different bacterial species to confirm broad spectrum antibiotic activity. We are working in collaboration with CSGID (Center for Structural Genomics of Infectious Diseases) at Northwestern, as well as Argonne National Laboratories, to expand the validation of DapE as a target for different types of bacterial infections.

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Acknowledgements

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Members of the Becker Research Lab

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