Office of Research and Sponsored Programs (ORSP)
2022 Summer Research Fellowship Program

Project title: Novel combination antibacterial therapy against carbapenem-resistant *Acinetobacter baumannii*
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ABSTRACT

Carbapenem-resistant Acinetobacter baumannii (CRAb) is an urgent public health threat, according to the CDC. This pathogen has few treatment options and causes severe nosocomial infections with >50% fatality rate. Since more than five decades ago, β-lactam class antibiotics have been the primary therapeutic treatment used to combat both gram-positive and negative bacterial infections. However, the emergence of β-lactam drug resistance bacterial pathogens has become a major public health threat against immune-compromised individuals, post-surgical patients, and the elderly in hospitals. The major goal of the project is to develop a novel class of potent β-lactamase inhibitors to rescue existing β-lactam antibiotic activities for combination antibacterial therapy. The ability to preserve the efficacy of existing β-lactam antibiotics arsenal provides maximum opportunity for combination antimicrobial therapy development. To achieve this objective, we will use a state-of-the-art drug design methodology involving computational molecular modeling, cell/enzyme-based study, and chemical synthesis to generate inhibitors of a β-lactamase protein. Estimating first-time novel class of compounds as potent irreversible/reversible inhibitors against β-lactamase with low micromolar inhibition activities comparable to established FDA approved β-lactamase inhibitors drugs.

BACKGROUND

Common occurrence of β-lactam antibiotic drug resistance is a global health concern to our antibiotic arsenals against bacterial infections. β-lactam antibiotic is a first line broad spectrum antibiotic use for the treatment of Gram-positive and Gram-negative bacterial infections. It consists of a strained β-lactam ring structure that irreversibly inhibits penicillin binding protein (PBP), an enzyme that facilitates the transpeptidation process in the biosynthesis of the peptidoglycan (PG) layer of the bacterial cell wall that is vital for maintaining structural integrity during cell growth and division. Inappropriate and unnecessary uses of antibiotics for maintaining human health and the health of food producing livestock are the leading causes for the selection, inoculation and spread of β-lactam antibiotic resistance bacterial pathogens. Their continued emergence in community and healthcare settings has become a serious health threat as nosocomial infections and secondary infections against immune deficient, weakened or suppressed patients.

Expression of β-lactamase (BL) from chromosomally encoded or acquired plasmid gene afford the development of β-lactam drug resistance. β-lactamase enzymatically cleaves open the β-lactam ring which renders β-lactam antibiotics inactive against their intended PBP target. Expression of broad spectrum β-lactamase or multiple classes of β-lactamases is one of the primary mechanisms for the development of multi-drug resistance against multiple classes of β-lactam antibiotics. There are several classes of β-lactam drug resistant pathogens including Klebsiella spp., Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, and Methicillin-resistant Staphylococcus aureus (MRSA) that have been identified as microorganisms with urgent or serious threat level in the United States. Up to 90% of ampicillin resistance in drug resistance E. coli is due to the expression of TEM-1 β-lactamase. Mycobacteria tuberculosis, the causative

agent for tuberculosis (TB), consists of chromosomally encoded β-lactamase (BlaC) which render them intrinsically resistant to β-lactam antibiotic drugs. A combination therapy with a β-lactamase inhibitor and a β-lactam antibiotic is the standard of care for treating β-lactam drug resistance, prior to prescribing alternative classes of non-β-lactam antibiotics such as fluoroquinolones, aminoglycosides, tetracyclines, or polymyxins that may be more toxic, less efficacious and may increase the risk of selecting and spreading of bacterial pathogens that are resistant to multiple classes of antibiotics. To date, there are only three β-lactam β-lactamase inhibitors (Clavulanic acid, Sulbactam, and Tazobactam) and one recently discovered non-β-lactam β-lactamase inhibitor (Avibactam) approved for the combination therapy against β-lactam drug resistant infections. A novel class of β-lactamase inhibitors that resuscitates and redeploys existing β-lactam antibiotic arsenals provides the maximum opportunity for developing novel combination therapies for combating β-lactam drug resistance. It may also provide opportunities for developing a new combination therapy for treating TB.

SIGNIFICANCE

β-lactam drug resistance remains a major public health threat. Developments of a single new β-lactamase inhibitor that resuscitate existing β-lactam antibiotic arsenals provide the maximum opportunity for novel combination antimicrobial therapy for combating drug-resistant pathogens. To achieve this objective, we will use a state-of-the-art drug design methodology involving computational molecular modeling, cell/enzyme-based study, and chemical synthesis to generate inhibitors of a β-lactamase protein. In addition, the use of non-toxic and abundant natural products as antibiotics suppresses the development of drug resistance, promising safer administration and lower side effects for patients.

GOALS AND OBJECTIVES FOR SUMMER RESEARCH PROJECT

Our group has demonstrated a new class of non-β-lactam β-lactamase inhibitors with reasonable therapeutic properties for combination therapy against A. baumannii infections, MRSA infections or infections caused by other bacterial strains having resistance to β-lactam antibiotics (penicillins, cephalosporins, carbapenems etc.), due to the presence of a resistance-conferring β-lactamase enzyme of the serine-type.

Patients afflicted with bacterial infections that are refractory to treatment with the β-lactam class of antibiotics, wherein the bacterial resistance mechanism involves the expression of a serine-type β-lactamase enzyme, can be treated by use of an effective amount of a β-lactam antibiotic administered in conjunction with an effective amount of a β-lactamase inhibitory sulfonyl oxadiazole compound.

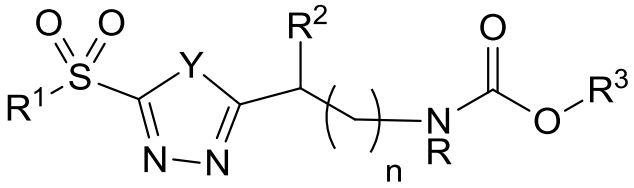


Figure 1. Structure of sulfonyl oxadiazole compound.

The long-term goal of the project is to develop our novel classes of sulfonyl oxadiazole compound as a potent β-lactamase inhibitor to rescue existing β-lactam antibiotic activities. The ability to preserve the efficacy of existing β-lactam antibiotics arsenal provide maximum opportunity for combination antimicrobial therapy development. As part of these long-term research projects, the short-term goals of the summer research project are:

Aim 1 Design and optimize a new potent sulfonyl oxadiazole analog structure that can be developed as a new drug candidate by using computer-aided drug design approaches based on the natural lead compounds.

Aim 2 Determine the structural activity relationships (SAR) of a novel class of non-β-lactam β-lactamase inhibitors in both biochemical and bacterial cell-based assay to examine the molecular basis for bioactivity in combination therapy.

RESEARCH METHODS FOR SUMMER PROJECT

- Molecular modeling and visualization
- Structure-based virtual screening
- Protein-ligand docking
- Enzyme kinetics
- MIC bacteria cell proliferation assay
- Human cell Toxicity assay
- Skin wound model *in vivo* test (optional)

PROPOSED METHODS OF DATA ANALYSIS

Drug design and discovery is an interdisciplinary, expensive and time-consuming process. Modern drug discovery owes to the scientific advancements during the past two decades thereby computers and computational methods became indispensable tools in the pipeline which could lead to a reduction of up to 50% in the cost of drug design.

Recently, our laboratory has been successfully established the pipeline of motif-guided drug screening and drug carrier protein design using CADD to *in vitro* & *in vivo* verification. In addition, our computational approach further demonstrated that quercetin and its analogs, once bound to the NDM-1 active site, could effectively block the activity of metallo beta-lactamase enzymes. (Fig 2). It focuses on the development and optimization of well-balanced methods with computational sequence/structure-based design and molecular cell biology experiment targets to the infectious disease.

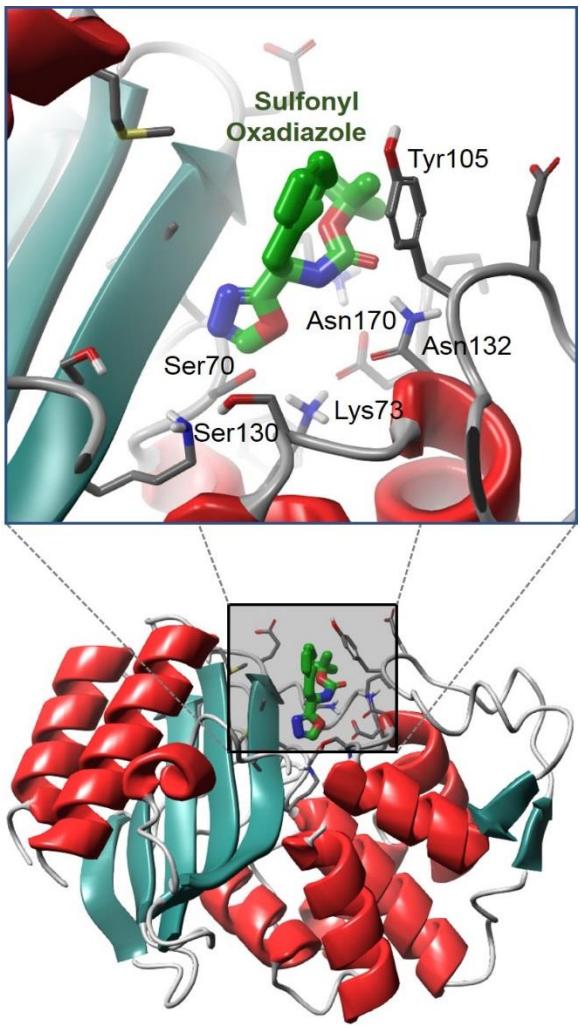


Figure 2. The predicted binding complex model of TEM-1 serine beta-lactamase (PDB ID:1JTG) and sulfonyl oxadiazole.

As early lead identification and lead optimization are our initial goals of the drug discovery process, advances in computational techniques have enabled *in-silico* methods, and in the particular structure-based drug design methods, to speed up new target selection through the identification of hits to the optimization of lead compounds.

CONTRIBUTION OF SUMMER RESEARCH FELLOW

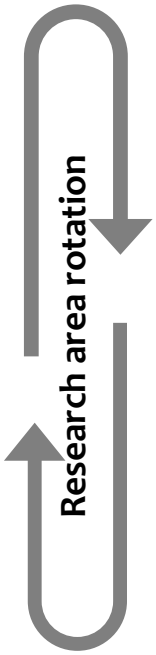
The new potential lead compound-based analog structure findings with its structural activity relationships (SAR) from the summer research fellow will contribute to elucidate the exact mechanism of inhibition and the specific protein-ligand interactions using state-of-the-art computer-aided drug design techniques.

And we will further demonstrate the therapeutic potential of this new potent metallo β-lactamase inhibitor with broad-spectrum activity and identify the stability with pharmacokinetics (pK) against *in vitro* system.

This short-term summer project that uses computing power to rapidly find lead compounds and validate the *in-silico* data with simple *in-vitro* experiments are exactly aligned with our laboratory's goal of drug design and will provide a great research experience to our professional level students.

STUDENT FELLOW TRAINING / MENTORING PLAN

The research timeline for summer students will be divided into computer-aided drug design and wet lab biology research sessions according to their respective research interests and background expertise. The students will learn the principles of drug design starting with basic training, participating in the subscale study, research poster presentation, writing research manuscript, and prepare of the grant proposal. The students will rotate in different research areas within a period of two to three weeks, learn various research approaches, and participate in the project. The topics of each research field that students will learn and participate in are as follows.



Computer-aided Drug Design

- Bioinformatics (ClastalW, Schrodinger)
- Molecular modeling and visualization (Maestro, VMD, SYBYL, Pymol)
- 3D Structure motif-based virtual screening (Phase)
- Protein-ligand docking and binding free energy calculation (Glide, AutoDock)
- Molecular dynamic simulation and analysis (NAMD, VMD)
- Prediction of protein structure (Prime, SWISS-Model)
- Pharmacophore modeling (PHASE)
- Huge scale multi-sequence data analysis (ClastalW, Schrodinger)

Biological Experiment

- Basic cell culture (neuroblastoma, biosensor cell line)
- Cell Lysis and protein extraction
- SDS/Native Gel PAGE (Western Blot / Coomassie blue staining)
- Human cell proliferation assay and toxicity testing
- Toxicity testing
- Protein crystallization study
- Enzyme kinetics
- Protein concentration measurement (BCA/ELIZA)
- Skin wound model in vivo test

Available Resource and Laboratory location

- Computer-aided Drug Design (RGE #401 write-up area) – Schrodinger CADD modeling package
- BSL 1 area (RGE #400) – Enzyme kinetics assay / inhibitor preparation / Proteomics study / SDS & Native Gel Page / Protein crystallization
- BSL 2 area (RGE #407 / RGE #416) – Human & bacteria culture environment / Toxicity test / Cell proliferation assay
- Mouse surgery (RGE#414) – Skin wound model in vivo test