# Drug Discovery Day 2023

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April 14, 2023
Hahn Hall South Atrium

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# TOPICAL AZOLE ANTIFUNGALS: IS THIS THE TREATMENT FOR *CLOSTRIDIOIDES DIFFICILE* INFECTION (CDI)?

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*Clostridioides difficile* is a prominent source of healthcare-associated infections and is regarded as an urgent public health problem globally with around 30,000 deaths in the U.S[1]. The only FDA-approved antibiotics for the treatment of *C. difficile* infection (CDI) are vancomycin and fidaxomicin. The high rate of treatment failure and recurrence linked to these antibiotics, as well as the rising number of infections, make CDI treatment extremely difficult. Therefore, it is imperative to find new, powerful anti-*C. difficile* drugs. The key factor in CDI is the use of antibiotics, which leads to an imbalance in the body's microbiota and encourages Candida species to proliferate and form biofilms, which in turn helps *C. difficile* produce toxins and spores[2, 3]. That inspired us to consider using antifungals to treat CDI in order to get around the limitations of antibiotics. Drug repurposing is a potential method for cutting costs and time while lowering the hazards connected with de novo drug discovery when compared to the development of new drugs from scratch. Using this method, we tested a library of azole antifungals against diverse range of pathogenic *C. difficile* strains, and the results showed that miconazole, econazole, and tioconazole displayed the most potent activity against *C. difficile* inhibiting the growth of 50% of tested isolates ($MIC_{50}$) at concentrations of 1 µg/ml, 2 µg/ml, and 2 µg/ml, respectively. Miconazole was selected for further investigation since it demonstrated the most potent anti-*C. difficile* activity, and it is orally bioavailable. In a time-kill kinetics study, miconazole showed a fast bactericidal activity outperforming vancomycin, where it decreased a high bacterial inoculum by more than 3 $\log_{10}$ within 2-4 hours and completely cleared the bacterial burden after 4 hours and regarding the spore inhibition, this antifungal offers promise sporocidal activity to overcome the infection recurrence. Furthermore, miconazole did not show inhibitory activity against key species that compose the host intestinal microbiota and showed a prolonged post-antibiotic effect (PAE) (>6 hours) exceeding that of the drug of choice, vancomycin. Physicochemical properties of miconazole including, the effects of pH, pre-exposure to simulated gastric fluid (SGF), and simulated intestinal fluid (SIF), were also examined. High pH values did not affect the miconazole's antibacterial action, and it retained the same potency after being exposed to SGF and SIF. Overall, these findings suggest that miconazole deserves more research as a powerful and focused anti-clostridial agent to replenish the depleted pipeline of new anti-*C. difficile* treatments with antifungal efficacy to regulate the entire CDI environment.

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**Acknowledgement:** Funding was provided by the National Institute of Allergy and Infectious Diseases to M. N. Seleem (Grant No. R01AI130186).

# DISCOVERY OF A NEW CLASS OF ANTIBIOTICS AGAINST MULTIDRUG-RESISTANT *NEISSERIA GONORRHOEAE*: CAN WE FINALLY SOLVE THIS SUPER-WICKED PROBLEM?

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*Neisseria gonorrhoeae* is an urgent public health threat worldwide that infects about 82.5 million patients annually. Due to the increasing incidence of infections that has been accompanied by an increase in the bacterial resistance to most antibiotics, the number of effective anti-gonorrheal therapeutic options is gradually diminishing. Currently, ceftriaxone is the only recommended antibiotic for treatment of *N. gonorrhoeae* infections. Yet, high-level gonococcal resistance to ceftriaxone is uprising. Without developing new anti-gonorrheal treatments, the world faces the real possibility of an untreatable gonococcal infection. Drug repurposing represents an attractive approach of drug discovery as it reduces the time, costs, and risks associated with traditional drug innovation. Utilizing this approach, we identified the FDA-approved carbonic anhydrase inhibitors (CAIs), acetazolamide (AZM) and ethoxzolamide (EZM), as potent anti-gonococcal agents. AZM and EZM displayed $MIC_{50}$, against a panel of *N. gonorrhoeae* isolates, of 1 µg/mL, and 0.125 µg/mL, respectively. Both agents exhibited a bacteriostatic activity against *N. gonorrhoeae*, demonstrated post-antibiotic effects up to 10 hours, and no resistant mutants were isolated against both in the presence of a high bacterial inoculum. A permeability assay indicated that the increased anti-gonococcal potency of EZM *in vitro* is attributed to its increased permeability in *N. gonorrhoeae* as compared to that of AZM. Mechanistic investigations revealed that AZM and EZM inhibit *N. gonorrhoeae* carbonic anhydrase (CA). *N. gonorrhoeae* CA has been identified as an essential gonococcal enzyme that is required to maintain carbon dioxide and pH homeostasis. Both molecules exhibited almost similar potency against the gonococcal CA *in vitro*, where AZM displayed an inhibition constant ($K_i$) of 74 nM, while EZM's $K_i$ was estimated to 94 nM. In addition, antisense peptide nucleic acids (PNAs) targeting the gonococcal CA indicated that the gene is essential for the bacteria and its inhibition by PNAs results in bacterial killing. Finally, the *in vivo* efficacy of AZM in a mouse model of *N. gonorrhoeae* genital tract infection was investigated. Compared to vehicle-treated mice, AZM significantly reduced the gonococcal burden by 90% in the vagina of infected mice after three days of treatment. Taken together, these results indicate that AZM and EZM warrant further investigation for translation into effective anti-*N. gonorrhoeae* agents to supplement the limited pipeline of anti-gonococcal therapeutics.

**ISOLATION AND STRUCTURAL DETERMINATION OF FORGOTTEN ANTIBIOTICS**

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The role of natural products has become increasingly important in drug discovery in recent years. Antimicrobial resistance poses an enormous threat to the health of humans around the world, and this necessitates finding more potent and effective antibiotics to help solve these problems. Thermorubin is a natural product that is known for its inhibition of bacterial protein synthesis, with the structure already determined, we are interested in isolating this active compound from Laceyella sacchari and being able to understand and further explore its function and modification.
XK-46 and litmocidin are other antibiotics believed to be similar in structure to thermorubin, but the structure of these antibiotics was never reported. They were however tested for antibiotic activity and found to be potent against pathogens and mycobacteria. Efforts are ongoing to optimize the growth of the organisms that produce these secondary metabolites, isolate the compounds, and characterize them to be able to understand what structures are responsible for the reported function. This information will be vital in subsequent semi-synthetic approaches to potentially develop these antibiotics as drugs.

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We thank the Department of Chemistry, Virginia Tech for supporting this project.

**RITONAVIR, A PROMISING COST-EFFECTIVE AMPHOTERICIN SYNERGIST AGAINST CRYPTOCOCCAL MENINGITIS INFECTION**

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*Cryptococcus neoformans* causing life-threatening meningitis and represents an alarming global health threat associated with high mortality among immunocompromised individuals and HIV patients. The current arsenal of antifungal drugs to combat the growing problem of cryptococcus is very limited. Amphotericin B is the front line treatment for cryptococcal meningitis. However, the treatment with amphotericin B is commonly associated with severe adverse effects. In this study, we used the combinatorial approach to minimize the toxicity and to enhance the efficacy of amphotericin B against *C. neoformans*. We evaluate the HIV-protease inhibitor, ritonavir, as a potential co-drug to work synergistically and to enhance the effectiveness of amphotericin B treatment. Ritonavir exhibits a potent in-vitro synergistic interactions when combined with amphotericin B against 100% (15/15) of the tested *C. neoformans* isolates with a fractional inhibitory concentration index (ΣFICI) ranging from 0.07 to 0.31. Notably, the combination of ritonavir with amphotericin B led to killing of all tested isolates within 3 hours as measured by time killing assays. As a part of the involved mechanistic study, ritonavir significantly interferes with glucose transport in *C. neoformans* reducing its uptake by 52%. These data highlight the potential of antifungal combination between amphotericin B and ritonavir to combat *C. neoformans* infections. Furthermore, these data will provide insight into the potential clinical usefulness of ritonavir because it is commonly administered in HIV-infected patients and cryptococcus is a leading cause of morbidity and mortality in those patients.

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# OPTIMIZING LINEAR SIALIC ACID-CONTAINING POLYMER PARAMETERS FOR ENHANCED INFLUENZA INHIBITION

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Influenza replication is mitigated by the interactions between hemagglutinin on the viral surface and sialic acid on the epithelial cell surface of the lungs. This polyvalent interaction that is seen in nature can be mimicked synthetically with sialic acid-containing polymers where each repeat unit acts as a place for potential binding. These "decoy" sialic acid receptors bind to the surface of influenza via hemagglutinin and reduce the available viral surface that can interact with the cell surface. This mechanism of action greatly reduces the amount of viral replication that can occur and therefore consequent infection.

Linear sialic acid-containing polymers have been extensively studied for influenza inhibition, but due to varied structural motifs and nonuniform experimental assay conditions, it is difficult to conclude how polymer parameters affect overall antiviral properties.[1] By synthesizing materials with controlled and well characterized polymer properties (molecular weight, sialic acid content, comonomer identity, linker length), we can better elucidate how these parameters impact the mechanism of inhibition and efficacy of the material. To do this, we have synthesized a library of sialic acid-containing polymers with systematically varied parameters and will measure their influenza inhibition using a variety of assays (hemagglutination inhibition, plague, etc.). We will use this data to study and optimize sialic acid-containing polymers for enhanced influenza inhibition.

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This work was supported by GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement DMR-1933525.

# THE POWER OF CONDUCTIVE QUINONES: ELECTRON SHUTTLING CAPABILITIES OF QUINONE CONTAINING NATURAL PRODUCTS AND SYNTHETIC ANALOGS

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A dire need for renewable energy sources has come to the forefront with concerns of fossil fuel carbon emissions causing climate change. A promising renewable energy source are Microbial Fuel Cells (MFCs) that utilize metal reducing bacteria. One particular bacterium, *Shewanella oneidensis* MR-1, utilizes a polar menaquinone analog 2-amino-3-carboxy-1,4-naphthoquinone (ACNQ) to shuttle electrons to an insoluble terminal electron acceptor, facilitating anaerobic cell growth. In this study, a library of natural product-mimicking quinone-containing metabolites has been assembled through a combination of commercial sources, isolation, and or semi-synthesis. Compounds in the library are given to knockout strains of metal reducing bacteria, unable to produce endogenous shuttling compounds, and their rate of electron transfer to a terminal electron acceptor is measured. The compound's rate of electron transfer is related to chemical properties, *i.e.,* redox potential, clogP, polarizability, and size. We hope to gain a better understanding of the key properties that lead to efficient activity. This will help answer outstanding questions about electron shuttling mechanisms, substrate specificity, and how to increase MFC energy output to a larger scale.

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Department of Defense MURI Grant (2022)

# 1,2,3-TRIAZOLE DERIVATIVES OF THE ANTIBIOTIC PLEUROMUTILIN

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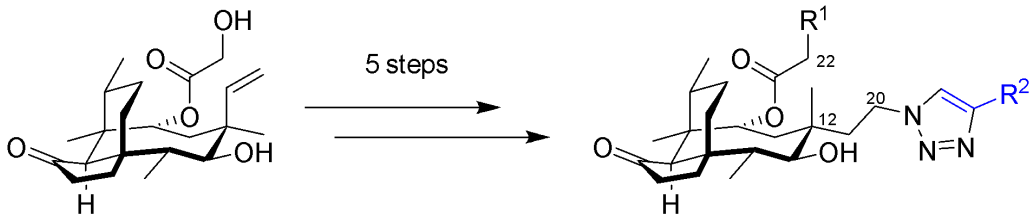
Pleuromutilin-derived semisynthetic drugs are used as critical human and veterinary antibiotics. The unique mechanism of action of the fungal natural products results in low development of resistance in targeted pathogens making them an attractive scaffold for continued drug development.[1] Our previous work functionalizing pleuromutilin with triazoles showed that C22 substituted compounds maintained activity while C20 substitution abolished it.[2] Guided by computation and structure-based drug design, we reveal here that epimerization of the C12 position gives potent lead compounds with C20 triazoles. In addition, electrophilic intermediates identified en route to our final compound showed enhanced activity. Currently, a series of electrophilic pleuromutilin analogs has been synthesized and is undergoing testing. These derivatives will show whether pharmacophore action or covalent binding to the target is causing the increase in activity.




Pleuromutilin 12-*epi*-20-triazolylpleuromutilins

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I would like to acknowledge funding from CeZAP IDEP Summers 2021 and 2022, as well as from the Graduate Research Assistantship Award, Fall 2022.

**REGIO- AND STEREOSELECTIVE COPPER-CATALYZED HYDROBORATION OF 1,3-ENYNES: ACCESS TO 2-BORYL-1,3-DIENES**

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Organoboron compounds are a novel chemical scaffold in drug development. To date, five organoboron drugs have been approved for the treatment of various maladies.[1] Additionally, boron-carbon bonds have been used in carbon-carbon, carbon-nitrogen and carbon-oxygen bond formatting reactions.[2,3] In fact, the Suzuki-Miyaura cross-coupling reaction was found to be in the top five most used reactions in drug discovery.[4] While many types of borylation reactions have been explored, the hydroboration of highly unsaturated molecules remains challenging due to various chemo-, regio- and stereoselectivities being possible. Previously reported 1,3-enyne hydroboration reactions resulted in allene formation (1,4-functionalization) or 1-boryl-1,3-diene formation (1,2-functionalization).[5-7] In this work, the (*Z*)-3,4-selective hydroboration of 1,3-enynes is disclosed. A kinetic study was conducted to investigate the reaction mechanism and justify the exclusive (*Z*)-isomer formation. Copper (I) acetate (5 mol%), Xantphos (5 mol %), and pinacolborane (2.0 equiv) were used in toluene at 40 °C to achieve this transformation. Aryl enynes bearing halides, electron donating functionality, electron withdrawing functionality and heterocycles were well tolerated. Up to 92% isolated yield of the resulting 2-boryl-1,3-dienes was achieved, and 24 substrates are represented.

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**THE FUNGUS AMONG US: COUNTERACTING AZOLE RESISTANCE IN ASPERGILLUS SPECIES WITH LOPINAVIR**

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*Aspergillus fumigatus* annually contributes to more than 300,000 infections globally, with an average mortality of 65%[1-2]. Invasive pulmonary infections arising from *Aspergillus fumigatus* are life-threatening fungal infections for immunosuppressed and cancer patients. However, the disease is much broader in scope, with cases of COVID-19 infections also containing aspergillus infections, raising the specter of more infections that are undiagnosed[3]. Triazoles, such as voriconazole, itraconazole, posaconazole, are primary antifungals for first-line therapies for infections arising due to *Aspergillus sp*[4-5]. Our predominant issue is the ever increasing incidence of broadly antifungal resistant strains of *A. fumigatus*[4]. These incidents are and will continue to increase and trigger alarm due to treatment failure and high mortality[5]. Mortality for these infections can range from 40-90%[2]. In recognition of this threat alongside other fungal pathogens, the CDC has generated a watch list. *Aspergillus fumigatus* has been placed within the critical priority group due to its rate of occurrence, predation of the ill and rapidly growing resistance. Therefore, our group is searching for a co-drug capable of enhancing frontline antifungal potency. Here our group sought to determine the mechanisms behind interactions between HIV protease inhibitors and frontline azole drugs. We challenged various pathogenic and clinically important *A. fumigatus* isolates. Through this extensive investigation lopinavir was identified to work with powerful synergy when in tandem with itraconazole and posaconazole against *A. fumigatus* isolates. Lopinavir demonstrated synergistic relationships with itraconazole against 16 *A. fumigatus* isolates and 4 *Aspergillus species*, *niger, flavus* and *brasiliensis;* ΣFICI values were between 0.188 and 0.375. An indifference effect was noted, with an ΣFICI ranging from 0.53 to 1.125. Surprisingly, once lopinavir was in growth media with posaconazole, effective synergistic outcomes were determined to be present in 22 isolates, their ΣFICI range was 0.091 to 0.188; an indifference effect was noted in 3 azole-resistant isolates, 0.53 to 0.62. Furthermore, lopinavir has shown itself capable of re-sensitizing azole-resistant *A. fumigatus* isolates, 731 and 733 from the CDC. These results generate novel routes for further investigation and tenable translatable treatments that can drastically reduced the azole payload necessary to remove *Aspergillus fumigatus* from an infected host.

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**EFFECTS OF CORAL PATHOGEN SECONDARY METABOLITES ON CORAL MICROBIOME HEALTH**

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Coral reefs are a critical ecosystem to the health and biodiversity of the Earth.[1] Unfortunately, numerous coral diseases have ravaged reefs in recent years, particularly in the Caribbean, wiping out over 80% of coral cover in 40 years.[2] These diseases are greatly exacerbated by ocean warming and acidification, dredging, agricultural runoff, tourism, loss of critical herbivores like sea urchins,[3] and other factors.[4] Although most diseases appeared several decades ago, scientists have yet to fully understand the pathogenicity of many of them, how the diseases progress, and the underlying chemical interplay causing the coral demise. It is suggested that these diseases do not follow a one-disease-one-pathogen model, but rather progress through disruption of the microbiome,[5] particularly the endosymbiotic dinoflagellate critical to the coral's health and survival.[6] This research project aims to understand the chemical exchanges between microbial pathogens and the coral microbiome that leads to dysbiosis. It has been found that the pathogen *Vibrio coralliilyticus* exhibits antibacterial activity against multiple coral symbiotic bacteria. Additionally, metabolite extracts from both pathogens and symbiotic bacteria have been shown to upregulate metabolites produced by coral's dinoflagellate, and metabolites of symbiotic bacteria also exhibited algistatic activity. These results indicate that the cascade of coral disease progression may begin with a pathogen disrupting the bacterial microbiome, inducing production of metabolites harmful to the dinoflagellate, which ultimately leads to coral tissue necrosis.

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The authors acknowledge the National Science Foundation Graduate Research Fellowship Program and the Department of Chemistry for funding that made this project possible.

# 12/15-LOX INHIBITION REVERSES NSAID-UNRESPONSIVE NEUROPATHIC PAIN STATES

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Chemotherapy-induced peripheral neuropathy (CIPN) elicited by therapeutic doses of anti-neoplastic drugs (*e.g.*, paclitaxel) manifests in nearly 70% of patients in the first month of cancer treatment. Painful neuropathy (tactile and cold allodynia) often persists long after chemotherapy is discontinued, substantially impacting patient quality of life. However, there are no FDA-approved treatments indicated for CIPN *per se*; off-label usage of the anti-depressant duloxetine offers limited relief, while opioids are associated with substantial risks including addiction. Thus, developing effective drugs for CIPN that are not addictive and do not interfere with the anti-tumorigenic effects of chemotherapeutics remains a critical unmet need for cancer patients. The acute to chronic transition of pain of CIPN is dependent in part upon activation of spinal Toll-like Receptor 4 (TLR4). This process is modeled in rodents by spinal (intrathecal, IT) delivery of the TLR4 agonist lipopolysaccharide (LPS), which dose-dependently elicits neuropathic-like pain hypersensitivity in both sexes in the absence of peripheral inflammation. Lipidomics analysis of lumbar spinal cord following IT LPS revealed significant increases in cyclooxygenase-mediated production of prostaglandins (PGs) and in metabolites of 12/15-Lipoxygenase (12/15-LOX) enzymes (12/15-LMs). Nonetheless, IT LPS-induced pain behaviors are not attenuated by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) at analgesic doses that inhibit spinal $PGE_2$ release. Notably, systemic delivery of *in vivo* active, CNS-permeant inhibitors of 12-LOX (ML-355, 30 mg/kg i.p.) or 15-LOX-1 (ML-351, 30 mg/kg i.p.) both prevent and reverse tactile and cold allodynia in mice following IT LPS. Similarly, ML-355 and ML-351 also reverse CIPN-induced allodynia. Accordingly, selective drugs inhibiting 12/15-LOX activity with minimal abuse liability may be useful in mitigation of chronic pain states that are refractory to treatment with NSAIDs.

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**Acknowledgement**

NIDA R01 DA035865 to MWB, NIAMS R01 AR075241 to AMG, NINDS R01 NS099338 and NIDA R01 DA002110 to TLY

**LPAR2 IS THE HIGH AFFINITY RECEPTOR FOR CART II NEUROPEPTIDE**

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Cocaine- and amphetamine-regulated transcript (CART) prepropeptide (CARTpt) has been implicated in multiple pathologies including obesity, nociception, mood disorders, diabetes mellitus, and drug addiction. However, the absence of validated receptor targets has hampered mechanistic interrogation of the two principal peptides, CART I (CART 55-102) and CART II (CART 62-102). Recently, the G-protein coupled receptor (GPCR) GPR160 was identified as the common target for both CART I and CART II. Using advanced high-throughput screening (PRESTO-Tango) and secondary functional assays (TRUPATH), we established that human Lysophosphatidic Acid Receptor 2 (LPAR2) is the high affinity GPCR for CART II and that GPR160 is a receptor for CART I (but not for CART II). Knockdown of endogenous LPAR2 in a native rat cell line (PC-12) attenuated both LPA- and CART II-induced increases in cell viability. Finally, using genetic (LPAR2 knockout mice) and pharmacological (LPAR2 antagonists H2L5186303 and LPA2 Antagonist 1) tools, we confirmed that LPAR2 mediates the anorexic and analgesic effects of CART II, but not of CART I *in vivo*. Collectively, these results suggest that the behavioral actions of CARTpt-derived neuropeptides are mediated by at least two GPCRs. Furthermore, the cross-species translatability of CART II-mediated actions at LPAR2 and potential relevance in multiple metabolic and neurological disease states justify progression to an early stage drug discovery campaign for novel biased therapeutics targeting LPAR2.

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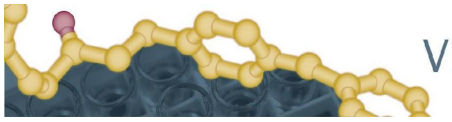
**Funding:**
NIDA grant DA035865 to MWB
NIAMS grant AR075241 to AMG
VTCDD seed grant to MWB and AMG (2021)



Virginia Tech Center for **Drug Discovery**

# IMPROVING POTENCY AND ORAL BIOAVAILABILITY OF SPNS2 INHIBITORS *VIA* TAIL MODIFICATIONS

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The sphingosine 1-phosphate (S1P) signaling pathway has caught the attention of the pharmaceutical industry in the last decade during which four drugs targeting the pathway received FDA approval for the treatment of relapsing-remitting multiple sclerosis. These drugs act through G-protein coupled receptors $S1P_{1\text{-}5}$ and are now called S1P modulators that all exhibit adverse on-target cardiovascular side effects.[1] An alternative therapeutic target within the S1P signaling pathway is the S1P transporter enzyme Spinster homologue 2 (Spns2), which has recently become an attractive target for the treatment of other autoimmune diseases.[2, 3] Namely, the inhibition of Spns2 has proven to mitigate the progression of renal fibrosis in mice.[4] Our laboratories performed a structure-activity relationship study and discovered **SLF80821178** bearing a decyl tail, a potent Spns2 inhibitor with an $IC_{50}$ of 92 ± 3 nM, which induces lymphopenia *in vivo* following IP administration (a hallmark of Spns2 inhibition) yet failed to elicit a similar response following PO administration, suggesting poor oral bioavailability. In this work, significant modifications were installed to the saturated aliphatic tail, yielding several examples of more potent and orally bioavailable small molecule inhibitors of Spns2.

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**Acknowledgement**
We thank NIH for funding (R01 AI144026).

# DISCOVERY OF TWO INHIBITORS OF THE TYPE IV PILUS ASSEMBLY ATPASE PilB AS POTENTIAL ANTIVIRULENCE COMPOUNDS

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With the pressing antibiotic resistance pandemic, antivirulence has been increasingly explored as an alternative strategy against bacterial infections. The bacterial type IV pilus (T4P) is a well-documented virulence factor and attractive target for small molecules for antivirulence purposes. The PilB ATPase is essential for T4P biogenesis because it catalyzes the assembly of monomeric pilins into the polymeric pilus filament. Here, we describe the identification of two PilB inhibitors by a high throughput screen (HTS) *in vitro* and their validation as effective inhibitors of T4P assembly *in vivo*. We used *Chloracidobacterium thermophilum* PilB (*Ct*PilB) as a model enzyme to optimize an ATPase assay for the HTS of a library of 2,320 compounds. Benserazide and levodopa, two widely used drugs for the treatment of Parkinson's disease, were identified and confirmed to be PilB inhibitors biochemically. We demonstrate that both compounds inhibited the T4P-dependent motility and T4P assembly of the bacterium *Myxococcus xanthus.* Additionally, benserazide and levodopa were shown to inhibit the T4P-mediated motility and biofilm formation in *Acinetobacter* spp. These results suggest that these two compounds are effective against the PilB protein *in vivo.* The potency of benserazide and levodopa as PilB inhibitors both *in vitro* and *in vivo* demonstrates the potential of the HTS and its two hits here for the development of anti-T4P chemotherapeutics.

This work was supported by the NSF and the Fralin Life Science Institute.

**HOPE ON THE HORIZON: HIV PROTEASE INHIBITOR, ATAZANAVIR OVERCOMES AZOLE RESISTANCE IN *CANDIDA AURIS* INFECTION**

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*Candida auris* represents an urgent public health threat that has been linked to numerous outbreaks around the world and is associated with a significantly high mortality rate. Therapeutic options are currently limited to 3 main classes of antifungals (azoles, polyenes, and echinocandins) to treat *C. auris* infections. The limited treatment options and the upsurge of drug resistance in *C. auris*, prompted us to evaluate a library of FDA-approved drugs for their ability to restore the anti-*Candida* activity of azole antifungal agents. We identified the HIV protease inhibitor atazanavir, as a co-drug that can overcome azole resistance in *C. auris*. Atazanavir displayed a remarkable *in vitro* synergistic activity with itraconazole against 19/19 *C. auris* isolates with a fractional inhibitory concentration index (ΣFICI) that ranged from 0.09 to 0.38. Moreover, atazanavir restored the fungistatic activity of itraconazole against *C. auris* in an *in vitro* time-kill assay. Mechanistic studies revealed that atazanavir significantly interfered with *C. auris* efflux pumps which resulted in an increase in the Nile red fluorescence by ~50%. Additionally, atazanavir inhibited glucose transport and ATP synthesis, which caused the glucose utilization and ATP content in *C. auris* to decrease by 30% and 20%, respectively. When evaluated in a mouse model of disseminated candidiasis, the combination of atazanavir/itraconazole, along with ritonavir that serves as a bioavailability booster, significantly reduced *C. auris'* burden in murine kidneys, generating a 1.15-$\log_{10}$ colony forming unit (CFU) (~93%) reduction. Altogether, the data indicate that atazanavir is a potent azole chemo-sensitizing agent that merits further investigation.

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We thank the CDC and Westerdijk Fungal Biodiversity Institute for providing us with the *C. auris* isolates used in this study.

# INVESTIGATING MATURATION OF IPSC-HEPATOCYTE-LIKE CELLS FOR HEPATOTOXICITY STUDIES

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Approximately 23% of Americans take five or more prescription drugs daily[1]. Adverse reactions to medications can cause drug-induced liver injury (DILI), which is the leading cause of acute liver failure in the U.S. Majority of DILI cases are idiosyncratic, where responses to medications vary amongst individuals.

Currently, *in vitro* studies ideally use primary human hepatocytes (PHHs). However, these cells are difficult to obtain. Induced pluripotent stem cell-hepatocyte like cells (iHLCs) exhibit significant potential as a more sustainable source for hepatotoxicity studies. iHLCs are retrieved non-invasively from skin cells and maintain host genotype, making them ideal for patient-specific studies[2]. Although, widespread use is limited due to their fetal phenotypic characteristics.

Therefore, our goal was to investigate whether interactions with hepatic non-parenchymal cells (NPCs) may improve liver functions in iHLCs[3]. We have assembled 3D liver organoids with Kupffer cells (KCs) and liver sinusoidal endothelial cells (LSECs), both NPCs, that recapitulate the *in vivo* hepatic cell ratios, environment, and architecture. This method can provide a more systematic, repeatable, and reproducible process for maturing iHLCs that could be used more consistently across the liver research community.

Briefly, iHLCs (**i**) or PHHs (**p**) are seeded on Type 1 collagen hydrogels. To assemble organoids, liver sinusoidal endothelial cells (LSECs) and Kupffer cells (KCs) are encapsulated in hydrogels of collagen and 1% (v/v) fibronectin on top (referred to as **i**3DHLK or **p**3DHLK). iHLCs cultured in a collagen sandwich serves as the control.

**i**3DHLK models exhibited a ~15% and ~18% increase in intracellular albumin and decrease in hepatic nuclear factor-4α expression after 7 and 14 days post-organoid assembly compared to control, indicating that iHLCs have matured with NPCs and time. Similar albumin expression is observed in **p**3DHLK organoids after 7 days post-organoid assembly. Expression of mature markers including CYP3A4 and CYP2E1 increase in **i**3DHLK models, comparable to **p**3DHLK organoids. An indirect enzyme activity analysis revealed that **i**3DHLK cultures after 7 days post-organoid assembly exhibited ~19% increase in apoptosis compared to untreated samples and ~70% live cells, respectfully, after administering 160 mM ethanol 24 hours prior. However, **p**3DHLK organoids exhibited ~50% live cells.

**i**3DHLK organoids exhibit increased hepatic mature markers and function compared to monocultures. Implementation of these organoids in the liver research community could serve as a more reproducible method to mature iHLCs. Quantification of the signaling factors secreted by the NPCs, including hepatocyte growth factor, is ongoing. Future work will include adding hepatic stellate cells (HSCs), another NPC, to further recapitulate the liver environment.

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**Acknowledgement**
We thank the NSF CCF-2200045, Virginia Tech Institute of Critical Technology and Applied Science, and the Computational Tissue Engineering Interdisciplinary Program.

**DETECTION OF MATRIX METALLOPROTEASES' ACTIVITY FOR CANCER THERANOSTICS**

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The tumor microenvironment plays a critical role in the in the development and the progression of cancer.[1] During carcinogenesis, the extracellular matrix (ECM) is remodeled in a way that favors the growth of the tumor and metastasis. Matrix metalloproteases (MMPs) are a group of enzymes that degrade the ECM and that have been reported to participate in many tumorigenic processes, such as the epithelial to mesenchymal transition (EMT), angiogenesis, and cancer cell intravasation and extravasation.[2] MMPs are overexpressed by most cancers and are found in very high concentrations in the tumor microenvironment, making them a promising cancer biomarker and a potential therapeutic target.[3,4] However, MMPs are diffused into the blood in very low concentrations, and the detection of its activity for cancer diagnostics requires a highly sensitive biosensor.[5] In this study, we analyzed the in-vitro activity of the matrix metalloproteases produced by colorectal cancer cells and by pancreatic cancer cells through zymography experiments.[6] We also designed a synthetic biomarker module that contains a substrate that is able to interact with matrix metalloproteases, resulting in the release of a reporter that can be measured with a highly sensitive carbon nanotube biosensor. By measuring the concentration of the reporter, the activity of the matrix metalloproteases in biofluids can be measured, which serves as a cancer indicator. Lastly, MMP inhibitors can potentially be attached to the synthetic biomarker module to build an *in vivo* cancer theranostic agent.

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This project was supported by the Luther and Alice Hamlett Undergraduate Research Support Award and by the Integrated Cellular Responses Laboratory.

**A LARGE SCREEN IDENTIFIES ANTIBIOTICS WHICH CAN BE REPURPOSED TO TARGET THE SYPHILIS AGENT AT LOW-NANOMOLAR CONCENTRATIONS**

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Since its discovery, penicillin has been the primary treatment option for syphilis, caused by the spirochete *Treponema pallidum*. Its efficacy against the pathogen is significantly greater compared to doxycycline, the only other viable treatment option thus far. Due to previous inability to culture *T. pallidum in vitro*, efforts to identify compounds that rival the efficacy of penicillin have been hindered. With up to 10% of the global population reporting penicillin allergies, global penicillin shortages, and growing concern about antibiotic resistance, alternative treatment options are needed. In this study a novel drug screen of almost 100 B-lactams was performed to determine their efficacy against *T. pallidum in vitro*. A multiphase, iterative approach of manual enumeration and qRT-PCR was used to identify a small subset of high performing compounds. The top 10% of compounds were further evaluated to determine their *in vitro* minimum inhibitory concentration (MIC). Finally, we used a fluorescent D-amino acid to visualize peptidoglycan synthesis in drug treated and untreated controls to validate the efficacy of the antibiotics on a cellular level. We identified multiple B-lactams with similar or lower *in vitro* MICs compared to benzathine penicillin G, the current standard of care. Of note, was nafcillin which had an *in vitro* MIC of 0.553 ng/mL and had several additional promising characteristics such as a long half-life, low cost:efficacy ratio, and its current use in clinical settings treating penicillin resistant bacteria. Additionally, we determined that *T. pallidum* incorporates peptidoglycan ubiquitously across the sacculus indicating a lateral mechanism of growth. All of the top performing antibiotics caused a decrease in peptidoglycan remodeling and growth. This is the first major drug screen conducted for the syphilis agent and was successful in identifying several potential therapeutics for future clinical investigation. In addition, this work provides new insights into *T. pallidum* peptidoglycan biology.

**EXPLOITING VIRUS-HOST INTERACTIONS TO DEVELOP NOVEL INHIBITORS AGAINST VENEZUELAN EQUINE ENCEPHALITIS VIRUS**

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Venezuelan equine encephalitis virus (VEEV) is a mosquito-borne, positive sense, single-stranded RNA virus that belongs to the genus *Alphavirus*. VEEV can infect both equines and humans, with associated neurological complications in ~14% of human cases. Due to its low infectious dose, ease of aerosolization and manipulation, this virus is classified as a select agent by both the CDC and USDA. However, there are currently no FDA-approved therapeutics or licensed vaccines against VEEV infection in humans. The VEEV capsid protein is an essential virulence factor of VEEV. The capsid protein can simultaneously bind to the host's nuclear import receptors, importin α/β1, and the host export receptor, CRM1 to form a tetrameric complex. This complex accumulates at the nuclear pore channel, halting nucleocytoplasmic trafficking, downregulating host transcription and inhibiting cellular antiviral response. Moreover, VEEV TC83 Cm, with a mutated non-functional nuclear localization sequence within the capsid, failed to downregulate cellular transcription and antiviral response. This suggests that the nuclear import of VEEV capsid is pertinent for pathogenesis and could be exploited as an attractive target for therapeutic development. We hypothesized that small molecule inhibitors capable of disrupting the interaction of capsid with importin α/β1 should increase cellular antiviral response, resulting in reduced viral titers and rescue of cells from VEEV-induced cell death. Two small molecule inhibitors, **I2** and **1564**, were designed to disrupt the interaction between capsid and importin α. These inhibitors were well tolerated by HMC3 microglia cells with $CC_{50}$ of >250 µM and >500 µM for **I2** and **1564**, respectively. These compounds impacted VEEV TC83 titer with >1 $\log_{10}$ decrease at 9 hpi. Furthermore, **I2** displayed an $EC_{50}$ of 2.96 µM and **1564** an $EC_{50}$ of 5.38 µM against VEEV. Both compounds also rescued infected cells from VEEV-induced cell death. In order to evaluate the impact of these compounds on the capsid-importin α interaction, we cloned two viruses that contain a V5 tag at the N-terminus of the capsid, TC83 V5-C and TC83 V5-Cm. The replication kinetics of these new viruses were similar to that of parental TC83 and TC83 Cm. Future studies will involve evaluating the impact of these compounds on capsid-importin interaction and capsid localization using co-immunoprecipitation and confocal microscopy.

We thank the NIAID, NIH (funding no: 419331), VMCVM Office of Research and Graduate Studies, and Fralin Life Science Institute for funding.

# *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF STREPTOMYCIN-RESISTANT MUTANT STRAINS OF *NEISSERIA GONORRHOEAE*

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Gonorrhea, a sexually transmitted disease caused by *Neisseria gonorrhoeae*, is the second most common sexually transmitted bacterial infection in the United States. The increasing prevalence of *N. gonorrhoeae* infections has been due to the emergence of antimicrobial-resistant strains. Most seriously, this uprising resistance to all classes of antibiotics could lead to a future with untreatable gonorrhea. Thus, the development of novel anti-*N. gonorrhoeae* drugs is urgently needed. *N. gonorrhoeae* FA1090 is the only strain reported to be used for *in vivo* mouse models because of its natural resistance to streptomycin. Streptomycin is a necessary antibiotic utilized in the mouse model to inhibit the commensal flora in the lower genital tract of mice to enhance *N. gonorrhoeae* colonization. However, this strain is susceptible to all antibiotics used to treat gonorrhea, and therefore, it is not suitable for drug discovery. To test the efficacy of new therapeutics against clinically important *N. gonorrhoeae* isolates, such as ceftriaxone-resistant and azithromycin-resistant strains *in vivo*, streptomycin resistance is a required phenotype for performing the *in vivo* mouse model. Thus, there is a requirement to develop *N. gonorrhoeae* strains that are simultaneously resistant to streptomycin as well as standard-of-care antibiotics, azithromycin and ceftriaxone. In this study, using allelic-exchange procedures, we constructed a *N. gonorrhoeae* mutant that is resistant to both streptomycin and azithromycin, and another *N. gonorrhoeae* mutant that is resistant to both streptomycin and ceftriaxone. The minimum inhibitory concentrations of standard antibiotics were determined against the newly constructed strains compared to their wild-type strains. When used in *N. gonorrhoeae* genital tract infection mouse model, mice were colonized with the new mutants for 14 days similar to *N. gonorrhoeae* FA1090. Overall, our results indicate that the newly constructed mutants are suitable to be utilized in the *N. gonorrhoeae* infection mouse models for drug discovery studies.

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**Acknowledgement**

Our thanks go to NIH and the VT College of Veterinary Medicine for providing the financial and logistic support. Our thanks also go to the CDC and WHO for providing the *N. gonorrhoeae* strains.

**TOTAL SYNTHESIS OF THERMORUBIN UTILIZING HAUSER-TYPE ANNULATIONS**

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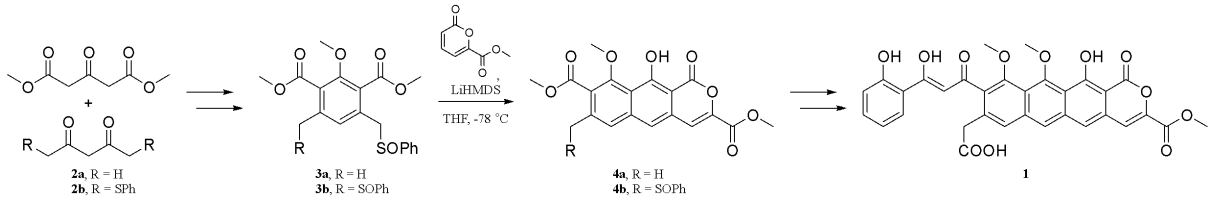
In order to overcome the growing problem of antibiotic resistance in hospital infections and clinical settings, new drugs must be discovered and tested. Previously discovered antibiotics warrant further investigation as a source of undeveloped drugs, as these molecules are already active against their targets. Thermorubin (**1**) is such an example, as it was first isolated in 1963 but has only seen a handful of derivatization tactics. It shows promising activity against Gram-positive and some Gram-negative strains of bacteria, although its oral bioavailability has to be improved before it could become a drug. Obtaining thermorubin for derivatization through a synthetic route will open the doorway to synthetic derivatives that will enable us to explore how to make compounds that are more effective than the initial product. For the proposed total synthesis,formation of the tetracyclic core is criticial and the key annulation steps are highlighted below. Utilizing either acetylacetone **2a** or its bis-phenyl sulfoxide (**2b**) and the dimethyl 3-oxopentanedioate, the resulting cyclized intermediates **3a** or **3b** will be reacted with the methyl-6-pyrone ester to create **4a** or **4b**, which will be subsequently converted to thermorubin.




**2a**, R = H
**2b**, R = SPh

**3a**, R = H
**3b**, R = SOPh

**4a**, R = H
**4b**, R = SOPh

**1**

# BIOASSAY-GUIDED ISOLATION OF PSEUDOCHELIN A FROM MARINE EGG MASS MICROBIOME

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Biofilms have been shown to represent the predominant form of existence of 40-80% of all bacterial cells.[1] Biofilms are a phenotype expressed during quorum sensing and has been described as surface-adhered communities of sessile bacteria that are enclosed in a matrix of extracellular polymeric substance (EPS).[2] In the biofilm state, bacteria are protected against antibiotics and the host's immune system. Biofilms commonly develop on medical devices in immunocompromised patients which leads to persistent infections that are difficult to treat.[3] Biofilms are responsible for infections such as cystic fibrosis and account for more than 65% of all bacterial infections and 80% of all chronic infections. Currently, efforts to target biofilms are focused on developing non-toxic compounds that do not threaten the viability of target pathogens but inhibit the formation of biofilms or disrupt already-formed biofilms. The goal of these methods is to have the pathogens remain in the planktonic state so they can be treated with low doses of antibiotics.[4]

In my research, I am using an ecology-based approach to study the underexplored marine egg mass microbiome for specialized metabolites that can be leveraged for the discovery of antibiofilm compounds. I have screened our library of >700 semi-crude bacterial fractions from moon snail egg masses for biofilm inhibition against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Biofilm formation in *P. aeruginosa* was inhibited by twenty-two fractions representing a hit rate of ~3% and thirty-eight fractions inhibited biofilm formation in *S. aureus*, representing a ~5% hit rate. Through bioassay-guided isolations, I have identified pseudochelin A, a compound that inhibits ~82% of biofilm formation in *S. aureus*. Identification of the possible mechanism of action of pseudochelin A is an on-going effort.

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NIH National Institute of General Medical Sciences

**REPURPOSING THE ANTI-INFLAMMATORY FDA-APPROVED DRUG, AURANOFIN, FOR COMBATING *NEISSERIA GONORRHOEAE***

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*Neisseria gonorrhoeae*, the second most common bacterial cause of sexually transmitted infections, is listed as an urgent-threat pathogen by the Centers for Disease Control and Prevention (CDC). Due to the growing prevalence of resistance development against the first-line treatment and several classes of antibiotics, the discovery of new anti-gonorrheal therapeutics is an urgent need. Drug repurposing significantly reduces the time and expense associated with traditional drug development. Herein, utilizing a drug repurposing approach, we screened 3,802 FDA-approved and clinical drugs against *N. gonorrhoeae* FA1090. A total of 14 novel non-antibiotic compounds were identified in the screening with significant anti-gonococcal activity. Auranofin, an FDA-approved anti-rheumatoid arthritis drug, was selected for further investigation due to its potent activity. A time-kill kinetics assay revealed that auranofin exhibited rapid bactericidal activity *in vitro* against *N. gonorrhoeae*, outperforming the drug of choice, azithromycin. Moreover, auranofin reduced the *N. gonorrhoeae* burden in a female murine model of vaginal infection by 91% and 96% after three and five days of treatment, respectively. In conclusion, our results indicate that auranofin merits further investigation for development as a future anti-gonorrheal therapeutic to replenish the dry pipeline of anti-gonorrhea medications.

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**EXPLORING THE DYNAMIC LANDSCAPE OF THE NS2B/NS3 PROTEASE IN THE DENGUE VIRUS USING ALL-ATOM MOLECULAR DYNAMICS SIMULATIONS**

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Positive-sense RNA viruses have a high rate of genetic recombination, which poses a significant challenge to drug discovery efforts *(1)*. Positive-sense RNA viruses including Dengue and Chikungunya viruses are global health threats that highlight a need for more efficient drug discovery methods, as well as the development of broad-spectrum antivirals to withstand the elevated rate of mutations. The integration of novel methodologies to functionalize and repurpose compounds with computational methods including virtual screening have been shown to increase the efficiency of the drug design process. Refining and elevating these screening methods and algorithms also requires a diverse set of structures to screen against to increase the potential success of computational hits. Proteases have emerged as a promising target for broad-spectrum antivirals due to their highly conserved catalytic site and crucial involvement in the replication and pathogenicity of RNA viruses *(2)*. Considering the deficiency of structural data for these proteases, this study aims to investigate the efficacy of molecular-dynamics-generated structures for drug design. Molecular dynamics simulations performed on the crystal structure of the NS2B/NS3 Dengue virus protease complex (PDB ID 2FOM) to highlight the dynamic behavior of the NS2B cofactor. During simulation, the NS2B cofactor did not transition to its active conformation, limiting the set of conformers for subsequent screening and mechanistic insight. Root-mean-square fluctuations (RMSF) for each individual residue reveal an important dynamic area in the protease involving the residues present in the two β-hairpins of the NS3 protein proximal to the catalytic residues where the cofactor should hypothetically anchor itself between in an active conformation. Future directions include molecular dynamics simulations with benzoyl-norleucine-Lys-Arg-Arg-aldehyde, a covalently bound peptide, to elucidate the mechanism of action of the NS2B/NS3 protease complex.

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*The authors acknowledge support from the Virginia Tech ICTAS Junior Faculty Award and computational resources from Virginia Tech Advanced Research Computing.*

**IDENTIFICATION OF REGULATORY TARGET SITES WITHIN THE NEGATIVE ARM OF THE CIRCADIAN CLOCK**

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The core clock component Period 2 (PER2) regulates the transcriptional activity and stability of the tumor suppressor p53 as well as the p53-mediated DNA damage response to genotoxic stress. In this study, we investigate the relevance of target phosphorylation sites in PER2 for their regulation in its binding to p53 and the PER2's counterpart CRY1. NIH3T3 cells were transfected with each of the indicated Myc-tagged PER2mutants (PER2mut), circadian synchronized with dexamethasone, treated with (or without controls) ionizing radiation, and monitored for ectopic expression of PER2mut by immunofluorescence microscopy. Images confirmed changes in PER2mut localization compared to PER2's wild-type in response to ionizing radiation. Expression of downstream genes of p53 was quantified using RT-qPCR and confirmed expression of cell cycle inhibitors. There were no changes in the expression of CCGs. Co-immunoprecipitation results showed PER2mut bind CRY1 in agreement with RT-qPCR data. Information regarding PER2's phosphorylation sites and their impact on binding behavior with p53 and CRY1 can identify possible targeting sites for future therapeutics correcting disrupted p53-mediated DNA damage response and steady CCG expression, respectively.

# SYNTHESIZING A LIBRARY OF HYPERBRANCHED GLYCOPOLYMERS VIA RAFT POLYMERIZATION FOR HUMAN NOROVIRUS INHIBITION

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With nearly 700 million infections annually, human norovirus (HNV) presents a potent global health burden that has yet to be properly addressed. Research into vaccines and antiviral compounds have not yielded any medications capable of suppressing viral infection, requiring a need for alternative approaches. Antiviral polymers may be able to fill that gap. Taking inspiration from nature and cell-virus interactions, we aim to decorate a polymer's surface with glycan moieties that HNV commonly utilizes for infection. Modified sialic acids (SAs) are chemically synthesized and armed with an acrylamide linker extending from the C2 position. Histo-blood group antigens (HBGAs), possessing a more complex structure, are synthesized via automated glycan assembly. Hyperbranched polymers are synthesized via reversible addition-fragmentation chain-transfer (RAFT) polymerization using either a tetrafluorophenyl 4-vinylbenzene sulfonate or tetrafluorophenyl acrylate monomer. To better elucidate our material's antiviral efficacy, structure-property relationships are investigated through the synthesis of a polymer library: molecular weight, degree of branching, and the hydrophobicity/hydrophilicity of the material's backbone are all systematically altered. The presence of terminal trithiocarbonate functional groups on the hyperbranched structures allows for the controlled insertion of glycans (SA or HBGAs) onto the terminal ends of polymers. Antiviral inhibition will be measured using hemagglutination assays.

This work was supported by GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement DMR-1933525.

**EXPLORING THE BIOSYNTHETIC POTENTIAL OF THE MILLIPEDES ISCHNOCYBE PLICATA**

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Millipedes consist of a diverse class (Diplopoda) of arthropods that are distributed worldwide and are a promising source of novel small molecules.[1] Millipedes have evolved repugnatorial glands that contain high concentrations of specialized small molecules that are predicted to have a role in chemical defense. The repugnatorial glands are capable of squirting these fluids over a distance of several inches when a potential hazard is identified.[2] *Ischnocybe plicata* (Platydesmida; Andrognathidae) is a monotypic millipede species of the Pacific Northwestern United States and feed in aggregations on fungus. When disturbed, *I. plicata* exudes a pine oil or citrus scent, suggestive of terpene production. This ecological observation and the lack of chemical studies on *I. plicata* led us to investigate the specialized metabolites produced by these millipedes. Approximately 300 *I. plicata* adults were collected in down woody debris in coniferous and mixed hardwood forests in Oregon. Extraction and chemical evaluation led to the identification of four new oxidized alkaloids – ischnocybine A-C and ischnocybinone. Full 2D NMR datasets ($^{1}$H, $^{13}$C, COSY, HSQC, H2BC, and HMBC) were acquired on each metabolite to elucidate their planar structures. The molecular formula, respectively, $C_{18}H_{29}NO_2$, $C_{18}H_{27}NO_3$, $C_{20}H_{31}NO_4$, and $C_{22}H_{37}NO_4$ were determined by high-resolution ESI-TOF mass spectrometry. The relative configuration of ischnocybine A-C and ischnocybinone were assigned using NMR and theoretical calculations. The metabolites showed ant deterrence activity in the ecological assay, causing paralysis in a couple of ants. This activity led us to evaluate the alkaloids in a variety of neurological receptor binding assays by the PDSP – Psychoactive Drug Screen Program and they showed promising neuromodulating activity.

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This work was funded by NIH [R35 GM146740] and Virginia Tech Startup funds.

# ENGINEERED PROBIOTIC YEAST STRAINS SECRETING ANTI-NOROVIRUS VHH TRANSIENTLY RESIDE IN THE GNOTOBIOTIC PIG GUT AND PRODUCE HIGH TITERS OF NANOBODIES

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Human norovirus (HuNV) is a leading cause of viral gastroenteritis, causing millions of illnesses per year. Despite the burden of disease, no specific therapeutics are available. Here, we evaluated the potential of engineered probiotic yeasts, *Saccharomyces boulardii* (*S. boulardii*) secreting nanobodies against HuNV, to reside in the intestines of gnotobiotic (Gn) pigs and produce nanobodies *in situ*. *S. boulardii* were engineered to constitutively produce either hetero-tetravalent M4M6 on the Fc of the human IgG1 or the monovalent nanobody M5. Beginning at 2 days of age, Gn pigs were orally inoculated twice daily with $10^9$ or $10^{10}$ colony forming units (CFU) of *S. boulardii* secreting M4M6 (Sb_M4M6-Fc), *S. boulardii* secreting M5 (Sb_M5), *S. boulardii* empty vector (Sb_EP, as control), or diluent (PBS) for 5 days. Rectal swabs were taken daily before the morning dose to determine titers of nanobodies shed in feces. At post-inoculation day (PID) 10, pigs were euthanized, and small and large intestinal contents were collected for determining yeast CFU and M4M6-Fc and M5 titers. Sections of the pig intestines were collected to detect M4M6-Fc and M5 in mucosal tissues. High titers of M4M6-Fc (up to 4096) and M5 (up to 256) were detected in the small intestinal contents of the engineered yeast-inoculated pigs, with higher titers corresponding with higher yeast doses. M4M6-Fc and M5 were also detected in the ileum, cecum, and colon mucosa of the engineered yeast-inoculated pigs. Pigs who received $10^{10}$ doses of yeast had higher yeast CFU counts isolated from intestinal contents. Based on these results, the $10^{10}$ dose was selected for the subsequent evaluation and the dose frequency was adjusted to once per day. Another litter of Gn pigs, divided into the same groups (n=3-4), was inoculated using this regime beginning at 5 days of age for 10 days. Rectal swabs were taken daily to evaluate fecal yeast shedding. At PID10, pigs were euthanized and small and large intestinal contents were collected for determining yeast CFU and the nanobody titers. Sb_M5 and Sb_EP were detected in fecal samples beginning at PID3 and Sb_M4M5-Fc was detected by PID4. The CFU counts in small and large intestinal contents were comparable across groups. Both M4M6-Fc and M5 titers were up to 256. The consistent nanobody production by the engineered *S. boulardii* strains indicates they are ready for efficacy testing against HuNV infection and disease in virus-challenge studies.

National Institute of Allergy and Infectious Disease, National Institutes of Health, R01AI148357 to L. Yuan and H.Feng.

**RIFT VALLEY FEVER VIRUS NSS PROTEIN INTERACTS WITH LC3 FAMILY MEMBERS TO MODULATE AUTOPHAGY**

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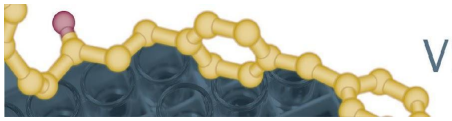
Rift Valley fever virus (RVFV) is a negative-sense RNA arbovirus (Phlebovirus genus, Phenuiviridae family, Bunyavirales order), endemic in sub-Saharan Africa, that infects both ruminants and humans. Transmission occurs via mosquitos, contact with blood or amniotic fluid of an infected animal, vertically from mother to offspring, or via virus-laden aerosols. Studies identified several competent RVFV vectors such as Cx. pipiens mosquitoes in the US and Europe. Impregnated ruminant infections are characterized by abortion storms and fetal malformations in which spontaneous abortion occurs in approximately 100% of cases. Ruminant disease is severe with mortality rates up to 100% in young ruminants and 30% in adults, perilously causing severe socio-economic impacts. In humans the infection is typically mild, with symptoms including headache, muscle pain, and fatigue. Ten percent of cases progress to a severe version of the disease that includes hemorrhagic fever or encephalitis. Despite its pathogenic potential and economic impact, there are no FDA approved therapeutics or vaccines to challenge the global spread of this infectious organism. The nonstructural small (NSs) protein is the main virulence factor of RVFV, making it an attractive antiviral target. We bioinformatically identified four potential LC3 interacting region motifs (LIR) in the RVFV NSs protein, suggesting that NSs may have polyvalent interactions with the host key autophagy protein LC3 to modulate the host autophagy systems. Autophagy is a homeostatic process in which cellular material is degraded and recycled and can be exploited by viruses to facilitate replication or can be antiviral. We hypothesize that RVFV NSs interacts with LC3-family members to inhibit antiviral autophagy, which could be therapeutically targeted. To determine whether NSs interacts with LC3-family proteins, peptides corresponding to each of the predicted LIRs were prepared and the dissociation constant for each of the six LC3-family members was determined using isothermal titration calorimetry (ITC) experiments. ITC experiments demonstrated that LIR4 interacts with similar affinity with all six LC3 proteins. Interaction of NSs with all human LC3-family members was additionally observed through co-immunoprecipitation in virally infected cells. RVFV NSs proteins and LC3 host proteins are found in both the nucleus and cytoplasm. LC3 is initially located in the nucleus and translocated to the cytoplasm upon autophagy initiation where it forms autophagosomes. Fractionation co-immunoprecipitation was preformed to determine NSs-LC3 interaction location; results showed a strong nuclear reaction. Confocal results demonstrated that NSs-LC3 colocalized in perinuclear and filamentous regions, interaction, and retention of LC3 in the nucleus further indicates that NSs is downregulating autophagy. LC3 subfamily members (A-C) are acetylated on two conserved lysine residues (K49 and K51), and acetylated LC3 proteins are retained in the nucleus, thereby down regulating autophagy. To determine the impact of acetylation of LC3 proteins on their interactions with NSs, plasmids expressing LC3A acetylation mimics were tested in cells. Results indicated that NSs preferentially interacts with de-acetylated LC3. Additionally, LC3 cellular assays demonstrated that NSs downregulates autophagy. Using Artificial intelligence, structural, and molecular studies, these results demonstrate that RVFV NSs inhibits antiviral autophagy through interaction with LC3. Competent mosquito vectors have been identified in the U.S and Europe leading to a high likelihood that RVFV will emerge in new locations or cause significant outbreaks. Thus, our research to understand the modulation of the conserved host autophagy pathway by RVFV NSs is critically important for development of therapeutics.

We thank the Biomedical and Veterinary Sciences Program, the Molecular and Cellular Biology Program, and the Center for Emerging, Zoonotic, and Arthropod-borne Pathogens at Virginia Polytechnic Institute and State University. This work was funded through Defense Threat Reduction Agency (DTRA) grant HDTRA1-22-1-0009 to KKH. Funders do not have any role in the design of the study and collection, analysis, and interpretation of data.



**CNS4 PREFERENTIALLY POTENTIATES HYPOGLUTAMATERGIC DISEASE-CAUSING MUTANT GLUN1/2A SUBTYPE OF NMDA RECEPTORS**

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The normal function of N-methyl D-aspartate receptors (NMDARs) plays a critical role in brain development and cognitive function. Children born with mutations in NMDAR subunits, that reduce glutamate potency, suffer from various neuropsychiatric disorders including epilepsy, major depression, developmental delay, and intellectual disability. Two disease-causing GluN2A mutants that are known to reduce glutamate potency to 4.4 (A548T) and 126 (V685G) fold were generated, sequenced and assayed using two-electrode voltage clamp electrophysiology technique. The results obtained from this experiment indicate that CNS4 significantly increased glutamate potency in A548T [(Glu EC50 in μM; 4.68 vs 1.93, p0.05] in the wild-type GluN1/2A receptors. These results reveal that CNS4 selectively modulates the hypoactive NMDARs. Previous findings demonstrated that CNS4 potentiated NMDAR currents disproportionately higher levels when activated by sub-saturating concentration of glutamate.

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**Acknowledgements**

Received FY 23 VCOM REAP-ICTAS grant. Received FY22 VCOM REAP grant.

# GENOME MINING OF BACTERIAL ISOLATES FROM MOON SNAIL EGG MASSES

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Natural products play an essential role in drug discovery and are responsible for over 60% of FDA approved drugs.[1] There are many sources of small molecules, most of them are related to the ecology of the producing organism. Moon snail egg masses are an understudied unique ecological niche. Studies on Hawaiian Bob tail squid egg masses have shown the presence of bacterial chemical defenses.[2] In recent years, the number of approved drugs, especially antibiotics, has substantially decreased making new techniques crucial to identify novel metabolites.[3] The establishment of genome mining has allowed the identification of new bacterial compounds in a faster and cheaper way. This work aims to use genome mining to identify uncharacterized natural product biosynthetic gene clusters from bacteria associated with moon snail egg masses collected in Florida and Puerto Rico. Initial genome mining work has identified a lanthipeptide gene cluster from a *Lysinibacillus* strain isolated from the egg masses. A lanthipeptide was chosen because they are excreted extracellularly and often exhibit antimicrobial properties. A knockout of the lanthipeptide gene cluster has allowed for presence/absence identification of the produced metabolite. Work is currently being done to isolate the lanthipeptide for structural characterization. Once the structure is has been determined, assays will be performed to determine the biological activity of the isolated lanthipeptide.

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**Acknowledgement**

We thank the national institutes of health (NIGMS) for funding.

# IDENTIFICATION AND CHARACTERIZATION OF ENZYMES IN THE THERMORUBIN BIOSYNTHETIC PATHWAY

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Thermorubin is a tetracyclic naphthoisocoumarin natural product produced by the *Laceyella sacchari* that shows great potential as an antibiotic however, the exact biosynthetic pathway and kinetics of its enzymes are not fully understood[1,2]. The tetracyclic structure is indicative of a type II polyketide synthase (PKS) module. While PKS enzymes are normally unstable at higher temperatures, initial testing has shown the PKS enzymes produced by the thermophile *L. sacchari* have shown remarkable heat stability along with rapid kinetics[1,3]. Previous in-depth bioinformatic analysis has tentatively identified the PKS genes in the biosynthetic module. Further experiments revealed that unlike other PKS modules which use acyl-CoA ester as a starter unit, this biosynthetic pathway uses a salicylic acid-CoA ester. Current work involves confirming the genes (*TheE*, *TheI TheG*, and *TheJ*) involved in the minimal PKS module along with determining the kinetics and stability of the associated enzymes.

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*This work was funded by Virginia Tech.*

# LANSOPRAZOLE POTENTIATES THE ANTIFUNGAL ACTIVITY OF AMPHOTERICIN B AGAINST MULTI-DRUG RESISTANT CANDIDA AURIS, TARGETING THE CYTOCHROME BC1 COMPLEX

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*Candida auris* has emerged as a problematic fungal pathogen associated with high morbidities and mortalities. Amphotericin B is the most effective and broad-spectrum antifungal agent used for treatment of invasive fungal candidiasis with extremely rare resistance among clinical isolates. However, the clinical efficacy of this drug has been impacted recently with the emergence of *C. auris* which possessed extraordinary resistant profile against all available antifungal drugs, including amphotericin B. There is an urgent need for novel antifungal agents or co-drugs capable of restoring/enhancing the antifungal activity of amphotericin B and reducing its toxicity. In this study, by screening a panel of ~3,400 FDA-approved drugs we identified the proton pump inhibitor, lansoprazole, as a potent enhancer for the activity of amphotericin B against *C. auris*. Lansoprazole exhibited potent synergistic interactions with amphotericin B against 18/20 (90%) *C. auris* isolates with ΣFICI ranged from 0.25 to 0.5. Proteome Integral Solubility Alteration (PISA) assay revealed that lansoprazole inhibits an essential target in the yeast cytochrome system (Rieske protein of the mitochondrial cytochrome bc1 complex) leading to increase in the oxidative stress in the fungal cells which consequently augment the oxidative damaging effect of amphotericin B on *C. auris* cells. The target was confirmed with the rotenone rescue assay and transcriptome sequencing (RNA-Seq) analysis. Most importantly, lansoprazole restored the *in vivo* efficacy of amphotericin B in an immunocompromised mouse model, resulting in a 1.7-log (~98%) CFU reduction in the kidney burden of *C. auris*. In conclusion, our results identified lansoprazole as a potent enhancer to the antifungal activity of amphotericin B in addition to identification of mitochondrial cytochrome bc1 as a novel drug target to overcome the antifungal resistance in *C. auris*.

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**Acknowledgement**

This work was supported by National Institute of Health Grant R01AI141439.

**DESIGN AND SYNTHESIS OF ANTI-INFLUENZA GLYCOPOLYPEPTIDES**

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Influenza is a family of highly contagious viruses. Current approaches to combat the viruses are limited by their frequent mutations of the viruses. Here, we are proposing to inhibit influenza viruses through an approach not constricted to specific virus strains—sialic acid appended synthetic polymers. These antiviral polymers utilize the polyvalent interactions between hemagglutinins (on the virus surface) and sialic acids (on the cell surface) to achieve virus inhibition. The merit of using synthetic polymers lies in their tunability. Previous research has looked into the impact of various polymer strutural parameters, but different conclusions were drawn as different polymers were used. In this research, we will investigate different parameters within the same system. We will systematically synthesize a library of sialic acid appended polypeptides to investigate how polymer structural parameters (i.e., sialic acid content, degree of polymerization, and polymer backbone conformation) covary with each other to inhibit the viruses. In the future, the antiviral efficacy of our sialic acid appended polypeptides will be tested *via* hemagglutinin inhibition assay and the results could later instruct us on designing antiviral polymers with maximum inhibition performance.

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This work was supported by GlycoMIP, a National Science Foundation Materials Innovation Platform funded through Cooperative Agreement DMR-1933525.

**BENZIMIDAZOLES AS SPINGOSINE-1-PHOSPHATE TRANSPORTER SPNS2 INHIBITORS**

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Sphingosine-1-phosphate (S1P) is a ubiquitous signaling molecule that is involved in multiple biological processes, including lymphocyte trafficking.[1] Sphingosine is phosphorylated by one of two kinases (SphK1 and SphK2) producing S1P. It can then be exported out of the cell by Spinster homolog 2 (Spns2) where S1P can interact with its G protein-coupled receptors $S1P_{1\text{-}5}$.[2] Its importance to lymphocyte trafficking and maintaining circulating S1P levels make Spns2 a valuable target as an immunosuppressant. Our lab recently discovered **SLB1122168** as a novel Spns2 inhibitor with an $IC_{50}$ of 94 ± 6 nM *in vitro*.[3] Administration of **SLB1122168** in mice *via* IP injection induces lymphopenia, which is a hallmark of Spns2 inhibition. However, it fails to elicit the same response when administered PO. In this study, we performed a structure-activity relationship study to test the importance of the benzoxazole ring. This study led to the discovery of **SLS2012201**, which replaces the benzoxazole ring with a benzimidazole and maintains the secondary amine headgroup. Testing of this compound *in vitro* showed an $IC_{50}$ of 332 ± 12 nM with activity seen in mice following oral administration. Our continued investigations will allow us to use small molecule inhibitors to better understand Spns2 and establish it as a viable drug target.

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**Acknowledgement**

We thank the NIH (R01 AI144026) for funding.

**A SEMISYNTHETIC APPROACH OF CHLORAMPHENICOL DERIVATIZATION**

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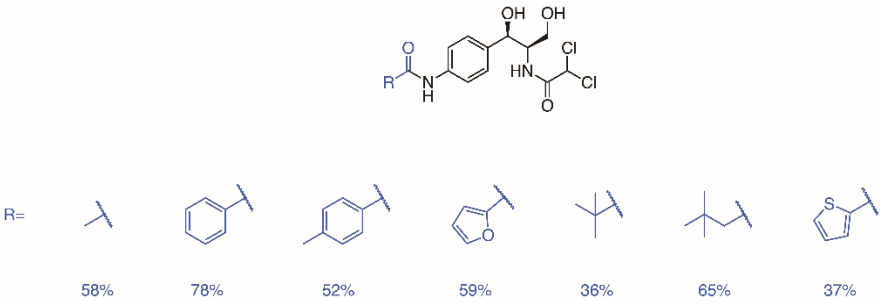
Chloramphenicol, a peptidyl transferase inhibitor, binds to the 50S ribosome and hinders protein synthesis. Among the different functional groups this molecule possesses, the p-nitro group is uncommon and a known toxicophoric, which may be responsible for the off-target effects of this antibiotic. Replacing the p-nitro groups has been a common strategy for Chloramphenicol derivatization, but these derivatizations have been done synthetically so far. To improve activity and lower toxicity, we are replacing the p-nitro group with an amide. We have synthesized novel amide derivatives by reducing the p-nitro group into aniline[1] and reacting it with several acyl chlorides. After creating a series of these derivatives, their activity and toxicity will be investigated. The most active and least toxic molecule will be subjected to further modification with a view to further improving the drug.

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# ACTIVATION OF CELL APOPTOSIS PATHWAY AS POTENTIAL ANTIVIRAL AGAINST NEUROVIRULENT VIRUSES

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Eastern and Venezuelan equine encephalitis virus (EEEV and VEEV) are new world alphaviruses that can cause mild flu-like illness which can progress into severe neurological deficits in both humans and equines.[1] The primary virulence factor of these viruses, the capsid protein has a variety of functions including binding to membrane glycoproteins, inhibiting transcription of host cells, and blocking nucleocytoplasmic transport.[2] Interestingly, the capsid protein of some other encephalitic viruses including West Nile virus has been demonstrated to mediate apoptosis through the p53/HDM2 cell apoptosis pathway.[3] The role of ubiquitination and other post-translational modifications in apoptosis specifically has only recently been highlighted as a contributing factor impacting viral proliferation in non-oncogenic viruses.[4] We hypothesize that HDM2 is a pro-viral factor which is activated during EEEV and VEEV infection with the goal of establishing HDM2 as a promising antiviral target against encephalitic alphavirus replication. Here, we investigated the antiviral potential of an HDM2 inhibitor, NVP-CGM097. Treatment of microglia cells (HMC3s) with NVP-CGM097 at nontoxic concentrations (>80% cell viability) resulted in up to 4 $\log_{10}$ reduction in viral titers of VEEV TC-83. Additional studies revealed that treatment of EEEV (FL93-939) infected microglia with NVP-CGM097 at nontoxic concentrations resulted in a >1$\log_{10}$ reduction of viral titers. LC-MS/MS analysis of capsid immunoprecipitated samples from VEEV infected cells identified p53 as a potential host interactor of VEEV capsid. Furthermore, Co-immunoprecipitation with a VEEV TC-83 V5 tagged capsid verified that there is an interaction between HDM2 and VEEV capsid protein as well as p53 and VEEV capsid protein. Further studies are aimed at determining the impact of VEEV capsid on HDM2 expression and the importance of HDM2 to viral proliferation. Ultimately, this study highlights the potential for HDM2 inhibition as an antiviral against encephalitic viruses.

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**Acknowledgements:**

We thank the Virginia Maryland College of Veterinary Medicine as well as Virginia Tech for funding and laboratory space. We also thank the Center for Emerging, Zoonotic, and Arthropod-Borne Pathogens at Virginia Tech for supporting our graduate students in their research exploration. Finally, we thank the Defense Threat Reduction Agency for funding and support. This work was funded through Defense Threat Reduction Agency (DTRA) grant HDTRA1-18-1-0040 to AN and KKH. The content of the information does not necessarily reflect the position or the policy of the federal government, and no official endorsement should be inferred.

# THE VIRGINIA TECH CENTER FOR DRUG DISCOVERY SCREENING LABORATORY

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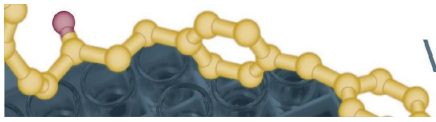
The Virginia Tech Center for Drug Discovery operates a laboratory for high-throughput screening that is available to Virginia Tech researchers. The facility can aid in adapting assays to a high-throughput format, running screening experiments, analyzing screening results, as well as provide letters of support for grant applications that involve screening. The lab is approved for BSL2-level experiments.

A number of compound libraries are available to researchers including a library of FDA-approved drugs, a unique transition-metal complex library developed by Dr. Joe Merola, a brominated-fragment library, a kinase-targeted library, two natural-products libraries, and libraries designed to maximize structural and chemical diversity. In total, the laboratory has over 43,000 compounds available for testing in biochemical and cell-based assays.

The laboratory instrumentation includes a robot capable of high-accuracy plate-to-plate transfers and plate replication, various liquid dispensers, and a plate washer that can be used on loosely-adherent cells and biofilms. Assay results can be determined using UV-Vis absorbance, fluorescence, time-resolved fluorescence, fluorescence polarization, and glow luminescence.

For more information about the laboratory or to discuss a screening project, contact the lab at 540-231-3525.

**Acknowledgments**: We thank the Fralin Life Sciences Institute at Virginia Tech, the Virginia Tech College of Science, and the Virginia Tech College of Agriculture and Life Sciences for financial support.



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