REVOLUTIONIZING BIOMEDICAL RESEARCH: INTEGRATING CUTTING-EDGE AI/ML TO UNLEASH INNOVATION IN DRUG DISCOVERY AND THERAPEUTICS DEVELOPMENT

Early-stage Investigation Of Small Molecule Inhibitors Targeting Eastern Equine Encephalitis Virus (EEV) And Western Equine Encephalitis Virus (WEEV) Non-structural Protein 2 (nsP2) Protease.

Victor Ogungbe The University of Alabama in Huntsville and Biomolecular Science LLC **Olawale Adeyinka** The University of Alabama in Huntsville **Damilohun Metibemu** The University of Alabama in Huntsville **Olamide Crown** The University of Alabama in Huntsville **Michael Barrera** George Mason University **Aarthi Narayanan** George Mason University

Emerging viral infections caused by mosquito-borne alphaviruses such as Venezuelan/Western/Eastern equine encephalitis (VEEV/WEEV/EEEV) are responsible for distinct emerging viral diseases that pose tremendous risks to public health systems globally including to warfighters. Alphaviruses cause life-threatening encephalitis and/or incapacitating acute and chronic arthritis. Alphaviruses have a single-stranded, positive-sense RNA genome that is ~11.4 kb in size and contains two open reading frames, one of which encodes the non-structural polyprotein (nsP) precursor nsP1234. The viral structural proteins, capsid protein (CP), small peptides (E3 and 6K), and the envelope glycoproteins (E1 and E2) are translated from the 26S subgenomic viral RNA. nsP2 regulates negative-strand RNA synthesis via its methyltransferase activity. It also facilitates the packaging of genomic RNA into virus particles. Proteolysis of nsP1234 by the virus-encoded cysteine protease nsP2, which has been shown to play essential roles in the virus lifecycle, yields the functional, viral non-structural proteins nsP1, nsP2, nsP3, and nsP4.

nsP2 protease is profoundly important to viral infectivity, replication, and host cell invasion. Because of its importance, the immediate focus of our work is to identify and advance small molecule inhibitors of WEEV and EEEV from discovery to preclinical investigations. Importantly, the nsP2 protease provide opportunities for targeting by small molecule therapeutics.

The methods currently applied to this work ranges from computational modeling, molecular and cell biology techniques, protein chemistry methods and enzyme kinetics, synthetic chemistry, in vitro and in vivo pharmacology methods.
Ongoing studies from our laboratory have identified inhibitors of the cysteine protease domain of WEEV and EEEV's non-structural protein 2 (nsP2) as promising scaffolds for antiviral development. The initial compounds in the series were found to have potent inhibitory activity against nsP2 and block the replication of VEEV and CHIKV as well. In addition, the initial hits were found to have promising ADME properties. Analogs of the initial hits were synthesized based on preditive homology modeling of the WEEV and EEEV's nsP2 structures.

Our current results provide structural insights into a new class of potent non-peptidic covalent inhibitors of nsP2 cysteine protease. Ongoing work is focused on structure-activity relationships to establish efficacy of key compounds. These studies may facilitate the evolution of the compounds into selective and broad-spectrum anti-alphaviral drug leads. We envision that nsP2 protease inhibitors can be used as countermeasures against several emerging/reemerging viral pathogens that threaten the warfighter and the civilian population.

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