BROAD-SPECTRUM THERAPEUTICS FOR VIRAL DISEASES: A MEDICAL COUNTERMEASURE PLATFORM FOR EMERGING THREATS

Investigation Of Non-structural Protein 2 (nsp2) Inhibitors As Therapeutics For Equine Encephalitis And Chikungunya Viral Infections

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Emerging viral infections caused by mosquito-borne alphaviruses such as Venezuelan/Western/Eastern equine encephalitis (VEEV/WEEV/EEEV) and Chikungunya virus (CHIKV) 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. Studies from several investigators have shown that 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. Therefore, nsP2 is profoundly important to viral infectivity, replication, and host cell invasion. Importantly, the nsPs provide opportunities for targeting by small molecule therapeutics.

Studies from our laboratory have identified inhibitors of the cysteine protease domain of VEEV's non-structural protein 2 (nsP2) and promising scaffolds against VEEV, WEEV, and CHIKV. The initial compounds in the series were found to have potent inhibitory activity against nsP2 and block the replication of VEEV, WEEV, CHIKV in infected cells in vitro. In addition, the initial hits were found to have promising but suboptimal ADME properties. Analogs of the initial hits were synthesized and evaluated against both viruses and found to be significantly more potent against CHIKV, have similar activity against VEEV, and have optimal in vitro ADME properties. Our current results provide structural insights into a new class of potent non-peptidic covalent inhibitors of nsP2 cysteine protease with validated antiviral activities. Ongoing work is focused on preliminary DMPK and efficacy studies of key compounds in murine models as well as multiparameter SAR investigations. These results may facilitate the evolution of the compounds into selective and broad-spectrum anti-alphaviral drug leads. We envision that potent nsP2 inhibitors can be used as countermeasures against several emerging/reemerging viral pathogens that threaten the warfighter and the civilian population.

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