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

A Structure-based Drug Design Approach To The Discovery Of Inhibitors Of Alphavirus Nsp2 Helicase

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Venezuelan, Eastern, and Western equine encephalitis viruses (VEEV, EEEV and WEEV) are potentially fatal New World alphaviruses that can cause fever, encephalitis, sustained neurological disorders, and mortality in humans. Surprisingly, only a few small molecule inhibitors have been reported to improve survival in VEEV mouse models of infection, and no therapeutic studies to date have been reported in nonhuman primates (NHPs). Our efforts to target a novel binding site led us to a structure-based drug design approach to develop inhibitors of the alphavirus nsP2 helicase. The nsP2 protein functions as protease and helicase and both are required for replication. The helicase 'unpacks' genes by using energy from ATP hydrolysis, thus inhibitors targeting the ATP binding site are expected to limit energy required for helicase unwinding activity and inhibit replication. Furthermore, the high similarities among VEEV, EEEV, and WEEV helicases make pan-inhibitors possible, and since the alphavirus helicases are structurally different from human helicase proteins, selectivity should not be an issue.

Using in silico models based on the structure of the CHIKV nsP2 helicase domain, a set of ~3M commercially available compounds was virtually screened to provide a prioritized set of a few hundred compounds predicted to fit and bind well in the ATP pocket. Validation of the virtual screen in vitro using VEEV cell culture and VEEV nsP2 helicase biochemical assays led to several small molecule lead compounds. Challenges in obtaining pure and active full length nsP2 protein and subsequent follow-up of lead compounds will be presented.