Hit-to-lead development of inhibitors of the Chikungunya virus nsP3 macrodomain using crystallographic fragment screening

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Chikungunya virus (CHIKV) is a mosquito-borne alphavirus, transmitted in urban areas by *Aedes aegypti* and *Aedes albopictus* mosquitoes [1]. It poses a significant health threat, as seen by recent outbreaks driven by the spread of the mosquito vectors to additional geographical areas [2]. Patients infected with CHIKV present with severe fever, rash, and debilitating joint pain [3]. In up to 60 % of patients, the joint pain will persist for months or even years after the initial infection, severely impacting the patient's quality of life [4].

The CHIKV nsP3 macrodomain functions as an ADP-ribosyl (ADPr) hydrolase by removing mono-ADPr from post-translationally modified aspartate and glutamate residues of viral and host-cell proteins, counteracting the innate immune response [5]. It is highly conserved between alphaviruses, and deficiency in the ADPr-binding and hydrolase function of the nsP3 macrodomain was shown to attenuate CHIKV virulence in mice and to lead to reduced viral replication in cell culture, making it an attractive target for antiviral research [6].

In this study, we performed a high-throughput, crystallographic fragment screen on the nsP3 macrodomain of CHIKV, screening a total of 1385 fragments, yielding 109 fragment hits covering the ADPr-binding site of CHIKV nsP3 macrodomain and close subsites nearby. To progress these fragment hits into lead-like molecules with on-scale potency, we applied interaction-driven algorithmic methodologies to design and curate >1500 fragment merges, which were either commercially available or could be rapidly synthesised in parallel by chemist-assisted robotic synthesis. These follow-up compounds will be evaluated for binding using high-throughput structural biology and grating-coupled interferometry (GCI) to rapidly inform the design-make-test cycle for developing effective alphavirus inhibitors as part of the NIH-funded Rapidly Emerging Antiviral Drug Development Initiative.

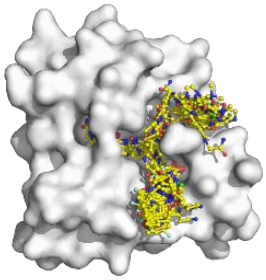

Figure 1. Fragments binding to the ADPr-binding site of CHIKV nsP3 macrodomain.

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