Title: Protease-Targeted Strategies Against Enterovirus-Induced Myocarditis: Discovery of a 2A/3C Inhibitor Through FRET-Based Screening and a P4 Medicine Perspective

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Abstract

Coxsackievirus B3 (CVB3) is a leading cause of viral myocarditis and dilated cardiomyopathy. Its 2A protease ($2A^{pro}$) is essential for viral replication and directly contributes to cardiac pathology by cleaving eukaryotic initiation factor 4G (eIF4G) and dystrophin. These events block host translation and disrupt sarcolemmal integrity, driving apoptosis, inflammation, and progressive heart damage. Although the related 3C protease ($3C^{pro}$) has been validated as a druggable target, efforts against $2A^{pro}$ are limited. Docking approaches underestimate ligand potential due to the enzyme's shallow substrate groove, and even known inhibitors often score below acceptable thresholds.

We aimed to identify small-molecule inhibitors of enteroviral proteases with potential to block both viral replication and protease-mediated cardiac injury. To this end, we developed a fluorescence resonance energy transfer (FRET)–based assay using peptide substrates that mimic natural cleavage sites of $2A^{pro}$ and $3C^{pro}$, enabling sensitive and quantitative measurement of protease activity.

Through this screening approach, we identified a novel compound that inhibits both proteases, demonstrating that empirical methods can overcome the limitations of in silico prediction and reveal dual-acting inhibitors. The compound provides a promising starting point for medicinal chemistry optimization.

These findings support dual inhibition of $2A^{pro}$ and $3C^{pro}$ as a therapeutic strategy capable of both suppressing viral replication and preventing cardiac damage. Within the framework of P4 medicine (predictive, preventive, personalized, and participatory) such inhibitors could help preserve cardiac function, stratify patient treatment, and support preventive measures in at-risk populations.