Tuberculosis is currently a leading cause of death from an infectious agent, second only to COVID-19. While advances in public health have contributed to a reduction in tuberculosis cases, the prevalence of multidrug-resistant Mycobacterium tuberculosis (MDR-TB) infections has created an urgent need to exploit novel drug targets. One such target is the ClpC1P1P2 protease, which degrades folded cytosolic proteins through the cooperation of the ATP-dependent unfoldase ClpC1 and the ClpP1P2 peptidase. Both protease components are strictly essential for Mtb viability and are validated therapeutic targets. However, efforts to develop anti-Mtb compounds are constrained by a limited understanding of Clp protease function and essentiality. Thus, it is crucial to identify physiological substrates and pathways regulated by this protease. More so, there is a related need to characterize regulators to understand the intricacies of regulation, and how this activity contributes to the physiology of mycobacteria and other actinobacterial species.

In this study, we characterized N-methylhydantoinase as a novel negative regulator of the unfoldase ClpC1. This protein, which appears in a potential bicistronic operon with 5-oxoprolinase, a Clp protease substrate, affects the ATPase and unfoldase activities of ClpC1. It also impacts its ability to stimulate the peptidase activity of ClpP1P2, thus inhibiting the proteolytic activity of the ClpC1P1P2 protease. This lays the groundwork for future efforts to expand on the roles potentially played by negative Clp protease regulators in mycobacteria. Future work will be required to delineate the exact mechanism of regulation, and how this activity contributes to the physiology of mycobacteria and other actinobacterial species.

**Abstract**

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