An anti-virulence strategy to treat *Salmonella* infections: Development of lead compounds targeting the transcriptional regulator HilD

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Salmonellosis is the second most commonly reported foodborne gastrointestinal infection in Europe. While most non-typhoidal *Salmonella* infections are self-limiting, hospitalization rates remain high among vulnerable individuals. Non-typhoidal *Salmonella* can cause systemic infections by invading the intestinal epithelium. The transcriptional regulator HilD is the central positive regulator of invasion-associated virulence genes. The intestinal colonization and systemic dissemination of *Salmonella* strains lacking *hilD* are strongly impaired in mice and in chicken infection models.

Here we unveil the structures of synthetic small molecules targeting HilD at low µM scale, and report advances in the optimization of their activity. Following the genetic and *in vitro* evidence of the specific binding of the inhibitors to HilD, we used Hydrogen deuterium exchange-mass spectrometry (HDX-MD) to identify the binding pocket, and deduce the conformational changes occurring after the binding. The inhibitors bind to a pocket localized at the interface between the N-terminal regulatory domain and the C-terminal DNA binding domain of HilD, without impairing its dimerization. Based on these experimental data and molecular dynamics simulations, a drug-target model was built to drive the structure-activity relationship analysis of over 180 synthesized compounds, and the successful rational design of optimized analogs. Finally, we will describe our approach combining machine learning prediction with the brute-force docking of a small fraction of active analogs to ultimately screen giga-scale libraries.

HilD inhibitors are being developed as standalone drugs to reduce the risk of systemic *Salmonella* infections in human patients, and to shorten hospitalization rate and length. HilD inhibitors could also be drug candidates to reduce the mortality rate among young boiler chicken.