

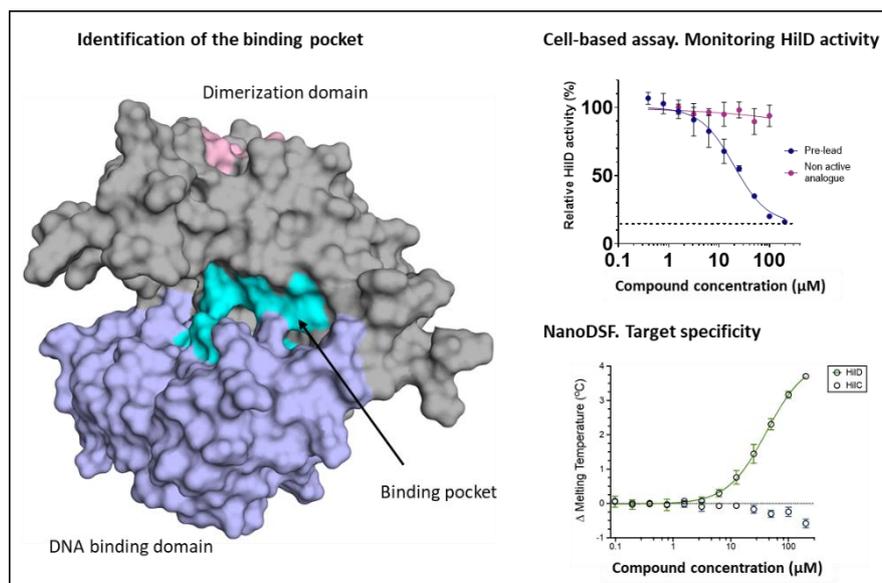
# Development of anti-virulence agents targeting HilD, the central regulator of *Salmonella* pathogenicity

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Salmonellosis is a major food-borne illness causing approximately 153 million cases and 300,000 deaths per year. The key virulence mechanism enabling non-typhoidal *Salmonella* to cause systemic infection is its ability to invade and propagate within the intestinal epithelium, thus increasing the risk of life-threatening bloodstream infections. The invasion-related pathogenicity of non-typhoidal *Salmonella* is mediated by several secretion systems which are mainly under control of the positive transcriptional regulator HilD. A *hilD*-deficient *Salmonella* strain is unable to express these pathogenicity factors and is therefore avirulent.

Here we report advances in the development of a series of synthetic small molecules targeting HilD at low  $\mu\text{M}$  scale, subsequently blocking the invasion of *Salmonella* into human host cells. Through biochemical, biophysical, and cell-based approaches, we provide evidence on the mode of action of the compounds and their binding mode to the target HilD. Based on the structural characterization of the binding pocket and 2 rounds of structure-activity relationship analysis, structural analogues were rationally designed, among which optimized compounds with improved activity were found. Pharmacological and mouse toxicity analyses of the selected pre-lead compound demonstrated its safety and suitability for oral administration. Finally, we provide an *in vivo* proof-of-concept using a mouse model of *Salmonella* gastro-intestinal infection.

HilD inhibitors will be designed as standalone drugs to reduce the risk of an invasive infection in patients developing an enteric *Salmonella* infection, and to shorten hospitalization time while avoiding the use of direct-acting antibiotics. A combination therapy with standard-of-care antibiotics could also be considered for the treatment of invasive and antibiotic-resistant *Salmonella* infections.



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