Anti-Virulence Strategy Targeting Sortase A

A Structural Investigation of the Sortase A Enzyme, and the Identification, Synthesis, and Evaluation of Sortase A Inhibitors

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Abstract

The emergence of multi-resistant bacteria and their continuous spread is one of the greatest challenges when treating bacterial infections. Increased understanding of bacterial pathogenesis has revealed new strategies for treating bacteria-mediated diseases. Targeting virulence factors or virulence-mediated mechanisms is one strategy which is believed to cause less selective pressure and thereby resistance development since it would not affect bacterial growth or survival. The bacterial enzyme sortase A (SrtA) anchors the majority of virulence associated proteins to the bacterial cell wall and is a promising target for development of anti-virulence drugs. This thesis describes the investigation of SrtA conformations, derived from MD simulations, and their performance in virtual screening (VS) using a diverse set of active inhibitors and their decoys. From the performance results, SrtA structures can be selected for further docking studies and VS. Further, novel SrtA inhibitors were discovered using high throughput and fragment based screening (HTS and FBS) as starting points for hit selection. Hits were synthetically modified and evaluated using several different biochemical and biophysical assays. The HTS resulted in the discovery of substituted thiadiazoles with inhibitory activities in the low micromolar range. They probably act by binding covalently to the active site cysteine of SrtA. The fragment screening resulted in the discovery of substituted pyrazoles and isoxazoles as promising starting points for further development into more potent SrtA inhibitors. A hybrid compound combining the knowledge from the HTS and FBS was developed. The hybrid is a potent non-covalent inhibitor as opposed to the HTS compounds. The flavone morin and its effects on SrtA were also investigated, showing that morin might act as both an inhibitor and an activator. Morin seems to bind to the SrtA dimer interface inducing a conformational change in the protein allowing various fragments to bind more efficiently to the active site. This sheds further light on the importance of investigating the inhibitory mechanism of already existing SrtA inhibitors as to get a better understanding of their mode of action, which will be crucial for the development of more potent SrtA inhibitors.

Keywords: Sortase A, structural investigation, molecular dynamics, virtual screening, SrtA inhibitors, fragment based lead generation, FBLG, high-throughput screening, HTS, allosteric modulation.