Abstract

The sirtuins is a family of NAD^+-dependent lysine deacylases that catalyze the hydrolysis of N^{ε}-acyllysine post translational modifications (PTMs) on a vast array of proteins. The seven human sirtuins (SIRT1-7) vary in subcellular localization, substrate- and acyl group preferences and have been shown to be involved in important cellular processes including metabolism, regulation of transcription, and DNA repair. However, these enzymes have also been implicated in various cancers, neurodegenerative diseases and metabolic disorders. Thus, the sirtuins has the potential of being novel drug targets and the development of sirtuin activity modulators has gained a lot of attention. Additionally, such compounds could be used to further study the individual functions and roles of the sirtuins. Among the sirtuin activity modulators, mechanism-based sirtuin inhibitors containing N^{ε}-acyllysine-mimicking moieties have been utilized extensively over the years. These inhibitors have been crucial in the elucidation of the sirtuin deacylation mechanism and are often highly potent, yet there is still a need for more potent and selective inhibitors.

A large series of novel mechanism-based SIRT5 inhibitors have been synthesized utilizing the N^{ε}-acyllysine preferences of SIRT5. Through an iterative structure-activity relationship approach potency was improved >100-fold from lead to final compound, affording the most potent SIRT5 inhibitor to date. The mode of binding has been elucidated through co-crystal structures with both zebrafish and human SIRT5. Additionally, the first co-crystal structures with thiourea-based sirtuin inhibitors have been obtained, validating their suggested mode of inhibition. Furthermore, kinetic studies have unveiled the first examples of slow, tight-binding behavior of SIRT5 inhibitors.

In a parallel project, several novel inhibitors have been developed against SIRT1 and SIRT2 deacylation by utilizing their respective N^{ε}-acyllysine preferences. Kinetic studies on long chain SIRT2 inhibitors have revealed an unprecedented slow, tight-binding mechanism of inhibition. Additionally, a unique behavior has been observed for thioacetamide sirtuin inhibitors, suggesting that these inhibitors are readily deacylated by SIRT1.

Finally, a novel sirtuin (lacSir2) has been discovered from the probiotic *Lactobacillus acidophilus NCFM* through bioinformatics in combination with a comprehensive substrate screen. The enzyme was also shown to be susceptible to inhibition with known sirtuin inhibitors, further substantiating its sirtuin-like features.