Abstract

Sirtuins are a family of enzymes catalyzing the removal of acyl groups from ε-*N*-acyllysine residues. In mammals seven sirtuins (SIRT1-7) are identified, which differ in sub-cellular localization and substrate specificity. Sirtuins are regulatory enzymes responsible for controlling important cellular processes such as metabolic pathways and DNA repair. High sirtuin activity is associated with protection against various age-related disorders. However, in some pathophysiologies sirtuin inhibition is advantageous, and sirtuins have been considered potential drug targets in neurological, metabolic and cancerous diseases.

This thesis investigates three different sirtuins, SIRT5-7, and the inhibition thereof. The first part investigates the possibility of exploiting the tyrosine-arginine motif present in the active site of SIRT5 to inhibit SIRT5 covalently. It was discovered that aryl fluorosulfate-based SIRT5 inhibitors were able to covalently label and inhibit SIRT5 in a time-dependent manner. Also, they exhibited high selectivity for SIRT5 over the six additional members of the sirtuin family and did not bind to denaturated SIRT5. Through mutational studies it was proved that the specific arginine residue present in the active site of SIRT5 was required for covalent labeling, however the covalent binding site was discovered to be divided onto two different tyrosine residues. The stability of the compounds was assessed in serum, which revealed greater metabolic stability compared to the parent reversible SIRT5 inhibitor. The developed aryl fluorosulfate-based inhibitors were discovered to be cell permeable and exhibited target engagement with endogenous SIRT5 both in mammalian cells and *in vivo*.

The second part of this thesis presents the expression and purification of catalytically active SIRT7, which was used to develop a high-throughput fluorogenic assay to investigate SIRT7 activity. Also, a structural study of SIRT7 using small angle X-ray scattering was performed. As SIRT7 is one of the least studied sirtuins, the substrate preferences for SIRT7 were initially explored, which revealed SIRT7 to mainly hydrolyze long-chain ε-*N*-acyllysine residues, when the modified lysine residue was positioned C-terminal of a positively charged amino acid residue. Furthermore, SIRT7 activity was increased in the presence of DNA and tRNA. The final assay conditions were used to test potential SIRT7 inhibitors, which showed warheads based on inverse amides or inverse thioamides to facilitate SIRT7 inhibition.

Finally, the last part of this thesis includes an investigation of SIRT6 inhibition. During the study of SIRT7 inhibitors, a compound was discovered to display more efficient inhibition of SIRT6 than SIRT7. A small selection of compounds was screened for SIRT6 inhibition, and two inverse thioamide-based compounds were identified as SIRT6 inhibitors. Kinetic insight proposed the SIRT6 inhibitors to act as non-competitive inhibitors, suggesting the inhibitors to bind both to the active site and to an allosteric site. Interestingly, substitution of the inverse thioamide moiety to an inverse amide in one of the SIRT6 inhibitors, changed the compound characteristics from a SIRT6 inhibitor to a SIRT6 activator.