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Modulating Sirtuin Activity
Design, Synthesis and Evaluation of Sirtuin 2 Inhibitors

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Abstract

Sirtuins (SIRT) are NAD^+ -dependent lysine deacetylating enzymes targeting histones and a multitude of non-histone proteins. The SIRT have been related to important cellular processes such as gene expression, cell proliferation, apoptosis and metabolism. They are proposed to be involved in the pathogenesis of e.g. cancer, neurodegeneration, diabetes and cardiovascular disorders. Thus, development of SIRT modulators has attracted an increased interest in recent years.

This thesis describes the design and synthesis of tri- and tetrasubstituted chroman-4-one and chromone derivatives as novel SIRT inhibitors. The chroman-4-ones have been synthesized via a one-pot procedure previously developed by our group. Further modifications of the chroman-4-ones using different synthetic strategies have increased the diversity of the substitution pattern. Chromones have been synthesized from the corresponding chroman-4-one precursors. Biological evaluation of these compounds has identified highly selective and potent SIRT2 inhibitors with IC_{50} values in the low μM range. Evaluation of selected compounds in cancer cell lines has shown an antiproliferative effect in breast cancer and lung carcinoma cells and an effect on the viability and morphology of brain tumor cells. A binding site for the SIRT2 inhibitors, i.e. the C-pocket of the NAD^+ binding site, has been proposed using molecular modeling that showed to be consistent with the structure-activity relationship data.

The proposed binding site has been further investigated using a photoaffinity labeling approach. For this, two photoactivatable chroman-4-ones containing either an azide or a diazirine moiety have been synthesized. The diazirine analog was a potent SIRT2 inhibitor. The light-induced incorporation of this photoprobe into SIRT2 followed by mass spectral analysis of the adducts has indicated that a stretch of eight amino acids has been labelled. The amino acids are located around the active site of SIRT2. One of the amino acids is a conserved histidine residue that is positioned at the part of the C-pocket to which the chroman-4-ones presumably bind. However, the low cross-linking yield has complicated the identification of the specific amino acid(s) modified by the probe.

The chroman-4-one scaffold has also been replaced with different analogous bicyclic frameworks, e.g. quinolones, saccharins and benzothiadiazine-1,1-dioxides. Most of the new compounds were less active than the chroman-4-one based inhibitors, but some were moderately potent. Interestingly, the new compounds also possessed moderate SIRT3 inhibitory activity. Thus, cyclic sulfonamides show potential as SIRT2 inhibitors and might also be valuable for the development of SIRT3 selective inhibitors

Keywords: Sirtuin, SIRT2, Inhibitors, Chroman-4-ones, Chromones, Benzothiadiazine-1,1-dioxides, Saccharin, Scaffold, Structure-activity relationship, Antiproliferative properties, Binding site, Homology modeling, Photoaffinity labeling, Diazirine, Mass spectrometry.