Inhibition of the Human SIRT5 Lysine Deacylase Enzyme

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The human sirtuin 5 (SIRT5) lysine deacylase is a mitochondrial protein, which serves as an important regulator of biological functions by hydrolysing ε-N-carboxyacyllysine posttranslational modifications in the cell. Inhibition of SIRT5 have been shown to be a promising target in for example acute myeloid leukaemia [1] and we have previously developed highly potent slow, tight-binding inhibitors of the enzyme.[2] However, despite being potent *in vitro*, these did not shown any efficacy in cells, likely due to poor cell permeability. By systematic replacement of the carboxylic acid moiety that is crucial for activity, we have screened a series of 26 isosteres, providing two hits that were equipotent to the lead inhibitor (K_i 0.5–7 nM). Employing the chloroalkane penetration assay (CAPA) we evaluated the cell permeability of the most potent isosteres. We also synthesized an ethyl ester prodrug of the carboxylic acid, which we anticipated to be cleaved inside the cells to give the parent carboxylic acid. Unsurprisingly, the prodrug had a superior CP_{50} value 6–9 fold better than of the isosteres and we therefore decided to mask the potent tetrazole isostere discovered to also optimize its cell permeability. Utilizing cellular thermal shift assays (CETSA) we identified the masked tetrazole as a potent and selective inhibitor of SIRT5 in cells. Finally, both the prodrug and the masked isostere-containing compound showed cyto-selective toxicity towards SIRT5-dependent AML cells (SKM-1) over HEK293T cells.

With these findings, we hope to inspire others for future inhibitor design and expand the functionalities that can be used to target SIRT5. Further, these isosteres and prodrugs may serve as tool compounds to enable a more detailed investigation of the biology of SIRT5 and its potential as a cancer target.

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