Development of Cell-Permeable Bisubstrate Inhibitors Against N-terminal Acetyltransferase D

Protein N-terminal acetyltransferase D (NatD) is a highly selective NAT because its only known substrates are histones H2A and H4. NatD expression is increased in primary human lung and colorectal cancer tissues and is associated with poor clinical outcomes. Knockdown of NatD slows human lung cancer progression by repressing the transcription factor Slug, which prevents the epithelial-to-mesenchymal transition. Furthermore, NatD depletion causes p53-independent apoptosis in colorectal cancer cells. Therefore, NatD appears to be a potential epigenetic target for lung and colorectal cancers. This study aims to identify potent and selective NatD inhibitors to unravel their roles in lung and colorectal cancers. Based on the acetyl transfer mechanism, we have designed and synthesized a series of potent and selective NatD bisubstrate inhibitors by covalently attaching coenzyme A to peptide substrates via a linker. To improve the cell permeability of these inhibitors, we designed and attached cell-penetrating peptides (CPPs) to bisubstrate inhibitors, which displayed Ki values in the nano-molar range. Meanwhile, we developed a continuous fluorescence-based acetyltransferase assay and applied this assay to perform high-throughput screening to discover small-molecule NatD inhibitors. We expect that the discovery of the inhibitors can significantly facilitate our understanding of the biological roles of NatD.