Discovery of inhibitors and covalent inactivators targeting proline cycle enzymes using focused fragment-based approaches

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The proline metabolic enzymes proline dehydrogenase (PRODH) and pyrroline-5-carboxylate reductase 1 (PYCR1) catalyze opposing reactions of a substrate cycle. PRODH catalyzes the FAD-dependent oxidation of proline to pyrroline-5-carboxylate, whereas PYCR1 catalyzes the NAD(P)H-dependent reduction of pyrroline-5-carboxylate to proline. Together, PRODH and PYCR1 form the proline cycle, a novel pathway that has been implicated in the metabolic alterations that occur in cancer cells. The proline cycle has been implicated in supporting ATP production, protein and nucleotide synthesis, anaplerosis, and redox homeostasis in cancer cells. In particular, PYCR1 is one of the most consistently upregulated genes in numerous cancers. We have used small libraries of target-focused fragments to explore structure-affinity relationships and discover inhibitors of PRODH and PYCR1. In crystallo screening of a library of 27 compounds generated the first inhibitor of PYCR1 that has been thoroughly validated by demonstrating the kinetic mechanism of action against the purified enzyme, the mode of binding to the enzyme by X-ray crystallography, and activity in cancer cells. A similar strategy targeting PRODH revealed synergism between inhibitor ring size and hydrogen bonding to a conserved water molecule, ultimately leading to the serendipitous discovery of a novel class of photoinduced inactivators that covalently modify the N5 of the FAD.