

## New horizons for structure-based discovery of PYCR1 inhibitors

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Recent years have seen mounting evidence linking proline metabolism to cancer cell survival, proliferation, and metastasis. In the final step of proline biosynthesis,  $\delta^1$ -pyrroline-5-carboxylate (P5C) reductase catalyzes the NAD(P)H-dependent conversion of P5C to proline. Among the three human P5C reductase isoforms—PYCR1, PYCR2, and PYCR3—PYCR1 has emerged as a particularly attractive therapeutic target. Since 2019, over 100 studies have associated elevated PYCR1 expression with poor prognosis across numerous cancer types. Functional studies further support its role in malignancy: PYCR1 knockdown or knockout reduces proliferation, impairs tumor growth, and induces cell cycle arrest and apoptosis. These findings are consistent with a large-scale 2014 transcriptomic analysis, which ranked PYCR1 among the most consistently overexpressed metabolic enzymes in tumors across 19 cancer types.

Despite its potential, the chemical space of PYCR1 inhibitors remains remarkably limited, with no lead-like candidates reported to date. To address this gap, we initiated a structure-guided discovery effort combining crystallographic fragment screening (XFS), high-throughput enzymatic screening (HTS), supported by structural analysis.

Here, we present results from the initial hit discovery phase, in which we performed the first XFS campaign targeting PYCR1 using a library of 96 chemically diverse small-molecules. Soaking experiments enabled binding to both the P5C and NADH binding pockets, resulting in several fragment hits with well-defined binding modes in one or both sites. Notably, some fragments induced unexpected conformational rearrangements, revealing previously uncharacterized structural plasticity in the active site. These findings provide a valuable starting point for the development of novel PYCR1 inhibitors. In parallel to XFS, we have conducted an HTS campaign, which revealed novel PYCR1 inhibitors of higher potency than those previously available. Our work significantly expands the chemical landscape of PYCR1 druggability with small molecules and lays the groundwork for the future development anticancer therapeutics targeting this underexplored enzyme.

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