Covalent Proximity Scanning of a Distal Cysteine to Target PI3Kα

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Inhibitors of the phosphatidylinositol 3-kinase (PI3K) – protein kinase B (PKB/Akt) - mechanistic target of rapamycin (mTOR) axis are considered valuable assets in cancer therapy.[1] A considerable effort has been dedicated to the development of drugs targeting class I PI3Ks, which are evaluated in preclinical and clinical studies.

Here we present a strategy to convert a phase II clinical candidate, a pan-PI3K inhibitor (PQR309, bimiralisib)[2], into a highly selective, covalent PI3Kα inhibitor with the aim to minimize off-target and on-target metabolic side effects of PI3K inhibitor cancer therapy. We exploited a rational approach to increase target selectivity by covalently targeting PI3Kα at the non-conserved nucleophilic Cys862.

A combination of warhead activity design, proximity screening and an optimized orientation allowed a tight control of reversible inhibitor binding in combination with an isoform-specific covalent reaction. To avoid off-target reactions, all warheads' reactivities were determined and optimize for selectivity and of Cys862 modification. An extensive Structure Activity Relationship (SAR) study was performed and a wide range of linear and restricted rotation linkers were introduced. A comprehensive understanding of the kinetics of irreversible inhibition acquired by kinetic TR-FRET assays and subsequent determination of kchem, kinact and calculated Ki allowed the establishment of a SAR, for compound selection with minimal off-target reactivity and high PI3Kα selectivity. X-ray crystallography and MS-based proteomics validated the covalent modification of Cys862. Our pilot compounds exceed specificity and potency over an experimental dimethyl-substituted enone, CNX-1351.[3] Moreover, our compounds display increased stability in rat liver microsomal assays and outperform the rapidly metabolized CNX-1351.

Our strategy to investigate and tune warheads' reactivity represents a major step forward in the rational design of covalent chemical tools. Moreover, we provide highly selective chemical tools to dissect PI3K isoform signaling in physiology and disease.

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