# Precision Drugs: A Rational Approach to Covalent PI3Kα Inhibitors

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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.[1-6] 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)[6,7], 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.[8] 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, overcoming the serendipity in the discovery of irreversible compounds. Moreover, we provide highly selective chemical tools to dissect PI3K isoform signaling in physiology and disease. A clarification of the role of the different PI3K isoforms in insulin signaling allows to address the challenges in isoform selectivity and to develop PI3K inhibitors showing ideal isoform specificity.

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