

**COMBINING MEDICINAL CHEMISTRY WITH CHEMOGENOMIC
AND COMPUTER-AIDED STRUCTURE-BASED DESIGN IN
DEVELOPMENT OF NOVEL KINASE INHIBITORS**

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Although only 6% of the estimated 518 protein kinases [1] encoded in the human genome have had structures of their catalytic domains determined by X-ray crystallography, structure-based design techniques can significantly aid in the discovery of novel kinase inhibitors across this protein class. Conservation of the ATP binding site between known kinase structures, together with knowledge of the human kinase genome and an abundance of selectivity data, allows for significant advances in understanding the relationship between kinase targets and inhibitors. Utilization of this information in discovery of kinase inhibitors defines practical chemogenomics [2]. Chemogenomics technologies at Lilly aid in lead generation strategies and in making informed decisions about advancement of chemical entities in the drug discovery pipeline. In turn, computational docking and scoring, aided by X-ray crystallography, is used to optimize chemical series discovered with the aid of chemogenomics. In this paper we present the Lilly Kinase portal and show examples of two projects that highlight the utility of integrated technologies. Specific examples include novel classes of kinase inhibitors directed at CDK2 and TGFBR1 kinases.

The implementation of a strategy of compound profiling across a kinase panel led to the identification of a group of benzimidazoles as inhibitors of several kinases. This finding led to the evaluation of aminoimidazo[1,2-*a*]pyridine [3] as a potentially novel ATP-competitive CDK2 inhibitor. Our exploration hypothesis was guided by docking experiments [4] that correctly predicted the binding mode of these compounds. Chemogenomic tools provided direction for selectivity exploration and determined a primary focused-kinase selectivity panel for the project. The hypothesis was tested through both SAR studies and X-ray crystallographic analysis. An initial lead against CDK2 with a potency of 320 nM was optimized to achieve a 28 nM inhibitor with improved cyclin selectivity and high selectivity against a representative set of other serine/threonine kinases. This compound selectively inhibited the CDK2-dependent phosphorylation of Rb in HCT116 tumor cells and showed an IC₅₀ for growth inhibition of 0.21 μM after a 72 hour exposure.

Another application in the use of integrated technologies was used to discover a group of aryl- and heteroaryl-substituted pyrazole inhibitors of the TGFBR1 kinase [5]. In this case, our efforts began with the *in vitro* screening of a large library of compounds. SAR exploration of identified diheteroaryl-substituted

pyrazole (TGFBR1 IC_{50} = 51 nM) was aided by docking studies that led to the emergence of two sub-series featuring substituted quinoline and phenyl groups at the 4-position of the pyrazole, and exhibiting differing selectivity versus p38 MAPK14 kinase. A common binding mode at the active site predicted by docking studies was subsequently confirmed by successful co-crystallization of potent inhibitors with the TGFBR1 kinase construct. The strength of kinase hinge interaction is the major determinant of selectivity with respect to p38 between the two sub-series, with the 4-aryl series being more selective, and exemplifies the chemogenomic relationship between the two targets. In one example, 3-(2-pyridinyl)-4-(4-hydroxyphenyl)-pyrazole inhibited the enzyme with an IC_{50} of 31 nM *in vitro* and exhibited more than 10-fold selectivity over p38 inhibition. Activities against TGF β -dependent luciferase production in mink lung cells (p3TP Lux, IC_{50} = 44 nM) and in the reversal of TGF β -stimulated growth in mouse fibroblasts (NIH 3T3, IC_{50} = 32 nM) were also strong.

In summary, we have demonstrated that the proper usage of the integrated technologies can be successfully applied to aid kinase inhibitor development efforts. Utilization of the inhibitors discovered against one target may be used to explore secondary targets, and thus represents the foundation of a practical application of chemogenomics.

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