

METHODS IN MOLECULAR BIOLOGY™

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Kinase Inhibitors

Methods and Protocols

Edited by

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Preface

Protein and lipid kinases are often the master regulators of cell signaling in eukaryotic systems. The human genome codes for more than 500 of these enzymes and their misregulation has been shown to be involved in the onset and progression of many diseases including cancer and inflammation. Therefore, small molecule kinase inhibitors have become important research tools for the elucidation of many biological roles of kinases and their mechanisms of action. In addition, kinase inhibitors are now successful drugs in a number of liquid and solid tumors. In fact, about one dozen molecules are currently approved for clinical use and 200 more molecules are in different stages of clinical evaluation. Kinase inhibitors thus contribute significantly to the drug pipelines of pharmaceutical and biotechnology industries and to the growing need for the treatment of cancer and inflammation.

There are many challenges in the discovery and development of kinase inhibitors both for research and clinical use and thus, many methods are being devised and applied to understand the often complex functional relationships between kinases and the respective inhibitors. In this book, experts in kinase biology, drug discovery, and clinical research present a series of exemplary methods that can be used to address these challenges.

To set the scene, two introductory reviews discuss how kinase inhibitors can be used to target cancer and inflammation. The themes covered span a wide range of topics from the structural basis of kinase inhibition, to mechanistic aspects, resistance formation, and animal models. The following three chapters present biochemical kinase activity assays for protein and lipid kinases. These include the classical recombinant enzyme assays which today still are workhorses in the evaluation of kinase inhibitor potency and selectivity. However, the discovery of novel kinase inhibitors increasingly attempts to target protein domains that are distinct from the kinase domain and its ATP pocket. The rationale for these approaches is to provide better selectivity of the inhibitors because they might better reflect the actual mechanisms of action of kinase activation and deactivation.

Two major and often related issues in the development of kinase inhibitors as drugs or research tools are their selectivity and toxicity which is why six chapters of this book are devoted to these topics. Apart from issues such as hepatotoxicity, which is a major problem in all areas of drug development, the structural conservation of kinase domains in general and ATP binding sites in particular pose a number of extra challenges as many small molecules have the propensity to inhibit many kinases. In cancer therapy, this may sometimes be advantageous because many cancers represent a molecularly heterogeneous group of diseases. However, multikinase inhibition may also lead to toxicity which may prevent the use of these agents particularly in chronic applications. Classically, selectivity profiling is performed using large panels of recombinant kinase assays. More recently, proteomic approaches are being followed as they provide a means to study inhibitor selectivity in cells or tissues, which is thought to represent a more realistic biological assay context than individual kinases tested in the absence of other cellular components.

Kinase inhibitors may impact a biological system very specifically or rather broadly. The full appreciation of the potential of a kinase inhibitor should therefore include an evaluation of its impact at the level of individual signaling pathways, cellular model systems, or the

entire biological system. As examples, the last five chapters in this book describe methods that identify individual kinase–substrate relationships, measure the phosphorylation status of proteins in response to kinase inhibitor treatment, and identify resistance mechanisms by which many tumors eventually escape therapeutic intervention.

It is obviously beyond the scope of this book to cover the field of kinase inhibitors in its entirety. However, the individual chapters aim to provide modern and relevant exemplary methods that scientists may implement in their laboratories to accelerate or strengthen their research and drug discovery programs.

Freising, Germany

Bernhard Kuster

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