Methods in Molecular Biology 795

Springer Protocols

Bernhard Kuster *Editor*

Kinase Inhibitors

Methods and Protocols

Humana Press

METHODS IN MOLECULAR BIOLOGY™

Series Editor
John M. Walker
School of Life Sciences
University of Hertfordshire
Hatfield, Hertfordshire, AL10 9AB, UK

For further volumes:
http://www.springer.com/series/7651

Editor
Bernhard Kuster
LS für Proteomik und Bioanalytik
Technische Universität München
Emil Erlenmeyer Forum 5
85354 Freising
Germany
kuster@tum.de

ISSN 1064-3745 e-ISSN 1940-6029
ISBN 978-1-61779-336-3 e-ISBN 978-1-61779-337-0
DOI 10.1007/978-1-61779-337-0
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011936748

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Kinase Inhibitors

Methods and Protocols

Edited by

Bernhard Kuster

LS für Proteomik und Bioanalytik, Technische Universität München, Freising, Germany

Humana Press

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 rational 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*

Contributors

Marcus Bantscheff • *Cellzome AG, Heidelberg, Germany*

Thorsten Berg • *University of Leipzig, Institute for Organic Chemistry, Leipzig, Germany*

Scott M. Brittain • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*

Elisa Ciraolo • *Department of Genetics, Biology and Biochemistry, Molecular Biotechnology Center, University of Torino, Torino, Italy*

Ivan Cornella-Taracido • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*

Sandra W. Cowan-Jacob • *Novartis Institutes for Biomedical Research, Structural Biology Platform, Basel, Switzerland*

Ted M. Dawson • *Department of Neurology, Neuroregeneration and Stem Cell Program, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MA, USA; Solomon H. Snyder Department of Neuroscience, Neuroregeneration and Stem Cell Program, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MA, USA*

Valina L. Dawson • *Department of Neurology, Neuroregeneration and Stem Cell Program, Institute for Cell Engineering, Baltimore, MA, USA; Solomon H. Snyder Department of Neuroscience, Neuroregeneration and Stem Cell Program, Institute for Cell Engineering, Baltimore, MA, USA; Department of Physiology, Johns Hopkins University School of Medicine, Baltimore, MA, USA*

Mathias Dreger • *caprotec bioanalytics GmbH, Berlin, Germany*

James S. Duncan • *Department of Biochemistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, ON, Canada; Department of Pharmacology, University of North Carolina, School of Medicine, Chapel Hill, NC, USA*

Margaret C. Dunn • *Pfizer Research Technology Center, Cambridge, MA, USA*

Doriano Fabbro • *Novartis Institutes for Biomedical Research, Expertise Platform Kinases, Basel, Switzerland*

Oleg Fedorov • *Structural Genomics Consortium, Oxford University, Oxford, UK*

Jenny J. Fischer • *caprotec bioanalytics GmbH, Berlin, Germany*

Matthias Gaestel • *Hannover Medical School, Institute of Biochemistry, Hannover, Germany*

Olivia Y. Graebner (neé Baessler) • *caprotec bioanalytics GmbH, Berlin, Germany*

Edmund Harrington • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*

Timothy A.J. Haystead • *Department of Pharmacology & Cancer Biology, Duke University Medical Centre, Durham, NC, USA*

Tanja Henzler • *Merck KGaA, Darmstadt, Germany*
Thomas Herget • *Merck KGaA, Darmstadt, Germany*
Emilio Hirsch • *Molecular Biotechnology Center, University of Torino, Torino, Italy*
Scott Hobson • *Cellzome AG, Heidelberg, Germany; Department for CNS Research, Boehringer Ingelheim GmbH & Co KG, Biberach an der Riß, Germany*
Thomas O. Joos • *NMI Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany*
Kian Kani • *University of Southern California, Los Angeles, CA, USA*
Marc Kirchner • *Department of Pathology, Children's Hospital Boston, John F. Enders Research Laboratories, Boston, MA, USA*
Stefan Knapp • *Structural Genomics Consortium, Oxford University, Oxford, UK*
Alexey Kotlyarov • *Hannover Medical School, Institute of Biochemistry, Hannover, Germany*
Bernhard Kuster • *TU München LS für Proteomik, Freising, Germany*
Mark R. Lackner • *Department of Development Oncology Diagnostics, Genentech Inc., South San Francisco, CA, USA*
Gabriela Lavezzari • *Theranostics Health, Rockville, MA, USA*
Byoung Dae Lee • *Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, USA*
Xiaojie Li • *Cell and Molecular Medicine Graduate Training Program, Johns Hopkins University School of Medicine, Baltimore, MA, USA*
David W. Litchfield • *Department of Biochemistry, Schulich School of Medicine & Dentistry, University of Western Ontario, London, ON, Canada*
Parag Mallick • *University of Southern California, Los Angeles, CA, USA; Stanford University, Stanford, CA, USA*
Georg Martiny-Baron • *Novartis Institutes for Biomedical Research, Expertise Platform Kinases, Basel, Switzerland*
Henrik Möbitz • *Novartis Institutes for Biomedical Research, Global Discovery Chermistry, Basel, Switzerland*
Frank H. Niesen • *Structural Genomics Consortium, Oxford University, Oxford, UK*
Nurhan Özlü • *Center for Life Science, F.M. Kirby Center for Neurobiology, Children's Hospital Boston, Istanbul, Turkey*
Alessia Perino • *Department of Genetics, Biology and Biochemistry, Molecular Biotechnology Center, University of Torino, Torino, Italy*
Eugene C. Petrella • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*
Oliver Pötz • *NMI Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany*
Wolfgang Reindl • *Department of Molecular Biology, and Center for Integrated Protein Science Munich (CIPSM), Max Planck Institute of Biochemistry, Martinsried, Germany*
Markus Schirle • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*
Nicole Schneiderhan-Marra • *NMI Natural and Medical Sciences Institute at the University of Tuebingen, Reutlingen, Germany*

David Schwalb • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*
Kevan M. Shokat • *Department of Cellular and Molecular Pharmacology, University of California San Francisco, San Francisco, CA, USA*
Arthur R. Smith • *Pfizer Research Technology Center, Cambridge, MA, USA*
Rafaella Sordella • *Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA*
Alexander V. Statsuk • *Department of Chemistry, Northwestern University, Evanston, IL, USA*
Judith Jebanathirajah Steen • *Center for Life Science, F.M. Kirby Center for Neurobiology, Children's Hospital Boston, Boston, MA, USA*
Klaus Strebhardt • *Department of Gynecology and Obstetrics, Johann Wolfgang Goethe-University, Medical School, Frankfurt, Germany*
John A. Tallarico • *Novartis Institutes for Biomedical Research, Cambridge, MA, USA*
Eric S. Tien • *Pfizer Research Technology Center, Cambridge, MA, USA*
Jinghai J. Xu • *Knowledge Discovery & Knowledge Management, Merck & Co., Inc., RY86-235, Rahway, NJ, USA*

Contents

Preface . . . . . *v*
Contributors . . . . . *ix*

1 Targeting Cancer with Small-Molecular-Weight Kinase Inhibitors 1
Doriano Fabbro, Sandra W. Cowan-Jacob, Henrik Möbitz, and Georg Martiny-Baron

2 Small-Molecule Protein and Lipid Kinase Inhibitors in Inflammation and Specific Models for Their Evaluation 35
Matthias Gaestel and Alexey Kotlyarov

3 Measuring the Activity of Leucine-Rich Repeat Kinase 2: A Kinase Involved in Parkinson's Disease 45
Byoung Dae Lee, Xiaojie Li, Ted M. Dawson, and Valina L. Dawson

4 Measuring PI3K Lipid Kinase Activity 55
Elisa Ciraolo, Alessia Perino, and Emilio Hirsch

5 A Fluorescence Polarization Assay for the Discovery of Inhibitors of the Polo-Box Domain of Polo-Like Kinase 1 69
Wolfgang Reindl, Klaus Strebhardt, and Thorsten Berg

6 Assessment of Hepatotoxicity Potential of Drug Candidate Molecules Including Kinase Inhibitors by Hepatocyte Imaging Assay Technology and Bile Flux Imaging Assay Technology 83
Jinghai J. Xu, Margaret C. Dunn, Arthur R. Smith, and Eric S. Tien

7 Kinase Inhibitor Selectivity Profiling Using Differential Scanning Fluorimetry 109
Oleg Fedorov, Frank H. Niesen, and Stefan Knapp

8 Chemoproteomic Characterization of Protein Kinase Inhibitors Using Immobilized ATP 119
James S. Duncan, Timothy A.J. Haystead, and David W. Litchfield

9 Proteome-Wide Identification of Staurosporine-Binding Kinases Using Capture Compound Mass Spectrometry 135
Jenny J. Fischer, Olivia Y. Graebner (neé Baessler), and Mathias Dreger

10 Affinity Purification of Proteins Binding to Kinase Inhibitors Immobilized on Self-Assembling Monolayers 149
Marcus Bantscheff, Scott Hobson, and Bernhard Kuster

11 Kinase Inhibitor Profiling Using Chemoproteomics 161
Markus Schirle, Eugene C. Petrella, Scott M. Brittain, David Schwalb, Edmund Harrington, Ivan Cornella-Taracido, and John A. Tallarico

12 Covalent Cross-Linking of Kinases with Their Corresponding Peptide Substrates 179
Alexander V. Statsuk and Kevan M. Shokat

13 Receptor Tyrosine Kinase Inhibitor Profiling Using Bead-Based Multiplex Sandwich Immunoassays 191
Oliver Pötz, Nicole Schneiderhan-Marra, Tanja Henzler, Thomas Herget, and Thomas O. Joos

14 Monitoring Phosphoproteomic Response to Targeted Kinase Inhibitors Using Reverse-Phase Protein Microarrays 203
Gabriela Lavezzari and Mark R. Lackner

15 Measuring Phosphorylation-Specific Changes in Response to Kinase Inhibitors in Mammalian Cells Using Quantitative Proteomics 217
Nurhan Özlü, Marc Kirchner, and Judith Jebanathirajah Steen

16 Investigation of Acquired Resistance to EGFR-Targeted Therapies in Lung Cancer Using cDNA Microarrays. 233
Kian Kani, Rafaella Sordella, and Parag Mallick

Index. *255*

Chapter 1

Targeting Cancer with Small-Molecular-Weight Kinase Inhibitors

Doriano Fabbro, Sandra W. Cowan-Jacob, Henrik Möbitz, and Georg Martiny-Baron

Abstract

Protein and lipid kinases fulfill essential roles in many signaling pathways that regulate normal cell functions. Deregulation of these kinase activities lead to a variety of pathologies ranging from cancer to inflammatory diseases, diabetes, infectious diseases, cardiovascular disorders, cell growth and survival. 518 protein kinases and about 20 lipid-modifying kinases are encoded by the human genome, and a much larger proportion of additional kinases are present in parasite, bacterial, fungal, and viral genomes that are susceptible to exploitation as drug targets. Since many human diseases result from overactivation of protein and lipid kinases due to mutations and/or overexpression, this enzyme class represents an important target for the pharmaceutical industry. Approximately one third of all protein targets under investigation in the pharmaceutical industry are protein or lipid kinases.

The kinase inhibitors that have been launched, thus far, are mainly in oncology indications and are directed against a handful of protein and lipid kinases. With one exception, all of these registered kinase inhibitors are directed toward the ATP-site and display different selectivities, potencies, and pharmacokinetic properties. At present, about 150 kinase-targeted drugs are in clinical development and many more in various stages of preclinical development. Kinase inhibitor drugs that are in clinical trials target all stages of signal transduction from the receptor protein tyrosine kinases that initiate intracellular signaling, through second-messenger-dependent lipid and protein kinases, and protein kinases that regulate the cell cycle. This review provides an insight into protein and lipid kinase drug discovery with respect to achievements, binding modes of inhibitors, and novel avenues for the generation of second-generation kinase inhibitors to treat cancers.

Key words: Kinase inhibitors, Cancer, CML, Oncogene, Small-molecular-weight compounds, Kinase inhibitor binding mode

Abbreviations

AML	Acute myeloid leukemia
B-ALL	B-cell acute lymphoblastic leukemia
CEL	Chronic eosinophilic leukemia

Bernhard Kuster (ed.), *Kinase Inhibitors: Methods and Protocols*, Methods in Molecular Biology, vol. 795,
DOI 10.1007/978-1-61779-337-0_1,

CML	Chronic myeloid leukemia
CMML	Chronic myelomonocytic leukemia
RCC	Renal cell cancer
NSCLC	Non-small-cell lung cancer
GIST	Gastrointestinal stromal cancer
CSF1R	colony-stimulating factor 1 receptor
EGFR	Epidermal growth factor receptor
FLT3	FMS-related tyrosine kinase 3
GIST	Gastrointestinal stromal tumor
NSCLC	Non-small-cell lung cancer
PDGFR	Platelet-derived growth factor receptor
RCC	Renal cell carcinoma
Ph+	Philadelphia chromosome positive
VEGFR	Vascular endothelial growth factor receptor

1. Introduction

Protein and lipid kinases represent, after GPCRs and proteases, one of the most important target classes for treating human disorders. In fact, one third of the protein targets under investigation by pharmaceutical companies are protein or lipid kinases. Deregulation of the reversible phosphorylation that governs cellular processes leads to the development of a number of malignant pathological disorders (1–7). Many human malignancies are associated with activated protein or lipid kinases or inactivated phosphatases due to mutations, chromosomal rearrangements, and/or gene amplification (1, 8–11). Approximately 50 of the 100 known genes that have been directly linked to the induction and maintenance of cancer encode protein kinases (8, 12–15).

Given the role played by various protein and lipid kinases in cancer, it is not surprising that the majority of kinase inhibitors are being developed in life-threatening oncology indications, a therapeutic area in which there is a greater tolerance for side effects. All protein and lipid kinases share a conserved catalytic kinase domain. The kinase domain is a bilobal structure where the N-terminal lobe consists mainly of β-sheets and the C-terminal domain contains alpha helices (8, 12–17). The two lobes are connected by the so-called hinge region, which lines the ATP-binding site that is targeted by the majority of small-molecular-weight kinase inhibitors (8, 12–17). In the past decade, 11 of these small-molecule kinase inhibitors have been approved for clinical use (Table 1). These registered kinase inhibitors target only a few members of the kinome, including the tyrosine receptor kinases EGFR, ERBB2, VEGFRs, Kit, PDGFRs, the nonreceptor tyrosine kinases ABL and SRC, and only one Ser/Thr-specific kinase, the atypical protein kinase mTOR (Table 1). The commercial success of imatinib has

Table 1
Registered kinase inhibitors

Compound	Kinase target	Cancer target	Company
Imatinib (Glivec, Gleevec, STI571)	ABL 1–2, PDGFR, KIT	CML, Ph+ B-ALL, MML, CEL, GIST	Novartis
Gefitinib (Iressa, ZD1839)	EGFR	NSCLC	AstraZeneca
Erlotinib (Tarceva, OSI-774)	EGFR	NSCLC, pancreatic cancer	OSI, Genentech Inc, Roche
Lapatinib (Tykerb, GW2016)	EGFR, ERBB2	Breast cancer	Glaxo SmithKline
Dasatinib (Sprycel, BM-354825)	ABL1–2, PDGFR, KIT, SRC	CML	Bristol Myers
Nilotinib (Tasigna, AMN107)	ABL1–2, PDGFR, KIT	CML	Novartis
Sunitinib (Sutent, SU11248)	VEGFR1–3, KIT, PDGFR, RET, CSF1R, FLT3	RCC, GIST	Pfizer
Sorafenib (Nexavar, Bay 43-9006)	VEGFR2, PDGFR, KIT, FLT3, BRAF	RCC	Onyx and Bayer Pharmaceuticals
Pazopanib (Votrient, GW-786034)	VEGFR1–3, PDGFR, KIT,	RCC	GlaxoSmithKline
Everolimus (Afinitor, Rad001)	mTOR	RCC	Novartis
Temsirolimus (Torisel, CCI-779)	mTOR	RCC	Wyeth

attracted considerable interest and has triggered many efforts leading to the identification of broad-spectrum inhibitors of BCR-ABL1 and its imatinib-resistant mutants, while targets such as phosphoinositide-3 kinases (PI3Ks), mTOR, AKT, and cell-cycle regulating kinases are getting increasing attention (18–22).

The major challenge that kinase drug discovery for the treatment of cancer is currently facing is related to the molecular mechanisms underlying the various forms of cancer, and research is mainly geared to identify the following: the "addiction of tumors" to the target kinase (4, 5), the emerging resistance to kinase inhibition (23–25), the patients most likely to respond to a given kinase inhibitor treatment as well as the identification and validation of novel kinase targets for the treatment of cancer. Genome-wide screening for kinase mutations has revealed how the mutational status of the inherited variants of the various kinome members may

be associated with increasing risk of various cancer conditions (8–10). Additional investigation of deregulated kinase activities in cancer by phosphoproteomics, analysis of gene amplifications/mutations will not only lead to the identification of new kinase targets but also allow a better prediction of drug responses in patients (1, 12, 13, 25, 26). The finding that protein kinases with a gain of function mutation appear to be either more sensitive or resistant to inhibition by kinase inhibitors compared to the wild type variant has opened new avenues to kinase drug discovery approaches (1, 23, 25, 27, 28). In addition, multiple clinical studies have revealed that various protein kinases escape resistance by either mutating key residues in their catalytic domains or by developing compensatory mechanisms allowing to bypass the kinase target by overexpression of alternative kinases or other oncogenes (3, 29–34). All of these issues have prompted the pharmaceutical industry and small biotech companies to come up with strategies that may allow them to override these various types of resistances including compounds capable of circumventing the target related drug resistance (35–40).

Another important area of kinase drug discovery is focused on understanding the selectivity profile of kinase inhibitors as well as of targeting kinases by novel approaches (41, 42). Although there is a continuous debate on how selective a kinase inhibitor needs to be when used in cancer indications, the development of kinase inhibitors with outstanding selectivity is likely to be important not only for minimizing side effects and allowing chronic treatment of non-life-threatening diseases but also to better understand the on- and off-target pharmacology of kinase inhibitors.

2. Current Status of Protein and Lipid Kinase Drug Discovery

2.1. Approved Protein Kinase Inhibitors for the Treatment of Cancer

To date, 11 small-molecular-weight kinase inhibitors have been launched for cancer indications (Table 1). These include imatinib (Glivec®, STI571, Novartis AG) (43), gefitinib (Iressa™, ZD1839. AstraZeneca Inc.) (44), erlotinib (Tarceva™, OSI-774, Genentech Inc.) (45, 46), Lapatinib (Tykerb®, GW2016, GlaxoSmithKline) (47), sorafenib (Nexavar®, Bay 43–9006, Onyx and Bayer Pharmaceuticals) (48), sunitinib (Sutent®, SU11248; Pfizer, Inc.) (49), dasatinib (SPRYCEL®, BM-354825, Bristol-Myers Squibb) (39), nilotinib (Tasigna®, AMN107, Novartis) (36), pazopanib (Votrient™, GW-786034, GlaxoSmithKline) (50), torisel (Temsirolimus®, CCI-779, Wyeth Pharmaceuticals) (51), and everolimus (Afinitor®, RAD001, Novartis AG) (52). Everolimus, an orally active and potent immunosuppressive agent that inhibits T cell proliferation (53), has been already approved as Certican® for the prophylaxis of organ rejection of heart and kidney (54).

This rapalog is to date the only kinase inhibitor that has been registered for nononcology indications. All other approved kinase drugs are active against more than one type of cancer where the pivotal registration trials are ongoing (55).

Imatinib, a phenyl-amino-pyrimidine derivative targeting the inactive conformation of the ABL1 kinase, was the first kinase inhibitor used in clinical trials for the treatment of chronic myelogenous leukemia (CML) (23, 56). The ABL1 kinase is constitutively activated in more than 95% of CML due to a chromosomal translocation leading to the Philadelphia (Ph) chromosome (43, 56). The success of imatinib in CML patients may be considered unique among cancers, as CML is caused by a single molecular abnormality and during its chronic phase can be regarded as a myeloproliferative disorder rather than leukemia (23, 57). Owing to its multitargeted nature, imatinib, has since then been approved for various other cancer indications including Ph+ALL (targeting ABL1) (57), gastrointestinal stromal tumors (GIST) (targeting mutant forms of KIT and PDGFR) (27, 58–61), recurrent and/or metastatic dermatofibrosarcoma protuberans (targeting PDGFR) (62), myelodysplastic and/or myeloproliferative diseases (targeting PDGFR, KIT, and/or ABL1) (57, 63), and hypereosinophilic syndrome (targeting PDGFR) (57). Imatinib has also been used in nononcology indications (targeting PDGFR) for the treatment of pulmonary arterial hypertension, a severe, incurable blood vessel disorder (64). Imatinib and other kinase inhibitors are known to generate target dependent resistance, in particular in advanced stages of GIST, CML, or non-small-cell lung cancer (NSCLC) (23, 24, 61). More potent drugs, such as nilotinib, another phenyl-amino-pyrimidine with a selectivity profile similar to imatinib or drugs with a binding mode different from imatinib, such as the amino-thiazole dasatinib, a potent BCR-ABL1 inhibitor with a broad selectivity spectrum, have been approved for imatinib-resistant CML (65).

The indolinone-derivative sunitinib, a multikinase inhibitor, with a broad selectivity spectrum targeting the active conformation of VEGFR, PDGFR, FGFR, KIT, and FLT3, was approved for the treatment of renal cancer as well as second-line therapy in imatinib-resistant GIST (66–68). Although sorafenib is a multikinase inhibitor like sunitinib, its kinase specificity, chemical structure and mode of binding are quite distinct from sunitinib. Sorafenib is a urea derivative that was originally designed to target the RAF kinase was found later to bind to the inactive conformation of the VEGFR kinase (69). Owing its potent activity against VEGFR and PDGFR, sorafenib was first approved for the treatment of renal cell and hepato-cellular carcinoma (70). Similarly, pazopanib, a 2-amino pyrimidine targeting VEGFR, PDGFR, and KIT has been approved for the treatment of advanced renal cell carcinoma (50) (Table 1).

Another important cancer target is the EGFR family of RTPKs, which comprises four members with various ligands, with a complex

signaling through their homo- or heterodimerization (71, 72). The 4-anilino-quinazolines gefitinib and erlotinib target the active conformation of the EGFR kinase, while lapatinib, a dual kinase inhibitor, binds to a particular inactive conformation of EGFR and ERBB2 (HER2/neu) (73–75). Both, gefitinib and erlotinib have been approved for the use in second- and third-line therapy for advanced NSCLC (73, 74). Interestingly, gefitinib demonstrated a much higher response rate and survival benefit in Asian than in American patients, due to specific activating mutations in the EGFR receptor in a small subset of the Asian patients conferring enhanced sensitivity to gefitinib (24). Recently, erlotinib in combination with gemcitabine has been approved for the treatment of patients with metastatic pancreatic cancer, while lapatinib has been approved for the treatment of a particular set of advanced or metastatic breast cancers overexpressing ERBB2 (74, 75). The clinical successes of these EGFR kinase inhibitors have been hampered by the fact that the late stage NSCLC displays many levels of cross talk along several pathways of signal transduction, allowing cancer cells to escape these types of targeted therapies in multiple ways.

The only Ser/Thr-specific kinase inhibitors approved to date are two immunosuppressant macrolide rapamycin derivatives (rapalogs) temsirolimus and everolimus, which without binding to the ATP-site block specifically the raptor function of mTOR (*m*ammalian *Target of R*apamycin), an atypical protein kinase that is the major downstream mediator of the PI3K/AKT pathway (21, 53). These two rapalogs are the most selective kinase inhibitors on the market as they target a particular allosteric site of the mTOR kinase that results in the abrogation of only one aspect of the many mTOR functions. The orally administered everolimus has been registered as an immunosuppressant for the treatment of transplant rejection in 2001 as Certican® (54). Temsirolimus as Torisel® was approved in 2007 for the treatment of renal cell carcinoma (76), while everolimus as Afinitor® has been registered for advanced metastatic renal cancer in 2008 (52, 77). Both rapalogs are currently being tested in multiple clinical Phase II/III studies in various cancer indications as single agents and in combination with other drugs (52, 77) (Table 1).

With the exception of the rapamycin derivatives, these first-generation kinase drugs bind to the hinge region of the target kinase and can be, therefore, considered broadly as ATP mimetics. Except for the two rapalogs and lapatinib, which display outstanding selectivity profiles, all of the other registered kinase drugs also have many other kinase targets as demonstrated by recently published comprehensive biochemical profiling (78, 79). Although these profiling data should be taken with a grain of salt, as not all of the biochemically identified protein kinases have been shown to be inhibited in cell based assays (80), they represent the most comprehensive selectivity profile with the broadest kinome coverage.