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DRUGS**

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KINASE INHIBITOR DRUGS

Edited by

RONGSHI LI
JEFFREY A. STAFFORD



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CONTENTS

PREFACE	ix
CONTRIBUTORS	xiii
 PART I GROWTH FACTOR INHIBITORS: VEGFR2, ERBB2, AND OTHER KINASES	 1
1 Discovery and Development of Sunitinib (SU11248): A Multitarget Tyrosine Kinase Inhibitor of Tumor Growth, Survival, and Angiogenesis	3
<i>Connie L. Sun, James G. Christensen, and Gerald McMahon</i>	
2 Tykerb Discovery: A Dual EGFR and ERBB2 Tyrosine Kinase Inhibitor	41
<i>Karen Lackey and G. Stuart Cockerill</i>	
3 Discovery of Pazopanib: A Pan Vascular Endothelial Growth Factor Kinase Inhibitor	57
<i>Philip A. Harris and Jeffrey A. Stafford</i>	
4 Road to ABT-869: A Multitargeted Receptor Tyrosine Kinase Inhibitor	79
<i>Michael Michaelides and Daniel H. Albert</i>	
5 Discovery of Motesanib	113
<i>Andrew S. Tasker and Vinod F. Patel</i>	

6	Discovery of Brivanib Alaninate: A Dual Vascular Endothelial Growth Factor and Fibroblast Growth Factor Receptor Inhibitor	131
	<i>Rajeev S. Bhide and Joseph Fargnoli</i>	
7	Structure-Based Design and Characterization of Axitinib	167
	<i>Robert S. Kania</i>	
PART II	GROWTH FACTOR INHIBITORS: MEK INHIBITORS	203
8	Road to PD0325901 and Beyond: The MEK Inhibitor Quest	205
	<i>Judith S. Sebolt-Leopold and Alexander J. Bridges</i>	
9	Discovery of Allosteric MEK Inhibitors	229
	<i>Eli Wallace and James F. Blake</i>	
PART III	CELL CYCLE KINASE INHIBITORS: AURORA KINASE AND PLK INHIBITORS	253
10	Discovery of MK-0457 (VX-680)	255
	<i>Julian M. C. Golec</i>	
11	Discovery of PHA-739358	281
	<i>Daniele Fancelli and Jürgen Moll</i>	
12	Discovery of AZD1152: A Selective Inhibitor of Aurora-B Kinase with Potent Antitumor Activity	309
	<i>Kevin M. Foote and Andrew A. Mortlock</i>	
13	Case Study of Aurora-A Inhibitor MLN8054	333
	<i>Christopher F. Claiborne and Mark G. Manfredi</i>	
14	Discovery of GSK461364: A Polo-like Kinase 1 Inhibitor for the Treatment of Cancer	351
	<i>Kevin W. Kuntz and Kyle A. Emmitte</i>	
PART IV	RELATED SPECIAL TOPICS	365
15	Pharmacogenomics of Dasatinib (Sprycel™)	367
	<i>Fei Huang and Edwin A. Clark</i>	
16	Practical Use of Computational Chemistry in Kinase Drug Discovery	403
	<i>James M. Veal</i>	
17	Approaches to Kinase Homology Modeling: Successes and Considerations for the Structural Kinome	433
	<i>Victoria A. Feher and J. David Lawson</i>	

CONTENTS	vii
18 Fragment-Based Drug Discovery of Kinase Inhibitors	461
<i>Daniel A. Erlanson</i>	
19 Protein Kinase Structural Biology: Methods and Strategies for Targeted Drug Discovery	485
<i>Clifford D. Mol, Kengo Okada, and David J. Hosfield</i>	
INDEX	501

PREFACE

The field of protein kinase drug discovery has advanced dramatically since the discovery of protein kinase activity in 1954 (G. Burnett, and E. P. Kennedy, *J Biol Chem.* **1954**, 211(2), 969–980). In a landmark paper published in 2002 in the journal *Science*, it was revealed that 518 protein kinases are encoded in the human genome (G. Manning, D. B. Whyte, R. Martinez, et al., *Science*. **2002**, 298, 1912–1934), and the “kinome” was catalogued according to sequence similarity in the catalytic domain and other protein structural comparisons. The protein kinases all share the same catalytic mechanism—the transfer of the terminal phosphate from an ATP molecule, an enzymatic reaction that we now understand controls many crucial cellular processes. With growth and proliferation pathways being regulated by protein kinase activity, the pharmacological inhibition of kinases has been an attractive option for many years. Nonetheless, owing to the high degree of overlapping homology and function, it is fair to acknowledge that, in the 1990s, the challenge to identify selective protein kinase inhibitors (PKIs) was met with a healthy dose of skepticism among industrial and academic drug discovery groups alike. The inhibition of protein kinases using small molecules took a step forward in 1994 when Parke-Davis scientists showed that 4-anilinoquinazolines in general and PD153035 specifically are potent inhibitors of epidermal growth factor receptor (EGFR) kinase, having concomitant cell-based effects on EGFR autophosphorylation (D. W. Fry, A. J. Kraker, A. McMichael, et al., *Science*. **1994**, 265, 1093–1095). The identification of the quinazoline scaffold opened the door for later discoveries leading to the launches of the important PKI drugs, gefitinib (IressaTM), erlotinib (TarcevaTM), and lapatinib (TykerbTM).

The impressive clinical success of the Bcr-Abl kinase inhibitor, imatinib (GleevecTM), in the treatment of chronic myelogenous leukemia (CML) validated the use of small molecules in the fledgling field of molecular targeted therapy (R. Capdeville, E. Buchdunger, J. Zimmermann, et al., *Nat Rev Drug Discov.* **2002**, 1(7), 493–502). With 2006 worldwide sales surpassing \$2.5 billion,

Gleevec has been rightfully hailed as a “poster child” for targeted cancer therapy and “cancer magic bullet” by drug discovery researchers in the protein kinase field. Its success provides compelling and unequivocal evidence that kinase inhibitors can be highly efficacious, especially in diseases caused by mutations of a protein kinase. The daunting task of discovering selective, ATP-competitive inhibitors has now been achieved in numerous cases, including those profiled in this volume. Since the approval of Gleevec in 2001, seven more PKIs have reached marketing approval (Figure 1), and it is estimated that there are presently well over 100 PKIs in clinical development. The worldwide market for kinase inhibitor drugs is expected to exceed US\$10 billion by 2011, and efforts to discover new inhibitors continue vigorously in both industry and academic laboratories.

Not surprisingly, the first marketed PKIs were witnessed in oncology, where kinase gene mutations or dysregulated kinase activity is often associated with hyperproliferation of cells, a hallmark phenotype of cancer. To date, there have been dozens of protein kinases implicated in human cancer, with undoubtedly more to be identified. Beyond the direct causal role of the Bcr-Abl kinase in CML, a noteworthy example is B-Raf kinase, a member of the Ras-Raf-Mek-Erk growth pathway. Somatic mutations in the B-Raf *gene* are observed in 66% of malignant melanomas and at lower frequency in a range of other human cancers (H. Davies, G. R. Bignell, C. Cox, et al., *Nature*. **2002**, 417, 949–954). Similarly, mutations in the phosphatidylinositol-3 kinase (PI3K) are associated with gastric and colon cancers (S. Velho, C. Oliveira, A. Ferreira, et al., *Eur J Cancer*. **2005**, 41(11), 1649–1654). Overexpression of the oncogene, human epidermal growth factor 2 (Her2, also known as erbB2), has been widely characterized in human breast carcinomas and its overexpression correlates with more aggressive disease (S. Menard, S. M. Pupa, M. Campiglio, et al., *Oncogene*. **2003**, 22(42), 6570–6578). These are only three examples among the dozens of protein kinases that have been implicated in human cancer to date, with undoubtedly more to be identified. In addition to oncology, kinase inhibitors are being investigated in other disease areas such as inflammation, diabetes, neuroscience, and virology.

Kinase Inhibitor Drugs represents our effort to compile, within a single volume, important discovery case studies of marketed kinase drugs and several of the more advanced kinase inhibitors. These case studies are authored by leading investigators and experts in the field of protein kinase research and provide a first-hand account of kinase inhibitor discovery. The reader is exposed to current thinking on kinase structure, biochemistry, and signaling. State-of-the-art technologies and tools such as structure-based and fragment-based drug discovery are also addressed in this book. The discoveries of the marketed inhibitors, sunitinib (Sutent™, Chapter 1) and lapatinib (Tykerb™, see Fig 1 Chapter 2), highlight a lineup of clinical-phase growth factor inhibitors, including several VEGFR2 kinase and multitargeted, antiangiogenic agents from investigators at Abbott, Amgen, BMS, GSK, and Pfizer. There are several chapters on inhibitors of the cell cycle kinases, Aurora and PLK, contributed

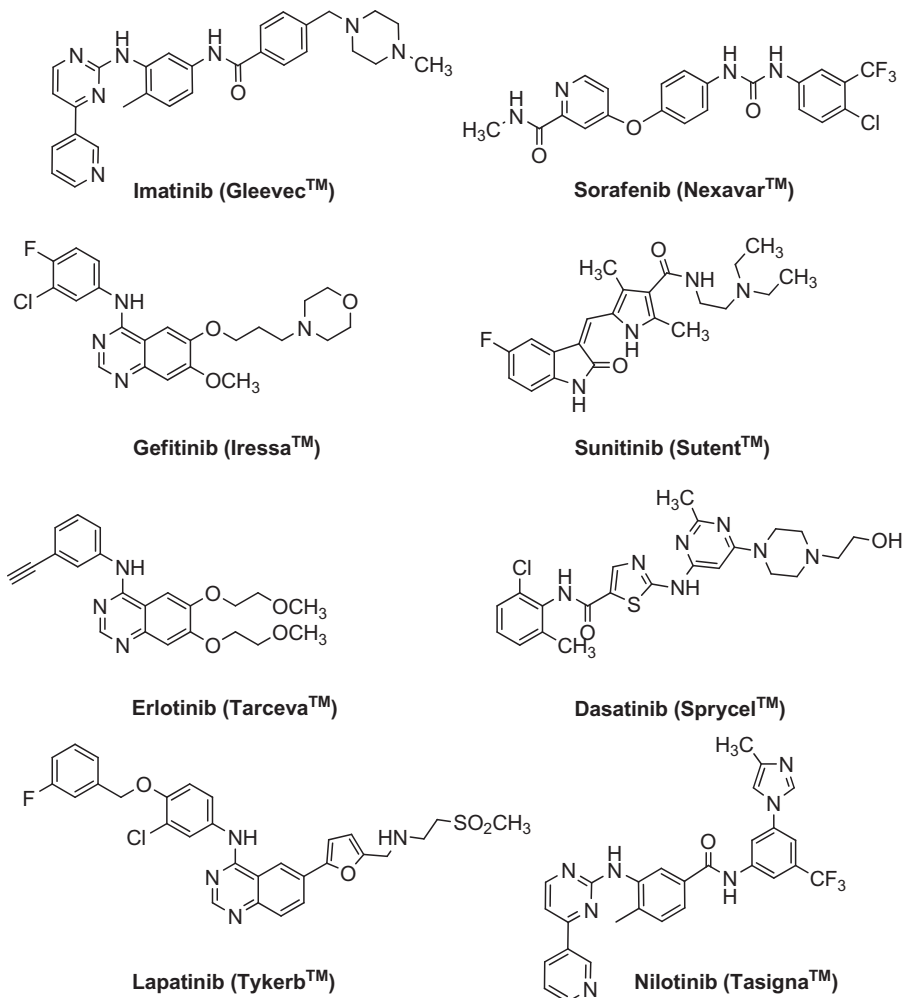


Figure 1. Kinase inhibitor drugs to reach market approval by the US Food and Drug Administration (2001–2008).

by investigators from AstraZeneca, GSK, Millenium, Nerviano, and Vertex. Additional case study chapters from Pfizer and Array Biopharma, respectively, are dedicated to the discovery of allosteric inhibitors of MEK kinase. Allosteric kinase inhibition engenders a kinase selectivity profile that is unequaled by inhibitors that occupy that ATP binding pocket. The field of pharmacogenomics and its application to kinase inhibitor clinical development is highlighted in the context of the marketed Src/Abl kinase inhibitor, dasatinib (Sprycel™). And finally, there are chapters dedicated to topics that are vital to efficient and intelligent kinase drug discovery, such as computational chemistry, fragment-based discovery, and methods in structural biology. We are grateful

to all of the authors for their dedication to this project, which we believe is unique even within the richly informed and documented field of protein kinase research. Our intent is that the present volume serves the reader as an informative text or supplementary reading on successful small-molecule drug discovery, specifically as it pertains to PKIs. The descriptions on contemporary applications of medicinal chemistry, including lead optimization and in vivo profiling, should find great interest among medicinal chemists and pharmacologists alike. Of course, it is fair to acknowledge that an undertaking of the present, limited scope will exclude informative case studies, including, notably, the Gleevec discovery story, case studies from the p38 MAPK inhibitor class, for example, SB203580 (A. M. Badger, J. N. Bradbeer, B. Votta, et al., *J Pharm Exp Ther.* **1996**, 279(3), 1453–1461) and BIRB796 (C. Pargellis, L. Tong, L. Churchill, et al., *Nat Structural Biol.* **2002**, 9(4), 268–272), and the search for second-generation inhibitors to kinases that have undergone active site mutation to become drug resistant (M. E. Gorre, M. Mohammed, K. Ellwood, et al., *Science.* **2001**, 293, 876–880; S. Kobayashi, T. Boggon, T. Dayaram, et al., *N Engl J Med.* **2005**, 352(8), 786–792).

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Color figures that supplement this book can be found at: ftp://ftp.wiley.com/public/sci_tech_med/kinase_drugs

RONGSHI LI, Ph.D.

Tampa, Florida

JEFFREY A. STAFFORD, Ph.D.

San Diego, California

August 2009

CONTRIBUTORS

Daniel H. Albert, Abbott Laboratories, Abbott Park, IL 60064

Rajeev S. Bhide, Research and Development, Bristol-Myers Squibb Company, Princeton, NJ 08543

James F. Blake, Drug Discovery, Array BioPharma, Boulder, CO 80301

Alexander J. Bridges, Oncovera Therapeutics, Inc., Ann Arbor, MI 48103

James G. Christensen, Pfizer Global Research and Development, La Jolla, CA 92121

Christopher F. Claiborne, Department of Oncology Chemistry, Millennium Pharmaceuticals, Inc., Cambridge, MA 02139

Edwin A. Clark, Oncology Clinical Biomarkers, Discovery Medicine & Clinical Pharmacology, Bristol-Myers Squibb Company, Princeton, NJ 08540

G. Stuart Cockerill, Arrow Therapeutics, London SE1 1DB, United Kingdom

Kyle A. Emmitte, GlaxoSmithKline R&D, Research Triangle Park, NC 27709

Daniel A. Erlanson, Sunesis Pharmaceuticals, Inc., South San Francisco, CA 94080

Daniele Fancelli, Congenia S.r.l. (Genextra Group), Via Adamello 16, I-20139 Milan, Italy

Joseph Fargnoli, Research and Development, Bristol-Myers Squibb Company, Princeton, NJ 08543

- Victoria A. Feher**, Department of Computational Sciences, Takeda San Diego, Inc., San Diego, CA 92121
- Kevin M. Foote**, Chemistry, AstraZeneca Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, United Kingdom
- Julian M. C. Golec**, Vertex Pharmaceuticals (Europe) Ltd, Oxfordshire OX14 4RY, United Kingdom
- Philip A. Harris**, GlaxoSmithKline R&D, Collegeville, PA 19426
- David J. Hosfield**, Department of Structural Biology, Takeda San Diego, Inc., San Diego, CA 92121
- Fei Huang**, Oncology Clinical Biomarkers, Discovery Medicine & Clinical Pharmacology, Bristol-Myers Squibb Company, Princeton, NJ 08540
- Robert S. Kania**, Pfizer Global Research and Development, San Diego, CA 92121
- Kevin W. Kuntz**, GlaxoSmithKline R&D, Research Triangle Park, NC 27709
- Karen Lackey**, Molecular Discovery Research, GlaxoSmithKline, Research Triangle Park, NC 27709
- J. David Lawson**, Department of Computational Sciences, Takeda San Diego, Inc., San Diego, CA 92121
- Mark G. Manfredi**, Department of Cancer Pharmacology, Millennium Pharmaceuticals, Inc., Cambridge, MA 02139
- Gerald McMahon**, Poniard Pharmaceuticals, Inc., South San Francisco, CA 94080
- Michael Michaelides**, Abbott Laboratories, Abbott Park, IL 60064
- Clifford D. Mol**, Department of Structural Biology, Takeda San Diego, Inc., San Diego, CA 92121
- Jürgen Moll**, Nerviano Medical Sciences S.r.l., Viale Pasteur 10, I-20014 Nerviano (Mi), Italy
- Andrew A. Mortlock**, Oncology, AstraZeneca Pharmaceuticals, Macclesfield, Cheshire SK10 4TG, United Kingdom
- Kengo Okada**, Department of Structural Biology, Takeda San Diego, Inc., San Diego, CA 92121
- Vinod F. Patel**, Chemistry Research and Discovery, Amgen, Inc., Cambridge, MA 02139
- Judith S. Sebolt-Leopold**, Oncovera Therapeutics, Inc., Ann Arbor, MI 48103
- Jeffrey A. Stafford**, Takeda San Diego, Inc., San Diego, CA 92121

Connie L. Sun, Poniard Pharmaceuticals, Inc., South San Francisco, CA 94080

Andrew S. Tasker, Chemistry Research and Discovery, Amgen, Inc., Thousand Oaks, CA 91320

James M. Veal, Structural Chemistry and Informatics, Serenex, Inc., Durham, NC 27701

Eli Wallace, Drug Discovery, Array BioPharma, Boulder, CO 80301

16

PRACTICAL USE OF COMPUTATIONAL CHEMISTRY IN KINASE DRUG DISCOVERY

JAMES M. VEAL

16.1. INTRODUCTION

Intense investigation of protein kinases as therapeutic targets has occurred over the past decade. The approval of Gleevec in 2001 for the treatment of leukemia validated kinases, more specifically their ATP-binding sites, as legitimate drug discovery targets. They now comprise a substantial fraction of the pharmaceutical discovery portfolio, particularly in the areas of cancer and inflammation. In coordination with the general increase in kinase discovery research has been an expansion in the application of computational chemistry, cheminformatics, and bioinformatics techniques to kinase drug discovery. Kinases as a target class are arguably the most favorable set of proteins for use of computational techniques. Although each is distinct, they nonetheless share broad overlapping features that allow learnings from past target efforts to be applied prospectively. The determinants of ligand binding to the ATP site are spatially concentrated, allowing for effective use of docking techniques, and the availability of hydrophobic pockets to target improves the probabilities of effective inhibitor design and modification. Selectivity considerations and, more recently, resistance have always been important in kinase drug discovery and modeling techniques have utility in evaluating and understanding these issues. Perhaps most importantly, protein kinases have proved highly amenable to crystallography and the wealth of X-ray data available has allowed for legitimate structure-aided drug discovery techniques to be employed.

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The goal of this chapter is to provide a survey of how computational techniques may be applied as part of a project team effort toward drug discovery. It incorporates specific examples from the literature as well as some personal experiences and viewpoints. It is beyond the scope of the chapter to be in any way comprehensive. In researching the chapter, PubMed searches were done for kinase inhibitors followed by secondary searches using keywords such as “modeling,” “docking,” “computational,” “structure-based,” and “structure–activity.” For late 2003 to the present or roughly the past 5 years, the number of references was in excess of 400! Instead, the hope is that the examples will illustrate how computational techniques can be effectively incorporated into the drug discovery paradigm. Particular objectives are to focus on how techniques can be used in a pragmatic time-bound manner and can be effectively integrated with the skills of other team members such as medicinal chemists and screening biologists.

16.2. OVERVIEW OF THE PROTEIN KINASE BINDING SITE AND CONFORMATIONAL VARIABILITY

16.2.1. Binding Site

The characteristics of the protein kinase ATP-binding site and its vicinity have recently been described in detail elsewhere (Liao, 2007; Liao and Andrews, 2007). Nonetheless, for the sake of completeness, a concise summary incorporating personal observations is provided here as well. As indicated, X-ray crystallography has been essential in understanding the range of possible conformations available for inhibitors to target (Marsden and Knapp, 2008). The insulin receptor bound to a nonhydrolyzable ATP analog (Figures 16.1 and 16.2) provides a starting reference point from which to understand binding modes (Hubbard, 1997). The protein is observed to be in a catalytically active conformation capable of phosphorylating a target peptide. The adenine moiety forms two hydrogen bonds, via N1 and exocyclic amine N6, to backbone peptide atoms that compose the “hinge” region of the protein. Over time, a jargon has developed around protein kinase structures and the hinge naming derives from the fact that it is a flexible linker region connecting the primary N and C terminal domains of the kinase. ATP and analogs reside in a binding cleft formed by these two domains with the adenine also being sandwiched by a series of hydrophobic residues both above and below the aromatic plane. The tetrahydrofuran portion of the ribose sugar also makes additional hydrophobic interactions, more so with the N-terminal domain region, and may engage in hydrogen bonding as well with a series of more polar residues that sit at the front of the binding site at the solvent interface.

It is worthwhile to note that the residues that form the adenine and ribose portions of the ATP-binding site are not conserved in sequence although some do show high homology. Instead, conserved residues occur in the

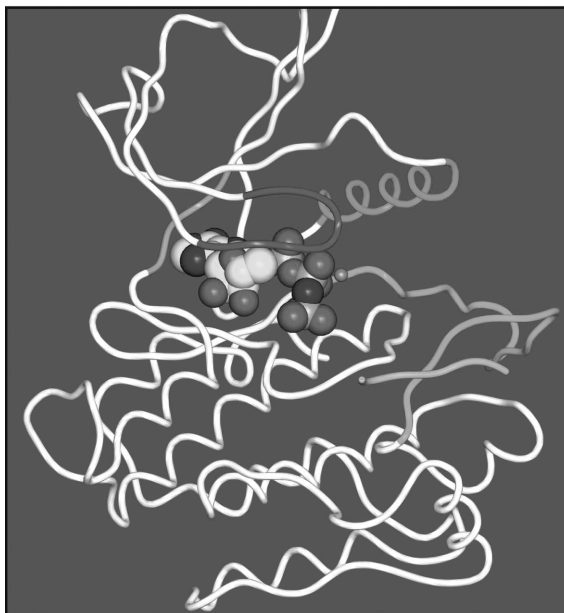


Figure 16.1. Representative protein kinase structure (Hubbard, 1997; pdb code 1IR3). ATP (nonhydrolyzable analog in this structure) binds into a cleft formed between the primary N (top) and C (bottom) terminal domains and engages in hydrogen bonding with the flexible hinge region (orange) that links the two domains. Key regulatory regions are the glycine-rich loop colored in magenta and draping over the phosphate-binding site; the activation loop colored in cyan; and the C-helix colored in green. A subset of kinase inhibitors target variations in conformation of these regulatory regions. Peptide inhibitor is shown in light green. (See color insert.)

phosphate-binding region (Figure 16.2), where they are responsible for both the correct phosphate group orientation and chemistry functions required for catalysis. A central question for protein kinases has always been the issue of attaining appropriate selectivity (Thaimattam et al., 2007), although increasingly what that means is less well defined. Regardless, strategies for modulating selectivity are apparent from analysis of ligand X-ray structures. A useful example to consider is that of the 4-anilinoquinazoline Tarceva bound to EGFR (Figure 16.2) (Stamos et al., 2002). Comparison to the insulin receptor structure shows that the ligand occupies substantial additional space relative to that occupied by ATP. In particular, the aniline moiety projects back into a hydrophobic region, past the so-called gatekeeper residue. This area is frequently targeted in kinase inhibitor design, shows sequence variability, and, although hydrophobic as indicated, also offers additional potential hydrogen-bonding opportunities. The gatekeeper residue derives its name because its steric size in part determines the availability of this back binding region. As can be seen, it is a methionine in IR versus a threonine in EGFR and the steric

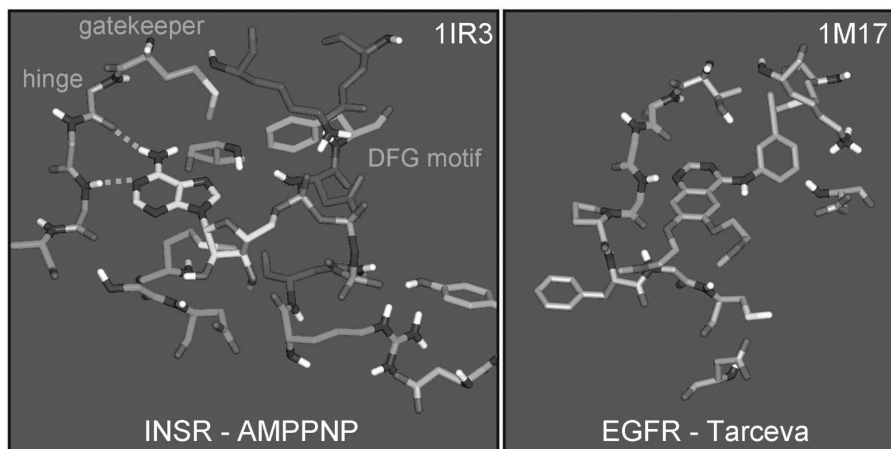


Figure 16.2. (Left) Close-up view of the insulin receptor ATP-binding site as determined by X-ray (Hubbard, 1997; pdb code 1IR3). Backbone atoms of the hinge region (orange) engage in hydrogen bonds with adenine N1 and exocyclic amine N6 while the adenine ring system lies sandwiched between hydrophobic residues. Conserved catalytic residues are shown in magenta. The phenylalanine of the DFG motif is highlighted in cyan and the conformation is DFG in. On bottom left a portion of an inhibitor peptide (light green) is shown with tyrosine hydroxyl group proximal to gamma phosphate of AMPPNP analog. (Right) EGFR-Tarceva active site as determined by X-ray (Stamos et al., 2002; pdb code 1M17). The anilino group projects into a back hydrophobic pocket made accessible by a small gatekeeper residue (Thr790). The catechol ether moiety of Tarceva also projects into a hydrophobic space, and then to solvent, at the front of the binding site. Both of these regions are unoccupied by ATP. (See color insert.)

bulk of the methionine side chain, if in an extended conformation, diminishes the ability to target this area. Even more pronounced examples are the CDKs and FLT3, which possess a phenylalanine at this position. The gatekeeper residue's importance to inhibitor binding is seen in the clinical manifestations of resistance to kinase inhibitors. T790M is a mutation (selected allele) that occurs in 50% of patients who initially respond to Tarceva and then relapse (Pao et al., 2005; Balak et al., 2006). Similarly, the T315I mutation in BCR-ABL confers resistance to Gleevec (Gorre et al., 2001).

In addition to the back hydrophobic pocket, there is also a second hydrophobic region that occurs in front of the binding site as shown in Figure 16.2, off the ATP C2 and N3 positions. Almost all kinases have a strongly hydrophobic residue (Ile, Val, Leu, Met, Phe, etc.) that forms the top of this site (the residue is not shown in the figure to keep from obscuring the ligand). The catechol ether moiety of Tarceva utilizes this binding area. This front region also shows a series of variable polar residues, proximal to the ribose hydroxyl's in ATP-bound structures, and additional affinity and selectivity may be gained by appropriate interactions. Another point is that the front of the site, where

the ends of the ether linkages of Tarceva project, is solvent exposed. Structurally related quinazoline analogs (e.g., Iressa, Zactima, Tykerb) as well as a wide range of other ligand classes incorporate an amine basic functionality in this vicinity for solubility and modulation of physicochemical properties. An exception to the above statements is the AGC family kinase members (PKA, AKT, etc.), which largely close off the front hydrophobic region through insertion of a phenylalanine residue from the end of the protein C-terminal domain.

16.2.2. Conformational Variability

Because of their central role in cellular signaling pathways, it is not surprising that protein kinases are extensively regulated. From a structural viewpoint, at least three distinct mechanisms are available to provide appropriate regulatory control, and targeting the resulting conformational states has been a central strategy in the design and optimization of kinase inhibitors (Bogoyevitch and Fairlie, 2007). The first mechanism is modulation of the conformation of the glycine-rich loop region of the N-terminal domain (Figure 16.1). Phosphorylation (e.g., CDK2 by Wee1) and interactions of Hsp90 with cochaperone CDC37 are examples of endogenous regulation (Den Haese et al., 1995; Terasawa et al., 2006). Owing to its inherent conformational plasticity, the glycine-rich loop has been observed to mold to various ligand inhibitors with a recent example being a pyrazolopyridazine analog targeting ERK2 (Kinoshita et al., 2006).

Perhaps the most important mode of regulation is via the “activation loop” (T-loop) that spans from a consensus DFG motif at the back of the ATP-binding site to residues just upstream of a second, albeit lesser conserved, APE motif. This loop undergoes a wide range of conformational motions spanning from the “DFG-in” conformation seen for insulin receptor to a “DFG-out” orientation in which the phenylalanine may dock into the ATP-binding region and autoinhibit the kinase. The activation loop is a primary site of regulatory phosphorylation and is central to the trans-activating autophosphorylation mechanisms that occur when receptor tyrosine kinases bind their peptide ligands and homo- or heterodimerize. The DFG-out conformation is targeted by a range of inhibitors including Gleevec and BIRB 796 (Figure 16.3) (Schindler et al., 2000; Pargellis et al., 2002). A common feature for these inhibitors is a diaryl amide, urea, or related isostere; one aromatic group binds into the region vacated by the phenylalanine and the amide/urea functionalities form multiple hydrogen bonds involving the conserved glutamate from the kinase C-helix (Figure 16.1) and backbone NH of the aspartic acid of the DFG triad. Portions of the binding regions occupied by these ligands are now well removed spatially from the area occupied by ATP. Different kinases seem to exhibit a range of propensities for adopting the DFG-out conformation, and it is important to view the range of conformations available to the activation loop as an equilibrium-based phenomenon in which the conformational energy to adopt any one orientation will be an essential consideration as to its likelihood of occurring and being targeted by an inhibitor.

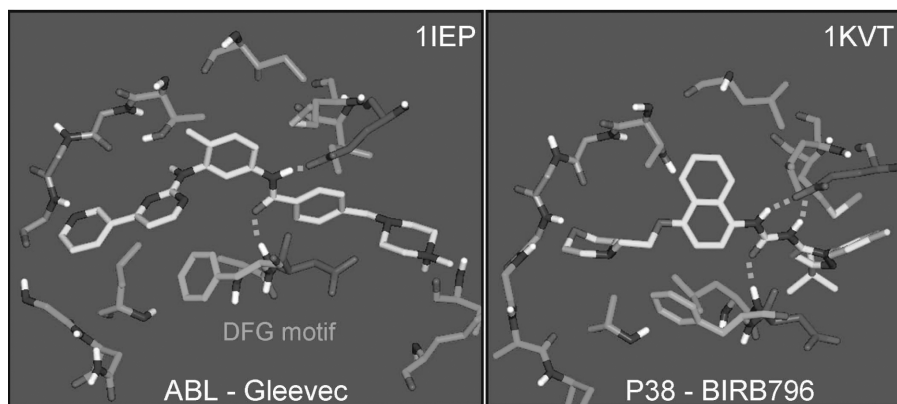


Figure 16.3. (*Left*) ABL–Gleevec structure as determined by X-ray crystallography (Schindler et al., 2000; pdb code 1IEP). The structure exemplifies the DFG-out kinase motif in which the phenylalanine is relocated from its position in a catalytically active kinase to, in this case, a position roughly adjacent to where the ATP ribose would be. The phenyl amide moiety occupies the region where the phenylalanine normally resides and makes hydrogen bonds to both the backbone NH of the DFG aspartate and carboxyl group of the catalytic glutamate from the C-helix. The piperazine group reaches solvent on the backside of the protein. (*Right*) P38–BIRB796 structure as a second example of the DFG-out binding motif (Pargellis et al., 2002; pdb code 1KVT). A *tert*-butyl-pyrazole-urea functionality provides pharmacophore elements analogous to the phenyl-amide group of Gleevec. Because of the strong interactions with the allosteric binding region, the ligand has a diminished need for strong interactions in the adenine-binding region, which is occupied by the morpholino group. (See color insert.)

A third mechanism of regulatory control involves modulation of the C-helix, which contains a conserved glutamic acid residue responsible in part for maintaining appropriate phosphate orientation prior to catalysis. Endogenous regulation (e.g., of Src kinase or CDK2) involves this helix being in an inactive form in the absence of appropriate factors such as cyclins. Recently, examples have emerged of inhibitors that likewise target an inactive C-helix formation. Notably, the dual EGFR/Her2 inhibitor Tykerb targets, at least for EGFR, a displaced C-helix conformation (Wood et al., 2004). Another class of allosteric ligands, which inhibit MEK kinase, exert this effect in part by again binding to a conformation in which the C-helix is inappropriately aligned for catalysis (Ohren et al., 2004). As with the activation loop, C-helix conformational changes represent an equilibrium process and as such they may or may not be readily targeted for a particular kinase.

16.2.3. Summary on Binding Site and Conformational Flexibility

Two general points emerge from the brief description just given. First, there are multiple regions of the protein kinase in the vicinity of the ATP-binding

site that may be targeted for improved affinity once a core scaffold of interest is identified. Additionally, the conformational variability of certain regions of the kinase structure, owing largely to regulatory mechanisms, provides an additional strategy for gaining both potency and selectivity beyond just targeting specific amino acids. A conceptual point is that there is sometimes a frustration among researchers that the high concentration of ATP in the cell (low millimolar, mM) makes the hurdles of achieving cellular activity and in vivo efficacy more difficult due to simple competitive effects. Binding affinities (K_d) of 100 nM or better are often required in order to begin to see low micromolar (μ M) or better cellular activities. The nature of the enzymatic assay, for example, IC_{50} only determination (vs. a true K_d) at low ATP concentrations, may further confuse the situation. However, it is argued here that the presence of a high intracellular ATP concentration ultimately is really a boon for the kinase drug discovery effort. Concomitant with the high ATP concentration is that the K_m for ATP is low (typically 20–100 μ M) for protein kinases, meaning that there is no strong natural pressure to have an optimized binding site for ATP for a given kinase. Thus there is high variability and “looseness” to the ATP-binding site in protein kinases and these properties can be properly exploited for the design of good inhibitors.

16.3. APPLICATION OF COMPUTATIONAL TECHNIQUES WITHIN THE DRUG DISCOVERY PROCESS

16.3.1. Overview

The goal of this main section is to provide recent examples of how different computational techniques have been utilized within the scope of the standard drug discovery process (Figure 16.4). For the discussion here, the process today

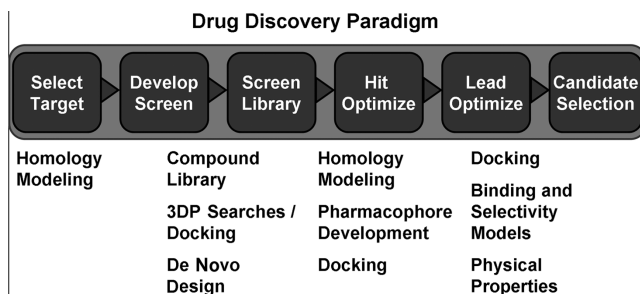


Figure 16.4. Overview of the typical process for a drug discovery program. The types of computational techniques that are most likely to be applied at different steps in the process are indicated. As described in the text, computational methods are particularly useful in developing libraries or novel templates for screening, and at the hit and lead optimization steps.

generally begins with identification of a target of interest via emerging literature or internal biological data. There are strong personal feelings as to whether this is the best strategy (or should be the only strategy), and these are a subject of a different discussion, but the “target-based approach” seems to have become a widely engrained methodology in the era of the human genome effort. In order to prosecute a target, a screen is then set up; the screen may vary from an enzymatic assay designed to assess a limited or focused set of compounds to a high-throughput screen (HTS) intended for an entire compound collection. Both types may actually be run with the focused screen typically providing early data and the HTS, which generally takes longer to set up, providing subsequent data. If successful, the screen will provide a series of compounds of interest or hits that show affinity for the target of interest and provide the initial input into medicinal chemistry efforts. Chemical analysis and exploration of these hits yields molecules that now possess more impressive qualities and can be termed leads. The hit versus lead distinction is arbitrary, and a lead definition can range from something having cellular activity to *in vivo* activity (pharmacokinetic or efficacy). Practically, multiple hits, each with limited medicinal chemistry evaluation, triage to one or two leads that are subjected to extended medicinal chemistry exploration. If all hurdles are met, one of the leads successfully advances to candidate status and enters development. What follows is how computational techniques may be applied within each of the above domains of the discovery process.

16.3.2. Target Selection

The view here is that, overall, computational techniques have relatively limited impact at the target selection stage for protein kinases. The chief technique that may be employed is binding site evaluation via either analysis of X-ray data or homology modeling, if such data are unavailable. As a general note, homology modeling, as defined here, is a term meant to include the underlying bioinformatics components of sequence alignment and so on in addition to the computational chemistry component of protein model building. Homology modeling and binding site assessment generally can allow for informed evaluation of the likely tractability of a target for small molecule inhibitor design. Since the central ATP-binding site of protein kinases is well established as a target for small molecules, this utility is not relevant. A possible use is to assess whether a kinase should be pursued as a target based on its homology to related kinases that could clearly be viewed as off-targets. Again, it is thought that this effort is generally not productive; a desirable biological target will be pursued regardless of selectivity issues and usually these issues can at least to some degree be overcome. A case in point is the effort to discover selective small molecule kinase inhibitors for the cancer target IGF-1R despite its high homology to insulin receptor; such efforts appear to have succeeded to a reasonable degree (García-Echeverría et al., 2004; Ji et al., 2007). Homology models and underlying sequence alignments are useful at this stage of discovery in flagging up what likely may be

off-targets to be concerned about and this effort may in turn allow early development of relevant counterscreens.

16.3.3. Focused Compound Collections, Library Design, and Novel Scaffolds for Screening

The design and selection of compound sets for screening has proved to be an area of high value for the application of computational chemistry and cheminformatics techniques. There are multiple reasons for finding particular utility here. First, the adenine moiety of ATP provides a defined scaffold from which to derive a range of core templates, which can then be incorporated into two-dimensional (2D) descriptor-based searches. This comment can actually be a bit more formalized to state that the protein kinase ATP-binding pocket provides a well-defined pharmacophore target in terms of a hydrophobic cleft, designed for an aromatic ring system, and an adjacent acceptor–donor–acceptor hydrogen-bonding motif afforded by the hinge region. The majority of kinase inhibitors, including many that target the DFG-out motif, contain an aromatic adenine isostere and utilize some element of hydrogen bonding to the hinge region. This motif leads to a list of templates as exemplified in Figure 16.5 as being suitable substructures around which to base library design and acquisition efforts. Additionally, the kinase-binding site beyond the hinge

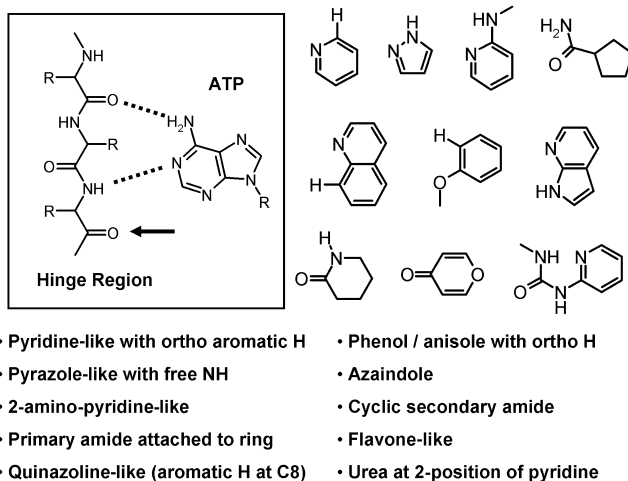


Figure 16.5. Representative substructures that can be used in developing screening libraries. The structures all have precedent as adenine mimetics for protein kinases. They offer the potential to satisfactorily replace the two hydrogen bonds formed by ATP to the backbone groups of the kinase hinge region. Sufficiently polar aromatic hydrogens may act as isosteres for the exocyclic N6 amino group of ATP. Note also that combining or substituting the various mimetics allows for targeting an additional backbone carbonyl group in the hinge region that is well positioned for hydrogen bonding.

region, as indicated in the binding site overview, is relatively well defined owing to the extensive X-ray crystallography efforts directed at kinases. Given that, for screening, the goal is generally to identify hit templates in the 100 nM to 5 μ M range, this information can be generalized and incorporated into docking or pharmacophore studies, including ones that are more three-dimensional (3D) in their nature. There is always a trade-off between the time, resources, and quality of results when comparing 2D descriptor searches versus 3D based full docking. Appropriate consideration of the goals and timelines is essential in evaluating which techniques are appropriate for the given situation.

Two side points are worth mentioning here as well. First, a somewhat unusual feature of the interactions between inhibitor and hinge region is that it frequently involves aromatic hydrogens from the ligand interacting at short distance (<2.5 Å) with a backbone carbonyl oxygen from the hinge region. The definition of this interaction is not formally a hydrogen bond, but the aromatic hydrogens are, nonetheless, also typically more “acidic” in nature as they are usually bonded to carbons that are in turn ortho, or otherwise polarized, by aromatic nitrogens. These interactions have recently been surveyed in some detail (Pierce et al., 2002; Tóth et al., 2007). A second note is that the emergence of DFG-out and inactive C-helix targeted ligands has diminished the absolute importance of having an adenine mimetic as part of the scaffold. Still, DFG-out ligands at least have their own defined pharmacophore as well, the aryl-amide and urea-type motifs, which may in turn be incorporated into design principles. Moreover, an attractive selectivity strategy (molecular weight considerations withstanding) is to attempt to span the full adenine and DFG-out binding regions with an inhibitor. If good hydrogen bonds that are remote in distance can be achieved, the prospects for selectivity are enhanced as even subtle conformational changes between two kinases will likely lead to a detuning and reduced value of such hydrogen bonds for the off-target.

16.3.3.1. Focused Screening. Kinases are particularly good targets for focused screening for the reasons outline previously. Corporate and academic focused screening sets are now common and are designed, augmented, and/or maintained via computational methods (Prien, 2005; Brenk et al., 2008). These methods typically cull and cluster proven inhibitors as well as incorporate new templates from internal synthetic efforts and external compound acquisition. A well-designed compound set that is on the order of 10–50K compounds should produce hits and can usually be screened rapidly, much more so than an HTS, which involves significant setup efforts and longer times to screen, analyze, and follow-up. However, these arguments are in no way meant to disparage HTS as a valuable mechanism for kinase hit discovery. Hit rates should be substantially lower, but an HTS may produce more unusual or novel templates that perturb the kinase protein in unexpected ways and thus provide valuable new chemical matter. This matter can then be iteratively incorporated

into new focused library designs. Thus focused screening and HTS are viewed as fully complementary and their combined and integrated use provides the highest probability for success. Virtual screening, in which a database is searched and evaluated, leading to a specific screening set for a specific target is now also routinely employed as a method to identify hits or starting compounds of interest for a given kinase. The methodology used may involve direct docking of compounds into a kinase X-ray or model structure or the development of a 3D pharmacophore model that is then used to search a database.

There are numerous representative examples from the literature since 2005 where computational methods have been used to provide a focused compound set for screening with subsequent identification of hits. Virtual screen docking was combined with a novel chromatography and mass spectrometry based screening technique to identify inhibitors of EphB2 (Toledo-Sherman et al., 2005). In silico docking using a receptor grid strategy produced a pyrazolo-pyrimidine as an EGFR inhibitor (Cavasotto et al., 2006). Chk1 inhibitors were identified by docking compounds to the ATP-binding site followed by selected screening of top scoring compounds (Foloppe et al., 2006). High-throughput docking led to CDK inhibitors, which were subsequently optimized into isoform selective binders (Thomas and McInnes, 2006) in one study and optimized for selectivity over GSK-3 in another effort (Richardson et al., 2007). High-throughput docking also was used to discover additional inhibitors for CDK2 as well as Plk1 and CK2 (Golub et al., 2008; Taylor et al., 2008). A 3D pharmacophore model was used to screen a corporate database and identified previously unreported scaffolds that targeted the DFG-out binding conformation of p38 (Angell et al., 2008b). Similarly, CDK2 and GSK-3 inhibitors were found via 3D pharmacophore models followed by virtual screening (Kim et al., 2007; Kim et al., 2008; Taha et al., 2008). Development of pharmacophore models for Src based on known inhibitors, followed by commercial database searches, yielded 1,3,4-thiadiazoles and pyrazolodine-3,5-diones as new inhibitors (Manetti et al., 2006). A detailed homology model for RSK1 was used to find inhibitors from the NCI repository (Nguyen et al., 2006). Receptor models were also utilized to identify compounds for synthesis that would bind to KGFR, yielding cell active analogs (Hackett et al., 2007). Aurora kinases have been cancer targets of much interest and fragment-based virtual screening followed by synthesis led to a pyrrolopyrimidine inhibitor (Warner et al., 2006), whereas a virtual screen of a 60K compound collection produced a 6,7-dihydro-4*H*-pyrazolo-[1,5-*a*]pyrrolo[3,4-*d*]pyrimidine-5,8-dione novel scaffold (Coumar et al., 2008). Pyrazolo[1,5-*a*][1,3,5]triazines were identified as a CK2 binding scaffold via computational screening methodologies and subsequently optimized to potent inhibitors (Nie et al., 2007). Recently, energy-based docking strategies yielded EphB4 inhibitors (Kolb et al., 2008a,b), and high-throughput docking to Pim-1 kinase successfully provided multiple hits on which to initiate optimization efforts (Pierce et al., 2008).

16.3.3.2. Novel Scaffold Identification. An increasing role for computational chemistry in the realm of kinase drug discovery is to assist in the design of novel scaffolds. The intellectual property environment for kinases is highly competitive, increasingly crowded, and now a central issue in discovery efforts. One method for addressing this challenge is to design and synthesize novel templates, including so-called scaffold morphing or hopping, and as such de novo design has arguably been used more in the kinase target space than in any other protein systems class. As with computational screening methods, there have been multiple publications in the last three years that demonstrate practical application. Scaffold morphing from a purine scaffold led to novel triazolo[1,5-*a*]pyrimidines targeting CDK2 (Richardson et al., 2006); similarly, migration of a thiazole core template to a pyrimidine based on matching electrostatic characteristics yielded new inhibitors, one of which ultimately progressed to clinical trials (Chu et al., 2006). New granulatimide analogs and macrocyclic pyridyl ureas, the latter using a *cis*-amide bond as an adenine isostere, have been designed as Chk1 inhibitors (Conchon et al., 2007; Tao et al., 2007). A macrocyclic design strategy based on X-ray data was also used to identify a novel constrained pyrazolo[1,5-*a*] [1,3,5]triazine inhibitor of CK2. For GSK-3, modeled alterations to a VEGFR2 furo[2,3-*d*]pyrimidine scaffold converted it to a GSK-3 inhibitor (Miyazaki et al., 2008) and de novo design yielded a 7-hydroxy-benzimidazole scaffold (Shin et al., 2007). Multiple p38 targeted novel scaffolds have been derived from assessment of BIRB 796 binding followed by chemical modification (Goldberg et al., 2007; Cogan et al., 2008; Montalban et al., 2008).

New scaffold design has also recently been directed at receptor tyrosine kinases. For ALK, one strategy that was successfully employed to identify novel pyridone inhibitors was to develop models that could identify known inhibitors and then apply that model to virtual libraries to prioritize them for syntheses (Li et al., 2006). Overlapping of distinct compounds into an X-ray structure allowed combining them to yield a modified oxindole FGF1R inhibitor (Kammasud et al., 2007). VEGF receptors have been the focus of much investigation with a range of new scaffolds, derived using structure and modeling components, being reported (Tripathy et al., 2006; Uno et al., 2008; Abou El Ella et al., 2008; Peifer et al., 2008). Finally, structure-guided modeling aided in the elaboration of a novel pyrimido-diazepine core scaffold that produced potent inhibitors of VEGFR2, Flt3, and c-Kit (Gracias et al., 2008).

16.3.4. Hit Optimization

After the screening process is complete, there will typically be a range of hits, hopefully with some diversity in structure that will provide initial input into medicinal chemistry efforts. The goal of the computational chemist at this point is to develop plausible models that can provide insight into binding modes, offer strategies for optimization, and suggest routes to pursue to improve selectivity if that is an issue. The goal is not to provide the designed candidate

molecule; actually that should never be the goal, rather it should be to facilitate the discovery process that leads a project team to a candidate molecule.

A central initial objective, if not done already as part of the screening process, is to develop reasonable protein models in which to dock ligands. The degree of effort needed is largely dependent on the X-ray data available. The possibilities range from the need to develop full homology models for the kinase of interest, to developing a DFG-out model when a DFG-in X-ray is available (or vice versa), to developing a series of models that take into account possible side chain rotamers of key residues (e.g., gatekeeper or catalytic lysine). There is a range of software available that offer the potential to build homology models in an automated fashion. That is fine and they may facilitate the process, but the homology model is a case where the computational chemist should put in detailed and often manual effort to develop as accurate as possible models. This effort requires thorough review of the available protein structures. Typically, the model will be based off one primary structure; however, significant components may best derive from alternative structures and evaluation of local homology is thus as important as global homology. The same rules apply to evaluation of side chain rotamer populations. The rationale for expending significant effort toward the development of models is that it is by and large a one-time effort for a given project; the week or two of effort to derive good models will be well served over the course of a two year medicinal chemistry discovery program.

Following development of the models as necessary, binding modes for compounds are then developed. The goal should be to establish all viable models and, if there are multiple plausible binding modes, strategies for distinguishing them. Because kinase pharmacophores are often rich in aromatic nitrogens and amine groups, there are often multiple legitimate binding models, and it is much preferred to present the variations until there is confidence about the correct model. An example of the potential pitfalls is a 3-amino-pyrazole series, which showed alternative binding modes to CDK2 depending on substituent patterns (Sato et al., 2006). Similarly, a pyrazolylpyrrole showed significantly different binding modes to ERK and JNK3 (Aronov et al., 2007), and the phenomenon of a kinase-dependent binding mode was also recently reported for the binding of an anilino-bipyridine to JNK1 and JNK3 (Swahn et al., 2006).

A particularly valuable objective, if it can be achieved, is to understand how different hit molecules overlap one another, as often that may provide rapid strategies for combining features into a single molecule with enhanced novelty and/or potency. The framework of a binding site is especially helpful as it is common to derive overlaps using protein structural information that would not necessarily be the most intuitive or computationally preferred in the absence of structural guidance. Table 16.1 provides a representative sampling of the recent use of hit optimization techniques to prospectively aid kinase programs. The designation of hit optimization versus lead optimization, as already noted, is somewhat arbitrary but the aim here was to identify efforts

TABLE 16.1. Summary of Representative Recent Literature References Where Computational Chemistry Techniques Were Applied to the Hit Optimization Process

Kinase	Core Scaffold	Reference	Comment
Aurora-A	Indazole-benzimidazole	Poulsen et al. (2008)	Improved potency from substitutions
B-RAF	Diaryl-imidazole	Wolin et al. (2008)	Incorporation of urea to target both ATP and allosteric binding sites
EphB4	Dianilino-pyrimidine	Bardelle et al. (2008)	Optimization from merging based on overlap of pyrimidine and quinazoline hits
CaMKII δ	Pyrimidine	Mavunkel et al. (2008)	Improved potency from substitutions
MK2	Indole	Xiong et al. (2008)	Improved potency from substitutions
Abl	Pyrazolo[3,4- <i>d</i>]pyrimidines	Manetti et al. (2008)	Improved potency from substitutions
CDK1/GSK3	9-Oxo-thiazolo[5,4- <i>f</i>]quinazoline	Loge et al. (2008)	Binding model to identify target positions for substitution
Chk1	Biphenyl pyrazole	Teng et al. (2007)	Modifications to maintain enzyme binding but improve cell activity
Abl	Benzotriazine	Cao et al. (2007)	Targeting C-helix glutamic acid
Chk1	Pyrazoloquinolinone	Brnardic et al. (2007)	Improved potency and solubility from substitutions
CDK5	Quinolin-2(1 <i>H</i>)-one	Zhong et al. (2007)	Improved potency using active site homology model
VEGFR2/TIE2	Benzimidazole	Hasegawa et al. (2007)	Modeling to understand and guide SAR
Lck	Pyrimidine	Bamborough et al. (2007)	Binding models to guide indazole isostere design for improved PK
EGFR/Her2	Pyrrolotriazine	Mastalerz et al. (2007)	Modeling to understand and guide SAR

TABLE 16.1. *Continued*

Kinase	Core Scaffold	Reference	Comment
ROCK II	Indazole	Iwakubo et al. (2007)	Further optimization of designed scaffold
p38	Benzimidazolone	Hammach et al. (2006)	Energy calculation-based modifications to improve potency
CDKs	Quinoxalin-2-one macrocycle	Kawanishi et al. (2006)	Improved potency from substitutions
VEGFR2	1,4-Dihydroindeno[1,2- <i>c</i>]pyrazole	Dinges et al. (2006)	Modeling to understand and guide SAR
IKK β	Thienopyridine	Morwick et al. (2006)	Pharmacophore model to aid transition to more tractable scaffold
TrkA	Isothiazole	Lippa et al. (2006)	Improved potency from substitutions
FAK	7 <i>H</i> -Pyrrolo[2,3- <i>d</i>]pyrimidine	Choi et al. (2006)	Improved potency from substitutions
EGFR	Anilino-quinazoline	Ballard et al. (2006)	Guided design of compounds targeting ribose pocket
PKB	Isoquinoline-5-sulfonamide	Collins et al. (2006)	Identification of potent substituents via screening virtual libraries
CDK2/CDK4	Pyrazolopyrimidinone	Rossi et al. (2005)	Improved potency from substitutions; selectivity SAR
CDKs	Imidazo[1,2- <i>a</i>]pyridine	Hamdouchi et al. (2005)	Improved selectivity from substitutions
VEGFR2	Aryl-oxazole	Harris et al. (2005)	Improved selectivity from substitutions
CDK1	Alsterpaullone	Kunick et al. (2005)	Improved potency from substitutions
PKB	Azepines	Breitenlechner et al. (2005)	Improved selectivity from substitutions, relative to PKA

that involved more hit series overlap and early optimization prior to the point of in vivo efficacy and other later stage type studies.

16.3.5. Lead Optimization

At the point lead optimization is initiated, there are typically one to two front running series that demonstrate structure–activity relationships that are presumably positive and tractable. From the computational chemistry perspective, there are hopefully well-defined binding models or sets of models that can be used to assist in optimization. Optimization should not be assumed to be synonymous with increased potency but may also reflect improving physicochemical properties (e.g., solubility or lowered molecular weight) while maintaining potency. Selectivity issues may also be an ongoing consideration that can be improved through an understanding of binding modes. Identifying strategies for introducing conformational restraint, to improve potency or other properties, is also an area where models are valuable.

High-throughput docking, or more specifically restrained docking, is a particularly useful computational technique to apply to the process of lead optimization. The scenario that occurs frequently in the lead optimization process is as follows. There is a lead molecule whose binding mode is reasonably well established. Medicinal chemists have identified a series of synthetic schemes that allow optimization at distinct positions on the molecule. The chemistries involved are sufficiently robust and involve starting materials that are widely available, say, amines or carboxylic acids or aromatic bromides. The objective for the computational chemist should be to first help in prioritizing which substitution positions are likely to be most beneficial and then, in cases where reagents are widely available, to prioritize a set of analogs for synthesis. Reagents such as carboxylic acids and amines number in the thousands of commercially available analogs, and even robust parallel syntheses procedures require reduced numbers of such analogs. Docking procedures thus require several steps. First, in silico synthesis of analogs of interest from the lead template and reagent class is carried out. Second, the product molecules are docked in a flexible but restrained mode, for example, based on established hydrogen bonds to the hinge region of the core scaffold. Conformational searching of the substituent region and possibly the entire molecule should then be pursued followed by minimization of preferred conformers. Scoring of different possible analogs is a subject of much literature incorporated in the referenced examples below. However, the personal view is that docking or scoring energies should be used as a filter to winnow down possible analogs of interest to a number on the order of 20% of the original possibilities and from that point both binding energies and visual inspection, often in conjunction with medicinal chemists, should be used to guide selection of the final set of analogs to pursue synthetically.

Recent examples of the use of computational techniques in lead optimization span a range of kinase targets and include reports on molecules now undergoing clinical development. Pan receptor tyrosine kinase profile

inhibitors are an area of active investigation; docking of thienopyrimidine and indazole core scaffolds helped guide optimization of urea moieties targeting the adjacent allosteric pocket of RTKs, leading to a clinical candidate (Dai et al., 2005, 2007). Discovery of a Src and VEGFR2 dual inhibitor clinical candidate incorporated design strategies for making interactions with the conserved C-helix glutamate (Palanki et al., 2008). For VEGFR2 specifically, modeling aided successful transition from a benzimidazole template to an optimized benzoxazole that showed in vivo activity (Potashman et al., 2007). Computational overlaps of quinazoline and pyrimidine scaffolds and subsequent docking studies also facilitated discovery of a VEGFR2 clinical candidate (Harris et al., 2008) as did modeling efforts to identify optimal locations for modifications to improve solubility and PK aspects of a pyrrolo[2,1-*f*][1,2,4]triazine (Bhide et al., 2006). Achieving selectivity for LCK tyrosine kinase over VEGFR2 for a pyrimido[1,2-*a*]benzimidazol-5(6*H*)-one template was accomplished through structural analysis of binding modes followed by targeting the gatekeeper threonine of LCK (Martin et al., 2008). IGF-1R computational models were used in discovery and optimization of orally active imidazo[1,5-*a*]pyrazines that utilize a quinoline to target the back binding region (Mulvihill et al., 2008). For serine–threonine kinases, computational techniques have also been recently applied in lead optimization. For example, docking studies identified substitution at the 8-position of a cyano quinoline template as a way to successfully establish selectivity for Tpl2 kinase versus EGFR (Green et al., 2007). Use of polar surface area calculations led to improvement in cellular potency of an indolylquinolinone scaffold targeting Chk1 (Huang et al., 2006). CoMFA models were used to help prioritize synthesis efforts targeting the MEK allosteric inhibitor site (Spicer et al., 2007), and binding models aided in optimization of biphenyl amides as allosteric inhibitors of p38 (Angell et al., 2008a).

16.4. TWO APPLICATION EXAMPLES

Two brief examples are provided from personal experience. One involves general design of a small focused screening library from commercial vendors. The library was intended for purine-binding proteins of which protein kinases are a major subset. The second effort involves lead optimization efforts toward CDK2 involving an oxindole scaffold.

16.4.1. Focused Library Development

The objective was to select and acquire from commercial vendors a quality set of 5–10K compounds with a biased likelihood for targeting the purine-binding proteome. The core methodology was to first select a comprehensive set of compounds that were sufficiently like a “modified” adenosine (adenosine plus a phenyl group to provide additional hydrophobicity). From that set, a maximally diverse and representative subset was then chosen. The methodology is outlined in Figure 16.6.

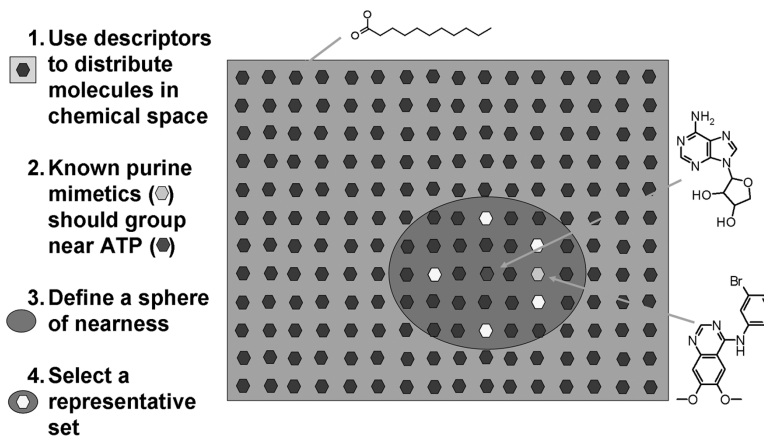


Figure 16.6. General schematic outlining strategy for development of a focused screening library. Descriptors are developed to allow distribution of compounds (blue hexagons) into a dimensional chemical space. If descriptor design is done successfully, known adenosine mimetics such as quinazolines will map close in space to adenosine, whereas non-mimics, for example, a fatty acid, will map far away. A cutoff defines a selection region which is then sampled representatively to provide a compound set for screening.

The first step was to obtain, in SDF format, vendor lists of compounds for purchase. A set of vendors can be obtained through the ACD directory lists, and SDF files for ~2.4 million compounds were compiled from 19 vendors. A central component of the entire compound selection process is the idea that two molecules can be related in terms of “similarity” or “diversity.” It is important to realize that these types of classifications are totally relative to the comparisons used. Typically, these descriptor measurements are derived from a dissection of molecular topology (bonds, connectivities, rings, etc.) and features such as hydrogen-bonding groups, hydrophobic moieties, or other functional groups. In-house algorithms were developed to analyze each molecule and determine these characteristics from the provided atom and bond information such that at the end of the process, a detailed characterization was available for each molecule. Included in the characterization was an analysis for adenine mimetics (Figure 16.5).

Molecular profile information was initially utilized as a way to filter molecules that had chemically reactive groups or other undesirable features related to general properties (e.g., molecular weight and rotatable bonds) and functional group counts. A set of 85 filters were used, leading to elimination of more than 50% of some vendor collections from the selection process. The calculated topologies and descriptors were then used to identify compounds that had sufficient similarity to the modified adenosine template. Fingerprints (arrays containing counts of each property) were weighted such that a group of ~30 known kinase inhibitors, inserted into the compound set as controls,

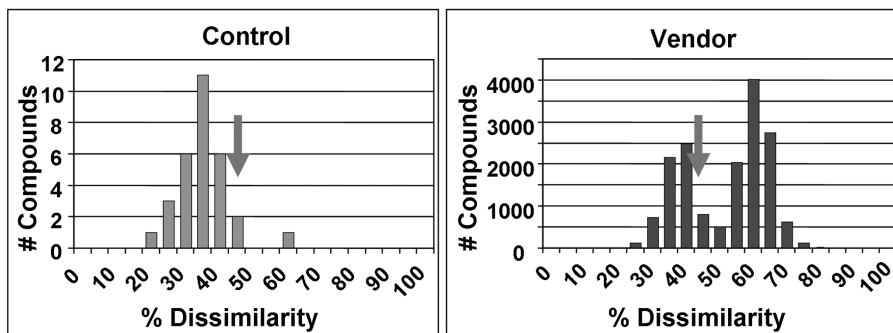


Figure 16.7. Profiling of compounds using descriptors. Optimized weighted descriptors are used to calculate a Sorensen coefficient measuring the likeness of a compound to an adenosine analog (0% dissimilarity would be an identical match). A cutoff value of ~45% captures >90% of a control group of known kinase inhibitors. Using this cutoff then allows selection of a set of compounds for purchase from a vendor (following clustering, representative selection, and visual inspection). The biphasic nature of the profiles derives from the inclusion of adenine isosteres as part of the compound profiling.

were preferentially selected from the set when Sorensen or Tanimoto coefficient calculations were performed. A cutoff criteria (Figure 16.7) that identified >90% of the kinase inhibitors returned a subset of approximately 150K molecules of interest.

Fingerprints were then used to pick as representative (i.e., dissimilar) a set of molecules as possible from this subset of molecules that had survived the selection process so far. A first molecule was arbitrarily picked and then the process was to proceed iteratively through the collection, evaluating at each step, all unselected molecules against all those already selected. A penultimate target set of 15K molecules was identified. The final, and perhaps most important, step was to use visual inspection to reduce the compound set down to approximately 8000 molecules. This step concluded the selection process and compounds were then purchased. The library was actually developed as a 5000 member initial screening set followed by a second round of 3000 compounds. Screening results versus 10 kinases are summarized in Figure 16.8; the screen yielded multiple hits of less than 5 μ M and less than 1 μ M versus all kinases tested. Also shown as part of Figure 16.8 are two examples of a novel 2,8-disubstituted naphthyridine scaffold identified as part of the screen. The scaffold was subsequently successfully expanded via medicinal chemistry efforts (Hanson et al., 2006).

16.4.2. Lead Optimization of a CDK2 Inhibitor

As part of a CDK2 inhibitor discovery program a 1*H*-indole-2,3-dione-3-phenylhydrazone scaffold was identified as an attractive lead series to pursue

Kinase	IC ₅₀ <5μM	IC ₅₀ <1μM	Kinase	IC ₅₀ <5μM	IC ₅₀ <1μM
ALK	37	6	EphB1	37	9
AXL	37	3	Her2	55	13
EGFR	46	8	PDK1	62	15
EphA2	64	15	SKY	24	4
EphA4	45	10	Tie2	50	14

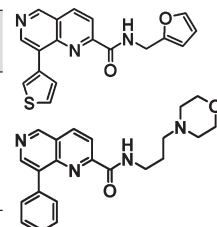


Figure 16.8. Summary screening results of 8000 member acquired library. Library was designed for purine-binding proteins generally but was profiled against 10 protein kinases and hits identified as summarized. Compounds at right are an example of a novel template identified from the screening process (2,8-disubstituted naphthyridines).

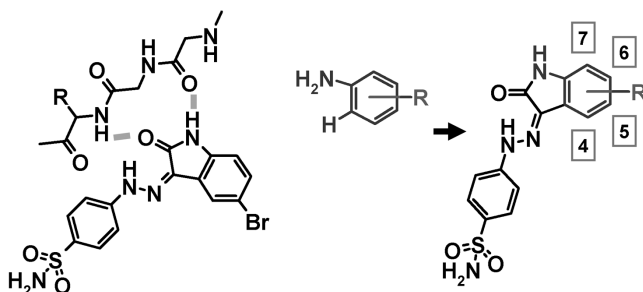


Figure 16.9. Oxindole CDK2 inhibitor. (Left) Schematic of initial lead oxindole structure showing hydrogen-bonding interactions with hinge region. (Right) Schematic showing incorporation of substituted aniline starting reagent into oxindole final product. Oxindole numbering is shown.

for further optimization (Bramson et al., 2001). In particular, a *para*-sulfonamide on the pendant phenyl ring, coupled with a 5-position bromo substitution on the oxindole moiety, was a 60 nM starting point (Figure 16.9). X-ray data were also available for this compound bound to CDK2 (but in absence of cyclin A). The objective was to replace the bromo group, while retaining its increase in potency relative to the unsubstituted oxindole, by exploring positions 4–7 of the oxindole. These substitutions derived from aniline precursors containing a free 2-H (Figure 16.9). The objective of the computational chemistry effort was to identify a subset of anilines for synthesis to facilitate rapid optimization of the template.

Anilines from commercial sources were compiled and filtered based on molecular weight and chemical reactivity criteria leading to a set of 410 anilines for evaluation in docking studies. SMILES string based virtual chemistry, using in-house developed algorithms, was employed to transform the anilines to corresponding benzene-sulfonamide derivatives. Note that for meta-substituted anilines, both 4- and 6-substituted oxindoles were possible synthetic products, and both were evaluated. Conformational searching (without protein) was

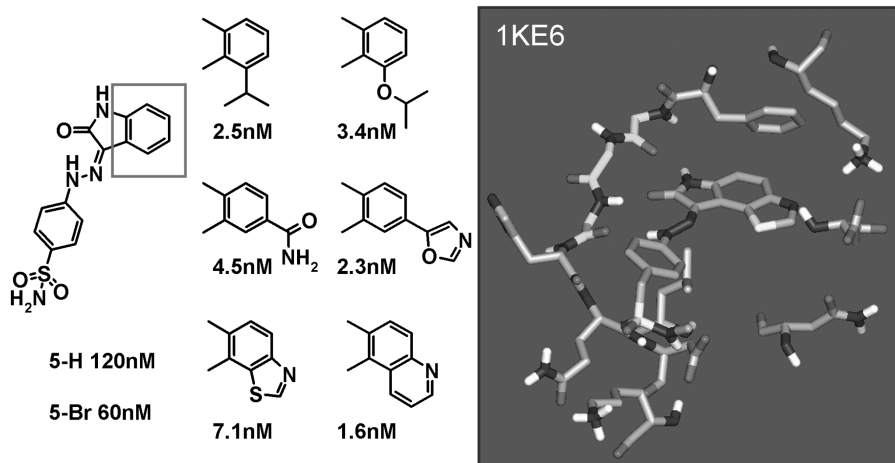


Figure 16.10. Oxindole derivatives potentially inhibit CDK2. (*Left*) Hydrophobic substitutions at 4-position or hydrogen bond acceptors at 5-position substantially improved potency, relative to unsubstituted oxindole and 5-Br lead. Fused analogs also showed high potency. (*Right*) X-ray of a fused thiazole derivative bound to CDK2 (Bramson et al., 2001; pdb code 1KE6).

done via a high-temperature molecular dynamics (MD) based script. One hundred conformations were generated by a repeated cycle consisting of: MD 2000 °C/0.5 ps; MD 100 °C/0.2 ps; minimization 200 steps. Additional rejection criteria were to discard conformations with energy of 7.5 kcal/mol above the lowest energy structure and identical conformations as defined by a sum root mean square (rms) deviation of less than 0.25 Å for heavy atoms. Partial atomic charges were established for each molecule via semiempirical PM3 minimization of low-energy conformer followed by a Hartree–Fock 3-21G* single-point calculation to set up for CHARGE charge determination.

Ligand docking was done to a protein conformation derived from an in-house X-ray complex of CDK2, cyclin A, and flavopiridol using the X-ray data from the 5-Br analog to position the oxindole scaffold initially. Energy minimization was performed with GB/SA solvation; proximal protein atoms were allowed free minimization but those beyond 8 Å from the ligand starting atomic coordinates were subjected to positional restraints. Solvation energy differences, ligand–protein interaction energies, and ligand only conformation energy differences were used to compute a binding energy score, which in turn was used to rank order possible substitutions. This ranking, coupled with visual inspection of the compounds docked into the CDK2 binding site, led to selection of a set of 40 anilines of which ~25 were ultimately incorporated into syntheses. Multiple analogs were identified with single-digit nanomolar potency and were primarily modeled to provide either additional favorable hydrophobic interactions with Val18 or hydrogen bonding to Lys33 via 4- and 5-position substitutions, respectively (Figure 16.10). 4,5-Fused thiazole and quinoline

analogs were particularly promising, providing the potential for both types of interactions. 6-Position analogs and especially 7-position analogs showed steric crowding due to the Phe80 gatekeeper residue. Ultimately, a clinical candidate was identified from this series.

16.5. SUMMARY

The purpose of this chapter has been to demonstrate how computational chemistry and cheminformatics techniques may be incorporated into the kinase drug discovery process to aid in the discovery of inhibitors and ultimately new medicines. Strategies and examples have been provided that span from new template generation to lead optimization. A central underlying theme has been how to use computational methodologies in a practical and *prospective* manner. To be clear, there is certainly value in post-rationalizations of binding modes and retrospective analyses of which scoring function best reproduced the data that were generated, but these efforts are, nonetheless, outside the direct process of drug discovery. There is now a substantial body of examples where computational techniques have provided significant contributions toward the development of screening libraries and discovery of new scaffolds, promising hits, and optimized leads that have ultimately become drug candidates. The wealth of protein structural data, coupled with ever-improving computational power, has made the past decade a particularly exciting one for computational chemistry-based research applied to kinases. Hopefully, kinase inhibitors will continue to progress through the clinic to become new medicines, and in doing so will promote continuing discovery efforts directed at kinases. Computational methodologies are well suited to play an integral role in those efforts.

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