



Drug discovery and the human kinome: Recent trends

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ABSTRACT

A major new trend in drugs targeted at protein kinases is the discovery of allosteric modulators. These compounds differ from ATP-centric drugs in that they do not compete with ATP for binding to the catalytic domain, generally acting by inducing conformational changes to modulate activity. They could provide a number of advantages over more classical protein kinase drugs. For example, they are likely to be more selective, since they bind to unique regions of the kinase and may be useful in overcoming resistance that has developed to drugs that compete with ATP. They offer the ability of activating the kinases either by removing factors that inhibit kinase activity or by simply producing changes to the enzyme to foster catalytic activity. Furthermore, they provide more subtle modulation of kinase activity than simply blocking ATP access to inhibit activity. One hurdle to overcome in discovering these compounds is that allosteric modulators may need to inhibit protein–protein interactions; generally difficult to accomplish with small molecules. Despite the technical problems of identifying allosteric modulators, major gains have been made in identifying allosteric inhibitors and activators of the growth factor receptors as well as soluble tyrosine and serine/threonine kinases and some of these drugs are now in various stages of clinical trials. This review will focus on the discovery of novel allosteric modulators of protein kinases and drug discovery approaches that have been employed to identify such compounds.

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Contents

1. Introduction	144
2. Discovering small molecules that act like large proteins	145
3. Allosteric modulators of soluble protein kinases	149
4. Assays to discover novel allosteric modulators of protein kinases	153
5. Summary	154
Acknowledgments	154
References	154

1. Introduction

Protein kinases are a family of enzymes involved in signal transduction in every human cell. The enzymes detect both external and internal stimuli to cells and produce their functions by phosphorylating proteins. This process initiates and propagates information flow to allow cells to respond to their changing environment. This family of proteins is essential for normal physiology and when dysfunctional leads to abnormal cellular activity and disease.

The protein kinase family is a major target for drug discovery by the pharmaceutical industry (Simpson et al., 2009; Eglen & Reisine, 2009, 2010). A large number of protein kinase inhibitors are either in clinical development or have been approved for marketing by the FDA to treat a wide variety of diseases including cancer, inflammation, diabetes, immunodeficiency and CNS disorders (see Eglen & Reisine,

Abbreviations: ATP, adenosine triphosphate; AKT, v-akt murine thymoma viral oncogene homolog; BRAF, v-raf murine sarcoma viral oncogene homolog B1; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; EGF, epidermal growth factor; EPO, erythropoietin; ERK, extracellular-signal-regulated kinase; FRET, Förster resonance energy transfer; IL, interleukin; JAK-STAT, Janus kinase-signal transducer and activator of transcription; MAP kinase, mitogen-activated protein kinase; NGF, nerve growth factor; NMR, nuclear magnetic resonance; PDK1, PDK1, 3-phosphoinositide-dependent kinase-1.

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2009, 2010). Many of these drugs have improved survival and quality of life of cancer patients as well as in individuals suffering from other complications.

In general, kinase inhibitors have been classified into four different types based on their mechanisms of action (see Zhang et al., 2009; Eglén & Reisine, 2010). Type 1 inhibitors work via a classical mechanism of action to block the access of ATP to the catalytic domain of the kinase in a competitive manner. Type 2 inhibitors interact with kinases in a somewhat different manner, specifically binding to the inactive form of the kinase to prevent the activation process, much in the manner of Gleevec. Type 3 inhibitors, which will be the major focus of this review act via allosteric sites to block the activity. As defined by Zhang et al. (2009) and others, allosteric modulators interact with “A site distinct from the enzyme active site... [to] regulate[s] enzyme activity”. This can mean interacting with sites near the active site but not within the catalytic domain or more distal sites such as regions involved in the regulatory subunit interaction with the catalytic domain of the cAMP dependent protein kinase, or even the growth factor binding sites on the N-terminal transmembrane domain receptors that affect conformational changes in the C-terminal catalytic domain to affect enzyme activity. Thus, allosterism encompasses a large gamut of mechanisms of kinase regulation. Finally, type 4 inhibitors are primarily covalent inhibitors of kinases that target active sites.

While much of the classical discovery of the protein kinase drugs has targeted regions around the ATP binding sites to identify inhibitors, emerging trends have focused on identifying allosteric modulators. The interest in developing allosteric modulators is that such drugs may provide unique advantages over more classically developed compounds (Li et al., 2004; Noble et al., 2004; Zhang et al., 2009; Eglén & Reisine, 2009, 2010).

First, they offer the possibility of greater selectivity because they target sites in kinases more unique in sequence and structure than those compounds that bind to the regions near the ATP binding domain, which are generally more conserved amongst protein kinases. Greater selectivity might be expected to reduce the side-effects compared to the more pervasive kinase inhibitors. The selectivity of the allosteric modulators can also provide approaches to differentially regulate the subtypes of a kinase within a subfamily, which may have similar or the same substrates and high overall sequence similarity.

Secondly, allosteric modulators hold a promise in selectively targeting mutant forms of disease causing protein kinases and in overcoming resistance of the kinases to the ATP binding competitive drugs. As described elsewhere (Cohen, 2002; Bardelli et al., 2003; Dancy & Sausville, 2003; Noble et al., 2004; Pearson et al., 2006; Zhang et al., 2009), many diseases, notably proliferative diseases such as cancer, are caused in part or completely by mutations that generate constitutive kinase activity. The mutations can change the conformation of the kinase and drugs that selectively interact with the mutant form of the kinase may block the activity of the disease causing enzyme while having less or no effect on the natural form of enzyme preserving normal function.

For example, this has been shown to be the case for the serine/threonine kinase BRAF^{V600E} which causes almost half of the malignant melanomas and is the most common disease causing mutant kinase (Tsai et al., 2008). The mutation causes constitutive activity of the kinase and continuous stimulation of the downstream MAPkinase/ERK signaling pathway. The small molecule drug PLX4032 (see Fig. 1 for structure) selectively inhibits BRAF^{V600E} and is much less potent against the wild type kinase or any other kinase and blocks the MAPkinase/ERK signaling pathway only in cells expressing the mutant kinase both in vitro and in vivo. This drug selectively targets the disease causing kinase providing an incredible level of specificity over any other protein in the body and this drug, PLX4032 is currently being tested in the clinic and has shown great promise in treating

melanomas which have previously not been effectively treated by other drugs (Flaherty et al., 2009). While this drug is not allosteric in action, the allosteric inhibitors are more likely to show this profile because in general they are much more selective in targeting kinase specific conformations than the more classical ATP competitive inhibitors.

Thirdly, while most of the drug discovery activities against protein kinases have focused on identifying the inhibitors of kinase activity and ATP binding, allosterism allows for the identification of compounds that could result in activators of kinases. This may be particularly relevant for the family of receptor kinases such as the growth factor receptors, where binding of large proteins to the extracellular domains induces conformational changes that activate the intracellular catalytic activity of the kinase. Some of these growth factors, such as BDNF and NGF, have an important therapeutic value in abrogating neurodegeneration due to a supporting role in neuronal survival and blocking disease progression in Alzheimer's and Parkinson's diseases. Large growth factors are not generally good drug candidates and are difficult to optimize for CNS penetration. Novel technologies have now been developed to allow for the identification of small molecules that bind to similar regions of the growth factors on their receptors and cause kinase activation, providing approaches to identify new growth factor receptor modulators with optimal pharmacokinetic properties.

Finally, small molecule allosteric modulators can provide subtle regulation of kinases controlled by multiple endogenous factors. For example, the cyclin dependent kinases (CDK) are regulated by both endogenous protein activators (cyclins) and inhibitors (CDKI) (Roy & Sausville, 2001). Small molecules could affect the balance of CDK control by these endogenous factors to cause cell apoptosis, something not easily done with the ATP-centric drugs.

Developing small molecule regulators of the endogenous factors controlling protein kinases can require the use of approaches to identify compounds that inhibit protein–protein interaction. Once considered a difficult, if not impossible approach, numerous examples have in fact become available (White et al., 2008; Arkin & Whitty, 2009). New technologies have been adapted to discover protein–protein inhibitors (PPI) in a high throughput screening (HTS) format, as discussed below. Furthermore, there is good evidence that allosteric sites are ‘druggable’ (Hajduk et al., 2005; Fuller et al., 2009) and that some of the same structure–function analysis the industry has employed to discover ATP binding site inhibitors can actually be used to develop the allosteric regulators.

The focus of this review, rather than discussing protein kinase drug discovery as a whole, will attempt to describe the innovations that provide the basis of drug development that targets allosteric regulators. We will be liberal in the use of the term allosteric modulator to encompass factors that affect kinase activity through mechanisms independent of a direct ATP binding site competition to include molecules binding to sites outside of the catalytic domain, such as the growth factors that affect kinase conformation or dimer formation to regulate activity. Importantly, using the knowledge of kinase function and its regulation, new technologies have been developed to exploit the utility of these advances to foster a new generation of drugs for the future that may not only provide advantages over the drugs developed to date, but may lead to new compounds as tools for defining their biological function.

2. Discovering small molecules that act like large proteins

Receptor tyrosine kinases (RTKs) are a major subfamily of kinases that mediate the biological effects of many growth factors. Unlike the soluble kinases, this family consists of the integral membrane proteins containing an extracellular domain that binds the growth factors and intracellular domains which contain the tyrosine kinase catalytic activity. The binding of the growth factor to the allosteric regions in

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