

Small Molecule Inhibitors of the PI3-Kinase Family

Zachary A. Knight

Contents

1	Introduction	263
2	LY294002	265
3	Wortmannin	267
4	p110 δ Inhibitors and the Selectivity Pocket	268
5	p110 β , DNA-PK, and ATM Inhibitors: A Shared Selectivity Mechanism?	270
6	p110 γ Inhibitors	272
7	Class I PI3-K Inhibitors	273
8	Conclusions	274
	References	274

Abstract The PI3-K family is one of the most intensely pursued classes of drug targets. This chapter reviews some of the chemical and structural features that determine the selectivity of PI3-K inhibitors, by focusing on a few key compounds that have been instrumental in guiding our understanding of how to design drugs against this family.

1 Introduction

PI3-K was first identified in the late 1980s as an enzyme activity associated with immunoprecipitates of oncogenic tyrosine kinases (Kaplan et al. 1986; Whitman et al. 1985, 1988) and activated growth factor receptors (Kaplan et al. 1987; Ruderman et al. 1990). In 1992, PI3-K (p110 α) was purified and cloned

Z.A. Knight (✉)

The Rockefeller University, 1230 York Ave, New York, NY 10065, USA

e-mail: zknight@rockefeller.edu

(Hiles et al. 1992), and it was shown to have sequence homology to VPS34, a yeast gene required for protein sorting (Robinson et al. 1988). This led to the rapid discovery of a family of 15 kinases, termed the PI3-K family, that share a conserved phosphoinositide kinase (PIK) domain but otherwise vary in their substrate specificity, expression pattern, and modes of regulation.

Most attention has focused on the four class I PI3-Ks, which contain a 110-kDa catalytic domain (termed p110 α , p110 β , p110 δ , or p110 γ) that heterodimerizes with a family of adaptor molecules (termed p85 or p101, among others). The class I enzymes are activated by receptor tyrosine kinases (RTKs) or G-protein coupled receptors (GPCRs) to synthesize phosphatidylinositol-3,4,5-trisphosphate (PIP₃), a lipid second messenger that acts as a recruitment site at the plasma membrane for downstream proteins, including Akt and PDK1. These downstream proteins in turn regulate a wide range of cellular processes that include growth, nutrient uptake, survival, chemotaxis, and proliferation.

In addition to the class I enzymes, the PI3-K family includes four further classes of kinases. These include the class II and class III PI3-Ks, which synthesize phosphatidylinositol-3-phosphate (PI(3)P) or phosphatidylinositol-3,4-bisphosphate (PI(3,4)P₂), and the PI4-Ks, which synthesize phosphatidylinositol-4-phosphate (PI(4)P). The biological roles of these kinases are still being elucidated, but all appear to participate in the regulation of the intracellular trafficking of proteins or vesicles. Finally, the PI3-K family encodes five kinases that phosphorylate proteins rather than lipids. These phosphoinositide 3-kinase-related kinases (PIKKs) include mTOR, which is a key regulator of cell growth, and ATM, ATR, hSmg1, and DNA-PK, which monitor genomic integrity and initiate the DNA damage response.

Recently, there has been considerable excitement about the potential of PI3-Ks as targets for the treatment of cancer and autoimmune disease. In the field of cancer, a key discovery was the finding that PIK3CA, the gene encoding p110 α , is a frequently mutated oncogene (Samuels et al. 2004). Recent estimates suggest that PIK3CA may be mutated at a cumulative frequency of up to 15% across all tumor types (Karakas et al. 2006), which would make p110 α the most frequently mutated kinase in cancer. In addition, the lipid phosphatase PTEN, which inhibits PI3-K signaling, is one of the most commonly inactivated tumor suppressors (Cantley and Neel 1999). Together, these and other data have led to the view that PI3-K is a critical node that controls cancer cell growth and survival (Chang et al. 1997; Shaw and Cantley 2006).

The potential of PI3-Ks as targets for autoimmune disease was suggested by the finding that the expression of p110 γ and p110 δ is restricted (primarily) to leukocytes. Inactivation of these kinases in mice (either by deletion of the gene or by substitution of a kinase-dead allele) demonstrated that they are required for the immune response but otherwise have a limited role in normal physiology (Hirsch et al. 2000; Okkenhaug et al. 2002; Sasaki et al. 2000). This indicated that inhibitors of these kinases might have anti-inflammatory activity with limited side effects, and, indeed, pharmacological inhibition of p110 γ or p110 δ has shown efficacy in preclinical models of arthritis (Camps et al. 2005), lupus (Barber et al. 2005), and the allergic response (Ali et al. 2004).

In response to this data, the pharmaceutical industry has made intense effort in recent years to develop potent and selective PI3-K inhibitors. As recently as 2003, only two PI3-K inhibitors were widely known – wortmannin and LY294002. Today, dozens of new chemotypes of PI3-K inhibitors have been described, either in the peer-reviewed or patent literature, and the first such compounds entered clinical trials in late 2007. These new chemotypes have been comprehensively reviewed (Marone et al. 2008).

In this chapter, I summarize what has been learned over the past few years about the chemical determinants of PI3-K inhibitor selectivity. I do this by focusing on a few key compounds that have been instrumental in guiding our understanding of how to design drugs that target this family.

2 LY294002

In the early 1990s, Eli Lilly initiated a screening effort to identify natural products that inhibit PI3-K. This led to the identification of the flavonoid quercetin (Fig. 1) as a PI3-K inhibitor with an IC_{50} of 3.8 μ M (Matter et al. 1992).

Flavonoids often display promiscuous biological activity (Davies et al. 2000; Knight and Shokat 2005), and, by 1992, quercetin had already been shown to inhibit several targets unrelated to PI3-K (Matter et al. 1992). To identify compounds with improved selectivity, analogs of quercetin were synthesized that replaced the catechol moiety with more drug-like substituents (Fig. 1). This led to the discovery in 1994 of LY294002, a reversible, ATP competitive inhibitor of most enzymes in the PI3-K family (Vlahos et al. 1994). LY294002 inhibits the class I PI3-Ks, mTOR, and DNA-PK in vitro with IC_{50} values in the 1–10 μ M range (Brunn et al. 1996; Knight et al. 2004; Vlahos et al. 1994). Unlike quercetin, LY294002 is selective for PI3-K relative to most protein kinases; exceptions include CK2 (Davies et al. 2000) and PLK1 (Liu et al. 2005).

The enhanced PI3-K selectivity of LY294002 relative to quercetin is due to the introduction of the morpholine ring in LY294002 (Fig. 1). In the crystal structure of LY294002 bound to p110 γ , the morpholine ring adopts a chair conformation that enables it to make close hydrophobic contacts with a complementary region of the ATP-binding pocket (Walker et al. 2000). The morpholine oxygen also makes a critical hydrogen bond to the backbone amide of V882. This hydrogen bond is made by the N1 of the adenine ring of ATP, as well as every PI3-K inhibitor that has been described to date, suggesting that this hydrogen bond is required for high-affinity binding. A requirement for a similar hydrogen bond is found among inhibitors of the protein kinase family. Remarkably, although LY294002 was discovered as an analog of quercetin, the crystal structures of these two compounds revealed that they bind to PI3-K in opposite orientations (Fig. 1).

The core pharmacophore in LY294002 is an aromatic ring linked to a morpholine. This “aryl morpholine” pharmacophore is a privileged structure for PI3-K inhibition: it is embedded within many newer classes of PI3-K inhibitors, including

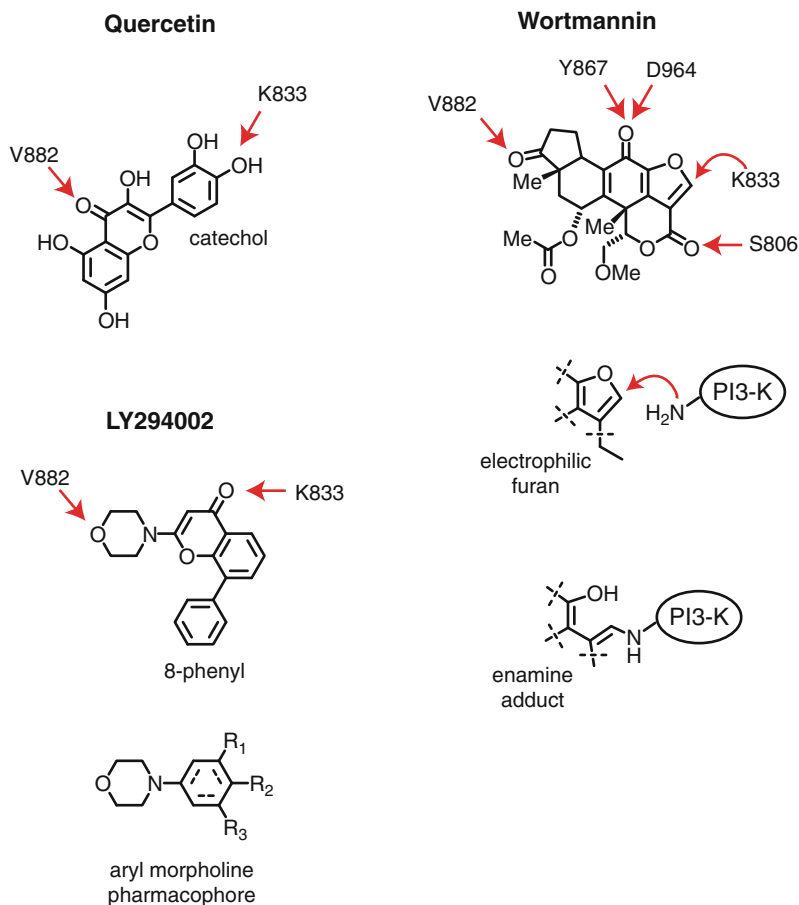


Fig. 1 Classical PI3-K inhibitors. Chemical structure and relative orientation in the ATP-binding pocket of p110 γ of the PI3-K inhibitors quercetin, LY294002, and wortmannin. Hydrogen bonds to residues in p110 γ are indicated by *straight arrows*. The nucleophilic attack of K833 on wortmannin is indicated by a *curved arrow*

molecules that target p110 α (Alexander et al. 2008; Folkes et al. 2008; Hayakawa et al. 2006, 2007; Knight et al. 2006; Perry et al. 2008a, b; Raynaud et al. 2007; Yaguchi et al. 2006), p110 β (Jackson et al. 2005; Knight et al. 2004), DNA-PK (Barbeau et al. 2007; Desage-El Murr et al. 2008; Griffin et al. 2005; Hardcastle et al. 2005; Hollick et al. 2007; Knight et al. 2004; Leahy et al. 2004), mTOR (Knight et al. 2006), and ATM (Hickson et al. 2004; Hollick et al. 2007). Structure–activity relationship (SAR) data and co-crystal structures indicate that the aryl morpholine in all of these compounds binds in an orientation similar to the aryl morpholine in LY294002. In this sense, these newer compounds are all analogs of LY294002.

LY294002 and its partner wortmannin (discussed below) have been two of the most widely used tool compounds in biological research. Much of what is known about PI3-K signaling was first learned by using these reagents. Nonetheless, it is now clear that the cellular selectivity of LY294002 is limited. To fully inhibit PI3-K activity in cells, LY294002 is often applied at concentrations above 10 μM . In this concentration range, LY294002 targets several unrelated proteins, including calcium channels (Welling et al. 2005), potassium channels (Sun et al. 2004; Wu et al. 2009), phosphodiesterases (Abbott and Thompson 2004), and the estrogen receptor (Pasapera Limon et al. 2003). Therefore, some of the cellular functions attributed to PI3-Ks based on the use of LY294002 are probably mediated by these secondary targets. For example, LY294002 induces apoptosis in some cell lines and acute toxicity in mice, which are not observed with more potent and selective PI3-K inhibitors.

3 Wortmannin

Wortmannin is a steroid-like natural product that was originally isolated from the fungus *Penicillium wortmanni* (Brian et al. 1957). Early experiments revealed that wortmannin had potent antiproliferative and anti-inflammatory activity (Wiesinger et al. 1974). The specificity of this activity was suggested by the finding that wortmannin blocked, at low nanomolar concentrations, the respiratory burst of neutrophils activated by fMLP, yet did not directly inhibit NADPH oxidase (Baggiolini et al. 1987). This led to the hypothesis that wortmannin may “interfere with the signal transduction sequence initiated by the particulate stimulus” in neutrophils, even though the components of that signaling pathway had not been fully characterized.

In 1993, PI3-K was identified as the direct target of wortmannin responsible for its inhibition of the neutrophil response to fMLP (Arcaro and Wymann 1993). Within the PI3-K family, the most potent targets of wortmannin are the class I enzymes, which are inhibited with IC_{50} values in the 1–10 nM range. Wortmannin also potently inhibits the class II enzyme PI3K-C2 β (IC_{50} = 1.6 nM), but is somewhat less active against PI3K-C2 α (IC_{50} = 420 nM), PI4-KIII β (IC_{50} = 320 nM), mTOR (IC_{50} = 200 nM), and DNA-PK (IC_{50} = 150 nM) (Arcaro et al. 1998; Balla et al. 2008b; Brunn et al. 1996; Domin et al. 1997; Hartley et al. 1995). Interestingly, the wortmannin sensitivity of VPS34, the class III PI3-K, is species dependent: the human and fly enzymes are sensitive (IC_{50} = 10 nM), whereas the yeast enzyme is resistant (IC_{50} = 3 μM) (Fruman et al. 1998; Stack and Emr 1994). Despite this striking difference in sensitivity between orthologs, efforts to use this information to alter the wortmannin sensitivity of p110 γ by mutagenesis were unsuccessful (Walker et al. 2000).

Wortmannin inhibits PI3-Ks by covalent inactivation of the enzyme (Walker et al. 2000; Wymann et al. 1996): the electrophilic furan ring of wortmannin is attacked by a lysine residue in the kinase active site (K833 in p110 γ), resulting in

ring opening and formation of a stable enamine (Fig. 1). This lysine residue is conserved in all PI3-Ks as a result of its role in catalysis, and this conservation may contribute to the potent inhibition of several PI3-Ks by wortmannin. Nonetheless, noncovalent interactions are also critical for the high-affinity binding of this molecule. The co-crystal structure of wortmannin bound to p110 γ revealed a network of five hydrogen bonds and extensive hydrophobic interactions between the natural product and the enzyme (Fig. 1) (Walker et al. 2000).

The electrophilic furan ring of wortmannin is sensitive to cellular nucleophiles, and wortmannin rapidly decomposes in tissue culture media (half-life \sim 10 min) (Holleran et al. 2003). Nonetheless, wortmannin treatment of cells results in durable PI3-K inhibition. This “wortmannin paradox” may reflect the slow reversibility of the covalent reaction of wortmannin with some cellular nucleophiles, which results in the gradual regeneration of active drug (Yuan et al. 2007). This observation has enabled the design wortmannin pro-drugs with improved pharmacological properties (Yuan et al. 2006).

4 p110 δ Inhibitors and the Selectivity Pocket

In 2003, scientists from ICOS described IC87114 (Fig. 2), an isoquinolinone purine that inhibits p110 δ with an IC₅₀ value of 0.5 μ M (Sadhu et al. 2003b). IC87114 displays remarkable selectivity for p110 δ relative to the rest of the PI3-K family: it is 100-fold more potent for p110 δ than p110 β or p110 γ , and it is essentially inactive against all other PI3-K family members. This molecule was the first selective PI3-K inhibitor to be described, and it has been widely used to probe the role of p110 δ in processes such as the allergic response (Ali et al. 2004, 2008; Zhang et al. 2008), neutrophil activation (Sadhu et al. 2003a, b), and leukemic cell proliferation (Billottet et al. 2006; Sujobert et al. 2005).

The impressive selectivity of IC87114 raised the question of how this molecule discriminates between the class I PI3-K isoforms. IC87114 is ATP competitive, but the ATP-binding pocket of the class I PI3-Ks is highly conserved: among the residues that directly contact the adenine of ATP, there is only one difference among the class I PI3-Ks (a conservative substitution of a valine for isoleucine in p110 γ). While these kinases do diverge in sequence on the periphery of the ATP-binding pocket, it is unclear how these differences could be targeted by a small molecule to achieve selectivity. Like most kinase inhibitors, IC87114 was discovered through chemical optimization of a screening hit, rather than through structure-based design.

In 2006, the crystal structure of an analog of IC87114 (called PIK-39) was reported in complex with p110 γ , along with crystal structures of several other novel PI3-K inhibitors (Knight et al. 2006). Comparison of the binding mode of PIK-39 with that of other inhibitors suggested a rationale for its unique selectivity. Most PI3-K inhibitors bind to the kinase in a flat orientation, in which the drug sits primarily within the plane defined by the adenine of ATP. By contrast, the PIK-39

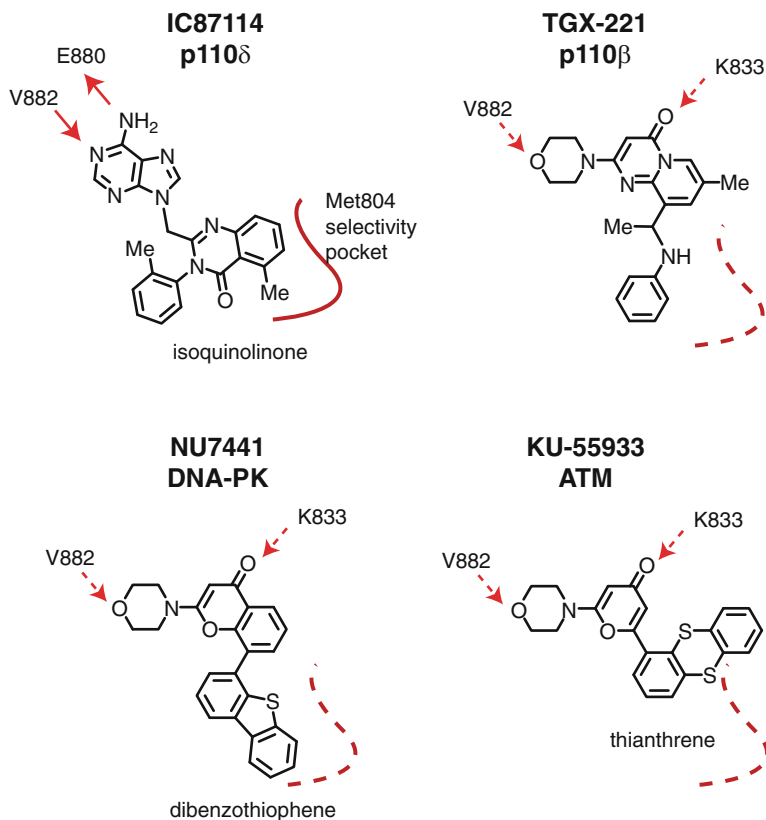


Fig. 2 Isoform selective PI3-K inhibitors. Chemical structure and relative orientation in the PI3-K ATP-binding site of four selective inhibitors. Hydrogen bonds observed in crystal structures are indicated by *solid red arrows*; those predicted from models are indicated by *dashed red arrows*

structure revealed that the isoquinolinone moiety of the drug projects upward toward the roof of the ATP-binding pocket (Fig. 3). To accommodate the drug and avoid a steric clash, the kinase undergoes a conformational rearrangement in which the side chain of Met804 moves downward, creating a new hydrophobic pocket at the entrance to the ATP binding site. This inducible pocket, which is not observed in any other PI3-K crystal structure, buries approximately 180 Å² of solvent-accessible surface area of the drug.

These structural data revealed the existence of an inducible drug-binding pocket, gated by Met804, located at the entrance to the PI3-K active site. To test whether this inducible pocket is required for the selectivity of PIK-39, resistance mutants were designed in which Met804 was mutated to a β -branched residue (isoleucine or valine); modeling indicated that a β -branched side chain would be unable to move and thereby accommodate the drug. These resistance mutations blocked the binding of PIK-39 (and IC87114) to p110 δ but had no effect on other classes of inhibitors

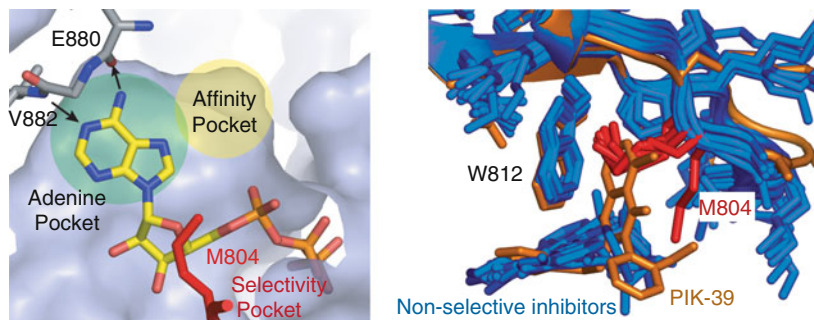


Fig. 3 Structure of the ATP-binding pocket of p110 γ . *Left*, crystal structure of ATP bound to p110 γ , indicating hydrogen bonds to the backbone of residues E880 and V882, and the relative location of the regions occupied by the adenine of ATP, the inducible selectivity pocket, and a deeper region occupied by potent inhibitors (affinity pocket). *Right*, an overlay of the crystal structures of structurally diverse PI3-K inhibitors bound to p110 γ . Multitargeted compounds are shown in *blue* and PIK-39 is shown in *orange*. Met804 (*red*) is highlighted to show the conformational change that occurs upon binding of PIK-39. These figures are reproduced with permission from (Knight et al. 2006)

(Knight et al. 2006). This confirmed that the inducible pocket is required for the unique selectivity of the isoquinolinone chemotype.

It is likely that different PI3-K isoforms vary in their ability to form this inducible pocket, as a result of sequence differences that are distal to the ATP-binding site (e.g., second and third shell interactions). If so, this provides an explanation for how PIK-39 can discriminate between PI3-K isoforms even though the residues that directly contact the drug are the same. This prediction has been supported by the recent crystal structure of p110 α , which showed that the loop containing the residue equivalent to Met804 in p110 α adopts an orientation that would preclude the conformational rearrangement of Met804 observed in the PIK-39 structure (Amzel et al. 2008). Finally, it is worth noting that this mechanism of drug selectivity, which is based on differences in conformational flexibility between targets, is well characterized in the field of protein kinase inhibitors. The first example of this was the drug imatinib, which distinguishes between the closely related kinases Abl and Src through binding to a differentially accessible inactive conformation (Schindler et al. 2000).

5 p110 β , DNA-PK, and ATM Inhibitors: A Shared Selectivity Mechanism?

In 2004 and 2005, a series of selective inhibitors of p110 β were described (Jackson et al. 2005; Knight et al. 2004). These compounds are analogs of LY294002 that replace the 8-phenyl substituent of LY294002 with bulkier and more extended groups (Fig. 2). A representative compound is TGX221, which is a potent inhibitor

of p110 β (IC_{50} = 0.005 μ M) and displays impressive selectivity for p110 β over p110 α and p110 γ (\sim 1,000-fold). The selectivity of these compounds for p110 β over p110 δ is much less (two- to tenfold) (Knight et al. 2004). However, the residual p110 δ activity of these compounds has not limited their usefulness, because highly selective p110 δ inhibitors such as IC87114 have been available. Thus, it has been possible to dissect the specific contribution of p110 β in cell-based experiments by using these two classes of inhibitors in combination. Compounds from the TGX series have been employed in this way to assign a role for p110 β in thrombus formation by platelets (Jackson et al. 2005), in fine-tuning insulin signaling in muscle cells (Knight et al. 2006), and in driving cell proliferation in PTEN-null tumors (Torbett et al. 2008), among other functions.

During the same time period, scientists from KuDOS published a series of papers describing their efforts to identify selective DNA-PK inhibitors (Griffin et al. 2005; Hollick et al. 2003, 2007; Leahy et al. 2004). These molecules were identified by synthesizing focused libraries that introduced diversity around the 8-phenyl group of LY294002 and then screening these compounds for selectivity within the PI3-K family. One of the most selective compounds to emerge from this effort was NU7441, which contains a sterically demanding dibenzothiophene in place of the 8-phenyl moiety of LY294002 (Fig. 2). This compound potently inhibits DNA-PK (IC_{50} = 0.020 μ M) and displays 100- to 1000-fold selectivity against other kinases in the PI3-K family (Leahy et al. 2004).

KU-55933 is a second important compound that originated from KuDOS (Fig. 2) (Hickson et al. 2004). KU-55933 is a potent inhibitor of ATM (IC_{50} = 0.013 μ M) and displays a high degree of selectivity relative to all other PI3-K family members (\sim 1,000-fold). Like TGX-221 and NU7441, KU-55933 introduces a bulky substituent (a thianthrene ring) into the approximate region occupied by the 8-phenyl of LY294002. This compound has become a widely used probe for ATM signaling. One early application of this compound demonstrated the potential of ATM as a therapeutic target for HIV, based on the fact that ATM mediates a DNA damage response required for viral integration (Lau et al. 2005).

Crystal structures have not been reported for NU7441, KU-55933, or the TGX series of p110 β inhibitors. Therefore, the precise structural basis for their selectivity is unknown. However, based on SAR data, it is clear that the aryl morpholine moiety in these drugs binds to PI3-Ks in the same orientation as LY294002. This has made it possible to generate models for how these drugs bind to PI3-Ks, by using the LY294002 crystal structure as a template (Knight et al. 2006). These models indicate that all three drugs project a large hydrophobic substituent out of the plane occupied by ATP and toward the region of the kinase that forms the inducible selectivity pocket in the PIK-39 structure (Fig. 2). In each case, it appears that a conformational change in the kinase would be necessary to accommodate the drug (although in the case of ATM, sequence differences in the residues in this region may also be relevant (Knight et al. 2006)). Thus, it is likely that these varied inhibitors achieve their selectivity, in part, by exploiting differences between PI3-Ks in conformational flexibility around the region that moves in the PIK-39 structure.

Data from mutagenesis of p110 β is consistent with this hypothesis (Frazzetto et al. 2008).

6 p110 γ Inhibitors

Serono has described a series of selective p110 γ inhibitors (Camps et al. 2005; Pomel et al. 2006). A representative compound is AS-605240 (Fig. 4), which has low nanomolar activity against p110 γ and \sim 30-fold selectivity relative to p110 β and p110 δ . The selectivity of AS-605240 for p110 γ over p110 α is less (\sim 10-fold), which is consistent with data from other chemotypes that it is unusually difficult to identify small molecules that discriminate between these two isoforms (Knight et al. 2006).

Compounds from this chemical series have two functional parts: (1) a hetero-aromatic ring that binds in the region of the kinase active site occupied by the adenine of ATP and makes a hydrogen bond to the backbone amide of V882; and (2) a thiazolidinedione that occupies a deeper region of the ATP-binding pocket and makes a hydrogen bond to K833 in p110 γ (Fig. 4). Crystal structures of several

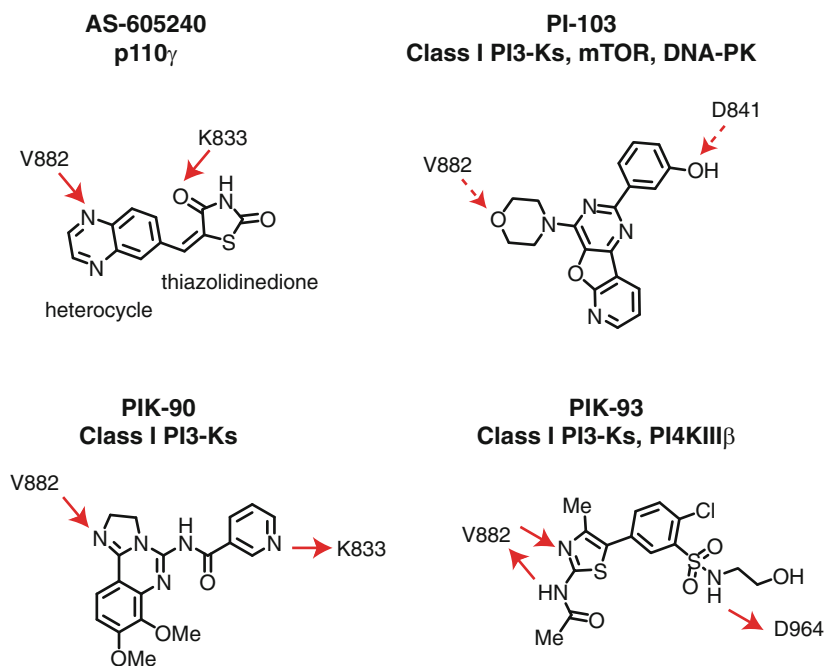


Fig. 4 Inhibitors of p110 γ and class I PI3-Ks. Chemical structure and relative orientation within the ATP binding site of several classes of PI3-K inhibitors. Hydrogen bonds observed in crystal structures are indicated by *solid red arrows*; those predicted from models are indicated by *dashed red arrows*

compounds from this series have been reported, but the interactions that determine the p110 γ selectivity of these compounds are not apparent (Camps et al. 2005). Unlike many classes of PI3-K inhibitors that have been reported, the p110 γ inhibitors from Serono have favorable pharmacological properties, including low molecular weight, high aqueous solubility, and oral bioavailability in mouse.

7 Class I PI3-K Inhibitors

Several classes of multitargeted drugs have been described that inhibit most or all of the class I PI3-Ks. Representative chemotypes are shown in Fig. 4. These compounds often also inhibit DNA-PK and, in some cases, have activity against mTOR. One compound, PIK-93, has unusually potent activity against PI4KIII β and has become a useful probe for that kinase (Balla et al. 2008a, b). By contrast, potent inhibition of ATM, ATR, or hSmg-1 by these drugs is uncommon.

An important early representative of this class was PI-103, an aryl morpholine developed by Piramed (Knight et al. 2006; Raynaud et al. 2007). PI-103 inhibits the class I PI3-Ks, DNA-PK, and mTOR at low- to mid-nanomolar concentrations *in vitro*. As a result of this broad selectivity, PI-103 effectively inhibits almost all PI3-K-dependent growth factor signaling, and PI-103 displays potent antiproliferative activity in most cells, typically as a result of a G₀G₁ cell cycle arrest rather than apoptosis (Fan et al. 2006; Raynaud et al. 2007).

The antiproliferative activity of PI-103 requires direct inhibition of mTOR in addition to the class I PI3-Ks in many cell types. This was unexpected, because it was widely assumed that potent inhibition of PI3-Ks would be sufficient to block tumor cell proliferation. However, a systematic comparison of a panel of structurally diverse PI3-K inhibitors with varying target selectivities revealed that PI-103 was uniquely active against several glioma cell lines (Fan et al. 2006). Compounds such as PIK-90 (Fig. 4) that inhibit the class I PI3-Ks but are less potent against mTOR were found to also have significantly less antiproliferative activity. By combining PIK-90 with the mTOR inhibitor rapamycin, it was possible to phenocopy the cell cycle arrest induced by PI-103, suggesting that inhibition of both PI3-Ks and mTOR is required (Fan et al. 2006). This result has been reproduced in a range of tumor cell lines (Apsel et al. 2008; Fan et al. 2007; Kharas et al. 2008; Park et al. 2008; Torbett et al. 2008) and is also observed with newer inhibitors that are structurally unrelated to PI-103 or PIK-90 (Apsel et al. 2008), arguing that direct mTOR inhibition may be generally required for blockade of cell proliferation by PI3-K family inhibitors. However, it remains unclear which mTOR targets mediate the additional antiproliferative effect caused by direct mTOR inhibition, since upstream inhibition of PI3-K is typically sufficient to block signaling through known mTOR pathway components. In addition, it is unknown to what extent these differences in the drug sensitivity of cell lines *in vitro* will predict the response to PI3-K inhibitors of tumors *in vivo*.

PI-103 has demonstrated activity *in vivo* against tumor xenografts (Fan et al. 2006; Raynaud et al. 2007) and in studies of insulin signaling (Knight et al. 2006).

However, the use of this compound *in vivo* is limited by its low aqueous solubility and rapid metabolism (Raynaud et al. 2007). Piramed has described medicinal chemistry efforts to optimize the pharmacological properties of this compound, resulting in an analog (GDC-0941) that has entered human clinical trials (Folkes et al. 2008). This compound joins class I PI3-K inhibitors such as NVP-BEZ235 (Maira et al. 2008; Serra et al. 2008), XL-147, and XL-765 that have advanced to clinical testing. These compounds are described in greater detail in a separate chapter in this book.

8 Conclusions

Much has been learned over the past 5 years about the chemical principles that control the selectivity PI3-K inhibitors, but some basic questions remain unanswered. Thus far, no highly specific inhibitor of p110 α has been described, despite the fact that this kinase is the primary target of most drug discovery efforts focusing on the PI3-K family. This may reflect the fact that p110 α is challenging to selectively inhibit with a small molecule; for example, SAR and structural data indicate that the selectivity pocket exploited by p110 δ inhibitors is particularly inaccessible in p110 α . Alternatively, it may be that highly selective inhibition of p110 α is not an optimal strategy for the treatment of cancer. Preclinical data clearly indicate that less selective PI3-K inhibitors, such as those that also target mTOR, have enhanced antitumor activity. This has led to efforts to identify small molecules whose selectivity has been expanded even further to target both tyrosine kinases and PI3-Ks (Apsel et al. 2008). Moreover, it seems unlikely that enhanced selectivity for p110 α would prevent the feared side effects of PI3-K inhibitors on glucose metabolism, since p110 α is the primary kinase responsible for insulin signaling (Foukas et al. 2006; Knight et al. 2006).

These questions can be resolved only through clinical testing, and, fortunately, several chemotypes of PI3-K inhibitors are now being evaluated in clinical trials. These human experiments should provide, at last, a verdict on the therapeutic potential of PI3-K inhibitors, at least for the treatment of cancer. Judging from the recent experience of other targeted therapies, however, these clinical data are also likely to supply surprises that will require re-examination of our understanding of PI3-K signaling.

References

- Abbott BM, Thompson PE (2004) *Bioorg Med Chem Lett* 14:2847–2851
- Alexander R, Balasundaram A, Batchelor M, Brookings D, Crepy K, Crabbe T, Deltent MF, Driessens F, Gill A, Harris S, Hutchinson G, Kulisa C, Merriman M, Mistry P, Parton T, Turner J, Whitcombe I, Wright S (2008) *Bioorg Med Chem Lett* 18:4316–4320

- Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B (2004) *Nature* 431:1007–1011
- Ali K, Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, Pasquali C, Chabert C, Rommel C, Vanhaesebroeck B (2008) *J Immunol* 180:2538–2544
- Amzel LM, Huang CH, Mandelker D, Lengauer C, Gabelli SB, Vogelstein B (2008) *Nat Rev Cancer* 8:665–669
- Apfel B, Blair JA, Gonzalez B, Nazif TM, Feldman ME, Aizenstein B, Hoffman R, Williams RL, Shokat KM, Knight ZA (2008) *Nat Chem Biol* 4:691–699
- Arcaro A, Wymann MP (1993) *Biochem J* 296(Pt 2):297–301
- Arcaro A, Volinia S, Zvelebil MJ, Stein R, Watton SJ, Layton MJ, Gout I, Ahmadi K, Downward J, Waterfield MD (1998) *J Biol Chem* 273:33082–33090
- Baggiolini M, Dewald B, Schnyder J, Ruch W, Cooper PH, Payne TG (1987) *Exp Cell Res* 169:408–418
- Balla A, Kim YJ, Varnai P, Szentpetery Z, Knight Z, Shokat KM, Balla T (2008a) *Mol Biol Cell* 19:711–721
- Balla A, Tuymetova G, Toth B, Szentpetery Z, Zhao X, Knight ZA, Shokat K, Steinbach PJ, Balla T (2008b) *Biochemistry* 47:1599–1607
- Barbeau OR, Cano-Soumillac C, Griffin RJ, Hardcastle IR, Smith GC, Richardson C, Clegg W, Harrington RW, Golding BT (2007) *Org Biomol Chem* 5:2670–2677
- Barber DF, Bartolome A, Hernandez C, Flores JM, Redondo C, Fernandez-Arias C, Camps M, Ruckle T, Schwarz MK, Rodriguez S, Martinez AC, Balomenos D, Rommel C, Carrera AC (2005) *Nat Med* 11:933–935
- Billett C, Grandage VL, Gale RE, Quattropiani A, Rommel C, Vanhaesebroeck B, Khwaja A (2006) *Oncogene* 25:6648–6659
- Brian PW, Curtis PJ, Hemming HG, Norris GLF (1957) *Transactions of the British Mycological Society* 40:365–368
- Brunn GJ, Williams J, Sabers C, Wiederrecht G, Lawrence JC Jr, Abraham RT (1996) *Embo J* 15:5256–5267
- Camps M, Ruckle T, Ji H, Ardisson V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Francon B, Martin T, Gretener D, Perrin D, Leroy D, Vitte PA, Hirsch E, Wymann MP, Cirillo R, Schwarz MK, Rommel C (2005) *Nat Med* 11:936–943
- Cantley LC, Neel BG (1999) *Proc Natl Acad Sci USA* 96:4240–4245
- Chang HW, Aoki M, Fruman D, Auger KR, Bellacosa A, Tsichlis PN, Cantley LC, Roberts TM, Vogt PK (1997) *Science* 276:1848–1850
- Davies SP, Reddy H, Caivano M, Cohen P (2000) *Biochem J* 351:95–105
- Desage-El Murr M, Cano C, Golding BT, Hardcastle IR, Hummersome M, Frigerio M, Curtin NJ, Menear K, Richardson C, Smith GC, Griffin RJ (2008) *Bioorg Med Chem Lett* 18:4885–4890
- Domin J, Pages F, Volinia S, Rittenhouse SE, Zvelebil MJ, Stein RC, Waterfield MD (1997) *Biochem J* 326(Pt 1):139–147
- Fan QW, Knight ZA, Goldenberg DD, Yu W, Mostov KE, Stokoe D, Shokat KM, Weiss WA (2006) *Cancer Cell* 9:341–349
- Fan QW, Cheng CK, Nicolaides TP, Hackett CS, Knight ZA, Shokat KM, Weiss WA (2007) *Cancer Res* 67:7960–7965
- Folkes AJ, Ahmadi K, Alderton WK, Alix S, Baker SJ, Box G, Chuckowree IS, Clarke PA, Depledge P, Eccles SA, Friedman LS, Hayes A, Hancox TC, Kugendradas A, Lensun L, Moore P, Olivero AG, Pang J, Patel S, Pergl-Wilson GH, Raynaud FI, Robson A, Saghir N, Salphati L, Sohal S, Ultsch MH, Valenti M, Wallweber HJ, Wan NC, Wiesmann C, Workman P, Zhyvoloup A, Zvelebil MJ, Shuttleworth SJ (2008) *J Med Chem* 51:5522–5532
- Foukas LC, Claret M, Pearce W, Okkenhaug K, Meek S, Peskett E, Sancho S, Smith AJ, Withers DJ, Vanhaesebroeck B (2006) *Nature* 441:366–370
- Frazzetto M, Suphioglu C, Zhu J, Schmidt-Kittler O, Jennings IG, Cranmer SL, Jackson SP, Kinzler KW, Vogelstein B, Thompson PE (2008) *Biochem J* 414:383–390

- Fruman DA, Meyers RE, Cantley LC (1998) *Annu Rev Biochem* 67:481–507
- Griffin RJ, Fontana G, Golding BT, Guiard S, Hardcastle IR, Leahy JJ, Martin N, Richardson C, Rigoreau L, Stockley M, Smith GC (2005) *J Med Chem* 48:569–585
- Hardcastle IR, Cockcroft X, Curtin NJ, El-Murr MD, Leahy JJ, Stockley M, Golding BT, Rigoreau L, Richardson C, Smith GC, Griffin RJ (2005) *J Med Chem* 48:7829–7846
- Hartley KO, Gell D, Smith GC, Zhang H, Divecha N, Connelly MA, Admon A, Lees-Miller SP, Anderson CW, Jackson SP (1995) *Cell* 82:849–856
- Hayakawa M, Kaizawa H, Moritomo H, Koizumi T, Ohishi T, Okada M, Ohta M, Tsukamoto S, Parker P, Workman P, Waterfield M (2006) *Bioorg Med Chem* 14:6847–6858
- Hayakawa M, Kaizawa H, Moritomo H, Koizumi T, Ohishi T, Yamano M, Okada M, Ohta M, Tsukamoto S, Raynaud FI, Workman P, Waterfield MD, Parker P (2007) *Bioorg Med Chem Lett* 17:2438–2442
- Hickson I, Zhao Y, Richardson CJ, Green SJ, Martin NM, Orr AI, Reaper PM, Jackson SP, Curtin NJ, Smith GC (2004) *Cancer Res* 64:9152–9159
- Hiles ID, Otsu M, Volinia S, Fry MJ, Gout I, Dhand R, Panayotou G, Ruiz-Larrea F, Thompson A, Totty NF et al (1992) *Cell* 70:419–429
- Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirolo L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP (2000) *Science* 287:1049–1053
- Holleran JL, Egorin MJ, Zuhowski EG, Parise RA, Musser SM, Pan SS (2003) *Anal Biochem* 323:19–25
- Hollick JJ, Golding BT, Hardcastle IR, Martin N, Richardson C, Rigoreau LJ, Smith GC, Griffin RJ (2003) *Bioorg Med Chem Lett* 13:3083–3086
- Hollick JJ, Rigoreau LJ, Cano-Soumillac C, Cockcroft X, Curtin NJ, Frigerio M, Golding BT, Guiard S, Hardcastle IR, Hickson I, Hummersone MG, Menear KA, Martin NM, Matthews I, Newell DR, Ord R, Richardson CJ, Smith GC, Griffin RJ (2007) *J Med Chem* 50:1958–1972
- Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM, Yuan Y, Sturgeon SA, Prabaharan H, Thompson PE, Smith GD, Shepherd PR, Daniele N, Kulkarni S, Abbott B, Saylik D, Jones C, Lu L, Giuliano S, Hughan SC, Angus JA, Robertson AD, Salem HH (2005) *Nat Med* 11:507–514
- Kaplan DR, Whitman M, Schaffhausen B, Raptis L, Garcea RL, Pallas D, Roberts TM, Cantley L (1986) *Proc Natl Acad Sci USA* 83:3624–3628
- Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, Cantley L, Roberts TM (1987) *Cell* 50:1021–1029
- Karakas B, Bachman KE, Park BH (2006) *Br J Cancer* 94:455–459
- Kharas MG, Janes MR, Scarfone VM, Lilly MB, Knight ZA, Shokat KM, Fruman DA (2008) *J Clin Invest* 118:3038–3050
- Knight ZA, Shokat KM (2005) *Chem Biol* 12:621–637
- Knight ZA, Chiang GG, Alaimo PJ, Kenski DM, Ho CB, Coan K, Abraham RT, Shokat KM (2004) *Bioorg Med Chem* 12:4749–4759
- Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, Loewith R, Stokoe D, Balla A, Toth B, Balla T, Weiss WA, Williams RL, Shokat KM (2006) *Cell* 125:733–747
- Lau A, Swinbank KM, Ahmed PS, Taylor DL, Jackson SP, Smith GC, O'Connor MJ (2005) *Nat Cell Biol* 7:493–500
- Leahy JJ, Golding BT, Griffin RJ, Hardcastle IR, Richardson C, Rigoreau L, Smith GC (2004) *Bioorg Med Chem Lett* 14:6083–6087
- Liu Y, Shreder KR, Gai W, Corral S, Ferris DK, Rosenblum JS (2005) *Chem Biol* 12:99–107
- Maira SM, Stauffer F, Bruegggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia-Echeverria C (2008) *Mol Cancer Ther* 7:1851–1863
- Marone R, Cmiljanovic V, Giese B, Wymann MP (2008) *Biochim Biophys Acta* 1784:159–185
- Matter WF, Brown RF, Vlahos CJ (1992) *Biochem Biophys Res Commun* 186:624–631

- Okkenhaug K, Bilancio A, Farjot G, Priddle H, Sancho S, Peskett E, Pearce W, Meek SE, Salpekar A, Waterfield MD, Smith AJ, Vanhaesebroeck B (2002) *Science* 297:1031–1034
- Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, Knight ZA, Shokat KM, Azar N, Viguie F, Ifrah N, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2008) *Leukemia* 22:1698–1706
- Pasapera Limon AM, Herrera-Munoz J, Gutierrez-Sagal R, Ulloa-Aguirre A (2003) *Mol Cell Endocrinol* 200:199–202
- Perry B, Alexander R, Bennett G, Buckley G, Ceska T, Crabbe T, Dale V, Gowers L, Horsley H, James L, Jenkins K, Crepy K, Kulisa C, Lightfoot H, Lock C, Mack S, Morgan T, Nicolas AL, Pitt W, Sabin V, Wright S (2008a) *Bioorg Med Chem Lett* 18:4700–4704
- Perry B, Beevers R, Bennett G, Buckley G, Crabbe T, Gowers L, James L, Jenkins K, Lock C, Sabin V, Wright S (2008b) *Bioorg Med Chem Lett* 18:5299–5302
- Pomel V, Klicic J, Covini D, Church DD, Shaw JP, Roulin K, Burgat-Charvillon F, Valognes D, Camps M, Chabert C, Gillieron C, Francon B, Perrin D, Leroy D, Gretener D, Nichols A, Vitte PA, Carboni S, Rommel C, Schwarz MK, Ruckle T (2006) *J Med Chem* 49:3857–3871
- Raynaud FI, Eccles S, Clarke PA, Hayes A, Nutley B, Alix S, Henley A, Di-Stefano F, Ahmad Z, Guillard S, Bjerke LM, Kelland L, Valenti M, Patterson L, Gowan S, de Haven Brandon A, Hayakawa M, Kaizawa H, Koizumi T, Ohishi T, Patel S, Saghir N, Parker P, Waterfield M, Workman P (2007) *Cancer Res* 67:5840–5850
- Robinson JS, Klionsky DJ, Banta LM, Emr SD (1988) *Mol Cell Biol* 8:4936–4948
- Ruderman NB, Kapeller R, White MF, Cantley LC (1990) *Proc Natl Acad Sci USA* 87:1411–1415
- Sadhu C, Dick K, Tino WT, Staunton DE (2003a) *Biochem Biophys Res Commun* 308:764–769
- Sadhu C, Masinovsky B, Dick K, Sowell CG, Staunton DE (2003b) *J Immunol* 170:2647–2654
- Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, Yan H, Gazdar A, Powell SM, Riggins GJ, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Velculescu VE (2004) *Science* 304:554
- Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I, Joza N, Mak TW, Ohashi PS, Suzuki A, Penninger JM (2000) *Science* 287:1040–1046
- Schindler T, Bornmann W, Pellicena P, Miller WT, Clarkson B, Kuriyan J (2000) *Science* 289:1938–1942
- Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, Botero ML, Llonch E, Atzori F, Di Cosimo S, Maira M, Garcia-Echeverria C, Parra JL, Arribas J, Baselga J (2008) *Cancer Res* 68:8022–8030
- Shaw RJ, Cantley LC (2006) *Nature* 441:424–430
- Stack JH, Emr SD (1994) *J Biol Chem* 269:31552–31562
- Sujobert P, Bardet V, Cornillet-Lefebvre P, Hayflick JS, Prie N, Verdier F, Vanhaesebroeck B, Muller O, Pesce F, Ifrah N, Hunault-Berger M, Berthou C, Villemagne B, Jourdan E, Audhuy B, Solary E, Witz B, Harousseau JL, Hemberlin C, Lamy T, Lioure B, Cahn JY, Dreyfus F, Mayeux P, Lacombe C, Bouscary D (2005) *Blood* 106:1063–1066
- Sun H, Oudit GY, Ramirez RJ, Costantini D, Backx PH (2004) *Cardiovasc Res* 62:509–520
- Torbett NE, Luna-Moran A, Knight ZA, Houk A, Moasser M, Weiss W, Shokat KM, Stokoe D (2008) *Biochem J* 415:97–110
- Vlahos CJ, Matter WF, Hui KY, Brown RF (1994) *J Biol Chem* 269:5241–5248
- Walker EH, Pacold ME, Perisic O, Stephens L, Hawkins PT, Wymann MP, Williams RL (2000) *Mol Cell* 6:909–919
- Welling A, Hofmann F, Wegener JW (2005) *Mol Pharmacol* 67:541–544
- Whitman M, Kaplan DR, Schaffhausen B, Cantley L, Roberts TM (1985) *Nature* 315:239–242
- Whitman M, Downes CP, Keeler M, Keller T, Cantley L (1988) *Nature* 332:644–646
- Wiesinger D, Gubler HU, Haefliger W, Hauser D (1974) *Experientia* 30:135–136
- Wu J, Ding WG, Matsuura H, Tsuji K, Zang WJ, Horie M (2009) *Br J Pharmacol* 156:377–387
- Wymann MP, Bulgarelli-Leva G, Zvelebil MJ, Pirola L, Vanhaesebroeck B, Waterfield MD, Panayotou G (1996) *Mol Cell Biol* 16:1722–1733

- Yaguchi S, Fukui Y, Koshimizu I, Yoshimi H, Matsuno T, Gouda H, Hirono S, Yamazaki K, Yamori T (2006) *J Natl Cancer Inst* 98:545–556
- Yuan H, Luo J, Weissleder R, Cantley L, Josephson L (2006) *J Med Chem* 49:740–747
- Yuan H, Barnes KR, Weissleder R, Cantley L, Josephson L (2007) *Chem Biol* 14:321–328
- Zhang TT, Okkenhaug K, Nashed BF, Puri KD, Knight ZA, Shokat KM, Vanhaesebroeck B, Marshall AJ (2008) *J Allergy Clin Immunol* 122:811–819, e2