Chemically targeting the PI3K family

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Abstract

PI3K (phosphoinositide 3-kinase) is a key regulator of cell growth, metabolism and survival. The frequent activation of the PI3K pathway in cancer has stimulated widespread interest in identifying potent and selective inhibitors of PI3K isoforms. The present paper highlights recent progress in identifying such molecules and the challenges that remain for efforts to pharmacologically target the PI3K family.

Introduction

PI3K (phosphoinositide 3-kinase) is activated by receptor tyrosine kinases and other cell-surface receptors to synthesize the lipid second messenger PIP_3 (phosphatidylinositol-3,4,5-trisphosphate) [1]. PIP_3, in turn, acts as a docking site at the plasma membrane that recruits and activates proteins containing phospholipid-binding domains. These downstream PI3K effectors include (i) protein kinases that promote cell growth, survival and proliferation [such as Akt, PDK1 (phosphoinositide-dependent kinase 1) and the Tec family kinases]; (ii) GAPs (GTPase-activating proteins) and GEFs (guanine nucleotide-exchange factors) that regulate GTPases mediating cell motility and membrane trafficking; and (iii) scaffolding proteins that nucleate the assembly of key signalling complexes. In this way, PI3K utilizes a single second messenger to link extracellular hormonal cues to intracellular signalling proteins that control diverse cellular processes.

The PI3K family consists of 15 proteins that share sequence homology within their kinase domains but have distinct substrate specificities and modes of regulation [1]. The most well characterized members of this family are the four class I PI3Ks that link PI3K activity to receptor tyrosine kinases (p110α, p110β and p110δ) or G-protein-coupled receptors (p110γ). These proteins synthesize exclusively PIP_3 and mediate most of the known signalling functions attributed to PI3K. In addition to these enzymes, class II and III PI3Ks have been described that also generate 3-phosphorylated inositol lipids, although the physiological roles of these proteins remain elusive. The PIKKs (PI3K-related protein kinases) are a subfamily of serine/threonine kinases that share sequence homology with PI3K but phosphorylate proteins rather than lipids. The PIKKs include key kinases that monitor genomic integrity [ATM (ataxia telangiectasia mutated), ATR (ATM- and Rad3-related), Smg-1 and DNA-PK (DNA-dependent protein kinase)] as well as mTOR (mammalian target of rapamycin), a kinase that integrates nutritional and growth factor signals in order to control protein synthesis and cell growth.

Recent interest in PI3K signalling has been fuelled by evidence that the PI3K pathway is among the most commonly activated signalling pathways in cancer. Several observations have been critical in establishing this connection. First, the p110α isoform of PI3K is activated by mutation at high frequency in a range of primary tumours [2]. Indeed, a recent analysis reported that p110α mutations occur at a cumulative frequency of 15% across all cancer types surveyed to date [3], suggesting that p110α is likely to be the most commonly mutated kinase in the human genome. Secondly, the phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome 10), which antagonizes PI3K signalling by dephosphorylating PIP_3, is a well-characterized tumour suppressor that is frequently inactivated by mutation, gene deletion or epigenetic silencing [4]. In addition, PI3K is allosterically activated by the oncogene Ras [5], and many tyrosine kinases that activate PI3K are themselves the target of mutations or amplification in cancer. Together, these observations reveal a nexus of genetic alterations in cancer that each stimulate PI3K signalling, suggesting that PI3K activation is likely to be an essential step in tumorigenesis.

The widespread activation of PI3K in cancer has stimulated intense interest in developing drugs that target the PI3K pathway. Nonetheless, significant uncertainties surround efforts to therapeutically target these kinases. Unlike protein kinases, class I PI3Ks all phosphorylate the same substrate in order to generate a single lipid product, PIP_3. For this reason, the kinase activity of individual PI3K isoforms cannot be readily distinguished in cells. As class I PI3Ks are essential for a range of normal physiological processes, including glucose homoeostasis and the immune response, broad spectrum PI3K inhibition is likely to be associated with significant toxicity. Isoform-selective PI3K inhibitors may overcome these difficulties, but in many cases it has been difficult to define the isoforms that mediate PI3K's critical physiological functions [6]. Moreover, the highly conserved ATP-binding site of the class I PI3Ks presents a significant challenge for medicinal chemists seeking to identify molecules that display high selectivity between these isoforms. This review briefly

Key words: cancer, mammalian target of rapamycin (mTOR), p110, phosphatidylinositol, phosphoinositide 3-kinase (PI3K).

Abbreviations used: ATM, ataxia telangiectasia mutated; ATR, ATM- and Rad3-related; DNA-PK, DNA-dependent protein kinase; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PIKK, PI3K-related protein kinase; PIP_3, phosphatidylinositol-3,4,5-trisphosphate.

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summarizes recent progress in developing isoform-selective PI3K inhibitors as well as challenges that remain for efforts to pharmacologically target this enzyme family.

The prototype PI3K inhibitors wortmannin and LY294002

The discovery of the pan-specific PI3K inhibitors wortmannin and LY294002 was a critical early event that enabled the rapid exploration of PI3K signalling. Wortmannin is a fungal natural product that was originally described as a potent inhibitor of the respiratory burst in neutrophils and monocytes [7]. PI3K was subsequently identified as the molecular target of wortmannin [8], and the mechanism of inhibition was shown to involve covalent attack of Lys^{833} within the ATP-binding site of PI3K at an electrophilic site on the natural product [9].

Shortly after the identification of PI3K as the target of wortmannin, the synthetic PI3K inhibitor LY294002 was reported [10]. The discovery of LY294002 demonstrated the feasibility of inhibiting PI3K with a reversible, drug-like small molecule at a time (1994) when few kinase inhibitors were known. Subsequent crystal structures of LY294002 and wortmannin bound to p110γ confirmed that both molecules occupy the ATP-binding site [11]. Despite the limitations of these inhibitors (wortmannin is a reactive electrophile, whereas LY294002 has micromolar affinity), the early availability of these molecules played an essential role in shaping our current understanding of PI3K signalling.

The development of isoform-specific PI3K inhibitors

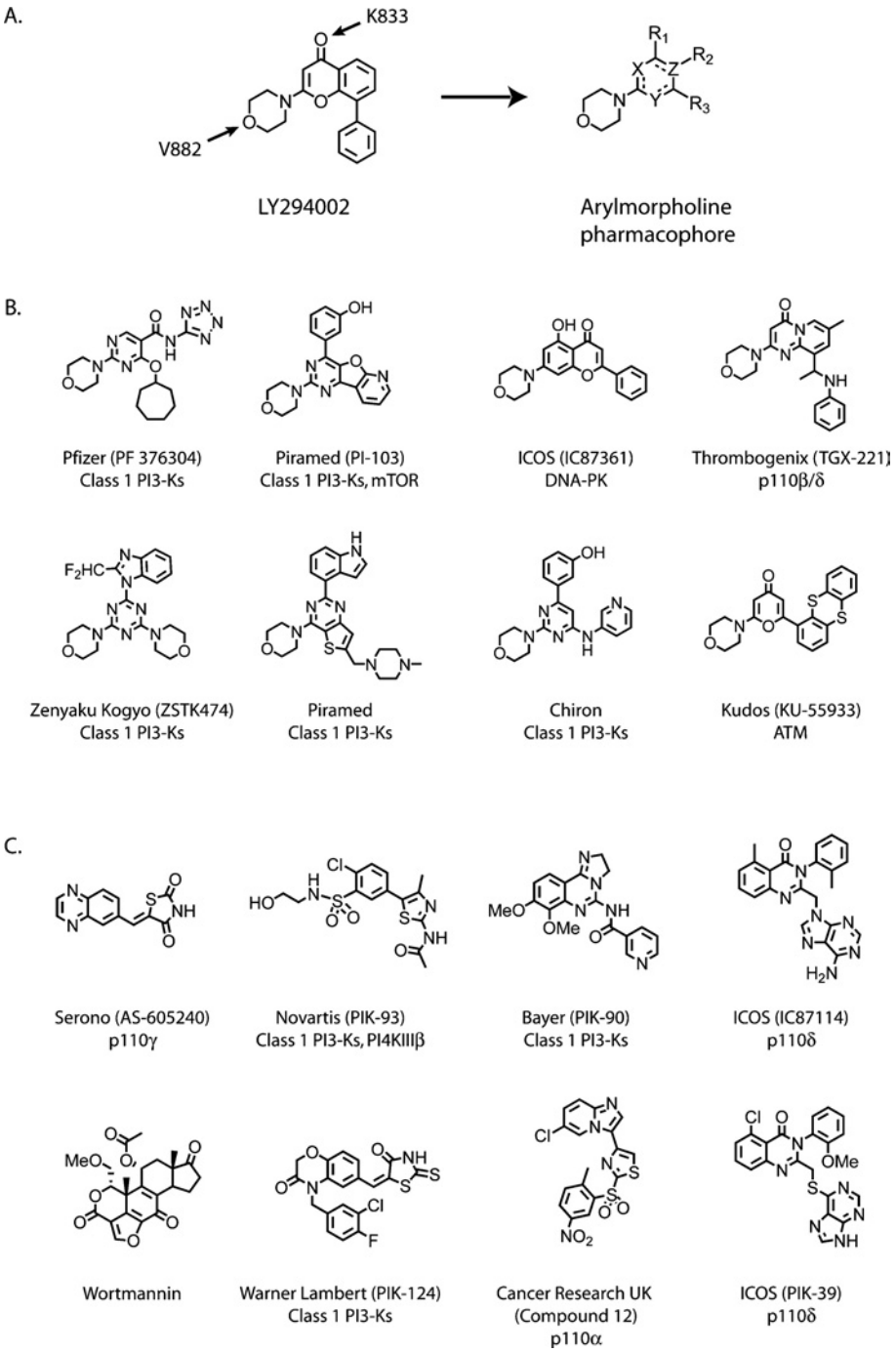
LY294002 and wortmannin show little selectivity within the PI3K family. For this reason, these molecules cannot be used to probe signalling by specific PI3Ks, and their structures provide little guidance for the design of selective inhibitors. Nonetheless, growing appreciation of the therapeutic potential of PI3K inhibitors has led to significant efforts in recent years within the pharmaceutical industry to identify new PI3K inhibitors with enhanced potency, selectivity and pharmacological properties. Recently, the first of these molecules has been reported.

IC87114 was the first isoform-selective PI3K inhibitor to be described [12] (Figure 1). This quinazolinone purine inhibits p110δ at mid-nanomolar concentrations and shows 100–1000-fold selectivity between p110δ and the other class I PI3Ks (p110α, p110β and p110γ). The selectivity of IC87114 is remarkable given that the residues that line the ATP-binding pocket of the class I PI3Ks are highly conserved. The co-crystal structure of an IC87114 analogue (PIK-39) bound to p110γ was recently reported, and this structure suggests a mechanism for the selectivity of PIK-39 [13]. This structure revealed that PIK-39 binding induces a conformational change in the kinase whereby Met^{804}, which forms the upper surface of the ATP-binding pocket, shifts downward to form a novel pocket at the entrance to the kinase active site. This induced pocket buries most of the hydrophobic surface area of the PIK-39 quinazolinone ring system and therefore is likely to be critical for the high-affinity binding of this compound to p110δ. The importance of this conformational shift for PIK-39 binding was tested by introducing mutations into p110δ that replace Met^{804} with β-branched amino acids (valine or isoleucine) that were predicted to restrict formation of the induced pocket [13]. Consistent with this prediction, these mutations impaired the binding of IC87114 and PIK-39 to p110δ, but had no effect on the binding of three other chemotypes of PI3K inhibitors that do not access the induced pocket formed by Met^{804}. Together, these results support a model in which quinazolinone purines such as IC87114 and PIK-39 selectively inhibit p110δ by targeting a residue (Met^{804}) that shows differential conformational mobility among PI3K isoforms. Modelling suggests that additional chemotypes of PI3K inhibitors may achieve their selectivity via similar interactions with this inducible pocket, [13] but this hypothesis remains to be crystallographically verified. Finally, it is worth noting that this mechanism of inhibitor binding shares similarities with protein kinase inhibitors such as imatinib, which distinguish between closely related kinases such as Abl and Src by selectively stabilizing differentially populated conformational states of these proteins [14].

In the three years since the description of IC87114, a series of small molecules have been reported that show varying degrees of selectivity for p110α [13,15,16], p110β [17,18], p110γ [19,20], DNA-PK [21–23] and ATM [24,25] (Figure 1). All of these compounds are drug-like, reversible inhibitors that are ATP-competitive and possess nanomolar affinity against their primary target. The extended biochemical selectivity of representatives from several of these compound classes has been determined [13] and co-crystal structures of several compounds bound to p110γ have been reported [13,19]. While no compound shows absolute selectivity for its primary target, impressive $\sim$100-fold selectivity has been achieved in a several cases. A detailed discussion of each chemotype is beyond the scope of the present paper, but several themes have emerged from this analysis. First, PI3K inhibitors share many features in common with well-characterized inhibitors of protein kinases. Like protein-kinase inhibitors, all known PI3K inhibitors satisfy the hydrogen bond that is made between the N1 of adenine in ATP and the hinge region of the kinase [11,13,19]. In protein kinases, the size of a single residue in the interior of the ATP-binding pocket, termed the gatekeeper, has been shown to control the potency of a wide range of inhibitors by regulating their access to a deeper hydrophobic pocket [26,27]. Co-crystal structures of potent PI3K inhibitors bound to p110γ revealed that these molecules also access a deeper hydrophobic pocket in lipid kinases that is essential for their high-affinity binding [13]. However, a slight shift in the orientation of the gatekeeper residue in PI3Ks relative to protein kinases appears to make this pocket accessible throughout the family, irrespective of the size of the gatekeeper residue [28]. Interestingly, despite these structural similarities between the ATP-binding sites of protein and lipid kinases,

Figure 1 | Chemical structures of PI3K inhibitors

(**A**) Structure of LY294002, indicating hydrogen bonds to Val^{882} and Lys^{833} of p110γ. (**B**) Structures of arylmorpholine PI3K inhibitors. (**C**) Structures of PI3K inhibitors from other chemical classes.



potent PI3K inhibitors from diverse chemotypes generally do not inhibit a wide range of protein kinases. This presumably reflects the fact that, despite their superficial similarities, PI3Ks and protein kinases share no sequence homology and therefore present unique surfaces within their ATP-binding sites that are recognized by high-affinity inhibitors.

Analysis of the selectivity profiles of chemically diverse PI3K inhibitors has revealed trends among molecules that target this enzyme family. In particular, certain PI3K family members show similar patterns of sensitivity to existing small molecule inhibitors. We have referred to these pairs of kinases as pharmalogs to reflect the fact that they show homology in their sensitivity to chemical inhibitors [13]. For example, among the class I PI3Ks, inhibitors of p110β often potently inhibit p110δ, whereas inhibitors of p110α often potently inhibit p110γ. In a similar fashion, inhibitors of class I PI3Ks

frequently inhibit DNA-PK potently, even though the same molecules show little or no activity against the PIKKs that are most closely related to DNA-PK in amino acid sequence (such as mTOR, ATM and ATR). The latter example is particularly striking because sequence alignments do not suggest obvious similarities between the class I PI3Ks and DNA-PK in the residues that line their ATP-binding pockets. This suggests that these kinases share a cryptic similarity in the three-dimensional structures of their active sites that is not obvious from sequence analysis. Alternatively, DNA-PK may be generally more druggable than other members of the PI3K family and therefore shows enhanced sensitivity to inhibitors that target any PI3K family member. Distinguishing between these possibilities will require the detailed biochemical characterization of chemically diverse inhibitors that target PI3K family members other than the class I PI3Ks.

Comparison of the chemical structures of PI3K inhibitors reveals that certain privileged scaffolds are frequently found in molecules targeting this enzyme family. The most common of these is the arylmorpholine, which forms the core structure of the original PI3K inhibitor LY294002 (Figure 1A). The crystal structure of LY294002 bound to p110γ revealed that the oxygen atom of morpholine satisfies the hinge region hydrogen bond to Val^{882} [11]. Furthermore, the morpholine ring makes extensive hydrophobic interactions with residues that form the top and bottom of the ATP-binding pocket. These extended interactions densely pack against the morpholine ring, explaining why subtle alterations in the morpholine structure result in a dramatic loss of affinity [10,29]. The versatility of this chemical class is highlighted by the diversity of arylmorpholines that have recently been described to inhibit p110α, p110β/δ, mTOR, ATM or DNA-PK (Figure 1B).

Questions for the development of PI3K inhibitors

The past three years have witnessed an explosion of information about small molecules that target the PI3K family. The original inhibitors LY294002 and wortmannin have now been joined by at least 15 new chemical classes of inhibitors, many of which have crystallographically defined binding modes and biochemically enumerated target selectivities. As a result of extensive medicinal chemistry efforts within the pharmaceutical industry, relatively selective inhibitors now exist for each of the class I PI3Ks as well as the PIKKs ATM and DNA-PK.

Despite these advances, our understanding of the rules that determine the selectivity and potency of PI3K inhibitors remains in its infancy. In this regard, protein kinase inhibitors, whose clinical development has preceded lipid kinase inhibitors by a decade, may provide useful guidance. For example, a key discovery in the development of protein kinase inhibitors was the finding that protein kinases shuttle between multiple conformational states, and that highly selective kinase inhibitors such as imatinib act by selectively trapping these states [30]. For many protein kinases, the equilibrium between these conformations is believed to be controlled by phosphorylation of key residues in the activation loop, such that these states correspond to natural active or inactive conformations of the kinase. By contrast, PI3K is not known to undergo activation loop phosphorylation, and the conformational changes that accompany PI3K activation are largely unknown. Do current PI3K inhibitors sense the activation status of these enzymes? If not, is it possible to identify molecules that stabilize the inactive conformation of a lipid kinase, in a manner analogous to protein kinase inhibitors that bind to protein kinases in a 'DFG out' orientation? The crystal structure of PIK-39 provides the first evidence that PI3K inhibitors can exploit the conformational flexibility of a lipid kinase to achieve highly selective binding, but the broader significance of this 'Met^{804} down' conformation, for the binding of other classes of inhibitors or for normal PI3K regulation, is unknown. The biochemical and structural exploration of these questions is likely to provide important insights into strategies for selectively targeting the PI3K family.

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Received 23 October 2006