Protein and lipid kinase inhibitors as targeted anticancer agents of the Ras/Raf/MEK and PI3K/PKB pathways

Carlos García-Echeverría

Abstract The identification and characterization of the components of individual signal transduction cascades, and advances in our understanding of how these biological signals are integrated in cancer initiation and progression, have provided new strategies for therapeutic intervention in solid tumors and hematological malignancies. To this end, pharmaceutical efforts have been directed to target different components of the Ras/Raf/MEK and PI3K/PKB pathways. This review article covers recent salient achievements in the identification and development of Raf, MEK, and PI3K inhibitors.

Keywords Cancer · Drug design · Kinase · MEK · PI3K · Raf · Targeted therapy

Abbreviations
HMVEC human dermal microvascular endothelial cells
i.v. intravenous
mTOR mammalian target of rapamycin
PD pharmacodynamic
PK pharmacokinetic
PKB protein kinase B (also known as Akt)
p.o. per os
PTEN phosphatase and tensin homolog deleted on chromosome 10

RCC renal cell carcinoma
RTKs receptor tyrosine kinases
VEGF vascular endothelial growth factor

Introduction

The growth factor-stimulated Ras/Raf/MEK (Fig. 1) and PI3K/PKB (Fig. 2) signal transduction pathways have been demonstrated to play central roles in the biology of human cancers. Whatever the mechanism, aberrant activation of these signaling cascades is a frequent event in human tumors due to their involvement in key biological processes like cancer cell survival, growth, proliferation, and invasion. Drug discovery activities to control the irregular activation of these pathways have led to the development of novel target-based agents for cancer treatment. To this end, several approaches have been adopted to inhibit or decrease pathway activity. Much of the original therapeutic efforts was directed to block receptor tyrosine kinases (RTKs), which are upstream activators of these pathways (Figs. 1 and 2). The clinical efficacy of antibodies that inhibit ligand-mediated activation of RTKs—e.g., Herceptin™, Erbitux™, or Avastin™—or low-molecular mass inhibitors that target the ATP-binding cleft of these enzymes—e.g., Iressa™, Tarceva™, or Tykerb™—have provided proof of concept of the therapeutic validity of this strategy. Parallel to the development of RTK inhibitors, pharmaceutical efforts have also been directed to target some of the cytosolic molecules of the Ras/Raf/MEK and PI3K/PKB pathways, in particular protein and lipid kinases. In this review, I will briefly summarize recent salient medicinal chemistry achievements in the identification and development of...
kinase inhibitors of these cytoplasmic pathways for the treatment of cancer. Several articles on individual topics are available and the reader is referred to the most recent publications in the text.

Modulators of the Ras/Raf/MEK signaling cascade

The Ras/Raf/MEK pathway (Fig. 1) is activated through the initial extracellular binding and stimulation of RTKs by their respective cognate ligands. Upon autophosphorylation of specific tyrosine residues in the cytosolic domain of RTKs, the Grb2-Sos complex translocates to the plasma membrane and converts the inactive Ras guanosine diphosphate (GDP) to active Ras guanosine triphosphate (GTP). The interaction between Grb2 and the activated kinases or the phosphorylated receptor-associated proteins is mediated by the SH2 domain of the signaling protein that recognizes specific phosphotyrosine sequences. Ras undergoes conformational changes upon GTP binding and causes the recruitment of Raf-1 to the cytoplasmic membrane where it is phosphorylated by several kinases and simultaneously diphosphorylated at key residues by protein phosphatase 2B. Activated Raf phosphorylates MEK on two serine residues in the activation loop, which results in the activation of this protein kinase. MEK then phosphorylates and activates ERK, allowing its translocation to the nucleus where it phosphorylates transcriptional factors permitting the expression of a variety of genes. As shown in this section, several components of the Raf/MEK/ERK cascade represent unique opportunities for cancer treatment [1, 2].

Inhibitors of the kinase activity of Raf

Raf kinases exist as three isoforms A-, B-, and C-Raf. As previously mentioned, activated Raf phosphorylates and activates MEK and downstream efforts [3]. B-Raf is one of the most frequently mutated genes in human cancers, with an overall frequency of 7–8% [4]. B-Raf mutations have been found in 27–70% of melanomas, approximately 30% of papillary thyroid cancers, and 10–15% of colorectal and ovarian cancers. Among the reported mutations, the most prominent one results in a change of the Val residue in the activation loop to Glu [5], leading to a constitutive active kinase [3]. Accumulating preclinical and clinical studies have indicated that tumors harboring B-Raf mutations may exhibit an oncogenic addiction and that this phenotype presents an unique opportunity for therapeutic interference by blocking the kinase activity of Raf [6].

Sorafenib (Nexavar™, BAY 43–9006, compound 1, Fig. 3) is the only Raf kinase modulator to receive marketing approval—December 2005—for treatment of advanced renal cell carcinoma (RCC). This biaryl urea derivative inhibits in vitro biochemical assays not only Raf kinase (e.g., IC₅₀=6 nM, 22 nM, and 40 nM for C-Raf, B-Raf, and B-RafV600E, respectively), but also shows potent activity for other protein kinases, including the proangiogenic receptor tyrosine kinases PDGF-β (IC₅₀=57 nM), VEGFR-2 (IC₅₀=90 nM), and FGFR-1 (IC₅₀=580 nM) [7]. The in vivo antitumor activity reported for this compound is most likely driven by its multikinase inhibitory profile. In this context, the relative broad-spectrum in vivo antitumor activity exhibited by sorafenib in preclinical animal models usually did not correlate with reduced ERK activation. Thus, tumor stasis was observed when the compound was administered to mice bearing the B-RafV600E mutant Colo205 tumor xenograft, but no significant effect on ERK phosphorylation was detected at the end of this efficacy study [7].

The resolution of the X-ray structure of the kinase domain of B-Raf in complex with sorafenib helped to understand some of the structural features of this kinase and provided the basis for the rational design of other Raf kinase modulators [3]. The inhibitor interacts with the ATP-binding cleft of the enzyme and stabilizes the protein in a conformation that prevents substrate binding and phosphorylation. In its binding mode, the distal pyridyl group occupies the ATP-binding pocket and the opposite trifluoromethyl-containing phenyl moiety inserts into a hydrophobic pocket. The interactions mediated by these groups are
complemented by two critical hydrogen bonds with the urea moiety: one via its amide nitrogen atom to the carboxylate side chain of the catalytic Glu500 residue and the second via the carbonyl moiety to the main chain nitrogen of Asp593 of the DFG moiety. Similar types of hydrogen bonds have been reported between c-Abl and p38 MAP kinase and their respective inhibitors Gleevec™ and BIRB-796, probing the importance of these type of interactions in the unique mechanism of action of these kinase inhibitors [8–10].

RAF265 (previously known as CHIR-265, compound 2, Fig. 3) is another orally bioavailable Raf modulator currently being investigated in phase I clinical trials. The compound inhibits in biochemical assays C-Raf, B-Raf, B-RafV600E mutant, VEGFR-2, and c-Kit with IC\textsubscript{50} values in the low nM range and has shown potent downregulation of the ERK pathway and tumorigenesis in relevant \textit{in vivo} models [11]. PLX4032 (structure not available), which was identified following a scaffold-based drug discovery strategy, has also recently started phase I clinical trials. The compound significantly inhibits V600E B-Raf (IC\textsubscript{50}=31 nM) compared with a panel of over 70 other protein kinases. Its kinase profile translates into selective antiproliferative activity against Raf- but not Ras-driven melanoma and colorectal cancer cell lines (IC\textsubscript{50}=0.04–0.4 vs 6.5–16 \textmu M against cell lines bearing mutant Ras). Statistically significant antitumor activity was observed in mice bearing human colorectal cancer Colo205 xenografts when given orally at 20 mg/kg per day, with no effect on body weight and strong inhibition of pERK as determined by \textit{ex vivo} analyses of tumor tissue at the end of the efficacy study. XL281 (structure not disclosed) is another potent B-Raf, V600E-B-Raf, and C-Raf inhibitor (IC\textsubscript{50}=4.5, 6, and 2.5 nM, respectively) that has initiated phase I clinical trials in 2007. Significant inhibition of pERK levels was observed in B-Raf-driven tumor xenograft models with ED\textsubscript{50} values ranging from 60 to 90 mg/kg.
Inhibitors of the kinase activity of MEK

The dual–specificity MEK1 and MEK2 kinases are capable of phosphorylating both serine/threonine and tyrosine residues of their physiological substrates ERK1 and ERK2. The observation that ERK is the only known MEK substrate and that ERK is commonly activated in tumor cells has triggered substantial interest in the identification and development of inhibitors of MEK as a means to block ERK activation in human cancers [1].

A common feature of the available MEK kinase modulators is their allosteric non-ATP-competitive inhibitory mechanism. The X-ray structure of the kinase domain of MEK in complex with one of the early MEK inhibitors—a derivative of compound 3, Fig. 3—has shown that the compound did not perturb ATP binding [12]. Instead, the molecule is bound to a unique, probably exclusive, pocket adjacent to the Mg/ATP-binding site locking the enzyme in a catalytically inactive conformation. The recognition of the amino acids involved in this pocket—Met143, Ile141, Leu118, and Phe209—and the conformation adopted by the enzyme upon compound binding may account for the exclusive target selectivity reported for the current non-ATP-competitive MEK kinase inhibitors.

CI-1040 (PD184352, compound 3, Fig. 3) was the first orally active, potent, and selective MEK1/2 inhibitor to enter clinical trials in cancer patients [13, 14]. Unfortunately, the compound was determined to have suboptimal human pharmacokinetic properties—low oral bioavailability and metabolic instability—and clinical investigations were
discontinued after negative phase II clinical studies in cancer patients with solid tumors [15]. Two second-generation MEK1- and 2-specific inhibitors—PD0325901 (compound 4, Fig. 3) and ARRY-142886/AZD6244 (compound 5, Fig. 3)—believed to have addressed some of the pharmacological deficiencies of CI-1040 have been developed and are currently undergoing clinical trials [16].

PD0325901 is a structurally related analog of CI-1040. The modifications introduced in the scaffold resulted in a significant increase in potency (> 50-fold, IC₅₀=1 nM), improved oral bioavailability, and better in vivo target coverage compared to CI-1040. ARRY-142886 is also an orally bioavailable, potent MEK1/2 inhibitor (IC₅₀=12 nM) that exploits a similar binding mode as CI-1040 and PD0325901. Preclinical evaluation of these compounds showed statistically significant antitumor activity in several tumor human xenograft models at tolerated doses [2]. The observed antitumor activity was found to correlate with suppression of pERK as determined by ex vivo analyses of tumor tissue. PD0325901 and ARRY-142886/AZD6244 are currently been evaluated in phase II clinical trials in patients with solid tumors [1].

The available X-ray structural information has also been used to design new, non-ATP-competitive MEK inhibitors. This approach has been nicely illustrated in a recent publication [17]. The pyridone derivative (compound 6, Fig. 3) was found to be more potent than CI-1040 (IC₅₀=4 vs 46 nM, colon26 pERK cellular assay) and more efficacious in in vivo settings as measured by response rate as well as tumor growth delay.

In addition to the non-ATP-competitive compounds described previously, a limited number of chemotypes have been described as classic ATP-MEK1/2-competitive inhibitors. Representative examples of this class of inhibitors are cyanoquinoline (compound 7, Fig. 3) [18] and resorcyclic acid (compound 8, Fig. 3) [19] derivatives. In addition to MEK1/2 modulation (e.g., IC₅₀=4 nM for L-783,277) [19], resorcyclic-based compounds have shown inhibitory activity against Fli-3, FLT-1, KDR, PDGFR, TRKB, ERK, and Src. The key pharmacophore in these broad-spectrum kinase modulators is the enone functionality, which is involved in the formation of a covalent adduct with Cys-166. The inherent chemical reactivity of the enone group to Michael addition poses a major liability to develop these derivatives as potential new therapeutic agents. For other examples of competitive and noncompetitive ATP MEK inhibitors, see [1].

**Modulators of the PI3K/PKB signaling cascade**

A substantial number of epidemiological and experimental studies support an important role of the PI3K/PKB signaling cascade in the biology of human cancer. Thus, the activation of this pathway (Fig. 2) has been clearly validated as an essential step for the initiation and maintenance of the tumorigenic phenotype. Upregulation can be the result of: (1) constitutively active mutants or overexpression of some receptor tyrosine kinases (e.g., EGFR or ErbB2) leading to constitutive recruitment and activation of PI3K; (2) amplification and/or overexpression of the p110α catalytic subunit; (3) presence of activating mutations in the PIK3CA gene encoding the p110α catalytic subunit; (4) overexpression of the downstream kinase PKB (also known as Akt); (5) loss or inactivating mutation of the tumor suppressor gene phosphatase and tensin homolog deleted on chromosome 10 (PTEN), a natural negative regulator of the PI3K pathway; or (6) constitutive recruitment and activation by mutant forms of the Ras oncogene. The preceding alterations trigger a cascade of biological events, from cell growth and proliferation to survival and migration, that drive tumor progression, survival, angiogenesis, and metastasis. In addition to its role in the biology of human tumors, this pathway also plays a major part in the resistance of tumor cells to conventional anticancer therapies. Blocking the PI3K/PKB pathway could therefore simultaneously inhibit the proliferation and growth of tumor cells and sensitize them toward programmed cell death. To this end, drug discovery activities have been directed to identify agents that effectively and specifically disrupt this pathway in tumor cells. Herein, the progress in the development of compounds designed to target PI3K, one of the main components of the pathway, is briefly reviewed [20, 21].

Inhibitors of the lipid kinase activity of PI3K

3-Phosphorylated inositol lipids are phosphorylated by members of a family of closely related enzymes known as PI3Ks. These lipid kinases are ubiquitously expressed in cells and function as signal transducers downstream of cell surface receptors. The products of PI3K-catalyzed reactions, phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P₃), phosphoinositidylinositol 3,4-bisphosphate (PtdIns(3,4)P₂), and phosphoinositidylinositol 3-phosphate (PtdIns(3)P), are second messengers and have central roles in a number of cellular processes, including cell growth, differentiation, mobility, proliferation, and survival.

The eight members of the PI3K family have been classified into three groups based on their primary sequence, in vitro substrate preference, domain structure, and mode of regulation. The subject of this section is class I PI3Ks, which is composed of two subgroups, IA and IB [22]. The class IA PI3K subgroup consists of there catalytic subunits, p110α, p110β, and p110δ, that form heterodimers with one of five regulatory domains: p85α, p85β, p85γ,
p50α, and p55α. As shown in Fig. 2, these PI3Ks are activated by cell surface receptor tyrosine kinases. The class IB PI3K consists of one member, a heterodimer of p110γ and a regulatory p101 domain, that is activated by G proteins βγ subunits following the stimulation of G protein-coupled receptors. PI3Ks IA and IB catalyze the formation of PtdIns(3,4,5)P3, a process that is reversed by the action of a phosphatase, PTEN.

Two well-known and isoform nonselective PI3K inhibitors are the fungal metabolite wortmannin (compound 9, Fig. 4) [23] and LY294002 (compound 10, Fig. 4) [24]. Although these two compounds bind to the ATP-binding cleft of the lipid kinase, they block the enzymatic activity of PI3K by different mechanisms. Thus, wortmannin is an irreversible inhibitor (IC50 ≈ 2 nM) that forms a covalent bond with a conserved lysine residue in the ATP-binding cleft of PI3K [25], while LY294002 is a classic ATP-competitive PI3K inhibitor (IC50 = 1.40 μM) [24, 26]. In spite of the crossover inhibition of other lipid and protein kinases and their unfavorable pharmaceutical properties, these two compounds have served as powerful research tools for more than a decade to elucidate the role of PI3Ks in human tumorigenesis and evaluate the potential utility of PI3K modulators as cancer therapeutics.

The scaffolds of wortmannin and LY294002 have been the subject of intense lead optimization efforts to overcome the pharmaceutical limitations of the parent compounds. Thus, using wortmannin as a starting point, broad-spectrum PI3K inhibitors with improved pharmaceutical properties and therapeutic indexes like PWT-458 (compound 11, Fig. 4) [27] and PX-866 (compound 12, Fig. 4) [28, 29] have been reported. PWT-458 is a PEGylated derivative of wortmannin that has shown to have a higher therapeutic index over its parent compound. Upon i.v. administration, the polyethylene-glycol moiety is cleaved and 17-hydroxywortmannin is released. In vivo antitumor activity has been
reported in several human xenograft models (e.g., U87MG, A549, and A498). PX-866, which is a semisynthetic viridin derivative, potently and irreversibly inhibits PI3Kα, γ, and δ (IC50=5.5, 9.0, and 2.7 nM, respectively), but unlike wortmannin shows a certain level of selectivity over PI3Kβ (IC50=300 nM) and higher biological stability. The compound has also shown antitumor activity against several human solid tumor models (e.g., OvCar-3, HT-29, or A-549) when used alone (2 mg/kg, p.o.) or in combination with chemotherapeutic or targeted anticancer agents (e.g., paclitaxel or gefitinib). In addition to a decrease in body weight and leukocytosis, a major toxicity of PX-866 administration to SCID mice was hyperglycemia with decreased glucose tolerance. These changes were dose dependent and reversible upon discontinuation of the drug. PX-866 is expected to enter phase I clinical trials in 2008.

Similar lead optimization approaches have been used for LY29002 and derivatives thereof. SF1126 (compound 13, Fig. 4) [30] is a water-soluble prodrug of LY294002. The compound, which is designed to target specific integrins, inhibits all paralogs of PI3K (IC50=0.5–5.7 μM) and other members of the PI3K family, including DNA-PK and mTOR. It blocks the phosphorylation and activation of PKB/Akt in cellular settings with IC50 values in the low μM range (e.g., IC50=2.4 μM, MM.1S). As in the case of the wortmannin derivatives, SF1126 has demonstrated significant antitumor activity in several xenograft models of brain, neuroblastoma, non-small cell lung, prostate, myeloma, renal, and colon carcinoma. In addition to its direct activity in cancer cells, microvessel density analysis of tumor tissue demonstrated that the compound also had significant antiangiogenic activity in vivo [30]. SF1126 is currently in phase I clinical trials.

In addition to the efforts made to overcome the pharmacological hurdles encountered with wortmannin and LY294002, high-throughput screening and medicinal chemistry activities have been utilized to discover pan- or paralog-selective PI3K inhibitors. Overall, the new generation of PI3K inhibitors have better pharmacological characteristics than the early compounds as well as improved PI3K selectivity profile. Some of these compounds are briefly reviewed in the following paragraphs.

NVP-BEZ235 (compound 14, Fig. 4) is a dual pan-PI3K/mTOR inhibitor: IC50=4, 76, 7, 5, and 21 nM against p110α, β, γ, δ, and mTOR, respectively [31, 32]. The compound exhibits potent antiproliferative activity against a broad panel of tumor cell lines (e.g., IC50=10 nM, U87MG cells) by specifically blocking the biological function of PI3K signaling components (e.g., IC50=10 ± 1 nM, Ser473-PKB/Akt inhibition in U87MG cells). The antiproliferative activity of this imidazoquinoline derivative in cellular settings translates well in in vivo models of human cancer (e.g., U87MG, PC3M, and A549). Thus, the compound displayed disease stasis or tumor regression when administered orally—25–50 mg/kg per day—and enhanced the efficacy of other anticancer agents when used in in vivo combination studies (e.g., docetaxel or temozolomide). Ex vivo PK/PD analyses of tumor tissues upon acute dose or after termination of in vivo efficacy studies showed a time-dependent correlation between compound concentration and inhibition of PKB/Akt phosphorylation. NVP-BEZ235 was well tolerated at the efficacious doses when compared with vehicle-treated animals, with no significant difference seen in the body weight. Unlike other modulators of the PI3K/PKB pathway, no elevated blood glucose levels were observed in the animals treated with NVP-BEZ235 after in vivo efficacy experiments in mice or rats. Moreover, no effect on insulin or glucose levels was reported for NVP-BEZ235 at efficacious doses in a 13-week rat toxicity study [32]. Phase I trials with this dual PI3K/mTOR inhibitor were initiated in patients with solid tumors at the end of 2006.

XL147 and XL765 (structures not disclosed) are also low-molecular mass PI3K inhibitors in phase I clinical trials. XL147 is active against class I PI3Ks (IC50=39, 383, 23, and 36 nM for p110α, β, γ, and δ, respectively) without inhibiting the kinase activity of Vps34 (IC50=6,975 nM), DNA-PK, or mTOR (IC50 > 15,000 nM) [33, 34]. The compound inhibits PI3K signaling in cultured tumor cells (IC50=0.40–1 μM, p-Thr308PKB) and blocks VEGF-induced tubule formation in cellular setting (IC50=529 nM, HMVEC-L). Oral dosing slows tumor growth or induces shrinkage in preclinical models of breast, lung, ovarian, prostate, and glioma tumors. Increased tumor growth inhibition over the corresponding single agents was obtained by combining this PI3K inhibitor with cytotoxic or targeted anticancer agents (e.g., carboplatin, paclitaxel, or erlotinib). XL765 has similar activity towards class I PI3K (IC50=39, 113, 9, and 43 nM for p110α, β, γ, and δ, respectively) as XL147, but, in addition, it also inhibits DNA-PK (IC50=150 nM) and mTOR (IC50=157 nM) [35, 36]. As in the case of XL147, tumor growth is delayed or halted in in vivo efficacy experiments using suitable xenograft tumor models. Interim analyses of ongoing phase I studies with these compounds have shown interpatient variability with terminal half-life values that ranged from 2.5 to 8 days for XL147 and 2 to 15 h for XL765. Preliminary signs of exposure-dependent pharmacodynamic modulation (changes in plasma insulin levels) were observed at 30 mg/60 mg for XL147 and 15 mg/30 mg b.i.d. for XL765. Analyses of other surrogate tissues, including PBMC lysates and hair follicles, for target modulation are also under investigation.

Imidazopyrimidines and pyridofuropyrimidines with class I PI3K activity have been disclosed in several...
publications. For examples, PI103 (compound 15, Fig. 4) is a pan-PI3K inhibitor with enzymatic IC$_{50}$ values in the 2 nM (PI3Kα) to 15 nM range (PI3Kγ) [37, 38]. Despite its fast in vivo metabolism, the compound, which is rapidly distributed to tumor tissue, delays the growth of different subcutaneous human cancer xenografts. Recently, it has been shown that PI-103 as other pan-PI3K modulators also inhibits both complexes of mTOR: the rapamycin-sensitive mTORC1 (IC$_{50}=0.02$ μM) and the rapamycin-insensitive mTORC2 (IC$_{50}=0.083$ μM). The mTOR biochemical activity was further confirmed in cells, where the compound blocks downstream markers of mTOR signaling (e.g., phosphorylation of p70 S6 kinase IC$_{50} < 0.1$ μM). PI-103 has shown a significant antitumor activity in human glioma xenografts with a significant therapeutic window.

The identification and optimization of the preceding PI3K modulators has certainly benefitted by having early access to structural information. The resolution of the X-ray structure of the p110 subunit of PI3Kγ [39, 40], and more recently of the human p110α/p85α complex [41], has been fundamental in providing a molecular map of the ATP-binding cleft of this special family of kinases.

Parallel to the drug discovery efforts to develop pan-PI3K modulators, there is an increase in interest to identify inhibitors with some level of selectivity against the different p110 enzymes. Sequence alignment and homology models have revealed that some amino acid differences exist between PI3K members, in particular, at the entrance of the ATP-binding pocket. Modulating and fine-tuning the interactions of the modified molecular template with these amino acids may provide paralog- or mutation-selective PI3K inhibitors for cancer treatment [42, 43]. To this end, selectivity trends among compound classes that target PI3K members have already been identified by extensive biochemical profiling against all the members of this lipid kinase family [44]. According to these results, inhibitors of p110β often tend to inhibit also p110δ, whereas inhibitors of p110αx seems to inhibit preferentially p110γ. Interestingly, and in spite of a clear sequence homology in the kinase domain, modulators of class I PI3K tend to inhibit also DNA-PK (e.g., SF1126, XL765). The in vivo exploration—efficacy and toxicity studies—of compounds with different selectivity profiles is likely to provide important insights into the optimal kinase activity profile to exploit for drug discovery. However, as with any other drug discovery project, the ultimate proof of the “most desirable lipid kinase activity profile” will come from clinical trials.

Conclusions and outlook

Rapid progress has occurred in the last few years in the development of kinase modulators that target different components of the Ras/Raf/MEK and PI3K/PKB pathways. As shown in the preceding sections, aberrant activation of these signaling cascades is a frequent event in human tumors providing unique opportunities for therapeutic intervention. Equally important as the epidemiological data, a better understanding of the different components and their roles in the Ras/Raf/MEK and PI3K/PKB pathways has allowed the identification and development of clinical candidates that control the irregular activation of these pathways. Medicinal chemists and molecular modelers have faced the challenge of identifying structural features around the ATP-binding site of the targeted enzyme to design specific kinase inhibitors. Despite the lack of complete specificity, with the exception of the MEK inhibitors that are among the most selective inhibitors reported to date, the compounds that have entered clinical trials have shown in preclinical models significant in vivo antitumor activities in the absence of limiting toxicities. It is still unclear if the potential therapeutic benefit and tolerability that direct inhibition of components of the Ras/Raf/MEK and PI3K/PKB pathways and potential concomitant inhibition of other off-targets will be beneficial in the treatment of cancer. We can only hope that novel and safe targeted anticancer agents will come from these drug discovery efforts.

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