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Inhibition of Akt with small molecules and biologics: historical perspective and current status of the patent landscape

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Abstract

Introduction—Akt plays a pivotal role in cell survival and proliferation through a number of downstream effectors; unregulated activation of the PI3K/PTEN/Akt pathway is a prominent feature of many human cancers. Akt is considered an attractive target for cancer therapy by the inhibition of Akt alone or in combination with standard cancer chemotherapeutics. Both preclinical animal studies and clinical trials in humans have validated Akt as an important target of cancer drug discovery.

Area covered—A historical perspective of Akt inhibitors, including PI analogs, ATP-competitive and allosteric Akt inhibitors, along with other inhibitory mechanisms are reviewed in this paper with a focus on issued patents, patent applications and a summary of clinical trial updates since the last review in 2007.

Expert opinion—A vast diversity of inhibitors of Akt, both small molecule and biologic, have been developed in the past 5 years, with over a dozen in various phases of clinical development, and several displaying efficacy in humans. While it is not yet clear which mechanism of Akt inhibition will be optimal in humans, or which Akt isoforms to inhibit, or whether a small molecule or biologic agent will be best, data to all of these points will be available in the near future.

Keywords

Akt; allosteric; apoptosis; ATP-competitive; cancer; chemotherapy; clinical trial; inhibitors; kinase; PH domain; PKB; schizophrenia

1. Introduction

Cancer, a complex disease driven by a combination of external (smoking, diet, etc.) and internal factors (hormones, genetics, etc.), remains the leading cause of death in

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Declaration of interest

Lindsley is a professor of phramacology at Vanderbilt University Medical Center. Mattmann is a postdoctoral fellow in the Lindsley laboratory. Sydney Stoops is a graduate student in the Lindsley laboratory. None of the authors have any financial stake or interest in Akt or any of the corporations discussed in this review.

economically developed countries and has quickly earned the second place in developing countries [1,2]. Based on GLOBOCAN 2008 statistics, there were 12.7 million cancer cases and 7.6 million cancer deaths in 2008, with both figures expected to climb annually due to population aging/growth and adoption of smoking and Western-style diets [1]. By 2030, cancer deaths are expected to exceed 11 million [1,2]. Currently, cancer is viewed as a term to encompass hundreds of distinct disorders, and clinicians treat each case on an individual basis based on the molecular signaling mechanisms driving the tumor versus the classical approach of administering general cytotoxic agents. Thus, drug development is aimed at targeted therapies (specific action at tumor cells vs healthy cells) and biased patient populations (personalized medicine) for clinical trials [1,2]. From the search for new mechanisms for targeted therapies, Akt emerged in the early 2000s as an exciting target for drug development, leading to a concentrated effort to develop inhibitors of Akt [3–12]. Akt has a key role in the regulation of cell survival, proliferation and growth. Activation of the Akt pathway is common in cancer cells and efforts to understand the role of Akt in the transformed phenotype has led to a large body of research that has been the subject of numerous basic science reviews [3–12] and a patent review in 2007 in this journal [13]. This review covers small molecule and biological inhibition of Akt from both a historical perspective as well as current status of the patent landscape and clinical trials.

Akt (protein kinase B or PKB) is a serine/threonine kinase that belongs to the AGC family of kinases and shares high homology with protein kinase A (PKA) and PKC. There are three isozymes of Akt (Akt1, 2 and 3, also referred to in the literature as PKB-α,-β and -γ), and each of the three Akt isozymes has distinct functions and expression profiles [3–15]. Moreover, the three isoforms share similar kinetic mechanisms, but differing kinetic constants, that is, the affinity of the three isoforms for ATP (with Akt 1 and Akt3 both comparable K_m values, but Akt2 ~ 5-fold weaker K_m) and catalytic efficiency [3–15]. Akt1 and 2 are found throughout the body, while Akt3 is predominantly expressed in the brain, kidneys and heart [16]. The functional differences between Akt isozymes have also been studied in knockout mice, where Akt1 was found to be important for overall growth (Akt1 knockout mice are healthy, but have impaired fetal and postnatal growth), Akt2 primarily involved in glucose metabolism (Akt2 knockout mice have normal growth characteristics, but have a mild to severe insulin resistance phenotype) and Akt3 a critical mediator of brain development [17–21]. Recent knock-out data with shRNA in tumor-bearing mice suggest that inhibition of all three Akt isoforms would afford maximum efficacy [22]; however, data with isoform-specific, allosteric small molecule inhibitors in capsase-3 assays suggest that inhibition of Akt1 and Akt2 would be optimal [23,24]. Akt2 is the most commonly observed isozyme overexpressed in tumors while Akt1 and Akt3 overexpression appear to be less common [21]. The identification of isozyme-specific inhibitors provides valuable tools to study Akt activity, although it remains to be determined if isozyme selective inhibitors will have a larger therapeutic window in humans. From a drug design perspective, differences in regional distribution, coupled with differing affinity for ligands at the ATP-binding site, could complicate ATP-competitive Akt inhibitor development.

Structurally, each Akt isozyme contains an *N*-terminal pleckstrin homology (PH) domain, a 39 amino-acid hinge region, a kinase domain, an ~ 260 amino-acid ATP-binding domain

and a 21 amino-acid C-terminal hydrophobic motif [3–15]. The PH domains of the three Akt isoforms contain ~ 110 amino acids and are only about 60% identical. The hinge regions of the three isoforms are only about 25% identical while the kinase domains have 90% homology. As expected, the residues of the ATP-binding site are highly conserved (estimates of 97 – 100% sequence identity) suggesting ATP-competitive ligands will be unable to distinguish the three Akt isozymes [3–16]. Moreover, due to the high homology with PKA, many reported ATP-competitive Akt inhibitors have little selectivity versus PKA (a master regulator of multiple, critical functions in the cell), and engineering selectivity for Akt has represented a required attribute and a formidable challenge [3–16,25].

Akt is a highly flexible protein [26–30]. In the cytoplasm, a dynamic equilibrium of ‘PH-in’ (or closed) and ‘PH-out’ (or open) conformations exist. On growth factor stimulation, PI3K phosphorylates PIP2 to PIP3, which recruits Akt to the plasma membrane. Here, the PH domain of ‘PH-out’ conformation engages PIP3 to enable phosphorylation of T308 (by PDK1) and Ser473 (by mammalian target of rapamycin (mTOR)C2, previously described as a putative PDK2) leading to kinase activation and downstream signaling pathways involved in cell survival, cell proliferation, glucose metabolism, neuroscience and protein synthesis (Figure 1) [3–32]. As such, Akt is a high profile target for both oncology and numerous CNS disorders, with oncology being the most advanced (*vide infra*).

Based on the strong rationale for targeting Akt inhibition, many efforts to identify Akt inhibitors with acceptable pharmaceutical properties have been pursued [3–16]. These attempts can be classified by where the compounds bind to Akt and which specific molecular functions are blocked; importantly, Akt has a number of specific binding pockets which can be targeted with small molecules (Figure 1). Binding in these pockets would be expected to block specific aspects of Akt function and signaling through Akt. The most clearly described pockets are the PI(3,4,5)P_3 binding pocket on the PH domain, the phosphorylated carboxy terminus binding hydrophobic pocket and the ATP/substrate pocket. In addition to these three, there may be small molecule binding sites which are created in the interface of the interacting PH domain/hinge region and the kinase domain or pockets created by conformational changes created by the interacting domains [3–16]. This review covers the primary, patent and clinical literature for PIP analogs, alkyl phosphocholines and sulfonamide PH domain inhibitors (ligands that have been shown to bind to the PH-domain and block PIP3 binding), ATP-competitive kinase inhibitors (ligands that complete for ATP and shown to bind to the activated state, ‘PH-out’ conformation) and allosteric kinase inhibitors (non-ATP competitive ligands that possess binding sites in both the PH and kinase domains, and stabilize the ‘PH-in’ conformation of the inactive enzyme).

2. Akt and cancer

Activation of the PI3K/phosphatase and tensin homolog (PTEN)/Akt pathway is common in cancer cells and efforts to understand the role of Akt in the transformed phenotype have led to a large body of research which has been the subject of numerous reviews [3–15,33–35]. The Akt pathway is often improperly regulated in cancer cells due to PTEN deletions or mutations, growth factor mutations leading to high levels of PI3K recruitment to the plasma membrane, mutationally-activated PI3K, Akt overexpression/gene amplification or

constitutive activity of Akt [33–41]. As would be expected, activation of the pathway upstream of Akt results in elevated levels of Akt phosphorylation, as observed in prostate cancer, breast cancer and colorectal carcinoma. Additional support for the Akt pathway target rationale has come from the analysis of patients treated with tyrosine kinase inhibitors [42–46]. These inhibitors include gleevec (inhibitor of BCR-ABL, PDGFR and c-Kit), iressa (EGFR inhibitor), tarceva (EGFR inhibitor) and herceptin (Her-2/Neu antibody). In general, patients who have the pathway activated are more likely to respond and those patients who respond have reduced phospho-Akt levels. In these studies, it was shown that patients with elevated phospho-Akt levels in tumors had a higher response rate and longer time to progression than patients with low levels of phospho-Akt. In another example, herceptin-resistant tumors were shown to have elevated levels of phospho-Akt and low levels of PTEN. Similar results have been observed with tarceva and gleevec [47,48]. Together, these results suggest that the transformed phenotype in some tumors is driven by activation of the Akt pathway as a result of receptor activation or overexpression. In these cases, inhibition of receptor function may have a therapeutic benefit. Additional evidence for the importance of the Akt pathway in tumorigenesis has been reported in several papers describing the effects of reducing the levels of PDK1 or Akt1 in $PTEN^{+/-}$ mice. Heterozygous $PTEN^{+/-}$ mice have elevated $PI(3,4,5)P_3$ levels, higher phospho-Akt levels and develop several tumor types including those of the prostate, endometrium, thyroid, adrenal medulla and the intestine [49–51]. Mice carrying a hypomorphic mutation in PDK1 express 80 – 90% less PDK1 than normal mice. PDK1 hypomorphic mice were crossed with $PTEN^{+/-}$ mice and the resulting mice had a greatly reduced tumor incidence [52]. In a similar set of experiments, $PTEN^{+/-}$ mice were mated with $Akt1^{-/-}$mice. The resulting progeny had dramatically reduced levels of endometrial and prostate neoplasia and there were reduced levels of adrenal medulla and thyroid tumors and intestinal polyps [53]. This large and growing body of genetic and inhibitor data strongly suggest that activation of the Akt pathway has an important role in generating and maintaining the transformed phenotype. Finally, recent preclinical data in animals [3–16] and, more importantly, clinical trial data [54] in humans with multiple Akt inhibitors possessing diverse mechanisms of kinase inhibition further substantiate Akt as a target for cancer therapy.

3. Akt and CNS disorders

In addition to oncology, Akt is center stage as a preclinical target for CNS drug discovery as research has shown that dopamine regulates Akt and its downstream substrate GSK-3β [55]. In particular, substantial neuropharmacological and genetic risk factor data have identified the dysregualtion of the Akt signaling pathway in both schizophrenia and bipolar disorder [55,56]. Interestingly, it has been shown that multiple anti-psychotic agents, such as clozapine and olanzapine, activate the Akt pathway, presumably through G protein; thus, for schizophrenia, Akt activators would be required [57]. Recently, Aamodt and co-workers showed that antipsychotic drugs activate the *Caenorhabditis elegans* Akt pathway via the DAF-2 insulin/IGF-1 receptor [58]. In addition, protein levels of Akt1 are reduced in post-mortem schizophrenic brains, and an Akt1 haloptype is associated with increased risk for the disease [59]. Disrupted in schizophrenia 1, a susceptibility gene for schizophrenia, is intimately linked to Akt and the pathogenesis of schizophrenia [60,61].

Dopamine hyperfunction has guided drug development for schizophrenia for decades, and both dopaminergic and noradrenergic signaling play critical roles in mood, memory, movement, cognition and reward [62]. Recent studies by Galli and co-workers have shown that Akt signaling is a regulator of norepinephrine transporter (NET) trafficking and norepinephrine homeostasis by controlling NET surface availability [63,64]. The related dopamine transporter is subject to Akt-dependent and isoform-specific (Akt2) regulation of cell surface expression and dopamine homeostasis [65]. Intense efforts are now focused on the pharmacological manipulation of Akt in the CNS, and this story will quickly evolve and therapeutic relevance will be evaluated. At issue is the pro-oncogenic potential of Akt activation by small molecules or biologics.

4. Small molecule Akt inhibitors

4.1 Inhibitors targeting the pleckstrin homology domain of Akt

An alternative approach to classical ATP competitive inhibitors would be to identify compounds that block and/or compete with $PI(3,4,5)P_3$ binding to the PH domain [3–16]. This mode of inhibition would prevent Akt translocation to the plasma membrane by trapping Akt in the cytoplasm and thus preventing activation [66,67]. The feasibility of this approach was suggested by the demonstration that D-3-deoxy-myo-inositols inhibited the growth of transformed cells, and it was subsequently found that the inositol derivative DPI (**1**) had an IC_{50} of 35 μM against HT-29 colon cancer growth [68,69]. As shown in Figure 2, replacement of the two ester functionalities with ether linkages led to improved cell stability and resulted in DPIEL (**2**) which showed improved inhibition (IC_{50} = 2.1 μM) [70].

Recently, a study examined a range of PI analog inhibitors and found that PIA5 (**3**), in addition to other simple analogs of DPIEL, was active at inhibiting Akt in H1603 cells (IC_{50} = 4.13 μM) [71]. PIA5 (**3**) did not affect phosphorylation of PDK-1 but was found to inhibit phosphorylation of proteins downstream of Akt and significantly increase apoptosis in cells with high levels of constitutive Akt activity. It was also shown to inhibit translocation of a fluorescent Akt-PH construct to the cell membrane which is consistent with the expected mechanism of these inhibitors.

A somewhat related class of Akt inhibitors, the alkyl phosphocholines (Figure 2), is the most advanced in the clinic with perifosine (**4**), a phospholipid derivative of alkyl phosphocholine, in which Phase III trials are underway [54,72–76]. As expected with this class, **4** also disrupts both MAPK and JNK pathways in addition to Akt signaling. Perifosine (**4**) is manufactured by AEterna Zentaris, and successfully moved through Phase I into Phase II trials, where **4** showed efficacy in hematological malignancies and solid tumors. Phase III trials are ongoing in multiple myeloma and metastatic colorectal cancer. Erucylphopshocholine (also referred to as eruphosphine or ErPC3) (**5**) is the back-up to **4**, which is in preclinical development. A recent report in 2010 highlighted that ErPC3 induces apoptotic cell death in prostate cancer cells and synergizes with short-term ionizing radiation [77]. The patent cooperation treaty (PCT) covering these compounds was published in 2004, and preferred embodiments are captured in markush **6**.

Researchers at the University of Arizona and M.D. Anderson set out to discover novel compounds that bind to the PH domain of Akt and thereby inhibit Akt activation [78–80]. By application of proprietary software, 22 scaffolds were identified and surface plasmon resonance was then used to measure binding of the compounds to the PH domain of Akt. From this effort, a series of 4-aliphatic-*N*-(1,3,4-thiadiazol-2-yl)benzensulfonamides was discovered, with PHT-427 (**7**) being the optimal compound. PHT-427 inhibited Akt activation and induced apoptosis at low micro-molar concentrations, inhibited Akt and downstream targets in cells and displayed efficacy in pancreatic tumor xenografts. SAR studies have also been reported surveying multiple domains of the PHT-427 core **8** [78–80]. In addition, a patent application appeared in 2009 covering PHT-427 with a very broad genus **9**, and this series also resulted in formation of a small company, PHusis Therapeutics, and a second patent application with a far more narrow genus **10** and describing a topical formulation of PHT-427 (Figure 2) [81,82].

While this approach obviates the selectivity issues associated with the ATP-binding domain homology among kinases, there is the potential to interfere with the function of other PH domain containing proteins [78–80]. Over 500 human proteins possess a 100 – 120 amino-acid PH domain, and the PH domain is the 11th most common domain in the proteome [83,84]. While the amino-acid sequence of PH domains is not universally conserved, the tertiary structure is highly conserved. A subset of ~ 40 PH domains display a remarkable selectivity for binding to phosphorylated PtdIns lipids, which are vital for signal transduction pathways involved in a multitude of critical cellular processes [78–80,83,84]. From all reports to date, little selectivity data are reported versus the ~ 40 PH domains of concern (except for PDK1 and PDPK1), and this leaves a question mark on this approach [78–80].

4.2 ATP-competitive inhibitors

Most kinase inhibitors bind in the enzyme active site and display ATP-competitive behavior [85]. Due to the high degree of homology in the ATP-binding pocket among Akt, PKA and PKC, many typical PKA and PKC inhibitors have been identified as inhibitors of Akt. Indeed, most ATP-competitive programs are driven by achieving selectivity versus PKA as a first tier, as inhibition of PKA, a master regulator of key cell functions with multiple feedback loops, is not acceptable in a development candidate [3–12,25]. As a notable example, the prototypical kinase inhibitor staurosporine has an Akt IC_{50} of 11 nM and bis-indolemaleimide analogs have subsequently been studied as Akt inhibitors [85]. There are numerous patent applications that list inhibitors of either Akt or PKB, which require searching multiple strings in patent databases. In general, these sources lack data on specificity and mechanism of inhibition; therefore, after a review of compounds in the primary literature, the reader will find tables of markush structures from the patent literature.

Based on a crystal structure of an analog (**11**) of balanol, a non-selective kinase inhibitor binding in PKA, isosteric analogs were rationally designed [86]. In particular, the diamide compound **12** has an IC_{50} against Akt of 4 nM and against PKA of 2 nM as well as improved plasma stability relative to **11** (Figure 3). Other ester replacements, including less constrained ether and amine analogs, were significantly less active than **11** and **12**. Another

known PKA inhibitor that has been the subject of optimization for Akt activity is H-89 (**13**). H-89 is a potent inhibitor of PKA (IC_{50} = 0.035 μM) with modest activity at Akt1 (IC_{50} = 2.5 μM) [87]. Libraries around the lipophilic amino-terminus of **13** identified NL-71 – 101 (**14**) with an Akt1 IC_{50} of 3.7 μM and a PKA IC_{50} of 9 μM. Both **13** and **14** induced apoptosis in OVCAR-3 ovarian carcinoma cells at high concentrations (> 25 μM). Structure–activity relationship (SAR) studies of H-89 showed that modification of the isoquinoline function, the sulfonamide moiety or the diamine linker resulted in loss of Akt1 inhibitory activity. A more potent and moderately selective analog of H-89, **15** inhibits Akt with an IC_{50} of 0.17 μM (PKC IC_{50} = 1.4 μM, PDK1 IC_{50} > 30 μM) [88].

High-throughput screening (HTS) of Abbott's sample collection identified *trans*-3,4′-bispyridinylethylene **16** as a weak inhibitor against Akt1 (IC_{50} = 5 μM), and this class of compounds is broadly characterized as ATP-competitive, reversible pan-Akt kinase inhibitors (Figure 4) [89–95]. Potency was improved by appending an aromatic group to the ether-linked side chain. In particular, incorporating an indole (**17**) led to a 350-fold enhancement in Akt1 activity relative to **16**. Cyclizing the olefin onto the central pyridine ring was not well tolerated; however, constraining the conformation of the olefin as a bicyclic isoquinoline significantly improved Akt potency to give compound **18** (IC_{50} = 1.3 nM). Given the high homology of the ATP-binding pocket, compound **18** showed poor selectivity over closely related kinases from the CMGC family and the other members of the AGC family of kinases, and was even more potent for PKA than either of the other two Akt isoforms, Akt2 (IC_{50} = 6.8 nM) and Akt3 (IC_{50} = 35 nM).

Abbott then replaced the isoquinoline in **18** with alternate azaheterocycles, which led to the identification of indazole **19**, the most potent Akt1 inhibitor reported from this class of compounds (IC_{50} = 0.16 nM) with an improved kinase selectivity profile. However, compound **19** displayed a poor PK profile with a short half-life, high clearance and no oral bioavailability. Tumor cells treated with **19** caused a dose-dependent decrease in cell growth and in Akt-mediated phosphorylation of downstream targets (including GSK3, FOXO3, TSC2 and mTOR) at concentrations comparable to staurosporine. Compound **19** showed antiproliferative activity on MiaPaCa-2 human pancreatic cancer cells with an IC_{50} = 100 nM and equal potency against all three isoforms of Akt in cells and also inhibited phosphorylation of GSKα/β and TSC2 with an EC_{50} of 0.3 uM [89–95].

Consistent with the *in vitro* studies, indazole **19** inhibited Akt activity *in vivo* in a dose-dependent manner and significantly slowed tumor growth as monotherapy in two mouse xenograft models, MiaPaCa-2 and 3T3-Akt1. Although Akt activity was not fully inhibited in tumors, concentrations of **19** above the cellular EC_{50} for 12 h were sufficient to effectively induce apoptosis in tumors. In addition to being effective as a monotherapy, **19** sensitized H460 human lung carcinoma cells to apoptosis when combined with several chemotherapeutic agents including doxorubicin, camptothecin and paclitaxel. Dosing of this class of Akt inhibitors was limited in both magnitude and duration by severe toxicities including lethargy and weight loss. The authors reported a narrow therapeutic window for **19** with maximum tolerated doses only twofold lower than the efficacious doses. Other observations from treated animals include an increase in the total amount of phosphorylated Akt and an increase in plasma insulin levels, a general concern with inhibition of Akt2 noted

in Akt2-knockout animals. In addition, all of these analogs suffered from CV toxicity (QT prolongation and hypotension) [89–95]. The addition of a nitrogen atom to the 6-position of the indazole ring provided **20**, a compound of comparable Akt inhibition (Akt1 IC_{50} = 0.6 nM), oral bioavailability (F = 25%) and exposure (AUC = 1.7 μM h); however, no statistically significant hypotension was observed when dosed orally at 150 mg/kg in conscious mice [94].

Chiron recently disclosed another series of ATP-competitive kinase inhibitors that show activity against Akt. Optimization of a weak Akt3 inhibitor (**21**) led to the identification of compound **22**, a potent inhibitor of Akt1 and Akt3 (IC_{50} = 6 and 2.6 nM, respectively) and a moderately potent inhibitor of Akt2 (IC_{50} = 23 nM) [96]. Compound **22** showed a poor selectivity profile with closely related kinases PKCα and Kit, and was 30- to 230-fold more potent on PKA than the three Akt isozymes. X-ray of a complex between **22** and PKA showed similar binding interactions as the Abbott compounds (**16 – 20**) [89–95]. Compound **22** inhibited cell proliferation in DOV13 ovarian carcinoma cells (EC_{50} = 1 uM) as well as phosphorylation of GSK3 (EC_{50} = 0.3 uM); however, the superior PKA activity over Akt was also observed in cells. No *in vivo* activity was reported for this series of kinase inhibitors [96]. GSK reported on a related series derived from HTS hit **23** (Akt1 IC_{50} = 0.6 nM) in 2009 [97]. SAR studies, aided by a co-crystal of **23** in Akt2, led to the identification of a potent Akt inhibitor **24** (Akt1 IC_{50} = 0.03 nM): a structure nearly identical to Chiron's **22**. Moreover, a dose-dependent reduction in pGSK3β levels was observed when **24** was dosed in BT474 tumor bearing xenograft mice [97].

In 2008, GSK described the development of a novel series of Akt inhibitors [98]. Once again, using modeling and co-crystallization approaches, optimized preclinical candidate Akt inhibitor **25** (GSK690693) was developed (Figure 4). **25** was a potent Akt inhibitor (Akt1 IC_{50} = 2 nM, Akt2 IC_{50} = 13 nM, Akt3 IC_{50} = 9 nM) which also inhibited the phosphorylation of the downstream target GSK3β in cells. Intraperitoneal administration of **25** in uncompromised mice resulted in the inhibition of GSK3β and repeated dosing of **25** in BT474 breast carcinoma xenograft mice produced a significant delay in tumor growth [98–100]. GSK690693 (**25**) entered Phase I clinical development as an intravenous formulation, but the trial was halted due to interim results suggesting transient drug-related hyperglycemia [54,98–100]. This, and additional preclinical data, point to GSK690693 causing peripheral insulin resistance. A second Akt inhibitor, GSK2141795, entered Phase I in January 2011 as an oral agent in 70 patients with solid tumors [54,101–103]. Very little is known about GSK2141795 and the structure in unavailable. Thus, it is unclear if GSK2141795 is the same chemical series as **25** or a novel series [54,98–103].

Pfizer disclosed a novel series of Akt inhibitors (Figure 5) based on HTS pyrrolopyrimidine lead **26** (Akt1 IC_{50} = 210 nM) [104]. Utilizing an X-ray co-crystal structure of several inhibitors in this series with Akt1, the team was able to identify key interactions and launch a structure-based design effort which culminated in **27**, with an ~ 100-fold increase in potency (Akt1 IC_{50} = 2.4 nM). In a cell-based Akt1 assay, **27** displayed an IC_{50} of 50 nM; however, ancillary kinase activity for **27** and this series was a key issue. None the less, mice xenografted with a Rat-1a tumor line, activated with human (Myr-Akt1), were dosed at 50 mg/kg orally for 10 days, which resulted in tumor growth inhibition of 68% versus vehicle

control [104]. In late 2010, Pfizer disclosed **28**, a more potent analog with an improved kinase selectivity profile (> 900-fold vs PKA) [105].

In 2006, Ko *et al.* described a unique series of ATP-competitive inhibitors based on a 1*H*-indazole-4,7-dione scaffold **29** [106]. Though weak (Akt1 IC_{50} values in the 4.9 – 20 μM range), prototypical inhibitor **29** (Akt1 IC_{50} = 4.9 μM) afforded a 27% reduction in tumor growth in a PC-3 tumor xenograft model when dosed 5 mg/kg/day for 16 days. Moreover, studies in their lab show that **29** and related congeners have dual inhibitory effects on both activity and phosphorylation of Akt in PC-3 cells (Figure 5) [106].

In 2010, Array Biopharma reported on a pyrrolopyrimidine class of Akt inhibitors represented by **30** (Figure 5) [107]. This series shows potent activity at all three Akt isoforms, modest selectivity versus PKA, robust knockdown of phosphor-PRAS40 in LNCaP cells and U87 tumor xenografts at 20 mg/kg. GSK has also described a third class of tetra-substituted pyridines as ATP-competitive Akt inhibitors exemplified by **31** [108]. Compound **31** displayed dose-dependent inhibition of the phosphorylation of GSK3β and robust efficacy in a BT474 tumor xenograft model in mice. Amgen has described a novel series of thiazole Akt inhibitors, such as **32**, which displayed good pharmacokinetic and efficacy in U87 tumor xenograft models [109,110]. In 2011, Array BioPharma and Genetech reported on a new chemotype of Akt inhibitor based on a spirochromane core [111,112]. A HTS campaign identified **33** with modest selectivity versus PKA and mid-micromolar potency against Akt1. Resolution of the hydroxyl diastereomers (they were unable to resolve the spiro core) indicated that the Akt inhibitory activity resided preferentially in the d1 diastereomer **34** (d1Akt1 IC_{50} = 2.1 μM vs d2Akt1 IC_{50} = 40 μM). Further optimization and replacement of the isoxazole ring with a 2,6-dimethyl phenyl moiety provided **35**, a 260 nM Akt1 inhibitor with > 385-fold selectivity versus PKA. More extensive changes led to the discovery of a 2,7-diazas-piro[4.5]decane core with an amino pyrimidine head group **36** that possessed single digit nanomolar potency (Akt1 IC_{50} = 8 nM) and > 1000-fold selectivity versus PKA (Figure 5) [111,112].

Figures 6 and 7 depict the markush structures **37 – 68** of granted US patents and patent applications claiming Akt (or PKB) inhibitors that are competitive with ATP [113–140]. Here, Eli Lilly, GSK, Pfizer, Abbott, Amgen, Vertex, Astex, Aventis and Exelixis have numerous patent applications and several issued US patents. A diversity of chemotypes are claimed, and the majority of these patents reflect series for which primary publications have described SAR and activity in detail (*vide supra*). Related to **30**, Array and Genentech have multiple patent applications claiming hydroxylated pyr-imidyl cyclopentanes, **54 – 59** [127–133], with an advanced pan-Akt inhibitor, GDC-0068 (structure not disclosed, though probably closely related to **59** based on narrowed patent claims), in Phase I clinical trials [54,133]. Similarly, Exe-lixis advanced XL-418, a dual Akt–p70S6K inhibitor, into Phase I trials in patients with solid tumors [134–136,141]. Due to low exposure, the trial was halted, and the structure of XL-418 has not been disclosed; however, based on the patent literature it is most likely within the **62** genus [136]. As mentioned above, potential issues with insulin regulation, due to Akt2 inhibition, persist for pan-Akt inhibitors, as well as the traditional issues with kinome selectivity, and PKA in particular, as it has been used as a basis for Akt ligand design in numerous instances [3–13,54].

4.3 Allosteric Akt kinase inhibitors

The study of Akt as a potential therapeutic target for cancer therapy has been hampered by a lack of small molecule kinase inhibitors specific for Akt as well as isoform selective inhibitors that would enable one to test the role of the three isozymes in tumor formation and maintenance. The development of Akt specific and isozyme selective inhibitors that bind to the catalytic kinase domain was predicted to be difficult due to high sequence identity [3–13,142–148]. Therefore, efforts to identify allosteric inhibitors, and thus potential isoform selective inhibitors, were undertaken primarily by Merck [3–13,23,24]. A moderate screening effort directed at identifying compounds capable of inhibiting activated full-length Akt1 produced compounds **60** and **61** (Figure 8) that were subsequently shown to exhibit isozyme selectivity [149]. Compound **60** was selective for Akt 1 (Akt1 IC_{50} = 4.6 μM, Akt2 IC_{50} > 250 μM) while compound **61** proved to be a dual Akt1–Akt2 inhibitor (Akt1 IC_{50} = 2.1 μM, Akt2 IC_{50} = 21 μM). A detailed kinetic analysis indicated that these inhibitors were not competitive with ATP or peptide substrate. Another unexpected finding was that neither compound inhibited Akt3, PH domain deleted mutants or any of the closely related AGC kinases (PKA, PKC, SGK) at concentrations up to 250 μM. Similar selectivity profiles were also observed in cell-based assays. The dependence on the PH domain for inhibition coupled with a kinetic analysis indicating that these inhibitors were noncompetitive with respect to ATP and peptide substrate supported an allosteric mode of inhibition for these compounds [149]. Further work demonstrated that these inhibitors can prevent phosphorylation of T308 by PDK1 in cells. It was also reported that antibodies bound to either the PH domain or hinge region did not affect enzymatic activity but did prevent inhibition. Based on these findings, a model has been proposed to describe this novel mode of inhibition wherein inhibitors bind to a site that exists only in the presence of the PH domain. This site could be at the interface of the two domains or could be formed as the result of conformational changes induced by domain interactions. In either case, inhibitor binding is proposed to stabilize Akt in a ‘PH-in’ conformation that is not capable of being activated by PDK1 (Figure 1) [149]. A definitive explanation of the mechanism of inhibition will be detailed later in the text as improved allosteric inhibitors became available.

In 2005, Merck disclosed the synthesis and SAR of allosteric Akt inhibitors (Figure 8) derived from the screening lead **61** [23,24]. A library approach generated the more potent dual Akt1–2 inhibitor **62** that still displayed PH domain-dependent inhibition. While a notable advance, **62** displayed poor solubility. Further rounds of library synthesis delivered **63**, a potent dual Akt1–2 allosteric inhibitor with improved physical properties and a potential proof of concept compound. Concurrently, monocyclic templates were being synthesized, and this effort surprisingly afforded Akt1- and Akt2-selective inhibitors, **64** and **65**, respectively. These isozyme selective tools demonstrated in caspase-3 assays that a maximal apoptotic response required inhibition of both Akt1 and Akt2 in multiple tumor cell lines. Based on these data, compound **63** was examined in a mouse pharmacodynamic assay to determine if it could inhibit Akt and inhibit Akt phosphorylation *in vivo*. By IP Western, both basal-and IGF-stimulated Akt1 and Akt2 were inhibited in mouse lung (plasma concentrations of 1.5 – 2 μM), with no effect on Akt3 phosphorylation. Similar selectivity profiles were also observed in cell-based C33a IPKA assays, and all of these compounds maintained the same allosteric mode of inhibition observed with **61** [23,24].

Recently, Calleja *et al.* reported on a mechanistic study on the mode of Akt inhibition by allosteric Akt1–2 dual inhibitor **63**, where they identified a critical residue in the PH-domain, Trp 80, which was consistent with the proposed model of PH-induced cavity formation formed in the kinase domain of Akt [150]. This cavity was found in Akt1, but not in Akt3, and hence the high selectivity of **63** for Akt1 versus Akt3. Further *in vivo* FRET data showed that the presence of **63** locked Akt into its 'PH-in' conformation, prohibiting the phosphorylation of Thr 308 and Ser 473 [150]. In late 2010, Brandhuber and co-workers [151] reported on the co-crystal structure of Akt1 with **63** bound, confirming the earlier postulates of Barnett *et al.* and Calleja *et al.* [149,150]. Here, they showed a two-site binding mode: the benzimidazolone moiety binds in the PH domain and the imidazoquinoxaline binds in the kinase domain, locking Akt in the 'PH-in' or 'closed' cytoplasmic conformation thus inhibiting the kinase as well as preventing activation (phosphorylation) of the kinase [151].

One further point of differentiation, Okuzumi *et al.* demonstrated that ATP-competitive Akt inhibitors such as **19**, induce hyperphosphorylation on binding at the ATP site of Akt and, therefore, impart regulatory phosphorylation of their target [152,153]. In contrast, allosteric Akt inhibitors, such as **63**, were found to inhibit both the physiological activation of Akt and the drug-induced Akt hyperphosphorylation. These data further highlight the distinct mechanistic differences between ATP-competitive and allosteric Akt inhibitors; however, the ramifications of these differences have yet to be determined in the clinic.

Due to compound liabilities, **63** could not be advanced further, and efforts focused on improving physical properties by the incorporation of a more basic nitrogen into the core ring system, that is, converting the quinoxaline core to a quinoline core (Figure 9) [154]. Both quinoline isomers, **66** and **67**, were prepared wherein either the N4 or N1 nitrogen atoms of the quinoxline core of **62** were excised, respectively. Quinoline **66**, with the N1 nitrogen intact, was equipotent to **62**, whereas quinoline **67** displayed moderate inhibition of Akt1 and almost no inhibition of Akt2. Indeed, the more basic **66** displayed improved physical properties; however, molecular mass was still an issue. To further increase basicity and reduce molecular mass, the quinoline core was truncated to provide a pyridine template. Subsequent rounds of library synthesis afforded pyridine **68**, a potent dual Akt1 – 2 allosteric inhibitor that displayed excellent aqueous solubility at pH 4.5. Of note, inhibitors in the pyridine series uniformly displayed greater inhibition of Akt2 than Akt1 in contrast to the bicyclic templates [154].

Following these initial disclosures, Merck published seven manuscripts during 2008 – 2009 describing additional refinements to their allosteric Akt inhibitors [155–161], culminating in a successful oral Phase I trial with MK-2206 **74**. The first manuscript described **69**, a congener of **62** with improved aqueous solubility and balanced biochemical and cell-based activity [155]. Then, reports of naphthyridine **70** and naphthyridonones **71** appeared in which **71** was efficacious in inhibiting Akt1 and Akt2 in lung tissue (80 and 75%, respectively) and in tumors (95 and 54%, respectively) in an A2780 tumor xenograft model [156–159]. A series of pyridopyrimidines soon followed, such as **72**, and SAR efforts addressed hERG liabilities [160]. The most recent primary literature report describes a series of [1,2,4]triazolo[3,4-f][1,6]naphthyridines, exemplified by **73**, with potent and balanced

Akt inhibition properties and devoid of hERG liabilities [161]. Further optimization and truncation led to MK-2206 **74** (Akt1 IC_{50} = 8 nM, Akt2 IC_{50} = 12 nM, Akt3 IC_{50} = 65 nM). Merck's MK-2206 is non-competitive with ATP and, like its progenitors, induces a conformational change in the Akt protein to the 'PH-in' cytoplasmic conformation which inhibits both the activity and activation of the kinase and blocks signal transduction through the PTEN pathway [12,54]. An oral Phase I study just completed with MK-2206 in patients with solid tumors was found to be generally well tolerated at doses up to 60 mg QOD at concentrations that align with activity in preclinical models [162–164]. Currently, MK-2206 is being studied in combination with various cytotoxic therapies in HER-2 overexpressing breast cancer [54]. In addition, Merck and AstraZeneca recently announced a collaboration around Merck's MK-2206 and AstraZeneca's AZD6244 (a highly selective ATP-uncompetitive MEK 1/2 inhibitor) as a novel combination therapy to treat cancer [165,166]. Recent reports detail how the combination of MK-2006 and AZD6244 is more effective than either drug alone in human NSCLC both *in vitro* and *in vivo*. Moreover, initial clinic reports on the combination, as well as MK-2206 alone, are quite promising.

Figure 10 depicts the markush structures **75 – 89** of granted US patents claiming allosteric Akt (or PKB) inhibitors based on HTS lead **61** [167–181]. A smaller group of US patents, markush structures **90 – 92**, claim analogs based on HTS hit **60** (Figure 11) [182–184]. Virtually all of these patents reflect series for which primary publications have described SAR and activity in detail (*vide supra*), and are exclusively developed by Merck with one definitive exception. A PCT has recently published from Bayer Schering describing an analog **98** [185] closely related to the Merck series **75 – 89** and **93 – 98** [186–190], but with a different core heterocycle (Figure 12). For all of these, there are multiple use patents describing co-treatment with other cytotoxic agents and chemosensitizers. Finally, in 2005, Imclone described a series of 2,3-dithienylquinoxalines **99 – 101** as Akt inhibitors with Akt1 IC_{50} values ranging from 66 μM to 300 nM [191]. Though no mechanism of action studies have ever been reported, based on the structural similarity to the Merck allosteric inhibitors, it is presumed that these are also allosteric; however, this presumption needs to be verified [191].

4.4 'Other' inhibitors of Akt activation

Triciribine (**102**), a DNA synthesis inhibitor that entered and failed clinical trials > 20 years ago as the phosphate salt **103**, has regained new interest after the discovery in an NCI screen that triciribine inhibited Akt activation (Figure 13) [192–199]. Championed, and patented, by Cheng at the University of South Florida [200–202], **102** was shown to block phosphorylation of all three Akt isoforms at both Ser473 and Thr308; interestingly, **102** did not directly inhibit Akt and was highly selective versus the AGC family of kinases as well as other kinases [199]. **102** also inhibited cell growth, induced apoptosis, decreased phosphorylation levels of downstream substrates and was efficacious in multiple tumor xenograft models. Since 2006, triciribine (**102**/**103**, VQD-002 or TCN-PM) has been in two clinical trials (one in adult subjects with metastatic cancer and one in adult patients with advanced hematologic malignancies) conducted by the H. Lee Moffitt Cancer Center in collaboration with VioQuest Pharmaceuticals [202,203]. No SAR or mechanism of action studies have been published. However, a recent study found that in prostate cancer cell lines

with constitutively active Akt, such as PC-3 and LNCaP, **102** was sensitized to death receptor-induced apoptosis with TRAIL [199]. Thus, trial design is focusing on patients with elevated Akt to avoid the toxicity seen with **102**/**103** in early high dose clinical trials as a DNA synthesis inhibitor [203,204].

5. Biologics

There have been a number of patents filed describing antibodies specific for individual Akt ioszymes as well as general Akt antibodies. Nuclea Biotechnologies has patented antibodies that immunospecifically bind to phospho-Akt and certain p-Akt substrates [205]. Nastech has disclosures on meroduplex ribonucleic acid molecules (mdRNA) capable of decreasing or silencing AKT gene expression [206], and Isis has been granted US patents for antisense modulation, via antisense oligonucleotides, of the expression of all three Akt isoforms [207–209]. Of the biologic approaches, antisense oligonucleotides are the most advanced, with clinical trials underway. Rexhan is advancing RX-0201, a 20-mer antisense oligonucleotide to the RNA encoding Akt1 through the clinic. RX-0201 has completed Phase I trials and is now in Phase II trials for pancreatic cancer in combination with gemcitabine [210–213].

6. Expert opinion

Recent progress in the field of Akt kinase inhibitors and the patent landscape has been reviewed. Since the first disclosures in 2003, our understanding of Akt (PKB) biology, mechanisms of Akt inhibition and the design of small molecule Akt inhibitors has grown tremendously. While the reports of Akt inhibition by ATP-competitive inhibitors and PH domain inhibitors have increased, key issues regarding Akt specificity (vs kinome, and PKA especially, as well as PH domain-containing proteins) and Akt isozyme selectivity remain. With the recent discovery of allosteric Akt kinase inhibitors, these issues have begun to be addressed. Several series of allosteric and PH domain-dependent inhibitors have demonstrated specificity for Akt versus other kinases. Data obtained with these inhibitors indicate that a development candidate should inhibit both Akt1 and Akt2, while shRNA suggests all three. It remains to be determined if a compound that only inhibits Akt1 or Akt2 will have an improved therapeutic window when compared to an inhibitor of all three isozymes, and there may be tumor types in which Akt3 activation is important. The distinct mechanistic underpinnings of the ATP-competitive versus allosteric approaches will probably not be definitively addressed until molecules have been studied in patients beyond the acute setting. Despite the issues and divergent approaches, there is tremendous interest in the target with Amgen, Abbott, Pfizer, GSK, Chiron, Bayer Schering, Eli Lilly, Vertex, Array, Genetech and Merck all publishing and possessing diverse patent portfolios of small molecule Akt inhibitors. Based on the extensive number of granted US patents, Merck is firmly in the lead advancing allosteric inhibitors. All of the other major efforts are focused on ATP-competitive inhibitors, and each with formidable, yet less extensive, patent suite. However, despite a large number of published PCT applications in the time period of 2005 – 2008, few have converted to US patents, suggesting the organizations halted development. Triciribine, a molecule that inhibits Akt activation by an as yet unknown mechanism, is progressing through clinical trials as well. Multiple biologic approaches (antibodies, mdRNA, antisense oligonucleotides) are also underway, with significant IP and clinical

success. At the time of this writing, there are > 10 ongoing clinical trials testing the hypothesis that inhibition of Akt will have therapeutic benefit in cancer patients, representing numerous mechanisms (small molecule and biologic) for Akt inhibition and drugs (perifosine, MK-2206, RX-0201, PBI-05204, GSK2141795, GDC-0068, triciribine and ErPC). In addition, many more are staging Phase I trials. Efficacy data from late stage Phase III clinical trials, and a new therapeutic agent for the treatment of cancer, are eagerly awaited. Within the next 3 years, many of the basic science questions will be answered from the human studies: this is a very exciting time for Akt.

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Article highlights

- Significant advancements have been made in the design and synthesis of both small molecule and biological inhibitors of Akt activity through multiple, distinct mechanisms. The benefits and concerns of each approach are discussed in detail.
- A review of the primary literature on the chemistry, structure–activity relationship and pharmacology of pleckstrin homology domain inhibitors of Akt, ATP-competitive inhibitors of Akt and allosteric inhibitors of Akt is described.
- Proof of concept has been achieved for Akt inhibition in multiple preclinical models as well as in humans in early Phase I and Phase II trials, both alone and in combination with other chemotherapeutics.
- The current status of preclinical and clinical biologic approaches (antibodies, mdRNA, antisense oligonucleotides) is discussed.
- Both a historical and an up-to-date review of the patent literature for inhibitors of Akt is presented for both small molecules and biologics. Companies have aligned themselves as either pursuing ATP-competitive or allosteric ligand design efforts.
- The present status of Akt inhibitors in various stages of clinical development is discussed.

This box summarizes key points contained in the article.

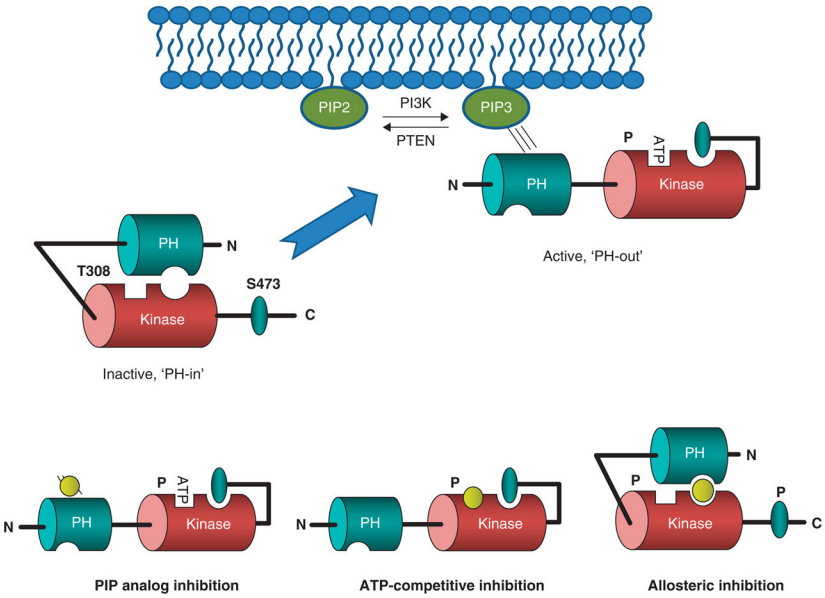


Figure 1. Akt activation

Extracellular signaling events such as growth factor-induced receptor dimerization result in receptor autophosphorylation and recruitment of PI3K. Once PI3K is at the membrane, it phosphorylates the 3′-OH of $PI(4,5)P_2$ and converts it to $PI(3,4,5)P_3$. The interfacial concentration of $PI(3,4,5)P_3$ is regulated by specific phosphatases. PTEN is the phosphatase which removes the phosphate from the 3′-postion and if PTEN function is abrogated through mutation, the PI $(3,4,5)P_3$ levels are elevated. Akt and PDK1 bind to $PI(3,4,5)P_3$ and this results in their colocalization and subsequent phosphorylation of a T308 in the activation loop of Akt by PDK1 and Sr473 by mTORC2. Akt exists in an inactive 'PH-in' conformation in the cytoplasm. On recruitment of the plasma membrane, Akt opens to an active, 'PH-out' conformation where PIP3 binds to the Akt PH domain allowing phosphorylation to occur. Inset. **A**) model of PIP analog inhibition, where the PIP analog binds to the PH domain; **B**) model of ATP competitive inhibition, where inhibitor competes for ATP-binding pocket and leaves kinase in 'PH-out' conformation; **C**) model for allosteric inhibition wherein inhibitor binds within both other PH and kinase domain, forcing the kinase to assume the 'PH-in' conformation and thereby inhibiting both the activity of Akt as well as the activation/phosphorylation.

mTOR: Mammalian target of rapamycin; PH: Pleckstrin homology; PTEN: Phosphatase and tensin homolog.

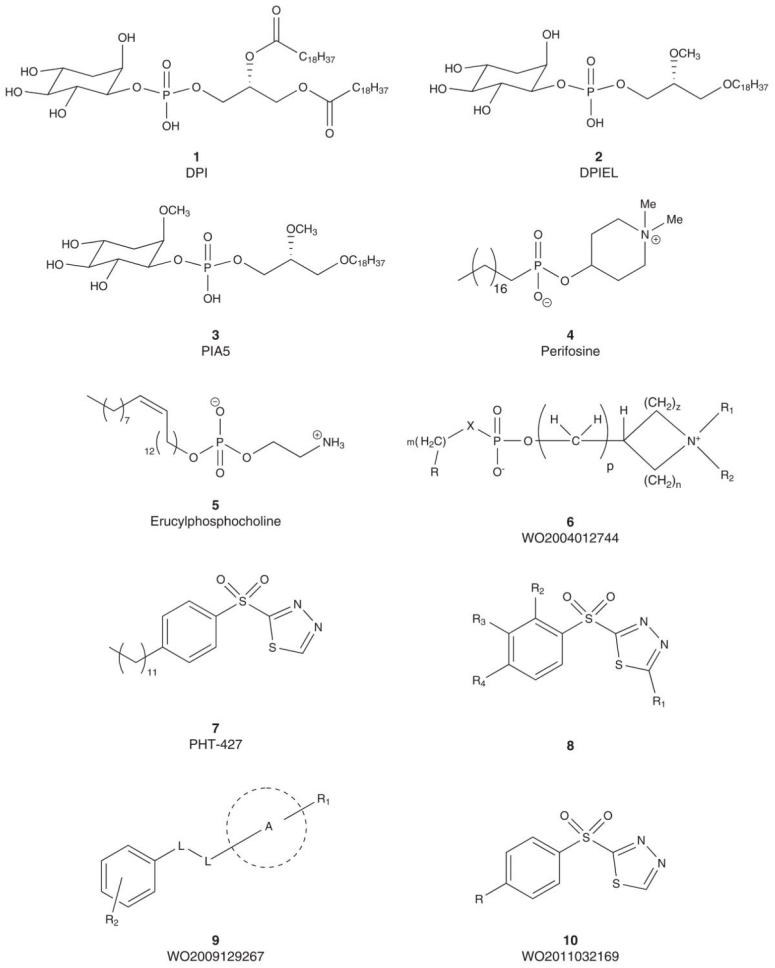


Figure 2.
Pleckstrin homology domain inhibitors 1 – 10 that inhibit Akt activation.

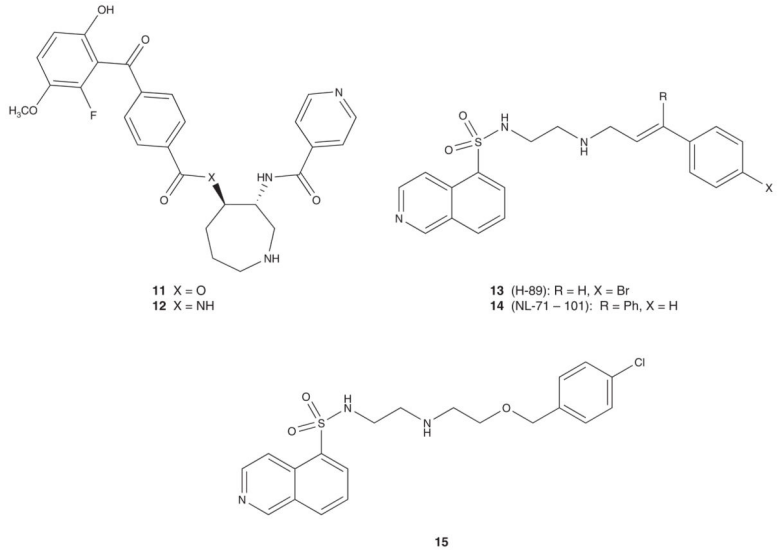


Figure 3.
Early ATP-competitive Akt kinase inhibitors.

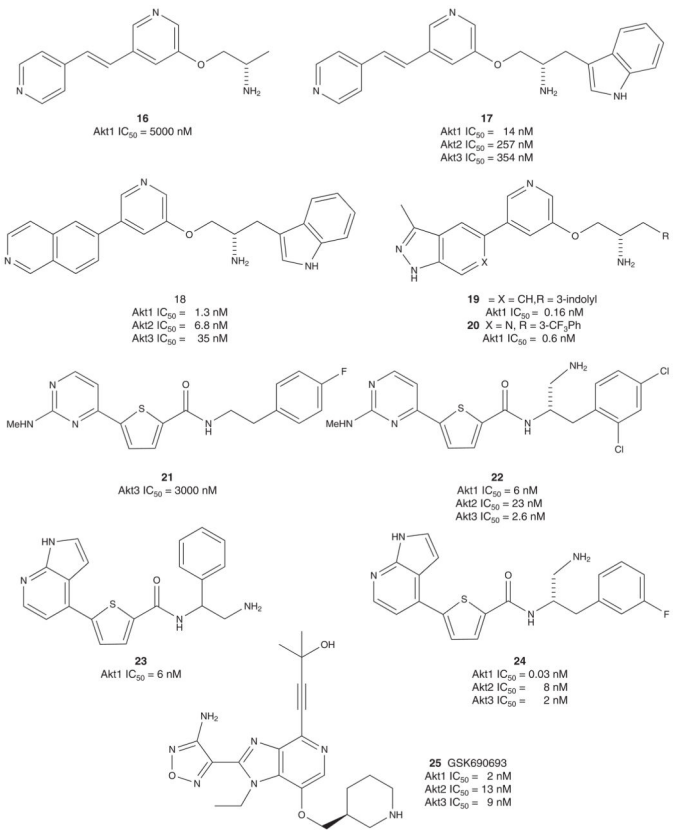
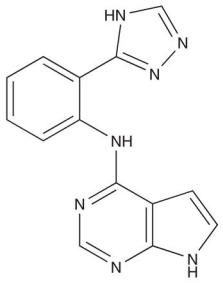
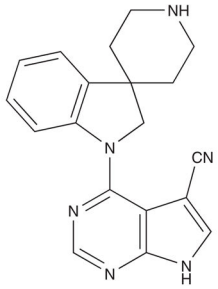


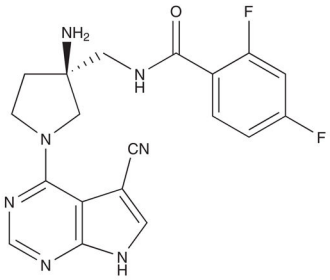
Figure 4.
Published ATP-competitive Akt inhibitors, part I.



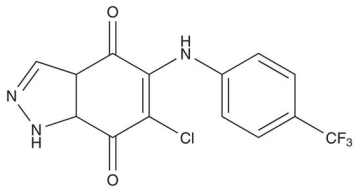
26
Akt1 IC_{50} = 210 nM



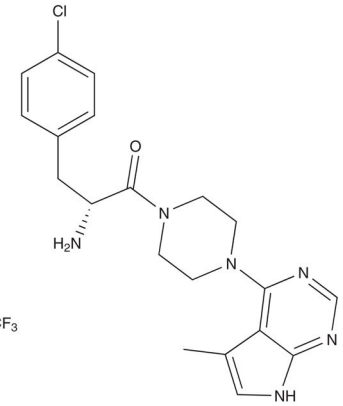
27
Akt1 IC_{50} = 2.4 nM



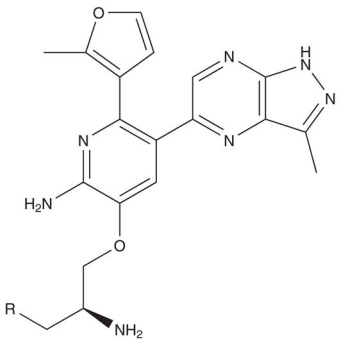
28
Akt1 IC_{50} = 0.5 nM



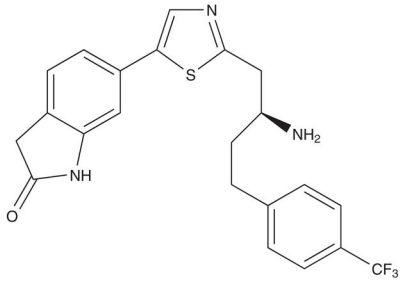
29
Akt1 IC_{50} = 4.9 μM



30
Akt1 IC_{50} = 1 nM



31 R = 3-indolyl
Akt1 IC_{50} = 0.001 nM



32
Akt1 IC_{50} = 18 nM

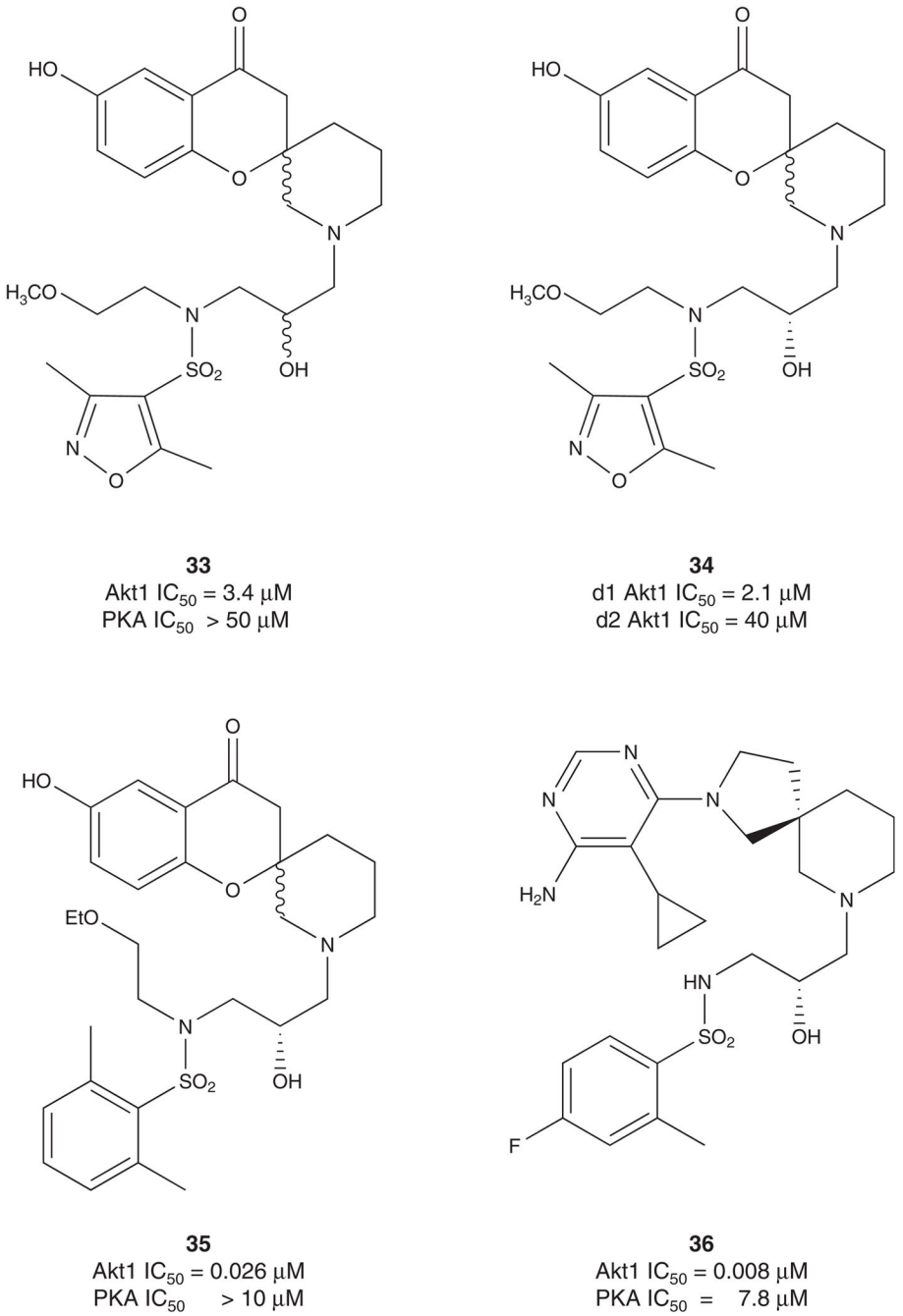
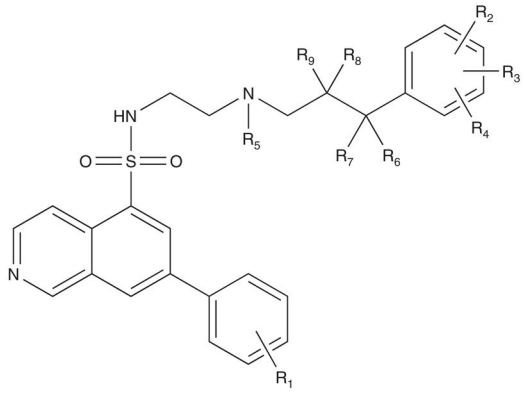
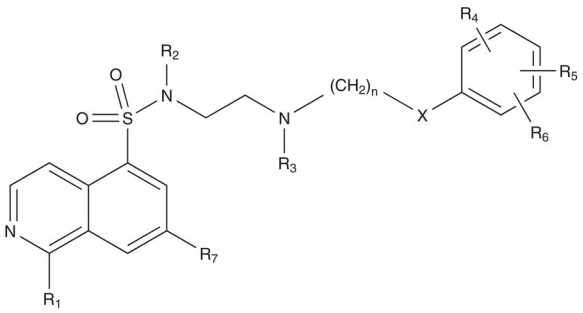


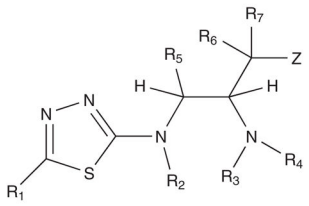
Figure 5.
Published ATP-competitive Akt inhibitors, part II.



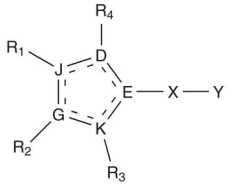
37 US7449477



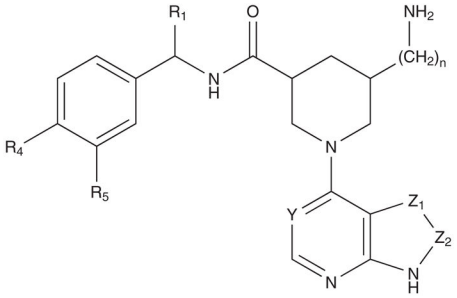
38 US7414063



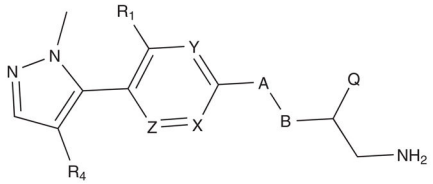
39 WO20090298836



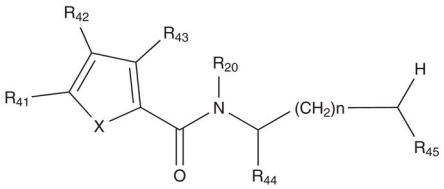
40 WO20090275592



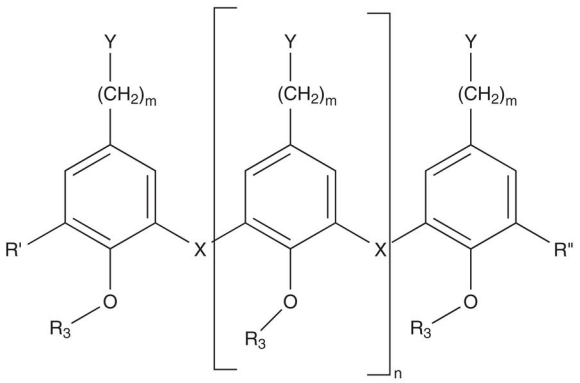
41 WO20090163524



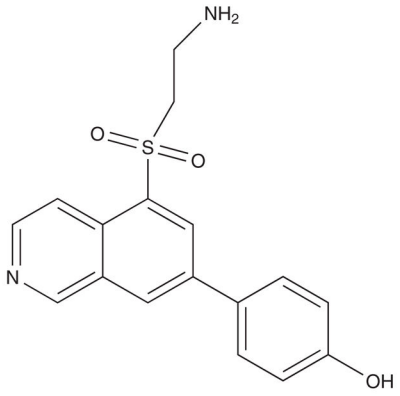
42 WO20100137338



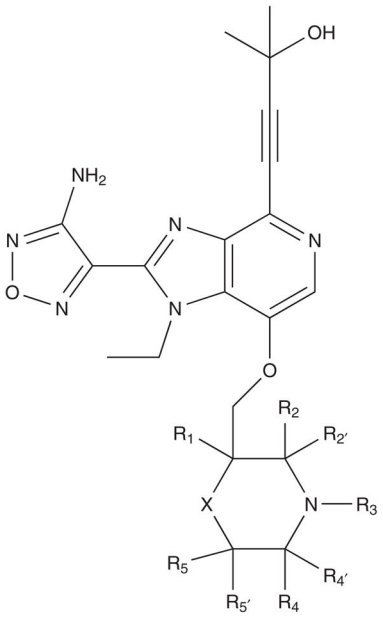
43 WO20100041726
*Same structure as WO20100267759



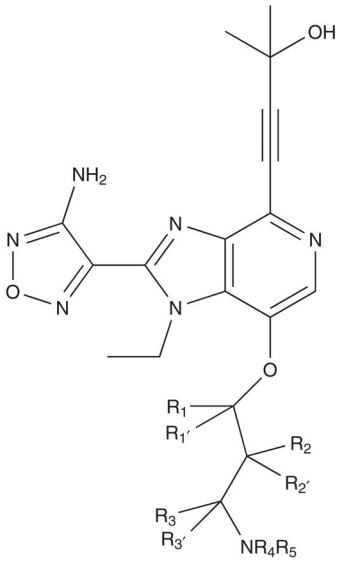
44 WO20080039527



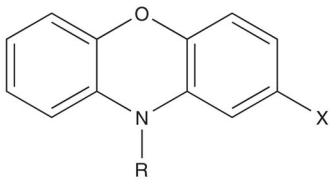
45 WO20090221633



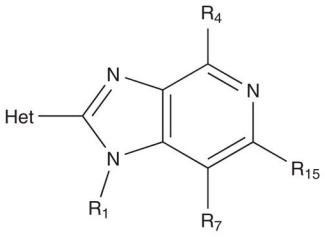
50 WO20100056523



51 WO20090209607
*Same structure as WO20100267759, and WO20100041726



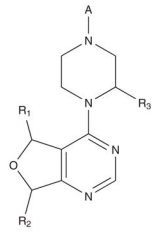
52 WO2006094207



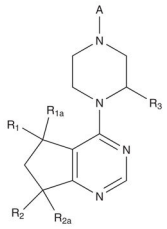
53 WO20080318947

Figure 6. Markush structures of issued patents and patent applications for ATP-competitive Akt (PKB) inhibitors, part I

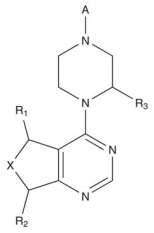
PKB: Protein kinase B.



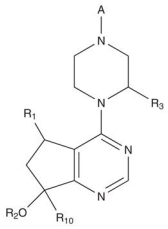
54 WO2008006025



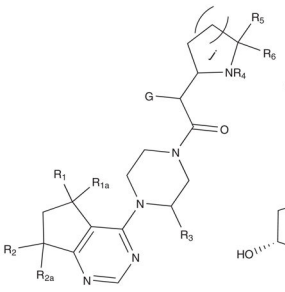
55 WO2008006032
WO2009006569



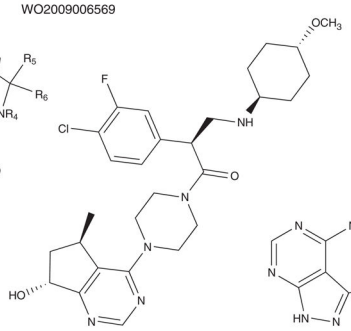
56 WO2008006039



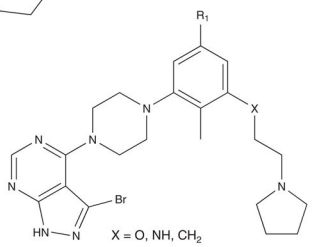
57 WO2008006040



58 WO2009006567

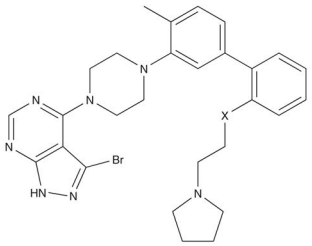


59 WO2009089453

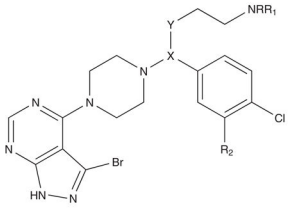


X = O, NH, CH_2

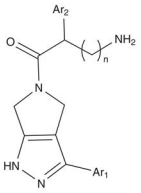
60 WO05117909



X = O, NH, CH_2
61 WO05117909



X = CH, CHO, NH
When X = N, Y = CO, SO_2
62 WO06071819



63 WO05030776

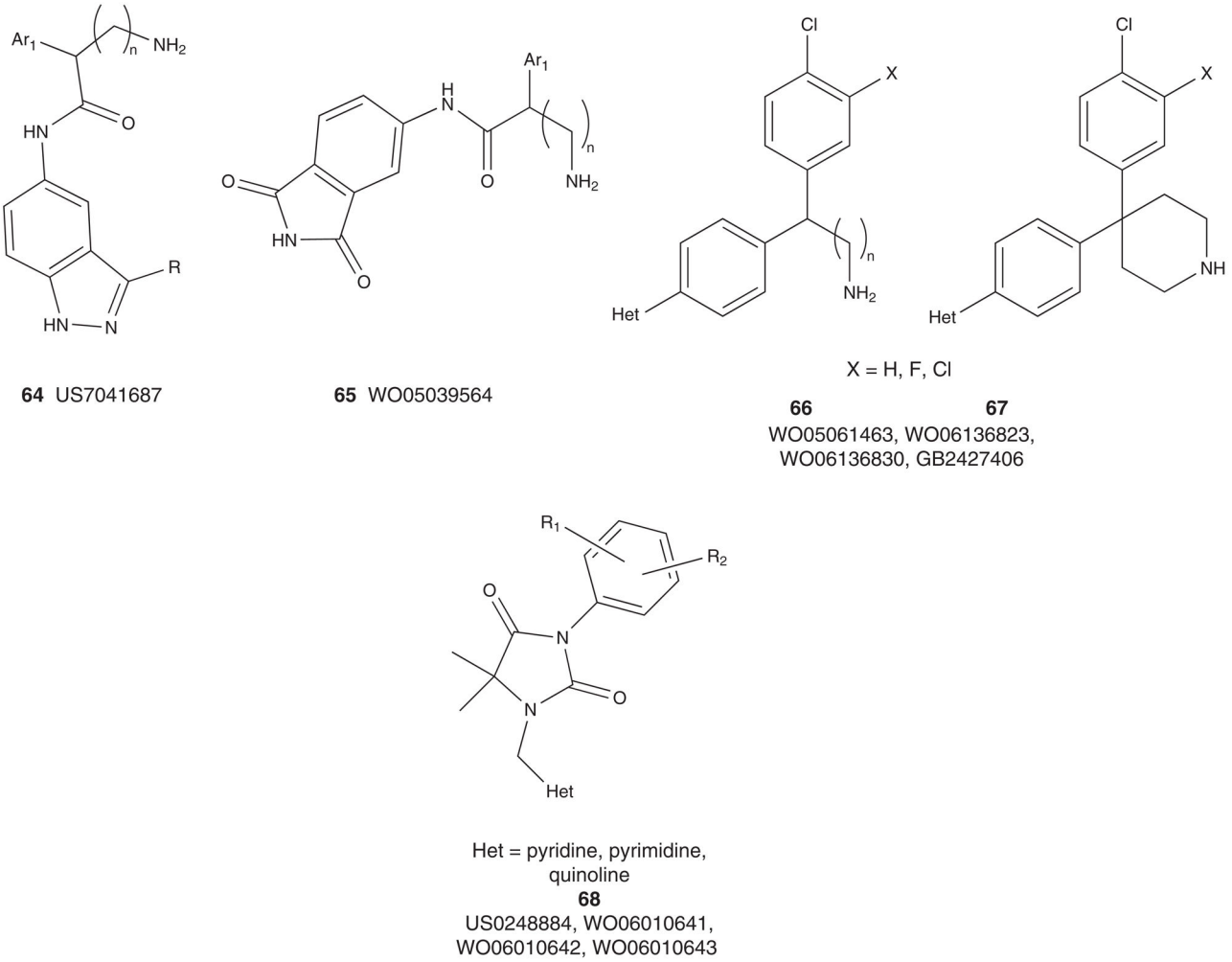
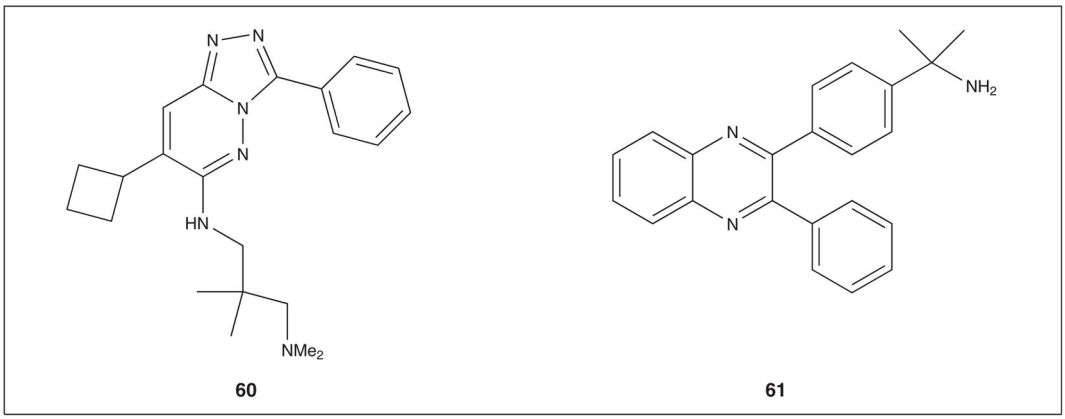
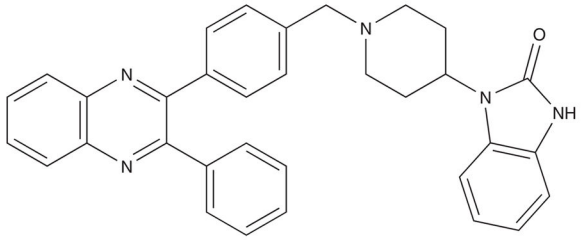


Figure 7. Markush structures of issued patents and patent applications for ATP-competitive Akt (PKB) inhibitors, part II

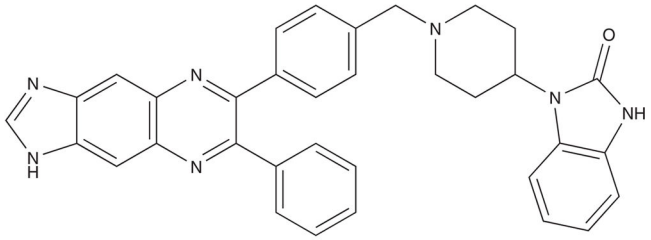
PKB: Protein kinase B.



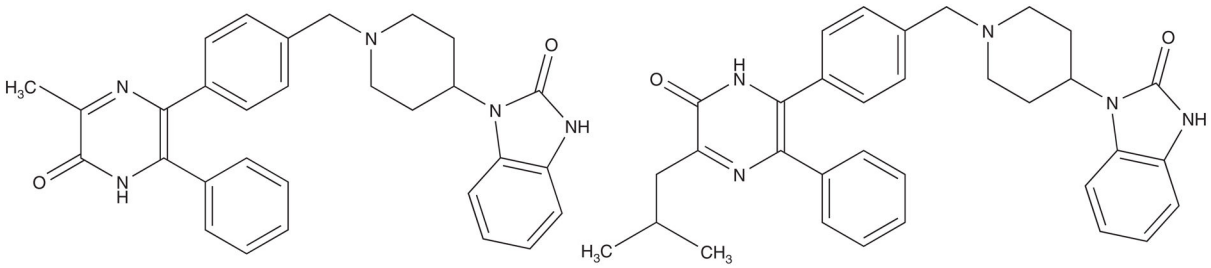
60 **61**



Dual Akt1/Akt2 selective
62
Akt 1 IC_{50} = 295 nM
Akt 2 IC_{50} = 2,057 nM
Akt 3 IC_{50} >50,000 nM



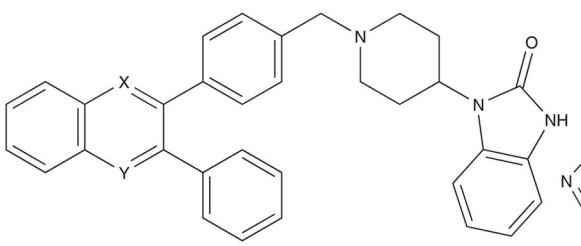
Dual Akt1/Akt2 selective
63
Akt 1 IC_{50} = 58 nM
Akt 2 IC_{50} = 210 nM
Akt 3 IC_{50} = 2,200 nM



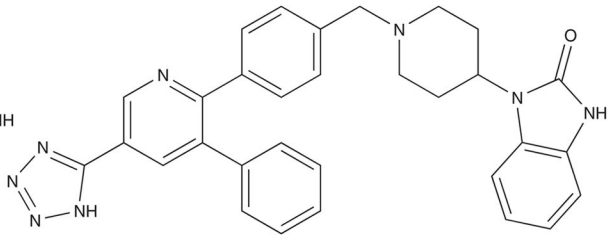
Akt1 selective
64
Akt1 IC_{50} = 760 nM
Akt2 IC_{50} > 20,000 nM
Akt3 IC_{50} > 20,000 nM

Akt2 selective
65
Akt1 IC_{50} > 20,000 nM
Akt2 IC_{50} = 325 nM
Akt3 IC_{50} > 20,000 nM

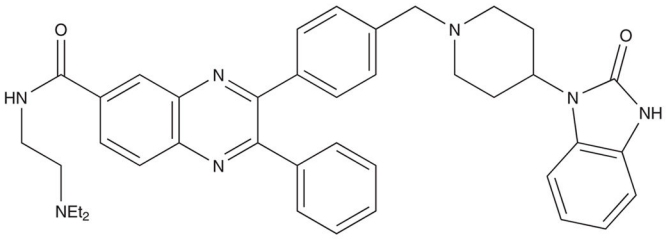
Figure 8.
Early allosteric, isoform selective Akt inhibitors from Merck that established roles for the individual isoforms and provided mechanistic understanding for inhibition.



66 X = N, Y = CH
Akt1 IC_{50} = 365 nM
67 X = CH, Y = N
Akt1 IC_{50} = 3136 nM

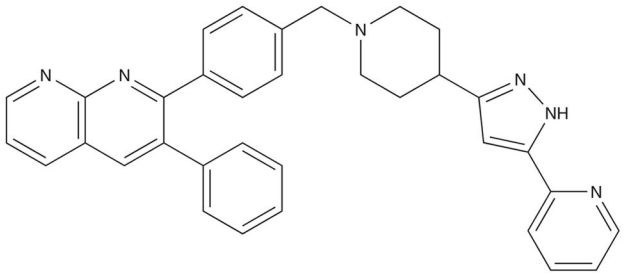


68
Akt1 IC_{50} = 273 nM
Akt2 IC_{50} = 157 nM
Akt3 IC_{50} > 50,000 nM



69

Biochemical	Cell
Akt1 IC_{50} = 138 nM	Akt1 IC_{50} = 253 nM
Akt2 IC_{50} = 212 nM	Akt2 IC_{50} = 276 nM
Akt3 IC_{50} = 7,200 nM	Akt3 IC_{50} >10,000 nM



70

Biochemical	Cell
Akt1 IC_{50} = 120 nM	Akt1 IC_{50} = 369 nM
Akt2 IC_{50} = 260 nM	Akt2 IC_{50} = 1,454 nM

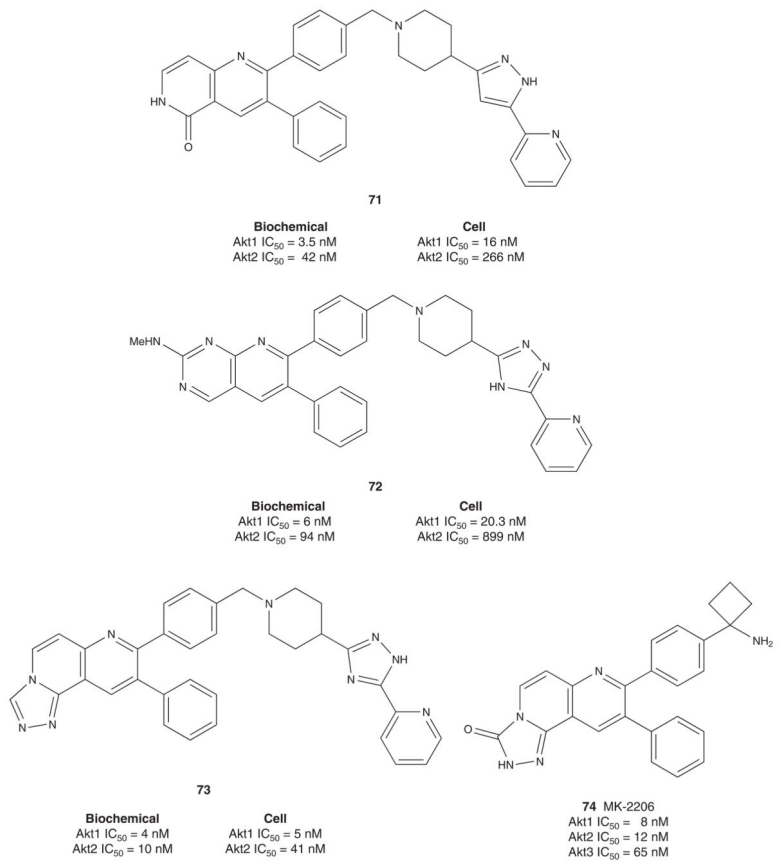
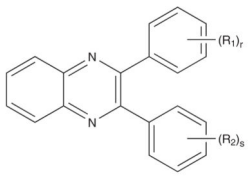
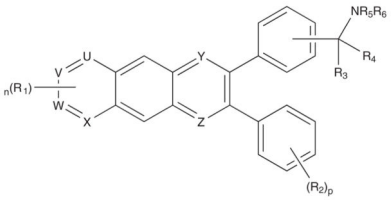


Figure 9.

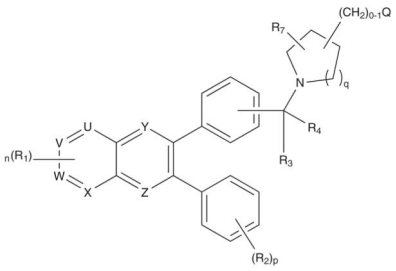
Advance iterations of allosteric, isoform selective Akt inhibitors from Merck culminating in MK-2206 in Phase I.



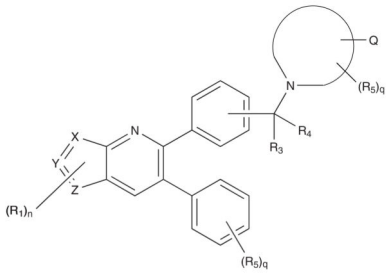
75 US7034026



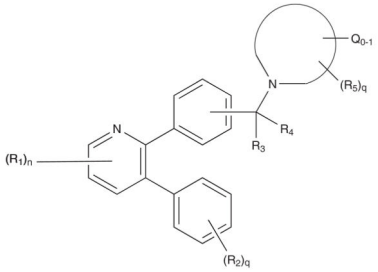
76 US7273869



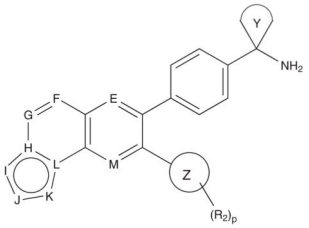
77 US7223738



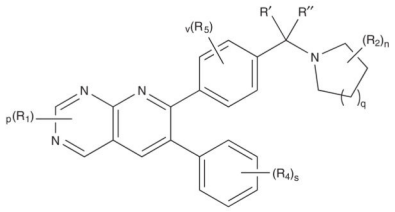
78 US7638530



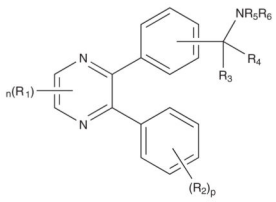
79 US7579355



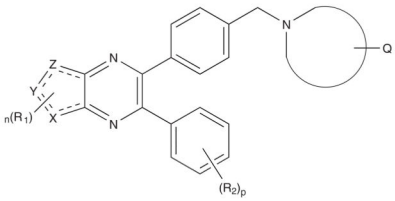
80 US7576209



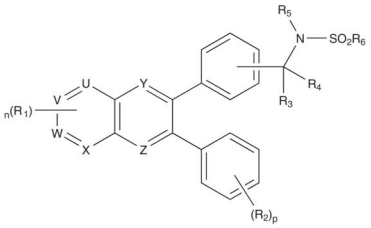
81 US7544677



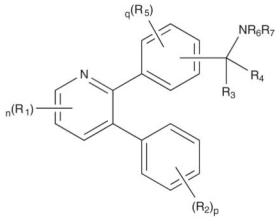
82 US7524850



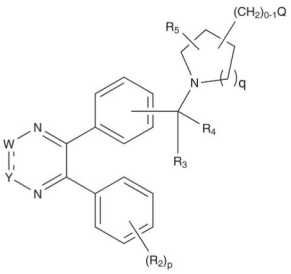
83 US7399764



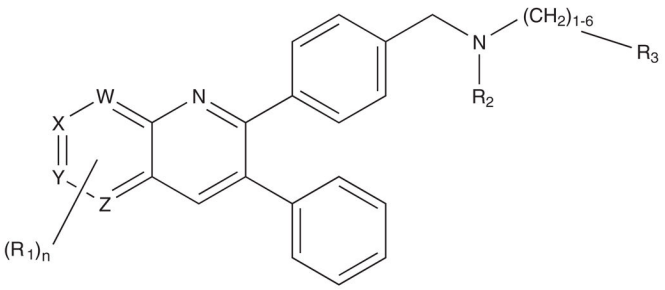
84 US7496832



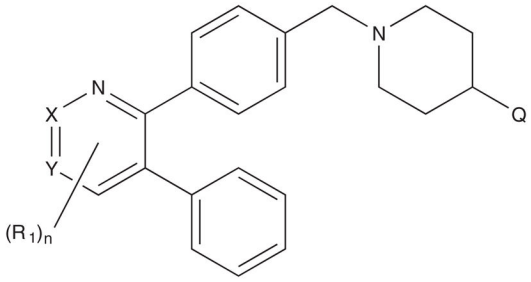
85 US7414055



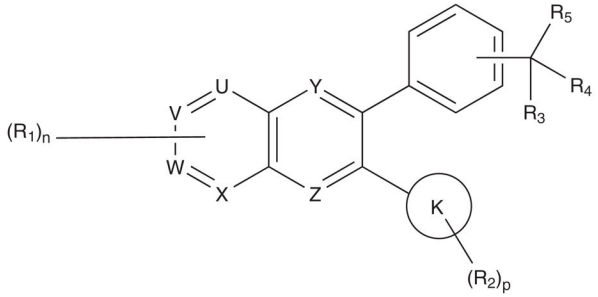
86 US7304063



87 US7750151

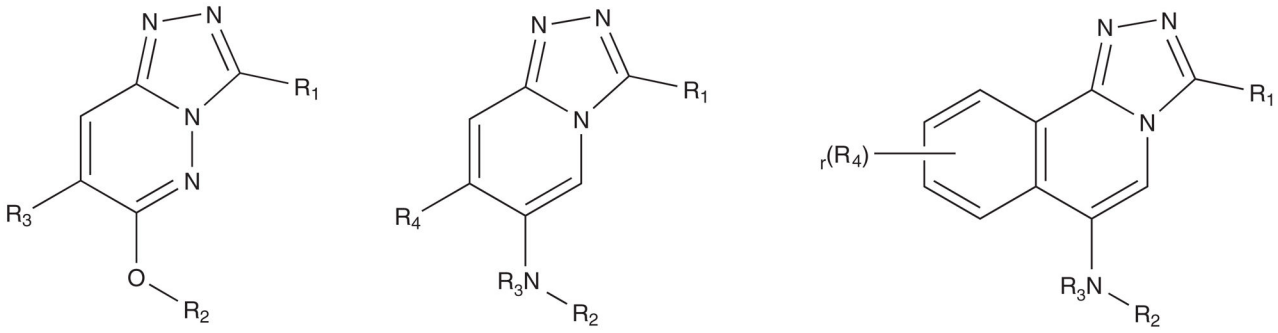


88 US7655649



89 US7589068

Figure 10. Markush structures of issued US patents for allosteric Akt (PKB) inhibitors
PKB: Protein kinase B.



90 US6960584 **91** US7098208 **92** US6958334

Figure 11. Markush structures of issued US patents for allosteric Akt (PKB) inhibitors
PKB: Protein kinase B.

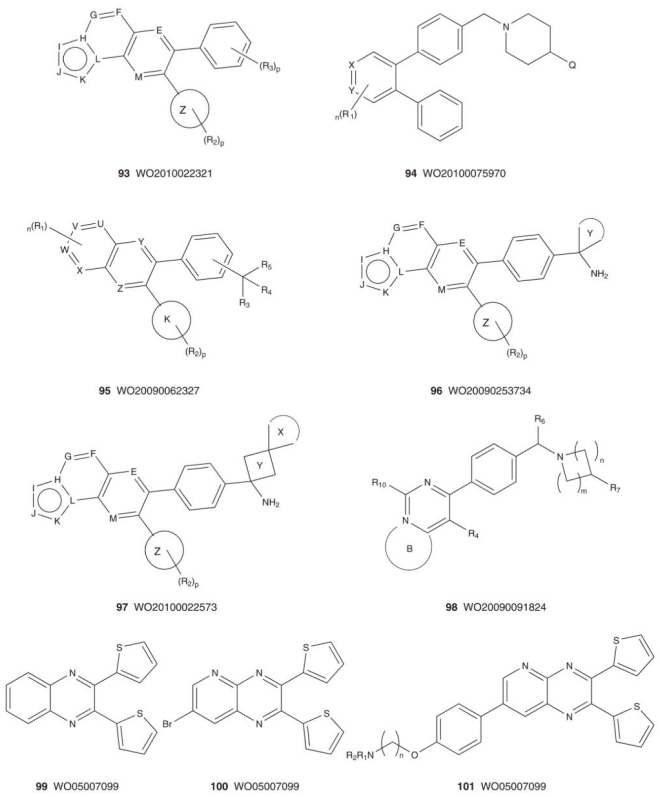
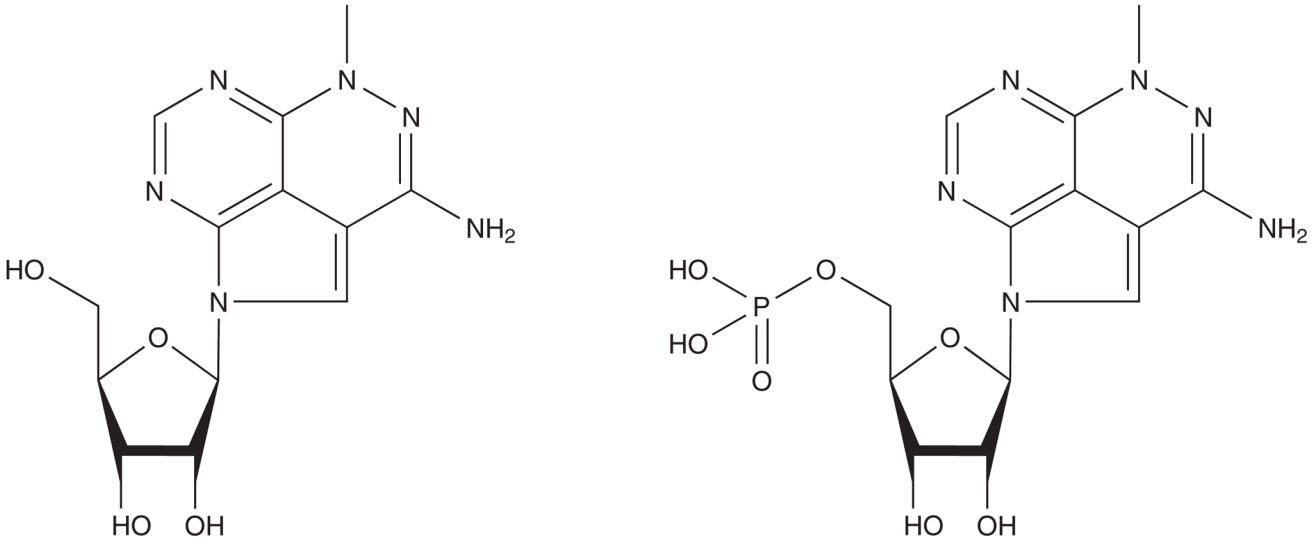


Figure 12. Markush structures of published patent applications for confirmed and presumed allosteric Akt (PKB) inhibitors

PKB: Protein kinase B.



102 Triciribine **103** Triciribinephosphate

Figure 13. Triciribine and the analogous phosphate congener

DNA synthesis inhibitors that have been shown to inhibit Akt activation through a yet undefined mechanism and currently in clinical trials.