Developments in Selective Small Molecule ATP-Competitive Inhibitors Targeting the Serine/Threonine Kinase Akt/PKB

P. Wang[1], L. Zhang[#,2], Q. Hao[1] and G. Zhao*[,1]

[1]*School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, PR China*

[2]*Qilu Hospital of Shandong University, Jinan, Shandong 250012, PR China*

Abstract: The serine/threonine kinase Akt, also known as protein kinase B (PKB), plays a key role in cell survival and proliferation through a number of downstream effectors. Recent studies indicate that unregulated activation of the Phosphatidylinositol 3-kinase (PI3K)/Akt pathway is a prominent feature of many human cancers and Akt is over-expressed or activated in all major cancers. For these reasons, Akt is considered as an attractive target for cancer therapy. In the past few years, several series of compounds with diverse structural features have been reported as Akt inhibitors, such as, ATP-competitive inhibitors, Phosphatidylinositol (PI) analogs, and allosteric inhibitors. Although most of the inhibitors exhibited potent inhibitory activities at nanomolar concentrations against Akt, some of them have shown unfavorable selectivity against other protein kinases especially PKA and PKC. This review will focus on the recent advances in the development and biological evaluation of selective ATP-competitive inhibitors for Akt. We will summarize the novel approaches toward the developments of selective ATP-competitive inhibitors, expecting to give information to design new ATP-competitive inhibitors with high selectivity, bioavailability, and potency.

Keywords: Akt, ATP-competitive inhibitors, PI3K/Akt signaling pathway, selective.

INTRODUCTION

The serine/threonine kinase Akt, also known as protein kinase B (PKB), has a key role in the regulation of cell survival, proliferation and growth [1]. Akt belongs to Phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and recent studies indicate that unregulated activation of the PI3K/Akt pathway is a crucial feature in many human cancers [2]. Akt is over-expressed or activated in all major cancers, such as ovarian, breast and pancreatic cancers [3-7]. The activated Akt phosphorylates a series of substrates, which could block apoptosis pathway, promote cell proliferation, and maintain the survival of tumor cells [5, 8]. Inhibition of Akt proves to be an effective way to cancer intervention. Several Akt inhibitors are now or once in clinical trials [9]. Perifosine, a phospholipid derivative of alkylphosphocholine targeting the PH-domain, is currently in Phase II study [10]. MK-2206, an allosteric inhibitor of Akt, is testing in Phase I [11]. RX-0201, an antisense oligonucleotide to mRNA encoding Akt1, is in Phase I [12]. GSK690693 is an ATP-competitive inhibitor which was once into Phase I study [13], it was terminated probably for the reason of causing hyperglycemia [14]. GSK2141795, an oral Akt inhibitor with undisclosed structure information currently under development by GlaxoSmithKline, is being assessed in a Phase I trial [15]. XL-418 is dual inhibitor of Akt and p70S6K, but it was suspended due to low drug exposure in Phase I trial [16]. The selectivity is the most important principle in designing Akt inhibitors. Otherwise unselective inhibitors will lead to side effects unexpected by blocking a wide range of kinases, such as protein kinase A (PKA) and protein kinase C (PKC) [17].

Akt belongs to AGC kinase super family (the term AGC kinase was coined by Steven Hanks and Tony Hunter1 in 1995 to define the subgroup of Ser/Thr protein kinases based on sequence alignments of their catalytic kinase domain), and has a high degree of homology with PKA and PKC which are also AGC kinases [18]. Many typical PKA and PKC inhibitors have been identified as inhibitors of Akt because of the high degree of homology in the ATP binding pocket [19, 20]. However, this is a double-edged sword, because ATP-competitive inhibitors face a challenge of selectivity over other kinases specially AGC family kinases for the reason of high degree of homology [21]. Numerous reviews [22-35] related to Akt physiological action and Akt inhibitors, but few focus on the selectivity. Since 2005, lots of drug design strategies were used leading to series of ATP-competitive inhibitors with potent selectivity. Some of ATP-competitive inhibitors were once into the clinical trials or had excellent pre-clinical properties. This review will focus primarily on the selective small molecule ATP-competitive inhibitors. We will provide research-related reference materials for the design of new anti-cancer drugs.

Structure of Akt

Akt is divided into three isoforms, Akt1/PKBα, Akt2/PKBβ, and Akt3/PKBγ, which share a high degree of structural similarity and approximately 85 % sequence homology. Especially in the ATP-binding site it is 100 % except for one non-crucial amino acid in Akt3. Because of these, the inhibitors targeting the ATP-binding site have potent activity to inhibit all of three isoforms. Akt contains

*Address correspondence to this author at the School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong, 250012, P.R. China;
Fax: (+86) 531 88382009; E-mail: guisenzhao@sdu.edu.cn
[#]Co-first author.

1389-5575/11 $58.00+.00

an amino terminal pleckstrin homology (PH) domain, a central kinase domain (catalytic domain) and a carboxyl-terminal regulatory domain [25]. The PH domain of Akt plays a significant role in recognition by upstream kinases and membrane translocation [36]. It interacts with membrane lipid product, phosphatidylinositol (3,4,5)trisphosphate (PIP3) produced by PI3K, to translate Akt from cytoplasm to the membrane where Akt is actived by the phosphoinositide-dependent kinase 1 (PDK1) and the rictor-mammalian TOR (mTOR) complex [37-39]. The catalytic domain locates in the central region of the molecule, including the ATP-binding site and the substrate binding site. A conserved threonine residue, whose phosphorylation is required for enzymatic activation, lies in the ATP-binding site region.

Activation and Function of Akt

The PI3K/Akt signaling pathway starts from receptor tyrosine kinases (RTKs). RTKs are activated by growth factors and directly activate PI3K on the membrane. RTKs also activate PI3K indirectly through the Ras signaling pathway. PI3K phosphorylates the lipid second messenger phosphatidylinositol (4, 5) isphosphate (PIP2) into phosphatidylinositol (3, 4, 5) triphosphate (PIP3) which departs from the membrane to cytoplasm and recruits and activates PDK1. Meanwhile, PIP3 binds directly to the PH domain of Akt, recruits Akt from cytoplasm to the membrane and induces a conformational change of Akt to expose its two phosphorylation sites, Thr308 and Ser473 (for Akt2). PDK1 partly activates Akt by phosphorylating Thr308. Full activation of Akt is associated with phosphorylation by rictor-mTOR complex (mTORC2) on the residue Ser473 within a C-terminal hydrophobic motif which is characteristic of the AGC kinase family [38]. Then, Akt is fully activated and dissociates from the membrane to the cytoplasm and the nucleus, where it phosphorylates numerous substrates [40].

In the nucleus, Akt controls gene transcription by inhibiting the activities of the forkhead (FOXO) transcription factors [41]. Phosphorylation of FOXO transcription factors by Akt results in the loss of function as mediators of apoptosis and cell-cycle arrest, leading to cell proliferation and survival [42]. Akt inactivates targets, such as pro-apoptotic protein Bad, apoptosis signal-regulating kinase 1 (ASK-1) and caspase-9, and also controls the cellular survival. Bad is a member of the Bcl-2 apoptosis-regulating proteins and is located on the mitochondria bound to Bcl-XL (a member of the BCL-2 family of proteins), inducing cell death. Phosphorylation by Akt leads to the loss of its pro-apoptotic properties [43]. ASK-1 is a positive regulator of cell apoptosis by activating p38 and Jun amino-terminal kinases (JNKs) [44]. Akt could phosphorylate ASK1 on Ser83 and inactivates the apoptotic function of ASK1, leading to the enhancement of cell survival [45]. Caspase-9 is an important protease in the intrinsic apoptotic pathway [46]. Akt inhibits its pro-apoptotic activity by phosphorylation on Ser196 [47, 48]. Cyclin-dependent kinase inhibitors such as p21 and p27 cease the cell cycle, but once phosphorylated by Akt, p21 and p27 lost the anti-cell cycle function [49, 50]. Akt also interferes in glycogen metabolism by inhibiting GSK-3 (GSK-3α and GSK-3β) activity, leading to dephosphorylation of glycogen synthase and the stimulation of glycogen synthesis [51-54]. mTOR is an important regulator of protein synthesis, and exists two multi-protein complexes, referred to as mTOR complex 1 (mTORC1) and mTORC2 [55]. The mTORC1 controls anabolic processes for promoting protein synthesis and cell growth [56]. Akt reduces the activity of inhibitors of mTORC1 to promote protein synthesis [57].

Negative Regulation of Akt

The tumor-suppressor phosphatase and tensin homolog deleted on chromosome ten (PTEN) is the most important negative regulator of PI3K/Akt signaling pathway [58-60]. It could dephosphorylate PIP3 back to PIP2, resulting in PI3K/Akt signaling pathway downregulation. PTEN inactivating mutations or deletion often occur in many human cancers leading to over-expressed or activated Akt [61]. Inhibition of PTEN causes activation of PI3K/Akt signaling pathway [62]. SHIP1 and SHIP2 (for Src homology domain-containing inositol phosphatase) are two phosphatases, removing the 5-phosphate from PIP3 yield PIP2 [63, 64]. They both are negative regulators of PI3K/Akt signaling pathway. Protein phosphatase 2A (PP2A) is identified as a directly negative regulator of Akt. It could directly dephosphorylate Akt to inactive Akt [65]. Pleckstrin homology domain leucine-rich repeat phosphatase (PHLPP-1 and -2) are reported to target the Thr308 and Ser473 residues of Akt, working as negative regulator of Akt [66].

ATP-COMPETETIVE INHIBITORS

Pyridylimidazoleoxadiazolyl Amines Derivatives

Researchers in GlaxoSmithKline [67] worked out a way to develop selective Akt inhibitors with the understanding of Akt structure in the ATP binding site. There is a back pocket in the binding site in Akt2 and ROCK1 (Rho-kinase 1), but in Akt, a leucine residue lies in the bottom of this pocket, whereas the corresponding residue in ROCK1 is a methionine. This difference was used to improve selectivity for Akt by introducing a steric element into the pocket at C-4 of the imidazopyridine core of compound **1** (Fig. **1**) which was a ROCK1 inhibitor. This strategy led to a series of pyridylimidazoleoxadiazolyl amines derivatives as ATP-competitive inhibitors (Fig. **1**). These compounds were evaluated for their kinase activities against Akt, ROCK1, and cellular activities by BT474 cells (human breast carcinoma). The results showed that, in terms of Akt activity, there was a clear preference for five-membered over six-membered aryl rings in the back pocket. Compounds **3** and **4** exhibited potent Akt activity as compared to compound **2** (IC_{50} values of 32, 79 and 331 nM). Compound 4 showed potent selectivity over ROCK1 with IC_{50} values of 79 and 562 nM for Akt1 and ROCK1. Replacement of furan ring by 2-methy-3-butyn-2ol group on compound **4** led to compound **5** with boosted Akt inhibitory activity, meanwhile the selective profile over ROCK1 was also increased (IC_{50} values of 6 nM for Akt1, 501 nM for ROCK1). The main difference of the furan ring and the 2-methy-3-butyn-2ol group was the steric property. The furan ring was too big to enter the back pocket, while the 2-methy-3-butyn-2ol group was suitable to penetrate into the back pocket in Akt. Compound **6** (GSK690693) with a cyclic constraint amino compared to **5**

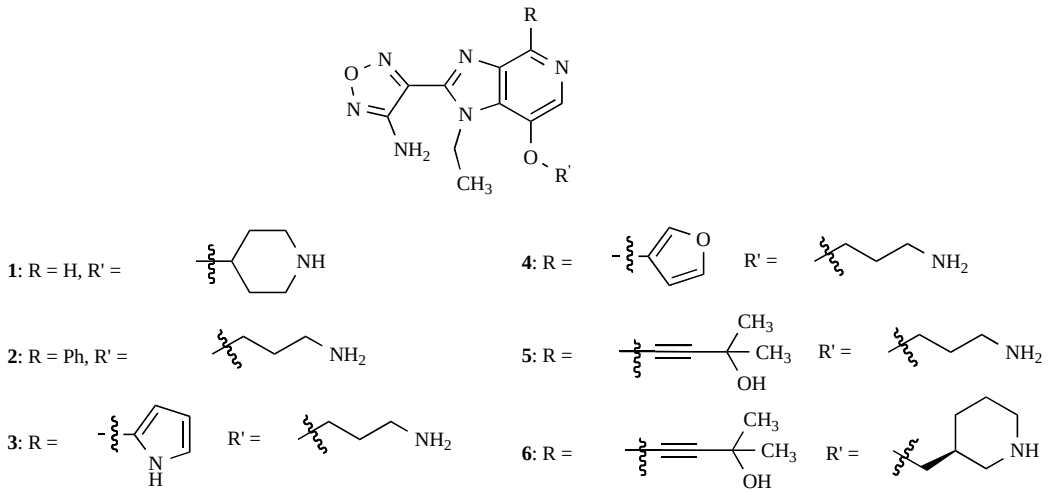


Fig. (1). Structures of Pyridylimidazoleoxadiazolyl Amines Derivatives.

was a highly selective Akt kinase inhibitor versus ROCK1, with IC_{50} values of 2, 13, and 9 nM against AKT1, 2, and 3, compared to IC_{50} values of 890 nM against ROCK1, nearly 450 folds more selective. An X-ray cocrystal structure was solved with GSK690693 and the kinase domain of Akt2, confirming that GSK690693 bound in the ATP binding site, and the 2-methy-3-butyn-2ol group heading into the back pocket as designed. It could significantly decrease GSK3β phosphorylation in BT474 tumor for up to 8 h after single ip dose in SCID mice at 20 mg/kg [68]. Furthermore, daily dosage of GSK690693 (ip, 30 mg/kg) resulted in 64% inhibition of tumor growth as compared to vehicle treated mice in BT474 xenografts [69]. It was also selective against some members of the AGC kinase family, such as MSK1 and RSK1. However, it showed less selectivity for other AGC kinases, such as PKA, PrkX, and PKC isozymes. With the understanding of the high degree of homology in the ATP binding site within PKA, PrkX, and the PKC isozymes, the poorer selectivity for these kinases was not entirely surprising. Other kinases inhibited by GSK690693 were AMPK and DAPK3 from the CAMK family and PAK4, 5, and 6 from the STE family. GSK690693 was the first ATP-competitive inhibitor getting in clinical trial as an IV agent used by patients with solid tumors or hematological malignancies, but it was suspended probably for the reason of causing hyperglycemia [14].

In order to improve pharmacokinetic or pharmaco-dynamic properties to that of GSK690693, Rouse *et al.* [70] investigated C-6 side chain of this scaffold that have afforded compounds (**7**~**11**, Fig. **2**) with comparable activity profiles to that of GSK690693. Compounds **7**, **8** and **9**, which contained aromatic substitution adjacent to the amine, offered significant enhancements in enzyme and cellular potency compared to compounds **10** and **11** (IC_{50} values against Akt1 kinase of 0.6, 1, 2, 4 and 3 nM, cellular activities of 0.08, 0.04, 0.041, 4 and 13 μM by determining inhibition of phosphorylation of GSK3β in BT474 cells). Compounds **7**, **8** and **9** displayed PK profiles suitable for IV dosing, but their oral administration properties were not ideal. Compound **8** showed statistically significant dose dependent inhibition, comparable to the response observed for GSK690693 in a mouse pharmacodynamic study of inhibition of GSK3β phosphorylation in BT474 xenografts *in vivo*. There was no report about clinical trials of these pyridylimidazoleoxadiazolyl amines derivatives to date.

This series of pyridylimidazoleoxadiazolyl amines derivatives had a 2-methy-3-butyn-2ol group which was observed to function as an important selectivity element to reduce ROCK1 inhibitory activity of this chemical class. This substituent could also significantly improve the activity against Akt. The studies on the C-6 and C-7 side chain showed that cellular potency was affected by the alicyclic amines [69, 70]. For the first time, these compounds were targeting the back pocket in the ATP-binding site, and modest selective profile was gained. Further studies on the back pocket probably lead to more selective inhibitors.

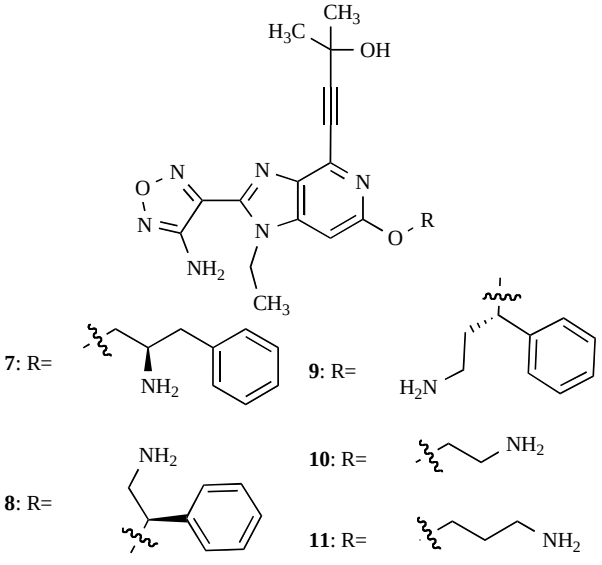


Fig. (2). Structures of compounds modified on the C-6 side chain.

Substituted-Pyridine Derivatives

Researchers in Abbott Laboratories [71, 72] found a series of 3,5-di-substituted-pyridine derivatives as ATP-

competitive Akt inhibitors (Fig. **3**). This series of 3,5-di-substituted-Pyridine derivatives displayed potent inhibitory activity against Akt, and the selectivity over other kinase were acceptable.

Compound **12** was identified as a promising Akt inhibitor lead from their compound library by high-throughput screening [71]. It was an ATP competitive inhibitor with an IC_{50} of 5.29 μM against Akt1. The structure-activity relationship (SAR) studies at the *trans*-3,4-bispyridinylethylene of **12** and modifition of the alkylamine led to a nanomolar Akt inhibitor **13** [73]. Compound **13** exhibited activity with IC_{50} value of 14 nM against Akt1, and excellent selectivity against distinct families of kinases such as TK and CAMK. But it displayed poor to marginal selectivity against the AGC and CMGC families of kinases. The cyclization studies [74, 75] at the stilbene double bond of **13** to lock the conformation were evaluated, providing 3-isoquinolinylpyridine compound **14** with a 10-fold boost in potency against Akt1 (IC_{50} value of 1.3 nM). Antiproliferative activity against FL5.12-Akt1 and MiaPaCa-2 cells were improved to 0.42 and 0.59 μM. The X-ray structures of **14** in complex with PKA in the ATP-binding site showed the nitrogen of the isoquinoline bond to the hinge *via* a hydrogen bond with the backbone NH of Val123. However, further SAR studies at the isoquinoline scaffold; found these analogs had poor pharmacokinetic profile, especially cellular toxicity. Metabolism at the C-1 position of the isoquinoline was responsible for the poor pharmacokinetic profile of this series of Akt inhibitors. However, blocking this site failed to provide potency against Akt [71].

Replacement of the metabolically labile isoquinoline of compound **14** to heterocyclic pharmacophores led to the discovery of indazole-pyridine derivative **15** (A-443654) [76], oxindole-pyridine derivative **16** [77] and pyrazolo-pyridine-pyridine derivative **17** [78]. The indazole, oxindole and pyrazolopyridine groups provided two hydrogen bonding interactions to the hinge-binding region. This change provided great improvement in potency against Akt1 (IC_{50} values of 0.16 nM, 0.17nM and 0.34 nM for **15**, **16** and **17**, respectively). A-443654 was 40-fold selective for Akt over PKA, 200-fold selective over PKCδ (IC_{50} values of 6.3 nM and 33 nM). The compound showed cellular activity against MiaPaCa cells (Soft Agar EC_{50} = 44 nM; MTT EC_{50} = 100 nM; GSK3β EC_{50} = 300 nM) and caused significant delay in the growth of tumors in mouse xenograft models. When given in combination with paclitaxel, A-443654 increased the efficacy of paclitaxel in a PC-3 xenograft mode which was a PTEN-deficient human prostate carcinoma cell line. When given in combination with rapamycin, an mTOR inhibitor, in the Mia-PaCa-2 model, rapamycin was significantly less toxic and could be maintained longer, leading to more durable responses. The efficacy observed for the combination of the Akt and mTOR inhibitors was better than that observed for either agent alone [72]. Tumor growth inhibition was examined *in vivo* efficacy in several mouse tumor models dosed subcutaneously at 7.5 and 15 mg/kg/day for 14 days, and the inhibitor was found to significantly slow the growth of the tumors. But the tumors regrew when compound administration was ceased. The deficiency of A-443654 was a short half-life ($t_{1/2}$ = 0.6 h in mouse) and no oral bioavailability. [76]

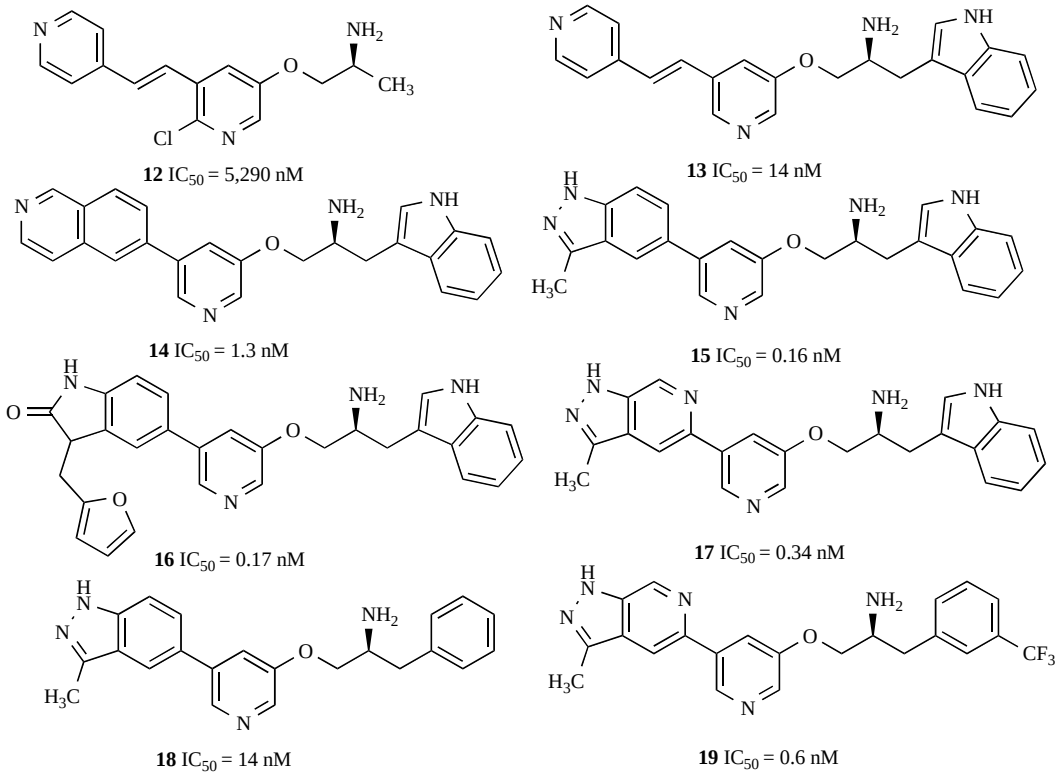


Fig. (3). Structures of 3,5-di-substituted-Pyridine derivatives.

Replacing the indole with a phenyl moiety in compound **15** (A-443654) led to compound **18** (A-674563), an orally available inhibitor [79]. Despite a nearly 100-fold loss in potency (IC_{50} values of 14 nM) compared to A-443654, compound **18** showed a comparable efficacy *in vivo* as A-443654 at higher doses and displayed an improved pharmacokinetic profile in several species (mouse, rat, and dog). Despite a relatively short half-life (IV $t_{1/2}$ =1 h in mouse), compound **18** showed 70% oral bioavailability in mice with respectable plasma drug exposure (PO auc = 2.0 μM ·h, 10 mg/kg). However, the selectivity over ROCK1 and PKA was poor with IC_{50} values of 79 nM against ROCK1, 16 nM against PKA [79, 80].

To improve the selectivity of compound **18** over other protein kinases, a nitrogen atom was incorporated into selected phenyl analogues of **18** at the C-6 position of the methyl indazole scaffold. Meanwhile, several substitutions were introduced into phenyl of **18**. These modifications resulted in the discovery of compound **19** which displayed excellent potency against Akt1 with an IC_{50} of 0.6 nM, improved selectivity over other protein kinases (22 folds selectivity over PKA, 120 folds over PKCδ), and improved cardiovascular safety profile [71]. Compound **19** was orally bioavailable in mice (*F*= 25%), with a longer half-life ($t_{1/2}$= 1.8 h) and similar plasma exposure (auc = 1.7 μM·h) at 10 mg/pk. When dosed orally in conscious mice up to 150 mg/kg, no statistically meaningful hypotension was observed. Compound **19** was negative in a dog Purkinje assay, indicating relatively less risk of cardiovascular QT prolongation.

Lin *et al.* [80-82] developed a new series of 2,3,5-trisubstituted pyridine derivatives (Figs. **4** and **5**) as selective Akt inhibitors based on compounds **6** (GSK690693), **15** (A-443654) and **18** (A-674563). They overlaid **6**, **15** and **18**, suggesting that a C-2 substitution of the core pyridine of **15** and **18** could occupy the space, where the 2-methy-3-butyn-2ol substituent resided in **6**. Substitution at the 2-position of the core pyridine with phenyl diminished ROCK1 inhibition, a more than 200-fold improvement in selectivity over ROCK1 was achieved when comparing compound **20** (IC_{50} values of 50 nM against Akt1, > 10000 nM against ROCK1) and compound **18** (IC_{50} values of 125 nM against Akt1, 79 nM against ROCK1) [80]. Compounds **20** and **21** demonstrated improved Akt1 potency (IC_{50} values of 15 nM for **21**). Besides, compounds **24, 25** and **26**, with the halogenated ortho-phenol, were observed as being potent and selective (IC_{50} values of 10, 3 and 63 nM for Akt1, 9800, 7600 and >10000 for Rock1, respectively). Especially, compound **25** demonstrated single digit nanomolar potency against AKT1 and greater than 2,000-fold selectivity over ROCK1. However, the *meta*- and *para*-phenol derivatives, compounds **22** and **23**, were less potent Akt1 inhibitors. The 3-furanyl derivative **27** was observed to be much more potent and selective than the 2-furanyl region isomer **28** with IC_{50} values of 10 nM compared to 790 nM. Compound **27** maintained the activity and increased the selectivity over ROCK1 (IC_{50} values of 5200 nM against ROCK1). However, direct introduction of 2-methy-3-butyn-2ol group in **18** led to compound **29** with decreased activity against Akt1 (IC_{50} values of 10500 nM for **29**). This study showed that the 3-furanyl group (compound **27**) was the most tolerance substituent on C-2 position of the core pyridine, leading to improved selectivity over ROCK1.

Compounds **30**-**37** (Fig. **5**) were synthesized to further improve enzymatic potency against Akt of this chemical class by replacing the *S*-phenylalaninol side chain with *S*-tryptophanol. Compound **30** exhibited activity with an IC_{50} value of 1 nM, which was a 10-fold increase of potency against Akt1 compared to **27**. The 2-methyl-3-furan group at 2-position of the core pyridine provided the most significant increase in activity (compound **31** with IC_{50} value of 0.8 nM against Akt1). Compound **31** was highly potent in cellular mechanistic assay with IC_{50} value of 84 nM measuring the reduction of phosphorylated GSK3β in BT474 cell line. Substitution of fluorine on the 5-, 6-, and 7-position of indole on **30** led to compounds **32**-**34**, which were equipotent in anti-Akt1 activity and cellular activity with compound **30** (IC_{50} values of 1, 0.8 and 1 nM against Akt1). This modification was not significantly influenced the activity. Introduction of nitrogen atom in indole on **30** led to less tolerated compounds **35**-**37**, their anti-Akt1 activity and cellular activity were decreased (IC_{50} values of 8, 13 and 10

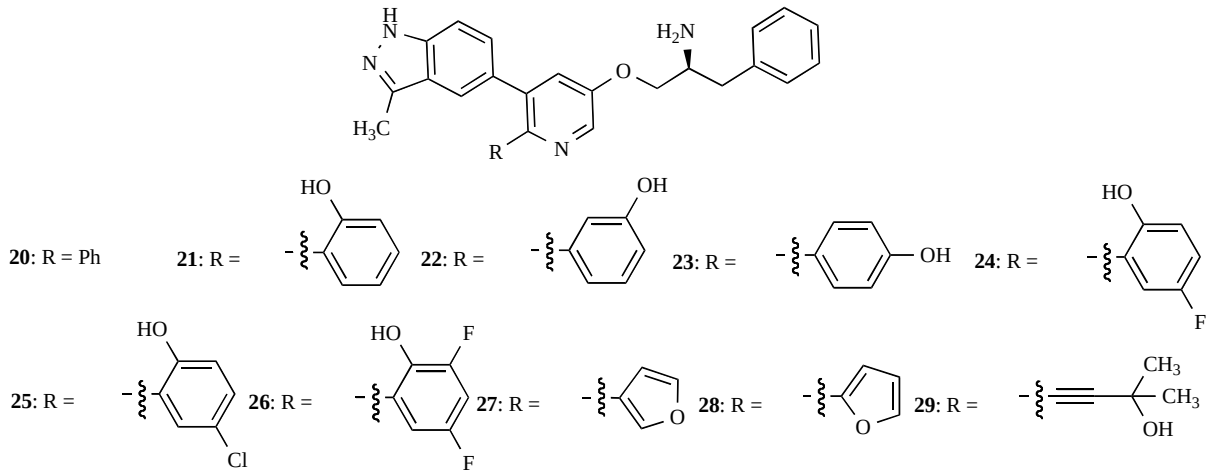


Fig. (4). Structures of 2,3,5-trisubstituted pyridine derivatives.

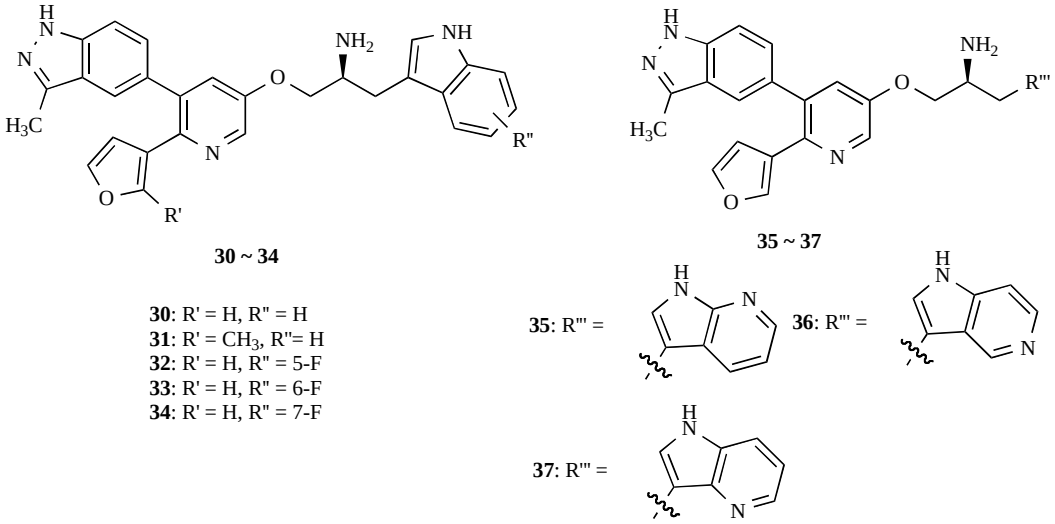


Fig. (5). Structures of 2,3,5-trisubstituted pyridine derivatives.

nM for Akt1, 930, 6800 and 4300 nM for cellular activity, respectively).

Compound **30** was profiled in an *in vivo* pharmacodynamic assay using a BT474 tumor xenograft model in mice for intracellular Akt activity by measuring the inhibition of GSK3β phosphorylation. However, because of the poor physical properties, high lipophilicity (clogP = 4.45) and high protein binding, compound **30** failed to show a pharmacodynamic effect. Besides, compound **30** was observed as very potent CYP450 3A4 inhibitor, displaying 50 nM potency. In order to improve drug-like properties and kinase selectivity, a series of azaindazole analogs **38**-**42** (Fig. **6**) were designed by introducing one or two nitrogen atoms in the indazole ring to increase polarity of the molecule and to lower the clogP values [82]. Compounds **39** and **42,** showed clogP values of 4.17 and 3.96, were not acceptable, without activity and selectivity improvement. Compounds **38**, **40** and **41** displayed lower clogP values (3.75, 3.31 and 3.31) than that of **30**, accompanied improved drug-like properties. The anti-Akt activity of three compounds was maintained with IC_{50} values of 1, 2 and 1 nM against Akt1. Compound **40** displayed reduced protein binding rate compared to **30** (90.9 % vs 95.9 %) and improved kinase selectivity, 10-fold decreased potency against PKA, almost 100-fold decreased potency against MSK1, CYP450 3A4 inhibitory activity decreased to 400 nM. When studied in a BT474 tumor xenograft model at a dose of 50 mg/kg (intraperitoneal administration), compound **40** exhibited more than 80% inhibition of GSK3β phosphorylation.

Introduction of a C-6 substitution on the core pyridine of 2,3,5-trisubstituted pyridine derivatives led to tetrasubstituted pyridines compounds **44**-**47** [81] (Fig. **7**). Compounds **44**-**47** maintained or slightly increased the enzymatic potency by introduction of an amino group (IC_{50} values of 3, 1, 1 and 1 nM against Akt1, 15, 15, 19 and 13 nM against Akt2, 1, 2, 2 and 3 nM against Akt3, respectively). Compound **46** showed reduced CYP450 3A4 inhibitory potency (IC_{50} value of 5000 nM) and hERG channel inhibitory potency (IC_{50} value of 10400 nM) compared to trisubstituted pyridine analog **43** (IC_{50} values of 630 nM against CYP450 3A4, 1400 nM against hERG channel). Compound **46** was not selective over PKA. In the pharmacokinetic study using mouse, rat, dog and monkey, compound **46** exhibited higher exposure and lower clearance in all species. Besides, it could significantly decrease GSK3β phosphorylation in BT474 tumor xenograft mouse model after single ip dose at 50 mg/kg. Furthermore, daily dosing of **46** (ip, 30 mg/kg) resulted in 59% inhibition of tumor growth compared to vehicle treated mouse in BT474 tumor xenografts.

By reviewing the evolutionary process of these substituted-pyridine derivatives, the structure of these

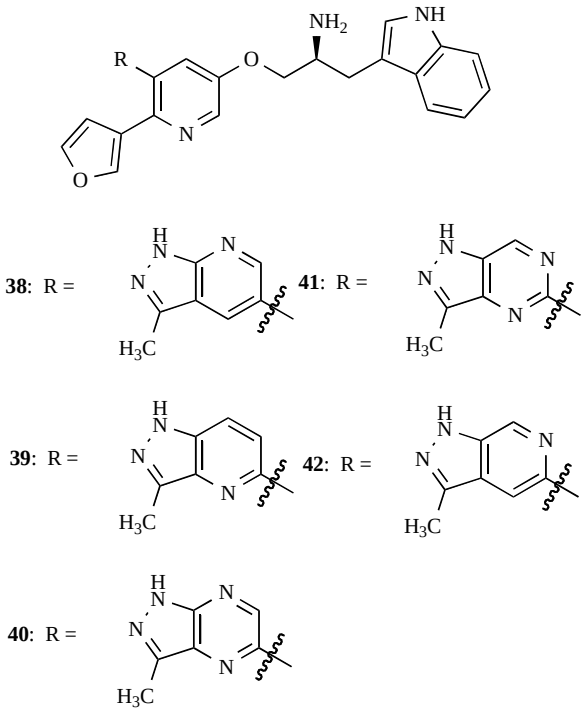
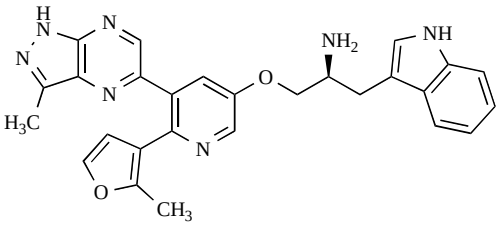
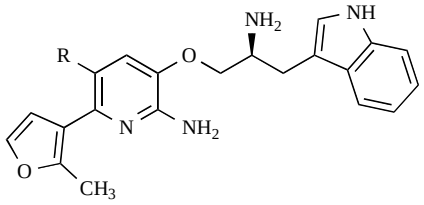


Fig. (6). Structures of compounds **38** to **42.**

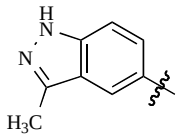
inhibitors can be outlined in a general formula (Fig. **8**) and their SAR could be summarized as bellow:



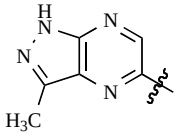
43



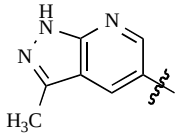
44: R =



46: R =



45: R =



47: R =

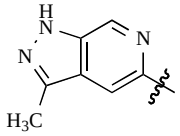


Fig. (7). Structures of compounds **43** to **47**.

1) The core pyridine was the key structure of these inhibitors, and can't be replaced by pyridazine or pyrazine [81].

2) The aliphatic amine with hydrophobic groups were crucial for activity and selectivity, especially (*S*)-2-amino-3-(1H-indol-3-yl)-1-propoxy group [71-73, 81].

3) When the "Ar" group was directly connected to core pyridine to lock the conformation, the inhibitory potency increased. The introduction of nitrogen atom on the "Ar" group was beneficial to the activity, selectivity and pharmacodynamic effect [82].

4) The substitution on C-2 (-R) and C-6 (-R') was helpful to increase selectivity over other kinase [80, 81].

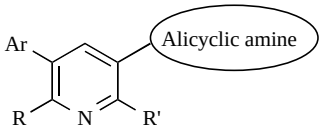


Fig. (8). General formula of substituted-pyridine derivatives.

Substituted-Thiophene Derivatives

Lin *et al.* [83] disclosed a 2,5-di-substituted-thiophene derivatives as ATP-competitive Akt inhibitors (Fig. **9**). High throughput screening of Akt3 inhibition identified compound **48** as a weak inhibitor with IC_{50} value of 3000 nM. Optimization of the halogen substitution on the terminal phenyl ring led to compound **49** with increased activity (IC_{50} value of 610 nM). Addition of an aminomethyl group to the amide linker got compounds **50** and **51**, which were enantiomers. The *S*-enantiomer **51** displayed optimal potency against Akt3 than the *R*-enantiomer **50** (IC_{50} values of 2.6 and 240 nM). When tested in DOV13 ovarian carcinoma cells, compound **51** inhibited cell proliferation with EC_{50} value of 1 μM. However, compound **51** showed a poor selectivity profile over PKA (IC_{50} value of 0.1 nM). The co-crystal structure of **51** in complex with PKA was determined, showing some key interactions including the aminopyrimidine with the hinge region, the primary amine with the Mg^{2+} binding site and the phenyl group with the glycine-rich loop. These interactions could explain the poor selectivity profile of compound **51**.

Recently, another series of substituted-thiophene derivatives (Fig. **9**) were developed by Seefeld *et al.* [84]. Through a screening of the GSK kinase inhibitor collection, Compound **52** was identified as low nanomolar ATP-competitive inhibitors of Akt1 (IC_{50} value of 6 nM). Structural optimization on **52** led to compounds **53**-**56**. Introduction of a bromo group (compound **53**) or a phenyl (compound **55**) alone on C-3 position of thiophene core was less tolerated (IC_{50} values of 8 and 50 nM). Modification of phenyl group on compound **53** improved the activity (IC_{50} values of 1 nM for **54**, 1 nM for **56**). Compound **56** inhibited cell proliferation with EC_{50} value of 0.24 μM in BT474 cells. Besides, it could significantly decrease GSK3β phosphorylation in BT474 tumor xenograft mice model for up to 4 h after single ip dose at 25 mg/kg. However, the poor selective potency over AGC family of kineses was observed, such as ROCK1 and PKCδ (IC_{50} values of 2 and 40 nM).

Substituted-Azole Derivatives

Zeng *et al.* [85, 86] reported a series of 2,5-di-substituted 1,3,4-thiadiazole derivatives as Akt inhibitors (Fig. **10**). Lead compound **57** was found to inhibit Akt1, PKA and cyclin-dependent kinase 2 (CDK2) at nanomolar (IC_{50} values of 76, 54 and 72 nM, respectively). Introduction of hydrophobic group on the C-3 and C-4 positions of terminal phenyl ring led to compounds **58**-**60**, which exhibited improved activity against Akt1 with IC_{50} values of 6.1, 8.9 and 5.5 nM, respectively. The inhibitory potency against PKA and CDK2 was also increased, no significant selectivity over PKA or CDK2 was observed. An X-ray co-crystal structure of **59** and PKA showed that there were two hydrophobic interactions between the methyl group on the indazole ring and two residues of PKA. The deletion of these interactions was helpful to the selectivity. Compound **61**, without methyl group of indazole ring, showed 20-fold selectivity over PKA, 4-fold selectivity over CDK2 (IC_{50} values of 6.0 nM against Akt1, 108 nM against PKA and 24 nM against CDK2). Compound **62** in which the isoquinoline provided two hydrophobic interactions as methyl group of indazole ring, lack selectivity over PKA (IC_{50} values of 3.0 nM against Akt1, 8 nM against PKA). When tested in U87MG cells, compound **61** inhibited cell proliferation with IC_{50} value of 0.44 μM by measuring phosphorylation of PRAS40 (Akt

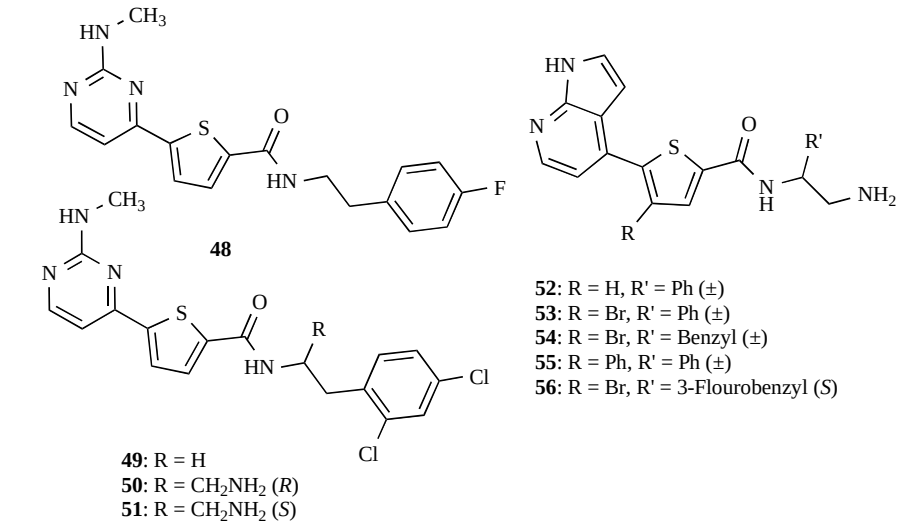


Fig. (9). Structures of substituted-thiophene derivatives.

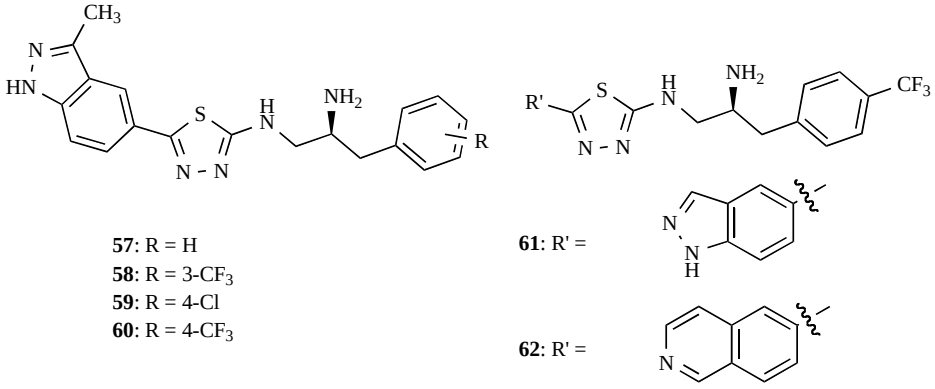


Fig. (10). Structures of 2,5-di-substituted 1,3,4-thiadiazole derivatives.

substrates) *via* an enzymelinked immunosorbent assay (ELISA), 0.15 μM by measuring the translocation of FKHRL1 (Akt substrates).

The effort to explore the SAR around the core azole ring while keeping the isoquinoline linker binder element of the potent thiadiazole analog **62** led to compounds **63**-**66** [86] (Fig. **11**). However, these compounds showed decreased inhibitor activity against Akt1 (IC_{50} values of 404, 17, 1600 and 85 nM, respectively). Compound **67** (Fig. **11**) proved that the thiazole core played the role as thiadiazole in compound **62**, maintaining the activity with IC_{50} values of 4.9 nM against Akt1. But **67** inherited the poor selectivity over PKA with IC_{50} values of 5.9 nM because of the isoquinoline group. Replacement of isoquinoline group generated compounds **68**, **69** and **70** (Fig. **11**) with significant selectivity over PKA (IC_{50} values of 8.5, 18.3 and 8.0 nM against Akt1, 39.9, 167 and 326 nM against PKA). This confirmed that the deletion of the hydrophobic interactions between the isoquinoline group and PKA was helpful to increase the selectivity. The cellular potency of compounds **69** and **70** was tested in U-87 MG cell with IC_{50} values of 0.3 and 0.5 μM. Compounds **67**, **69** and **70** were advanced into *in vivo* experiments. Compound **67** was orally bioavailable in male Sprague Dawley rats (F = 20 %) with longer half-life ($t_{1/2}$ = 2.9 h). Compound **70** had poor bioavailability (F = 11 %) and longer half-life ($t_{1/2}$ = 3.8 h). Compound **69** exhibited 64% oral bioavailability with respectable plasma drug exposure (auc = 2600 ng·h/mL, 5 mg/kg) and longer half-life ($t_{1/2}$ = 4.3 h). All of these compounds were cleared *via* oxidation of the thiazole ring [87], therefore their half-lives were similar. *In vivo* pharmacodynamic (PD) studies in mice six hours after a 30 mg/kg dose, plasma concentration of **69** was about 10-fold over its cellular IC_{50}, and significant inhibition of PRAS40 phosphorylation was observed (43%). Furthermore, daily dosage of **69** (po, 30 mg/kg) resulted in 93% inhibition of tumor growth compared to vehicle treated control in U-87 MG tumor xenografts in nude mice.

Substituted-Piperidine Derivatives

Caldwell *et al.* [88] reported a series of substituted-piperidine derivatives (Fig. **12**). Their previous study [89] identified compound **71** as an unselective Akt inhibitor over PKA with IC_{50} value of 20 nM. In order to get the selective inhibitors, modifications of **71** were employed leading to compounds **72**-**76**. Compound **72** shared the most structural

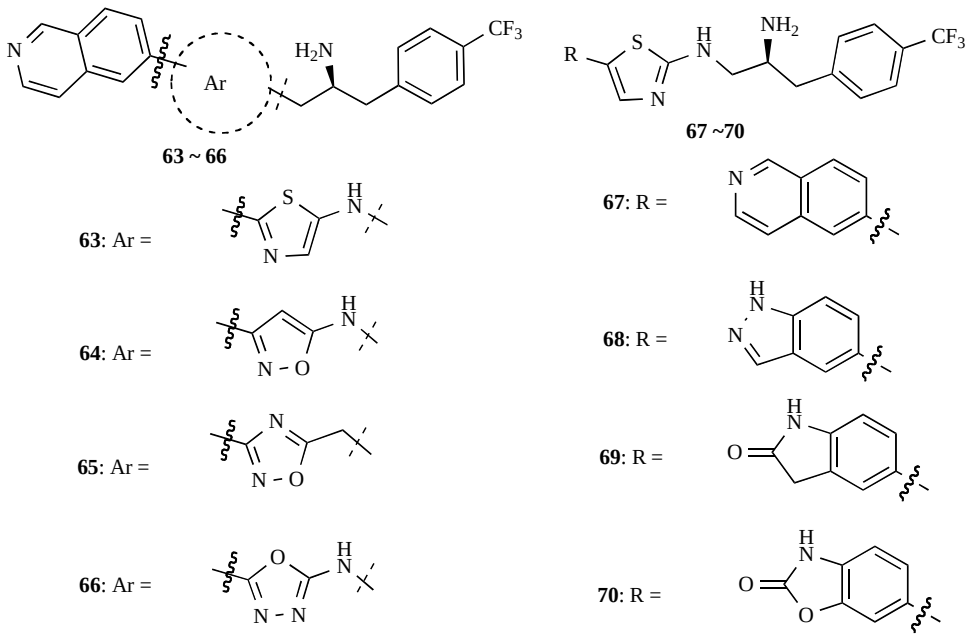


Fig. (11). Structures of substituted-azole derivatives.

similarity to **71** with IC_{50} values of 25 nM against Akt2, 54 nM against PKA. When 4-chlorophenyl substituent or 4-chlorobenzyl substituent directly connected to piperidine core compared to **72**, the inhibitory potency against Akt2 and PKA was both increased (compound **73** with IC_{50} values of 8 nM against Akt2, 5 nM against PKA, compound **74** with IC_{50} values of 5 nM against Akt2, 9 nM against PKA). With the 4-chlorophenyl substituent and amino group directly attached to the 4-position of the piperidine, compound **75** were potent Akt2 inhibitors with no selectivity over PKA (IC_{50} values of 7 nM against Akt2, 15 nM against PKA). However, replacement of the 4-chlorophenyl substituent to the 4-chlorobenzyl substituent in **76** (CCT128930) introduced 30-fold selectivity for Akt2 over PKA (IC_{50} values of 6 nM against Akt2, 168 nM against PKA). The X-ray structures of **73**, **74**, and **76** suggested that the piperidine linker, the terminal amine and lipophilic group in this chemical series appear to work together to achieve the selectivity over PKA by exploiting the relatively subtle differences between PKA and PKB in the ATP-binding site. The cell growth inhibition of compound **76** was tested in PC-3M human prostate cancer cells with IC_{50} values of 12 μM using sulforhodamine B colorimetric assay, 3.0μM measuring GSK3β phosphorylation. In the pharmacokinetic study in mice, compound **76** exhibited good distribution to tissue (Vss = 0.25 L), short half-life ($t_{1/2}$ = 0.95 h, iv, 25mg/kg) and low bioavailability (F_{oral} = 8.5 %).

Recently, McHardy *et al.* [90] reported modifications of compound **76** to the identification of selective and orally bioavailable inhibitors of Akt2 with *in vivo* antitumor activity (Fig. **13**). Variation of the substituents on the benzyl group of **76** led to compounds **77** and **78** with potent activity and increased selectivity over PKA. Compound **77** showed inhibitory potency with IC_{50} values of 27 nM against Akt2, 3400 nM against PKA (126-fold). Compound **78** was more potent than **77** with IC_{50} values of 8.5 nM against Akt2, and was 153-fold selective over PKA. The selectivity seemed to be connected with the lipophilic group, so compound **79** with 2-napthyl group was synthesized with intermediate level of selectivity (70-fold) and IC_{50} value of 7.0 nM against Akt2. The X-ray crystal structure of compound **77** bound to Akt2 showed that the tert-butyl substituent occupied the lipophilic pocket formed by the P-loop of Akt, and the 4-amino substituent interacted with Glu236 and Glu279 in the ribose pocket. Modification of lipophilic group and the chain length between the 4-aminopiperidine and lipophilic group led to compounds **80-83**. The 4-chlorobenzyl derivative **80** exhibited the most potent inhibitory activity with IC_{50} value of 2.2 nM against Akt2, but the selectivity over PKA was decreased (14-fold). Variation of the position of the chlorine atom in the aromatic ring (compounds **81**, **82** and **83**) showed decreased Akt2 inhibitory activity (IC_{50} values of 36, 4.9 and 5.7 nM, respectively). The azaindole analog **84** and 8-oxopurine analog **85** also showed decreased activity and selectivity compared to compound **80** (IC_{50} values of 12 and 5.0 nM). In the cellular activity assay, compound **80** exhibited potent inhibitory activity with IC_{50} values of 2.3 and 0.93 μM using cellular ELISA for inhibition of GSK3β phosphorylation in PC-3M cells and U87MG (Glioblastoma) cells. In the pharmacokinetic properties study, compound **80** showed very good oral bioavailability in mice (F_{oral}= 58 %). When tested in mice bearing established subcutaneous U87MG human glioblastoma xenografts, doses of **80** up to 200 mg/kg (5 days dosing in 7) were well tolerated with no effects on mouse body weight and resulted in 23% inhibition of tumor growth compared to control groups. At 4 h after a single dose at 200 mg/kg *po*, high levels of **80** were found in plasma and tumor samples (20 and 43 μM, respectively).

Substituted-Pyrrolidine Derivatives

Compound **86** (Fig. **14**) was identified as Akt inhibitor by Lippa *et al.* [91] with IC_{50} value of 2.4 nM against Akt1.

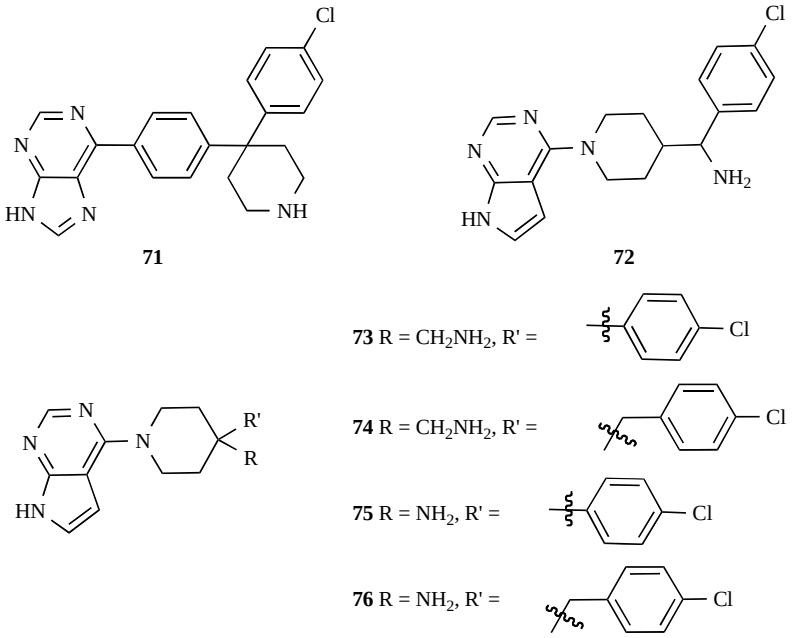


Fig. (12). Structures of compounds **71** to **76**.

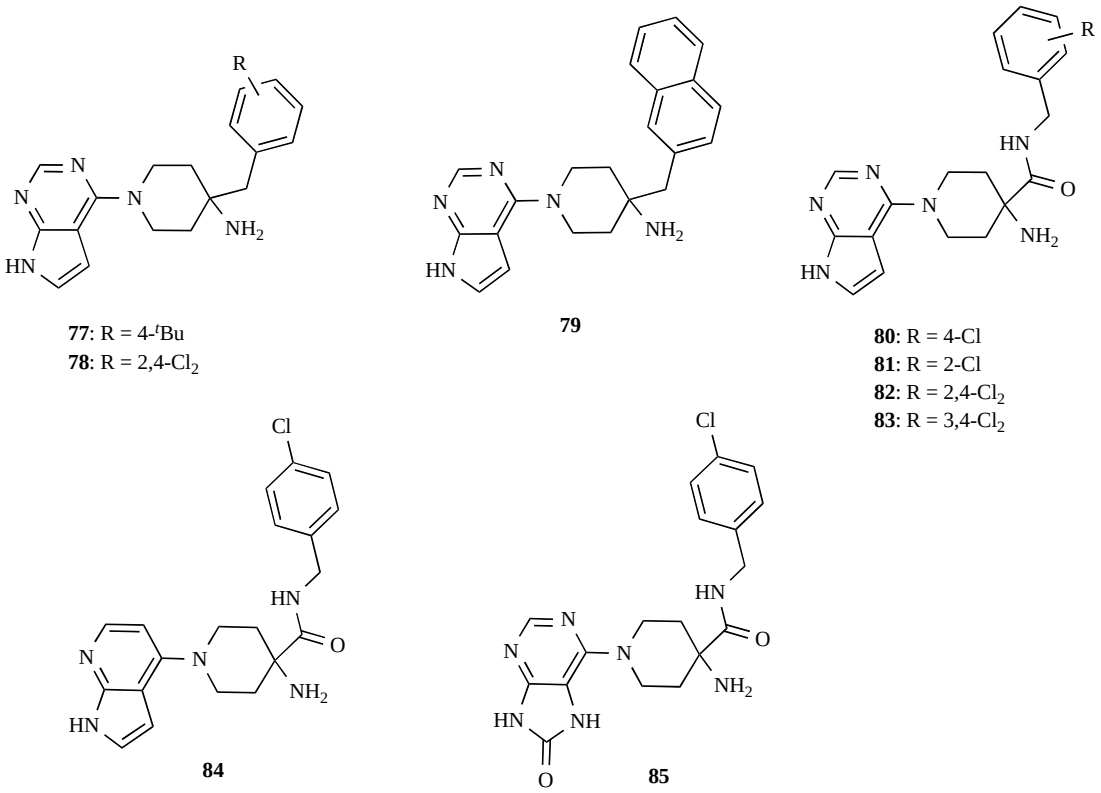


Fig. (13). Structures of compounds **77** to **85**.

However, compound **86** exhibited no selectivity over PKA with IC_{50} value of 3.6 nM against PKA. Freeman-Cook *et al*. [92] reported a series of substituted-pyrrolidine derivatives (Fig. **11**) based on the study of compound **86**. Investigation on the potential new scaffolds by replacing the spiroindoline substituent of **86** by various structurally diverse amines led to selective compounds **87** and **88** (19- and 17-fold selective for Akt1 over PKA, respectively), although their inhibitory activity was decreased (IC_{50} values of 68 and 180 nM). The (3*R*)-aminopyrrolidine core was responsible for conferring selectivity. X-ray structures of **88** bound to Akt1 and PKA were determined, showing that C-3 primary amine of the

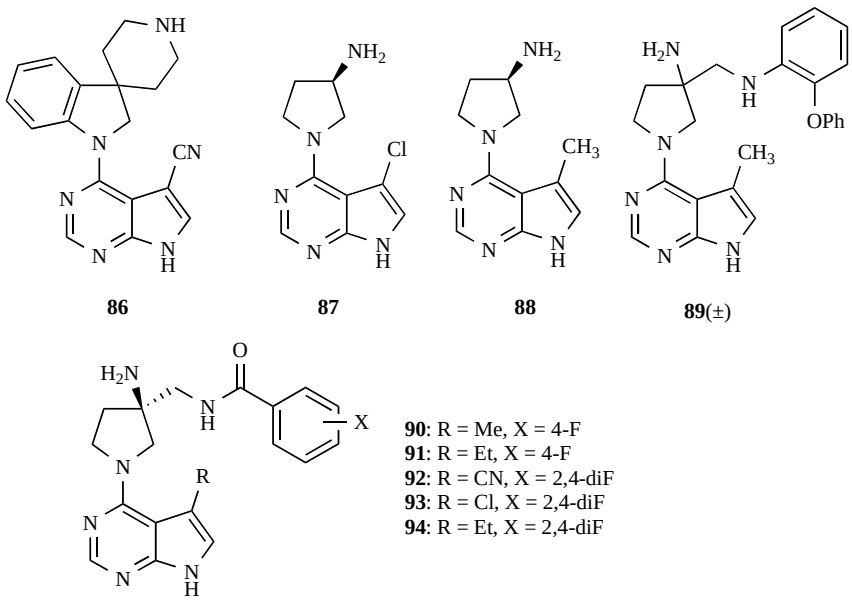


Fig. (14). Structures of compounds **86** to **94**.

(3*R*)-aminopyrrolidine core was displayed in a pseudoaxial orientation and formed a salt bridge to Glu-234 in Akt1. Introduction of substitution at the C-3 methine would lock the amine into the required pseudoaxial conformation in Akt1. However, any extension at that position in PKA would likely result in a steric clash with nearby residues. This strategy of introducing substitution at the C-3 methine of 3-aminopyrrolidine core generated racemic compound **89** with potent inhibitory activity and selectivity (IC_{50} values of 16 nM, 51-fold selective over PKA). But the high lipophilicity of compound **89** (clogP = 4.4) led to increased clearance and increased hERG binding. The effort to decrease the lipophilicity while maintain the activity and selectivity got compounds **90**-**94** with IC_{50} values at single nanomolar concentration. The IC_{50} values of compounds **90**-**94** were 3.0, 1.4, 7.7, 1.1 and 0.5 nM against Akt1, respectively. These compounds showed high selectivity, at least 50-fold selective over PKA. Compound **94** demonstrated the most kinase inhibitory activity, cellular activity (0.31 μM) and selectivity, which achieved 900-fold selectivity for Akt1 over PKA. When tested in 226 kinases, compound **94** exhibited at least 100-fold selectivity for Akt over other kinases. In the pharmacokinetic study in dog, compound **94** exhibited moderate clearance (11.6 (mL/min)/kg) and volume of distribution (4.8 L/kg), well absorbed (F = 54%) and a half-life of 4.4 h. The tumor growth inhibition (TGI) assays were tested in PC-3 prostate carcinoma xenografts (at 100 mg/kg b.i.d. dosing for 10 days) and colorectal carcinoma (Colo205) xenografts (at 150 mg/kg b.i.d. after 10 days), with 75% and 60% TGI observed, respectively. Compound **94** showed 98% TGI at a dose of 75 mg/kg b.i.d. (10 days) combining with rapamycin (10 mg/kg, ip) in PC-3 prostate carcinoma xenograf, compared to single use with TGI of 56% of **94**, 66% of rapamycin. Except modest weight loss in the highest dosing groups, no significant toxicological side effects were observed in these studies. Compound **94** was nominated for clinical development.

Substituted-Azepane Derivatives

Breitenlechner *et al*. [93] found compounds **96** ~ **99** as selective Akt inhibitors from compound **95** which showed no selectivity over PKA (Fig. **15**). The pyridine, amide, azepane and second amide part occupied the ATP-binding site, while the benzophenone part stretched out of the ATP-binding site. They used two different amino acid residues outside of the ATP-binding site, the phenylalanine 187 in PKA and leucine 187 in Akt. The leucine had smaller isobutyl group than the benzyl group in Phenylalanine. In compound **95**, the dimethylamino group was fit to interact with both isobutyl group and benzyl group, so **95** showed no selectivity over PKA (IC_{50} Values of 30 nM anginst PKA, 23 nM against Akt). A series of bulky groups were introduced in to this position leading to compounds **96** ~ **99** with modest selective profile. Compounds **98** was the most selective for Akt versus PKA (IC_{50} Values of 1900 nM anginst PKA, 20 nM against Akt). Compared to other groups in **96**, **97** and **98**, the 3,3-dimethylpiperidine group was steric suitable to form hydrophobic interaction with leucine in Akt, while it was so big that some unfavorable steric contacts existed between 3,3-dimethylpiperidine group and Phenylalanine in PKA. This was the steric reason for selectivity.

CONCLUSIONS

Recent progress in the field of selective ATP-competitive inhibitors of Akt kinase has been reviewed. Akt has a key role in the regulation of cell survival, proliferation and growth. Several series of ATP-competitive inhibitors with diverse structural features have been reported as Akt inhibitors and hold the promise of being broadly effective agents in combination therapy with various standard chemotherapeutics. However, Akt inhibitors are facing the challenge of selectivity over other kinases. Some selective Akt inhibitors have been identified with the understanding of crystal structure. The full crystal structures of Akt and the difference between Akt and other kinases have been

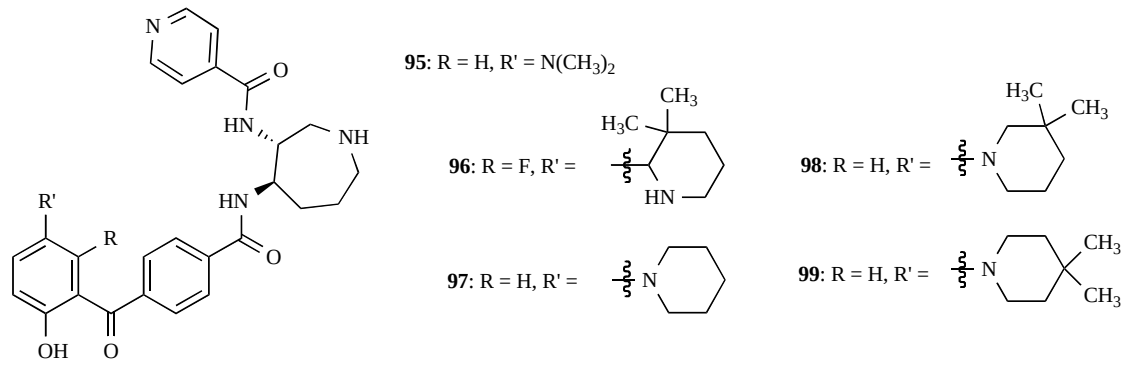


Fig. (15). Structures of compounds **95** to **99**.

determined, and various cocrystal structures of Akt and ATP-competitive inhibitors disclosed the binding interactions of Akt and inhibitors [67, 92, 93]. The different amino acid residues between Akt and other kinases show different properties, such as space size, hydrophobic property, static electricity and so on. This is helpful to design new selective inhibitors using structure-based drug design. Several selective ATP-competitive inhibitors have been successfully evaluated such as GSK690693 (compound **6**), compound **94** and compound **98** in this way. New selective ATP-competitive inhibitors on Akt will be explored with further studies.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No. 21072115) and Shandong Natural Science Foundation (No. ZR2011HM042).

REFERENCES

[1] Wendel, H.G.; de Stanchina, E.; Fridman, J.S.; Malina, A.; Ray, S.; Kogan, S.; Cordon-Cardo, C.; Pelletier, J.; Lowe, S.W. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature*, **2004**, *428*, 332-7.

[2] Omar, B.; Zmuda-Trzebiatowska, E.; Manganiello, V.; Goransson, O.; Degerman, E. Regulation of AMP-activated protein kinase by cAMP in adipocytes: Roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. *Cell Signal.*, **2009**, *21*, 760-6.

[3] Lawlor, M.A.; Alessi, D.R. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J. Cell. Sci.*, **2001**, *114*, 2903-10.

[4] Tang, J.M.; He, Q.Y.; Guo, R.X.; Chang, X.J. Phosphorylated Akt overexpression and loss of PTEN expression in non-small cell lung cancer confers poor prognosis. *Lung Cancer*, **2006**, *51*, 181-91.

[5] Al-Bazz, Y.O.; Underwood, J.C.; Brown, B.L.; Dobson, P.R. Prognostic significance of Akt, phospho-Akt and BAD expression in primary breast cancer. *Eur. J. Cancer*, **2009**, *45*, 694-704.

[6] Zhang, M.; Fang, X.; Liu, H.; Guo, R.; Wu, X.; Li, B.; Zhu, F.; Ling, Y.; Griffith, B.N.; Wang, S.; Yang, D. Bioinformatics-based discovery and characterization of an AKT-selective inhibitor 9-chloro-2-methylellipticinium acetate (CMEP) in breast cancer cells. *Cancer Lett.*, **2007**, *252*, 244-58.

[7] Zhang, M.; Fang, X.; Liu, H.; Wang, S.; Yang, D. Blockade of AKT activation in prostate cancer cells with a small molecule inhibitor, 9-chloro-2-methylellipticinium acetate (CMEP). *Biochem. Pharmacol.*, **2007**, *73*, 15-24.

[8] Shtilbans, V.; Wu, M.; Burstein, D.E. Current overview of the role of Akt in cancer studies *via* applied immunohistochemistry. *Ann. Diagn. Pathol.*, **2008**, *12*, 153-60.

[9] Pal, S.K.; Reckamp, K.; Yu, H.; Figlin, R.A. Akt inhibitors in clinical development for the treatment of cancer. *Expert Opin. Investig. Drugs*, **2010**, *19*, 1355-66.

[10] Clinicaltrials.gov. Search for clinical trials. Use Perifosine as search term. Available from: http://clinicaltrials.gov/ct2/results?term=Perifosine (Accessed September 29, 2010).

[11] Clinicaltrials.gov. Search for clinical trials. Use MK-2206 as search item. Available from: http://clinicaltrials.gov/ct2/results?term=MK-2206 (Accessed September 29, 2010).

[12] Clinicaltrials.gov. Search for clinical trials. Use RX-0201 as search item. Available from: http://clinicaltrials.gov/ct2/results?term=RX-0201 (Accessed September 29, 2010).

[13] Clinicaltrials.gov. Search for clinical trials. Use GSK690693 as search item. Available from: http://clinicaltrials.gov/ct2/results?term=GSK690693 (Accessed September 29, 2010).

[14] Crouthamel, M.C.; Kahana, J.A.; Korenchuk, S.; Zhang, S.Y.; Sundaresan, G.; Eberwein, D.J.; Brown, K.K.; Kumar, R. Mechanism and management of AKT inhibitor-induced hyperglycemia. *Clin. Cancer Res.*, **2009**, *15*, 217-25.

[15] Clinicaltrials.gov. Search for clinical trials. Use GSK2141795 as search item. Available from: http://clinicaltrials.gov/ct2/results?term=GSK2141795 (Accessed September 29, 2010).

[16] Clinicaltrials.gov. Search for clinical trials. Use XL-418 as search item. Available from: http://clinicaltrials.gov/ct2/results?term=XL-418 (Accessed September 29, 2010).

[17] Davies, T.G.; Verdonk, M.L.; Graham, B.; Saalau-Bethell, S.; Hamlett, C.C.F.; McHardy, T.; Collins, I.; Garrett, M.D.; Workman, P.; Woodhead, S.J.; Jhoti, H.; Barford, D. A structural comparison of inhibitor binding to PKB, PKA and PKA-PKB chimera. *J. Mo. Biol.*, **2007**, *367*, 882-94.

[18] Pearce, L.R.; Komander, D.; Alessi, D.R. The nuts and bolts of AGC protein kinases. *Nat. Rev. Mol. Cell Biol.*, **2010**, *11*, 9-22.

[19] Reuveni, H.; Livnah, N.; Geiger, T.; Kleid, S.; Ohne, O.; Cohen, I.; Benhar, M.; Gellerman, G.; Levitzki, A. Toward a PKB inhibitor: Modification of a selective PKA inhibitor by rational design. *Biochemistry*, **2002**, *41*, 10304-14.

[20] Collins, I.; Caldwell, J.; Fonseca, T.; Donald, A.; Bavetsias, V.; Hunter, L.J.; Garrett, M.D.; Rowlands, M.G.; Aherne, G.W.; Davies, T.G.; Berdini, V.; Woodhead, S.J.; Davis, D.; Seavers, L.C.; Wyatt, P.G.; Workman, P.; McDonald, E. Structure-based design of isoquinoline-5-sulfonamide inhibitors of protein kinase B. *Bioorg. Med. Chem.*, **2006**, *14*, 1255-73.

[21] Saxty, G.; Woodhead, S.J.; Berdini, V.; Davies, T.G.; Verdonk, M.L.; Wyatt, P.G.; Boyle, R.G.; Barford, D.; Downham, R.; Garrett, M.D.; Carr, R.A. Identification of inhibitors of protein kinase B using fragment-based lead discovery. *J. Med. Chem.*, **2007**, *50*, 2293-6.

[22] Yang, J.; Cron, P.; Good, V.M.; Thompson, V.; Hemmings, B.A.; Barford, D. Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP. *Nat. Struct. Biol.*, **2002**, *9*, 940-4.

[23] Chen, Y.L.; Law, P.Y.; Loh, H.H. Inhibition of PI3K/Akt signaling: an emerging paradigm for targeted cancer therapy. *Curr. Med. Chem. Anticancer Agents*, **2005**, *5*, 575-89.

[24] Barnett, S.F.; Bilodeau, M.T.; Lindsley, C.W. The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation. *Curr. Top. Med. Chem.*, **2005**, *5*, 109-25.

[25] Hanada, M.; Feng, J.; Hemmings, B.A. Structure, regulation and function of PKB/AKT--a major therapeutic target. *Biochim. Biophys. Acta*, **2004**, *1697*, 3-16.

[26] Liao, J.J. Molecular recognition of protein kinase binding pockets for design of potent and selective kinase inhibitors. *J. Med. Chem.*, **2007**, *50*, 409-24.

[27] Donald, A.; McHardy, T.; Rowlands, M.G.; Hunter, L.J.; Davies, T.G.; Berdini, V.; Boyle, R.G.; Aherne, G.W.; Garrett, M.D.; Collins, I. Rapid evolution of 6-phenylpurine inhibitors of protein kinase B through structure-based design. *J. Med. Chem.*, **2007**, *50*, 2289-92.

[28] Lindsley, C.W.; Barnett, S.F.; Yaroschak, M.; Bilodeau, M.T.; Layton, M.E. Recent progress in the development of ATP-competitive and allosteric Akt kinase inhibitors. *Curr. Top. Med. Chem.*, **2007**, *7*, 1349-63.

[29] Lindsley, C.W.; Barnett, S.F.; Layton, M.E.; Bilodeau, M.T. The PI3K/Akt pathway: recent progress in the development of ATP-competitive and allosteric Akt kinase inhibitors. *Curr. Cancer Drug Targets*, **2008**, *8*, 7-18.

[30] Cheng, G.Z.; Park, S.; Shu, S.; He, L.; Kong, W.; Zhang, W.; Yuan, Z.; Wang, L.H.; Cheng, J.Q. Advances of AKT pathway in human oncogenesis and as a target for anti-cancer drug discovery. *Curr. Cancer Drug Targets*, **2008**, *8*, 2-6.

[31] Lindsley, C.W. The Akt/PKB Family of Protein Kinases: A Review of Small Molecule Inhibitors and Progress Towards Target Validation: A 2009 Update. *Curr. Top. Med. Chem.*, **2010**, *10*, 458-77.

[32] Kawauchi, K.; Ogasawara, T.; Yasuyama, M.; Otsuka, K.; Yamada, O. The PI3K/Akt Pathway as a Target in the Treatment of Hematologic Malignancies. *Anti-Cancer Agents Med. Chem.*, **2009**, *9*, 550-9.

[33] Martelli, A.M.; Tabellini, G.; Bortul, R.; Nyakern, M.; Tazzari, P.L.; Evangelisti, C.; Cocco, L. The phosphoinositide 3-kinase (PI3K)/AKT signaling pathway as a therapeutic target for the treatment of human acute myeloid leukemia (AML). *Curr. Signal Transduct. Ther.*, **2007**, *2*, 246-56.

[34] Dos Santos, C.; Recher, C.; Demur, C.; Payrastre, B. The PI3K/Akt/mTOR pathway: a new therapeutic target in the treatment of acute myeloid leukemia. *Bull. Cancer*, **2006**, *93*, 445-7.

[35] West, K.A.; Castillo, S.S.; Dennis, P.A. Activation of the PI3K/Akt pathway and chemotherapeutic resistance. *Drug Resist. Updat*, **2002**, *5*, 234-48.

[36] Rong, S.B.; Hu, Y.; Enyedy, I.; Powis, G.; Meuillet, E.J.; Wu, X.; Wang, R.; Wang, S.; Kozikowski, A.P. Molecular modeling studies of the Akt PH domain and its interaction with phosphoinositides. *J. Med. Chem.*, **2001**, *44*, 898-908.

[37] Sarbassov, D.D.; Guertin, D.A.; Ali, S.M.; Sabatini, D.M. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, **2005**, *307*, 1098-101.

[38] Bayascas, J.R.; Alessi, D.R. Regulation of Akt/PKB Ser473 phosphorylation. *Mol. Cell*, **2005**, *18*, 143-5.

[39] Okuzumi, T.; Fiedler, D.; Zhang, C.; Gray, D.C.; Aizenstein, B.; Hoffman, R.; Shokat, K.M. Inhibitor hijacking of Akt activation. *Nat. Chem. Biol.*, **2009**, *5*, 484-93.

[40] Wang, R.W.; Brattain, M.G. AKT can be activated in the nucleus. *Cell. Signal.*, **2006**, *18*, 1722-31.

[41] Huang, H.J.; Tindall, D.J. Dynamic FoxO transcription factors. *J. Cell Sci.*, **2007**, *120*, 2479-87.

[42] Cully, M.; You, H.; Levine, A.J.; Mak, T.W. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat. Rev. Cancer*, **2006**, *6*, 184-92.

[43] Datta, S.R.; Dudek, H.; Tao, X.; Masters, S.; Fu, H.; Gotoh, Y.; Greenberg, M.E. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. *Cell*, **1997**, *91*, 231-41.

[44] Ichijo, H.; Nishida, E.; Irie, K.; ten Dijke, P.; Saitoh, M.; Moriguchi, T.; Takagi, M.; Matsumoto, K.; Miyazono, K.; Gotoh, Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science*, **1997**, *275*, 90-4.

[45] Pan, J.J.; Chang, Q.S.; Wang, X.; Son, Y.; Zhang, Z.; Chen, G.; Luo, J.; Bi, Y.Y.; Chen, F.; Shi, X.L. Reactive Oxygen Species-Activated Akt/ASK1/p38 Signaling Pathway in Nickel Compound-Induced Apoptosis in BEAS 2B Cells. *Chem. Res. Toxicol.*, **2010**, *23*, 568-77.

[46] Li, J.; Yuan, J. Caspases in apoptosis and beyond. *Oncogene*, **2008**, *27*, 6194-206.

[47] Cardone, M.H.; Roy, N.; Stennicke, H.R.; Salvesen, G.S.; Franke, T.F.; Stanbridge, E.; Frisch, S.; Reed, J.C. Regulation of cell death protease caspase-9 by phosphorylation. *Science*, **1998**, *282*, 1318-21.

[48] Cantarelli, B.; Duca, L.; Blanchevoye, C.; Poitevin, S.; Martiny, L.; Debelle, L. Elastin peptides antagonize ceramide-induced apoptosis. *FEBS Lett.*, **2009**, *583*, 2385-91.

[49] Zheng, T.S.; Meng, X.Z.; Wang, J.B.; Chen, X.; Yin, D.L.; Liang, Y.J.; Song, X.A.; Pan, S.H.; Jiang, H.C.; Liu, L.X. PTEN- and p53-Mediated Apoptosis and Cell Cycle Arrest by FTY720 in Gastric Cancer Cells and Nude Mice. *J. Cell. Biochem.*, **2010**, *111*, 218-28.

[50] Qu, Y.; Wang, J.H.; Sim, M.S.; Liu, B.Y.; Giuliano, A.; Barsoum, J.; Cui, X.J. Elesclomol, counteracted by Akt survival signaling, enhances the apoptotic effect of chemotherapy drugs in breast cancer cells. *Breast Cancer Res. Treat.*, **2010**, *121*, 311-21.

[51] Doble, B.W.; Woodgett, J.R. GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.*, **2003**, *116*, 1175-86.

[52] Frame, S.; Cohen, P. GSK3 takes centre stage more than 20 years after its discovery. *Biochemical Journal*, **2001**, *359*, 1-16.

[53] Grimes, C.A.; Jope, R.S. The multifaceted roles of glycogen synthase kinase 3 beta in cellular signaling. *Prog. Neurobiol.*, **2001**, *65*, 391-426.

[54] Li, Y.-C.; Gao, W.-J. GSK-3β activity and hyperdopamine-dependent behaviors. *Neurosci. Biobehav. Rev.*, **2010**, *35*, 645-54.

[55] Martelli, A.M.; Evangelisti, C.; Chiarini, F.; Grimaldi, C.; Cappellini, A.; Ognibene, A.; McCubrey, J.A. The emerging role of the phosphatidylinositol 3-kinase/Akt/mammalian target of rapamycin signaling network in normal myelopoiesis and leukemogenesis. *Biochim Biophys Acta*, **2010**, *1803*, 991-1002.

[56] Tamburini, J.; Green, A.S.; Chapuis, N.; Bardet, V.; Lacombe, C.; Mayeux, P.; Bouscary, D. Targeting translation in acute myeloid leukemia A new paradigm for therapy? *Cell Cycle*, **2009**, *8*, 3893-9.

[57] Bai, X.C.; Jiang, Y. Key factors in mTOR regulation. *Cell. Mol. Life Sci.*, **2010**, *67*, 239-53.

[58] Salmena, L.; Carracedo, A.; Pandolfi, P.P. Tenets of PTEN tumor suppression. *Cell*, **2008**, *133*, 403-14.

[59] Stocker, H.; Andjelkovic, M.; Oldham, S.; Laffargue, M.; Wymann, M.P.; Hemmings, B.A.; Hafen, E. Living with lethal PIP3 levels: Viability of flies lacking PTEN restored by a PH domain mutation in Akt/PKB. *Science*, **2002**, *295*, 2088-91.

[60] Stambolic, V.; Suzuki, A.; de la Pompa, J.L.; Brothers, G.M.; Mirtsos, C.; Sasaki, T.; Ruland, J.; Penninger, J.M.; Siderovski, D.P.; Mak, T.W. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. *Cell*, **1998**, *95*, 29-39.

[61] Hou, P.; Ji, M.J.; Xing, M.Z. Association of PTEN Gene Methylation With Genetic Alterations in the Phosphatidylinositol 3-Kinase/AKT Signaling Pathway in Thyroid Tumors. *Cancer*, **2008**, *113*, 2440-7.

[62] Fine, B.; Hodakoski, C.; Koujak, S.; Su, T.; Saal, L.H.; Maurer, M.; Hopkins, B.; Keniry, M.; Sulis, M.L.; Mense, S.; Hibshoosh, H.; Parsons, R. Activation of the PI3K Pathway in Cancer Through Inhibition of PTEN by Exchange Factor P-REX2a. *Science*, **2009**, *325*, 1261-5.

[63] Suwa, A.; Kurama, T.; Shimokawa, T. SHIP2 and its involvement in various diseases. *Expert Opin. Ther. Targets*, **2010**, *14*, 727-37.

[64] Kalesnikoff, J.; Sly, L.M.; Hughes, M.R.; Büchse, T.; Rauh, M.J.; Cao, L.P.; Lam, V.; Mui, A.; Huber, M.; Krystal, G. The role of SHIP in cytokine-induced signaling. *Rev. Physiol., Biochem. Pharmacol.*, **2003**, *149*, 87-103.

[65] Mistafa, O.; Ghalali, A.; Kadekar, S.; Hogberg, J.; Stenius, U. Purinergic Receptor-mediated Rapid Depletion of Nuclear Phosphorylated Akt Depends on Pleckstrin Homology Domain Leucine-rich Repeat Phosphatase, Calcineurin, Protein Phosphatase 2A, and PTEN Phosphatases. *J. Biol. Chem.*, **2010**, *285*, 27900-10.

[66] Brognard, J.; Newton, A.C. PHUPPing the switch on Akt and protein kinase C signaling. *Trends Endocrinol. Metab.*, **2008**, *19*, 223-30.

[67] Heerding, D.A.; Rhodes, N.; Leber, J.D.; Clark, T.J.; Keenan, R.M.; Lafrance, L.V.; Li, M.; Safonov, I.G.; Takata, D.T.; Venslavsky, J.W.; Yamashita, D.S.; Choudhry, A.E.; Copeland, R.A.; Lai, Z.; Schaber, M.D.; Tummino, P.J.; Strum, S.L.; Wood, E.R.; Duckett, D.R.; Eberwein, D.; Knick, V.B.; Lansing, T.J.; McConnell, R.T.; Zhang, S.; Minthorn, E.A.; Concha, N.O.; Warren, G.L.; Kumar, R. Identification of 4-(2-(4-amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]o xy}-1H-imidazo[4,5-c]pyridin-4-yl)-2-methyl-3-butyn-2-ol (GSK690693), a novel inhibitor of AKT kinase. *J. Med. Chem.*, **2008**, *51*, 5663-79.

[68] Rhodes, N.; Heerding, D.A.; Duckett, D.R.; Eberwein, D.J.; Knick, V.B.; Lansing, T.J.; McConnell, R.T.; Gilmer, T.M.; Zhang, S.Y.; Robell, K.; Kahana, J.A.; Geske, R.S.; Kleymenova, E.V.; Choudhry, A.E.; Lai, Z.; Leber, J.D.; Minthorn, E.A.; Strum, S.L.; Wood, E.R.; Huang, P.S.; Copeland, R.A.; Kumar, R. Characterization of an Akt Kinase Inhibitor with Potent Pharmacodynamic and Antitumor Activity. *Cancer Res.*, **2008**, *68*, 2366-74.

[69] Levy, D.S.; Kahana, J.A.; Kumar, R. AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines. *Blood*, **2009**, *113*, 1723-9.

[70] Rouse, M. Aminofurazans as potent inhibitors of AKT kinase. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 1508-11.

[71] Zhu, G.D.; Gandhi, V.B.; Gong, J.; Thomas, S.; Woods, K.W.; Song, X.; Li, T.; Diebold, R.B.; Luo, Y.; Liu, X.; Guan, R.; Klinghofer, V.; Johnson, E.F.; Bouska, J.; Olson, A.; Marsh, K.C.; Stoll, V.S.; Mamo, M.; Polakowski, J.; Campbell, T.J.; Martin, R.L.; Gintant, G.A.; Penning, T.D.; Li, Q.; Rosenberg, S.H.; Giranda, V.L. Syntheses of potent, selective, and orally bioavailable indazole-pyridine series of protein kinase B/Akt inhibitors with reduced hypotension. *J. Med. Chem.*, **2007**, *50*, 2990-3003.

[72] Luo, Y.; Shoemaker, A.R.; Liu, X.; Woods, K.W.; Thomas, S.A.; de Jong, R.; Han, E.K.; Li, T.; Stoll, V.S.; Powlas, J.A.; Oleksijew, A.; Mitten, M.J.; Shi, Y.; Guan, R.; McGonigal, T.P.; Klinghofer, V.; Johnson, E.F.; Leverson, J.D.; Bouska, J.J.; Mamo, M.; Smith, R.A.; Gramling-Evans, E.E.; Zinker, B.A.; Mika, A.K.; Nguyen, P.T.; Oltersdorf, T.; Rosenberg, S.H.; Li, Q.; Giranda, V.L. Potent and selective inhibitors of Akt kinases slow the progress of tumors *in vivo*. *Mol. Cancer Ther.*, **2005**, *4*, 977-86.

[73] Li, Q.; Li, T.; Zhu, G.-D.; Gong, J.; Claibone, A.; Dalton, C.; Luo, Y.; Johnson, E.F.; Shi, Y.; Liu, X. Discovery of trans-3,4′-bispyridinylethylenes as potent and novel inhibitors of protein kinase B (PKB/Akt) for the treatment of cancer: Synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 1679-85.

[74] Li, Q.; Woods, K.W.; Thomas, S.; Zhu, G.-D.; Packard, G.; Fisher, J.; Li, T.; Gong, J.; Dinges, J.; Song, X. Synthesis and structure–activity relationship of 3,4′-bispyridinylethylenes: Discovery of a potent 3-isoquinolinylpyridine inhibitor of protein kinase B (PKB/Akt) for the treatment of cancer. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 2000-7.

[75] Zhu, G.-D.; Gong, J.; Claiborne, A.; Woods, K.W.; Gandhi, V.B.; Thomas, S.; Luo, Y.; Liu, X.; Shi, Y.; Guan, R. Isoquinoline–pyridine-based protein kinase B/Akt antagonists: SAR and *in vivo* antitumor activity. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3150-5.

[76] Woods, K.W.; Fischer, J.P.; Claiborne, A.; Li, T.; Thomas, S.A.; Zhu, G.-D.; Diebold, R.B.; Liu, X.; Shi, Y.; Klinghofer, V. Synthesis and SAR of indazole-pyridine based protein kinase B/Akt inhibitors. *Bioorg. Med. Chem.*, **2006**, *14*, 6832-46.

[77] Zhu, G.-D.; Gandhi, V.B.; Gong, J.; Luo, Y.; Liu, X.; Shi, Y.; Guan, R.; Magnone, S.R.; Klinghofer, V.; Johnson, E.F. Discovery and SAR of oxindole–pyridine-based protein kinase B/Akt inhibitors for treating cancers. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3424-9.

[78] Zhu, G.-D.; Gong, J.; Gandhi, V.B.; Woods, K.; Luo, Y.; Liu, X.; Guan, R.; Klinghofer, V.; Johnson, E.F.; Stoll, V.S. Design and synthesis of pyridine–pyrazolopyridine-based inhibitors of protein kinase B/Akt. *Bioorg. Med. Chem.*, **2007**, *15*, 2441-52.

[79] Thomas, S.A.; Li, T.; Woods, K.W.; Song, X.; Packard, G.; Fischer, J.P.; Diebold, R.B.; Liu, X.; Shi, Y.; Klinghofer, V. Identification of a novel 3,5-disubstituted pyridine as a potent, selective, and orally active inhibitor of Akt1 kinase. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 3740-4.

[80] Lin, H.; Yamashita, D.S.; Zeng, J.; Xie, R.; Wang, W.; Nidarmarthy, S.; Luengo, J.I.; Rhodes, N.; Knick, V.B.; Choudhry, A.E. 2,3,5-Trisubstituted pyridines as selective AKT inhibitors—Part I: Substitution at 2-position of the core pyridine for ROCK1 selectivity. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 673-8.

[81] Lin, H.; Yamashita, D.S.; Xie, R.; Zeng, J.; Wang, W.; Leber, J.; Safonov, I.G.; Verma, S.; Li, M.; LaFrance, L. Tetrasubstituted pyridines as potent and selective AKT inhibitors: Reduced CYP450 and hERG inhibition of aminopyridines. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 684-8.

[82] Lin, H.; Yamashita, D.S.; Zeng, J.; Xie, R.; Verma, S.; Luengo, J.I.; Rhodes, N.; Zhang, S.; Robell, K.A.; Choudhry, A.E. 2,3,5-Trisubstituted pyridines as selective AKT inhibitors. Part II: Improved drug-like properties and kinase selectivity from azaindazoles. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 679-83.

[83] Lin, X.; Murray, J.M.; Rico, A.C.; Wang, M.X.; Chu, D.T.; Zhou, Y.; Rosario, M.D.; Kaufman, S.; Ma, S.; Fang, E. Discovery of 2-pyrimidyl-5-amidothiophenes as potent inhibitors for AKT: Synthesis and SAR studies. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 4163-8.

[84] Seefeld, M.A.; Rouse, M.B.; McNulty, K.C.; Sun, L.; Wang, J.; Yamashita, D.S.; Luengo, J.I.; Zhang, S.; Minthorn, E.A.; Concha, N.O. Discovery of 5-pyrrolopyridinyl-2-thiophenecarboxamides as potent AKT kinase inhibitors. *Bioorg. Med. Chem. Lett.*, **2009**, *19*, 2244-8.

[85] Zeng, Q.; Bourbeau, M.P.; Wohlhieter, G.E.; Yao, G.; Monenschein, H.; Rider, J.T.; Lee, M.R.; Zhang, S.; Lofgren, J.; Freeman, D.; Li, C.; Tominey, E.; Huang, X.; Hoffman, D.; Yamane, H.; Tasker, A.S.; Dominguez, C.; Viswanadhan, V.N.; Hungate, R.; Zhang, X. 2-Aminothiadiazole inhibitors of AKT1 as potential cancer therapeutics. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 1652-6.

[86] Zeng, Q.; Allen, J.G.; Bourbeau, M.P.; Wang, X.; Yao, G.; Tadesse, S.; Rider, J.T.; Yuan, C.C.; Hong, F.T.; Lee, M.R.; Zhang, S.; Lofgren, J.A.; Freeman, D.J.; Yang, S.; Li, C.; Tominey, E.; Huang, X.; Hoffman, D.; Yamane, H.K.; Fotsch, C.; Dominguez, C.; Hungate, R.; Zhang, X. Azole-based inhibitors of AKT/PKB for the treatment of cancer. *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 1559-64.

[87] Subramanian, R.; Lee, M.R.; Allen, J.G.; Bourbeau, M.P.; Fotsch, C.; Hong, F.T.; Tadesse, S.; Yao, G.M.; Yuan, C.C.; Surapaneni, S.; Skiles, G.L.; Wang, X.H.; Wohlhieter, G.E.; Zeng, Q.P.; Zhou, Y.H.; Zhu, X.C.; Li, C. Cytochrome P450-Mediated Epoxidation of 2-Aminothiazole-Based AKT Inhibitors: Identification of Novel GSH Adducts and Reduction of Metabolic Activation through Structural Changes Guided by in Silico and *in vitro* Screening. *Chem. Res. Toxicol.*, **2010**, *23*, 653-63.

[88] Caldwell, J.J.; Davies, T.G.; Doiiald, A.R.R.; McHardy, T.; Rowlands, M.G.; Aherne, G.W.; HLinter, L.K.; Taylor, K.; Ruddle, R.; Raynaud, F.I.; Verdonk, M.; Workman, P.; Garrett, M.D.; Collins, I. Identification of 4-(4-aminopiperidin-1-yl)-7H-pyrrolo[2,3-d]pyrimidines as selective inhibitors of protein kinase B through fragment elaboration. *J. Med. Chem.*, **2008**, *51*, 2147-57.

[89] Donald, A.; McHardy, T.; Rowlands, M.G.; Hunter, L.J.K.; Davies, T.G.; Berdini, V.; Boyle, R.G.; Aherne, G.W.; Garrett, M.D.;

Collins, I. Rapid evolution of 6-phenylpurine inhibitors of protein kinase B through structure-based design. *J. Med. Chem.*, **2007**, *50*, 2289-92.

[90] McHardy, T.; Caldwell, J.J.; Cheung, K.M.; Hunter, L.J.; Taylor, K.; Rowlands, M.; Ruddle, R.; Henley, A.; de Haven Brandon, A.; Valenti, M.; Davies, T.G.; Fazal, L.; Seavers, L.; Raynaud, F.I.; Eccles, S.A.; Aherne, G.W.; Garrett, M.D.; Collins, I. Discovery of 4-amino-1-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)piperidine-4-carboxamides as selective, orally active inhibitors of protein kinase B (Akt). *J. Med. Chem.*, **2010**, *53*, 2239-49.

[91] Lippa, B.; Pan, G.; Corbett, M.; Li, C.; Kauffman, G.S.; Pandit, J.; Robinson, S.; Wei, L.; Kozina, E.; Marr, E.S. Synthesis and structure based optimization of novel Akt inhibitors. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 3359-63.

[92] Freeman-Cook, K.D.; Autry, C.; Borzillo, G.; Gordon, D.; Barbacci-Tobin, E.; Bernardo, V.; Briere, D.; Clark, T.; Corbett, M.; Jakubczak, J.; Kakar, S.; Knauth, E.; Lippa, B.; Luzzio, M.J.; Mansour, M.; Martinelli, G.; Marx, M.; Nelson, K.; Pandit, J.; Rajamohan, F.; Robinson, S.; Subramanyam, C.; Wei, L.; Wythes, M.; Morris, J. Design of Selective, ATP-Competitive Inhibitors of Akt. *J. Med. Chem.*, **2010**, *53*, 4615-22.

[93] Breitenlechner, C.B.; Friebe, W.G.; Brunet, E.; Werner, G.; Graul, K.; Thomas, U.; Kunkele, K.P.; Schafer, W.; Gassel, M.; Bossemeyer, D.; Huber, R.; Engh, R.A.; Masjost, B. Design and crystal structures of protein kinase B-selective inhibitors in complex with protein kinase A and mutants. *J. Med. Chem.*, **2005**, *48*, 163-70.

Received: March 13, 2011 Revised: May 30, 2011 Accepted: June 25, 2011