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Discovery of new azaindole-based PI3Kα inhibitors: Apoptotic and antiangiogenic effect on cancer cells

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ABSTRACT

Phosphatidylinositol-3-kinase alpha (PI3Kα) is an important target in cancer due to the deregulation of the PI3K/AKT signaling pathway in many tumors. In this study, we designed [3,5-*d*]-7-azaindole analogs as PI3Kα inhibitors through the fragment-growing strategy. By varying groups at the 3,5-positions of azaindole, we developed the SAR (Structure–activity relationship) and identified a series of potent PI3Kα inhibitors. Representative azaindole derivatives showed activity in a cellular proliferation and apoptosis assays. Moreover, **B3** exhibited strong antiangiogenic effects on cancer cells.

Solid tumors contain hypoxic regions that have considerably lower oxygen tension than the normal tissues. The transcription factor hypoxia-inducible factor 1 (HIF-1) is a major regulator of tumor cell adaptation to hypoxic stress. In addition to HIF-1 association in tumor progression, HIF-1 has been implicated in the upregulation of genes involved in angiogenesis including vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (NOS) and heme oxygenase-1. Angiogenesis is an essential process for tumor growth and metastasis, and VEGF is a chemical signal stimulates the growth of new blood vessels from pre-existing vessels. Recently, numerous studies have shown that PI3K/Akt pathway plays critical roles in angiogenesis and expression of HIF-1α and VEGF.[1]

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that catalyze phosphorylation of the 3-hydroxyl position of PIP2 (phosphatidylinositol 4,5-diphosphate) to PIP3 (phosphatidylinositol 3,4,5-triphosphate). The resulting second messenger, PIP3 can regulate multiple physiological processes, including cell growth, differentiation, survival, and motility.[2] Deregulation of PI3K pathway leads to elevated PIP3 levels and downstream activation of Akt, which might be involved in the pathology of cancer, inflammation, immune disorders, and cardiovascular diseases.[3] In particular, the *PIK3CA* gene that encodes p110a catalytic subunit is frequently mutated and overexpressed in a range of primary cancers.[4]

Together, these observations strongly suggest that PI3K pathway is among the most commonly activated signaling pathway in cancer and the inhibition of the PI3K/Akt pathway (PI3K/Akt, HIF-1α, VEGF) may lead to greater therapeutic potential. Herein, we report the identification of new azaindole-based PI3K inhibitors that demonstrate apoptotic and antiangiogenic effects on cancer cell lines.

Fragment-based growing strategy,[5] which creates novel structures by adding interacting moieties to a starting scaffold, is an alternative approach to high-throughput screening for the identification of active compounds for therapeutic targets. For inhibiting kinases, the most exemplified approach targets the kinase domain by competitive inhibition of the ATP binding site. Some heterocyclic fragments mimicking the pyrimidine portion of ATP can bind to the hinge region of the kinase via hydrogen bonds with the backbone and represent start points for a fragment-growing approach. As substantial structural and biochemical information is available, PI3 kinases may represent one of good targets for structure-based drug design to facilitate the discovery of new potent inhibitors. With the goal of discovery of a new structural class of inhibitors PI3K, we initiated a pharmacophore-directed design. Among many hetero-aromatics, we selected 7-azaindole as a scaffold for a PI3K hinge region binding moiety. 7-Azaindole possesses both H-donor and H-acceptor for interaction with the hinge region of the kinase[6] and also has a strong advantage in that it can be easily diversified in 3 and 5-positions via simple organic reactions. To assess back pocket (DFG-motif, gate keeper and catalytic lysine), we thought to take advantage of the information known in the literature[7] and combine a suitable pharmacophore

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to yield novel molecules with potent activity. In fact, a number of inhibitors show various moieties which can interact in this back pocket and the pyridyl sulfonamide group profoundly influenced the activity of PI3K inhibitors by binding this region.[7f] Thus, our design strategy prioritized the incorporation of the pyridyl sulfonamide pharmacophore into azaindole at C5 to generate novel hybrid molecules with the expectation of combining high affinity for PI3 kinases (Fig. 1).

The general synthetic route for the preparation of substituted azaindole derivatives is shown in Scheme 1. In all cases, the C3 position of the azaindole was functionalized prior to performing a Susuki coupling with the C5 aryl bromide. The starting material bromo-azaindole (**1**) was treated with *N*-iodosuccinimide in acetone room temperature for 2 h to give the corresponding 5-bromo-3-iodo-azaindole. After protection of NH group with benzenesulfonylcholoride, various aryl groups were attached to the 3-position of the azaindole core using palladium-mediated cross-coupling, and benzenesulfonyl group was then deprotected to yield the desired product **3**. To facilitate the exploration of C5 position, compound **3** underwent another palladium catalyzed Suzuki coupling with different boronic acid derivatives. Finally, the various sulfonyl groups were introduced to build the target products, by treatment with arylsulfonylcholoride and pyridine at room temperature. To build acetyl group at C3 position, Friedel–Crafts acetylation of **1** with aluminium chloride and acetyl chloride provided intermediate **5** as a white solid in 98% yield. The aryl group at C5 was then installed by Suzuki coupling in a similar manner.

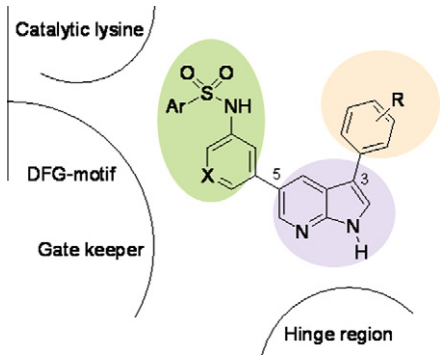
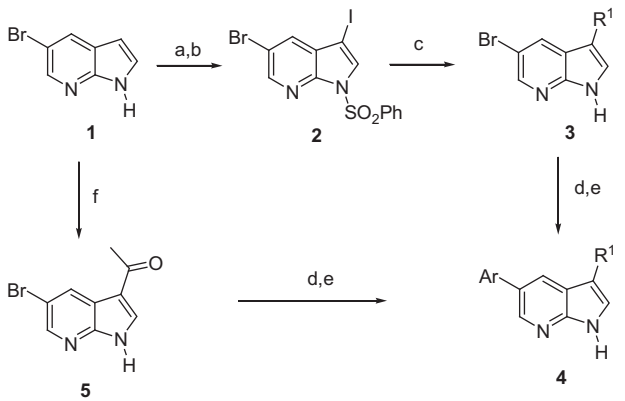


Figure 1. Design of an azaindole scaffold as PI3K inhibitor and opportunities for modification.



Scheme 1. Reagents and conditions: (a) NIS, acetone, rt, 2 h; (b) benzenesulfonylchloride, 50% NaOH, Bu_4NHSO_4, DCM, rt, 12 h; (c) aryl boronic acid, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/H_2O = 3:1, 80 °C, 3 h, then 4 N KOH, MeOH, rt, 1 h; (d) aryl boronic acid, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/H_2O = 3:1, 100 °C, 5 h; (e) arylsulfonylchloride, pyridine, rt, 12 h; (f) AcCl, $AlCl_3$, DCM, rt, 6 h.

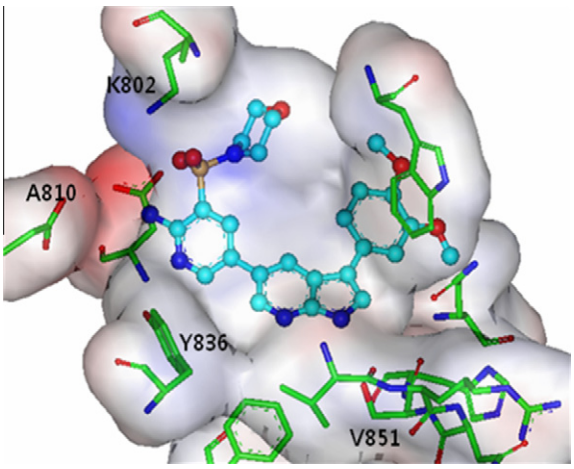
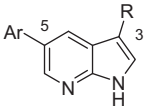


Figure 2. Putative binding mode of **B3** with the PI3Kα homology model, based on PI3Kγ crystal structures. In this model, the azaindole forms a key hinge region hydrogen bond with the backbone of Val851, and the sulfonamide bind to the catalytic lysine (Lys802). Selected residues (A810 and Y836) are shown for possible interaction with pyridyl sulfonamide subunit.

In the resulting compound **A1**, an pyridyl sulfonamide was grown from the C5 position of the azaindole to enalble into a hydrophobic region in the back pocket of the enzyme. Compound

Table 1
Inhibition of PI3Kα of selected azaindole derivatives: structure–activity relationship with various Ar and R groups



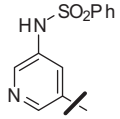
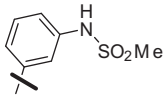
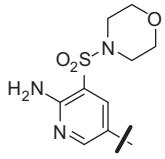
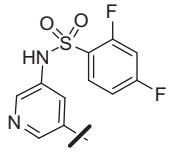
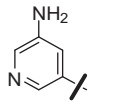
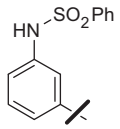
Compd	R^1	R^2	PI3Kα IC_{50} (μM)
A1		H	5.2
A2		Acetyl	0.62
A3		3-Cyanophenyl	0.031
A4		3-Pyridyl	0.012
A5		3,4-Dimethoxyphenyl	0.012
A6			0.95
B1		Acetyl	0.73
B2		3-Cyanophenyl	0.13
B3		3,4-Dimethoxyphenyl	0.016
C1		3-Cyanophenyl	0.027
C2		3,4-Dimethoxyphenyl	0.003
D1		3,4-Dimethoxyphenyl	0.46
E1		3,4-Dimethoxyphenyl	1.6

Table 2
Cancer cell proliferation of selected azaindole derivatives

Compd	Growth IC_{50} (μM)		
	T47D	SK-BR3	MCF7
A4	3.6	12	6.8
A5	8.5	22	–
B3	0.7	3.7	–

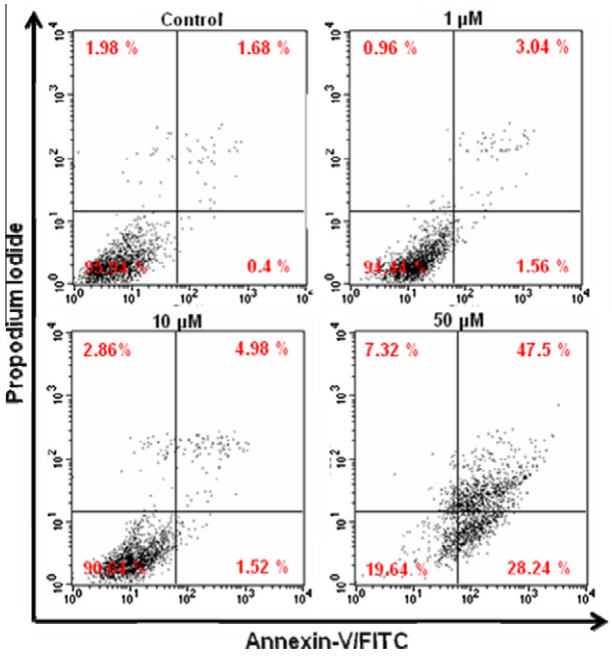


Figure 3. **B3** promotes early-apoptosis of T47D breast cancer cells. T47D cells were treated with various concentrations of **B3** for 24 h and apoptosis was determined by flow cytometric analysis using Annexin V-FITC and PI staining.

A1 had an IC_{50} of 5.2 μM for inhibition of PI3Kα. Analysis of the docking model[8] reveals that the azaindole anlogues maintains the hydrogen-bonding pattern the hinge (Val851), while the pyridyl sulfonamide group is lined by Y836 and A810 (Fig. 2). Building upon the success observed with pyridyl sulfonamide group, an investigation of the effect of the appendage at C3 position was conducted. Profound effects on potency were achieved with compounds incorporating the C3-aryl group, and **A3** was the earliest example in the azaindole series to display potency over PI3Kα enzyme at double-digit nanomolar concentration (IC_{50} = 31 nM). The significant increase in activity afforded by C3-aryl group prompted further investigation through synthesis of a series of related analogs. Some heteroaromatic groups were well tolerated, and in pyridyl analog **A4** modest increase in activity was observed. The presence of dimethoxyl groups resulted in compounds which were generally of equivalent or better enzyme potency than the corresponding unsubstituted derivatives, and possesses good activity in a cellular proliferation assay. In the next design cycle, we examined the structure–activity relationship (SAR) of the sulfonamide moiety, and the highest PI3Kα potency was obtained with 2,4-diF substituted phenyl groups (IC_{50} of **C2** = 3 nM). After mixing and matching, reversal of the sulfonamide connectivity and addition of morpholine led to **B3** which showed increased solubility and provided an advantage in terms of cellular activity. The pyridyl moiety at the 5 position was found to be essential for enhanced potency, and replacement of pyridyl group with phenyl group resulted in a significant loss of activity suggesting that it was deemed important to preserve the intermolecular hydrogen bonds that the pyridyl nitrogen makes with a conserved active site water molecule[7f] (Table 1).

Given the impressive enzyme activity profile, several compounds from this series were further tested for cellular proliferation activity against cancer cell lines. To measure the inhibitory effect of compounds on cell growth, cell viability was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

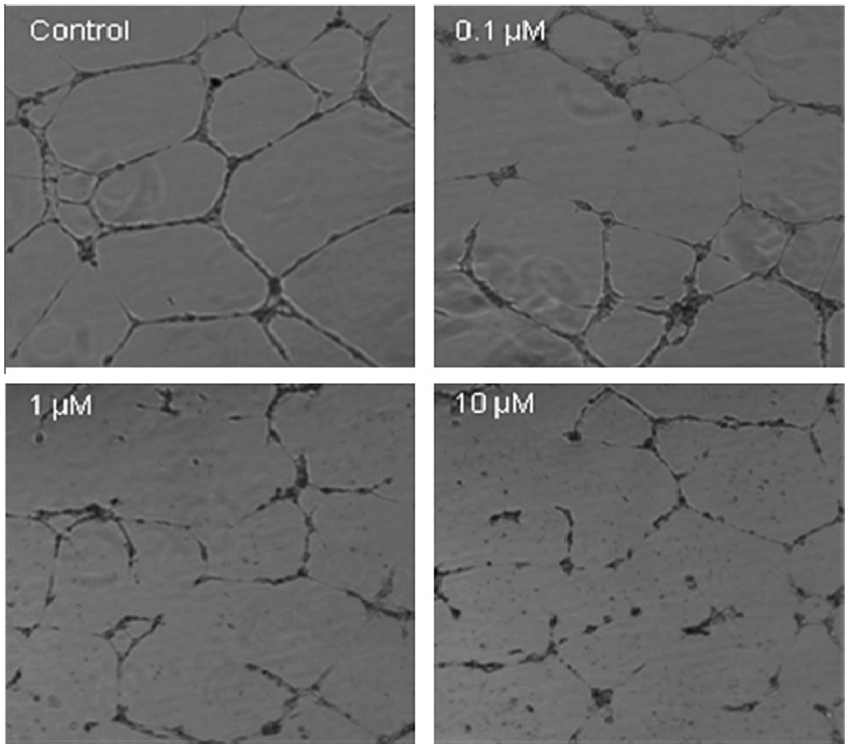


Figure 4. Representative images depicting the formation of capillary-like tubular network formation of HUVECs by treatment with DMSO (control) and **B3** (0.1 μM, 1 μM and 10 μM).

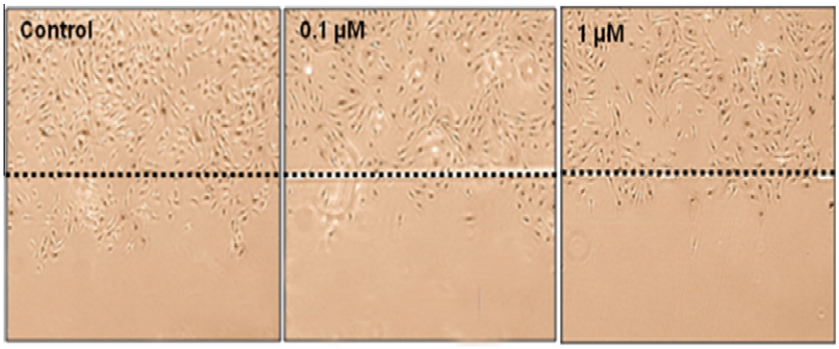


Figure 5. Inhibition of HUVECs migration by **B3** in a wound-induced migration assay. Migration was quantified by counting the number of cells that moved beyond the reference line.

assay in T47D, SK-BR3, and MCF-7 human breast cancer cell cultures. Notably, **B3** showed potent antiproliferative effects at submicromolar concentration (IC_{50} = 0.7 μM) as shown in Table 2.

Activation of the PI3K pathway leads to phosphorylation of Thr^{308} and Ser^{473} of AKT and subsequently a number of downstream substrates, such as IKK, GSK and Caspase-9. To ensure that new series of compounds were inhibiting PI3K signaling in cells, the most active compound based on growth assays, **B3** was further profiled for its ability to suppress cellular biomarker. **B3** demonstrated the ability to inhibit *p*-AKT (at Ser^{473}) in T47D cancer cells and promotes apoptosis of cancer cells, inducing cleaved-PARP (cPARP) as a marker of apoptosis (data not shown). The percentage of early-apoptotic cells was measured by the percentage of Annexin V-positive/PI-negative cells after incubation with various concentration of **B3** for 24 h. Exposure to 50 μM of **B3** resulted in a 28.4% increase in early-apoptotic cells (Annexin V-positive/PI-negative), a 47.5% increase in late-apoptotic cells after 24 h (Fig. 3).

Next, the antiangiogenic activity[1] of the azaindole PI3Kα inhibitor was evaluated using assays for a wound induced migration of human umbilical vein endothelial cells (HUVEC) and tubular formation.[9] While HUVECs were plated onto Matrigel where they aligned with one another and formed tubes resembling a capillary plexus, **B3** produced significant inhibition of tubular network formation of HUVECs on Matrigel beds in a dose-dependent manner (Fig. 4).

Cell migration is important for angiogenesis and was examined whether azaindole analog controls HUVEC motility using a wound migration assay.[10] Cell migration was quantified by counting the number of HUVECs that migrated into the non-wounded region. As shown in Figure 5, **B3** reduced the migration of HUVECs in a dose-dependent manner. These results together suggest that **B3** represses angiogenesis by inhibiting migration and tube formation of endothelial cells.

In conclusion, a novel series of azaindole-based PI3Kα inhibitors have been developed by the fragment-growing strategy. When incorporated with the pyridyl sulfonamide moiety, azaindole analogs show potency over PI3Kα. Introduction of the 3,4-dimethoxyphenyl group onto azaindole scaffold at C3 position enhanced solubility and potency in both enzyme and antiproliferative cellular assays. Through our fragment-based approach, we rapidly developed potent inhibitors, and **B3** demonstrates that inhibition of PI3K leads to blocking of phosphorylation of AKT and subsequent cancer cell growth. It also was found to inhibit tubular network formation and a wound-induced migration of HUVECs. These results clearly provide useful insight in the design of new inhibitors with more potent antiangiogenic and antiproliferative effects.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.108.

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