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Discovery of new aminopyrimidine-based phosphoinositide 3-kinase beta (PI3Kβ) inhibitors with selectivity over PI3Kα

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ABSTRACT

Phosphatidylinositol-3-kinase beta (PI3Kβ) is an important therapeutic target in arterial thrombosis and special types of cancer. In this study, a new series of aminopyridine-based PI3Kβ selective inhibitors have been developed by the structure-based design strategy. When incorporated with the phenyl ring on sulfonamide moiety, aminopyrimidine analogs showed good potency on PI3Kβ and selectivity over PI3Kα. Intriguingly, replacement of phenyl group on sulfonamide with naphthyl group enhanced selectivity over PI3Kα while retaining submicromolar PI3Kβ potency. Molecular modeling suggests that increased PI3Kβ specificity is caused by the interaction with salt bridge (Lys782–Asp923) and Asp862 that creat a unique pocket in PI3Kβ. These results clearly provide useful insight in the design of new PI3Kβ inhibitors with high potency and selectivity.

Phosphoinositide 3-kinases (PI3Ks) are a family of evolutionarily conserved lipid kinases that catalyze phosphorylation of the 3-hydroxyl position of the inositol ring of phosphatidylinositol 4,5-diphosphate (PIP2) to produce phosphatidylinositol 3,4,5-triphosphate (PIP3). The resulting second messenger, PIP3 mediates a variety of physiological processes, including cell growth, differentiation, survival, and motility.[1] The PI3K signaling pathway is negatively regulated by the lipid-phosphatase PTEN (phosphatase and tension homolog deleted on chromosome 10). Aberrant upregulation of the PI3K pathway leads to an extraordinarily elevated PIP3 level and downstream activation of Akt, which has been known to be responsible for cancer, inflammation, immune disorders, and cardiovascular diseases.[2]

Class I PI3Ks are subdivided into class IA and class IB based on different regulatory subunits and activation mechanisms. Class IA includes three isoforms, PI3Kα, β, and δ. Class IB PI3K contains only one isoform, PI3Kγ. PI3Kα and δ isoforms are activated by receptor tyrosine kinases/cytokine receptor activation, while PI3Kγ is activated by G protein βγ subunits (Gβγ), which are usually derived from G-protein-coupled receptors (GPCRs). In contrast, the PI3Kβ isoform is regulated by both receptor tyrosine kinases and by Gβγ-subunits. The p110α is reported to correlate most strongly with cancer progress because the p110α-encoding PIK3CA gene is overexpressed or frequently mutated in many human cancers.[3] The PI3Kδ and PI3Kγ isoforms are mainly restricted to hematopoietic systems and their impairment has been linked to immunodeficiency and inflammatory disorder. Among different subtype of PI3Ks, the p110β isoform potentiates integrin-mediated platelet aggregation and arterial thrombosis.[4] In addition, recent studies on genetically engineered mouse models and chemical inhibitors indicate that tumors driven by loss of PTEN may be more sensitive to inhibition of p110β rather than p110α.[5] Therefore, it would be beneficial to consider the generation of p110β selective compounds for special type of cancer or cardiovascular treatment in order to minimize the risk of potential toxicity and insulin resistance associated with the inhibition of PI3Kα. Although many PI3K inhibitors have been identified, only a few isoform-specific PI3Kβ inhibitors have been reported. Recently, patent disclosure has described TGX series as selective PI3Kβ inhibitors (patent WO 2004016607).[6] The structure of TGX-221 was modified based on structure and function analysis of LY294002 (Fig. 1). Here, we report the identification of a new series of aminopyrimidine-based PI3Kβ specific inhibitors.

The recent availability of the three-dimensional structure of therapeutic targets has enhanced opportunities for the rapid development of active compounds utilizing structure-based design. For

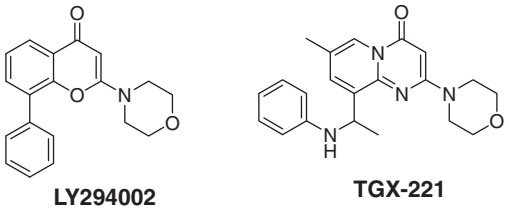

Figure 1. Structure of selective PI3Kβ inhibitor, TGX-221.

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inhibiting kinases, the most exemplified approach targets the kinase domain by competitive inhibition of the ATP binding site. Some heterocyclic fragments mimicking the purine portion of ATP can bind to the hinge region of the kinase via hydrogen bonds with the backbone. During the past decade, substantial structural and biochemical information on PI3Ks has greatly improved the potency and selectivity of PI3K inhibitors. Based on these premises, our aim was to identify new structural class of PI3Kβ selective inhibitors by a pharmacophore-directed design. First, we planned to identify the template of efficient PI3Kβ inhibitors, which would offer opportunities for future optimization of selectivity by exploiting differences in the conformational plasticity of PI3K isoforms. To assess the back pocket (DFG-motif, gate keeper and catalytic lysine), we took advantage of the information in the literature and combine a suitable pharmacophore to yield potent PI3Kβ inhibitor. Recently, it is reported that a number of PI3K inhibitors have pyridyl sulfonamides to enhance potency by filling this pocket.[7] While fixing a *N*-(pyridin-3-yl)benzenesulfonamide moiety, our initial round of analogues was focused around incorporation of a variety of heterocycles to generate potent PI3Kβ inhibitors (Fig. 2). With the identified PI3Kβ inhibitors, we planned to enhance selectivty by exploiting extended interactions with PI3Kβ unique residues (Lys782, Asp923, and Asp862) at the selectivity pocket.

The preparation of the target compounds is described in Scheme 1. The synthesis begins with treatment of the commercially available bromopyridine amine (**1**) with benzene sulfonyl chloride in CH_2Cl_2, followed by borylation with bis(pinacolato) diboron to afford the corresponding boronic ester **2**. Finally, various heteroaryl groups were then attached to the pyridyl sulfonyl moiety using a palladium-catalyzed cross-coupling to furnish the target compounds **3** as shown in Scheme 1.

The resulting compounds in which various heterocycles were grown from the pyridyl sulfonamide moiety, were tested over PI3Kβ and PI3Kα at 10 μM concentration in a high-throughput binding assay (KINOMEscan, Ambit Biosciences).[8] To our delight, several compounds were discovered as hits for PI3Kβ [POC (percent of control) values <10] as shown in Table 1. Of particular significance is the observation that a profound potency on PI3Kβ and selectivity over PI3Kα was achieved by the combination of 2-aminopyrimidine with the pyridyl sulfonamide moiety (**9**, PI3Kβ, PI3Kα: POC = 1.4, 60, respectively). Considering tolerance within the PI3Kβ active site, selectivity over PI3Kα and low molecular weight of 327, compound **9** was expected to serve as a good scaffold from which much more potent and selective inhibitors can be derivatized. For these reasons, compound **9** was selected for further optimization to embark upon in-depth structural modification.

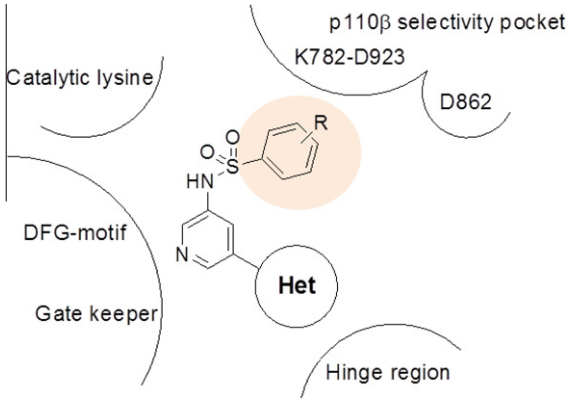
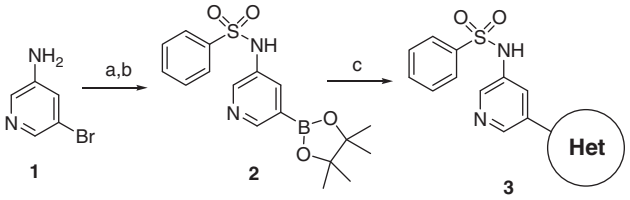
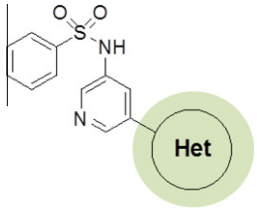


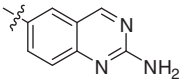
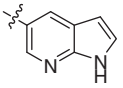
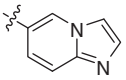
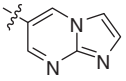
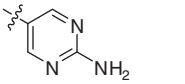
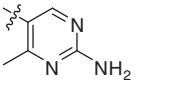
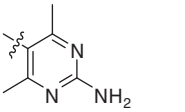
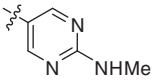
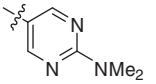
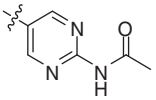
Figure 2. Design of aminopyrimidine scaffolds as PI3Kβ inhibitors, and opportunities for modification.



Scheme 1. Reagents and conditions: (a) $PhSO_2Cl$, Pyridine, CH_2Cl_2, rt; (b) bis(pinacolato)diboron, $Pd(dppf)Cl_2$, KOAc, 1,4-dioxane, 100 °C; (c) Aryl bromide, $Pd(dppf)Cl_2$, K_2CO_3, 1,4-dioxane/water = 3:1, 100 °C.

Table 1
Array of various heterocycles and inhibition data against PI3Kα and PI3Kβ



Compd	Het	PI3Kβ (POC) [a]	PI3Kα (POC) [a]
4		0	0
5		4.9	56
6		0.6	4
7		39	95
8		7.4	52
9		1.4	60
10		5.2	66
11		18	89
12		5.2	39
13		66	100
14		34	100

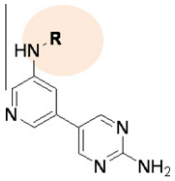
[a] Lower numbers of POC (percent of control) indicate stronger hits. Values show an average of duplicate measurements.

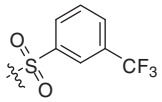
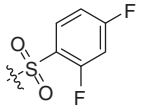
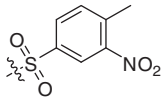
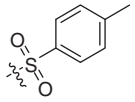
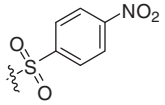
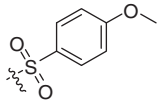
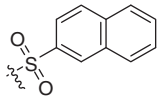
Building upon the success observed with aminopyrimidine scaffold, a further investigation of the effect of sulfonamide subunit was then conducted (Table 2). The sulfonamide moiety was found to be essential for enhanced potency, and replacement of

sulfonamide group with hydrogen or amide group resulted in a significant loss of activity over all PI3Ks. Introduction of small size sulfonamide, such as methylsulfonamide also decreased all PI3Ks inhibitory activity, implying the importance of the role of aryl ring for PI3K inhibition. The increase in activity afforded by toluene sulfonamide group (K_d of **22** = 0.1 μM) prompted further investigation through synthesis of a series of substituted aryl sulfonamide analogs. The presence of *meta*-, or *para*-substituents on aryl ring resulted in compounds which were generally of equivalent or better enzyme potency than the corresponding unsubstituted derivatives. Compound **23**, having a *p*-OMe group had amplified selectivity verses PI3Kα. Intriguingly, profound effect on selectivity for PI3Kβ over PI3Kα was achieved with compound incorporating the naphthyl group, leading to **24** with excellent specificity profiles (PI3Kβ, PI3Kα: POC = 1.0, 92; K_d = 0.23, >40 μM, respectively). In addition, this series of PI3Kβ inhibitors possess excellent selectivity over mTOR.

To obtain structural insight into the inhibitory and selectivity mechanisms of the identified PI3Kβ inhibitors, their binding modes in the ATP-binding site were investigated in a comparative fashion. As the structure of PI3Kβ has not been established, a homology model was built based on p110γ complex with staurosporine (PDB code: 1E8Z) as the template using the program MODELER to mimic the conformational rearrangement. Subsequent docking studies were performed using AutoDock 4.0.[9] Docking studies were either performed without constraints or with a hydrogen bonding constraint to the backbone-NH of Val854. In the calculated PI3Kβ–**9** complex shown in Figure 3, compound **9** appeared to be in close contact with residues Val854–Val853, Tyr839–Asp813, Lys805, Asp862, Ser781, and Lys782–Asp923, respectively. From the overall structural features derived from docking simulations, the inhibitory activity of **9** is likely to stem from the multiple hydrogen bonds and hydrophobic interactions. Thus, examination of the hinge-region/aminopyrimidine interaction in the PI3Kβ homology model showed that the 2-aminopyrimidine of **9** maintains the hydrogen-bonding pattern with Val854. The pyridyl group is anchored by a hydrogen bond to the Tyr839 and the sulfonamide participates in hydrogen bonding with the catalytic lysine (Lys805), as shown in Figure 3. These three hydrogen bonds seem to play a role of anchor for binding of **9** with PI3Kβ.

Table 2
Structure–activity relationship with R groups and selectivity data against PI3K isoforms



Compd	R	PI3Kβ K_d (μM)	POC [a]	
			PI3Kβ/α/γ/δ	mTOR
15	–H	>40	86/98/100/82	53
16	–COMe	>20	69/97/98/65	79
17	$-SO_2Me$	5.2	28/62/43/38	67
9	$-SO_2Ph$	0.36	1.4/60/24/4.2	74
18		0.43	1.2/30/9.6/0.9	41
19		0.22	0.7/22/7.2/4.7	56
20		0.25	0.7/42/5.3/0.1	78
21		0.10	0.5/17/8.2/3.4	76
22		0.55	1.6/18/6.7/9.5	68
23		0.50	1.7/87/20/4.3	39
24		0.23	1.0/92/25/4.2	96

[a] Lower numbers of POC (percent of control) indicate stronger hits. Values show an average of duplicate measurements.

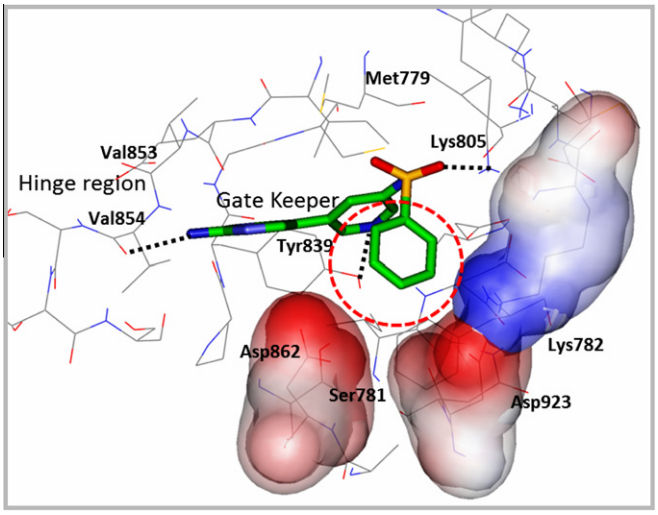


Figure 3. Calculated binding mode of **9** in the ATP-binding site of the PI3Kβ homology model. In this model, the aminopyrimidine unit forms a key hinge region hydrogen bond with the backbone of Val854. Selected residues (Try839 and Lys805) are shown for possible hydrogen bonding interaction with pyridyl sulfonamide subunit. Each dotted line indicates a hydrogen bond.

Compound **9** may be further stabilized in the ATP-binding site via the hydrophobic interactions among its nonpolar groups with the side chains in the back pocket (DFG-motif, gate keeper and catalytic lysine). The selectivity enhancement of **9** seems to be the result of accessing the more hydrophobic region within PI3Kβ. Docking modeling suggests that the difference of several residues between PI3Kβ and PI3Kα causes a difference in the depth of the phenyl group binding pockets that is responsible for the increased selectivity observed for the aminopyrimidine analogs with arylsulfonamide subunit. It was expected that the presence of aryl ring on sulfonamide group (in red circle, Fig. 3) would allow for a favorable hydrophobic interaction with salt bridge (Lys782–Asp923) which is missing in alpha isoform (PI3Kα: Ala775 and Ser919).[10] This hydrophobic interaction seems to introduce affinity for PI3Kβ and explains weaker binding affinity for PI3Kα. Moreover, phenyl group is also directed toward a hydrophobic pocket located behind Asp862 whch is unique to PI3Kβ (α, Gln859; δ, Asn836; γ, Lys890).[10] These amino acid residues may create a deeper binding pocket in PI3Kβ, which accommodates the aryl ring of sulfonamide derivatives without causing unfavorable steric contacts. The modeling analysis similarly applies to analogs with larger aryl groups (e.g., naphthyl group) which seem to be accommodated by this binding pocket in PI3Kβ.

Given the impressive enzyme activity profiles, several compounds from this series were further tested for cellular proliferation activity. 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) assay in T47D human breast cancer cell cultures. Notably, compounds **9**, **19**, and **21** showed promising inhibitory activity against T47D at micromolar concentration (Table 3).

In conclusion, a new series of aminopyridine-based PI3Kβ inhibitors Ref.[11] have been developed by the structure-based design. When incorporated with the phenyl sulfonamide moiety, aminopyrimidine analogs showed good potency on PI3Kβ and selectivity over PI3Kα. Intriguingly, replacement of phenyl group on sulfonamide with larger groups, such as naphtyl group enhanced selectivity over PI3Kα while retaining submicromolar PI3Kβ potency. Molecular modeling suggests that increased PI3Kβ selectivity is caused by salt bridge (Lys782–Asp923) and Asp862 that creat a deeper pocket in PI3Kβ. These results clearly provide useful insight in the design of new PI3Kβ inhibitors with high potency and selectivity.

Table 3
Inhibition of breast cancer cell (T47D) proliferation by representative aminopyrimidine derivatives

	9	19	21
T47D IC_{50} (μM)	9.6	8.5	4.7

Acknowledgments

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11. *Spectra data of representative compounds.* ***9***: ^{1}H NMR (300 MHz, MeOD-d_6) δ 7.50 (m, 4H), 7.65 (s, 1H), 7.75 (s, 1H), 7.78 (s, 1H), 8.16 (s, 1H), 8.41 (m, 2H). **18**: ^{1}H NMR (300 MHz, MeOD-d_6) δ 7.68 (m, 2H), 7.87 (d, J = 7.4, 1H), 8.01 (d, J = 7.6, 2H), 8.15 (d, J = 2.3, 1H), 8.43 (d, J = 2.6, 3H). **19**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.93 (s, 2H), 7.25 (m, 1H), 7.53 (m, 1H), 7.61 (s, 1H), 7.92 (m, 1H), 8.21 (d, J = 1.9, 1H), 8.47 (s, 2H), 8.51 (s, 1H), 11.02 (s, 1H). **20**: ^{1}H NMR (300 MHz, MeOD-d_6) δ 2.53 (s, 3H), 7.55 (d, J = 8.2, 1H), 7.68 (m, 1H), 7.88 (dd, J = 2.0, 8.1, 1H), 8.17 (s, 1H), 8.30 (d, J = 1.8, 1H), 8.42(m, 3H). **21**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.33 (s, 3H), 6.92 (s, 2H), 7.34 (s, 1H), 7.36 (s, 1H), 7.59 (t, J = 2.2, 1H), 7.67 (s, 1H), 7.70 (s, 1H), 8.17 (d, J = 2.4, 1H), 8.46 (s, 2H), 8.48 (d, J = 2.0, 1H), 10.60 (s, 1H). **22**: ^{1}H NMR (300 MHz, MeOD-d_6) δ 7.71 (t, J = 2.2, 1H), 8.01 (m, 2H), 8.19 (d, J = 2.3, 1H), 8.31 (m, 2H), 8.44 (d, J = 1.9, 1H), 8.45 (s, 2H). **23**: ^{1}H NMR (300 MHz, MeOD-d_6) δ 3.76 (s, 3H), 6.96 (dd, J = 2.0, 6.9, 2H), 7.67 (m, 3H), 8.15 (d, J = 2.3, 1H), 8.37 (d, J = 1.9, 1H), 8.42 (s, 2H). **24**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.91 (s, 2H), 7.66 (m, 3H), 7.80 (dd, J = 1.7, 8.7, 1H), 7.99 (d, J = 7.6, 1H), 8.12 (m, 2H), 8.20(d, J = 2.3, 1H), 8.42 (s, 3H), 8.51 (s, 1H), 10.74 (s, 1H).