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Brief Article

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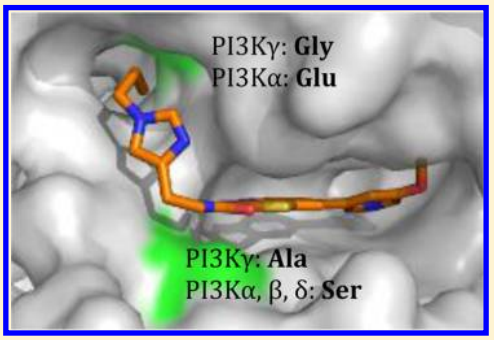
Structural Basis for Isoform Selectivity in a Class of Benzothiazole Inhibitors of Phosphoinositide 3-Kinase γ

Philip N. Collier,* Gabriel Martinez-Botella,§ Mark Cornebise,‡ Kevin M. Cottrell, John D. Doran, James P. Griffith, Sudipta Mahajan, François Maltais, Cameron S. Moody, Emilie Porter Huck, Tiansheng Wang, and Alex M. Aronov*

Vertex Pharmaceuticals Inc., 50 Northern Avenue, Boston, Massachusetts 02210, United States

Supporting Information

ABSTRACT: Phosphoinositide 3-kinase γ (PI3Kγ) is an attractive target to potentially treat a range of disease states. Herein, we describe the evolution of a reported phenylthiazole pan-PI3K inhibitor into a family of potent and selective benzothiazole inhibitors. Using X-ray crystallography, we discovered that compound **22** occupies a previously unreported hydrophobic binding cleft adjacent to the ATP binding site of PI3Kγ, and achieves its selectivity by exploiting natural sequence differences among PI3K isoforms in this region.



Phosphoinositide 3-kinases (PI3Ks), a family of enzymes that act as lipid and protein kinases, have been linked to numerous cellular functions including cell growth, differentiation, proliferation, survival, migration, and intracellular trafficking.[1−3] The eight known mammalian PI3Ks have been divided into three classes based on their structure, regulatory subunits, and substrate specificity. The most extensively studied class I PI3Ks are heterodimeric complexes comprising a 110 kDa catalytic subunit and a smaller associated regulatory subunit. Class Ia PI3Ks (α, β, and δ) containing the catalytic subunits p110α, p110β, and p110δ, respectively, are activated through tyrosine kinase signaling. In contrast, the sole class Ib member, PI3Kγ, contains catalytic subunit p110γ associated with either a p101 or p84 regulatory subunit, and is mostly activated through GPCRs.[4]

While there is growing evidence that inhibitors of PI3Kγ may have utility in treating cancer and cardiovascular disease, much of the validation for PI3Kγ as a potential drug target has been performed in the area of inflammation and autoimmune disease because of its important role in such processes as lymphocyte chemotaxis and mast cell degranulation.[3,5−8] The compelling biology surrounding this target, with its potential for disease modification, has attracted the attention of the pharmaceutical industry. A number of selective inhibitors of PI3Kγ have now been published.[4,9−15] Whereas expression of PI3Kγ and PI3Kδ is mainly confined to the hematopoietic system, the other PI3K isoforms are ubiquitously expressed. If PI3Kγ is to be targeted for a chronic indication, any cross-activity against the α and β isoforms in particular should be avoided. It is known, for example, that activation of class Ia PI3Ks is important in insulin signaling.[16] PI3Kγ knockout mice are viable and show no reproductive abnormalities.[17−19]

Selective inhibition of PI3Kγ over the other PI3K isoforms has proven to be a challenging endeavor, in part due to the high sequence homology within the ATP binding site of the PI3Ks. In this report, we describe a series of benzothiazole isoform-selective inhibitors of PI3Kγ. Binding modes revealed from X-ray crystallographic studies, combined with analysis of residue differences among PI3K isoforms, shed light on some of the selectivity determinants within the ATP binding site.

Upon analysis of the reported crystal structure of pan-PI3K inhibitor PIK-93 (PI3Kγ K_i = 7 nM) in the active site of PI3Kγ, we investigated the possibility of morphing from the phenylthiazole core of PIK-93 to a benzothiazole, as depicted in **1** (Figure 1).[20] We suspected that a 6-substituted benzothiazole would better project into the deep ATP pocket, occupying space adjacent to Tyr867 side chain. We were pleased to observe submicromolar PI3Kγ affinity for 6-phenylbenzothiazole **2** (Table 1). Interestingly, a Novartis group has recently reported identification of a similar benzothiazole scaffold from a HTS campaign in their search for selective inhibitors of PI3Kα.[21] We quickly discovered that replacement of the phenyl group at C-6 of benzothiazole **2** with a 3-pyridyl substituent led to a 20-fold improvement in PI3Kγ affinity (Table 1, **3**). A crystal structure of pyridine **3** in complex with PI3Kγ confirmed the presence of two hydrogen bonds to the PI3Kγ hinge region, specifically backbone amide nitrogen and backbone carbonyl of Val882 within the ATP binding site (Figure 2a). The interaction with Val882 is apparent in all known ligand-

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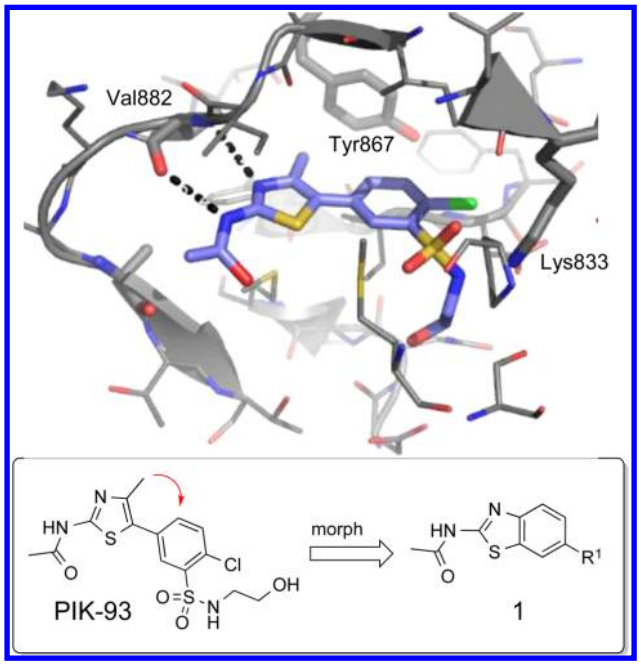
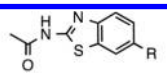
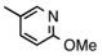
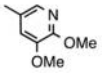
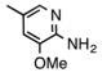


Figure 1. X-ray crystal structure of reported pan-PI3K phenylthiazole inhibitor PIK-93 bound to PI3Kγ (PDB code 2chz).[20] Ample space is available within the ATP binding site allowing for the possibility of a ring fusion to benzothiazole core **1**.

Table 1. SAR at Benzothiazole C6 Position[22]



Cpd	R =	PI3Kγ Ki (μM)	Cpd	R =	PI3Kγ Ki (μM)
2		0.77	8		0.044
3		0.039	9		0.036
4		0.013	10		0.001
5		0.004	11		0.019
6		0.006	12		0.013
7		0.036	13		0.021

bound structures of PI3Kγ. The dramatic improvement in potency observed with pyridine **3** can be rationalized by the observation of key water-mediated hydrogen bonds to Tyr867 and Asp841. These residues are conserved across PI3K isoforms; increased activity against PI3Kγ (K_i = 39 nM) is mirrored by increased activity against PI3Kα (K_i = 19 nM).

SARs at the benzothiazole C-6 position were further explored, and the data are summarized in Table 1.[22] A 10-fold improvement in PI3Kγ affinity was obtained by incorporating a methoxy group at the C-5 position on the

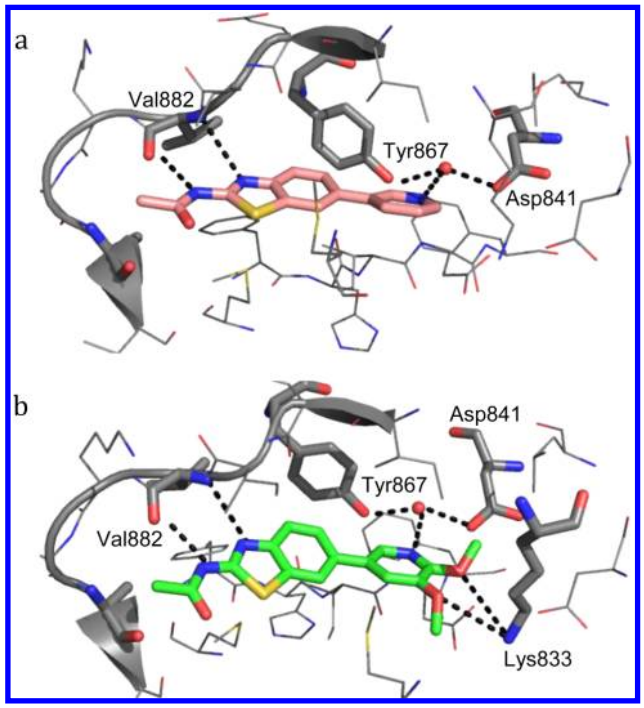


Figure 2. (a) X-ray structure of pyridine **3** bound to PI3Kγ (PDB code 4PS7). Val882 forms a bidentate hinge-binding interaction with the ligand. Water-mediated hydrogen bonds are formed from the pyridine nitrogen atom to Tyr867 and Asp841. (b) X-ray structure of dimethoxypyridine **10** bound to PI3Kγ (PDB code 4PS8). The dimethoxy moiety coordinates Lys833.

pyridine ring (Table 1, **5**). Electron-withdrawing groups at the C5-position had a neutral effect on PI3Kγ affinity (Table 1, **7** and **8**), while 4,5-dimethoxypyridine **10** was the highest affinity compound of the set. This substitution pattern is consistent with recent findings from Cellzome for a related triazolopyridine scaffold.[11] Overall, a remarkable gain in affinity (almost 800-fold) was realized on transitioning from phenyl analogue **2** to dimethoxypyridine analogue **10** as a result of forming three favorable interactions to the ATP binding site of PI3Kγ.

Structural information collected for **10** in complex with PI3Kγ indicated that the likely explanation for the increased affinity of compounds possessing 5-alkoxy substituents was due to a favorable hydrogen bonding interaction with the side chain of catalytic lysine (Lys833) in PI3Kγ (Figure 2b).

As a result of SAR exploration in the region of the scaffold occupied by the acetamide and replacement of the acetamide with chain-extended ureas, we discovered compounds with isoform selectivity, an observation subsequently reported in the literature by other groups.[23,24] The urea functional group was chosen, since it allowed rapid structure−activity profiling via straightforward coupling reactions with amines (see Supporting Information). Increasing the urea chain length resulted in compounds with improved PI3Kγ affinity (Table 2, **17**, **18** vs **14**−**16**). However, we also made the serendipitous discovery that increasing the chain length of trifluoroethylamide **17** by one methylene unit, giving **18**, dramatically improved selectivity for PI3Kγ over PI3Kα by 72-fold while maintaining PI3Kγ activity. We observed a similar selectivity trend in a small family of alkylated imidazoles (**19**−**22**). Structural studies were initiated in order to understand these observations.

Propylimidazole **22**, which was crystallized with PI3Kγ to a resolution of 2.9 Å, adopted a U-shaped conformation with its

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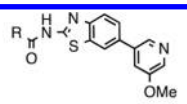
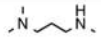
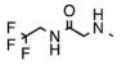
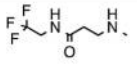
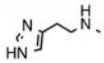
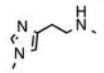
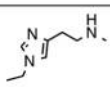
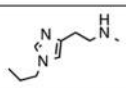
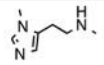
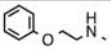
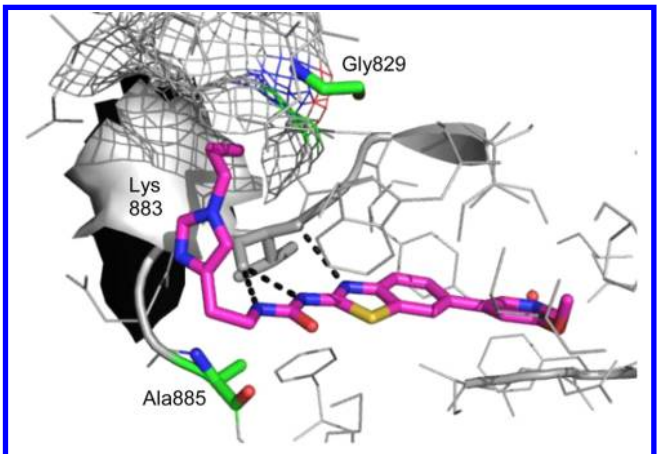
Table 2. Urea SAR and Corresponding PI3Kα Selectivity



Cpd	R =	PI3Kγ Ki (μM)	PI3Kα Ki (μM)	PI3Kα/ PI3Kγ
14		0.009	0.016	2
15		0.007	0.052	7
16		0.19	0.056	0.3
17		0.006	0.007	1
18		0.001	0.072	72
19		0.005	0.02	4
20		0.007	0.105	15
21		0.002	0.113	57
22		0.002	0.132	66
23		0.022	0.067	3
24		0.019	>4	>211

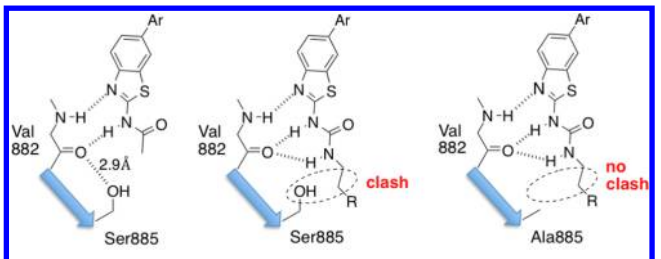
propyl group occupying a previously unreported binding cleft adjacent to the ATP binding site (Figure 3). In the X-ray structures of inhibitors **3** and **10** described earlier in this report, hydrogen-bonding interactions are observed from Lys883 to Thr827 (3.2 Å to side chain hydroxyl group of **10**) and to Glu814 (3.4 Å for **10**). Similar interactions are observed in many of the reported ligand bound structures of PI3Kγ.[25,26] However, in the structure of **22** bound to PI3Kγ, neither of these interactions of Lys883 are present; the pocket stitched up by Lys883 interactions is open. Evidently, the energy cost of this rearrangement is more than compensated by interactions created by **22** in the newly formed binding cleft.

PI3K isoform sequence on the periphery of the ATP binding site is divergent, in contrast to the conservation of residues within the ATP binding site. By comparison of amino acid residues within and surrounding this newly identified binding cleft, an explanation for the observed selectivities of the urea analogues was proposed. A degree of selectivity over all PI3K class Ia isoforms is imparted by a functional group change from amide to urea (Figure 4, **5** vs **15** and **22**). In a published X-ray structure of PI3Kδ, a 2.9 Å hydrogen bond is present between ATP binding site residue Val828 (corresponding residue in PI3Kγ is numbered Val882) and Ser831.[27] We hypothesize that this internal hydrogen bonding interaction is conserved across



residue	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
829	*E*	G	G	G
885	S	S	*A*	S

Figure 3. X-ray structure of **22** bound to PI3Kγ (PDB code 4PS3). Propylimidazole unit occupies a hydrophobic cleft adjacent to the ATP binding site. Residue differences (Gly829 and Ala885) that drive isoform selectivity are highlighted.



Cpd	PI3Kγ Ki (μM)	PI3Kα/ PI3Kγ	PI3Kβ/ PI3Kγ	PI3Kδ/ PI3Kγ
5	0.004	1	10	3
15	0.007	7	26	26
22	0.002	66	21	61

Figure 4. Mechanistic hypothesis for isoform selectivity of ureas. Hydrogen bonding interaction between Val882 and Ser885 (PI3Kγ numbering) in PI3K class Ia isoforms bound to acetamide **5** is eliminated in the case of urea binding (**15** and **22**). This disruption sets up a negative interaction between free Ser885 hydroxyl group and the hydrophobic urea side chain.

class Ia PI3Ks and is disrupted upon urea binding (Figure 4). The urea forms a bidentate interaction with the backbone carbonyl of Val882, liberating the Ser885 hydroxyl group, which negatively interacts with the lipophilic side chain of the ureas. No such negative interaction exists on urea binding to PI3Kγ where residue 885 is alanine.

Further improvement in PI3Kα selectivity of the ureas was realized with increasing chain length. This can be explained by a key residue difference inside the newly described selectivity pocket: an unfavorable interaction of the propyl group of **22** with an Asp residue in PI3Kα (Gly829 in PI3Kγ). Protrusion into the hydrophobic pocket has little effect on PI3Kγ

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selectivity over the β and δ isoforms (Figure 4, **15** vs **22**), consistent with our hypothesis. The 200-fold PI3Kα selectivity seen with phenoxyethylamide **24** (Table 2) again is consistent with a detrimental interaction of the phenyl group of **24** with the Asp residue of PI3Kα. A striking combination of the two aforementioned effects accounts for the dramatic PI3Kα selectivity difference observed between **17** and homologue **18**. Presumably, the amide carbonyl group of **17** makes a positive interaction with the liberated Ser885 side chain of PI3Kα while the clash between the shorter side chain of **17** and Asp829 of PI3Kα is reduced.

In summary, we have discovered potent and isoform selective inhibitors of PI3Kγ based around a benzothiazole core. To our knowledge, this is the first report that details structural determinants of PI3Kγ selectivity around a newly defined binding cleft adjacent to the ATP binding site and provides a framework to examine PI3K isoform selectivity.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic schemes and experimental procedures, characterizaton of organic molecules, biochemical assays, crystallographic information. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*P.N.C.: phone, 1-617-341-6921; e-mail, philip_collier@vrtx.com.

*A.M.A.: phone, 1-617-341-6804; e-mail, alex_aronov@vrtx.com.

Present Addresses

‡M.C.: AstraZeneca R&D Boston, 35 Gatehouse Drive, Waltham, Massachusetts 02451, United States.

§G.M.-B.: Sage Therapeutics, 215 First Street, Cambridge, Massachusetts 02142, United States.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

GPCR, G-protein-coupled receptor

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