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Class II but Not Second Class—Prospects for the Development of Class II PI3K Inhibitors

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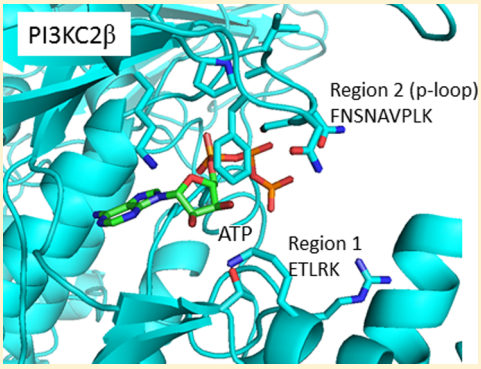
ABSTRACT: The Class II PI3 kinases are emerging from the shadows of their Class I cousins. The data emerging from PIK3C2 genetic modification studies and from siRNA knockdown suggest important roles in physiology and pathology. With some well-studied Class I isoform inhibitors showing strong Class II activity and a wealth of crystallographic information available, the structural similarity of these isoforms to Class I provides both the opportunity and the challenge in design of selective pharmacological inhibitors.



KEYWORDS: *PI3 kinase, Class II, isoform selectivity*

The phosphatidylinositol 3-kinases (PI3Ks) are among the most prominent of contemporary drug targets, with over 25 PI3K inhibitors currently under clinical investigation. PI3Ks are key players in cell signaling and as such are involved in a diverse array of physiological and pathological cell processes.[1] There are eight mammalian PI3K isoforms, grouped into three classes on the basis of their substrate specificity and structure. Of the eight PI3Ks, just the four Class I isoforms have been the targets of drug discovery programs.[2] The other four, the Class II PI3Ks (PI3KC2α, 2β, and 2γ) and the Class III isoform (Vps34), have been largely neglected as therapeutic targets.

The Class II PI3Ks are now emerging as important signaling enzymes with gene knockdown studies, suggesting key regulatory roles for the enzymes in diverse cellular contexts.[3] It is important to note the fundamental differences between Class II PI3Ks and Class I PI3Ks. The substrate preferences of the PI3Ks are a major point of divergence. For Class I PI3Ks, the in vivo substrate is PtdIns(4,5)P2, yielding PtdIns(3,4,5)P3. The Class II PI3Ks on the other hand do not catalyze PtdIns(4,5)P2 phosphorylation, but rather the formation of either PtdIns3P or PtdIns(3,4)P2; the in vivo substrate remains controversial. The Class III PI3K (Vps34) catalyzes the formation of PtdIns3P. Note also that a Class IV series of PI3K-related enzymes are protein kinases, including mTOR, ATM, ATR, and DNA-PK. The Class II PI3Ks also have different architecture from the Class I PI3Ks, retaining the central C2 and the helical and catalytic domains of the Class I forms, but having marked variation in N- and C-terminal domains that dictate constitutive and transient binding partners. The Class II PI3Ks have a C-terminal PX domain and a second C2 domain that seem to direct membrane associations.

The precise cellular functions of the three Class II PI3Ks are still poorly defined but have been the subject of much recent interest. PI3KC2α and PI3KC2β are widely expressed, are activated downstream of cell surface receptors, and play a role in cell migration, survival, glucose transport, and endocytosis.[3,4] PI3KC2α has been shown to have an essential role in angiogenesis and vascular barrier function and cilium organization.[4,5] Altered transcription and/or mutation in the PIK3C2B gene (that expresses PI3KC2β) may contribute to the pathogenesis of various human cancers.[4,6] It has also been shown that PI3KC2β regulates the hepatitis C virus replication cycle.[7] PI3KC2γ has a narrowed tissue distribution and has not been studied in as much detail, although a role in apolipoprotein B100 degradation in hepatocytes has been postulated.[8]

The above data has been basically obtained through genetic studies involving knockdown of gene expression. Only two papers directly identify Class II PI3K inhibitors.[6,9] Otherwise, Class II PI3K inhibition has been relegated to an occasional off-target screen in studies of Class I inhibitors. There is clearly a

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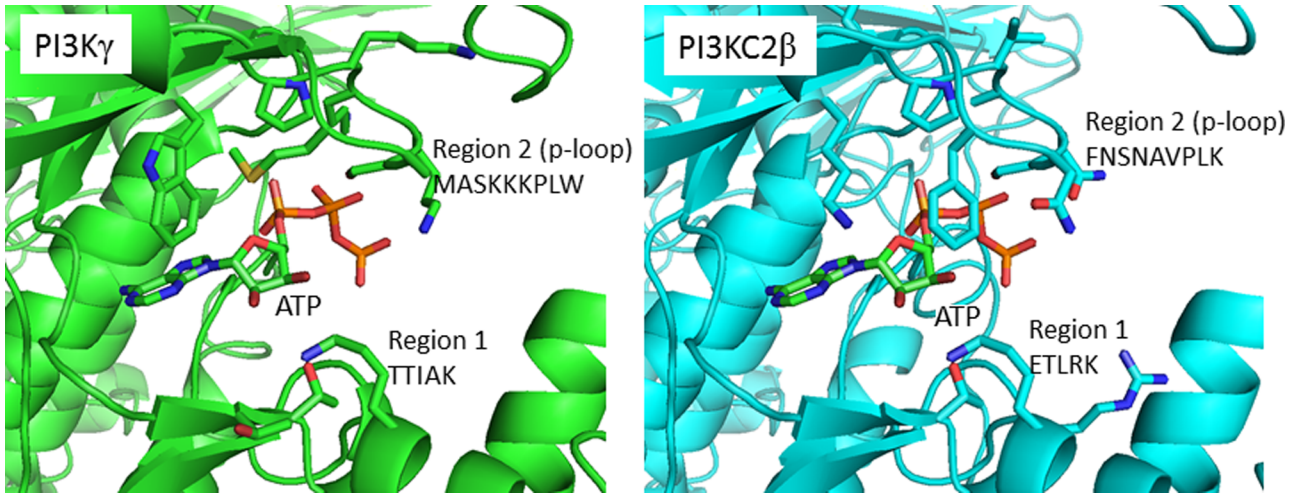
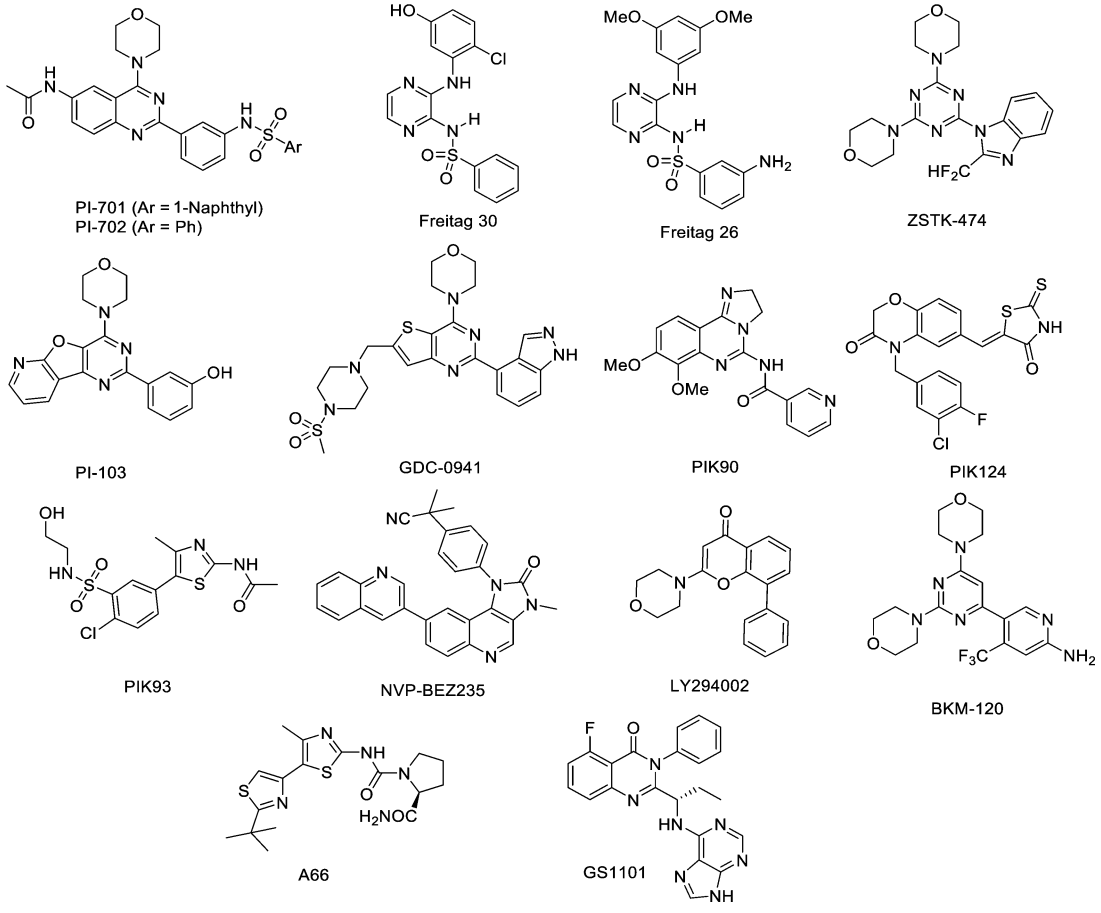


Figure 1. Class I PI3Kγ (X-ray, PDB 1e8x, ATP bound) and Class II PI3KC2β (homology model) compared. Key residues in regions of heterogeneity are shown by sequence and have key side chains visible.

Chart 1. Chemical Structures of Class II PI3K Inhibitors



shortfall of potent, isoform selective Class II PI3K inhibitors with which to validate the enzymes as therapeutic targets.

Against a background of an emerging important set of roles for this class of enzymes and a dearth of compounds that can be applied to their study, the purpose of this Viewpoint is to identify the key inputs that might drive the identification of isoform selective inhibitors of Class II PI3Ks. These include an analysis of the heterogeneity of the PI3K binding sites, gained from sequence alignment and homology modeling and discussion of the structure−activity relationships that can be sketched from the existing published data.

1. The Class II PI3 Kinase Active Site—Structural and Sequence Comparison to Class I PI3 Kinases. The extensive analysis of the active site of Class I PI3Ks provides a robust template for the analysis of Class II PI3K structure and also the development of homology models of the Class II isoforms (Figure 1).[10] Sequence alignment shows the capacity of the catalytic domain of the Class I and Class II isoform

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Table 1. Current Class I Inhibitors Including Clinical Candidates[a]

#	PI3KC2α	PI3KC2β	PI3KC2γ	Class I	Class III	Class IV
			Selective			
PI701[6]	n.d.	0.53	n.d.	>10	n.d.	>100
PI702[6]	n.d.	0.63	n.d.	>10	n.d.	>100
Freitag-30[9]	>10	2.7	23	>10	>10	n.d.
Freitag-26[9]	>10	29	0.34	>10	>10	n.d.
			Nonselective			
ZSTK474[13]	>100	0.18	n.d.	0.006 (δ)	>100	0.37 (mTOR)
PI-103[12]	1.0	0.026	n.d.	0.008 (α)	2.3	0.002 (DNA-PK)
GDC0941[13]	>100	0.59	n.d.	0.007 (δ)	>100	0.41 (mTOR)
PIK90[12]	0.047	0.064	n.d.	0.011 (α)	0.83	0.013 (DNA-PK)
PIK124[12]	0.14	0.37	n.d.	0.023 (α)	10	1.5 (DNA-PK)
PIK93[12]	16	0.14	n.d.	0.016 (γ)	0.32	0.064 (DNA-PK)
NVP-BEZ235[13]	0.034	0.044	n.d.	0.007 (α)	0.45	0.002 (mTOR)
LY294002[13]	27	10.4	n.d.	0.60 (α)	3.5	3.9 (mTOR)
A-66[14]	>5	0.462	n.d.	0.032 (α)	>5	>5 (mTOR)
GS1101[15]	n.d.	>1	n.d.	0.025 (δ)	978	6.7 (DNA-PK)

[a]IC_{50} values (in μM) for reported inhibitors of Class II PI3K inhibitors and Class I (most potently inhibited isoform) and Class III and IV PI3K isoforms (most potent sub-type).

PI3Ks to adopt a common fold with a canonical ATP binding site architecture. Of 12 residues that have been shown to interact with ATP in the Class I PI3K crystal structure, 8 are absolutely conserved across the family and most changes are conservative. The most prominent exception is a Met [M804γ] ↔ Phe change between Class I and Class II.

The lipid binding site does vary between the classes consistent with the different lipid substrate preferences. Basic residues in the activation loop and the P-loop (eg Lys807−Lys809 in PI3Kγ) are key mediators of presentation of 4- and 5-phosphates of PIP-4,5-P2 in Class I isoforms but absent in the Class II isozymes (Figure 1). Solved dsVps34 crystal structures also provide a useful reference point for comparing the isoform structures. Miller et al. showed a difference in the P-loops of Class III and Class I structures dictated by sequence length and sequence, and a relatively restricted binding site in the Class III catalytic pocket.[11] The methionine and tryptophan residues that present the p-loop in Class I PI3Ks are also modified, replaced by phenylalanine and lysine, respectively, in Class II (phenylalanine and glutamine, respectively, in Class III).

These and other residues are known to contribute to the generation of inhibitor isoform selectivity within the Class I isoforms.[12] The p-loop is quite heterogeneous, as is another region (termed "region 1" in our earlier studies) which is at the outer edge of the ATP pocket (Figure 1). There is a mix of conserved and nonconserved substitutions spanning the family here which potentially complicates the classification. Overall, the levels of heterogeneity between the Class I, Class II, and Class III PI3K catalytic sites can be classified into three groups: (i) those conserved within the class but different between classes; (ii) those conserved within one class but variable in the other; and (iii) those generally nonconserved but with commonalities between certain Class I and Class II isoforms. The heterogeneity of the PI3K binding sites is sufficient to anticipate that selective inhibition will be possible but also the interesting prospect of dual but cross-class inhibition which might have an interesting influence on compound pharmacology.

2. A Brief SAR of Class II PI3Ks. Virtually nothing is known about what makes compounds inhibit Class II PI3Ks, let alone what might render them selective. Two recent reports describe PI3KC2β inhibitors, PI701 and PI702 retrieved from earlier analogue sets developed at Yamanouchi[6] and very recently some inhibitors of PI3KC2γ and PI3KC2β derived by adaptation of the Class I inhibitor XL147.[9] Most of the data that can be retrieved relates to the Class II PI3Ks as a potential "off-target" activity in assessment of just 10 Class I inhibitors (Chart 1, Table 1). While the Class II inhibition profile has been neglected or ignored in the assessment of most of the current PI3K inhibitors under clinical investigation, it seems plausible that inhibition of these isoforms may contribute to either the efficacy or side-effect potential of these compounds.

Despite the paucity of biochemical data relating Class II inhibition, binding models can be built with some confidence because the main Class II inhibitors (or close analogues) have been cocrystallized with Class I enzymes. For example, one of the most potent PI3KC2α inhibitors is PIK90 (IC_{50} = 47 nM). PIK90 has been cocrystallized with both PI3Kγ and dsVps34, providing a cogent template for the likely Class II binding mode.[11,12]

The collected data can be used to draw together the following inferences, although some caveats apply: the rigor of off-target screening may not be to the same level as on-target testing, and there is no cellular marker of Class II PI3K activity. Three features characterize inhibitor binding.

(1) PI3KC2β is quite readily inhibited. Most of the Class I inhibitors tested block PI3KC2β to some degree with IC_{50} values just 3−30-fold higher than at the target Class I isoform. PI-103 is most potent, with an IC_{50} of 26 nM. The exceptions are the PI3Kδ inhibitors PIK39 and IC87114 and the PI3Kβ inhibitors TGX286 and PIK108, each of which inhibit PI3KC2β poorly.[12] The feature known to dictate the selectivity of these compounds in Class I PI3Ks is the cryptic "selectivity" pocket formed by methionine (M804γ) and tryptophan (W812γ). In Class II PI3Ks these residues are phenylalanine (F) and lysine (K), respectively. This implies that in Class II PI3K the equivalent pocket cannot form or cannot support binding.

(2) A number of the compounds above show distinct selectivity for PI3KC2β over PI3KC2α, including the morpholino substituted compounds and the carbonylamino-

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thiazoles. Against crystallographic models, it is hard to reconcile the selectivity shown by these compounds. Nor is it simple to explain the absolute PI3KC2β selectivity shown by PI-701 and PI-702. One possibility is that these two compounds adopt a "flipped" pose where the acetamidophenyl group is placed in the affinity pocket[12] and the arylsulfonanmide projects to the mouth of the binding site.

(3) Compounds with strong potency at both PI3KC2α and PI3KC2β isoforms (PIK90, PIK124, and NVP-BEZ235) are potent also against Class III PI3K's and the Class IV kinases. Notably these are all "non-morpholino" compounds. PIK90 has been cocrystallized with both PI3Kγ and dsVps34 and sits very deep in the catalytic pocket, tight against the hinge region, projecting the nicotinamide group into the affinity pocket, but making little interaction with the p-loop. In Class I isoform selective compounds, projection of substituents from the purine binding site can engender major changes in isoform selectivity.[14]

The lack of information available at present from which to build an understanding of Class II inhibition juxtaposed against thousands of compounds prepared in Class I inhibitor campaigns suggests that a robust data set could be developed for Class II isoforms from existing libraries, with modest synthetic effort. Indeed, PI701 and PI702 appear to have emerged from such a process. The tools to delineate Class II PI3K function may be quite close at hand.

CONCLUSION

The Class II PI3Ks are classic "poor cousins" in the PI3 kinase field. They have been left behind as a research target by the chronology of their discovery and the apparent complexity or ignorance of the signaling pathways they regulate. But just like their more famous relatives, there seems to be a ripe opportunity for the development of new molecules to unravel the physiology and pathology of the enzymes. Indeed, it would not be surprising to find that Class II PI3K inhibition is already showing a clinical, if off-target, effect. The prospects for development of selective inhibitors seem very strong, built on a wealth of adjacent information unavailable in the first phase of PI3K research and providing a rational basis for their discovery.

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Notes

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