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PI3Kδ and PI3Kγ as Targets for Autoimmune and Inflammatory Diseases

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INTRODUCTION

Autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and asthma are chronic and often progressive diseases associated with a dysregulated or an overactive immune system, respectively. The causes and the drivers of these diseases remain ill-defined. They are characterized by complex cellular interactions between multiple inflammatory cells of the innate and adaptive immune system. The approval of rituximab (Rituxan) for RA and belimumab (Benlysta) for SLE indicates an important role for B cells in the pathology of immune-mediated diseases. However, the heterogeneity and complexity of the disease etiology of these conditions make the search for new good cellular targets challenging, as it is unclear who in the cellular infiltrate is a primary player of the pathology versus an "innocent" bystander. Therefore, targeting signaling molecules that are required for the activation of multiple immune cells may be the more likely route to success in combating these chronic, immune cell-mediated diseases. This review will highlight two such signaling molecules, the related lipid kinases, class I PI3K (phosphoinositide 3-kinase) δ and γ, both of which are primarily expressed in leukocytes and seem to play important roles in their function and activation.

This review will compare and contrast these two kinases as targets for autoimmune and inflammatory diseases and summarize efforts toward the development of small molecule inhibitors of PI3Kδ and PI3Kγ.[1−3]

Phosphoinositides (PIs) act as second messengers in a wide variety of cellular roles including signal transduction, control of membrane trafficking and transport, cytoskeleton organization, cell survival and death, among many other functions. The target of PI3K kinase activity is the phosphoinositides within cell membranes. Phosphoinositides are incorporated within the lipid bilayer of the membrane via two fatty acids that are attached to the cytosolic inositol ring via a glycerol phosphate linker. Cytosolic PI3K phosphorylates the inositol ring at the 3-position, converting PIP2 to PIP3[4] (Figure 1). It is through the phosphorylation of phosphoinositides that class I PI3Ks control growth, survival, and proliferation of the cells. PIP3 serves as the membrane anchoring site for multiple protein kinases[5] that contain pleckstrin homology (PH) domains, such as the serine/threonine kinases AKT (or PKB), PDK1, and the tyrosine kinases of the Tec family, BTK, ETK, and ITK.[6] Membrane bound AKT activates further downstream proteins such as mTOR, FOXO3a, and others. Calcium mobilization and gene transcription are dependent on the activity of Tec family members and other PIP3 binding effectors control cytoskeleton organization, membrane trafficking, and signaling efficiency. The phosphatase PTEN returns PIP3 to its precursor PIP2, serving as a regulator of PI3K activity.

PI3Ks are divided into three classes (I, II, III) based on functional and sequence homology. Although there is increasing interest in the class II and class III PI3Ks and their products, the majority of pharmaceutical research and the focus of this review are on the class I PI3Ks. Class I PI3Ks are further divided based on signaling pathways and regulatory proteins into class IA and class IB. The class IA PI3Ks comprise three closely related kinases, PI3Kα, PI3Kβ, and PI3Kδ, which exist as heterodimers composed of a catalytic subunit (p110α, p110β, or p110δ) and a regulatory subunit. These respond to signaling generally through receptor tyrosine kinases (RTKs). The class IB PI3Kγ signals through G-protein-coupled receptors (GPCRs) and is composed of a p110γ catalytic domain that can associate with regulatory subunits distinct from the class IA isoforms. Expression of the PI3Kα and PI3Kβ isoforms is ubiquitous, while the expression pattern of PI3Kδ and PI3Kγ seems more restricted, with both isoforms found primarily in leukocytes. The relatively restricted expression pattern of PI3Kδ and PI3Kγ, in addition to data accumulated from studies in mice, where PI3Kδ and/or PI3Kγ was either genetically or pharmacologically inactivated, suggests that these two isoforms play a major role in the adaptive and innate immune systems, respectively.

SMALL MOLECULE TOOLS FOR ELUCIDATING THE ROLE OF VARIOUS PI3K ISOFORMS

Two broad spectrum oral inhibitors, **1** (wortmannin)[17] and **2** (LY294002)[18] (Figure 2, Table 2), have been used to study the role of PI3K in various functions and disease states. With the identification of PI3K isoforms and the development of specific activity assays, inhibitors with varying selectivity profiles for one isoform over another have been discovered. Select compounds that have served as tool molecules to elucidate the role of PI3K isoforms are listed in Figure 3 and Table 2 along with their relative potencies against the various isoforms. Inhibitors with 60- to 1000-fold selectivity for PI3Kδ over the other three class I isoforms are **3** (IC87114)[19] and **4** (PIK-39).[19] Compounds have also been discovered that are selective for PI3Kγ such as **5** (AS-252424)[20] and **6** (AS-605240).[21] In addition, profiles that can be characterized as dual activity are observed for compounds such as **7** (TG100-115).[22] A challenge of drug

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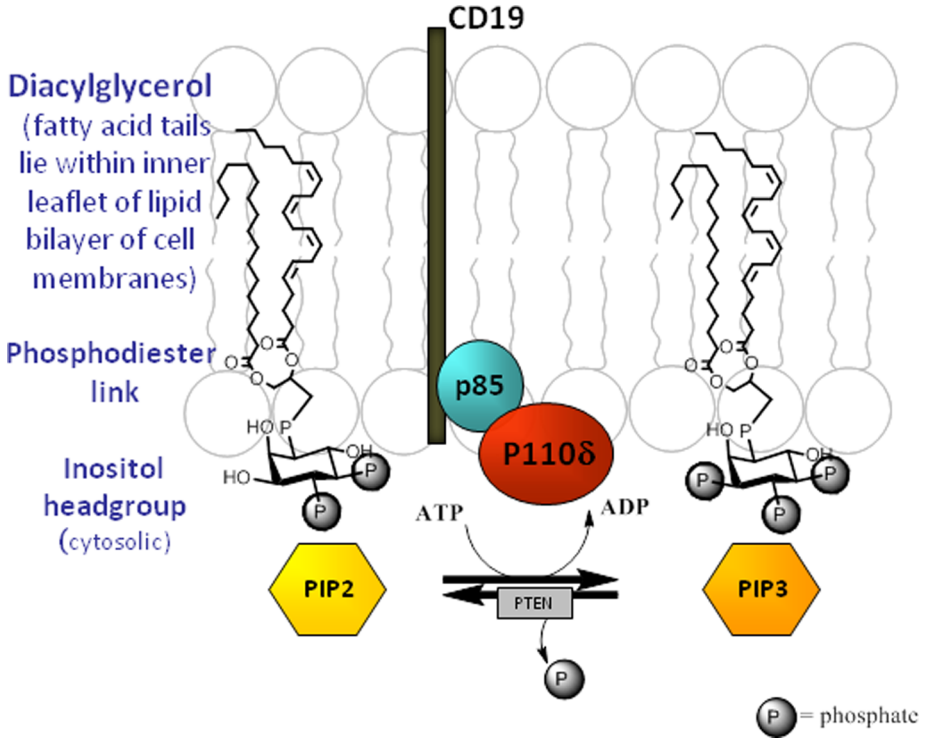
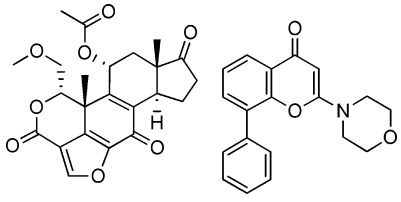


Figure 1. The function of PI3K (p85 regulatory, p110 catalytic domain) is to phosphorylate PIP2, converting it to the phosphoinositide PIP3. The phosphatase PTEN reverses the process. There are four types of class I PI3Ks: class IA, PI3Kα, -β, -δ; class 1B, PI3Kγ. PI3Kα and PI3Kβ are ubiquitously expressed, but PI3Kδ and PI3Kγ are primarily found in leukocytes.



(**1**) Wortmannin (**2**) LY294002

Figure 2. Nonselective PI3K inhibitors used in early biological studies.

Table 1. Cells and Cytokines Implicated in Pathogenesis of Disease

disease	main cell types and cytokines implicated in pathogenesis of disease
RA[7]	CD4$^+$ T-cells, macrophages, B-cells, SFs, IFN-γ, TNF-α, IL-6 , IL-12, IL-17, IL-23
SLE[8]	B-cells autoantibodies, T-cells, neutrophils, macrophages, basophils,
MS[9]	T-cells, B-cells, macrophages
psoriasis[10]	T-cells (T_h1), dendritic cells, neutrophils, IL-23, IL-17, IL-22, TNF-α, IFN-γ
COPD[11]	CD8$^+$ T-cells, neutrophils, macrophages, IgG autoantibodies, eosinophils
ALI/ARDS[12]	neutrophils, T-cells
asthma[13]	T_h2 cells, B-cells, mast cells, macrophages, IgE, histamine, IL-4, IL-5, IL-13, TNF-α, IL-1, eosinophils, macrophages, allergen-specific CD4$^+$ T-cells
IPF[14]	increases in collagen, fibrosis, TGF-β
IBD[15]	neutrophils, macrophages, CD4$^+$ T-cells
MI[16]	leukocyte migration

discovery is to discern which profile will lead to efficacy along with safety appropriate for the particular disease indication.

Table 2. Published IC_{50} (μM) Values for Tool Compounds

	PI3K isoform, IC_{50} (μM)			
compd	δ	γ	β	α
1[a]	0.009	0.005	0.014	0.001
2[b]	1.05	6.60	0.31	0.73
3[c]	0.13	61	16	>200
4[c]	0.18	17	11	>200
5[d]	>20	0.03	>20	0.940
6[a]	0.3	0.008	0.27	0.06
7[e]	0.235	0.83	1.2	1.3
8[a]	>20	0.25	>20	4.5
9[e]	1.7	0.07	1.45	0.24
10[c]	0.058	0.018	0.35	0.011
11[c]	0.048	0.15	0.088	0.008

[a]ATP concentration equals K_m.[21] [b]ATP concentration equals K_m.[33] [c]Tested at 10 μM ATP.[34] [d]Determined in triplicate.[20] [e]Commercial analysis.[35]

Highly PI3K-isoform selective compounds are likely to provide the safest bet for becoming drug candidates in conditions that require chronic dosing such as many autoimmune disorders. Although selectivity measurements are an important parameter to consider when developing a selective PI3K small molecule antagonist for chronic inflammatory conditions, physical parameters such as solubility and lipophilicity are variables that have an impact on overall ADME properties and also have to be considered when choosing a compound for further profiling. For example, although the PI3Kγ versus PI3Kα selectivity of **8** (AS-604850)[21] was superior to **6** (18 vs 7.5 respectively), compound **6** had a much improved PK profile and was hence chosen for further examination.[21]

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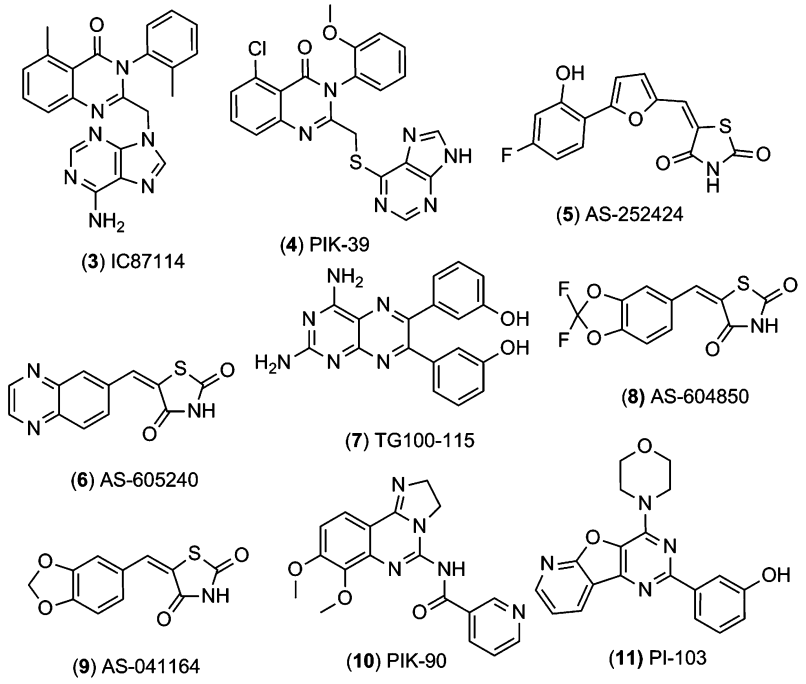

Figure 3. Early inhibitors of PI3Kδ and PI3Kγ.

The nonredundant roles of PI3Kδ and PI3Kγ in leukocyte function make them each attractive targets for intervention of immune-mediated diseases. Much has been learned from studies with mice where either or both isoforms were genetically disabled,[23−26] and knowledge of the KDKI or KO phenotypes informs interpretation of results in in vivo experiments using PI3K small molecule inhibitors.[27]

The PI3Kδ KDKI[28] mice show a leukocyte phenotype, with the most pronounced effects seen in B cell function. Thus, in the absence of δ-kinase activity, B-cell proliferation in response to B cell receptor stimulation is inhibited and their function as antigen presenting cells (APC) is impaired. Nearly 50% of the peripheral B-cells and follicular splenic B-cells are eliminated, and there are no MZ or B1 B-cells.[23] Effects observed in the PI3Kδ KO mice are similar though less severe, exhibiting impaired lymphocyte activation with developmental defects in B-cells and other cells.

B-CELLS AND PI3Kδ AS TARGETS FOR DISEASE THERAPY

Rituximab (Rituxan) treatment results in the depletion of B-cells, proving efficacious as therapy for non-Hodgkin's lymphoma. More recently, rituximab was also approved for treatment of RA,[29] for the first time implicating B-cells as drivers in this disease. The mechanism underlying the success of rituximab's ability to ameliorate autoimmune disease such as RA[30] is complex and not completely understood,[31] but B-cells are involved in cytokine production and the production of autoantibodies.[32] Their function as professional APCs in chronic inflammatory conditions may also be underappreciated. While PI3Kδ signaling is required for many functions of multiple leukocytes, its strong impact on B cell signaling alone suggests that inhibition of this PI3K isoform may impact the treatment of RA and other autoimmune disorders with B cell components such as lupus. Interestingly, PI3Kγ does not appear to have a role in B-cell activation or function.

T-CELLS: PI3Kδ AND PI3Kγ, TARGETS FOR DISEASE THERAPY

T-Cell function is profoundly affected in PI3Kγ and PI3Kδ KDKI and KO mice. For example, the differentiation of T_h1 cells, T_h2 cells, and T-cell proliferation to antigen is compromised in PI3Kδ KDKI mice, as is IL-10 mediated regulatory T-cell function, although no apparent effect on T-cell development has been noted.[23]

PI3Kδ plays a nonredundant role in T-cell function, making PI3Kδ inhibition an even more attractive target for RA, as well as other autoimmune diseases where T-cells are thought to play a key role in disease pathology.[36]

Work involving PI3Kγ KO mice demonstrated that PI3Kγ controls both the survival of thymocytes (progenitors of mature T cells) and the activation of mature T cells.[37] There is also a direct role for PI3Kγ in eliciting a TCR stimulated response sustaining T-cell and APC interaction.[38] Additionally, the migration of T-cells occurs after stimulation of PI3Kγ or other class 1A PI3Ks by certain chemokines.[39,40]

NEUTROPHILS AND OTHER LEUKOCYTES: PI3Kδ AND PI3Kγ

The role of neutrophils in inflammatory diseases such as RA, COPD, and SLE is well established.[28] Neutrophil migration to inflamed tissue and subsequent release of proteases and reactive oxygen species (ROS) cause tissue damage and further activation and recruitment of leukocytes. Although all four PI3K isoforms are expressed in neutrophils, experiments were performed using neutrophils from KO mice and antibodies specific for p110δ indicating that PI3Kδ and PI3Kγ are the main players in chemoattractant stimulated neutrophil or macrophage migration. Compound **3** was used to demonstrate that PI3Kδ plays an active role in the directional chemotaxis of neutrophil migration by limiting the (direct fMLP induced) formation of PIP3.[41] In vivo studies with **3** and PI3Kδ KO mice demonstrated a similar decrease of extravasation of neutrophils

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Table 3. PI3Kδ, PI3Kγ: In Vivo Models of Disease

model	species[a]	modality[b]	PI3K	notes, disease biomarkers	outcome	disease	ref
ovalbumin	m	KDKI	δ	IL-4, IL-5, IL-13	decreased	asthma	59
ovalbumin	m	**3**	δ	IgE levels, OVA-bound IgE	increased	asthma	59
ovalbumin	m	**3**	δ	IgE levels, AHR, IL-4, IL-5, IL-13	decreased	asthma	53
ovalbumin	m	**3**	δ	IL-17 expression	decreased	asthma	60
cigarette-smoke-induced glucocorticoid	m	KDKI	δ	(GR-α)/(HDAC-2)[c]	function restored	COPD	61
				lung neutrophils	≥50% decreased	asthma	
		KO	γ		no effects		
ovalbumin[d]	m	**7**	γ/δ	AHR	50% decrease	asthma	49
				lung eosinophils	79% decrease		
cigarette-smoke-induced pulmonary[d]	m	**7**	γ/δ	pulmonary neutrophilia	≥81% decrease	COPD	22, 49
LPS-induced pulmonary[d]	m	**7**	γ/δ	pulmonary neutrophilia	42−95% decrease	COPD	22, 49
OVA-specific pulmonary	m	KO	γ	cell infiltration (neutrophils, eosinophils, lymphocytes, macrophages)	decreased	asthma	46, 47
passive cutaneous anaphylaxis	m	**3**	δ	PCA response (anti-DNP IgE stimulation) and vascular permeability	decreased	allergy	44
		5	γ	PCA response (anti-DNP IgE stimulation) and vascular permeability	no effect	allergy	44
bleomycin pulmonary fibrosis	r	**6**	γ	BALF leukocyte content	decreased	IPF	62
				collagen content	decreased		
DSS colitis	m	KDKI	γ	clinical score vs control	decreased	IBD	63
				IFN-γ, TNF-α	increased		
DSS colitis	m	KO	γ	clinical score vs control	decreased	IBD	64
				IL-1β, TNF-α	decreased		
DSS colitis	m	**6**	γ	clinical score vs control	decreased	IBD	64
				IFN-γ, IL-1β, TNF-α	decreased		
K/BxN serum transfer	m	**3**	δ	paw diameter, histopath clinical score	strong response	RA	74
		3/KO	δ/γ	paw diameter, histopath clinical score	synergistic response		
		KO/KO	γ/δ	paw diameter, histopath clinical score	synergistic response		
CIA	m	**6**	γ	clinical score vs control	decreased	RA	21
CIA	r	**6**	γ	clinical score vs control	decreased	RA	21
αCII1A	m	**6**	γ	clinical score vs control	similar to WT	RA	21
AIA	m	**6**	γ	clinical score vs control	40% lower	RA	65
CIA	r	**3**	δ	clinical score vs control	decreased	RA	66
CIA	m	INK055[e]	γ/δ	clinical score vs control	decreased	RA	67
RANTES activation	m	**2**, **9**	γ	neutrophil migration into peritoneal cavity	decreased	RA	33
carrageenan paw edema	m	**9**	γ	paw swelling	decreased	RA	33
cytokine-dependent hTNFtg arthritis	m	KO × hTNFtg	γ	synovial mesenchymal fibroblasts	decreased prolif	RA	68
				MMP3	decreased prolif		
				cartilage damage	decreased		
				subchondral, bone erosion	decreased		
Miles assay vascular permeability	r	**7**	γ/δ	VEGF-induced vascular permeability	inhibited		69
				hindpaw volume	reduced ≥62%		
MI	r	**7**	γ/δ	60 min coronary artery occlusion[f]	≥40%[g]	MI	69
MI	p	**7**	γ/δ	90 min coronary artery occlusion[f]	35%[g]	MI	69
MI	m	**6**	γ	coronary artery ligation and infarct measurement at 14 days	increased infarct size	MI	70
LPS induced ALI/ARDS	m	**6**, KO	γ	aerosolized LPS; PMN transmigration, vascular permeability	similar effects	ALI/ARDS	12
EAE	m	**6**, KO	γ	MOG induced; CCL2, CCL5 levels in CNS	decreased	MS	71
				leukocyte rolling or adhesion	no effect		
MRL-lpr lupus	m	**6**	γ	glomerulonephritis	decreased	SLE	72

[a]Species m: mouse, r: rat, p: pig. [b]Inhibition modality. [c]GR-α: Glucocorticoid receptor-α, HDAC-2: Histone deacetylase 2. [d]Aerosolized delivery. [e]INK055 (structure not disclosed). [f]Reperfusion and infarct measurement at 24 h. [g]Percent reduction in infarct size.

toward sites of inflammation and the prevention of neutrophil adhesion to endothelium cells.[42] Nonetheless, PI3Kγ and PI3Kδ do act in concert in the migration, adhesion, and ultimate antimicrobial function of neutrophils. Neutrophils, T-

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cells, and B-cells are among many cell types that have been implicated in autoimmune and inflammatory diseases. Other cells that have been thought to play a role, specifically with asthma and atopic disorders, are mast cells and basophils, both of which are activated through their Fcε receptor (FcRε) by IgE bound to allergen. FcRε signaling is dependent on PI3Kδ, and its inhibition prevents mast cell and basophil degranulation.[43,44]

Comparable to PI3Kδ, the inhibition of PI3Kγ both pharmacologically and genetically had similar inhibitory effects on mast cell degranulation in vitro. For example, comparable inhibitory effect was observed for degranulation of bone-marrow derived mast cells (BMMC) stimulated with Ag/IgE in the presence of inhibitors **3** (PI3Kδ selective) and **5** (PI3Kγ selective, Figure 3 and Table 2). Degranulation of stimulated mast cells derived from KO or KDKI mice was similarly diminished.[20,45] PI3Kγ and PI3Kδ have effects on other leukocytes such as eosinophils. Eosinophil infiltration profoundly reduced in PI3Kγ KO animals compared to WT mice in a model of ovalbumin (OVA) specific pulmonary inflammation,[46,47] while PI3Kδ inhibition reduces eosinophil rolling and adhesion to endothelium.[48] The dual inhibitor **7** was found to be efficacious in an AHR response OVA-murine asthma model, reducing the level of lung eosinophils by 79%, similar to the effects with dexamethasone.[49] Other leukocytes have been implicated in inflammatory disorders (Table 1). In-depth discussions of how P3Kδ and PI3Kγ inhibition may ameliorate activity of these cells have recently been reviewed.[50−52]

TARGETED DISEASES AND EFFECTS OF PI3Kδ AND PI3Kγ INHIBITORS

The role of PI3Kδ and PI3Kγ signaling in leukocytes is complex. While it is clear that both isoforms play important roles in all leukocytes, the function of each isoform is often associated with signaling through specific cell surface receptors, which gives PI3Kδ and PI3Kγ distinct and often complementary functions within each cell type.

Several animal models, primarily for rodents, have been used in an attempt to establish a disease association with PI3Kδ and PI3Kγ signaling whereby kinase activity of one of the two isoforms was either genetically (KO, KDKI) or pharmaceutically (selective compounds) impaired. Several rodent models of RA have been used to show disease amelioration in the absence of either isoform.[21,33] Similarly, simulating aspects of asthma, acute lung inflammation models using OVA or fMLP as the stimulus were used to show disease modulating effects through inhibition of either PI3K isoform.[43,53−55] And last, transgenic mice with enhanced class IA PI3K activity have a lymphoproliferative disorder with systemic lupus-like symptoms[56] while PI3Kγ selective inhibitor **6** showed efficacy in a spontaneous mouse model of lupus. Many other models of autoimmune and inflammatory diseases have been tried, and disease modifying effects have been shown by inhibiting either PI3K isoform. Some of these are summarized in Table 3. Even though there are many preclinical data showing the impact of PI3Kδ or PI3Kγ in rodent models of human disease, we have to bear in mind that rodents are in every aspect quite distinct from humans. In addition, few of these rodent models are spontaneous and chronic in nature but are instead induced by administration of specific antigens leading to acute inflammation. While these models may reflect certain aspects of human disease, they do not accurately reflect the complexity and heterogeneity associated with human disease pathology. So despite the intriguing biology associated with both PI3K isoforms that holds promise for inflammatory disorders and despite the multitude of preclinical models that seem to substantiate that promise, we will have to await the outcome of clinical trials to determine whether selective inhibitors of PI3Kδ or PI3Kγ are indeed safe and efficacious.

SAFETY CONCERNS FOR PI3Kγ AND PI3Kδ AS A TARGET FOR INFLAMMATION AND AUTOIMMUNE DISEASE

Death of the PI3Kγ KO mice was observed when high doses of vaccinia virus (VV) were employed in a model examining the migratory ability of CD8 T-cells. In contrast, WT mice had little trouble clearing the infectious agents at the same viral loads. Infection is a concern for PI3Kγ inhibitors in any indication.[57] There are also potential concerns with targeting PI3Kδ, as the PI3Kδ deficient mice develop a mild, subclinical IBD/colitis phenotype possibly due to suppression of IL-10 in these mice.[23] Whether this phenotype would be seen with a selective compound and whether it will translate into humans are currently not known. There is evidence in PI3Kδ KDKI mice that points to suppression of some T_{reg} cell functions; this could impact autoimmunity.[58] There are several publications suggesting that PI3Kδ inhibition will lead to an increase in IgE, which may be counterindicative to using these inhibitors for the treatment of allergic diseases.[59] However, there does seem to be conflicting evidence for this outcome[53] (Table 3).

DUAL INHIBITION OF PI3Kδ AND PI3Kγ

Although PI3Kγ and PI3Kδ inhibitors have shown promise in preclinical disease models and suggest that a dual inhibitor may have synergistic affects, it remains unclear whether a dual inhibitor would be safe. Mice in which both isoforms are inactive have profound reductions in thymus size, and total thymus cell count. Their peripheral T cell compartments are almost completely absent,[73] while mice with individual KOs of p110γ and p110δ have relatively normal thymi and thymocytes. In addition, crossing the p110γ KO with a p110δ KDKI[26] leads to not only severe lymphopenia but also homeostatic peripheral expansion of T_h2 cells, with subsequent type 2 inflammation in various organs. This is characterized by increases in IgE secretion in serum and eosinophilic infiltration into the mucosal organs leading to pronounced inflammation. However, although the PI3Kγ/δ deficient mice developed homeostatic peripheral expansion of T_h2 cells, dual pharmacological inhibition did not increase the T_h2 response but decreased the T_h1 cytokine release. It is possible therefore that the observations in genetically dual-deficient mice could result from developmental defects that would not be recapitulated in mature animals under pharmacological intervention. To substantiate that notion, in a murine inflammatory arthritis model using PI3Kγ KO mice dosed with **3** a pronounced synergistic effect on disease amelioration was shown.[74]

INHIBITORS OF PI3Kγ AND PI3Kδ

A great deal of effort in the past several years has been directed at discovering selective inhibitors of PI3Kδ and PI3Kγ. Many institutions have entered the fray, and the field has expanded rapidly. Additionally, dual inhibitors have also become an attractive target for some.[75]

Table 4. Potency and Isoform Selectivity: Examples of PI3Kδ Inhibitors from the Literature[a]

compd	organization	biochemical PI3Kδ, IC_{50} (μM)	biochemical selectivity γ/δ	α/δ	β/δ	cellular PI3Kδ, IC_{50} (μM)	refs and notes
3	Gilead[152]	0.5	58	>200	150		200 μM ATP
12	Roche	0.003	25	1	11		
13	Roche	<0.1	>20				
14	Roche	0.0049 (K_i)		101		0.017	cell: HWB CD69; WO2006046035
15	Roche	0.00046 (K_i)		256		0.003	cell: HWB CD69; WO2012007493
16	Roche	0.000576 (K_i)		175		0.0052	cell: HWB CD69; WO2012007493
17	Roche	0.00343		>350		0.041	cell: HWB CD69; WO2011101429
18	Roche	0.00274		64		0.0178	cell: HWB CD69; WO2010138589
19	Novartis	0.005	10−40	10−40	10−40	0.015	WO2008000421
20	Lilly	0.040	187	1447	126		*S*-isomer preferred
21	Lilly	0.006	28	528	123		*S*-isomer preferred
22	Lilly		≥10	≥10	≥10	0.665	cell: neutrophil (PMN) elastase release
23	Lilly		≥10	≥10	≥10	0.665	as above (typical example)
24	Amgen					0.002	cell: B-cell proliferation; WO08118454
25	Amgen					0.0005	cell: B-cell proliferation; WO09118468
26	Amgen	0.00285 (K_i)					WO2010151791
27	Amgen	0.00105 (K_i)					WO2010151737
28	Amgen	0.00226 (K_i)					WO2010151737
29	Amgen	0.005 (K_i)					WO2010151735
30	Amgen	0.1059					US 7705018, WO2008118455
31	Amgen	0.0002 (K_i)					WO2011123751
37	Takeda	<0.1	≥1				(typical example) WO09046448
38	Takeda	<0.1	≥1	≥10	≥100		potent in a rat CIA model 60 mg/kg b.i.d.
39	Takeda	<0.1	>100	>100	>100	<0.1	Cell: B-cell proliferation; WO2010129816
40	Takeda	<0.050		≥20	≥20		WO2010006086, potent against m-TOR
41	Takeda	0.040	2.8	1.3	16.6		ref 111
42	Takeda	0.001	185	788	23		ref 111WO2010036380
43	Takeda	0.0005	8	274	18		ref 111, WO2010036380
44	Takeda	0.0003	24.7	3643	1157	0.500	cell: (EC_{50}) proliferation assay[109]
45	UCSF	0.010	16	1000	49		ref 111
46	UCSF	0.0007	47	1857	314		ref 111
47	UCSF	0.007	186	12000	107		ref 111
52	AUL[b]	>0.1		1.0−10	≥10		WO2009120094

[a]For structures, see Figures 3−6. [b]Auckland Uniservices Ltd.

It is difficult to discern what constitutes a selective inhibitor. In general, selectivities from 10- to 50-fold seem a reasonable starting point, but some examples may fall below this threshold. The categorization is complicated by the fact that precise IC_{50} values are lacking from the many published patent applications. Refer to Tables 4−7 for the summary of data (references) for structures found in Figures 4−9.

PI3Kδ SELECTIVE INHIBITORS

Roche/Genentech/Piramed has been active in developing PI3K inhibitors, although most patent applications disclose compounds specifically targeting PI3Kα. Compound **12** (GDC-0941)[76] (Figure 4 and Table 4), which entered phase II clinical trials for the treatment of metastatic breast cancer and non-small-cell lung cancer,[77] is a prominent example.[78] Several patent applications showcase a series of structurally related thienopyrimidines as PI3Kδ selective inhibitors such as **13** (Figure 5).[79,80] Some of these compounds are highly soluble (40−100 μM) with low hepatocyte clearance, reasonable permeability, and low CYP-450 inhibition and induction activity. They are potent in a biochemical assay (PI3Kδ, IC_{50} < 0.1 μM) with generally <90% plasma protein binding and selectivity of >20-fold over PI3Kγ in some cases. Morpholinothienopyrimidine **14**[81] also has low micromolar activity in a PI3Kδ human whole blood (HWB) assay. These compounds have efficacy in a CIA model, although no detail is provided.[82] Additional compounds from Genentech/Roche, benzoimidazolpurine **15**,[83] indolpurine **16**,[83] and indolpyridopyrimidine **17**[84] are very potent in enzyme and HWB assays. Morpholinofuropyrimidine **18**[85] is one of the more potent analogues described in an application exemplified by 11 analogues having IC_{50} values in a HWB assay of 0.0178−0.0659 μM. Additionally, selectivities over PI3Kα in an enzyme assay are reported, from 14- to >300-fold.

Other PI3Kδ selective inhibitors have been disclosed by Novartis, Lilly, Amgen, Takeda (Intellikine) UCSF, UCB Pharma, Auckland Uniservices, Zenyaku Kogyo, Xcovery (Tyrogenex), Chemical Diversity Research Institute, Serono Astellas Pharma, Karus Therapeutics, GlaxoSmithKline, Incyte, Incozen/Rhizen, Infinity, Exelixis, Respirvert, and Gilead (formerly Calistoga, formerly ICOS). Novartis has claimed a group of compounds exemplified by thiazolylurea **19**.[86] This compound is potent in a scintillation assay of PI3Kδ activity (IC_{50} = 0.005 μM) with a selectivity profile over the other isoforms of 10- to 40-fold. Additionally, **19** has a potency of 0.015 μM in a B-cell proliferation assay. Lilly described

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Table 5. Potency and Isoform Selectivity: Examples of PI3Kδ Inhibitors from the Literature[a]

compd	organization	biochemical PI3Kδ, IC_{50} (μM)	biochemical selectivity γ/δ	α/δ	β/δ	cellular PI3Kδ, IC_{50} (μM)	refs and notes
53	Zenyaku Kogyo	0.0046	10.6	3.5	9.5		WO2002088112
54	Xcovery	<0.03	>0.1	>0.1	>0.1		WO2010005558
55	CDRI[b]	2.1	33.8	6.0	38		WO2009011617
56	EMD Serono	0.027				0.016	cell: pAKT of B-cells; WO2007023186
57	EMD Serono	0.090	111	4.4	111		ref 111, WO2007023186
58	EMD Serono	0.034	588	101	124		ref 111
59	Astellas Pharma	0.0036		444		0.00062	cell: anti-IgM B-cell thymidine incorporation
61	Karus	0.26	>38	>38	13		WO2011135351
62	GlaxoSmithKline	pIC_{50} > 7.0	>10	>10	>10		WO2010125082
64	GlaxoSmithKline	pIC_{50} > 7.0	>10	>10	>10		WO2010125082
65	Incozen/Rhizen	<0.25	*d*	*d*	*d*		
66	Respivert	0.004	72	492		0.57	cell: H_2O_2 induced pAKT; WO 2011048111
67	Incyte	<0.05	>10	>10	>10	<0.05	cell: B-cell proliferation; WO2011008487
68	Incyte	<0.2				<0.5	cell: B-cell proliferation; WO2011075643
69	Incyte	<0.1				<0.1	cell: B-cell proliferation; WO2011130342
70	Incyte	<0.05					US20110312979
71	Incyte	<0.05				<0.05	cell: B-cell proliferation; WO2011075630
XL499[c]	Exelixis	0.0075	190	96	111	0.066	cell: inhibition of pAKT(T308) (Raji cells)
72	Exelixis	<0.05					WO2012037226
73	Exelixis	<0.05					WO2012037204
74	Gilead	0.009	21	248	49	0.0061	cell: (EC_{50}) human B lymphocyte
75	Gilead	0.007 (K_i)	61	2963	119		WO2011011550

[a]For structures, see Figure 7. [b]Chemical Diversity Research Institute. [c]Structure not disclosed. [d]<25% inhibition at 1 μM

Table 6. Potency and Isoform Selectivity: Examples of PI3Kγ Inhibitors from the Literature[a]

compd	organization	biochemical PI3Kγ, IC_{50} (μM)	biochemical selectivity δ/γ	α/γ	β/γ	refs and notes
76	Chemical Diversity Research Institute	0.0059		6339	5915	WO2009011617
77	Serono	0.013				US20080188531
78	Pfizer	0.054	6.3	0.4	20.4	10 μM ATP[34]
		0.001				20 μM ATP, WO2004052373
79	Pfizer	0.003				20 μM ATP, WO2004108713
80	Pfizer	0.197				Streptococcal cell-wall-induced rat paw edema model ID_{50} = 16.3 mg/kg (30 mg/kg 52% inhibition)
81	Cellzome	<0.1	>10	>10	>100	WO2010092015, WO2008025821
82	Vertex	0.1 (K_i)				WO2008027584
83	Vertex	0.003 (K_i)				WO2010096389
84	Vertex	0.01 (K_i)		1−10		WO2009129211
85	Vertex	0.003 (K_i)		<33		WO2010135014
86	Novartis	<2.0				WO2007134828
87	Novartis	0.016	40	189		WO2005021519, US2008280871
88	Novartis	0.012				WO2009115517
89	Bayer	<0.1			1− 5	WO2004029055
90	Chroma Therapeutics	<0.1				WO2007129048
91	Boehringer Ingelheim	<0.6				US20090093474
92	Applied Research Systems	0.006				cell: pAKT macrophages, ELISA; <0.010 μM, WO2006024666
94	Shionogi	0.027				WO2010027002
95	Exelixis	0.018	24.2	38.3	114.4	cell IC_{50} = 0.773 μM[170]
96	Exelixis	0.034	10.1	12.2	33.8	cell IC_{50} = 0.805 μM[170]

[a]For structures, see Figure 8.

thienopyrimidinones **20**, **21**,[87] pyridopyrimidinone **22**, and pyrazolopyrimidinone **23**,[88] similar in structure and isoform selectivity to **3**. Amgen has compounds containing a central core quinoxaline **24**[89] or quinoline **25**[90] and a purine or pyrazolopyrimidine side chain. No selectivity data are given though the compounds listed have cellular activity down to 0.0005 μM. Compounds with biochemical potency against PI3Kδ (K_i) in the single digit nanomolar range are exemplified by quinolines **26**,[91] **27**,[92] **28**,[93] and pyridopyrimidinone **29**.[94] Compounds **28** and **29** contain an unusual aminopyrimidine

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Table 7. Potency and Isoform Selectivity: Examples of PI3Kδ/γ Inhibitors from the Literature[a]

compd	organization	biochemical PI3Kδ, IC_{50} (μM)	biochemical selectivity γ/δ	α/δ	β/δ	cellular PI3Kδ, IC_{50} (μM)	refs and notes
7	TargeGen	0.235	3.5	5.5	5.1		
97	UCB Group	0.032	2.4	9.2	16.9	0.111	cell: neutrophil inhibition superoxide
98	UCB Group	0.139	0.77	5.3	9.5		
99	UCB Group	0.014	3.7				
100	Millenium	<0.1[b]					WO2009154741
101	Millenium	<0.1[b]					WO2009154741
102	GlaxoSmithKline	$pIC_{50} \geq 5$[b]				$pIC_{50} \geq 5$[c]	cell: human PBMC
103	GlaxoSmithKline	$pIC_{50} \geq 5$[b]				$pIC_{50} \geq 5$[d]	cell: pAKT T-cells; WO2009147190
104	GlaxoSmithKline	$pIC_{50} \geq 5$[b]				$pIC_{50} \geq 6$[d]	cell: pAKT T-cells; WO2009147189
105	GlaxoSmithKline	$pIC_{50} \geq 5$[b]					WO200912958
106	GlaxoSmithKline	$pIC_{50} \geq 5$[b]				$pIC_{50} \geq 6$[d]	cell: pAKT T cells; WO2009147188
107	GlaxoSmithKline	$pIC_{50} \geq 5$[b]					WO2011067365
108	GlaxoSmithKline	$pIC_{50} \geq 5$[b]					WO2011067366
109	UCSF	0.009	2.2	1000	77		ref 111
110	Takeda	<0.1	IC_{50} < 0.1	>10	>10	<0.1	WO2009088990
INK055[e]	Takeda	0.005	1.8	>2000	>2000		refs 67, 109

[a]For structures, see Figures 3 and 9. [b]PI3Kδ and/or PI3Kγ. [c]Presumed PI3Kδ. [d]Presumed PI3Kγ. [e]Structure not disclosed.

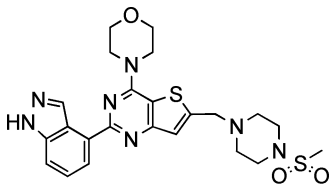

Figure 4. 12 (GDC-0941).

side chain. Amgen has also published patent applications on many other novel structures, some with a pyrimidine instead of a purine but all with diverse core moieties. These are exemplified by quinoline **30**,[95] benzimidazole **31**,[96] thienopyridine **32**,[97] 1,5-naphthyridine-4-carboximide **33**,[98] and quinolines **34**,[99] **35**,[100] and **36**,[101] although no biological data were provided. Amgen has advanced one PI3Kδ inhibitor AMG-319 (structure not disclosed) into phase I clinical trials for lymphoid malignancies.[102] Intellikine (now Takeda) specialized in PI3K and m-TOR inhibition and indicated that they have selective PI3Kδ inhibitors.[103] The relevant structures were not disclosed, but they have described various substituted quinazolines such as 2-methylaminoquinazoline **37**.[104] Selectivity for PI3Kδ and PI3Kγ relative to the other isoforms and also m-TOR is claimed, although no specific biological data are provided. Methylisoquinolinone **38**[105] is the most studied analogue in an application that deals with a series of isoquinolinones, although **38** could be considered a dual PI3Kδ/PI3Kγ inhibitor. There is some interesting biological data, including a CIA model with a pronounced reduction in mouse ankle diameter and histopathological score at the 60 mg/kg dose. Other examples in the applications have a greater level of selectivity favoring PI3Kδ. Another application describes isoquinolines such as **39**[106] with potencies of <100 nM against PI3Kδ and selectivities over the other isoforms of >100-fold in an enzyme assay. A series of compounds similar in structure to **38** but with a small group such as an isopropyl or cyclopentane replacing the isoquinolinone functionality have also been claimed by Takeda (Intellikine). These are exemplified by pyrazolopyrimidine **40**.[107] Although **40** is selective against PI3Kα and PI3Kβ, it is also potent toward mTOR (<0.05 μM). Compounds of this type were reported to be active in a lupus model, perhaps indicating that they are not all that selective over PI3Kγ.[108] An example with a more extensive profile is the isoquinolinone **44** (INK007).[109] This compound was found to be potent and selective and inhibited the growth of PDGF induced FLS (fibroblast-like synoviocytes). Shokat and co-workers at UCSF have revealed a series of selective PI3Kδ inhibitors, exemplified by quinazolinones **45**,[110] **46** (SW-13),[110] and **47** (SW-30),[110] that directly target the PI3K affinity pocket, enhancing potency considerably.[111] UCB Pharma has described a series of quinolines, as PI3Kδ inhibitors exemplified by **48**,[112] **49**,[113] **50**,[114] and **51**,[115] but no biological data were provided. Auckland Uniservices Ltd., working to develop PI3Kα inhibitors containing triazine and pyrimidine, also discovered several compounds that have reasonable selectivity toward PI3Kδ, as exemplified by the morpholinotriazine **52**.[116] Zenyaku Kogyo has been developing compound **53** (ZSTK474)[117] for oncology that has potent antitumor activity in vivo.[118] It has some selectivity toward the PI3Kδ isoform.[119] Compound **53** also demonstrated efficacy in a CIA model of RA, both in a semitherapeutic and therapeutic setting.[120] Xcovery has disclosed compounds with selectivity toward PI3Kδ. One of their most potent and selective compounds is the morpholinopurine **54**.[121] The Chemical Diversity Research Institute revealed substituted pyrimidinones, including pan inhibitors as judged by the selectivity data. There are examples, however, that exhibit strong selectivity toward PI3Kδ or PI3Kγ exemplified by furopyrimidinones **55** and **76**[122] (Figures 7 and 8, respectively). This is an interesting example of a small change in structure having a large impact on the selectivity characteristics, the difference being a 4-OMe-phenyl group versus an unsubstituted benzyl group. The changes in **76** increased potency toward PI3Kγ and increased selectivity over the PI3Kα and PI3Kβ isoforms. Serono has been active in discovering

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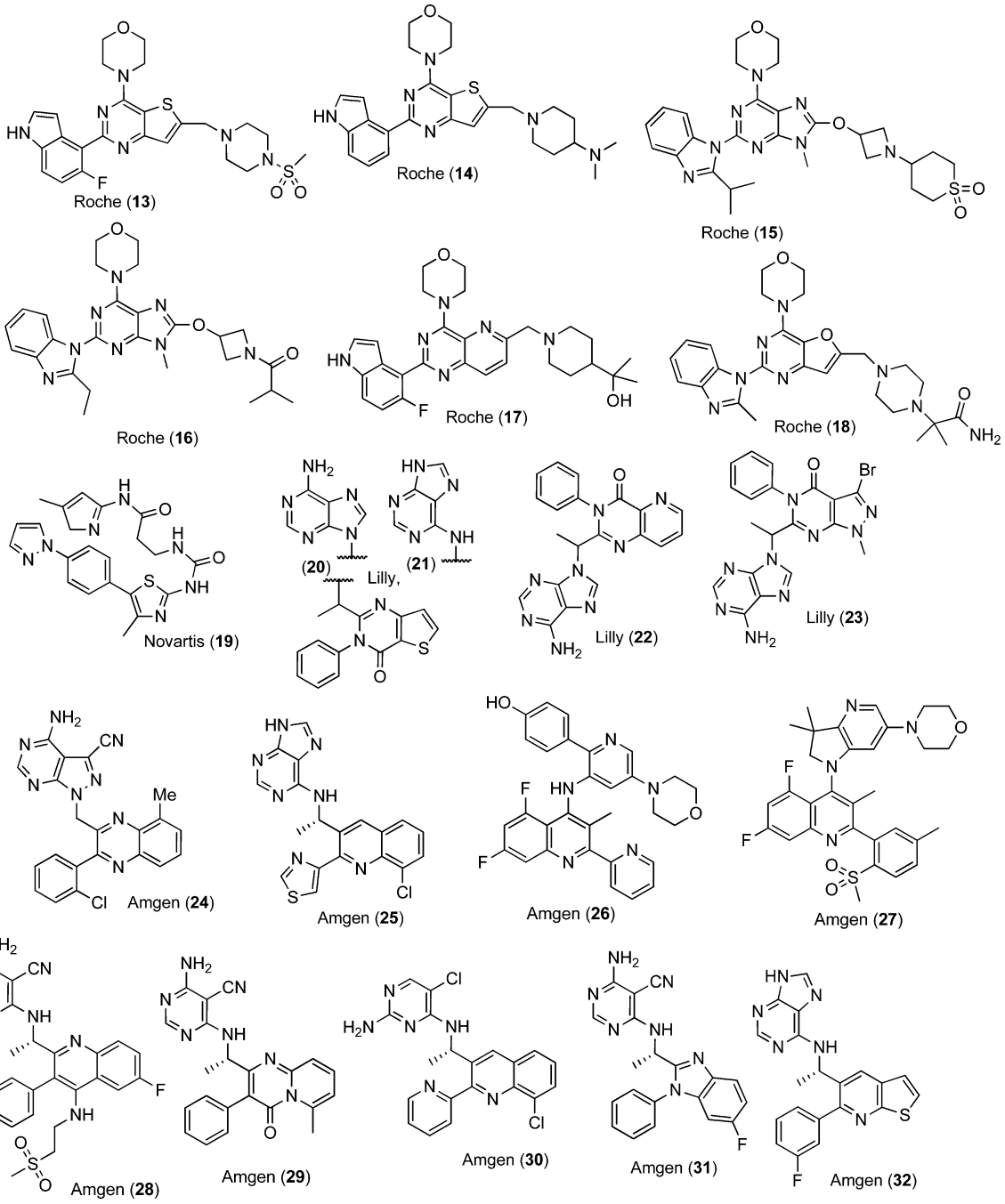

Figure 5. Examples of PI3Kδ inhibitors from the recent literature (see Table 4).

compounds that inhibit PI3Kδ and PI3Kγ. Quinoxaline **56**[123] is a typical example, which is potent in a biochemical assay and also active in an anti-IgM stimulated peripheral blood mononuclear cell (PBMC) cellular assay. Several compounds including **41** (DL06),[111] **42** (INK654),[124] **43** (INK666),[124] **46**, **47**, **57** (AS5),[123] and **58** (AS15)[111] have been extensively profiled including multiple PI3Kδ cocrystal structures from researchers at the University of Cambridge, UCSF, Takeda (Intellikine), and Merck-Serono.[111] Quinoxaline **57** has a high level of PI3Kδ selectivity over the PI3Kγ and PI3Kβ isoforms. In addition these workers also studied **42** a quinoline analogue that has PI3Kδ selectivity relative to PI3Kγ and PI3Kα, less so to PI3Kβ. Tetrahydroquinazolinone **58** has an unusual five-bond linkage from the core moiety to a pendent quinoxaline (see next section). Astellas Pharma has examples of potent PI3Kδ inhibitors. One of their most potent is morpholino-triazine **59**[125] with an IC_{50} in B-cells of <1 nM. Karus has disclosed compounds such as morpholinobenzoxazinone **60**[126] and indoylisoquinoline **61**[127] that may be PI3Kδ and/or dual PI3Kδ/PI3Kβ inhibitors. No biological data were provided for **60**, but **61** is 13-fold selective over PI3Kβ and >38-fold over PI3Kγ and PI3Kα. Karus has revealed that they are pursuing two inhibitors KAR4139 (structure not disclosed), a dual PI3Kδ/PI3Kβ inhibitor, and KAR4141(structure not disclosed), a selective PI3Kδ inhibitor for cancer/inflammation and inflammation, respectively.[128] GlaxoSmithKline has disclosed compounds[129] such as indoylindazole **62**[130] and furo[3,2-*b*]pyridineindazole **63**[131] that are potent toward

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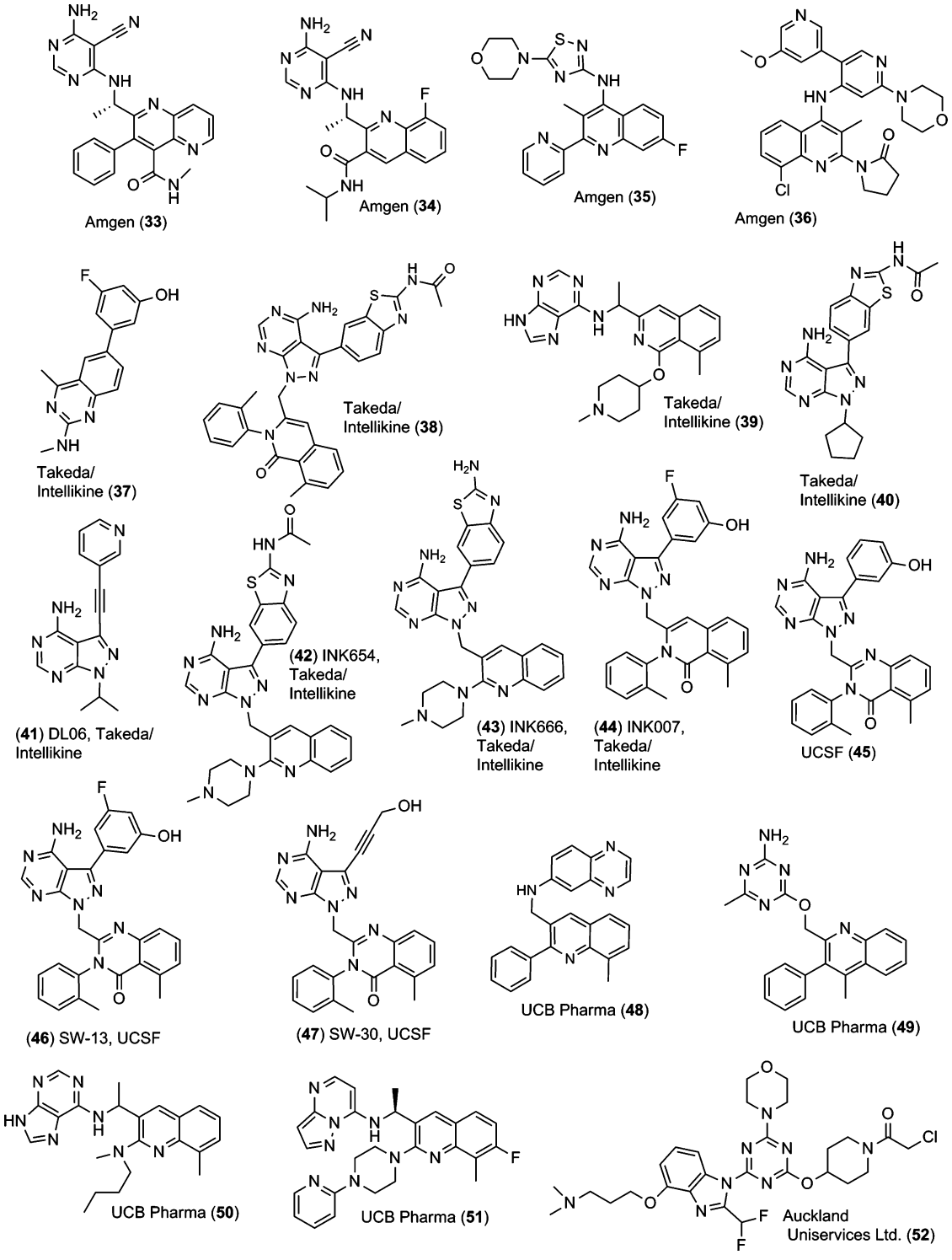

Figure 6. Examples of PI3Kδ inhibitors from the recent literature (see Table 4).

PI3Kδ. Compound **62** is at least 10-fold selective over the other isoforms. Additionally, they have recently disclosed various salt forms and polymorphs of oxazoloindazole **64**,[130,132] another inhibitor with greater than 10-fold PI3Kδ selectivity. Interestingly, GlaxoSmithKline has started a phase I clinical trial targeting asthma with GSK2269557 (structure not disclosed).[133] Incozen/Rhizen has described a patent that showcases a series of 2,3-substituted 4*H*-chromen-4-ones such as **65**[134] with potency toward the PI3Kδ isoform. Respirvert disclosed quinoxoline compounds such as **66**,[135] which is potent against PI3Kδ and selective over PI3Kγ and PI3Kα. Incyte has also been active in discovering PI3Kδ inhibitors. They describe at least five compounds, which have potency in a B-cell proliferation assay of less than 50 nM. These compounds, including the thiazolopyrimidinone **67**,[136] are potent on an enzyme assay with at least 10-fold selectivity over all the other isoforms. Compound **67** was potent (<50 nM) in a proliferation assay using a diffuse large B-cell lymphoma cell line. Incyte has additional patent applications with a six−five fused core, including both imidazo[1,2-*a*]pyridine **68**[137] and

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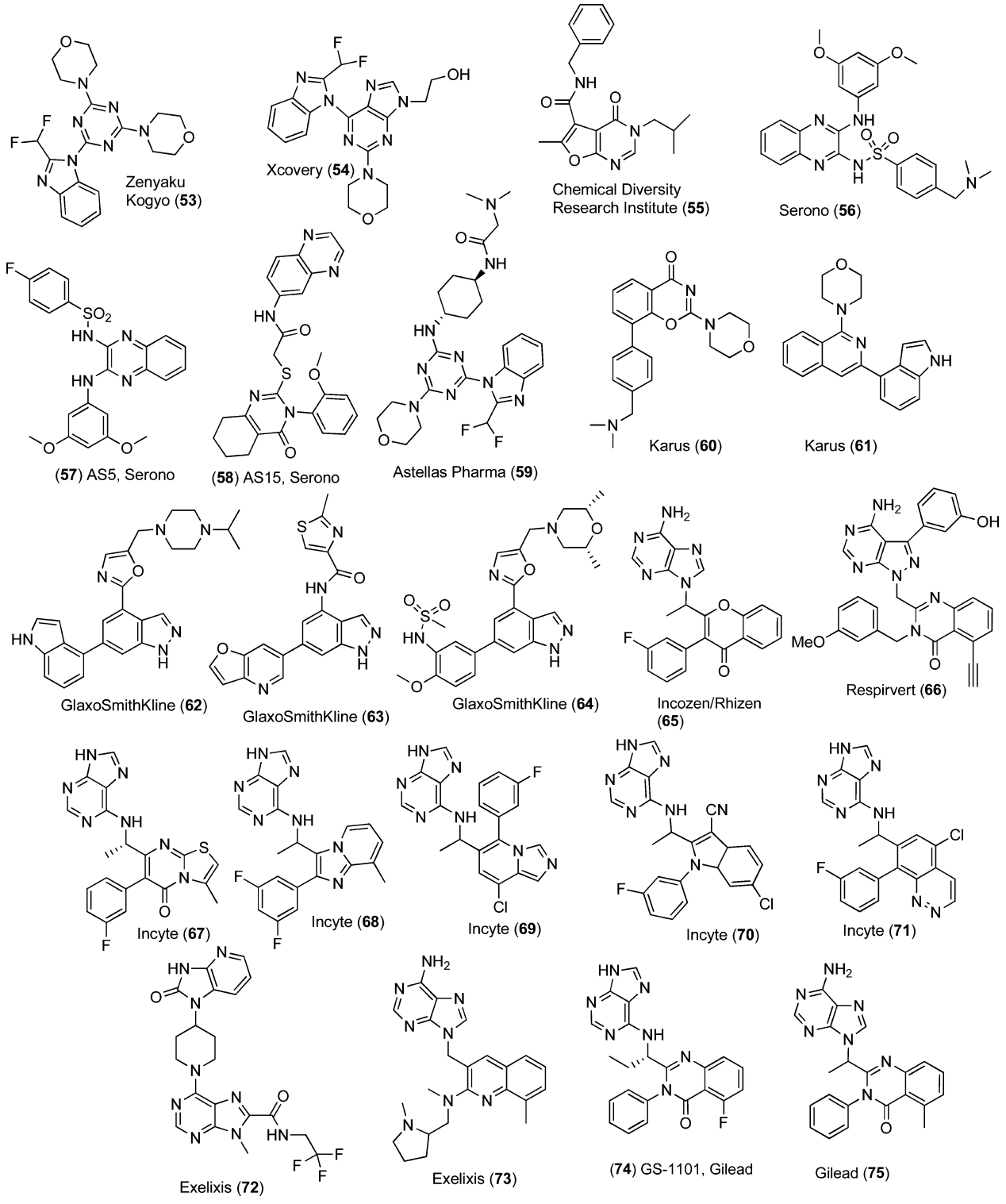

Figure 7. Examples of PI3Kδ inhibitors from the recent literature (see Table 5).

imidazo[1,4-*a*]pyridine **69**.[138] These were found to be potent in a B-cell proliferation assay at <500 and <50 nM, respectively. Additional patent applications from Incyte have exemplified indolylpurine **70** and cinnolinylpurine **71**.[139] The key feature in these applications remains the pendent ethylaminopurine. Pathway Therapeutics claims to have an inhibitor that is potent against PI3Kδ and >100-fold selective.[140] No structures are disclosed, but their previous patent literature indicates compounds that have pan-inhibitor character reminiscent of **53** and **54**. Exelixis has partnered with Merck in the development of XL499 (structure not disclosed), a selective PI3Kδ inhibitor targeting hematological malignancies and inflammation. Although the structure has not been disclosed, data surrounding this compound have been released.[141] The compound demonstrated high selectivity and reasonable PK, *F* of 48−99.9% in animals (cynomolgus monkey, rat, mouse, dog). It was potent in cell assays and displayed effectiveness in a passive cutaneous anaphylaxis mouse model. The compound

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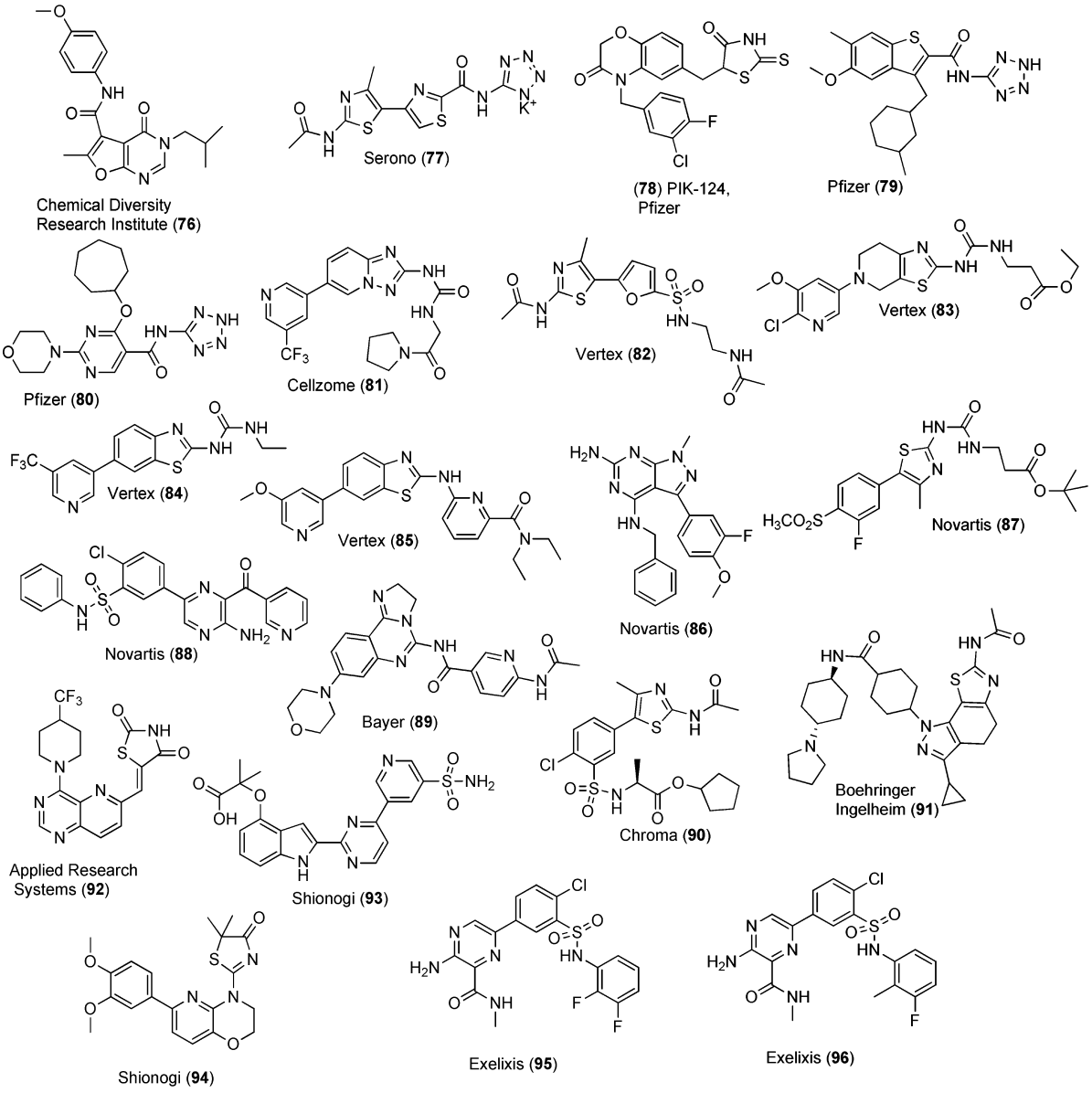

Figure 8. Examples of PI3Kγ inhibitors from the recent patent and other literature (see Table 6).

appeared well tolerated in a 14-day rat toxicology study with no clinical signs or significant change in animal weight. Exelixis has described specific PI3Kδ inhibitors in two patent applications. The first lists a series of 6,9-disubstituted purines that generally incorporate a 6-piperidinylimidazopyridine such as **72**.[142] Of the 397 compounds described, 146 inhibit PI3Kδ at <50 nM. The other patent application describes 191 analogues of which 40 have potency toward the PI3Kδ isoform of less than 50 nM. Quinoline **73**[143] is such an example.

The most advanced group remains Gilead (formerly Calistoga) with two compounds in clinical trials. Quinazolinone **74** (GS-1101 formerly CAL-101)[144] is a PI3Kδ selective compound that has been in several phase I/II trials for hematologic malignancies, including combination therapy with rituximab.[145,146] Promising results have been disclosed. In a phase I study involving 57 patients with hematological malignancies (NHL, CLL, AML) significant clinical response rates were seen at doses of 50, 100, 200, and 350 mg b.i.d., although there was variability among the types of malignancies. For example, although there was no improvement in the AML cohort, response rates of 56% was found in the NHL cohort. The dose limiting toxicity was a reversible increase of serum transaminases.[147] More clinical data relating to **74** are emerging, but some aspects of the compound itself require elaboration. The compound was found to be well tolerated at 400 mg q.d. and 200 mg b.i.d. for up to 7 days of dosing in healthy volunteers. In patients with lymphoid malignancies dose levels of 350 mg were tolerated for several months. An MTD was not reached, but exposures were not dose proportional at these levels. There were minimal gains in plasma exposure beyond a dose of 150 mg b.i.d. Steady state trough levels from b.i.d. dosing of ≥150 mg could be maintained at >10-fold over the HWB EC_{50} of 62 nM.[148] The PI3Kδ inhibitor **74** has entered phase II trials for CLL in combination therapy with rituximab. It was also examined in a phase I trial of allergic rhinitis involving doses of 100 mg b.i.d. for 7 days.[149] Another compound CAL-263 (structure not disclosed) presumed to be an analogue of **74** seems to be intended primarily for inflammatory diseases. This drug candidate was also entered in a similar allergic rhinitis phase I

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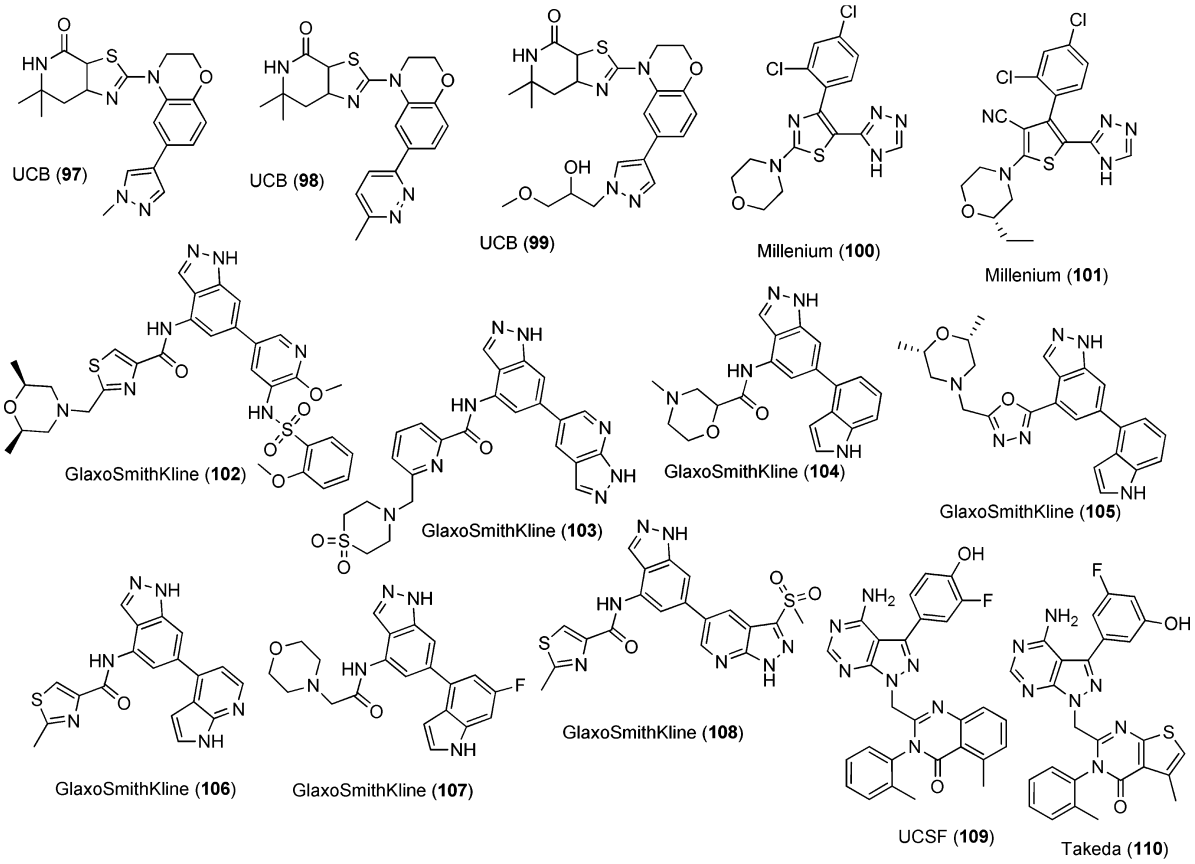

Figure 9. Examples of PI3Kδ/γ inhibitors from the literature (see Table 7).

trial and at >10-fold lower dose (10 mg q.d.).[150] Gilead (Calistoga) has recently published a patent application exemplified by the potent and isoform selective quinazolinone **75**[151] for use in certain liver disorders.

■ PI3Kγ SELECTIVE INHIBITORS

Selective inhibitors of PI3Kγ are also of great interest. In addition to the Serono compounds **5**, **6**, **8**, and **9** (AS-041164), Serono has also published on bis-thiazoles. Tetrazole **77**[153] is one of their potent examples. Pfizer/Warner Lambert has several published patent applications, with benzoxazinone **78**[154] and benzothiophene **79**[155] as representative examples. The selectivity over PI3Kα may be an issue, as **78** indicates (α/γ = 0.4, Table 6). However, pyrimidine **80**[156] demonstrated reasonable activity in a Streptococcal cell wall (SCW) paw edema model of arthritis and had activity in a CIA model at 30 mg/kg (52% reduction in paw edema). Cellzome has described compounds that have potency against PI3Kγ (<0.1 μM) and various selectivity over the other isoforms, with some examples of dual PI3Kγ/δ inhibitors. Many of these compounds have selectivity over the PIKK DNA-PK. Their most potent and selective analogue reported is triazolopyridine **81**.[157] Vertex has examples of potentially PI3Kγ selective inhibitors such as furylthiazole **82**,[158] tetrahydrothiazolopyridine **83**,[159] benzothioazolyl-urea **84**,[160] and benzothiazolylamine **85**.[161] Novartis has patent submissions on PI3Kγ since 2003. The diaminopyrazolopyrimidine **86**[162] had some potency toward the PI3Kγ isoform, but thiazole **87**[163] had an IC_{50} of 16 nM and was 39-fold selective over PI3Kδ and 189-fold selective over PI3Kα, although no selectivity data were provided against PI3Kβ. In 2009 they disclosed a series of substituted pyridines or pyrazines such as aminopyrazine **88**[164] that appear quite potent against PI3Kγ. Bayer, Chroma Therapeutics, and Boehringer Ingelheim have programs aimed at discovering PI3Kγ inhibitors. Although representative compounds dihydroimidazoquinazoline **89**,[54] thiazole **90**,[165] and dihydrothiazoloindazole **91**[166] may be quite potent, no selectivity data have been published. Applied Research Systems has described a series of pyridinemethyleneazolidinones. A representative example is **92**[167] with single digit nanomolar potency in a biochemical assay against PI3Kγ and IC_{50} < 10 nM in a p-AKT ELISA cell assay. Shionogi has been active in developing PI3Kγ inhibitors. The pyrimidine **93**[168] is potent in a whole cell assay with ≥95% inhibition of AKT phosphorylation at 10 μM. The compound showed reasonable physical chemical properties as well; however, no selectivity data were provided. They have also claimed a series of pyridooxazines such as **94**[169] with potency in a biochemical assay in the double digit nanomolar range. Exelixis has published a series of analogues exemplified by *N*-methylpyrazine-2-carboxamides **95** and **96**.[170] These compounds, while exhibiting considerable selectivity over the other isoforms, had excellent physicochemical properties. The high oral bioavailability exhibited by **95** (76.7%) and **96** (66.2%) in rats was attributed to the high solubility and permeability of the analogues. Both compounds also demonstrated effectiveness in a mast cell degranulation in vivo mouse model.

■ DUAL PI3Kγ AND PI3Kδ INHIBITORS

Though it is occasionally difficult to discern from the patent literature whether a program is targeting selective PI3Kδ, PI3Kγ, or dual inhibition, there are several examples where a dual inhibitor is being specifically pursued. TargeGen[35]

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produced **7**, a dual inhibitor of PI3Kγ and PI3Kδ in phase I/II clinical trials against ischemic reperfusion injury following myocardial infarction. As mentioned previously, compound **7** was also studied for use in asthma and COPD, where it was effective in a cigarette-smoke-induced pulmonary mouse model. In the course of 3 days over several dosing regimens, it was found that neutrophil accumulation in BALF was decreased significantly relative to smoke treatment alone. Another in vivo experiment found that induction by LPS for enhancement of pulmonary neutrophilia could be ameliorated by aerosolized **7**. In this model decreases in BALF neutrophils of 42% were found compared to dexamethasone (70% reduction).[49]

The UCB group has reported a few compounds with noted selectivity for PI3Kδ over the other isoforms, although generally these compounds had similar potency between PI3Kγ and PI3Kδ.[171] For example, tetrahydrothiazolopyridinone **97**[172] has a PI3Kδ IC_{50} of 32 nM but is only 2.4-fold less potent toward PI3Kγ. The compound has reasonable PK, as demonstrated in Han−Wistar rats (Cl = 7 mL min^{-1} kg^{-1}, F = 97%). The structurally related **98** displayed similar selectivity and PK in Han−Wistar rats (Cl = 13 mL min^{-1} kg^{-1}, F = 66%). However, both these compounds have poor solubility and were less than 10-fold selective over PI3Kα. Incorporation of a solubilizing group providing **99** increased the solubility and decreased the plasma protein binding. Inhibitor **99** has an ED_{50} of 5 mg/kg in a rat in vivo model of CD3 induced IL-2 release.[173] Wilex has partnered with UCB to develop WX-037 (structure not disclosed), a dual PI3Kδ/α inhibitor for solid tumors. Millennium has disclosed thiazole **100** and thiophene **101** for targeting inflammation.[174] Few biological data were disclosed, other than a range of IC_{50} values against the isoforms. GlaxoSmithKline has disclosed a series of carboxamide substituted indazoles claimed to be useful for COPD, asthma, and other inflammatory diseases. Several compounds such as pyridylindazole **102**[129] are potent in an enzyme assay against various PI3K isoforms and against PI3Kδ in a human PBMC cell assay with a mean pIC_{50} of 5 or greater. Similar compounds, for example, picolinamide **103**,[175] indazole **104**,[176] oxadiazole **105**,[177] thiazolecarboxamide **106**,[178] morpholinoacetamide **107**,[179] and pyrozolopyridine **108**,[180] may have activity against PI3Kγ and PI3Kδ in an enzyme assay or a T-cell assay (presumably PI3Kγ) with a pIC_{50} of 5−6 or greater. Shokat and co-workers discovered an interesting selectivity differential. A few minor structural changes with a phenol moiety in the affinity pocket caused a shift in the PI3Kδ/γ selectivity, as a comparison of **45** and quinazolinone **109** indicates. They also published a detailed treatise on the relative merits of the PI3Kδ inhibitor **47**, **109**, and pan-inhibitor **10** (PIK-90). By use of 2 different human cell lines, 7 stimulating agents, and up to 20 different expressed proteins known to be involved in inflammation, they found that the greatest suppression of these cytokines and other inflammatory second messengers came from the dual PI3Kδ/γ inhibitor **109**. They also compared these results to several known anti-inflammatory agents and found that **109** most closely matches prednisolone in a functional way. On the basis of this in vitro treatment, the team concluded that the targeting of RA and other diseases of inflammation would be most effective with a dual PI3Kδ/γ inhibitor such as **109**.[181] Takeda/Intellikine is championing the development of a dual PI3Kγ and PI3Kδ inhibitor for inflammatory diseases and hematologic malignancies.[75] They disclose at least nine compounds, exemplified by thienopyrimidinone **110**,[182] with PI3Kδ and PI3Kγ potency of less than 100 nM in enzyme assays. The compounds were weakly potent (>10 μM) toward PI3Kα and PI3Kβ but active with EC_{50} < 100 nM in a B-cell proliferation assay. The structures all contain a phenolic substituted pyrazolopyrimidine side chain as found in **45** and **46**.

Takeda/Intellikine has put forward two dual PI3Kγ/δ inhibitors. INK055 (structure not disclosed) was dosed therapeutically at 30 mg/kg in a CIA model of RA with clinical scoring >2-fold lower than vehicle control at day 34 (dosing started on day 30). Interestingly, anti type II collagen antibody levels were unaffected, consistent with a PI3Kγ driven response.[67,109] Prior to their acquisition by Takeda, Intellikine licensed the dual PI3Kγ/δ inhibitor IPI-145 (formerly INK1197, structure not disclosed) to Infinity Pharmaceuticals Inc., which is currently in phase I for hematological malignancies.[183]

STRUCTURAL ASPECTS OF THE PI3Kδ AND PI3Kγ INHIBITORS

Development of class I PI3K isoform specific inhibitors is challenging because of the high sequence identity exhibited between catalytic p110 subunits and the even higher level of conservation of amino acids within the ATP-binding site (Table 8).[184] However, as the preceding sections demonstrate, inhibitors with varying isoform selectivities exist, and indeed, some are quite selective. Central to our understanding of the structure of the p110 proteins and of their complexes with small molecule ATP-competitive inhibitors were the publications of the crystal structures of porcine and human class IB p110γ proteins.[185,186]

Table 8. Amino Acid Sequence Identity (%) between Human PI3K Isoforms

kinase domain	p110δ	p110γ	p110α	p110β
p110δ		42	47	73
p110γ			44	43
p110α				48
active site	p110δ	p110γ	p110α	p110β
p110δ		68	62	81

These structures elucidated the key features of the active site and provided detailed views of how classic pan-PI3K inhibitors such as **1** and **2** inhibited the PI3Ks. Briefly, inhibitors bound to the active site by making one or more hydrogen bonds to residues in the hinge region of the kinase domain, analogous to interactions made by ATP-competitive inhibitors of protein kinases. Hydrogen bonds in the hinge region are often the strongest contact that ATP competitive inhibitors make, estimated to account for 40−60% of the overall binding energy for inhibitors of certain protein kinases.[187] Most PI3K inhibitors also interact with the large, flat hydrophobic face of a conserved tyrosine residue (Tyr867 in p110γ), and many extend back into the affinity pocket where additional hydrophobic interactions can be gained to boost potency and where, with the presence of an appropriate heteroatom, inhibitors can form hydrogen bonds with the catalytic lysine (Lys833 in p110γ) or other hydrophilic residues.[34] In the case of **2** (Figure 10A), the morpholine ring hydrogen-bonds to hinge residue Val882 and the edge of the morpholine ring interacts with the face of Tyr867. The chromanone carbonyl group points toward the affinity pocket, and the phenyl group sitting orthogonal to the chromanone ring occupies the ribose

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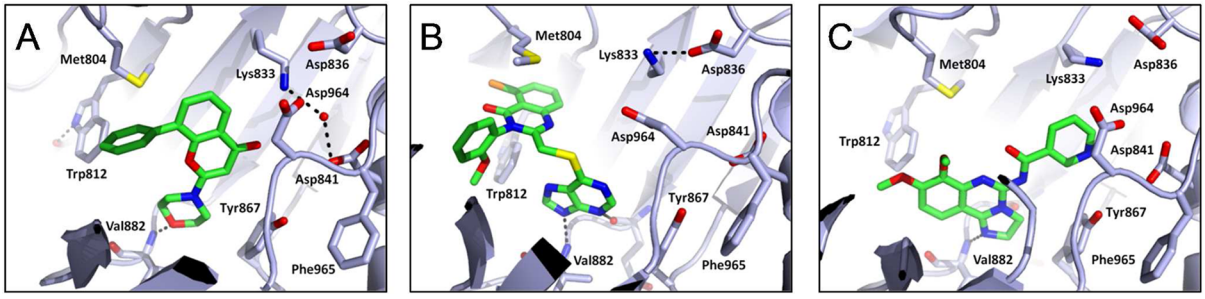

Figure 10. (A) Crystal structure of porcine p110γ and **2** (PDB code 1E7V). (B) Crystal structure of human p110γ and **4** (PDB code 2CHW), a propeller-shaped inhibitor. (C) Crystal structure of human p110γ and **10** (PDB code 2CHX), a flat inhibitor.

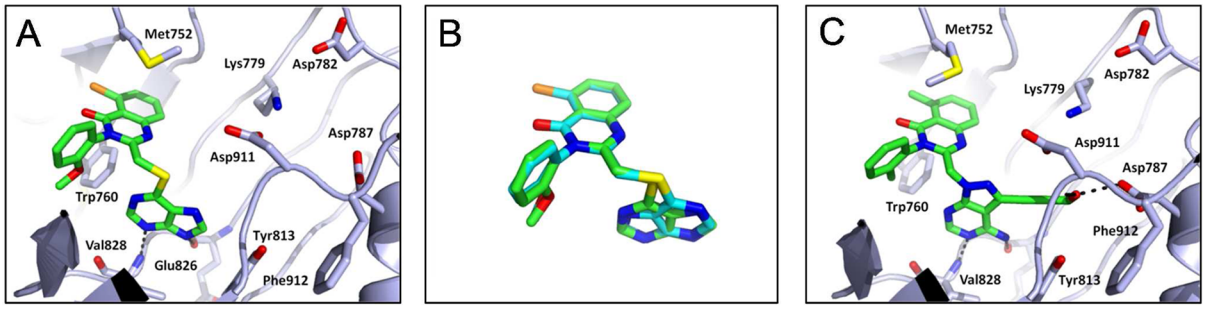

Figure 11. (A) Crystal structure of murine p110δ and **4** (PDB code 2WXF). (B) Overlay of **4** from murine p110δ (green) and human p110γ (blue). The purine binding mode depicted in green is likely the energetically more favorable conformation for both isoforms. (C) Crystal structure of murine p110δ and **47** (PDB code 2WXI) showing an alkynol group in the affinity pocket.

pocket. Subsequent work by numerous groups has resulted in the structure elucidation of many additional p110γ crystal structures in complex with small molecule inhibitors. More recently, crystal structures of the class IA p110α, p110β, and p110δ enzymes have been published.[111,188−191] The availability of multiple inhibitor-bound p110δ crystal structures in particular has allowed for the detailed analysis of binding interactions of pan- and selective class I PI3K inhibitors.

Crystal structure analysis combined with data from biochemical and cellular assays can be used to understand the molecular basis for the observed inhibitor selectivities. In notable work,[34] extensive biochemical profiling of various inhibitor chemotypes combined with crystal structures of three distinct molecules in PI3Kγ allowed the authors to correlate and explain the observed selectivities for various structural classes of PI3K inhibitors. The diverse inhibitors clustered into several different classes but bound to p110γ in one of two principal binding modes: (I) a propeller shaped binding mode exemplified by quinazolinones **3** and **4** (Figure 10B) and (II) a flatter, more classical kinase binding mode exemplified by **10** (Figure 10C). There is a distinct difference in the overall selectivity between these binding modes. Flat inhibitors such as **10** can occupy the binding pocket without any significant amino acid rearrangements. They extend into the affinity pocket (**10** uses a pyridine ring), and they are typically pan-PI3K inhibitors. On the other hand, PI3Kδ selective quinazolinone **3** binds only when a key methionine residue (Met804 PI3Kγ, conserved in all isoforms) adopts an alternative conformation that opens a hydrophobic pocket (specificity pocket) sitting orthogonal to the position of residues in the p110γ hinge region (Figure 10B). This ligand-induced methionine reorientation, first observed in the crystal structure of p110γ and **4**, also induces a shift of the peptide backbone that propagates through the adjacent loop (equivalent to the P-loop in protein kinases, amino acids 803−811 in PI3Kγ). It was postulated that this conformational change, although possible in all class I PI3Ks, was most facile in PI3Kδ, thereby accounting for the higher binding affinity of propeller shaped inhibitors on p110δ relative to the other isoforms. Following publication of the first p110α crystal structure, an examination of **4** binding to this isoform concluded that differences in the conformation of the P-loop precluded binding of **4** because of steric reasons, even with a conformational change in Met772.[192] However, a recent crystal structure of murine p110α showing a ligand-induced Met772 reorientation calls that conclusion into question.[189] In addition to the crystallographic work, computational approaches have also been used to examine issues of PI3K inhibitor selectivity, and they have reached varying conclusions.[193,194]

Crystal structures of both flat and propeller-shaped molecules in p110δ have reinforced and extended the initial selectivity arguments. Crystal structures of the most selective analogues, **3** and **4** (Figure 11), provide an explanation of selectivity that is similar but perhaps more thorough than what was argued based on the PI3Kγ structures. The N-terminal lobe of the kinase domain in p110 proteins contains a loop spanning the kα1 and kα2 helices. This loop is longer in PI3Kγ than in PI3Kδ, and it sits on top of the P-loop, limiting its flexibility and leading to less facile rearrangement of the active site in PI3Kγ relative to PI3Kδ. Molecular dynamics simulations based on the crystal structures demonstrated a lower degree of flexibility in p110γ versus p110δ, thus supporting this argument. Also contributing to lowered flexibility in p110γ is a hydrogen bond network that restricts the movement of Trp812, an essential component of the hydrophobic pocket into which the propeller shaped molecules bind. The nearly identical conformation of **4** in p110δ versus p110γ (Figure 11B) further bolsters the argument that differences in the

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ability of the active sites to accommodate the inhibitor account for potency differences rather than an altered binding conformation of the inhibitor in each isoform. The PI3Kδ crystal structures also demonstrated that simultaneously occupying the selectivity and affinity pockets is possible, and indeed, propeller shaped analogues **42**, **43**, **44**, **46**, **47**, and **109** take advantage of this strategy to impart an additional level of potency toward PI3Kδ. For example, in **47** the pendent alkynol hydrogen-bonds to Asp787 and to the backbone NH of Asp911(Figure 11C), resulting in significantly improved potency relative to **3** and **4**. Even among the more selective propeller shaped molecules, however, a divergence of selectivity is obtainable by only minor modification. For example, **46** forms hydrogen bonds between the phenol −OH group and Tyr813 and between the meta-fluoro substituent and Lys779, whereas **109**, a constitutional isomer of **46**, forms only the hydrogen bond with Lys779. As a consequence, **46** is a more PI3Kδ selective inhibitor than **109**. This example highlights the fact that although propeller shaped compounds can be very PI3Kδ selective, care must be taken to monitor the selectivity of such compounds, as variations are made on the periphery of the molecule.

Among the many crystal structures of p110γ now available in the public realm, only a few contain inhibitors reported to be PI3Kγ selective. The structures of **6** and **8** from Serono provide insight into the structural features that promote selectivity for PI3Kγ over the other isoforms. Although PI3Kγ can be inhibited by propeller shaped molecules as discussed above, such molecules seemed destined to carry along PI3Kδ activity. In contrast, the Serono structures show compact molecules that form a hydrogen bond with hinge residue Val882 and extend toward the affinity pocket where they form an additional hydrogen bond to catalytic Lys833 using their thiazolidinedione moieties (Figure 12). These modestly sized molecules appear

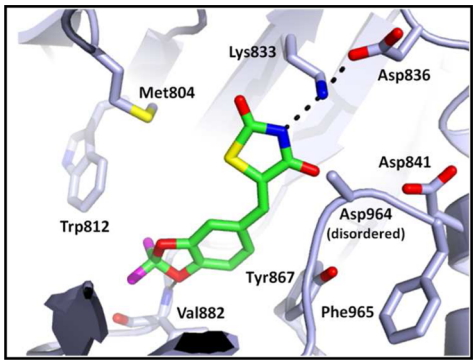

Figure 12. Crystal structure p110γ-selective inhibitor **8** and human p110γ (PDB code 2A4Z).

to have the optimal dimensions to span this distance when Lys833 is in a conformation that sits lower in the affinity pocket, constricting its size. A computational analysis of the selectivity of these molecules using homology models of class IA PI3Ks reinforced the idea that these compounds are ideally sized for PI3Kγ, as identical binding interactions were not reproduced in the other isoforms.[194] A separate series of PI3Kγ selective inhibitors reported recently follows this paradigm.[170] From the crystal structures of the class IA enzymes, it appears that the kinase domain conformation may also play a role in modulating the affinity of these molecules against various isoforms, as p110δ is distinct from p110α and p110γ in the orientation of its N-lobe with respect to its C-lobe in the kinase domain and molecules **6**, **8**, and **9** are significantly less potent on PI3Kδ. By the same argument, it seems likely that achieving selectivity over PI3Kα will be the most challenging aspect of designing PI3Kγ selective inhibitors, an idea supported by the inhibitor profiling already reported[34] and borne out in a recently published work.[170]

Whereas the selectivity profiles of many PI3K inhibitors are easy to understand based on our current knowledge of class I PI3K inhibitor binding modes, rational design of exquisitely selective PI3K inhibitors still presents a challenge. Future work is sure to produce further insights into mechanisms of achieving isoform selective inhibitors, as the recent publication of hexahydroquinoxalinone compound **58** illustrates.[111] This compound is PI3Kδ selective and yet does not adopt a propeller shape. The crystal structure of **58** in p110δ shows that rather than sitting sandwiched between the Met752 and Trp760 in the selectivity pocket, the compound sits adjacent to these two amino acids in a small pocket formed by Thr750. The other isoforms do not have such a pocket because the residue at this position is a much larger arginine or lysine. Indeed, the recent advances in molecular biology and protein expression that enabled the crystallization of the class IA isoforms may provide further novel insights into the selectivities of PI3K inhibitors as more structures are solved.[188,189,195]

■ DISCUSSION AND CONCLUSION

Teasing out the differences that PI3Kδ and PI3Kγ have in the immune system is not a trivial endeavor. Clearly the adaptive immune system has evolved in proximity to the innate system, and this relationship is illustrated by the interplay between these two key kinases. For example, both PI3Kδ and PI3Kγ over multiple steps influence neutrophil responses to sites of infection or inflammation such that in diseases that activate this cell type, targeting one or the other of these two kinases could prove advantageous. Mast cell degranulation is affected by both PI3Kγ and PI3Kδ, as has been shown in several genetic and pharmacologic studies. Conflicting data (IgE levels, tracheal smooth muscle hyperresponsiveness) in some asthma model studies are indicative of overlapping outcomes, in spite of the distinct functions of PI3Kδ and PI3Kγ. The inhibition of PI3Kδ has a great impact on B-cell proliferation, antibody production, and Ig class switching, but the inhibition of PI3Kγ has negligible effects on B-cells. This is an advantage for drugs targeting PI3Kδ for diseases that have a significant B-cell component such as shown to be the case for RA. Leukocyte infiltration is a hallmark of asthma, ALI/ARDS, IPF, RA, and colitis (IBD), and PI3Kγ controls much of this chemotaxis. A case can be made for targeting RA with PI3Kγ inhibitors by inhibition of leukocyte migration. Also, a PI3Kγ inhibitor was effective in a lupus model over several months and in a MS model, but no such data have been described for PI3Kδ inhibitors. Dual PI3Kγ and PI3Kδ inhibitors have been getting increasing scrutiny, although the data from animal models rely mainly on **7**. The use of this compound in asthma and COPD models showed promise. In spite of the deleterious outcomes of the dual PI3Kγ KO and PI3Kδ KDKI mice, the severe lymphopenia and other adverse effects were not replicated in the study involving PI3Kγ KO mice and **3**. Instead, there was a further reduction in the mean ankle thickness and an improved clinical score relative to **3** dosed WT mice.

It is tempting to make judgments of the utility of the two targets based on the number of studies reported for the various

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animal disease models. Using this approach, researchers have been interested in selective PI3Kδ inhibitors for asthma, COPD, and selective PI3Kγ inhibitors for IBD, SLE, and RA. The sheer number of reported studies targeting PI3Kγ would indicate that this is the preferred target, but this is refuted somewhat by the dearth of chemical matter related to this target. There are many more patent applications for PI3Kδ inhibitors than for PI3Kγ inhibitors, although many PI3Kγ inhibitors could be found in patents that are more closely aligned to mTOR as the dominant target. In this review we have ignored for the most part the vast IP space that mentions mTOR as co-target or PI3Kα for that matter. Finally, the prevalence of PI3Kδ inhibitors verses PI3Kγ inhibitors could simply be due to the relative ease of finding isoform and kinase selective PI3Kδ inhibitors, as the structural data indicate. Dual inhibitors are less frequently mentioned in the literature and are possibly masked in related patents, as most applications are written to cover all isoforms. Interestingly, there are several dual inhibitors that have advanced toward the clinic.

A perusal of the selectivity data in Tables 4 and 5 indicates that the propeller shape seems to dominate inhibitor design. Of the 18 compounds with PI3Kγ/PI3Kδ selectivity of greater than 20, 11 are of this type. The remaining seven compounds, while all having PI3Kγ/PI3Kδ selectivity of greater than 20, fall considerably short in the selectivity over the other isoforms (see, for example, **12**, **55**, and **57**). On the other hand, 9 of the 11 propeller shaped analogues exhibit PI3Kδ selectivity over the other isoforms of greater than 100-fold. However, as was pointed out earlier, the Serono compound **58** is not propeller shaped and does have similar selectivity compared to these compounds. Whether these structural data have been utilized in further inhibitor design is unknown. Roche/Genentech has shown that the more flat molecules such as **14**−**17**, while structurally very similar to **12**, have considerably higher selectivity over PI3Kα and may have selectivity over PI3Kγ. Less can be said about the PI3Kγ inhibitor class, as there are fewer published reports dealing with PI3Kγ-selective compounds, although the best examples of these are relatively compact molecules that do not include any propeller shaped compounds.

One paradox of drug discovery is that the initial biological hypothesis is of necessity based on limited understanding of complex systems. Fundamental biological research is facilitated by progress in the development of small molecule tools that help address understanding of target coverage and selectivity requirements as well as issues such as target redundancy or compensation. As this understanding develops, the drug product profile may change considerably from that initially proposed on the basis of a one target−one effect model. Additionally, animal disease models mentioned in this review have uncertain relevance to actual human disease states and their utility as predictors of clinical success is in question. The question of final target validation is likely to remain unanswered until one or more compounds targeting either PI3Kγ or PI3Kδ complete clinic trials for diseases of autoimmunity/inflammation.

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The authors declare the following competing financial interest(s): The authors are all employees of Amgen Inc.

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ABBREVIATIONS USED

ADME, absorption, distribution, metabolism, and excretion; AHR, airway hyperresponsiveness; AIA, antigen-induced arthritis; ALI/ARDS, acute lung injury/acute respiratory distress syndrome; AML, acute myeloid leukemia; APC, antigen presenting cells; ATP, adenosine triphosphate; B1, a

class of B cell lymphocytes; BALF, bronchoalveolar lavage fluid; BCR, B-cell receptor; b.i.d., twice a day; BLM, bleomycin; BMMC, bone-marrow-derived mast cell; BTK, Bruton's tyrosine kinase; cAMP, cyclic adenosine monophosphate; CCL2, chemokine (C−C motif) ligand 2; CCL5, chemokine (C−C motif) ligand 5; CD, cluster of differentiation; CIA, collagen induced arthritis; Cl, clearance; CLL, chronic lymphocytic leukemia; CNS, central nervous system; COPD, chronic obstructive pulmonary disease; DNA-PK, DNA-dependent protein kinase; DNP, dinitrophenyl; DSS, dextran sodium sulfate; ETK, cryptic autophosphorylating protein tyrosine kinase; fMLP, *N*-formylmethionylleucylphenylalanine; FO, follicular; FOXO3a, forkhead box O3; GPCR, G-protein-coupled receptor; GR-α, glutocorticoid receptor α; HWB, human whole blood; IBD, inflammatory bowel disease; IFN-γ, interferon γ; Ig, immunoglobulin; IL, interleukin; IPF, Idiopathic pulmonary fibrosis; ITK, interleukin-2-inducible T-cell kinase; KDKI, kinase dead knock-in; KO, knockout; LPS, lipopolysaccharide; MI, myocardial infarction; MMP3, matrix metalloproteinase 3; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; MTD, maximum tolerated dose; mTOR, mammalian target of rapamycin; MZ, marginal zone; NHL, non-Hodgkin's lymphoma; OVA, ovalbumin; PCA, passive cutaneous anaphylaxis; PBMC, peripheral blood mononuclear cell; PDGF, platelet-derived growth factor; PDK1, phosphoinositide dependent kinase 1; PIKK, phosphatidylinositol 3-kinase-related kinase; PKB/Akt, protein kinase B; PMN, polymorphonuclear leukocyte (neutrophil); PTEN, phosphatase and tensin homologue; q.d., once a day dosing; RA, rheumatoid arthritis; RTK, receptor tyrosine kinase; ROS, reactive oxygen species; SCW, Sstreptococcal cell wall; SF, synovial fibroblast; SLE, systemic lupus erythematosus; TCR, T-cell receptor; T_h1, T helper cell type 1; T_h2, T helper cell type 2; TLR, Toll-like receptor; TNF-α, tumor necrosis factor α; T_{reg}, T regulatory cell; VEGF, vascular endothelial growth factor; VV, vaccinia virus; WT, wild type

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