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Discovery, Optimization, and in vivo Evaluation of Benzimidazole Derivatives AM-8508 and AM-9635 as Potent and Selective PI3Kδ Inhibitors

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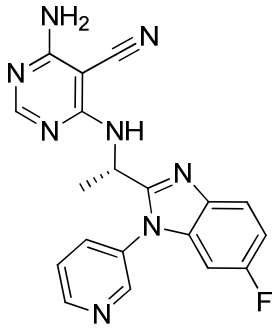
ABSTRACT

Lead optimization efforts resulted in the discovery of two potent, selective, and orally bioavailable PI3Kδ inhibitors, **1** (AM-8508) and **2** (AM-9635) with good pharmacokinetic properties. The com-

pounds inhibit B cell receptor (BCR)-mediated AKT phosphorylation (pAKT) in PI3Kδ-dependent in vitro cell based assays. These compounds which share a benzimidazole bicycle are effective when administered in vivo at unbound concentrations consistent with their in vitro cell potency as a consequence of improved unbound drug concentration with lower unbound clearance. Furthermore, the compounds demonstrated efficacy in a Keyhole Limpet Hemocyanin (KLH) study in rats, where the blockade of PI3Kδ activity by inbibitors **1** and **2** led to effective inhibition of antigen-specific IgG and IgM formation after immunization with KLH.



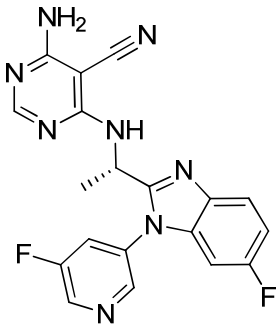
1 (AM-8508)

HWB,u (pAKT) IC_{50} = 1.0 nM

Rat KLH

IgG ED_{50} = 0.08 mg/kg

IgM ED_{50} = 0.12 mg/kg



2 (AM-9635)

HWB,u (pAKT) IC_{50} = 5.7 nM

Rat KLH

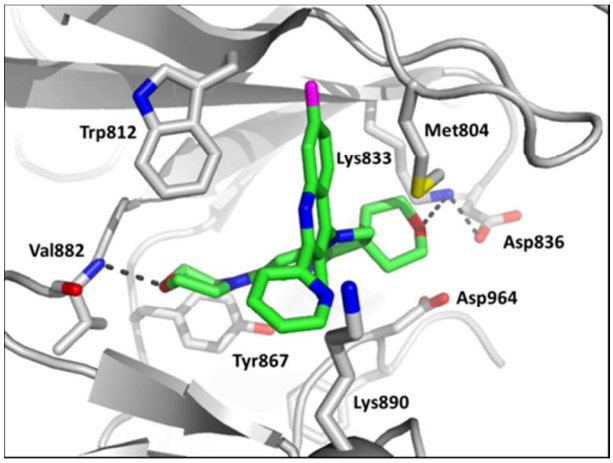
IgG ED_{50} = 0.11 mg/kg

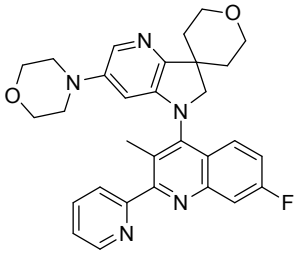
IgM ED_{50} = 0.04 mg/kg

INTRODUCTION

Phosphoinositide 3-kinases (PI3Ks) belong to a large family of lipid kinases that regulate numerous biological functions by generating lipid second messengers.[1] PI3Ks utilize ATP to phosphorylate the 3-OH of the inositol ring moiety, converting the phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3).[2] PIP3 induces AKT phosphorylation, which acts as a secondary messenger in the control of a wide number of cellular functions including metabolism, cell growth and motility.[3] PI3Ks are divided into three classes (I, II, and III) based on functional and sequence homology.[4,1] Class I PI3Ks are further divided into subclasses IA and IB based on their signaling pathways

and the regulatory proteins to which they bind. The class IA isoforms PI3Kα, PI3Kβ, and PI3Kδ, are primarily activated by protein tyrosine kinase-coupled receptors,[5] whereas the class IB member PI3Kγ is activated by G-protein coupled receptors (GPCRs), such as the chemokine receptors.[6] PI3Kα and PI3Kβ are ubiquitously expressed and play a role in cell growth, division and survival.[7] The expression pattern of PI3Kδ and PI3Kγ is more restricted, with both isoforms found primarily in leukocytes.[8] PI3Kδ and PI3Kγ have been identified as promising therapeutic targets for the treatment of immune cell-mediated diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and hematological malignancies.[9] Given these findings, PI3Kδ or PI3Kγ selective inhibitors and dual inhibitors of PI3Kβ/δ and PI3Kδ/γ have been utilized in research both to understand the biological roles of PI3Ks, and as potential drugs for the treatment of inflammatory disorders.[10] We recently disclosed the discovery of dual PI3Kβ/δ inhibitors[10c, 11] and PI3Kδ isoform selective inhibitors with a diverse range of core moieties, including pyrazolopyrimidine,[12] quinoline and quinoxaline,[13] reversed quinoline,[14] 4-substituted-3-linked-quinoline and quinoxaline,[15] naphthyridine,[16] pyridopyrimidinone,[17] benzimidazole,[18] imidazopyridine,[18] and thienylpyridine.[19] Among these, AMG 319[20] has advanced into clinical development for the treatment of lymphoid malignancies and AMG 357[21] has similarly advanced for the treatment of inflammatory disease. Herein, we communicate the discovery and optimization of a unique series[18] of PI3Kδ selective inhibitors and their potential application in the treatment of inflammatory disease.





3

PI3Kα[a] IC_{50} = 4610 nM
PI3Kβ[a] IC_{50} = 78 nM
PI3Kδ[a] IC_{50} = 20 nM
PI3Kγ[a] IC_{50} = 825 nM
B Cell[b] IC_{50} = 9 nM
pAKT[c] IC_{50} = 36.2 nM

Figure 1. Crystal structure of PI3Kγ in complex with **3** (PDB code 4FJZ). Amino acid labels correspond to the PI3Kγ isoform. Dashed lines indicate hydrogen bonds. [a]Biochemical: Alphascreen assay. [b]Cellular: In vitro anti-IgM/CD40L-induced B cell proliferation (as measured by thymidine incorporation) assay. [c]PI3Kβ counterscreen assay: phosphorylation of AKT in MDA-MB-468 cells.

RESULTS AND DISCUSSION

Earlier reports from our PI3Kδ program have demonstrated that the dual PI3Kβ/δ inhibitor **3** was well tolerated and efficacious in animal models of inflammation.[10c] A crystal structure of **3** in complex with PI3Kγ indicates that the compound adopts a propeller-like conformation in which the quinoline and hexahydrospiro[pyran-4,3'-pyrrolo[3,2-b]pyridine] ring are at an angle of approximately 90° (Figure 1). Previous reports have stated that similar propeller-shaped inhibitors induce a conformational change in the ATP-binding pocket where a methionine (Met804) is displaced to accommodate the quinoline ring in a newly-formed hydrophobic pocket.[22] This ligand-induced conformational change is critical for achieving the selectivity of **3** for PI3K β/δ over other protein kinases.

In an attempt to optimize **3** as a PI3Kδ selective inhibitor, alternative hinge binders and bicyclic heteroaryl moieties were further explored and have been described in previous reports.[16-19] These studies identified a 4-aminopyrimidine-5-carbonitrile moiety as an alternative hinge binder that favorably interacts with Val882 that shows potency and selectivity similar to the established morpholine and purine

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analogs.[17,14c] Analogs that contain this promising hinge binder along with quinoline and quinoxaline core moieties were designed,[13c] and as predicted, the compounds showed good PI3Kδ potency and improved selectivity over other PI3K isoforms when compared to compound **3**.

As part of our medicinal chemistry efforts towards the identification of inhibitors of the CXCR3 receptor, we showed that five-six fused heterocyclic systems, such as benzimidazole and imidazopyridine, could be suitable replacements of the six-six fused heterocycles.[23] It was therefore hypothesized that these heterocyclic moieties could serve as isosteres of the quinoline and quinoxaline heterocycles within the context of the PI3K program.

Early SAR

To test this hypothesis, the benzimidazole analog **5** was first synthesized and tested in an *in vitro* Alphascreen biochemical assay (Table 1). Gratifyingly, it was found that this analog with the aminopyrimidine as a hinge binder exhibited good inhibition of the PI3Kδ enzyme as well as promising selectivity over other PI3K isoforms, especially the PI3Kβ isoform and the replacement of quinoline bicycle by the benzimidazole isostere substantially improved selectivity over the PI3Kγ isoform compared to compound **3**. Consistent with our previous results, the *R*-enantiomers, **4** and **6**, were much less potent towards PI3Kδ than their corresponding *S*-enantiomers, **5** and **7**.[20b] This experimental result can be rationalized by invoking a steric clash between the methyl group of *R*-enantiomers and the protein, which is not present in the corresponding *S*-enantiomers. This initial optimization work also revealed that the 6-fluoro analogs, **4** and **5,** were more potent than the corresponding 5-fluoro analogs **6** and **7**, a trend that continued throughout the series.

Table 1. SAR of benzimidazole analogs.

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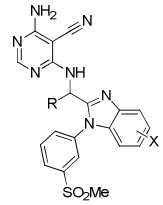

			Biochemical potency[a] IC_{50} (μM)				Selectivity		
cmpd	R	X	PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ	PI3Kα/ PI3Kδ	PI3Kβ/ PI3Kδ	PI3Kγ/ PI3Kδ
3			4.61 ± 5.46	0.0786 ± 0.0917	0.0202 ± 0.0139	0.825 ± 0.698	230	4	42
4	*R*-Me	6-F	49.6	>125	18.3	29.5	3	>7	2
5	*S*-Me	6-F	>125	3.58	0.069	2.88	>1800	52	42
6	*R*-Me	5-F	>125	>125	>125	>125			
7	*S*-Me	5-F	33.2	31.8	0.1760 ± 0.0732	14.0 ± 9.16	189	181	79

[a]Biochemical: Alphascreen assay.

The exceptional selectivity of **5** over other PI3K isoforms can be explained by examining the X-ray crystal structure of the compound in complex with PI3Kγ (Figure 2). The aminopyrimidine ring interacts with the Val882 residue in the hinge region of PI3Kγ and the nitrile is projected into the affinity pocket. The benzimidazole ring occupies the specificity pocket and adopts the desired propeller-like conformation in which the aminopyrimidine and benzimidazole rings are at an angle of approximately 90°, a conformation that has been shown previously to result in PI3Kδ-selective inhibitors.[22b] The 3-methylsulfonyl-phenyl ring sits in the ribose pocket, orthogonal to the benzimidazole ring. Having demonstrated the potential of the benzimidazole moiety as a promising isostere of the quinoline, subsequent optimization efforts were pursued to further improve upon the lead compound **5**.

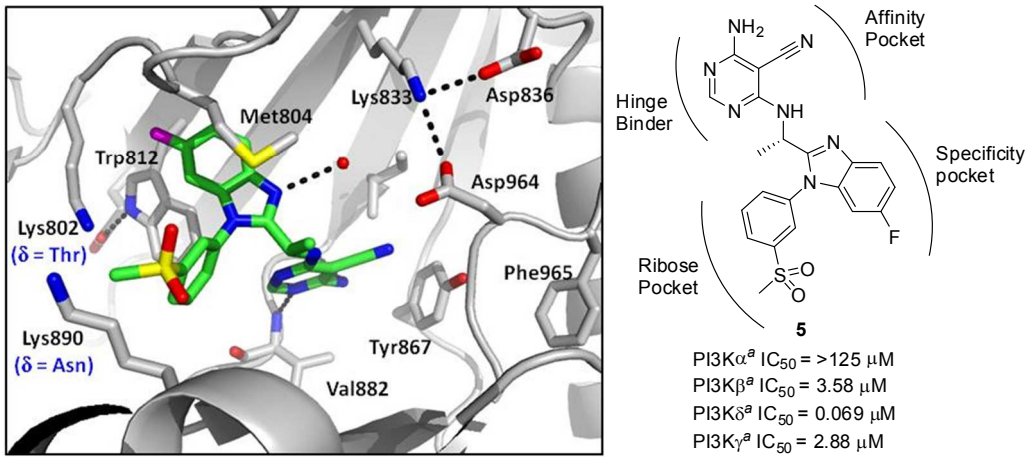

Figure 2. Crystal structure of PI3Kγ in complex with **5** (PDB code 5EDS). Amino acid labels correspond to the PI3Kγ isoform; blue labels indicate the corresponding residue in PI3Kδ. Dashed lines indicate hydrogen bonds. [a]Biochemical: Alphascreen assay.

Chemistry

A general synthetic route for the benzimidazole analogs of **5** is described in Scheme 1. Nucleophilic aromatic substitution of 2,4-difluoro-1-nitrobenzene **(8)** with the relevant anilines was followed by reduction of the nitro group in **9a-g** using tin chloride dihydrate to provide **10a-g**. Coupling of **10a-c,e-g** with Boc-L-alanine afforded **11a-c,e-g** which then underwent cyclodehydration in refluxing acetic acid. During the cyclodehydration, the Boc-protecting group was cleaved and subsequently acylated in situ to provide benzimidazole intermediate **12a-c,e-g**. Epimerization was later found to occur at the stereogenic carbon attached to the methyl group. Alternatively, **10d** was coupled to Fmoc-L-alanine to give **11d**, which was cyclodehydrated in refluxing acetic acid to produce **12d**.

2-(Methylthio)phenyl analog **12b** was oxidized to the sulfone analog **12b'**. Deacylation of the N-acetates **12a-c, e-g** with hydrochloric acid afforded **13a-c, e-g**. The Fmoc group on **12d** was removed by piperidine to provide **13d**. Treatment of **13a-g** with 4-amino-6-chloropyrimidine-5-carbonitrile afforded the racemic coupling products **14a-g**, which were then subjected to chiral separation to provide **5, 15-18,**

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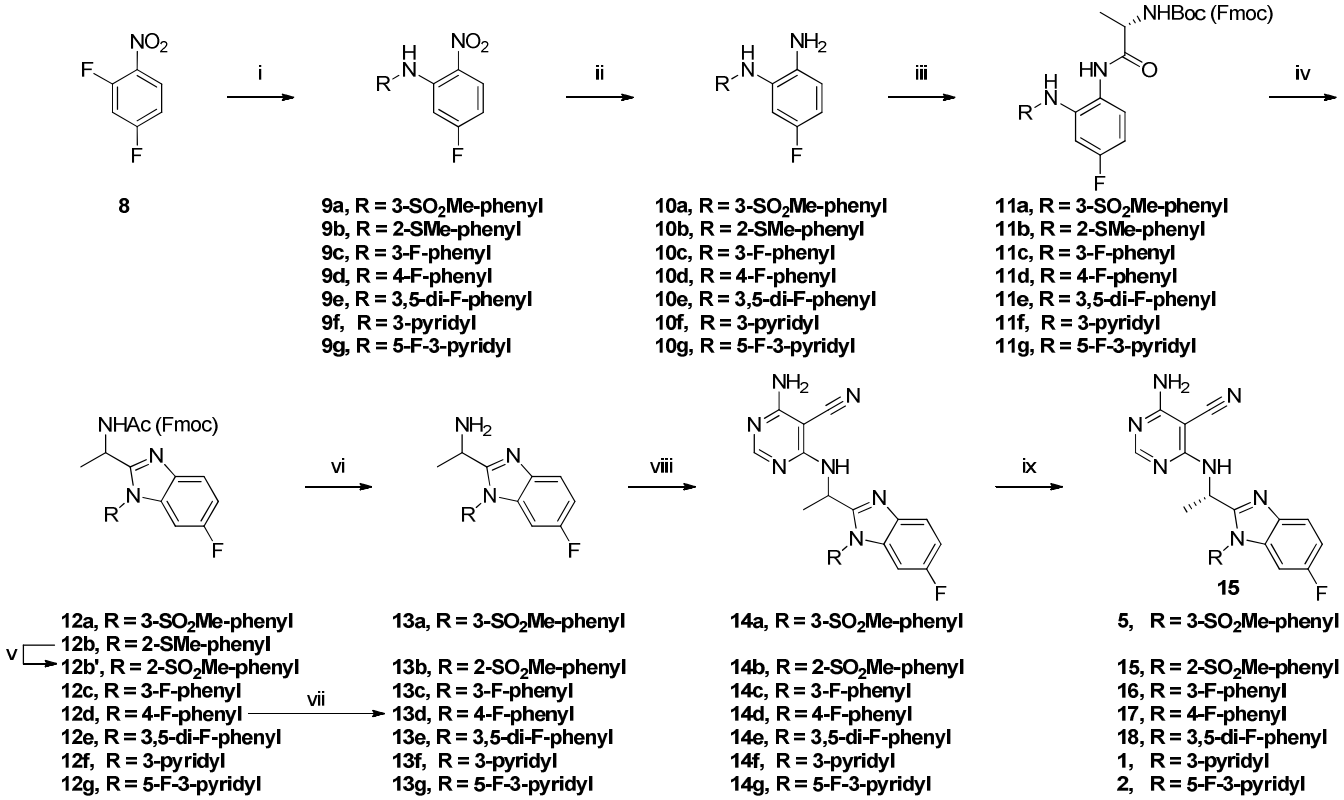
1, and 2. This route was designed as a regio-and stereo-selective synthetic route; however the stereoselectivity was not retained due to the epimerization during cyclodehydration.

Scheme 1[a]



[a]Reagents and conditions: (i) Aniline, 130 °C; (ii) $SnCl_2 \cdot 2H_2O$, EtOAc, 90 °C; (iii) N-methylmorpholine, isobutyl chloroformate, Boc-L-Ala-OH, DCM (for 11d, Fmoc-L-Ala-OH), -10 °C; (iv) AcOH, 100 °C; (v) Oxone, THF-water (3:1), rt; (vi) 2 N HCl, 100 °C; (vii) piperidine, DCM, rt; (viii) 4-amino-6-chloropyrimidine-5-carbonitrile, DIPEA, microwave, n-butanol, 120 °C; (ix) chiral SFC chromatography.

A second synthetic route solved the stereoselectivity issue, but the regioselectivity was not addressed (Scheme 2). Cyclodehydration of Boc-L-Ala-OH and **19** afforded **20**, which was then coupled with the relevant boronic acids to give intermediates **21a-b** as a mixture of regioisomers. The Boc-protecting group on **21a-b** was cleaved with TFA to provide the amines **22a-b**. Coupling of **20** with 2-fluoropyridine at 150 °C afforded deprotected amine **22c**. Treatment of **22a-c** with 4-amino-6-

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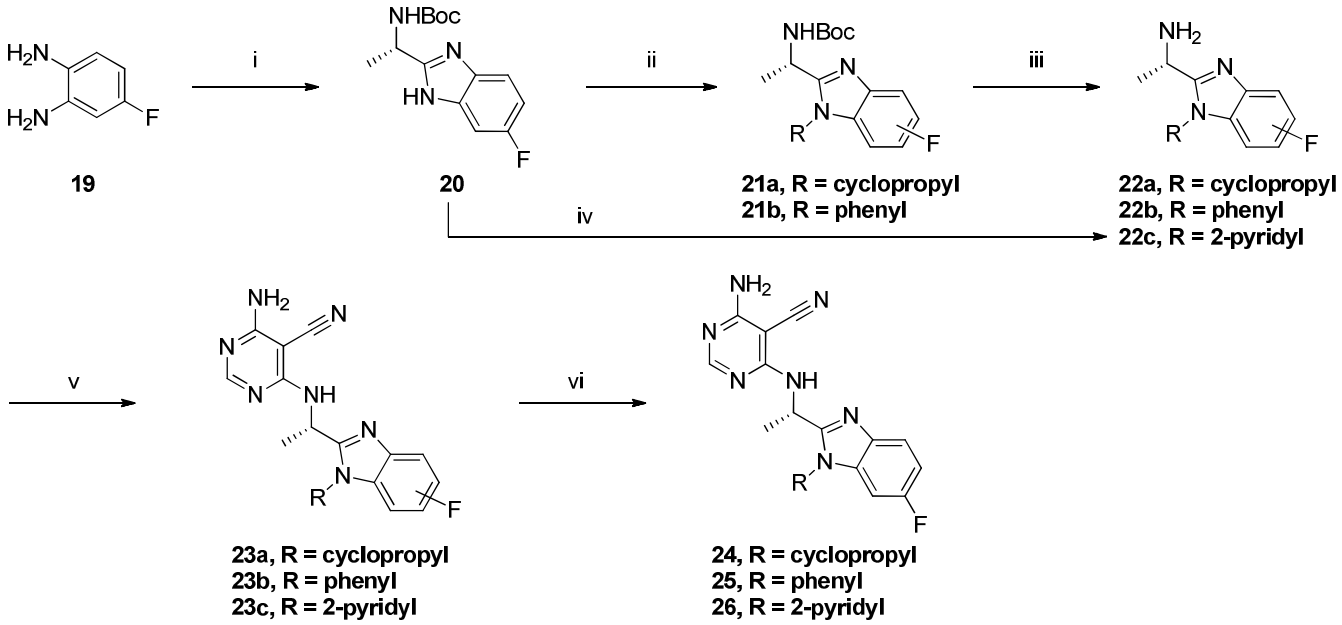
chloropyrimidine-5-carbonitrile afforded a mixture of 5 and 6- fluorosubstituted benzimidazoles **23a-c**, which were then separated by SFC purification to give 6-fluorobenzimidazoles **24-26**.

Scheme 2[a]



[a]Reagents and conditions: (i) Boc-L-Ala-OH, EDC·HCl, pyridine, rt; (ii) $RB(OH)_2$, Cu $(OAc)_2$, 2,2'-bipyridyl, Cs_2CO_3, dichloro-ethane, 70 °C; (iii) TFA, DCM, rt; (iv) 2-fluoropyridine, DMA, Cs_2CO_3, 150 °C; (v) 4-amino-6-chloropyrimidine-5-carbonitrile, DIPEA, microwave, n-butanol, 120 °C; (vi) SFC separation.

Scheme 3[a]

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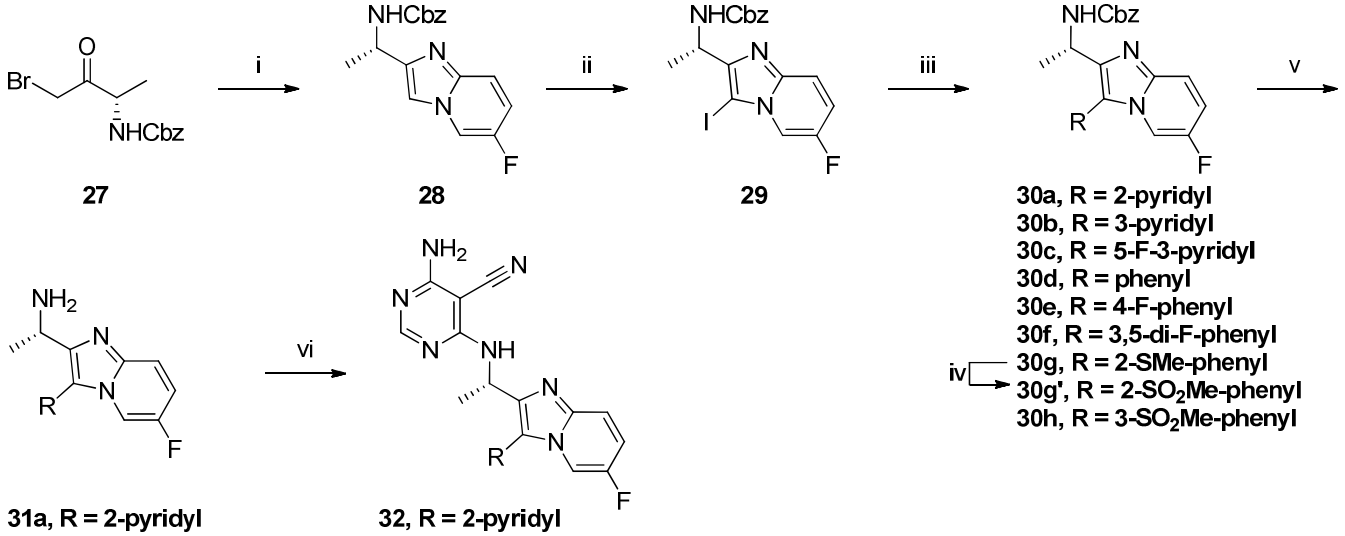

31a, R = 2-pyridyl
31b, R = 3-pyridyl
31c, R = 5-F-3-pyridyl
31d, R = phenyl
31e, R = 4-F-phenyl
31f, R = 3,5-di-F-phenyl
31g, R = 2-SO_2Me-phenyl
31h, R = 3-SO_2Me-phenyl

32, R = 2-pyridyl
33, R = 3-pyridyl
34, R = 5-F-3-pyridyl
35, R = phenyl
36, R = 4-F-phenyl
37, R = 3,5-di-F-phenyl
38, R = 2-SO_2Me-phenyl
39, R = 3-SO_2Me-phenyl

[a]Reagents and conditions: (i) 2-amino-5-fluoropyridine, EtOH, reflux; (ii) NIS, CH_3CN, rt; (iii) $RB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3, CH_3CN-water (3:1), 100 °C or $RSnBu_3$, $Pd(PPh_3)_4$, toluene, 110 °C; (iv) Oxone, THF-water (4:1), rt; (v) DMS, TFA, rt; (vi) 4-amino-6-chloropyrimidine-5-carbonitrile, DIPEA, n-butanol, 110 °C.

The regio- and stereoselective synthetic route employed for the synthesis of the imidazopyridine series is shown in Scheme 3. Condensation of **27** with 2-amino-5-fluoropyridine afforded the protected imidazopyridine **28**, which was subsequently iodinated with NIS to give **29**. Palladium-catalyzed coupling of **29** with the relevant boronic acids or esters afforded **30a-h**. 2-(Methylthio)phenyl analog **30g** was oxidized to the sulfone **30g'**. The Cbz-protecting group on **30a-h** was cleaved with dimethyl sulfide and TFA to provide **31a-h**. Nucleophilic displacement of 4-amino-6-chloropyrimidine-5-carbonitrile with the amine **31a-h** generated the desired imidazopyridine analogs **32-39**.

Table 2. SAR: Potency and selectivity of analogs at 1-position of benzimidazole

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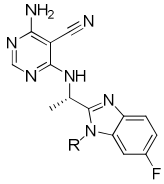


		Biochemical potency[a] IC_{50} (μM)				Human B cell[b]	Mouse B cell (pAKT)[c]	HWB (CD69)[d]	HWB (pAKT)[e]	HWB_unbound (pAKT)[f]
cmpd	R	PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (nM)
5	3-SO_2Me-phenyl	>125	3.58	0.069	2.9	0.0291	-	-		
15	2-SO_2Me-phenyl	55.9	8.96	0.107	61.9	0.25	-	-		
16	3-F-phenyl	8.6	0.51	0.0008	0.118	0.0083	-	-		
17	4-F-phenyl	30.6	0.45	0.0036	0.191	0.0573	-	-		
18	3,5-di-F-phenyl	>125	0.54	0.0004	0.27	0.0044	0.0045	0.019	0.084	10.1
24	cyclopropyl	2.5	0.03	0.0019	0.144	-	-	-		
25	phenyl	40.4	1.08	0.0022	1.4	0.0019	0.0025	0.022	0.032	2.4
26	2-pyridyl	29.4	3.53	0.012	12.6	0.016	-	0.107	0.131	35.4
1	3-pyridyl	58.2	1.78	0.016	5.8	0.0064	0.0046	-	0.0027	1.0
2	5-F-3-pyridyl	27.2	2.33	0.019	5.9	0.0065	0.0042	0.0195	0.0191	5.7

[a]Biochemical: Alphascreen assay. [b]Cellular: In vitro anti-IgM/CD40L-induced human B cell proliferation (as measured by thymidine incorporation) assay. [c]Cellular: Ability of compound to inhibit anti-IgM induced AKT phosphorylation ($pAKT^{Ser473}$) in mouse B cells; phospho-AKT (pAKT) expression on B220+gated B cells was determined by flow cytometry in mouse splenocytes. [d]Cellular: Compound pretreated human whole blood (HWB) was stimulated with anti-IgD to induce CD-69 expression on B cells (6 hours) and was evaluated by flow cytometry. [e]Compound pretreated human whole blood (HWB) was stimulated with anti-IgD to induce phosphorylation of AKT ($pAKT^{Ser473}$). [f]The unbound human whole blood (HWB_unbound) potency was derived by multiplying the human plasma protein binding (PPB) fraction unbound by the total HWB potency (f_u × HWB). f_u values are found in Table 4. [g]See Supporting Information for standard deviations.

SAR

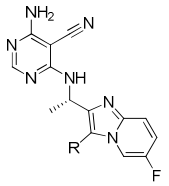
Attempts to optimize the benzimidazole series were focused on modification of the 3-methylsulfonylphenyl group in **5** (Table 2). Introduction of a 2-sulfonylmethyl group (**15)** decreases PI3Kδ activity (<2-fold). Subsequent replacement of 3-sulfonylmethyl group in **5** with a fluorine atom in **16** led to substantial improvement of both biochemical potency and cellular potency, while maintaining good selectivity over other PI3K isoforms (>10,000-fold over PI3Kα, 643-fold over PI3Kβ, and 148-fold over PI3Kγ). 4-Fluorophenyl **17** exhibited good biochemical potency and reasonably good selectivity (8500-fold over PI3Kα, 126-fold over PI3Kβ, and 53-fold over PI3Kγ), but showed reduced cellular potency. 3,5-Difluorophenyl **18** was very potent in both biochemical and cellular assays and showed great selectivity over other PI3K isoforms (>312,500-fold over PI3Kα, 1343-fold over PI3Kβ,

and 675-fold over PI3Kγ). Cyclopropyl analog **24** showed good biochemical potency, but the selectivity over PI3Kβ dropped (1337-fold over PI3Kα, 15-fold over PI3Kβ, and 76-fold over PI3Kγ). Unsubstituted phenyl analog **25** was potent in both biochemical and cellular assays and showed good selectivity (18,364-fold over PI3Kα, 491-fold over PI3Kβ, and 614-fold over PI3Kγ). As we observed in an earlier report,[10c] phenyl **25** and substituted phenyl analogs **5**, **15-18** significantly improved selectivity over other PI3K isoforms when compared to the cyclopropyl analog **24**. A 2-pyridyl analog **26** was well tolerated, showing reasonable biochemical potency, cellular potency, and good selectivity (2450-fold over PI3Kα, 294-fold over PI3Kβ, and 1050-fold over PI3Kγ). 3-Pyridyl analog **1** and 5-fluoro-3-pyridyl analog **2** were also well tolerated, and demonstrated a high level of potency, and selectivity over other PI3K isoforms (3638-fold and 1432-fold over PI3Kα, 111-fold and 123-fold over PI3Kβ, and 363-fold and 312-fold over PI3Kγ, respectively). Both **1** and **2** showed excellent kinase selectivity (KINOME*scan*'s selectivity score, S-Score(35) = 0) in a large panel of 442 protein kinases tested at 10 μM drug concentration.[24] The analogs **18**, **25**, **26**, **1,** and **2** that showed good cellular potency in B cell proliferation assay were further profiled in additional cellular assays, including anti-IgM-induced phosphorylation of AKT in mouse B cells (pAKTSer473), anti-IgD-induced CD-69 expression on B cells in 90% human whole blood [HWB (CD69)], and anti-IgD-induced phosphorylation of AKT ($pAKT^{Ser473}$) in 90% human whole blood [HWB (pAKT)]. **18**, **25**, **1,** and **2** showed good cellular potency (in vitro pAKT IC_{50} = 4.5 nM, 2.5 nM, 4.6 nM, and 4.2 nM, respectively), which were similar to potency in the B cell proliferation assay. **18**, **25**, **1**, and **2** were potent in HWB (CD69 and pAKT) assays, but **26** showed weaker HWB cellular potency compared to other analogs. In particular, **1** exhibited excellent HWB potency (HWB (pAKT) IC_{50} = 2.7 nM).

An alternative series of imidazopyridines was also explored (Table 3). While imidazopyridine analogs showed promising PI3Kδ enzyme potencies and increased selectivities over other isoforms, they were

consistently less potent, in both biochemical and cellular assays, compared to their benzimidazole counterparts.

Table 3. SAR of Imidazopyridine substitution



		Biochemical potency[a] IC_{50} (μM)				B Cell[b]
cmpd	R	PI3Kα	PI3Kβ	PI3Kδ	PI3Kγ	IC_{50} (μM)
32	2-pyridyl	46.1	10.40 ± 4.46	0.103 ± 0.144	7.92 ± 3.08	0.103 ± 0.0872
33	3-pyridyl	>125	24.7	0.612	82.5	-
34	5-F-3-pyridyl	>125	18.1 ± 13.4	0.57 ± 0.68	24.8 ± 18.6	0.0355
35	phenyl	>125	18.7	0.109	27.5	0.0576
36	4-F-phenyl	>125	5.93	0.017	1.42	0.0905
37	3,5-di-F-phenyl	>125	14.10 ± 5.44	0.0228 ± 0.00956	3.550 ± 0.988	0.036
38	2-SO_2Me-phenyl	>125	30.2	0.08	63.4	0.282
39	3-SO_2Me-phenyl	>125	5.51	0.113	3.34	0.142

[a]Biochemical: Alphascreen assay. [b]Cellular: In vitro anti-IgM/CD40L-induced B cell proliferation (as measured by thymidine incorporation) assay.

In Vivo PK

The pharmacokinetic profiles of several promising analogs were evaluated and despite their desirable potency and selectivity, the phenyl and substituted phenyl analogs (**5**, **16, 17, 18, and 25)** were found unsuitable for advancement due to a poor PK profile, namely undesirable clearance in the rat (Table 4). Alternatively, it was observed that replacing the phenyl moiety with a pyridine ring, as with **26**, **1**, and **2**, resulted in compounds with good total clearance and good oral bioavailability (F = 58%, 45%, and 41%, respectively). In fact, the unbound clearance of these pyridyl analogs was around 10-fold lower than the phenyl analogs. All analogs showed good solubility and did not inhibit CYP3A4.

Table 4. Physicochemical Properties of the Benzimidazole leads

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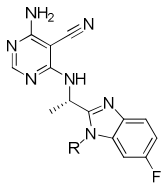
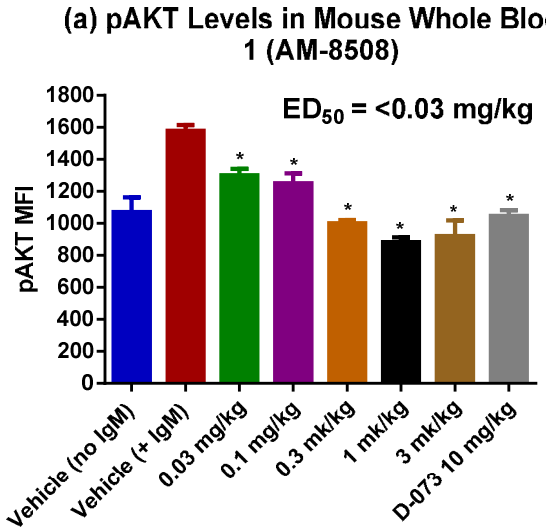
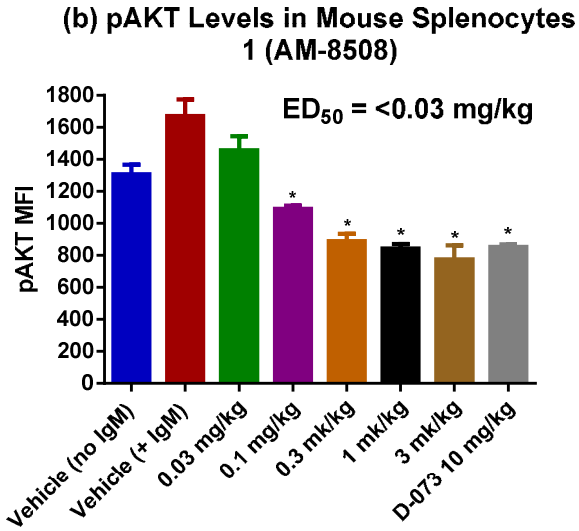


cmpd	R	Solubility[a] (μM)	CYP3A4[b] (% inhibition)	Microsomal Stability[c] (%Turnover) human	rat	Protein Binding (f_u) human[d]	rat[e]	Rat PK[f] IV[g] CL (L/h/Kg)	CL$_u$[k] (L/h/Kg)	PO[l] %F
5	3-SO_2Me-phenyl	500	20	<10	<10	0.40	0.35	4.3[h]	12.3	-
16	3-F-phenyl	401	15	15	28	0.12	0.10	2.76[i]	27.6	-
17	4-F-phenyl	353	<10	<10	22	0.11	0.10	1.78	18.0	-
18	3,5-di-F-phenyl	305	16	11	30	0.12	0.13	2.64	20.3	-
25	Phenyl	374	<10	<10	26	0.07	0.09	2.41	26.8	-
26	2-pyridyl	500	<10	<10	<10	0.27	0.22	0.97	4.4	58
1	3-pyridyl	474	<10	<10	<10	0.36	0.36	0.93[j]	2.6	45
2	5-F-3-pyridyl	500	<10	<10	<10	0.30	0.32	0.99	3.1	41

[a]Single experimental values. Aqueous equilibrium solubility assay: compound was equilibrated at room temperature for 72 hours in PBS buffer (pH 7.4), supernatant was centrifuged and analyzed by HPLC for reporting solubility as μM. [b]CYP: cytochrome P450 assay competitive (midazolam as probe substrate, 5 μM) reported as % inhibition at 3 μM (LC/MS). [c]Single experimental values. %Turnover was measured by LC/MS after incubation of parent compound (1 μM) in liver microsome (0.25 mg/mL) in potassium phosphate (66.7 mM) buffered with NADPH (1 mM) at 37 °C for 30 min. [d]5 μM concentration in human plasma, protein binding measured by ultracentrifugation and LC/MS. [e]5 μM concentration in rat plasma, protein binding measured by ultracentrifugation and LC/MS. [f]Pharmacokinetic parameters following administration in male Sprague-Dawley rats: two animals per study for IV and three animals per study for PO. [g]Dosed at 0.5 mg/kg as a solution in 100% DMSO. [h]Dose IV, 0.8 mg/kg. [i]Dose IV, 0.72 mg/kg. [j]Dose IV, 0.71 mg/kg. [k]Unbound clearance equals the total clearance divided by the rat fraction unbound (CL$_u$ = CL/f_u). [l]Dosed at 2.0 mg/kg as a suspension in 1% Tween 80, 0.5% methylcellulose, 98.5% water.





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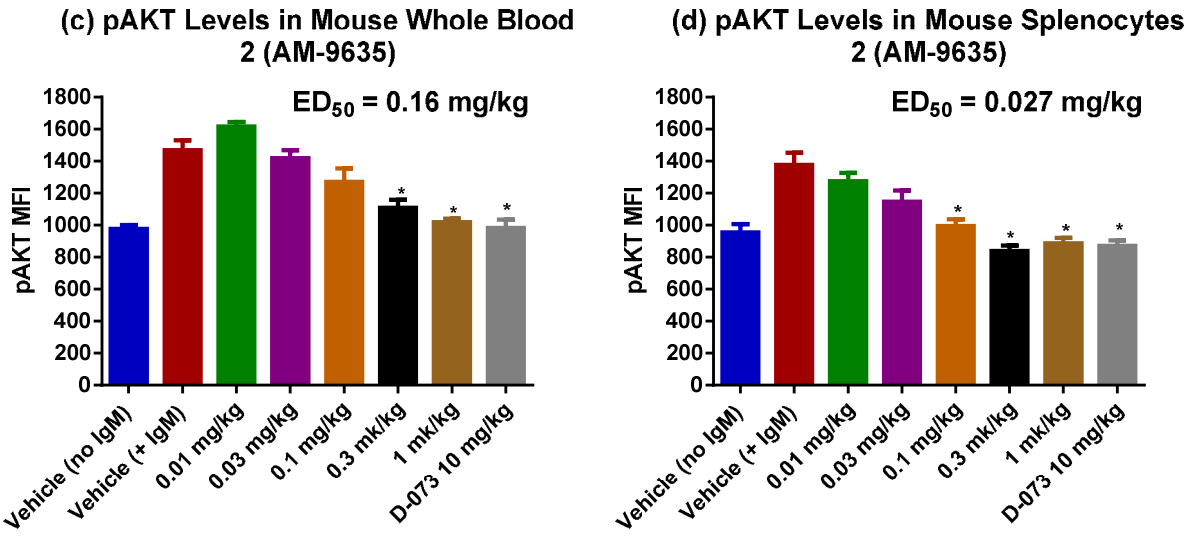

Figure 3. Inhibition of activation induced AKT phosphorylation in B cells in vivo. Vehicle, **1** (**AM-8508**), **2** (**AM-9635**), and benchmark compound (D-073)[25] in 2%HPMC+1%Tween80, pH 2.0, was administered to IgM_m mice orally 15 minutes before intravenous (IV) injection of FITC-labeled anti-IgM to stimulate B cells. Mice were sacrificed 30 minutes after stimulation to measure pAKT in the spleen and whole blood of FITC-IgM positive B cells by flow cytometry. (a) pAKT of **1 (AM-8508)** in whole blood. (b) pAKT of **1 (AM-8508)** in splenocytes. (c) pAKT of **2 (AM-9635)** in whole blood. (d) pAKT of **2 (AM-9635)** in splenocytes. t-Test was used to evaluate differences between groups. The asterisk (*) denotes statistical significance ($p < 0.05$) when compared to the control group.

In Vivo Pharmacology

Given the potency, selectivity, and pharmacokinetic profile of **1** and **2**, these compounds were advanced for evaluation in our in vivo animal models of inflammation. To establish a correlation between the in vitro and in vivo activity of **1** and **2**, a single-dose in vivo assay was established that simulated the in vitro anti-IgM stimulated pAKT assay. In this assay, the murine B cell-transgenic line 3751, which has only surface-bound IgM and lacks secreted/circulating IgM was employed ($IgM_{membrane}$ only; IgM_m).[26] These transgenic mice were used to avoid IgM/anti-IgM immune complex formation that could promote serum sickness in normal mice. Compound **1** or **2** was administered to IgM_m mice orally 15 minutes before intravenous (IV) injection of FITC-labeled anti-IgM to stimulate B cells. Mice were sacrificed 30 minutes after stimulation to measure pAKT in the spleen and whole blood of FITC-IgM positive B cells by flow cytometry (Figure 3). **1** and **2** showed dose-dependent inhibition of AKT phosphorylation in anti-IgM stimulated B cells isolated from the whole blood and spleen compartments of IgM_m mice.

The calculated ED_{50} values of **1** in whole blood and spleen were <0.03 mg/kg (Figure 3a and Figure 3b). The calculated ED_{50} values of **2** in mouse whole blood and spleen were 0.16 mg/kg and 0.027 mg/kg, respectively (Figure 3c and Figure 3d). Data from the in vivo pAKT assay was subsequently utilized to select doses for the multi-dose rat protein-antigen (Keyhole Limpet Hemocyanin, KLH) studies. A multiple dose rat study was performed to determine whether **1** and **2** impacted B cell function in vivo. For this purpose, the effect of **1** on a humoral immune response was studied at oral doses of 0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg per day and the effect of **2** on a humoral immune response was studied at oral doses of 0.01, 0.03, 0.1, 0.3, and 1 mg/kg per day to determine whether the compounds inhibit antigen-specific Ig secretion by B cells. **1** inhibited KLH-specific IgG and IgM in a dose-dependent manner (Figure 4a and Figure 4b).[a] The calculated ED_{50} values were 0.08 mg/kg and 0.12 mg/kg, respectively. Exposure at these doses would be expected to significantly inhibit PI3Kδ given the free drug concentrations achieved relative to the HWB_unbound pAKT IC_{50} (1.0 nM) and PI3Kδ in vitro mouse pAKT_unbound IC_{50} (3.1 nM) over the 24 hours dosing interval (Figure 4c). **2** inhibited KLH-specific IgG and IgM in a dose-dependent manner (Figure 5a and Figure 5b).[b] The calculated ED_{50} values were 0.11 mg/kg and 0.04 mg/kg, respectively. Exposure at these doses would be expected to significantly inhibit PI3Kδ given the free drug concentrations achieved relative to the HWB_unbound pAKT IC_{50} (5.7 nM) and PI3Kδ in vitro mouse pAKT_unbound IC_{50} (3.2 nM) over the 24 hours dosing interval (Figure 5c). Exposure at all doses of **1** and **2** were below PI3Kβ in vitro human pAKT_unbound IC_{50} (650 nM and 1928 nM, respectively) over a 24 hours dosing interval (Figure 4c and Figure 5c). Both compounds **1** and **2** were well tolerated at all doses and exhibited significantly reduced IgG and IgM

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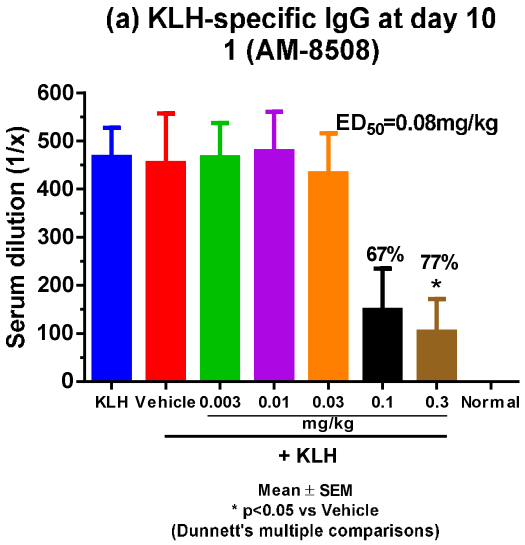
[a] The effect on IgM was not statistically significant.

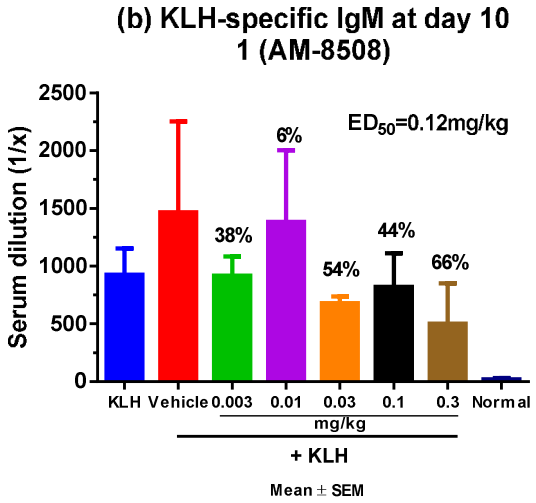
[b] The effect on IgM was only statistically significant at the 1 mg/kg dose.

specific antibodies (Figure 4 and Figure 5). The KLH-mediated humoral immune response allowed us to select **1** and **2** as candidates for further animal disease studies.

CONCLUSION

A novel series of potent and selective PI3Kδ inhibitors were designed by introducing an optimized 4-aminopyrimidine-5-carbonitrile moiety as a hinge binder on a benzimidazole ring in the specificity pocket. The alternative hinge binder 4-aminopyrimidine-5-carbonitrile moiety substantially improved selectivity over the PI3Kβ isoform. The replacement of the quinoline ring to benzimidazole ring significantly improved selectivity over the PI3Kγ isoform. The 90° angle conformation of the aminopyrimidine and benzimidazole rings resulted in excellent kinase selectivity. SAR efforts led to the optimized compounds **1** (AM-8508) and **2** (AM-9635). Notably, analogs **1** and **2** had excellent PI3Kδ potency and selectivity over other PI3K isoforms and protein kinases, as well as acceptable PK properties, which made them suitable for evaluation in in vivo efficacy studies. These experiments demonstrated that both **1** and **2** could inhibit KLH-specific antibodies in animal models, signifying their potential for the treatment of human inflammatory diseases.





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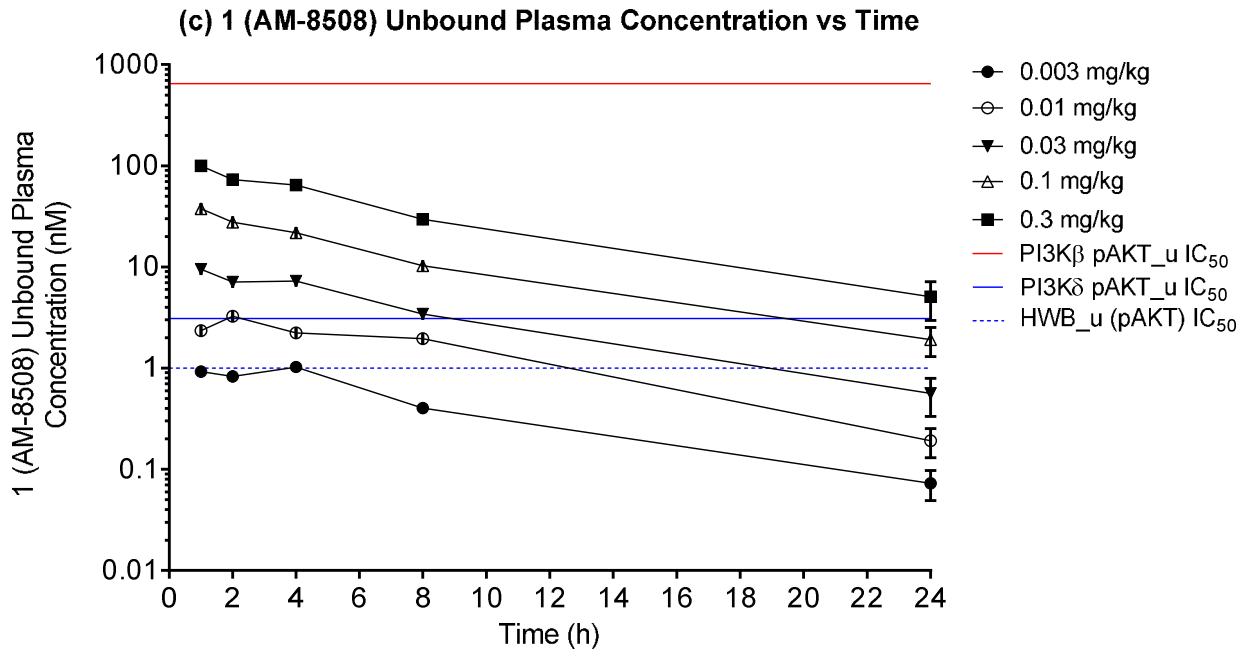
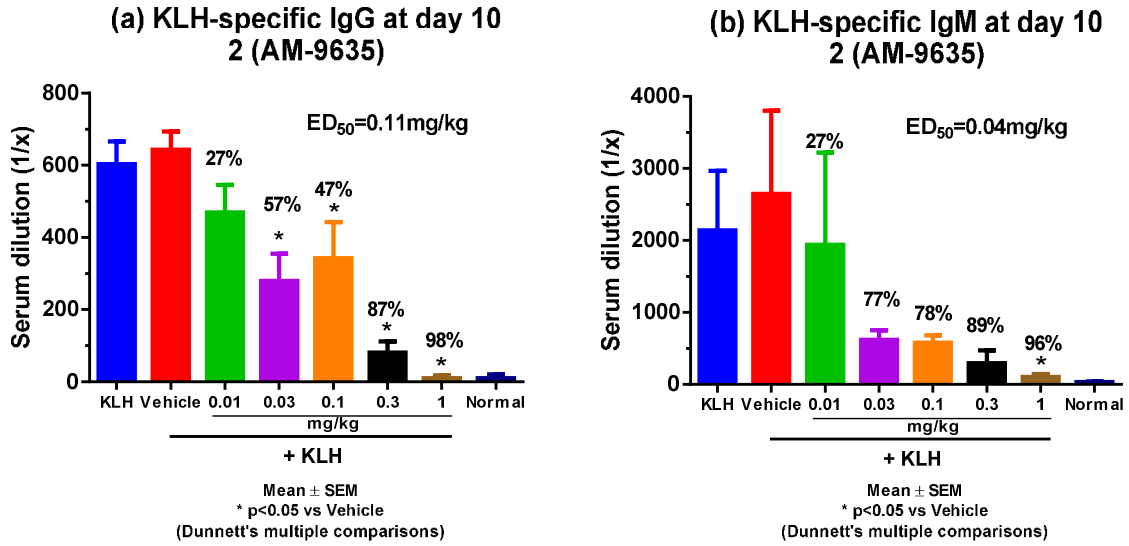


Figure 4. Inhibition of KLH-specific antibodies and delta-specific coverage. Vehicle or **1** (**AM-8508**) in 2% HPMC, 1% Pluronic F68, 10% Captisol, pH 2.0, was administered (0.003, 0.01, 0.03, 0.1, and 0.3 mg/kg) q.d. po for 10 days in female Lewis rats (N = 8/dose group). 2 hours after the first dosing, 200 μL of PBS containing 60 μg of KLH was administered to each rat intravenously. Ten days after the KLH priming, blood was collected for the measurement of KLH specific IgG (a) and IgM (b) by ELISA. The y-axis is represented as a mean serum dilution factor. Error bars represent the standard error of the mean (SEM) of eight rats. (c) After administration of **1** (**AM-8508**), plasma was also harvested at day 10 to assess exposures in each dose group. Unbound drug concentrations were measured by LC−MS/MS and plotted relative to HWB_unbound pAKT IC_{50} = 1.0 nM (in Table 2) represented as a blue-dotted line, PI3Kδ in vitro mouse pAKT_unbound IC_{50} 3.1 nM [calculated from 4.6 nM (PI3Kδ in vitro mouse pAKT IC_{50}) × 0.674 (f_u in pAKT assay media)] represented as a blue line, and PI3Kβ in vitro human pAKT_unbound IC_{50} 650 nM [calculated from 964 nM (PI3Kβ in vitro human pAKT IC_{50}) × 0.674 (f_u in pAKT assay media)] represented as a red line.



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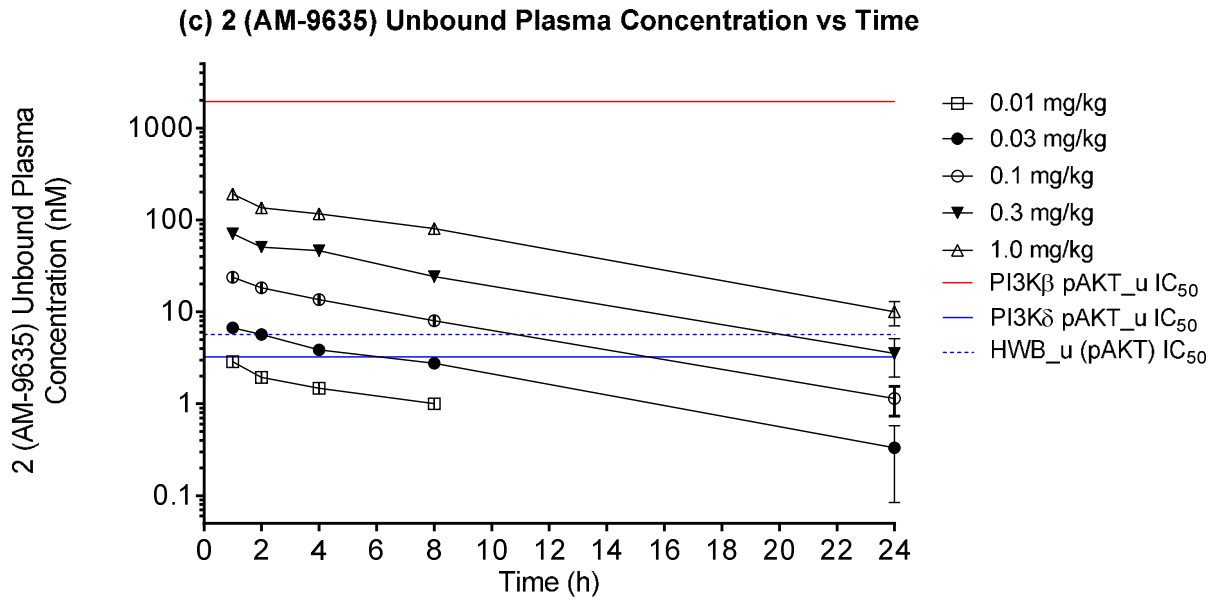

Figure 5. Inhibition of KLH-specific antibodies and delta-specific coverage. Vehicle or **2** (**AM-9635**) in 2% HPMC, 1% Pluronic F68, 10% Captisol, pH 2.0, was administered (0.01, 0.03, 0.1, 0.3, and 1 mg/kg) q.d. po for 10 days in female Lewis rats (N = 8/dose group). 2 hours after the first dosing, 200 µL of PBS containing 60 µg of KLH was administered to each rat intravenously. Ten days after the KLH priming, blood was collected for the measurement of KLH specific IgG (a) and IgM (b) by ELISA. The y-axis is represented as a mean serum dilution factor. Error bars represent the standard error of the mean (SEM) of eight rats. (c) After administration of **2** (**AM-9635**), plasma was also harvested at day 10 to assess exposures in each dose group. Unbound drug concentrations were measured by LC−MS/MS and plotted relative to HWB_unbound pAKT IC_{50} = 5.7 nM (in Table 2) represented as a blue-dotted line, PI3Kδ in vitro mouse pAKT_unbound IC_{50} 3.2 nM [calculated from 4.2 nM (PI3Kδ in vitro mouse pAKT IC_{50}) × 0.771 (f_u in pAKT assay media)] represented as a blue line, and PI3Kβ in vitro human pAKT_unbound IC_{50} 1928 nM [calculated from 2500 nM (PI3Kβ in vitro human pAKT IC_{50}) × 0.771 (f_u in pAKT assay media)] represented as a red line.

EXPERIMENTAL

General Chemistry

All solvents and chemicals used were reagent grade. Anhydrous solvents were purchased from Sigma-Aldrich and used as received. Analytical thin layer chromatography (TLC) and silica gel column chromatography were performed on Merck silica gel 60 (230-400 mesh). Removal of solvents was conducted by using a rotary evaporator and residual solvents were removed from nonvolatile compounds using a vacuum manifold maintained at approximately 1 Torr. All yields reported are isolated yields. Preparative reverse–phase high pressure liquid chromatography (RP–HPLC) was performed using an Agilent 1100 series HPLC and Phenomenex Gemini C18 column (5 µm, 100 mm × 30 mm i.d.), eluting with a

binary solvent system, A and B, using a gradient elution [A, H_2O with 0.1% TFA, B, CH_3CN with 0.1% TFA] with UV detection at 220 nm. All final compounds were purified to ≥95% purity as determined by an Agilent 1100 series HPLC with UV detection at 220 nm using the following method: Zorbax SB-C8 column (3.5 μm, 150 mm × 4.6 mm i.d.), eluting with a binary solvent system, A and B, using a 5-95% B (0-15 min) gradient elution [A, H_2O with 0.1% TFA, B, CH_3CN with 0.1% TFA]; flow rate 1.5 mL/min. Mass spectral data was recorded on an Agilent 1100 series LCMS with UV detection at 254 nm. All accurate mass data (High-resolution mass spectra: HRMS) were acquired on a Synapt G2 ToF instrument operating in positive electrospray ionization mode, over the *m/z* range 50-1200. Lock mass correction was performed on the leucine-enkephalin ion *m/z* 556.2771. The instrument resolution was 28,000 at FWHM. The compounds were introduced into the mass spectrometer using an Agilent 1200 operated with a C4 Bridged-ethyl-hybrid (BEH) analytical column (2.1 x 50 mm) at 0.25 mL/min. NMR spectra were recorded on a Bruker Avance 400 MHz and 500 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) relative to residual undeuterated solvent as internal reference and coupling constants (*J*) are reported in hertz (Hz). Splitting patterns are indicated as follows: s = singlet; d = doublet; t = triplet; q = quartet; qn = quintet; dd = doublet of doublets; dt = doublet of triplets; tt = triplet of triplets; m = multiplet; br = broad peak.

Benzimidazole Analogs

General Procedure for the Synthesis of 9a-g. A mixture of 2,4-difluoronitrobenzene **8** (1 equiv) and substituted aniline **9a-g** (1 equiv) was stirred at 130 °C for 24 hours. The mixture was cooled to room temperature and precipitates were noted. The mixture was dissolved in DCM and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by chromatography on a silica gel column using 0-50% gradient of EtOAc in hexane as eluent to give **9a-g**.

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5-Fluoro-N-(3-(methylsulfonyl)phenyl)-2-nitroaniline (9a). 50.5 % yield as a bright yellow solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.65 (1 H, s), 8.25 (1 H, dd, *J*=9.6, 6.1 Hz), 7.85 - 7.89 (1 H, m), 7.64 - 7.75 (3 H, m), 6.98 (1 H, dd, *J*=11.4, 2.6 Hz), 6.79 - 6.87 (1 H, m), 3.24 (3 H, s). Mass spectrum (ESI) *m/z* 311.0 $[M+H]^+$.

5-Fluoro-N-(2-(methylthio)phenyl)-2-nitroaniline (9b). 43.3 % yield as an orange solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.52 (1 H, s), 8.26 (1 H, dd, *J*=9.4, 6.1 Hz), 7.34 - 7.45 (3 H, m), 7.26 - 7.32 (1 H, m), 6.67 - 6.75 (1 H, m), 6.35 (1 H, dd, *J*=11.6, 2.6 Hz), 2.43 (3 H, s). Mass spectrum (ESI) *m/z* 279.0 $[M+H]^+$.

5-Fluoro-N-(3-fluorophenyl)-2-nitroaniline (9c). 20.9% yield as an orange solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.54 (1 H, s), 8.24 (1 H, dd, *J*=9.4, 6.1 Hz), 7.40 - 7.50 (1 H, m), 7.18 - 7.27 (2 H, m), 7.05 (1 H, td, *J*=8.5, 2.5 Hz), 6.94 (1 H, dd, *J*=11.6, 2.6 Hz), 6.79 (1 H, ddd, *J*=9.6, 7.3, 2.6 Hz). Mass spectrum (ESI) *m/z* 251.1 $[M+H]^+$.

5-Fluoro-N-(4-fluorophenyl)-2-nitroaniline (9d). 80 % yield as an orange solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.56 (1 H, s), 8.24 (1 H, dd, *J*=9.5, 6.2 Hz), 7.36 - 7.44 (2 H, m), 7.25 - 7.33 (2 H, m), 6.67 - 6.74 (1 H, m), 6.64 (1 H, dd, *J*=11.8, 2.6 Hz). Mass spectrum (ESI) *m/z* 251.1 $[M+H]^+$.

N-(3,5-Difluorophenyl)-5-fluoro-2-nitroaniline (9e). 17.6% yield as an orange solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.48 (1 H, s), 8.23 (1 H, dd, *J*=9.4, 6.1 Hz), 7.05 - 7.17 (3 H, m), 6.99 (1 H, tt, *J*=9.4, 2.3 Hz), 6.84 - 6.91 (1 H, m). Mass spectrum (ESI) *m/z* 267.0 $[M-H]^-$.

N-(5-Fluoro-2-nitrophenyl)pyridin-3-amine (9f). 38.3 % yield as an orange solid. Mass spectrum (ESI) *m/z* 234.1 $[M+H]^+$.

5-Fluoro-N-(5-fluoro-2-nitrophenyl)pyridin-3-amine (9g). 52.5 % yield as an orange solid. Mass spectrum *m/z* 252.1 $[M+H]^+$.

General Procedure for the Synthesis of 10a-g. A mixture of **9a-g** (1 equiv) and Tin(II) chloride dihydrate (5 equiv) in EtOAc (0.15 M) was stirred at reflux. After 5 hours, the mixture was cooled to room temperature, and poured into 10 M aqueous NaOH solution. The mixture was extracted with EtOAc. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography on a silica gel column using a 0-20% gradient of EtOAc in hexane as eluent to give **10a-g**.

5-Fluoro-N1-(3-(methylsulfonyl)phenyl)benzene-1,2-diamine (10a). 100% yield as a colorless syrup. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.82 (1 H, s), 7.36 - 7.43 (1 H, m), 7.18 - 7.25 (2 H, m), 7.01 (1 H, ddd, *J*=8.2, 2.2, 1.1 Hz), 6.83 - 6.89 (1 H, m), 6.71 - 6.79 (2 H, m), 4.71 (2 H, s), 3.14 (3 H, s). Mass spectrum (ESI) *m/z* 281.0 $[M+H]^+$.

5-Fluoro-N1-(2-(methylthio)phenyl)benzene-1,2-diamine (10b). 92% yield as a pale yellow syrup. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.35 (1 H, dd, *J*=7.7, 1.5 Hz), 7.11 (1 H, td, *J*=7.7, 1.5 Hz), 6.91 (1 H, td, *J*=7.5, 1.2 Hz), 6.78 (1 H, dd, *J*=8.0, 1.4 Hz), 6.71 (1 H, dd, *J*=8.6, 5.9 Hz), 6.65 (1 H, s), 6.52 - 6.62 (2 H, m), 4.61 (2 H, s), 2.40 (3 H, s). Mass spectrum (ESI) *m/z* 249.0 $[M+H]^+$.

5-Fluoro-N1-(3-fluorophenyl)benzene-1,2-diamine (10c). 69.4% yield as an orange oil. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.54 (1 H, s), 7.12 - 7.22 (1 H, m), 6.83 (1 H, dd, *J*=10.4, 2.7 Hz), 6.65 - 6.77 (2 H, m), 6.57 - 6.63 (1 H, m), 6.43 - 6.53 (2 H, m), 4.66 (2 H, s). Mass spectrum (ESI) *m/z* 221.1 $[M+H]^+$.

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5-Fluoro-N1-(4-fluorophenyl)benzene-1,2-diamine (10d). 94% yield as a brown syrup. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.18 (1 H, s), 6.98 - 7.07 (2 H, m), 6.81 - 6.90 (2 H, m), 6.65 - 6.76 (2 H, m), 6.54 - 6.62 (1 H, m), 4.61 (2 H, s). Mass spectrum (ESI) *m/z* 221.1 $[M+H]^+$.

N1-(3,5-Difluorophenyl)-5-fluorobenzene-1,2-diamine (10e). 96 % yield as a light yellow syrup. ^{1}H-NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.85 (1 H, s), 6.85 (1 H, dd, *J*=10.0, 2.3 Hz), 6.72 - 6.81 (2 H, m), 6.41 (1 H, tt, *J*=9.4, 2.3 Hz), 6.24 - 6.33 (2 H, m), 4.71 (2 H, s). Mass spectrum (ESI) *m/z* 239.1 $[M+H]^+$.

5-Fluoro-N1-(pyridin-3-yl)benzene-1,2-diamine (10f). 77 % yield as a black solid. Mass spectrum (ESI) *m/z* 204.1 $[M+H]^+$.

5-Fluoro-N1-(5-fluoropyridin-3-yl)benzene-1,2-diamine (10g). 95% yield as a dark solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.98 (1 H, t, *J*=2.0 Hz), 7.83 - 7.91 (2 H, m), 6.84 - 6.91 (1 H, m), 6.72 - 6.79 (3 H, m), 4.75 (2 H, s). Mass spectrum (ESI) *m/z* 222.1 $[M+H]^+$.

General Procedure for the Synthesis of 11a-c, 11e-g. To a -10 °C solution (NaCl-ice bath) of Boc-L-Ala-OH (2 equiv) and N-methylmorpholine (2.1 equiv) in DCM (0.2 M) was added isobutyl chloroformate (2 equiv). The resulting cloudy colorless mixture was stirred at -10 °C. After 1 hour, a solution of **10a-c, 10e-g** (1 equiv) in DCM (0.2 M). was added to the mixture. The resulting mixture was stirred at -10 °C for 40 minutes, then allowed to warm to room temperature. After 24 hours, saturated aqueous NH_4Cl solution was added to the mixture. and the organic layer separated. The aqueous mixture was extracted with DCM. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography on a silica gel column using 0-50% gradient of EtOAc in hexane as eluent to give **11a-c, 11e-g**.

(S)-tert-Butyl 1-(4-fluoro-2-(3-(methylsulfonyl)phenylamino)phenylamino)-1-oxopropan-2-ylcarbamate (11a). 84 % yield as a solid. ^{1}H NMR (500 MHz, *DMSO-d*$_6$) δ ppm 9.40 (1 H, s), 7.86 (1 H, s), 7.48 (2 H, q, *J*=8.0 Hz), 7.40 (1 H, br. s.), 7.35 (1 H, d, *J*=7.3 Hz), 7.23 (1 H, d, *J*=7.8 Hz), 7.13 (1 H, d, *J*=6.4 Hz), 7.09 (1 H, dd, *J*=10.5, 2.7 Hz), 6.87 (1 H, td, *J*=8.2, 1.5 Hz), 4.05 - 4.11 (1 H, m), 3.16 (3 H, s), 1.35 (9 H, s), 1.16 - 1.21 (3 H, m). Mass spectrum (ESI) m/z 452.1 $[M+H]^+$ and m/e 450.1 $[M-H]^-$.

(S)-tert-Butyl 1-(4-fluoro-2-(2-(methylthio)phenylamino)phenylamino)-1-oxopropan-2-ylcarbamate (11b). 94% yield as a white solid. ^{1}H-NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.56 (1 H, br. s.), 7.36 (1 H, dd, *J*=7.8, 1.4 Hz), 7.26 (1 H, dd, *J*=8.6, 6.3 Hz), 7.12 - 7.19 (1 H, m), 7.10 (1 H, d, *J*=6.8 Hz), 6.97 - 7.07 (3 H, m), 6.70 (1 H, td, *J*=8.2, 2.3 Hz), 6.61 (1 H, d, *J*=11.0 Hz), 4.14 (1 H, quin, *J*=6.8 Hz), 2.37 (3 H, s), 1.34 (9 H, s), 1.26 (3 H, d, *J*=7.2 Hz). Mass spectrum (ESI) *m/z* 420.1 $[M+H]^+$.

(S)-tert-Butyl (1-((4-fluoro-2-((3-fluorophenyl)amino)phenyl)amino)-1-oxopropan-2-yl)carbamate (11c). 93% yield as a white solid. Mass spectrum (ESI) *m/z* 390.1 $[M-H]^-$.

(S)-(9H-Fluoren-9-yl)methyl 1-(4-fluoro-2-(4-fluorophenylamino)phenylamino)-1-oxopropan-2-ylcarbamate (11d). To a -10 °C solution (NaCl-ice bath) of Fmoc-L-Ala-OH (2.1 equiv) and N-methylmorpholine (2.1 equiv) in DCM (0.4 M) was added isobutyl chloroformate (2 equiv). The resulting cloudy colorless mixture was stirred at -10 °C for 30 minutes. To the mixture was then added a solution of 5-fluoro-N1-(4-fluorophenyl)benzene-1,2-diamine (1 equiv) in DCM (0.5 M). The resulting mixture was stirred at -10 °C. After 1 hour, to the cold mixture was added saturated NH_4Cl. The resulting precipitate was collected by filtration and washed the solid with water to give **11d** (quantitave yield) as an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 9.42 (1 H, s), 7.89 (2 H, d, *J*=7.6 Hz), 7.63

- 7.78 (3 H, m), 7.22 - 7.46 (6 H, m), 6.96 - 7.14 (4 H, m), 6.83 (1 H, dd, *J*=11.2, 2.7 Hz), 6.67 (1 H, td, *J*=8.5, 2.8 Hz), 4.11 - 4.33 (4 H, m), 1.28 (3 H, d, *J*=7.0 Hz). Mass spectrum (ESI) *m/z* 514.2 $[M+H]^+$.

(S)-tert-butyl 1-(2-(3,5-difluorophenylamino)-4-fluorophenylamino)-1-oxopropan-2-ylcarbamate (11e). 98 % yield as a white solid. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 9.41 (1 H, br. s.), 7.87 (1 H, br. s.), 7.50 (1 H, dd, *J*=8.6, 6.5 Hz), 7.07 - 7.20 (2 H, m), 6.87 - 6.96 (1 H, m), 6.43 - 6.62 (3 H, m), 3.95 - 4.13 (1 H, m), 1.37 (9 H, s), 1.17 (3 H, d, *J*=7.0 Hz). Mass spectrum (ESI) *m/z* 410.2 $[M+H]^+$.

(S)-Methyl 5-(2-(2-(tert-butoxycarbonylamino)propanamido)-5-fluorophenylamino)nicotinate (11f). 99% yield as a dark brown solid. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 9.44 (1 H, br s), 8.28 (1 H, br s), 8.08 (1 H, br d, *J*=4.1 Hz), 7.32 - 7.68 (1 H, m), 7.31 - 7.81 (1 H, m), 7.24 (1 H, dd, *J*=8.1, 4.6 Hz), 7.16 (1 H, br s), 6.99 (1 H, dd, *J*=10.8, 2.9 Hz), 6.72 - 6.85 (1 H, m), 4.07 (1 H, br s), 1.35 (9 H, s), 1.20 (3 H, br d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 375.1 $[M+H]^+$.

(S)-tert-Butyl 1-(4-fluoro-2-(5-fluoropyridin-3-ylamino)phenylamino)-1-oxopropan-2-ylcarbamate (11g). 79 % yield as a light yellow solid. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 9.45 (1 H, s), 8.11 (1 H, br s), 7.99 (1 H, d, *J*=2.0 Hz), 7.92 (1 H, s), 7.44 - 7.54 (1 H, m), 7.02 - 7.19 (2 H, m), 6.91 (1 H, br t, *J*=7.1 Hz), 2.60 - 2.86 (1 H, m), 1.30 - 1.42 (9 H, m), 1.14 - 1.19 (3 H, m). Mass spectrum (ESI) *m/z* 393.1 $[M+H]^+$.

General Procedure for the Synthesis of 12a-g. A solution of **11a-g** in AcOH (0.3 M) was stirred and heated at 100 °C. After 24 hours, the mixture was cooled to room temperature and poured into DCM and saturated aqueous $NaHCO_3$ solution. The aqueous layer was separated. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, washed with water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **12a-g**.

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[NOTE]: Epimerization occurred during cyclization, and Boc-protecting group was cleaved and the free amine was aceylated under reaction conditions.

(S)-N-(1-(6-Fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12a). 83% yield as a pink solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.40 (1 H, d, *J*=8.0 Hz), 8.07 - 8.14 (1 H, m), 8.04 (1 H, br. s.), 7.89 (2 H, d, *J*=4.5 Hz), 7.75 (1 H, dd, *J*=8.8, 4.9 Hz), 7.15 (1 H, ddd, *J*=9.9, 8.9, 2.5 Hz), 7.01 (1 H, dd, *J*=9.0, 2.3 Hz), 4.98 - 5.13 (1 H, m), 3.32 (3 H, s), 1.59 (3 H, s), 1.45 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 376.0 $[M+H]^+$.

N-(1-(6-fluoro-1-(2-(methylthio)phenyl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12b). 29.3 % yield as a pink solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.16 - 8.45 (1 H, m), 7.72 (1 H, dd, *J*=8.8, 4.9 Hz), 7.56 - 7.64 (1 H, m), 7.49 - 7.55 (1 H, m), 7.33 - 7.48 (2 H, m), 7.10 (1 H, ddd, *J*=9.8, 8.8, 2.5 Hz), 6.61 - 6.70 (1 H, m), 4.73 - 5.04 (1 H, m), 2.35 - 2.43 (3 H, m), 1.63 - 1.72 (3 H, m), 1.34 - 1.44 (3 H, m). Mass spectrum (ESI) *m/z* 344.0 $[M+H]^+$.

N-(1-(6-fluoro-1-(2-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12b'). To a mixture of **12b** (1 equiv) in THF-water (3:1, 1 M) was added Oxone (2.5 equiv) and the mixture was stirred at room temperature. After 48 hours, to the mixture was diluted with water and extracted with DCM. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give **12b'** (98 % yield) as an orange solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.13 - 8.30 (2 H, m), 7.84 - 8.02 (2 H, m), 7.48 - 7.80 (2 H, m), 6.82 - 7.18 (2 H, m), 4.85 - 5.03 (1 H, m), 2.81 - 3.02 (3 H, m), 1.53 - 1.73 (3 H, m), 1.32 - 1.50 (3 H, m). Mass spectrum (ESI) *m/z* 376.0 $[M+H]^+$.

N-(1-(6-Fluoro-1-(3-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12c). Quantitative yield as pink solid. Mass spectrum (ESI) *m/z* 316.1 $[M+H]^+$.

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(S)-(9H-fluoren-9-yl)methyl 1-(6-fluoro-1-(4-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethylcarbamate (12d). Quantitative yield as a dark brown syrup. Mass spectrum (ESI) *m/z* 496.1 $[M+H]^+$.

N-(1-(1-(3,5-Difluorophenyl)-6-fluoro-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12e). 86% yield as a brown solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.38 (1 H, d, *J*=7.4 Hz), 7.72 (1 H, dd, *J*=8.8, 4.9 Hz), 7.51 (1 H, tt, *J*=9.5, 2.3 Hz), 7.41 (2 H, dd, *J*=7.9, 1.7 Hz), 7.13 (1 H, ddd, *J*=9.8, 8.8, 2.5 Hz), 7.03 - 7.09 (1 H, m), 5.07 (1 H, qd, *J*=7.1, 6.8 Hz), 1.64 (3 H, s), 1.44 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 334.1 $[M+H]^+$.

N-(1-(6-Fluoro-1-(pyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12f). 99 % yield as a brown foam. Mass spectrum (ESI) *m/z* 299.1 $[M+H]^+$.

N-(1-(6-Fluoro-1-(5-fluoropyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)acetamide (12g). 69.7% yield as a dark brown solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.20 - 8.39 (1 H, m), 8.13 (1 H, s), 7.98 (1 H, d, *J*=2.3 Hz), 7.47 (1 H, dd, *J*=8.8, 6.3 Hz), 7.02 - 7.18 (2 H, m), 6.88 (1 H, td, *J*=8.5, 2.7 Hz), 4.28 (1 H, br d, *J*=7.2 Hz), 1.83 (3 H, s), 1.18 (3 H, d, *J*=7.0 Hz). Mass spectrum (ESI) *m/z* 317.1 $[M+H]^+$.

General Procedure for the Synthesis of 13a-c, 13e-g. A solution of **12a-c, 12e-g** (1 equiv) and 2 N HCl (16 equiv) was stirred and heated at 100 °C. After 24 hours, the mixture was cooled to room temperature. The acidic aqueous mixture was washed with DCM to remove organic impurities and then basified to pH 10 with aqueous 10 N NaOH and extracted with DCM. The combined organic layers were washed with water, brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **13a-c, 13e-g**.

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1-(6-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethanamine (13a). 80% yield as white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.16 - 8.20 (1 H, m), 8.09 - 8.15 (1 H, m), 7.97 - 8.03 (1 H, m), 7.89 - 7.95 (1 H, m), 7.71 (1 H, dd, *J*=8.8, 4.9 Hz), 7.12 (1 H, ddd, *J*=10.0, 8.8, 2.5 Hz), 7.01 (1 H, dd, *J*=9.0, 2.3 Hz), 3.96 (1 H, q, *J*=6.7 Hz), 3.35 (3 H, s), 1.95 (2 H, br. s.), 1.36 (3 H, d, *J*=6.7 Hz). Mass spectrum (ESI) *m/z* 334.0 $[M+H]^+$.

(S)-1-(6-Fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethanamine (13a). 80 % yield as a white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.16 - 8.20 (1 H, m), 8.09 - 8.15 (1 H, m), 7.97 - 8.03 (1 H, m), 7.89 - 7.95 (1 H, m), 7.71 (1 H, dd, *J*=8.8, 4.9 Hz), 7.12 (1 H, ddd, *J*=10.0, 8.8, 2.5 Hz), 7.01 (1 H, dd, *J*=9.0, 2.3 Hz), 3.96 (1 H, q, *J*=6.7 Hz), 3.35 (3 H, s), 1.95 (2 H, br. s.), 1.36 (3 H, d, *J*=6.7 Hz). Mass spectrum (ESI) *m/z* 334.0 $[M+H]^+$.

1-(6-Fluoro-1-(2-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethanamine (13b). 80 % yield as a tan solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.20 - 8.29 (1 H, m), 7.89 - 8.03 (2 H, m), 7.64 - 7.81 (2 H, m), 7.04 - 7.14 (1 H, m), 6.81 - 6.91 (1 H, m), 3.62 - 3.75 (1 H, m), 2.80 - 2.98 (3 H, m), 1.57 - 1.93 (2 H, m), 1.19 - 1.48 (3 H, m). Mass spectrum (ESI) *m/z* 334.0 $[M+H]^+$.

1-(6-Fluoro-1-(3-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethanamine (13c). 81% yield an off-white solid. Mass spectrum (ESI) *m/z* 274.1 $[M+H]^+$.

1-(6-Fluoro-1-(4-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethanamine (13d). To a solution of **12d** (1 equiv) in DCM (0.2 M) was added piperidine (3.6 equiv). The mixture was stirred at room temperature for 5 hours, then concentrated under reduced pressure to give a brown solid. The brown solid was purified by silica gel column chromatography using 0-100% gradient of DCM:MeOH:NH_4OH (89:9:1) in DCM as eluent to give **13d** (46.5 % yield) over three steps as a pink semi-solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.67 (3 H, td, *J*=8.8, 5.0 Hz), 7.48 (2 H, t, *J*=8.8 Hz), 7.09 (1 H, ddd, *J*=9.8, 8.8, 2.5

Hz), 6.88 (1 H, dd, *J*=9.0, 2.3 Hz), 3.93 (1 H, q, *J*=6.8 Hz), 1.92 (2 H, br. s.), 1.33 (3 H, d, *J*=6.7 Hz). Mass spectrum (ESI) *m/z* 274.0 $[M+H]^+$.

1-(1-(3,5-Difluorophenyl)-6-fluoro-1H-benzo[d]imidazol-2-yl)ethanamine (13e). 79 % yield as a pink solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.69 (1 H, dd, *J*=8.8, 4.9 Hz), 7.49 - 7.58 (3 H, m), 7.04 - 7.15 (2 H, m), 4.01 (1 H, q, *J*=6.6 Hz), 1.95 (2 H, br. s.), 1.36 (3 H, d, *J*=6.5 Hz). Mass spectrum (ESI) *m/z* 292.0 $[M+H]^+$.

1-(6-fluoro-1-(pyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethanamine (13f). 61.2% yield as as a brown foam. Mass spectrum (ESI) *m/z* 257.1 $[M+H]^+$.

1-(6-Fluoro-1-(5-fluoropyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethanamine (13g). 95% yield as a black tar. Mass spectrum (ESI) *m/z* 275.1 $[M+H]^+$.

General Procedure for the Synthesis of 14a-g. A mixture of 4-amino-6-chloropyrimidine-5-carbonitrile (1 equiv), **13a-g** (1 equiv), and DIPEA (3 equiv) in n-butanol (0.1 M) was stirred at 120 °C. After 24 hours, the mixture was cooled to room temperature and concentrated under reduced pressure to give a brown solid. The brown solid was suspended in water, filtered, and washed with water to give a tan solid. The tan solid was suspended in EtOAc-hexane (1:4), filtered, washed with EtOAc-hexane (1:4), and dried to give **14a-g** as a racemic mixture.

4-Amino-6-(1-(6-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14a). 62.8 % yield as a tan solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.14 (1 H, t, *J*=1.9 Hz), 8.03 (1 H, dt, *J*=7.8, 1.4 Hz), 7.87 - 7.96 (1 H, m), 7.84 (1 H, s), 7.72 - 7.83 (3 H, m), 7.09 - 7.25 (3 H, m), 6.97 (1 H, dd, *J*=8.9, 2.4 Hz), 5.50 (1 H, quin, *J*=7.0 Hz), 3.26 (3 H, s), 1.55 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$.

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4-Amino-6-(1-(6-fluoro-1-(2-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14b). 65.4 % yield as a yellow solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.05 - 8.26 (1 H, m), 7.86 - 8.00 (1 H, m), 7.60 - 7.83 (4 H, m), 6.82 - 7.53 (5 H, m), 5.30 - 5.74 (1 H, m), 2.82 - 2.91 (3 H, m), 1.38 - 1.61 (3 H, m). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$.

4-Amino-6-(1-(6-fluoro-1-(3-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14c). 67.7% yield an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.86 (1 H, s), 7.65 - 7.78 (2 H, m), 7.52 - 7.60 (1 H, m), 7.48 (1 H, br d, *J*=9.6 Hz), 7.35 - 7.40 (1 H, m), 7.31 (1 H, td, *J*=8.4, 2.2 Hz), 7.06 - 7.24 (3 H, m), 6.93 (1 H, dd, *J*=9.0, 2.3 Hz), 5.56 (1 H, quin, *J*=6.8 Hz), 1.54 (3 H, d, *J*=6.7 Hz). Mass spectrum (ESI) *m/z* 392.1 $[M+H]^+$.

4-Amino-6-(1-(6-fluoro-1-(4-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14d). 81% yield as an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.86 (1 H, s), 7.72 (1 H, dd, *J*=8.8, 4.9 Hz), 7.67 (1 H, d, *J*=7.2 Hz), 7.54 - 7.63 (2 H, m), 7.36 (2 H, t, *J*=8.8 Hz), 7.19 (2 H, br. s.), 7.10 (1 H, ddd, *J*=9.8, 8.9, 2.4 Hz), 6.86 (1 H, dd, *J*=8.9, 2.4 Hz), 5.46 (1 H, quin, *J*=6.9 Hz), 1.52 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 392.0 $[M+H]^+$.

4-Amino-6-(1-(1-(3,5-difluorophenyl)-6-fluoro-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14e). 72.7 % yield as a tan solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.87 (1 H, s), 7.68 - 7.77 (2 H, m), 7.29 - 7.41 (3 H, m), 7.09 - 7.25 (3 H, m), 7.05 (1 H, dd, *J*=9.0, 2.3 Hz), 5.59 - 5.70 (1 H, m), 1.56 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 410.0 $[M+H]^+$.

4-Amino-6-(1-(6-fluoro-1-(pyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14f). 79% yield as an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.73 (1 H, d, *J*=2.2 Hz), 8.65 (1 H, dd, *J*=4.9, 1.6 Hz), 8.00 (1 H, dt, *J*=8.3, 1.7 Hz), 7.82 (1 H, s), 7.75 (2 H, dd,

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J=8.9, 5.0 Hz), 7.55 (1 H, dd, *J*=8.1, 4.8 Hz), 7.07 - 7.25 (3 H, m), 6.93 (1 H, dd, *J*=8.9, 2.4 Hz), 5.48 (1 H, quin, *J*=6.9 Hz), 1.55 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 375.1 $[M+H]^+$.

4-Amino-6-(1-(6-fluoro-1-(5-fluoropyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (14g). 65.7% yield as an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.66 (1 H, d, *J*=2.5 Hz), 8.62 (1 H, s), 8.07 (1 H, br d, *J*=8.6 Hz), 7.83 (1 H, s), 7.72 - 7.78 (2 H, m), 7.11 - 7.26 (3 H, m), 7.07 (1 H, dd, *J*=9.0, 2.5 Hz), 5.59 (1 H, quin, *J*=7.0 Hz), 1.58 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 393.0 $[M+H]^+$.

Preparative SFC method for Chiral separation. Sample was dissolved in DCM-MeOH (2:1, 35 mg/mL). Column: AD-H (250 × 21 mm, 5 μm), IA-H (250 × 21 mm, 5 μm), Lux column (250 × 30mm, 5 mm). Mobile Phase: A=Liquid CO_2; B=MeOH, EtOH, or isopropanol, Flow Rate: 70 mL/min. Column/Oven temperature: 40 ^{o}C. 220 nm. 20.7 mg/injection. 200 - 206 bar inlet pressure.

(R)-4-amino-6-(1-(6-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (4). The racemic mixture **14a** was purified with AD-H (250 × 21mm, 5 μm) column using pure methanol as additive B in supercritical CO_2. Compound **4** was the first eluting enantiomer isolated as a light yellow solid (40.6% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.14 (1 H, t, *J*=2.0 Hz), 8.03 (1 H, ddd, *J*=8.0, 1.3, 1.1 Hz), 7.91 (1 H, d, *J*=7.2 Hz), 7.84 (1 H, s), 7.80 (1 H, t, *J*=7.9 Hz), 7.71 - 7.78 (2 H, m), 7.08 - 7.25 (3 H, m), 6.97 (1 H, dd, *J*=8.9, 2.4 Hz), 5.45 - 5.55 (1 H, m), 3.26 (3 H, s), 1.55 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1298.

(S)-4-amino-6-((1-(6-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (5). The racemic mixture **14a** was purified with AD-H (250 × 21mm, 5 μm) column using pure MeOH as additive B in supercritical CO_2. Compound **5** was the se-

cond eluting enantiomer isolated as light-yellow solid (43% yield). ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 8.14 (1 H, t, J=1.9 Hz), 8.00-8.05 (1 H, m), 7.91 (1 H, d, J=7.4 Hz), 7.84 (1 H, s), 7.80 (1 H, t, J=7.9 Hz), 7.72-7.78 (2 H, m), 7.10-7.24 (3 H, m), 6.97 (1 H, dd, J=8.7,2.4 Hz), 5.50 (1 H, qd, J=6.9,6.7 Hz), 3.26 (3 H, s), 1.55 (3 H, d, J=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1301.

(R)-4-amino-6-(1-(5-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (6) and (S)-4-amino-6-(1-(5-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (7).

Compound **6** and **7** were prepared from 1,4-difluoro-2-nitrobenzene using procedures analogous to that used to obtain **4 and 5**.

(R)-4-amino-6-(1-(5-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (6). 48.7 % yield as a brown solid. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 8.15 (1 H, t, *J*=1.9 Hz), 8.04 (1 H, dt, *J*=7.9, 1.4 Hz), 7.92 (1 H, d, *J*=7.0 Hz), 7.74 - 7.86 (3 H, m), 7.55 - 7.60 (1 H, m), 7.07 - 7.26 (4 H, m), 5.50 (1 H, qd, *J*=7.0, 6.8 Hz), 3.26 (3 H, s), 1.56 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1303.

(S)-4-amino-6-(1-(5-fluoro-1-(3-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (7). 49% yield as a brown solid. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 8.15 (1 H, t, *J*=1.9 Hz), 8.01 - 8.06 (1 H, m), 7.92 (1 H, d, *J*=7.6 Hz), 7.74 - 7.86 (3 H, m), 7.54 - 7.60 (1 H, m), 7.05 - 7.27 (4 H, m), 5.50 (1 H, quin, *J*=6.8 Hz), 3.26 (3 H, s), 1.56 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1304.

4-Amino-6-((1S)-1-(6-fluoro-1-(2-(methylsulfonyl)phenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (15). The racemic mixture **14b** was purified with AD-H column using pure MeOH as additive B in supercritical CO_2. Compound **15** was the second eluting enantiomer isolated as a brown solid (24.2% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.05 - 8.11 (1 H, m), 7.78 (1 H, dd, *J*=8.8, 4.9 Hz), 7.66 - 7.73 (2 H, m), 7.60 - 7.66 (2 H, m), 7.45 - 7.51 (1 H, m), 7.13 (3 H, ddd, *J*=9.8, 8.9, 2.4 Hz), 6.88 (1 H, dd, *J*=8.8, 2.5 Hz), 5.62 - 5.72 (1 H, m), 2.85 (3 H, s), 1.56 (3 H, d, *J*=6.8 Hz). Mass spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1306.

(S)-4-amino-6-(1-(6-fluoro-1-(3-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (16). The racemic mixture **14c** was purified with AD-H column using pure EtOH as additive B in supercritical CO_2. Compound **16** was the second eluting enantiomer isolated as a white solid (50% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.86 (1 H, s), 7.66 - 7.78 (2 H, m), 7.52 - 7.60 (1 H, m), 7.48 (1 H, d, *J*=9.2 Hz), 7.37 (1 H, d, *J*=7.8 Hz), 7.31 (1 H, td, *J*=8.6, 2.4 Hz), 7.05 - 7.25 (3 H, m), 6.94 (1 H, dd, *J*=8.9, 2.4 Hz), 5.56 (1 H, quin, *J*=6.9 Hz), 1.53 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 392.2 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{15}F_2N_7$ $[M+H]^+$: 392.1435, mass measured: 392.1431.

(S)-4-amino-6-(1-(6-fluoro-1-(4-fluorophenyl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (17). The racemic mixture **14d** was purified with AD-H column using pure MeOH as additive B in supercritical CO_2. Compound **17** was the second eluting enantiomer isolated as an off-white solid (42.6% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.86 (1 H, s), 7.72 (1 H, dd, *J*=8.8, 4.9 Hz), 7.67 (1 H, d, *J*=7.2 Hz), 7.55 - 7.63 (2 H, m), 7.36 (2 H, t, *J*=8.8 Hz), 7.19 (2 H, br. s.), 7.10 (1 H, ddd, *J*=9.7, 8.9, 2.5 Hz), 6.86 (1 H, dd, *J*=8.9, 2.4 Hz), 5.46 (1 H, quin, *J*=6.9 Hz), 1.52 (3 H, d,

J=6.8 Hz). Mass spectrum (ESI) *m/z* 392.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{15}F_2N_7$ $[M+H]^+$: 392.1435, mass measured: 392.1435.

(S)-4-amino-6-(1-(1-(3,5-difluorophenyl)-6-fluoro-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (18). The racemic mixture **14e** was purified with IA-H column using pure isopropanol as additive B in supercritical CO_2. Compound **18** was the second eluting enantiomer isolated as an off-white solid (28.8% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.87 (1 H, s), 7.68 - 7.78 (2 H, m), 7.29 - 7.40 (3 H, m), 7.09 - 7.24 (3 H, m), 7.05 (1 H, dd, *J*=8.9, 2.4 Hz), 5.59 - 5.69 (1 H, m), 1.56 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 410.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{14}F_3N_7$ $[M+H]^+$: 410.1341, mass measured: 410.1338.

(S)-4-amino-6-((1-(6-fluoro-1-(pyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (1). The racemic mixture **14f** was purified with AD-H column using pure MeOH as additive B in supercritical CO_2. Compound **1** was the second eluting enantiomer isolated as an off-white solid (50% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.73 (1 H, d, *J*=2.3 Hz), 8.65 (1 H, dd, *J*=4.8, 1.5 Hz), 8.00 (1 H, d, *J*=8.2 Hz), 7.82 (1 H, s), 7.70 - 7.78 (2 H, m), 7.56 (1 H, dd, *J*=8.0, 4.9 Hz), 7.07 - 7.33 (3 H, m), 6.94 (1 H, dd, *J*=8.9, 2.4 Hz), 5.48 (1 H, quin, *J*=6.9 Hz), 1.55 (3 H, d, *J*=6.7 Hz). Mass Spectrum (ESI) *m/z* 375.2 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{19}H_{15}FN_8$ $[M+H]^+$: 375.1482, mass measured: 375.1489.

(S)-4-amino-6-((1-(6-fluoro-1-(5-fluoropyridin-3-yl)-1H-benzo[d]imidazol-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (2). The racemic mixture **14g** was purified with Lux column using pure MeOH as additive B in supercritical CO_2. Compound **2** was the first eluting enantiomer isolated as a white solid (45% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.66 (1 H, d, *J*=2.5 Hz), 8.62 (1 H, s), 8.07 (1 H, d, *J*=8.6 Hz), 7.83 (1 H, s), 7.75 (2 H, dt, *J*=8.6, 4.0 Hz), 7.10 - 7.29 (3 H, m),

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7.07 (1 H, dd, *J*=9.0, 2.5 Hz), 5.59 (1 H, quin, *J*=7.0 Hz), 1.58 (3 H, d, *J*=6.7 Hz). Mass Spectrum (ESI) *m/z* 393.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{19}H_{14}F_2N_8$ $[M+H]^+$: 393.1388, mass measured: 393.1380.

(S)-tert-Butyl l-(6-fluoro-lH-benzo[d]imidazol-2-yl)ethylcarbamate (20). Boc-L-Ala-OH (3.00 g, 15.86 mmol) and 3,4-diamino-1-fluorobenzene **19** (2.00 g, 15.86 mmol) were stirred in pyridine (52.9 mL). EDC·HCl (9.12 g, 47.6 mmol) was added and the mixture was stirred at ambient temperature for 20 min. The reaction mixture was diluted with EtOAc, washed with water, 1.0 N HCl and brine. The organic layer was dried with Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography using 0-100% gradient of EtOAc in hexane as eluent. The desired fractions were combined and concentrated under reduced pressure to give the crude amide intermediate (4.0 g). The amide was dissolved in acetic acid (26.9 mL) and heated in an oil bath at 70 °C for about 30 min. The reaction mixture was diluted with EtOAc and carefully neutralized with aqueous K_2CO_3. The organic layer was washed with NaCl, dried with Na_2SO_4, and filtered. The filtrate was concentrated to dryness to yield **20** (3.69 g, 98 %yield). ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 12.23 (1 H, br. s.), 7.15 - 7.61 (3 H, m), 6.98 (1 H, t, *J*=8.5 Hz), 4.78 - 4.89 (1 H, m), 1.46 (3 H, d, *J*=7.0 Hz), 1.40 (9 H, s). Mass Spectrum (ESI) *m/z* 280.1 $[M+H]^+$.

General Procedure for the Synthesis of 21a-b. To a solution of **20** (1 equiv), boronic acid (2 equiv), and cesium carbonate (2 equiv) in dichloroethane (0.3 M) was added 2,2'-bipyridyl (1 equiv) and copper (II) acetate (1 equiv). The solution was stirred at 70 °C for 90 minutes then at room temperature overnight. The solution was poured into 10% aqueous ammonium chloride and extracted with DCM. The combined organic extracts were concentrated in vacuo and purified by silica gel column chromatography using 0-10% gradient of methanol in DCM as eluent to give a mixture of regioisomers, **21a-b**.

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(S)-tert-butyl 1-(1-cyclopropyl-5-fluoro-1H-benzo[d]imidazol-2-yl)ethylcarbamate and (S)-tert-butyl 1-(1-cyclopropyl-6-fluoro-1H-benzo[d]imidazol-2-yl)ethylcarbamate (21a). 38.5 % yield as a tan solid.

(S)-tert-butyl 1-(6-fluoro-1-phenyl-1H-benzo[d]imidazol-2-yl)ethylcarbamate and (S)-tert-butyl 1-(5-fluoro-1-phenyl-1H-benzo[d]imidazol-2-yl)ethylcarbamate (21b). 24.2% yield as light-yellow solid. Mass Spectrum (ESI) *m/z* 356.1 $[M+H]^+$.

General Procedure for the Synthesis of 22a-b. To a solution of **21a-b** (mixture of regioisomers, 0.220 g, 0.688 mmol) in DCM (0.2 M) was added trifluoroacetic acid (20 equiv). The solution was stirred at room temperature for two hours then concentrated under reduced pressure. The resulting residue was diluted with DCM and washed with saturated aqueous sodium bicarbonate; organic extracts were dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a mixture of **22a-b**.

(S)-1-(1-Cyclopropyl-6-fluoro-1H-benzo[d]imidazol-2-yl)ethanamine and (S)-1-(1-cyclopropyl-5-fluoro-1H-benzo[d]imidazol-2-yl)ethanamine (22a). 94% yield as tan solid. Mass Spectrum (ESI), m/z 220.1 $[M+H]^+$.

(S)-1-(6-fluoro-1-phenyl-1H-benzo[d]imidazol-2-yl)ethanamine and (S)-1-(5-fluoro-1-phenyl-1H-benzo[d]imidazol-2-yl)ethanamine (22b). 90% yield as a light-yellow syrup. ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 7.55 - 7.72 (11 H, m), 7.47 - 7.53 (1 H, m), 6.99 - 7.13 (3 H, m), 6.85 (1 H, dd, *J*=9.0, 2.3 Hz), 3.91 - 4.01 (2 H, m), 1.94 (4 H, br. s.), 1.32 (6 H, dd, *J*=6.8, 3.1 Hz). Mass Spectrum (ESI) *m/z* 256.1 $[M+H]^+$.

(S)-1-(5-Fluoro-1-(pyridin-2-yl)-1H-benzo[d]imidazol-2-yl)ethanamine and (S)-1-(6-fluoro-1-(pyridin-2-yl)-1H-benzo[d]imidazol-2-yl)ethanamine (22c). To a microwave vial was added **20** (1.0040 g, 3.59 mmol), N,N-dimethylacetamide (7.99 mL), 2-fluoropyridine (0.371 mL, 4.31 mmol),

and cesium carbonate (5.86 g, 17.97 mmol). The mixture was heated at 150 °C for 40 minutes in the microwave reactor. The mixture was poured into EtOAc (100 mL), filtered, and concentrated under reduced pressure. The crude material was purified by silica gel chromatography using 0-50% gradient of MeOH in DCM:MeOH:NH_4OH (89:9:1) as eluent to give **22c** (0.40 g, 1.58 mmol, 43.9 % yield) as light-yellow solid. Mass Spectrum (ESI) *m/z* 257.0 $[M+H]^+$.

General Procedure for the Synthesis of 23a-c. A mixture of 4-amino-6-chloropyrimidine-5-carbonitrile (1 equiv), **22a-c** (1 equiv), and DIPEA (3 equiv) in n-butanol (0.1 M) was stirred at 120 °C. After 24 hours, the mixture was allowed to cool to room temperature. The mixture was concentrated under reduced pressure to give a yellow syrup. The residue was dissolved in DCM (50 mL). The solution was washed with water (30 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using 0-50% gradient of DCM:MeOH:NH_4OH (89:9:1) in DCM as eluent to give **23a-c** as racemic mixtures.

(S)-4-Amino-6-(1-(1-cyclopropyl-6-fluoro-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (24). The mixture **23a** was purified with OJ-H column using pure isopropanol as additive B in supercritical CO_2. Compound **24** was the first eluting enantiomer isolated as a white solid (24% yield). ^1H NMR (500 MHz, *DMSO-d_6*) δ ppm 8.04 (1 H, s), 7.70 (1 H, d, *J*=7.1 Hz), 7.60 (1 H, dd, *J*=8.8, 4.9 Hz), 7.36 (1 H, ddd, *J*=9.3, 3.7, 2.4 Hz), 7.29 (2 H, br. s.), 7.03 (1 H, ddd, *J*=9.9, 8.8, 2.6 Hz), 5.79 (1 H, quin, *J*=6.9 Hz), 3.32 - 3.35 (1 H, m), 1.60 (3 H, d, *J*=6.8 Hz), 1.00 - 1.22 (4 H, m). Mass Spectrum (ESI) *m/z* 336.1 $[M-H]^-$. HRMS (ESI) *m/z* calculated for $C_{17}H_{16}FN_7$ $[M+H]^+$: 338.1529, mass measured: 338.1538.

(S)-4-Amino-6-(1-(6-fluoro-1-phenyl-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (25). The mixture **23b** was purified with AD-H column using pure MeOH as additive B in supercritical CO_2. Compound **25** was the first eluting enantiomer isolated as a brown solid (37.6%

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yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 7.86 (1 H, s), 7.73 (1 H, dd, *J*=8.8, 4.9 Hz), 7.67 (1 H, d, *J*=7.0 Hz), 7.45 - 7.59 (5 H, m), 7.19 (2 H, br. s.), 7.11 (1 H, ddd, *J*=9.9, 8.9, 2.5 Hz), 6.84 (1 H, dd, *J*=8.9, 2.4 Hz), 5.46 (1 H, quin, *J*=6.7 Hz), 1.50 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 374.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{16}FN_7$ $[M+H]^+$: 374.1529, mass measured: 374.1532.

(S)-4-Amino-6-(1-(6-fluoro-1-(pyridin-2-yl)-1H-benzo[d]imidazol-2-yl)ethylamino)pyrimidine-5-carbonitrile (26). The mixture **23c** was purified with AD-H column using pure MeOH as additive B in supercritical CO_2. Compound **26** was the second eluting enantiomer isolated as a tan solid (17.3% yield). ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.60 (1 H, dd, *J*=4.8, 1.3 Hz), 8.06 (1 H, td, *J*=7.7, 1.8 Hz), 7.86 (1 H, s), 7.75 (1 H, dd, *J*=8.8, 4.9 Hz), 7.70 (2 H, d, *J*=7.8 Hz), 7.47 - 7.54 (1 H, m), 7.10 - 7.26 (4 H, m), 5.82 (1 H, quin, *J*=6.9 Hz), 1.53 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 375.1 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{19}H_{15}FN_8$ $[M+H]^+$: 375.1482, mass measured: 375.1485.

Imidazopyridines

(S)-Benzyl 4-bromo-3-oxobutan-2-ylcarbamate (27). To a solution of *N*-((benzyloxy) carbonyl) L-alanine (20 g, 89.64 mmol) in THF (400 mL) at -20 °C was added NMM (13.5 g, 133.2 mmol), followed by isobutyl chloroformate (14.6 g, 107 mmol). After stirring for 1.5 h at -20 °C, the mixture was filtered while cold and the filtrate was treated with excess freshly prepared diazomethane/Et_2O solution (prepared from 30.0 g of *N*- methyl nitroso urea, 200 mL 40 % KOH/100 mL Et_2O) with stirring until a yellow color persisted. The mixture was allowed to warm to 0 °C and the solution was then warmed to room temperature and stirred for 1 hour. The solution was cooled to 0 °C and treated with a solution of HBr (45 % aqueous)/acetic acid (1/1 (v/v), 50 mL) for 30 minutes. The reaction was monitored by TLC. Upon completion, the reaction was diluted with EtOAc (500 mL) and water (200 mL). The organic phase was separated, washed with water, dried over Na_2SO_4, filtered and concentrated under reduced

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pressure. The crude product was purified by silica gel column chromatography using hexanes/EtOAc (2:1) as the eluent to give **27** (17.0 g, 63 % yield). ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 7.35-7.38 (5 H, m), 5.4 (1 H, br. s), 5.0-5.1 (2 H, s), 4.6-4.7 (1 H, q), 4.01-4.09 (1 H, q), 1.41-1.43 (3 H, d). Mass Spectrum (ESI) *m/z* 300.1 [M+H (^{79}Br)]$^+$ and 302.1 [M+H (^{81}Br)]$^+$.

(S)-benzyl 1-(6-fluoroimidazo[1,2-a]pyridin-2-yl)ethylcarbamate (28). A mixture of **27** (23.32 mmol) and 2-amino-5-fluoropyridine (23.32 mmol) in EtOH was heated to reflux overnight. The mixture was cooled to room temperature and the EtOH was removed under reduced pressure. The residue was dissolved in EtOAc and washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography using a 0-8 % gradient of MeOH in DCM as eluent to give **28** as a brown solid (3.80 g, 12.11 mmol, 52%). ^{1}H NMR (400 MHz, *DMSO-d_6*) δ ppm 8.01-8.07 (2 H, m), 9.03 (1 H, s), 7.75-7.84 (2 H, m), 7.34-7.37 (5 H, m), 5.01-5.11 (2 H, m), 4.95-4.98 (1 H, m), 1.50 (3 H, d, *J=6.8Hz*). Mass Spectrum (ESI) *m/z* 313.9 [M+H]$^+$.

(S)-benzyl 1-(6-fluoro-3-iodoimidazo[1,2-a]pyridin-2-yl)ethylcarbamate (29). To a solution of **28** (3.80 g, 12.11 mmol) in acetonitrile (60 mL) was added N-iodosuccinimide (2.73 g, 12.11 mmol). The reaction mixture was stirred overnight at room temperature. The mixture was diluted with water and extracted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography using a 0-50 % gradient of EtOAc in hexane as eluent to afford **29** as a pink solid (4.36 g, 9.92 mmol, 82%). Mass Spectrum (ESI) *m/z* 440.0 [M+H]$^+$.

(S)-Benzyl 1-(6-fluoro-3-(pyridin-2-yl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30a). A mixture of **29** (1 equiv), 2-(tributylstannyl)pyridine (1.2 equiv), and $Pd(PPh_3)_4$ (0.1 equiv) in toluene (0.3 M)

was stirred at 110 °C. After stirring overnight, the reaction mixture was cooled to room temperature, and concentrated under reduced pressure. The crude material was purified by silica gel column chromatography using a 0-40 % gradient of EtOAc in hexane as eluent to give **30a** (10% yield) as a colorless syrup. Mass Spectrum (ESI) *m/z* 391.1 $[M+H]^+$.

General Procedure B: Suzuki Coupling for the Synthesis of 30b-30h. A mixture of boronic acid (0.854 mmol), **24** (0.569 mmol), $Pd(Ph_3P)_4$ (0.028 mmol), and Na_2CO_3 (1.138 mmol) in acetonitrile:water (3:1) was stirred and heated at 100 °C for 2 hours. The reaction mixture was partitioned between water and EtOAc. The organic layer was separated, dried with Na_2SO_4, filtered, and concentrated. The residue was dissolved in DCM and loaded onto a silica gel cartridge. The compound was purified by silica gel column chromatography using a 0-100% gradient of EtOAc in hexane as eluent to give **30b-h**.

(S)-Benzyl (1-(6-fluoro-3-(pyridine-3-yl)imidazo[1,2-a]pyridine-2-yl)ethyl)carbamate (30b). 46.6% yield. Mass Spectrum (ESI) *m/z* 391.0 $[M+H]^+$.

(S)-Benzyl 1-(6-fluoro-3-(5-fluoropyridin-3-yl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30c). 76% yield as an off-white foam. Mass Spectrum (ESI) *m/z* 409.1 $[M+H]^+$.

(S)-Benzyl (1-(6-fluoro-3-phenylimidazo[1,2-a]pyridin-2-yl)ethyl)carbamate (30d). 64% yield as a brown oil. Mass Spectrum (ESI) *m/z* 390.1 $[M+H]^+$.

(S)-Benzyl 1-(6-fluoro-3-(4-fluorophenyl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30e). 96% yield. Mass Spectrum (ESI) *m/z* 408.2 $[M+H]^+$.

(S)-Benzyl 1-(3-(3,5-difluorophenyl)-6-fluoroimidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30f). 83% yield. Mass Spectrum (ESI) *m/z* 426.1 $[M+H]^+$.

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Benzyl (1S)-1-(6-fluoro-3-(2-(methylthio)phenyl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30g). 24.2% yield as amber residue. Mass Spectrum (ESI) *m/z* 436.1 $[M+H]^+$.

Benzyl (1S)-1-(6-fluoro-3-(2-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30g'). To a solution of **30g** (1 equiv) in THF-water (4:1, 0.1 M) was added Oxone (2.5 equiv). The solution was stirred at room temperature overnight, then was quenched with $NaHCO_3$. The product was extracted with EtOAc, dried with Na_2SO_4, filtered and concentrated under reduced pressure to give **30g'** as a clear residue (90% yield). Mass Spectrum (ESI) *m/z* 468.0 $[M+H]^+$.

(S)-Benzyl 1-(6-fluoro-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethylcarbamate (30h). Quantitave yield as a colorless oil. Mass Spectrum (ESI) *m/z* 468.1 $[M+H]^+$.

General Procedure C: Deprotection of Cbz for the Synthesis of 31a-31h. The mixture of the **30a-h** (1 equiv), dimethyl sulfide (5 equiv) in TFA (0.7 M) was stirred at room temperature overnight. The mixture was concentrated under vacuum, dissolved in EtOAc, and washed with saturated aqueous $NaHCO_3$ solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated to yield the crude product **31a-h**, which was carried on crude without further purification.

(S)-1-(6-Fluoro-3-(pyridin-2-yl)imidazo[1,2-a]pyridin-2-yl)ethanamine (31a). 77% yield as an amber residue. Mass Spectrum (ESI) *m/z* 257.1 $[M+H]^+$.

(S)-1-(6-Fluoro-3-(5-fluoropyridin-3-yl)imidazo[1,2-a]pyridin-2-yl)ethanamine (31c). Quantitative yield as a tan solid. Mass Spectrum (ESI) *m/z* 275.1 $[M+H]^+$.

(S)-1-(6-Fluoro-3-phenylimidazo[1,2-a]pyridin-2-yl)ethanamine (31d). Quantitative yield as a yellow solid. Mass Spectrum (ESI) *m/z* 256.1 $[M+H]^+$.

(S)-1-(6-Fluoro-3-(4-fluorophenyl)imidazo[1,2-a]pyridin-2-yl)ethanamine (31e). Mass Spectrum (ESI) *m/z* 274.1 $[M+H]^+$.

(S)-1-(3-(3,5-Difluorophenyl)-6-fluoroimidazo[1,2-a]pyridin-2-yl)ethanamine (31f). 99% yield as a clear oil. Mass Spectrum (ESI) *m/z* 292.0 $[M+H]^+$.

(1S)-1-(6-Fluoro-3-(2-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethanamine (31g). 97% yield. Mass Spectrum (ESI) *m/z* 334.0 $[M+H]^+$.

(S)-1-(6-Fluoro-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethanamine (31h). Quantitative yield. Mass Spectrum (ESI) *m/z* 334.0 $[M+H]^+$.

General Procedure D for the Synthesis of 32-29. A mixture of 4-amino-6-chloropyrimidine-5-carbonitrile (1.0 eq.), **31a-h** (1.0 eq.) and DIPEA (3.0 eqv.) in n-butanol was stirred at 110 °C for 12 hours. The reaction mixture was concentrated under reduced pressure. The residue was purified by silica gel column chromatography on using a 0-10 % gradient of MeOH in DCM as eluent to give **32-29**.

(S)-4-amino-6-((1-(6-fluoro-3-(pyridin-2-yl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (32). 28.6% yield as white solid. ^{1}H NMR (400 MHz, *MeOH-d4*) δ ppm 8.98 - 9.06 (1 H, m), 8.85 - 8.91 (1 H, m), 8.11 (1 H, td, *J*=7.8, 1.9 Hz), 7.97 - 8.04 (2 H, m), 7.82 - 7.89 (1 H, m), 7.72 - 7.80 (1 H, m), 7.58 (1 H, ddd, *J*=7.6, 4.9, 1.2 Hz), 5.84 (1 H, q, *J*=7.0 Hz), 1.68 (3 H, d, *J*=7.0 Hz). Mass Spectrum (ESI) *m/z* 375.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{19}H_{15}FN_8$ $[M+H]^+$: 375.1482, mass measured: 375.1486.

(S)-4-amino-6-((1-(6-fluoro-3-(pyridin-3-yl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (33). 5% yield as a white solid. ^{1}H NMR (400 MHz, *DMSO-d6*) δ ppm 8.72 (1 H, d, J=1.2 Hz), 8.66-8.68 (1 H, m), 8.32-8.33 (1 H, m), 8.01 (1 H, d, *J*=8 Hz), 7.73-7.77 (1 H, m), 7.55-7.58

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(1 H, m), 7.40-7.45 (1 H, m), 7.23 (2 H, br s), 7.04 (1 H, d, *J*=7.6 Hz), 5.40-5.47 (1 H, m), 1.52 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 375.1 $[M+H]^+$. HRMS (ESI) m/z calculated for $C_{19}H_{15}FN_8$ $[M+H]^+$: 375.1482, mass measured: 375.1488.

(S)-4-amino-6-((1-(6-fluoro-3-(5-fluoropyridin-3-yl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (34). 38.3% yield as an off-white solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.66 (1 H, d, *J*=2.7 Hz), 8.56 (1 H, s), 8.43 (1 H, dd, *J*=4.5, 2.2 Hz), 7.96 - 8.06 (1 H, m), 7.89 (1 H, s), 7.74 (1 H, dd, *J*=9.8, 5.3 Hz), 7.38 - 7.50 (1 H, m), 7.22 (2 H, br. s.), 7.09 (1 H, d, *J*=7.2 Hz), 5.47 (1 H, quin, *J*=6.9 Hz), 1.54 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 393.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{19}H_{14}F_2N_8$ $[M+H]^+$: 393.1388, mass measured: 393.1388.

(S)-4-amino-6-((1-(6-fluoro-3-phenylimidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (35). 51.5% yield as a beige solid. ^{1}H NMR (400 MHz, *DMSO-d*$_6$) δ ppm 8.25 (1 H, dd, *J*=4.7, 2.3 Hz), 7.96 (1 H, s), 7.74 (1 H, dd, *J*=9.9, 5.2 Hz), 7.57 (4 H, d, *J*=4.3 Hz), 7.50 (1 H, dq, *J*=8.9, 4.2 Hz), 7.40 (1 H, ddd, *J*=10.1, 8.1, 2.3 Hz), 7.26 (2 H, br. s.), 6.90 (1 H, d, *J*=7.4 Hz), 5.46 (1 H, quin, *J*=6.8 Hz), 1.49 (3 H, d, *J*=6.7 Hz). Mass Spectrum (ESI) *m/z* 374.1 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{16}FN_7$ $[M+H]^+$: 374.1529, mass measured: 374.1531.

(S)-4-Amino-6-((1-(6-fluoro-3-(4-fluorophenyl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (36). 50.6% yield as an off-white solid. ^{1}H NMR (400 MHz, *MeOH-d*$_4$) δ ppm 8.02 (1 H, dd, *J*=3.7, 2.3 Hz), 7.92 (1 H, s), 7.59 - 7.65 (1 H, m), 7.51 - 7.57 (2 H, m), 7.27 - 7.37 (3 H, m), 5.58 (1 H, q, *J*=6.9 Hz), 1.61 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 392.2 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{15}F_2N_7$ $[M+H]^+$: 392.1435, mass measured: 392.1436.

(S)-4-amino-6-((1-(3-(3,5-difluorophenyl)-6-fluoroimidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (37). Quantitative yield as a white solid. ^{1}H NMR (400 MHz, *MeOH-d4*) δ ppm 8.17 (1 H, ddd, *J*=4.5, 2.3, 0.8 Hz), 7.94 (1 H, s), 7.60 - 7.66 (1 H, m), 7.37 (1 H, ddd, *J*=9.9, 7.9, 2.3 Hz), 7.19 - 7.27 (2 H, m), 7.11 (1 H, tt, *J*=9.2, 2.3 Hz), 5.64 (1 H, q, *J*=6.8 Hz), 1.62 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 410.1 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{20}H_{14}F_3N_7$ $[M+H]^+$: 410.1341, mass measured: 410.1336.

4-Amino-6-(((1S)-1-(6-fluoro-3-(2-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (38). 83% yield as a white solid. ^{1}H NMR (500 MHz, *MeOH-d4*) δ ppm 8.19 - 8.23 (1 H, m), 7.75 - 7.84 (3 H, m), 7.69 (1 H, ddd, *J*=4.3, 2.4, 0.9 Hz), 7.62 - 7.66 (1 H, m), 7.51 - 7.55 (1 H, m), 7.35 (1 H, ddd, *J*=10.0, 7.9, 2.3 Hz), 5.65 (1 H, q, *J*=7.0 Hz), 2.92 (3 H, s), 1.65 (3 H, d, *J*=6.8 Hz). Mass Spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1307.

(S)-4-amino-6-((1-(6-fluoro-3-(3-(methylsulfonyl)phenyl)imidazo[1,2-a]pyridin-2-yl)ethyl)amino)pyrimidine-5-carbonitrile (39). 23.7% yield as a white solid. ^{1}H NMR (400 MHz, *MeOH-d4*) δ ppm 8.31 (1 H, s), 8.12 - 8.16 (1 H, m), 8.09 (1 H, dt, *J*=7.6, 1.6 Hz), 8.00 (1 H, s), 7.87 - 7.90 (1 H, m), 7.80 - 7.85 (1 H, m), 7.65 (1 H, dd, *J*=10.0, 5.1 Hz), 7.37 (1 H, ddd, *J*=10.1, 7.9, 2.3 Hz), 5.61 (1 H, q, *J*=7.2 Hz), 3.21 (3 H, s), 1.63 (3 H, d, *J*=7.0 Hz). Mass Spectrum (ESI) *m/z* 452.0 $[M+H]^+$. HRMS (ESI) *m/z* calculated for $C_{21}H_{18}FN_7O_2S$ $[M+H]^+$: 452.1305, mass measured: 452.1299.

Crystallography

Human p110γ (144-1102) was expressed, purified, crystallized, and inhibitor complexes were prepared according to published procedures.[27] Diffraction data for p110γ + **5** were collected at the Canadian Light Source, beam line CMCF1, using λ = 0.9793 Å and a Mar225 CCD detector. Data were processed

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using the HKL software suite[28] and the structures were refined using REFMAC starting from previously solved models of PI3Kγ.[29] Model building was performed with COOT.[30]

ASSOCIATED CONTENT

Supporting Information

(i) Biological assays. (ii) Enzyme selectivity data of compounds **1** (AM-8508) and **2** (AM-9635). This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The cocrystal structure of PI3Kγ with compound **5** has been deposited in the Protein Data Bank with PDB code 5EDS.

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Notes

The authors declare no competing finalcial interest. All authors were employed by Amgen Inc at the time this work was done.

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ABBREVIATIONS USED

AKT, (PKB) protein kinase B; CD, Cluster of differentiation; CXCR3, chemokine (C-X-C motif) receptor 3; CL, clearance; CYP, Cytochrome P450; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMSO, dimethyl sulfoxide; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EtOAc, ethyl acetate; FBS, Fetal bovine serum; FITC, Fluorescein isothiocyanate; GPCR, G protein-coupled receptor*;* HLM, human liver microsomal; HWB, human whole blood; IgD, Immunoglobulin D; IgG, Immunoglobulin G; IgM, Immunoglobulin M; KLH, Keyhole limpet hemycin; mTOR, mammalian target of rapamycin; MeOH, methanol; NIS, N-Iodosuccinimide; NMM, *N*-Methylmorpholine; PBMC, peripheral blood mononuclear cell ; PBS, phosphate buffer saline; PI, Phosphoinositides; PI3Ks, Phosphoinositide 3-kinases; PIP2, phosphatidylinositol 4, 5- bisphosphate; PIP3, phosphatidylinositol 3, 4, 5-trisphosphate; POC, percent of *control;* P70S6, serine/threonine kinase target substrate is S6 ribosomal protein; RA, Rheumatoid arthritis; RLM, Rat liver microsomal; RTK, Receptor tyrosine kinase; SLE, Systemic Lupus Erythematosus;

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SEM, standard error of the mean; SFC, Supercritical Fluid Chromatography; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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