Discovery and in Vivo Evaluation of the Potent and Selective PI3Kδ Inhibitors 2-((1S)-1-((6-Amino-5-cyano-4-pyrimidinyl)amino)ethyl)-6-fluoro-N-methyl-3-(2-pyridinyl)-4-quinolinecarboxamide (AM-0687) and 2-((1S)-1-((6-Amino-5-cyano-4-pyrimidinyl)amino)ethyl)-5-fluoro-N-methyl-3-(2-pyridinyl)-4-quinolinecarboxamide (AM-1430)

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ABSTRACT: Optimization of the potency and pharmacokinetic profile of 2,3,4-trisubstituted quinoline, 4, led to the discovery of two potent, selective, and orally bioavailable PI3Kδ inhibitors, 6a (AM-0687) and 7 (AM-1430). On the basis of their improved profile, these analogs were selected for in vivo pharmacodynamic (PD) and efficacy experiments in animal models of inflammation. The in vivo PD studies, which were carried out in a mouse pAKT inhibition animal model, confirmed the observed potency of 6a and 7 in biochemical and cellular assays. Efficacy experiments in a keyhole limpet hemocyanin model in rats demonstrated that administration of either 6a or 7 resulted in a strong dose-dependent reduction of IgG and IgM specific antibodies. The excellent in vitro and in vivo profiles of these analogs make them suitable for further development.

INTRODUCTION

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that phosphorylate the 3′ position of the inositol ring in phosphatidylinositol.1 The resulting phosphoinositides (PIs) are secondary messengers that play central roles in the regulation of cellular processes including signal transduction, cell survival, and control of membrane trafficking. Thus, inhibition of PI3K activity has been regarded as a suitable strategy to modulate these key cellular processes.2,3

Among the different types of PI3Ks, class I PI3Ks play a central role in the regulation of phosphatidylinositol (3,4,5)-triphosphate (PIP3) levels. Importantly, PIP3 functions as a membrane anchoring site for key protein kinases including serine/threonine kinases such as AKT (or PKB), PDK1, and tyrosine kinases of the Tec family. Regulation of the PIP3 level in the cell is controlled by two different subclasses of class I PI3Ks: class IA, which is comprised of PI3Kα, β, and δ isoforms and class IB, which is comprised of PI3Kγ.4,5 In terms of tissue distribution, PI3Kα and PI3Kβ are ubiquitously expressed, whereas PI3Kδ and PI3Kγ are primarily found in leukocytes.6 The key role of class I PI3Ks in signal transduction in combination with its tissue distribution and mouse genetic studies have made small molecule inhibition of class I PI3Ks a prominent area of research within the pharmaceutical industry.7,8 Despite this interest, only one PI3Kδ inhibitor...
(Idelalisib) has been approved for the treatment of certain types of cancer. The limited success in this field is a testimony to not only the vast amount of research necessary to bring a new medicine to patients but also the importance of flexibility during clinical trial design and execution. In particular, within the PI3K field, mouse genetic studies and preclinical animal models of disease established PI3Kα and PI3Kβ as plausible targets for the treatment of human cancer, whereas PI3Kγ and PI3Kδ, which have a central role in leukocyte biology, were viewed as attractive targets for the treatment of inflammatory diseases. Despite this data, clinical trials in cancer patients with PI3Kα and PI3Kβ inhibitors are still in progress, and results from pivotal phase 2 and 3 clinical trials for drug approval are still pending. Concurrent with these studies, the realization from pivotal phase 2 and 3 clinical trials for drug approval are combination with Rituximab,12 relapsed follicular non-Hodgkin lymphoma, and relapsed small lymphocytic lymphoma (Idelalisib) has been approved for the treatment of certain types of leukemias led to the repositioning of PI3Kδ inhibitors as potential therapeutics for the treatment of oncologic diseases. This change in development strategy proved critical for the successful outcome of the PI3Kδ field and has resulted in the accelerated approval of Idelalisib for the treatment of patients suffering from relapsed chronic lymphocytic leukemia (in combination with Rituximab),12 relapsed follicular non-Hodgkin lymphoma, and relapsed small lymphocytic lymphoma. Our own research efforts in this field have focused on a rational design approach to identify PI3K inhibitors with built-in kinase and isoform selectivity for the treatment of both oncologic and inflammatory human diseases. Some of the initial work in this area described the identification and in vivo evaluation of PI3Kα, dual PI3Kβ/δ, and selective PI3Kδ inhibitors (Figure 1). Recently, the identification of the clinically candidate 3 (AMG 319), which is currently being evaluated in phase 2 clinical trials for the treatment of human papillomavirus (HPV) and negative head and neck squamous cell carcinoma (HNSCC), has also been reported. Herein we describe the discovery and in vivo preclinical studies of 6a and 7, two potent and selective PI3Kδ inhibitors containing a unique 4-carboxamide quinoline pharmacophore. These studies are part of an effort to identify a clinical candidate with suitable profile for the treatment of inflammatory diseases.

## RESULTS AND DISCUSSION

### Optimization Strategy

Efforts to identify a second clinical candidate within the PI3Kδ program focused on further improving the isoform selectivity profile of 3 while maintaining its favorable PK parameters. A number of different strategies were pursued to achieve this goal, including exploration of new chemical series (e.g., benzimidazoles,14 naphthyridines,16 pyridopyrimidinones,17 or thienopyridines18) as well as small modifications of the quinoline ring present in the 3 series (Chart 1). The latter approach led to the identification of the 2,3,4-substituted quinoline pharmacophore exemplified by 4, a reasonably potent and selective PI3Kδ inhibitor. At the outset, this compound was viewed as a suitable lead for further optimization and work focused on improving its intrinsic microsomal stability and PXR activation profile (Chart 1). To address these issues, the strategy focused on reducing the clogP of these analogues by replacing the pyridine ring at C4 with more polar groups.

Due to the synthetic accessibility of a C3 phenyl-substituted quinoline penultimate intermediate (carboxylic acid 15, Scheme 1), initial optimization of the C4 group was performed within this series. Preliminary work focused on replacing the pyridine ring at C4 with small functional groups that would also contain a H-bond acceptor (Figure 2a, general structure 5). After a suitable replacement of the pyridine ring was identified, our attention was refocused on improvement of the PXR activation profile by further reducing clogP. Among the compounds synthesized, the series in which the C3 pyridine substituent had been reintroduced was found to display the best overall profile (Figure 2b, general structure 6). Finally, modification of the 6-F-quinoline substitution pattern was also explored since previous optimization of the 3 series suggested that this modification could improve the overall profile of these inhibitors (Figure 2c, representative structure 7).

### Chemistry

With this strategy in mind, a rapid synthesis of 3-phenylquinoline derivatives was developed (Scheme 1). Pfitzinger condensation between 5-fluoroisatin (8) and 1-phenylbutan-2-one provided 4-quinoline acid 9.22 Next, exposure of 9 to thionyl chloride followed by a methanol quench afforded methyl ester 10. Treatment of 10 with 1,3-dibromo-5,5-dimethylhydantoin in the presence of a radical initiator provided benzyl bromide 11, which was converted to the corresponding amine intermediate 12 in two steps including: (1) substitution using sodium azide and (2)
hydrogenation under Pd/C catalysis. Key carboxylic acid 14 was obtained through a S_NAr reaction between 4-amino-6-chloropyrimidine-5-carbonitrile followed by LiI-mediated methyl ester hydrolysis. Chiral acid 15 was obtained by SFC purification of this racemate (using isopropanol/hexane gradient on an AD column) and the final analogs 5a−c and 5f−i were synthesized through treatment of 15 with various amines and the coupling reagent PyBop in DMF.

The synthesis of analogs 5d and 5e began with the Boc-protection of racemic amine 12. The resultant intermediate was purified by chiral SFC to afford chiral carbamate 16. The methyl ester substituent of 16 was then hydrolyzed under aqueous basic conditions to give the corresponding acid, which was subsequently coupled with either ethylamine or isopropylamine to provide 17d and 17e, respectively. The final analogs 5d and 5e were obtained after HCl-mediated Boc-deprotection and S_NAr of the corresponding amines with 4-amino-6-chloropyrimidine-5-carbonitrile.

The synthesis of the C3 pyridine compounds was performed following two different routes (Schemes 2 and 3). Analog 6d was synthesized following a synthetic route similar to that described in Scheme 1 (albeit in lower yield). The methyl ester 19 was obtained by Pfitzinger condensation of 8 with 1-(pyridin-2-yl)butan-2-one and subsequent esterification with TMSCHN_2. An analogous three step benzylic amination procedure was followed, which included: (1) bromination, (2) substitution, and (3) azide reduction to yield amine 21. Coupling of this intermediate with 4-amino-6-chloropyrimidine-5-carbonitrile and hydrolysis of the resultant methyl ester provided acid 23. Finally, the analog 6d was obtained via PyBop-mediated amide coupling with azetidin-3-ol as a racemate.
The low yield of the previous route prompted the development of an alternative approach for the synthesis of analogs 6a, 6b, and 6e (Scheme 3). This route commenced with known ketone 24 (prepared in one step from the corresponding Weinreb amide), which when refluxed with 5-fluoroisatin 8 and potassium hydroxide in ethanol gave acid 25. This intermediate was then coupled to a variety of different amines under the previously described PyBop conditions to deliver the corresponding amides 26a, 26b, and 26e. Amides 26a, 26b, and 26e were then treated with 4 N HCl to remove the Boc protecting group. Subsequent S_NAr reaction of the crude amine with 4-amino-6-chloropyrimidine-5-carbonitrile provided the final products 6a, 6b, and 6e after chiral separation (Scheme 3, top). The synthesis of 6c (Scheme 3, bottom) began with the coupling of key acid intermediate 25 with benzyl piperazine-1-carboxylate under standard conditions. Deprotection of the Boc-group and a S_NAr reaction of the resultant amine with 4-amino-6-chloropyrimidine-5-carbonitrile afforded 27. Final deprotection of the Cbz group in 27 provided analog 6c.

An analogous synthetic route to that described in Scheme 3 enabled the synthesis of the 5-fluoro analog 7. This sequence started with commercially available 4-fluoroisatin and N-Boc-protected ketoamino intermediate 24, which provided acid...
intermediate 28 via the previously described Pfützinger condensation (Scheme 4). Coupling of intermediate 28 with methylamine using PyBop and Hunig’s base provided Boc-protected intermediate 29. Deprotection of the Boc-group with 4 N HCl in dioxane and subsequent S$_N$Ar reaction of the amino intermediate with 4-amino-6-chloropyrimidine-5-carbonitrile (Scheme 4).

Table 1. SAR of 3-Phenyl Analogs 5a–i

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“Biochemical: Alphascreen assay. Cellular: In vitro anti-IgM/CD40L-induced human B cell proliferation (as measured by thymidine incorporation) assay. "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. ”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. "CYP: cytochrome P450 assay competitive (midazolam as probe substrate, 5 µM) reported as % inhibition at 3 µM (LC/MS). "Human pregnane X receptor. Data are shown as percent of control. ”PK experiments were carried out using male Sprague–Dawley rats (n = 3). Test compounds formulated at appropriate concentrations for either IV (0.5 mL/kg) or oral (2–10 mL/kg) administration. Unbound clearance equals the total clearance divided by the rat fraction unbound (CL$_{unb}$ = CL/f$_{u}$). "Racemic compound.
provided the desired racemic 7. Chiral separation by SFC led to the isolation of 7.

**Structure Activity Relationship (SAR).** Initial replacement of the pyridine ring at the C4 position in 4 with a primary amide led to an improvement in both biochemical and cellular potencies (5a, Table 1). This analog also displayed improved in vitro microsomal stability and PXR activation profile relative to the parent bis-pyridine compound 4. A further improvement in biochemical and cellular potency was achieved by replacing the primary amide in 5a with a methylamide (5b, Table 1). Compound 5b also had improved PI3Kα/γ isoform selectivity relative to 5a. The low microsomal intrinsic clearance correlated well with a favorable PK profile (Cl = 0.5 L/h/kg), and the Clu (clearance corrected by free fraction) was comparable to that observed in 3 (4.5 vs 2.7 L/h/kg, Table 1). Introduction of a dimethylamide at the C4 position (analogs 5c, Table 1) led to a marginal improvement in biochemical potency, but the PXR activation profile was unfavorable (75 and 151 POC at 2 and 10 μM, respectively). The secondary ethylamide substitution (analog 5d, Table 1) was significantly less potent than the corresponding methylamide 5b (51 vs 1.3 nM) in the biochemical assay. Cyclic tertiary amides were also evaluated (analogs 5f–h, Table 1). Among these, the piperazine amide 5g displayed the most favorable potency profile (0.2 nM against PI3Kδ in the biochemical assay), but the rat PK was significantly worse (Clu = 60 L/h/kg).

With the goal of improving the unfavorable PXR activation profile observed in some of the most promising analogs (e.g., 5a–e), a strategy directed toward introducing polarity in 5a–h was pursued. Consistent with the structure of bis-pyridine analog 4, it was predicted that replacement of the phenyl group at C3 in analogs 5a–I with a pyridine ring would not be detrimental to potency and could potentially address the PXR activation liability. To test this hypothesis analogs 6a–e and 7 were synthesized.

Table 2 illustrates the in vitro and in vivo data for the C3 pyridine analogs; consistent with our hypothesis, the PXR activation profile was significantly improved. The reduction in the clogP of these analogs did have an impact in their biochemical potency, although the overall ligand efficiency was generally very good (lipE > 7 for most analogs). Among these PI3K inhibitors, the methyl amide-substituted compound (6a, Table 2) displayed excellent levels of biochemical and cellular potency and similar PK profile relative to 3 (Clu = 2.3 vs 2.7). The potency profile of the ethyl amide analog 6b was also very good, although the Clu was about 3-fold higher that the parent inhibitor 6a. The overall parameters of tertiary amides 6c and 6d were also satisfactory, although the cellular potency of these analogs was reduced relative to 6a. Having established the

Table 2. SAR of Analogs 6a–e and 7

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<th>Cmpd</th>
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Biochemical: Alphascreen assay. aCellular: In vitro anti-IgM/CD40L-induced human B cell proliferation (as measured by thymidine incorporation) assay. bCellular: Ability of compound to inhibit anti-IgM induced AKT phosphorylation (pAKTSer473) in mouse B cells; phospho-AKT (pAKT) expression on B220 + gated B cells was determined by flow cytometry in mouse splenocytes. cSingle experimental values. dTurnover 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. eCYP: cytochrome P450 assay competitive (midazolam as probe substrate, 5 μM) reported as % inhibition at 3 μM (LC/MS). fHuman pregnane X receptor. Data are shown as percent of control. gPK experiments were carried out using male Sprague–Dawley rats (n = 3). Test compounds formulated at appropriate concentrations for IV (0.5 mL/kg). Unbound clearance equals the total clearance divided by the rat fraction unbound (Clu = Cl/fu). hRacemic compound.
methyl amide in 6a as the optimal substituent, the fluorination pattern of the quinoline ring was also explored. Contemporary work within the 3 series suggested that the C5 fluoroquinoline substitution might be beneficial. Accordingly, 7 was prepared and had excellent potency and selectivity profile in biochemical and cellular assays as well as optimal rat PK profile. The potency and selectivity profiles of 3, 6a, and 7 in biochemical, cellular, and human whole blood assays are shown in Table 3. These data highlight the improved potency and isoform selectivity of 6a and 7 relative to 3 in both biochemical and cellular assays. In the case of 6a, the selectivity ratios over other isoforms were found to be improved relative to 3. The intrinsic human whole blood assay data for 6a and 7 were also determined and were found to be within 2.7 and 4.5-fold relative to 3 respectively, which was deemed suitable for further progression into in vivo studies.

The physicochemical properties and oral PK of these analogs are shown in Table 4. The solubility of both 6a and 7 in PBS was comparable to that of 3, and these compounds also exhibited good passive permeability. The oral bioavailability of 6a in the rat was found to be superior to both 3 and 7, while the Clu remained low for all three analogs.

### Kinase Selectivity Profile and Crystallography.

Consistent with the profile of the 3 series, both quinolines, 6a and 7, were found to retain excellent kinase selectivity (KINOMEscan’s selectivity score: S-Score(35) = 0.018 for 6a, S-Score(35) = 0.01 for 7) in a large panel of 442 protein kinases tested at 10 μM drug concentration. The selectivity can be attributed to a common propeller-shaped conformation displayed by these molecules, illustrated by the X-ray co-crystal structure of analog 5g with PI3Kα (Figure 3). The improved biochemical potency of these analogs can also be rationalized

<table>
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<th>Table 3. Profile of Selected Analogs</th>
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*Biochemical: Alphascreen assay. aCellular: In vitro anti-IgM/CD40L-induced human B cell proliferation (as measured by thymidine incorporation) assay. cCellular: Ability of compound to inhibit anti-IgM induced AKT phosphorylation (pAKTSer473) in mouse B cells; phospho-AKT (pAKT) expression on B220 + gate. bCells was determined by flow cytometry in mouse splenocytes; compound pretreated human whole blood (HWB) was stimulated with anti-IgD to induce phosphorylation of AKT (pAKTSer473). dThe unbound human whole blood (HWB_unbound) potency was derived by multiplying the human plasma protein binding (PPB) fraction unbound by the total HWB potency (fu × HWB). eCellular: Compound pretreated HWB was stimulated with anti-IgD to induce CD-69 expression on B cells (6 h) and was evaluated by flow cytometry.

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*Single experimental values. Aqueous equilibrium solubility assay: compound was equilibrated at room temperature for 72 h in PBS buffer (pH 7.4), and supernatant was centrifuged and analyzed by HPLC for reporting solubility as µg/mL. aPassive permeability in LLC-PK1 cells, apparent permeability (Papp) measured by mass balance (LC/MS). b5 µM concentration in human plasma, protein binding measured by ultracentrifugation and LC/MS. c5 µM concentration in rat plasma, protein binding measured by ultracentrifugation and LC/MS. dPharmacokinetic parameters following administration in male Sprague–Dawley rats: two animals per study, dosed at 0.5 mg/kg as a solution in 100% DMSO. eUnbound clearance equals the total clearance divided by the rat fraction unbound (Clu = CI/fu). fDosed at 2.0 mg/kg in 0.5% Tween 80, 1% methylcellulose, 98.5% water. gDosed at 2.0 mg/kg in 0.5% methylcellulose, 1% Tween 80, 98.5% water.
by this cocrystal structure; the C4 substituent of the inhibitor makes additional interactions with the face of the Trp residue adjacent to the quinoline moiety (Trp812 in PI3Kγ, Trp760 in PI3Kδ). Added flexibility in the position of this Trp residue in PI3Kδ relative to the other class I isoforms likely allows for optimization of these additional interactions, thereby enhancing selectivity primarily through a greater potency boost to PI3Kδ compared to the other isoforms.

**In Vivo Pharmacology.** Given the improved selectivity of 6a and 7, these analogs were advanced for evaluation in our in vivo animal models of inflammation. The in vivo experiments were performed with analogs 6a and 7, which were orally dosed to IgMm mice26 15 min before an intravenous (IV) injection of FITC-labeled anti-IgM to stimulate circulation of B cells. The mice were sacrificed 30 min after stimulation to measure pAKT levels in spleen and whole blood of FITC-IgM positive B cells by flow cytometry (Figure 4). Both analogs 6a and 7 showed dose-dependent inhibition of AKT phosphorylation in anti-IgM stimulated B cells isolated from the whole blood and spleen compartments of IgMm mice. The calculated ED50 values for 6a in spleen and whole blood were <0.01 and <0.04 mg/kg, respectively (Figure 4a,b). The calculated ED50 values for 7 in splenocytes and whole blood were <0.003 and 0.005 mg/kg, respectively (Figure 4c,d).

In a second in vivo experiment, the data from the in vivo pAKT assay were utilized to select doses for multidose rat protein-antigen (keyhole limpet hemocyanin, KLH) studies. A multiple dose rat study was performed to determine whether 6a and 7 could impact B cell function in vivo. For this purpose, the effect of 6a and 7 on a humoral immune response, which is a measure of whether a compound inhibits antigen-specific Ig secretion by B cells, was studied via oral administration at multiple doses (Figures 5 and 6). These experiments demonstrated that analog 6a inhibited KLH-specific IgG and

![Figure 3. Crystal structure of PI3Kγ in complex with compound 5g (PDB code: 5KAE). Residues that differ in PI3Kδ are labeled. Dashed lines indicate hydrogen bonds.](image-url)

![Figure 4. Inhibition of activation induced AKT phosphorylation in B cells in vivo. Vehicle, 6a, 7 in 2% HPMC + 1% Tween 80, pH 2.0, was administered to IgMm mice orally 15 min before IV injection of FITC-labeled anti-IgM to stimulate B cells. Mice were sacrificed 30 min after stimulation to measure pAKT in the spleen and whole blood of FITC-IgM positive B cells by flow cytometry. (a) pAKT of 6a in splenocytes. (b) pAKT Levels in Mouse Whole Blood (6a). (c) pAKT Levels in Mouse Splenocytes (7). (d) pAKT Levels in Mouse Whole Blood (7).](image-url)
IgM in a dose-dependent manner (Figure 5a,b). The calculated ED50 values were 0.026 and 0.016 mg/kg, respectively. Exposure at these doses was expected to significantly inhibit PI3Kδ given the free drug concentrations achieved relative to the in vitro-derived pAKT_unbound IC50 (0.61 nM) over the 24 h dosing interval (Figure 5c). Analog 7 inhibited KLH-specific IgG and IgM in a dose-dependent manner (Figure 6a,b). The calculated ED50 values were 0.016 and 0.015 mg/kg, respectively. Exposure at these doses was expected to significantly inhibit PI3Kδ given the free drug concentrations achieved relative to in vitro-derived pAKT_unbound IC50 (0.67 nM) over the 24 h dosing interval (Figure 6c).

Exposure at all doses of 6a and 7 were below in vitro PI3Kβ/pAKT_unbound IC50 (2882 and 3500 nM, respectively) over 24 h dosing interval (Figures 5c and 6c). Additionally, both compounds, 6a and 7, were well tolerated at all doses. Ultimately, the KLH-mediated humoral immune response allowed us to select 6a and 7 as candidates for further development.

CONCLUSION

A novel series of potent, selective, and metabolically stable PI3Kδ inhibitors have been identified by optimizing the properties of the trisubstituted quinoline 4 (Figure 7). The successful strategy to improve the profile of this inhibitor involved modification of the pyridine ring at C4 and initial optimization within the C3 phenyl quinoline scaffold leading to the identification of a new series of amide analogs (e.g., 5b). These new compounds had improved microsomal stability and isoform selectivity but unfavorable PXR activation profile. The latter issue was addressed by reducing the clogP of these molecules, which was accomplished by replacing the phenyl group at C3 (Figure 7, compound 5b) with a pyridine ring. This strategy resulted in the identification of 6a, which upon further modification of the halogenation pattern led to the discovery of 7. Both of these analogs showed satisfactory activity in both mice pAKT and rat KLH efficacy studies, which in combination with their optimal pharmacokinetic and selectivity profile made them suitable candidates for further development.

EXPERIMENTAL SECTION

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, H2O with 0.1% TFA; B, CH3CN 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, H2O with 0.1% TFA; B, CH3CN with 0.1% TFA]; flow rate 1.5 mL/min. Mass spectral data were recorded on an Agilent 1100 series LC-MS with UV detection at 254 nm. All accurate mass data (high-resolution mass spectra: HRMS) were acquired on a Synapt G2 Q-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 × 50

Figure 6. Inhibition of KLH-specific antibodies and delta-specific coverage. Vehicle or 7 in 1% Pluronic F68, 2% HPMC, 10% Captisol, and 87% water 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). Two 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 SEM of eight rats. (c) After administration of 7, 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 PI3Kδ in vitro mouse pAKT_unbound IC50 0.67 nM [calculated from 0.8 nM (PI3Kδ in vitro mouse pAKT IC50) × 0.842 (fu in pAKT assay media)] represented as a blue solid line; PI3Kβ in vitro human pAKT_unbound IC50 3500 nM [calculated from 4110 nM (PI3Kβ in vitro human pAKT IC50) × 0.842 (fu in pAKT assay media)] represented as a red line.

Figure 7. Evolution of the SAR leading to the identification of 6a and 7.
mm) at 0.25 mL/min. NMR spectra were recorded on a Bruker Avance 400 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.

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), AS-H (250 × 30 mm, 5 μm), IA-H (250 × 21 mm, 5 μm), Lux Column (250 × 30 mm, 5 mm). Mobile Phase: A = liquid CO2; B = MeOH, EtOH, or isopropanol, flow rate: 70 mL/min. Column/oven temperature: 40 °C. UV detection wavelength 220 nm; 20.7 mg/injection; 200–206 bar inlet pressure.

2-Ethyl-6-fluoro-3-phenylquinoline-4-carboxylic acid (9). A mixture of 5-fluorosoratin 8 (5.00 g, 30.3 mmol), 1-phenylbutan-2-one (4.99 mL, 33.3 mmol), and potassium hydroxide (5.10 g, 91 mmol) in MeOH (100 mL) was heated to reflux for 3 h. After cooling to rt, the reaction mixture was concentrated in vacuo. The residue was diluted with water and acidified with 1 N HCl to pH 5. The resulting solid was collected by filtration, washed with water, and dried to give 2-ethyl-6-fluoro-3-phenylquinoline-4-carboxylic acid 9 (8.60 g, 29.1 mmol, 96% yield) as a fine tan powder. Mass spectrum (ESI) m/z 296.2 [M + H]+.

Methyl 2-ethyl-6-fluoro-3-phenylquinoline-4-carboxylate (10). To a solution of 2-ethyl-6-fluoro-3-phenylquinoline-4-carboxylic acid 9 (8.60 g, 29.1 mmol) in DCM (100 mL) was added thionyl chloride (11.1 mL, 151 mmol), and the resulting mixture was stirred at rt overnight. The solvent was removed, MeOH (50 mL) was added, and the resulting solution was stirred at rt for 2 h. The solvent was removed, and the crude residue was purified by column chromatography on silica gel using a 0–40% gradient of EtOAc/hexane as eluent to give methyl 2-ethyl-6-fluoro-3-phenylquinoline-4-carboxylate 10 (2.00 g, 6.47 mmol, 21% yield) as an oil. Mass spectrum (ESI) m/z 409.2 [M + H]+.

Methyl 2-(1-azidoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate (11). Methyl 2-ethyl-6-fluoro-3-phenylquinoline-4-carboxylate 10 (1.00 g, 3.2 mmol) and 1,3-dibromo-5,5-dimethylhydantoin (647 mg, 2.26 mmol) were suspended in carbon tetrachloride (30 mL) and treated with benzoyl peroxide (78 mg, 0.32 mmol), and the mixture was heated at reflux for 3 h. The reaction mixture was cooled to rt, and treated with a saturated aqueous sodium bicarbonate solution. The mixture was separated, and the aqueous layer was extracted with DCM twice. The combined organic layers were washed with brine, dried over Na2SO4, filtered, and concentrated in vacuo to give methyl 2-(1-bromoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate 11 (1.40 g, 3.60 mmol, 56% yield) as a yellow solid. Mass Spectrum (ESI) m/z 388.0 [M + H] +; 390.0 [M + H (Br)2]+.

Methyl 2-(1-aminoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate (12). To a solution of methyl 2-(1-bromoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate 11 (1.17 g, 3.00 mmol) in DMF (6 mL) was added NaN3 (0.293 g, 4.5 mmol) at rt. After 2 h, the reaction mixture was diluted with water and extracted twice with EtOAc. The organic layers were combined, washed with water and brine, dried over Na2SO4, filtered, and concentrated in vacuo to give methyl 2-(1-azidoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate as a tan oil. Mass spectrum (ESI) m/z 351 [M + H]+.

A solution of methyl 2-(1-azidoethyl)-6-fluoro-3-phenylquinoline-4-carboxylate (3.00 mmol) in MeOH (10 mL) was treated with 10% Pd/C (100 mg), and the mixture was stirred under an atmosphere of hydrogen gas at rt overnight. The reaction mixture was filtered through a Celite pad and concentrated in vacuo to give methyl 2-(1-aminooethyl)-6-fluoro-3-phenylquinoline-4-carboxylate 12 (0.92 g, 2.85 mmol, 95% yield) as a yellow oil. Mass spectrum (ESI) m/z 325.2 [M + H]+.

Methyl 2-(1-(6-amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylate (13). To the solution of methyl 2-(1-aminooethyl)-6-fluoro-3-phenylquinoline-4-carboxylate 12 (660 mg, 2.04 mmol) in n-BuOH (6 mL) were added 4-amino-6-chloropyrimidine-5-carbonitrile (314 mg, 2.04 mmol) and DIPEA (0.43 mL, 2.44 mmol), and the resulting mixture was heated to 120 °C for 3 h. The reaction mixture was cooled to rt, diluted with water, and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water and brine, dried over Na2SO4, filtered, and concentrated by column chromatography on silica gel (DCM/MeOH/NH3, 20/1/0.1) to give methyl 2-(1-(6-amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylate (747 mg, 1.69 mmol, 83% yield) as an off-white solid.1 H NMR (400 Hz, DMSO-d6) δ 8.16 (dd, J = 8.0, 4.0 Hz, 1H), 7.91 (s, 1H), 7.81 (td, J = 8.0, 4.0 Hz, 1H), 7.45–7.52 (m, 5H), 7.35 (dd, J = 8.0, 4.0 Hz, 1H), 7.25 (s, br, 2H), 5.30–5.40 (m, 1H), 3.59 (s, 3H), 1.30 (t, J = 8.0 Hz, 3H). Mass spectrum (ESI) m/z 443.2 [M + H]+.

2-(1-(6-Amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylic acid (14). To a suspension of methyl 2-(1-(6-amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylate 13 (1.09 g, 2.47 mmol) in pyridine (20 mL) was added lithium iodide (0.99 g, 7.40 mmol), and the resulting mixture was stirred at 100 °C overnight. The mixture was concentrated in vacuo. The residue was suspended in water, and the aqueous mixture was acidified with 1 N HCl to pH 5. The resulting solid was collected by filtration, washed with water, and dried to give 2-(1-(6-amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylic acid (1.04 g, 2.43 mmol, 98% yield) as a white solid.1 H NMR (400 Hz, DMSO-d6) δ 8.16 (dd, J = 8.0, 4.0 Hz, 1H), 7.97 (s, 1H), 7.81 (td, J = 8.0, 4.0 Hz, 1H), 7.72 (dd, J = 8.0, 4.0 Hz, 1H), 7.38–7.52 (m, 8H), 5.30–5.40 (m, 1H), 1.30 (t, J = 8.0 Hz, 3H). Mass spectrum (ESI) m/z 429.2 [M + H]+.

General Procedure for the Synthesis of 5a-c and f-i. To a solution of 5-(1-(6-Amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylic acid (15). The racemic mixture was purified by an AD-H column using 22% isopropanol (0.2% diethylamine) as additive B in supercritical CO2. Compound 15 was the first eluting enantiomer and isolated as a yellow solid (45% yield). Mass spectrum (ESI) m/z 429.2 [M + H]+.

5-(1-(6-Amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylic acid (15). The racemic mixture was purified by an AD-H column using 22% isopropanol (0.2% diethylamine) as additive B in supercritical CO2. Compound 15 was the first eluting enantiomer and isolated as a yellow solid (45% yield). Mass spectrum (ESI) m/z 429.2 [M + H]+.

5a-c and f-i. To a solution of 5-(1-(6-Amino-5-cyano-4-ylamo)ethyl)-6-fluoro-3-phenylquinoline-4-carboxylic acid (15). The racemic mixture was purified by an AD-H column using 22% isopropanol (0.2% diethylamine) as additive B in supercritical CO2. Compound 15 was the first eluting enantiomer and isolated as a yellow solid (45% yield). Mass spectrum (ESI) m/z 429.2 [M + H]+.
General Procedure for the Synthesis of $5d,e$. To a crude residue of $17d$ or $17e$ (0.051 mmol) was added HCl (0.5 mL, 2.00 mmol), and the resulting mixture was stirred at r.t. for 1 h. The mixture was concentrated in vacuo, and the crude product was carried on crude for next step.

To a solution of (S)-2-(1-aminoethyl)-N-ethyl-6-fluoro-3-phenylquinoline-4-carboxamide or (S)-2-(1-aminoethyl)-6-fluoro-N-isopropyl-3-phenylquinoline-4-carboxamide in n-BuOH (0.5 mL) were added DIEA (0.026 mL, 0.152 mmol) and 4-amino-6-chloropyrimidine-5-carbonitrile (7.81 mg, 0.051 mmol). The resulting mixture was stirred and heated at 100 °C overnight. The reaction mixture was cooled to r.t., diluted with water, and extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with water and brine, dried over Na$_2$SO$_4$, filtered, concentrated, and purified by column chromatography on silica gel (DCM/MeOH/NH$_3$, 20/1/0.1) to give $5d$, e.

$2$-((1S)-1-((6-Amino-5-cyano-4-pyrimidinyl)amino)ethyl)-6-fluoro-3-phenylquinoline-4-carboxamide ($5d$). Obtained in 31% yield as a white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ ppm 8.84 (1 H, br, J = 5.4 Hz), 8.19 (1 H, dd, J = 9.2, 5.3 Hz), 8.10 (1 H, s), 7.65–7.71 (1 H, m), 7.58–7.54 (6 H, m), 5.70 (1 H, q, J = 6.8 Hz), 3.11–3.22 (2 H, m), 1.43 (3 H, d, J = 6.7 Hz), 0.79 (3 H, d, J = 7.2 Hz). Mass spectrum (ESI) m/z 456.2 [M + H]$^+$. HRMS (ESI) m/z calculated for C$_{24}$H$_{21}$FN$_{7}$O$_3$: 456.1408; mass measured, 456.1402.

$2$-((1S)-1-((6-Amino-5-cyano-4-pyrimidinyl)amino)ethyl)-6-fluoro-3-phenylquinoline-4-carboxamide ($5e$). Obtained in 68% yield as a white solid. $^1$H NMR (400 MHz, CD$_3$OD) δ ppm 8.19 (1 H, dd, J = 9.2, 5.3 Hz), 7.92–7.97 (1 H, m), 7.56 (1 H, td, J = 8.6 Hz), 7.38–7.54 (6 H, m), 5.45 (1 H, q, J = 6.5 Hz), 3.14 (3 H, d, J = 6.5 Hz), 0.73 (3 H, br, d, J = 2.8 Hz). Mass spectrum (ESI) m/z 470.2 [M + H]$^+$. HRMS (ESI) m/z calculated for C$_{26}$H$_{24}$FN$_{7}$O: 470.1755; mass measured, 470.1757.

$2$-Ethyl-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid ($18$). A mixture of compound 8 (22.0 g, 133 mmol), 1-(pyridin-2-yl)butan-2-one (19.9 g, 133 mmol), KOH (22.5 g, 400 mmol), EtOH (100 mL), and water (100 mL) was stirred and heated at 90 °C overnight. After cooling to r.t., the reaction mixture was concentrated in vacuo. The residue was diluted with water and acidified with 1 N HCl to pH 5. The resulting solid was collected by filtration, washed with water, and dried to give 2-ethyl-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid 18 (389.0 g, 32.0 mmol, 99% yield) as an off-white solid.

Methyl 2-Ethyl-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid ($19$). To a solution of 18 (138.4 g, 32.0 mmol) in MeOH (75 mL) at 0 °C was added TMSCHN$_2$ (2 M in DCM, 62 mL, 93 mmol), and the mixture was allowed to warm to r.t. After 2 h, the mixture was concentrated in vacuo and purified by column chromatography on silica gel using 30% EtOAc/hexane to give methyl 2-ethyl-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid 19 (46.0 g, 14.82 mmol, 29% yield) as a pink solid. $^1$H NMR (400 MHz, CHLOROFORM-d) δ ppm 8.71–8.82 (1 H, m), 8.08–8.19 (1 H, m), 7.82 (1 H, td, J = 7.7, 1.8 Hz), 7.49–7.60 (2 H, m), 7.43 (1 H, dt, J = 7.6, 1.0 Hz), 7.36 (1 H, ddd, J = 7.6, 4.9, 1.2 Hz), 3.63 (3 H, s), 2.92 (2 H, q, J = 7.5 Hz), 1.23 (3 H, t, J = 7.5 Hz).

Methyl 2-(Bromoethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylate ($20$). To a solution of 19 (0.300 g, 0.967 mmol) in carbon tetrachloride (9 mL) were added 1,3-dibromo-5,5-dimethylimidazolidine-2,4-dione (0.193 g, 0.677 mmol) followed by benzoyl peroxide (0.023 g, 0.097 mmol), and the mixture was heated to reflux overnight. The mixture was washed with a saturated sodium bicarbonate, brine, and dried over sodium sulfate and activated carbon. The mixture was filtered and concentrated in vacuo to give methyl 2-(1-bromoethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylate 20 (302 mg, 0.776 mmol, 80% yield). Mass spectrum (ESI) m/z 389.0 [M + H (Br)]$^+$ and 391.1 [M + H (Br)]$^+$.
Methyl 2-(1-(6-Amino-5-cyanopyrimidin-4-ylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylate (2.85 g, 8.11 mmol) was dissolved in MeOH (20 mL). The reaction mixture was quenched with a saturated NH₄Cl solution added dropwise using a cannula before warming to r.t. overnight. The mixture was concentrated in vacuo, the crude residue was suspended in water, and the aqueous mixture was acidified with concentrated HCl to pH 2. The mixture was extracted twice with EtOAc (350 mL) was stirred at r.t. for 30 min before the addition of (S)-tert-buty1 3-oxo-4-phenylbutan-2-ylcarbamate (2.00 g, 4.51 mmol, 98% yield) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ ppm 8.71 (1 H, d, J = 6.5 Hz), 8.21–8.28 (1 H, m), 7.68–7.78 (3 H, m), 7.59 (1 H, ddd, J = 7.1, 5.8, 1.0 Hz), 5.62 (1 H, q, J = 6.5 Hz), 1.46 (3 H, d, J = 6.7 Hz). Mass spectrum (ESI) m/z 504.2 [M + H]⁺.

(S)-tert-Butyl 3-oxo-4-(pyridin-2-yl)butan-2-ylcarbamate (24). To a solution of picoline (31.9 mL, 323 mmol) in THF (300 mL) was added MeLi (1.6 M in hexanes, 202 mL, 324 mmol) dropwise at −40 °C under nitrogen. The reaction mixture was allowed to warm to −20 °C, stirred for 10 min, and then cooled to −40 °C, and magnesium bromide (59.4 g, 323 mmol) was added in three portions. The reaction mixture was allowed to warm to r.t. and stirred for 30 min to provide bromo-(pyridin-2-ylmethyl)magnesium (323 mmol).

-Benzyl 4-(2-(1-(6-Amino-5-cyanopyrimidin-4-ylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinolin-2-yl)ethylcarbamate (26a). Obtained in 84% yield as a light-yellow oil. Mass spectrum (ESI) m/z 439.2 [M + H]⁺.

-Benzyl 1-(4-(2-(Dimethylamino)ethylcarbamoyl)-6-fluoro-3-(pyridin-2-yl)quinolin-2-yl)ethylcarbamate (26b). Obtained in 37% yield as a yellow solid. Mass spectrum (ESI) m/z 482.2 [M + H]⁺.

-Benzyl 4-(2-(1-(tert-Butyloxycarbonylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinolin-2-yl)ethylcarbamate (26c). To a solution of 2-(1-(tert-butyloxycarbonylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid (25) (0.15 g, 0.37 mmol) in DMF (2 mL) were added benzyl piperazine-1-carboxylate (0.16 mL, 0.82 mmol), Pybop (429 mg, 0.82 mmol), and DIPEA (0.20 mL, 1.12 mmol), and the resulting mixture was stirred at r.t. After 1 h, the mixture was diluted with EtOAc, washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using 0–10% EtOAc/hexane as an eluent to give benzyl 4-(2-(1-(6-Amino-5-cyanopyrimidin-4-ylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinolin-2-yl)ethylcarbamate (26d). To a solution of picoline (31.9 mL, 323 mmol) in THF (300 mL) was added MeLi (1.6 M in hexanes, 202 mL, 324 mmol) dropwise at −40 °C under nitrogen. The reaction mixture was allowed to warm to −20 °C, stirred for 10 min, and then cooled to −40 °C, and magnesium bromide (59.4 g, 323 mmol) was added in three portions. The reaction mixture was allowed to warm to r.t. and stirred for 30 min to provide bromo-(pyridin-2-ylmethyl)magnesium (323 mmol).

-Tert-Butyl 1-(methoxy(methyl)amino)-1-oxopropan-2-ylcarbamate (50.0 g, 215 mmol) in THF (450 mL) was cooled to −40 °C (dry ice/acetone) and slowly charged with isopropylmagnesium chloride (2.0 M in Et₂O, 102 mL, 204 mmol). After a clear solution was obtained (became clear at −20 °C and milky again at −40 °C), bromo-(pyridin-2-ylmethyl)magnesium solution (323 mmol) was added dropwise using a cannula before warming to r.t. overnight. The reaction mixture was quenched with a saturated NH₄Cl solution and extracted with EtOAc twice. The combined organic layers were washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on a silica gel column using 0–30% gradient of EtOAc/hexane.

The combined organic extracts was washed with water and brine, and dried over sodium sulfate. The resulting suspension was stirred under hydrogen at r.t. After 45 min, the reaction mixture was filtered through a Celite pad, and the solvent was removed under reduced pressure to afford methyl 2-(1-azidoethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylate (2.85 g, 8.11 mmol) was dissolved in MeOH (20 mL). The solution was added 10% Pd/C (0.129 g), and the resulting suspension was stirred under hydrogen at r.t. After 45 min, the reaction mixture was filtered through a Celite pad, and the solvent was removed under reduced pressure to afford methyl 2-(1-aminooethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylate (71.70 g, 174 mmol, 78% yield) as a pale yellow solid.

H NMR (500 MHz, CDCl₃-δ₀) ppm 8.70 (1 H, br, d, J = 4.6 Hz), 8.19 (1 H, dd, J = 9.2, 5.5 Hz), 7.96 (1 H, d, J = 7.7, 1.5 Hz), 7.79 (1 H, td, J = 8.8, 2.7 Hz), 7.51–7.63 (2 H, m), 7.47 (1 H, dd, J = 7.2, 5.3 Hz), 7.08 (1 H, br, d, J = 7.6 Hz), 4.86 (1 H, br, t, J = 7.1 Hz), 1.30 (9 H, s), 1.22 (3 H, br, d, J = 6.8 Hz). Mass spectrum (ESI) m/z 412.2 [M + H]⁺.

General Procedure for the Synthesis of 26a,b,e. To a solution of 2-(1-(tert-butyloxycarbonylamino)ethyl)-6-fluoro-3-(pyridin-2-yl)quinoline-4-carboxylic acid (25) (3.16 mmol) in DMF (10 mL) were added amine (2.0 equiv), DIPEA (1.2 equiv) and Pybop (2.5 equiv), and the resulting mixture was stirred at r.t. overnight. The mixture was concentrated in vacuo, and the crude residue was dissolved in EtOAc, washed with 1% NaOH, 1 N HCl, water, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using 0–100% EtOAc/hexane as an eluent to give 26a,b,e.

-Benzyl 1-(6-Fluoro-4-(methylcarbamoyl)-3-(pyridin-2-yl)quinolin-2-yl)ethylcarbamate (26a). Obtained in 62% yield as a green solid. H NMR (400 MHz, CDCl₃-δ₀) ppm 8.71 (1 H, br, d, J = 4.7 Hz), 8.20 (1 H, dd, J = 9.2, 5.3 Hz), 7.96 (1 H, td, J = 7.8, 1.9 Hz), 7.66 (1 H, ddd, J = 9.2, 8.4, 2.9 Hz), 7.61 (1 H, br, d, J = 8.0 Hz), 7.47–7.53 (2 H, m), 4.90–4.99 (1 H, m), 2.66 (3 H, s), 1.38 (12 H, s). Mass spectrum (ESI) m/z 425.2 [M + H]⁺.
mass spectrum (ESI) \( \text{m/z} = 457.2 [M + H]^+ \). HRMS (ESI) calculated for C26H26FN9O \([M+H]^+\): 443.1749; mass measured, 443.1735.

To a solution of \( \text{benzyl 4-((1-(6-amino-5-cyano-4-pyrimidinyl)amino)ethyl)-6-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide} \) (6d) in DMF (3 mL) were added DIPEA (0.66 mL, 3.77 mmol) and 4-amino-6-chloropyrimidine-5-carbonitrile (146 mg, 0.94 mmol, 86% yield) as white solid. The mixture was stirred and heated at 50 °C overnight. The reaction mixture was allowed to cool to r.t. and concentrated in vacuo. The mixture was dissolved in DCM, 2 M NH3 in MeOH/DCM for 5 min, and the crude residue was dissolved in EtOAc, washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give 2-(1-(1-tetrafluorobutyl)iminobenzyl)-5-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide (7). To a solution of 2-(1-(1-tetrafluorobutyl)iminobenzyl)-5-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide (7) in DCM (3 mL) were added DIPEA (0.58 mL, 3.3 mmol) and the reaction mixture was stirred at r.t. overnight. The mixture was concentrated in vacuo, and the crude residue was dissolved in EtOAc and washed with 1 N NaOH, 1 N HCl, water, and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using 0–100% EtOAc/hexane as an eluent to give 2-\((1S)-1-(((6-Amino-5-cyano-4-pyrimidinyl)amino)ethyl)-5-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide\) (6e). To a solution of \( \text{benzyl 4-((1-(6-amino-5-cyano-4-pyrimidinyl)amino)ethyl)-6-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide} \) (6d) and \( \text{4N HCl in 1,4-dioxane} \) (1 mL, 4.00 mmol) were added. The mixture was acidiﬁed with 3 N HCl to pH 2 and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo to give 2-(1-(1-tetrafluorobutyl)iminobenzyl)-5-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide (6f). Obtained in 36% yield as a tan solid. \( \text{1H NMR} \) (400 MHz, DMSO-d6) \( \delta \) ppm 8.78–8.84 (1 H, m), 8.24 (1 H, dd, \( J = 9.2, 5.3 \) Hz), 8.17 (1 H, s), 8.09–8.15 (1 H, m), 7.70–7.80 (2 H, m), 7.60–7.68 (2 H, m), 7.54 (1 H, q, \( J = 6.2 \) Hz), 3.49–3.63 (2 H, m), 2.99–3.08 (2 H, m), 2.86 (6 H, s), 1.51 (3 H, \( J = 6.7 \) Hz). Mass spectrum (ESI) \text{m/z} = 502.0 \([M + H]^+\); HRMS (ESI) \text{m/z} calculated for C23H19FN8O \([M + H]^+\): 502.2332; mass measured, 502.2312.

To a solution of \( \text{2-(1-tetrafluorobutyl)iminobenzyl)-5-fluoro-3-(2-pyridinyl)-4-quinolinecarboxamide} \) (28) in DCM (10 mL) were added 4-fluorofuran-2,3-dione (673 mg, 4.1 mmol) and KOH (486 mg, 8.0 mL, 12.2 mmol). The resulting mixture was heated to 95 °C overnight. The reaction mixture was cooled to 50 °C and the mixture was diluted with EtOAc. The mixture was washed with three times with water and brine, dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using 30% [10:1 MeOH/DCM] in DCM and further purified by chiral HPLC (isopropanol/hexane gradient, AD column) to give \( \text{6b, a, e.} \)
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00827.

(i) Biological assays; (ii) enzyme selectivity data of compounds 6a (AM-0687) and 7 (AM-1430); (iii) molecular formula strings and the associated biochemical and biological data; and (iv) crystal structure of PI3Kγ in complex with compound 5g (PDF)

Accession Codes
The cocrystal structure of PI3Kδ with compound 5g has been deposited in the protein data bank with PDB code: 5KAЕ.

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Notes
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ABBREVIATIONS USED

AKT, (PKB) protein kinase B; Cl, clearance; CYP, cytochrome P450; DCM, dichloromethane; DIPEA, N,N-diisopropylethylamine; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; EtOAc, ethyl acetate; FBS, fetal bovine serum; FITC, fluorescein isothiocyanate; HLM, human liver microsomal; HBW, human whole blood; IgD, immunoglobulin D; IgG, immunoglobulin G; IgM, immunoglobulin M; KLH, keyhole limpet hemocyanin; mTOR, mammalian target of rapamycin; MeOH, methanol; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffer saline; PI, phosphoinositides; PI3Ks, phosphoinositide 3-kinases; PIP3, phosphatidylinositol 3,4,5-trisphosphate; POC, percent of control; RLM, rat liver microsomal; SEM, standard error of the mean; SFC, supercritical fluid chromatography; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

REFERENCES

(13) Idealisib (Zidelig) is intended to be used in patients who have received at least two prior systemic therapies.


(23) This result contrasts with the observation from the following on which the microsomal stability of related cyano-aminopyrimidine-containing PI3K inhibitors was not a good predictor of in vivo clearance: Patel, L.; et al. J. Med. Chem. 2016, 59, 3532−3548, DOI: 10.1021/acs.jmedchem.6b00213.

(24) See Supporting Information for kinase selectivity profile for these analogs.