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Selective Class I Phosphoinositide 3-Kinase Inhibitors: Optimization of a Series of Pyridyltriazines Leading to the Identification of a Clinical Candidate, AMG 511

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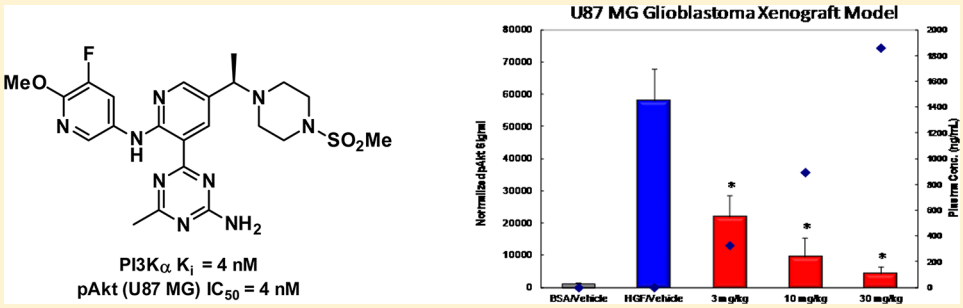
Supporting Information



ABSTRACT: The phosphoinositide 3-kinase family catalyzes the phosphorylation of phosphatidylinositol-4,5-diphosphate to phosphatidylinositol-3,4,5-triphosphate, a secondary messenger which plays a critical role in important cellular functions such as metabolism, cell growth, and cell survival. Our efforts to identify potent, efficacious, and orally available phosphatidylinositol 3-kinase (PI3K) inhibitors as potential cancer therapeutics have resulted in the discovery of 4-(2-((6-methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**1**). In this paper, we describe the optimization of compound **1**, which led to the design and synthesis of pyridyltriazine **31**, a potent pan inhibitor of class I PI3Ks with a superior pharmacokinetic profile. Compound **31** was shown to potently block the targeted PI3K pathway in a mouse liver pharmacodynamic model and inhibit tumor growth in a U87 malignant glioma glioblastoma xenograft model. On the basis of its excellent in vivo efficacy and pharmacokinetic profile, compound **31** was selected for further evaluation as a clinical candidate and was designated AMG 511.

INTRODUCTION

The phosphatidylinositol 3-kinase (PI3K) signaling network has a central role in several cellular processes critical to the initiation and progression of cancer, including growth, survival, and metabolism. Class I PI3Ks, separated from other classes of PI3Ks by structure and function, are the most clearly implicated in cancer.[1] Class I PI3Ks are subdivided into class I_A (PI3Kα, β, and δ) and class I_B (PI3Kγ) isoforms. Class I_A PI3Ks are heterodimers composed of a p85 regulatory subunit and an isoform-specific catalytic p110 subunit, which define their individual identity. The PIK3R1 gene encodes the p85 regulatory subunit, whereas the catalytic, p110α, p110β, and p110δ, subunits are the products of PIK3CA, PIK3CB, and PIK3CD genes, respectively. All cell types express PI3Kα and PI3Kβ, in contrast to PI3Kδ and PI3Kγ, which are predominantly expressed in leukocytes.

PI3K-dependent signaling is frequently activated in human cancers through a variety of genetic and epigenetic alterations, including activating somatic mutations in PIK3CA, the gene

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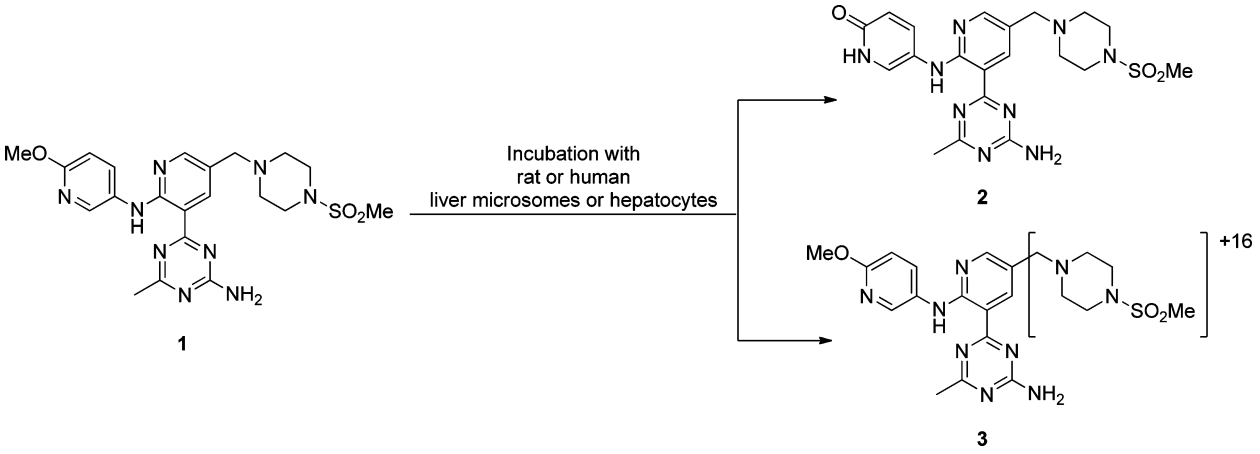

Figure 1. Lead compound **1** and major oxidative metabolites of compound **1**.

encoding the catalytic subunit of PI3Kα, and the loss of function of the lipid phosphatase PTEN (a negative regulator of PI3K).[2,3] For example, oncogenic mutations in PIK3CA are a frequent event in many types of human tumors, notably in breast and colon cancer; and amplification of PIK3CA has also been observed in a significant number of ovarian and non-small-cell lung tumors. In addition, loss-of-function mutations of PTEN commonly occur in prostate cancers and glioblastomas.[4] Consequently, significant efforts have been applied to discover and develop PI3K inhibitors as potential cancer therapeutics,[5] and several compounds have entered clinical trials in recent years (e.g., SF1126,[6] PX-866,[7] BEZ235,[8] BGT226,[9] GDC-0941,[10] XL765,[11] and GSK-615[12]).

We recently reported the identification of a highly selective series of pyridyltriazine-based inhibitors of the class I PI3Ks, exemplified by **1** (Figure 1), in our efforts to identify novel inhibitors of this pathway.[13,14] Pyridyltriazine **1** showed potent activities in both the biochemical (K_i values of 12, 5, 2, and 4 nM for PI3Kα, PI3Kβ, PI3Kδ, and PI3Kγ, respectively) and cellular (U87 MG IC_{50} = 16 nM) assays. In addition, compound **1** was active in vivo as determined by the inhibition of AKT (Ser473) phosphorylation in a mouse liver pharmacodynamic assay (EC_{50} = 137 ng/mL). It was also shown to inhibit tumor growth in a U87 MG glioblastoma tumor xenograft model in mice with an ED_{50} of 6.0 mg/kg (AUC_{0-24h} = 7.6 μM·h). Although pyridyltriazine **1** proved to be a potent, selective, and efficacious PI3K inhibitor that represented a promising novel lead, it had rapid iv clearance in both rat and mouse (CL = 1.7 and 2.5 L/h/kg, respectively; approximately 50% of liver blood flow in both species) and a short mean residence time (MRT = 1.6 h for both rat and mouse). Therefore, this compound was not suitable for further advancement, and we sought to improve the pharmacokinetic profile of this series to enhance the in vivo efficacy.

As an initial step toward improving the overall pharmacokinetic profile of these PI3K inhibitors, we examined compound **1** in more detail. A single intravenous dose of compound **1** was administered to bile-duct-cannulated rats, and the concentration of the parent drug was quantified in the excreta (urine, bile, and feces) collected over 24 h postdose.[15] The analysis revealed that less than 2.5% of the administered dose of compound **1** was excreted as intact parent compound, which led us to infer that metabolism was the dominant clearance pathway. Therefore, metabolite identification studies were carried out by incubating compound **1** with rat and human liver microsomes or hepatocytes.[16] LC–MS metabolite profiling indicated that the majority of observed metabolites resulted from oxidative metabolism. Two prominent metabolic pathways were identified: one arising from O-demethylation, giving pyridone **2**, and another arising from oxidation around the benzylic piperazine region, giving compound **3** (although the exact site of oxidation was not ascertained). With this information in hand, we prepared a series of analogues designed to block the sites of metabolism of compound **1**, focusing initially on modifying the methoxypyridine to reduce O-demethylation and, subsequently, on examining substitutions around the benzylic piperazine region of the molecule. These investigations culminated in the identification of the clinical candidate AMG 511 (compound **31**).

CHEMISTRY

The synthetic methods outlined below are organized by one of three general target classes: analogues wherein the methoxypyridine is modified (Scheme 1), benzylic or piperazine monomethyl-substituted (or trifluoromethyl-substituted) derivatives (Scheme 2), and benzylic or piperazine dimethyl-substituted analogues (Scheme 3).

The syntheses of analogues of compound **1** wherein the methoxypyridine moiety was modified are shown in Scheme 1. These derivatives were prepared from one of two known intermediates, *tert*-butyl 4-((5-(4-(bis(4-methoxybenzyl)-amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)-methyl)piperazine-1-carboxylate (**4**) or 2-(2-fluoro-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-4-methyl-6-(methylthio)-1,3,5-triazine (**22**).[14] For example, the reaction of fluoropyridine **4** with 6-methoxypyridin-3-amine, 2-methoxypyrimidin-5-amine, or 5-fluoro-6-methoxypyridin-3-amine provided intermediates **5**–**7**, respectively. TFA-mediated Boc removal of **5**–**7** followed by sulfonylation of the resulting piperazines with methanesulfonyl chloride gave the corresponding methylsulfonamides **9**–**11**. Alternatively, fluoropyridine **4** was converted to methylsulfonamide **8**, which was subsequently treated with 6-chloropyridin-3-amine, 6-methylpyridin-3-amine, or 5-fluoropyridin-3-amine to give pyridine–triazine intermediates **12**–**14**. The final products (**1** and **15**–**19**) were obtained by removal of the bis(*p*-methoxybenzyl) (bis-PMB) protecting groups of **9**–**14**. Compound **1** was also demethylated by treatment with iodotrimethylsilane to give the pyridone metabolite **2**. The final two compounds illustrated in Scheme 1 (**20** and **21**) were derived from thiomethyl

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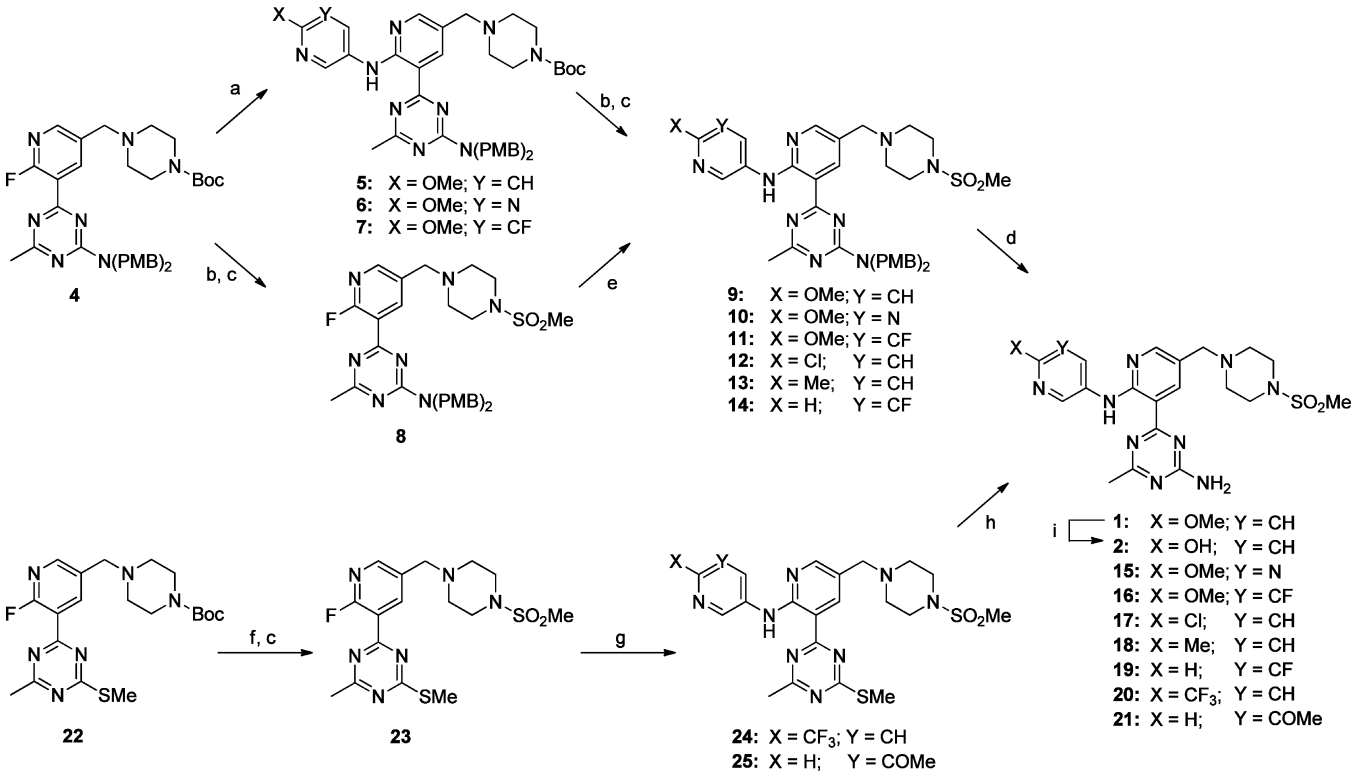
Scheme 1[a]



[a]Reagents and conditions: (a) 6-methoxypyridin-3-amine, 2-methoxypyrimidin-5-amine, or 5-fluoro-6-methoxypyridin-3-amine, LiHMDS, THF, 0 °C; (b) TFA, CH_2Cl_2, rt; (c) MsCl, TEA, CH_2Cl_2, 0 °C; (d) TFA, TfOH, Δ; (e) 6-chloropyridin-3-amine, 6-methylpyridin-3-amine, or 5-fluoropyridin-3-amine, THF, NaHMDS, or LiHMDS, 0 °C to rt; (f) TFA, CH_2Cl_2, 0 °C to rt; (g) 6-(trifluoromethyl)pyridin-3-amine or 5-methoxypyridin-3-amine, LiHMDS, THF, 0 °C; (h) 2 M NH_3 in *i*-PrOH, sealed tube, 90 °C; (i) (TMS)I, $CHCl_3$, 70 °C.

intermediate **22**. Deprotection of the Boc group followed by conversion of the resulting free amine to the corresponding sulfonamide yielded **23**. Treatment of triazine **23** with 6-(trifluoromethyl)pyridin-3-amine or 5-methoxypyridin-3-amine provided intermediates **24** and **25**, which were heated with ammonia in a sealed tube to give the requisite trifluoromethyl and methoxy derivatives **20** and **21**, respectively.

The preparation of derivatives containing a monosubstitution on either the benzylic position or the piperazine ring is outlined in Scheme 2. Both the methyl- and trifluoromethyl-substituted benzylic derivatives **30**–**33** were prepared from a common intermediate, fluoropyridine **26**.[14]Introduction of the 5-fluoro-6-methoxypyridin-3-amine group to **26** followed by hydrolysis of the acetal group provided aldehyde **27**. Addition of methylmagnesium bromide to aldehyde **27** and subsequent conversion of the resulting alcohol to the mesylate gave intermediate **29**. Displacement of the mesyl group with 1-(methylsulfonyl)piperazine followed by removal of the PMB protecting groups gave the racemic product, which was separated and purified by chiral preparative supercritical fluid chromatography (SFC) to provide both **30** and **31** in >98% ee. The corresponding trifluoromethyl derivatives were prepared by hydrolysis of acetal **26** followed by treatment of the resulting aldehyde with trimethyl(trifluoromethyl)silane. Subsequent addition of 5-fluoro-6-methoxypyridin-3-amine yielded alcohol **34**. Intermediate **36** was prepared by conversion of **34** to the corresponding chloride **35** followed by displacement with *tert*-butyl 1-piperazinecarboxylate. Deprotection of **36** followed by reaction with methanesulfonyl chloride provided methylsulfonamide **37**. The final trifluoromethyl derivatives (**32** and **33**) were prepared similarly to the corresponding methyl derivatives by treatment with trifluoroacetic acid and subsequent purification by chiral preparative SFC.

The two enantiomers containing monomethyl substitutions on the piperazine ring (**42** and **43**) were derived from a common intermediate, **38**,[14] where the key steps involved the addition of a potassium trifluoroborate intermediate by the method reported by Molander et al.[17] (Scheme 2). The potassium trifluoroborate reagents, prepared by the treatment of potassium (bromomethyl)trifluoroborate and (*R*)- or (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate amine, were coupled with chloropyridine intermediates (**38** or **44**) under palladium-catalyzed cross-coupling conditions. The synthesis of each enantiomer differed only in the sequence of the reaction steps performed. For the *R*-enantiomer (**42**), the trifluoroborate reagent was added first, followed by methylsulfonamide formation and addition of the 5-fluoro-6-methoxypyridin-3-amine group, whereas with the *S*-enantiomer (**43**), the 5-fluoro-6-methoxypyridin-3-amine was introduced initially followed by the addition of the trifluoroborate reagent and subsequent elaboration to the desired sulfonamide.

The syntheses of the derivatives that contain two methyl groups α to the basic piperazine nitrogen (**56**–**59**) are outlined in Scheme 3. The addition of methyl Grignard to 6-fluoronicotinaldehyde (**47**) followed by treatment of the resulting alcohol with thionyl bromide provided bromide **49**. Fluoropyridine **50** was prepared by the displacement of the bromine in **49** with (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate in good yield. Deprotonation of **50** at the position *ortho* to the fluoro group with *n*-butyllithium followed by

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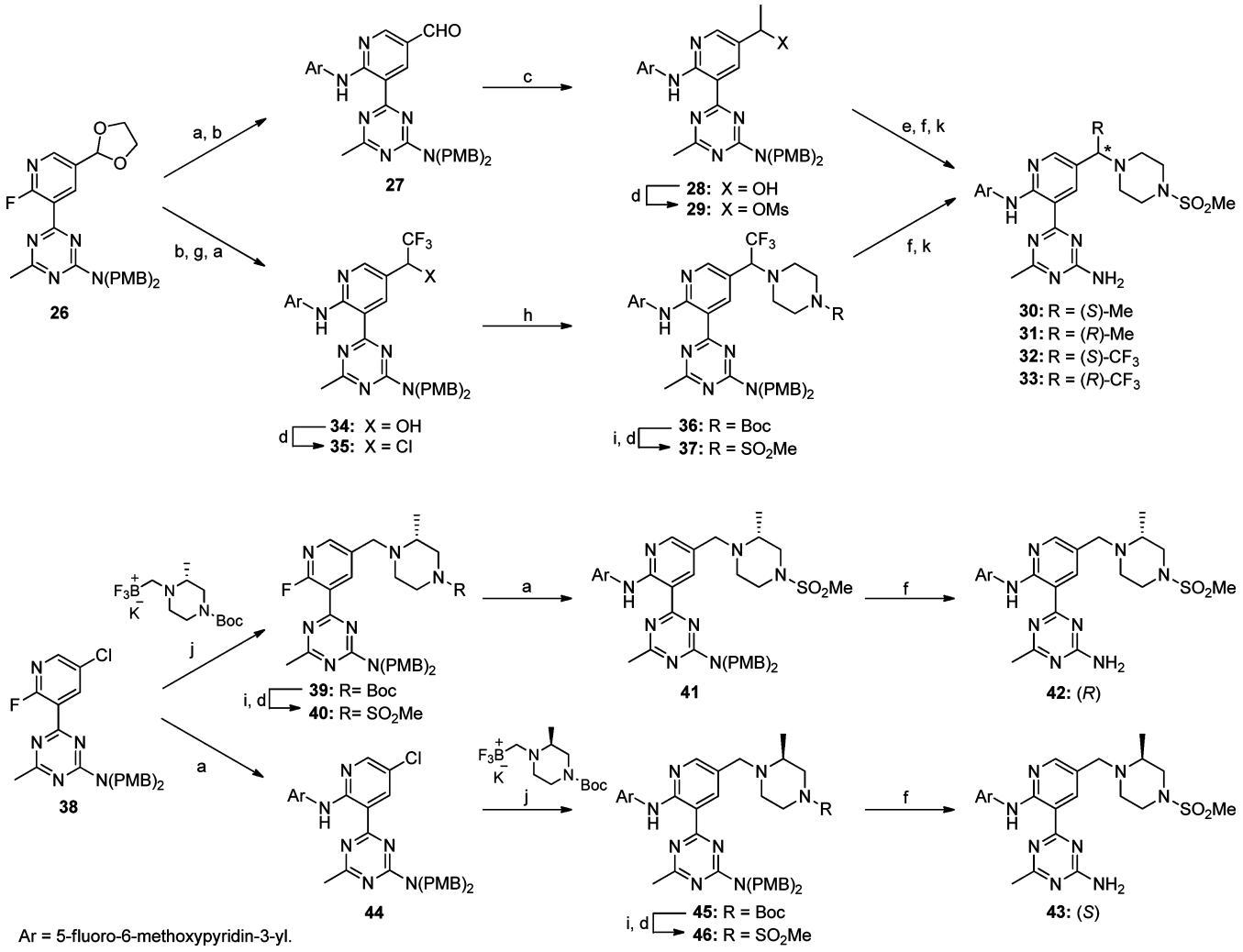
Scheme 2[a]



[a]Reagents and conditions: (a) 5-fluoro-6-methoxypyridin-3-amine, LiHMDS, THF, 0 °C; (b) HCl, MeOH, 0 °C to rt; (c) MeMgBr, THF, 0 °C; (d) MsCl, TEA, CH_2Cl_2, rt or 0 °C; (e) 1-(methylsulfonyl)piperazine, TEA, CH_2Cl_2; (f) TFA, 75 °C, or TFA, TfOH, 70−80 °C; (g) $(TMS)CF_3$, THF, 0 °C; (h) *tert*-butyl 1-piperazinecarboxylate, CH_3CN, TEA; (i) TFA, CH_2Cl_2, rt; (j) $Pd(OAc)_2$, X-Phos, Cs_2CO_3, THF, H_2O, microwave, 85−140 °C; (k) chiral separation by preparative SFC on a Chiralcel OJ-H column.

quenching with triisopropyl borate gave the corresponding boronic acid **51**. Suzuki−Miyaura coupling of **51** with 4-chloro-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine and subsequent addition of 5-fluoro-6-methoxypyridin-3-amine gave compound **53** as a mixture of two diastereomers epimeric at the benzylic position. Deprotection of the Boc group followed by methylsulfonamide formation with methanesulfonyl chloride provided the penultimate intermediates **54** and **55**, which were separable by column chromatography on silica gel. Acid deprotection of **54** and **55** provided the first two desired diastereomers, **56** and **57**.

An alternative route was employed to prepare the final two diastereomers, **58** and **59**. Addition of 5-fluoro-6-methoxypyridin-3-amine to fluoropyridine **38** followed by the Stille coupling with tributyl(1-ethoxyvinyl)stannane provided ketone **60** in good yield (Scheme 3). Mesylate **62** was obtained by reduction of **60** with sodium borohydride and subsequent treatment with methanesulfonyl chloride. Reaction of mesylate **62** with (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate provided **63** as a mixture of two diastereomers epimeric at the benzylic position. Compound **63** was converted to the methylsulfonamide derivative by the methods described previously, and the resulting two diastereomers were separated by column chromatography before deprotection to yield the aminotriazines **58** and **59**.

The final analogue required for our investigation was the benzylic *gem*-dimethyl analogue **69**. This compound was prepared from intermediate **38** as shown in Scheme 3. Addition of 5-fluoro-6-methoxypyridin-3-amine followed by Suzuki−Miyaura coupling of **38** with isopropenylboronic acid pinacol ester provided propenyl intermediate **66**. The benzylic amine was introduced by the reaction of **66** with sodium azide in trifluoroacetic acid to give azide **67**, which was subsequently reduced to the corresponding primary amine **68** by hydrogenation over palladium on carbon. Bisalkylation of the benzylic amine by treatment with ((methylsulfonyl)azanediyl)-bis(ethane-1,2-diyl) dimethanesulfonate formed the piperazine sulfonamide ring, and final deprotection of the PMB groups provided the requisite *gem*-dimethyl derivative **69**.

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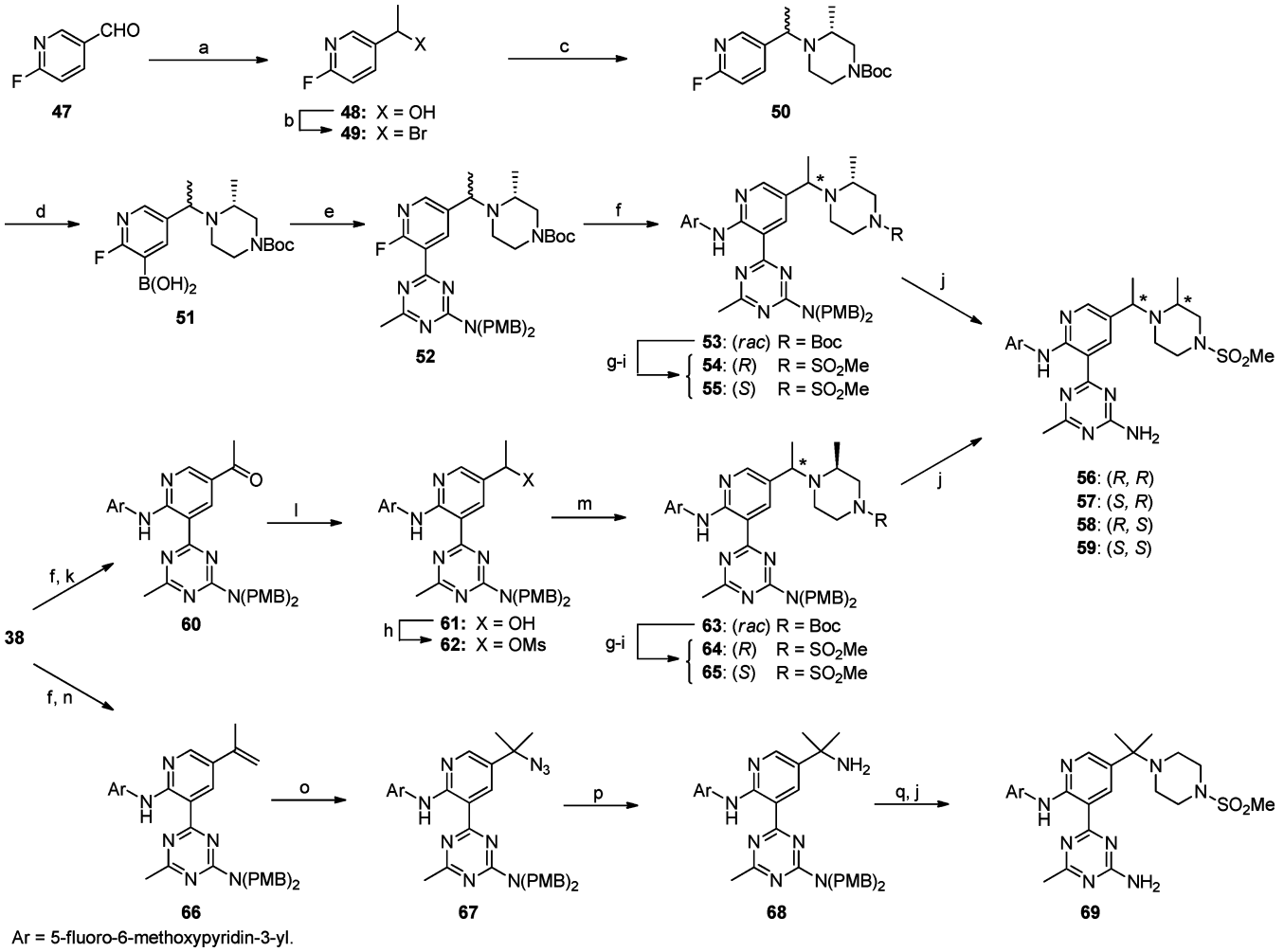
Scheme 3[a]



[a]Reagents and conditions: (a) MeMgBr, THF, 0 °C; (b) $SOBr_2$, CH_2Cl_2, 25 °C; (c) (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate, K_2CO_3, KI, CH_3CN, 70 °C; (d) *n*-BuLi, $B(O\text{-}i\text{-}Pr)_3$, THF; (e) 4-chloro-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine,[14] $PdCl_2$(A-Phos)$_2$, KOAc, dioxane−H_2O, 100 °C; (f) 5-fluoro-6-methoxypyridin-3-amine, LiHMDS, THF, 0 °C; (g) TFA, CH_2Cl_2, rt; (h) MsCl, TEA, CH_2Cl_2, 0 °C; (i) diastereomeric separation; (j) TFA, TfOH, rt to 80 °C; (k) X-Phos, $Pd(OAc)_2$, CsF, dioxane, tributyl(1-ethoxyvinyl)stannane, 110 °C; (l) $NaBH_4$, THF, 0 °C; (m) (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate, 2,2,6,6-tetramethylpiperidine, CH_3CN, reflux; (n) isopropenylboronic acid pinacol ester, K_2CO_3, $PdCl_2$(A-Phos)$_2$, 4:1 dioxane/water, sealed tube, Δ; (o) NaN_3, TFA, $CHCl_3$, −15 °C to rt; (p) H_2, Pd/C, THF, rt; (q) ((methylsulfonyl)azanediyl)bis(ethane-1,2-diyl) dimethanesulfonate, DIPEA, sealed vial, 130 °C.

RESULTS AND DISCUSSION

The compounds described in this paper were inhibitors of all class I PI3Ks, and the data, which were determined using modified in vitro AlphaScreen assays, are reported in Tables 1 and 2.[18] Cell-based activity was measured by the inhibition of AKT (Ser473) phosphorylation in U87 MG glioblastoma cells. In addition to the enzyme and cellular assays, the metabolic stabilities of the compounds were evaluated in rat and human liver microsomal (RLM and HLM) preparations for an initial assessment of metabolism; these data are reported as intrinsic clearance (CL_{int}).[19] These microsomal data were used to prioritize potent compounds for additional pharmacokinetic studies (vide infra).

In the initial phase of this investigation, we examined a small set of analogues that contained replacements of the methoxypyridine group in an attempt to increase potency and reduce or eliminate the O-demethylation observed with compound **1** (Table 1). To aid in the design of novel inhibitors that could address this metabolic liability, we first analyzed the crystal structure of compound **1** bound to the active site of the PI3Kγ protein, which is highly homologous to the other class I PI3K isoforms.[14,20] The key interactions of compound **1** within the active site of the enzyme are illustrated in Figure 2. In the region of the active site referred to as the affinity pocket (Figure 2A), a hydrogen bond network exists between the pyridyl nitrogen and a water molecule that is bridged between the Asp841 carboxylate and the Tyr867 hydroxyl groups, as we have observed in an earlier series of PI3K inhibitors.[14,21] In addition, the methyl of the methoxy group occupies a small channel that projects toward Asp836, and the methoxy oxygen atom forms a favorable interaction with Lys833. Since we had established that the position of the pyridine nitrogen was important for PI3K activity from our earlier SAR studies, we retained this nitrogen and focused our modifications in the latter two areas (i.e., exploring the pocket near Asp836 and probing potential interactions with Lys833) as potential ways to both increase potency and improve metabolic stability.

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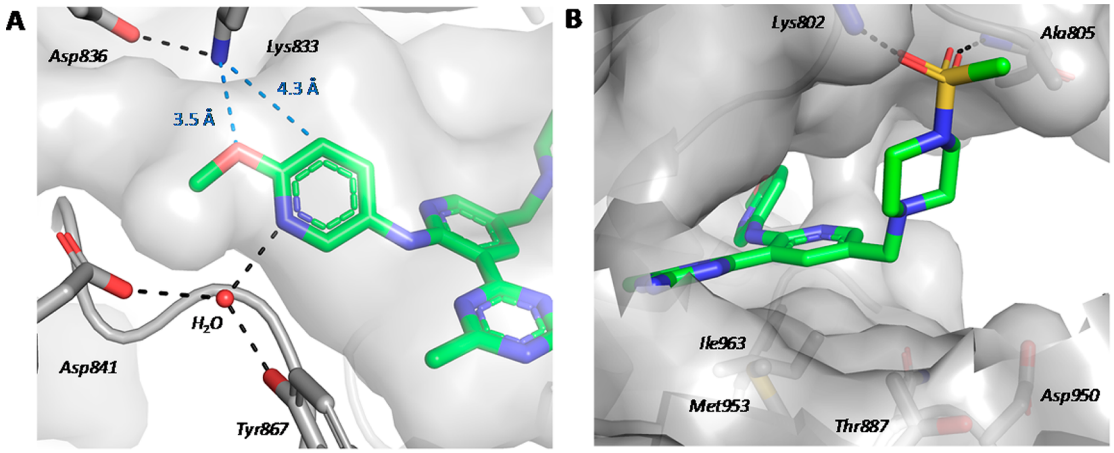

Figure 2. X-ray crystal structure of compound **1** bound to PI3Kγ determined at 2.95 Å resolution (PDB code 1E8Y). The gray surface indicates the van der Waals surface area, and hydrogen bonds (or potential hydrogen bonds) are shown by dashed lines. (A) Affinity pocket region. (B) Ribose pocket region. Note: Lys890 has been cut away in the surface view for clarity.

The first five compounds shown in Table 1 examined substitutions on the 2-position of the pyridyl ring that project into the Asp836 channel. A significant loss in PI3K activity was observed when compound **1** was demethylated to give pyridone **2**. Presumably, compound **2** exists predominantly in the form of the pyridone tautomer (vs the hydroxypyridine tautomer), and as such, the favorable interaction observed between the pyridyl nitrogen of compound **1** and the bridged water molecule between Asp841 and Tyr867 is disrupted, thereby resulting in diminished activity. Replacement of the methoxy group of compound **1** with small hydrophobic groups, such as chloro (**17**) or methyl (**18**), was tolerated and gave only a slight loss in activity on both the enzymatic and cellular levels; however, a significant (~10−15-fold) loss in potency was observed with the larger trifluoromethyl substituent (compound **20**). This result is consistent with the narrow dimensions of the Asp836 channel observed in the crystal structure.

The next two compounds shown in Table 1 were designed to alter the electron density in the ring (which we postulated may affect the compound's propensity for O-demethylation) as well as to add a potential hydrogen bond with Lys833 (through interactions with the 3-N pyrimidinyl nitrogen of compound **15** or with the 3-fluoro group of compound **16**). The PI3K activities of pyrimidine **15** were comparable to those of compound **1**; however, its cellular activity diminished by approximately 4-fold. On the other hand, an increase in cellular activity was obtained by the addition of a fluorine atom at the 3-position of the pyridine ring (compound **16**). Unfortunately, the relative degrees of O-demethylation for compounds **1**, **15**, and **16** could not be ascertained from the microsomal data, since the intrinsic clearances of compounds **15** and **16** were comparable to that of compound **1**. However, when compound **16** was incubated in rat hepatocytes, metabolite profiling revealed that oxidation on the methoxy group was significantly reduced and that the dominant metabolite resulted from oxidation on the core and not from demethylation of the methoxy group. Although this was only a qualitative observation, as a small amount of the demethylated metabolite was still observed, the result was encouraging and suggested that the added fluoro substituent could improve the overall metabolic profile of the compound.

Surprisingly, only a minimal loss in activity was observed by the elimination of the 2-methoxy group while the 3-fluoro group was maintained (**19**) or by moving the methoxy group from the 2-position to the 3-position (**21**). As seen in the cocrystal structure of **1** with PI3Kγ (Figure 2A), the distance between Lys833 and the 3-position of the pyridine ring was 4.3 Å. Modeling studies suggested that Lys833 could adopt multiple conformations, including one where it was positioned to favorably interact with the 3-position of the pyridine ring. Taken together, the SAR results for the pyridine ring suggested that interactions of the 2-methoxy group within the channel were not critical for binding affinity, as a range of replacements were tolerated.

Although the exact position of metabolism in compound **3** was not determined from our metabolism studies with compound **1**, oxidation on either side of the basic piperazine nitrogen was probable, as benzylic C−H bonds and carbons adjacent to a nitrogen atom are particularly prone to oxidation by cytochrome P450 enzymes.[22] In addition, the benzylic position of compound **1** was also predicted to be the most likely site of metabolism using MetaSite prediction software, which was consistent with literature precedent.[23,24] Therefore, we examined the effect of methyl (and trifluoromethyl) substitutions on the benzylic position and/or on the piperazine ring in the second phase of our SAR investigations as a potential means of blocking metabolism in this region of the molecule. Figure 2B highlights the area of the enzyme being explored with these derivatives and suggests that there would be sufficient room to accommodate additional substituents in this region. In addition, we postulated that we may also be able to increase potency with these substituted analogues by gaining additional hydrophobic interactions with the Thr887, Met953, and Asp950 residues on the floor of the pocket (Figure 2B).

Since compound **16** had the best combination of cellular potency and microsomal stability, the 5-fluoro-6-methoxypyridin-3-amine moiety was retained in the next set of analogues (Table 2). These derivatives were designed to systematically examine the combinations of both monomethyl (**30**, **31**, **42**, and **43**) and dimethyl (**56**−**59**, and **69**) substitutions on the benzylic and piperazine positions. In general, all of the substitutions were well tolerated within the PI3K enzymes, as predicted by modeling studies. Both sets of monomethyl derivatives (**30** and **31**; **42** and **43**) showed a slight increase in activities at the PI3Kα and PI3Kγ isoforms as compared to compound **16**. Although no significant stereochemical prefer-

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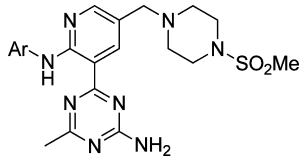
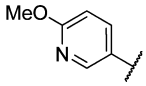
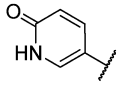
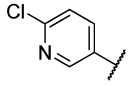
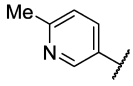
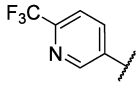
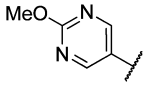
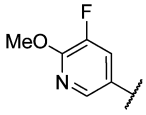
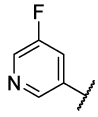
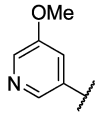
Table 1. Structure–Activity Relationships of Affinity Pocket Derivatives[a]



Compd No.	Ar	PI3Kα K_i (nM)	PI3Kβ K_i (nM)	PI3Kδ K_i (nM)	PI3Kγ K_i (nM)	U87 MG IC_{50} (nM)[b]	RLM (μL/min/mg)[c]	HLM (μL/min/mg)[c]
1		12	5	4	8	16	26	<14
2		5320	4810	5580	2350	>10000	<14	-----
17		24	17	20	21	35	24	<14
18		34	20	30	31	44	22	<14
20		106	143	84	136	237	22	<14
15		17	10	16	16	62	14	14
16		18	8	6	21	6	30	<14
19		37	19	40	24	23	30	<14
21		16	55	20	7	30	29	14

[a]The inhibition of PI3K activity was determined using a modified in vitro AlphaScreen assay with human p110*α*, p110*β*, p110*δ*, and p110*γ* enzymes. The data represent an average of at least two determinations. Standard deviations are reported in the Supporting Information. [b]Cellular IC_{50} values were determined by an AKT (Ser473) phosphorylation assay using U87 MG glioblastoma cells. [c]Single experimental values.[19]

ence was observed with regard to PI3K inhibition, the (*R*)-methyl benzylic (**31**) and the (*S*)-methylpiperazine (**43**) derivatives showed greater rat microsomal stabilities than their corresponding enantiomers (**30** and **42**, respectively). Increasing the size of the benzylic substituent to a trifluoromethyl group (**32** and **33**) was detrimental to activity, which was consistent with the steric constraints imposed by the enzyme in this region of the binding site.

Combining methyl substitutions on both the benzylic and piperazine positions provided the four diastereomers **56**–**59**. In

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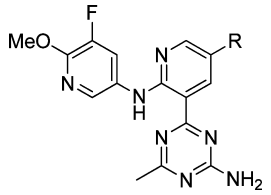
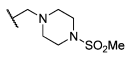
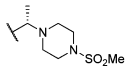
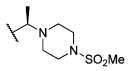
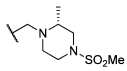
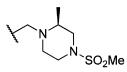
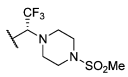
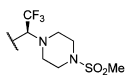
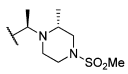
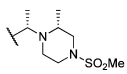
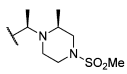
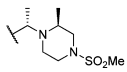
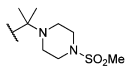
Table 2. Structure–Activity Relationships of Mono- and Disubstituted Derivatives[a]



Compd No.	R	PI3Kα K_i (nM)	PI3Kβ K_i (nM)	PI3Kδ K_i (nM)	PI3Kγ K_i (nM)	U87 MG IC_{50} (nM)[b]	RLM (μL/min/mg)[c]	HLM (μL/min/mg)[c]
16		18	8	6	21	6	30	<14
30		4	3	1	7	7	98	34
31		4	6	2	1	4	20	22
42		3	4	1	9	1	120	29
43		2	5	1	9	7	54	41
32		9	12	3	20	18	17	23
33		31	21	7.6	34	44	22	25
56		13	6	2	12	12	113	64
57		2	8	2	12	31	174	54
58		1	3	12	8	1	69	67
59		4	2	14	9	3	128	80
69		4	2	14	9	3	58	81

[a]The inhibition of PI3K activity was determined using a modified in vitro AlphaScreen assay with human p110*α*, p110*β*, p110*δ*, and p110*γ* enzymes. The data represent an average of at least two determinations. Standard deviations are reported in the Supporting Information. [b]Cellular IC_{50} values were determined by an AKT (Ser473) phosphorylation assay using U87 MG glioblastoma cells. [c]Single experimental values.[19]

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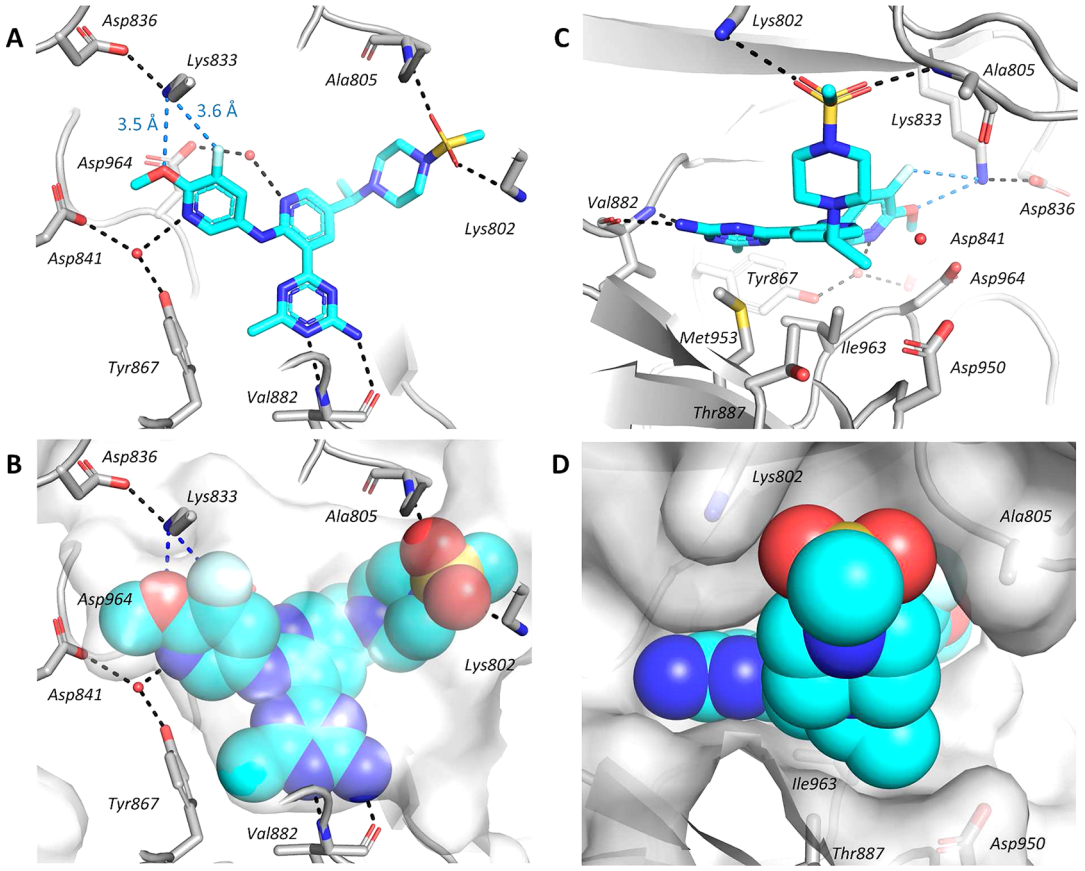

Figure 3. X-ray cocrystal structure of compound **31** bound to PI3Kγ determined at 2.64 Å resolution (PDB code 4FLH). The gray surface indicates the van der Waals surface area, and hydrogen bonds (or potential hydrogen bonds) are shown by dashed lines. (A, B) Side views highlighting the linker and affinity pocket regions. (C, D) End views highlighting the ribose pocket region. Note: Lys890 has been cut away in the surface view for clarity.

general, the bismethyl substitutions were also tolerated, with the (*S*)-methylpiperazine diastereomers **58** and **59** being preferred (with U87 MG IC_{50} values of 1−3 nM). As was observed with the monomethyl derivatives, the stereochemistries of the methyl groups had an impact on microsomal stability within this series, with the *R,S*-isomer being more stable (compound **58**). The *gem*-dimethyl substitution on the benzylic position was also tolerated and provided a derivative (**69**) that exhibited excellent potencies in both the enzyme and cellular assays.

All of the class I PI3K inhibitors prepared in this investigation were highly selective over other members of the closely related PIKK family of kinases, including the protein kinase mTOR and the lipid kinases hVPS34 and PI4Ks (biochemical IC_{50} values >10 μM). In addition, a selected set of derivatives also showed excellent selectivity against several protein kinases when evaluated in an Ambit KINOME*scan* selectivity screen.[25,26] For example, compound **31** showed no significant binding affinity toward 372 protein kinases when tested at 1 μM. As noted previously,[14] the high degree of selectivity over protein kinases for this series of compounds may be attributable, in large part, to the 2-methyl substituent on the triazine hinge binder that occupies a small hydrophobic pocket found in the PI3Ks (near Tyr867) which is generally not present in protein kinases (excluding mTOR). The additional structural features of the compounds optimized from this investigation can be exemplified in the X-ray cocrystal structure of compound **31** bound to PI3Kγ (Figure 3). As observed previously for **1**, the aminotriazine of compound **31** forms two hydrogen bonds to Val882 in the hinge region, and the methoxypyridine nitrogen atom makes a hydrogen bond to an ordered water molecule sitting between Tyr867 and Asp841 (Figure 3A,B). The methoxy oxygen atom and the additional 3-fluoro substituent both form favorable interactions with Lys833 within the affinity pocket. The nitrogen in the pyridine central core forms a hydrogen bond to a water molecule that is associated with Asp964, an interaction that was not resolved in the crystal structure with compound **1**. Finally, the additional methyl group on the benzylic position of compound **31** effectively fills the hydrophobic pocket formed by Thr887, Ile963, and Asp950 residues on the floor of the enzyme, while the methylsulfonamide oxygens engage in hydrogen bonds to Lys802 and Ala805 (Figure 3C,D).

The SAR investigations described above enabled the identification of several potent pan class 1 PI3K inhibitors (<10 nM in the U87 MG cellular assay) that also showed good metabolic stability in human and rat liver microsomes (CL_{int} < 100 μL/min/mg) (i.e., **16**, **30**, **31**, **43**, **58**, and **69**). This set of compounds was evaluated further in pharmacokinetic experiments, and the results of these studies are shown in Table 3. Although there was not an exact correlation between in vitro rat microsomal clearance values and the actual clearance observed in vivo, generally, the rank order between the two measurements held within this series. For example, compound **30**, which was the least stable in rat liver microsomes (CL_{int} = 98 μL/min/mg), also demonstrated the highest in vivo clearance

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Table 3. Pharmacokinetic Profiles of Selected Compounds[a]

	iv[b]			po[c]	
compd	CL (L/kg/h)	Vdss (L/kg)	MRT (h)	*F* (%)	AUC (μM·h)
1	1.7	2.6	1.6	77	0.68
16	2.0	3.3	1.7	45	0.95
30	2.9	6.4	2.2	57	0.35
31	0.4	1.7	3.9	60	5.0
43	1.0	3.2	3.2	60	2.3
58	1.3	4.0	3.1	49	1.4
69	0.7	3.5	4.9	24	1.3

[a]Pharmacokinetic parameters following administration in male Sprague–Dawley rats: three animals per study. [b]Dosed at 1 mg/kg as a solution in DMSO. [c]Dosed at 2 mg/kg as a solution or suspension in 15% (hydroxypropyl)-β-cyclodextrin (HPβCD), 2% hydroxypropyl methylcellulose (HPMC), and 1% Pluronic F68, pH 2.2, with methanesulfonic acid (MSA).

in rats (CL = 2.9 L/kg/h), while the compound that was most stable in microsomes (compound **31**; CL_{int} = 20 μL/min/mg) showed the lowest clearance in vivo (CL = 0.4 L/kg/h). In general, the compounds reported in Table 3 demonstrated moderate to high volumes of distribution (Vdss ≈ 2–6 L/kg) and moderate mean residence times (MRT ≈ 2–5 h). The majority of the compounds also demonstrated good oral bioavailabilities (*F* = 45–77%) (albeit with varying exposure levels), with the exception of compound **69**, which exhibited oral bioavailability of only 24%. Overall, the benzylic (*R*)-methyl analogue **31** had a superior pharmacokinetic profile with low clearance (0.4 L/h/kg, 12% of liver blood flow), good oral bioavailability (*F* = 60%), and a commensurate high oral exposure (AUC = 5.0 μM·h).

With its excellent in vitro cellular potency and favorable pharmacokinetic profile, pyridyltriazine **31** was evaluated further in pharmacodynamic and xenograft studies. Initially, compound **31** was tested in a mouse liver pharmacodynamic model in which the inhibition of hepatocyte growth factor (HGF)-induced AKT (Ser473) phosphorylation was measured.[27] The compound was dosed orally at 3, 10, and 30 mg/kg, and after 6 h the mice were administered HGF to activate PI3K-dependent AKT phosphorylation in the liver. The levels of AKT (Ser473) phosphorylation were determined 5 min following the administration of HGF by a quantitative electrochemiluminescence immunoassay. The results are shown in Figure 4. Compound **31** significantly suppressed PI3K signaling, as indicated by a dose-dependent decrease in phosphorylated AKT (p-AKT) at Ser473 (Figure 4), and a nonlinear regression analysis revealed a plasma EC_{50} of 228 ng/mL. Inhibition of AKT phosphorylation directly correlated with plasma concentrations in this study.

Given the potent pharmacodynamic effect of compound **31**, this compound was evaluated for its ability to inhibit tumor growth in a mouse U87 MG glioblastoma xenograft model.[28] In this model, compound **31** was administered orally at 1, 3, or 10 mg/kg daily for 12 days (Figure 5). Treatment with compound **31** at 1 mg/kg QD resulted in significant inhibition of tumor growth of approximately 70% compared to the vehicle control group. Tumor stasis was observed in the cohort treated with 3 mg/kg, and tumor regression was observed in the 10 mg/kg

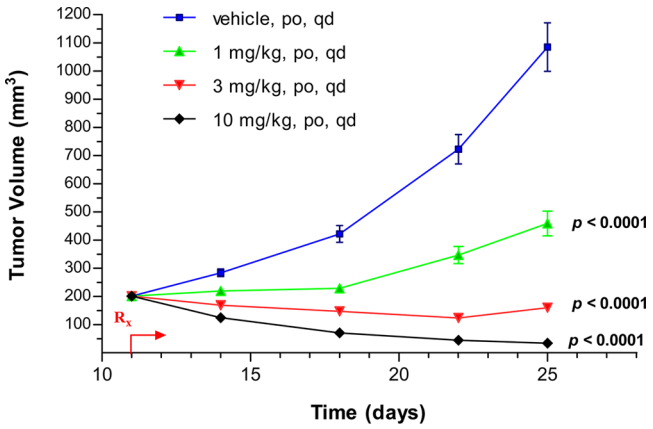

Figure 5. Effect of daily dosing of pyridine–triazine **31** in an established U87 MG glioblastoma xenograft model grown in female CD1 NU/NU mice. The arrow denotes the first day of dosing. Statistical significance was evaluated by repeated measures analysis of variance followed by Dunnett's post hoc test.

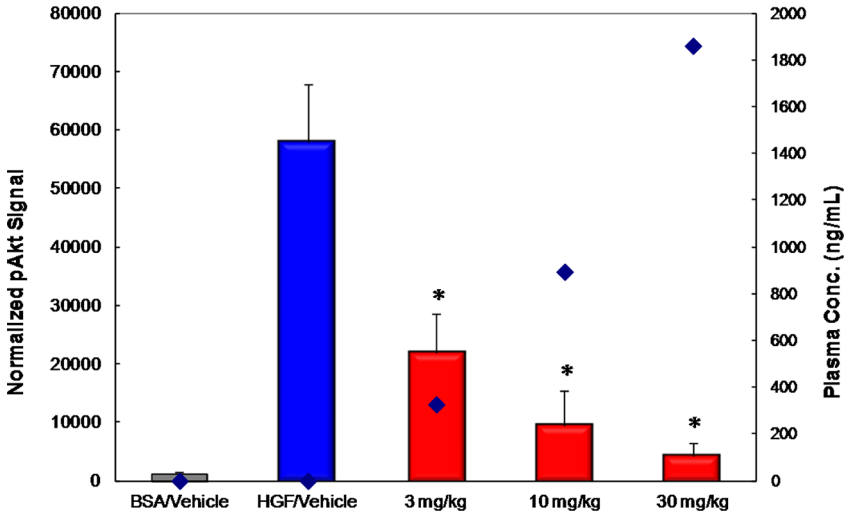

Figure 4. Effect of compound **31** in a mouse liver pharmacodynamic model measuring the inhibition of HGF-stimulated AKT (Ser473) phosphorylation 6 h postdose. Asterisks denote $p < 0.0001$ compared with the vehicle/HGF group. Statistical significance was evaluated by Dunnett's method. Bars represent the average ± SD (n = 3). Tilted squares represent mean plasma concentrations.

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cohort. The ED_{50} of compound **31** was 0.6 mg/kg with an AUC at EC_{50} of 3.6 μg·h/mL.

SUMMARY

In this investigation, we examined compounds designed to block the proposed sites of metabolism of compound **1**, focusing initially on modifying the methoxypyridine to reduce O-demethylation and then subsequently blocking metabolism at the benzylic piperazine region of the molecule. In the first phase of this investigation, we found that the 5-fluoro-6-methoxypyridin-3-amine group in the affinity pocket provided the best combination of both cellular potency and microsomal stability (compound **16**). Further SAR investigations revealed that enhanced stability and improved potencies could be obtained by adding a methyl group on the benzylic position. Ultimately, this work led to identification of pyridyltriazine **31**, a potent pan inhibitor of class I PI3Ks with a superior pharmacokinetic profile. Compound **31** was shown to potently block the targeted PI3K pathway in a mouse liver pharmacodynamic model with an EC_{50} of 228 ng/mL. Furthermore, **31** potently inhibited tumor growth in a U87 MG glioblastoma xenograft model (ED_{50} = 0.6 mg/kg). On the basis of its excellent pharmacokinetic properties and in vivo efficacy, compound **31** was selected for further evaluation as a clinical candidate for the treatment of cancer and was designated AMG 511. A more detailed biological profile of AMG 511 will be reported in due course.

EXPERIMENTAL SECTION

General Procedures. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich, Acros, or EM Science and used directly. All reactions involving air- or moisture-sensitive reagents were performed under a nitrogen or argon atmosphere. Microwave-assisted reactions were conducted with either an Initiator from Biotage, Uppsala, Sweden, or an Explorer from CEM, Matthews, NC. Silica gel chromatography was performed using either glass columns packed with silica gel (200−400 mesh, Aldrich Chemical) or prepacked silica gel cartridges (Biotage or Redisep). NMR spectra were determined with a Bruker 400 MHz spectrometer. Chemical shifts were reported in parts per million (ppm, δ units). All final compounds were purified to >95% purity as determined by LC−MS obtained on an Agilent 1100 spectrometer using the following methods: (A) Agilent SB-C18 column (50 × 3.0 mm, 2.5 μm) at 40 °C with a 1.5 mL/min flow rate using a 5−95% gradient of 0.1% TFA in CH_3CN in 0.1% TFA in water over 3.5 min; (B) Phenomenex Gemini NX C18 column (50 × 3.0 mm, 3 μm) at 40 °C with a 1.5 mL/min flow rate using a 5−95% gradient of 0.1% formic acid in CH_3CN in 0.1% formic acid in water over 3.5 min. Low-resolution MS data were obtained at the same time as the purity determination on the LC−MS instrument using the ES ionization mode (positive). Compound **1** and intermediates **4**, **5**, **9**, **22**, **26**, and **38** were prepared as previously described.[14]

5-((3-(4-Amino-6-methyl-1,3,5-triazin-2-yl)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-2-yl)amino)-pyridin-2-ol (2). 4-(2-((6-Methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**1**; 1.00 g, 2.06 mmol), $CHCl_3$ (16 mL), and iodotrimethylsilane (0.88 mL, 6.18 mmol) were added to a resealable vial. The reaction mixture was heated and stirred at 70 °C for 3 h, and then an additional amount of iodotrimethylsilane (0.88 mL, 6.18 mmol) was added. After being stirred at 70 °C for a total of 19 h, the solution was pipetted into a 1:1 saturated sodium bicarbonate/$Na_2S_2O_3$ aqueous solution (100 mL). The mixture was filtered, and the filtercake was washed with water and dried under vacuum to give the crude product as a dark-yellow solid. The crude material was purified by preparative HPLC (Phenomenex Gemini C18, 100 × 30 mm, 5 μm) using a gradient of 5−40% CH_3CN (0.1% TFA)/H_2O (0.1% TFA) over 20 min and then 100% CH_3CN (0.1% TFA) for 3 min. The fractions containing product were concentrated under vacuum, and the resulting solid was suspended in aqueous saturated sodium bicarbonate, sonicated, and centrifuged. The supernatant liquid was decanted, and the solid was washed with aqueous saturated sodium bicarbonate (1×) and water (2×) and then dried under vacuum to afford the title compound (0.46 g, 47%) as a yellow solid. MS (ESI positive ion): *m/z* calcd for $C_{20}H_{25}N_9O_3S$ 471, found 472 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.41 (s, 1H), 11.36 (br s, 1H), 8.68 (d, *J* = 2.1 Hz, 1H), 8.17 (d, *J* = 2.1 Hz, 1H), 7.99 (br s, 1H), 7.83 (br s, 1H), 7.59−7.76 (m, 2H), 6.37 (d, *J* = 9.5 Hz, 1H), 3.47 (br s, 2H), 3.10 (t, *J* = 4.0 Hz, 4H), 2.86 (s, 3H), 2.47 (br s, 4H), 2.42 (s, 3H).

4-(2-((2-Methoxypyrimidin-5-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (15). *tert-Butyl 4-((5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((2-methoxypyrimidin-5-yl)amino)pyridin-3-yl)methyl)piperazine-1-carboxylate (6).* A solution of *tert*-butyl 4-((5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)methyl)piperazine-1-carboxylate (**4**; 0.500 g, 0.78 mmol) and 2-methoxypyrimidin-5-amine (0.117 g, 0.93 mmol) in THF (10 mL) was cooled to 0 °C and treated dropwise via syringe with LiHMDS in THF (1.0 M, 2.33 mL, 2.33 mmol). The reaction mixture was stirred at 0 °C for 1 h and quenched with water (10 mL). The reaction mixture was diluted with water (15 mL) and EtOAc (100 mL). The organic layer was separated, dried over sodium sulfate, filtered, and concentrated in vacuo to give the title compound (0.42 g, 72%). MS (ESI positive ion): *m/z* calcd for $C_{40}H_{48}N_{10}O_5$ 748, found 749 $(M + H)^+$.

N,N-Bis(4-methoxybenzyl)-4-(2-((2-methoxypyrimidin-5-yl)-amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (10). TFA (4 mL) was added to a cooled (ice bath) solution of **6**(0.413 g, 0.55 mmol) in CH_2Cl_2 (5 mL). The resulting mixture was stirred at room temperature for 1 h and partially concentrated under vacuum. The residue was dissolved in CH_2Cl_2 (5 mL), and triethylamine (0.38 mL, 2.76 mmol) and methanesulfonyl chloride (0.13 mL, 1.65 mmol) were slowly added at 0 °C. The mixture was stirred at 0 °C for 1 h and concentrated under vacuum. The crude product was partitioned between 1 M NaOH and CH_2Cl_2 (20 mL each), and the separated aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to give the crude product. The crude product was purified by silica gel chromatography (0−3% 2 M NH_3/MeOH in CH_2Cl_2) to give the title compound (0.277 g, 69%) as a brown foam. MS (ESI positive ion): *m/z* calcd for $C_{36}H_{42}N_{10}O_5S$ 726.3, found 727.2 $(M + H)^+$.

4-(2-((2-Methoxypyrimidin-5-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (15). Two drops of trifluoromethanesulfonic acid were added to a solution of **10** (0.301 g, 0.41 mmol) in TFA (0.6 mL) at room temperature. The mixture was heated at 80 °C for 15 h. The reaction mixture was allowed to cool to room temperature, and the solvent was removed under vacuum. The crude product was first purified by silica gel chromatography (0−3% MeOH in CH_2Cl_2). The isolated yellow solid was dissolved in DMSO and subjected to a reversed-phase preparative HPLC purification (Shimadzu using Phenomenex, Gemini C18, 100 Å, 150 × 30 mm, 5 μm, 10−100% CH_3CN/H_2O with 0.1% TFA). The fractions containing the product were combined, basified with saturated aqueous sodium bicarbonate, and extracted with CH_2Cl_2 (3 × 20 mL), and the combined organic layers were dried over sodium sulfate, filtered, and concentrated in vacuo to give the title compound (0.065 g, 32%) as a bright yellow solid. MS (ESI positive ion): *m/z* calcd for $C_{20}H_{26}N_{10}O_3S$ 486, found 487 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.78 (s, 1H), 9.03 (s, 2H), 8.73 (d, *J* = 1.6 Hz, 1H), 8.23 (d, *J* = 1.8 Hz, 1H), 7.91 (br s, 1H), 7.75 (br s, 1H), 3.91 (s, 3H), 3.50 (s, 2H), 3.11 (br s, 4H), 2.86 (s, 3H), 2.46−2.49 (m, 4H), 2.44 (s, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-

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1,3,5-triazin-2-amine (16). *tert-Butyl 4-((5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)methyl)piperazine-1-carboxylate (7).* A solution of **4** (25 g, 38.8 mmol) and 5-fluoro-6-methoxypyridin-3-amine (8.28 g, 58.3 mmol) in THF (400 mL) contained in an oven-dried, 1 L, three-necked, round-bottomed flask equipped with a nitrogen inlet was stirred at a temperature between −5 and −10 °C and treated dropwise with LiHMDS in THF (1.0 M, 117 mL, 117 mmol). The dark pink solution was stirred between −5 and −10 °C for 1 h. The reaction was quenched with water (100 mL) and saturated aqueous ammonium chloride (100 mL) and diluted with EtOAc (350 mL). The separated aqueous layer was extracted with EtOAc (2 × 300 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel column chromatography (10−50% EtOAc in hexanes) to give the title compound (26.8 g, 90%). MS (ESI positive ion): m/z calcd for $C_{41}H_{48}FN_9O_5$ 765, found 766 $(M + H)^+$.

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (11). A solution of **7** (25 g, 32.6 mmol) in CH_2Cl_2 (150 mL) and TFA (150 mL) was stirred at room temperature for 1 h. The solution was concentrated under vacuum, and the residue was dissolved in CH_2Cl_2 and carefully neutralized with saturated aqueous sodium bicarbonate. The aqueous layer was extracted with CH_2Cl_2, and the organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo to give 4-(2-((5-fluoro-6-methoxypyridin-3-yl)amino)-5-(piperazin-1-ylmethyl)-pyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (20 g, 92%). The crude product was taken on to the next step without purification. A 1 L, three-necked, round-bottomed flask equipped with a thermometer was charged with 4-(2-((5-fluoro-6-methoxypyridin-3-yl)amino)-5-(piperazin-1-ylmethyl)pyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (25.1 g, 37.7 mmol) and CH_2Cl_2 (500 mL). The suspension was stirred at −15 °C and treated dropwise with triethylamine (52.4 mL, 0.38 mol). The resulting solution was treated with methanesulfonyl chloride (8.92 mL, 113 mmol) and stirred at 0 °C for 1 h. The solvent was removed under vacuum, and the crude product was washed with water (300 mL), suspended in water, stirred, and sonicated. The resulting suspension was collected, washed with water, and dried to give the title compound (26.5 g, 94%). MS (ESI positive ion): m/z calcd for $C_{37}H_{42}FN_9O_5S$ 743, found 744 $(M + H)^+$.

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (16). A three-necked 500 mL flask equipped with an overhead stirrer, a thermocouple, and a nitrogen inlet was charged with **11** (26.9 g, 36.2 mmol) and TFA (175 mL). Upon complete dissolution, the solution was warmed to 45 °C, and trifluoromethanesulfonic acid (16.1 mL, 181 mmol) was added dropwise. The reaction mixture was stirred for 1 h and then cooled to 20 °C. A separate 2 L flask equipped with an overhead stirrer and thermocouple was charged with 10% aqueous trisodium citric acid (500 mL) and cooled to 0 °C. The reaction mixture was added dropwise to the cooled aqueous solution. The product precipitated out of solution, CH_2Cl_2 was added, and the slurry was stirred at 20 °C for 16 h. The solid was collected by filtration, washed with copious water and copious ethanol, and dried to give the title compound (15.06 g, 83%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{21}H_{26}FN_9O_3S$ 503, found 504 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.73 (d, J = 2.1 Hz, 1H), 8.42 (d, J = 2.1 Hz, 1H), 8.37 (dd, J = 12.7, 2.1 Hz, 1H), 8.26 (d, J = 2.1 Hz, 1H), 7.93 (br s, 1H), 7.78 (br s, 1H), 3.93 (s, 3H), 3.50 (s, 2H), 3.11 (br s, 4H), 2.87 (s, 3H), 2.45−2.49 (m, 4H), 2.44 (s, 3H).

4-(2-((6-Chloropyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (17). *4-(2-Fluoro-5-((4-(methylsulfonyl)piperazin-1-yl)-methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (8).* A 100 mL round-bottomed flask was charged with **4** (0.246 g, 0.383 mmol) and CH_2Cl_2 (3 mL). TFA (3.0 mL, 38.9 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 45 min. The mixture was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (5 mL), and TEA (0.40 mL, 2.9 mmol) was added. The mixture was cooled in an ice−water bath, and methanesulfonyl chloride (0.100 mL, 1.28 mmol) was added dropwise. The solution was stirred in an ice−water bath for 2 h and then concentrated in vacuo. The crude product was purified by silica gel column chromatography (0−6.25% 2-propanol with 10% NH_4OH in $CHCl_3$) to give the title compound as a light yellow solid (0.223 g, 94%). MS (ESI positive ion): m/z calcd for $C_{31}H_{36}FN_7O_4S$ 621, found 622 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.46 (dd, J = 9.0, 2.1 Hz, 1H), 8.24 (d, J = 1.4 Hz, 1H), 7.23 (dd, J = 8.5, 6.9 Hz, 4H), 6.86 (t, J = 8.7 Hz, 4H), 4.81 (d, J = 2.0 Hz, 4H), 3.81 (d, J = 6.3 Hz, 6H), 3.60 (s, 2H), 3.23 (s, 4H), 2.74−2.80 (m, 3H), 2.56−2.61 (m, 4H), 2.55 (s, 3H).

4-(2-((6-Chloropyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (12). A 10 mL vial was charged with **8** (0.110 g, 0.17 mmol), 6-chloropyridin-3-amine (0.046 g, 0.36 mmol), and THF (1 mL). NaHMDS in THF (1.0 M, 0.61 mL, 0.61 mmol) was added dropwise at 0 °C, and the dark red solution was stirred at that temperature for 1 h. The reaction was quenched with saturated aqueous ammonium chloride (0.2 mL) and partitioned between water (20 mL) and EtOAc (20 mL). The aqueous phase was extracted with EtOAc (20 mL). The combined organic extracts were washed with brine (40 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (0−6.25% *i*-PrOH (with 10% NH_4OH) in $CHCl_3$) and then again by silica gel chromatography (50−100% EtOAc in hexanes) to afford the title compound (0.104 g, 81%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{36}H_{40}ClN_9O_4S$ 729, found 730 $(M + H)^+$.

4-(2-((6-Chloropyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (17). Trifluoromethanesulfonic acid (0.02 mL, 0.22 mmol) was added to a solution of **12** (0.103 g, 0.14 mmol) in TFA (1 mL). The reaction mixture was stirred at room temperature for 3.5 h. The solvent was partially evaporated, and solid sodium carbonate was added followed by saturated aqueous sodium bicarbonate. The resulting precipitate was collected by filtration, and the crude product was purified by preparative HPLC (Gilson using Phenomenex, Gemini C18, 100 × 30 mm, 5 μm, 1−90% CH_3CN with 0.1% TFA in H_2O with 0.1% TFA in 10 min). The fractions containing the desired compound were combined and concentrated under vacuum, and the residue was treated with saturated aqueous sodium bicarbonate. The resulting solid was collected by filtration, washed with water, and dried in a vacuum oven to yield the title compound (0.047 g, 68%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{20}H_{24}ClN_9O_2S$ 489, found 490 $(M + H)^+$. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H), 8.88 (d, J = 2.7 Hz, 1H), 8.75 (d, J = 2.1 Hz, 1H), 8.47 (dd, J = 8.7, 2.8 Hz, 1H), 8.30 (d, J = 2.1 Hz, 1H), 7.94 (br s, 1H), 7.79 (br s, 1H), 7.45 (d, J = 8.8 Hz, 1H), 3.52 (s, 2 H), 3.11 (br s, 4H), 2.86 (s, 3H), 2.45 (s, 3H). Note: The four hydrogens on the piperazine ring were under the DMSO signal and could not be assigned/integrated.

4-Methyl-6-(2-((6-methylpyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-1,3,5-triazin-2-amine (18). *N,N-Bis(4-methoxybenzyl)-4-methyl-6-(2-((6-methylpyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)-methyl)pyridin-3-yl)-1,3,5-triazin-2-amine (13).* LiHMDS in THF (1.0 M, 0.72 mL, 0.72 mmol) was added dropwise to a solution of **8** (0.149 g, 0.24 mmol) and 6-methylpyridin-3-amine (0.039 g, 0.36 mmol) in THF (5 mL) at 0 °C under an atmosphere of argon. The solution was allowed to stir at 0 °C for 2 h, and then the reaction was quenched by the addition of saturated aqueous ammonium chloride. The reaction mixture was extracted with EtOAc (50 mL), dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (1−15% MeOH in CH_2Cl_2) to give the title compound (0.138 g, 81%) as a yellow semisolid. MS (ESI positive ion): m/z calcd for $C_{37}H_{43}N_9O_4S$ 709, found 710 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.92 (s, 1H), 8.71 (d, J = 2.5 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 8.23 (d, J = 2.3 Hz, 1H), 8.07 (dd, J = 8.3, 2.6 Hz, 1H), 7.17−7.24 (m, 4H), 7.07 (d, J = 8.4 Hz, 1H), 6.86 (dd, J = 12.4, 8.7 Hz, 4H), 4.84 (d, J = 5.3 Hz, 4H),

3.81 (s, 3H), 3.79 (s, 3H), 3.50 (s, 2H), 3.18 (d, J = 4.3 Hz, 4H), 2.70 (s, 3H), 2.59 (s, 3H), 2.55 (t, J = 4.6 Hz, 4H), 2.51 (s, 3H).

*4-Methyl-6-(2-((6-methylpyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-1,3,5-triazin-2-amine (**18**).* **13** (0.138 g, 0.19 mmol) was treated with TFA (5 mL) and heated at 80 °C for 16 h. The excess TFA was removed under reduced pressure, and the crude residue was treated with ice and a saturated aqueous solution of sodium bicarbonate. The resulting yellow suspension was filtered through a medium-porosity sintered glass frit and washed with water. The yellow solid was washed with a small amount of *i*-PrOH, and the resulting yellow amorphous solid was purified by silica gel chromatography (0−3% MeOH in CH_2Cl_2), affording the title compound (0.051 g, 56%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{21}H_{27}N_9O_2S$ 469, found 470 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.83 (d, J = 2.7 Hz, 1H), 8.73 (d, J = 2.3 Hz, 1H), 8.22−8.28 (m, 2H), 7.91 (br s, 1H), 7.77 (br s, 1H), 7.20 (d, J = 8.6 Hz, 1H), 3.50 (s, 2H), 3.33 (br s, 4H), 3.11 (br s, 4H), 2.87 (s, 3H), 2.45 (s, 3H), 2.43 (s, 3H).

4-(2-((5-Fluoropyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (19). *4-(2-((5-Fluoropyridin-3-yl)amino)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**14**).* LiHMDS in THF (1.0 M, 0.45 mL, 0.45 mmol) was added dropwise to a solution of **8** (0.080 g, 0.13 mmol) and 3-amino-5-fluoropyridine (0.029 g, 0.26 mmol) in THF (5 mL) at 0 °C under an atmosphere of argon. The solution was allowed to stir at 0 °C for 20 min and quenched by the addition of saturated aqueous ammonium chloride. The mixture was extracted with EtOAc (2 × 15 mL), dried over magnesium sulfate, filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (25−50% CH_2Cl_2 in 4% MeOH/EtOAc) to give the title compound (0.072 g, 78%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{36}H_{40}FN_9O_4S$ 713, found 714 $(M + H)^+$.

*4-(2-((5-Fluoropyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**19**).* A microwave vial was charged with **14** (0.070 g, 0.10 mmol) and TFA (1.5 mL). The mixture was sealed under argon and heated in a microwave reactor at 120 °C for 30 min. The solvent was removed under vacuum, and the residue was purified by preparative HPLC (Phenomenex, Gemini C18, 100 × 30 mm, 10 μm, 10−40% CH_3CN/H_2O with 0.1% TFA). Product-containing fractions were concentrated and treated with saturated aqueous sodium bicarbonate and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3×). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo to give the title compound (0.027 g, 58%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{20}H_{24}FN_9O_2S$ 473, found 474 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.33 (s, 1H), 8.82 (s, 1H), 8.77 (s, 1H), 8.56 (d, J = 12.1 Hz, 1H), 8.35 (s, 1H), 8.17 (d, J = 2.0 Hz, 1H), 7.97 (br s, 1H), 7.81 (br s, 1H), 3.54 (s, 2H), 3.12 (br s, 4H), 2.87 (s, 3H), 2.46 (br s, 7H).

4-Methyl-6-(5-((4-(methylsulfonyl)piperazin-1-yl)methyl)-2-((6-(trifluoromethyl)pyridin-3-yl)amino)pyridin-3-yl)-1,3,5-triazin-2-amine (20). *2-(2-Fluoro-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-4-methyl-6-(methylthio)-1,3,5-triazine (**23**).* TFA (4 mL) was slowly added to a stirred solution of *tert*-butyl 4-((6-fluoro-5-(4-methyl-6-(methylthio)-1,3,5-triazin-2-yl)pyridin-3-yl)-methyl)piperazine-1-carboxylate (0.853 g, 1.96 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The mixture was concentrated, and the sticky residue obtained was dissolved in CH_2Cl_2 (10 mL) and cooled in an ice bath. Triethylamine (1.37 mL, 9.82 mmol) followed by methanesulfonyl chloride (0.46 mL, 5.89 mmol) was added to this solution. The reaction mixture was stirred at 0 °C for 1 h, and the solvent was removed under vacuum. The crude product was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2). The product was washed with *i*-PrOH to give the title compound (0.640 g, 79%) as a white solid. MS (ESI positive ion): m/z calcd for $C_{16}H_{21}FN_6O_2S_2$ 412, found 413 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 8.58 (dd, J = 9.3, 2.2 Hz, 1H), 8.37 (s, 1H), 3.67 (s, 2H), 3.01−3.19 (m, 4H), 2.87 (s, 3H), 2.61 (s, 3H), 2.60 (s, 3H), 2.47−2.49 (m, 4H).

*3-(4-Methyl-6-(methylthio)-1,3,5-triazin-2-yl)-5-((4-(methylsulfonyl)piperazin-1-yl)methyl)-N-(6-(trifluoromethyl)-pyridin-3-yl)pyridin-2-amine (**24**).* A stirred solution of **23** (0.109 g, 0.26 mmol) and 6-(trifluoromethyl)pyridin-3-amine (0.064 g, 0.39 mmol) in THF (1.5 mL) at 0 °C was treated with LiHMDS in THF (1.0 M, 0.79 mL, 0.79 mmol), and the mixture was stirred for 1 h. The reaction mixture was diluted with water (10 mL) and EtOAc (15 mL). The separated aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the title compound (0.098 g, 67%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{22}H_{25}F_3N_8O_2S_2$ 554, found 555 $(M + H)^+$.

*4-Methyl-6-(5-((4-(methylsulfonyl)piperazin-1-yl)methyl)-2-((6-(trifluoromethyl)pyridin-3-yl)amino)pyridin-3-yl)-1,3,5-triazin-2-amine (**20**).* **24** (0.087 g, 0.16 mmol) and 2 M NH_3 in *i*-PrOH (2 mL) were added to a 10 mL reaction vial. The tube was sealed and heated at 90 °C overnight. After cooling, the mixture was adsorbed onto silica gel, and the material was purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2) to give the title compound (0.033 g, 40%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{21}H_{24}F_3N_9O_2S$ 523, found 524 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.49 (s, 1H), 9.12 (d, J = 2.5 Hz, 1H), 8.79 (d, J = 2.1 Hz, 1H), 8.74 (dd, J = 8.7, 2.0 Hz, 1H), 8.36 (d, J = 2.1 Hz, 1H), 7.97 (br s, 1H), 7.84 (d, J = 8.6 Hz, 2H), 3.55 (s, 2H), 3.12 (br s, 4H), 2.87 (s, 3H), 2.48−2.50 (m, 4H), 2.47 (s, 3H).

4-(2-((5-Methoxypyridin-3-yl)amino)-5-((4-(methylsulfonyl)-piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (21). A stirred solution of **23** (0.200 g, 0.48 mmol) and 5-methoxypyridin-3-amine (0.090 g, 0.73 mmol) in DMF (5 mL) was treated with LiHMDS in THF (1.0 M, 1.94 mL, 1.94 mmol) at 0 °C, and the mixture was stirred for 1 h. The reaction mixture was diluted with water (10 mL) and EtOAc (15 mL). The separated aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the crude residue, which was heated with 2 M NH_3 in *i*-PrOH (3 mL) in a sealed tube at 90 °C overnight. After cooling, the mixture was adsorbed onto silica gel, and the material was purified by silica gel chromatography (0−5% MeOH in CH_2Cl_2) to give the title compound (0.044 g, 19%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{21}H_{27}N_9O_3S$ 485, found 486 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.13 (s, 1H), 8.74 (s, 1H), 8.55 (s, 1H), 8.31 (s, 1H), 8.19 (br s, 1H), 7.93 (br s, 2H), 7.78 (br s, 1H), 3.86 (s, 3H), 3.52 (s, 2H), 3.11 (br s, 4H), 2.87 (s, 3H), 2.46−2.50 (m, 4H), 2.46 (s, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amines 30 and 31. *5-(4-(Bis(4-methoxybenzyl)-amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)nicotinaldehyde (**27**).* 4-(5-(1,3-Dioxolan-2-yl)-2-fluoropyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**26**; 1.69 g, 3.26 mmol) and 5-fluoro-6-methoxypyridin-3-amine (0.477 g, 3.36 mmol) were dissolved in THF (28 mL), and the reaction flask was cooled in an ice−water bath. The solution was placed under a nitrogen atmosphere, and LiHMDS in THF (1.0 M, 9.5 mL, 9.5 mmol) was added. The reaction mixture was stirred for 35 min. Aqueous HCl (5 M, 4.5 mL) and MeOH (5.8 mL) were added, and the reaction was allowed to warm to room temperature and stirred. After 10 min, the reaction was treated with water (20 mL) and extracted with CH_2Cl_2. The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated in vacuo. Purification by silica gel chromatography (0−10% MeOH in CH_2Cl_2) gave the product. This material was dissolved in THF (20 mL) and MeOH (4 mL), and aqueous HCl (5 M, 2 mL) was added. The reaction mixture was stirred at room temperature for 25 min. The reaction was diluted with water (20 mL) and CH_2Cl_2 (20 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 (10 mL). The organic extracts were combined, dried over sodium sulfate, filtered, concentrated, and dried under high vacuum overnight to give the title compound (1.10 g, 57%). MS (ESI positive ion): m/z calcd for $C_{32}H_{30}FN_7O_4$ 595, found 596 $(M + H)^+$. ^1H NMR (400 MHz, $CDCl_3$): δ 12.58 (s, 1H), 9.92 (s, 1H), 9.25 (d, J = 2.3 Hz, 1H), 8.75

(d, J = 2.1 Hz, 1H), 8.03−7.97 (m, 2H), 7.21 (dd, J = 15.1, 8.4 Hz, 4H), 6.87 (dd, J = 11.1, 8.6 Hz, 4H), 4.89 (s, 2H), 4.85 (s, 2H), 4.04 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 2.61 (s, 3H).

*1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethanol (**28**).* **27** (1.054 g, 1.77 mmol) was suspended in THF (16 mL), and the reaction flask was cooled to 0 °C. A solution of methylmagnesium bromide in diethyl ether (3.0 M, 1.80 mL, 5.40 mmol) was added via syringe, and the reaction was stirred at 0 °C for 25 min. The reaction was treated with saturated aqueous ammonium chloride (3 mL, dropwise at first as gas evolution was observed) and water (20 mL). The reaction was diluted with EtOAc, and the layers were separated. The aqueous phase was extracted with EtOAc, and the organic extracts were combined, dried over sodium sulfate, filtered, concentrated, and purified by silica gel chromatography (1−5% MeOH in CH_2Cl_2) to give the title compound (0.894 g, 83%). MS (ESI positive ion): m/z calcd for $C_{33}H_{34}FN_7O_4$ 611, found 612 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.86 (s, 1H), 8.83 (d, J = 2.3 Hz, 1H), 8.32 (d, J = 2.3 Hz, 1H), 8.01 (dd, J = 12.1, 2.1 Hz, 1H), 7.96 (d, J = 2.3 Hz, 1H), 7.21 (dd, J = 10.8, 8.6 Hz, 4H), 6.87 (dd, J = 12.1, 8.8 Hz, 4H), 4.81−4.94 (m, 5H), 4.02 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 2.59 (s, 3H), 1.73 (d, J = 3.7 Hz, 1H), 1.53 (d, J = 6.5 Hz, 3H).

*1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethyl Methanesulfonate (**29**).* Triethylamine (0.90 mL, 6.5 mmol) and methanesulfonyl chloride (0.41 mL, 5.3 mmol) were added to a solution of **28** (0.867 g, 1.42 mmol) in CH_2Cl_2 (22 mL) at 0 °C under a nitrogen atmosphere. The reaction mixture was stirred at 0 °C for 20 min, diluted with CH_2Cl_2 (125 mL), and treated with water (25 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated in vacuo to give the title compound, which was used without further purification.

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine. The crude product from the previous step was dissolved in CH_2Cl_2 (15 mL) and treated with triethylamine (0.90 mL, 6.5 mmol) and 1-(methylsulfonyl)-piperazine (0.754 g, 4.59 mmol). The reaction mixture was stirred under nitrogen at room temperature overnight and treated with water (20 mL) and CH_2Cl_2 (10 mL). The layers were separated, and the aqueous phase was extracted with CH_2Cl_2. The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated, and the crude material was purified by silica gel chromatography (2% MeOH in CH_2Cl_2 and then a gradient of 3.5−10% 2 M NH_3/MeOH in CH_2Cl_2) to give the title compound (0.457 g, 42%). MS (ESI positive ion): m/z calcd for $C_{38}H_{44}FN_9O_5S$ 757, found 758 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.87 (s, 1H), 8.72 (d, J = 2.3 Hz, 1H), 8.25 (d, J = 2.3 Hz, 1H), 8.07 (d, J = 12.3, 2.1 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 7.22 (t, J = 8.5 Hz, 4H), 6.82−6.91 (m, 4H), 4.76−4.93 (m, 4H), 4.02 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.55 (q, J = 6.8 Hz, 1H), 3.16 (t, J = 4.6 Hz, 4H), 2.69 (s, 3H), 2.60 (s, 3H), 2.49−2.58 (m, 4H), 1.38 (d, J = 6.6 Hz, 3H).

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amines **30** and **31**.* 4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (0.457 g, 0.61 mmol) was dissolved in TFA (9.5 mL) and heated at 75 °C for 16 h. The reaction was allowed to cool to room temperature and concentrated under vacuum. The crude product was suspended in CH_2Cl_2 (40 mL) and treated with saturated aqueous sodium bicarbonate and NaOH (5 M) to raise the pH of the aqueous phase to ∼8. The layers were separated, and the aqueous phase was extracted with CH_2Cl_2 and CH_2Cl_2/MeOH (10:1). The organic extracts were combined, dried over sodium sulfate, filtered, and concentrated in vacuum. The crude product was purified by silica gel column chromatography (3−20% 2 M NH_3/MeOH in CH_2Cl_2) to give the racemic compound (0.195 g, 62%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{22}H_{28}FN_9O_3S$ 517, found 518 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.93 (s, 1H), 8.71 (d, J = 2.5 Hz, 1H), 8.31 (t, J = 2.2 Hz, 1H), 8.27 (d, J = 2.3 Hz, 1H), 8.05 (d, J = 2.1 Hz, 1H), 5.42 (br s, 2H), 4.04 (s, 3H), 3.56 (q, J = 6.6 Hz, 1H), 3.24 (t, J = 4.7 Hz, 4H), 2.78 (s, 3H), 2.54−2.68 (m, 7H), 1.44 (t, J = 6.8 Hz, 3H).

The racemic mixture of 4-(2-((5-fluoro-6-methoxypyridin-3-yl)-amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine was separated using chiral preparative SFC on a Chiralcel OJ-H column (250 × 20 mm) using 78:22 supercritical CO_2/MeOH (1% DEA). The two separate peaks were collected, concentrated, and dried under high vacuum to afford the two enantiomers (**30** and **31**). The absolute stereochemistries of the two enantiomers were assigned on the basis of the X-ray crystal structure of compound **31** cocrystallized with PI3Kγ (see Figure 3).

(*R*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**30**) was the second eluting peak and was isolated as a yellow powder. MS (ESI positive ion): m/z calcd for $C_{22}H_{28}FN_9O_3S$ 517, found 518 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.93 (s, 1H), 8.71 (d, J = 2.3 Hz, 1H), 8.26−8.32 (m, 2H), 8.05 (d, J = 2.1 Hz, 1H), 5.41 (br s, 2H), 4.04 (s, 3H), 3.56 (q, J = 6.4 Hz, 1H), 3.24 (t, J = 4.5 Hz, 4H), 2.78 (s, 3H), 2.53−2.68 (m, 4H), 2.59 (s, 3H), 1.44 (d, J = 6.6 Hz, 3H).

(*S*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**31**) was the first eluting peak and was isolated as a yellow powder. MS (ESI positive ion): m/z calcd for $C_{22}H_{28}FN_9O_3S$ 517, found 518 $(M + H)^+$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.93 (s, 1H), 8.71 (d, J = 2.3 Hz, 1H), 8.26−8.32 (m, 2H), 8.05 (d, J = 2.3 Hz, 1H), 5.40 (br s, 2H), 4.03 (s, 3H), 3.56 (q, J = 7.0 Hz, 1H), 3.24 (t, J = 4.9 Hz, 4H), 2.78 (s, 3H), 2.53−2.68 (m, 4H), 2.59 (s, 3H), 1.44 (d, J = 6.6 Hz, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amines 32 and 33. *1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)-2,2,2-trifluoroethanol.* To a stirred solution of 5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoronicotinaldehyde (3.78 g, 7.99 mmol) in THF (50 mL) was added trimethyl(trifluoromethyl)silane (1.70 g, 11.98 mmol) followed by CsF (0.243 g, 1.60 mmol) at 0 °C, and the mixture was stirred for 10 min before being allowed to warm to room temperature followed by stirring for 3.5 h. The reaction was quenched with 1 M HCl and stirred for another 30 min, and then EtOAc (10 mL) was added. The separated aqueous layer was extracted with EtOAc (2 × 20 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the title compound as a brown foam, which was used without further purification.

*1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-2,2,2-trifluoroethanol (**34**).* LiHMDS in THF (1 M, 42.7 mL, 42.7 mmol) was added dropwise to a stirred mixture of 5-fluoro-6-methoxypyridin-3-amine (2.43 g, 17.07 mmol) and 1-(5-(4-(bis(4-methoxybenzyl)-amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)-2,2,2-trifluoroethanol (4.64 g, 8.54 mmol) in THF (50 mL) at −10 °C. The mixture was stirred for 30 min. The reaction was quenched with water (25 mL) and saturated aqueous ammonium chloride (25 mL) and diluted with EtOAc (25 mL). The separated aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the crude product. Purification by silica gel column chromatography (0−50% EtOAc in hexanes) gave the title compound (5.14 g, 91%) as a brown foam.

*4-(5-(1-Chloro-2,2,2-trifluoroethyl)-2-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**35**).* Triethylamine (0.54 mL, 3.87 mmol) and methanesulfonyl chloride (0.24 mL, 3.09 mmol) were added to a solution of **34** (1.03 g, 1.55 mmol) in CH_2Cl_2 (20 mL) at 0 °C, and the mixture was stirred for 3 h. The reaction mixture was allowed to warm to room temperature and stirred for an additional 2 h. The reaction was quenched with saturated aqueous ammonium chloride and water. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL).

The combined organic extracts were dried over sodium sulfate, concentrated, and dried under vacuum to give the crude product, which was used without further purification. MS (ESI positive ion): *m/z* calcd for $C_{33}H_{30}ClF_4N_7O_3$ 683, found 684 $(M + H)^+$.

*tert-Butyl 4-(1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)-pyridin-3-yl)-2,2,2-trifluoroethyl)piperazine-1-carboxylate (**36**).* A solution of **35** (1.059 g, 1.55 mmol) in CH_3CN (10 mL) was treated with *tert*-butyl 1-piperazinecarboxylate (0.577 g, 3.10 mmol) and triethylamine (1.08 mL, 7.74 mmol), and the mixture was heated at 100 °C for 3 h. The reaction mixture was concentrated, and the crude product was purified by silica gel column chromatography (0−5% MeOH/2 M NH_3 in CH_2Cl_2) to give the title compound (1.24 g, 96%) as a brown foam. MS (ESI positive ion): *m/z* calcd for $C_{42}H_{47}F_4N_9O_5$ 833, found 834 $(M + H)^+$.

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**37**).* TFA (3 mL) was added to a solution of **36** (1.24 g, 1.49 mmol) in CH_2Cl_2 (5 mL), and the reaction mixture was stirred at room temperature for 1 h. The mixture was concentrated under vacuum to remove as much TFA as possible. The sticky residue was dissolved in a solution of CH_2Cl_2 (5 mL), and triethylamine (1.04 mL, 7.44 mmol) and methanesulfonyl chloride (0.35 mL, 4.46 mmol) were slowly added at 0 °C. The mixture was stirred for 1 h. The crude product was partitioned between 1 M NaOH (20 mL) and CH_2Cl_2 (20 mL). The separated aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the title compound (1.20 g, 99%) as a dark foam.

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amines **32** and **33**.* Trifluoromethanesulfonic acid (0.5 mL) was added to a solution of **37** (1.20, 1.48 mmol) in TFA (5 mL), and the reaction mixture was heated at 70 °C for 2 h. The reaction was allowed to cool to room temperature and concentrated under vacuum. The crude product was purified by silica gel column chromatography (0−5% 2 M NH_3/MeOH in CH_2Cl_2), followed by preparative TLC (EtOAc/CH_2Cl_2, 3:2) to give the racemic compound (0.841 g, 100%) as a yellow solid. MS (ESI positive ion): *m/z* calcd for $C_{22}H_{25}F_4N_9O_3S$ 571, found 572 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.84 (d, J = 2.1 Hz, 1H), 8.40 (d, J, = 2.3 Hz, 1H), 8.29−8.39 (m, 2H), 8.03 (br s, 1H), 7.88 (br s, 1H), 4.86 (q, J = 9.4 Hz, 1H), 3.95 (s, 3H), 3.13 (t, J = 4.6 Hz, 4H), 2.86 (s, 3H), 2.69−2.78 (m, 2H), 2.58−2.69 (m, 2H), 2.45 (s, 3H).

The racemic mixture of 4-(2-((5-fluoro-6-methoxypyridin-3-yl)-amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)-pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine was separated using chiral preparative SFC on a Chiralcel OJ-H column (250 × 20 mm) using 78:22 supercritical CO_2/MeOH (1% DEA). The two separate peaks were collected, concentrated, and dried under high vacuum to afford the two enantiomers (**32** and **33**). The absolute stereochemistries were assigned on the basis of eluting peak order and by analogy to compounds **30** and **31**.

(*R*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**32**) was the second eluting peak and was isolated as a yellow powder. MS (ESI positive ion): *m/z* calcd for $C_{22}H_{25}F_4N_9O_3S$ 571, found 572 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 12.01(s, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.40 (d, J = 2.3 Hz, 1H), 8.29−8.39 (m, 2H), 7.93 (br s, 1H), 7.79 (br s, 1H), 4.84 (q, J = 9.6 Hz, 1H), 3.94 (s, 3H), 3.12 (t, J = 4.7 Hz, 4H), 2.86 (s, 3H), 2.69−2.78 (m, 2H), 2.58−2.69 (m, 2H), 2.45 (s, 3H).

(*S*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2,2,2-trifluoro-1-(4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**33**) was the first eluting peak and was isolated as a yellow powder. MS (ESI positive ion): *m/z* calcd for $C_{22}H_{25}F_4N_9O_3S$ 571, found 572 $(M + H)^+$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.96 (s, 1H), 8.84 (d, J = 2.1 Hz, 1H), 8.40 (d, J = 2.3 Hz, 1H), 8.29−8.39 (m, 2H), 8.03 (br s, 1H), 7.88 (br s, 1H), 4.86 (q, J = 9.4 Hz, 1H), 3.95 (s, 3H), 3.13 (t, J = 4.6 Hz, 4H), 2.86 (s, 3H), 2.69−2.78 (m, 2H), 2.58−2.69 (m, 2H), 2.45 (s, 3H).

(*R*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (42). *Potassium (R)-((4-(tert-Butoxycarbonyl)-2-methylpiperazin-1-yl)methyl)trifluoroborate.* A mixture of potassium (bromomethyl)trifluoroborate (1.20 g, 5.38 mmol) and (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (1.12 g, 5.65 mmol) in THF (7 mL) was heated at 80 °C under nitrogen for 24 h. The mixture was allowed to cool to room temperature and concentrated under vacuum. The residue was dissolved in acetone (125 mL) and treated with potassium carbonate (1 equiv). The suspension was stirred at room temperature for 30 min and then filtered through a short plug of diatomaceous earth. The filter cake was washed with additional acetone, and the combined organic extracts were concentrated to give the crude product as a colorless foam (1.51 g, 88%). The pure material was obtained as a beige powder by adding acetone/diethyl ether (2:1) to the foam and then slowly adding hexanes while sonicating the mixture. ^{19}F NMR (377 MHz, acetone-d_6): δ −141.36.

*(R)-tert-Butyl 4-((5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)methyl)-3-methylpiperazine-1-carboxylate (**39**).* 4-(5-Chloro-2-fluoropyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine[14] (**38**; 1.00 g, 2.08 mmol), 2-(dicyclohexylphosphino)-2′,4′,6′,-triisopropyl-1,1′-biphenyl (0.100 g, 0.21 mmol), cesium carbonate (2.04 g, 6.25 mmol), potassium (*R*)-((4-(*tert*-butoxycarbonyl)-2-methylpiperazin-1-yl)-methyl)trifluoroborate (0.701 g, 2.19 mmol), and palladium(II) acetate (0.023 g, 0.104 mmol) were added to a 20 mL microwave tube, and the tube was purged with argon. The solids were then treated with THF (10 mL) and water (1 mL) and heated under microwave irradiation at 85 °C for 16 h. The mixture was filtered through a short plug of diatomaceous earth, washed with EtOAc (3×), and concentrated. The crude product was purified by silica gel chromatography (0−5% MeOH in CH_2Cl_2) to obtain the title compound (1.01 g, 74%) as a yellow foam. MS (ESI positive ion): *m/z* calcd for $C_{36}H_{44}FN_7O_4$ 657, found 658 $(M + H)^+$.

*(R)-4-(2-Fluoro-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)-methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**40**).* TFA (4 mL) was added to a cooled solution of **39** (1.01 g, 1.53 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated, and the sticky residue was dissolved in CH_2Cl_2 (10 mL), to which triethylamine (2.14 mL, 15.35 mmol) and methanesulfonyl chloride (0.60 mL, 7.68 mmol) were slowly added at 0 °C. The mixture was stirred at 0 °C for 1 h and concentrated, and the crude product was partitioned between 1 M NaOH and CH_2Cl_2 (20 mL each). The separated aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the crude residue. Purification by silica gel column chromatography (10−70% EtOAc in hexanes) gave the title compound (0.478 g, 49%) as a pale yellow foam. MS (ESI positive ion): *m/z* calcd for $C_{32}H_{38}FN_7O_4S$ 635, found 636 $(M + H)^+$.

*(R)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**41**).* LiHMDS in THF (1 M, 2.26 mL, 2.26 mmol) was added dropwise to a stirred mixture of 5-fluoro-6-methoxypyridin-3-amine (0.118 g, 0.83 mmol) and **40** (0.478 g, 0.75 mmol) in THF (10 mL) at −10 °C, and the mixture was stirred for 30 min. The reaction was quenched with water and saturated aqueous ammonium chloride (25 mL each) and diluted with EtOAc (25 mL). The separated aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated to give the crude product. Purification by silica gel column chromatography (0−1% MeOH in CH_2Cl_2) followed by washing with *i*-PrOH gave the title compound (0.377 g, 66%).

*(R)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**42**).* **41** (0.377 g, 0.48 mmol), TFA (2 mL),

and trifluoromethanesulfonic acid (0.22 mL) were added to a 25 mL round-bottomed flask. The reaction mixture was stirred at 70 °C for 1 h and allowed to cool to room temperature. The volatiles were removed under vacuum, the residual sticky oil was cooled to 0 °C, and ice (∼100 g) was added followed by 1 M NaOH until basic. The resulting yellow precipitate was filtered and purified by silica gel column chromatography (0−5% MeOH with 2 M NH_3 in CH_2Cl_2) to give the title compound (0.125 g, 48%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{22}H_{28}FN_9O_3S$ 517, found 518 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.71 (d, J = 2.3 Hz, 1H), 8.41 (d, J = 2.1 Hz, 1H), 8.33−8.40 (m, 1H), 8.27 (d, J = 2.1 Hz, 1H), 7.90 (br s, 1H), 7.76 (br s, 1H), 3.93 (s, 3H), 3.91 (s, 1H), 3.13−3.30 (m, 3H), 2.87−2.95 (m, 1H) 2.85 (s, 3H), 2.53−2.79 (m, 3H), 2.44 (s, 3H), 2.14−2.28 (m, 1H), 1.16 (d, J = 6.3 Hz, 3H).

(*S*)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (43). *Potassium (S)-((4-(tert-Butoxycarbonyl)-2-methylpiperazin-1-yl)methyl)trifluoroborate.* A mixture of potassium (bromomethyl)trifluoroborate (1.20 g, 5.38 mmol) and (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (1.13 g, 5.65 mmol) in THF (7 mL) was heated at 80 °C under nitrogen for 24 h. The mixture was allowed to cool to room temperature, and the solvent was removed under vacuum. The residue was redissolved in acetone (125 mL) and treated with potassium carbonate (1 equiv). The suspension was stirred at room temperature for 30 min and then filtered through a short plug of diatomaceous earth. The filter cake was washed with acetone, and the combined organic phases were concentrated to give the crude product as a colorless foam (1.51 g, 88%). The pure material was obtained as a beige powder by adding acetone/diethyl ether (2:1) to the foam and then slowly adding hexanes while sonicating the mixture. ^{19}F NMR (377 MHz, acetone-d_6): δ −141.28.

4-(5-Chloro-2-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (44). A stirred mixture of **38**[14] (2.53 g, 5.28 mmol) and 5-fluoro-6-methoxypyridin-3-amine (0.834 g, 5.87 mmol) in THF (50 mL) was treated dropwise with LiHMDS in THF (1 M, 16.74 mL, 16.74 mmol) at −15 °C and stirred for 40 min. The reaction was quenched with water and saturated ammonium chloride (25 mL each) and diluted with EtOAc (25 mL). The separated aqueous layer was extracted with EtOAc (2 × 50 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel chromatography (0−1% MeOH in CH_2Cl_2) followed by washing with *i*-PrOH to give the title compound (2.07 g, 88%). MS (ESI positive ion): m/z calcd for $C_{31}H_{29}ClFN_7O_3$ 601, found 602 (M + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.77 (d, J = 2.5 Hz, 1H), 8.23 (d, J = 2.5 Hz, 1H), 7.95 (d, J = 2.0 Hz, 1H), 7.90 (d, J = 12.1 Hz, 1H), 7.18 (dd, J = 17.4, 8.4 Hz, 4H), 6.86 (t, J = 8.1 Hz, 4H), 4.86 (s, 2H), 4.82 (s, 2H), 4.01 (s, 3H); 3.81 (s, 3H), 3.79 (s, 3H), 2.60 (s, 3H).

(S)-tert-Butyl 4-((5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)-pyridin-3-yl)methyl)-3-methylpiperazine-1-carboxylate (45). **44** (0.197 g, 0.33 mmol), 2-(dicyclohexylphosphino)-2′,4′,6′,-triisopropyl-1,1′-biphenyl (0.016 g, 0.03 mmol), cesium carbonate (0.32 g, 0.98 mmol), potassium (*S*)-((4-(*tert*-butoxycarbonyl)-2-methylpiperazin-1-yl)methyl)trifluoroborate (0.126 g, 0.39 mmol), and palladium acetate (4 mg, 0.02 mmol) were added to a 10 mL reaction vial. The vial was sealed and purged with argon and then treated with THF (1 mL) and water (0.1 mL). The mixture was heated in a microwave reactor at 140 °C for 30 min. After cooling, the mixture was filtered through a short plug of diatomaceous earth, washed with EtOAc (3×), and concentrated in vacuo. The crude product was purified by silica gel column chromatography (0−5% MeOH in CH_2Cl_2) to give the title compound (0.230 g, 90%) as a yellow syrup. MS (ESI positive ion): m/z calcd for $C_{42}H_{50}FN_9O_5$ 779, found 780 $(M + H)^+$.

(S)-tert-Butyl 4-((5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)-pyridin-3-yl)methyl)-3-methylpiperazine-1-carboxylate (46). **45** (0.230 g, 0.29 mmol), CH_2Cl_2 (3 mL), and TFA (3 mL) were added to a 25 mL round-bottomed flask. The reaction mixture was stirred at room temperature for 30 min. The solvent was removed under vacuum, and the brown oil obtained was redissolved in CH_2Cl_2 (5 mL). The mixture was cooled to −15 °C, and triethylamine (0.42 mL, 2.94 mmol) and methanesulfonyl chloride (0.14 mL, 1.77 mmol) were added sequentially. The reaction mixture was stirred at −15 °C for 1 h. The reaction was quenched with water and saturated aqueous sodium bicarbonate (10 mL each) and diluted with EtOAc (10 mL). The separated aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated. The crude product was used without further purification.

(S)-4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((2-methyl-4-(methylsulfonyl)piperazin-1-yl)methyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (43). **46** (0.219 g, 0.29 mmol), TFA (2 mL), and trifluoromethanesulfonic acid (0.1 mL) were added to a 25 mL round-bottomed flask. The reaction mixture was stirred at 80 °C for 16 h and allowed to cool to room temperature. The volatiles were removed under vacuum, and the crude product was purified by silica gel column chromatography [0 −5% MeOH (with NH_3 in CH_2Cl_2)] to give the title compound (0.098 g, 53%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{22}H_{28}FN_9O_3S$ 517, found 518 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6, mixture of rotamers): δ 12.06 (d, J = 5.1 Hz, 1H), 9.44−9.97 (m, 1H), 8.76−9.09 (m, 1H), 8.21−8.54 (m, 2H), 7.97 (br s, 1H), 7.87 (br s, 1H), 4.08−4.96 (m, 2H), 3.95 (s, 3H), 3.61−3.87 (m, 2H), 2.79−3.36 (m, 8H), 2.45 (s, 3H), 1.34−1.65 (m, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((*R*)-1-((*R*)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (56). *1-(6-Fluoropyridin-3-yl)-ethanol (48).* A clear solution (prepared by filtration of a slightly cloudy suspension) of 6-fluoronicotinaldehyde (**47**; 9.88 g, 79 mmol) in THF (100 mL) was added dropwise via an addition funnel to a solution of methylmagnesium bromide in diethyl ether (3 M, 31.6 mL, 95 mmol) in THF (280 mL) at −6 °C. The addition was completed over about 20 min, and the reaction temperature was kept below −5 °C during the addition. MeOH (10 mL) was added dropwise, followed by saturated aqueous ammonium chloride (300 mL) and a sufficient amount of water to dissolve the precipitate. EtOAc (200 mL) was then added, and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 150 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuo to provide the title compound (10.55 g, 95%) as a light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, J = 2.0 Hz, 1H), 7.85 (td, J = 8.1, 2.5 Hz, 1H), 6.92 (dd, J = 8.5, 2.8 Hz, 1H), 4.98 (q, J = 6.5 Hz, 1H), 2.21 (br s, 1H), 1.53 (d, J = 6.5 Hz, 3H).

5-(1-Bromoethyl)-2-fluoropyridine (49). Thionyl bromide (11.60 mL, 149 mmol) was added dropwise over a 15 min period to a solution of **48** (10.55 g, 74.7 mmol) in CH_2Cl_2 (300 mL) at 25 °C, and the resulting orange solution was stirred at 25 °C for 3 h. The excess of thionyl bromide was carefully quenched with water (150 mL) at 0 °C with vigorous stirring, and NaOH (5 M, 100 mL) was then carefully added at 0 °C (over ∼10 min). The pH was adjusted to ∼9 by the addition of saturated aqueous sodium bicarbonate. The resulting mixture was vigorously stirred at 0 °C for 5 min and partitioned between CH_2Cl_2 (200 mL) and aqueous sodium bicarbonate (300 mL of saturated sodium bicarbonate and 300 mL of water). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (300 mL). The combined organic extracts were sequentially washed with saturated aqueous sodium bicarbonate (500 mL) and brine (500 mL), dried over sodium sulfate, filtered, and concentrated in vacuo to provide the title compound (14.58 g, 96%) as a yellow oil. This material was used without further purification.

(3R)-tert-Butyl 4-(1-(6-Fluoropyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (50). **49** (3.90 g, 19.11 mmol), CH_3CN (75 mL), (*R*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (4.02 g, 20.07 mmol), potassium carbonate (3.16 g, 22.94 mmol), and potassium iodide (0.634 g, 3.82 mmol) were added to a 100 mL round-bottomed flask. The reaction mixture was stirred at 70 °C for 16 h. The reaction mixture was diluted with water (50 mL) and CH_2Cl_2 (50 mL), and the organic layer was separated. The separated aqueous layer was extracted

with CH_2Cl_2 (3 × 50 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated. The residue was purified by silica gel column chromatography (0−50% EtOAc in hexanes) to provide the title compound (3.05 g, 49%).

*(5-(1-((R)-4-(tert-Butoxycarbonyl)-2-methylpiperazin-1-yl)ethyl)-2-fluoropyridin-3-yl)boronic Acid (**51**).* **50** (3.0 g, 9.28 mmol) and THF (100 mL) were added to a 150 mL round-bottomed flask. The reaction mixture was cooled to −78 °C and treated dropwise with *n*-butyllithium in hexanes (2.5 M, 4.08 mL, 10.20 mmol). The solution was stirred at −78 °C for 1 h and treated in one portion with triisopropyl borate (2.55 mL, 11.13 mmol). The mixture was stirred at −78 °C for 1 h, 1 M NaOH (30 mL) was added, and the solution was stirred at room temperature for 30 min. The aqueous layer was separated, and the organic layer was extracted with additional 1 M NaOH (30 mL). The combined aqueous layers were carefully acidified with 5 M HCl (12 mL) to a final pH of ∼4−5. The reaction mixture was extracted with EtOAc (3 × 100 mL), and the combined organic extracts were dried over sodium sulfate, filtered, and concentrated under vacuum to give the title compound (2.5 g, 73%). This material was used without further purification.

*(3R)-tert-Butyl 4-(1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-fluoropyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (**52**).* A glass microwave reaction vessel was charged with **51** (1.40 g, 3.81 mmol), 4-chloro-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (1.46 g, 3.81 mmol), 1,4-dioxane (10 mL), potassium acetate (1.12 g, 11.44 mmol), water (1 mL), and Pd(A-Phos)Cl_2 (0.27 g, 0.38 mmol). The solution was stirred and heated in a microwave reactor at 120 °C for 20 min. The reaction mixture was diluted with water (20 mL) and CH_2Cl_2 (25 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were dried over sodium sulfate, filtered, and concentrated in vacuum. The crude product was purified by silica gel chromatography (0−50% EtOAc in hexanes) to provide the title compound (1.26 g, 49%).

*(3R)-tert-Butyl 4-(1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)-pyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (**53**).* **52** (1.0 g, 1.49 mmol), 5-fluoro-6-methoxypyridin-3-amine (0.317 g, 2.23 mmol), and THF (20 mL) were added to a 250 mL round-bottomed flask. The solution was cooled to −40 °C and treated dropwise via an addition funnel with LiHMDS in THF (1 M, 4.47 mL, 4.47 mmol). The solution was stirred at −40 °C for 30 min, quenched with water (20 mL) and saturated aqueous ammonium chloride (20 mL), and diluted with EtOAc (20 mL). The organic layer was separated, and the aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with brine (20 mL), dried over sodium sulfate, and concentrated. The crude product was purified by silica gel chromatography (40 g, 5−50% EtOAc in hexanes) to provide (*R*)-*tert*-butyl 4-((*R*)-1-(5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-ethyl)-3-methylpiperazine-1-carboxylate (0.45 g, 38%) and (*R*)-*tert*-butyl 4-((*S*)-1-(5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-ethyl)-3-methylpiperazine-1-carboxylate (0.35 g, 30%).

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((R)-1-((R)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-N,N-bis-(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**54**).* (*R*)-*tert*-Butyl 4-((*R*)-1-(5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-ethyl)-3-methylpiperazine-1-carboxylate (0.200 g, 0.25 mmol), CH_2Cl_2 (5 mL), and TFA (5 mL) were added to a 100 mL round-bottomed flask. The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (10 mL) and slowly washed with a saturated aqueous sodium bicarbonate solution. The organic phase was separated, dried over sodium sulfate, filtered, concentrated, and dried in a vacuum oven. The residue was dissolved in CH_2Cl_2 (5 mL) and cooled to −50 °C. To the reaction mixture were added triethylamine (0.35 mL, 2.52 mmol) and methanesulfonyl chloride (0.10 mL, 1.26 mmol). The solution was stirred at −50 °C for 30 min, and the solvent was removed under vacuum. The crude product was washed with water (30 mL), stirred, and sonicated. The resulting solid was collected, washed with water, and dried in a vacuum oven to give the title compound (0.150 g, 77%).

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((R)-1-((R)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**56**).* **54** (0.130 g, 0.168 mmol), TFA (5 mL), and trifluoromethanesulfonic acid (0.2 mL) were added to a 50 mL round-bottomed flask. The solution was stirred at 70 °C for 30 min. The volatiles were removed under vacuum, and the residue was cooled in an ice bath and neutralized with 1 M NaOH. The mixture was stirred at room temperature for 16 h, and the precipitate obtained was filtered and washed with water. Purification by preparative TLC using 5% MeOH in CH_2Cl_2 gave the title compound (0.047 g, 52%). MS (ESI positive ion): *m*/*z* calcd for $C_{23}H_{30}FN_9O_3S$ 531, found 532 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.93 (s, 1H) 8.70−8.74 (m, 1H), 8.37−8.43 (m, 2H), 8.31−8.34 (m, 1H), 7.88−7.92 (m, 1H), 7.74−7.78 (m, 1H), 4.00 (d, *J* = 6.8 Hz, 1H), 3.94 (s, 3H), 3.01−3.18 (m, 3H), 2.84 (s, 5H), 2.45−2.55 (m, 5H), 1.38 (d, *J* = 6.6 Hz, 3H), 1.07 (d, *J* = 6.3 Hz, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((*S*)-1-((*R*)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (57). *4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((S)-1-((R)-2-methyl-4-(methylsulfonyl)-piperazin-1-yl)ethyl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**55**).* (*R*)-*tert*-Butyl 4-((*S*)-1-(5-(4-(bis-(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (0.150 g, 0.19 mmol), CH_2Cl_2 (5 mL), and TFA (5 mL) were added to a 100 mL round-bottomed flask. The reaction mixture was stirred at room temperature for 1 h, and the solvent was removed under vacuum. The residue was dissolved in CH_2Cl_2 (10 mL) and slowly washed with a saturated aqueous solution of sodium bicarbonate. The organic phase was separated, dried over sodium sulfate, filtered, and concentrated. The residue was dried in a vacuum oven, dissolved in CH_2Cl_2 (5 mL), and cooled to −50 °C. Triethylamine (0.27 mL, 0.19 mmol) and methanesulfonyl chloride (0.08 mL, 0.19 mmol) were added, the solution was stirred at −50 °C for 30 min, and the solvent was removed under vacuum. The crude product was washed with water (30 mL), stirred, and sonicated. The resulting solid was collected, washed with water, and dried in a vacuum oven to give the title compound (0.100 g, 69%), which was used without further purification.

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((S)-1-((R)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**57**).* **55** (0.095 g, 0.12 mmol), TFA (5 mL), and trifluoromethanesulfonic acid (1 mL) were added to a 50 mL round-bottomed flask. The solution was stirred at 70 °C for 30 min. The volatiles were removed under vacuum, and the residue was cooled in an ice bath and neutralized with 1 M NaOH. The mixture was stirred at room temperature for 16 h, and the resulting precipitate was filtered and washed with water. Purification by preparative TLC using 5% MeOH in CH_2Cl_2 gave the title compound (0.031 g, 47%). MS (ESI positive ion): *m*/*z* calcd for $C_{23}H_{30}FN_9O_3S$ 531, found 532 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.95 (br s, 1H), 8.75 (br s, 1H), 8.32−8.49 (m, 3H), 7.90 (br s, 1H), 7.76 (br s, 1H), 4.05 (d, *J* = 5.7 Hz, 1H), 3.94 (s, 3H), 3.25 (br s, 2H), 3.04 (br s, 2H), 2.85 (br s, 5H), 2.45 (s, 4H), 2.32 (d, *J* = 7.2 Hz, 1H), 1.38 (d, *J* = 6.6 Hz, 3H), 1.16 (d, *J* = 5.7 Hz, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((*R*)-1-((*S*)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (58). *1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethanone (**60**).* A stirred mixture of **38** (0.602 g, 1.00 mmol), X-Phos-Pd$(OAc)_2$ remilled mix (1:1, 0.070 g, 0.10 mmol), and cesium fluoride (0.532 g, 3.50 mmol) in 1,4-dioxane (5 mL) was treated with tributyl(1-ethoxyvinyl)-stannane (0.507 mL, 1.50 mmol) under nitrogen. The mixture was sealed and heated at 110 °C for 16 h, allowed to cool to room temperature, and filtered through a short plug of diatomaceous earth. The filter cake was washed with EtOAc (3 × 20 mL) and

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concentrated. The crude product was purified by silica gel chromatography (0−5% MeOH in CH_2Cl_2) to give a mixture of the desired product and a small amount of dechlorinated product. This mixture was subjected to flash column chromatographic purification (30% EtOAc in hexanes) to give the title compound (0.482 g, 79%) as a light yellow solid. MS (ESI positive ion): m/z calcd for $C_{33}H_{32}FN_7O_4$ 609, found 610 $(M + H)^+$.

*1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethanol (**61**).* A stirred solution of **60** (0.135 g, 0.22 mmol) in THF (5 mL) was treated with sodium borohydride (0.042 g, 1.11 mmol) at 0 °C, and the mixture was stirred for 10 min before being allowed to warm to room temperature for 1 h. The suspension was quenched with 1 M NaOH, and the separated aqueous layer was extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give the title compound, which was used directly in the next step without further purification.

*1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethyl methanesulfonate (**62**).* A solution of **61** (0.436 g, 0.71 mmol) in CH_2Cl_2 (10 mL) was treated with triethylamine (0.30 mL, 2.14 mmol) and methanesulfonyl chloride (0.14 mL, 1.78 mmol). The mixture was stirred for 2 h and then quenched with water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), and the combined organic extracts were washed with 1 M HCl, saturated aqueous sodium bicarbonate, and brine and dried over sodium sulfate. Removal of the solvent under vacuum gave the title compound as a yellow residue, which was used directly in the next step without further purification.

*(3S)-tert-Butyl 4-(1-(5-(4-(Bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)-pyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (**63**).* A stirred solution of crude **62** (0.492 g, 0.71 mmol) in CH_3CN (10 mL) was treated with 2,2,6,6-tetramethylpiperidine (0.18 mL, 1.07 mmol) and (*S*)-*tert*-butyl 3-methylpiperazine-1-carboxylate (0.186 g, 0.93 mmol). The mixture was heated at reflux overnight. The reaction mixture was allowed to cool to room temperature, and the resulting suspension was diluted with water and CH_2Cl_2 (20 mL each). The separated aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL), and the combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by silica gel column chromatography (0−15% EtOAc in CH_2Cl_2 followed by 5% MeOH in CH_2Cl_2) to give the two separated diasteromers (*S*)-*tert*-butyl 4-((*S*)-1-(5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-ethyl)-3-methylpiperazine-1-carboxylate (**65**) (first eluting diastereomer; 0.101 g, 18%) followed by (*S*)-*tert*-butyl 4-((*R*)-1-(5-(4-(bis(4-methoxybenzyl)amino)-6-methyl-1,3,5-triazin-2-yl)-6-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)ethyl)-3-methylpiperazine-1-carboxylate (**64**) (second eluting diastereomer; 0.101 g, 18%). The absolute stereochemistries at the diastereomeric centers of the two diastereomers were assigned by the cocrystal structure of compound **58** (derived from the second eluting distereomer) in complex with PI3Kγ at 2.9 Å resolution.

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((R)-1-((S)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**58**).* A solution of **64** (0.101 g, 0.13 mmol) in CH_2Cl_2 (3 mL) was treated with TFA (3 mL), and the mixture was stirred at room temperature for 30 min. The solution was concentrated under vacuum to give a brown oil. This residue was redissolved in CH_2Cl_2 (3 mL) and treated with triethylamine (0.09 mL, 0.64 mmol) followed by the slow addition of methanesulfonyl chloride (0.03 mL, 0.38 mmol) at −15 °C. The reaction mixture was stirred for 1 h and then quenched with saturated aqueous sodium bicarbonate and water. The separated aqueous layer was extracted with CH_2Cl_2 (2×), and the combined organic extracts were dried over sodium sulfate and concentrated. The crude product was treated with TFA (2 mL) and heated at 80 °C overnight. The resulting mixture was allowed to cool to room temperature and concentrated, and the resulting residue was purified by silica gel column chromatography (0−5% MeOH in CH_2Cl_2) to give the title compound (0.029 g, 43%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{23}H_{30}FN_9O_3S$ 531, found 532 $(M + H)$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.93 (s, 1H), 8.72 (d, J = 2.4 Hz, 1H), 8.35−8.45 (m, 3H), 7.89 (br s, 1H), 7.76 (br s, 1H), 3.96−4.08 (m, 1H), 3.93 (s, 3H), 3.07−3.15 (m, 2H), 3.05 (dd, J = 10.9, 2.8 Hz, 1H), 2.73−2.90 (m, 5H), 2.51−2.55 (m, 2H), 2.44 (s, 3H), 1.37 (d, J = 6.8 Hz, 3H), 1.07 (d, J = 6.1 Hz, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-((*S*)-1-((*S*)-2-methyl-4-(methylsulfonyl)piperazin-1-yl)ethyl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (59). A solution of **65** (0.101 g, 0.13 mmol) in CH_2Cl_2 (3 mL) was treated with TFA (3 mL), and the mixture was stirred at room temperature for 30 min before being concentrated to give a brown oil. This was redissolved in CH_2Cl_2 (3 mL) and treated with triethylamine (0.09 mL, 0.64 mmol) followed by slow addition of methanesulfonyl chloride (0.03 mL, 0.38 mmol) at −15 °C. The mixture was stirred for 1 h and then quenched with saturated aqueous sodium bicarbonate and water. The separated aqueous layer was extracted with CH_2Cl_2 (2×), and the combined organic extracts were dried over sodium sulfate and concentrated. The crude product was treated with TFA (2 mL) and heated at 80 °C overnight. The resulting mixture was allowed to cool to room temperature and concentrated, and the crude product was purified by silica gel chromatography (0−5% MeOH in CH_2Cl_2) to give the title compound (0.029 g, 43%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{23}H_{30}FN_9O_3S$ 531, found 532 $(M + H)$. ^1H NMR (400 MHz, DMSO-d_6): δ 11.95 (s, 1H), 8.74 (d, J = 2.5 Hz, 1H), 8.27−8.46 (m, 2H), 7.90 (br s, 1H), 7.77 (br s, 1H), 3.99−4.09 (m, 1H), 3.93 (s, 3H), 3.20−3.30 (m, 1H), 2.94−3.11 (m, 2H), 2.84 (s, 3H), 2.75−2.84 (m, 2H), 2.45 (s, 3H), 2.40 −2.45(m, 1H), 2.29−2.35 (m, 1H), 1.28 (d, J = 6.6 Hz, 3H), 1.15 (d, J = 6.3 Hz, 3H).

4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2-(4-(methylsulfonyl)piperazin-1-yl)propan-2-yl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (69). *4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(prop-1-en-2-yl)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**66**).* A mixture of isopropenylboronic acid pinacol ester (0.214 mL, 1.14 mmol), potassium carbonate (0.316 g, 2.28 mmol), bis(4-(di-*tert*-butylphosphino)-*N*,*N*-dimethylbenzenamine)palladium dichloride (0.040 g, 0.057 mmol), and **44** (0.344 g, 0.571 mmol) in 4:1 1,4-dioxane/water (3.75 mL) was sealed in a vial and heated at 80 °C for 24 h. The reaction mixture was allowed to cool to room temperature and partitioned between water and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The material was purified by silica gel chromatography (0−30% EtOAc/hexane) to afford the title compound (0.282 g, 81%) as an orange solid. MS (ESI positive ion): m/z calcd for $C_{34}H_{34}FN_7O_3$ 607, found 608 $(M + H)$. ^1H NMR (400 MHz, $CDCl_3$): δ 11.92 (br s, 1H), 8.94 (d, J = 2.5 Hz, 1H), 8.46 (d, J = 2.7 Hz, 1H), 8.05 (dt, J = 12.4, 1.1 Hz, 1H), 7.97 (d, J = 2.3 Hz, 1H), 7.18−7.26 (m, 4H), 6.82−6.91 (m, 4H), 5.34 (s, 1H), 5.02 (t, J = 1.5 Hz, 1H), 4.89 (s, 2H), 4.83 (s, 2H), 4.02 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 2.60 (s, 3H), 2.14 (s, 3H).

*4-(5-(2-Azidopropan-2-yl)-2-((5-fluoro-6-methoxypyridin-3-yl)-amino)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**67**).* A slurry of sodium azide (0.151 g, 2.32 mmol) and **66** (0.282 g, 0.464 mmol) in $CHCl_3$ (1 mL) at −15 °C (MeOH/ice) was treated with a solution of TFA (0.36 mL, 4.64 mmol) in $CHCl_3$ (1 mL) via a syringe over 10 min. The reaction was sealed in a vial and heated at 60 °C. After 2 h, the temperature was increased to 80 °C. After 2 h, an additional 2.5 equiv of sodium azide and 5 equiv of TFA were added. The reaction was sealed and heated at 80 °C for 20 min. The reaction was allowed to cool to room temperature and then placed in a freezer over the weekend. The reaction was allowed to warm to ambient temperature and then heated at 80 °C for 1 h. The reaction mixture was treated with ice and 10 N NaOH until basic, and the solution was partitioned between water and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3×), and the combined organic extracts were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo to give the title compound (0.280 g, 93%) as an orange oil. MS (ESI positive ion): m/z calcd for $C_{34}H_{35}FN_{10}O_3$ 650, found 651 $(M + H)$. ^1H NMR (400 MHz, $CDCl_3$): δ 11.89 (br s, 1H), 8.88−8.93 (m, 1H), 8.38−8.43 (m, 1H), 8.03 (dt, J = 12.1, 1.2 Hz,

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1H), 7.90−7.98 (m, 1H), 7.23 (t, J = 8.6 Hz, 4H), 6.82−6.92 (m, 4H), 4.91 (s, 2H), 4.82 (s, 2H), 4.02 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 2.60 (s, 3H), 1.64 (s, 6H).

*4-(5-(2-Aminopropan-2-yl)-2-((5-fluoro-6-methoxypyridin-3-yl)amino)pyridin-3-yl)-N,N-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (**68**).* A mixture of **67** (0.280 g, 0.430 mmol) and palladium on 10% carbon with 50% water (0.366 g) was flushed with nitrogen and treated with THF (7.5 mL). The atmosphere was replaced with hydrogen from a balloon. The reaction was stirred rapidly for 1.5 h and flushed with nitrogen. The solution was filtered through diatomaceous earth, which was then rinsed with CH_2Cl_2/MeOH. The filtrate was concentrated in vacuo to give the title compound (0.272 g, 100%), which was used without further purification. MS (ESI positive ion): m/z calcd for $C_{34}H_{37}FN_8O_3$ 624, found 625 (M + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.79 (s, 1H), 8.94 (d, J = 2.7 Hz, 1H), 8.51 (d, J = 2.7 Hz, 1H), 8.00−8.10 (m, 1H), 7.93 (d, J = 2.3 Hz, 1H), 7.13−7.25 (m, 4H), 6.80−6.93 (m, 4H), 4.89 (s, 3H), 4.82 (s, 2H), 4.00 (s, 3H), 3.81 (s, 3H), 3.77 (s, 3H), 2.58 (s, 2H), 1.55 (s, 6H).

*4-(2-((5-Fluoro-6-methoxypyridin-3-yl)amino)-5-(2-(4-(methylsulfonyl)piperazin-1-yl)propan-2-yl)pyridin-3-yl)-6-methyl-1,3,5-triazin-2-amine (**69**).* A mixture of **68** (0.272 g, 0.435 mmol) and ((methylsulfonyl)azanediyl)bis(ethane-1,2-diyl) dimethanesulfonate (0.222 g, 0.653 mmol) in DIPEA (3 mL) was added to a 50 mL round-bottomed flask. The flask was fitted with a water-cooled reflux condenser and placed in a 130 °C oil bath. After 2.5 h, the reaction mixture was allowed to cool to room temperature and partitioned between saturated aqueous sodium bicarbonate and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The crude material was purified by silica gel chromatography (0−100% EtOAc/hexane) to give 4-(2-((5-fluoro-6-methoxypyridin-3-yl)amino)-5-(2-(4-(methylsulfonyl)piperazin-1-yl)-propan-2-yl)pyridin-3-yl)-*N*,*N*-bis(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (0.180 g, 54%) as an orange oil. MS (ESI positive ion): m/z calcd for $C_{39}H_{46}FN_9O_5S$ 771, found 772 (M + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.87 (br s, 1H), 8.91 (dd, J = 2.2, 0.4 Hz, 1H), 8.47 (dd, J = 2.3, 0.4 Hz, 1H), 8.11 (dt, J = 12.5, 1.2 Hz, 1H), 7.91−8.01 (m, 1H), 7.18−7.25 (m, 4H), 6.77−6.94 (m, 4H), 4.77−4.89 (m, 4H), 4.02 (s, 3H), 3.82 (s, 3H), 3.79 (s, 3H), 3.10−3.16 (m, 4H), 2.56−2.65 (m, 10H), 1.37 (s, 6H).

A solution of 4-(2-((5-fluoro-6-methoxypyridin-3-yl)amino)-5-(2-(4-(methylsulfonyl)piperazin-1-yl)propan-2-yl)pyridin-3-yl)-*N*,*N*-bis-(4-methoxybenzyl)-6-methyl-1,3,5-triazin-2-amine (0.180 g, 0.233 mmol) in TFA (2 mL) was treated with trifluoromethanesulfonic acid (0.104 mL, 1.17 mmol). The reaction was sealed and heated to 80 °C for 30 min. The reaction was cooled with ice and treated with ice and 10 N NaOH until basic. The solution was partitioned between water and CH_2Cl_2. The aqueous layer was extracted with CH_2Cl_2 (3×), and the combined organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The material was treated with CH_2Cl_2 and purified by silica gel chromatography (0−10% MeOH in CH_2Cl_2). The product-containing fractions were concentrated, and the residue was sonicated in diethyl ether (2 mL), filtered, and rinsed with diethyl ether (1 mL). The resulting solid was dried in vacuo to give the title compound (0.067 g, 54%) as a yellow solid. MS (ESI positive ion): m/z calcd for $C_{23}H_{30}FN_9O_3S$ 531, found 532 (M + H). ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.91 (s, 1H), 8.86 (d, J = 2.5 Hz, 1H), 8.51 (d, J = 2.5 Hz, 1H), 8.37−8.45 (m, 2H), 7.88 (br s, 1H), 7.74 (br s, 1H), 3.93 (s, 3H), 3.09 (m, 4H), 2.86 (s, 3H), 2.51−2.58 (m, 4H), 2.44 (s, 3H), 1.37 (s, 6H).

■ ASSOCIATED CONTENT

Supporting Information

Biological assays, in vivo study protocols, selectivity data, MetaSite metabolite predictions for compound **1**, standard deviation information, X-ray experimental procedures, and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

The cocrystal structure of PI3Kγ + compound **31** has been deposited in the Protein Data Bank with PDB code 4FLH.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

Ac, acetyl; AKT, serine−threonine kinase Akt; A-Phos, di-*tert*-butyl(4-(dimethylamino)phenyl)phosphine; DIPEA, diisopropylethylamine; DEA, diethylamine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethyl sulfoxide; EC, effective concentration; ED, effective dose; HGF, hepatocyte growth factor; HLM, human liver microsome; HPβCD, (hydroxypropyl)-β-cyclodextrin; HPMC, hydroxypropyl methylcellulose; iv, intravenous; LiHMDS, lithium hexamethyldisilazide; mTOR, mammalian target of rapamycin; MG, malignant glioma; MSA, methanesulfonic acid; NADPH, nicotinamide adenine dinucleotide phosphate; PD, pharmacodynamic; PIP2, phosphatidylinositol-4,5-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; PI3K, phosphoinositide 3-kinase; PK, pharmacokinetic; po, per os (orally); PTEN, phosphatase and tensin homologue gene; RLM, rat liver microsome; rt, room temperature; SAR, structure−activity relationship; SFC, supercritical fluid chromatography; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; X-Phos, 2-(dicyclohexylphosphino)-2′,4′,6′-triisopropyl-1,1′-biphenyl

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collected at 0–4, 4–8, and 8–24 h postdose. The parent drug concentrations were determined in each excreta from each collection period using an LC–MS/MS assay.

(16) Compound **1** (1 μM) was incubated in rat and human liver microsomes in potassium phosphate buffer (66.6 μM, pH 7.4) with NADPH (1 mM) and $MgCl_2$ (3 mM) at 37 °C for 1 h. Hepatocytes (rat and human) were suspended in Krebs–Henseleit buffer (2 million cells/mL, pH 7.4) and incubated with compound **1** (3 μM) in a humidified atmosphere of 95% oxygen and 5% CO_2 (95% relative humidity) at 37 °C for 4 h.

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(27) Experimental details of the PD study can be found in the Supporting Information.

(28) Experimental details of the U87 MG glioblastoma xenograft model can be found in the Supporting Information.