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Drug Annotation

Discovery of AZD8154, a Dual PI3Kγδ Inhibitor for the Treatment of Asthma

Matthew W. D. Perry,* Karin Björhall, Peter Bold, Mikael Brűlls, Ulf Börjesson, Johan Carlsson, Hui-Fang Amy Chang, Yunhua Chen, Anders Eriksson, Britt-Marie Fihn, Rebecca Fransson, Linda Fredlund, Hongbin Ge, Haijuan Huang, Kostas Karabelas, Eva Lamm Bergström, Sarah Lever, Helena Lindmark, Mickael Mogemark, Antonios Nikitidis, Anna-Pia Palmgren, Nils Pemberton, Jens Petersen, Mio Rodrigo Blomqvist, Reed W. Smith, Matthew J. Thomas, Victoria Ullah, Christian Tyrchan, Tiiu Wennberg, Annika Westin Eriksson, Wenzhen Yang, Shuchun Zhao, and Linda Öster

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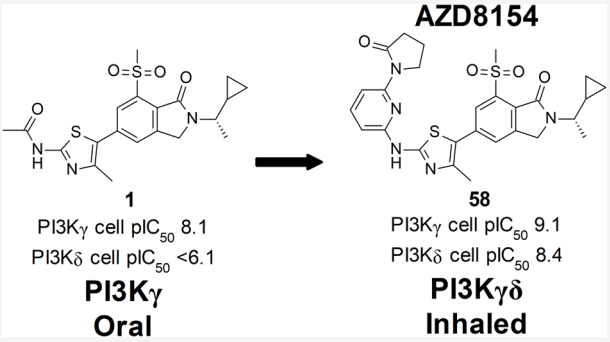
ABSTRACT: Starting from our previously described PI3Kγ inhibitors, we describe the exploration of structure−activity relationships that led to the discovery of highly potent dual PI3Kγδ inhibitors. We explored changes in two positions of the molecules, including macrocyclization, but ultimately identified a simpler series with the desired potency profile that had suitable physicochemical properties for inhalation. We were able to demonstrate efficacy in a rat ovalbumin challenge model of allergic asthma and in cells derived from asthmatic patients. The optimized compound, AZD8154, has a long duration of action in the lung and low systemic exposure coupled with high selectivity against off-targets.



INTRODUCTION

Phosphoinositol 3-kinases (PI3Ks) are a group of lipid kinases that phosphorylate the 3′-hydroxyl of the inositol ring of phosphatidyl inositol lipids. They have been divided into three classes, I, II, and III, based on their preferred substrates. The most intensively studied of these classes are the four class I enzymes, known as PI3Kα, β, γ, and δ. These enzymes exist as heterodimers consisting of a catalytic p110 subunit and a regulatory subunit. PI3Kα, β, and δ, which share p85 regulatory subunits and principally act downstream of tyrosine kinase receptors, are known as class IA. PI3Kγ, in contrast, has its own regulatory subunits (p101 and p84) and is found downstream of GPCRs through the agency of GTPases; it is the only member of class IB PI3Ks. The class I PI3Ks principally convert the membrane lipid phosphoinositol-4,5-bis phosphate (PIP_2) to phosphoinositol-3,4,5-tris phosphate (PIP_3). PIP_3 is recognized by proteins containing a pleckstrin homology domain which are recruited to and activated by PIP_3. Pleckstrin homology domains are found in a variety of proteins that activate multiple pathways leading to cell proliferation, differentiation, and other effects. PI3Ks have been widely studied, and extensive reviews cover the biology in detail.[1−3]

Unlike PI3Kα and β, which are ubiquitously or widely distributed throughout the body,[4−6] PI3Kγ and δ have a much more restricted distribution and are principally found in leukocytes.[7,8] Inhibitors of PI3Ks have been investigated for the treatment of cancers, with four agents now on the market, but the roles of PI3Kγ and δ in leukocytes have also resulted in investigation of inhibitors of these isoforms for the treatment of inflammatory diseases.[9,10] PI3Kδ has been shown to be responsible for cytokine release from Th2 and Th17 cells,[11] while PI3Kγ has been shown to be involved in the activation of both eosinophils and neutrophils.[12] These mechanisms are believed to be important in asthma, both for allergic (Type 2) asthma but also to less defined non-Th2 asthmatics with mixed T cell subsets driving the inflammatory responses.[13]

The oral dual PI3Kδγ inhibitor Duvelisib has been investigated in clinical trials for asthma[14] and rheumatoid arthritis,[15] where it has shown positive effects, but further

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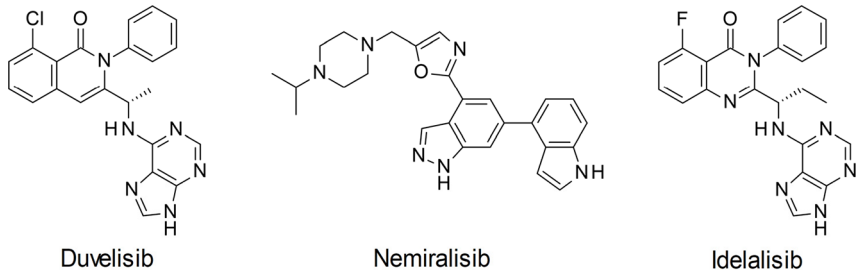

Figure 1. Marketed and investigational PI3Kδ and PI3K$\delta\gamma$ inhibitors referred to in the text.

development for these indications has not been pursued, possibly because of side effects. The inhaled selective PI3Kδ inhibitor Nemiralisib has been evaluated in clinical trials with asthmatics,[16] COPD patients,[17] and patients with activated PI3Kδ syndrome,[18] though development of this compound appears to have stopped, potentially based on a lack of clinical efficacy (Figure 1).

Oral PI3Kδ inhibitors, such as Idelalisib (Figure 1) and Duvelisib, have been effective in the treatment of B cell lymphomas, but dose-limiting toxicity has been a problem,[19] and it is notable that Duvelisib was not further investigated for inflammatory diseases beyond two small studies. Inhaled administration has been used to target drugs to the lung where systemic side effects might otherwise result in unacceptable toxicity.[20] We were thus interested to discover a dual PI3K$\gamma\delta$ inhibitor with properties suitable for inhaled administration which we hoped would be able to display profound immune modulation while minimizing undesired systemic effects.

We have previously described our earlier series of PI3Kδ inhibitors[21,22] and the optimization of one of them for inhalation. We have also described the discovery of a series of PI3Kγ inhibitors[23] with an unprecedented mode of action and selectivity[24] that led to the identification of **1** (Figure 2) as

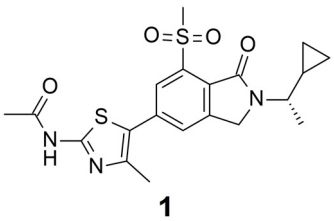

Figure 2. PI3Kγ selective inhibitor **1**.

a potential development candidate. Given the roles of both PI3Kγ and PI3Kδ in hematopoetic cells, it was of great interest to us to try to identify compounds that combined these activities in a single molecule. In this work, we describe our exploration of the chemistry around our PI3Kγ selective series that led to the identification of features that gave inhibition of PI3Kδ while retaining activity against PI3Kγ and the optimization of the compounds for inhaled delivery.

CHEMISTRY

Synthesis of the compounds reported began with preparation of the sulfonyl chloride **4**, via displacement of chlorine from chloride **2**[23] by benzyl thiolate followed by oxidative chlorination of thioether **3** with sulfuryl chloride. Condensation of **4** with amines gave sulfonamides **5**–**15** (Scheme 1).

In order to explore substitution on the aminothiazole, we began by hydrolyzing the acetamide of **1** and **5** to give the amines **16** and **17**. These could then be used to displace a halogen from a heterocycle under palladium-catalyzed conditions to give compounds **18**–**22** (Scheme 2).

Synthesis of the macrocycles **32**–**37** used the sulfonyl chloride **4** and readily assembled pyridine amide moieties (**25**, **27**–**31**) that were joined together through formation of a sulfonamide. Deprotection of the aminothiazole enabled macrocyclization via an intramolecular Buchwald-Hartwig coupling to give the target compounds (Scheme 3).

The substituted pyridine derivatives **52**–**72** were synthesized by palladium-catalyzed amination of **16**, **17**, or analogues thereof. The required bromopyridine derivatives were either commercial or synthesized simply. Heterocycles **38**, **39**, and **40** were built up from the available acid or acid chloride. Most of the reversed amides were made by amination of 2,6-dibromopyridine with the appropriate lactam, though in some cases acylation of 2-amino-6-bromopyridine followed by cyclization was used. Alcohols were protected as TBS ethers and free amines with carbobenzyloxy groups (Scheme 4).

RESULTS AND DISCUSSION

Despite the very high selectivity displayed by (**2**) and the close analogue (**5**), it is noticeable that there is more activity against PI3Kδ than against either PI3Kα or β; thus, we wondered if we could discover modifications that gave a dual PI3K$\gamma\delta$ inhibitory profile while retaining selectivity against PI3Kα and β.

As part of the SAR exploration of this series of molecules and, having access to the sulfonyl chloride (**4**), we made a series of sulfonamides through reaction with a variety of amines. Small aliphatic amines had been explored previously[23] and did not show any significant changes in potency, but heterocyclic sulfonamides and, particularly, sulfonanilides exhibited a marked jump in inhibition of PI3Kδ, though with considerable variation, depending on substitution. The most interesting analogues are shown in Table 1. It should be noted that the ceiling for activity in our enzyme assays was approximately pIC_{50} 9.1, and any activity greater than about pIC_{50} 8.9 may be an underestimate. This was particularly an issue for the PI3Kγ activity, and we regarded the potency figure from the cell assays as indicative of the true inhibition achieved.

The gain in activity at PI3Kδ was modest for the parent sulfonanilide (**6**); however, two different sulfonamide substitution patterns were found to be favored for increasing potency at PI3Kδ, *meta*-electron withdrawing groups (sulfone **7**, cyano **8**); however, this could not be enhanced by 3,5-disubstitution (**10**). Alternatively, para-amino methyl substitution gave an improvement in PI3Kδ potency but at a slight cost to that at PI3Kγ (**12**). The specificity of these substitutions was shown by the *meta*-dimethylaminomethyl

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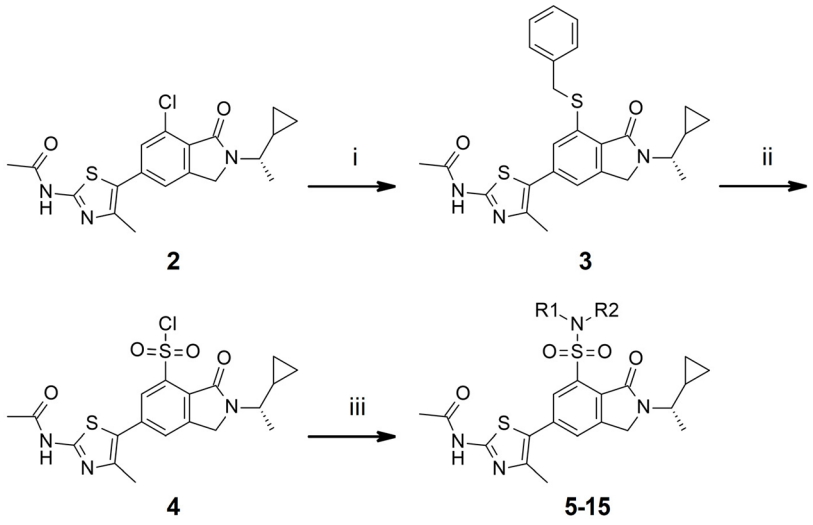
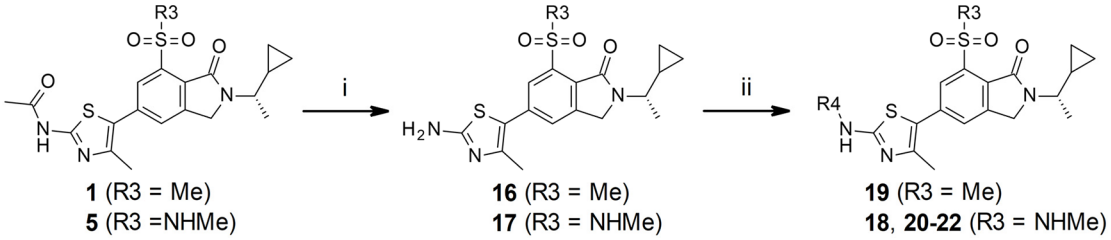
Scheme 1. Synthesis of Sulfonamides 5−15[a]



[a]i, $PhCH_2SH$, NaO*t*-Am, DMF, 110 °C; ii, SO_2Cl_2, MeCN, AcOH, H_2O, 0−5 °C; iii, R1R2NH, pyridine, CH_2Cl_2.

Scheme 2. Synthesis of *N*-Heterocyclic Derivatives 18−22[a]



[a]i, HCl, EtOH; ii, R4X, $Pd(OAc)_2$, Xantphos, dioxane, 120 °C.

analogue (**11**) and the *para*-cyano and sulfone analogues (**14**, **15**). *N*-Methylation of the sulfonamide was not beneficial (**9**, **13**). For all these compounds, an appreciable drop in potency in the cell assay of about 10-fold was unsurprising but left us needing to find additional potency to be useful.

We were able to obtain X-ray crystal structures of **7** and **12** in a PI3Kδ construct. The protein structure observed matched closely to that seen previously for **1**, with the anilide moiety fitting nicely into the space available. For the sulfone of **7**, we were unable to identify any specific interactions made that could account for the improved potency of this group relative to others. The *para*-dimethylaminomethyl substituent of **12**, however, was close to, though probably beyond direct contact with, the side chain carboxylate group of Asp897 (Figure 3).

Among the changes that we made to the parent compounds (**1** and analogues) during SAR exploration was to replace the acetamide group with a heterocycle (Table 2). Of the various heterocyclic rings that we attached, it was noticeable that pyridyl (**18**) particularly with an amide substituent (**19**) gave significantly increased activity against PI3Kδ. Changing the position of the amide (**21**) appeared to have little effect, while disubstitution on the amide (**22**) appeared not to be favorable for activity at PI3Kδ.

Crystallization of **20** in PI3Kδ showed that the pyridine ring was coplanar with the thiazole and with the nitrogen of the pyridine pointing inward; we interpreted this as a lone pair-sulfur interaction[25] stabilizing this conformation. The amide side chain continued along the same axis, leaving the terminal dimethylamino group in proximity to Asp897, which in turn rotates slightly to optimize the interaction, though the observed distance is slightly too long (~5.4 Å) for a direct interaction (Figure 4). Inspection of the crystal structure of **20** in comparison to that of **12** showed that the two amino functions both pointed toward the same Asp897 carboxylate. This raised the prospect that a macrocycle might be an effective way forward.

Our initial design of macrocycle hybridized between the amines **12** and **20**. These compounds (**32**, **33**) showed very good potency at PI3Kδ but lost activity against PI3Kγ (Table 3). We continued to investigate macrocyclic analogues, and after a preliminary study using ring closing metathesis established that the optimal linker length was five atoms between the amide and sulfonamide (Supplementary Table 1), we made a series with purely aliphatic linkers (**34**−**37**). These compounds had excellent activity at both PI3Kγ and PI3Kδ, with good cellular activity at both isoforms as well, though activity against PI3Kα also increased, resulting in lower selectivity against this isoform than we desired. Activity against PI3Kβ (not shown) was weaker.

In parallel with our exploration of macrocycles, we continued to explore SAR of compounds related to the pyridyl amides. We synthesized isosteres to amides as a possible way to further improve δ cell potency (Table 4). Heterocycles (**52**−**54**) were similar to or poorer than earlier compounds, and likewise reversing the amide (**55**) had little effect until we *N*-alkylated (**56**, **57**), which gave a significant improvement in potency against PI3Kδ, in contrast to the effect seen previously with the alternate orientation (**22**).

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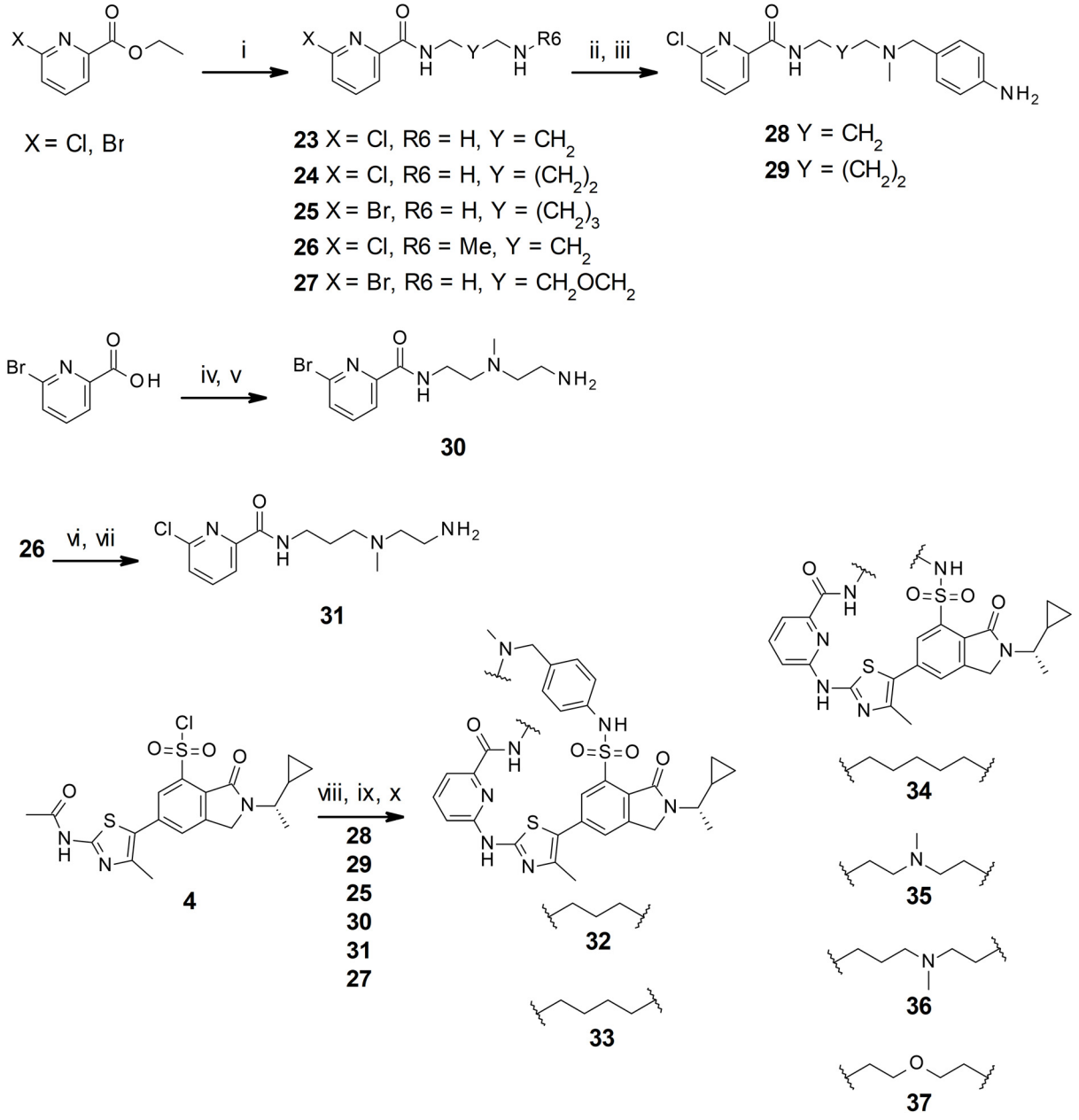
Scheme 3. Macrocycle Synthesis *via* Buchwald–Hartwig Amination[a]



[a]i, diamine, CH_2Cl_2; ii, 4-acetamidobenzaldehyde, $NaBH_3CN$, AcOH, MeOH then aq CH_2O; iii, HCl (3 M aq), EtOH; iv, $H_2N(CH_2)_2N(Me)(CH_2)_2NHBOC$, T3P, Et_3N, EtOAc; v, HCl, dioxane, CH_2Cl_2; vi, **30**, $BOCNHCH_2CHO$, $NaBH_3CN$, AcOH, MeOH; vii, TFA, CH_2Cl_2; viii amine (**28**, **29**, **25**, **30**, **31**, **27**), Et_3N, CH_2Cl_2; ix, HCl (3 M aq), EtOH; x, second or third generation Xantphos precatalyst, Na_2CO_3, DMF, 140 °C.

In following up on the results for the reversed amides, we made a series of lactams, cyclic carbamates, and ureas. In this exploration, we also investigated the combination of the sulfone or sulfonamide substituent on the isoindolinone with the pyridine substituent. Many of the resulting compounds exhibited the levels of PI3Kδ cell activity that we had been hoping to see without any loss of PI3Kγ activity (Table 5).

We were able to crystallize **58** in PI3Kδ (Figure 5; Supplementary Figure S2); the molecule binds in the same general orientation as **20**. The lactam moiety is almost coplanar with the pyridine ring and is not within contact distance of any residues of the protein. The carbonyl group is oriented antiparallel to the C═N of the pyridine, as expected from electrostatic effects, and it appears that the carbonyl group can make a water-mediated hydrogen bond to the terminal amino group of the nonconserved asparagine 836 (PI3Kα Gln859, PI3Kβ Asp856, PI3Kγ Lys890). This hydrogen bond is not possible for the earlier amide compounds and may account for the improved potency observed at PI3Kδ with this motif. Consistent with the crystal structures, the SAR for PI3Kδ activity was flat, with only subtle changes apparent upon varying this group. Inhibition of PI3Kα, however, showed larger variations, and some compounds had quite high activity against that isoform. Inhibition of PI3Kβ was always low (data not shown).

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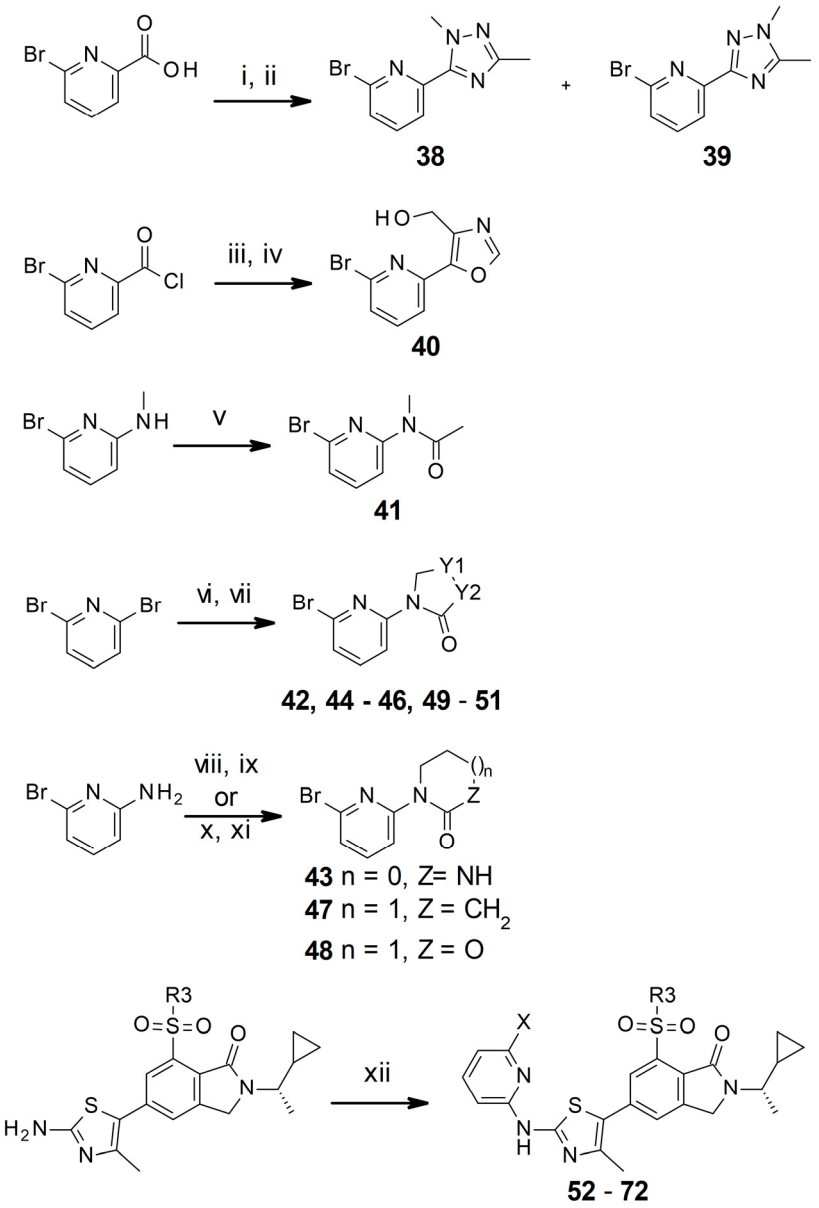
Scheme 4. Synthesis of Amide Isosteres and Reversed Amides[a]



[a]i, acetamidine hydrochloride, HATU, DIPEA, DMF; ii, $MeNHNH_2$, AcOH, DMF, 80 °C; iii, ethyl 2-isocyanoacetate, Et_3N, DMAP, THF; iv, $NaBH_4$, EtOH, 0 °C; v, Ac_2O, py, 120 °C; vi, second or third generation Xantphos precatalyst, Na_2CO_3, DMF, 140 °C; vii, deprotection, if required; viii, $ClCH_2CH_2NCO$, toluene; ix, NaH, DMF, THF; x, $Cl(CH_2)_nZCOCl$, pyridine; xi, Cs_2CO_2, DMF; xii second or third generation Xantphos precatalyst, Na_2CO_3, DMF, 120 °C.

Given the broad range of compounds with good primary potency against PI3Kγ and PI3Kδ, we focused attention on the margin of selectivity against PI3Kα, for which we hoped to achieve two log units (based on the enzyme inhibition values) and the PI3Kδ cell potency, which we wanted to be as potent as possible, giving as a good dual PI3K$\gamma\delta$ profile as we could attain. On the basis of these criteria, compounds **58** and **68** were selected for further profiling, with compound **59**, synthesized slightly later in our program, as a back-up.

From the outset of this program, we had intended to administer our molecule by inhalation, and thus we had not concerned ourselves with metabolic stability, being hopeful instead of achieving low systemic exposure. As our program developed, we found ourselves with a set of compounds with high lipophilicity and often high crystallinity as well. Indeed, some compounds we made, principally those with amide NH groups in the X substituent (Table 5), were extremely problematic to work with as they precipitated from DMSO stock solution.

Several approaches have been described by which extended duration of action for inhaled molecules can be achieved.[26] Given the physicochemical properties that we had, we decided that we would focus our attention on a low-solubility approach, whereby slow dissolution of the drug substance in the lung is used to maintain an efficacious concentration over a prolonged period, thus providing a long duration of action. To this end, we were determined to carry out our pharmacokinetic studies by nebulization of suspensions of crystalline material in order that the effect of the dissolution rate could be observed. In order to achieve timely availability of suitable material for PK

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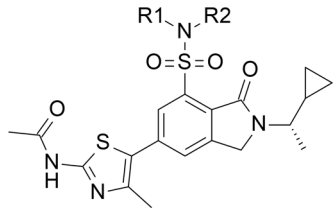
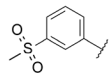
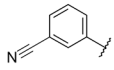
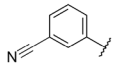
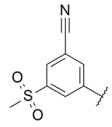
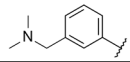
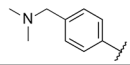
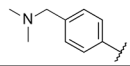
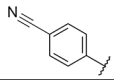
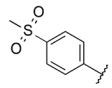
Table 1. Data for Sulfonanilides



Compound	R1	R2	PI3Kγ pIC_{50}[a,d]	PI3Kδ pIC_{50}[a,d]	PI3Kγ cell pIC_{50}[a,e]	PI3Kδ cell pIC_{50}[a,f]
1			9.1	6.5	8.1	<6.1
5	Me	H	9.1	6.8	8.3	6.4
6	Ph	H	9.0	7.3		
7		H	8.9	8.5	7.8	7.5
8		H	9.1	8.0	8.2	6.9
9		Me	9.1[c]	7.3[b]		
10		H	8.9[b]	7.6[b]		
11		H	8.7[c]	7.8[c]		
12		H	8.6	8.2	7.7	7.3
13		Me	8.6[b]	7.6[b]		
14		H	9.0[b]	6.8[c]		
15		H	9.0[c]	6.8[c]		

[a]All values are $n \geq 3$ unless otherwise shown. [b]$n = 2$. [c]$n = 1$. [d]Inhibition of the production of ADP by recombinant human PI3K detected by ADP-Glo (Promega). [e]Inhibition of phosphorylation of AKT (at Ser473) in RAW264 cells stimulated by C5a. [f]Inhibition of phosphorylation of AKT (at Ser473) in JeKo-1 cells stimulated anti-IgM.

experiments, we initiated a procedure whereby, as soon as synthesis was complete and primary assays requested, all compounds were investigated for crystallinity by X-ray powder diffraction. Unless the material was already crystalline, we then slurried it in multiple solvent systems to identify a crystalline form that could be used for inhalation dosing, though at the initial stage we did not explicitly seek a preferred polymorph. With crystalline material available, we were able to determine crystalline solubility and melting onset to give an indication of the likely behavior of the material in the lung. Our hypothesis was that solubility of 1 μM or less would give an extended duration upon dosing of a nebulized suspension. Compounds were selected for further study based on their *in vitro* profile and dosed by nebulization to rats to study the pharmacokinetics. We also ran conventional iv and po pharmacokinetic studies in order to understand the behavior of compound once it was absorbed into the systemic circulation and the fate of any fraction of the dose that was swallowed (Table 6). We were interested to understand the permeabilities of these molecules and tested them in CaCO-2 permeability assays; however, low recovery of the compounds meant that we were unable to define precise values with confidence (data not shown). The results we did get suggested that the macrocycles (**35**, **37**) had low permeability and moderate efflux, while the nonmacrocycles (**58**, **68**) had moderate permeability and little efflux.

We were pleased to find that our design hypothesis was borne out in reality for the compounds that we tested and that we could obtain an extensive residence in the lung through very low solubility. Of note, this apparent terminal $t_{1/2}$ (inhaled $t_{1/2}$) was longer in every case than the plasma half-life measured from iv dosing, which implies that a depot effect was accounting for the prolonged half-life, in accordance with our hypothesis. Compounds **35**, **37**, and **68** had very high *in vivo* clearance, being measured at or above liver blood flow, probably as a result of biliary clearance (observed for other

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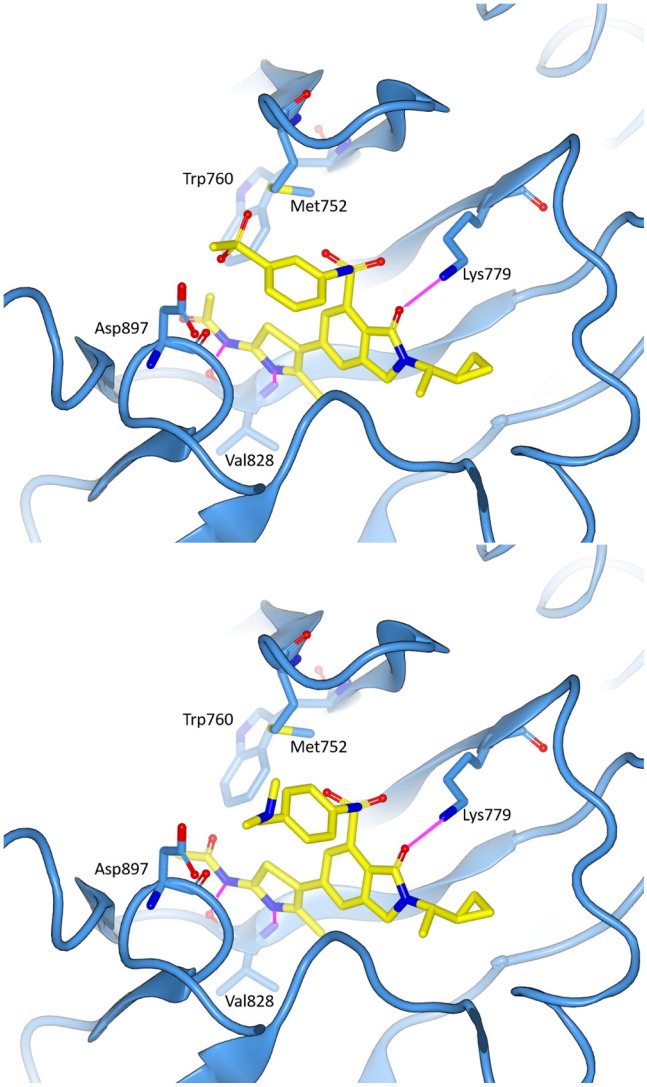
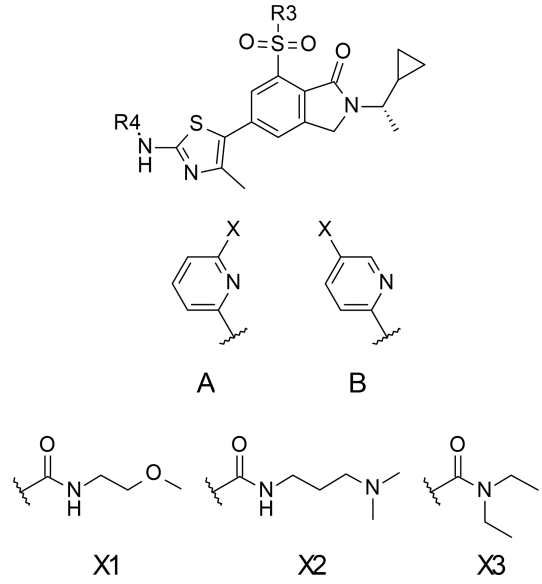


Figure 3. Crystal structures of compounds **7** and **12** bound in PI3Kδ. (Top) **7** bound in mPI3Kδ (PDB 7oi4, 1.8 Å). (Bottom) **12** bound in mPI3Kδ (PDB 7ois, 2.3 Å) Note that for each compound hydrogen bonds can be seen from the acetylaminothiazole to the hinge Val828 and from the carbonyl of the isoindolinone to the terminal amino of Lys779.

compounds in this series, data not shown) and, for **35** and **68**, undetectable oral bioavailability. In contrast, **58** had a more modest, though still quite high, clearance; despite this clearance, bioavailability of **58** was very low. For **58** biliary clearance was only a minor factor (about 2% total clearance); however, Caco-2 permeability ($P_{app} \approx 0.4$ but poor recovery of the lipophilic compound makes the precise value uncertain) was low, which, coupled with the very low solubility, places it into BCS category 4.[27] In combination with the moderately high clearance, the low bioavailability is thus unsurprising.

With compounds available that gave extended lung exposure through inhaled dosing, we were in a position to explore pharmacodynamic effects. Disease models for asthma, particularly severe asthma, are in their infancy, so we opted to use a mechanistic model of allergic lung inflammation driven by an allergic response to ovalbumin,[28] even though we realized that this simplistic model did not recapitulate the full complexity of asthma. Rats were sensitized by ip administration of ovalbumin, then primed by inhalation of ovalbumin,

Table 2. SAR of Heterocyclic Substituents



		R4		pIC$_{50}$ PI3K[a,c]	
compound	R3	Het	X	γ	δ
1	Me		Ac	9.1	6.5
5	NHMe		Ac	9.1	6.8
18	NHMe	A	Me	9.0	7.3
19	Me	A	X1	9.1[b]	8.3[b]
20	NHMe	A	X2	8.5	8.4
21	NHMe	B	X2	8.9[b]	8.2[b]
22	NHMe	A	X3	8.5	6.8[b]

[a]all values are $n \geq 3$ unless otherwise shown. [b]$n = 2$. [c]Inhibition of the production of ADP by recombinant human PI3K detected by ADP-Glo (Promega).

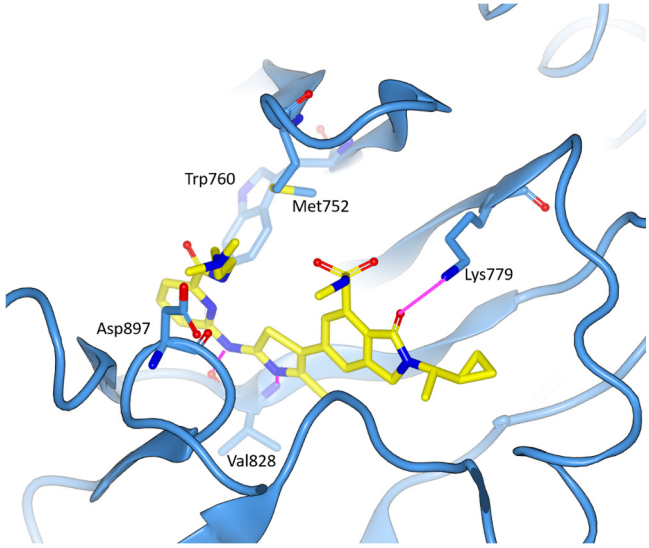


Figure 4. Crystal structure of **20** in PI3Kδ. Figure 4 X-ray structure of compound **20** bound in mPI3Kδ (PDB 7oij, 1.7 Å). Note how the amine at the terminus of the side chain ends up close to the sulfonamide on the isoindolinone suggesting the possibility for macrocyclization.

expected to result in recruitment of antigen specific T cells and granulocytes to the lung. On the day of challenge test, compound was administered to the lung 1 h prior to

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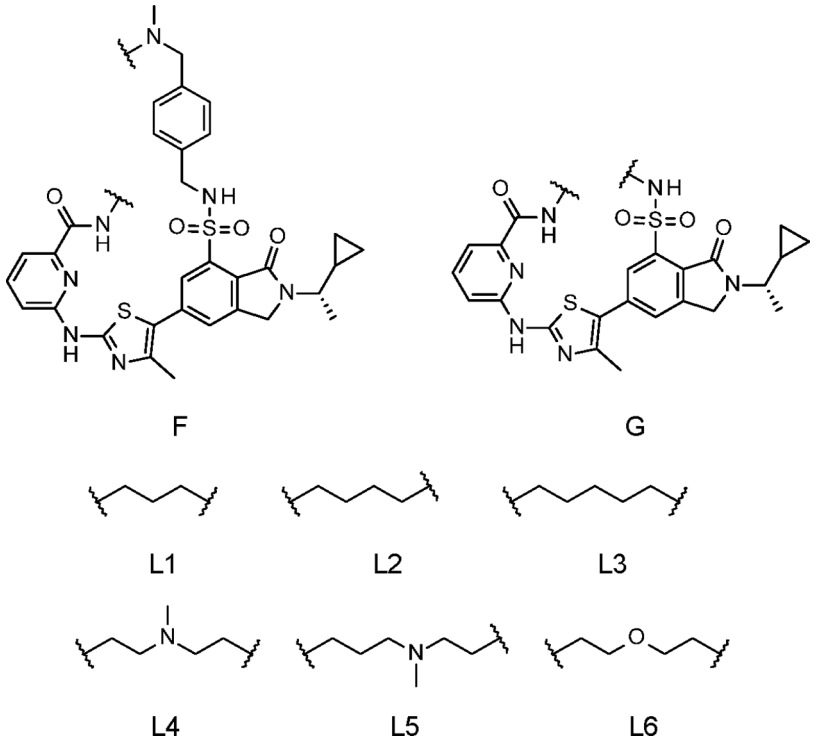
Table 3. Activities of Macrocyclic Dual PI3K$\gamma\delta$ Inhibitors



compound	core	linker	PI3K enzyme IC_{50} [a,c] γ	δ	α	PI3K cell IC_{50} [a] γ [d]	δ [e]
32	F	L1	8.1	9.1		8.4	8.8[b]
33	F	L2	8.0	9.0		8.3	9.0[b]
34	G	L3	8.9	9.3	7.5	8.8	8.9
35	G	L4	8.8	9.3	7.4	8.8	8.5
36	G	L5	9.1	9.2	7.3	9.0	8.5
37	G	L6	8.6	9.2	7.4	9.1	8.7

[a]Al values are $n \geq 3$ unless otherwise shown. [b]$n = 1$. [c]Inhibition of the production of ADP by recombinant human PI3K detected by ADP-Glo (Promega). [d]Inhibition of phosphorylation of AKT (at Ser473) in RAW264 cells stimulated by C5a [e]Inhibition of phosphorylation of AKT (at Ser473) in JeKo-1 cells stimulated anti-IgM.

administration of ovalbumin to the lung. Following drug treatment and ovalbumin challenge we waited for 48 h until the animals were terminated and the recruitment of eosinophils to the lung was measured by bronchioalveolar lavage (BAL) to give an indication of the anti-inflammatory effect of the compound. (Figure 6)

To further analyze the effects of the compounds, we could track the progress of target engagement by measurement of the degree of phosphorylation of the downstream target S6 ribosomal protein in the lymphocyte population at 2 h post dosing. We were further able to measure the levels of inflammatory cytokines in lung lavage fluid. In both cases, the data were consistent with the picture obtained from the eosinophil level (Supplementary Figures S3 and S4).

In order to probe the pharmacodynamic duration of the compounds, we ran an experiment where the compound was dosed at various time points prior to the ovalbumin challenge (Figure 7).

In this experiment, we saw that we obtained statistically significant inhibition with dosing 7 h prior to challenge and a weaker effect with 12 h predosing. Dosing 24 h before challenge had no effect. These data suggested that twice-daily dosing of compound **58** might be required to achieve a durable effect in man.

Comparison of the four compounds that we had evaluated *in vivo* was based on a balance of factors. The weakly basic compound **35** (pK_a 6.4) had the least attractive profile when tested in a panel of 150 diverse targets, showing activity at 1 μM at 16 of these, consistent with the common experience that lipophilic amines are more likely to exhibit off-target effects. In contrast, the neutral compounds **37** (8 targets inhibited at 1 μM), **58** (7), and **68** (5) were less promiscuous (Supplementary Table S2).

Kinase selectivity for the compounds was evaluated in a panel of 348 protein kinases at 1 μM; however, none of the compounds showed >50% activity against any of the tested kinases (full details in Supplementary Data Table S3 and Figure S5). Compounds **37**, **58**, and **68** were also evaluated in a panel of lipid kinases; beyond the activity at class I PI3Ks, we also observed some inhibition of class 2 PI3Ks (Table 7). The activity against class 2 PI3Ks, PIK3C2B, and PIK3C2G was significant; however, we decided to take a compound forward with these activities as a potential risk.

The synthetic complexity of the macrocycles was an additional factor in our prioritization since this was anticipated

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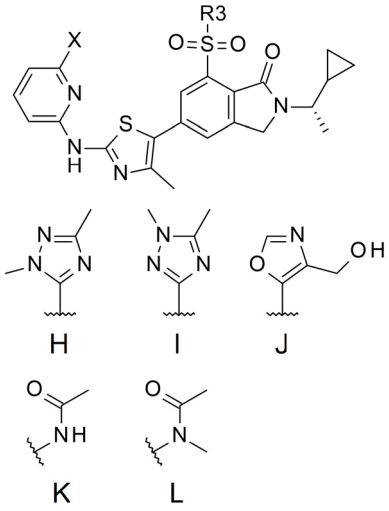
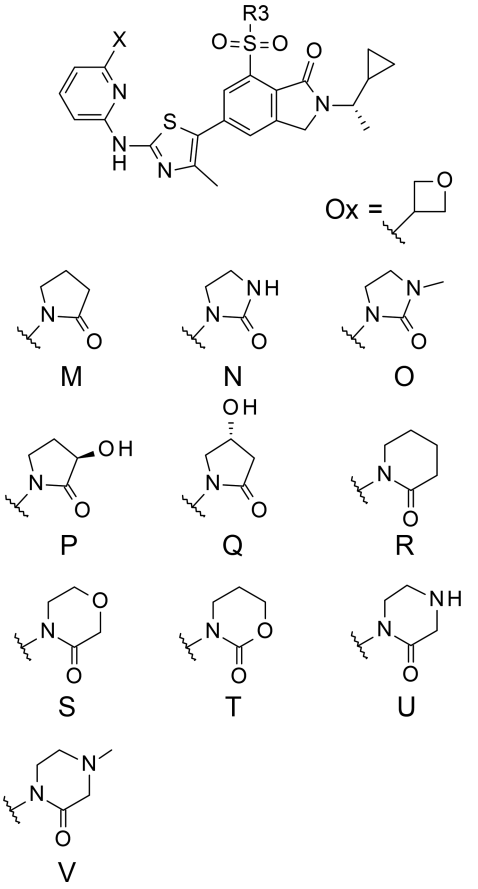
Table 4. Amide Replacements



			pIC50[a,b]			
compound	X	R3	PI3Kγ	PI3Kδ	PI3Kγ cell	PI3Kδ cell
52	H	Me	9.0	8.8		
53	I	Me	9.0	7.6		
54	J	Me	9.2	8.7		
55	K	Me	9.0	7.8		
56	L	Me	9.1	8.9		
57	L	NHMe	9.1	9.1	9.0	8.2

[a]All values are $n \geq 3$. [b]Inhibition of the production of ADP by recombinant human PI3K detected by ADP-Glo (Promega).

Table 5. Lactams, Carbamates, and Ureas



			PI3K enzyme IC50[a,b,d]		PI3K cell IC50[b]	
compound	X	R3	δ	α	γ[e]	δ[f]
58	M	Me	9.2	7.2	9.1	8.4
59	M	NHOx	9.1	6.9	9.0	8.3
60	N	NHOx	9.2	7.5	8.7	8.5
61	O	Me	9.1	7.4	9.0	8.4
62	P	NHMe	9.3	7.8	8.6	8.4
63	Q	NHMe	8.9	7.4	8.7	8.1
64	R	Me	8.9	7.5	8.8	7.9
65	S	NHMe	9.0	7.7	9.0	8.2
66	S	Me	9.0	7.3	9.0	8.1
67	T	NHMe	9.2	7.4	9.1	8.4
68	T	NHOx	9.0	7.0	9.0	8.4
69	T	Me	8.9[c]	7.7	8.9[c]	8.4[c]
70	U	NHMe	9.2	7.7	9.0	8.6
71	U	NHMe	9.1	7.4	8.4	8.2
72	V	NHMe	8.8	7.4	8.9	8.2

[a]All compounds reached the maximum inhibition in the PI3Kγ enzyme inhibition assay. [b]All values are $n \geq 3$ unless otherwise shown. [c]$n = 2$. [d]Inhibition of the production of ADP by recombinant human PI3K detected by ADP-Glo (Promega). [e]Inhibition of phosphorylation of AKT (at Ser473) in RAW264 cells stimulated by C5a. [f]Inhibition of phosphorylation of AKT (at Ser473) in JeKo-1 cells stimulated anti-IgM.

to result in delays and costs during the development process. Compound **58** was selected as having the best overall profile and was advanced to preclinical studies.

In order to build confidence in our inhibitor for the treatment of human disease, we carried out studies using blood obtained from GINA 3 or 4 asthmatic patients. In peripheral blood mononuclear cells (PBMCs), a PI3Kδ response was obtained through stimulation of T cell antigen receptor with a mixture of antibodies to CD2, 3, and 28 for 24 h. Inhibition of this response was measured by detection of the impact of δ, γ and dual γ δ inhibitors on the release of IL-5 and IL-17. In contrast, eosinophils from the same patients' blood was treated with eotaxin-3 (CCL26) to produce a PI3Kγ-driven effect, observable by the appearance of CD11b expression, that can be measured by flow cytometry. Both these effects could be inhibited by the dual PI3Kγδ inhibitor **58**, in contrast to a pure PI3Kδ inhibitor (PI3Kδ pK_i 9.9; PI3Kγ pIC_{50} 5.2)[29] or a pure PI3Kγ inhibitor (PI3Kδ $pIC_{50} < 6.1$; PI3Kγ pIC_{50} 9.1) (**1**), each of which could only effectively inhibit one of the responses (Figure 8).

CONCLUSION

We have described how, through SAR exploration and paying particular attention to cellular activity, we went from a highly selective PI3Kγ inhibitor to a series of dual PI3Kγδ inhibitors. On the basis of the physicochemical properties of the series and our desire for inhaled administration, we focused on making crystalline material and understanding solid state properties. Administration of compounds to rats by nebulization showed prolonged retention of compounds in the lungs and extended pharmacodynamic effects in a mechanistic model, building confidence in the suitability of the identified compounds for investigation as inhaled agents. Studies using human patient derived blood cells demonstrated a potential benefit of dual PI3Kγδ inhibition. On the basis of excellent potency and good in vivo PK with long duration of action

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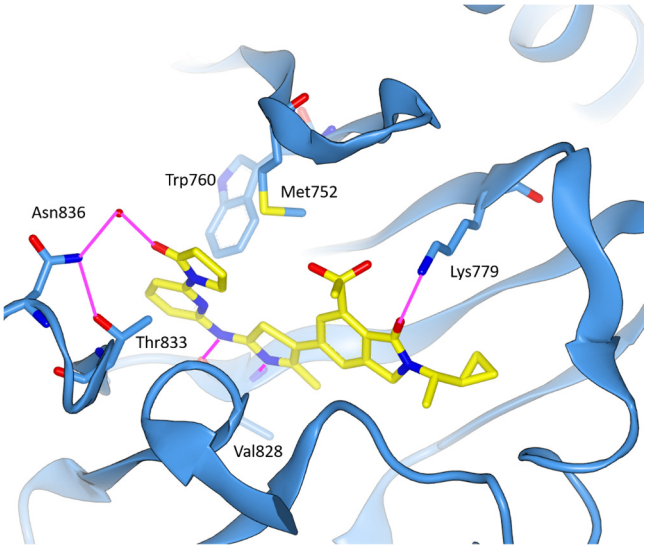

Figure 5. Crystal structure of **58** bound to PI3Kδ. X-ray view of **58** bound in PI3Kδ (PDB code 7oil, 2.0 Å). The binding mode is essentially identical to earlier compounds. The lactam can be seen to make a water-mediated hydrogen bond to the terminal NH_2 of Asp836, which itself is in a network of hydrogen bonds with Thr833. Other residues shown are Trp760 and Met752 that form the other side of the pocket around the lactam and Lys779 that forms H-bonds with the carbonyl of the isoindolinone and the sulfone.

coupled with an acceptable off-target profile, compound **58** (AZD8154) was selected for development.

EXPERIMENTAL SECTION

All reagents obtained from commercial sources were used without further purification; anhydrous solvents were used without further drying. All compounds were purified to ≥95% purity as judged by HPLC with UV and MS analysis. When Cl or Br was present, expected isotopic distribution patterns were observed. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at ambient temperature. Solutions were typically prepared in deuterated dimethyl sulfoxide (DMSO-d_6), deuteromethanol (CD_3OD), or deuterochloroform ($CDCl_3$) with chemical shifts referenced to solvent as an internal standard. ^{1}H NMR data are reported indicating the chemical shift (δ), the multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; dd, doublet of doublets; etc.), the coupling constant (J) in Hz, and the integration (e.g., 1H). ^{13}C NMR spectra are reported with proton decoupling.

General Procedure A – Synthesis of Sulfonamides from Sulfonyl Chloride 4. (*S*)-6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonyl chloride[23] (4, 80 mg) was dissolved in a mixture of dichloromethane (3 mL) and pyridine (42 μL, 2 equiv). The appropriate aniline (1 equiv) was added, and the mixture was stirred overnight. Solvent was evaporated, and the residue was purified by preparative reverse phase HPLC. Typically a 5 μM Xbridge C-18 19 × 150 mm column was used running a gradient of acetonitrile in 0.05% TFA in water at 20 mL/min.

The following compounds were prepared according to general procedure A and the appropriate aniline:

*N-(5-{2-[(1S)-1-Cyclopropylethyl]-1-oxo-7-(phenylsulfamoyl)-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (**6**).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.30 (s, 1H; exchangeable NH and reduced to ∼60%), 10.14 (s, 1H; exchangeable NH and reduced to ∼72%), 7.96 (d, J = 1.5 Hz, 1H), 7.81 (d, J = 1.5 Hz, 1H), 7.23 (dd, J = 8.5, 7.3 Hz, 2H), 7.12−7.07 (m, 2H), 7.07−7.01 (m, 1H), 4.80−4.67 (m, 2H), 3.70 (dq, J = 9.3, 6.8 Hz, 1H), 2.36 (s, 3H), 2.16 (s, 3H), 1.36 (d, J = 6.8 Hz, 3H), 1.24−1.13 (m, 1H), 0.67−0.58 (m, 1H), 0.50−0.40 (m, 2H), 0.39−0.30 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 168.8, 164.6, 156.4, 146.1, 144.9, 137.1, 136.1, 135.8, 129.4, 127.4, 126.9, 125.8, 125.0, 121.5, 120.5, 52.8, 46.5, 22.5, 18.1, 16.3, 15.7, 4.0, 3.4.

ES+ $(M + H)^+$ observed 511.1487 expected 511.1474.

*N-[5-(2-[(1S)-1-Cyclopropylethyl]-7-{[3-(methylsulfonyl)phenyl]-sulfamoyl}-1-oxo-2,3-dihydro-1H-isoindol-5-yl)-4-methyl-1,3-thiazol-2-yl]acetamide (**7**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.29 (s, 1H), 10.55 (s, 1H), 7.97 (d, J = 1.7 Hz, 1H), 7.90 (d, J = 1.7 Hz, 1H), 7.63 (t, J = 2.0 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 8.0 Hz, 1H), 7.48−7.43 (m, 1H), 4.80−4.65 (m, 2H), 3.69 (dq, J = 9.3, 6.8 Hz, 1H), 3.12 (s, 3H), 2.37 (s, 3H), 2.16 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.22−1.12 (m, 1H), 0.65−0.55 (m, 1H), 0.52−0.38 (m, 2H), 0.38−0.29 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.74, 164.31, 156.44, 146.21, 144.93, 141.86, 138.14, 135.81, 135.59, 130.74, 127.71, 127.08, 125.82, 124.41, 122.90, 121.44, 117.69, 52.75, 46.47, 43.37, 22.45, 17.96, 16.33, 15.57, 3.95, 3.37.

ES+ $(M + H)^+$ observed 589.1263 expected 589.1249.

*N-(5-{7-[(3-Cyanophenyl)sulfamoyl]-2-[(1S)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)-acetamide (**8**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.30 (s, 1H), 10.48 (s, 1H), 7.98 (d, J = 1.5 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.52−7.40 (m, 4H), 4.78−4.68 (m, 2H), 3.72−3.64 (m, 1H), 2.38 (s, 3H), 2.16 (s, 3H), 1.36 (d, J = 6.8 Hz, 3H), 1.22−1.14 (m, 1H), 0.65−0.58 (m, 1H), 0.49−0.40 (m, 2H), 0.40−0.32 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.8, 164.3, 156.5, 146.2, 144.9, 138.2, 135.8, 135.4, 130.9, 128.22, 127.8, 127.2, 125.9, 124.5, 122.8, 121.4, 118.0, 112.1, 52.7, 46.5, 22. 5, 18.0, 16.3, 15.7, 3.9, 3.3.

ES+ $(M + H)^+$ observed 536.1440 expected 536.1426.

*N-(5-{7-[(3-Cyanophenyl)(methyl)sulfamoyl]-2-[(1S)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (**9**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.27 (s, 1H), 7.99 (d, J = 1.5 Hz, 1H), 7.78 (d, J = 1.5 Hz, 1H), 7.73 (t, J = 1.9 Hz, 1H), 7.66 (dt, J = 7.8, 1.4 Hz, 1H), 7.63 (ddd, J = 8.3, 2.4, 1.1 Hz, 1H), 7.51 (t, J = 8.0 Hz, 1H), 4.67−4.59 (m, 2H), 3.58 (dq, J = 9.2, 6.8 Hz, 1H), 3.41 (s, 3H), 2.29 (s, 3H), 2.16 (s, 3H), 1.28 (d, J = 6.8 Hz, 3H), 1.15−1.08 (m, 1H), 0.62−0.55 (m, 1H), 0.47−0.36 (m, 2H), 0.28−0.21 (m, 1H).

Table 6. Physicochemical Properties and PK Data for Advanced Compounds

				rat PK[b]				
				inhaled	iv			po
compound	crystalline solubility (phosphate buffer pH 7.4)[c] μM	melting onset °C	LogD pH 7.4	$t_{1/2}$ h	V_{ss} (L/kg)	CL mL/min/kg	$t_{1/2}$ h	F %
35	1.09	349	3.9	7.5	7.3	83	1.8	ND[a]
37	0.084	377	3.7	6.9	8.6	110	1.8	NT
58	0.054	285	4.4	7.1	4.0	20	2.9	<4
68	0.337	187	3.5	4.2	5.4	136	0.7	ND

[a]ND = not detected; NT = not tested. [b]PK experiments are the mean of values obtained for at least two animals. Compound was dosed at 0.5 mg/kg iv and 1.0 mg/kg po. [c]Material of known crystallinity was suspended in pH 7.4 phosphate buffer at 1 mg/mL and stirred at 1100 rpm for 24 h. Solid material was removed by centrifugation and the concentration of substrate was determined by HPLC MS.

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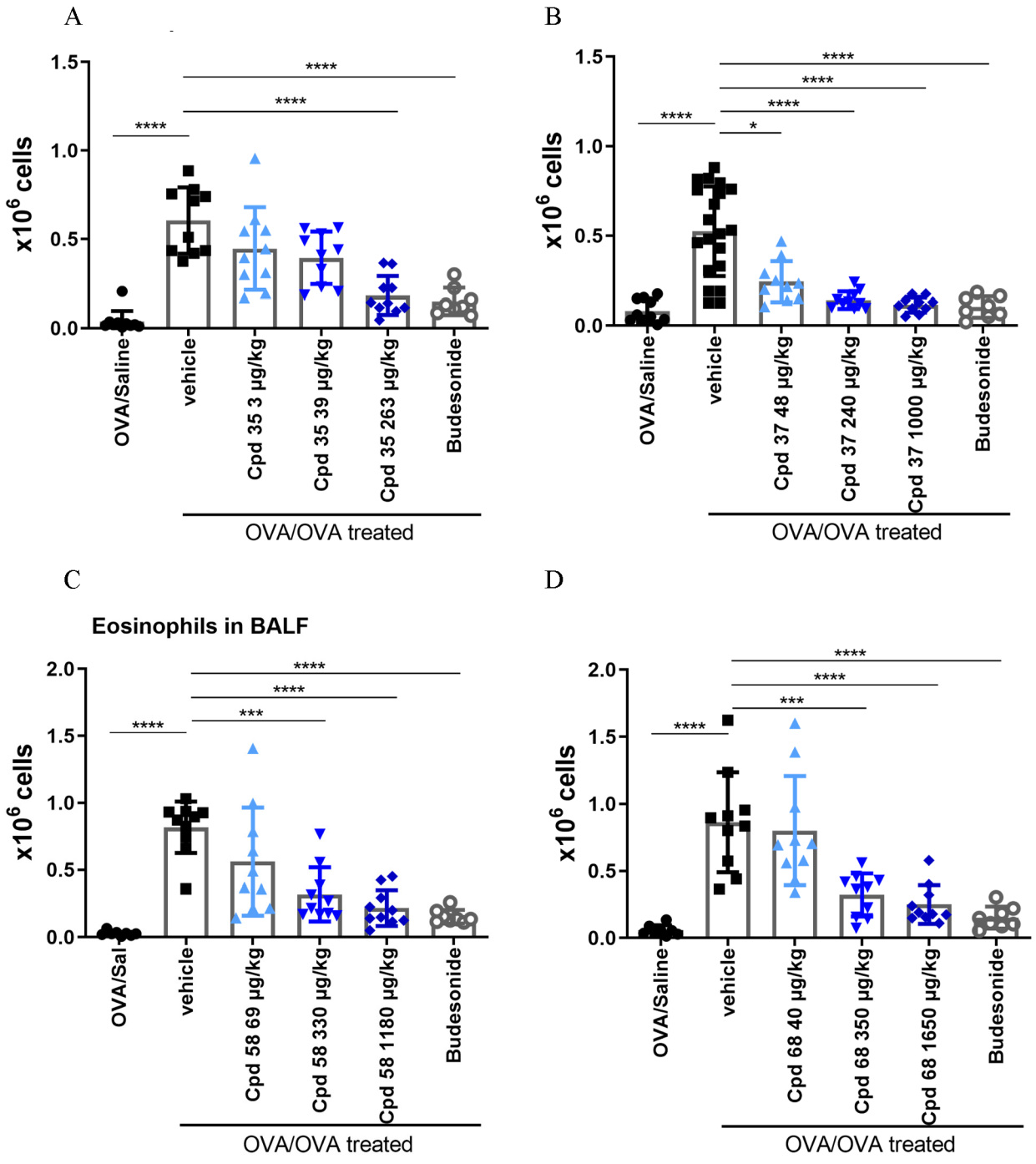

Figure 6. (A–D) Inhibition of eosinophil migration in response to an inhaled ovalbumin challenge. Rats (BN, male, 10 per group) were sensitized to ovalbumin by ip administration on two separate occasions, then challenged with aerosolized administration of ovalbumin and, after a week dosed with compound by nebulization before subsequent challenge with aerosolized ovalbumin. After 48 h the animals were terminated, the lungs lavaged, and the numbers of eosinophils in the BAL fluid were measured by flow cytometry. Budesonide (300 μg, dosed i.t.) was used as positive control. Individual animals are represented by points with the average effect shown in bars. Compound amounts represent the administered dose of compound.

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 162.7, 156.3, 146.4, 144.5, 142.0, 135.1, 134.3, 130.3, 130.2, 129.9, 129.4, 128.3, 128.0, 127.8, 121.6, 118.1, 111.8, 51.9, 45.5, 37.9, 22.4, 18.0, 16.1, 15.6, 3.9, 3.3.

ES+ $(M + H)^+$ observed 550.1573 expected 550.1583.

N-[5-(7-{[3-Cyano-5-(methylsulfonyl)phenyl]sulfamoyl}-2-[(1S)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1H-isoindol-5-yl)-4-methyl-1,3-thiazol-2-yl]acetamide (***10***). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.30 (s, 1H), 10.90 (s, 1H), 7.67–8.13 (m, 5H), 4.70 (s, 2H), 3.66 (p, J = 6.8 Hz, 1H), 3.22 (s, 3H), 2.38 (s, 3H), 2.17 (s, 3H), 1.34 (d, J = 6.7 Hz, 3H), 1.17 (dd, J = 8.2, 4.4 Hz, 1H), 0.61 (dt, J = 8.6, 4.1 Hz, 1H), 0.38–0.48 (m, 2H), 0.3–0.37 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.2, 164.5, 156.9, 146.7, 145.1, 144.1, 143.5, 134.8, 127.9, 127.0, 126.0, 123.4, 121.9, 119.3, 115.4, 52.7, 43.4, 22.9, 18.4, 16.8, 16.1, 4.4, 3.8.

ES+ $(M + H)^+$ observed 614.1219 expected 614.1202.

N-(5-{2-[(1S)-1-Cyclopropylethyl]-7-({3-[(dimethylamino)-methyl]phenyl}sulfamoyl)-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (***11***). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.28 (s, 1H), 10.01 (s, 1H), 7.95 (d, J = 1.2 Hz,

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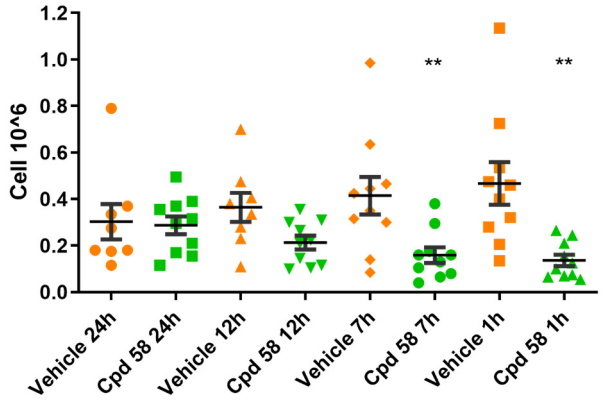

Figure 7. Experiment showing duration of effect of nebulized **58** in rats. Compound **58** was dosed by nebulization (240 μg/kg administered dose) to male rats (BN, 8−10 per group) that had been sensitized to ovalbumin at varying intervals prior to ovalbumin challenge. 48 h after challenge, the animals were terminated, the lungs lavaged, and the numbers of eosinophils in the lung lavage fluid were measured by flow cytometry, and the results were compared to time-matched controls.

Table 7. Inhibition of Lipid Kinases

	pIC_{50} [b,d]					
example	PIK3C2A	PIK3C2B	PIK3C2G	PIK3C3	PI4KA	PI4KB
37	6.5[c]	9.1[c]	NT[a]	7.6[c]	<5.0[c]	7.7[c]
58	5.4	8.5	7.2	6.3	<5.0[c]	5.5[c]
68	5.5[c]	9.0[c]	NT	7.5[c]	<5.0[c]	6.3[c]

[a]NT = not tested. [b]n = 3 unless otherwise shown. [c]n = 1. [d]FRET Adapta assays measuring inhibition of phosphorylation of a synthetic substrate run by Thermo Fisher Scientific.

1H), 7.78 (d, J = 1.4 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 7.03−7.01 (m, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.92 (d, J = 7.6 Hz, 1H), 4.73 (s, 2H), 3.75−3. 67 (m, 1H), 3.23 (q, J = 13.2 Hz, 2H), 2.35 (s, 3H), 2.16 (s, 3H), 1.96 (s, 6H), 1.37 (d, J = 6.8 Hz, 3H), 1.23−1.15 (m,1H), 0.66−0.60 (m, 1H), 0.50−0.43 (m, 2H), 0.37−0.31 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.2, 165.1, 156.9, 146.5, 145.2, 140.7, 137.5, 136.3, 136.2, 129.5, 127.8, 127.6, 126.3, 125.8, 121.9, 121.1, 119.9, 63.4, 53.2, 46.9, 45.2, 22.9, 18.5, 16.7, 16.1, 4.5, 3.9.

ES+ $(M + H)^+$ observed 568.2058 expected 568.2052.

N-(5-{2-[(1S)-1-Cyclopropylethyl]-7-({4-[(dimethylamino)-methyl]phenyl}sulfamoyl)-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (***12***). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.28 (s, 1H), 10.10 (s, 1H), 7.96 (d, J = 1.6 Hz, 1H), 7.79 (d, J = 1.6 Hz, 1H), 7.15−7.11 (m, 2H), 7.05−7.01 (m, 2H), 4.80−4.70 (m, 2H), 3.71 (dq, J = 9.4, 6.8 Hz, 1H), 3.21 (s, 2H), 2.35 (s, 3H), 2.16 (s, 3H), 2.03 (s, 6H), 1.37 (d, J = 6.8 Hz, 3H), 1.24−1.15 (m, 1H), 0.64−0.59 (m, 1H), 0.51−0.41 (m, 2H), 0.38−0.31 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 164.7, 156.4, 146.1, 144.8, 136.1, 135.8, 135.7, 135.7, 129.6, 127.4, 126.8, 125.8, 121.5, 120.5, 62.6, 52.7, 46.5, 44.9, 22. 5, 18.0, 16.3, 15.7, 4.0, 3.4.

ES+ $(M + H)^+$ observed 568.2070 expected 568.2052.

N-(5-{2-[(1S)-1-cyclopropylethyl]-7-[{4-[(dimethylamino)-methyl]phenyl}(methyl)sulfamoyl]-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)acetamide (***13***). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.24 (s, 1H), 7.93 (d, J = 1.6 Hz, 1H), 7.65 (d, J = 1.6 Hz, 1H), 7.21−7.16 (m, 2H), 7.16−7.11 (m, 2H), 4.67−4.58 (m, 2H), 3.63 (dq, J = 9.2, 6.8 Hz, 1H), 3.43 (s, 3H), 3.35 (s, 2H), 2.22 (s, 3H), 2.15 (s, 3H), 2.08 (s, 6H), 1.30 (d, J = 6.8 Hz, 3H), 1.18−1.09 (m, 1H), 0.63−0.56 (m, 1H), 0.47−0.37 (m, 2H), 0.32−0.24 (m, 1H).

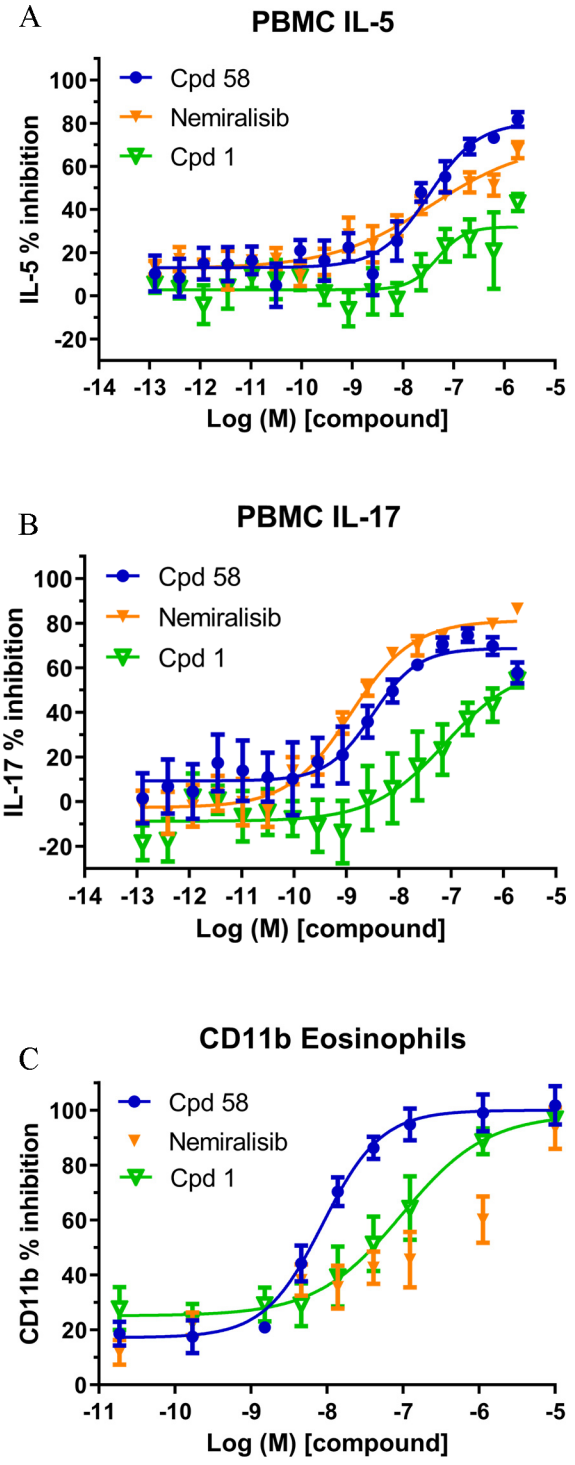

Figure 8. PI3K isoform specific inhibition of T cell cytokine release and eosinophil surface CD11b expression, using PBMC and purified granulocytes from severe asthma patients (GINA 3 and 4). Effects of PI3Kγ (Cpd **1**), PI3Kδ (Nemiralisib), or PI3K$\gamma\delta$ dual (Cpd **58**) selective inhibitors on (A) IL-5, (B) IL-17 release after anti-CD2/3/28 stimulation of PBMCs, and (C) CD11b surface expression on blood-derived eosinophils stimulated with eotaxin-3 measured by flow cytometry. The impact of treatment is visualized as %-inhibition. Error bars depicts SEM, asthmatic donors N = 6−10.

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.7, 163.7, 162.9, 156.2, 146.2, 144.3, 139.9, 136.8, 135.2, 134.8, 129.4, 127.7, 127.4, 125.5, 121.7, 62.3, 51.9, 45.4, 44.6, 38.7, 22.4, 19.0, 16.1, 15.6, 4.0, 3.3.

ES+ $(M + H)^+$ observed 582.2244 expected 582.2209.

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*N-(5-{7-[(4-cyanophenyl)sulfamoyl]-2-[(1S)-1-cyclopropylethyl]-1-oxo-2,3-dihydro-1H-isoindol-5-yl}-4-methyl-1,3-thiazol-2-yl)-acetamide (**14**).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.32 (s, 1H), 10.63 (br. s, 1H), 7.99 (d, J = 1.5 Hz, 1H), 7.93 (d, J = 1.6 Hz, 1H), 7.74−7.67 (m, 2H), 7.31−7.25 (m, 2H), 4.78−4.65 (m, 2H), 3.66 (dq, J = 9.2, 6.8 Hz, 1H), 2.38 (s, 3H), 2.17 (br. s, 3H), 1.33 (d, J = 6.8 Hz, 3H), 1.20−1.11 (m, 1H), 0.65−0.56 (m, 1H), 0.48−0.36 (m, 2H), 0.36−0.27 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 168.8, 164.1, 156.5, 146.3, 145.0, 141.8, 135.7, 135.6, 133.8, 127.8, 127.2, 126.0, 121.5, 119.2, 118.6, 106.2, 52.7, 46.4, 22.5, 18.0, 16.4, 15.7, 3.9, 3.4.

ES+ (M + H)$^+$ observed 536.1389 expected 536.1426.

*N-[5-(2-[(1S)-1-Cyclopropylethyl]-7-{[4-(methylsulfonyl)phenyl]-sulfamoyl}-1-oxo-2,3-dihydro-1H-isoindol-5-yl)-4-methyl-1,3-thiazol-2-yl]acetamide (**15**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.69 (s, 1H), 8.00 (s, 1H), 7.95 (d, J = 1.2 Hz, 1H), 7.80 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 8.7 Hz, 2H), 4.73 (s, 2H), 3.73−3. 65 (m, 1H), 3.12 (s, 3H), 2.40 (s, 3H), 2.18 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.18−1.14 (m,1H), 0.65−0.59 (m, 1H), 0.48−0.40 (m, 2H), 0.37−0.31 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.2, 164.7, 156.9, 151.8, 146.8, 145.4, 142.4, 136.4, 136.2, 129.2, 128.2, 127.6, 126.4, 122.0, 119.5, 53.1, 46.9, 44.0, 22.9, 18.4, 16.8, 16.1, 4.4, 3.9.

ES+ (M + H)$^+$ observed 589.1236 expected 589.1249.

*(S)-5-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**).* **1** (2.28 g, 5.26 mmol) was suspended in EtOH (11.5 mL). Hydrochloric acid (3.8 M, 13.8 mL, 52.6 mmol) was added to the slurry. The reaction mixture was stirred at reflux for 20 h. The reaction mixture was allowed to come to RT and was then cooled to 0 °C. The mixture was neutralized with 2 M NaOH and was then extracted with DCM (100 mL; 2 × 50 mL), and the combined organic layers were dried through a phase separator and concentrated to dryness to give the title compound (1.76 g, 86%) as a yellow solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.9−7.86 (m, 2H), 7.31 (s, 2H), 4.66 (s, 2H), 3.61 (m, 4H), 2.30 (s, 3H), 1.29 (d, J = 6.8 Hz, 3H), 1.17−1.09 (m, 1H), 0.62−0.56 (m, 1H), 0.46−0.36 (m, 2H), 0.29−0.23 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.9, 163.7, 146.8, 145.8, 137.5, 136.3, 126.3, 125.8, 125.7, 115.4, 51.9, 45.8, 43.0, 17.9, 16.8, 15.5, 3.8, 3.4.

ES+ (M + H)$^+$ 392.

*(S)-6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-N-methyl-3-oxoisoindoline-4-sulfonamide (**17**).* Prepared from **5** analogously to **16**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.25 (s, 1H), 7.98 (d, J = 1.4 Hz, 1H), 7.80 (d, J = 1.5 Hz, 1H), 7.56 (q, J = 5.1 Hz, 1H), 4.75 (s, 2H), 3.69−3.61 (m, 1H), 2.51 (s, 3H), 2.34 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.21−1.13 (m, 1H), 0.65−0.57 (m, 1H), 0.48−0.38 (m, 2H), 0.31−0.24 (m, 1H).

ES+ (M + H)$^+$ 407.

General Procedure B, Arylations of Amines 16 and 17. (*S*)-6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (150 mg, 0.37 mmol), haloheterocycle (0.44 mmol, 1.2 equiv), potassium phosphate (157 mg, 0.74 mmol, 2 equiv), $PdOAc_2$ (41 mg, 0.18 mmol, 0.5 equiv), and Xantphos (214 mg, 0.37 mmol. 1 equiv) were mixed in dioxane (10 mL) and sealed into a microwave tube. The reaction was heated to 120 °C for 60 min in a microwave reactor. The solvent was removed under reduced pressure, and the crude product was purified by preparative HPLC.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-methylpyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**18**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.46 (s, 1H), 7.98 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.65−7.58 (m, 2H), 6.87 (d, J = 8.2 Hz, 1H), 6.82 (d, J = 7.3 Hz, 1H), 4.76 (s, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 2.52 (d, J = 5.2 Hz, 3H), 2.48 (s, 3H), 2.44 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.20−1.12 (m, 1H), 0.64−0.57 (m, 1H), 0.48−0.38 (m, 2H), 0.31−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.7, 158.08, 155.3, 150.5, 145.9, 144.9, 138.3, 136.7, 136.0, 126.7, 126.3, 125.1, 119.7, 115.3, 107.8, 52.3, 46.3, 29.2, 23.4, 17.9, 16.7, 15.5, 3.9, 3.4.

ES+ (M + H)$^+$ observed 498.1617 expected 498.1633.

*(S)-6-((5-(2-(1-Cyclopropylethyl)-7-(methylsulfonyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)-N-(2-methoxyethyl)-picolinamide (**19**).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.88 (s, 1H; exchangeable NH and reduced to ∼57%), 8.15 (br. t, J = 5.6 Hz, 1H), 8.08 (s, 2H), 7.92 (t, J = 7.9 Hz, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.26 (d, J = 8.3 Hz, 1H), 4.83−4.57 (m, 2H), 3.67−3.59 (m, 4H), 3.59−3.54 (m, 2H), 3.54−3.48 (m, 2H), 3.04 (s, 3H), 2.47 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.24−1.09 (m, 1H), 0.67−0.52 (m, 1H), 0.51−0.35 (m, 2H), 0.34−0.20 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 163.7, 163.5, 157.2, 150.4, 147.1, 145.8, 145.2, 139.5, 137.7, 135.8, 127.4, 126.9, 126.6, 119.5, 114.7, 114.5, 70.4, 57.6, 52.1, 45.9, 43.1, 38.5, 18.0, 16.6, 15.6, 3.9, 3.4.

ES+ (M + H)$^+$ observed 570.1837 expected 570.1844.

*(S)-6-((5-(2-(1-Cyclopropylethyl)-7-(N-methylsulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)-N-(3-(dimethylamino)-propyl)picolinamide (**20**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.90 (bs, 1H), 9.47 (bs, 1H), 8.24 (t, J = 6.3 Hz, 1H), 8.08 (d, J = 1.3 Hz, 1H), 7.96 (d, J = 1.5 Hz, 1H), 7.96−7.92 (m, 1H), 7.63 (q, J = 5.2 Hz, 1H), 7.62−7.59 (m, 1H), 7.30−7.27 (m, 1H), 4.76 (s, 2H), 3.69−3.63 (m, 1H), 3.51 (q, J = 6.7 Hz, 2H), 3.16−3.08 (m, 2H), 2.74 (s, 3H), 2.73 (s, 3H), 2.53 (d, J = 5.2 Hz, 3H), 2.51 (s, 3H), 2.00−1.92 (m, 2H), 1.33 (d, J = 6.8 Hz, 3H), 1.21−1.14 (m, 1H), 0.65−0. 59 (m, 1H), 0.48−0.4 (m, 2H), 0.31−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 165.1, 164.8, 157.7, 150.8, 147.8, 146.4, 145.9, 140.0, 136.6, 136.6, 127.3, 126.6, 125.8, 119.9, 115.4, 114.9, 55.2, 52.9, 46.9, 42.7, 36.7, 29.7, 25.2, 18.4, 17.2, 16.0, 4.4, 3.8.

ES+ (M + H)$^+$ observed 612.2409 expected 612.2427.

*(S)-6-((5-(2-(1-Cyclopropylethyl)-7-(N-methylsulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)-N-(3-(dimethylamino)-propyl)nicotinamide (**21**).* ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.81 (s, 1H), 8.84 (d, J = 2.3 Hz, 1H), 8.44 (t, J = 5.6 Hz, 1H), 8.12 (dd, J = 8.7, 2.4 Hz, 1H), 8.01 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.61 (s, 1H), 7.09 (d, J = 8.6 Hz, 1H), 4.75 (s, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 3.27 (td, J = 7.2, 5.6 Hz, 2H), 2.52 (s, 3H), 2.47 (s, 3H), 2.25 (t, J = 7.1 Hz, 2H), 2.13 (s, 6H), 1.65 (p, J = 7.1 Hz, 2H), 1.32 (d, J = 6.8 Hz, 3H), 1.20−1.11 (m, 1H), 0.64−0.57 (m, 1H), 0.47−0.37 (m, 2H), 0.32−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.7, 164.3, 157.6, 152.7, 146.2, 145.9, 145.1, 137.0, 136.3, 136.0, 126.7, 126.3, 125.3, 122.7, 120.4, 110.3, 56.8, 52.4, 46.4, 45.2, 37.6, 29.2, 27.1, 18.0, 16.6, 15.5, 3.9, 3.4.

ES+ (M + H)$^+$ observed 612.2415 expected 612.2427.

*(S)-6-((5-(2-(1-Cyclopropylethyl)-7-(N-methylsulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)-N,N-diethylpicolinamide (**22**).* ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.75 (s, 1H), 7.98 (d, J = 1.5 Hz, 1H), 7.86 (d, J = 1.5 Hz, 1H), 7.84 (t, J = 7.8 Hz, 1H), 7.59 (q, J = 5.2 Hz, 1H), 7.11 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 7.4 Hz, 1H), 4.75 (s, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 3.49 (q, J = 7.0 Hz, 2H), 3.35−3.30 (m, 2H), 2.51 (s, 3H), 2.47 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.22−1.12 (m, 4H), 1.03 (t, J = 7.1 Hz, 3H), 0.65−0.56 (m, 1H), 0.50−0.37 (m, 2H), 0.34−0.23 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 167.3, 164.7, 157.8, 152.3, 150.1, 145.9, 145.1, 139.1, 136.4, 136.1, 126.8, 126.0, 125.2, 120.1, 114.1, 111.1, 52.4, 46.3, 42.3, 38.5, 29.1, 17.9, 16.6, 15.5, 14.3, 12.8, 3.9, 3.4.

ES+ (M + H)$^+$ observed 583.2151 expected 583.2161.

*N-(3-Aminopropyl)-6-chloropicolinamide (**23**).* Propane-1,3-diamine (1.6 g, 21.5 mmol) was added to ethyl 6-chloropicolinate (1.0 g, 5.4 mmol) in DCM (10 mL). The resulting mixture was stirred at RT overnight, and then the solvent was evaporated. The residue was purified by reverse phase C18-flash chromatography, elution gradient 0 to 100% MeCN in water to give the title compound (1.1 g, 96%) as a brown oil.

ES+ [M + H]$^+$ 214/216.

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*N-(4-Aminobutyl)-6-bromopicolinamide hydrochloride (**24**).* Step 1: *tert*-Butyl (4-(6-bromopicolinamido)butyl)carbamate

6-Bromopicolinic acid (2.0 g, 9.90 mmol) was dissolved in ethyl acetate (20 mL). Triethylamine (3.0 mL, 21.8 mmol) was added followed by *tert*-butyl (4-aminobutyl)carbamate (2.05 g, 10.9 mmol) and then 1-propanephosphonic acid cyclic anhydride (50% in EtOAc) (8.8 mL, 14.8 mmol). The resultant hazy solution was stirred overnight at RT. The reaction was quenched with water, and the layers were separated. The organic layer was washed with water and then with a sat. solution of sodium bicarbonate and then finally with dilute hydrochloric acid (<1 N). The organic layer was dried over sodium sulfate, filtered, and concentrated to obtain the subtitle compound (3.50 g, 95%) as a light yellow colored gum which slowly crystallized into a solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (dd, J = 7.6, 1.0 Hz, 1H), 7.86 (d, J = 6.8 Hz, 1H), 7.70 (t, J = 7.7 Hz, 1H), 7.60 (dd, J = 7.9, 1.0 Hz, 1H), 4.60 (s, 0H), 4.11 (q, J = 7.1 Hz, 1H), 3.47 (q, J = 6.8 Hz, 2H), 3.15 (d, J = 7.1 Hz, 2H), 2.03 (s, 1H), 1.52−1.71 (m, 4H), 1.25 (t, J = 7.1 Hz, 2H).

ES+ $[M + H]^+$ 372/374.

Step 2: *N*-(4-Aminobutyl)-6-bromopicolinamide hydrochloride

tert-Butyl (4-(6-bromopicolinamido)butyl)carbamate (3.5 g, 9.40 mmol) was dissolved in CH_2Cl_2 (20 mL). Dioxane-HCl 4 M (5 mL, 20 mmol) was added and the solution was stirred overnight at RT. The resulting precipitate was collected and was washed with CH_2Cl_2 to give the title compound (2.80 g, 97%) as a white colored solid.

^{1}H NMR (500 MHz, DMSO-*d6*) δ 8.72 (t, J = 6.2 Hz, 1H), 8.03 (dd, J = 7.6, 0.9 Hz, 1H), 7.94 (t, J = 7.7 Hz, 1H), 7.83−7.9 (m, 3H), 5.76 (s, 1H), 3.31 (d, J = 6.1 Hz, 2H), 2.79 (q, J = 6.2 Hz, 2H), 1.52−1.61 (m, 4H).

ES+ $[M + H]^+$ 272/274.

*N-(5-Aminopentyl)-6-bromopicolinamide (**25**).* Prepared analogously to **23** using pentane-1,5-diamine and methyl 6-bromopicolinate.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (t, J = 5.7 Hz, 1H), 8.03 (dd, J = 7.5, 1.0 Hz, 1H), 7.94 (t, J = 7.7 Hz, 1H), 7.85 (dd, J = 7.9, 0.9 Hz, 1H),3.32−3.23 (m, 2H), 2.55−2.51 (m, 1H), 1.53 (p, J = 7.4 Hz, 3H), 1.42−1.22 (m, 6H).

ES+ $[M + H]^+$ 286/288.

*6-Chloro-N-(3-(methylamino)propyl)picolinamide (**26**).* Prepared analogously to **23** using *N*-1-methylpropane-1,3-diamine.

^{1}H NMR (300 MHz, $CDCl_3$ δ 8.46 (s, 1H), 8.14 (dd, J = 7.5, 1.1 Hz, 1H), 7.83 (td, J = 7.8, 1.2 Hz, 1H), 7.47 (dd, J = 7.8, 1.1 Hz, 1H), 3.57 (q, J = 6.0 Hz, 2H), 2.74 (td, J = 6.7, 1.2 Hz, 2H), 2.49 (s, 3H), 1.92−1.83 (m, 2H).

ES+ $[M + H]^+$ 228/230.

*N-(2-(2-Aminoethoxy)ethyl)-6-bromopicolinamide (**27**).* 2-(2-Aminoethoxy)ethanamine (4.82 g, 46.3 mmol) was added to a solution of methyl 6-bromopicolinate (5.0 g, 23.1 mmol) in THF (100 mL) at 25 °C over a period of 15 min. The resulting solution was heated to 50 °C for 45 h. The solvent was evaporated, and the residue was purified by flash silica chromatography, elution gradient 10 to 10% MeOH in DCM to give the title compound (5.0 g, 75%) as a brown oil.

1H NMR (400 MHz, $CDCl_3$) δ 8.22 (s, 1H), 8.15 (dd, J = 7.6, 1.0 Hz, 1H), 7.72 (t, J = 7.7 Hz, 1H), 7.61 (dd, J = 8.0, 1.0 Hz, 1H), 3.73−3.62 (m, 4H), 3.55 (t, J = 5.1 Hz, 2H), 2.91 (t, J = 5.1 Hz, 2H).

ES+ $[M + H]^+$ 288/290.

*N-(3-((4-Aminobenzyl)(methyl)amino)propyl)-6-chloropicolinamide (**28**).* Step 1 *N*-(3-aminopropyl)-6-chloropicolinamide (1.1 g, 5.15 mmol), *N*-(4-formylphenyl)acetamide (0.924 g, 5.66 mmol), acetic acid (0.309 mL, 5.41 mmol) and sodium cyanotrihydroborate (0.971 g, 15.44 mmol) were dissolved in MeOH (20 mL) to give a brown solution. The resulting mixture was stirred at RT for 1 h. Aqueous formaldehyde (4.73 mL, 51.48 mmol) was added. The resulting mixture was stirred at RT for a further 30 min. The crude product was added to a silica gel column and was eluted with DCM:EA:MeOH 5:5:1 to give *N*-(3-((4-acetamidobenzyl)(methyl)-amino)propyl)-6-chloropicolinamide (0.550 g, 28%) as a pale tan oil.

ES+ $[M + H]^+$ 375/377.

Step 2 *N*-(3-((4-acetamidobenzyl)(methyl)amino)propyl)-6-chloropicolinamide (500 mg, 1.33 mmol) was dissolved in a mixture of hydrochloric acid (3 M aq, 4.45 mL, 13.34 mmol) and ethanol (4.45 mL) to give a colorless solution. The resulting mixture was stirred at 85 °C for 3 h. The solvent was removed under reduced pressure. The crude product was neutralized with saturated aqueous $NaHCO_3$, and evaporated to dryness. The residue was purified by C18-flash chromatography, elution gradient 0 to 100% MeCN in water to give the title compound (460 mg, 100%) as a pale yellow oil.

ES+ $[M + H]^+$ 333/335.

*N-(4-((4-Aminobenzyl)(methyl)amino)butyl)-6-chloropicolinamide (**29**).* Prepared analogously to **28**.

ES+ $[M + H]^+$ 347/349.

*N-(2-((2-Aminoethyl)(methyl)amino)ethyl)-6-bromopicolinamide (**30**).* Methyl 6-bromopicolinate (2.0 g, 9.26 mmol) was dissolved in THF (20 mL). *N*-1-(2-Aminoethyl)-*N*-1-methylethane-1,2-diamine (2.17 g, 18.52 mmol) was added, and the mixture was heated to 50 °C for 16 h. The mixture was allowed to cool, and the volatiles were evaporated. The residue was purified using a Biotage KP-SIL 100 g column eluting with a gradient of 0−8% methanolic ammonia (2 M) in DCM to give the title compound (2.40 g, 86%) as a light yellow colored viscous oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (br. s, 1H), 8.12 (dd, J = 7.6, 0.9 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.57 (dd, J = 7.9, 1.0 Hz, 1H), 3.52 (q, J = 5.82 Hz, 2H), 2.78 (dd, J = 6.4, 5.1 Hz, 2H), 2.61 (t, J = 6.1 Hz, 2H), 2.49 (dd, J = 6.4, 5.0 Hz, 2H), 2.28 (s, 3H).

ES+ $[M + H]^+$ 301/303.

*N-(3-((2-Aminoethyl)(methyl)amino)propyl)-6-chloropicolinamide (**31**).* Step 1: 6-Chloro-*N*-(3-(methylamino)propyl)-picolinamide (**26**, 1.0 g, 4.39 mmol), *tert*-butyl (2-oxoethyl)carbamate (1.05 g, 6.59 mmol), and acetic acid (0.025 mL, 0.44 mmol) were dissolved in MeOH (50 mL) to give a yellow solution which was stirred at 25 °C for 30 min, and then sodium cyanoborohydride (0.828 g, 13.18 mmol) was added, and the resulting mixture was stirred at 25 °C for 12 h. The solvent was evaporated, and the residue was purified by flash silica chromatography, elution gradient 0 to 5% MeOH in DCM to give *tert*-butyl (2-((3-(6-chloropicolinamido)-propyl)(mmethyl)amino)ethyl)carbamate (1.00 g, 61%) as a yellow liquid. ES+ $[M + H]^+$ 371/373.

Step 2: *tert*-Butyl (2-((3-(6-chloropicolinamido)propyl)(methyl)-amino)ethyl)carbamate (600 mg, 1.62 mmol) was dissolved in DCM (10 mL) to give a colorless solution. TFA (5 mL, 64.9 mmol) was added, and the resulting mixture was stirred at 25 °C for 1 h. The volatiles were evaporated, and the residue was dissolved in DCM (150 mL) and washed with 2 M NaOH (50 mL) and saturated brine (100 mL × 2). The organic phase was dried over Na_2SO_4, filtered, and evaporated to give the title compound (400 mg, 91%) as a yellow liquid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.03 (bs, 1H), 8.12 (d, J = 7.6 Hz, 1H), 7.80 (t, J = 7.8 Hz, 1H), 7.43 (d, J = 7.9 Hz, 1H), 3.56 (q, J = 6.0 Hz, 2H), 2.86 (t, J = 5.9 Hz, 2H), 2.56−2.42 (m, 4H), 2.26 (d, J = 4.9 Hz, 3H), 1.90−1.77 (m, 4H).

ES+ $[M + H]^+$ 271/273.

*(S)-1^2-(1-Cyclopropylethyl)-2^4,12-dimethyl-1^2,1^3-dihydro-1^{1}H-16-thia-3,6,12,15-tetraaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridina-14(1,4)-benzenacyclohexadecaphane-1^3,5-dione 16,16-dioxide (**32**).* Step 1: (*S*)-6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonyl chloride (**4**, 558 mg, 1.23 mmol), *N*-(3-((4-aminobenzyl)(methyl)amino)propyl)-6-chloropicolinamide (**28**, 450 mg, 1.35 mmol), and triethylamine (373 mg, 3.69 mmol) were dissolved in DCM (10 mL) to give a yellow solution. The resulting mixture was stirred at RT for 3 h, and then the solvent was evaporated. The residue was purified by flash C18-flash chromatography, elution gradient 0 to 100% MeCN in water to give (*S*)-*N*-(3-((4-(6-(2-acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)benzyl)(methyl)amino)-propyl)-6-chloropicolinamide (400 mg, 43%) as a yellow solid.

ES+ $[M + H]^+$ 750/752.

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Step 2: (*S*)-*N*-(3-((4-(6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)benzyl)(methyl)-amino)propyl)-6-chloropicolinamide.

(*S*)-*N*-(3-((4-(6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)benzyl)(methyl)-amino)propyl)-6-chloropicolinamide (390 mg, 0.52 mmol) was dissolved in ethanol (8.7 mL) to give a colorless solution. Hydrogen chloride (3 M aq, 8.66 mL, 26 mmol) was added, and the reaction was heated under reflux for 3 h. The solvent was evaporated, and the residue was diluted with MeOH (5 mL), basified with saturated $NaHCO_3$, and purified by flash C18-flash chromatography, elution gradient 0 to 100% MeCN in water to give the subtitle compound (320 mg, 87%) as a yellow solid.

ES+ $[M + H]^+$ 708/710.

Step 3: (*S*)-*N*-(3-((4-(6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)benzyl)(methyl)-amino)propyl)-6-chloropicolinamide (320 mg, 0.45 mmol), $PdOAc_2$ (51 mg, 0.23 mmol), 2-di-*tert*-butylphosphino-2′,4′,6′-triisopropylbiphenyl (211 mg, 0.50 mmol) and DIPEA (0.473 mL, 2.71 mmol) were dissolved in DMF (40 mL) to give a brown solution. The resulting mixture was stirred at 130 °C overnight. The solvent was evaporated, and the residue was purified by preparative HPLC (X Bridge RP18, 19 × 150 mm, 5 μm; mobile phase A: water/10 nmol NH_4HCO_3, mobile phase B: MeCN; flow rate: 30 mL/min; gradient: 48% B to 55% B over 8 min) to give the title compound (30 mg, 10%) as a yellow solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.82 (s, 1H), 10.08 (s, 1H), 8.00 (s, 1H), 7.91−7.85 (m, 2H), 7.59−7.56 (m, 1H), 7.51 (d, J = 7.4 Hz, 1H), 7.20 (t, J = 8.7 Hz, 3H), 7.04 (d, J = 8.4 Hz, 2H), 4.81 (s, 2H), 3.78−3.72 (m, 1H), 3.32 (s, 6H), 2.36 (s, 3H), 2.30 (s, 3H), 2.01−1.92 (m, 2H), 1.40 (d, J = 6.8 Hz, 3H), 1.26−1.19 (m,1H), 0.67−0.61 (m, 1H), 0.54−0.45 (m, 2H), 0.42−0.35 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 165.4, 163.8, 157.7, 150.6, 147.9, 146.4, 140.0, 137.7, 136.5, 136.3, 135.9, 130.6, 129.0, 128.3, 126.7, 122.1, 119.5, 114.8, 114.6, 61.6, 53.4, 53.3, 47.1, 44.0, 39.3, 27.5, 18.5, 16.1, 156.0, 4.5, 3.8.

ES+ $(M + H)^+$ observed 672.2441 expected 672.2427.

*(S)-1^2-(1-Cyclopropylethyl)-2^4,10-dimethyl-1^2,1^3-dihydro-1^{1}H-14-thia-3,6,10,13-tetraaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridina-12(1,4)-benzenacyclotetradecaphane-1^3,5-dione 14,14-dioxide (**33**).* Prepared analogously to compound **32** using **4** and **29**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.81 (s, 1H), 9.96 (s, 1H), 7.98 (s, 1H), 7.93−7. 87 (m, 1H), 7.84 (t, J = 5.8 Hz, 1H), 7.73−7.69 (m, 1H), 7.55 (d, J = 7.2 Hz, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 8.4 Hz, 2H), 4.73 (s, 2H), 3.76−3.73 (m, 1H), 3.21 (d, J = 5.6 Hz, 2H), 2.36 (s, 3H), 2.17 (s, 3H), 1.79 (t, J = 6.6 Hz, 2H), 1.40 (d, J = 6.8 Hz, 3H), 1.33−1.26 (m, 2H), 1.25−1.17 (m, 3H), 0.67−0.61 (m, 1H), 0.53−0.44 (m, 2H), 0.39−0.33 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 165.3, 164.1, 157.7, 150.8, 148.1, 146.1, 145.6, 139.9, 136.5, 136.5, 136.1, 136.0, 130.1, 128.6, 128.4, 126.4, 121.9, 119.4, 115.0, 114.7, 62.0, 54.0, 53.3, 46.9, 43.2, 39.3, 26.9, 24.1, 18.6, 16.3, 16.1, 4.5, 3.8.

ES+ $(M + H)^+$ observed 686.257 expected 686.2583.

*(S)-1^2-(1-Cyclopropylethyl)-2^4-methyl-1^2,1^3-dihydro-1^{1}H-13-thia-3,6,12-triaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridinacyclotridecaphane-1^3,5-dione 13,13-dioxide (**34**).* Prepared analogously to compound **32** using **4** and **25**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.90 (s, 1H), 8.06 (s, 1H), 7.92 (t, J = 7.8 Hz, 1H), 7.90−7.85 (m, 2H), 7.57 (d, J = 7.3 Hz, 1H), 7.23 (d, J = 8.3 Hz, 1H), 7.15−7.11 (m, 1H), 4.75 (s, 2H), 3.67−3.60 (m, 1H), 3.26 (dt, J = 11.4, 5.8 Hz, 2H), 2.80−2.73 (m, 2H), 2.48 (s, 3H), 1.61−1.52 (m, 2H), 1.51−1.43 (m, 2H), 1.32 (d, J = 6.8 Hz, 3H), 1.27−1.20 (m, 2H), 1.20−1.12 (m, 1H), 0.64−0.58 (m, 1H), 0.47−0.38 (m, 2H), 0.30−0.25 (m, 1H).

ES+ $(M + H)^+$ observed 581.2052 expected 581.2004.

*(S)-1^2-(1-cyclopropylethyl)-2^4,9-dimethyl-1^2,1^3-dihydro-1^{1}H-13-thia-3,6,9,12-tetraaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridinacyclotridecaphane-1^3,5-dione 13,13-dioxide (**35**).* Prepared analogously to compound **32** using **4** and **30**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.89 (s, 1H), 8.07 (s, 1H), 7.94−7. 89 (m, 3H), 7.58 (d, J = 7.4 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 7.10 (t, J = 4.8 Hz, 1H), 4.75 (s, 2H), 3.68−3.61 (m, 1H), 3.45−3.37 (m, 2H), 2.93−2. 82 (m, 2H), 2.62 (t, J = 7.9 Hz, 2H), 2.50 (s, 3H), 2.49−2.44 (m, 2H), 2.21 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.19−1.13 (m, 1H), 0.63−0.58 (m, 1H), 0.47−0.38 (m, 2H), 0.30−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.6, 163.9, 157.6, 150.7, 147.7, 146.6, 144.5, 139.9, 136.3, 136.0, 129.2, 127.4, 126.8, 120.3, 115.2, 114.8, 56.9, 55.1, 52.6, 46.8, 43.5, 37.7, 18.4, 16.4, 16.0, 4.4, 3.8.

ES+ $(M + H)^+$ observed 596.2120 expected 596.2114.

*(S)-1^2-(1-cyclopropylethyl)-2^4,10-dimethyl-1^2,1^3-dihydro-1^{1}H-14-thia-3,6,10,13-tetraaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridinacyclotetradecaphane-1^3,5-dione 14,14-dioxide (**36**).* Prepared analogously to compound **32** using **4** and **31**.

Step 1: (*S*)-*N*-(3-((2-(6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)ethyl)(methyl)-amino)propyl)-6-chloropicolinamide.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.57 (bs, 1H), 8.62 (bs, 1H), 8.11 (s, 1H), 8.08 (d, J = 7.6 Hz, 1H), 7.77 (t, J = 7.8 Hz, 2H), 7.65 (s, 1H), 7.38 (d, J = 7.9 Hz, 1H), 4.70−4. 45 (m, 2H), 3.87−3.70 (m, 1H), 3.47 (q, J = 6.1 Hz, 2H), 3.17 (bs, 2H), 2.66−2.44 (m, 3H), 2.42 (s, 3H), 2.30 (s, 3H), 2.18 (bs, 3H), 1.67 (bs, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.11−0.94 (m, 1H), 0.73−0.59 (m, 1H), 0.52−0.29 (m, 3H).

ES+ $(M + H)^+$ 688/690.

Step 2: (*S*)-*N*-(3-((2-(6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonamido)ethyl)(methyl)-amino)propyl)-6-chloropicolinamide.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.98 (s, 1H), 7.80−7.70 (m, 2H), 7.55 (s, 1H), 7.37 (d, J = 7.9 Hz, 1H), 5.21−5.06 (m, 2H), 4.45−4.68 (m, 2H), 3.81−3.71 (m, 1H), 3.50−3.41 (m, 2H), 3.21−3.10 (m, 2H), 2.57 (bs, 2H), 2.45 (bs, 2H), 2.35 (d, J = 1.4 Hz, 3H), 2.17 (s, 3H), 1.34 (d, J = 6.8 Hz, 3H), 1.25 (s, 1H), 1.06−0.96 (m, 1H), 0.90−0.79 (m, 1H), 0.69−0.60 (m, 1H), 0.49−0.30 (m, 3H).

ES+ $(M + H)^+$ 646/648.

Step 3: (*S*)-1^2-(1-cyclopropylethyl)-2^4,10-dimethyl-1^2,1^3-dihydro-1^1*H*-14-thia-3,6,10,13-tetraaza-2(5,2)-thiazola-1(6,4)-isoindolina-4-(2,6)-pyridinacyclotetradecaphane-1^3,5-dione 14,14-dioxide.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.88 (s, 1H), 7.98 (s, 1H), 7.95−7. 89 (m, 2H), 7.79 (d, J = 1.2 Hz, 1H), 7.68 (t, J = 5.9 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 4.76 (s, 2H), 3.71−3. 64 (m, 1H), 3.29−3.23 (m, 2H), 3.22−3.13 (m, 2H), 2.45 (s, 3H), 2.38−2.29 (m, 2H), 2.26−2.18 (m, 2H), 1.75 (s, 3H), 1.59 (p, J = 7.8 Hz, 2H), 1.35 (d, J = 6.8 Hz, 3H), 1.21−1.14 (m, 1H), 0.65−0.58 (m, 1H), 0.46−0.40 (m, 2H), 0.32−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 165.4, 163.9, 157.4, 150.7, 147.8, 146.0, 144.6, 140.4, 140.0, 136.1, 126.7, 126.3, 125.7, 120.1, 114.9, 114.7, 54.9, 52.79, 52.77, 46.7, 43.4, 41.8, 38.4, 26.1, 18.5, 16.2, 16.1, 4.5, 3.7.

ES+ $(M + H)^+$ observed 610.2282 expected 610.2270.

*(S)-1^2-(1-Cyclopropylethyl)-2^4-methyl-1^2,1^3-dihydro-1^{1}H-9-oxa-13-thia-3,6,12-triaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridinacyclotridecaphane-1^3,5-dione 13,13-dioxide (**37**).* Prepared analogously to compound **32** using **4** and **27**.

Step 1: (*S*)-*N*-(2-(2-((6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline)-4-sulfonamido)ethoxy)ethyl)-6-bromopicolinamide.

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (s, 1H), 8.26−8.17 (m, 1H), 8.14 (d, J = 1.4 Hz, 1H), 8.12 (dd, J = 7.5, 1.0 Hz, 1H), 8.05−7.97 (m, 1H), 7.95 (t, J = 5.9 Hz, 1H), 7.74−7.65 (m, 2H), 7.58 (dd, J = 7.9, 1.0 Hz, 1H), 4.72−4.49 (m, 2H), 3.87−3.73 (m, 1H), 3.60−3.41 (m, 6H), 3.22 (q, J = 5.6 Hz, 2H), 2.43 (s, 3H), 2.30 (s, 3H), 1.35 (d, J = 6.8 Hz, 3H), 1.11−0.95 (m, 1H), 0.70−0.60 (m, 1H), 0.51−0.40 (m, 1H), 0.4−0.31 (m, 2H).

ES+ $(M + H)^+$ 705/707.

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Step 2: (S)-*N*-(2-(2-((6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline)-4-sulfonamido)ethoxy)ethyl)-6-bromopicolinamide.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.49 (s, 1H), 7.99 (dd, J = 7.4, 1.1 Hz, 1H), 7.91 (t, J = 7.6 Hz, 2H), 7.83 (dd, J = 7.8, 1.1 Hz, 1H), 7.78 (d, J = 1.4 Hz, 1H), 7.71 (d, J = 1.5 Hz, 1H), 4.70 (s, 2H), 3.67−3. 53 (m, 1H), 3.37 (t, J = 5.1 Hz, 2H), 3.26 (m, 4H), 3.03 (s, 2H), 2.28 (s, 3H), 1.33−1.20 (m, 4H), 1.13−1. 06 (m, 1H), 0.89−0.81 (m, 1H), 0.59−0.47 (m, 1H), 0.46−0.29 (m, 2H), 0.28−0.17 (m, 1H).

ES+ $(M + H)^+$ 663/665.

Step 3: (*S*)-1^2-(1-Cyclopropylethyl)-2^4-methyl-1^2,1^3-dihydro-1^1*H*-9-oxa-13-thia-3,6,12-triaza-2(5,2)-thiazola-1(6,4)-isoindolina-4(2,6)-pyridinacyclotridecaphane-1^3,5-dione 13,13-dioxide.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.07−8.03 (m, 2H), 7.94−7.90 (m, 2H), 7.59−7.62 (m, 1H), 7.43−7.39 (m, 1H), 7.24 (d, J = 8.3 Hz, 1H), 4.72 (s, 2H), 3.66−3.60 (m, 1H), 3.60−3. 51 (m, 6H), 3.17−3. 08 (m, 2H), 1.31 (d, J = 6.8 Hz, 3H), 1.19−1.12 (m, 1H), 0.63−0.58 (m, 1H), 0.47−0.38 (m, 2H), 0.31−0.25 (m, 1H). 3H obscured.

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.1, 163.9, 157.7, 150.9, 147.8, 146.4, 145.3, 139.9, 137.5, 136.0, 127.7, 127.0, 126.8, 120.5, 115.3, 114.9, 68.8, 68.6, 52.4, 46.4, 42.0, 39.0, 18.4, 16.9, 16.0, 4.4, 3.8.

ES+ $(M + H)^+$ observed 583.1800 expected 583.1797.

*2-Bromo-6-(1,3-dimethyl-1H-1,2,4-triazol-5-yl)pyridine (**38**). 2-Bromo-6-(1,5-dimethyl-1H-1,2,4-triazol-3-yl)pyridine (**39**).* Step 1: 6-Bromopicolinic acid (200 mg, 0.99 mmol), acetimidamide hydrochloride (140 mg, 1.49 mmol) and DIPEA (0.692 mL, 3.96 mmol) were dissolved in DMF (5 mL) at 25 °C under nitrogen. HATU (565 mg, 1.49 mmol) was added, and the resulting mixture was stirred for 12 h. The reaction mixture was poured onto water and was then extracted with ethyl acetate (3 × 10 mL), and the organic phases were combined, dried, filtered, and evaporated to give a product that was used without purification (240 mg).

Step 2: Methylhydrazine sulfate (0.536 g, 3.72 mmol) was added to (*Z*)-*N*-(1-aminoethylidene)-6-bromopicolinamide (previous step, 0.60 g, 2.48 mmol) and acetic acid (1.49 g, 24.8 mmol) in DMF (20 mL) at 25 °C over a period of 15 min. The resulting mixture was heated to 80 °C for 12 h. The reaction mixture was quenched with water (25 mL) and extracted with DCM (3 × 20 mL), and the organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash silica chromatography, eluting with 30% EtOAc in petroleum ether, to give the title compounds (0.060 g, 9.6%) and (0.180 g, 29%) as yellow solids.

38 ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (d, J = 7.7 Hz, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 4.27 (s, 3H), 2.40 (s, 3H).

ES+ $(M + H)^+$ 253/255.

39 ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.77 (d, J = 7.6 Hz, 1H), 7.60 (t, J = 7.8 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 3.63 (s, 3H), 2.23 (s, 3H).

ES+ $(M + H)^+$ 253/255.

*(5-(6-Bromopyridin-2-yl)oxazol-4-yl)methanol (**40**).* Step 1: Ethyl 5-(6-bromopyridin-2-yl)oxazole-4-carboxylate

Oxalyl chloride (0.942 g, 7.43 mmol) was added to 6-bromopicolinic acid (1 g, 4.95 mmol) and *N*,*N*-dimethylformamide (0.036 g, 0.50 mmol) in DCM (15 mL) at 0 °C. The resulting mixture was stirred at r.t. for 5 h. The volatiles were evaporated to leave a brown solid (1.10 g, 100%) that was used without purification.

6-Bromopicolinoyl chloride (500 mg, 2.27 mmol), ethyl 2-isocyanoacetate (308 mg, 2.72 mmol), DMAP (14 mg, 0.11 mmol), and TEA (1.31 g, 12.9 mmol) were dissolved in THF (20 mL), and the mixture was heated to 60 °C for 12 h. The reaction mixture was allowed to cool and was filtered. The filtrate was diluted with EtOAc, and the resultant solution was washed with water and brine. The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash silica chromatography, elution gradient 0 to 20% EtOAc in petroleum ether to give the title compound (270 mg, 40%) as a brown solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.17 (d, J = 7.6 Hz, 1H), 7.94 (t, J = 7.8 Hz, 1H), 7.78 (d, J = 7.9 Hz, 1H), 4.29 (q, J = 7.1 Hz, 2H), 1.26 (t, J = 7.1 Hz, 3H).

ES+ $(M + H)^+$ 297/299.

Step 2 (5-(6-Bromopyridin-2-yl)oxazol-4-yl)methanol

Ethyl 5-(6-bromopyridin-2-yl)oxazole-4-carboxylate (200 mg, 0.67 mmol) was dissolved in EtOH (20 mL), and the solution was cooled to 0 °C. $NaBH_4$ (153 mg, 4.04 mmol) was added, and the resulting mixture was heated to 70 °C for 2 h. Water (15 mL) was added to the cooled reaction mixture which was then extracted with EtOAc (3 × 15 mL), and the organic phases were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash alumina chromatography, elution gradient 0 to 80% EtOAc in petroleum ether to give the title compound (110 mg, 64%) as a yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.50 (s, 1H), 7.89 (t, J = 7.8 Hz, 1H), 7.82−7.75 (m, 1H), 7.63 (dd, J = 7.9, 0.7 Hz, 1H), 5.21 (t, J = 5.9 Hz, 1H), 4.76 (d, J = 5.8 Hz, 2H).

ES+ $(M + H)^+$ 255/257.

*N-(6-Bromopyridin-2-yl)-N-methylacetamide (**41**).* 6-Bromo-*N*-methylpyridin-2-amine (200 mg, 1.07 mmol) was dissolved in acetic anhydride (303 μL, 3.2 mmol) and pyridine (259 μL, 3.2 mmol). The resulting solution was heated to 120 °C for 2 h. The reaction mixture was concentrated, and then EtOAc (100 mL) was added. The solution was washed with saturated $NaHCO_3$ (75 mL × 2), 0.1 M HCl (75 mL × 2) and saturated brine (75 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and evaporated to give the title compound (180 mg, 73%) as a yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (t, J = 7.8 Hz, 1H), 7.45−7.31 (m, 2H), 3.40 (s, 3H), 2.19 (s, 3H).

ES+ $(M + H)^+$ 229/231.

*1-(6-Bromopyridin-2-yl)pyrrolidin-2-one (**42**).* Pyrrolidin-2-one (1.61 mL, 21.1 mmol) was added to a solution of 2,6-dibromopyridine (5.0 g, 21.1 mmol) in dioxane (5 mL). Xantphos (3.66 g, 6.3 mmol) and Cs_2CO_3 (20.63 g, 63.3 mmol), second Generation XantPhos precatalyst (3.75 g, 4.2 mmol) were added. The resulting mixture was stirred at 105 °C for 5 h. The crude product was purified by flash silica chromatography, elution gradient 0 to 1:20 EtOAc in petroleum ether to give the title compound (4.00 g, 79%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.33−8.26 (m, 1H), 7.79−7.71 (m, 1H), 7.4−7.33 (m, 1H), 3.97−3.89 (m, 2H), 2.58 (t, J = 8.1 Hz, 2H), 2.09−1.97 (m, 2H).

ES+ $(M + H)^+$ 241/243.

*1-(6-Bromopyridin-2-yl)imidazolidin-2-one (**43**).* Step 1: 1-(6-Bromopyridin-2-yl)-3-(2-chloroethyl)urea 6-bromopyridin-2-amine (3.0 g, 17.3 mmol) was added to a solution of 1-chloro-2-isocyanatoethane (2.20 g, 20.8 mmol) in toluene (20 mL. The resulting mixture was heated to 70 °C for 2 h. The reaction mixture was filtered through a Celite pad, and the crude product was purified by crystallization from EtOAc/petroleum ether = 1/10 to give the subtitle compound (3.10 g, 64%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 9.59 (s, 1H), 7.70−7.57 (m, 2H), 7.30 (t, J = 5.9 Hz, 1H), 7.16 (dd, J = 6.7, 1.7 Hz, 1H), 3.68 (t, J = 5.9 Hz, 2H), 3.48 (q, J = 5.9 Hz, 2H).

ES+ $(M + H])^+$ = 278/280/282.

Step 2:1-(6-Bromopyridin-2-yl)imidazolidin-2-one

A solution of 1-(6-bromopyridin-2-yl)-3-(2-chloroethyl)urea (3.0 g, 10.8 mmol) in DMF (25 mL) was added to a stirred suspension of 60% sodium hydride (0.775 g, 19.39 mmol) in THF (25 mL) at 0 °C under nitrogen. The resulting mixture was stirred at 25 °C for 1 h. The reaction mixture was quenched by the addition of water (10 mL), filtered, and evaporated to give the title compound (2.10 g, 81%) white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (d, J = 8.3 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.33−7.39 (m, 1H), 7.18 (d, J = 7.5 Hz, 1H), 3.93 (dd, J = 8.9, 7.1 Hz, 2H), 3.40 (t, J = 8.0 Hz, 2H).

ES+ $(M + H])^+$ = 242/244.

*1-(6-Bromopyridin-2-yl)-3-methylimidazolidin-2-one (**44**).* Prepared following the method of example **42** using 1-methylimidazolidin-2-one.

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^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27−8.17 (m, 1H), 7.48−7. 37 (m, 1H), 7.08−6.98 (m, 1H), 4.06−3.96 (m, 2H), 3.51−3.40 (m, 2H), 2.92−2.85 (m, 3H).

ES+ $(M + H])^+$ = 256/258.

*(R)-1-(6-bromopyridin-2-yl)-3-((tert-butyldimethylsilyl)oxy)-pyrrolidin-2-one (**45**).* Step 1: (*R*)-3-((*tert*-butyldimethylsilyl)oxy)-pyrrolidin-2-one

tert-Butylchlorodimethylsilane (2.24 g, 14.8 mmol) was added to a solution of (*R*)-3-hydroxypyrrolidin-2-one (1.0 g, 9.89 mmol) and imidazole (1.35 g, 19.8 mmol) in DMF (15 mL). The resulting mixture was stirred at RT for 2 h. The reaction mixture was diluted with EtOAc (50 mL) and washed sequentially with water (20 mL × 3), saturated brine (20 mL × 2). The aqueous layer was dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash silica chromatography, elution gradient 0 to 2% DCM in MeOH to give the subtitle compound (1.90 g, 89%) as a pale yellow solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.72 (s, 1H), 4.19 (t, J = 8.0 Hz, 1H), 3−3.22 (m, 2H), 2.21−2.37 (m, 1H), 1.77 (dq, J = 12.4, 8.4 Hz, 1H), 0.86 (s, 9H), 0.08 (d, J = 1.4 Hz, 6H).

ES+ $(M\text{-}t\text{-}Bu)^+$ = 158.

Step 2: (*R*)-1-(6-bromopyridin-2-yl)-3-((*tert*-butyldimethylsilyl)-oxy)pyrrolidin-2-one

Prepared following the method of example **42** using (R)-3-((*tert*-butyldimethylsilyl)oxy)pyrrolidin-2-one

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.31 (dd, J = 8.2, 0.7 Hz, 1H), 7.81−7. 73 (m, 1H), 7.40 (dd, J = 7.6, 0.7 Hz, 1H), 4.62 (dd, J = 9.7, 8.0 Hz, 1H), 4.02−3.89 (m, 1H), 3.73−3.58 (m, 1H), 2.48−2.37 (m, 1H), 1.95−1.77 (m, 1H), 0.90 (s, 9H), 0.14 (s, 3H), 0.13 (s, 3H).

ES+ $(M + H)^+$ = 371/373.

*(R)-1-(6-Bromopyridin-2-yl)-4-((tert-butyldimethylsilyl)oxy)-pyrrolidin-2-one (**46**).* Step 1: (*R*)-4-((*tert*-butyldimethylsilyl)oxy)-pyrrolidin-2-one.

Prepared from (*R*)-4-hydroxypyrrolidin-2-one following the method of example **45** Step 1.

ES+ $(M + H])^+$ = 216.

Step 2: (*R*)-1-(6-bromopyridin-2-yl)-4-((*tert*-butyldimethylsilyl)-oxy)pyrrolidin-2-one.

Prepared from (*R*)-4-((*tert*-butyldimethylsilyl)oxy)pyrrolidin-2-one following the method of example **45** Step 2.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.30 (d, J = 8.3 Hz, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 7.7 Hz, 1H), 4.65−4.52 (m, 1H), 4.11 (dd, J = 11.6, 5.1 Hz, 1H), 3.80 (d, J = 11.6 Hz, 1H), 3.00 (dd, J = 17.1, 6.0 Hz, 1H), 2.39 (d, J = 17.1 Hz, 1H), 0.85 (s, 9H), 0.09 (s, 6H).

ES+ $(M + H])^+$ = 371/373.

*1-(6-Bromopyridin-2-yl)piperidin-2-one (**47**).* Step 1: *N*-(6-Bromopyridin-2-yl)-5-chloropentanamide

5-Chloropentanoyl chloride (1.08 g, 6.94 mmol) was added to a solution of 6-bromopyridin-2-amine (1.0 g, 5.78 mmol) and pyridine (1,37 g, 17.3 mmol) in MeCN (10 mL). The resulting mixture was stirred at RT for 5 h. The solvent was removed under reduced pressure to give the subtitle compound (1.68 g, 100%) as a colorless oil which crystallized on standing. Used without further purification.

ES+ $(M + H])^+$ = 291/293/295.

Step 2:1-(6-Bromopyridin-2-yl)piperidin-2-one.

Cs_2CO_3 (11.26 g, 34.6 mmol) was added to a solution of *N*-(6-bromopyridin-2-yl)-5-chloropentanamide (1.68 g, 5.76 mmol) in DMF (20 mL). The resulting suspension was stirred at 80 °C for 2 h. The reaction mixture was allowed to cool and was then diluted with DCM (75 mL) and washed sequentially with water (50 mL × 2) and saturated brine (75 mL × 2). The organic layer was dried over Na_2SO_4, filtered and evaporated to give the title compound (1.48 g, 100%) as a colorless oil.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (dd, J = 8.1, 1.1 Hz, 1H), 7.72 (dd, J = 8.1, 7.3 Hz, 1H), 7.42 (dd, J = 7.4, 1.0 Hz, 1H), 3.80 (t, J = 5.7 Hz, 2H), 2.53−2.46 (m, 2H), 1.91−1.75 (m, 4H).

ES+ $(M + H])^+$ = 255/257.

*4-(6-Bromopyridin-2-yl)morpholin-3-one (**48**).* Prepared following the method of example **42** using morpholin-3-one.

^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (d, J = 8.1 Hz, 1H), 7.78 (t, J = 8.0 Hz, 1H), 7.46 (d, J = 7.7 Hz, 1H), 4.27 (s, 2H), 3.98 (dd, J = 6.0, 3.6 Hz, 2H), 3.91 (dd, J = 6.0, 3.6 Hz, 2H).

ES+ $(M + H])^+$ = 257/259.

*3-(6-Bromopyridin-2-yl)-1,3-oxazinan-2-one (**49**).* Step 1: 3-Chloropropyl (6-bromopyridin-2-yl)carbamate

Pyridine (3.51 mL, 43.4 mmol) was added dropwise to a solution of 6-bromopyridin-2-amine (5.0 g, 28.9 mmol) and 3-chloropropyl carbonochloridate (5.44 g, 34.7 mmol) in acetonitrile (50 mL) cooled in ice over a period of 15 min. The resulting solution was stirred at RT for 4 h. The reaction mixture was diluted with EtOAc(150 mL) and was then washed with 0.1 M HCl (3 × 100 mL) and brine (2 × 100 mL). The organic layer was dried over Na_2SO_4, filtered, and evaporated to give the subtitle compound (8.20 g, 97%) as a pale yellow oil which solidified on standing.

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.50 (s, 1H), 7.83 (dd, J = 8.3, 0.7 Hz, 1H), 7.71 (t, J = 7.9 Hz, 1H), 7.25−7.3 (m, 1H), 4.20 (t, J = 6.2 Hz, 2H), 3.66−3.77 (m, 2H), 2.06 (p, J = 6.3 Hz, 2H).

ES+ $(M + H)^+$ = 293/295/297.

Step 2:3-(6-Bromopyridin-2-yl)-1,3-oxazinan-2-one

Cs_2CO_3 (1.89 g, 5.79 mmol) was added to a solution of 3-chloropropyl (6-bromopyridin-2-yl)carbamate (1.70 g, 5.79 mmol) and KI (0.96 g, 5.79 mmol) in DMF (20 mL). The resulting suspension was stirred at 60 °C for 1 h. The reaction mixture was filtered through Celite and then diluted with EtOAc (100 mL). The resulting suspension was washed sequentially with water (100 mL × 3) and saturated brine (100 mL × 2). The organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash silica chromatography, elution gradient 0 to 50% EtOAc in petroleum ether to give the title compound (1.20 g, 81%) as a white solid.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.87 (dd, J = 8.2, 0.7 Hz, 1H), 7.74 (dd, J = 8.2, 7.6 Hz, 1H), 7.42 (dd, J = 7.6, 0.7 Hz, 1H), 4.35 (t, J = 5.4 Hz, 2H), 3.87 (t, J = 6.2 Hz, 2H), 2.11 (qd, J = 6.1, 4.7 Hz, 2H).

ES+ $(M + H)^+$ = 257/259.

*Benzyl 4-(6-bromopyridin-2-yl)-3-oxopiperazine-1-carboxylate (**50**).* Prepared from benzyl 3-oxopiperazine-1-carboxylate following the method of example **42**.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.53−7.6 (m, 1H), 7.31−7.42 (m, 5H), 7.29 (d, J = 7.6 Hz, 1H), 5.18 (s, 2H), 4.35 (s, 2H), 4.08−4.18 (m, 2H), 3.79−3.85 (m, 2H), 1.23−1.34 (m, 1H).

ES+ $(M + H)^+$ = 390/392.

*1-(6-Bromopyridin-2-yl)-4-methylpiperazin-2-one (**51**).* Prepared from 4-methylpiperazine-2-one following the method of Example **42**.

^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (d, J = 8.2 Hz, 1H), 7.77 (t, J = 7.9 Hz, 1H), 7.47 (d, J = 7.7 Hz, 1H), 3.83 (t, J = 5.6 Hz, 2H), 3.19 (s, 2H), 2.73 (t, J = 5.5 Hz, 2H), 2.27 (s, 3H).

ES+ $(M + H)^+$ = 270/272.

*(S)-2-(1-Cyclopropylethyl)-5-(2-((6-(1,3-dimethyl-1H-1,2,4-triazol-5-yl)pyridin-2-yl)amino)-4-methylthiazol-5-yl)-7-(methylsulfonyl)isoindolin-1-one (**52**).* (*S*)-5-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**, 100 mg, 0.26 mmol), 2-bromo-6-(1,3-dimethyl-1*H*-1,2,4-triazol-5-yl)pyridine (**38**, 64 mg, 0.26 mmol), Xantphos (44 mg, 0.08 mmol), second Generation Xantphos precatalyst (45 mg, 0.05 mmol), and Na_2CO_3 (81 mg, 0.77 mmol) were dissolved in DMF (5 mL) and sealed into a microwave tube. The reaction was heated to 120 °C for 1 h in a microwave reactor and cooled to RT. The reaction mixture was poured into water (10 mL) and extracted with DCM (3 × 10 mL), and the organic layer was dried over Na_2SO_4, filtered, and evaporated. The residue was purified by preparative HPLC (XBridge Prep Phenyl OBD column, 5 μ silica, 19 mm diameter, 150 mm length) using decreasingly polar mixtures of water (containing 0.5% NH_4HCO_3) and MeCN as eluents to give the title compound (44 mg, 30%) as a yellow solid.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.75 (s, 1H), 8.03 (d, J = 1.6 Hz, 1H), 8.01 (d, J = 1.7 Hz, 1H), 7.90 (dd, J = 8.4, 7.4 Hz, 1H), 7.51 (dd, J = 7.3, 0.8 Hz, 1H), 7.24 (d, J = 8.3 Hz, 1H), 4.75−4.65 (m, 2H), 4.19 (s, 3H), 3.68−3.58 (m, 4H), 2.47 (s, 3H), 2.30 (s, 3H),

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1.30 (d, J = 6.9 Hz, 3H), 1.18−1.11 (m, 1H), 0.64−0.56 (m, 1H), 0.48−0.37 (m, 2H), 0.31−0.23 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 163.65, 158.72, 157.46, 152.31, 151.15, 145.98, 145.31, 144.68, 139.12, 137.65, 135.75, 126.89, 126.68, 126.21, 119.83, 117.25, 112.48, 52.00, 45.95, 43.05, 37.57, 17.95, 16.61, 15.55, 13.45, 3.86, 3.40.

ES+ (M + H)$^+$ observed 564.1871 expected 564.1851.

(S)-2-(1-Cyclopropylethyl)-5-(2-((6-(1,5-dimethyl-1H-1,2,4-triazol-3-yl)pyridin-2-yl)amino)-4-methylthiazol-5-yl)-7-(methylsulfonyl)isoindolin-1-one (***53***). Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and 2-bromo-6-(1,5-dimethyl-1*H*-1,2,4-triazol-3-yl)pyridine (**39**) following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.62 (s, 1H), 8.15 (d, J = 1.6 Hz, 1H), 8.06 (d, J = 1.6 Hz, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.57 (d, J = 7.4 Hz, 1H), 7.07 (d, J = 8.2 Hz, 1H), 4.76−4.66 (m, 2H), 3.90 (s, 3H), 3.69−3.60 (m, 4H), 2.50 (s, 3H), 2.48 (s, 3H), 1.31 (d, J = 6.8 Hz, 3H), 1.20−1.12 (m, 1H), 0.64−0.56 (m, 1H), 0.48−0.38 (m, 2H), 0.31−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 163.77, 158.70, 158.40, 153.31, 150.83, 147.26, 145.90, 145.04, 138.69, 137.75, 136.74, 126.24, 126.19, 125.93, 120.58, 113.47, 110.61, 52.00, 45.93, 43.11, 35.33, 17.96, 17.02, 15.55, 11.49, 3.85, 3.42.

ES+ (M + H)$^+$ observed 564.1898 expected 564.1851.

(S)-2-(1-Cyclopropylethyl)-5-(2-((6-(4-(hydroxymethyl)oxazol-5-yl)pyridin-2-yl)amino)-4-methylthiazol-5-yl)-7-(methylsulfonyl)-isoindolin-1-one (***54***). Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and (5-(6-bromopyridin-2-yl)oxazol-4-yl)methanol (**40**) following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.67 (s, 1H), 8.46 (s, 1H), 8.11 (d, J = 1.6 Hz, 1H), 8.08 (d, J = 1.6 Hz, 1H), 7.86 (t, J = 7.9 Hz, 1H), 7.36 (d, J = 7.4 Hz, 1H), 7.10 (d, J = 8.2 Hz, 1H), 5.25 (t, J = 5.9 Hz, 1H), 4.84 (d, J = 5.3 Hz, 2H), 4.75−4.65 (m, 2H), 3.67−3.60 (m, 4H), 2.49 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.19−1.11 (m, 1H), 0.65−0.55 (m, 1H), 0.49−0.36 (m, 2H), 0.30−0.22 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 163.70, 157.94, 151.35, 151.32, 145.92, 145.13, 144.42, 139.00, 138.05, 137.65, 136.14, 126.79, 126.44, 125.86, 120.11, 114.08, 110.89, 55.13, 51.99, 45.88, 43.07, 18.00, 16.82, 15.58, 3.89, 3.41. (one signal obscured).

ES+ (M + H)$^+$ observed 566.1558 expected 566.1532.

(S)-N-(6-((5-(2-(1-Cyclopropylethyl)-7-(methylsulfonyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)acetamide (***55***). Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and *N*-(6-bromopyridin-2-yl)acetamide following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.42 (s, 1H), 10.06 (s, 1H), 8.08 (d, J = 1.6 Hz, 1H), 8.03 (d, J = 1.7 Hz, 1H), 7.68−7.62 (m, 2H), 6.75 (dd, J = 7.4, 1.4 Hz, 1H), 4.76−4.66 (m, 2H), 3.69−3.59 (m, 4H), 2.42 (s, 3H), 2.17 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.21−1.13 (m, 1H), 0.63−0.57 (m, 1H), 0.47−0.38 (m, 2H), 0.3−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.38, 163.73, 158.56, 150.42, 149.71, 145.70, 145.07, 139.66, 137.55, 136.39, 127.47, 126.60, 126.52, 119.82, 106.08, 105.11, 52.07, 45.89, 43.17, 24.26, 17.95, 16.52, 15.53, 3.85, 3.44.

ES+ (M + H)$^+$ observed 526.1610 expected 526.1583.

(S)-N-(6-((5-(2-(1-Cyclopropylethyl)-7-(methylsulfonyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)-N-methylacetamide (***56***). Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**), and *N*-(6-bromopyridin-2-yl)-*N*-methylacetamide (**41**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.68 (br. s, 1H), 8.03 (d, J = 1.6 Hz, 1H), 8.00 (d, J = 1.6 Hz, 1H), 7.78 (t, J = 7.9 Hz, 1H), 7.04 (d, J = 7.6 Hz, 1H), 6.95 (d, J = 8.1 Hz, 1H), 4.75−4.67 (m, 2H), 3.67−3.57 (m, 4H), 3.36 (s, 3H), 2.44 (s, 3H), 2.09 (s, 3H), 1.31 (d, J = 6.9 Hz, 3H), 1.18−1.10 (m, 1H), 0.63−0.56 (m, 1H), 0.48−0.37 (m, 2H), 0.31−0.24 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.71, 163.68, 157.68, 153.27, 150.67, 145.95, 145.04, 139.89, 137.66, 136.00, 127.09, 126.69, 126.24, 119.83, 112.00, 108.55, 52.01, 45.95, 43.08, 34.98, 22.98, 17.96, 16.58, 15.56, 3.87, 3.40.

ES+ (M + H)$^+$ observed 540.1755 expected 540.1739.

(S)-N-(6-((5-(2-(1-Cyclopropylethyl)-7-(N-methylsulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)-N-methylacetamide (***57***). Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**), and *N*-(6-bromopyridin-2-yl)-*N*-methylacetamide (**41**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.69 (s, 1H), 7.96 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.79 (t, J = 7.9 Hz, 1H), 7.61 (q, J = 5.2 Hz, 1H), 7.05 (d, J = 7.6 Hz, 1H), 6.96 (d, J = 8.1 Hz, 1H),), 4.85−4.69 (m, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 3.37 (s, 3H), 2.51 (d, J = 5.3 Hz, 3H), 2.44 (s, 3H), 2.09 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.19−1.11 (m, 1H), 0.64−0.57 (m, 1H), 0.48−0.38 (m, 2H), 0.31−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 169.70, 164.65, 157.60, 153.26, 150.51, 145.89, 145.09, 139.91, 136.25, 136.03, 126.74, 126.37, 125.30, 119.87, 112.07, 108.41, 52.33, 46.35, 34.97, 29.16, 22.99, 17.91, 16.56, 15.53, 3.89, 3.34.

ES+ (M + H)$^+$ observed 564.1866 expected 564.1848.

(S)-2-(1-Cyclopropylethyl)-5-(4-methyl-2-((6-(2-oxopyrrolidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-7-(methylsulfonyl)isoindolin-1-one (***58***). Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and 1-(6-bromopyridin-2-yl)pyrrolidin-2-one (**42**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.54 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 8.01 (d, J = 1.6 Hz, 1H), 7.84 (d, J = 8.0 Hz, 1H), 7.71 (t, J = 8.0 Hz, 1H), 6.76 (d, J = 8.0 Hz, 1H), 4.70 (s, 2H), 4.23 (t, J = 7.1 Hz, 2H), 3.64 (s, 4H), 2.59 (t, J = 8.0 Hz, 2H), 2.45 (s, 3H), 2.10 (p, J = 7.7 Hz, 2H), 1.31 (d, J = 6.8 Hz, 3H), 1.15 (tt, J = 8.1, 3.9 Hz, 1H), 0.56−0.64 (m, 1H), 0.37−0.48 (m, 2H), 0.24−0.31 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 174.6 (s), 163.7 (s), 157.5 (s), 149.9 (d, J = 11.4 Hz), 146.0 (s), 145.3 (s), 139.6 (s), 137.7 (s), 136.1 (s), 126.5 (d, J = 11.2 Hz), 126.1 (s), 119.6 (s), 105.7 (s), 105.3 (s), 52.0 (s), 48.5 (s), 46.0 (s), 43.1 (s), 33.0 (s), 18.0 (s), 17.3 (s), 16.8 (s), 15.6 (s), 3.9 (s), 3.4 (s).

ES+ (M + H)$^+$ observed 552.1739 expected 552.1739.

(S)-2-(1-Cyclopropylethyl)-6-(4-methyl-2-((6-(2-oxopyrrolidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-N-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide (***59***). Step 1: (*S*)-*N*-(5-(2-(1-Cyclopropylethyl)-7-(N-(oxetan-3-yl)sulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)acetamide

(*S*)-6-(2-Acetamido-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-3-oxoisoindoline-4-sulfonyl chloride (1.80 g, 4.0 mmol) was dissolved in DCM (30 mL). DMAP (1.45 g, 11.9 mmol) and $MgSO_4$ (0.48 g, 4.0 mmol) were added followed by oxetan-3-amine (0.435 g, 6.0 mmol). The resulting mixture was stirred at r.t. for 2 h. The reaction mixture was filtered through Celite. The solvent was evaporated, and the residue was purified by flash silica chromatography, elution gradient 0 to 20% MeOH in DCM to give the subtitle compound (1.80 g, 93%) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.33 (s, 1H), 8.57 (d, J = 7.0 Hz, 1H), 8.02 (d, J = 1.6 Hz, 1H), 7.84 (d, J = 1.6 Hz, 1H), 4.75 (s, 2H), 4.53 (q, J = 6.4 Hz, 2H), 4.44 (dt, J = 13.7, 6.8 Hz, 1H), 4.35 (q, J = 6.0 Hz, 2H), 3.72−3.61 (m, 1H), 2.44 (s, 3H), 2.17 (s, 3H), 1.33 (d, J = 6.8 Hz, 3H), 1.22−1.12 (m, 1H), 0.65−0.56 (m, 1H), 0.48−0.38 (m, 2H), 0.32−0.25 (m, 1H).ES+ (M + H)$^+$ 491.

Step 2: (*S*)-6-(2-Amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide

(*S*)-*N*-(5-(2-(1-Cyclopropylethyl)-7-(*N*-(oxetan-3-yl)sulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)acetamide (1.80 g, 3.67 mmol) was dissolved in MeOH (20 mL). A solution of LiOH (0.879 g, 36.7 mmol) in water (0.2 mL) was added, and the resulting solution was heated to 80 °C for 10 h. The reaction mixture was allowed to cool, and then the solvent was evaporated. The residue was dissolved in DCM (100 mL) and washed sequentially with water (50 mL × 2) and saturated brine (50 mL × 2). The organic layer was

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dried over Na_2SO_4, filtered, and evaporated to give the subtitle compound (1.50 g, 91%) as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.59 (s, 1H), 7.83 (d, J = 1.6 Hz, 1H), 7.70 (d, J = 1.6 Hz, 1H), 7.37 (s, 2H), 4.71 (s, 2H), 4.52 (q, J = 6.8 Hz, 2H), 4.49−4.37 (m, 1H), 4.37−4.30 (m, 2H), 3.71−3.59 (m, 1H), 2.30 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.20−1.10 (m, 1H), 0.66−0.55 (m, 1H), 0.48−0.36 (m, 2H), 0.32−0.22 (m, 1H).

ES+ (M + H)$^+$ 449.

Step 3: (*S*)-2-(1-Cyclopropylethyl)-6-(4-methyl-2-((6-(2-oxopyrrolidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-N-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide Prepared from (S)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-N-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide, and 1-(6-bromopyridin-2-yl)pyrrolidin-2-one (**42**), following the method used for **52**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.56 (s, 1H), 8.56 (d, J = 7.1 Hz, 1H), 7.98 (d, J = 1.4 Hz, 1H), 7.90 (d, J = 1.5 Hz, 1H), 7.88−7.84 (m, 1H), 7.72 (t, J = 8.0 Hz, 1H), 6.79−6.76 (m, 1H), 4.75 (s, 2H), 4.57−4.50 (m, 2H), 4.48−4.39 (m, 1H), 4.40−4. 34 (m, 2H), 4.25 (t, J = 7.1 Hz, 2H), 3.72−3. 63 (m, 1H), 2.60 (t, J = 8.1 Hz, 2H), 2.45 (s, 3H), 2.10 (p, J = 7.9 Hz, 2H), 1.34 (d, J = 6.8 Hz, 3H), 1.21−1.12 (m, 1H), 0.65−0.56 (m, 1H), 0.49−0.39 (m, 2H), 0.33−0.25 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 174.6, 164.7, 157.6, 149.9, 149.8, 146.1, 145.4, 139.6, 137.4, 136.4, 126.1, 125.9, 124.8, 119.5, 105.7, 105.4, 76.9, 76.8, 52.5, 48.5, 47.2, 46.5, 33.0, 18.0, 17.3, 16.7, 15.6, 3.9, 3.3.

ES+ (M + H)$^+$ observed 609.1938 expected 609.1954.

*(S)-2-(1-Cyclopropylethyl)-6-(4-methyl-2-((6-(2-oxoimidazolidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-N-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide (**60**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide (**59**, step 2), and 1-(6-bromopyridin-2-yl)-imidazolidin-2-one (**43**), following the method used for **52**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.45 (s, 1H), 8.54 (d, J = 7.2 Hz, 1H), 7.97 (s, 1H), 7.89 (d, J = 1.3 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.62 (t, J = 8.0 Hz, 1H), 7.22 (s, 1H), 6.62 (d, J = 7.8 Hz, 1H), 4.74 (s, 2H), 4.57−4.50 (m, 2H), 4.44 (h, J = 6.6 Hz, 1H), 4.39−4. 32 (m, 2H), 4.31−4.23 (m, 2H), 3.72−3. 62 (m, 1H), 3.46 (t, J = 8.1 Hz, 2H), 2.44 (s, 3H), 1.34 (d, J = 6.8 Hz, 3H), 1.22−1. Thirteen (m, 1H), 0.66−0.57 (m, 1H), 0.47−0.39 (m, 2H), 0.33−0.25 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 164.7, 158.1, 157.8, 151.1, 149.9, 146.1, 145.3, 139.1, 137.4, 136.4, 126.2, 125.9, 124.8, 119.3, 103.5, 103.2, 76.9, 76.8, 52.4, 47.2, 46.4, 45.0, 36.6, 18.0, 16.7, 15.6, 3.9, 3.3.

ES+ (M + H)$^+$ observed 610.1912 expected 610.1906.

*(S)-2-(1-Cyclopropylethyl)-5-(4-methyl-2-((6-(3-methyl-2-oxoimidazolidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-7-(methylsulfonyl)-isoindolin-1-one (**61**).* Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**), and 1-(6-bromopyridin-2-yl)-3-methylimidazolidin-2-one (**44**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6)) δ 11.44 (s, 1H), 8.07 (d, J = 1.6 Hz, 1H), 8.01 (d, J = 1.4 Hz, 1H), 7.74−7.71 (m, 1H), 7.63 (t, J = 8.0 Hz, 1H), 6.61 (d, J = 7.9 Hz, 1H), 4.70 (s, 2H), 4.20 (dd, J = 8.9, 7.2 Hz, 2H), 3.64 (s, 4H), 3.48−3.52 (m, 2H), 2.81 (s, 3H), 2.45 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.19−1.12 (m, 1H), 0.64−0.58 (m, 1H), 0.47−0.39 (m, 2H), 0.31−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.2, 158.1, 157.1, 151.5, 150.4, 146.4, 145.7, 139.7, 138.1, 136.6, 127.0, 126.9, 126.6, 119.9, 103.7, 103.7, 52.5, 46.4, 43.9, 43.5, 42.9, 31.1, 18.5, 17.2, 16.1, 4.4, 3.9.

ES+ (M + H)$^+$ observed 567.1859 expected 567.1848.

*2-((S)-1-Cyclopropylethyl)-6-(2-((6-((R)-3-hydroxy-2-oxopyrrolidin-1-yl)pyridin-2-yl)amino)-4-methylthiazol-5-yl)-N-methyl-3-oxoisoindoline-4-sulfonamide (**62**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**) and (*R*)-1-(6-bromopyridin-2-yl)-3-((*tert*-butyldimethylsilyl)oxy)pyrrolidin-2-one (**45**), following the method used for **52**, followed by deprotection of the alcohol: (triethylamine trihydrofluoride (2 mL, 12.28 mmol) was added dropwise to 6-(2-((6-((*S*)-3-((*tert*-butyldimethylsilyl)oxy)-2-oxopyrrolidin-1-yl)-pyridin-2-yl)amino)-4-methylthiazol-5-yl)-2-((*S*)-1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (95 mg, 0.14 mmol) in THF (6 mL) at 25 °C over 15 min. The resulting solution was stirred at 25 °C for 3 h. The solvent was evaporated, and the residue was purified by preparative HPLC.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.58 (s, 1H), 7.98 (s, 1H), 7.92 (s, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.61−7.56 (m, 1H), 6.80 (d, J = 8.0 Hz, 1H), 4.76 (s, 2H), 4.44−4.39 (m, 1H), 4.31−4.25 (m, 1H), 4.02−3.94 (m, 1H), 3.69−3.62 (m, 1H), 2.53 (d, J = 5.2 Hz, 3H), 2.48−2.41 (m, 4H), 1.94−1.85 (m, 1H), 1.33 (d, J = 6.8 Hz, 3H), 1.23 (bs, 1H), 1.21−1.13 (m, 1H), 0.65−0.58 (m, 1H), 0.49−0.39 (m, 2H), 0.32−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 175.2, 165.1, 158.0, 150.6, 150.2, 146.4, 145.7, 140.2, 136.8, 136.5, 127.2, 126.5, 125.7, 120.1, 106.5, 105.7, 70.8, 52.8, 46.9, 44.4, 29.6, 28.2, 18.4, 17.1, 16.0, 4.4, 3.8.

ES+ (M + H)$^+$ observed 583.1816 expected 583.1797.

*2-((S)-1-Cyclopropylethyl)-6-(2-((6-((R)-4-hydroxy-2-oxopyrrolidin-1-yl)pyridin-2-yl)amino)-4-methylthiazol-5-yl)-N-methyl-3-oxoisoindoline-4-sulfonamide (**63**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**), and (*R*)-4-((*tert*-butyldimethylsilyl)oxy)-pyrrolidin-2-one (**46**), following the method used for **62**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.97 (s, 1H), 7.92 (d, J = 1.5 Hz, 1H), 7.87 (d, J = 8.1 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.57 (s, 1H), 6.78 (d, J = 8.0 Hz, 1H), 5.30 (d, J = 3.1 Hz, 1H), 4.75 (s, 2H), 4.41 (bs, 1H), 4.34−4.27 (m, 1H), 4.17 (d, J = 11.6 Hz, 1H), 3.69−3.60 (m, 1H), 3.00−2.61 (m, 1H), 2.52 (s, H), 2.44 (s, 3H), 2.36 (d, J = 17.1 Hz, 1H), 1.33 (d, J = 6.8 Hz, 3H), 1.21−1.19 (m, 1H), 0.66−0. 57 (m, 1H), 0.49−0.38 (m, 2H), 0.32−0.24 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 173.3, 164.7, 157.6, 150.0, 149.9, 145.9, 145.2, 139.7, 136.3, 136.1, 126.9, 126.4, 125.3, 119.6, 105.7, 105.3, 62.4, 57.5, 52.4, 46.4, 43.0, 29.2, 18.0, 16.6, 15.6, 4.0, 3.4.

ES+ (M + H)$^+$ observed 583.1812 expected 583.1797.

*(S)-2-(1-Cyclopropylethyl)-5-(4-methyl-2-((6-(2-oxopiperidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-7-(methylsulfonyl)isoindolin-1-one (**64**).* Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**), and 1-(6-bromopyridin-2-yl)piperidin-2-one (**47**), following the method used for **52**.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.62 (s, 1H), 8.10−8. 02 (m, 2H), 7.72 (t, J = 8.0 Hz, 1H), 7.31 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 4.71 (s, 2H), 4.11−4.02 (m, 2H), 3.69−3.58 (m, 4H), 2.48 (s, 3H), 2.00−1.92 (m, 2H), 1.90−1.81 (m, 2H), 1.32 (d, J = 6.8 Hz, 3H), 1.21−1.12 (m, 1H), 0.64−0.54 (m, 1H), 0.48−0.37 (m, 2H), 0.31−0.23 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 170.1, 163.7, 157.6, 151.9, 150.1, 146.0, 145.3, 138.9, 137.7, 136.1, 126.5, 126.1, 119.5, 112.0, 107.0, 76.9, 52.0, 48.0, 46.0, 43.1, 33.3, 22.6, 20.4, 18.0, 16.9, 15.6, 3.9, 3.4.

ES+ (M + H)$^+$ observed 566.1915 expected 566.1896.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-(2-oxopiperidin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**65**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**) and 1-(6-bromopyridin-2-yl)piperidin-2-one (**47**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.00 (d, J = 1.3 Hz, 1H), 7.92 (d, J = 1.5 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.57 (q, J = 5.2 Hz, 1H), 7.32−7.29 (m, 1H), 6.86 (d, J = 8.0 Hz, 1H), 4.76 (s, 2H), 4.09−4.04 (m, 2H), 3.69−3.62 (m, 1H), 2.52 (d, J = 5.3 Hz, 3H), 2.48 (s, 3H), 1.99−1.93 (m, 2H), 1.89−1.83 (m, 2H), 1.33 (d, J = 6.8 Hz, 3H), 1.21−1.14 (m, 1H), 0.64−0.58 (m, 1H), 0.48−0.39 (m, 2H), 0.31−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 170.6, 165.2, 158.1, 152.3, 150.5, 146.4, 145.8, 139.4, 136.8, 136.5, 127.1, 126.3, 125.6, 120.0, 112.5, 107.4, 52.8, 48.4, 46.9, 33.7, 29.6, 23.1, 20.8, 18.4, 17.2, 16.0, 4.4, 3.8.

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ES+ $(M + H)^+$ observed 581.1995 expected 581.2004.

*(S)-4-(6-((5-(2-(1-Cyclopropylethyl)-7-(methylsulfonyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)morpholin-3-one (**66**).* Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and 4-(6-bromopyridin-2-yl)morpholin-3-one (**48**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.07−8.03 (m, 2H), 7.76 (t, J = 8.0 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 4.71 (s, 2H), 4.29 (s, 2H), 4.19−4.15 (m, 2H), 4.09−4.05 (m, 2H), 3.64 (s, 3H), 3.6−3.63 (m, 1H), 2.47 (s, 3H), 1.31 (d, J = 6.8 Hz, 3H), 1.19−1.12 (m, 1H), 0.63−0.57 (m, 1H), 0.46−0.38 (m, 2H), 0.30−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 163.7, 157.4, 150.4, 150.1, 146.0, 145.3, 139.4, 137.7, 136.0, 126.5, 126.5, 126.1, 119.6, 110.4, 107.4, 67.9, 63.4, 52.0, 46.7, 46.0, 43.1, 18.0, 16.8, 15.6, 3.9, 3.4.

ES+ $(M + H)^+$ observed 568.1710 expected 568.1688.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-(3-oxomorpholino)pyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**67**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**), and 4-(6-bromopyridin-2-yl)morpholin-3-one (**48**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H), 7.98 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 1.6 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.63−7.54 (m, 2H), 6.88 (d, J = 8.0 Hz, 1H), 4.80−4.70 (m, 2H), 4.29 (s, 2H), 4.21−4.14 (m, 2H), 4.09−4.04 (m, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 2.51 (d, J = 4.9 Hz, 3H), 2.46 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.19−1.12 (m, 1H), 0.64−0.56 (m, 1H), 0.47−0. 38 (m, 2H), 0.32−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 167.55, 165.5, 158.0, 150.9, 150.6, 146.4, 145.8, 139.9, 136.8, 136.5, 127.1, 126.3, 125.6, 120.0, 110.9, 108.0, 68.4, 63.9, 52.8, 47.2, 46.9, 29.6, 18.4, 17.2, 16.0, 4.4, 3.8.

ES+ $(M + H)^+$ observed 583.1825 expected 583.1797.

*(S)-2-(1-Cyclopropylethyl)-6-(4-methyl-2-((6-(3-oxomorpholino)-pyridin-2-yl)amino)thiazol-5-yl)-N-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide (**68**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-(oxetan-3-yl)-3-oxoisoindoline-4-sulfonamide (**59**, step 2) and 4-(6-bromopyridin-2-yl)morpholin-3-one (**48**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.56 (d, J = 6.3 Hz, 1H), 7.99 (d, J = 1.6 Hz, 1H), 7.89 (d, J = 1.6 Hz, 1H), 7.76 (t, J = 8.0 Hz, 1H), 7.59 (d, J = 7.9 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 4.81−4.70 (m, 2H), 4.54 (dt, J = 11.1, 6.7 Hz, 2H), 4.48−4.41 (m, 1H), 4.37 (dt, J = 9.9, 6.3 Hz, 2H), 4.30 (s, 2H), 4.20−4.15 (m, 2H), 4.09−4.04 (m, 2H), 3.67 (dq, J = 9.4, 6.8 Hz, 1H), 2.46 (s, 3H), 1.34 (d, J = 6.8 Hz, 3H), 1.14−1.22 (m, 1H), 0. 64−0.58 (m, 1H), 0.47−0.40 (m, 2H), 0.32−0.26 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 167.0, 164.7, 157.5, 150.4, 150.1, 146.1, 145.5, 139.4, 137.4, 136.3, 126.0, 125.9, 124.8, 119.5, 110.5, 107.5, 76.9, 76.8, 67.9, 63.4, 52.5, 47.7, 46.7, 46.5, 17.9, 16.8, 14.5, 4.8, 2.7.

ES+ $(M + H)^+$ observed 625.1906 expected 625.1903.

*(S)-3-(6-((5-(2-(1-Cyclopropylethyl)-7-(methylsulfonyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)-1,3-oxazinan-2-one (**69**).* Prepared from (*S*)-5-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-7-(methylsulfonyl)isoindolin-1-one (**16**) and 3-(6-bromopyridin-2-yl)-1,3-oxazinan-2-one (**49**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (s, 1H), 8.06 (d, J = 1.6 Hz, 1H), 8.03 (d, J = 1.6 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 6.84 (d, J = 8.0 Hz, 1H), 4.66−4.76 (m, 2H), 4.39 (t, J = 5.4 Hz, 2H), 4.12 (t, J = 6.2 Hz, 2H), 3.66−3.59 (m, 4H), 2.47 (s, 3H), 2.23−2.17 (m, 2H), 1.31 (d, J = 6.8 Hz, 3H), 1.19−1.11 (m, 1H), 0.63−0.56 (m, 1H), 0.47−0.37 (m, 2H), 0.31−0.24 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 164.5, 157.5, 152.5, 151.5, 149.9, 146.4, 145.3, 139.3, 137.7, 136.0, 126.4, 126.1, 119.5, 110.7, 106.7, 67.0, 51.1, 46.0, 45.9, 43.1, 21.9, 18.0, 16.8, 15.6, 3.9, 3.4. One C obscured—probably overlap of two peaks at 126.4.

ES+ $(M + H)^+$ observed 568.1713 expected 568.1688.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-(2-oxo-1,3-oxazinan-3-yl)pyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**70**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**) and 3-(6-bromopyridin-2-yl)-1,3-oxazinan-2-one (**49**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.61 (s, 1H), 8.00 (d, J = 1.6 Hz, 1H), 7.92 (d, J = 1.6 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.56 (q, J = 5.2 Hz, 1H), 7.37 (d, J = 7.8 Hz, 1H), 6.85 (d, J = 7.9 Hz, 1H), 4.80−4.68 (m, 2H), 4.39 (t, J = 5.4 Hz, 2H), 4.13 (t, J = 6.2 Hz, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 2.51 (d, J = 5.2 Hz, 3H), 2.47 (s, 3H), 2.22−2.15 (m, 2H), 1.32 (d, J = 6.9 Hz, 3H), 1.20−1.12 (m, 1H), 0.64−0.57 (m, 1H), 0.48−0.39 (m, 2H), 0.32−0.24 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 165.1, 157.6, 152.5, 151.5, 149.9, 145.9, 145.3, 139.6, 136.3, 136.0, 126.7, 125.9, 124.4, 120.8, 113.3, 107.7, 67.0, 52.8, 46.4, 44.6, 29.2, 21.9, 18.0, 16.7, 15.0, 4.6, 2.8.

ES+ $(M + H)^+$ 583.1803 expected 583.1792.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-(2-oxopiperazin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**71**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**) and benzyl 4-(6-bromopyridin-2-yl)-3-oxopiperazine-1-carboxylate (**50**), following the method used for **52**, followed by deprotection of the carbamate: (BBr_3 (0.185 mL, 1.96 mmol) was added to (*S*)-benzyl 4-(6-((5-(2-(1-cyclopropylethyl)-7-(*N*-methylsulfamoyl)-1-oxoisoindolin-5-yl)-4-methylthiazol-2-yl)amino)pyridin-2-yl)-3-oxopiperazine-1-carboxylate (140 mg, 0.20 mmol) in DCM (10 mL) at 0 °C over 0.5 h. The resulting mixture was stirred at RT for 4 h. The reaction mixture was poured onto ice (20 mL), extracted with DCM (2 × 20 mL), and the aqueous layer was adjusted to pH = 9 with saturated Na_2CO_3 and extracted with DCM (3 × 50 mL). The crude product was purified by preparative HPLC.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.60 (s, 1H), 7.98 (d, J = 1.6 Hz, 1H), 7.91 (d, J = 1.6 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.60−7.54 (m, 1H), 7.48 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 8.0 Hz, 1H), 4.80−4.71 (m, 2H), 4.06 (t, J = 5.5 Hz, 2H), 3.65 (dq, J = 9.1, 6.8 Hz, 1H), 3.48 (s, 2H), 3.10 (t, J = 5.5 Hz, 2H), 2.52 (d, J = 4.0 Hz, 3H), 2.46 (s, 3H), 1.32 (d, J = 6.8 Hz, 3H), 1.20−1.13 (m, 1H), 0.64−0.57 (m, 1H), 0.49−0.39 (m, 2H), 0.31−0.25 (m, 1H).

^{13}C NMR (151 MHz, DMSO-d_6) δ 168.8, 164.7, 157.5, 150.9, 150.0, 145.9, 145.3, 139.1, 136.3, 136.1, 126.7, 125.9, 125.1, 119.5, 111.2, 107.1, 52.4, 51.2, 48.5, 46.4, 42.7, 29.2, 18.3, 16.8, 16.0, 4.7, 3.4.

ES+ $(M + H)^+$ observed 582.1983 expected 582.1957.

*(S)-2-(1-Cyclopropylethyl)-N-methyl-6-(4-methyl-2-((6-(4-methyl-2-oxopiperazin-1-yl)pyridin-2-yl)amino)thiazol-5-yl)-3-oxoisoindoline-4-sulfonamide (**72**).* Prepared from (*S*)-6-(2-amino-4-methylthiazol-5-yl)-2-(1-cyclopropylethyl)-*N*-methyl-3-oxoisoindoline-4-sulfonamide (**17**),and 1-(6-bromopyridin-2-yl)-4-methylpiperazin-2-one (**51**), following the method used for **52**.

^{1}H NMR (600 MHz, DMSO-d_6) δ 11.58 (s, 1H), 8.00 (d, J = 1.7 Hz, 1H), 7.90 (d, J = 1.7 Hz, 1H), 7.75 (t, J = 8.0 Hz, 1H), 7.58 (br. q, J = 5.2 Hz, 1H), 7.44 (d, J = 8.0 Hz, 1H), 6.88 (d, J = 8.0 Hz, 1H), 4.80−4.72 (m, 2H), 4.08 (dd, J = 6.7, 4.6 Hz, 2H), 3.65 (dq, J = 9.2, 6.8 Hz, 1H), 3.21 (s, 2H), 2.77−2.85 (m, 2H), 2.52 (d, J = 4.3 Hz, 3H), 2.46 (s, 3H), 2.29 (s, 3H), 1.33 (d, J = 6.8 Hz, 3H), 1.20−1.13 (m, 1H), 0.64−0.58 (m, 1H), 0.48−0.38 (m, 2H), 0.32−0.26 (m, 1H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.9, 164.7, 157.5, 151.0, 150.1, 146.0, 145.3, 139.3, 136.3, 136.1, 126.8, 126.0, 125.2, 119.6, 110.8, 107.4, 59.8, 52.4, 51.3, 47.3, 46.4, 44.3, 29.2, 18.0, 16.7, 15.6, 4.0, 3.4.

ES+ $(M + H)^+$ observed 596.2106 expected 596.2114.

Biological Assays. All animal procedures were approved by the ethical committee in Gothenburg, Sweden and were carried out under the appropriate license.

Enzymatic inhibition assays for PI3K isoforms were run as previously described.[22]

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Cellular Assays. Inhibition of activity in JeKo-1 cells (PI3Kδ) was run as previously described.[22]

Inhibition of p-Akt in RAW-264 cells was run as previously described.[23]

Pharmacokinetic Studies. Pharmacokinetic studies were carried out as described previously.[30]

Rat Ovalbumin Challenge Study. All rat experiments were approved by the by the regional ethics committee in Gothenburg with approval number 94−2014 and the approved site number was 31−5373/11.

Rats (male BN, Charles River Laboratories; male, 200−230 g; 8−10 per group) were sensitized by subcutaneous injection of a mixture of ovalbumin (100 μg) and aluminum hydroxide (100 mg) in saline (1 mL total volume) on days 1 and 8. On day 15 the animals were placed into a closed plexiglass box and were exposed to an aerosolized solution of 1% ovalbumin for 20 min. Compounds were administered by nebulization (compounds under investigation) or intratracheal instillation (budesonide positive control) on day 22. After a gap, normally 1 h, but of variable length for the time-course study, the animals were again exposed to aerosolized 1% ovalbumin. Rats were terminated either 2 h post challenge (for determination of phosphorylation of S6 ribosomal protein) or 48 h post challenge (for measurement of eosinophils and cytokines). Bronchioalveolar lavage (BAL) was collected using 4 mL PBS (without $Ca2^+$ or $Mg2^+$) in a twice in, twice out manner. BAL fluid was centrifuged and the supernatant was divided into aliquots and stored at −70 °C until analysis. Eosinophil levels were determined using a Sysmex flow cytometer.

Cytokine and mediator levels were determined by a MSD v-plex proinflammatory rat panel (Meso Scale Discovery), except for IL-17 which was determined using an ELISA with AbCam antibody 119536.

S6 ribosomal protein levels were determined by immunofluorescence of pS6RP+ lymphocytes which were measured using XPRabbit mAb (Alexa Fluor 488 Conjugate) #4803. Samples were processed by flow cytometry on a LSR Fortessa (BD Biosciences), and the lymphocyte population was selected based on FSC-A and SSC-A properties. Collected data were analyzed with FlowJo Software (TreeStar).

Human Peripheral Blood Studies. *Patients and Sample Collection.* Fresh venous blood samples were obtained from asthmatic blood donors in the West Sweden Asthma Study (WSAS).[31] The study was approved by the Regional Ethical Approval Committee in Gothenburg, Sweden, and all subjects had signed informed consents.

PBMC T Cell Cytokine Responses. Human PBMCs were isolated from six asthmatic donors classified as moderate to severe asthma. All asthmatic donors except one were treated with inhaled corticosteroids and β2 agonists. PBMCs were isolated by density gradient centrifugation using Polymorphoprep (Axis-Shield) and cultured in U-shaped 96-well plates at 200 000 cells/well in RPMI1640 media (Gibco) supplemented with 10% heat-inactivated FBS (hiFBS; Gibco) and penicillin-streptomycin (100 U/mL; Gibco). Cells were preincubated for 1 h with compounds in 3-fold serial dilutions (duplicates) at final concentrations of 1.84 μM to 0.13 pM at 37 °C, 5% CO_2 and incubated for 24 h with anti-CD2/CD3/CD28 T cell activation beads (100 000 beads/well; Miltenyi Biotec). Supernatants were collected by centrifugation and analyzed for cytokines by a V-PLEX custom IL-5 and IL-17A assay (Meso Scale Discovery).

Eosinophil CD11b Assay. Primary human eosinophils were isolated from fresh venous blood samples from asthmatic donors classified moderate to severe asthma (N = 6−10 depending on compound). All asthmatic donors were treated with inhaled corticosteroids and β2 agonists.

Granulocytes were isolated from whole blood by polymorphprep (Axis-Shield), seeded in U-shaped 96-well plates at 1×10^5 cells/well in Hank's Balanced Salt solution media (HBSS, Gibco) with 0.1% bovine serum albumin (BSA, Sigma-Aldrich), and preincubated with inhibitors for 30 min at 37 °C, 5% CO_2 followed by stimulation with 3 nM eotaxin-3 (Peprotech) for 30 min at RT.

Stimulations were stopped by staining with APC-conjugated antihuman CD11b (Clone Vim12, Life technologies) and PE-conjugated antihuman CD16 (Clone 3G8, BD Biosciences) antibodies diluted in Streck cell preservative (Streck) for 30 min, in the dark at RT. Thereafter, residual red blood cells were lysed by addition of Optilyse B (Beckman Coulter), and samples were washed and resuspended in DPBS (Gibco), 2 mM EDTA (Invitrogen), 2% FBS (Gibco) prior to analysis on an Accuri C6 Cytometer (BD Biosciences). Data were analyzed with FlowJo Software (TreeStar, Ashland, OR). Eosinophils were gated by CD16-CD11b+ staining and SSC-A properties versus CD16+CD11b+ neutrophils, and median fluorescence intensity (MFI) values for CD11b were collected for each population.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.1c00434.

Details of the preliminary macrocyclization experiments and results; a ligand interaction diagram for the crystal structure of **58**; additional *in vivo* experiment results; off-target and kinase screening results, copies of ^{1}H and ^{13}C NMR spectra and HPLC traces for final compounds (PDF)

Table of molecular formula strings (CSV)

Accession Codes

Crystal structures of PI3Kδ small molecule complexes have been deposited in the PDB under the following accession codes **7** (7oi4); **12** (7ois); **20** (7oij); **58** (7oil). Authors will release the atomic coordinates and experimental data upon article publication. PDB IDs have been provided in figure legends.

■ AUTHOR INFORMATION

Corresponding Author

Matthew W. D. Perry − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* orcid.org/0000-0002-9799-0746; Phone: +46725827070; Email: matthew.perry@astrazeneca.com

Authors

Karin Björhall − *Bioscience COPD/IPF, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Peter Bold − *DMPK, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Mikael Brűlls − *Early Product Development & Manufacturing, Pharmaceutical Sciences R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Ulf Börjesson − *Computational Chemistry, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Johan Carlsson − *Discovery Biology, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* Present Address: (J.C.) AstraZeneca Operations, Sweden Biomanufacturing Center (SBC), Gärtunavägen 14, Gärtuna, SE-15257 Södertälje, Sweden.

Hui-Fang Amy Chang − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Yunhua Chen − *Pharmaron Beijing Co., Ltd., Beijing 100176, P.R. China*
Anders Eriksson − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Britt-Marie Fihn − *DMPK, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Rebecca Fransson − *Advanced Drug Delivery, Pharmaceutical Sciences R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Linda Fredlund − *Discovery Biology, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* orcid.org/0000-0002-2104-5617
Hongbin Ge − *Pharmaron Beijing Co., Ltd., Beijing 100176, P.R. China*
Haijuan Huang − *Pharmaron Beijing Co., Ltd., Beijing 100176, P.R. China*
Kostas Karabelas − *Projects, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* Present Address: (K.K.) Bioglan AB, Borrgatan 31, SE-202 13 Malmö, Sweden.
Eva Lamm Bergström − *DMPK, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Sarah Lever − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Helena Lindmark − *Discovery Biology, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Mickael Mogemark − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Antonios Nikitidis − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Anna-Pia Palmgren − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Nils Pemberton − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* orcid.org/0000-0002-8875-7221
Jens Petersen − *Structure & Biophysics, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Mio Rodrigo Blomqvist − *Bioscience Cough and In Vivo, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Reed W. Smith − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Matthew J. Thomas − *Bioscience COPD/IPF, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* Present Address: (M.J.T.) Boehringer-Ingelheim Pharma, Birkendorf Strasse, Biberach 88397, Germany.
Victoria Ullah − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Christian Tyrchan − *Medicinal Chemistry, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden;* orcid.org/0000-0002-6470-984X
Tiiu Wennberg − *Bioscience COPD/IPF, Research and Early Development, Respiratory and Immunology (R&I), BioPharmaceuticals R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Annika Westin Eriksson − *Clinical Pharmacology and Safety Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*
Wenzhen Yang − *Pharmaron Beijing Co., Ltd., Beijing 100176, P.R. China;* orcid.org/0000-0003-2226-0869
Shuchun Zhao − *Pharmaron Beijing Co., Ltd., Beijing 100176, P.R. China*
Linda Öster − *Structure & Biophysics, Discovery Sciences, R&D, AstraZeneca, Gothenburg SE-431 83, Sweden*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jmedchem.1c00434

Notes

The authors declare the following competing financial interest(s): All authors were employees and/or shareholders of AstraZeneca or Pharmaron when this work was carried out. M.W.D.P., P.B., U.B., K.K:, M.M., A.N., J.P, N.P. and C.T. are inventors of patents assigned to AstraZeneca covering inhibitors of PI3Ks.

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■ ABBREVIATIONS USED

BSA, bovine serum albumin; CCR2, CC chemokine receptor 2; CCL2, CC chemokine ligand 2; CCR5, CC chemokine receptor 5; DCM, dichloromethane; DIPEA, diisopropylethylamine; DPBS, Dulbecco's phosphate-buffered saline; HBSS, Hank's balanced salt solution media; PBMC, peripheral blood mononuclear cells; T3P, 1-propanephosphonic anhydride

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