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Article

Discovery of (*S*)-2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3*H*)-one (IHMT-PI3K*δ*-372) as a Potent and Selective PI3K*δ* Inhibitor for the Treatment of Chronic Obstructive Pulmonary Disease

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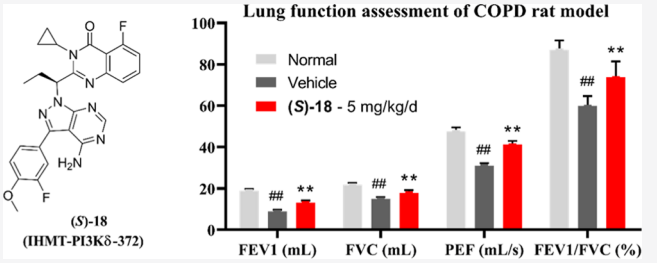
ABSTRACT: Accumulated pieces of evidence have shown that PI3K*δ* plays a critical role in chronic obstructive pulmonary disease (COPD). Using a fragment-hybrid approach, we discovered a potent and selective PI3K*δ* inhibitor (***S*)-18**. In the biochemical assay, (***S*)-18** inhibits PI3K*δ* (IC_{50} = 14 nM) with high selectivity over other class I PI3Ks (56∼83 fold). (***S*)-18** also achieves good selectivity over other protein kinases in the kinome (*S*-score (35) = 0.015). In the cell, (***S*)-18** selectively and potently inhibits the PI3K*δ*-mediated phosphorylation of AKT T308 but not other class I PI3K-mediated signaling. Additionally, (***S*)-18** exhibits no apparent inhibitory effect on CYP isoforms except for a moderate effect on CYP2C9. Furthermore, it shows no apparent inhibitory activity against hERG (IC_{50} > 10 *μ*M). In vivo, (***S*)-18** displays favorable PK properties for inhaled delivery and improves lung function in a rodent model of pulmonary inflammation. These results suggest that (***S*)-18** might be a new potential therapeutic candidate for COPD.



INTRODUCTION

The Global Burden of Disease study 2017 reports that chronic obstructive pulmonary disease (COPD) is becoming a major global health problem and it has been ranked as the fourth cause of death.[1−4] COPD, characterized by airflow limitation[5] and associated with abnormal inflammatory response of the lung, is a highly prevalent chronic progressive and not fully reversible respiratory disease. The common symptoms of COPD include emphysema, airway fibrosis, chronic bronchitis, etc., which can lead to innate immune response of inflammatory cells. With disease progression, inflammatory associated cells such as macrophages, neutrophils, lymphocytes, and dendritic cells would be elevated in the microenvironment. Increased inflammatory responses in turn promote the development of COPD.[6−8] Currently, most of the COPD therapies do not directly target the pathogenic mechanisms of chronic inflammation.[8] Therefore, new approaches such as targeting the inflammatory process have attracted extensive interests for the new COPD therapy.

PI3K*δ* belongs to the class I Phosphoinositide 3-kinases (PI3Ks), which include the PI3K*α*, PI3K*β*, and PI3K*γ* isoforms.[9] The expressions of PI3K*α* and PI3K*β* are ubiquitous,[10] and they both play essential roles in cell growth, survival, and proliferation.[11] In addition, PI3K*α* and PI3K*β* knockout (KO) mice are embryonically lethal.[12] PI3K*γ* is primarily present in leukocytes[13] and to a lesser extent in cardiomyocytes. Although PI3K*γ* KO mice are viable and fertile, they have a reduced ability to cope with cardiac distress.[14−16] The expression and function of PI3K*δ* are predominantly restricted to immune cells. Many studies indicated the importance of isoform-specific inhibitors for clinical applications. Inhibition of PI3K*δ* has been shown to be beneficial to inflammatory diseases.[10,17−25] Several small-molecule PI3K*δ* inhibitors such as **1** (GSK2269557),[23] **2** (GSK2292767),[23] and **3** (LAS191954)[26] have been investigated either in the clinical trial or preclinical stages of COPD (Figure 1). In this study, we adopted a fragment hybridization

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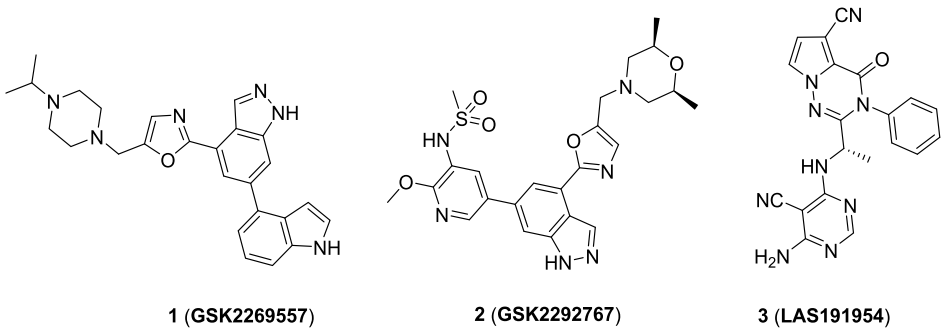

Figure 1. Chemical structures of representative PI3Kδ inhibitors for COPD.

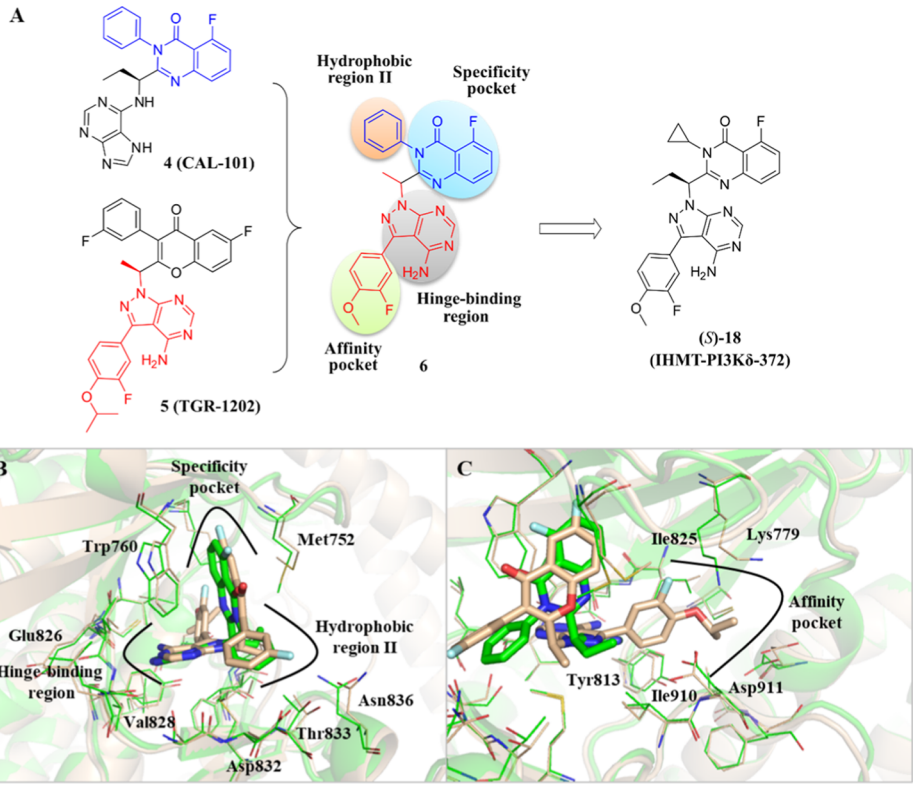

Figure 2. (A) Schematic illustration of the discovery of (*S*)-**18** (IHMT-PI3Kδ-372). (B) Overlay of **4** (green; PDB code: 4XE0[28]) and **5** (wheat; PDB code: 5M6U[24]) bound to PI3Kδ in the specificity pocket (Trp760 and Met752), hydrophobic region II (Asp832, Thr833, and Asp836), and hinge binding region (Glu826 and Val828). (C) Binding modes of **4** and **5** with PI3Kδ in the affinity pocket (Ile825, Tyr813, Ile910, and Asp911).

strategy starting from two propeller-shaped PI3Kδ inhibitors **4** (CAL-101)[27,28] and **5** (TGR-1202),[29] which have been applied to cancer. Through medicinal chemistry effort, we have discovered a novel PI3Kδ inhibitor compound (*S*)-**18** (IHMT-PI3Kδ-372), which exhibited anti-COPD efficacy in vivo (Figure 2A).

RESULTS AND DISCUSSION

Structure−Activity Relationship (SAR) Exploration. In general, there are four distinct inhibitor binding regions in the ATP-binding pocket of PI3Kδ,[27,30−32] i.e., a hinge binding region with hydrogen-bond interaction, an affinity pocket deep in the protein not occupied by ATP, a specificity pocket, and a hydrophobic region II located at the entrance of the active site. Compound **4**, which adopts a special propeller shape, occupies the hydrophobic region II, the specificity pocket, and the hinge region, but not the affinity pocket,[27,28] which is potentially essential for improving the potency and isoform selectivity to PI3Kδ (Figure 2B,C). The quinazolinone moiety of **4** is sandwich-folded into the induced hydrophobic specificity pocket between Trp760 and Met752 with a hydrophobic interaction with the kinase. It is worthy to note that this specificity pocket is not present in the apo PI3Kδ kinase structure in which Trp760 and Met752 are packed against each other. The purine moiety of **4** locates in the hinge binding region and forms hydrogen bonds with the backbone amide of Val828 and the backbone carbonyl oxygen of Glu826 (PDB: 4XE0[28]). The propeller-shaped selective PI3Kδ inhibitor **5** adopts a similar binding mode except that the fluoro-isopropoxyphenyl moiety interacts with the affinity pocket, which is not occupied by **4** (Figure 2B,C). Therefore, we tried to combine the binding elements of **4** and **5** to design novel PI3Kδ inhibitors and exploited the hydrophobic region II and the affinity pocket for the SAR study.

We initially synthesized a series of compounds as racemic mixtures to quickly explore the SAR of hydrophobic region II.

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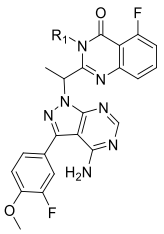
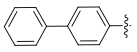
Table 1. SAR Exploration of the R_1 Moiety[c]



Compd	R_1	PI3Kδ[a] (IC_{50}: nM)	class I PI3K isoform selectivity (ratio)[a,b]		
			α/δ	β/δ	γ/δ
4	-	6 ± 0.04	65	141	11
5	-	48.4±2.8	>1000[c]	>30-50[c]	>15-50[c]
6		61 ± 3.8	>164	>164	1.6
7		>10000	>1	>1	>1
8		2368 ± 178	>4	>4	2
9		53 ± 11.7	85	>188	24
10		608 ± 2.2	14	>16	2
11		26 ± 0.9	195	157	54
12		33 ± 1.7	217	82	106
13		18 ± 3.5	95	72	12
14		40 ± 7	94	37	114
15		51 ± 3.1	63	16	129
16		45 ± 1.8	39	10	90
17		53 ± 1	29	8	59

[a]All IC_{50} values were obtained by triplet testing. [b]Determined from isoform-specific PI3K IC_{50} values. [c]Data from ref 33.

The kinase inhibitory activity and isoform selectivity of the new compounds were profiled using an ADP-Glo kinase assay. Compound **6** was generated by appending a modified tail of **5** to the 5-fluoro-3-phenylquinazolin-4(3*H*)-one fragment of **4**.

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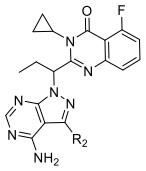
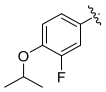
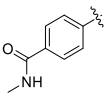
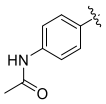
Table 2. SAR Exploration of the R_2 Moiety[b]



Compd	R_2	PI3Kδ[a] (IC_{50}: nM)	class I PI3K isoform selectivity (ratio)[a,b]		
			α/δ	β/δ	γ/δ
18		17 ± 1.8	130	50	62
19	H	>10000	>1	>1	>1
20		2190 ± 148	>5	>5	>5
21		29 ± 4.6	50	48	21
22		32 ± 6	30	90	27
23		85 ± 22.9	37	39	16
24		1749 ± 82	>6	>6	>6
25		532 ± 69.3	>19	>19	3
26		156 ± 25.9	13	1	5
27		1520 ± 329.5	>6	2	>6
28		2234 ± 7.5	>4	1.6	>4
29		34 ± 3.9	>294	37	>294
30		868 ± 82.4	>11	7	6

[a]All IC_{50} values were obtained by triplet testing. [b]Determined from isoform-specific PI3K IC_{50} values.

The results showed that **6** displayed decreased potency against PI3Kδ and lost selectivity against PI3Kγ, while it maintained selectivity over PI3Kα and PI3Kβ compared with **4**. Meanwhile, it displayed comparable inhibitory activity against PI3Kδ relative to **5** (Table 1). Unfortunately, introduction of larger moieties such as biphenyl (**7**) at R_1 resulted in loss of potency against all of the class I PI3K isoforms. Replacement of the phenyl group of **6** with the benzyl group (**8**) also decreased the activity against PI3Kδ and other class I PI3K isoforms. The smaller methylpyrazole group (**9**) at R_1 retained the potency against PI3Kδ and isoform selectivity in comparison with **6**. These results indicated that the hydrophobic region II could not accommodate the larger hydrophobic groups such as the biphenyl or benzyl group. Switching the phenyl group at R_1 to the aliphatic cyclohexyl group (**10**) resulted in significant activity loss against PI3Kδ. Replacement of aromatic substituents with smaller cyclic aliphatic rings such as the cyclopentyl group (**11**), the cyclobutyl group (**12**), and the

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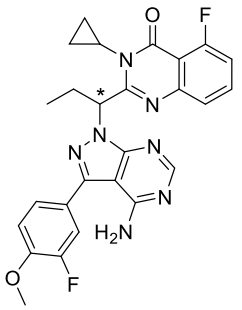
cyclopropyl group (**13**) led to improved potencies against PI3Kδ and retained the selectivity against other class I PI3K members. This suggested that the hydrophobic region II could accommodate the small cyclic aliphatic substituents. In addition, introduction of the isobutyl group (**14**) at R_1 did not affect the potency against PI3Kδ or the overall selectivity over other class PI3K members. Replacement of the phenyl group at R_1 with the isopropyl group (**15**), the ethyl group (**16**), and the methyl group (**17**) retained the activities against PI3Kδ and the selectivity against PI3Kγ but lost the selectivity over PI3Kα and PI3Kβ.

Among the tested compounds, **13** displayed the highest inhibitory activity against PI3Kδ and also retained isoform selectivity. Therefore, we kept the cyclopropyl group at R_1 and further explored the SAR of other positions. Considering that the ethyl group of **4** binds in a hydrophobic pocket formed by Ile910, Met-900, and Met752,[22] we also examined the ethyl analogue and found that **18** retained the potency against PI3Kδ and selectivity over PI3Kα and PI3Kβ (Table 2). Additionally, it exhibited increased selectivity over PI3Kγ. Therefore, we went forward to investigate the SAR of the R_2 moiety in **18**, which interacts with the affinity pocket of PI3Kδ. Removal of the fluoromethoxyphenyl moiety in **18** (**19**) led to complete loss of activity against class I PI3K members, suggesting that the hydrophobic interactions between these compounds and the affinity pocket of PI3Kδ are critical to the potencies against PI3Kδ. Replacing the fluoromethoxyphenyl moiety with a phenyl group (**20**) led to significant activity loss against PI3Kδ, indicating that the substituents in the phenyl moiety are necessary to form the hydrophobic interactions with PI3Kδ. Removal of the fluorine atom (**21**) in the R_2 moiety of **18** or replacement of the fluorine atom by the methoxyl group (**22**) or the methyl group (**23**) resulted in slightly decreased activities against PI3Kδ and selectivity over other class I PI3K members. Replacing the methoxy group in **18** with the isopropoxy group (**24**) led to significant activity loss against PI3Kδ, indicating that the methoxy group played an important role in the interactions with PI3Kδ. Mono-substituted phenyl moieties at R_2 such as **25** and **26** also led to decreased potencies against PI3Kδ. Further increasing the size and hydrophobicity of the R_2 moiety (**27**−**28**) did not improve the potency to PI3Kδ or the isoform selectivity. Interestingly, replacement of the fluoromethoxyphenyl moiety with a smaller methylpyrozole moiety (**29**) retained both the potency against PI3Kδ and isoform selectivity. However, installation of the thienyl moiety at R_2 (**30**) led to dramatic activity loss against PI3Kδ.

Since the racemic compound **18** exhibited optimum potency against PI3Kδ and isoform selectivity, we then evaluated the two stereoisomers of **18** (Table 3). The *S*-enantiomer ((***S***)-**18**) was slightly more active than the *R*-enantiomer ((***R***)-**18**). In addition, (***S***)-**18** showed better selectivity over PI3Kβ and PI3Kγ than (***R***)-**18** and thus it was selected for further examination.

Selectivity Evaluation of Compound (*S*)-18. We next used the DiscoverX KINOMEscan technology to further characterize the kinome-wide selectivity profile of (***S***)-**18**. The results showed that it exhibited high selectivity with an *S*-score (35) = 0.015 among 468 kinases/mutant tested at 1 μM concentration (Figure 3 and Supporting Table 1). Besides strong binding to PI3Kδ, (***S***)-**18** also displayed binding to PI3Kβ, γ, and PI4Kβ in the PIK family as well as Chk2 kinase.

Table 3. Evaluation of the Enantiomers of 18 against PI3Kδ



	PI3Kδ[a]	class I PI3K isoform selectivity (ratio)[a,b]		
compd	(IC_{50}: nM)	α/δ	β/δ	γ/δ
(***R***)-**18**	19 ± 7.0	124	41	74
(***S***)-**18**	14 ± 1.2	83	56	81

[a]All IC_{50} values were obtained by triplet testing. [b]Determined from isoform-specific PI3K IC_{50} values.

Since KINOMEscan is a binding-based assay that may not really reflect the actual inhibitory activity of the kinase inhibitor, we also examined the cellular potencies of (***S***)-**18** against all four class I PI3K isoforms (α, β, γ, δ) using a series of PI3K isoform-specific in vitro cell-based assays to better evaluate the isoform selectivity (Figure 4). Upon anti-IgM stimulation, the PI3Kδ-mediated AKT T308 phosphorylation in Raji cells was inhibited by (***S***)-**18** with an EC_{50} value of 67 nM. In addition, (***S***)-**18** did not show apparent inhibitory effects on AKT phosphorylation controlled by PI3Kα (NIH-3T3, PDGF-BB stimulation), PI3Kβ (NIH-3T3, LPA stimulation), and PI3Kγ (RAW 264.7, human C5a stimulation) up to 3 μM.

To further understand the binding mode of (***S***)-**18**, the inhibitor was docked into the PI3Kδ structure (PDB: 5M6U[24]) that contains a propeller compound via molecular modeling (Figure 5). The model revealed that (***S***)-**18** binds to the ATP-binding site of the kinase domain of PI3Kδ with a propeller shape. Two hydrogen bonds are formed between the amine pyrazolopyrimidine moiety and residues Glu826 and Val828 in the hinge binding region (Figure 5A). The fluoroquinazolinone moiety is sandwiched into the induced specificity pocket between Trp760 and Met752. The cyclopropyl group is located at hydrophobic region II. The methoxyphenyl moiety interacts with the affinity pocket formed by Ile825, Tyr813, Ile910, and Asp911 (Figure 5B). The oxygen atom of the methoxyphenyl forms a hydrogen bond with the backbone of Ile910. The contribution of these interactions is consistent with the observed potency difference between **19** and (***S***)-**18**.

Primary Safety Evaluation of Compound (*S*)-18. To evaluate the potential drug−drug interactions, (***S***)-**18** was tested for inhibition of the most important hepatic metabolic enzymes (Table 4). (***S***)-**18** showed moderate inhibition of CYP2C9 (IC_{50} = 2.7 μM) and no apparent inhibition against CYP1A2, CYP2B6, CYP2C19, and CYP3A4 (IC_{50}s > 10 μM). The potential heart toxicity of (***S***)-**18** was also examined with the electrophysiology functional hERG assay with a patch clamp (Figure 6). The results revealed a very weak inhibitory activity of (***S***)-**18** (IC_{50} = 13.8 μM), which was considered in a safe range (>10 μM).

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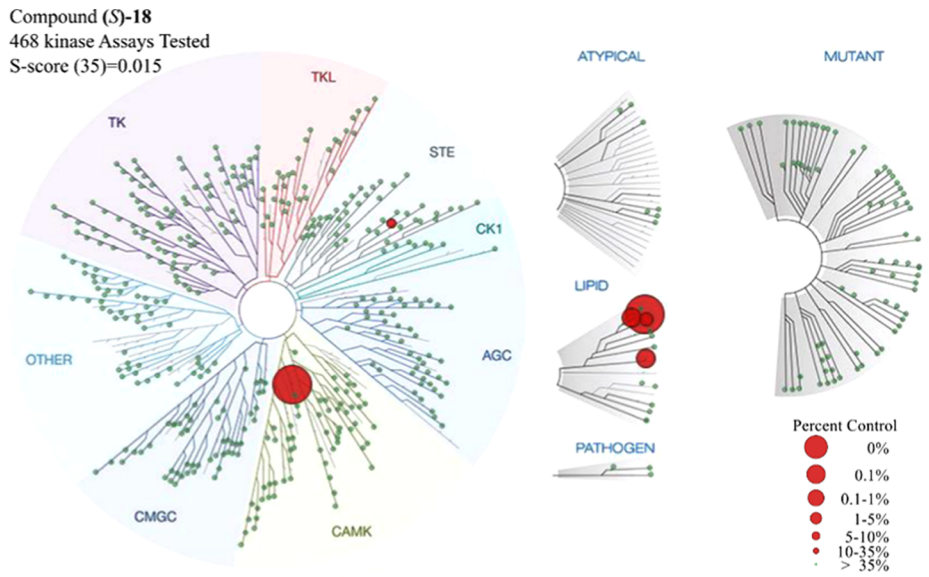

Figure 3. Kinome-wide selectivity profiling of (*S*)-**18** with the DiscoverX KINOMEscan technology. Measurements were performed at a concentration of 1 μM (*S*)-**18**. TREEspot interaction maps for (*S*)-**18** against 468 kinases. *S*-score is a quantitative measure of compound selectivity. It is calculated by dividing the number of kinases that compounds bind to by the total number of distinct kinases tested, excluding mutant variants. *S* is the number of hits/number of assays.

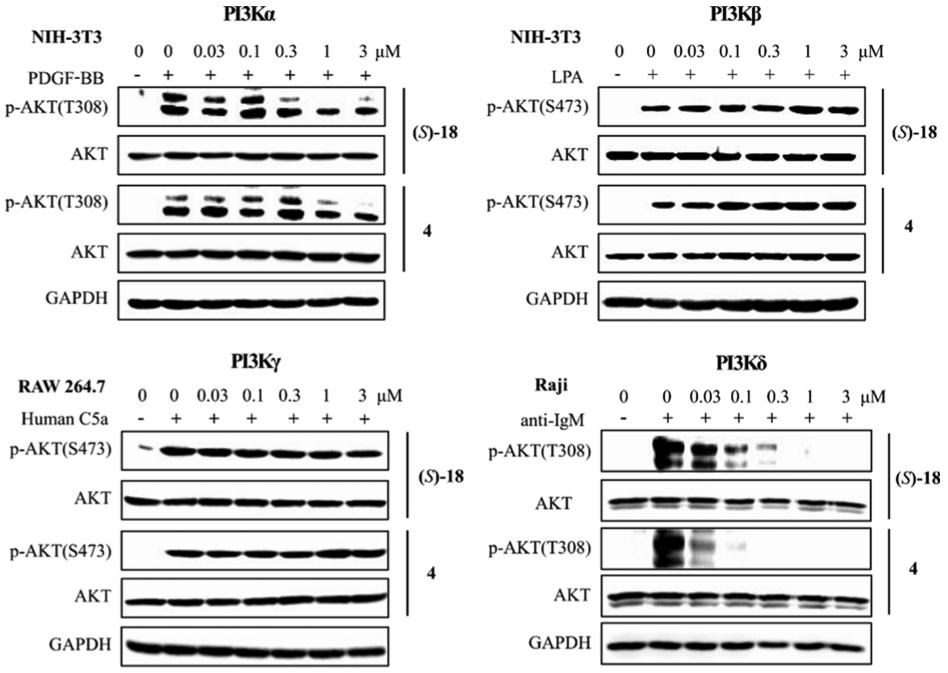

Figure 4. Inhibitory efficacies of (*S*)-**18** against class I PI3K isoforms in cell-based assays.

Pharmacokinetic Profiles of Compound (*S*)-18. We next evaluated the in vivo pharmacokinetic profiles of (*S*)-**18** in rats. At an oral dose of 10 mg/kg, (*S*)-**18** showed high in vivo clearance of 375.9 mL/min/kg and poor oral bioavailability of 5.2% (Table 5), indicating that it might not be a good candidate for oral delivery. Given the fact that COPD is a respiratory disease, we then investigated inhalation as an alternative route of administration. In rats, inhalation of 5 mg/kg dose of (*S*)-**18** displayed a half-life of 2.3 h, low exposure of 66 ng/mL, and high clearance of 348.5 mL/min/kg in plasma (Table 5) but high exposure of 5599 ng/g (6 h after inhalation) in lung tissue (Figure 7). These data indicated that (*S*)-**18** is suitable for inhaled delivery.[23,24] In addition, we also evaluated the metabolic stability of (*S*)-**18** in liver microsomes among different species. The results showed that (*S*)-**18** is stable in human, rat, and mouse liver microsomes, while it has moderate stability in monkey and dog liver microsomes (Table 6).

In Vivo Efficacy Evaluation of Compound (*S*)-18. The in vivo efficacy of (*S*)-**18** was evaluated in an animal model. The cigarette-smoke- and LPS-induced rodent model mimics the characteristic features of COPD, including lung function decline and increased lung inflammation. Aerosolized (*S*)-**18** was dosed at 1 mg/kg/day (low dose), 3 mg/kg/day (medium

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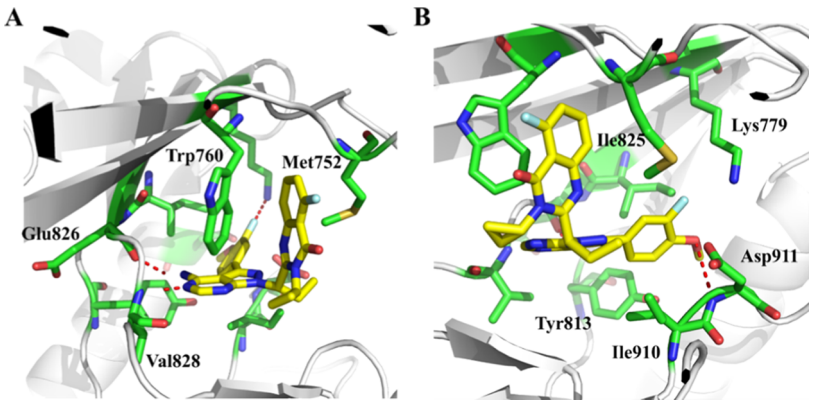

Figure 5. Molecular modeling of (*S*)-**18** (yellow) with PI3Kδ kinase (PDB: 5M6U). The key binding amino acid residues from the protein were labeled as follows: carbon in green, nitrogen in blue, and oxygen in red. Hydrogen bonds are indicated by red dashed lines to the key amino acid residues. (A) Cartoon view of the specificity pocket formed by Trp760, Met752, and the hinge binding region. (B) Cartoon view of the affinity pocket formed by Ile825, Tyr813, Ile910, and Asp911 to accommodate the methoxyphenyl moiety.

Table 4. Inhibition of (*S*)-18 against Five Cytochrome P450s

CYP enzyme	CYP1A2	CYP2B6	CYP2C9	CYP2C19	CYP3A4
(*S*)-**18** (IC_{50}, μM)	>10	>10	2.7	>10	>10

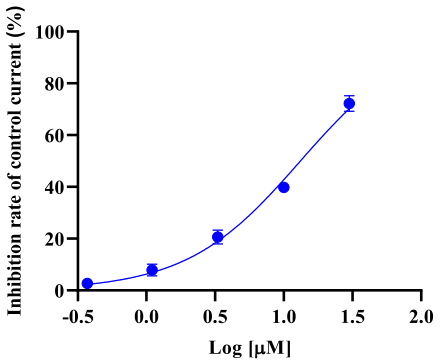

Figure 6. Patch-clamp functional hERG assay of (*S*)-**18**.

dose), and 5 mg/kg/day (high dose) to Sprague-Dawley (SD) rats via the inhalation route for 28 consecutive days (Figure 8). The results showed that the lung function parameters such as forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), and peak expiratory flow (PEF) were improved dose-dependently (Figure 8A and Supporting Table 2). Blood-gas analysis showed that the partial pressure of arterial oxygen (PaO_2) and the arterial oxygen saturation (SaO_2) were also improved in the drug-treated groups compared with the vehicle group, and meanwhile the partial pressure of arterial carbon dioxide ($PaCO_2$) decreased (Figure 8B and Supporting Table 3). Cell counting of bronchoalveolar lavage fluid (BALF) demonstrated that the abnormally high level of leukocytes including the alveolar macrophages, neutrophils, and lymphocytes were reduced by drug treatment

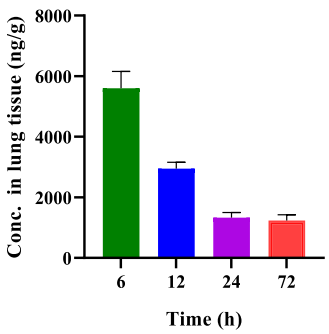

Figure 7. Concentration of (*S*)-**18** in the lung tissue of rats via an inhaled route.

Table 6. Metabolic Stability of (*S*)-18 in Liver Microsomes

species[a]	$t_{1/2}$ (min)	CL_{int} (μL/min/mg)	CL^{hep} (mL/min/kg)
HLM	187	7.40	5.04
MkLM	18.6	74.6	30.6
DLM	36.5	38.0	19.7
RLM	94.9	14.6	20.1
MLM	365	3.80	12.9

[a]Species: HLM, human liver microsomes; MkLM, monkey liver microsomes; DLM, dog liver microsomes; RLM, rat liver microsomes; MLM, mouse liver microsomes.

(Figure 8C and Supporting Table 4). Meanwhile, the inflammatory cytokines such as IL-6, IL-8, TNF-α, and IL-1β were also downregulated in both BALF (Figure 8D and Supporting Table 5) and plasma (Figure 8E and Supporting Table 6). In addition, histopathological analysis of the rat lung tissue demonstrated that (*S*)-**18** decreased the inflammatory cell infiltration in a dose-dependent manner (HE staining in Figure 8F). Furthermore, apoptosis detection showed that the number of TUNEL-positive cells (stained brown or tan)

Table 5. Pharmacokinetic Profiles of (*S*)-18 in Rats[a]

dose (mg/kg)	administration route[b]	$t_{1/2}$ (h)	C_{max} (ng/mL)	AUC_{0-t} (h*ng/mL)	Cl (mL/min/kg)	*F* (%)
1	i.v.	1.7 ± 0.8	942.3 ± 34.0	904 ± 264	18.1 ± 5.9	
10	p.o.	2.3 ± 0.3	122.0 ± 130.9	521.1 ± 241.2	375.9 ± 190.3	5.2 ± 2.4
5	inhal.	2.3 ± 0.9	65.5 ± 23.2	240.2 ± 93.5	348.5 ± 137.1	

[a]Mean ± SD, n = 3. [b]Route of administration: i.v., intravenous administration; p.o., oral administration; inhal., inhaled administration.

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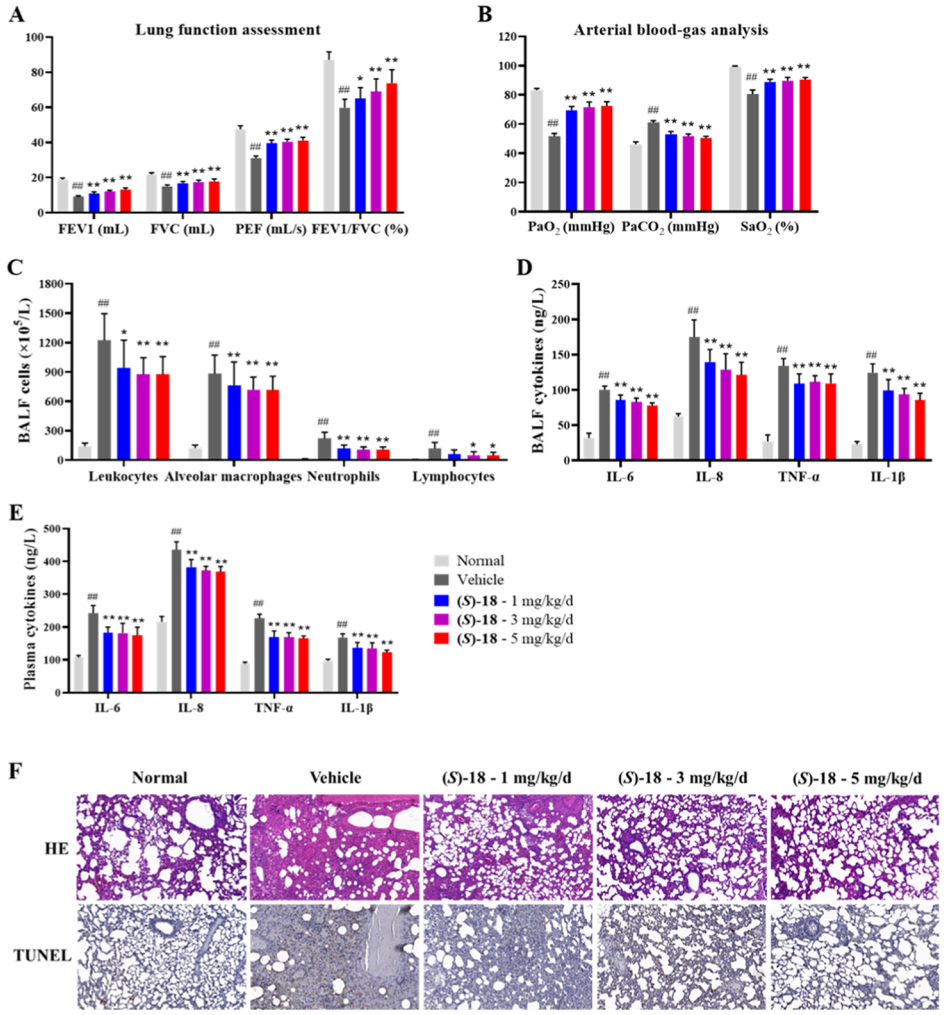

Figure 8. In vivo efficacy evaluation of (*S*)-**18** in a cigarette-smoke-induced and LPS-induced lung inflammation rodent model. (A) Lung function assessment. (B) Blood-gas analysis. (C) BALF cell counts. (D) Measurement of the inflammatory cytokines in BALF. (E) Measurement of the inflammatory cytokines in plasma. Mean±SD, $n = 10$. *$p < 0.05$, **$p < 0.01$ vs the vehicle group; ## for $p < 0.01$ vs the normal group. (F) Histopathological examination of the lung tissue by HE staining and apoptosis detection by the TUNEL assay.

gradually decreased in the low-, medium-, and high-dose (*S*)-**18** groups compared with the vehicle group (TUNEL assay in Figure 8F). Overall, the in vivo results indicated that (*S*)-**18** improved lung function and reduced the inflammatory patterns characteristic of COPD (Figure 8).

CHEMISTRY

The synthetic routes are depicted in Schemes 1−4. The intermediates **32a−l** were synthesized by the Suzuki−Miyaura coupling reaction (Scheme 1). Benzoic acid **33** was reacted with different amines under standard 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU)-mediated coupling condition to yield the amides **34a−g**, followed by treatment with acyl chloride to afford the diamides **35a−g**. Hexamethyldisilazane (HMDS)/$ZnCl_2$-mediated cyclization of **35a−g** in acetonitrile provided the quinazolinone analogues **36a−g**,[34] which were then converted to the target compounds **6−7**, **9**, and **14−17** by nucleophilic substitution as racemic mixtures.

As shown in Scheme 2, benzoic acid **37** was treated with oxalyl dichloride followed by coupling with a set of amines to yield the corresponding amides **38a−e** (Scheme 2). Propionylation or butyrylation of **38a−e** with excess propionic or butyric anhydride followed by reduction of the nitro group with zinc in acetic acid and spontaneous cyclization afforded the quinazolinones **39a−e**. Bromination of **39a−e** with NBS and AIBN provided the intermediates **40a−e**. Subsequent nucleophilic substitution reaction offered the target compounds **8**, **10−12**, and **18−30** as racemic mixtures.

Compound **13** was synthesized from **33** through the amide coupling reaction, acylation, nucleophilic substitution, and cyclization in four steps as racemic mixtures (Scheme 3). (*R*)-2-Chlorobutanoic acid and (*S*)-2-chlorobutanoic acid (**44**) were treated with oxalyl dichloride and then coupled with **41** to give the corresponding diamides **45a−b** (Scheme 4). The quinazolinones **46a−b** were obtained via cyclization of **45a−b**, and subsequent nucleophilic substitution with **32a** afforded the two stereoisomers (*R*)-**18** and (*S*)-**18**.

CONCLUSIONS

In summary, we have discovered a novel potent and isoform-selective PI3Kδ inhibitor compound (*S*)-**18** through a fragment hybridization strategy. It exhibited an IC_{50} value of 14 nM against PI3Kδ with high selectivity over other class I PI3Ks (56−83 fold). By comparison, (*S*)-**18** was slightly less potent than **4** but more potent than **5**, and meanwhile it

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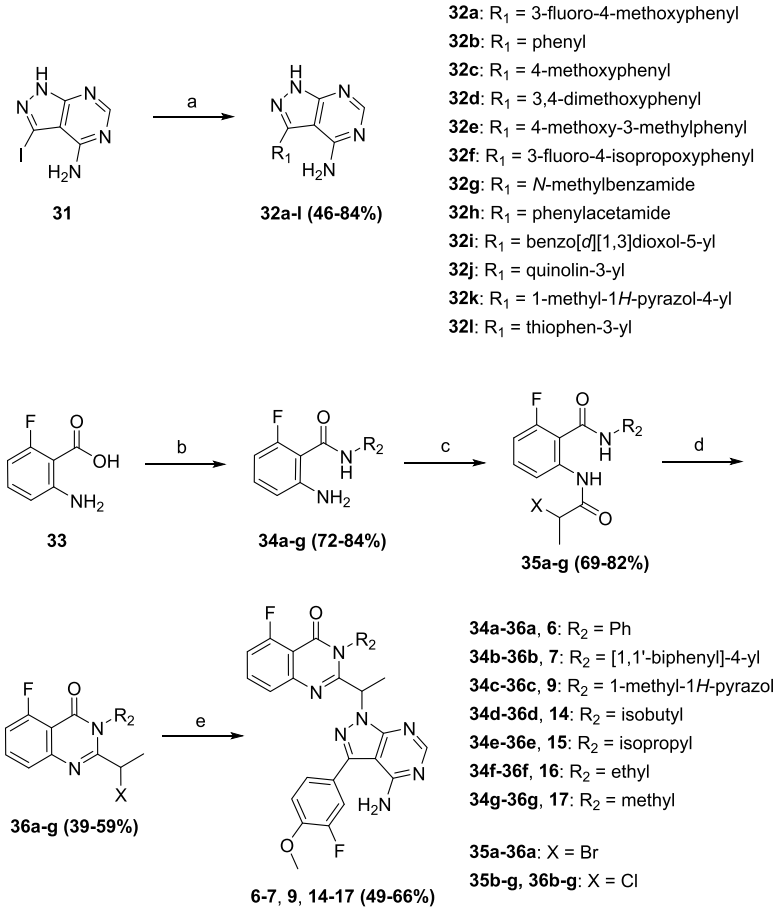
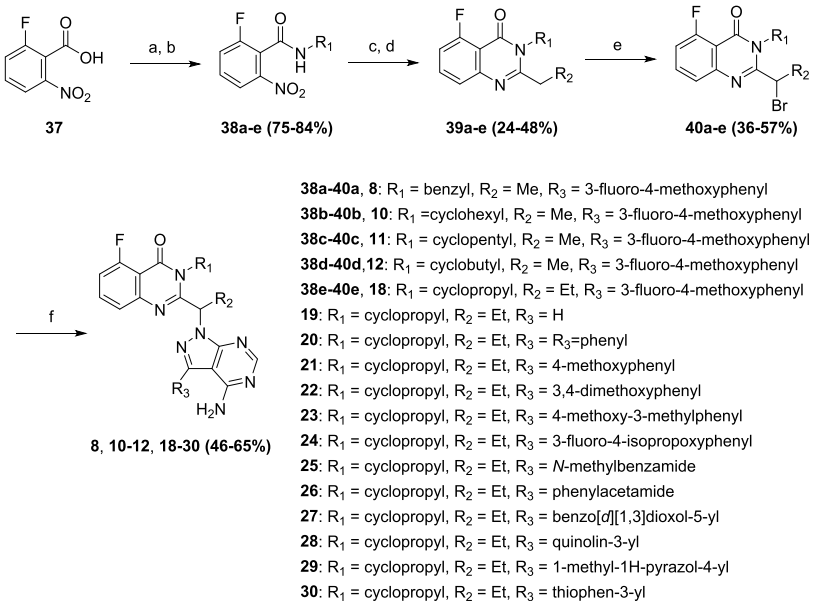
Scheme 1. Synthetic Route to Racemic Compounds 6−7, 9, and 14−17[a]



[a]Reagents and conditions: (a) boronic acid or ester, $Pd(PPh_3)_4$, K_2CO_3, dioxane, H_2O, 135 °C, 14 h; (b) amine, HATU, *N*,*N*-diisopropylethylamine (DIPEA), tetrahydrofuran (THF), rt, 6 h; (c) acyl chloride, DIPEA, THF, 0 °C to rt, 6 h; (d) HMDS, $ZnCl_2$, MeCN, reflux, 5 h; (e) **32a**, K_2CO_3, DMF, 60 °C, 8 h.

Scheme 2. Synthetic Route to Racemic Compounds 8, 10−12, and 18−30[a]



[a]Reagents and conditions: (a) $(COCl)_2$, DMF, THF, 0 °C to rt, 5 h; (b) amine, DIPEA, THF, 0 °C to rt, 6 h; (c) acid anhydride, microwave, 140 °C, 3 h; (d) Zn, AcOH, rt, 8 h; (e) NBS, AIBN, CCl_4, reflux, 10 h; (f) **32a−l**, K_2CO_3, DMF, 60 °C, 8 h.

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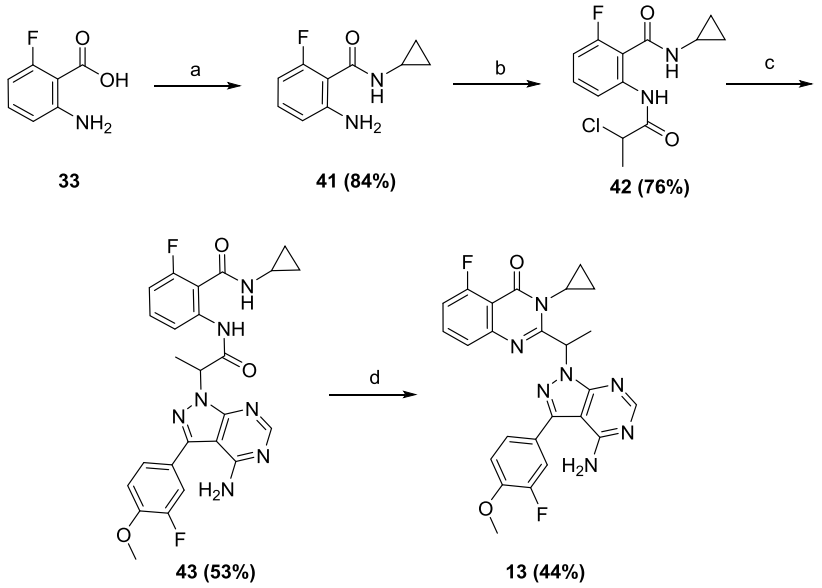
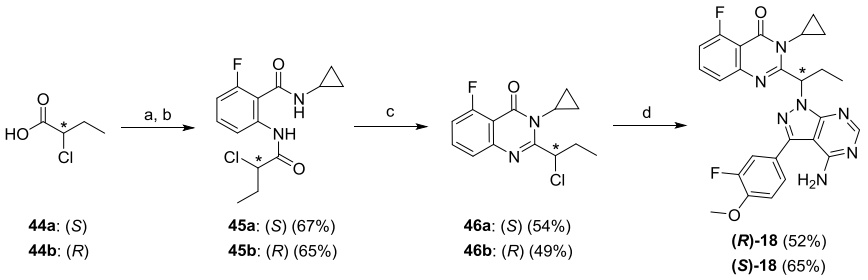
Scheme 3. Synthetic Route to Racemic Compound 13[a]



[a]Reagents and conditions: (a) cyclopropanamine, HATU, DIPEA, THF, rt, 6 h; (b) 2-chloropropanoyl chloride, DIPEA, THF, 0 °C to rt, 6 h; (c) **32a**, K_2CO_3, DMF, 60 °C, 8 h; (d) HMDS, $ZnCl_2$, MeCN, reflux, 5 h.

Scheme 4. Synthetic Route to Compounds (*R*)-18 and (*S*)-18[a]



[a]Reagents and conditions: (a) $(COCl)_2$, DMF, THF, 0 °C to rt, 5 h; (b) **41**, DIPEA, THF, 0 °C to rt, 6 h; (c) HMDS, $ZnCl_2$, MeCN, reflux, 5 h; (d) **32a**, K_2CO_3, DMF, 60 °C, 8 h.

displayed higher selectivity against PI3Kγ. (**S**)-**18** also exhibited high selectivity over other protein kinases, which was revealed in the KINOMEscan profiling (*S*-score (35) = 0.015). Furthermore, the CYP enzymatic assay indicated a low potential for induction of the drug−drug interactions, and the patch-clamp electrophysiology functional assay implied a low risk of hERG-mediated cardiac toxicity.

Notably, the pharmacokinetic profiles of (**S**)-**18** by oral delivery, such as high plasma clearance and low oral bioavailability, indicated that it is a poor oral drug candidate compared with most of the reported PI3Kδ inhibitors. However, administration of (**S**)-**18** by the inhaled route showed high exposure in rat lung tissue upon 6 h, and meanwhile high plasma clearance and low exposure in blood plasma were retained. This is actually an advantage if the inhibitor is aiming for lung disease because it has been reported that oral administration of PI3Kδ inhibitor **4** exhibited adverse effects as an anticancer drug in the clinic,[35] whereas delivering drugs directly to the site of action, e.g., inhaled drug delivery for respiratory disease, may result in a rapid onset of desired effects, the use of smaller doses, and with a potential reduction or avoidance of systemic side effects.[24] Therefore, although not designed purposely, the pharmacokinetic profile of (**S**)-18 happened to make it a good candidate for inhaled delivery. The present favorable in vitro biochemical properties combined with the PK properties and in vivo anti-COPD efficacy indicated that (**S**)-**18** might be a suitable potential inhaled drug candidate for clinical investigation of COPD.

EXPERIMENTAL SECTION

Chemistry. All reagents and solvents were purchased from commercial sources and were used as received unless specified otherwise or prepared as described in the literature. All moisture-sensitive reactions were carried out using dry solvents under ultrapure argon protection. Glassware was dried in an oven at 140 °C for at least 12 h prior to use and then assembled quickly while hot, sealed with rubber septa, and allowed to cool under a stream of argon. Reactions were stirred magnetically using Teflon-coated magnetic stirring bars. Commercially available disposable syringes were used for transferring the reagents and solvents. High-resolution mass spectrometry (HRMS) and high-performance liquid chromatography (HPLC) analyses were performed on an Agilent 6224 TOF using an ESI source coupled to an Agilent 1260 Infinity HPLC system operating in the reverse mode with an Agilent HC-C18 column (4.6 mm × 250 mm, 5 μm) using a water/acetonitrile (each with 0.3% (v/v) formic acid) gradient at a flow rate at 0.8 mL/min. ^1H and ^{13}C spectra were recorded with a Bruker 500 MHz NMR spectrometer and referenced to deuterated methanol (CD_3OD), deuterated

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dimethyl sulfoxide (DMSO-d_6), or deuterated chloroform ($CDCl_3$). Chemical shifts are expressed in ppm. In the NMR tabulation, s indicates singlet; d, doublet; t, triplet; q, quartet, and m, multiplet. Flash column chromatography was conducted using silica gel (Silicycle 40−64 μm). The purities of all final compounds were determined to be ≥95% by HPLC.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoro-3-phenylquinazolin-4(3H)-one (**6**).* To a solution of **36a** (100 mg, 0.29 mmol, 1.0 equiv) in anhydrous DMF (3 mL) was added **32a** (114 mg, 0.44 mmol, 1.5 equiv) and K_2CO_3 (80 mg, 0.58 mmol, 2.0 equiv). Then, the reaction mixture was heated to 60 °C for 8 h. The resulting mixture was concentrated to dryness to give the crude product. The residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent afforded the crude product, which was purified by flash chromatography (eluting with 0−3% MeOH in dimethyl chloride (DCM)) to give **6** as a white solid (81 mg, 53%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93−7.87 (m, 2H), 7.64 (d, J = 8.2 Hz, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.46−7.40 (m, 2H), 7.37 (qd, J = 6.2, 4.4 Hz, 2H), 7.29 (t, J = 8.7 Hz, 1H), 7.10 (t, J = 7.5 Hz, 1H), 6.85 (td, J = 7.8, 1.4 Hz, 1H), 6.35 (d, J = 8.6 Hz, 1H), 5.92 (q, J = 6.6 Hz, 1H), 3.89 (s, 3H), 1.74 (d, J = 6.7 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.96 (d, $J_{C\text{-}F}$ = 264.0 Hz), 158.86 (d, $J_{C\text{-}F}$ = 4.0 Hz), 158.14, 155.71, 154.72, 153.85, 152.02 (d, $J_{C\text{-}F}$ = 244.6 Hz), 148.87, 148.09 (d, $J_{C\text{-}F}$ = 10.5 Hz), 143.73, 136.09 (d, $J_{C\text{-}F}$ = 10.6 Hz), 135.59, 129.99, 128.67, 128.41, 128.13, 127.74, 125.82 (d, $J_{C\text{-}F}$ = 6.9 Hz), 125.34 (d, $J_{C\text{-}F}$ = 3.0 Hz), 124.26 (d, $J_{C\text{-}F}$ = 3.4 Hz), 116.35 (d, $J_{C\text{-}F}$ = 19.0 Hz), 114.83 (d, $J_{C\text{-}F}$ = 1.8 Hz), 114.46 (d, $J_{C\text{-}F}$ = 20.4 Hz), 110.89 (d, $J_{C\text{-}F}$ = 5.5 Hz), 97.63, 56.57, 53.97, 18.95. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{28}H_{22}F_2N_7O_2$, 526.1803; found, 526.1798. HPLC retention time 5.75 min, >98% pure.

Compounds **7**−**12**, **14**−**30**, (***R***)-**18**, (***S***)-**18**, and **43** were prepared following the synthetic procedure of **6**.

*3-([1,1′-Biphenyl]-4-yl)-2-(1-(4-amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoroquinazolin-4(3H)-one (**7**).* Yield 49%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (dd, J = 13.7, 8.1 Hz, 1H), 7.80 (s, 1H), 7.68−7.64 (m, 3H), 7.49−7.35 (m, 8H), 7.26 (t, J = 8.7 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.30 (d, J = 7.4 Hz, 1H), 6.01 (q, J = 6.4 Hz, 1H), 3.88 (s, 3H), 1.76 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.41 (d, $J_{C\text{-}F}$= 266.7 Hz), 159.60 (d, $J_{C\text{-}F}$ = 4.2 Hz), 157.37, 155.47, 153.96, 153.52, 152.64 (d, $J_{C\text{-}F}$ = 248.9 Hz), 148.79, 148.69 (d, $J_{C\text{-}F}$ = 10.7 Hz), 143.86, 142.29, 139.89, 134.89 (d, $J_{C\text{-}F}$ = 10.2 Hz), 134.17, 128.80, 128.75, 127.97, 127.73, 127.67, 127.41, 127.23, 125.70 (d, $J_{C\text{-}F}$ = 6.7 Hz), 124.49 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.20 (d, $J_{C\text{-}F}$ = 3.9 Hz), 116.35 (d, $J_{C\text{-}F}$ = 19.3 Hz), 114.22 (d, $J_{C\text{-}F}$ = 20.6 Hz), 114.01 (d, $J_{C\text{-}F}$ = 1.6 Hz), 111.10 (d, $J_{C\text{-}F}$ = 5.6 Hz), 98.05, 56.41, 54.09, 18.67. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{34}H_{26}F_2N_7O_2$, 602.2116; found, 602.2113. HPLC retention time 15.17 min, >97% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-benzyl-5-fluoroquinazolin-4(3H)-one (**8**).* Yield 54%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.28 (s, 1H), 7.64 (td, J = 8.1, 5.4 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.23−7.15 (m, 2H), 7.12−7.06 (m, 1H), 7.00−6.91 (m, 4H), 6.72 (s, 2H), 6.24 (q, J = 6.6 Hz, 1H), 5.42 (d, J = 16.0 Hz, 1H), 5.25 (d, J = 16.1 Hz, 1H), 3.84 (s, 3H), 1.82 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.21 (d, $J_{C\text{-}F}$ = 266.4 Hz), 158.57 (d, $J_{C\text{-}F}$ = 4.1 Hz), 156.58, 154.71, 152.95, 152.84, 151.45 (d, $J_{C\text{-}F}$ = 248.2 Hz), 147.67, 147.52 (d, $J_{C\text{-}F}$ = 10.4 Hz), 143.12, 133.64 (d, $J_{C\text{-}F}$ = 10.3 Hz), 127.01, 125.67, 124.52 (d, $J_{C\text{-}F}$ = 6.9 Hz), 124.17, 123.46 (d, $J_{C\text{-}F}$ = 3.5 Hz), 123.13 (d, $J_{C\text{-}F}$ = 4.0 Hz), 115.43 (d, $J_{C\text{-}F}$ = 18.9 Hz), 112.98 (d, $J_{C\text{-}F}$ = 20.6 Hz), 112.75 (d, $J_{C\text{-}F}$ = 1.8 Hz), 109.61 (d, $J_{C\text{-}F}$ = 5.5 Hz), 97.33, 55.32, 52.57, 44.83, 18.03. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{29}H_{24}F_2N_7O_2$, 540.1960; found, 540.1965. HPLC retention time 14.17 min, >98% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoro-3-(1-methyl-1H-pyrazol-4-yl)-quinazolin-4(3H)-one (**9**).* Yield 57%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.13 (s, 1H), 7.89 (dd, J = 13.6, 8.1 Hz, 1H), 7.62 (d, J = 8.2 Hz, 1H), 7.41 (d, J = 12.1 Hz, 1H), 7.36 (t, J = 9.2 Hz, 2H), 7.28 (t, J = 8.6 Hz, 1H), 6.88 (s, 2H), 6.10 (q, J = 6.3 Hz, 1H), 3.89 (s, 3H), 3.56 (s, 3H), 1.75 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.75 (d, $J_{C\text{-}F}$ = 265.9 Hz), 160.49 (d, $J_{C\text{-}F}$ = 4.3 Hz), 158.44, 155.71, 154.60, 153.90, 153.15 (d, $J_{C\text{-}F}$ = 248. 4 Hz), 149.33 (d, $J_{C\text{-}F}$ = 10.4 Hz), 149.04, 144.92, 137.09, 135.95 (d, $J_{C\text{-}F}$ = 10.4 Hz), 128.89, 125.78 (d, $J_{C\text{-}F}$ = 6.7 Hz), 125.11 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.70 (d, $J_{C\text{-}F}$ = 4.1 Hz), 116.82 (d, $J_{C\text{-}F}$ = 19.4 Hz), 116.79, 114.92 (d, $J_{C\text{-}F}$ = 20.9 Hz), 114.63 (d, $J_{C\text{-}F}$ = 1.6 Hz), 110.98 (d, $J_{C\text{-}F}$ = 6.0 Hz), 98.52, 56.77, 54.81, 39.45, 18.96. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{22}F_2N_9O_2$, 530.1865; found, 530.1866. HPLC retention time 11.59 min, >95% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-cyclohexyl-5-fluoroquinazolin-4(3H)-one (**10**).* Yield 56%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.37 (s, 1H), 7.85−7.77 (m, 1H), 7.54 (d, J = 8.1 Hz, 1H), 7.32−7.23 (m, 4H), 6.51 (q, J = 6.3 Hz, 1H), 3.89 (t, J = 9.8 Hz, 1H), 3.85 (s, 3H), 2.45 (dt, J = 21.4, 7.3 Hz, 1H), 2.18 (td, J = 12.1, 9.2 Hz, 1H), 1.79 (d, J = 6.4 Hz, 3H), 1.77−1.66 (m, 2H), 1.37 (d, J = 12.5 Hz, 1H), 1.23−1.14 (m, 2H), 0.92 (q, J = 13.2 Hz, 1H), 0.12 (d, J = 11.1 Hz, 1H), 0.00 (q, J = 12.9 Hz, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.57 (d, $J_{C\text{-}F}$ = 265.3 Hz), 160.98 (d, $J_{C\text{-}F}$ = 3.9 Hz), 158.49, 156.42, 153.91, 153.77, 153.02 (d, $J_{C\text{-}F}$ = 248.1 Hz), 149.22 (d, $J_{C\text{-}F}$ = 10.8 Hz), 148.70, 145.11, 134.79 (d, $J_{C\text{-}F}$= 10.4 Hz), 125.69 (d, $J_{C\text{-}F}$ = 7.0 Hz), 125.04 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.23 (d, $J_{C\text{-}F}$ = 4.2 Hz), 116.80 (d, $J_{C\text{-}F}$ = 19.4 Hz), 114.49 (d, $J_{C\text{-}F}$ = 1.8 Hz), 114.27 (d, $J_{C\text{-}F}$ = 21.2 Hz), 112.32 (d, $J_{C\text{-}F}$ = 4.6 Hz), 98.75, 60.44, 56.78, 54.82, 29.06, 28.34, 26.72, 26.11, 25.38, 19.98. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{28}H_{28}F_2N_7O_2$, 532.2273; found, 532.2270. HPLC retention time 16.37 min, >97% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-cyclopentyl-5-fluoroquinazolin-4(3H)-one (**11**).* Yield 55%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.85−7.77 (m, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.36−7.25 (m, 4H), 6.58 (q, J = 6.5 Hz, 1H), 4.65−4.55 (m, 1H), 3.87 (s, 3H), 2.19−2.09 (m, 1H), 1.84 (t, J = 11.0 Hz, 5H), 1.73−1.62 (m, 2H), 1.40 (dt, J = 19.7, 7.1 Hz, 1H), 1.10−1.01 (m, 1H), 0.22 (dd, J = 15.9, 7.5 Hz, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.01 (d, $J_{C\text{-}F}$ = 264.9 Hz), 159.78 (d, $J_{C\text{-}F}$ = 3.9 Hz), 157.94, 155.92, 153.75, 153.54, 152.48 (d, $J_{C\text{-}F}$ = 248.3 Hz), 148.68 (d, $J_{C\text{-}F}$ = 10.7 Hz), 148.23, 144.54, 134.25 (d, $J_{C\text{-}F}$ = 10.5 Hz), 125.22 (d, $J_{C\text{-}F}$ = 6.8 Hz), 124.55 (d, $J_{C\text{-}F}$ = 3.3 Hz), 123.75 (d, $J_{C\text{-}F}$ = 3.9 Hz), 116.31 (d, $J_{C\text{-}F}$ = 19.4 Hz), 113.95 (d, $J_{C\text{-}F}$ = 1.8 Hz), 113.70 (d, $J_{C\text{-}F}$ = 21.2 Hz), 111.60 (d, $J_{C\text{-}F}$ = 4.8 Hz), 98.27, 58.99, 56.26, 54.59, 28.52, 27.94, 25.84, 25.73, 19.23. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{27}H_{26}F_2N_7O_2$, 518.2116; found, 518.2118. HPLC retention time 15.63 min, >96% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-cyclobutyl-5-fluoroquinazolin-4(3H)-one (**12**).* Yield 54%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.31 (s, 1H), 7.55−7.50 (m, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.28−7.20 (m, 2H), 7.00 (dd, J = 10.1, 8.7 Hz, 1H), 6.95 (t, J = 8.4 Hz, 1H), 6.26 (q, J = 6.6 Hz, 1H), 4.58 (p, J = 8.6 Hz, 1H), 3.81 (s, 3H), 3.14 (p, J = 9.8 Hz, 1H), 2.72 (p, J = 9.9 Hz, 1H), 2.16 (dd, J = 18.9, 8.6 Hz, 1H), 1.89 (d, J = 6.6 Hz, 3H), 1.80 (q, J = 10.5 Hz, 1H), 1.48−1.35 (m, 1H), 1.08−0.99 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.04 (d, $J_{C\text{-}F}$ = 266.5 Hz), 160.67 (d, $J_{C\text{-}F}$ = 3.9 Hz), 158.11, 156.12, 153.92, 153.63, 152.50 (d, $J_{C\text{-}F}$ = 248.4 Hz), 148.63 (d, $J_{C\text{-}F}$ = 10.4 Hz), 148.18, 144.24, 134.13 (d, $J_{C\text{-}F}$ = 10.3 Hz), 125.50 (d, $J_{C\text{-}F}$ = 6.7 Hz), 124.55 (d, $J_{C\text{-}F}$ = 3.2 Hz), 123.68 (d, $J_{C\text{-}F}$ = 4.1 Hz), 116.35 (d, $J_{C\text{-}F}$ = 19.3 Hz), 113.92 (d, $J_{C\text{-}F}$ = 1.6 Hz), 113.70 (d, $J_{C\text{-}F}$ = 21.1 Hz), 111.76 (d, $J_{C\text{-}F}$ = 4.8 Hz), 98.33, 56.32, 54.49, 52.27, 26.85, 26.81, 19.11, 14.48. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{24}F_2N_7O_2$, 504.1960; found, 504.1963. HPLC retention time 15.13 min, >99% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**13**).* To a solution of **43** (50 mg, 0.1 mmol, 1.0 equiv) in anhydrous MeCN (3 mL) were added HMDS (0.07 mL, 0.33 mmol, 3.3 equiv) and $ZnCl_2$ (45 mg, 0.33 mmol, 3.3 equiv). Then, the reaction mixture was heated at reflux for 5 h. The resulting mixture was concentrated to dryness to give the crude product. The residue was diluted with water and extracted with DCM. The combined

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organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−10% EtOAc in hexane) to give **13** as a white solid (21 mg, yield 44%). ^{1}H NMR (500 MHz, CD_3OD) δ 8.23 (d, J = 5.6 Hz, 1H), 7.56 (td, J = 8.2, 5.4 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.32−7.23 (m, 2H), 7.04−6.98 (m, 2H), 6.71 (q, J = 6.8 Hz, 1H), 3.85 (s, 3H), 2.22 (dq, J = 7.1, 4.2 Hz, 1H), 1.93 (d, J = 6.8 Hz, 3H), 1.24 (ddd, J = 11.9, 8.0, 5.9 Hz, 1H), 1.20−1.15 (m, 1H), 0.98−0.85 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.25 (d, $J_{C\text{-}F}$ = 3.9 Hz), 161.23 (d, $J_{C\text{-}F}$ = 266.1 Hz), 158.34, 156.59, 156.19, 154.71, 152.95 (d, $J_{C\text{-}F}$ = 248.2 Hz), 149.06 (d, $J_{C\text{-}F}$ = 10.7 Hz), 148.72, 144.59, 134.87 (d, $J_{C\text{-}F}$ = 10.3 Hz), 125.92 (d, $J_{C\text{-}F}$ = 7.1 Hz), 125.08 (d, $J_{C\text{-}F}$ = 3.4 Hz), 124.13 (d, $J_{C\text{-}F}$ = 3.9 Hz), 116.86 (d, $J_{C\text{-}F}$ = 19.4 Hz), 114.38 (d, $J_{C\text{-}F}$ = 1.9 Hz), 114.24 (d, $J_{C\text{-}F}$ = 21.0 Hz), 111.47 (d, $J_{C\text{-}F}$ = 6.0 Hz), 98.75, 56.76, 54.31, 26.86, 18.92, 11.30, 10.39. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{25}H_{22}F_2N_7O_2$, 490.1803; found, 490.1804. HPLC retention time 13.39 min, >97% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoro-3-isobutylquinazolin-4(3H)-one (**14**).* Yield 66%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.81 (dd, J = 13.7, 8.2 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.37 (t, J = 9.9 Hz, 2H), 7.33−7.27 (m, 2H), 6.32 (q, J = 6.5 Hz, 1H), 3.93 (dd, J = 14.0, 7.8 Hz, 1H), 3.88 (s, 3H), 3.74 (dd, J = 13.7, 7.3 Hz, 1H), 1.99 (dt, J = 13.6, 6.7 Hz, 1H), 1.87 (d, J = 6.6 Hz, 3H), 0.86 (d, J = 6.6 Hz, 3H), 0.68 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.17 (d, $J_{C\text{-}F}$ = 265.7 Hz), 159.69 (d, $J_{C\text{-}F}$ = 4.3 Hz), 157.96, 156.08, 154.30, 153.92, 152.56 (d, $J_{C\text{-}F}$ = 248.3 Hz), 148.63, 148.61 (d, $J_{C\text{-}F}$ = 10.2 Hz), 144.14, 134.30 (d, $J_{C\text{-}F}$ = 10.3 Hz), 125.74 (d, $J_{C\text{-}F}$ = 7.1 Hz), 124.58 (d, $J_{C\text{-}F}$ = 3.4 Hz), 123.95 (d, $J_{C\text{-}F}$ = 4.1 Hz), 116.50 (d, $J_{C\text{-}F}$ = 19.3 Hz), 113.89 (d, $J_{C\text{-}F}$ = 1.7 Hz), 113.69 (d, $J_{C\text{-}F}$ = 20.9 Hz), 110.79 (d, $J_{C\text{-}F}$ = 5.2 Hz), 98.56, 56.35, 53.53, 49.03, 28.58, 20.05, 19.99, 18.99. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{26}F_2N_7O_2$, 506.2116; found, 506.2120. HPLC retention time 15.33 min, >99% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoro-3-isopropylquinazolin-4(3H)-one (**15**).* Yield 61%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.85−7.75 (m, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.37−7.24 (m, 4H), 6.50 (q, J = 6.5 Hz, 1H), 4.48 (dt, J = 13.3, 6.6 Hz, 1H), 3.87 (s, 3H), 1.82 (d, J = 6.4 Hz, 3H), 1.51 (d, J = 6.6 Hz, 3H), 0.71 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.15 (d, $J_{C\text{-}F}$ = 263.8 Hz), 160.08 (d, $J_{C\text{-}F}$ = 1.8 Hz), 157.93, 156.27, 153.94, 153.44, 152.58 (d, $J_{C\text{-}F}$ = 248.18 Hz), 148.70 (d, $J_{C\text{-}F}$ = 10.7 Hz), 148.35, 144.23, 134.07 (d, $J_{C\text{-}F}$ = 10.4 Hz), 125.54 (d, $J_{C\text{-}F}$ = 7.0 Hz), 124.55 (d, $J_{C\text{-}F}$ = 3.4 Hz), 123.77 (d, $J_{C\text{-}F}$ = 4.0 Hz), 116.41 (d, $J_{C\text{-}F}$ = 19.3 Hz), 113.96 (d, $J_{C\text{-}F}$ = 1.7 Hz), 113.68 (d, $J_{C\text{-}F}$ = 21.2 Hz), 111.96 (d, $J_{C\text{-}F}$ = 4.7 Hz), 98.44, 56.38, 54.29, 51.39, 19.72, 19.39, 18.50. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{25}H_{24}F_2N_7O_2$, 492.1960; found, 492.1962. HPLC retention time 14.68 min, >99% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-3-ethyl-5-fluoroquinazolin-4(3H)-one (**16**).* Yield 64%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.32 (s, 1H), 7.81 (dd, J = 13.7, 8.2 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.41−7.33 (m, 2H), 7.29 (dd, J = 19.3, 10.4 Hz, 2H), 6.40 (q, J = 6.5 Hz, 1H), 4.07−3.91 (m, 2H), 3.88 (s, 3H), 1.85 (d, J = 6.6 Hz, 3H), 0.68 (t, J = 6.9 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 161.08 (d, $J_{C\text{-}F}$ = 265.5 Hz), 159.27 (d, $J_{C\text{-}F}$ = 4.3 Hz), 157.93, 156.19, 154.25, 153.54, 152.58 (d, $J_{C\text{-}F}$ = 248.5 Hz) 148.70, 148.62, 144.17, 134.26 (d, $J_{C\text{-}F}$ = 10.3 Hz), 125.66 (d, $J_{C\text{-}F}$ = 7.0 Hz), 124.56 (d, $J_{C\text{-}F}$ = 3.4 Hz), 123.95 (d, $J_{C\text{-}F}$ = 4.1 Hz), 116.50 (d, $J_{C\text{-}F}$ = 19.4 Hz), 113.91 (d, $J_{C\text{-}F}$ = 1.7 Hz), 113.64 (d, $J_{C\text{-}F}$ = 20.8 Hz), 110.83 (d, $J_{C\text{-}F}$ = 5.5 Hz), 98.60, 56.37, 53.68, 38.77, 19.11, 13.23. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{24}H_{22}F_2N_7O_2$, 478.1803; found, 478.1799. HPLC retention time 13.96 min, >98% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)ethyl)-5-fluoro-3-methylquinazolin-4(3H)-one (**17**).* Yield 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.82 (dd, J = 13.7, 8.1 Hz, 1H), 7.53 (d, J = 8.2 Hz, 1H), 7.41−7.25 (m, 4H), 6.41 (q, J = 6.5 Hz, 1H), 3.87 (s, 3H), 3.20 (s, 3H), 1.83 (d, J = 6.6 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 161.45 (d, $J_{C\text{-}F}$ = 264.0 Hz), 160.64 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.77, 156.49, 154.50, 154.38, 153.06 (d, $J_{C\text{-}F}$ = 247.0 Hz), 149.28 (d, $J_{C\text{-}F}$ = 10.5 Hz), 149.13, 145.36, 135.29 (d, $J_{C\text{-}F}$ = 10.5 Hz), 125.75 (d, $J_{C\text{-}F}$ = 6.9 Hz), 125.22 (d, $J_{C\text{-}F}$ = 3.5 Hz), 124.44 (d, $J_{C\text{-}F}$ = 4.2 Hz), 116.90 (d, $J_{C\text{-}F}$ = 19.4 Hz), 114.53 (d, $J_{C\text{-}F}$ = 2.2 Hz), 114.35 (d, $J_{C\text{-}F}$ = 21.0 Hz), 110.77 (d, $J_{C\text{-}F}$ = 5.6 Hz), 98.88, 56.72, 54.98, 30.35, 18.97. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{23}H_{20}F_2N_7O_2$, 464.1647; found, 464.1644. HPLC retention time 13.07 min, >99% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**18**).* Yield 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.78 (dd, J = 13.6, 8.1 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.40 (t, J = 9.2 Hz, 2H), 7.34−7.25 (m, 2H), 6.53 (dd, J = 8.9, 5.0 Hz, 1H), 3.90 (s, 3H), 2.52−2.38(m, 2H), 2.21−2.13 (m, 1H), 1.22−1.14 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H), 0.84−0.81 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 263.3 Hz), 159.76 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.74, 156.94, 156.44, 155.77, 151.96 (d, $J_{C\text{-}F}$ = 244.4 Hz), 148.32, 148.11 (d, $J_{C\text{-}F}$ = 10.4 Hz), 143.98, 135.38 (d, $J_{C\text{-}F}$ = 10.6 Hz), 125.79 (d, $J_{C\text{-}F}$ = 6.8 Hz), 125.38 (d, $J_{C\text{-}F}$ = 2.8 Hz), 123.81 (d, $J_{C\text{-}F}$ = 3.4 Hz), 116.37 (d, $J_{C\text{-}F}$ = 19.1 Hz), 114.81, 114.01 (d, $J_{C\text{-}F}$ = 20.6 Hz), 110.90 (d, $J_{C\text{-}F}$ = 5.7 Hz), 97.63, 59.28, 56.54, 26.75, 25.94, 11.26, 10.83, 10.09. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{24}F_2N_7O_2$, 504.1960; found, 504.1965. HPLC retention time 14.14 min, >99% pure.

*(R)-2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo-[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one ((**R**)-**18**).* Yield 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.78 (td, J = 8.1, 5.7 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.43−7.37(m, 2H), 7.34−7.25 (m, 2H), 6.54 (dd, J = 9.0, 5.0 Hz, 1H), 3.90 (s, 3H), 2.53−2.37 (m, 2H), 2.21−2.13 (m, 1H), 1.25−1.10 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H), 0.87−0.78 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 261.1 Hz), 159.76 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.74, 156.94, 156.44, 155.77, 151.97 (d, $J_{C\text{-}F}$ = 245.3 Hz), 148.32, 148.11 (d, $J_{C\text{-}F}$ = 10.3 Hz), 143.99, 135.37 (d, $J_{C\text{-}F}$ = 10.5 Hz), 125.80 (d, $J_{C\text{-}F}$ = 7.1 Hz), 125.38 (d, $J_{C\text{-}F}$ = 3.0 Hz), 123.81 (d, $J_{C\text{-}F}$ = 3.4 Hz), 116.37 (d, $J_{C\text{-}F}$ = 19.0 Hz), 114.80, 114.01 (d, $J_{C\text{-}F}$ = 20.6 Hz), 110.91 (d, $J_{C\text{-}F}$ = 5.8 Hz), 97.64, 59.28, 56.53, 26.75, 25.94, 11.25, 10.83, 10.09. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{24}F_2N_7O_2$, 504.1960; found, 504.1963. HPLC retention time 14.13 min, >99% pure.

*(S)-2-(1-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo-[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one ((**S**)-**18**).* Yield 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.30 (s, 1H), 7.78 (td, J = 8.1, 5.7 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.42−7.38 (m, 2H), 7.34−7.25 (m, 2H), 6.53 (dd, J = 9.0, 5.0 Hz, 1H), 3.90 (s, 3H), 2.53−2.37 (m, 2H), 2.20−2.13 (m, 1H), 1.25−1.10 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H), 0.86−0.79 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 260.1 Hz), 159.76 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.74, 156.94, 156.44, 155.77, 151.96 (d, $J_{C\text{-}F}$ = 248.1 Hz), 148.32, 148.11 (d, $J_{C\text{-}F}$ = 10.4 Hz), 143.99, 135.38 (d, $J_{C\text{-}F}$ = 10.4 Hz), 125.79 (d, $J_{C\text{-}F}$ = 6.8 Hz), 125.38 (d, $J_{C\text{-}F}$ = 2.9 Hz), 123.81 (d, $J_{C\text{-}F}$ = 3.5 Hz), 116.37 (d, $J_{C\text{-}F}$ = 19.0 Hz), 114.81, 114.01 (d, $J_{C\text{-}F}$ = 20.5 Hz), 110.90 (d, $J_{C\text{-}F}$ = 5.8 Hz), 97.63, 59.28, 56.54, 26.75, 25.94, 11.26, 10.83, 10.09. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{24}F_2N_7O_2$, 504.1960; found, 504.1965. HPLC retention time 14.13 min, >97% pure.

*2-(1-(4-Amino-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**19**).* Yield 58%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.20 (s, 1H), 8.13 (s, 1H), 7.79 (dd, J = 13.7, 8.2 Hz, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.3, 8.7 Hz, 1H), 6.44 (dd, J = 9.0, 5.0 Hz, 1H), 2.48−2.40 (m, 1H), 2.39−2.29 (m, 1H), 2.00−1.93 (m, 1H), 1.18−1.04 (m, 3H), 0.86 (t, J = 7.3 Hz, 3H), 0.77−0.70 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 263.8 Hz), 159.74 (d, $J_{C\text{-}F}$ = 4.1 Hz), 158.63, 157.01, 156.58, 154.61, 148.34, 135.43 (d, $J_{C\text{-}F}$ = 10.5 Hz), 133.54, 123.79 (d, $J_{C\text{-}F}$ = 3.6 Hz), 114.04 (d, $J_{C\text{-}F}$ = 20.6 Hz), 110.90 (d, $J_{C\text{-}F}$ = 5.6 Hz), 100.16, 59.06, 26.62, 26.05, 11.11, 10.78, 10.01. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{19}H_{19}FN_7O$, 380.1635; found, 380.1631. HPLC retention time 9.66 min, >98% pure.

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*2-(1-(4-Amino-3-phenyl-1H-pyrazolo[3,4-d]pyrimidin-1-yl)-propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**20**).* Yield 60%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.28 (s, 1H), 7.78 (td, J = 8.1, 5.5 Hz, 1H), 7.59 (d, J = 7.1 Hz, 2H), 7.54−7.43 (m, 4H), 7.28 (dd, J = 10.5, 8.5 Hz, 1H), 6.52 (dd, J = 9.1, 4.9 Hz, 1H), 2.49−2.36 (m, 2H), 2.14−2.09 (m, 1H), 1.20−1.08 (m, 3H), 0.93 (t, J = 7.3 Hz, 3H), 0.82−0.76 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.84 (d, $J_{C\text{-}F}$ = 265.41 Hz), 160.63 (d, $J_{C\text{-}F}$ = 3.9 Hz), 158.03, 155.90, 155.71, 155.45, 148.38, 145.35, 134.17 (d, $J_{C\text{-}F}$ = 10.3 Hz), 132.92, 129.26, 129.20, 128.61, 123.76 (d, $J_{C\text{-}F}$ = 4.0 Hz), 113.65 (d, $J_{C\text{-}F}$ = 20.8 Hz), 111.16 (d, $J_{C\text{-}F}$ = 5.8 Hz), 98.30, 59.20, 26.49, 25.92, 11.35, 11.04, 10.23. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{25}H_{23}FN_7O$, 456.1948; found, 456.1945. HPLC retention time 13.83 min, >99% pure.

*2-(1-(4-Amino-3-(4-methoxyphenyl)-1H-pyrazolo[3,4-d]-pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**21**).* Yield 63%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.78 (dd, J = 13.6, 8.1 Hz, 1H), 7.53 (d, J = 8.6 Hz, 2H), 7.46 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.3, 8.7 Hz, 1H), 7.08 (d, J = 8.6 Hz, 2H), 6.52 (dd, J = 9.0, 5.0 Hz, 1H), 3.81 (s, 3H), 2.52−2.37 (m, 2H), 2.14−2.08 (m, 1H), 1.21−1.09 (m, 3H), 0.94 (t, J = 7.3 Hz, 3H), 0.82−0.75 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 263.2 Hz), 160.16, 159.77 (d, $J_{C\text{-}F}$ = 3.7 Hz), 158.77, 157.00, 156.39, 155.72, 148.33, 144.98, 135.41 (d, $J_{C\text{-}F}$ = 10.4 Hz), 130.13, 125.42, 123.81 (d, $J_{C\text{-}F}$ = 3.2 Hz), 115.05, 114.02 (d, $J_{C\text{-}F}$ = 20.6 Hz), 110.90 (d, $J_{C\text{-}F}$ = 5.6 Hz), 97.59, 59.21, 55.69, 26.74, 25.97, 11.26, 10.81, 10.06. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{25}FN_7O_2$, 486.2054; found, 486.2056. HPLC retention time 13.71 min, >98% pure.

*2-(1-(4-Amino-3-(3,4-dimethoxyphenyl)-1H-pyrazolo[3,4-d]-pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**22**).* Yield 57%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.78 (dd, J = 13.6, 8.1 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.3, 8.7 Hz, 1H), 7.14−7.08 (m, 3H), 6.52 (dd, J = 8.9, 5.0 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.52−2.35 (m, 2H), 2.13−2.07 (m, 1H), 1.22−1.06 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H), 0.86−0.77 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 263.2 Hz), 159.81 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.75, 157.02, 156.39, 155.70, 149.80, 149.41, 148.32, 145.21, 135.40 (d, $J_{C\text{-}F}$ = 10.4 Hz), 125.51, 123.80 (d, $J_{C\text{-}F}$ = 3.6 Hz), 121.23, 114.01 (d, $J_{C\text{-}F}$ = 20.4 Hz), 112.66, 112.30, 110.88 (d, $J_{C\text{-}F}$ = 5.8 Hz), 97.65, 59.28, 56.01, 55.82, 26.75, 25.96, 11.28, 10.81, 10.05. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{27}H_{27}FN_7O_3$, 516.2159; found, 516.2161. HPLC retention time 12.51 min, >98% pure.

*2-(1-(4-Amino-3-(4-methoxy-3-methylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**23**).* Yield 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.78 (td, J = 8.1, 5.7 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.38 (s, 1H), 7.28 (dd, J = 10.4, 8.6 Hz, 1H), 7.08 (d, J = 8.4 Hz, 1H), 6.52 (dd, J = 9.0, 5.0 Hz, 1H), 3.84 (s, 3H), 2.52−2.37 (m, 2H), 2.20 (s, 3H), 2.14−2.08 (m, 1H), 1.23−1.10 (m, 3H), 0.93 (t, J = 7.3 Hz, 3H), 0.85−0.78 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.43 (d, $J_{C\text{-}F}$ = 264.4 Hz), 159.77 (d, $J_{C\text{-}F}$ = 3.9 Hz), 158.74, 158.31, 157.01, 156.38, 155.68, 148.32, 145.21, 135.40 (d, $J_{C\text{-}F}$ = 10.6 Hz), 130.80, 127.70, 126.87, 124.99, 123.81 (d, $J_{C\text{-}F}$ = 3.5 Hz), 114.01 (d, $J_{C\text{-}F}$ = 20.6 Hz), 111.23, 110.90 (d, $J_{C\text{-}F}$ = 5.6 Hz), 97.58, 59.19, 55.84, 26.75, 25.98, 16.51, 11.25, 10.84, 10.06. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{27}H_{27}FN_7O_2$, 500.2210; found, 500.2207. HPLC retention time 14.33 min, >96% pure.

*2-(1-(4-Amino-3-(3-fluoro-4-isopropoxyphenyl)-1H-pyrazolo-[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**24**).* Yield 48%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.81−7.74 (m, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.36 (d, J = 12.9 Hz, 1H), 7.33−7.25 (m, 3H), 6.50 (dd, J = 9.0, 4.9 Hz, 1H), 4.68 (dt, J = 12.0, 6.0 Hz, 1H), 2.49−2.33 (m, 2H), 2.15−2.06 (m, 1H), 1.31 (d, J = 6.0 Hz, 6H), 1.20−1.06 (m, 3H), 0.92 (t, J = 7.3 Hz, 3H), 0.81−0.73 (m, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.90 (d, $J_{C\text{-}F}$ = 3.8 Hz), 160.72 (d, $J_{C\text{-}F}$ = 264.1 Hz), 157.83, 155.67, 155.52, 155.14, 153.57 (d, $J_{C\text{-}F}$ = 247.7 Hz), 148.24, 146.82 (d, $J_{C\text{-}F}$ = 10.3 Hz), 144.33, 134.41 (d, $J_{C\text{-}F}$ = 10.2 Hz), 125.64 (d, $J_{C\text{-}F}$ = 7.0 Hz), 124.52 (d, $J_{C\text{-}F}$ = 3.4 Hz), 123.69 (d, $J_{C\text{-}F}$ = 4.0 Hz), 117.61 (d, $J_{C\text{-}F}$ = 2.3 Hz), 116.72 (d, $J_{C\text{-}F}$ = 20.0 Hz), 113.74 (d, $J_{C\text{-}F}$ = 20.9 Hz), 110.92 (d, $J_{C\text{-}F}$ = 5.8 Hz), 98.09, 72.46, 59.12, 26.50, 25.82, 21.86, 21.84, 11.20, 10.87, 10.12. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{28}H_{28}F_2N_7O_2$, 532.2273; found, 532.2275. HPLC retention time 15.49 min, >98% pure.

*4-(4-Amino-1-(1-(3-cyclopropyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)propyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-methylbenzamide (**25**).* Yield 64%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.62−8.55 (m, 1H), 8.33 (s, 1H), 8.00 (d, J = 8.3 Hz, 2H), 7.78 (td, J = 8.2, 5.7 Hz, 1H), 7.70 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.4, 8.6 Hz, 1H), 6.57 (dd, J = 9.0, 5.0 Hz, 1H), 2.83 (d, J = 4.4 Hz, 3H), 2.53−2.39 (m, 2H), 2.23−2.17 (m, 1H), 1.26−1.11 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H), 0.87−0.81 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.58, 160.43 (d, $J_{C\text{-}F}$ = 263.5 Hz), 159.76 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.66, 156.92, 156.41, 155.89, 148.30, 144.48, 135.47, 135.41 (d, $J_{C\text{-}F}$ = 10.6 Hz), 134.88, 128.69, 128.33, 123.82 (d, $J_{C\text{-}F}$ = 3.5 Hz), 114.04 (d, $J_{C\text{-}F}$ = 20.5 Hz), 110.91 (d, $J_{C\text{-}F}$ = 5.6 Hz), 97.76, 59.38, 26.76, 25.94, 11.28, 10.86, 10.13. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{27}H_{26}FN_8O_2$, 513.2163; found, 513.2162. HPLC retention time 10.68 min, >96% pure.

*N-(4-(4-Amino-1-(1-(3-cyclopropyl-5-fluoro-4-oxo-3,4-dihydroquinazolin-2-yl)propyl)-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)-acetamide (**26**).* Yield 62%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.24 (s, 1H), 8.29 (s, 1H), 7.82−7.73 (m, 3H), 7.53 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.4, 8.6 Hz, 1H), 6.52 (dd, J = 9.0, 5.0 Hz, 1H), 2.51−2.35 (m, 2H), 2.15−2.11 (m, 1H), 2.08 (s, 3H), 1.23−1.10 (m, 3H), 0.93 (t, J = 7.3 Hz, 3H), 0.83−0.75 (m, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 169.02, 160.43 (d, $J_{C\text{-}F}$ = 263.0 Hz), 159.78 (d, $J_{C\text{-}F}$ = 3.8 Hz), 158.68, 156.98, 156.33, 155.73, 148.31, 145.01, 140.41, 135.43 (d, $J_{C\text{-}F}$ = 10.3 Hz), 129.25, 127.50, 123.82 (d, $J_{C\text{-}F}$ = 3.8 Hz), 119.72, 114.04 (d, $J_{C\text{-}F}$ = 20.6 Hz), 110.91 (d, $J_{C\text{-}F}$ = 5.7 Hz), 97.59, 59.23, 26.76, 25.96, 24.52, 11.26, 10.85, 10.09. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{27}H_{26}FN_8O_2$, 513.2163; found, 513.2158. HPLC retention time 10.71 min, >99% pure.

*2-(1-(4-Amino-3-(benzo[d][1,3]dioxol-5-yl)-1H-pyrazolo[3,4-d]-pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**27**).* Yield 55%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.26 (s, 1H), 7.81−7.74 (m, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.5, 8.5 Hz, 1H), 7.08−7.01 (m, 3H), 6.49 (dd, J = 9.0, 5.0 Hz, 1H), 6.08 (s, 2H), 2.49−2.33 (m, 2H), 2.12−2.05 (m, 1H), 1.19−1.06 (m, 3H), 0.92 (t, J = 7.3 Hz, 3H), 0.82−0.74 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.84 (d, $J_{C\text{-}F}$ = 265.0 Hz), 160.62 (d, $J_{C\text{-}F}$ = 3.9 Hz), 157.97, 155.95, 155.71, 155.38, 148.56, 148.44, 148.37, 145.01, 134.18 (d, $J_{C\text{-}F}$ = 10.3 Hz), 126.76, 123.74 (d, $J_{C\text{-}F}$ = 3.8 Hz), 122.39, 113.66 (d, $J_{C\text{-}F}$ = 20.9 Hz), 111.15 (d, $J_{C\text{-}F}$ = 5.8 Hz), 109.09, 108.94, 101.51, 98.18, 59.09, 26.49, 25.89, 11.36, 11.02, 10.24. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{26}H_{23}FN_7O_3$, 500.1846; found, 500.1849. HPLC retention time 13.65 min, >99% pure.

*2-(1-(4-Amino-3-(quinolin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**28**).* Yield 46%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.05 (d, J = 1.9 Hz, 1H), 8.56 (d, J = 1.4 Hz, 1H), 8.32 (s, 1H), 8.09 (dd, J = 11.2, 8.6 Hz, 2H), 7.82 (t, J = 7.6 Hz, 1H), 7.77 (dd, J = 13.7, 8.2 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.27 (dd, J = 10.6, 8.5 Hz, 1H), 6.58 (dd, J = 9.1, 5.0 Hz, 1H), 2.58−2.53 (m, 1H), 2.49−2.42 (m, 1H), 2.27−2.19 (m, 1H), 1.24−1.12 (m, 3H), 0.96 (t, J = 7.3 Hz, 3H), 0.83 (dd, J = 10.8, 5.5 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.83 (d, $J_{C\text{-}F}$ = 265.6 Hz), 160.56 (d, $J_{C\text{-}F}$ = 4.1 Hz), 157.95, 156.18, 155.79, 155.67, 149.84, 148.32, 147.92, 142.09, 135.57, 134.25 (d, $J_{C\text{-}F}$ = 10.3 Hz), 130.45, 129.39, 128.12, 127.59, 127.46, 126.08, 123.72 (d, $J_{C\text{-}F}$ = 3.9 Hz), 113.73 (d, $J_{C\text{-}F}$ = 21.0 Hz), 111.12 (d, $J_{C\text{-}F}$ = 5.6 Hz), 98.75, 59.27, 26.55, 25.89, 11.40, 11.10, 10.34. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{28}H_{24}FN_8O$, 507.2057; found, 507.2058. HPLC retention time 13.25 min, >99% pure.

*2-(1-(4-Amino-3-(1-methyl-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-d]-pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**29**).* Yield 57%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.24 (s, 1H), 8.02 (s, 1H), 7.78 (dd, J = 13.7, 8.2 Hz, 1H), 7.65 (s, 1H), 7.45 (d, J = 8.2 Hz, 1H), 7.28 (dd, J = 10.3, 8.7 Hz, 1H), 6.46 (dd, J = 9.1, 5.0 Hz, 1H), 3.87 (s, 3H), 2.48−2.31 (m, 2H), 2.08−2.04 (m, 1H), 1.20−

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1.06 (m, 3H), 0.93−0.86 (m, 3H), 0.81−0.72 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 158.9 (d, $J_{C\text{-}F}$ = 265.3 Hz), 158.74 (d, $J_{C\text{-}F}$ = 3.9 Hz), 156.32, 154.06, 153.72, 153.29, 146.43, 136.41, 135.64, 132.28 (d, $J_{C\text{-}F}$ = 10.3 Hz), 128.10, 121.84 (d, $J_{C\text{-}F}$ = 4.1 Hz), 112.43, 111.76 (d, $J_{C\text{-}F}$ = 20.8 Hz), 109.26 (d, $J_{C\text{-}F}$ = 5.7 Hz), 96.77, 57.09, 37.30, 24.55, 24.05, 9.48, 9.05, 8.28. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{23}H_{23}FN_9O$, 460.2010; found, 460.2008. HPLC retention time 10.22 min, >98% pure.

*2-(1-(4-Amino-3-(thiophen-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**30**).* Yield 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.27 (s, 1H), 7.81 (dd, J = 2.8, 1.2 Hz, 1H), 7.78 (dt, J = 13.6, 6.9 Hz, 1H), 7.70 (dd, J = 4.9, 2.9 Hz, 1H), 7.44 (d, J = 8.2 Hz, 1H), 7.32 (dd, J = 4.9, 1.2 Hz, 1H), 7.27 (dd, J = 10.3, 8.5 Hz, 1H), 6.50 (dd, J = 9.0, 5.0 Hz, 1H), 2.48−2.36 (m, 2H), 2.15−2.09 (m, 1H), 1.21−1.07 (m, 3H), 0.92 (t, J = 7.3 Hz, 3H), 0.83−0.75 (m, 1H). ^{13}C NMR (126 MHz, CD_3OD) δ 160.88 (d, $J_{C\text{-}F}$ = 4.2 Hz), 160.72 (d, $J_{C\text{-}F}$ = 265.3 Hz) 157.93, 155.70, 155.50, 154.92, 148.24, 140.99, 134.39 (d, $J_{C\text{-}F}$ = 10.5 Hz), 133.39, 127.62, 127.59, 124.89, 123.67 (d, $J_{C\text{-}F}$ = 3.9 Hz), 113.73 (d, $J_{C\text{-}F}$ = 20.8 Hz), 110.95 (d, $J_{C\text{-}F}$ = 5.8 Hz), 98.42, 59.13, 26.49, 25.84, 11.21, 10.86, 10.11. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{23}H_{21}FN_7OS$, 462.1512; found, 462.1513. HPLC retention time 13.50 min, >96% pure.

*3-(3-Fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32a**).* To a solution of **31** (2.00 g, 7.7 mmol, 1.0 equiv) in dioxane (30 mL)/H_2O (10 mL) were added (3-fluoro-4-methoxyphenyl)boronic acid (3.9 g, 23.1 mmol, 3.0 equiv), $Pd(PPh_3)_4$ (924 mg, 0.8 mmol, 0.1 equiv), and K_2CO_3 (2.1 g, 15.4 mmol, 2.0 equiv) at room temperature under argon. The reaction mixture was degassed by argon, and then it was heated to 135 °C for 14 h. The resulting mixture was concentrated to dryness. The residue was diluted with water and filtered, and the precipitate was washed with DCM to give **32a** as an off-white solid (1.6 g, 79%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.49−7.42 (m, 2H), 7.33 (t, J = 8.7 Hz, 1H), 3.92 (s, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{12}H_{11}FN_5O$, 260.0948; found, 260.0953.

Compounds **32b−l** were prepared following the synthetic procedure of **32a**.

*3-Phenyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32b**).* Yield 84%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.25 (s, 1H), 7.70 (d, J = 7.4 Hz, 2H), 7.58−7.55 (m, 2H), 7.49 (t, J = 7.3 Hz, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.52, 156.71, 156.17, 144.79, 133.96, 129.55, 128.91, 128.70, 97.46. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{11}H_{10}N_5$, 212.0936; found, 212.0937.

*3-(4-Methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32c**).* Yield 77%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.61 (d, J = 8.2 Hz, 2H), 7.11 (d, J = 8.2 Hz, 2H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.90, 158.55, 156.73, 156.03, 144.56, 130.01, 126.35, 114.99, 97.44, 55.68. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{12}H_{12}N_5O$, 242.1042; found, 242.1046.

*3-(3,4-Dimethoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32d**).* Yield 80%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.27−7.17 (m, 2H), 7.12 (d, J = 8.2 Hz, 1H), 3.83 (s, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.55, 156.78, 155.99, 149.51,149.46, 144.73, 126.54, 120.98, 112.63, 112.24, 97.45, 56.02, 55.89. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{14}N_5O_2$, 272.1147; found, 272.1142.

*3-(4-Methoxy-3-methylphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32e**).* Yield 79%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.22 (s, 1H), 7.47−7.46 (m, 2H), 7.10 (d, J = 8.0 Hz, 1H), 3.86 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.56, 158.10, 156.47, 156.17, 144.80, 130.82, 127.51, 126.80, 125.78, 111.19, 97.40, 55.84, 16.59. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{14}N_5O$, 256.1198; found, 256.1201.

*3-(3-Fluoro-4-isopropoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32f**).* Yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.45 (t, J = 10.2 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.31 (t, J = 8.4 Hz, 1H), 6.73 (s, 2H), 4.70 (dd, J = 11.6, 5.7 Hz, 1H), 1.32 (t, J = 13.5 Hz, 6H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{15}FN_5O$, 288.1261; found, 288.1263.

*4-(4-Amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)-N-methylbenzamide (**32g**).* Yield 71%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.27 (s, 1H), 8.04 (d, J = 7.5 Hz, 2H), 7.80 (d, J = 7.5 Hz, 2H), 6.85 (s, 2H), 2.86 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 166.78, 158.53, 156.95, 156.14, 144.12, 136.43, 134.54, 128.55, 128.28, 97.56, 26.79. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{13}N_6O$, 269.1151; found, 269.1150.

*N-(4-(4-Amino-1H-pyrazolo[3,4-d]pyrimidin-3-yl)phenyl)-acetamide (**32h**).* Yield 77%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.56 (s, 1H), 10.16 (s, 1H), 8.25 (s, 1H), 7.79 (d, J = 8.2 Hz, 2H), 7.63 (d, J = 8.2 Hz, 2H), 2.10 (d, J = 13.6 Hz, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{13}N_6O$, 269.1151; found, 269.1153.

*3-(Benzo[d][1,3]dioxol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32i**).* Yield 63%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.53 (s, 1H), 8.23 (s, 1H), 7.19 (s, 1H), 7.15 (d, J = 7.8 Hz, 1H), 7.08 (d, J = 7.9 Hz, 1H), 6.12 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.52, 156.43, 156.22, 148.30, 148.10, 144.64, 127.67, 122.63, 109.29, 109.03, 101.80, 97.38. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{12}H_{10}N_5O_2$, 256.0834; found, 256.0831.

*3-(Quinolin-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32j**).* Yield 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.22 (d, J = 1.4 Hz, 1H), 8.61 (s, 1H), 8.28 (s, 1H), 8.11 (d, J = 8.3 Hz, 2H), 7.83 (t, J = 7.6 Hz, 1H), 7.68 (t, J = 7.4 Hz, 1H), 7.06 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.74, 157.12, 156.27, 150.50, 147.56, 142.13, 135.52, 130.26, 129.23, 129.15, 128.04, 127.45, 127.16, 97.94. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{11}N_6$, 263.1045; found, 263.1047.

*3-(1-Methyl-1H-pyrazol-4-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32k**).* Yield 46%^{1}H NMR (500 MHz, DMSO-d_6) δ 13.42 (s, 1H), 8.20 (s, 1H), 8.08 (s, 1H), 7.76 (s, 1H), 6.86 (s, 2H), 3.93 (s, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for C9H10N7, 216.0998; found, 216.0997.

*3-(Thiophen-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**32l**).* Yield 56%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.55 (s, 1H), 8.22 (s, 1H), 7.84 (d, J = 1.5 Hz, 1H), 7.75 (dd, J = 4.9, 2.9 Hz, 1H), 7.45 (dd, J = 4.9, 1.2 Hz, 1H), 6.83 (s, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_9H_8N_5S$, 218.0500; found, 218.0503.

*2-Amino-6-fluoro-N-phenylbenzamide (**34a**).* To a solution of **33** (1.0 g, 6.4 mmol, 1.0 equiv) in anhydrous THF (20 mL) were added aniline (0.6 mL, 6.4 mmol, 1.0 equiv), HATU (2.9 g, 7.7 mmol, 1.2 equiv), and DIPEA (3.4 mL, 19.2 mmol, 3.0 equiv). Then, the reaction mixture was stirred at room temperature for 6 h. The resulting mixture was concentrated to dryness to give the crude product. The residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−25% EtOAc in hexane) to give **34a** as a white solid (1.1 g, yield 73%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.26 (s, 1H), 7.73 (d, J = 7.8 Hz, 2H), 7.34 (t, J = 7.8 Hz, 2H), 7.12 (dt, J = 15.0, 7.7 Hz, 2H), 6.57 (d, J = 8.2 Hz, 1H), 6.40 (t, J = 9.1 Hz, 1H), 5.76 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.51, 160.66 (d, $J_{C\text{-}F}$ = 243.0 Hz), 149.78 (d, $J_{C\text{-}F}$ = 6.2 Hz), 139.42, 131.82 (d, $J_{C\text{-}F}$ = 11.1 Hz), 129.13, 124.16, 120.28, 111.87 (d, $J_{C\text{-}F}$ = 2.0 Hz), 109.06 (d, $J_{C\text{-}F}$ = 19.3 Hz), 102.42 (d, $J_{C\text{-}F}$ = 22.8 Hz). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{12}FN_2O$, 231.0934; found, 231.0937.

Compounds **34b−g** and **41** were prepared following the synthetic procedure of **34a**.

*N-([1,1′-Biphenyl]-4-yl)-2-amino-6-fluorobenzamide (**34b**).* Yield 75%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.85 (d, J = 8.4 Hz, 2H), 7.68−7.66 (m, 4H), 7.47−7.44 (m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.15 (dd, J = 14.9, 8.1 Hz, 1H), 6.60 (d, J = 8.2 Hz, 1H), 6.46−6.38 (m, 1H), 5.83 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.54, 160.68 (d, $J_{C\text{-}F}$ = 243.0 Hz), 149.84 (d, $J_{C\text{-}F}$ = 6.2 Hz), 140.19, 138.94, 135.80, 131.89 (d, $J_{C\text{-}F}$ = 11.1 Hz), 129.39, 127.55, 127.34, 126.77, 120.61, 111.89, 108.99 (d, $J_{C\text{-}F}$ = 19.3 Hz), 102.39 (d, $J_{C\text{-}F}$ = 22.8 Hz). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{19}H_{16}FN_2O$, 307.1247; found, 307.1244.

*2-Amino-6-fluoro-N-(1-methyl-1H-pyrazol-4-yl)benzamide (**34c**).* Yield 72%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H),

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7.99 (s, 1H), 7.52 (s, 1H), 7.18−7.05 (m, 1H), 6.56 (d, J = 8.2 Hz, 1H), 6.39 (dd, J = 9.8, 8.7 Hz, 1H), 5.87 (s, 2H), 3.82 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.66, 160.65 (d, J_{C-F} = 242.9 Hz), 150.14 (d, J_{C-F} = 5.9 Hz), 131.84 (d, J_{C-F} = 11.2 Hz), 130.51, 122.04, 121.84, 111.89 (d, J_{C-F} = 1.6 Hz), 107.91 (d, J_{C-F} = 19.1 Hz), 102.31 (d, J_{C-F} = 23.1 Hz), 39.12. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{11}H_{12}FN_4O$, 235.0995; found, 235.0999.

*2-Amino-6-fluoro-N-isobutylbenzamide (**34d**).* Yield 84%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.06 (dd, J = 14.8, 8.0 Hz, 1H), 6.50 (d, J = 8.2 Hz, 1H), 6.32 (dd, J = 10.0, 8.6 Hz, 1H), 5.79 (s, 2H), 3.06 (t, J = 6.4 Hz, 2H), 1.81 (dp, J = 13.4, 6.7 Hz, 1H), 0.89 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.67, 160.75 (d, J_{C-F} = 242.8 Hz), 150.04 (d, J_{C-F} = 6.4 Hz), 131.32 (d, J_{C-F} = 11.2 Hz), 111.71 (d, J_{C-F} = 2.1 Hz), 108.42 (d, J_{C-F} = 18.9 Hz), 102.33 (d, J_{C-F} = 23.5 Hz), 46.78, 28.46, 20.57. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{11}H_{16}FN_2O$, 211.1247; found, 211.1244.

*2-Amino-6-fluoro-N-isopropylbenzamide (**34e**).* Yield 83%. ^{1}H NMR (500 MHz, DMSO-d_6) δ8.04 (d, J = 7.2 Hz, 1H), 7.05 (dd, J = 14.8, 8.1 Hz, 1H), 6.49 (d, J = 8.2 Hz, 1H), 6.35−6.26 (m, 1H), 5.74 (s, 2H), 4.11−4.01 (m, 1H), 1.13 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.69, 160.66 (d, J_{C-F} = 242.8 Hz), 149.88 (d, J_{C-F} = 6.4 Hz), 131.23 (d, J_{C-F} = 11.2 Hz), 111.64 (d, J_{C-F} = 2.2 Hz), 108.70 (d, J_{C-F} = 19.2 Hz), 102.32 (d, J_{C-F} = 23.2 Hz), 41.15, 22.69. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{10}H_{14}FN_2O$, 197.1090; found, 197.1087.

*2-Amino-N-ethyl-6-fluorobenzamide (**34f**).* Yield 79%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.06 (q, J = 7.5 Hz, 1H), 6.50 (d, J = 8.2 Hz, 1H), 6.34−6.29 (m, 1H), 5.87 (s, 2H), 3.25 (p, J = 6.7 Hz, 2H), 1.09 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.48, 160.76 (d, J_{C-F} = 242.8 Hz), 150.14 (d, J_{C-F} = 6.4 Hz), 131.40 (d, J_{C-F} = 11.3 Hz), 111.76 (d, J_{C-F} = 2.0 Hz), 108.04 (d, J_{C-F} = 18.6 Hz), 102.21 (d, J_{C-F} = 23.3 Hz), 34.20, 15.09. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_9H_{12}FN_2O$, 183.0934; found, 183.0938.

*2-Amino-6-fluoro-N-methylbenzamide (**34g**).* Yield 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.07 (dd, J = 15.3, 7.5 Hz, 1H), 6.51 (d, J = 8.2 Hz, 1H), 6.35−6.30 (m, 1H), 5.96 (s, 2H), 2.75 (d, J = 4.6 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 165.28, 160.87 (d, J_{C-F} = 243.0 Hz), 150.29 (d, J_{C-F} = 6.4 Hz), 131.51 (d, J_{C-F} = 11.6 Hz), 111.85 (d, J_{C-F} = 2.2 Hz), 107.59 (d, J_{C-F} = 18.8 Hz), 102.15 (d, J_{C-F} = 23.7 Hz), 26.46. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_8H_{10}FN_2O$, 169.0777; found, 169.0776.

*2-(2-Bromopropanamido)-6-fluoro-N-phenylbenzamide (**35a**).* To a solution of **34a** (737 mg, 3.2 mmol, 1.0 equiv) in anhydrous THF (20 mL) was added DIPEA (1.7 mL, 9.6 mmol, 3.0 equiv), and then 2-bromopropanoyl chloride (0.38 mL, 3.8 mmol, 1.2 equiv) was slowly added to the mixture at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then warmed up to room temperature for 6 h. The resulting mixture was concentrated to dryness to give the crude product. The residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−15% EtOAc in hexane) to give **35a** as a yellow solid (920 mg, yield 77%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 1H), 10.06 (s, 1H), 7.72 (d, J = 7.8 Hz, 2H), 7.63 (d, J = 8.2 Hz, 1H), 7.55−7.48 (m, 1H), 7.38−7.32 (m, 2H), 7.18 (t, J = 8.8 Hz, 1H), 7.11 (t, J = 7.4 Hz, 1H), 4.84 (q, J = 6.7 Hz, 1H), 1.69 (d, J = 6.7 Hz, 3H). HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{16}H_{15}BrFN_2O_2$, 365.0301; found, 365.0304/367.0284.

Compounds **35b−g** and **42** were prepared following the synthetic procedure of **35a**.

*N-([1,1′-Biphenyl]-4-yl)-2-(2-chloropropanamido)-6-fluorobenzamide (**35b**).* Yield 82%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.71 (s, 1H), 10.18 (s, 1H), 7.81 (d, J = 8.6 Hz, 2H), 7.70−7.64 (m, 5H), 7.54 (dd, J = 14.7, 8.2 Hz, 1H), 7.47−7.44(m, 2H), 7.34 (t, J = 7.4 Hz, 1H), 7.20 (t, J = 8.8 Hz, 1H), 4.82 (q, J = 6.7 Hz, 1H), 1.57 (d, J = 6.8 Hz, 3H). HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{22}H_{19}ClFN_2O_2$, 397.1119; found, 397.1122.

*2-(2-Chloropropanamido)-6-fluoro-N-(1-methyl-1H-pyrazol-4-yl)benzamide (**35c**).* Yield 69%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.67 (s, 1H), 10.22 (s, 1H), 7.99 (s, 1H), 7.76 (d, J = 8.2 Hz, 1H), 7.56−7.47 (m, 2H), 7.17 (t, J = 8.9 Hz, 1H), 4.81 (q, J = 6.8 Hz, 1H), 3.83 (s, 3H), 1.59 (d, J = 6.8 Hz, 3H). HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{14}H_{15}ClFN_4O_2$, 325.0868; found, 325.0870.

*2-(2-Chloropropanamido)-6-fluoro-N-isobutylbenzamide (**35d**).* Yield 81%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.29 (s, 1H), 8.71 (t, J = 5.4 Hz, 1H), 7.83 (d, J = 8.2 Hz, 1H), 7.48 (dd, J = 15.2, 7.6 Hz, 1H), 7.11 (t, J = 9.0 Hz, 1H), 4.84 (q, J = 6.7 Hz, 1H), 3.17−3.03 (m, 2H), 1.83 (dp, J = 13.3, 6.6 Hz, 1H), 1.62 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.7 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.32, 163.06, 159.33 (d, J_{C-F} = 245.9 Hz), 137.38 (d, J_{C-F} = 5.2 Hz), 131.68 (d, J_{C-F} = 9.9 Hz), 118.80 (d, J_{C-F} = 1.9 Hz), 117.15 (d, J_{C-F} = 19.7 Hz), 112.35 (d, J_{C-F} = 22.6 Hz), 55.51, 47.09, 28.39, 22.00, 20.54. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{14}H_{19}ClFN_2O_2$, 301.1119; found, 301.1121.

*2-(2-Chloropropanamido)-6-fluoro-N-isopropylbenzamide (**35e**).* Yield 74%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.57 (d, J = 7.5 Hz, 1H), 7.77 (d, J = 8.2 Hz, 1H), 7.46 (dd, J = 15.3, 7.6 Hz, 1H), 7.10 (t, J = 8.9 Hz, 1H), 4.86 (q, J = 6.7 Hz, 1H), 4.13−4.02 (m, 1H), 1.62 (d, J = 6.8 Hz, 3H), 1.14 (d, J = 6.6 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.29, 161.96, 159.24 (d, J_{C-F} = 246.0 Hz), 137.07 (d, J_{C-F} = 5.4 Hz), 131.48 (d, J_{C-F} = 9.6 Hz), 119.07 (d, J_{C-F} = 2.5 Hz), 117.85 (d, J_{C-F} = 19.9 Hz), 112.43 (d, J_{C-F} = 22.4 Hz), 55.44, 41.67, 22.54, 22.47, 21.99. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{13}H_{17}ClFN_2O_2$, 287.0963; found, 287.0960.

*2-(2-Chloropropanamido)-N-ethyl-6-fluorobenzamide (**35f**).* Yield 70%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.34 (s, 1H), 8.67 (s, 1H), 7.82 (d, J = 8.2 Hz, 1H), 7.48 (dd, J = 15.3, 7.6 Hz, 1H), 7.11 (t, J = 9.0 Hz, 1H), 4.85 (q, J = 6.7 Hz, 1H), 3.35−3.22 (m, 2H), 1.62 (d, J = 6.8 Hz, 3H), 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.31, 162.79, 160.32, 158.36, 137.42, 137.38, 131.73, 131.66, 118.92, 118.90, 117.18, 117.03, 112.44, 112.26, 55.49, 34.59, 21.97, 14.86. ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.31, 162.79, 159.34 (d, J_{C-F} = 246.2 Hz), 137.40 (d, J_{C-F} = 5.2 Hz), 131.70 (d, J_{C-F} = 9.7 Hz), 118.91 (d, J_{C-F} = 2.5 Hz), 117.11 (d, J_{C-F} = 19.4 Hz), 112.35 (d, J_{C-F} = 22.5 Hz), 55.50, 34.59, 21.98, 14.86. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{12}H_{15}ClFN_2O_2$, 273.0806; found, 273.0811.

*2-(2-Chloropropanamido)-6-fluoro-N-methylbenzamide (**35g**).* Yield 73%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.45 (s, 1H), 8.58 (d, J = 3.8 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.48 (dd, J = 15.3, 7.6 Hz, 1H), 7.11 (t, J = 9.1 Hz, 1H), 4.84 (q, J = 6.7 Hz, 1H), 2.79 (d, J = 4.5 Hz, 3H), 1.61 (t, J = 7.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.34, 163.55, 159.43 (d, J_{C-F} = 246.2 Hz), 137.63 (d, J_{C-F} = 5.1 Hz), 131.86 (d, J_{C-F} = 10.0 Hz), 118.81 (d, J_{C-F} = 2.5 Hz), 116.56 (d, J_{C-F} = 19.3 Hz), 112.30 (d, J_{C-F} = 22.7 Hz), 55.50, 26.70, 21.95. HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{11}H_{13}ClFN_2O_2$, 259.0650; found, 259.0653.

Compounds **36a−g** and **46a−b** were prepared following the synthetic procedure of **13**.

*2-(1-Bromoethyl)-5-fluoro-3-phenylquinazolin-4(3H)-one (**36a**).* Yield 54%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.67−7.63 (m, 1H), 7.54−7.51 (m, 2H), 7.49−7.46 (m, 3H), 7.11−7.07 (m, 2H), 4.45 (q, J = 6.8 Hz, 1H), 1.95 (d, J = 6.7 Hz, 3H). HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{16}H_{13}BrFN_2O$, 347.0195; found, 347.0192/349.0173.

*3-([1,1′-Biphenyl]-4-yl)-2-(1-chloroethyl)-5-fluoroquinazolin-4(3H)-one (**36b**).* Yield 52%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.93−7.88 (m, 3H), 7.79 (d, J = 7.3 Hz, 2H), 7.64 (t, J = 9.3 Hz, 2H), 7.58−7.56 (m, 1H), 7.53 (t, J = 7.6 Hz, 2H), 7.44 (t, J = 7.4 Hz, 1H), 7.39 (dd, J = 10.4, 8.6 Hz, 1H), 4.68 (q, J = 6.5 Hz, 1H), 1.82 (d, J = 6.5 Hz, 3H). HRMS (ESI, m/z): [M + H]$^+$ calcd for $C_{22}H_{17}ClFN_2O$, 379.1013; found, 379.1014.

*2-(1-Chloroethyl)-5-fluoro-3-(1-methyl-1H-pyrazol-4-yl)-quinazolin-4(3H)-one (**36c**).* Yield 39%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.00 (s, 1H), 7.88 (dd, J = 13.6, 8.0 Hz, 1H), 7.61−7.55 (m, 2H), 7.39−7.32 (m, 1H), 4.88 (q, J = 6.3 Hz, 1H), 3.94 (s, 3H), 1.79 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.87 (d, J_{C-F} = 264.0 Hz), 158.59 (d, J_{C-F} = 4.1 Hz), 156.88, 148.67, 138.13, 136.21 (d, J_{C-F} = 10.5 Hz), 130.65, 124.10 (d, J_{C-F} = 3.8 Hz), 116.18, 114.70 (d, J_{C-F} = 20.5 Hz), 110.53 (d, J_{C-F} = 5.7 Hz), 53.95,

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22.20. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{13}ClFN_4O$, 307.0762; found, 307.0764.

*2-(1-Chloroethyl)-5-fluoro-3-isobutylquinazolin-4(3H)-one (**36d**).* Yield 57%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.84 (td, J = 8.2, 5.6 Hz, 1H), 7.54 (d, J = 8.2 Hz, 1H), 7.34 (dd, J = 10.7, 8.5 Hz, 1H), 5.52 (q, J = 6.3 Hz, 1H), 4.24 (dd, J = 14.1, 7.3 Hz, 1H), 3.83 (dd, J = 14.2, 8.0 Hz, 1H), 2.19−2.08 (m, 1H), 1.92 (d, J = 6.4 Hz, 3H), 0.95−0.91 (m, 6H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{17}ClFN_2O$, 283.1013; found, 283.1009.

*2-(1-Chloroethyl)-5-fluoro-3-isopropylquinazolin-4(3H)-one (**36e**).* Yield 55%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.80 (td, J = 8.1, 5.5 Hz, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.30 (dd, J = 10.8, 8.4 Hz, 1H), 5.62 (q, J = 5.9 Hz, 1H), 4.86−4.72 (m, 1H), 1.90 (d, J = 6.3 Hz, 3H), 1.63−1.59 (m, 6H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{15}ClFN_2O$, 269.0857; found, 269.0858.

*2-(1-Chloroethyl)-3-ethyl-5-fluoroquinazolin-4(3H)-one (**36f**).* Yield 59%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.83 (td, J = 8.2, 5.7 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.33 (dd, J = 10.8, 8.3 Hz, 1H), 5.53 (q, J = 6.3 Hz, 1H), 4.32 (dq, J = 14.2, 7.1 Hz, 1H), 4.05 (dq, J = 14.1, 7.0 Hz, 1H), 1.91 (d, J = 6.4 Hz, 3H), 1.32 (t, J = 7.0 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.67 (d, $J_{C\text{-}F}$ = 263.9 Hz), 158.33 (d, $J_{C\text{-}F}$ = 4.3 Hz), 155.97, 148.66, 135.64 (d, $J_{C\text{-}F}$ = 10.5 Hz), 123.94 (d, $J_{C\text{-}F}$ = 4.0 Hz), 114.30 (d, $J_{C\text{-}F}$ = 20.4 Hz), 110.44 (d, $J_{C\text{-}F}$ = 5.7 Hz), 53.84, 38.81, 22.66, 14.47. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{12}H_{13}ClFN_2O$, 255.0700; found, 255.0687.

*2-(1-Chloroethyl)-5-fluoro-3-methylquinazolin-4(3H)-one (**36g**).* Yield 52%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.82 (td, J = 8.2, 5.6 Hz, 1H), 7.52 (d, J = 8.2 Hz, 1H), 7.33 (dd, J = 10.9, 8.3 Hz, 1H), 5.55 (q, J = 6.4 Hz, 1H), 3.62 (s, 3H), 1.89 (d, J = 6.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.62 (d, $J_{C\text{-}F}$ = 263.8 Hz), 158.73 (d, $J_{C\text{-}F}$ = 4.4 Hz), 156.20, 148.59, 135.62 (d, $J_{C\text{-}F}$ = 10.7 Hz), 123.95 (d, $J_{C\text{-}F}$ = 3.9 Hz), 114.30 (d, $J_{C\text{-}F}$ = 20.7 Hz), 110.20 (d, $J_{C\text{-}F}$ = 5.6 Hz), 54.33, 29.93, 22.14. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{11}H_{11}ClFN_2O$, 241.0544; found, 241.0543.

*N-Benzyl-2-fluoro-6-nitrobenzamide (**38a**).* To a solution of **37** (14 g, 75.6 mmol, 1.0 equiv) in anhydrous THF (50 mL) was added DMF (0.5 mL), and then oxalyl dichloride (7.7 mL, 90.7 mmol, 1.2 equiv) was slowly added to the mixture at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then warmed up to room temperature for 5 h. The resulting mixture was concentrated to dryness to give the crude product, which was used in the next step without further purification. To a solution of phenylmethanamine (8.3 mL, 75.6 mmol, 1.0 equiv) in anhydrous THF (50 mL) was added DIPEA (26.3 mL, 151.2 mmol, 2.0 equiv), and then the previous crude product in anhydrous THF was slowly added to the mixture at 0 °C under argon. Then, the reaction mixture was stirred at room temperature for 6 h. The resulting mixture was concentrated to dryness. The residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−20% EtOAc in hexane) to give **38a** as a yellow solid (17.4 g, yield 84%). ^1H NMR (500 MHz, DMSO-d_6) δ 9.29 (t, J = 5.6 Hz, 1H), 8.02 (d, J = 8.0 Hz, 1H), 7.81−7.70 (m, 2H), 7.39−7.33 (m, 4H), 7.31−7.25 (m, 1H), 4.51 (d, J = 5.9 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.08, 158.93 (d, $J_{C\text{-}F}$ = 248.9 Hz), 147.13 (d, $J_{C\text{-}F}$ = 5.4 Hz), 138.95, 132.01 (d, $J_{C\text{-}F}$ = 8.9 Hz), 128.81, 127.82, 127.49, 122.50 (d, $J_{C\text{-}F}$ = 22.5 Hz), 122.20 (d, $J_{C\text{-}F}$ = 24.2 Hz), 120.95 (d, $J_{C\text{-}F}$ = 2.9 Hz), 43.14. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{12}FN_2O_3$, 275.0832; found, 275.0829.

Compounds **38b−e** were prepared following the synthetic procedure of **38a**.

*N-Cyclohexyl-2-fluoro-6-nitrobenzamide (**38b**).* Yield 81%. ^1H NMR (500 MHz, DMSO-d_6) δ 8.63 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.8 Hz, 1H), 7.77−7.68 (m, 2H), 3.74 (td, J = 14.1, 7.0 Hz, 1H), 1.89−1.80 (m, 2H), 1.72−1.69 (m, 2H), 1.57−1.55 (m, 1H), 1.36−1.28 (m, 2H), 1.26−1.12 (m, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.91, 158.89 (d, $J_{C\text{-}F}$ = 248.6 Hz), 147.06 (d, $J_{C\text{-}F}$ = 5.5 Hz), 131.63 (d, $J_{C\text{-}F}$ = 8.8 Hz), 122.66 (d, $J_{C\text{-}F}$ = 24.5 Hz), 122.39 (d, $J_{C\text{-}F}$ = 22.6 Hz), 120.77 (d, $J_{C\text{-}F}$ = 2.8 Hz), 48.58, 32.31, 25.63, 24.76. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{16}FN_2O_3$, 267.1145; found, 267.1146.

*N-Cyclopentyl-2-fluoro-6-nitrobenzamide (**38c**).* Yield 75%. ^1H NMR (500 MHz, $CDCl_3$) δ 7.92 (dd, J = 8.2, 0.9 Hz, 1H), 7.54 (td, J = 8.3, 5.5 Hz, 1H), 7.43 (td, J = 8.3, 1.0 Hz, 1H), 4.49−4.39 (m, 1H), 2.14−2.06 (m, 2H), 1.74−1.65 (m, 4H), 1.61−1.54 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{12}H_{14}FN_2O_3$, 253.0988; found, 253.0990.

*N-Cyclobutyl-2-fluoro-6-nitrobenzamide (**38d**).* Yield 81%. ^1H NMR (500 MHz, DMSO-d_6) δ8.99 (d, J = 7.2 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 7.79−7.70 (m, 2H), 4.38−4.28 (m, 1H), 2.30−2.21 (m, 2H), 2.00−1.91 (m, 2H), 1.73−1.66 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{11}H_{12}FN_2O_3$, 239.0832; found, 239.0828.

*N-Cyclopropyl-2-fluoro-6-nitrobenzamide (**38e**).* Yield 77%. ^1H NMR (500 MHz, DMSO-d_6) δ 8.80 (d, J = 2.2 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.79−7.70 (m, 2H), 2.79 (tq, J = 7.4, 3.8 Hz, 1H), 0.74−0.71 (m, 2H), 0.52−0.47 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{10}H_{10}FN_2O_3$, 225.0675; found, 225.0672.

*3-Benzyl-2-ethyl-5-fluoroquinazolin-4(3H)-one (**39a**).* A solution of **38a** (2.0 g, 7.3 mmol, 1.0 equiv) in propionic anhydride (14 mL) was stirred at 140 °C in a microwave reactor for 3 h. The resulting mixture was concentrated to dryness to give the crude product, which was used in the next step without further purification. To a solution of the former crude product in anhydrous AcOH (20 mL) was added zinc (2.4 g, 36.5 mmol, 5.0 equiv) at room temperature under argon. Then, the reaction mixture was stirred at room temperature for 8 h. The resulting mixture was concentrated to dryness. The residue was diluted with water and extracted with DCM. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−10% EtOAc in hexane) to give **39a** as a white solid. Yield 48%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.80 (td, J = 8.2, 5.7 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.37−7.34 (m, 2H), 7.32−7.24 (m, 2H), 7.21 (d, J = 7.3 Hz, 2H), 5.37 (s, 2H), 2.77 (q, J = 7.2 Hz, 2H), 1.19 (t, J = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.89 (d, $J_{C\text{-}F}$ = 263.5 Hz), 159.66, 158.96 (d, $J_{C\text{-}F}$ = 4.0 Hz), 149.53, 136.94, 135.53 (d, $J_{C\text{-}F}$ = 10.6 Hz), 129.23, 127.71, 126.63, 123.47 (d, $J_{C\text{-}F}$ = 3.8 Hz), 113.23 (d, $J_{C\text{-}F}$ = 20.5 Hz), 109.93 (d, $J_{C\text{-}F}$ = 5.5 Hz), 45.64, 27.79, 10.99. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{17}H_{16}FN_2O$, 283.1247; found, 283.1252.

Compounds **39b−e** were prepared following the synthetic procedure of **39a**.

*3-Cyclohexyl-2-ethyl-5-fluoroquinazolin-4(3H)-one (**39b**).* Yield 24%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.72 (td, J = 8.1, 5.6 Hz, 1H), 7.38 (d, J = 8.2 Hz, 1H), 7.18 (dd, J = 10.8, 8.4 Hz, 1H), 4.20−3.00 (m, 1H), 2.92 (q, J = 7.3 Hz, 2H), 2.58 (d, J = 11.0 Hz, 2H), 1.81 (d, J = 13.0 Hz, 2H), 1.71 (d, J = 11.2 Hz, 2H), 1.65 (d, J = 12.9 Hz, 1H), 1.39 (q, J = 13.1 Hz, 2H), 1.27 (t, J = 7.3 Hz, 3H), 1.24−1.13 (m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{16}H_{20}FN_2O$, 275.1560; found, 275.1557.

*3-Cyclopentyl-2-ethyl-5-fluoroquinazolin-4(3H)-one (**39c**).* Yield 28%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.72 (td, J = 8.2, 5.5 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 7.23−7.15 (m, 1H), 4.83−4.73 (m, 1H), 2.96 (q, J = 7.3 Hz, 2H), 2.23−2.13 (m, 2H), 2.03−1.95 (m, 2H), 1.93−1.86 (m, 2H), 1.66−1.56 (m, 2H), 1.29 (t, J = 7.3 Hz, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{15}H_{18}FN_2O$, 261.1403; found, 261.1406.

*3-Cyclobutyl-2-ethyl-5-fluoroquinazolin-4(3H)-one (**39d**).* Yield 34%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.72 (td, J = 8.2, 5.6 Hz, 1H), 7.37 (d, J = 8.2 Hz, 1H), 7.24−7.15 (m, 1H), 4.87 (p, J = 8.6 Hz, 1H), 3.02−2.91 (m, 2H), 2.86 (q, J = 7.3 Hz, 2H), 2.37−2.27 (m, 2H), 1.93−1.83 (m, 1H), 1.82−1.70 (m, 1H), 1.24 (t, J = 7.3 Hz, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{16}FN_2O$, 247.1247; found, 247.1245.

*3-Cyclopropyl-5-fluoro-2-propylquinazolin-4(3H)-one (**39e**).* Yield 41%. ^1H NMR (500 MHz, DMSO-d_6) δ 7.71 (td, J = 8.2, 5.6 Hz, 1H), 7.36 (d, J = 8.1 Hz, 1H), 7.18 (ddd, J = 11.1, 8.2, 0.8 Hz, 1H), 3.00−2.92 (m, 3H), 1.87−1.77 (m, 2H), 1.22−1.16 (m, 2H),

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1.01 (t, J = 7.4 Hz, 3H), 0.86−0.81 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{16}FN_2O$, 247.1247; found, 247.1251.

*3-Benzyl-2-(1-bromoethyl)-5-fluoroquinazolin-4(3H)-one (**40a**).* To a solution of **39a** (1.0 g, 3.5 mmol, 1.0 equiv) in CCl_4 (30 mL) were added NBS (0.75 g, 4.2 mmol, 1.2 equiv) and AIBN (54 mg, 0.35 mmol, 0.1 equiv) at room temperature under argon. Then, the reaction mixture was stirred at reflux for 10 h. The resulting mixture was concentrated to dryness. The residue was diluted with water and extracted with DCM. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−8% EtOAc in hexane) to give **40a** as a white solid (740 mg, yield 57%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.88 (td, J = 8.2, 5.6 Hz, 1H), 7.58 (d, J = 8.2 Hz, 1H), 7.39−7.34 (m, 3H), 7.28 (t, J = 7.3 Hz, 1H), 7.21 (d, J = 7.4 Hz, 2H), 5.67 (d, J = 16.7 Hz, 1H), 5.26 (q, J = 6.4 Hz, 1H), 5.19 (d, J = 16.7 Hz, 1H), 1.98 (d, J = 6.5 Hz, 3H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{17}H_{15}BrFN_2O$, 361.0352; found, 361.0357/363.0335.

Compounds **40b−e** were prepared following the synthetic procedure of **40a**.

*2-(1-Bromoethyl)-3-cyclohexyl-5-fluoroquinazolin-4(3H)-one (**40b**).* Yield 36%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.79 (td, J = 8.1, 5.5 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.29 (dd, J = 10.7, 8.5 Hz, 1H), 5.66 (q, J = 6.2 Hz, 1H), 4.30−4.16 (m, 1H), 2.70−2.53 (m, 2H), 2.05 (d, J = 6.3 Hz, 3H), 1.86−1.77 (m, 4H), 1.70−1.65 (m, 1H), 1.50 (dt, J = 16.2, 8.4 Hz, 1H), 1.34 (q, J = 13.0 Hz, 1H), 1.19 (dd, J = 25.6, 12.6 Hz, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{16}H_{19}BrFN_2O$, 353.0665; found, 353.0668/355.0647.

*2-(1-Bromoethyl)-3-cyclopentyl-5-fluoroquinazolin-4(3H)-one (**40c**).* Yield 37%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66−7.59 (m, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.14−7.05 (m, 1H), 5.12−5.06 (m, 1H), 4.84 (dq, J = 17.0, 8.4 Hz, 1H), 2.46−2.31 (m, 2H), 2.20−2.08 (m, 5H), 2.08−2.00 (m, 1H), 1.93−1.84 (m, 1H), 1.66 (tdd, J = 14.4, 7.4, 4.9 Hz, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{15}H_{17}BrFN_2O$, 339.0508; found, 339.0513/341.0484.

*2-(1-Bromoethyl)-3-cyclobutyl-5-fluoroquinazolin-4(3H)-one (**40d**).* Yield 40%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (td, J = 8.2, 5.5 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.30 (dd, J = 11.0, 8.2 Hz, 1H), 5.67 (q, J = 6.3 Hz, 1H), 5.04 (p, J = 8.6 Hz, 1H), 3.13−3.05 (m, 2H), 2.39−2.28 (m, 2H), 2.04 (d, J = 6.3 Hz, 3H), 1.99−1.90 (m, 1H), 1.84−1.74 (m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{15}BrFN_2O$, 325.0352; found, 325.0351/327.0335.

*2-(1-Bromopropyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**40e**).* Yield 45%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.79 (dd, J = 13.6, 8.1 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.30 (dd, J = 10.7, 8.4 Hz, 1H), 5.67 (t, J = 7.1 Hz, 1H), 3.01−2.96 (m, 1H), 2.50−2.40 (m, 1H), 2.33−2.24 (m, 1H), 1.33−1.23 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H),1.00−0.96 (m, 1H), 0.91−0.86 (m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{15}BrFN_2O$, 325.0352; found, 325.0349/327.0336.

*2-Amino-N-cyclopropyl-6-fluorobenzamide (**41**).* Yield 84%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.21 (s, 1H), 7.05 (dd, J = 14.8, 8.1 Hz, 1H), 6.50 (d, J = 8.2 Hz, 1H), 6.33−6.26 (m, 1H), 5.79 (s, 2H), 2.83 (tq, J = 7.7, 4.0 Hz, 1H), 0.70−0.64 (m, 2H), 0.54−0.49 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{10}H_{12}FN_2O$, 195.0934; found, 195.0932.

*2-(2-Chloropropanamido)-N-cyclopropyl-6-fluorobenzamide (**42**).* Yield 76%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.22 (s, 1H), 8.68 (d, J = 3.5 Hz, 1H), 7.74 (d, J = 8.2 Hz, 1H), 7.46 (dd, J = 14.8, 8.2 Hz, 1H), 7.09 (t, J = 8.9 Hz, 1H), 4.85 (q, J = 6.8 Hz, 1H), 2.84 (tq, J = 7.7, 4.0 Hz, 1H), 1.62 (d, J = 6.8 Hz, 3H), 0.73−0.67 (m, 2H), 0.57−0.52 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 168.30, 164.15, 159.34 (d, $J_{C\text{-}F}$ = 246.1 Hz), 137.15 (d, $J_{C\text{-}F}$ = 5.4 Hz), 131.60 (d, $J_{C\text{-}F}$ = 9.7 Hz), 119.25 (d, $J_{C\text{-}F}$ = 2.0 Hz), 117.65 (d, $J_{C\text{-}F}$ = 19.6 Hz), 112.44 (d, $J_{C\text{-}F}$ = 22.4 Hz), 55.41, 23.30, 21.95, 6.22, 6.14. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{13}H_{15}ClFN_2O_2$, 285.0806; found, 285.0811.

*2-(2-(4-Amino-3-(3-fluoro-4-methoxyphenyl)-1H-pyrazolo[3,4-d]pyrimidin-1-yl)propanamido)-N-cyclopropyl-6-fluorobenzamide (**43**).* Yield 53%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.73 (s, 1H), 8.56 (d, J = 4.3 Hz, 1H), 8.26 (s, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.60 (dd, J = 12.1, 1.8 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.43 (dd, J = 14.8, 8.3 Hz, 1H), 7.34 (t, J = 8.7 Hz, 1H), 7.01 (t, J = 8.8 Hz, 1H), 5.65 (q, J = 7.2 Hz, 1H), 3.92 (s, 3H), 2.59 (tt, J = 7.9, 4.0 Hz, 1H), 1.83 (d, J = 7.2 Hz, 3H), 0.62−0.54 (m, 2H), 0.45−0.41 (m, 1H), 0.36−0.33(m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{25}H_{24}F_2N_7O_3$, 508.1909; found, 508.1912.

*(S)-2-(2-Chlorobutanamido)-N-cyclopropyl-6-fluorobenzamide (**45a**).* To a solution of **44a** (0.84 mL, 8.2 mmol, 1.0 equiv) in anhydrous THF (20 mL) was added DMF (0.05 mL), and then oxalyl dichloride (0.8 mL, 9.8 mmol, 1.2 equiv) was slowly added to the mixture at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then warmed up to room temperature for 5 h. The resulting mixture was concentrated to dryness to give the crude product, which was used in the next step without further purification. To a solution of **41** (0.57 mL, 8.2 mmol, 1.0 equiv) in anhydrous THF (20 mL) was added DIPEA (4.3 mL, 24.6 mmol, 3.0 equiv), and then the crude product of the former step (8.2 mmol, 1.0 eq) was slowly added to the mixture at 0 °C. The reaction mixture was stirred at 0 °C for 1 h and then warmed up to room temperature for 6 h. The resulting mixture was concentrated to dryness to give the crude product. The residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and brine and dried over anhydrous Na_2SO_4. Evaporation of the solvent provided the crude product, which was purified by flash chromatography (eluting with 0−15% EtOAc in hexane) to give **45a** as a yellow solid (1.6 g, yield 67%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.68 (d, J = 3.7 Hz, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.46 (dt, J = 14.8, 7.4 Hz, 1H), 7.09 (t, J = 8.8 Hz, 1H), 4.69 (t, J = 6.6 Hz, 1H), 2.84 (tq, J = 7.7, 4.0 Hz, 1H), 2.08−1.98 (m, 1H), 1.94−1.85 (m, 1H), 0.98 (t, J = 7.3 Hz, 3H), 0.73−0.66 (m, 2H), 0.56−0.51 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 167.75, 164.14, 159.31 (d, $J_{C\text{-}F}$ = 246.0 Hz), 136.99 (d, $J_{C\text{-}F}$ = 5.4 Hz), 131.54 (d, $J_{C\text{-}F}$ = 9.8 Hz), 119.49 (d, $J_{C\text{-}F}$ = 2.4 Hz), 117.96 (d, $J_{C\text{-}F}$ = 19.7 Hz), 112.51 (d, $J_{C\text{-}F}$ = 22.5 Hz), 61.51, 28.55, 23.29, 10.71, 6.20, 6.11. HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{17}ClFN_2O_2$, 299.0963; found, 299.0962.

Compounds **45b** were prepared following the synthetic procedure of **45a**.

*(R)-2-(2-Chlorobutanamido)-N-cyclopropyl-6-fluorobenzamide (**45b**).* Yield 65%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.18 (s, 1H), 8.68 (d, J = 3.2 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.46 (dt, J = 14.8, 7.4 Hz, 1H), 7.10 (t, J = 8.9 Hz, 1H), 4.69 (t, J = 6.6 Hz, 1H), 2.83 (tt, J = 7.7, 3.9 Hz, 1H), 2.08−1.96 (m, 1H), 1.93−1.85 (m, 1H), 0.98 (t, J = 7.3 Hz, 3H), 0.72−0.68 (m, 2H), 0.56−0.50 (m, 2H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{17}ClFN_2O_2$, 299.0963; found, 299.0966.

*(S)-2-(1-Chloropropyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**46a**).* Yield 54%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (td, J = 8.2, 5.6 Hz, 1H), 7.47 (d, J = 8.1 Hz, 1H), 7.30 (dd, J = 10.4, 8.4 Hz, 1H), 5.62 (dd, J = 7.9, 6.2 Hz, 1H), 3.04 (qd, J = 7.0, 4.2 Hz, 1H), 2.38 (dp, J = 14.0, 7.2 Hz, 1H), 2.20 (dp, J = 14.8, 7.4 Hz, 1H), 1.33−1.24 (m, 2H), 1.08 (t, J = 7.3 Hz, 3H), 1.00−0.96 (m, 1H), 0.94−0.89 (m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{15}ClFN_2O$, 281.0857; found, 281.0861.

*(R)-2-(1-Chloropropyl)-3-cyclopropyl-5-fluoroquinazolin-4(3H)-one (**46b**).* Yield 49%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.80 (td, J = 8.2, 5.6 Hz, 1H), 7.48 (d, J = 8.1 Hz, 1H), 7.30 (dd, J = 10.3, 8.3 Hz, 1H), 5.62 (dd, J = 7.9, 6.2 Hz, 1H), 3.04 (qd, J = 7.1, 4.2 Hz, 1H), 2.38 (dp, J = 14.1, 7.2 Hz, 1H), 2.20 (dp, J = 14.8, 7.3 Hz, 1H), 1.34−1.23 (m, 2H), 1.08 (t, J = 7.3 Hz, 3H), 1.00−0.96 (m, 1H), 0.95−0.87 (m, 1H). HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{14}H_{15}ClFN_2O$, 281.0857; found, 281.0855.

Cell Lines, Cell Culture, and Chemicals. The cell lines NIH-3T3, RAW 264.7, and Raji were purchased from Cobioer Biosciences Co., Ltd. (Nanjing, China). NIH-3T3 and RAW 264.7 cell lines were cultured in DMEM media (Corning) with 10% fetal bovine serum (FBS) and supplemented with 2% L-glutamine and 1% penicillin/streptomycin. Raji cells were cultured in RPMI 1640 media (Corning) with 10% fetal bovine serum (FBS), supplemented with 2% L-

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glutamine and 1% penicillin/streptomycin. All cell lines were maintained in humidified incubators at 37 °C with 5% CO_2.

Compounds **4** and **5** were purchased from MedChemExpress (Shanghai, China).

PI3K Isoform ADP-Glo Assays. The ADP-Glo kinase assay (Promega) was used to screen the compounds for their phosphoinositide kinase inhibition activities. Kinase reaction systems contain 3 μL of PI3Kα (0.24 μg/mL), PI3Kβ (3 μg/mL), PI3Kδ (3.3 μg/mL), or PI3Kγ (12 μg/mL) (Invitrogen), 3 μL of serially diluted compound, and 6 μL of substrate PIP2:PS (0.1 mM) (Invitrogen) with 100 μM ATP (200 μM ATP for PI3Kβ). Reactions were started immediately by adding ATP and continued for an hour at 37 °C. After the reaction, tubes were cooled for 5 min at room temperature, and 5 μL of the reaction solution was transferred to a 384-well plate. Then, 5 μL of ADP-Glo reagent was added into each well to stop the reaction and consume the remaining ADP within 40 minutes. At the end, 10 μL of the kinase detection reagent was added into each well and incubated for 30 min to produce a luminescence signal, which was measured with an automated plate reader (Perkin-Elmer Envision), and each measurement was performed in triplicates.

PI3K Isoform Cellular Selectivity Assays. NIH-3T3, RAW 264.7, and Raji cells were treated with DMSO, serially diluted compound **(*S*)-18**, and 1 μM compound **4** for 1 h, after which the cells were treated with and without different stimulating factors. All cells were then washed with 1×PBS buffer and lysed in cell lysis buffer at 4 °C for 30 min. The lysates were cleared by centrifugation, and the protein concentrations were measured by BCA analysis (Beyotime, China). Then, 50 μg of total protein lysates was mixed with 5× loading buffer and heated in a metal bath for 10 min at 100 °C. The cell lysates were analyzed by Western blotting with the following antibodies purchased from Cell Signaling Technology (Danvers, MA): P-AKT Thr308 (#2965S), P-AKT Ser473 (#4060S), and total AKT (#4691S). The antibodies were used at 1:1000.

CYP Inhibition Assay. The CYP enzyme assays were performed according to P450-Glo Assay instructions (Promega). Briefly, assays were assembled and performed in opaque white 96-well plates. Compound **(*S*)-18** at 10 different final concentrations descending 3-fold from 10 μmol/L was added into the plates. After incubation for 20 or 30 min at 37 °C or room temperature (20−23 °C), 50 μL of the luciferin detection reagent was added to each 50 μL CYP reaction to stop the reactions and initiate luminescence, which was measured 20 min later using the Bio-Rad Microplate reader (Bio-Rad). Data were normalized to control groups (DMSO), and IC_{50} values were calculated using Prism 7.0 (GraphPad Software, San Diego, CA).

Patch-Clamp Functional hERG Assay. The human ether-á-go-go-related gene (hERG) assay was provided by PharmaCore Labs (Nantong, China). In short, the assay was performed with a stable Kv11.1 transfected human embryonic kidney cell line (HEK293) by whole-cell patch clamp carried out at room temperature. The Kv11.1 (hERG) ion channel blocker quinidine was used as the reference compound, and the effects of compound **(*S*)-18** and quinidine were normalized to the corresponding vehicle control. Whole-cell recordings were carried out with the patch-clamp device (HEKA Instruments Inc., D-67466 Lambrecht, Pfalz, Germany) with commercial amplifiers (EPC10, HEKA Elektronik Dr. Schulze GmbH, Germany). Patch-clamp measurements were run on silicate-coated chips with a hole of a defined diameter. GI_{50} values were calculated using Origin 8.5 (OriginLab Corporation, Northampton, MA).

Pharmacokinetic Study. This study protocol was approved by the Animal Ethics Committee of Hefei Institutes of Physical Science, Chinese Academy of Sciences (Hefei, China). The pharmacokinetic study was performed on male Sprague-Dawley (SD) rats. All of the rats were allowed to acclimatize to the research facilities for one week followed by fasting for 12 h prior to drug administration. **(*S*)-18** was formulated as a solution in DMSO/5% of glucose solution (70:30, v/v) at a concentration of 2 mg/mL (intravenous) or a solution in DMSO/0.5% of CMC-Na (4:96, v/v) at a concentration of 2 mg/mL (oral). For inhaled formulation, gel-like HP-β-CD was first prepared by mixing with ultrapure water (1:2−1:3, m/v), followed by further dilution with ultrapure water (1:1, m/v) under continuous stirring. **(*S*)-18** was mixed with the HP-β-CD gel at a ratio of 1:7 and then stirred in the dark at 4 °C for 6 h. After prefreezing at −80 °C for 2 h, the mixture was freeze-dried overnight and redissolved in saline for further animal study. The rats were randomly and equally divided into three groups (3 animals/group) by different routes of administration. One group was injected with **(*S*)-18** at a dosage of 1 mg/kg; the second group was treated by oral administration of **(*S*)-18** at a dosage of 10 mg/kg; and the third group was treated by inhalation of nebulized **(*S*)-18** at a dosage of 5 mg/kg. Blood samples were collected into heparinized tubes at 2, 5, 15, and 30 min and 1, 2, 4, 6, 9, 12, and 24 h after intravenous injection and at 5, 15, and 30 min and 1, 2, 4, 6, 9, 12, 24, and 72 h after oral or inhaled administration. Plasma (100 μL) was harvested by centrifuging the blood sample at 4 °C and 8000 rpm for 5 min and then stored at −80 °C until analysis. An aliquot of 100 μL of each plasma sample was mixed with 20 μL of the internal standard working solution (200 ng/mL of caffeine), and then 400 μL of methanol was added for protein precipitation. After vortexing for 5 min and centrifuging at 14 000 rpm for 10 min, 10 μL of the supernatant was injected for liquid chromatography−mass spectrometry (LC−MS)/MS analysis.

For lung tissue distribution study, the rats receiving **(*S*)-18** at a dose of 5 mg/kg by inhalation were sacrificed by bleeding the femoral artery at 6, 12, 24, or 72 h (three rats per time point). After that, lung tissue samples were excised and then washed using iced physiological saline solution three times, blotted on filter paper, and stored at −80 °C until analysis. Before analysis, the weighed tissues were homogenized in iced physiological saline solution (1:4, w/v) to prepare homogenates by Tissuelyser-24 (Jingxin Industrial Development Co. Ltd, Shanghai, China). An aliquot of 100 μL of each lung tissue sample was mixed with 20 μL of an internal standard working solution (200 ng/mL of caffeine); then, 400 μL of methanol was added for protein precipitation. After vortexing for 5 min and centrifuging at 14 000 rpm for 10 min, 10 μL of the supernatant was injected for LC−MS/MS analysis.

The in vitro metabolic stability of **(*S*)-18** was performed using human, monkey, dog, rat, and mice liver microsomes, respectively, in triplicate. The incubation mixtures (200 μL) consisted of human liver microsomes (0.5 mg/mL), **(*S*)-18** (1 mM), NADPH (1 mM), $MgCl_2$ (4 mM), and PBS. Reactions were initiated with the addition of NADPH and kept in a water-bath at 37 °C. Aliquots were withdrawn at 0, 5, 10, 15, 30, 60, and 90 min. The reaction was terminated with acetonitrile containing caffeine (internal standard, 40 ng/mL) and then centrifugated. The supernatant was subjected to HPLC-mass spectrometry (MS/MS) analysis. The in vitro elimination half-life ($t_{1/2}$), intrinsic hepatic clearance, and extrapolated hepatic clearance were determined as described by Obach.[36]

Drug Efficacy Evaluation, Histopathological Analysis, and Apoptosis Detection in a Rodent Model of COPD. The in vivo assessment of drug efficacy, histopathological analysis, and apoptosis detection against COPD in rodent model were provided by Nanjing Bosgene Biotechnology Co., Ltd. (Nanjing, China). All animal care and experimental studies were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Laboratory Animal Ethics Committee of BOSGENE Biotech (Nanjing, China). Five-week-old male Sprague-Dawley rats purchased from Qinglongshan Animal Breeding Base (SCXK2017−001) were used for this study and were fed under controlled environmental conditions of a 12 h light/dark cycle and maintained on standard food pellets and tap water. A week later, 10 animals were randomly selected as the normal group and exposed to fresh air only. The rest of the animals were exposed to cigarette smoke from day 2 to day 42 (6 days per week) and tracheal infusion of LPS on day 1 and day 20, respectively. After 42 days, the animals were selected and divided (10 animals per group) and administered with different doses of aerosolized compound **(*S*)-18** by inhalation once a day for 28 days. The vehicle group (10 animals) was treated with an aerosolized equal volume of vehicle solution every day for 28 days.

After the end of the treatments, the rats were sacrificed by anesthesia and autopsied. Lung tissue was removed, fixed in 4%

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glutaraldehyde, and embedded in paraffin. Sections were then cut at 5 μm, stained with HE, and imaged using a Leica light microscope (DM500, Germany) for histological analysis. Apoptosis detection in the lung tissue was carried out using a Promega TUNEL staining kit (Madison, Wisconsin) according to the manufacturer's instructions. Sections were prepared and then imaged using a Leica light microscope (DM500, Germany) to identify the TUNEL-positive cells.

Molecular Docking. Docking simulations were performed using the Induced Fit Docking (IFD) protocol in Schrodinger Suite 2017. Prior to docking, the PI3K-delta receptor structure (PDB ID: 5M6U) was prepared using the Protein Preparation Wizard with the OPLS3 force field. The ligands were prepared using LigPrep.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c01544.

KINOMEScan profiling data of (*S*)-**18**, in vivo efficacy evaluation of (*S*)-**18** in a COPD rat model, NMR spectra of newly synthesized compounds, and HPLC traces of final compounds (PDF)
Molecular formula strings (CSV)
Docking pose for (*S*)-**18** with PI3Kδ kinase (PDB)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

COPD, chronic obstructive pulmonary disease; DCM, dimethyl chloride; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; HATU, 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate; HE, hematoxylin-eosin; HMDS, hexamethyldisilazane; LPS, lipopolysaccharide; PI3K, phosphatidylinositol 3 kinase; SAR, structure−activity relationship; THF, tetrahydrofuran; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling

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