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Modification of a dihydropyrrolopyrimidine phosphoinositide 3-kinase (PI3K) inhibitor to improve oral bioavailability

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ABSTRACT

Phosphoinositide 3-kinase (PI3K) is activated in various human cancer cells and well known as a cancer therapy target. We previously reported a dihydropyrrolopyrimidine derivative as a highly potent PI3K inhibitor that has strong tumor growth inhibition in a xenograft model. In this report, we describe further optimization to improve its bioavailability.

1. Introduction

Phosphoinositide 3-kinases (PI3K) are a family of intracellular lipid kinases. Activated by receptor tyrosine kinases and G-protein-coupled receptors, PI3K converts phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) to phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P3).[1,2] PI(3,4,5)P3 activates serine/threonine kinase AKT as a second messenger and regulates various cellular functions, including cell cycle, apoptosis, and glucose metabolism. Oncogenic gene mutations and gene amplifications of PI3K, especially class IA PI3K, are found in various human cancers, as are loss of heterozygosity and mutations of the tumor suppressor PTEN, which controls the cellular concentration of PI(3,4,5)P3 by converting PI(3,4,5)P3 to PI(4,5)P2.[3–5] Therefore, the PI3K pathway is a promising target for cancer therapeutics and several inhibitors are now in clinical trials.[6]

We have already reported the design of a dihydropyrrolopyrimidine-type PI3K inhibitor by superimposing Piramed's PI103 and Chiron's PI3K inhibitors and have also reported the optimization of the lead compound.[7] Because of the rapid glucuronidation of phenol, our lead compound was metabolically unstable, and bioavailability was extremely low in mice (1.6%). To overcome this issue, an aminopyrimidine was designed as a bioisostere of phenol based on a FlexSIS docking simulation and structure–activity relationship information. Finally we identified compound **1** as a metabolically stable and potent PI3K inhibitor. The key amino acids of PI3Kγ that interact with the phenol-type inhibitor are D841 and Y867, but the X-ray structure of PI3Kγ and **1** revealed that compound **1** formed hydrogen bonding with K833 and D836. Compound **1** had oral bioavailability of 41% in mice and showed strong tumor growth inhibition in a KPL-4 xenograft model.[8]

In this paper, we report further optimization of the pyridine moiety of compound **1** to improve its low oral bioavailability in monkey (5.6%). Compound **3** was identified by the following optimizations: (1) adding a solubilizing group (2) removing the hydrogen bonding acceptor (3) introducing an ortho-substituent (4) optimizing the solubilizing group. The solubility in FaSSIF (fasted state simulated intestinal fluid),[9] liver microsome (LM) stability, and permeability of compound **3** were better than in compound **1**, and the oral bioavailability of compound **3** in monkey was 8 times better than that of compound **1** (Fig. 1).

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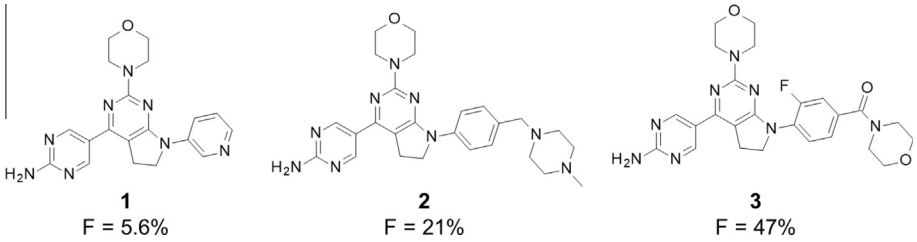


Figure 1. Improvement of bioavailability in monkey.

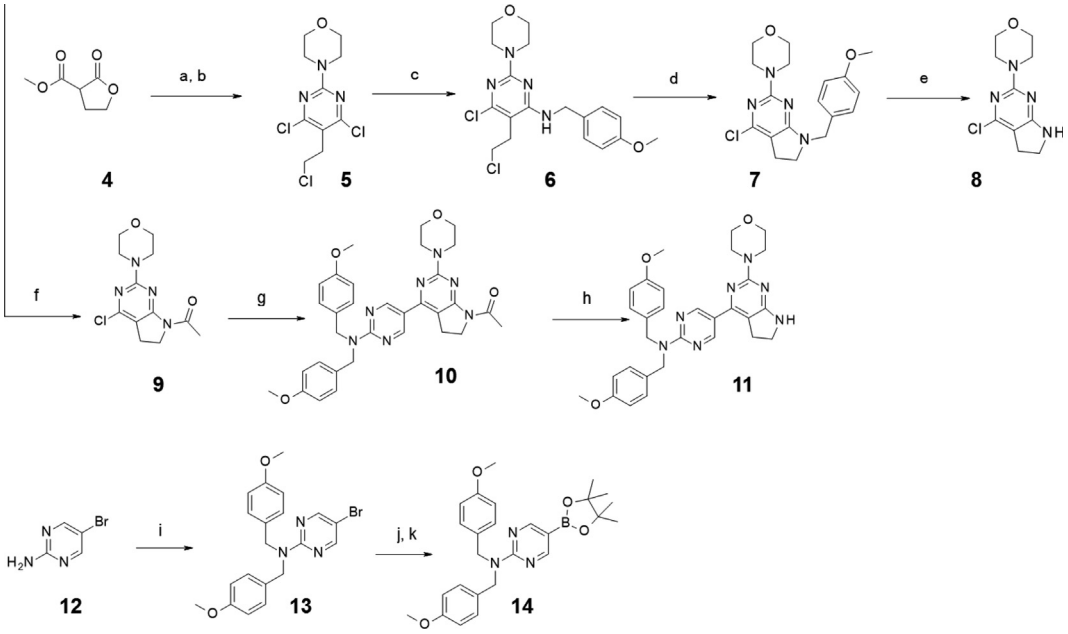
2. Chemistry

Compound **11** was selected as a key intermediate to prepare new derivatives, because various aryl functions can be introduced by the Buchwald–Hartwig reaction. The preparation of this intermediate from the commercially available diester **4** is described in Scheme 1. Treatment of **4** with morpholinoformamidine under a basic condition, and subsequent chlorination of the resulting triol by $POCl_3$ afforded the trichloride **5**. Next, 4-methoxybenzylamine was coupled with **5** as a nitrogen atom source and a dihydropyrrolopyrimidine skeleton was constructed by the intramolecular cyclization of **6** under a basic condition. The *p*-methoxybenzyl (PMB) group of **7** was removed by trifluoroacetic acid in the presence of conc. H_2SO_4 and replaced with acetyl group to afford **9**. Suzuki-Miyaura coupling with the pinacol ester **14** followed by the deprotection of the acetyl group afforded the desired key compound **11**. This compound was coupled with various aryl halides by the Buchwald–Hartwig reaction.

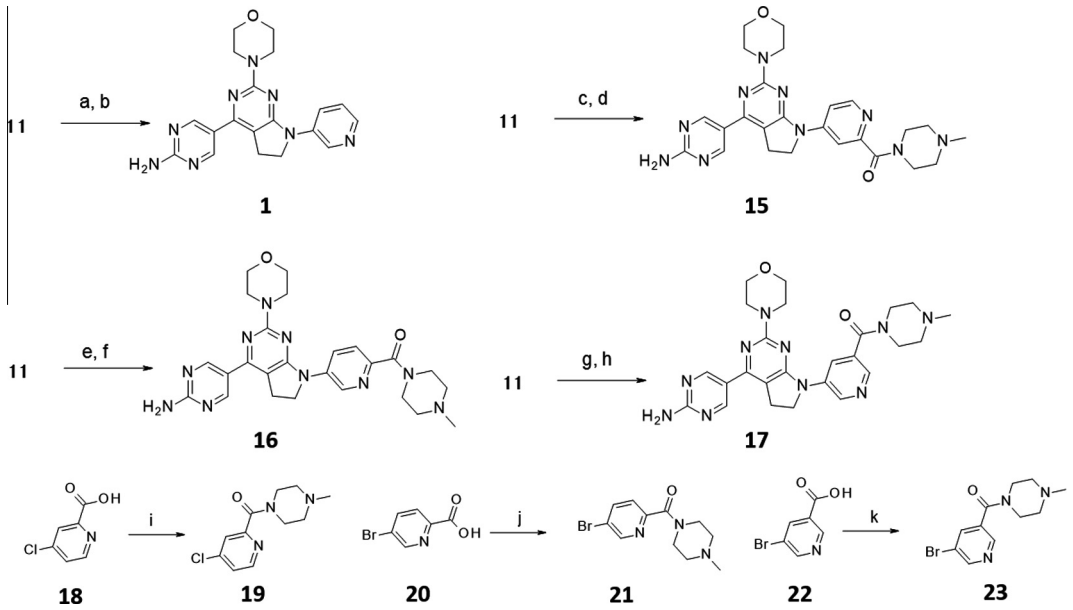
The coupling reaction of **11** with halopyridine derivatives is summarized in Scheme 2. The amidation of 4-chloropicolinic acid (**18**), 5-bromopicolinic acid (**20**), and 5-bromonicotinic acid (**22**) with *N*-methylpiperazine afforded the corresponding derivatives: **19**, **21** (picolinamides), and **23** (nicotinamide). The palladium–catalyzed Buchwald–Hartwig reaction with **11** and the subsequent deprotection of PMB groups by TFA afforded the desired pyridine derivatives **1**, **15**, **16**, and **17**.

The preparation of compound **24** and its benzamide derivatives is summarized in Scheme 3. Compound **24** was prepared by the coupling reaction of **11** with bromobenzene and the subsequent deprotection of the PMB groups. For the synthesis of **27**, 3-bromobenzoic acid methyl ester was coupled with key intermediate **11**, and the methyl ester was cleaved under a basic condition. Amidation of the resulting benzoic acid by *N*-methylpiperazine and subsequent deprotection of PMB groups afforded **27**. Compound **28** was synthesized by the coupling reaction of **11** with **30** and following deprotection of PMB groups. To prepare compound **3**, compound **11** was coupled with 4-bromo-3-fluoro-benzoic acid. Amide bond formation with *N*-methylpiperazine followed by deprotection of PMB groups afforded compound **3**.

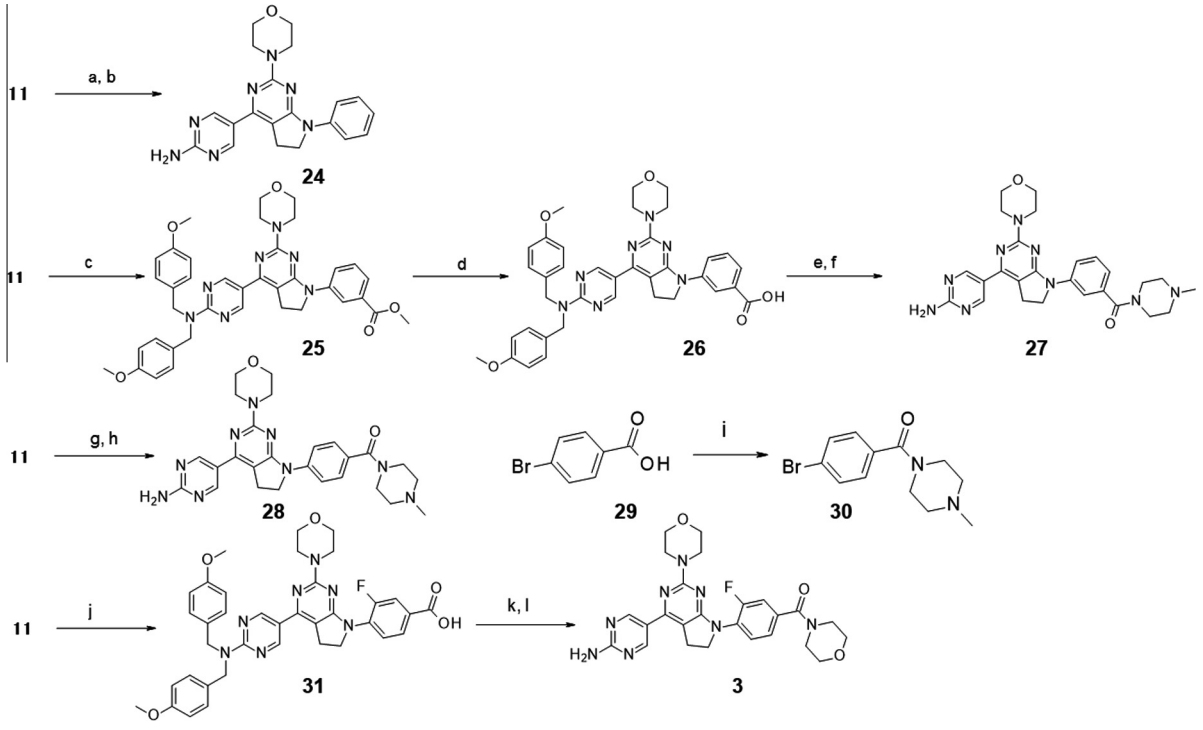
The synthesis of benzylamine derivatives is shown in Scheme 4. The coupling reaction of **11** with 2-(4-bromophenyl)-1,3-dioxolane and deprotection under a mild acidic condition gave benzaldehyde derivative **32**. Reductive amination with *N*-methylpiperazine or morpholine, followed by deprotection of PMB groups afforded **2** or **33**, respectively. To synthesize compound **35**, the key intermediate **11** was coupled with 4-bromo-3-fluorobenzaldehyde to afford **34**. Reductive amination with morpholine and subsequent deprotection of PMB groups gave **35**.



Scheme 1. Preparation of key intermediate **11**. Reagents and conditions: (a) morpholinoformamidine hydrochloride, NaOMe, MeOH, reflux; (b) $POCl_3$, 100 °C, 28% in 2 steps; (c) 4-methoxybenzylamine, DIPEA, CH_3CN, reflux, 68%; (d) Cs_2CO_3, NaI, CH_3CN, reflux; (e) TFA, conc.H_2SO_4, reflux; (f) AcCl, pyridine, DMAP, CH_3CN, 0 °C to rt, 92% in 3 steps; (g) **14**, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C, quant.; (h) 5 M NaOH, THF, reflux, 85%; (i) 4-methoxybenzyl chloride, NaH, NaI, THF, rt, 92%; (j) $B(OiPr)_3$, *n*BuLi, toluene/THF, −78 °C; (k) pinacol, $MgSO_4$, DME, rt, 88% in 2 steps.



Scheme 2. Preparation of pyridine derivatives. Reagents and conditions: (a) 3-bromopyridine, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C; (b) TFA, *N*-acetylcysteine (NAC), 70 °C, 18% in 2 steps; (c) **19**, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C; (d) TFA, NAC, 70 °C, 38% in 2 steps; (e) **21**, $Pd_2(dba)_3$, S-Phos, K_3PO_4, DMF, 100 °C; (f) TFA, NAC, 70 °C, 58% in 2 steps; (g) **23**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, DMF, 100 °C; (h) TFA, NAC, 70 °C, 91% in 2 steps; (i) *N*-methylpiperazine, EDC, HOBt, CH_2Cl_2, 83%; (j) *N*-methylpiperazine, EDC, HOBt, CH_2Cl_2, 28%; (k) *N*-methylpiperazine, EDC, HOBt, CH_2Cl_2, 78%.

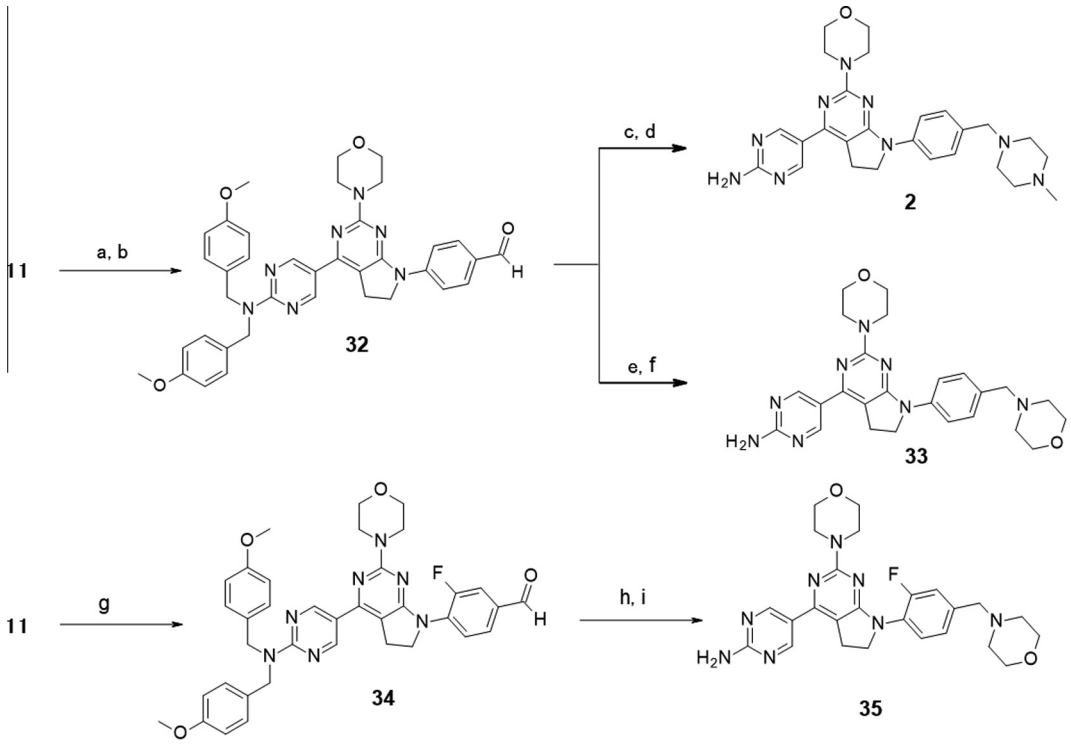


Scheme 3. Preparation of benzamide derivatives. Reagents and conditions: (a) bromobenzene, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C; (b) TFA, NAC, 70 °C, 80% in 2 steps; (c) 3-bromobenzoic acid methyl ester, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C, 86%; (d) 5 M NaOH, THF, 30% aq. H_2O_2 reflux; (e) *N*-methylpiperazine, EDC, HOBt, DIPEA, DMF, 60 °C, 71% in 2 steps; (f) TFA, 70 °C, 96%; (g) **30**, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 °C; (h) TFA, NAC, 70 °C, 87% in 2 steps; (i) *N*-methylpiperazine, EDC, HOBt, CH_2Cl_2, 97%; (j) 4-bromo-3-fluoro-benzoic acid, $Pd_2(dba)_3$, X-Phos, K_3PO_4, DMF, 100 °C, 80%; (k) morpholine, EDC, HOBt, CH_2Cl_2; (l) TFA, reflux, 96% in 2 steps.

3. Results and discussion

We previously reported the lead optimization of a virtually designed PI3K inhibitor and identified the aminopyrimidine moiety as an alternative to the metabolically unstable phenol.[8] As described in Table 1, oral bioavailability of compound **1** was good in mouse (F = 41%) but poor in monkey (F = 5.6%), which indicated exposure in human would be poor. In order to improve this low oral bioavailability, compound **1** was optimized further.

First, because the solubility of compound **1** in FaSSIF was low (Table 2), an amide function was introduced as a solubilizing group. After analyzing the X-ray structure of **1**, the solubilizing group was connected to the pyridine, since this substituent is located at the solvent-exposed region of PI3Kα.[8] As expected, all

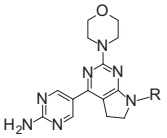


Scheme 4. Preparation of benzylamine derivatives. Reagents and conditions: (a) 2-(4-Bromophenyl)-1,3-dioxolane, $Pd_2(dba)_3$, X-Phos, K_3PO_4, DMF, 100 °C; (b) THF, 1 M HCl, rt, 96% in 2 steps; (c) *N*-methylpiperazine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt, 51%; (d) TFA, NAC, 70 °C, 90%; (e) morpholine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt; (f) TFA, NAC, 70 °C, 50% in 2 steps; (g) 4-bromo-3-fluorobenzaldehyde, $Pd(OAc)_2$, X-Phos, K_3PO_4, DMF, 100 °C, 65%; (h) morpholine, $NaBH(OAc)_3$, AcOH, CH_2Cl_2, rt; (i) TFA, NAC, 70 °C, 52% in 2 steps.

Table 1
Pharmacokinetic data of compound **1** in mouse and monkey

Species	i.v.					p.o.			*F* (%)
	Dose (mg/kg)	C_0 (ng/mL)	AUC_{inf} (ng·h/mL)	CL (mL/min/kg)	$T_{1/2}$ (h)	dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	
Mouse	10	10,400	4690	35.9	10.3	100	4190	19,400	41
Monkey	5	3120	3700	24.4	1.36	10	41.6	417	5.6

Table 2
In vitro and in vivo profiles of pyridine derivatives



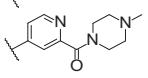
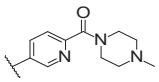
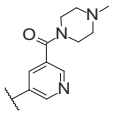
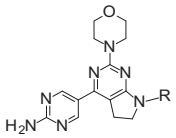
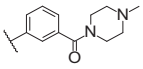
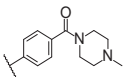
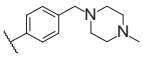
Compound	R	PI3Kα IC_{50} (μM)	FaSSIF (μg/ml)	LM stability		PAMPA (10^{-6} cm/s)	Mouse PK (50 mg/kg po)	
				mouse (μL/min/mg)	human (μL/min/mg)		C_{max} (ng/mL)	AUC_{0-t} (ng·mL/h)
1		0.033	<12	11.5	9.7	1.90	4250	7640
15		0.040	439	27.3	15.3	1.13	128	188
16		0.025	29	3.5	13.4	1.64	326	1650
17		0.032	376	19.7	15.2	0.73	158	455

Table 3
In vitro and in vivo profiles of phenyl derivatives



Compound	R	PI3Kα IC50 (μM)	FaSSIF (μg/ml)	LM stability		PAMPA (10^{-6} cm/s)	Mouse PK (50 mg/kg po)	
				mouse (μL/min/mg)	human (μL/min/mg)		C_{max} (ng/mL)	AUC_{0-t} (ng·mL/h)
24		0.035	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
27		0.12	454	6.2	13.8	1.79	1500	4030
28		0.014	331	10.8	12.6	1.24	2840	13,200
2		0.048	153	13.3	13.3	0.92	1730	13,400

three compounds (**15**, **16**, and **17**) had improved solubility in FaSSIF, without a significant loss of enzyme inhibitory activity. Since the pharmacokinetic profiles of these compounds in mice were much poorer than compound **1**, the pharmacokinetics in monkeys was not tested.

Next, because the number of hydrogen bonding acceptors had been increased by introducing an amide bond and an amine (the number of hydrogen bonding acceptors in compounds **15**, **16**, and **17** is 11), we replaced the pyridine with a phenyl moiety to decrease the number of hydrogen bonding acceptors. In our previous study, this replacement resulted in the significant loss of enzyme inhibitory activity in the phenol derivatives, and the presence of the nitrogen atom in the pyridine is critical.[8] But Table 3 shows, the nitrogen atom in the pyridine was not important for the aminopyrimidine derivatives, which suggests that the dominant part for enzyme affinity is the aminopyrimidine. In addition to their strong inhibitory activity, compounds **27** and **28** also had good solubility in FaSSIF, and their C_{max} and AUC in mice were much better than those of **15** and **16**. The benzylamine derivative **2** also showed a good pharmacokinetic profile, and the results in monkeys are described in Table 4. The oral bioavailability of **2** (21%) was 4 times greater than that of **1** (5.6%), but its clearance (CL) was slightly increased, and C_{max} and AUC were still low.

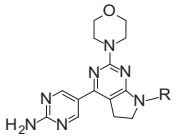
Our next goal was to overcome the high CL of compound **2** and simultaneously improve the oral bioavailability even further by improving its permeability.[10] As shown in Table 5, morpholine

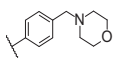
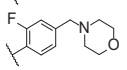
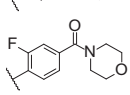
Table 4
Pharmacokinetic data of compound **2** in mouse and monkey

Species	i.v.					p.o.			*F* (%)
	Dose (mg/kg)	C_0 (ng/mL)	AUC_{inf} (ng·h/mL)	CL (mL/min/kg)	$T_{1/2}$ (h)	dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	
Mouse	10	3940	4400	38.0	5.03	50	1730	13,400	61
Monkey	5*	430	2540	33.9	10.5	10	95.9	1080	21

* $n = 1$.

Table 5
In vitro and in vivo profiles of morpholine derivatives



Compound	R	PI3Kα IC_{50} (μM)	FaSSIF (μg/ml)	LM stability		PAMPA (10^{-6} cm/s)	Mouse PK (50 mg/kg po)	
				mouse (μL/min/mg)	human (μL/min/mg)		C_{max} (ng/mL)	AUC_{0-t} (ng·mL/h)
33		0.046	<4	4.32	1.97	4.52	N.D.	N.D.
35		0.22	107	35.9	19.1	4.18	16,900	50,900
3		0.042	37	0.14	2.87	3.16	10,000	64,500

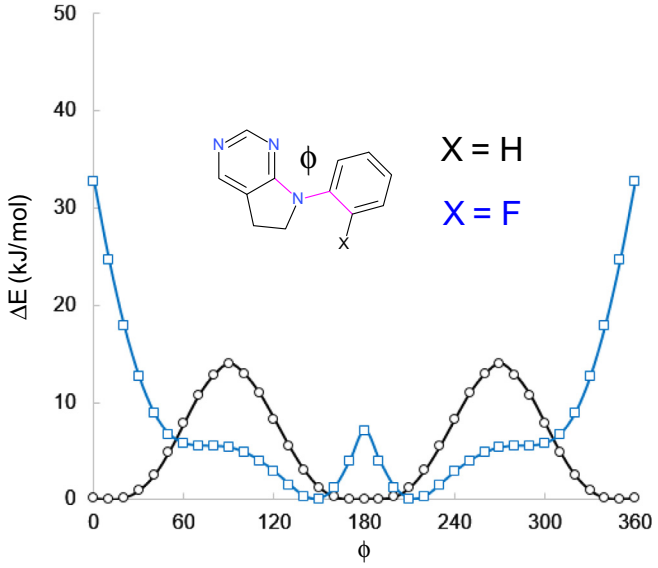


Figure 2. B3LYP/6-31+G(d,p) level calculation of *N*-phenyl dihydropyrrolopyrimidine derivatives. Potential energies were calculated with fixing the torsion ϕ between 0 and 350 at 10° intervals while other variables were optimized. All calculations were done by the program Gaussian 09.

was the means to this goal. By replacing *N*-methylpiperazine of **2** with morpholine, the LM stability and passive permeability were significantly improved, but the solubility in FaSSIF was lost (**33**). To recover the water solubility, an 'ortho-substituent' was introduced in the phenyl group. The introduction of an ortho-substituent in a bicyclic compound disrupts the molecular planarity and improves water solubility, as was demonstrated by Ishikawa et al., who introduced an ortho-fluoro atom into an *N*-phenylpiperidine derivative to improve the water solubility.[11,12] As described in Fig. 2, B3LYP/6-31 + G(d,p) level calculation[13] of the *N*-phenyl dihydropyrrolopyrimidine derivatives showed that the most stable conformation of the unsubstituted one is planar (ϕ = 0, 180°) and that of the ortho-fluoro derivative is twisted (ϕ = 150, 210°). In fact, ortho-fluorinated compound **35** showed good FaSSIF solubility and remarkable pharmacokinetic profiles in mice. On the other hand, the inhibitory activity of **35** against PI3Kα was 5 times weaker than that of **33**, and its LM stability was low. As shown in Table 3, the PI3Kα IC_{50} value of benzamide **28** was more than 3 times stronger than that of benzylamine **2**, suggesting that the carbonyl group contributes as a hydrogen bonding acceptor and that this structure–activity relationship could be applied to **35**. As shown in Table 5, the benzoylmorpholine derivative **3** recovered not only the enzyme inhibitory activity but also the LM stability. Although its solubility in FaSSIF decreased, it was 3 times greater than that of compound **1** and acceptable for further examination. The pharmacokinetic profiles of compound **3**, as summarized in Table 6, show that C_{max}, AUC, and CL were good, and oral bioavailability in monkeys was greatly improved compared to **1** (F in compound **1** = 5.6% vs F in compound **3** = 47%).

4. Conclusion

We examined various strategies to overcome the low oral bioavailability of compound **1** in monkey (*F* = 5.6%). As the solubility of **1** in FaSSIF was low, a solubilizing group was introduced at the pyridine, which is located at the solvent-exposed region of PI3Kα. This method improved water solubility without losing inhibitory activity, but the increased number of hydrogen bonding acceptors resulted in a poor pharmacokinetic profile in mice. Reducing the number of hydrogen bonding acceptors by replacing the pyridine with phenyl improved the pharmacokinetic profile, and the oral bioavailability of compound **2** in monkey was more than 4 times greater than that of **1**. The LM stability and passive permeability were improved by substituting *N*-methylpiperazine with morpholine, but this change lowered the water solubility (**33**). This antimony was then resolved by introducing an ortho-fluoro atom. Finally, based on the structure–activity relationship, enzyme inhibitory activity was recovered and compound **3** showed good pharmacokinetic profiles (*F* = 47%) with strong PI3Kα inhibition (IC_{50} = 0.042 μM).

5. Experimental

5.1. Chemistry

All solvents and reagents were obtained commercially. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz), JEOL JNM-EX400 (400 MHz), or JNM-GSX400 (400 MHz), and chemical shifts are expressed as δ units using tetramethylsilane as an internal standard. The spectral splitting patterns are described as follows: s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; and bs, broad singlet peak. High resolution mass spectra (HRMS) were measured with a Thermo Fisher Scientific LTQ Orbitrap XL MS spectrometer using an ESI source coupled to a Waters HPLC system operating in reversed phase with an ACQUITY UPLC BEH C18 (1.7 μm, 2.1 mm × 50 mm) column. Flash column chromatography was performed with Biotage SNAP cartridges or SILICYCLE SiliaSep packed columns.

5.1.1. 4-[4,6-Dichloro-5-(2-chloroethyl)-pyrimidin-2-yl]-morpholine (5)

To a solution of Na (3.19 g, 139 mmol) in MeOH (140 mL) was added morpholinoformamidine hydrochloride (15.3 g, 92.0 mmol) and **4** (13.3 g, 92.0 mmol). After refluxing for 2 h, the mixture was cooled to an ambient temperature and concentrated under reduced pressure, which afforded 5-(2-hydroxyethyl)-2-morpholin-4-yl-pyrimidine-4,6-diol as a crude product. The residue was dissolved in $POCl_3$ (90 ml) and the resulting mixture was stirred at 100 °C for 10 h. After cooling to ambient temperature, the mixture was concentrated under reduced pressure. After ice (100 g) was added at 0 °C, the crude mixture was neutralized with 5 M NaOH and extracted with EtOAc. The combined organic extract was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc, 20/1 to 10/1) to afford the titled compound as a yellow solid (8.4 g, 28% in 2 steps). ^{1}H

Table 6
Pharmacokinetic data of compound **3** in mouse and monkey

Species	i.v.					p.o.			*F* (%)
	Dose (mg/kg)	C_0 (ng/mL)	AUC_{inf} (ng·h/mL)	CL (mL/min/kg)	$T_{1/2}$ (h)	dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-t} (ng·h/mL)	
Mouse	10	9180	15,000	11.1	1.27	50	10,000	64,500	86
Monkey	2.5*	2660	5570	7.54	4.50	10	1060	10,400	47

* *n* = 1.

NMR (400 MHz, DMSO-d_6) δ: 3.76 (2H, t, J = 7.3 Hz), 3.65 (8H, s), 3.12 (2H, t, J = 7.3 Hz).

5.1.2. 6-Chloro-5-(2-chloroethyl)-*N*-[(4-methoxyphenyl)-methyl]-2-morpholino-pyrimidin-4-amine (6)

To a solution of **5** (5.78 g, 19.47 mmol) in CH_3CN (80 mL) was added 4-aminomethyl-anisole (5.04 mL, 38.9 mmol) and DIPEA (8.50 mL, 48.7 mmol). After being refluxed for 17 h, the reaction mixture was cooled, and then evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with saturated aqueous NH_4Cl and then brine. The combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc, 1/0 to 5.5/1) afforded titled compound as a yellow solid (68%, 5.28 g). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.76 (1H, t, J = 5.9 Hz), 7.23 (2H, d, J = 8.8 Hz), 6.86 (2H, d, J = 8.8 Hz), 4.44 (2H, d, J = 5.9 Hz), 3.71 (3H, s), 3.60 (2H, t, J = 7.8 Hz), 3.55 (8H, s), 2.98 (2H, t, J = 7.6 Hz); HRMS (ESI), m/z calcd for $C_{18}H_{22}Cl_2N_4O_2$ + H: 397.1198, found 397.1195.

5.1.3. 1-(4-Chloro-2-morpholino-5,6-dihydropyrrolo[2,3-*d*] pyrimidin-7-yl)ethanone (9)

To a solution of **6** (5.26 g, 13.24 mmol) in CH_3CN (200 ml) was added Cs_2CO_3 (12.94 g, 39.7 ml) and NaI (3.97 g, 26.5 mmol). After being refluxed for 24 h, the reaction mixture was cooled, and then evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with H_2O and then brine. Combined organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure, affording 4-[4-chloro-7-[(4-methoxyphenyl) methyl]-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-2-yl] morpholine (**7**) as a crude product. This crude product was used in the next reaction without further purification. To a solution of the crude compound in TFA (14 ml) was added conc. H_2SO_4 (672 μL, 12.6 mmol). After being refluxed for 5 h, the reaction mixture was cooled, and then evaporated under reduced pressure. The residue was diluted by CH_2Cl_2, poured into iced water, and neutralized by 5 M NaOH and extracted with EtOAc/THF (4/1). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure, which afforded 4-(4-chloro-6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidin-2-yl)morpholine (**8**) as a crude product. This crude product was used in the next reaction without further purification. To a solution of the crude compound, pyridine (2.42 mL, 29.9 mmol), DMAP (29 mg, 0.239 mmol) in CH_3CN (50 ml) was added acethyl chloride (1.70 mL, 23.9 mmol) dropwise at 0 °C. After being stirred for 15 min at an ambient temperature, the reaction mixture was diluted with H_2O (200 ml) and EtOAc (200 ml), filtered through Celite. The filtrate was extracted with EtOAc twice, and the combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc, 1/0 to 3/1) to afford the titled compound as a light yellow solid (3.13 g, 92% in 3 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.92 (2H, t, J = 8.3 Hz), 3.64 (8H, s), 2.85 (2H, t, J = 8.3 Hz), 2.53 (3H, s); HRMS (ESI), m/z calcd for $C_{12}H_{15}ClN_4O_2$ + H: 283.0962, found 283.0962.

5.1.4. 1-[4-[2-[Bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholino-5,6-dihydro- pyrrolo[2,3-*d*]pyrimidin-7-yl] ethanone (10)

A suspension of **9** (2.00 g, 7.07 mmol), **14** (3.92 g, 8.49 mmol), $Pd(OAc)_2$ (32 mg, 0.141 mmol), 2-dicyclohexylphosphino-2',6'-dimethoxy-1,1′-biphenyl (S-Phos) (116 mg, 0.283 mmol) and K_3PO_4 (3.00 g, 14.2 mmol) in DMF (70 ml) was degassed under ultrasonic irradiation and stirred for 30 min at 100 °C in a nitrogen atmosphere. After cooling to ambient temperature, H_2O (200 ml) was added to afford precipitate. The precipitate was dissolved in CH_2Cl_2 and washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 50:1) to afford the titled compound as a white solid (4.26 g, quant.). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.99 (2H, s), 7.20 (4H, d, J = 8.8 Hz), 6.88 (4H, d, J = 8.3 Hz), 4.79 (4H, s), 3.93 (2H, t, J = 8.3 Hz), 3.72-3.68 (14H, m), 3.19 (2H, t, J = 8.3 Hz), 2.58 (3H, s); HRMS (ESI), m/z calcd for $C_{32}H_{35}N_7O_4$ + H: 582.2828, found 582.2842.

5.1.5. *N*,*N*-Bis[(4-methoxyphenyl)methyl]-5-(2-morpholino-6,7-dihydro-5H-pyrrolo[2,3-*d*]pyrimidin-4-yl)pyrimidin-2-amine (11)

To a solution of **10** (4.00 g, 6.86 mmol) in THF was added 5 M NaOH (69 ml) at ambient temperature. After being refluxed for 10 h, the reaction mixture was cooled to ambient temperature and neutralized by conc. HCl to afford precipitate. The precipitate was dissolved in CH_2Cl_2 and washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 50:1) to afford the titled compound as a white solid (3.16 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.88 (2H, s), 7.19 (4H, d, J = 8.3 Hz), 6.88 (4H, d, J = 8.3 Hz), 4.78 (4H, s), 3.73 (6H, s), 3.65-3.63 (8H, br m), 3.59-3.57 (2H, br m), 3.18 (2H, t, J = 7.8 Hz); HRMS (ESI), m/z calcd for $C_{30}H_{33}N_7O_3$ + H: 540.2723, found 540.2722.

5.1.6. 5-Bromo-*N*,*N*-bis[(4-methoxyphenyl)methyl]pyrimidin-2-amine (13)

To a suspension of 5-bromopyrimidin-2-amine (5.00 g, 28.7 mmol) and NaI (431 mg, 2.87 mmol) in DMF (60 ml) was added NaH (60% dispersion in mineral oil, 2.87 g, 71.8 mmol) at 0 °C. After stirring for 15 min at 0 °C, 1-(chloromethyl)-4-methoxy-benzene (8.57 ml, 63.2 mmol) was added at 0 °C. After stirring for 30 min at ambient temperature, the reaction mixture was neutralized by saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc, 1/0 to 9/1) to afford the titled compound as a white solid (10.9 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.49 (2H, s), 7.16 (4H, d, J = 8.8 Hz), 6.87 (4H, d, J = 8.8 Hz), 4.68 (4H, s), 3.72 (6H, s); HRMS (ESI), m/z calcd for $C_{20}H_{20}BrN_3O_2$ + H: 414.0817, found 414.0814.

5.1.7. *N*,*N*-Bis[(4-methoxyphenyl)methyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin -2-amine (14)

To a solution of **13** (5.85 g, 14.1 mmol) and triisopropyl borate (6.48 ml, 28.2 mmol) in toluene/THF = 4/1 (66 ml) was added *n*-BuLi (1.6 M in hexane, 10.6 ml, 28.2 mmol) at −78 °C. After stirring for 15 min at −78 °C, the reaction mixture was neutralized by saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure affording [2-[bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]boronic acid as a crude product. This crude product was used in the next reaction without further purification. To a suspension of the crude compound and $MgSO_4$ (6.79 g, 56.4 mmol) in 1,2-dichloroethane was added pinacol (3.33 g, 28.2 mmol). After stirring for 1.5 h at ambient temperature, the reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by flash column chromatography (*n*-hexane/EtOAc, 4/1) to afford the titled compound as a yellow solid (5.71 g, 88% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.53 (2H, s), 7.15 (4H, d, J = 8.6 Hz), 6.86 (4H, d, J = 8.6 Hz), 4.73 (4H, s), 3.72 (6H, s), 1.28 (12H, s); HRMS (ESI), m/z calcd for $C_{26}H_{32}BN_3O_4$ + H: 462.2564, found 462.2565.

5.1.8. 5-[2-Morpholino-7-(3-pyridyl)-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-4-yl]pyrimidin-2-amine (1)

A mixture of **11** (50 mg, 0.093 mmol), 3-bromopyridine (13 ul, 0.14 mmol), $Pd(OAc)_2$ (1.0 mg, 4.6 μmol), S-Phos (3.8 mg, 9.3 μmol), K_3PO_4 (39 mg, 0.19 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred at 100 °C for 8 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, diluted with EtOAc, washed with half-brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. This crude product and *N*-acetyl-cysteine (NAC) (30 mg, 0.185 mmol) were dissolved in TFA (1 ml) and the resulting reaction mixture was stirred at 70 °C for 3 h. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by amino silica gel flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 9/1) to afford the titled compound as a white solid (6.3 mg, 18% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.08 (1H, s), 8.82 (2H, s), 8.25-8.23 (2H, br m), 7.41 (1H, dd, J = 8.3, 4.9 Hz), 7.07 (2H, s), 4.12 (2H, t, J = 9.0 Hz), 3.71 (8H, d, J = 4.4 hz), 3.33 (2H, t, J = 9.0 Hz); HRMS (ESI), m/z calcd for $C_{19}H_{20}N_8O$ + H: 377.1838, found 377.1835.

5.1.9. [4-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]-2-pyridyl]-(4-methylpiperazin-1-yl)methanone (15)

Compound **15** was prepared from **11** and **19** by following the same procedure as described for **1** (yellow solid, 38% in 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.89 (2H, s), 8.47 (1H, d, J = 6.0 Hz), 8.09 (1H, dd, J = 6.0, 2.1 Hz), 7.79 (1H, d, J = 1H, d, J = 2.1 Hz), 5.28 (2H, s), 4.12 (2H, t, J = 8.8 Hz), 3.85 (10H, m), 3.69 (2H, m), 3.33 (2H, t, J = 8.8 Hz), 2.57 (2H, m), 2.47 (2H, m), 2.36 (3H, s); HRMS (ESI), m/z calcd for $C_{25}H_{30}N_{10}O_2$ + H: 503.2631, found 503.2628.

5.1.10. [5-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]-2-pyridyl]-(4-methylpiperazin-1-yl)methanone (16)

A mixture of **11** (50 mg, 0.093 mmol), **21** (32 mg, 0.11 mmol), $Pd_2(dba)_3$ (4.2 mg, 4.6 μmol), S-Phos (7.6 mg, 19 μmol), K_3PO_4 (39 mg, 0.19 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred at 100 °C for 4 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, diluted with CH_2Cl_2, washed with saturated aqueous NH_4Cl, H_2O. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. This crude product and *N*-acetyl-cysteine (NAC) (30 mg, 0.185 mmol) was dissolved in TFA (1 ml) and the resulting reaction mixture was stirred at 70 °C for 4 h. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by amino silica gel flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 33/1) to afford the titled compound as a white solid (27 mg, 58% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.05 (1H, d, J = 2.0 Hz), 8.83 (2H, s), 8.38 (1H, dd, J = 9.0, 2.0 Hz), 7.65 (1H, d, J = 9.0 Hz), 7.13 (2H, s), 4.15 (2H, t, J = 8.1 Hz), 3.71 (8H, br s), 3.59 (4H, m), 3.34 (2H, t, J = 8.1 Hz), 2.33 (4H, m), 2.20 (3H, s); HRMS (ESI), m/z calcd for $C_{25}H_{30}N_{10}O_2$ + H: 503.2631, found 503.2627.

5.1.11. [5-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]-3-pyridyl]-(4-methylpiperazin-1-yl)methanone (17)

A mixture of **11** (50 mg, 0.093 mmol), **23** (40 mg, 0.14 mmol), $Pd_2(dba)_3$ (4.3 mg, 4.7 μmol), X-Phos (8.9 mg, 19 μmol), K_3PO_4 (39 mg, 0.19 mmol) in DMF (2 ml) was stirred at 100 °C for 18 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, diluted with CH_2Cl_2, washed with saturated aqueous NH_4Cl, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. This crude product and *N*-acetyl-cysteine (NAC) (30 mg, 0.185 mmol) was dissolved in TFA (2 ml) and the resulting reaction mixture was stirred at 70 °C for 3 h. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by amino silica gel flash column chromatography (CH_2Cl_2/MeOH/8 M NH_3 in MeOH, 30/1/0 to 10/1/0, then 50/5/1) to afford the titled compound as a light yellow solid (42 mg, 91% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.18 (1H, s), 8.83 (2H, s), 8.26 (2H, s), 7.13 (2H, s), 4.14 (2H, t, J = 8.0 Hz), 3.71 (8H, s), 3.34 (10H, s), 2.40 (3H, s); HRMS (ESI), m/z calcd for $C_{25}H_{30}N_{10}O_2$ + H: 503.2631, found 503.2631.

5.1.12. (4-Chloro-2-pyridyl)-(4-methylpiperazin-1-yl)methanone (19)

To a solution of 4-chloropyridine-2-carboxylic acid (18, 400 mg, 2.54 mmol), HOBt (686 mg, 5.08 mmol) and 1-methyl piperazine (0.309 ml, 2.79 mmol) in CH_2Cl_2 was added EDC (584 mg, 3.05 mmol). After stirring for 30 min at ambient temperature, the reaction mixture was diluted with saturated $NaHCO_3$ and extracted with EtOAc (3 times). The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 9/1) to afford the titled compound as a brown oil (506 mg, 83%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.57 (1H, d, J = 5.4 Hz), 7.71-7.71 (1H, m), 7.65-7.63 (1H, m), 3.64 (2H, t, J = 5.1 Hz), 3.35 (2H, t, J = 5.1 Hz), 2.38 (2H, t, J = 5.1 Hz), 2.28 (2H, t, J = 5.1 Hz), 2.20 (3H, s); HRMS (ESI), m/z calcd for $C_{11}H_{14}ClN_3O$ + H: 240.0903, found 240.0902.

5.1.13. (5-Bromo-2-pyridyl)-(4-methylpiperazin-1-yl)methanone (21)

Compound **21** was prepared from 5-bromopyridine-2-carboxylic acid by following the same procedure as described for **19** (brown oil, 28%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.73 (1H, dd, J = 2.4, 1.0 Hz), 8.19 (1H, dd, J = 8.3, 2.4 Hz), 7.55 (1H, d, J = 7.8 Hz), 3.63 (2H, t, J = 5.1 Hz), 3.36 (2H, t, J = 5.1 Hz), 2.37 (2H, t, J = 5.1 Hz), 2.27 (2H, t, J = 5.1 Hz), 2.19 (3H, s); HRMS (ESI), m/z calcd for $C_{11}H_{14}BrN_3O$ + H: 284.0398, found 284.0393.

5.1.14. (5-Bromo-3-pyridyl)-(4-methylpiperazin-1-yl)methanone (23)

Compound **23** was prepared from 5-bromopyridine-3-carboxylic acid by following the same procedure as described for **19** (yellow solid, 78%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.79 (1H, d, J = 2.0 Hz), 8.59 (1H, d, J = 1.5 Hz), 8.12 (1H, t, J = 2.2 Hz), 3.62 (2H, br s), 3.34 (2H, br s), 2.37 (2H, br s), 2.28 (2H, br s), 2.20 (3H, s); HRMS (ESI), m/z calcd for $C_{11}H_{14}BrN_3O$ + H: 284.0398, found 284.0395.

5.1.15. 5-(2-Morpholino-7-phenyl-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-4-yl)pyrimidin-2-amine (24)

Compound **24** was prepared from **11** and bromobenzene by following the same procedure as described for **1** (yellow solid, 80% in 2 steps). ^{1}H NMR (400 MHz, d-TFA) δ: 9.10 (2H, s), 7.59-7.42 (4H, m), 7.35 (1H, t, J = 7.7 Hz), 4.41 (2H, t, J = 7.4 Hz), 4.08-3.89 (8H, m), 3.31 (2H, t, J = 7.4 Hz); HRMS (ESI), m/z calcd for $C_{20}H_{21}N_7O$ + H: 376.1886, found 376.1882.

5.1.16. Methyl 3-[4-[2-[bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]benzoate (25)

A mixture of **11** (100 mg, 0.19 mmol), 3-bromobenzoic acid methyl ester (48 mg, 0.22 mmol), $Pd(OAc)_2$ (2.1 mg, 9.3 μmol), S-Phos (7.6 mg, 19 μmol), K_3PO_4 (79 mg, 0.37 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred at 100 °C

for 3 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature and H_2O was added. The resulting filter cake was washed by H_2O, *n*-hexane/EtOAc = 2/1 to afford the titled compound as a yellow solid (108 mg, 86%). The residue was used in the next reaction without further purification.

5.1.17. 3-[4-[2-[Bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholino-5,6-dihydro-pyrrolo[2,3-*d*]pyrimidin-7-yl]benzoic acid (26)

A mixture of **25** (60 mg) in THF/5 M NaOH/30%-H_2O_2 aq = 4/2/1 (1.4 ml) was refluxed for 48 h. The reaction mixture was then cooled to ambient temperature, acidified with 6 M HCl aq. The resulting filter cake was washed by H_2O, *n*-hexane/EtOAc = 2/1 to afford the titled compound as a yellow solid (56 mg, 95%). The residue was used in the next reaction without further purification.

5.1.18. [3-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]phenyl]-(4-methylpiperazin-1-yl)methanone (27)

To a solution of **26** (38 mg, 0.058 mmol), HOBt (8.0 mg, 0.059 mmol), 1-methyl piperazine (12 mg, 0.117 mmol) and EDC (17 mg, 0.087 mmol) in DMF (1 ml) was added DIPEA (9.8 μl, 0.056 mmol). After stirring for 90 min at 60 °C, 1-methyl piperazine (12 mg, 0.117 mmol) and EDC (17 mg, 0.087 mmol) was added. After stirring for 90 min at 60 °C, the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O, then concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH) to afford [3-[4-[2-[bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholin-4-yl-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]phenyl]-(4-methylpiperazin-1-yl)methanone (31 mg, 71%). This residue was dissolved in TFA (1 ml). After being refluxed for 6 h, TFA was evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/ MeOH, 30/1 to 10/1) to afford the titled compound as a yellow solid (20 mg, 96%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.82 (2H, s), 8.02 (1H, d, J = 8.1 Hz), 7.87 (1H, s), 7.50 (1H, dd, J = 8.2, 7.6 Hz), 7.18-7.04 (3H, m), 4.11 (2H, *t*, 7.9 Hz), 3.79-3.65 (8H, m), 3.43-2.99 (10H, m), 2.83 (3H, s); HRMS (ESI), m/z calcd for $C_{26}H_{31}N_9O_2$ + H: 502.2679, found 502.2681.

5.1.19. [4-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]phenyl]-(4-methylpiperazin-1-yl)methanone (28)

Compound **24** was prepared from **11** and **30** by following the same procedure as described for **1** (white solid, 87% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.80 (2H, s), 7.89 (2H, d, J = 8.8 Hz), 7.42 (2H, d, J = 8.8 Hz), 7.04 (2H, s), 4.10 (2H, t, J = 8.3 Hz), 3.74-3.65 (8H, m), 3.55-3.45 (4H, br m), 3.45-3.42 (2H, m), 2.34-2.27 (4H, br m), 2.18 (3H, s); HRMS (ESI), m/z calcd for $C_{26}H_{31}N_9O_2$ + H: 502.2679, found 502.2683.

5.1.20. (4-Bromophenyl)-(4-methylpiperazin-1-yl)methanone (30)

Compound **30** was prepared from 4-bromobenzoic acid by following the same procedure as described for **19** (white solid, 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.64 (2H, dt, J = 8.6, 2.1 Hz), 7.34 (2H, dt, J = 8.6, 2.1 Hz), 3.59 (2H, br s), 3.30 (2H, br s), 2.34 (2H, br s), 2.27 (2H, br s), 2.19 (3H, s); HRMS (ESI), m/z calcd for $C_{12}H_{15}BrN_2O$ + H: 283.0446, found 283.0445.

5.1.21. 4-[4-[2-[Bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholino-5,6-dihydro-pyrrolo[2,3-*d*]pyrimidin-7-yl]-3-fluoro-benzoic acid (31)

A mixture of **11** (100 mg, 0.185 mmol), 4-bromo-3-fluorobenzoic acid (48.7 mg, 0.222 mmol), $Pd_2(dba)_3$ (4.24 mg, 4.63 μmol), X-Phos (8.83 mg, 18.5 μmol), K_3PO_4 (118 mg, 0.556 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred at 100 °C for 24 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, and then 1 M HCl (6 ml) was added. After being stirred for 1 hour at ambient temperature, the resulting filter cake was washed by H_2O, *n*-hexane/EtOAc = 1/1 to afford the titled compound as a light yellow solid (101 mg, 80%). The residue was used in the next reaction without further purification.

5.1.22. [4-[4-(2-Aminopyrimidin-5-yl)-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl]-3-fluoro-phenyl]-morpholino-methanone (3)

To a solution of **31** (120 mg, 0.18 mmol), HOBt (24 mg, 0.15 mmol) and morpholine (31 μl, 0.35 mmol) in CH_2Cl_2 (4 ml) was added EDC (68 mg, 0.35 mmol) at ambient temperature. After stirring for 1 hour at ambient temperature, H_2O was added. The organic layer was separated and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude product was dissolved in TFA (2 ml) and the resulting reaction mixture was refluxed for 4 h. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 9/1) to afford the titled compound as a white solid (86 mg, 96% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.81(2H, s), 7.75 (1H, t, J = 8.1 Hz), 7.41 (1H, m), 7.29 (1H, m), 7.09 (2H, s), 4.13-4.06 (2H, m), 3.71-3.45 (10H, m), 3.36-3.33 (8H, m); ^{13}C NMR (d-TFA) δ: 171.0, 165.8, 158.0 (d, J = 238.0 Hz), 156.8, 155.4, 154.6, 136.2, 133.6 (d, J = 6.9 Hz), 127.4, 127.1 (d, J = 12.2 Hz), 123.6 (d, J = 3.1 Hz), 116.4, 114.0 (d, J = 31.3 Hz), 66.1, 65.4, 52.0, 48.0, 44.6, 43.3, 21.6; HRMS (ESI), m/z calcd for $C_{25}H_{27}FN_8O_3$ + H: 507.2268, found 507.2280.

5.1.23. 4-[4-[2-[Bis[(4-methoxyphenyl)methyl]amino]pyrimidin-5-yl]-2-morpholino-5,6-dihydro-pyrrolo[2,3-*d*]pyrimidin-7-yl]benzaldehyde (32)

A mixture of **11** (300 mg, 0.556 mmol), 2-(4-boromopheyl)-1,3-dioxolane (178 ul, 0.778 mmol), $Pd_2(dba)_3$ (13 mg, 14 μmol), X-Phos (27 mg, 56 μmol), K_3PO_4 (236 mg, 1.11 mmol) in DMF (5 ml) was degassed under ultrasonic irradiation and stirred at 100 °C for 14 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, diluted with EtOAc and washed with saturated aqueous NH_4Cl. The collected organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next reaction without further purification. To a solution of the residue in THF (6 ml) was added 1 M HCl aq. (2 ml). After stirring for 3 h at ambient temperature, the reaction mixture was diluted with saturated aqueous NH_4Cl and extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/ MeOH, 1/0 to 19/1) to afford the titled compound as a brown solid (345 mg, 96% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.87 (1H, s), 8.99 (2H, s), 8.06 (2H, d, J = 8.8 Hz), 7.91 (2H, d, J = 8.8 Hz), 7.21 (4H, d, J = 8.3 Hz), 6.89 (4H, d, J = 8.3 Hz), 4.79 (4H, s), 4.14 (2H, t, J = 8.2 Hz), 3.77-3.65 (16H, brm); HRMS (ESI), m/z calcd for $C_{37}H_{37}N_7O_4$ + H: 644.2985, found 644.2987.

5.1.24. 5-[7-[4-[(4-Methylpiperazin-1-yl)methyl]phenyl]-2-morpholino-5,6-dihydro-pyrrolo[2,3-*d*]pyrimidin-4-yl]pyrimidin-2-amine (2)

The mixture of **32** (76 mg, 0.118 mmol), *N*-methylpiperazine (40 μl, 0.354 mmol), $NaBH(OAc)_3$ (100 mg, 472 mmol) and AcOH (13 μl, 0.236 mmol) in CH_2Cl_2 (2 ml) was stirred for 40 h at ambient temperature. The mixture was diluted with saturated aqueous

NH_4Cl and extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/2 M NH_3 in MeOH) to afford *N*,*N*-bis(4-methoxybenzyl)-5-(7-(4-((4-methylpiperazin-1-yl)methyl)phenyl)-2-morpholino-6,7-dihydro-5H-pyrrolo[2,3-*d*] pyrimidin-4-yl)pyrimidin-2-amine (44 mg, 51%). To a solution of this residue in TFA (1 ml) was added NAC (20 mg, 0.120 mmol). After being refluxed for 4 h, TFA was evaporated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/2 M NH_3 in MeOH, 30/1 to 10/1) to afford the titled compound as a yellow solid (26 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.81 (2H, s), 7.82 (2H, d, J = 8.3 Hz), 7.33 (2H, d, J = 8.3 Hz), 7.08 (2H, s), 4.08 (2H, t, J = 7.3 Hz), 3.70 (4H, s), 3.56 (2H, s), 3.34 (10 H, br s), 3.00 (4H, s), 2.63 (3H, s); HRMS (ESI), m/z calcd for $C_{26}H_{33}N_9O$ + H: 488.2886, found 488.2881.

5.1.25. 5-[2-Morpholino-7-[4-(morpholinomethyl)phenyl]-5,6-dihydropyrrolo[2,3-*d*]pyrimidin-4-yl]pyrimidin-2-amine (33)

Compound **33** was prepared from **32** and morpholine by following the same procedure as described for **2** (light brown solid, 50% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.82 (2H, s), 7.95 (2H, d, J = 8.6 Hz), 7.51 (2H, d, J = 8.6 Hz), 7.09 (2H, s), 4.27 (2H, s), 4.11 (2H, s), 3.68-3.55 (14H, m), 3.35 (2H, s), 3.19 (2H, s); HRMS (ESI), m/z calcd for $C_{25}H_{30}N_8O_2$ + H: 475.2570, found 475.2572.

5.1.26. 4-(4-(2-(Bis(4-methoxybenzyl)amino)pyrimidin-5-yl)-2-morpholino-5H-pyrrolo[2,3-*d*]pyri-midin-7(6H)-yl)-3-fluorobenzaldehyde (34)

A mixture of **11** (50 mg, 0.093 mmol), 4-bromo-3-fluorobenzaldehyde (23 ul, 0.11 mmol), $Pd(OAc)_2$ (1.0 mg, 4.6 μmol), X-Phos (4.4 mg, 9.3 μmol), K_3PO_4 (39 mg, 0.19 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred at 100 °C for 3 h under a nitrogen atmosphere. The reaction mixture was then cooled to ambient temperature, diluted with EtOAc, washed with half-brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (CH_2Cl_2/MeOH, 1/0 to 19/1) to afford the titled compound as a yellow solid (40 mg, 65%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.94 (1H, d, J = 1.5 Hz), 8.99 (2H, s), 8.00 (1H, t, J = 7.9 Hz), 7.81 (1H, s), 7.78 (1H, t, J = 1.8 Hz), 7.21 (4H, d, J = 8.6 Hz), 6.89 (4H, d, J = 8.6 Hz), 4.79 (4H, s), 4.17 (2H, t, J = 7.8 Hz), 3.73 (6H, s), 3.67-3.60 (8H, m), 3.37 (2H, t, J = 7.8 Hz); ^{13}C NMR (DMSO-d_6) δ:191.5, 166.4, 161.8, 161.2, 158.9, 158.0, 156.8, 154.3, 153.1, 134.4, 134.3, 133.7, 133.7, 130.2, 129.2, 126.6, 126.5, 125.1, 125.1, 120.6, 117.2, 117.0, 114.4, 105.4, 66.5, 55.5, 50.9, 50.8, 48.9, 44.8, 25.3; HRMS (ESI), m/z calcd for $C_{37}H_{36}FN_7O_4$ + H: 662.2891, found 662.2896.

5.1.27. 5-[7-[2-Fluoro-4-(morpholinomethyl)phenyl]-2-morpholino-5,6-dihydropyrrolo[2,3-*d*]pyri-midin-4-yl]pyrimidin-2-amine (35)

Compound **35** was prepared from **34** and morpholine by following the same procedure as described for **2** (light brown solid, 52% in 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.80 (2H, s), 7.58 (1H, t, J = 8.1 Hz), 7.23 (1H, d, J = 12.2 Hz), 7.17 (1H, d, J = 8.1 Hz), 7.05 (2H, s), 4.02 (2H, t, J = 7.8 Hz), 3.59 (12H, br s), 3.48 (2H, s), 3.31 (2H, t, J = 7.8 Hz), 2.38 (4H, br s); HRMS (ESI), m/z calcd for $C_{25}H_{29}FN_8O_2$ + H: 493.2476, found 493.2472.

5.2. In vitro kinase enzyme assay

The inhibitory activity on PI3Kα (p110α/p85α) (Life Technologies) was determined by Adapta Universal Kinase Assay Kit with PIP2:PS Lipid Kinase Substrate (Life Technologies).

5.3. LM stability assay

1 μM of each compound was incubated with human (or mouse) LM (0.5 mg protein/mL) in 50 mM phosphate buffer (pH 7.4) containing 1 mM NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) at 37 °C for 30 min. After the enzyme reaction was terminated with the addition of a three-fold volume of acetonitrile, the reaction mixture was centrifuged at 1500 rpm for 10 min. The resultant supernatant was used as a test sample to measure the stability in human (or mouse) LM by measuring the compound in the sample using LC–MS/MS.

5.4. Permeability assay

10 mM stock solutions of each compounds were diluted with donor buffer. The resulting mixture was checked for precipitation and then filtered. The donor plate was filled with the resulting filtrate. The filter plate was coated with phosphor-lipids and placed into the donor plate, and was then filled with acceptor buffer. After a certain time, donor and acceptor concentration was analyzed by UV. A donor buffer of 0.05 M MOPSO (3-(*N*-morpholino)-2-hydroxy-1-propanesulfonic acid) containing 0.01 M GCA (glycocholic acid) was used (pH = 6.5). An acceptor buffer of 0.05 M MOPSO was used (pH = 6.5). Permeability of PAMPA is calculated by using the following equation:

$$P_e = \frac{V_D}{A\ M_D(0)}\left(\frac{\Delta M_A}{\Delta t}\right)$$

P_e = Permeations coefficient (10^{-6} cm/s)
V_D = Volumes in donor (cm^3)
A = Surface area of the film (cm^2)
$M_D(0)$ = Concentration in donor (mol/cm^3)
ΔM_A = Concentration in acceptor (mol/cm^3)
Δt = Permeation times (s)

5.5. Solubility assay

An aliquot of 50 μl of 4 mM or 1 mM sample in dimethylsulfoxide (DMSO) was freeze-dried to remove DMSO. To the resulting residue was added 50 μM of FaSSIF (pH = 6.5), which was then irradiated ultrasonically for 10 min, shaken for 2 h, centrifuged for 10 min (3000 rpm), and filtered by Whatman Unifilter. Concentration of the filtrate was analyzed by HPLC-UV based on the calibration curve of each sample. Composition of FaSSIF: Sodium taurocholate (1.61 g), Lecithin (0.59 g), KH_2PO_4 (3.9 g), KCl (7.7 g) and NaOH (pH 6.5) per 1 L.

5.6. Pharmacokinetic study in mice and monkeys

Pharmacokinetic study: Female BALB/c-nu mice and male cynomolgus monkeys (n = 2–3 per treatment group) were given the solutions of test compounds by intravenous (iv) or oral (po) route at doses of 10 mg/kg (iv) and 50 or 100 mg/kg (po) in mice, and 2.5 or 5 mg/kg (iv) and 10 mg (po) in monkeys. Blood samples of each animal were collected with heparin as an anticoagulant at 0.08, 0.25, 1, 2, 4, 7, and 24 h following iv dosing and at 0.5, 1, 2, 4, 7, and 24 h following po dosing. Samples were centrifuged to obtain plasma and stored at −80 °C until analysis. Plasma concentrations were determined by using LC–MS/MS system. The pharmacokinetic parameters were calculated by non-compartmental analysis using WATSON ver. 7.1 (Thermo Fisher Scientific, Wayne, PA).

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