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Optimization of the phenylurea moiety in a phosphoinositide 3-kinase (PI3K) inhibitor to improve water solubility and the PK profile by introducing a solubilizing group and ortho substituents

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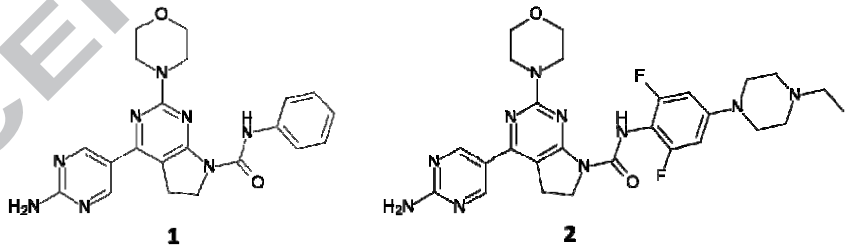
ABSTRACT
Phosphoinositide 3-kinase (PI3K) is a promising anti-cancer target, because various mutations and amplifications are observed in human tumors isolated from cancer patients. Our dihydropyrrolopyrimidine derivative with a phenylurea moiety showed strong PI3K enzyme inhibitory activity, but its pharmacokinetic property was poor because of lack of solubility. Herein, we report how we improved the solubility of our PI3K inhibitors by introducing a solubilizing group and ortho substituents to break molecular planarity.

1. Introduction

Phosphoinositide 3-kinases (PI3Ks) are lipid kinases that control various functions, including cell survival, gene expression, and metabolism.[1] The PI3K family comprises classes IA, IB, II, and III, and class IA PI3Ks phosphorylate phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) to generate phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P3),[2] which is an important lipid second messenger that activates serine/threonine kinase AKT, by which many cellular processes are regulated.[1] In addition, numerous cases of oncogenic gene mutations and gene amplification of class IA PI3Ks have been reported in human cancer.[2, 3, 4] Furthermore, efficient inhibition of PI3K{ (an isozyme of class IA) in PTEN-negative cancer cell lines was also reported.[5] Thus, PI3Ks are promising cancer therapeutic targets; several small molecule inhibitors are now being developed in clinical trials,[6] and idelalisib was approved as a selective inhibitor of PI3Kδ(another isozyme of class IA) in 2014.[7]

We have previously reported lead identification and optimization of our dihydropyrrolopyrimidine PI3K inhibitors.[8, 9] A lead compound was identified by superimposing Piramed's PI3K inhibitor PI103 and Chiron's inhibitor. Based on information from a docking simulation, the metabolically unstable phenol of our lead compound was replaced by aminopyrimidine, and strong in vivo antitumor efficacy was observed. During our lead optimization process, we found that the phenylurea derivative (**1**) also shows strong PI3K enzyme inhibitory activity, but its solubility was quite low and pharmacokinetic profiles were poor. In this report, we describe how we improved the low solubility by introducing a solubilizing group and breaking the molecular planarity.[10] After the optimization, compound **2** showed much better AUC (p.o.) in mice and showed strong tumor growth inhibition in vivo against PC-3 xenograft model.

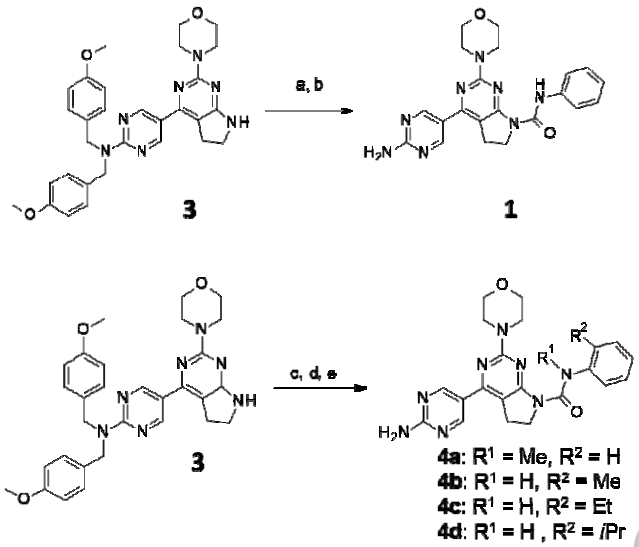


1 **2**

Figure 1. PI3K inhibitors of phenylurea derivatives

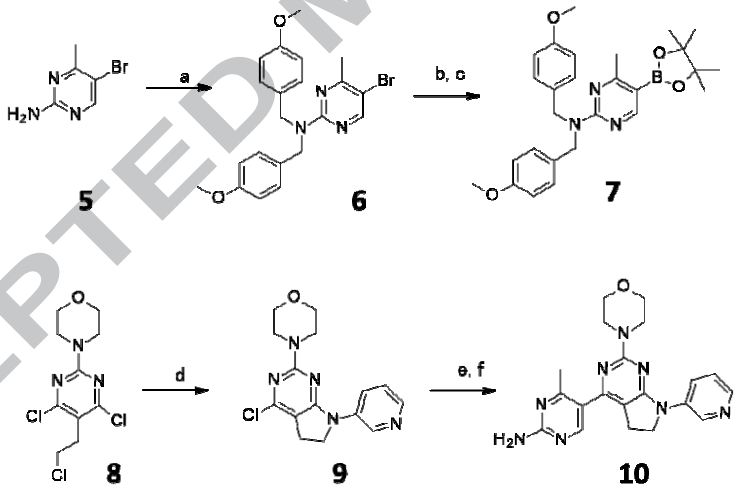
2. Chemistry

Compound **1** was prepared by the urea formation of dihydropyrrolopyrimidine derivative **3** [11] with phenyl isocyanate in the presence of triethylamine (TEA), followed by deprotection of paramethoxybenzyl (PMB) under an acidic condition (Scheme 1). Compounds **4a**–**4d** were synthesized from the same key intermediate **3**. First, compound **3** was treated with triphosgene to afford a carbamoyl chloride intermediate. The resulting crude mixture was treated with four different aniline derivatives to give corresponding phenylurea derivatives. Deprotection of PMB by trifluoroacetic acid (TFA) in the presence of N-acetylcysteine as a cation trap reagent gave compounds **4a**–**4d,** respectively.



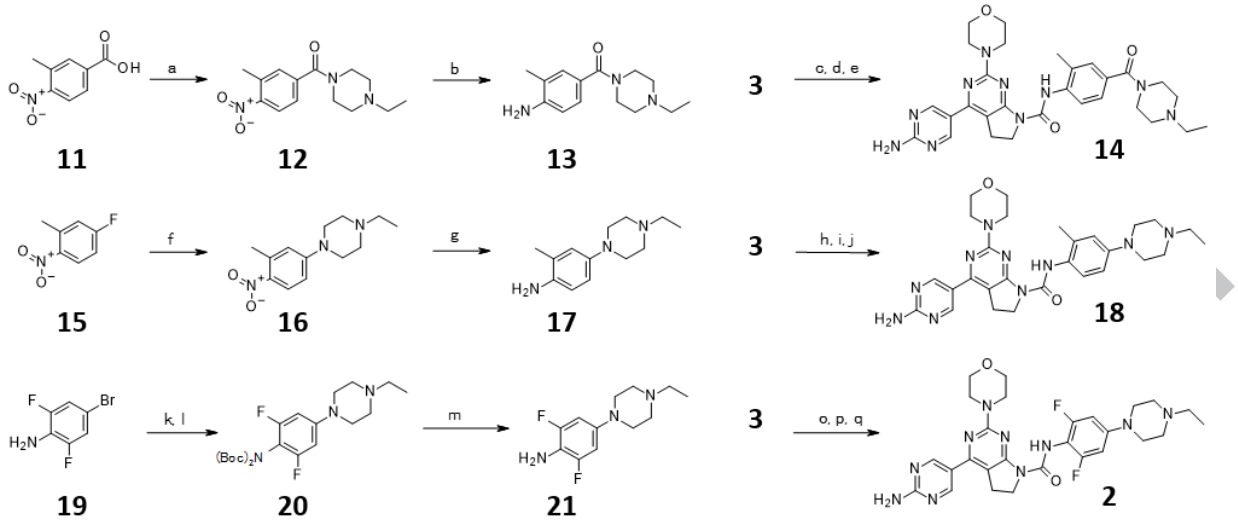
Scheme 1. Preparation of phenyl urea derivatives**.** Reagents and conditions: (a) phenyl isocyanate, TEA, DCE, reflux, 51%; (b) TFA, conc.H_2SO_4, 40 ˚C, 82%; (c) triphosgene, pyridine, DCM, rt; (d) aniline, DCM, rt; (e) TFA, N-acetylcysteine, reflux, 49% in 3 steps (**4a**), 68% in 3 steps (**4b**), 56% in 3 steps (**4c**), 57% in 3 steps (**4d**).

Synthesis of ortho methyl aminopyrimidine derivative **10** is described in Scheme 2. Based on our knowledge of preparing another aminopyrimidine derivative,[11] 2-amino-5-bromo-4-methylpyrimidine (**5**) was protected by PMB. After the boronic acid formation using *n*BuLi and triisopropyl borate, the resulting boronic acid was converted to boronic acid pinacol ester **7** by treatment with pinacol and Mg_2SO_4. A coupling partner **9** was prepared from trichloride **8** [7]. 3-aminopyridine was treated with NaH and coupled with trichloride **8**. In situ cyclization afforded the desired dihydropyrrolopyrimidine derivative **9**. Suzuki-Miyaura coupling of **7** and **9** under conventional conditions, and the subsequent deprotection of PMB gave **10**.



Scheme 2. Preparation of compound **10.** Reagents and conditions: (a) 4-methoxybenzyl chloride, NaH, NaI, THF, rt, 79%; (b) B(O*i*Pr)$_3$, *n*BuLi, toluene/THF, –78 ˚C; (c) pinacol, $MgSO_4$, DME, rt, 78% in 2 steps; (d) 3-aminopyridine, NaH, THF, reflux, 67%; (e) **7**, $Pd(OAc)_2$, S-Phos, K_3PO_4, DMF, 100 ˚C; (f) TFA, conc.H_2SO_4, 40 ˚C, 43% in 2 steps.

Preparation of ortho-substituted phenylurea derivatives with a solubilizing group is shown in Scheme 3. Amidation of 3-methyl-4-nitrobenzoic acid **11** with N-ethylpiperazine in the presence of a condensation reagent gave **12**. Following hydrogenation of the nitro group by Pd/C under a H_2 gas atmosphere afforded the desired aniline derivative **13**. 4-Fluoro-2-methyl-nitrobenzene **15** was treated with N-ethylpiperazine in the presence of K_2CO_3 in DMSO at 80 ˚C to afford **16**. Then, the nitro group was reduced by zinc to give **17**. 4-Bromo-2,6-difluoro-aniline was treated with $(Boc)_2O$ and a catalytic amount of DMAP to protect the amino group by Boc. Following the Buchwald-Hartwig reaction using N-ethylpiperazine under conventional coupling conditions gave **20**. Deprotection of Boc under an acidic condition afforded the desired aniline derivative **21**. These aniline derivatives **13**, **17**, and **21** were treated with carbamoyl chloride of **3**, which was prepared by a method similar to that described above, and deprotection of PMB gave **14**, **18**, and **2**, respectively.

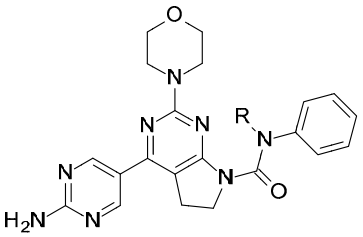


Scheme 3. Preparation of phenyl urea derivatives with a solubilizing group. Reagents and conditions: (a) N-ethylpiperazine, EDC•HCl, DMAP, DCM, rt, 79%; (b) Pd/C, H_2 gas, MeOH, rt, 96%; (c) triphosgene, pyridine, DCM, rt; (d) **13**, DCM, rt; (e) TAF, N-acetylcysteine, reflux, 19% in 3 steps; (f) N-ethylpiperazine, K_2CO_3, DMSO, 80 ˚C; (g) Zn, NH_4Cl, MeOH, rt, 90% in 2 steps; (h) phosgene, DCM/sat.$NaHCO_3$ aq. = 1/1, rt; (i) **17**, TEA, DCE, rt; (j) TFA, reflux, 76% in 3 steps; (k) $(Boc)_2O$, DMAP, DMF, rt; (l) N-ethylpiperazine, $Pd(OAc)_2$, S-Phos, *t*BuOK, toluene, 60 ˚C, 20% in 2 steps; (m) 6M HCl/EtOAc, rt, 91%; (o) triphosgene, pyridine, DCM, rt; (p) **21**, DCM, rt; (q) TAF, N-acetylcysteine, reflux, 52% in 3 steps.

3. Result and Discussion

Previously, we have reported our dihydropyrrolopyrimidine PI3K inhibitor **22** (IC_{50} for PI3Kαis 0.033 μM) and described its hydrogen bonding patterns with PI3Kαaccording to an X-ray crystal structure of PI3Kγ[6,7]. We have revealed that morpholine of compound **22** interacts with Val851 of the hinge region, that aminopyrimidine makes a key interaction with both Asp805 and Lys802, and that pyridine is located at the solvent-exposed region of PI3Kα which allows the pyridine part to be used for various structural modifications without significant loss of inhibitory activity [11]. On the other hand, the phenylurea derivative **1** inhibited PI3Kαwith an IC_{50} value of 0.013 μM (Table 1). This enhanced inhibitory activity could be explained from the docking simulation of compound **1** with the PI3KαX-ray structure. As shown in Figure 2, phenyl group can be expected to make a favorite lipophilic interaction with W780 (the distance is somewhat far for good interaction) and cation-πinteraction with R770. We could find the side chain of R770 near the phenyl ring of compound **1** in two PI3KαX-ray structures (PDB: 3ZIM, 4A55) but we could not find the side chain of R770 at the phenyl ring side in the other two PI3KαX-ray structures (PDB: 4L23, 4L2Y). Thus the amino acid side chain of R770 is flexible and induced fit of this side chain could be expected for our phenylurea derivatives. Compound **22** can also make these interactions using the pyridine part, but the pyridine of compound **22** is relatively far from R770. Thus, the urea linker would place the aromatic ring close enough to R770. The importance of the aromatic ring position is also suggested by compound **4a**, which has a methylated nitrogen atom in the urea. As shown in Table 1, the enzyme inhibitory activity of **4a** was 10 times weaker than that of compound **1**, indicating that the phenyl ring of **4a** is not positioned suitably to interact with R770 and W780. This methyl group was introduced in an attempt to improve the quite low solubility and poor pharmacokinetics profiles of **1**. As described in Table 1, the solubility of compound **1** in FaSSIF (fasted state simulated intestinal fluid)[12] was extremely low and pharmacokinetic profiles in mice were poor. One reason for this low water solubility would be the highly planar structure of compound **1**. As shown in Figure 3, intramolecular hydrogen bonding of urea NH with the pyrimidine hetero atom would induce a flat configuration that increases the crystallization energy and results in poor solubility. Although we tried breaking this intramolecular hydrogen bond to reduce the crystallization energy in N-methylated compound **4a**, this methylation did not improve the solubility, as Table 1 shows.

Table 1
In vitro and in vivo profiles of phenylurea derivatives



Compound	R	PI3Kα IC_{50} (μM)	FaSSIF (μg/ml)	Mouse PK (50 mg/kg po)	
				Cmax (ng/mL)	AUC (ng·h/mL)
1	H	0.013	<3	104	437
4a	Me	0.13	<3	N.T.	N.T.

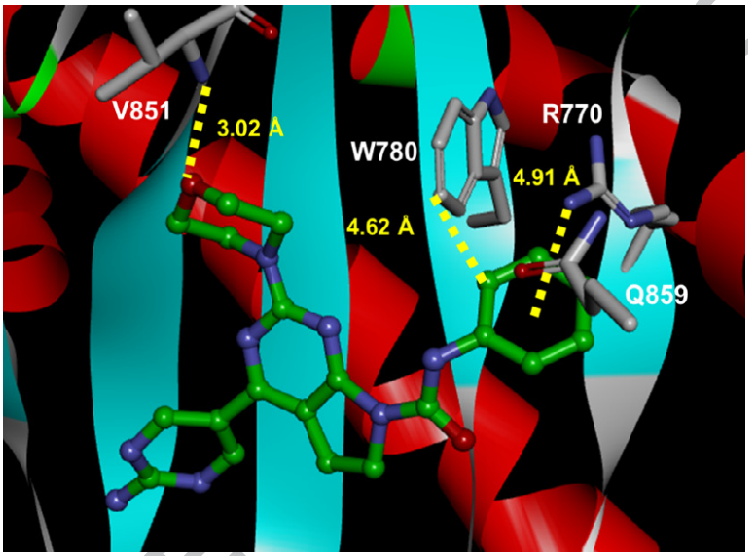


Figure 2. Docking simulation of phenylurea compound **1** with PI3Kα Based on the X-ray structure information of PI3Kα(PDB: 3ZIM) and the X-ray structure of our dihydropyrrolopyrimidine PI3K inhibitor with PI3Kγ(PDB: 3APD), compound **1** (green) was docked with PI3Kα X-ray structure (ribbon). V851 and amino acid side chains of R770 and W780 were shown in stick form.

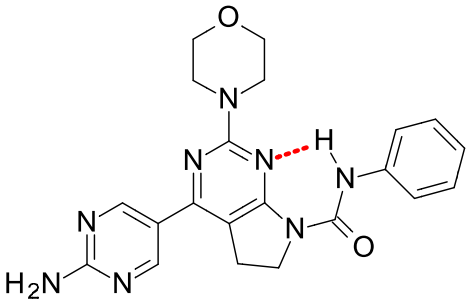
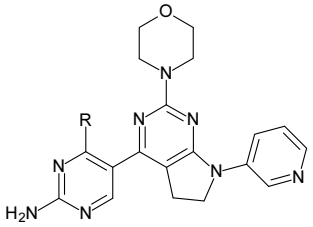


Figure 3. Estimated intramolecular hydrogen bonding

Ishikawa and Hashimoto reported that disrupting the molecular planarity by introducing an ortho substituent can improve the solubility because its decreases the efficiency of the crystal packing.[10] Thus, we introduced an ortho methyl group on the biaryl moiety of our inhibitor, and compound **10** showed good FaSSIF solubility (Table 2). But the IC_{50} value of compound **10** against PI3Kαwas decreased 8-fold compared to compound **1**, because (as mentioned above) both aminopyrimidine and morpholine are essential for making key interactions with the PI3K enzyme, and disrupting the planarity at this point was not acceptable for keeping these interactions. As changes in the dihedral angle at the aminopyrimidine part resulted in a loss of inhibitory activity, ortho subsutituents were introduced on the aromatic ring of the phenylurea. In the case of phenylurea compounds, alkyl substituents at the ortho position could be

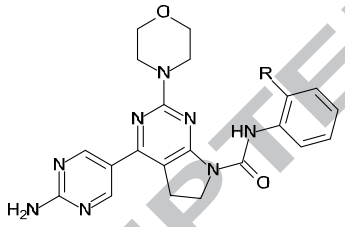
placed opposite the urea carbonyl to avoid steric repulsion. As shown in Figure 4a, the most stable structure of ortho methyl phenylurea derivatives without morpholine, as calculated at B3LYP/6-31+G(d,p) level, [13] is a flat conformation; however, the most stable structure in the ortho methyl phenylurea derivatives with morpholine (ϕ=33˚, Figure 4b) was a twisted conformation that avoided the steric repulsion between ortho methyl and the methylene of morpholine. As summarized in Table 3, the enyzme inhibitory activity of ortho methylated compound **4b** was decreased 4-fold (IC_{50}=0.052 μM). In the case of an ethyl group, which is larger than a methyl group, the IC_{50} value was 0.064 μM (**4c**). A more bulky isopropyl group was also tested, and compound **4d** inhibited PI3Kαwith an IC_{50} value of 0.081 μM. These results indicate that the ortho substituent at this point gradually disrupts the molecular planarity, and the reduced inhibitory activity would depend on the position of the aromatic ring or the bulk of the introduced alkyl substituents. Unfortunately, solubility in FaSSIF was still low even in compound **4d**, so we attempted further derivatizations to compound **4b,** which had shown the most potent IC_{50} value out of **4a–4d**.

Table 2
In vitro profiles of ortho substituted compounds (1)



Compound	R	PI3Kα IC_{50} (μM)	FaSSIF (μg/ml)
22	H	0.033	<12
10	Me	0.26	56

Table 3
In vitro profiles of ortho substituted compounds (2)

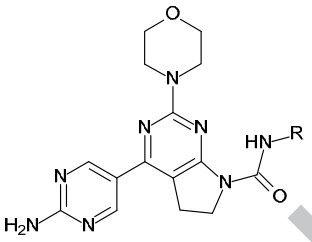


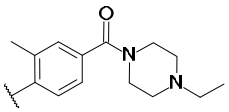
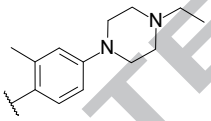
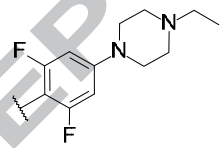
Compound	R	PI3Kα IC_{50} (μM)	FaSSIF (μg/ml)
4b	Me	0.052	<14
4c	Et	0.064	N.T.
4d	*i*Pr	0.081	13

As described in Table 4, solubilizing groups and other ortho substituents were introduced to **4b**. First, N-ethylpiperazine was introduced through an amide linker (**14**), which increased the enzyme inhibitory activity to 0.026 μM, but solubility in FaSSIF was not improved. Next, N-ethylpiperazine was directly connected to the phenyl ring, and the IC_{50} value of compound **18** was stronger than with an amide linker, but solubility was not enough. Simply introducing amines as a solubilizing group was not effective, so other ortho substituents were tested. We evaluated ortho difluorinated compound **2**, in which electrostatic repulsion between one fluoro atom and the urea carbonyl can be expected. As shown in Figure 4c, the most stable conformation of the mono fluorinated compound was flat, because of a favorable electrostatic interaction of the fluoro atom with urea NH; however, the most stable difluorinated compound had the twisted conformation that avoided, as expected, the electrostatic repulsion of the second fluoro atom with urea carbonyl (ϕ=55˚, Figure 4d). In fact, FaSSIF solubility of **2** was slightly improved to 20 μg/ml, indicating that the molecular planarity had been broken more than compound **18**.

The PI3Kαenzyme inhibitory activity of compound **2** was lower than the ortho methyl compound **18**, but its IC_{50} value was still potent (IC_{50}=0.022 μM). Compound **2** was an inhibitor that balanced both inhibitory activity and solubility, which was achieved by appropriately disrupting the molecular planarity and introducing a solubilizing group. In addition, liver microsomal stability of compound **2** was good in both mouse (7.6 μL/min/mg) and human (2.2 μL/min/mg), and permeability was also acceptable (1.1 x 10^{-6} cm/sec in PAMPA). Following these results, the pharmacokinetic profile of compound **2** in mice was also evaluated. Compound **2** was orally administered at a dose of 50 mg/kg, and its AUC value was more than 10 times better than the non-substituted phenylurea lead compound **1**. As shown in Table 4, compound **2** inhibited PI3Kβwith an IC_{50} value of 7 nM. PI3Kβis an antitumor target in PTEN-negative cancer cell lines,[5] and compound **2** showed antiproliferative activity in vitro against the PC-3 prostate cancer cell lines, which are known to be PTEN-negative. Finally, the antitumor efficacy of compound **2** was evaluated in a PC3 xenograft model. As described in Figure 5, compound **2** showed strong tumor growth inhibition (82%) at the low dose (6.25 mg/kg) without significant body weight loss.

Table 4
In vitro and in vivo profiles of ortho substituted compounds with a solubilizing group



Compound	R	PI3Kα IC_{50} (μM)	PI3Kβ IC_{50} (μM)	PC3[1] IC_{50} (μM)	FaSSIF (μg/ml)	Mouse PK (50 mg/kg po) Cmax (ng/mL)	Mouse PK (50 mg/kg po) AUC (ng·h/mL)
14		0.026	N.T.	0.25	<18	N.T.	N.T.
18		0.010	N.T.	0.21	11	N.T.	N.T.
2		0.022	0.007	0.058	20	460	5680

[1] Prostate cancer, PTEN negative

Figure 4a **Figure 4b**

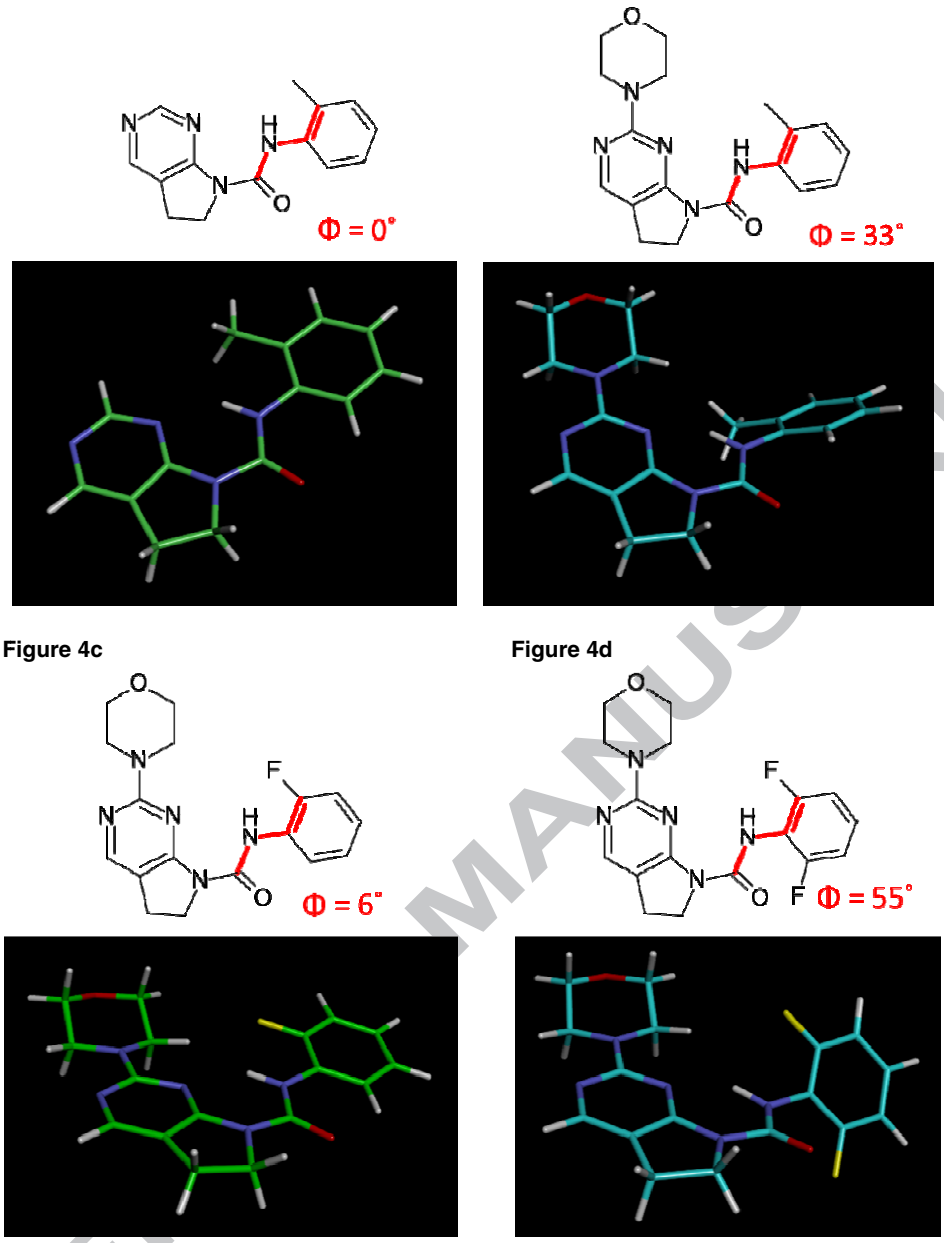


Figure 4. B3LYP/6-31+G(d,p) level calculation of phenylurea derivatives. The most stable conformations and torsions of each compound are shown. All calculations were done by the program Gaussian 09.

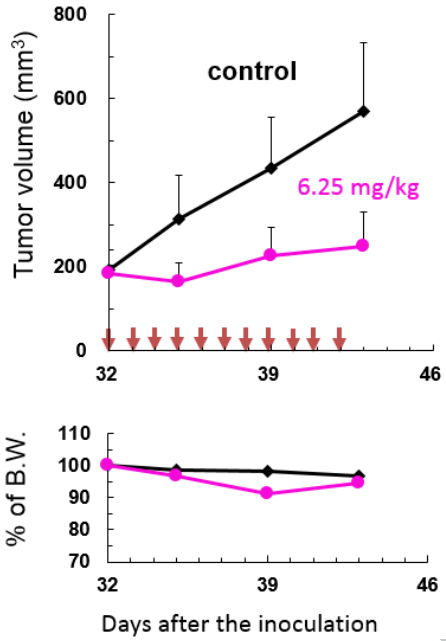


Figure 5. In vivo antitumor effect of **2** in PC-3 xenograft mice models. Mice (four mice per group) bearing PC-3 were administered compound **2** orally once daily for 11 days at 6.25 mg/kg (82% TGI).

4. Conclusion

By the combination of disrupting molecular planarity and introducing a solubilizing group, the solubility and pharmacokinetic profiles of a phenylurea derivative were improved. Ortho methylation at the biaryl part was effective in improving solubility, but it was not acceptable in our case, because of significant loss of inhibitory activity (**10**). On the other hand, ortho alkylation at the phenylurea moiety slightly decreased inhibitory activity but did not improve solubility (**4b**, **4c**, and **4d**). Combinations of ortho substitutions at the phenylurea moiety that introduced N-ethylpiperazine as a solubilizing group were also tested. Although ortho methylated compound **18** did not improve the solubility, ortho difluorinated compound **2** showed moderate solubility. In the case of the ortho difluorinated compound, one fluoro atom is arranged in the carbonyl site of urea, which increases the dihedral angle to avoid electrostatic repulsion between the fluoro atom and urea carbonyl. As a result, solubility in FaSSIF of compound **2** was slightly improved to 20 μg/ml and the mouse AUC was more than 10 times better than **1**. Furthermore, compound **2** showed strong tumor growth inhibition in vivo against a PC3 xenograft model without significant body weight loss.

5. Experimental

5.1 Chemistry

All solvents and reagents were obtained commercially. 1H and 13C NMR spectra were recorded on a JEOL JNM-EX270 (270 MHz), JEOL JNM-EX400 (400 MHz), or JNM-GSX400 (400 MHz), or Bruker Ascend400 (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 brs, broad singlet peak. High resolution mass spectra (HRMS) were measured with a I-Class/Xevo G2S TOF (Waters) using an ESI source. Flash column chromatography was performed with Biotage SNAP cartridges or SILICYCLE SiliaSep packed columns.

5.1.1 4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid phenylamide (1)

To a solution of **3** (54.0 mg, 0.100 mmol) and TEA (41.7 μL, 0.300 mmol) in DCE (1 mL) was added phenyl isocyanate (33.0 μL, 0.300 mmol). After refluxing for 2 hours, the mixture was cooled to an ambient temperature and washed with saturated aqueous NH_4Cl and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography (*n*-hexane/EtOAc, 1/0 to 1/1) to afford the

4-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)-2-morpholino-N-phenyl-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidine-7-carboxamide (33.7 mg, 51%). To a solution of this intermediate in TFA (1 mL) was added conc. H_2SO_4 (0.53 μL, 10.0 μmol), and the mixture was stirred at 70 ˚C for 3 hours. After cooling to ambient temperature, the mixture was diluted with H_2O (1 mL) and neutralized by 5M NaOH. The resulting filter cake was washed with MeOH to afford the titled compound as a light brown powder (17.1 mg, 82%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.85 (2H, s), 7.53 (2H, d, J = 7.8 Hz), 7.36 (2H, t, J = 8.1 Hz), 7.19 (2H, s), 7.07 (1H, t, J =7.6 Hz), 4.05 (2H, t, J = 8.5 Hz), 3.79-3.73 (8H, m), 3.23 (2H, t, J = 8.5 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.1, 163.6, 159.9, 158.4, 155.0, 151.0, 138.7, 129.7, 123.6, 120.0, 119.4, 106.3, 66.4, 46.4, 45.0, 23.5 ; HRMS (ESI), m/z Calcd for $C_{21}H_{22}N_8O_2$+H: 419.1944, Found 419.1943.

5.1.2

4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid methyl-phenyl-amide (4a)

To a solution of **3** (54.0 mg, 0.100 mmol) and pyridine (16 μL, 0.200 mmol) in DCM (1 mL) was added triphosgene (59.4 mg, 0.200 mmol) at 0 ˚C. After stirring for 1 hour at an ambient temperature, DCM was evaporated under reduced pressure. The residue was dissolved in DCM (1 mL) again and N-methylaniline (13 μL, 0.120 mmol) was added to the mixture. After stirring for 4 hours, the mixture was directly charged on silica gel and purified (DCM/MeOH, 1/0 to 95/5) to afford 4-[2-[bis(4-methoxybenzyl)amino]pyrimidin-5-yl]-N-methyl-2-morpholino-N-phenyl-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidine-7-carboxamide (52.2 mg, 78% in 2 steps). This residue and NAC (25.2 mg, 0.155 mmol) was dissolved in TFA (1 mL) and the mixture was refluxed for 6 hours. After cooling to an ambient temperature, H_2O (1 mL) and 5M NaOH (1 mL) was added. The resulting filter cake was washed by MeOH and purified by silica gel flash column chromatography (DCM/MeOH, 1/0 to 95/5) to afford titled compound as a white powder (21.1 mg, 63%). ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 8.71 (2H, s), 7.32-7.27 (4H, m), 7.14-7.11 (1H, m), 7.11-7.08 (2H, m), 3.87 (2H, t, J = 8.2 Hz), 3.64-3.59 (8H, brm), 3.35 (3H, s), 3.09 (2H, t, J =8.2 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.9, 163.9, 160.7, 158.1, 155.5, 153.5, 144.8, 129.1, 125.7, 125.0, 120.3, 105.1, 66.6, 48.4, 44.7, 38.8, 24.5 ; HRMS (ESI-TOF), m/z Calcd for $C_{22}H_{24}N_8O_2$+H: 433.2100, Found 433.2094.

5.1.3

4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid *o*-tolylamide (4b)

Compound **4b** was prepared from **3** and 2-methylaniline by following the same procedure as described for **4a** (white powder, 68% in 3 steps). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.29 (1H, s), 8.84 (2H, s), 7.59 (1H, d, J = 8.1 Hz), 7.26 (1H, d, J = 7.0 Hz), 7.23-7.18 (3H, m), 7.09 (1H, t, J = 7.5 Hz), 4.05 (2H, t, J = 8.3 Hz), 3.74-3.65 (8H, m), 3.24 (2H, t, J = 8.3 Hz), 2.27 (3H, s); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.1, 163.9, 160.1, 158.4, 154.8, 151.6, 136.3, 130.8, 130.4, 126.6, 125.1, 124.9, 120.0, 106.5, 66.4, 46.5, 45.1, 23.5, 18.1 ; HRMS (ESI-TOF), m/z Calcd for $C_{22}H_{24}N_8O_2$+H: 433.2100, Found 433.2097.

5.1.4

4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid (2-ethyl-phenyl)-amide (4c)

Compound **4c** was prepared from **3** and 2-ethylaniline by following the same procedure as described for **4a** (white powder, 56% in 3 steps). ^{1}H-NMR (DMSO-d_6) δ: 10.29 (1H, s), 8.85 (2H, s), 7.52 (1H, d, J = 7.5 Hz), 7.29 (1H, d, J = 7.5 Hz), 7.24-7.13 (4H, m), 4.05 (2H, t, J = 8.3 Hz), 3.72-3.65 (8H, m), 3.24 (2H, t, J = 8.3 Hz), 2.63 (2H, q, J = 7.5 Hz), 1.16 (3H, t, J = 7.5 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.1, 163.9, 160.0, 158.4, 154.8, 151.9, 136.9, 135.5, 128.8, 126.6, 126.1, 125.8, 120.0, 106.5, 66.3, 46.5, 45.1, 24.1, 23.5, 14.5 ; HRMS (ESI-TOF), m/z Calcd for $C_{23}H_{26}N_8O_2$+H: 447.2257, Found 447.2255.

5.1.5

4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid (2-isopropyl-phenyl)-amide (4d)

Compound **4d** was prepared from **3** and 2-isopropylaniline by following the same procedure as described for **4a** (white powder, 57% in 3 steps). ^{1}H-NMR (DMSO-d_6) δ: 10.33 (1H, s), 8.85 (2H, s), 7.46-7.33 (2H, m), 7.24-7.17 (4H, m), 4.05 (2H, t, J = 8.3 Hz), 3.67 (8H, brs s), 3.24 (2H, t, J = 8.3 Hz), 3.18 (1H, q, J = 7.0 Hz), 1.17 (6H, d, J = 7.0 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.1, 163.9, 160.0, 158.4, 154.8, 152.1, 142.2, 134.5, 126.9, 126.4, 126.3, 126.1, 120.0, 106.5, 66.3, 46.5, 45.0, 28.0, 23.7, 23.5 ; HRMS (ESI-TOF), m/z Calcd for $C_{24}H_{28}N_8O_2$+H: 461.2413, Found. 461.2413

5.1.6 5-Bromo-N,N-bis(4-methoxybenzyl)-4-methylpyrimidin-2-amine (6)

To a suspension of 5-bromo-4-methylpyrimidin-2-amine (500 mg, 2.66 mmol) in DMF (5 mL) was added NaH (60% dispersion in mineral oil, 266 mg, 6.65 mmol) at 0 ˚C. After stirring for 30 minutes at 0 °C, 4-methoxybenzyl chloride (0.797 ml, 5.85 mmol) and NaI (39.9 mg, 0.266 mmol) was added at 0 °C. After stirring for 4 hours at ambient temperature, 4-methoxybenzyl chloride (0.400 ml, 2.94 mmol) and NaH (60% dispersion in mineral oil, 80 mg, 2.00 mmol) was added at 0 ˚C. After stirring for 1 hour at ambient temperature, the reaction mixture was neutralized by saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc/*n*-hexane=2/1. The combined organic layer was washed with brine twice, 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 colorless oil (898 mg, 79%). ^{1}H-NMR ($CDCl_3$) δ: 8.24 (1H, d, J = 2.7 Hz), 7.16 (4H, dd, J = 8.6, 2.7 Hz), 6.84 (4H, dd, J = 8.6, 2.1 Hz), 4.73 (4H, d, J = 3.2 Hz), 3.79 (6H, s), 2.45 (3H, d, J = 2.1 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{21}H_{22}BrN_3O_2$+H: 428.0974, Found 428.0959.

5.1.7
N,N-Bis(4-methoxybenzyl)-4-methyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyrimidin-2-amine (7)

To a solution of **6** (400 mg, 0.934 mmol) and triisopropyl borate (0.431 ml, 1.87 mmol) in toluene/THF=4/1 (10 ml) was added *n*-BuLi (2.6 M in *n*-hexane, 0.718 ml, 1.87 mmol) at −78 °C. After stirring for 30 minutes 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-methoxybenzyl)amino]-4-methylpyrimidin-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 $MgSO_4$ (450 mg, 3.74 mmol) in 1,2-dichloroethane (10 mL) was added pinacol (221 mg, 187 mmol). After stirring for 15 hours 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 (346 g, 78% in 2 steps). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 8.43 (1H, s), 7.16 (4H, d, J = 6.8 Hz), 6.86 (4H, dt, J = 9.2, 2.4 Hz), 4.72 (4H, s), 3.72 (6H, s), 2.46 (3H, s), 1.28 (12H, s) ; HRMS (ESI-TOF), m/z Calcd for $C_{27}H_{34}BN_3O_4$+H: 476.2721, Found 476.2739.

5.1.8 4-(4-Chloro-7-(pyridin-3-yl)-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-2-yl)morpholine (9)

NaH (60% dispersion in mineral oil, 2.00 g, 50.0 mmol) was washed with *n*-hexane twice. 3-aminopyridine (4.70 g, 50.0 mmol) in THF (80 mL) was added to the NaH above in one portion at ambient temperature. The mixture was stirred at 50 ˚C for 1 hour and the resulting purple solution was cooled to ambient temperature. A solution of **8** (3.00 g, 10.1 mmol) in THF (15 mL) was added to the mixture and the resulting mixture was stirred at ambient temperature for 6.5 hours. The mixture was poured onto saturated aqueous NH_4Cl (250 mL) at 0 ˚C. The resulting filter cake was washed with *n*-hexane to afford the titled compound as a light yellow powder (2.12 g, 67%). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 9.06 (1H, d, J = 2.6 Hz), 8.31 (1H, dd, J = 4.7, 1.4 Hz), 8.06 (1H, dq, J = 8.5, 1.4 Hz), 7.31 (1H, dd, J = 8.5, 4.7, 0.7 Hz), 4.09 (2H, t, J = 8.3 Hz), 3.81-3.73 (8H, m), 3.11 (2H, t, J = 8.3 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{15}H_{16}ClN_5O$+H: 318.1122, Found 318.1115.

5.1.9
4-Methyl-5-(2-morpholin-4-yl-7-pyridin-3-yl-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)-pyrimidin-2-ylamine (10)

A suspension of **9** (64.0 mg, 0.201 mmol), **7** (105 mg, 0.221 mmol), $Pd(OAc)_2$ (2.3 mg, 10.2 μmol), 2-dicyclohexylphosphino-2',6'-dimethoxy-1,1'-biphenyl (S-Phos) (8.2 mg, 20.0 μmol) and K_3PO_4 (85.0 mg, 0.400 mmol) in DMF (2 ml) was degassed under ultrasonic irradiation and stirred for 10 hours at 100 °C in a nitrogen atmosphere. After cooling to ambient temperature, the reaction mixture was diluted with EtOAc/THF=4/1 (80 mL), washed with saturated aqueous $NaHCO_3$, 1% aqueous NAC and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH, 1/0 to 50/1) to afford Bis-(4-methoxy-benzyl)-[4-methyl-5-(2-morpholin-4-yl-7-pyridin-3-yl-6,7-dihydro-5H-pyrrolo[2,3-d]pyrimidin-4-yl)-pyrimidin-2-yl]-amine as a yellow amorphous (78 mg). This residue and NAC (78.0 mg, 0.478 mmol) was dissolved in TFA (5 mL) and the mixture was refluxed for 3 hours. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by amino silica gel flash column chromatography (DCM/MeOH, 1/0 to 50/1) to afford the titled compound as a light yellow powder (34 mg, 43% in 2 steps). ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 9.07 (1H, d, J = 2.3 Hz), 8.23 (1H, s),

8.23 (1H, dd, J = 8.7, 3.0 Hz), 7.67 (1H, td, J = 5.4, 2.7 Hz), 7.42 (1H, dd, J = 8.7, 4.8 Hz), 6.77 (2H, s), 4.08 (2H, t, J = 8.2 Hz), 3.67 (8H, brs), 3.04 (2H, t, J = 8.2 Hz), 2.33 (3H, s); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 166.0, 165.6, 163.2, 161.3, 157.9, 156.5, 143.0, 139.9, 138.1, 124.9, 124.0, 120.4, 108.9, 66.5, 48.6, 45.0, 23.3, 23.1 ; HRMS (ESI-TOF), m/z Calcd for $C_{20}H_{22}N_8O$+H: 391.1995, Found 391.1991.

5.1.10 (4-Ethylpiperazin-1-yl)(3-methyl-4-nitrophenyl)methanone (12)

To a solution of 3-methyl-4-nitrobenzoic acid (500 mg, 2.76 mmol), EDC•HCl (794 mg, 4.14 mmol) and DMAP (33.7 mg, 0.276 mmol) in DCM (10 mL) was added 1-ethylpiperazine (0.420 mL, 3.31 mmol). After stirring for 2 hours at ambient temperature, the reaction mixture was diluted with saturated NH_4Cl and 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 silica gel flash column chromatography (DCM/MeOH, 1/0 to 9/1) to afford the titled compound as a light yellow powder (606 mg, 79%). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.00 (1H, d, J = 8.3 Hz), 7.39 (1H, s), 7.35 (1H, dd, J = 8.3, 1.2 Hz), 3.81 (2H, brs), 3.40 (2H, brs), 2.63 (3H, s), 2.54 (2H, brs), 2.46 (2H, q, J = 7.2 Hz), 2.39 (2H, brs), 1.10 (3H, t, J = 7.2 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{14}H_{19}N_3O_3$+H: 278.1505, Found 278.1505.

5.1.11 4-Amino-3-methylphenyl)(4-ethylpiperazin-1-yl)methanone (13)

A suspension of **12** (300 mg, 1.08 mmol) and palladium (10% on carbon, 30.0 mg, 28.0 μmol) in MeOH (5.40 mL) was stirred under H2 atmosphere for 3.5 hours at ambient temperature. The reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH, 1/0 to 9/1) to afford the titled compound as a white amorphous (245 mg, 96%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.00 (1H, s), 6.97 (1H, dd, J = 8.2, 2.0 Hz), 6.58 (1H, d, J = 8.2 Hz), 5.24 (2H, s), 3.49-3.44 (4H, m), 2.36-2.29 (6H, m), 2.05 (3H, s), 1.00 (3H, t, J = 7.2 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{14}H_{21}N_3O$+H: 248.1754, Found 248.1754.

5.1.12
4-(2-Aminopyrimidin-5-yl)-N-(4-(4-ethylpiperazine-1-carbonyl)-2-methylphenyl)-2-morpholino-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidine-7-carboxamide (14)

Compound **14** was prepared from **3** and **13** by following the same procedure as described for **4a** (white powder, 19% in 3 steps). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.35 (1H, s), 8.85 (2H, s), 7.71 (1H, d, J = 8.1 Hz), 7.29 (1H, s), 7.25-7.19 (3H, m), 4.06 (2H, t, J = 8.3 Hz), 3.75-3.65 (8H, m), 3.61-3.40 (2H, br m), 3.28-3.20 (4H, m), 2.39-2.32 (6H, m), 2.30 (3H, s), 1.00 (3H, t, J = 7.3 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 169., 164.1, 163.8, 160.1, 158.4, 154.9, 151.4, 137.5, 132.2, 130.0, 129.7, 125.5, 124.0, 120.0, 106.5, 66.4, 52.8, 52.0, 46.6, 45.2, 23.5, 18.1, 12.3 ; HRMS (ESI-TOF), m/z Calcd for $C_{29}H_{36}N_{10}O_3$+H: 573.3050, Found 573.3065.

5.1.13 1-Ethyl-4-(3-methyl-4-nitrophenyl)piperazine (16)

To a solution of 4-fluoro-2-methylnitrobenzene (0.394 mL, 3.22 mmol) in DMSO (8 mL) was added 1-ethylpiperazine (0.612 mL, 4.82 mmol) and K_2CO_3 (668 mg, 4.83 mmol). The resulting mixture was stirred at 80 ˚C for 90 minutes. After cooling to ambient temperature, the mixture was diluted with H_2O (16 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude product was used in the next reaction without further purification.

5.1.14 4-(4-Ethylpiperazin-1-yl)-2-methylaniline (17)

To a solution of **16** (803 mg, 3.22 mmol) in MeOH (15 mL) was added NH_4Cl (517 mg, 9.66 mmol) and zinc (1.05 g, 16.1 mmol). After stirring for 2 hours, the reaction mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH, 1/0 to 9/1) to afford the titled compound as a brown powder (638 mg, 90% in 2 steps). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 6.75-6.67 (2H, m), 6.62 (1H, d, J = 8.3 Hz), 3.36 (2H, brs), 3.10-3.06 (4H, m), 2.64-2.59 (4H, m), 2.48 (2H, q, J = 7.2 Hz), 2.16 (3H, s), 1.13 (3H, t, J = 7.2 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{13}H_{21}N_3$+H: 220.1814, Found 220.1811.

5.1.15
4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid [4-(4-ethyl-piperazin-1-yl)-2-methyl-phenyl]-amide (18)

To a solution of **3** (150 mg, 0.278 mmol) in DCM (4 mL) was added saturated aqueous $NaHCO_3$ (3.5 mL). After cooling to 0 ˚C, phosgene (20% in toluene, 0.45 mL, 0.834 mmol) was added to the mixture dropwise. After stirring for 40 minutes at 0 ˚C, the organic layer was separated, washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was dissolved in DCM (4 mL) and TEA (58.0 μl, 0.417 mmol) was added. To this mixture was added **17** (67 mg, 0.306

mmol) in DCM (1 mL) dropwise and stirred overnight at ambient temperature. The mixture was quenched by 1M HCl, then extracted with DCM/MeOH (50/1). 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 (DCM/MeOH, 50/1 to 20/1) to afford 4-(2-(bis(4-methoxybenzyl)amino)pyrimidin-5-yl)-N-(4-(4-ethylpiperazin-1-yl)-2-methylphenyl)-2-morpholino-5,6-dihydro-7H-pyrrolo[2,3-d]pyrimidine-7-carboxamide as a yellow powder (205 mg). This residue was dissolved in TFA (1.5 mL) and the mixture was refluxed for 4 hours. After cooling to ambient temperature, TFA was evaporated under reduced pressure. The residue was purified by amino silica gel flash column chromatography (DCM/MeOH, 100/1 to 50/1) to afford the titled compound as a light yellow powder (110 mg, 76% in 3 steps). ^{1}H-NMR (270 MHz, CDCl3) δ: 10.20 (1H, s), 8.90 (2H, s), 7.43 (1H, d, J = 8.8 Hz), 6.82 (2H, m), 5.32 (2H, s), 4.22 (2H, t, J = 8.5 Hz), 3.77 (8H, m), 3.22 (6H, m), 2.62 (4H, m), 2.49 (2H, q, J = 7.2 Hz), 1.14 (3H, t, J = 7.2 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 163.5, 163.4, 159.4, 157.7, 154.1, 151.3, 148.4, 131.3, 127.2, 125.8, 119.5, 116.9, 113.2, 105.8, 65.7, 52.3, 51.5, 48.4, 45.9, 44.4, 40.1, 39.9, 39.7, 39.4, 39.2, 39.0, 38.8, 22.9, 18.0, 11.9 ; HRMS (ESI-TOF), m/z Calcd for $C_{28}H_{36}N_{10}O_2$+H: 545.3101, Found 545.3119.

5.1.16 N,N-Di-Boc-4-bromo-2,6-fluoro-phenylamine

To a solution of 4-bromo-2,5-difluoroniline (10.0 g, 4.81 mmol) in DMF (10 mL) was added $(Boc)_2O$ (2.31 g, 10.6 mmol) and DMAP (29 mg, 0.240 mmol). After stirring overnight at ambient temperature, the mixture was diluted with H_2O (20 mL) and extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. This crude product was used in the next reaction without further purification.

5.1.17 *tert*-Butyl(4-(4-ethylpiperazin-1-yl)-2,6-difluorophenyl)carbamate (20)

A suspension of N,N-di-boc-4-bromo-2,6-fluoro-phenylamine (220 mg, 0.539 mmol), 1-ethylpiperazine (0.273 mL, 2.16 mmol), $Pd(OAc)_2$ (12.1 mg, 54.0 μmol), S-Phos (44.2 mg, 108 μmol) and Cs_2CO_3 (351 mg, 1.08 mmol) in toluene (5 ml) was degassed under ultrasonic irradiation and refluxed for 2 hours in a nitrogen atmosphere. After cooling to ambient temperature, the reaction mixture was diluted with EtOAc, washed with saturated aqueous NH_4Cl and then brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (DCM/MeOH, 1/0 to 95/5). The resulting residue was washed with *n*-hexane to afford the titled compound as a light brown powder (37.7 mg, 20% in 2 steps). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.42 (2H, dt, J = 17.4, 3.3 Hz), 5.74 (1H, s), 3.21-2.15 (4H, m), 2.60-2.54 (4H, m), 2.46 (2H, q, J = 7.3 Hz), 1.49 (9H, s), 1.12 (3H, t, J = 7.3 Hz) ; HRMS (ESI-TOF), m/z Calcd for $C_{17}H_{25}F_2N_3O_2$+H: 342.1993, Found 342.2002.

5.1.18 4-(4-ethylpiperazin-1-yl)-2,6-difluoroaniline (21)

To a solution of **20** (30 mg, 88.0 μmol) in THF (343 μL) was added 6M HCl (117 μL). After stirring for 30 minutes at ambient temperature, the mixture was added 8M NaOH and 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 silica gel flash column chromatography (DCM/MeOH, 1/0 to 95/5) to afford the titled compound as a colorless oil (19.2 mg, 91%). ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 6.44 (2H, ddd, J = 20.4, 10.7, 2.9 Hz), 3.37 (2H, s), 3.07-3.04 (4H, m), 2.61-2.55 (4H, m), 2.46 (2H, q, 7.2 Hz), 1.12 (3H, t, J = 7.2 Hz); HRMS (ESI-TOF), m/z Calcd for $C_{12}H_{17}F_2N_3$+H: 242.1469, Found 242.1478.

5.1.19
4-(2-Amino-pyrimidin-5-yl)-2-morpholin-4-yl-5,6-dihydro-pyrrolo[2,3-d]pyrimidine-7-carboxylic acid [4-(4-ethyl-piperazin-1-yl)-2,6-difluoro-phenyl]-amide (2)

Compound **2** was prepared from **3** and **21** by following the same procedure as described for **4a** (white powder, 52% in 3 steps). ^{1}H-NMR (270 MHz, DMSO-d_6) δ: 10.15 (1H, s), 8.84 (2H, s), 7.22 (2H, s), 6.73 (2H, d, J = 11.7 Hz), 4.01 (2H, t, J = 8.0 Hz), 3.67 (8H, s), 3.26 (2H, t, J = 8.0 Hz), 3.22-3.16 (4H, m), 2.48-2.44 (4H, m), 2.36 (2H, q, J = 7.1 Hz), 1.03 (3H, t, J = 7.1 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6) δ: 164.1, 163.7, 159.8, 158.4, 157.7, 155.1, 151.7, 128.5, 126.0, 120.0, 106.1, 98.5, 98.2, 66.3, 52.2, 51.9, 47.5, 46.4, 44.8, 23.7, 12.1 ; HRMS (ESI-TOF), m/z Calcd for $C_{27}H_{32}F_2N_{10}O_2$+H: 567.2756, Found 567.2773.

5.2 In vitro kinase enzyme assay

Inhibitory activities on human PI3Kα (p110α/p85α) or PI3Kβ (p110β/p85α) (expressed in baculovirus and purified) were determined by AlphaScreen competition assay. Test compound solutions were mixed with PI3K, and incubated for 20 minutes at room temperature. Substrate solution 4 μM PI(4,5)P2

(echelon) and 10 μM ATP solution (5 mM Hepes pH7.5, 2.5 mM MgCl2) were added, and incubated for 15 minutes at 37 ˚C. Detection of the reaction product PI(3,4,5)P3 was done using AlphaScreen GST Detection Kit (PerkinElmer) according to the manufacture's recommendation. GST-GRP1 (expressed in E.coli and purified), Anti-GST Acceptor Beads, Streptavidin Donor Beads, biotin-PI(3,4,5)P3 (echelon) (10 mM Tsir-HCl pH7.4, 150 mM NaCl, 7.5 mM EDTA, 1 mM DTT, 0.1% Tween20) were added, and incubated for 1 hour at room temperature. Signal was measured with an EnVision plate reader (PerkinElmer).

5.3 In vitro cell proliferation assay

The prostate cancer PC-3 (ATCC) cells were treated with various concentrations of assay compounds for 96 hours. Cell growth inhibition was determined by Cell Counting Kit-8 solution (Dojindo Laboratories).

5.4 Solubility assay

An aliquot of 50 μl of a 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 minutes, shaken for 2 hours, centrifuged for 10 minutes (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 (1.61g), Lecithin (0.59 g), KH2PO4 (3.9 g), KCl (7.7 g) and NaOH (pH 6.5) per 1 L.

5.5 Pharmacokinetic study in mice

Pharmacokinetic study: Female BALB/c-nu mice (n=2 per treatment group) were given a solution of the test compounds by oral (po) route at doses of 50 mg/kg (po) in mice. Vehicle was 10% DMSO, 10% Cremophor EL, 15% PEG400, and 15% HPCD (2-hydroxypropyl-β-cyclodextrin). Blood samples of each animal were collected with heparin as an anticoagulant at 0.25, 2, 4, 7, and 24 hours following po dosing. Samples were centrifuged to obtain plasma and stored at –80 °C until analysis. Plasma concentrations were determined using a LC–MS/MS system. The pharmacokinetic parameters were calculated by non-compartmental analysis using WATSON ver. 7.1 (Thermo Fisher Scientific, Wayne, PA).

5.6 In vivo antitumor effect on PC-3 in nude mice

A cell line (PC-3) was used to evaluate antitumor activity of **2** in vivo. It was grown as sc tumors in nude mice (Charles River Laboratories Japan, Inc.). Therapeutic experiments were started (day 0) when the tumor reached around 130 mm^3. Mice (four mice per group) were randomized to treatment groups to receive vehicle or **2** (oral, QD) on days 0 to 10. Vehicle was 10% DMSO, 10% Cremophor EL, 15% PEG400, and 15% HPCD (2-hydroxypropyl-β-cyclodextrin). Compound **2** was given to mice by forced oral administration using a sonde tube. The length (L) and width (W) of the tumor mass were measured, and the tumor volume (TV) was calculated as: $TV = (L\ W^2)/2$. Body weight change rate (BW) was calculated using the following formula: $BW = W/W_0 \times 100$, where W and W_0 are the body weight on a specific experimental day and on the first day of treatment, respectively. Tumor growth inhibition (TGI) was calculated using the following formula: $TGI = [1 - (T - T_0)/(C - C_0)] \times 100$, where T and T_0 are the mean tumor volumes on a specific experimental day and on the first day of treatment, respectively, for the experimental groups and, likewise, where C and C_0 are the mean tumor volumes for the control group.

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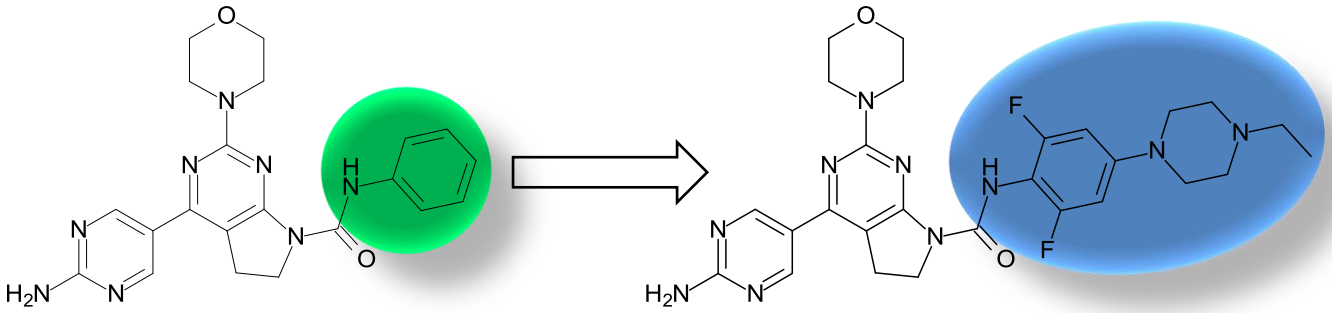
***Graphical Abstract (for review)**

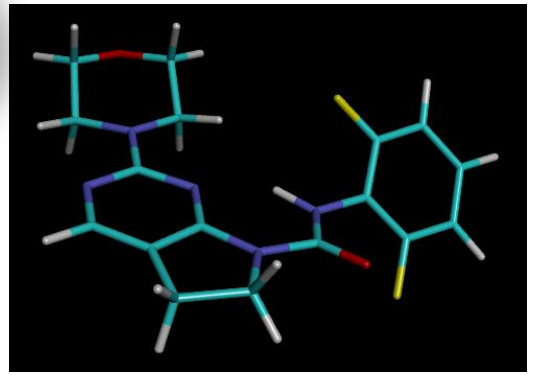


1

PI3Kα IC_{50}: 0.013 μM
Solubility in FaSSIF: < 3 μg/ml
Cmax: 104 ng/mL
AUC: 437 ng x h/mL

2

PI3Kα IC_{50}: 0.022 μM
Solubility in FaSSIF: 20 μg/ml
Cmax: 460 ng/mL
AUC: 5680 ng x h/mL
82% TGI in PC-3 xenograft model



Disruption of molecular planarity