Title: Synthesis and biological evaluation of 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives as novel PI3Kδ inhibitors

Author: Jia-Lin Guo Yun-Yong Liu Ya-Zhong Pei

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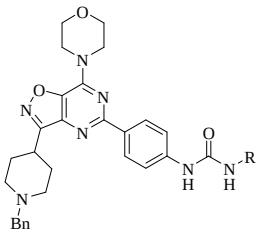
Graphical Abstract

Synthesis and biological evaluation of 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives as novel PI3Kδ inhibitors

Jia-Lin Guo[a], Yun-Yong Liu[b], Ya-Zhong Pei[a,]*

[a] *The Center for Combinatorial Chemistry and Drug Discovery of Jilin University, School of Pharmaceutical Sciences, Jilin University, Changchun 130021, China*
[b] *Nanjing Gator MediTech Company, Ltd., Nanjing 211300, China*



Compd.	R	IC_{50} (μmol/L) PI3K *d*	BT-474
20	$-CH_2CH_2F$	0.286	1.565
21	$-CH_3$	0.452	1.311

An efficient synthesis of 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives has been designed and developed. A series of novel PI3Kδ inhibitors with potent antitumor activities have been identified.

Original article

Synthesis and biological evaluation of 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives as novel PI3Kδ inhibitors

Jia-Lin Guo[a], Yun-Yong Liu[b], Ya-Zhong Pei[a,*]

[a] *The Center for Combinatorial Chemistry and Drug Discovery of Jilin University, School of Pharmaceutical Sciences, Jilin University, Changchun 130021, China*
[b] *Nanjing Gator MediTech Company, Ltd., Nanjing 211300,. China*

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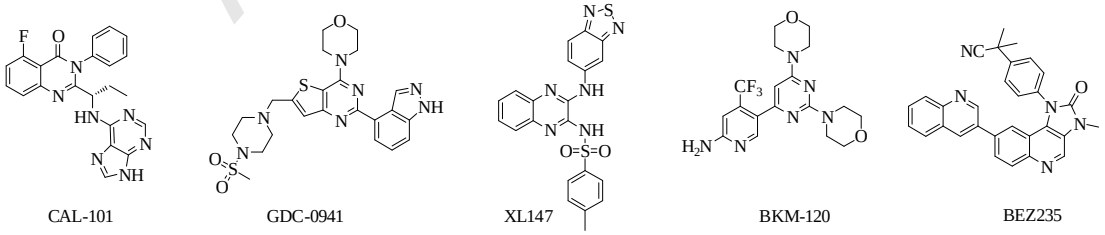
ABSTRACT

An efficient synthesis of novel 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine scaffold has been designed and deveopled. A series of 5-phenylurea derivatives was synthesized using this method. Their cytotoxic activities against breast cancer cell line BT-474 were evaluated by CCK-8 assay. Most of them showed potent anti-proliferative activities, of which compound **20** and **21** exhibited IC_{50}s of 1.565 μmol/L and 1.311 μmol/L, respectively. Furthermore, compound **20** and **21** also showed potent inhibitory activities against PI3Kδ with IC_{50}s of 0.286 μmol/L and 0.452 μmol/L, respectively. These results indicate that these 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives are novel antitumor agents through the inhibition of PI3Kδ.

1. Introduction

Phosphoinositide-3-kinases (PI3Ks) are members of lipid kinase family that phosphorylate inositol lipids at the 3-hydroxy group [1]. They play key regulatory roles in many cellular processes, including cell growth, survival, proliferation, motility, metabolism, and differentiation [2-4]. They are the central components of the PI3K/Akt/mTOR signalling pathway and activated by receptor tyrosine kinases (RTKs) and G-protein coupled receptors (GPCRs). The activated PI3Ks transduce signals from various growth factors and cytokines into intracellular messages by generating phospholipids. As the key second messengers, these phospholipids activate the serine/threonine protein kinase Akt and other downstream signaling pathways, promoting cell growth and proliferation [2]. PI3Ks can be divided into three classes according to their structures, substrates and functions, including class I (IA and IB), II, and III. The class I are heterodimers composed of a regulatory subunit and a catalytic subunit. The class IA consists of p85 and p110 (α, β and δ), whereas the class IB is p101 and p110γ. The class II consists of a monomeric catalytic subunit, including three isoforms (PI3KC2α, PI3KC2β and PI3KC2γ). The class III consists of a single heterodimer of a catalytic (Vps34) and a regulatory (Vsp15) subunit [5,6]. Aberrations of PI3K signaling are found in various tumors, including breast, prostate, colon, liver, and pancreatic cancers. The most frequently observed abnormalities are the loss or attenuation of PTEN function and mutations of the gene that encodes p110α [7]. The overexpression of p110β, δ, and γ has also been implicated in various forms of human tumors [4]. In addition, PI3K pathway activations also lead to resistance to current cytotoxic agents as well as targeted anticancer therapies [8]. Idelalisib (CAL-101 or GS-1101), a PI3Kδ inhibitor, is the first in its class that has gained the approvals of the FDA and EMA for the treatment of relapsed chronic lymphocytic leukemia (CLL) in July 2014. A number of other PI3K inhibitors are currently being studied in clinics, including GDC-0941, XL-147, BKM-120 and BEZ235 (Fig. 1) (http://ClinicalTrials.gov, [4]). These data indicate that regulating the activities of PI3Ks is an effective strategy for anticancer therapy.



CAL-101 GDC-0941 XL147 BKM-120 BEZ235

* Corresponding author.
E-mail address: peiyz@jlu.edu.cn (Y.-Z. Pei).

Fig. 1. Reported PI3K inhibitors.

PI3K inhibitors from several structural classes, such as GDC-0941, BKM-120, and PKI-402 [9] contain a common 2,4-disubstituted pyrimidine fragment with 4-morpholino group at the 4-position. Their binding data indicated that 2- and 4-substituents could pick up key hydrogen bond interactions with PI3Ks, whereas the pyrimidine ring mainly serve as a scaffold [9-11]. Based on this hypothesis, we designed the novel 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidines (Fig. 2). This bicyclic template allows us to maintain the 2,4-disubstituented pyrimidine structure while investigating the effect of the fused 3-(piperidin-4-yl)isoxazole. This report describes the syntheses of a series of novel 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives. Their anti-proliferative activities were evaluated in a cellular CCK-8 assay against breast cancer cell line BT-474. The lead compounds, **20** and **21**, were found to be potent inhibitors of PI3K*δ*.

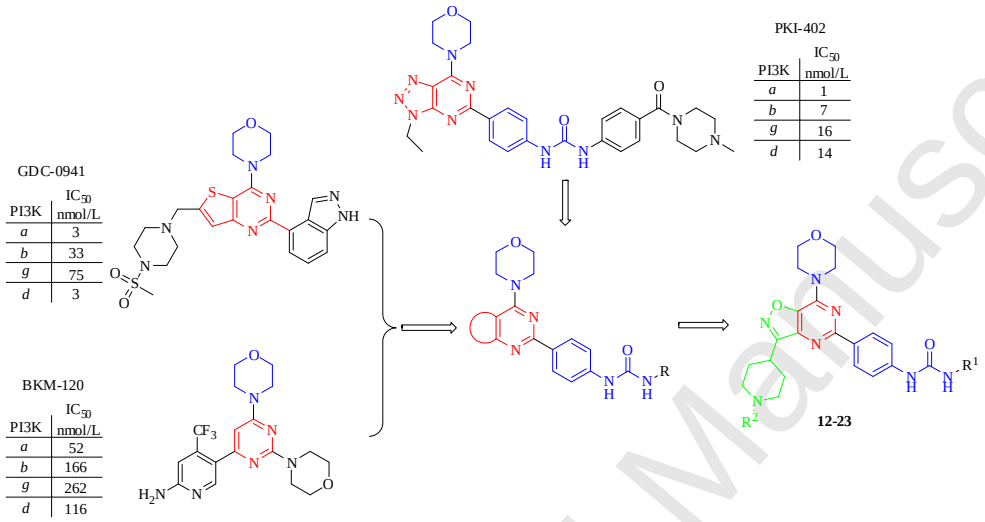


Fig. 2. Design considerations.

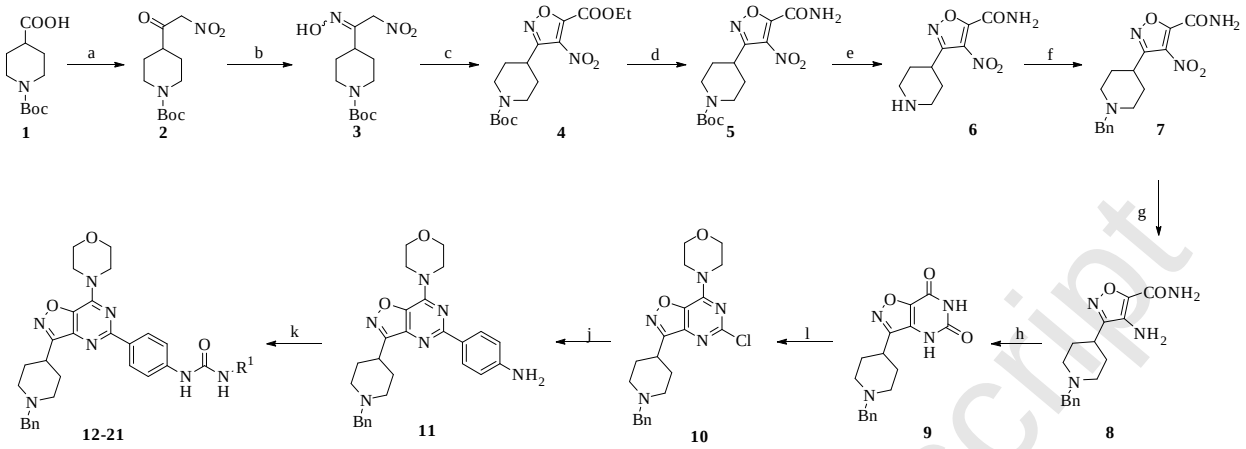
2. Experimental

2.1 Syntheses

The syntheses of compounds **2-21** are outlined in Scheme 1. Details of reaction conditions and characterization data related to compounds **2-11** are provided in the Supplementary data [12-19].

To a solution of N^1-(2-(dimethylamino)ethyl)-N^1-methylbenzene-1,4-diamine (116 mg, 0.6 mmol) and TEA (182 mg, 1.8 mmol) in anhydrous DCM (4 mL) at -18 °C was added dropwise a solution of triphosgene (89 mg, 0.3 mmol) in anhydrous DCM (2 mL). After 15 min., a solution of compound **11** (94 mg, 0.2 mmol) in anhydrous DCM (2 mL) was added. The mixture was stirred overnight and allowed to warm to room temperature. The reaction mixture was diluted with water (5 mL) and extracted with DCM (5 mL × 3). The combined organic layers were subsequently washed with H_2O (8 mL×3) and brine (8 mL×3), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified on a silica gel column (DCM/MeOH/TEA = 60/1/1, *v*/*v*/*v*) to give compound **12** (50 mg, 36% yield) as a yellow solid.

General procedure for compounds **13-21**: To a solution of compound **11** (94 mg, 0.2 mmol) and TEA (61 mg, 0.6 mmol) in anhydrous DCM (2 mL) at -18 °C was added dropwise a solution of triphosgene (30 mg, 0.1 mmol) in anhydrous DCM (2 mL). After 15 min., a solution of amine (5 eq.) in anhydrous DCM (2 mL) or amine hydrochloride (5 eq.) and TEA (6 eq.) was added. The mixture was stirred overnight and allowed to warm to room temperature. The reaction mixture was diluted with water (5 mL) and extracted with DCM (5 mL × 3). The combined organic layers were subsequently washed with H_2O (6 mL×3) and brine (6 mL×3), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified on a silica gel column or preparative thin-layer chromatography to give compounds **13-21** [20]. All data of compounds **12-21** are summarized in the Supplementary data.

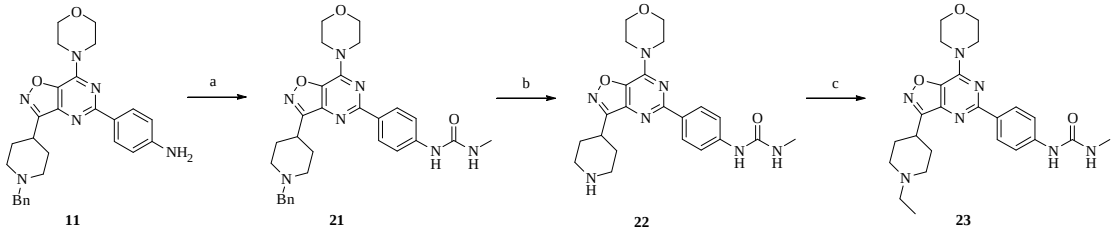


Scheme 1. Syntheses of compounds **2**–**21**. Reagents and conditions: (a) (1) CDI, anhydrous THF, N_2, r.t., 1-2 h; (2) DBU, nitromethane, r.t., 36 h, 96%. (b) hydroxylamine hydrochloride, $NaHCO_3$, EtOH, 50 °C, overnight, 95%. (c) (1) ethyl 2-chloro-2-oxoacetate, anhydrous ether, r.t., 24 h; (2) TEA, 0 °C-r.t., 60 h, 58%. (d) NH_3/MeOH, r.t., 3 h, 96%. (e) TFA, DCM, r.t., 2 h, 100% (TFA salt). (f) BnBr, DIPEA, anhydrous MeCN, 0 °C-r.t., 6 h, 81%. (g) Zn, NH_4Cl, EtOH/water = 2/1, 0 °C-r.t., 4 h, 78%. (h) triphosgene, anhydrous THF, reflux, 1 h, 90% (hydrochloride salt). (i) (1) $POCl_3$, reflux, 24 h; (2) morpholine, TEA, DCM, -18 °C, 10 min., 62%. (j) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, $Pd(Pcy_3)_2Cl_2$, CsF, NMP/water = 9/1, Ar, 100 °C, 48 h, 34%. (k) (1) TEA, triphosgene, anhydrous DCM, -18 °C, 15 min; (2) amine or amine hydrochloride and TEA, -18 °C-r.t., overnight, 30%-56%.

The syntheses of compounds **22** and **23** are outlined in Scheme 2. To a solution of compound **11** (471 mg, 1 mmol) and TEA (126 mg, 1.25 mmol) in anhydrous DCM (4 mL) was slowly added a solution of methylcarbamic chloride (103 mg, 1.1 mmol) in anhydrous DCM (3 mL). The mixture was then refluxed for 3 days. The reaction mixture was diluted with water (5 mL) and extracted with DCM (8 mL×3). The combined organic layers were subsequently washed with H_2O (10 mL×3) and brine (10 mL×3), dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The crude product was purified on a silica gel column (DCM/MeOH = 20/1, *v*/*v*) to give compound **21** (447 mg, 71% yield) [21].

To a solution of compound **21** (1.342 g, 2.54 mmol) in DCE (40 mL) was added ACE-Cl (2.910 g, 20.35 mmol) and stirred overnight at room temperature. The mixture was concentrated to dryness. The residue was refluxed in MeOH (20 mL) for 2 h and concentrated under vacuum to give the crude 1-methyl-3-(4-(7-morpholino-3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidin-5-yl)phenyl)urea hydrochloride (1.218 g) [22]. This crude intermediate was suspended in DCM (50 mL) at 0 °C. TEA (936 mg, 9.25 mmol) and a solution of $(Boc)_2O$ (618 mg, 2.83 mmol) in anhydrous DCM (10 mL) was then added. The mixture was stirred for 3 h, diluted with water (20 mL) and extracted with DCM (20 mL×3). The combined organic layers were washed with H_2O (25 mL×3) and brine (25 mL×3), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The crude product was purified on a silica gel column (DCM/MeOH = 50/1, *v*/*v*) to give *tert*-butyl-4-(5-(4-(3-methylureido)phenyl)-7-morpholinoisoxazolo[4,5-d]pyrimidin-3-yl)piperidine-1-carboxylate (1.037 g, 76% yield) as a white solid. The solution of the above intermediate (330 mg, 0.61 mmol) in DCM (1 mL) and TFA (3 mL) was stirred for 2 h at room temperature. The mixture was concentrated to dryness *in vacuo* to give compound **22** as its di-TFA salt (405 mg, 99% yield) as a yellow solid, which was used in the next step without further purification. Note: Compound **22** was very difficult to purify "as is". The crystallization of its hydrochloride salt did not furnish a product with satisfactory yield and purity. The *t*-Boc-protection/deprotection steps were for the ease of purification only.

To a suspension of compound **22** di-TFA salt (100 mg, 0.15 mmol) and DIPEA (71 mg, 0.55 mmol) in MeCN (3 mL) at -18 °C was added iodoethane (29 mg, 0.18 mmol). The mixture was stirred for 1 h at the same temperature followed by stirring at room temperature for 5 h. TLC showed that reaction was not complete. The mixture was stirred at 65 °C for 11 h and followed by refluxing for 8 h. The reaction mixture was concentrated *in vacuo* and diluted with water (3 mL). The aqueous layer was adjusted to pH 14 using 6 mol/L NaOH(aq.) and extracted with DCM/MeOH = 20/1 (5 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4. The crude product was purified on a silica gel column (DCM/MeOH/TEA = 55/1/1, *v*/*v*/*v*) to give compound **23** (48 mg, 69% yield) as a yellow solid [16,23]. All analytical data of compounds **22** and **23** were summarized in the Supplementary data. A small amount of quaternary salt was also observed during the reaction by LC-MS. It was easily removed during the work-up and purification.



Scheme 2. Syntheses of compounds **22** and **23**. Reagents and conditions: (a) methylcarbamic chloride, TEA, anhydrous DCM, reflux, 3 d, 71%. (b) (1) ACE-Cl, DCE, r.t., overnight; (2) MeOH, reflux, 2 h; (3) $(Boc)_2O$, TEA, DCM, 0 °C, 3 h, 76%; (4) TFA, DCM, r.t., 2 h, 99% (di-TFA salt). (c) iodoethane, DIPEA, MeCN, -18 °C-reflux, 69%.

2.2 Biological evaluation

The anti-proliferative activities of compounds **11–23** were assessed against BT-474 cells (human breast ductal carcinoma) by CCK-8 assay. BT-474 cells were plated in 96-well flat-bottomed microtiter plates (cells suspended in 100 µL culture medium per well) and incubated at 37 °C for 24 h under a 5% CO_2 and 100% relative humidity atmosphere. A 25 µL aliquot of culture medium containing the tested compound was added to the wells. The plates were incubated for an additional 72 h. After removal of culture medium, fresh culture medium containing 10% CCK-8 was added and incubated at 37 °C for 2-4 h. The absorbance was measured on a SpectraMax M5 Microplate Reader at 450 nm. The percentage of inhibition at each compound concentration was calculated according to formula: The percentage of inhibition = $(A_{sample}-A_{negative})/(A_{blank}-A_{negative})\times100\%$. Compounds were studied for dose-response relationship at 100 µmol/L, 25 µmol/L, 6.25 µmol/L, 1.563 µmol/L, 0.391 µmol/L,0.098 µmol/L, 0.024 µmol/L, 0.006 µmol/L, 0.0015 µmol/L, 0.0004 µmol/L. Their IC_{50}s were calculated using GraphPad Prism 5.

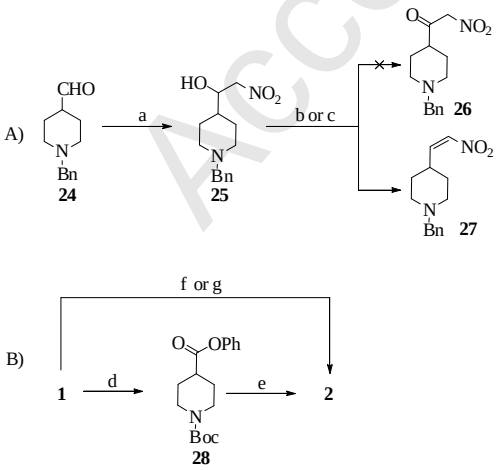
PI3Kδ kinase activity was determined using a PI3-Kinase (human) HTRF™ Assay kit in 384 well opaque black plates. CAL-101 was used as a positive control. Typically, 5 µL of ATP solution (40 µmol/L) was added into a mixture of 10 µL of PIP2 solution (20 µmol/L) containing PI3Kδ enzyme (80 ng) and 5 µL of test compound solution. The negative control and blank control were composed of the same mixed solutions except replacing test compound with DMSO. The blank control did not contain PI3Kδ enzyme. After a 30 min. incubation at room temperature, the reaction was stopped by the addition of 5 µL of stop buffer (stop A/stop B = 3/1) followed by detection buffer (5 µL, DMC/DMA/DMB = 18/1/1). After 1 h incubation at room temperature, emission signal was measured on EnVision® Multilabel Reader. Emission Ratio (ER) of each well was calculated according to the formula: Emission Ratio (ER) = 665 nm Emission signal/620 nm Emission signal. The percentage of inhibition at each compound concentration was calculated according to formula: The percentage of inhibition = $(ER_{sample}-ER_{negative})/(ER_{blank}-ER_{negative})\times100\%$. Compounds were studied for dose-response relationship at 100 µmol/L, 25 µmol/L, 6.25 µmol/L, 1.563 µmol/L, 0.391 µmol/L, 0.098 µmol/L, 0.024 µmol/L, 0.006 µmol/L, 0.0015 µmol/L, 0.0004 µmol/L. Their IC_{50}s were calculated using GraphPad Prism 5.

3. Results and discussion

3.1 Chemistry

A facile synthesis of novel 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine scaffold was developed, starting from *N*-Boc-piperidine-4-carboxylic acid (Scheme 1). Using this route, fifteen derivatives were synthesized for their anti-proliferative activity evaluation and initial structure activity relationship development.

We at first attempted a two-step process for the synthesis of intermediate **26** as shown in Scheme 3A, which has the potential of avoiding the exchange of *N*-substitution on piperidine from Boc- to benzyl. Condensation of aldehyde **24** and nitromethane in the presence of KF worked well, giving the alcohol **25** in 96% yield. Oxidation of **25**, under both Dess-Martin periodinane and Swern conditions, gave nitro olefin **27** in nearly quantitative yield (86% and 81%, respectively) instead of the α-nitroketone **26**. These *N*-benzyl piperidine derivatives were also difficult to purify due to their good aqueous solubility. For the ease of intermediate isolation, we therefore decided to use the *N*-Boc-piperidines during the early part of the syntheses. Details of reaction conditions and characterization data related to compounds **24**, **25** and **27** are summarized in the Supplementary data.

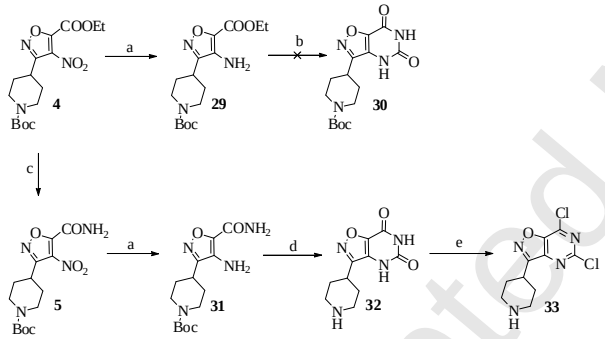


Scheme 3. Syntheses of α-nitroketone **2**. Reagents and conditions: (a) nitromethane, KF, 2-propanol, r.t., overnight, 96%. (b) Dess-Martin periodinane, anhydrous DCM, r.t., 35 min., 86%. (c) (1) oxalyl chloride, DCM, -78 °C, DMSO, 15 min.; (2) compound **25**, DCM, -78 °C, 1 h; (3) TEA, -78 °C, 15 min., r.t., 1 h, 81%. (d) (1) $(COCl)_2$, DMF, anhydrous DCM, 0 °C, 2 h; (2) PhOH, TEA, anhydrous DCM, 0 °C, 3 h, 43%. (e) nitromethane, *t*-BuOK, DMSO, r.t., 8 h, 77%. (f) (1) CDI, anhydrous THF, N_2, reflux, 1 h; (2) nitromethane, *t*-BuOK, reflux, 24 h, 75%. (g) CDI, anhydrous THF, N_2, r.t., 1-2 h; (2) nitromethane, DBU, N_2, r.t., 36 h, 96%.

As shown in Scheme 3B, key intermediate **2** can be obtained from *N*-Boc-piperidine-4-carboxylic acid (**1**) in a two-step process in moderate overall yield. The condensation of phenol ester **28** with nitromethane gave **2** in high yield (77%). However, the conversion of **1** to **28** in the presence of oxalyl chloride was only 43%. Alternatively, **1** was first activated with CDI followed by addition of *t*-BuOK and nitromethane in anhydrous THF to give **2** in high overall yield (75%) in a one-pot procedure. Using DBU instead of *t*-BuOK as base, **2** was obtained in excellent yield (96%). This optimized condition was used in the preparation of 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine derivatives, as shown in Scheme 1. Details of reaction conditions and characterization data of compound **28** are summarized in the Supplementary data.

Under reflux, the reaction of compound **2** with hydroxylamine hydrochloride in EtOH did not yield **3,** but the *t*-Boc deprotection product of **3**. Most likely, the deprotection was due to the *in situ* generated hydrochloride during the condensation. When the reaction was carried out in the presence of $NaHCO_3$, **3** was obtained in 62% yield. We also found this reaction was highly temperature dependent. At 60 ^oC and 50 ^oC, **3** was isolated in 77% and 95% yield, respectively. Notably, intermediate **3** was an inseparable mixture of *cis*- and *trans*-isomers (*cis*/*trans* = 6/1 by ^1H NMR) and used "as is" in the next step. In a one-pot procedure, **3** was first condensed with 2-chloro-2-oxoacetate in the presence of TEA. Complete disappearance of **3** was observed by TLC after 24 h, suggesting both *cis*- and *trans*-**3** reacted with 2-chloro-2-oxoacetate. Only the intermediate derived from the *cis*-isomer cyclized to furnish isoxazole **4**. No inter-conversion between *cis*- and *trans*-isomers was observed at any stage of the reaction.

As shown in Scheme 4, intermediate **4** was reduced to **29**. Direct cyclization of **29** with urea at high temperature failed to yield any appreciable amount of **30** due to charring. Intermediate **4** was converted to amide **5** by aminolysis using NH_3 in methanol. Intermediate **5** was reduced to **31** in high yield (90%). Reaction of **31** with triphosgene gave **32** as its hydrochloride salt in high yield (85%). Treatment of **32** with $POCl_3$ and *N*,*N*-dimethylaniline under reflux gave **33** cleanly, in which the *N*-Boc group was removed during the reaction (confirmed by LC-MS). Compound **33** exhibited excellent aqueous solubility and could not be isolated during workup. Based on these results, we decided to exchange the *N*-substituent on the piperidine from *t*-Boc- to benzyl (**5** to **7** *via* **6**) as shown in Scheme 1. Details of reaction conditions and characterization data related to compounds **29**, **31**, and **32** are summarized in the Supplementary data.



Scheme 4. Constructing the fused pyrimidine. Reagents and conditions: (a) Zn, NH_4Cl, EtOH/water = 1/1, 0 ^oC-r.t., 3 h, compound **29** (79% yield), compound **31** (90% yield). (b) urea, 210 ^oC. (c) NH_3/MeOH, r.t., 3 h, 96%. (d) triphosgene, anhydrous dioxane, reflux, 1 h, 85% (hydrochloride salt). (e) *N*,*N*-dimethylaniline, $POCl_3$, reflux, 8 h.

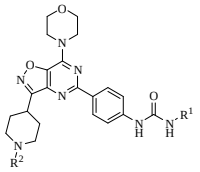
The transformation of **10** to **11** using Suzuki coupling was a problematic step. We examined the commonly used catalysts, such as $Pd(PPh_3)_4$, $Pd(OAc)_2$, $PdCl_2(dppf)$, and $Pd_2(dba)_3$, but all failed to produce product **11**. $Pd(OAc)_2$ in combination with different phosphine ligands, such as 2-(di-*tert*-butylphosphino)biphenyl, butyldi-1-adamantylphosphine, and 2-(dicyclohexylphosphino)biphenyl, also did not yield any desired product. The starting material was recovered in all cases. Wang Shen [19] reported $Pd(PCy_3)_2Cl_2$ was an effective catalyst for the activation of the aryl chlorides in the Suzuki reaction. He speculated that the electron-rich nature of PCy_3 might enhance the oxidative insertion of palladium into the Ar-C1 bond and that the increased steric encumbrance of the ligand also might facilitate the dissociation of the ligands from the palladium complex. Using $Pd(PCy_3)_2Cl_2$ as the catalyst in the presence of CsF at 100 ^oC for 48 hours (Scheme 1), **11** was obtained in 34% yield with 21% **10** recovered from the reaction mixture. Some isoxazole ring-opening product *via* the reductive cleavage of the N-O bond was also observed. Further optimization, such as prolonging reaction time, higher reaction temperature and various bases (K_3PO_4, K_2CO_3 and KF) did not improve the yield. Higher reaction temperature and/or longer reaction time all led to decreased yield due to greater extent of the isoxazole ring-opening reaction.

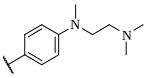
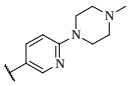
3.2 Biological evaluation

Cytotoxicity of compounds **11-23** against BT-474 cells was evaluated by CCK-8 assay. As summarized in Table 1, compared to other derivatives, **11** showed lower toxicity to BT-474 cells, indicating the urea moiety on the phenyl ring was required for activity. While keeping R^2 constant (R^2 = benzyl), various substituent on the urea (R^1) were examined. With large aromatic groups, **12**, **13**, and **14** showed substantial cytotoxicities, with IC_{50}s in the low to mid single digit micromolar range. In comparison, most alkyl derivatives, including the unsubstituted derivative **15**, showed greater potency, with IC_{50}s in the low single digit micromolar range, except the branched alkyl (**17**, R^1 = -$CH(CH_3)_2$, IC_{50} = 8.148 μmol/L). While keeping R^1 = CH_3, we also briefly investigated the effects of *N*-

substituents on the piperidine ring. *N*-ethyl substitution (**23**) and unsubstituted piperidine (**22**) all yield potent inhibitors, with IC_{50}s in the low single digit micromolar range, indicating this site is amendable for further modifications.

Table 1. The cytotoxicity of compounds **11-23** in BT-474 cells.



Compd.	R^1	R^2	IC_{50} (μmol/L)
11	See Scheme 1	-Bn	22.12
12		-Bn	5.242
13		-Bn	1.922
14		-Bn	6.896
15	-H	-Bn	1.724
16	$-CH_2CH_2N(CH_3)_2$	-Bn	1.895
17	$-CH(CH_3)_2$	-Bn	8.148
18	cyclopropyl	-Bn	2.759
19	$-CH_2CH_3$	-Bn	2.229
20	$-CH_2CH_2F$	-Bn	1.565
21	$-CH_3$	-Bn	1.311
22	$-CH_3$	-H	1.347
23	$-CH_3$	$-CH_2CH_3$	2.343

In combination with other data in hand, compounds **20** and **21** were further tested in biochemical assay to confirm their inhibition against PI3Kδ. As shown in Table 2, both **20** and **21** are potent PI3Kδ inhibitors with IC_{50}s values of 0.286 μmol/L and 0.452 μmol/L, respectively. In the same assay, CAL-101 showed an IC_{50} of 0.036 μmol/L [24]. These data indicate their anti-proliferative activities are most likely due to their inhibition of the PI3Kδ kinase.

Table 2 The inhibitory activities of compounds **20** and **21** against PI3Kδ

Compd.	PI3Kδ, IC_{50} (μmol/L)
20	0.286
21	0.452
CAL-101	0.036

4. Conclusion

In summary, an efficient syntheses of novel 3-(piperidin-4-yl)isoxazolo[4,5-d]pyrimidine scaffold was designed and developed. Using this method, a series of isoxazolo[4,5-d]pyrimidine derivatives has been prepared. Their cytotoxicity to BT-474 cells was evaluated by CCK-8 assay. Most of the derivatives displayed inhibitory potency against the proliferation of BT-474 cells. Preliminary SAR information from these compounds can be used to guide further exploration. Although the lead compounds were also shown to be potent inhibitors of PI3Kδ kinase, indicating their anti-cancer activity could be through PI3K pathway, further studies are needed to optimize their activities and to confirm their mechanism of action.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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