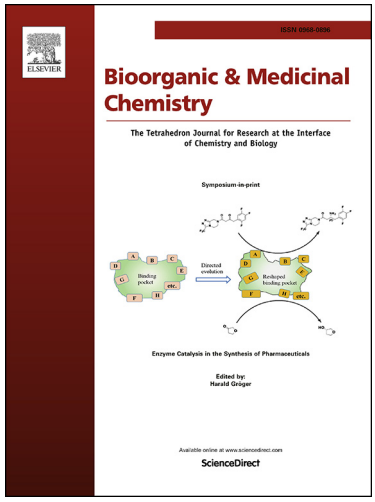
Journal Pre-Proof



Synthesis and Biological Evaluation of 4-(Piperid-3-yl)amino Substituted 6-Pyridylquinazolines as Potent PI3Kδ Inhibitors

Yifan Feng, Weiming Duan, Shu Fan, Hao Zhang, San-Qi Zhang, Minhang Xin

PII: S0968-0896(19)31006-5
DOI: https://doi.org/10.1016/j.bmc.2019.07.051
Reference: BMC 15035

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 19 June 2019
Revised Date: 29 July 2019
Accepted Date: 30 July 2019

Please cite this article as: Feng, Y., Duan, W., Fan, S., Zhang, H., Zhang, S-Q., Xin, M., Synthesis and Biological Evaluation of 4-(Piperid-3-yl)amino Substituted 6-Pyridylquinazolines as Potent PI3Kδ Inhibitors, *Bioorganic & Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.bmc.2019.07.051

Synthesis and Biological Evaluation of 4-(Piperid-3-yl)amino Substituted 6-Pyridylquinazolines as Potent PI3Kδ Inhibitors

Yifan Feng[†], Weiming Duan[†], Shu Fan, Hao Zhang, San-Qi Zhang, Minhang Xin *

Department of Medicinal Chemistry, School of Pharmacy, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an, Shaanxi 710061, P.R. China

---

Footnotes

* Corresponding author: Minhang Xin, E-mail: xmhcpu@163.com

[†] These authors contributed equally to this work.

Abstract: PI3Kδ is an intriguing target for developing anti-cancer agent. In this study, a new series of 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline derivatives were synthesized. After biological evaluation, compounds **A5** and **A8** were identified as potent PI3Kδ inhibitors, with IC_{50} values of 1.3 and 0.7 nM, respectively, which are equivalent to or better than idelalisib (IC_{50} = 1.2 nM). Further PI3K isoforms selectivity evaluation showed that compound **A5** afforded excellent PI3Kδ selectivity over PI3Kα, PI3Kβ and PI3Kγ. **A8** exhibited superior PI3Kδ/γ selectivity over PI3Kα and PI3Kβ. Moreover, compounds **A5** and **A8** selectively exhibited anti-proliferation against SU-DHL-6 *in vitro* with IC_{50} values of 0.16 and 0.12 μM. Western blot analysis indicated that **A8** could attenuate the AKT^{S473} phosphorylation. Molecular docking study suggested that **A8** formed three key H-bonds action with PI3Kδ, which may account for its potent inhibition of PI3Kδ. These findings indicate that 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline derivatives were potent PI3Kδ inhibitors with distinctive PI3K-isoforms and anti-proliferation profiles.

Keywords: PI3Kδ inhibitors; PI3K inhibitors; 6-pyridylquinazolines; anti-proliferation.

1. Introduction

Phosphoinositide 3-kinases (PI3Ks) are members of a family of key intracellular enzymes. PI3Ks mediate the phosporylation procedure, and trigger the downstream signal transducer AKT (protein kinase B) and mTOR (Mannalian target of rapamycin) to regulate cellular activities such as cell proliferation, differentiation, migration and survival. PI3K family consists of three classes. The class I PI3Ks are well characterized, which are further individed into PI3Kα, β, δ and γ.[1] PI3Kα and β are widely expressed, whereas PI3Kδ and γ are predominantly restricted in hematopoietic cells.[2] Consequently, inhibition of PI3Kδ (or mixed PI3Kδ/γ) was thought to enable to combat cancers, immunological and inflammatory conditions and development of small molecule PI3Kδ inhibitors receives significant interest from industrial and academic community.[3]

Many classes of PI3Kδ inhibitors have been investigated, and some have been approved or entered into clinical trials for treatment of leukemia, lymphoma, asthma, chronic obstructive pulmonary disease (COPD), sjogren's syndrome, activated PI3Kδ syndrome (APDS), and rheumatoid arthritis.[4] The first-in-class orally potent selective PI3Kδ inhibitor idelalisib (**1**) was approved by FDA (US Food and Drug Administration) in 2014 for treating chronic lymphocytic leukemia (CLL), follicular lymphoma (FL) and small lymphocytic lymphoma (SLL).[5] Although idelalisib gave significant efficacy in clinic, it showed severe toxicity leading to its approval with black-box warning. [6] Later in 2018, the selective PI3Kδ/γ inhibitors duvelisib (**2**) was approved by FDA also for treating CLL, FL and SLL, which was structurally similar to idelalisib.[7] Duvelisib was reported to show lower side effects than idelalisib. Umbralisib (**3**) was another potent selective PI3Kδ inhibitor, studied in phase III clinical trials for its efficacy against B-cell lymphoma. [8] Leniolisib (**4**), developed by Novartis, was an orally potent selective PI3Kδ inhibitor which recently entered phase III clinical evaluation for rare autoimmune diseases such as APDS and Sjogren's syndrome.[9, 10] Nemiralisib (**5**), developed by GSK (GlaxoSmithKline), was in phase II trials for treatment of asthma and COPD [11] (**Figure 1**). Overall, these approved and late-stage clinical selective PI3Kδ inhibitors are categorized into two classes and exemplified as follows: (1) propeller-shaped inhibitors such as idelalisib, duvelisib, and umbralisib, (2) flat-shaped inhibitors such as leniolisib and nemiralisib.[12] Considering the reported propeller-shaped PI3Kδ inhibitors

demonstrating severe side effects, there is greater need to develop new classes of PI3Kδ inhibitors differentiated from propeller-shaped ones, so as to investigate the medication regimen and improve the safety.[13] Herein, we reported our drug effort on development of PI3Kδ inhibitors which differed from propeller-shaped ones.

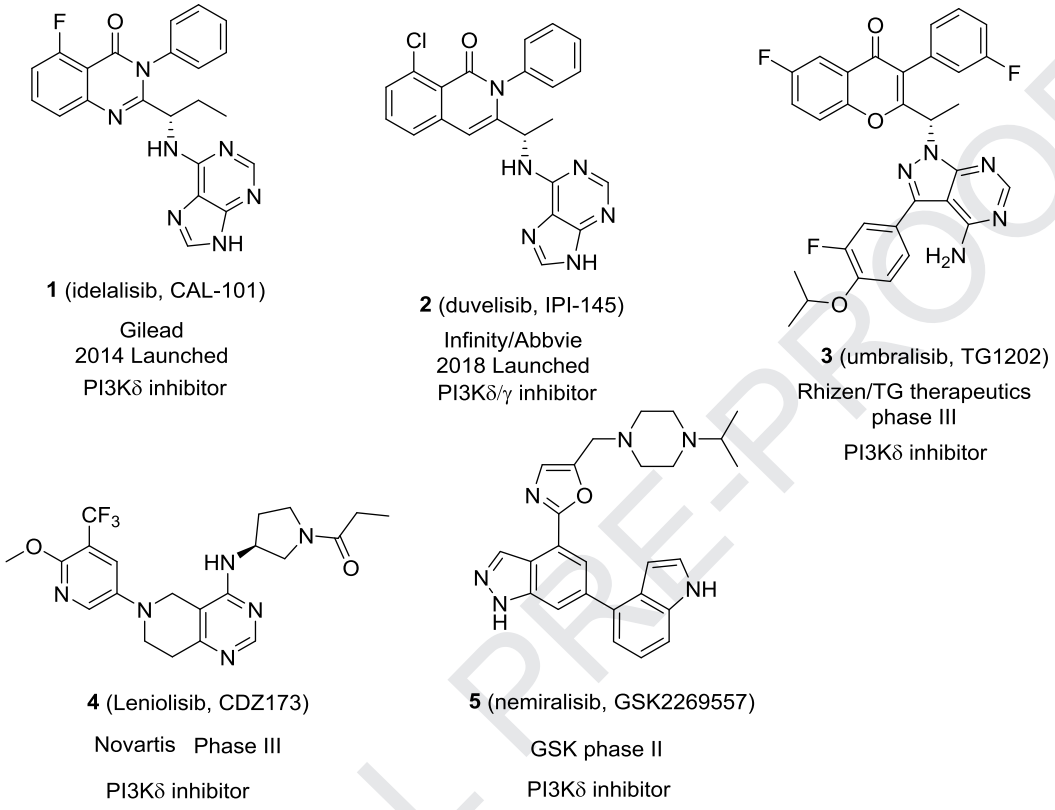
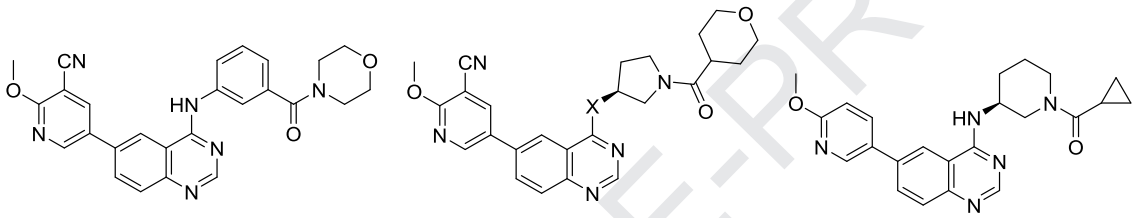


Figure 1. Representative structures of approved and late-stage clinical PI3Kδ inhibitors.

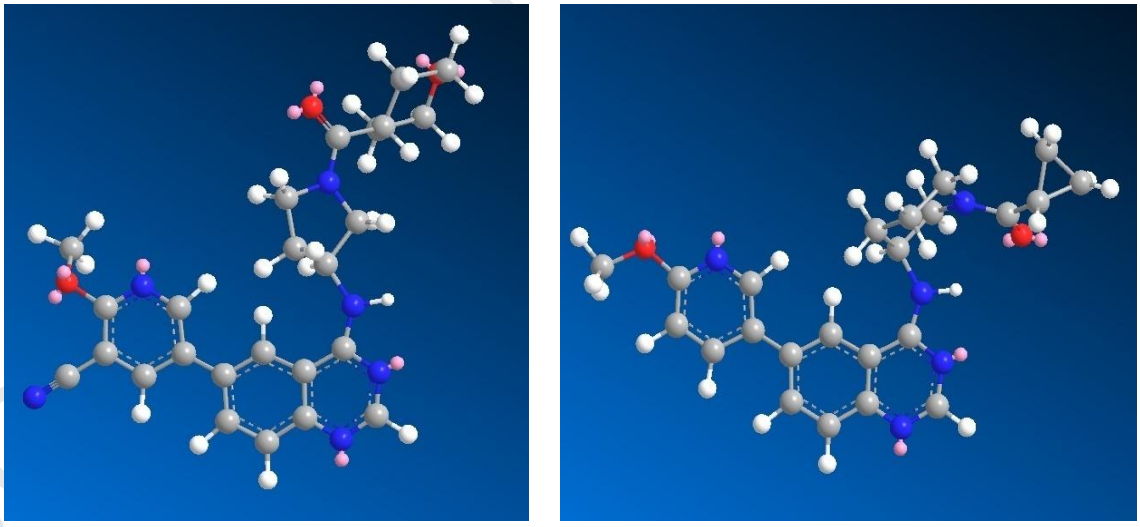
Recently, we disclosed a series of potential PI3Kδ inhibitors containing a quinazoline scaffold which were significantly different from the propeller-shaped inhibitors.[14] Exploring the 4-substitutents of 6-pyridylquinazoline led to the 4-aniline-6-pyridylquinazolines exemplified by **6** (PI3Kδ: IC_{50} = 9.3 nM) and the 4-pyrrolidineamino-6-pyridylquinazolines exemplified by **7** (PI3Kδ: IC_{50} = 2.7 nM).[15, 16] Simply switching the 4-pyrrolidineamino to 4-pyrrolidineoxy (or 4-(piperid-3-yl)amino) group made the PI3Kδ inhibitory activity retained or enhanced, such as **8** (PI3Kδ: IC_{50} = 4.9 nM) and **9** (PI3Kδ: IC_{50} = 3.0 nM).[17] In particular, we speculated that larger 4-(piperid-3-yl)amino moiety had a bigger twist angle compared to the 4-pyrrolidineamino motif linked to 6-pyridylquinazoline flat, which may contribute to

balance the PI3Kδ-selectivity (**Figure 2**). Therefore, we carry out a further structural investigation by introducing H-bond acceptor groups such as cyano, nitro and trifluoromethyl at 5-position of 6-pyridyl moiety and several side chains such as hydrophilic and hydrophobic groups as the tails linked to the 4-(piperid-3-yl)amino moiety, so as to establish a comprehensive structure-activity relationship (SAR) and the bioactive profiles. We now report the synthesis and biological evaluation of this series of new 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline analogues as potent PI3Kδ inhibitors, notably, of which several compounds showed significant selectivity in both PI3Kδ inhibition and anti-proliferative activity of cells lines (**Figure 3**).

(a)



(b)



compound **7** compound **9**

Figure 2. (a) Chemical structures of **6**, **7**, **8** and **9**; (b) 3D structures of **7** and **9**.

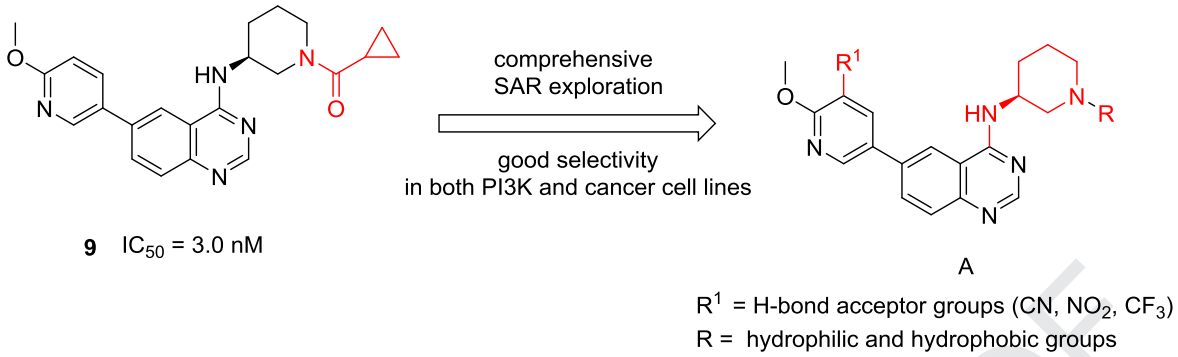
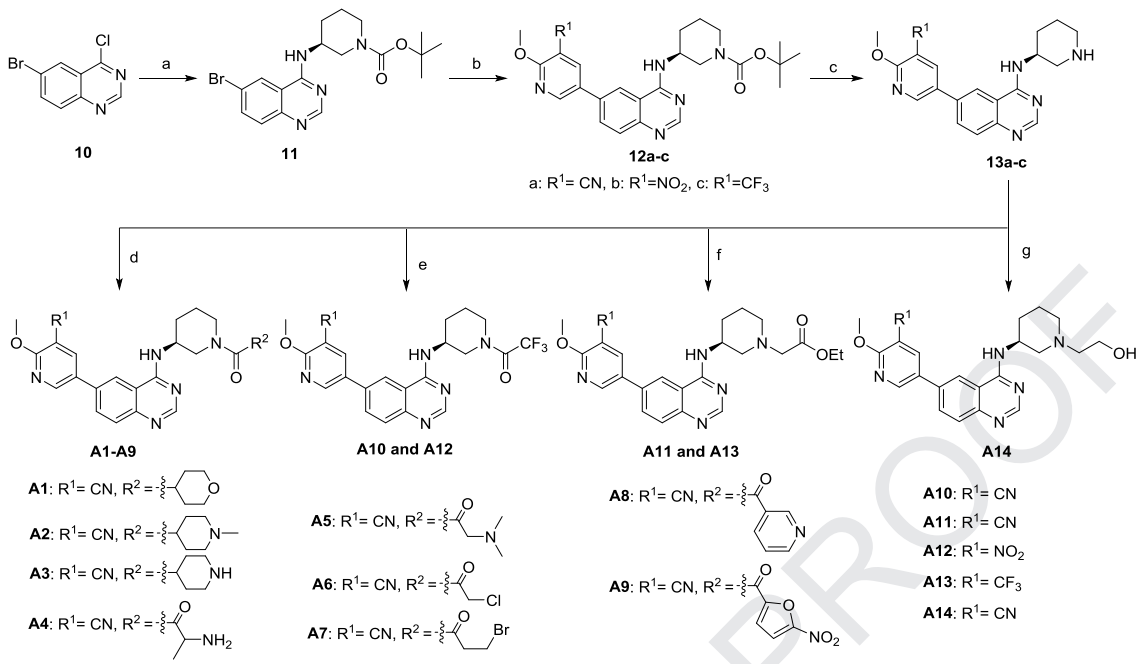


Figure 3. Current design of 4-(piperid-3-yl)amino substituted 6-pyridylquinazolines as PI3Kδ inhibitors.

2. Results and discussion

2.1 Chemistry

The synthetic route of theses 4-(piperid-3-yl)amino substituted quinazoline derivatives **A1-14** was outlined in Scheme 1. 6-Bromo-4-chloroquinazoline (**10**) reacted with (S)-1-Boc-3-aminopiperidine in the presence of *N*, *N*-diisopropylethylamine (DIPEA) in refluxing i-PrOH gave intermediate **11**. Subsequently, R^1-substituted 2-methoxy-3-bromopyridine reacted with bis(pinacolato)diboron under the Miyaura borylation condition without further separation to produce R^1-substituted 6-methoxypyridinylboronate, which were immediately treated with **11** using Pd-catalysized Suzuki-coupling reaction to provide **12a-c** in one pot. Removal of Boc-group of **12a-c** by TFA at room temperature afforded **13a-c**. **13a** was acylated with varied acids to generate compounds **A1-A9**. Compounds **A10** and **A12** were prepared following acylation procedure by TFAA, and compounds **A11**, **A13** and **A14** were prepared following nucleophilic substitution procedure by ethyl bromoacetate and bromoethanol, respectively (Scheme 1).



Scheme 1. Reagents and conditions: (a) (S)-1-Boc-3-aminopiperidine, DIPEA, i-PrOH, reflux, 85%; (b) (i) R^1-substituted 3-bromo-6-methoxypyridine KOAc, $PdCl_2$(dppf), dioxane, reflux, 2.5 h; (ii) R^1-substituted 6-methoxypyridinylboronate, Na_2CO_3, $PdCl_2$(dppf), dioxane/H_2O, reflux, 2.5 h; 57-79 % for two steps; (c) CH_2Cl_2:TFA = 5:1, rt, 2 h, 23-91 %; (d) diverse acids, DMF, HATU, DIPEA, rt, 12 h, 34-73 %; (e) TFAA, DMF, DIPEA, rt, 55 % for **A10** and 57% for **A12**; (f) $BrCH_2CO_2Et$, CH_2Cl_2, DIPEA, reflux, 43 % for **A11** and 57 % for **A13**; (g) $BrCH_2CH_2OH$, CH_3CN, DIPEA, reflux, 23 %.

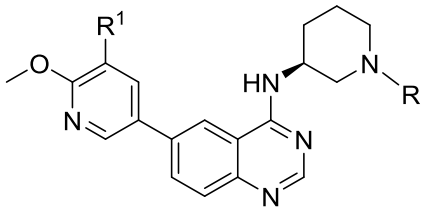
2.2 PI3Kδ inhibitory activities and structure-activity relationship (SAR) study

All the newly synthesized 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline analogues were firstly assessed for their inhibitory activities against PI3Kδ. Idelalisib was employed as the positive control. The IC_{50} values were illustrated in Table 1. As expected, all the 4-(piperid-3-yl)amino substituted 6-pyridylquinazolines showed significantly potent PI3Kδ inhibitory activities with IC_{50} values < 100 nM, suggesting the 4-(piperid-3-yl)amino-6-(6-methoxylpyridin-3-yl)quinazoline moiety is critical for maintaining the PI3Kδ inhibition. Based on the previously observed SAR from 4-pyrrolidineamino-6-6-pyridylquinazoline series, the cyano group was firstly fixed at the 5-position of 6-methoxylpyridin-3-yl moiety, several R side chains including hydrophilic and hydrophobic group were investigated. Compound **A1** bearing a hydrophilic tetrahydro-2*H*-pyran-4-carbonyl tail showed potent PI3Kδ inhibitory activity with an IC_{50} value of 7.7 nM, while replacement of the tetrahydro-2*H*-pyranyl group with

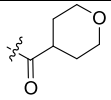
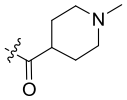
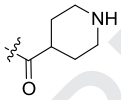
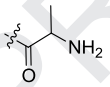
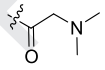
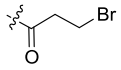
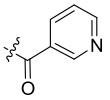
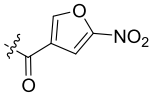
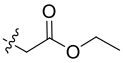
4-methylpiperidyl group gave equivalent potency (**A2**, IC_{50} = 9.4 nM). Removal of the methyl group led to compound **12c** showing 4-fold improved potency with IC_{50} value of 2.5 nM, whereas replacement with the 2-aminopropanoyl group retained approximately potent PI3Kδ activity (**A4**, IC_{50} = 3.7 nM). Tuning R to *N*,*N*-dimethylaminoacetyl group resulted in improving PI3Kδ inhibitory activity (**A5**: IC_{50} = 1.3 nM) which was equal to idelalisib (IC_{50} = 1.2 nM). Subsequently, compound **A6** bearing a hydrophobic chloroacetyl side chain afforded an IC_{50} value of 8.9 nM, while compound **A7** bearing bromopropionyl tail gave lower PI3Kδ inhibition with an IC_{50} value of 14.3 nM. Moreover, the heteroaromatic tails were attempted. Compound **A8** with a nicotinic terminal showed the most potent PI3Kδ inhibitory activity with an IC_{50} value of 0.7 nM, two-fold higher than idelalisib. Introduction of the 5-nitro-2-furoyl group gave compound **A9** showing PI3Kδ IC_{50} value of 3.9 nM. Furthermore, two hydrophobic terminal group were synthesized leading to compounds **A10** and **A11** tailed trifluoroacetyl and ethoxylcarbonylmethyl group, respectively, which displayed comparable potency, with IC_{50} value of both 3.2 nM, whereas further changing the cyano group into nitro (**A12**, IC_{50} = 27 nM) and trifluoromethyl group (**A13**, IC_{50} = 6.8 nM) on 6-pyridinyl moiety led to the potency 9- and 2-fold reduced, respectively. An attempt to introduce the hydroxyethyl tail led to compound **A14** showed PI3Kδ inhibition retainable, with an IC_{50} value of 4.7 nM. Overall, in this first PI3Kδ inhibition evaluation, two compounds **A5** and **A8** showed IC_{50} values of < 2 nM, which was equivalent to or better than idelalisib, and six compounds including **A3**, **A4**, **A9**, **A10**, **A11** and **A14** demonstrated IC_{50} values between 2 and 5 nM, which was comparable to idelalisib.

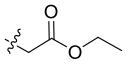
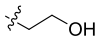
All the target compounds were subsequently examined for their cellular anti-proliferative activity against PI3Kδ-susceptible human malignant B-cell line SU-DHL-6. The results were summarized in Table 1. In general, except for compound **A12** with weak anti-proliferative efficacy against SU-DHL-6, the other compounds showed favorable anti-proliferation with micromolar IC_{50} values or below. Both of compounds **A5** and **A8**, the most potent PI3Kδ inhibitors, exhibited significantly anti-proliferative activity against SU-DHL-6 cell line with IC_{50} values of 0.16 and 0.12 μM, respectively.

Table 1. PI3Kδ inhibitory activity and SAR study of 4-(piperid-3-yl)amino substituted quinazolines [a]



A1-A14

Compds	R^1	R	PI3Kδ IC_{50} (nM)	SU-DHL IC_{50} (μM)
A1	CN		7.7 ± 2.1	0.15 ± 0.01
A2	CN		9.4 ± 0.1	0.42 ± 0.06
A3	CN		2.5 ± 0.3	2.44 ± 0.43
A4	CN		3.7 ± 0.2	0.44 ± 0.02
A5	CN		1.3 ± 0.4	0.16 ± 0.05
A6	CN		8.9 ± 1.0	0.38 ± 0.14
A7	CN		14.3 ± 1.0	0.015 ± 0.00
A8	CN		0.7 ± 0.1	0.12 ± 0.05
A9	CN		3.9 ± 0.7	0.14 ± 0.02
A10	CN		3.2 ± 0.2	0.21 ± 0.02
A11	CN		3.2 ± 0.9	0.50 ± 0.12
A12	NO_2		27.0 ± 3.1	127 ± 32

A13	CF_3		6.8 ± 1.8	0.23 ± 0.05
A14	CN		4.7 ± 2.1	2.05 ± 0.32
Idelalisib	-	-	1.2 ± 0.2	0.033 ± 0.10

[a] The IC_{50} values are shown as the mean ± SD (nM) from two separate experiments.

2.3 Isoform selectivity of the new PI3Kδ inhibitors

To further evaluated the selectivity of the title compounds, four compounds **A4**, **A5**, **A8**, and **A9** were evaluated for their inhibitory activities against a panel of PI3K kinases. The data were listed in Table 2. Obviously, compounds **A4** and **A5** were selective PI3Kδ inhibitors which showed weak inhibition against PI3Kβ and PI3Kγ, and moderate inhibition against PI3Kα. In particular, **A5** displayed 17-fold, 115-fold and 192-fold PI3Kδ selectivity over PI3Kα, PI3Kβ and PI3Kγ, respectively. **A8** was a PI3Kγ/δ inhibitor with IC_{50} values of 2.0 and 0.7 nM against PI3Kγ and PI3Kδ, respectively. Notably, **A8** exhibited 170-fold and 13-fold selectivity of PI3Kδ over PI3Kα and PI3Kβ, respectively. **A9** was a PI3Kα/δ inhibitor with IC_{50} values of 1.8 and 3.9 nM against PI3Kα and PI3Kδ, respectively, and showed moderate inhibition against PI3Kβ and PI3Kγ. Compounds selectively targeting PI3Kδ were thought to be capable of attenuating side effects. However, the orally potent PI3Kδ inhibitor idelalisib afforded severe toxicity in the clinical validation but the approved pan-PI3K inhibitor copanlisib demonstrated lower toxicity and fewer side effects compared to idelalisib. Therefore, the PI3K isoforms selectivity is still an open question. In this series, particularly, compound **A5** was a selective PI3Kδ inhibitor, and **A8** was a PI3Kγ/δ inhibitor (Table 2).

Table 2. PI3K isoform selectivity of compounds against Class I PI3Ks [a]

compds	IC_{50} (nM)			
	PI3Kα	PI3Kβ	PI3Kγ	PI3Kδ
A4	18.2 ± 1.4	147.4 ± 9.5	210.7 ± 7.4	3.7 ± 0.2
A5	21.8 ± 2.1	150.1 ± 5.4	250.4 ± 8.7	1.3 ± 0.4
A8	119.5 ± 6.2	9.1 ± 1.2	2.0 ± 0.2	0.7 ± 0.1
A9	1.8 ± 0.3	31.4 ± 2.1	19.3 ± 2.1	3.9 ± 0.7
Idelalisib	177.2 ± 4.4	139.1 ± 5.2	145.3 ± 7.9	1.2 ± 0.2

[a] The IC_{50} values are shown as the mean ± SD (nM) from two separate experiments.

2.4 Cellular anti-proliferation evaluation

Either the pan-PI3K or selective PI3Kδ (or PI3Kδ/γ) inhibitor mainly aimed to treat cancers. Consequently, furthermore, four compounds **A4**, **A5**, **A8**, and **A9** were tested for their anti-proliferative activities against five cancer lines including B cell lines SU-DHL-6, Ramos, Raji, and solid cancer lines HCT-116 and MCF-7 *in vitro*. Idelalisib and BEZ235 (a clinical PI3K/mTOR dual inhibitor) were used as positive control drugs. The data were shown in Table 3. It was found four compounds as well as idelalisib showed significantly selective anti-proliferative activity. They were effective only against SU-DHL-6 but no efficacy against Ramos, Raji, HCT-116 and MCF-7. Contrarily, BEZ235 exhibited potent anti-proliferation against all five cell lines. In a word, four compounds **A4**, **A5**, **A8**, and **A9** showed similar anti-proliferative profiles to idelalisib, which remarkably differed from PI3K/mTOR dual inhibitor BEZ235.

Table 3. Anti-proliferative activities of selected compounds *in vitro* [a]

compounds	IC_{50} (μM)				
	SU-DHL-6	Ramos	Raji	HCT-116	MCF-7
A4	0.44 ± 0.02	>10	>10	>10	>10
A5	0.16 ± 0.05	>10	>10	>10	>10
A8	0.12 ± 0.05	>10	>10	>10	>10
A9	0.14 ± 0.02	>10	>10	2.12 ± 0.97	>10
Idelalisib	0.033 ± 0.10	>10	>10	>10	>10
BEZ235	-	0.40 ± 0.00	0.52 ± 0.20	0.30 ± 0.12	0.2 ± 0.02

[a] The IC_{50} values are average ± SD (nM) of at least three independent experiments in triplicate.

2.5 PI3Kδ pathway cascade evaluation by AKT phosphorylation

Compound **A8** was a selective PI3Kδ inhibitor, which was further investigated for its effects on PI3K-mediated signaling in SU-DHL-6 cell line by western blot analysis. As shown in Figure 4, compound **A8** displayed good reduction of phosphorylation of AKT (Ser473) with a concentration-dependent manner. This result indicated that compound **A8** enable to inhibit the PI3K/AKT/mTOR signaling pathway (Figure 4).

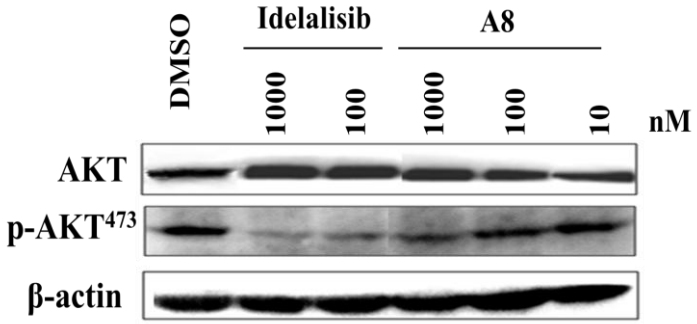


Figure 4. Effect of compound **A8** on AKT and p-AKT^{473}. SU-DHL-6 cells were treated with various concentration of **A8** for 8h and western blot for indicated proteins was performed. β-actin was used as a loading control.

2.6 Molecular modeling study

The molecular docking analysis of compound **A8** within human PI3Kδ enzyme were carried out to understand the potent PI3Kδ inhibition. As shown in Figure 5, the docked pose of **A8** showed the favorable interactions within the PI3Kδ binding pocket (PDB code: 6G6W). The quinazoline scaffold was engaged in H-bond contacts with Val828 in the hinge region, while the cyano group linked to 6-pyridyl moiety formed another H-bond with Lys779 in the affinity pocket. Moreover, the 4-(piperid-3-yl)amino side chain extended to solvent region and the carbonyl group generated an H-bond with Asn836. This region was close to the PI3Kδ isoform specificity pocket and had a hydrogen bond with Asn836 which were key factors to generate specific PI3Kδ selectivity [15]. After all, these results may account for the potent PI3Kδ inhibition for **A8** (Figure 5).

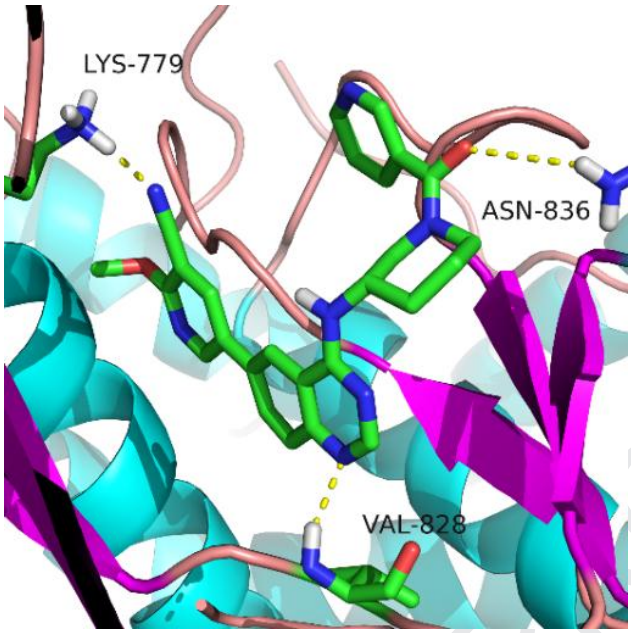


Figure 5. Molecular docking studies of compound **A8** into the site of PI3Kδ (PDB code: 6G6W). Compound is shown as sticks. H-bonds within 2.5 Å are shown as yellow dashed lines.

3. Conclusion

In summary, we described the design, synthesis and biological evaluation of a novel series of 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline derivatives as PI3Kδ inhibitors. The structure-activity relationship was discussed and many derivatives showed nanomolar PI3Kδ inhibitory activities. Compounds **A5** and **A8** displayed significantly potent PI3Kδ inhibitory activities with IC_{50} values of 1.3 and 0.7 nM, respectively, which was equivalent to or better than idelalisib (IC_{50} = 1.2 nM). Compounds **A5** and **A8** showed highly efficacious anti-proliferation against PI3Kδ-sensitive human malignant B-cell line SU-DHL-6 *in vitro* with IC_{50} values of 0.16 and 0.12 μM. Compounds **A5** showed superior isoform selectivity over PI3Kα, PI3Kβ and PI3Kγ, and **A8** showed favorable PI3Kγ/δ selectivity over PI3Kα and PI3Kβ. Interestingly, both **A5** and **A8** exhibited similarly potent anti-proliferation profiles to idelalisib. Western blot analysis of **A8**-treated SU-DHL-6 cell line indicated that **A8** reduced the AKT^{S473} phosphorylation. Molecular docking simulation suggested that the high potency of **A8** was attributed to the formation of three key H-bonds. These findings indicate the 4-(piperid-3-yl)amino substituted 6-pyridylquinazoline derivatives were potent PI3Kδ inhibitors with distinctive PI3K-isoforms and anti-proliferation profiles.

4. Experimental section

4.1 General methods

^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a 400 Bruker NMR spectrometer with TMS as an internal reference. Mass spectra (MS) were routinely recorded on an API 3200 LC/MS spectrometer. High-resolution MS (HRMS) were measured by electrospray ionization (positive mode) on a quadrupole time-of-flight (QTOF) mass spectrometer (Maxis Q-TOF, Bruker Inc.). The reagents and solvents were purchased commercially and used without further purification unless specially described otherwise. The starting material 6-bromo-4-chloroquinazoline (**10**) was prepared according to our previously reported methods [15].

4.1.1 (S)-tert-Butyl 3-((6-bromoquinazolin-4-yl) amino) piperidine-1-carboxylate (11)

To a solution of (S)-1-Boc-3-aminopiperidine (6.20 g, 30.99 mmol) in iso-propanol (20 mL), was slowly added 6-bromo-4-chloroquinazoline (5.00 g, 20.66 mmol) and DIPEA (5.33 g, 41.32mmol). The mixture was stirred at 85℃ for 2.5 h, and cooled to room temperature. After the saturated NaCl solution was added, the reaction mixture was extracted by EtOAc. Dried over anhydrous Na_2SO_4, the liquid was concentrated and purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 100:1) to produce compound **11** (7.15 g, 85 %) as a yellow solid. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H, ArH), 7.98 (s, 1H, ArH), 7.89-7.79 (m, 2H, ArH), 6.98 (s, 1H, NH), 4.41-4.39 (m, 1H, CH), 3.82-3.32 (m, 4H, 2×CH_2), 2.45-2.28 (m, 2H, CH_2), 1.98-1.75 (m, 2H, CH_2), 1.48 (s, 9H, 3×CH_3) ppm. MS (ESI) m/z: $[M+H]^+$ =406.9, 408.9.

4.1.2 (S)-tert-Butyl 3-((6-(6-methoxypyridin-3-yl)quinazolin-4-yl) amino) piperidine-1-carboxylate (12a)

Compound **12a** was prepared according our reported method. To a solution mixture of **11** (1.20 g, 2.95mmol) in DME/H_2O (8 mL/2 mL), were added the 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)nicotinonitrile (1.01 g, 3.9 mmol), $PdCl_2$(dppf) (270 mg, 0.12 mmol) and Na_2CO_3 (636 mg, 6.0 mmol). The mixture was refluxed under argon atmosphere for 4 h, cooled to room temperature and concentrated. The residue was purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 20:1) to give compound **12a** (1.10 g, 80 %) as a brown solid. MS (ESI) m/z: $[M+H]^+$

=461.2.

4.1.3 (S)-tert-Butyl 3-((6-(6-methoxy-5-nitropyridin-3-yl)quinazolin-4-yl)amino) piperidine-1-carboxylate (12b)

To a solution mixture of **11** (500 mg, 1.23mmol) in DME/H_2O (8 mL/2 mL), were added 2-methoxy-3-nitro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (518.2 g, 1.85 mmol), $PdCl_2$(dppf) (75 g, 0.12 mmol) and Na_2CO_3 (182 mg, 1.72 mmol). The mixture was refluxed under N_2 atmosphere for 4 h and then the mixture was concentrated and purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 20:1) to give compound **12b** (531 mg, 90 %) as a brown solid. MS (ESI) m/z: $[M+H]^+$ =481.2.

4.1.4 (S)-tert-Butyl 3-((6-(6-methoxy-5-(trifluoromethyl)pyridin-3-yl)quinazolin-4-yl)amino)piperidine-1-carboxylate (12c)

To a solution mixture of **11** (350 mg, 0.86 mmol) in DME/H_2O (8 mL/2 mL), were added the 2-methoxy-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3-(trifluoromethyl) pyridine (561 mg, 1.85 mmol), $PdCl_2$(dppf) (73.2 mg, 0.1 mmol) and Na_2CO_3 (182 mg, 1.72 mmol). The mixture was refluxed under N_2 atmosphere for 4 h and then the mixture was concentrated and purified by silica gel chromatography (CH_2Cl_2/CH_3OH, 20:1) to give compound **12c** (510 mg, 81 %) as a brown solid. MS (ESI) m/z: $[M+H]^+$ = 504.2.

4.1.5 (S)-2-Methoxy-5-(4-((1-(tetrahydro-2H-pyran-4-carbonyl)piperidin-3-yl)amino)quinazolin-6-yl)nicotinonitrile (A1)

To a mixture of **12a (**200 mg, 0.43 mmol) in CH_2Cl_2 (5 mL), was added TFA (1 mL). The mixture was stirred at room temperature for 2h. The mixture was concentrated, and then the residue was dissolved in CH_2Cl_2 (5 mL), were added tetrahydro-2H-pyran-4-carboxylic acid (85 mg, 0.63 mmol) and HATU (326 mg, 0.86 mmol) and DIPEA (77.4 mg, 0.6mmol). The reaction mixture was stirred at room temperature for 12h. The mixture concentrated and purified by silica gel chromatography to give compound **A1** (107 mg, 41 %) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H, Ar-H), 8.73 (s, 1H, NH), 8.63 (d, J = 19.0 Hz, 1H, Ar-H), 8.50 (d, J = 7.4 Hz, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 8.09 – 8.00 (m, 1H, Ar-H), 7.79 (s, 1H Ar-H), 4.30-4.25 (m, 1H, CH), 4.08 (s, 3H, OCH_3), 4.03 – 3.81 (m, 2H,

CH_2), 3.47 – 3.36 (m, 2H, CH_2), 3.22-3.15 (m, 1H, CH_2), 3.05-2.72 (m, 2H, CH_2), 2.60–2.50 (m, 1H, CH_2), 2.15-2.10 (m, 1H, CH_2), 1.90-1.78 (m, 2H, CH_2), 1.71 – 1.46 (m, 4H CH_2×2), 1.29-1.20 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 176.1, 172.7, 163.3, 159.5, 155.5, 150.2, 142.2, 132.6, 131.3, 128.9, 128.7, 120.9, 115.7, 115.4, 96.2, 66.8, 66.7, 55.4, 55.2, 54.1, 49.3, 37.3, 29.0, 18.5, 17.2, 13.0. ESI-HRMS $C_{26}H_{28}N_6O_3$, calcd $[M+H]^+$: 473.2296, found: 473.2306.

4.1.6 (S)-2-Methoxy-5-(4-((1-(1-methylpiperidine-4-carbonyl)piperidin-3-yl)amino)quinazolin-6-yl)nicotinonitrile (A2)

Using the similar procedure of **A1**, **A2** was prepared s a white solid. Yield: 56 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (d, *J* = 2.2 Hz, 1H, Ar-H), 8.70 (d, *J* = 2.3 Hz, 1H, NH), 8.61 (d, *J* = 14.0 Hz, 1H, Ar-H), 8.47 (d, *J* = 4.4 Hz, 1H, Ar-H), 8.15 (d, *J* = 8.6 Hz, 1H, Ar-H), 8.04 (dd, *J* = 18.6, 6.8 Hz, 1H, Ar-H), 7.76 (d, *J* = 8.6 Hz, 1H, Ar-H), 4.30 – 4.23 (m, 1H, CH), 4.06 (s, 3H, OCH_3), 3.18 – 2.92 (m, 4H CH_2×2), 2.80 – 2.53 (m, 4H CH_2×2), 2.16 (s, 3H CH_3), 2.13 – 1.85 (m, 3H CH_2+CH), 1.84 – 1.70 (m, 2H CH_2), 1.67 – 1.58 (m, 2H CH_2), 1.52 – 1.31 (m, 2H CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.8, 163.4, 159.5, 155.8, 155.7, 150.3, 149.4, 142.3, 132.7, 131.4, 128.9, 121.0, 115.7, 115.5, 96.3, 55.2, 53.6, 53.4, 49.3, 47.4, 46.1, 43.5, 42.1, 35.4, 30.2, 26.4, 24.4. ESI-HRMS $C_{27}H_{31}N_7O_2$, calcd $[M+H]^+$: 486.2612, found: 486.2617.

4.1.7 (S)-2-Methoxy-5-(4-((1-(piperidine-4-carbonyl)piperidin-3-yl)amino)quinazolin-6-yl)nicotinonitrile (A3)

Using the similar procedure of **A1**, **A3** was prepared as a white solid. Yield: 56 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (t, *J* = 1.8 Hz, 1H, Ar-H), 8.85 – 8.80 (m, 1H, NH), 8.76 (d, *J* = 1.8 Hz, 1H, Ar-H), 8.65 – 8.60 (m, 1H, Ar-H), 8.39 – 8.34 (m, 2H, Ar-H), 7.88 (dd, *J* = 5.7, 2.6 Hz, 1H, Ar-H), 4.40 – 4.35 (m, 1H, CH), 4.26 – 4.20 (m, 1H, NH), 4.06 (s, 3H, OCH_3), 4.05 – 3.95 (m, 2H, CH_2), 3.40 – 3.20 (m, 2H, CH_2), 3.18 – 2.92 (m, 4H, CH_2×2), 2.80 – 2.53 (m, 4H, CH_2), 2.20 – 2.10 (m, 1H CH), 1.95 – 1.65 (m, 6H CH_2×3), 1.60 – 1.40 (m, 1H, CH_2). ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.9, 163.3, 159.5, 158.6, 155.7, 150.2, 142.2, 132.6, 131.3, 128.9, 128.7, 121.1, 119.2, 115.7, 115.5, 96.2, 55.2, 49.3, 46.0, 43.1, 42.9, 42.6, 35.7, 34.6, 25.8, 25.5, 16.0. ESI-HRMS $C_{26}H_{29}N_7O_2$, calcd $[M+H]^+$: 472.2456, found: 472.2461.

4.1.8 (S)-5-(4-((1-Alanylpiperidin-3-yl)amino)quinazolin-6-yl)-2-methoxynicotinonitrile (A4)

Using the similar procedure of **A1**, **A4** was prepared as a white solid. Yield: 64 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.96 (s, 1H, Ar-H), 8.86 (d, *J* = 6.4 Hz, 1H, Ar-H), 8.73 (d, *J* = 2.0 Hz, 1H, Ar-H), 8.38 (d, *J* = 8.7 Hz, 1H, Ar-H), 8.17 (s, 2H, NH_2^+), 7.92 (dd, *J* = 8.6, 4.8 Hz, 1H, Ar-H), 4.67 – 4.38 (m, 3H, CH+NH_2), 4.08 (s, 3H OCH_3), 4.04 –3.89 (m, 1H, CH), 3.20-2.72 (m, 2H, CH_2), 2.20-2.00 (m, 1H, CH_2), 2.00-1.80 (m, 2H, CH_2), 1.55 – 1.45 (m, 1H CH_2), 1.40-1.30 (m, 3H, CH_3), 1.30-1.25 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.8, 163.7, 159.4, 155.5, 155.3, 149.0, 145.6, 138.2, 134.8, 131.4, 128.8, 128.7, 120.4, 115.5, 111.1, 53.8, 49.3, 48.8, 46.5, 42.4, 30.3, 24.3, 21.1. ESI-HRMS $C_{23}H_{25}N_7O_2$, calcd $[M+H]^+$: 432.2143, found: 432.2140.

4.1.9 (S)-5-(4-((1-(Dimethylglycyl)piperidin-3-yl)amino)quinazolin-6-yl)-2-methoxynicotinonitrile (A5)

Using the similar procedure of **A1**, **A5** was prepared as a white solid. Yield: 64 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (d, *J* = 2.2 Hz, 1H Ar-H), 8.70 (s, 1H NH), 8.62 (d, *J* = 10.4 Hz, 1H Ar-H), 8.56 – 8.47 (m, 1H Ar-H), 8.18 – 8.13 (m, 1H Ar-H), 8.04 (d, *J* = 6.6 Hz, 1H Ar-H), 7.76 (dd, *J* = 8.6, 5.4 Hz, 1H Ar-H), 4.25 – -4.10 (m, 1H, CH), 4.06 (s, 3H, OCH_3), 4.00 – 3.50 (m, 2H, CH_2), 3.45 – 3.20 (m, 2H, CH_2), 3.10 – 2.70 (m, 2H, CH_2), 2.45 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 2.11 – 1.75 (m, 2H, CH_2), 1.74 – 1.43 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3, 159.4, 159.4, 155.8, 150.3, 149.3, 142.3, 132.6, 131.4, 129.0, 128.8, 121.0, 115.7, 115.4, 96.2, 55.4, 55.2, 49.1, 48.5, 47.35, 46.0, 44.9, 44.7, 24.7, 24.1. ESI-HRMS $C_{24}H_{27}N_7O_2$, calcd $[M+H]^+$: 446.2299, found: 446.2301.

4.1.10 (S)-5-(4-((1-(2-Chloroacetyl)piperidin-3-yl)amino)quinazolin-6-yl)-2-methoxynicotinonitrile (A6)

Using the similar procedure of **A1**, **A6** was prepared as a white solid. Yield: 70 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.94 (s, 1H Ar-H), 8.71 (d, *J* = 2.3 Hz, 1H, NH), 8.62 (d, *J* = 11.2 Hz, 1H, Ar-H), 8.51 (d, *J* = 11.9 Hz, 1H, Ar-H), 8.16 (d, *J* = 8.4 Hz, 1H, Ar-H), 8.07 (dd, *J* = 11.6, 7.4 Hz, 1H Ar-H), 7.79 – 7.74 (m, 1H Ar-H), 4.55 – 4.50 (m,

1H, CH), 4.44 – 4.29 (m, 2H CH_2), 4.26-4.18 (m, 2H, CH_2), 4.07 (s, 3H OCH_3), 3.17 – 3.06 (m, 1H, CH_2), 2.82 – 2.65 (m, 1H, CH_2), 2.15 – 1.75 (s, 2H, CH_2), 1.78 – 1.51 (m, 2H CH_2).^{13}C NMR (100 MHz, DMSO-d_6) δ 165.2, 163.3, 159.4, 155.8, 150.3, 149.2, 142.3, 132.7, 131.3, 129.0, 129.0, 128.7, 121.0, 115.7, 115.4, 96.2, 55.4, 55.2, 49.1, 47.4, 42.7, 42.5, 30.3, 24.0. ESI-HRMS $C_{22}H_{21}ClN_6O_2$, calcd $[M+H]^+$: 437.1487, found: 437.1487.

4.1.11

(S)-5-(4-((1-(2-Bromopropionyl)piperidin-3-yl)amino)quinazolin-6-yl)-2-methoxynicotinonitrile (A7)

Using the similar procedure of **A1**, **A7** was prepared as a white solid. Yield: 35 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (d, J = 2.4 Hz, 1H, Ar-H), 8.69 (d, J = 1.8 Hz, 1H, Ar-H), 8.59 (s, 1H, NH), 8.51 (d, J = 12.8 Hz, 1H, Ar-H), 8.15 (dd, J = 8.7, 1.3 Hz, 1H Ar-H), 8.05 – 8.01 (m, 1H, Ar-H), 7.76 (d, J = 8.7 Hz, 1H Ar-H), 6.89 – 6.74 (m, 1H, CH_2), 6.15 – 6.08 (m, 1H, CH_2), 5.74 – 5.60 (m, 1H, CH_2), 4.67 – 4.20 (m, 3H CH + CH_2), 4.06 (s, 3H OCH_3), 3.17 – 2.68 (m, 2H, CH_2), 2.14 – 1.85 (m, 2H, CH_2), 1.85 – 1.53 (m, 2H, CH_2), 1.28-1.07 (m, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 164.9, 163.3, 155.8, 150.2, 149.2, 142.2, 132.6, 131.3, 129.0, 128.7, 127.7, 120.9, 115.7, 115.4, 96.20, 55.2, 49.7, 48.7, 47.5, 30.3, 25.4, 24.1. ESI-HRMS $C_{23}H_{24}BrN_6O_2$, calcd $[M+H]^+$: 495.1139, found: 495.1158.

4.1.12 (S)-2-Methoxy-5-(4-((1-nicotinoylpiperidin-3-yl)amino)quinazolin-6-yl) nicotinonitrile (A8)

Using the similar procedure of **A1**, **A8** was prepared as a white solid. Yield: 67 %; ^{1}H-NMR (400 MHz, DMSO-d_6) δ 9.30 – 8.97 (m, 2H, Ar-H), 8.74 – 8.48 (m, 5H, NH+Ar-H), 8.29 – 8.15 (m, 1H Ar-H), 7.90 – 7.76 (m, 2H, Ar-H), 7.52 – 7.40 (m, 1H Ar-H), 4.30 – 4.25 (m, 1H, CH), 4.04 (s, 3H OCH_3), 3.90 – 3.51 (m, 2H CH_2), 3.25 – 2.90 (m, 2H, CH_2), 2.23 – 2.00 (m, 1H, CH_2), 2.00 – 1.75 (m, 2H, CH_2), 1.75 – 1.60 (m, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.5, 163.4, 159.7 154.1, 154.0, 150.8, 150.7, 150.7, 150.3, 148.1, 147.9, 142.3, 135.2, 132.5, 132.2, 128.5, 126.2, 123.0, 121.7, 121.5, 115.6, 114.8, 96.2, 55.2, 53.8, 49.0, 42.1, 18.5, 17.2. ESI-HRMS $C_{26}H_{23}N_7O_2$, calcd $[M+H]^+$: 466.1986, found: 466.1984.

4.1.13 (S)-2-Methoxy-5-(4-((1-(5-nitrofuran-2-carbonyl)piperidin-3-yl)amino)

quinazolin-6-yl)nicotinonitrile (A9)

Using the similar procedure of **A1**, **A9** was prepared as a white solid. Yield: 36 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H, Ar-H), 8.70 (s, 1H, Ar-H), 8.60 (s, 1H, Ar-H), 8.50 (s, 1H, NH), 8.15 (d, J = 8.4 Hz, 1H, Ar-H), 8.01 (s, 1H, Ar-H), 7.79 (d, J = 3.8 Hz, 1H, Ar-H), 7.75 (d, J = 8.0 Hz, 1H, Ar-H), 7.58 (s, 1H Ar-H), 4.38 – 4.30 (m, 1H, CH), 4.29 – 4.09 (m, 2H, CH_2), 4.06 (s, 3H, OCH_3), 3.40 – 3.30 (m, 2H CH_2), 2.20 – 2.13 (m, 1H, CH_2), 1.98 – 1.75 (m, 2H, CH_2), 1.70 – 1.65 (m, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.3, 159.3, 157.4, 155.4, 151.6, 150.3, 149.3, 142.3, 140.8, 132.7, 131.3, 129.0, 128.8, 121.0, 116.9, 115.7, 115.3, 113.3, 96.2, 55.4, 55.2, 50.6, 43.5, 29.8, 23.8. ESI-HRMS $C_{25}H_{21}N_7O_5$, calcd $[M+H]^+$: 500.1677, found: 500.1679.

4.1.14 (S)-2-Methoxy-5-(4-((1-(2,2,2-trifluoroacetyl)piperidin-3-yl)amino) quinazolin-6-yl)nicotinonitrile (A10)

Using the similar procedure of **A1**, **A10** was prepared as a white solid. Yield: 20 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (d, J = 1.5 Hz, 1H, Ar-H), 8.83 (d, J = 2.3 Hz, 1H, Ar-H), 8.65 (dd, J = 6.4, 1.6 Hz, 1H, Ar-H), 8.52 (d, J = 4.0 Hz, 1H, NH), 8.20 (dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 8.19 – 8.10 (m, 1H, Ar-H), 7.80 (d, J = 8.7 Hz, 1H, Ar-H), 4.38 – 4.30 (m, 1H, CH), 4.11 (s, 3H OCH_3), 3.33 – 3.10 (m, 2H CH_2), 2.15 – 2.05 (m, 1H, CH_2), 2.00 – 1.94 (m, 1H, CH_2), 1.88 – 1.75 (m, 1H, CH_2), 1.70 – 1.55 (m, 1H, CH_2), 1.30 – 1.20 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.4, 159.6, 159.5, 155.4, 150.3, 142.4, 133.0, 133.0, 131.7, 129.0, 128.2, 128.1, 121.1, 115.7, 115.2, 96.3, 55.2, 54.1, 42.3, 18.5, 17.2, 13.0. ESI-HRMS $C_{22}H_{19}F_3N_6O_2$, calcd $[M+H]^+$: 457.1594, found: 457.1597.

4.1.15 Ethyl (S)-2-(3-((6-(5-cyano-6-methoxypyridin-3-yl)quinazolin-4-yl)amino) piperidin-1-yl)acetate (A11)

Using the similar procedure of **A1**, **A11** was prepared as a white solid. Yield: 57 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (d, J = 2.5 Hz, 1H), 8.69 (d, J = 2.5 Hz, 1H, Ar-H), 8.58 (d, J = 1.6 Hz, 1H, Ar-H), 8.47 (s, 1H, NH), 8.13 (dd, J = 8.7, 1.8 Hz, 1H, Ar-H), 7.91 (d, J = 7.8 Hz, 1H, Ar-H), 7.73 (d, J = 8.7 Hz, 1H, Ar-H), 4.43 – 4.33 (m, 1H, CH), 4.12 – 4.06 (m, 2H CH_2), 4.06 (s, 3H, OCH_3), 3.32 – 3.25 (m, 2H, CH_2), 3.08 – 3.00 (m, 1H, CH_2), 2.81 – 2.75 (m, 1H, CH_2), 2.36 – 2.30 (m, 2H, CH_2), 2.01 – 1.91 (m, 1H, CH_2), 1.79 – 1.71 (m, 1H, CH_2), 1.64 – 1.38 (m, 2H CH_2), 1.18 (t, J = 7.1 Hz, 3H, CH_3). ^{13}C

NMR (100 MHz, DMSO-d_6) δ 170.7, 163.3, 159.3, 155.9, 150.2, 149.2, 142.2, 132.5, 131.1, 129.0, 128.7, 120.8, 115.7, 115.4, 96.2, 60.3, 58.9, 57.3, 55.2, 52.4, 47.9, 29.9, 24.5, 14.6. ESI-HRMS $C_{24}H_{26}N_6O_3$, calcd $[M+H]^+$: 447.2139, found: 447.2132.

4.1.16 Ethyl (S)-2,2,2-trifluoro-1-(3-((6-(6-methoxy-5-nitropyridin-3-yl)quinazolin-4-yl) amino)piperidin-1-yl)ethan-1-one (A12)

Using the similar procedure of **A1**, **A12** was prepared as a white solid. Yield: 52 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.99 (d, *J* = 1.5 Hz, 1H, Ar-H), 8.82 (d, *J* = 2.3 Hz, 1H, NH), 8.65 (dd, *J* = 6.4, 1.6 Hz, 1H, Ar-H), 8.52 (d, *J* = 4.0 Hz, 1H, Ar-H), 8.20 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar-H), 8.15 – 8.06 (m, 1H, Ar-H), 7.79 (d, *J* = 8.7 Hz, 1H, Ar-H), 4.43 – 4.33 (m, 1H, CH), 4.10 (s, 3H OCH_3), 3.33 – 3.11 (m, 2H CH_2), 2.14 – 2.00 (m, 1H, CH_2), 2.00 – 1.93 (m, 1H, CH_2), 1.90 – 1.75 (m, 1H, CH_2), 1.65 – 1.50 (m, 1H, CH_2), 1.27 – 1.20 (m, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.5, 159.4, 155.8, 155.4, 150.2, 149.3, 134.3, 133.4, 132.4, 131.5, 129.3, 128.8, 121.2, 121.2, 115.4, 55.3, 47.9, 47.2, 43.8, 24.8, 23.4. ESI-HRMS $C_{21}H_{19}F_3N_6O_4$, calcd $[M+H]^+$: 477.1493, found: 477.1493.

4.1.17 Ethyl (S)-2-(3-((6-(5-cyano-6-methoxypyridin-3-yl)quinazolin-4-yl)amino)piperidin-1-yl)acet ate (A13)

Using the similar procedure of **A1**, **A13** was prepared as a white solid. Yield: 50 %; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H Ar-H), 8.62 (s, 1H NH), 8.49 (s, 1H Ar-H), 8.45 (s, 1H Ar-H), 8.17 (d, *J* = 8.5 Hz, 1H Ar-H), 7.96 (d, *J* = 7.6 Hz, 1H Ar-H), 7.76 (d, *J* = 8.6 Hz, 1H Ar-H), 4.43 – 4.33 (m, 1H, CH), 4.12 – 4.06 (m, 2H CH_2), 4.06 (s, 3H, OCH_3), 3.32 – 3.25 (m, 2H, CH_2), 3.08 – 3.00 (m, 1H, CH_2), 2.81 – 2.75 (m, 1H, CH_2), 2.36 – 2.30 (m, 2H, CH_2), 2.01 – 1.91 (m, 1H, CH_2), 1.79 – 1.71 (m, 1H, CH_2), 1.64 – 1.38 (m, 2H CH_2), 1.19 (t, *J* = 7.1 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.7, 160.0, 159.3, 155.8, 149.8, 149.2, 135.7, 133.1, 131.5, 128.8, 128.7, 122.2, 121.0, 115.5, 111.8, 60.3, 58.9, 57.3, 54.8, 52.4, 47.9, 29.9, 24.5, 14.6. ESI-HRMS $C_{24}H_{26}F_3N_5O_3$, calcd $[M+H]^+$: 490.2061, found: 490.2058.

4.1.18 (S)-5-(4-((1-(2-Hydroxyethyl)piperidin-3-yl)amino)quinazolin-6-yl)-2-methoxynicotino nitrile (A14)

Using the similar procedure of **A1**, **A14** was prepared as a white solid. Yield: 53 %; ^{1}H NMR (400 MHz, DMSO-*d*$_6$) δ 8.95 (s, 1H Ar-H), 8.74 – 8.68 (m, 1H Ar-H), 8.64 – 8.58 (m, 1H Ar-H), 8.51 – 8.47 (m, 1H Ar-H), 8.19 – 8.13 (m, 1H Ar-H), 8.07 – 7.95 (m, 1H NH), 7.78 – 7.73 (m, 1H Ar-H), 4.55 – 4.35 (m, 1H CH), 4.07 (s, 3H OCH_3), 3.60 – 3.50 (m, 2H CH_2), 3.40 – 3.30 (m, 2H CH_2), 3.07 (s, 1H OH), 2.75 – 2.65 (m, 2H CH_2), 2.40 – 2.25 (m, 2H, CH_2), 2.05 – 1.90 (m, 1H, CH_2), 1.85 – 1.75 (m, 1H, CH_2), 1.70 – 1.45 (m, 1H, CH_2). ^{13}C NMR (100 MHz, DMSO-*d*$_6$) δ 163.5, 160.1, 153.8, 150.4, 142.4, 134.1, 133.1, 128.5, 121.6, 119.0, 116.0, 115.6, 114.6, 113.1, 96.3, 58.9, 55.4, 55.3, 52.3, 46.08, 39.9, 27.8, 21.6. ESI-HRMS $C_{22}H_{24}N_6O_2$, calcd $[M+H]^+$: 405.2034, found: 405.2062.

4.2 PI3K enzymatic activity assay

The PI3K isoform activity assay was performed as our previous procedures.[18] All the PI3K isoform reaction was conducted at 30 ºC for 60 min. The 50 μL reaction mixture contains 50 mM HEPES (pH 7.5), 50 mM NaCl, 3 mM $MgCl_2$, 0.025 mg/mL BSA, 0.2 μg/mL PI3K, 10 μM ATP and 100 μM PI substrate. The tested compounds were diluted in 10% DMSO and 5 μL of the dilution was added to a 50 μL reaction so that the final concentration of DMSO is 1% in all the reactions. After the plate was cooled at room temperature for 5 min, 5 μL of ADP-Glo reagent was added into each well to stop the reaction and consume the remaining ADP within 40 min. At the end, 10 μL of kinase detection reagent was added into the well and incubated for 30 min. The assay was performed using Kinase-Glo Plus luminescence kinase assay. The luminescence signal was detected by VICTOR-X multi-label plate reader. The IC_{50} values were calculated using nonlinear regression with normalized dose−response fit using GraphPad Prism 5.0 sofeware.

4.3 Anti-proliferative assays using a MTT method i*n vitro*

Cellular proliferation was determined using a MTT method *in vitro*.[19]

4.4 Western blot assay

The levels of Akt and phospho-Akt (p-Akt, S473) in SU-DHL-6 cells were determined by Western blot. SU-DHL-6 cells were treated with final concentrations of 1000 nM, 100 nM, and 10 nM of **A8** and with the concentrations of 1000 and 100 nM of idelalisib for 8 h. Cell lysates were clarified by centrifugation at 12,000 rpm for 20 min at

4 ℃, and supernatant was collected. Equal amounts of protein were subjected to SDS-PAGE and transferred to nitrocellulose membranes (Merck Millipore, Billerica, MA, USA). The membranes were blocked and incubated overnight at 4 ℃ with primary antibodies against p-Akt, Akt, and beta-actin, followed by incubation with appropriate secondary antibodies. Antibody binding was detected with chemiluminescence reagents (Sigma-Aldrich, St. Louis, MO, USA).

4.5 Molecular docking simulation

The molecular docking was performed as our previous reported methods.[19]

Acknowledgements

This work was supported by the Natural Science Foundation of Shaanxi Province, China (2019JM-023). The authors also thank the Talent funding Program of Health Science Center, Xi'an Jiaotong University.

Appendix A. Supplementary data

Supplementary data to this article can be found attached.

References

[1] Liu P, Cheng H, Roberts TM, Zhao J. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat. Rev. Drug. Disc. **2009**, 8(8): 627-644.
[2] Garces AE, Stocks MJ, Class 1 PI3K clinical candidates and recent inhibitor design strategies: a medicinal chemistry perspective. J. Med. Chem. **2019**, 62(10): 4815-4850.
[3] Wei M, Wang X, Song Z, Jiao M, Ding J, Meng LH, Zhang A. Targeting PI3Kδ: emerging therapy for chronic lymphocytic leukemia and beyond. Med. Res. Rev. **2015**, 35(4): 720-752.
[4] Perry MWD, Abdulai R, Mogemark M, Petersen J, Thomas MJ, Valastro B, Westin Eriksson A. Evolution of PI3Kγ and δ inhibitors for inflammatory and autoimmune diseases. J. Med. Chem. **2019**, 62(10): 4783-4814.
[5] Fruman DA, Cantley LC. Idelalisib - a PI3Kδ inhibitor for B-cell cancers. N. Eng. J. Med. **2014**, 370(11): 1061-1062.
[6] Markham A. Idelalisib: first global approval. Drugs **2014**, 74(14): 1701-1707.
[7] Blair HA. Duvelisib: first global approval. Drugs **2018**, 78(17): 1847-1853.
[8] Barrientos JC. Can umbralisib bring PI3Kδ out of the shadows? Lancet Oncol. **2018**, 19(4): 432-434.
[9] Hoegenauer K, Soldermann N, Stauffer F, Furet P, Graveleau N, Smith AB, Hebach C, Hollingworth GJ, Lewis I, Gutmann S, Rummel G, Knapp M, Wolf RM, Blanz J, Feifel R, Burkhart C, Zecri F. Discovery and pharmacological characterization of novel quinazoline-based PI3K delta-selective inhibitors. ACS

Med. Chem. Lett. **2016**, 7(8): 762–767.

[10] Hoegenauer K, Soldermann N, Zecri F, Strang RS, Graveleau N, Wolf RM, Cooke NG, Smith AB, Hollingworth GJ, Blanz J, Gutmann S, Rummel G, Littlewood-Evans A, Burkhart C. Discovery of CDZ173 (Leniolisib), representing a structurally novel class of PI3K delta-selective inhibitors, ACS Med. Chem. Lett. 2017, 8(9): 975-980.

[11] Down K, Amour A, Baldwin IR, Cooper AW, Deakin AM, Felton LM, Guntrip SB, Hardy C, Harrison ZA, Jones KL, Jones P, Keeling SE, Le J, Livia S, Lucas F, Lunniss CJ, Parr NJ, Robinson E, Rowland P, Smith S, Thomas DA, Vitulli G, Washio Y, Hamblin JN. Optimization of Novel Indazoles as Highly Potent and Selective Inhibitors of Phosphoinositide 3-Kinase δ for the Treatment of Respiratory Disease. J Med Chem. **2015**, 58(18): 7381-7399.

[12] Berndt A, Miller S, Williams O, Le DD, Houseman BT, Pacold JI, Gorrec F, Hon WC, Liu Y, Rommel C, Gaillard P, Rückle T, Schwarz MK, Shokat KM, Shaw JP, Williams RL.
The p110 delta structure: mechanisms for selectivity and potency of new PI(3)K inhi bitors. Nat Chem Biol. **2010**, 6(2):117-24.

[13] Hoegenauer K, Soldermann N, Hebach C, Hollingworth GJ, Lewis I, Von Matt A, Burkhart C. Discovery of novel pyrrolidineoxy-substituted heteroaromatics as potent and selective PI3K delta inhibitors with improved physicochemical properties. Bioorg. Med. Chem. Lett, **2016**, 26(23): 5657-5662.

[14] Xin M, Hei YY, Zhang H, Xie XX, Mao S, Zuo SJ, Zhang SQ. Discovery of 6-benzamide containing 4-phenylquinazoline derivatives as novel PI3Kδ inhibitors. Lett. Drug Des. Dis. **2017**, 14(2): 167-174.

[15] Xin M, Hei YY, Zhang H, Shen Y, Zhang SQ. Design and synthesis of novel 6-aryl substituted 4-anilinequinazoline derivatives as potential PI3Kδ inhibitors. Bioorg. Med. Chem. Lett. **2017**, 27(9): 1972-1977.

[16] Xin M, Hei YY, Zhang H, Shen Y, Zhao HY, Mao S, Zhang SQ. Novel 6-aryl substituted 4-pyrrolidineaminoquinazoline derivatives as potent phosphoinositide 3-kinase delta (PI3Kδ) inhibitors Bioorg. Med. Chem. 2018, 26(8): 2028-2040.

[17] Xin M, Duan W, Feng Y, Hei YY, Zhang H, Shen Y, Zhao HY, Mao S, Zhang SQ. Introduction of pyrrolidineoxy or piperidineamino group at the 4-position of quinazoline leading to novel quinazoline-based phosphoinositide 3-kinase delta (PI3Kδ) inhibitors. J. Enzyme Inhib. Med. Chem, **2018**, 33(1): 651-656.

[18] Hei YY, Zhang SQ, Feng Y, Wang J, Duan W. Zhang H, Mao S, Sun H, Xin M. Alkylsulfonamide-containing quinazoline derivatives as potent and orally bioavailable PI3Ks inhibitors. Bioorg. Med. Chem. **2019**, https://doi.org/10.1016/j.bmc.2019.05.043

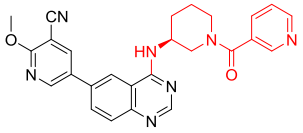
[19] Hei YY, Xin M, Zhang H, Xie XX, Mao S, Zhang SQ. Synthesis and antitumor activity evaluation of 4,6-disubstituted quinazoline derivatives as novel PI3K inhibitors. Bioorg. Med. Chem. Lett. **2016**, 26(18): 4408-4413.

Synthesis and Biological Evaluation of 4-(Piperid-3-yl)amino Substituted 6-Pyridylquinazolines as Potent PI3Kδ Inhibitors

Yifan Feng[†], Weiming Duan[†], Shu Fan, Hao Zhang, San-Qi Zhang, Minhang Xin *

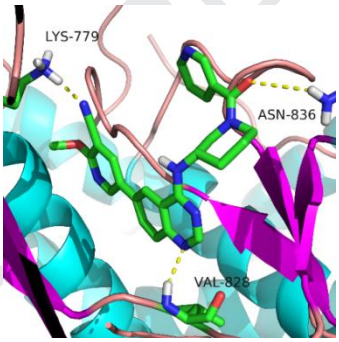
Department of Medicinal Chemistry, School of Pharmacy, Health Science Center, Xi'an Jiaotong University, 76 West Yanta Road, Xi'an, Shaanxi 710061, P.R. China

Graphical Abstract:



A8

IC_{50} = 0.7 nM (PI3Kdelta)
IC_{50} = 0.12 uM (SU-DHL-6)



* Corresponding author: E-mail: xmhcpu@163.com.