Introduction of pyrrolidineoxy or piperidineamino group at the 4-position of quinazoline leading to novel quinazoline-based phosphoinositide 3-kinase delta (PI3Kδ) inhibitors

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1. Introduction

Phosphoinositide 3-kinases (PI3Ks) play a pivotal role in multiple cellular functions including cell growth, development, migration, angiogenesis, and survival. Upon stimulation of cytokine signaling, PI3Ks function as an intracellular secondary messenger transforming phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3) via phosphorylation catalysis, thereafter activation of the downstream signal transducer (Akt, mTOR) and subsequent activator of transcription (Akt). PI3Ks function as an intracellular secondary messenger transduction and constitutive studies show activation of BCR signaling pathway is a hallmark of B-cell malignancies such as chronic lymphocytic leukemia (CLL), follicular lymphoma (FL), mantle cell lymphoma (MCL), small lymphocytic lymphoma (SLL), diffuse large B-cell lymphoma (DLBCL), and indolent non-Hodgkin’s lymphoma (INHL). Therefore, PI3Kδ is thought as a suitable target for the potential treatment of certain B-cell malignancies, as well as immunologic disorders (due to its specific role in controlling immune cell function). Notably, small molecules selective PI3Kδ inhibitor idelalisib (Compound 1) has been recently approved by Food and Drug Administration (FDA) for treatment of CLL, FL, and SLL, which solidify the therapeutic concept of PI3Kδ inhibitor (Figure 1).

Despite the first-in-class approved, potent and oral selective PI3Kδ inhibitor, idelalisib was tagged with black-box warning and demonstrated struggling with severe adverse events in the later clinical validation. Therefore, there is an urgent need to develop second generation PI3Kδ inhibitor with lower toxicity and fewer side effects. Duvelisib (Compound 2), another potent PI3Kδ inhibitor, shared chemical similarity to idelalisib, however, this was
recently terminated in the phase III clinical trials due to under-neath efficiency. Many other analogues derived from the chemical structure of idelalisib were recently reported and showed strong PI3Kδ efficacy and selectivity, for instances Compounds 3 (PI3Kδ): half maximal inhibitory concentration (IC50) = 2.2 nM, 4 (PI3Kδ): IC50 = 1.0 nM, and 5 (PI3Kδ): IC50 = 4.6 nM9-11. Nevertheless, our drug discovery efforts are engaged into the development of PI3Kδ inhibitors with novel and distinct chemotypes. Recently, we patented Compound 6 (PI3Kδ: IC50 = 9 nM) with potent PI3Kδ inhibition and selectivity13. A subsequent structural modification was carried out and a series of 4-anilinequinazolines was synthesised, exemplified by Compound 7 (PI3Kδ: IC50 = 17 nM)12, derived from the Novartis’s patented Compound 6 (PI3Kδ: IC50 = 9 nM) with potent PI3Kδ inhibition and selectivity13-15. A subsequent structural modification was carried out and a series of 4-anilinequinazolines was synthesised, exemplified by Compound 8 (PI3Kδ: IC50 = 9.3 nM) showing improved PI3Kδ inhibition16. Later, further structural investigation by replacing the 4-aniline with a 4-pyrrolidineamino moiety led to a series of potent and selective PI3Kδ inhibitors, such as Compound 9 (PI3Kδ: IC50 = 2.7 nM), showing equivalence to idelalisib in our examination (Figure 1)17. Encouraged by these fantastic findings, we decided to develop a new series of quinazoline based PI3Kδ inhibitors by introducing functionalised pyrrolidineoxy or piperidineamino group at the 4-position of quinazoline instead of the pyrrolidineamino moiety. Herein, we disclose the synthesis, biological evaluation of this series of 4-pyrrolidineoxy and 4-piperidineamino substituted quinazolines as potent and selective PI3Kδ inhibitors (Figure 2).

2. Results and discussion

2.1. Chemistry

The 4-pyrrolidineoxy and 4-piperidineamino substituted quinazoline derivatives were synthesised according to the synthetic routes outlined in Scheme 1. Treatment of 6-bromo-4-chloroquinazoline (Compound 10) with (S)-1-Boc-3-hydroxypyrrolidine in the presence of sodium hydride (NaH) gave (S)-4-pyrrolidineoxyquinazoline (Compound 11) in 70% yield, which was subsequently reacted with 6-methoxy-3-pyridinylboronic acid using Suzuki coupling condition to generate Compound 12a16,19. Compound 12a reacted with TFA at room temperature to get rid of the tert-butloxy carbonyl protecting group (Boc group) and then was acylated with diverse acids to afford Compounds 12(b-e). Compounds 14(a-f) and 16(a-c) were prepared by employing the similar synthetic procedures10. Compound 10 was treated with (S)-1-Boc-3-aminopiperidine or 1-Boc-4-aminopiperidine to give intermediate Compounds 13 and 15, respectively, which in turn underwent Suzuki coupling reaction, deprotection, and condensation to produce Compounds 14(a-f) and 16(a-c) successfully (Scheme 1).

![Figure 1. Representative structures for previously reported potent PI3Kδ inhibitors.](image1)

![Figure 2. Design of the 4-pyrrolidineoxy and 4-piperidineamino substituted quinazolines as PI3Kδ inhibitors.](image2)
2.2. PI3K\textsubscript{d} inhibitory activity for the title compounds

All the newly synthesised compounds were assessed for their PI3K\textsubscript{d} inhibitory activities and idelalisib was employed as the positive control. The 4-pyrrolidineoxy substituted quinazoline analogs were firstly examined and the results are shown in Table 1. It was found all the 4-pyrrolidineoxy substituted quinazoline analogues displayed significant PI3K\textsubscript{d} inhibitory activities under the concentration of 100 nM. The initial Compound 12a bearing a (S)-4-(1-Boc-pyrrolidin-3-yl)oxy side chain showed an inhibitory ratio of 79% at the concentration of 100 nM, while replacement of the tert-butoxy group with cyclopropyl group (Compound 12b: 90%) afforded enhanced PI3K\textsubscript{d} inhibitory activity, showing an IC\textsubscript{50} value of 9.3 nM. Switch of the cyclopropyl group (Compound 12b) to cyclobutyl (Compound 12c: IC\textsubscript{50} = 6.1 nM) and tetrahydro-2H-pyran-4-yl (Compound 12d: IC\textsubscript{50} = 4.9 nM) groups led to higher PI3K\textsubscript{d} inhibitory activity, whereas replacement with the branched tert-butyl (Compound 12e: 53%) resulted in weak PI3K\textsubscript{d} potency. In the 4-pyrrolidineoxy subseries, Compound 12d bearing tetrahydro-2H-pyran-4-yl side chain afforded the most potent PI3K\textsubscript{d} inhibitory activity.

**Table 1. PI3K\textsubscript{d} inhibitory activity of 4-pyrrolidineoxy substituted quinazolines.**

| Compounds | R          | PI3K\textsubscript{d} Inhibition (%)\textsuperscript{b} | PI3K\textsubscript{d} IC\textsubscript{50} (nM)\textsuperscript{c} |
|-----------|------------|----------------------------------------------------------|---------------------------------------------------------------|
| 12a       |            | 79                                                       | ND                                                            |
| 12b       |            | 90                                                       | 9.3                                                           |
| 12c       |            | 96                                                       | 6.1                                                           |
| 12d       |            | 98                                                       | 4.9                                                           |
| 12e       |            | 53                                                       | ND                                                            |
| 1         |            | 96                                                       | 2.7                                                           |

\textsuperscript{a}All the data are shown as the mean for at least two experiments.
\textsuperscript{b}PI3K\textsubscript{d} inhibition at the concentration of 100 nM.
\textsuperscript{c}The IC\textsubscript{50} values for PI3K\textsubscript{d} inhibition.

ND: not detected.

2.2. PI3K\textsubscript{d} inhibitory activity for the title compounds

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**Table 2. PI3K\textsubscript{d} inhibitory activity of 4-piperidineamino substituted quinazolines.**

| Compounds | R          | PI3K\textsubscript{d} Inhibition (%)\textsuperscript{b} | PI3K\textsubscript{d} IC\textsubscript{50} (nM)\textsuperscript{c} |
|-----------|------------|----------------------------------------------------------|---------------------------------------------------------------|
| 14a       |            | 51                                                       | ND                                                            |
| 14b       |            | 94                                                       | 3.0                                                           |
| 14c       |            | 91                                                       | 3.9                                                           |
| 14d       |            | 91                                                       | 8.7                                                           |
| 14e       |            | 88                                                       | 5.2                                                           |
| 14f       |            | 54                                                       | ND                                                            |
| 16a       |            | 72                                                       | ND                                                            |
| 16b       |            | 70                                                       | ND                                                            |
| 16c       |            | 48                                                       | ND                                                            |
| 1         |            | 96                                                       | 2.7                                                           |

\textsuperscript{a}All the data are shown as the mean for at least two experiments.
\textsuperscript{b}PI3K\textsubscript{d} inhibition at the concentration of 100 nM.
\textsuperscript{c}The IC\textsubscript{50} values for PI3K\textsubscript{d} inhibition.

ND: not detected.
inhibitory activity, which was approximately equivalent to control drug idelalisib (IC$_{50}$ = 2.7 nM; Table 1).

Subsequently, the 4-piperidineamino substituted quinazoline analogues were evaluated and the data are shown in Table 2. The initial (S)-4-(1-Boc-piperidin-3-yl)amino Compound 14a showed weak PI3K inhibitory activity with an inhibitory ratio of 51% at the concentration of 100 nM. However, replacement of tert-butoxy group with diverse cyclic aliphatic substituents afforded highly improved PI3K inhibitory activity. Analogue of Compound 14b bearing a cyclopropyl group gave an IC$_{50}$ value of 3 nM, and analogue of Compound 14c tailed with a cyclobutyl group showed almost comparable potency, with an IC$_{50}$ value of 3.9 nM, whereas analogues of Compound 14d with a cyclopropyl terminal and Compound 14e containing a tetrahydro-2H-pyran-4-yl tail showed a little less potent PI3K inhibition than that of Compound 14b, with IC$_{50}$ values of 8.7 and 5.2 nM, respectively. Again, an attempt to shift the cyclic group to non-cyclic alkyl group such as tert-butyl (Compound 14f: 54%) resulted in PI3K inhibition largely reduced. Otherwise, an exploration of changing the (S)-4-(piperidin-3-yl)amino side chain into 4-(piperidin-4-yl)amino group was also conducted, and three analogues were synthesised. However, unfortunately, Compound 16a bearing a Boc group (Compound 16a: 72%) and Compound 16b with a cyclopropyl group (Compound 16b: 70%) showed moderate PI3K inhibition, while Compound 16c incorporated with tetrahydro-2H-pyran-4-yl group (Compound 16c: 48%) produced unsatisfactorily weak potency. This suggested the spatial orientation of the tailed acyl substituents was critical for PI3K inhibition, which was consistent to the structure-activity relationship of our previously reported 6-aryl substituted 4-anilinequinazoline series. In this preliminary PI3K inhibition evaluation, three compounds 12d, 14b, and 14c showed IC$_{50}$ values beneath 5 nM, being approximately comparable to idelalisib, which were picked out for further evaluation.

### 2.3. Isoform selectivity of the new PI3K$\alpha$ inhibitors

Based on the above preliminary PI3K$\alpha$ inhibitory activity results, Compounds 12d, 14b, and 14c were subsequently evaluated for their selectivity among PI3K$\alpha$, PI3K$\beta$, and PI3K$\gamma$. As shown in Table 3, all three compounds 12d, 14b, and 14c showed much lower potency against other three PI3K isoforms than that of PI3K$\alpha$, although they displayed moderate PI3K$\alpha$ inhibition. Compound 12d with an IC$_{50}$ value of 4.5 nM against PI3K$\alpha$ demonstrated 11-fold, 131-fold, and 103-fold selectivity over PI3K$\alpha$, PI3K$\beta$, and PI3K$\gamma$, respectively, whereas Compounds 14b and 14c displayed the similar PI3K$\alpha$ selectivity which were 12- and 15-fold over PI3K$\alpha$, 105- and 96-fold over PI3K$\beta$, and 34- and 31-fold over PI3K$\gamma$, respectively. Moreover, it was noted that selectivity of compound 12d against the PI3K$\beta$ and PI3K$\gamma$ isoforms was much higher than idelalisib, although the poor selectivity against PI3K$\alpha$ was observed (Table 3).

### 2.4. In vitro anti-proliferative assays of the new PI3K$\alpha$ inhibitors

Furthermore, Compounds 12d, 14b, and 14c were tested for their anti-proliferative activities against four human B cell lines including Ramos, Raji, RPMI-8226, and SU-DHL-6 with idelalisib and SAHA as reference compounds. As shown in Table 4, Compound 12d exhibited most potent anti-proliferation against RPMI-8226 (IC$_{50}$ = 44 nM) among these four cell lines, whereas Compound 14b showed significantly potent anti-proliferative activity against Ramos, Raji, and SU-DHL-6, but moderate anti-proliferation against RPMI-8226 and Compound 14c also showed strong anti-proliferative activity against SU-DHL-6 with an IC$_{50}$ value of 1.49 nM. It was found that the reference PI3K$\alpha$ inhibitor idelalisib displayed markedly anti-proliferative activity against SU-DHL-6, whereas another reference drug SAHA (vorinostat) afforded significantly anti-proliferation against Ramos, Raji, and RPMI-8226. In a word, three Compounds 12d, 14b, and 14c as well as idelalisib were observed showing different anti-proliferative profiles in the four human B cell lines (Table 4).

### 2.5. Molecular modeling study

To further understand the potent PI3K$\alpha$ inhibition, molecular docking simulations of Compounds 12d, 14b, and 14c within human PI3K$\alpha$ enzyme were performed. As shown in Figure 3, the docked pose of each Compound (12d, 14b, and 14c) ma es the similarly favorable interactions with the PI3K$\alpha$ binding pocket of structure 2WXP as expected, namely, three key hydrogen bonds with the hinge residue, the quinazoline scaffold with Vai828, the methoxypyridyl moiety with Lys779, as well as the carbonyl group with Asn836. Moreover, it was observed that, although, the oxygen of the tetrahydro-2H-pyrane-4-yl group in Compound 20a formed an additional hydrogen bond with Asp753, it seemed to show little contribution for improving the inhibitory activity in this case (Figure 3).

### 3. Conclusion

In summary, we have synthesised and evaluated a novel series of quinazoline derivatives by introducing a functionalised 4-pyridineoxy or 4-piperidineamine groups as potent PI3K$\alpha$ inhibitors. The structure-activity relationship (SAR) was discussed and many derivatives showed nanomolar PI3K$\alpha$ inhibitory activities, particularly, Compounds 12d, 14b, and 14c demonstrating preferably potent PI3K$\alpha$ inhibitory activities with IC$_{50}$ values of 4.5, 3, and 3.9 nM, respectively, approximately comparable to idelalisib (IC$_{50}$ = 2.7 nM). Moreover, Compounds 12d, 14b, and 14c showed excellent PI3K$\alpha$ isoform selectivity over PI3K$\alpha$, PI3K$\beta$, and PI3K$\gamma$. These three compounds also displayed different anti-proliferative

### Table 3. Isoform selectivity of compounds against PI3K (p110$\alpha$, p110$\beta$, p110$\gamma$, and p110$\delta$)

| Compounds | IC$_{50}$ (nM) |
|-----------|----------------|
| 12d       | 14b            | 14c            | 1   |
| p110$\alpha$ | 50.4          | 36.6           | 58.9 | 306.4 |
| p110$\beta$ | 592.5         | 317.2          | 375.9 | 120.1 |
| p110$\gamma$| 467.6         | 104.0          | 121.1 | 139.4 |
| p110$\delta$| 4.9           | 3.0            | 3.9   | 2.7   |

*The IC$_{50}$ values are shown as the mean for at least two experiments.

### Table 4. Anti-proliferative activities of new compounds in vitro

| Compounds | IC$_{50}$ (mM) | SU-DHL-6 |
|-----------|----------------|----------|
| 12d       | 1.34           | 0.44     | 3.23   |
| 14b       | 1.34           | 0.81     | 8.66   | 1.04   |
| 14c       | ND             | ND       | 1.49   |
| 1         | >10            | 9.95     | 5.49   | 0.65   |
| SAHA      | 0.52           | 0.97     | 0.66   | ND     |

*The IC$_{50}$ values are shown as the mean for at least two experiments.

*Anti-proliferative activities were determined by[3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] tetrazolium (MTT) reduction method.

*Anti-proliferative activities were determined by CCK-8 method.

ND: not detected.
profiles against a panel of four human B cell lines. The molecular docking study indicated several key hydrogen bonding interactions formations, which may explain their higher PI3Kδ.

This study suggests the introduction of pyrrolidineoxy or piperidineamino groups into the 4-position of quinazoline leads to new potent and selective PI3Kδ inhibitors.

**Disclosure statement**

The authors declare no conflict of interest.

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**Figure 3.** Molecular docking studies of Compounds 12d (a), 14b (b) as well as 14c (c) into the site of PI3Kδ (PDB code: 2WXP). Compound is shown as sticks. Hydrogen bonds within 2.5Å are shown as yellow dashed lines.
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