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Discovery and optimization of a series of 2-aminothiazole-oxazoles as potent phosphoinositide 3-kinase γ inhibitors

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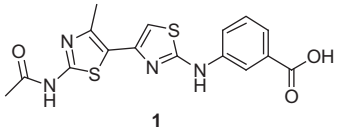
ABSTRACT

A novel series of 2-aminothiazole-oxazoles was designed and synthesized as part of efforts to develop potent phosphoinositide 3-kinase γ (PI3Kγ) inhibitors. The modification of a high-throughput screening hit, compound **1**, resulted in the identification of compounds **10** and **15**, which displayed potent inhibitory activities in enzyme-based and cell-based assays.

The phosphoinositide 3-kinase family (PI3Ks) of lipid kinases is involved in a diverse set of cellular functions, including cell growth, proliferation, motility, differentiation, glucose transport, survival intracellular trafficking, and membrane ruffling.[1] PI3Ks can be categorized as class I, II, or III molecules, depending on their subunit structure, regulation, and substrate selectivity. Class I PI3Ks are subdivided into class IA and class IB. Class IA PI3Ks contain p110α, p110β, and p110δ as catalytic subunits, and these subunits are activated in tyrosine kinase receptor signaling. Class IB PI3Ks contain only p110γ as a catalytic subunit, which is mostly activated by seven-transmembrane G-protein-coupled receptors (GPCRs) via the regulatory subunit p101 and the G-protein βγ subunits.[2,3] While PI3Kα and PI3Kβ are ubiquitously expressed, the expressions of PI3Kγ and PI3Kδ are mainly restricted to the hematopoietic system. Genetic studies show that PI3Kγ plays a crucial role in mediating leukocyte chemotaxis as well as mast cell degranulation, making it a potentially interesting target for autoimmune and inflammatory diseases such as psoriasis and rheumatoid arthritis.[4–10] Herein, we describe our initial efforts in this field, which led to the discovery and optimization of a series of 2-aminothiazole-oxazoles as potent PI3Kγ inhibitors.

We conducted a high-throughput screening (HTS) and identified compound **1** as an initial hit (Fig. 1). In response to this result, which had a promising enzyme activity (IC_{50} = 5 nM), compound **1** was docked into a molecular model that was constructed based on the X-ray structure of PI3Kγ co-crystallized with its inhibitor.[11,12] As shown in Figure 2, we speculated that nitrogen atoms in the



1

PI3Kγ IC_{50} = 5 nM

Figure 1. Structure and inhibitory activity of the initial hit compound **1**.

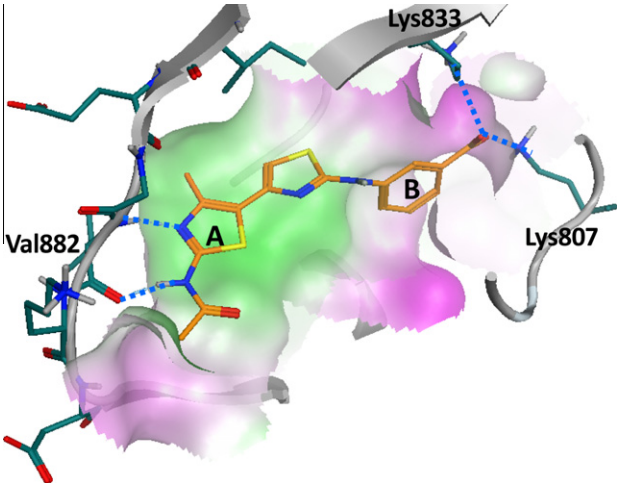


Figure 2. Predicted binding model of HTS hit compound **1** (orange) bound to the ATP site of PI3Kγ (PDB code:2CHZ).[11] The protein surface is colored according to the residue type (magenta, hydrophilic; light green, lipophilic; white, neutral). Oxygen, nitrogen and sulfur atoms are shown in the structure in red, blue, and yellow, respectively. Each hydrogen bond is shown by dotted lines (light blue).

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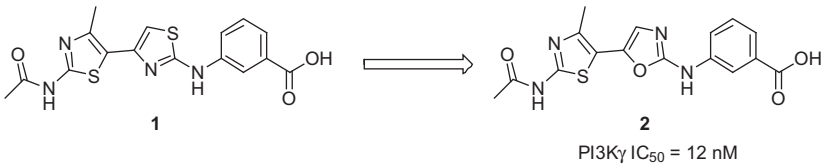


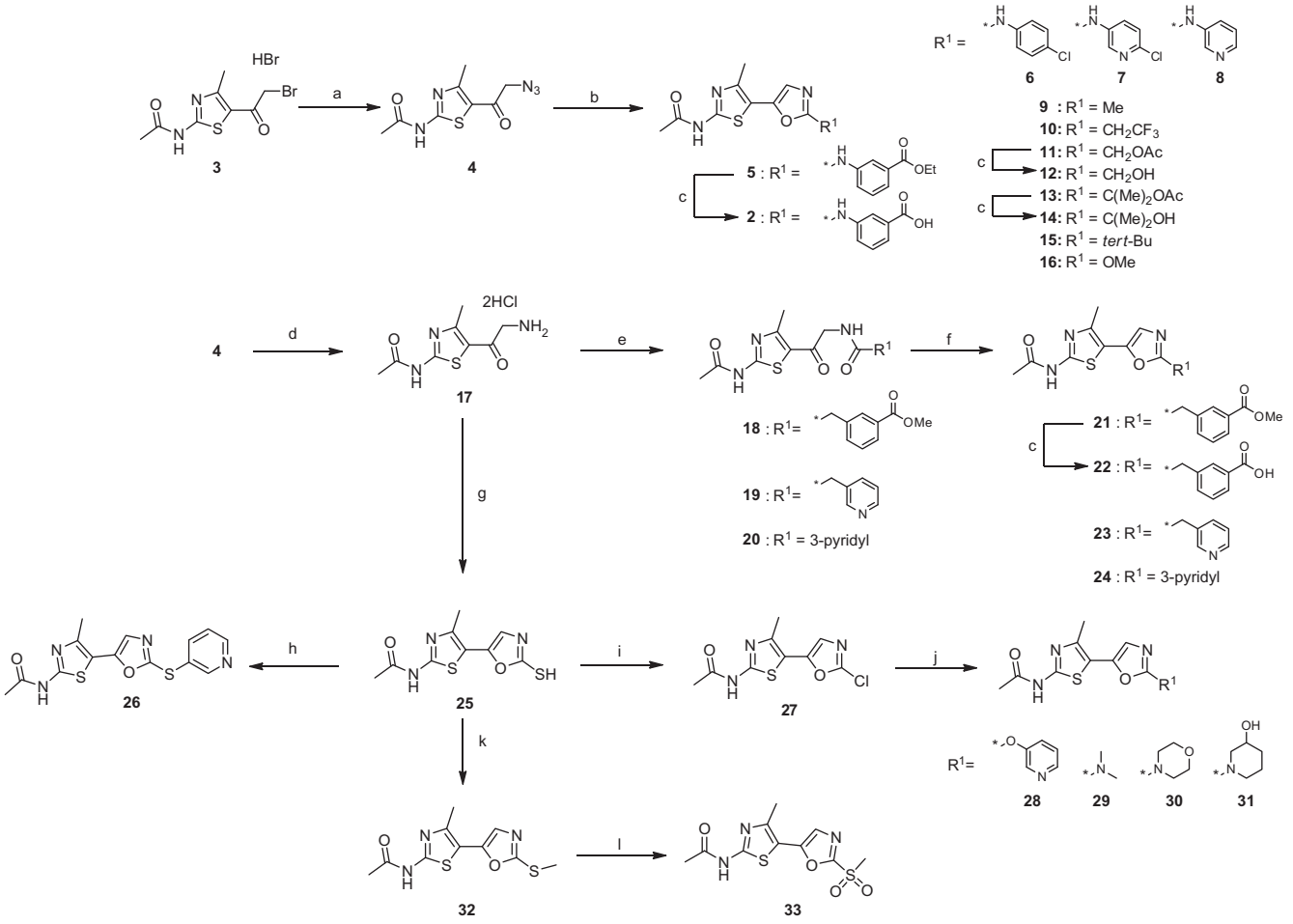
Figure 3. Modification of central heterocycle and 2-aminothiazole-oxazole derivative **2**.

aminothiazole formed known hydrogen bonds to the hinge Val882 and that the benzoic acid moiety interacted with Lys807 and Lys833 via hydrogen bonds.

Thus, our design strategy prioritized the incorporation of the benzoic acid pharmacophore (B ring in Fig. 2) into acetylaminothiazole (A ring in Fig. 2) through central heterocycles to generate novel molecules with the expectation of a high affinity for PI3Kγ. For this purpose, we synthesized various kinds of central heterocycles (data not shown) and identified oxazole derivative **2**, with an IC_{50} value of 12 nM (Fig. 3). This molecule appeared to be a potential lead for further optimization.

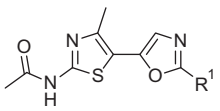
The general synthesis of 2-aminothiazole-oxazole derivatives is illustrated in Scheme 1.

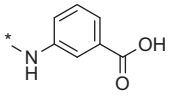
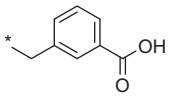
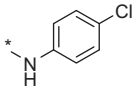
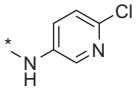
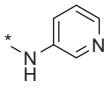
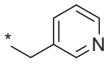
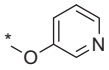
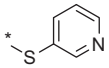
The first synthetic approach was as follows. Bromoacetylthiazole **3** was reacted with sodium azide to yield **4**. The following construction of the oxazole ring with acid chloride or isothiocyanate (as appropriate) using aza-Wittig reaction[13] provided target compounds **6**–**10**, **15**, and **16** and intermediates **5**, **11**, and **13**. The ester of **5** was hydrolyzed to the corresponding carboxylic acid **2**. Deprotection of the acetyl groups of **11** and **13** yielded the desired products **12** and **14**, respectively. In the second approach, reduction of the azide moiety of **4** under acidic condition yielded **17**, which was coupled with an appropriate carboxylic acid, followed by dehydration of **18**–**20** to yield target compounds **23**, **24**, and the intermediate **21**. Compound **22** was synthesized in a manner similar to the method described for the synthesis of **2**. Finally, in the third approach, **17** was converted to 2-mercapto-1,3-oxazole **25**, which served as a versatile intermediate for the preparation of several analogues, as shown in Scheme 1. Compound **25** was coupled with 3-iodopyridine to yield **26**. Chlorination of **25** with phosphorous oxychloride yielded 2-chlorooxazole **27**. Coupling or S_NAr displacement of **27** furnished the desired products **28**–**31**, respectively. Methylation of **25** yielded **32**, which was converted to **33** via oxidation. As shown, the synthetic availability allowed the rapid exploration of the SAR outlined below.



Scheme 1. Synthesis of 2-aminothiazole-oxazole derivatives. Reagents and conditions: (a) NaN_3, TEA, MeOH, rt, 90%; (b) R^1COCl, PPh_3, THF or dioxane, rt, or R^1NCS, PPh_3, dioxane, 100 °C; (c) aq KOH, THF–MeOH, 60 °C; (d) H_2, 10% Pd/C, *c* HCl, MeOH, rt, 75%; (e) R^1COOH, PyBOP®, TEA, DMF, rt, or R^1COCl, TEA, THF–$CHCl_3$, rt; (f) $POCl_3$, reflux, or Burgess reagent, THF, 80 °C; (g) CS_2, aq Na_2CO_3, EtOH, 80 °C, 57%; (h) 3-iodopyridine, NaO*t*-Bu, CuI, 1,10-phenanthroline, DMF, 100 °C, 56%; (i) $POCl_3$, 120 °C, 52%; (j) 3-hydroxypyridine, K_2CO_3, DMSO, 100 °C, 26%, or amine, THF, 80 °C; (k) MeI, K_2CO_3, DMF, rt, 45%; (l) H_2O_2, $Mo_7O_{24}(NH_4)_6{\cdot}4H_2O$, THF–EtOH, rt, 62%.

Table 1
SAR of oxazole derivatives with R^1-substitution modifications



Compound	R^1	PI3Kγ IC_{50}[a] (nM)	PI3Kα IC_{50}[a] (nM)	Selectivity (α/γ)	Akt IC_{50}[a] (nM)
2		12	328	27	>10,000
22		223	NT[b]		>10,000
6		346	NT[b]		NT[b]
7		24	NT[b]		1040
8		12	102	9	431[c]
23		95	NT[b]		NT[b]
28		117	NT[b]		1666[c]
26		16	112	7	279
24		3	46	15	59

[a] The IC_{50} value represents the mean of at least two independent experiments.
[b] NT = not tested.
[c] Data from one experiment ($n = 1$).

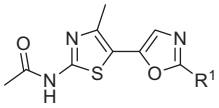
The series of 2-aminothiazole-oxazoles was screened using an enzyme assay,[14] and the effects of the derivatives on C5a-mediated PKB/Akt phosphorylation were investigated in Raw-264 murine macrophages.[15] This assay allowed the potency of PI3Kγ inhibition to be assessed in cells (Table 1).

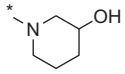
Compound **2** exhibited a good enzyme potency but it did not exhibit any inhibitory activity against PI3Kγ in cells. The discrepancy between enzyme and cellular activities was considered to be due to the limited cell permeability of **2** (PAMPA permeability of **2** was 1.4×10^{-6} cm/s at pH 6.2). The replacement of the NH linker with a carbon-linkage in compound **22** resulted in a loss of enzyme potency and did not improve the cellular activity. An attempt to remove carboxylic acid (**6**) to change the cell permeability diminished the enzyme potency. However, the conversion from benzene to pyridine (**7**) resulted in an increased enzyme activity and consistent cellular potency. This data showed that the inhibitory activity could be increased without focusing on the interaction with Lys807, 833. The elimination of a chlorine atom (**8**) improved the potency. Further exploration of the linker pattern, such as modifications of the nitrogen-linked analogue **8** to a carbon-linked analogue **23** or an oxygen-linked analogue **28**, reduced the activity, but the sulfur-linked analogue **26** exhibited a potency equivalent to that of compound **8**. Interestingly, the removal of a linkage atom (**24**) provided a substantial boost in enzyme and cellular potencies (IC_{50} = 3, 59 nM, respectively). Compound **24** exhibited high permeability in the PAMPA assay (Pe 77.3×10^{-6} cm/s at pH 6.2) as to the cell activity and **24** had an 15-fold selectivity against PI3Kα. However, **24** had poor physicochemical properties (the solubility of **24** was 0.39 μg/mL [in water]).

To further improve poor physicochemical properties, we attempted to incorporate aliphatic groups to produce potent PI3Kγ inhibitors with an improved solubility. Aliphatic compounds with PI3Kγ IC_{50} values <50 nM in the enzyme assay were tested for the inhibition of phosphorylation of Akt (Table 2).

Methyl (**9**) and hydroxymethyl (**12**) derivatives resulted in a decreased potency, but the trifluoroethyl derivative **10**, which was equivalent to a bulky alkyl group, exhibited an improved potency. Compound **14**, which was substituted with two methyl groups at the benzylic position of **12**, also exhibited an increased potency, and the *tert*-butyl derivative **15**, in which a hydroxyl substituent was replaced with the methyl moiety of **14**, exhibited an 8-fold improvement in enzyme activity. Membrane permeability of **15** was good (PAMPA, Pe 88.4×10^{-6} cm/s at pH 6.2) and the cell activity of **15** exhibited an IC_{50} value of 113 nM. We reasoned that filling the empty space near the central heterocycle using a lipophilic substituent would lead to inhibitors with improved potency. Nitrogen derivatives exhibited modest activities against PI3Kγ. The introduction of polar functional groups, such as ether (**30**) or hydroxyl group (**31**) at the distal position, also

Table 2
SAR of oxazole derivatives with aliphatic R^1 group



Compound	R^1	PI3Kγ IC_{50}[a] (nM)	PI3Kα IC_{50}[a] (nM)	Selectivity (α/γ)	Akt IC_{50}[a] (nM)
9	Me	181	NT[b]		NT[b]
10		10	43	4	88
12		161	NT[b]		NT[b]
14		25	NT[b]		369[c]
15		3	15	5	113
29		46	NT[b]		1222[c]
30		24	NT[b]		599[c]
31		14	NT[b]		237
16		250	NT[b]		NT[b]
32		22	NT[b]		322[c]
33		12	195	16	222

[a] The IC_{50} value represents the mean of at least two independent experiments.
[b] NT = not tested.
[c] Data from one experiment ($n = 1$).

improved the potency. The oxygen derivative **16** showed a reduced activity, whereas sulfur derivatives (**32** and **33**) displayed potent enzyme activities. However, **33** was unstable under basic conditions.

As can be seen, **10** and **15** were optimal, and these compounds tended to be equipotent with **24**. Compound **15** was found to have better physicochemical properties compared with **24** (the solubility of **15** was 35.20 μg/mL [in water]).

In summary, we have described the lead generation, synthesis, and initial SAR study for a novel series of 2-aminothiazole-oxazoles. Compounds **10** and **15** which were produced through the optimization of **2** displayed good enzymatic and cellular activities. Further modifications of this series to improve α/γ selectivity are currently in progress.

Acknowledgment

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References and notes

1. Engelman, J. A.; Luo, J.; Cantley, L. C. *Nat. Rev. Genet.* **2006**, *7*, 606.
2. Katso, R.; Okkenhaug, K.; Ahmandi, K.; White, S.; Timms, J. *Annu. Rev. Cell Dev. Biol.* **2000**, *17*, 615.
3. Vanhaesebroeck, B.; Leevers, S. J.; Ahmadi, K.; Timms, J.; Katso, R.; Driscoll, P. C.; Woscholski, R.; Parker, P. J.; Waterfield, M. D. *Annu. Rev. Biochem.* **2001**, *70*, 535.
4. Sasaki, T.; Irie-Sasaki, J.; Jones, R. G.; Oliveira-dos-Santos, A. J.; Stanford, W. L.; Bolon, B.; Wakeham, A.; Itie, A.; Bouchard, D.; Kozieradzki, I.; Joza, N.; Mak, T. W.; Ohashi, P. S.; Suzuki, A.; Penninger, J. M. *Science* **2000**, *287*, 1040.
5. Hirsch, E.; Katanaev, V. L.; Garlanda, C.; Azzolino, O.; Pirola, L.; Silengo, L.; Sozzani, S.; Mantovani, A.; Altruda, F.; Wymann, M. P. *Science* **2000**, *287*, 1049.
6. Li, Z.; Jiang, H.; Xie, W.; Zhang, Z.; Smrcka, A. V.; Wu, D. *Science* **2000**, *287*, 1046.
7. Laffargue, M.; Calvez, R.; Finan, P.; Trifilieff, A.; Barbier, M.; Altruda, F.; Hirsch, E.; Wymann, M. P. *Immunity* **2002**, *16*, 441.
8. Camps, M.; Rückle, T.; Ji, H.; Ardissone, V.; Rintelen, F.; Shaw, J.; Ferrandi, C.; Chabert, C.; Gillieron, C.; Francon, B.; Martin, T.; Gretener, D.; Perrin, D.; Leroy, D.; Vitte, P. A.; Hirsch, E.; Wymann, M. P.; Cirillo, R.; Schwarz, M. K.; Rommel, C. *Nat. Med. (NY)* **2005**, *11*, 936.
9. Wymann, M. P.; Bjoerkloef, K.; Calvez, R.; Finan, P.; Thomast, M.; Trifilieff, A.; Barbier, M.; Altruda, F.; Hirsch, E.; Laffargue, M. *Biochem. Soc. Trans.* **2003**, *31*, 275.
10. Vincent, P.; Jasna, K.; David, C.; Dennis, D. C.; Jeffrey, P. S.; Karen, R.; Fabienne, B.-C.; Delphine, V.; Montserrat, C.; Christian, C.; Corinne, G.; Bernard, F.; Dominique, P.; Didier, L.; Denise, G.; Anthony, N.; Pierre, A. V.; Susanna, C.; Christian, R.; Matthias, K. S.; Thomas, R. *J. Med. Chem.* **2006**, *49*, 3857.
11. Knight, Z. A.; Gonzalez, B.; Feldman, M. E.; Zunder, E. R.; Goldenberg, D. D.; Williams, O.; Loewith, R.; Stokoe, D.; Balla, A.; Toth, B.; Balla, T.; Weiss, W. A.; Williams, R. L.; Shokat, K. M. *Cell* **2006**, *125*, 733.
12. The binding model was examined and visualized using MOE™ (Molecular Operating Environment) Version 2010.10, Chemical Computing Group: Montreal, Canada.
13. Typical procedure for preparation of 2-aminothiazole-oxazole derivatives (**5**–**16**). To a mixture of **4** (2.94 g, 12.3 mmol) and triphenylphosphine (4.84 g, 18.4 mmol) in dioxane (50 ml) was added pivaloyl chrolide (1.63 g, 13.5 mmol) slowly, stirred at room temperature for 15 h. Sat. $NaHCO_3$ was added, and the mixture was extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography to afford **15** (475 mg, 1.70 mmol) as a colorless powder. ^1H NMR (200 MHz, DMSO-d_6): δ 1.36 (s, 9H) 2.16 (s, 3H) 2.40 (s, 3H) 7.19 (s, 1H) 12.26 (br s, 1H). MS ESI/APCI Dual m/z 280 $[M+H]^+$.
14. Human PI3Kγ (20 ng, Millipore) was incubated for 2 h at 30 °C with kinase buffer (20 mM Tris–HCl [pH 7.4], 5 mM $MgCl_2$, 5 mM DTT and 10 μM ATP/10 μCi γ[^{33}P]ATP, final concentrations) and lipid vesicles containing 5 μM of PtdIns (Calbiochem) and 25 μM of PtdSer (Sigma) (final concentrations) in the presence of inhibitors or DMSO. The kinase reaction was stopped by the addition of 25 mM of EDTA, and the samples were incubated using a FlashPlate® (a phospholipid 96-well scintillant-coated microplate; PerkinElmer) with substrate coating buffer (PerkinElmer). After the incubation step, the wells were washed with PBS. The bound radioactivity was determined by measuring the counts per second (CPS) using a packard top

count microplate scintillation counter (PerkinElmer). A human PI3Kα (20 ng, Millipore) assay was performed using the same procedure.

15. C5a-Mediated PKB/Akt phosphorylation in macrophages. Raw264.7, a murine macrophage-like cell line, was seeded on 6-well plates at a density of 8×10^6 cells per well and grown for 24 h. After replacing the medium with fresh serum-free 0.1% BSA medium and incubating for 3 h at 37 °C, the cells were pretreated with or without various concentration of inhibitors for 30 min and then stimulated with 5 nM of C5a (R&D Systems) for 5 min at 37 °C. The PKB/Akt phosphorylation levels were determined using a phospho-Ser-473 Akt-specific antibody kit (CST), according to the manufacturer's protocol.