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Discovery of novel PI3K γ/δ inhibitors as potential agents for inflammation

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ABSTRACT

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Keywords: PI3Kγ/δ Triazolopyridine Ureas Inflammation Asthma Dual PI3K γ/δ inhibitors have recently been shown to be suitable targets for inflammatory and respiratory diseases. In a recent study we described the discovery of selective PI3K γ inhibitors based on a triazolopyridine scaffold. Herein, we describe the elaboration of this structural class into dual PI3K γ/δ inhibitors with excellent selectivity over the other PI3K isoforms and the general kinome. Structural optimization led to the identification of two derivatives which showed significant efficacy in an acute model of lung inflammation.

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Phosphatidylinostol-3 kinases (PI3Ks) are a family of enzymes which phosphorylate the 3-hydroxyl position of the inositol ring of phosphatidylinositol upon activation by various cell surface receptors.¹ The resultant phosphoinositides play important roles in lipid and cell signaling and membrane trafficking, hence the PI3Ks have been implicated in many cellular functions such as growth, proliferation, survival, apoptosis, adhesion and migration.^{2,3} As such the development of inhibitors has generated vast interest in many areas such as oncology, metabolic, cardiovascular and, in particular, inflammatory diseases where they can be potentially targeted towards conditions such as systemic lupus erythematosus, psoriasis, rheumatoid arthritis, asthma and chronic obstructive pulmonary disorder (COPD).^{3,4}

The most extensively investigated are the Class I PI3Ks, which can be further subdivided into class IA isoforms (α , β , and δ) and the sole class IB member (γ). PI3K α and β are ubiquitously expressed and mice deficient in their catalytic sub-units are embry-onically lethal. In contrast, the expression of δ and γ are limited to hematopoietic and endothelial cells and loss of either or both isoforms yields viable offspring, albeit with compromised immunity.^{3,5} The limited expression pattern of PI3K γ and PI3K δ as well as the viability of the genetically modified, kinase dead mice makes the dual inhibitor approach attractive and yet there are only a few examples of such compounds in the literature.^{6–10} One compound, TG100-115 **1** (Fig. 1) has entered clinical trials as an intravenously administered drug to restore blood flow following acute myocardial

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Figure 1. TG100-115 with published PI3K activity (pIC₅₀).

infarction⁷ and has been investigated in inhaled models of asthma and COPD.¹¹

In a recent publication we described the elaboration of lead compound CZC19091 **2** to CZC19945 **4** (Fig. 2). The former suffered from poor cellular activity and bioavailability whereas **4** had good cellular potency, pharmacokinetics and in vivo efficacy in inflammatory models. Although it was only moderately selective over the PI3K family **4** was selective over the general kinome.¹² This account describes the use of this lead in order to develop potent PI3K γ/δ inhibitors that would be suitable for indications such as asthma and COPD.

Analysis of the crystal structure that we obtained of CZC19091 **2** complexed to PI3K γ^{13} (Fig. 3) revealed that, as in all PI3K γ inhibitors with structural data, the compound made a back-bone interaction with the hinge region (Val 882)^{6,14} and that the acetyl group pointed toward bulk solvent, on the periphery of the ATP pocket. It has been reported that although the interior of the ATP binding pocket is highly conserved between the PI3K family members, there are major differences in the residues on the boundary, giving significant changes in both shape and charge in this region.¹⁴ We

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Figure 2. PI3K activity (pIC₅₀) of triazolopyridine lead compounds.



Figure 3. Crystal structure of CZC19091 with PI3K γ showing the acetyl group on the periphery of the ATP binding pocket, pointing toward bulk solvent.

therefore postulated that the acetyl moiety, as well as being an amenable synthetic handle, would be a logical area to modify in order to obtain PI3K inhibitors with diverse selectivity profiles suitable for further characterization. Ureas were selected in preference to amides to circumvent the in vivo instability that had been observed for CZC19091.¹² Our approach was complimented by our Kinobeads technology where binding to the target protein is evaluated in a cell lysate, avoiding the need for recombinant protein and allowing access to all required isoforms in a single experiment. This allowed selectivity to be evaluated early in the assay cascade so that it could be rapidly incorporated into inhibitor design. Cellular activity was assessed in an fMLP-induced human neutrophil migration assay.¹²

We chose the CZC19945 **4** in preference to the CZC19463 **3** substructure as our starting scaffold as this was the most potent (in vitro and in vivo) and had the most desirable selectivity profile with regards to undesired off-targets. We had also developed a reliable and scalable route to this intermediate devoid of any column chromatography (Scheme 1). Preparation of sulfonamide **6** was achieved by reaction of 5-bromopyridine-3-sulfonyl chloride **5** with *tert*-butylamine, in pyridine. Subsequent Suzuki coupling with 2-aminopyridine-5-boronic acid pinacol ester gave aminopyridine **7** which underwent reaction with ethoxycarbonyl isothiocyanate to give the corresponding thiourea **8**. Cyclization with hydroxylamine in a 1:1 mixture of refluxing ethanol/methanol afforded **4** which could be isolated directly from the reaction mixture, with high purity, by simple filtration.

In a facile two step urea formation process, **4** was first reacted with trisphosgene in pyridine to afford the intermediate isocyanate, which was subsequently dissolved in DMF and transferred into reaction vials containing the desired amines. The reactions were then heated at 65 °C overnight and the desired ureas isolated by preparative HPLC directly from the reaction mixture. This method enabled the rapid, high-purity synthesis of the desired ureas in a parallel fashion. The glycinamide ureas were accessed *via* intermediate acid **10** which was synthesized via reaction of the in-situ generated isocyanate with glycine. Standard amide bond formation with HATU then furnished the desired products.¹⁵

To probe the SAR a diverse range of amines were selected; key examples are shown in Table 1. The simple methyl urea **9a** was tolerated whereas the morpholine **9b** showed a drop in both PI3K potency and cell-based activity. This was also observed for other simple trisubstituted ureas (data not shown) and therefore disubstituted ureas became the focus of our synthetic efforts.



Scheme 1. Reagents and conditions: (a) *tert*-Butylamine, pyridine, 0–40 °C; (b) 2-aminopyridine-5-boronic acid pinacol ester, Pd(dppf)(Cl)₂·DCM, Na₂CO₃, DME:EtOH:H₂O (5:3:2), µwave, 120 °C, 1 h; (c) ethoxycarbonyl isothiocyanate, DCM, 35 °C, 24 h; (d) NH₂OH-HCl, DIPEA, MeOH:EtOH (1:1), 80 °C, 18 h; (e) triphosgene, THF:pyridine, 0–35 h, 2 h; (f) R¹R²NH, DMF:pyridine (10:1), 65 °C, 18 h; (g) R³R⁴NH, HATU, DIPEA, DMF, 65 °C, 18 h.

Table 1

Ureas synthesized via Scheme 1 with PI3K potency as measured in the Kinobeads assay



Compound	NR ¹ ,R ²		PI3K	Neu		
		γ^{a}	δ^{a}	α^{a}	β^{a}	mig (pIC ₅₀) ª
4	n/a	7.6	5.8	5.6	6.7	6.0
9a	N H	7.9	6.1	<5.3	6.4	6.1
9b	Ň	6.9	5.5	<4.6	5.4	5.1
9c	`. _N ~~ H	6.9	5.9	<5.3	<6.0	4.9
9d	N H	7.8	7.7	5.9	6.7	7.3
9e	N.N.H	6.6	<4.6	<4.0	<4.0	_
9f	N H	7.2	5.8	<4.7	5.5	5.8
9g	N H	7.5	7.6	<5.5	6.0	6.4
9h	N H H	7.6	7.9	5.7	7.1	6.6
11a		8.5	7.1	4.9	5.9	6.1

^a The values are averages of at least two independent experiments.

Although the dimethylamino ethyl moiety **9c** showed a drop in potency, this was restored with the corresponding morpholine **9d** which also showed increased affinity for PI3K δ as well as moderate selectivity over the other isoforms. Aryl **9e** and benzyl ureas **9f** showed a drop in PI3K δ activity, but the latter was restored with analogues **9g** and **9h** with the ethyl linker which again showed a PI3K γ/δ dual inhibition profile. Finally glycinamide **11a**, was our first example with PI3K γ pIC₅₀ greater than eight. PI3K δ activity was moderate and selectivity over the other isoforms was more than 100 fold.

Encouraged by the PI3K γ/δ profile obtained and the potential therapeutic applications for dual inhibitors,^{2,9,16} we decided to elaborate on two of the urea sub-series optimizing activity and selectivity for both isoforms. Data for selected compounds is shown in Table 2, initially for the alkyl heterocycles (**9j-9o**) and then the glycinamides (**11b–11e**).

The pyrazole **9j** retained similar PI3K γ potency to the parent **4**, whereas thiazole **9k**, pyrazine **9l** and oxadiazole **9m** showed a moderate increase (pIC₅₀>8) with δ activity within 10 fold. In general, selectivity for the aminoethyl heterocycles was 100 fold over PI3K α but moderate over PI3K β . The most potent example was tetrazole **9n** which also showed good cell based activity;

Table 2

Heterocyclic and glycinamide ureas synthesized via Scheme 1 with PI3K potency, as measured in the Kinobeads assay



Compound	NR ¹ , R ²	PI3K (pIC ₅₀)				Neu
		γ^{a}	δ^{a}	α^{a}	β^a	mig (pIC ₅₀) ^a
9j	N= N= N= N= N= N= N= N= N= N= N= N= N= N	7.6	6.7	6.2	6.2	6.8
9k	N H	8.1	7.8	6.1	<6.7	6.4
91		8.2	7.6	5.9	6.9	6.9
9m	N-N N-V H	8.0	7.1	5.5	6.6	6.5
9n	N=N NNNN	8.6	8.2	6.1	7.2	7.1
90	N H N=N	7.2	7.3	<5.2	5.6	6.6
11b	`N H O	7.8	6.1	<4.8	<5.7	4.7
11c		8.2	7.2	6.0	5.9	5.8
11d		8.4	7.8	6.4	6.4	6.3
11e		8.6	7.9	6.1	6.0	5.9

^a The values are averages of at least two independent experiments.

homologation to the propyl spacer **90** gave a drop in activity across all isoforms.

The glycinamides (**11a**, Table 1 and **11b–e**, Table 2) generally showed enhanced selectivity over PI3K β than the heterocyclic subseries and retained excellent potency for PI3K γ and δ . Tertiary amides, **11a**, **c–e**, showed superior cell-based activity relative to their secondary counterparts (**11b**), but in general there were no gross differences in PI3K activity between them. To further assess selectivity, in a competition binding experiment in which over 100 kinases were detected, aside from the Class I PI3Ks, **11d** only exhibited off-target activity against 1 kinase (PIK4C α) demonstrating that the high selectivity extended into the general kinome.¹⁷

Compounds from each sub-series were then further investigated for microsomal stability, solubility and permeability (Table 3). Perhaps unsurprising, given the high PSA and molecular weight of the compounds, Caco-2 data suggested low permeability and high

Table 3						
Physicochemical	data	and in	vitro	PK for	selected	ureas

	Mwt	PSA	clog P	Cl _{int} a (human)	Cl _{int} a (rat)	Caco-2 ^b	Kin sol ^c
9d	503	142	2.3	1.4	0.9	n.d.	65
9n	526	176	2.3	0.5	0.9	0.54/109	<6.5
11a	474	161	1.5	0	0.4	0.08/252	>100
11d	517	160	1.6	0	0.8	n.d.	>100

^a Microsomal clearance (mL/min/g liver).

^b Papp, A–B ($\times 10^{-6}$ cm s⁻¹)/efflux ratio.

^c Kinetic solubility precipitation range (µM), 1% DMSO.

efflux.¹⁸ When **11d** was evaluated in rat pharmacokinetics no measurable exposure was obtained upon oral administration, and rapid elimination (>200 mL/min/kg) was observed on intravenous dosing. Plasma samples were analyzed for parent **4** or possible hydrolysis product, acid **10** but no trace of either, potentially active metabolite, was detected. As this was not in-line with the in vitro microsomal stability, the in vivo clearance is most likely attributed to a transporter mediated clearance mechanism. Of all of the urea sub-series, the glycinamides exhibited the highest kinetic solubility.

We postulated that the physico-chemical properties of the ureas made them suitable for inhaled delivery as this would enable the drug to be delivered directly to the airways, thus bypassing the GI tract. Low absorption and high clearance is also desirable in this delivery method to minimize systemic exposure and thus potential side effects.¹⁹ Efficacy of PI3K inhibitors had also been previously demonstrated in murine respiratory models.¹¹

A molecule from each subseries was progressed to an LPS induced pulmonary neutrophilia model as a preliminary in vivo surrogate for asthma.²⁰ The LPS challenge rapidly produces an inflammatory response, dominated by neutrophils. Compounds were assessed by their ability to reduce the accumulation of these immune cells.

9d, 9n, 11d and dexamethasone (0.5 mg) were dosed intratracheally, 1 hour prior to LPS challenge to BalbC, non-fasted mice (eight mice per group). Eight hours later the bronchoalveolar lung fluid (BALF) was collected and neutrophils were counted (Fig. 4).



Figure 4. Bar graphs representing the total number of neutrophils in BALF of LPS exposed mice pre-treated with test compounds or dexamethasone. Each column represents the mean of eight animals. Changes were compared to the vehicle control animals using ANOVA followed by Dunnets test: *P <0.05 and **P <0.01.

All compounds showed a reduction in neutrophil count, with statistical significance demonstrated for **9n** and **11d** which showed a 37% and 34% reduction in neutrophil accumulation resepectively (P < 0.05), thus providing validation for these chemotypes in a respiratory inflammation model.

In conclusion, a series of urea triazolopyrimidines were synthesized with excellent γ/δ potency and high selectivity over the other isoforms and the general kinome. Significant efficacy for two compounds from different sub-series was observed in a preliminary, inhaled, asthma model. The physicochemical properties of these molecules preclude them from oral absorption but lend themselves to an inhaled method of delivery and therefore respiratory diseases which represent a considerable area of unmet medical need.

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