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Discovery of novel PI3Kγ/δ inhibitors as potential agents for inflammation

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ABSTRACT

Dual PI3Kγ/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 PI3Kγ inhibitors based on a triazolopyridine scaffold. Herein, we describe the elaboration of this structural class into dual PI3Kγ/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.

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). PI3Kα and β are ubiquitously expressed and mice deficient in their catalytic sub-units are embryonically 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 PI3Kγ and PI3Kδ 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 infarction[7] and has been investigated in inhaled models of asthma and COPD.[11]

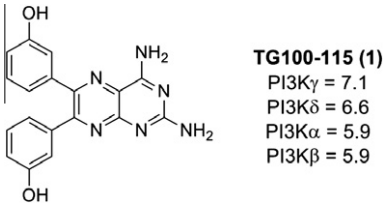

Figure 1. TG100-115 with published PI3K activity (pIC_{50}).

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 PI3Kγ/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 PI3Kγ[13] (Fig. 3) revealed that, as in all PI3Kγ 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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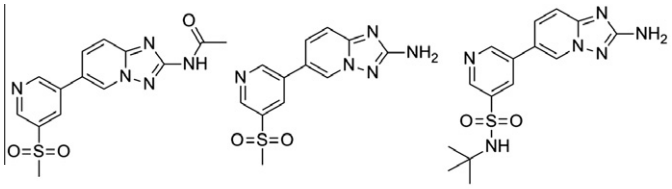


CZC19091 (2)	CZC19463 (3)	CZC19945 (4)
PI3Kγ = 7.1	PI3Kγ = 7.0	PI3Kγ = 7.6
PI3Kδ = 5.3	PI3Kδ = 5.2	PI3Kδ = 5.8
PI3Kα = 5.4	PI3Kα = 5.0	PI3Kα = 5.6
PI3Kβ = n.d	PI3Kβ = 5.6	PI3Kβ = 6.7

Figure 2. PI3K activity (pIC_{50}) of triazolopyridine lead compounds.

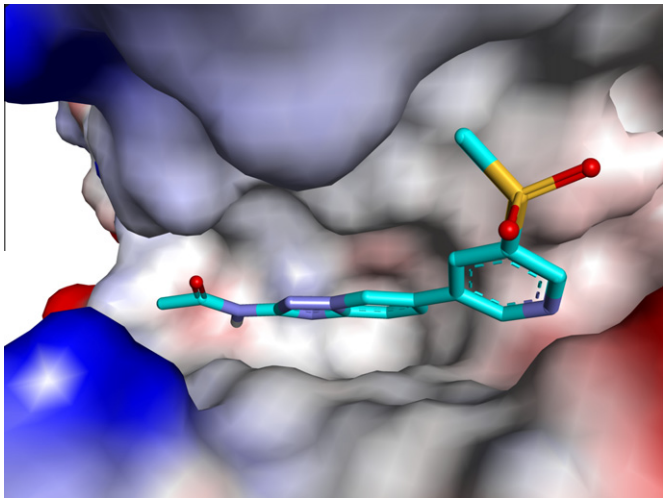


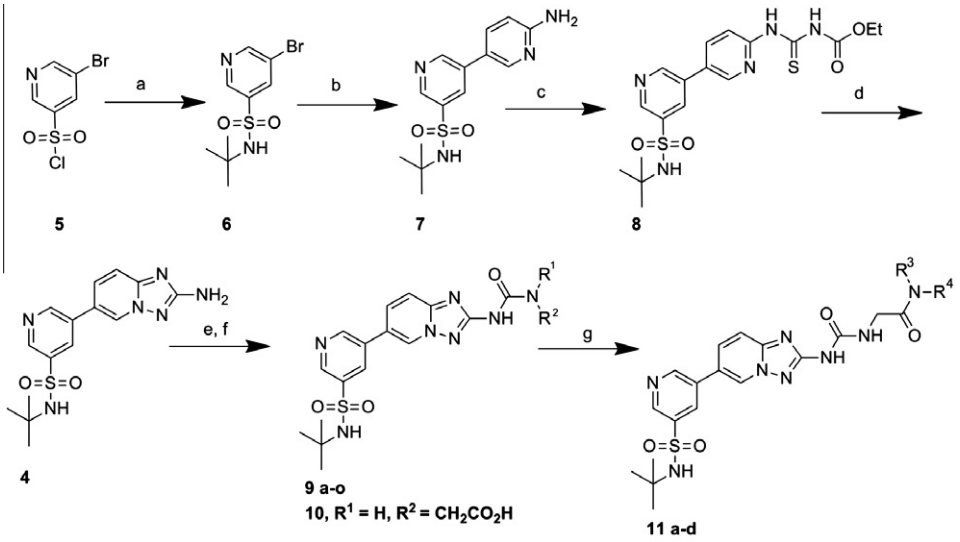
Figure 3. Crystal structure of CZC19091 with PI3Kγ showing the acetyl group on the periphery of the ATP binding pocket, pointing toward bulk solvent.

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, Na_2CO_3, DME:EtOH:H_2O (5:3:2), μwave, 120 °C, 1 h; (c) ethoxycarbonyl isothiocyanate, DCM, 35 °C, 24 h; (d) NH_2OH·HCl, DIPEA, MeOH:EtOH (1:1), 80 °C, 18 h; (e) triphosgene, THF:pyridine, 0–35 h, 2 h; (f) R^1R^2NH, DMF:pyridine (10:1), 65 °C, 18 h; (g) R^3R^4NH, HATU, DIPEA, DMF, 65 °C, 18 h.