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Discovery of 5-(2-amino-[1,2,4]triazolo[1,5-a]pyridin-7-yl)-*N*-(*tert*-butyl) pyridine-3-sulfonamide (CZC24758), as a potent, orally bioavailable and selective inhibitor of PI3K for the treatment of inflammatory disease

Mihiro Sunose [a,*], Kathryn Bell [a], Katie Ellard [a], Giovanna Bergamini [b], Gitte Neubauer [b], Thilo Werner [b], Nigel Ramsden [a]

[a] Cellzome Ltd, Chesterford Research Park, Little Chesterford CB10 1XL, UK
[b] Cellzome AG, Meyerhofstr. 1, 69117 Heidelberg, Germany

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ABSTRACT

Herein, we disclose the discovery of a series of 7-substituted triazolopyridines which culminated in the identification of **14** (CZC24758), a potent, orally bioavailable small-molecule inhibitor of PI3Kγ, an attractive drug target for inflammatory and autoimmune disorders. Compound **14** has excellent selectivity across the kinome, demonstrates good potency in cell based assays and furthermore exhibits in vivo efficacy in a collagen induced arthritis model in mouse after oral dosing.

Phosphoinositide 3-kinases (PI3Ks) represent a family of dual specificity enzymes that, by acting as both lipid and protein kinases, regulate numerous biological processes, including cell growth, differentiation, survival, proliferation, migration, and metabolism.[1] These kinases are activated by a wide variety of different stimuli such as growth factors, inflammatory mediators, and antigens. Deregulation of class I PI3Ks, is involved in the pathogenesis of various diseases, representing attractive targets for oncology, inflammatory and cardiovascular diseases, with several molecules being evaluated in early clinical trials in oncology and inflammation.[2]

In our efforts to target the PI3K family for the treatment of inflammatory disease, we were interested in developing selective ligands for the PI3Kγ isoform. This PI3Kγ subtype has been shown to play a role in several immune functions such as granulocyte migration, activation of mast cells, and dendritic cells, as well as the development and differentiation of lymphocytes, thereby suggesting that its inhibition might be beneficial in both inflammatory and autoimmune conditions.[3] Given the high sequence homology within the PI3K family we were aware that achieving substantial selectivity (e.g., >100-fold) for PI3Kγ alone would be challenging. However, as literature evidence suggests that both PI3Kβ and PI3Kδ also play a role in immune function and therefore may have a synergistic anti-inflammatory effect,[4] we were particularly interested in achieving high selectivity over PI3Kα, a target with known antiproliferative effects,[5] and hence more of interest in the cancer area.[6]

Several PI3K inhibitors such as AS-605240,[7] the first small-molecule known to inhibit PI3Kγ, are known in the literature (Fig. 1). The suboptimal pharmacokinetics and the limited selectivity window of this inhibitor, particularly over the PI3Kα isoform as well as other kinases such as DNAPK, severely limited its developability. Therefore we,[8] and others[9] set out to primarily target PI3Kγ inhibitory activity, with selectivity particularly over PI3Kα as well as the rest of the kinome, with novel compounds possessing drug-like properties that would be suitable for oral dosing in vivo.

As part of our efforts to identify potent and selective PI3Kγ inhibitors, we recently described the 6-substituted triazolopyridine **1** (Fig. 1) which showed efficacy in a number of inflammatory models.[10] The X-ray crystal structure of the analogous triazolopyridine **2**[10,11] (Fig. 2) showed that the scaffold occupies the ATP binding pocket with the aminotriazole portion making the usual donor/acceptor interaction with the Val882 of the PI3Kγ protein backbone (hinge). Furthermore, the triazolopyridine scaffold and the pyridine ring efficiently fill the hydrophobic pocket formed by a number of residues that include Ile963 and Tyr867. The methylsulfone and the acetamide groups extend towards the solvent front. We envisioned that the regioisomeric 7-substituted

* Corresponding author. Tel.: +44 179 953 2832; fax: +44 179 953 2801.
E-mail address: sunosem@hotmail.co.jp (M. Sunose).

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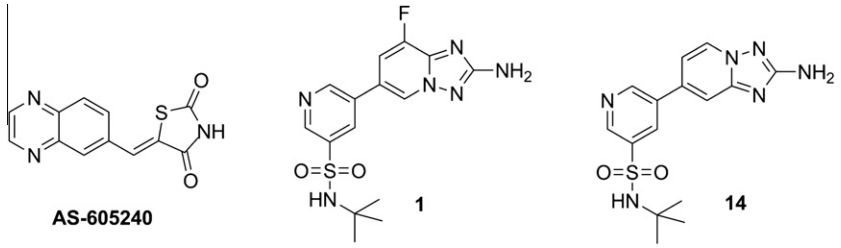

Figure 1. AS-605240, 6-substituted triazolopyridine 1 and 7-substituted triazolopyridine **14**.

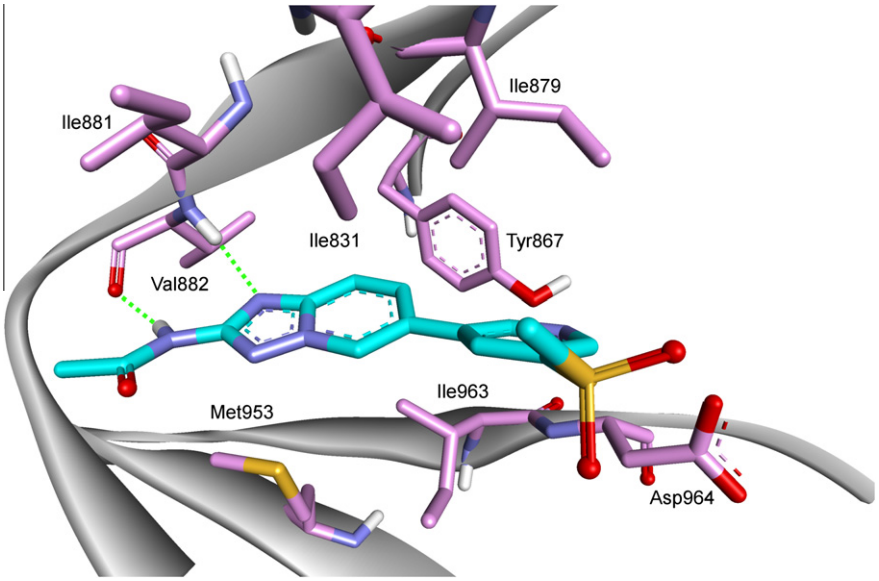

Figure 2. X-ray cocrystal structure (PDB ID: 4AOF) of **2** bound to the ATP pocket of the PI3Kγ kinase domain.

triazolopyridine scaffold should keep all the key interactions shown by the 6-substituted series and hence embarked on exploring the SAR in this scaffold (Fig. 3). This work culminated in the discovery of **14** (CZC24758) an orally bioavailable, potent and selective PI3K inhibitor which showed efficacy in a mouse collagen induced arthritis (CIA) model.

The convergent route for the preparation of 7-substituted triazolopyridines which involved coupling of boronic acids with the key intermediate **6** met with little success[12] (Scheme 1). A more robust synthetic route for the preparation of these analogues involved Suzuki coupling prior to the cyclisation to form the triazolopyridines **9–21** (Scheme 2). The synthesis began by coupling commercially available sulfonyl chlorides with amines to form sulfonamides **23**. We observed inconsistent yields (29% to 60%) and significant impurities in the coupling when the HCl salt of the sulfonyl chloride **22** was used. Using the free base, the sulfonamide intermediates **23** were isolated in almost quantitative yield after simple precipitation from the reaction mixture. Boronic esters were prepared in situ followed by subsequent Suzuki coupling reaction which yielded the intermediates **24**. Thiourea formation and cyclisation with hydroxylamine yielded **9–21** in 79% yield with high purity. This synthetic approach was developed to enable the preparation of multigram quantities required for later-stage experiments in vivo. Compound **9** was synthesized using commercially available 3-bromo-5-(methylsulfonyl)pyridine. The 5-fluorinated triazolopyridine **21** was synthesized in an analogous manner using commercially available 2-amino-4-bromo-6-fluoropyridine.

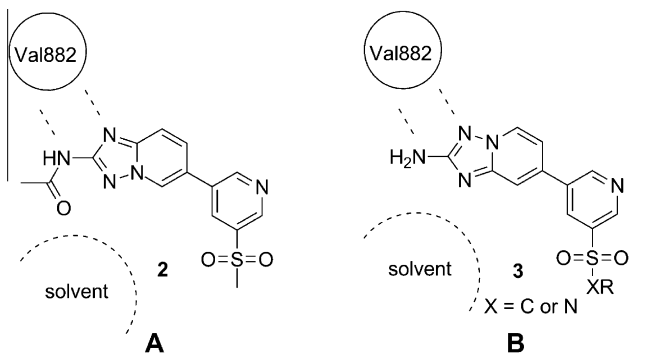

Figure 3. Cartoon representation showing hydrogen bonding interactions of 2 in PI3Kγ (**A**) and proposed binding of 7-substituted triazolopyridine scaffold (**B**).

The compounds **9–21** were initially tested using the *Kinobeads* assay developed for lipid kinases[10] to determine potency and selectivity followed by their cellular potency in a PI3Kγ/PI3Kδ dependent neutrophil migration assay (Table 1).[10] SAR analysis was carried out with a focussed set of compounds using the prior knowledge from the 6-substituted series.[10] As in the previous series the 7-substituted series showed good potency for PI3Kγ. The methyl sulfonamide showed a small increase in potency compared to the methyl sulfone (pIC_{50} **9**: 7.1 vs pIC_{50} **11**: 7.6), but larger substituents on the sulfonamide, although tolerated, failed to give a significant increase in potency (pIC_{50}: 7.5–7.9). The diethylsulfonamide **19** was the most potent compound identified (pIC_{50}: 8.1) although disappointingly it showed a lower potency in the cellular assay (pIC_{50}: 5.9). The majority of analogues gave a similar selectivity profile across the PI3K family. As was observed in the