Bioorganic & Medicinal Chemistry Letters 21 (2011) 6586–6590

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fragment based discovery of a novel and selective PI3 kinase inhibitor

Samantha J. Hughes [a,*], David S. Millan [a,*], Iain C. Kilty [b], Russell A. Lewthwaite [a], John P. Mathias [a], Mark A. O'Reilly [b], Andrew Pannifer [a], Anne Phelan [b], Frank Stühmeier [b], Darren A. Baldock [a], David G. Brown [a]

[a] Worldwide Medicinal Chemistry, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK
[b] Allergy and Respiratory Research Unit, Pfizer Global Research and Development, Sandwich Laboratories, Ramsgate Road, Sandwich, Kent CT13 9NJ, UK

ARTICLE INFO

Article history:
Received 7 July 2011
Revised 29 July 2011
Accepted 31 July 2011
Available online 6 August 2011

Keywords:
Fragment
Kinase

ABSTRACT

We report the use of fragment screening and fragment based drug design to develop a PI3γ kinase fragment hit into a lead. Initial fragment hits were discovered by high concentration biochemical screening, followed by a round of virtual screening to identify additional ligand efficient fragments. These were developed into potent and ligand efficient lead compounds using structure guided fragment growing and merging strategies. This led to a potent, selective, and cell permeable PI3γ kinase inhibitor with good metabolic stability that was useful as a preclinical tool compound.

Fragment based drug discovery is a powerful method to identify novel lead molecules. It starts from the identification of one or more low molecular weight, low complexity molecules called fragments. Suitable starting fragments are identified through screening a fragment collection by sensitive biophysical techniques such as nuclear magnetic resonance, surface plasmon resonance and X-ray crystallography, or by high concentration biochemical screening.[1,2] Although fragments typically have potencies in the millimolar to micromolar range, they often have high ligand efficiency (LE)[3] and can be developed into leads by growing, linking or merging strategies, usually guided by one or more protein X-ray crystal structures. Kinases, as drug targets, present numerous challenges including selectivity, cell penetration and crowded patent space. However, fragment screening has shown to be a successful means of identifying novel, efficient lead compounds in this area.[4]

Phosphatidylinositol-3 kinases (PI3Ks) are a family of lipid kinases whose activity is central to a plethora of cellular signalling pathways, and therefore ultimately to the maintenance of physiological homeostasis. Specifically, PI3Ks belonging to the class 1 group have been linked to a number of patho-physiologies of key interest to the pharmaceutical industry, such as cancer, rheumatoid arthritis, cardiovascular disease and respiratory disease.[5,6] This class 1 group of PI3Ks contain four isoforms (α, β, γ and δ) that are activated upon ligation of particular cell surface receptors which can subsequently generate a diverse range of cellular activities, including proliferation, differentiation, adhesion, migration, apoptosis and phagocytosis. Of particular note, the γ and δ isoforms are of significant interest in the pharmaceutical arena due to the increasing evidence of their role in inflammatory diseases together with their largely haematopoietic restricted tissue distribution that may ultimately afford a more acceptable safety toleration profile over the more ubiquitously expressed α and β isoforms.[5]

In an effort to identify novel leads for PI3γ, the Pfizer fragment library collection (5960 fragments) was screened using a high concentration biochemical assay.[7] An advantage of biochemical screening is that it provides an initial estimate of the K_i value even for weak binding compounds. A large number of hits were identified, 312 of which were selected for IC_{50} determination, resulting in 150 confirmed hits (or dose response curves). Selected hits were then further confirmed by orthogonal methods, namely isothermal denaturation and where possible by protein X-ray crystallography. A total of five X-ray structures of the fragment complexes were obtained. Hits that made it through the screening sequence to this point, with a protein co-crystal structure, were then considered on the basis of other properties, such as LE, potential for kinase selectivity and synthetic tractability. Compound **1**, an amino pyrazolopyrimidine, was identified as an inhibitor with good LE. An X-ray crystal structure of **1** in complex with PI3γ was obtained which demonstrates that **1** binds at the ATP site with the amino group acting as a hydrogen bond donor to the main chain carbonyl of Val882, and the pyrazole N3 accepting a hydrogen bond from Val882 main chain NH. The 5-methyl group is buried in a hydrophobic pocket, packing against the gatekeeper residue Tyr867 as

* Corresponding authors. Tel.: +44 1304 644965 (S.J.H); +44 1304 643497 (D.S.M.).
E-mail addresses: samantha.hughes@pfizer.com (S.J. Hughes), david.millan@pfizer.com (D.S. Millan).

0960-894X/$ - see front matter © 2011 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2011.07.117

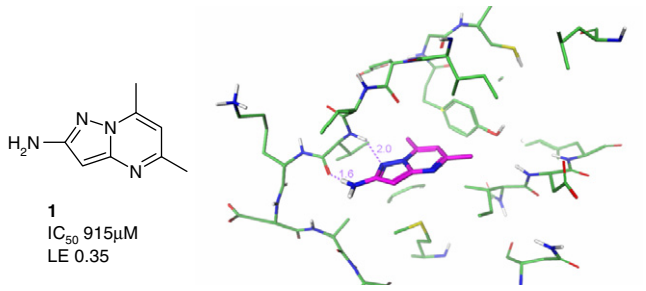


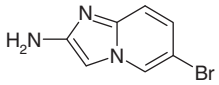
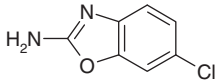
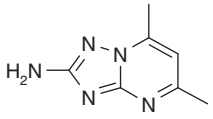
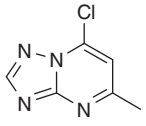
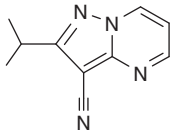
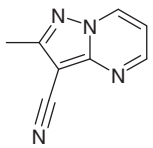
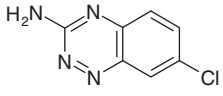
Figure 1. Structure of compound **1** a fragment screening hit, and X-ray crystal structure of compound **1** in PI3Kγ.

well as Val882, Phe 961 and Glu880 (Fig. 1), and may potentially offer wider kinase selectivity.[8]

The initial fragment hit **1** was followed up by virtual screening of the Pfizer solid compound collection (MW <300 Da) to identify similar compounds, using a custom written, proprietory, close-in-mining tool that uses both substructure and nearest neighbour (ECFP4 fingerprints) searches.[9] The searches were restricted to small changes in the heavy atom count in order to explore the chemical space around the fragment hit. Virtual screening identified a further round of compounds for biochemical dose–response screening (~20 compounds), resulting in a hit rate of ~50%. Several structurally differentiated fragments were identified as a result (Table 1).

Pleasingly compound **2**, an amino imidazopyridine, proved to be both more potent and had greater LE relative to compound **1**. The potency increase was achieved without any change in the heavy atom count but it should be noted that the LE of bromine containing compounds does tend to be higher than methyl analogues, as bromine is more lipophilic and capable of achieving a greater binding affinity than a carbon atom. The amino benzoxazole **3** is another similarity search hit with improved potency and LE relative to the initial hit **1**. Compound **3** demonstrates that removing the ring junction nitrogen is therefore not deleterious to the potency, whereas addition of a nitrogen atom to form a triazolopyrimidine as in **4** and **5**, reduces activity. Interestingly, the pyrazolopyrimidine substructure hits **6** and **7** retain activity, despite replacement of the amino substituent in **1**. The most potent fragment from the close-in-mining **8** is also the most ligand efficient. The 7-chloro substituent in **8** is likely to offer a different vector for growth compared to the other cores substituents. However, it was not possible to obtain an X-ray crystal structure of the complex with **8**, and therefore this was not pursued.

Table 1
Close-in-mining hits around compound **1**

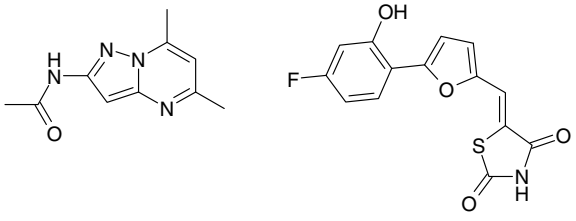
Compound	Structure	Kinase-Glo™ IC_{50} (μM)	LE
2		156	0.47
3		380	0.42
4		>1600	<0.32
5		>1670	<0.34
6		395	0.33
7		773	0.35
8		53	0.49

As the data above suggests, the close in virtual screening process revealed several useful SAR data points and identified structurally differentiated fragments with improved potency and increased LE. At this point we began exploration around hit **1**, and in particular to investigate binding interactions of the inhibitor with the hinge region of the kinase. Addition of just three heavy atoms to compound **1**, to produce the acetamide **9** led to a significant improvement in both potency and LE. As was noted for the close-in-mining results, it is worth exploring small changes to the initial fragment ahead of larger changes. The identification of good initial starting points through fragment screening is clearly a useful strategy but to leverage the power of this technique the fragment hit must be synthetically tractable such that further SAR around the fragment can be explored. Moreover, being able to explore several different vectors from a single fragment is also important. Fragment hit **1** was unfortunately not synthetically tractable to the point where we could rapidly explore the SAR around it but fortunately hit **2** had greater potential. We adopted fragment growing and merging strategies on hit **2** to further optimise for potency whilst attempting to maintain the excellent LE of the starting point.

Fragment merging is an alternative fragment elaboration strategy, where a fragment is merged with another compound that has a binding mode that overlaps with that of the fragment. X-ray crystallography showed that compound **1** and a literature compound **10**[10] overlayed well in the ATP-binding site of PI3γ (Fig. 2a). Therefore hybridisation of **1** with the methylene thiazolidinedione (TZD) moiety of compound **10** was hypothesised to improve potency, but as mentioned above **1** was synthetically difficult to manipulate. The more LE and synthetically amenable hit **2** is predicted to retain the same binding mode as **1** and therefore addition of the methylene TZD moiety onto **2** led to compound **11**. Compound **11** was significantly more potent in the biochemical assay, relative to **2**, and even more interestingly was improved in LE, which is not usually seen when fragments are significantly increased in molecular weight.[11] Gratifyingly, incorporation of an acetamide onto the pyrazolopyridine of **11** led to a sevenfold increase in potency in **12** with a similar LE. An X-ray crystal structure of the complex of hybrid **12** with PI3γ was obtained and the compound was observed to bind as anticipated from the component fragments (Fig. 2b).[8]

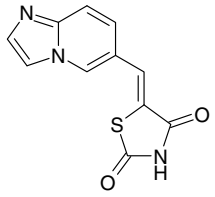
Compound **9** was prepared by a simple acetylation procedure from the fragment hit **1**, in good yield, which was originally prepared by the procedure of Ried et al. (Scheme 1).[12]

The imidazopyridine **11** was prepared by first reacting commercially available methyl 6-aminonicotinate with chloroacetaldehyde, followed by reduction of the methyl ester **13** with lithium aluminium hydride to afford the alcohol **14**. The alcohol **14** was then oxidised to the aldehyde **15** with manganese dioxide, followed by condensation with commercially available 1,3-thiazolidine-2,4-dione to afford the desired product **11** (Scheme 2).

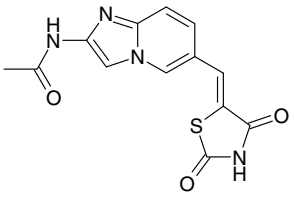


9
HTRF IC_{50} 19uM
LE 0.46

10
HTRF IC_{50} 30nM



11
HTRF IC_{50} 230nM
LE 0.53



12
HTRF IC_{50} 34nM
LE 0.48

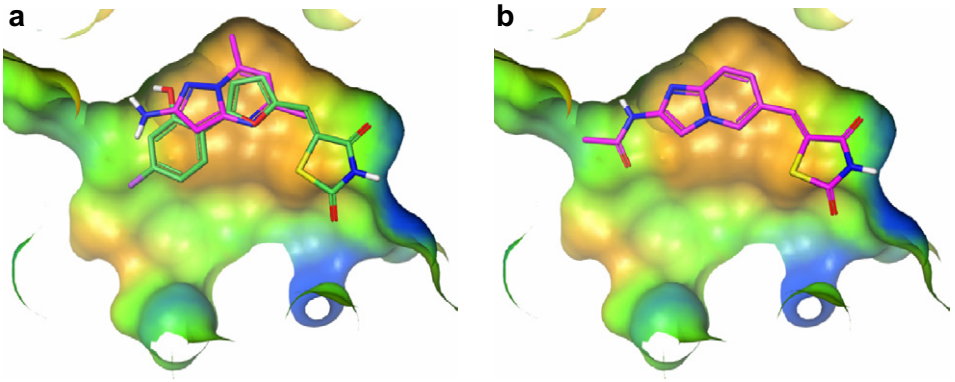
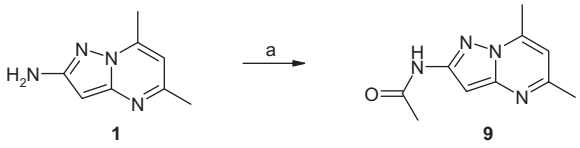
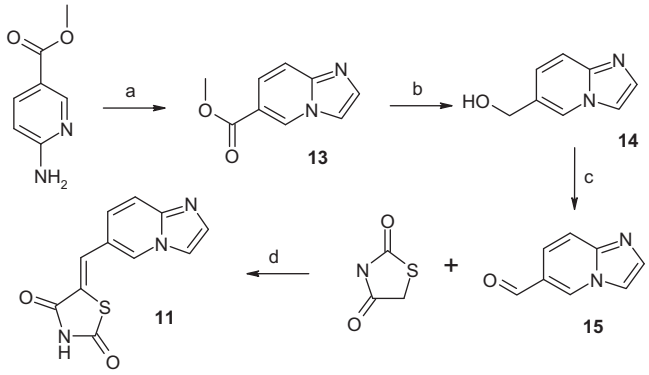


Figure 2. X-ray structures of (a) compound **1** (pink) and **10** (green), and (b) the hybrid compound **12** in PI3γ.



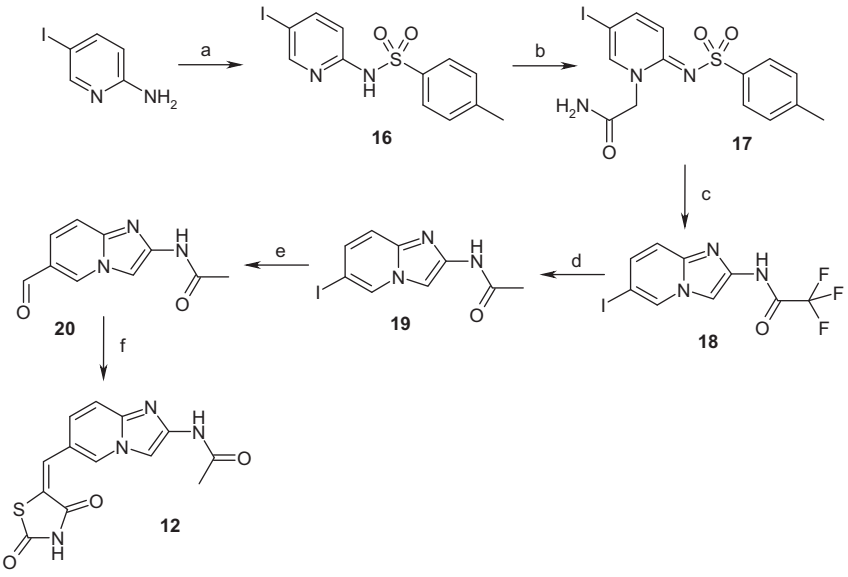
Scheme 1. Reagents and conditions: (a) acetic anhydride, reflux, 2 h, 100%.



Scheme 2. Reagents and conditions: (a) chloroacetaldehyde, $NaHCO_3$, MeOH, reflux, 8 h, 80%; (b) $LiAlH_4$, THF, 0 °C, 2 h, 32%; (c) MnO_2, CH_2Cl_2, reflux, 5 h, 66%; (d) β-alanine, AcOH, 100 °C, 2 h, 33%.

The imidazopyridine core in **12** was prepared according to the procedure of Hamdouchi et al. by first reacting the commercially available 2-amino-5-iodopyridine with tosyl chloride, affording **16**, followed by alkylation with iodoacetamide to give **17**.[13] Ring closure with trifluoroacetic anhydride afforded the imidazopyridine **18**. The trifluoroacetyl group in **18** was swapped for an acetyl group to afford **19**, followed by metallation and quenching with *N*,*N*-dimethyl formamide to afford **20**. The aldehyde **20** was then condensed with 1,3-thiazolidine-2,4-dione, as before, to afford the desired product **12** (Scheme 3).[14]

As compound **12** proved to be a potent and LE lead we were keen to assess its general kinase selectivity, and therefore potential utility as a tool compound. Kinase selectivity of **12** was assessed in an in-house panel of 43 kinases by measuring single point inhibition at 1 μM. Pan-kinase selectivity in this panel was impressive with only 3 kinases exhibiting >50% inhibition at 1 μM, namely CLK1 (94%), Caesin kinase II alpha chain (CKIIa) (95%) and Tao kinase 3 (TAO3) (57%) (Fig. 3). This selectivity is notable for a compound that binds in the ATP site through a standard



Scheme 3. Reagents and conditions: (a) TsCl, pyridine, rt, 16 h, 92%; (b) iodoacetamide, di-*iso*-propylethylamine, DMF, rt, 16 h, 53%; (c) TFAA, CH_2Cl_2, reflux, 16 h, 44%; (d) (i) NaOH, rt, 16 h, 100%, (ii) acetic anhydride, AcOH, 16 h, rt, 77%; (e) *i*PrMgCl, DMF, THF, −40 °C, 2 h, 68%; (f) β-alanine, 1,2-thiazolidinedione, AcOH, 100 °C, 3.5 h, 18%.

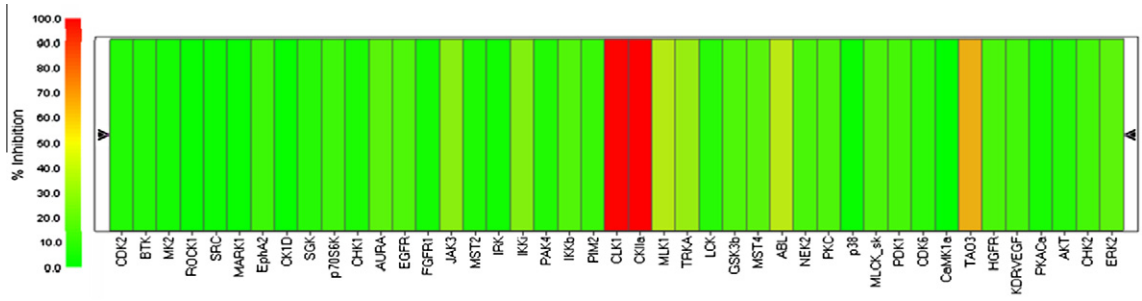


Figure 3. Heatmap showing single point activity of compound **12** against 43 kinases at 1 μM.

kinase donor/acceptor interaction at the hinge. However, the compound did not display any selectivity versus the PI3α kinase isoform (HTRF IC_{50} 25 nM). The hybrid **12** displayed good metabolic stability in both human and rat liver microsomes (10 μl/min/mg and 15 μL/min/mg, respectively), and crucially demonstrated good membrane permeability in a parallel artificial membrane permeability assay (PAMPA) (13.0×10^{-6} cm/s). In line with the good PAMPA data, the compound has shown activity (IC_{50} 0.760 μM) in a primary whole-cell assay, where compounds were evaluated for their ability to inhibit fMLP stimulated superoxide production by human isolated neutrophils.

In conclusion, screening of the Pfizer fragment library against PI3γ by a high concentration biochemical assay led to the identification of a weakly potent, but LE amino pyrazolopyrimidine fragment hit **1**. Close in virtual screening allowed exploration of chemical space around this initial hit, generating several structurally differentiated fragment cores with improved potencies and LE. Initial fragment growing efforts were restricted to small changes, and afforded significant gains in potency. Subsequent merging of the fragment with a literature compound **10** led to the identification of a novel, potent and ligand efficient PI3γ inhibitor **12** which shows activity in a cell based assay, and with promising general kinase selectivity and metabolic stability. The strategies outlined in this paper have allowed the rapid identification of a tool compound for further preclinical investigation.

Acknowledgments

We thank Sara Chunn and Sandra Newman for synthesis of compounds.

References and notes

1. Rees, D. C.; Congreve, M.; Murray, C. W.; Carr, R. *Nat. Rev. Drug Disc.* **2004**, *3*, 660.
2. Alex, A. A.; Flocco, M. M. *Curr. Top. Med. Chem.* **2007**, *7*, 1544.
3. Hopkins, A. L.; Groom, C. R.; Alex, A. A. *Drug Discovery Today* **2004**, *9*, 430.
4. Erlanson, D. A.; Arndt, J. W.; Cancilla, M. T.; Cao, K.; Elling, R. A.; English, N.; Friedman, J.; Hansen, S. K.; Hession, C.; Joseph, I.; Kumaravel, G.; Lee, W.-C.; Lind, K. E.; McDowell, R. S.; Miatkowski, K.; Nguyen, C.; Nguyen, T. B.; Park, S.; Pathan, N.; Penny, D. M.; Romanowski, M. J.; Scott, D.; Silvian, L.; Simmons, R. L.; Tangonan, B. T.; Yang, W.; Sun, L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3078.
5. Rückle, T.; Schwarz, M. K.; Rommel, C. *Nat. Rev. Drug Disc.* **2006**, *5*, 903.
6. Kong, D.; Yamori, T. *Cancer Sci.* **2008**, *99*, 1734.
7. The initial primary screen of the fragment library against recombinant human His-PI3γ was performed by quantifying the depletion of ATP using the Kinase-Glo™ luciferase assay format. The assay tolerated DMSO concentrations of up to 15%, thereby enabling primary screening at compound loads of 0.5–1.5 mM. The fragment library was screened in duplicate, with an excellent correlation between the first and the second data set and typical *Z′* values of greater than 0.6. The assay, which was used for the profiling of the primary hits in a concentration response format, allowed the quantification of K_i values in a range from 500 nM to 500 μM. During later stages of hit to lead optimisation the cost efficient and robust Kinase-Glo™ luciferase assay was replaced by a HTRF based assay allowing the quantitative determination of K_i values down to 1 nM, with typical *Z′* values of greater than 0.8.

8. The coordinates of compounds **1** and **12** bound to PI3γ have been deposited with the Protein Data Bank and assigned PDB ID codes 3zvv (2.5 Å) and 3zw3 (2.8 Å), respectively.
9. Rogers, D.; Hahn, M. *J. Chem. Inf. Model.* **2010**, *50*, 742.
10. Pomel, V.; Klicic, J.; Covini, D.; Church, D. D.; Shaw, J. P.; Roulin, K.; Burgat-Charvillon, F.; Valognes, D.; Camps, M.; Chabert, C.; Gillieron, C.; Francüon, B.; Perrin, D.; Leroy, D.; Gretener, D.; Nichols, A.; Vitte, P. A.; Carboni, S.; Rommel, C.; Schwarz, M. K.; Rückle, T. *J. Med. Chem.* **2006**, *49*, 3857.
11. Reynolds, C. H.; Bembenekb, S. D.; Tounge, B. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4258.
12. Ried, W.; Peuchert, K. P. *Jus. Liebigs Ann. der Chem.* **1965**, *682*, 136.
13. Hamdouchi, C.; Zhong, B.; Mendoza, J.; Collins, E.; Jaramillo, C.; Eugenio De Diego, J.; Robertson, D.; Spencer, C. D.; Anderson, B. D.; Watkins, S. A.; Zhanga, F.; Brooks, H. B. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1943.
14. All compounds showed ^{1}H NMR and mass spectra consistent with their structure and were >95% purity by HPLC.