Bioorganic & Medicinal Chemistry Letters 22 (2012) 5445–5450

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Development of isoform selective PI3-kinase inhibitors as pharmacological tools for elucidating the PI3K pathway

Ian Bruce *, Mohammed Akhlaq, Graham C. Bloomfield, Emma Budd, Brian Cox, Bernard Cuenoud, Peter Finan, Peter Gedeck, Julia Hatto, Judy F. Hayler, Denise Head, Thomas Keller, Louise Kirman, Catherine Leblanc, Darren Le Grand, Clive McCarthy, Desmond O'Connor, Charles Owen, Mrinalini S. Oza, Gaynor Pilgrim, Nicola E. Press, Lilya Sviridenko, Lewis Whitehead

Novartis Institutes for Biomedical Research, Respiratory Disease Area, Wimblehurst Road, Horsham, West Sussex RH12 5AB, UK

ARTICLE INFO

Article history:
Received 6 June 2012
Revised 8 July 2012
Accepted 9 July 2012
Available online 17 July 2012

Keywords:
PI3-Kinase inhibitors
Pharmacological tools
Aminothiazoles
Parallel synthesis
Isoform selectivity

ABSTRACT

Using a parallel synthesis approach to target a non-conserved region of the PI3K catalytic domain a pan-PI3K inhibitor **1** was elaborated to provide alpha, delta and gamma isoform selective Class I PI3K inhibitors **21**, **24**, **26** and **27**. The compounds had good cellular activity and were selective against protein kinases and other members of the PI3K superfamily including mTOR and DNA-PK.

Phosphatidylinositol 3-kinases (PI3K's), a family of enzymes which catalyse the phosphorylation of the 3′–OH of the inositol ring, play a central role in regulating a wide range of cellular processes including metabolism, survival, motility and cell activation.[1] These lipid kinases are divided into 3 major classes, I, II & III, according to their structure and in vitro substrate specificity.[2] The most widely understood class I family is further subdivided into subclasses IA and IB. Class IA PI3K's consist of an 85 kDa regulatory/adapter protein and three 110 kDa catalytic subunits (p110α, p110β and p110δ) which are activated in the tyrosine kinase system whilst class IB consists of a single p110γ isoform which is activated by G protein-coupled receptors. The three members of class II PI3K (C2α, C2β and C2γ) and single member of class III PI3K (Vps34) are less well understood. In addition there are also four PI4K's and several PI3-kinase related protein kinases (termed PIKK's or class IV) including DNA-PK, mTOR, ATM and ATR, all of which have a similar catalytic domain.[3]

There is currently an intense focus by pharmaceutical companies to develop PI3K inhibitors as therapy for cancer, cardiovascular, respiratory, autoimmune and inflammatory diseases.[4–6] The first generation of PI3-Kinase inhibitors, Wortmannin and LY29 4002, have proven to be invaluable tools with which to investigate the functional relevance of PI3-kinase inhibition although they inhibit multiple isoforms.

Progress in the field led to a second generation of structurally diverse PI3-kinase inhibitors (Fig. 1) which have shown efficacious effects in animal models.[7] Whilst selectivity over protein kinases appears to be readily achievable, many second generation compounds were either pan-PI3K inhibitors (e.g., PIK-90)[8] or were only moderately selective, often displaying additional inhibition of the PIKK's such as mTOR and DNA-PK which seems to favour flat hydrophobic scaffolds.[9] A number of examples do show specificity for single isoforms, e.g. AS-605240 (p110γ),[10] TGX-221 (p110β),[11] and CAL101 (p110δ),[12] however, reports of selective inhibitors such as these remain uncommon.

The situation is often not clear as to which isoform selectivities would provide the optimum balance between a desired therapeutic effect and appropriate safety profile. Indeed, it has been postulated that, for some targets, inhibition of more than one isoform may be a requirement for efficacy.[13] There is a continuing demand by researchers for suitable isoform selective class I PI3K inhibitors to use as pharmacological tools, especially PI3Kγ inhibitors, to further enhance the biological validation of these isoforms as drug targets.

In this paper we describe progression from a pan-PI3K lead molecule to isoform selective inhibitors with good cellular activity. All

* Corresponding author. Tel.: +44 (0) 1403 323080.
E-mail address: ian.bruce@novartis.com (I. Bruce).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.07.042

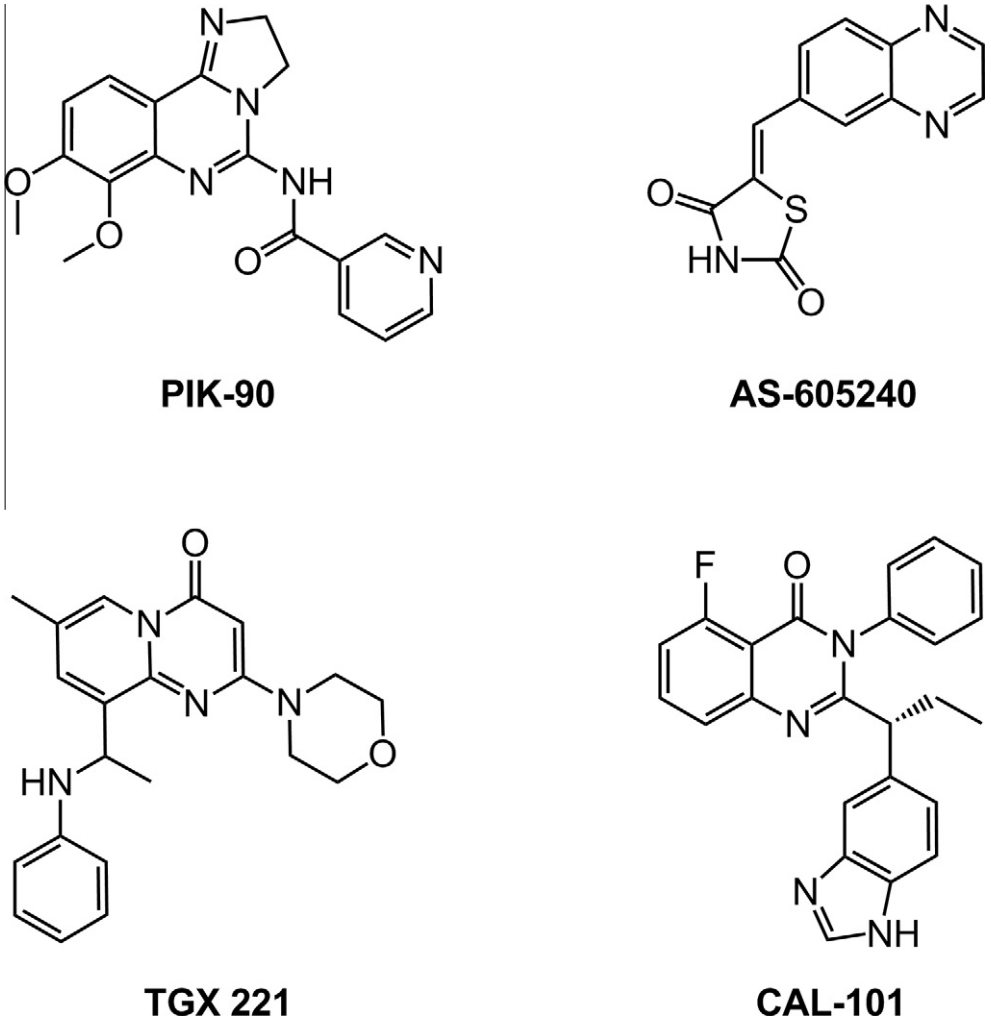


Figure 1. Second generation PI3-Kinase inhibitors

compounds were screened for in vitro PI3-kinase activity against p110α, p110β, p110γ and p110δ isoforms.[14] For secondary cellular assays the inhibitory effect of the compounds on respiratory burst in human neutrophils and B cell proliferation was determined. For the respiratory burst assay, the chemotactic peptide f-Met-Leu-Phe (fMLP) is used to mimic the action of *N*-formyl peptides released during bacterial infection which contribute to neutrophil recruitment and release of highly reactive oxygen species. This is primarily a PI3Kγ and PI3Kδ dependent process[15] whilst B-cell proliferation[16] is a PI3Kδ dependent process; selected results are shown in Tables 1 and 3.

Aminothiazole **1** (Fig. 2), identified from an HTS campaign using a PI3Kγ biochemical assay, was selected as a suitable lead for optimization as it lacked activity against a panel of protein kinases.

Table 1
Inhibition of PI3K by **1**, **7a–f, 11** and **15**

Compd	PI3-Kinase Inhibition (K_i, μM)[a]				fMLP
	P110γ	p110α	p110δ	p110β	IC_{50} (μM)
1	0.11	0.11	0.21	1.43	2.6
7a	0.23	0.20	0.19	1.36	1.93
7b	0.057	0.035	0.079	1.30	1.93
7c	0.032	0.011	0.061	0.55	0.34
7d	0.183	0.122	0.247	1.41	0.22
7e	0.062	0.045	0.079	0.76	0.30
7f	0.061	0.028	0.074	0.87	0.29
11	0.110	0.030	0.019	0.41	0.32
15	0.260	0.190	0.260	1.94	1.76

[a] Values are means of at least two experiments.

Table 2
Isoform selective PI3K inhibitors

Compd	Thiazole precursor	Amine precursor	PI3-Kinase Inhibition (Ki, μM)[a]			
			P110γ	p110α	p110δ	p110β
18	**6a**	**A**	5.81	0.187	3.62	>10
19	**6d**	**A**	3.06	0.050	2.90	>10
20	**6f**	**A**	1.14	0.020	1.67	>10
21	**10**	**A**	0.53	0.005	0.22	2.0
22[b]	**—**	**A**	1.19	0.04	0.54	2.94
23	**6d**	**B**	0.12	0.11	0.003	0.86
24	**10**	**B**	1.02	0.15	0.004	0.34
25	**6d**	**C**	0.02	2.13	0.38	0.09
26	**14**	**D**	0.088	1.89	0.74	0.25
27	**6d**	**E**	0.036	1.40	0.47	0.32

[a] Values are means of at least two experiments.
[b] Values quoted for **22** are IC_{50}'s measured in a Kinase–Glo luminescent assay.

Table 3
In vitro cellular and selectivity data for compounds **21**, **24**, **26** and **27**

Compd	fMLP IC_{50}, μM[a]	BCP IC_{50}, μM[a]		PI3K related assays. IC_{50}, μM[a]		Vps34	PI4 Kβ
	Human	Mouse	Human	mTOR	DNA-PK		
21	0.93	0.37	0.32	>9.1	>10	>9.1	0.92
24	0.72	0.014	0.005	>9.1	>10	>9.1	0.04
26	0.22	0.76	1.16	>9.1	>10	>9.1	0.24
27	0.27	0.59	1.61	>9.1	>10	>9.1	1.33

[a] Values are means of at least two experiments

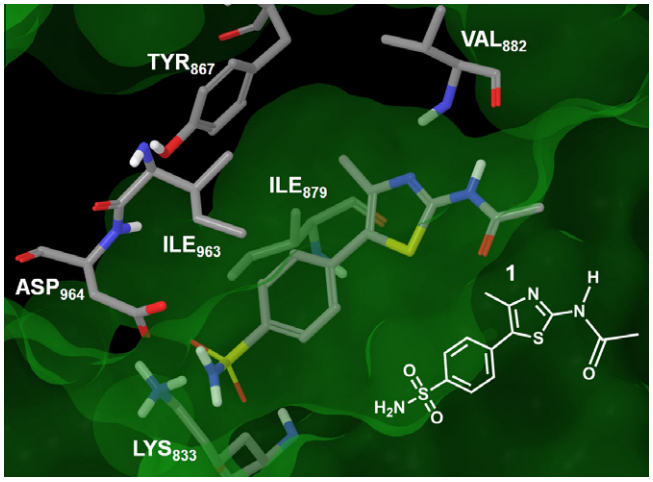


Figure 2. HTS hit **1** from screening against a PI3Kγ enzyme inhibition assay. Docking of **1** in the p110γ crystal structure is shown.

Docking studies of **1** and the kinase domain of p110γ showed the methyl group nicely occupies a small hydrophobic pocket in the ATP binding site formed by Tyr-867, Val-882 and Ile-963 with hydrophobic residues Ile-879 and Ile-576 located above and below the phenyl ring.[17] The thiazole nitrogen and amide hydrogen form a bidentate donor–acceptor pair with the NH and carbonyl of Val-882 whilst the sulfonamide oxygen appears to form a hydrogen bond to the side chain of Lys-883. The acyl substituent points to a channel leading to solvent space. This binding mode is consistent with the reported X-ray co-crystal structure of a related aminothiazole PIK-93 bound to p110γ.[18]

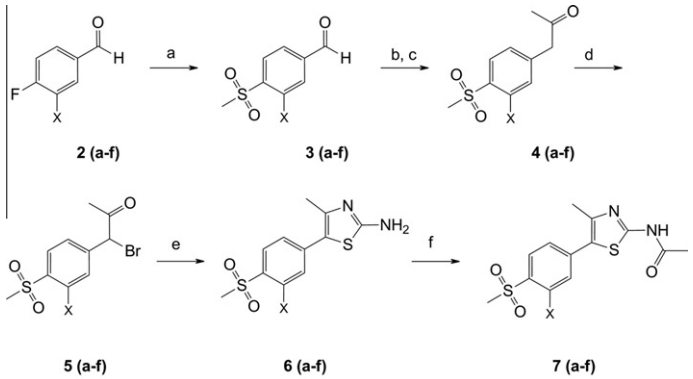
The interior of the ATP binding site is highly conserved between class I PI3K isoforms so the lack of selectivity with compound **1** was not unexpected (Table 1). However, residues lining the binding pocket are less conserved. We hypothesized that isoform selectivity might be achievable by extending or replacing the acyl moiety with diverse substituents to exploit these differences.[19] In an attempt to improve cellular potency by reducing the Polar Surface Area of **1** (PSA = 102 Å) the primary sulfonamide was replaced with methylsulfone (**7a**, PSA = 76.13 Å) pyrazole (**11**, PSA = 62.28 Å) and methyl ketone (**15**, PSA = 59.06 Å).

Synthesis of *p*-methylsulfone analogues from *p*-fluorobenzaldehydes is shown in Scheme 1. The methyl sulfone moiety was introduced by nucleophilic substitution using the sodium salt of methane sulfinic acid to provide analogues **3**.[20] Reaction with nitroethane gave nitrostyrenes which were reduced with iron in acetic acid to give benzyl methyl ketones **4**.[21] Bromination was effected by slow addition of bromine to a rapidly stirred solution of **4** in dioxane to give bromo derivatives **5**. Reaction with thiourea in ethanol gave aminothiazoles **6** which were acetylated to give products **7a–f**.

The pyrazole analogue **11** (Scheme 2) was prepared from **8** by a similar route. Attempted bromination of ketone **9** in this case gave a complex mixture but aminothiazole **10** could be accessed from **9** in a one-pot procedure using iodine and thiourea in pyridine.[22] Acetylation of **10** as before gave **11**.

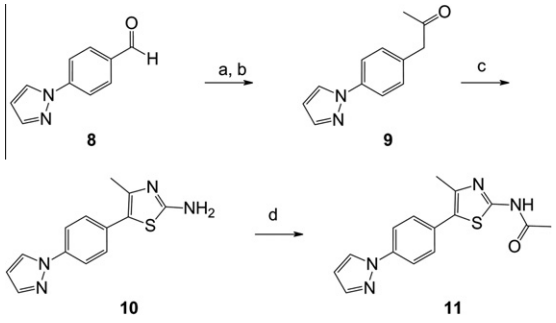
Ketone analogue **15** (Scheme 3) was prepared by Suzuki coupling of the commercial boronic acid with protected bromoaminothiazole **13** followed by deprotection and acetylation.

Replacing the primary sulfonamide of **1** by methylsulfone to give **7a** led to a reduction in binding activity and a minor improvement in cellular potency in the fMLP stimulated neutrophil assay (Table 1). Introducing electron withdrawing groups ortho to the sulfone (compounds **7b–f**) was found to restore potency in the biochemical assay by up to 8 fold and further improve cellular

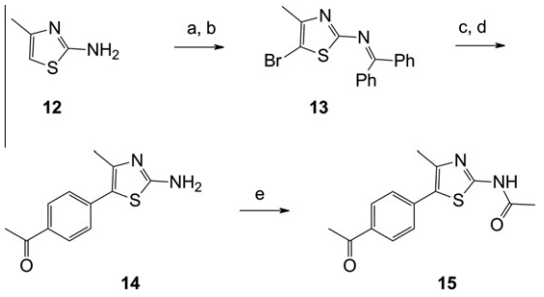


a X = H, **b** X = Cl, **c** X = Br, **d** X = F, **e** X = CN, **f** X = CF_3

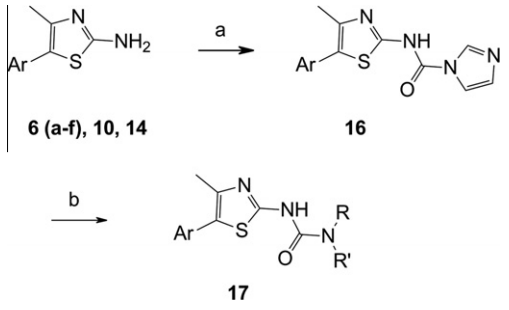
Scheme 1. Reagents and conditions: (a) $MeSO_2Na$, DMSO, 70 °C; (b) $EtNO_2$. NH_4OAc, reflux; (c) Fe, AcOH, 100 °C; (d) Br_2, dioxane, 5–10 °C; (e) Thiourea, EtOH, reflux; (f) Ac_2O, 70 °C, 10 min.



Scheme 2. Reagents and conditions: (a) $EtNO_2$. NH_4OAc, reflux, 18 h (95%); (b) Fe, AcOH, 100 °C, 2 h (91%); (c) I_2, pyridine, thiourea, 80 °C, 8 h (26%); (d) Ac_2O, 70 °C, 10 min (83%).



Scheme 3. Reagents and conditions: (a) Ph_2C = NH, toluene, reflux, 68 h; (b) NBS, AcOH, rt, 1 h (96%); (c) 4-acetylphenylboronic acid, $(PPh_3)_4Pd$, Cs_2CO_3, dioxane–water, reflux, 6 h (100%); (d) 2 M HCl, THF, rt, 1 h (82%); (e) Ac_2O, 70 °C, 10 min.



Scheme 4. Reagents and conditions: (a) CDI, CH_2Cl_2, reflux, 5 h; (b) RR'NH, Et_3N, rt to 70 °C, 30 min – 24 h.

potency. Pyrazole and ketone analogues (**11** and **15**) gave similar results to the sulfones **7a–f**. All examples were least potent against PI3Kβ.

With cellularly active compounds available we required an efficient procedure for generating a large number of analogues to test our hypothesis that substituents extending out to the non-conserved region of the binding site might result in isoform selective inhibitors. Our approach was to synthesize urea libraries from aminothiazoles using a parallel synthesis strategy. The general 2-step protocol is shown in Scheme 4. Reacting aminothiazoles **6a–f**, **10** and **14** with carbonyl di-imidazole in dichloromethane gave intermediates **16** which were either isolated crude by removing the solvent in vacuo or pure by filtration of the precipitate from the cooled reaction. Intermediates **16** were then allowed to react with an excess of an amine in tetrahydrofuran or dimethylformamide. The final products were isolated by filtering the

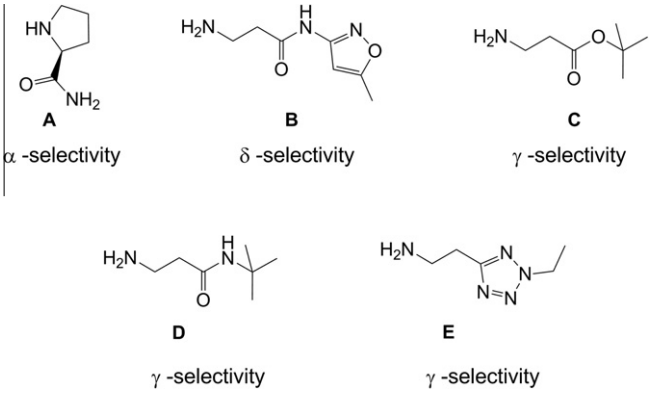


Figure 3. Amines RR'NH, which when combined with selected aminothiazole scaffolds to give ureas, provided Class 1 isoform-selective PI3-kinase inhibitors.

crude reaction mixture through a plug of polymer supported isocyanate (Argonaut Technologies), to remove the excess amine, or by preparative HPLC.

This method was particularly amenable to automation; using a Bohdan Synthesizer enabled us to generate hundreds of final compounds per week. Since it was not clear how to achieve selectivity for any particular isoform the first iteration utilised a diverse set of 80 amines (MW <200), mostly from commercial sources, leading to approximately 400 isolated examples. For compounds showing a trend towards isoform selectivity the process was repeated using the appropriate aminothiazoles and smaller more focused subsets of amines derived from compound archives or synthesis. Results for selected examples are shown in Table 2. Whilst the majority of final compounds were inactive or weak pan-PI3K inhibitors, three types of amines were identified which led to potent isoform selective inhibitors (Fig. 3). In particular, (*S*)-pyrrolidine carboxamide (**A**) conferred PI3Kα selectivity on the aminothiazole core scaffolds used herein as exemplified by compounds **18**–**21** (Table 2) as well as a related benzothiazole core to give analogue **22** (Fig. 4).[23] The binding of A-66, a related aminothiazole taken from a Novartis patent[24] and which contains the *(S)*-pyrrolidine carboxamide substituent, has recently been characterized by others using molecular modelling[25] and in vitro mutagenesis.[26] Results suggest that the

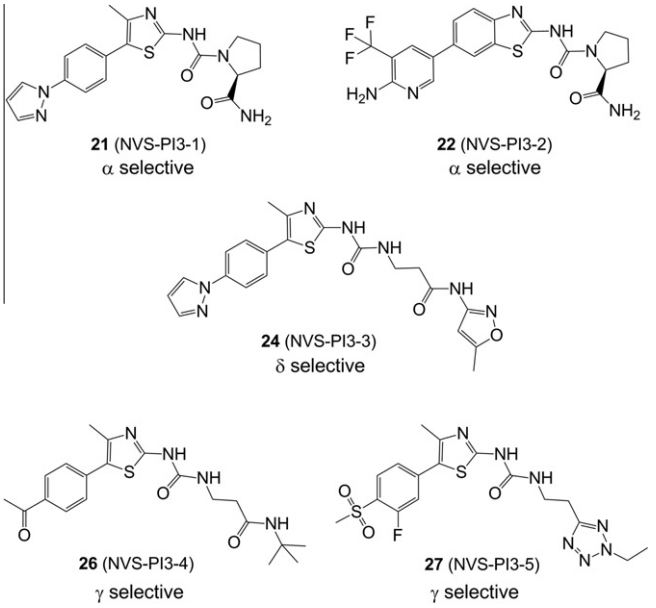


Figure 4. Isoform selective PI3-kinase inhibitors

Table 4
PK profiles of isoform selective PI3K inhibitors

Compd[a]	Dose[b] (mg/kg)	T_{max} (h)	C_{max} (μM)	AUC (μM.h)	F (%)	CL (ml/min/kg)	$T_{1/2}$ (h)	V_{ss} (L/kg)
21	10	4	0.28	4.1	21	21	0.7	1.29
24	10	8	0.05	0.69	3	18	0.6	0.4
26	10	05	1.72	10.8	57	23	0.8	1.4
27	5	1	0.49	4.3	24	4.0	1.8	0.6

[a] Results are mean of $n = 4$ experiments for 21 and 22 and n = 3 experiments for 26 & 27.
[b] Dosed p.o. as a 1% suspension in carboxymethyl cellulose (CMC) to female Wistar rats.

carboxamide group makes favorable contacts with residues in p110α, especially the non-conserved residue Gln^{859}.

Aminopropionic acid derivatives led to either delta or gamma selective compounds depending on the exact nature of the substituent. For PI3Kδ selectivity, screening aryl and heteroaryl amides led to optimized fragment (**B**) which was used to provide compounds **23** and **24**. These were both low nM PI3Kδ inhibitors showing >30-fold selectivity over the other three class 1 isoforms.[27] Moderately selective PI3Kγ inhibitors were produced from alkyl aminopropionic esters; maximum potency and selectivity was achieved when the *t*-butyl ester (**C**) was combined with aminothiazole **6d** to give **25**.

To address the potential hydrolytic and metabolic instability of **25** a series of ester replacements was investigated. Fragments **D** and **E** provided a favourable compromise between potency, selectivity and PK profile, particularly when combined with aminothiazoles **6d** and **14** to give urea analogues **26** and **27**.[28,29] Both PI3Kγ inhibitors showed good selectivity (>20 fold) against PI3Kα although were somewhat less selective against the δ and β isoforms. Interestingly no PI3Kβ selective examples were identified from this process. Molecular modeling and structural studies indicated that δ and γ isoform selectivity of compounds containing fragments (**B**) and (**C**) is most likely derived from interactions between the terminal functional groups of the urea side chain and non-conserved amino acids at the outer edge of the binding site. These findings will be disclosed in due course.

Cellular assay data for selected examples is shown in Table 3. The PI3Kα selective inhibitor **21** was moderately potent in all three cellular assays; this result is thought be accounted for by activity against other isoforms (PI3Kγ & PI3Kδ) at higher concentrations. The PI3Kδ selective inhibitor **24** was very potent in both mouse and human B-cell proliferation (BCP) assays which correlates well with the p110δ binding data. The potent PI3Kγ inhibitors **26** and **27** showed good activity in the oxidative burst (fMLP) assay.

Compounds **21**, **24**, **26** and **27** were inactive against an in-house panel of 20 protein kinases. In addition, **27** was also screened against an external panel of 230 protein kinases and showed minimal activity on all kinases in the panel with the exception of two (CLK1, IC_{50} = 118 nM, and CLK2, IC_{50} = 117 nM); the functional relevance of this is unclear. When screened against other members of the PI3K superfamily no significant inhibition was observed against mTOR, DNA-PK or Vps34 for the compounds in Table 3 although all showed activity in the PI4KIIIβ assay.

The in vivo rat pharmacokinetic profiles for selected compounds are shown in Table 4. The oral bioavailability (%*F*) of compound **24** was low as anticipated from its low aqueous solubility (<0.002 g/L @ pH 6.8). By contrast, the other examples showed greater plasma exposure.

In conclusion we have described progress towards isoform selective PI3K inhibitors using an automated parallel synthesis strategy. The compounds in Table 4[30] are pharmacological tools exhibiting good isoform selectivity and cellular activity that should prove valuable in dissecting the relative contributions of individual isoforms in PI3K signaling pathways.

Acknowledgments

We would like to thank Doriano Fabbro for screening compounds in the PIKK assays, Roger Williams of the University of Cambridge for the PI3Kγ crystal structure, Greg Hollingworth for assistance in preparation of the manuscript and our colleagues in synthetic development and analytical services for support.

References and notes

1. Vanhaesebroeck, B.; Leevers, S.; Ahmadi, K.; Timms, J.; Katso, R.; Driscoll, P. C.; Woscholski, R.; Parker, P. J.; Waterfield, M. D. *Annu. Rev. Biochem.* **2001**, *70*, 535.
2. Wymann, M.; Pirola, L. *Biochim. Biophys. Acta* **1998**, *1436*, 127.
3. Abraham, R. T. *DNA Repair* **2004**, *3*, 883.
4. Stein, R. C.; Waterfield, M. D. *Mol. Med. Today* **2000**, *6*, 347.
5. Ward, S.; Sotsios, Y.; Dowden, J.; Bruce, I.; Finan, P. *Chem. Biol.* **2003**, *10*, 207.
6. Thomas, M.; Owen, C. *Cur. Opin. Pharmacol.* **2008**, *8*, 267.
7. (a) Marone, R.; Cmiljanovic, V.; Giese, B.; Wymann, M. P. *Biochim. Biophys. Acta Proteins Proteomics* **2008**, *1784*, 159; (b) Shuttleworth, S. J.; Silva, F. A.; Cecil, A. R. L.; Tomassi, C. D.; Hill, T. J.; Raynaud, F. I.; Clarke, P. A.; Workman, P. *Curr. Med. Chem.* **2011**, *18*, 2687.
8. Shimada, M.; Murata, T.; Fuchikami, K.; Tsujishita, H.; Omori, N.; Kato, I.; Miura, M.; Urbahns, K.; Gantner, F.; Bacon, K. WO 2004029055, 2004. See also reference 18 which describes structural studies with PIK-90.
9. Hollick, J. J.; Rigoreau, L. J. M.; Cano-Soumillac, C.; Cockcroft, X.; Curtin, N. J.; Frigerio, M.; Golding, B. T.; Guiard, S.; Hardcastle, I. R.; Hickson, I.; Hummersone, M. G.; Menear, K. A.; Martin, N. M. B.; Matthews, I.; Newell, D. R.; Ord, R.; Richardson, C. J.; Smith, G. C. M.; Griffin, R. J. *J. Med. Chem.* **1958**, *2007*, 50.
10. Camps, M.; Ruckle, 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.; Schwartz, M. K.; Rommel, C. *Nat. Med.* **2005**, *11*, 936.
11. Robertson, A. D.; Jackson, S.; Kenche, A.; Yaip, C.; Parbaharan, H.; Thompson, P. WO 200153266, 2001.
12. Herman, S. E.; Gordon, A. L.; Wagner, A. J.; Heerema, N. A.; Zhao, W.; Flynn, J. M.; Jones, J.; Andritsos, L.; Puri, K. D.; Lannutti, B. J.; Giese, N. A.; Zhang, X.; Wei, L.; Byrd, J. C.; Johnson, A. J. *Blood.* **2010**, *116*, 2078.
13. (a) Chaisuparat, R.; Hu, J.; Jham, B. C.; Knight, Z. A.; Shokat, K. M.; Montaner, S. *Cancer Res.* **2008**, *68*, 8361; (b) Rommel, C. *Curr. Top. Microbiol. Immunol.* **2010**, *1*, 279.
14. The inhibitory activities of compounds against the recombinant PI3-kinase enzymes were determined by using a high throughput assay based on Amersham Pharmacia Biotech's Scintillation Proximity Assay (SPA) that measures the transfer of the terminal phosphate of adenosine triphosphate to phosphatidylinositol. Each well contains 10 μl test compound in 5% dimethylsulphoxide and 20 μl assay mix (40 mM Tris, 200 mM NaCl, 2 mM ethyleneglycol-aminoethyl-tetraacetic acid (EGTA), 15 μg/ml phosphatidylinositol, 12.5 μM adenosine triphosphate (ATP), 25 mM $MgCl_2$, 0.1 μCi [^{33}P]ATP). The reaction is started by the addition of 20 μl of enzyme mix (40 mM Tris, 200 mM NaCl, 2 mM EGTA containing recombinant GST-p110γ). The plate is incubated at room temperature for 60 min and the reaction terminated by the adding 150 μl of WGA-bead stop solution (40 mM Tris, 200 mM NaCl, 2 mM EGTA, 1.3 mM ethylene diamine tetraacetic acid (EDTA), 2.6 μM ATP and 0.5 mg of Wheat Germ Agglutinin–SPA beads (Amersham Biosciences) to each well. The plate is sealed, incubated at room temperature for 60 min, centrifuged at 1200 rpm and then counted for 1 min using a scintillation counter. Total activity is determined by adding 10 μl of 5% dimethylsulphoxide (DMSO) and non-specific activity is determined by adding 10 μl 50 mM EDTA in place of the test compound.
15. Condliffe, A. M.; Davidson, K.; Anderson, K. E.; Ellson, C. D.; Crabbe, T.; Okkenhaug, K.; Vanhaesebroeck, B.; Turner, M.; Webb, L.; Wymann, M. P.; Hirsch, E.; Ruckle, T.; Camps, M.; Rommel, C.; Jackson, S. P.; Chilvers, E. R.; Stephens, L. R.; Hawkins, P. T. *Blood* **2005**, *106*, 1432.
16. Julius, M. H.; Heusser, C. H.; Hartmann, K. H. *Eur. J. Immunol* **1984**, *14*, 753.
17. Walker, E. H.; Perisic, O.; Reid, C.; Stephens, L.; Williams, R. L. *Nature* **1999**, *402*, 313.
18. 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.
19. Knight, Z. A.; Chiang, G. G.; Alaimo, P. J.; Kenski, D. M.; Ho, C. B.; Coan, K.; Abraham, R. T.; Shokat, K. M. *Bioorg. Med. Chem.* **2004**, *12*, 4749.
20. Ulman, A.; Urankar, E. *J. Org. Chem.* **1989**, *54*, 4691.
21. Sargent, T., III; Shulgin, A. T.; Mathis, C. A. *J. Med. Chem.* **1984**, *1071*, 27.
22. King, L. C.; Hlavacek, R. J. *J. Am. Chem. Soc.* **1950**, *72*, 3722.
23. Ni, Z-J.; Pecchi, S.; Burger, M.; Han, W.; Smith, A.; Atallah, G.; Bartulis, S.; Frazier, K.; Verhagen, J.; Zhang, Y.; Iwanowicz, E.; Hendrickson, T.; Knapp, M.; Merritt, H.; Voliva, C.; Wiesmann, M.; Le Grand, D. M.; Bruce, I.; Dale, J.; Lan, J.; Levine, B.; Costales, A.; Liu, J.; Pick, T.; Menezes, D. WO 2007095588, 2007.

24. R. H. Fairhurst, R. H.; P. Imbach, P. WO 2009080705, 2009.
25. Jamieson, S.; Flanagan, J. U.; Kolekar, S.; Buchanan, C.; Kendall, J. D.; Lee, W. J.; Rewcastle, G. W.; Denny, W. A.; Singh, R.; Dickson, J.; Baguley, B. C.; Shepherd, P. R. *Biochem. J.* **2011**, *438*, 53.
26. Zheng, Z.; Amran, S. I.; Zhu, J.; Schmidt-Kittler, O.; Kinzler, K. W.; Vogelstein, B.; Shepherd, P. R.; Thompson, P. E.; Jennings, I. *Biochem. J.* **2012**, *444*, 529.
27. Bruce, I.; Weitz, G.S. WO 2008000421, 2008.
28. Budd, E.; Hatto, J. D. I.; Hayler, J. F.; Le Grand, D. M.; Valade, B. WO 2007068473, 2007.
29. Bloomfield, G. C.; Bruce, I.; Hayler, J. F.; Leblanc, C.; Le Grand, D. M.; McCarthy, C. WO 2005021519, 2005.
30. Detailed synthetic procedures for compounds **21, 24, 26** & **27** are provided in the folowing patents; Compound **24** is Example 2 in WO2008000421 (Ref.[27]). compound **21** is prepared as described for compound **24** using (*S*)-pyrrolidine carboxamide in the final step. Compound **22** is Example 2–33 in WO2007095588 (Ref.[23]). Compound **26** is Example 1 in WO2007068473 (Ref.[28]) and compound **27** is Example 110 in WO2005021519 (Ref.[29]).