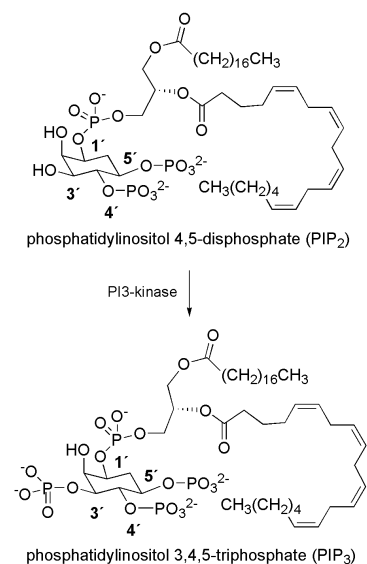


Inhibitors of Phosphatidylinositol 3-kinases: The Next Wave of Anti-Cancer Drugs?

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Phosphatidylinositol 3-kinases (PI3Ks) are a family of lipid kinase enzymes, which catalyse the phosphorylation of the 3'-hydroxyl position of the inositol ring of phosphatidylinositol 4,5-diphosphate (PIP₂) to give the messenger molecule phosphatidylinositol 3,4,5-triphosphate (PIP₃) (Scheme 1). This then participates in a variety of physiological processes, including cell growth and differentiation.¹ The PI3Ks are divided into three classes (I-III) based on their structure, mode of regulation, and substrate specificity. Class 1A PI3Ks are comprised of three isoforms (p110 α , p110 β and p110 δ) that share a common regulatory subunit (p85) activated by signals from receptor protein tyrosine kinases, while the Class IB PI3K (p110 γ) is structurally similar but lacks a regulatory subunit, and is activated by G protein-coupled receptors.² The pathway through p110 α is the most frequently activated signalling pathway in human cancer, and its corresponding gene (*PIK3CA*) undergoes amplification in tumours, with activating *PIK3CA* mutations being relatively common in late-stage colon, brain, breast, and gastric cancers.^{3,4}



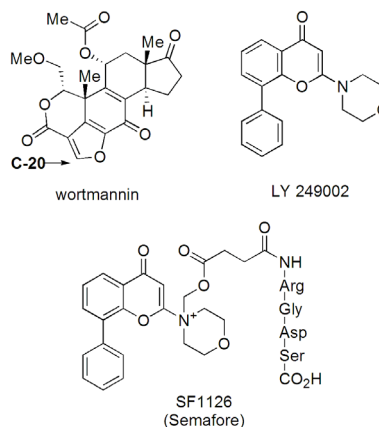
Scheme 1. PI3K phosphorylation of phosphatidylinositol 4,5-diphosphate (PIP₂).

Early investigations into the mechanism of PI3K inhibition were aided by two compounds, the fungal natural product wortmannin, first isolated⁵ from *Penicillium wortmanni* in 1957, and the synthetic inhibitor LY294002 (Chart 1), which was first synthesized by Eli Lilly in the early nineties.⁶

Wortmannin is a potent and irreversible inhibitor in which the furan ring adds to the amino group of a lysine residue in the ATP binding pocket of PI3K giving an enamine

at C20. X-ray studies with the p110 γ isoform confirmed that this is with the amino group of Lys-833 and they also showed an H-bond between the C17 carbonyl oxygen and the backbone NH of Val-882.² However, since similar amino acid residues are found in all of the PI3K isoforms, wortmannin shows very poor isoform selectivity, and displays considerable liver toxicity at low doses in animal studies. Several wortmannin analogues have been prepared in an attempt to reduce this toxicity⁷ but, since they all function as prodrugs of wortmannin itself, they show no advantage in terms of PI3K selectivity.

Chart 1



LY294002 binds reversibly with moderate potency and has proved useful as a tool due to its stability. It was the first synthetic PI3K inhibitor to have its complex with PI3K γ structurally elucidated.² The morpholine oxygen makes an H-bond with the backbone amide NH of Val-882, the same residue that forms an H-bond with wortmannin and, in fact, this is a much conserved interaction that is now known to be shared by all current PI3K inhibitors and ATP itself. LY294002 is too insoluble for investigation as a drug, although a prodrug derivative, SF 1126 (Chart 1) has now entered human clinical trials as a pan-PI3K inhibitor, targeting cell growth, proliferation and angiogenesis.⁸

The issue of isoform selectivity is potentially important since each of the isoforms have a suite of significant biological effects; the p110 β isoform is important in thrombus formation, while the p110 δ and p110 γ isoforms are important in aspects of inflammation. However, despite high-quality crystal structure data on both the α - and γ -isoforms, obtaining compounds with high selectivity for p110 α has proved difficult. This is illustrated (Table 1) by the IC₅₀ values (concentration of drug for 50% inhibition of PIP₂ phosphorylation) by LY294002, wortmannin, and the first of the other new PI3K inhibitors that have

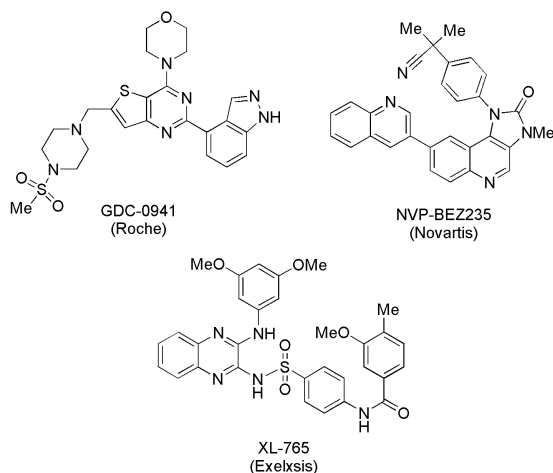
begun clinical trial; GDC-0941, NVP-Bez235, XL-765 (Chart 2).

Table 1. Isoform selectivity of PI3K inhibitors.

Compound	IC ₅₀ (nM) p110			ratio
	- α	- β	- δ	
Wortmannin	~4	~4	~4	~1
LY294002	800	1000	700	1.2
SF-1126	NA*	NA*	NA*	NA*
GDC-0941	3	33	11	11
NVP-Bez235	20	160	12	8.0
XL-765	13	113	43	8.7

*NA - not applicable; prodrug

Chart 2



GDC-0941 (Genentech/Roche) is the result of much study with other morpholine-containing analogues of LY294002, and is currently undergoing Phase I human cancer clinical trials.⁹ Heteroaromatic nitrogen atoms can also participate in hydrogen bonding to the NH of Val-882 and examples of this class include the Phase I clinical agent NVP-Bez235 (Novartis),¹⁰ where it is believed that the primary H-bond is via the quinoline nitrogen of the imidazo[4,5-*c*]quinoline core of the molecule. Another azaheterocycle that is reported¹¹ to have entered clinical studies for the treatment of solid tumours is the quinoxaline derivative XL-765 (Chart 2), although few details are available. There is, therefore, high interest in the development of PI3K inhibitors as anticancer agents,^{4,11,12} although most of the current compounds are pan-inhibitors, rather than specific inhibitors of p110 α , the PI3K isoform most often mutated in human cancers.

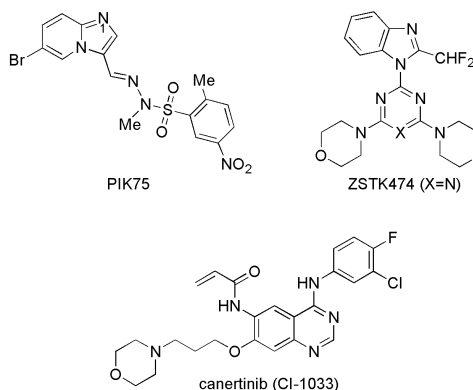
From its outset in 2005, our programme has thus been focused on the development of more selective inhibitors of p110 α as anticancer drugs, and began by studying the literature to see where we could make positive improvements.¹³ We started with the imidazo[1,2-*a*]pyridine derivative PIK75 (Chart 3), which is a moderately selective inhibitor of p110 α compared to the other Class I PI3K isoforms (p110 β , p110 δ and p110 γ) (Table 2),¹⁴ and is also active in human cancer xenograft models.¹⁵ Our systematic study of the changes to the imidazopyridine chromophore indicated tight structure-activity relationships (SAR),¹⁶ but did lead us to a new chromophore that had

both high potency and much higher selectivity for p110 α . A patent application has been filed on this new class of inhibitor,¹⁷ and we are continuing to optimise the structure. To date, we have been able to retain high potency and improve p110 α selectivity.

Table 2. Inhibitory effects of PIK75 on PI3K isoforms.

Compound	IC ₅₀ (nM) p110			ratio
	- α	- β	- δ	
PIK75	20	300	1000	15

Chart 3



In order to try and rationalize the high p110 α specificity of the imidazo[1,2-*a*]pyridines and the new chromophore, we studied the binding mode of the known inhibitor PIK75 to p110 α using a molecular modelling approach.¹⁶ For this work it was necessary to develop a p110 α homology model,^{16,18} since prior to December 2007 structural data were available only for the p110 γ isoform.² We used the high level of sequence identity shared across the PI3K isoforms around the ATP binding cleft to develop this model.^{16,18} As expected, the primary interaction involves an H-bond between the *N*-1 of PIK75 and the backbone NH of Val-851 (equivalent to Val-882 in p110 γ) but, in addition, a possible hydrogen bonding interaction between one of the oxygen atoms of the sulfonyl group and the NH of a histidine residue (His-855) was identified.¹⁶ Since this histidine is unique to the p110 α isoform, it was proposed that the additional interaction could account for the high selectivity of PIK75 against p110 α . However, in December 2007 the structure for the full length human p110 α catalytic subunit in conjunction with a portion of its p85 α regulatory subunit was published,¹⁹ and demonstrated that while most of the ATP binding site residues had a similar 3D structure, there were some notable differences at certain positions. Significantly, the most notable difference from our homology model related to His-855 which was *tied-back* due to an H-bond with Asp-925 and therefore not accessible to the sulfonyl oxygen atoms of PIK75. Whether this is a crystallization artefact or a real phenomenon remains to be determined, but in the interim we are developing a refined model based on this new data.²⁰

The second literature lead that we investigated in detail, was the dimorpholino-1,3,5-triazine derivative ZSTK474 (X=N; Chart 3) that is reported to be a reversible and non-selective PI3K inhibitor, but with excellent oral activity against human xenografts in mice.^{21,22} This is a very competitive field, with a Japanese patent application filed by

Zenyaku Kogyo Kabushiki Kaisha²³ in 2006 that covers both the triazine and its 2-pyrimidine derivatives (X=CH), where the second morpholine has been replaced by a piperazine group, and a suite of 12 patent applications from AstraZeneca covering a variety of different morpholine replacements, and all three possible pyrimidine isomers.²⁴ We modelled the binding of ZSTK474 (X=N) in the ATP-binding site of the p110 γ crystal structure,²⁵ and identified a binding mode in which the key H-bond with the NH of Val-882 was with the oxygen atom of one of the morpholine groups, rather than with the benzimidazole nitrogen as proposed,²² with the latter nitrogen actually H-bonding to the NH₂ group of Lys-833 (the amino group responsible for the irreversible interaction with wortmannin). Our binding model allowed us to design new analogues that are not predicted by the published model, and enabled us to identify several potent new lead structures.

With the exception of wortmannin and its analogues, all of the approaches discussed so far have involved reversible PI3K inhibitors that must compete with ATP for binding in the catalytic site of the enzyme. Irreversible inhibitors have advantages in that they allow for longer-term inhibition of the enzyme, promising greater therapeutic effect, while allowing for longer times between treatments, as shown by the erbB irreversible inhibitor canertinib (CI-1033; Chart 3) that we developed earlier to Phase II clinical trial.²⁶ Thus, our aim was to develop compounds able to bind irreversibly to the p110 α site, but only reversibly to the other isoforms. Such specific p110 α irreversible inhibitors should have better therapeutic potential than pan-PI3K irreversible inhibitors based on wortmannin. Preliminary results suggest this approach is feasible.²⁷

Our work in the PI 3-kinase area began in 2005 with in-house funding and support from the government-funded Maurice Wilkins Centre for Molecular Biodiscovery. A successful 2006 HRC grant application, coupled with 2007 support from Auckland's Faculty Research Development Fund, enabled sufficient results to be obtained for the commercialization arm of the University (Auckland UniServices Ltd.) to set up the spinout company *Pathway Therapeutics Ltd.*, which has recently successfully raised \$A10 million from two Australian-based venture capital companies, CM Capital Investments (Brisbane) and GBS Venture Partners (Melbourne), and the new Trans-Tasman Commercialisation Fund.

Our initial PI3K research team consisted of the authors with cell biologists Bruce Baguley and Elaine Marshall, and biochemist Peter Shepherd. More recent additions to the team include chemistry PhD student Andrew Marshall, biologist Claire Chaussade, molecular modellers Raphael Frederick and Jack Flanagan, pharmacologist Phil Kestell, and technicians Claire Mawson and Mindy Chao. New additions to the team resulting from the Pathway funding are chemists Swarna Gamage, Anna Giddens and Sophia Tsang, and five technical positions are to be filled.

The pharmaceutical development of PI3K inhibitors has taken great strides during the last five years. Several compounds are now in clinical trial, and large amounts of structural and biological data are becoming available. We

are hopeful that the future will see even better therapeutic results being achieved with more selective inhibitors.

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