The Development of PI3K Inhibitors as Anticancer Drugs

Andrew James Marshall

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Auckland Cancer Society Research Centre
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Abstract

Phosphatidylinositol 3-kinases (PI3Ks) are a lipid enzyme family that are vitally important regulators of intracellular signalling pathways which control cellular activities including cell survival, growth and proliferation. Deregulation of the PI3K signalling cascade has been observed in a broad range of human diseases including cancer, diabetes, thrombosis, immunity and inflammatory disorders. With the discovery of PI3K's link to a variety of diseases, there has been a race to produce ATP competitive inhibitors as therapeutic agents against the Class I PI3K isozymes. Herein, compounds from two structurally distinct chemotypes were synthesised and their activity and specificity characterized against isolated Class PI3K enzymes and two cellular lines.

The aryl morpholine containing pyrido[1,2-*a*]pyrimidines probed the requirements of the Class IA PI3K active sites through modification of the pendant C9 position. Interestingly, no compound synthesised exhibited superior activity towards the p110β enzyme than TGX-221 (**1.14**). The second series of compounds probed the requirements of the thiazole-linked pyrazolo[1,5-*a*]pyridine **4.41B**, identified through scaffold hopping studies using the novel p110α selective inhibitor PIK-75 (**1.34**). Although **4.41B** was not synthetically accessible, analogues explored alternative linkers and substitution of the 2-methyl-5-nitrobenzene ring, to investigate the effect on p110α selectivity and potency. The sulfone-pyrazole linker group in (**5.5**) was found to be critical, with alternative linker groups in the thiazole series SO_2CH_2 **4.123**, CH_2 **4.122**, CHOH **4.114** and linker absent **4.108** ablating activity, while activity was retained by thiazole-CH_2SO_2 **4.124**.

As the complexes between the pyrido[1,2-*a*]pyrimidine and pyrazolo[1,5-*a*]pyridine chemotypes with the active sites of p110β and p110α respectively are not known, docking simulations were performed using structural p110β models and p110α (pdb:2RD0) respectively to understand the molecular basis for the isoform selectivity exhibited by the two chemotypes. Suitable docking methods were obtained by first investigating the ability of three docking protocols GOLD, SURFLEX and AutoDock to find and correctly rank an experimentally derived conformation both retrospectively (rescoring), where the compounds were docked back into the p110γ crystal, and prospectively, where the ligands were docked into the apo p110α (2RD0).

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I was very fortunate to work alongside my supervisor Associate Professor Gordon Rewcastle an active bench chemist and group leader. His boyish enthusiasm for chemistry, amazing memory and hard work, to make a difference for cancer patients was a real inspiration for me. Most of all Gordon has been a great friend, confident and chauffeur to my mother and me for many years.

It was a pleasure to work with Dr Jackie Kendall who always kind, ready to listen and encouraging and greatly supported my work on the thiazole linked-pyrazolo[1,5-*a*]pyridines.

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Declaration and Preface

This is to certify that:

1. the thesis comprises only my original work towards the PhD except where indicated below;
2. due acknowledgment has been made in the text to all other material used.

My project was part of an established and multi-disciplinary drug development program exploring inhibititors of PI3K, comprised of Chemistry, Biology (Prof. Bill Denny and Prof. Bruce Baguely, ACSRC) and Molecular Biology (Prof. Peter Sheperd, MMP). To indicate the rationale for exploring the sulfonyl-thiazole linker moiety in Chapters 4 and the observed SAR presented in Chapter 5, these Chapters include data from compounds not synthesised by the Author: Chapter 4 (Tables 4.1 and 4.2) and Chapter 5 (Table 5.1) present data from a series of compounds synthesised by Jackie Kendall (ACSRC). Furthermore, molecular modelling work conducated by Raphael Frederick (ACSRC) was included to illustrate a hypothetical binding mode for the series, presented in Chapter 4 (Figures 4.2 and 4.3).

Compounds synthesised in the PI3K program were screening against isolated enzyme panel consisting of p110α, p110β and p110δ. The IC_{50} values are obtained from a kinase assay that measures radioactive phosphatidylinositol 3-phosphate (PIP), produced by the p110-p85 heterodimer phosphorylation of phosphatidylinositol (PI). Claire Mawson, Jo Yu (ACSRC), Kitty Cho, Sharada Kolekar, Woo-Jeong Lee and Alisha Malik (MMP) ran these assays under the supervision of Prof Peter Sheperd.

Cellular antiproliferative data were also obtained from two human tumour cell lines, by measuring radioactive thymidine incoperation. NZB5 is derived from a brain cancer, and has wild-type PI3K, while NZOV9 (an ovarian tumour cell line) contains mutation Y1021C in the p110α helical domain. Emma Richardson, Wayne Joseph, Laura Broome, and Elaine Marshall carried out the cellular assays, under the supervision of Prof. Bruce Baguely.

Abbreviations Chemistry

Ac:	Acetyl
AcOH:	Acetic acid
APCI:	Atmospheric pressure chemical ionization
aq.:	Aqueous
Ar:	Aromatic
Boc / BOC:	*tert*-Butyloxycarbonyl
br:	Broad
Bu:	Butyl
C:	Celsius
cat.:	Catalytic
cm:	Centimetre
Conc.:	Concentrated
COSY:	Correlation spectroscopy
d:	Doublet
dd:	Doublet of doublets
ddd:	Doublet of doublet of doublets
dil.:	Dilute
DIPEA:	Diisopropylethylamine
DMAP:	4-Dimethylaminopyridine
DMF:	*N*,*N*-Dimethylformamide
DMSO:	Dimethyl sulfoxide
dq:	Doublet of quartets
dt:	Doublet of triplets
EDG:	Electron donating group
EDTA:	Ethylenediaminetetraacetic acid
EI:	Electron impact ionization
Et:	Ethyl
EtOAc:	Ethyl acetate
EtOH:	Ethanol
eV:	Electron volt
g:	Gram
hr:	Hour
HPLC:	High performance liquid chromatography

HRMS:	High resolution mass spectroscopy
HSQC:	Heteronuclear single quantum coherence
Hz:	Hertz
^{i}Pr / *i*-Pr:	*iso*-Propyl
LDA:	Lithium diisopropylamide
LHMDS:	Lithium hexamethyldisilazide
lit.:	Literature
m.p.:	Melting point
m:	Multiplet (NMR)
m-CPBA:	*meta*-Chloroperoxybenzoic acid
Me:	Methyl
MeOH:	Methanol
mg:	Milligram
MHz:	Megahertz
min:	Minute
mmol:	Millimol
Ms:	Methanesulfonyl, Mesyl
^{n}Bu / *n*-Bu:	*n*-Butyl
NMR:	Nuclear magnetic resonance
NO:	Nitric oxide
NOE:	Nuclear Overhauser effect
Ph:	Phenyl
ppm:	Part per million
Pr:	Propyl
py:	Pyridine
q:	Quartet
qd:	Quartet of Doublets
r.t.:	Room temperature
s:	Singlet (NMR)
T / temp.:	Temperature
t:	Triplet
TBAB:	Tetrabutylammonium Bromide
^{t}Bu / *t*-Bu:	*tert*-Butyl
td:	Triplet of doublets
TFA:	Trifluoroacetic acid
THF:	Tetrahydrofuran

TLC:	Thin layer chromatography
TMS:	Trimethylsilyl, tetramethylsilane
Ts:	*para*-Toluenesulfonyl, Tosyl

Amino acids

Ala	(A)	Alanine
Arg	(R)	Arginine
Asn	(N)	Asparagine
Asp	(D)	Aspartic acid
Cys	(C)	Cysteine
Gln	(Q)	Glutamine
Glu	(E)	Glutamic acid
Gly	(G)	Glycine
His	(H)	Histidine
Ile	(I)	Isoleucine
Leu	(L)	Leucine
Lys	(K)	Lysine
Met	(M)	Methionine
Phe	(F)	Phenylalanine
Pro	(P)	Proline
Ser	(S)	Serine
Thr	(T)	Threonine
Try	(W)	Tryptophan
Tyr	(Y)	Tyrosine
Val	(V)	Valine

Abbreviations (Molecular and Cellular biology)

Akt/PKB:	v-akt murine thymoma viral oncogene homolog 1
ATM:	Ataxia telangiectasia mutated gene product
AML:	Acute myeloid leukemia
BAD:	Bcl2-antagonist of cell death
CDK:	Cyclin dependent kinase
CK2:	Casein kinase 2
cDNA:	Complimentary Deoxyribonucleic acid

DNA:	Deoxyribonucleic acid
DNA-PK:	DNA-dependent protein kinase
EGFR:	Epidermal growth factor receptor
GBM:	Glioblastoma multiforme
GPCR:	G-protein-coupled receptor
GSK3:	Glycogen synthesis kinase 3
HIF-1α:	Hypoxia-inducible factor 1α
IRS:	Insulin receptor substrate
mTOR:	Mammalian target of rapamycin
MLCK:	Myosin light chain kinase
n.d	Not determined
PAF:	Platelet activated factor
PH:	Pleckstrin homology
PI3K :	Phosphatidylinositol 3-kinase
PI:	Phosphatidylinositol
PIP:	Phosphatidylinositol 4-phosphate
PIP_2:	Phosphatidylinositol 4,5-bisphosphate
PIP_3:	Phosphatidylinositol 3,4,5-trisphosphate
PKB/Akt:	Protein kinase B
PKC:	Protein kinase C
PIKK:	PI3K-related kinase
PLK:	Polo-like kinase
PTEN:	Phosphatase and tensin homologue
PIK3CA:	cDNA for the human p110α subunit of PI3K
PIK3CB:	cDNA for the human p110β subunit of PI3K
PIK3CD:	cDNA for the human p110δ subunit of PI3K
PIK3CG:	cDNA for the human p110γ subunit of PI3K
PK:	Pharmacokinetics
RAS:	oncogenes causing Rat sarcoma
RTK:	Receptor tyrosine kinase
VEGF:	Vascular endothelial growth factor
Vps34:	Vacuolar protein sorting 34