

# Novel pyrazolo[1,5-*a*]pyridines as PI3K inhibitors: variation of the central linker group†

Cite this: *Med. Chem. Commun.*, 2014, 5, 41

Jackie D. Kendall,<sup>\*ab</sup> Andrew J. Marshall,<sup>a</sup> Anna C. Giddens,<sup>a</sup> Kit Yee Tsang,<sup>‡a</sup> Maruta Boyd,<sup>a</sup> Raphaël Frédérick,<sup>§a</sup> Claire L. Lill,<sup>c</sup> Woo-Jeong Lee,<sup>c</sup> Sharada Kolekar,<sup>c</sup> Mindy Chao,<sup>c</sup> Alisha Malik,<sup>c</sup> Shuqiao Yu,<sup>c</sup> Claire Chaussade,<sup>¶bc</sup> Christina M. Buchanan,<sup>bc</sup> Gordon W. Rewcastle,<sup>ab</sup> Bruce C. Baguley,<sup>ab</sup> Jack U. Flanagan,<sup>ab</sup> William A. Denny<sup>ab</sup> and Peter R. Shepherd<sup>bc</sup>

As part of our investigation into the pyrazolo[1,5-*a*]pyridines as novel PI3K inhibitors, we report a range of analogues where the central linker portion of the molecule was varied while retaining the pyrazolo[1,5-*a*]pyridine and arylsulfonyl or arylcarbonyl groups. Isostere generating software BROOD was used to assist with producing ideas. The isoform selectivity of the compounds varied from pan-PI3K for compound **41** to p110 $\alpha$ -selective for compound **58** or p110 $\delta$ -selective for compound **57**. The latter two compounds varied only in their sulphur oxidation state.

Received 30th July 2013  
Accepted 26th October 2013

DOI: 10.1039/c3md00221g

www.rsc.org/medchemcomm

## 1. Introduction

Phosphoinositide 3-kinases (PI3Ks) are a group of lipid kinases which phosphorylate the 3'-hydroxyl group of phosphatidylinositol diphosphate (PIP2) to phosphatidylinositol triphosphate (PIP3). PIP3 then recruits protein kinase B (PKB, otherwise known as AKT) to the cell membrane where it is in turn phosphorylated and activated, leading to a cascade of cell signalling which controls a range of cellular processes like cell proliferation, growth and survival.<sup>1</sup> PI3Ks are often over-expressed, and the lipid phosphatase PTEN (which dephosphorylates PIP3) is often deleted or inactivated in many cancer types, leading to increased levels of PIP3 and increased tumour survival.<sup>2</sup>

The PI3Ks are split into three sub-families (class I, II and III), and class I is further split into class Ia and Ib based upon their mechanism of activation. The class Ia PI3Ks are heterodimeric, consisting of a catalytic subunit (p110 $\alpha$ , p110 $\beta$  or p110 $\delta$ ) in complex with a regulatory subunit.<sup>1</sup> PIK3CA, the gene encoding for p110 $\alpha$ , is often over-expressed and mutated in many cancer

types. Two of the most common of these mutations (E545K and H1047R) have been confirmed as activating mutations and hence increase levels of PIP3.<sup>3</sup> Mutations in p110 $\beta$  and p110 $\delta$  have not been reported.<sup>4,5</sup> Inhibitors of PI3K, and in particular, selective inhibitors of p110 $\alpha$  could prove to be an important new strategy in cancer treatment.<sup>6</sup> A number of inhibitors of PI3K are currently in clinical trials, although none have yet reached market.<sup>7,8</sup>

We have shown previously that pyrazolo[1,5-*a*]pyridines such as **1** (ref. 9) and **2** (ref. 10) (Fig. 1) are potent and selective inhibitors of the p110 $\alpha$  isoform of PI3K. However, since the sulfonohydrazide central portion of the molecule is a known structural alert,<sup>11</sup> we were interested in replacing this with an alternative linker group. This has been investigated to a limited extent by Hayakawa *et al.*<sup>12</sup> with their related imidazo[1,2-*a*]pyridine series of compounds, however PI3K isoform selectivity data was not reported for all compounds.

Molecular modelling of the pyrazolo[1,5-*a*]pyridines showed that the pyrazolo nitrogen formed a key hydrogen bond with Val851 of p110 $\alpha$ ;<sup>9</sup> a residue interaction characteristic of many other PI3K inhibitor complexes.<sup>13</sup> Furthermore the sulfonyl

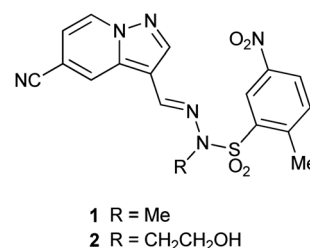


Fig. 1 Structures of our previous pyrazolo[1,5-*a*]pyridine PI3K inhibitors.

<sup>a</sup>Auckland Cancer Society Research Centre, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand. E-mail: j.kendall@auckland.ac.nz

<sup>b</sup>Maurice Wilkins Centre for Molecular Biodiscovery, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

<sup>c</sup>Department of Molecular Medicine and Pathology, School of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3md00221g

‡ Present address: Universal Display Corporation, Hong Kong.

§ Present address: Université Catholique de Louvain (UCL), Louvain Drug Research Institute, Medicinal Chemistry Research Group, 73 Avenue E. Mounier, bte B1.73.10, B-1200 Brussels, Belgium.

¶ Present address: Centre Hospitalier Universitaire de Nice, Nice, France.

group formed a hydrogen bond with the p110 $\alpha$  specific residue Gln859,<sup>9</sup> and the nitro group was also critical for good potency and selectivity.<sup>10</sup> We postulated that linking the pyrazolo[1,5-*a*]-pyridine and nitroaryl sulfonyl moieties in an appropriate way would generate a p110 $\alpha$  selective PI3K inhibitor with lower toxicity.

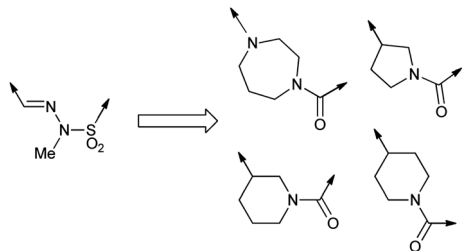
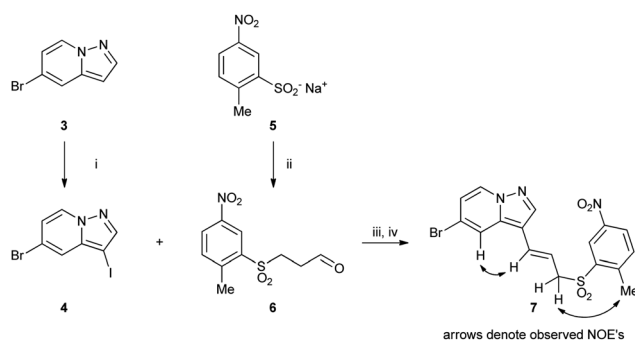


Fig. 2 Filtered set of sulfonohydrazide isosteres generated by BROOD v1.1.1.



Scheme 1 Reagents: (i) NIS, MeCN; (ii) acrolein, AcOH; (iii) <sup>i</sup>PrMgCl·LiCl, THF, −40 °C then 6; (iv) MsCl, DBU, MeCN.

## 2. Results and discussion

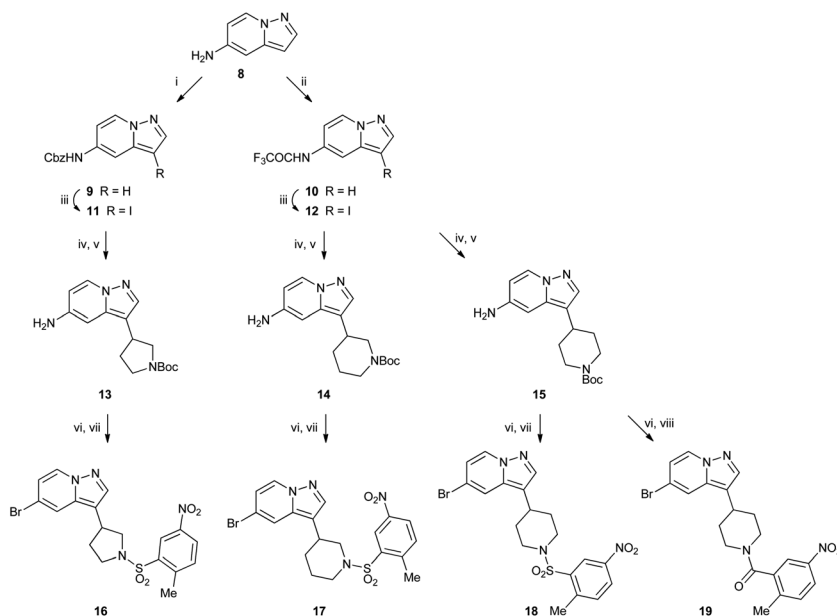
### 2.1. Selection of sulfonohydrazide replacements

To increase our sulfonohydrazide replacement ideas, the bio-isostere generating programme BROOD (v1.1.1; Openeye Scientific Software) was used to select a set of alternative linking groups that fit the conformation of the sulfonohydrazide in a model of the pyrazolo[1,5-*a*]pyridines bound to p110 $\alpha$ . Of the top 200 replacement fragments retrieved, only 51 unique isosteres were considered useful after visual inspection. Of these, only 42 hits contained a functional group we considered capable of hydrogen-bonding to the side chain amine of the p110 $\alpha$  specific amino acid Gln859. The group was further narrowed down to 11 based on lipophilicity of the whole candidate compound, determined by comparison of the *c* log *P* values (calculated using ACD/PhysChem v. 7.10, Advanced Chemistry Development, Inc.) with the *c* log *P* value for **1** of 2.43. Compounds with *c* log *P* greater than 2.43 were discarded since such compounds would be more lipophilic and hence are likely to have lower aqueous solubility than **1**. The group was finally reduced to 4 (Fig. 2) by assessment of the number of hydrogen-bond acceptors, in accordance with Lipinski rules.<sup>14</sup>

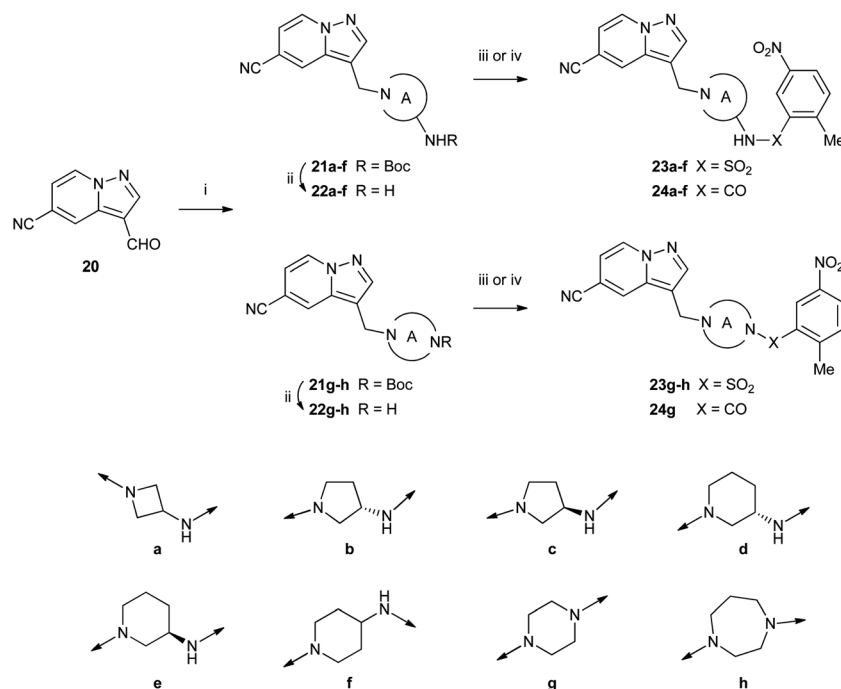
We also chose some linker groups of our own devising: an allylic sulfone to mimic the shape of the sulfonohydrazide without containing the two nitrogens, a set of amine-containing linkers which could improve water solubility, and other heterocyclic linking groups.

### 2.2. Chemistry

Allylic sulfone **7** was made starting from pyrazolo[1,5-*a*]pyridine **3** (ref. 9) by iodination at the 3-position with *N*-iodosuccinimide (NIS) to form **4** (Scheme 1). Sulfinate **5** was reacted with acrolein



Scheme 2 Reagents: (i) CbzCl, Na<sub>2</sub>CO<sub>3</sub>, acetone; (ii) TFAA, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (iii) NIS, MeCN; (iv) boronic ester, PdCl<sub>2</sub>(dppf), aq. Na<sub>2</sub>CO<sub>3</sub>, toluene, EtOH; (v) H<sub>2</sub>, Pd/C, DMF; (vi) NaNO<sub>2</sub>, H<sub>2</sub>O, 47% HBr then CuBr, 47% HBr; (vii) 2-methyl-5-nitrobenzenesulfonyl chloride, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (viii) 2-methyl-5-nitrobenzoyl chloride, NEt<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>.



**Scheme 3** Reagents: (i) amine, AcOH, CH<sub>2</sub>Cl<sub>2</sub> then Na(CN)BH<sub>3</sub>, EtOH; (ii) TFA, CH<sub>2</sub>Cl<sub>2</sub>; (iii) 2-methyl-5-nitrobenzenesulfonyl chloride, NEt<sub>3</sub>, DMF or MeCN or CH<sub>2</sub>Cl<sub>2</sub>; (iv) 2-methyl-5-nitrobenzoyl chloride, NEt<sub>3</sub>, DMF or MeCN or CH<sub>2</sub>Cl<sub>2</sub>.

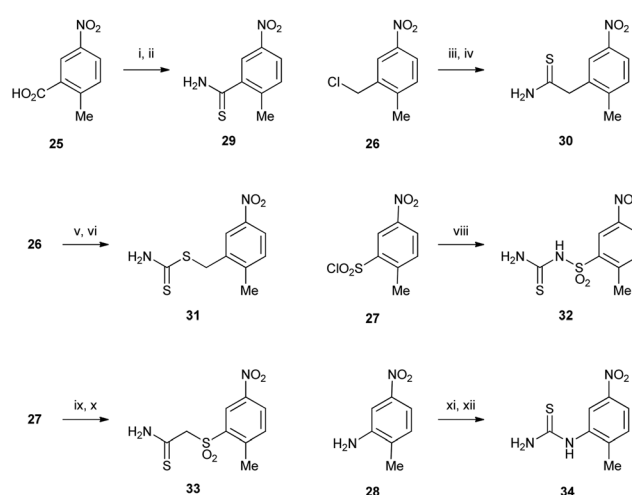
to form aldehyde **6**, which was subsequently reacted with the Grignard reagent derived from **4** followed by dehydration with MsCl and DBU to afford **7**. The position of the double bond was confirmed by NOE interactions observed between the pyrazolo [1,5-*a*]pyridine C4-H (7.65 ppm) and alkene CH (6.52 ppm), and between the allylic CH<sub>2</sub> (4.07 ppm) and aryl methyl protons (2.85 ppm).

Cyclic amine derivatives **16–19** were made starting from amine **8** (ref. 9) (Scheme 2). Amine **8** was protected as either benzyl carbamate **9** or trifluoroacetamide **10**, then iodinated at the 3-position to afford **11** and **12**. Next, a palladium catalysed coupling with a cyclic vinyl boronate installed the amine ring. When **12** was used, the trifluoroacetamide protecting group was lost during this step. Hydrogenation over Pd/C then reduced the double bond from the amine ring and removed the benzyl carbamate to leave Boc protected compounds **13–15**. Then diazotisation converted the free amine to a bromide and also removed the Boc group under the acidic conditions used, and finally sulfonylation or acylation afforded compounds **16–19**.

Aminomethylene-linked compounds **23a–h** and **24a–g** which contain a basic amine to increase aqueous solubility were made by reductive amination of aldehyde **20** (ref. 9) to afford amines **21a–h** (Scheme 3). Removal of the Boc group was then followed by sulfonylation to give sulfonamides **23a–h** or acylation to give carboxamides **24a–g**.

Thiazoles were made using a Hantzsch thiazole synthesis from an  $\alpha$ -bromoketone and a thioamide. The syntheses of the required thioamides **29–34** are depicted in Scheme 4. Carboxylic acid **25** was converted to thioamide **29** by formation of the acid chloride followed by treatment with ammonia to give the primary amide, followed by thionation with Lawesson's reagent

to give **29** in good yield. Benzylic chloride **26** was displaced with potassium cyanide to give the phenylacetone nitrile intermediate, which was subsequently reacted with phosphorous pentasulfide in ethanol under mild conditions to give thioamide **30**.<sup>15</sup> Dithiocarbamate **31** was also prepared from **26** by firstly converting it to the more reactive bromide followed by displacement with a large excess of ammonium dithiocarbamate.<sup>16</sup> Sulfonyl chloride **27** was reacted with cyanamide, followed by



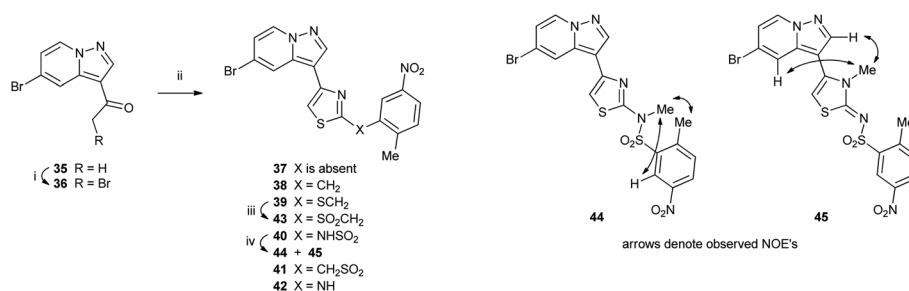
**Scheme 4** Reagents: (i) SOCl<sub>2</sub> then aq. NH<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (ii) Lawesson's reagent, MeOH, reflux; (iii) KCN, DMSO; (iv) P<sub>2</sub>S<sub>5</sub>, EtOH, reflux; (v) LiBr, THF, reflux; (vi) H<sub>2</sub>NCS<sub>2</sub><sup>−</sup> NH<sub>4</sub><sup>+</sup>, EtOH, reflux; (viii) Na<sub>2</sub>NCN, H<sub>2</sub>O, 40 °C then Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, 4.5 M aqueous H<sub>2</sub>SO<sub>4</sub>; (ix) Na<sub>2</sub>SO<sub>3</sub>, NaHCO<sub>3</sub>, H<sub>2</sub>O then bromoacetonitrile, DMF; (x) H<sub>2</sub>S, NEt<sub>3</sub>, THF; (xi) PhCONCS, acetone, reflux; (xii) KOH, MeOH, 40 °C.

thiolysis using *in situ* generation of thiosulfuric acid to give thiourea **32**. Thioamide **33** was accessed by displacement of bromoacetonitrile with the sulfinate salt of **27** to give the nitrile, which underwent thiolysis with hydrogen sulfide. Thiourea **34** was prepared from aniline **28** by reaction with benzoyl isothiocyanate followed by hydrolysis according to published routes.<sup>17,18</sup>

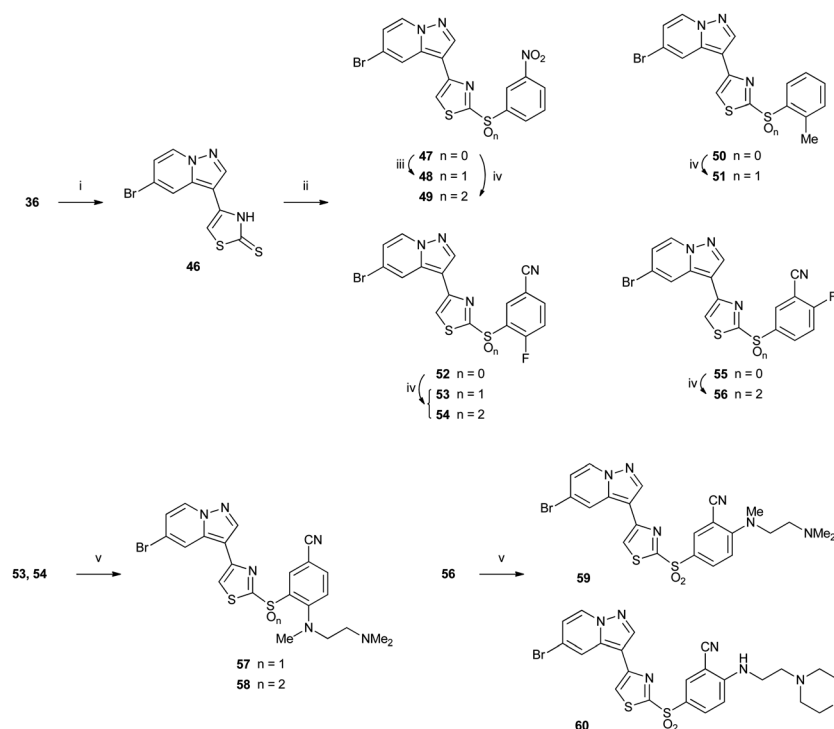
Methylketone **35** (ref. 9) was brominated under standard conditions to give versatile intermediate  $\alpha$ -bromoketone **36**, which underwent a modified Hantzsch reaction with thioamides **29–34** to give thiazoles **37–42** (Scheme 5). Thioether **39** was oxidized with MMPP (magnesium bis(monoperoxyphthalate)) to give sulfone **43**. Sulfonamide **40** was methylated with methyl iodide under mild conditions to give two regioisomeric products **44** and **45**. The sites of methylation were identified by NMR experiments. *N*-Methylsulfonamide **44** showed NOE interactions between the *N*-methyl protons (3.65 ppm) and those of the aryl methyl protons (2.67 ppm) and aryl

C6-H (8.88 ppm), whereas *N*-methylthiazole **45** had NOE interactions between the *N*-methyl protons (3.46 ppm) and pyrazolo[1,5-*a*]pyridine C2-H (7.97 ppm) and C4-H (7.65 ppm).

2-Thiothiazoles **47**, **50**, **52** and **55** were accessed *via* copper-mediated coupling<sup>19</sup> of thione **46** with phenyl boronic acids to give moderate yields of the desired thioethers (Scheme 6). Thione **46** could be prepared in good yield by reaction of  $\alpha$ -bromoketone **36** with fresh ammonium dithiocarbamate. Oxidation with either Oxone<sup>®</sup> or MMPP gave the sulfoxide and/or sulfone, depending on the substrate and choice of oxidant. Oxone<sup>®</sup> oxidation of **47** gave exclusively sulfoxide **48**, and MMPP oxidation afforded sulfone **49**. MMPP oxidation of the more sterically hindered 2-methylphenyl compound **50** gave only sulfoxide **51** under microwave heating, whereas 5-cyano-2-fluorophenyl compound **52** afforded a mixture of sulfoxide **53** and sulfone **54** after reaction with the same reagent at room temperature. In contrast, **55** which did not bear a 2-phenyl substituent gave exclusively sulfone **56**, suggesting that the



Scheme 5 Reagents: (i) Br<sub>2</sub>, 33% HBr/AcOH, (ii) **29–34**, EtOH; (iii) MMPP, CH<sub>2</sub>Cl<sub>2</sub>, EtOH, H<sub>2</sub>O, (iv) MeI, DMF.



Scheme 6 Reagents: (i) H<sub>2</sub>NCS<sub>2</sub><sup>−</sup> NH<sub>4</sub><sup>+</sup>, MeOH then AcOH, reflux; (ii) aryl boronic acid, Cu(OAc)<sub>2</sub>, 1,10-phenanthroline, 1,2-dichloroethane, reflux; (iii) Oxone<sup>®</sup>, solvent; (iv) MMPP, CH<sub>2</sub>Cl<sub>2</sub>, EtOH; (v) amine, THF.

Table 1 Inhibition of class Ia PI3K isoforms

| Cmpd                  | IC <sub>50</sub> (μM) |       |             | Cmpd      | IC <sub>50</sub> (μM) |             |             |
|-----------------------|-----------------------|-------|-------------|-----------|-----------------------|-------------|-------------|
|                       | p110α                 | p110β | p110δ       |           | p110α                 | p110β       | p110δ       |
| <b>1</b> <sup>a</sup> | 0.0009                | 0.046 | 0.049       | <b>37</b> | >1                    | >1          | >1          |
| <b>7</b>              | 0.14 ± 0.05           | >1    | 3.35 ± 0.41 | <b>38</b> | >1                    | >1          | >1          |
| <b>16</b>             | 3.00 ± 0.80           | >1    | >1          | <b>39</b> | >1                    | >1          | >1          |
| <b>17</b>             | 0.59 ± 0.20           | >1    | >1          | <b>40</b> | 1.26 ± 0.41           | >1          | >1          |
| <b>18</b>             | >1                    | >1    | >1          | <b>41</b> | 0.24 ± 0.13           | 0.14 ± 0.01 | 0.09 ± 0.05 |
| <b>19</b>             | >1                    | >1    | >1          | <b>42</b> | >1                    | >1          | >1          |
| <b>23a</b>            | >1                    | >1    | >1          | <b>43</b> | >1                    | >1          | >1          |
| <b>23b</b>            | >1                    | >1    | >1          | <b>44</b> | >1                    | >1          | 1.55 ± 0.27 |
| <b>23c</b>            | >1                    | >1    | >1          | <b>45</b> | >1                    | >1          | >1          |
| <b>23d</b>            | >1                    | >1    | >1          | <b>47</b> | >1                    | >1          | >1          |
| <b>23e</b>            | >1                    | >1    | >1          | <b>48</b> | 0.74 ± 0.01           | 4.42 ± 1.83 | 0.32 ± 0.17 |
| <b>23f</b>            | >1                    | >1    | >1          | <b>49</b> | 0.63 ± 0.18           | 5.45 ± 0.30 | 0.25 ± 0.05 |
| <b>23g</b>            | >1                    | >1    | >1          | <b>51</b> | 1.28 ± 0.22           | >1          | >1          |
| <b>23h</b>            | >1                    | >1    | >1          | <b>53</b> | 0.30 ± 0.01           | >1          | 0.20 ± 0.03 |
| <b>24a</b>            | >1                    | >1    | >1          | <b>54</b> | 0.72 ± 0.02           | >1          | 0.44 ± 0.03 |
| <b>24b</b>            | >1                    | >1    | >1          | <b>56</b> | 0.17 ± 0.02           | >1          | 0.15 ± 0.12 |
| <b>24c</b>            | >1                    | >1    | >1          | <b>57</b> | 4.03 ± 1.65           | 4.34 ± 1.76 | 0.25 ± 0.03 |
| <b>24d</b>            | >1                    | >1    | >1          | <b>58</b> | 0.37 ± 0.15           | 3.56 ± 0.08 | 2.00 ± 0.48 |
| <b>24e</b>            | >1                    | >1    | >1          | <b>59</b> | 2.47 ± 0.69           | >1          | 0.64 ± 0.14 |
| <b>24f</b>            | >1                    | >1    | >1          | <b>60</b> | 2.13 ± 0.29           | >1          | 0.24 ± 0.13 |
| <b>24g</b>            | >1                    | >1    | >1          |           |                       |             |             |

<sup>a</sup> Data from ref. 9.

presence of an *ortho*-phenyl substituent had a detrimental effect on sulfur oxidation. Nucleophilic displacement of the activated 2- or 4-fluorobenzenes (**53**, **54** and **56**) with alkyl amines at room temperature gave the corresponding amino derivatives **57–60**.

### 2.3. Biological activity

We have shown previously that both 5-bromo- and 5-cyanopyrazolo[1,5-*a*]pyridines are highly potent and selective inhibitors of the p110α isoform of PI3K,<sup>9,10</sup> so these building blocks were chosen for further elaboration around the central hydrazone portion of the molecule. Compounds were assayed for their inhibition of the three class Ia isoforms of PI3K: p110α, p110β and p110δ (Table 1).

Replacement of the hydrazone with allylic sulfone **7**, where the hydrazone nitrogens atoms were replaced by carbons, was 150-fold less active against p110α than **1**, while retaining some selectivity over p110β and p110δ. None of the cyclic amine derivatives **16–19**, **23a–h** and **24a–g** had IC<sub>50</sub> less than 1 μM for any of the class Ia PI3K isoforms, with the sole exception of 3-substituted piperidine **17** with a modest p110α IC<sub>50</sub> of 0.59 μM. These compounds were not investigated further, and we instead focussed our efforts on aromatic heterocyclic linker groups.

Of the 2-methyl-5-nitrophenyl compounds **37–45**, only **41** had p110α IC<sub>50</sub> less than 1 μM, however this compound lost all selectivity for p110α over the other class Ia PI3K isoforms (p110α/β/δ IC<sub>50</sub> 0.24/0.14/0.09 μM). By comparison of 3-nitrophenyl compounds **47**, **48** and **49**, the addition of at least one oxygen on sulfur was necessary for p110α IC<sub>50</sub> less than 1 μM although there was little difference between sulfoxide **48** and sulfone **49** (p110α IC<sub>50</sub> **48**: 0.74 μM and **49**: 0.63 μM). Both **48**

and **49** were less than 10-fold selective over p110β but were slightly more potent against p110δ than p110α.

The removal of the nitro group (**51**) decreased p110α activity in comparison to **48** by 2-fold. Benzonitrile derivatives **53**, **54** and **56–60** all demonstrated PI3K IC<sub>50</sub> of less than 1 μM against at least one of the class Ia isoforms, however the selectivity varied with the nature of the other phenyl substituent and sulfur oxidation state. Fluorophenyl compounds **53**, **54** and **56** all had p110α IC<sub>50</sub> under 1 μM and were all similarly active against p110δ. In contrast, amine-containing compounds **57**, **59** and **60** all demonstrated modest selectivity, albeit for p110δ over p110α (by 16-fold, 4-fold and 9-fold, respectively). In contrast **58**, the sulfone analogue of sulfoxide **57**, was 5-fold selective for p110α over p110δ.

## 3. Conclusions

A range of pyrazolo[1,5-*a*]pyridines were prepared where the central portion of the molecule was varied with cyclic and acyclic, aromatic and aliphatic linking groups to investigate the PI3K potencies. We found that the thiazole group was preferred, however the isoform selectivity could be varied by changes of the sulfur oxidation state and phenyl ring substituents. These compounds were not sufficiently potent in comparison with our earlier sulfonylhydrazides, and were not investigated further.

## 4. Experimental

### 4.1. Molecular modelling

The bioisostere generating programme BROOD (v1.1.1; Open-eye Scientific Software) was used with a 3-dimensional model of the sulfonylhydrazide core based on the top ranked predicted



binding mode of *N'*-((5-bromopyrazolo[1,5-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonylhydrazide (compound **5m** in ref. 9) in the ATP binding site of p110 $\alpha$ . BROOD settings were kept at default values, and the electrostatic tanimoto similarity value was calculated. The f5 multi-conformer fragment library consisting of 3-dimensional models of 140 000 fragments was searched. The electrostatic tanimoto similarity hit list was ranked according to the combined electrostatic shape and electrostatic tanimoto values then visually inspected. The 2D scheme of the full compounds was generated for further analysis.

## 4.2. Chemistry

Methods and characterisation for all novel compounds are detailed in the ESI.†

## 4.3. Enzyme assays

The Class Ia PI3K assays were performed using a basic thin layer chromatography technique, as described previously.<sup>20</sup> The p110 $\delta$ /p85 PI3K was from Upstate and the p110 $\alpha$ /p85 and p110 $\beta$ /p85 isoforms were prepared as described previously.<sup>20</sup> Reactions were made containing 0.1  $\mu$ g recombinant enzyme, 10  $\mu$ g 1- $\alpha$ -phosphatidylinositol, inhibitor (DMSO only or DMSO + inhibitor to a final concentration of 1%), 2 $\times$  lipid kinase buffer (40 mM Tris-HCl pH 7.4, 200 mM NaCl, 1 mM EDTA), and activated upon the addition of an ATP mix (5 mM MgCl<sub>2</sub>, 100  $\mu$ M ATP, 0.1  $\mu$ L [ $\gamma$ -<sup>33</sup>P]ATP). Reactions were incubated at room temperature for 1 h following which the reactions were stopped by the addition of 1 M HCl. The lipids were then extracted using a two step procedure. Firstly, 200  $\mu$ L of chloroform : methanol (1 : 1) was added, the biphasic reactions mixed and centrifuged briefly, and the inorganic phase was removed and discarded. Following this 80  $\mu$ L of methanol : hydrochloric acid (1 : 1) was added and the same procedure followed. Next, 70  $\mu$ L of the organic phase was transferred to a clean 1.6 mL tube and the reactions were dried using a speed vac, with no heating, for 30 min. The reactions were spotted onto TLC plates (Merck Ltd) and developed for 1 h in 1-propanol : 2 M acetic acid (13 : 7). The TLC plates were then dried at room temperature and quantified using a phosphor-imager (StormImager, Amersham). Nine inhibitor concentrations were used to determine the IC<sub>50</sub>. Each experiment was performed twice and the average IC<sub>50</sub> value used.

## Acknowledgements

The authors would like to thank the Health Research Council of New Zealand, Maurice Wilkins Centre for Molecular Biodiscovery, and Pathway Therapeutics for their financial assistance.

## References

- 1 S. Shuttleworth, F. Silva, C. Tomassi, A. Cecil, T. Hill, H. Rogers and P. Townsend, *Prog. Med. Chem.*, 2009, **48**, 81–131.
- 2 M. C. Hollander, G. M. Blumenthal and P. A. Dennis, *Nat. Rev. Cancer*, 2011, **11**, 289–301.
- 3 C. Chaussade, K. Cho, C. Mawson, G. W. Rewcastle and P. R. Shepherd, *Biochem. Biophys. Res. Commun.*, 2009, **381**, 577–581.
- 4 J. M. Cleary and G. I. Shapiro, *Curr. Oncol. Rep.*, 2010, **12**, 87–94.
- 5 P. Liu, H. Cheng, T. M. Roberts and J. J. Zhao, *Nat. Rev. Drug Discovery*, 2009, **8**, 627–644.
- 6 S. Jamieson, J. U. Flanagan, S. Kolekar, C. Buchanan, J. D. Kendall, W.-J. Lee, G. W. Rewcastle, W. A. Denny, R. Singh, J. Dickson, B. C. Baguley and P. R. Shepherd, *Biochem. J.*, 2011, **438**, 53–62.
- 7 P. Wu and Y. Hu, *Med. Chem. Commun.*, 2012, **3**, 1337–1355.
- 8 B. Markman, J. J. Tao and M. Scaltriti, *Curr. Pharm. Des.*, 2013, **19**, 895–906.
- 9 J. D. Kendall, P. D. O'Connor, A. J. Marshall, R. Frédérick, E. S. Marshall, C. L. Lill, W.-J. Lee, S. Kolekar, M. Chao, A. Malik, S. Yu, C. Chaussade, C. Buchanan, G. W. Rewcastle, B. C. Baguley, J. U. Flanagan, S. M. F. Jamieson, W. A. Denny and P. R. Shepherd, *Bioorg. Med. Chem.*, 2012, **20**, 69–85.
- 10 J. D. Kendall, A. C. Giddens, K. Y. Tsang, R. Frédérick, E. S. Marshall, R. Singh, C. L. Lill, W.-J. Lee, S. Kolekar, M. Chao, A. Malik, S. Yu, C. Chaussade, C. Buchanan, G. W. Rewcastle, B. C. Baguley, J. U. Flanagan, S. M. F. Jamieson, W. A. Denny and P. R. Shepherd, *Bioorg. Med. Chem.*, 2012, **20**, 58–68.
- 11 S. D. Nelson, *J. Med. Chem.*, 1982, **25**, 753–765.
- 12 M. Hayakawa, H. Kaizawa, K.-i. Kawaguchi, N. Ishikawa, T. Koizumi, T. Ohishi, M. Yamano, M. Okada, M. Ohta, S.-i. Tsukamoto, F. I. Raynaud, M. D. Waterfield, P. Parker and P. Workman, *Bioorg. Med. Chem.*, 2007, **15**, 403–412.
- 13 A. Berndt, S. Miller, O. Williams, D. D. Le, B. T. Houseman, J. I. Pacold, F. Gorrec, W.-C. Hon, Y. Liu, C. Rommel, P. Gaillard, T. Rückle, M. K. Schwarz, K. M. Shokat, J. P. Shaw and R. L. Williams, *Nat. Chem. Biol.*, 2010, **6**, 117–124.
- 14 C. A. Lipinski, F. Lombardo, B. W. Dominy and P. J. Feeney, *Adv. Drug Delivery Rev.*, 1997, **23**, 3–25.
- 15 B. Kaboudin and D. Elhamifar, *Synthesis*, 2006, 224–226.
- 16 M. Yokoyama and H. Hatanaka, *Synthesis*, 1985, 891–892.
- 17 C. R. Rasmussen, F. J. Villani, L. E. Weaner, B. E. Reynolds, A. R. Hood, L. R. Hecker, S. O. Nortey, A. Hanslin, M. J. Costanzo, E. T. Powell and A. J. Molinari, *Synthesis*, 1988, 456–459.
- 18 S. Mahboobi, S. Dove, A. Sellmer, M. Winkler, E. Eichhorn, H. Pongratz, T. Ciossek, T. Baer, T. Maier and T. Beckers, *J. Med. Chem.*, 2009, **52**, 2265–2279.
- 19 H. Prokopcová and C. O. Kappe, *J. Org. Chem.*, 2007, **72**, 4440–4448.
- 20 C. Chaussade, G. W. Rewcastle, J. D. Kendall, W. A. Denny, K. Cho, L. M. Gronning, M. L. Chong, S. H. Anagnostou, S. P. Jackson, N. Daniele and P. R. Shepherd, *Biochem. J.*, 2007, **404**, 449–458.