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Synthesis, biological evaluation and molecular modelling of sulfonohydrazides as selective PI3K p110α inhibitors

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Abstract—A series of 2-methyl-5-nitrobenzenesulfonohydrazides were prepared and evaluated as inhibitors of PI3K. An isoquinoline derivative shows good selectivity for the p110α isoform over p110β and p110δ, and also demonstrates good in vitro activity in a cell proliferation assay. Molecular modelling provides a rationalisation for the observed SAR.

1. Introduction

Phosphoinositide-3-kinases (PI3Ks) are a group of lipid kinases which phosphorylate the 3-hydroxyl group of phosphoinositides. They are split into three classes (Class I, II and III) and play an important role in cellular signaling.[1] Class I is further split into Class Ia and Ib based on their mechanism of activation. The Class Ia PI3Ks are heterodimeric, consisting of a catalytic subunit (p110α, p110β or p110δ) in complex with a regulatory subunit.[2] There is a great deal of interest in PI3Ks as cancer targets, particularly for the p110α isoform which is mutated and/or over-expressed in more than 30% of tumours.[3,4] Many of these cancer-specific mutations lead to a gain in function which results in oncogenicity.[4] A recent review notes that 22 different chemotypes of PI3K inhibitors have been reported, with several compounds approaching clinical trial.[5]

Phosphoinositide 3,4,5-triphosphate (PIP3), the product of PI3K phosphorylation, recruits protein kinase B (PKB) which in turn is phosphorylated by 3-phosphoinositide dependent protein kinase 1 (PDK1). Phosphorylated PKB is subsequently involved in a range of processes which inhibit apoptosis and promotes cell growth and cell motility.[6,7] The lipid phosphatase PTEN dephosphorylates PIP3 and acts as a tumour suppressor. It is deleted or inactivated in a range of tumours, leading to increased levels of PIP3 and increased tumour survival.[8]

The exact roles of the individual isoforms are beginning to emerge,[9,10] and hence more potent and selective compounds of this type would not only be useful as potential drugs, but also as biological tools to help establish the roles of individual PI3K isoforms in cellular systems.

Two inhibitors, wortmannin and LY-294002, have been studied extensively; however, neither shows selectivity within the Class I PI3K isoforms (Fig. 1). Wortmannin, a fungal metabolite, has a low nanomolar IC_{50} against PI3K and binds covalently in the ATP-binding pocket. It is unstable in solution and has limited activity in tumour xenograft models.[11] LY-294002 is chemically stable but inhibits only at micromolar concentrations.

There is considerable interest in obtaining selective inhibitors of the p110α isoform as novel cancer therapeutics; however, few selective inhibitors have been

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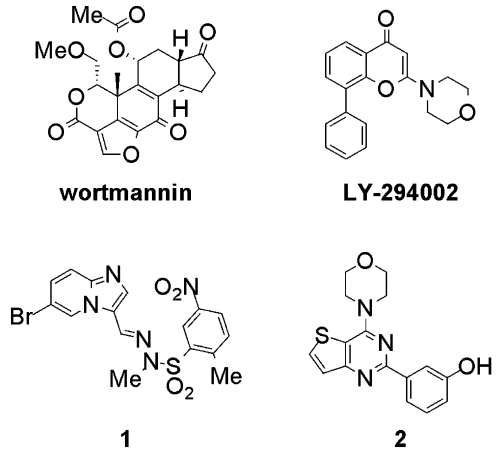


Figure 1. Structures of PI3K inhibitors.

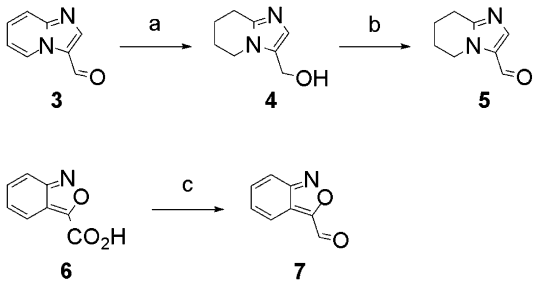
reported in the literature. Imidazo[1,2-*a*]pyridines have recently been demonstrated to show excellent p110α selectivity.[12–14] In particular, imidazo[1,2-*a*]pyridine **1** is reported to be a sub-nanomolar p110α inhibitor with >100-fold selectivity over p110β and p110γ. In addition **1** showed activity in a human cancer xenograft model.[14] Thieno[3,2-*d*]pyrimidine **2** is also a very potent p110α inhibitor (IC_{50} 2.0 nM) with 8-fold selectivity over p110β.[15,16]

The work we report here investigates the structure–activity relationship (SAR) around **1** exploring major changes in the two-ring chromophore. We wanted to determine whether the position of the two nitrogens in the ring system was crucial and whether the ring size was important. We then used that information to validate the molecular modelling results to determine whether the model we had was consistent with the observed data.

2. Results and discussion

2.1. Preparation of sulfonohydrazides

The sulfonohydrazides were prepared from the corresponding heteroaryl aldehydes. Aldehyde **5** was prepared by hydrogenation of the 6-membered ring of **3** followed by reoxidation of the benzylic alcohol with MnO_2 (Scheme 1). Benzo[*c*]isoxazole **7** was prepared by reduction of carboxylic acid **6** via the benzylic alcohol.



Scheme 1. Reagents and conditions: (a) H_2, Pd/C, MeOH; (b) MnO_2, CH_2Cl_2; (c) i—CDI, THF; ii—$NaBH_4$; iii—MnO_2.

The syntheses of bromoisoquinolines **15a–c** are depicted in Scheme 2. Bromoacetophenones **8a** and **8b** were converted to phenylpropionitriles **9a** and **9b** with TosMIC under basic conditions.[17] Borane reduction of the nitriles followed by formylation afforded protected amines **11a** and **11b** in excellent yield. Cyclisation to isoquinolines **13a–c** was achieved in two steps by heating with P_2O_5 in polyphosphoric acid, followed by MnO_2 oxidation to install the remaining double bond. Oxidation of the methyl group of **13a–c** to the aldehyde proved to be problematic. Direct oxidation with SeO_2 was unsuccessful and returned only unreacted starting material. It was eventually achieved by radical bromination followed by immediate substitution with NaOAc in one pot, then basic hydrolysis to afford alcohols **14a–c**. The intermediate bromides were unstable to isolation. Finally MnO_2 oxidation provided the required aldehydes **15a–c**.

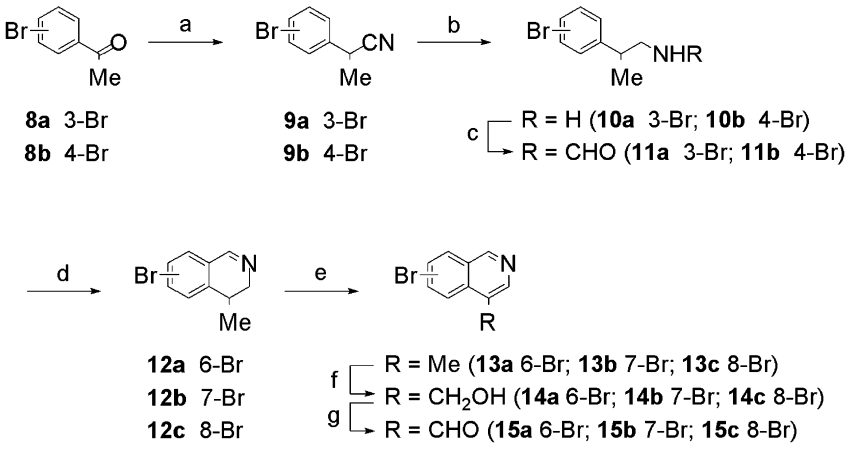
Sulfonohydrazides **16a–p** were prepared from the corresponding aldehydes by one of three methods as depicted in Scheme 3 and Table 1. Reaction of the aldehyde with methylhydrazine followed by sulfonylation (Method A) was used for the majority of the aldehydes. However in our hands we found this method to be somewhat capricious and irreproducible, which led us to investigate alternative routes. More reliable methods were condensation of the aldehyde with 2-methyl-5-nitrobenzenesulfonohydrazide followed by methylation under phase transfer conditions with iodomethane (Method B) or with diazomethane under neutral conditions (Method C). In all cases only one stereoisomer was observed.

This also led to the development of a higher yielding synthesis of sulfonohydrazide **1** (Scheme 3). The previous synthesis gave a 8% yield of **1** from aldehyde **17**.[14] We have improved this yield to 40% by reaction of **17** using Method C.

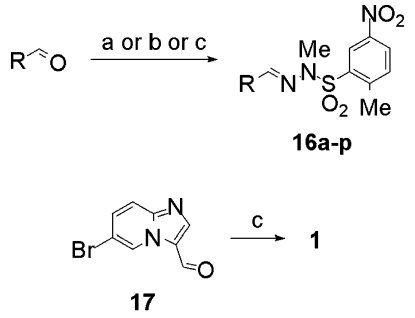
2.2. Biological activity

The biological activity of the compounds was evaluated using an isolated enzyme inhibition assay. All compounds were assayed against the three Class I a PI3K isoforms, and the results are summarised in Table 1.

Removal of the bromo substituent of **1** (**16a**) gave a 100-fold decrease in potency for p110α compared to **1**, but still retained selectivity over p110β and p110*δ*. This was deemed sufficient activity to allow use of unsubstituted (more easily accessible) chromophores for the initial comparative studies. Partial reduction of the imidazo[1,2-*a*]pyridine ring (**16b**) and removal of the pyridyl ring (**16c**) led to a complete loss of p110α activity (IC_{50} > 10 μM against p110α), as did several heterocyclic replacements that still retained the 6,5-ring system (**16d–f**, **16h**, **16i**). Benzo[*c*]isoxazole **16g** possessed weak activity against p110α (IC_{50} 5.0 μM). Quinoline **16k** was only 2.7-fold less active than **16a**, however moving the position of the sulfonohydrazide group (**16l**) again led to a complete loss of activity for p110α. The isomeric isoquinoline **16m** was the best unsubstituted hetereocyclic replacement tested, with a similar PI3K inhibition profile to **16a**.



Scheme 2. Reagents and conditions: (a) TosMIC, KO^{t}Bu, ^{t}BuOH, DME; (b) i—BH_3·SMe_2, THF; ii—6 M HCl; (c) ethyl formate; (d) P_2O_5, PPA, 160 °C; (e) MnO_2, dioxane; (f) i—NBS, AIBN, benzene; ii—BHT; iii—NaOAc, DMF; iv—NaOH, MeOH; (g) MnO_2, CH_2Cl_2.



Scheme 3. Reagents and conditions: (a) i—methylhydrazine, EtOH; ii—2-methyl-5-nitrobenzenesulfonyl chloride, NEt_3, CH_2Cl_2; (b) i—2-methyl-5-nitrobenzenesulfonohydrazide, MeOH; ii—MeI, $PhMe_3$ N^+I^-, 15% aq NaOH, CH_2Cl_2; (c) i—2-methyl-5-nitrobenzenesulfonohydrazide, MeOH; ii—CH_2N_2, Et_2O, THF.

We then investigated three bromo substituted isoquinolines analogues of **16m** (**16n–p**) to establish whether a similar dramatic increase in p110α activity to that seen between **1** and **16a** could be seen. The three regioisomers were made because it was not immediately obvious from a simple overlay of the structures which position on the isoquinoline ring would be optimal. The 6-bromo isomer **16n** was the most active against p110α of these compounds, but was 3-fold less active than **16m**.

The compounds were also evaluated in a cellular assay measuring inhibition of proliferation using two early passage human cancer cell lines in a thymidine depletion assay (Table 1). The NZB5 cell line was derived from a brain tumour (medulloblastoma), and has the wild-type gene for p110α. The NZOV9 cell line was derived from an ovarian tumour (poorly differentiated endometrioid adenocarcinoma), and contains a mutant p110α enzyme with a single amino acid substitution in the kinase domain (Y1021C).

There is overall good agreement between the two different cell lines; there is at most a 2-fold difference between NZB5 and NZOV9 for the same compound. Hence the mutation in p110α (as found in NZOV9) does not significantly affect the results. The compounds which are most potent against p110α are also active in both cell lines (**1**, **16a**, **16g**, **16k**, **16m**, **16n**). This would also suggest that this series of compounds has good cell penetration. Furthermore, **16a** and **16m** both inhibit cell proliferation at <1 μM. Five compounds (**16d**, **16e**, **16l**, **16o** and **16p**) all have activity in at least one of the two cell lines at <10 μM, but do not possess activity against the PI3K isoforms at the same concentration. This may be due to effects on kinase targets other than PI3K.

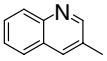
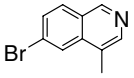
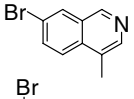
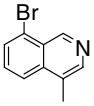
We have developed a novel series of sulfonohydrazides. Isoquinoline **16m** shows good selectivity for p110α over p110β and p110δ, and in addition inhibits cell proliferation in two human cancer cell lines.

2.3. Molecular modelling and discussion

In order to rationalise the affinity and selectivity observed for the lead structure **1**, we studied its binding mode within PI3K by means of molecular modelling. The structure of p110α has not yet been experimentally solved. However, the X-ray crystal structure of the p110γ isoform, alone or in complex with different ligands such as wortmannin and LY-294002, is available in the Protein Data Bank (www.pdb.org),[10,18,19] and the p110α and p110γ isoforms share good sequence identity (~35%). The alignment of their sequences (Fig. 2a) clearly shows that residues lying in the active site region (within 15 Å around the ATP-binding site, denoted by * in Fig. 2a) are mostly identical (coloured in cyan) or similar (coloured in yellow). Only a few amino acids are different (coloured in magenta). In addition to being a highly potent inhibitor of p110α, **1** also exhibits good inhibitory potency against p110γ (IC_{50} 76 nM).[10] These observations prompted us to investigate the binding of **1** within the p110γ active site with a view to explain its activity and selectivity profile.

Compound **1** was docked within the p110γ binding site (PDB code: 2CHX) by means of the automated *GOLD* program.[20] In order to take into account protein

Table 1. Synthetic methods, and inhibition of PI3K isoforms and cell proliferation for compounds **1** and **16a**–**p**

Compound	R	Method[a]	PI3K IC_{50}[b] (μM)			Cell line IC_{50}[c] (μM)	
			p110α	p110β	p110δ	NZB5	NZOV9
1		C	0.0078	0.34	0.91	0.069	0.066
16a		A	0.86	>10	5.4	0.35	0.68
16b		A	>10	>10	>10	10	10
16c		A	>10	>10	>10	>20	>20
16d		A	>10	>10	>10	2.1	1.2
16e		A	>10	>10	>10	3.3	n.d.[d]
16f		A	>10	>10	>10	15	>20
16g		C	5.0	>10	>10	3.9	3.3
16h		A	>10	>10	>10	12	8.5
16i		A	>10	>10	>10	>20	>20
16j		A	>10	>10	>10	>20	>20
16k		A	2.3	>10	>10	1.5	0.8
16l		B	>10	>10	>10	3.0	6.0
16m		B	0.80	>10	3.6	0.89	0.50
16n		C	2.2	>10	9.0	5.1	4.2
16o		C	>10	>10	>10	6.6	11
16p		C	>10	>10	>10	5.7	12

[a] Synthetic method from Scheme 3.
[b] Values are means of two experiments, variation between experiments is no more than ±20%.
[c] Values are means of one to four experiments; variation between experiments is no more than ±32%.
[d] n.d., not determined.

flexibility, the conformation with the highest score (GoldScore) was further refined using the MINIMIZE module as implemented in *SYBYL* version 7.3 (Tripos force field and Gasteiger–Hückel charges).[21] The key interactions which stabilise **1** within the cleft are depicted in Figure 2b. The imidazo[1,2-*a*]pyridine moiety is deeply inserted in the ATP-binding site, interacting through a *T-shape* interaction with the phenol of Tyr-867. The heterocycle is further stabilised via a hydrogen bond contact between *N*-1 of **1** and the backbone NH of

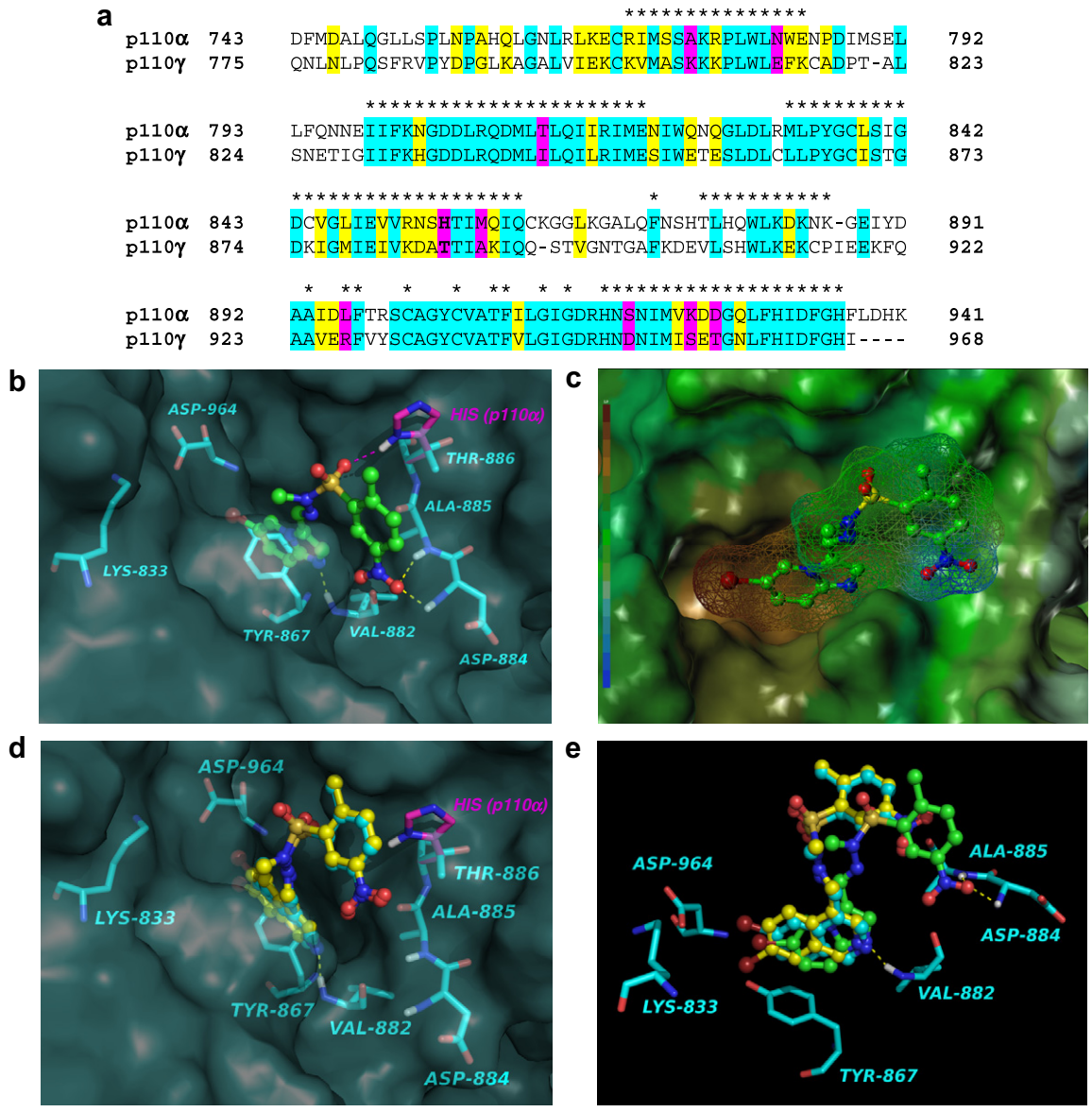


Figure 2. (a) Sequence alignment between p110α and p110γ isoforms. *, Amino acid residue within 15 Å of the ATP-binding site; cyan, conserved residue; yellow, similar residue; magenta, different residue; (b) docking of **1** within the active site of p110γ (carbon atom in cyan), in magenta = histidine residue in p110α; (c) representation of lipophilic properties mapped onto the Connolly surface around p110γ and **1** (picture made using Molcad);[21] (d) docking of **16n** (carbon atom in cyan) and **16o** (carbon atom in yellow) within the active site of p110γ, in magenta = histidine residue in p110α; (e) superimposition of **1** (carbon atom in green), **16n** (carbon atom in cyan) and **16o** (carbon atom in yellow) within the active site of p110γ. (Pictures made using Pymol.)[22]

Val-882. This interaction appears to be crucial as it is observed in all p110γ-inhibitor complexes described.[10,18,19] This is consistent with compounds **16d**, **16e**, **16h–j** which do not possess a nitrogen at this position available for hydrogen bonding being essentially inactive (IC_{50}s >10 μM for all Class Ia PI3Ks). Compound **1** is also stabilised through hydrogen bond interactions between the nitro group on the arylsulfonyl moiety and the backbone NH of Asp-884 and Ala-885.

Aiming to identify key amino acids that could explain the selectivity of **1** for p110α, we also analysed the non-conserved residues between both isoforms (Fig. 2a in magenta). Although most of these are situated far from the hinge region, the residue in position 886 (according to the p110γ numbering) is not and could play a crucial role in selectivity. The Thr-886 in p110γ corresponds to a histidine in p110α. To evaluate the potential implication of this difference on the binding of **1**, we virtually mutated Thr-886 into a histidine in the p110γ-compound **1** complex (the His residue is represented in magenta in Fig. 2b). A new hydrogen bonding interaction between the histidine NH and one of the oxygen atoms of the sulfonyl group of **1** could then be formed, which would further stabilise the ligand in the p110α cavity. This additional interaction could clearly account for the high selectivity of **1** against p110α.

As expected, the bromine atom also plays a critical role in the binding. The active site cavity is particularly

lipophilic (Fig. 2c), and the hydrophobic bromine atom fits neatly in a lipophilic pocket.

We have also studied the binding of the bromoisoquinolines **16n** and **16o** with a view to rationalise the weak p110α activity observed in that series compared to **1**. The docking of **16n** and **16o** in the p110γ active site revealed a slightly different binding conformation. Similarly to **1**, the critical hydrogen bond interaction between the backbone NH of Val-882 and the N atom of the isoquinoline ring system is formed; however, the arylsulfonyl moieties of **16n** and**16o** are less deeply inserted in the active site cavity (Fig. 2d). Importantly, in that orientation, the hydrogen bond interaction between the histidine NH (in p110α) and one oxygen atom of the sulfonyl group could not be formed. Moreover, the favourable interactions between the nitro group on the arylsulfonyl moiety and the backbone NH of Asp-884 and Ala-885 are also lost. Finally, the superimposition of **16n** and **16o** with **1** (Fig. 2e) clearly shows that in the isoquinoline series the bromo substituent cannot adopt the same orientation as observed with the imidazo[1,2-*a*]pyridine ring system. All together, these differences probably account for the weaker activity in this series compared to **1**.

From this model it becomes evident that none of the isoquinolines (**16n**, **16o**, **16p**) can place a bromo substituent in the same position as does the imidazo[1,2-*a*]pyridine, thus accounting for the lack of p110α activity for the bromoisoquinolines.

3. Conclusion

A range of heterocyclic analogues of **1** have been prepared and were evaluated against the Class Ia PI3K isoforms. Isoquinoline **16m** exhibited selectivity for p110α over p110β and p110δ. The compounds were also assayed for inhibition of cell proliferation in two different human cancer cell lines, and furthermore compounds with good p110α potency also showed good activity in the cellular assay.

Modelling of **1** in the active site of p110γ helps to rationalise the observed SAR. In particular, a suitably positioned nitrogen on the heterocycle available for hydrogen bonding is required, and the bromine of **1** fits neatly into a lipophilic pocket to provide significant stabilisation of the complex. Mutation of Thr-886 in p110γ to His, as it would appear in p110α, provides a rationalisation for the observed selectivity of this series of compounds where the sulfonyl group is now involved in a hydrogen bond with the histidine. Further work in this area is ongoing and will be reported in future publications.

4. Experimental

4.1. Chemistry

NMR spectra were recorded on a Bruker Avance 400 spectrometer; chemical shifts are reported in δ using $SiMe_4$ as the internal standard when measured in $CDCl_3$, and the residual DMSO as standard when measured in DMSO-d_6. Low resolution mass spectra were recorded on a Thermo Finnigan MSQ single quadrupole mass spectrometer. Silica gel chromatography was performed using 200–320 mesh silica gel obtained from APS Finechem Ltd. THF was distilled from the sodium benzophenone ketyl immediately before use. BHT is 2,6-di-*tert*-butyl-4-methylphenol; TosMIC is *p*-toluenesulfonylmethyl isocyanide. Yields have not been optimised.

4.1.1. (5,6,7,8-Tetrahydroimidazo[1,2-*a*]pyridin-3-yl)methanol (4). A solution of imidazo[1,2-*a*]pyridine-3-carbaldehyde[23] **3** (93 mg, 0.64 mmol) in MeOH (20 mL) was hydrogenated at 40 psi in the presence of 10% Pd/C (20 mg) at room temperature for 18 h. The reaction mixture was filtered through Celite, washed with MeOH and the solvent removed from the filtrate in vacuo to leave **4** as a white solid (91 mg, 94%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 6.89 (s, 1H), 4.59 (s, 2H), 3.95–4.00 (m, 2H), 2.84–2.89 (m, 2H), 1.86–2.05 (m, 4H). LC–MS (APCI$^+$) 153 (MH^+, 100%).

4.1.2. 5,6,7,8-Tetrahydroimidazo[1,2-*a*]pyridine-3-carbaldehyde (5). MnO_2 (520 mg, 6.0 mmol) was added to a solution of **4** (91 mg, 0.60 mmol) in CH_2Cl_2 (10 mL) and stirred at room temperature for 18 h. The reaction mixture was filtered through Celite, washed with CH_2Cl_2 and the solvent removed from the filtrate in vacuo to leave **5** as a white solid (85 mg, 94%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.66 (s, 1H), 7.71 (s, 1H), 4.29–4.35 (m, 2H), 2.92–2.97 (m, 2H), 1.88–2.04 (m, 4H). LC–MS (APCI$^+$) 151 (MH^+, 100%).

4.1.3. Benzo[*c*]isoxazole-3-carbaldehyde (7). A mixture of benzo[*c*]isoxazole-3-carboxylic acid[24] **6** (3.31 g, 20 mmol) and 1,1′-carbonyldiimidazole (4.86 g, 30 mmol) in dry THF (200 mL) was stirred at room temperature for 12 h, when a clear solution was obtained. The solution was added to a stirred solution of sodium borohydride (3.4 g, 90 mmol) in water (100 mL) at room temperature. After 15 min the mixture was treated dropwise with concd HCl until hydrogen evolution ceased. The THF was removed in vacuo and the residue extracted with EtOAc, washed with dilute ammonia solution and dried (Na_2SO_4). MnO_2 (5 g) was added and the mixture was heated under reflux for 3 h. After filtering, the solvent was removed and the residue was chromatographed on silica, eluting with CH_2Cl_2, to give 1.03 g (44%) of **7**. ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.38 (s, 1H), 8.02 (br d, *J* 8.8 Hz, 1H), 7.78 (br d, *J* 9.1 Hz, 1H), 7.45 (ddd, *J* 9.1, 6.5, 0.9 Hz, 1H), 7.33 (ddd, *J* 8.8, 6.5, 0.6 Hz, 1H).

4.1.4. 2-(3-Bromophenyl)propanenitrile (9a). A solution of KO^{t}Bu (2.26 g, 20.1 mmol) in dry ^{t}BuOH (10 mL) was added to a solution of 3′-bromoacetophenone **8a** (2.00 g, 10.0 mmol) and TosMIC (2.35 g, 12.0 mmol) in dry 1,2-dimethoxyethane (30 mL) under an atmosphere of N_2, while cooled in an ice bath, at a rate to ensure the reaction temperature remained <10 °C. After 1 h the ice bath was removed, the reaction mixture stirred at room temperature for a further 1 h, then diluted with water and extracted twice with hexanes. The organic extracts were combined, dried (Na_2SO_4) and the solvent removed in vacuo. The crude product was

distilled on a Kugelrohr apparatus (oven temperature 130 °C, oil pump) to afford **9a** as a colourless oil (1.24 g, 59%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.51 (m, 1H), 7.47 (m, 1H), 7.24–7.33 (m, 2H), 3.87 (q, *J* 7.3 Hz, 1H), 1.65 (d, *J* 7.3 Hz, 3H).

4.1.5. 2-(4-Bromophenyl)propanenitrile (9b). Compound **9b** was prepared from 4′-bromoacetophenone **8b** by the same procedure as that of **9a**. Compound **9b** was obtained as a colourless oil (2.30 g, 73%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.52 (d, *J* 8.4 Hz, 2H), 7.24 (d, *J* 8.4 Hz, 2H), 3.86 (q, *J* 7.3 Hz, 1H), 1.63 (d, *J* 7.3 Hz, 3H).

4.1.6. 2-(3-Bromophenyl)propan-1-amine (10a). $BH_3{\cdot}SMe_2$ (1.77 mL, 17.7 mmol) was added to a solution of **9a** (1.24 g, 5.90 mmol) in dry THF (15 mL) at room temperature under an atmosphere of N_2, and then refluxed for 18 h. The reaction was quenched by the dropwise addition of 6 M aqueous HCl (4 mL). After refluxing for a further 2 h, the solution was made basic with 6 M aqueous NaOH then extracted three times with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Column chromatography, eluting with CH_2Cl_2/MeOH/concd aqueous NH_3 99:1:0.1 to 98:2:0.2 to 97:3:0.3 gave **10a** as a colourless oil (1.18 g, 94%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.32–7.37 (m, 2H), 7.10–7.20 (m, 2H), 2.63–2.88 (m, 3H), 1.24 (d, *J* 6.9 Hz, 3H). LC–MS (APCI$^+$) 214 (MH^+ with ^{79}Br, 85%), 216 (MH^+ with ^{81}Br, 100%).

4.1.7. 2-(4-Bromophenyl)propan-1-amine (10b). Compound **10b** was prepared from **9b** by the same procedure as that of **10a**. Compound **10b** was obtained as a colourless oil (1.99 g, 86%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 7.43 (d, *J* 8.4 Hz, 2H), 7.09 (d, *J* 8.4 Hz, 2H), 2.67–2.88 (m, 3H), 1.23 (d, *J* 6.9 Hz, 3H). LC–MS (APCI$^+$) 214 (MH^+ with ^{79}Br, 90%), 216 (MH^+ with ^{81}Br, 100%).

4.1.8. *N*-(2-(3-Bromophenyl)propyl)formamide (11a). Compound **10a** (1.18 g, 5.51 mmol) was refluxed in ethyl formate (2.3 mL, 28 mmol) for 40 h. After cooling to room temperature, the solvent was removed in vacuo to afford **11a** as a colourless oil (1.33 g, 100%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.12 (s, 0.85H, major rotamer), 7.93 (d, *J* 11.9 Hz, 0.15H, minor rotamer), 7.31–7.40 (m, 2H), 7.08–7.23 (m, 2H), 5.20–5.50 (m, 1H), 3.62–3.72 (m, 1H), 3.22–3.38 (m, 1H), 2.81–3.01 (m, 1H), 1.25–1.31 (m, 3H). LC–MS (APCI$^+$) 242 (MH^+ with ^{79}Br, 100%), 244 (MH^+ with ^{81}Br, 90%).

4.1.9. *N*-(2-(4-Bromophenyl)propyl)formamide (11b). Compound **11b** was prepared from **10b** by the same procedure as that of **11a**. Compound **11b** was obtained as a colourless oil (2.25 g, 100%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.10 (s, 0.85H, major rotamer), 7.90 (d, *J* 11.9 Hz, 0.15H, minor rotamer), 7.42–7.47 (m, 2H), 7.01–7.13 (m, 2H), 5.25–5.40 (m, 1H), 3.59–3.74 (m, 1H), 3.21–3.40 (m, 1H), 2.81–2.99 (m, 1H), 1.24–1.29 (m, 3H). LC–MS (APCI$^+$) 242 (MH^+ with ^{79}Br, 100%), 244 (MH^+ with ^{81}Br, 90%).

4.1.10. 6-Bromo-4-methyl-3,4-dihydroisoquinoline (12a) and 8-bromo-4-methyl-3,4-dihydroisoquinoline (12c). A mixture of **11a** (1.33 g, 55.0 mmol) and polyphosphoric acid (13.4 g) were heated to 160 °C, then P_2O_5 (3.14 g, 11.1 mmol) was added and the resulting mixture heated at 160 °C for 18 h. After cooling to room temperature, ice was added and the mixture stirred with a glass rod until all of the ice was melted. The resulting solution was made basic with 6 M aqueous NaOH then extracted three times with CH_2Cl_2. The combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Column chromatography, eluting with hexanes/EtOAc 9:1 to 4:1 to 3:1 gave first **12c** as a yellow oil (468 mg, 38%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.70 (m, 1H), 7.48 (d, *J* 7.8 Hz, 1H), 7.14–7.25 (m, 2H), 3.79 (ddd, *J* 16.1, 6.1, 2.3 Hz, 1H), 3.60 (ddd, *J* 16.1, 8.1, 2.2 Hz, 1H), 2.85 (m, 1H), 1.23 (d, *J* 7.0 Hz, 3H). LC–MS (APCI$^+$) 224 (MH^+ with ^{79}Br, 100%), 226 (MH^+ with ^{81}Br, 100%). Second to elute was **12a**, obtained as an off-white solid (618 mg, 50%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.30 (m, 1H), 7.45 (dd, *J* 8.0, 1.8 Hz, 1H), 7.39 (d, *J* 1.8 Hz, 1H), 7.30 (d, *J* 8.0 Hz, 1H), 3.82 (ddd, *J* 16.2, 6.2, 2.2 Hz, 1H), 3.52 (ddd, 16.2, 9.0, 2.3 Hz, 1H), 2.88 (m, 1H), 1.25 (d, *J* 7.0 Hz, 3H). LC–MS (APCI$^+$) 224 (MH^+ with ^{79}Br, 90%), 226 (MH^+ with ^{81}Br, 100%).

4.1.11. 7-Bromo-4-methyl-3,4-dihydroisoquinoline (12b). Compound **12b** was prepared from **11b** by the same procedure as that of **12a**. Compound **12b** was obtained as a brown oil (914 mg, 99%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.28 (dd, *J* 2.2, 2.2 Hz, 1H), 7.52 (dd, *J* 8.1, 2.0 Hz, 1H), 7.42 (d, *J* 2.0 Hz, 1H), 7.12 (d, *J* 8.1 Hz, 1H), 3.83 (ddd, *J* 16.3, 6.2, 2.2 Hz, 1H), 3.56 (ddd, 16.3, 8.7, 2.2 Hz, 1H), 2.85 (m, 1H), 1.23 (d, *J* 7.0 Hz, 3H). LC–MS (APCI$^+$) 224 (MH^+ with ^{79}Br, 90%), 226 (MH^+ with ^{81}Br, 100%).

4.1.12. 6-Bromo-4-methylisoquinoline (13a). Compound **12a** (618 mg, 2.76 mmol) and MnO_2 (3.60 g, 41.4 mmol) were refluxed in dioxane (50 mL) for 18 h, then filtered through a plug of Celite and washed with CH_2Cl_2. The solvent was removed from the filtrate in vacuo. Column chromatography, eluting with hexanes/EtOAc 9:1 to 85:15 gave **13a** as a yellow oil (212 mg, 35%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.09 (s, 1H), 8.40 (s, 1H), 8.12 (d, *J* 1.8 Hz, 1H), 7.84 (d, *J* 8.7 Hz, 1H), 7.70 (dd, *J* 8.7, 1.8 Hz, 1H), 2.59 (s, 3H). LC–MS (APCI$^+$) 222 (MH^+ with ^{79}Br, 100%), 224 (MH^+ with ^{81}Br, 95%).

4.1.13. 7-Bromo-4-methylisoquinoline (13b). Compound **13b** was prepared from **12b** by the same procedure as that of **13a**. Compound **13b** was obtained as a yellow solid (342 mg, 38%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.04 (s, 1H), 8.40 (s, 1H), 8.13 (d, *J* 1.9 Hz, 1H), 7.84 (d, *J* 8.9 Hz, 1H), 7.80 (dd, *J* 8.9, 1.9 Hz, 1H), 2.62 (s, 3H). LC–MS (APCI$^+$) 222 (MH^+ with ^{79}Br, 90%), 224 (MH^+ with ^{81}Br, 100%).

4.1.14. 8-Bromo-4-methylisoquinoline (13c). Compound **13c** was prepared from **12c** by the same procedure as that of **13a**. Compound **13c** was obtained as a white solid (198 mg, 43%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.51 (s, 1H), 8.46 (s, 1H), 7.93 (d, *J* 8.4 Hz, 1H), 7.85 (d, *J* 7.5 Hz, 1H), 7.56 (dd, *J* 8.4, 7.5 Hz, 1H), 2.64 (s, 3H). LC–MS (APCI$^+$) 222 (MH^+ with ^{79}Br, 100%), 224 (MH^+ with ^{81}Br, 95%).

4.1.15. (6-Bromoisoquinolin-4-yl)methanol (14a). A solution of **13a** (212 mg, 0.95 mmol), NBS (187 mg, 1.05 mmol) and AIBN (16 mg, 0.097 mmol) in benzene (25 mL) was refluxed under an atmosphere of N_2 for 1 h. After cooling to room temperature, 2,6-di-*tert*-butyl-4-methylphenol (105 mg, 0.48 mmol) was added and the solution stirred further 30 min. The reaction was then diluted with DMF (25 mL), then NaOAc (392 mg, 4.78 mmol) was added and the solution heated at 60 °C for 1 h. The solvent was removed in vacuo, the residue taken up in MeOH (40 mL) and 1 M aqueous NaOH (20 mL) and refluxed for 1 h. The MeOH was removed in vacuo, the aqueous residue neutralised with 1 M aqueous HCl, extracted twice with CH_2Cl_2, the combined extracts were dried (Na_2SO_4) and the solvent removed in vacuo. Column chromatography, eluting with hexanes/EtOAc 2:1 to 1:1 to EtOAc gave **14a** as a yellow solid (76 mg, 33%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.19 (s, 1H), 8.54 (s, 1H), 8.36 (s, 1H), 7.88 (d, *J* 8.7 Hz, 1H), 7.74 (d, *J* 8.7 Hz, 1H), 5.09 (s, 2H). LC–MS (APCI$^+$) 238 (MH^+ with ^{79}Br, 95%), 240 (MH^+ with ^{81}Br, 100%).

4.1.16. (7-Bromoisoquinolin-4-yl)methanol (14b). Compound **14b** was prepared from **13b** by the same procedure as that of **14a**. Compound **14b** was obtained as a yellow solid (89 mg, 32%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.15 (s, 1H), 8.55 (s, 1H), 8.17 (d, *J* 2.0 Hz, 1H), 8.07 (d, *J* 9.0 Hz, 1H), 7.84 (dd, *J* 9.0, 2.0 Hz, 1H), 5.12 (s, 2H). LC–MS (APCI$^+$) 238 (MH^+ with ^{79}Br, 100%), 240 (MH^+ with ^{81}Br, 95%).

4.1.17. (8-Bromoisoquinolin-4-yl)methanol (14c). Compound **14c** was prepared from **13c** by the same procedure as that of **14a**. Compound **14a** was obtained as an off-white solid (52 mg, 42%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.63 (s, 1H), 8.62 (s, 1H), 8.30 (d, *J* 8.3 Hz, 1H), 7.89 (d, *J* 7.5 Hz, 1H), 7.60 (dd, *J* 8.3, 7.5 Hz, 1H), 5.14 (d, *J* 5.8 Hz, 2H), 1.79 (t, *J* 5.8 Hz, 1H). LC–MS (APCI$^+$) 238 (MH^+ with ^{79}Br, 95%), 240 (MH^+ with ^{81}Br, 100%).

4.1.18. 6-Bromoisoquinoline-4-carbaldehyde (15a). Compound **14a** (74 mg, 0.31 mmol) and MnO_2 (541 mg, 6.22 mmol) were stirred in CH_2Cl_2 (20 mL) for 3 days, then filtered through a plug of Celite and the solvent removed from the filtrate in vacuo to afford **15a** as a yellow solid (36 mg, 49%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.35 (s, 1H), 9.45 (d, *J* 1.8 Hz, 1H), 9.40 (s, 1H), 8.96 (s, 1H), 7.94 (d, *J* 8.7 Hz, 1H), 7.84 (dd, *J* 8.7, 1.8 Hz, 1H). LC–MS (APCI$^+$) 236 (MH^+ with ^{79}Br, 70%), 238 (MH^+ with ^{81}Br, 85%), 268 (MH^+ with ^{79}Br + MeOH, 100%), 270 (MH^+ with ^{81}Br + MeOH, 90%).

4.1.19. 7-Bromoisoquinoline-4-carbaldehyde (15b). Compound **15b** was prepared from **14b** by the same procedure as that of **15a**. Compound **15b** was obtained as a yellow solid (54 mg, 61%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.37 (s, 1H), 9.36 (s, 1H), 9.11 (d, *J* 9.1 Hz, 1H), 8.96 (s, 1H), 8.24 (d, *J* 2.0 Hz, 1H), 7.98 (dd, *J* 9.1, 2.0 Hz, 1H). LC–MS (APCI$^+$) 236 (MH^+ with ^{79}Br, 50%), 238 (MH^+ with ^{81}Br, 45%), 268 (MH^+ with ^{79}Br + MeOH, 90%), 270 (MH^+ with ^{81}Br + MeOH, 100%).

4.1.20. 8-Bromoisoquinoline-4-carbaldehyde (15c). Compound **15c** was prepared from **14c** by the same procedure as that of **15a**. Compound **15c** was obtained as an off-white solid (43 mg, 60%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 10.41 (s, 1H), 9.87 (s, 1H), 9.22 (d, *J* 8.6 Hz, 1H), 9.02 (s, 1H), 7.98 (d, *J* 7.6 Hz, 1H), 7.74 (dd, *J* 8.6, 7.6 Hz, 1H). LC–MS (APCI$^+$) 236 (MH^+ with ^{79}Br, 75%), 238 (MH^+ with ^{81}Br, 75%), 268 (MH^+ with ^{79}Br + MeOH, 100%), 270 (MH^+ with ^{81}Br + MeOH, 85%).

4.1.21. *N*′-((6-Bromoimidazo[1,2-*a*]pyridin-3-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (1) Method C. A mixture of 6-bromoimidazo[1,2-*a*]pyridine-3-carbaldehyde[14] **17** (2.25 g, 10 mmol) and 2-methyl-5-nitrobenzenesulfonohydrazide[25] (3.0 g, 13 mmol) in MeOH (200 mL) was stirred at room temperature for 4 h to give a precipitate that was collected, washed with MeOH and dried to give *N*′-((6-bromoimidazo[1,2-*a*]pyridin-3-yl)methylene)-2-methyl-5- nitrobenzenesulfonohydrazide (2.11 g, 48%). ^{1}H NMR δ (400 MHz, DMSO-d_6) 12.16 (br, exchangeable with D_2O, 1H), 9.00 (dd, *J* 2.0, 0.7 Hz, 1H), 8.76 (d, *J* 2.5 Hz, 1H), 8.41 (dd, *J* 8.4, 2.5 Hz, 1H), 8.30 (s, 1H), 8.04 (s, 1H), 7.75 (d, *J* 8.5 Hz, 1H), 7.69 (dd, *J* 9.5, 0.7 Hz, 1H), 7.55 (dd, *J* 9.5, 2.0 Hz, 1H), 2.77 (s, 3H). A solution of the crude hydrazone (1.30 g, 3 mmol) in THF (150 mL) was treated with excess CH_2N_2 in ether until the yellow colour persisted. The solvent was removed in vacuo and the residue recrystallized from ethanol to give **1** as a yellow solid (1.12 g, 83%). Mp 221–223 °C. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.03 (d, *J* 2.4 Hz, 1H), 9.01 (dd, *J* 2.0, 0.8 Hz, 1H), 8.35 (dd, *J* 8.4, 2.4 Hz, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.55 (d, *J* 8.9 Hz, 1H), 7.53 (d, *J* 7.7 Hz, 1H), 7.38 (dd, *J* 9.5 Hz, 1H), 3.51 (s, 3H), 2.73 (s, 3H). LC–MS (APCI$^+$) 452 (MH^+ with ^{79}Br, 95%), 454 (MH^+ with ^{81}Br, 100%). Anal. Calcd for $C_{16}H_{14}BrN_5O_4S$: C, 42.49; H, 3.12; N, 15.48. Found: C, 42.65; H, 3.15; N, 15.52.

4.1.22. *N*′-(Imidazo[1,2-*a*]pyridin-3-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16a) Method A. Methylhydrazine (19 mg, 0.41 mmol) was added to a solution of imidazo[1,2-*a*]pyridine-3-carbaldehyde[23] (40 mg, 0.27 mmol) in EtOH (2 mL). After 6 h at room temperature, the solvent was removed in vacuo. The residue was taken up in CH_2Cl_2 (5 mL), then NEt_3 (0.07 mL, 0.50 mmol) and 2-methyl-5-nitrobenzenesulfonyl chloride (129 mg, 0.55 mmol) were added. After 2 h, water was added. The layers were separated, the aqueous layer was extracted with CH_2Cl_2, the combined organic layers dried (Na_2SO_4) and the solvent removed in vacuo. Column chromatography, eluting with hexanes/EtOAc 1:1 to EtOAc gave **16a** as a yellow solid (26 mg, 25%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.06 (d, *J* 2.4 Hz, 1H), 8.98 (m, 1H), 8.31 (dd, *J* 8.4, 2.4 Hz, 1H), 7.97 (s, 1H), 7.87 (s, 1H), 7.68 (m, 1H), 7.52 (d, *J* 8.4 Hz, 1H), 7.36 (m, 1H), 6.93 (m, 1H), 3.49 (s, 3H), 2.74 (s, 3H). LC–MS (APCI$^+$) 374 (MH^+, 100%). Anal. Calcd for $C_{16}H_{15}N_5O_4S\cdot$ 0.15EtOAc: C, 51.57; H, 4.22; N, 18.11. Found: C, 51.62; H, 4.23; N, 18.12.

4.1.23. *N*,2-Dimethyl-5-nitro-*N*′-((5,6,7,8-tetrahydroimidazo[1, 2-*a*]pyridin-3-yl)methylene)benzenesulfonohydrazide (16b). Compound **16b** was prepared from **5** by Method A. Compound **16b** was obtained as a yellow solid (44 mg, 42%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.91 (d, *J* 2.4 Hz, 1H), 8.30 (dd, *J* 8.4, 2.4 Hz, 1H), 7.60 (s, 1H), 7.51 (d, *J* 8.4 Hz, 1H), 7.19 (s, 1H), 3.86–3.93 (m, 2H), 3.38 (s, 3H), 2.83–2.87 (m, 2H), 2.72 (s, 3H), 1.77–1.91 (m, 4H). LC–MS (APCI$^+$) 378 (MH^+, 100%). Anal. Calcd for $C_{16}H_{19}N_5O_4S{\cdot}0.25EtOAc$: C, 51.12; H, 5.30; N, 17.53. Found: C, 51.26; H, 5.25; N, 17.78.

4.1.24. *N*,2-Dimethyl-*N*′-((1-methyl-1*H*-imidazol-5-yl)methylene)-5-nitrobenzenesulfonohydrazide (16c). Compound **16c** was prepared from 1-methyl-1*H*-imidazole-5-carbaldehyde[26] by Method A. Compound **16c** was obtained as a yellow solid (43 mg, 16%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.89 (d, *J* 2.3 Hz, 1H), 8.30 (dd, *J* 8.4, 2.3 Hz, 1H), 7.63 (s, 1H), 7.51 (d, *J* 8.4 Hz, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 3.64 (s, 3H), 3.42 (s, 3H), 2.72 (s, 3H). LC–MS (APCI$^+$) 338 (MH^+, 100%). Anal. Calcd for $C_{13}H_{15}N_5O_4S$: C, 46.28; H, 4.48; N, 20.76. Found: C, 46.16; H, 4.55; N, 20.46.

4.1.25. *N*′-(Imidazo[1,5-*a*]pyridin-3-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16d). Compound **16d** was prepared from imidazo[1,5-*a*]pyridine-3-carbaldehyde[27] by Method A. Compound **16d** was obtained as an orange solid (35 mg, 25%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.07 (d, *J* 2.4 Hz, 1H), 8.85 (m, 1H), 8.30 (dd, *J* 8.4, 2.4 Hz, 1H), 8.02 (s, 1H), 7.49–7.58 (m, 3H), 6.93 (ddd, *J* 9.1, 6.6, 0.9 Hz, 1H), 6.74 (m, 1H), 3.51 (s, 3H), 2.73 (s, 3H). LC–MS (APCI$^+$) 374 (MH^+, 100%). Anal. Calcd for $C_{16}H_{15}N_5O_4S$: C, 51.47; H, 4.05; N, 18.76. Found: C, 51.70; H, 4.18; N, 18.65.

4.1.26. *N*′-(Imidazo[1,5-*a*]pyridin-1-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16e). Compound **16e** was prepared from imidazo[1,5-*a*]pyridine-1-carbaldehyde[27] by Method A. Compound **16e** was obtained as an orange solid (127 mg, 34%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.04 (d, *J* 2.4 Hz, 1H), 8.27 (dd, *J* 8.4, 2.4 Hz, 1H), 8.03 (s, 1H), 7.99 (s, 1H), 7.93 (m, 1H), 7.80 (m, 1H), 7.49 (d, *J* 8.4 Hz, 1H), 6.91 (ddd, *J* 9.2, 6.5, 0.9 Hz, 1H), 6.70 (m, 1H), 3.44 (s, 3H), 2.75 (s, 3H). LC–MS (APCI$^+$) 374 (MH^+, 100%). Anal. Calcd for $C_{16}H_{15}N_5O_4S$: C, 51.47; H, 4.05; N, 18.76. Found: C, 51.78; H, 4.12; N, 18.91.

4.1.27. *N*,2-Dimethyl-*N*′-((1-methyl-1*H*-indol-3-yl)methylene)-5-nitrobenzenesulfonohydrazide (16f). Compound **16f** was prepared from 1-methyl-1*H*-indole-3-carbaldehyde by Method A. Compound **16f** was obtained as an orange solid (113 mg, 36%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* 2.4 Hz, 1H), 8.28 (dd, *J* 8.4, 2.4 Hz, 1H), 8.08 (s, 1H), 7.86 (d, *J* 8.0 Hz, 1H), 7.46 (d, *J* 8.4 Hz, 1H), 7.25–7.33 (m, 3H), 7.13 (m, 1H), 3.79 (s, 3H), 3.34 (s, 3H), 2.74 (s, 3H). LC–MS (APCI$^+$) 387 (MH^+, 100%). Anal. Calcd for $C_{18}H_{18}N_4O_4S$: C, 55.95; H, 4.70; N, 14.50. Found: C, 56.15; H, 4.74; N, 14.76.

4.1.28. *N*′-(Benzo[*c*]isoxazol-3-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16g). Compound **16g** was prepared from **7** by Method C. Compound **16g** was obtained as a yellow solid (300 mg, 41%). Mp 168–170 °C. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.04 (d, *J* 2.4 Hz, 1H), 8.32 (dd, *J* 8.4, 2.4 Hz, 1H), 7.92 (s, 1H), 7.61 (br d, *J* 8.8 Hz, 1H), 7.56 (br d, *J* 9.1 Hz, 1H), 7.51 (br d, *J* 8.4 Hz, 1H), 7.32 (ddd, *J* 9.1, 6.5, 0.8 Hz, 1H), 7.05 (dd, *J* 8.7, 6.5 Hz, 1H), 3.55 (s, 3H), 2.76 (s, 3H). LC–MS (APCI$^+$) 375 (MH^+, 100%). Anal. Calcd for $C_{16}H_{14}N_4O_5S$: C, 51.33; H, 3.77; N, 14.97; Found: C, 51.45; H, 3.77; N, 14.93.

4.1.29. *N*′-(Benzofuran-3-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16h). Compound **16h** was prepared from benzofuran-3-carbaldehyde[28] by Method A. Compound **16h** was obtained as a yellow solid (292 mg, 76%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 8.53 (d, *J* 2.4 Hz, 1H), 8.28 (dd, *J* 8.4, 2.4 Hz, 1H), 7.56 (s, 1H), 7.36–7.44 (m, 3H), 7.16–7.32 (m, 3H), 3.86 (s, 3H), 2.56 (s, 3H). LC–MS (APCI$^+$) 374 (MH^+, 100%). Anal. Calcd for $C_{17}H_{15}N_3O_5S$: C, 54.68; H, 4.05; N, 11.25. Found: C, 54.86; H, 4.03; N, 11.16.

4.1.30. *N*′-(Benzo[*b*]thiophen-3-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16i). Compound **16i** was prepared from benzo[*b*]thiophene-3-carbaldehyde by Method A. Compound **16i** was obtained as an orange solid (126 mg, 53%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.08 (d, *J* 2.4 Hz, 1H), 8.29 (dd, *J* 8.4, 2.4 Hz, 1H), 8.22 (d, *J* 8.0 Hz, 1H), 7.91 (s, 1H), 7.80 (d, *J* 8.0 Hz, 1H), 7.61 (s, 1H), 7.47 (d, *J* 8.4 Hz, 1H), 7.27–7.39 (m, 2H), 3.48 (s, 3H), 2.74 (s, 3H). LC–MS (APCI$^+$) 390 (MH^+, 100%). Anal. Calcd for $C_{17}H_{15}N_3O_4S_2$: C, 52.43; H, 3.88; N, 10.79. Found: C, 52.68; H, 3.90; N, 10.82.

4.1.31. *N*,2-Dimethyl-*N*′-(naphthalen-1-ylmethylene)-5-nitrobenzenesulfonohydrazide (16j). Compound **16j** was prepared from 1-naphthaldehyde by Method A. Compound **16j** was obtained as a yellow solid (272 mg, 74%). ^{1}H NMR δ (400 MHz, DMSO-d_6) 8.75 (d, *J* 2.5 Hz, 1H), 8.55 (d, *J* 8.5 Hz, 1H), 8.39–8.44 (m, 2H), 7.92–7.97 (m, 2H), 7.73–7.78 (m, 2H), 7.51–7.56 (m, 2H), 7.41 (m, 1H), 3.54 (s, 3H), 2.72 (s, 3H). LC–MS (APCI$^+$) 384 (MH^+, 100%). Anal. Calcd for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96. Found: C, 59.69; H, 4.66; N, 11.06.

4.1.32. *N*,2-Dimethyl-5-nitro-*N*′-(quinolin-4-ylmethylene)-benzenesulfonohydrazide (16k). Compound **16k** was prepared from quinoline-4-carbaldehyde by Method A. Compound **16k** was obtained as an orange solid (87 mg, 24%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.01 (d, *J* 2.4 Hz, 1H), 8.91 (d, *J* 4.5 Hz, 1H), 8.37 (dd, *J* 8.5, 0.8 Hz, 1H), 8.31 (dd, *J* 8.4, 2.4 Hz, 1H), 8.13 (d, *J* 8.5, 0.7 Hz, 1H), 8.08 (s, 1H), 7.72 (m, 1H), 7.47–7.56 (m, 3H), 3.59 (s, 3H), 2.77 (s, 3H). LC–MS (APCI$^+$) 385 (MH^+, 100%). Anal. Calcd for $C_{18}H_{16}N_4O_4S$: C, 56.24; H, 4.20; N, 14.57. Found: C, 56.50; H, 4.20; N, 14.57.

4.1.33. *N*,2-Dimethyl-5-nitro-*N*′-(quinolin-3-ylmethylene)-benzenesulfonohydrazide (16l) Method B. A mixture of quinoline-3-carbaldehyde (157 mg, 1 mmol) and of 2-methyl-5-nitrobenzenesulfonohydrazide[25] (230 mg, 1 mmol) in MeOH (5 mL) was stirred at room temperature for 2 h to give a precipitate of 0.32 g (86% yield) of 2-methyl-5-nitro-*N*′-(quinolin-3-ylmethylene)benzenesulfonohydrazide.

The crude hydrazide was combined with a mixture of iodomethane (0.25 g), phenyltrimethylammonium iodide (25 mg), 15% NaOH (3 mL) and CH_2Cl_2 (5 mL), and the two-phase solution was stirred overnight at room temperature. The organic layer was separated, dried (Na_2SO_4) and eluted through a column of alumina to remove polar impurities. The solvent was removed and the residue was recrystallized from MeOH to give **16l** as white crystals (148 mg, 45%). Mp 130–132 °C. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.08 (d, *J* 2.1 Hz, 1H), 8.93 (d, *J* 2.4 Hz, 1H), 8.30 (dd, *J* 8.4, 2.4 Hz, 1H), 8.24 (d, *J* 2.0 Hz, 1H), 8.08 (br d, *J* 8.5 Hz, 1H), 7.83 (dd, *J* 8.1, 1.1 Hz, 1H), 7.76–7.72 (m, 2H), 7.57 (td, *J* 7.5, 1.1 Hz, 1H), 7.50 (d, *J* 8.4 Hz, 1H), 3.50 (s, 3H), 2.85 (s, 3H). LC–MS ($APCI^+$) 385 (MH^+, 100%). Anal. Calcd for $C_{18}H_{16}N_4O_4S$: C, 56.24; H, 4.20; N, 14.57; Found: C, 56.03; H, 4.28; N, 14.33.

4.1.34. *N*′-(Isoquinolin-4-ylmethylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16m). Compound **16m** was prepared from isoquinoline-4-carbaldehyde[29] by Method B. Compound **16m** was obtained as a white solid (32%). Mp (MeOH) 160–162 °C. ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.17 (s, 1H), 9.07 (d, *J* 2.4 Hz, 1H), 8.65 (d, *J* 8.3 Hz, 1H), 8.55 (s, 1H), 8.30 (dd, *J* 8.4, 2.4 Hz, 1H), 8.00–7.96 (m, 2H), 7.71–7.62 (m, 2H), 7.49 (d, *J* 8.4 Hz, 1H), 3.58 (s, 3H), 2.76 (s, 3H). LC–MS ($APCI^+$) 385 (MH^+, 100%). Anal. Calcd for $C_{18}H_{16}N_4O_4S$: C, 56.24; H, 4.20; N, 14.57; Found: C, 56.31; H, 4.30; N, 14.85.

4.1.35. *N*′-((6-Bromoisoquinolin-4-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16n). Compound **16n** was prepared from **15a** by Method C. Compound **16n** was obtained as a cream-coloured solid (8 mg, 11%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.14 (s, 1H), 9.01 (d, *J* 2.4 Hz, 1H), 8.93 (d, *J* 1.8 Hz, 1H), 8.58 (s, 1H), 8.31 (dd, *J* 8.4, 2.4 Hz, 1H), 7.96 (s, 1H), 7.83 (d, *J* 8.7 Hz, 1H), 7.72 (dd, *J* 8.7, 1.8 Hz, 1H), 7.50 (d, *J* 8.4 Hz, 1H), 3.59 (s, 3H), 2.76 (s, 3H). LC–MS ($APCI^+$) 463 (MH^+ with ^{79}Br, 85%), 465 (MH^+ with ^{81}Br, 100%). Anal. Calcd for $C_{18}H_{15}BrN_4O_4S{\cdot}1/3$ EtOAc: C, 47.13; H, 3.61; N, 11.38. Found C, 47.32; H, 3.56; N, 11.50.

4.1.36. *N*′-((7-Bromoisoquinolin-4-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16o). Compound **16o** was prepared from **15b** by Method C. Compound **16o** was obtained as a cream-coloured solid (39 mg, 37%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.10 (s, 1H), 9.01 (d, *J* 2.4 Hz, 1H), 8.64 (d, *J* 9.1 Hz, 1H), 8.56 (s, 1H), 8.31 (dd, *J* 8.4, 2.4 Hz, 1H), 8.14 (d, *J* 2.0 Hz, 1H), 7.94 (s, 1H), 7.78 (dd, *J* 9.1, 2.0 Hz, 1H), 7.50 (d, *J* 8.4 Hz, 1H), 3.57 (s, 3H), 2.76 (s, 3H). LC–MS ($APCI^+$) 463 (MH^+ with ^{79}Br, 90%), 465 (MH^+ with ^{81}Br, 100%). Anal. Calcd for $C_{18}H_{15}BrN_4O_4S$: C, 46.66; H, 3.26; N, 12.09. Found: C, 46.83; H, 3.44; N, 11.99.

4.1.37. *N*′-((8-Bromoisoquinolin-4-yl)methylene)-*N*,2-dimethyl-5-nitrobenzenesulfonohydrazide (16p). Compound **16p** was prepared from **15c** by Method C. Compound **16p** was obtained as a cream-coloured solid (25 mg, 30%). ^{1}H NMR δ (400 MHz, $CDCl_3$) 9.61 (s, 1H), 9.04 (d, *J* 2.4 Hz, 1H), 8.70 (d, *J* 8.6 Hz, 1H), 8.63 (s, 1H), 8.30 (dd, *J* 8.4, 2.4 Hz, 1H), 7.98 (s, 1H), 7.88 (d, *J* 7.5 Hz, 1H), 7.54 (dd, *J* 8.6, 7.5 Hz, 1H), 7.49 (d, *J* 8.4 Hz, 1H), 3.58 (s, 3H), 2.75 (s, 3H). LC–MS ($APCI^+$) 463 (MH^+ with ^{79}Br, 90%), 465 (MH^+ with ^{81}Br, 100%). Anal. Calcd for $C_{18}H_{15}BrN_4O_4S$: C, 46.66; H, 3.26; N, 12.09. Found: C, 46.74; H, 3.42; N, 11.96.

4.2. Enzyme assays

The Class Ia PI3K assays were performed using a basic thin-layer chromatography technique, as described previously.[9] The p110δ/p85 PI3K was from Upstate and the p110α/p85 and p110β/p85 isoforms were prepared as described previously.[9] Reactions were made containing 0.1 μg recombinant enzyme, 10 μg L-α-phosphatidylinositol, inhibitor (DMSO only or DMSO + inhibitor to a final concentration of 1%), 2× 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 $MgCl_2$, 100 μM ATP, 0.1 μL [γ^{33}-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. First, 200 μL 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 μL of methanol/hydrochloric acid (1:1) was added and the same procedure followed. Next, 70 μL 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 phosphorimager (StormImager, Amersham). Nine inhibitor concentrations were used to determine the IC_{50}. Each experiment was performed twice and the average IC_{50} value used.

4.3. Cellular assay

The early passage cell lines used in this study were developed in this laboratory and cultured as previously described.[30] Cell lines were grown in α-modified minimal essential growth medium supplemented with insulin, transferrin, selenite and 5% foetal bovine serum. Individual wells of 96-well tissue culture plates contained 1000 cells in a volume of 150 μL. Drugs were added at 10-fold concentration steps to a maximum of 20 μM and plates were incubated under an atmosphere of 5% O_2, 5% CO_2 and 90% N_2 for five days, with [^{3}H]thymidine (0.04 μCi per well) being added over the last 6 h. Cells were harvested and the incorporated radioactivity was measured. Duplicate samples were analysed for each drug dose with multiple control samples and data were fitted to a least-squares regression of the form $y = y_0 + ae^{-bx}$, where y is the incorporated radioactivity, x is the drug concentration and y_0, a and b are variables. The IC_{50} value was defined as the drug concentration reducing [^{3}H]thymidine incorporation by 50%.

4.4. Molecular modelling

Molecular modelling studies were carried out using *SYBYL* software, version 7.3, running on a Linux worksta-

tion.[21] Compound **1** was built using the Sketch module and optimised using the Tripos force field. Docking simulations were performed on human p110γ (PDB code 2CHX) with the automated *GOLD* program.[20] 20 conformations were generated. In order to take into account protein flexibility, the conformation with the best score (GoldScore) was further refined using the *MINIMIZE* module. The minimization process used the Powell method[31] with the Tripos force field (dielectric constant 1*r*) to reach a final convergence of 0.01 kcal mol^{-1}.

Mutation of Thr-886 into histidine was done using the *BIOPOLYMER* module. The side chain conformation was set according to the Lovell rotamer library[32] and refined using the *MINIMIZE* module to a final convergence of 0.01 kcal mol^{-1}.

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