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Discovery of potent, selective small molecule inhibitors of α-subtype of type III phosphatidylinositol-4-kinase (PI4KIIIα)

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ABSTRACT

The discovery and optimisation of novel, potent and selective small molecule inhibitors of the α-isoform of type III phosphatidylinositol-4-kinase (PI4Kα) are described. Lead compounds show cellular activity consistent with their PI4Kα potency inhibiting the accumulation of IP1 after PDGF stimulation and reducing cellular PIP, PIP2 and PIP3 levels. Hence, these compounds are useful in vitro tools to delineate the complex biological pathways involved in signalling through PI4Kα.

Phosphatidylinositol-4-kinases (PI4Ks) catalyse the phosphorylation of phosphatidylinositol (PI) at the D4 position of the inositol head-group to form phosphatidylinositol-4 phosphate (PI4P).[1] PI4P acts as a binding partner for a wide range of proteins with pleckstrin homology (PH) or related lipid-binding domains.[2,3] PI4P is also an important signalling molecule as a precursor to other phosphoinositides such as phosphatidylinositol-4,5-diphosphate $PI(4,5)P_2$ and phosphatidylinositol-3,4,5-triphosphate $PI(3,4,5)P_3$ (Scheme 1).[3,4] Four PI4K isoforms have been identified in mammals and are classified as type II (PI4KIIα and β) and type III (PI4KIIIα and β, hereafter referred to as PI4Kα and β). PI4Kα is the main isoform responsible for PI4P generation at the plasma membrane and is localised at the endoplasmic reticulum. PI4Kβ is mainly associated with the Golgi complex where it functions in the generation of Golgi-derived carriers.[5] Recent reports suggest the implication of PI4Kα and PI4P in viral replication.[3,6]

Pharmacological manipulation of cellular PI4P levels has been a challenge due to the non-specificity and low potency of known PI4K inhibitors such as wortmannin, LY294002 and phenylarsine oxide[7]; although more recently, reports have emerged describing PI4K inhibitors from several groups.[8–12]

Recently, we disclosed two distinct series of potent and selective α- and β-subtype PI4K inhibitors and described their effect on cellular concentration of PI4P and $PI(4,5)P_2$ and cellular proliferation in a panel of cancer cell lines.[13] In this communication we report the results of our work that led to the identification of potent and selective small molecule inhibitors of PI4Kα.

To optimise for suitable probe compounds that would allow us to evaluate the effect of PI4Kα inhibition on the PI signalling pathway, we first screened compounds against PI4Kα and β Compounds active in PI4Kα were subsequently evaluated against other related enzymes on the PI pathway, including PI3Kα/β/γ/δ and PIP5Kα/β/γ. Finally, with potent and selective PI4Kα inhibitors in hand, we assessed their effect on accumulation of inositol-1-phosphate (IP1) in NIH3T3-PDGFRβ cells.[13]

We identified the original PI4Kα-selective hit from a screen against recombinant PI4Kβ of approximately 100,000 compounds selected from the AstraZeneca compound collection based on structures known to have lipid kinase activity. Active compounds from the screen then had their IC_{50} values determined against PI4Kβ and PI4Kα.[14] From this screening campaign we identified a moderately selective 2-aminobenzothiazole derivative **1** (Table 1) as a promising starting point for further evaluation. To get more detailed insight into structure activity relationships (SAR) of 2-aminobenzothiazoles, we first investigated the effect of substitution of the pyridine ring in **1** on PI4Kα potency and selectivity (Table 1). Replacement of the ethoxy group with smaller substituents such as 6-methoxy (**2**) or 6-methyl (**10**) led to reduction of PI4Kα potency and selectivity over PI4Kβ and PI3Ks.

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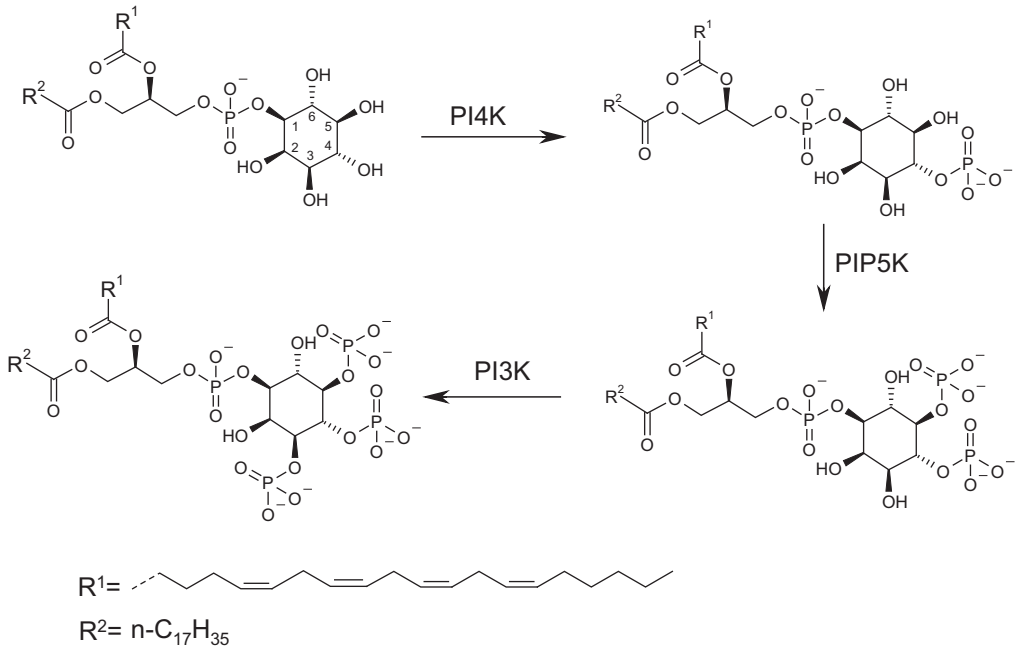
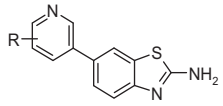


Scheme 1. The PI4K cascade.

Table 1
SAR of substituted 2-amino-6-(pyrid-3-yl)-benzothiazoles (**1**–**18**)[17]

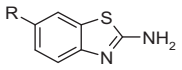


1-18

No	R	PI4Kα pIC_{50}	PI4Kβ pIC_{50}	PI3Kα/β/γ/δ pIC_{50}	PIP5Kγ pIC_{50}
1	6-EtO–	6.8	6.2	5.8/5.2/5.7/5.4	<4
2	6-MeO–	6.2	6.0	6.1/5.8/6.4/6.1	4.6
3	6-EtS–	7.0	6.2	5.9/5.3/6.0/5.7	<4
4	6-$MeOCH_2$–	6.0	6.5	5.6/4.8/ND/5.2	<4
5	6-$MeNHCH_2$–	5.6	5.5	4.2/4.1/4.3/4.2	<4
6	6-^{i}PrO–	7.1	5.9	5.3/4.5/4.9/4/9	<4
7	6-EtNH–	6.2	6.0	5.7/4.9/5.4/5.2	4.5
8	6-Et(Me)N–	6.2	5.8	5.2/4.4/5.4/4.9	<4
9	6-$HOCH_2$–	5.8	6.1	5.5/ND/ND/ND	<4
10	6-Me–	5.8	6.3	6.0/5.6/5.9/5.9	<4
11	6-CF_3–	4.8	5.8	5.7/5.4/6.0/6.0	<4
12	6-$MeSO_2$–	4.6	5.1	3.8/4.4/5.0/4.9	5.0
13	6-$HO(CH_2)_2O$–	6.5	6.3	5.9/5.2/5.9/5.7	<4
14	6-$MeO(CH_2)_2O$–	6.5	6.0	5.6/5.1/5.5/5.2	<4
15	6-MeNHC(O)–	5.5	5.6	5.7/ND/ND/ND	4.5
16	6-$Me_2NC(O)$–	5.9	4.4	4.4/4.1/4.7/4.2	<4
17	5-*i*-PrO–	6.0	6.0	6.3/5.9/6.6/5.9	4.5
18	5-$MeSO_2$–	6.4	5.4	6.6/6.6/6.9/6.5	<4

The 6-ethylthio analogue **3** was found to have a similar profile to the ethoxy compound **1**. Increasing the size of the alkoxy substituent was tolerated in several cases (e.g., in **6**, **13** and **14**), but this did not improve the selectivity profile for these compounds. However, the 6-methoxymethyl derivative **4** was more potent at PI4Kβ than PI4Kα. Diminished selectivity was also observed with 6-aminopyridyl derivatives **7** and **8**. The presence of electron withdrawing groups such as a trifluoromethyl (**11**), methylsulfonyl (**12**) and carbamoyl (**15**, **16**) at the 6-position of pyridine ring gave lower PI4Kα IC_{50} values for these compounds and hence, in most cases, reduced their selectivity over PI4Kβ and PI3K kinases. An exception to this was the amide **16**, which although less potent

Table 2
SAR of substituted 2-amino-6-heteroaryl-benzothiazoles (**19**–**35**)[17]



19-35

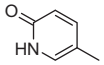
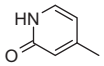
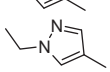
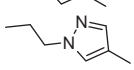
No	R	PI4Kα pIC_{50}	PI4Kβ pIC_{50}	PI3Kα/β/γ/δ pIC_{50}	PIP5Kγ pIC_{50}
19	Ph	5.6	6.3	4.9/ND/ND/ND	<4
20		5.0	5.6	5.0/ND/4.9/4.1	<4
21		5.8	6.2	5.7/ND/ND/5.9	4.5
22		5.6	6.0	5.6/ND/ND/ND	5.6
23		4.8	5.2	<4/ND/4.5/4.3	4.5
24		5.1	5.2	5.7/5.2/5.4/5.1	<4
25		5.5	5.4	4.6/4.3/5.0/4.5	4.4
26		6.5	6.2	5.4/5.2/5.9/5.0	4.8
27		5.6	5.9	5.2/4.8/5.6/4.9	4.2
28		4.9	6.0	4.9/4.5/5.6/4.6	<4
29		5.6	5.9	5.1/4.8/5.6/5.1	<4
30		5.5	5.8	4.9/4.5/5.5/4.7	<4
31		5.8	5.7	4.9/4.9/5.8/4.9	4.6

Table 2 (*continued*)

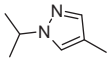
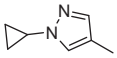
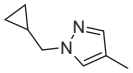
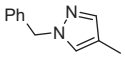
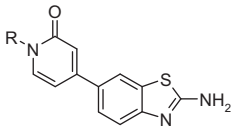
No	R	PI4Kα pIC_{50}	PI4Kβ pIC_{50}	PI3Kα/β/γ/δ pIC_{50}	PIP5Kγ pIC_{50}
32		4.6	5.6	4.8/ND/5.4/4.7	4.2
33		5.1	5.7	5.0/5.0/6.0/5.4	<4
34		6.2	5.7	4.9/4.8/5.9/4.7	ND
35		6.8	6.1	5.1/4.6/5.7/4.7	<4

Table 3
Effect of *N*-substitution of the 2-pyridone in **26** on PI4Kα potency and selectivity[17]



26, 36-39

No	R	PI4Kα pIC_{50}	PI4Kβ pIC_{50}	PI3Kα/β/γ/δ pIC_{50}	PIP5Kγ pIC_{50}
26	H	6.5	6.2	5.4/5.2/5.9/5.0	4.8
36	Me	6.3	6.5	5.6/4.2/ND/5.3	<4
37	Et	6.5	6.5	5.2/4.3/5.5/5.0	<4
38	*c*-$PrCH_2$	8.2	5.8	5.2/<3.9/5.8/4.9	<4.1
39	2-$MeO(CH_2)_2O$	6.2	5.0	4.6/<3.7/5.0/<3.7	<4

at PI4Kα showed an encouraging selectivity profile. Selectivity against PIP5Kα/β/γ was very good across the series.[15]

Next, we explored the replacement of the pyrid-3-yl substituent at the 6-position of 2-aminobenzothiazole ring (Table 2). The unsubstituted 3-pyridyl analogue **21** was an order of magnitude less potent than **1** in PI4Kα but showed equipotent PI4Kβ activity to **1**. The corresponding phenyl derivative **19** showed a similar profile. The replacement of the pyrid-3-yl substituent in **21** with other 6-membered heterocyclic rings generally led to reduction of PI4Kα potency and selectivity. Interestingly, 2-hydroxypyrid-4-yl (2-pyridone) derivative **26** was about 10-fold more potent and selective when compared to the corresponding pyrid-4-yl analogue **22**.

The replacement of the pyrid-4-yl substituent in **22** with 1-methyl-pyrazol-3-yl in **27** resulted in retention of PI4Kα potency and selectivity. Isomeric pyrazoles **28** and **29** showed a comparable profile to **27**. However, increasing the size of the *N*-alkyl substituent in **29** with β-branching (relative to the N1 pyrazole nitrogen), as exemplified with the *N*-cyclopropylmethyl (**34**) and *N*-benzyl (**35**) analogues, resulted in improved PI4Kα inhibitory activity and enhanced selectivity against other kinases.

A similar effect on potency and selectivity was observed with *N*-alkylated analogues of the 2-pyridone **26** (Table 3). While small substituents such as methyl (**36**) or ethyl (**37**) revealed a similar profile to the reference compound **26**, the corresponding *N*-cyclopropylmethyl analogue **38** showed excellent potency at PI4Kα (pIC_{50} 8.2) and good selectivity over PI4Kβ, PI3K and PIP5K isoforms (>100 fold). Moreover, **38** demonstrated a very good selectivity profile when tested in the Millipore kinase panel, consisting of 377 different kinases and related targets (Fig. 1), showing activity against only 3 kinases (FGR 98%, ZIPK 72% and STK17A 68% of inhibition at 1 μM concentration). Pleasingly, **38** inhibited the accumulation of IP_1 (IC_{50} 0.52 μM) and reduced cellular PIP, PIP_2 and PIP_3 levels in NIH3T3-PDGFRβ cells.[13] Furthermore, **38** was tested for growth inhibition in the panel of 183 cancer cell lines inhibiting 91 cell lines with pGI_{50} >5.0.[13]

In order to understand the observed differences in selectivity for these compounds, several compounds were docked into homology models of PI4Kα and PI4Kβ. Homology models were built using the MOE[16] software package based on available crystal structures of PI3Kγ, as described previously.[13] Ligand docking was also performed in MOE with the two kinase-hinge hydrogen bonds set as a pharmacophore restraint. An induced-fit protocol was employed to allow limited movement of protein side-chains within 6 Å of the

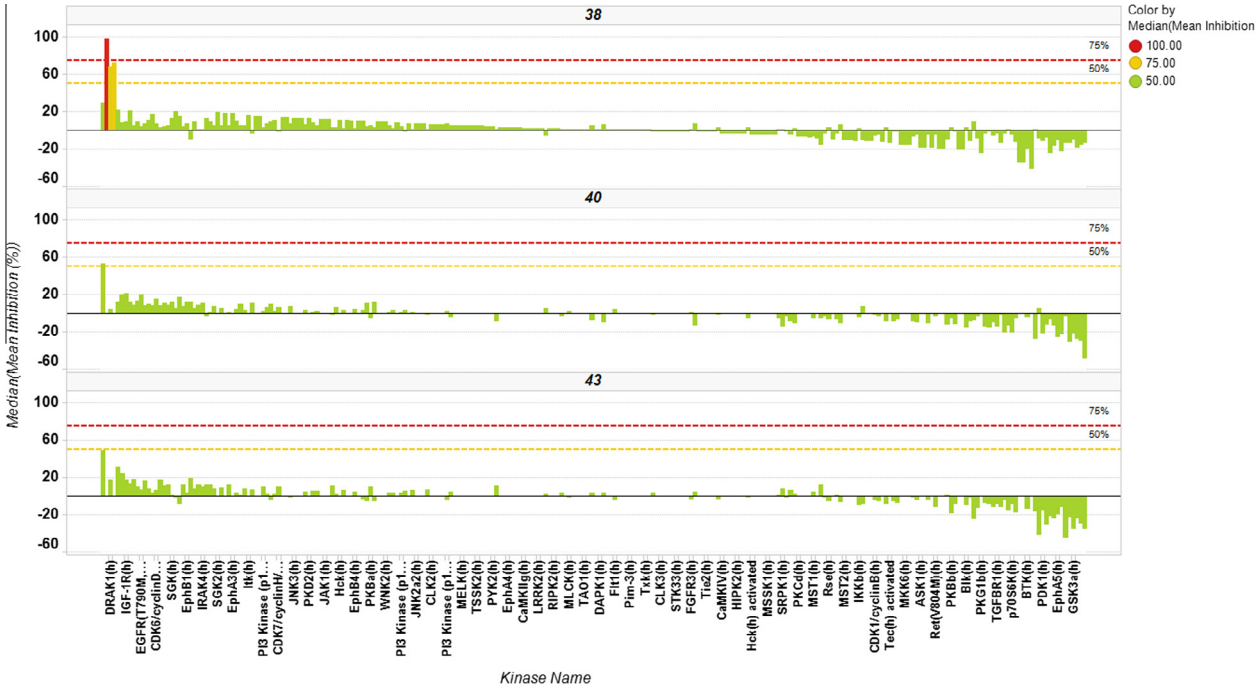


Figure 1. Selectivity profile of **38**, **40** and **43** in the kinase panel (% inhibition at 1 μM concentration).

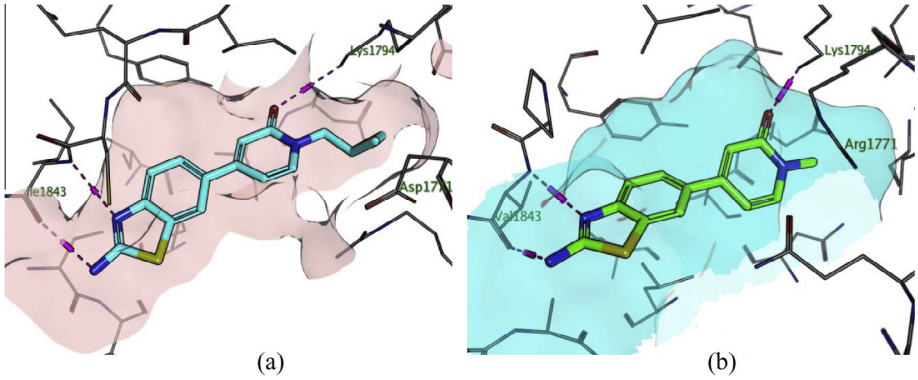
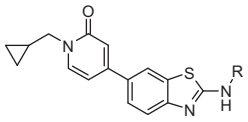
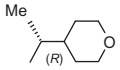
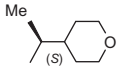
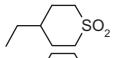
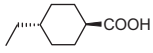


Figure 2. (a) Compound **38** docked into the PI4Kα homology model. (b) Compound **36** docked into the PI4Kβ homology model. Notice that the subpocket occupied by the cyclopropyl moiety of **38** is much smaller in β than in α. Larger groups occupying this pocket confer isoform selectivity.

Table 4
N-Substituted analogues of 2-pyridone **38**[17]

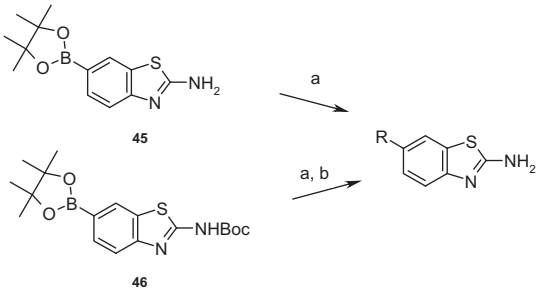


38, 40-44

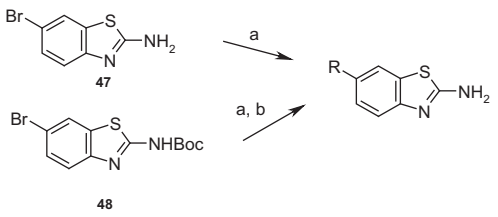
No	R	PI4Kα pIC_{50}	PI4Kβ pIC_{50}	PI3Kα/β/γ/δ pIC_{50}	PIP5Kγ pIC_{50}	IP1 pIC_{50}
38	H	8.2	5.8	5.2/<3.9/5.8/4.9	<4.1	6.3
40		8.3	6.4	<3.7/<3.7/4.8/4.0	<4	6.1
41		8.1	6.5	<3.7/<3.7/4.2/<3.7	<4	6.2
42		7.4	5.6	<3.7/<3.7/<3.7/<3.7	4.6	5.1
43		8.7	6.2	4.1/<3.7/4.3/<3.7	<4	6.2
44		9.0	6.6	4.0/<3.7/5.0/<4.1	ND	6.5

ligand. Figure 2 shows the common binding mode of this series to both PI4K isoforms. The amino-benzothiazole unit binds to the 'hinge' of the kinase, making two strong hydrogen-bonds. A third hydrogen-bond is made to the catalytic lysine at the back of the pocket (K1794, conserved between isoforms). We postulated that the crucial difference in structure that determines selectivity of **38**, compared to the unselective **36**, is the presence of an arginine (R1771) in beta, near the back of the pocket; the equivalent residue in the alpha being aspartic acid (D1771), see Figure 2. The arginine, being a bulkier residue, reduces the size of the subpocket near the catalytic lysine. This, together with some small structural differences between isoforms, means that while there is a pocket in PI4Kα large enough to bind the cyclopropyl moiety of **38** with high affinity, no such pocket exists in PI4Kβ. Compounds with smaller substituents, such as **36**, are accommodated in both structures and as such bind with approximately equal potency to both isoforms. Docking of **38** in the β-isoform indicates that the ligand shifts to accommodate the larger group in the smaller pocket and the hydrogen-bond to K1794 cannot be made, hence lower PI4Kβ affinity.

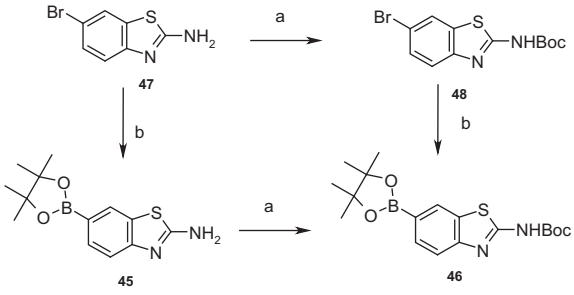
The potent and selective probe **38** could be optimised further by substitution of the NH_2 group (Table 4). A number of substituents were tolerated at this position as represented by the



Scheme 2. Reagents and conditions: (a) R-X (X = Br,Cl), Pd-118, K_2CO_3 or K_3PO_4, MeCN–H_2O, 80–100 °C, 1–2 h, MW; (b) 20% CF_3COOH in CH_2Cl_2, room temperature, 1–2 h (overall yields: 8–53%).

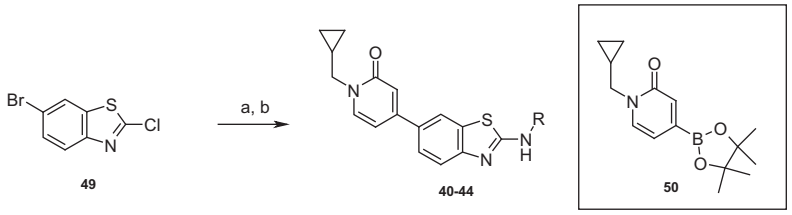


Scheme 3. Reagents and conditions: (a) R-B(OH)$_2$ or R-BPin, Pd-118, K_2CO_3 or K_3PO_4, MeCN–H_2O, 80–100 °C, 1–2 h, MW; (b) 20% CF_3COOH in CH_2Cl_2, room temperature, 1–2 h (overall yields: 4–64%).



Scheme 4. Reagents and conditions: (a) Boc_2O, Et_3N, DMAP, CH_2Cl_2, rt, 3 days, 70–89%; (b) Pin_2B_2, Pd(dppf)$_2Cl_2$, AcOK, 1,4-dioxane, 95 °C, 5 h, 83–100%.

tetrahydropyranyl methyl derivative **40**. The chiral (*R*) analogue **41** was equipotent with **40**, whereas for the corresponding (*S*) enantiomer **42** PI4Kα potency was reduced by 6-fold. However, a gain in PI4Kα potency and further improvement in selectivity



Scheme 5. Reagents and conditions: (a) R-NH_2, Et_3N, MeCN, 180 °C, 2 h, MW; (b) **50**, Pd-118, K_2CO_3, MeCN–H_2O, 120 °C, 1–2 h, MW (33–76% for 2 steps).

was achieved with the corresponding sulfone derivative **43** and the acid **44**. Benzothiazoles **40** and **43** were tested in the Millipore kinase panel consisting of 124 different kinases (Fig. 1) showing only weak activity against human PAK2 (49% and 53% inhibition at 1 μM concentration for **40** and **43**, respectively). Additionally, both **40** and **43** exhibit reduced STK17A inhibition (17% and 4% inhibition for **40** and **43**, respectively vs 68% inhibition for **38**) and FGFR1 activity was removed entirely. Compounds **40**, **41**, **43** and **44** displayed a similar inhibitory effect to **38** in the IP_1 cellular assay suggesting, that these compounds may also be considered as useful in vitro tools to study the biological pathways involved in signalling through PI4Kα and PI4P.

All compounds described in this communication were prepared using, as a key step, Suzuki cross-coupling reaction between selected heteroaryl halides or boronic acids/boronates and corresponding 2-amino-benzothiazole derived bromides or boronate esters (**45**, **46**, **47** or **48**) (Schemes 2 and 3).

Required building blocks **45**, **46** and **48** were synthesised from the commercially available 2-amino-5-bromobenzothiazole **47** by Boc protection of the amino group and the boronic ester were introduced by prior or subsequent palladium catalysed coupling (Scheme 4).

N-Substituted analogues of **38** (compounds **40**–**44**) were made from commercial 2-chloro-5-bromobenzothiazole **49** in a 2-step, one-pot sequence that starts with nucleophilic substitution of the chlorine at the 2-position of the benzothiazole ring with a corresponding amine following by the Suzuki cross-coupling reaction with the 2-pyridone boronate **50** (Scheme 5).

In conclusion, a novel series of potent and selective small molecule inhibitors of PI4Kα was discovered. Several compounds showed excellent PI4Kα potency and selectivity against other kinases. These compounds might serve as good quality biochemical tools for evaluation and interpretation of complex biological cascades involved in signalling through PI4Kα.

References and notes

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14. Protocols for PI4 Kα, PI4 Kβ, PI3 Kα, PIP5 Kγ and IP1assays are described in the supplementary information of Ref. 13. Protocols for PI3 Kβ/γ/δ assays were as reported for PI3 Kα.
15. Data presented for PIP5 Kγ isoform are indicative of activity against PIP5 Kαβ isoforms.
16. Molecular Operating Environment (MOE), 2013.08; Chemical Computing Group Inc., 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2015.
17. pIC_{50}s are mean values of up to nine independent experiments. ND means no data available.