Bioorganic & Medicinal Chemistry Letters 25 (2015) 3569–3574

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Identification and optimisation of a 4′,5-bisthiazole series of selective phosphatidylinositol-3 kinase alpha inhibitors

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ARTICLE INFO

*Article history:*
Available online 27 June 2015

*Keywords:*
Oncology
Kinase inhibitor
Phosphatidylinositol-3-kinase-alpha

ABSTRACT

Exploring the affinity-pocket binding moiety of a 2-aminothiazole (*S*)-proline-amide-urea series of selective PI3Kα inhibitors using a parallel-synthesis approach led to the identification of a novel 4′,5-bisthiazole sub-series. The synthesis and optimisation of both the affinity pocket and (*S*)-proline amide moieties within this 4′,5-bisthiazole sub-series are described. From this work a number of analogues, including **14** (A66) and **24**, were identified as potent and selective PI3Kα inhibitor in vitro tool compounds.



Of the lipid kinase family the class I phosphatidylinositol-3-kinases (PI3Ks): PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ have been the most extensively investigated to date. The work in this area has highlighted the class I PI3K's to be potential targets for the treatment of a wide range of diseases.[1] In an effort to fulfil these therapeutic opportunities, the search for isoform selective agents has become increasingly important to supersede the first generation of pan-PI3 K inhibitors.[2] The main drive for increasing isoform selectivity being to enhance tolerability, by dialing out unnecessary activities, and to facilitate a better understanding of PI3K signalling pathways. In particular, PI3Kα is amplified, or overexpressed, in a range of cancers, and has been identified as one of the most commonly mutated genes in human cancer.[3] Additionally, these mutations typically lead to enhanced, or constitutive, PI3Kα kinase-activity. As a result, identifying selective PI3Kα inhibitors has become an area of active research to explore for the treatment of a range of cancers.[4]

Previously, an (*S*)-proline-amide aminothiazole-urea series had been identified within Novartis and shown to have a high level of selectivity for the PI3Kα isoform.[5] The origin of this selectivity was determined to be derived from the proline amide-moiety targeting an interaction with the non-conserved glutamine 859 residue, situated at the entrance to the ATP-pocket of PI3Kα. This series was identified using a parallel synthesis approach which explored the 2-amino thiazole-substituent structure activity relationships (SAR) starting from the corresponding pan-PI3K inhibitor 2-aminothiazole-acetamide analogues, as exemplified by **1** leading to **2** in Figure 1. Following on from this activity we have already described the optimisation of the closely related starting point **3**, in which the pyrimidyl affinity-pocket binding moiety was optimised to provide the clinical candidate alpelisib (NVP-BYL719), Figure 2.[6] In this Letter we describe a parallel approach from **3** that was taken to optimise the (*S*)-proline-amide aminothiazole series to more broadly understand the affinity-pocket and proline SAR surrounding PI3K inhibition.

In the early stages of the project the affinity pocket SAR had been developed primarily around substituted phenyl and 4-pyrimidyl residues, as exemplified by the 3-fluoro-4-methylsulphonylphenyl in **2** and 2-*tert*-butylpyrimidyl in **3**. Additionally, within these residues a range of substituents were found to be tolerated in the positions *meta* and *para* to the point of attachment to

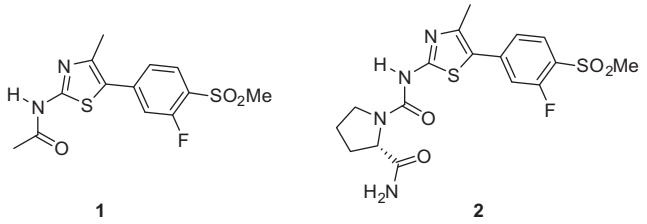


**Figure 1.** Structures of the *N*-acetyl pan-PI3K inhibitor **1** and the (*S*)-proline-amide-urea PI3Kα selective inhibitor **2**.

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http://dx.doi.org/10.1016/j.bmcl.2015.06.078
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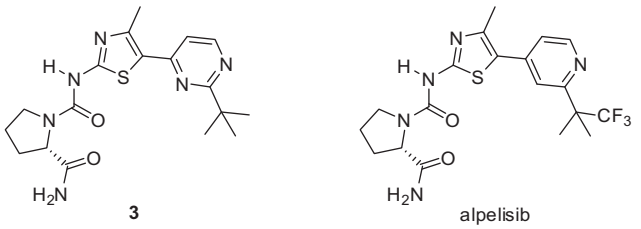



**Figure 2.** Structures of the PI3Kα selective inhibitors **3** and alpelisib.

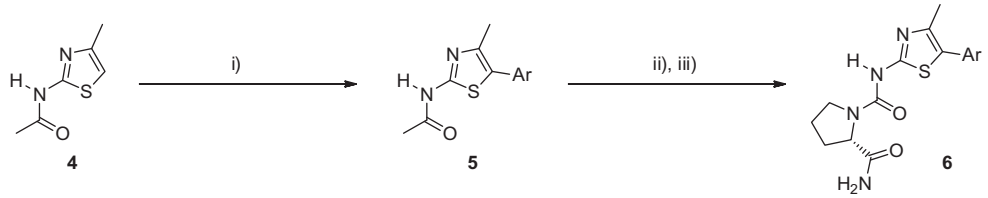
the thiazole 5-position. Therefore, to rapidly expand the SAR in this region, the feasibility of a parallel synthesis approach was investigated that would enable the introduction of a range of aromatic residues into the 5-position of the thiazole core. The synthetic route that was identified to support this approach was a *C–H* arylation of the unsubstituted 5-position of 2-acetamido-4-methylthiazole **4**.[7] This reaction enabled a range of aryl chlorides, bromides and iodides to be coupled to give the *N*-acylated aminothiazole analogues **5**, as shown in Scheme 1. The aryl halides used to prepare the analogues **5** were selected from internal and commercially available sources. At this point, any interesting aryl groups would be anticipated to be pan-PI3K inhibitors, based upon analogy with **1**, and biochemical screening was carried to assess the extent of inhibition of PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ.[8] The analogues **5** exhibiting an interesting level of activity (<10 μM) were then hydrolysed and converted to the corresponding (*S*)-proline-amide-urea analogues **6**, via the *N*-acylimidazole intermediates.

Following the above approach a small number of heterocycles were identified as 5-thiazole substituents with PI3K biochemical activities <1 μM, including: 2-substituted 4-thiazoyl, 5-substituted 2-pyrazinyl, 6-substituted 2-pyrazinyl, and 6-substituted 4-pyrimidinyl. Of these, the 4-(2-isopropylthiazoyl) was selected as a particularly interesting opportunity for further optimisation based upon the pan-PI3K and PI3Kα activities of **7** and **8**, Figure 3 and Table 1. Additionally, when the selection was made some precedent already existed for the 4′,5-bisthiazole biaryl core from a related series of pan-PI3K inhibitors.[9] Based upon these data compound **8** was selected as the starting point for further optimisation. More recently a related 4′,5-bisthiazole series of PI3Kγ selective inhibitors has been reported originating from a high throughput screening hit.[10]

Docking the 4′,5-bisthiazole **8** into a homology model of PI3Kα, derived from a single-crystal X-ray structure of an aminothiazole based inhibitor bound into PI3Kγ,[11] suggested that modifying the nature of the 2-substituent in the 4-linked thiazole moiety could further enhance the interaction within the affinity pocket. More specifically, the 2-isopropyl group was determined not to fully occupy the portion of the affinity pocket formed by the residues I800, I848, P778 and K802, and highlighted the possibility for larger groups to better fill this region, as depicted in Figure 4. Additionally, opportunities were seen for substituting the proline ring to either: increase the interaction with the ATP site of PI3Kα, or to modify the physicochemical properties of the inhibitors without negatively impacting the ATP-site interaction. Towards the end of the project, the modelling used to generate the above hypotheses could be shown to be in good agreement with a single-crystal X-ray structure of alpelisib bound into the ATP pocket of PI3Kα.[12]

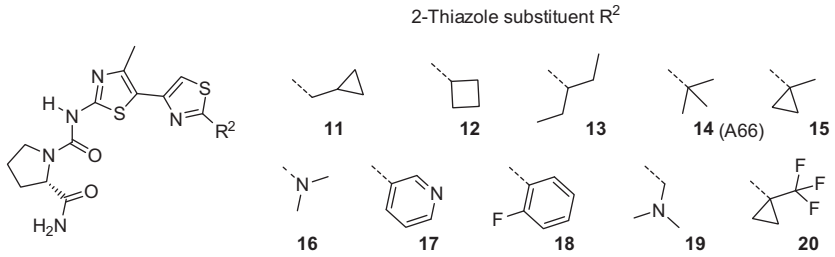
To explore the above possibilities, alternative synthetic approaches were investigated to improve upon the flexibility of the *C–H* arylation route used in the identification of **8**. The sequence that was identified is shown in Scheme 2, and proved to be more flexible with respect to the range of 2-substituents ($R^2$) that could be readily introduced into the 4′-linked thiazole moiety.[13] Starting from commercially available 5-acetyl-2-(*N*-acetylamino)-4-methylthiazole **9**, the derived bromoketone was readily cyclised to the corresponding thiazoles **10** upon reaction with a range of thioamides and thioureas, catalysed by ammonium phosphomolybdate.[14] Acetamide hydrolyses of **10** were followed by activation as the acylimidazole intermediates which could be conveniently isolated by filtration from the reaction mixtures when dichloromethane was used as the solvent. These shelf-stable intermediates were then reacted with the corresponding proline amide derivatives to give the final products **11**–**33**.

Table 1 shows the biochemical and cellular data for the reference compounds and the representative analogues **11** to **20** from the series in which the affinity pocket SAR is explored through variation of the 2-substituent ($R^2$) in the 4′-linked thiazole moiety.[15] From the analogues incorporating alkyl residues, **11** to **15**, the most active examples have been shown to contain a quaternary centre at the point of attachment to the 4′-thiazole 2-position. Both the *tert*-butyl and methylcyclopropyl analogues, **14** and **15**, have been shown to give the highest levels of PI3Kα activity and selectivity. Selectivities of greater than 20-fold were determined with the PI3Kδ-isoform typically being the closest off-target to the PI3Kα activity. This SAR is in line with our findings in the related pyrimidyl and pyridyl series, as exemplified by the *tert*-butyl analogue **3**.[6] For the most active examples in Table 1 the biochemical activities translated well into cellular activities, with $IC_{50}$ shifts of less than 10-fold, and the cellular data were used as the primary assays to drive the PI3K SAR. To better understand the origin of the cellular potency shifts, the variation in plasma protein binding (PPB) within the series was assessed using a chromatography method measuring the affinity for immobilised human and rat serum albumin (HSA and RSA),[16] selected physicochemical and in vitro pharmacokinetic (PK) data are shown in Table 2. In the case of alpelisib a good correlation was observed between PPB predicted by HSA and RSA binding compared with PPB measured directly by a rapid equilibrium dialysis method (94.4% and 94.3%).[6] Analysing the data, the similar and modest cellular $IC_{50}$ shifts observed across the series are consistent with the predict less than 2-fold variation in unbound fraction from the HSA and RSA data. Interestingly, the *tert*-butyl analogue **14** has been identified from our published claims by Vogt et al, and shown independently to be a potent and selective PI3Kα-inhibitor.[17] As a result, **14** has been assigned the identifier A66, and has found use as a reference PI3Kα-selective




**Scheme 1.** Library approach to exploring the SAR of the thiazole 5-position. Reagents and conditions: (i) Ar-X (X = Cl, Br, I), $Pd(OAc)_2$, $P(t\text{-}Bu)_3HBF_4$, $Cs_2CO_3$, DMF, 160 °C Biotage Initiator® microwave reactor, 1 h; (ii) 1.25 M HCl in MeOH, 50 °C, 17 h; (iii) carbonyl diimidazole (CDI), $Et_3N$, DMF, 50 °C, 17 h, then (*S*)-proline amide, DMF, 50 °C, 17 h.

**Table 1**
PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ activities for the reference compounds and for the 4′-thiazole 2-substituted analogues **11**–**20** exploring the affinity pocket SAR




| Compound | Biochemical $IC_{50}$ (μM) | | | | Cellular $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|---|
| | PI3Kα | PI3Kβ | PI3Kγ | PI3Kδ | PI3Kα | PI3Kβ | PI3Kδ |
| **1** | 0.18 | 1.6 | 0.87 | 0.41 | n.d. | n.d. | n.d. |
| **2** | 0.28 | >9.1 | >10 | 4.7 | >10 | >10 | >10 |
| **3** | 0.007 | 1.9 | 0.23 | 0.38 | 0.039 | 3.1 | 1.5 |
| Alpelisib | 0.005 | 1.2 | 0.25 | 0.29 | 0.074 | 2.2 | 1.2 |
| **7** | 0.21 | 1.8 | 1.2 | 0.62 | 0.99 | 0.95 | 1.9 |
| **8** | 0.020 | >9.1 | n.d. | n.d. | 0.51 | >10 | >10 |
| **11** | 0.070 | 8.5 | 6.3 | 1.5 | 0.52 | >10 | 6.3 |
| **12** | 0.033 | 7.0 | 6.1 | 1.6 | 0.36 | >10 | >10 |
| **13** | 0.088 | >9.1 | >10 | 1.8 | 0.61 | >10 | >10 |
| **14** (A66) | 0.015 | 6.5 | 8.0 | 1.5 | 0.20 | >10 | >10 |
| **15** | 0.014 | 4.9 | n.d. | n.d. | 0.37 | >10 | >10 |
| **16** | 0.55 | >9.1 | >10 | 8.1 | 1.0 | >10 | >10 |
| **17** | 0.044 | 8.6 | 3.0 | 0.63 | 0.44 | >10 | >10 |
| **18** | 0.039 | >9.1 | >10 | 7.4 | 1.0 | >10 | >10 |
| **19** | 0.16 | 8.9 | n.d. | n.d. | 8.2 | >10 | >10 |
| **20** | 0.026 | 5.5 | 2.9 | 0.81 | 0.18 | >10 | 4.2 |

n.d. not determined.

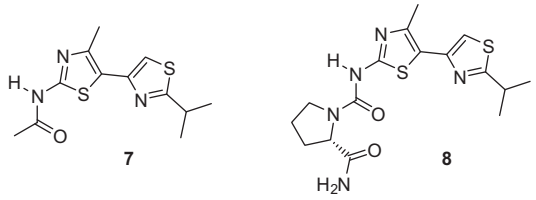



**Figure 3.** Structure of the 4′,5-bisthiazole PI3K inhibitors **7** and **8**.

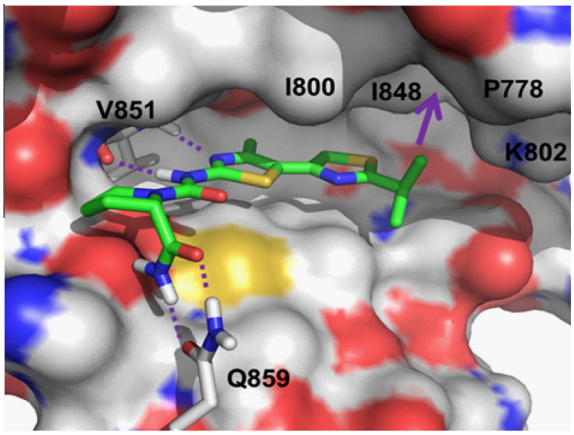
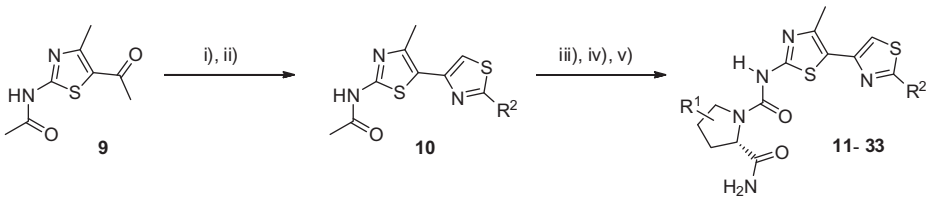



**Figure 4.** Model of compound **8** bound into the ATP pocket of PI3Kα. Key hydrogen bonds are represented by dashed lines. The purple arrow indicates the region of the affinity pocket formed by the side chains of residues I800, I848, P778 and K802 which is incompletely filled by the 2-isopropyl group.

inhibitor.[18] The dimethylamino analogue **16**, isosteric with the isopropyl starting point **8**, has been shown to be 10-fold less active, and this result focused the attention on *C*-substituted analogues. Aryl 2-substituents have also been found to be well tolerated, as exemplified by **17** and **18**, leading to activities within 2-fold of the most active quaternary alkyl substituted examples. The dimethylaminomethyl analogue **19** led to a much greater 50-fold shift between biochemical and cellular activity, and was representative of our findings when a basic group, of sufficient p*K*a to be extensively protonated at physiological pH, was present within the 2-substituent. Introduction of fluorine into the most active example **15** was well tolerated, as demonstrated by the trifluoromethylcyclopropyl analogue **20** retaining an equivalent level of potency and selectivity.

Evaluating the biophysical profiles of the analogues from Table 1 with the most interesting levels of PI3Kα activity: all showed high levels of permeability,[19] but with limited solubility (<130 μM).[20] In particular the aryl-substituted analogues, **17** and **18**, were found to be very poorly soluble (<4 μM). Unsurprisingly, the tertiary amine containing **19** was shown to possess a much higher solubility, but as discussed above, this was accompanied by an unacceptably low level of cellular activity, and also a reduced level of permeability. In the case of the *tert*-butyl analogue **14**, an in vivo PK study in the rat was representative of our findings for the series of compounds exemplified within Table 1. When orally dosed as a suspension (3.0 mg $kg^{-1}$), a bioavailability of only 2% was determined for compound **14**.[21] Interpreting these data, a metabolic first-pass effect was anticipated to contribute to some extent to the measured low oral-bioavailability. This was based upon the rat in vivo clearance approximating to 75% of liver blood flow, which for **14** was consistent with the rat in vitro microsomal clearance.[22] High microsomal clearance was also observed for the most interesting examples in Table 1. Only the poorly soluble 2-aryl analogue **18** and weakly active 2-dimethylaminomethyl **19** exhibited modest stability in rat liver microsomes. However, the limited aqueous solubility was also considered to be a major contributor to the low oral bioavailability, at what was considered to




**Scheme 2.** Synthesis of the 4′,5-bisthiazole analogues **11**–**33**. Reagents and conditions: (i) $Br_2$, 1,4-dioxane, 50 °C, 18 h (62–75%); (ii) $R_2C(S)NH_2$, 0.1 equiv $(NH_4)_3PMo_{12}O_{40}$, EtOH, reflux, 48 h (16–76%); (iii) conc. HCl, EtOH, reflux, 3 h (83–95%); (iv) CDI, DCM, 25 °C, 6 h (47–93%); (v) proline-amide derivative, DMF, 25 °C, 18 h (22–90%).

**Table 2**
Physicochemical and in vitro PK data for the reference compounds and selected 4′-thiazole 2-substituted analogues

| Compound | *c*log*P*/PSA (Å$^2$) | Sol. pH 6.8 (μM) | HDM FA (%) | Caco-2 A-B/B-A ($10^{-6}$ cms$^{-1}$) | Rat microsome Cl (μl min$^{-1}$ mg$^{-1}$) | HSA/RSA (%) |
|---|---|---|---|---|---|---|
| **2** | 0.8/122 | 435 | 28 | 1.4/7.7 | 19 | n.d. |
| Alpelisib | 2.1/101 | 53 | 89 | 3.8/18.3 | 29 | 90.0/92.7 |
| **8** | 2.1/101 | 285 | 82 | n.d. | 132 | 84.4/n.d. |
| **12** | 2.2/101 | 113 | 81 | n.d. | 136 | 90.8/92.8 |
| **13** | 3.2/101 | 124 | 96 | n.d. | 283 | 92.2/94.0. |
| **14** (A66) | 2.5/101 | 90 | 96 | 12.3/15.6 | 126 | n.d. |
| **15** | 2.2/101 | 27 | 95 | n.d. | 68 | 92.2/93.5 |
| **18** | 3.0/101 | <4 | 93 | n.d. | 18 | n.d. |
| **19** | 0.5/104 | 977 | 39 | n.d. | 30 | n.d. |
| **21** | 3.1/101 | 5 | 99 | 14.5/17.4 | 140 | n.d. |
| **23** | 3.1/101 | 151 | 99 | 11.1/17.4 | 89 | n.d. |
| **24** | 2.3/101 | 29 | 92 | n.d. | 62 | 93.9/93.5 |
| **25** | 1.4/121 | 141 | 27 | 0.9/12.4 | 67 | n.d. |
| **26** | 0.7/121 | 711 | 29 | n.d. | n.d | n.d |
| **27** | 2.4/101 | 304 | 91 | 5.5/20.7 | 70 | 92.2/n.d. |
| **28** | 1.5/121 | 10 | 25 | 0.7/8.4 | 51 | n.d. |
| **29** | 1.5/121 | 258 | 46 | 1.2/20.8 | 70 | n.d. |
| **30** | 2.4/104 | >1000 | 48 | n.d. | 139 | n.d. |
| **31** | 2.1/104 | >1000 | 41 | n.d. | 52 | n.d. |
| **32** | 2.4/104 | 973 | 31 | n.d. | 91 | n.d. |
| **33** | 2.3/101 | 167 | 91 | n.d. | 375 | 90.2/91.8 |

*c*log*P* was calculated using *c*log*P* version 7.4, BioByte Corporation.

be a sub-efficacious exposure in the rat PK studies. As a result, approaches were sought to increase the solubility and metabolic stability of the most interesting analogues from Table 1, as a means to increase oral exposure, whilst retaining, or improving, their favourable PI3Kα potency and selectivity profiles.

One area which was explored, as a means to improve upon the profiles of the analogues **11** to **20**, was the introduction of substituents into the proline moiety. Previous work had shown the primary amide to be essential for PI3Kα activity and the corresponding 4-membered azetidine analogues to be less active.[6] The larger 6-membered analogues were found to be chemically unstable, and unsuitable for pharmacological evaluation.[23] Therefore, the effort was focused on modifications of the pyrrolidine nucleus, and Table 3 shows the biochemical and cellular data for the analogues **21** to **33** bearing substituents in the proline 2-, 3- and 4-positions. Substituting the 5-position was not explored because molecular modelling indicated insufficient space to be available to accommodate a substituent on either face of the pyrrolidine ring when docked within the ATP pocket of PI3Kα. The SAR for the series is exemplified in Table 3 using the three most promising 2-thiazole substituents ($R^2$): *tert*-butyl, methylcyclopropyl and trifluoromethylcyclopropyl. The substituted proline-amide building-blocks used to prepare **21** to **33** were readily synthesised from commercially-available proline-analogues, or as outlined below, and introduced using the synthetic sequence in Scheme 2.[13] The non-commercial intermediates were: the 2-methylproline analogue, which was prepared by the alkylation of proline using the self-reproduction of chirality concept;[24] and the *cis*-3-methylproline analogue, which was synthesised using an amino–zinc-enolate-cyclisation.[25]

The 2-methyl analogues, **21** and **22**, both retained comparable PI3Kα activity and selectivity compared to the parent compounds **14** and **20**. Similarly, a *cis* or *trans*, methyl or hydroxyl group in the 3-position, entries **23** to **26**, were also shown to be well tolerated. Of these analogues, the *cis*-3-methyl derivative **24** provided a 4-fold increase in the cellular potency, whilst maintaining a high level of selectivity for the other PI3K isoforms, and proved to be the most cellular active example prepared within the series. The *trans*-3-hydroxy analogue **25** also provided a similar favourable PI3Kα activity and isoform selectivity profile, comparable to the parent compound **14**. Fluoro and hydroxyl substituents in the 4-position were also well tolerated, entries **27** to **29**. Interestingly, the *cis*-4-hydroxy analogue **29** has been shown to lead to a decrease in the relative PI3Kβ cellular selectivity, whilst maintaining a similar level of selectivity versus the other isoforms. Introduction of a dimethylamino group into the 4-position on either face of the proline ring was tolerated to a greater extent than the introduction of a basic group in the 4′-thiazole 2-position, but still resulted in a 5-fold loss of cellular PI3Kα activity for the more potent *trans* analogue **30**. The 2,3-cyclopropyl analogue **33** showed no PI3K activity advantage over the corresponding compounds mono-methylated at the proline 2- and 3-positions.

Evaluating the biophysical profiles of the analogues from Table 3: of the methylated prolines, only the *trans*-3-methyl analogue **23** potentially led to some degree of increased solubility (150 μM), compared to the unsubstituted parent compound **14**. However, the methylated analogues showed no improvement in microsomal clearance, as might be anticipated from their increased lipophilicity. This increased metabolic instability was evident in vivo in a mouse pharmacokinetic study with **21**, where plasma

concentrations were found to fall rapidly, to below 50 nM, within 2 h of dosing (bolus intravenous administration, 10 mg kg$^{-1}$).[21] The hydroxylated analogues also led to modest increases in solubility (up to 710 μM), with slightly improved levels of in vitro metabolic stability compared to the unsubstituted parent compounds. However, introducing the secondary hydroxyl group resulted in a reduced level of permeability, which was likely driven primarily by the increased polarity (polar surface area of 121 Å$^2$).[19] Additionally, data for the hydroxylated analogues in Caco-2 monolayers showed a 10-fold higher basolateral to apical flux, supporting the low passive permeability from the hexadecane membrane (HDM) assay, and indicating a significant level of efflux to be operating. This permeability profile was only evident to a marginal extent in the cellular assays, as assessed by the biochemical to cellular potency shifts for **25**, **26**, **28** and **29**, but was anticipated to be a further limitation to achieving an acceptable level of oral bioavailability. The dimethylamino analogues, **30**, **31** and **32**, as anticipated, led to a higher level of solubility (>1 mM), and a rat PK with compound **31** showed that an improvement in oral exposure was possible for the series. Oral dosing of a suspension of **31** (3.0 mg kg$^{-1}$) resulted in 59% bioavailability, in addition to an improved terminal half-life of 1.9 h.[21] The increased half-life being a result of a lower clearance (27 ml min$^{-1}$ kg$^{-1}$), consistent with the microsomal data,[22] and a larger volume of distribution (3.3 L kg$^{-1}$) compared to other non-basic members of the series.

To further characterise the potential of the series, the most potent analogue **24** was tested against an internal panel of 35 kinase biochemical assays, including the lipid kinases phosphatidylinositol-4-kinase beta (PI4Kβ) Vps34 and mTor. No significant inhibition at concentrations up to 10 μM were measured in these assays, with the exception of PI4Kβ, where an $IC_{50}$ of 0.25 μM was determined. Indicating a similar level of selectivity over PI4Kβ as compared to the selectivity over the other class 1 PI3K's, which was found to be a consistent finding across the 4′,5-bithiazole series. In addition, compound **24** was also tested against a panel of 28 receptor, ion channel, transporter and enzyme assays. No significant inhibition was observed at concentrations up to 10 μM in these screens, supporting a favourable level of specificity for the series. Of particular interest, and in contrast to a number of reported class 1 PI3K inhibitors, the absence of mTor activity determined for **24** was also reproduced for other 4′,5-bisthiazole analogues tested from Tables 1 and 3, and appeared to be characteristic of the series.

To better understand the origin of the above activity changes the analogues **11**–**33** were modelled in the ATP pocket of PI3Kα derived from the X-ray structure solved with alpelisib in which the urea-*H* and adjacent thiazole-*N* make a bidentate *H*-bonding interaction with V851 in the hinge region.[12] From these insights the higher potency of **24** can be rationalised through favourable contributions from both the *cis*-3-methylproline-amide and

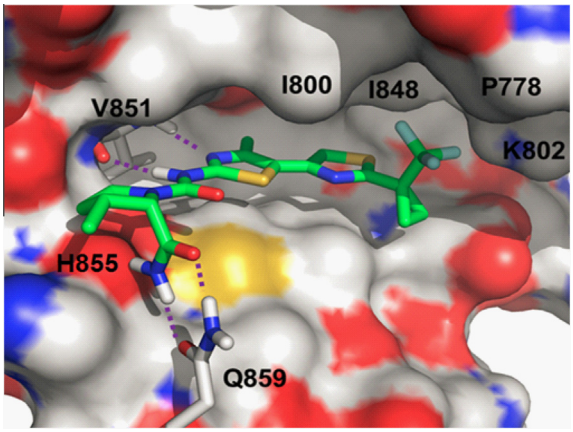
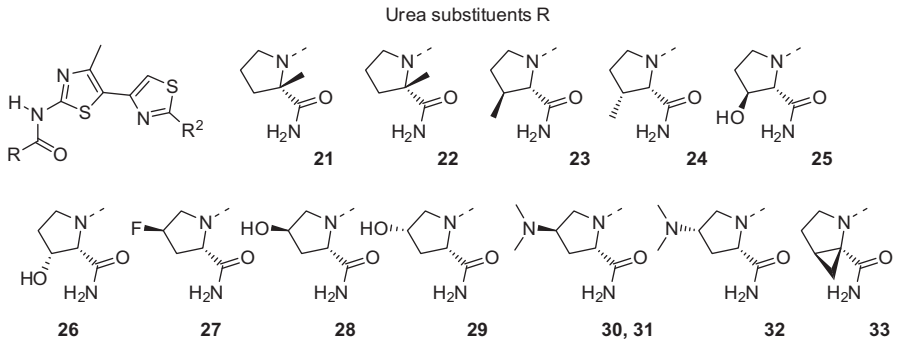



**Figure 5.** Proposed binding mode of compound **24** in the ATP pocket of PI3Kα. Key hydrogen bonds are indicated as dashed lines.

**Table 3**
PI3Kα, PI3Kβ, PI3Kγ and PI3Kδ activities for the substituted proline-amide derivatives **21**–**33**




| Compound | $R^2$ | Biochemical $IC_{50}$ (μM) | | | | Cellular $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|---|---|---|
| | | PI3Kα | PI3Kβ | PI3Kγ | PI3Kδ | PI3Kα | PI3Kβ | PI3Kδ |
| **21** | *tert*-Bu | 0.030 | 5.0 | n.d. | n.d. | 0.20 | 5.6 | >10 |
| **22** | $CF_3$cPr | 0.10 | 2.1 | n.d. | n.d. | 0.40 | >10 | >10 |
| **23** | *tert*-Bu | 0.022 | 8.1 | n.d. | n.d. | 0.14 | 8.1 | 3.4 |
| **24** | $CF_3$cPr | 0.014 | 4.6 | 2.5 | 1.5 | 0.047 | >10 | 3.9 |
| **25** | *tert*-Bu | 0.024 | 7.1 | >10 | >10 | 0.18 | 6.2 | >10 |
| **26** | $CF_3$cPr | 0.043 | >9.1 | 5.0 | 3.9 | 0.32 | >10 | 9.9 |
| **27** | *tert*-Bu | 0.039 | 8.0 | 8.3 | 3.8 | 0.45 | 5.8 | 3.6 |
| **28** | *tert*-Bu | 0.071 | >9.1 | 7.9 | 4.0 | 0.12 | 4.5 | 2.5 |
| **29** | *tert*-Bu | 0.084 | >9.1 | >10 | 7.9 | 0.15 | 1.3 | 5.2 |
| **30** | *tert*-Bu | 0.17 | >9.1 | >10 | 9.2 | 0.98 | >10 | >10 |
| **31** | MecPr | 0.52 | >9.1 | >10 | >10 | 3.0 | >10 | >10 |
| **32** | *tert*-Bu | 1.2 | >9.1 | n.d. | n.d. | 5.5 | >10 | >10 |
| **33** | *tert*-Bu | 0.18 | >9.1 | >10 | 3.3 | 0.54 | >10 | >10 |

trifluoromethylcyclopropyl moieties, as highlighted in Figure 5. More specifically, the proline *cis*-3-methyl substituent is assumed to make van der Waals contact with the side chain of H855, and the trifluoromethylcyclopropyl group makes further van der Waals contacts with the side chains of residues I800, I848 and P778. A hydrogen bond between one of the fluorine atoms and the side chain amino group of K802 is also hypothesised.

In conclusion, exploring the affinity pocket SAR of an (*S*)-proline-amide aminothiazole-urea series of selective PI3Kα inhibitors led to the identification a 4′,5-bisthiazole sub-series. The most active examples from this sub-series contained a quaternary alkyl 2-substituent in the 4′-thiazole affinity-pocket moiety with a *cis*-3-methyl substituent in the proline ring, and achieved comparable levels of activity and PI3K-isoform selectivity to the previous best (*S*)-proline-amide aminothiazole-urea examples. However, further profiling revealed minimal levels of oral exposure for the most interesting 4′,5-bisthazole examples, and low aqueous solubility was assigned as a primary reason for this limitation. Attempts to address this pharmacokinetic deficiency identified no examples with the required combination of potency, solubility, permeability and metabolic stability to move forward into more extensive pre-clinical profiling. As a result, our attention turned to alternative ways to improve the series, and identify analogues with the targeted profiles.[26]

## Acknowledgments

The authors would like to thank Francesca Blasco, Peter Wipfli and Christian Schnell for conducting pharmacokinetic studies, and Aurore Roustan, Romain Gambert, Peter Loewenberg and Esther Roehn-Carnemolla for their excellent technical assistance.

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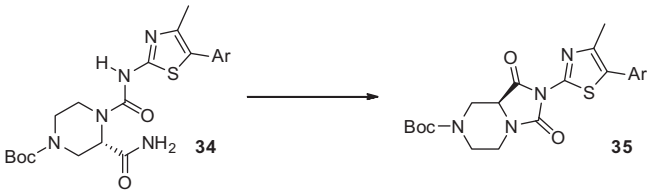
22. In vitro metabolic stability was measured, using the compound depletion approach, in rat liver microsomes from pooled rat hepatic microsome preparations, microsomes (protein concentration 0.5 mg/mL) were incubated at 37 °C with the test compounds (1 μM) and the co-factor NADPH. Aliquots were removed after incubation times of 0, 5, 15 and 30 min. Reactions were stopped by addition of acetonitrile and samples were subsequently analysed by LC–MS/MS after protein precipitation. The data were analysed as percentage disappearance of parent relative to the zero time sample, from which hepatic extraction ratios were determined.
23. As a representative example of the stability of the 6-membered ring containing analogues: a product consistent with the targeted piperazine analogue **34** could be detected in the final urea-forming step, following Scheme 2. However, attempts to isolate **34** in a pure form at room temperature were not possible, due to the formation a major byproduct over time. The isolated byproduct was consistent with the further reaction of **34** to form the internally cyclised hydantoin **35**, as shown below.

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