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Identification and optimisation of a series of *N*-(4-anilino-2-pyridyl)acetamide activin receptor-like kinase 1 (ALK1) inhibitors†‡

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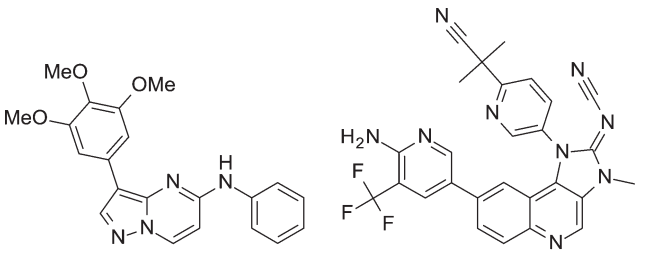
A novel class of *N*-(4-anilino-2-pyridyl)amide based activin receptor-like kinase (ALK1) inhibitors are disclosed, which were rapidly optimised to a ligand efficient probe compound **21** with good potency in enzyme (4 nM) and cell (45 nM) assays and favourable physical and pharmacokinetic properties (24 h free cover over cell IC_{50} after a single 50 mg kg^{-1} dose in nude mice). This was achieved by identifying a small, ligand efficient group in the solvent channel (C2) whilst optimising the selectivity pocket (C4) group for enzyme and cell potency, using related SAR that has been observed previously for Src inhibitors.

Introduction

Activin receptor-like kinase (ALK1, also referred to as ACVRL1) is a serine/threonine kinase of interest for oncology, due to its reported linkage to angiogenesis *in vivo*.[1,2] It is structurally related to the TGF-β type I receptor (also known as activin receptor-like kinase 5, ALK5) for which numerous small molecule inhibitors have been published. By contrast, only one series of small molecule inhibitors of ALK1 have been disclosed[3] (Fig. 1), although the mTOR inhibitor Panulisib (P-7170, Piramal, phase I) has been reported to have ALK1 activity,[4–6] monoclonal antibodies have also been described (PF-3446962, Pfizer)[7] and Acceleron are in phase II with Dalantercept (ACE-041),[8] a fusion protein comprising a portion of the human ALK1 protein and IgG1 Fc region. Furthermore, a series of ALK2 inhibitors has recently been reported, some of which are also reported to have ALK1 activity,[9] which is not surprising given the close structural homology between ALK1 and ALK2.

In a previous publication[10] we reported the discovery of a series of ALK5 (TGFβR1) inhibitors. This series did not have significant ALK1 activity however (compounds were typically ~100× selective for ALK5 *vs.* ALK1) so a new series was required to develop probe compounds for ALK1 inhibition. To identify hits a small subset (1000 compounds) of the AstraZeneca collection was tested for ALK1 inhibition in a fluorescence polarization (FP) enzyme assay, based on selecting compounds we knew to be structurally related to inhibitors of ALK5. This work identified compound **3** (Fig. 2), which was potent (48 nM) in the enzyme assay. Although we later demonstrated that this compound only had modest activity in an ALK1 cell assay (3.6 μM, measuring translocation of pSMAD1/5 in HMEC-1 cell line), we were attracted to this compound as a start point due to its excellent ligand efficiency[11] (LE) of 0.48. Interestingly, the C4 group, which was assumed to bind into the selectivity pocket[12] of the ATP binding site, is the same selectivity pocket group as in Src inhibitor Saracatinib **4** (AZD0530),[13] although the 2-amidopyridine hinge binder and acetamide solvent channel group differ significantly. Although the related 2,4-bisanilino-pyrimidine hinge binder is well known in the kinase field,[14–16] 2-amido-4-anilinopyridine hinge binders were unknown to us at the time, although since this work was performed two examples with similar hinge binders have been reported within a quinoline-based series of ALK5 inhibitors.[17]



1 Panulisib **2**

Fig. 1 An example from a Bayer-Schering ALK1 patent **1** and Panulisib **2**.

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† The authors declare no competing interests.

‡ Electronic supplementary information (ESI) available: Additional experimental protocols are given in the supporting information. See DOI: 10.1039/c6md00039h

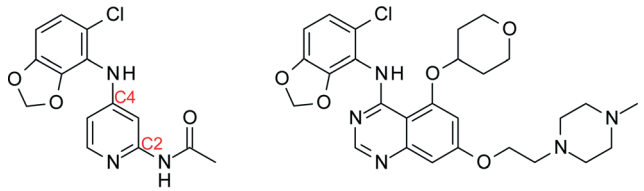


Fig. 2 Structures of initial ALK1 hit compound **3** and Saracatinib **4**.

Chemistry

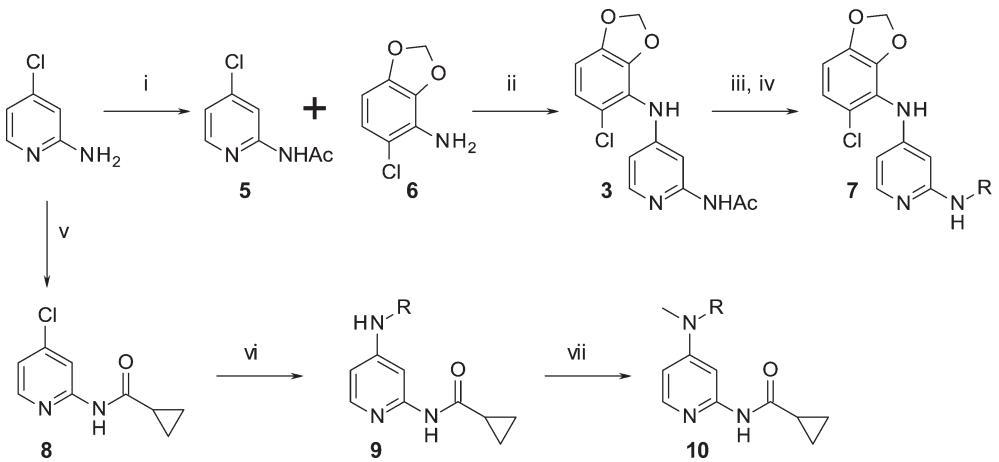
The initial hit **3** and variations of the solvent channel aniline were accessed *via* Buchwald–Hartwig amination of 4-chloropyridine **5** with 5-chloro-1,3-benzodioxol-4-amine **6**. The amide group could then be varied by removal of the acetyl group and HATU-mediated amide coupling with the relevant carboxylic acid to form **7** (Scheme 1). In order to assess the SAR of the C4 group, the order of the steps could be reversed to put the C4 group in last. Thus, a Buchwald–Hartwig amination was performed on 4-chloropyridine **8** with a range of arylamines to give **9**, which generally worked well although a very low yield was obtained for addition of 4-aminopyridine (for compound **20**). The C4 aniline could then be selectively methylated on the C4 nitrogen if desired with Cs_2CO_3/MeI to give **10**.

Results and discussion

Compound **3** was optimised for cell potency whilst keeping molecular weight low and maintaining $\log D_{7.4} < 4$. We felt that this strategy would give us the best chance of identifying a probe compound that would be suitable for studies to establish the effects on an ALK1 inhibitor on angiogenesis *in vivo*. There was no advantage for increasing the size to ethyl (**11**, Table 1) or iso-propyl (**12**), but cyclopropyl **13** led to increased enzyme potency. Encouragingly, cell potency also improved (albeit with a drop-off from enzyme to cell) so compound **13** had good cell potency (0.24 μM). Increasing the size of the cycloalkyl group further to cyclobutyl or cyclopentyl led to decreased potency, as did changing the group to phenyl. The physical properties of compound **13** (measured $\log D_{7.4}$ = 3.3, aq. solubility = 46 μM) were acceptable, so **13** was selected for further optimisation.

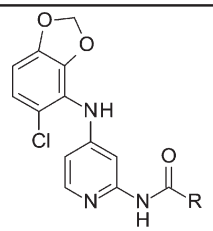
Switching focus onto the C4 group we started by investigating whether the chlorine and dioxole groups were both required for potency (Table 2) with simple C4 substituents to identify a more efficient compound. We found that removing either the dioxole (**17**) or the chlorine (**18**), or both (**19**), significantly reduced potency. However, we also observed that a 4-pyridyl group **20** was more potent (at lower clog *P*) than the corresponding phenyl **19**. Thus we investigated adding in the 4-pyridyl nitrogen to the 5-chloro-1,3-benzodioxol-4-amine group in **13** to give **21**. Compound **21** has excellent potency in both biochemical (4 nM) and cell (45 nM) assays with moderate lipophilicity ($\log D_{7.4}$ = 2.7), and therefore an improved LLE (ligand lipophilicity efficiency, defined as enzyme $pIC_{50} - \log D_{7.4}$) of 5.7 *vs.* 4.5 for **13**. Methylating the NH group as in **22** was tolerated but decreased potency, so was not explored further. Achieving such high levels of potency with low MW (332) and moderate lipophilicity gave us confidence that this was an efficient scaffold for further optimisation, and that compound **21** should be profiled further as a potential *in vitro* and *in vivo* probe compound for the effects of ALK1 inhibition.

Unfortunately we did not have access to an ALK1 crystal structure, so in order to confirm the predicted binding mode of **21** we obtained a crystal structure bound to ALK5. As expected the amidopyridine picks up the familiar donor–acceptor pair interaction to the hinge residues Tyr-282 (3.0 Å) and His-283 (2.8 Å), which places the cyclopropyl group into the solvent-exposed channel (Fig. 3a). The chlorodioxolopyridine orientates itself into the hydrophobic "selectivity pocket" where it makes a water-mediated hydrogen bond to the protein through residues Tyr-249 and Glu-245



Scheme 1 Reagents and conditions: (i) AcCl, pyridine, 83%; (ii) $Pd(OAc)_2$, xantphos, Cs_2CO_3, DMA, chlorobenzo[*d*][1,3]dioxol-4-amine, 83% (iii) NaOH, $EtOH/H_2O$, 100%; (iv) HATU, RCO_2H, $^iPr_2NEt$, DMA, 31–82%; (v) ^cPrCOCl, pyridine, 53%; (vi) $Pd(OAc)_2$, xantphos, Cs_2CO_3, DMA, RNH_2, 3–51%; (vii) MeI, Cs_2CO_3, DMF, 61%.

Table 1 Optimisation of the amide group



Ex.	R	ALK1 enz IC_{50}[a] (μM)	ALK1 cell IC_{50}[a] (μM)	LE[b]	clog *P*	log *D*[c]
3	Methyl	0.048	3.6	0.48	3.7	2.7
11	Ethyl	0.040	0.98	0.46	4.2	
12	Iso-propyl	0.80		0.36	4.5	
13	Cyclopropyl	0.017	0.24	0.46	4.2	3.3
14	Cyclobutyl	0.23		0.38	4.6	
15	Cyclopentyl	0.94		0.33	5.1	
16	Phenyl	0.34		0.34	5.2	

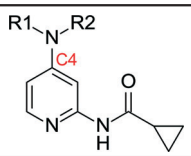
[a] Geometric mean of at least 2 independent measurements. [b] Ligand efficiency, defined in this article as LE = (enzyme pIC_{50}/heavy atom count) × 1.37. [c] Measured log *D* at pH 7.4.

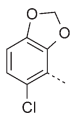
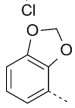
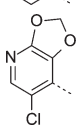
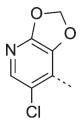
(Fig. 3b), similar water-mediated interactions as described in our previous publication with the ALK5 protein.[10]

The general kinase selectivity of **21** was assessed by submission to a panel of 55 representative kinases[18] at Dundee University at 1 μM (see ESI‡ for complete dataset). At that concentration none showed inhibition >80%, and only two kinases showed >50% inhibition (EPHA2 and YES1). In subsequent testing we also found that **21** was selective *vs.* Src (4.4 μM in a HTRF enzyme assay, and inactive at 1 μM in the Dundee university panel) despite the structural similarity to **4**. However, in addition to ALK1 it also inhibited ALK5 (0.12 μM in an ALK5 cell assay, measuring translocation of GFP-smad2 in a recombinant MDA-MB-468 cell line). To assess ALK family selectivity more generally, compound **21** was submitted to a panel of FRET enzyme assays at Thermofisher, where it showed similarly high levels of potency against ALK1 (1.1 nM, n = 3), ALK2 (1.2 nM, n = 2), ALK3 (2.4 nM, n = 2) and ALK5 (1.9 nM, n = 3), but had some degree of selectivity against ALK4 (31 nM, n = 2) and ALK6 (1.3 μM, n = 2).

The *in vivo* PK data from Han Wistar rats (AUC = 3.8 μM h at 5 μmol kg^{-1}, CL = 8.4 ml min^{-1} kg^{-1}, F = 38%) were promising so **21** was assessed in mouse PK. In nude mice, dosing orally at 50 mg kg^{-1}, **21** provided >10× free cover over the cell IC_{50} 24 h after a single dose (free C_{max} = 31.7 μM, free concentration after 24 h = 0.37 μM, cell IC_{50} = 0.045 μM, protein

Table 2 Optimisation of the C4 group (selectivity pocket group)



Ex.	R1	R2	ALK1 enz. IC_{50}[a] (μM)	ALK1 cell IC_{50}[a] (μM)	LLE[b]	clog *P*	log *D*[c]	Solubility[d] (μM)
13		H	0.017	0.24	4.5	4.2	3.3	46
17		H	4.6		1.6	3.5	3.1	49
18		H	0.12 (n = 1)	2.3	3.8	4.4	3.7	
19		H	2.3 (n = 1)		2.3	3.5	3.3	
20		H	1.0	3.8		2.4		
21		H	0.004	0.045	5.7	3.9	2.7	63
22		Me	0.026	0.32		3.7		

[a] Geometric mean of at least 2 independent measurements, unless otherwise stated. [b] Ligand lipophilicity efficiency (LLE), defined in this article as LLE = enzyme pIC_{50} − log $D_{7.4}$. [c] Measured log *D* at pH 7.4. [d] Solubility performed under thermodynamic conditions from a solid sample.

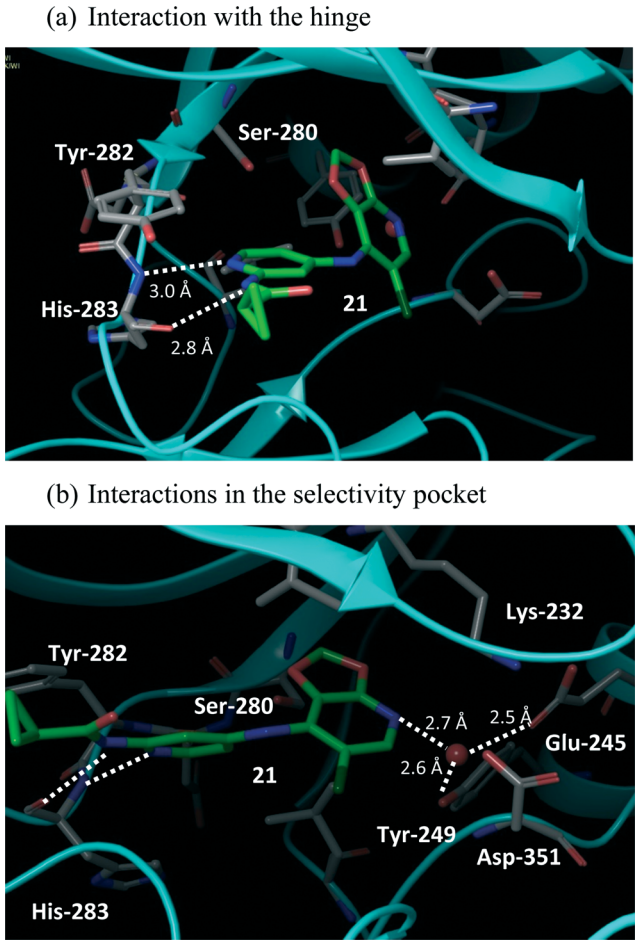


Fig. 3 Crystal structure of **21** bound to ALK5, pdb code 5FRI, showing (a) the hinge interactions and (b) the water-mediated hydrogen bond in the selectivity pocket. The ATP binding sites of ALK1 and ALK5 are similar, although one notable difference is that the gatekeeper residue (Ser280 in ALK5) is Thr in ALK1.

binding in murine plasma = 27% free) so **21** is a suitable probe compound to study the effects of ALK1 inhibition in both *in vitro* and *in vivo* model systems.

Experimental section

General

All solvents and chemicals used were reagent grade. Flash column chromatography was carried out using prepacked silica cartridges from Redisep, Biotage, or Crawford and eluted using an Isco Companion system. Purity and characterization of compounds were established by a combination of liquid chromatography-mass spectroscopy (LC-MS) and NMR analytical techniques and was >95% for all compounds. Chemical shifts are reported in ppm relative to tetramethylsilane (TMS) (0 ppm) or solvent peaks as the internal reference, *J* values are given in Hz. Merck precoated thin layer chromatography (TLC) plates (silica gel 60 F254, 0.25 mm, art. 5715) were used for TLC analysis. Preparative HPLC was performed on C18 reversed-phase silica on a Waters or Phenomenex column using decreasingly polar mixtures of water (containing 1% formic acid or 1% aq. NH_4OH) and MeCN. All reactions were performed under nitrogen unless otherwise stated. All *in vivo* experiments were performed in compliance with the relevant laws and institutional guidelines.

Synthesis of compound 21 (see ESI‡ for other compounds)

***N*-(4-Chloropyridin-2-yl)cyclopropanecarboxamide 8.** To a solution of 4-chloropyridin-2-amine (25 g, 194.5 mmol) in pyridine (200 mL) at 0 °C, cyclopropanecarbonyl chloride (20.33 g, 194.5 mmol) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C then was allowed to warm to rt. The reaction mixture was stirred for 1 h at rt before being concentrated *in vacuo*. The resulting residue was dissolved in DCM (50 mL), washed with water and dried over $MgSO_4$. The resulting crude product was triturated with 9 : 1 iso-hexane/diethyl ether to afford the title compound **8** (20.41 g, 53%) as a cream solid; ^{1}H NMR (300 MHz, DMSO) 0.78–0.90 (4H, m), 1.97–2.07 (1H, m), 7.20 (1H, dd), 8.16 (1H, s), 8.31 (1H, d), 11.07 (1H, s); *m*/*z* MH^+ 197.

***N*-[4-[(6-Chloro-[1,3]dioxolo[4,5-*b*]pyridin-7-yl)amino]-2-pyridyl]cyclopropanecarboxamide 21.** To a stirred solution of *N*-(4-chloropyridin-2-yl)cyclopropanecarboxamide **8** (15 g, 76.3 mmol) in 1,4-dioxane (200 mL) was added 6-chloro-[1,3]dioxolo[4,5-*b*]pyridin-7-amine (13.16 g, 76.3 mmol), cesium carbonate (62.1 g, 190.7 mmol), (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine) (4.86 g, 8.39 mmol) and diacetoxypalladium (1.37 g, 6.10 mmol). The reaction mixture was degassed with N_2 and heated at reflux for 4 h, then was allowed to cool to rt, diluted with DCM (200 mL) and filtered. The filtrate was concentrated and the residue was purified by flash silica chromatography, eluting with 2% MeOH in DCM. The resulting residue was recrystallised from DCM and diethyl ether, washed with diethyl ether and dried *in vacuo* to afford the title compound **21** (15.00 g, 59%) as a white powder, m.p. 206–207 °C; ^{1}H NMR (400 MHz, DMSO) 0.76–0.79 (4H, m), 1.97–2.01 (1H, m), 6.15 (2H, s), 6.57–6.59 (1H, m), 7.62 (1H, d, *J* 2.3), 7.75 (1H, s), 7.99 (1H, d, *J* 5.7), 8.99 (1H, s), 10.59 (1H, s); ^{13}C NMR (175 MHz, DMSO) 8.5 (2C), 16.0, 101.1, 102.1, 108.8, 117.8, 126.5, 129.6, 138.8, 148.1, 148.7, 152.6, 158.5, 172.6; HRMS (ESI) calc. for $C_{15}H_{14}N_4O_3Cl$ (MH^+) 333.0754, found 333.0744.

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