Design, Synthesis and Optimization of 7-substituted- pyrazolo[4,3-b]pyridine ALK5 (activin receptor-like kinase 5) inhibitors

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ABSTRACT: A series of potent ALK5 inhibitors were designed using a SBDD approach and subsequently optimized to improve drug likeness. Starting with a 4-substituted quinoline screening hit SAR was conducted using a ALK5 binding model to understand the binding site and optimize activity. The resulting inhibitors displayed excellent potency but were limited by high in vitro clearance in rat and human microsomes. Using a scaffold morphing strategy, these analogs were transformed into a related pyrazolo[4,3-b]pyridine series with improved ADME properties.

ALK5 (activin-like kinase 5, also known as TGF- β RI / transforming growth factor β receptor I) is a receptor serine-threonine kinase that has a role in the regulation of a variety of physiological processes.^{1a} TGF- β ligands initially bind TGF- β RII which recruits and phosphorylates ALK5 (TGF- β RI). The activated ALK5 then phosphorylates SMAD2/SMAD3 proteins which dissociate from the receptor and form a heteromeric complex with SMAD4. This complex eventually translocates to the nucleus and interacts with DNA cofactors to regulate gene expression controlling cell growth, differentiation and development. Thus, modulation of TGF- β signaling via small-molecule ALK5 inhibitors has been considered a potential treatment of fibrotic disease^{1j,k} and cancer. The role of TGF- β in cancer biology is complex with both promoter and suppressor functions depending on cancer stage. During late stages of carcinogenesis tumor cells utilize the TG signaling system to promote metastatic spread and overcome immune surveillance.^{1b,c} This later role of TGF- β makes it a particularly attractive target to combat the most invasive and metastatic cancers particularly in brain and pancreas.

Modulating ALK5 with small molecule inhibitors has received pharmaceutical industry attention, however to date only two small molecules LY2157299 and EW-7197 have entered clinical trials.^{1b,d,2} In this paper, we describe our efforts to identify potent and selective small molecule inhibitors of ALK5 and their subsequent optimization to yield an orally bio-available candidate which advanced into toxicological testing.

Figure1. Structures of ALK5 inhibitor LY2157299 (1) and screening hit based lead (2)

Screening of our internal compound collection followed by deconstruction afforded the early lead molecule **2** which displayed reasonable potency in the ALK5 enzymatic assay (ALK5 pIC_{50} = 6.2)³. Compound **2** was docked into the ATP binding site of the ALK5 protein using coordinates disclosed in the literature⁴ (see Fig 2). Compound **2** was predicted to adopt a binding pose that positions the quinoline ring nitrogen to make an H-bond with the backbone NH of His₂₈₃ at the kinase's hinge region. In this pose, a potential pseudo hydrogen bond is also formed

between the C-H at position C2 of the quinoline ring and the carbonyl oxygen of Asp₂₈₁. The pyridyl ring of compound **2** is positioned to hydrogen bond with the side chain amino group of Lys₂₃₂ as well as with a conserved water molecule that is coordinated by Asp₃₅₁, Glu₂₄₅ and Tyr₂₄₉. Finally, the phenyl ring (which is attached at the C2' position of pyridine ring) is oriented towards the back hydrophobic pocket.

Figure 2. Molecular model of early quinoline screening lead (**2**) docked into the ALK5 nucleotide binding site.

Using the hypothesized binding insights, initial optimization efforts on this series were targeted at improving enzymatic potency and obtaining a better understanding of the importance of the various hydrophobic pocket groups (described in the ALK5 literature⁵) (Table 1). Additionally we also attempted to optimally fill a small hydrophobic pocket (observed in the docked model of lead molecule **2** in ALK5) located directly below the C5 position of the pyridyl ring of compound **2** (see Fig 2).

Synthetic methodology used to access the elaborated quinolines (**2-11**) is described in Scheme 1.⁶

Scheme 1

Scheme 1. Reagents and conditions: (a) $\text{Pd}_2(\text{PPh}_3)_4$, K_2CO_3 aq, 1,4-dioxane, 120 °C 0.5h MW; (b) $\text{Pd}_2(\text{dba})_3$, NaOtBu , XANTPHOS, 1,4-dioxane, 100 °C 1h MW

Substitution at the meta position on the phenyl ring in **2** with a Cl atom gave a modest increase in potency (**3**). Installation of a CH_3 group at R^1 further improved potency by filling a small lipophilic groove in the ALK5 model. Installation of a 5-chloro-2-fluorophenyl at R of the core further improved potency (compound **5**). Modeling suggested the incorporation of a F atom (compound **5**) helped to improve the orientation of the aromatic ring in the hydrophobic pocket. Next we looked at substituent size at position R^1 (compounds **6-8**) holding the ring at position R (5-chloro-2-fluorophenyl) constant. Unfortunately increased size at R^1 resulted in decreased enzyme potency (compare **6** vs. **7** & **8**) and thus a methyl group at R^1 was judged optimal. Exchange of the 5-Cl for a methyl group in analogs **9** and **10** afforded similarly potent analogs (compare **10** vs. **6**). Finally introduction of a 5-fluoro-2-methylpyridine group at position R afforded a potent analog with improved efficiency (compare **9** vs. **11**; LLE^{7a} 3.6 vs. 4.1).

Table 1. Selected data for 4-substituted quinoline analogues

Cmpd	R	R ¹	LLE	pIC ₅₀
2	Phenyl	H	2.6	6.2
3	3-chlorophenyl	H	2.4	6.5
4	3-chlorophenyl	CH ₃	3.2	7.5
5	5-chloro-2-fluorophenyl	H	3.6	7.7
6	5-chloro-2-fluorophenyl	CH ₃	3.5	7.9
7	5-chloro-2-fluorophenyl	CH ₃ CH ₂		7.6
8	5-chloro-2-fluorophenyl		2.6	7.3
9	5-methyl-2-fluorophenyl	H	3.6	7.6
10	5-methyl-2-fluorophenyl	CH ₃	3.7	7.9
11	3-fluoro-6-methylpyridin-2-yl	H	4.1	7.4

LLE = p(IC₅₀) - logD see ref. 7a

Although the quinoline series of compounds showed good ALK5 enzyme and cellular potency (data not shown) most examples displayed high in vitro clearance in rat and human microsomes and substantial

hERG^{14b} inhibition. As an example analog **6** displayed an in vitro clearance of 302(R) and 86(H) mL/min/kg. Additionally the series suffered elevated hERG inhibition with compound **6** showing 94% inhibition at 10uM. Both liabilities were partially attributed to the high lipophilicity of the series (compound **6** cLogD = 4.4).

Using a scaffold morphing strategy, the quinoline series was transformed into the related 7-substituted-pyrazolo[4,3-b]pyridine series. The replacement of a phenyl ring with a pyrazole resulted in a reduction of lipophilicity (contrast LLE values between Tables 1 & 2)

The chemistry methodology used to access the appropriate 7-substituted-pyrazolo[4,3-b]pyridines (**12-18**) is described in Scheme 2⁸, and their biological properties are disclosed in Table 2.

Scheme 2

Scheme 2. Reagents and conditions: (a) Lithium diisopropylamide, I₂, THF, -78 to 0°C; (b) NH_2NH_2 , n-BuOH, 105°C; (c) NaNO_2 , HI, AcOH, DME, 0 to 80°C; (d) NaH, 1-(chloromethyl)-4-methoxybenzene, DMF; (e) NaOtBu, BINAP, $\text{Pd}(\text{OAc})_2$; (f) TFA, DCM

Table 2. Selected data for 7-substituted-pyrazolopyridine

Cmpd	R	R ¹	LLE	pIC ₅₀	pEC ₅₀ [*]	pEC ₅₀ [†]
12	5-chloro-2-fluorophenyl	H	5.3	8.2	7.3	-
13	5-methyl-2-fluorophenyl	H	5.1	7.8	-	-
14	2,5-difluorophenyl	H	5.8	8.1	7.4	6.7
15	5-chloro-2-fluorophenyl	CH ₃	4.7	7.8	7.5	-
16	2,5-difluorophenyl	CH ₃	6.5	9.0	8.1	7.5
17	6-methylpyridin-2-yl	CH ₃	6.5	8.3	7.5	6.7
18	3-fluoro-6-methylpyridin-2-yl	CH ₃	6.5	8.6	-	7.5

LLE = p(IC₅₀) - logD see ref. 7a

^{*}Reporter Inh Cell Line 293T SBE-bla; [†]pSMAD2/3Calu-6

The utilization of a pyrazolo[4,3-b]pyridine hinge binding element in place of the quinoline afforded more efficient inhibitors of ALK5 as evidenced by lipophilic ligand efficiencies values (compare analog LLEs for **2-11** vs. **12-18**) as well as improved enzyme and cell potencies.

Installation of either a 5-chloro-2-fluorophenyl or 5-methyl-2-fluorophenyl group (analogs **12** & **13**) at position R of the pyrazolo[4,3-b]pyridine core afforded improved potency with lower lipophilicity and a marked improvement in LLE (compare **12** vs. **5** and **13** vs. **9**). Addi-

tionally, exchange of a F atom for Cl or CH₃ (analogs **13** & **14**) resulted in comparable potency with a decrease in lipophilicity. Installation of a CH₃ group at R¹ similar to analogs in the quinoline series at first did not offer an increase in potency (compare **15** vs **12**); however in case of the R= 2,5-difluorophenyl analogs, a 8x increase in potency was observed (compare **16** vs. **14**). Keeping CH₃ at position R¹ constant and installing 2-methylpyridine (analog **17**) or 5-fluoro-2-methylpyridine at position R afforded slightly less potent but equally efficient ALK5 inhibitors. Inhibitors **12-18** additionally showed cellular potency in both a functional assay (Reporter Inh Cell Line 293T SBE-bla) and in a MOA (mechanism of action) cell assay (pSMAD).⁹

The most promising analogs from the 7-substituted- pyrazolo[4,3-b]pyridine series (highest cellular potency) were further profiled to assess their *in vitro* ADME properties. Compounds **15-16** & **18** displayed high metabolic clearance and *in vitro* extraction ratios. Additionally these analogs showed elevated Cyp profiles particularly isoforms 2C8 and 3A4 (Table 3) despite the reduction in lipophilicity.

Table 3. Selected In Vitro ADME data for Compounds **15-18**

Cmpd	cLogD	E _h (H/R) mL/min/kg	CYP2C8/ CYP3A4
15	2.9	.57/.75	6.4/5.5
16	2.5	.74/.84	6.5/5.8
18	2.2	.57/.74	5.6/5.2

E_h = Cl_h/Q_h where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow see ref. 7b-c

Cytochromes P450 (CYPs) family (2C8 & 3A4) pIC₅₀

To improve metabolic stability and reduce Cyp inhibition we decided to further reduce lipophilicity through the installation of polar substituent onto the lead examples (molecules **15-16** & **18**, Table 2). Based on the binding model developed for these compounds in ALK5 protein, both the quinoline ring nitrogen (required for good hinge binding) and the pyridine nitrogen (needed for interaction with the conserved water) did not allow for nearby substitutions. Only the N1 and N2 positions of the pyrazolo[4,3-b]pyridine in this series appeared to be open for modification. We were gratified that many analogs having substituents at these positions (Tables 4-7) showed enzyme and cell potency along with improved metabolic profiles. The chemistry to access the N1 and N2 substituent involved simple alkylation, separation and characterization and is described in our patent application.⁸

Table 4. Selected data for Compounds N1 substituted compounds

Cmpd	X	R ¹	R ²	pIC ₅₀	pEC ₅₀ SBE-bla	pEC ₅₀ pSMAD2/3	E _h (H/R)
19	F		H	7.3	6.7	5.9	.53/.57
20	F		H	8.6	7.7	6.9	.55/.27
21	F		H	8.3	7.5	6.8	.53/.29

22	F		CH ₃	9.0	7.6	7.7	<.23/.2
23	F		CH ₃	8.5	8.1	7.7	.64/.54
24	Cl		H	8.5	7.6	7.2	.57/.31
25	Cl		CH ₃	7.8	7.5	-	.51/.52
26	Cl		CH ₃	7.9	7.7	7.5	.54/.44
27	Cl		H	5.6	-	-	.91/.93

E_h = Cl_h/Q_h where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow see ref. 7b-c

Substitution at R¹ with acetamide (**19**), N-methylcarboxamide (**20**) and N-ethylcarboxamide (**21**) to the N1 position of the difluorophenyl analogs indicated that N-methyl was optimal for enzyme potency. Incorporation of a CH₃ group at R² (compounds **22** and **23**) further boosted potency and reduced *in vitro* clearance for compound **22**. A similar set of design changes for the 5-chloro-2-fluorophenyl analogs **24-26** did not have the same potency effect in regard to the CH₃ group at R² (data consistent with trend seen in Table 2 ex. **15**)

A similar set of changes at position R² of the pyridine ring (Table 5) was also tolerated with compound **31** showing the highest cellular potency in this series.

Table 5. Selected data for Compounds N2 substituted compounds

Cmpd	X	R ¹	R ²	pIC ₅₀	pEC ₅₀ SBE-bla	pEC ₅₀ pSMAD2/3	E _h (H/R)
28	F		H	7.6	6.6	5.8	.44/.42
29	F		CH ₃	7.7	6.9	6.8	.79/.68
30	Cl		H	7.9	7.0	-	.52/.54
31	Cl		CH ₃	7.8	7.9	7.5	.46/.56
32	Cl		H	7.0	6.0	-	.36/.16

E_h = Cl_h/Q_h where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow see ref. 7b-c

The piperidine analogs **27** and **32** were made to increase solubility and enhance the probability of obtaining a crystal structure of the chemotype bound to ALK5. The basic nitrogen of the piperidine ring could also form a salt bridge with Asp₂₉₀ and the edge of the piperidine ring could also make a mainly hydrophobic interaction with the backbone of Gly₃₂₂ and Thr₃₂₃ in models.

The team was pleased that a structure was successfully determined with 2.6Å resolution with compound **32**. Examination of the co-structure in ALK5¹⁰ confirmed the predicted binding mode of the series (Figure 3). The structure shows the N4 of the pyrazolo[4,3-b]pyridine accepting an H-bond from the backbone NH of His₂₈₃ at the kinase's hinge region while the C5 C-H makes a pseudo hydrogen bond^{10g} to the carbonyl oxygen of Asp₂₈₁. The pyridyl ring of compound **32** is positioned to hydrogen bond with the conserved water molecule that is coordinated by Asp₃₅₁, Glu₂₄₅ and Tyr₂₄₉. The phenyl ring, attached at the C2' position of pyridine ring, is oriented towards the back hydrophobic pocket. As compared to our model of compound **2**, Lys₂₃₂ now shifts away from the ligand, no longer H-bonding to the pyridyl N. This is likely due to subtle shift in the H-bonding network induced by an additional H-bond acceptor on the ligand of the parent crystal structure (ref 4) used for modeling compound **2**. The parent structure's ligand has a 2-pyridyl ring in the place of compound **32**'s phenyl ring.

Figure 3. Co crystal structure of compound **32** in ALK5 protein

Several representative analogs from the 7-substituted- pyrazolo[4,3-b]pyridine series with substituents at the N1 and N2 positions (Tables 6 & 7) which displayed the highest cellular potency were further profiled to assess their *in vitro* properties. Analog **22** which initially appeared promising due to cell potency unfortunately had high Cyp 3A4 inhibition ($pIC_{50} = 6.1$) and was no longer considered useful.

Compounds **26**, **30-31**, did not display elevated Cyps (3A4 values of 4.9, 4.9, and <4.3 respectively). Compounds **26** displayed moderate *in vivo* rat clearance (42 mL/kg/min) with good oral bioavailability ($F\% = 71$). Unfortunately compounds **26**, **30-31** all displayed elevated hERG signal (59% & 41% @ 10uM respectively).

To overcome the hERG inhibition observed in these analogs, a further reduction in lipophilicity was achieved by introducing a 5-fluoro-2-methylpyridine group in place of the 2,5-difluorophenyl or 5-chloro-2-fluorophenyl group (see analog **18** in Table 3 and derivatives thereof in Tables 4 & 5).

Substituent groups R were better tolerated at position N1 (Table 6) than N2 (Table 7) in this series. Simple alkyl groups at position R (**33-34** & **40-41**) were similarly potent as the parent **18** with no improvement in *in vitro* clearance. More polar substitution particularly those in **36-39** and **43-46** appeared to mitigate clearance, and many were advanced to *in vivo* PK experiments. The most promising analogs (**36** $F\% = 38$; **37** $F\% = 21$ and **43** $F\% = 40$) were tested for hERG inhibition and unfortunately showed elevated hERG signal (42%, 51% and 56% @ 10uM respectively).

Table 6. Selected data for Compounds N1 substituted compounds

Cmpd	R	pIC ₅₀	pEC ₅₀ SBE-bla	pEC ₅₀ pSMAD2/3	E _h (H/R)
33	-CH ₃	8.4	8.2	7.4	.56/.61
34	-CH ₂ CH ₃	8.7	8.1	7.3	.69/.69
35		7.9	7.7	-	-
36		8.1	7.0	6.3	.3/.3
37		8.1	7.2	6.4	.4/.3
38		8.5	-	6.3	<.23/.21
39		7.8	6.9	5.8	.28/<.15

$E_h = Cl_h/Q_h$ where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow see ref. 7b-c

Table 7. Selected data for Compounds N2 substituted compounds

Cmpd	R	pIC ₅₀	pEC ₅₀ SBE-bla	pEC ₅₀ pSMAD2/3	E _h (H/R)
40	-CH ₃	8.4	7.1	6.5	.66/.46
41	-CH ₂ CH ₃	8.0	7.8	6.8	-
42		8.1	6.6	-	.38/.43
43		8.1	6.8	6.3	.29/.3
44		8.1	7.1	6.5	.4/.3
45		8.0	-	6.6	.25/.16
46		7.5	6.9	6.3	.37/.24

$E_h = Cl_h/Q_h$ where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow see ref. 7b-c

At this point in the program we learned of a potential cardiac toxicity associated with modulating ALK5.¹¹ The chemistry team was advancing additional ALK5 inhibitors described in a previous article in this journal^{12a} and had only begun to fully examine compound **36** (limited selectivity¹³ and *in vitro* safety data was obtained) when a decision was made to do *in vitro* safety testing thus necessitating a choice of lead molecule. Although the on target cardiac valvulopathy that was hypothesized from ALK5 inhibition was unrelated to hERG signal^{14a}, the teams nevertheless choose an alternative molecule to compound **36** for the toxicological studies to remove any potential to confound the results.^{12b}

In summary, a novel series of ALK5 inhibitors was identified and developed. Starting from screening hit **2**, by using SBDD and medicinal chemistry knowledge, the team optimized potency, ascertained binding pose (compound **32**) and finally improved metabolic stability and bioavailability of this series, culminating in the identification of lead compound **36**. However, inhibition of ALK5 was shown to produce significant cardiac toxicity¹² and lead to the program's termination.

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- Representative experimental for analogs in Table 1. Compound **3** step a: 2-chloropyridin-4-amine (200mg, 1.56 mmol), 3-chlorophenylboronic acid (243 mg, 1.56 mmol) and Pd(PPh₃)₄ (450 mg, 0.39 mmol) were mixed in dioxane (4mL). K₂CO₃ saturated solution (2 mL) was added and the mixture was heated at 120 °C for 30 minutes in a microwave reactor. The solvent was removed and the crude residue was purified on a silica column eluted with a 0-5% MeOH in DCM gradient. Concentration of product containing fractions gave 2-(3-chlorophenyl)pyridin-4-amine (150mg, 47.1 % yield) as a light yellow oil. Compound **3** step b: 4-bromo-5-methoxyquinoline (174 mg, 0.733 mmol), 2-(3-chlorophenyl)pyridin-4-amine (150 mg, 0.733 mmol), Pd₂(dba)₃ (168 mg, 0.183 mmol), XANTPHOS (106 mg, 0.183 mmol) and sodium 2-methylpropan-2-olate (211 mg, 2.199 mmol) were mixed in 1,4-Dioxane (4mL) and heated at 140 °C for 1h in a microwave reactor. The reaction was concentrated; the crude residue was taken up in MeOH, filtered and subjected to preparative HPLC. N-(2-(3-chlorophenyl)pyridin-4-yl)-5-methoxyquinolin-4-amine (compound **3**) was obtained as a light yellow oil (55mg, 0.152 mmol, 20.74 % yield). ¹H NMR (400 MHz, METHANOL-d₄) δ ppm 4.09 (s, 3 H) 7.26 (d, J=8.08 Hz, 1 H) 7.42 - 7.53 (m, 4 H) 7.71 - 7.81 (m, 2 H) 7.84 - 7.93 (m, 2 H) 8.08 (d, J=2.27 Hz, 1 H) 8.49 (d, J=6.82 Hz, 1 H) 8.63 (d, J=6.32 Hz, 1 H)
- a) LLE= p(IC₅₀) - logD see Leeson and Springthorpe *Nat. Rev. Drug Discovery* **2007**, 6(11) 881; LE $\approx$ 1.37 $\times$ p(IC₅₀)/HA where HA = Heavy Atoms see Hopkins *et al.*, *Drug Discov. Today* **2004**, 9, 430. b) Obach *Curr. Top. Med Chem* **2011**, 11, 334. c) Davies and Morris *Pharm. Res.* **1993**, 10, (7), 1093.
- For a more detailed experimental see WO/2011/146287
- Test compounds were screened for cellular TGFBR1 ALK5 inhibition by use of a CellSensor® SBE-bla HEK 293T (cat # K1550, Invitrogen Corp. Madison, WI) cell line. The CellSensor® SBE-bla HEK 293T cell line contains a beta-lactamase reporter gene under control of the SBE response element stably integrated into HEK 293T cells. The SBE response element is a DNA region that binds activated SMADs which results from the binding of TGF β to, and subsequent activation of ALK5.
- a) PDB ID: **5USQ** b) The ALK5 catalytic domain (residues 192-500) was cloned into the pFastBachTb vector and recombinant baculovirus was generated and expressed in fusion with an N-terminal 6x poly-histidine tag and TEV cleavage site. Large scale production of recombinant protein was carried out in Spodoptera frugiperda Sf9 cells. The cell pellet was suspended into lysis buffer consisting of 25 mM HEPES (pH 8.0), 1 M NaCl, 20 mM imidazole, 3 Roche Complete tablets. The lysate was centrifuged, and clarified supernatant was batch bound with 10 ml of Probond Ni resin (Invitrogen). The protein was then eluted with buffer containing 25 mM HEPES (pH 7.6), 150 mM NaCl, and 250 mM imidazole. After tag cleavage, the protein was further purified by size-exclusion chromatography utilizing a Superdex 200 column equilibrated in 25 mM HEPES (pH 7.6), 150 mM NaCl, 5% Glycerol. Fractions containing the protein of interest were pooled, concentrated to ~2 mg/ml and flash-frozen in liquid nitrogen for storage at -80°C. Crystals suitable for data collection were obtained by vapor diffusion in sitting drops at 20 °C. Reservoirs contained 14 -18% PEG 8K and 100 mM CHES pH 9.0 -9.4. Crystals that took 7 -10 days to grow were immersed in mother liquor solution containing 22% ethylene glycol for cryoprotection and flash frozen in liquid nitrogen. Crystals of ALK5 complex grew in the orthorhombic space group P212121 and contain one molecule in the asymmetric unit. Diffraction data to 2.55 Å were collected from single cryogenically protected crystals at beam line 5.0.3 of the Advanced Light Source at Lawrence Berkeley National Laboratory. Data was reduced using the HKL2000 software package. The structure was determined by molecular replacement with either

- MOLREP2 or PHASER3 of the CCP4 program suite and refined with the program REFMAC.4 several cycles of model building with XtalView5 and refinement were performed for improving the quality of the model. The coordinates and structure factors have been deposited in Protein Data Bank with accession code **5USQ**. c) Otwinowski, Z.; Minor, W. **1997**, *Methods Enzymol.* 276, 307. d) Vagin, A.; Teplyakov, A. **1997**, MOLREP: an automated program for molecular replacement. *J. Appl. Cryst.* 30, 1022. e) McCoy, A. J.; Grosse-Kunstleve, R. W.; Adams, P. D.; Winn, M. D.; Storoni, L. C.; Read, R. J.; **2007**, Phaser Crystallographic Software. *J. Appl. Cryst.* 40, 658. f) Murshudov, G. N.; Vagin, A. A.; Dodson, E. J. **1997**, *Acta Crystallogr. Sect. D* 53, 240–255. g) McRee, D. E.; **1999** *J. Struct. Biol.* 125, 156. h) In accord with Definition of the hydrogen bond (IUPAC Recommendations **2011**) *Pure Appl. Chem.*, 83, 8, 1637.
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 13. Compound **36** ALK5 family isozyme profiling selectivity: ALK4 is 39-fold, ALK2 and ALK3 (BMPRI α) are each >200-fold, based on 2pt screening done at 10 & 1 uM, and compared to the in-house determined of ALK5 IC₅₀ value.
 14. a) We do not believe hERG activity has any relationship to cardio valvulopathy however we did not want potential AE to be possibly clouded by or attributed to hERG risk. b) Automated whole cell patch-clamp (Qpatch 16) or conventional whole cell patch-clamp technique was used to record outward potassium currents from a single cell. Conducted at: www.cerep.com

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$pIC_{50} = 6.2$
 $E_h = .9$
 $LE = .3$
 $LLE = 2.5$

X-ray Co-crystal Structure
 in ALK5

$pIC_{50} = 8.1$
 $E_h = .3$ H/R
 $LLE = 5.6$
 $F\% = 38$

LLE= ligand-lipophilicity efficiency $p(IC_{50}) - \log D$

LE = ligand efficiency $LE \approx 1.37 \times p(IC_{50})/HA$ where HA = heavy atoms

$E_h = Cl_h/Q_h$ where E_h = Hepatic extraction ratio; Cl_h = Clearance hepatic (mL/min/kg); Q_h = Hepatic blood flow