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2-Phenyl and 2-heterocyclic-4-(3-(pyridin-2-yl)-1*H*-pyrazol-4-yl)pyridines as inhibitors of TGF-β1 and activin A signalling

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ABSTRACT

Novel inhibitors of TGF-β1 and activin A signalling based on a 2-aryl-4-(3-(pyridin-2-yl)-1*H*-pyrazol-4-yl)pyridine pharmacophore have been synthesised. Compounds containing phenyl or aromatic nitrogen heterocycle substituents inhibited both types of signalling with HEK-293T cells in culture, with a selectivity preference for TGF-β1. Synthetic compounds containing pyridin-3-yl, pyrazol-4-yl, pyrazol-1-yl or 1*H*-imidazoyl-1-yl substituents exhibited structural and functional attributes suitable for further investigation related to the development of more potent TGF-β inhibitors.

The transforming growth factor-β (TGF-β) superfamily of cytokines includes a large number of signalling proteins, including the archetypal members TGF-β1 and activin A. These proteins modulate many different biological processes,[1] including cell proliferation,[2] cell differentiation,[3] extracellular matrix production,[4] apoptosis[5] and wound repair.[6] Their biological effects are manifested by signalling through transmembrane serine/threonine receptor kinases. Both TGF-β1 and activin A are known to bind to the type II serine/threonine receptors TGF-βRII and ActRII, respectively, which sequentially leads to recruitment, phosphorylation and activation of a type I serine/threonine receptor, activin-like kinase (ALK), ALK5 and ALK4, respectively.[7] Upon activation, the ALK receptors phosphorylate a subset of cytoplasmic Smad proteins (Smad 2 and Smad 3), which then bind to a common-partner, Smad 4, to form a complex that induces regulation of gene transcription.[7]

Increased TGF-β1 signalling has been implicated in pathological fibrosis and associated with the growth of highly malignant cancers, whilst activin A has also been implicated in numerous malignancies as well as in apoptosis, wound repair, cell differentiation and proliferation, and endocrine functions.[8] Consequently, ALK5 has been proposed as a therapeutic target for these diseases and investigations with different competitive inhibitors of ATP-binding to ALK5 have been previously undertaken based on five-membered 6-nitrogen heterocycles containing either a pyridin-2-yl or methylpyridin-2-yl substituent and an *ortho* aromatic substituent (Fig. 1). Examples of these compound classes include 2,4,5-substituted imidazoles (**1**)[9], 1,3,5-substituted 1,4,5-triazoles (**2**)[10], 2,4,5-substituted 1,2,3-triazoles (**3**)[11], 4,5-substituted 2-aminothiazoles (**4**)[12], 1,2,4-substituted imidazoles (**5**)[13], 1,3,5-substituted pyrazoles (**6**)[13], 2,3-substituted pyryolo-pyrazoles (**7**)[12,14] and 4,5-substituted pyrazoles (**8** and **9**).[15]

Recent clinical and biological investigations have also shown increased levels of activin A signalling are involved in pathological fibrosis, the progression from pre-malignancy to advanced cancers as well as pre-eclampsia.[16] Therefore ALK4 inhibitors may be potentially useful in treating these diseases. Given the high sequence homology between the ATP-binding sites of ALK4 and ALK5 (>90%)[17] it is feasible that ATP-competitive ALK5 inhibitors may also inhibit activin A signalling. Indeed, recent work has shown that specific members from the pharmacophore series **5** and **6** are capable of inhibiting both ALK4 and ALK5 kinase activity with different selectivities.[13]

Compounds based on the 2-aryl-4-(3-(pyridin-2-yl)-1*H*-pyrazol-4-yl)pyridine pharmacophore **9** containing a *para*-substituted phenyl ring as the aromatic substituent are potent inhibitors of ALK5/TGF-β1 signalling, and can prevent kidney fibrosis in rats and mice.[15b,18] However, structure–activity relationships for inhibition of activin A signalling have not been explored in detail for this class of compound. Here we present work on the synthesis

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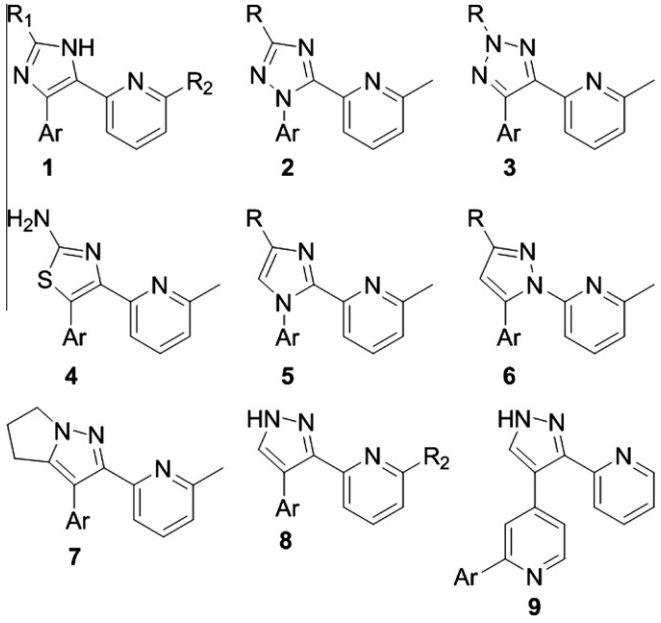
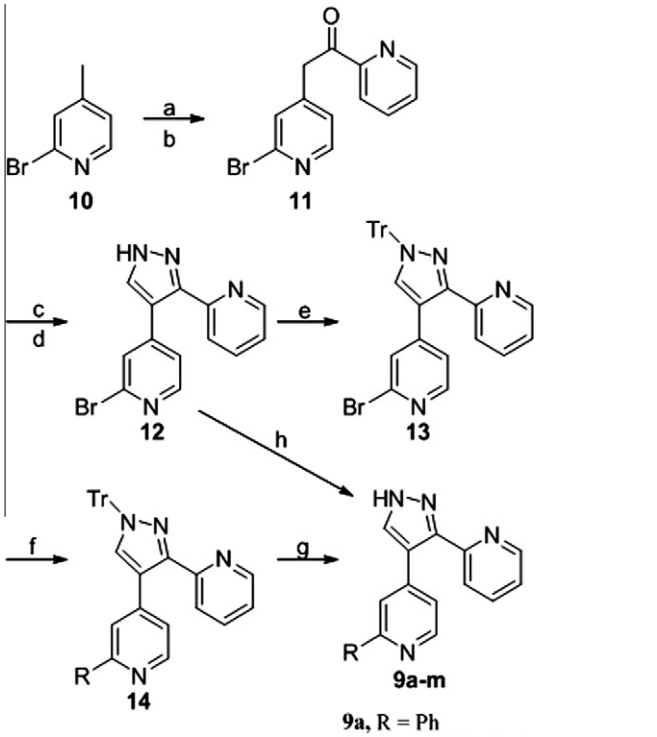


Figure 1. Structures of ATP-competitive inhibitors of ALK-5.

of novel analogues containing different nitrogen heterocycle substituents on the 4-pyridine ring and document their activities, along with other synthetic compounds containing phenyl substituents, as inhibitors of both TGF-β1 and activin A signalling using human embryonic kidney (HEK)-293T cells in culture.

To synthesise analogues of the pharmacophore series **9** containing nitrogen heterocycle substituents at position 2 of the 4-pyridine ring, the precursor 2-bromo-4-(3-(pyridin-2-yl)-1-trityl-1*H*-pyrazol-4-yl)pyridine (**13**) was first synthesised from 2-bromo-4-methyl pyridine (**10**) and ethyl picolinate with some improved modifications (Scheme 1).[19] Firstly, treatment of **10** with KHDMS and then ethyl picolinate gave 2-(2-bromopyridin-4-yl)-1-(pyridin-2-yl)ethanone (**11**). We found that on the multigram scale, mild heating following addition of ethyl picolinate gave an improved yield of **11** and removed the need for purification by column chromatography as previously reported.[15b] Treatment of **11** with dimethylformamide dimethyl acetal followed by hydrazine gave crude **12** which was purified on a multigram scale by simply tituration with hexane/dichloromethane to give **12** an excellent yield. Protection of **12** with the trityl group in acetone containing K_2CO_3 gave poor yields of **13** and significant impurities in our hands. Hence, we protected **12** in dichloromethane using triethylamine as the base which gave an improved yield of **13** and removed the need for subsequent column chromatography. The trityl intermediate **13** was reacted with different aromatic heterocyclic boronic acids in a Suzuki–Miyaura coupling followed by trityl deprotection to yield the corresponding arylated derivatives **9a–g**. Alternatively, trityl intermediate **13** was reacted with different aromatic or aliphatic heterocyclic secondary amines at high temperature in a focused microwave reactor followed by trityl deprotection to give the corresponding aminated derivatives **9h–l**. Moreover, the precursor **12** was readily hydrodebrominated to give the unsubstituted analogue **9m**.

The compounds were assessed as inhibitors of TGF-β signalling in HEK-293T cell culture by measuring their inhibition of TGF-β1 and activin A dependent luciferase transcription (Table 1).[20] This cell line has previously been shown to endogenously express the ALK-4, ALK-5 and associated type II co-receptors required for TGF-β1 and activin A signalling.[21a,b] The cells were transfected with a luciferase expression vector (A3-LUX) containing the activin



Scheme 1. Reagents and conditions: (a) KHMDS, −50 °C; (b) ethyl picolinate, −50 °C to rt 18 h, 45 °C 1 h; (c) DMF–DMA, AcOH; (d) $NH_2NH_2{\cdot}H_2O$, 50 °C 2 h to rt 22 h; (e) trityl chloride, Et_3N; (f) $ArB(OH)_2$, $Pd(PPh_3)_4$, Na_2CO_3 or heterocyclic secondary amine, K_2CO_3, microwave heating; (g) methanolic 1 M HCl; (h) H_2, Pd/C.

Table 1
Inhibition of TGF-β1 and activin A signalling by compounds **9a–m**

Compound	TGF-β1[a] activity (% of control)[b]		Activin A[a] activity (% of control)[b]	
	1 μM	100 nM	1 μM	100 nM
9a	14 (2–26)	na	59 (41–77)	na
9b	1 (0–6)	63 (55–71)	58 (42–74)	na
9c	7 (3–11)	72 (49–95)	62 (53–70)	na
9d	29 (22–36)	69 (55–83)	na	na
9e	46 (32–60)	na	na	na
9f	58 (55–61)	na	na	na
9g	1 (0–2)	3 (0–7)	1 (0–5)	83 (80–86)
9h	1 (0–9)	42 (37–47)	37 (32–42)	na
9i	11 (9–13)	50 (45–55)	62 (51–73)	na
9j	34 (30–38)	70 (61–79)	62 (61–63)	na
9k	80 (66–94)	na	na	na
9l	90 (89–91)	na	na	na
9m	50 (44–56)	87 (86–88)	na	na

[a] 100 pM.
[b] Values are means of duplicate experiments, 95% confidence intervals are given in parentheses (na = not active).

responsive binding elements (ARE) in the promoter region, and a fork head activin signal transducer-2 (FAST-2) expression vector.[21a,22] The smad 2/3-smad 4 complex, arising from TGF-β signalling through ALK receptors, targets the ARE elements which in turn

are mediated by direct interaction with FAST-2.[21] Hence, the assay system used in these investigations enables TGF-β and activin A signalling via the ALK-smad pathway to be selectively measured in cell culture by quantifying the level of luciferase expression. The results of these assays showed that compounds containing pyridinyl (**9b** and **9c**) or the smaller 1-methyl-1*H*-pyrazol-4-yl (**9g**), 1*H*-pyrazol-1-yl (**9h**) or 1*H*-imidazoly-1-yl (**9i**) substituents were more potent inhibitors of TGF-β1 signalling compared to the phenyl derivative **9a**, and had comparable potency to **9a** as inhibitors of activin A signalling, except for **9g** which had significantly greater potency. The compounds containing quinolinyl substituents (**9d** and **9e**), the pyrmidin-5-yl substituent (**9f**) or no substituent (**9m**) were comparable to **9a** as inhibitors of TGF-β1 signalling but were weaker inhibitors of activin A signalling. In addition, the benzimidazol-1-yl derivative **9j** showed comparable activity against both types of signalling. The compounds containing aliphatic piperidine or piperazine substituents (**9k** and **9l**) had much reduced or no activity compared to **9a**. Overall, most of the compounds that inhibited TGF-β1 signalling showed more selective inhibition of TGF-β1 relative to activin A in these initial screening assays.

Compounds in the pharmacophore class **9** containing *para*-2-(dimethylamino)ethoxyphenyl (**9n**) or *para*-*N*-*(tetrahydro-2H*-pyran-4-yl)benzamide (**9o**) substituents have been shown to be considerably more potent inhibitors of ALK5 kinase activity and TGF-β1 signalling compared to **9a** whereas analogues containing *ortho* or *meta* substituents are known to be relatively poor inhibitors.[15b] However, the effect of different substituents or substitution pattern on inhibition of activin A signalling has not been explored in detail for this class. Hence we have synthesised compounds containing *ortho*, *meta* and *para* substituents on the phenyl ring along with novel pyridin-3-yl analogues containing 3-(dimethylamino)propan-1-oxy (**9r**) and 4-methyl-piperazine (**9s**) substituents at position 6 of the pyridin-3-yl ring and assessed their activity as inhibitors of TGF-β1 and activin A signalling (Table 2).[19,20]

Compound **9q** containing the *ortho*-methoxyphenyl substituent inhibited both types of signalling with potency comparable to **9a** but did not show any selectivity. The *meta*-methoxyphenyl derivative **9p** was not active at all. Compounds **9n** and **9o** containing the *para*-substituted phenyl substituents were significantly more potent as inhibitors of TGF-β1 and activin A signalling compared to **9a**. We also found that the novel substituted pyrdin-3-yl analogues **9r** and **9s** inhibited both types of signalling with potency comparable to **9n**. As observed with the active compounds in the initial screening assays, most of these more potent compounds also showed selectivity toward TGF-β1 (Fig. 2).

To obtain a more accurate assessment of structure–activity relationships, the IC_{50} values for signalling inhibition were determined from dose–response curves of the more potent phenyl and pyridin-3-yl derivatives, and the potent inhibitors containing the smaller five-membered nitrogen heterocycle substituents identified in the initial screening assays (Table 3).[20] The compounds containing *para*-substituted phenyl or 6-substituted pyridin-3-yl substituents were of similar potency and had a slight selectivity toward TGF-β1, except for **9r** which had a comparable IC_{50} value for inhibition of both types of signalling. Compounds containing the relatively smaller five-membered nitrogen heterocycle substituents were also potent inhibitors of TGF-β1 signalling and showed greater selectivity toward TGF-β1 over activin A compared to the compounds with the larger aromatic substituents.

In conclusion, the 2-aryl-(4-(pyridin-4-yl)-1*H*-pyrazol-3-yl)pyridine pharmacophore **9** incorporating different aromatic nitrogen heterocycles as aryl substituents to give compounds that inhibit both TGF-β1 and activin A signalling, with selectivity for the former. In terms of drug development, these different structural features and selectivities are expected to be important determinants of pharmacokinetics, metabolism and side-effects. We have also shown that compounds containing five-membered aromatic nitrogen heterocycle substituents are comparatively potent to those containing larger aromatic substituents and are more selective for TGF-β1. Given that specific substitutions of the larger phenyl and pyridin-3-yl substituents lead to improved potency, it can be concluded that similar substitutions of the smaller five-membered nitrogen heterocycles may also provide compounds with improved activity. The synthesis of a library of these compounds is the focus of our current research and will be reported in due course.

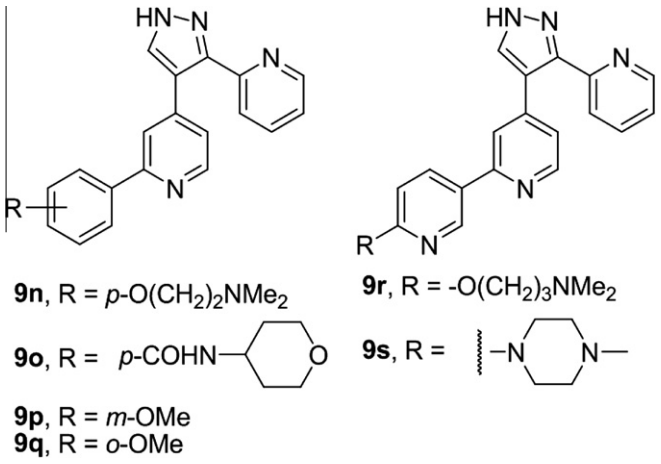


Figure 2. Structures of synthetic compounds **9n–s**.

Table 2
Inhibition of TGF-β1 and activin A signalling by compounds containing different phenyl and pyrdin-3-yl substituents

Compound	TGF-β1[c] activity (% of control)[d]	Activin A[c] activity (% of control)[d]
9n[a]	1 (0–2)	4 (0–30)
9o	52 (48–56)[a]	42 (31–53)[b]
9p[b]	na	na
9q[b]	54 (46–62)	71 (53–89)
9r[a]	1 (0–3)	38 (28–48)
9s[a]	1 (0–5)	31 (27–45)

[a] 100 nM.
[b] 1 μM.
[c] 100 pM.
[d] Values are means of duplicate experiments, 95% confidence intervals are given in parentheses (na = not active).

Table 3
IC_{50} values for inhibition of TGF-β1 and activin A signalling

Compound	TGF-β1[a] activity IC_{50}[b] (nM)	Activin A[a] activity IC_{50}[b] (nM)
9g	68 (49–95)	224 (155–322)
9h	77 (58–103)	882 (357–3021)
9i	88 (33–234)	956 (368–2495)
9n	19 (16–24)	46 (30–70)
9o	446 (288–689)	>1000
9r	11 (6–19)	18 (11–30)
9s	32 (23–43)	108 (46–256)

[a] 100 pM.
[b] Calculated from non-linear regression analysis of dose–response curves, 95% confidence intervals are given in parentheses.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.12.120.

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