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Letters

Synthesis and Activity of New Aryl- and Heteroaryl-Substituted Pyrazole Inhibitors of the Transforming Growth Factor-β Type I Receptor Kinase Domain

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Abstract: Pyrazole-based inhibitors of the transforming growth factor-β type I receptor kinase domain (TβR-I) are described. Examination of the SAR in both enzyme- and cell-based in vitro assays resulted in the emergence of two subseries featuring differing selectivity versus p38 MAP kinase. A common binding mode at the active site has been established by successful cocrystallization and X-ray analysis of potent inhibitors with the TβR-I receptor kinase domain.

The multifunctional cytokine transforming growth factor-β (TGF-β) is a member of a large family of growth factors involved in the regulation of a diverse array of biological processes including cell growth and differentiation, matrix modulation, and embryonic development. Cell surface binding of TGF-β ligand leads to the formation of a heteromeric complex involving type I (TβR-I) and type II (TβR-II) receptors, each of which are transmembrane-spanning proteins featuring a serine/threonine kinase domain. Downstream signal transduction is mediated by the TβR-I kinase domain through the phosphorylation of Smad proteins, which as oligomeric complexes translocate to the nucleus and regulate gene response via association with DNA transcription factors.[1] The TGF-β signaling pathway may play a role in a number of disease states involving inflammation, angiogenesis, and immune function, including fibrosis,[2] wound healing,[3] Alzheimer's disease,[4] atherosclerosis,[5] hypertension,[6] restenosis,[7] and cancer.[8]

Our efforts in this area began with the in vitro screening of a large library of compounds as potential inhibitors in a TGF-β-dependent cell-based assay. Promising hits were then evaluated as inhibitors of autophosphorylation of the isolated human TβR-I kinase domain in the form of a constitutively active construct (T204D mutation)[9] produced in Sf9 insect cells and purified by nickel-affinity chromatography. Diheteroaryl-substituted pyrazole **1** was quickly identified as a potent inhibitor (IC_{50} = 51 nM, Table 1) and was chosen as a platform for SAR development. Compounds were further evaluated as inhibitors of TGF-β-dependent luciferase production in mink lung cells (p3TP Lux)[10] and growth in mouse fibroblasts (NIH 3T3).[11]

The 4-(quinolin-4-yl)-substituted pyrazole analogues (Table 1) were prepared as illustrated for the syntheses of compounds **2** and **10** (Scheme 1). Lepidine was deprotonated and condensed with an appropriate picolinic ester to provide the intermediate ketone, which generally was not purified but treated directly with DMF dimethyl acetal and hydrazine to provide final product **2**. Alternatively, **10** was obtained through treatment of the intermediate ketone with the appropriate hydrazide followed by thermal cyclization of the resulting hydrazone hydrochloride salt. Treatment of the ketone intermediate with benzylhydrazine hydrate in the presence of 2 equiv of base followed by DMF dimethyl acetal provided the *N*-benzyl analogue **12**

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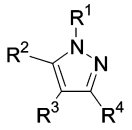
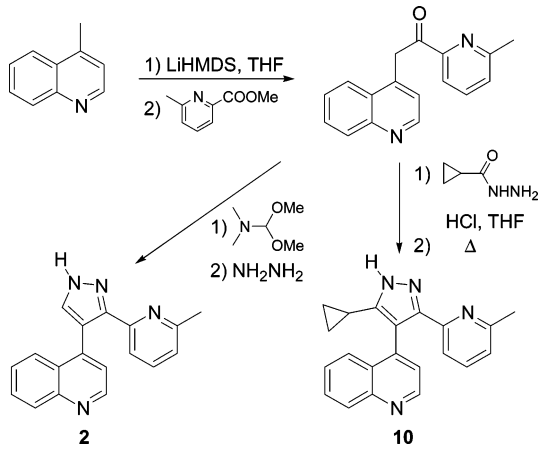
Table 1. 4-Heteroaryl-Substituted Pyrazole Inhibitors



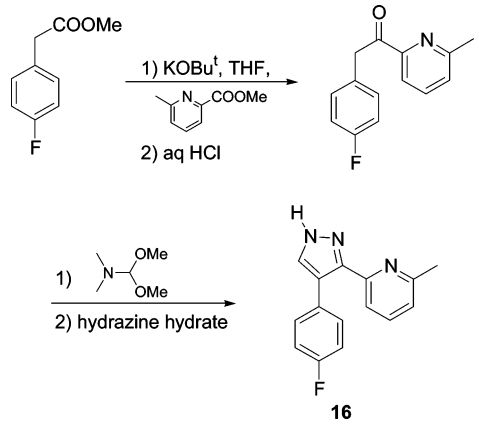
cmpd	R^1	R^2	R^3	R^4
1	H	H		
2	H	H		
3	H	H		
4	H	H		
5	H	H		
6	H	H		
7	H	H		
8	H	H		
9	H	CH_3		
10	H			
11	H	H		
12	$PhCH_2$-	H		

(Table 1). In the case of the 4-phenyl-substituted series (Table 3), the intermediate ketones were obtained after Claisen condensation and decarboxylation of the corresponding β-ketoesters or β-ketonitriles, which were then cyclized as described above (e.g., **16**, Scheme 2). For **9** and **20**, the corresponding aryl- or heteroaryl-substituted acetone was used in an initial aldol condensation with 2-formyl-6-methylpyridine, followed by Lewis acid mediated addition of a polymer-supported sulfonylhydrazide to affect cyclization and provide the 5-methyl-substituted pyrazole ring system.

Scheme 1. Representative Synthesis of 4-(Quinolin-4-yl)-Substituted Pyrazoles



Scheme 2. Synthesis of 4-Aryl-Substituted Pyrazoles



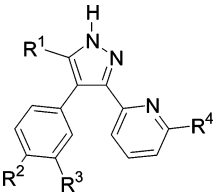
A limited exploration of the SAR of the 4-(quinolin-4-yl)-substituted pyrazoles (Table 2) revealed a number of interesting observations. Replacement of the pyridin-2-yl group with phenyl (**3**) or thiophene-2-yl (**11**) led to a loss of activity in both the enzyme and cellular assays. However, the addition of a trifluoromethyl group to **3** (**4**) restored some of this loss. Substitution of fluorine at the 5-position of pyridine (**5**) maintained good activity, but chloro analogue **6** exhibited a noticeable drop in potency. In addition, substitution of methyl (**2**) or bromo (**7**) at the 6-position of pyridine improved activity, particularly in the NIH 3T3 assay, relative to **1**. Bromo-substituted **7** was the most potent inhibitor tested with an IC_{50} in the cellular assays of about 2 nM. Taken together, these results appear to implicate the pyridine nitrogen in providing a key interaction at the TβR-I

Table 2. In Vitro Activity of 4-Heteroaryl-Substituted Pyrazole TGF-β RI Kinase Inhibitors

compd	TGF-β RIK, IC_{50},[a] μM (n)	p3TP Lux, IC_{50},[a] μM (n)	NIH 3T3, IC_{50},[a] μM (n)	p38 MAPK, inhibition,[b] μM
1	0.051 ± 0.0015 (258)	0.047 ± 0.003 (55)	0.089 ± 0.010 (68)	0.74
2	0.034 ± 0.003 (4)	0.0029 ± 0.0004 (3)	0.0071 ± 0.003 (4)	0.078
3	0.382 ± 0.045 (4)	1.20 ± 0.25 (7)	2.92 ± 0.70 (4)	0.025
4	0.217 ± 0.011 (6)	0.355 ± 0.041 (10)	0.524 ± 0.103 (6)	0.0068
5	0.091 ± 0.016 (5)	0.094 ± 0.022 (6)	0.076 ± 0.011 (6)	1.3
6	1.76 ± 0.160 (5)	>10 (2)	4.67 ± 1.22 (5)	2.3
7	0.031 ± 0.0035 (3)	0.0029 ± 0.0013 (3)	0.0012 ± 0.0001 (3)	0.11
8	0.150 ± 0.018 (3)	0.683 ± 0.164 (9)	1.68 ± 0.39 (4)	7.7
9	0.082 ± 0.0058 (3)	0.024 ± 0.004 (5)	0.148 ± 0.046 (3)	0.25
10	0.203 ± 0.046 (3)	0.094 ± 0.013 (3)	0.538 ± 0.248 (3)	0.28
11	0.353 ± 0.098 (4)	1.66 ± 0.94 (4)	2.81 ± 0.48 (3)	0.40
12	0.369 ± 0.044 (3)	0.399 ± 0.055 (3)	1.34 ± 0.09 (4)	0.51

[a] Mean values ± SEM for a minimum of three determination. [b] Single point determination.

Table 3. 4-Aryl-Substituted Pyrazole Inhibitors



compd	R^1	R^2	R^3	R^4
13	H	H	H	H
14	H	F	H	H
15	H	H	H	CH_3
16	H	F	H	CH_3
17	H	Cl	H	CH_3
18	H	OH	H	CH_3
19	H	F	F	CH_3
20	CH_3	OCH_3	H	CH_3

kinase domain pharmacophore. The electronic disposition of the pyrazole ring also effects activity because the "reversed" substitution pattern results in a 5-fold drop in potency (**8**). Activity was somewhat maintained when methyl or cyclopropyl was substituted for hydrogen at the 5-position of the pyrazole ring (**9** and **10**), while substitution of benzyl on the 1-position (**12**) led to lower activity.

Compounds in the quinolin-4-yl series exhibited a range of activity as inhibitors of p38 MAP kinase, with the most potent examples having phenyl in place of pyridine (**3** and **4**), a result we found unsurprising given the activity of known structurally related p38 inhibitors.[12] We addressed this issue of selectivity by substituting the quinolin-4-yl group with aryl while keeping the pyridin-2-yl moiety intact (Table 4). While phenyl analogue **13** exhibited a significant loss of activity in kinase inhibition, the substitution of a 4-fluorophenyl group provided **14**, which possessed moderate activity in the enzyme assay.[13] Compound **15**, the simple 6-methyl-substituted pyrindin-2-yl analogue of **13**, exhibited similar activity relative to **14**. Combining the observed SAR into **16** gave a potent inhibitor with an IC_{50} in the enzyme assay of 70 nM and activity against p38 of greater than 20 μM. Since incorporation of fluoro at the 4-position of the phenyl group had such a pronounced effect on activity, further substitutions were examined. Although a chloro substituent (**17**) failed to improve cellular activity relative to **16**, hydroxyl-substituted **18** proved to be extremely active in all assays examined, while still maintaining moderate selectivity against p38. Placement of an additional fluoro at the 3-position of **16** (**19**) was detrimental, but excellent activity and selectivity were observed with **20**, which incorporates a 4-methoxy group at phenyl and a 5-methyl group on the pyrazole ring.

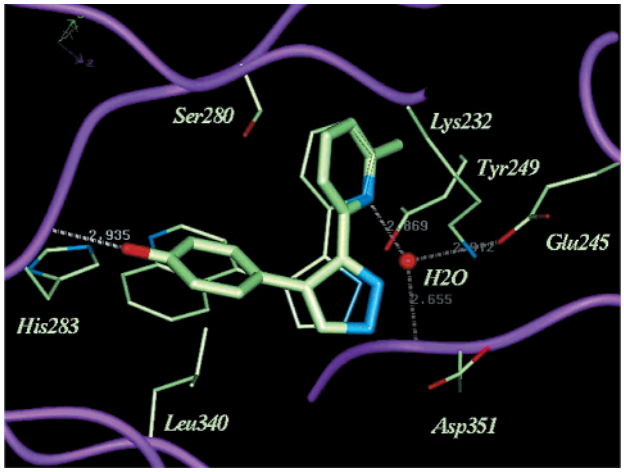


Figure 1. X-ray crystal structures of **1** and **18** bound to the ATP-binding site of the TβR-I kinase domain.

Assessing the SAR from each series suggests that the minimum requirements for tight binding at the active site consist of the presence of a 2-pyridyl ring on the 3-position of pyrazole and an aryl or heteroaryl substituent at the 4-position featuring a hydrogen bond acceptor. This hypothesis is supported further by the X-ray crystal structures of **1** and **18** bound to the ATP-binding site of the TβR-I kinase domain (Figure 1). In each structure, the pyridyl nitrogen is committed to a hydrogen bond to a water molecule held in place by additional hydrogen bonds to Asp-351, Glu-245, and Tyr-249. For quinolin-4-yl **1**, the quinoline nitrogen atom also acts as a hydrogen bond acceptor from the backbone N−H of residue His-283. This same interaction is also observed in the case of **18**, where the hydrogen bond acceptor is the oxygen of the hydroxyl substituent. Also, there is enough flexibility at the active site Asp-351 and Lys-232 side chains to allow the shift needed to accommodate the additional spacing requirement demanded by the 4-substituted aryl substructure.

This hydrogen-bonding interaction corresponds to that observed for the pyridyl nitrogen of SB203580 with the N−H residue of Met-109 in the active site of p38 MAP kinase,[14] while the pyrazol-3-yl substituent from each of our series occupies the hydrophobic binding pocket corresponding to that filled by the 4-fluorophenyl group of known p38 inhibitors. The difference in TβR-I/p38 selectivity observed for our two series is best explained by the His-283 hydrogen-bonding interaction. Owing to differences in the hydrogen-bonding environment attributed to sequence/structure variations, the TβR-I hinge tolerates weaker acceptors, such as aromatic fluorine, while the more flexible p38 hinge requires a stronger acceptor such as quinoline nitrogen.

Table 4. In Vitro Activity of 4-Aryl-Substituted Pyrazole TGF-β RI Kinase Inhibitors

compd	TGF-β RIK, IC_{50},[a] μM (n)	p3TP Lux, IC_{50},[a] μM (n)	NIH 3T3, IC_{50},[a] μM (n)	p38 MAPK, inhibition,[b] μM
13	3.99 ± 0.322 (5)	>9.0 (3)	9.54 ± 3.6 (3)	>20
14	0.275 ± 0.022 (6)	3.53 ± 1.69 (5)	2.59 ± 0.57 (4)	6.8
15	0.543 ± 0.033 (4)	3.37 ± 0.474 (4)	2.16 ± 0.037 (3)	>20
16	0.070 ± 0.005 (6)	0.139 ± 0.029 (5)	0.041 ± 0.010 (4)	>20
17	0.057 ± 0.007 (6)	0.551 ± 0.145 (4)	0.421 ± 0.104 (4)	>20
18	0.031 ± 0.004 (6)	0.044 ± 0.016 (4)	0.032 ± 0.007 (4)	0.56
19	0.151 ± 0.025 (5)	0.788 ± 0.246 (5)	0.683 ± 0.163 (5)	>20
20	0.044 ± 0.004 (6)	0.047 ± 0.018 (5)	0.061 ± 0.011 (3)	5.4

[a] Mean values ± SEM for a minimum of three determination. [b] Single point determination.

Furthermore, the interactions of the pyridine nitrogen present in both series with active site water molecules, together with the presence of different hydrophobic pocket gate-keeper residues (Ser-280 in TβR-I versus Thr-106 in p38), would also be expected to affect selectivity depending on how deeply the pyrazole-3-yl group is embedded. This in turn may depend on whether the backbone warhead is quinoline-4-yl or the more sterically demanding 4-substituted phenyl group.

In summary, we have developed an interesting series of pyrazole-based inhibitors of the TβR-I kinase domain. Two subclasses of inhibitors, involving different substituents at the 4-position of the pyrazole ring, were identified. The quinolin-4-yl series was found to have good to excellent activity at the active site and in relevant cellular assays but shown to possess only moderate selectivity against p38 MAP kinase. Alternatively, the aryl-substituted series exhibited equally good in vitro activity with generally high selectivity against p38 MAP kinase. The SAR of these compounds, together with an active site model derived from selective enzyme/inhibitor cocrystallization and X-ray crystal structure analysis, will be used to further elucidate the pharmacophore of the TβR-I kinase domain.

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Supporting Information Available: Representative experimental procedures and spectral data for the preparation and characterization of pyrazole derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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