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Synthesis and activity of new aryl- and heteroaryl-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole inhibitors of the transforming growth factor-β type I receptor kinase domain

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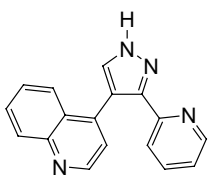
Abstract—We have expanded our previously reported series of pyrazole-based inhibitors of the TGF-β type I receptor kinase domain (TβR-I) to now include new 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole analogues. Limited examination of the SAR of this new series in both enzyme and cell based in vitro assays has revealed selectivity differences with respect to p38 MAP kinase (p38 MAPK) depending on the nature of the 'warhead' group on the dihydropyrrolopyrazole ring. As with our original pyrazole series, phenyl substituents tended to show greater selectivity against p38 MAPK than those comprised of the quinoline-4-yl moiety. We have also achieved co-crystallization and X-ray analysis of compounds **3** and **15**, two potent examples of this new series, with the TβR-I receptor kinase domain.

Transforming growth factor-β (TGF-β) has been postulated to be involved in a number of disease states, including inflammation, fibrosis, cancer, asthma, and cardiovascular disease.[1] The complex function of this cytokine, which mediates pathways involving the regulation of gene response and DNA transcription factors, is dependent upon the activation of the type I (TβR-I) and type II (TβR-II) receptors, transmembrane-spanning proteins containing serine/threonine kinase domains.[2] Inhibition of these domains with small molecule inhibitors would be expected to interrupt downstream signal transduction and potentially provide palliative effects on diseases modulated via TGF-β. Only a few such inhibitors have been described in the literature, including a series of triaryl-substituted imidazole-based compounds, members of which are selective against p38 MAPK.[3]

Recently, we reported on a series of pyrazole-based inhibitors of the TGF-β type I receptor kinase domain (TβR-I) featuring either phenyl- or quinoline-4-yl warheads that were shown to hydrogen bond with the peptide backbone in the ATP binding pocket.

Limited examination of the SAR of this series in both enzyme and cell based in vitro assays resulted in the emergence of two sub-series featuring differing selectivity versus p38 MAP kinase. Specifically, our group was the first to report the co-crystallization and X-ray analysis of **1** (LY364947, $IC_{50} = 51$ nM) and **2**



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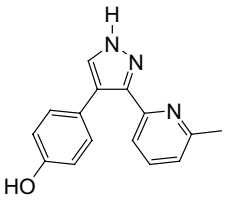

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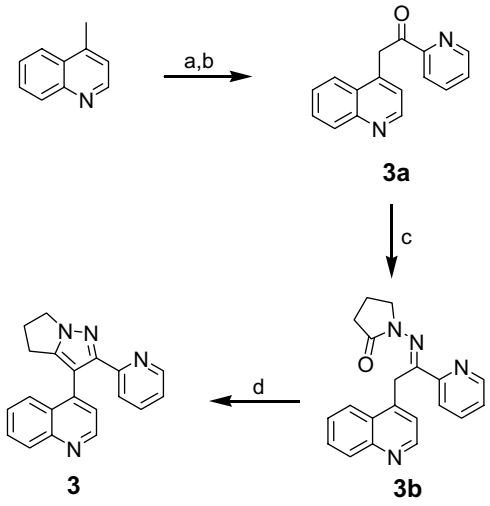
($IC_{50} = 31$ nM) from each of these sub-series with the TβR-I receptor kinase domain.[4] Recently, the X-ray co-crystallization analysis of **1** with the TβR-I receptor kinase domain was independently confirmed.[5]

We now disclose a new platform based on the 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole scaffold that features additional substitutions at the warhead ring systems as well as the 2-pyridyl group. As with our earlier work, compounds were evaluated as inhibitors of autophosphorylation of the isolated human TβR-I kinase domain in the form of a constitutively active construct (T204D mutation)[6] produced in Sf9 insect cells and purified by nickel-affinity chromatography. The synthesis and evaluation of di-heteroaryl-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole **3** revealed a potent inhibitor ($IC_{50} = 104$ nM, Table 1) and the platform was therefore chosen for further SAR development. The resulting compounds were also evaluated as inhibitors of TGF-β-dependent luciferase production in mink lung cells (p3TP Lux)[7] and growth in mouse fibroblasts (NIH 3T3).[8] Compounds were also evaluated as inhibitors of p38 MAP kinase as described previously.[4]

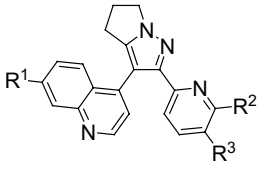


Scheme 1. Representative synthesis of 4-(quinolin-4-yl)-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazoles: (a) LiHMDS, THF; (b) methyl picolinate, 75% (for two steps); (c) 1-amino-2-pyrrolodinone, EtOH, py, 50%; (d) NaH, DMF, 50%.

The 4-(quinoline-4-yl)-substituted dihydropyrrolopyrazole analogues (Table 1) were prepared as illustrated for the synthesis of **3** (Scheme 1). Lepidine was deprotonated and condensed with an appropriate picolinic ester to provide the intermediate ketone **3a**, which was subsequently condensed with 1-amino-2-pyrrolidinone to provide the corresponding acyl hydrazone **3b**. Base-mediated cyclization of **3b** provided final compound **3**. Sulfone **12** was prepared via Oxone®-mediated oxidation of thioether **11**, which was made in turn by treatment of the corresponding 7-bromo-substituted quinoline[9] with ethanethiol. For the phenyl-substituted examples (Table 2), the corresponding ketones were made by deprotonation of the requisite benzyl nitriles followed by condensation with picolinic ester and subsequent decarboxylation (e.g., **15a**, Scheme 2). Similar to the quinolin-4-yl examples above, hydrazone formation and cyclization provided the final target compounds. The methoxy group in **21** was introduced via treatment of **15** with methoxide, which could then be demethylated with boron tribromide to provide **22**.

Exploration of the SAR of the quinolin-4-yl compounds (Table 1) revealed a number of interesting observations. While good enzyme activity was observed with the unsubstituted pyridyl-2-yl analogue **3**, better overall cellular activity was observed after the addition of a 6-methyl or 6-ethyl group (**4** and **5**). However, larger branched groups at this position (**6**) led to reduced activity. As in the original pyrazole series, substitution

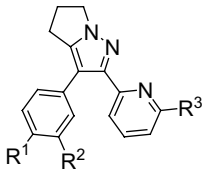
Table 1. 4-(Quinolin-4-yl)-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole inhibitors



Compd	R^1	R^2	R^3	TGF-βRIK IC_{50}, μM[a] (*n*)	p3TP Lux IC_{50}, μM[a] (*n*)	NIH 3T3 IC_{50}, μM[a] (*n*)	p38 MAPK IC_{50}, μM[b]
3	H	H	H	0.104 ± 0.033 (4)	0.345 ± 0.316 (11)	5.1 ± 8.2 (4)	7.9
4	H	Me	H	0.078 ± 0.017 (5)	0.094 ± 0.048 (8)	0.280 ± 0.176 (3)	0.68
5	H	Et	H	0.071 ± 0.048 (3)	0.143 ± 0.082 (3)	0.500 ± 0.310 (3)	0.62
6	H	Pr^i	H	1.3	2.0	2.0	15
7	H	H	F	0.151 ± 0.032 (4)	0.475 ± 0.148 (3)	0.664 ± 0.232 (3)	11
8	Cl	H	H	0.054 ± 0.018 (4)	0.196 ± 0.078 (4)	0.282 ± 0.067 (3)	1.8
9	Cl	Me	H	0.048 ± 0.024 (3)	0.093 ± 0.039 (5)	0.727 ± 0.500 (4)	0.44
10	OEt	Me	H	0.088 ± 0.061 (3)	0.086 ± 0.0017 (4)	0.152 ± 0.063 (3)	0.059
11	SEt	H	H	0.110 ± 0.017 (3)	0.285 ± 0.127 (4)	0.326 ± 0.058 (3)	1.6
12	SO_2Et	H	H	9.29	>20	—	9.8

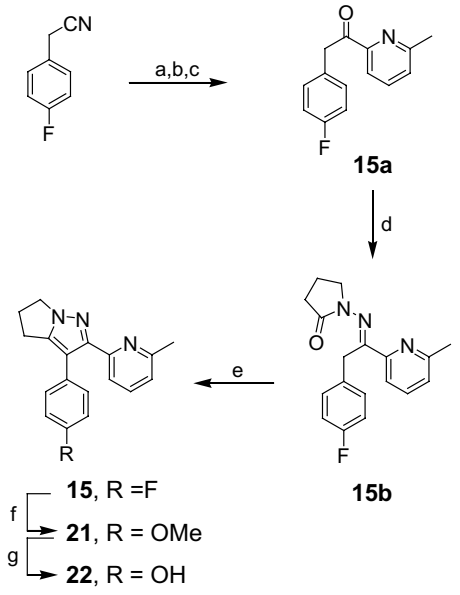
[a] Mean values ± SEM for a minimum of three determinations.
[b] 10-point IC_{50} determination.

Table 2. 4-Phenyl-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole inhibitors



Compd	R^1	R^2	R^3	TGF-βRIK IC_{50}, μM[a] (*n*)	p3TP Lux IC_{50}, μM[a] (*n*)	NIH 3T3 IC_{50}, μM[a] (*n*)	p38 MAPK IC_{50}, μM
13	Me	H	Me	4.0	>20	>20	>20[b]
14	F	H	H	1.32 ± 0.291 (3)	3.86 ± 2.57 (4)	3.33 ± 0.294 (3)	>20[b]
15	F	H	Me	0.175 ± 0.088 (4)	0.096 ± 0.016 (3)	0.339 ± 0.349 (4)	>20[b]
16	F	F	Me	0.343 ± 0.185(4)	1.51 ± 0.697(4)	1.04 ± 0.108 (3)	>20[b]
17	F	H	CF_3	0.877 ± 0.445 (4)	2.55 ± 1.15 (4)	1.20 ± 0.528 (3)	>20[b]
18	F	H	CH_2OH	2.62 ± 0.991 (4)	11.8 ± 3.73 (3)	5.62 ± 0.778 (3)	>20[b]
19	SO_2Me	H	Me	19.5	>20	>20	>20[b]
20	OMe	H	H	0.646 ± 0.288 (3)	6.83 ± 2.74 (4)	4.71 ± 2.76 (3)	>20[b]
21	OMe	H	Me	0.121 ± 0.074 (3)	0.304 ± 0.069 (4)	0.397 ± 0.123 (3)	4.6[c]
22	OH	H	Me	0.047 ± 0.006 (3)	0.022 ± 0.0036 (4)	0.105 ± 0.089 (4)	4.3[c]

[a] Mean values ± SEM for a minimum of three determinations.
[b] Single point determination.
[c] 10-point IC_{50} determination.



Scheme 2. Representative synthesis of 4-phenyl-substituted 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazole inhibitors: (a) NaH, THF; (b) 6-methylpicolinic methyl ester; (c) aq HCl, 50% for three steps; (d) 1-amino-2-pyrrolodinone, AcOH, py; (e) NaH, DMF, 25% for two steps; (f) NaOMe, 74%; (g) BBr_3, CH_2Cl_2, 71%.

of fluorine at the 5-position of pyridine (**7**) maintained good activity. The best overall activity was observed for compounds having electron-donating substituents at the 7-position of the quinolin-4-yl moiety (**8**–**11**), while oxidation of thioether **11** to the corresponding sulfone **12** led to a significant loss of activity. The general trend observed with the quinolin-4-yl substituted compounds was that greater activity against the TGF-β enzyme also correlated with greater p38 MAP kinase activity. With the exception of **8**, activity below 100 nM against TGF-β RI kinase corresponded to p38 activity of less than 1 μM, with the 7-ethoxy-substituted analogue **10** displaying an IC_{50} of 59 nM. Nitrogen substitutions at the 7-position have also been examined.[10]

As discussed above, compounds in the quinoline-4-yl series exhibited a range of activity as inhibitors of p38 MAPK. We addressed the issue of selectivity by substituting the quinolin-4-yl group with phenyl while keeping the pyridin-2-yl moiety intact (Table 2). While the 4-methylphenyl analogue **13** exhibited a significant loss of activity in kinase inhibition, the substitution of a 4-fluorophenyl group provided **15**, which possessed good activity in the enzyme assay. In addition, greatly decreased activity against p38 was noted with these compounds. Des-methyl **14** lost significant TβR-I activity relative to **15**. Lower cell activity was observed with the addition of a second fluorine (**16**), and overall activity was decreased when the 6-pyridyl substituent was modified (**17** and **18**).

As the above results were very similar to the original pyrazole series, we decided to once again focus on substitutions at the 4-position of the phenyl group. While the strongly electron-withdrawing sulfonyl group led to greatly decreased activity (**19**), substitution with methoxy gave good activity (**21**), while the hydroxy analogue **22** proved to be highly potent and selective against p38 MAP kinase. Relative to 6-methyl-substituted **21**, 6-hydrido-substituted **20** displayed decreased activity, as predicted.

Assessment of our previous pyrazole series suggested that the minimum requirements for tight binding at the active site consist of the presence of a 2-pyridyl group on the 3-position of the pyrazole ring 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 the 5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazoles **3** and **15** bound to the ATP site

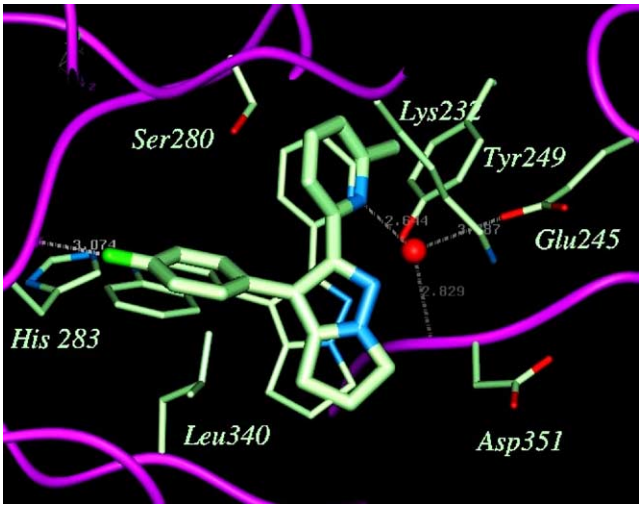


Figure 1. X-ray crystal structure of **3** (thin bonds) and **15** (thick bonds) bound to the ATP-binding site of the TβR-I kinase domain.

of the TβR-I kinase domain (Fig. 1).[11] In each structure, the pyridyl nitrogen is committed to a hydrogen bond at a water molecule held in place by additional hydrogen bonds to Asp-351, Glu-245, and Tyr-249. For quinoline-4-yl **3**, the quinoline nitrogen 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 **15**, where the hydrogen bond acceptor is the fluorine atom. There is enough flexibility at the active site to allow the shift needed to accommodate the additional spacing requirement demanded by the 4-substituted aryl substructure. The selectivity observed for the 4-phenyl-substituted series over p38 MAPK follows from the argument we advanced in our earlier study of the pyrazole scaffold.[4] Variations in the structure of the TβR-I hinge region allow for the key His-283 hydrogen bond to be formed with weaker acceptors, such as fluorine, while the hinge region of p38 requires a stronger acceptor, in this case quinoline nitrogen. There are also differences in the hydrophobic pocket gate-keeper residues between the two enzyme active sites that facilitate binding of the 4-phenyl-substituted series in the TβR-I domain in preference to that of p38.

Acknowledgements

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References and notes

1. (a) Nakamura, T.; Miller, D.; Ruoslahti, E.; Border, W. A. *Kidney Int.* **1992**, *41*, 1213; (b) Derynk, R.; Akhurst, R. J.; Balmain, A. *Nature Genet.* **2001**, *29*, 117; (c) Ray, S.; Broor, S. L.; Vaishnav, Y.; Sarkar, C.; Girish, R.; Dar, L.; Seth, P.; Broor, S. *J. Gastroent. Hepatol.* **2003**, *18*, 393; (d) McCaffrey, T. A. *Cytokine Growth Factor Rev.* **2000**, *11*, 103; (e) Chamberlain, J. *Cardiovasc. Drug Rev.* **2001**, *19*, 329; (f) Duvernelle, C.; Freund, V.; Frossard, N. *Pul. Pharmacol. Ther.* **2003**, *16*, 181.
2. (a) Huse, M.; Muir, R. W.; Xu, L.; Chen, Y.-G.; Kuriyan, J.; Massague, J. *Mol. Cell.* **2001**, *8*, 671; (b) Zimmerman, C. M.; Padgett, R. W. *Gene* **2000**, *249*, 17.
3. (a) Callahan, J. F.; Burgess, J. L.; Fornwald, J. A.; Gaster, L. M.; Harling, J. D.; Harrington, F. P.; Heer, J.; Kwon, C.; Lehr, R.; Mather, A.; Olson, B. A.; Weinstock, J.; Laping, N. J. *J. Med. Chem.* **2002**, *45*, 999; (b) Inman, G. J.; Nicolas, F. J.; Callahan, J. F.; Harling, J. D.; Gaster, L. M.; Reith, A. D.; Laping, N. J.; Hill, C. S. *Mol. Pharmacol.* **2002**, *62*, 65.
4. Sawyer, J. S.; Anderson, B. D.; Beight, D. W.; Campbell, R. M.; Jones, M. L.; Herron, D. K.; Lampe, J. W.; McCowan, J. R.; McMillen, W. T.; Mort, N.; Parsons, S.; Smith, E. C. R.; Vieth, M.; Weir, L. C.; Yan, L.; Zhang, F.; Yingling, J. M. *J. Med. Chem.* **2003**, *46*, 3953.
5. Singh, J.; Chuaqui, C. E.; Boriack-Sjodin, P. A.; Lee, W.-C.; Pontz, T.; Corbley, M. J.; Cheung, H.-K.; Arduini, R. M.; Mead, J. N.; Newman, M. N.; Papadatos, J. L.; Bowes, S.; Josiah, S.; Ling, L. E. *BioMed. Chem. Lett.* **2003**, *13*, 4355.
6. Wieser, R.; Wrana, J. L.; Massague, J. *EMBO J.* **1995**, *14*, 2199.
7. Wrana, J. L.; Attisano, L.; Carcamo, J.; Zentella, A.; Doody, J.; Laiho, M.; Wang, X. F.; Massague, J. *Cell* **1992**, *71*, 1003.
8. Leof, E. B.; Proper, J. A.; Goustin, A. S.; Shipley, G. D.; DiCorleto, P. E.; Moses, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 2453.
9. Beight, D. W.; Ciapetti, P.; DeCollo, T. V.; Godfrey, A. G.; Goodson, T.; Herron, D. K.; Li, H.-Y.; Liao, J.; McMillen, W. T.; Miller, S. C.; Mort, N. A.; Sawyer, J. S.; Smith, E. C. R.; Yingling, J. M. PCT Int. Appl. WO 0294833 A1, 2002; *Chem. Abstr.* **2002**, *138*, 4598.
10. Li, H.-Y.; Wang, Y.; Yan, L.; Campbell, R. M.; Anderson, B. D.; Wagner, J. R.; Yingling, J. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, following paper in this issue. doi:10.1016/j.bmcl.2004.04.065.
11. Crystals were grown in a hanging drop plate by mixing 4.5 mg/mL TβR-I kinase at a 1:1 (v/v) ratio with a solution containing 30% (w/v) PEG-4000, 100 mM Tris–HCL, pH 8.5, 200 mM Li_2SO_4, 5 mM DTT at room temperature. Compounds were soaked into the crystals at 10 M excess for 48 h. X-ray diffraction data were collected at −170 °C with a Mar CCD detector at the IMCA beam line ID-17 at Advanced Photon Source in Argonne National Laboratories. The structure was solved by molecular replacement and refined by program CNX.