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Novel and potent transforming growth factor beta type I receptor kinase domain inhibitor: 7-amino 4-(2-pyridin-2-yl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-3-yl)-quinolines

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Abstract—A novel series of 7-amino 4-(2-pyridin-2-yl-5,6-dihydro-4*H*-pyrrolo[1,2-*b*]pyrazol-3-yl)-quinolines was synthesized and their TβR-1 inhibitory, p38 MAPK inhibitory, and TβR-1-dependent cellular activity were evaluated. Compound **5a** was found to be a highly potent in the enzyme assay and TβR-1-dependent cellular assays. In addition, dimer (**4g**), with a urea linker, shows a similar enzyme and cellular activity despite a bulky substitution.

In the preceding paper[1] we have described SAR studies of a dihydropyrrolo-pyrazole-quinoline series of the transforming growth factor beta Type I receptor kinase domain (TβR-1) inhibitors, including compounds with *S* and *O* linkages at 7-positions of quinoline-4-yl. In the further elaboration of this series, we have discovered that dimer **4g**, containing a urea linkage, shows excellent activity at the TβR-1 active site. We therefore concluded that the 7-position of the quinolin-4-yl had the potential to accommodate a variety of substitutions, which might improve the potency and ADME profile of our series. In this paper, we would like to report the systematic SAR studies of 7-amino-derivatives of the quinoline-4-yl group.[2]

The general synthetic approach to the 7-amino derivatives is outlined in Scheme 1. The key step in the synthesis of these compounds involved the conversion of the 7-bromo-substituted **1** to the 7-amino-substituted **2**. This was accomplished by conversion of bromide **1**[2b] to the benzophenone enamine with benzophenone imine in the presence of sodium *tert*-butoxide, $Pd_2(dba)_3$, and BINAP at 80 °C in toluene for one day under argon atmosphere.[3] Subsequently, the benzophenone enamine was hydrolyzed to amine **2** by refluxing in 1 N HCl. The overall yield for two steps was 95%. The starting materials for the preparation of **3a** and **3b**, dimethylamino-acetyl chloride and 3-dimethylamino-propionyl chloride, were synthesized by the treatment of dimethylamino-acetic acid and 3-dimethylamino-propionic acid with $SOCl_2$, respectively. The preparation of methanesulfonamide **3c** and acetamide **3d** was straightforward. However, the synthesis of amides **3a** and **3b** required the harsh conditions of refluxing amine **2** in pyridine with acyl chlorides (~50 equiv) in the presence of catalytic DMAP for three days. The reaction yields were 30–70% due to the decomposition of amine **2** under these conditions. Similarly, the key intermediate for the synthesis of ureas **4a**–**f**, carbamic chloride, required a large excess phosgene (50 equiv). A small amount dimer **4g** was still detected by LC–MS spectrometer under these conditions. With 2 or 3 equiv of phosgene, dimer **4g** was

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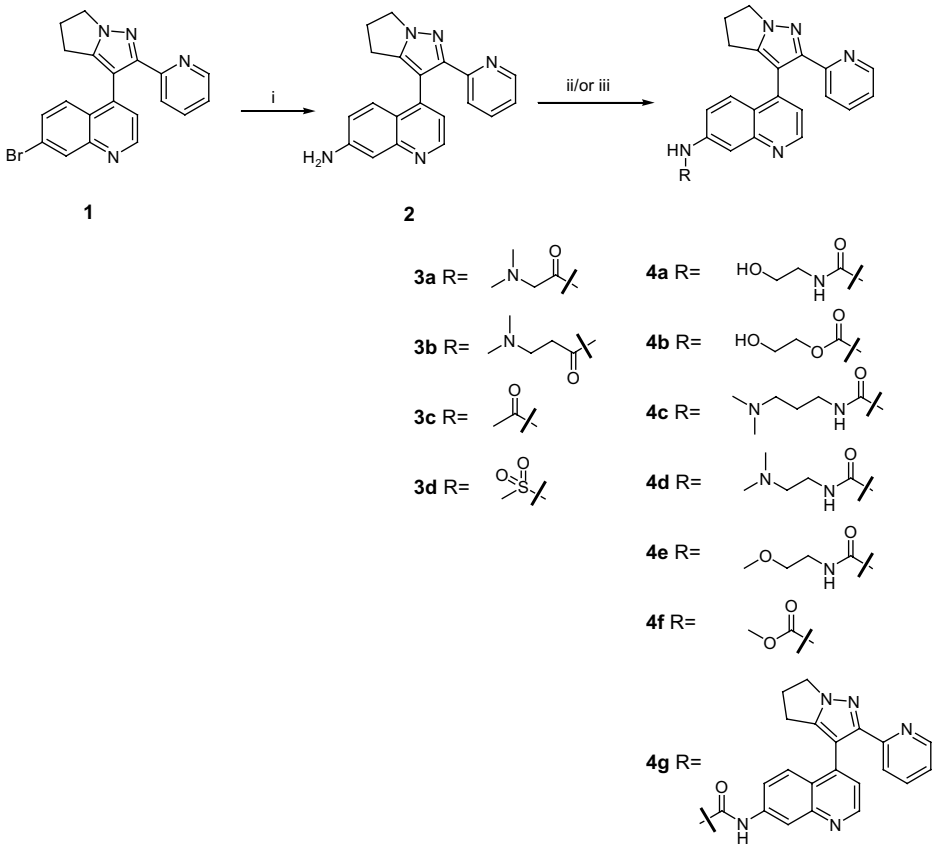
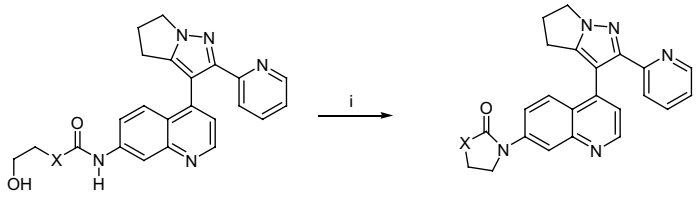


Scheme 1. Reagents and conditions: (i) (a) $(C_6H_5)_2C{=}NH$, $Pd_2(dba)_3$, BINAP, $NaOCH(CH_3)_2$, toluene (95%); (b) 1 N HCl, refluxed; (ii) for compound **3**, R–COCl, DMAP, pyridine, refluxed (20–70%); (iii) for compound **4** (a) ClCOCl, DMAP, pyridine, refluxed; (b) $R–NH_2$/R–OH (50–70%).

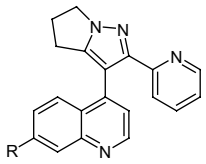
isolated as the major product. The ureas **4a** and **4b** were cyclized under standard Mitsunobu conditions to give **5a** and **5b** in 30–40% isolated yield (Scheme 2).

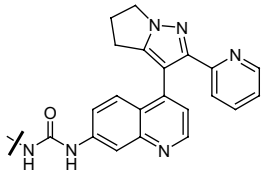
The TβR-1 enzyme inhibitory activity of amine **2** and its derivatives were determined by measuring the phosphorylation of the isolated human TβR-1 kinase domain in the form of a constitutively active construct (T204D mutation) produced in Sf9 insect cells and purified by nickel-affinity chromatography.[4] A standard compound, 4-(3-pyridin-2-yl-1*H*-pyrazol-4-yl)-quinoline was used for the calibration of assay results.[5] Table 1 summarizes the TβR-1 inhibitory activity of 7-derivatives of amine **2**.[6] The most important SAR finding was that the 7-position of quinoline-4-yl tolerates an array of substitutions including amides, ureas, cyclized ureas and carbamates, in addition to a dimer through a urea linkage. Three amides (**3a**–**c**) did show TβR-1 activities ($IC_{50} = 64$, 25, and 87 nM, respectively), that were very similar to the activity of the parent amine **2** ($IC_{50} = 59$ nM). Ureas **4a**–**f**, except for **4c**, exhibited less potency ($IC_{50} = 255$ nM), while the remaining compounds showed inhibitory activities of less than 100 nM. Cyclized compounds **5a** and **5b** had activity similar to their precursors **4a** and **4b**. More significantly, dimer **4g** demonstrated excellent TβR-1 inhibition, indicating that the 7-position may be oriented out from the binding pocket toward solvents. This result is consistent with the X-ray crystallography analysis of TβR-1 pyrazole inhibitors.[1,2b] The low potency for compound **3d** ($IC_{50} = 2040$ nM) may imply the importance of electron density on the quinoline nitrogen for TβR-1 inhibitory activity.



Scheme 2. Reagents and conditions: (i) PPh_3, DEAD, THF (30–40%).

Table 1. Kinase and cellular activity



Compd	R	TβR-1 IC_{50}, μM[a]	p38 MAPK IC_{50}, μM[a]	p3TP Lux IC_{50}, μM (n)[b]	NIH 3T3 IC_{50}, μM (n)[b]
2	NH_2	0.059	2.57	0.072 (3)	0.170 (3)
3a	$NHCOCH_2N(CH_3)_2$	0.064	1.46	0.039 (2)	0.137 (2)
3b	$NHCOCH_2CH_2N(CH_3)_2$	0.025	2.46	0.034 (2)	0.305 (2)
3c	$NHCOCH_3$	0.087	1.34	0.224 (2)	0.727 (2)
3d	$NHS(O)_2CH_3$	2.34	nt	nt	nt
4a	$NHCONH(CH_2)_2OH$	0.032	1.08	0.085 (2)	0.710 (2)
4b	$NHCOO(CH_2)_2OH$	0.079	0.616	0.541 (3)	0.375 (3)
4c	$NHCONH(CH_2)_3N(CH_3)_2$	0.255	nt	3.36 (3)	2.34 (3)
4d	$NHCONH(CH_2)_2N(CH_3)_2$	0.018	0.867	0.129 (3)	3.46 (3)
4e	$NHCONH(CH_2)_2OCH_3$	0.034	1.05	0.330 (3)	0.567 (3)
4f	$NHCOOCH_3$	0.040	0.526	0.843 (3)	0.245 (3)
4g		0.106	4.64	>20 (2)[c]	0.176 (2)
5a		0.043	2.06	0.016 (3)	0.059 (3)
5b		0.028	nt	0.185 (3)	1.47 (3)

nt = not tested.
[a] 10 points IC_{50} determination.
[b] 12 points IC_{50} determination.
[c] Single point test in duplication.

The p38 MAP kinase selectivity issue has been one of the major challenges for the development of TβR-1 inhibitors.[1,5] By introducing substitutions at the 7-position of quinoline-4-yl, moderate to good selectivity was achieved. All 12 potent TβR-1 inhibitors (IC_{50} <100 nM) showed >15-fold selectivity versus p38 MAPK. Compounds **2**, **3b**, and **4d** were especially good, exhibiting about 50-fold selectivity.

In addition to the TβR-1 inhibitory activity discussed above, the synthesized 7-amino-derivatives were also studied for their cellular activities in two cell lines p3TP Lux[7] and NIH3T3.[8] The enzyme potency of this series did not always correlate with the observed cellular activities. Compounds **3c**, **4a**, **4d**, and **5b** were very active in the p3TP Lux assay, while compounds **4f** and **4g** exhibited poor activity. Only compounds **2**, **3a**, **3b**, and **5a** showed good activity on both cellular assays. It is worth mentioning that the excellent cellular activities (p3TP Lux: $IC_{50} = 16$ nM and NIH 3T3: $IC_{50} = 59$ nM) were observed for **5a**. The above results support the theory that the compounds with different substitutions at the 7-position of quinoline-4-yl affect cell permeability properties, and therefore not all of the highly potent enzyme inhibitors show good activity in cells.

In summary, we have described a synthetic method for making 7-amino-analogues of quinoline-4-yl. These compounds were found to represent a very potent series of TβR-1 inhibitors with reasonable selectivity over p38 MAPK. In addition, good cellular activity was observed for several compounds, which indicates that small molecules might have the potential to be active in vivo.

Acknowledgements

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References and notes

1. For other TβR-1 inhibitors, see: Sawyer, J. S.; Beight, D. W.; Britt, K. S.; Anderson, B. D.; Campbell, R. M.; Goodson, T., Jr.; Herron, D. K.; Li, H.-Y.; McMillen, W. T.; Mort, N.; Parsons, S.; Smith, E. C. R.; Wagner, J. R.; Yan, L.; Zhang, F.; Yingling, J. M. *Bioorg. Med. Chem. Lett*. **2004**, *14*, preceding paper, doi:10.1016/j.bmcl.2004.04.007.

2. (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*, 999–1001; (b) 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*, 3953–3956; (c) Sawyer, J. S.; Beight, D. W.; Ciapetti, P.; Decollo, T. V.; Godfrey, A. G.; Goodson, T.; Herron, D. K.; Li, H.; Liao, J.; McMillen, W. T.; Miller, S. C.; Yingling, J.; Smith, E. C. PCT Int. Appl. WO 0294833, 2002.
3. Prashad, M.; Hu, B.; Lu, Y.; Draper, R.; Bar, D.; Repic, O.; Blacklock, T. J. *J. Org. Chem.* **2000**, *65*, 2612–2614.
4. Wieser, R.; Wrana, J. L.; Massague, J. *EMBO J.* **1995**, *14*, 2199–2208.
5. (a) Minami, N.; Sato, M.; Hasumi, K.; Yamamoto, N.; Keino, K.; Matsui, T.; Kanada, A.; Ohta, S.; Saito, T.; Sato, S.; Asagarasu, A.; Doi, S.; Kobayashi, M.; Sato, J.; Asano, H. PCT Int. Appl. WO 0075131, 2000; (b) Anantanarayan, A.; Clare, M.; Collins, P.; Crich, J.; Devraj, R.; Flynn, D. L.; Geng, L.; Graneto, M. J.; Hanau, C. E.; Hanson, G. J.; Hartmann, S. J.; Hepperle, M.; Huang, H.; Khanna, I. K.; Koszyk, F. J.; Liao, S.; Metz, S.; Partis, R. A.; Perry, T. D.; Rao, S. N.; Selness, S. R.; South, M. S.; Stealey, M. A.; Talley, J. J.; Vazquez, M. L.; Weier, R. M.; Xu, X.; Yu, Y. PCT Int. Appl. WO 0031063, 2000; (c) Salituro, F. G.; Germann, U. A.; Wilson, K. P.; Bemis, G. W.; Fox, T.; Su, S.-S. *Curr. Med. Chem.* **1999**, *6*, 807–823.
6. Differences in rank-order potency between the enzyme and cell-based assays are due to physiochemical differences among the compounds tested, in addition to the nature of the enzyme assay (autophosphorylation) versus that of the cellular assays (substrate-phosphorylation).
7. Wrana, J. L.; Attisano, L.; Carcamo, J.; Zentella, A.; Doody, J.; Laiho, M.; Wang, X. F.; Massague, J. *Cell* **1992**, *71*, 1003–1014.
8. Leof, E. B.; Prpper, J. A.; Goustin, A. S.; Shipley, G. D.; DiCorleto, P. E.; Moses, H. L. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *71*, 1003–1014.