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Design, synthesis, and evaluation of novel 4-thiazolylimidazoles as inhibitors of transforming growth factor-β type I receptor kinase

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ABSTRACT

A novel series of 4-thiazolylimidazoles was synthesized as transforming growth factor-β (TGF-β) type I receptor (also known as activin receptor-like kinase 5 or ALK5) inhibitors. These compounds were evaluated for their ALK5 inhibitory activity in an enzyme assay and their TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay. *N*-{[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}butanamide **20**, a potent and selective ALK5 inhibitor, exhibited good enzyme inhibitory activity (IC_{50} = 8.2 nM) as well as inhibitory activity against TGF-β-induced Smad2/3 phosphorylation at a cellular level (IC_{50} = 32 nM).

Transforming growth factor-β (TGF-β) is a cytokine that plays important roles in the regulation of a variety of physiological processes. It forms part of a family of cytokines that are involved in cell growth and differentiation, matrix expression, and embryonic development. TGF-β belongs to the TGF-β superfamily, which includes TGF-β1, TGF-β2, TGF-β3, activins, inhibins, and bone morphogenetic proteins. TGF-β signals through two types of transmembrane serine/threonine kinase receptors, namely the TGF-β type I receptor and the type II receptor (TGF-βRI and TGF-βRII, respectively). TGF-βRI is also known as activin receptor-like kinase 5 (ALK5). TGF-β binding to TGF-βRII recruits and is followed by its association with ALK5. Activated ALK5 in turn phosphorylates and activates transcription factors Smad2/3, allowing them to bind to the commonly mediated Smad4. These Smad complexes translocate into the nucleus to affect gene transcription.[1–3] The deregulation of TGF-β signaling has been implicated in various human diseases, such as, fibrosis,[4] atherosclerosis,[5] and cancer.[6] Therefore, the inhibition of ALK5 seems to be a good strategy for the treatment of these diseases.

Many research groups have reported small-molecule inhibitors of ALK5 (Fig. 1).[3,7–15] According to the published literature on this topic, a typical hydrogen bond acceptor, usually the 2-pyridyl group, forms a water-mediated hydrogen bond with the side chains of Tyr-249 and Glu-245 as well as the backbone of Asp-351. Initially, we took into consideration of the hydrogen bond ability and the van der Waals' radius and designed five- and six-membered heterocycles as an alternative for 2-pyridyl group. Using a docking model, which we built based on the X-ray structure of ALK5 co-crystallized with its inhibitors,[13,16] we predicted that the thiazolyl group might be able to bind in a manner similar to that of the 2-pyridyl group (Fig. 2). Therefore, we synthesized a series of 4-thiazolylimidazoles to verify this hypothesis.

The compounds appearing in Table 1 were synthesized according to Scheme 1. Commercially available 5-ethynyl-1,3-benzodioxole **1** was used as the starting material and was reacted with either bromothiazoles (**2a**, **2c**–**e**) or iodothiazole **2b** under reflux to give acetylenes **3a–e**. The oxidation of acetylenes **3a–e** with DMSO and $PdCl_2$ afforded α-diketones **4a–e**. The resulting α-diketones **4a–e** were reacted with 4-formylbenzonitrile and ammonium acetate in acetic acid under reflux to give imidazole analogues **5a–e**. The cyano group in **5a–e** was hydrolyzed with KOH in *t*-BuOH under reflux to afford benzamide analogues **6a–e**.

The synthesis of 2-substituted-4-(4-methylthiazol-2-yl)imidazole compounds is summarized in Schemes 2 and 3. Cyclization of α-diketone **4b** with aldehydes **7a–c** and ammonium acetate under reflux followed by deprotection of the phthalimido group with hydrazine monohydrate gave amine analogues **8a–c**. The resulting amines **8a–c** were coupled to carboxylic acids (R^2-CO_2H) in the presence of EDC·HCl or DCC and HOBt·H_2O to give reverse amide analogues **9a–d**. The reaction of α-diketone **4b** with aldehydes **10a–d** and ammonium acetate under reflux followed by hydrolysis with NaOH gave carboxylic acids **11a–d**. The carboxylic acids

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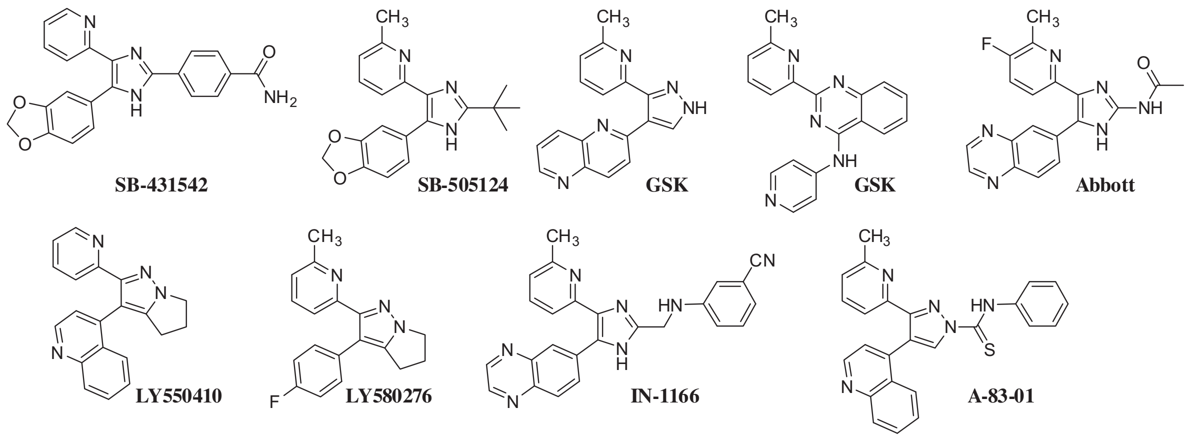


Figure 1. Representative ALK5 inhibitors.

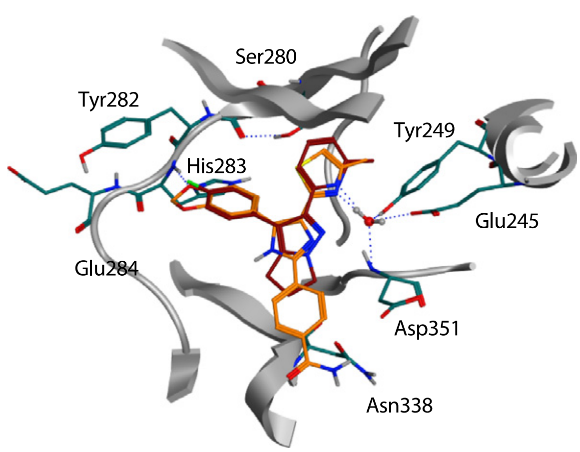
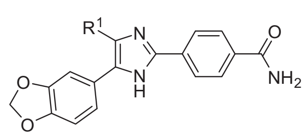
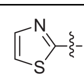
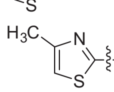
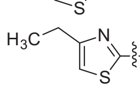
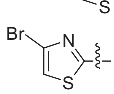
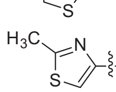


Figure 2. X-ray crystal structure of LY580276 (brown) (PDB code: 1RW8[13]) and the predicted binding mode of **6b** (orange) bound to the ATP site of ALK5.

Table 1
Inhibitory profile of 4-thiazolylimidazoles **6a**–**e**



Compound	R^1	IC_{50} (nM)	
		ALK5[a]	Smad2/3[a]
6a		1100	2700
6b		54	170
6c		340	760
6d		270	390
6e		370	680

[a] Values are the mean of two or more separate experiments.

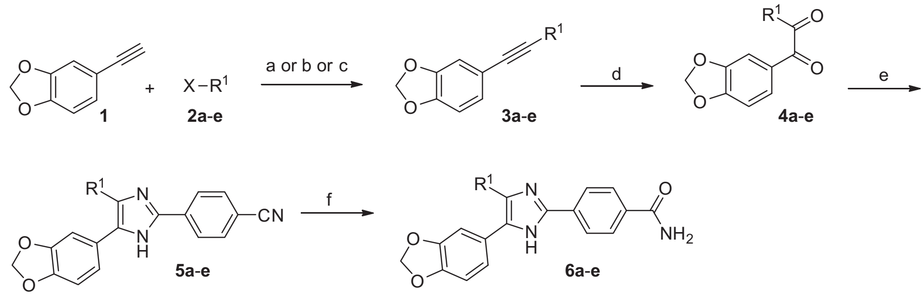
11a–d were reacted with amines (R^4-NH_2) to afford amide analogues **12a–f**. Alternatively, the carboxylic acid **11d** was reacted with $SOCl_2$ under reflux to give the corresponding acid chloride. The reaction of the resulting acid chloride with aqueous ammonia gave pentanamide analogue **12g** (Scheme 2).

Commercially available 2-(1,3-benzodioxol-5-yl)acetic acid **13** was converted to Weinreb amide. The Weinreb amide was reacted with an anion of 4-methylthiazole generated by treatment with *n*-BuLi to obtain ketone **14**. Treatment of **14** with copper(II) bromide followed by reaction with *N*-acetylguanidine under reflux gave imidazole analogue **15**. Deprotection of the acetyl group under acidic conditions gave 2-aminoimidazole analogue **16**. Acylation of the amino group in **16** with the corresponding acid chloride (R^5-COCl) gave **17a** and **17b** (Scheme 3).

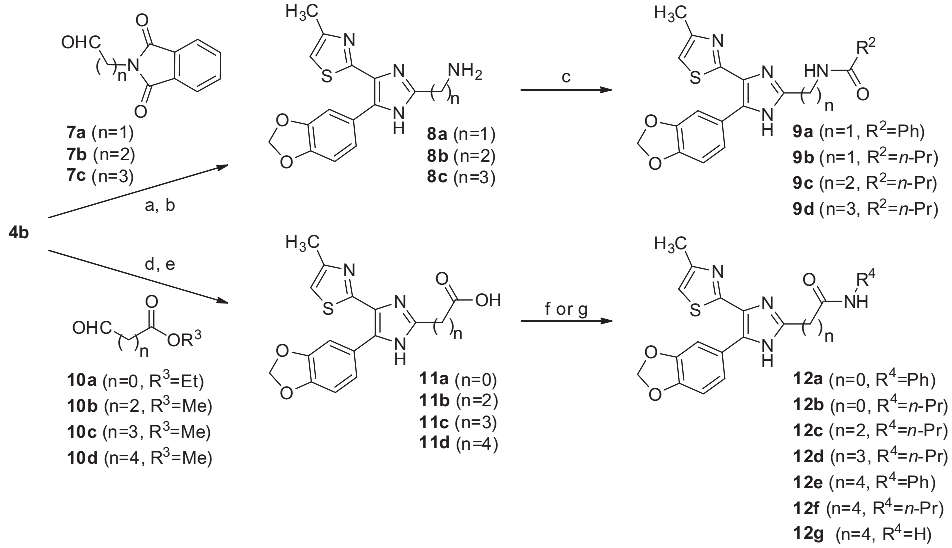
The imidazole analogues **18**–**20** were prepared from 2-ethynyl-4-methylthiazole **21**, 1-ethynyl-4-fluorobenzene **22**, 6-ethynyl-1,3-benzothiazole **23**, and the corresponding reagents using the same reaction conditions shown in Schemes 1 and 2. However, the reaction of acetylene **24c** with DMSO in the presence of $PdCl_2$ gave α-diketone **25c** in a very low yield (6%). The oxidation of **24c** was accomplished by reaction with $KMnO_4$ in a mixed solvent of acetone and buffer ($NaHCO_3$, $MgSO_4$ in H_2O) to afford α-diketone **25c** in 73% yield (Scheme 4).[17]

All the compounds were evaluated for their ALK5 inhibitory activity in an enzyme assay[18] and their TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay.[19] The unsubstituted thiazole ring analogue **6a** showed a tolerable potency. The introduction of a methyl group at the 4-position of the thiazole ring markedly increased ALK5 inhibition in the enzyme inhibitory activity and cellular activity assays (**6b**). The introduction of either an ethyl group or a bromo group at this position resulted in a moderate potency (**6c** and **6d**). Replacement of the 4-methylthiazol-2-yl group with a 2-methylthiazol-4-yl group led to a decrease in the enzyme inhibitory activity and the cellular activity. Therefore, we identified **6b** as the initial lead compound (Table 1).

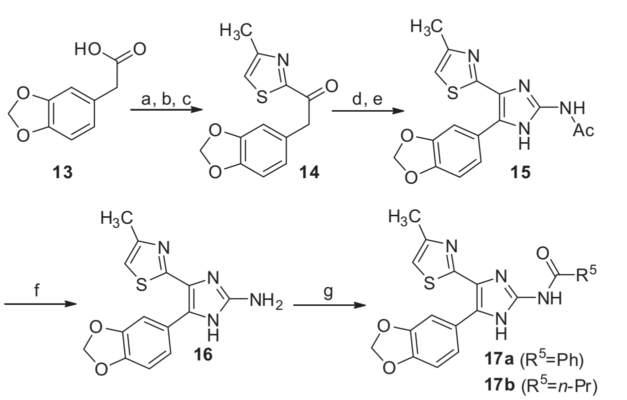
Subsequently, we focused on developing SAR with respect to the benzamide moiety of the initial lead **6b**. Replacement of the benzamide moiety was expected to improve the physicochemical properties. The SAR observed with a substituent at the 2-position of the imidazole ring are summarized in Table 2. The introduction of aminoalkyl groups significant decreased the inhibition activity, even though the potency of the 2-aminoimidazole analogue **16** was maintained (**8a–c** vs **16**). These results suggest that the aromatic amine **16** with a weak basicity is stronger for ALK5 kinase inhibition than the aliphatic amines **8a–c** with basicity. Conversion from the amino group in **16** to either a benzamido group or an



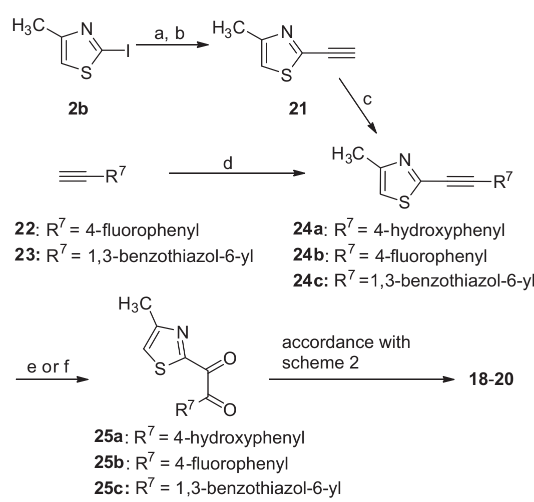
Scheme 1. Reagents and conditions: (a) preparation of **3a**: $PdCl_2(PPh_3)_2$, CuI, NEt_3, $CHCl_3$, reflux; (b) preparation of **3b**, **3c**, and **3e**: $Pd(PPh_3)_4$, NEt_3, CH_3CN, reflux; (c) preparation of **3d**: $Pd(PPh_3)_4$, CuI, $NH(i\text{-}Pr)_2$, THF, rt; (d) $PdCl_2$, DMSO, 125 °C; (e) 4-formylbenzonitrile, NH_4OAc, AcOH, reflux; (f) KOH, *t*-BuOH, reflux.



Scheme 2. Reagents and conditions: (a) **7a–c**, NH_4OAc, THF-MeOH, reflux; (b) $N_2H_4{\cdot}H_2O$, EtOH, reflux; (c) R^2-CO_2H, EDC·HCl or DCC, HOBt·H_2O, DMF, rt; (d) **10a–d**, NH_4OAc, THF–MeOH, rt or reflux; (e) NaOH, MeOH–H_2O, reflux; (f) preparation of **12a–f**: R^4-NH_2, EDC·HCl or DCC, HOBt·H_2O, DMF, rt; (g) preparation of **12g**: (i) $SOCl_2$, $CHCl_3$, reflux; (ii) NH_4OH, $CHCl_3$, rt.



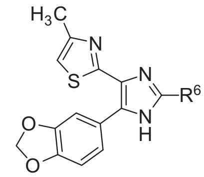
Scheme 3. Reagents and conditions: (a) $SOCl_2$, toluene, 60 °C; (b) *N*,*O*-dimethylhydroxylamine hydrochloride, NaOH, H_2O–toluene, 0 °C-rt; (c) 4-methylthiazole, *n*-BuLi, THF, −78 °C; (d) $CuBr_2$, AcOEt, reflux; (e) *N*-acetylguanidine, CH_3CN, reflux; (f) H_2SO_4, MeOH–H_2O, reflux; (g) R^5-COCl, pyridine, rt or reflux.

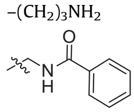
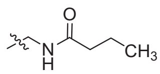
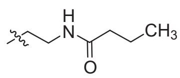
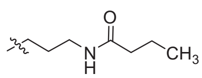
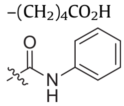
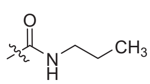
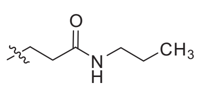
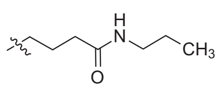
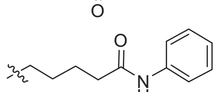
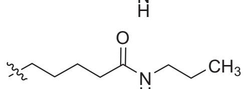
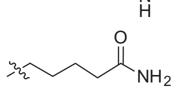
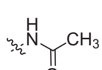
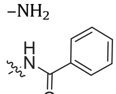
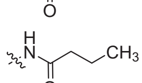
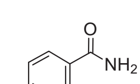


Scheme 4. Reagents and conditions: (a) trimethylsilylacetylene, $PdCl_2(PPh_3)_2$, CuI, NEt_3, reflux; (b) K_2CO_3, MeOH, rt; (c) 4-iodophenol, $PdCl_2(PPh_3)_2$, CuI, NEt_3, reflux; (d) **2b**, $Pd(PPh_3)_4$, NEt_3, CH_3CN, reflux; (e) $PdCl_2$, DMSO, 125 °C; (f) $KMnO_4$, acetone-buffer ($NaHCO_3$, $MgSO_4$, H_2O), rt.

alkanamido group resulted in a large loss in enzyme and cellular potency (**15**, **17a**, and **17b**). Interestingly, replacement of the amino group in **8a** with either a benzamido or a butanamido group that had a non-basicity resulted in good potency (**9a** and **9b**). However butanamide analogues **9c** and **9d** with a longer linker chain compared with **9b** showed a decreased potency. On the other hand, the aromatic carboxylic acid analogue **11a** showed a moderate enzyme inhibitory activity. As the linker chain grew in length, the aliphatic carboxylic acid analogues **11b–d** displayed an

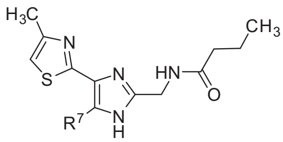
Table 2
Inhibitory profile of 4-(4-methylthiazol-2-yl)imidazoles **8a–c**, **9a–d**, **11a–d**, **12a–g**, **15**, **16**, **17a**, and **17b**

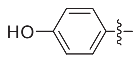
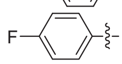
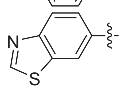
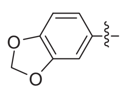


Compound	R^6	IC_{50} (nM)	
		ALK5[a]	Smad2/3[a]
8a	$-CH_2NH_2$	790	3600
8b	$-(CH_2)_2NH_2$	2200	1900
8c	$-(CH_2)_3NH_2$	810	1000
9a		150	680
9b		63	350
9c		420	1600
9d		600	1400
11a	$-CO_2H$	190	NE[b]
11b	$-(CH_2)_2CO_2H$	530	NE[b]
11c	$-(CH_2)_3CO_2H$	130	NE[b]
11d	$-(CH_2)_4CO_2H$	30	NE[b]
12a		>1000[c]	1700
12b		>1000[c]	3900
12c		380	1000
12d		510	1700
12e		170	830
12f		130	750
12g		110	730
15		1100	1900
16	$-NH_2$	90	280
17a		>4000	NE[b]
17b		>4000	2100
6b		54	170

[a] Values are the mean of two or more separate experiments.
[b] Not evaluated.
[c] The right IC_{50} values could not be calculated because of their low solubility.

Table 3
Inhibitory profile of 4-(4-methylthiazol-2-yl)imidazoles **18**–**20**



Compound	R^7	IC_{50} (nM)	
		ALK5[a]	Smad2/3[a]
18		130	1200
19		1800	6400
20		8.2	32
9b		63	350

[a] Values are the mean of two or more separate experiments.

increase in potency. The amide group, which was directly linked to the imidazole ring, displayed a significant loss in enzyme and cellular potency (**12a** and **12b**). Conversion from the most potent aliphatic carboxylic acid analogue **11d** to pentanamide analogue **12g** was tolerable with regard to the enzyme inhibition activity. The introduction of either a phenyl group or a *n*-propyl group to a terminally carbamoyl group in **12g** resulted in a slight decrease in potency (**12g** vs **12e** and **12f**). The *N*-propylamide analogues **12c** and **12d** showed weak potency compared with the corresponding **12f**. We thought that the position of the amide linkage and an appropriate number of atoms in the linker were very important for potency (**9b** vs **9c**, **9d**, **12b**–**d**, **12f**, **15**, and **17b**). Compound **9b** maintained the inhibitory activities; furthermore, **9b** (15.7 μg/mL) was about 170 times more soluble in water than **6b** (0.09 μg/mL).

Next, we investigated the effect of the substituent at the 5-position of the imidazole ring. The ALK5 inhibitory activities of these compounds are summarized in Table 3. Replacement of the 1,3-benzodioxol-5-yl group with a 4-hydroxyphenyl group led to the loss of inhibition activity in a cell-based assay, even though it showed a potent enzyme inhibitory activity (**18**). This result might be due to its low membrane permeability. The introduction of a 4-fluorophenyl group displayed poor activities in both the enzyme and the cell-based assay (**19**). The introduction of a 1,3-benzothiazol-6-yl group resulted in an approximately eightfold increase in the enzyme inhibition activity and an elevenfold increase in the cellular activity, compared with the 1,3-benzodioxol-5-yl group (**20** vs **9b**). Compound **20** (18.1 μg/mL) had a solubility in water that was about 200 times higher than that of the initial lead **6b**.

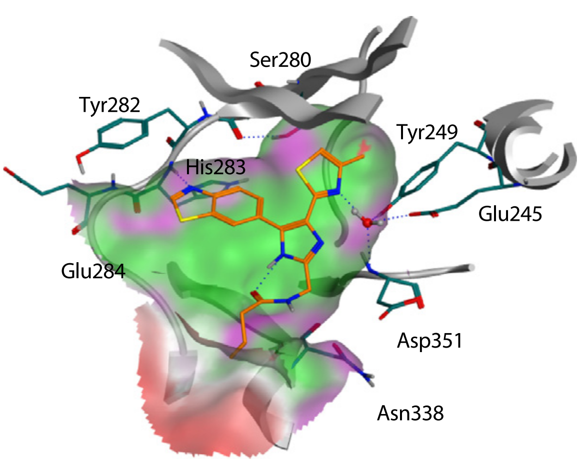


Figure 3. Predicted binding mode of **20** bound to the ATP site of ALK5. Hydrogen bonds are shown as dotted lines. The protein surface is colored according to the residue type (magenta, polar; green, hydrophobic; red, exposed).

To examine the binding mode of **20** in the ATP binding site of ALK5, **20** was docked into the molecular model.[13,16] As shown in Figure 3, the benzothiazole ring binds to the hinge region and accepts a hydrogen bond from the backbone NH of His-283. The 4-methylthiazol-2-yl nitrogen atom forms water-mediated networks of hydrogen bonds with the carboxy oxygen of Glu-245, the hydroxy hydrogen of Tyr-249, and the backbone NH of Asp-351. The amide moiety at the 2-position of the imidazole ring is assumed to form a ring through an intramolecular hydrogen bond in the sugar region. Alternatively, many polar residues exist near the entrance of the pocket, and the amide moiety is considered to be committed to an additional hydrogen bond to polar residues or a water-mediated hydrogen bond to them without ring formation.[20]

Compound **20**, the most potent analogue, was evaluated for selectivity using a diverse kinase panel.[21] Compound **20** was highly selective against most of the kinases; however, moderate inhibition was observed for KDR (94%), HGK (75%), CK1δ (63%), LYN (60%), LCK (58%) and p38α (57%) at 10 μM.

In conclusion, we have described that the synthesis, enzyme inhibitory activity, inhibitory activity against TGF-β-induced Smad2/3 phosphorylation at the cellular level, SAR, and proposed ALK5 binding mode of a novel series of 4-thiazolylimidazoles. We found that the thiazolyl group could be replaced with 2-pyridyl group. The improvement of the physicochemical properties was achieved by replacement of the benzamide moiety with an alkylamide moiety. Compound **20** showed the most ALK5 inhibitory activity and also was highly selective for other kinases.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.066.

References and notes

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18. A description of the assay conditions for ALK5 inhibitory activity at the enzyme level can be found in Supplementary data.
19. A description of the assay conditions for inhibitory activity against TGF-β-induced Smad2/3 phosphorylation in whole cells can be found in Supplementary data.
20. The calculated conformation of **20** can be found in Supplementary data.
21. A description of the assay conditions for the kinase selectivity profile can be found in Supplementary data.