Synthesis and Biological Evaluation of 4(5)-(6-Alkylpyridin-2-yl)imidazoles as Transforming Growth Factor-β Type 1 Receptor Kinase Inhibitors

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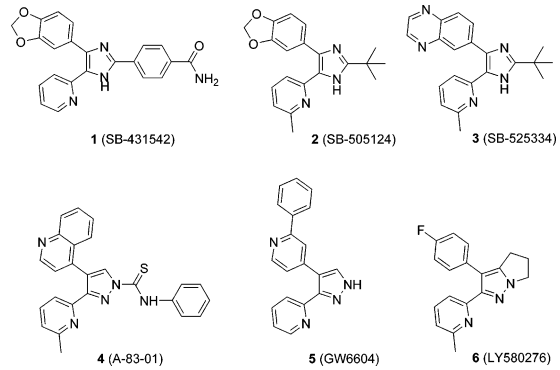
A series of 4(5)-(6-alkylpyridin-2-yl)imidazoles **13a**−**p**, **17a**, and **17b** have been synthesized and evaluated for ALK5 inhibitory activity in an enzyme assay and in cell-based luciferase reporter assays. The quinoxalinyl analogue **13e** inhibited ALK5 phosphorylation with an IC_{50} of 0.012 μM and showed more than 90% inhibition at 0.05 μM in a luciferase reporter assay using HaCaT cells transiently transfected with p3TP-luc reporter construct. The binding mode of **13e** generated by flexible docking studies shows that **13e** fits well into the active site cavity of ALK5 by forming several tight interactions.

Introduction

The transforming growth factor-β (TGF-β[a]) family has a pivotal role in the regulation of a variety of physiological processes. Three TGF-β isoforms (TGF-β1, TGF-β2, and TGF-β3) are expressed in mammals, and each is encoded by a unique gene and expressed in a tissue-specific manner. TGF-β1 is the prototypic member of this family of cytokines that signals through two highly conserved single transmembrane serine/threonine kinases, the type I and type II TGF-β receptors (TβR-I and TβR-II, respectively). Upon ligand-induced oligomerization, TβR-II hyperphosphorylates serine/threonine residues in the GS region of the TβR-I or activin receptor-like kinase 5 (ALK5), which leads to activation of TβR-I by creating a binding site for Smad2/Smad3 proteins. The activated ALK5 in turn phosphorylates Smad2/Smad3 proteins at the C-terminal SSXS-motif, thereby causing dissociation from the receptor and heteromeric complex formation with Smad4. These Smad complexes translocate into the nucleus and assemble with specific DNA-binding cofactors and co-modulators to finally regulate the transcription of specific genes involved in cell growth, differentiation, development, and the immune response.[1] Deregulation of TGF-β has been implicated in the pathogenesis of various diseases including fibrosis, atherosclerosis, and cancer.[1c,2] Numerous studies have consistently indicated the role of TGF-β as a potent fibrogenic cytokine evoking pathological fibrosis in various organs such as kidney, liver, lung, heart, and skin.[3] Attempts to block the effects of TGF-β contributed to the development of molecules that inhibit TGF-β binding to its receptor including decorin,[4] soluble chimeric TGF-β receptor,[5] and neutralizing antibodies.[6] The extensive knowledge regarding TGF-β-mediated ALK5-dependent signaling pathway as an initiating point at the receptor level has highlighted the therapeutic potential of TGF-β signaling antagonist. Recent studies have shown that several small molecule ATP-competitive ALK5 inhibitors such as **1** (SB-431542),[7] **2** (SB-505124),[8] **3** (SB-525334),[9] **4** (A-83−01),[10] **5** (GW6604),[11] **6** (LY580276),[12] and SD-208[13] inhibited autophosphorylation of ALK5 and TGF-β-induced transcription of matrix genes in reporter assays at submolar concentrations. Among them, **3**, **5**, and SD-208 effectively retarded progressive fibrosis in kidney, liver, and lung, respectively, and SD-208 also strongly inhibited growth and invasiveness of cancer cells in animal models.



To develop a more potent and selective ALK5 inhibitor, we have synthesized a series of 4(5)-(6-alkylpyridin-2-yl)imidazoles **13a**−**p** that have a carbonitrile- or carboxamide-substituted phenylaminomethyl or phenylmethylamino moiety at the 2-position of the imidazole ring. It was of particular interest to us whether incorporation of a nitrogen atom between an imidazole ring and a phenyl in this series of compounds may serve as an additional binding site to the ATP binding site of ALK5.

Chemistry

A series of 4(5)-(6-alkylpyridin-2-yl)imidazoles **13a**−**p** was prepared as shown in Scheme 1. Treatment of 2,6-lutidine (**7a**) and 6-ethyl-2-methylpyridine (**7b**) with lithium bis(trimethylsilyl)amide in anhydrous THF at −60 °C followed by reaction with benzo[1,3]dioxole-5-carboxylic acid methyl ester (**8a**) gave the 2-(6-alkylpyridin-2-yl)-1-(benzo[1,3]dioxol-5-yl)ethanones **9a**[14] and **9b** in 76% and 36% yields, respectively. Alternatively, the 2-(6-alkylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanones **9c**[15] and **9d** were prepared by treatment of **7a** and **7b** with *n*-BuLi and Et_2AlCl in anhydrous THF at −60 °C, followed by reaction with quinoxaline-6-carbonyl chloride (**8b**) in 65% and 29% yields, respectively.

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[a] Abbreviations: TGF-β, transforming growth factor-β; ALK5, activin receptor-like kinase 5.

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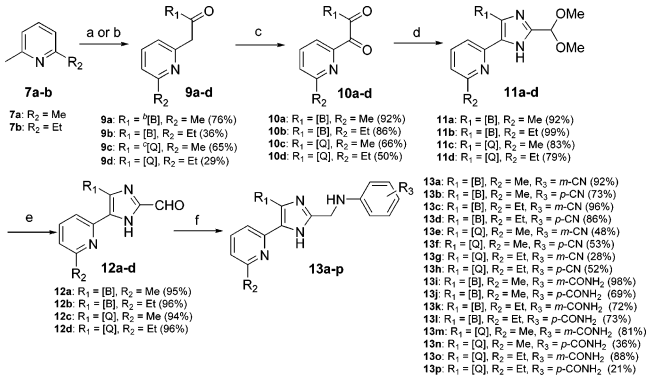
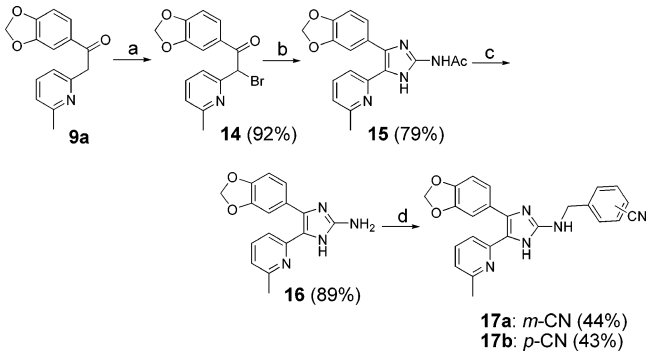
Scheme 1[a]



[a] Reagents and conditions: (a) lithium bis(trimethylsilyl)amide, benzo[1,3]dioxole-5-carboxylic acid methyl ester (**8a**), anhydrous THF, −60 °C, Ar atmosphere; (b) *n*-BuLi, Et_2AlCl, quinoxaline-6-carbonyl chloride (**8b**), anhydrous THF, −60 °C, Ar atmosphere; (c) HBr, DMSO, 60−70 °C, 2 h; (d) glyoxal dimethyl acetal, $CH_3CO_2NH_4$, *t*-BuOMe/MeOH, rt, 3 h; (e) 1 N HCl, 70 °C, 3 h; (f) 3- or 4-aminobenzonitrile or 3- or 4-aminobenzamide, AcOH, 1,2-dichloroethane, rt, 1 h, then sodium triacetoxyborohydride, rt, overnight. [b][B] represents benzo[1,3]dioxol-5-yl. [c][Q] represents quinoxalin-6-yl.

Scheme 2[a]

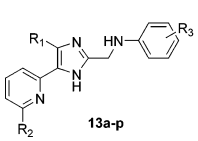


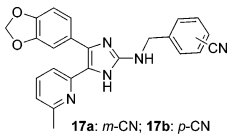
[a] Reagents and conditions: (a) *N*-bromosuccinimide, CH_2Cl_2, rt, 30 min; (b) 1-acetylguanidine, anhydrous DMF, rt, 96 h; (c) H_2SO_4, MeOH, H_2O, reflux, 24 h; (d) 3- or 4-cyanobenzaldehyde, K_2CO_3, anhydrous MeOH, rt, 3 h, Ar atmosphere, then $NaBH_4$, anhydrous THF, reflux, 15 h, Ar atmosphere.

Oxidation of the monoketones **9a**−**d** with HBr in DMSO afforded the diketones **10a**−**d** in 50−92% yields. Condensation of the diketones **10a**−**d** with glyoxal dimethyl acetal and $CH_3CO_2NH_4$ in *t*-BuOMe/MeOH produced 4(5)-(6-alkylpyridin-2-yl)-2-(dimethoxymethyl)imidazoles **11a**−**d** in 79−99% yields. The acetal protecting group of the **11a**−**d** was removed in an acidic condition to generate the imidazole-2-carbaldehydes **12a**−**d** in high yields. Coupling of the **12a**−**d** with an appropriate aminobenzonitrile or aminobenzaldehyde in the presence of AcOH in CH_2Cl_2 followed by reduction of the resulting unstable imines with sodium triacetoxyborohydride afforded the 4(5)-(6-alkylpyridin-2-yl)imidazoles **13a**−**p** in 21−98% yields.

The 3- and 4-{[4-(benzo[1,3]dioxol-5-yl)-5-(6-methylpyridin-2-yl)-1*H*-imidazol-2- ylamino]methyl}benzonitrile, **17a** and **17b**, were prepared as shown in Scheme 2. Bromination of **9a** with *N*-bromosuccinimide in CH_2Cl_2 gave 1-(benzo[1,3]dioxol-5-yl)-2-bromo-2-(6-methylpyridin-2-yl)ethanone (**14**)[16] in 92% yield. Cyclization of **14** with 1-acetylguanidine in anhydrous DMF for 96 h yielded *N*-[4-(benzo[1,3]dioxol-5-yl)-5-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl]acetamide (**15**) in 79% yield, which was subsequently hydrolyzed in an acidic condition to give the amine **16** in 89% yield. Coupling of **16** with 3- or 4-aminobenzonitrile in the presence of K_2CO_3 in anhydrous MeOH followed by reduction of the resulting imines with $NaBH_4$ in anhydrous THF afforded **17a** or **17b** in 44 or 43% yield, respectively.

Table 1. Inhibitory Activity of 4(5)-(6-Alkylpyridin-2-yl)imidazoles **13a**−**p**, **17a**, and **17b** on ALK5





					luciferase activity[b,c] (% control)		
compd	R_1	R_2	R_3	IC_{50}[a] (μM)	p3TP-luc	SBE-luc	ARE-luc
mock					5 ± 1	18 ± 3	2
TGF-β					100 ± 23	100 ± 11	100 ± 8
13a	[B][d]	Me	*m*-CN	0.046	14 ± 6	36 ± 4	62 ± 6
13b	[B]	Me	*p*-CN	0.174	43 ± 3	35 ± 5	123 ± 26
13c	[B]	Et	*m*-CN	0.073	21 ± 4	33 ± 6	75 ± 6
13d	[B]	Et	*p*-CN	0.360	67 ± 12	99 ± 12	136 ± 17
13e	[Q][e]	Me	*m*-CN	0.012	7 ± 1	25 ± 9	13 ± 1
13f	[Q]	Me	*p*-CN	0.042	16 ± 3	23 ± 7	55 ± 6
13g	[Q]	Et	*m*-CN	0.119	33 ± 7	62 ± 24	79 ± 8
13h	[Q]	Et	*p*-CN	0.047	32 ± 9	40 ± 7	61 ± 4
13i	[B]	Me	*m*-$CONH_2$	0.021	28 ± 10	54 ± 18	79 ± 11
13j	[B]	Me	*p*-$CONH_2$	0.262	55 ± 28	67 ± 2	119 ± 12
13k	[B]	Et	*m*-$CONH_2$	0.046	30 ± 2	59 ± 9	64 ± 3
13l	[B]	Et	*p*-$CONH_2$	0.385	125	96	133 ± 11
13m	[Q]	Me	*m*-$CONH_2$	0.010	63 ± 14	95 ± 33	99 ± 5
13n	[Q]	Me	*p*-$CONH_2$	0.075	84 ± 8	101 ± 40	127 ± 12
13o	[Q]	Et	*m*-$CONH_2$	0.015	67 ± 13	95 ± 23	96 ± 10
13p	[Q]	Et	*p*-$CONH_2$	0.092	104 ± 7	112 ± 23	98 ± 8
17a				0.024	33 ± 8	52 ± 18	95 ± 10
17b				0.153	61 ± 13	66 ± 9	129 ± 15
1				1.542	69 ± 9	68 ± 20	128 ± 20

[a] ALK5 was expressed in Sf9 insect cells as human recombinant GST-fusion protein by means of the baculovirus expression system. A proprietary radioisotopic protein kinase assay (33PanQinase Activity Assay) was performed at ProQinase GmbH (Freiburg, Germany) using casein as a substrate. [b] Activity is given as the mean ± SD of three independent experiments run in triplicate relative to control incubations with DMSO vehicle. [c] Luciferase activity was determined at a concentration of 0.1 μM of inhibitor. [d] [B] represents benzo[1,3]dioxol-5-yl. [e] [Q] represents quinoxalin-6-yl.

Results and Discussion

To investigate whether these potential inhibitors **13a**−**p**, **17a**, and **17b** could inhibit ALK5, a kinase assay was performed using the purified human ALK5 kinase domain produced in Sf9 insect cells (Table 1). Among them, the quinoxalinyl analogue **13e** and its carboxamide derivative **13m** showed the most potent ALK5 inhibition with IC_{50} of 0.012 μM and 0.010 μM, respectively, while **1** inhibited ALK5 with IC_{50} of 1.542 μM. All of the quinoxalinyl analogues exhibited approximately 2−4-fold more potent ALK5 inhibitory activity than the corresponding benzo[1,3]dioxolyl analogues. The alkyl substituent at the 6-position of the pyriding ring also seemed to affect ALK5 inhibition, thus, 6-methylpyridyl analogues were equipotent or slightly more potent than the corresponding 6-ethylpyridyl analogues, except that the 6-methylpyridyl compound **13e** was even 10-fold more potent in ALK5 inhibition than the corresponding 6-ethylpyridyl compound **13g** (IC_{50} = 0.119 μM). Although it was previously demonstrated that introduction of a methyl group at the 6-position of the pyriding ring in the pyridyl-substituted imidazoles, thiazoles, and pyrazoles significantly increased ALK5 inhibitory activity,[7,8,17] a bulkier group than a methyl group seems to reduce binding of those inhibitors in the ATP binding site of ALK5. Compounds with either a carbonitrile or a caboxamide substituent in the *meta*-position displayed 3−5-fold or 6−12-fold more potent ALK5 inhibitory

activity than the corresponding compounds, which have substituents in the *para*-position. The position of a nitrogen atom in the linker moiety also influenced ALK5 inhibition, thus, the benzo[1,3]dioxolyl analogues **17a** (IC_{50} = 0.024 μM) having $NHCH_2$ as a linker showed approximately 2-fold more potent ALK5 inhibition than the corresponding **13a** (IC_{50} = 0.046 μM) having CH_2NH as a linker. To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **13a**−**p**, **17a**, and **17b** was measured using HaCaT cells transiently transfected with three different luciferase receptor genes, p3TP-luciferase reporter,[18] SBE-luciferase reporter,[19] and ARE-luciferase reporter[20] at a concentration of 0.1 μM (Table 1). The quinoxalinyl analogues **13e** (p3TP-luciferase, 93%; SBE-luciferase, 75%; ARE-luciferase, 87%) and **13f** (p3TP-luciferase, 84%; SBE-luciferase, 77%; ARE-luciferase, 45%), with a carbonitrile functionality in the phenyl ring, displayed the most significant ALK5 inhibition at 0.1 μM compared to that of control, which is higher than that of **1** (p3TP-luciferase, 31%; SBE-luciferase, 32%; ARE-luciferase, 0%). Although the quinoxalinyl analogue **13m** with a carboxamide functionality in the phenyl ring was one of the most potent ALK5 inhibitors in this series of compounds in the kinase assay; it exhibited only marginal inhibition in the cell-based luciferase assay (p3TP-luciferase, 37%; SBE-luciferase, 5%; ARE-luciferase, 1%). It was also observed that introduction of a carbonitrile functionality in the phenyl ring rather than a carboxamide functionality increased the ALK5 inhibitory activity in all the cases. These findings suggest that the carbonitrile-containing analogues have better cellular permeability than the carboxamide-containing analogues. The benzo-[1,3]dioxolyl analogues with a carbonitrile functionality (**13a**, **13b**, and **13d**) showed lower ALK5 inhibition compared to the quinoxalinyl analogues with a carbonitrile functionality (**13e**, **13f**, and **13h**), however, the benzo[1,3]dioxolyl analogues with a carboxamide functionality (**13i**, **13j**, and **13k**) showed higher ALK5 inhibition compared to the quinoxalinyl analogues with a carboxamide functionality (**13m**, **13n**, and **13o**). As observed in the kinase assay, the 6-methylpyridyl analogues (**13a**, **13b**, **13e**, **13f**, and **13j**) displayed higher ALK5 inhibitory activity than the corresponding 6-ethylpyridyl analogues (**13c**, **13d**, **13g**, **13h**, and **13l**). Compounds with either a carbonitrile or a caboxamide substituent in the *meta*-position, except **13g**, showed higher ALK5 inhibition compared to the corresponding compounds that have either of those substituents in the *para*-position. Although the ALK5 inhibitory activity of **13a** and **13b** was lower than that of **17a** and **17b** in a kinase assay, in cell-based luciferase reporter assays, **13a** (p3TP-luciferase, 86%; SBE-luciferase, 64%; ARE-luciferase, 38%) and **13b** (p3TP-luciferase, 57%; SBE-luciferase, 65%; ARE-luciferase, 0%) exhibited higher ALK5 inhibition than the corresponding **17a** (p3TP-luciferase, 67%; SBE-luciferase, 48%; ARE-luciferase , 5%) and **17b** (p3TP-luciferase, 39%; SBE-luciferase, 34%; ARE-luciferase, 0%).

The most potent compounds **13e** and **13f** in cell-based luciferase reporter assays were chosen, and their ALK5 inhibitory activity was compared with **1**, **2**, and **3** at four different concentrations (0.01, 0.05, 0.1, and 0.5 μM) using HaCaT cells transiently transfected with p3TP-luc reporter construct.

As shown in Figure 1, **13e** and **13f** inhibited ALK5 in a dose-dependent manner, and both compounds were more potent than **1**, **2**, and **3** in all the four different concentrations. Compound **13e** showed more than 90% inhibition at 0.05 μM, whereas **1** showed no inhibition, and **2** and **3** showed only 23% and 9% inhibition, respectively.

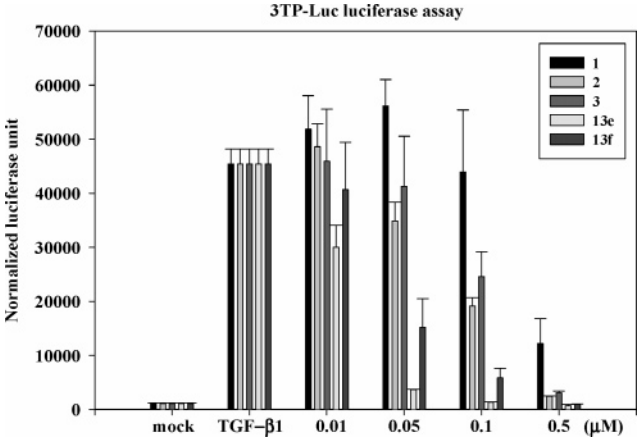


Figure 1. Effect of **13e** and **13f** on the activity of TGF-β-induced ALK5. HaCaT cells were transiently transfected with p3TP-luc reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as the mean ± SD of three independent experiments run in triplicate relative to control.

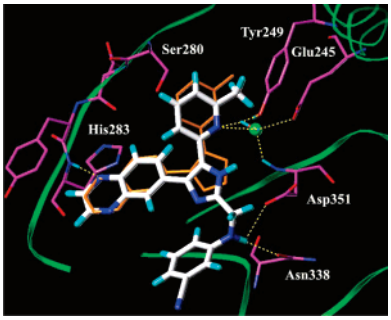


Figure 2. Binding pose of **13e** in the active site of ALK5, in comparison with the X-ray pose of 1,5-naphthyrine inhibitor (orange stick). A trapped water molecule in the X-ray structure is shown in the green sphere. Key amino acid residues within the binding site are represented in line form, and the docked **13e** is rendered in capped stick. Yellow dotted lines are hydrogen-bonding interactions (<2.5 Å).

The selectivity of **13e** for ALK5 versus other kinases was evaluated using a panel of 38 kinases (ProQinase GmbH (Germany) and MDS Pharma Services (Taiwan)). Compound **13e** inhibited ALK5 phosphorylation with an IC_{50} of 0.012 μM and inhibited JNK2α2 by 79% at 1 μM. Except JNK2α2, p38α (IC_{50} = 1.03 μM), and PKCμ (IC_{50} = 1.0 μM), **13e** was at least 100-fold more selective for ALK5 than the rest of 35 kinases (ABL1, AKT1, Ca2+/Calmodulin-Dep. II, Cdk1/Cyclin B, Cdk2/Cyclin A, Cdk5/p35, CHK1, CK2α1, CSK, EGF receptor, ERBB2, ERK1, GSK-3β, IKK-2(β), insulin receptor, JNK1α1, JNK3, Lck, LynA, MAPKAPK5, MEK1, p38β, p38γ, p38δ, PDK1, PKA (nonselective), PKCα, PKCβ1, PKCβ2, PKCδ, PKCγ, $PP60^{C-SRC}$, RPS6KB1(S6K), RPS6KB2, and SGK1).

To account for strong and selective ALK5 inhibitory activity of **13e**, we built a docking model of the ALK5/**13e** complex based on the X-ray crystal structure of ALK5 complexed with 1,5-naphthyrine inhibitor[17] (PDB id: 1VJY). As demonstrated in Figure 2, **13e** is well superimposed over the X-ray pose of 1,5-naphthyrine compound (orange color) lying both in the cavity for ATP and the backside of the ATP binding pocket containing Ser280.[17,21,22] The substituents attached to the central imidazole ring of **13e** form several hydrogen bonds (H-bonds) with amino acid residues in the binding pocket, acting as anchors, and helping the ligand bind deeper into active site pockets. The cavity for quinoxaline ring includes the hinge region (residues 281 to 283) of the kinase, normally occupied

by the adenine ring of ATP,[17] and the main interaction observed in this region is a H-bond of quinoxaline ring nitrogen with backbone NH of His283. The 6-methylpyridine moiety is positioned in what is called the "selectivity pocket" of ALK5 containing Ser280, which is critical for the selectivity of **13e** for ALK5 over p38 MAP kinase.[17,21,22] In case of p38 MAP kinase, Thr106 residue lies in the equivalent position to Ser280 of ALK5. Therefore, a close contact between 6-methylpyridine ring and Thr106 would not be formed in the binding pocket of p38 MAP kinase due to the steric clash. In addition, the H-bond between the pyridine nitrogen and the water molecule is also maintained in this model, which is commonly observed in inhibitors containing pyridyl moiety in this position.[17,21] The secondary amino group of the linker forms a bidentate H-bond with residues, Asp351 and Asn338. If the secondary amino group is protonated in the biological system, the H-bond will be stronger and contribute to the increase in the binding selectivity toward this site. The H-bond with Asp351 is observed in the X-ray structures of ALK5 complexed with other inhibitors. The reported data suggest that the loop containing Asp351 is quite flexible, and usually Asp351 is located close to bound inhibitors in the inactivated conformation of ALK5.[17,21,22] Therefore, the amino linker group of **13e** may contribute the drug to stabilize inactive conformation of ALK5 by pinning the loop containing Asp351 and Asn338. Conclusively, the binding mode of **13e** generated by flexible docking studies shows that the structure of the ligand fits well into the binding cavity of ALK5 by forming tight interactions and well elucidates potent and selective activity of **13e** for ALK5.

In this report, a series of 4(5)-(6-alkylpyridin-2-yl)imidazoles has been synthesized and evaluated for ALK5 inhibitory activity in an enzyme assay and in cell-based luciferase reporter assays. The structure−activity relationships in this series of compounds have been established and discussed. Compound **13e** showed the most significant ALK5 inhibitory activity in the series of compounds that is much higher than those of **1**, **2**, and **3**. A docking model of ALK5/**13e** complex shows that **13e** fits well into the active site cavity of ALK5. The highly potent and selective ALK5 inhibitor **13e** might represent a valuable lead for developing effective drugs to treat fibrosis and cancer.

Experimental Section

^{1}H NMR spectra were recorded on a Varian Unity 400 spectrophotometer. The chemical shifts are reported in parts per million (ppm) relative to internal tetramethylsilane in $CDCl_3$, CD_3OD, or DMSO-d_6. Infrared spectra were recorded on a FT-infrared spectrometer (Bio-Rad). Electrospray ionization mass spectra (ESI-MS) were obtained on a Q-Tof2 mass spectrometer (Micromass). All melting points were taken in Pyrex capillaries using an electrothermal digital melting point apparatus (Buchi) and are not corrected. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60F-254 glass plates. Medium-pressure liquid chromatography (MPLC) was performed using Merck silica gel 60 (230−400 mesh) with a YFLC-540 ceramic pump (Yamagen). Elemental analyses were performed on a Carlo Erba 1106 elemental analyzer.

General Procedure for the Preparation of the 2-(6-Alkylpyridin-2-yl)-1-(benzo[1,3]dioxol-5-yl)ethanones 9a and 9b. A stirred solution of pyridine **7a** or **7b** (13 mmol) in anhydrous THF (100 mL) at −60 °C under an Ar atmosphere was treated dropwise with a solution of lithium bis(trimethylsilyl)amide in THF (1.0 M, 39 mmol), and then the solution was transferred via cannula to a stirred solution of benzo[1,3]dioxole-5-carboxylic acid methyl ester (**8a**; 10.3 mmol) in anhydrous THF (100 mL) at −60 °C. Stirring was continued for 20 min, and the reaction mixture was quenched with saturated aqueous NH_4Cl solution. The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by MPLC on silica gel to afford the title compound **9a** or **9b**.

General Procedure for the Preparation of the 2-(6-Alkylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanones 9c and 9d. A stirred solution of pyridine **7a** or **7b (**13mmol) in anhydrous THF (100 mL) at −60 °C under an Ar atmosphere was treated dropwise with a solution of *n*-BuLi in hexanes (2.0 M, 13 mmol). After 30 min, a solution of Et_2AlCl in hexanes (1.0 M, 14 mmol) was added dropwise to the reaction mixture, and the reaction mixture was allowed to warm to room temperature. The reaction mixture was cooled to −60 °C and transferred via cannula to a stirred solution of quinoxaline-6-carbonyl chloride (**8b**; 10.3 mmol) in anhydrous THF (100 mL) at −60 °C. Stirring was continued for 20 min, and the reaction mixture was quenched with saturated aqueous NH_4Cl solution. The mixture was filtered through a pad of Celite, and the filtered residue was washed with EtOAc (100 mL). The combined filtrate was concentrated under reduced pressure, and the residue was purified by MPLC on silica gel to afford the title compound **9c** or **9d** as a solid.

General Procedure for the Preparation of the 1-Aryl-2-(6-alkylpyridin-2-yl)ethane-1,2-diones 10a−d. A stirred suspension of **9a**−**d** (13 mmol) in DMSO was treated dropwise with HBr (48 wt % in water, 6 mL), and the mixture was heated to 60−70 °C. After 2 h, the reaction mixture was cooled to 0 °C, poured onto ice water, and brought to pH 10 with K_2CO_3. The mixture was extrated with EtOAc (30 mL × 3), and the EtOAc solution was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by MPLC on silica gel to afford the title compound **10a**−**d** as a solid.

General Procedure for the Preparation of the 4-Aryl-5-(6-alkylpyridin-2-yl)-2-(dimethoxymethyl)-1*H*-imidazoles 11a−d. Compound **10a**−**d** (1.8 mmol) was dissolved in *t*-BuOMe (10 mL) and treated with glyoxal dimethyl acetal (60 wt. % solution in water, 3.6 mmol). $CH_3CO_2NH_4$ (4.5 mmol) in MeOH (5 mL) was added to it, and the mixture was stirred at room temperature for 3 h. The pH of the reaction was adjusted to pH 8 with saturated aqueous $NaHCO_3$ solution. The reaction mixture was partitioned between CH_2Cl_2 (50 mL) and water (50 mL). The CH_2Cl_2 layer was separated, dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel to afford the title compounds **11a** and **11b** as an oil or **11c** and **11d** as a solid.

General Procedure for the Preparation of the 4-Aryl-5-(6-alkylpyridin-2-yl)-1*H*-imidazole-2-carbaldehydes 12a−d. Compound **11a**−**d** (0.59 mmol) was dissolved in 1 N HCl solution (4 mL), and the mixture was heated at 70 °C for 3 h. The cooled reaction mixture was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2 (50 mL × 3). The CH_2Cl_2 solution was dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel to afford the title compound **12a**−**d** as a solid.

General Procedure for the Preparation of the 3- or 4-{[5-(6-Alkylpyridin-2-yl)-4-aryl-1*H*-imidazol-2-ylmethyl]amino}-benzonitriles 13a−h and the 3- or 4-{[5-(6-Alkylpyridin-2-yl)-4-aryl-1*H*-imidazol-2-ylmethyl]amino}benzamides 13i−p. A mixture of the imidazole-2-carbaldehyde **12a**−**d** (0.56 mmol), AcOH (0.56 mmol), and 3-aminobenzonitrile, 4-aminobenzonitrile, 3-aminobenzamide, or 4-aminobenzamide (0.84 mmol) in 1,2-dichloroethane (10 mL) was stirred at room temperature for 1 h. Sodium triacetoxyborohydride (0.84 mmol) was added to it, and the mixture was stirred at room temperature overnight. The reaction mixture was neutralized with 10% K_2CO_3 aqueous solution to pH 8 at 0 °C. The mixture was extracted with 20% MeOH in $CHCl_3$ (50 mL × 5). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using a mixture of MeOH and CH_2Cl_2 as eluent to afford the titled compound **13a**−**p** as a solid.

Cellular Assays to Measure Anti-TGF-β Activity of ALK5 Inhibitors. Biological activity of the test compounds was deter-

mined by measuring their ability to inhibit TGF-β1-induced p3TP-luciferase reporter activity, SBE-luciferase reporter activity, and ARE-luciferase reporter activity in HaCaT cells. HaCaT cells were seeded at concentrations of 5×10^4 in 24-well plates. And the next day, when they reach approximately 90% confluence, cells were transfected with 0.1 μg of p3TP-Luc reporter construct, SBE-Luc reporter construct, or ARE-Luc reporter construct and 0.1 μg of β-galactosidase, using lipofectamine 2000 (Invitrogen). At 24 h after transfection, various concentrations of ALK5 inhibitors were added to the cells. After 2 h, cells were treated with 5 ng/mL of TGF-β for 18−24 h. Cell lysates were harvested according to the manufacturer's instruction, and luminescence was measured by a luminometer VICTOR (Perkin-Elmer Life).

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Supporting Information Available: Analytical data for new compounds, synthetic procedures for compounds **15**, **16**, **17a**, **17b**, and procedures of docking experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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