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Synthesis and biological evaluation of 5-(pyridin-2-yl)thiazoles as transforming growth factor-β type1 receptor kinase inhibitors

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Abstract—A series of 5-(pyridin-2-yl)thiazoles (**14a**–**l** and **15a**–**l**) has been synthesized and evaluated for their ALK5 inhibitory activity in cell-based luciferase reporter assays. Among them, 3-[[5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)thiazol-2-ylamino]methyl]benzamide (**15i**) and 3-[[5-(6-ethylpyridin-2-yl)-4-(quinoxalin-6-yl)thiazol-2-ylamino]methyl]benzamide (**15k**) showed more than 95% inhibition at 0.1 μM in luciferase reporter assays using HaCaT cells transiently transfected with p3TP-luc reporter construct and ARE-luciferase reporter construct.

The transforming growth factor-β (TGF-β) superfamily comprises TGF-βs, activins, and bone morphogenetic proteins (BMPs) and regulates a wide range of responses including cell proliferation, differentiation, adhesion, migration, and apoptosis. The three major 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 and is often the major isoform in fibrosis and cancer metastasis. TGF-β1 signals through a complex of two related but structurally and functionally distinct transmembrane receptor serine/threonine kinases, the type I and type II TGF-β receptors (TβR-I and TβR-II, respectively). Binding of the homodimeric TGF-β to homodimer of TβR-II enables the formation and stabilization of TβR-I/TβR-II receptor complexes. The TβR-II kinase then phosphorylates 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. This complex is shuttled into the nucleus and regulates transcription of specific target genes involved in cell growth, differentiation, development, and the immune response.[1] Deregulated signaling of TGF-β participates in various human pathologies including fibrosis, vascular disorders, and carcinogenesis.[1c,2] TGF-β also plays important roles in wound healing and is possibly deregulated in the related pathologies of hypertrophic scars and keloids.[3,4] There are a number of reports that the therapeutic administration of TGF-β binding proteins including the proteoglycan decorin,[5] soluble chimeric TGF-β receptors,[6] and neutralizing antibodies[7] ameliorates experimental fibrosis. 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),[8] **2** (SB-505124),[9] **3** (SB-525334),[10] **4** (A-83-01),[11] **5** (GW6604),[12] **6** (LY580276),[13] and SD-208[14] 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. We have also prepared the 2-pyridyl-substituted triazoles[15,16] and imidazoles[17,18] having a carbonitrile- or carboxamide-substituted phenyl or benzyl moiety as ALK5 inhibitors and found that both

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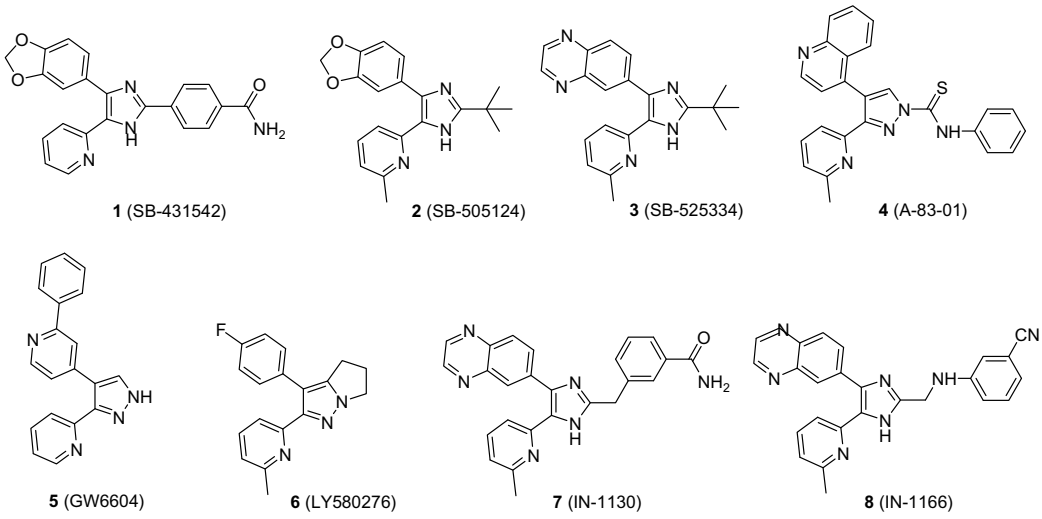
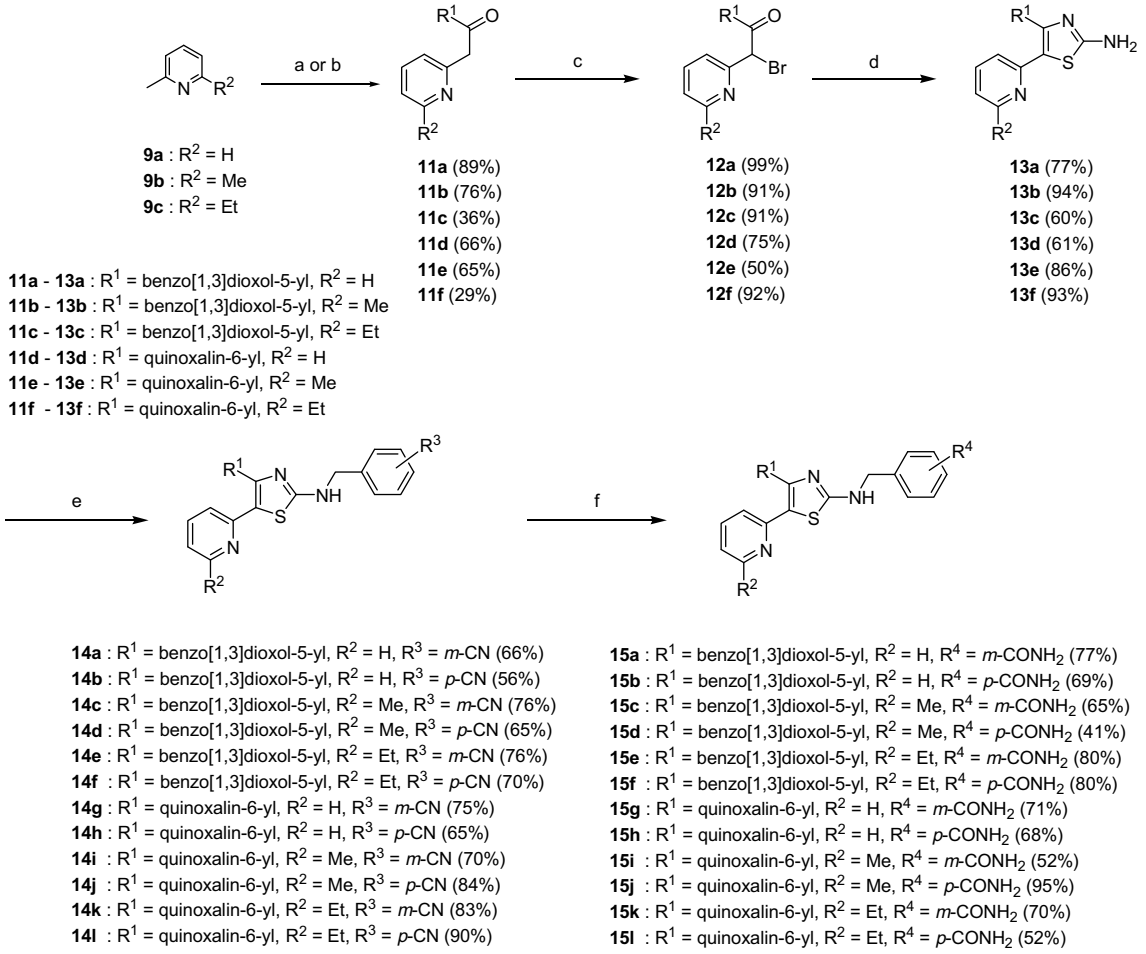
introduction of a carbonitrile or carboxamide group at *meta*- or *para*-position in the phenyl ring and incorporation of a methylene, a methyleneamino, or an aminomethylene linkage between a central triazole or an imidazole ring and a phenyl ring significantly increased ALK5 inhibitory activity. Among them, **7** (IN-1130) effectively suppressed renal fibrosis induced by unilateral ureteral obstruction (UUO) in rats[17] and ameliorated experimental autoimmune encephalomyelitis (EAE) in SBE-luc and GFAP-luc mice immunized with $MOG_{35–55}$,[19] and **8** (IN-1166) was found to be a highly potent and selective ALK5 inhibitor.[18]



On the basis of these findings, we have synthesized a series of 5-(pyridin-2-yl)thiazoles, **14a**–**l** and **15a**–**l**, that have a *meta*- or *para*-carbonitrile- or carboxamide-substituted phenylmethylamino moiety at the 2-position of the thiazole ring and evaluated for their ALK5 inhibitory activity in cell-based luciferase reporter assays.

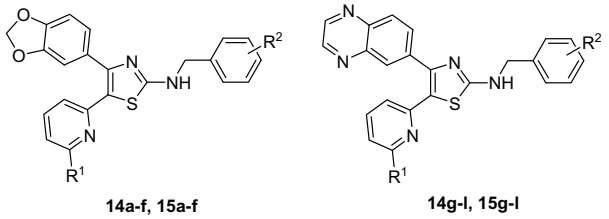
A series of 5-(pyridin-2-yl)thiazoles, **14a**–**l** and **15a**–**l**, was prepared as shown in Scheme 1.[20] Treatment of 2-picoline (**9a**), 2,6-lutidine (**9b**), and 6-ethyl-2-methylpyridine (**9c**) with lithium bis(trimethylsilyl)amide in anhydrous THF at −60 °C followed by reaction with methyl benzo[1,3]dioxole-5-carboxylate (**10a**) gave the 1-(benzo[1,3]dioxol-5-yl)-2-(pyridin-2-yl)ethanones **11a**,[18] **11b**,[18] and **11c** in 89%, 76%, and 36% yields, respectively. Alternatively, the 2-(pyridin-2-yl)-1-(quinoxalin-6-yl)ethanones **11d**,[18] **11e**,[18] and **11f** were prepared by treatment of **9a**–**c** with *n*-BuLi and Et_2AlCl in anhydrous THF at −60 °C followed by reaction with quinoxaline-6-carbonyl chloride (**10b**) in 66%, 65%, and 29% yields, respectively. In the case of **9c**, lithiation occurred at both a 2-methyl group and a methylene of 6-ethyl group, thus, compounds **11c** and **11f** were obtained along with their corresponding regioisomers 1-benzo[1,3]dioxol-5-yl-2-(6-methylpyridin-2-yl)propan-1-one and 2-(6-methylpyridin-2-yl)-1-quinoxalin-6-yl-propan-1-one in 12% and 14% yields, respectively. Bromination of the monoketones **11a**–**f** with *N*-bromosuccinimide in CH_2Cl_2 at 0 °C afforded the monobromo ketones **12a**–**f** in 50–99% yields. Condensation of **12a**–**f** with thiourea in DMF at 120 °C produced 2-amino-5-(pyridin-2-yl)thiazoles **13a**–**f** in 60–93% yields. The 2-aminothiazoles **13a**–**f** reacted with 3- or 4-cyanobenzyl bromide in the presence of Cs_2CO_3 in DMF to generate the 2-[(cyanophenylmethyl)amino]-5-(pyridin-2-yl)thiazoles **14a**–**l** in 56–90% yields. Conversion of the nitrile functionality in compounds **14a**–**l** to the corresponding carboxamide functionality was accomplished by treatment with 28% H_2O_2 and 1 N NaOH in a mixture of MeOH/EtOH/$CHCl_3$ (v/v/v, 2:3:2) at 40 °C to afford the 2-[(carboxamidophenylmethyl)amino]-5-(pyridin-2-yl)thiazoles **15a**–**l** in 41–95% yields.

To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **14a**–**l** and **15a**–**l** was measured using HaCaT cells transiently transfected with three different luciferase reporter genes (p3TP-luciferase reporter, ARE-luciferase reporter, and SBE-luciferase reporter) at a concentration of 0.1 μM (Table 1).[21] The p3TP-luc reporter construct contains three AP-1 binding elements and the plasminogen-activator inhibitor-1 (PAI-1) promoter.[22] The ARE-luc reporter construct consists of three copies of activin response element sequence derived from the Mix.2 homeobox gene promoter. When cotransfected with the forkhead activin signal transducer FAST-1, ARE-luc construct is induced by TGF-β- or activin-activated Smad2/4 complexes.[23] The SBE-luc reporter construct contains four tandem copies of the CAGA Smad-binding element cloned upstream of the adenovirus major late promoter (MLP).[24] The quinoxalinyl analogs, **14g**–**l** and **15g**–**l**, all displayed more potent ALK5 inhibitory activity than the corresponding benzo[1,3]dioxolyl analogs, **14a**–**f** and **15a**–**f**, respectively. 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.[8,9,18,25] In this series of compounds, the methyl substituent at the 6-position of the pyriding ring also markedly increased ALK5 inhibition, but the 6-ethylpyridyl analogs were equipotent or slightly less potent than the corresponding 6-methylpyridyl analogs. In all



Scheme 1. Reagents and conditions: (a) For **11a–c**, lithium bis(trimethylsilyl)amide, methyl benzo[1,3]dioxole-5-carboxylate (**10a**), anhydrous THF, −60 °C, Ar atmosphere; (b) For **11d–f**, *n*-BuLi, Et_2AlCl, quinoxaline-6-carbonyl chloride (**10b**), anhydrous THF, −60 °C, Ar atmosphere; (c) *N*-bromosuccinimide, CH_2Cl_2, 0 °C, 30 min; (d) thiourea, DMF, 120 °C, 1 h; (e) 3- or 4-cyanobenzyl bromide, Cs_2CO_3, DMF, 120 °C, 1 h; (f) 28% H_2O_2, 1 N NaOH, MeOH/EtOH/$CHCl_3$ (v/v/v, 2:3:2), 40 °C, overnight.

Table 1. Inhibitory activity of 5-(pyridin-2-yl)thiazoles, **14a–l** and **15a–l**, on ALK5



Compound	R^1	R^2	Activity (% control)[a]		
			p3TP-luciferase[b]	ARE-luciferase[b]	SBE-luciferase[b]
Mock			2 ± 0	5 ± 1	19 ± 4
TGF-β			100 ± 8	100 ± 5	100 ± 8
14a	H	*m*-CN	8 ± 1	12 ± 1	39 ± 2
14b	H	*p*-CN	33 ± 1	68 ± 1	64 ± 9
14c	Me	*m*-CN	5 ± 2	6 ± 1	35 ± 1
14d	Me	*p*-CN	13 ± 2	46 ± 3	39 ± 6
14e	Et	*m*-CN	11 ± 2	45 ± 1	41 ± 4
14f	Et	*p*-CN	27 ± 7	84 ± 13	57 ± 9
14g	H	*m*-CN	5 ± 0	5 ± 1	47 ± 2
14h	H	*p*-CN	9 ± 2	16 ± 2	40 ± 4

Table 1 (*continued*)

Compound	R^1	R^2	Activity (% control)[a]		
			p3TP-luciferase[b]	ARE-luciferase[b]	SBE-luciferase[b]
14i	Me	*m*-CN	2 ± 0	4 ± 1	32 ± 1
14j	Me	*p*-CN	2 ± 0	5 ± 0	33 ± 3
14k	Et	*m*-CN	4 ± 0	13 ± 1	33 ± 5
14l	Et	*p*-CN	8 ± 1	26 ± 3	35 ± 5
15a	H	*m*-$CONH_2$	17 ± 1	37 ± 2	40 ± 2
15b	H	*p*-$CONH_2$	56 ± 7	84 ± 7	82 ± 14
15c	Me	*m*-$CONH_2$	7 ± 1	17 ± 2	36 ± 1
15d	Me	*p*-$CONH_2$	9 ± 2	39 ± 4	34 ± 1
15e	Et	*m*-$CONH_2$	9 ± 3	32 ± 2	34 ± 2
15f	Et	*p*-$CONH_2$	26 ± 4	74 ± 4	59 ± 3
15g	H	*m*-$CONH_2$	10 ± 2	15 ± 2	41 ± 5
15h	H	*p*-$CONH_2$	26 ± 2	47 ± 3	45 ± 4
15i	Me	*m*-$CONH_2$	2 ± 0	4 ± 1	22 ± 2
15j	Me	*p*-$CONH_2$	9 ± 2	14 ± 0	29 ± 2
15k	Et	*m*-$CONH_2$	2 ± 0	1 ± 0	32 ± 2
15l	Et	*p*-$CONH_2$	9 ± 1	27 ± 2	31 ± 5
1			25 ± 2	82 ± 6	49 ± 5
2			3 ± 0	16 ± 0	25 ± 4
3			5 ± 1	29 ± 4	26 ± 1

[a] Activity is given as means ± SD of three independent experiments run in triplicate relative to control incubations with DMSO vehicle.
[b] Luciferase activity was determined at a concentration of 0.1 μM of inhibitor.

cases, compounds with either a carbonitrile or a caboxamide substituent in the *meta*-position showed higher ALK5 inhibition compared to the corresponding compounds having either of those substituents in the *para*-position. Between the compounds **14a**–**l** and **15a**–**l**, the quinoxalinyl analogs **15i**[26] (p3TP-luciferase, 98%; ARE-luciferase, 96%; SBE-luciferase, 78%) and **15k**[26] (p3TP-luciferase, 98%; ARE-luciferase, 99%; SBE-luciferase, 68%) with a carboxamide functionality in the phenyl ring exhibited the most significant ALK5 inhibition at 0.1 μM compared to that of controls **1** (p3TP-luciferase, 75%; ARE-luciferase, 18%; SBE-luciferase, 51%), **2** (p3TP-luciferase, 97%; ARE-luciferase, 84%; SBE-luciferase, 75%), and **3** (p3TP-luciferase, 95%; ARE-luciferase, 71%; SBE-luciferase, 74%).

The most potent compound **15i** was chosen, and its 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-luciferase reporter construct. As shown in Figure 1, **15i** inhibited ALK5 in a dose-dependent manner, and it was more potent than **1**, **2**, and **3** at all four concentrations tested.

In this report, a series of 5-(pyridin-2-yl)thiazoles containing a *meta*- or *para*-carbonitrile- or carboxamide-substituted phenylmethylamino moiety at the 2-position of the thiazole ring was synthesized and evaluated for ALK5 inhibitory activity in cell-based luciferase reporter assays. The structure–activity relationships in this series of compounds have been established and discussed. The most potent compound in this series, **15i**, inhibited TGF-β-induced ALK5 activity 98, 96, and 78% at 0.1 μM in luciferase reporter assays using HaCaT cells transiently transfected with p3TP-luc reporter construct, ARE-luciferase reporter construct, and SBE-luciferase reporter construct, respectively.

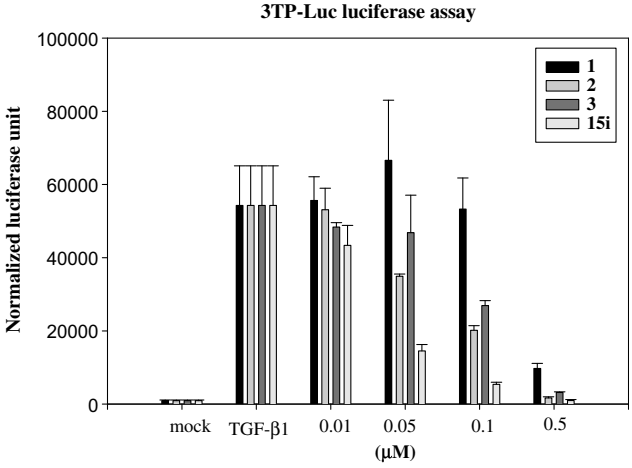


Figure 1. Effect of **15i** on the activity of TGF-β-induced ALK5. HaCaT cells were transiently transfected with p3TP-luciferase reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as means ± SD of three independent experiments run in triplicate relative to control.

Acknowledgment

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20. *General synthetic procedure for preparation of the 2-(6-alkylpyridin-2-yl)-1-(benzo[1,3]dioxol-5-yl)ethanones* ***11a–c***. A stirred solution of pyridine **9a–c** (13 mmol) in anhydrous THF (100 mL) at −60 °C under 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 methyl benzo[1,3]dioxole-5-carboxylate (**10a**) (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 titled compounds **11a–c**.
General synthetic procedure for preparation of the 2-(6-alkylpyridin-2-yl)-1-(quinoxalin-6-yl)ethanones ***11d–f***. A stirred solution of pyridine **9a–c** (13 mmol) in anhydrous THF (100 mL) at −60 °C under 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 (**10b**) (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 titled compounds **11d–f** as a solid.
General synthetic procedure for preparation of the 2-bromo-2-(6-alkylpyridin-2-yl)ethanones ***12a–f***. To a stirred solution of **11a–f** (0.78 mmol) in CH_2Cl_2 at 0 °C was added *N*-bromosuccinimide (0.86 mmol), and the mixture was stirred for an additional 30 min. To the mixture, 5% aqueous sodium thiosulfate solution (20 mL) was added, and the mixture was extracted with CH_2Cl_2 (3× 30 mL). The CH_2Cl_2 solution was washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by MPLC on silica gel to afford the titled compounds **12a–f** as a solid.
General synthetic procedure for preparation of the 2-amino-5-(6-alkylpyridin-2-yl)thiazoles ***13a–f***. A stirred solution of **12a–f** (1.20 mmol) and thiourea (2.52 mmol) in DMF (6 mL) was heated at 120 °C for 1 h and then cooled to 0 °C. To the mixture, cold water (20 mL) was added, and the resulting precipitates were collected by filtration and washed thoroughly with water to afford the titled compounds **13a–f** as a powder.
General synthetic procedure for preparation of the 2-[(cyanophenylmethyl)amino]-5-(pyridin-2-yl)thiazoles ***14a–l***. A stirred solution of **13a–f** (0.65 mmol), 3- or 4-cyanobenzyl bromide (0.78 mmol), and Cs_2CO_3 (0.85 mmol) in DMF (16 mL) was heated at 120 °C for 1 h and then cooled to room temperature and diluted with water (50 mL). The mixture was extracted with EtOAc (70 mL), 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 titled compounds **14a–l** as a solid.
General synthetic procedure for preparation of the 2-[(carboxamidophenylmethyl)amino]-5-(pyridin-2-yl)thiazoles ***15a–l***. To a stirred solution of **14a–l** (0.27 mmol) in MeOH/EtOH/$CHCl_3$ (2:3:2 ratio, 7 mL) were added 1 N NaOH (0.95 mmol) and 28% H_2O_2 (1.20 mmol). The mixture was warmed to 40 °C and stirred overnight, and to the mixture, 1 N HCl was added to adjust to pH 7 at 0 °C. The mixture was diluted with water and extracted with $CHCl_3$ (3× 40 mL). The $CHCl_3$ solution was dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The residue was purified by crystallization from hot MeOH/CH_2Cl_2 to afford the titled compounds **15a–l**.
21. *Cellular assays to measure anti-TGF-β activity of ALK5 inhibitors:* biological activity of the test compounds was determined by measuring their ability to inhibit TGF-β-induced p3TP-luciferase reporter activity, ARE-luciferase

reporter activity, and SBE-luciferase reporter activity in HaCaT cells. HaCaT cells were seeded at concentrations of 5×10^4 in 24-well plates. The next day, when they reach approximately 90% confluence, cells were transfected with 0.1 μg of p3TP-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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26. *Compound **15i***. ^{1}H NMR (DMSO-d_6) δ 2.43 (s, 3 H), 4.58 (br d, 2 H, J = 5.6 Hz, NCH_2), 6.89 (d, 1 H, J = 8.0 Hz), 7.02 (d, 1 H, J = 7.6 Hz), 7.37 (br s, 1 H, NH), 7.40 (t, 1 H, J = 7.6 Hz), 7.45 (dd, 1 H, J = 8.0, 7.6 Hz), 7.57 (d, 1 H, J = 8.0 Hz), 7.79 (d, 1 H, J = 8.0 Hz), 7.94 (m, 2 H), 7.98 (br s, 1 H, NH), 8.08 (d, 1 H, J = 8.8 Hz), 8.18 (d, 1 H, J = 1.6 Hz), 8.54 (br t, 1 H, J = 5.6 Hz, NH), 8.95 (m, 2H); IR (neat) 3427 (NH_2), 3255 (NH_2), 1643 (CO) cm^{-1}; MS (EIS) *m*/*z* 453 (MH^+); mp 196–197 °C; Anal. Calcd for $C_{25}H_{20}N_6OS$: C, 66.35; H, 4.45; N, 18.57. Found: C, 66.13; H, 4.59; N, 18.49.
*Compound **15k***. ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7.6 Hz), 2.69 (q, 2H, J = 7.6 Hz), 4.59 (br d, 2H, J = 6.0 Hz, NCH_2), 6.92 (d, 1H, J = 8.0 Hz), 7.03 (d, 1H, J = 7.2 Hz), 7.36 (br s, 1H, NH), 7.44 (m, 2 H), 7.57 (m, 1H), 7.78 (m, 1H), 7.94 (m, 2H), 7.97 (br s, 1H, NH), 8.08 (d, 1H, J = 8.8 Hz), 8.18 (d, 1H, J = 1.6 Hz), 8.52 (br t, 1H, J = 6.0 Hz, NH), 8.94 (m, 2H); IR (neat) 3359 (NH_2), 3206 (NH_2), 1666 (CO) cm^{-1}; MS (EIS) *m*/*z* 467 (MH^+); mp 198–199 °C; Anal. Calcd for $C_{26}H_{22}N_6OS$: C, 66.93; H, 4.75; N, 18.01. Found: C, 66.68; H, 4.88; N, 17.89.