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Synthesis and biological evaluation of 5-(fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)imidazoles as inhibitors of transforming growth factor-β type I receptor kinase

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ABSTRACT

To further optimize a clinical candidate **5** (EW-7197), a series of 5-(3-, 4-, or 5-fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)imidazoles **19a–l** have been synthesized and evaluated for their TGF-β type I receptor kinase (ALK5) and p38α MAP kinase inhibitory activity in an enzyme assay. The 5-(5-fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)-imidazoles **19h–l** displayed the similar level of potency to that of **5** against both ALK5 (IC_{50} = 7.68–13.70 nM) and p38α MAP kinase (IC_{50} = 1240–3370 nM). Among them, **19j** inhibited ALK5 with IC_{50} value of 7.68 nM in a kinase assay and displayed 82% inhibition at 100 nM in a luciferase reporter assay.

The transforming growth factor-β (TGF-β) is a member of a large family of dimeric polypeptide growth factors that include TGF-βs (TGF-β1, TGF-β2, TGF-β3), activins, inhibins, and bone morphogenetic proteins. TGF-β signals through heteromeric complexes of type I and type II TGF-β receptors (TβR-I and TβR-II, respectively). The signaling cascade is initiated by the binding of ligand to the constitutively active type II receptor. Successively, the type I receptor (ALK5) is phosphorylated in the juxtamembrane GS domain, stimulating its kinase activity. The activated ALK5 propagates the signals through phosphorylation of the receptor-regulated Smads, Smad2 and Smad3 that in turn form complexes with the common mediator Smad, Smad4. These Smad complexes, when translocated into the nucleus, regulate the expression of several hundred genes involved in cell proliferation, differentiation, growth, migration, adhesion, immune response, apoptosis, and extracellular matrix production.[1–3] TGF-β plays an essential role in the initiation and progression of fibrosis in various organ systems such as liver,[4] lung,[5] kidney,[6] and heart.[7] Deregulation of TGF-β signaling has been implicated in various human diseases, including cancer,[8] pancreatic diseases,[9] and hematological malignancies.[10]

Current studies have shown that blocking the TGF-β signaling pathway with several small-molecule ATP-competitive ALK5 inhibitors such as **1** (SB-505124),[11] **2** (SD-208),[12] **3** (GW6604),[13] **4** (LY-2157299),[14] and **5** (EW-7197)[15] inhibited autophosphorylation of ALK5 and TGF-β-induced transcription of matrix genes in reporter assays at sub-micromolar concentrations. Among them, **4** and **5** have progressed to phase II and phase I clinical trials for cancer, respectively.

Previously, we reported a number of the 2-pyridyl-substituted triazoles,[16] thiazoles,[17] pyrazoles,[18–20] and imidazoles[21,22] as potential ALK5 inhibitors. Very recently, we have prepared a series of 2-substituted-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)-5-(6-methylpyridin-2-yl)imidazoles and found that **5** is a highly potent, selective, and orally bioavailable ALK5 inhibitor.[15] The **5** has demonstrated its pronounced anticancer and antifibrotic efficacy in various animal models.[23–25]

Recently, Bonafoux et al. reported a series of 2-aminoimidazoles as ALK5 inhibitors that possessing a F substituent at the 5-position of the 6-methyl-2-pyridyl moiety.[26] They showed that a 5-fluoro-substituted compound **6** was 1.5-fold more inhibitory in TGF-β-induced PAI-luciferase assay and 2.1-fold less inhibitory in p38α MAP kinase assay compared to a parent compound, indicating improved selectivity profile (Fig. 1).

On the basis of this finding, we decided to further investigate the effect of a F substituent at the three different positions (3, 4, or 5) of the 6-methyl-2-pyridyl moiety in **5** and its analogues on ALK5 inhibitory activity and selectivity. The target compounds

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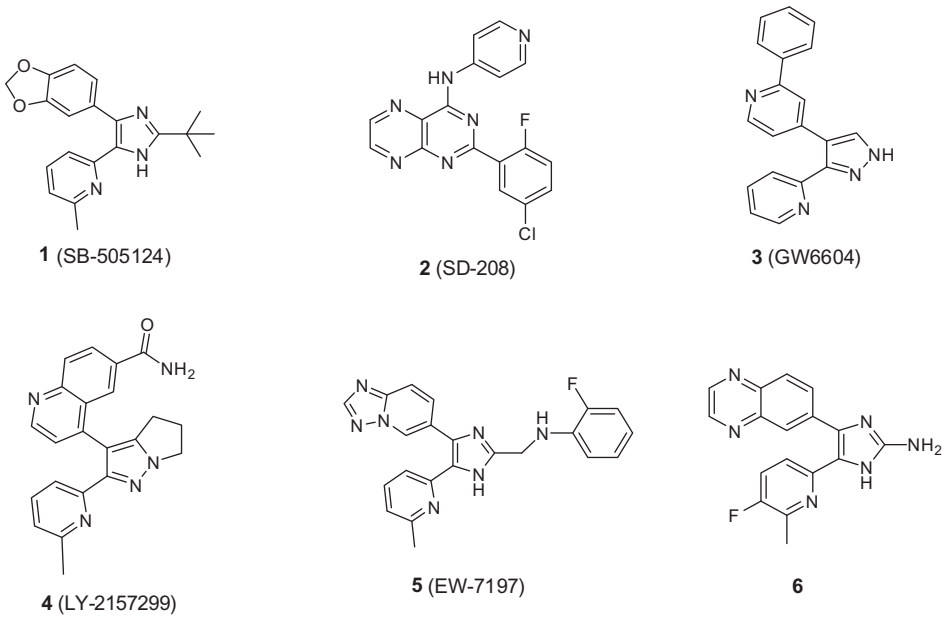
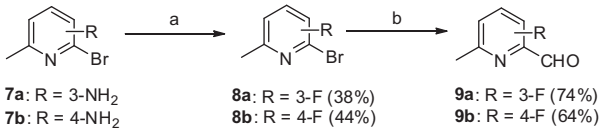
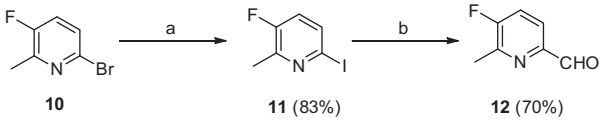


Figure 1. ALK5 inhibitors under development.



Scheme 1. Reagents and conditions: (a) (i) concn HCl, $NaNO_2$, 0 °C, 10 min; (ii) 65% HPF_6 in H_2O, 100 °C, 10 min; (b) *n*-BuLi (1.6 M in hexane), anhydrous DMF, toluene, −78 °C, 2 h.



Scheme 2. Reagents and conditions: (a) TMSCl, NaI, CH_3CN, 90 °C, 2 d; (b) *i*-PrMgCl (2.0 M in THF), CH_2Cl_2, anhydrous DMF, rt, overnight.

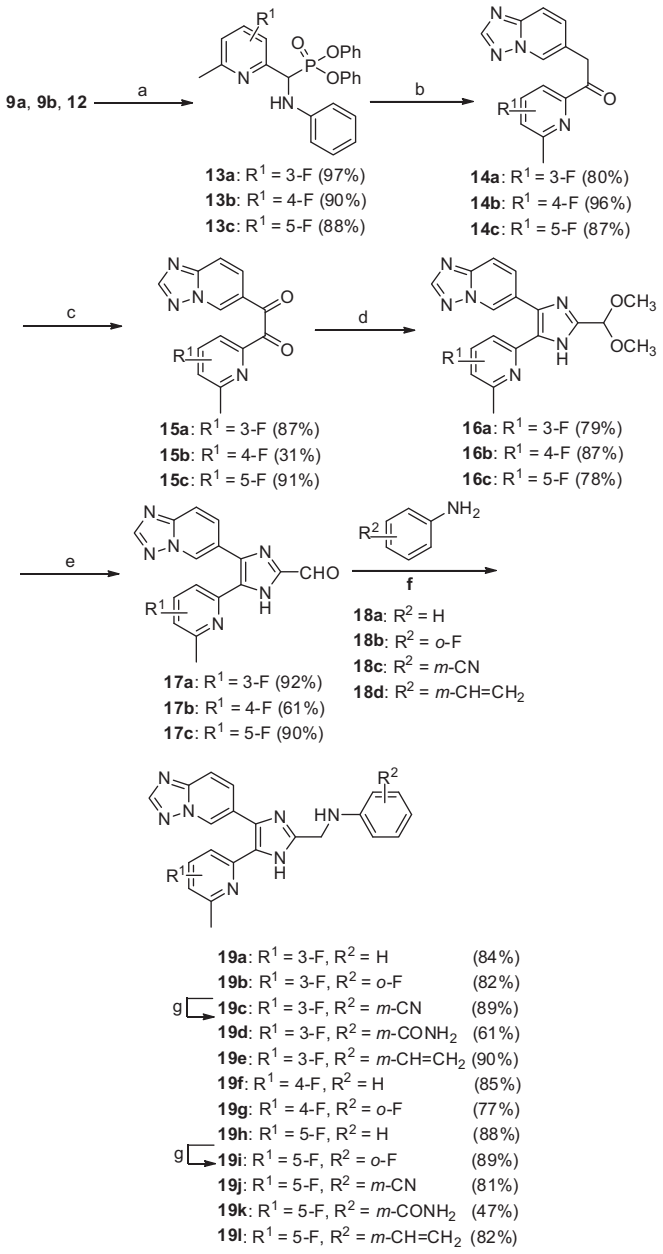
19b–e, **19g**, and **19i–l** possess a substituent, either *o*-F, *m*-CN, *m*-$CONH_2$, or *m*-CH=CH_2 in a phenyl ring because they were found to be the most beneficial to the ALK5 inhibitory activity and selectivity in a previous study.[15]

The requisite 3-, 4-, and 5-fluoro-6-methyl-2-pyridinecarboxaldehydes **9a**, **9b**, and **12** were prepared as shown in Schemes 1 and 2. Treatment of commercially available 3-amino-2-bromo-6-methylpyridine (**7a**) and 4-amino-2-bromo-6-methylpyridine (**7b**)[27] with aqueous $NaNO_2$ at 0 °C in the presence of concn HCl and followed by fluorination with 65% aqueous HPF_6 solution gave the corresponding fluoro compounds **8a** and **8b** in 38% and 44% yields, respectively. Lithiation of **8a** and **8b** with *n*-BuLi at −78 °C in toluene followed by treatment with anhydrous *N*,*N*-dimethylformamide gave the aldehydes **9a** and **9b** in 74% and 64% yields, respectively (Scheme 1).

The attempted conversion of the commercially available 2-bromo-5-fluoro-6-methylpyridine (**10**) to the aldehyde **12** under the similar reaction condition for the **9a** and **9b** shown in Scheme 1 was failed. Thus, alternatively, the bromo atom of the **10** was exchanged to the iodo atom with NaI in the presence of chlorotrimethylsilane (TMSCl) in CH_3CN at 90 °C to afford the **11** in 83% yield, which was subsequently converted to the aldehyde **12** in 70% yield by reaction with *i*-PrMgCl (2.0 M in THF) in CH_2Cl_2 and followed by treatment with anhydrous DMF (Scheme 2).

A series of 5-(fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)imidazoles **19a–l** was prepared as shown in Scheme 3. The aldehydes **9a**, **9b**, and **12** were treated with aniline and diphenyl phosphite in *i*-PrOH at room temperature to give the (phenylamino)methylphosphonates **13a–c** in 65–97% yields. Coupling of the **13a–c** with [1,2,4]triazolo[1,5-*a*]pyridine-6-carbaldehyde[28] in a mixture of THF and *i*-PrOH (4:1) at room temperature in the presence of Cs_2CO_3 and followed by hydrolysis with 3 N HCl gave the corresponding monoketones **14a–c** in 66–87% yields.

Oxidation of the **14a–c** with 48% HBr in DMSO at either 40 °C (for **15b**) or 70 °C (for **15a** and **15c**) afforded the diketones **15a–c** in 31–91% yields. It was found that the 4-fluoro atom in the pyridine ring was rather labile in this strong acidic condition, thus affording the **15b** in much lower yield compared to the **15a** and **15c** even at a lower reaction temperature. The condensation of the diketones **15a–c** with 60% 2,2-dimethoxyacetaldehyde and NH_4OAc in a mixture of *t*-BuOMe and MeOH (2:1) at room temperature produced the imidazoles **16a–c** in good yields. The acetal protecting group of the **16a** and **16c** was cleaved in 1 N HCl solution at 70 °C to give the imidazole-2-carboxaldehydes **17a** and **17c** in 92% and 90% yields, respectively. But, in the same reaction condition, the **16b** gave the **17b** along with the inseparable mixture of by-products as major products in which a F atom in the **17b** was replaced with a Cl atom or a OH group (determined by HRMS). Thus, deprotection of the acetal group in the **16b** was accomplished in a mild condition with *p*-toluenesulfonic acid monohydrate in anhydrous DMF at 50 °C to give the **17b** in 61% yield. Coupling of the **17a–c** with appropriately substituted anilines **18a–d** in 1,2-dichloroethane in the presence of AcOH and followed by reduction of the resulting imines with $NaBH_4$ in a mixture of THF and MeOH (1:3) gave the target compounds **19a–c**, **19e–j**, and **19l** in good yields (77–90%). Conversion of the nitrile functionality in compounds **19c** and **19j** to the corresponding carboxamide was achieved by treatment with 34.5% H_2O_2 and 1 N NaOH in EtOH to afford the **19d** and **19k** in 61% and 47% yields, respectively.

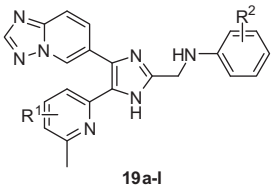


Scheme 3. Reagents and conditions: (a) aniline, diphenyl phosphite, *i*-PrOH, rt, 12–19 h; (b) (i) [1,2,4]triazolo[1,5-*a*]pyridine-6-carbaldehyde, Cs_2CO_3, THF/*i*-PrOH (4:1), rt, 24 h; (ii) 3 N HCl, rt, 1 h; (c) 48% HBr in H_2O, DMSO, 40 °C (for **15b**), 1.5 h or 70 °C (for **15a** and **15c**), 2 h; (d) 60% 2,2-dimethoxyacetaldehyde in H_2O, NH_4OAc, *t*-BuOMe/MeOH (2:1), rt, 4–6 h; (e) 1 N HCl, 70 °C, 2 h (for **17a** and **17c**) or PTSA·H_2O, anhydrous DMF, 50 °C, 24 h (for **17b**); (f) (i) **18a–d**, AcOH, 1,2-dichloroethane, reflux, 3–12 h; (ii) $NaBH_4$, MeOH/THF (3:1), rt, 0.5–4 h; (g) 34.5% H_2O_2, 1 N NaOH, EtOH, rt, 2.5–4 h.

To evaluate whether these potential inhibitors **19a–l** could inhibit ALK5, a kinase assay was performed using the purified human ALK5 kinase domain produced in Sf9 insect cells and casein as a substrate (Table 1). Because the kinase domain of p38α MAP kinase is known to be one of the most homologous to that of ALK5,[29] it was also chosen for a kinase assay to study the selectivity profile of this series of compounds.

All the imidazoles having a fluoro-substituent at the 3-position in the pyridine ring, **19a–e** (IC_{50} = 9.23–12.10 nM) showed the similar level of potency against ALK5 to that of **5** (IC_{50} = 9.67 nM). But, **19a–e** (IC_{50} = 171–766 nM) were 2.8–12.7-fold more potent in

Table 1
ALK5 and p38α inhibitory activity of 5-(fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)imidazoles **19a–l** in kinase assay



19a-l

Compd	R^1	R^2	IC_{50} (nM)		Selectivity index[c]
			ALK5[a]	p38α[b]	
19a	3-F	H	9.76	399	41
19b	3-F	*o*-F	12.10	766	63
19c	3-F	*m*-CN	9.23	209	23
19d	3-F	*m*-$CONH_2$	11.00	251	23
19e	3-F	*m*-CH=CH_2	9.81	171	17
19f	4-F	H	72.50	>10000	>137
19g	4-F	*o*-F	41.90	>10000	>238
19h	5-F	H	9.23	2040	221
19i	5-F	*o*-F	12.50	3370	267
19j	5-F	*m*-CN	7.68	1240	161
19k	5-F	*m*-$CONH_2$	10.80	1480	137
19l	5-F	*m*-CH=CH_2	13.70	1810	132
1 (SB-505124)			34.90	668	19
4 (LY-2157299)			69.40	405	6
5 (EW-7197)			9.67	2180	225

[a] ALK5 was expressed in Sf9 insect cells as human recombinant GST-fusion protein by means of the vaculovirus expression system. A proprietary radioisotopic protein kinase assay (^{33}PanQinase® Activity Assay) was performed at ProQinase GmbH (Freiburg, Germany) using casein as a substrate.
[b] p38α MAP kinase was expressed as untagged human recombinant protein in *E. coli*. The enzyme was purified by Ni–NTH–agarose (Qiagen). A proprietary radioisotopic protein kinase assay (^{33}PanQinase® Activity Assay) was performed at ProQinase GmbH (Freiburg, Germany) using ATF2 as a substrate.
[c] IC_{50} of p38α/IC_{50} of ALK5.

Table 2
ALK5 inhibitory activity of **19j**, **1**, **4**, and **5** in luciferase assay

Compd	p3TP-luciferase activity[a,b] (% control)
Mock[c]	3 ± 0.3
TGF-β	100 ± 10.3
19j	18 ± 4.3
1 (SB-505124)	45 ± 1.3
4 (LY-2157299)	140 ± 3.8
5 (EW-7197)	35 ± 4.5

[a] Luciferase activity was determined at a concentration of 100 nM of inhibitor.
[b] Activity is given as the mean ± SD of three independent experiments run in triplicate relative to control incubations without DMSO vehicle.
[c] Luciferase activity was determined without treatment of TGF-β and inhibitor.

p38α MAP kinase inhibition than **5** (IC_{50} = 2180 nM), thus showing that the selectivity indices of the former (17–63) were much lower than that of the latter (225). Introduction of a fluoro-substituent at the 4-position in the pyridine ring had negative impact on ALK5 inhibition, thus, **19f** (R^2 = H, IC_{50} = 72.50 nM) and **19g** (R^2 = *o*-F, IC_{50} = 41.90 nM) displayed 7.5- and 4.3-fold lower ALK5 inhibitory activity than **5**, respectively. They also did not inhibit p38α MAP kinase up to a concentration of 10 μM. The imidazoles having a fluoro-substituent at the 5-position in the pyridine ring, **19h–l** displayed the similar level of potency to that of **5** against both ALK5 (IC_{50} = 7.68–13.70 nM) and p38α MAP kinase (IC_{50} = 1240–3370 nM) regardless of R^2 substituent. Consequently, the selectivity indices of the compounds (132–267) were comparable to that of **5**. All the 3- and 5-fluoro-substituted compounds were more potent in ALK5 inhibition than the competitors **1** (IC_{50} = 34.90 nM) and **4** (IC_{50} = 69.40 nM). And, the 4- and 5-fluoro-substituted

compounds showed much higher selectivity indices against p38α MAP kinase than **1** (19) and **4** (6).

Because **19j** showed slightly higher level of potency in ALK5 inhibition in a kinase assay (IC_{50} = 7.68 nM) than **5**, a cell-based luciferase assay was performed using HaCaT cells permanently transfected with p3TP-luciferase reporter construct[30] at a concentration of 100 nM to compare their ALK5 inhibitory activity with competitors **1** and **4** (Table 2). Similar to the kinase assay, **19j** (82% inhibition) was more inhibitory than **1** (55% inhibition), **4** (no inhibition), and **5** (65% inhibition) in a cell-based luciferase assay.

In this letter, we have synthesized and evaluated a series of 5-(3-, 4-, or 5-fluoro-substituted-6-methylpyridin-2-yl)-4-([1,2,4]-triazolo[1,5-*a*]pyridin-6-yl)imidazoles **19a–l** to further optimize an ALK5 inhibitor **5**. The 3-fluoro-substituted compounds showed the similar level of potency against ALK5 to that of **5**, but were much less selective against p38α MAP kinase than **5**. The 4-fluoro-substituted compounds were chemically rather unstable and displayed much lower ALK5 and p38α MAP kinase inhibitory activity than **5**. The 5-fluoro-substituted compounds displayed the similar level of potency to that of **5** against both ALK5 and p38α MAP kinase. One of the 5-fluoro-substituted compounds, **19j** was found to be more inhibitory than the parent compound **5** in ALK5 inhibition in both kinase assay and cell-based luciferase assay with a high selectivity index of 161 against p38α.

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Supplementary data

Supplementary data (synthetic procedures and analytical data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.09.058.

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