European Journal of Medicinal Chemistry 46 (2011) 3917–3925

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of 1-substituted-3(5)-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)pyrazoles as transforming growth factor-β type 1 receptor kinase inhibitors

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ARTICLE INFO

Article history:
Received 7 March 2011
Received in revised form
9 May 2011
Accepted 26 May 2011
Available online 21 June 2011

Keywords:
TGF-β
ALK5 inhibitor
Fibrosis
Cancer
Kinase assay
Cell-based luciferase reporter assay
Docking

ABSTRACT

A series of 1-substituted-3(5)-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)pyrazoles **14a–d**, **15a–d**, **17a**, **17b**, **18a–d**, **19a**, and **19b** has been synthesized and evaluated for their ALK5 inhibitory activity in an enzyme assay and in a cell-based luciferase reporter assay. The 2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1*H*-pyrazol-1-yl]-*N*-phenylethanethioamide (**18a**) inhibited ALK5 phosphorylation with an IC_{50} value of 0.013 μM and showed 80% inhibition at 0.1 μM in a luciferase reporter assay using HaCaT cells permanently transfected with p3TP-luc reporter construct.

1. Introduction

The transforming growth factor-β (TGF-β) superfamily members, which include TGF-βs, activins, bone morphogenetic proteins (BMPs), growth and differentiation factors, and Mullerian inhibiting substance are structurally related secreted cytokines found in species ranging from worms and insects to mammals. A wide range of cellular functions such as cell proliferation, differentiation, adhesion, migration, and apoptosis are regulated by TGF-β superfamily members. The TGF-βs include the three major TGF-β isoforms, TGF-β1, TGF-β2, and TGF-β3 which are expressed in mammals. Among them, TGF-β1 is the prototypic member of this family of cytokines and is the major isoform. TGF-β1 transduces signals through a complex of two related but structurally and functionally distinct serine/threonine kinase receptors, termed type 1 and type II [1–3]. The signaling cascade is promoted by the binding of ligand to the constitutively active type II receptors on the cell surface, which further recruits the type I receptors, also called as activin receptor-like kinase 5 (ALK5), into the complex. Subsequently, ALK5 is phosphorylated in the juxtamembrane GS domain by the type II receptors thereby creating a binding site for Smad proteins and stimulating its kinase activity. The activated ALK5 phosphorylates Smad2 and Smad3 proteins thereby causing their dissociation from the receptor and heteromeric complex formation with Smad4. These Smad complexes translocate to the nucleus, assemble with specific DNA-binding co-factors and co-modulators to finally activate several hundred genes involved in cell differentiation, proliferation, apoptosis, migration, and extracellular matrix production [4]. TGF-β1 plays a critical role in the initiation and progression of fibrosis in various organ systems such as kidney [5], heart [6], lung [7], and liver [8]. Deregulation of TGF-β signaling has been also implicated in various human diseases including cancer [9], pancreatic diseases [10], and hematological malignancies [11].

There are a number of reports that the therapeutic administration of TGF-β binding proteins including the proteoglycan decorin [12], soluble chimeric TGF-β receptors [13], and neutralizing antibodies

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doi:10.1016/j.ejmech.2011.05.063

[14] ameliorates experimental fibrosis. Recent studies have shown that several small molecules ATP-competitive ALK5 inhibitors (Fig. 1) such as **1** (SB-505154) [15], **2** (SB-525334) [16], **3** (GW6604) [17], **4** (SD-208) [18,19], and **5** (LY-2157299) [20] inhibited autophosphorylation of ALK5 and TGF-β induced transcription of matrix genes in receptor assays at sub micromolar concentrations. Among them **2**, **3**, and **4** effectively retarded progressive fibrosis in kidney, liver and lung, respectively, and **4** and **5** also strongly inhibited growth and invasiveness of cancer cells in animal models.

We have prepared a number of the 2-pyridyl-substituted triazoles, pyrazoles, imidazoles, and thiazoles as ALK5 inhibitors and found that insertion of a methylene, a methyleneamino, or an aminomethylene linkage between a central five-membered heterocyclic ring and a phenyl ring significantly increased ALK5 inhibitory activity and selectivity [21–29]. Among them, the imidazole IN-1130 (**6**), one of our preclinical candidate, demonstrated its remarkable activity as a suppressor of fibrogenic process of unilateral ureteral obstruction in rats [30] and ameliorated experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, by inhibition of TGF-β signaling [31]. It also reduced tunical fibrosis and corrected penile curvature in rats [32], inhibited cancer metastasis in MMTV/c-Neu breast cancer mice, and enhanced CTL response in cancer mice [33]. The imidazole IN-1233 (**7**), another preclinical candidate, effectively prevented development and progression of pulmonary arterial hypertension in monocrotaline rat model through inhibition of TGF-β signaling [34] and reduced granulation tissue formation after bare metallic stent placement in a rat urethral model [35].

A novel class of ALK5 inhibitors possessing a thioamide linkage between a pyrazole ring and a phenyl ring has been reported, and among them, A-83-01 (**8**) exhibited significant inhibition of the transcriptional activity induced by ALK5 [36]. We also recently reported a new class of 2-pyridyl-substituted pyrazoles possessing a thioamide linkage, and one of the compounds in this series, 3-[3-(6-methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazol-1-carbothioamido]benzamide (**9**), showed more than 90% inhibition at 0.1 μM in luciferase reporter assays [26]. Although insertion of a thioamide linkage between a pyrazole ring and a phenyl ring markedly increased ALK5 inhibitory activity as shown in previous reports [26,36], the thioamide linkage was rather unstable and slowly cleaved to generate a pyrazole ring on long-term storage.

Therefore, in this report, we attempted to replace a thioamide linkage with a chemically stable thioamidomethylene linkage, thus, prepared the compounds **18a**–**d** as target molecules. To compare the influence of the thioamidomethylene linkage of **18a**–**d** on ALK5 activity, their counterpart derivatives **14a**–**d** possessing the amidomethylene linkage were prepared. The compounds **17a** and **17b** possessing the amidoethylene linkage were also prepared to optimize the length between the pyrazole ring and a phenyl ring. A docking model of ALK5:**7** complex showed that the nitrogen atom of the quinolin-6-yl moiety formed an H-bond with backbone amide NH of His283 in the hinge region of the kinase, originally a binding pocket for the adenine ring of ATP [27]. To examine whether the capability of the *N*-1 of the quinoxalin-6-yl moiey as an H-bond acceptor could be increased by aromatic substitution, compounds having a 2,3-dimethylquinoxalin-6-yl moiety were prepared for comparison (**14b**, **14d**, **15b**, **15d**, **17b**, **18b**, **18d**, and **19b**).

2. Results and discussion

2.1. Chemistry

The 3-(6-methylpyridin-2-yl)-1-(phenylcarboxamidomethyl)-4-(quinoxalin-6-yl)pyrazoles **14a**–**d** were synthesized as shown in Scheme 1. The quinoxaline-6-carbaldehyde (**10a**) [37] and 2,3-dimethylquinoxaline-6-carbaldehyde (**10b**) [37] were coupled with diphenyl (6-methylpyridin-2-yl)(phenylamino)methylphosphonate [38] in a mixture of THF and *i*-PrOH (4:1) in the presence of Cs_2CO_3 at room temperature and followed by treatment with 3 N HCl to afford the corresponding ketones **11a** and **11b** in 79% and 86% yields. Treatment of **11a** and **11b** with *N*,*N*-dimethylformamide dimethyl acetal (DMF·DMA) in DMF at 80 °C in the presence of AcOH and followed by cyclization with hydrazine monohydrate in absolute EtOH produced the pyrazoles **12a** [39] and **12b** in 79% and 75% yields. The pyrazoles **12a** and **12b** were alkylated with 2-chloro-*N*-phenylacetamide (**13a**) [40] or 2-chloro-*N*-(3-cyanophenyl)acetamide (**13b**) [41] in the presence of NaH in anhydrous DMF to yield the target compounds **14a**–**d** and their positional isomers **15a**–**d** in 41–70% and 4–10% yields, respectively. The positional isomers were separated by MPLC, and their structures were confirmed by NOE experiments. In NOE experiments, irradiation of the methylene protons of the **14a** at δ 5.14 gave an enhancement of the proton *H*-5 in pyrazole ring at δ 7.86, while

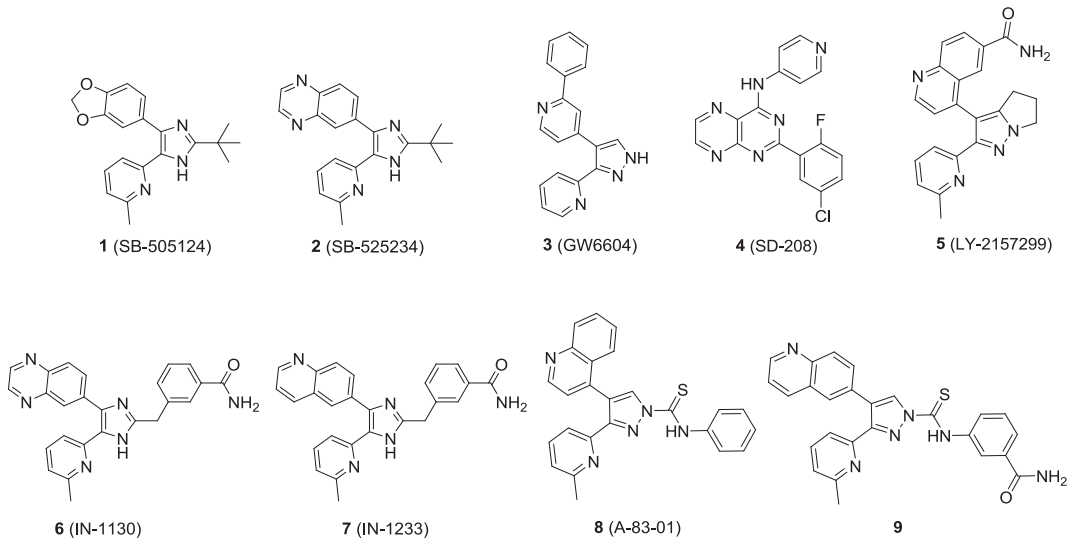
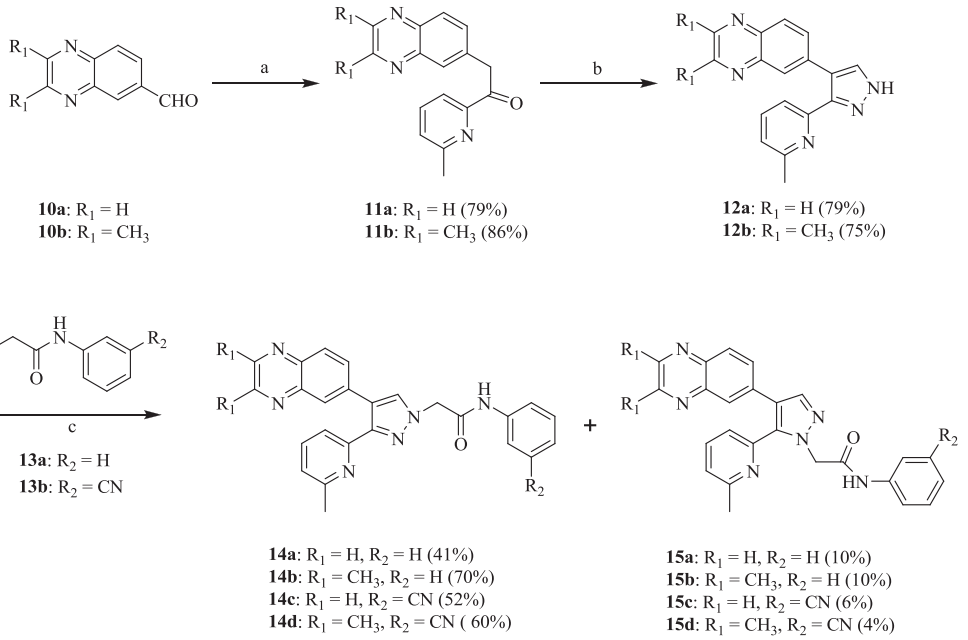


Fig. 1. Small molecule ATP-competitive ALK5 inhibitors.



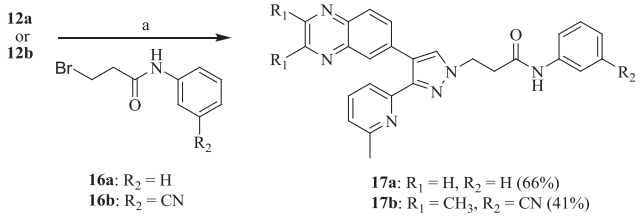
Scheme 1. Reagents and conditions: (a) diphenyl (6-methylpyridin-2-yl)(phenylamino)methylphosphonate, Cs_2CO_3, THF: *i*-PrOH (4:1), rt, 16 h; (ii) 3 N HCl 1 h; (b) (i) DMF·DMA, DMF, AcOH, 80 °C, 2 h; (ii) $N_2H_4 \cdot H_2O$, EtOH, reflux, 4 h; (c) NaI (cat.), NaH, anhydrous DMF, rt, 2 h.

irradiation of the methylene protons of the **15a** at δ 5.06 gave no enhancement of the proton *H*-5 in pyrazole ring at δ 7.95, confirming the respective positions of alkylation.

The 3-(6-methylpyridin-2-yl)-1-(phenylcarboxamidoethyl)-4-(quinoxalin-6-yl)pyrazoles **17a** and **17b** were synthesized as shown in Scheme 2. Reaction of aniline or 3-aminobenzonitrile with 3-bromopropanoyl chloride in the presence of K_2CO_3 in CH_2Cl_2 gave 3-bromo-*N*-phenylpropanamide (**16a**) [42] and 3-bromo-*N*-(3-cyanophenyl)propanamide (**16b**) in 58% and 70% yields, respectively. Alkylation of the pyrazoles **12a** and **12b** with **16a** or **16b** in the presence of Cs_2CO_3 in anhydrous DMF at 120 °C afforded the target compounds **17a** and **17b** in 66% and 41% yields, respectively. In this case, the positional isomers of the **17a** and **17b** were not isolated after column chromatography.

Thionation of the **14a**–**d** with Lawesson's reagent in anhydrous DME at reflux condition produced the thioamides **18a**–**d** in 24–68% yields (Scheme 3). It was observed in the ^{1}H NMR that the methylene protons attached to the *N*-1 in the thioamides **18a**–**d** were shifted to the downfield by around ca. 0.4 ppm relative to those in the amides **14a**–**d**.

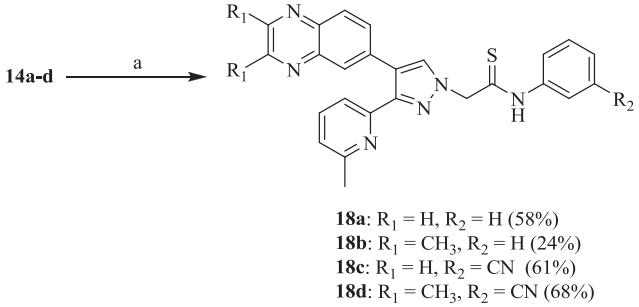
Conversion of the nitrile functionality in compounds **18c** and **18d** to the corresponding carboxamide functionality was attempted by treatment with 28% H_2O_2 and 1 N NaOH in EtOH at 40 °C, however, both hydrolysis and dethionation took place to generate the **19a** and **19b** in 23% and 30% yields, respectively (Scheme 4). As expected, all of the target compounds we prepared were quite stable at room temperature on long-term storage.



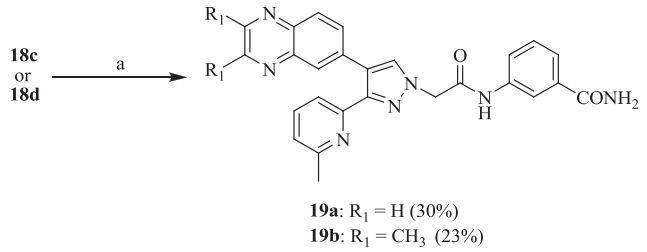
Scheme 2. Reagents and conditions: (a) Cs_2CO_3, anhydrous DMF, 120 °C, 2 h.

2.2. ALK5 inhibitory activity in an enzyme assay and in a cell-based luciferase reporter assay

To investigate whether these compounds **14a**–**d**, **15a**–**d**, **17a**, **17b**, **18a**–**d**, **19a**, and **19b** could inhibit ALK5, a kinase assay was performed using the purified human ALK5 kinase domain produced in Sf9 insect cells. All the compounds possessing a quinoxalin-6-yl moiety (**14a**, **14c**, **15a**, **15c**, **17a**, **18a**, **18c**, and **19a**) displayed potent ALK5 inhibition (IC_{50} = 0.013–0.737 μM), whereas those possessing a 2,3-dimethylquinoxalin-6-yl moiety (**14b**, **14d**, **15b**, **15d**, **17b**, **18b**, **18d**, and **19b**) displayed no significant ALK5 inhibitory activity up to a concentration of 1 μM (Table 1). We envisioned that introduction of electron-donating groups in the 6-quinoxalinyl moiety may increase the capability of the *N*-1 in that moiety as an H-bond acceptor, thus, potentiating ALK5 inhibitory activity. But, the result showed that a 2,3-dimethylquinoxalin-6-yl moiety seemed to be not accommodated favorably into the ATP binding pocket of ALK5. The 3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)pyrazoles **14a** (IC_{50} = 0.038 μM) and **14c** (IC_{50} = 0.153 μM) displayed 3.8-fold and 4.8-fold higher ALK5 inhibitory activity than their respective positional isomers, 5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-pyrazoles **15a** (IC_{50} = 0.143 μM) and **15c** (IC_{50} = 0.737 μM). The length of alkyl chain between the *N*-1 of the central pyrazole and



Scheme 3. Reagents and conditions: (a) Lawesson's reagent, anhydrous DME, 85 °C, 12 h.

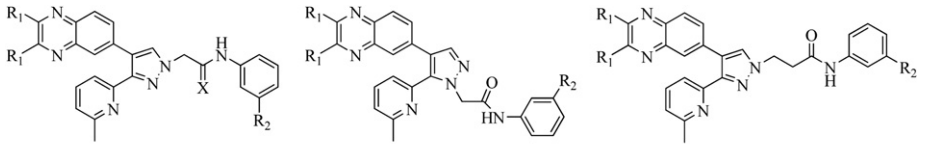


Scheme 4. Reagents and conditions: (a) 28% H_2O_2, 1 N NaOH, EtOH, 40 °C, 4 h.

a phenyl ring also influenced the ALK5 inhibition. Thus, the pyrazole **17a** (IC_{50} = 0.082 μM) possessing an amidoethylene linkage was 2.2-fold less potent than the corresponding **14a** possessing an amidomethylene linkage. We have reported that introduction of a carbonitrile or a carboxamide group at *meta*-position of the phenyl or benzyl moiety in the 2-pyridinyl-substituted pyrazoles, triazoles, imidazoles, and thiazoles significantly increased their ALK5 inhibitory activity. However, it is not the case in this series of compounds. The unsubstituted pyrazole **14a** was slightly more potent than the respective carboxamide-substituted **19a** (IC_{50} = 0.048 μM) and 4.0-fold more potent than the carbonitrile-substituted **14c**, and the unsubstituted **18a** (IC_{50} = 0.013 μM) was 2.0-fold more potent than the respective carbonitrile-substituted **18c** (IC_{50} = 0.026 μM). It was previously demonstrated by Tojo et al. [36] and us (unpublished work) that the pyrazoles possessing a thioamido linkage between a pyrazole ring and a phenyl ring were much more potent in ALK5 inhibition than those possessing a respective carboxamido linkage. As expected, the pyrazoles **18a** and **18c** possessing a thioamidomethylene linkage was 2.9-fold and 5.9-fold more potent than the corresponding **14a** and **14c** possessing an amidomethylene linkage. The pyrazole **18a** showed the most potent ALK5 inhibitory activity in this series of compounds. It was slightly more potent than **6** (IC_{50} = 0.017 μM) and 4.2-fold more potent than **1** (IC_{50} = 0.054 μM).

To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of all target

Table 1
Inhibitory activity of 1-substituted-3(5)-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)pyrazoles **14a–d**, **15a–d**, **17a**, **17b**, **18a–d**, **19a**, and **19b** on ALK5.



Compound	R_1	R_2	X	IC_{50} (μM)		Selectivity index[c]	p3TP-luciferase activity (% control)[d,e]
				ALK5[a]	p38α[b]		
Mock							9 ± 1
TGF–β							100 ± 4
14a	H	H	O	0.038	>1.0	>26	36 ± 3
14b	CH_3	H	O	>1.0	>1.0		99 ± 10
14c	H	CN	O	0.153	>1.0	>7	36 ± 3
14d	CH_3	CN	O	>1.0	>1.0		92 ± 3
15a	H	H		0.143	>1.0	>7	80 ± 3
15b	CH_3	H		>1.0	>1.0		112 ± 8
15c	H	CN		0.737	>1.0	>1	90 ± 4
15d	CH_3	CN		>1.0	>1.0		127 ± 9
17a	H	H		0.082	>1.0	>12	34 ± 0
17b	CH_3	CN		>1.0	>1.0		113 ± 15
18a	H	H	S	0.013	>1.0	>77	20 ± 1
18b	CH_3	H	S	>1.0	>1.0		97 ± 6
18c	H	CN	S	0.026	>1.0	>38	24 ± 3
18d	CH_3	CN	S	>1.0	>1.0		92 ± 12
19a	H	$CONH_2$	O	0.048	>1.0	>21	46 ± 4
19b	CH_3	$CONH_2$	O	>1.0	>1.0		97 ± 8
1(SB–505124)				0.054	0.594	11	34 ± 0
6 (IN-1130)				0.017	0.480	28	20 ± 3

Elemental Analysis Data**14a:** $C_{25}H_{24}N_7O$ Found: C, 68.22; H, 5.60; N, 22.44. Requires: C, 68.48; H, 5.52; N, 22.36. **14b**: $C_{27}H_{25}N_6O$ Found: C, 72.01; H, 5.68; N, 18.61. Requires: C, 72.14; H, 5.61; N, 18.70. **14c:** $C_{26}H_{20}N_7O$ Found: C, 70.13; H, 4.44; N, 21.74. Requires: C, 69.94; H, 4.52; N, 21.96. **14d:** $C_{28}H_{24}N_7O$ Found: C, 70.67; H, 5.22; N, 20.73. Requires: C, 70.87; H, 5.10; N, 20.66. **15a:** $C_{25}H_{24}N_7O$ Found: C, 68.55; H, 5.36; N, 22.22. Requires: C, 68.48; H, 5.52; N, 22.36. **15b:** $C_{27}H_{25}N_6O$ Found: C, 72.01; H, 5.77; N, 18.59. Requires: C, 72.14; H, 5.61; N, 18.70. **15c:** $C_{26}H_{20}N_7O$ Found: C, 69.88; H, 4.58; N, 21.79. Requires: C, 69.94; H, 4.52; N, 21.96. **15d:** $C_{28}H_{24}N_7O$ Found: C, 71.03; H, 4.88; N, 20.56.Requires: C, 70.87; H, 5.10; N, 20.66. **17a:** $C_{26}H_{23}N_6O$ Found: C, 71.62; H, 5.52; N, 19.18.Requires: C, 71.71; H, 5.32; N, 19.30. **17b:** $C_{29}H_{26}N_7O$ Found: C, 71.44; H, 5.28; N, 19.95. Requires: C, 71.29; H, 5.36; N, 20.07. **18a:** $C_{25}H_{21}N_6S$ Found: C, 68.42; H,4.99; N,19.01. Requires: C, 68.63; H,4.84; N,19.21. **18b:** $C_{27}H_{25}N_6S$ Found: C, 69.43; H, 5.63; N, 17.99. Requires: C, 69.65; H, 5.41; N, 18.05. **18c:** $C_{26}H_{20}N_7S$ Found: C, 67.82; H, 4.12; N, 21.02. Requires: C, 67.51; H, 4.36; N, 21.20. **18d:** $C_{28}H_{24}N_7S$ Found: C, 68.34; H, 5.14; N, 19.92. Requires: C, 68.55; H, 4.93; N, 19.99. **19a:** $C_{26}H_{22}N_7OS$ Found: C, 64.77; H, 4.74; N, 20.63. Requires: C, 64.98; H, 4.61; N, 20.40. **19b:** $C_{28}H_{26}N_7OS$ Found: C, 65.88; H, 5.31; N, 19.11. Requires: C, 66.12; H, 5.15; N, 19.28.

[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.

[d] Luciferase activity was determined at a concentration of 0.1 μM of inhibitor.

[e] Activity is given as the mean ± SD of three independent experiments run in triplicate relative to control incubations with DMSO vehicle.

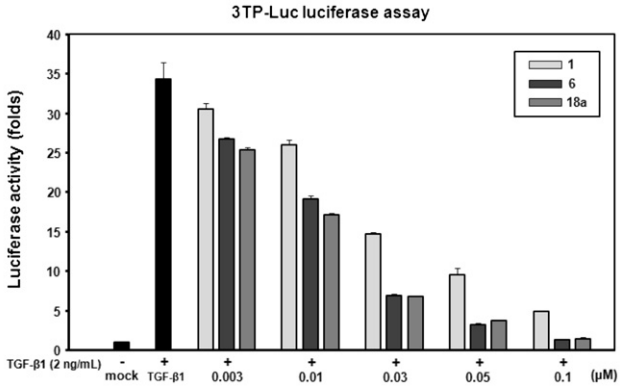


Fig. 2. Effect of **18a** on the activity of TGF-β-induced ALK5. HaCaT cells were permanently transfected with p3TP-luciferase reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as the mean ± SD of three independent experiments run in triplicate relative to control.

molecules was measured using HaCaT cells permanently transfected with p3TP-luciferase reporter construct at a concentration of 0.1 μM (Table 1). The p3TP-luciferase reporter construct contains three AP-1 binding elements and the plasminogen-activator inhibitor-1 (PAI-1) promoter [43]. Similar to kinase assay, the compounds possessing a 2,3-dimethylquinoxalin-6-yl moiety, **14b**, **14d**, **15b**, **15d**, **17b**, **18b**, **18d**, and **19b** showed no significant ALK5 inhibitory activity, and inhibition of the luciferase activity by most compounds consisted with that of kinase assay. The pyrazole derivative **18a** was the most inhibitory, showing 80% inhibition that is comparable to that of **6** (80%) and higher than that of **1** (66%).

The most potent compound **18a** was chosen, and its ALK5 inhibitory activity was compared with those of **1** and **6** at five different concentrations (0.003, 0.01, 0.03, 0.05, and 0.1 μM) using HaCaT cells permanently transfected with p3TP-luciferase reporter construct. As shown in Fig. 2, **18a** inhibited ALK5 in a dose-dependent manner and was equipotent to **6** and more potent than **1**.

2.3. p38α MAP kinase assay

The kinase domain of p38α MAP kinase is known to be one of the most homologous to that of ALK5 [44], therefore, it was chosen to examine the selectivity profile of this series of compounds. All the target molecules we prepared were devoid of p38α MAP kinase inhibitory activity up to the maximum concentration of 1 μM tested. Compound **18a** was found to be the most selective in this series, showing the selectivity index of >77 that is higher than those of **6** (28) and **1** (11).

2.4. Binding mode of *18a* in the ALK5 active site

To examine whether compound **18a** will bind into the same binding pocket that is occupied by **9**, we compared docking poses between **18a** and **9**. As shown in Fig. 3, both **18a** and **9** fit well into the ALK5 active site, being superimposed over the X-ray pose of 1,5-naphthyrine inhibitor [45] (yellow). The quinoxalinyl ring of **18a** and the corresponding 6-quinolinyl moiety of **9** occupy the pocket for adenine ring of ATP, with forming a hydrogen bond backbone NH of His283. The thioamide NH group of the linker in **18a** forms an H-bond with Lys337, one of the conserved lysines in the catalytic domain of ALK5. Instead, the thioamide NH group of **9** is hydrogen-bonded to Asp351, and the carboxamide-substituent forms additional H-bonds with Lys337 and Ser287. Although the calculated binding energy scores (FlexX score) of both compounds show that **18a** (−31.4 kcal/mol) forms less stable complex with ALK5 than **9** (−41.4 kcal/mol), **18a** is more desirable than **9** in terms of chemical stability.

3. Conclusion

In this report, a series of 1-substituted-3(5)-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)pyrazoles has been synthesized and evaluated for their ALK5 inhibitory activity in an enzyme assay and in a cell-based luciferase reporter assay. The structure–activity relationships in this series of compounds have been established and discussed. The pyrazole derivative **18a** displayed the most significant ALK5 inhibitory activity in the series of compounds that is much higher than that of **1** and is equipotent to **6**. This series of compounds were selective for ALK5 inhibition than p38α MAP kinase inhibition. The selectivity index of **18a** against p38α MAP kinase is >77 that is much higher than those of **1** (11) and **6** (28).

4. Experimental section

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded on a varian Unity 400 spectrophotometer. The chemical shifts are reported in parts per

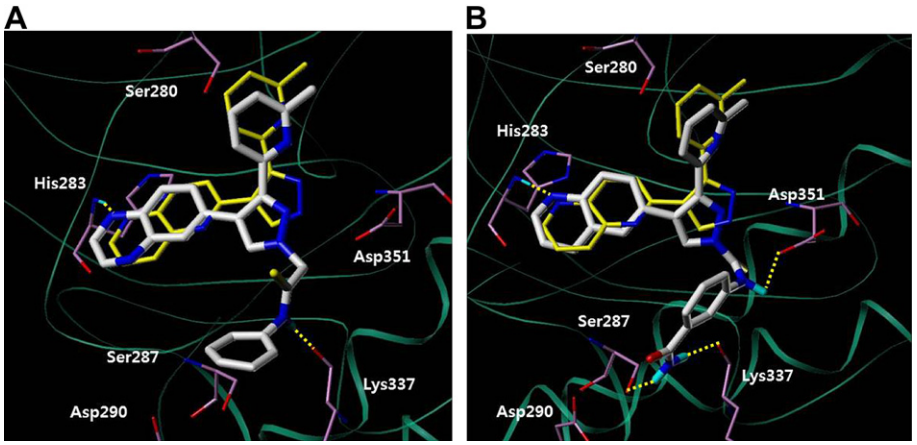


Fig. 3. Binding pose of **18a** (A) and **9** (B) in the active site of ALK5, superimposed over the X-ray pose of 1,5-naphthyrine inhibitor (yellow carbon). Key amino acid residues within the binding site are represented in line form, and the bound ligand is rendered in capped stick (grey carbon). Yellow dotted lines are hydrogen bonding interactions (<2.5 Å).

million (ppm) relative to internal tetramethylsilane as an internal standard. Infrared spectra were recorded on an FT-infrared spectrometer (Bio-Rad). High resolution mass spectra electro spray ionization (HRMS-ESI) was obtained on an Agilent technologies 6220 TOF LC/MS spectrometer. All melting points were taken in Pyrex capillaries using an electrothermal digital melting point apparatus (Buchi) and are not correct. 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 an YFLC-540 ceramic pump (Yamagen). All chemicals and solvents were purchased from Aldrich or TCI Laboratory Chemicals.

*4.1.1. General procedure for the preparation of 1-(6-methylpyridin-2-yl)-2-(quinoxalin-6-yl)ethanone (**11a**) and 2-(2,3-dimethylquinoxalin-6-yl)-1-(6-methylpyridin-2-yl)ethanone (**11b**)*

To a stirred solution of **10a** or **10b** (18.97 mmol) in a mixture of THF (40 mL) and *i*-PrOH (10 mL), diphenyl (6-methylpyridin-2-yl)(phenylamino)methylphosphonate (18.97 mmol) and Cs_2CO_3 (24.65 mmol) were added. The mixture was stirred at room temperature for 16 h, and to it, 3 N HCl (25 mL) was added dropwise over a period of 5 min. The reaction mixture was diluted with MTBE (30 mL). The aqueous layer was separated, and the organic layer was extracted with 1 N HCl (2 × 50 mL). The combined aqueous layer was neutralized with 50% aqueous KOH solution (pH 7∽8) and extracted with EtOAc (3 × 100 mL). The EtOAc solution 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 EtOAc/hexane as eluent to give the titled compound **11a** or **11b** as a light yellow solid.

*4.1.1.1. 1-(6-Methylpyridin-2-yl)-2-(quinoxalin-6-yl)ethanone (**11a**).* Yield 79%; mp 128.6 °C; IR (KBr) 1696 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.68 (s, 3H), 4.81 (s, 2H), 7.35 (dd, 1H, J = 8.0, 0.8 Hz), 7.72 (t, 1H, J = 7.8 Hz), 7.80 (dd, 1H, J = 8.8, 1.6 Hz), 7.88 (dd, 1H, J = 8.0, 0.8 Hz), 8.06 (d, 1H, J = 1.6 Hz), 8.07(d, 1H, J = 8.8 Hz), 8.81 (d, 1H, J = 2.0 Hz), 8.82 (d, 1H, J = 2.0 Hz); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{16}H_{14}N_3O$: 264.1131, found 264.1135.

*4.1.1.2. 2-(2,3-Dimethylquinoxalin-6-yl)-1-(6-methylpyridin-2-yl)ethanone (**11b**).* Yield 86%; mp 195.5 °C; IR (KBr) 1698 (CO) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.67 (s, 3H), 2.73 (s, 6H), 4.76 (s, 2H), 7.33 (br d, 1H, J = 7.6 Hz), 7.68 (dd, 1H, J = 8.6, 1.8 Hz), 7.70 (t, 1H, J = 7.6 Hz), 7.86 (br d, 1H, J = 7.6 Hz), 7.94–7.96 (m, 2H)); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{18}H_{18}N_3O$: 292.1444, found 292.1454.

*4.1.2. General procedure for the preparation of 6-[3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]quinoxaline (**12a**) and 2,3-dimethyl-6-[3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]quinoxaline (**12b**)*

To a stirred solution of **11a** or **11b** (2.66 mmol) in anhydrous DMF (7 mL), AcOH (9.58 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (7.98 mmol) were added. The mixture was heated at 80 °C for 2 h. After cooled to room temperature, the reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in EtOH (10 mL), and to it, hydrazine monohydrate (54.88 mmol) was added. The mixture was heated at reflux temperature for 4 h, then cooled to room temperature, and evaporated to dryness under reduced pressure. The residue was diluted with $CHCl_3$ (100 mL) and washed with water (30 mL) and brine (30 mL). The $CHCl_3$ solution 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 MeOH/CH_2Cl_2 as eluent to give the titled compound **12a** or **12b** as a light yellow foam.

*4.1.2.1. 6-[3-(6-Methylpyridin-2-yl)-1H-pyrazol-4-yl]quinoxaline (**12a**).* Yield 79%; mp 105.4 °C; IR (KBr) 3159 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.59 (s, 3H), 7.10 (d, 1H, J = 7.6 Hz), 7.20 (d, 1H, J = 7.6 Hz), 7.45 (t, 1H, J = 7.6 Hz), 7.81 (s, 1H), 7.84 (dd, 1H, J = 8.4, 2.0 Hz), 8.12 (d, 1H, J = 8.4 Hz), 8.19 (d, 1H, J = 1.6 Hz), 8.85 (d, 1H, J = 2.0 Hz), 8.86 (d, 1H, J = 2.0 Hz).

*4.1.2.2. 2,3-Dimethyl-6-[3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]-quinoxaline (**12b**).* Yield 75%; mp 119.2 °C; IR (KBr) 3164 (NH) cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.65 (s, 3H), 2.75 (s, 3H), 2.76 (s, 3H), 7.11 (d, 1H, J = 7.6 Hz), 7.20 (d, 1H, J = 7.6 Hz), 7.46 (t, 1H, J = 7.6 Hz), 7.70 (dd, 1H, J = 8.8, 2.0 Hz), 7.78 (s, 1H), 7.98 (d, 1H, J = 8.8 Hz), 8.05 (d, 1H, J = 2.0 Hz)); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{19}H_{18}N_5$: 316.1557, found 316.1557.

*4.1.3. General procedure for the preparation of 2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylacetamide (**14a–d**) and 2-[5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylacetamide (**15a–d**)*

To a solution of pyrazole **12a** or **12b** (0.348 mmol) in anhydrous DMF (5 mL), a catalytic amount of sodium iodide, NaH (0.42 mmol), and 2-chloro-*N*-phenylacetamide **13a** or **13b** (0.42 mmol) were added. The mixture was stirred at room temperature for 2 h and then evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using MeOH/$CHCl_3$ as eluent to give the two positional isomers **14a–d** and **15a–d** as white solids.

*4.1.3.1. 2-[3-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-y]-N-phenylacetamide (**14a**).* Yield 41%; mp 182.3 °C; IR (KBr) 3288, 1687, 1603, 1552 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H), 5.14 (s, 2H), 7.09 (br t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.28 (t, 2H, J = 7.6 Hz), 7.39 (d, 1H, J = 7.6 Hz), 7.49 (d, 2H, J = 7.6 Hz), 7.58 (t, 1H, J = 7.6 Hz), 7.76 (dd, 1H, J = 8.8, 2.0 Hz), 7.86 (s, 1H), 8.01 (d, 1H, J = 8.8 Hz), 8.11 (d, 1H, J = 2.0 Hz), 8.81(d, 1H, J = 2.0 Hz), 8.83 (d, 1H, J = 2.0 Hz), 8.90 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.49, 56.12, 120.35 (2C), 120.63, 121.76, 123.02, 124.90, 128.01, 128.36, 129.09 (2C), 132.08, 132.56, 134.76, 136.98, 137.36, 142.36, 143.24, 144.82, 145.40, 150.36, 151.08, 158.82, 164.76; HRMS-ESI m/z $[M + NH_4]^+$ calcd. for $C_{25}H_{24}N_7O$: 438.2037, found 438.2028.

*4.1.3.2. 2-[5-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylacetamide (**15a**).* Yield 10%; mp 272.3 °C; IR (KBr) 3278, 1685, 1601 1553 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.71 (s, 3H), 5.06 (s, 2H), 7.10–7.13 (m, 2H), 7.28 (br d, 1H, J = 7.6 Hz), 7.33 (br t, 2H, J = 8.0 Hz), 7.57–7.62 (m, 3H), 7.64 (dd, 1H, J = 8.8, 2.0 Hz), 7.95 (s, 1H), 8.00 (d, 1H, J = 2.0 Hz), 8.03 (d, 1H, J = 8.8 Hz), 8.81 (d, 1H, J = 2.0 Hz), 8.82 (d, 1H, J = 2.0 Hz), 9.82 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.53, 55.51, 120.01 (2C), 121.53, 123.35, 123.97, 124.47, 127.97, 129.16 (2C), 129.94, 131.03, 134.76, 137.82, 138.13, 139.45, 140.14, 142.30, 143.39, 144.93, 145.56, 147.72, 159.47, 165.59; HRMS-ESI m/z $[M + NH_4]^+$ calcd. for $C_{25}H_{24}N_7O$: 438.2037, found 438.2019.

*4.1.3.3. 2-[4-(2,3-Dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]-N-phenylacetamide (**14b**).* Yield 70%; mp 227.9 °C; IR (KBr) 3277, 1689, 1603, 1553 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 2.72 (s, 3H), 2.74 (s, 3H), 5.08 (s, 2H), 7.12 (br t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.28–7.32 (m, 2H), 7.34 (d, 1H, J = 8.0 Hz), 7.47–7.50 (m, 2H), 7.55 (t, 1H, J = 7.8 Hz), 7.63 (dd, 1H, J = 8.8, 2.0 Hz), 7.80 (s, 1H), 7.88 (d, 1H, J = 8.8 Hz), 7.98 (d, 1H, J = 2.0 Hz), 8.62 (br s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{27}H_{25}N_6O$: 449.2084, found 449.2093.

*4.1.3.4. 2-[4-(2,3-Dimethylquinoxalin-6-yl)-5-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]-N-phenylacetamide (**15b**).* Yield 10%; mp 224.3 °C; IR (KBr) 3275, 1684, 1600, 1554 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.71 (s, 3H), 2.79 (s, 3H), 2.80 (s, 3H), 5.05 (s, 2H), 7.08–7.12 (m, 2H), 7.28 (d, 1H, J = 8.0 Hz), 7.32 (t, 1H, J = 8.0 Hz), 7.56 (dd, 1H, J = 8.8, 1.6 Hz), 7.58–7.62 (m, 3H), 7.93 (s, 1H), 8.00–8.02 (m, 2H), 9.86 (br s, 1H); HRMS-ESI m/z $[M + Na]^+$ calcd. for $C_{27}H_{24}N_6NaO$: 471.1904, found 471.1920.

*4.1.3.5. N-(3-Cyanophenyl)-2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl] acetamide (**14c**).* Yield 52%; mp 280.6 °C; IR (KBr) 3274, 2233, 1700, 1617, 1557 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 5.17 (s, 2H), 7.18 (br d, 1H, J = 8.0 Hz), 7.33 (br d, 1H, J = 7.6 Hz), 7.36–7.38 (m, 2H), 7.58 (t, 1H, J = 7.8 Hz), 7.62–7.66 (m, 1H), 7.76 (dd, 1H, J = 8.8, 2.0 Hz), 7.85 (s, 1H), 7.90 (br s, 1H), 8.05 (d, 1H, J = 8.8 Hz), 8.12 (d, 1H, J = 2.0 Hz), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz), 9.55 (s, 1H); HRMS-ESI m/z $[M + K]^+$ calcd. for $C_{26}H_{19}KN_7O$: 484.1283, found 484.1303.

*4.1.3.6. N-(3-Cyanophenyl)-2-[5-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl] acetamide (**15c**).* Yield 6%; mp 129.3 °C; IR (KBr) 3277, 2232, 1699, 1617, 1558 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.69 (s, 3H), 5.04 (s, 2H), 7.12 (d, 1H, J = 7.6 Hz), 7.30 (d, 1H, J = 7.6 Hz), 7.39 (dt, 1H, J = 7.6, 1.6 Hz), 7.43 (t, 1H, J = 7.8 Hz), 7.61 (t, 1H, J = 7.8 Hz), 7.63 (dd, 1H, J = 8.8, 2.0 Hz), 7.84 (ddd, 1H, J = 8.0, 2.4, 1.2 Hz), 7.95 (s, 1H), 8.00–8.05 (m, 3H), 8.82 (s, 2H), 10.47 (br s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{26}H_{20}N_7O$: 446.1724, found 446.1720.

*4.1.3.7. N-(3-Cyanophenyl)-2-[4-(2,3-dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]acetamide (**14d**).* Yield 60%; mp 196.3 °C; IR (KBr) 3261, 2231, 1702, 1592, 1557 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 2.73 (s, 3H), 2.74 (s, 3H), 5.16 (s, 2H), 7.15 (br d, 1H, J = 7.6 Hz), 7.25 (br d, 1H, J = 7.6 Hz), 7.36–7.39 (m, 2H), 7.53 (t, 1H, J = 7.8 Hz), 7.60 (dd, 1H, J = 8.4, 2.0 Hz), 7.62–7.65 (m, 1H), 7.80 (s, 1H), 7.89–7.92 (m, 2H), 7.98 (d, 1H, J = 2.0 Hz), 9.63 (d, 1H, J = 2.0 Hz); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{28}H_{24}N_7O$: 474.2037, found 474.2046.

*4.1.3.8. N-(3-Cyanophenyl)-2-[4-(2,3-dimethylquinoxalin-6-yl)-5-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]acetamide (**15d**).* Yield 4%; mp 189.3 °C; IR (KBr) 3281, 2232, 1699, 1591, 1558, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.69 (s, 3H), 2.75 (s, 3H), 2.76 (s, 3H), 5.04 (s, 2H), 7.10 (br d, 1H, J = 7.6 Hz), 7.28 (br d, 1H, J = 7.6 Hz), 7.38 (dt, 1H, J = 8.0, 1.4 Hz), 7.43 (t, 1H, J = 7.8 Hz), 7.52 (dd, 1H, J = 8.8, 2.0 Hz), 7.59 (t, 1H, J = 7.6 Hz), 7.83 (dt, 1H, J = 8.0, 1.8 Hz), 7.91 (s, 1H), 7.93–7.95 (m, 2H), 8.03 (t, 1H, J = 1.8 Hz), 10.54 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{28}H_{24}N_7O$: 474.2037, found 474.2036.

*4.1.4. 3-Bromo-N-(3-cyanophenyl)propanamide (**16b**)*

3-Bromopropanoyl chloride (40.63 mmol) was added dropwise to a mixture of 3-aminobenzonitrile (33.86 mmol) and anhydrous K_2CO_3 (40.63 mmol) in CH_2Cl_2 at room temperature. The resulting mixture was heated at reflux temperature for 4 h, then cooled to room temperature, and slowly poured into cold water (120 mL). The aqueous solution was extracted with CH_2Cl_2 (2 × 100 mL), and the CH_2Cl_2 solution was dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure to give a white solid which was purified by crystallization from Et_2O/hexane to give the titled compound **16b**. Yield 70%; mp 98.4 °C; IR (KBr) 3222, 2233, 1676 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.99 (t, 2H, J = 6.4 Hz), 3.70 (t, 2H, J = 6.4 Hz), 7.39–7.45 (m, 2H), 7.75 (dt, 1H, J = 7.6, 2.0 Hz), 7.94 (br s, 1H), 7.97 (br s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{10}H_{10}BrN_2O$: 252.9971, found 252.9980.

*4.1.5. General procedure for the preparation of 3-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylpropanamide (**17a**) and N-(3-cyanophenyl)-3-[4-(2,3-dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]propanamide (**17b**)*

To a stirred mixture of **12a** or **12b** (0.174 mmol) and Cs_2CO_3 (0.23 mmol) in anhydrous DMF (3 mL), 3-bromo-*N*-phenylpropanamide (**16a**) or 3-bromo-*N*-(3-cyanophenyl)propanamide (**16b**) (0.21 mmol) was added. The mixture was heated at 120 °C for 2 h and then cooled to room temperature. The reaction mixture was diluted with water (10 mL) and extracted with $CHCl_3$ (2 × 40 mL). The $CHCl_3$ solution 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/$CHCl_3$ as eluent to give the titled compound **17a** or **17b** as a white solid.

*4.1.5.1. 3-[3-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylpropanamide (**17a**).* Yield 66%; mp 221.2 °C; IR (KBr) 3315, 1681, 1548, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.62 (s, 3H), 3.11 (t, 2H, J = 6.0 Hz), 4.66 (t, 2H, J = 6.0 Hz), 7.05 (br t, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.23–7.27 (m, 3H), 7.51 (d, 2H, J = 8.0 Hz), 7.55 (t, 1H, J = 7.8 Hz), 7.64 (dd, 1H, J = 8.8, 2.0 Hz), 7.78 (s, 1H), 7.97 (d, 1H, J = 8.8 Hz), 8.02 (d, 1H, J = 2.0 Hz), 8.47 (br s, 1H), 8.79 (d, 1H, J = 2.0 Hz), 8.80 (d, 1H, J = 2.0 Hz); ^{13}C NMR ($CDCl_3$/CD_3OD) δ 23.84, 37.41, 48.64, 119.98, 120.07 (2C), 121.13, 122.94, 124.17, 127.11, 128.59, 128.65 (2C), 131.56, 131.72, 135.11, 137.11, 137.94, 141.56, 142.73, 144.24, 145.02, 148.59, 151.06, 158.54, 169.25; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{26}H_{23}N_6O$: 435.1928, found 435.1921.

*4.1.5.2. N-(3-Cyanophenyl)-3-[4-(2,3-dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-y]propanamide (**17b**).* Yield 41%; mp 207.1 °C; IR (KBr) 3334, 2231, 1691, 1589, 1553 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.62 (s, 3H), 2.71 (s, 3H), 2.72 (s, 3H), 3.11 (t, 2H, J = 5.8 Hz), 4.62 (t, 2H, J = 5.8 Hz), 7.09 (d, 1H, J = 7.6 Hz), 7.15 (d, 1H, J = 7.6 Hz), 7.28–7.33 (m, 2H), 7.45 (t, 1H, J = 7.6 Hz), 7.46 (dd, 1H, overlapped, J = 8.8, 2.0 Hz), 7.67 (s, 1H), 7.77 (dt, 1H, J = 7.6, 2.0 Hz), 7.85 (d, 1H, overlapped, J = 8.8 Hz), 7.86 (d, 1H, overlapped, J = 2.0 Hz), 7.89 (br s, 1H), 9.57 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{29}H_{26}N_7O$: 488.2193, found 488.2208.

*4.1.6. General procedure for the preparation of 2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylethanethioamide **18a–d***

A stirred mixture of **14a–d** (0.45 mmol), Lawesson's reagent (0.45 mmol), and anhydrous DME (10 mL) in a dry sealed tube was heated at 85 °C for 12 h. After cooled to room temperature, the solvent was evaporated to dryness under reduced pressure, and the residue was purified by MPLC on silica gel using MeOH/$CHCl_3$ as eluent to give the titled compound **18a–d** as a light yellow solid.

*4.1.6.1. 2-[3-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]-N-phenylethanethioamide (**18a**).* Yield 58%; mp 182 °C; IR (KBr) 3209, 1553, 1421, 1171 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 5.49 (s, 2H), 7.17 (d, 1H, J = 7.6 Hz), 7.25 (tt, 1H, J = 7.6, 1.6 Hz), 7.35–7.41 (m, 3H), 7.61 (t, 1H, J = 7.6 Hz), 7.74–7.77 (m, 3H), 7.90 (s, 1H), 8.03 (d, 1H, J = 8.8 Hz), 8.12 (d, 1H, J = 2.0 Hz), 8.82 (d, 1H, J = 2.0 Hz), 8.83 (d, 1H, J = 2.0 Hz), 10.61(s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.50, 63.97, 120.55, 122.11, 123.24, 123.36 (2C), 127.28, 128.59, 129.13 (2C), 129.18, 132.12, 132.39, 134.53, 137.25, 138.41, 142.49, 143.28, 144.97, 145.50, 150.61, 150.76, 158.87, 192.94; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{25}H_{21}N_6S$: 437.1543, found 437.1553.

*4.1.6.2. 2-[4-(2,3-Dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]-N-phenylethanethioamide (**18b**).* Yield 24%; mp 205.5 °C; IR (KBr) 3266, 1554, 1494, 1160 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.53 (s, 3H), 2.72 (s, 3H), 2.74 (s, 3H), 5.47 (s, 1H), 7.14

(dd, 1H, J = 7.6, 0.4 Hz), 7.23–7.27 (m, 1H), 7.32 (br d, 1H, J = 7.6 Hz), 7.35–7.40 (m, 2H), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 8.8, 2.0 Hz), 7.72–7.75 (m, 2H), 7.83 (d, 1H), 7.88 (d, 1H, J = 8.8 Hz), 7.98 (d, 1H, J = 2.0 Hz), 10.54 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{27}H_{25}N_6S$: 465.1856, found 465.1862.

*4.1.6.3. N-(3-Cyanophenyl)-2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]ethanethioamide (**18c**).* Yield 61%; mp 213.9 °C; IR (KBr) 3271, 2232, 1431, 1186 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 5.55 (s, 2H), 7.18 (d, 1H, J = 7.6 Hz), 7.28 (d, 1H, J = 8.0 Hz), 7.41 (t, 1H, J = 8.0 Hz), 7.49 (dt, 1H, J = 7.6, 1.2 Hz), 7.57 (t, 1H, J = 7.8 Hz), 7.75 (dd, 1H, J = 8.8, 2.0 Hz), 7.91 (s, 1H), 7.96 (ddd, 1H, J = 8.0, 2.0, 1.2 Hz), 8.06 (d, 1H, J = 8.8 Hz), 8.11 (d, 1H, J = 2.0 Hz), 8.25 (t, 1H, J = 2.0 Hz), 8.84 (d, 1H, J = 2.0 Hz), 8.85 (d, 1H, J = 2.0 Hz), 11.50 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{26}H_{20}N_7S$: 462.1495, found 462.1508.

*4.1.6.4. N-(3-Cyanophenyl)-2-[4-(2,3-dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]ethanethioamide (**18d**).* Yield 68%; mp 219.4 °C; IR (KBr) 3263, 2232, 1588, 1553, 1163 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.54 (s, 3H), 2.73 (s, 3H), 2.74 (s, 3H), 5.48 (s, 2H), 7.15 (d, 1H, J = 7.6 Hz), 7.24 (d, 1H, J = 7.6 Hz), 7.44 (t, 1H, J = 8.0 Hz), 7.51 (dt, 1H, overlapped, J = 7.6, 1.6 Hz), 7.52 (t, 1H, overlapped, J = 7.6 Hz), 7.60 (dd, 1H, J = 8.4, 2.0 Hz), 7.83 (s, 1H), 7.90 (d, 1H, J = 8.4 Hz), 7.97 (d, 1H, J = 1.6 Hz), 8.00 (ddd, 1H, J = 8.0, 2.4, 1.2 Hz), 8.25 (t, 1H, J = 1.6 Hz), 11.27 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{28}H_{24}N_7S$: 490.1808, found 490.1817.

*4.1.7. General procedure for the preparation of 3-{2-[3-(6-methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]acetamido}benzamide (**19a**) and 3-{2-[4-(2,3-dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]acetamido}benzamide (**19b**)*

A stirred solution of **18c** or **18d** (0.11 mmol), 1 N NaOH (0.31 mmol), and 28% H_2O_2 (0.38 mmol) in absolute EtOH (3 mL) was heated at 40 °C for 4 h. The reaction mixture was cooled to 0 °C and neutralized with 1 N HCl to pH ~8. The mixture was extracted with 10% MeOH in $CHCl_3$ (2 × 25 mL), and the combined organic layer 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 MeOH/$CHCl_3$ as eluent to afford the titled compound **19a** or **19b** as a white solid.

*4.1.7.1. 3-{2-[3-(6-Methylpyridin-2-yl)-4-(quinoxalin-6-yl)-1H-pyrazol-1-yl]acetamido}benzamide (**19a**).* Yield 30%; mp 221.9 °C; IR (KBr) 3349, 3059, 1648, 1464, cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H), 4.98 (s, 2H), 7.06 (d, 1H, J = 7.6 Hz), 7.18 (d, 1H, J = 7.6 Hz), 7.27 (t, 1H, J = 7.6 Hz), 7.45–7.49 (m, 2H), 7.62 (dd, 1H, overlapped, J = 8.8, 2.0 Hz), 7.63 (ddd, 1H, overlapped, J = 8.0, 2.0, 0.8 Hz), 7.84 (t, 1H, J = 1.8 Hz), 7.86 (d, 1H, J = 8.8 Hz), 7.90 (s, 1H), 7.92 (d, 1H, J = 2.0 Hz), 8.66 (d, 1H, J = 2.0 Hz), 8.67 (d, 1H, J = 2.0 Hz); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{26}H_{22}N_7OS$: 464.1829, found 464.1841.

*4.1.7.2. 3-{2-[4-(2,3-Dimethylquinoxalin-6-yl)-3-(6-methylpyridin-2-yl)-1H-pyrazol-1-yl]acetamido}benzamide (**19b**).* Yield 23%; mp 293.9 °C; IR (KBr) 3419, 3225, 1661, 1441 cm^{-1}; ^1H NMR ($CDCl_3$/CD_3OD) δ 2.72 (s, 3H), 2.73 (s, 3H), 2.74 (s, 3H), 5.22 (s, 2H), 7.29 (br d, 2H, J = 8.0 Hz), 7.38 (t, 1H, J = 8.0 Hz), 7.59 (dd, 1H, J = 8.8, 2.0 Hz), 7.66 (dt, 1H, J = 7.6, 1.4 Hz), 7.70 (t, 1H, J = 7.8 Hz), 7.89 (br d, 1H, overlapped, J = 8.0 Hz), 7.92–7.94 (m, 3H), 8.02 (br s, 1H), 10.06 (s, 1H); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{28}H_{26}N_7OS$: 492.2142, found 492.2155.

4.2. Luciferase reporter assay

Biological activity of the test compounds was determined by measuring their ability to inhibit TGF-β-induced p3TP-luciferase reporter activity in HaCaT stable cells transfected with p3TP-Luc. HaCaT cells were seeded at concentrations of 3 × 10^4 in 96-well plates. The next day, when they reach approximately 90% confluency, various concentrations of ALK5 inhibitors and 2 ng/mL of TGF-β were added to the cells. After 24 h, cell lysates were harvested using Luciferase assay kit (Promega) according to the manufacturer's instruction, and luminescence was measured by a luminometer Micro Lumat Plus (Berthold).

4.3. Docking experiments

Docking experiments have been performed with the Sybyl 8.1 software package (Tripos, Inc., St. Louis, MO, USA) based on CentOS Linux 5.4.

4.3.1. Preparation of ligand database

The structures of ligands were prepared in MOL2 format using the sketcher module, and Gasteiger-Huckel charges were assigned to the ligand atoms. The structures of molecules were optimized by energy minimization, and molecular dynamics using simulated annealing method. The conformer library for each ligand was prepared by random selection of 200 conformers from the molecular dynamics output.

4.3.2. Flexible docking

The X-ray coordinate of ALK5 in complex with 1,5-naphthyrine inhibitor (PDB id: 1VJY) [45] was retrieved from the PDB, and all crystallographic water molecules were removed. The active site was defined as all the amino acid residues enclosed within 6.5 Å radius sphere centered by the bound ligand. The docking and subsequent scoring were performed using the default parameters of the FlexX programs implanted in the Sybyl 8.1. For the docking of ligand database, the main settings are 1000 solutions per iteration during the incremental construction algorithm. Final scores for all FlexX solutions were calculated by a consensus scoring function (CScore), and used for database ranking. One of the poses with the highest FlexX score and high consensus score (CScore = 5 or 4) was selected, and docked with ALK5.

Acknowledgment

This work was supported by RP-Grant 2009 of Ewha Womans University and a grant from Ministry of Education, Science and Technology, Korea (20100029596).

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