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

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ABSTRACT

A series of 4(5)-(6-methylpyridin-2-yl)imidazoles **16–19** and -pyrazoles **22–29**, **33**, and **34** have been synthesized and evaluated for their ALK5 inhibitory activity in an enzyme assay and in cell-based luciferase reporter assays. The 6-quinolinyl imidazole analogs **16** and **18** inhibited ALK5 phosphorylation with IC_{50} values of 0.026 and 0.034 μM, respectively. In a luciferase reporter assay using HaCaT cells transiently transfected with p3TP-luc reporter construct, **18** displayed 66% inhibition at 0.05 μM, while competitor compounds **2** and **3** showed 44% inhibition. The binding mode of **18** generated by flexible docking studies with ALK5:**18** complex shows that it fits well into the active site cavity of ALK5 by forming broad and tight interactions.

1. Introduction

Transforming growth factor-β (TGF-β) is the superfamily of proteins which includes TGF-β1, TGF-β2, and TGF-β3. These cytokines are pleiotropic modulators of cell proliferation and differentiation, wound healing, extracellular matrix production, and immunosuppression.[1] Other members of this superfamily include activins, inhibins, bone morphogenetic proteins, growth and differentiation factors, and Mullerian inhibiting substance. TGF-β1 represents the prototypic member of the TGF-β superfamily. TGF-β1 transduces signals through two highly conserved transmembrane serine/threonine kinases, the type I (ALK5) and type II TGF-β receptors. The ligand induced oligomerization promotes the type II receptor to hyperphosphorylate serine/threonine residues in the GS region of the ALK5.[2] This phosphorylation leads to activation of the ALK5 by creating a binding site for Smad proteins. The activated ALK5 in turn phosphorylates Smad2 and Smad3 proteins thereby causing their dissociation from the receptor and heteromeric complex formation with Smad4. Smad complexes translocate to the nucleus, assemble with specific DNA-binding co-factors and co-modulators to finally activate transcription of extracellular matrix components and inhibitors of matrix-degrading proteases.[3] Numerous studies have demonstrated an association of TGF-β with the initiation and progression of fibrosis in a variety of organ systems such as kidney,[4] heart,[5] lung,[6] and liver.[7] TGF-β signaling and over-expression of TGF-β receptors are linked to various human diseases including cancer,[8] pancreatic diseases,[9] and hematological malignancies.[10] Inhibition of the catalytic activity of ALK5 by the use of small molecule inhibitors is one of the strategies used to normalize TGF-β signaling. Several ALK5 inhibitors such as **1** (SB-431542),[11] **2** (SB-505124),[12] **3** (SB-525334),[13] **4** (GW6604),[14] **5** (SD-208),[15,16] and **6** (LY580276),[17] are under preclinical development (Fig. 1). These and other similar inhibitors have become a subject of intense investigations.[11,18–21]

We have also prepared the 2-pyridinyl-substituted triazoles,[22,23] imidazoles such as **7** (IN-1166),[24,25] and thiazoles[26] having a carbonitrile-, carboxamide-, or sulfonamide-substituted phenyl or benzyl moiety as ALK5 inhibitors and found that both introduction of a carbonitrile, carboxamide, or sulfonamide group at *meta*- or *para*-position in the phenyl ring and incorporation of a methylene, a methyleneamino, or an aminomethylene linkage between a central heterocyclic ring and a phenyl ring significantly increased ALK5 inhibitory activity. Among them, **8** (IN-1130) effectively suppressed renal fibrosis induced by unilateral ureteral obstruction (UUO) in rats[27] and ameliorated experimental autoimmune encephalomyelitis (EAE) in SBE-luc and GFAP-luc mice immunized with MOG_{35-55}.[28] Additionally, it has been observed that the injection of **8** into the tunica albuginea in rats lessened tunical fibrosis and corrected penile curvature.[29]

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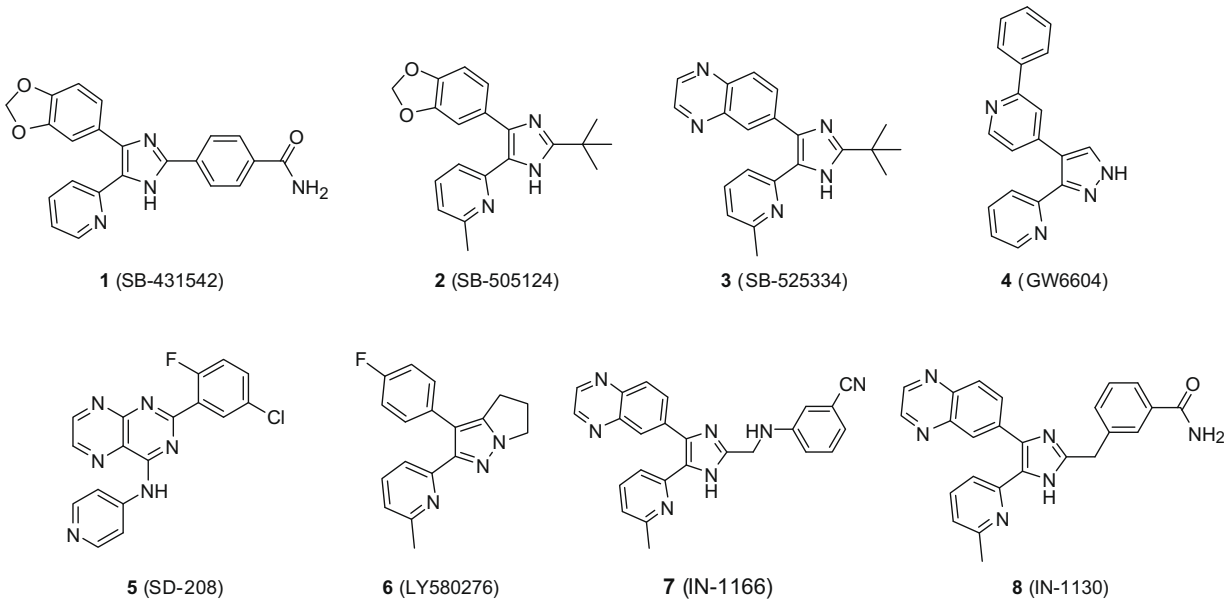
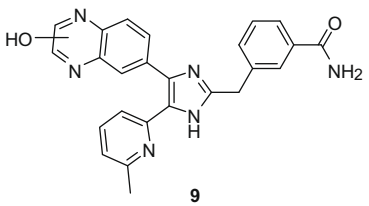


Figure 1. ALK5 inhibitors under development.

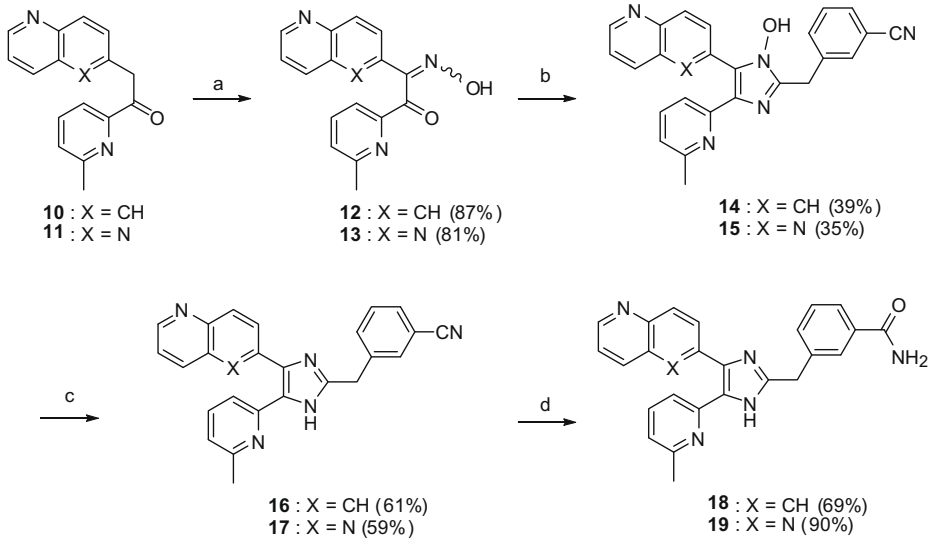


In pharmacokinetic studies with **8**, a major metabolite of **8** was detected in the systemic circulation of rat and mouse and was purified and tentatively characterized as 3-((4-(2-hydroxyquinoxalin-6-yl)-5-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzamide or 3-((4-(3-hydroxyquinoxalin-6-yl)-5-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzamide) (**9**) based on its ^{1}H NMR spectra and LC-MS/MS spectra.[30] Metabolic hydroxylation of **8** at the 2- or 3-position of the 6-quinoxaliny moiety significantly reduced its biological activity. Therefore, it was of particular interest to us whether replacement of the 6-quinoxalinyl of **8** with a different heteroaryl moiety could reduce metabolic hydroxylation. Since the *N*-1 in the 6-quinoxalinyl of **8** is essential for hydrogen-bond interaction in the ATP binding site, we attempted to replace the 6-quinoxalinyl of **8** with a 6-quinoliny or 1,5-naphthyridin-2-yl moiety. We prepared the imidazole derivatives **16–19** and the pyrazole derivatives **22–29**, **33**, and **34** to examine the effect of the central heterocyclic ring and optimal positioning of a carbonitrile- or carboxamide-substituted benzyl moiety on ALK5 inhibition.

2. Results and discussion

2.1. Chemistry

The 4(5)-(6-methylpyridin-2-yl)imidazoles **16–19** were prepared as shown in Scheme 1. Treatment of **10**[31] and **11**[32] with

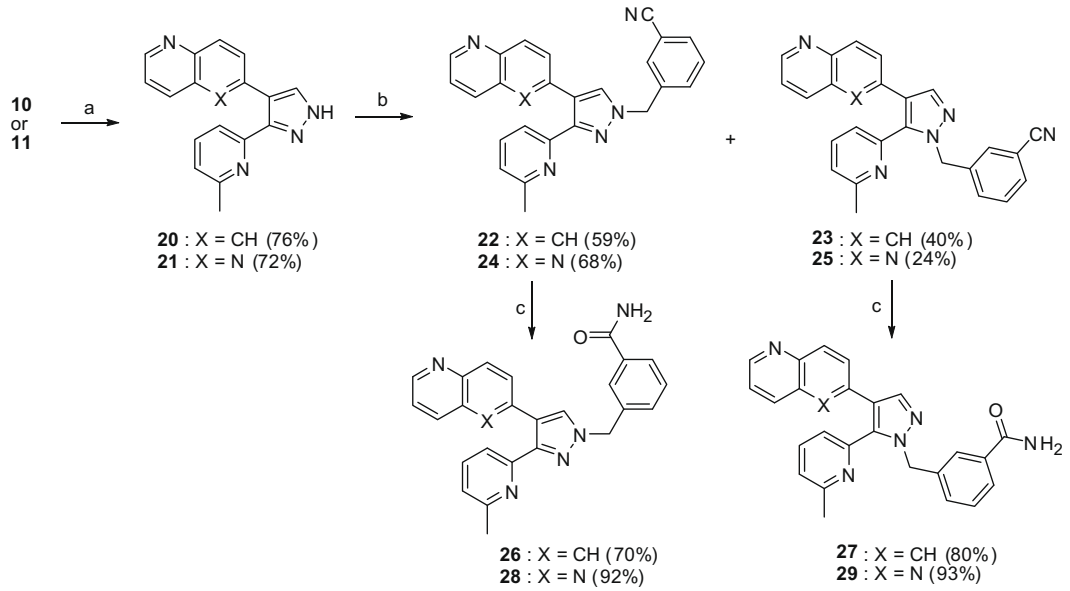


Scheme 1. Reagents and conditions: (a) $NaNO_2$, 18% HCl, 0 °C then rt, 30 min; (b) 3-cyanophenylacetaldehyde, NH_4OAc, AcOH, 110 °C, 16 h; (c) triethyl phosphite, anhydrous DMF, 110 °C, 16 h; (d) 28% H_2O_2, 1 N NaOH, 95% EtOH, 60 °C, 2 h.

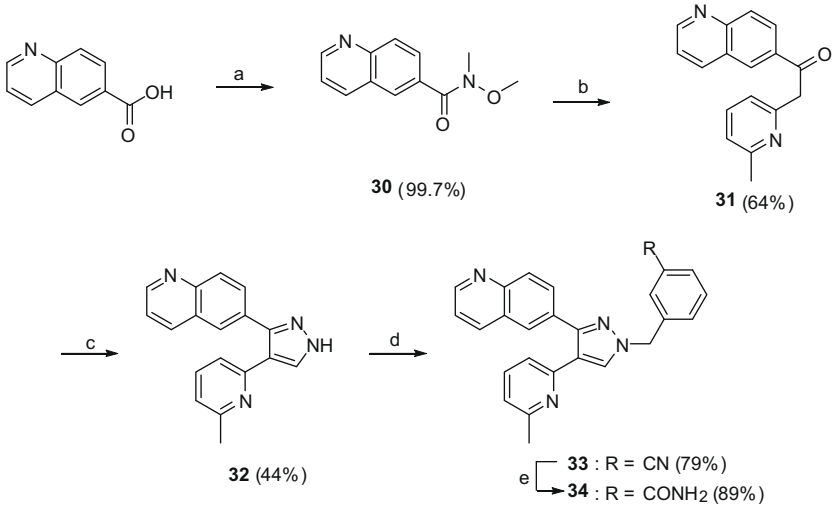
$NaNO_2$ in 18% HCl solution at room temperature gave the respective hydroxyimino ketones **12** and **13**. Cyclization of these compounds with 3-cyanophenylacetaldehyde in the presence of NH_4Ac in AcOH afforded the hydroxyl imidazoles **14** and **15** in 39% and 35% yields, respectively. Dehydroxylation of the resulting imidazoles with triethyl phosphite in anhydrous DMF at 110 °C produced 1*H*-imidazoles **16** and **17** in 61% and 59% yields. Conversion of the benzonitriles **16** and **17** to the carboxamide derivatives **18** and **19** was accomplished by treatment of 28% H_2O_2 and 1 N NaOH in 95% ethanol.

The counterpart pyrazole derivatives **22–29** were prepared as shown in Scheme 2. Treatment of the ketones **10** and **11** with *N*,*N*-dimethylformamide dimethyl acetal (DMF·DMA) in the presence of AcOH in DMF, followed by reaction with hydrazine monohydrate, produced the pyrazoles **20**[31] and **21**.[32] Alkylation of **20** or **21** with α-bromo-*m*-tolunitrile in anhydrous DMF in the presence of Cs_2CO_3 gave the two positional isomers **22** and **23** or **24** and **25**, respectively. The positional isomers were separated by flash column chromatography and identified by NOE experiments. In NOE experiments, irradiation of the benzylic methylene protons of the derivatives **22** and **24** gave enhancement for proton *H*-5 in pyrazole ring, while irradiation of the similar methylene protons for compounds **23** and **25** gave enhancement of methyl protons attached at 6-position of pyridyl ring, confirming the respective positions of benzylic substitutions. Then the nitrile group was hydrolyzed to carboxamide as mentioned above for Scheme 1.

The 6-quinolinyl substituted pyrazole isomers **33** and **34** were prepared as shown in Scheme 3. Commercially available quinoline-6-carboxylic acid was converted to Weinreb amide **30**[33] in a quantitative yield using peptide coupling conditions. The amide **30** was coupled with an anion of 2,6-lutidine generated by treatment with *n*-BuLi to obtain the ketone **31** in 64% yield. The ketone



Scheme 2. Reagents and conditions: (a) (i) DMF·DMA, AcOH, DMF, rt, 1 h; (ii) $N_2H_4·H_2O$, 50 °C, 2 h; (b) α-bromo-*m*-tolunitrile, Cs_2CO_3, anhydrous DMF, rt, 2 h; (c) 28% H_2O_2, 1 N NaOH, 95% EtOH, 60 °C, 1 h.



Scheme 3. Reagents and conditions: (a) *N*,*O*-dimethylhydroxylamine hydrochloride, PYBOP, 1-hydroxybenzotriazole (HOBt), Et_3N, anhydrous DMF, rt, 16 h; (b) 2,6-lutidine, *n*-BuLi, anhydrous THF, Ar atmosphere, −78 °C for 10 min then rt; (c) (i) DMF·DMA, THF, 48 h; (ii) $N_2H_4·H_2O$, EtOH, rt, 12 h; (d) α-bromo-*m*-tolunitrile, Cs_2CO_3, anhydrous DMF, 80 °C, 12 h; (e) 28% H_2O_2, 1 N NaOH, 95% EtOH, 60 °C, 1 h.

31 was cyclized to the desired pyrazole **32** which was alkylated with α-bromo-*m*-tolunitrile to give the benzonitrile **33** using the conditions mentioned for Scheme 2. Conversion of the nitrile functionality of the compound **33** to carboxamide was accomplished as mentioned above to give the pyrazole **34**.

2.2. Biological evaluation

To investigate whether these inhibitors **16–19**, **22–29**, **33**, and **34** could inhibit ALK5, a kinase assay was performed using the purified human ALK5 kinase domain produced in Sf9 insect cells. Among them, 1-benzyl-5-(pyridin-2-yl)pyrazole derivatives **23**, **25**, **27**, and **29** displayed no significant ALK5 inhibitory activity up to a concentration of 10 μM (Table 1). The compounds possessing a 6-quinolinyl moiety showed higher ALK5 inhibitory activity than the compounds possessing a 1,5-naphthyridin-2-yl moiety. In the case of imidazole derivatives, the 6-quinolinyl analogs **16** (IC_{50} = 0.026 μM) and **18** (IC_{50} = 0.034 μM) are 87- and 76-fold more potent than the corresponding 1,5-naphthyridin-2-yl analogs **17** (IC_{50} = 2.25 μM) and **19** (IC_{50} = 2.60 μM). However, the difference in inhibitory activity with respect change in pyrazole derivatives was less profound. The 6-quinolinyl analogs **22** (IC_{50} = 0.231 μM) and **26** (IC_{50} = 0.131 μM) displayed 1.4- and 1.9-fold higher ALK5 inhibitory activity than the 1,5-naphthyridin-2-yl analogs **24** (IC_{50} = 0.318 μM) and **28** (IC_{50} = 0.255 μM). In the 6-quinolinyl analogs, the imidazole derivatives **16** and **18** exhibited higher inhibitory activity than their counterpart pyrazole derivatives **22**, **23** and **33** (IC_{50} = 0.034 μM), and **26**, **27** and **34** (IC_{50} = 0.061 μM), respectively. In this series, the most potent compound **16** is approximately 1.4-fold more potent ALK5 inhibitor than **8**, and compound **18** showed the similar level of inhibitory activity to that of **8**. These two compounds **16** and **18** displayed much higher ALK5 inhibitory activity than **2** (IC_{50} = 0.169 μM) and **3** (IC_{50} = 0.162 μM).

To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **16–19**,

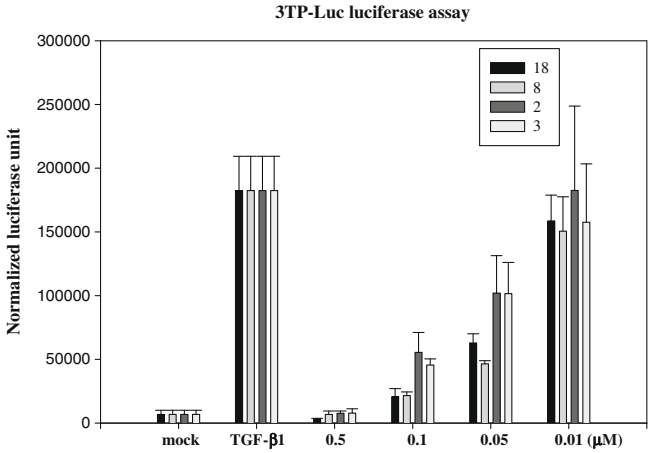
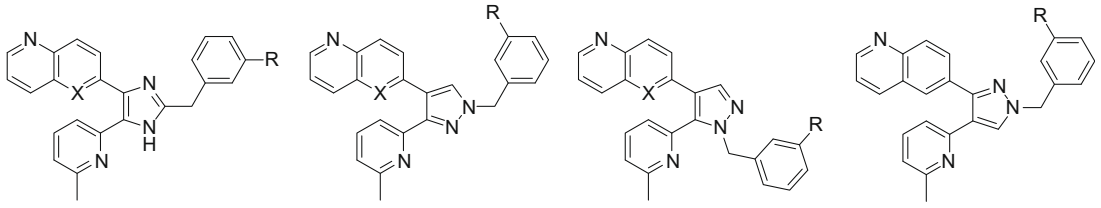


Figure 2. Effect of **18** on the activity of TGF-β-induced ALK5. HaCaT cells were transiently transfected with p3TP-luc reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as the mean ± SD of three independent experiments run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

Table 1
Inhibitory activity of imidazoles **16–19** and pyrazoles **22–29**, **33**, and **34** on ALK5



Compd	X	R	IC_{50}[a] (μM)	Luciferase activity[b,c] (% control)		
				p3TP-luc	SBE-luc	ARE-luc
Mock				2	16 ± 3	3 ± 0
TGF-β				100 ± 13	100 ± 37	100 ± 32
16	CH	CN	0.026	4 ± 1	16 ± 1	13 ± 4
17	N	CN	2.25	104 ± 26	74 ± 7	87 ± 38
18	CH	$CONH_2$	0.034	7 ± 1	22 ± 5	22 ± 8
19	N	$CONH_2$	2.60	74 ± 16	74 ± 31	74 ± 23
22	CH	CN	0.231	15 ± 4	38 ± 12	35 ± 19
23	CH	CN	>10	114 ± 33	95 ± 11	86 ± 58
24	N	CN	0.318	21 ± 2	29 ± 16	38 ± 13
25	N	CN	>10	132 ± 31	94 ± 4	86 ± 24
26	CH	$CONH_2$	0.131	9 ± 2	18 ± 0	22 ± 11
27	CH	$CONH_2$	>10	147 ± 42	106 ± 24	87 ± 52
28	N	$CONH_2$	0.255	20 ± 4	30 ± 9	43 ± 20
29	N	$CONH_2$	>10	95 ± 12	105 ± 30	85 ± 85
33		CN	0.034	49 ± 13	74 ± 54	63 ± 36
34		$CONH_2$	0.061	87 ± 28	80 ± 28	50 ± 15
2			0.169	26 ± 3	22 ± 1	47 ± 24
3			0.162	29 ± 4	26 ± 5	55 ± 31
8			0.036	6 ± 2	16 ± 3	21 ± 9

[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 (^{33}PanQinase 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.

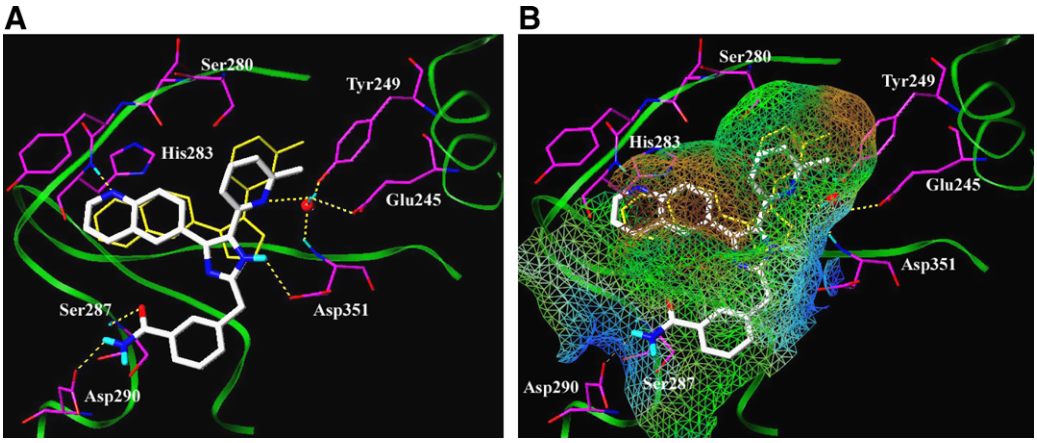


Figure 3. (A) Docked pose of **18** in the active site of ALK5. Bound 1,5-naphthyridine inhibitor (yellow) and a trapped water molecule (red sphere) in the X-ray structure (PDB id: 1VJY) are shown for comparison. Hydrogen bonding interactions between **18** and key amino acid residues within the binding site are represented in yellow dotted lines (<2.5 Å). (B) Lipophilic potential surface map of the ATP-binding pocket of ALK5 is demonstrated in the docking model of **18**. Lipophilicity increases from blue (hydrophilic) to brown (lipophilic).

22–29, **33**, and **34** was measured using HaCaT cells transiently transfected with three different luciferase reporter genes, p3TP-luciferase reporter,[34] SBE-luciferase reporter,[35] and ARE-luciferase reporter[36] at a concentration of 0.1 μM (Table 1). Similar to kinase assay, 1-benzyl-5-(pyridin-2-yl)pyrazole derivatives **23**, **25**, **27**, and **29** showed no ALK5 inhibitory activity, and the luciferase activity of most compounds consisted with that of kinase assay. However, the pyrazole **34** (IC_{50} = 0.061 μM) is 2.1-fold more inhibitory than the pyrazole **26** (IC_{50} = 0.131 μM) in a kinase assay, the former displayed much lower inhibition than the latter in luciferase assays suggesting that **26** has better cellular permeability than **34**. Consistent with the kinase assay, **16** showed the most potent luciferase inhibitory activity, and **18** showed the similar level of inhibitory activity to **8**. Compound **18** (14.3 mg/mL) has twofold higher aqueous solubility than compound **16** (7.1 mg/mL) in buffered solution (pH 1.2), hence it was chosen for advanced studies.

The ALK5 inhibitory activity of **18** was compared with those of **2**, **3**, and **8** 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 2, **18** inhibited ALK5 in a dose-dependent manner and was equipotent to **8** and more potent than **2** and **3**. Compound **18** showed 66% inhibition at 0.05 μM, while competitor compounds **2** and **3** showed 44% inhibition.

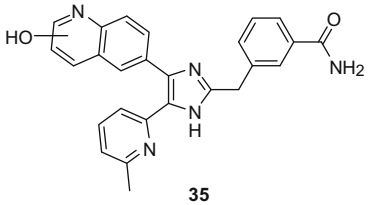
The selectivity of **18** for ALK5 versus other kinases was evaluated using a panel of 156 kinases (ProQinase GmbH (Germany)). Compound **18** inhibited ALK5 phosphorylation with an IC_{50} of 0.034 μM. Except p38α (IC_{50} = 0.54 μM), PKCμ (IC_{50} = 0.65 μM), VEGFR-1 (IC_{50} = 2.1 μM), VEGFR-2 (IC_{50} = 2.2 μM), VEGFR-3 (IC_{50} = 0.94 μM), and PDGFRα (IC_{50} = 3.0 μM), **18** was at least 100-fold more selective for ALK5 than the rest of 149 kinases.[37]

2.3. Molecular modeling

To examine the binding mode of **18** in the active site of ALK5, we built a docking model of ALK5:**18** complex based on the X-ray structure of ALK5 co-crystallized with 1,5-naphthyridine inhibitor[32] (PDB id: 1VJY). As shown in Figure 3, **18** fits well both in the cavity for ATP and the hinge region (residues 281–283) that link the *N*- and *C*-terminal domains of kinase.[32] The central imidazole ring and its substituents form several interactions with amino acid residues in the binding pocket, facilitating binding of the ligand deep into the active site. The NH of imidazole ring forms a hydrogen bond (H-bond) with Asp351, which is one of key interactions observed in the X-ray structures of ALK5 complexed with other inhibitors.[32,38,39] It was reported that Asp351 is usually located close to bound inhibitors in the inactivated conformation of ALK5.[32] The H-bond between imidazole NH of **18** and Asp351 may contribute to hold the loop containing Asp351 and stabilize inactive conformation of ALK5. The 6-methylpyridin-2-yl moiety occupies the hydrophobic pocket containing Ser280, which is critical for the selectivity of **18** for ALK5 over p38 MAP kinase.[24,32,39] This model also implies that the pyridinyl nitrogen would form a H-bond with water molecule, a center of H-bond network in the binding pocket, which is crystallographically determined in other inhibitors containing pyridinyl moiety at this position.[17,32] The nitrogen atom of the 6-quinolinyl group forms a 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, and it is another major interaction of inhibitor with the binding site.[32,38] In addition, the carboxamide group attached to the benzyl ring contacts the α-helix next to the hinge region and forms H-bonds with backbone NH of Ser287 and carboxyl side chain of Asp290. The other active carbonitrile derivative **16** exhibited the binding mode very similar to that of **18**, except that the CN group forms H-bond only with side chain OH of Ser 287, not with Asp290 (results not shown). The present docking model demonstrates a possible broad and tight interaction of **18** with the left side of ALK5 active site ranging from Ser280 to Asp290, and this may be related to the excellent activity of **18**. Conclusively, the binding mode of **18** generated by docking studies supports the strong and selective activity of this compound and provides the insights for further modification to develop analogs with more desirable biological activity.

2.4. Metabolism

To compare the metabolic stability of **8** and **18**, these compounds were incubated with nine human CYP supersomes™ in the presence of an NADPH generating system (Table 2).



35

Table 2
Comparison between compound **8** metabolite (**9**) and compound **18** metabolite (**35**) by supersomal CYP

Metabolite[a,b] (peak area)	CYP isozymes								
	CYP1A2	CYP2A6	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
9	ND[c]	ND	ND	33.40 ± 1.68	ND	12.37 ± 0.58	15.39 ± 1.89	ND	17.00 ± 1.75
35	ND	ND	ND	1.99 ± 0.38	ND	11.31 ± 0.70	ND	ND	10.6 ± 1.2

[a] 120 μM of **8** or **18** was incubated with nine human CYP supersomes™ in the presence of an NADPH generating system. HPLC analysis was performed as described in Section 4.
[b] Data represent the mean ± SD (n = 3).
[c] ND, not detected.

A major metabolite of **18** was isolated, purified and tentatively characterized as **35** based on its ^{1}H NMR spectra and LC-MS/MS spectra. Because standards for metabolites of both compounds **8** and **18** were not available, amounts of metabolite **9** and **35** were expressed as peak area comparing with those of compound **8** and **18** of which concentrations were known. When amounts of major metabolites of compounds **8** (**9**) and **18** (**35**) were compared, metabolite **35** was much less (30% of **9**) than **9**, which indicated that both *N*-1 and *N*-4 at compound **8** were important to be metabolized to **9** (Table 2). Compound **8** seems to be metabolized by CYP2C8, CYP3A4, CYP2C19, and CYP2D6 whereas compound **18** seems to be metabolized mainly by CYP3A4 and CYP2C19 and minimally by CYP2C8. In other words, CYP2D6 metabolize only compound **8** suggesting that *N*-1 might need for CYP2D6 metabolism as well as maximal metabolism by CYP2C8.

3. Conclusion

In this report, a series of 4(5)-(6-methylpyridin-2-yl)imidazoles and -pyrazoles have been synthesized and evaluated for their 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. The imidazole analogs **16** and **18** having a 6-quinolinyl moiety showed the most significant ALK5 inhibitory activity in the series of compounds that is much higher than those of **2** and **3** and is equipotent to **8**. The in vitro metabolism study of compounds **8** and **18** in human CYP supersomes™ revealed that the 6-quinolinyl analog **18** is more stable than the 6-quinoxalinyl analog **8**. A docking model of ALK5:**18** complex shows that **18** fits well into the active site cavity of ALK5.

Recent studies demonstrated that **18** effectively prevented the development and progression of pulmonary arterial hypertension in the monocrotaline rat model through the inhibition of TGF-β signaling[40] and also prevented granulation tissue formation after bare metallic stent placement in a rat urethral model.[41]

On the basis of these results, the regulatory preclinical studies of **18** are currently under way.

4. Experimental

4.1. General methods

^{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 (ESIMS) 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).

4.2. Chemistry

4.2.1. General procedure for the preparation of the 2-(hydroxyimino)-1-(6-methylpyridin-2-yl)ethanones 12 and 13

A solution of **10** or **11** (0.58 mmol) in aqueous HCl (18%, 2.3 mL) was cooled to 0 °C using an ice-NaCl bath. To this solution was added sodium nitrite (0.70 mmol), while the reaction temperature was maintained at 0 °C during this addition. After addition was complete, the ice-NaCl bath was removed, and the reaction mixture was allowed to warm to room temperature and stirred for an additional 30 min. The reaction mixture was neutralized with 6 N NaOH solution, and the precipitates were filtered, washed with water, and dried in vacuo to afford the title compound **12** or **13** as a solid.

4.2.1.1. 2-(Hydroxyimino)-1-(6-methylpyridin-2-yl)-2-(quinolin-6-yl)ethanone (mixture of *E* & *Z* form) (12). Yield 87%; mp 223 °C; ^{1}H NMR (DMSO-d_6) δ 2.42 (s, 3H), 2.49 (s, 3H), 7.46 (d, 1H, J = 7.6 Hz), 7.53 (dd, 1H, J = 8.2, 4.4 Hz), 7.56–7.59 (m, 2H), 7.72 (d, 1H, J = 7.6 Hz), 7.81 (d, 1H, J = 2.0 Hz), 7.87–7.90 (m, 2H), 7.95–8.01 (m, 2H), 8.06–8.10 (m, 2H), 8.12 (d, 1H, J = 2.0 Hz), 8.15 (d, 1H, J = 1.6 Hz), 8.39 (dd, 1H, J = 8.4, 1.6 Hz), 8.46 (dd, 1H, J = 8.4, 1.6 Hz), 8.91 (ddd, 1H, J = 4.4, 2.0, 1.6 Hz), 8.96 (ddd, 1H, J = 4.4, 2.0, 1.6 Hz), 11.7 (s, 1H), 12.8 (s, 1H); IR ($CHCl_3$) 3411 cm^{-1}; MS (ESI) m/z 292.11 (MH^+).

4.2.1.2. 2-(Hydroxyimino)-1-(6-methylpyridin-2-yl)-2-(1,5-naphthyridin-2-yl)ethanone (13). Yield 81%; mp 216−219 °C; ^{1}H NMR (DMSO-d_6) δ 2.36 (s, 3H), 7.52 (dd, 1H, J = 6.0, 2.4 Hz), 7.70 (dd, 1H, J = 8.4, 4.4 Hz), 7.93−7.98 (m, 2H), 8.15 (dt, 1H, J = 8.4, 0.8 Hz), 8.34 (d, 1H, J = 9.2 Hz), 8.50 (dd, 1H, J = 9.2, 0.6 Hz), 8.97 (dd, 1H, J = 4.0, 1.6 Hz), 12.19 (s, 1H); IR (MeOH) 3401, 1498 cm^{-1}; MS (ESI) m/z 293.11 (MH^+).

4.2.2. General procedure for the preparation of the 3-((1-hydroxy-4-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl)methyl)-benzonitriles 14 and 15

A mixture of NH_4OAc (9.40 mmol), **12** or **13** (0.47 mmol), and 3-cyanophenylacetaldehyde (0.94 mmol) in AcOH (5 mL) was heated at 110 °C for 16 h. The reaction mixture was cooled to room temperature and neutralized with NH_4OH to pH ~7. The mixture was extracted with CH_2Cl_2 (40 mL × 3), and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel with CH_2Cl_2/MeOH as eluent to afford the title compound **14** or **15** as a solid.

4.2.2.1. 3-((1-Hydroxy-4-(6-methylpyridin-2-yl)-5-(quinolin-6-yl)-1*H*-imidazol-2-yl)methyl)benzonitrile (14). Yield 39%; mp 135 °C; ^{1}H NMR ($CDCl_3$) δ 2.43 (s, 3H), 4.24 (s, 2H), 7.04 (d, 1H,

J = 7.6 Hz), 7.16 (d, 1H, J = 8.4 Hz), 7.31–7.38 (m, 2H), 7.49 (d, 2H, J = 7.6 Hz), 7.52–7.56 (m, 1H), 7.64 (s, 1H), 7.69 (d, 1H, J = 8.0 Hz), 7.84 (d, 1H, J = 8.8 Hz), 7.98 (s, 1H), 8.05 (d, 1H, J = 8.0 Hz), 8.71 (s, 1H); IR ($CHCl_3$) 3400, 3024, 2938, 2230 cm^{-1}; MS (ESI) m/z 418.17 (MH^+).

4.2.2.2. 3-((1-Hydroxy-5-(6-methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzonitrile (15). Yield 35%; mp 120 °C; ^1H NMR ($CDCl_3$) δ 2.59 (s, 3H), 4.27 (s, 2H), 7.15 (d, 1H, J = 6.8 Hz), 7.37 (t, 1H, J = 8.0 Hz), 7.49 (dt, 1H, J = 8.0, 1.6 Hz), 7.60–7.66 (m, 2H), 7.69 (t, 1H, J = 7.6 Hz), 7.72 (s, 1H), 7.78 (d, 1H, J = 7.6 Hz), 8.23 (d, 1H, J = 8.4 Hz), 8.32 (dd, 1H, J = 9.2, 0.4 Hz), 8.79 (d, 1H, J = 9.2 Hz), 8.90 (dd, 1H, J = 4.4, 1.6 Hz); IR (CH_2Cl_2) 3406, 2229 cm^{-1}; MS (ESI) m/z 419.14 (MH^+).

4.2.3. General procedure for the preparation of the 3-((4-(6-methylpyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzonitrile 16 and 17

To a stirred solution of **14** or **15** (0.14 mmol) in anhydrous DMF (1.5 mL) was added triethyl phosphite (0.21 mmol) at room temperature. The mixture was heated at 110 °C for 16 h and then cooled to room temperature. The reaction mixture was diluted with EtOAc (100 mL) and washed with water (10 mL × 8) and brine (20 mL). The 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 with CH_2Cl_2/MeOH as eluent to afford the title compound **16** or **17** as a solid.

4.2.3.1. 3-((4-(6-Methylpyridin-2-yl)-5-(quinolin-6-yl)-1*H*-imidazol-2-yl)methyl)benzonitrile (16). Yield 61%; mp 114 °C; ^1H NMR ($CDCl_3$) δ 2.52 (s, 3H), 4.25 (s, 2H), 7.00 (d, 1H, J = 7.2 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.41–7.46 (m, 3H), 7.55 (d, 1H, J = 7.6 Hz), 7.61 (d, 1H, J = 7.6 Hz), 7.65 (s, 1H), 7.95 (dd, 1H, J = 8.8, 2.0 Hz), 8.13 (d, 1H, J = 8.8 Hz), 8.19 (m, 2H), 8.93 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3405, 3180, 2230 cm^{-1}; MS (ESI) m/z 402.17 (MH^+). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44. Found: C, 77.58; H, 4.81; N, 17.40.

4.2.3.2. 3-((5-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzonitrile (17). Yield 59%; mp 148 °C; ^1H NMR ($CDCl_3$) δ 2.62 (s, 3H), 4.29 (s, 2H), 7.17 (d, 1H, J = 8.0 Hz), 7.40 (t, 1H, J = 8.0 Hz), 7.51 (dt, 1H, J = 7.6, 1.2 Hz), 7.63–7.68 (m, 2H), 7.72 (t, 1H, J = 7.6 Hz, overlapped), 7.74 (s, 1H), 7.81 (d, 1H, J = 7.6 Hz), 8.27 (d, 1H, J = 8.4 Hz), 8.35 (d, 1H, J = 9.2 Hz), 8.81 (d, 1H, J = 9.2 Hz), 8.92 (dd, 1H, J = 4.0, 1.6 Hz); IR (CH_2Cl_2) 2228 cm^{-1}; MS (ESI) m/z 403.16 (MH^+). Anal. Calcd for $C_{25}H_{18}N_6$: C, 74.61; H, 4.51; N, 20.88. Found: C, 74.47; H, 4.67; N, 20.68.

4.2.4. General procedure for the conversion of carbonitrile derivatives 16, 17, 22–25, and 33 to carboxamide derivatives 18, 19, 26–29, and 34

A stirred solution of carbonitrile compound **16**, **17**, **22**–**25** or **33** (0.06 mmol), 1 N NaOH (0.21 mL, 0.21 mmol), and 28% H_2O_2 (0.008 mL, 0.06 mmol) in 95% EtOH (2 mL) was heated at 60 °C for 2 h. The reaction mixture was cooled to 0 °C and neutralized with 2 N HCl (0.1 mL) to pH ~8. The mixture was extracted with $CHCl_3$ (30 mL × 3), and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel with $CHCl_3$/MeOH or CH_2Cl_2/MeOH as eluent to afford the title compound **18**, **19**, **26**–**29**, or **34** as a solid.

4.2.4.1. 3-((4-(6-Methylpyridin-2-yl)-5-(quinolin-6-yl)-1*H*-imidazol-2-yl)methyl)benzamide (18). Yield 69%; mp 151.5 °C; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3H), 4.29 (s, 2H), 5.55 (br s, 1H), 6.56 (br s, 1H), 7.03 (d, 1H, J = 7.6 Hz), 7.31 (d, 1H, J = 8.0 Hz), 7.37 (t, 1H, J = 7.6 Hz), 7.43 (dd, 1H, J = 8.0, 4.4 Hz), 7.45–7.52 (m, 2H), 7.74 (d, 1H, J = 7.6 Hz), 7.89 (dd, 1H, J = 8.8, 2.0 Hz), 8.05 (s, 1H), 8.11 (d, 1H, J = 8.8 Hz), 8.16–8.18 (m, 2H), 8.93 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3433, 1636 cm^{-1}; MS (ESI) m/z 420.18 (MH^+). Anal. Calcd for $C_{26}H_{21}N_5O$: C, 74.44; H, 5.05; N, 16.70. Found: C, 74.39; H, 5.15; N, 16.54.

4.2.4.2. 3-((5-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-imidazol-2-yl)methyl)benzamide (19). Yield 90%; mp 229 °C; ^1H NMR ($CDCl_3$) δ 2.60 (s, 3H), 4.31 (s, 2H), 5.72 (br s, 1H), 6.31 (br s, 1H), 7.16 (d, 1H, J = 7.2 Hz), 7.38 (t, 1H, J = 7.6 Hz), 7.59 (d, 1H, J = 7.6 Hz), 7.64 (dd, 1H, J = 8.4, 4.4 Hz), 7.67–7.73 (m, 2H), 7.78 (d, 1H, J = 7.6 Hz), 7.87 (s, 1H), 8.23 (d, 1H, J = 8.8 Hz), 8.32 (d, 1H, J = 9.2 Hz), 8.74 (d, 1H, J = 9.2 Hz), 8.92 (d, 1H, J = 2.8 Hz); IR (CH_2Cl_2) 3299, 3180, 1655 cm^{-1}; MS (ESI) m/z 421.17 (MH^+). Anal. Calcd for $C_{25}H_{20}N_6O$: C, 71.41; H, 4.79; N, 19.99. Found: C, 71.35; H, 4.77; N, 19.74.

4.2.4.3. 3-((3-(6-Methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzamide (26). Yield 70%; mp 198.5 °C; ^1H NMR (CD_3OD) δ 2.44 (s, 3H), 5.54 (s, 2H), 7.26 (dd, 1H, J = 7.8, 0.6 Hz), 7.38 (dd, 1H, J = 7.2, 0.4 Hz), 7.48–7.51 (m, 2H), 7.60 (m, 1H), 7.67 (dd, 1H, J = 8.8, 2.0 Hz), 7.70 (t, 1H, J = 7.8 Hz), 7.84 (dt, 1H, J = 8.0, 2.0 Hz), 7.87 (d, 1H, J = 2.0 Hz), 7.90 (d, 1H, J = 8.8 Hz), 7.94–7.95 (m, 1H), 8.17 (s, 1H), 8.22 (dd, 1H, J = 8.0, 0.8 Hz), 8.78 (dd, 1H, J = 4.4, 1.8 Hz); IR ($CHCl_3$) 3357, 3180, 1670 cm^{-1}; MS (ESI) m/z 420.18 (MH^+). Anal. Calcd for $C_{26}H_{21}N_5O$: C, 74.44; H, 5.05; N, 16.70. Found: C, 74.22; H, 5.25; N, 16.64.

4.2.4.4. 3-((5-(6-Methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzamide (27). Yield 80%; mp 97 °C; ^1H NMR ($CDCl_3$) δ 2.67 (s, 3H), 5.62 (s, 2H), 6.90 (d, 1H, J = 8.0 Hz), 7.18 (d, 1H, J = 7.6 Hz), 7.34–7.36 (m, 2H), 7.46 (t, 2H, J = 7.6 Hz), 7.55 (dd, 1H, J = 7.2, 1.6 Hz), 7.67–7.69 (m, 2H), 7.76 (s, 1H), 7.85 (s, 1H), 8.09 (br s, 1H), 8.16 (br s, 1H), 8.87 (dd, 1H, J = 4.8, 1.8 Hz); IR ($CHCl_3$) 3426, 1664 cm^{-1}; MS (ESI) m/z 420.18 (MH^+). Anal. Calcd for $C_{26}H_{21}N_5O$: C, 74.44; H, 5.05; N, 16.70. Found: C, 74.65; H, 5.01; N, 16.49.

4.2.4.5. 3-((3-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-pyrazol-1-yl)methyl)benzamide (28). Yield 92%; mp 86−88 °C; ^1H NMR ($CDCl_3$) δ 2.49 (s, 3H), 5.41 (s, 2H), 6.07 (br s, 1H), 6.83 (br s, 1H), 7.12 (d, 1H, J = 7.6 Hz), 7.36 (t, 1H, J = 7.6 Hz), 7.47 (d, 1H, J = 6.8 Hz), 7.49 (d, 1H, J = 7.6 Hz), 7.53−7.61 (m, 2H), 7.72 (d, 1H, J = 9.2 Hz), 7.75 (d, 1H, J = 7.6 Hz), 7.87 (s, 1H), 8.12 (s, 1H), 8.16 (d, 1H, J = 9.2 Hz), 8.26 (m, 1H), 8.86 (dd, 1H, J = 4.4, 1.6 Hz); IR (CH_2Cl_2) 3341, 3191, 1671 cm^{-1}; MS (ESI) m/z 421.17 (MH^+). Anal. Calcd for $C_{25}H_{20}N_6O$: C, 71.41; H, 4.79; N, 19.99. Found: C, 71.28; H, 4.92; N, 19.74.

4.2.4.6. 3-((5-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-pyrazol-1-yl)methyl)benzamide (29). Yield 93%; mp 88−90 °C; ^1H NMR ($CDCl_3$) δ 2.66 (s, 3H), 5.52 (s, 2H), 5.96 (br s, 1H), 6.37 (br s, 1H), 7.07 (d, 1H, J = 7.6 Hz), 7.22 (d, 1H, J = 8.0 Hz), 7.25–7.31 (m, 2H), 7.36 (d, 1H, J = 8.8 Hz), 7.50–7.58 (m, 2H), 7.62 (s, 1H), 7.66 (dt, 1H, J = 6.4, 2.2 Hz), 8.16 (d, 1H, J = 8.8 Hz), 8.19 (s, 1H), 8.22 (d, 1H, J = 8.0 Hz), 8.85 (dd, 1H, J = 4.4, 1.6 Hz); IR (CH_2Cl_2) 3352, 3191, 1671 cm^{-1}; MS (ESI) m/z 421.17 (MH^+). Anal. Calcd for $C_{25}H_{20}N_6O$: C, 71.41; H, 4.79; N, 19.99. Found: C, 71.35; H, 4.87; N, 19.79.

4.2.4.7. 3-((4-(6-Methylpyridin-2-yl)-3-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzamide (34). Yield 89%; mp 96 °C; ^1H NMR ($CDCl_3$) δ 2.66 (s, 3H), 5.46 (s, 2H), 6.99 (d, 1H, J = 7.6 Hz), 7.07 (d, 1H, J = 8.0 Hz), 7.26 (1H, overlapped with residual $CHCl_3$), 7.41

(dd, 1H, J = 8.4, 4.4 Hz), 7.45 (t, 2H, J = 7.8 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.83 (d, 2H, J = 8.0 Hz), 7.94 (br s, 1H), 8.07–8.09 (m, 2H), 8.14 (d, 1H, J = 8.0 Hz), 8.92 (dd, 1H, J = 4.2, 1.8 Hz); IR ($CHCl_3$) 3367, 3196, 1674 cm^{-1}; MS (ESI) m/z 420.18 (MH^+). Anal. Calcd for $C_{26}H_{21}N_5O$: C, 74.44; H, 5.05; N, 16.70. Found: C, 74.69; H, 4.87; N, 16.44.

4.2.5. General procedure for the preparation of the 3-(6-methylpyridin-2-yl)-1*H*-pyrazoles 20 and 21

To a stirred solution of **10** or **11** (0.33 mmol) in anhydrous DMF (3 mL) were added AcOH (0.84 mmol) and DMF·DMA (0.70 mmol). After the mixture was stirred for 1 h at room temperature, hydrazine monohydrate (5.14 mmol) was added, and the mixture was heated at 50 °C for 2 h. The cooled reaction mixture was poured into water (10 mL) and extracted with EtOAc (100 mL). The organic layer was washed with water (10 mL × 8) and saturated aqueous $NaHCO_3$ (10 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel with CH_2Cl_2/MeOH as eluent to afford the compounds **20** or **21** as a solid.

4.2.6. General procedure for the alkylation of 20, 21, and 32

A solution of pyrazole **20**, **21**, or **32** (0.69 mmol) in anhydrous DMF (4 mL) was added dropwise over 15 min to a stirred suspension of Cs_2CO_3 (0.75 mmol) in anhydrous DMF (1 mL) at room temperature. The mixture was stirred for 15 min, and to it, α-bromo-*m*-tolunitrile (0.69 mmol) was added. After 2 h (for **33**, 12 h at 80 °C), the reaction mixture was diluted with EtOAc (120 mL), washed with water (12 mL × 8) and brine (12 mL). The 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 with CH_2Cl_2/MeOH as eluent to afford the title compound **22**–**25** or **33** as a solid.

4.2.6.1. 3-((3-(6-Methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzonitrile (22). Yield 59%; mp 76 °C; ^1H NMR ($CDCl_3$) δ 2.52 (s, 3H), 5.49 (s, 2H), 7.10 (dd, 1H, J = 8.0, 0.4 Hz), 7.31 (dd, 1H, J = 7.6, 0.4 Hz), 7.41 (dd, 1H, J = 8.0, 4.4 Hz), 7.48–7.54 (m, 2H), 7.57–7.60 (m, 1H), 7.63–7.65 (m, 3H), 7.68 (dd, 1H, J = 8.8, 2.0 Hz), 7.86 (d, 1H, J = 2.0 Hz), 8.05 (d, 1H, J = 8.8 Hz), 8.11 (d, 1H, J = 8.0 Hz), 8.88 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3384, 3061, 2927, 2231 cm^{-1}; MS (ESI) m/z 402 (MH^+). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44. Found: C, 77.45; H, 4.97; N, 17.24.

4.2.6.2. 3-((5-(6-Methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzonitrile (23). Yield 40%; mp 83 °C; ^1H NMR ($CDCl_3$) δ 2.65 (s, 3H), 5.64 (s, 2H), 6.88 (dd, 1H, J = 8.0, 0.4 Hz), 7.17 (dd, 1H, J = 7.6, 0.4 Hz), 7.37–7.45 (m, 3H), 7.46–7.48 (m, 2H), 7.51–7.54 (m, 2H), 7.75 (d, 1H, J = 2.0 Hz), 7.85 (s, 1H), 8.04 (d, 1H, J = 8.8 Hz), 8.11 (d, 1H, J = 8.0 Hz), 8.87 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3443, 3067, 2230 cm^{-1}; MS (ESI) m/z 402 (MH^+). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44. Found: C, 77.58; H, 4.87; N, 17.23.

4.2.6.3. 3-((3-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-pyrazol-1-yl)methyl)benzonitrile (24). Yield 68%; mp 49–50 °C; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H), 5.42 (s, 2H), 7.11 (d, 1H, J = 7.6 Hz), 7.42 (t, 1H, J = 7.6 Hz), 7.48 (d, 1H, J = 7.6 Hz), 7.53–7.63 (m, 5H), 7.75 (d, 1H, J = 8.8 Hz), 8.10 (s, 1H), 8.16 (d, 1H, J = 8.8 Hz), 8.26 (d, 1H, J = 8.4 Hz), 8.85 (dd, 1H, J = 4.4, 1.6 Hz); IR (CH_2Cl_2) 2231 cm^{-1}; MS (ESI) m/z 403.13 (MH^+). Anal. Calcd for $C_{25}H_{18}N_6$: C, 74.61; H, 4.51; N, 20.88. Found: C, 74.79; H, 4.35; N, 20.62.

4.2.6.4. 3-((5-(6-Methylpyridin-2-yl)-4-(1,5-naphthyridin-2-yl)-1*H*-pyrazol-1-yl)methyl)benzonitrile (25). Yield 23%; mp 149–151 °C; ^1H NMR ($CDCl_3$) δ 2.66 (s, 3H), 5.55 (s, 2H), 7.08 (d, 1H, J = 7.6 Hz), 7.23 (d, 1H, J = 7.6 Hz), 7.35 (dd, 1H, J = 7.6, 7.6 Hz), 7.40 (d, 1H, J = 8.8 Hz), 7.43–7.47 (m, 2H), 7.50 (dt, 1H, J = 7.6, 1.6 Hz), 7.54 (t, 1H, J = 7.6 Hz), 7.59 (dd, 1H, J = 8.8, 4.0 Hz), 8.20 (s, 1H), 8.21 (d, 1H, J = 8.8 Hz), 8.26 (d, 1H, J = 8.8 Hz), 8.87 (dd, 1H, J = 4.0, 1.6 Hz); IR (CH_2Cl_2) 2230 cm^{-1}; MS (ESI) m/z 403.16 (MH^+). Anal. Calcd for $C_{25}H_{18}N_6$: C, 74.61; H, 4.51; N, 20.88. Found: C, 74.43; H, 4.77; N, 20.74.

4.2.6.5. 3-((4-(6-Methylpyridin-2-yl)-3-(quinolin-6-yl)-1*H*-pyrazol-1-yl)methyl)benzonitrile (33). Yield 79%; mp 70 °C; ^1H NMR ($CDCl_3$) δ 2.61 (s, 3H), 5.45 (s, 2H), 6.98 (d, 1H, J = 7.6 Hz), 7.04 (d, 1H, J = 8.0 Hz), 7.26 (1H, overlapped with residual $CHCl_3$), 7.41–7.45 (m, 2H), 7.49–7.53 (m, 1H), 7.61–7.65 (m, 3H), 7.86 (dd, 1H, J = 8.8, 1.6 Hz), 8.09–8.11 (m, 2H), 8.16 (d, 1H, J = 7.6 Hz), 8.93 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3399, 2231 cm^{-1}; MS (ESI) m/z 402 (MH^+). Anal. Calcd for $C_{26}H_{19}N_5$: C, 77.79; H, 4.77; N, 17.44. Found: C, 77.93; H, 4.56; N, 17.23.

4.2.7. 2-(6-Methylpyridin-2-yl)-1-(quinolin-6-yl)ethanone (31)

To a stirred solution of 2,6-lutidine (2.33 mmol) in anhydrous THF (8 mL) at −78 °C under Ar was treated dropwise with *n*-BuLi (2.5 M solution in hexane, 3.50 mmol). After 10 min, a solution of *N*-methoxy-*N*-methylquinoline-6-carboxamide (2.80 mmol) in anhydrous THF (7 mL) was added dropwise to the reaction mixture, and the solution was allowed to warm to room temperature. The solution was quenched with saturated aqueous NH_4Cl solution (7 mL) and extracted with EtOAc (300 mL). The organic layer was washed with H_2O (30 mL × 2) and brine (30 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel with CH_2Cl_2/MeOH (19:1 (v/v)) as eluent to afford the compound **31** (389 mg, 64%) as a solid. Mp 87 °C; ^1H NMR ($CDCl_3$) δ 2.57 (s, 3H), 6.23 (s, 1H), 6.85 (d, 1H, J = 7.6 Hz), 6.95 (d, 1H, J = 8.0 Hz), 7.46 (dd, 1H, J = 8.0, 4.2 Hz), 7.55 (t, 1H, J = 7.8 Hz), 8.09–8.16 (m, 2H), 8.23 (dd, 1H, J = 8.0, 1.6 Hz), 8.26–8.29 (m, 1H), 8.37 (d, 1H, J = 1.6 Hz), 8.92 (dd, 1H, J = 4.2, 1.8 Hz); IR ($CHCl_3$) 3432, 1690 cm^{-1}; MS (ESI) m/z 263 (MH^+).

4.2.8. 6-(4-(6-Methylpyridin-2-yl)-1*H*-pyrazol-3-yl)quinoline (32)

To a stirred solution of **31** (1.37 mmol) in THF (10 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (10.98 mmol). After 48 h, the mixture was concentrated in vacuo. The residue was dissolved in EtOH (10 mL), and to it, hydrazine monohydrate (32.94 mmol) was added. The mixture was stirred for 12 h at room temperature and concentrated. The residue was diluted with CH_2Cl_2 (200 mL) and washed with H_2O (40 mL). 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 with CH_2Cl_2/MeOH (15:1 (v/v)) as eluent to afford the compound **32** (173 mg, 44%) as a solid. Mp 95 °C; ^1H NMR ($CDCl_3$) δ 2.55 (s, 3H), 7.01 (dd, 2H, J = 6.8, 6.4 Hz), 7.42 (m, 2H), 7.85 (dd, 1H, J = 8.8, 1.6 Hz), 8.08 (t, 3H, J = 4.4 Hz), 8.13 (dd, 1H, J = 8.4, 0.8 Hz), 8.92 (dd, 1H, J = 4.4, 1.6 Hz); IR ($CHCl_3$) 3384, 3151, 2927 cm^{-1}; MS (ESI) m/z 287 (MH^+).

4.3. Docking experiments

Protein modeling and docking experiments have been performed with the SYBYL 8.0 software package (Tripos, Inc., St. Louis, MO, USA)[42] based on Linux CentOS 4.0.

4.3.1. Preparation of molecular structures

The structure of **18** was prepared in MOL2 format using the sketcher module and Gasteiger-Huckel charges were assigned to the ligand atoms. The structure of **18** was optimized by energy minimization until a convergence value of 0.001 kcal/(Å mol), and molecular dynamics using simulated annealing method. The conformer database for **18** was prepared by random selection of 50 conformers from molecular dynamics output.

4.3.2. Preparation of target protein structure and flexible docking

The X-ray coordinate of ALK5 complexed with 1,5-naphthyridine inhibitor (PDB id: 1VJY)[32] was retrieved from the PDB, and all crystallographic water molecules were removed except one involved in H-bond with ligand inside the binding pocket. After the hydrogen atoms and atomic charges were added to the receptor, side chain amides of ALK5 were fixed. The active site was defined as all the amino acid residues enclosed within 6.5 Å radius sphere centered by the bound 1,5-naphthyridine inhibitor. The docking was performed using the default parameters of the FlexX programs implanted in the SYBYL 8.0, and subsequent scoring for FlexX solution was conducted by a consensus scoring function (CScore). One of the conformers having the highest consensus score (CScore = 5) was selected and complexed with ALK5, resulting in a final model shown in Figure 3.

4.4. CYP-mediated metabolism

Compound **8** or **18** (120 μM) was incubated with recombinant human CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4 (1 mg protein/mL supersomes™), and the NADPH generating system (0.065 mM $NADP^+$, 3.3 mM glucose-6-phosphate, 0.4 U/mL of glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium chloride) in potassium phosphate buffer (pH 7.4, 100 mM) for 30 min at 37 °C. The reaction was then quenched with three volumes of MeOH, chilled, and centrifuged. The supernatants were directly assayed by HPLC with UV detection. The reaction mixture without the NADPH generating system was used as negative control. HPLC chromatographic separation was carried out with a Shiseido Capcell Pak UG120 C_{18} column (250 × 4.6 mm, particle size 5 μM, Shiseido, Tokyo, Japan) using the Hewlett Packard HP 1100 system (Waldbrone, Germany). The ratio of mobile phase A to B (mobile phase A, CH_3CN:TFA = 100:0.1 (v/v); mobile phase B, H_2O:TFA = 100:0.1 (v/v)) was 5–95% in the beginning and gradually changed in a linear manner to 90–10% at 20 min after running at a flow rate of 1 mL/min. The column effluent was monitored by an UV detector at 240 nm. All the procedures were performed at room temperature. The detection limit of compounds **8** and **18** in samples was 0.25 μM, and the retention times of metabolites **9** and **35** were 8.7 and 8.0 min, respectively.

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