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

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ABSTRACT

A series of 2-benzylamino-4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazoles **12a**–**ab**, **13a**, **13b**, and **18a**–**d** has been synthesized and evaluated for their ALK5 inhibitory activity in an enzyme assay and in a cell-based luciferase reporter assay. The *N*-(3-fluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazol-2-amine (**12b**) inhibited ALK5 phosphorylation with an IC_{50} value of 7.01 nM and showed 61% inhibition at 30 nM in a luciferase reporter assay using HaCaT cells permanently transfected with p3TP-luc reporter construct.

1. Introduction

The transforming growth factor-β (TGF-β) is the most potent and ubiquitous profibrogenic cytokine. TGF-β transduces signals through two distinct serine/threonine kinase receptors, the type I and type II receptors [1]. Following the binding of ligand to the constitutively active type II receptor, the type I receptor, also called as activin receptor-like kinase 5 (ALK5), is phosphorylated, which further phosphorylates Smad2/Smad3 proteins. Phosphorylated Smad2/Smad3 proteins form a heteromeric complex with Smad4, which translocates into the nucleus, assembles with specific DNA-binding cofactors and co-modulators, and binds to the promoters of TGF-β target genes involved in cell differentiation, proliferation, apoptosis, migration, and extracellular matrix production [2]. TGF-β plays a pivotal role in the development of fibrosis in various organ systems such as kidney [3], heart [4], lung [5], and liver [6]. Perturbation of TGF-β signaling has been also implicated in various human diseases including cancer [7], pancreatic diseases [8], and hematological malignancies [9]. Recent studies have shown that blocking the TGF-β signaling pathway with several small molecule ATP-competitive ALK5 inhibitors such as **1** (SB-505154) [10], **2** (GW6604) [11], **3** (SD-208) [12], and **4** (LY-2157299) [13] (Fig. 1) inhibited autophosphorylation of ALK5 and TGF-β-induced transcription of matrix genes in reporter assays at sub-micromolar concentrations. Among them, **2** and **3** effectively retarded progressive fibrosis in liver and lung, respectively, and **3** and **4** also strongly inhibited growth and invasiveness of cancer cells in animal models.

We have also reported a number of the 2-pyridyl-substituted five-membered heterocycles as ALK5 inhibitors such as IN-1130 (**5**) and IN-1233 (**6**) [14,15] (Fig. 1). One of our preclinical candidates, **5**, displayed its pronounced activity as a suppressor of fibrogenic process of unilateral ureteral obstruction (UUO) in a rat renal fibrosis model [16]. Another preclinical candidate, **6**, effectively prevented development and progression of pulmonary arterial hypertension in a monocrotaline rat model [17].

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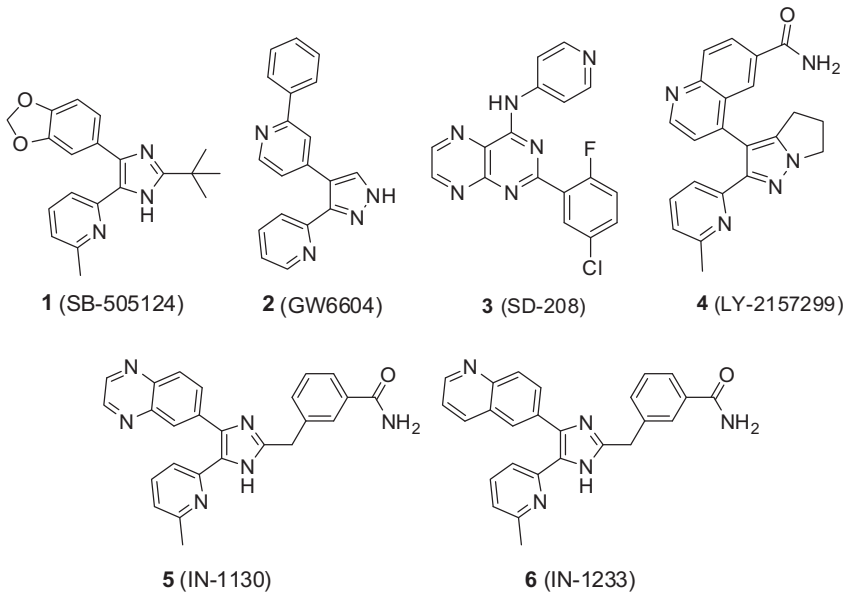


Fig. 1. ALK5 inhibitors under development.

To develop a more potent and selective ALK5 inhibitor, we have prepared a series of 2-benzylamino-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazoles **12a**–**ab**, **13a**, and **13b** possessing an aminomethylene linkage between a thiazole ring and a phenyl ring. It was of particular interest to us whether the amino group at the linkage may serve as an additional binding group to the ATP binding site of ALK5. The target molecules have a [1,2,4]triazolo[1,5-*a*]pyridin-6-yl moiety rather than a quinoxalin-6-yl moiety of **5** or a quinolin-6-yl moiety of **6** at the 5-position of the thiazole and have various substituents such as F, Cl, Me, OMe, OCF_3, CN, and $CONH_2$ in the phenyl ring. In pharmacokinetic studies, the major metabolite of **5** was detected in the systemic circulation of rat and mouse and was identified 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 [18].

To overcome metabolic oxidation of the quinoxalin-6-yl moiety of **5**, we adopted a [1,2,4]triazolo[1,5-*a*]pyridin-6-yl moiety since metabolic oxidation of 2-position of this moiety is assumed to be difficult due to the presence of two adjacent nitrogen atoms. To examine the influence of the position of two hetero atoms in a thiazole ring on ALK5 inhibition, we have prepared 2-benzylamino-5-(6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]-pyridin-6-yl)thiazoles **18a**–**d**, respective thiazole ring isosteres of **12a**, **12g**, **13a**, and **12n** for comparison.

2. Results and discussion

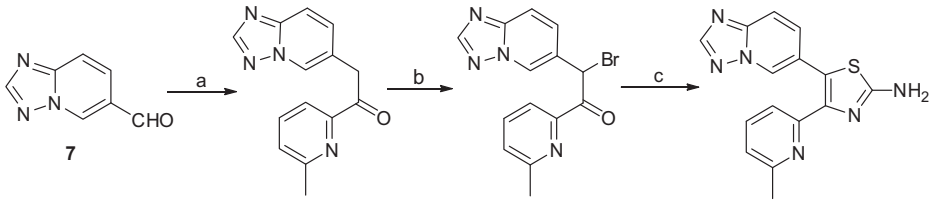
2.1. Chemistry

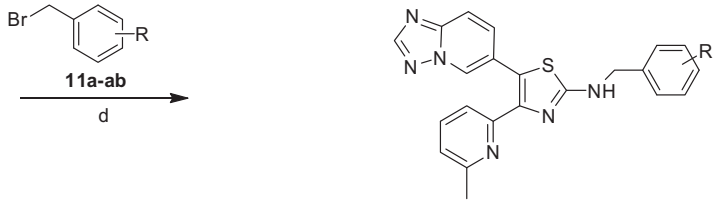
A series of 2-benzylamino-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-*a*]pyridin-6-yl)thiazoles **12a**–**ab** was prepared as shown in Scheme 1. Coupling of the [1,2,4]triazolo[1,5-*a*]pyridine-6-carbaldehyde (**7**) [19] with diphenyl (6-methylpyridin-2-yl)(phenylamino)methylphosphonate [20] in a mixture of THF and *i*-PrOH (4:1) in the presence of Cs_2CO_3 at room temperature and followed by hydrolysis with 3 N HCl afforded the ketone **8** [21] in 90% yield. The ketone **8** was brominated with Br_2 in 1,4-dioxane at room temperature to give the monobromo ketone **9** in 83% yield. Condensation of **9** with thiourea in DMF at 120 °C produced 4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-*a*]-pyridin-6-yl)thiazol-2-amine (**10**) in 82% yield. The 2-aminothiazole **10** was alkylated with an appropriately substituted benzyl bromide (**11a**–**ab**) in the presence of Cs_2CO_3 in DMF at 120 °C to afford the target compounds **12a**–**ab** in various yields (24–95%). Conversion of the nitrile functionality in **12g** and **12m** to the corresponding carboxamide was accomplished by treatment of 35% H_2O_2 and 1 N NaOH in absolute ethanol to afford **13a** and **13b** in 60% and 71% yields, respectively (Scheme 2).

For comparison, the 2-benzylamino-5-(6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazoles **18a**–**d**, the respective thiazole ring isosteres of **12a**, **12g**, **13a**, and **12n**, were prepared as shown in Scheme 3. The aldehyde **7** was treated with aniline and diphenyl phosphite in *i*-PrOH at room temperature to afford diphenyl ([1,2,4]triazolo[1,5-*a*]pyridin-6-yl(phenylamino)methyl)-phosphonate (**14**) in 86% yield. Coupling of **14** with 6-methylpyridine-2-carboxaldehyde in a mixture of THF and *i*-PrOH (4:1) in the presence of Cs_2CO_3 and followed by treating with 3 N HCl afforded the monoketone **15** in 83% yield. Bromination of **15** with *N*-bromosuccinimide (NBS) in CH_2Cl_2 at 0 °C gave the monobromo ketone **16** in 81% yield. Condensation of **16** with thiourea in the same reaction condition as described in Scheme 1 afforded 5-(6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazol-2-amine (**17**) in 67% yield. The 2-aminothiazole **17** was alkylated with benzyl bromides (**11a**, **11g**, and **11n**) to afford the target compounds **18a**, **18b**, and **18d** in 55%, 28%, and 49% yields, respectively. The nitrile derivative **18b** was converted to the carboxamide derivative **18c** in 42% yield in the same reaction condition as mentioned in Scheme 2.

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

To evaluate whether these potential inhibitors **12a**–**ab**, **13a**, **13b**, and **18a**–**d** 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). The ALK5 inhibitory activity of the 2-(benzylamino)thiazole derivatives **12b**–**ab**, **13a**, and **13b** having various substituents such as F, Cl, Me, OMe, OCF_3, CN, and $CONH_2$ in the phenyl ring was compared with that of the unsubstituted 2-(benzylamino)thiazole derivative **12a**.



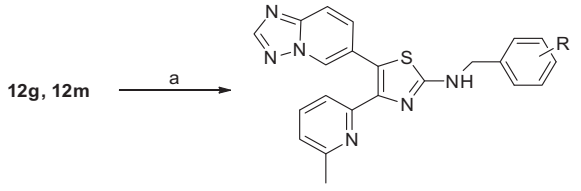


a: R = H
b: R = *m*-F
c: R = *m*-Cl
d: R = *m*-Me
e: R = *m*-OMe
f: R = *m*-OCF_3
g: R = *m*-CN
h: R = *p*-F
i: R = *p*-Cl
j: R = *p*-Me
k: R = *p*-OMe
l: R = *p*-OCF_3
m: R = *p*-CN
n: R = *o*-F
o: R = *o*-Cl
p: R = *o*-Me
q: R = *o*-OCF_3
r: R = 2,3-F_2
s: R = 3,4-F_2
t: R = 3,5-F_2
u: R = 2,3-Cl_2
v: R = 3,4-Cl_2
w: R = 3,5-Cl_2
x: R = 2,3-Me_2
y: R = 3,5-Me_2
z: R = 2,3-$(OMe)_2$
aa: R = 3,4-$(OMe)_2$
ab: R = 3,5-$(OMe)_2$

12a: R = H (55%)
12b: R = *m*-F (89%)
12c: R = *m*-Cl (38%)
12d: R = *m*-Me (26%)
12e: R = *m*-OMe (61%)
12f: R = *m*-OCF_3 (64%)
12g: R = *m*-CN (72%)
12h: R = *p*-F (95%)
12i: R = *p*-Cl (57%)
12j: R = *p*-Me (46%)
12k: R = *p*-OMe (41%)
12l: R = *p*-OCF_3 (47%)
12m: R = *p*-CN (78%)
12n: R = *o*-F (52%)
12o: R = *o*-Cl (67%)
12p: R = *o*-Me (56%)
12q: R = *o*-OCF_3 (29%)
12r: R = 2,3-F_2 (54%)
12s: R = 3,4-F_2 (60%)
12t: R = 3,5-F_2 (87%)
12u: R = 2,3-Cl_2 (46%)
12v: R = 3,4-Cl_2 (53%)
12w: R = 3,5-Cl_2 (39%)
12x: R = 2,3-Me_2 (38%)
12y: R = 3,5-Me_2 (31%)
12z: R = 2,3-$(OMe)_2$ (51%)
12aa: R = 3,4-$(OMe)_2$ (57%)
12ab: R = 3,5-$(OMe)_2$ (24%)

Scheme 1. Reagents and conditions: (a) (i) diphenyl (6-methylpyridin-2-yl)(phenylamino)methylphosphonate, Cs_2CO_3, THF/*i*-PrOH (4:1), rt, 16 h; (ii) 3 N HCl, rt, 1 h; (b) Br_2, 1,4-dioxane, rt, 1 h; (c) thiourea, DMF, 120 °C, 2 h; (d) Cs_2CO_3, DMF, 120 °C, 2 h.

All of the 2-(benzylamino)thiazole derivatives except the *p*-OCF_3 substituted compound **12l** (IC_{50} = 60.90 nM) exhibited more potent ALK5 inhibitory activity (IC_{50} = 7.01–47.70 nM) than the competitor **1** (IC_{50} = 54.40 nM). Among the *meta*-substituted compounds **12b**–**g**, the *m*-F substituted compound **12b** (IC_{50} = 7.01 nM) displayed 1.6-fold more potent ALK5 inhibition than the unsubstituted compound **12a** (IC_{50} = 11.30 nM). The *m*-CN substituted compound **12g** (IC_{50} = 9.26 nM) and *m*-Cl substituted compound **12c** (IC_{50} = 10.20 nM) were slightly more potent than **12a**, and the *m*-Me substituted compound **12d** (IC_{50} = 11.20 nM) and *m*-OMe substituted compound **12e** (IC_{50} = 11.40 nM) were equipotent to **12a**. Among the *para*-substituted compounds **12h**–**m**, the *p*-F substituted compound **12h** (IC_{50} = 8.61 nM) and *p*-Me substituted compound **12j** (IC_{50} = 8.75 nM) were 1.3-fold more inhibitory in ALK5 inhibition than **12a**. Introduction of a substituent at the *ortho* position was proved to be not beneficial, thus, the *o*-F substituted compound **12n** (IC_{50} = 10.40 nM) only displayed the comparable ALK5 inhibitory activity to that of the **12a**. In overall, structure–activity relationships (SAR) of this series of compounds clearly showed that introduction of a fluoro atom at *meta*- or *para*-position in the phenyl ring was the most beneficial in ALK5 inhibition. This SAR was extended to the di-substituted derivatives, thus, the 3,4-F_2 substituted compound **12s** (IC_{50} = 7.43 nM) and 3,5-F_2 substituted compound **12t** (IC_{50} = 8.92 nM) displayed 1.5-fold and 1.3-fold more potent ALK5 inhibitory activity than **12a**.

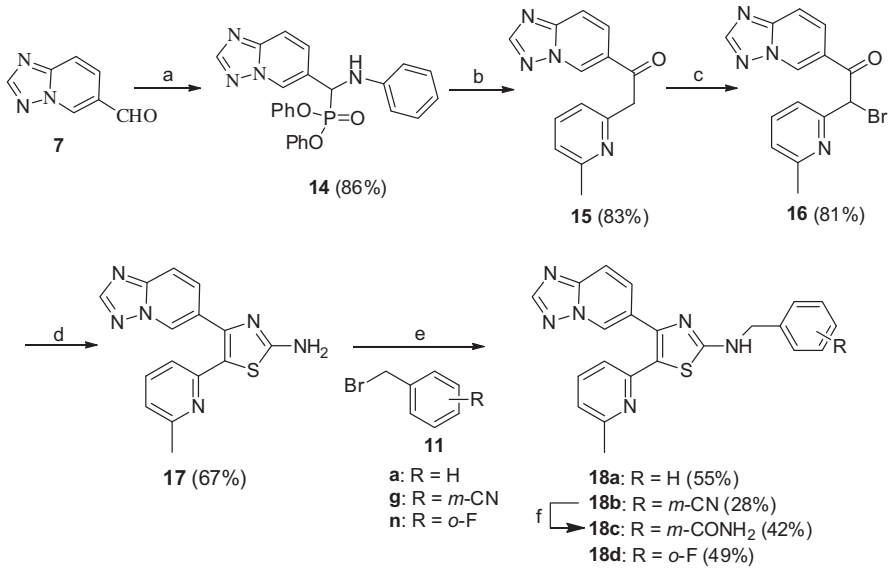


13a: R = *m*-$CONH_2$ (60%)
13b: R = *p*-$CONH_2$ (71%)

Scheme 2. Reagents and conditions: (a) 35% H_2O_2, 1 N NaOH, EtOH, rt, 4 h.

The position of hetero atoms in the central thiazole ring also influenced the ALK5 inhibitory activity. Although, the *m*-$CONH_2$ substituted compound **18c** (IC_{50} = 12.00 nM) showed the similar level of potency to that of the respective positional isomer **13a** (IC_{50} = 13.60 nM), the unsubstituted compound **18a** (IC_{50} = 24.50 nM), *m*-CN substituted compound **18b** (IC_{50} = 25.10 nM), and *o*-F substituted compound **18d** (IC_{50} = 21.20 nM) were 2.2-fold, 2.7-fold, and 2.0-fold less inhibitory than the corresponding positional isomers **12a**, **12g**, and **12n**. Compound **12b** showing the most potent ALK5 inhibitory activity in this series of compounds was 7.8-fold and 2.5-fold more potent than the competitors **1** and **5** (IC_{50} = 17.20 nM), respectively.

To examine TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **12a**–**ab**, **13a**, **13b**, and **18a**–**d** was measured using HaCaT cells permanently transfected with p3TP-luciferase reporter construct at a concentration of 30 nM (Table 1). The p3TP-luciferase reporter construct contains three AP-1 binding elements and the plasminogen-activator inhibitor-1 (PAI-1) promoter [22]. Similar to kinase assay, compound **12b** displayed the most significant ALK5 inhibition (61%). Compounds **12g** (60%), **12c** (58%), **12h** (57%), and **12t** (57%) exhibited the similar level of inhibition to that of **12a** (58%), while **1** and **5** exhibited 44% and 51% of inhibition in this assay.



Scheme 3. Reagents and conditions: (a) aniline, diphenyl phosphite, *i*-PrOH, rt, 3 h; (b) (i) 6-methylpyridine-2-carboxaldehyde, Cs_2CO_3, THF/*i*-PrOH (4:1), rt, 12 h; (ii) 3 N HCl, rt, 1 h; (c) NBS, CH_2Cl_2, 0 °C, 30 min; (d) thiourea, DMF, 120 °C, 2 h; (e) Cs_2CO_3, DMF, 120 °C, 2 h; (f) 35% H_2O_2, 1 N NaOH, EtOH, rt, 4 h.

2.3. Effect of **12b** on the phosphorylation of Smad2

To examine the effect of **12b** on the phosphorylation of Smad2, we have performed Western blot analysis of the phosphorylated Smad2 protein in the NMuMG cells treated with either **12b** or **1** in the presence of TGF-β1. As shown in Fig. 2, the level of phosphorylated Smad2 protein was rapidly increased by TGF-β1 treatment in NMuMG cells, whereas **12b** treatment inhibited the TGF-β1-stimulated level of the phosphorylated Smad2. The comparison of the inhibitory potency of **12b** and **1** showed that **12b** resulted in the more potent inhibition than **1**.

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

To rationalize the SAR shown in Table 1, we examined the binding modes of three representative ligands including **12b**, **12g**, and **18b**. Docking analyses were conducted using the recently reported X-ray structure of the kinase domain of human ALK5 in complexed with quinazoline ALK5 inhibitor [23] (PDB id: 3GXL.pdb). As shown in Fig. 3, the [1,2,4]triazolo[1,5-*a*]pyridine ring binds to the hinge region with forming a hydrogen bond (H-bond) with the backbone NH of His 283. The methylpyridine ring occupies the hydrophobic pocket, and pyridine nitrogen forms a H-bond with Ser 280 which is critical for selectivity of ligands toward ALK5 over p38α MAP kinase where the corresponding residue is Thr 106 [24]. For **12b** and **12g**, the nitrogen atom of thiazole core forms a H-bond with Lys 232, a gatekeeper residue for the specificity pocket of ALK5. Furthermore, the amino group in the linker forms a H-bond with side-chain carboxyl group of Asp 351, as we expected. However, the binding conformation of **18b** (a thiazole ring isostere of **12g**), is a little changed, and these two H-bonds with Lys 232 and Asp 351 are missing (Fig. 3C).

2.5. p38α MAP kinase assay

The kinase domain of p38α MAP kinase is known to be one of the most homologous to that of ALK5 [25], therefore, it was chosen to examine the selectivity profile of this series of compounds. All the target compounds in this series did not inhibit p38α MAP kinase efficiently, showing IC_{50} values of >3280 nM and selectivity indices of >164. Compounds **12b** and **12g** showed selectivity indices of 504 and 408 that are much higher than those of **1** (11) and **5** (28).

3. Conclusion

Compound **12g** efficiently inhibited TGF-β1-induced Smad signaling, epithelial-to-mesenchymal transition (EMT), cell motility, and breast tumor metastasis to the lung in xenografted Balb/c mice, demonstrating that this series of compounds has a high therapeutic potential for cancer metastasis and fibrosis [26].

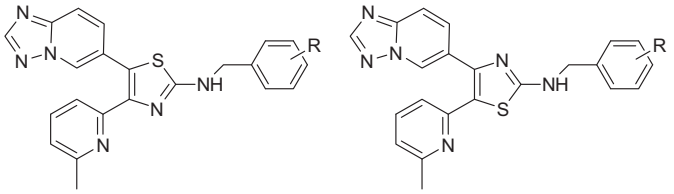
In this report, a series of 2-benzylamino-4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazoles has been prepared and evaluated for their ALK5 inhibitory activity in an enzyme assay and in a cell-based luciferase reporter assay. It has been proved that incorporation of the [1,2,4]triazolo[1,5-*a*]pyridin-6-yl moiety and benzyl moiety at the 4(5)- and 2-positions of the thiazole ring, respectively, significantly increased both ALK5 inhibitory activity and selectivity. Introduction of fluoro atom at *meta*- or *para*-position in the phenyl ring also increased ALK5 inhibitory activity. The most potent compound **12b** inhibited ALK5 phosphorylation with IC_{50} value of 7.01 nM and showed 61% inhibition at 30 nM in a luciferase reporter assay using HaCaT cells permanently transfected with p3TP-luc reporter construct. The selectivity index of **12b** against p38α MAP kinase is 504 that is much higher than those of **1** (11) and **5** (28).

4. Experimental section

4.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on a Varian Unity 400 spectrophotometer. The chemical shifts are reported in parts per million (ppm). For ^1H NMR spectra, $CDCl_3$ was used as solvent, and it served as the internal standard at δ 7.26. For ^{13}C NMR spectra, $CDCl_3$, $CDCl_3/CD_3OD$, or DMSO-d_6 was used as solvent, and it served as the internal standard at δ 77.23 ($CDCl_3$, $CDCl_3/CD_3OD$) or 39.50 (DMSO-d_6). Infrared spectra were recorded on a FT-infrared spectrometer (Bio-Rad). All melting points were taken in Pyrex capillaries using an electrothermal digital melting point apparatus (Buchi) and are not correct. High resolution mass spectra electro

Table 1
Inhibitory activity of 2-benzylamino-4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)thiazoles **12a**–**ab**, **13a**, **13b**, and **18a**–**d** on ALK5 and p38α.



12a-ab, 13a, 13b 18a-d

Compound	R	IC_{50} (nM)		Selectivity index[c]	p3TP-luciferase activity[d,e] (% control)
		ALK5[a]	p38α[b]		
Mock					5 ± 0
TGF-β					100 ± 5
12a	H	11.30	5530	489	42 ± 8
12b	*m*-F	7.01	3530	504	39 ± 4
12c	*m*-Cl	10.20	3280	322	42 ± 1
12d	*m*-Me	11.20	5370	479	62 ± 2
12e	*m*-OMe	11.40	>10,000	>877	44 ± 4
12f	*m*-OCF_3	19.30	7390	383	81 ± 1
12g	*m*-CN	9.26	3780	408	40 ± 2
12h	*p*-F	8.61	9200	1069	43 ± 3
12i	*p*-Cl	15.80	>10,000	>633	48 ± 0
12j	*p*-Me	8.75	>10,000	>1143	58 ± 4
12k	*p*-OMe	24.30	>10,000	>412	56 ± 6
12l	*p*-OCF_3	60.90	>10,000	>164	90 ± 12
12m	*p*-CN	30.80	>10,000	>325	59 ± 3
12n	*o*-F	10.40	>10,000	>962	51 ± 1
12o	*o*-Cl	15.50	5860	378	49 ± 1
12p	*o*-Me	16.90	>10,000	>592	56 ± 4
12q	*o*-OCF_3	35.90	>10,000	>279	80 ± 5
12r	2,3-F_2	29.40	7200	245	45 ± 2
12s	3,4-F_2	7.43	3580	482	49 ± 1
12t	3,5-F_2	8.92	3820	428	43 ± 3
12u	2,3-Cl_2	34.30	>10,000	>292	66 ± 6
12v	3,4-Cl_2	21.90	>10,000	>457	75 ± 5
12w	3,5-Cl_2	15.10	>10,000	>662	65 ± 4
12x	2,3-Me_2	22.10	>10,000	>452	72 ± 6
12y	3,5-Me_2	22.00	>10,000	>455	69 ± 3
12z	2,3-$(OMe)_2$	43.50	>10,000	>230	64 ± 3
12aa	3,4-$(OMe)_2$	47.70	>10,000	>210	75 ± 8
12ab	3,5-$(OMe)_2$	16.50	7990	484	59 ± 4
13a	*m*-$CONH_2$	13.60	6190	455	66 ± 4
13b	*p*-$CONH_2$	25.70	>10,000	>389	75 ± 6
18a	H	24.50	8820	360	84 ± 5
18b	*m*-CN	25.10	>10,000	>398	86 ± 6
18c	*m*-$CONH_2$	12.00	>10,000	>833	46 ± 2
18d	*o*-F	21.20	>10,000	>472	82 ± 5
1		54.40	594	11	56 ± 2
5		17.20	480	28	49 ± 0

[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] 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 30 nM of inhibitor.

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

spray ionization (HRMS-ESI) was obtained on an Agilent Technologies 6220 TOF LC/MS spectrometer.

The HPLC analyses were accomplished with an Agilent 1100 series HPLC system (Agilent, Germany) consisting of a solvent degasser (G1322A degasser), a quaternary pump (G1311A liquid chromatography), and an auto sampler injector (G1313A). Separations were carried out using a reversed-phase C_{18} analytical column (Capcell-pak C_{18} MG120 5 μm, 3.0 × 250 mm) (Shiseido,

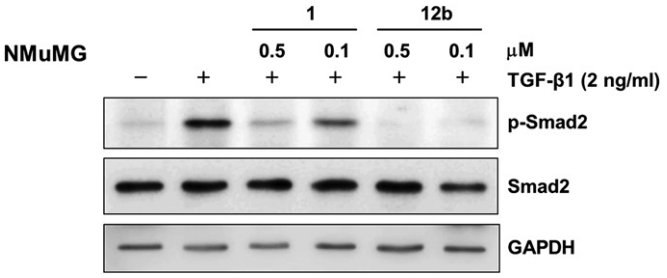


Fig. 2. 12b inhibits TGF-β-induced Smad2 phosphorylation. NMuMG cells were treated with TGF-β1 (2 ng/mL) in the presence of **12b** or **1** for 2 h. Total protein extracts from treated cell were immunoblotted with anti-Smad2, anti-phospho-Smad2, and anti-GAPDH antibodies.

Tokyo, Japan) with a solvent system consisting of an isocratic solvent. The solvent contained 20–35% acetonitrile in distilled water containing 0.1% trifluoroacetic acid and was delivered at 0.5 mL/min over 25 min. The column was maintained at 40 °C with a column oven (G1316A). All compounds were detected at 230 nm with a G1315A diode array detector (DAD). The HPLC system was controlled by a ChemStation Software (Agilent). The purity of the each compound was calculated from a surface integral of detected peaks and is described as percent (%). All compounds were found to be >95% pure except for **12q** with the purity of 94.31%.

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 Chemical or TCI Laboratory chemicals.

*4.1.1. 2-Bromo-1-(6-methylpyridin-2-yl)-2-([1,2,4]triazolo[1,5-a]-pyridin-6-yl)ethanone (**9**)*

To a stirred solution of 1-(6-methylpyridin-2-yl)-2-([1,2,4]triazolo[1,5-*a*]pyridin-6-yl)ethanone (**8**) (2.86 g, 11.34 mmol) in 1,4-dioxane, bromine (0.64 mL, 12.47 mmol) was added. The mixture was stirred at room temperature for 1 h and neutralized with aqueous $NaHCO_3$ solution. The reaction mixture was extracted with EtOAc (3 × 100 mL), and the EtOAc solution was washed with water (50 mL) and brine (50 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 using EtOAc/Hex (1:2) as eluent to afford the titled compound as a brown syrup. Yield 83%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.65 (s, 3H), 7.31 (s, 1H), 7.38 (d, 1H, J = 7.6 Hz), 7.76 (t, 1H, overlapped, J = 7.8 Hz), 7.77 (dd, 1H, overlapped, J = 9.2, 0.8 Hz), 7.88 (dd, 1H, J = 9.2, 1.6 Hz), 7.95 (br d, 1H, overlapped, J = 8.0 Hz), 8.35 (s, 1H), 8.89 (dd, 1H, J = 1.6, 0.8 Hz); IR (KBr) 3001, 1710, 1644, 1533 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{14}H_{12}BrN_4O$: 331.0189, found 331.0174.

*4.1.2. Diphenyl ([1,2,4]triazolo[1,5-a]pyridin-6-yl(phenylamino)-methyl)phosphonate (**14**)*

To a mixture of **7** (0.50 g, 3.40 mmol) and aniline (0.37 mL, 4.08 mmol) in *i*-PrOH (10 mL), diphenyl phosphite (1.04 mL, 5.44 mmol) was added. The mixture was stirred at room temperature for 3 h and then evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using EtOAc/Hex (1:1) as eluent to afford the titled compound as a white solid. Yield 86%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.96 (dd, 1H, J = 10.4, 7.6 Hz), 5.22 (dd, 1H, J = 24.8, 7.6 Hz), 6.63–6.66 (m, 2H), 6.78–6.82 (m, 1H), 6.99–7.02 (m, 2H), 7.08–7.11 (m, 2H), 7.13–7.19 (m, 4H),

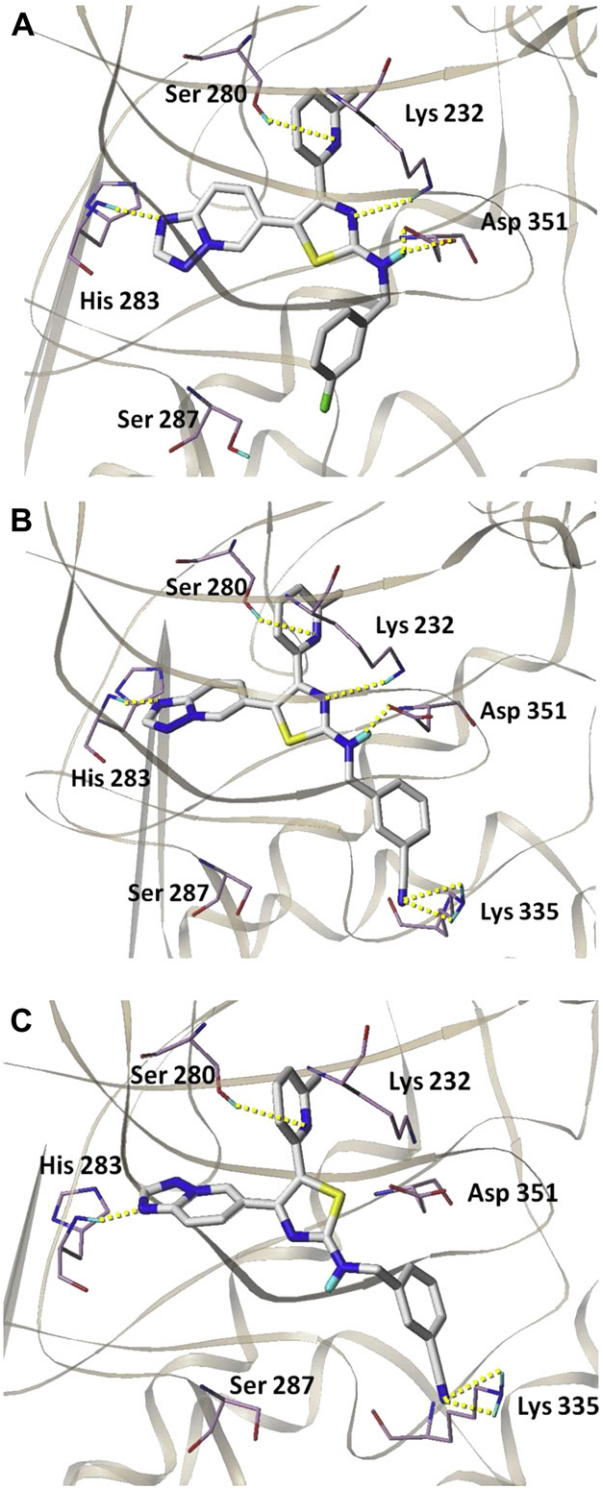


Fig. 3. The top-scored docking poses of **12b** (A), **12g** (B), and **18b** (C) generated by Surflex-Dock program. The key amino acid residues in the ALK5 binding site are represented in line form. Yellow dotted lines are hydrogen bonding interactions (<2.5 Å). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

7.22–7.30 (m, 4H), 7.73 (d, 1H, J = 9.2 Hz), 7.77 (td, 1H, J = 9.2, 1.6 Hz), 8.32 (s, 1H), 8.77–8.78 (m, 1H); IR (KBr) 3313, 3054, 1597, 1492 cm^{-1} HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{25}H_{22}N_4O_3P$: 457.1424, found 457.1432.

*4.1.3. 2-(6-Methylpyridin-2-yl)-1-([1,2,4]triazolo[1,5-a]pyridin-6-yl)ethanone (**15**)*

To a stirred solution of 6-methylpyridine-2-carboxaldehyde (3.18 g, 26.31 mmol) in a mixture of THF (120 mL) and *i*-PrOH (30 mL), **14** (12.00 g, 26.31 mmol) and Cs_2CO_3 (11.11 g, 34.21 mmol) were added. The mixture was stirred at room temperature for 16 h, and to it, 3 N HCl (40 mL) was added dropwise over a period of 10 min. After 1 h, the reaction mixture was diluted with MTBE (100 mL). The aqueous layer was separated, and the organic layer was extracted with 1 N HCl (3 × 250 mL). The combined aqueous layer was neutralized with 50% aqueous NaOH solution (pH 7–8). The precipitates were collected by filtration using a Buchner funnel and washed thoroughly with water (4 × 250 mL). The yellow solid was dried in air for 48 h and dried for an additional 24 h in a vacuum oven over P_2O_5 to give the titled compound as a yellow sold. Yield 83%; ^1H NMR (400 MHz, $CDCl_3$): (enol form, 82%); δ 2.56 (s, 3H), 6.03 (s, 1H), 6.82 (d, 1H, J = 7.6 Hz), 6.91 (d, 1H, J = 8.0 Hz), 7.55 (t, 1H, overlapped, J = 7.8 Hz), 7.74 (dd, 1H, J = 9.6, 0.8 Hz), 7.94 (dd, 1H, J = 9.6, 1.6 Hz), 8.37 (s, 1H), 9.13 (dd, 1H, J = 1.6, 0.8 Hz), 16.38 (br s, 1H); IR (KBr) 3112, 3086, 2919, 1645 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): (keto form, 18%); δ 2.54 (s, 3H), 4.45 (s, 2H), 7.05 (d, 1H, J = 7.6 Hz), 7.13 (d, 1H, J = 7.6 Hz), 7.55 (t, 1H, overlapped, J = 7.6 Hz), 7.77 (d, 1H, J = 9.6, 0.8 Hz), 8.16 (dd, 1H, J = 9.6, 1.6 Hz), 8.45 (s, 1H), 9.59 (dd, 1H, J = 1.6, 0.8 Hz); HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{14}H_{13}N4O$: 253.1084, found 253.1096.

*4.1.4. 2-Bromo-2-(6-methylpyridin-2-yl)-1-([1,2,4]triazolo[1,5-a]-pyridin-6-yl)ethanone (**16**)*

To a stirred solution of **15** (5.40 g, 22.36 mmol) in CH_2Cl_2 (250 mL) at 0 °C, *N*-bromosuccinimide (4.36 g, 24.54 mmol) was added. After 30 min, the reaction mixture was quenched with 5% aqueous $Na_2S_2O_3$ solution and extracted with $CHCl_3$ (4 × 150 mL). The organic layer was washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and evaporated to dryness under reduced pressure. The residue was purified by MPLC on silica gel using EtOAc/Hex (2:1) as eluent to afford the titled compound as yellow syrup. Yield 81%; ^1H NMR (400 MHz, $CDCl_3$): δ 2.52 (s, 3H), 6.33 (s, 1H), 7.11 (d, 1H, J = 7.6 Hz), 7.54 (d, 1H, J = 8.0 Hz), 7.67 (t, 1H, J = 7.8 Hz), 7.78 (dd, 1H, J = 9.2, 0.8 Hz), 8.12 (dd, 1H, J = 9.2, 2.0 Hz), 8.45 (s, 1H), 9.49 (dd, 1H, J = 2.0, 0.8 Hz); IR (KBr) 3075, 1708, 1631 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{14}H_{12}BrN_4O$: 331.0189, found 331.0184.

*4.1.5. General procedure for the preparation of the 4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amines **10** and **17***

A mixture of **9** or **16** (2.52 g, 7.61 mmol) and thiourea (1.22 g, 15.98 mmol) in DMF (20 mL) was heated at 120 °C for 2 h and then cooled to 0 °C. To the mixture, cold water (100 mL) was added, and the resulting precipitates were collected by filtration and washed thoroughly with water to afford the titled compound as a brown solid.

*4.1.5.1. 4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**10**).* Yield 82%; mp 279.0 °C; ^1H NMR (400 MHz, $CDCl_3/CD_3OD$): δ 2.27 (s, 3H), 6.98 (d, 1H, J = 7.6 Hz), 7.26 (d, 1H, J = 7.6 Hz), 7.36 (dd, 1H, J = 9.2, 1.6 Hz), 7.47 (t, 1H, overlapped, J = 7.6 Hz), 7.49 (dd, 1H, overlapped, J = 9.2, 0.8 Hz), 8.18 (s, 1H), 8.58 (dd, 1H, J = 1.6, 0.8 Hz); IR (KBr) 3295, 3147, 2924, 1544 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{15}H_{13}N_6S$: 309.0917, found 309.0913.

*4.1.5.2. 5-(6-Methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**17**).* Yield 67%; mp 275.3 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.54 (s, 3H), 5.42 (br s, 2H), 6.98 (d, 1H, overlapped,

J = 7.6 Hz), 7.01 (d, 1H, overlapped, J = 7.6 Hz), 7.40 (t, 1H, J = 7.6 Hz), 7.68 (dd, 1H, J = 9.6, 1.6 Hz), 7.73 (dd, 1H, J = 9.2, 1.2 Hz), 8.36 (s, 1H), 8.90 (dd, 1H, J = 1.6, 1.2 Hz); IR (KBr) 3316, 3134, 2923, 1516 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{15}H_{13}N_6S$: 309.0917, found 309.0913.

4.1.6. General procedure for the preparation of the 2-benzylamino-4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-a]pyridin-6-yl)-thiazoles ***12a–ab***, ***18a***, ***18b***, *and* ***18d***

A stirred solution of 2-aminothiazole **10** or **17** (0.08 g, 0.27 mmol), an appropriately substituted benzyl bromide (**11a–ab**) (0.03 mL, 0.29 mmol), and Cs_2CO_3 (0.11 g, 0.35 mmol) in DMF (5 mL) was heated at 120 °C for 1 h and then cooled to room temperature. The reaction mixture was diluted with water (25 mL) and extracted with $CHCl_3$ (3 × 60 mL). 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/$CHCl_3$ (1:40) as eluent to afford the titled compounds as a solid.

*4.1.6.1. N-Benzyl-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]-pyridin-6-yl)thiazol-2-amine (****12a****).* Yield 55%; mp 237.9 °C; Purity by HPLC: 96.65% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.52 (s, 2H), 6.20 (br s, 1H), 7.03 (d, 1H, J = 7.6), 7.31–7.41 (m, 5H), 7.46–7.49 (m, 2H), 7.53 (t, 1H, J = 7.6 Hz), 7.60 (dd, 1H, J = 9.2, 0.8 Hz), 8.31 (s, 1H), 8.72 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.45, 50.04, 115.36, 117.92, 120.49, 120.77, 122.77, 127.84 (2C), 128.18, 128.42, 129.06 (2C), 133.21, 137.07, 137.15, 146.53, 149.66, 152.28, 154.44, 158.26, 167.71; IR (KBr) 3221, 2924, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{19}N_6S$: 399.1386, found 399.1389.

*4.1.6.2. N-(3-Fluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12b****).* Yield 89%; mp 171.2 °C; Purity by HPLC: 98.56% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 4.48 (s, 2H), 6.70 (br s, 1H), 6.95–6.98 (m, 1H), 7.01 (dd, 1H, J = 7.6, 0.8 Hz), 7.05–7.08 (m, 1H), 7.12–7.15 (m, 1H), 7.28–7.33 (m, 1H), 7.45 (dd, 1H, J = 9.2, 1.6 Hz), 7.46 (d, 1H, J = 1.6 Hz), 7.49 (t, 1H, overlapped, J = 7.6 Hz), 7.59 (dd, 1H, J = 9.2, 0.8 Hz), 8.29 (s, 1H), 8.70 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.40, 49.30 (d, J = 1.5 Hz), 114.59 (d, J = 21.2 Hz), 114.98 (d, J = 21.2 Hz), 115.35, 120.43, 120.81, 122.79, 123.22 (d, J = 2.9 Hz), 128.39, 129.82, 130.54 (d, J = 8.1 Hz), 133.16, 137.07, 140.01 (d, J = 6.6 Hz), 146.67, 149.64, 152.31, 154.42, 158.23, 163.25 (d, J = 245.4 Hz), 167.67; IR (KBr) 3215, 3000, 1552 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}FN_6S$: 417.1292, found 417.1284.

*4.1.6.3. N-(3-Chlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12c****).* Yield 38%; mp 191.3 °C; Purity by HPLC: 99.23% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.53 (s, 2H), 5.94 (br s, 1H), 7.04 (d, 1H, J = 7.6 Hz), 7.28–7.30 (m, 3H), 7.40 (br s, 1H), 7.48–7.50 (m, 2H), 7.54 (t, 1H, J = 7.8 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.55, 49.12, 115.70, 118.18, 119.97, 121.03, 123.60, 125.90, 127.81, 128.21, 128.45, 130.34, 133.04, 134.80, 138.42, 139.06, 149.48, 150.50, 154.16, 155.13, 157.92, 168.17; IR (KBr) 3221, 2924, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}ClN_6S$: 433.0997, found 433.0994.

*4.1.6.4. N-(3-Methylbenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12d****).* Yield 26%; mp 191.3 °C; Purity by HPLC: 98.53% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 2.39 (s, 3H), 4.51 (d, 2H, J = 5.6 Hz), 5.78 (br s, 1H), 7.04 (dd, 1H, J = 7.6, 0.8 Hz), 7.14 (br d, 1H, J = 7.6 Hz), 7.19–7.22 (m, 2H), 7.27 (t, 1H, J = 7.4 Hz), 7.48–7.51 (m, 2H), 7.55 (t, 1H, J = 7.8 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.62, 24.42, 50.13, 115.46, 117.63, 120.28, 120.71, 122.90, 124.92, 128.49, 128.54, 128.96, 129.01, 133.13, 136.76, 137.15, 138.85, 145.79, 149.69, 151.79, 154.48, 158.34, 167.83; IR (KBr) 3225, 2981, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{21}N_6S$: 413.1543, found 413.1538.

*4.1.6.5. N-(3-Methoxybenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12e****).* Yield 61%; mp 166.2 °C; Purity by HPLC: 99.38% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 3.81 (s, 3H), 4.51 (d, 2H, J = 4.4 Hz), 5.84 (br s, 1H), 6.86 (ddd, 1H, J = 8.0, 2.4, 0.8 Hz), 6.95 (t, 1H, J = 2.0 Hz), 6.98 (ddd, 1H, J = 7.6, 1.6, 1.2 Hz), 7.02–7.05 (m, 1H), 7.29 (t, 1H, J = 8.0 Hz), 7.48–7.50 (m, 2H), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.31 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.40, 49.92, 55.45, 113.36, 113.44, 115.31, 117.87, 119.98, 120.52, 120.77, 122.72, 128.36, 130.04, 133.20, 137.04, 138.81, 146.56, 149.61, 152.33, 154.39, 158.19, 160.17, 167.85; IR (KBr) 3220, 2928, 1552 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{21}N_6OS$: 429.1492, found 429.1504.

*4.1.6.6. 4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)-N-(3-(trifluoromethoxy)benzyl)thiazol-2-amine (****12f****).* Yield 64%; mp 145.7 °C; Purity by HPLC: 97.18% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.59 (d, 2H, J = 5.2 Hz), 5.67 (t, 1H, J = 5.2 Hz), 7.04 (ddd, 1H, J = 7.6, 1.2, 0.4 Hz), 7.18 (br d, 1H, J = 7.6 Hz), 7.28 (br s, 1H), 7.36 (br d, 1H, J = 8.0 Hz), 7.41 (t, 1H, J = 7.8 Hz), 7.49–7.51 (m, 2H), 7.55 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.74 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.57, 46.79, 114.23, 117.24, 119.51, 119.92, 120.05, 120.13 (q, J = 254.9 Hz), 120.26, 122.01, 126.65, 128.57, 130.36, 133.69, 137.03, 142.17, 145.08, 148.49, 148.90, 152.35, 154.22, 156.45, 165.86; IR (KBr) 3220, 3012, 1553, 1258 cm^{-1}; HRMS-ESI m/z $[M - H]^+$ calcd. for $C_{23}H_{16}F_3N_6OS$: 481.1064, found 481.1069.

*4.1.6.7. 3-(((4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzonitrile (****12g****).* Yield 72%; mp 201.6 °C; Purity by HPLC: 99.77% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.39 (s, 3H), 4.61 (s, 2H), 6.57 (br s, 1H), 7.06 (d, 1H, J = 7.6 Hz), 7.46 (d, 1H, overlapped, J = 8.0 Hz), 7.47–7.51 (m, 2H), 7.55 (t, 1H, J = 7.8 Hz), 7.61 (dt, 1H, overlapped, J = 7.6, 1.4 Hz), 7.63 (dd, 1H, J = 9.2, 0.8 Hz), 7.66 (dd, 1H, J = 8.0, 0.8 Hz), 7.71 (br s, 1H), 8.32 (s, 1H), 8.73 (dd, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.35, 48.77, 113.04, 115.40, 118.37, 118.73, 120.28, 120.86, 122.87, 128.41, 129.76, 131.12, 131.62, 132.03, 133.10, 137.17, 139.36, 146.70, 149.62, 152.25, 154.41, 158.20, 167.39; IR (KBr) 3222, 3012, 2230, 1550 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{18}N_7S$: 424.1339, found 424.1341.

*4.1.6.8. N-(4-Fluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12h****).* Yield 95%; mp 205.0 °C; Purity by HPLC: 98.88% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 4.52 (d, 2H, J = 5.0 Hz), 5.78 (br s, 1H), 7.03–7.09 (m, 3H), 7.36–7.40 (m, 2H), 7.47–7.50 (m, 2H), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 24.07, 48.85, 115.05, 115.68 (2C, d, J = 21.8 Hz), 117.38, 120.78, 120.84, 122.84, 128.09, 129.42 (2C, d, J = 8.4 Hz), 133.07 (d, J = 2.8 Hz), 133.39, 137.20, 146.62, 149.24, 152.19, 153.87, 158.10, 162.44 (d, J = 244.2 Hz), 167.96; IR (KBr) 3217, 3001, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}FN_6S$: 417.1292, found 417.1287.

*4.1.6.9. N-(4-Chlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (****12i****).* Yield 57%; mp 248.3 °C; Purity by HPLC: 99.43% (30% acetonitrile); ^{1}H NMR

(400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.50 (s, 2H), 6.19 (br s, 1H), 7.03 (br d, 1H, J = 7.6 Hz), 7.33 (br s, 4H), 7.46 (d, 1H, J = 8.0 Hz), 7.47 (dd, 1H, J = 9.2, 2.0 Hz), 7.52 (t, 1H, J = 7.8 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.31 (s, 1H), 8.71 (dd, 1H, J = 2.0, 0.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.45, 46.72, 114.03, 117.01, 119.92, 120.13, 121.78, 128.10 (2C), 128.22, 129.19 (2C), 131.70, 133.32, 136.83, 137.82, 144.95, 148.75, 152.21, 153.83, 156.23, 165.84; IR (KBr) 3229, 2928, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}ClN_6S$: 433.0997, found 433.0993.

*4.1.6.10. N-(4-Methylbenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12j**).* Yield 46%; mp 238.3 °C; Purity by HPLC: 98.93% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 2.38 (s, 3H), 4.50 (d, 2H, J = 5.2 Hz), 5.78 (br s, 1H), 7.04 (dd, 1H, J = 7.6, 0.8 Hz), 7.19 (d, 2H, J = 8.0 Hz), 7.30 (d, 2H, J = 8.0 Hz), 7.48–7.51 (m, 2H), 7.55 (t, 1H, J = 7.8 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 20.99, 23.86, 49.28, 114.86, 116.96, 120.86, 120.95, 122.81, 127.60 (2C), 127.91, 129.38 (2C), 133.43, 134.07, 137.22, 137.53, 146.53, 149.03, 152.15, 153.62, 157.99, 168.30; IR (KBr) 3221, 2923, 1551 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{21}N_6S$: 413.1543, found 413.1543.

*4.1.6.11. N-(4-Methoxybenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12k**).* Yield 41%; mp 219.0 °C; Purity by HPLC: 98.30% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 3.82 (s, 3H), 4.47 (d, 2H, J = 4.8 Hz), 5.69 (br s, 1H), 6.91 (m, 2H), 7.04 (d, 1H, J = 7.6 Hz), 7.33 (m, 2H), 7.48–7.50 (m, 2H), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.93, 49.03, 55.29, 114.13 (2C), 114.90, 117.03, 120.84, 120.92, 122.81, 127.95, 129.05 (2C), 129.18, 133.43, 137.20, 146.52, 149.08, 152.17, 153.68, 158.02, 159.23, 168.18; IR (KBr) 3220, 2927, 1551, cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{21}N_6OS$: 429.1492, found 429.1510.

*4.1.6.12. 4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)-N-(4-(trifluoromethoxy)benzyl)thiazol-2-amine (**12l**).* Yield 47%; mp 177.0 °C; Purity by HPLC: 99.29% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.45 (s, 3H), 4.60 (d, 2H, J = 3.6 Hz), 7.11 (d, 1H, J = 7.6 Hz), 7.24 (m, 2H), 7.43 (br d, 1H, J = 7.6 Hz), 7.46 (m, 2H), 7.50 (dd, 1H, J = 9.2, 1.6 Hz), 7.57 (t, 1H, J = 7.6 Hz), 7.69 (dd, 1H, J = 9.2, 0.8 Hz), 8.37 (s, 1H), 8.77 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.37, 48.98, 115.33, 117.97, 120.44, 120.59 (q, J = 255.6 Hz), 120.82, 121.41 (2C), 122.74, 128.30, 129.02 (2C), 133.08, 136.21, 137.01, 146.87, 148.88, 149.58, 152.45, 154.38, 158.23, 167.76; IR (KBr) 3220, 3017, 1552, 1260 cm^{-1}; HRMS-ESI m/z $[M - H]^+$ calcd. for $C_{23}H_{16}F_3N_6OS$: 481.1064, found 481.1067.

*4.1.6.13. 4-(((4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzonitrile (**12m**).* Yield 78%; mp 225.0 °C; Purity by HPLC: 99.05% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 4.63 (s, 2H), 6.19 (br s, 1H), 7.03 (d, 1H, J = 7.6 Hz), 7.45 (d, 1H, overlapped, J = 8.0 Hz), 7.47 (dd, 1H, overlapped, J = 9.2, 1.6 Hz), 7.50–7.54 (m, 3H), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 7.66 (m, 2H), 8.31 (s, 1H), 8.72 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.83, 48.51, 111.18, 114.96, 117.60, 118.71, 120.72, 120.89, 122.91, 128.05, 128.16 (2C), 132.52 (2C), 133.39, 137.35, 143.66, 146.44, 149.12, 152.00, 153.71, 157.94, 167.61; IR (KBr) 3221, 2926, 2229, 1550 cm^{-1}; HRMS-ESI m/z $[M - H]^+$ calcd. for $C_{23}H_{16}N_7S$: 422.1193, found 422.1192.

*4.1.6.14. N-(2-Fluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12n**).* Yield 52%; mp 238.6 °C; Purity by HPLC: 96.25% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 4.61 (s, 2H), 6.49 (br s, 1H), 7.04–7.12 (m, 2H), 7.15 (td, 1H, J = 7.6 Hz, 1.2 Hz), 7.29–7.34 (m, 1H), 7.45–7.52 (m, 3H), 7.55 (t, 1H, J = 7.6 Hz), 7.63 (d, 1H, J = 9.2 Hz), 8.33 (s, 1H), 8.75 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.13, 44.62, 116.07 (d, J = 20.6 Hz), 116.99, 119.88, 121.28 (d, J = 25.9 Hz), 124.06, 124.84, 125.20 (d, J = 3.8 Hz), 129.73, 130.05 (d, J = 3.1 Hz), 130.94, 131.02, 132.03, 138.26, 145.71, 150.28, 155.22, 155.68, 159.46, 161.02 (d, J = 246.5 Hz), 168.55; IR (KBr) 3226, 2927, 1554 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}FN_6S$: 417.1292, found 417.1297.

*4.1.6.15. N-(2-Chlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12o**).* Yield 67%; mp 174.4 °C; Purity by HPLC: 98.18% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 4.64 (d, 2H, J = 4.4 Hz), 6.01 (br s, 1H), 7.03 (dd, 1H, J = 7.6, 0.8 Hz), 7.25–7.30 (m, 2H), 7.39–7.42 (m, 1H), 7.47–7.52 (m, 3H), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.31 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.89, 47.21, 114.92, 117.32, 120.86, 120.90, 122.86, 127.07, 128.03, 129.16, 129.41, 127.73, 133.46, 133.62, 134.75, 137.29, 146.61, 149.11, 152.13, 153.70, 157.98, 167.96; IR (KBr) 3221, 2968, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{18}ClN_6S$: 433.0997, found 433.1009.

*4.1.6.16. N-(2-Methylbenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12p**).* Yield 56%, mp 159.8 °C; Purity by HPLC: 99.41% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 2.41 (s, 3H), 4.52 (d, 2H, J = 5.2 Hz), 5.58 (br s, 1H), 7.04 (dd, 1H, J = 7.6, 0.8 Hz), 7.20–7.26 (m, 3H), 7.38 (dd, 1H, J = 7.6, 1.6 Hz), 7.49–7.51 (m, 2H), 7.55 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.74 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.24, 24.40, 48.07, 115.23, 117.75, 120.65, 120.78, 122.63, 126.45, 128.25, 128.30, 128.43, 130.83, 133.23, 134.99, 136.60, 136.98, 146.89, 149.57, 152.55, 154.34, 158.12, 167.55; IR (KBr) 3228, 2982, 1553 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{23}H_{21}N_6S$: 413.1543, found 413.1541.

*4.1.6.17. 4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)-N-(2-(trifluoromethoxy)benzyl)thiazol-2-amine (**12q**).* Yield 29%; mp 185.4 °C; Purity by HPLC: 94.31% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.65 (d, 2H, J = 6.4 Hz), 5.68 (br s, 1H), 7.04 (dd, 1H, J = 7.6, 1.0 Hz), 7.28–7.39 (m, 3H), 7.49 (dd, 1H, J = 9.2, 1.6 Hz), 7.51 (dd, 1H, J = 7.8, 0.8 Hz), 7.53–7.58 (m, 2H), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.74 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 24.15, 44.12, 115.10, 117.69, 120.70, 120.71 (q, J = 256.3 Hz), 120.76, 120.82, 122.79, 127.22, 128.21, 129.29, 129.57, 129.99, 133.38, 137.15, 146.76, 147.46, 149.35, 152.28, 154.02, 158.08, 167.82; IR (KBr) 3221, 3009, 1551, 1255 cm^{-1}; HRMS-ESI m/z $[M - H]^+$ calcd. for $C_{23}H_{16}F_3N_6OS$: 481.1064, found 481.1068.

*4.1.6.18. N-(2,3-Difluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12r**).* Yield 54%; mp 162.7 °C; Purity by HPLC: 98.35% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 4.66 (d, 2H, J = 4.8 Hz), 5.72 (br s, 1H), 7.04 (dd, 1H, J = 7.6, 1.2 Hz), 7.05–7.15 (m, 2H), 7.21–7.25 (m, 1H), 7.50 (dd, 1H, J = 9.2, 2.0 Hz), 7.52 (br d, 1H, overlapped, J = 7.6 Hz), 7.55 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.75 (dd, 1H, J = 2.0, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.84, 42.65, 114.95, 116.73 (d, J = 16.8 Hz), 117.56, 120.81, 120.90, 122.90, 124.27 (dd, J = 6.8, 4.6 Hz), 124.42 (t, J = 3.1 Hz), 127.23 (d, J = 11.4 Hz), 128.09, 133.48, 137.40, 146.43, 148.97 (dd, J = 246.4, 12.2 Hz), 149.15, 150.55 (dd, J = 247.2, 12.2 Hz), 152.05, 153.73, 157.92, 167.62; IR (KBr) 3224, 3012, 1551 cm^{-1}; HRMS-ESI m/z $[M + H]^+$ calcd. for $C_{22}H_{17}F_2N_6S$: 435.1198, found 435.1191.

*4.1.6.19. N-(3,4-Difluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12s**).* Yield 60%; mp 197.9 °C; Purity by HPLC: 95.64% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.50 (s, 2H), 6.04 (br s, 1H), 7.04 (d, 1H, J = 7.6 Hz), 7.11–7.16 (m, 2H), 7.19–7.25 (m, 1H), 7.47 (d, 1H, overlapped, J = 7.6 Hz), 7.48 (dd, 1H, J = 9.2, 1.6 Hz), 7.54 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 23.82, 48.01, 114.83, 116.44 (d, J = 17.6 Hz), 117.34 (d, J = 17.6 Hz), 120.83, 120.86, 122.80, 123.51 (t, J = 3.1 Hz), 123.58, 127.95, 133.41, 134.84 (t, J = 4.6 Hz), 137.18, 146.59, 149.03, 149.72 (dd, J = 246.5, 12.2 Hz), 150.35 (dd, J = 247.2, 12.2 Hz), 152.16, 153.61, 157.95, 167.75; IR (KBr) 3201, 2993, 1551 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{22}H_{17}F_2N_6S$: 435.1198, found 435.1193.

*4.1.6.20. N-(3,5-Difluorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12t**).* Yield 87%; mp 202.5 °C; Purity by HPLC: 97.80% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.56 (br s, 2H), 5.87 (br s, 1H), 6.75 (tt, 1H, J = 9.0, 2.4 Hz), 6.93–6.96 (m, 2H), 7.04 (dd, 1H, J = 7.6, 0.8 Hz), 7.49 (dd, 1H, J = 7.6, 0.8 Hz), 7.50 (dd, 1H, J = 9.2, 2.0 Hz), 7.55 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 1.2 Hz), 8.32 (s, 1H), 8.74 (dd, 1H, J = 2.0, 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 24.04, 48.44, 103.07 (t, J = 25.2 Hz), 110.20 (2C, dd, J = 18.3, 6.8 Hz), 115.03, 117.66, 120.71, 120.87, 122.83, 128.14, 133.39, 137.19, 141.97 (t, J = 8.8 Hz), 146.72, 149.23, 152.21, 153.87, 158.07, 163.28 (2C, dd, J = 247.2, 12.2 Hz), 167.74; IR (KBr) 3197, 3090, 1550 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{22}H_{17}F_2N_6S$: 435.1198, found 435.1193.

*4.1.6.21. N-(2,3-Dichlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12u**).* Yield 46%; mp 206.3 °C; Purity by HPLC: 97.60% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 4.63 (s, 2H), 6.32 (br s, 1H), 7.02 (dd, 1H, J = 7.6, 0.8 Hz), 7.20 (t, 1H, J = 8.0 Hz), 7.42 (d, 2H, J = 8.0 Hz), 7.45 (br d, 1H, J = 8.0 Hz), 7.46 (dd, 1H, J = 9.2, 2.0 Hz), 7.51 (t, 1H, J = 7.6 Hz), 7.60 (dd, 1H, J = 9.2, 1.2 Hz), 8.30 (s, 1H), 8.72 (dd, 1H, J = 2.0, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 24.13, 48.01, 115.28, 117.96, 120.45, 120.85, 122.94, 127.39, 127.61, 128.31, 129.95, 131.79, 133.24, 133.59, 137.21, 137.34, 146.37, 149.43, 151.96, 154.15, 158.09, 167.68; IR (KBr) 3221, 2924, 1549 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{22}H_{17}Cl_2N_6S$: 467.0607, found 467.0604.

*4.1.6.22. N-(3,4-Dichlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12v**).* Yield 53%; mp 205.5 °C; Purity by HPLC: 97.39% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.52 (s, 2H), 6.00 (br s, 1H), 7.04 (d, 1H, J = 7.6 Hz), 7.24 (dd, 1H, J = 8.4, 2.0 Hz), 7.44 (d, 1H, J = 8.4 Hz), 7.47 (br d, 1H, J = 7.6 Hz), 7.48 (dd, 1H, J = 9.2, 1.6 Hz), 7.50 (d, 1H, overlapped, J = 2.0 Hz), 7.54 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 2.0, 0.8 Hz), ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 23.75, 29.68, 115.04, 117.68, 120.69, 120.96, 123.02, 126.98, 128.09, 129.52, 130.66, 131.63, 132.74, 133.39, 137.56, 138.07, 146.17, 149.16, 151.82, 153.74, 157.92, 167.74; IR (KBr) 3221, 2924, 1549 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{22}H_{17}Cl_2N_6S$: 467.0607, found 467.0599.

*4.1.6.23. N-(3,5-Dichlorobenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12w**).* Yield 39%; mp 244.4 °C; Purity by HPLC: 96.99% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.37 (s, 3H), 4.52 (s, 2H), 6.09 (br s, 1H), 7.04 (d, 1H, J = 7.6 Hz), 7.28–7.31 (m, 3H), 7.48 (d, 1H, J = 7.6 Hz), 7.49 (dd, 1H, J = 9.2, 2.0 Hz), 7.54 (t, 1H, J = 7.6 Hz), 7.62 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.74 (dd, 1H, J = 2.0, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3/CD_3OD$) δ 24.00, 48.28, 115.13, 117.87, 120.66, 120.93, 122.96, 126.04 (2C), 127.93, 128.21, 133.41, 135.35 (2C), 137.40, 141.31, 146.49, 149.28, 152.02, 153.91, 158.05, 167.62; IR (KBr) 3216, 2924, 1551 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{22}H_{17}Cl_2N_6S$: 467.0607, found 467.0597.

*4.1.6.24. N-(2,3-Dimethylbenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12x**).* Yield 38%, mp 210.6 °C; Purity by HPLC: 99.17% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27 (s, 3H), 2.31 (s, 3H), 2.36 (s, 3H), 4.50 (s, 2H), 5.80 (br s, 1H), 7.02 (dd, 1H, J = 7.6, 2.4 Hz), 7.07–7.14 (m, 2H), 7.22 (br d, 1H, J = 7.6 Hz), 7.47–7.54 (m, 3H), 7.60 (dd, 1H, J = 9.2, 1.6 Hz), 8.28 (s, 1H), 8.74 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.23, 20.67, 24.44, 48.90, 115.31, 117.75, 120.58, 120.77, 122.73, 125.96, 126.86, 128.40, 130.19, 133.25, 134.73, 135.38, 137.06, 137.77, 146.62, 149.65, 152.34, 154.42, 158.21, 167.33; IR (KBr) 3228, 2972, 1551 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{24}H_{23}N_6S$: 427.1699, found 427.1703.

*4.1.6.25. N-(3,5-Dimethylbenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]-triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12y**).* Yield 31%; mp 225.2 °C; Purity by HPLC: 97.68% (35% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.31 (s, 6H), 2.38 (s, 3H), 4.44 (d, 2H, J = 4.0 Hz), 5.93 (br s, 1H), 6.95 (s, 1H), 7.00 (s, 2H), 7.03 (dd, 1H, J = 7.6, 0.8 Hz), 7.47–7.49 (m, 2H), 7.53 (t, 1H, J = 7.6 Hz), 7.60 (dd, 1H, J = 9.2, 0.8 Hz), 8.30 (s, 1H), 8.72 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.50 (2C), 24.45, 50.18, 115.49, 117.63, 120.29, 120.71, 122.93, 125.65 (2C), 128.50, 129.92, 133.15, 136.64, 137.15, 138.77 (2C), 145.78, 149.72, 151.78, 154.51, 158.39, 167.79; IR (KBr) 3228, 3008, 1553 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{24}H_{23}N_6S$: 427.1699, found 427.1699.

*4.1.6.26. N-(2,3-Dimethoxybenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12z**).* Yield 51%; mp 156.1 °C; Purity by HPLC: 96.84% (29% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 3.87 (s, 3H), 3.91 (s, 3H), 4.53 (d, 2H, J = 5.2 Hz), 5.98 (br s, 1H), 6.89 (dd, 1H, J = 8.0, 2.0 Hz), 6.98–7.06 (m, 3H), 7.46–7.50 (m, 2H), 7.52 (t, 1H, J = 7.8 Hz), 7.59 (dd, 1H, J = 9.2, 0.8 Hz), 8.30 (s, 1H), 8.72 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.38, 45.36, 55.97, 60.96, 112.52, 115.17, 117.75, 120.70, 120.76, 121.13, 122.57, 124.29, 128.31, 131.02, 133.28, 136.97, 146.79, 147.39, 149.57, 152.56, 152.88, 154.33, 158.05, 167.74; IR (KBr) 3228, 2930, 1553, 1481 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{24}H_{23}N_6O_2S$: 459.1598, found 459.1616.

*4.1.6.27. N-(3,4-Dimethoxybenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12aa**).* Yield 57%; mp 157.5 °C; Purity by HPLC: 98.10% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.36 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 4.41 (br s, 2H), 6.30 (br s, 1H), 6.82 (d, 1H, J = 8.0 Hz), 6.88 (d, 1H, J = 2.0 Hz), 6.91 (dd, 1H, J = 8.0, 2.0 Hz), 7.01 (dd, 1H, J = 7.6, 0.8 Hz), 7.44–7.47 (m, 2H), 7.50 (t, 1H, J = 7.6 Hz), 7.59 (dd, 1H, J = 9.2, 0.8 Hz), 8.29 (s, 1H), 8.70 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.39, 49.77, 56.07 (2C), 110.89, 111.31, 115.27, 117.84, 120.19, 120.58, 120.73, 122.64, 128.26, 129.63, 133.16, 136.96, 146.74, 148.90, 149.42, 149.55, 152.47, 154.34, 158.18, 167.78; IR (KBr) 3225, 2937, 1552 cm^{-1}; HRMS-ESI m/z [M + H]$^+$ calcd. for $C_{24}H_{23}N_6O_2S$: 459.1598, found 459.1619.

*4.1.6.28. N-(3,5-Dimethoxybenzyl)-4-(6-methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**12ab**).* Yield 24%; mp 149.0 °C; Purity by HPLC: 98.05% (30% acetonitrile); ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.38 (s, 3H), 3.79 (s, 6H), 4.47 (d, 2H, J = 4.4 Hz), 5.90 (br s, 1H), 6.40 (t, 1H, J = 2.4 Hz), 6.55 (d, 2H, J = 2.4 Hz), 7.04 (d, 1H, J = 7.6 Hz), 7.48 (br d, 1H, J = 7.6 Hz), 7.49 (dd, 1H, J = 9.2, 1.6 Hz), 7.53 (t, 1H, J = 7.6 Hz), 7.61 (dd, 1H, J = 9.2, 0.8 Hz), 8.32 (s, 1H), 8.73 (dd, 1H, J = 1.6, 0.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.44, 50.16,

55.62 (2C), 99.92, 105.71 (2C), 115.41, 117.98, 120.42, 120.74, 122.81, 128.46, 133.20, 137.10, 139.44, 146.34, 149.68, 152.13, 154.46, 158.29, 161.41 (2C), 167.72; IR (KBr) 3207, 2928, 1550 cm^{-1}; HRMS-ESI *m/z* $[M - H]^+$ calcd. for $C_{24}H_{21}N_6O_2S$: 457.1452, found 457.1457.

*4.1.6.29. N-Benzyl-5-(6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-a]-pyridin-6-yl)thiazol-2-amine (**18a**).* Yield 55%; mp 177.4 °C; Purity by HPLC: 98.35% (30% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$): δ 2.51 (s, 3H), 4.51 (d, 2H, *J* = 5.2 Hz), 6.19 (t, 1H, *J* = 5.2 Hz), 6.93 (d, 1H, *J* = 8.0 Hz), 6.94 (d, 1H, *J* = 7.6 Hz), 7.28–7.37 (m, 6H), 7.63–7.68 (m, 2H), 8.33 (s, 1H), 8.88 (t, 1H, *J* = 1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.61, 50.05, 116.30, 118.90, 121.74, 122.70, 124.38, 127.80 (2C), 128.23, 129.02, 129.10 (2C), 131.53, 136.87, 137.00, 141.69, 150.20, 150.33, 154.73, 159.02, 168.99; IR (KBr) 3221, 2971, 1558, 1452 cm^{-1}; HRMS-ESI *m/z* $[M + H]^+$ calcd. for $C_{22}H_{19}N_6S$: 399.1386, found 399.1398.

*4.1.6.30. 3-(((5-(6-Methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzonitrile (**18b**).* Yield 28%; mp 187.6 °C; Purity by HPLC: 97.24% (30% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$): δ 2.52 (s, 3H), 4.62 (d, 2H, *J* = 4.8 Hz), 5.99 (br s, 1H), 6.96 (d, 1H, *J* = 7.6 Hz), 6.98 (d, 1H, *J* = 7.6 Hz), 7.38 (t, 1H, *J* = 7.8 Hz), 7.48 (t, 1H, *J* = 7.6 Hz), 7.59–7.65 (m, 2H), 7.67–7.70 (m, 3H), 8.35 (s, 1H), 8.89 (dd, 1H, *J* = 1.6, 1.2 Hz); IR (KBr) 3226, 2925, 2231, 1558 cm^{-1}; HRMS-ESI *m/z* $[M + H]^+$ calcd. for $C_{23}H_{18}N_7S$: 424.1339, found 424.1344.

*4.1.6.31. N-(2-Fluorobenzyl)-5-(6-methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-amine (**18d**).* Yield 49%; mp 229.9 °C; Purity by HPLC: 98.08% (30% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$): δ 2.52 (s, 3H), 4.62 (d, 2H, *J* = 5.6 Hz), 5.84 (br t, 1H, *J* = 5.6 Hz), 6.95 (d, 1H, *J* = 7.6 Hz), 6.96 (d, 1H, *J* = 7.6 Hz), 7.06–7.11 (m, 1H), 7.14 (td, 1H, *J* = 7.6, 1.0 Hz), 7.27–7.31 (m, 1H), 7.37 (t, 1H, *J* = 7.6 Hz), 7.45 (td, 1H, *J* = 7.4, 1.8 Hz), 7.65–7.70 (m, 2H), 8.35 (s, 1H), 8.90 (pseudo t, 1H, *J* = 1.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.59, 43.74 (d, *J* = 3.6 Hz), 115.70 (d, *J* = 20.5 Hz), 116.05, 118.96, 121.56, 123.19, 124.35, 124.48 (d, *J* = 14.7 Hz), 124.48 (d, *J* = 3.0 Hz), 128.91, 129.75, 129.81 (d, *J* = 3.0 Hz), 131.61, 136.78, 142.48, 150.07, 150.51, 154.58, 158.93, 161.10 (d, *J* = 244.6 Hz), 168.82; IR (KBr) 3223, 2925, 1558 cm^{-1}; HRMS-ESI *m/z* $[M + H]^+$ calcd. for $C_{22}H_{18}FN_6S$: 417.1292, found 417.1302.

*4.1.7. General procedure for the preparation of the 3(4)-(((4(5)-(6-methylpyridin-2-yl)-5(4)-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzamide **13a**, **13b**, and **18c***

To a stirred solution of **12g**, **12m**, or **18b** (0.11 g, 0.26 mmol) in absolute EtOH (3 mL), 1 N NaOH (0.78 mL, 0.78 mmol), and 35% H_2O_2 (0.09 mL, 0.91 mmol) were added. The mixture was stirred at room temperature for 3 h, cooled to 0 °C, and then neutralized with 1 N HCl to pH 8. The reaction mixture was extracted with 10% MeOH/$CHCl_3$ (2 × 50 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/$CHCl_3$ (1:25) as eluent to yield the titled compound as a white solid.

*4.1.7.1. 3-(((4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzamide (**13a**).* Yield 60%; mp 242.5 °C; Purity by HPLC: 96.66% (20% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$): δ 2.35 (s, 3H), 4.53 (s, 2H), 6.36 (br s, 1H), 6.57 (br s, 1H), 6.98 (br s, 1H), 7.01 (d, 1H, *J* = 7.6 Hz), 7.38 (t, 1H, *J* = 7.8 Hz), 7.41–7.44 (m, 2H), 7.48–7.52 (m, 2H), 7.58 (dd, 1H, *J* = 9.2, 0.8 Hz), 7.70 (br d, 1H, *J* = 8.0 Hz), 7.91 (br s, 1H), 8.29 (s, 1H), 8.67 (dd, 1H, *J* = 2.0, 0.8 Hz); ^{13}C NMR (100 MHz, DMSO-d_6/$CDCl_3$) δ 23.84, 29.74, 115.20, 117.57, 120.64, 120.99, 123.06, 126.65, 127.01, 128.10, 129.02, 131.35, 133.29, 133.86, 137.58, 138.10, 146.09, 149.22, 151.81, 153.86, 158.02, 168.03, 170.30; IR (KBr) 3202, 2923, 1667, 1551 cm^{-1}; HRMS-ESI *m/z* $[M + H]^+$ calcd. for $C_{23}H_{20}N_7OS$: 442.1445, found 442.1453.

*4.1.7.2. 4-(((4-(6-Methylpyridin-2-yl)-5-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzamide (**13b**).* Yield 71%; mp 214.3 °C; Purity by HPLC: 98.50% (20% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$/CD_3OD): δ 2.16 (s, 3H), 4.41 (s, 2H), 6.89 (d, 1H, *J* = 8.0 Hz), 7.23–7.30 (m, 4H), 7.38 (dd, 1H, *J* = 9.2, 1.2 Hz), 7.40 (t, 1H, *J* = 7.6 Hz), 7.64 (m, 2H), 8.09 (s, 1H), 8.47 (dd, 1H, *J* = 1.6, 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 23.88, 48.81, 114.96, 117.31, 120.80, 120.91, 122.86, 127.61 (2C), 127.94 (2C), 127.98, 132.60, 133.37, 137.28, 141.85, 146.54, 149.10, 152.13, 153.71, 157.99, 167.97, 170.18; IR (KBr) 3203, 2922, 1666, 1551 cm^{-1}; HRMS-ESI *m/z* $[M - H]^+$ calcd. for $C_{23}H_{18}N_7OS$: 440.1299, found 440.1298.

*4.1.7.3. 3-(((5-(6-Methylpyridin-2-yl)-4-([1,2,4]triazolo[1,5-a]pyridin-6-yl)thiazol-2-yl)amino)methyl)benzamide (**18c**).* This compound was prepared according to the same procedure for **13a** using **18b** as the starting material. Yield 42%; mp 258.4 °C; Purity by HPLC: 96.94% (20% acetonitrile); ^1H NMR (400 MHz, $CDCl_3$): δ 2.49 (s, 3H), 4.53 (br s, 2H), 6.32 (br s, 1H), 6.53 (br s, 1H), 6.90 (d, 1H, *J* = 8.0 Hz), 6.93 (d, 1H, *J* = 7.6 Hz), 6.94 (br s, 1H, overlapped), 7.33 (t, 1H, overlapped, *J* = 7.8 Hz), 7.37 (d, 1H, overlapped, *J* = 8.0 Hz), 7.47 (d, 1H, *J* = 7.6 Hz), 7.62 (m, 2H), 7.67 (d, 1H, *J* = 7.6 Hz), 7.87 (br s, 1H), 8.31 (s, 1H), 8.85 (t, 1H, *J* = 1.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$/CD_3OD) δ 24.16, 29.74, 115.72, 119.42, 121.82, 123.31, 123.49, 126.62, 126.94, 128.78, 129.02, 131.30, 132.01, 133.88, 137.04, 138.15, 142.06, 149.72, 150.26, 154.08, 158.86, 169.11, 170.34; IR (KBr) 3352, 3213, 2924, 1668, 1557 cm^{-1}; HRMS-ESI *m/z* $[M + H]^+$ calcd. for $C_{23}H_{20}N_7OS$: 442.1445, found 442.1453.

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-Lux. 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. Immunoblot assay

Recombinant human TGF-β1 was purchased from R&D Systems (Minneapolis, MN, USA). Mouse mammary epithelial cells (NMuMG) were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), insulin (10 μg/mL), penicillin (100 U/mL), and streptomycin (100 μg/mL, Invitrogen life technology, Grand Island, NY, USA). Total cell lysates were prepared by RIPA buffer (50 mM Tris, 0.5% sodium deoxycholate, 150 mM NaCl, and 81% NP-40) containing protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany) and sodium orthovanadate (Sigma–Aldrich, St. Louis, Mo, USA) for 20 min on ice. The concentration of proteins was determined by using Bradford assay (Bio-Rad, Hercules, CA, USA). Equal amounts of proteins were electrophoresed in 10% SDS-PAGE gel and transferred to a PVDF membrane (Millipore, Billerica, MA, USA). The membranes were blocked in 5% nonfat dry milk solution and then incubated for 1 h at room temperature with one of following antibodies: anti-Smad2, anti-phospho-Smad2, and anti-GAPDH (Cell Signaling Technology, Beverly, MA, USA) in Tris-buffered saline. Following three washes in Tris-buffered saline,

the membranes were incubated with either horseradish peroxidase-conjugated goat anti-rabbit or goat anti-mouse antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in Tris-buffered saline for 1 h. Proteins were detected using an ECL kit (Amersham Biosciences/GE Healthcare, Piscataway, NJ, USA).

4.4. 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.

4.4.1. 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 performed 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.

4.4.2. FlexiDock and Surflex-Dock docking

Compounds (**12b**, **12g**, and **18b**) were docked into active site of ALK5 (PDB id: 3GXL) by the FlexiDock module. The binding site was defined around important residues (Lys 232, Ser 280, and Ser 287) in the radius of 3 Å. Single bonds of these residues (Lys 232, Ser 280, and Ser 287) and each compound were allowed to be flexible in docking process, while the backbone and remaining bonds were held rigid. The ligand was located within the distance that these residues of ALK5 were capable of forming H-bonds. Through this process, the results gained 20 (maximum number of generations to allow: 30,000) solutions, and these structures were energy-minimized to remove bad energy and steric position. Most stable complex between ALK5 and each compound was selected as a receptor structure for further docking experiments using Surflex-Dock program. The Surflex-Dock program uses an idealized binding site called protomol. The protomol was built from the hydrogen-containing protein mol2 file. The construction was based on protein residues that constitute the binding site using parameters to produce a small and buried docking target (proto_thresh = 0.5 and proto_bloat = 0). We select 0.1 Å radius around each of ligands to generate protomols. The docking and subsequent scoring were performed using the default parameters of the Surflex-Dock programs implanted in the Sybyl 8.1. Final scores for all Surflex-Dock solutions were calculated by a consensus scoring function (CScore), and used for database ranking. One of the poses with the highest Surflex-Dock score (in $-\log(K_d)$ unit) and high consensus score (CScore 5 or 4) was selected, and docked with ALK5.

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Appendix A. Supplementary material

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.011.

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