Bioorganic & Medicinal Chemistry 20 (2012) 7128–7138

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazole derivatives as potent and selective transforming growth factor-β type I receptor inhibitors

Hideaki Amada [a,*], Yoshinori Sekiguchi [a], Naoya Ono [a], Takeshi Koami [a], Tetsuo Takayama [a], Tetsuya Yabuuchi [a], Hironori Katakai [a], Akiko Ikeda [b], Mari Aoki [b], Takumi Naruse [b], Reiko Wada [b], Akiko Nozoe [c], Masakazu Sato [a]

[a] Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403, Yoshino-Cho, Kita-Ku, Saitama, Saitama 331-9530, Japan
[b] Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403, Yoshino-Cho, Kita-Ku, Saitama, Saitama 331-9530, Japan
[c] Pharmaceutical Technology Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403, Yoshino-Cho, Kita-Ku, Saitama, Saitama 331-9530, Japan

ARTICLE INFO

Article history:
Received 3 September 2012
Revised 25 September 2012
Accepted 26 September 2012
Available online 12 October 2012

Keywords:
ALK5 inhibitor
Transforming growth factor-β
Solubility
Topical application
Alopecia

ABSTRACT

A series of 5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazole derivatives was synthesized as transforming growth factor-β (TGF-β) type I receptor (also known as activin-like kinase 5 or ALK5) inhibitors. These compounds were evaluated for their ALK5 inhibitory activity in an enzyme assay and for their TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay. As a representative compound, **16i** was a potent and selective ALK5 inhibitor, exhibiting a good enzyme inhibitory activity (IC_{50} = 5.5 nM) as well as inhibitory activity against TGF-β-induced Smad2/3 phosphorylation at a cellular level (IC_{50} = 36 nM). Furthermore, the topical application of 3% **16i** lotion significantly inhibited Smad2 phosphorylation in Mouse skin (90% inhibition compared with vehicle-treated animals).

1. Introduction

Transforming growth factor-β (TGF-β) belongs to the TGF-β superfamily, which includes TGF-β1, TGF-β2, TGF-β3, activins, inhibins and bone morphogenetic proteins. TGF-β is a cytokine that plays important roles in the regulation of a variety of physiological processes. It forms part of a family of cytokines that are involved in cell growth and differentiation, matrix expression and embryonic development. TGF-β signals through two types of transmembrane serine/threonine kinase receptors, namely the TGF-β type I receptor and the type II receptor (TGF-βRI and TGF-βRII, respectively). TGF-βRI is also known as activin receptor-like kinase 5 (ALK5). TGF-β binds to the constitutively active TGF-βRII. Subsequently, ALK5 is recruited into the complex and is activated by TGF-βRII through the mediated phosphorylation in the juxtamembrane GS domain. The activated ALK5 in turn phosphorylates and activates transcription factors Smad2/3, allowing them to bind to the commonly mediated Smad4. These Smad complexes translocate into the nucleus to affect gene transcription.[1–3] The overactivation of TGF-β signaling has been implicated in various human diseases, such as, fibrosis,[4] atherosclerosis[5] and cancer.[6] Therefore, the inhibition of ALK5 seems to be a good strategy for the treatment of these diseases.

On the other hand, TGF-β is known to exhibit a significant growth inhibitory action against various cells, such as epithelial cells, vascular endothelial cells, hematocytes or lymphocytes.[7] As for hair follicles, it has been reported that TGF-β hyperexpression induces growth suppression/apoptosis in hair follicle cells, shifting the hair cycle from anagen to catagen; thus, TGF-β appears to be deeply involved in the progression of alopecia.[8–10] Furthermore, it has been reported that TGF-β/Smad2 signaling pathway is intimately involved in hair growth suppression based on analyses involving transgenic mice overexpressing Smad2 in the epidermis under the control of keratin 14 promoter.[11]

Many research groups have reported small molecule inhibitors of ALK5 (Fig. 1).[3,12–19] We previously discovered that a novel series of 4-(4-methylthiazol-2-yl)imidazole derivatives, such as **1** (ALK5 IC_{50} = 8.2 nM, Smad2/3 IC_{50} = 32 nM), acted as potent inhibitors of ALK5.[20] Moreover, we recently discovered that the most potent ALK5 inhibitor **3a**, which was used as a tool compound, inhibited Smad2 phosphorylation in Mouse skin in such a way as to block the TGF-β/Smad signals after the topical application.[21] Based on these findings, compounds that block the TGF-β/Smad signaling

* Corresponding author. Tel.: +81 48 669 3029; fax: +81 48 652 7254.
E-mail address: hideaki.amada@po.rd.taisho.co.jp (H. Amada).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.066

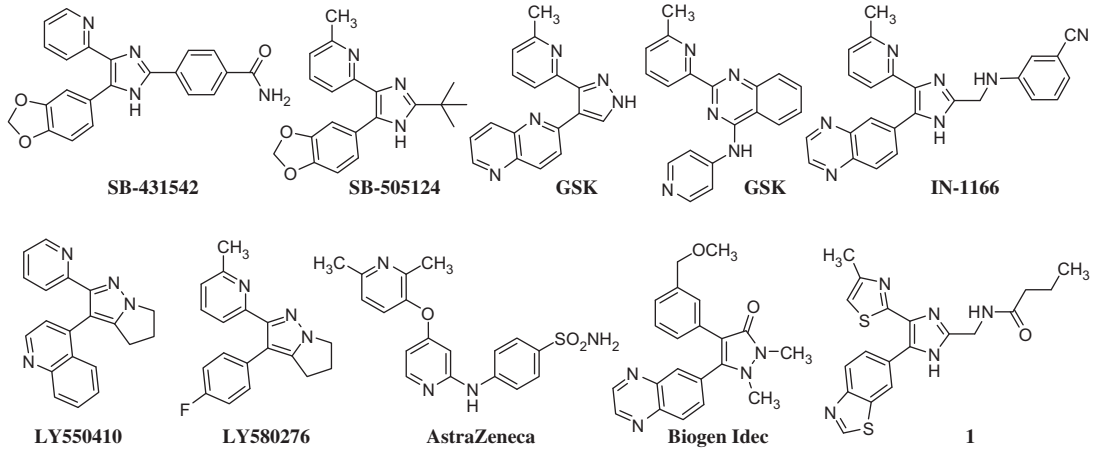
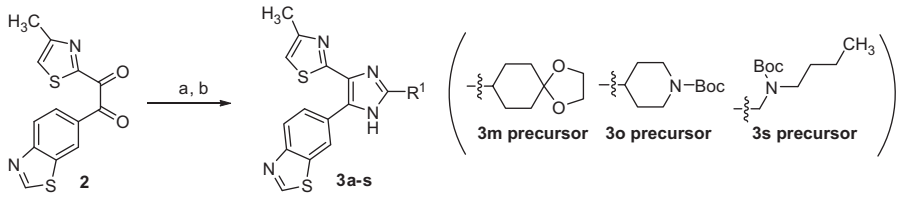
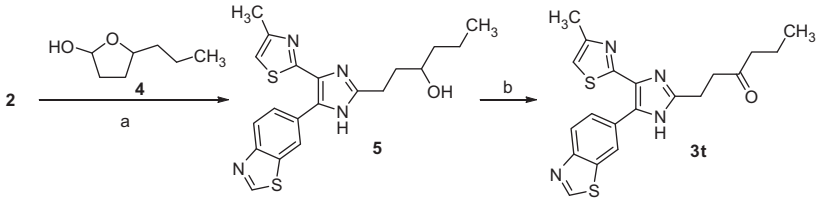


Figure 1. Representative ALK5 inhibitors.



Scheme 1. Reagents and conditions: (a) various aldehydes, NH_4OAc, THF-MeOH, rt or reflux, 36–98%; (b) preparation of **3m**, **3o** and **3s**: HCl, THF-H_2O, rt, 31–90%.



Scheme 2. Reagents and conditions: (a) **4**, NH_4OAc, THF-MeOH, rt, 46%; (b) Dess-Martin periodinane, CH_2Cl_2, rt, 66%.

pathway can be expected to possibly elongate anagen and ameliorate alopecia. As a formula for the topical application of these compounds in an in vivo study, we selected a lotion base that could be used topically on human skin. However, the solubility of **3a** remains inadequate for inclusion in this lotion base. If the solubility could be improved, the pharmacological efficacy could likely be increased. In the present study, we attempted to modify the substituent chemically at the 2-position of the 5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazole ring, which is the most potent template, so as to further improve the inhibitory activity and the solubility in the lotion base for an in vivo study.

2. Chemistry

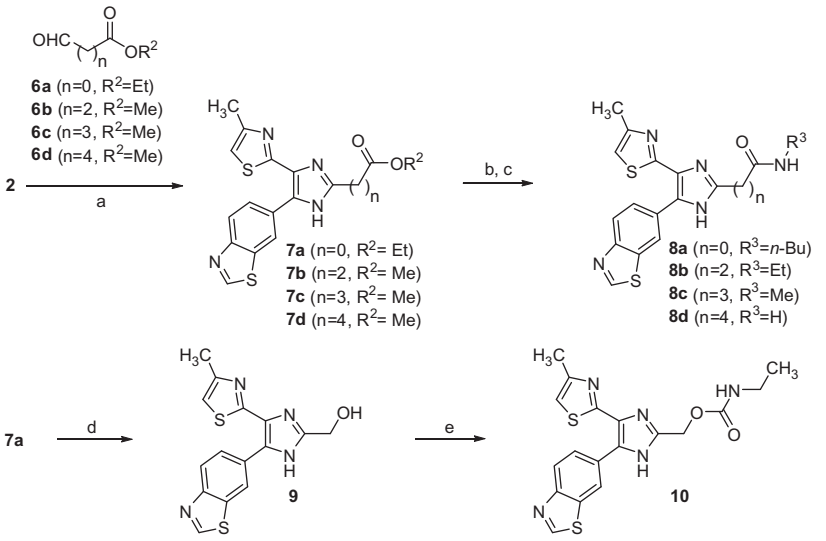
The imidazole analogues **3a**–**s** were prepared by the reaction of the α-diketone **2**[20] with the corresponding aldehydes and ammonium acetate in THF-MeOH at room temperature or under reflux (Scheme 1). For **3m**, **3o** and **3s**, the deprotection of the cyclic acetal moiety and Boc group under acidic conditions was performed to give the corresponding ketone **3m**, amine **3o** and amine **3s**, respectively.

The ketone **3t** was prepared as shown in Scheme 2. The treatment of α-diketone **2** with lactol **4**, which was synthesized in the same manner as described in the literature,[22] and ammonium acetate in THF-MeOH at room temperature gave secondary alcohol **5**. The oxidation of **5** was accomplished by reaction with Dess-Martin periodinane in CH_2Cl_2 at room temperature to afford **3t**.

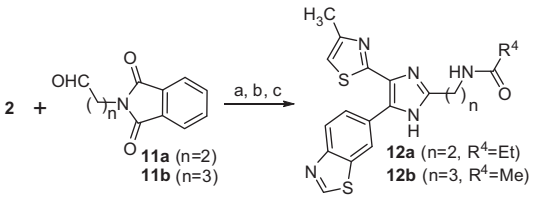
The preparations of the amides **8a**–**d** and the carbamate **10** are shown in Scheme 3. The esters **7a**–**d** were prepared using the same reaction conditions shown in Scheme 1. The hydrolysis of **7a**–**d** under basic conditions afforded the corresponding carboxylic acids. The resulting carboxylic acids were reacted with amines (R^3-NH_2) in the presence of EDC· HCl and HOBt ·H_2O to afford **8a**–**d**. The treatment of **7a** with lithium aluminium hydride in THF at -40 to 0 °C gave alcohol **9**. The resulting alcohol **9** was reacted with isocyanatoethane to afford **10**.

The preparations of the reverse amides **12a** and **12b** are shown in Scheme 4. The cyclization of α-diketone **2** with aldehyde **11a** or **11b** and ammonium acetate followed by the deprotection of phthalimido group with hydrazine monohydrate gave the corresponding primary amines. The resulting primary amines were coupled with acid chlorides (R^4-COCl) to give **12a** and **12b**.

The urea **16a**, the sulfonamide **16b** and the carbamates **16c**–**j** were prepared as shown in Scheme 5. The imidazole derivative **14** was prepared in a similar manner to that shown in Scheme 1. The deprotection of Boc group in **14** under acidic conditions gave 2-aminomethylimidazole **15**. The treatment of **15** with isocyanatoethane, 1-propanesulfonyl chloride and alkyl chloroformates in the presence of NEt_3 in $CHCl_3$ gave **16a**, **16b** and **16c**–**j**, respectively.



Scheme 3. Reagents and conditions: (a) **6a**–**d**, NH_4OAc, THF-MeOH, rt or reflux, 40–100%; (b) KOH, THF-MeOH-H_2O, rt; (c) R^3-NH_2, EDC·HCl, HOBt ·H_2O, NEt_3, $CHCl_3$, rt, 23–74%; (d) LAH, THF, −40–0 °C, 72%; (e) CH_3CH_2NCO, CuCl, pyridine, toluene, 50 °C, 52%.

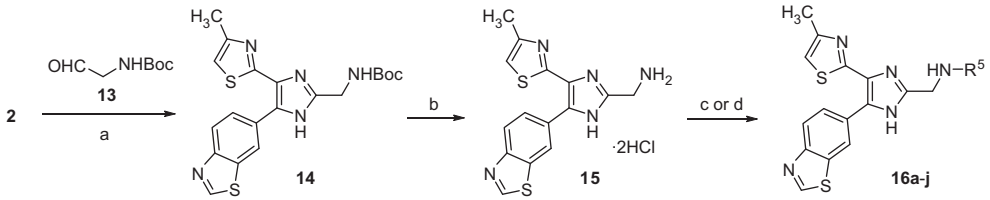


Scheme 4. Reagents and conditions: (a) NH_4OAc, THF-MeOH, rt; (b) $N_2H_4·H_2O$, MeOH, rt; (c) R^4-COCl, NEt_3, $CHCl_3$, rt, 20–37%.

3. Results and discussion

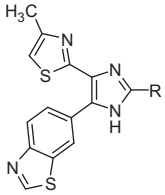
All the compounds were evaluated for their ALK5 inhibitory activity in an enzyme assay and for their TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay. The SAR observed using an alkyl substituent at the 2-position of the imidazole ring are summarized in Table 1. The unsubstituted imidazole ring analogue **3a** showed the most potent activities in both the enzyme assay and the cell-based assay. All the alkyl-substituted analogues displayed relatively potent ALK5 inhibitory activities. However, the bulky analogues with higher lipophilicity, such as **3g** (*tert*-Bu), **3h** (*n*-Hex) and **3l** (*c*-Hex), displayed a decrease in cellular activity. These results might be due to their poor physicochemical property. The potent alkyl-substituted analogues were evaluated for their solubilities in the lotion base (1,3-butylene glycol/EtOH/H_2O = 10 g/79 mL/diluted to 100 mL). The branched isopropyl analogue **3e** and the isobutyl analogue **3f** had a solubility that was about twofold higher than the unsubstituted analogue **3a** and the straight alkyl analogues **3b**–**d**. However, the *tert*-butyl analogue **3g** had a poor solubility. Although the trifluoromethyl analogue **3i** was equipotent with **3a**–**f** with regard to inhibitory activity, the solubility of **3i** was significantly decreased. A series of cycloalkyl analogues, **3j**–**l**, had moderate solubilities. The introduction of neutral substituents, such as alkyl substituents at the 2-position of the imidazole ring, did not adequately improve either the ALK5 inhibitory activity or the solubility in the lotion base.

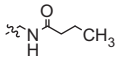
Subsequently, the polar-substituent effects at the 2-position of the imidazole ring were studied (Table 2). The cyclic ketone analogue **3m**, the cyclic ether analogue **3n** and the cyclic amide analogue **3p** were slightly less potent than the corresponding cycloalkyl analogues **3l** and **3k**. The activity of the cyclic amine analogue **3o** was decreased. These compounds with a cyclic substituent had moderate solubilities in the lotion base. Compound **3p** without the amide proton, and compound **3q** that was introduced a methyl group to the amide linkage in **1**, resulted in a decreased activity. Both the amine analogue **3s** and the ketone analogue **3t** without the amide linkage maintained the inhibitory activities. These results suggested that the proton on the atom at the 2nd position from the imidazole ring might contribute to the inhibitory activity. Next, modifications focused on the position of the amide linkage and different types of substituent modes in the linker were attempted. Compounds **8a** and **8c** were less potent than **1**. Compounds **8b** and **8d** showed a decreased potency in the cell-based assay, while they were equipotent to **1** with regard to their enzyme inhibitory activity. Compounds **12a** and **12b** displayed a decreased potency in both the enzyme and cellular activities. The reverse carbamate analogue **10**, the urea analogue **16a** and the sulfonamide analogue **16b** maintained the enzyme inhibitory activity; however, they exhibited a loss of inhibitory activity in the cell-based assay. It was delightful to find that the carbamate analogue **16c** displayed



Scheme 5. Reagents and conditions: (a) **13**, NH_4OAc, THF-MeOH, rt, 88%; (b) HCl, dioxane–$CHCl_3$, rt, 99%; (c) preparation of **16a**: CH_3CH_2NCO, NEt_3, $CHCl_3$, rt, 40%; (d) preparation of **16b**-**j**: R^5-Cl, NEt_3, $CHCl_3$, rt or 50 °C, 15–42%.

Table 1
Inhibitory profile and physicochemical properties of imidazoles **3a–l**



Compound	R	IC_{50} (nM)		Solubility[b] (mg/mL)
		ALK5[a]	Smad2/3[a]	
3a	H	2.7	8.7	14.9
3b	Me	5.8	17	18.3
3c	Et	9.0	35	10.6
3d	*n*-Pr	8.1	33	14.9
3e	*i*-Pr	8.2	48	27.3
3f	*i*-Bu	9.0	43	28.0
3g	*t*-Bu	14	68	3.49
3h	*n*-Hex	7.0	120	NE[c]
3i	CF_3	5.8	49	2.40
3j	*c*-Pr	8.8	45	23.6
3k	*c*-Pen	8.7	40	19.8
3l	*c*-Hex	12	75	>31.8
1		8.2	32	43.6

[a] Values are the mean of two or more separate experiments.
[b] 1,3-Butylene glycol/EtOH/H_2O (10/79/11).
[c] Not evaluated.

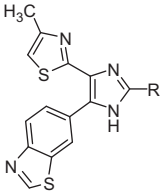
an IC_{50} of 4.5 nM in the enzyme assay, which was about twice as potent as **1**. Furthermore, **16c** showed a potent cellular activity and had a good solubility.

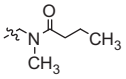
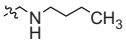
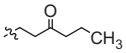
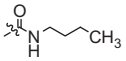
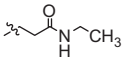
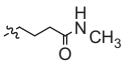
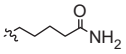
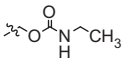
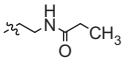
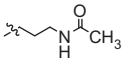
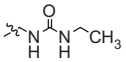
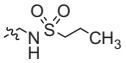
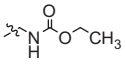
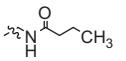
Finally, the substituent effect of the carbamate linker was studied (Table 3). The cyclic carbamate analogue **3r**, the methyl carbamate analogue **16d** and the *n*-propyl carbamate analogue **16e** were slightly less potent than **16c** and they displayed a significant decrease in solubility. As the linker chain grew in length, the straight alkyl carbamate analogues **16f** and **16g** showed a decrease in potency. Regarding the branched alkyl carbamate analogues, the isopropyl carbamate analogue **16h** and the neopentyl carbamate analogue **16j** displayed a slightly decrease potency, compared with **16c**. The isobutyl carbamate analogue **16i** showed a similar potency to **16c**; furthermore, **16i** was more than three times more soluble in the lotion base than **16c**. The introduction of branched substituents produced a good solubility in the lotion base that was higher than that of the straight substituted analogues. This tendency applied equally to 2-alkylsubstituted imidazoles.

Compound **16i** was evaluated for selectivity using a diverse kinase panel (96 kinases). Compound **16i** was highly selective against most of the kinases, and **16i** exhibited about a 200-fold selectivity against KDR (IC_{50} = 1.08 μM), which was the kinase that was most potently inhibited by **16i**.

Compound **16i**, which showed a potent ALK5 inhibitory activity and a high selectivity for other kinases in addition to being quite soluble in the lotion base, was assessed in a Smad2 phosphorylation inhibitory activity test using a Mouse model in which the hair cycle was synchronized by wax depilation.[23] It has been reported that TGF-β1 expression in hair follicles is hair cycle-dependent and increases during late anagen and the onset of catagen development.[9] Therefore, 16 days after depilation, when the hair follicles in the depilated area were in the late anagen phase, lotions containing 1% and 3% **16i** were applied to the Mouse skin. The skin was harvested after 8 h of the application and was processed for the western blot analysis of the phosphorylated-Smad2 level. As

Table 2
Inhibitory profile and physicochemical properties of imidazoles **3m–q**, **3s**, **3t**, **8a–d**, **10**, **12a**, **12b** and **16a–c**

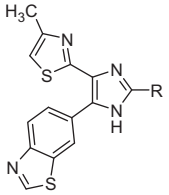


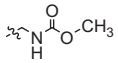
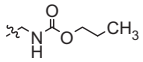
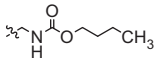
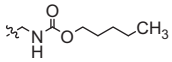
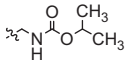
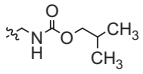
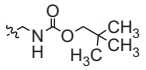
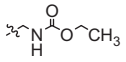
Compound	R	IC_{50} (nM)		Solubility[b] (mg/mL)
		ALK5[a]	Smad2/3[a]	
3m		23	89	16.7
3n		21	99	26.1
3o		240	NE[c]	NE[c]
3p		26	130	9.89
3q		28	110	NE[c]
3s		13	72	NE[c]
3t		9.0	42	20.0
8a		45	420	NE[c]
8b		12	71	18.4
8c		37	230	NE[c]
8d		8.3	190	NE[c]
10		7.2	93	NE[c]
12a		25	130	NE[c]
12b		26	150	NE[c]
16a		11	130	14.4
16b		17	150	NE[c]
16c		4.5	35	50.0
1		8.2	32	43.6

[a] Values are the mean of two or more separate experiments.
[b] 1,3-Butylene glycol/EtOH/H_2O (10/79/11).
[c] Not evaluated.

shown in Figure 2, the topical application of 1% and 3% **16i** inhibited the phosphorylation of Smad2 significantly (59% and 90% inhibition, compared with vehicle-treated animals, respectively). These data indicated that **16i** definitely inhibited ALK5 not only in experiments in vitro, but also in vivo. Moreover, the improvement in the solubility made it possible to produce a higher dose of **16i** in a

Table 3
Inhibitory profile and physicochemical properties of imidazoles **3r** and **16d–j**



Compound	R	IC_{50} (nM)		Solubility[b] (mg/mL)
		ALK5[a]	Smad2/3[a]	
3r		8.1	92	1.79
16d		7.7	38	3.93
16e		7.3	36	9.80
16f		9.1	74	NE[c]
16g		11	140	NE[c]
16h		8.6	41	18.2
16i		5.5	36	>151
16j		9.3	79	NE[c]
16c		4.5	35	50.0

[a] Values are the mean of two or more separate experiments.
[b] 1,3-Butylene glycol/EtOH/H_2O (10/79/11).
[c] Not evaluated.

Smad2 phosphorylation inhibitory activity test in Mouse skin; as a result, the pharmacological efficacy of the 3% **16i** lotion was increased. Hence, **16i** may be a good topical drug for alopecia with a single daily dosing schedule.

4. Conclusion

In this study, a series of 5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl) -1*H*-imidazole derivatives was synthesized and evaluated as ALK5 inhibitors. Elaborate SAR studies of the 2-position of the imidazole ring showed that 2-unsubstituted imidazole, 2-lower alkyl substituted imidazoles and 2-carbamate substituted imidazoles were more potent than **1**. Furthermore, the introduction of branched substituents at the 2-position of the imidazole ring produced a good solubility in the lotion base that was higher than that of the straight substituted analogues. Compound **16i** exhibited a potent ALK5 inhibitory activity and selectivity for ALK5 among other kinases and had a good solubility in the lotion base. Moreover, **16i** showed an inhibitory activity against Smad2 phosphorylation in Mouse skin. Compound **16i** may be useful for the amelioration of alopecia, although more detailed findings regarding the pharmacological properties of **16i** remain to be studied.

5. Experimental

5.1. Chemistry

All starting materials and reagents were commercial products that were used without further purification. The reaction progresses were usually monitored by TLC using Merck silica gel 60 F_{254} plates or Fuji Silysia chromatorex NH plates. Column chromatography was performed using silica gel Wako Pure Chemical C-200 and NH-silica gel Fuji Silysia chromatorex DM1020. Melting points were determined on a Mettler FP-61 or a Yanaco MP-500D melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded at 200 MHz, 300 MHz or 600 MHz using a Varian Instruments Gemini 2000, a Varian Instruments INOVA 300 or a JEOL ECA600 with TMS as an internal standard, and proton chemical shifts were expressed in parts per million (ppm) in the indicated solvent. Multiplicity was defined as s (singlet), d (doublet), t (triplet), q (quartet), dd (double doublet), m (multiplet), br s (broad singlet), br d (broad doublet) or br t (broad triplet). Analytical LC was performed on an Agilent 1290 Infinity LC (Waters Acquity CSH C18 column, 1.7 μm, 2.1 × 50 mm; 0.1% formic acid in water/0.1% formic acid in acetonitrile gradient; UV detection at 210 and 254 nm). Mass spectra (MS) were recorded on a Micromass Platform LC mass spectrometer with electrospray ionization (ESI). High resolution mass spectra (HRMS) were recorded on a Shimadzu LCMS-IT-TOF mass spectrometer with electrospray

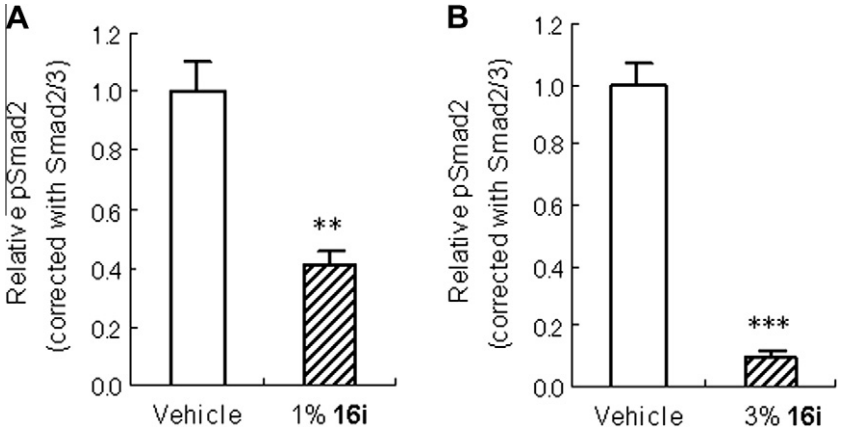


Figure 2. Smad2 phosphorylation inhibitory activity of **16i** in Mouse skin. (A) 1% **16i** lotion; (B) 3% **16i** lotion, topical application. Data are expressed as the means ± SEM ($n = 5$). $^{**}p < 0.01$, $^{***}p < 0.001$, compared with vehicle-treated animals (Welch's test).

ionization (ESI)/atmospheric pressure chemical ionization (APCI) dual source. Elemental analyses were performed on a Perkin-Elmer 2400, a Yanaco MT-6 or an Elementar vario MICRO cube elemental analyzers, and the results were within 0.4% of the calculated values.

5.1.1. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3a)

To a solution of **2** (304 mg, 1.05 mmol) and paraformaldehyde (305 mg, 3.39 mmol) in THF (10 mL) was added NH_4OAc (829 mg, 10.8 mmol) in MeOH (5 mL). The reaction mixture was stirred at room temperature for 4 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture and extracted with EtOAc twice. The combined organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with EtOAc to 10% MeOH/$CHCl_3$. The powder obtained was recrystallized from MeOH/$CHCl_3$/*n*-Hexane to afford **3a** as a colorless powder (122 mg, 39%): mp 210.0–211.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.35 (d, J = 0.9 Hz, 3H), 7.16 (d, J = 0.9 Hz, 1H), 7.88 (s, 1H), 8.07 (br d, J = 8.5 Hz, 1H), 8.14 (dd, J = 8.5, 0.5 Hz, 1H), 8.87 (br s, 1H), 9.44 (s, 1H), 12.95 (br s, 1H); MS (ESI) m/z 299 $[M+H]^+$, 100%, 321 $[M+Na]^+$, 75%, 297 $[M-H]^-$, 100%; Anal. Calcd for $C_{14}H_{10}N_4S_2$: C, 56.35; H, 3.38; N, 18.78. Found: C, 56.12; H, 3.60; N, 18.45.

5.1.2. 6-[2-Methyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3b)

Yield: 65%, a colorless powder: mp 217.5–218.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.43 (d, J = 0.9 Hz, 3H), 2.52 (s, 3H), 6.71 (s, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.41 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 313 $[M+H]^+$, 100%, 335 $[M+Na]^+$, 30%, 311 $[M-H]^-$, 100%; Anal. Calcd for $C_{15}H_{12}N_4S_2$: C, 57.67; H, 3.87; N, 17.93. Found: C, 57.41; H, 4.03; N, 17.82.

5.1.3. 6-[2-Ethyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole hydrochloride (3c)

To a solution of **2** (400 mg, 1.39 mmol) and propionaldehyde (121 mg, 2.08 mmol) in THF (14 mL) was added NH_4OAc (1.07 g, 13.9 mmol) in MeOH (7 mL). The reaction mixture was stirred at room temperature for 6 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture and extracted with $CHCl_3$ twice. The combined organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with 4–7% MeOH/$CHCl_3$ to afford free base of **3c** as a pale yellow amorphous (301 mg, 66%). To a solution of the amorphous obtained in MeOH (10 mL) was added 4 M HCl in EtOAc (0.45 mL). The mixture was stirred at room temperature for 10 min and concentrated. The residue was recrystallized from MeOH/$CHCl_3$/EtOAc to afford **3c** as a colorless powder: ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.40 (t, J = 7.6 Hz, 3H), 2.44 (d, J = 0.9 Hz, 3H), 3.04 (q, J = 7.6 Hz, 2H), 7.36 (d, J = 0.9 Hz, 1H), 7.84 (dd, J = 8.5, 1.8 Hz, 1H), 8.29 (dd, J = 8.5, 0.5 Hz, 1H), 8.60 (dd, J = 1.8, 0.5 Hz, 1H), 9.58 (s, 1H); MS (ESI) m/z 327 $[M+H]^+$, 100%, 325 $[M-H]^-$, 100%; Anal. Calcd for $C_{16}H_{14}N_4S_2 \cdot 1.9HCl \cdot 1.1H_2O$: C, 46.25; H, 4.39; N, 13.48. Found: C, 46.01; H, 4.30; N, 13.38.

5.1.4. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-2-propyl-1*H*-imidazol-5-yl]-1,3-benzothiazole (3d)

Yield: 36%, a colorless powder: mp 144.5–146.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.04 (t, J = 7.4 Hz, 3H), 1.77–1.93 (m, 2H), 2.44 (d, J = 1.1 Hz, 3H), 2.79 (t, J = 7.4 Hz, 2H), 6.69 (s, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.17 (dd, J = 8.5, 0.5 Hz, 1H), 8.39 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 341 $[M+H]^+$, 100%, 339 $[M-H]^-$, 100%; Anal. Calcd for $C_{17}H_{16}N_4S_2$: C, 59.97; H, 4.74; N, 16.46. Found: C, 59.82; H, 4.79; N, 16.31.

5.1.5. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-2-(propan-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3e)

Yield: 52%, a colorless powder: mp 155.0–155.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.44 (d, J = 7.0 Hz, 6H), 2.44 (d, J = 0.9 Hz, 3H), 3.10–3.26 (m, 1H), 6.68 (br s, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.17 (dd, J = 8.5, 0.6 Hz, 1H), 8.35 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 341 $[M+H]^+$, 100%, 363 $[M+Na]^+$, 15%, 339 $[M-H]^-$, 100%; Anal. Calcd for $C_{17}H_{16}N_4S_2$: C, 59.97; H, 4.74; N, 16.46. Found: C, 59.64; H, 4.64; N, 16.27.

5.1.6. 6-[2-(2-Methylpropyl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole hydrochloride (3f)

Yield: 55%, a colorless powder: mp 212.0–214.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 0.99 (d, J = 6.7 Hz, 6H), 2.16–2.33 (m, 1H), 2.44 (d, J = 0.9 Hz, 3H), 2.88 (d, J = 7.3 Hz, 2H), 7.35 (d, J = 0.9 Hz, 1H), 7.87 (dd, J = 8.5, 1.8 Hz, 1H), 8.29 (dd, J = 8.5, 0.5 Hz, 1H), 8.64 (d, J = 1.8 Hz, 1H), 9.57 (s, 1H); MS (ESI) m/z 355 $[M+H]^+$, 100%, 353 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{18}N_4S_2 \cdot 1.1HCl \cdot 0.5H_2O$: C, 53.57; H, 5.02; N, 13.88. Found: C, 53.44; H, 4.84; N, 13.82.

5.1.7. 6-[2-*tert*-Butyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3g)

Yield: 46%, a colorless powder: mp 239.5–240.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.49 (s, 9H), 2.44 (d, J = 0.9 Hz, 3H), 6.64 (s, 1H), 7.85 (dd, J = 8.5, 1.6 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.29 (d, J = 1.6 Hz, 1H), 9.02 (s, 1H), 9.91 (br s, 1H); MS (ESI) m/z 355 $[M+H]^+$, 100%, 353 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{18}N_4S_2 \cdot 0.1CHCl_3$: C, 59.33; H, 4.98; N, 15.29. Found: C, 59.14; H, 4.97; N, 15.06.

5.1.8. 6-[2-Hexyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3h)

Yield: 36%, a colorless powder: mp 129.0–130.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 0.88 (t, J = 7.0 Hz, 3H), 1.23–1.42 (m, 6H), 1.65–1.79 (m, 2H), 2.34 (s, 3H), 2.69 (t, J = 7.5 Hz, 2H), 7.13 (d, J = 0.9 Hz, 1H), 8.03–8.19 (m, 2H), 8.90 (br s, 1H), 9.42 (s, 1H), 12.48 (br s, 1H); MS (ESI) m/z 383 $[M+H]^+$, 100%, 381 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{22}N_4S_2 \cdot 0.2H_2O$: C, 62.21; H, 5.85; N, 14.51. Found: C, 62.32; H, 5.81; N, 14.46.

5.1.9. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-2-(trifluoromethyl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3i)

To a solution of **2** (500 mg, 1.73 mmol) in AcOH (10 mL) were added 1-ethoxy-2,2,2-trifluoroethanol (1.26 g, 8.74 mmol) and NH_4OAc (666 mg, 8.64 mmol). The reaction mixture was stirred at reflux temperature for 4 h. A saturated aqueous solution of $NaHCO_3$ was added to the reaction mixture and extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with 45% EtOAc/*n*-Hexane. The powder obtained was recrystallized from EtOAc/*n*-Hexane to afford **3i** as a colorless powder (234 mg, 37%): mp 253.0–255.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.31 (s, 3H), 7.25 (s, 1H), 8.04 (br d, J = 8.5 Hz, 1H), 8.17 (d, J = 8.5 Hz, 1H), 8.78 (br s, 1H), 9.49 (s, 1H), 14.32 (br s, 1H); MS (ESI) m/z 367 $[M+H]^+$, 100%, 389 $[M+Na]^+$, 15%, 365 $[M-H]^-$, 100%; Anal. Calcd for $C_{15}H_9F_3N_4S_2$: C, 49.17; H, 2.48; N, 15.29. Found: C, 48.99; H, 2.65; N, 15.02.

5.1.10. 6-[2-Cyclopropyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3j)

Yield: 60%, a colorless powder: mp 190.5–191.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.00–1.15 (m, 4H), 1.93–2.06 (m, 1H), 2.43 (d, J = 1.1 Hz, 3H), 6.67 (s, 1H), 7.83 (dd, J = 8.4, 1.7 Hz, 1H), 8.16 (dd, J = 8.4, 0.6 Hz, 1H), 8.34 (br s, 1H), 9.02 (s, 1H); MS (ESI) m/z

339 $[M+H]^+$, 100%, 337 $[M-H]^-$, 15%; Anal. Calcd for $C_{17}H_{14}N_4S_2$: C, 60.33; H, 4.17; N, 16.55. Found: C, 60.05; H, 4.00; N, 16.56.

5.1.11. 6-[2-Cyclopentyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3k)

Yield: 56%, a colorless powder: mp 180.5–182.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.61–2.00 (m, 6H), 2.02–2.26 (m, 2H), 2.42 (d, J = 0.9 Hz, 3H), 3.11–3.33 (m, 1H), 6.67 (s, 1H), 7.84 (dd, J = 8.4, 1.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.35 (br s, 1H), 9.02 (s, 1H); MS (ESI) m/z 367 $[M+H]^+$, 100%, 365 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{18}N_4S_2$: C, 62.27; H, 4.95; N, 15.29. Found: C, 62.24; H, 4.91; N, 15.29.

5.1.12. 6-[2-Cyclohexyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3l)

Yield: 63%, a colorless powder: mp 195.0–196.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.20–1.47 (m, 3H), 1.51–1.74 (m, 3H), 1.76–1.87 (m, 2H), 1.92–2.03 (m, 2H), 2.33 (s, 3H), 2.66–2.82 (m, 1H), 7.12 (d, J = 0.9 Hz, 1H), 8.08–8.15 (m, 2H), 8.85 (br s, 1H), 9.42 (s, 1H), 12.36 (br s, 1H); MS (ESI) m/z 381 $[M+H]^+$, 100%, 403 $[M+Na]^+$, 10%, 379 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{20}N_4S_2$: C, 63.13; H, 5.30; N, 14.72. Found: C, 63.00; H, 5.33; N, 14.72.

5.1.13. 4-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]cyclohexanone (3m)

To a solution of **2** (668 mg, 2.32 mmol) and 1,4-dioxaspiro[4.5]decane-8-carbaldehyde (550 mg, 3.23 mmol) in THF (25 mL) was added NH_4OAc (1.79 g, 23.2 mmol) in MeOH (17 mL). The reaction mixture was stirred at room temperature for 1.5 h. Water was added to the reaction mixture and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with 50–99% EtOAc/*n*-Hexane to afford 6-[2-(1,4-dioxaspiro[4.5]dec-8-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole as a pale yellow amorphous (865 mg, 85%): ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.63–2.00 (m, 6H), 2.11–2.22 (m, 2H), 2.43 (d, J = 0.9 Hz, 3H), 2.87–3.01 (m, 1H), 3.96–4.00 (m, 4H), 6.67 (br s, 1H), 7.86 (dd, J = 8.4, 1.7 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.30 (br s, 1H), 9.03 (s, 1H), 10.02 (br s, 1H); MS (ESI) m/z 439 $[M+H]^+$, 100%, 437 $[M-H]^-$, 100%.

To a solution of 6-[2-(1,4-dioxaspiro[4.5]dec-8-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (825 mg, 1.88 mmol) in THF (10 mL) was added 2 M HCl (10 mL). The reaction mixture was stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc and washed with 2 M NaOH and brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was recrystallized from EtOAc/*n*-Hexane to afford **3m** as a colorless powder (371 mg, 50%): mp 207.5–208.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 2.10–2.26 (m, 2H), 2.40–2.67 (m, 6H), 2.44 (d, J = 0.9 Hz, 3H), 3.24–3.37 (m, 1H), 6.71 (br s, 1H), 7.85 (dd, J = 8.5, 1.7 Hz, 1H), 8.18 (d, J = 8.5 Hz, 1H), 8.32 (br s, 1H), 9.04 (s, 1H); MS (ESI) m/z 395 $[M+H]^+$, 100%, 393 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{18}N_4OS_2$: C, 60.89; H, 4.60; N, 14.20. Found: C, 60.68; H, 4.59; N, 14.40.

5.1.14. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-2-(tetrahydro-2*H*-pyran-4-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3n)

Yield: 64%, a colorless powder: mp 204.5–205.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.80–2.14 (m, 4H), 2.44 (d, J = 0.9 Hz, 3H), 3.00–3.21 (m, 1H), 3.46–3.63 (m, 2H), 4.03–4.17 (m, 2H), 6.69 (s, 1H), 7.84 (dd, J = 8.4, 1.7 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.35 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 383 $[M+H]^+$, 100%, 405 $[M+Na]^+$, 30%, 381 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{18}N_4OS_2$: C, 59.66; H, 4.74; N, 14.65. Found: C, 59.53; H, 4.86; N, 14.40.

5.1.15. 6-[4-(4-Methyl-1,3-thiazol-2-yl)-2-(piperidin-4-yl)-1*H*-imidazol-5-yl]-1,3-benzothiazole (3o)

Yield: 30%, a colorless powder: mp 199.5–200.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.78–2.16 (m, 4H), 2.43 (d, J = 0.9 Hz, 3H), 2.72–2.85 (m, 2H), 2.96–3.09 (m, 1H), 3.21–3.31 (m, 2H), 6.68 (br s, 1H), 7.85 (dd, J = 8.5, 1.7 Hz, 1H), 8.17 (dd, J = 8.5, 0.5 Hz, 1H), 8.33 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 382 $[M+H]^+$, 100%, 380 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5S_2$: C, 59.81; H, 5.02; N, 18.36. Found: C, 59.46; H, 5.01; N, 18.74.

5.1.16. 1-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}pyrrolidin-2-one (3p)

Yield: 51%, a colorless powder: mp 209.5–210.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.90–2.04 (m, 2H), 2.29 (t, J = 8.2 Hz, 2H), 2.33 (s, 3H), 3.42 (t, J = 7.3 Hz, 2H), 4.50 (s, 2H), 7.15 (d, J = 1.1 Hz, 1H), 8.00–8.11 (m, 1H), 8.13 (d, J = 8.5 Hz, 1H), 8.81 (br s, 1H), 9.43 (s, 1H), 12.83 (br s, 1H); MS (ESI) m/z 396 $[M+H]^+$, 100%, 418 $[M+Na]^+$, 85%, 394 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{17}N_5OS_2$: C, 57.70; H, 4.33; N, 17.71. Found: C, 57.43; H, 4.41; N, 17.50.

5.1.17. *N*-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}-*N*-methylbutanamide (3q)

Yield: 54%, a pale yellow powder: mp 175.0–175.5 °C; ^{1}H NMR (600 MHz, $CDCl_3$, δ): 0.98 (t, J = 7.2 Hz, 3H), 1.61–1.77 (m, 2H), 2.35 (t, J = 7.4 Hz, 2H), 2.44 (d, J = 0.9 Hz, 3H), 3.18 (s, 3H), 4.60 (s, 2H), 6.76 (br s, 1H), 7.86 (dd, J = 8.7, 1.7 Hz, 1H), 8.15 (d, J = 8.7 Hz, 1H), 8.60 (br s, 1H), 9.03 (s, 1H), 10.53 (br s, 1H); MS (ESI) m/z 412 $[M+H]^+$, 100%, 434 $[M+Na]^+$, 95%, 410 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{21}N_5OS_2 \cdot 0.1H_2O$: C, 58.12; H, 5.17; N, 16.94. Found: C, 58.11; H, 5.00; N, 16.55.

5.1.18. 3-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}-1,3-oxazolidin-2-one (3r)

Yield: 38%, a colorless powder: mp 232.0–233.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.34 (s, 3H), 3.60–3.68 (m, 2H), 4.27–4.36 (m, 2H), 4.49 (s, 2H), 7.16 (d, J = 0.9 Hz, 1H), 8.09 (br s, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.82 (br s, 1H), 9.44 (s, 1H), 12.93 (br s, 1H); MS (ESI) m/z 398 $[M+H]^+$, 10%, 420 $[M+Na]^+$, 70%, 396 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{15}N_5O_2S_2$: C, 54.39; H, 3.80; N, 17.62. Found: C, 54.26; H, 3.90; N, 17.51.

5.1.19. *N*-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}butan-1-amine dihydrochloride (3s)

Yield: 62%, a colorless powder: ^{1}H NMR (300 MHz, DMSO-d_6, δ): 0.91 (t, J = 7.4 Hz, 3H), 1.30–1.45 (m, 2H), 1.62–1.74 (m, 2H), 2.38 (d, J = 0.9 Hz, 3H), 3.00–3.13 (m, 2H), 4.31–4.42 (m, 2H), 7.25 (d, J = 0.9 Hz, 1H), 8.12 (dd, J = 8.6, 1.7 Hz, 1H), 8.19 (dd, J = 8.6, 0.6 Hz, 1H), 8.94 (d, J = 1.7 Hz, 1H), 9.49 (s, 1H), 9.69 (br s, 2H); MS (ESI) m/z 384 $[M+H]^+$, 100%; HRMS (ESI/APCI Dual) m/z Calcd for $C_{19}H_{21}N_5S_2$ $[M+H]^+$, 384.1311; Found 384.1302; 100% pure by LC–MS.

5.1.20. 1-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]hexan-3-ol (5)

To a solution of **2** (537 mg, 1.86 mmol) and **4** (290 mg, 2.23 mmol) in THF (18 mL) was added NH_4OAc (1.43 g, 18.6 mmol) in MeOH (12 mL). The reaction mixture was stirred at room temperature for 13 h. Water was added to the reaction mixture and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on silica gel eluting with EtOAc to 3% MeOH/$CHCl_3$ to afford **5** as a pale yellow amorphous (343 mg, 46%): ^{1}H NMR (300 MHz, DMSO-d_6, δ): 0.89 (t, J = 6.5 Hz, 3H), 1.21–1.50 (m, 4H), 1.63–1.79 (m, 1H), 1.80–1.94 (m, 1H), 2.34 (s,

3H), 2.64–2.91 (m, 2H), 3.43–3.59 (m, 1H), 7.13 (s, 1H), 8.04–8.16 (m, 2H), 8.89 (br s, 1H), 9.42 (s, 1H); MS (ESI) *m*/*zm*/*z* 399 $[M+H]^+$, 100%, 421 $[M+Na]^+$, 45%, 397 $[M-H]^-$, 100%; HRMS (ESI/APCI Dual) *m*/*z* Calcd for $C_{20}H_{22}N_4OS_2$ $[M+H]^+$, 399.1308; Found 399.1312; 100% pure by LC–MS.

5.1.21. 1-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]hexan-3-one (3t)

To a solution of **5** (342 mg, 0.858 mmol) in CH_2Cl_2 (7 mL) was added Dess-Martin periodinane (400 mg, 0.944 mmol). The reaction mixture was stirred at room temperature for 2 h. Water was added to the reaction mixture and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with 50–90% EtOAc/*n*-Hexane to afford **3t** as a colorless powder (225 mg, 66%): mp 145.0–146.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.93 (t, *J* = 7.4 Hz, 3H), 1.57–1.73 (m, 2H), 2.42–2.50 (m, 2H), 2.44 (d, *J* = 0.9 Hz, 3H), 2.95–3.01 (m, 2H), 3.05–3.11 (m, 2H), 6.72 (s, 1H), 7.86 (dd, *J* = 8.5, 1.8 Hz, 1H), 8.16 (dd, *J* = 8.5, 0.6 Hz, 1H), 8.48 (br s, 1H), 9.03 (s, 1H); MS (ESI) *m*/*z* 397 $[M+H]^+$, 100%, 395 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{20}N_4OS_2{\cdot}0.2H_2O$: C, 60.03; H, 5.14; N, 14.00. Found: C, 59.97; H, 4.96; N, 13.85.

5.1.22. Ethyl 5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazole-2-carboxylate (7a)

Yield: 40%, a colorless powder: mp 238.5–239.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.37 (t, *J* = 7.1 Hz, 3H), 2.30 (s, 3H), 4.39 (q, *J* = 7.1 Hz, 2H), 7.24 (s, 1H), 8.03 (br d, *J* = 8.5 Hz, 1H), 8.14 (d, *J* = 8.5 Hz, 1H), 8.74 (s, 1H), 9.47 (s, 1H), 14.01 (br s, 1H); MS (ESI) *m*/*z* 371 $[M+H]^+$, 55%, 393 $[M+Na]^+$, 100%, 369 $[M-H]^-$, 100%; Anal. Calcd for $C_{17}H_{14}N_4O_2S_2$: C, 55.12; H, 3.81; N, 15.12. Found: C, 54.93; H, 3.77; N, 14.84.

5.1.23. Methyl 3-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]propanoate (7b)

Yield: 72%, a pale yellow powder: mp 168.5–170.5 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 2.44 (d, *J* = 0.9 Hz, 3H), 2.81–2.90 (m, 2H), 3.07–3.18 (m, 2H), 3.74 (s, 3H), 6.72 (br s, 1H), 7.86 (dd, *J* = 8.4, 1.8 Hz, 1H), 8.15 (d, *J* = 8.4 Hz, 1H), 8.35 (br s, 1H), 9.02 (s, 1H); MS (ESI) *m*/*z* 385 $[M+H]^+$, 55%, 407 $[M+Na]^+$, 20%, 383 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{16}N_4O_2S_2{\cdot}0.6H_2O$: C, 54.69; H, 4.39; N, 14.17. Found: C, 54.88; H, 4.24; N, 13.84.

5.1.24. Methyl 4-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]butanoate (7c)

Yield: 82%, a yellow amorphous: ^{1}H NMR (600 MHz, DMSO-d_6, δ): 1.97–2.03 (m, 2H), 2.34 (br s, 3H), 2.45 (t, *J* = 7.4 Hz, 2H), 2.73 (t, *J* = 7.4 Hz, 2H), 3.60 (s, 3H), 7.13 (d, *J* = 1.2 Hz, 1H), 8.08–8.16 (m, 2H), 8.90 (br s, 1H), 9.42 (s, 1H), 12.55 (br s, 1H); MS (ESI) *m*/*z* 399 $[M+H]^+$, 100%, 421 $[M+Na]^+$, 20%, 397 $[M-H]^-$, 100%; HRMS (ESI/APCI Dual) *m*/*z* Calcd for $C_{19}H_{18}N_4O_2S_2$ $[M+Na]^+$, 421.0763; Found 421.0778; 100% pure by LC–MS.

5.1.25. Methyl 5-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]pentanoate (7d)

Yield: quantitative, a yellow amorphous: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.68–1.95 (m, 4H), 2.35–2.43 (m, 2H), 2.43 (d, *J* = 0.9 Hz, 3H), 2.84 (t, *J* = 7.1 Hz, 2H), 3.69 (s, 3H), 6.72 (br s, 1H), 7.91 (br s, 1H), 8.16 (d, *J* = 8.5 Hz, 1H), 8.40 (br s, 1H), 9.02 (s, 1H); MS (ESI) *m*/*z* 413 $[M+H]^+$, 55%, 435 $[M+Na]^+$, 20%, 411 $[M-H]^-$, 100%; HRMS (ESI/APCI Dual) *m*/*z* Calcd for $C_{20}H_{20}N_4O_2S_2$ $[M+H]^+$, 413.1100; Found 413.1084; 100% pure by LC–MS.

5.1.26. 5-(1,3-Benzothiazol-6-yl)-*N*-butyl-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazole-2-carboxamide (8a)

To a solution of **7a** (282 mg, 0.762 mmol) in THF (10 mL) was added KOH (128 mg, 2.28 mmol) in a mixed solvent of water (1 mL) and MeOH (1 mL). The reaction mixture was stirred at room temperature for 20 h. The reaction mixture was neutralized with 4 M HCl in dioxane and concentrated. The residue was dissolved in $CHCl_3$ (10 mL) and *n*-butylamine (67 mg, 0.92 mmol), HOBt H_2O (103 mg, 0.762 mmol), NEt_3 (115 mg, 1.14 mmol) and EDC HCl (218 mg, 1.14 mmol) were added. The reaction mixture was stirred at room temperature for 10 h. The reaction mixture was diluted with $CHCl_3$ and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on NH–silica gel eluting with 5% MeOH/$CHCl_3$. The powder obtained was recrystallized from EtOAc/*n*-Hexane to afford **8a** as a pale yellow powder (71 mg, 23%): ^{1}H NMR (600 MHz, DMSO-d_6, δ): 0.92 (t, *J* = 7.4 Hz, 3H), 1.30–1.39 (m, 2H), 1.52–1.59 (m, 2H), 2.31 (br s, 3H), 3.27–3.33 (m, 2H), 7.22 (br s, 1H), 8.02 (br d, *J* = 8.7 Hz, 1H), 8.11 (d, *J* = 8.7 Hz, 1H), 8.48 (br s, 1H), 8.74 (br s, 1H), 9.45 (s, 1H), 13.71 (br s, 1H); MS (ESI) *m*/*z* 398 $[M+H]^+$, 100%, 420 $[M+Na]^+$, 55%, 396 $[M-H]^-$, 100%; HRMS (ESI/APCI Dual) *m*/*z* Calcd for $C_{19}H_{19}N_5OS_2$ $[M+H]^+$, 398.1104; Found 398.1091; 100% pure by LC–MS.

5.1.27. 3-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]-*N*-ethylpropanamide (8b)

Yield: 57%, a colorless powder: mp 208.0–209.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.02 (t, *J* = 7.2 Hz, 3H), 2.34 (s, 3H), 2.53–2.60 (m, 2H), 2.88–2.96 (m, 2H), 3.04–3.14 (m, 2H), 7.13 (d, *J* = 1.1 Hz, 1H), 7.95 (br t, *J* = 5.4 Hz, 1H), 8.09–8.15 (m, 2H), 8.91 (br s, 1H), 9.43 (s, 1H), 12.57 (br s, 1H); MS (ESI) *m*/*z* 398 $[M+H]^+$, 100%, 420 $[M+Na]^+$, 100%, 396 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5OS_2{\cdot}0.1H_2O$: C, 57.15; H, 4.85; N, 17.54. Found: C, 56.92; H, 4.73; N, 17.50.

5.1.28. 4-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]-*N*-methylbutanamide (8c)

Yield: 74%, a pale yellow amorphous: ^{1}H NMR (600 MHz, DMSO-d_6, δ): 1.92–1.98 (m, 2H), 2.17 (t, *J* = 7.4 Hz, 2H), 2.33 (br s, 3H), 2.57 (d, *J* = 5.0 Hz, 3H), 2.69 (t, *J* = 7.6 Hz, 2H), 7.13 (s, 1H), 7.77 (br s, 1H), 8.09–8.16 (m, 2H), 8.91 (br s, 1H), 9.43 (s, 1H), 12.52 (br s, 1H); MS (ESI) *m*/*z* 398 $[M+H]^+$, 100%, 420 $[M+Na]^+$, 20%, 396 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5OS_2$: C, 57.41; H, 4.82; N, 17.62. Found: C, 57.29; H, 4.87; N, 17.41.

5.1.29. 5-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]pentanamide (8d)

Yield: 59%, a pale yellow amorphous: ^{1}H NMR (600 MHz, DMSO-d_6, δ): 1.54–1.62 (m, 2H), 1.68–1.76 (m, 2H), 2.11 (t, *J* = 7.4 Hz, 2H), 2.33 (d, *J* = 0.9 Hz, 3H), 2.70 (t, *J* = 7.6 Hz, 2H), 6.73 (br s, 1H), 7.13 (d, *J* = 0.9 Hz, 1H), 7.27 (br s, 1H), 8.12–8.14 (m, 2H), 8.91 (s, 1H), 9.43 (s, 1H), 12.50 (s, 1H); MS (ESI) *m*/*z* 398 $[M+H]^+$, 100%, 420 $[M+Na]^+$, 85%, 396 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5OS_2{\cdot}0.5H_2O$: C, 56.14; H, 4.96; N, 17.23. Found: C, 56.48; H, 4.90; N, 16.85.

5.1.30. [5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methanol (9)

To a solution of lithium aluminium hydride (134 mg, 2.84 mmol) in THF (30 mL) at -40 °C was added **7a** (700 mg, 1.89 mmol). The reaction mixture was stirred at 0 °C for 0.5 h. 2 M HCl was added to the reaction mixture and stirred for 5 min. The reaction mixture was diluted with EtOAc and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was

purified by column chromatography on silica gel eluting with 5–10% MeOH/$CHCl_3$ to afford **9** as a pale orange powder (448 mg, 72%): mp 222.0–223.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.34 (d, J = 0.9 Hz, 3H), 4.53 (s, 2H), 5.50 (br s, 1H), 7.15 (d, J = 0.9 Hz, 1H), 8.09 (br d, J = 8.5 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H), 8.87 (br s, 1H), 9.43 (s, 1H), 12.79 (br s, 1H); MS (ESI) m/z 329 $[M+H]^+$, 100%, 327 $[M-H]^-$, 100%; Anal. Calcd for $C_{15}H_{12}N_4OS_2{\cdot}0.2H_2O$: C, 54.26; H, 3.76; N, 16.87. Found: C, 54.20; H, 3.55; N, 16.79.

5.1.31. [5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl ethylcarbamate (10)

To a solution of **9** (239 mg, 0.728 mmol), CuCl (7 mg, 0.07 mmol) and pyridine (1 mL) in toluene (2 mL) was added isocyanatoethane (57 mg, 0.80 mmol). The reaction mixture was stirred at 50 °C for 2 h. Water was added to the reaction mixture and the reaction mixture was extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on silica gel eluting with 3% MeOH/$CHCl_3$. The amorphous obtained was crystallized from EtOAc/*n*-Hexane to afford **10** as a colorless powder (151 mg, 52%): mp 144.0–145.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.03 (t, J = 7.2 Hz, 3H), 2.34 (s, 3H), 2.99–3.11 (m, 2H), 5.03 (s, 2H), 7.18 (d, J = 0.8 Hz, 1H), 7.33 (br t, J = 5.7 Hz, 1H), 8.08 (br d, J = 8.5 Hz, 1H), 8.15 (d, J = 8.5 Hz, 1H), 8.90 (br s, 1H), 9.45 (s, 1H), 13.06 (br s, 1H); MS (ESI) m/z 400 $[M+H]^+$, 10%, 422 $[M+Na]^+$, 20%, 398 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{17}N_5O_2S_2{\cdot}0.2H_2O$: C, 53.63; H, 4.35; N, 17.37. Found: C, 53.92; H, 4.28; N, 17.08.

5.1.32. *N*-{2-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]ethyl}propanamide (12a)

To a solution of **2** (300 mg, 1.04 mmol) and **11a** (317 mg, 1.56 mmol) in THF (30 mL) was added NH_4OAc (802 mg, 10.4 mmol) in MeOH (30 mL). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was diluted with EtOAc and washed with a saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on silica gel eluting with 0–3% MeOH/$CHCl_3$ to afford 2-{2-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]ethyl}-1*H*-isoindole-1,3(2*H*)-dione as a yellow solid (437 mg, 89%): MS (ESI) m/z 472 $[M+H]^+$, 100%, 470 $[M-H]^-$, 100%.

To a solution of 2-{2-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]ethyl}-1*H*-isoindole-1,3(2*H*)-dione (437 mg, 0.928 mmol) in MeOH (50 mL) was added hydrazine monohydrate (500 mg, 9.99 mmol). The reaction mixture was stirred at room temperature for 20 h and concentrated. The residue was diluted with $CHCl_3$ and washed with water four times. The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by column chromatography on NH–silica gel eluting with 3–10% MeOH/$CHCl_3$ to afford 2-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]ethanamine (177 mg, 56%): MS (ESI) m/z 342 [M+H]+, 100%, 340 $[M-H]^-$, 100%.

To a solution of 2-[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]ethanamine (177 mg, 0.519 mmol) in $CHCl_3$ (2 mL) were added propionyl chloride (72 mg, 0.78 mmol) and NEt_3 (104 mg, 1.03 mmol). The reaction mixture was stirred at room temperature for 0.5 h. The reaction mixture was diluted with EtOAc and washed with a saturated aqueous solution of $NaHCO_3$. The organic layer was dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography on NH–silica gel eluting with 0–5% MeOH/$CHCl_3$ to afford **12a** as a pale yellow amorphous (153 mg, 74%): ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.16 (t, J = 7.5 Hz, 3H), 2.26 (q, J = 7.5 Hz, 2H), 2.45 (d, J = 0.9 Hz, 3H), 3.03–3.14 (m, 2H), 3.73–3.85 (m, 2H), 6.34 (br s, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.94 (dd, J = 8.4, 1.8 Hz, 1H), 8.18 (d, J = 8.4 Hz, 1H), 8.60 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 398 $[M+H]^+$, 100%, 396 $[M-H]^-$, 15%; Anal. Calcd for $C_{19}H_{19}N_5OS_2{\cdot}0.9H_2O$: C, 55.16; H, 5.07; N, 16.93. Found: C, 55.30; H, 5.07; N, 16.65.

5.1.33. *N*-{3-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]propyl}acetamide (12b)

Yield: 20%, a pale yellow amorphous: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.80–2.01 (m, 2H), 2.10 (s, 3H), 2.46 (d, J = 1.3 Hz, 3H), 2.80–2.92 (m, 2H), 3.35–3.51 (m, 2H), 5.96 (br s, 1H), 6.76 (s, 1H), 8.02 (d, J = 8.8 Hz, 1H), 8.17 (d, J = 8.8 Hz, 1H), 9.01 (s, 1H); MS (ESI) m/z 398 $[M+H]^+$, 100%; HRMS (ESI/APCI Dual) m/z Calcd for $C_{19}H_{19}N_5OS_2$ $[M+H]^+$, 398.1104; Found 398.1094; 100% pure by LC–MS.

5.1.34. *tert*-Butyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (14)

Yield: 88%, a pale yellow amorphous: ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.47 (s, 9H), 2.43 (d, J = 0.9 Hz, 3H), 4.43 (d, J = 6.2 Hz, 2H), 5.50 (br t, J = 6.2 Hz, 1H), 6.74 (s, 1H), 7.82 (dd, J = 8.5, 1.7 Hz, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.41–8.61 (m, 1H), 9.02 (s, 1H); MS (ESI) m/z 428 $[M+H]^+$, 100%, 426 $[M-H]^-$, 100%; HRMS (ESI/APCI Dual) m/z Calcd for $C_{20}H_{21}N_5O_2S_2$ $[M+H]^+$, 428.1209; Found 428.1183; 96% pure by LC–MS.

5.1.35. 1-[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methanamine dihydrochloride (15)

To a solution of **14** (22.0 g, 51.5 mmol) in $CHCl_3$ (800 mL) was added 4 M HCl in dioxane (130 mL). The reaction mixture was stirred at room temperature for 16 h. The reaction mixture was concentrated and the residue was recrystallized from MeOH/Et_2O to afford **15** as a light brown powder (20.5 g, 99%): mp 229.0–233.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.37 (d, J = 0.9 Hz, 3H), 4.16–4.25 (m, 2H), 7.22 (d, J = 0.9 Hz, 1H), 8.08 (dd, J = 8.6, 1.8 Hz, 1H), 8.18 (d, J = 8.6 Hz, 1H), 8.55–8.65 (m, 3H), 8.90 (d, J = 1.8 Hz, 1H), 9.47 (s, 1H); MS (ESI) m/z 328 $[M+H]^+$, 100%, 326 $[M-H]^-$, 100%; Anal. Calcd for $C_{15}H_{13}N_5S_2{\cdot}2HCl{\cdot}2H_2O$: C, 41.29; H, 4.39; N, 16.05. Found: C, 41.10; H, 4.09; N, 16.12.

5.1.36. 1-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}-3-ethylurea (16a)

To a solution of **15** (500 mg, 1.25 mmol) and NEt_3 (379 mg, 3.75 mmol) in $CHCl_3$ (10 mL) at 0 °C was added isocyanatoethane (108 mg, 1.52 mmol). The reaction mixture was stirred at 0 °C for 1.5 h and concentrated. The crude product was purified by column chromatography on NH–silica gel eluting with 5% MeOH/$CHCl_3$. The powder obtained was recrystallized from MeOH/EtOAc/*n*-Hexane to afford **16a** as a colorless powder (199 mg, 40%): mp 209.0–210.5 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.01 (t, J = 7.2 Hz, 3H), 2.34 (s, 3H), 2.99–3.10 (m, 2H), 4.30 (d, J = 5.6 Hz, 2H), 6.08 (t, J = 5.5 Hz, 1H), 6.33 (t, J = 5.6 Hz, 1H), 7.15 (d, J = 0.9 Hz, 1H), 8.01–8.11 (m, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.86 (br s, 1H), 9.43 (s, 1H), 12.69 (br s, 1H); MS (ESI) m/z 399 $[M+H]^+$, 100%, 421 $[M+Na]^+$, 15%, 397 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{18}N_6OS_2{\cdot}0.2H_2O$: C, 53.77; H, 4.61; N, 20.90. Found: C, 53.70; H, 4.53; N, 20.80.

5.1.37. *N*-{[5-(1,3-Benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}propane-1-sulfonamide (16b)

Yield: 15%, a light brown powder: ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.06 (t, J = 7.5 Hz, 3H), 1.80–1.96 (m, 2H), 2.43 (d, J = 0.9 Hz, 3H), 3.03–3.14 (m, 2H), 4.44 (d, J = 6.2 Hz, 2H), 5.46 (br t, J = 6.2 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.83 (dd, J = 8.5, 1.8 Hz, 1H), 8.16 (dd, J = 8.5, 0.5 Hz, 1H), 8.44 (br s, 1H), 9.04 (s, 1H); MS (ESI) m/z 434

$[M+H]^+$, 100%, 456 $[M+Na]^+$, 50%, 432 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{19}N_5O_2S_3 \cdot 0.3H_2O$: C, 49.25; H, 4.50; N, 15.95. Found: C, 49.16; H, 4.25; N, 15.70.

5.1.38. Ethyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16c)

Yield: 39%, a colorless powder: mp 183.0–184.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.27 (t, J = 7.1 Hz, 3H), 2.44 (d, J = 0.9 Hz, 3H), 4.18 (q, J = 7.1 Hz, 2H), 4.47 (d, J = 6.2 Hz, 2H), 5.56 (br t, J = 6.2 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.85 (dd, J = 8.5, 1.7 Hz, 1H), 8.16 (dd, J = 8.5, 0.6 Hz, 1H), 8.49 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 400 $[M+H]^+$, 100%, 422 $[M+Na]^+$, 45%, 398 $[M-H]^-$, 100%; Anal. Calcd for $C_{18}H_{17}N_5O_2S_2$: C, 54.12; H, 4.29; N, 17.53. Found: C, 54.12; H, 4.28; N, 17.46.

5.1.39. Methyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16d)

Yield: 22%, a colorless powder: mp 207.0–209.0 °C; ^{1}H NMR (300 MHz, DMSO-d_6, δ): 2.34 (s, 3H), 3.58 (s, 3H), 4.31 (d, J = 5.9 Hz, 2H), 7.15 (d, J = 0.9 Hz, 1H), 7.67 (br t, J = 5.9 Hz, 1H), 8.08 (br s, 1H), 8.14 (d, J = 8.5 Hz, 1H), 8.84 (br s, 1H), 9.43 (s, 1H), 12.72 (br s, 1H); MS (ESI) m/z 386 $[M+H]^+$, 100%, 408 $[M+Na]^+$, 60%, 384 $[M-H]^-$, 100%; Anal. Calcd for $C_{17}H_{15}N_5O_2S_2 \cdot 0.2H_2O$: C, 52.48; H, 3.99; N, 18.00. Found: C, 52.69; H, 3.93; N, 17.65.

5.1.40. Propyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16e)

Yield: 34%, a colorless powder: mp 200.5–201.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.94 (t, J = 7.4 Hz, 3H), 1.57–1.73 (m, 2H), 2.44 (d, J = 0.9 Hz, 3H), 4.08 (t, J = 6.7 Hz, 2H), 4.47 (d, J = 6.1 Hz, 2H), 5.54 (br t, J = 6.1 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.16 (dd, J = 8.5, 0.5 Hz, 1H), 8.50 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 414 $[M+H]^+$, 100%, 436 $[M+Na]^+$, 65%, 412 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5O_2S_2 \cdot 0.15H_2O$: C, 54.83; H, 4.67; N, 16.83. Found: C, 55.10; H, 4.65; N, 16.54.

5.1.41. Butyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16f)

Yield: 24%, a colorless powder: mp 161.0–162.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.92 (t, J = 7.3 Hz, 3H), 1.22–1.49 (m, 2H), 1.50–1.73 (m, 2H), 2.44 (d, J = 0.9 Hz, 3H), 4.13 (t, J = 6.6 Hz, 2H), 4.47 (d, J = 6.2 Hz, 2H), 5.52 (br t, J = 6.2 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.85 (dd, J = 8.4, 1.8 Hz, 1H), 8.16 (d, J = 8.4 Hz, 1H), 8.49 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 428 $[M+H]^+$, 100%, 426 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{21}N_5O_2S_2$: C, 56.18; H, 4.95; N, 16.38. Found: C, 55.85; H, 5.10; N, 16.34.

5.1.42. Pentyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16g)

Yield: 42%, a colorless powder: mp 177.5–178.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.89 (t, J = 7.1 Hz, 3H), 1.28–1.39 (m, 4H), 1.56–1.71 (m, 2H), 2.44 (d, J = 0.9 Hz, 3H), 4.12 (t, J = 6.7 Hz, 2H), 4.48 (d, J = 6.1 Hz, 2H), 5.48 (br t, J = 6.1 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.17 (dd, J = 8.5, 0.5 Hz, 1H), 8.51 (br s, 1H), 9.04 (s, 1H); MS (ESI) m/z 442[M+H]$^+$, 100%, 464 $[M+Na]^+$, 40%, 440 $[M-H]^-$, 100%; Anal. Calcd for $C_{21}H_{23}N_5O_2S_2$: C, 57.12; H, 5.25; N, 15.86. Found: C, 56.96; H, 5.27 N, 15.53.

5.1.43. Propan-2-yl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16h)

Yield: 30%, a colorless powder: mp 180.0–181.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 1.25 (d, J = 6.6 Hz, 6H), 2.44 (d, J = 0.9 Hz, 3H), 4.46 (d, J = 6.2 Hz, 2H), 4.85–5.05 (m, 1H), 5.49 (br t, J = 6.2 Hz, 1H), 6.74 (d, J = 0.9 Hz, 1H), 7.85 (dd, J = 8.4, 1.8 Hz, 1H), 8.15 (d, J = 8.4 Hz, 1H), 8.50 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 414 $[M+H]^+$, 100%, 436 $[M+Na]^+$, 35%, 412 $[M-H]^-$, 100%; Anal. Calcd for $C_{19}H_{19}N_5O_2S_2$: C, 55.19; H, 4.63; N, 16.94. Found: C, 54.93; H, 4.63; N, 16.92.

5.1.44. 2-Methylpropyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16i)

Yield: 21%, a colorless powder: mp 142.5–144.0 °C; ^{1}H NMR (200 MHz, $CDCl_3$, δ): 0.93 (d, J = 6.6 Hz, 6H), 1.81–2.03 (m, 1H), 2.44 (d, J = 0.9 Hz, 3H), 3.92 (d, J = 6.6 Hz, 2H), 4.48 (d, J = 6.2 Hz, 2H), 5.49 (br t, J = 6.2 Hz, 1H), 6.75 (d, J = 0.9 Hz, 1H), 7.86 (dd, J = 8.4, 1.8 Hz, 1H), 8.17 (d, J = 8.4 Hz, 1H), 8.51 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 428[M+H]$^+$, 100%, 426 $[M-H]^-$, 100%; Anal. Calcd for $C_{20}H_{21}N_5O_2S_2$: C, 56.18; H, 4.95; N, 16.38. Found: C, 55.87; H, 4.90 N, 16.05.

5.1.45. 2,2-Dimethylpropyl {[5-(1,3-benzothiazol-6-yl)-4-(4-methyl-1,3-thiazol-2-yl)-1*H*-imidazol-2-yl]methyl}carbamate (16j)

Yield: 34%, a colorless powder: mp 170.5–171.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.93 (s, 9H), 2.44 (d, J = 0.9 Hz, 3H), 3.83 (s, 2H), 4.48 (d, J = 6.1 Hz, 2H), 5.55 (br t, J = 6.1 Hz, 1H), 6.75 (s, 1H), 7.86 (dd, J = 8.5, 1.7 Hz, 1H), 8.16 (d, J = 8.5 Hz, 1H), 8.50 (br s, 1H), 9.03 (s, 1H); MS (ESI) m/z 442[M+H]$^+$, 100%, 464 $[M+Na]^+$, 45%, 440 $[M-H]^-$, 100%; Anal. Calcd for $C_{21}H_{23}N_5O_2S_2$: C, 57.12; H, 5.25; N, 15.86. Found: C, 57.01; H, 5.26 N, 15.51.

5.2. Solubility

An excess amount of each compound was added to 1,3-butylene glycol/ethanol/water (10 g/79 mL/diluted to 100 mL) and shaken on a shaker (model SA31; Yamato Kagaku) at room temperature for 24 h. The suspension was filtrated with 0.45 μm filter (Ekicrodisc 13CR; Pall) and diluted with 50% aqueous acetonitrile solution; the concentrations were then measured by HPLC. The HPLC analysis was performed using a Shimadzu HPLC system composed of a LC-10AD, SPD-10AV and SIL-10A. The conditions for HPLC were as follows: mobile phase, 10 mM ammonium acetate aqueous solution/acetonitrile = 6:4; flow rate, 1.0 mL/min; column, reversed-phase (Capcell Pak C18 UG120, 5 μm, 4.6 × 150 mm; Shiseido) at 40 °C; and detection wavelength, 270 nm.

5.3. In vitro pharmacological studies

5.3.1. ALK5 inhibitory activity in an enzyme assay

ALK5 inhibitory activity of the compounds was measured using a QS S Assist TGFβR1 (ALK5) ELISA Kit (Carna Biosciences Inc., Japan) according to the manufacturer's instructions. Briefly, a GST-tagged kinase domain of human ALK5 was incubated with 125 nM GST-tagged Samd3 in 15 mM Tris–HCl (pH 7.5), 0.01% Tween 20, 2 mM DTT and 5 mM $MnCl_2$ containing 2 μM ATP and different concentrations of compounds for 30 min at room temperature. The reaction was stopped with EDTA, and the reaction mixture was transferred to glutathione coated 96-well plates. Each well was treated with anti-phosphorylated Smad3 antibody and HRP labeled secondary antibody, followed by incubation with TMB substrate for 30 min at room temperature. After the addition of the stop solution, the absorbance at 450 nm of each well was measured using an Infinite 200 Pro (TECAN). The IC_{50} values were calculated by analyzing the concentration-response curves.

5.3.2. TGF-β-induced Smad2/3 phosphorylation inhibitory activity in a cell-based assay

A549 cells were cultured in plates overnight at 37 °C in a 5% CO_2-95% air atmosphere and pretreated with various concentrations of compounds or DMSO as a control for 2 h followed by the

addition of 1 ng/mL of TGF-β1 (R&D Systems). After 1 h of incubation, the cells were washed with PBS and lysed with RIPA solution, then mixed with biotinylated anti-Smad2/3 antibody (Santa Cruz). The mixture was transferred to a streptavidin-coated 96-well plate and left to stand for 2 h. Then, the mixture was discarded, and each well was treated with Rabbit anti-phosphorylated serine antibody (Zymed Laboratories Inc.), followed by Eu-labeled anti-Rabbit IgG antibody and DELFIA Enhancement Solution (Perkin Elmer Life Sciences). The developed fluorescence was measured using an ARVO multi-label counter (Perkin Elmer Life Sciences). The IC_{50} values were determined by analyzing the concentration-response curves.

5.3.3. Kinase selectivity assay

Microfluidics-based technology was used in this assay for kinase profiling. The base components of the screening were a Lab Chip® 3000 instrument (Caliper Life Sciences) and a biochemical assay using the ProfilerPro™ Kinase Selectivity Assay Kit (Caliper Life Sciences). The assay was carried out in a final volume of 25 μL containing fluorescently labeled peptide substrate, enzyme, ATP and test compound. This technology also used the charge or shift in electrophoretic mobility of the labeled substrates upon enzymatic conversion to its product. As a result, this assay system eliminated the need for radioactive reagents or other secondary reagents, such as antibodies.

Briefly, recombinant enzyme was preincubated with or without the test compounds (final concentration 10 μM) at 28 °C for 15 min in 100 mM HEPES (pH7.5) containing 10 mM $MgCl_2$, 4% DMSO, 0.003% Brij 35, 0.004% Tween 20 and 1 mM dithiothreirescentol. Fluorescently labeled peptide substrate (final concentration, 1.5 μM) and ATP (at the ATP Km apparent) were added and incubated at 28 °C for 90 min. The kinase reaction was terminated by the addition of 3 mM EDTA. The phosphorylated peptide was separated from the substrate peptide and quantified using the Lab Chip® 3000, then directly used to quantify the product conversion rate.

5.4. In vivo pharmacological studies

5.4.1. Animals

C57BL/6 mice (8-week-old female mice; Charles River, Yokohama, Japan) were maintained under a 12-h light/dark cycle in a temperature and humidity-controlled holding room. The animals were given free access to food and water. All the studies were reviewed by the Taisho Pharmaceutical Co., Ltd. Animal Care Committee.

5.4.2. Smad2 phosphorylation inhibitory activity in Mouse skin

Under anesthetization with pentobarbital, the dorsal hairs of 8-week-old C57BL/6 mice were depilated using depilatory wax. Sixteen days after depilation, when the hair follicles in the depilated area were in the late anagen phase, 200 μL of **16i** lotion or vehicle was applied topically to the dorsal area. At 8 h after application, the skin tissues were harvested. Each skin sample was homogenized with 50 mM Tris–HCl buffer (pH 7.6) containing 150 mM NaCl and 1% NP-40, then centrifuged at 3000 rpm for 15 min. The supernatant was subjected to SDS-PAGE, followed by the transfer of the proteins to the PVDF membrane. The membranes were probed with Rabbit anti-phosphorylated Smad2 antibody (Cell Signaling), followed by HRP-labeled anti-Rabbit IgG secondary antibody. Finally, ECL Western Blotting Detection Reagents (GE Healthcare) was used to detect the protein bands. The light-emitting amount of each band was measured using a Lumi-Imager F1 (Roche Diagnostics). Subsequently, the membranes were stripped and reprobed with Rabbit anti-Smad2/3 antibody (Cell Signaling) as a loading control. After treatment with HRP-labeled anti-Rabbit IgG secondary antibody and ECL Western Blotting Detection Reagents, the light-emitting amount of each band was measured using a Lumi-Imager F1.

References and notes

1. Heldin, C.-H.; Miyazono, K.; ten Dijke, P. *Nature* **1997**, *390*, 465.
2. Shi, Y.; Massague, J. *Cell* **2003**, *113*, 685.
3. Yingling, J. M.; Blanchard, K. L.; Sawyer, J. S. *Nat. Rev. Drug Disc.* **2004**, *1011*, 3.
4. Leask, A.; Abraham, D. J. *FASEB J.* **2004**, *18*, 816.
5. Agrotis, A.; Kalinina, N.; Bobik, A. *Curr. Vasc. Pharmacol.* **2005**, *3*, 55.
6. Bierie, B.; Moses, H. L. *Nat. Rev. Cancer* **2006**, *6*, 506.
7. Massague, J. *Annu. Rev. Cell Biol.* **1990**, *6*, 597.
8. Soma, T.; Ogo, M.; Suzuki, J.; Takahashi, T.; Hibino, T. *J. Invest. Dermatol.* **1998**, *111*, 948.
9. Foitzik, K.; Lindner, G.; Mueller-Roever, S.; Maurer, M.; Botchkareva, N.; Botchkarev, V.; Handjiski, B.; Metz, M.; Hibino, T.; Soma, T.; Dotto, G. P.; Paus, R. *FASEB J.* **2000**, *14*, 752.
10. Inui, S.; Fukuzato, Y.; Nakajima, T.; Yoshikawa, K.; Itami, S. *FASEB J.* **1967**, *2002*, 16.
11. Ito, Y.; Sarkar, P.; Mi, Q.; Wu, N.; Bringas, P., Jr.; Liu, Y.; Reddy, S.; Maxson, R.; Deng, C.; Chai, Y. *Dev. Biol.* **2001**, *236*, 181.
12. Callahan, J. F.; Burgess, J. L.; Fornwald, J. A.; Gaster, L. M.; Harling, J. D.; Harrington, F. P.; Heer, J.; Kwon, C.; Lehr, R.; Mathur, A.; Olson, B. A.; Weinstock, J.; Laping, N. J. *J. Med. Chem.* **2002**, *45*, 999.
13. Inman, G. J.; Nicolas, F. J.; Callahan, J. F.; Harling, J. D.; Gaster, L. M.; Reith, A. D.; Laping, N. J.; Hill, C. S. *Mol. Pharmacol.* **2002**, *62*, 65.
14. Gellibert, F.; Woolven, J.; Fouchet, M.-H.; Mathews, N.; Goodland, H.; Lovegrove, V.; Laroze, A.; Nguyen, V.-L.; Sautet, S.; Wang, R.; Janson, C.; Smith, W.; Krysa, G.; Boullay, V.; de Gouville, A.-C.; Huet, S.; Hartley, D. *J. Med. Chem.* **2004**, *47*, 4494.
15. Gellibert, F.; Fouchet, M.-H.; Nguyen, V.-L.; Wang, R.; Krysa, G.; de Gouville, A.-C.; Huet, S.; Dodic, N. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2277.
16. Guckian, K.; Carter, M. B.; Lin, E. Y.-S.; Choi, M.; Sun, L.; Boriack-Sjodin, P. A.; Chuaqui, C.; Lane, B.; Cheung, K.; Ling, L.; Lee, W.-C. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 326.
17. Sawyer, J. S.; Beight, D. W.; Britt, K. S.; Anderson, B. D.; Campbell, R. M.; Goodson, T., Jr.; Herron, D. K.; Li, H.-Y.; McMillen, W. T.; Mort, N.; Parsons, S.; Smith, E. C. R.; Wagner, J. R.; Yan, L.; Zhang, F.; Yingling, J. M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3581.
18. Kim, D.-K.; Jang, Y.; Lee, H. S.; Park, H.-J.; Yoo, J. *J. Med. Chem.* **2007**, *50*, 3143.
19. Goldberg, F. W.; Ward, R. A.; Powell, S. J.; Debreczeni, J. É.; Norman, R. A.; Roberts, N. J.; Dishington, A. P.; Gingell, H. J.; Wickson, K. F.; Roberts, A. L. *J. Med. Chem.* **2009**, *52*, 7901.
20. Amada, H.; Sekiguchi, Y.; Ono, N.; Matsunaga, Y.; Koami, T.; Asanuma, H.; Shiozawa, F.; Endo, M.; Ikeda, A.; Aoki, M.; Fujimoto, N.; Wada, R.; Sato, M. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2024.
21. Naruse, T.; Aoki, M.; Fujimoto, N.; Ueda, Y.; Sato, M.; Amada, H.; Koami, T.; Wada, R.; Ikeda, A. Manuscript in preparation.
22. Bates, H. A.; Farina, J. *J. Org. Chem.* **1985**, *50*, 3843.
23. Müller-Röver, S.; Handjiski, B.; van der Veen, C.; Eichmüller, S.; Foitzik, K.; Mckay, I. A.; Stenn, K. S.; Paus, R. *J. Invest. Dermatol.* **2001**, *117*, 3.