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Synthesis and biological evaluation of 2-pyridyl-substituted pyrazoles and imidazoles as transforming growth factor-β type 1 receptor kinase inhibitors

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ABSTRACT

A series of 2-pyridyl-substituted pyrazoles (**16a–d**, **17**, **18**, and **28a–e**) and imidazoles (**22** and **23**) has been synthesized and evaluated for their ALK5 inhibitory activity in cell-based luciferase reporter assays. Among them, 3-(3-(6-methylpyridin-2-yl)-4-(quinolin-6-yl)-1*H*-pyrazole-1-carbothioamido)benzamide (**28c**) showed 96% and 93% inhibition at 0.1 μM in luciferase reporter assays using HaCaT cells transiently transfected with p3TP-luc reporter construct and ARE-luc reporter construct, respectively.

Preceding its purification and cloning in the 1980s transforming growth factor (TGF)-β1 has become a subject of intense investigation, initially by virtue of its potential to direct the diverse phenotypic changes in normal cells and later due to the finding that it could inhibit growth in epithelial and immune cells.[1] TGF-β1 represents the prototypic member of the TGF-β superfamily. TGF-βs signal through a heterotetrameric receptor complex that consists of two 'type II' and two 'type I' transmembrane serine/threonine kinase receptors. The signaling cascade is initiated by the binding of ligand to the constitutively active type II receptor which further recruits the type I receptor, also named as activin-like kinase 5 (ALK5), into the complex. Subsequently, ALK5 is phosphorylated in the juxtamembrane GS domain by the type II receptor thereby stimulating its kinase activity.[2] The activated ALK5 propagates the signals through phosphorylation of receptor-regulated Smads that in turn form complexes with the common-mediator Smads. These Smad complexes, when delivered to the nucleus regulate the expression of several hundred genes involved in cell differentiation, proliferation, apoptosis, migration, and extracellular matrix production.[3]

TGF-β plays a pivotal role in the initiation and progression of fibrosis in a variety of organ systems such as kidney,[4] heart,[5] lung,[6] and liver.[7] Perturbation of TGF-β signaling has been implicated in various human diseases including cancer,[8] pancreatic diseases,[9] and hematological malignancies.[10]

One of the strategies used to inhibit TGF-β signaling is to block the catalytic activity of ALK5 by the use of small molecule inhibitors. Due to growing interest of pharmaceutical industries, several inhibitors such as **1** (SB-431542),[11] **2** (SB-505124),[12] **3** (SB-525334),[13] **4** (GW6604),[14] **5** (SD-208),[15] and **6** (LY580276)[16] are in various stages of clinical development (Fig. 1). These inhibitors have indicated palliative effects on diseases modulated by TGF-β.[11,17–19]

We have reported a number of 2-pyridyl-substituted heterocycles as ALK5 inhibitors such as IN-1166 (**7**), IN-1130 (**8**), and IN-1233 (**9**) and found that insertion of a methylene, an aminomethylene, or a methyleneamino linkage between a central five-membered heterocyclic ring and a phenyl group markedly increased ALK5 inhibitory activity and selectivity.[20–30] The compound **8**, one of our preclinical candidates, has shown activity as a suppressor of fibrogenic process of unilateral ureteral obstruction in rats underscoring the potential clinical benefits in the treatment of renal fibrosis.[27] This compound reduced not only TGF-β signaling in the CNS but also the onset and severity of paralytic disease.[28] Additionally, it has been observed that the injection of **8** into the tunica albuginea in rats lessened tunical fibrosis and corrected penile curvature.[29] Recent studies demonstrated that **9** effectively prevented the development and progression of pulmonary arterial hypotension in the monocrotaline rat model through the inhibition of TGF-β signaling.[30]

A novel class of ALK5 inhibitors that possess a carbothioamide linkage between a pyrazole ring and a phenyl group has been reported by Tojo et al.[31] One of the representative compounds, A-83-01 (**10**), from this carbothioamide series exhibited significant inhibition of the transcriptional activity induced by ALK5. The compound **10** is more potent ALK5 inhibitor than **1** and was found to be more efficacious to prevent phosphorylation of Smad2/3 and the growth inhibition induced by TGF-β.[31]

It has been established by us that a carbonitrile- or carboxamide-substituted phenyl substitution on a central five-membered

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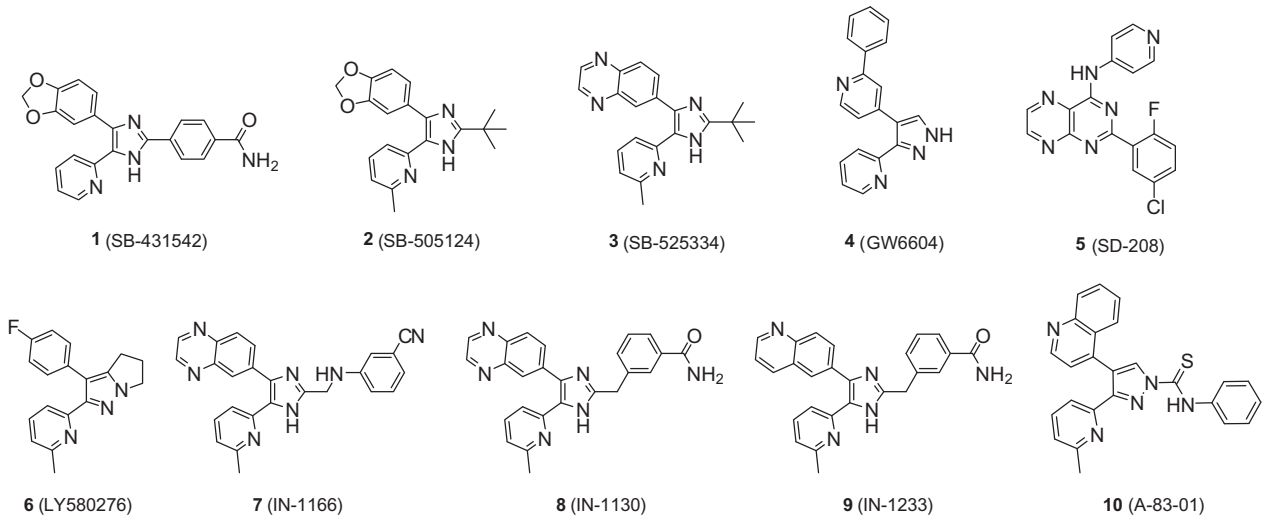


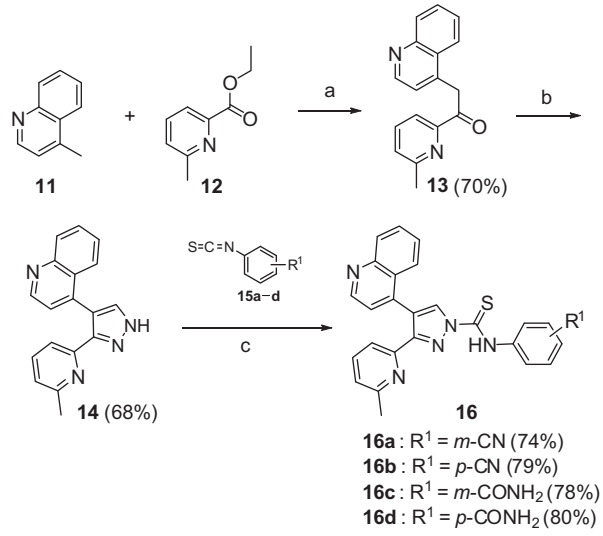
Figure 1. ALK5 inhibitors under development.

heterocyclic ring in ALK5 inhibitors increased activity and selectivity.[20,23–27] On the basis of these results, a new series of chimeric molecules **16a–d** and **28a–e** possessing a carbonitrile- or carboxamide-substituted phenyl substituent on a pyrazole and an imidazole ring, derived from **9** and **10**, were designed as target molecules (Fig. 2). Since the 6-quinolinyl moiety of **9** was proved to be one of the most suitable warhead group,[24] we replaced the 4-quinolinyl moiety on **16a–d** with the 6-quinolinyl moiety (**28a–d**) for comparison.

To compare the influence of the carbothioamide linkage of **16a** and **16c**, their counterpart derivatives **17** and **18** having the methylene linkage were prepared. We also prepared the imidazole derivatives **22** and **23** along with the pyrazoles **17** and **18** to investigate the effect of the central heterocycles in ALK5 inhibition. Expectedly, this strategy was successful and provided us with more active ALK5 inhibitors than **10**. Here, we are now reporting the valuable structure–activity relationships of these new chimeric molecules.

The 4-quinolinyl pyrazole derivatives **16a–d** were prepared as shown in Scheme 1.

Treatment of lepidine **11** with sodium bis(trimethylsilyl)amide in anhydrous THF at −60 °C followed by reaction with ethyl 6-methylpicolinate (**12**) gave 1-(6-methylpyridin-2-yl)-2-(quinolin-4-yl)ethanone (**13**) in 70% yield.[32] Cyclization of the ethanone **13** by reaction with dimethylformamide dimethylacetal and hydrazine monohydrate in the presence of acetic acid in DMF provided



Scheme 1. Reagents and conditions: (a) sodium bis(trimethylsilyl)amide, anhydrous THF, −60 °C, Ar atmosphere; (b) DMF?DMA, acetic acid, DMF, $N_2H_4{\cdot}H_2O$; (c) 3- or 4-isothiocyanatobenzonitrile (**15a** or **15b**), 3- or 4-isothiocyanatobenzamide (**15c** or **15d**), NaH, anhydrous THF.

the pyrazole **14** in 68% yield.[32] The pyrazole **14** was alkylated with carbonitrile- or carboxamide-substituted phenyl isothiocynates **15a–d** using NaH in anhydrous THF to afford the triaryl-substituted derivatives **16a–d** with a carbothioamide linkage between pyrazole and phenyl moiety in 74–80% yields. The cyanophenyl isothiocynates **15a** and **15b** were commercially available, and the carboxamide-substituted phenyl isothiocynates **15c** and **15d** were prepared[33] in 78% and 82% yields, respectively, from the reaction of *meta*- or *para*-aminobenzamide with thiophosgene in the presence of $NaHCO_3$ in water and chloroform.

The 4-quinolinyl pyrazole derivatives **17** and **18** having a methylene linkage were prepared as shown in Scheme 2.

The pyrazole **14** was alkylated using α-bromo-*m*-tolunitrile to afford the carbonitrile derivative **17** in 75% yield. Hydrolysis of the nitrile **17** with 28% H_2O_2 and 1 N NaOH solution gave the carboxamide **18** in 90% yield.

To synthesize the 4-quinolinyl imidazole derivatives **22** and **23**, the ketone **13** was converted to the α-oximinoketone **19** which was obtained as a mixture of oxime regioisomers (Scheme 3).

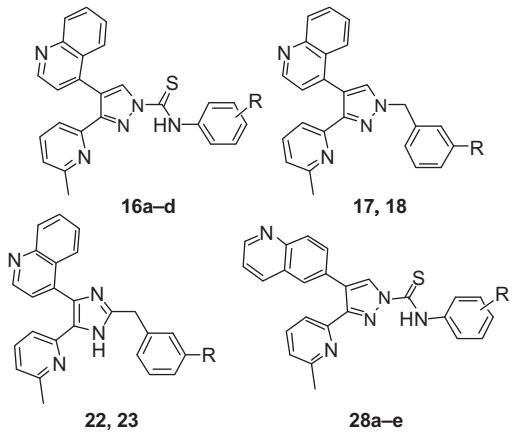
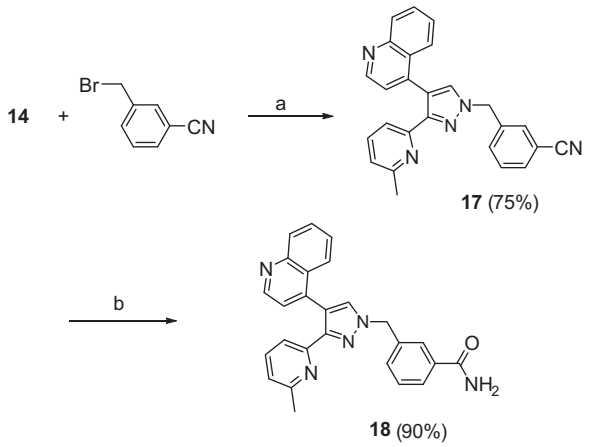
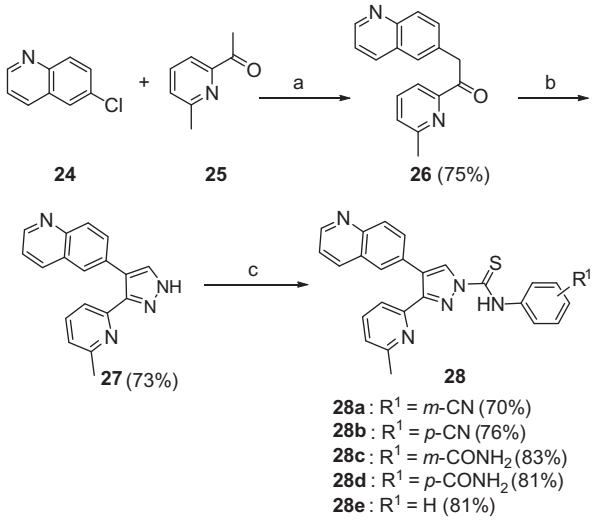


Figure 2. Target compounds.



Scheme 2. Reagents and conditions: (a) NaH, anhydrous THF; (b) 28% H_2O_2, 1 N NaOH, EtOH, 40 °C.



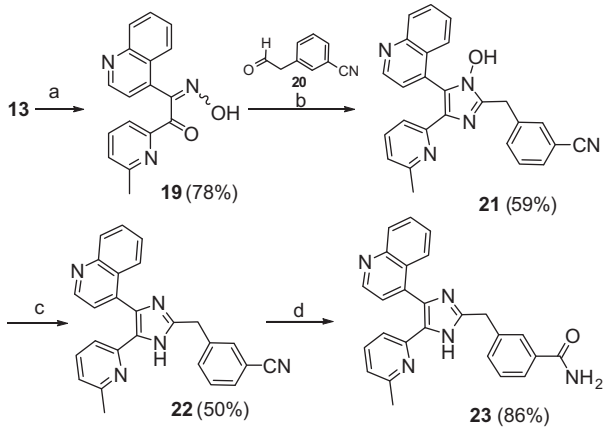
Scheme 4. Reagents and conditions: (a) palladium acetate, 2-dicyclohexylphosphino-2′-(*N*,*N*-dimethylamino)biphenyl, potassium *tert*-butoxide, anhydrous THF, 80 °C, Ar atmosphere; (b) DMF?DMA, acetic acid, DMF, $N_2H_4{\cdot}H_2O$; (c) **15a–d** or isothiocyanatobenzene (**15e**), NaH, anhydrous THF.

The resulted oximinoketone **19** was coupled with *m*-cyanophenylacetaldehyde (**20**) and ammonium acetate to give the *N*-hydroxy imidazole **21** in 59% yield. The imidazole **21** was reduced to **22** by reaction with triethyl phosphite[34] in 50% yield, and then the carbonitrile group of **22** was hydrolyzed to give the carboxamide **23** in 86% yield.

The 6-quinolinyl moiety of **9** was introduced into the scaffold of **10** using the synthetic strategy shown in Scheme 4.

The 6-chloroquinoline (**24**) was coupled with 2-acetyl-6-methylpyridine (**25**) using palladium catalyzed coupling reaction to obtain the ketone **26** in 75% yield.[35,36] This ketone was further cyclized by the treatment with dimethylformamide dimethylacetal and hydrazine monohydrate to give the pyrazole **27** in 73% yield, which was further alkylated with phenyl isothiocynates **15a–d** or isothiocyanatobenzene (**15e**) to afford the target compounds **28a–e** in 70–83% yields.

To evaluate TGF-β-induced downstream transcriptional activation to ALK5 signaling, cell-based luciferase activity of **16a**–**d**, **17**, **18**, **22**, **23**, and **28a**–**e** was measured using HaCaT cells transiently transfected with three different luciferase reporter genes (p3TP-luciferase reporter, ARE-luciferase reporter, and SBE-luciferase reporter) at a concentration of 0.1 μM (Table 1).[37] The p3TP-luc reporter construct contains three AP-1 binding elements and the plasminogen-activator inhibitor-1 (PAI-1) promoter.[38] The ARE-luc reporter construct consists of three copies of activin response element sequence derived from the Mix.2 homeobox gene promoter. When cotransfected with the forkhead activin signal transducer FAST-1, ARE-luc construct is induced by TGF-β- or activin-activated Smad2/4 complexes.[39] The SBE-luc reporter construct contains four tandem copies of the CAGA Smad-binding element cloned upstream of the adenovirus major late promoter (MLP).[40]



Scheme 3. Reagents and conditions: (a) $NaNO_2$, 2 N HCl, EtOH; (b) NH_4Ac, *tert*-butyl methyl ether/MeOH; (c) $P(OEt)_3$, DMF; (d) 28% H_2O_2, 1 N NaOH, EtOH, 40 °C.

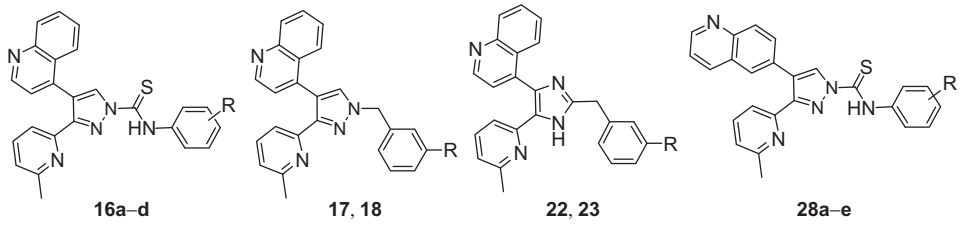
All the pyrazole derivatives having a carbothioamide linkage, **16a–d** and **28a–e**, displayed more than 85% inhibition at 0.1 μM in p3TP-luciferase reporter assay.

As we expected, introduction of a carboxamide-substituent at the *meta*-position on the phenyl ring increased ALK5 inhibitory activity, thus, showing that **16c** (p3TP-luciferase, 94%; ARE-luciferase, 74%) and **28c** (p3TP-luciferase, 96%; ARE-luciferase, 93%) were more inhibitory than the unsubstituted **10** (p3TP-luciferase, 87%; ARE-luciferase, 60%) and **28e** (p3TP-luciferase, 88%; ARE-luciferase, 88%), respectively. The pyrazole derivatives having a carbothioamide linkage, **16a** (p3TP-luciferase, 92%; ARE-luciferase, 64%; SBE-luciferase, 76%) and **16c** (p3TP-luciferase, 94%; ARE-luciferase, 74%; SBE-luciferase, 77%), displayed much higher ALK5 inhibition compared to the corresponding derivatives having a methylene linkage, **17** (p3TP-luciferase, 48%; ARE-luciferase, 11%; SBE-luciferase, 24%) and **18** (p3TP-luciferase, 43%; ARE-luciferase, 1%; SBE-luciferase, 14%).

Among the compounds having a methylene linkage, the pyrazole derivatives **17** and **18** were more potent than the respective imidazole derivatives **22** (p3TP-luciferase, 9%; ARE-luciferase, 15%; SBE-luciferase, 5%) and **23** (p3TP-luciferase, 2%; ARE-luciferase, 2%; SBE-luciferase, 10%). The compounds **28a**–**e** possessing a 6-quinolinyl moiety as a warhead group on the central pyrazole ring exhibited higher ALK5 inhibitory activity than the **16a**–**d** possessing a 4-quinolinyl moiety. This implies that the 6-quinolinyl of **9** is a better functionality as a warhead group in these kinds of ALK5 inhibitors than the 4-quinolinyl of **10**.

The most potent compound **28c** (p3TP-luciferase, 96%; ARE-luciferase, 93%; SBE-luciferase, 80%) possessing a carboxamide-substituent at the *meta*-position on the phenyl ring and a 6-quinolinyl moiety was chosen, and its ALK5 inhibitory activity was compared with **2**, **3**, **8**, and **10** at four different concentrations (0.01, 0.05, 0.1, and 0.5 μM) using HaCaT cells transiently transfected with p3TP-luciferase reporter construct. As shown in Figure 3, **28c** inhibited ALK5 in a dose-dependent manner, and it was more potent than **2**, **3**, **8**, and **10** at all four concentrations tested.

Table 1
Inhibitory activity of trisubstituted imidazole and pyrazole derivatives **15**, **17**, **18**, **21**, **22** and **27**



Compd	R	Activity[a] (% control)		
		p3TP-luciferase[b]	ARE-luciferase[b]	SBE-luciferase[b]
Mock[c]		3 ± 0	6 ± 2	20 ± 2
TGF-β		100 ± 23	100 ± 48	100 ± 10
16a	*m*-CN	8 ± 2	36 ± 6	24 ± 4
16b	*p*-CN	11 ± 3	39 ± 6	24 ± 4
16c	*m*-$CONH_2$	6 ± 3	26 ± 11	23 ± 4
16d	*p*-$CONH_2$	15 ± 9	42 ± 14	25 ± 2
17	CN	52 ± 27	89 ± 10	76 ± 9
18	$CONH_2$	57 ± 20	99 ± 7	86 ± 11
22	CN	91 ± 43	85 ± 29	95 ± 15
23	$CONH_2$	98 ± 6	98 ± 14	90 ± 15
28a	*m*-CN	5 ± 1	14 ± 7	21 ± 1
28b	*p*-CN	6 ± 1	12 ± 8	21 ± 1
28c	*m*-$CONH_2$	4 ± 1	7 ± 0	20 ± 1
28d	*p*-$CONH_2$	6 ± 1	12 ± 1	22 ± 1
28e	H	12 ± 11	12 ± 2	22 ± 2
2 (SB-505124)		35 ± 5	93 ± 2	56 ± 6
3 (SB-525334)		39 ± 8	54 ± 0	54 ± 9
8 (IN-1130)		20 ± 13	43 ± 41	42 ± 20
10 (A-83-01)		13 ± 3	40 ± 5	28 ± 5

[a] Activity is given as means ± SD of three independent experiments run in triplicate relative to control incubation with DMSO vehicle.
[b] Luciferase activity was determined at a concentration of 0.1 μM of inhibitor.
[c] Luciferase activity was determined without treatment of TGF-β and inhibitor.

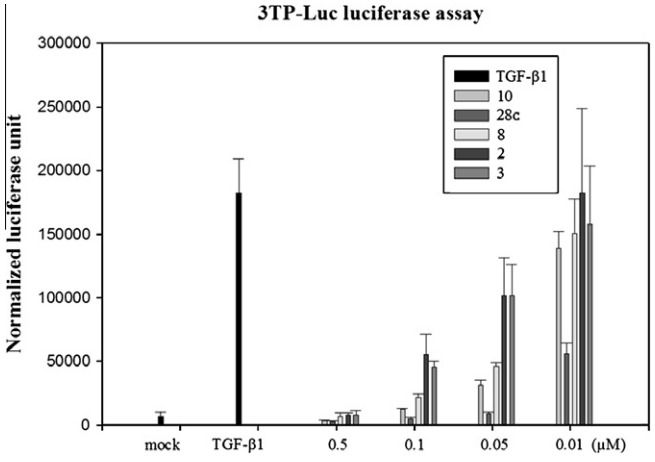


Figure 3. Effect of **28c** on the activity of TGF-β-induced ALK5. HaCaT cells were transiently transfected with p3TP-luc reporter construct. Luciferase activity was determined in the presence of different concentrations of each compound and is given as the mean ± SD of three independent experiments run in triplicate relative to control.

In this Letter, a series of 2-pyridyl-substituted pyrazoles and imidazoles containing a carbothioamide or a methylene linkage between a phenyl moiety and a central 5-membered heterocycle was synthesized and evaluated for their ALK5 inhibitory activity in cell-based luciferase reporter assays. The structure–activity relationships in this series of compounds have been established and discussed. The most potent compound in this series, **28c**, inhibited TGF-β-induced ALK5 activity 96%, 93%, and 80% at 0.1 μM in luciferase reporter assays using HaCaT cells transiently transfected with p3TP-luciferase reporter construct, ARE-luciferase reporter construct, and SBE-luciferase reporter construct, respectively.

Acknowledgments

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37. *Cellular assays to measure anti-TGF-β activity of ALK5 inhibitors:* biological activity of the test compounds was determined by measuring their ability to inhibit TGF-β-induced p3TP-luciferase reporter activity, ARE-luciferase reporter activity, and SBE-luciferase reporter activity in HaCaT cells. HaCaT cells were seeded at concentrations of 5×10^4 in 24-well plates. The next day, when they reach approximately 90% confluence, cells were transfected with 0.1 μg of p3TP-Luc reporter construct, ARE-Luc reporter construct, or SBE-Luc reporter construct and 0.1 μg of β-galactosidase, using Lipofectamine 2000 (Invitrogen). At 24 h after transfection, various concentrations of ALK5 inhibitors were added to the cells. After 2 h, cells were treated with 5 ng/mL of TGF-β for 18–24 h. Cell lysates were harvested according to the manufacturer's instruction, and luminescence was measured by a luminometer VICTOR (Perkin–Elmer Life).
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