Discovery of 7-Methoxy-6-[4-(4-methyl-1,3-thiazol-2-yl)-1H-imidazol-5-yl]-1,3-benzothiazole (TASP0382088): A Potent and Selective Transforming Growth Factor-β Type I Receptor Inhibitor as a Topical Drug for Alopecia

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7-Methoxy-6-[4-(4-methyl-1,3-thiazol-2-yl)-1H-imidazol-5-yl]-1,3-benzothiazole 11 (TASP0382088) was synthesized and evaluated as transforming growth factor-β (TGF-β) type I receptor (also known as activin receptor-like kinase 5 or ALK5) inhibitor. Compound 11, a potent and selective ALK5 inhibitor, exhibited good enzyme inhibitory activity (IC_{50}=4.8 nM) as well as inhibitory activity against TGF-β-induced Smad2/3 phosphorylation at a cellular level (IC_{50}=17 nM). The introduction of a methoxy group to the benzothiazole ring in 1 and the break up of the planarity between the imidazole ring and the thiazole ring improved the solubility in the lotion base of 11. Furthermore, the topical application of 3% 11 lotion significantly inhibited Smad2 phosphorylation in mouse skin at 8h after application (71% inhibition, compared with vehicle-treated animals).

Key words activin receptor-like kinase 5; kinase inhibitor; transforming growth factor-β; solubility; topical application; alopecia

Transforming growth factor-β (TGF-β) is a member of a large superfamily of multifunctional polypeptide factors. The TGF-β superfamily includes activins, inhibins, bone morphogenetic proteins, and TGF-βs. Three isoforms of TGF-β (TGF-β1, TGF-β2, and TGF-β3) have been identified in mammals; these isoforms are pleiotropic modulators of cell growth and differentiation, embryonic and bone development, extracellular matrix formation, hematopoiesis, and immune and inflammatory responses. 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 with a high affinity to TGF-βRII, and the ligand-bound TGF-βRII phosphorylates ALK5 in a glycine/serine rich domain. The activated ALK5, in turn, phosphorylates and activates downstream signaling molecules, the transcription factors Smad2/3, allowing them to bind to the commonly mediated Smad4. The entire hetero-Smad complex translocates to the nucleus and regulates the transcription of various TGF-β-responsive genes.5–7 The hyperactivity of the TGF-β signaling pathway underlies many human disorders, such as the excess deposition of the extracellular matrix,6 fibrotic disorders,5 and progressive cancers.6

Therefore, the inhibition of ALK5 seems to be a good strategy for the treatment of these disorders.

On the other hand, TGF-β is well known to exhibit significant growth inhibitory activities against various cells, such as epithelial cells, vascular endothelial cells, hematocytes or lymphocytes.5 Regarding 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, and TGF-β has been strongly implicated in the progression of alopecia.2–5 In addition, it has been reported that TGF-β/Smad2 signaling pathway has a strong relationship with hair growth suppression based on research involving transgenic mice overexpressing Smad2 in the epidermis under the control of keratin 14 promoter.10 Based on these findings, compounds that inhibit ALK5 and block the TGF-β/Smad signaling pathway might have the potential to ameliorate alopecia.

Many research groups have reported small molecule inhibitors of ALK5.3,11–20 In a previous paper, we described the discovery of 121 (ALK5 IC_{50}=2.7 nM, Smad2/3 IC_{50}=8.7 nM), which was the most potent inhibitor of ALK5. Moreover, we reported that 1 inhibited Smad2 phosphorylation in mouse skin in such a way as to block the TGF-β/Smad signals after the topical application.22 We selected a lotion base (1,3-butylen glycol/ethanol (EtOH)/H_{2}O 10 g/79 mL/diluted to 100 mL) that could be used topically on human skin as a formula for the topical application of compounds in an in vivo study. However, the solubility of 1 remains inadequate for inclusion in this lotion base. We also reported that the introduction of polar-substituents at the 2-position of the imidazole ring in 1 significantly increased the solubility in the lotion base. Among them, 2 had an approximately 10-fold higher solubility in the lotion base than 1 with almost the same inhibitory activities; as a result, the pharmacological efficacy of 2 was increased.21

In the present study, we tried another approach to improve solubility in the lotion base by using the most potent template. Initially, we focused on the planarity between the imidazole ring and the thiazole ring in 1 from the X-ray crystallographic analysis (Fig. 1A). This result might be one of the major reasons for the low solubility of 1 in the lotion base. Next, using a docking model, which we built based on the X-ray structure of ALK5 co-crystallized with its inhibitors,16,23 we predicted that the introduction of a substituent at the 7-position of the benzothiazole ring might be able to maintain the inhibitory activities. To verify this hypothesis, the compounds were

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designed by means other than the introduction of a substituent at the 2-position of the imidazole ring in 1. Therefore, we synthesized a closer analogue of 1, which contained a substituent at the 7-position of the benzothiazole ring, and examined the influence of both the introduction of substituents and the relaxation of planarity on the solubility in the lotion base.

Chemistry

The imidazole derivative 11 was prepared as shown in Chart 1. Commercially available 5,6-dihydro-1,3-benzothiazol-7(4H)-one 3 was used as a starting material and was reacted with bromine in hydrobromic acid and acetic acid (AcOH) at 45°C to give 4. Treatment of 4 with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in tetrahydrofuran (THF) at room temperature gave 5. Compound 5 was reacted with iodomethane in the presence of K₂CO₃ in N,N-dimethylformamide (DMF) at room temperature to afford 6. The reaction of 6 with trimethylsilylacetylene in the presence of Pd(PPh₃)₄, CuI and triethylamine (NEt₃) in acetonitrile (MeCN) under reflux gave 7. Treatment of 7 with K₂CO₃ in methanol (MeOH) at room temperature afforded 8. Compound 8 was reacted with 2-iodo-4-methylthiazole, Pd(PPh₃)₄ and NEt₃ in MeCN under reflux to give 9. The oxidation of 9 was accomplished by reaction with KMnO₄ in a mixed solvent of acetone and buffer (NaHCO₃ and MgSO₄ in H₂O) at room temperature to afford 10. The resulting α-diketone 10 was reacted with paraformaldehyde and ammonium acetate in THF-MeOH under reflux to give 11.

Results and Discussion

The ALK5 inhibitory activity of compound 11 was evaluated using an enzyme assay, while its TGF-β-induced Smad2/3 phosphorylation inhibitory activity was evaluated using a cell-based assay. The inhibitory activities and solubilities of 1, 2, and 11 are summarized in Table 1. The potency of compound 11 was maintained in both the enzyme and cellular assays, and 11 was more than three times more soluble in the lotion base than 1. However, the introduction of either neutral-substituents or polar-substituents that were expected to improve the solubility, such as a methyl group, a hydroxy group, a dimethylaminoethoxy group, a methoxyethoxy group, and so on, at the 7-position of the benzothiazole ring did not adequately improve either the ALK5 inhibitory activity or the solubility in the lotion base (data not shown). As shown in Fig. 1B, the dihedral angle between the imidazole ring and the thiazole ring in 11 changed significantly in the X-ray crystallographic analysis, and the planarity between the imidazole ring and the thiazole ring was broken up completely. These findings suggest that this result is one of the major factors responsible for the improvement in solubility. It has been reported that the introduction of a substituent group and breaking the planarity of the compound resulted in improvement of solubility. 24–26)

Compound 11 was evaluated for selectivity using a diverse kinase panel (96 kinases). Compound 11 was more than 100 times selective against 95 different kinases and exhibited about a 14-fold selectivity against only kinase insert domain receptor (IC₅₀=68 nm).

To examine the binding mode of 11 in the ATP binding site of ALK5, 11 was docked into the molecular model. 16,23) As
shown in Fig. 2, the nitrogen atom of the benzothiazole ring binds to the backbone NH of His-283. The 4-methylthiazol-2-yl nitrogen atom forms water-mediated hydrogen bonds to protein in the side chains of Tyr-249 and Glu-245 as well as the backbone NH of Asp-351. In general, breaking the planarity of compounds improves solubility greatly. However, the crystal state conformation does not necessarily correspond to the solution state conformation and various conformations have been considered in the solution. 27) We thought that only the binding mode of \( \text{I1} \) was essential to an inhibitory activity against ALK5. Moreover, the conformation in this binding mode was different from those of both \( \text{I} \) and \( \text{I1} \) in the X-ray crystallographic analysis. Therefore, we believe \( \text{I1} \) binds in a manner similar to \( \text{I} \) and both compounds, as a result, had a potent inhibitory activity against ALK5.

Compound \( \text{I1} \), 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. 28) It has been reported that TGF-\( \beta \)1 expression in hair follicles is hair cycle-dependent and increases during late anagen and the onset of catagen development. 8) Therefore, 16 d after depilation, when the hair follicles in the depilated area were in the late anagen phase, lotions containing 1% and 3% \( \text{I1} \) were applied to the mouse skin. The skin was harvested after 1 h and 8 h of the application and was processed for the western blot analysis of the phosphorylated-Smad2 level. As shown in Figs. 3A and B, the topical application of 1% and 3% \( \text{I1} \) inhibited the phosphorylation of Smad2 significantly at 1 h after application (75% and 74% inhibition, compared with vehicle-treated animals, respectively). These results indicated that \( \text{I1} \) was absorbed by the skin immediately and inhibited potently TGF-\( \beta \) signals in the skin. As shown in Figs. 3C and D, although the effect of the topical application of 1% \( \text{I1} \) was insignificant at 8 h after application, the topical application of 3% \( \text{I1} \) inhibited the phosphorylation of Smad2 significantly at 8 h after application (4% and 71% inhibition, compared with vehicle-treated animals, respectively). These results suggested that the improvement in the solubility made it possible to produce a higher dose of \( \text{I1} \) in a Smad2 phosphorylation inhibitory activity test in mouse skin, and the pharmacological efficacy of the 3% \( \text{I1} \) lotion persisted for 8 h, compared with the 1% \( \text{I1} \) lotion. Hence, \( \text{I1} \) may be a good topical drug for the treatment of alopecia when administered according to a once daily dosing schedule.

### Conclusion

In this paper, we reported a new approach to improving the solubility of \( \text{I} \) in the lotion base and discovered \( \text{I1} \) (TASP0382088), which exhibits a potent ALK5 inhibitory activity and selectivity for other kinases. The introduction of a methoxy group at the 7-position of the benzothiazole ring in \( \text{I} \) resulted in the break up of the planarity between the imidazole ring and the thiazole ring in \( \text{I1} \), based on an X-ray crystallographic analysis. As a result, \( \text{I1} \) was more than three times more soluble in the lotion base than \( \text{I} \) with almost the same inhibitory activities. Furthermore, \( \text{I1} \) showed the long-lasting inhibitory activity against Smad2 phosphorylation in mouse skin. We think that \( \text{I1} \) (TASP0382088) may be useful for the amelioration of alopecia, although more detailed
findings regarding the pharmacological properties of 11 remain to be studied.

Experimental
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 C-200 (Wako Pure Chemical Industries, Ltd.) and NH-silica gel Fuji Silysia chromatorex DM1020. Melting points were determined on a Yanaco MP-500D melting point apparatus and were uncorrected. 1H-NMR spectra were recorded at 600 MHz using a JEOL ECA600 with tetramethylsilane 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), m (multiplet) or br s (broad singlet). Mass spectra (MS) were recorded on a Micromass Platform LC mass spectrometer with electrospray ionization (ESI) or a Shimadzu LCMS-2010EV mass spectrometer with electrospray ionization (ESI)/atmospheric pressure chemical ionization (APCI) dual source. Elemental analyses were performed on a Yanaco MT-6 elemental analyzer, and the results were within ±0.4% of the calculated values.

6,6-Dibromo-5,6-dihydro-1,3-benzothiazol-7-ol (4)
To a solution of 3 (100.0 g, 0.653 mol) in AcOH (880 mL) was added 48% HBr (35.4 mL, 0.313 mol) dropwise. To the resulting suspension was dropped bromine (70.2 mL, 1.37 mol) as a light brown powder (102.6 g, 96%): 1H-NMR (600 MHz, CDCl 3) δ: 0.28 (9H, s), 4.21 (3H, s), 7.54 (1H, d, J = 8.4 Hz), 9.01 (1H, s); MS (ESI/APCI Dual) m/z 262 (M+H)+, 100%.

6-Bromo-1,3-benzothiazole (5)
To a solution of 4 (154.4 g, 0.496 mol) in THF (2.5 L) at 0°C was dropped DBU (222 mL, 1.49 mol) over a 20-min period. The reaction mixture was stirred at room temperature for 1.5 h. 1 N HCl (1.6 L) was added to the reaction mixture and extracted with EtOAc (5.0 L). The organic layer was washed with brine, dried over MgSO 4 and concentrated to afford 5 as a brown powder (110.5 g, 97%): 1H-NMR (600 MHz, DMSO-d 6) δ: 7.54 (1H, d, J = 8.4 Hz), 7.64 (1H, d, J = 8.4 Hz), 9.33 (1H, s), 10.69 (1H, br s); MS (ESI/APCI Dual) m/z 230 (M+H)+, 98%, 232 (M+2+H)+, 100%, 228 (M−H)−, 27%, 230 (M+2−H)−, 33%.

6-Bromo-7-methoxy-1,3-benzothiazole (6)
To a solution of 5 (116.0 g, 0.504 mol) in DMF (1.1 L) at 0°C was added K 2 CO 3 (139.0 g, 1.01 mol). Methyl iodide (34.5 mL, 0.554 mol) was dropped to the mixture at 0°C. The reaction mixture was stirred at room temperature for 12.5 h. Water was added to the reaction mixture and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over MgSO 4 and concentrated to afford 6 as a light brown powder (100.0 g, 83%): 1H-NMR (600 MHz, CDCl 3) δ: 4.05 (3H, s), 7.67 (1H, d, J = 8.4 Hz), 7.77 (1H, d, J = 8.4 Hz), 8.96 (1H, s); MS (ESI/APCI Dual) m/z 244 (M+H)+, 95%, 246 (M+2+H)+, 100%.

7-Methoxy-6-[(trimethylsilyl)ethynyl]-1,3-benzothiazole (7)
A mixture of 6 (100.0 g, 0.410 mol), trimethylsilylecylacetonate (289.6 mL, 2.05 mol), Pd (PPh 3) 4 (47.4 g, 41.0 mmol), CuI (7.80 g, 41.0 mmol) and NEt 3 (720 mL) in MeCN (1.44 L) was stirred at reflux temperature for 18 h. The reaction mixture was evaporated and the residue was purified by column chromatography on silica gel eluting with 50% EtOAc/CHCl 3 and on silica gel eluting with 10–30% EtOAc/n-hexane to afford 7 as a light brown powder (102.6 g, 96%). 1H-NMR (600 MHz, CDCl 3) δ: 0.28 (9H, s), 4.21 (3H, s), 7.54 (1H, d, J = 8.3 Hz), 7.76 (1H, d, J = 8.3 Hz), 8.99 (1H, s); MS (ESI/APCI Dual) m/z 262 (M+H)+, 100%.

6-Ethynyl-7-methoxy-1,3-benzothiazole (8)
To a solution of 7 (102.6 g, 0.392 mol) in MeOH (1.0 L) was added K 2 CO 3 (59.7 g, 0.432 mol). The reaction mixture was stirred at room temperature for 30 min. A saturated aqueous solution of NH 4 Cl and 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% EtOAc/CHCl 3 and on silica gel eluting with 10–30% EtOAc/n-hexane to afford 8 as a light brown powder (116.0 g, 96%). 1H-NMR (600 MHz, CDCl 3) δ: 1.06 (9H, s), 4.21 (3H, s), 7.54 (1H, d, J = 8.3 Hz), 7.67 (1H, d, J = 8.3 Hz), 8.99 (1H, s); MS (ESI/APCI Dual) m/z 262 (M+H)+, 100%.

7-Methoxy-6-[(4-methyl-1,3-thiazol-2-yl)ethynyl]-1,3-benzothiazole (9)
A mixture of 8 (59.6 g, 0.315 mol), 2-iodo-4-methyl-1,3-thiazole (85.1 g, 0.378 mol), Pd (PPh 3) 4 (21.8 g, 18.9 mmol) and NEt 3 (600 mL) in MeCN (1.2 L) was stirred at reflux temperature for 7 h. The resulting precipitate was filtered off and the filtrate was concentrated.

Fig. 3. Smad2 Phosphorylation Inhibitory Activity of 11 in Mouse Skin
(A) 1% 11 lotion; (B) 3% 11 lotion, topical application (1h); (C) 1% 11 lotion; (D) 3% 11 lotion, topical application (8h). Data are expressed as means±S.E.M. (n=5).

*p<0.05, **p<0.01 compared with vehicle-treated animals (Welch’s test).
puriﬁed by column chromatography on NH-silica gel eluting with EtOAc and on silica gel eluting with EtOAc/CHCl 3/n-hexane (25/0/75) to EtOAc/CHCl 3/n-hexane (37.5/25/37.5) to afford 9 as a light brown powder (66.7 g, 74%): mp 128.0–129.0°C; 1H-NMR 600 MHz, CDCl 3) δ: 2.51 (3H, d, J=9.9 Hz), 4.29 (3H, s), 6.96 (1H, q, J=9.9 Hz), 7.65 (1H, d, J=8.3 Hz), 7.83 (1H, d, J=8.3 Hz), 9.03 (1H, s); MS (ESI) m/z 287 (M+H)+, 100%, 309 (M+Na), 20%.

1-(7-Methoxy-1,3-benzothiazol-6-yl)-2-(4-methyl-1,3-thiazol-2-yl)ethane-1,2-dione (10) To a solution of 9 (23.2 g, 81.0 mmol) in a mixed solvent of acetone (1.5 L) and hexane (25/0/75) to EtOAc/CHCl 3/n-hexane (37.5/25/37.5) to afford 10 as a light brown powder (24.9 g, 97%); mp 204.0–206.0°C; 1H-NMR (600 MHz, DMSO-d 6) δ: 2.39 (3H, d, J=9.9 Hz), 3.83 (3H, s), 7.35 (1H, d, J=9.9 Hz), 8.01 (1H, d, J=8.7 Hz), 8.23 (1H, d, J=8.7 Hz), 9.17 (1H, s); MS (ESI) m/z 319 (M+H)+, 40%, 341 (M+Na)+, 20%.

7-Methoxy-6-[4-(4-methyl-1,3-thiazol-2-yl)-1H-imidazol-5-yl]-1,3-benzothiazole (11) To a suspension of 10 (34.0 g, 0.107 mol) in THF (800 mL) and MeOH (400 mL) were added paraformaldehyde (34.0 g, 0.377 mol) and NH 4OAc (66.0 g, 0.856 mol). The reaction mixture was stirred at 60°C for 125 h. A saturated aqueous solution of NaHCO 3 was added to the reaction mixture to adjust the pH to 2–3. The reaction mixture was transferred to a glutathione 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 Smad3 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 immunoglobulin G (IgG) antibody and dissociation-enhanced lanthanide ﬂuoroimmunoassay (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.

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 dimethyl sulfoxide (DMSO) as a control for 2 h followed by the addition of 1 ng/mL of TGF-β (R&D Systems). After 1 h of incubation, the cells were washed with phosphate buffered saline (PBS) and lysed with radioimmunoprecipitation assay (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 immunoglobulin G (IgG) antibody and dissociation-enhanced lanthanide ﬂuoroimmunoassay (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.

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 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) (pH 7.5) containing 10 mM MgCl2, 4% DMSO, 0.003% Brij 35, 0.004% Tween 20 and 1 mM dithiothreireoscentul. 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.

**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.

**Smad2 Phosphorylation Inhibitory Activity in Mouse Skin** Under anesthetization with pentobarbital, the dorsal haires 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 11 ligation or vehicle was applied topically to the dorsal area. At 1 h and 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% Nonidet P-40, then centrifuged at 3000 rpm for 15 min. The supernatant was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), followed by the transfer of the proteins to the polyvinylidene difluoride (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.

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**References and Notes**

23) The binding model was examined and visualized using MOE™ (Molecular Operating Environment) Version 2009.10, Chemical Computing Group: Montreal, Canada.