NOTE

5-Prenyltryptophol, a new inhibitor of bone morphogenetic protein-induced alkaline phosphatase expression in myoblasts, produced by *Streptomyces colinus* subsp. *albescens* HEK608

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Fibrodysplasia ossificans progressiva (FOP) is a congenital disorder of progressive and widespread postnatal ossification of soft tissues.¹ Patients with classical FOP have congenital malformations that result in profoundly decreased mobility.^{2,3} Recently, a recurrent mutation (R206H) within ACVR1/ALK2, bone morphogenetic protein (BMP) type I receptor, was identified in both familial and sporadic patients with FOP.⁴ Because this mutation induced the activation of BMP signaling in ectopic bone formation, BMP signaling inhibitors could offer therapeutic benefits for FOP.^{5,6} Therefore, we screened inhibitors of BMP-induced alkaline phosphatase (ALP) activity using a stable ALK2(R206H)-expressing C2C12 cell line (abbreviated as C2C12(R206H) cells).⁷ During the course of this screening program, we reported pyrrolidine-containing compounds produced by fungal strain *Fusarium* sp. B88⁷ and trichocyalides produced by fungal strain *Trichoderma* sp. FKI-5513⁸ as potential inhibitor candidates for osteoblastic differentiation. Continuing this screening, a new active compound, designated 5-prenyltryptophol (5-PT, Figure 1), was isolated from the culture broth of actinomycete strain *Streptomyces colinus* subsp. *albescens* HEK608.

The producing microorganism *S. colinus* subsp. *albescens* HEK608, classified on the basis of the partial sequence of 16S rRNA genes (data not shown), was isolated from soil collected in Hegurajima, Wajima-shi, Ishikawa Prefecture, Japan. A loopful of spores of strain HEK608 was inoculated into 100 ml seed medium consisting of 1.0% starch, 0.5% glucose, 0.3% NZ-Case (Sigma-Aldrich, St Louis, MO, USA), 0.2% yeast extract (Oriental Yeast, Itabashi-ku, Tokyo, Japan), 0.5% Bacto-tryptone (Becton Dickinson and Company, Franklin Lakes, NJ, USA), 0.1% K₂HPO₄, 0.05% MgSO₄ 7H₂O, 0.3% CaCO₃ (adjusted to pH 7.0 before sterilization) in a 500-ml Erlenmeyer flask. The inoculated Erlenmeyer

flask was incubated in a rotary shaker (210 r.p.m.) at 30 °C for 3 days to obtain the seed culture. For the production of 5-PT, the culture was initiated by transferring 3 ml seed culture into 25 × 500 ml Erlenmeyer flasks containing production medium (0.5% glucose, 2.0% glycerol, 2.0% soluble starch, 1.5% pharmamedia (Southern Cotton Oil, Richmond, TX, USA), 0.3% yeast extract, 1% Diaion HP-20 (Mitsubishi Chemical, Tokyo, Japan), non-adjusted pH) and fermentation was carried out in a rotary shaker (210 r.p.m.) at 30 °C for 7 days.

The culture broth (2.5 l) was centrifuged at 8000 r.p.m. for 15 min to obtain the mycelium. The mycelium was treated with acetone (2.5 l) for 1 h, and acetone extracts were filtered to remove the mycelium. After concentration of the extracts to remove acetone, the aqueous solution was adjusted to pH 9.0 and extracted with EtOAc (2.5 l). The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure to give brown materials (1.24 g). The materials were dissolved in a small amount of MeOH and purified by octa decyl silyl (ODS) column chromatography (i.d. 3.5 × 16 cm, Chromatorex ODS, 100–200 mesh; Fuji Silysia Chemical, Aichi-ken, Japan). The active materials were eluted stepwise with CH₃CN–H₂O (0, 20, 40, 60, 80 and 100%, 160 ml each). 5-PT was recovered from the 80% CH₃CN–H₂O fraction, which was concentrated under reduced pressure to give brown oily materials (48.8 mg). The materials containing 5-PT were dissolved in MeOH and finally purified by HPLC under the following conditions: column, CAPCELL PAK C18 (i.d. 20 × 250 mm; Shiseido, Tokyo, Japan); mobile phase, a linear gradient from 40 to 70% CH₃CN for 45 min; flow rate, 8 ml min^{−1}; detection, UV 210 nm. 5-PT was eluted as a peak with a retention time of 24 min. The fraction of the peak was collected and concentrated to dryness to give pure 5-PT (1.2 mg) as a yellow powder.

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Figure 1 Structure of 5-prenyltryptophol.

Table 1 Physico-chemical properties of 5-prenyltryptophol

Appearance	Yellow powder
Molecular formula	C ₁₅ H ₁₉ NO
MW	229
HREI-MS	
Calcd	229.1467 [M] ⁺
Found	229.1460 [M] ⁺
UV λ _{max} in nm (log ε) in MeOH	225 (1.46), 280 (0.27), 341 (0.02)
IR ν _{max} in cm ⁻¹ (KBr)	3408, 2926, 1655, 1439, 1376, 1214, 1180, 798
Solubility	
Soluble	CHCl ₃ , EtOAc, CH ₃ CN, CH ₃ OH
Insoluble	H ₂ O

The physico-chemical properties of 5-PT are summarized in Table 1. In UV spectra, 5-PT showed absorption maxima at 225, 280 and 341 in MeOH, suggesting the presence of an indole chromophore. In IR spectra, broad OH and/or NH absorption near at 3408 cm⁻¹, typical C–H (CH₂) stretching absorptions at 2926 cm⁻¹ and aromatic C–C stretch absorptions (for carbon–carbon bonds in the aromatic ring) at 1655 and 1439 cm⁻¹ were observed.

5-PT showed a molecular ion peak at *m/z* 229 [M]⁺ in EIMS, and the molecular formula C₁₅H₁₉NO was assigned on the basis of its HR-EIMS [*m/z* 229.1460 [M]⁺, Δ –0.7 m.m.u.], indicating 7 degrees of unsaturation. The ¹H and ¹³C NMR spectra of **1** in CD₃OD (Table 2) showed 17 proton and 15 carbon signals, which were confirmed by analysis of 2D NMR correlations. The multiplicity of the carbon signals was classified into two methyl carbons, two *sp*³ methylene carbons, one *sp*³ oxygenated methylene carbon, four *sp*² methine carbons, one *sp*² nitrogenated methine carbon, four *sp*² quaternary carbons and one *sp*² nitrogenated quaternary carbon by analysis of DEPT and HMQC data. The connectivity of proton and carbon atoms was established by HMQC (Table 2). As shown in Figure 2, the partial structures I to III were elucidated by ¹H–¹H COSY spectra. The ¹³C–¹H long-range couplings of ²*J* and ³*J* in the HMBC spectra (Figure 2) proved the presence of the following linkages: (1) The cross peaks from the *sp*² methine proton H-4 (δ 7.29) to the *sp*² quaternary carbons C-3a (δ 129.1) and C-7a (δ 136.7) and to the *sp*² methine carbon C-6 (δ 123.2), the *sp*² methine proton H-6 (δ 6.90) to the *sp*² methine carbon C-4 (δ 118.2), and from the *sp*² methylene proton H-7 (δ 7.22) to C-3a and the *sp*² quaternary carbon C-5 (δ 132.9) suggested the presence of a 3a,5,7a-trisubstituted benzene (ring A) containing the partial structure I. Further long-range couplings from the *sp*² methine proton H-2 (δ 7.02) to the *sp*² quaternary carbon C-3 (δ 112.3), C-3a and C-7a and from H-4 to C-3 showed the presence of an indole moiety containing the ring A, supported by the UV absorption and the ¹H-NMR chemical shifts of

Table 2 ¹H and ¹³C NMR chemical shifts of 5-prenyltryptophol in CD₃OD

Position	δ _C ^a	Multiplicity	δ _H ^b	Multiplicity	<i>J</i> (Hz)
2	123.7	d	7.02	s	—
3	112.3	s	—	—	—
3a	129.1	s	—	—	—
4	118.2	d	7.29	s	—
5	132.9	s	—	—	—
6	123.2	d	6.90	dd	8.1, 1.5
7	112.0	d	7.22	d	8.1
7a	136.7	s	—	—	—
8	29.8	t	2.94	dt	7.3, 0.8
9	63.7	t	3.79	t	7.3
10	35.5	t	3.41	br.d	7.8
11	126.1	d	5.37	tm	7.8
12	132.0	s	—	—	—
13	25.9	q	1.76	s	—
14	17.6	q	1.75	d	0.9

^a¹³C Chemical shifts are shown in δ-values (p.p.m.) relative to CD₃OD at 49.0 p.p.m.

^b¹H Chemical shifts are shown in δ-values (p.p.m.) relative to CD₃OD at 3.31 p.p.m. followed by multiplicity and coupling constants *J* (Hz).

Figure 2 Key correlations in ¹H–¹H COSY and HMBC spectra of 5-prenyltryptophol.

3,5-disubstituted indole moiety;⁹ (2) Long-range couplings from the *sp*³ methylene proton H₂-10 (δ 3.41) to the *sp*² quaternary carbon C-12 (δ 132.0), from the methyl protons H₃-13 (δ 1.76) and H₃-14 (δ 1.75) to the *sp*² methine carbon C-11 (δ 126.1), the *sp*² quaternary carbon C-12 and the methyl carbon C-13 (δ 25.9) and from the methyl proton H₃-14 to C-11, C-12 and the methyl carbon C-14 (δ 17.6) suggested the presence of indole group containing the partial structure II. This isoprenyl group was connected to the indole moiety at C-5 by observation of the ³*J* cross peaks from H-4 and H-6 to the *sp*³ methylene carbon C-10 (δ 35.5), from H-10 to C-4, C-5 and C-6 and from the *sp*² methine proton H-11 (δ 5.37) to C-5 in HMBC experiments; (3) Long-range couplings from the *sp*³ methylene proton H₂-8 (δ 2.94) to the *sp*² methine carbon C-2 (δ 123.7), C-3 and C-3a and from the *sp*³ oxygenated methylene proton H-9 (δ 3.79) to C-3 showed that a 9-oxyethyl group containing the partial structure III attached to the indole moiety at C-3. Furthermore, the chemical shifts of C-9 (δ_C 63.7, δ_H 3.79) indicated that a hydroxyl group is attached to C-9. Taking these observations into consideration, the structure of 5-PT was elucidated

as shown in Figure 1, which fulfilled the molecular formula and the degrees of unsaturation.

BMP-induced ALP inhibitory activity and cytotoxicity of 5-PT in C2C12(R206H) cells were measured according to the previously reported method (details in Supplementary Information).^{7,8} C2C12 cells expressed ALP activity as an osteogenic phenotype in the presence of high concentrations of recombinant BMP. Therefore, ALP activity was used as a typical marker of BMP signaling in the C2C12 cell line. As a result, 5-PT exhibited ALP inhibitory activity and cytotoxicity with IC₅₀ values of 81.3 and 263 μ M, respectively. Furthermore, in order to examine the direct effect of 5-PT on BMP signaling, we determined Id1WT4F-luc activity in C2C12 cells according to the previously reported method (details in Supplementary Information).^{7,8} However, 5-PT showed no effect on luciferase activity even at 43.6 μ M, suggesting that the target of 5-PT does not lie within early events of Smads⁶ but within late steps, the BMP signaling pathway.⁵ The target of 5-PT in osteoblastic differentiation remains to be demonstrated.

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