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BRIEF COMMUNICATION

# A new protein tyrosine phosphatase 1B inhibitory α-pyrone-type polyketide from Okinawan plant-associated *Aspergillus* sp. TMPU1623

Hiroyuki Yamazaki[1] · Kenta Takahashi[1] · Natsuki Iwakura[1] · Tatsuki Abe[1] · Masanari Akaishi[1] · Satomi Chiba[1] · Michio Namikoshi[1] · Ryuji Uchida[1]

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## Abstract

A new polyenyl-α-pyrone polyketide, aspopyrone A (**1**), was isolated from a culture broth of Okinawan plant-associated *Aspergillus* sp. TMPU1623 by solvent extraction, ODS column chromatography, and preparative HPLC (ODS). The structure of **1** was assigned based on NMR experiments. Compound **1** exhibited protein tyrosine phosphatase (PTP) 1B and T-cell PTP (TCPTP) inhibitory activities with $IC_{50}$ values of 6.7 and 6.0 μM, respectively.

Natural products are known to be a rich source of useful substances with unprecedented skeletons and diverse bioactivities [1]. Microbial metabolites have contributed to drug development for several diseases [2, 3]; however, the number of new drug candidates from microorganisms has been decreasing each year [1, 2, 4]. Therefore, unutilized microorganisms derived from various environments have recently been attracting increasing attention. Among these, plant-associated fungi are producers of unique secondary metabolites [5].

In the course of our screening study on a new class of protein tyrosine phosphatase (PTP) 1B inhibitors of microbial origin, we found that a culture broth of the fungal strain *Aspergillus* sp. TMPU1623, which was obtained from a root part of an Okinawan plant, exhibited PTP1B inhibitory activity. The bioassay-guided separation of the EtOAc extract from the culture broth led to the isolation of a new α-pyrone-containing polyketide, aspopyrone A (**1**), as an active component (Fig. 1a). PTP1B is widely considered as a key negative regulator in the insulin and leptin signaling pathways [6, 7], and, thus, its inhibitor is expected to be a promising lead compound for the prevention and treatment of type 2 diabetes mellitus and obesity [8, 9]. We herein describe the isolation, structural elucidation, and biological activity of compound **1**.

Fungal strain TMPU1623 was isolated from a root sample of an unidentified plant collected at Ishigaki Island, Okinawa, Japan, in September 2016. After initial washing with sterile water, a part of the roots was minced in 1 ml of sterile water using a mortar and a pestle, and approximately 100 μl of the solution was spread on a potato dextrose agar (PDA) plate (BD, Franklin Lakes, NJ, USA) containing 0.005% rose bengal (Wako, Osaka, Japan) and 0.01% kanamycin (Wako). The plate was incubated at 25 °C for 7 days, and strain TMPU1623 was isolated and inoculated onto another PDA plate. The 241-bp ITS1 rDNA sequence of strain TMPU1623 was identical to 154 known species of the genus *Aspergillus* and, thus, the strain was identified as *Aspergillus* sp. The ITS1 rDNA sequence of strain TMPU1623 has been deposited in DDBJ under the accession number LC369134.

The mycelia of strain TMPU1623, maintained on a PDA plate, were inoculated into a 100-ml Erlenmeyer flask containing 50 ml of seed medium [2.0% glucose (Wako), 0.50% polypeptone (Nihon Pharmaceutical Co., Ltd., Tokyo, Japan), 0.050% $MgSO_4 \cdot 7H_2O$ (Wako), 0.20% yeast extract (BD), 0.10% $KH_2PO_4$ (Wako), and 0.10% agar (Wako) in water and adjusted to pH 6.0 before sterilization].

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✉ Hiroyuki Yamazaki
yamazaki@tohoku-mpu.ac.jp

✉ Ryuji Uchida
uchidar@tohoku-mpu.ac.jp

[1] Faculty of Pharmaceutical Sciences, Tohoku Medical and Pharmaceutical University, Sendai 981-8558, Japan

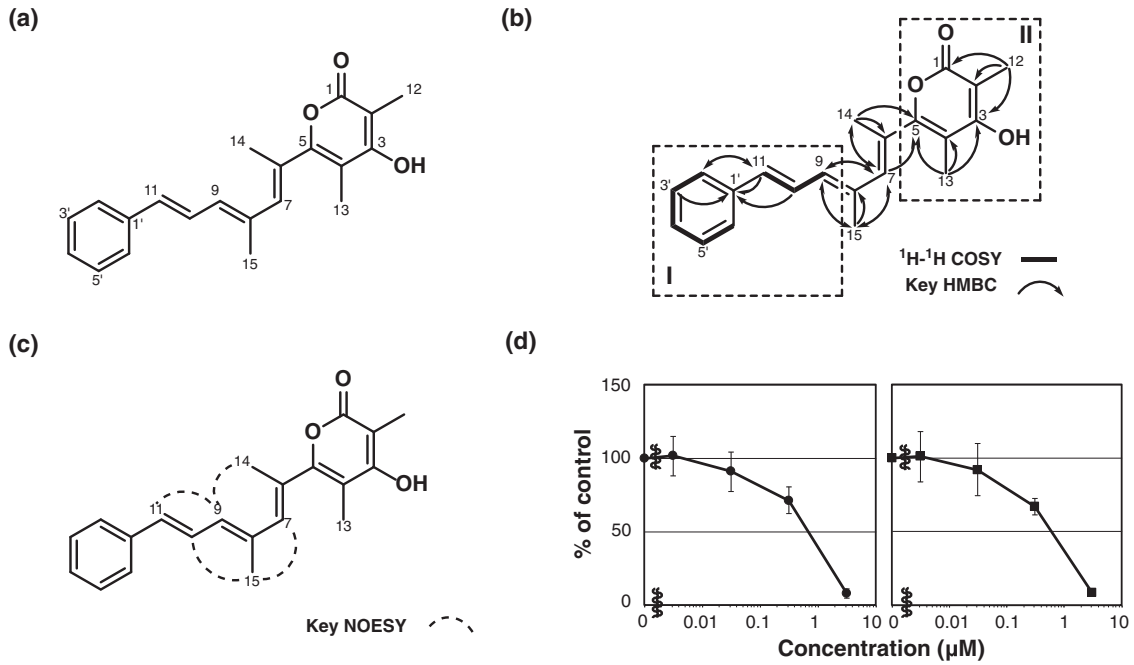


**Fig. 1** (**a**) Structure of **1** produced by Okinawan fungus *Aspergillus* sp. TMPU1623. (**b**) $^1$H-$^1$H COSY and key HMBC correlations for **1**. (**c**) Key NOESY correlations for **1**. (**d**) Inhibitory activities of **1** against PTP1B (●) and TCPTP (■)

**Table 1** $^{13}$C (100 MHz) and $^1$H (400 MHz) NMR data for **1** in DMSO-$d_6$

| C# | $\delta_C$ | $\delta_H$, mult. ($J$ in Hz) |
|---|---|---|
| 1 | 163.8 | |
| 2 | 98.1 | |
| 3 | 164.8 | |
| 3-OH | – | 10.5, s |
| 4 | 106.6 | |
| 5 | 158.2 | |
| 6 | 127.1 | |
| 7 | 137.9 | 6.19, br s |
| 8 | 134.4 | |
| 9 | 133.3 | 6.37, d (11.1) |
| 10 | 125.0 | 7.22, dd (15.5, 11.1) |
| 11 | 133.6 | 6.68, d (15.5) |
| 12 | 9.3 | 1.85, s |
| 13 | 11.8 | 1.95, s |
| 14 | 16.79 | 2.06, s |
| 15 | 16.75 | 2.10, s |
| 1′ | 137.1 | |
| 2′ | 126.5 | 7.53, d (8.0) |
| 3′ | 128.6 | 7.32, t (8.0) |
| 4′ | 127.6 | 7.23, t (8.0) |
| 5′ | 128.6 | 7.32, t (8.0) |
| 6′ | 126.5 | 7.53, d (8.0) |

The flask was shaken intermittently for 3 days at 25 °C to obtain the seed culture, which was then transferred to production medium [3.0% sucrose (Wako), 3.0% soluble starch (Wako), 1.0% malt extract (BD), 0.30% Ebios (Asahi Food & Healthcare Co., Ltd., Tokyo, Japan), 0.50% $KH_2PO_4$, and 0.050% $MgSO_4{\cdot}7H_2O$ in water and adjusted to pH 6.0 before sterilization]. This main fermentation was performed at 25 °C for 7 days under agitation on a rotary shaker (150 rpm). The culture broth (2.4 l) was treated with acetone (2.4 l), and the residue was filtered. The filtrate was concentrated in vacuo to remove acetone, and the aqueous solution was extracted three times with EtOAc (2.4 l). After the evaporation of EtOAc, the extract (4.5 g) was suspended in 30% $CH_3OH$ in $H_2O$, applied to an ODS column (100 g, Wako), and eluted stepwise with 30, 50, 70, 85, and 100% $CH_3OH$ in $H_2O$ (200 ml each × 2) to divide into ten fractions (Fr. 1–Fr. 10). Fr. 6 (the second 200 ml of the 70% $CH_3OH$ eluate) was concentrated to give a dark yellow oil (110 mg), which was purified by preparative HPLC [column; Inertsil ODS-P (GL Science, Inc., Tokyo, Japan), 10 × 250 mm; mobile phase, 70% $CH_3CN$ in $H_2O$; detection, UV at 210 nm; flow rate, 2.0 ml min$^{-1}$] to obtain 24 mg of compound **1** ($t_R$ = 23.2 min).

Compound **1** showed UV absorption at 348 nm (log $\varepsilon$ 4.6) in $CH_3OH$, suggesting the presence of a polyene moiety. In the IR spectrum of **1**, the presence of hydroxy and carbonyl groups was confirmed by bands at 3402 and 1675 cm$^{-1}$, respectively (the physicochemical properties of **1** are summarized in detail in Supplementary Information).

The molecular formula of **1** was deduced as $C_{21}H_{22}O_3$ from its HREIMS (*m/z* 322.1574 $[M]^+$, Δ +0.5 mmu) and NMR data (Table 1). The $^1$H NMR spectrum of **1** (in DMSO-$d_6$) displayed 22 proton signals, one of which was suggested to be a hydroxy proton (δ 10.5). The $^{13}$C NMR spectrum of **1** (in DMSO-$d_6$) indicated 21 carbon signals, which were classified into four methyl, nine $sp^2$ methine, five $sp^2$ quaternary, two $sp^2$ oxygenated quaternary, and one carbonyl carbons by analyzing the DEPT and HMQC spectra of **1** (Table 1). The aromatic proton signals [(δ 7.23 t, 1H), (δ 7.32 t, 2H), and (δ 7.53 d, 2H)] in the $^1$H NMR spectrum revealed the presence of a monosubstituted benzene ring, and partial structure I was established from $^1$H-$^1$H COSY correlations as shown by the bold line and HMBC data observed from H-10 (δ 7.22) to C-1′ (δ 137.1), from H-11 (δ 6.68) to C-1′ and C-2′/C-6′ (δ 126.5), from H-2′/H-6′ (δ 7.53) to C-11 (δ 133.6), and from H-3′/H-5′ (δ 7.32) to C-1′ (Fig. 1b). HMBC correlations from $H_3$-12 (δ 1.85) to C-1 (δ 163.8), C-2 (δ 98.1), and C-3 (δ 164.8) and from $H_3$-13 (δ 1.95) to C-3, C-4 (δ 106.6), and C-5 (δ 158.2) revealed the 2,4-dimethyl-α-pyrone ring moiety as partial structure II (Fig. 1b). The position of a hydroxy group in **1** was noted at C-3 from the chemical shift (δ 164.8). The connections of the two partial structures I and II with the remaining carbons were assigned by HMBC correlations from H-7 (δ 6.19) to C-5, C-9 (δ 133.3), C-14 (δ 16.79), and C-15 (δ 16.75), from H-9 (δ 6.37) to C-7 (δ 137.9) and C-15, from $H_3$-14 (δ 2.06) to C-5, C-6 (δ 127.1), and C-7, and from $H_3$-15 (δ 2.10) to C-7, C-8 (δ 134.4), and C-9 (Fig. 1b). The geometry of each double bond was assigned to be trans by the large coupling constants ($J = 15.5$ Hz) for the olefinic protons H-10 and H-11 and the NOESY correlations of **1** between H-7/$H_3$-15, H-9/H-11, H-9/$H_3$-14, and H-10/$H_3$-15 (Table 1 and Fig. 1c). Thus, the structure of **1** was elucidated as shown in Fig. 1a, and compound **1** was named aspopyrone A.

The PTP1B inhibitory activity of **1** was evaluated using the enzyme inhibition assay [10]. Compound **1** inhibited enzyme activity with an $IC_{50}$ value of 6.7 μM (Fig. 1d and Table S1). A positive control, oleanolic acid [11] (Tokyo Chemical Industry, Tokyo, Japan), had an $IC_{50}$ value of 0.9 μM in the same experiment (Table S1).

Insulin and leptin signaling pathways are negatively regulated not only by PTP1B, but also by other PTPs such as T-cell PTP (TCPTP) [12]. Although TCPTP shares high sequence similarities with PTP1B in their catalytic domains [13], both enzymes clearly possess different biological functions. Accordingly, the effects of compound **1** on TCPTP activity are also an important property, and its inhibitory activity was examined using an enzyme-based in vitro assay [14]. Compound **1** inhibited TCPTP activity with equivalent potency ($IC_{50} = 6.0$ μM) (Fig. 1d and Table S1). Based on the data described above, compound **1** is a dual inhibitor of PTP1B and TCPTP. Previous studies using PTP1B (*ptp1B*$^{-/-}$) knockout mice demonstrated improvements in insulin resistance and glucose homeostasis [15, 16], whereas TCPTP knockout (*tcptp*$^{-/-}$) mice died at 3–5 weeks old due to serious inflammatory phenotypes [17, 18]. However, recent studies indicated no significant abnormalities in *ptp1B*$^{+/-}$ or *tcptp*$^{+/-}$ mice with the deletion of single copies of PTP1B and TCPTP [19]. Consequently, dual inhibitors against PTP1B and TCPTP may also be drug candidates for the treatment of type 2 diabetes and obesity.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

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