A new biphenyl ether derivative produced by Indonesian ascidianderived Penicillium albobiverticillium

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Submission date: 20-Aug-2019 10:05AM (UTC+0700)
Submission ID: 1161607747
File name: 2017_DASumilat_Penicillium_albobiverticillium.pdf (390.71K)
Word count: 2620
Character count: 13146

J Nat Med (2017) 71:776–779
DOI 10.1007/s11418-017-1094-5

NOTE

A new biphenyl ether derivative produced by Indonesian ascidian-derived *Penicillium albobiverticillium*

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Received: 14 March 2017 / Accepted: 12 May 2017 / Published online: 26 May 2017

Abstract A new biphenyl ether derivative, 2-hydroxy-6-(2′-hydroxy-3′-hydroxymethyl-5-methylphenoxy)-benzoic acid (**1**), was isolated together with the known benzophenone derivative, monodictyphenone (**2**), from a culture broth of Indonesian ascidian-derived *Penicillium albobiverticillium* TPU1432 by solvent extraction, ODS column chromatography, and preparative HPLC (ODS). The structure of **1** was elucidated based on NMR experiments. Compound **2** exhibited moderate inhibitory activities against protein tyrosine phosphatase (PTP) 1B, T cell PTP (TCPTP), and CD45 tyrosine phosphatase (CD45), whereas compound **1** modestly inhibited CD45 activity.

Keywords Biphenyl ether · Ascidian-derived fungus · *Penicillium albobiverticillium* · Protein tyrosine phosphatase 1B · Inhibitor

Introduction

Microbial secondary metabolites have continued to provide structurally and biologically novel compounds, some of which have contributed to the development of new drugs [1, 2]. Natural products obtained from marine microorganisms have recently been receiving increasing attention. Marine-derived fungi are an attractive source of pharmacologically active substances [3–5] and, thus, a large number of metabolites have been reported and expected to become candidates for clinical medicines and chemical reagents.

In the course of our screening study on new metabolites produced by marine-derived fungi, a new biphenyl ether, 2-hydroxy-6-(2′-hydroxy-3′-hydroxymethyl-5-methylphenoxy)-benzoic acid (**1**), and known benzophenone, monodictyphenone (**2**) [6], were isolated from a culture broth of Indonesian ascidian-derived *Penicillium albobiverticillium* TPU1432 (Fig. 1). Compound **2** moderately inhibited the activities of protein tyrosine phosphatase (PTP) 1B, T-cell PTP (TCPTP), and CD45 tyrosine phosphatase (CD45), while compound **1** exhibited modest CD45 inhibitory activity. Protein phosphorylation by PTPs is an important regulatory system in cell growth and signaling pathways [7]. Among the PTP family of enzymes, PTP1B is a promising target for the treatment of type II diabetes and obesity because of its negative regulation of insulin and leptin cascades [8, 9]. In the present study, we describe the fermentation, isolation, structural elucidation, and biological activities of compounds **1** and **2**.

Results and discussion

Fungal strain TPU1432 was isolated from an unidentified coronal ascidian collected at Manado, Indonesia in 2014 and identified as *P. albobiverticillium* from the ITS1 rDNA sequence (224 nucleotides) (100% similarity).

A culture broth of strain TPU1432 was treated with acetone and filtered, and the residue obtained after evaporation was extracted with EtOAc. The EtOAc extract was subjected to ODS column chromatography followed by

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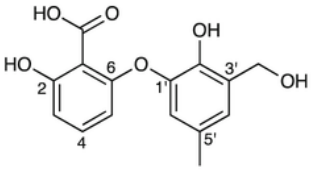

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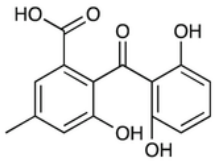

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Fig. 1 Structures of **1** and **2** produced by Indonesian ascidian-derived *Penicillium albobiverticillium* TPU1432

Table 1 ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR data for **1** (CD_3OD)

C#	δ_C	δ_H, mult. (*J* in Hz)
1	105.7	
2	159.8	
3	105.7	6.07, d (8.3)
4	135.4	7.18, t (8.3)
5	111.8	6.57, d (8.3)
6	163.5	
1-COOH	172.7	
1′	150.5	
2′	137.9	
3′	136.2	
4′	121.0	6.83, br s
5′	137.7	
6′	117.8	6.72, br s
3′-CH_2	60.3	4.48, s
5′-CH_3	21.3	2.31, s

preparative HPLC (ODS) in order to yield compounds **1** (0.7 mg) and **2** (1.0 mg). The productivity of compound **1** was markedly reduced in freshwater medium.

Compound **2** was identified as monodictyphenone by comparing its spectroscopic data with reported values [6].

The molecular formula of compound **1** was established as $C_{15}H_{14}O_6$ from HREIMS data (*m/z* 290.0787 $[M]^+$, Δ −0.3 mmu). The EIMS of **1** showed a fragment ion at *m/z* 246 corresponding to the $[M - CO_2]^+$ ion, which suggested the presence of a carboxylic acid in **1**. The ^{1}H and

Table 2 Effects of compounds **1** and **2** on four protein tyrosine phosphatases (PTP1B, TCPTP, CD45, and VHR)

Compound	Protein tyrosine phosphatases (IC_{50}, μM)			
	PTP1B	TCPTP	CD45	VHR
1	>35	>35	43	>35
2	36	20	21	>35
Oleanolic acid[a]	1.0	0.9	0.8	4.5

[a] Positive control [12]

^{13}C NMR spectra (in CD_3OD) showed 10 proton and 15 carbon signals (Table 1), which were classified into one methyl, one oxygenated methylene, five sp^2 methine, three sp^2 quaternary, four sp^2 oxygenated quaternary, and one carbonyl carbon in an analysis of the HMQC and DEPT spectra of **1**. The presence of OH and phenyl groups was revealed from the IR band at 3402 cm^{-1} and UV absorption at 284 nm, respectively. These spectroscopic data indicated that compound **1** possessed a biphenyl ether skeleton [10], and ^{1}H–^{1}H COSY and HMBC correlations of **1** revealed its structure, as shown in Fig. 2a.

The structure of **1** was confirmed in an analysis of its NOESY spectrum. Compound **1** showed NOE correlations between H-5 (δ_H 6.57)/H-6′ (6.72), H-4′ (6.83)/3′-CH_2 (4.48), H-4′/5′-CH_3 (2.31), and H-6′/5′-CH_3 (Fig. 2b). The structure of **1** was consequently assigned, as shown in Fig. 1.

The PTP1B inhibitory activities of **1** and **2** were assessed using the enzyme assay method [11]. Compound **2** inhibited PTP1B with an IC_{50} value of 36 μM, whereas compound **1** was inactive at 35 μM (Table 2). The IC_{50} value of oleanolic acid [12], a positive control, in the same experiment was 1.0 μM (Table 2). We previously reported that fungal biphenyl ether metabolites inhibited PTP1B activity (IC_{50} = 13–17 μM) [10]. Comparisons of the structures of **1** and **2** with those of the active metabolites [10], carboxyl and/or hydroxymethyl groups, may be unfavorable for their inhibitory activities against PTP1B.

The PTP family is a large enzyme group that comprises >100 members, including PTP1B, and controls various

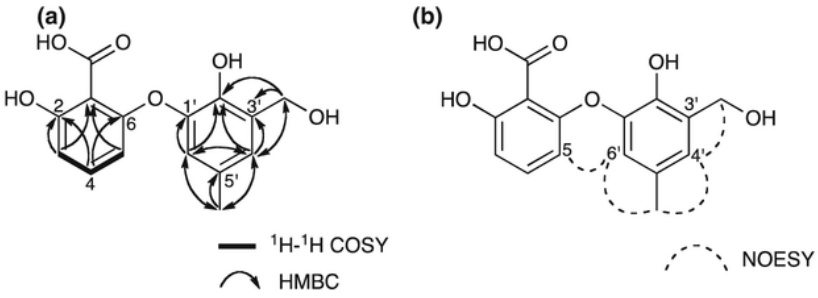

Fig. 2 ^{1}H–^{1}H COSY and HMBC data (**a**) and NOESY correlations (**b**) of **1**

cellular functions [7]. Therefore, selectivity against PTP1B over other PTPs is an important point. The inhibitory effects of compounds **1** and **2** on TCPTP as one of the non-transmembrane PTPs, CD45 as one of the receptor-like PTPs, and *vaccinia* H-1-related phosphatase (VHR) as one of the dual-specificity phosphatases were evaluated. Although compound **2** moderately inhibited TCPTP and CD45 activities, similar to PTP1B activity, that of VHR was not affected by **2** at 35 μM (Table 2). On the other hand, compound **1** inhibited CD45 with an IC50 value of 43 μM. CD45 plays a critical role in lymphocyte signaling, and recent studies have suggested that this enzyme is a potential target for the treatment of autoimmune diseases [7].

Materials and methods

General experimental procedures

UV spectra were measured on a U-3310 UV–visible spectrophotometer (Hitachi, Ltd., Tokyo, Japan) and IR spectra on a PerkinElmer Spectrum One Fourier transform infrared spectrometer (PerkinElmer, Waltham, MA, USA). NMR spectra were recorded on a JEOL JNM-AL-400 NMR spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C) in CD_3OD (δ_H 3.31, δ_C 49.0). EIMS and HREIMS were performed using a JMS-MS 700 mass spectrometer (JEOL, Tokyo, Japan). Preparative HPLC was performed using a Hitachi L-6200 system (Hitachi, Ltd.).

Materials

Human recombinant PTP1B, CD45, and VHR were purchased from Enzo Life Sciences (Farmingdale, NY, USA). Human recombinant TCPTP was purchased from R&D Systems (Minneapolis, MN, USA). *p*-Nitrophenyl phosphate (*p*NPP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Oleanolic acid was purchased from Tokyo Chemical Industry (Tokyo, Japan). Potato dextrose agar (PDA) was purchased from BD (Franklin Lakes, NJ, USA). Ebios was purchased from Asahi Food & Healthcare Co., Ltd. (Tokyo, Japan). Plastic plates (96-well) were purchased from Corning Inc. (Corning, NY, USA). All other chemicals including organic solvents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Isolation and identification of strain TPU1432

Fungal strain TPU1432 was isolated from an unidentified ascidian collected at Manado, Indonesia, in August 2014. A piece of the ascidian was minced using a pestle and mortar with 1 ml of sterilized seawater, and approximately 100 μl of the solution was spread on a PDA plate containing 0.005% rose bengal and 0.01% kanamycin. The plate was incubated at 25 °C for 1 week, and strain TPU1432 was isolated and inoculated onto another PDA plate. Strain TPU1432 was identified as *P. verruculosum* in a comparison of the 224-bp ITS1 rDNA sequence (100% match).

Fermentation

Strain TPU1432 grown on a PDA plate was inoculated into a 100-ml Erlenmeyer flask containing 50 ml of the seed medium (2.0% glucose, 0.50% polypeptone, 0.050% $MgSO_4{\cdot}7H_2O$, 0.20% yeast extract, 0.10% KH_2PO_4, and 0.10% agar in natural seawater; adjusted to pH 6.0 before sterilization). The flask was shaken reciprocally at 25 °C for 3 days in order to obtain the seed culture, which was then transferred to the production medium (3.0% sucrose, 3.0% soluble starch, 1.0% malt extract, 0.30% Ebios, 0.50% KH_2PO_4, and 0.050% $MgSO_4{\cdot}7H_2O$ in seawater and adjusted to pH 6.0 before sterilization). The production culture was conducted at 25 °C for 7 days under agitation.

Isolation of compounds 1 and 2

Acetone (2.4 l) was added to the culture broth (2.4 l) after 7 days and filtered. The filtrate was concentrated to remove acetone and extracted with EtOAc. The EtOAc extract was concentrated in vacuo to dryness, and the brown residue (2.8 g) was suspended in 30% CH_3OH and adsorbed on an ODS column (100 g). The ODS column was eluted stepwise with 30, 50, 70, 85, and 100% CH_3OH in H_2O (each 200 ml × 2) to separate into ten fractions (Fr. 1–Fr. 10). Fr. 2 (second 200 ml of the 30% CH_3OH eluate) exhibited inhibitory activity against PTP1B and was concentrated in vacuo to dryness to give a dark black oil (48.2 mg), which was purified by preparative HPLC [column; PEGASIL ODS (Senshu Scientific Co., Ltd., Tokyo, Japan), 10 × 250 mm; mobile phase, 30% CH_3OH containing 0.05% TFA; detection, UV at 210 nm; flow rate, 2.0 ml/min] to give compounds **1** (0.7 mg, t_R = 33.9 min) and **2** (1.0 mg, t_R = 21.3 min).

2-Hydroxy-6-(2′-hydroxy-3′-hydroxymethyl-5-methylphenoxy)-benzoic acid (**1**): a brown oil; IR (KBr) ν_{max} 3402, 1676, 1611, 1459, 1219 cm^{-1}; UV (CH_3OH) λ_{max} nm (log ε) 284 (3.6); EIMS *m/z* 290 $[M]^+$; HREIMS *m/z* 290.0787 ($[M]^+$; calcd for $C_{15}H_{14}O_6$, 290.0790); ^{1}H and ^{13}C NMR (CD_3OD), see Table 1.

Inhibitory activity against PTPs

The effects of compounds **1** and **2** on PTPs were examined by measuring the rate of hydrolysis of the substrate, *p*NPP, according to the method previously described with slight

modifications [10, 11, 13]. PTP1B (100 µl of a 0.5 µg/ml stock solution), TCPTP (100 µl of a 0.5 µg/ml stock solution), CD45 (100 µl of a 0.5 µg/ml stock solution) or VHR (100 µl of a 1.0 µg/ml stock solution) in 50 mM citrate buffer (pH 6.0) containing 0.1 M NaCl, 1 mM dithiothreitol (DTT), and 1 mM EDTA was added to each well of a 96-well plastic plate. A sample (2.0 µl in CH_3OH) was added to each well to make the final concentration and was then incubated at 37 °C for 10 min. The reaction was initiated by the addition of *p*NPP in citrate buffer (100 µl of a 4.0 mM stock solution), incubated at 37 °C for 30 min, and then terminated using 10 µl of a stop solution (10 M NaOH). Optical density in each well was measured at 405 nm using an MTP-500 microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan). PTP1B inhibitory activity (%) was defined as $[1 - (ABS_{sample} - ABS_{blank})/(ABS_{control} - ABS_{blank})] \times 100$. ABS_{blank} is the absorbance of wells containing only the buffer and *p*NPP. $ABS_{control}$ is the absorbance of *p*-nitrophenol liberated by the enzyme in the assay system without a test sample, whereas ABS_{sample} is that with a test sample. Assays were performed in three duplicate experiments for all test samples. Oleanolic acid, a known phosphatase inhibitor [12], was used as a positive control.

Acknowledgements This work was supported in part by Grants-in-Aid for Scientific Research (25870660 and 16K21310) from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan to HY, the Takeda Science Foundation to HY, the Kanae Foundation for the Promotion of Medical Science to HY, and a grant for Basic Science Research Projects from the Sumitomo Foundation to HY. We express our thanks to Mr. T. Matsuki and S. Sato of Tohoku Medical and Pharmaceutical University for measuring mass spectra and to Mr. T. Miura, Y. Bunya, T. Togashi, and Ms. S. Sugai of Tohoku Medical and Pharmaceutical University for their technical assistance.

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