

Short Communication

Manzamenones Inhibit T-Cell Protein Tyrosine Phosphatase

Yusuke Wakuda¹, **Takaaki Kubota**², **Hiroshi Shima**^{1,3}, **Tadashi Okada**¹, **Shinya Mitsuhashi**¹, **Naohito Aoki**⁴, **Kunimi Kikuchi**¹ and **Jun'ichi Kobayashi**^{2,*}

¹ Division of Biochemical Oncology and Immunology, Institute for Genetic Medicine, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060-0815, Japan

² Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan.
Tel. 81 11 706 3239. Fax 81 11 706 4989. E-mail: jkobay@pharm.hokudai.ac.jp.

³ Division of Cancer Chemotherapy, Miyagi Cancer Center Research Institute, Natori, Miyagi 981-1293, Japan.

⁴ Department of Life Sciences, Faculty of Bioresources, Mie University, 1577 Kurimamachiya-cho, Tsu, Mie 514-8507 Japan

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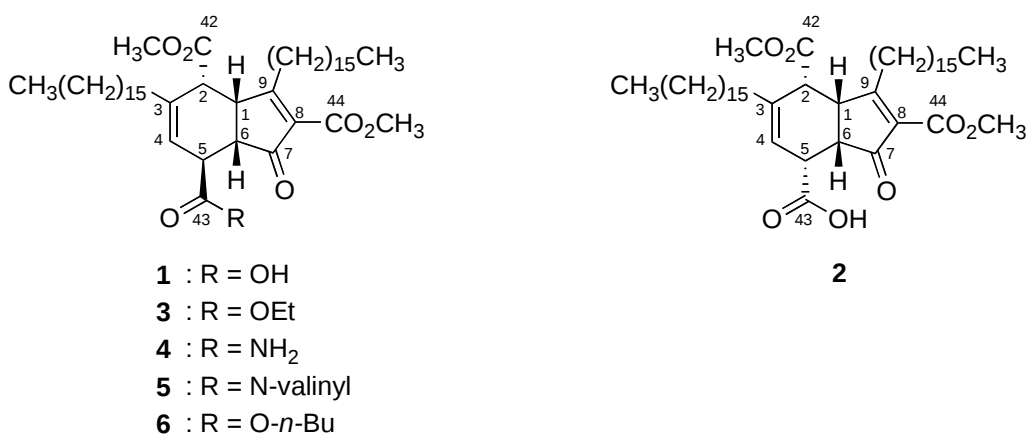
Abstract: Manzamenones A~C (1~3) and E~F (5~6), unique oxylipin metabolites isolated from a marine sponge *Plakortis* sp., have been found to exhibit inhibitory activity against T-cell protein tyrosine phosphatase (TCPTP). The inhibitory activity of **2** and **5** against TCPTP was 4 times more potent than that against protein tyrosine phosphatase-1B (PTP1B).

Keywords: Protein tyrosine phosphatases; TCPTP; Inhibitors; Manzamenones

1. Introduction

Protein tyrosine phosphatases (PTPs) are responsible for selective dephosphorylation of tyrosine residues, and regulate a wide variety of cellular processes including cell growth, differentiation, metabolism, cell-cell communication, cell migration, the immune response, and apoptosis/survival decisions [1-3]. Since recent studies have demonstrated that loss of protein tyrosine phosphatase-1B (PTP1B) activity resulted in enhancement of insulin sensitivity in addition to decrease in susceptibility to diet-induced obesity [4-5], specific PTP1B inhibitors have been developed as therapeutic agents in type-2 diabetes and obesity [6-7]. On the other hand, little attention has been paid to other PTPs such as T-cell PTP (TCPTP), CD45, SHP-1, and SHP-2. Among them TCPTP is the most homologous

phosphatase to PTP1B [8], and recent biochemical analysis revealed that TCPTP regulates hematopoietic development, cytokine response, and insulin signaling *in vitro* [9-10]. In our screening of a number of sponge metabolites against TCPTP and PTP1B, manzamenones B (2) and E (5) [11-12] were found to be relatively potent inhibitors, and more potent against TCPTP rather than PTP1B. In this paper we describe inhibitory activity of manzamenones A~F (1~6) [11-12] against TCPTP and PTP1B, and show manzamenones B (2) and E (5) to be relatively potent inhibitors against TCPTP.



2. Results and Discussion

The inhibitory effects against TCPTP of manzamenones A~F (1~6) were examined as follows. The hydrolysis of *p*-nitrophenyl phosphate (pNPP) by TCPTP was monitored by absorbance at 405 nm in the presence and absence of 5 μ M of compounds 1~6 (Fig.1). Manzamenones A (1) and B (2) showed inhibitory activities against TCPTP, while manzamenone D (4) did not show such activity. The IC₅₀ values of compounds 1~6 are shown in Table 1. The IC₅₀ values of relatively potent compounds 2 and 5, possessing a carboxylic acid functionality in the side chain, were 2.5 and 3.2 μ g/ml, respectively, while compound 1, the C-5 epimer of 2, and compounds 3 and 6, the ethyl or *n*-butyl ester of 1, respectively, showed less inhibitory activity than 2. On the other hand, compound 4, a simple amide at C-5 of 1, did not show such activity. Thus, the presence of a carboxylic acid or ester functionality and the stereochemistry at C-5 may be important for the inhibitory activity.

Next, inhibitory activities of manzamenones A~F (1~6) against PTP1B were examined (Table1). Compounds 2 and 5 showed inhibitory activities against PTP1B with IC₅₀ values of 10.8 and 13.5 μ M, respectively, whereas the other compounds did not show such activity. Although many PTP inhibitors have been reported so far, compounds 2 and 5 are the first inhibitors showing significant selectivity to TCPTP over PTP1B. No inhibitory effects of compounds 1~6 against PP1 and PP2A, Ser/Thr protein phosphatases, and A-kinase, one of Ser/Thr protein kinase (Table 1) were observed. Therefore,

compounds **2** and **5** may interact mainly with the catalytic site of PTPs, with preference to TCPTP rather than PTP1B.

Figure 1. Progress of *p*-nitrophenyl phosphate hydrolysis by T-Cell protein tyrosine phosphatase in the absence and presence of manzamenones (**1**, **2**, and **4**). 0.125 μ M TCPTP was added to the reaction buffer containing 1mM pNPP, and incubated in the presence of 5 μ M each of **1** ($\square$), **2** ($\circ$) and **4** ($\triangle$), or absence of inhibitor ($\times$) at 30 °C. The reaction was continuously monitored at 405 nm by spectrophotometer.

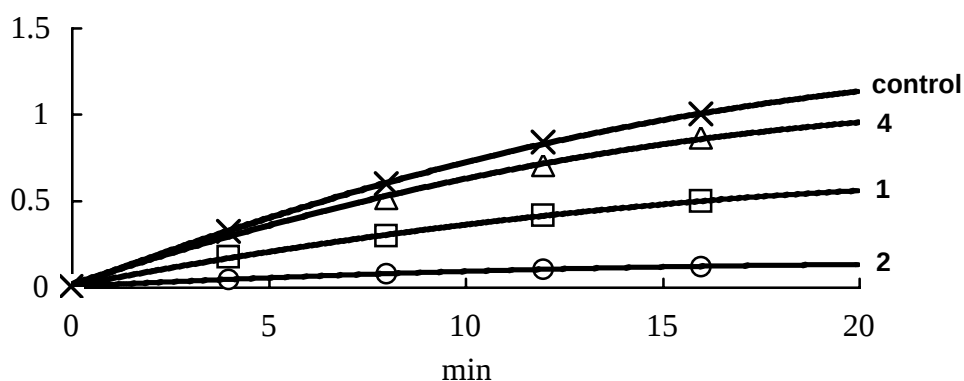


Table 1. Inhibitory Activities of Manzamenones A~F (**1**~**6**) against Protein Tyrosine Phosphatases.

Compound	IC ₅₀ values (μ M)			
	TCPTP	PTP1B	PP1/PP2A	A-kinase
1	9.1	>20	>50	>50
2	2.5	10.8	>50	>50
3	7.6	>20	>50	>50
4	>20	>20	-	-
5	3.2	13.5	>50	>50
6	12.6	>20	-	-

Since manzamenone B (**2**) showed relatively potent inhibitory activity against TCPTP, its inhibition kinetics were analyzed (Fig. 2). The inhibition mode of **2** was a mixed type one as shown in Fig. 2A, suggesting that **2** might interact with the catalytic center and peripheral site(s) of TCPTP. The K_i value for inhibition of TCPTP by **2** was calculated to be 2.2 μ M (Fig.2B).

In this study, manzamenones B (**2**) and E (**5**) were found to be relatively potent inhibitors against TCPTP rather than TCP1B. Compounds **2** and **5** may be useful bioprobes for studies of TCPTP functions as well as drug leads of selective TCPTP inhibitors.

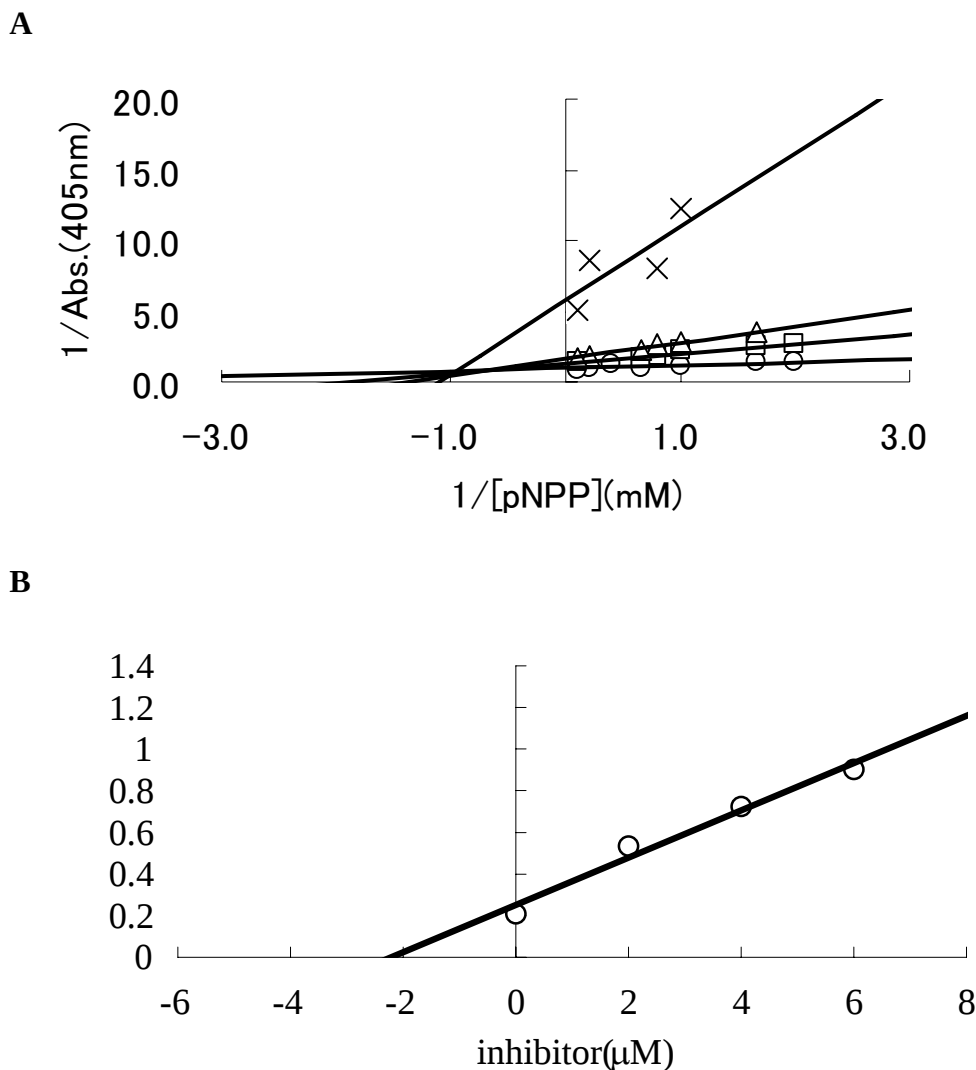


Figure 2. Manzamenone B (2) behaves as a mixed-type inhibitor of TCPTP. (A) Lineweaver-Burk plot. The activity of TCPTP was measured as described in the experimental section in the presence of the following concentrations of 2: ($\times$) 0 μM ; ($\triangle$) 2 μM ; ($\square$) 4 μM ; ($\circ$) 6 μM . Substrate concentrations used were 0.60, 0.70, 0.75, 0.85, 0.90, 1.0, 1.25, 1.5, 2.0 and 2.5 mM. (B) Secondary replots of the Lineweaver-Burk plot: K_m versus various concentrations of 2.

3. Experimental Section

3.1. Compounds

Manzamenones A~F (1~6) were supplied as described previously [11].

3.2. Bacterial expression of recombinant proteins

For bacterial expression of TCPTP as GST (glutathione S-transferase)-fusion protein, the coding regions, amplified by PCR, were subcloned into pGEX-6P-3 (Amersham Biosciences, Piscataway, NJ, U.S.A.). GST-PTP-1B [13] was expressed in *Escherichia coli* and purified as previously described [13].

3.3. Inhibitory activity against protein tyrosine phosphatases

The inhibitory activity of sponge metabolites against TCPTP and PTP1B was examined using *p*-nitrophenyl phosphate (pNPP) as a substrate. An assay mixture (100 μ l) containing 100 mM sodium acetate (pH5.0)/1.6 mM dithiothreitol/1 mM pNPP, and enzyme was incubated at 30 °C for 10min. Reactions were terminated by addition of 125 μ l of 1M NaOH and 225 μ l of each quenched reaction mixture was then added to a well of a 96-well titer plate where the absorbance was read at 405 nm using a microplate spectrophotometer (TECAN). IC₅₀ values of inhibitors were obtained on the basis of the data at the concentrations of 1, 2, 5 and 10 μ M of compounds **1**~**6**. Activities for PP1, PP2A and A-kinase were analyzed as previously described [14].

3.4. Determination of Ki values

The initial rate at 10 different substrate concentrations (0.60, 0.70, 0.75, 0.85, 0.90, 1.0, 1.25, 1.5, 2.0 and 2.5 mM) was measured at three different inhibitor concentrations (2, 4 and 6 μ M). The inhibition pattern was evaluated using Lineweaver-Burk plot, and the Ki values were obtained as the X-intercept by linear regression of the plot of Km versus inhibitor concentration.

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Sample availability: Not available.