J. Antibiot. 59(4): 251–253, 2006

NOTE

THE JOURNAL OF ANTIBIOTICS

A New Nucleoside Derivative, AJP117510, as an Inhibitor of Integrin $\alpha_2\beta_1$-Collagen Binding

Seiichi Sato†, Fumie Futaki, Naoyuki Fukuchi, Kenichi Kaida††, Masako Hiraga††, Seikichi Kobaru, Takashi Tsuji†

Received: March 6, 2006 / Accepted: April 7, 2006

Abstract A new nucleoside derivative, AJP117510 (**1**) was isolated from unidentified fungus AJ117510. The structure of **1** was elucidated by spectroscopic analyses. Nucleoside **1** inhibited the binding of integrin $\alpha_2\beta_1$ to collagen in a dose dependent manner with an IC_{50} value of 5.9 μM.

Keywords AJP117510, integrin $\alpha_2\beta_1$, collagen, inhibitor, nucleoside

The platelet membrane glycoprotein integrin $\alpha_2\beta_1$ is an important collagen receptor in hemostasis [1]. Platelet adhesion to subendothelial collagen that is exposed upon damage of the vessel wall is one of the initiating steps in thrombus formation [2]. In our search for novel antiplatelet agents based on the interaction between integrin $\alpha_2\beta_1$ and extracellular matrix collagen, a novel nucleoside derivative, AJP117510 (**1**) was isolated from unidentified fungus AJ117510 (Fig. 1). **1** inhibited the binding of integrin $\alpha_2\beta_1$ to collagen in a dose dependent manner *in vitro*. We describe isolation, structure elucidation, and inhibitory activity of integrin $\alpha_2\beta_1$-collagen binding of **1**.

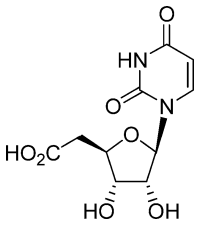

Fig. 1 Structure of AJP117510 (**1**).

Unidentified fungus AJ117510 was isolated from fruiting body of unidentified discomycete collected at Yamanashi Prefecture, Japan. A slant culture of strain AJ117510 was maintained on an agar slant. A 28-day culture at 25°C of the agar slant was transferred into twenty of Roux flasks containing 100 ml of a producing medium composed of glucose 0.2%, fructose 0.5%, sucrose 0.8%, NZ-Case (Humco) 0.2%, $MgSO_4 \cdot 7H_2O$ 0.05%, KCl 0.05%, $ZnSO_4 \cdot 7H_2O$ 0.05%, and KH_2PO_4 0.1% (pH 6.0). The fermentation was carried out at 25°C for 14 days. The mycelium of AJ117510 was extracted with acetone (4 liters) at room temperature. The acetone extract was concentrated *in vacuo* to give an aqueous suspension. The concentrate was partitioned between *n*-BuOH and H_2O. The aqueous layer was dried *in vacuo*. The residue was applied to a Dowex 1X8 (AcO^-) column (3.0 i.d.×16 cm). After washing with deionized water, the active compound was eluted with 5～10% aqueous acetic acid. The active fraction was subjected to a Dowex 50WX4 (H^+) column (2.2 i.d.×20 cm), and eluted with deionized water. Further purification was performed by DEAE-TOYOPEARLPAK 650S (AcO^-) (2.2 i.d.×20 cm) with a linear gradient from 0 to 10% aqueous acetic acid at a flow rate of 4.0 ml/minute to give **1** (510 mg) (Scheme 1).

S. Sato (Corresponding author)**, F. Futaki, N. Fukuchi, K. Kaida, M. Hiraga, S. Kobaru, T. Tsuji:** Pharmaceutical Research Laboratories, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki 210-8681, Japan, E-mail: seiichi_sato@ajinomoto.com

† Present address: AminoScience Laboratories
†† Present address: Institute of Life Sciences

The physico-chemical properties of **1** are shown in Table 1. The UV spectrum of **1** showed absorption maximum at 261 nm. The molecular formula of **1** was established to be $C_{10}H_{12}O_7N_2$ by HR-FAB MS. **1** was negative to ninhydrin and $FeCl_3$ reagent. The ^1H (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** are shown in Table 2.

The ^1H NMR spectrum showed a methylene proton (δ 2.65 and 2.81), three carbinyl protons (δ 4.02, 4.23, and 4.26), an anomeric proton (δ 5.70), and two aromatic protons. When considered together, the UV spectrum and the ^1H NMR signals in the aromatic region indicated the presence of uracil moiety. These results suggested that **1** was nucleoside derivative related to uridine. The difference between **1** and uridine was that the chemical shift values of the protons at C-5′ methylene of **1** were observed at higher field (δ 2.65 and 2.81) than those of uridine (δ 3.81 and 3.92). The large J value between the geminal protons (16.4 Hz) indicated that the C-5′ methylene was in the α position of the carbonyl group. ^{13}C NMR spectrum supported a presence of uracil moiety (δ 104.6, 144.3, 153.7, and 168.4), furanose moiety (δ 39.7, 74.5, 75.1, 82.0, and 92.5), and carboxylic acid (δ 176.9). Connectivities from C-1′ to C-5′, and C-5 to C-6 were established by the ^1H-^1H COSY spectrum. Furthermore, the HMBC correlation data connected C-1′ and N-1, and C-5′ and carboxylic acid moiety. Thus, the structure of **1** was determined as carboxylic acid analogue in place of the hydroxyl group at C-5′ of uridine (Figure 2). The J values in the sugar moiety of **1** were good agreement with those of uridine (Table 3). The elucidated structure of **1** is similar to the nucleoside skeleton of polyoxins [3]. Biosynthesis of similar 5′-elongated nucleoside polyoxins have been reported previously [4]. Analogously, **1** would be biosynthsized *via* 5′-aldehyde of uridine and the absolute configuration of **1** was deduced to be same as uridine.

Effect of **1** on the interaction of integrin $\alpha_2\beta_1$ and

Acetone extract of AJ117510
↓ Partitioned between *n*-BuOH and H_2O
H_2O
↓ Dowex 1X8 (AcO^-)
5-10 % aq. AcOH
↓ Dowex 50WX4 (H^+)
H_2O
↓ DEAE-TOYOPEARLPAK 650 S (AcO^-)
↓ aq. AcOH
1

Scheme 1 Isolation procedure for **1**.

Table 1 Physico-chemical properties of **1**

Appearance	colorless needles
Melting point	202~205°C
Molecular formula	$C_{10}H_{12}O_7N_2$
HR FAB-MS (*m/z*)	
Found $(M-H)^-$	271.0551
Calcd	271.0566
UV $\lambda_{max}^{H_2O}$ nm (ε)	261 (19100)
$[\alpha]_D^{23}$	+30.8°(*c* 0.5, H_2O)

Table 2 ^1H and ^{13}C NMR spectral data of **1** recorded in D_2O

Position	**1**		Uridine
	^1H	^{13}C	^1H
2	—	153.7	—
4	—	168.4	—
5	5.75 (d, *J*=8.0[a])	104.6	5.90 (d, *J*=8.4)
6	7.52 (d, *J*=8.0)	144.3	7.88 (d. *J*=8.4)
1′	5.70 (d, *J*=4.4)	92.5	5.92 (d, *J*=4.8)
2′	4.26 (dd, *J*=4.4, 5.6)	75.1	4.36 (dd, *J*=4.8, 5.2)
3′	4.02 (t, *J*=5.6)	74.5	4.23 (dd, *J*=5.2, 5.6)
4′	4.23 (ddd, *J*=4.4, 5.6, 8.8)	82.0	4.14 (ddd, *J*=2.8, 4.4, 5.6)
5′a	2.65 (dd, *J*=8.8, 16.4)	39.7	3.92 (dd, *J*=2.8, 12.8)
5′b	2.81 (dd, *J*=4.4, 16.4)		3.81 (dd, *J*=4.4, 12.8)
6′	—	176.9	—

[a] Multiplicity, *J* in Hz.

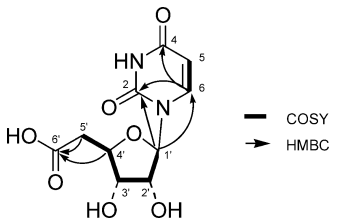

Fig. 2 ^{1}H-^{1}H COSY and selected HMBC correlations of **1**.

Table 3 ^{1}H-^{1}H coupling constants of sugar moiety of **1** and uridine

	$J_{1'-2'}$	$J_{2'-3'}$	$J_{3'-4'}$
1	4.4	5.6	5.6
Uridine	4.8	5.2	5.6

immobilized collagen was evaluated by using dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) method [5]. **1** and europium (Eu)-labeled integrin $\alpha_2\beta_1$ in assay buffer (Wallac Inc.) including 2 mM $MgCl_2$ were applied to each well of a collagen-coated microtiterplate. The plate was incubated for 2 hours. After washing the plate, enhancement solution (Wallac Inc.) was added. Time-resolved fluorometry of Eu was used to measure the level of the binding. The Eu signal was detected with excitation at 340 nm and emission at 615 nm. Nucleoside **1** inhibited the binding of integrin $\alpha_2\beta_1$ to collagen in a dose dependent manner with an IC_{50} value of 5.9 μM. Uridine exhibited no activity at a dose of 120 μM in this assay.

No cytotoxicity against P388 murine leukemia cells was observed at a dose of 294 μM. The absolute configuration and synthesis of its analogues are under investigation.

Acknowledgement We wish to thank Dr. Hideyuki Nagao (University of Tsukuba) for providing the strain AJ117510. We are also grateful to Reiko Yuji, Naoko Shimba, and Mina Nakamura for measurement of mass and NMR spectra.

References

1. Knight CG, Morton LF, Onley DJ, Peachey AR, Messent AJ, Smethurst PA, Tuckwell DS, Farndale RW, Barnes MJ. Identification in collagen type I of an integrin $\alpha_2\beta_1$-binding site containing an essential GER sequence. J Biol Chem 273: 33287–33294 (1998)
2. Depraetere H, Wille C, Gansemans Y, Stanssens P, Lauwereys M, Baruch D, De Reys S, Deckmyn H. The integrin $\alpha_2\beta_1$ (GPIa/IIa)-I-domain inhibits platelet-collagen interaction. Thromb Haemost 77: 981–985 (1997)
3. Isono K, Asahi K, Suzuki S. Studies on polyoxins, antifungal antibiotics. XIII. J Am Chem Soc 91: 7490–7505 (1969)
4. Isono K, Sato T, Hirakawa K, Funayama S, Suzuki S. Biosynthesis of the nucleoside skeleton of polyoxins. J Am Chem Soc 100: 3937–3939 (1978)
5. Waddleton D, Ramachandran C, Wang Q. Development of a time-resolved fluorescent assay for measuring tyrosine-phosphorylated proteins in cells. Anal Biochem 309: 150–157 (2002)