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A bile acid derivative with PPARγ-mediated anti-inflammatory activity

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ABSTRACT

During our search for bioactive secondary metabolites in the jellyfish-derived fungus *Penicillium chrysogenum* J08NF-4, several bile acid derivatives (**2–6**) were isolated along with a new steroidal artifact (**1**). An *in vitro* anti-inflammatory assay showed that pretreatment with **1** suppressed NO production and the gene expressions of the pro-inflammatory mediators iNOS and TNF-α in LPS-induced RAW 264.7 macrophages. Docking analysis of **1** revealed that it might bind to the ligand binding domain (LBD) of PPARγ in a manner similar to that of the synthetic steroid mifepristone (**7**), which is used clinically to treat hypercortisolism and was recently reported to be a PPARγ agonist. Compound **1** activated PPARγ in murine Ac2F liver cells and suppressed the LPS-induced phosphorylation of the NF-κB p65 subunit leading to downregulation of pro-inflammatory mediators.

Our findings suggest that **1** acts as a steroidal PPARγ activator that downregulates the expressions of pro-inflammatory mediators by suppressing the NF-κB signaling pathway.

1. Introduction

Peroxisome proliferator-activated receptors (PPARs) are members of the ligand-activated nuclear receptor family, members of which function as transcription factors. The PPAR family consists of three subtypes (α, δ/β, and γ) which are encoded by separate genes and play essential roles in variety of physiological functions including glucose absorption, lipid balance, inflammation, and cell differentiation [1]. PPARγ is predominantly expressed in adipose and colon tissues and in macrophages, and is an essential modulator of adipocyte differentiation, fatty acid storage, and glucose metabolism. Thiazolidinediones (TZDs) are synthetic PPARγ ligands that activate PPARγ receptor, and these activations lead to the assembly of coactivators and retinoid X receptor (RXR), which bind to the peroxisome proliferator response elements (PPRE) of DNA and up-regulate genes, such as, adiponectin, ap2 (adipocyte protein 2), and ADRP (adipose differentiation-related protein) that ameliorate hyperglycemia. Because of their insulin-sensitizing properties, the TZDs rosiglitazone and pioglitazone have been approved for the treatment of type-2 diabetes by the FDA.

PPARγ also acts as a key regulator of inflammatory and immune responses, and PPARγ agonists inhibit pro-inflammatory gene expression by suppressing NF-κB [2] or AP-1 (activator protein 1) [3], modulate kinase activities [4], interfere with the aggregation of coactivators (SRC-1, TIF2, and AIB-1) [5], and interact with corepressors (HDAC3, TBL1, and TBLR1) [6]. Endogenous PPARγ agonists, such as, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15d-PGJ_2), potently block inflammatory responses by suppressing the expressions of various transcription factors [NF-κB, STAT (signal transducer and activator of transcription-1), and AP-1] and of pro-inflammatory mediators, such as inducible nitric oxide synthase (iNOS) and tumor necrosis factor (TNF-α) [7,8].

During our investigation of the bioactive components from the jellyfish-derived fungus *Penicillium chrysogenum* J08NF-4, we isolated a bile acid trifluoroacetate (**1**) and five known bile acids (**2–6**) (Fig. 1), and bile acid derivatives compose a steroid class with anti-inflammatory, anticancer, neuroprotective, antifungal, and antimicrobial activities [9–13]. As regards to their anti-inflammatory properties, they have been reported to suppress immune response by inhibiting NF-κB and related pro-inflammatory cytokines [14]. The synthetic steroid mifepristone (**7**) has been used clinically to treat hypercortisolism, and was recently reported to act as a PPARγ agonist with anti-diabetic activity in animal models, in which it reduced blood glucose levels and improved insulin sensitivity [15–19]. In view of the structural similarity between the steroidal skeletons of **1** and mifepristone, we performed *in*

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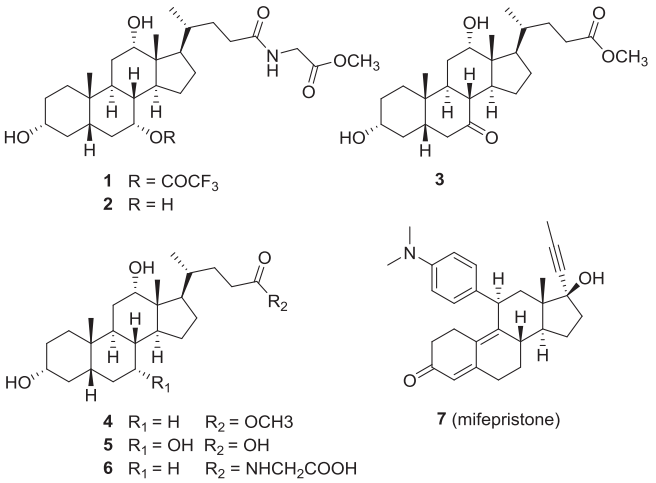

Fig. 1. Chemical structures of isolated steroids **1**–**6** and of mifepristone (**7**).

silico analysis of **1** by docking simulation. This analysis indicated that hydrogen bonding and hydrophobic interactions between **1** and the LBD of PPARγ may stabilize helices 3 and 5, and the β-sheet region of the LBD of PPARγ in a manner similar to mifepristone.

Our *in vitro* assay revealed that **1** modulated inflammatory responses by activating PPARγ and suppressing the NF-κB signaling pathway and the expressions of pro-inflammatory mediators (NO, iNOS, and TNF-α). Herein, we describe the structural elucidation and PPARγ/NF-κB-mediated anti-inflammatory activity of compound **1**.

2. Experimental

2.1. General experimental procedures

1D and 2D NMR spectra were recorded using Varian UNITY 400 and Varian INOVA 500 spectrometers, respectively. Chemical shifts are reported with respect to respective residual solvents or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD). HRESIMS data were obtained using an Agilent 1200 UHPLC accurate-mass Q-TOF MS spectrometer. HPLC was performed using a Gilson 307 pump, an ODS column (YMC-triart C18, 250 × 10 mm, i.d. 5 μm) and a Shodex RI-101 detector.

2.2. Fungal strain

The fungal strain *P. chrysogenum* J08NF-4 was isolated from the marine jellyfish *Nemopilema nomurai*, which was collected off the southern coast of South Korea in June 2008. A specimen has been deposited at the Marine Natural Product Laboratory, Pusan National University. After rinsing with filtered and sterilized seawater (National Institute of Fisheries Science), small pieces of surface and inner tissues were homogenized and then inoculated on malt extract agar (MEA) in Petri dishes. The sterilized MEA medium used was prepared using seawater containing glucose (20 g/L), malt extract (20 g/L), agar (20 g/L), peptone (1 g/L), and antibiotics (final concentrations: 50 μg/mL penicillin and 50 μg/mL streptomycin). Emerging fungal colonies were transferred to the same medium in a Petri dish and incubated at 25 °C for 10–14 days to allow colony development [20]. Twelve pure fungal strains (J08NF-1 ~ J08NF-12) were isolated. The fungal strain J08NF-4 was selected and identified as *P. chrysogenum* based on its morphologic characteristics and ITS gene sequences (Gene Bank accession No. KR011759). *P. chrysogenum* J08NF-4 was then cultured in 44 L of MEA medium (prepared with 75% seawater) containing glucose (20 g/L), malt extract (20 g/L), and peptone (1 g/L) at 30 °C on a shaker platform at 130 rpm for 21 days.

2.3. Extraction and isolation

After culturing *P. chrysogenum* J08NF-4 for 21 days, culture medium and mycelia were extracted with EtOAc at room temperature. The

Table 1
^1H (500 MHz) and ^{13}C NMR (100 MHz) Data for Compounds **1** and **2** (CD_3OD).

Position	**1**			**2**		
	δ_C type	δ_H (J in Hz)	HMBC(H → C)	δ_C type	δ_H (J in Hz)	HMBC(H → C)
1	36.2, CH_2	1.43, m; 1.62, m		36.5, CH_2	1.42, m; 1.76, m	
2	31.0, CH_2	1.44, m; 1.60, m		31.2, CH_2	1.40, m; 1.56, m	
3	72.4, CH	3.40, m		72.9, CH	3.37, m	
4	39.7, CH_2	1.53, m; 1.91, m		40.4, CH_2	1.64, m; 2.27, m	
5	42.3, CH	1.46, m		43.2, CH	1.37, m	
6	32.3, CH_2	1.66, m; 2.18, m		35.8, CH_2	1.51, m; 1.95, m	
7	78.8, CH	5.13, m	5, 6, 8, 9, 1″	69.0, CH	3.79, m	5, 6, 8, 9
8	39.4, CH	1.83, m		41.0, CH	1.53, m	
9	29.4, CH_2	2.31, m		27.9, CH_2	2.23, m	
10	35.4, C			35.9, C		
11	29.8, CH_2	1.59, m; 1.62, m		29.6, CH_2	1.51, m; 1.55, m	
12	73.2, CH	3.98, t (3.0)	9, 13, 14, 18	74.0, CH	3.95, t (3.0)	9, 13, 14, 18
13	47.8, C			47.5, C		
14	43.2, CH	1.99, m		43.0, CH	1.96, m	
15	24.0, CH_2	1.18, m; 1.32, m		24.2, CH_2	1.10, m; 1.72, m	
16	28.4, CH_2	1.31, m; 1.87, m		28.6, CH_2	1.29, m; 1.89, m	
17	47.8, CH	1.87, m		48.0, CH	1.87, m	
18	12.9, CH_3	0.73, s	12, 14, 17	13.0, CH_3	0.71, s	12, 14, 17
19	23.0, CH	0.96, s	1, 5, 9	23.2, CH	0.90, s	1, 5, 9
20	36.6, CH	1.44, m		36.8, CH	1.44, m	
21	17.6, CH_3	1.03, d (6.5)	17, 20, 22	17.7, CH_3	1.02, d (6.5)	17, 20, 22
22	33.0, CH_2	1.35, m; 1.79, m		33.1, CH_2	1.35, m; 1.79, m	
23	33.6, CH_2	2.17, m; 2.30, m		33.7, CH_2	2.17, m; 2.28, m	
24 (CONH)	177.3, C			177.2, C		
1′	41.8, CH_2	3.90, s	2′, 24	41.8, CH_2	3.89, s	2′, 24
2′ (C═O)	171.9, C			173.1, C		
OCH_3	52.6, CH_3	3.70, s	2′	52.6, CH_3	3.70, s	2′
1″ (C═O)	157.8, C					
2″ (CF_3)	117.2, C					