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An Antitumor Component from *Fomitiporia ellipsoidea*

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A natural furan derivative was isolated from the methanolic extract of the fruit bodies of *Fomitiporia ellipsoidea*. Its chemical structure was elucidated as methyl 3,5-dioxo-1,3,5,7-tetrahydrobenzo[1,2-c:4,5-c']difuran-4-carboxylate by means of extensive NMR and MS data analysis, and named as fomitiporiaester A (1). Compound 1 showed significant antitumor activity to hepatoma H_{22} *in vivo*, and the inhibition rates were 42.94%, 49.17%, and 58.15% at concentrations of 5, 10, and 20 mg/kg, respectively. Compound 1 showed weak cytotoxic activities against the human hepatoblastoma (HepG-2) and human oophoroma (Skov 3) cell lines with IC_{50} values of more than 100 μM.

Keywords: *Fomitiporia ellipsoidea*, furan ester, antitumor

The fungal kingdom includes many species that produce various classes of structurally unique and biologically active metabolites [3, 7, 8]. *Fomitiporia ellipsoidea* (Hymenochaetaceae), a giant polypore, was first recorded in 2008 by Professor Bao-kai Cui in the Wanmulin Nature Reserve of Fujian Province, China [1]. Previous chemical investigation indicated that this fungus produced a large amount of common ergosterol and its derivatives, which had been reported to exhibit anti-inflammatory or tumor growth inhibition activities [5]. Mushroom belonging to the genus *Fomitiporia* and similar genus *Phellinus* or *Inonotus* have been used as traditional medicines for the treatment of gastrointestinal cancer or heart disease [4]. In the course of our ongoing screening for antitumor components from macrofungi resources, methyl 3,5-dioxo-1,3,5,7-tetrahydrobenzo[1,2-c:4,5-c']difuran-4-carboxylate was isolated and identified from *Fomitiporia ellipsoidea*; we named it as fomitiporiaester A (**1**). In this paper, we present the isolation, and structural determination of **1** and its antitumor activities *in vitro* and *in vivo*. In addition, to evaluate the safety of this compound, the effects on the body weight, immune organs (spleen and thymus), and interleukin-2 (IL-2) of KM mice were examined at the same time.

The ground fruiting body (1.4 kg) was extracted with MeOH at room temperature for 5×12 h. After the solvent was removed under reduced pressure at 35°C, a dark brown residue was obtained. The residue was suspended in water and then partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc fraction was separated by open column chromatography on silica gel eluting with a gradient MeOH (0–20%) in CH_2Cl_2 to give 7 fractions (Fr.1–7). Fraction 2 was chromatographed on silica gel eluting with CH_2Cl_2: MeOH (30:1), and further purified by Sephadex LH-20 eluted with CH_2Cl_2: MeOH (6:4) to afford compound **1** (76 mg).

Fomitiporiaester A (**1**) was obtained as a colorless square crystal with molecular formula of $C_{12}H_8O_6$ established base on positive high-resolution electron-impact mass spectra (HR-EI-MS) at *m/z* 248.0337[M]$^+$ (Calcd 248.0321). The IR spectrum (KBr) implies the presence of aromatic rings (1,622, 1,512, 1,446.8 cm^{-1}), and a carbonyl group (1,772 cm^{-1}). The ^{1}H-NMR spectrum of compound **1** showed signals attributable to the ABX system at δ8.05 (1H, *t*, H-3), a methoxyl moiety at δ3.98 (3H, *s*, -OCH_3), and oxygenated methylene δ5.59 (4H, *d*, H-7,12). The ^{13}C-NMR spectrum of compound **1** (Table 1) showed signals for 8 carbons (five quaternary, one methine, one methylene, and one methyl carbon atoms). Comprehensive analyses of the 2D-NMR data, including the HMQC and HMBC, were used to elucidate the planar structure of the compound. The HMQC spectrum showed the following correlations: from δ_H5.59 to δ_C70.10, δ_H8.05 to δ_C118.95, and δ_H3.98 to

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Table 1. ^{1}H- and ^{13}C-NMR spectra data for fomitiporiaester A.

No.	δ_H	δ_C	No.	δ_H	δ_C
1 (5)	-	130.31	7 (12)	5.59 (4H, *d*)	70.10
2 (4)	-	153.46	9 (10)	-	167.60
3	8.05 (1H, *t*)	118.95	13	-	164.01
6	-	123.49	$-OCH_3$	3.98 (3H, *s*)	52.71

Compound **1** was measured in CD_3COCD_3 at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR with TMS as the internal standard.

Table 2. *In vivo* antitumor activities of compound **1** on H_{22} in KM mice (n = 10).

Group	Dose (mg/kg)	Tumor weight (g)	Inhibitory rate (%)	IL-2
Normal group				69.471 ± 5.543
NaCl		1.8013 ± 0.7941		53.405 ± 2.971
[a]CTX	20	0.8546 ± 0.4557**	52.56%	48.517 ± 3.852
Compound	5	1.0279 ± 0.4240*	42.94%	58.650 ± 2.302
	10	0.9156 ± 0.3317**	49.17%	62.285 ± 3.212*
	20	0.7539 ± 0.4779**	58.15%	53.970 ± 3.368

[a]CTX was used as the positive control. *$p < 0.05$, **$p < 0.01$ compared with the negative control or blank group.

δ_C52.71. Key correlations were observed in the HMBC spectrum (Fig. 2) from δ3.98 ($-OCH_3$) to C-13; from δ5.59 (H-7) to C-3, C-5, and C-9; and from δ8.05 (H-3) to C-4, C-5, and C-7. Taking into account the molecular formular of $C_{12}H_8O_6$ generated by HR-MS, the structure of compound **1** was unambiguously established as methyl 3,5-dioxo-1,3,5,7-tetrahydrobenzo[1,2-c:4,5-c']difuran-4-carboxylate (Fig. 1), and we named it fomitiporiaester A.

The cytotoxicity activities of compound **1** against proliferation of HepG-2 (human hepatoblastoma) and Skov-3 (human ovarian cancer) cells were conducted with the SRB assay, as previously described. Compound **1** showed weak cytotoxic effect against HepG-2 and Skov-3 with an IC_{50} value of more than 100 μM.

In vivo antitumor activity was evaluated using H_{22}-bearing female Kunming strain mice (20±2.0 g). The H_{22} tumor cells (1×10^6 cells per mouse) were inoculated subcutaneously to mice at the axillary region besides the normal group. At the 24 h time point of post-inoculation, the H_{22}-bearing mice were randomly assigned to the following six groups (10 per group): normal group, positive control (CTX, 20 mg/kg), negative control (0.9% sodium chloride), and test samples (5 mg/kg group, 10 mg/kg group, and 20 mg/kg group). The test samples and 0.9% sodium chloride were administered intragastrically once daily for 10 consecutive days. The cyclophosphamide (CTX) was injected into the mice (0.1 ml/mouse) by intraperitoneal injection, once every two days for five times. Mice were sacrificed on the 11th day of post-inoculation and the tumors were excised and weighed, along with the spleen and thymus. The tumor inhibition was calculated as follows: tumor inhibitory rate (%) = $(1-W_{sample}/W_{negative\ control}) \times 100\%$, where W is the average tumor weight of each group. To evaluate the immunocompetencies of each sample, the relative spleen weight (mg/g, W_{spleen}/W_{mouse}) and relative thymus weight

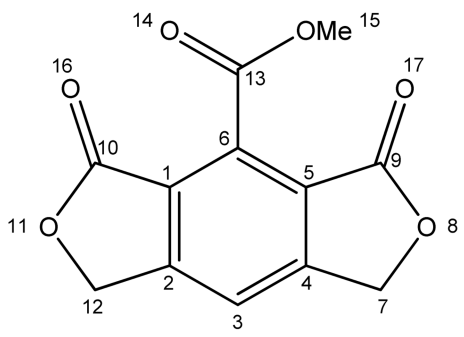

Fig. 1. Chemical structure of compound **1**.

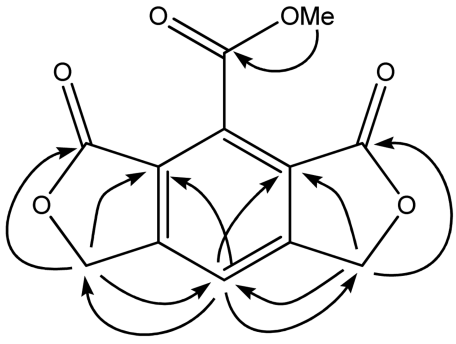

Fig. 2. Key HMBC correlations of compound **1**.

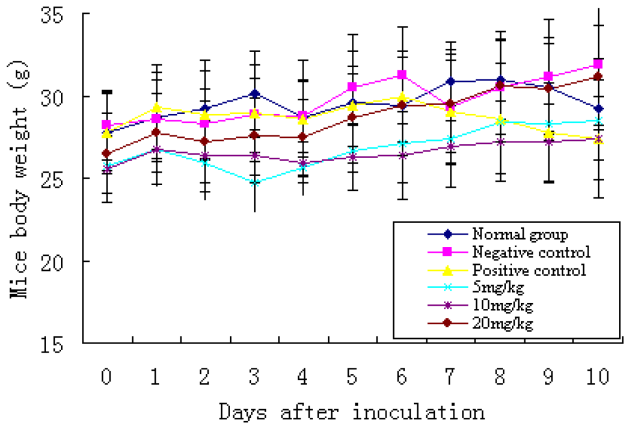

Fig. 3. Curve of body weight changing of H_{22} mice.

(mg/g, W_{thymus} /W_{mouse}) were calculated from a comparison between the treated groups and the control group. Sera collected from the H_{22}-bearing mice were measured using a murine enzyme-linked immunosorbent assay (ELISA) kit for interleukin-2 (IL-2) according to the instruction of the manufacturer.

As shown in Table 2, significant tumor inhibitions were observed on the three doses of compound treatment group compared with the control group, and inhibition rates were calculated as 42.94%, 49.17%, and 58.15% at concentrations of 5, 10, and 20 mg/kg, respectively. The positive drug (CTX, 20 mg/kg) exhibited a moderate inhibitory rate at 52.56%, but at the same time, it decreased the body weight in the tumor-bearing mice compared with the test sample groups. Fomitiporiaester A remarkably increased the organ weight of spleen and thymus in H_{22}-bearing mouse (Fig. 4). As shown in Table 2, the IL-2 content of mice in the CTX group decreased significantly. Contrary to the CTX group, the IL-2 content in the fomitiporiaester A treatment groups increased slightly, and there was no significant difference between these three groups. It was also found that, except for the positive control group, there was no significant change in body weight in all treatment groups (Fig. 3) and this result suggested that compound **1** did not affect the normal metabolism of the animals.

Currently, chemotherapy is considered as the most effective method of cancer treatment. Intervention with chemopreventive agents at the early stage in carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumors with chemotherapeutic drugs. However, most chemotherapeutic drugs severely affect the normal cells of the host [6]. The use of lower cytotoxic natural products has been contemplated to be of exceptional value in the treatment of cancer. In this note, we have isolated the compound, namely fomitiporiaester A, from *Fomitiporia ellipsoidea*, which showed potent antitumor activity against H_{22} tumors *in vivo*, with lower direct cytotoxic activity against HepG 2. A significant increase of treatment group in the relative spleen and thymus weights was observed compared with the model control group. The relative spleen and thymus weights was an important index for nonspecific immunity. An immunopotentiator could increase the spleen and thymus weights. Immunosuppressive agents could induce weight decrease of the spleen and thymus or even the decline of immune function. CTX inhibited the growth of tumor in mice, but at the same time, it damaged the immunity of the mice. IL-2 plays an important role in the development and expansion of effector T cells, which is also critical for the establishment and maintenance of immune tolerance [9]. Compared with the positive control CTX treatment, the IL-2 content in compound **1** treatment groups increased slightly further, indicating that fomitiporiaester A could potentially serve as an antitumor agent without disturbing normal metabolism.

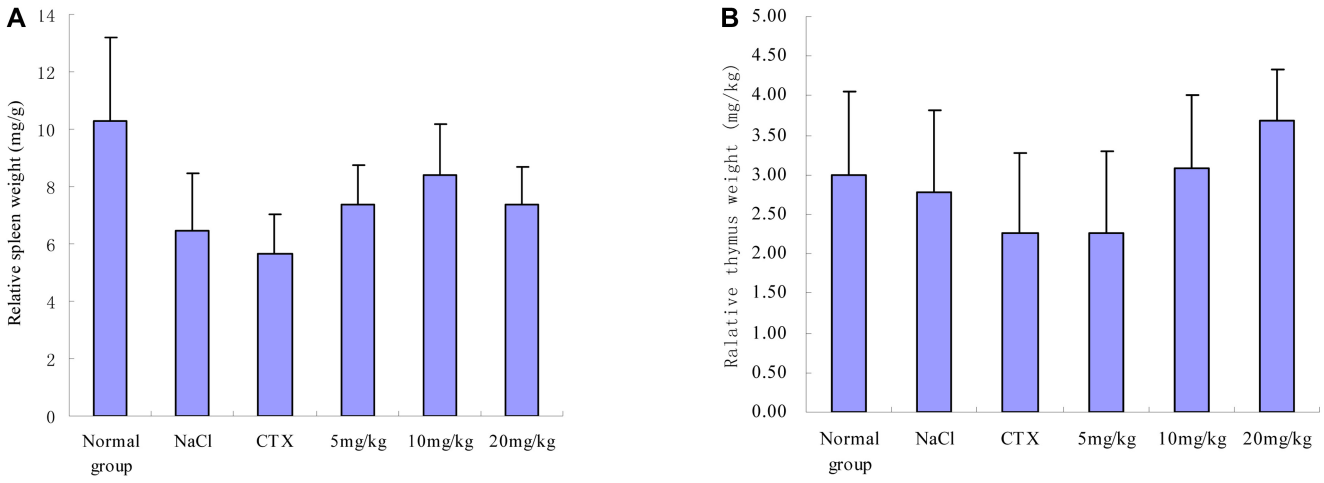

Fig. 4. Spleen index (**A**) and thymus index (**B**) of H_{22} mice treated with fomitiporiaester A from *F. ellipsoidea* (n = 10).

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