Isoindolones from *Lasiosphaera fenzlii* REICH. and Their Bioactivities

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Two new, **1** and **2**, along with one known isoindolone, **3**, were isolated from the AcOEt extract of *Lasiosphaera fenzlii* REICH. The structures of these compounds were determined as 4,6-dihydroxy-1*H*-isoindole-1,3(2*H*)-dione (**1**), 4,6-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one (**2**), and clitocybin A (**3**) on the basis of chemical and spectroscopic evidences. The bioactivity assays revealed that all of them were devoid of significant cytotoxicities against tumor cells, whereas **1** exhibited potent antiangiogenic activity by inhibiting the secretion of vascular endothelial growth factor (VEGF) in A549 cells.

1. Introduction. – *Lasiosphaera fenzlii* REICH., a fungus widely distributed in all parts of China, has been used as a remedy for bleeding in Traditional Chinese Medicine. A number of studies have been reported on the chemical constituents and biological activities of *L. fenzlii* [1–3]. Several natural products isolated from *L. fenzlii* have received increased interest for their potential biological activities as anticancer agents in recent years [4–6]. In our efforts to search for inhibitors of tumors from natural sources, *L. fenzlii* was collected for a systematic investigation, which led to the isolation of two new and one known isoindolones from the AcOEt extract (*Fig. 1*). The structures of the two new compounds were determined as 4,6-dihydroxy-1*H*-isoindole-1,3(2*H*)-dione (**1**), 4,6-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one (**2**) by spectroscopic methods. The known compound was identified as clitocybin A (**3**). Here, the isolation and structure determination of compounds **1** and **2**, and their cytotoxicities against tumor cells are presented.

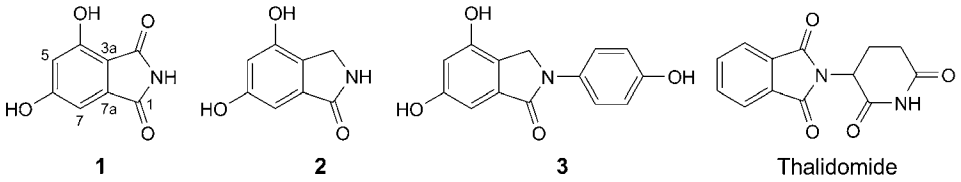

Fig. 1. *Structures of compounds* **1**–**3** *and thalidomide*

Compound **1** contains a phthalimide moiety, like that responsible for the antitumor and antiangiogenic activities of thalidomide [7] (*Fig. 1*). Angiogenesis plays a critical

role in the growth of tumor cells, and tumor angiogenesis inhibition represents a promising strategy for the treatment of cancer [8]. Among multiple signals that can contribute to angiogenesis, vascular endothelial growth factor (VEGF), highly expressed in tumors, can be the indirect target for therapeutic intervention against angiogenesis [9–12]. Therefore, effects of these compounds on VEGF secretion by tumor cells are also reported to indirectly evaluate their antitumor properties.

2. Results and Discussion. – 2.1. *Structure Elucidation.* Compound **1** was obtained as pale-yellow crystals from MeOH, and its molecular formula, $C_8H_5NO_4$, was determined by negative-ion-mode HR-ESI-MS (*m/z* 178.0154 ($[M-H]^-$; calc. 178.0140)). The UV spectrum of **1** showed absorption maxima at 219.7, 240.9, and 348.9 nm, suggesting the presence of a benzene ring structure. The ^{1}H-NMR (300 MHz, (D_6)DMSO; *Table*) data indicated the presence of two phenolic OH groups (δ 10.75 (br. *s*, 4,6-OH)) and one NH group (δ 10.65 (br. *s*, NH)). Two aromatic H-atom signals at δ 6.57 (*d*, $J = 1.8$, H–C(7)), 6.50 (*d*, $J = 1.8$, H–C(5)) revealed the presence of a disubstituted benzene moiety. The ^{13}C-NMR (75 MHz, (D_6)DMSO; *Table*) spectrum showed eight C-atom signals, including those of two C=O C-atoms (δ 170.0 and 168.9), two oxygenated sp^2 C-atoms (δ 165.3 and 157.9), and four sp^2 C-atoms (δ 108.7, 137.8, 108.5, and 103.5). In the HMBC spectrum, the aromatic H-atom signals at δ 6.57 and 6.50 showed long-range correlations with the C-atom signals at δ 170.0, 165.3, 157.9, and 108.5, and at δ 168.9, 165.3, 157.9, 108.7, and 103.5, respectively. The long-range correlations of these aromatic H-atoms revealed the presence of a 4,6-disubstituted isoindolone moiety. Thus, the structure of compound **1** was identified as 4,6-dihydroxy-1*H*-isoindole-1,3(2*H*)-dione.

Table. *^{1}H-* (300 MHz) *and ^{13}C-NMR* (75 MHz) *Data for Compounds* **1** *and* **2** *in (D_6)DMSO.* δ in ppm, *J* in Hz.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	170.0	–	170.4
3	–	168.9	4.11 (*s*, 2 H)	42.5
4	–	157.9	–	153.4
5	6.50 (*d*, $J = 1.8$, 1 H)	108.5	6.44 (*d*, $J = 1.8$, 1 H)	105.6
6	–	165.3	–	158.7
7	6.57 (*d*, $J = 1.8$, 1 H)	103.5	6.50 (*d*, $J = 1.8$, 1 H)	100.0
3a	–	108.7	–	121.0
7a	–	137.8	–	135.1
4,6-OH	10.75 (br. *s*, 2 H)	–	9.53 (br. *s*, 2 H)	–
NH	10.65 (br. *s*, 1 H)	–	8.37 (*s*, 1 H)	–

Compound **2** was isolated as pale-yellow crystals from MeOH, and its molecular formula, $C_8H_7NO_3$, was determined by negative-ion-mode HR-ESI-MS (*m/z* 164.0357 ($[M-H]^-$; calc. 164.0348)). The UV spectrum of **2** showed absorption maxima at 206.8, 246.8, and 302.5 nm, suggesting the presence of a benzene ring structure. The ^{1}H-NMR (300 MHz, (D_6)DMSO; *Table*) data indicated the presence of two phenolic OH groups (δ 9.53 (br. *s*, 4,6-OH)) and one NH group (δ 8.37 (*s*, NH)), and one CH_2

group (δ 4.11 (*s*, CH_2(3))). Two aromatic H-atom signals at δ 6.50 (*d*, $J = 1.8$, H–C(7)) and 6.44 (*d*, $J = 1.8$, H–C(5)) revealed the presence of a disubstituted benzene moiety. The ^{13}C-NMR spectrum of **2** was similar to that of **1**, except for the C=O C-atom signal at δ 168.9 of **1** which was replaced by those of a CH_2 group at δ 42.5 and 4.11 (*s*, CH_2(3)). In the HMBC spectrum, CH_2 H-atom signal at δ 4.11 showed correlations with the C-atom signals at δ 170.4, 153.4, 105.6, 158.7, 100.0, 121.0, and 135.1. Accordingly, the structure of compound **2** was elucidated as 4,6-dihydroxy-2,3-dihydro-1*H*-isoindol-1-one.

In addition, one known isoindolone was identified as clitocybin A (**3**) by comparing the ^{1}H-NMR, ^{13}C-NMR, and MS data with those previously reported in [13].

2.2. *Biological Evaluation.* 2.2.1. *Cytotoxic Activity.* All the compounds were tested for their antiproliferative effects against A549, PC-3, U87, and HeLa tumor cells. The results revealed that all of them were devoid of evident cytotoxic activities in the concentration range of 12.5 – 100 μM (only data with respect to A549 cells are shown in *Fig. 2*).

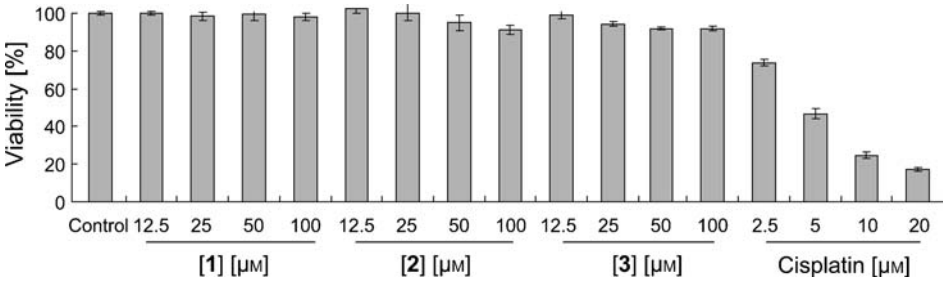

Fig. 2. *Effects of compounds* **1**–**3** *on viability of A549 cells after 48-h incubation.* Viable cells were examined by MTT assay. The data are mean ± SD ($n = 3$). Cisplatin: positive control, A549: human lung adenocarcinoma cells.

2.2.2. *Antiangiogenic Activity.* Among these compounds, different concentrations of **1** (25, 50, and 100 μM) inhibited the secretion of VEGF in A549 cells (*Fig. 3*). Thalidomide had been shown to inhibit VEGF produced by A549 cells [14], and our results suggested that **1** exhibited a higher antiangiogenic activity than thalidomide (*Fig. 3*).

Experimental Part

1. *General.* All solvents used were either spectral grade or distilled from glass prior to use. UV Spectra: *Shimadzu UV-2501* spectrophotometers; λ in nm. NMR Spectra: *Bruker Avance 300* or *600* NMR spectrometers with Me_4Si as an internal standard; chemical shifts (δ) in ppm and coupling constants (J) in Hz. HR-ESI-MS: *Waters LCT Premier XE* mass spectrometer; *m*/*z*.

2. *Plant Material. Lasiosphaera fenzlii* REICH. was collected in March 2010 at *Jian Ren Medicine Co., Ltd.* (Hebei, P. R. China). It was identified by Prof. *Qi-Shi Sun* at Shenyang Pharmaceutical University. A voucher specimen (A20100326L) was deposited with the Department of Pharmacy, General Hospital of Shenyang Military Area Command.

3. *Extraction and Isolation.* The dried *L. fenzlii* (5.0 kg) was extracted three times with 45% EtOH under reflux. The EtOH extract was concentrated to 5 l of aq. soln. under reduced pressure. The aq. soln. was partitioned successively with petroleum ether (12.1 g), $CHCl_3$ (8.6 g), AcOEt (9.7 g), and BuOH

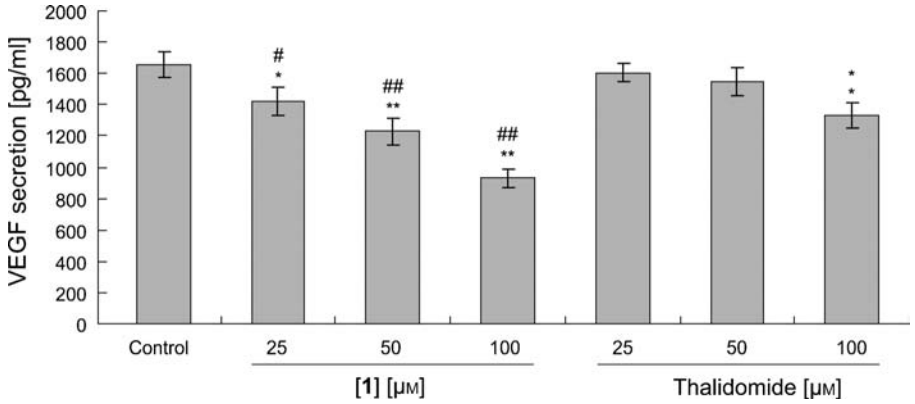

Fig. 3. *Effect on the secretion of VEGF in A549 cells by compound* **1**. A549 Cells were pretreated with compound **1** at doses ranging from 25 to 100 μM for 48 h, followed by ELISA assay. The data are mean ± SD ($n = 3$). *: $p < 0.05$, **: $p < 0.01$ compared with the control; #: $p < 0.05$, ##: $p < 0.01$ compared with thalidomide. Thalidomide: positive control.

(6.4g). Part of AcOEt extract (9.7 g) was subjected to column chromatography (CC) using silica gel and eluted with gradient $CHCl_3$/MeOH. The collected fractions were combined on the basis of their TLC characteristics, and grouped into seven fractions, *Frs. 1 – 7. Fr. 4* was further purified by CC (silica gel and *Sephadex LH-20*), to afford **1** (100 mg). *Fr. 5* was subjected to CC (ODS; MeOH/H_2O gradient), then further purified by *Sephadex LH-20* and repeated pHPLC (25% MeOH) to give **3** (34 mg) and **2** (33 mg).

3.1. *4,6-Dihydroxy-1*H-*isoindole-1,3(2*H*)-dione* (**1**). Pale-yellow crystals. UV (MeOH): 219.7, 240.9, 348.9. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 178.0154 ($[M - H]^-$, $C_8H_5NO_4^-$; calc. 178.0140).

3.2. *4,6-Dihydroxy-2,3-dihydro-1*H-*isoindol-1-one* (**2**). Pale-yellow crystals. UV (MeOH): 206.8, 246.8, 302.5. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 164.0357 ($[M - H]^-$, $C_8H_7NO_3^-$; calc. 164.0348).

4. *Cytotoxicity Assay.* Cytotoxicity was assessed using the standard MTT (= 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) assay. Briefly, cancer cell lines were seeded into 96-well plates for 24 h. Then, different concentrations of the tested compounds were added, and incubation was continued for 48 h. The absorbance was determined *via* a MTT assay with a microplate reader at 492 nm. For the positive and negative control groups, the cells were incubated with cisplatin and 0.1% DMSO, resp. The percentage of cell viability was calculated using the following equation: $A_S/A_C \times 100\%$, where A_C is the absorbance of the negative control, and A_S is the one of samples.

5. *Antiangiogenic Activity.* A549 Cells were placed in 96-well plate at a density of 5×10^3 cells per well and treated with various concentrations of compounds for 48 h. The VEGF levels in culture supernatant were determined with the human VEGF ELISA kit (*Saier Si Biotechnology Co., Ltd.*, Yantai, P. R. China) according to the manufacturer's instruction.

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