J Appl Biol Chem (2013) 56(3), 179–181
http://dx.doi.org/10.3839/jabc.2013.028

Online ISSN 2234-7941
Print ISSN 1976-0442

Short Communication: Bioactive Materials

Cytotoxicity of Neolignans from *Magnolia obovata* Fruits

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Received: 24 December 2012 / Accepted: 30 May 2013 / Published Online: 30 September 2013

Abstract Repeated SiO_2 and octadecyl silica gel (ODS) column chromatographies of the EtOAc fraction from *Magnolia obovata* fruits, 10 neolignans, named magnolol (**1**), honokiol (**2**), isoobovatol (**3**), isomagnolol (**4**), obovatol (**5**), obovatal (**6**), 9-methoxyobovatol (**7**), magnobovatol (**8**), obovaaldehyde (**9**), and 2-hydroxyobovaaldehyde (**10**) were isolated and identified. All isolated compounds were evaluated for *in vitro* cytotoxicity against seven human cancer cell lines.

Keywords cancer cell · cytotoxicity · honokiol · *Magnolia obovata* · magnolol · neolignan

Magnolia obovata Thunb. is a deciduous tree that is distributed throughout Korea, China, and Japan. This plant has been used for the treatment of fever, headache, diarrhea, anxiety, and relief of asthma in Chinese medicine. *M. obovata* has been reported to have anti-platelet (Pyo et al., 2002a; 2002b), anti-gastritic (Cho et al., 2008), anti-inflammatory (Tzeng and Liu, 2004; Seo et al., 2013) and cytotoxic (Min et al., 2008; Youn et al., 2008) activities. So far, magnolol, honokiol, and obovatol have been reported to show cytotoxic activities against human cancer cells (Kim and Ryu, 1999; Lin et al., 2001; Yang et al., 2003; Youn et al., 2008; Patrick et al., 2011). However cytotoxic effects of neolignans from *M. obovata* fruits have never been reported. Therefore, in the present study, the neolignans from *M. obovata* fruits were evaluated for cytotoxic effects against human cancer cell lines *in vitro* using the micro culture tetrazolium (MTT) assay. We isolated 10 neolignans from *M. obovata* fruits using SiO_2 and octadecyl silica gel (ODS) column chromatography and their phytochemical structures were identified using spectroscopic methods.

M. obovata Thunb. fruits were collected at Kyung Hee University, Korea in September 2010 and identified by Prof. Seung-Woo Lee, Department of Horticultural Biotechnology, Kyung Hee University. A voucher specimen (KHU-NPCL-201009) was deposited in the Laboratory of Natural Products Chemistry, Kyung Hee University.

Human colon adenocarcinoma (HCT-116), human breast adenocarcinoma (MCF-7), human breast adenocarcinoma (SK-BR-3), human ovarian adenocarcinoma (SK-OV-3), human cervix adenocarcinoma (HeLa), human hepatoma (HepG2), and human melanoma (SK-MEL-5) were obtained from the Korean Cell Line Bank (KCLB, Korea). Cells were grown at 37°C with 5% CO_2 in RPMI 1640 medium with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin except for HeLa, HepG2, which was grown in Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) FBS. All cell culture media and reagents were purchased from Thermo Scientific Hyclone (USA).

The cytotoxicities of neolignans from *M. obovata* fruits were measured using an 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) colorimetric assay. Cell culture and cytotoxic assays against seven human cancer cells were performed employing the MTT assay as described in the literature (Park et al. 2011).

During our search for cytotoxic compounds from natural sources, the MeOH extract of the fruits of *M. obovata* was found

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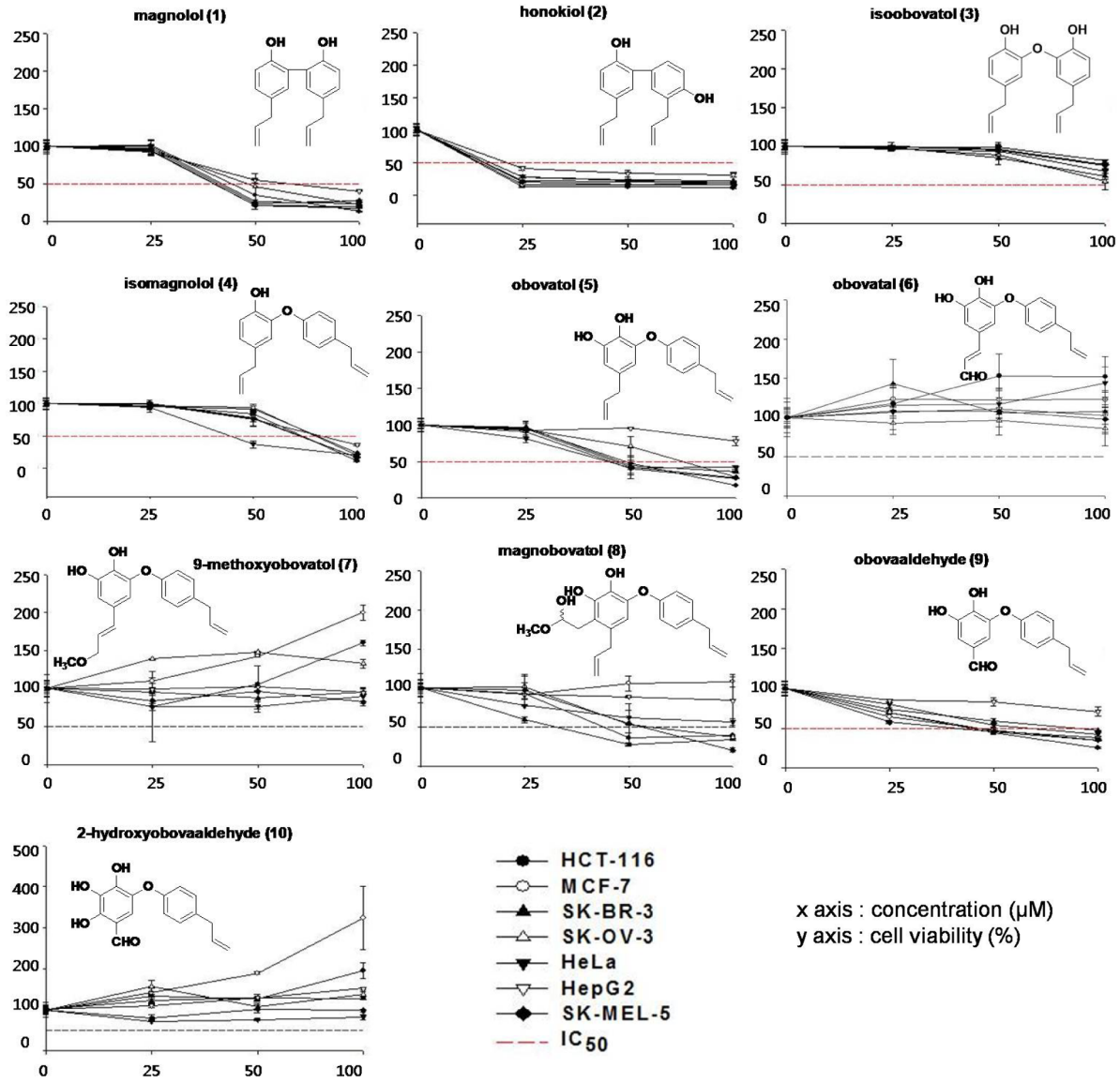

Fig. 1 The cytotoxicity of neolignans 1–10 isolated from the fruits of *Magnolia obovata* Thunb. against HCT-116, MCF-7, SK-BR-3, SK-OV-3, HeLa, HepG2, and SK-MEL-5 cancer cell lines.

to show significant cytotoxic effects on human cancer cell lines. Repeated SiO_2 and ODS column chromatographies for the EtOAc fraction of the MeOH extract yielded 10 neolignans, which were identified as magnolol (**1**), honokiol (**2**), isoobovatol (**3**), isomagnolol (**4**), obovatol (**5**), obovatal (**6**), 9-methoxyobovatol (**7**), magnobovatol (**8**), obovaaldehyde (**9**), and 2-hydroxy-obovaaldehyde (**10**) (Seo et al. 2013). Thus, the isolated compounds were tested for their cytotoxic activity against seven human cancer cell lines *in vitro* by the modified MTT assay method (Fig. 1). Compound **1** showed cytotoxicity with IC_{50} values of 39.2 μM (SK-MEL-5), 39.5 μM (MCF-7), 41.4 μM (SK-BR-3), 42.8 μM (HeLa), and 44.6 μM (HCT-116). Compound **2** showed IC_{50} values of 13.3 μM (SK-BR-3), 13.5 μM (MCF-7), 15.5 μM (SK-MEL-5), 15.6 μM (SK-OV-3), 17.1 μM (HCT-116), 17.1 μM (HeLa), and 20.3 μM (HepG2). Compound **4** showed IC_{50} values of 44.6 μM for HeLa, and moderate cytotoxicity with IC_{50} values over 70 μM for other cancer cell lines. Compound **5** showed cytotoxicity against all cancer cells except for HepG2 with IC_{50} values of 42.8-74.1 μM. Compound **8** showed IC_{50} values of 30.2 μM (SK-BR-3), 42.5 μM (HCT-116), 57.8 μM (SK-MEL-5), and 61.4 μM (SK-OV-3). Compound **9** showed IC_{50} values of 40.7 μM (SK-MEL-5), 44.5 μM (HCT-116), 46.1 μM (MCF-7), 46.3 μM (SK-OV-3), HeLa 63.1 μM (HeLa), and 87.1 μM (SK-BR-3). Compounds **3**, **6**–**7**, and **10** showed no cytotoxic activities against the seven cell lines even at 100 μM. Even though the tested neolignans have similar chemical structure, they showed different cytotoxicity according to a few variance in structure. The inactive neolignans such as compounds **3**–**10** have the ether bond

between monomer phenylpropanoids, while active ones such as compounds **1** and **2** have C-C bond. Therefore, the distance between two phenylpropanoid moieties could be a conclusive factor to show the cytotoxicity (Park et al. 2011; Lee et al. 2010). Magnolol (**1**), honokiol (**2**), and obovatol (**5**) were reported to show cytotoxic activity against the HeLa and HCT-116 cancer cell lines with IC_{50} values ranging from 8.6±1.4 to 16.4±1.7 μg/mL (Youn et al., 2008). However, the structures of isolated neolignans from *M. obovata* fruits are very similar, they showed different cytotoxic potencies on the cancer cells used in this study.

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