Synthesis of Obovatol Derivatives and Their Preliminary Evaluation as Antitumor Agents

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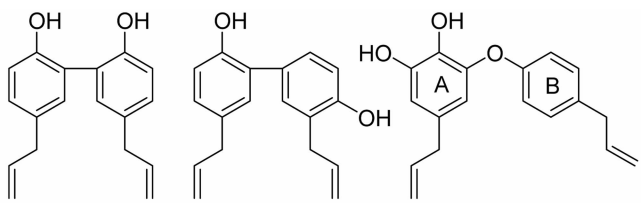
The bark of *Magnolia obovata* is widely used as a folk remedy for gastrointestinal disorders, cough, anxiety and allergic diseases in East Asia.[1] Major components, isolated from *Magnolia* species, are bisphenolic neolignans such as magnolol (**1**), honokiol (**2**) and obovatol (**3**) (Figure 1).[2] While considerable progress for biological activities has been made on both magnolol (**1**) and honokiol (**2**),[3] obovatol (**3**) possessing diaryl ether skeleton, has attracted a little attention.[3g,4,5] For example, magnolol (**1**) and honokiol (**2**) have shown to possess anti-platelet aggregation, anti-bacterial, anti-carcinogenic, and anti-metastatic activities.[3]

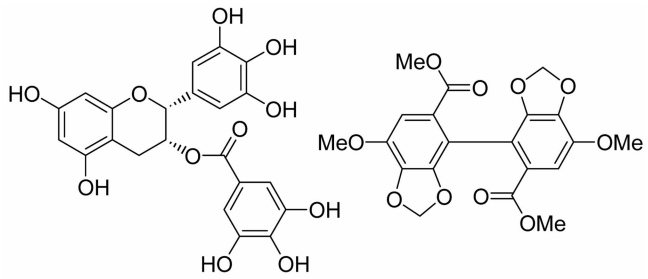
Recently, it was revealed that obovatol (**3**) has anti-inflammatory and antitumor activity through inhibition of NF-κB, significant transcriptional factor to control of cancer cell growth activity.[5c] Especially, obovatol inhibited cancer cell growth in prostate cancer (LNcap and PC-3) and colon cancer (SW620 and HCT116) cells, and also induced apoptotic cell death without any cytotoxic activity in normal cells (up to 50 μM). It is also well-known that NF-κB mediates tumor promotion, angiogenesis, and metastasis through the expression of genes participating in malignant conversion and tumor promotion, particularly in inflammation associated cancer models.[6]

The exciting biological roles of obovatol, combined with our continuing efforts toward diaryl ether compounds[7] possessing potential biological activities, led us to explore syntheses of obovatol derivatives. Herein, we report the synthesis and preliminary evaluation of a series of novel obovatol derivatives as antitumor agents. In considering the options for design of obovatol derivatives, we focused on a structural similarity between A-ring of obovatol and gallic acid, which is a key pharmacophore of several anticancer



Magnolol (**1**) Honokiol (**2**) Obovatol (**3**)

Figure 1. Representative Compounds isolated from *Magnolia* sp.



(-)-Epigallocatechin-3-gallate (**4**) α-DDB (**5**)

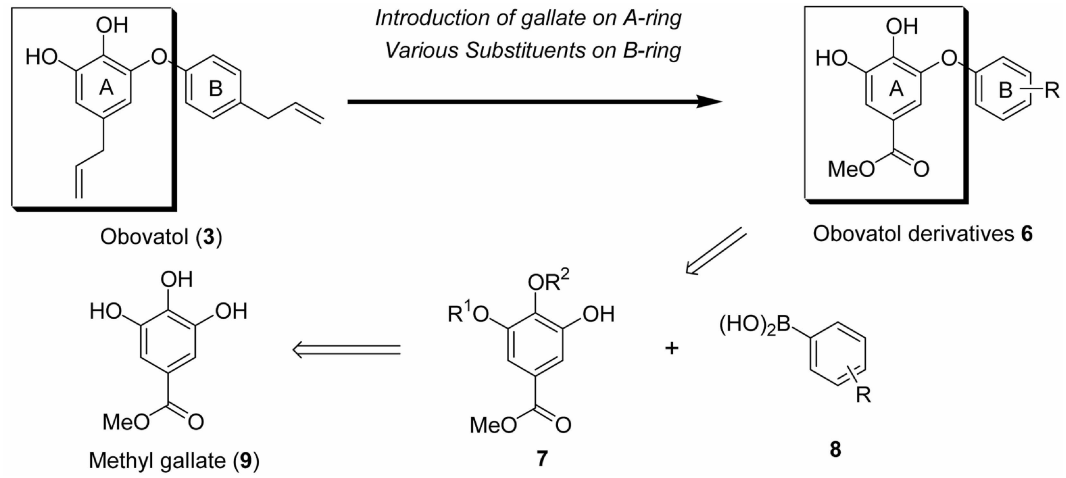
Figure 2. Structures of Natural Compounds Possessing Gallate Moiety.

agents (*e.g.* (−)-epigallocatechin gallate (**4**)[8] and 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxy-2,2'-dimethoxy-carbonyl-biphenyl (α-DDB, **5**)[9]), as shown in Figure 2.

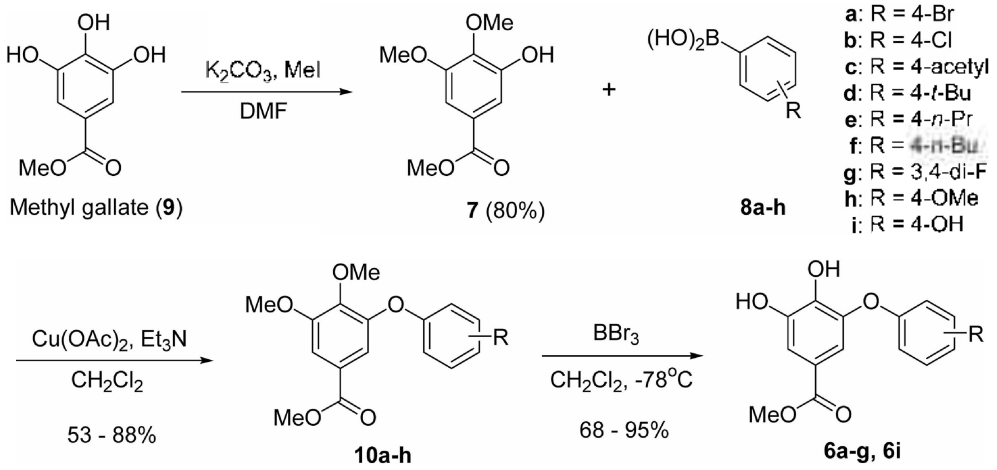
Thus, we decided to introduce a gallate moiety to the A-ring of obovatol and change the 4-allyl group on B-ring of obovatol with various substituents, from which a preliminary structure-activity relationship could be revealed (Scheme 1). To facilitate the efficient and diverse synthesis of the key diaryl ether[10] skeleton, the approach that emerged as being most attractive was the $Cu(OAc)_2$-mediated coupling reaction[11] between the protected gallate **7** and boronic acids **8**, due to its mild reaction conditions and functional group tolerance.

As illustrated in Scheme 2, our studies commenced with the preparation of the protected gallate **7**[9a] as a precursor for the formation of the obovatol derivatives **6**. Selective methylation of the methyl gallate (**9**) using MeI and basic treatment (K_2CO_3, DMF, 0 °C) provided the known compound **7**[9a] in 80% yield. Next, a variety of diaryl ethers **10** were prepared using Evans' protocol. Treatment of 4- or 3,4-substituted arylboronic acids **8** and phenol **7** with $Cu(OAc)_2$ in the presence of Et_3N and molecular sieve at room temperature afforded the desired diaryl ethers **10a-h** in modest to high yields.[11] Finally, deprotection of the coupling products **10** with BBr_3 furnished the desired derivatives **6a-g** and **6i** in good yields.

Newly synthesized diaryl ethers **10a-h**, **6a-g**, and **6i** were screened for their NF-κB luciferase activities in prostate LNCaP and colon cancer HCT116 cells, respectively.



Scheme 1. Synthetic Plan of Gallate-Introduced Obovatol derivatives.



Scheme 2. Synthesis of Obovatol derivatives.

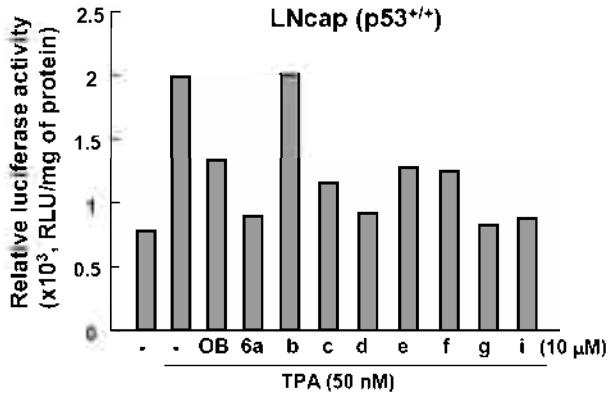


Figure 3. Luciferase Activity in Prostate Cancer LNCaP Cells (OB = obovatol).

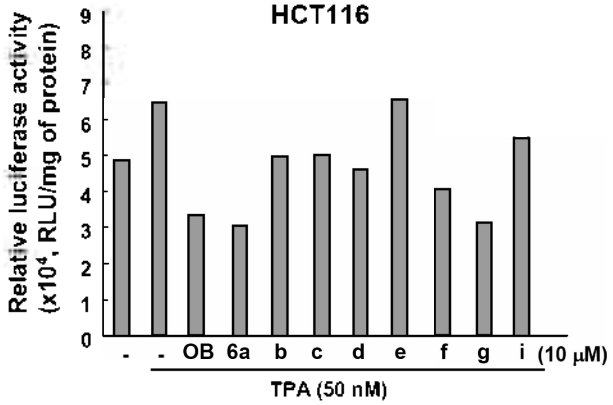


Figure 4. Luciferase Activity in Colon Cancer HCT116 Cells (OB obovatol).

Obovatol shows an IC_{50} of 18 μM on the TPA (50 nM)-induced prostate and colon cancer cell growth,[5c] and was utilized as a reference compound. As expected, all of the catechol-protected compounds **10a-h** displayed poor inhibitory activity. This result implies that phenol groups of obovatol would be essential for inhibition of NF-κB luciferase activity. Preliminary results in prostate cancer LNCaP and colon cancer HCT116 cells of **6a-i** are shown in Figure 3 and 4, respectively.

Analogues **6a** and **6g** with halogen group at the 4 position of ring B exhibited compatible activities with obovatol in both assay systems. On the other hand, chloro derivative **6b**

displayed poor inhibitory activity. Interestingly, 4-alkyl substituted derivatives **6d-f** show considerable activities in only prostate cancer LNCaP cells, whilst moderate activities in colon cancer cells. 4-Hydroxy derivative **6i** also displayed potent activity in prostate cancer cells. These results suggested that introduction of both the gallate moiety on ring A and the electronegative atom at the 4 position of ring B (*e.g.* F, Br, OH) would be positive effect on NF-κB luciferase activities. Furthermore, all of active compounds **6a**, **6c**, **6d**, **6g**, and **6i** didn't show any cytotoxic activity in cell viability assay.

In conclusion, a series of novel obovatol derivatives possessing gallate moiety on ring A were synthesized and preliminary evaluation of NF-κB luciferase activities in prostate LNCaP and colon cancer HCT116 cells showed that **6a**, **6c**, **6d**, **6g**, and **6i** had potent to moderate inhibitory activities. Taken together, our findings provide important information of the structural requirement for antitumor activities and offer new possibilities for further explorations to improve potency. Further study including IC_{50} values of these diaryl ethers is under good progress.

Experimental

General Procedure for the Preparation of Diary Ethers 10a-h. To a solution of the protected gallate **7** (0.1 mmol), arylboronic acid **8** (0.1 mmol), and copper acetate (0.1 mmol) in the presence of 4 A molecular sieves in CH_2Cl_2 (3 mL) was added triethylamine (0.5 mmol). The reaction mixture was vigorously stirred for 3-18 h at ambient temperature and filtered through a pad of Celite. The filtrate was concentrated *in vacuo* and the residue was purified *via* flash column chromatography on silica gel to afford the desired coupling product **10**.

Compound 10a: Yield 88%; IR (thin film) 1725 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.44 (s, 1H), 7.41 (d, 2H, J = 8.8 Hz), 7.31 (s, 1H), 6.84 (d, 2H, J = 8.8 Hz) 3.94 (s, 3H) 3.87 (s, 6H). **Compound 10b:** Yield 75%; IR (thin film) 1722 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (s, 1H), 7.29 (s, 1H), 7.25 (d, 1H, J = 8.8 Hz), 6.87 (d, 2H, J = 8.8 Hz) 3.92 (s, 3H) 3.86 (s, 3H), 3.85 (s, 3H); MS (FAB$^-$) m/z 322 (M^+). **Compound 10c:** Yield 53%; IR (thin film) 1718, 1670 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.93 (d, 2H, J = 8.8 Hz), 7.50 (s, 1H), 7.40 (s, 1H), 6.96 (d, 2H, J = 8.8 Hz), 3.95 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 2.57 (s, 3H); MS (FAB$^-$) m/z 330 (M^+). **Compound 10d:** Yield 60%; IR (thin film) 1722 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.41 (s, 1H), 7.33 (d, 2H, J = 8.8 Hz), 7.27 (s, 1H), 6.90 (d, 2H, J = 8.8 Hz), 3.95 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 1.31 (s, 9H); MS (FAB$^+$) m/z 344 (M^+). **Compound 10e:** Yield 82%; IR (thin film) 1714 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.39 (s, 1H), 7.29 (s, 1H), 7.12 (d, 2H, J = 8.8 Hz), 6.88 (d, 2H, J = 8.8 Hz), 3.93 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H), 2.48-2.58 (m, 2H), 1.53-1.68 (m, 2H), 0.85-0.96 (m, 3H); MS (FAB$^-$) m/z 330 (M^+). **Compound 10f:** Yield 81%; IR (thin film) 1720 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.39 (s, 1H), 7.29 (s, 1H), 7.12 (d, 2H, J = 8.8 Hz), 6.68 (d, 2H, J = 8.8 Hz), 3.93 (s, 3H), 3.91 (s, 3H), 3.85 (s, 3H), 2.50-2.60 (m, 2H), 1.46-1.60 (m, 2H), 1.31-1.41 (m, 2H), 0.85-0.94 (m, 3H); MS (FAB$^-$) m/z 344 (M^+). **Compound 10g:** Yield 65%; IR (thin film) 1733 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.46 (s, 1H), 7.32 (s, 1H), 7.05-7.14 (m, 1H), 6.75-6.82 (m, 1H), 6.65-6.69 (m, 1H), 3.95 (s, 3H), 3.88 (s, 6H); MS (FAB$^-$) m/z 324 (M^-). **Compound 10h:** Yield 66%; IR (thin film) 1718 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.37 (s, 1H), 7.20 (s, 1H), 6.94 (d, 2H, J = 9.1 Hz) 6.87 (d, 2H, J = 9.1 Hz), 3.94 (s, 6H), 3.84 (s, 3H), 3.80 (s, 3H); MS (FAB$^+$) m/z 318 (M^-).

General Procedure for the Preparation of Compounds 6a-g and 6i. To a solution of **10** (0.052 mmol) in dry methylene chloride (1 mL) was added BBr_3 (1.0 M solution, 0.16 mL, 0.16 mmol) at −78 °C. The reaction mixture was stirred at −78 °C for 1 hr and then at room temperature for 2 h, and was quenched by adding 1 mL of anhydrous MeOH dropwise. The solution was stirred for 30 min. The reaction mixture was then extracted with EtOAc and washed with $NaHCO_3$. The combined organic layers were dried over anhydrous $MgSO_4$, filtered through paper and concentrated. The residue was purified by flash column chromatography to afford **6**.

Compound 6a: Yield 95%; IR (thin film) 3396, 1698 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.44 (d, 2H, J = 8.9 Hz), 7.33 (s, 1H), 7.11 (s, 1H), 6.85 (d, 2H, J = 8.9 Hz), 3.81 (s, 3H). **Compound 6b:** Yield 70%; IR (thin film) 3318, 1700 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.46 (s, 1H), 7.33 (d, 2H, J = 8.8 Hz), 7.15 (s, 1H), 6.98 (d, 2H, J = 8.8 Hz), 5.94 (s, 1H), 5.60 (s, 1H), 3.81 (s, 3H); MS (FAB$^+$) m/z 294 (M^-). **Compound 6c:** Yield 68%; IR (thin film) 3335, 1690, 1665 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.95 (d, 2H, J = 8.4 Hz), 7.35 (s, 1H), 7.16 (s, 1H), 6.94 (d, 2H, J = 8.4 Hz), 3.79 (s, 3H), 2.53 (s, 3H); MS (FAB$^+$) m/z 302 (M^-). **Compound 6d:** Yield 72%; IR (thin film) 3313, 1700 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (s, 1H), 7.37 (d, 2H, J = 8.6 Hz), 7.19 (s, 1H), 6.96 (d, 2H, J = 8.6 Hz), 5.96 (s, 1H), 5.52 (s, 1H), 3.83 (s, 3H), 1.33 (s, 9H); MS (FAB$^+$) m/z 316 (M^-). **Compound 6e:** Yield 78%; IR (thin film) 3340, 1689 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (s, 1H), 7.16 (d, 2H, J = 8.4 Hz), 7.16 (s, 1H), 6.94 (d, 2H, J = 8.4 Hz), 5.99 (s, 1H), 5.56 (s, 1H), 3.81 (s, 3H), 2.57 (t, 2H, J = 7.6 Hz), 1.57-1.70 (m, 2H), 0.95 (t, 3H, J = 7.3 Hz); MS (FAB$^+$) m/z 302 (M^-). **Compound 6f:** Yield 70%; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.42 (s, 1H), 7.16 (d, 2H, J = 8.4 Hz), 7.16 (s, 1H), 6.94 (d, 2H, J = 8.4 Hz), 5.95 (s, 1H), 5.49 (s, 1H), 3.82 (s, 3H), 2.60 (t, 2H, J = 7.7 Hz), 1.55-1.65 (m, 2H), 1.25-1.42 (m, 2H), 0.85 (t, 3H, J = 8.0 Hz); MS (FAB$^-$) m/z 316 (M^-). **Compound 6g:** Yield 91%; IR (thin film) 3315, 1697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.47 (s, 1H), 7.12-7.21 (m, 2H), 6.86-6.92 (m, 1H), 6.76-6.81 (m, 1H), 5.87 (s, 1H), 5.54 (s, 1H), 3.85 (s, 3H); MS (FAB$^+$) m/z 296 (M^-). **Compound 6i:** Yield 77%; IR (thin film) 3345, 1692 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.17 (s, 1H), 6.88 (s, 1H), 6.82 (d, 2H, J = 8.9 Hz), 6.73 (d, 2H, J = 8.9 Hz), 4.87 (s, 3H), 3.72 (s, 3H); MS (FAB$^-$) m/z 276 (M^+).

Biological Assay

Cell culture: Prostate and colon cancer cells were obtain-

ed from the American Type Culture Collection (Manassas, VA 20108 USA). Dulbecco's modified Eagle medium (DMEM), penicillin, streptomycin, and fetal bovine serum were purchased from Gibco Life Technologies (Grand Island, NY). Prostate and colon cancer cells were grown in RPMI1640 medium with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin at 37 °C in 5% CO_2 humidified air.

Cell viability assay: Prostate and colon cancer cells were plated at a density of 10^4 cells/well in 96 well plates. The cytotoxic effect was evaluated in the cells cultured for 24 and 48 hr using the cell counting kit-8 (an assay kit) according to the manufacturer's instructions (Dojindo, Maryland, USA). Briefly, 10 μL of the CCK-8 solution was added to cell cultured for designed time. The plates incubate for 1-4 hr in the incubator. Metabolic activity was quantified by measuring light absorbance at 450 nm.

Transfection and assay of luciferase activity: Prostate and colon cancer cells (2.5×10^5 cells/cm^2) were plated in 24-well plates and transiently transfected with pNF-κB-Luc plasmid (5x NF-κB; Stratagene, CA, USA) using a mixture of plasmid and lipofectAMINE PLUS in OPTI-MEN according to manufacture's specification (Invitrogen, Carlsbad, CA, USA). The transfected cells were treated with TNF-α, TPA and 10 μM of obovatol derivatives for 8 hr. Luciferase activity was measured by using the luciferase assay kit (Promega) according to the manufacturer's instructions (WinGlow, Bad Wildbad, Germany).

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