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Ailanthoidol Derivatives and their *Anti*-inflammatory Effects

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Ailanthoidol showed a strong *anti*-inflammatory effect in a previous result. Ailanthoidol derivatives were prepared for the *anti*-inflammatory test using Sonogashira coupling, iodine induced cyclization and Wittig reaction. *Anti*-inflammatory effects of the prepared ailanthoidol derivatives were examined in lipopolysaccharide (LPS)-stimulated RAW 264-7 macrophages. The results showed that some ailanthoidol derivatives inhibited significantly the production of inflammatory mediator nitric oxide.

Key Words : Ailanthoidol, 2-Arylbenzo[*b*]furan, *Anti*-inflammatory, Nitric oxide, Iodine-induced cyclization

Introduction

Ailanthoidol, a natural 3-deformylated 2-arylbenzo[*b*]-furan, was isolated from the chloroform-soluble fraction of the tree of *Zanthoxylum ailanthoides*.[1] While there have been no reports on this compound's biological activities, extracts of the bark and leaves of this tree have been used in folk medicine. The 2-arylbenzo[*b*]furan structures in natural and unnatural sources showed a wide variety of biological activities including anticancer,[2] antiproliferative,[3] *anti*-inflammatory,[4] antiviral,[5] antifungal,[6] immunosuppressive,[7] antiplatelet,[8] antioxidative,[9] antifeedent,[10] and insecticidal activities.[11] The investigation of structure-activity relationships for 2-arylbenzo[*b*]furan substituents is still attractive due to a variety of biological activities. Recently, we synthesized ailanthoidol **1** (Fig. 1) in five steps reaction procedures in 72% overall yield from vanillin by mainly using Sonogashira coupling reaction with iodine-induced cyclization, and examined its *anti*-inflammatory activities.[12] The key features of our synthesis of benzofuran nucleus were regioselective halogenation, Sonogashira coupling and iodine-induced cyclization including optimization of the synthetic sequences. In this report, we describe the synthesis of ailanthoidol derivatives **2-8** (Fig. 2), and the comparison of *anti*-inflammatory effects of seven derivatives with **1**.

Results and Discussion

In order to prove the importance of 2-arylbenzo[*b*]furans for their biological activities, we prepared ailanthoidol

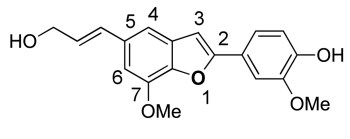

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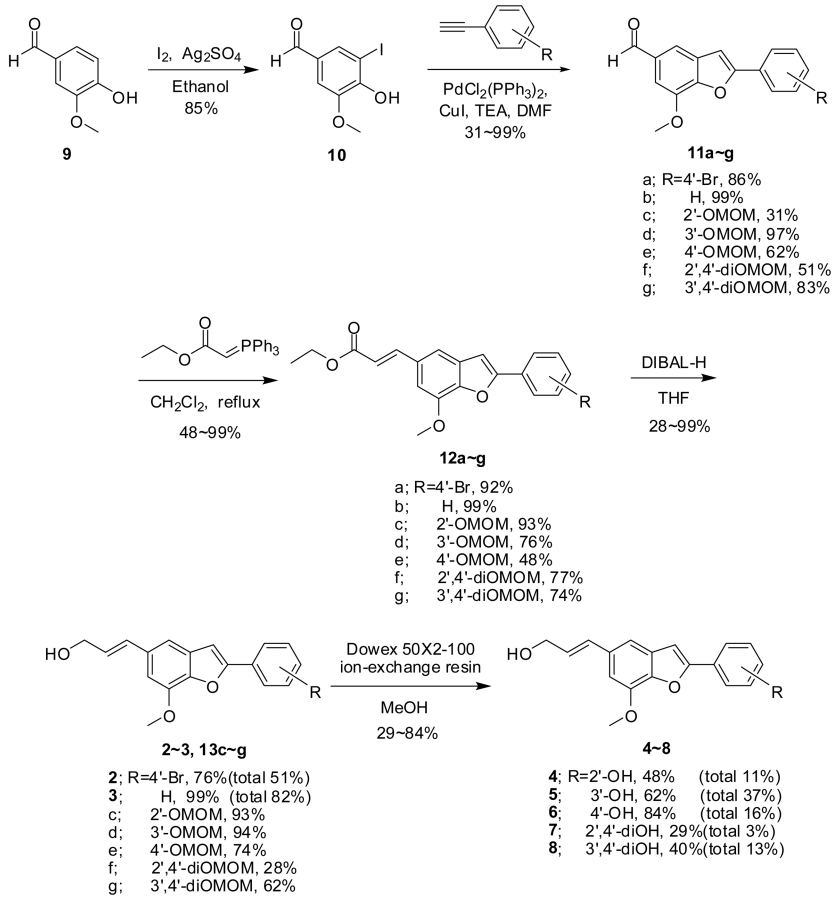
Figure 1. Structure of ailanthoidol.

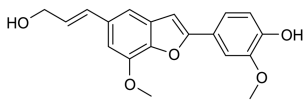
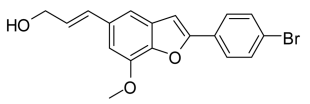
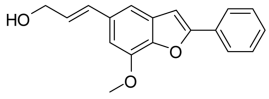
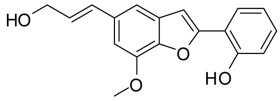
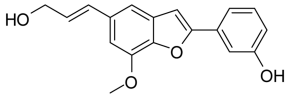
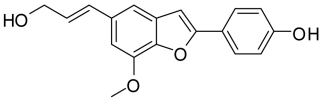
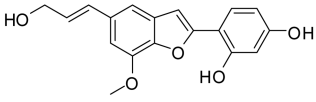
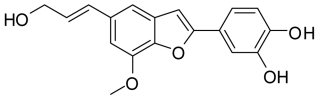
derivatives **2-8** (Scheme 1). The 3-iodo substituent **10**, which was regioselectively prepared from vanillin **9** as described in the synthesis of XH-14,[13] was easily coupled and cyclized to the benzofurans **11a-g** in 31-99% yields by Sonogashira coupling reaction using $Pd(PPh_3)_4$/CuI/TEA/DMF with appropriated acetylene derivatives. Direct cyclization of the coupled product in one-flask is due to the presence of free 4-OH group of iodovanillin **10**. The Wittig-Horner reaction of the coupled benzofurans **11a-g** in methylene chloride under reflux condition with (carbethoxymethylene)triphenylphosphorane produced only (*E*)-**12a-g** isomers in 48-99% yields, which stereochemistry was easily confirmed by coupling constants of trans olefin (J = 15-16 Hz) in ^{1}H NMR spectra. The esters **12a-g** were then reduced to allylic alcohols **2-3** and **13c-g** with DIBAL-H in 28-99% yields. The methoxymethyl (MOM) geoups on benzofurans **13c-g** were deprotected using Dowex 50X2-100 ion-exchange resin to give the ailanthoidol derivatives **4-8** in 29-84% yields. Seven ailanthoidol derivatives, which structures were summarized in Table 1, were prepared from vanillin overall four or five steps with 3-82% overall yields depend on substituent groups.

Inflammation is a beneficial host response to a foreign challenge or tissue injury that leads ultimately to the restoration of normal tissue structure and function, however, prolonged inflammation contributes to the pathogenesis of many inflammatory diseases.[14] In order to investigate the *anti*-inflammatory properties of the prepared seven benzofurans (**2-8**) to compare with which of ailanthoidol **1**, we measured the amount of nitric oxide (NO), which is one of essential mediators on inflammation, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages (Fig. 2 and Table 2).[15] Ailanthoidol derivatives **2**, **3**, **5**, **7**, **8** significantly suppressed the production of NO in LPS-stimulated RAW264.7 cells at 10 μM, and **5** and **7** inhibited even at 1 μM. The strong inhibitory activity was shown in **2**, **5**, **7** and **8**. Among these active compounds, only **5** showed better inhibition effect than ailanthoidol **1**. The suppression effect of ailanthoidol **1** and derivative **5** for NO production showed



Scheme 1. Synthesis of ailanthoidol derivatives **2-8** from vanillin **9**.

Table 1. Structure of ailanthoidol and its derivatives **1-8**

	
1 (Ailanthoidol)	**2**
	
3	**4**
	
5	**6**
	
7	**8**

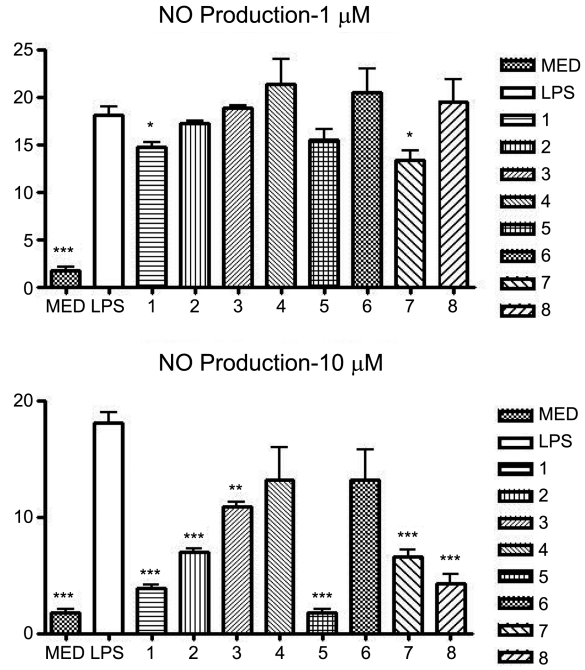


Figure 2. Effects of ailanthoidol **1** and derivatives **2-8** on LPS-induced NO production. RAW264.7 cells were treated with (a) 1 μM and (b) 10 μM of benzofurans in the presence of 1 μg mL^{-1} of LPS, and NO production was determined. Statistical significance is based on the difference when compared with LPS-stimulated cells (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

78% and 90% at 10 μM, and 19% and 14% at 1 μM, respectively. As shown in Figure 3, however, the cell viability was affected by the derivatives **5** and **8** at 10 μM, indicating cytotoxicity. The derivative **7** showed comparable *anti-*

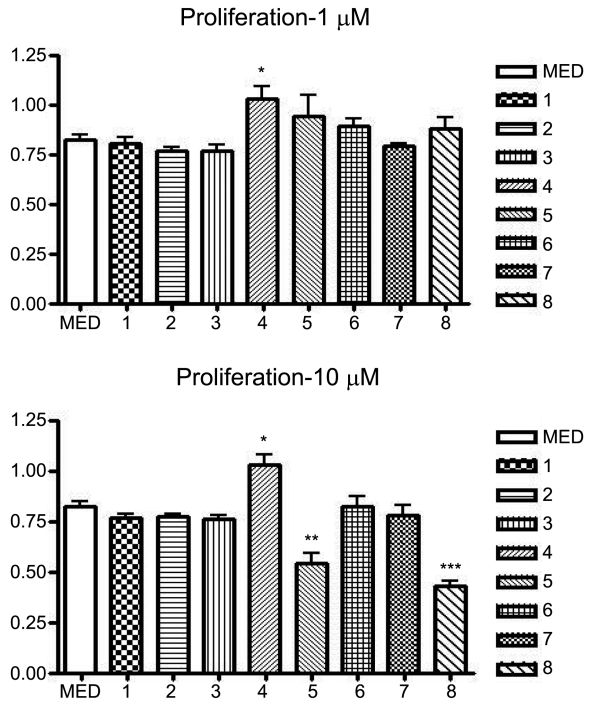


Figure 3. Cell viability assay of ailanthoidol **1** and derivatives **2-8** at 1 and 10 μM.

Table 2. Anti-inflammatory activities of ailanthoidol **1** and derivatives **2-8**

Compound	NO Production	(% Inhibition)
	1 μM	10 μM
MED	9.7 ± 0.4 (90.3)	9.7 ± 0.4 (90.3)
1	81.2 ± 0.7 (18.8)	21.6 ± 1.2 (78.4)
2	95.0 ± 0.5 (5.0)	38.5 ± 0.3 (61.5)
3	104.6 ± 0.3 (-4.6)	60.4 ± 0.4 (39.6)
4	118.0 ± 3.8 (-18.0)	73.0 ± 0.5 (27.0)
5	85.9 ± 1.6 (14.1)	9.9 ± 4.0 (90.1)
6	113.5 ± 3.5 (-13.5)	73.0 ± 3.7 (27.0)
7	73.7 ± 1.5 (26.3)	36.5 ± 0.8 (63.5)
8	91.6 ± 3.3 (8.4)	23.5 ± 1.2 (76.5)
LPS	100.0 ± 0.7 (0.0)	100.0 ± 0.7 (0.0)

The results are reported as mean value ± SEM for n = 3. % Inhibition is based on LPS as shown in parenthesis.

inflammatory activity with the ailanthoidol without any cytotoxicity at 10 μM, and even better effect at 1 μM showing 26% inhibition.

In conclusion, the practical and optimized four or five steps reaction procedures produced ailanthoidol derivatives **2-8** in 3-82% overall yields from vanillin. The derivatives **2-8** were examined their *anti*-inflammatory activity in lipopolysaccharide (LPS)-stimulated RAW 264-7 macrophages to compare with ailanthoidol. Among these benzofurans, derivative **7** showed 64% inhibition of NO production at 10 μM and 26% inhibition even at 1 μM without any cytotoxicity, however, derivative **5** showed cytotoxicity at 10 μM even though its better *anti*-inflammatory activity than ailanthoidol.

Experimental

All chemicals used were purchased from commercial sources and used as received unless otherwise stated. NMR spectra were recorded at Varian Mercury-300 MHz FT-NMR for ^{1}H and 75 MHz for ^{13}C, with the chemical shifts (δ) reported in parts per million (ppm) relative to TMS and the coupling constants (*J*) quoted in Hz. $CDCl_3$ was used as a solvent and an internal standard. Flash chromatography was carried out using silica gel Merck 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on DC-Plastikfolien 60, F_{254} (Merck, layer thickness 0.2 mm) plastic-backed silica gel plates with visualization by UV light (254 nm) or by treatment with *p*-anisaldehyde. Melting points were measured on a MEL-TEMP II apparatus and were uncorrected. LC-MS were measured on a Thermo Finnigan LCQ Advantage Max System. LPS derived from Escherichia coli and Salmonella typhosa was obtained from Sigma (St Louis, Mo, USA). The Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin used in this study were obtained from Hyclone (Logan, Utah, USA).

4-Hydroxy-3-iodo-5-methoxybenzaldehyde (10). Vanillin **9** (1.00 g, 6.57 mmol) was dissolved in dry EtOH (50 mL) under N_2 atmosphere and iodine (2.08 g, 7.89 mmol), silver sulfate (2.46 g, 7.89 mmol) was slowly added and stirred for 1 h at rt. The reaction was quenched by adding water and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried over Na_2SO_4 and filtered. The filtrate was concentrated *in vacuo* to give a yellow solid. The solid was chromatographed on silica gel to give a yellow solid (1.55 g, 85%). R_f 0.34 (EtOAc:hexane = 1:3); mp 178-181 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.97 (3H, s, Ar-OMe), 6.66 (1H, s, Ar-OH), 7.36 (1H, d, *J* = 1.5 Hz, Ar-H), 7.81 (1H, d, *J* = 1.8 Hz, Ar-H), 9.75 (1H, s, CHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.8, 80.7, 108.8, 131.2, 136.4, 146.6, 151.5, 189.7.

General Procedure of Sonogashira Coupling Reaction. To a solution of iodovanillin (**10**) (0.06 g, 0.22 mmol), $PdCl_2(PPh_3)_2$ (0.014 g, 0.02 mmol), acetylene derivative (0.32 mmol) and CuI (0.001 g, 0.004 mmol) in DMF (3 mL) under nitrogen atmosphere was added Et_3N (0.03 mL, 0.22 mmol) and stirred for 10 h at rt. The organic product was extracted with CH_2Cl_2, washed with brine, dried and concentrated to give the solid. The solid was chromatographed (EtOAc:Hexane = 1:4) to give the yellow solid **11a-g**.

2-(4-Bromophenyl)-7-methoxybenzofuran-5-carbaldehyde (11a): Yield (86%). R_f 0.56 (EtOAc:hexane = 1:3); mp 134-137 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.11 (3H, s), 7.07 (1H, s), 7.35 (1H, br s), 7.56 (2H, d, *J* = 8.7 Hz), 7.68 (1H, br s), 7.72 (2H, d, *J* = 8.1 Hz), 9.96 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 102.6, 105.0, 119.5, 123.5, 126.8, 128.6, 130.8, 132.2, 133.8, 146.2, 147.8, 156.8, 191.7.

2-Phenyl-7-methoxybenzofuran-5-carbaldehyde (11b): Yield (99%). R_f 0.54 (EtOAc:hexane = 1:2); mp 135-136 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.07 (3H, s), 7.05 (1H, s), 7.38 (4H, m), 7.67 (1H, d, *J* = 1.5 Hz), 7.85 (1H, s), 7.87

(1H, d, J = 1.2 Hz), 9.96 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 101.7, 104.5, 118.9, 124.9, 128.6, 128.9, 129.3, 130.6, 133.3, 145.8, 147.4, 157.5, 191.3.

2-(2-Methoxymethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (11c): Yield (31%). R_f 0.38 (EtOAc:hexane = 1:4); mp 90-92 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53 (3H, s), 4.06 (3H, s), 5.36 (2H, s), 7.10 (1H, br t, J = 6.9 Hz), 7.22 (1H, br t, J = 7.5 Hz), 7.28 (1H, dd, J = 7.2, 1.8 Hz), 7.32 (1H, d, J = 0.9 Hz), 7.39 (1H, s), 7.69 (1H, d, J = 0.9 Hz), 8.08 (1H, dd, J = 7.8, 1.5 Hz), 9.96 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.5, 94.7, 104.9, 106.9, 114.7, 119.3, 119.6, 122.0, 122.1, 127.6, 130.1, 131.5, 133.5, 146.1, 146.9, 154.1, 154.3, 191.7.

2-(3-Methoxymethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (11d): Yield (97%). R_f 0.56 (EtOAc:hexane = 1:2); m.p. 88-90 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.51 (3H, s), 4.09 (3H, s), 5.25 (2H, s), 7.06 (1H, dd, J = 8.1, 1.8 Hz), 7.09 (1H, s), 7.35 (1H, br s), 7.36 (1H, t, J = 8.1 Hz), 7.53 (2H, m), 7.70 (1H, d, J = 0.9 Hz), 9.98 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 56.1, 94.3, 102.1, 104.6, 112.9, 116.9, 118.7, 119.1, 129.8, 130.6, 130.7, 133.3, 145.8, 147.5, 157.3, 157.4, 191.4.

2-(4-Methoxymethoxyphenyl)-7-methoxybenzofurna-5-carbaldehyde (11e): Yield (62%). R_f 0.54 (EtOAc:hexane = 1:2); mp 125-127 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (3H, s), 4.10 (3H, s), 5.20 (2H, s), 6.89 (1H, s), 7.07 (2H, d, J = 8.9 Hz), 7.28 (1H, br s), 7.60 (1H, d, J = 0.9 Hz), 7.75 (2H, d, J = 8.7 Hz), 9.98 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 56.1, 94.2, 100.4, 104.3, 116.4, 118.8, 123.3, 126.5, 130.9, 133.2, 145.6, 157.6, 157.8, 191.4.

2-(2,4-Bismethoxymethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (11f): Yield (51%). R_f 0.14 (EtOAc:hexane = 1:4); mp 68-70 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.43 (3H, s), 3.47 (3H, s), 3.97 (3H, s), 5.13 (2H, s), 5.28 (2H, s), 6.74 (1H, d, J = 1.8 Hz), 6.84 (1H, d, J = 1.8 Hz), 7.17 (1H, s), 7.19 (1H, d, J = 9.3 Hz), 7.56 (1H, s), 7.90 (1H, d, J = 9.0 Hz), 9.86 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 56.5, 56.7, 94.6, 94.8, 103.5, 104.6, 105.2, 109.3, 113.5, 119.3, 128.4, 131.7, 133.5, 145.9, 146.6, 154.2, 155.4, 158.9, 191.8.

2-(3,4-Bismethoxymethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (11g): Yield (83%). R_f 0.53 (EtOAc:hexane = 1:2); mp 108-110 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.54 (3H, s), 3.57 (3H, s), 4.08 (3H, s), 5.29 (2H, s), 5.33 (2H, s), 7.00 (1H, s), 7.23 (1H, d, J = 7.8 Hz), 7.34 (1H, d, J = 0.9 Hz), 7.52 (1H, dd, J = 8.4, 2.1 Hz), 7.63 (1H, d, J = 2.1 Hz), 7.67 (1H, d, J = 1.5 Hz), 9.98 (1H, s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2, 56.3, 56.4, 95.3, 95.4, 101.0, 113.5, 116.0, 116.4, 118.4, 119.6, 120.4, 127.2, 133.2, 145.7, 146.6, 147.2, 148.3, 157.3, 191.4.

General Procedure of Wittig-Horner Reaction. To a solution of **11** (0.17 mmol) in CH_2Cl_2 (5 mL) under nitrogen atmosphere was added (carbethoxymethylene)triphenylphosphorane (0.09 g, 0.25 mmol) and refluxed for 8 h. The organic product was extracted with CH_2Cl_2, washed with brine, dried and concentrated to give the solid. The solid was chromatographed (EtOAc:hexane = 1:3) to give the white solid **12a-g**.

2-(4-Bromophenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12a): Yield (92%). R_f 0.73 (EtOAc:hexane = 1:3); mp 121-123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (3H, t, J = 7.2 Hz), 4.04 (3H, s), 4.28 (2H, q, J = 7.0 Hz), 6.39 (1H, d, J = 15.6 Hz), 6.95 (2H, br s), 7.29 (1H, d, J = 0.9 Hz), 7.53 (2H, d, J = 8.4 Hz), 7.69 (2H, d, J = 8.7 Hz), 7.72 (1H, d, J = 15.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 56.3, 60.8, 102.4, 105.8, 114.9, 117.4, 123.1, 126.7, 128.9, 131.0, 131.1, 132.1, 145.3, 145.5, 145.6, 156.0, 167.2.

2-Phenyl-5-(carbethoxyethenyl)-7-methoxybenzofuran (12b): Yield (99%). R_f 0.57 (EtOAc:hexane = 1:2); mp 150-151 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (3H, t, J = 6.9 Hz), 4.04 (3H, s), 4.27 (2H, q, J = 7.5 Hz), 6.39 (1H, d, J = 16.2 Hz), 6.95 (1H, s), 6.96 (1H, s), 7.36 (4H, m), 7.73 (2H, d, J = 15.6 Hz), 7.84 (2H, d, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 56.0, 60.3, 101.4, 105.3, 114.5, 116.8, 124.8, 128.5, 128.6, 129.6, 130.4, 130.9, 145.0, 145.1, 145.2, 156.8, 166.8.

2-(2-Methoxymethoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12c): Yield (93%). R_f 0.42 (EtOAc:hexane = 1:4); mp 91-93 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.44 (3H, t, J = 7.5 Hz), 3.61 (3H, s), 4.13 (3H, s), 4.36 (2H, q, J = 7.2 Hz), 5.45 (2H, s), 6.48 (1H, d, J = 15.6 Hz), 7.04 (1H, br s), 7.18 (1H, t, J = 7.8 Hz), 7.30 (1H, t, J = 8.1 Hz), 7.36 (1H, dd, J = 7.5, 1.5 Hz), 7.40 (1H, s), 7.43 (1H, br s), 7.83 (1H, d, J = 15.6 Hz), 8.17 (1H, dd, J = 7.8, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 56.5, 56.7, 60.7, 94.6, 105.8, 106.7, 114.6, 115.2, 116.9, 122.0, 122.1, 127.5, 129.8, 130.6, 131.9, 144.6, 145.5, 145.6, 153.4, 154.2, 167.3.

2-(3-Methoxymethoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12d): Yield (76%). R_f 0.64 (EtOAc:hexane = 1:2); mp 89-91 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (3H, t, J = 6.9 Hz), 3.51 (3H, s), 4.06 (3H, s), 4.27 (2H, q, J = 7.2 Hz), 5.24 (2H, s), 6.40 (1H, d, J = 15.6 Hz), 6.97 (1H, d, J = 1.5 Hz), 6.99 (1H, s), 7.04 (1H, dd, J = 7.8, 1.5 Hz), 7.34 (2H, m), 7.51 (2H, m), 7.74 (1H, d, J = 16.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 56.0, 56.1, 60.3, 94.3, 101.9, 105.4, 112.8, 114.5, 116.5, 116.9, 118.6, 129.7, 130.4, 130.9, 131.0, 145.0, 145.2 (x2), 156.5, 157.3, 166.8.

2-(4-Methoxymethoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12e): Yield (48%). R_f 0.44 (EtOAc:hexane = 1:3); mp 139-141 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (3H, t, J = 6.9 Hz), 3.50 (3H, s), 4.06 (3H, s), 4.27 (2H, q, J = 8.1 Hz), 5.21 (2H, s), 6.40 (1H, d, J = 15.6 Hz), 6.87 (1H, s), 6.95 (1H, d, J = 1.5 Hz), 7.09 (2H, br d, J = 9.0 Hz), 7.31 (1H, d, J = 1.5 Hz), 7.73 (1H, d, J = 16.2 Hz), 7.80 (2H, br d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 56.0, 56.1, 60.3, 94.2, 100.1, 105.2, 114.3, 116.2, 116.3, 116.7, 123.6, 126.4, 130.3, 131.2, 144.9, 145.1, 156.8, 157.6, 166.8.

2-(2,4-Bismethoxymethoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12f): Yield (76%). R_f 0.25 (EtOAc:hexane = 1:4); mp 71-74 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.28 (3H, t, J = 7.5 Hz), 3.40 (3H, s), 3.45 (3H, s), 3.99 (3H, s), 4.19 (2H, q, J = 6.6 Hz), 5.09 (2H, s), 5.24 (2H, s), 6.31 (1H, d, J = 15.6 Hz), 6.70 (1H, br d, J = 8.7 Hz), 6.82 (2H, br s), 7.08 (1H, s), 7.19 (1H, s), 7.64 (1H, d, J =

16.2 Hz), 7.87 (1H, d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 55.9, 56.1, 56.4, 60.3, 94.4, 94.5, 103.2, 103.3, 104.8, 108.9, 113.6, 114.6, 116.5, 127.9, 130.2, 131.8, 144.1, 145.1, 145.4, 153.2, 155.1, 158.5, 166.9.

2-(3,4-Bismethoxymethoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (12g): Yield (74%). R_f 0.54 (EtOAc:hexane = 1:2); mp 120-122 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (3H, t, J = 6.9 Hz), 3.53 (3H, s), 3.56 (3H, s), 4.06 (3H, s), 4.27 (2H, q, J = 6.9 Hz), 5.28 (2H, s), 5.32 (2H, s), 6.40 (1H, d, J = 15.6 Hz), 6.90 (1H, s), 6.95 (1H, d, J = 0.9 Hz), 7.21 (1H, d, J = 8.7 Hz), 7.30 (1H, d, J = 1.2 Hz), 7.49 (1H, dd, J = 8.3, 1.8 Hz), 7.61 (1H, d, J = 1.8 Hz), 7.74 (1H, d, J = 16.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.4, 56.1, 56.2, 56.3, 60.3, 95.2, 95.5, 100.7, 105.2, 113.5, 114.4, 116.5, 116.8, 119.6, 124.3, 130.4, 131.1, 131.8, 145.0, 145.1, 147.2, 147.9, 156.6, 166.9.

General Procedure of DIBAL-H Reduction. To a solution of **12** (0.11 mmol) in THF (4 mL) under nitrogen atmosphere at −78 °C was added DIBAL-H (1.0 M, 0.25 mL) and stirred for 1.5 h at rt. The reaction was quenched by addition of aqueous $Na_2CO_3 \cdot 10H_2O$. The organic product was extracted with CH_2Cl_2, washed with brine, dried and concentrated to give the solid. The solid was chromatographed (MeOH:$CHCl_3$ = 1:15) to give the yellow solid **2-3** and **13c-g**.

2-(4-Bromophenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (2): Yield (76%). R_f 0.49 (EtOAc:hexane = 3:1); mp 91-93 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 3.99 (3H, s), 4.12 (2H, dd, J = 6.0, 1.5 Hz), 6.27 (2H, dt, J = 15.7, 5.4 Hz), 6.56 (1H, d, J = 16.2 Hz), 6.91 (1H, d, J = 1.5 Hz), 7.10 (1H, d, J = 0.9 Hz), 7.12 (1H, s). 7.52 (2H, br d, J = 8.4 Hz), 7.70 (2H, br d, J = 8.7 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 56.5, 63.2, 103.6, 106.2, 112.5, 123.1, 127.2, 127.3, 129.7, 130.2, 131.0, 131.8, 132.9, 134.8, 146.2, 155.9; LC-MS (ESI) m/z 716.03 [$(2M)^+$].

2-Phenyl-5-(3-hydroxypropenyl)-7-methoxybenzofuran (3): Yield (99%). R_f 0.28 (EtOAc:hexane = 1:2); mp 122-123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.05 (3H, s), 4.34 (2H, t, J = 4.2 Hz), 6.33 (1H, dt, J = 15.6, 5.7 Hz), 6.67 (1H, d, J = 15.6 Hz), 6.87 (1H, s), 6.96 (1H, s), 7.16 (1H, s), 7.33 (1H, t, J = 7.2 Hz), 7.42 (2H, t, J = 7.2 Hz), 7.85 (2H, d, J = 7.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 63.7, 101.5, 104.8, 111.8, 124.8, 127.2, 128.4, 128.5, 129.9, 130.8, 131.6, 132.6, 143.8, 144.9, 156.3; LC-MS (ESI) m/z 263.20 [$(M-OH)^+$].

2-(2-Methoxymethoxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (13c): Yield (93%). R_f 0.39 (EtOAc:hexane = 1:1); mp 82-84 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.52 (3H, s, MOM), 4.05 (3H, s, OMe), 4.33 (2H, d, J = 6.0 Hz, =CH$\underline{CH_2}$OH), 5.35 (2H, s), 6.32 (1H, dt, J = 15.6, 5.7 Hz, =$\underline{CH}CH_2OH$), 6.66 (1H, d, J = 15.6 Hz, $\underline{CH}$=$CHCH_2OH$), 6.86 (1H, d, J = 1.5 Hz), 7.09 (1H, br t, J = 7.8 Hz), 7.17 (1H, d, J = 1.2 Hz), 7.20 (1H, br t, J = 7.5 Hz), 7.25 (1H, dd, J = 7.5, 1.5 Hz), 7.28 (1H, s), 8.08 (1H, dd, J = 7.8, 1.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 56.3, 63.8, 94.3, 104.9, 106.4, 111.9, 114.3, 119.6, 121.7, 127.0, 127.2, 129.1, 131.4, 131.7, 132.7, 142.9, 144.8, 152.5, 153.7.

2-(3-Methoxymethoxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (13d): Yield (94%). R_f 0.12 (EtOAc:hexane = 1:2); mp 102-104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (3H, s), 4.00 (3H, s), 4.30 (2H, br d, J = 6 Hz), 5.21 (2H, s), 6.28 (1H, dt, J = 16.2, 5.4 Hz), 6.60 (1H, d, J = 15.9 Hz), 6.82 (1H, d, J = 1.2 Hz), 6.88 (1H, s), 7.00 (1H, dd, J = 8.1, 1.5 Hz), 7.06 (1H, br s), 7.30 (1H, t, J = 8.1 Hz), 7.46 (1H, s), 7.48 (1H, d, J = 2.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.9, 56.0, 63.6, 94.3, 101.9, 105.0, 111.8, 112.7, 116.3, 118.5, 127.3, 129.6, 130.7, 131.3, 131.4, 132.7, 143.7, 144.9, 155.9, 157.3.

2-(4-Methoxymethoxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (13e): Yield (74%). R_f 0.14 (EtOAc:hexane = 1:2); mp 95-97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50 (3H, s), 4.05 (3H, s), 4.34 (2H, dd, J = 6.4, 0.9 Hz), 5.21 (2H, s), 6.33 (1H, dt, J = 15.6, 6.0 Hz), 6.66 (1H, d, J = 15.6 Hz), 6.84 (1H, s), 6.85 (1H, d, J = 1.2 Hz), 7.08 (2H, br d, J = 8.7 Hz), 7.13 (1H, d, J = 1.5 Hz), 7.78 (2H, br d, J = 8.7 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 56.1, 63.8, 94.2, 100.2, 104.6, 111.6, 116.2, 123.9, 126.3, 127.1, 131.0, 131.7, 132.5, 143.6, 144.9, 156.3, 157.4.

2-(2,4-Bismethoxymethoxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (13f): Yield (28%). R_f 0.38 (EtOAc:hexane = 1:1); mp 54-57 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (3H, s), 3.51 (3H, s), 3.99 (3H, s), 4.30 (2H, br d, J = 4.8 Hz), 5.17 (2H, s), 5.31 (2H, s), 6.29 (1H, dt, J = 15.6, 5.7 Hz), 6.62 (1H, d, J = 15.6 Hz), 6.80 (1H, dd, J = 8.7, 2.4 Hz), 6.81 (1H, d, J = 1.5 Hz), 6.89 (1H, d, J = 2.4 Hz), 7.12 (1H, br s), 7.14 (1H, s), 7.98 (1H, d, J = 8.4 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 56.5, 56.7, 63.9, 94.7, 103.6, 104.9, 105.0, 105.1, 109.3, 112.1, 114.2, 127.5, 128.3, 131.8, 131.9, 132.8, 142.9, 145.1, 152.9, 155.2, 158.5.

2-(3,4-Bismethoxymethoxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (13g): Yield (62%). R_f 0.14 (EtOAc:hexane = 1:2); mp 97-99 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.53 (3H, s), 3.56 (3H, s), 4.05 (3H, s), 4.34 (2H, dd, J = 6.2, 1.0 Hz), 5.27 (2H, s), 5.31 (2H, s), 6.33 (1H, dt, J = 15.6, 5.7 Hz), 6.67 (1H, d, J = 15.6 Hz), 6.86 (1H, br s), 6.87 (1H, s), 7.13 (1H, br s), 7.20 (1H, d, J = 9.0 Hz), 7.49 (1H, dd, J = 8.4, 1.8 Hz), 7.61 (1H, d, J = 1.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 56.2, 56.3, 63.8, 95.2, 95.5, 100.8, 104.6, 111.6, 113.4, 116.4, 116.4, 119.4, 124.6, 127.1, 130.9, 131.6, 132.6, 144.8, 147.1, 147.6, 156.0.

General Procedure of Deprotection. Dowex 50X2-100 cation exchange resin (70 mg) was added to the compound **13** (0.12 mmol) in MeOH (2 mL) and stirred at rt for 8 h. The Dowex resin was filtered and MeOH was concentrated *in vacuo*, and the crude reaction mixture was purified by *silica* gel column chromatography (EtOAc:hexane = 1:3) to give the yellow product **4-8**.

2-(2-Hydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (4): Yield (48%). R_f 0.68 (EtOAc:hexane = 1:1); (liquid); ^{1}H NMR (300 MHz, CD_3OD) δ 4.03 (3H, s), 4.09 (2H, dd, J = 6.3, 0.9 Hz), 6.26 (1H, dt, J = 16.2, 6.3 Hz), 6.67 (1H, d, J = 16.0 Hz), 6.92 (2H, br t, J = 8.4 Hz), 6.94 (1H, d, J = 1.5 Hz), 7.14 (1H, dd, J = 7.2, 1.8 Hz), 7.18 (1H, d, J = 1.5 Hz), 7.33 (1H, s), 7.91 (1H, dd, J = 7.8, 2.1

Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 58.1, 74.4, 106.1, 106.9, 113.0, 116.8, 118.5, 120.5, 125.2, 127.4, 130.3, 132.9, 134.1, 134.7, 144.1, 146.3, 154.6, 155.9; LC-MS (ESI) *m/z* 279.05 [$(M\text{-}OH)^+$].

2-(3-Hydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (5): Yield (62%). R_f 0.22 (EtOAc:hexane = 1:1); mp 152-153 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.05 (3H, s), 4.12 (2H, br d, *J* = 5.4 Hz), 6.39 (1H, dt, *J* = 15.9, 5.1 Hz), 6.67 (1H, d, *J* = 15.6 Hz), 6.85 (1H, br s), 7.05 (1H, br s), 7.21 (2H, m), 7.32 (1H, m), 7.39 (2H, m). ^{13}C NMR (75 MHz, CD_3OD) δ 56.0, 62.9, 102.4, 105.5, 111.8, 111.9, 112.0, 115.3, 116.3, 116.6, 129.7, 130.0, 130.1, 130.5, 145.5, 145.9, 157.2, 158.2; LC-MS (ESI) *m/z* 279.05 [$(M\text{-}OH)^+$].

2-(4-Hydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (6): Yield (84%). R_f 0.50 (EtOAc:hexane = 2:1); mp 131-133 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.00 (3H, s), 4.22 (2H, br d, *J* = 5.1 Hz), 6.29 (1H, dt, *J* = 15.6, 6.0 Hz), 6.61 (1H, d, *J* = 15.6 Hz), 6.82 (2H, br d, *J* = 8.4 Hz), 6.83 (1H, s), 6.89 (1H, d, *J* = 1.2 Hz), 7.08 (1H, br s), 7.67 (2H, br d, *J* = 8.7 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 55.6, 62.7, 99.2, 104.7, 111.4, 115.5, 122.1, 126.3, 127.4, 131.2, 131.6, 133.4, 143.6, 145.1, 157.0, 158.1; LC-MS (ESI) *m/z* 279.11 [$(M\text{-}OH)^+$].

2-(2,4-Dihydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (7): Yield (29%). R_f 0.31 (EtOAc:hexane = 1:1); (liquid); ^{1}H NMR (300 MHz, CD_3OD) δ 4.05 (3H, s), 4.11 (2H, d, *J* = 6.8 Hz), 6.22 (1H, dt, *J* = 15.2, 6.3 Hz), 6.43 (2H, m), 6.67 (1H, d, *J* = 15.6 Hz), 6.84 (1H, d, *J* = 1.5 Hz), 7.14 (1H, d, *J* = 1.5 Hz), 7.15 (1H, s), 7.80 (1H, d, *J* = 8.4 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 57.8, 73.4, 102.9, 103.3, 104.5, 107.4, 109.9, 120.0, 123.9, 128.0, 132.0, 132.3, 133.9, 142.1, 144.7, 154.4, 155.7, 158.2.

2-(3,4-Dihydroxyphenyl)-5-(3-hydroxypropenyl)-7-methoxybenzofuran (8): Yield (40%). R_f 0.41 (EtOAc:hexane = 2:1); mp 171-173 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 4.03 (3H, s), 4.24 (2H, dd, *J* = 6.0, 1.5 Hz), 6.32 (1H, dt, *J* = 15.6, 5.7 Hz), 6.65 (1H, d, *J* = 15.6 Hz), 6.83 (1H, d, *J* = 8.9 Hz), 6.85 (1H, s), 6.92 (1H, d, *J* = 0.9 Hz), 7.14 (1H, d, *J* = 0.9 Hz), 7.23 (1H, dd, *J* = 8.4, 1.8 Hz), 7.28 (1H, d, *J* = 2.1 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ 56.6, 63.8, 100.3, 105.7, 112.3, 112.9, 116.5, 118.0, 123.6, 128.5, 132.2, 132.6, 134.4, 144.6, 146.2, 146.5, 147.4, 152.1.

Cell Culture and Cell Viability Assay. RAW264.7 murine macrophages were obtained from the Korean Cell Bank (Seoul, Korea) and cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in 5% CO_2. The effects of the various licorice extracts on cell viability were tested using the CellTiter 96® AQueous One Solution Assay of cell proliferation (Promega, Madison, WI), which uses colorimetry to count the number of viable cells. This assay was used to determine the number of viable cells remaining after the culturing process was complete. RAW264.7 cells were plated at a density of 2×10^4 cells in a 96-well flat-bottom plate, and the ailanthoidol derivatives were added to each plate at concentrations of 0, 1 and 10 μM. After a 24 h incubation period, the number of viable cells was counted according to the manufacturer's instructions. This assay is based on the reduction of a tetrazolium compound, MTS, to formazan, which has an optimum absorption at 490 nm. Thus, the quantity of the product in the cell culture is indicated by the optical density of formazan at 490 nm, which is directly proportional to the number of living cells.

Measurement of NO. The amount of NO produced by the mouse macrophage was indicated by the amount that was measured in the RAW264.7 cell culture supernatant. RAW264.7 cells were plated at a density of 5×10^5 cells in a 24-well cell culture plate with 500 μL of culture medium and incubated for 12 h. They were then treated with 1 or 10 μM of each compound in 1 μg/mL of LPS and incubated for another 18 h. The amount of NO produced was measured using the Griess reagent system (Promega).

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