DOI 10.5012/bkcs.2010.31.04.965

Facile Preparation of 2-Arylbenzo[*b*]furan Molecules and Their Anti-inflammatory Effects

Jung Woon Hwang,[†] Da Hye Choi,[†] Jae-Ho Jeon,[‡] Jin-Kyung Kim,[§] and Jong-Gab Jun[†,‡,§,*]

[†]Department of Chemistry and Institute of Applied Chemistry, [‡]Institute of Natural Medicine, and [§]Center for Efficacy Assessment and Development of Functional Foods and Drugs, Hallym University, Chuncheon 200-702, Korea. [*]E-mail: jgjun@hallym.ac.kr
Received November 25, 2009, Accepted March 2, 2010

An efficient and practical preparation of 2-arylbenzo[*b*]furan molecules including natural egonol, XH-14, ailanthoidol, and unnatural derivatives is demonstrated using Sonogashira coupling, iodine induced cyclization and Wittig reaction. Anti-inflammatory effects of the prepared benzo[*b*]furans were examined in lipopolysaccharide (LPS)-stimulated RAW 264-7 macrophages. The results showed that ailanthoidol, XH-14 and three other unnatural derivatives (**9-10**, **13**) inhibited significantly the production of inflammatory mediator nitric oxide without showing cytotoxicity.

Key Words: Ailanthoidol, XH-14, 2-Arylbenzo[*b*]furan, Anti-inflammatory, Nitric oxide

Introduction

The 2-arylbenzo[*b*]furan structure is prevalent in a wide variety of biologically active natural and unnatural compounds.[1] Many 2-arylbenzo[*b*]furan derivatives are well-known to exhibit broad range 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 variety of biological activities. A number of synthetic approaches to the 2-arylbenzo[*b*]furan derivatives have been introduced in recent years.[12] Recently, we synthesized egonol **2**, isolated from the seed oil of *Styrax japonicum*, in 5 step reaction procedures in 74% overall yield from vanillin **1**, and also prepared its derivatives **3-7** (Fig. 1).[13]

Another 2-arylbenzo[*b*]furan natural product XH-14 (**8**, Fig. 2), isolated from *Salvia miltiorrhiza* Bunge (Chinese name "Danshen") which has been widely used in China for the treatment of cardiovascular diseases such as acute myocardial infarction and angina pectoris,[14] was also synthesized in 9 steps overall 23% yield from vanillin by mainly using Sonogashira coupling reaction with iodine induced cyclization.[15] The key features of our synthesis of benzofuran nucleus were regioselective halogenation, Sonogashira coupling and halogen-induced cyclization in different substituents including optimiza-

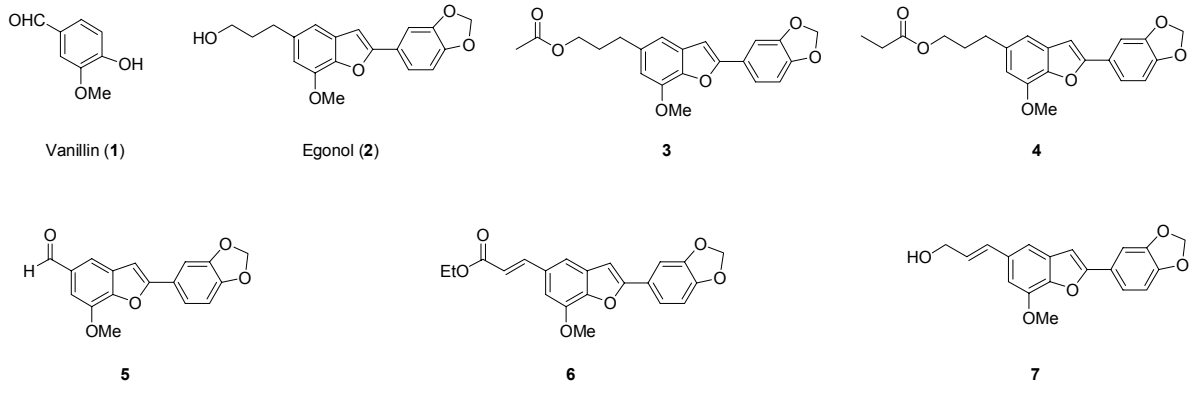


Figure 1. Structures of vanillin, egonol and egonol derivatives.

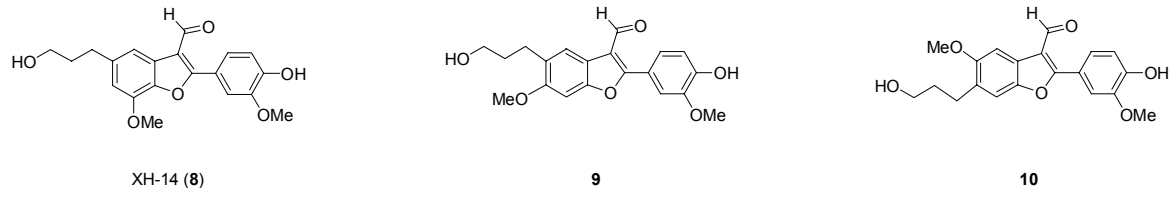


Figure 2. Structures of XH-14 and derivatives.

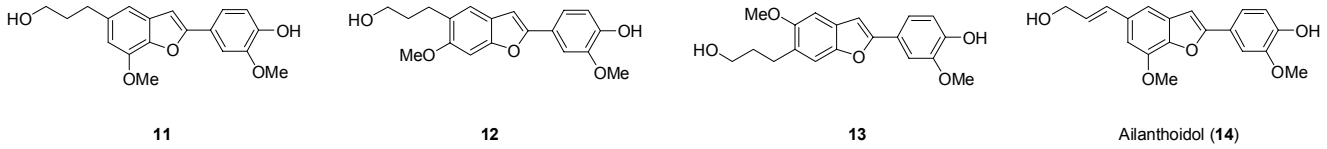


Figure 3. Structures of 3-deformylated 2-arylbenzo[*b*]furans.

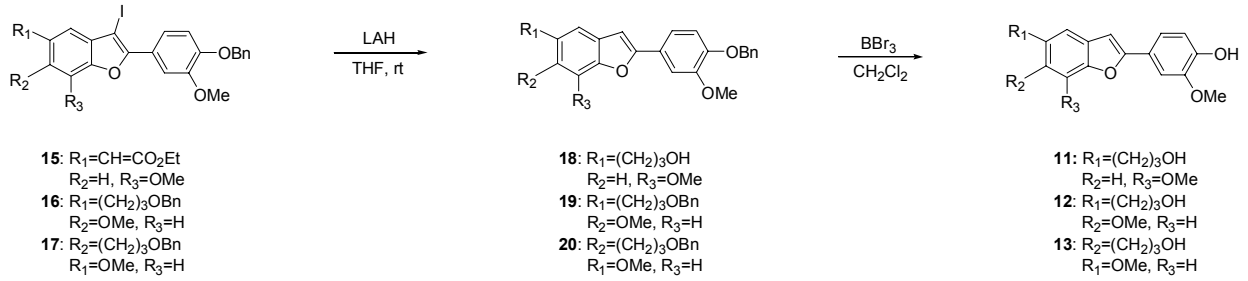
tion of the synthetic sequences. The modifications of XH-14 were given only to the synthesis of C-2 and C-3 substituted analogs. Due to its high selectivity for the A_1 receptor subtype, the preparation of analogs for SAR tests was clearly of interest. In order to prove the role of other substituents on XH-14 in biological selectivity, the derivatives of XH-14, **9** and **10**, were prepared using similar procedures in 9 steps overall 30% and 55% yield, respectively.[16] In this report, we describe the synthesis of 3-deformylated benzofurans **11-14** (Fig. 3) including ailanthoidol **14** and the comparison of anti-inflammatory effects for the prepared 13 benzofurans **2-14**.

Results and Discussion

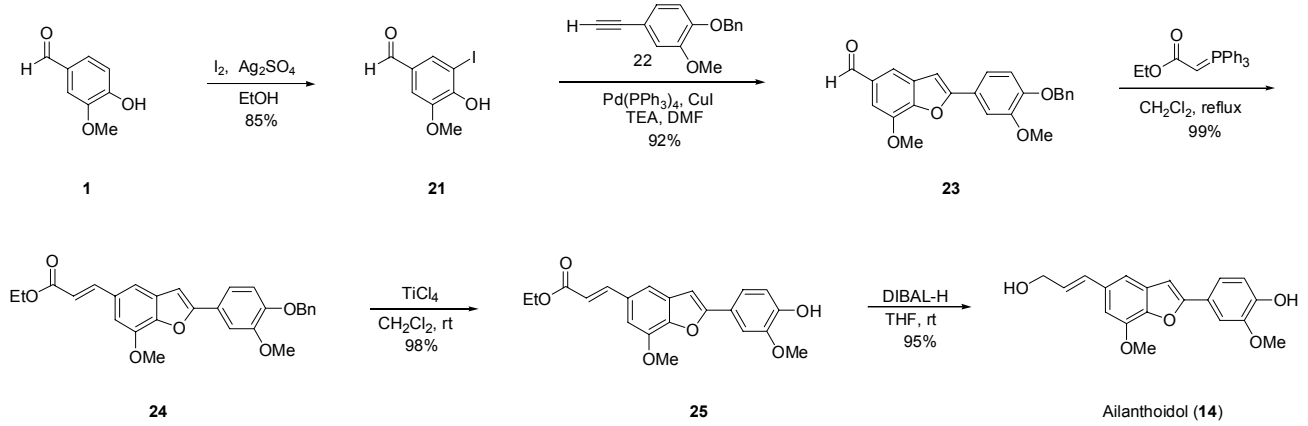
In order to prove the importance of 3-formyl substituent in 2-arylbenzo[*b*]furans for their biological activities, we prepared 3-deformylated derivatives of XH-14 (**8**) and analogues **9-10**. The synthetic intermediates **15-17**,[15,16] which have 3-iodo substituent, for the syntheses of XH-14 and analogues **9-10** were easily transformed to deiodo-benzofurans **18-20** by using $LiAlH_4$ reduction, and which were then debenzylated with BBr_3 to afford the desired 3-deformylated benzofurans **11-13** in high yields (Scheme 1).

The ailanthoidol **14**, which is a natural 3-deformylated 2-arylbenzo[*b*]furan, was isolated from the chloroform-soluble fraction of the tree of *Zanthoxylum ailanthoidos*.[17] 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.

Previous synthetic strategies for XH-14, basically, were applied for the ailanthoidol synthesis as shown in Scheme 2. Regioselective iodination of vanillin **1** using I_2-Ag_2SO_4 in EtOH at rt gave 3-iodovanillin **21** in 85% yield. Sonogashira coupling reaction of the iodovanillin with the acetylene **22** by using $Pd(PPh_3)_4$/CuI/TEA/DMF resulted in a coupled and cyclized benzofuran product **23** in 92% yield. Direct cyclization of the coupled product in one-flask is due to the presence of free 4-OH group of iodovanillin **21**. The only coupled product without cyclization can be isolated with the protection of the 4-OH of iodovanillin in the reaction. The Wittig reaction of the coupled benzofuran **23** in methylene chloride at reflux with (carbethoxymethylene)triphenylphosphorane produced 99% yield of only (*E*)-**24** which was then debenzylated to **25** (98%) with $TiCl_4$ and reduced to ailanthoidol **14** (95%) using DIBAL-H.



Scheme 1. Syntheses of deformylated 2-arylbenzo[*b*]furans **11-13**



Scheme 2. Total synthesis of ailanthoidol **14** from vanillin **1**

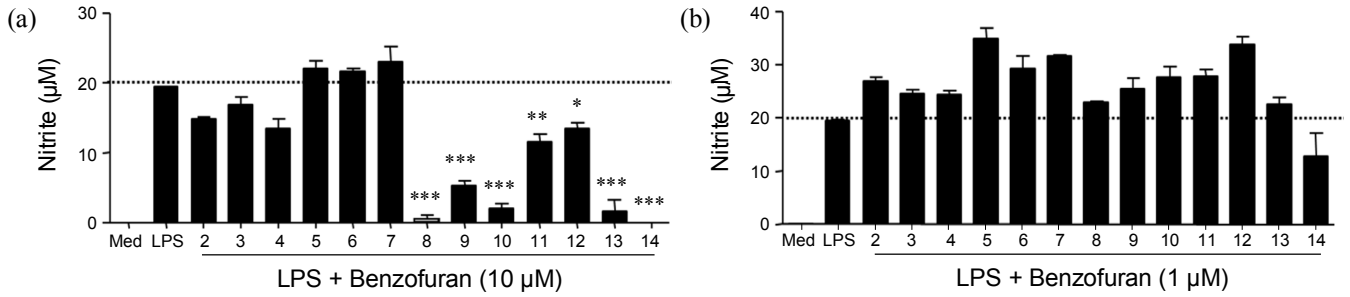


Figure 4. Effects of 2-arylbenzo[*b*]furans **2-14** on LPS-induced NO production. RAW264.7 cells were treated with (a) 10 μM and (b) 1 μM of benzofurans in the presence of 1 μg mL^{-1} of LPS, and NO production was determined. Statistical significance is based on the difference when compared with LPS-stimulated cells (**P* < 0.05, ***P* < 0.01, ****P* < 0.001).

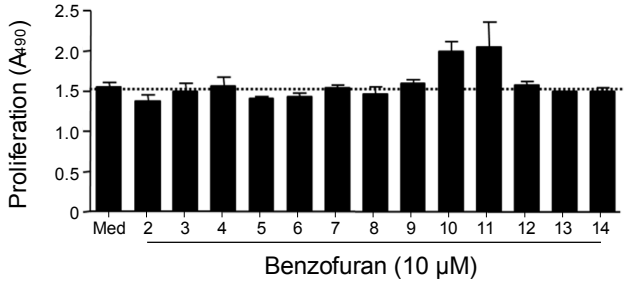


Figure 5. Cell viability assay of 2-arylbenzo[*b*]furans **2-14** at 10 μM.

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.[18] In order to investigate the anti-inflammatory properties of the prepared 13 benzofurans **2-14**, we measured the amount of nitric oxide (NO), which is one of the essential mediators on inflammation, in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages (Fig. 4 and Table 1).[19] Benzofurans **8-14** (Fig. 4a, 10 μM) significantly suppressed the production of NO in LPS-stimulated RAW264.7 cells. The strong inhibitory activity was shown in benzofurans **8**, **10**, **13** and **14**. Among these active compounds, ailanthoidol **14** showed 100% inhibition of NO production at 10 μM and 35% inhibition even at 1 μM (Fig. 4b). As shown in Fig. 5, the cell viability was not affected by the all synthetic benzofurans **2-14**, indicating no cytotoxicity.

In conclusion, the practical and optimized 5 step reaction procedures produced ailanthoidol (**14**) in 72% overall yield from vanillin. Ailanthoidol, egonol, XH-14 and their derivatives were examined their anti-inflammatory activity in lipopolysaccharide (LPS)-stimulated RAW 264-7 macrophages. Among these benzofurans, ailanthoidol showed 100% inhibition of NO production at 10 μM and 35% inhibition even at 1 μM. The cell viability assay at 10 μM indicated that the all synthetic benzofurans **2-14** did not show any significant cytotoxicity.

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 con-

Table 1. Inhibitory activities of 2-arylbenzo[*b*]furans **2-14**

Compound	NO production (% inhibition)	
	10 μM	1 μM
Egonol (**2**)	76.1 ± 0.5 (23.9)	138.1 ± 0.9 (−38.1)
5-(3-Actyloxypropyl)-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**3**)	86.9 ± 1.4 (13.1)	126.3 ± 1.1 (−26.3)
5-(3-Propanoyloxypropyl)-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**4**)	69.6 ± 1.8 (30.4)	125.0 ± 1.0 (−25.0)
5-(3-Hydroxyprop-1-en-yl)-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**5**)	113.4 ± 1.5 (−13.4)	179.1 ± 2.8 (−79.1)
5-Formyl-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**6**)	110.9 ± 0.7 (−10.9)	150.3 ± 3.3 (−50.3)
5-Carbethoxyethenyl-7-methoxy-2-(3,4-methylenedioxyphenyl)benzofuran (**7**)	118.3 ± 3.1 (−18.3)	162.4 ± 0.4 (−62.4)
XH-14 (**8**)	2.7 ± 0.7 (97.3)	117.1 ± 0.3 (−17.1)
2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofuran-3-carbaldehyde (**9**)	27.0 ± 1.0 (73.0)	130.3 ± 2.6 (−30.3)
2-(4-Hydroxy-3-methoxyphenyl)-6-(3-hydroxypropyl)-5-methoxybenzofuran-3-carbaldehyde (**10**)	10.3 ± 1.0 (89.7)	142.0 ± 2.5 (−42.0)
2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzofuran (**11**)	59.6 ± 1.5 (40.4)	143.1 ± 1.8 (−43.1)
2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofurane (**12**)	69.0 ± 1.1 (31.0)	173.4 ± 2.2 (−73.4)
2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofurane (**13**)	8.3 ± 2.2 (91.7)	116.2 ± 1.7 (−16.2)
Ailanthoidol (**14**)	0.0 ± 0.0 (**100.0**)	65.1 ± 6.1 (**34.9**)
LPS	100 ± 0.2 (0.0)	100 ± 0.2 (0.0)

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

stants (*J*) quoted in Hz. $CDCl_3$ was used as a solvent and an internal standard. Infrared spectra were recorded on a Shimadzu IR-435 spectrometer. GC-MS analyses were performed using a HP-5890/JMS-AM 150, JEOL. 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. LPS derived from *Escherichia coli* 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).

General procedure of deiodination.

2-(4-Benzyloxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzofuran (18): 2-(4-Benzyloxy-3-methoxyphenyl)-5-carbethoxyethenyl-3-iodo-7-methoxybenzofuran (0.089 g, 0.152 mmol) was dissolved in dry THF (10 mL) under N_2 atmosphere and $LiAlH_4$ (2.0 M, 0.38 mL) was slowly added *via* syringe. The solution was stirred for 24 hr at 50 °C, warmed to room temperature and stirred for 1 hr. The reaction was quenched by adding 3 mL water slowly. After stirring for 20 min, the water-phase was extracted with CH_2Cl_2 (5 mL × 3). The organic layer was washed with water and brine, dried over $MgSO_4$, removed by filtration. The filtrate was concentrated in vacuo to give a yellow solid. The solid was chromatographed on silica gel to give a white solid (0.045 g, 55%). R_f 0.43 (EtOAc:Hexane = 1:1); mp 120 - 123 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94 (2H, m, propyl C2-H), 2.78 (2H, t, *J* = 8.1 Hz, propyl C1-H), 3.70 (2H, t, *J* = 6.3 Hz, propyl C3-H), 3.98 (3H, s), 4.02 (3H, s), 5.19 (2H, s), 6.61 (1H, s, C3-H), 6.81 (1H, s), 6.91 (1H, d, *J* = 8.1 Hz), 6.96 (1H, s), 7.28-7.46 (7H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.8 (propyl C2-H), 35.0 (propyl C1-H), 56.4 (OCH_3), 56.5 (OCH_3), 62.6 (OCH_2), 71.3 (OCH_2Ph), 100.6, 107.4, 108.8, 112.5, 114.1, 118.2, 124.1, 127.5 (x2), 128.1, 128.8 (x2), 131.3, 137.0, 137.7, 142.7, 144.9, 148.8, 149.9, 156.4.

2-(4-Benzyloxy-3-methoxyphenyl)-5-(3-benzyloxypropyl)-6-methoxybenzofuran (19): Yield (92%). R_f 0.42 (EtOAc:Hexane = 1:3); mp 119 - 121 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.94 (2H, m), 2.77 (2H, t, *J* = 7.2 Hz), 3.52 (2H, t, *J* = 6.3 Hz), 3.86 (3H, s), 3.98 (3H, s), 4.52 (2H, s), 5.20 (2H, s), 6.76 (1H, s, C3-H), 6.91 (1H, d, *J* = 8.7 Hz, C4'-H), 7.01 (1H, s, C7-H), 7.23 (1H, s), 7.28 (1H, dd, *J* = 1.8, 8.1 Hz, C5'-H), 7.32-7.40 (10H, m), 7.43 (1H, br s). ^{13}C NMR (75MHz, $CDCl_3$) δ 27.6, 30.3, 55.9, 56.4, 703, 71.3, 73.1, 94.1, 100.2, 108.3, 114.3, 117.4, 120.9, 122.0, 124.7, 126.6, 127.5 (x2), 127.7, 127.9, 128.1, 128.5 (x2), 128.8 (x2), 129.3, 137.1, 138.9, 148.3, 149.9, 154.5, 154.8, 155.9.

2-(4-Benzyloxy-3-methoxyphenyl)-6-(3-benzyloxypropyl)-5-methoxybenzofuran (20): Yield (95%). R_f 0.45 (EtOAc:Hexane = 1:3); mp 94 - 97 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (2H, m), 2.79 (2H, t, *J* = 7.2 Hz), 3.51 (2H, t, *J* = 6.3 Hz), 3.81 (3H, s), 3.95 (3H, s), 4.49 (2H, s), 5.14 (2H, s), 6.76 (1H, s, C3-H), 6.87 (1H, d, *J* = 8.4 Hz, C4'-H), 6.90 (1H, s, C7-H), 7.25 (1H, dd, *J* = 1.8, 5.4 Hz, C5'-H), 7.27-7.35 (10H, m), 7.40-7.42 (2H, m). ^{13}C NMR (75MHz, $CDCl_3$) δ 27.9, 30.3, 56.1, 56.5, 70.2, 71.3, 73.2, 100.6, 101.3, 108.6, 112.2, 114.3, 117.8, 124.5, 127.5, 127.7, 127.9 (x2), 128.0, 128.1, 128.6, 128.8, 137.1, 138.9, 148.6, 149.6, 149.9, 154.5, 155.9.

General procedure of debenzylation.

2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzofuran (11): To a 2-(4-benzyloxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzofuran (0.05 g, 0.119 mmol) in dried CH_2Cl_2 (5 mL) under N_2 at −78 °C was slowly added *via* syringe boron tribromide (1.0 M in CH_2Cl_2, 0.13 mL, 1 mmol per benzyloxy group). The solution was stirred for 1 hr at −78 °C, warmed to room temperature and stirred for 1 hr. The reaction was quenched by adding 2 mL water slowly. After stirring for 20 min, the solvent was evaporated, extracted with EtOAc (5 mL × 3), dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on silica gel to give a white solid (0.034 g, 88%). R_f 0.21 (EtOAc:Hexane = 1:1); mp 102 - 104 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.95 (2H, m, propyl C2-H), 2.80 (2H, t, *J* = 8.1 Hz, propyl C1-H), 3.70 (2H, t, *J* = 6.3 Hz, propyl C3-H), 3.97 (3H, s, C3'-OMe) 4.01 (3H, C7-OMe), 6.65 (1H, s, C6-H), 6.77 (1H, s, C2'-H), 6.99 (1H, d, *J* = 7.8 Hz, C5'-H), 7.07 (1H, s, C3-H), 7.37 (1H, dd, *J* = 1.8, 8.4 Hz, C6'-H), 7.40 (1H, d, *J* = 1.5 Hz, C4-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.8 (propyl C2), 35.0 (propyl C1), 56.3 (C3'-OCH_3), 56.4 (C7-OCH_3), 62.6 (CH_2OH), 100.3 (C3), 107.3 (C2'), 107.8 (C6), 112.5 (C4), 115.0 (C6'), 118.9 (C5'), 123.2 (C3a), 131.3 (C1'), 137.6 (C5), 142.5 (C7), 144.9 (C4'), 146.4 (C3'), 146.9 (C7a), 156.6 (C2).

2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-6-methoxybenzofuran (12): Yield (82%). R_f 0.21 (EtOAc:Hexane = 1:1); mp 128 - 131 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.87 (2H, m, propyl C2-H), 2.76 (2H, t, *J* = 7.2 Hz, propyl C1-H), 3.62 (2H, t, *J* = 6.6 Hz, CH_2OH), 6.76 (1H, s, C7-H), 6.92 (1H, d, *J* = 9 Hz, C5'-H), 7.03 (1H, s, C3-H), 7.27 (1H, s, C2'-H), 7.29 (1H, dd, *J* = 1.8, 6.6 Hz, C6'-H), 7.32 (1H, s, C4-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.6 (propyl C2), 33.7 (propyl C1), 56.1 (C3'-OCH_3), 56.3 (C6-OCH_3), 62.3 (CH_2OH), 94.2 (C7), 99.7 (C3), 107.3 (C6'), 114.9 (C2'), 118.3 (C5'), 121.0 (C5), 122.5 (C4), 123.6 (C3a), 126.2 (C1'), 145.9 (C3'), 146.9 (C4), 154.4 (C7a), 155.2 (C6), 155.7 (C2).

2-(4-Hydroxy-3-methoxyphenyl)-6-(3-hydroxypropyl)-5-methoxyben zofuran (13): Yield (90%). R_f 0.25 (EtOAc:Hexane = 1:1); mp 136 - 142 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.89 (2H, m, propyl C2-H), 2.86 (2H, t, *J* = 7.2 Hz, propyl C1-H), 3.62 (2H, t, *J* = 6 Hz, CH_2OH), 3.88 (3H, s, C3'-OCH_3), 3.99 (3H, s, C5-OCH_3), 6.80 (1H, d, *J* = 0.9 Hz, C5'-H), 6.95 (1H, d, *J* = 8.4 Hz, C6'-H), 7.26 (1H, s, C3-H), 7.27 (1H, s, C4-H), 7.29 (1H, s, C7-H), 7.31 (1H, s, C2'-H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.0 (propyl C2), 33.2 (propyl C1), 55.7 (C3'-OCH_3), 55.9 (C5-OCH_3), 61.7 (CH_2OH), 99.8 (C4), 100.9 (C2'), 107.6 (C5'), 111.6 (C6), 115.2 (C7), 118.1 (C6'), 122.9 (C3a), 127.4 (C1'), 128.5 (C3), 146.5 (C4'), 147.2 (C3'), 149.2 (C5), 154.1 (C7a), 155.8 (C2).

4-Hydroxy-3-iodo-5-methoxybenzaldehyde (21): To a solution of vanillin (1.0 g, 6.572 mmol), iodine (2.081 g, 7.887 mmol) and silver sulfate (2.459 g, 7.887 mmol) in EtOH (50 mL) was stirred for 1 hr at room temperature. The solvent was evaporated, extracted with CH_2Cl_2 (5 mL × 3), and the organic layer was washed with water (20 mL) and brine (20 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and

concentrated. The residue was chromatographed on silica gel to give a white solid (1.552 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, OCH_3), 6.69 (1H, s, OH), 7.36 (1H, d, J = 1.5 Hz, C6-H), 7.81 (1H, d, J = 1.5 Hz, C2-H), 9.75 (1H, s, CHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.8 (OMe), 80.7 (C3-I), 108.8 (C6), 131.2 (C1), 136.4 (C2), 146.6 (C5), 151.5 (C4), 189.7 (C=O).

2-(4-Benzyloxy-3-methoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (23): To a solution of 4-hydroxy-3-iodo-5-methoxybenzaldehyde **21** (0.145 g, 0.523 mmol), $Pd(PPh_3)_4$ (0.018 g, 0.026 mmol), acetylene derivative (**22**) (0.187 g, 0.784 mmol) and CuI (0.005 g, 0.026 mmol) in DMF (8 mL) under N_2 was added Et_3N (0.146 mL, 1.046 mmol), and stirred for 14 hr at room temperature. The organic-phase was extracted with CH_2Cl_2 (5 mL × 4), washed with water several times, and the combined organic layer was dried over anhydrous $MgSO_4$ and concentrated. The residue was chromatographed on silica gel to give a yellow solid (0.19 g, 92%). R_f 0.3 (EtOAc:Hexane = 1:2); mp 160 - 162 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (3H, s, C3'-OCH_3), 4.06 (3H, s, C7-OCH_3), 5.19 (2H, s), 6.91 (1H, s, C3-H), 6.93 (1H, s, C2'-H), 7.29-7.45 (8H, m), 7.65 (1H, s, C4-H), 9.96 (1H, s, CHO). ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.5 (x2), 71.2, 100.9, 104.5, 108.9, 114.0, 118.5, 119.2, 123.1, 127.5, 128.2, 128.8, 131.2, 133.6, 136.8, 146.0, 147.6, 149.3, 149.9, 158.0, 191.9 (C=O).

2-(4-Benzyloxy-3-methoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (24): To a solution of 2-(4-benzyloxy-3-methoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **23** (0.270 g, 0.75 mmol) in CH_2Cl_2 (20 mL) under N_2 was added (carbethoxymethylene)triphenylphosphorane (0.264 g, 0.99 mmol). The reaction mixture was refluxed for 6 hr, and warmed to room temperature. The solution was extracted with CH_2Cl_2 (5 mL × 3), washed with water (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$, and concentrated. The residue was chromatographed on silica gel to give a white solid (0.341 g, 99%). R_f 0.2 (EtOAc:Hexane = 1:4); mp 142 - 144 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.28 (3H, t, J = 7.1 Hz, CH_3), 3.92 (3H, s), 4.07 (3H, s), 4.20 (2H, q, J = 7.1 Hz, OCH_2), 5.15 (2H, s), 6.52 (1H, d, J = 16.0 Hz, *trans* ethenyl C1-H), 6.52 (1H, d, J = 16 Hz), 7.12 (1H, d, J = 8.3 Hz), 7.15 (1H, s), 7.26 (1H, d, J = 1.4 Hz), 7.31-7.52 (8H, m), 7.72 (1H, d, J = 16.0 Hz, *trans* ethenyl C2-H). ^{13}C NMR (75 MHz, acetone-d_6) δ 14.8 (CH_3), 56.5 (OCH_3), 56.7 (OCH_3), 60.8 (OCH_2), 71.5 (OCH_2), 101.7, 106.5, 109.8, 115.1, 118.1 (*trans* ethenyl-C1), 118.8, 124.3, 128.7, 128.9, 129.4, 131.9, 132.5, 138.4, 146.1 (*trans* ethenyl-C2), 146.1, 146.6, 150.5, 151.3, 158.1, 167.4 (C=O).

2-(4-Hydroxy-3-methoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran (25): To a solution of 2-(4-benzyloxy-3-methoxyphenyl)-5-(carbethoxyethenyl)-7-methoxybenzofuran **24** (0.29 g, 0.63 mmol) in CH_2Cl_2 (25 mL) was added $TiCl_4$ (0.7 mL, 0.7 mmol, 1.0 M in CH_2Cl_2) dropwise at ambient temperature. The reaction was monitored by TLC and quenched by treatment with MeOH. The solvent was removed and the residue was chromatographed on silica gel to give a white solid (0.228 g, 98%) R_f 0.5 (EtOAc:Hexane = 1:2); mp 149 - 151 °C; ^{1}H NMR (300 MHz, acetone-d_6) δ 1.27 (3H, t, J = 7.1 Hz, CH_3), 3.91 (3H, s), 4.07 (3H, s), 4.20 (2H, q, J = 7.1 Hz, OCH_2), 6.52 (1H, d, J = 16.0 Hz, *trans* ethenyl C1-H), 6.94 (1H, d, J = 8.2 Hz), 7.07 (1H, s), 7.24 (1H, d, J = 1.4 Hz), 7.41(1H, dd, J = 8.2, 2.0 Hz), 7.70 (1H, d, J = 16.0 Hz, *trans* ethenyl C2-H), 8.07 (1H, s). ^{13}C NMR (75 MHz, acetone-d_6) δ 14.8 (CH_3), 56.6 (OCH_3), 56.6 (OCH_3), 60.9 (OCH_2), 100.1, 106.5, 109.4, 115.5, 116.6 (*trans* ethenyl-C1), 118.0, 119.5, 123.0, 129.5, 131.9, 132.6, 146.1 (*trans* ethenyl-C2), 146.6, 148.9 (x2), 158.4, 167.5 (C=O).

Ailanthoidol (14): To a solution of 2-(4-hydroxy-3-methoxyphenyl)-5-(carbethoxyethenyl)-7-methoxy benzofuran **25** (0.170 g, 0.46 mmol) in THF (10 mL) was added a solution of DIBAL-H (0.25 mL, 1.90 mmol, 1.0 M in CH_2Cl_2) at −78 °C under N_2 atmosphere. After being stirred at the same conditions for 2 hr, the reaction was quenched with saturated $Na_2CO_3 \cdot 10H_2O$ (5mL), and the resulting mixture was partitioned between CH_2Cl_2 (50 mL) and water (30 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$ and concentrated. The residue was chromatographed on silica gel to give a white solid (0.143 g, 95%). R_f 0.6 (EtOAc:Hexane = 3:1); mp 199 - 201 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.87 (3H, s, C7-OCH_3), 3.93 (3H, s, C3'-OCH_3), 4.13 (2H, d, J = 4.7 Hz, CH_2OH), 5.59 (1H, s), 6.31 (1H, dt, J = 5.0, 15.8 Hz, *trans* ethenyl C2-H), 6.57 (1H, d, J = 15.8 Hz, *trans* ethenyl C1-H), 6.86 (1H, d, J = 8.2 Hz, C5'-H), 6.90 (1H, s, C2'-H), 7.03 (1H, d, J = 1.4 Hz, C3-H), 7.10 (1H, s), 7.29 (1H, dd, J = 1.4, 8.2 Hz, C6'-H), 7.33 (1H, s, C4-H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.7 (C3'-OCH_3, C7-OCH_3), 61.6 (CH_2OH), 100.1 (C3), 104.3 (C4), 108.6 (C6), 110.8 (C2'), 115.8 (C6'), 117.8 (C5'), 121.0 (*trans* ethenyl C2), 128.9 (C1'), 129.5 (C3a), 130.7 (*trans* ethenyl C1), 133.1 (C5), 142.3 (C7), 144.5 (C4'), 147.5 (C3'), 147.8 (C7a), 156.1 (C2).

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 prepared 13 benzofurans **2-14** 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 prepared 13 benzofurans **2-14** 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.

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).

Acknowledgments. This research was financially supported by the Ministry of Education, Science Technology (MEST) and Korea Industrial Technology Foundation (KOTEF) through the Human Resource Training Project for Regional Innovation

(4R09-0301-003-S000100), and by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094072).

References

1. Katritzky, A. R.; Rees, C. W.; Scriven, E. F. V. *Comprehensive Heterocyclic Chemistry II*; Pergamon Press: Oxford, UK, 1996; Vol. 2, pp 259-321.
2. Navarro, E.; Alanso, S. J.; Trujillo, J.; Jorge, E.; Perez, C. *J. Nat. Prod.* **2001**, *64*, 134.
3. Ikeda, R.; Nagao, T.; Okabe, H.; Nakano, Y.; Matsunaga, H.; Katano, M.; Mori, M. *Chem. Pharm. Bull.* **1998**, *46*, 871.
4. Day, S. H.; Chiu, N. Y.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *J. Nat. Prod.* **2000**, *63*, 1560.
5. Craigo, J.; Callahan, M.; Huang, R. C. C.; DeLucia, A. L. *Antiviral Res.* **2000**, *47*, 19.
6. Zacchino, S.; Rodriguez, G.; Pezzenati, G.; Orellana, G.; Enriz, R.; Gonzalez, S. M. *J. Nat. Prod.* **1997**, *60*, 659.
7. Gordaliza, M.; Castro, M.; del Corral, J. M.; Lopez-Vazquez, M.; Feliciano, A. S.; Faircloth, G. T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2781.
8. Chen, C. C.; Hsin, W. C.; Ko, F. N.; Huang, Y. L.; Ou, J. C.; Teng, C. M. *J. Nat. Prod.* **1996**, *59*, 1149.
9. Silva, D. H. S.; Pereira, F. C.; Zanoni, M. V. B.; Yoshida, M. *Phytochemistry* **2001**, *57*, 437.
10. Ward, R. S. *Nat. Prod. Rep.* **1995**, *12*, 183.
11. Brader, G.; Greger, H.; Bacher, M.; Kalchhauser, H.; Hofer, O.; Vajrodaya, S. *J. Nat. Prod.* **1998**, *61*, 1482.
12. (a) Yue, D.; Yao, T.; Larock, R. C. *J. Org. Chem.* **2005**, *70*, 10292. (b) Lin, S.-Y.; Chen, C.-L.; Lee, Y.-J. *J. Org. Chem.* **2003**, *68*, 2968. (c) Kao, C.-L.; Chern, J.-W. *J. Org. Chem.* **2002**, *67*, 6772. (d) Katritzky, A. R.; Ji, Y.; Fang, Y.; Prakash, I. *J. Org. Chem.* **2001**, *66*, 5613. (e) Lutjens, H.; Scammells, P. J. *Tetrahedron Lett.* **1998**, *39*, 6581.
13. Choi, D. H.; Hwang, J. W.; Lee, H. S.; Yang, D. M.; Jun, J.-G. *Bull. Korean Chem. Soc.* **2008**, *29*, 1594.
14. Chang, H. M.; Cheng, K. P.; Choang, T. F.; Chow, H. F.; Chui, K. Y.; Hon, P. M.; Tan, F. W. L.; Yang, Y.; Zhong, Z. P. *J. Org. Chem.* **1990**, *55*, 3537.
15. Bang, H. B.; Han S. Y.; Choi, D. H.; Yang D. M.; Hwang, J. W.; Lee, H. S.; Jun, J.-G. *Synth. Commun.* **2009**, *39*, 506.
16. Bang, H. B.; Han S. Y.; Choi, D. H.; Hwang, J. W.; Jun, J.-G. *Arkivoc* **2009**, *(ii)*, 112.
17. Sheen, W.-S.; Tsai, I.-L.; Teng, C.-M.; Chen, I.-S. *Phytochemistry* **1994**, *36*, 213.
18. (a) Vernooy, J. H.; Dentener, M. A.; Suylen, R. J. van.; Buurman, W. A.; Wouters, E. F. *Am. J. Respir Cell Mol. Biol.* **2002**, *26*, 152. (b) Sakagami, T.; Vella, J.; Dixon, M. F.; Rourke, J.; Radcliff, F.; Sutton, P.; Shimoyama, T.; Beagley, K.; Lee, A. *Infect. Immun.* **1997**, *65*, 3310.
19. Kwon, K. H.; Murakami, A.; Hayashi, R.; Ohigashi, H. *Biochem. Biophys. Res. Commun.* **2005**, *337*, 647.