JOURNAL OF NATURAL PRODUCTS

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Article

Synthesis and Cytotoxicity Studies of Bioactive Benzofurans from *Lavandula agustifolia* and Modified Synthesis of Ailanthoidol, Homoegonol, and Egonol

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Cite This: *J. Nat. Prod.* 2020, 83, 3354−3362

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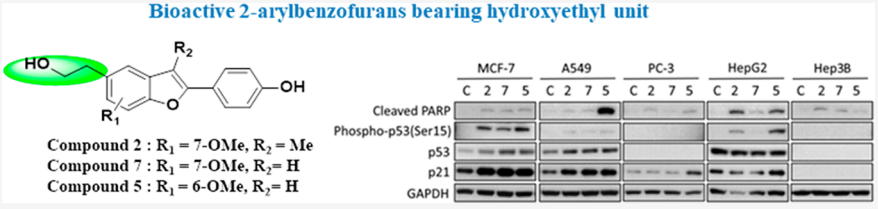

ABSTRACT: 2-Aryl/alkylbenzofurans, which constitute an important subclass of naturally occurring lignans and neolignans, have attracted extensive synthetic efforts due to their useful biological activities and significant pharmacological potential. Herein, we report a general and efficient approach to divergent 2-arylbenzofurans through a one-pot synthesis of versatile 2-bromobenzofurans as key intermediates. Using this approach, the first total synthesis of a series of trisubstituted and tetrasubstituted benzofurans bearing the hydroxyethyl unit, including the natural compounds isolated from *Lavandula agustifolia* (**1**−**3**) and their non-natural derivatives (**4**−**8**), was accomplished. We also report a modified synthesis of ailanthoidol, homoegonol, and egonol that enables the divergent synthesis of their derivatives for future exploration. Among these, the representative phenolic natural compound **2** and its derivatives **7** and **5** induced apoptotic cell death related poly(ADP-ribose) polymerase (PARP) cleavage in MCF74, A549, PC3, HepG2, and Hep3B cancer cell lines. Additionally, the tumor suppressor protein p53 was also induced in p53 wild type cancer cells.

2-Arylbenzo[*b*]furans are intriguing because of their diverse biological and pharmaceutical properties. Exploration benzofuran-based compounds from both natural and non-natural sources and evaluation of their broad range of biological activities are highly desirable in medicinal chemistry. Furthermore, new synthetic approaches that lead to more divergent synthesis of novel benzofuran compounds are needed for further exploitation of arylbenzofuran scaffolds.

Recently, three new arylbenzofurans **1**, **2**, and **3** were isolated from *Lavandula agustifolia*, and they exhibit significant anti-TMV (tobacco mosaic virus) activity and cytotoxicity against the NB4, A549, SHSY5Y, PC3, and MCF7 cancer cell lines.[1] Ailanthoidol (**9**), a representative member of the neoligan family, was isolated from the $CHCl_3$-soluble fraction of the woody stem of *Zanthoxylum ailanthoides* and exhibits antiviral, antioxidant, and antifungal activities.[2−4] Homoegonol (**10**) and egonol (**11**), which exhibit cytostatic activity in human leukemic H-60 cells, were isolated from the glycosidic fraction of *Styrax officinalis* and *S. japonica* seeds, respectively[5−9] (Figure 1). Our earlier structure−activity relationship study on homoegonol (**10**) also identified novel benzofuran derivatives that mitigate lung inflammation by blocking MAPK/AP-1 and AKT/mTOR signaling *in vitro* and *in vivo*.[10,11]

Thus far, several different 2-arylbenzo[*b*]furan synthesis strategies have been published.[2,12−25] Most involve the synthesis of the benzofuran core in the later stages using Sonogashira coupling, McMurry coupling, or domino cyclization of *gem*-dibromovinylphenol with triarylbismuth reagents. These methods have several drawbacks including reagent cost, tedious workup, the need for additional protection and deprotection techniques, and their limited application in rapid and divergent synthesis processes. Alternative strategies involving the synthesis of a common core intermediate (benzo[*b*]furan) that can react with complementary structural partners to provide a pool of synthetic targets in a straightforward and concise manner is highly desirable.

We previously developed a useful strategy for the one-pot synthesis of 2-bromo-6-hydroxybenzofurans that results in versatile syntheses of moracins and chromone compounds.[26] This mild, operationally simple methodology overcomes the

Received: June 23, 2020
Published: October 19, 2020

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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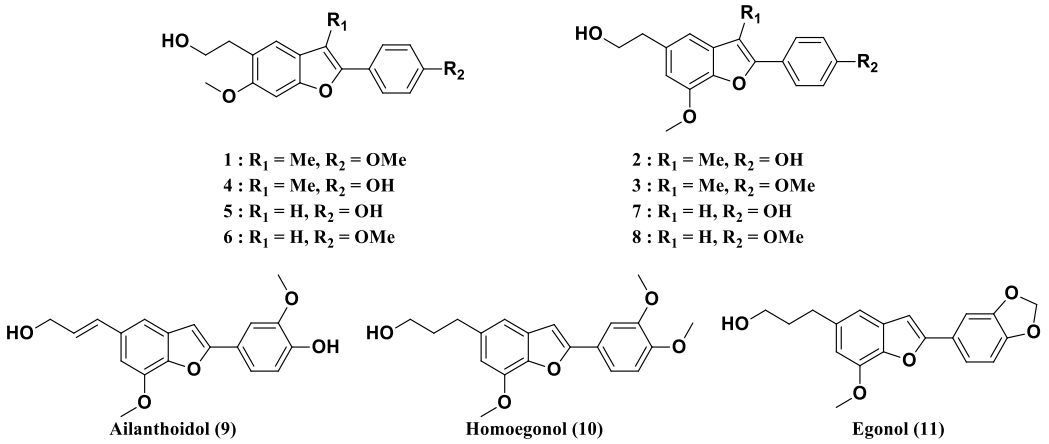


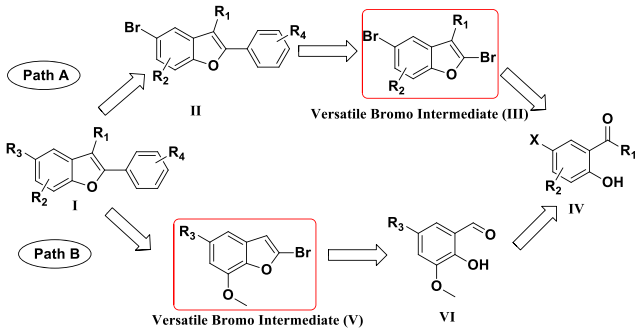
Figure 1. Benzofuran-containing natural products and derivatives.

drawbacks of previous strategies and enables the use of coupling partners without protecting groups in Pd-catalyzed coupling reactions. In the aforementioned approach, we reported an expeditious synthesis of 2-arylbenzo[*b*]furans via a 2-bromobenzofuran skeleton bearing key substituents, which enabled further elaborations in multitarget synthesis. We used this strategy for the divergent synthesis of less explored tetrasubstituted benzofuran compounds, as exemplified by the first total synthesis of a series of hydroxyethyl-bearing tetrasubstituted benzofuran compounds (natural **1**−**3** and non-natural **4**−**8**). Additionally, we report a modified synthesis of ailanthoidol (**9**), homoegonol (**10**), and egonol (**11**) that enables the divergent synthesis of their derivatives for future structure−activity relationship (SAR) explorations. In addition, the synthesized benzofurans **1**−**8** were evaluated for their cytotoxic effects against several cancer cell lines.

RESULTS AND DISCUSSION

Chemistry. A retrosynthetic analysis for the target series (**I**) is depicted in Scheme 1. Two different routes that involve

Scheme 1. Retrosynthetic Analysis



2-bromobenzofurans as versatile intermediates are shown. Path A allows introduction of various substituents on the benzofuran core (R_3) during the later stages using C-2 arylated bromo intermediate (**II**). Intermediate (**II**) can be obtained by C-2 selective arylation of the corresponding dibromobenzofurans (**III**) obtained via one-pot synthesis of the corresponding 2-hydroxy-benzaldehydes/-acetophenones (**IV**). In Path B, different C-2 aryl groups are introduced at the later stages using transition-metal-catalyzed coupling reactions from the substituted 2-bromobenzofuran intermediate (**V**) that may be obtained from the corresponding aldehydes via a one-pot *gem*-dibromo olefination followed by cyclization. The methyl group/substituents at C-3 can be introduced either by the cyclization of keto compounds initially or the transition-metal-catalyzed coupling reactions during the later stages.

The synthetic approaches used to generate the benzofuran compounds are described in Schemes 2−6. The hydroxyethyl compounds (**1**−**8**) were synthesized via Path A, and ailanthoidol (**9**), homoegonol (**10**), and egonol (**11**) were synthesized via Path B. Initially, the synthesis of hydroxyethyl bearing trisubstituted benzofurans **5** and **6** was examined via Path A. 5-Bromo-2-hydroxy-4-methoxybenzaldehyde (**12a**) was obtained from 2-hydroxy-4-methoxybenzaldehyde, as reported previously.[27] One-pot *gem*-dibromo olefination and cyclization gave 2,5-dibromo-6-methoxybenzofuran (**13a**) in 60% yield, which yielded in 4-(5-bromo-6-methoxybenzofuran-2-yl)phenol (**14a**) after selective C-2 arylation[28,29] under Suzuki conditions. Vinylation of **14a**, followed by hydroboration/oxidation,[10,11,30] afforded compound **5** (23% yield), and subsequent methylation[31] gave compound **6** in 16% yield (Scheme 2).

Tetrasubstituted compounds **1** and **4** were subsequently synthesized using a similar protocol. The introduction of a C-3 methyl group by direct cyclization of an acetophenone is less common.[26] Initial attempts at cyclizing 1-(5-bromo-2-hydroxy-4-methoxyphenyl)ethanone[32] yielded particularly low amounts of the corresponding 2-bromobenzofuran compound. However, promising results were obtained using 1-(5-bromo-2,4-dihydroxyphenyl)ethanone (**12b**)[33] to produce 2,5-dibromo-3-methylbenzofuran-6-ol (**13b**), which was subsequently methylated to form 2,5-dibromo-6-methoxy-3-methylbenzofuran (**13c**). Using the aforementioned reaction sequence, compounds **4** and **1** were obtained in overall yields of 6 and 5%, respectively (Scheme 2).

A similar method was used to synthesize compounds **7** and **8**. Starting with 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**16**), one-pot cyclization afforded **17** that, via selective C-2 arylation, gave **18** and, subsequently, 4-(7-methoxy-5-vinylbenzofuran-2-yl)phenol (**19**) via vinylation. Hydroboration/oxidation of **19** yielded compound **7** (22% yield), which was subsequently methylated to produce compound **8** in an overall yield of 16% (Scheme 3).

Additional synthetic steps were needed for the synthesis of the methyl ketone, 1-(5-bromo-2,3-dihydroxyphenyl)-

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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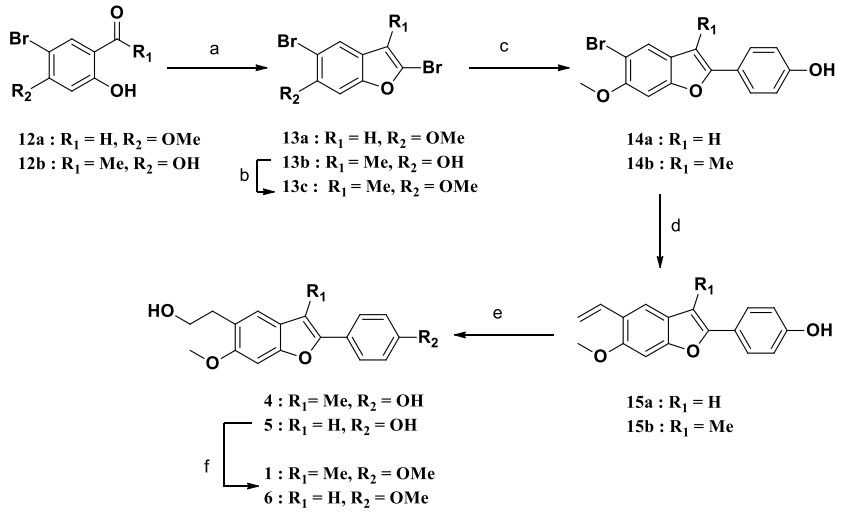
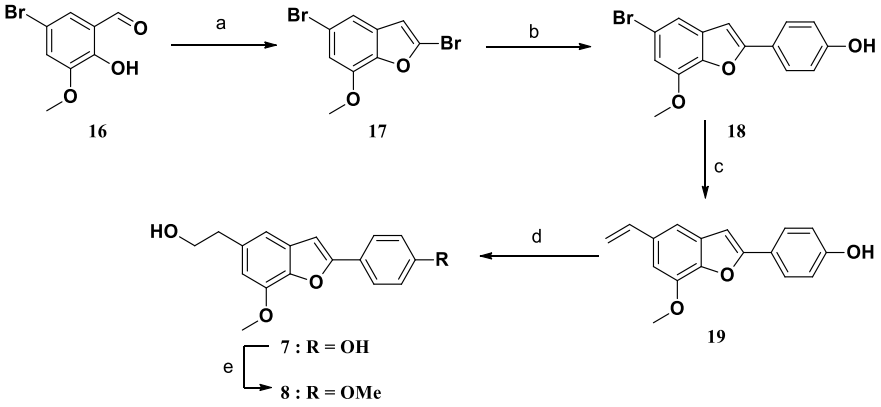
Scheme 2. Synthesis of Compounds 1, 4, 5, and 6[a]



[a](a) CBr_4, TPP, Zn, NH_4Cl, Cs_2CO_3, CuI, CH_3CN:DCM (4:1), 85 °C, 2 h, 60% for **13a** and CBr_4, TPP, Zn, 60 °C, 0.5 h, then NH_4Cl, Cs_2CO_3, CuI, 85 °C, 2 h, 40% for **13b**. (b) K_2CO_3, MeI, acetone, 60 °C, 1 h, 64%. (c) K_2CO_3, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol, $Pd(PPh_3)_4$, THF:EtOH:H_2O (20:1:1), 80 °C, 16 h, 62% for **14a** and 54% for **14b**. (d) K_3PO_4, vinylboronic acid, $Pd(dppf)Cl_2$·DCM, DMF:H_2O (4:1), 90 °C, 2 h, 88% for **15a** and 81% for **15b**. (e) BH_3·THF, THF, 2M NaOH−H_2O_2, 0 °C to rt, 2 h, 53% for **4** and 68% for **5**. (f) K_2CO_3, MeI, acetone, 60 °C, 1 h, 72% for **1** and 72% for **6**.

Scheme 3. Synthesis of Compounds 7 and 8[a]



[a](a) CBr_4, TPP, Zn, NH_4Cl, Cs_2CO_3, CuI, CH_3CN:DCM (4:1), 85 °C, 72%. (b) K_2CO_3, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol, $Pd(PPh_3)_4$, THF:EtOH:H_2O (20:1:1), 80 °C, 16 h, 57%. (c) K_3PO_4, vinylboronic acid, $Pd(dppf)Cl_2$·DCM, DMF:H_2O (4:1), 90 °C, 2 h, 90%. (d) BH_3·THF, THF, 2 M NaOH−H_2O_2, 0 °C to rt, 2 h, 60%. (e) K_2CO_3, MeI, acetone, 60 °C, 1 h, 70%.

ethanone, to prepare the tetrasubstituted compounds **2** and **3**, as in Scheme 2. Therefore, the C-3 methyl group was introduced by transition-metal-catalyzed reactions that can also provide an alternate divergent route for the tetrasubstituted benzofuran compounds. From the previously prepared compound **7**, initial diacetylation afforded **20** which, upon iodination,[34] gave the corresponding C-3 iodinated compound 4-[5-(2-acetoxyethyl)-3-iodo-7-methoxybenzofuran-2-yl]-phenyl acetate (**21**). Under Suzuki conditions,[35] the corresponding *C*-methylated compound 4-[5-(2-acetoxyethyl)-7-methoxy-3-methylbenzofuran-2-yl]phenyl acetate (**22**) was produced in 74% yield. Deprotection under basic conditions followed by *O*-methylation yielded compounds **2** and **3** in overall yields of 10 and 8%, respectively (Scheme 4).

The synthesis of ailanthoidol (**9**) was investigated via path B. The acrylate ester (**24**) was obtained from *o*-vanilin (**23**) by iodination followed by Heck coupling, as reported previously.[15] A one-pot *gem*-dibromoolefination followed by cyclization[26] yielded (*E*)-ethyl 3-(2-bromo-7-methoxybenzofuran-5-yl)acrylate (**25**), which afforded the C-2 arylated compound (*E*)-ethyl 3-[2-(4-hydroxy-3-methoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate (**26**) under standard Suzuki conditions. Reduction of **26** with DiBAlH, as reported previously,[19] gave ailanthoidol (**9**) in an overall yield of 36% (total of five steps from **23**), without any protection or deprotection steps that were required in previous methods (Scheme 5).

The syntheses of homoegonol (**10**) and egonol (**11**) were completed via a common route (Scheme 6). Hydrogenation of intermediate **24** gave **27** which, upon one-pot cyclization, afforded ethyl 3-(2-bromo-7-methoxybenzofuran-5-yl)-propanoate (**28**). Standard Suzuki coupling with the respective boronic acids followed by reduction[10,11] of **29a** and **29b**

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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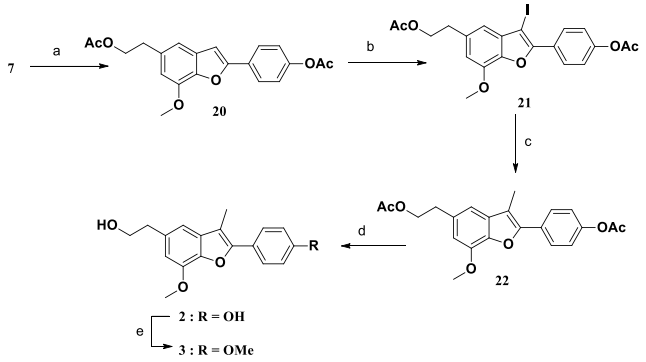
Scheme 4. Synthesis of Compounds 2 and 3[a]

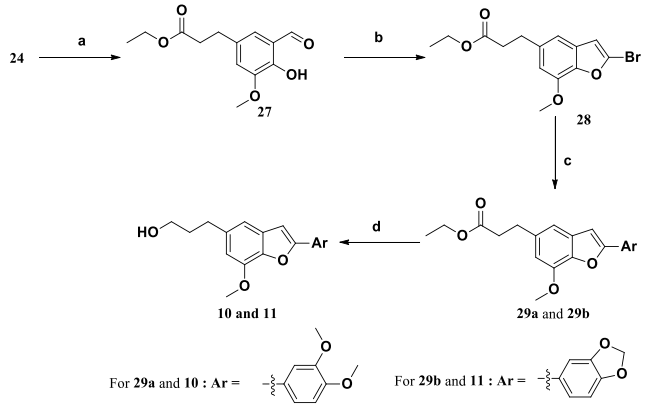


[a](a) AcCl, 4-DMAP, TEA, DCM, 0 °C to rt, 2 h, 88%. (b) NIS, PTSA, CH_3CN, 0 °C to rt, 2 h, 80%. (c) K_3PO_4, boronic acid, Pd(dppf)Cl_2·DCM, 1,4-dioxane:H_2O (100:1), 110 °C, 2 h (MW irradiation), 73%. (d) LiOH·H_2O, THF:EtOH:H_2O (4:4:1), 0 °C to rt, 2 h, 89%. (e) K_2CO_3, MeI, acetone, 60 °C, 1 h, 76%.

afforded homoegonol (**10**) and egonol (**11**) with overall yields of 26 and 27%, respectively (Scheme 6).

Biological Evaluation. Natural products **1**−**3**, isolated from *L. agustifolia* and their derivatives **4**−**8**, were investigated for their cytotoxic activities against human cancer cell lines, MCF-7 (breast carcinoma), A549 (lung carcinoma), PC3 (prostate carcinoma), HepG2 (liver carcinoma), and Hep3B (hepatocellular carcinoma) (Figure 1, Supporting Information). Among the tested compounds, the phenolic natural product **2** and its analogues **4**, **5**, and **7** showed significantly low cell counts. However, compounds **1**, **3**, **6**, and **8** displayed weak cytotoxicities compared to those of the phenolic compounds suggesting that the phenolic group might be important for the cytotoxic effect. The biological activities of compound **2** and its synthetic derivatives **7** and **5** on cancer cell viabilities and various protein levels using microscopic examinations and Western blot analysis were examined. Experiments were carried out at 6 μM which is close to the IC_{50} values of the compounds (Figure 2). Poly(ADP-ribose) polymerase (PARP) enzyme has a caspase cleavage site which is cleaved when cell death occurs by caspase activation. Interestingly, compounds **2**, **7**, and **5** induced PARP cleavage which reflects their effects on an apoptotic related pathway. In addition, because these compounds exhibited cytotoxic effects against various cancer cells, their p53 pathway responses were checked. The p53 S15 phosphorylation and the stabilization with these compounds were observed in p53 wild type (MCF-7, A549, and HepG2) but not in p53 mutant (PC-3 and Hep3B) type cancer cells. p21, one of the p53 target genes, was induced in both p53 wild type and p53 mutant PC-3 cells. Of note, p53 stabilization and target gene p21 induction were similar for all compounds, even though each cell line had diverse sensitivities. The above results suggest that compound **2** and its derivatives induced apoptotic cell death related PARP cleavage and p53 induction in various cancer cells.

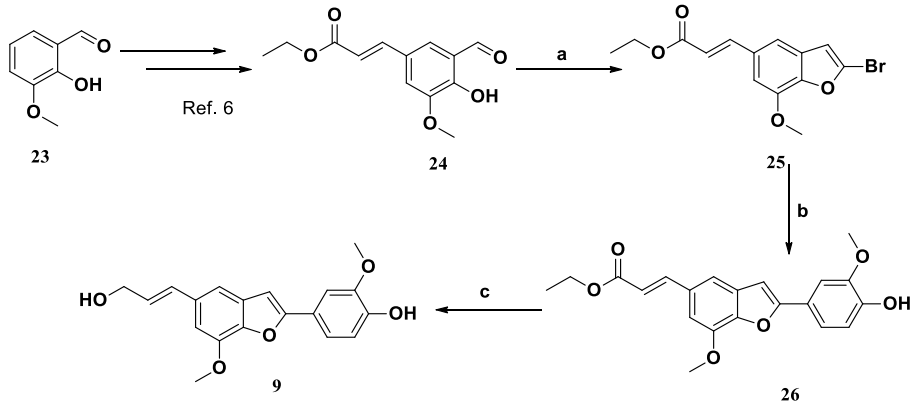
Scheme 6. Synthesis of Homoegonol (10) and Egonol (11)[a]



[a](a) Pd/C, H_2 balloon, EtOAc:THF (3:1), 2 h, 70%. (b) CBr_4, TPP, Zn, NH_4Cl, Cs_2CO_3, CuI, CH_3CN:DCM (4:1), 85 °C, 2 h, 70%. (c) K_3PO_4, boronic acid, Pd(dppf)Cl_2·DCM, DMF:H_2O (4:1), 70 °C, 1 h, 82% for **29a** and 83% for **29b**. (d) $LiAlH_4$, THF, 0 °C to rt, 1 h, 75% for homoegonol (**10**) and 77% for egonol (**11**).

In conclusion, the first total synthesis of a series of hydroxyethyl-bearing trisubstituted and tetrasubstituted benzofuran compounds, both natural and non-natural, was completed. In addition, a modified approach to the bioactive benzofurans ailanthoidol (**9**), homoegonol (**10**), and egonol (**11**) was achieved using 2-brombenzofurans as key intermediates. This common pooled strategy provides a unified route for the multitarget divergent synthesis of biologically important trisubstituted and tetrasubstituted benzofuran

Scheme 5. Synthesis of Ailanthoidol (9)[a]



[a](a) CBr_4, TPP, Zn, NH_4Cl, Cs_2CO_3, CuI, CH_3CN:DCM (4:1), 85 °C, 2 h, 62%. (b) K_3PO_4, 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol, Pd(dppf)Cl_2·DCM, DMF:H_2O (4:1), 70 °C, 1 h, 76%. (c) DiBAlH, THF, −30 to 0 °C, 2 h, 90%.

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
J. Nat. Prod. 2020, 83, 3354−3362

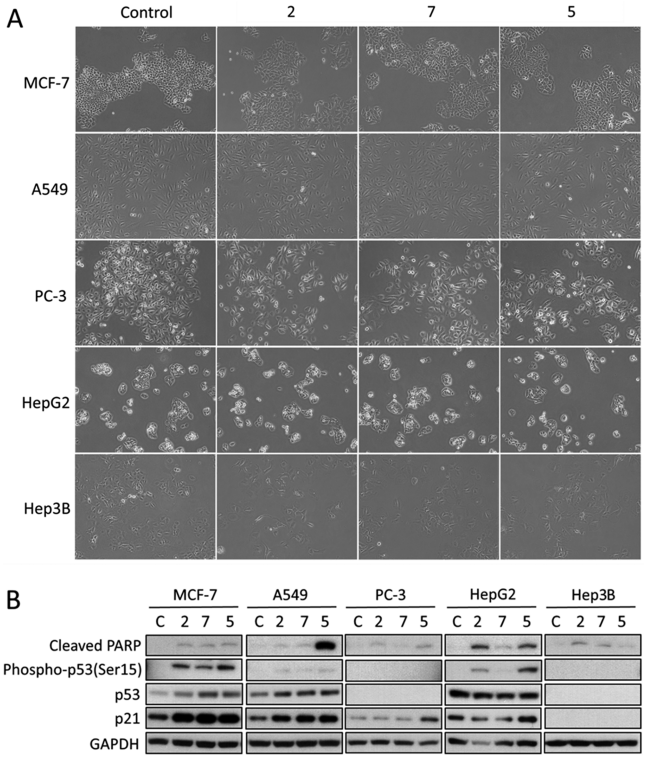


Figure 2. Effects of natural products and derivatives in cancer cell lines. (A) Light micrographs (10×) of cancer cells treated with compounds (6 μM) for 72 h. The control was treated with DMSO. (B) Western blot analysis of the indicated proteins by compound treatment, as described in part A.

compounds, which will be useful for further development of this scaffold as potential druggable compounds. The synthesized compounds **1**−**8** were evaluated for their *in vitro* cytotoxic effects. Among the series, the natural phenolic compound **2** and synthetic derivatives **7** and **5** showed cytotoxicities against various cancer cells. Furthermore, these compounds resulted in apoptotic cell death related PARP cleavage and stabilized p53 protein and its target gene p21 induction in both p53 wild type and mutant cells. Collectively, these compounds affect cancer cell survival in both p53 dependent and independent manners, providing an important framework for the future therapeutic development of benzofuran-based chemotherapeutic agents.

EXPERIMENTAL SECTION

General Experimental Procedures. All commercial chemicals were reagent grade and were used without further purification. Solvents were dried with standard procedures. All reactions were carried out under an atmosphere of dried argon in flame-dried glassware. ^{1}H NMR spectra were recorded on a Varian 400 MHz spectrometer (Varian Medical Systems, Inc., Palo Alto, CA, USA). ^{13}C NMR spectra were recorded on a Varian 100 MHz spectrometer. Chemical shifts are in parts per million (ppm) with coupling constants in Hz. The high resolution electrospray ionization mass spectrometry (HR-ESIMS) data were recorded on a JMS-700 mass spectrometer (Jeol, Japan) or by HR-ESIMS data recorded on a G2 QTOF mass spectrometer. The products of all reactions were purified by FCC using silica gel 60 (230−400 mesh Kieselgel 60). Additionally, TLC on 0.25 mm silica plates (E. Merck; silica gel 60 F254) was used to monitor the reactions. Product purity was determined by reversed-phase high performance liquid chromatography (RP-HPLC) using a Waters Corp. HPLC system equipped with a UV detector set at 254 nm. The mobile phases used were: (A) H_2O containing 0.05% TFA and (B) CH_3CN. HPLC employed a YMC Hydrosphere C_{18} (HS-302) column (5 μm particle size, 12 nm pore size) that was 4.6 mm in diameter × 150 mm in size with a flow rate of 1.0 mL/min. The compound purity was assessed using either a gradient of 25% B or 100% B in 30 min (method A). Melting points were measured on a Fisherbrand digital melting point apparatus.

General Procedure for the One-Pot Synthesis of 2-Bromobenzofurans. A solution of CBr_4 (4 equiv) in CH_3CN was added dropwise to a stirred suspension of aldehyde (1 equiv), TPP (4 equiv), and Zn powder (4 equiv) in CH_3CN:DCM (4:1) at 0 °C. The mixture was stirred for 30 min, while the temperature was slowly elevated to rt. To this mixture, NH_4Cl (5 equiv) was added, and the mixture was stirred vigorously until it became a clear solution. To this solution, Cs_2CO_3 (3.5 equiv) and CuI (0.05 equiv) were added, and the mixture was heated to 85 °C for 2 h, cooled to rt, filtered through Celite, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Suzuki Coupling (Method A). Pd(dppf)Cl_2·DCM (5 mol %) was added to a degassed solution of aryl bromide (1 equiv), boronic ester/acid (1.2 equiv), and K_3PO_4 (2.0 equiv) in DMF:H_2O (4:1). The reaction mixture was heated at 70 °C for 1 h, cooled to rt, filtered through Celite, and partitioned between EtOAc and water. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Suzuki Coupling (Method B). Pd$(PPh_3)_4$ (5 mol %) was added to a degassed solution of aryl bromide (1 equiv), boronic ester (1.1 equiv), and K_2CO_3 (2.0 equiv) in THF:EtOH:H_2O (20:1:1). The reaction mixture was heated at 80 °C for 16 h, cooled to rt, filtered through Celite, and partitioned between EtOAc and water. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Alkylation (Methylation). MeI (1.5 equiv) and K_2CO_3 (2.0 equiv) were added to a solution of phenol (1.0 equiv) in acetone, and the mixture was heated to 60 °C for 1 h. After consumption of the starting material, the mixture was cooled to rt, filtered through Celite, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Vinylation. Pd(dppf)Cl_2·DCM (5 mol %) and 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane (2 equiv) were added to a degassed solution of aryl bromide (1 equiv) and K_3PO_4 (2.0 equiv) in DMF:H_2O (4:1). The reaction mixture was heated at 90 °C for 2 h, cooled to rt, filtered through Celite, and partitioned between EtOAc and water. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Reduction of Ester to Alcohol. $LiAlH_4$ (1 M in THF, 1.5 equiv) was added dropwise to a solution of ester (1 equiv) in dry THF at 0 °C. The resulting mixture was stirred for 1 h, while the temperature was allowed to slowly rise to rt. The reaction mass was quenched with 1.5 N HCl and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

General Procedure for Hydroboration/Oxidation. BH_3 (1 M in THF, 5 equiv) was added dropwise to a solution of vinyl compound (1 equiv) in dry THF at 0 °C. The resulting mixture was stirred for 2 h, while the temperature was allowed to slowly rise to rt. The reaction mixture was then cooled again to 0 °C, and a mixture of 2 M NaOH and 30% H_2O_2 in H_2O (1:1) (10 equiv) was added top the mixture and stirred for another 30 min. The mixture was acidified with saturated citric acid solution and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc).

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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*2-[6-Methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl]-ethanol (**1**).* The title compound was prepared from **4** according to the general alkylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 7:3) gave **1** as an off-white solid (32.0 mg, 72%). mp = 149−150 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69 (d, J = 8.4 Hz, 2H), 7.26 (overlapped, s, 1H), 7.01 (s, 1H), 7.00 (d, J = 8.4 Hz, 2H), 6.82 (s, 1H), 3.88 (s, 1H), 3.87 (overlapped, q, J = 7.2 Hz, 2H), 3.86 (s, 3H), 3.00 (t, J = 6.0 Hz, 2H), 2.40 (s, 3H), 1.65 (t, J = 7.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.95, 155.78, 153.47, 149.88, 127.66, 124.41, 124.14, 122.21, 120.37, 114.06, 109.43, 93.97, 63.08, 55.78, 55.23, 34.47, 9.46. HR-ESIMS: $[M + H]^+$ $C_{19}H_{21}O_4$ calcd 313.1440, found 313.1440; purity ≥95.16% (as determined by RP-HPLC, method A, t_R = 18.14 min).

*4-[5-(2-Hydroxyethyl)-7-methoxy-3-methylbenzofuran-2-yl]-phenol (**2**).* LiOH·H_2O (0.03 g, 0.76 mmol) was added to a solution of **22** (60.0 mg, 0.16 mmol) in THF:EtOH:H_2O (4:4:1) at 0 °C, and the reaction mixture was stirred for 2 h; meanwhile, the temperature was allowed to slowly rise to rt. The mixture was acidified using saturated citric acid solution and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (*n*-hexane:EtOAc = 3:2), and it gave **2** as an off-white solid (40.0 mg, 89%). mp = 66−68 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.71 (d, J = 8.4 Hz, 2H), 6.96 (s, 1H), 6.87 (d, J = 8.4 Hz, 2H), 6.67 (s, 1H), 3.98 (s, 3H), 3.72 (t, J = 7.6 Hz, 2H), 2.91 (t, J = 7.6 Hz, 2H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4): δ 157.82, 155.96, 142.74, 141.93, 131.50, 130.94, 125.88, 122.09, 119.83, 115.19, 108.65, 97.88, 62.34, 55.23, 36.17, 13.36. HR-ESIMS: $[M + H]^+$ $C_{18}H_{19}O_4$ calcd 299.1283, found 299.1283; purity ≥93.2% (as determined by RP-HPLC, method A, t_R = 11.31 min).

*2-[7-Methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl]-ethanol (**3**).* The title compound was prepared from **2** according to the general alkylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 7:3) gave **3** as an off-white solid (21.0 mg, 76%). mp = 86−88 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.81 (d, J = 8.8 Hz, 2H), 7.03 (s, 1H), 7.00 (d, J = 8.8 Hz, 2H), 6.68 (s, 1H), 3.98 (s, 1H), 3.84 (s, 3H), 3.72 (t, J = 7.6 Hz, 2H), 2.91 (t, J = 7.6 Hz, 2H), 2.41 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4): δ 160.02, 155.56, 142.79, 142.06, 131.42, 131.05, 125.76, 123.23, 119.92, 113.83, 108.78, 98.48, 62.32, 57.66, 54.35, 36.16, 13.35. HR-ESIMS: $[M + H]^+$ $C_{19}H_{21}O_4$ calcd 313.1440, found 313.1440; purity ≥92.06% (as determined by RP-HPLC, method A, t_R = 16.64 min).

*4-[5-(2-Hydroxyethyl)-6-methoxy-3-methylbenzofuran-2-yl]-phenol (**4**).* The title compound was prepared from **15b** according to the general hydroboration/oxidation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 3:2) gave **4** as an off-white solid (71.0 mg, 53%). mp = 195−198 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.58 (d, J = 8.4 Hz, 2H), 7.27 (s, 1H), 7.06 (s, 1H), 6.87 (d, J = 8.4 Hz, 2H), 3.87 (s, 3H), 3.73 (t, J = 7.2 Hz, 2H), 2.92 (t, J = 7.2 Hz, 2H), 2.37 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4): δ 156.84, 155.88, 153.31, 149.87, 127.31, 123.81, 123.11, 122.08, 119.67, 115.00, 108.43, 93.19, 61.80, 54.81, 33.91, 7.97. HR-ESIMS: $[M + H]^+$ $C_{18}H_{19}O_4$ calcd 299.1283, found 299.1288; purity ≥99.56% (as determined by RP-HPLC, method A, t_R = 12.61 min).

*4-[5-(2-Hydroxyethyl)-6-methoxybenzofuran-2-yl]phenol (**5**).* The title compound was prepared from **15a** according to the general hydroboration/oxidation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 3:2) gave **5** as an off-white solid (90.0 mg, 68%). mp = 217−219 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.65 (d, J = 8.4 Hz, 2H), 7.29 (s, 1H), 7.10 (s, 1H), 6.83 (d, J = 8.4 Hz, 2H), 6.82 (s, 1H), 3.87 (s, 3H), 3.72 (t, J = 7.2 Hz, 2H), 2.90 (t, J = 7.2 Hz, 2H). ^{13}C NMR (100 MHz, methanol-d_4): δ 157.39, 155.67, 155.26, 154.43, 125.49, 122.63, 122.43, 122.10, 121.21, 115.18, 98.35, 93.41, 61.76, 54.77, 33.81. HR-ESIMS: $[M + H]^+$ $C_{17}H_{17}O_4$ calcd 285.1127, found 285.1129; purity ≥98.87% (as determined by RP-HPLC, method A, t_R = 11.66 min).

*2-[6-Methoxy-2-(4-methoxyphenyl)benzofuran-5-yl]ethanol (**6**).* The title compound was prepared from **5** according to the general alkylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 7:3) gave **6** as an off-white solid (42.0 mg, 71%). mp = 167−169 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 8.0 Hz, 2H), 7.31 (s, 1H), 7.05 (s, 1H), 6.96 (d, J = 8.0 Hz, 2H), 6.78 (s, 1H), 3.89 (s, 1H), 3.88 (overlapped, t, J = 7.2 Hz, 2H), 3.85 (s, 3H), 2.97 (t, J = 7.2 Hz, 2H), 1.64 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.54, 155.61, 155.13, 154.61, 125.90, 125.78, 123.62, 122.82, 121.81, 114.19, 99.34, 94.15, 63.03, 55.77, 55.25, 34.34. HR-ESIMS: $[M + H]^+$ $C_{18}H_{19}O_4$ calcd 299.1283, found 299.1280; purity ≥98.51% (as determined by RP-HPLC, method A, t_R = 16.95 min).

*4-[5-(2-Hydroxyethyl)-7-methoxybenzofuran-2-yl]phenol (**7**).* The title compound was prepared from **19** according to the general hydroboration/oxidation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 3:2) gave **7** as an off-white solid (0.20 g, 60%). mp = 146−148 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.70 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 1.2 Hz, 1H), 6.86 (s, 1H), 6.85 (d, J = 8.8 Hz, 2H), 6.71 (d, J = 1.2 Hz, 1H), 4.00 (s, 3H), 3.79 (t, J = 8.0 Hz, 2H), 2.87 (t, J = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, methanol-d_4): δ 157.91, 156.50, 144.67, 142.41, 134.51, 131.24, 125.96, 122.01, 115.21, 122.57, 107.43, 98.78, 63.21, 55.16, 39.10. HR-ESIMS: $[M + H]^+$ $C_{17}H_{17}O_4$ calcd 285.1127, found 285.1129; purity ≥97.49% (as determined by RP-HPLC, method A, t_R = 10.41 min).

*2-[7-Methoxy-2-(4-methoxyphenyl)benzofuran-5-yl]ethanol (**8**).* The title compound was prepared from **7** according to the general alkylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 7:3) gave **8** as an off-white solid (50.0 mg, 70%). mp = 97−99 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (d, J = 8.8 Hz, 2H), 7.01 (s, 1H), 6.96 (d, J = 8.8 Hz, 2H), 6.82 (s, 1H), 6.64 (s, 1H), 4.04 (s, 1H), 3.89 (q, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.93 (t, J = 6.0 Hz, 2H), 1.43 (t, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.98, 156.54, 144.96, 142.78, 133.90, 131.40, 126.53, 123.16, 144.17, 133.07, 107.58, 99.82, 63.95, 56.20, 55.38, 39.48. HR-ESIMS: $[M + H]^+$ $C_{18}H_{19}O_4$ calcd 299.1283, found 299.1294; purity ≥95.82% (as determined by RP-HPLC, method A, t_R = 15.42 min).

*Ailanthoidol (**9**).* Diisobutylaluminum hydride (1 M in THF, 0.4 mL) was added dropwise to a solution of **26** (50.0 mg, 0.14 mmol) in dry THF at −30 °C. The resulting mixture was stirred for 2 h, while the temperature was slowly allowed to rise to 0 °C. The reaction mass was quenched with saturated Na_2SO_4 solution and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (*n*-hexane:EtOAc = 3:2) to afford **9** as an off-white solid (41.0 mg, 90%). mp = 171−173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.45 (s, 1 H), 7.38 (s, 1 H), 7.32 (d, J = 8.0 Hz, 1 H), 7.19 (s, 1H), 7.17 (s, 1H), 7.00 (s, 1 H), 6.89 (d, J = 8.0 Hz, 1 H), 6.61 (d, J = 15.6 Hz, 1 H), 6.38 (dt, J = 15.6, 6.0 Hz, 1 H), 4.91 (br, s, 1 H), 4.13 (d, J = 4.8 Hz, 2 H), 3.98 (s, 3 H), 3.87 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 156.69, 148.39, 148.19, 145.07, 142.89, 133.69, 131.29, 129.98, 129.56, 121.53, 118.40, 116.29, 111.42, 109.00, 104.02, 100.66, 62.03, 56.25, 56.09. HR-ESIMS: $[M + H]^+$ $C_{19}H_{19}O_5$ calcd 327.1232, found 327.1231.

*Homoegonol (**10**).* The title compound was prepared from **29a** according to the general reduction procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **10** as an off-white solid (40.0 mg, 75%). mp = 125−127 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (dd, J = 8.0, 2.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 6.98 (s, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.83 (s, 1H), 6.64 (s, 1H), 4.04 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.71 (q, J = 6.0 Hz, 2H), 2.78 (t, J = 7.6 Hz, 2H), 1.95 (pentet, J = 7.6 Hz, 2H), 1.30 (t, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.35, 149.52, 149.11, 144.78, 142.49, 137.50, 131.13, 123.50, 118.07, 112.28, 111.24, 108.12, 107.21, 107.19, 100.28, 62.33, 56.10, 56.08, 55.98, 34.70, 32.45. HR-ESIMS: $[M + H]^+$ $C_{20}H_{23}O_5$ calcd 343.1545, found 343.1560.

*Egonol (**11**).* The title compound was prepared from ethyl **29b** according to the general reduction procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **11** as an off-white solid (30.0 mg, 77%). mp = 111−113 °C. ^{1}H NMR (400

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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MHz, $CDCl_3$): δ 7.40 (dd, J = 8.4, 1.6 Hz, 1H), 7.32 (d, J = 1.6 Hz, 1H), 6.97 (s, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.79 (s, 1H), 6.63 (s, 1H), 6.00 (s, 2H), 4.03 (s, 3H), 3.71 (q, J = 6.0 Hz, 2H), 2.78 (t, J = 7.6 Hz, 2H), 1.94 (pentet, J = 7.6 Hz, 2H), 1.28 (t, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.09, 148.04, 147.97, 144.78, 142.45, 137.51, 131.04, 124.70, 119.21, 112.32, 108.62, 107.43, 105.54, 101.31, 100.37, 100.35, 62.30, 56.16, 34.67, 32.43. HR-ESIMS: $[M + H]^+$ $C_{19}H_{19}O_5$ calcd 327.1232, found 327.1242.

*2,5-Dibromo-6-methoxybenzofuran (**13a**).* The title compound was prepared from 5-bromo-2-hydroxy-4-methoxybenzaldehyde (**12a**) according to the general procedure for the one-pot synthesis of 2-bromobenzofurans. Purification via silica gel column chromatography (*n*-hexane) afforded **13a** as a white solid (1.20 g, 60%). mp = 123−124 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.67 (s, 1H), 7.03 (s, 1H), 6.61 (s, 1H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.51, 153.42, 127.09, 123.59, 122.79, 107.79, 107.47, 95.34, 56.62. HR-ESIMS: $[M + H]^+$ $C_9H_7O_2Br_2$ calcd 304.8813, found 304.8801.

*2,5-Dibromo-3-methylbenzofuran-6-ol (**13b**).* A solution of CBr_4 (2.88 g, 8.69 mmol) in CH_3CN was added dropwise to a stirred suspension of 1-(5-bromo-2,4-dihydroxyphenyl)ethanone (**12b**) (0.50 g, 2.17 mmol), TPP (2.28 g, 8.69 mmol), and Zn powder (0.56 g, 8.69 mmol) in CH_3CN:DCM (4:1) at 60 °C. The reaction mass was stirred for 30 min. NH_4Cl (0.58 g, 10.8 mmol) was added to the mixture, and the mixture was stirred until it became a clear solution. Cs_2CO_3 (2.82 g, 8.69 mmol) and CuI (20.6 mg, 0.11 mmol) were added, and the mixture was heated to 85 °C for another 2 h. The mixture was cooled to rt, filtered through Celite, and concentrated under reduced pressure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 20:1) afforded **13b** as a white solid (0.25 g, 40%). mp = 67−69 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.52 (s, 1H), 7.10 (s, 1H), 2.13 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.03, 149.41, 125.67, 124.27, 120.95, 114.46, 106.20, 98.66. 8.74. HR-ESIMS: $[M + H]^+$ $C_9H_7O_2Br_2$ calcd 304.8813, found 304.8821.

*2,5-Dibromo-6-methoxy-3-methylbenzofuran (**13c**).* The title compound was prepared from **13b** according to the general alkylation procedure. Purification via silica gel column chromatography (*n*-hexane) afforded **13c** as a white solid (0.50 g, 64%). mp = 96−98 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.60 (s, 1H), 6.99 (s, 1H), 3.92 (s, 3H), 2.14 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.76, 153.36, 125.12, 123.64, 122.43, 114.60, 107.23, 95.37, 56.62, 8.73. HR-ESIMS: $[M + H]^+$ $C_{10}H_9O_2Br_2$ calcd 318.8969, found 318.8983.

*4-(5-Bromo-6-methoxybenzofuran-2-yl)phenol (**14a**).* The title compound was prepared from **13a** and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol according to the general Suzuki coupling procedure (method B). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **14a** as a light yellow solid (0.62 g, 62%). mp = 196−198 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 9.86 (s, 1 H), 7.80 (s, 1 H), 7.68 (d, J = 8.8 Hz, 2 H), 7.44 (s, 1H), 7.06 (s, 1H), 6.87 (d, J = 8.8 Hz, 2 H), 3.89 (s, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 158.55, 155.37, 154.45, 153.06, 126.46, 124.04, 121.15, 116.28, 106.69, 98.96, 96.79, 57.08. HR-ESIMS: $[M + H]^+$ $C_{15}H_{12}O_3Br$ calcd 318.9968, found 318.9970.

*4-(5-Bromo-6-methoxy-3-methylbenzofuran-2-yl)phenol (**14b**).* The title compound was prepared from **13c** and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol according to the general Suzuki coupling procedure (method B). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **14b** as an off-white solid (0.28 g, 54%). mp = 170−172 °C. ^1H NMR (400 MHz, DMSO-d_6): δ 9.83 (s, 1 H), 7.82 (s, 1 H), 7.58 (d, J = 8.8 Hz, 2 H), 7.38 (s, 1H), 6.91 (d, J = 8.8 Hz, 2 H), 3.89 (s, 3 H), 2.34 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.92, 153.29, 151.03, 128.03, 125.74, 122.95, 121.85, 116.19, 108.68, 106.24, 96.53, 57.08, 9.43. HR-ESIMS: $[M + H]^+$ $C_{16}H_{14}O_3Br$ calcd 333.0126, found 333.0120.

*4-(6-Methoxy-5-vinylbenzofuran-2-yl)phenol (**15a**).* The title compound was prepared from **14a** according to the general vinylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **15a** as an off-white solid (0.22 g, 88%). mp = 161−163 °C. ^1H NMR (400 MHz, methanol-d_4): δ 7.66 (d, J = 8.8 Hz, 2H), 7.62 (s, 1H), 7.11 (s, 1H), 7.07 (dd, J = 18.0, 11.2 Hz, 1H), 6.86 (s, 1H), 6.84 (d, J = 8.8 Hz, 2H), 5.70 (d, J = 18.0 Hz, 1H), 5.15 (d, J = 11.2 Hz, 1H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4): δ 157.55, 155.77, 155.29, 155.13, 131.93, 125.61, 122.50, 122.19, 116.93, 115.22, 111.74, 111.57, 98.47, 93.63, 54.99. HR-ESIMS: $[M + H]^+$ $C_{17}H_{15}O_3$ calcd 267.1021, found 267.1021.

*4-(6-Methoxy-3-methyl-5-vinylbenzofuran-2-yl)phenol (**15b**).* The title compound was prepared from **14b** according to the general vinylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **15b** as an off-white solid (0.15 g, 81%). mp = 153−155 °C. ^1H NMR (400 MHz, methanol-d_4): δ 7.58 (d, J = 8.8 Hz, 2H), 7.57 (s, 1H), 7.08 (dd, J = 18.0, 11.2 Hz, 1H), 7.05 (s, 1H), 6.88 (d, J = 8.8 Hz, 2H), 5.73 (d, J = 18.0 Hz, 1H), 5.16 (d, J = 11.2 Hz, 1H), 3.88 (s, 3H), 2.38 (s, 3H). ^{13}C NMR (100 MHz, methanol-d_4): δ 156.99, 155.35, 154.21, 150.37, 132.05, 127.40, 124.21, 122.87, 122.68, 115.35, 115.28, 115.06, 111.56, 108.57, 55.00, 7.97. HR-ESIMS: $[M + H]^+$ $C_{18}H_{17}O_3$ calcd 281.1178, found 281.1179.

*2,5-Dibromo-7-methoxybenzofuran (**17**).* The title compound was prepared from 5-bromo-2-hydroxy-3-methoxybenzaldehyde (**16**) according to the general procedure for the one-pot synthesis of 2-bromobenzofurans. Purification via silica gel column chromatography (*n*-hexane) gave **17** as a white solid (1.9 g, 72%). mp = 70−72 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.24 (d, J = 1.6 Hz, 1H), 6.89 (d, J = 1.6 Hz, 1H), 6.65 (s, 1H), 3.98 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.96, 144.02, 131.33, 129.30, 116.67, 115.00, 110.15, 108.14, 56.37. HR-ESIMS: $[M + H]^+$ $C_9H_7O_2Br_2$ calcd 304.8813, found 304.8813.

*4-(5-Bromo-7-methoxybenzofuran-2-yl)phenol (**18**).* The title compound was prepared from **17** and 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol according to the general Suzuki coupling procedure (method B). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **18** as an off-white solid (0.60 g, 57%). mp = 167−169 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, J = 8.4 Hz, 2H), 7.28 (s, 1H), 6.90 (d, J = 8.4 Hz, 2H), 6.88 (s, 1H), 6.79 (s, 1H), 4.97 (s, 1H), 4.02 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.12, 156.29, 145.41, 142.72, 132.32, 126.91, 122.90, 115.86, 115.74, 115.71, 109.84, 99.36, 56.36. HR-ESIMS: $[M + H]^+$ $C_{15}H_{12}O_3Br$ calcd 318.9970, found 318.9976.

*4-(7-Methoxy-5-vinylbenzofuran-2-yl)phenol (**19**).* The title compound was prepared from **18** according to the general vinylation procedure. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **19** as an off-white solid (0.38 g, 90%). mp = 139−141 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 8.8 Hz, 2H), 7.17 (s, 1H), 6.89 (d, J = 8.8 Hz, 2H), 6.88 (s, 1H), 6.81 (s, 1H), 6.77 (dd, J = 17.2, 11.2 Hz, 1H), 5.71 (d, J = 17.2 Hz, 1H), 5.22 (d, J = 11.2 Hz, 1H), 4.95 (s, 1H), 4.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.61, 156.06, 144.97, 143.77, 137.28, 133.72, 131.19, 126.76, 123.28, 115.68, 112.60, 111.59, 104.26, 100.11, 56.14. HR-ESIMS: $[M + H]^+$ $C_{17}H_{15}O_3$ calcd 267.1021, found 267.1022.

*4-[5-(2-Acetoxyethyl)-7-methoxybenzofuran-2-yl]phenyl Acetate (**20**).* A solution of acetyl chloride (0.15 g, 1.84 mmol) in dry DCM was added dropwise to a solution of **7** (0.18 g, 0.62 mmol), 4-DMAP (15.0 mg, 0.12 mmol), and TEA (0.31 mg, 3.07 mmol) in DCM at 0 °C. The resulting mixture was stirred for 2 h, while the temperature was allowed to slowly rise to rt. The reaction was quenched with a saturated $NaHCO_3$ solution and extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) and afforded the title compound (**20**) as an off-white solid (0.20 g, 88%). mp = 84−86 °C. ^1H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 8.8 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 7.02 (s, 1H), 6.92 (s, 1H), 6.66 (s, 1H), 4.32 (t, J = 7.2 Hz, 2H), 4.04 (s, 3H), 3.00 (t, J = 7.2 Hz, 2H), 2.32 (s, 3H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.04, 169.29, 155.56, 150.75, 144.93, 143.07, 133.48, 130.92, 128.06, 126.11, 121.93, 113.16, 107.95, 101.52, 65.26, 56.15, 35.37. 21.14, 21.02. HR-ESIMS: $[M + H]^+$ $C_{21}H_{21}O_6$ calcd 369.1338, found 369.1336.

*4-[5-(2-Acetoxyethyl)-3-iodo-7-methoxybenzofuran-2-yl]phenyl Acetate (**21**).* NIS (0.10 g, 0.45 mmol) and PTSA (0.08 g, 0.45 mmol) were added to a solution of **20** (0.15 g, 0.40 mmol) in CH_3CN

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
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at 0 °C. The resulting mixture was stirred for 2 h, while the temperature was allowed to slowly rise to rt. The reaction mass was quenched with saturated sodium thiosulfate solution and extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) and afforded **21** as an off-white solid (0.16 g, 80%). mp = 146−149 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.90 (d, J = 8.4 Hz, 2H), 7.19 (d, J = 8.4 Hz, 2H), 6.92 (s, 1H), 6.75 (s, 1H), 4.30 (t, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.16 (t, J = 7.2 Hz, 2H), 2.33 (s, 3H), 2.07 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.00, 169.27, 155.47, 151.05, 145.19, 141.39, 136.39, 135.74, 127.51, 126.27, 122.04, 108.99, 105.59, 80.42, 64.03, 56.34, 39.23, 21.15, 21.08. HR-ESIMS: $[M + H]^+$ $C_{21}H_{20}O_6I$ calcd 495.0305, found 495.0307.

*4-[5-(2-Acetoxyethyl)-7-methoxy-3-methylbenzofuran-2-yl]-phenyl Acetate (**22**).* Pd(dppf)Cl_2·DCM (12.4 mg, 0.02 mmol) was added to a degassed solution of **22** (0.15 g, 0.30 mmol), methyl boronic acid (36.3 mg, 0.61 mmol), and K_3PO_4 (0.16 g, 0.76 mmol) in 1,4-dioxane:H_2O (100:1). The mixture was heated to 110 °C for 2 h in a microwave. The reaction mixture was cooled to rt, filtered through Celite, and partitioned between EtOAc and water. The organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) to afford **22** as an off-white solid (0.09 g, 73.4%). mp = 125−128 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.89 (d, J = 8.4 Hz, 2H), 7.17 (d, J = 8.4 Hz, 2H), 6.98 (s, 1H), 6.63 (s, 1H), 4.26 (t, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.02 (t, J = 7.2 Hz, 2H), 2.44 (s, 3H), 2.32 (s, 3H), 2.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.06, 169.36, 155.04, 150.64, 143.00, 142.63, 131.35, 130.14, 128.17, 126.05, 121.92, 120.75. 109.16, 100.72, 64.60, 56.28, 32.51, 21.17, 21.07, 14.77. HR-ESIMS: $[M + H]^+$ $C_{22}H_{23}O_6$ calcd 383.1495, found 383.1495.

*(E)-Ethyl 3-(2-Bromo-7-methoxybenzofuran-5-yl)acrylate (**25**).* The title compound was prepared from **24** according to the general procedure for the one-pot synthesis of 2-bromobenzofurans. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 9:1) afforded **25** as an off-white solid (0.40 g, 62%). mp = 99−101 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 15.6 Hz, 1H), 7.26 (overlapped, s, 1H), 6.95 (s, 1H), 6.73 (s, 1H), 6.39 (d, J = 15.6 Hz, 1H), 4.27 (q, J = 7.2 Hz, 2H), 4.02 (s, 3H), 1.34 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.89, 146.20, 144.86, 131.08, 130.51, 129.14, 117.76, 113.51, 108.47, 105.29, 60.49, 56.14, 14.32. HR-ESIMS: $[M + H]^+$ $C_{14}H_{14}O_4Br$ calcd 325.0075, found 325.0074.

*(E)-Ethyl 3-(2-(4-Hydroxy-3-methoxyphenyl)-7-methoxybenzofuran-5-yl)acrylate (**26**).* The title compound was prepared from **25** and 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenol according to the general Suzuki coupling procedure (method A). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **26** as an off-white solid (0.13 g, 76%). mp = 148−150 °C. ^{1}H NMR (400 MHz, methanol-d_4): δ 7.73 (d, J = 16.0 Hz, 1H), 7.41 (s, 1H), 7.36 (s, 1H), 7.36 (d, J = 8.0 Hz, 1H), 7.10 (s, 1H), 6.99 (s, 1H), 6.87 (d, J = 8.0 Hz, 1H), 6.49 (d, J = 16.0 Hz, 1H), 4.25 (q, J = 7.2 Hz, 2H), 4.04 (s, 3H), 3.95 (s, 3H), 1.33 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 167.15, 157.29, 146.71, 146.58, 145.25, 145.05, 131.38, 130.51, 122.43, 119.01, 116.96, 114.78, 114.47, 107.59, 105.03, 100.13, 60.42, 56.09, 55.97, 14.35. HR-ESIMS: $[M + H]^+$ $C_{21}H_{21}O_6$ calcd 369.1338, found 369.1346.

*Ethyl 3-(3-Formyl-4-hydroxy-5-methoxyphenyl)propanoate (**27**).* Pd/C (41.5 mg, 0.39 mmol) was added to a solution of **24** (1.0 g, 3.99 mmol) in EtOAc:THF (3:1) and stirred under H_2 pressure for 2 h. The mixture was filtered through Celite and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane:EtOAc = 7:3) to afford **27** as an off-white solid (0.70 g, 70%). mp = 42−45 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.99 (s, 1H), 9.88 (s, 1H), 7.01 (s, 1H), 6.98 (s, 1H), 4.12 (q, J = 7.2 Hz, 2H), 3.92 (s, 3H) 2.94 (t, J = 8.0 Hz, 2H), 2.63 (t, J = 8.0 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.55, 172.56, 150.05, 148.16, 132.03, 123.45, 120.36, 118.34, 60.57, 56.25, 35.76, 30.22, 14.21. HR-ESIMS: $[M + H]^+$ $C_{13}H_{17}O_5$ calcd 253.1076, found 253.1077.

*Ethyl 3-(2-Bromo-7-methoxybenzofuran-5-yl)propanoate (**28**).* The title compound was prepared from **27** according to the general procedure for the one-pot synthesis of 2-bromobenzofurans. Purification via silica gel column chromatography (*n*-hexane:EtOAc = 9:1) afforded **28** as a colorless oil (0.45 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.91 (s, 1H), 6.64 (s, 1H), 6.62 (s, 1H), 4.13 (q, J = 7.2 Hz, 2H), 3.98 (s, 3H), 2.99 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.6 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.83, 144.33, 143.84, 136.91, 130.25, 128.17, 111.50, 108.49, 107.15, 50.46, 56.03, 36.40, 31.26, 14.22. HR-ESIMS: $[M + H]^+$ $C_{14}H_{15}BrO_4$ calcd 327.0233, found 327.0238.

*Ethyl 3-[2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl]-propanoate (**29a**).* The title compound was prepared from **28** and (3,4-dimethoxyphenyl)boronic acid according to the general Suzuki coupling procedure (method A). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 4:1) afforded **29a** as an off-white solid (45.0 mg, 82%). mp = 110−112 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (dd, J = 8.4, 2.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 6.98 (s, 1H), 6.92 (d, J = 8.4 Hz, 1H), 6.84 (s, 1H), 6.63 (s, 1H), 4.16 (q, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.02 (t, J = 8.0 Hz, 2H), 2.67 (t, J = 8.0 Hz, 2H), 1.24 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.00, 156.38, 149.52, 149.09, 144.77, 142.61, 136.30, 131.15, 123.43, 118.06, 112.24, 111.22, 108.09, 107.06, 100.23, 60.43, 56.07, 56.04, 55.97, 36.56, 31.36, 14.24. HR-ESIMS: $[M + H]^+$ $C_{22}H_{25}O_6$ calcd 385.1651, found 385.1649.

*Ethyl 3-{2-(Benzo[d][1,3]dioxol-5-yl)-7-methoxybenzofuran-5-yl}propanoate (**29b**).* The title compound was prepared from **28** and benzo[d][1,3]dioxol-5-ylboronic acid according to the general Suzuki coupling procedure (method A). Purification via silica gel column chromatography (*n*-hexane:EtOAc = 9:1) afforded **29b** as an off-white solid (47.0 mg, 83%). mp = 102−104 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39 (dd, J = 8.0, 1.6 Hz, 1H), 7.38 (d, J = 1.6 Hz, 1H), 6.97 (s, 1H), 6.86 (d, J = 8.0 Hz, 1H), 6.79 (s, 1H), 6.63 (s, 1H), 6.00 (s, 2H), 4.13 (q, J = 7.6 Hz, 2H), 4.02 (s, 3H), 3.01 (t, J = 8.0 Hz, 2H), 2.66 (t, J = 8.0 Hz, 2H), 1.24 (t, J = 7.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.99, 156.14, 148.04, 147.99, 144.79, 142.58, 136.32, 131.06, 124.64, 119.21, 112.27, 108.60, 107.24, 105.53, 101.31, 100.38, 60.43, 56.14, 36.55, 31.35, 14.24. HR-ESIMS: $[M + H]^+$ $C_{21}H_{21}O_6$ calcd 369.1338, found 369.1336.

Cell Culture and Cell Viability Assay. MCF-7, PC-3, HepG2, and Hep3B cell lines were obtained from the Korean Cell Line Bank (KCLB, Seoul, Korea). The A549 cell line was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). All cells were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS) (Gibco). The cultures were maintained in an atmosphere containing 5% CO_2 at 37 °C.

Cell viability was assessed by cell count. Cells were plated in 96-well plates (1 × 10^3 cells/well) and incubated overnight at 37 °C. Then, cells were treated with different concentrations of compounds in RPMI1640 medium containing 5% FBS for 72 h, and Hoechst 33342 staining was used to examine cell number. Cellular analysis was performed using Gen 5 software and was determined by measuring Hoechst 33342 stained cell nuclei.

Western Blotting Analysis. Cells were lysed in 1X cell lysis buffer (Cell Signaling Technology) supplemented with Xpert Protease inhibitor cocktail solution (GenDEPOT), and proteins were separated on 4−12% BOLT gels (Invitrogen) and transferred to PVDF membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% nonfat milk and then immunoblotted with primary antibodies at 4 °C overnight. After being washed three times with TBST, membranes were incubated for 1 h with appropriate secondary antibodies at room temperature, followed by chemiluminescence detection. Antibodies used include: Cleaved PARP (#9541), p-p53(Ser15) (#9284), and p21(#2947) from Cell Signaling Technology; GAPDH (ab128915) from abcam; and p53(sc-126) from Santa Cruz. Antimouse IgG and antirabbit IgG secondary antibodies conjugated to peroxidase were purchased from GenDEPOT.

https://dx.doi.org/10.1021/acs.jnatprod.0c00697
J. Nat. Prod. 2020, 83, 3354−3362

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c00697.

Cell viability assay, ^{1}H NMR and ^{13}C NMR spectra of compounds, and HPLC and HR-ESIMS data (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded with a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (NRF 2018R1A5A2023127) and National Cancer Center Research Grants [1810030 to K.K. and J.-W.P.].

■ REFERENCES

(1) Tang, S.; Shi, J.; Liu, C.; Zhang, F.; Xiang, N.; Liu, X.; Chen, Y.; You, J.; Shen, Q.; Miao, M.; Liu, Z.; Yang, G. *Phytochem. Lett.* **2017**, *19*, 60−63.

(2) Kao, C. L.; Chern, J. W. *J. Org. Chem.* **2002**, *67*, 6772−6787.

(3) Sheen, W. S.; Tsai, I. L.; Teng, C. M.; Chen, I. S. *Phytochemistry* **1994**, *36*, 213−215.

(4) Fuganti, C.; Serra, S. *Tetrahedron Lett.* **1998**, *39*, 5609−5610.

(5) Segal, R.; Milo-Goldzweig, I.; Sokoloff, S.; Zaitschek, D. V. *J. Chem. Soc. C* **1967**, 2402−2404.

(6) Takanashi, M.; Takizawa, Y. *Jpn. Oil Chem. Soc.* **1988**, *37*, 1164−1164.

(7) Takanashi, M.; Takizawa, Y.; Mitsuhashi, T. *Chem. Lett.* **1974**, *3*, 869−871.

(8) Takanashi, M.; Takizawa, Y. *Phytochemistry* **1988**, *27*, 1224−1226.

(9) Hirano, T.; Gotoh, M.; Oka, K. *Life Sci.* **1994**, *55*, 1061−1069.

(10) Xu, X.; Kwon, O. K.; Shin, I. S.; Mali, J. R.; Harmalkar, D. S.; Lim, Y.; Lee, G.; Lu, Q.; Oh, S. R.; Ahn, K. S.; Jeong, H. G.; Lee, K. *Sci. Rep.* **2019**, *9*, 862.

(11) Lee, K.; Ahn, K. S.; Kwon, O. K.; Kim, D. Y.; Ryu, H. W.; Kim, J. H.; Xu, X. US2017/0174645A1, 2017.

(12) Chen, P.; Wang, J.; Liu, K.; Li, C. *J. Org. Chem.* **2008**, *73*, 339−341.

(13) Duan, X. F.; Zeng, J.; Zhang, Z. B.; Zi, G. F. *J. Org. Chem.* **2007**, *72*, 10283−10286.

(14) Duan, X.-F.; Shen, G.; Zhang, Z.-B. *Synthesis* **2010**, *7*, 1181−1187.

(15) Rao, M. L. N.; Murty, V. N. *Eur. J. Org. Chem.* **2016**, *2016*, 2177−2186.

(16) Duan, X. F.; Feng, J. X.; Zhang, Z. B. *Synthesis* **2010**, *3*, 515−519.

(17) Wang, E. C.; Wein, Y. S.; Kuo, Y. H. *Tetrahedron Lett.* **2006**, *47*, 9195−9197.

(18) More, K. R.; Mali, R. S. *Tetrahedron* **2016**, *72*, 7496−7504.

(19) Kao, C. L.; Chern, J. W. *Tetrahedron Lett.* **2001**, *42*, 1111−1113.

(20) Rao, M. L.N.; Awasthi, D. K.; Banerjee, D. *Tetrahedron Lett.* **2010**, *51*, 1979−1981.

(21) Sharma, U.; Naveen, T.; Maji, A.; Manna, S.; Maiti, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 12669−12673.

(22) Heravi, M. M.; Zadsirjan, V. *Adv. Heterocycl. Chem.* **2015**, *117*, 0065−2725.

(23) Heravi, M. M.; Zadsirjan, V.; Hamidi, H.; Tabar Amiri, P. H. *RSC Adv.* **2017**, *7*, 24470−24521.

(24) Chen, W.; Zhang, Y.; Zhang, L.; Wang, M.; Wang, L. *Chem. Commun.* **2011**, *47*, 10476−10478.

(25) Ji, Y.; Li, P.; Zhang, X.; Wang, L. *Org. Biomol. Chem.* **2013**, *11*, 4095−4101.

(26) Sivaraman, A.; Harmalkar, D. S.; Kang, J.; Choi, Y.; Lee, K. *Org. Biomol. Chem.* **2019**, *17*, 2153.

(27) Schmidt, B.; Krehl, S.; Kelling, A.; Schilde, U. *J. Org. Chem.* **2012**, *77*, 2360−2367.

(28) Hussain, M.; Thai Hung, N.; Abbas, N.; Khera, R. A.; Malik, I.; Patonay, T.; Kelzhanova, N.; Abilov, Z. A.; Villinger, A.; Langer, P. *J. Heterocyclic Chem.* **2015**, *52*, 497.

(29) Rao, M. L. N.; Awasthi, D. K.; Talode, J. B. *Tetrahedron Lett.* **2012**, *53*, 2662−2666.

(30) Harmalkar, D. S.; Lu, Q.; Lee, K. *J. Nat. Prod.* **2018**, *81*, 798−805.

(31) Archer, S.; Michael, J.; Osei-Gyimah, P.; Seyed-Mozaffari, A.; Zukin, S.; Maneckjee, R.; Simon, E. J.; Gioannini, T. L. *J. Med. Chem.* **1985**, *28*, 1950−1953.

(32) Huang, L.; Zhang, B.; Yang, Y.; Gong, X.; Chen, Z.; Wang, Z.; Zhang, P.; Zhang, Q. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 5218−5221.

(33) Vasas, A.; Patonay, T.; Konya, K.; Silva, A. M. S.; Cavaleiro, A. S. *Aust. J. Chem.* **2011**, *64*, 647−657.

(34) Vo, D. D.; Elofsson, M. *Adv. Synth. Catal.* **2016**, *358*, 4085−4092.

(35) Chao, J.; Jain, R.; Hu, L.; Lewis, J. G.; Baribault, H.; Caldwell, J. WO2018/039386Al, 2018.