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Novel benzofuran derivative DK-1014 attenuates lung inflammation via blocking of MAPK/AP-1 and AKT/mTOR signaling in vitro and in vivo

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Benzofuran derivatives have wide range of biological activities as anti-oxidant, anti-inflammatory and anticonvulsant agent. In this study, we investigated whether the novel benzofuran derivative, DK-1014 has the anti-inflammatory effects on macrophage and lung epithelial cells and anti-asthmatic effects on ovalbumin-treated mice. A series of 2-arylbenzofuran analogues were synthesized and evaluated for NO and interleukin-6 (IL-6) inhibition in LPS-stimulated Raw264.7 cells. Of these analogues, compounds 8, 22a, 22d, and 22 f (DK-1014) exhibited notable inhibitory activity with respect to IL-6 and NO production. In particular, compound DK-1014 strongly reduced IL-6, IL-8, and MMP-9 mRNA expression and IL-6, IL-8, and MCP-1 production in phorbol myristate acetate stimulated A549 cells, reduced MAPKs phosphorylation and c-fos translocation, and attenuated AKT, p70S6K and GSK phosphorylation. *In vivo* experiments were also performed on ovalbumin-sensitized and challenged BALB/c mice. DK-1014 reduced the airway hyperresponsiveness, inflammatory cell counts and cytokine levels (IL-4, 5, 13) in bronchial alveolar lavage fluid (BALF) and immunoglobulin E in serum, and attenuated inflammatory cell infiltration and mucus hypersecretion in lung tissue. These findings indicate that DK-1014 can protect against allergic airway inflammation through the AP-1 and AKT/mTOR pathways and could be useful source for the development of a therapeutic agent for asthma.

Asthma is a serious health concern that affects up to 18% of the population worldwide¹. It is a common and long-term inflammatory disorder of the airway and lung that often causes inflammation and excessive mucus build up in response to allergens or other triggers. Pulmonary inflammation is characterized by elevated serum IgE and cytokine levels, airway hyper-responsiveness (AHR), airway eosinophilia, and mucus accumulation^{2,3}. These inflammatory mediators, including proinflammatory cytokines and monocyte chemoattractant protein-1 (MCP-1), are produced by macrophages and airway epithelial cells via a series of inducible genes, leading to the infiltration of inflammatory cells into airway inflammation⁴. In addition, inflammatory cells produce nitric oxide (NO) or inflammatory cytokines and chemokines, which can be useful markers for airway inflammation^{5,6}. Thus, the models for treatment of murine macrophage (Raw 264.7 cells) and human lung epithelial cells (A549 cells) have been widely used to evaluate anti-inflammatory agents for asthma and to elucidate their mechanism^{7–10}.

Recent studies have proposed MMPs (matrix metalloproteinase) as important inflammation regulators¹¹. It has been reported that their expression can be stimulated by various agents, such as growth factors, inflammatory cytokines, and phorbol myristate acetate (PMA) PMA-induced MMP-9 overexpression is modulated by

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transcription factor activation such as activator protein-1 (AP-1) and NF- κ B through MAPKs (e.g., ERK, JNK and p38) and phosphoinositide 3-kinase (PI3K)/AKT signaling pathways^{12–14}. Eventually, MMPs damages the normal alveolar structure, leading to emphysema. Therefore, agents that can suppress AP-1 and MMP-9 activity could be useful for asthma treatment¹⁵.

It has been reported that the PI3K-AKT-mTOR signaling pathway is mutated or hyper-activated in human cancer. Since the effect of PI3K on intracellular calcium activates cell migration, PI3Kgamma is also believed to be the primary target for chemokine-induced neutrophil movement and chemotactic eosinophilia in several lung disorders, including chronic obstructive pulmonary disorder (COPD)¹⁶ and asthma¹⁷. PI3K activates the AKT serine/threonine kinase pathway via direct TSC2 phosphorylation. RSK kinase also phosphorylates mTORC1, Raptor, and p70S6 kinase subunits as well as eEF2k and its downstream effectors. RAS-RAF-MKK1/2-Erk1/2 pathway activation triggers subsequent TSC2 phosphorylation. p38 also activates mTORC- and ERK-mediated TSC2 phosphorylation¹⁸. Recent studies have found that several pan PI3K inhibitors, including LY294002 and mTOR inhibitor rapamycin, attenuate allergic airway inflammation by suppressing mTOR and p70S6K phosphorylation in mice that have inhaled ovalbumin (OVA)¹⁹.

Benzofuran heterocycles are key components found in a variety of natural products, particularly in the *Styrax* family, belonging to the class of dibenzylbutane derivatives called neolignans or norneolignans²⁰. Some benzofurans have been investigated closely and found to display anticancer, antimicrobial, immune modulatory, antioxidant, and anti-inflammatory activity²¹. The 2-arylbenzofuran scaffold is commonly found in naturally occurring egonols, including homoeogonol. Homoeogonol isolated from *Styrax japonica*, a medicinal plant widely used for the treatment of inflammatory diseases in Korea. Reportedly, homoeogonol exhibits an inhibitory effect on cyclooxygenase-1 and 2 and is also known as a proinflammatory mediator²². Our earlier studies have shown that homoeogonol effectively suppresses asthmatic responses induced by OVA challenge²³.

Considering the multifarious potential applications of homoeogonol for development of anti-inflammatory agent, we report in this study synthesis and structure-activity relationship of a series of homoeogonol analogues with 2-arylbenzofuran scaffolds and *in vitro* assays of IL-6 and NO production inhibitory activity in Raw264.7 cells. But the novel 2-arylbenzofuran analogue DK-1014 is demonstrated to have protective effects against the inflammation of human lung epithelial cells and in asthmatic mice, making it a particularly interesting candidate for the treatment of inflammatory disease.

Results and Discussion

Chemistry. Many approaches to the synthesis of homoeogonol have been reported^{24–28}, with most based on the construction of a benzofuran backbone using the Sonogashira coupling of *O*-halophenols with a palladium catalyst, e.g. PdCl₂(PPh₃)₂²⁴. Other synthetic routes include cross-McMurry coupling of a substituted salicylaldehyde with an aromatic aldehyde using low-valent titanium, e.g. TiCl₄/Zn and TiCl₄/Mn followed by the oxidative cyclization of *O*-vinylphenols^{25,26}, domino cyclization of dibromo vinylphenol with triarylbismuth reagents using Pd(PPh₃)₄ in the presence of Cs₂CO₃²⁷ and cross pinacol-type coupling of a substituted salicylaldehyde with aryl aldehyde using TiCl₄/Mn followed by acid-promoted diol cyclization²⁸. However, these methods have a number of drawbacks, including expensive reagents, tedious workup, and metallic waste.

Our synthetic strategy was modified from that used by Chen *et al.*²⁴, which involves the Sonogashira coupling reaction of *o*-iodophenol with alkynes in the presence of Pd/C as a catalyst. Utilizing this process, a series of 2-arylbenzofurans, including homoeogonol (**8**) and the analogues **12–15**, **17a–b**, and **22a–q**, were readily synthesized. (Supplementary Information).

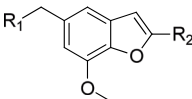
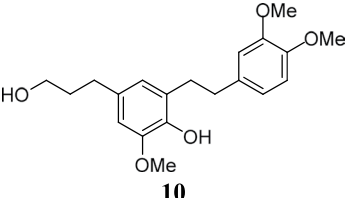
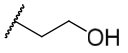
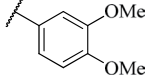
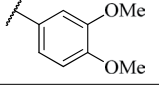
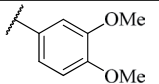
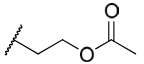
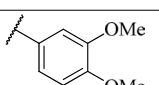
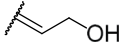
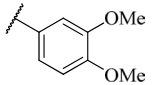
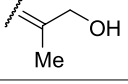
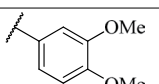
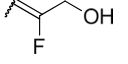
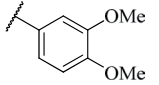
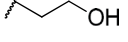
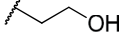
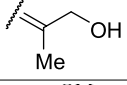
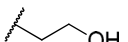
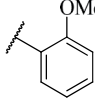
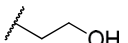
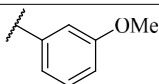
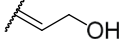
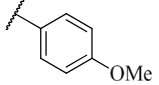
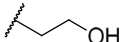
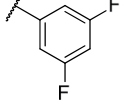
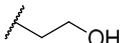
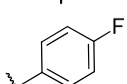
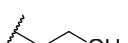
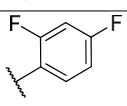
Benzofuran derivative effects on LPS-induced inflammation in Raw264.7 cells. Homoeogonol **8** and its newly synthesized 2-arylbenzofurans (**12–15**, **17a–b**, and **22a–q**) were evaluated for their inhibitory effects on NO and IL-6 production in LPS-stimulated Raw264.7 cells. The Raw264.7 cells were first co-treated with or without the compounds at a concentration of 40 μ M for 1 h and LPS (0.5 μ g/ml) for 24 h, after which the NO concentration in the supernatant was determined using Griess assays, as summarized in Table 1. Cell viability measured using MTT assays demonstrated that some compounds had no significant cytotoxicity at concentrations where they effectively inhibited NO and IL-6 production.

Our optimization strategy for the 2-arylbenzofuran analogues was to first modify the hydroxyl alkyl side chain (R₁), and aryl substitution (R₂), as shown in Table 1, and then combine the best substituents into a single compound with an improved overall profile. The detailed SAR study have shown in the supporting information.

Among the 2-arylbenzofuran derivatives, **22f** (referred to as **DK-1014**) was selected as the representative compound for subsequent testing because it demonstrated the potent inhibition of IL-6 and NO production with little effect on cell viability at 40 μ M.

DK-1014 effects on PMA-induced expression and production of cytokines. Cell viability at different **DK-1014** concentrations was investigated to confirm the appropriate concentration prior to verifying its anti-inflammatory effects in A549 cells. **DK-1014** did not affect cell viability up to 20 μ M (Fig. 1a) and then decreased IL-6, IL-8, and MCP-1 production in a dose-dependent manner, all of which were significantly higher in the PMA-treated cell supernatant (Fig. 1b–d). Because **DK-1014** inhibited cytokine production, we then investigated cytokine gene expression. mRNA expression of IL-6, IL-8, and MMP-9 only increased with PMA treatment but was significantly inhibited by **DK-1014** pretreatment (Fig. 1e).

DK-1014 effects on PMA-induced MAPK and AP-1 activation. MAP kinase has been implicated in inflammatory mediator production in various cells. ERK activation induces NF- κ B activation and cross-talk between the ERK and AP-1 pathways²⁹. AP-1 is major transcription factor that regulates the expression of pro-inflammatory cytokine³⁰, thus we investigated whether **DK-1014** affected on AP-1 and MAP kinase signaling

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Code No.	Structure		at 40 μ M		IL-6 inhibition (IC ₅₀ , μ M)
	R ₁	R ₂	NO inhibition (%)	Viability (%)	
8			13.26 $\pm$ 0.84	43.69 $\pm$ 0.49	19.94 $\pm$ 1.26
10	—	—	16.59 $\pm$ 3.83	56.55 $\pm$ 3.50	N.D.
12			23.08 $\pm$ 5.97	76.92 $\pm$ 4.85	48.69 $\pm$ 4.55
13			33.84 $\pm$ 1.83	62.34 $\pm$ 1.60	28.30 $\pm$ 0.07
14			33.53 $\pm$ 2.50	95.45 $\pm$ 15.18	35.61 $\pm$ 0.12
15			30.22 $\pm$ 0.27	7.21 $\pm$ 0.01	>40
17a			−3.40 $\pm$ 6.03	36.19 $\pm$ 2.20	>40
17b			−1.05 $\pm$ 0.17	25.71 $\pm$ 0.69	>40
22a			12.94 $\pm$ 2.29	95.28 $\pm$ 2.25	15.89 $\pm$ 0.06
22b			41.88 $\pm$ 2.67	92.76 $\pm$ 8.04	>40
22c			24.58 $\pm$ 2.96	105.93 $\pm$ 0.55	>40
22d			17.99 $\pm$ 0.65	103.31 $\pm$ 3.41	20.31 $\pm$ 0.01
22e			12.86 $\pm$ 3.53	94.80 $\pm$ 1.20	>40
22f (DK-1014)			30.36 $\pm$ 9.82	101.30 $\pm$ 1.48	16.19 $\pm$ 0.20
22g			93.32 $\pm$ 1.88	8.34 $\pm$ 3.42	8.34 $\pm$ 3.42
22h			68.52 $\pm$ 2.14	8.44 $\pm$ 1.44	>40
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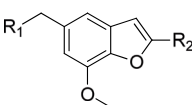
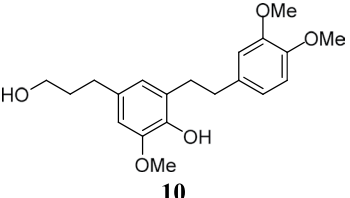
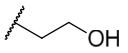
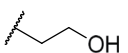
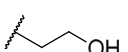
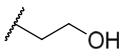
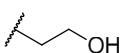
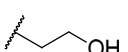
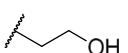
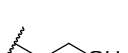
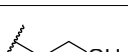
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Code No.	Structure		at 40 μ M		IL-6 inhibition (IC ₅₀ , μ M)
	R ₁	R ₂	NO inhibition (%)	Viability (%)	
22i			78.37 $\pm$ 1.01	8.48 $\pm$ 0.69	>40
22j			18.91 $\pm$ 6.94	98.93 $\pm$ 14.41	>40
22k			25.25 $\pm$ 8.16	95.99 $\pm$ 6.08	>40
22l			86.23 $\pm$ 0.45	0.49 $\pm$ 0.02	24.19 $\pm$ 6.48
22m			21.92 $\pm$ 2.35	114.97 $\pm$ 1.09	>40
22n			36.51 $\pm$ 5.44	55.62 $\pm$ 2.36	>40
22o			17.58 $\pm$ 0.50	77.04 $\pm$ 12.72	>40
22p			53.83 $\pm$ 2.70	5.21 $\pm$ 3.61	>40
22q			90.12 $\pm$ 2.03	10.82 $\pm$ 5.2	>40

Table 1. Inhibitory activity of benzofuran derivatives in LPS-induced inflammation^a. ^aValues are the mean of three experiments. N.D., not determined.

pathway for IL-6 production using western blot analysis. **DK-1014** significantly suppressed PMA-induced ERK and p38 kinase phosphorylation as dexamethasone (DEX) (Fig. 2a,b). Additionally, DK-1014 attenuated the translocation of c-fos and c-jun to the nucleus and the AP-1 luciferase activity by PMA (Fig. 2c,d). Thus, the inhibition of IL-6 expression by **DK-1014** is the result of not only ERK and p38 phosphorylation but also AP-1 activation blockade in PMA-treated A549 cells.

DK-1014 effects on PMA-induced AKT and mTOR pathway phosphorylation. AKT overexpression is associated with inflammation³¹ and the mTOR pathway is important in allergic asthma³². Therefore, we used immunoblot assays and immunocytochemistry to establish whether **DK-1014** suppresses the AKT/mTOR signaling pathway. Figure 3a shows that **DK-1014** inhibited AKT phosphorylation and mTOR (GSK and 70S6K) downstream components in PMA-treated A549 cells in a dose-dependent manner. Immunocytochemistry analysis found that levels of phosphor-AKT were lower in **DK-1014**-pretreated cells than in PI3K inhibitors (LY294002 and AS605240) treated cells, compared to PMA-treated A549 cells.

DK-1014 effects on airway hyperresponsiveness in OVA-sensitized/challenged mice. To investigate the effects of **DK-1014** on OVA-induced asthmatic mice, the mice were sensitized and nebulized with OVA and orally administered DK-1014 by scheme (Fig. 4a). In asthmatic (OVA) mice, AHR was markedly elevated with an increase in methylcholine concentrations compared with the controls. However, montelukast-treated

(Mon) mice exhibited significantly reduced AHR compared with OVA mice for methylcholine concentrations of 25 and 50 mg/ml. Similarly, **DK-1014**-treated mice exhibited significantly lower AHR compared with OVA mice at these same concentrations (Fig. 4b).

DK-1014 effects on the number of inflammatory cells and Th2 cytokine production in BALF. Asthmatic mice exhibited a marked increase in inflammatory cell count, particularly for eosinophils, in bronchial alveolar lavage fluid (BALF) compared to normal mice (Fig. 5a), whereas Mon mice had a significantly lower inflammatory cell count compared with OVA mice. Compound **DK-1014**-treated mice also exhibited a significant reduction in inflammatory cell count compared to OVA mice.

Levels of Th2 cytokines (IL-4, 5 and 13) markedly increased in OVA mice compared to normal mice. However, Mon mice exhibited markedly lower Th2 cytokine levels compared with OVA mice. Compound **DK-1014**-treated mice also had a significant reduction in cytokine levels compared to OVA mice (Fig. 5b).

DK-1014 effects on total IgE and OVA-specific IgE in serum. Total IgE and OVA-specific IgE in serum significantly increased in OVA mice (Fig. 5c). In contrast, Mon and **DK-1014**-treated mice exhibited significantly reduced total IgE and OVA-specific IgE production.

DK-1014 effects on airway inflammation and mucus production in lung tissue. The lung tissue of asthmatic mice exhibited extensive airway inflammation following H&E staining for peribronchiolar and perivascular lesions. However, **DK-1014**-treated mice exhibited less inflammatory cell infiltration into the peribronchiolar and perivascular lesions compared to OVA mice (Fig. 6a). OVA mice exhibited an overproduction of mucus under PAS staining whereas sections from **DK-1014**-treated mice exhibited a significant reduction in mucus hyperproduction in the airway compared to asthmatic mice (Fig. 6b).

Conclusion

In summary, we designed and synthesized a series of homoeogonol analogues (8–22). Among the synthesized compounds, homoeogonol **8**, phenyl analogue **22a**, and 3,5-fluorophenyl analogue **DK-1014** exhibited excellent IL-6 suppression and moderate NO inhibition in Raw264.7 cells. Compound **DK-1014** exhibited more potent inhibitory activity of IL-6 (IC_{50} $16.19 \pm 0.20 \mu M$) than homoeogonol **8** ($IC_{50} = 19.94 \pm 1.26 \mu M$). Therefore, we investigated the anti-inflammatory effects of **DK-1014** in PMA-treated A549 cells in more detail.

Inflammation is a complex reaction against many pathological conditions including tissue injury and these inflammatory responses have critical functions in host immune defense, signal transduction pathways, and airway regulation. In this study, PMA-treated A549 cells exhibited an increase in IL-6, IL-8 and MCP-1 protein and IL-6 and IL-8 (containing MMP-9) mRNA expression whereas their expression in **DK-1014**-treated cells was lower in a dose-dependent manner (Fig. 1).

MAPKs have a major influence on the expression of inflammatory mediators³³. Previous research has shown that IL-8 activates ERK and p38 but not JNK³⁴, and our data indicates that PMA activates ERK and p38. In contrast, pretreatment with **DK-1014** inhibited the phosphorylation of p38 and ERK but not JNK (data not shown) and significantly downregulated the translocation of c-fos more than c-jun. This inhibition appears to be associated with **DK-1014**'s effect to inactivate multiple cellular transcription factors and signaling proteins such as AP-1, p38, and ERK (Fig. 2).

Previous studies have demonstrated that airway inflammation is suppressed by reducing the activation of the mTOR pathway in asthma³⁵ and that the AKT/mTOR/p70S6K/GSK pathways play an important role in regulating the cell cycle of lung epithelial cells³⁶. Therefore, we explored the effects of **DK-1014** on the AKT pathway and found that **DK-1014** suppressed the phosphorylation of downstream effectors (i.e., mTOR, p70S6K and GSK) and AKT phosphorylation. Additionally, **DK-1014** strongly alleviated AKT phosphorylation more than LY294002³⁴ and AS605240³⁷ (a selective PI3Kgamma inhibitor) did, based on the results of immunofluorescent imaging (Fig. 3c). Together, this data suggests that the effect of **DK-1014** contributes to the regulation of several complex regulatory pathways in PMA-treated A549 cells.

Asthma is chronic disease accompanied by airway inflammation and airway hyperresponsiveness as a result of various mechanisms. Of the various inflammatory cells, eosinophils in particular have cytotoxic proteins, lipid mediators, and cytokines which damage epithelial cells, lead to mucus hyperplasia, and cause AHR. In our animal study, the administration of **DK-1014** effectively suppressed airway hyper-responsiveness (Fig. 4b), the production of Th2 cytokines and inflammatory cells in BALF, IgE production in serum (Fig. 5), the infiltration of inflammatory cells, and mucus overproduction (Fig. 6) compared with OVA-sensitized/challenged mice.

These anti-asthmatic effects of **DK-1014** might be illustrated by inhibitory mechanism on mRNA and protein expression of inflammatory mediators (cytokines, MMP-9 and MUC5AC), which blocking the translocation of AP-1 and the phosphorylation of MAPK and AKT/mTOR/p70S6K/GSK3 in PMA-treated lung epithelial cells (Fig. 7).

This study thus indicates that **DK-1014** has a significant effect on pro-inflammatory cytokines, provides a molecular/biochemical basis for its anti-inflammatory actions, and suggests future avenues for drug discovery and development in the treatment of various inflammatory diseases, including asthma.

Material and Methods

Synthesis. All commercial chemicals were of reagent grade and were used without further purification. Solvents were dried using standard procedures. All reactions were performed under dried argon atmosphere in flame-dried glassware. Proton nuclear magnetic resonance (¹H-NMR) spectra were analyzed using a Varian (400 MHz) spectrometer (Varian Medical Systems, Inc., Palo Alto, CA, USA), while ¹³C-NMR spectra were recorded using a Varian (100 MHz) spectrometer. Chemical shifts were provided in parts per million (ppm)

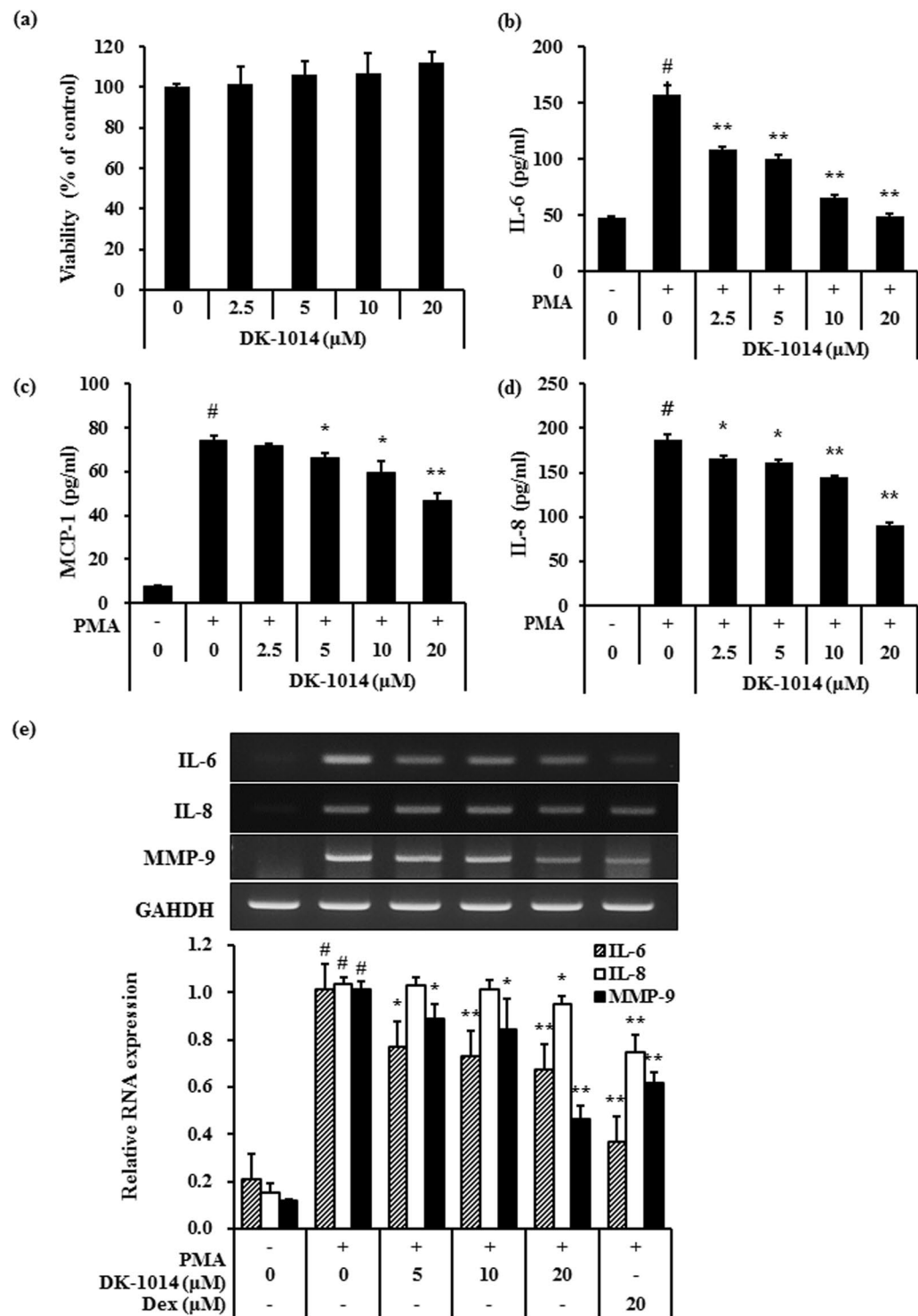


Figure 1. Cell viability and cytokine expression in A549 cells treated with DK-1014. (a) A549 cells were incubated with DK-1014 for 24 h and cell viability was detected using MTT; the A549 cells were pre-treated with DK-1014 for 1 h and then incubated for 12 h with PMA (5 nM). Vehicle control cells were treated with 0.1% DMSO in PBS. (b–d) Cytokines released into the supernatant quantified using ELISA. (e) The mRNA levels of IL-6, 8, and MMP-9 quantified using RT-PCR. The data is expressed as the mean $\pm$ SD from three independent experiments. ^{*}p < 0.05 compared to DMSO-treated cells; ^{*}p < 0.05 and ^{**}p < 0.005 compared to PMA-treated cells.

downfield with coupling constants in hertz (Hz). Mass spectra were recorded using high resolution mass spectrometry (HRMS; electron ionization MS) obtained on a JMS-700 mass spectrometer (Jeol, Japan) or HRMS (electrospray ionization MS) obtained on a G2 QTOF mass spectrometer. The products from all reactions were purified by flash-column chromatography using silica gel 60 (230–400 mesh Kieselgel 60). Thin-layer

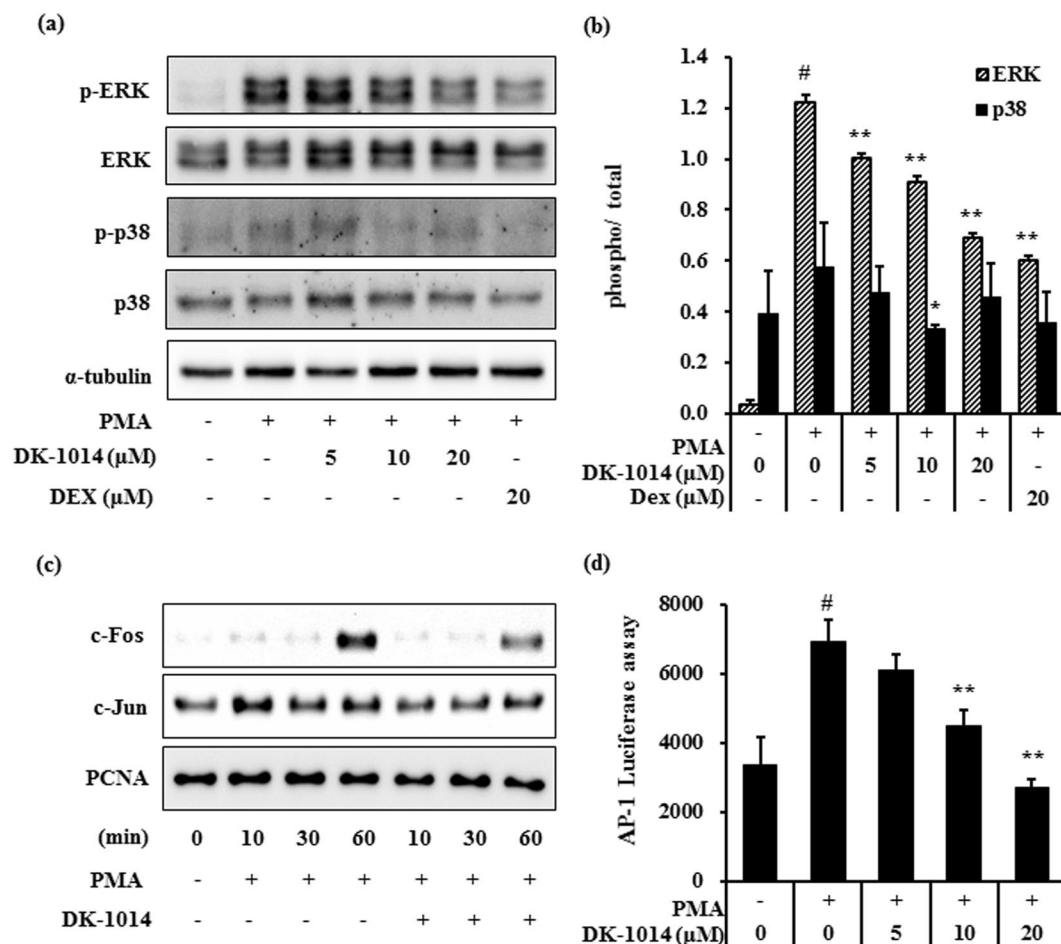


Figure 2. Phosphorylation of MAPK and AP-1 activation in A549 cells treated with DK-1014. A549 cells were pretreated with DK-1014 or Dexamethasone (DEX) for 1 h and then incubated for 30 min with PMA (5 nM). (a) Phosphorylation of MAPKs was evaluated using western blot analysis and (b) quantified using ImageJ. (c) Nuclear extracts were isolated from the cells using a Pierce kit. (d) AP-1 promoter activity detected using luciferase assays. The data is expressed as the mean $\pm$ SD from three independent experiments. [#] $p < 0.05$ compared to DMSO-treated cells; ^{**} $p < 0.005$ compared to PMA-treated cells.

chromatography on 0.25-mm silica plates (E. Merck; silica gel 60 F254) was used to monitor the reactions. Final product purity was determined using reversed-phase high pressure liquid chromatography (RP-HPLC), performed on a Waters Corp. HPLC system equipped with an ultraviolet (UV) detector at 254 nm. The mobile phases used were (A) H₂O containing 0.05% trifluoroacetic acid and (B) CH₃CN. The HPLC employed a YMC hydro-sphere C18 (HS-302) column (5-μm particle size, 12-nm pore size) with a diameter of 4.6 mm and length of 150 mm at a flow rate of 1.0 ml/min. Compound purity was assessed using method A with a gradient of 25% B to 100% B in 35 min. All of the biologically evaluated compound purities were >95% using method A. Details of the synthetic schemes, methods, and characterization of the compounds are described in Supplementary Information.

Cell culture. Human lung epithelial A549 cells and murine macrophage Raw 264.7 cells were purchased from ATCC (Rockville, MD). The cells were maintained at 37 °C in a humidified incubator containing 5% CO₂ in Dulbecco's modified Eagle's medium (Welgene, Korea) or RPMI containing 10% fetal bovine serum (GibcoBRL, Invitrogen) and supplemented with 100 unit/ml penicillin and 100 μg/ml of streptomycin (GibcoBRL, Invitrogen). Cells were seeded onto culture plates and adhered for several hours. Cells were stimulated with LPS or PMA after preincubation with the various compounds (20 mM in DMSO) which was diluted with 1X PBS. Vehicle control cells was treated with 0.1% DMSO in PBS. Cells and the supernatant were harvested and stored at -80 °C until used.

Cell viability assay. We used colorimetric MTT assays to study the effects of benzofuran derivatives on cell proliferation. Cells (1×10^4 cells/well) were seeded onto a 96-well plate and treated with various concentrations of the compounds for 24 h. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Amresco, OH, USA) was dissolved in PBS at 5 mg/ml then added to the well plate and incubated for 4 h at 37 °C. The supernatant

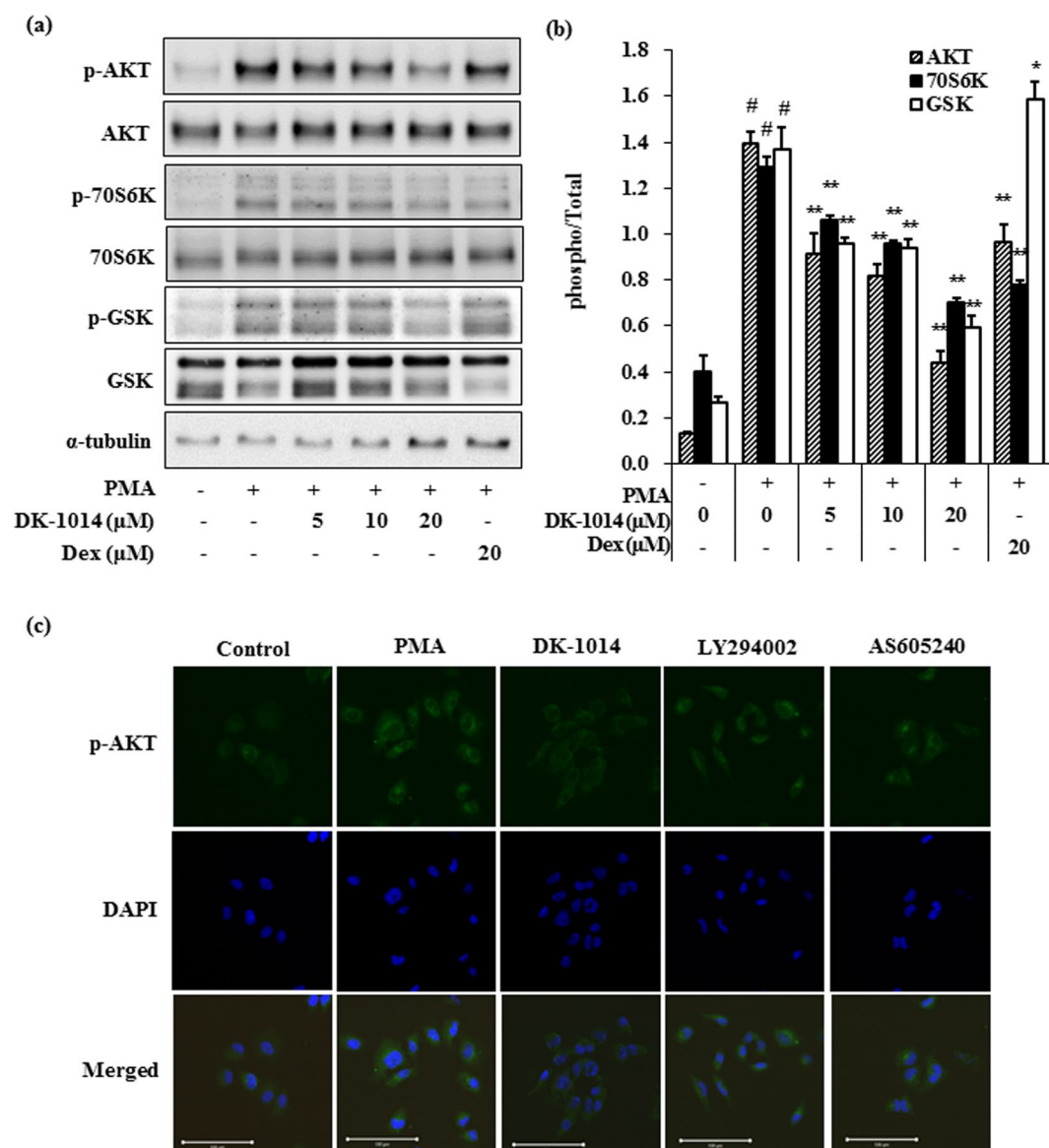


Figure 3. Phosphorylation of AKT/p70S6K in A549 cells treated with **DK-1014**. A549 cells were pre-treated with **DK-1014** or DEX for 1 h and then incubated for 30 min with PMA (5 nM). (a) Phosphorylation of AKT/mTOR protein evaluated using western blot analysis and (b) quantified using ImageJ. (c) Immunocytochemical staining with anti-phosphoAKT antibodies for A549 cells that were **DK-1014** treated or inhibitors (magnification = $\times 100$, bar; 100 μm). * $p < 0.05$ compared to DMSO-treated cells; ** $p < 0.005$ compared to PMA-treated cells.

was suctioned, and formazan crystals were dissolved in 100 μl DMSO. Optical density was measured using a microplate reader (SPARK10, TECAN, Switzerland) at 570 nm and cell viability was expressed as the proportion of untreated cells.

Nitric oxide assays. We used the modified Griess reaction to estimate the nitric oxide content in the cell culture supernatant³⁸. The cells (5×10^4 cells/well) were treated with various concentrations of the compound for 1 h followed by incubation with 0.5 $\mu\text{g}/\text{ml}$ LPS for 24 h. The supernatant was mixed with the same volume of Griess reagent (1% sulfanilamide and 0.1% *N*-[1-naphthyl]-ethylenediamine dihydrochloride in 5% phosphoric acid, Sigma) and reacted at room temperature for 10 min. Optical density was measured at 540 nm with a microplate reader and LPS-induced Raw264.7 cells using a sodium nitrite standard.

RT-PCR analysis. Total RNA was extracted from A549 cells using Trizol reagent (Invitrogen, CA, USA). The concentration and purity of the RNA were measured with a NanoDropTM 2000c spectrophotometer (ThermoFisher Scientific, Inc., Waltham, MA). Equal amounts of mRNA were converted by reverse-transcription into cDNA using a QuantiTect Reverse Transcription kit (Qiagen, Hilden, Germany). Polymerase chain reaction

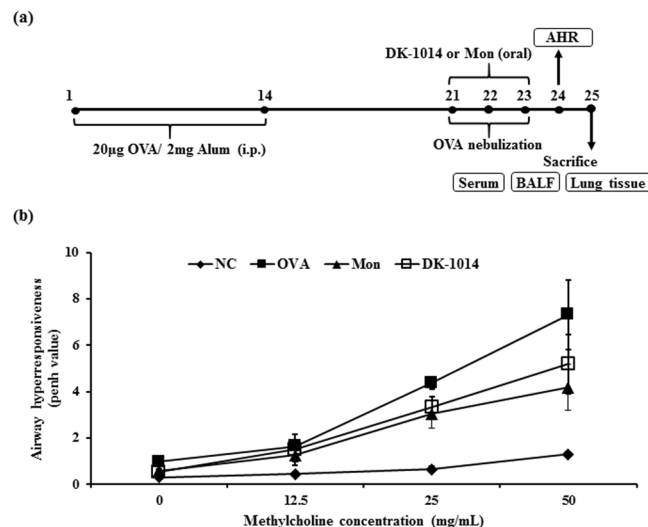


Figure 4. Airway inflammatory mice model and airway hyperresponsiveness in mice exposed to ovalbumin and DK-1014. Mice were sensitized with a 20- μ g intraperitoneal injection of OVA in aluminium hydroxide in PBS on days 1 and 14: (a) airway challenge performed using an ultrasonic nebulizer that administered 30 mg/kg/day of DK-1014 or Montelukast on days 21, 22, and 23. (b) AHR indirectly assessed 24 h after the last challenge using single-chamber, whole-body plethysmography. NC = normal control mice treated with PBS only; OVA = ovalbumin-sensitized/challenged mice; Mon = ovalbumin sensitized/challenged mice treated with montelukast (30 mg/kg); DK-1014 = ovalbumin sensitized/challenged mice treated with DK-1014 (30 mg/kg). ## $p < 0.005$ compared with the normal control; * $p < 0.05$ and ** $p < 0.005$ compared to OVA mice.

(PCR) analysis was conducted using specific primers and a premix (Fermentas, Germany) according to the manufacturer's protocols, with the following PCR conditions: pre-denaturation at 94 °C for 5 min, 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 45 s for 30 cycles and then a final extension phase at 72 °C for 10 min. GAPDH expression was included as an internal standard. PCR products were separated by electrophoresis on 1.5% agarose gel and visualized with a UV transilluminator (CoreBioSystem, Seoul, Korea). Images were captured with an Olympus C4000 camera (Olympus America Inc., Melville, NY, USA) and RT-PCR band intensities were measured using ImageJ software.

Luciferase reporter assays. We used a dual luciferase reporter assay system (Promega, WI, USA) to analyze promoter activity. Cells were seeded into 48-well plates and incubated at 37 °C at 70–80% confluence. The cells were then incubated with serum-free media for 6 h and transiently co-transfected with an AP-1 promoter luciferase construct and pRL-SV plasmid (Promega, WI, USA) using LipofectAMINE 2000 reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The medium was replaced with a basal medium after 5 h. The cells were lysed and luciferase activity was measured using SPARK10M. The luciferase signal for each sample was normalized to Renilla luciferase activity and expressed relative to the control.

Western blot analysis. Western blot analysis was conducted as described previously⁹. Proteins were isolated using NP-40 (ELPIS-Biotech. Inc., Daejeon, Korea) at a concentration determined with a Pierce BSA kit. Equal amount of protein was resolved using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to a polyvinylidene difluoride (PVDF, Millipore) membrane, incubated for 1 h in 5% skim milk in a TBS-T buffer, then incubated with primary antibodies, including phospho and total ERK, AKT, GSK, 70S6K (1:1000 dilution; Cell signaling, Danvers MA), phospho and total p38 (1:500 dilution; Santacruz, Dallas, TX) and alpha-tubulin (1:1000 dilution; Millipore, Boston, MA). The membrane was washed with TBS-T buffer and incubated with secondary antibodies; goat anti-mouse (Santacruz), goat anti-rabbit (Jackson ImmunoResearch Laboratories) for 1 h. Each protein was detected using an enhanced chemiluminescence (ECL) detection system (Bio-Rad Laboratories, Inc.) following the manufacturer's protocol. Western blot band intensities were measured using ImageJ software.

Immunocytochemical analysis. A549 cells were adhered to chamber slides and treated with chemicals. The cells were immobilized in cold ethanol for 10 min and then washed with cold PBS three times. Slides were blocked with 2% (w/v) BSA in PBS and then incubated with anti-phospho-AKT overnight at 4 °C. The cells were subsequently washed with PBS and incubated with Alexa Fluor 488 coated anti-rabbit IgG secondary antibodies for 2 h at room temperature. After staining with Gold Antifade reagent containing DAPI (Invitrogen) for 5 min, the cells were washed and fluorescent images obtained using LSM 510 m confocal microscopy (Carl Zeiss AG, Germany).

Animal treatment. Specific pathogen-free female 6-week-old BALB/c mice were purchased from Koatech Co. (Pyeongtaek, Korea) and used after 2 weeks quarantine and acclimatization in an air-conditioned room at approximately 22 °C and 55% RH. The mice were allowed sterilized tap water and standard rodent chow ad libitum.

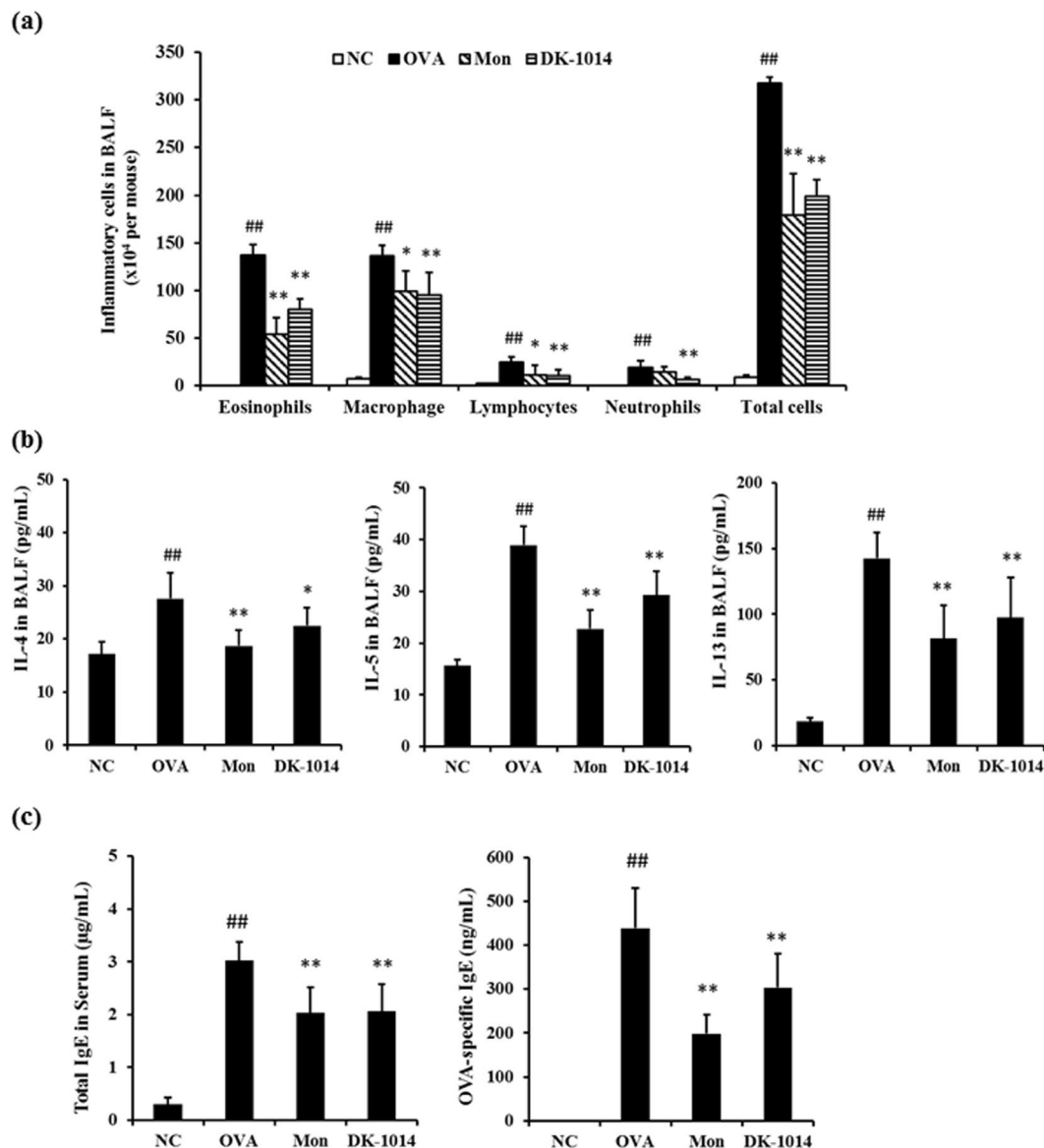


Figure 5. Inflammatory cells, cytokines, and IgE in sensitized mice exposed to ovalbumin and DK-1014. Inflammatory cells were collected using centrifugation and stained with Diff-Quik stain reagent. (a) Cell count was determined by counting the cells in at least five hemocytometer squares using a light microscope after excluding dead cells using Trypan blue. (b) Cytokines in BALF quantified using an ELISA kit. (c) Total IgE and OVA-specific IgE in serum quantified using an ELISA kit. NC = normal control mice treated with PBS only; OVA = ovalbumin sensitized/challenged mice; Mon = ovalbumin sensitized/challenged mice treated with montelukast (30 mg/kg); DK-1014 = ovalbumin sensitized/challenged mice treated with DK-1014 (30 mg/kg). ^{##} $p < 0.005$ compared to the normal control; ^{*} $p < 0.05$ and ^{**} $p < 0.005$ compared to OVA mice.

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Korea Research Institute of Bioscience and Biotechnology and were performed in compliance with National Institutes of Health Guidelines for the care and use of laboratory animals and Korean national animal welfare laws.

Immunization and challenge. Mice were sensitized on days 1 and 14 with a 20- μ g intraperitoneal (i.p.-) injection of ovalbumin (OVA) emulsified in 2 mg of aluminium hydroxide in 200 μ l of PBS buffer (pH 7.4). After initial sensitization, the mice received airway challenge with OVA (1%, w/v, in PBS) using an ultrasonic nebulizer (NE-U12; Omron Corp., Tokyo, Japan) for 1 h per day for days 21–23. DK-1014 and montelukast (Mon) were dissolved in dimethylacetamide and aliquoted into one dose. Samples were prepared in PBS containing 10% Tween-80 and sonicated for 3 min fresh daily before each treatment and were administered daily for days 21–23 by oral gavage (30 mg/kg) using feeding needle. Normal control mice (NC) were sensitized and challenged with PBS without ovalbumin and positive control mice were administered Mon by oral gavage (30 mg/kg)²³.

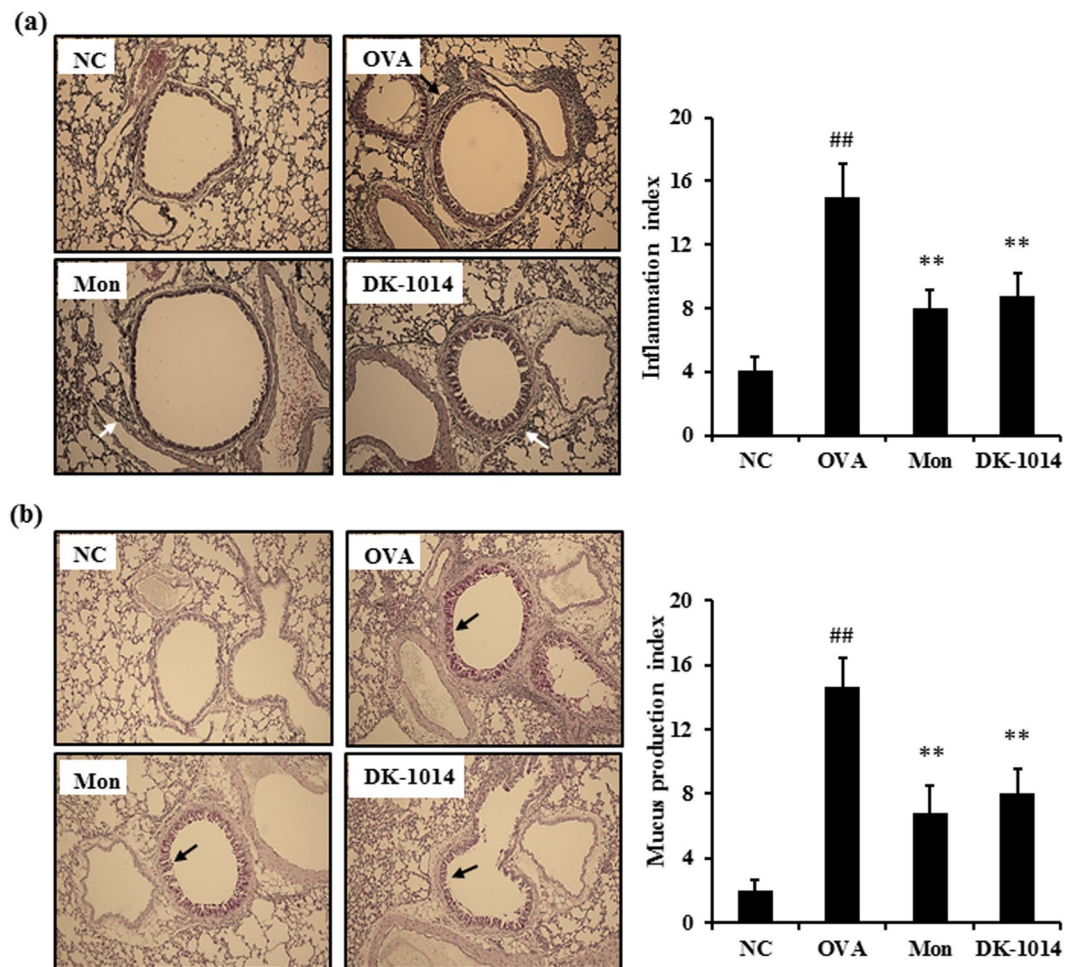


Figure 6. Lung tissue in sensitized mice exposed to ovalbumin and DK-1014. (a) Histological examination of lung tissue performed using H&E staining for airway inflammation (arrows indicate inflammatory cells). (b) PAS staining for mucus secretion (arrows indicate mucus cells). Original magnification was $\times 100$. NC = normal control mice treated with PBS only; OVA = ovalbumin sensitized/challenged mice; Mon = ovalbumin sensitized/challenged mice treated with montelukast (30 mg/kg); DK-1014 = ovalbumin sensitized/challenged mice treated with DK-1014 (30 mg/kg). ^{##} $p < 0.005$ compared to the normal control; ^{**} $p < 0.005$ compared to OVA mice.

Airway hyperresponsiveness measurement. Twenty-four hours after the final OVA challenge, AHR was measured in conscious and unrestrained mice through whole-body plethysmography (OCP3000; Allmedicus, Seoul, Korea). Each mouse was placed in a plastic chamber and exposed to methylcholine aerosols at increasing concentrations (12.5–50 mg/mL in PBS) for 3 min. After each methylcholine challenge, enhanced pause (Penh) values were measured over 3 min. The results were expressed as the percentage Penh increase following each methylcholine dose, where the baseline Penh after PBS challenge was expressed as 100%²³.

BAL fluid collection and leukocyte count. Forty-eight hours after the final OVA challenge, mice were anesthetized via an intraperitoneal pentobarbital injection (50 mg/kg; Hanlim Pharm. Co., Seoul, Korea) and bronchoalveolar lavage fluid (BALF) was collected via lavage of the lung through the trachea with 0.4 ml of ice-cold PBS three times. The total number of inflammatory cells was measured with a hemacytometer. BALF was centrifuged at 200 g for 5 min at 4 °C to determine differential cell counts. The cell pellet was resuspended in PBS and centrifuged onto a slide using Cytospin (Hanil Science Industrial, Seoul, Korea). The slides were dried and cells stained with a Diff-Quik staining kit (B4132-1A; IMEB Inc., Deerfield, IL, USA). Supernatant from the BALF was stored at -70°C for subsequent cytokine and IgE analysis.

Cytokine and IgE assays. Enzyme linked immunosorbent assay (ELISA) kits were used to measure the inhibitory effects of the compounds on cytokine production. Cytokines were analyzed following the manufacturer's instructions.

Histology. After BALF sampling, lung tissue was excised from the mice and fixed with 10% (v/v) neutral formaldehyde solution for 24 h at room temperature. Fixed tissues were embedded in paraffin and then cut into

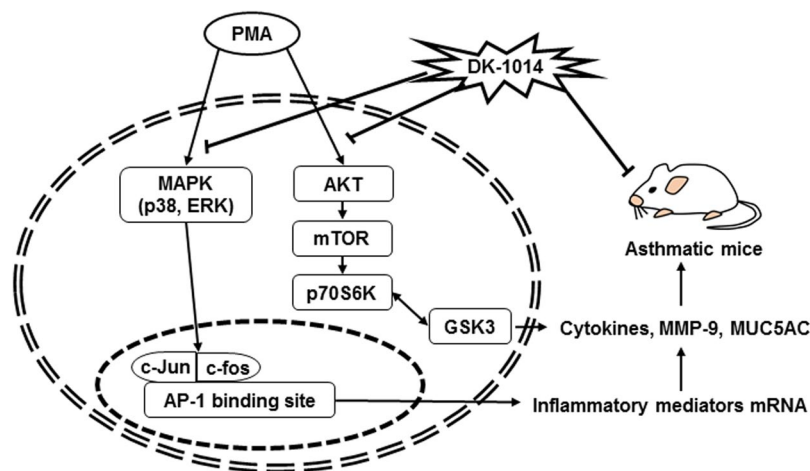


Figure 7. Proposed mechanism of DK-1014 in PMA-treated lung epithelial cells and asthmatic mice. PMA activates the phosphorylation of MAPK (p38 and ERK) and AKT/mTOR/p70S6K/GSK3, which in turn AP-1 activation and eventually induces the mRNA and protein expression of inflammatory mediators associated with inflammatory cytokines, MMP-9, and MUC5AC. Downregulation of these inflammatory mediators by DK-1014 leads to attenuation the recruitment of inflammatory cells, AHR, and the production of inflammatory cytokine and mucus in asthmatic mice (↓; activation, ↑; inhibition).

4- μ m sections using a microtome (Leica, Nussloch, Germany). The resulting slides were stained with hematoxylin (MHS-16, Sigma) and eosin (HT110-1-32, Sigma) to estimate inflammatory cell infiltration into the peribronchial connective tissue. Periodic acid-Schiff (PAS, IMEB Inc., San Marcos, CA, USA) was used to evaluate mucus production in the lung tissue. Airway inflammation and mucus production were quantified using an image analyzer (Molecular Devices Ins., CA, USA).

Statistical Analyses. All experiments were repeated at least three times and results were reported as means and standard deviations. Statistical significance was determined using a one-way analysis of variance (ANOVA) followed by a Tukey-Kramer test, with $p < 0.05$ as the standard for significance.

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Author Contributions

K.L. and H.G.J. designed the study and wrote the manuscript. X.X., D.S.H., Q.L. and J.R.M. performed chemical synthesis and analyzed the relevant data. O.K.K., I.S.S., Y.L., G.L., S.R.O. and K.S.A. performed the biological experiments and analyzed the relevant data. All authors have reviewed the manuscript.

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Novel benzofuran derivative DK-1014 attenuates the lung inflammation via blocking of MAPK/AP-1 and AKT/mTOR signaling in vitro and in vivo

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Synthesis.

The key intermediates and final compounds were prepared following the synthetic pathways described in Schemes S1-7 (Supplementary Information). One of the major intermediates, 3,4-dimethoxyphenylacetylene **3**, was obtained by Corey-Fuchs reaction from 3,4-dimethoxybenzaldehyde **1**. First, 3,4-dimethoxybenzaldehyde was converted into dibromide **2** in 80% yield using Ramirez olefination. Treating dibromide **2** with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) generated a bromoalkyne intermediate via dehydrohalogenation, which underwent subsequent lithium-halogen exchange followed by α -elimination to give alkyne **3** in 90% yield. Sonogashira coupling/cyclization of 5-iodovaniline **4** with 3,4-dimethoxyphenylacetylene **3** was conducted to give 2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** in good yield (70%). In our study we used 10% Pd/C, which is readily available and cheap, as a catalyst, along with PPh₃ and CuI in aqueous conditions at 90°C. The HWE olefination of **5** with methyl (triphenylphosphoranylidene) acetate gave methoxybenzofuran acrylate **6** in 90% yield, followed by catalytic hydrogenation with Pd/C in acetic acid and THF at ambient temperature to give compound **7** in 95% yield. Compound **7** was reduced with LiAlH₄ to afford homoegonol **8** in 95% yield (overall yield = 40.9 %, Scheme S1).

As shown in Scheme S2, acrylic ester **6** was subjected to catalytic hydrogenation with 10% Pd/C in THF at ambient temperature to give the ring-opened product **9** in 70% yield. The phenolic analogue **9** was then reduced using LiAlH₄ to give 2-(3,4-dimethoxyphenethyl)-(3-hydroxypropyl)-6-methoxyphenol **10** in 90% yield (Scheme S2). The acyclic structure of compound **10** was confirmed using spectral analysis.

The synthesis of the methanol and ethanol analogues of homoegonol is described in Scheme S3. Aldehyde **5** was reduced with NaBH₄ to give the desired methanol analogue **12**. For ethanol analogue **13**, compound **5** was subjected to Wittig olefination with methyltriphenylphosphonium iodide using sodium hydride in DMF at ambient temperature to afford vinyl benzofuran **11**, followed by hydroboration-oxidation with BH₃ and basic hydrogen peroxide in THF gave compound **13** in 85% yield. The treatment of the hydroxyl group of **8** with acetyl chloride and triethylamine at ambient temperature gave *O*-acetyl compound **14** in 87% yield (Scheme S4). The acrylic ester analogue **6** underwent chemoselective reduction with DIBAL-H gave the allylic alcohol compound **15** in 48% yield

(Scheme S5). Compounds **16a** and **16b** were prepared by Wittig olefination of **5** with (carbethoxymethylene) triphenylphosphorane and triethyl 2-fluoro-2-phosphonoacetate, respectively, followed by reduction with DIBAL-H to yield the methyl prophenol compounds **17a** and **17b**, respectively (Scheme S6).

For homoeogonol derivatives with various 2-aryl substituents **22a-q**, the Sonogashira coupling/cyclization of 5-iodovaniline **4** with alkynes **18a-q** in the presence of 10% Pd/C, PPh₃, and CuI in aqueous conditions was performed to give compounds **19a-q**, respectively, with 50-80% yields (Scheme S7). The synthesis of compounds **20a-q**, **21a-q**, and **22a-q** were achieved using a similar sequence for homoeogonol **8**.

Structure activity relationship study.

Homoeogonol **8** initially exhibited a very low inhibition of NO production ($13.26 \pm 0.84\%$) but exhibited a potent IC₅₀ value for IL-6 ($19.94 \pm 1.26 \mu\text{M}$) in Raw264.7 cells. Acyclic compound **10** exhibited moderate NO inhibition related to homoeogonol **8** but did not decrease IL-6 production, indicating that the benzofuran backbone led to a higher inhibitory effect on IL-6. When a hydroxyl alkyl chain was substituted at the R₁ position, homoeogonol **8** exhibited weaker NO inhibition and stronger IL-6 inhibitory activity related to the shorter side chain and O-acylated compounds such as **12**, **13**, and **14**. On the other hand, unsaturated side chain compounds **15**, **17a**, and **17b** reduced both the release of IL-6 and the inhibitory activity of NO production, suggesting that the R₁ group, i.e. the hydroxylpropyl side chain, led to potent NO and IL-6 inhibition.

It was found that side-chain length and structural features based on the benzofuran skeleton had a significant effect on inhibitory potency. Accordingly, further modification focused on 2-aryl substitution in homoeogonol moiety by replacing the R₂ aryl substituent i.e., the dimethoxy group, with methoxy, fluorine, and trifluoromethyl groups. The compounds (**22a-e**) were evaluated for NO production inhibition and IL-6 activity in LPS-induced Raw264.7 cells. Amongst them, phenyl analogue **22a** and methoxyphenyl analogue **22d** exhibited good IC₅₀ values for IL-6 (15.89 ± 0.06 , and $20.31 \pm 0.01 \mu\text{M}$, respectively). Of note, compound **22a** was more potent than homoeogonol **8** and 3-methoxyphenyl analogue **22d**. Therefore, we examined dimethoxy group substitution effects in the aryl ring of **8** on NO inhibition and IL-6 activity by replacing it with a fluoro group. NO inhibitory effects of the 3,5-difluorophenyl analogue, **22f**, (named as DK-1014) and 3,4-difluorophenyl analogue,

22g, (30.36±9.82% and 93.32±1.88, respectively) and IC₅₀ value for IL-6 (16.19±0.20 μM and 8.34±3.42, respectively) were higher than that for homoeogonol 8 (13.26±0.84% and IC₅₀ value for IL-6 = 19.94±1.26 μM), whereas compound 22g displayed higher cytotoxicity (8.34±3.42%). These findings suggest that the two fluoro groups at the *meta* position in the aryl ring enhance the inhibitory effects on NO and IL-6 production.

Following these results, we explored the effects on NO and IL-6 production of fluoro and trifluoromethyl substitution at the *o*, *m*, and *p*-positions in the phenyl ring (**22i-n**). Trifluoromethylphenyl analogue **22l** exhibited a higher NO inhibition (86.23±0.45%) and IC₅₀ value for IL-6 (24.19±6.48 μM), indicating that having a CF₃ group at the *ortho* position in the phenyl ring was more favorable for NO and IL-6 inhibition. However, compound **22l** exhibited the strongest cell viability (0.49±0.02%), and compounds **22o** and **22p** had lower NO and IL-6 inhibition. Compound **22q** also exhibited potent NO inhibition (90.12±2.03%) but did not improve the inhibitory effect of IL-6 at the same concentration. This indicates that dimethylamine, hydroxymethyl group installation, and ethereal linkage at the aryl ring had almost no observable effect on IL-6 inhibition.

Experimental Section.

General.

All the commercial chemicals were reagent grade and were used without further purification. Solvents were dried with standard procedures. All the reactions were carried out under an atmosphere of dried argon in flame-dried glassware. The proton nuclear magnetic resonance (¹H-NMR) spectra were determined on a Varian (400 or 500 MHz) spectrometer (Varian Medical Systems, Inc. Palo Alto, CA, USA). ¹³C-NMR spectra were recorded on a Varian (100 or 125 MHz) spectrometer. The chemical shifts are provided in parts per million (ppm) downfield with coupling constants in hertz (Hz). The mass spectra were recorded using high-resolution mass spectrometry (HRMS) (electron ionization MS) obtained on a JMS-700 mass spectrometer (Jeol, Japan) or using HRMS (electrospray ionization MS) obtained on a G2 QTOF mass spectrometer. The products from all the reactions were purified by flash column chromatography using silica gel 60 (230-400 mesh Kieselgel 60). Additionally, thin-layer chromatography on 0.25-mm silica plates (E. Merck; silica gel 60 F254) was used to monitor reactions. The purity of the final products was checked by reversed phase high-pressure liquid chromatography (RP-HPLC), which was performed on a Waters Corp. HPLC system

equipped with an ultraviolet (UV) detector set at 254 nm. The mobile phases used were (A) H₂O containing 0.05% trifluoroacetic acid, and (B) CH₃CN. HPLC employed an YMC Hydrosphere C18 (HS-302) column (5- μ m particle size, 12-nm pore size) that was 4.6 mm in diameter $\times$ 150 mm in size with a flow rate of 1.0 mL/min. The compound purity was assessed either using (Method A) a gradient of 20% B to 100% B in 35 min or (Method B) a gradient of 25% B to 100% B in 35 min.

4-(2,2-Dibromovinyl)-1,2-dimethoxybenzene (2).

To a mixture of PPh₃ (20.95 g, 80.0 mmol), CBr₄ (6.62 g, 20.0 mmol) in dichloromethane (20 mL) was added 3,4-dimethoxybenzaldehyde **1** (1.60 g, 10.0 mmol) dropwise at 0 °C, then the reaction mixture was stirred at room temperature for 30 minutes and quenched with water. The mixture was extracted with dichloromethane and washed with brine. The organic layers were dried over anhydrous MgSO₄, filtered and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 9:1) to give 4-(2,2-dibromovinyl)-1,2-dimethoxybenzene as a yellow oil (3.72 g, 95.9% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.41 (1 s, H), 7.18 (d, *J* = 2.0 Hz, 1H), 7.09 (1 dd, *J* = 1.4 Hz, 8.8 Hz, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 3.89 (s, 3H), 3.88 (s, 3H).

4-Ethynyl-1,2-dimethoxybenzene (3).

To a solution of 4-(2,2-Dibromovinyl)-1,2-dimethoxybenzene **2** (3.72 g, 10.0 mmol) in dry tetrahydrofuran (16 mL) was added LDA (29 mL, 60.0 mmol) dropwise at -80 °C, then the mixture was stirred at same temperature for 2 h. After completion of the reaction, the reaction mixture was quenched with NH₄Cl solution and extracted with dichloromethane. The organic layers were washed with brine and dried over anhydrous MgSO₄. The mixture was filtered and concentrated in vacuum and the crude product was purified by column chromatography on silica gel column chromatography (Hexanes:EtOAc = 8:2) to give 4-ethynyl-1,2-dimethoxybenzene as yellow solid (1.70 g, 90.9% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.26 (s, 1H), 7.11 (1 d, *J* = 1.6 Hz, 4.8 Hz, H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.81 (1 d, *J* = 5.6 Hz, 1H), 3.89 (3 s, H), 3.87 (s, 3H).

2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (5).

To a 50.0 mL sealed tube equipped with a stir bar was added 5-iodovaniline **4** (0.30g, 1.08 mmol), 10 % Pd/C (0.03g, 0.03 mmol), PPh₃ (0.03g, 0.13 mmol), CuI (0.01g, 0.06 mmol), L-prolinol or Et₃N (0.32g or 0.32g, 3.00 mmol) and water, degassed with argon for about 1 hour. The 4-Ethynyl-1,2-dimethoxybenzene **5** (0.43g, 2.70 mmol) was added degassed continued for another 15 minutes, then the mixture was refluxed for 24 h. After completion of the reaction, the reaction mixture was partitioned between EtOAc and brine. The organic layer was separated, washed with water, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 7:3) to give 2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde as a yellow solid (0.36g, 62.5% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 10.01 (s, 1H), 7.70 (1 d, *J* = 1.2 Hz, 1H), 7.49 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 7.38 (d, *J* = 2.0 Hz, 1H), 7.36 (1 s, 1H), 6.99 (s, 1H), 6.95 (d, *J* = 8.8 Hz, 1H), 4.10 (s, 3H), 4.00 (s, 3H), 3.95 (s, 3H).

(E)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate (6).

To a solution of 2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** (0.10g, 0.32 mmol) in dichloromethane (5 mL) was added methyl(triphenylphosphoranylidene)acetate (1.07 g, 3.20 mmol), then the reaction mixture was refluxed for 12 h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 8:2) to give (E)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate as white solid (0.10g, 85.5% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.77 (d, *J* = 16.0 Hz, 1H), 7.47 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.33 (s, 1H), 6.97 (s, 1H), 6.94 (d, *J* = 1.2 Hz, 1H), 6.90 (s, 1H), 6.42 (d, *J* = 15.6 Hz, 1H), 4.08 (s, 3H), 3.99 (s, 3H), 3.94 (s, 3H), 3.83 (s, 3H).

Methyl-3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propanoate (7).

To a solution of (E)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate **6** (0.05g, 0.14 mmol) in THF (5 mL) was added Pd/C (0.03g, 0.014 mmol, 10 wt %), followed by one or two drops of CH₃COOH and bubbled with hydrogen at room temperature for about 30 minutes under stirring. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel

column chromatography (Hexanes:EtOAc = 7:3) to give methyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propanoate as white solid (0.05g, 95.4% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.45 (dd, *J* = 2.0 Hz, 8.8 Hz, 1H), 7.36 (d, *J* = 2.0 Hz, 1H), 6.99 (s, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 6.83 (s, 1H), 6.63 (s, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 3.68 (s, 3H), 3.02 (t, *J* = 8.0 Hz, 1H), 2.68 (d, *J* = 7.8 Hz, 1H).

3-[2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl]propan-1-ol (Homoegonol, 8).

To a solution of methyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propanoate **7** (0.06g, 0.15 mmol) in THF (5 mL) was added LiAlH₄ in THF (2.0 M, 0.15 mL, 0.31 mmol) dropwise at 0 °C, then the mixture was stirred at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 5:5) to give 3-[2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl]propan-1-ol as a white solid (0.05g, 87.5% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.45 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 6.98 (d, *J* = 1.2 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.84 (s, 1H), 6.64 (d, *J* = 1.2 Hz, 1H), 4.04 (s, 3H), 3.99 (s, 3H), 3.93 (s, 3H), 3.72 (q, *J* = 5.6 Hz, 2H), 2.79 (t, *J* = 8.0 Hz, 2H), 1.92-1.99 (m, 2H); ¹³C-NMR (CDCl₃, 125 MHz) δ 148.7, 147.1, 146.2, 141.6, 135.1, 132.7, 127.3, 122.2, 120.3, 112.0, 111.2, 108.7, 62.3, 35.6, 34.5, 32.3, 31.8, 29.7; HRMS [*M* + *H*]⁺ calcd [C₂₀H₂₃O₅], 343.1545; found, 343.1537; Purity 99.99% (as determined by RP-HPLC, method A, *t_R* = 14.45 min).

Methyl-3-(3-(3,4-dimethoxyphenethyl)-4-hydroxy-5-methoxyphenyl)propanoate (9).

To a solution of (*E*)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate **6** (0.01g) in THF (5 mL) was added Pd/C (0.5 equiv., 10 wt %), stirred for 10 h under hydrogen atmosphere at room temperature. After completion of the reaction, the reaction mixture was filtered through a Celite pad and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 7:3) to give Methyl-3-(3-(3,4-dimethoxyphenethyl)-4-hydroxy-5-methoxyphenyl)propanoate as a white solid (0.098g, 98.4% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 6.74-6.80 (m, 2H), 6.71 (d, *J* = 2.0 Hz, 1H), 6.58 (d, *J* = 2.0 Hz, 1H), 6.53 (d, *J* = 1.6 Hz, 1H), 5.58 (s, 1H), 3.87 (s, 3H),

3.86 (s, 3H), 3.85 (s, 3H), 3.67 (s, 3H), 2.82-2.89 (m, 6H), 2.57 (d, $J = 7.8$ Hz, 1H).

2-(3,4-Dimethoxyphenethyl)-4-(3-hydroxypropyl)-6-methoxyphenol (10).

To a solution of Methyl-3-(3-(3,4-dimethoxyphenethyl)-4-hydroxy-5-methoxyphenyl)propanoate **9** (0.08g equiv.) in THF (10 ml) was added LiAlH₄ in THF (2.0 M, 1.0 equiv) dropwise at 0 °C, then the mixture was stirred at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 7:3) to obtain 2-(3,4-dimethoxyphenethyl)-4-(3-hydroxypropyl)-6-methoxyphenol as a White solid (0.068g, 91.2% yield). ¹H-NMR (CDCl₃, 500 MHz) δ 7.45 (dd, $J = 2.0$ Hz, 8.5 Hz, 1H), 7.37 (d, $J = 2.0$ Hz, 1H), 6.98 (s, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 6.84 (s, 1H), 6.64 (d, $J = 0.5$ Hz, 1H), 4.04 (s, 3H), 3.99 (s, 3H), 3.93 (s, 3H), 3.72 (t, $J = 6.5$ Hz, 2H), 2.79 (t, $J = 7.5$ Hz, 2H), 1.93-1.98 (m, 2H); ¹³C-NMR (CDCl₃, 125 MHz) δ 156.4, 149.6, 149.2, 144.8, 137.5, 131.2, 123.6, 118.8, 112.3, 111.4, 108.3, 107.3, 100.3, 62.3, 56.1, 56.0, 34.7, 32.4; HRMS [M + H]⁺ calcd [C₂₀H₂₆O₅], 347.1858; found, 347.1855; Purity 99.99% (as determined by RP-HPLC, method A, $t_R = 11.81$ min).

2-(3,4-Dimethoxyphenyl)-7-methoxy-5-vinylbenzofuran (11).

To a mixture of 2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** (60 mg, 0.19 mmol), methyltriphenylphosphonium iodide (116 mg, 0.29 mmol) in DMF (5 mL) was added sodium hydride (0.023 g, 0.95 mmol) at 0 °C, then the mixture was stirred for 12 h at room temperature. The reaction mixture was then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 7:3) to give 2-(3,4-Dimethoxyphenyl)-7-methoxy-5-vinylbenzofuran as a white solid (0.05 g, 83.3% yield). ¹H-NMR (CD₃OD, 500 MHz) δ 7.47 (dd, $J = 2.0$ Hz, 8.0 Hz, 1H), 7.44 (d, $J = 2.0$ Hz, 1H), 7.18 (s, 1H), 7.04 (d, $J = 8.5$ Hz, 1H), 7.02 (s, 1H), 6.98 (s, 1H), 6.79 (dd, $J = 10.5$ Hz, 17.5 Hz, 1H), 5.74 (d, $J = 17.5$ Hz, 1H), 5.18 (d, $J = 11.0$ Hz, 1H), 4.04 (s, 3H), 3.93 (s, 3H), 3.88 (s, 3H).

[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]methanol (12).

To a solution of 2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** (0.1 g, 0.32 mmol) in THF (5 mL) was added NaBH₄ (0.036 g, 0.96 mmol) at 0 °C, then the mixture was stirred at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 6:4) to give [2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]methanol as a white solid (0.09g, 84.6% yield). ¹H-NMR (CDCl₃, 500 MHz) δ 7.50 (dd, *J* = 2.0 Hz, 8.0 Hz, 1H), 7.47 (d, *J* = 1.5 Hz, 1H), 7.15 (s, 1H), 7.12 (s, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 6.92 (s, 1H), 4.68 (d, *J* = 6.0 Hz, 2H), 4.14 (t, *J* = 6.0 Hz, 1H), 4.02 (s, 3H), 3.92 (s, 3H), 3.87 (s, 3H); ¹³C-NMR (CDCl₃, 125 MHz) δ 156.7, 149.7, 149.2, 145.2, 143.5, 136.8, 131.1, 123.4, 118.2, 111.5, 111.4, 108.3, 105.8, 100.4, 65.9, 56.1, 56.0; HRMS [M + H]⁺ calcd [C₁₈H₁₈O₅], 315.1232; found, 315.1223; Purity >99.98 % (as determined by RP-HPLC, method A, *t*_R = 12.37 min).

2-[2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl]ethanol (13).

To a solution of 2-(3,4-Dimethoxyphenyl)-7-methoxy-5-vinylbenzofuran **11** (30 mg, 0.10 mmol) in THF (2mL) was added BH₃·THF in THF (1.0 M, 0.11 mL, 0.11 mmol) dropwise at 0 °C, then the mixture was stirred at room temperature for 3 h. After 3 h of stirring at room temperature, added 0.1 mL of 10 % NaOH solution and 0.1 ml of 30 % H₂O₂ and stirred for 30 minutes at room temperature and at 55 °C for 1 h. The reaction mixture was then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 5:5) to give 2-[2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl]ethanol as a white solid (0.014 g, 43.8% yield). ¹H-NMR (CD₃OD, 500 MHz) δ 7.46 (dd, *J* = 2.0 Hz, 8.5 Hz, 1H), 7.44 (d, *J* = 2.0 Hz, 1H), 7.03 (d, *J* = 8.0 Hz, 1H), 7.01 (s, 1H), 6.98 (s, 1H), 6.74 (d, *J* = 0.5 Hz, 1H), 4.01 (s, 3H), 3.92 (s, 3H), 3.87 (s, 3H), 3.79 (t, *J* = 7.0 Hz, 2H), 2.88 (t, *J* = 7.0 Hz, 2H); ¹³C-NMR (CDCl₃, 125 MHz) δ 156.5, 149.7, 149.2, 145.0, 134.0, 131.4, 123.4, 118.2, 113.1, 111.4, 108.3, 107.6, 100.2, 63.9, 56.2, 56.1, 56.0, 39.5; HRMS [M + H]⁺ calcd [C₁₉H₂₀O₅], 329.1389; found, 329.1376; Purity 99.98 % (as determined by RP-HPLC, method A, *t*_R = 13.26 min).

3-(2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propyl acetate (14).

To a solution of 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propan-1-ol **8** (0.5g, 1.46 mmol) in MC (15 mL) Acetyl chloride (0.229 g, 2.90 mmol) and TEA (0.455 g, 4.5 mmol) were added at 0 °C, then the mixture was stirred at room temperature 12 hours. The reaction mixture was stopped by water then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 8:2) to give 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propyl acetate as a white solid (0.49g, 87.5% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.45 (dd, *J* = 2.0 Hz, *J* = 2.0 Hz, 1H), 7.36 (d, *J* = 2.0 Hz, 1H), 6.96 (d, *J* = 1.2 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.84 (s, 1H), 6.61 (d, *J* = 1.2 Hz, 1H), 4.13 (d, *J* = 6.8 Hz, 2H), 4.04 (s, 3H), 3.99 (s, 3H), 3.93 (s, 3H), 2.76 (d, *J* = 7.6 Hz, 2H), 2.07 (s, 3H), 1.97-2.04 (m, 2H); ¹³C-NMR (CDCl₃, 100 MHz) δ 171.2, 156.4, 149.5, 149.1, 144.8, 142.8, 142.5, 136.9, 131.1, 123.5, 118.1, 112.3, 111.2, 108.1, 107.1, 100.3, 63.9, 56.1, 56.1, 56.0, 32.5, 30.7, 21.0; HRMS [M + H]⁺ calcd [C₂₂H₂₄O₆], 385.1651; found, 385.1651; Purity 99.99% (as determined by RP-HPLC, method A, *t_R* = 21.61 min).

(E)-3-(2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl)prop-2-en-1-ol (15).

To a solution of (E)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate **6** (0.04g, 0.11 mmol) in THF (10ml) was added DIBAL-H in Toluene (1.0 M, 2.0 equiv) dropwise at 0 °C then the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction, the reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 6:4) to give (E)-Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl]acrylate as a white solid (0.02g, 48% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.46 (dd, *J* = 1.8 Hz, 8.6 Hz, 1H), 7.36 (d, *J* = 1.6 Hz, 1H), 7.15 (s, 1H), 6.93 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 1.2 Hz, 1H), 6.86 (s, 1H), 6.68 (d, *J* = 7.6 Hz, 1H), 6.35 (dt, *J* = 5.9 Hz, 15.7 Hz, 1H), 4.35 (t, *J* = 1.6 Hz, 2H), 4.06 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 1.41 (t, *J* = 6.0 Hz, 1H); ¹³C-NMR (CDCl₃, 100 MHz) δ 156.7, 149.7, 149.2, 145.1, 143.8, 132.8, 131.9, 131.3, 127.3, 123.3, 118.2, 111.7, 111.3, 108.2, 104.6, 100.4, 63.9,

56.1, 56.0; HRMS $[M + H]^+$ calcd $[C_{20}H_{21}O_5]$, 341.1389; found, 341.1389; Purity 99.99% (as determined by RP-HPLC, method A, t_R = 14.67 min).

(E)-Ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-methylacrylate (16a).

To a solution of 2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** (0.10g, 0.32 mmol) in dichloromethane (10ml) was added (carbethoxymethylene)triphenylphosphorane (0.23g, 0.64 mmol), then the reaction mixture was refluxed for 12 h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 8:2) to give (E)-ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-methylacrylate as a white solid (0.10g, 83.8% yield). ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.77 (d, J = 1.2 Hz, 1H), 7.47 (d, J = 8.4 Hz, 1H), 7.37 (s, 1H), 7.23 (s, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.91 (s, 1H), 6.82 (s, 1H), 4.29 (q, J = 6.9 Hz, 2H), 4.06 (s, 3H), 3.99 (s, 3H), 3.94 (s, 3H), 2.18 (s, 3H).

(Z)-Ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-fluoroacrylate (16b).

To a solution of 2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde **5** (0.10g, 0.32 mmol) in THF (10ml) was added Triethyl 2-fluoro-2-phosphonoacetate (0.12g, 0.48 mmol), DBU (0.08g, 0.55 mmol) and LiCl (0.02g, 0.48 mmol) then the reaction mixture was stirred for 12 h. After completion of the reaction, the reaction mixture was stopped by water then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous $MgSO_4$, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 8:2) to give (Z)-ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-fluoroacrylate as a white solid (105mg, 82.7% yield). ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.46 (d, J = 8.8 Hz, 1H), 7.37 (s, 1H), 7.31 (s, 1H), 7.10 (s, 1H), 7.02 (t, J = 8.8 Hz, 1H), 6.95 (d, J = 5.6 Hz, 1H), 6.92 (d, J = 5.2 Hz, 1H), 6.88 (s, 1H), 4.28 (q, J = 14.0 Hz, 2H), 4.05 (s, 3H), 3.99 (s, 3H), 3.93 (s, 3H), 2.27 (t, J = 6.8 Hz, 3H).

(E)-3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-methylprop-2-en-1-ol (17a).

To a solution of (E)-ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-methylacrylate **16a** (0.07g, 0.18 mmol) in THF (10ml) was added DUBAL in Toluene (1.0 M, 2.0 equiv) dropwise at 0 °C then the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 6:4) to give (E)-3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-methylprop-2-en-1-ol as a white solid (0.03g, 47% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.46 (dd, *J*₁ = 1.8 Hz, *J*₂ = 8.6 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.07 (s, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.87 (s, 1H), 6.70 (s, 1H), 6.60 (s, 1H), 4.22 (s, 2H), 4.04 (s, 3H), 3.98 (s, 3H), 3.93 (s, 3H), 2.04 (s, 3H), 0.83-0.89 (m, 1H); ¹³C-NMR (CDCl₃, 100 MHz) δ 156.5, 149.6, 149.1, 144.5, 142.7, 136.9, 133.4, 130.9, 125.6, 123.3, 118.1, 113.2, 111.2, 108.1, 107.8, 100.4, 69.1, 56.1, 56.0, 15.4. HRMS [M + H]⁺ calcd [C₂₁H₂₃O₅], 355.1545; found, 355.1544; Purity 99.97% (as determined by RP-HPLC, method A, *t*_R = 16.02 min).

(Z)-3-(2-(3,4-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-fluoroprop-2-en-1-ol (17b).

To a solution of (Z)-ethyl 3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-fluoroacrylate **16b** (0.04g, 0.10 mmol) in THF (10ml) was added DIBAL in Toluene (1.0 M, 2.0 equiv) dropwise at 0 °C then the reaction mixture was stirred for 2 h at room temperature. After completion of the reaction. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography (Hexanes:EtOAc = 6:4) to give (Z)-3-(2-(3,4-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)-2-fluoroprop-2-en-1-ol as a white solid (0.02g, 50% yield). ¹H-NMR (CDCl₃, 400 MHz) δ 7.46 (dd, *J* = 2.0 Hz, 8.4 Hz, 1H), 7.37 (d, *J* = 2.0 Hz, 1H), 7.30 (s, 1H), 7.02 (s, 1H), 6.93 (d, *J* = 7.6 Hz, 1H), 6.88 (s, 1H), 6.85 (d, *J* = 38.4 Hz, 1H), 4.22 (dd, *J* = 6.6 Hz, 15.0 Hz, 2H), 4.05 (s, 3H), 3.99 (s, 3H), 3.93 (s, 3H), 1.84 (t, *J* = 6.2 Hz, 1H); NMR (CDCl₃, 100 MHz) δ 158.5, 156.7, 155.9, 149.6, 149.1, 144.8, 143.3, 131.1, 128.5, 128.4, 123.2, 118.2, 113.8, 113.7, 111.2, 108.1, 108.1, 107.2, 107.2, 100.4, 62.3, 62.0, 56.1, 56.0. HRMS [M + H]⁺ calcd [C₂₀H₂₀O₅F], 359.1295; found, 359.1296;

Purity 99.96% (as determined by RP-HPLC, method A, t_R = 15.73 min).

General procedure of Sonogashira reaction for synthesis of 19a-q.

To a 50.0 mL sealed tube equipped with a stir bar was added 5-iodovaniline (1 equiv), 10 % Pd/C (0.03 equiv), PPh₃ (0.12 equiv), CuI (0.06 equiv), L-prolinol or Et₃N (3.0 equiv) and water, degassed with argon for about 1 hour. The acetylenecompound (2.5 equiv) was added degassed continued for another 15 minutes, then the mixture was refluxed for 24 h. After completion of the reaction, the reaction mixture was partitioned between EtOAc and brine. The organic layer was separated, washed with water, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography to obtain **19a-q**.

7-Methoxy-2-phenylbenzofuran-5-carbaldehyde (19a).

Yellow solid 0.350g, 82.8% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 10.01 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.73 (s, 1H), 7.47 (t, J = 7.6 Hz, 2H), 7.41 (d, J = 7.6 Hz, 1H), 7.38 (s, 1H), 7.12 (s, 1H), 4.10 (s, 3H).

2-(3,5-Dimethoxyphenyl)-7-methoxybenzofuran-5-carbaldehyde (19b).

Yellow solid 0.30 g, 53.4% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 10.01 (s, 1H), 7.72 (d, J = 0.8 Hz, 1H), 7.38 (s, 1H), 7.09 (s, 1H), 7.04 (d, J = 2.8 Hz, 2H), 6.51 (t, J = 2.2 Hz, 2H), 4.10 (s, 3H), 3.88 (s, 6H).

7-Methoxy-2-(2-methoxyphenyl)benzofuran-5-carbaldehyde (19c).

Yellow solid 0.35g, 69.1% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 10.01 (d, J = 0.8 Hz, 1H), 8.13 (dd, J = 1.6, 7.6 Hz, 1H), 7.73 (d, J = 1.6 Hz, 1H), 7.43 (s, 1H), 7.37 (dd, J = 1.7, 8.1 Hz, 2H), 7.07-7.11 (m, 1H), 7.03 (d, J = 8.4 Hz, 1H), 4.10 (s, 3H), 4.02 (s, 3H).

7-Methoxy-2-(3-methoxyphenyl)benzofuran-5-carbaldehyde (19d).

Yellow solid 0.34g, 71.8% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 10.01 (s, 1H), 7.72 (d, J = 1.5 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.43 (t, J = 2.0 Hz, 1H), 7.38 (t, J = 8.0 Hz, 2H), 7.11 (s, 1H), 6.95 (dd, J = 2.0 Hz, 8.0 Hz, 1H), 4.10 (s, 3H), 3.90 (s, 3H).

7-Methoxy-2-(4-methoxyphenyl)benzofuran-5-carbaldehyde (19e).

Yellow solid 0.40g, 80% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.00 (s, 1H), 7.83 (d, J = 9.2 Hz, 2H), 7.69 (d, J = 1.2 Hz, 1H), 7.34 (d, J = 0.8 Hz, 1H), 6.98 (d, J = 8.4 Hz, 2H) 6.96 (s, 1H), 4.09 (s, 3H), 3.87 (s, 3H).

2-(3,5-Difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19f).

Yellow solid 0.07g, 54.1% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 7.74 (s, 1H), 7.41 (d, J = 4.8, 3H), 7.15 (s, 1H), 6.83 (t, J = 8.8, 1H), 4.10 (s, 3H).

2-(3,4-Difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19g).

Yellow solid 0.15g, 75.6% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 7.69-7.74 (m, 2H), 7.75 (s, 1H), 7.40 (s, 1H), 6.94-7.04 (m, 2H), 4.10 (s, 3H).

2-(2,4-Difluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19h).

Yellow solid 0.17g, 78.5% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 8.09 (q, J = 8.4, 1H), 7.61-7.65 (m, 1H), 7.39 (s, 1H), 7.26 (q, J = 8.4, 1H), 7.07 (s, 1H), 4.10 (s, 3H).

2-(2-Fluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19i).

Yellow solid (2.00g, 90.0 % yield). $^1\text{H NMR}$ (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 8.10 (t, J = 7.8 Hz, 1H), 7.75 (s, 1H), 7.40 (s, 1H), 7.36 (t, J = 7.2 Hz, 1H), 7.33 (d, J = 3.2 Hz, 1H), 7.28 (d, J = 7.6 Hz, 1H), 7.20 (dd, J = 8.2 Hz, 11.4 Hz, 1H), 4.10 (s, 3H).

2-(3-Fluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19j).

Yellow solid 0.37g, 77.8% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 7.74 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.58-7.62 (m, 1H), 7.44 (q, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 7.09 (s, 1H), 4.10 (s, 1H).

2-(4-Fluorophenyl)-7-methoxybenzofuran-5-carbaldehyde (19k).

Yellow solid 0.30 g, 61.7% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 10.01 (s, 1H), 7.89 (dd, J = 5.2 Hz, 8.8 Hz, 2H), 7.72 (d, J = 1.2 Hz, 1H), 7.38 (d, J = 1.2, 1H), 7.16 (t, J = 8.8, 2H), 7.05 (s, 1H), 4.09 (s, 3H).

7-Methoxy-2-(2-(trifluoromethyl)phenyl)benzofuran-5-carbaldehyde (19l).

Brown solid (0.43 g, 74.6 % yield). ^1H NMR (CDCl_3 , 400 MHz) δ 10.03 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.78 (s, 1H), 7.67 (t, J = 7.6 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), 7.42 (s, 1H), 7.15 (s, 1H), 4.09 (s, 3H).

7-Methoxy-2-(3-(trifluoromethyl)phenyl)benzofuran-5-carbaldehyde (19m).

Yellow solid 0.30g, 52.1% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 8.14 (s, 1H), 8.07(d, J = 7.2 Hz, 1H), 7.75 (d, J = 1.6 Hz, 1H), 7.62 (q, J = 8.0 Hz, 1H), 7.41 (d, J = 1.2 Hz, 1H), 7.21 (s, 1H), 4.11 (s, 3H).

7-Methoxy-2-(4-(trifluoromethyl)phenyl)benzofuran-5-carbaldehyde (19n).

Yellow solid 0.25g, 73.0% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 10.02 (s, 1H), 8.01 (d, J = 8.4, 1H), 7.75 (d, J = 0.8 Hz, 1H), 7.72 (d, J = 8.4, 2H), 7.41 (d, J = 1.2 Hz, 1H), 7.23 (s, 1H), 4.11 (s, 3H).

2-(4-(Dimethylamino)phenyl)-7-methoxybenzofuran-5-carbaldehyde (19o).

Yellow solid 0.24g, 45.8% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 9.99 (d, J = 0.8 Hz, 1H), 7.70 (d, J = 7.2 Hz, 2H), 7.66 (t, J = 1.2 Hz, 1H), 7.32 (d, J = 0.8 Hz, 1H), 6.87 (s, 1H), 6.76 (d, J = 8.8 Hz, 2H), 4.09 (s, 3H), 3.03 (s, 6H).

2-(4-(Hydroxymethyl)phenyl)-7-methoxybenzofuran-5-carbaldehyde (19p).

Yellow solid 0.21g, 54.6% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 10.01 (s, 1H), 7.91 (d, J = 8.4 Hz, 2H), 7.73 (d, J = 0.8 Hz, 1H), 7.48 (d, J = 8.0 Hz, 2H), 7.38 (s, 1H), 7.12 (s, 1H), 4.77 (d, J = 6.0 Hz, 2H), 4.10 (s, 3H), 1.74 (t, J = 6.0 Hz, 1H).

7-Methoxy-2-(4-phenoxyphenyl)benzofuran-5-carbaldehyde (19q).

Yellow solid 0.18g, 75.8% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 10.01 (s, 1H), 7.84-7.87 (m, 2H), 7.71 (d, J = 1.6 Hz, 1H), 7.36-7.40 (m, 3H), 7.15-7.18 (m, 1H), 7.06-7.09 (m, 4H), 7.02 (s, 1H), 4.09 (s, 3H), 1.74 (t, J = 6.0 Hz, 1H).

General procedure of Wittig reaction for synthesis of 20a-q.

To a solution of aldehyde (1.0 equiv.) in dichloromethane (10 volume) was added

methyl(triphenylphosphoranylidene)acetate (3.0 equiv.), then the reaction mixture was refluxed for 12 h. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography to yielded white solid **20a-q**.

(E)-Methyl 3-(7-methoxy-2-phenylbenzofuran-5-yl)acrylate (20a).

Yellow solid 0.15g, 81.9% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.89 (d, $J = 7.2$ Hz, 2H), 7.77 (d, $J = 16.4$ Hz, 1H), 7.45 (t, $J = 7.6$ Hz, 2H), 7.38 (d, $J = 7.2$ Hz, 1H), 7.35 (s, 1H), 7.02 (s, 1H), 6.98 (d, $J = 1.2$ Hz, 1H), 6.42 (d, $J = 16.0$ Hz, 1H), 4.08 (s, 3H), 3.82 (s, 3H).

(E)-Methyl-3-(2-(3,5-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)acrylate (20b).

Yield white solid 0.11g, 58.5% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.77 (d, $J = 16$ Hz, 1H), 7.35 (s, 1H), 7.03 (t, $J = 1.2$, 2H), 6.99 (d, $J = 8.4$ Hz, 2H), 6.49 (t, $J = 2$, 1H), 6.42 (d, $J = 16.4$ Hz, 1H), 4.07 (s, 3H), 3.87 (s, 6H), 3.82 (s, 3H).

(E)-Methyl-3-(7-methoxy-2-(2-methoxyphenyl)benzofuran-5-yl)acrylate (20c).

Yellow solid 0.20g, 53.8% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 8.10 (dd, $J = 2.0$ Hz, 8.0 Hz, 1H), 7.77 (d, $J = 15.6$ Hz, 1H), 7.32-7.36 (m, 3H), 7.05-7.09 (m, 1H), 7.01 (d, $J = 8.0$, 1H), 6.97 (d, $J = 1.6$ Hz, 1H), 6.41 (d, $J = 16.0$ Hz, 1H), 4.07 (s, 3H), 4.01 (s, 3H), 3.82 (s, 3H).

(E)-Methyl-3-(7-methoxy-2-(3-methoxyphenyl)benzofuran-5-yl)acrylate (20d).

Yellow solid 0.22g, 57.6% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.77 (d, $J = 16.0$ Hz, 1H), 7.47 (d, $J = 7.6$ Hz, 1H), 7.41 (d, $J = 2.4$ Hz, 1H), 7.34-7.38 (m, 2H), 7.01 (s, 1H), 6.98 (d, $J = 1.2$ Hz, 1H), 6.92 (dd, $J = 1.4$ Hz, 8.2 Hz, 1H), 6.42 (d, $J = 16.0$ Hz, 1H), 4.08 (s, 3H), 3.89 (s, 3H), 3.82 (s, 3H).

(E)-Methyl 3-(7-methoxy-2-(4-methoxyphenyl)benzofuran-5-yl) acrylate (20e).

Yellow solid 0.28g, 60.7% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.82 (t, $J = 2.4$ Hz, 1H), 7.80 (t, $J = 2.4$ Hz, 1H), 7.76 (d, $J = 15.6$ Hz, 1H), 7.32 (s, 1H), 6.98 (t, $J = 2.4$ Hz, 1H), 6.96 (t, $J = 2.2$ Hz, 2H), 6.76 (s, 1H), 6.41 (d, $J = 15.6$ Hz, 1H), 4.07 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H).

(E)-Methyl-3-(2-(3,5-difluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20f).

Yellow solid 0.03g, 28.9% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.77 (d, J = 16.0 Hz, 1H), 7.36-7.39 (m, 3H), 7.05 (s, 1H), 7.01 (d, J = 1.0 Hz, 1H), 6.81 (t, J = 2.0 Hz, 8.5 Hz, 1H), 6.43 (d, J = 16.0 Hz, 1H), 4.08 (s, 3H), 3.83 (s, 3H).

(E)-Methyl 3-(2-(3,4-difluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20g).

Yellow solid 0.18g, 78.9% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.77 (d, J = 16.0 Hz, 1H), 7.66-7.71 (m, 1H), 7.59-7.62 (m, 1H), 7.35 (s, 1H), 7.21-7.27 (m, 1H), 7.00 (s, 1H), 6.97 (s, 1H), 6.43 (d, J = 16.0 Hz, 1H), 4.07 (s, 3H), 3.83 (s, 3H).

(E)-Methyl 3-(2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20h).

Yellow solid 0.19g, 75.9% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 8.03-8.09 (m, 1H), 7.77 (d, J = 16.4 Hz, 1H), 7.38 (s, 1H), 7.16 (d, J = 3.6 Hz, 1H), 6.92-7.02 (m, 3H), 6.43 (d, J = 16.0 Hz, 1H), 4.07 (s, 3H), 3.83 (s, 3H).

(E)-Methyl-3-(2-(2-fluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20i).

Brown solid (0.44 g, 81.2 % yield). ^1H NMR (CDCl_3 , 400 MHz) δ 8.08 (dt, J = 1.5 Hz J = 7.6 Hz, 1H), 7.78 (d, J = 16.0 Hz, 1H), 7.38 (s, 1H), 7.32-7.37 (m, 1H), 7.28 (s, 1H), 7.23 (d, J = 3.2 Hz, 1H), 7.21-7.16 (m, 1H), 7.0 (s, 1H), 6.43 (d, J = 16.0 Hz, 1H), 4.08 (s, 3H), 3.83 (s, 3H).

(E)-Methyl-3-(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20j).

Pale yellow solid 0.34g, 88.7% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.77 (d, J = 16.0 Hz, 1H), 7.65 (dt, J = 1.1 Hz, 7.9 Hz, 1H), 7.58 (dt, J = 2.2 Hz, 9.6 Hz, 1H), 7.41 (dt, J = 5.6 Hz, 9.6 Hz, 1H), 7.36 (d, J = 1.6 Hz, 1H), 7.08 (dt, J = 5.2 Hz, 8.8 Hz, 1H), 7.03 (s, 1H), 7.00 (d, J = 1.2 Hz, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.08 (3H, s, CH_3), 3.82 (3H, s, CH_3).

(E)-Methyl-3-(2-(4-fluorophenyl)-7-methoxybenzofuran-5-yl)acrylate (20k).

Yellow solid 0.20g, 81.6% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.86 (dd, J = 8.6 Hz J = 5.4 Hz, 2H), 7.77 (d, J = 15.6 Hz, 1H), 7.34 (s, 1H), 7.14 (t, J = 8.6 Hz, 2H), 6.98 (s, 1H), 6.95 (s, 1H), 6.42 (d, J = 15.6 Hz, 1H), 4.07 (s, 3H), 3.82 (s, 3H).

(E)-Methyl-3-(7-methoxy-2-(2-(trifluoromethyl)phenyl)benzofuran-5-yl)acrylate (20l).

Brown solid (0.31 g, 84.9 % yield). ^1H NMR (CDCl_3 , 400 MHz) δ 7.90 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.2 Hz, 1H), 7.76 (s, 1H), 7.64 (t, J = 8.0 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.40 (s, 1H), 7.26 (s, 1H), 7.03 (d, J = 6 Hz, 2H), 6.43 (d, J = 15.6 Hz, 1H), 4.07 (s, 3H), 3.83 (s, 3H).

(E)-Methyl-3-(7-methoxy-2-(3-(trifluoromethyl)phenyl)benzofuran-5-yl)acrylate (20m).

Yellow solid 0.24g, 86.3% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 8.12 (s, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.77 (d, J = 15.6 Hz, 1H), 7.59 (t, J = 7.6 Hz, 2H), 7.37 (d, J = 1.2 Hz, 1H), 7.11 (s, 1H), 7.01 (d, J = 1.2 Hz, 1H), 6.43 (d, J = 15.6 Hz, 1H), 4.08 (s, 3H), 3.83 (s, 3H).

(E)-Methyl-3-(7-methoxy-2-(4-(trifluoromethyl)phenyl)-1H-inden-5-yl)acrylate (20n).

Yellow solid 0.15g, 87.2% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.98 (d, J = 8.0 Hz, 2H), 7.77 (d, J = 16.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 2H), 7.38 (d, J = 1.6 Hz, 1H), 7.13 (s, 1H), 7.02 (d, J = 1.2 Hz, 1H), 6.43 (d, J = 15.6 Hz, 1H), 4.08 (s, 3H), 3.82 (s, 3H).

(E)-Methyl-3-(2-(4-(dimethylamino)phenyl)-7-methoxybenzofuran-5-yl)acrylate (20o).

Yellow solid 0.22g, 79.2% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.7 (t, J = 7.4 Hz, 3H), 7.30 (s, 1H), 6.93 (s, 1H), 6.79 (s, 3H), 6.40 (d, J = 16.4 Hz, 1H), 4.07 (s, 3H), 3.82 (s, 3H), 3.03 (s, 6H).

(E)-Methyl 3-(2-(4-(hydroxymethyl)phenyl)-7-methoxybenzofuran-5-yl)acrylate (20p).

Yellow solid 0.21g, 75.2% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.88 (d, J = 8.0 Hz, 1H), 7.77 (d, J = 16.0 Hz, 1H), 7.34-7.40 (m, 3H), 7.16 (t, J = 7.2 Hz, 1H); 7.07 (d, J = 8.8 Hz, 4H), 6.97 (d, J = 1.2 Hz, 1H), 6.93 (s, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.07 (s, 3H), 3.82 (s, 3H).

(E)-Methyl 3-(7-methoxy-2-(4-phenoxyphenyl)benzofuran-5-yl)acrylate (20q).

Yellow solid 0.24g, 75.2% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.84 (q, J = 2.0 Hz, 1H), 7.77 (d, J = 16.0 Hz, 1H), 7.45 (d, J = 8.4 Hz, 2H), 7.35 (s, 1H), 7.01 (s, 1H), 6.98 (s, 1H), 6.42 (d, J = 16.0 Hz, 1H), 4.76 (d, J = 10.0 Hz, 2H), 4.08 (s, 3H), 3.82 (s, 3H), 1.74 (t, J = 6.0 Hz, 1H).

General procedure of hydrogenation reaction for synthesis of 21a-q.

To a solution of acrylate (1 equiv.) in THF (10 volume) was added Pd/C (0.1 equiv., 10 wt%), followed by one or two drops of CH₃COOH and bubbled with hydrogen at room temperature for about 30 minutes under stirring. After completion of the reaction, the reaction mixture was concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography to give white solid 21a-q.

Methyl 3-(7-methoxy-2-phenylbenzofuran-5-yl)propanoate (21a).

White solid 0.13g, 93.3% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.87 (d, *J* = 7.6 Hz, 2H), 7.87 (t, *J* = 7.6 Hz, 2H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.00 (s, 1H), 6.96 (s, 1H), 6.65 (s, 1H), 4.04 (s, 3H), 3.68 (s, 3H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H).

Methyl 3-(2-(3,5-dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propanoate (21b).

Yield white solid 0.15g, 87.2% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.01 (q, *J* = 3.3 Hz, 3H), 6.94 (s, 1H), 6.65 (d, *J* = 1.2 Hz, 1H), 6.46 (t, *J* = 2.4, 1H), 4.03 (s, 3H), 3.86 (s, 6H), 3.68 (s, 3H), 3.02 (t, *J* = 8.0, 2H), 2.68 (t, *J* = 7.8, 2H).

Methyl 3-(7-methoxy-2-(2-methoxyphenyl)benzofuran-5-yl)propanoate (21c).

White solid 0.11g, 86.6% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 8.10 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.29-7.34 (m, 1H), 7.28 (s, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 2H), 6.64 (d, *J* = 1.2 Hz, 1H), 4.04 (s, 3H), 4.00 (s, 3H), 3.68 (s, 3H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H).

Methyl 3-(7-methoxy-2-(3-methoxyphenyl)benzofuran-5-yl)propanoate (21d).

Yellow solid 0.12 mg, 89.5% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.46 (d, *J* = 7.6 Hz, 2H), 7.40 (s, 1H), 7.34 (t, *J* = 7.8 Hz, 1H), 7.00 (s, 1H), 6.94 (s, 1H), 6.90 (dd, *J* = 2.2 Hz, 7.8 Hz, 1H), 6.65 (s, 1H), 4.03 (s, 3H), 3.88 (s, 3H), 3.68 (s, 3H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.68 (t, *J* = 7.6 Hz, 2H).

Methyl 3-(7-methoxy-2-(4-methoxyphenyl)benzofuran-5-yl)propanoate (21e).

Yellow solid 0.13g, 87.9% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.80 (q, *J* = 2.8 Hz, 2H), 6.95-6.98 (m, 3H), 6.81 (s, 1H), 6.62 (d, *J* = 1.2 Hz, 1H), 4.03 (s, 3H), 3.85 (s, 3H), 3.68 (s,

3H), 3.02 (t, $J = 8.0$ Hz, 2H), 2.68 (t, $J = 7.8$ Hz, 2H).

Methyl-3-(2-(3,5-difluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21f).

Yellow solid 0.11g, 89.5% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.35-7.38 (m, 2H), 7.01 (d, $J = 0.8$ Hz, 1H), 6.98 (s, 1H), 6.75-6.80 (m, 1H), 6.68 (d, $J = 1.6$ Hz, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.03 (t, $J = 7.8$ Hz, 2H), 2.68 (t, $J = 7.8$ Hz, 2H).

Methyl 3-(2-(3,4-difluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21g).

Yellow solid 0.15g, 90.5% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.64-7.70 (m, 1H), 7.57-7.59 (m, 1H), 7.20 (t, $J = 7.8$ Hz, 1H), 7.01 (d, $J = 0.8$ Hz, 1H), 7.00 (s, 1H), 6.90 (s, 1H), 6.70 (s, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.03 (t, $J = 7.8$ Hz, 2H), 2.68 (t, $J = 7.8$ Hz, 2H).

Methyl 3-(2-(2,4-difluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21h).

Yellow solid 0.26g, 95.5% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 8.02-8.08 (m, 1H), 7.10 (d, $J = 3.6$ Hz, 1H), 7.02 (s, 1H), 6.90-7.00 (m, 2H), 6.67 (s, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.03 (t, $J = 7.8$ Hz, 2H), 2.69 (t, $J = 7.8$ Hz, 2H).

Methyl-3-(2-(2-fluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21i).

White solid 0.07 g, 56.1 % yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 8.09-8.05 (m, 1H), 7.33-7.28 (m, 1H), 7.23 (d, $J = 7.2$ Hz, 1H), 7.16 (dd, $J = 8.2$ Hz, 11.4 Hz, 2H), 7.03 (s, 1H), 6.67 (s, 1H), 4.04 (s, 3H), 3.69 (s, 3H), 3.03 (t, $J = 7.8$ Hz, 2H), 2.68 (t, $J = 7.8$ Hz, 2H).

Methyl-3-(2-(3-fluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21j).

White solid 0.16g, 80.3% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.64 (d, $J = 8.0$ Hz, 1H), 7.57 (dt, $J = 2.0$ Hz, 9.9 Hz, 1H), 7.39 (dt, $J = 5.9$ Hz, 8.0 Hz, 1H), 7.04 (dd, $J = 2.2$ Hz, 8.2 Hz, 1H), 7.01 (d, $J = 0.8$ Hz, 1H), 6.97 (s, 1H), 6.67 (d, $J = 1.2$ Hz, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.03 (t, $J = 7.8$ Hz, 2H), 2.69 (t, $J = 7.8$ Hz, 2H).

Methyl-3-(2-(4-fluorophenyl)-7-methoxybenzofuran-5-yl)propanoate (21k).

Yellow solid 0.08g, 80.0% yield. $^1\text{H-NMR}$ (CDCl_3 , 400 MHz) δ 7.84 (dd, $J = 5.4$ Hz, $J = 8.6$ Hz, 2H), 7.12 (t, $J = 8.6$ Hz, 2H), 7.00 (s, 1H), 6.88 (s, 1H), 6.65 (s, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.02 (t, $J = 7.8$ Hz, 2H), 2.69 (t, $J = 7.8$ Hz, 2H).

Methyl-3-(7-methoxy-2-(2-(trifluoromethyl)phenyl)benzofuran-5-yl)propanoate (21l).

Yellow liquid 0.06 g, 58.7 % yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.89 (d, *J* = 7.6 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.62 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H), 7.05 (s, 1H), 6.97 (s, 1H), 6.70 (s, 1H), 4.03 (s, 3H), 3.69 (s, 3H), 3.04 (t, *J* = 7.8 Hz, 2H), 2.70 (t, *J* = 7.6 Hz, 2H).

Methyl-3-(7-methoxy-2-(3-(trifluoromethyl)phenyl)benzofuran-5-yl)propanoate (21m).

White solid 0.09g, 68.2% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 8.10 (s, 1H), 8.03 (d, *J* = 7.2 Hz, 1H), 7.57 (t, *J* = 5.4 Hz, 1H), 7.04 (s, 1H), 7.02 (d, *J* = 0.08 Hz, 1H), 6.68 (s, 1H), 4.04 (s, 3H), 3.69 (s, 3H), 3.03 (t, *J* = 7.8 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H).

Methyl-3-(7-methoxy-2-(4-(trifluoromethyl)phenyl)benzofuran-5-yl)propanoate (21n).

White solid 0.09g, 68.2% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.97 (d, *J* = 8.4 Hz, 2H), 7.68 (d, *J* = 8.4 Hz, 2H), 7.07 (s, 1H), 7.03 (d, *J* = 1.2 Hz, 1H), 6.69 (d, *J* = 1.6 Hz, 1H), 4.04 (s, 3H), 3.68 (s, 3H), 3.03 (t, *J* = 7.8 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H).

Methyl-3-(2-(4-(dimethylamino)phenyl)-7-methoxybenzofuran-5-yl)propanoate (21o).

White solid 0.10g, 67.6% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.74 (d, *J* = 9.2 Hz, 2H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.75 (d, *J* = 8.8 Hz, 2H), 6.72 (s, 1H), 6.59 (d, *J* = 2.0 Hz, 1H), 4.03 (s, 3H), 3.68 (s, 3H), 3.01 (t, *J* = 7.8 Hz, 2H), 3.01 (s, 6H), 2.67 (t, *J* = 7.8 Hz, 2H).

Methyl 3-(2-(4-(hydroxymethyl)phenyl)-7-methoxybenzofuran-5-yl)propanoate (21p).

White solid 0.15g, 73.6% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.87 (d, *J* = 8.4 Hz, 2H), 7.44 (d, *J* = 8.0 Hz, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 6.65 (s, 1H), 4.74 (d, *J* = 6.0 Hz, 2H), 4.04 (s, 3H), 3.68 (s, 3H), 3.03 (t, *J* = 7.8 Hz, 2H), 2.69 (t, *J* = 7.8 Hz, 2H), 1.70 (t, *J* = 6.0 Hz, 1H).

Methyl 3-(7-methoxy-2-(4-phenoxyphenyl)benzofuran-5-yl)propanoate (21q).

White solid 0.17g, 90.6% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.83 (q, *J* = 2.2 Hz, 2H), 7.35-7.39 (m, 2H), 7.12-7.16 (m, 1H), 7.04-7.07 (m, 4H), 6.99 (d, *J* = 1.2 Hz, 1H), 6.86 (s, 1H), 6.64 (d, *J* = 1.6 Hz, 2H), 4.03 (s, 3H), 3.68 (s, 3H), 3.02 (t, *J* = 7.8 Hz, 2H), 2.68 (t, *J* = 7.8 Hz, 2H).

General procedure of reduction ester for synthesis of (22a-q).

To a solution of ester (1.0 equiv.) in THF (10 volume) was added LiAlH₄ in THF (2.0 M, 1.0 equiv) dropwise at 0 °C, then the mixture was stirred at room temperature until the reaction was complete as judged by TLC. The reaction mixture was then acidified with 10% HCl and then partitioned between EtOAc and brine. The organic layer was separated, dried over anhydrous MgSO₄, filtered, and concentrated under *vacuo*. The resulting residue was purified by silica gel column chromatography to obtain white solid **22a-q**.

3-(7-Methoxy-2-phenylbenzofuran-5-yl)propan-1-ol (22a).

White solid 0.06g, 78.5% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.89 (d, *J* = 0.8 Hz, 1H), 7.87 (s, 1H), 7.43 (t, *J* = 7.6 Hz, 2H), 7.35 (d, *J* = 7.6 Hz, 1H), 7.01 (s, 1H), 6.96 (s, 1H), 6.66 (s, 1H), 4.04 (s, 3H), 3.72 (q, *J* = 9.2 Hz, 2H), 2.79 (t, *J* = 7.6 Hz, 2H), 1.92-1.99 (m, 2H), 1.29 (t, *J* = 5.2 Hz, 1H); ¹³C-NMR (CDCl₃, 100 MHz) δ 156.2, 144.9, 142.7, 137.5, 130.9, 130.4, 128.7, 128.5, 125.0, 112.5, 107.5, 101.5, 62.3, 56.2, 56.1, 34.7, 32.4; HRMS [M + H]⁺ calcd [C₁₈H₁₉O₃], 283.1334; found, 283.1336; Purity 99.97% (as determined by RP-HPLC, method A, *t*_R = 16.60 min).

3-(2-(3,5-Dimethoxyphenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22b).

White solid 0.04g, 90.1% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 7.02 (d, *J* = 2.0 Hz, 1H), 7.00 (d, *J* = 1.2 Hz, 1H), 6.94 (s, 1H), 6.66 (d, *J* = 0.8 Hz, 1H), 6.46 (t, *J* = 6.4 Hz, 1H), 4.04 (s, 3H), 3.87 (s, 6H), 3.72 (q, *J* = 5.7 Hz, 2H), 2.79 (t, *J* = 7.8 Hz, 2H), 1.91-1.98 (m, 2H), 1.31 (t, *J* = 5.0 Hz, 1H); ¹³C-NMR (CDCl₃, 100 MHz) δ 161.0, 156.0, 144.9, 142.7, 132.1, 130.8, 112.5, 107.7, 103.1, 102.0, 101.0, 62.3, 56.1, 55.5, 34.6, 32.4; HRMS [M + H]⁺ calcd [C₂₀H₂₂O₅], 343.1545; found, 343.1548; Purity 99.98% (as determined by RP-HPLC, method A, *t*_R = 17.02 min).

3-(7-Methoxy-2-(2-methoxyphenyl)benzofuran-5-yl)propan-1-ol (22c).

White solid 0.07g, 85% yield. ¹H-NMR (CDCl₃, 400 MHz) δ 8.11 (dd, *J* = 1.6 Hz, 8.0 Hz, 1H), 7.29-7.31 (m, 1H), 7.28 (s, 1H), 7.06 (t, *J* = 7.6 Hz, 1H), 7.01 (d, *J* = 0.8, 1H), 7.00 (d, *J* = 8.4, 1H), 6.64 (s, 1H), 4.04 (s, 3H), 3.99 (s, 3H), 3.71 (t, *J* = 6.4 Hz, 2H), 2.78 (t, *J* = 7.6 Hz, 2H), 1.91-1.97 (m, 2H), 0.885 (t, *J* = 5.0 Hz, 1H); ¹³C-NMR (CDCl₃, 100 MHz) δ 156.3,

152.4, 144.7, 141.7, 137.1, 131.5, 129.1, 127.2, 120.7, 119.2, 112.6, 110.9, 107.5, 106.4, 62.3, 56.1, 55.4, 34.7, 32.4; HRMS $[M + H]^+$ calcd $[C_{19}H_{20}O_4]$, 313.1440; found, 313.1437; Purity 99.99% (as determined by RP-HPLC, method A, t_R = 17.32 min).

3-(7-Methoxy-2-(3-methoxyphenyl)benzofuran-5-yl)propan-1-ol (22d).

White solid 0.07g, 75.8% yield. ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.47 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 2.4 Hz, 1H), 7.34 (t, J = 8.0 Hz, 1H), 7.00 (s, 1H), 6.95 (s, 1H), 6.90 (dd, J = 1.8 Hz, 7.8 Hz, 1H), 6.66 (s, 1H), 4.04 (s, 3H), 3.89 (s, 3H), 3.72 (q, J = 6.0 Hz, 2H), 2.79 (t, J = 8.0 Hz, 2H), 1.93-1.99 (m, 2H), 1.27 (t, J = 5.4 Hz, 1H); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ 159.8, 156.0, 144.9, 142.7, 137.5, 131.6, 130.8, 129.7, 117.6, 114.4, 112.5, 110.2, 107.7, 101.8, 62.3, 56.1, 55.4, 34.6, 32.4; HRMS $[M + H]^+$ calcd $[C_{19}H_{21}O_4]$, 313.1440; found, 313.1439; Purity 99.99% (as determined by RP-HPLC, method A, t_R = 17.10 min).

3-(7-Methoxy-2-(4-methoxyphenyl)benzofuran-5-yl)propan-1-ol(22e).

White solid 0.08g, 73.8% yield. ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.80 (t, J = 5.6 Hz, 2H), 6.99 (d, J = 6.0 Hz, 2H), 6.95 (s, 1H), 6.63 (s, 1H), 4.04 (s, 3H), 3.85 (s, 3H), 3.72 (q, J = 6.0 Hz, 2H), 2.78 (t, J = 7.6 Hz, 2H), 1.91-1.98 (m, 2H), 1.26 (t, J = 6.0 Hz, 1H); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ 159.9, 156.4, 144.7, 142.4, 137.4, 131.2, 126.4, 123.2, 114.1, 112.2, 107.2, 99.8, 62.3, 56.1, 55.3, 34.7, 32.4; HRMS $[M + H]^+$ calcd $[C_{19}H_{20}O_4]$, 313.1440; found, 313.1433; Purity 99.99% (as determined by RP-HPLC, method A, t_R = 16.84 min).

3-[2-(3,5-Difluorophenyl)-7-methoxybenzofuran-5-yl]propan-1-ol (22f).

White solid 0.05g, 28.5% yield. ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.35-7.40 (m, 1H), 7.02 (s, 1H), 6.98 (s, 1H), 6.74-6.80 (m, 1H), 6.69 (s, 1H), 4.06 (s, 3H), 3.72 (q, J = 6.0 Hz, 2H), 2.79 (t, J = 7.8 Hz, 2H), 1.87-2.04 (m, 2H), 1.26 (t, J = 5.0 Hz, 1H); ^{13}C -NMR ($CDCl_3$, 100 MHz) δ 164.8, 162.2, 145.0, 142.9, 138.0, 133.4, 130.3, 112.7, 108.3, 107.8, 103.5, 62.2, 56.1, 34.6, 32.4; HRMS $[M + H]^+$ calcd $[C_{18}H_{16}O_3F_2]$, 319.1146; found, 319.1146; Purity 99.98% (as determined by RP-HPLC, method A, t_R = 19.00 min).

3-(2-(3,4-Difluorophenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22g).

White solid 0.08g, 75.9% yield. ^1H -NMR ($CDCl_3$, 400 MHz) δ 7.65-7.70 (m, 1H), 7.57-7.60 (m, 1H), 7.22 (q, J = 8.4 Hz, 1H), 7.00 (s, 1H), 6.90 (s, 1H), 6.67 (s, 1H), 4.03 (s, 3H), 3.72

(q, J = 6.0 Hz, 2H), 2.79 (t, J = 7.8 Hz, 2H), 1.91-1.98 (m, 2H), 1.30 (t, J = 5.2 Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 154.1, 154.0, 151.9, 151.8, 151.7, 151.6, 149.4, 149.3, 149.2, 149.0, 144.9, 142.9, 137.9, 130.6, 127.6, 127.6, 127.6, 127.5, 121.2, 121.2, 121.1, 121.1, 114.1, 114.0, 112.6, 107.9, 102.2, 62.3, 56.1, 34.6, 32.4 ; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{18}\text{H}_{17}\text{O}_3\text{F}_2]$, 319.1146; found, 319.1159; Purity 99.99% (as determined by RP-HPLC, method A, t_{R} = 18.34 min).

3-(2-(2,4-Difluorophenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22h).

White solid 0.07g, 75.4% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 8.02-8.08 (m, 1H), 7.10 (d, J = 4.0 Hz, 1H), 7.03 (s, 1H), 6.90-7.01 (m, 2H), 6.68 (s, 1H), 4.04 (s, 3H), 3.72 (q, J = 5.2 Hz, 2H), 2.79 (t, J = 7.6 Hz, 2H), 1.92-1.99 (m, 2H), 1.29 (t, J = 5.2 Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 144.9, 142.1, 137.8, 130.9, 128.3, 128.2, 128.1, 112.8, 111.8, 111.6, 107.7, 106.1, 106.0, 104.8, 104.6, 104.3, 62.3, 56.1, 34.8, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{18}\text{H}_{16}\text{O}_3\text{F}_2]$, 319.1146; found, 319.1162; Purity 99.98% (as determined by RP-HPLC, method A, t_{R} = 18.54 min).

3-(2-(2-Fluorophenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22i).

Yellow solid (0.036 g, 80 % yield). ^1H NMR (CDCl_3 , 400 MHz) δ = 8.09-8.05 (m, 1H), 7.34-7.28 (m, 1H), 7.23 (dd, J = 1.2 Hz, 7.6 Hz, 1H), 7.17 (dd, J = 8.2 Hz, 11.4 Hz, 2H), 7.04 (s, 1H), 6.68 (d, J = 0.8 Hz, 1H), 4.05 (s, 3H), 3.72 (t, J = 5.2 Hz, 2H), 2.80 (t, J = 7.8 Hz, 2H), 1.99-1.92 (m, 2H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 160.7, 158.2, 150.1, 150.0, 144.8, 142.1, 137.6, 131.0, 129.5, 129.4, 124.4, 124.3, 118.7, 118.6, 116.1, 115.8, 112.8, 107.9, 106.8, 106.7, 62.3, 56.1, 34.7, 32.4, HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{18}\text{H}_{17}\text{O}_3\text{F}]$, 301.1240; found, 301.1245; Purity 99.99% (as determined by RP-HPLC, method A, t_{R} = 17.70 min).

3-(2-(3-Fluorophenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22j).

White solid 115mg , 95.8% yield . ^1H -NMR (CDCl_3 , 400 MHz) δ 7.64 (d, J = 8.0 Hz, 1H) 7.57 (d, J = 9.6 Hz, 1H), 7.39, (q, J = 7.3 Hz, 1H), 7.01-7.05 (m, 2H), 6.97 (s, 1H), 4.04 (s, 3H), 3.72 (q, J = 6.0 Hz, 2H), 2.79 (t, J = 7.8 Hz, 2H), 1.92-1.99 (m, 2H), 1.27 (q, J = 5.2 Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 164.3, 161.8, 154.8, 144.9, 142.8, 137.7, 132.5, 130.5, 120.6, 115.2, 112.6, 111.9, 107.9, 102.5, 62.2, 56.1, 34.6, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{18}\text{H}_{20}\text{O}_3\text{F}]$, 301.1240; found, 301.1241; Purity 99.99% (as determined by RP-HPLC,

method A, t_R = 17.77 min).

3-(2-(4-Fluorophenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22k).

Yellow solid 0.05g, 69.4% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.82-7.86 (m, 2H) 7.12 (t, J = 8.8 Hz, 2H), 7.00 (s, 1H), 6.88 (s, 1H), 6.65 (s, 1H), 4.04 (s, 3H), 3.71 (t, J = 6.4 Hz, 2H), 2.79 (t, J = 7.8 Hz, 2H), 1.91-1.98 (m, 2H), 1.27(d, J = 8.8 Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 164.1, 161.6, 155.4, 144.9, 137.7, 130.9, 126.9, 126.8, 126.7, 126.7, 115.9, 115.7, 112.5, 107.6, 101.2, 62.3, 56.1, 34.7, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{18}\text{H}_{17}\text{O}_3\text{F}]$, 301.1240; found, 301.1248; Purity 99.99% (as determined by RP-HPLC, method A, t_R = 17.52 min).

3-(7-Methoxy-2-(2-(trifluoromethyl)phenyl)benzofuran-5-yl)propan-1-ol (22l).

Transparent liquid (0.04 g, 67.2 % yield). ^1H NMR (CDCl_3 , 400 MHz) δ = 7.90 (d, J = 7.6 Hz, 1H), 7.79 (d, J = 8.0 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.50 (t, J = 7.8 Hz, 1H), 7.05 (s, 1H), 6.98 (s, 1H), 6.70 (d, J = 0.8 Hz, 1H), 4.04 (s, 3H), 3.73 (t, J = 6.4 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 2.00-1.93 (m, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 152.6, 144.9, 143.0, 137.7, 131.7, 131.1, 130.5, 129.5, 129.4, 127.7, 128.1, 128.0, 127.8, 127.4, 127.1, 126.7, 126.6, 126.5, 126.5, 125.2, 122.5, 62.2, 56.3, 34.7, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{19}\text{H}_{17}\text{O}_3\text{F}_3]$, 351.1208; found, 351.1209; Purity 99.98% (as determined by RP-HPLC, method A, t_R = 18.42 min).

3-(7-Methoxy-2-(3-(trifluoromethyl)phenyl)benzofuran-5-yl)propan-1-ol (22m).

White solid 0.05g, 79.4% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 8.10 (s, 1H) 8.03 (d, J = 7.2 Hz, 1H), 7.52-7.59 (m, 2H), 7.04 (s, 1H), 7.02 (d, J = 1.2 Hz, 1H), 6.69 (d, J = 1.2 Hz, 1H), 4.05 (s, 3H), 3.72 (d, J = 2.4 Hz, 2H), 2.80 (t, J = 7.6 Hz, 2H), 1.92-1.99 (m, 2H), 1.28(d, J = 29.2 Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 154.5, 145.0, 142.9, 137.9, 131.1, 130.5, 129.2, 1273.9, 124.9, 121.6, 112.6, 108.0, 102.8, 62.2, 56.1, 34.6, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{19}\text{H}_{17}\text{O}_3\text{F}_3]$, 351.1208; found, 351.1214; Purity 99.97% (as determined by RP-HPLC, method A, t_R = 15.93 min).

3-(7-methoxy-2-(4-(trifluoromethyl)phenyl)benzofuran-5-yl)propan-1-ol (22n).

White solid 0.50g, 79.4% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.97 (d, J = 8.8 Hz, 2H) 7.67

(d, $J = 8.4$ Hz, 2H), 7.06 (s, 1H), 7.03 (d, $J = 0.8$ Hz, 1H), 6.69 (d, $J = 1.2$ Hz, 1H), 4.04 (s, 3H), 3.72 (t, $J = 6.2$ Hz, 2H), 2.80 (t, $J = 7.6$ Hz, 2H), 1.92-1.99 (m, 2H), 1.35 (s, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 154.5, 145.0, 143.1, 137.9, 133.6, 130.5, 125.7, 125.0, 122.7, 112.7, 108.1, 103.4, 62.2, 56.1, 34.6, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{19}\text{H}_{17}\text{F}_3\text{O}_3]$, 351.1208; found, 351.1220; Purity 99.99% (as determined by RP-HPLC, method A, $t_R = 20.44$ min).

3-(2-(4-(dimethylamino)phenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22o).

White solid 0.06g, 78.6% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.74 (q, $J = 3.2$ Hz, 2H), 6.95 (d, $J = 1.2$ Hz, 1H), 6.75 (d, $J = 8.8$ Hz, 2H), 6.72 (s, 1H), 6.59 (d, $J = 1.6$ Hz, 1H), 4.04 (s, 3H), 3.71 (q, $J = 6.0$ Hz, 2H), 3.01 (s, 6H), 2.77 (t, $J = 7.6$ Hz, 2H), 1.91-1.98 (m, 2H), 1.25 (t, $J = 5.4$ Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 157.4, 150.5, 144.6, 142.1, 137.1, 131.5, 126.2, 118.5, 112.0, 106.7, 98.2, 62.3, 56.1, 40.3, 34.7, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{20}\text{H}_{23}\text{NO}_3]$, 326.1756; found, 326.1753; Purity 99.97% (as determined by RP-HPLC, method A, $t_R = 10.64$ min).

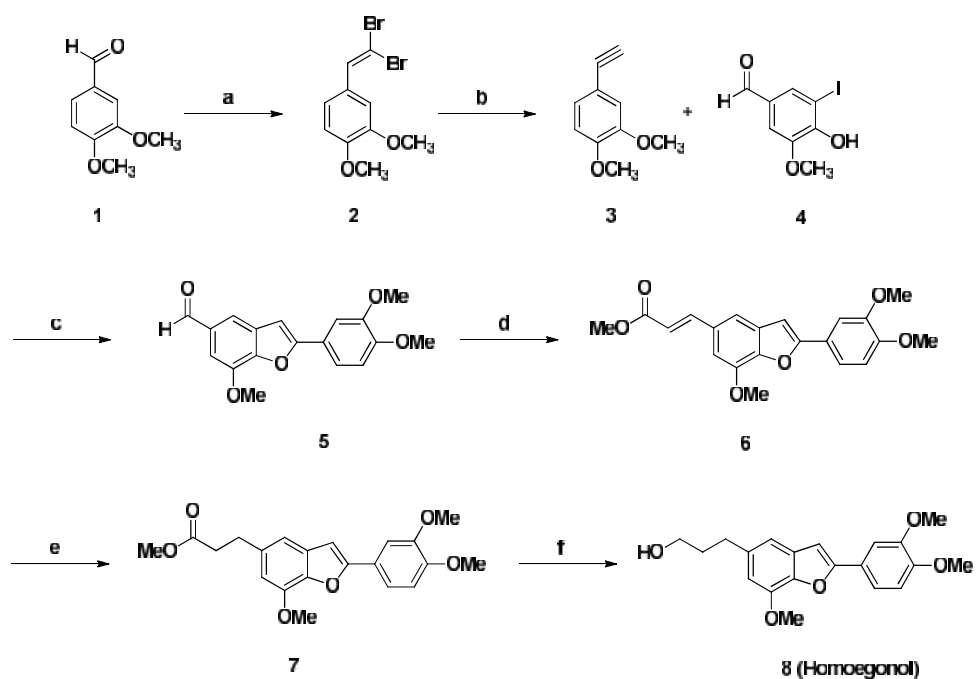
3-(2-(4-(Hydroxymethyl)phenyl)-7-methoxybenzofuran-5-yl)propan-1-ol (22p).

White solid 0.05g, 64.6% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.87 (d, $J = 8.4$ Hz, 2H), 7.44 (d, $J = 8.0$ Hz, 2H), 7.00 (s, 1H), 6.95 (s, 1H), 6.66 (s, 1H), 4.74 (d, $J = 6.0$ Hz, 1H), 4.04 (s, 3H), 3.72 (q, $J = 6.2$ Hz, 2H), 2.79 (t, $J = 7.8$ Hz, 2H), 1.93-1.99 (m, 2H), 1.07 (t, $J = 5.8$ Hz, 1H), 1.30 (d, $J = 1.2$ Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 156.0, 144.9, 142.7, 141.2, 137.6, 130.9, 129.8, 127.3, 125.2, 112.5, 107.6, 101.5, 65.1, 62.3, 56.2, 34.7, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{19}\text{H}_{21}\text{O}_4]$, 313.1440; found, 313.1443; Purity 99.97% (as determined by RP-HPLC, method A, $t_R = 10.92$ min).

3-(7-Methoxy-2-(4-phenoxyphenyl)benzofuran-5-yl)propan-1-ol (22q)

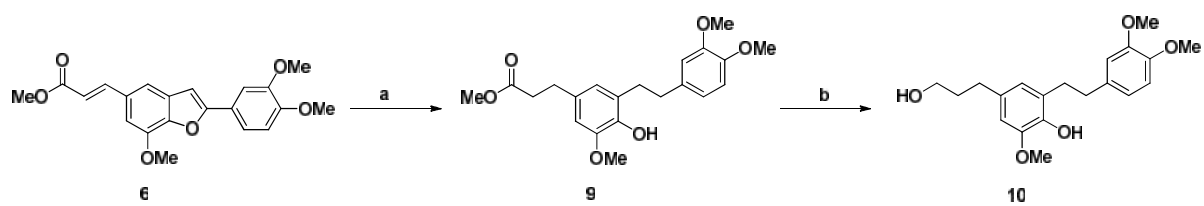
White solid 0.07g, 90.5% yield. ^1H -NMR (CDCl_3 , 400 MHz) δ 7.81-7.85 (m, 2H), 7.35-7.39 (m, 2H), 7.12-7.16 (m, 1H), 7.03-7.07 (m, 4H), 7.00 (s, 2H), 6.87 (s, 1H), 6.65 (d, $J = 2.0$ Hz, 1H), 4.04 (s, 3H), 3.72 (q, $J = 6.2$ Hz, 2H), 2.79 (t, $J = 7.8$ Hz, 2H), 1.92-1.99 (m, 2H), 1.27 (t, $J = 5.4$ Hz, 1H); ^{13}C -NMR (CDCl_3 , 100 MHz) δ 157.7, 156.7, 155.9, 144.9, 142.6, 137.6, 131.1, 129.9, 126.6, 125.5, 123.7, 119.2, 118.8, 112.4, 107.5, 100.7, 62.3, 56.2, 34.7, 32.4; HRMS $[\text{M} + \text{H}]^+$ calcd $[\text{C}_{24}\text{H}_{23}\text{O}_4]$, 375.1596; found, 375.1594; Purity 99.98% (as determined by RP-HPLC, method A, $t_R = 22.00$ min).

Scheme S1



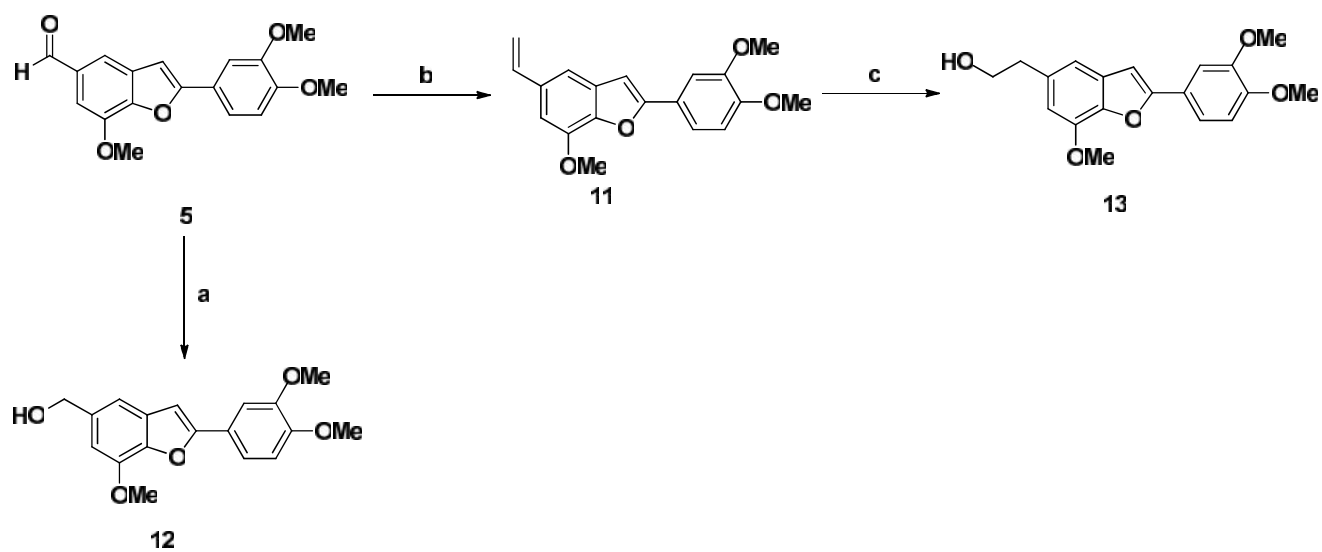
^a**Reagents and conditions:** a) CBr₄, PPh₃, DCM; b) LDA, THF; c) 10% Pd/C, PPh₃, CuI, Et₃N, H₂O; d) Methyl (triphenylphosphoranylidene)acetate, DCM; e) 10% Pd/C, H₂, CH₃COOH, THF; f) LiAlH₄, THF.

Scheme S2



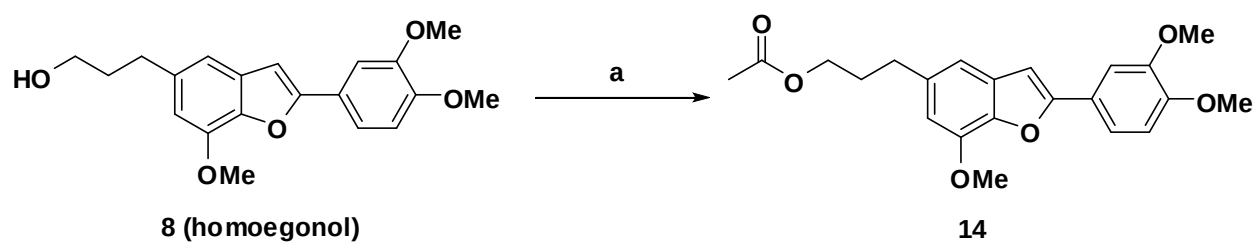
^a**Reagents and conditions:** a) 10% Pd/C, H₂, THF; b) LiAlH₄, THF.

Scheme S3



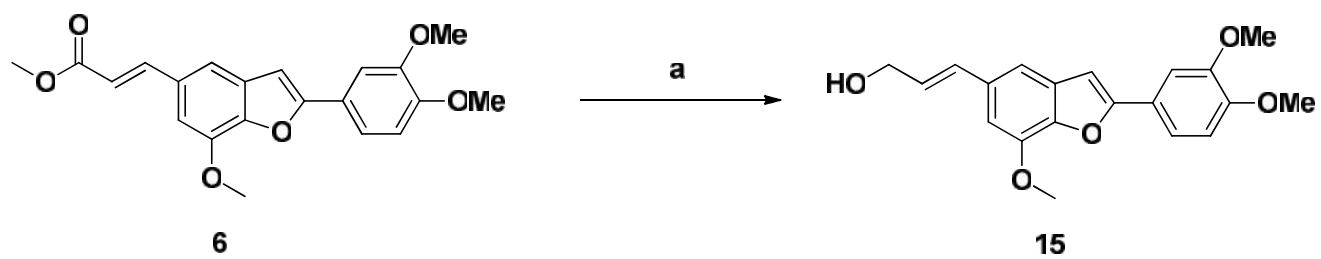
^aReagents and conditions: a) NaBH₄, THF; b) PPh₃CH₃I, NaH, DMF; c) BH₃, THF, H₂O₂, NaOH.

Scheme S4



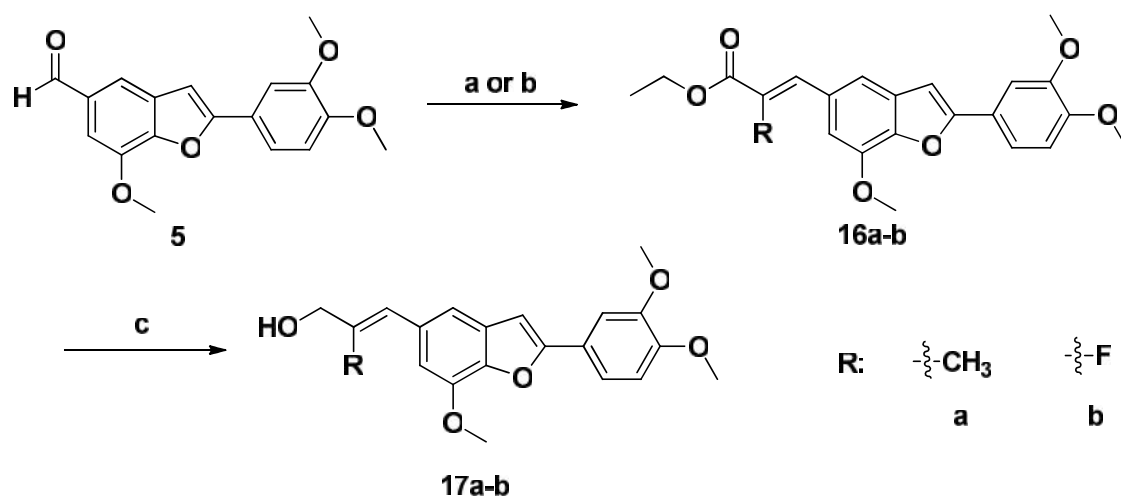
^aReagents and conditions: a) Acetyl chloride, TEA, DCM

Scheme S5



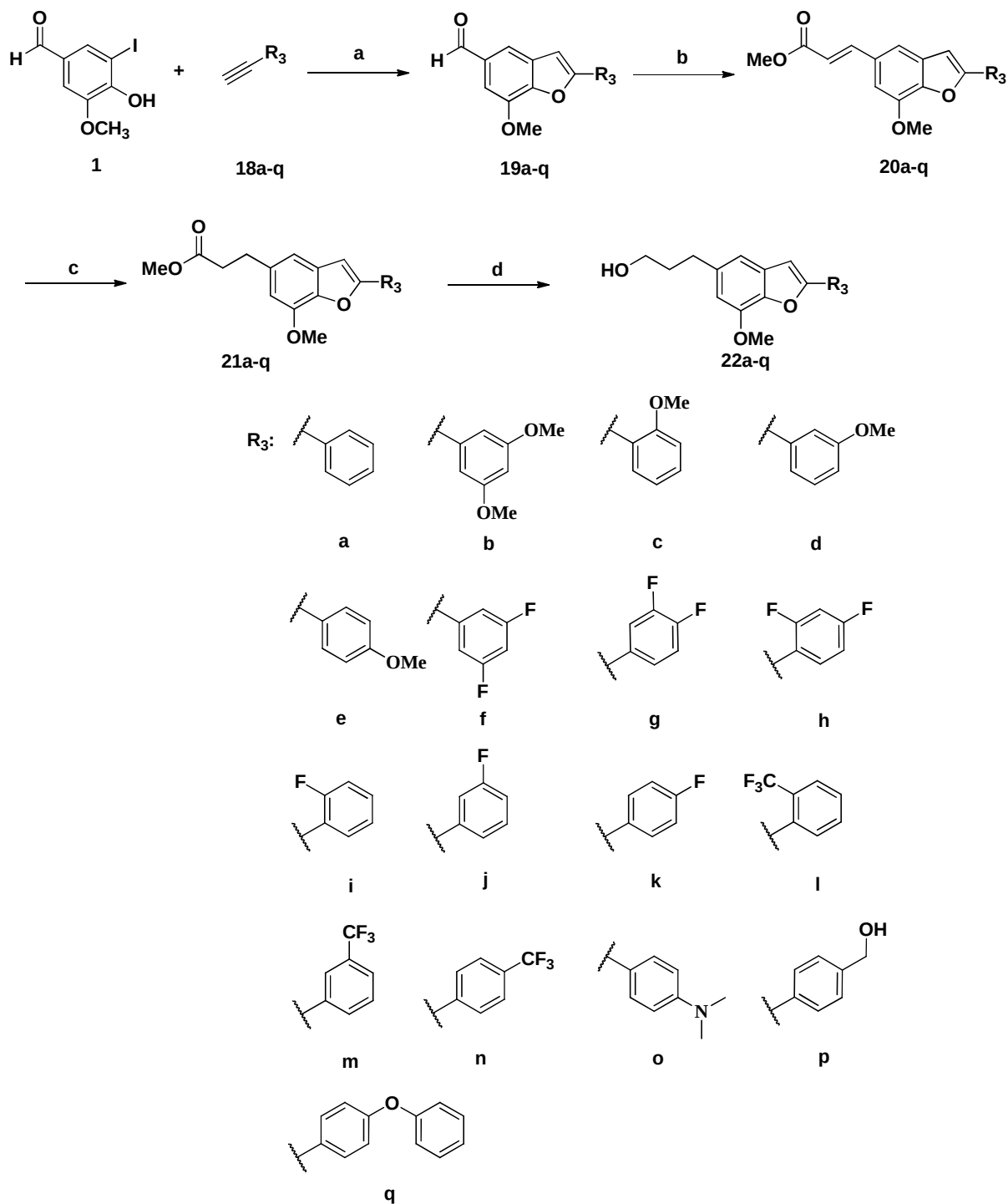
^aReagents and conditions: a) DIBAL-H, THF.

Scheme S6



^a**Reagents and conditions:** a) (Carbethoxymethylene)triphenylphosphorane, DCM; b) Triethyl 2-fluoro-2-phosphonoacetate, DBU, LiCl, THF; c) DICAL-H, THF.

Scheme S7



^a**Reagents and conditions:** a) 10% Pd/C, PPh₃, CuI, Et₃N, H₂O; b) Methyl (triphenylphosphoranylidene)acetate, CH₂Cl₂; c) 10% Pd/C, H₂, CH₃COOH, THF; d) LiAlH₄, THF.

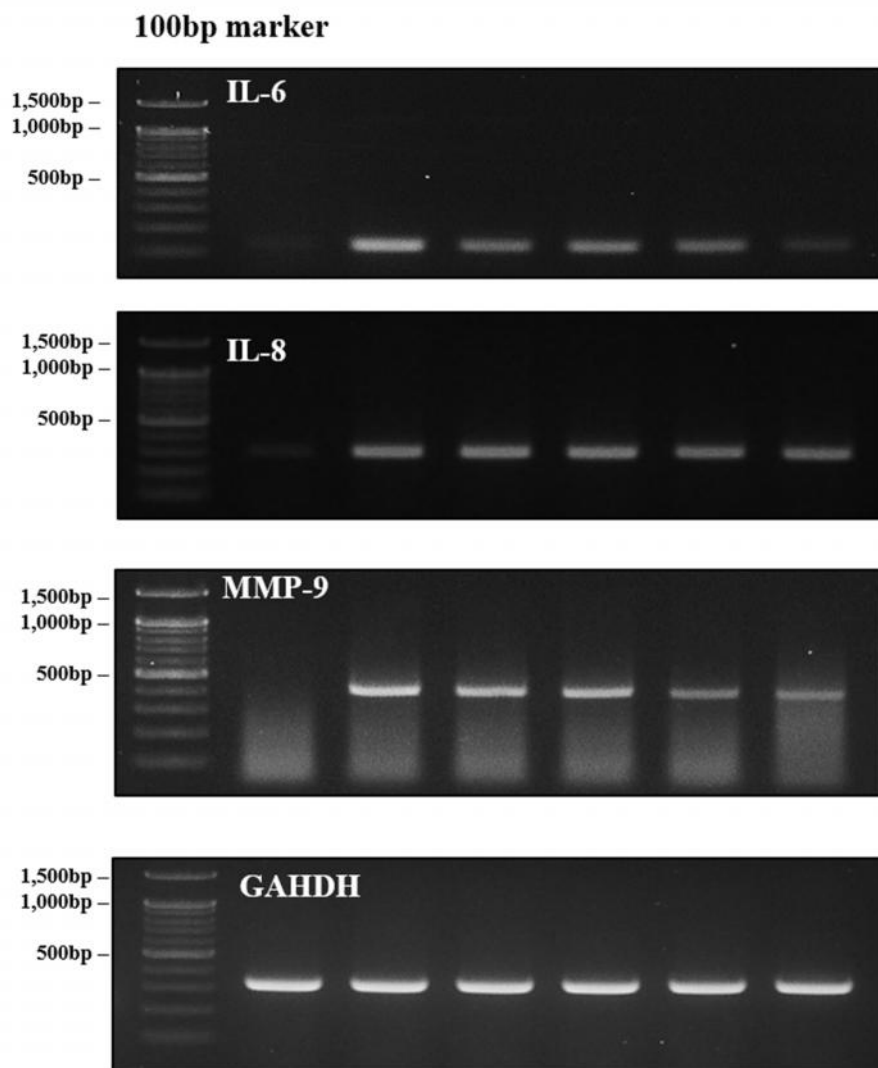


Fig S1. Original PCR for image used in Fig 1e.

Each PCR product was a clear band and detected at the expected size.

For detection of PCR product and GAPDH, relevant portions of the gel were scanned using the imaging system.

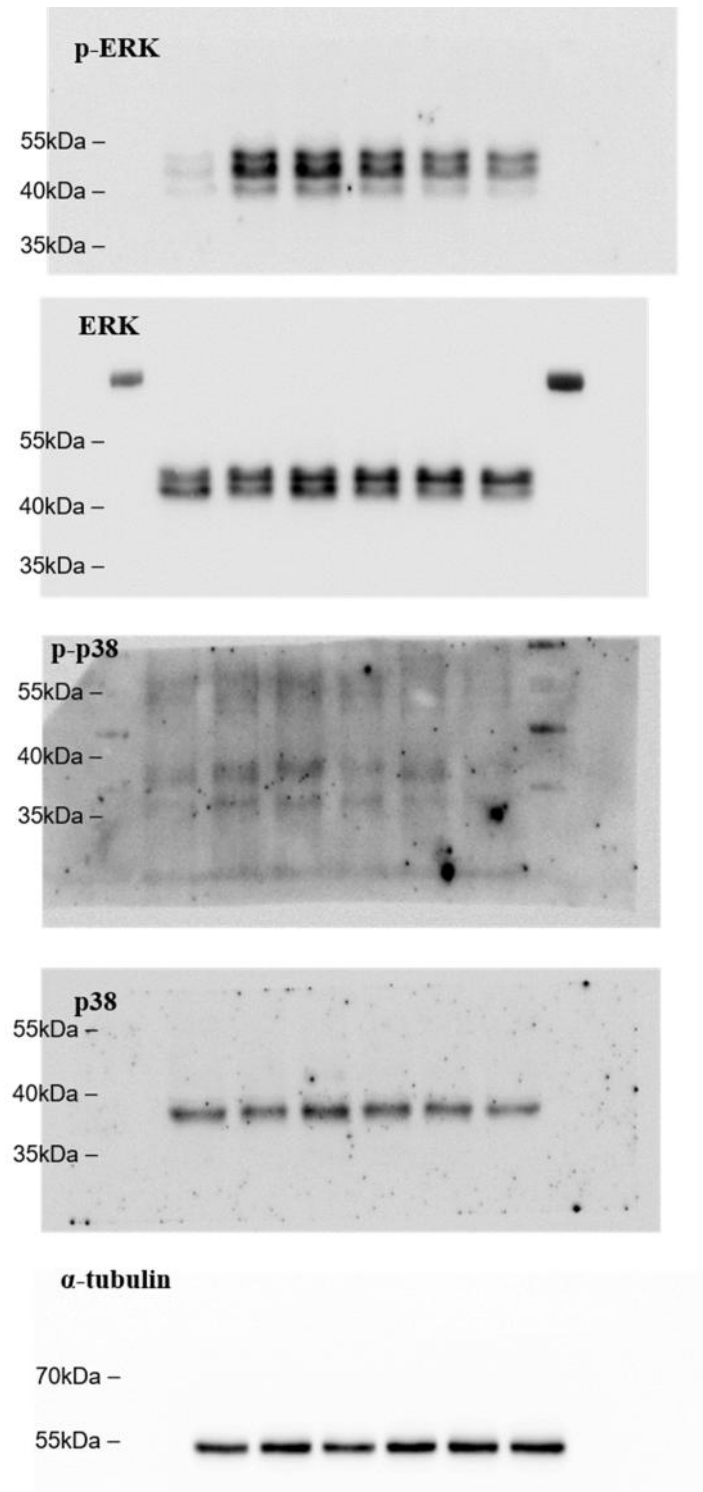


Fig S2. Original western blots for image used in Fig 2a.

Each antibody produced a clear band at the expected size.

For detection of phosphor and total proteins, the imaging system was used and relevant portions of the blot were scanned

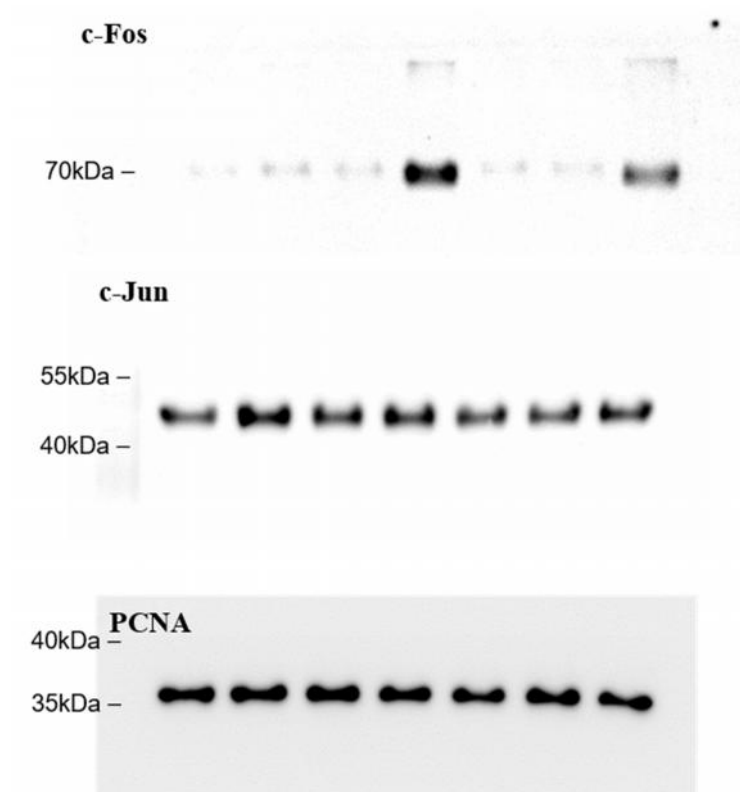


Fig S3. Original western blots for image used in Fig 2c.

Nuclear extracts were isolated from the cells using a Pierce kit and proteins analyzed by western blot. Each antibody produced a clear band at the expected size.

For detection of proteins and PCNA, the imaging system was used and relevant portions of the blot were scanned

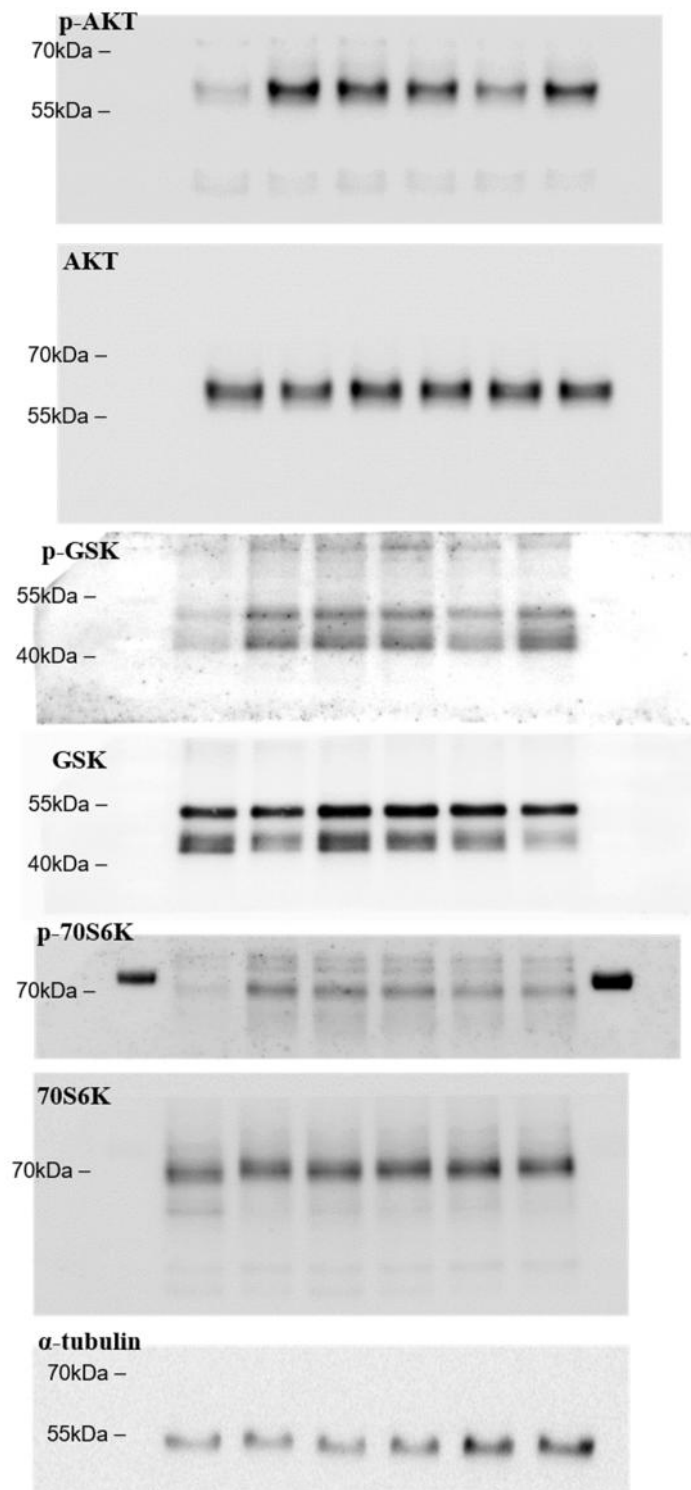


Fig S4. Original western blots for image used in Fig 3a.

Each antibody produced a clear band at the expected size.

For detection of phospho and total proteins, standard, the imaging system was used and relevant portions of the blot were scanned