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# Synthesis of Thio-lignan Analogues, Bioequivalent Salvinal without Unfavored Aldehyde

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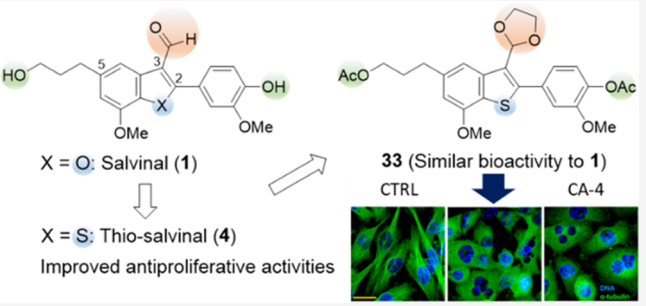
**ABSTRACT:** The oxygen in the benzofuran (BF) of three antiproliferative natural neolignans, salvinal (**1**), obovaten (**2**), and 2-[7-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl]ethanol (**3**), was replaced with sulfur to form the new biological scaffold benzothiophene (BT) thio-lignans **4**−**6**. Compounds **1**−**6** and **18** synthesized derivatives were evaluated for antiproliferative activity against five human cancer cell lines, including a multidrug-resistant cell line. Thio-salvinal (**4**) displayed significant antiproliferative effects with half-maximal inhibitory concentration ($IC_{50}$) values of 0.57−0.95 $\mu$M against all tested cell lines, except for the HER2 negative breast cancer cell line MCF-7. This thio-lignan was 6.5−9.4 times more potent than parent **1**. However, the related thio-lignans, **5** and **6**, showed much weaker antiproliferative effects than **4** and were less potent than the parent natural benzofuran lignans **2** and **3**. Newly synthesized thio-lignan **33** affected cell cycle progression at 24 and 48 h in the G2/M transition and S phase, respectively, as well as promoted sub-G1 induction by stimulating microtubule depolymerization and nuclear fragmentation. Since a highly reactive aldehyde in salvinal is generally not appropriate for drug development, we have successfully found nonaldehyde derivative **33** showing biological activity similar to salvinal by replacing BF with BT and an aldehyde with 1,3-dioxolane.




## INTRODUCTION

Lignans, which are biosynthesized by the oxidative coupling of two phenylpropanoids formed through the shikimate pathway, are distributed abundantly in plants. Various skeletons, including diphenylfurofuran, dibenzocyclooctadiene, arylnaphthalene, alkyl aryl ether, benzodioxane, and benzofuran (BF), can be formed depending on the coupling pattern. Salvinal (**1**) isolated from *Salvia miltiorrhiza* Burge (Danshen),[1] ovovaten (**2**) isolated from *Persea obovatifolia*,[2] and 2-[7-methoxy-2-(4-methoxyphenyl)-3-methylbenzofuran-5-yl]ethanol (**3**) isolated from *Lavandula angustifolia*[3] are bioactive lignans containing BF, a bicyclic heteroaromatic unit with a 10$\pi$-electron system. The above three BF lignans show significant antiproliferative activity against several human tumor cell lines (HTCLs) with half-maximal inhibitory concentration ($IC_{50}$) values of 5.0−10.0,[4] 0.7−2.2,[2] and 2.2−5.5[3] $\mu$M, respectively. The mechanism of action of antiproliferative salvinal was well-investigated;[4,5] however, an aldehyde in the structure is not a drug-like functional group because it binds randomly with nucleophiles in biomolecules such as proteins and nucleotides[6] and is likely metabolized by metabolic enzymes in vivo, such as aldehyde dehydrogenase, aldehyde oxidase, and P450.

Like BF, benzothiophene (BT) is a bicyclic 10$\pi$-heteroaromatic compound. The structures differ only by the heteroatom in the five-membered ring, sulfur in BT and oxygen in BF, which could affect the biological profile of compounds containing these privileged skeletons due to the differences in electronegativities and atomic/ionic radii. Abundant BT-based drug discovery has been conducted in diverse fields of bioactivity, such as antitumor, antimicrobial, anti-human immunodeficiency virus (anti-HIV), antitubercular, anti-inflammatory, antioxidant, antidiabetic, anticonvulsant, and others.[7] Raloxifene, a clinically used selective estrogen receptor modulator contains BT as a core skeleton, and several BT compounds, such as sertaconazole, mobam, zileuton, and benocyclidine, are marketed.[7] We also found that TEDB-TB, a BT analogue of triethyldesmosdumotin, dramatically increased the antiproliferative activity against human tumor cell lines ($IC_{50}$ 0.06−0.16 $\mu$M) acting to inhibit tubulin assembly.[8] Thus, BT now attracts special attention in the field of medicinal chemistry.

Our recent unpublished study showed that the replacement of BF by BT on a specific flavonoid skeleton clearly increased the antiproliferative activity, which prompted us to further

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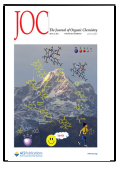







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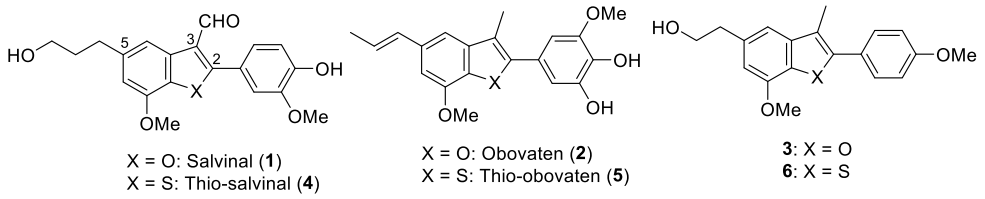
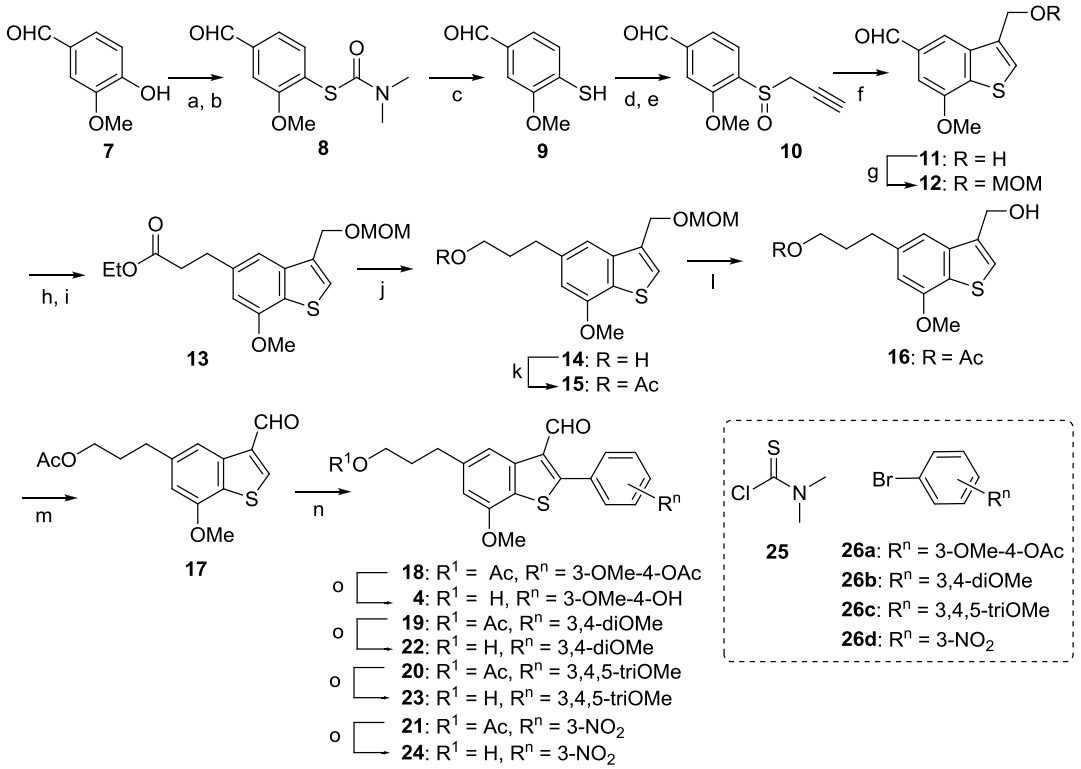



**Figure 1.** Cytotoxic natural benzofuran lignans **1**−**3** and their thio-derivatives **4**−**6**.

**Scheme 1. Preparation of Thio-salvinal (4) and Its Derivatives**[a]




[a]Reagents and conditions: (a) **25**, 1.0 M aq KOH, tetrahydrofuran (THF), 0 °C, 3 h, 86%; (b) 240 °C, 30 min, 77% or PhOPh, microwave, 220 °C, 15 min, 97%; (c) 5 M aq KOH, MeOH, reflux, 1 h, 99%; (d) propargyl bromide, tetrabutylammonium bromide (TBAB), 2 M aq NaOH, PhH, 0 °C, 1 h, 97%; (e) $H_2O_2$, $HCO_2H$, 0 °C, to room temperature (rt), 3 h, 68%; (f) dioxane, reflux, 1 h, then *p*-TsOH, $H_2O$, reflux, 2 h, 74%; (g) *N*,*N*-diisopropylethylamine (DIPEA), MOMCl, $CH_2Cl_2$, 0−40 °C, 1.5 h, 75%; (h) $Ph_3P$ = $CHCO_2Et$, $K_2CO_3$, $CH_2Cl_2$, reflux, 3.5 h, 96%; (i) Pd/C, $H_2$, EtOAc, rt, 8 h, 89%; (j) $LiBH_4$, THF, 60 °C, 3 h, 97%; (k) $Ac_2O$, 4-dimethylaminopyridine (DMAP), Py, $CH_2Cl_2$, rt, 3 h, 100%; (l) 1.0 M HCl in diethyl ether, isopropanol, 55 °C, $Ac_2O$, 4 h, 68%; (m) 2-iodoxybenzoic acid (IBX), dimethyl sulfoxide (DMSO), rt, 2 h, 100%; (n) (except for **21**) **26a**−**c**, $Pd(OAc)_2$, $PCy_3$, PivOH, $K_2CO_3$, toluene, reflux, 13 h, 65% for **18**, 56% for **19**, 85% for **20**; (n) (for **21**) **26d**, $Pd(OAc)_2$, KOAc, dimethylacetamide (DMA), 150 °C, 8 h, 33%; and (o) $K_2CO_3$, MeOH, rt, 1 h, 95% for **4**, 99% for **22**, 78% for **23**, 100% for **24**.

investigate BT to BF conversions. We report here the synthesis, antiproliferative activity, and structure−activity relationship (SAR) correlations of thio-lignans **4**−**6**, in which the oxygen in the BF lignans **1**−**3** was replaced by sulfur, as well as their derivatives (Figure 1). Furthermore, we finally succeeded in synthesizing an analogue with bioactivity similar to salvinal without an aldehyde group by replacement of BF to BT as well as aldehyde to dioxolane.

## RESULTS AND DISCUSSION

**Synthesis of Thio-lignans.** Thio-salvinal **4** was prepared from vanillin (**7**) as shown in Scheme 1. The treatment of vanillin (**7**) with dimethylcarbamothioic chloride, followed by Newman−Kwart rearrangement of the resulting *O*-thiocarbamate to an *S*-thiocarbamate under microwave conditions, and hydrolysis gave thiophenol **9**.[9] Propargylation, oxidation, and cyclization under an acidic condition produced 3-hydroxymethyl-BT **11**,[10] which was converted to the methoxymethyl (MOM) ether **12**. The side chain at C-5 was inserted using the conventional methods of a Wittig reaction and reduction[11] to give alcohol **14**. After protection of the terminal alcohol as an acetate, the resulting **15** was treated with 1 M HCl/$Et_2O$ in isopropanol to yield alcohol **16**, which was oxidized to the related aldehyde **17**. The direct arylation[12] of **17** with aryl bromide **26a** followed by hydrolysis generated the desired thio-salvinal (**4**). The related derivatives **19**, **20**, and **21** were also prepared similarly from **17** using other aryl bromides, **26b**, **26c**, and **26d**, respectively. The hydrolysis of **19**, **20**, and **21** generated **22**, **23**, and **24**, respectively.

Thio-obovaten (**5**) and thio-lignan **6** were obtained using the following reaction procedure (Scheme 2). Phenol **27** was converted to thiophenol **28** through the same reaction sequence for the preparation of **9**. Allylation of **28** using the Mizoroki−Heck reaction gave 3-methyl BT **29**, which was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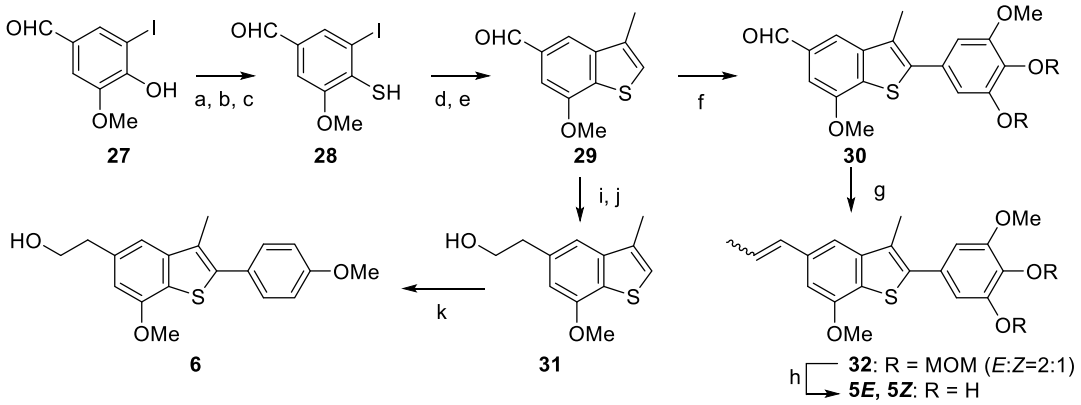
**Scheme 2. Preparation of Thio-lignans 5 and 6**[a]




[a]Reagents and conditions: (a) **25**, 1.0 M aq KOH, THF, 0 °C, 2 h, 92%; (b) PhOPh, microwave, 220 °C, 15 min, 70%; (c) 5.0 M aq KOH, MeOH, reflux, 3 h, 36%, 60% brms; (d) allyl bromide, TBAB, 2 M aq NaOH, PhH, 0 °C, 1 h, 79%; (e) $NEt_3$, $Pd(PPh_3)_4$, MeCN, 140 °C, 22 h, 42%; (f) 5-iodo-1-methoxy-2,3-bis(methoxymethoxy)benzene, $Pd(OAc)_2$, $Ag_2O$, NaOAc, hexafluoro-2-propanol (HFIP), 35 °C, 18 h, 51% (100% brsm); (g) $EtPPh_3Br$, PhLi, −78 °C to rt, 1 h, 80%; (h) 1.0 M HCl, MeOH, rt, 48 h, 93%; (i) $MePPh_3Br$, *n*-BuLi, THF, 0 °C, 45%; (j) 9-borabicyclo[3.3.1]nonane (9-BBN), THF, reflux, 1 h, then 0 °C, EtOH, 7 M NaOH, $H_2O_2$, 50 °C, 60%; and (k) 4-iodoanisole, $Pd(OAc)_2$, $Ag_2O$, NaOAc, HFIP, 35 °C, 18 h, 57% (61% brsm).

reacted with 5-iodo-1-methoxy-2,3-bis(methoxymethoxy)-benzene under direct arylation conditions.[13] The resulting 2-aryl BT **30** was treated with $EtPPh_3Br$ to obtain **32** as cis−trans isomers (*E*/*Z* = 2:1). Deprotection with 1 M HCl followed by separation with chiral chromatography gave thio-obovaten (**5**(*E*) and **5**(*Z*)). In addition, 3-methyl BT **29** was converted to **31** through vinylation, hydroboration, and oxidation. Then, direct arylation with 4-iodoanisole produced thio-lignan **6**.

As mentioned earlier, an aldehyde is a highly reactive functional group that is generally not appropriate for drug development because it binds randomly with nucleophiles in biomolecules such as proteins and nucleotides.[6] To find a suitable functional group at C-3, various 3-substituted derivatives were synthesized (Scheme 3). The acetal **33** was prepared from **18** by the protection of the aldehyde with ethylene glycol. Acetylation of **16** followed by direct arylation with **26a** provided 3-acetoxymethyl-BT **34**. Reduction of the aldehyde in **18** with $NaBH_4$ and hydrolysis of the acetate in **34** with aq NaOH gave 3-hydroxymethyl-BTs **35** and **36**, respectively. Oxidation of **18** with $KMnO_4$ produced a 3-carboxylic acid as well as unexpected deacetylation of the phenolic acetate to give guaiacol; reprotection with $Ac_2O$ gave **37**. Separate esterification with MeI and amidation with $MeNH_2$ of **37** provided 3-methylcarboxylate-BT **38** and 3-(*N*-methyl)-carboxamide-BT **39**, respectively. The reaction of **4** with MeMgBr generated 3-(1-hydroxyethyl)-BT **40**. Wittig reactions of **4** and 3-carbaldehyde-BT **23** with methyl (triphenylphosphoranylidene)acetate provided methyl acrylates **41** and **42**, respectively.

Deprotection of the MOM group on **13**, followed by IBX oxidation of the resultant alcohol provided 3-carbaldehyde-BT **43** (Scheme 4). Direct arylation of **43** with **26a** gave **44** with ethyl propionate as the C-5 side chain rather than propanol or propyl acetate as found in **4** and **18**, respectively. The dimeric compound (**45**) was also prepared through direct arylation.

**Synthesis of Salvinal.** The parent molecule, salvinal (**1**), was also prepared for comparison. The synthesis was achieved by modifying the reported method.[14] Briefly, methyl ferulate **46** was dimerized in the presence of $Ag_2O$ to give **47**, which was converted to **48** through acetylation and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation (Scheme 5). Hydrogenation of a double bond on **48** under acidic conditions, followed by the treatment of filtrate with sat. $NaHCO_3$ provided a phenol intermediate, which was protected to yield benzyl ether **49**. Reduction of methyl esters by $LiAlH_4$ and selective oxidation of benzyl alcohol by activated $MnO_2$ afforded **50**. Deprotection of the benzyl group gave salvinal (**1**). Acetylation of the hydroxy group on **1** followed by the protection of aldehyde on resultant **51** with ethylene glycol provided **52**, which is an oxygen analogue of compound **33**.

**Evaluation of Antiproliferative Activities against Human Tumor Cell Lines.** All synthesized thio-lignans were evaluated for antiproliferative activities against five human tumor cell lines (HTCLs), including lung carcinoma (A549), triple-negative breast cancer (MDA-MB-231), estrogen receptor-positive and HER2 negative breast cancer (MCF-7), cervical cancer cell line HeLa derivative (KB), and its multidrug-resistant (MDR) subline with P-glycoprotein (P-gp) overexpression (KB-VIN) (Table 1).

Antiproliferative activity of our newly synthesized salvinal (**1**) showed a profile similar to that previously reported.[4] As anticipated, thio-salvinal (**4**) showed significant antiproliferative effects with $IC_{50}$ values of 0.57−0.95 μM against all tested HTCLs, except for the HER2 negative breast cancer cell line MCF-7, and was 6.5−9.4 times more potent than parent compound **1**. This result indicates that, compared to BF, BT is preferred for the activity of the salvinal skeleton, although a similar effect was not observed with **2** and **3** (compare **2** vs **5** and **3** vs **6**). Thus, we decided to study SAR correlations based on the thio-salvinal structure.

The synthesized compounds were divided roughly into four groups based on their antiproliferative activity. Group-I (**4** and **18**) exhibited submicromolar $IC_{50}$ values. Group-II (**19**, **22**, **23**, **33**, **41**, and **44**), group-III (**5**(*E*), **21**, and **24**), and group-IV (**5**(*Z*), **6**, **34**−**40**, **42**, and **45**) displayed values of 1−10, 10−20, and over 20 μM, respectively (Figure 2). Compounds **4** and **18** in group-I exhibited an interesting antiproliferative activity profile, i.e., they were over 10-fold less potent against MCF-7 ($IC_{50}$: 11.9 μM) than the other four cell lines ($IC_{50}$: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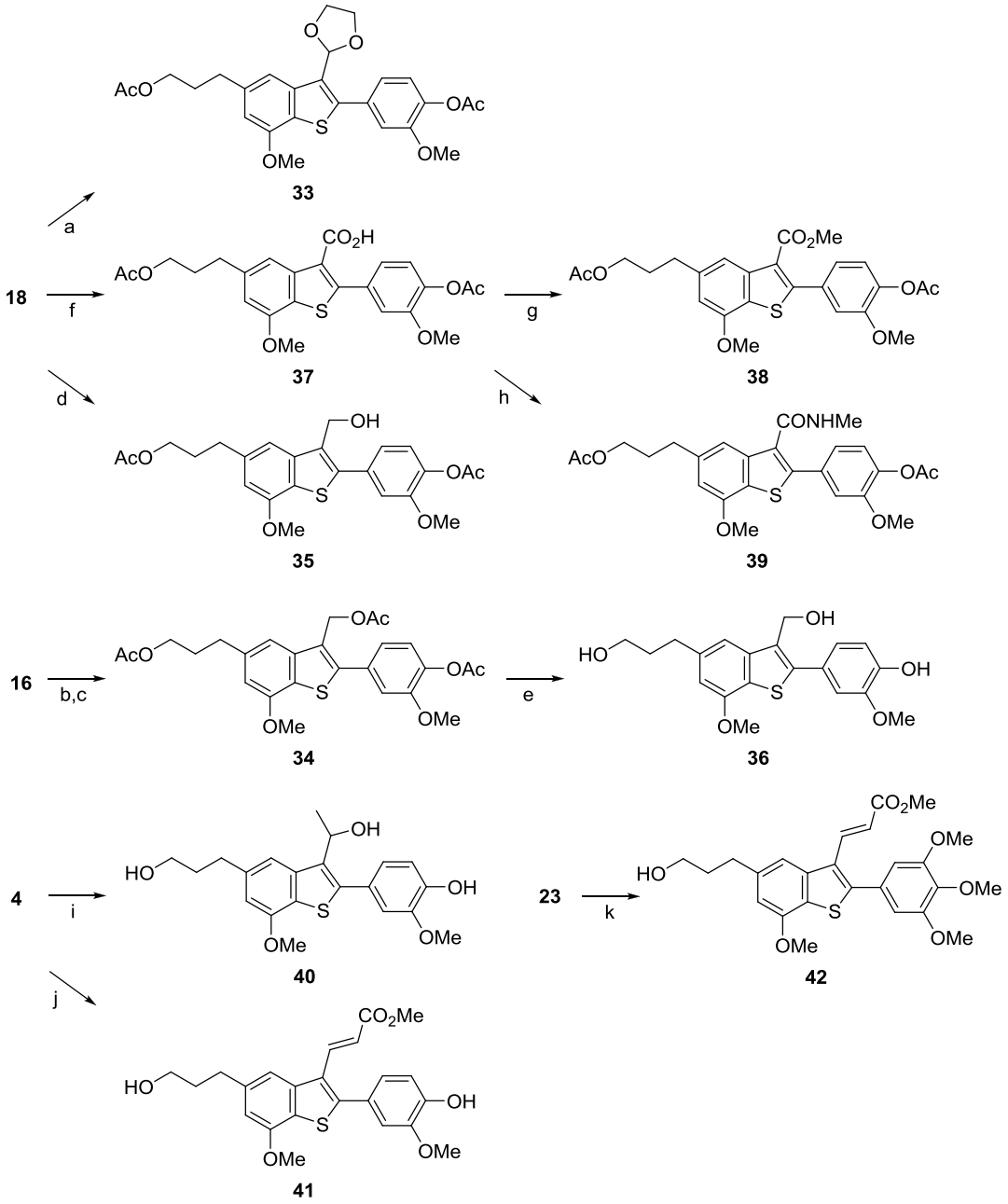
**Scheme 3. Preparation of Thio-lignan Derivatives 33−42**[a]




[a]Reagents and conditions: (a) ethylene glycol, *p*-TsOH, molecular sieves (MS) 4 Å, PhH, reflux, 100%; (b) $Ac_2O$, Py, DMAP, $CH_2Cl_2$, rt, 100%; (c) **26a**, $Pd(OAc)_2$, $PCy_3$, PivOH, $K_2CO_3$, toluene, reflux, 49%; (d) $NaBH_4$, THF, 0 °C, 85%; (e) 2.0 M NaOH, MeOH/THF (1:1, v/v), rt, 73%; (f) 0.4 M aq $KMnO_4$, acetone, 40 °C, then (b), 30%; (g) MeI, $K_2CO_3$, dimethylformamide (DMF), rt, 45%; (h) DIPEA, 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), MeCN, then $MeNH_2$, 78%; (i) MeMgBr, THF, 0 °C, 37%; (j) $Ph_3PCH_2CO_2Me$, *t*-BuOK, $CH_2Cl_2$, 60 °C, 78%; and (k) $Ph_3PCH_2CO_2Me$, $K_2CO_3$, $CH_2Cl_2$, 60 °C, 93%.

0.57−0.95 $\mu$M). Although the reason is unclear at this stage, compounds **4** and **18** might be metabolized quickly intracellularly or MCF-7 cells might have a low expression level of the molecular target of the thio-lignans. Also, the activities of acetates **18**, **19**, and **24** were much like those of the corresponding alcohols, **4**, **22**, and **21**, respectively, suggesting immediate hydrolysis by an intracellular esterase.

In the overall comparisons, the substituent at C-3 was a key factor to the antiproliferative activity; the aldehyde (**4**) was the most preferred functional group, followed by the related acetal (**33**) and methyl acrylate (**41**) in group-II. Regarding the substitution pattern on the pendant phenyl at C-2, the combination of 3-methoxy and 4-hydroxy or 4-acetoxy groups (**4** and **18**) on the pendant phenyl ring was more beneficial to activity than 3,4-dimethoxy (**19** and **22**), 3,4,5-trimethoxy (**23**), or 3-nitro groups (**21** and **24**). Based on the limited investigations, electron-donating groups (**4**, **18**, **19**, **22**, and **23**) might be more suitable than an electron-withdrawing group (**21** and **24**). Comparison of **4** and **44** disclosed that a terminal aliphatic alcohol (**4**) was more effective than an aliphatic ester (**44**) in the C-5 side chain. Meanwhile, the BT dimer (**45**) was much less active than the monomeric thio-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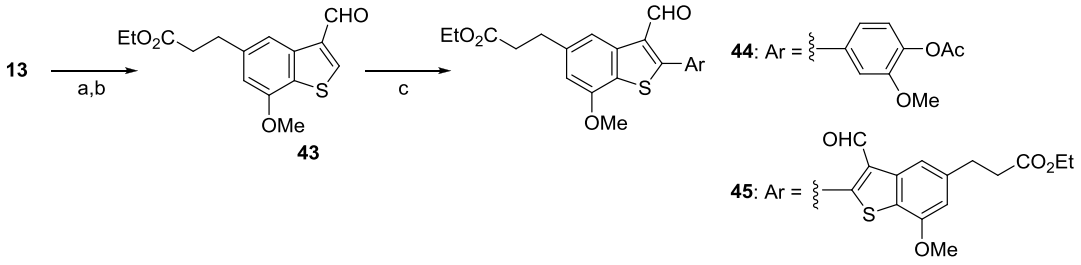
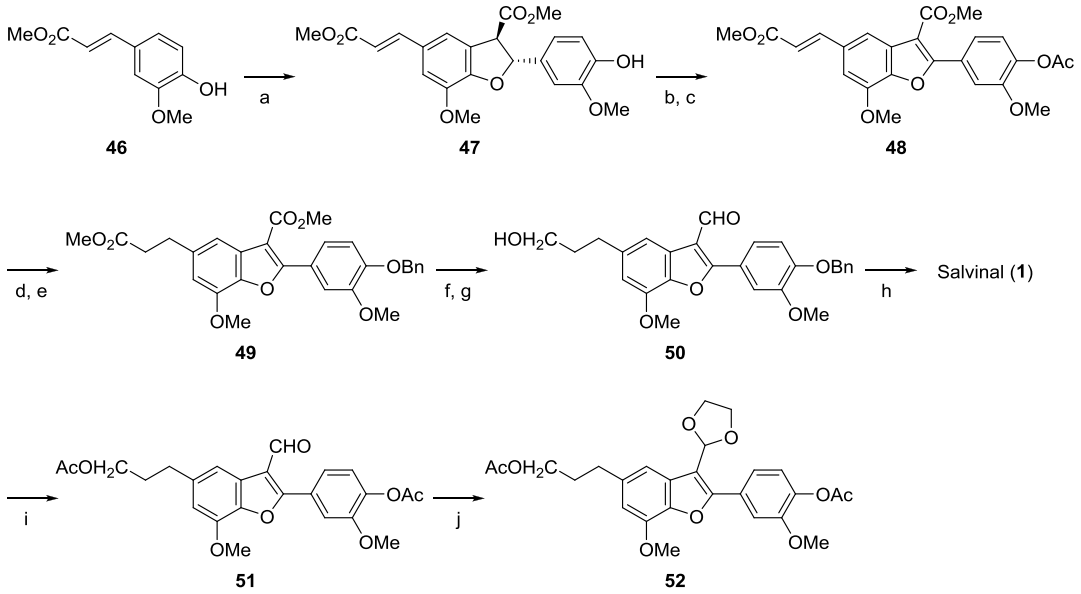
**Scheme 4. Preparation of Thio-lignan Derivatives 44 and 45**[a]




[a]Reagents and conditions: (a) 1.0 M HCl in diethyl ether, isopropanol, 55 °C, 88%; (b) IBX, DMSO, rt, 100%; and (c) ArBr, $Pd(OAc)_2$, $PCy_3BF_4$ for **44** or $PCy_3$ for **45**, PivOH, $K_2CO_3$, toluene, reflux, 80% for **44**, 26% for **45**.

**Scheme 5. Preparation of Salvinal (1)**[a]




[a]Reagents and conditions: (a) $Ag_2O$, benzene/acetone = 2:1, rt, 48%; (b) $Ac_2O$, DMAP, pyridine, rt; (c) DDQ, benzene, reflux, 50% in two steps; (d) *p*-TsOH, $H_2$, Pd−C, rt; (e) BnBr, $K_2CO_3$, 2-butanone, reflux, 87% in two steps; (f) $LiAlH_4$, THF, −5 °C to rt; (g) $MnO_2$, EtOAc, rt, 88% in two steps; (h) $TiCl_4$, $CH_2Cl_2$, rt, 90%; (i) $Ac_2O$, DMAP, pyridine, $CH_2Cl_2$ 98%; and (j) ethylene glycol, *p*-TsOH, toluene, reflux, 75%.

lignan **44**, which indicates that the combination of BT and an appropriately substituted phenyl was significant for the antiproliferative activity.

Excluding the thio-lignans with a C-3 aldehyde, compound **33** with a 1,3-dioxolane, an aldehyde equivalent, was the most potent with $IC_{50}$ values of 4.94−8.78 $\mu$M, comparable to salvinal (**1**), against all tested HTCLs. The compound **33** was also more effective than compound **52**, oxygen analogue of compound **33**, suggesting that BT is preferred for the activity. In the comparison of C-3 methyl acrylates **41** and **42**, trimethoxy substitution on the phenyl ring decreased the potency, which was consistent with the above-mentioned comparison (**4** and **23**).

Importantly, most tested compounds, including **33**, exhibited similar or greater antiproliferative activity against drug transporter P-gp overexpressing MDR subline KB-VIN cells compared to that against the parent chemosensitive cell line KB, suggesting that all compounds were not the substrates of P-gp.

**Mechanism of Action Study of Thio-lignan 33.** Based on the previous report, the parent natural product **1** inhibits tubulin polymerization and induced G2/M accumulation weakly about 20 or 30% of cells at 12 ($3 \times IC_{50}$) or 20 $\mu$M ($5 \times IC_{50}$) concentration, respectively.[4] The reason for the mild effect of **1** on G2/M accumulation was probably due to its weak specificity or affinity for tubulin caused by an aldehyde moiety. We have obtained **33**, which contained no aldehyde group and showed the same antiproliferative activity as **1** with the aldehyde group. However, a slightly different functional group can affect the activity or even change the target protein. For example, while the well-known lignan podophyllotoxin inhibits tubulin, its derivative etoposide inhibits topoisomerase-II. We also reported that a slight change in the functional group caused big differences in the activity of desmosdumotin derivatives.[8] Therefore, we needed to confirm the mechanism of action of the active thio-lignan derivatives. The most active compound, BT-salvinal (**4**), contains a reactive aldehyde, which might interact with numerous cellular proteins non-specifically and cause false positives. Therefore, we selected the most active compound **33** with an aldehyde equivalent for further investigation. Flow cytometric analysis of **1**, **4**, and **33** in MDA-MB-231 and KB-VIN showed G2/M accumulation at 24 h in both cell lines and dose-dependent G2/M cell cycle arrest in KB-VIN. Sub-G1 accumulation, which is a typical pattern of apoptosis, was obvious in cells treated for 48 h (Figure 3). Overall, the effects of compounds **1**, **4**, and **33** on


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**Table 1. Antiproliferative Activity of Synthesized Thio-lignans**

| | cell lines/$IC_{50}$ ($\mu$M)[a] | | | | |
|---|---|---|---|---|---|
| compound | A549 | MDA-MB-231 | MCF-7 | KB | KB-VIN |
| **1** (synthesized) | 6.84 | 7.49 | 8.47 | 6.14 | 5.37 |
| **1**[4] | | | 9.2 | 5.0 | 3.7 |
| **2**[2] | 1.1 | | | 2.3 (KB16) | |
| **3**[3] | 3.5 | | 5.0 | | |
| **4** | 0.81 | 0.95 | 11.9 | 0.95 | 0.57 |
| **5**(*E*) | 8.74 | 11.3 | 10.5 | 10.7 | 14.1 |
| **5**(*Z*) | 21.4 | 20.7 | 22.1 | 20.3 | 21.0 |
| **6** | 27.9 | >40 | 31.1 | 30.5 | 31.2 |
| **18** | 0.94 | 0.94 | 11.9 | 0.89 | 0.56 |
| **19** | 6.13 | 7.42 | 7.97 | 5.98 | 4.58 |
| **21** | 5.07 | 21.1 | 13.7 | 10.3 | 7.70 |
| **22** | 7.28 | 8.82 | 9.51 | 7.88 | 5.58 |
| **23** | 5.13 | 6.56 | 6.66 | 4.53 | 1.00 |
| **24** | 5.34 | 23.1 | 21.0 | 15.4 | 14.4 |
| **33** | 7.05 | 8.50 | 8.78 | 5.69 | 4.94 |
| **34** | >40 | >40 | 39.3 | >40 | >40 |
| **35** | 19.1 | 22.3 | 21.5 | 23.3 | 17.7 |
| **36** | 22.4 | 30.8 | 25.4 | 28.1 | 22.8 |
| **37** | >40 | >40 | >40 | >40 | >40 |
| **38** | 23.0 | 36.2 | 34.1 | 26.5 | 21.9 |
| **39** | >40 | >40 | >40 | >40 | >40 |
| **40** | >40 | >40 | >40 | >40 | >40 |
| **41** | 7.18 | 12.9 | 12.1 | 9.67 | 6.24 |
| **42** | 20.6 | 18.7 | 20.2 | 27.5 | 22.0 |
| **44** | 5.52 | 7.69 | 9.67 | 4.52 | 3.89 |
| **45** | 23.0 | 36.2 | 34.1 | 26.5 | 21.9 |
| **52** | 22.3 | 16.9 | 21.5 | 21.5 | 7.88 |
| paclitaxel (nM) | 6.34 | 9.96 | 11.4 | 5.64 | 1902 |

[a]Antiproliferative activity expressed as $IC_{50}$ values for each cell line, the concentration of the compound that caused 50% reduction relative to untreated cells determined by the SRB assay ($n$ = 6).

the cell cycle progression in both MDA-MB-231 and KB-VIN, especially on G2/M phase accumulation, were almost the same. However, the effect of compounds **1** and **4** at low concentrations on KB-VIN cells was dramatically weaker than that of compound **33**. These results suggested that, like salvinal (**1**), the new thio-lignan probably targets tubulin and subsequently induces apoptosis. Accordingly, we can say that the bioactivity of compound **33** on the cell cycle was improved compared with **1**. However, the cell cycle distribution pattern was distinguishable from that in cells treated with combretastatin A-4 (CA-4). Because CA-4 is a standard microtubule-destabilizing agent that binds to the colchicine site, we presume that **33** might bind to another site on tubulin. In addition, S phase accumulation was observed in MDA-MB-231 cells after 48 h treatment with low dose, suggesting that **1**, **4**, and **33** potentially affect DNA replication.

To see if **1** or **33** functions as a tubulin inhibitor in MDA-MB-231 cells, an immunocytochemical approach was employed. The cells were treated with **1** or **33** for 24 h at a concentration of 3-fold of the $IC_{50}$ value, which was subjected to double labeling with monoclonal antibody to $\alpha$-tubulin and 4′,6-diamidino-2-phenylindole (DAPI) for nuclei (Figure 4). It was clearly observed that the majority of microtubules were depolymerized by **33** like colchicine site agent CA-4 treated cells. In cells treated with **33**, a limited number of partially polymerized microtubules could be observed; comparing **1** and **33**, the effect of microtubule depolymerization was stronger in **33**. In addition, the cells with mitotic spindles were undetectable, suggesting that both **1** and **33** induced cell cycle arrest at the interphase by inhibiting tubulin polymerization. In addition, **1** or **33** treated cells were not stained with antibody to serine 10-phosphorylated histone H3 (p-H3), a marker for mitotic chromosome condensation (data not shown), which demonstrated that the cells were arrested in the G2 phase. Furthermore, nuclear fragmentation was often observed in cells treated with these compounds. These results supported that bioactivity of **33** was the same as that of salvinal (**1**), while the effect of **1** on tubulin depolymerization was significantly improved by **33**.

Herein, we have achieved to synthesize a bioequivalent salvinal analogue without an aldehyde group.

## CONCLUSIONS

We applied the medicinal chemistry concept of bioisosteres to convert natural products containing BF (lignans) to new synthetic derivatives containing BT (thio-lignans). The synthetic routes using Pd-catalyzed cross-coupling reactions are effective for the synthesis of other derivatives with different substituents on BT because of easy handling and high overall yields. Except for **4**, the new thio-lignans showed lower antiproliferative activities than the parent lignans against HTCLs. Since the aldehyde found in **4** is not applicable to drug development, we prepared derivatives containing ethylenedioxy, acetoxymethyl, hydroxymethyl, carboxyl, methyl ester, monomethylamide, hydroxyethyl, and methyl acrylate groups. However, almost all compounds without an aldehyde exhibited dramatically decreased antiproliferative activity. Based on our biological evaluation of **1**, **4**, and **33** side-by-side, we presume that both compounds mainly interfere with G2/M transition and DNA replication as well as induces apoptosis time- and dose-dependently. Immunostaining of $\alpha$-tubulin in MDA-MB-231 cells revealed that **33** functioned as a tubulin polymerization inhibitor more evidently than **1**. Thus, the biological profile of thio-salvinal analogue **33** without an aldehyde appears to be the same as that of salvinal with aldehyde, while its bioactivity, particularly against tubulin, was improved. The molecular mechanism of **33**, especially against DNA replication, will be further investigated. As the antiproliferative activity of the BT derivatives could be improved by differences in substituents, drug discovery studies on compounds possessing a BT ring will also be continued.

## EXPERIMENTAL SECTION

**General Procedures.** All reactions were conducted under an argon atmosphere and monitored by thin-layer chromatography (TLC) silica gel $60F_{254}$ (Merck). All reagents were purchased commercially and used without further purification unless otherwise indicated. Crude materials were purified by column chromatography on silica gel 60N (63−210 $\mu$m, Kanto Chemical). NMR spectra were measured on JEOL JMN-ECA600 and JMN-ECS400 spectrometers with tetramethylsilane as an internal standard, and chemical shifts are stated as $\delta$ values. High-resolution mass spectrometry (HRMS) data were recorded using a JEOL JMS-700 MStation mass spectrometer.

**Experimental Procedure for Novel Compounds.** *3-Methoxy-4-(prop-2-yn-1-ylsulfinyl)benzaldehyde (**10**).* Sodium hydroxide (5.0 mL, 9.5 mmol, 1.9 M in water) and benzaldehyde **9**[15] (934 mg, 5.6 mmol) were stirred for 25 min, after which the reaction mixture was cooled to 0 °C and propargyl bromide (8.7 mL, 8.3 mmol, 8% solution in benzene) was added dropwise. Tetrabutylammonium


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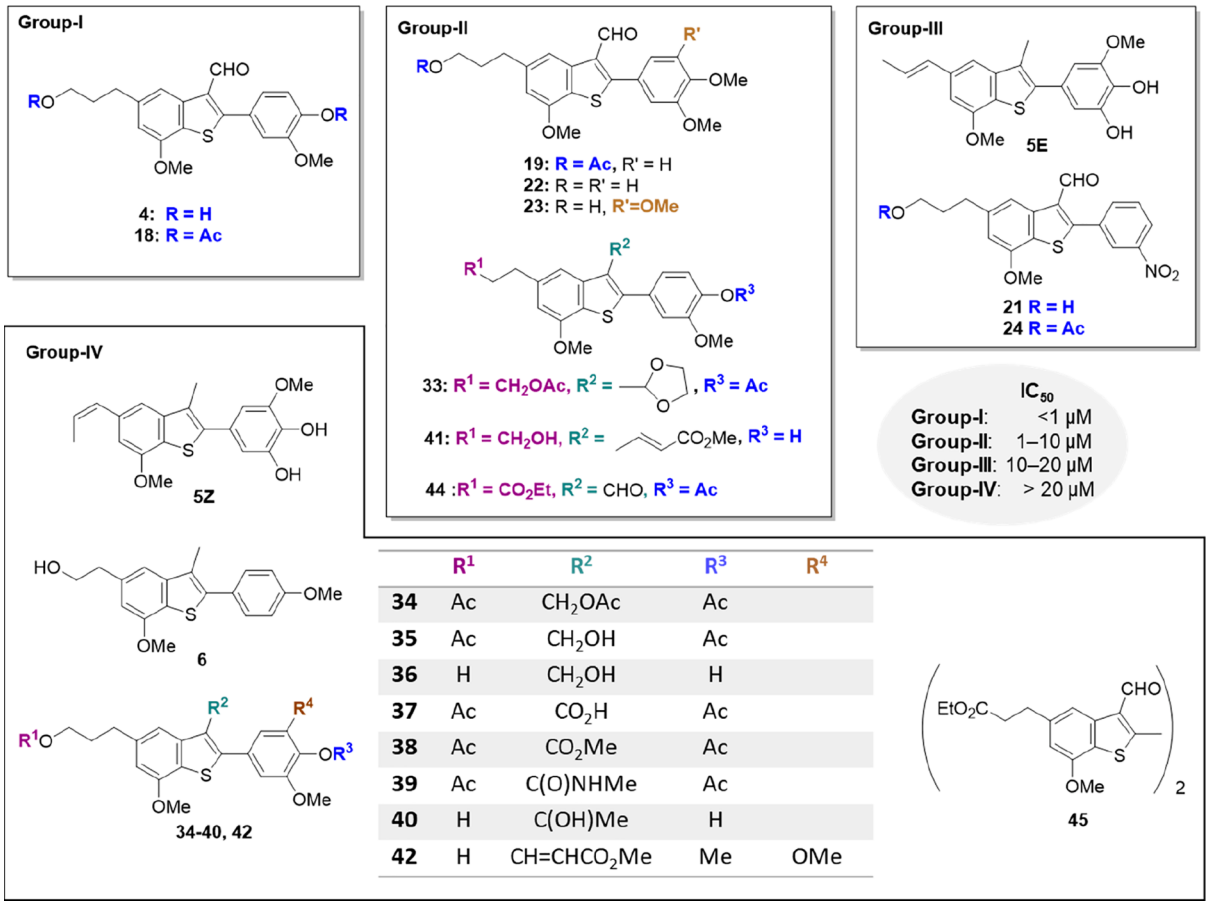


| | R1 | R2 | R3 | R4 |
|---|---|---|---|---|
| 34 | Ac | $CH_2OAc$ | Ac | |
| 35 | Ac | $CH_2OH$ | Ac | |
| 36 | H | $CH_2OH$ | H | |
| 37 | Ac | $CO_2H$ | Ac | |
| 38 | Ac | $CO_2Me$ | Ac | |
| 39 | Ac | C(O)NHMe | Ac | |
| 40 | H | C(OH)Me | H | |
| 42 | H | $CH{=}CHCO_2Me$ | Me | OMe |

**Figure 2.** Rough classification of the synthesized thio-lignans by $IC_{50}$ values.

bromide (181 mg, 0.56 mmol) was added, and the mixture was stirred vigorously for 1.0 h. The reaction mixture was poured into water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (1:1) to give 3-methoxy-4-(prop-2-yn-1-yl-thio)-benzaldehyde (1.11 g, 97%) as a yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 9.93 (s, 1H), 7.46 (s, 2H), 7.35 (s, 1H), 3.96 (s, 3H), 3.71(d, 2H, $J$ = 2.4 Hz), 2.24 (t, 1H, $J$ = 2.6 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 191.3, 156.5, 135.0, 133.6, 126.5, 125.0, 107.8, 78.7, 71.8, 56.1, 19.4; HRMS-fast atom bombardment (FAB) ($m/z$): [M + H]$^+$ calcd for $C_{11}H_{11}O_2S$, 207.0480; found, 207.0472. To a solution of 3-methoxy-4-(prop-2-yn-1-ylthio)benzaldehyde (1.83 g, 8.88 mmol) in 50.0 mL of $HCO_2H$/water (18:1, v/v) was slowly added 30% $H_2O_2$ (1.52 mL, 13.3 mmol) at 0 °C, and the mixture was allowed to reach rt. After being stirred for 5.0 h, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (1:1) to give **10** (1.34 g, 68%) as a pale yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 10.05 (s, 1H), 8.02 (d, 1H, $J$ = 7.6), 7.70 (dd, 1H, $J$ = 7.6, 1.1 Hz), 7.45 (d, 1H, $J$ = 1.1 Hz), 3.98 (s, 3H), 3.95 (dd, 1H, $J$ = 16.3, 2.6 Hz), 3.75 (dd, 1H, $J$ = 16.3, 2.6 Hz), 2.30 (t, 1H, $J$ = 2.0 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 191.3, 155.5, 139.9, 137.1, 127.5, 124.8, 108.6, 76.2, 72.5, 56.2, 43.9; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{11}H_{11}O_3S$, 223.0429; found, 223.0408.

*3-(Hydroxymethyl)-7-methoxybenzo[b]thiophene-5-carbaldehyde (**11**).* A solution of **10** (912 mg, 4.1 mmol) in 13.0 mL of 1,4-dioxane was refluxed. After the mixture was stirred for 1.0 h, *p*-TsOH·$H_2O$ (264 mg, 1.4 mmol) and 1.2 mL of water were added, followed by refluxing for a further 1.0 h. The mixture was cooled to ambient temperature and treated with saturated $NaHCO_3$ solution and extracted with EtOAc (4 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (7:3) to give **11** (675 mg, 74%) as a yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 10.05 (s, 1H), 7.97 (d, 1H, $J$ = 1.0 Hz), 7.49 (s, 1H), 7.30 (s, 1H), 4.99 (s, 2H), 4.06 (s, 3H), 1.96 (s, 1H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 192.2, 155.2, 139.3, 137.2, 136.2, 135.3, 125.7, 120.8, 101.0, 59.7, 56.0; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{11}H_{11}O_3S$, 223.0429; found, 223.0426.

*7-Methoxy-3-[(methoxymethoxy)methyl]benzo[b]thiophene-5-carbaldehyde (**12**).* To a solution of **11** (587 mg, 2.6 mmol) in anhydrous $CH_2Cl_2$ (8.0 mL) was added *N*,*N*-diisopropylethylamine (2.3 mL, 13.1 mmol) at 0 °C, and the mixture was stirred for 20 min under $N_2$. MOMCl (1.0 mL, 13.1 mmol) was added, and stirring was continued at 40 °C in an oil bath for 1.5 h. The reaction was quenched with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (7:3) to afford **12** (492 mg, 75%) as a pale yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 10.07 (s, 1H), 7.96 (d, 1H, $J$ = 1.0 Hz), 7.51 (s, 1H), 7.32 (s, 1H), 4.88 (d, 2H, $J$ = 1.0 Hz), 4.74 (s, 2H), 4.06 (s, 3H), 3.45 (s, 3H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 192.1, 155.2, 139.7, 136.1, 135.4, 134.2, 126.9, 120.9, 100.9, 95.5, 63.1, 56.0, 55.6; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{13}H_{15}O_4S$, 267.0686; found, 267.0686.

*Ethyl-3-{7-methoxy-3-[(methoxymethoxy)methyl]benzo[b]-thiophen-5-yl}propanoate (**13**).* A solution of **12** (365 mg, 1.4 mmol) and (carbethoxymethylene)triphenylphosphorane (734 mg, 2.1 mmol) in $CH_2Cl_2$ (10 mL) was refluxed for 3.5 h. The reaction mixture was cooled to rt, poured into water, and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (5:1) to


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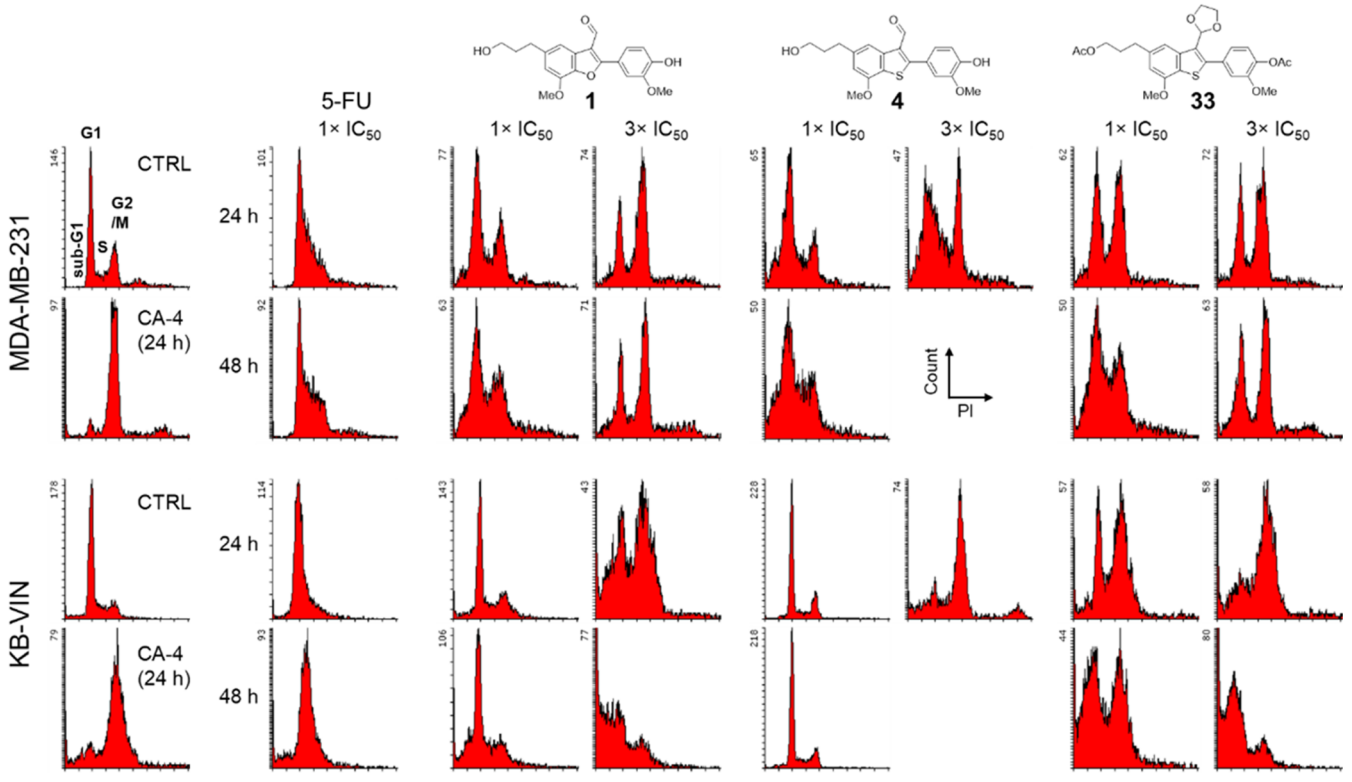


**Figure 3.** Effects of salvinal (**1**), **4**, and **33** on cell cycle progression. Triple-negative breast cancer MDA-MB-231 (upper two rows) or MDR subline KB-VIN (lower two rows) cells were treated with **1**, **4**, or **33** for 24 (upper row) and 48 h (lower row) as indicated. DMSO was used as a vehicle control (CTRL). **1**, **4**, and **33** were used at 1 × $IC_{50}$ and 3 × $IC_{50}$ concentrations. 5-Fluorouracil (5-FU), an inhibitor for DNA replication (S phase), was used at 1 × $IC_{50}$ and tubulin polymerization inhibitor combretastatin A-4 (CA-4), an inhibitor for mitotic onset (G2/M), was used at 3 × $IC_{50}$. Cell cycle distributions of treated cells were analyzed by flow cytometry after staining with propidium iodide (PI) in the presence of RNase.

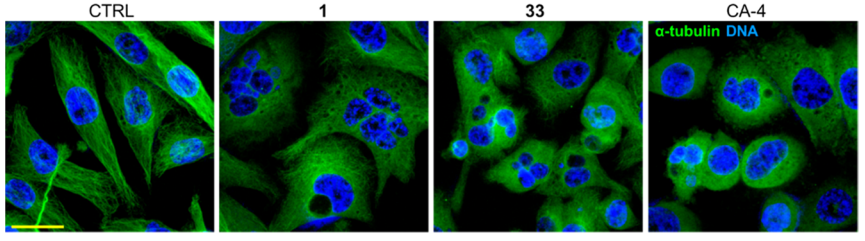


**Figure 4.** Compound **33** inhibited tubulin polymerization. MDA-MB-231 cells were treated with compound **1**, **33**, or CA-4 for 24 h at 3 × $IC_{50}$. DMSO was used as a vehicle control (CTRL). The cells were labeled with monoclonal antibody to $\alpha$-tubulin (green) and DAPI for DNA (blue), stained cells were observed by a confocal laser-scanning microscope. The represented merged image was a projection of 16−18 optical sections. Bar, 0.025 mm.

give ethyl-3-{7-methoxy-3-[(methoxymethoxy)methyl]benzo[*b*]-thiophen-5-yl}acrylate (440 mg, 96%) as a colorless solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.81 (d, 1H, $J$ = 15.8 Hz), 7.60 (s, 1H), 7.43 (s, 1H), 6.96 (s, 1H), 6.49 (d, 1H, $J$ = 15.8 Hz), 4.82 (s, 2H), 4.72 (s, 2H), 4.29 (dd, 2H, $J$ = 14.3, 7.1 Hz), 4.02 (s, 3H), 3.44 (s, 3H), 1.36 (t, 3H, $J$ = 14.3, 7.1 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 167.0, 154.8, 145.2, 140.0, 133.6, 132.6, 131.8, 126.3, 117.7, 116.4, 102.2, 95.5, 63.1, 60.5, 55.7, 55.5, 14.4; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{17}H_{21}O_5S$, 337.1110; found, 337.1079. Ethyl-3-{7-methoxy-3-[(methoxymethoxy)methyl]benzo[*b*]thiophen-5-yl}acrylate (440 mg, 1.3 mmol) was dissolved in EtOAc (9.0 mL), and 30% Pd/C (137 mg) was added. The mixture was shaken under $H_2$ for 8.0 h. The mixture was then filtered through a pad of Celite and chromatographed on silica gel, eluting with hexane−EtOAc (4:1) to give **13** (394 mg, 89%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.38 (s, 1H), 7.29 (s, 1H), 6.67 (s, 1H), 4.79 (s, 2H), 4.71 (s, 2H), 4.14 (dd, 2H, $J$ = 14.5, 7.0 Hz), 3.98 (s, 3H), 3.44 (s, 3H), 3.08 (t, 2H, $J$ = 7.9 Hz), 2.69 (t, 2H, $J$ = 7.9 Hz), 1.25 (t, 3H, $J$ = 7.0 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 172.9, 154.5, 140.0, 138.8, 133.0, 127.5, 125.7, 114.0, 105.3, 95.4, 63.3, 60.5, 55.6, 55.5, 36.4, 31.5, 14.2; HRMS-FAB ($m/z$): [M + Na]$^+$ calcd for $C_{17}H_{22}O_5SNa$, 361.1086; found, 361.1065.

*3-{7-Methoxy-3-[(methoxymethoxy)methyl]benzo[b]thiophen-5-yl}propan-1-ol (**14**).* $LiBH_4$ (1.5 mL, 3.0 mmol, 2.0 M in THF) was added to a solution of **13** (333 mg, 1.0 mmol) in THF (8.0 mL) under $N_2$ and stirred for 8.0 h at 60 °C in an oil bath. The mixture was adjusted to pH 6−7 by the addition of saturated $NH_4Cl$ solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to give **14** (286 mg, 97%) as a colorless solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.39 (s, 1H), 7.29 (s, 1H), 6.67 (s, 1H), 4.86−4.72 (m, 4H), 3.99 (s, 3H), 3.72 (t, 2H, $J$ = 6.0 Hz), 3.44 (s, 3H), 2.85 (t, 2H, $J$ = 7.7 Hz), 1.20−1.95 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 154.5, 140.0, 132.9, 127.2, 125.6, 114.0, 105.5, 95.3, 63.2, 62.3, 62.2, 55.6, 55.5, 34.6, 32.7; HRMS-FAB ($m/z$): [M + Na]$^+$ calcd for $C_{15}H_{20}O_4SNa$, 319.0980; found, 319.0952.



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*3-{7-Methoxy-3-[(methoxymethoxy)methyl]benzo[b]thiophen-5-yl}propyl Acetate (**15**).* To a solution of **14** (286 mg, 0.96 mmol) in $CH_2Cl_2$ (10.0 mL) were added pyridine (90.0 μL, 0.98 mmol), DMAP (12 mg, 0.10 mmol), and $Ac_2O$ (0.11 mL, 1.2 mmol), and the mixture was stirred at rt. After stirring for 2.5 h, DMAP (15 mg, 0.12 mmol) and $Ac_2O$ (45.0 μL, 0.48 mmol) were added and stirred for 30 min. The reaction mixture was poured into water and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (4:1) to give **15** (324 mg, 100%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): δ 7.39 (s, 1H), 7.27 (s, 1H), 6.64 (s, 1H), 4.81 (s, 2H), 4.72 (s, 2H), 4.13 (t, 2H, *J* = 6.6 Hz), 3.99 (s, 3H), 3.44 (s, 3H), 2.82 (t, 2H, *J* = 7.8 Hz), 2.07 (s, 3H), 2.06−2.01 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 171.2, 154.5, 140.0, 139.4, 132.9, 127.4, 125.7, 114.0, 105.4, 95.3, 63.9, 63.3, 55.6, 55.5, 32.8, 30.6, 21.0; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{17}H_{23}O_5S$, 339.1266; found, 339.1268.

*3-[3-(Hydroxymethyl)-7-methoxybenzo[b]thiophen-5-yl]propyl Acetate (**16**).* To a stirred solution of **15** (324 mg, 0.96 mmol) in dry isopropyl alcohol (10.0 mL) was added 0.8 mL of 1.0 M hydrochloric acid/diethyl ether (0.8 mmol), and the mixture was stirred at 55 °C in an oil bath for 4.0 h under $N_2$. The reaction mixture was cooled to rt, adjusted to pH 7−8 by the addition of saturated $NaHCO_3$ solution, and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (3:1) to give **16** (190 mg, 68%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): δ 7.36 (s, 1H), 7.29 (s, 1H), 6.65 (s, 1H), 4.89 (d, 2H, *J* = 5.1 Hz), 4.12 (t, 2H, *J* = 6.4 Hz), 3.99 (s, 3H), 2.82 (t, 2H, *J* = 7.6 Hz), 2.07−2.01 (m, 5H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 171.3, 154.6, 139.6, 139.4, 136.2, 127.5, 124.5, 114.0, 105.4, 63.8, 59.9, 55.6, 32.7, 30.6, 21.0; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{15}H_{19}O_4S$, 295.1004; found, 295.0977.

*3-(3-Formyl-7-methoxybenzo[b]thiophen-5-yl)propyl Acetate (**17**).* To a solution of **16** (190 mg, 0.65 mmol) in anhydrous DMSO (6.0 mL) was added 2-iodoxybenzoic acid (>39%, 633 mg, 0.88 mmol), and the mixture was stirred at rt under $N_2$. After being stirred for 2.5 h, the mixture was treated with water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water, aq $NaHCO_3$, and brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **17** (190 mg, 100%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): δ 10.12 (s, 1H), 8.29 (s, 1H), 8.10 (s, 1H), 6.73 (s, 1H), 4.12 (t, 2H, *J* = 6.7 Hz), 4.01 (s, 3H), 2.85 (t, 2H, *J* = 7.8 Hz), 2.09−2.01 (m, 5H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 185.6, 171.2, 154.0, 143.7, 141.7, 137.0, 136.7, 127.4, 116.5, 106.9, 63.8, 55.7, 32.8, 30.6, 21.0; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{15}H_{17}O_4S$, 293.0848; found, 293.0819.

**General Synthetic Procedure for Direct Arylation of Aryl Bromide and BT.** To a solution of **17** (251 mg, 0.86 mmol) in anhydrous toluene (8.0 mL) were added 4-bromo-2-methoxyphenyl acetate **26a** (382 mg, 1.8 mmol), $Pd(OAc)_2$ (37 mg, 0.16 mmol), $PCy_3$(65 mg, 0.23 mmol), PivOH (81 mg, 0.79 mmol), and $K_2CO_3$ (325 mg, 2.4 mmol), and the mixture was stirred at 110 °C in an oil bath under $N_2$. After being stirred for 13.0 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (4:1) to give **18** (175 mg, 65%).

*3-[2-(4-Acetoxy-3-methoxyphenyl)-3-formyl-7-methoxybenzo-[b]thiophen-5-yl]propyl Acetate (**18**).* A yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 10.07 (s, 1H), 8.20 (d, 1H, *J* = 1.4 Hz), 7.17−7.16 (m, 3H), 6.73 (d, 1H, *J* = 1.4 Hz), 4.13 (t, 2H, *J* = 6.6 Hz), 4.01 (s, 3H), 3.89 (s, 3H), 2.86 (t, 2H, *J* = 7.9 Hz), 2.37 (s, 3H), 2.10 (s, 3H), 2.08−2.03 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 186.9, 171.2, 168.8, 160.3, 153.6, 151.3, 141.9, 141.3, 138.7, 130.5, 130.5, 124.8, 123.3, 123.1, 117.0, 114.4, 106.8, 63.8, 56.1, 55.7, 32.9, 30.6, 21.0, 20.7; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{24}H_{25}O_7S$, 457.1321; found, 457.1307.

*3-[2-(3,4-Dimethoxyphenyl)-3-formyl-7-methoxybenzo[b]-thiophen-5-yl]propyl Acetate (**19**).* Compound **17** (15 mg, 0.052 mmol) and **26b** (46 mg, 0.21 mmol) were treated in the same manner as described above to produce **19** (13 mg, 56%) as a yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): δ 10.10 (s, 1H), 8.19 (s, 1H), 7.16 (dd, 1H, *J* = 8.2, 1.8 Hz), 7.09 (d, 1H, *J* = 1.8 Hz), 6.84 (d, 1H, *J* = 8.2 Hz), 6.72 (s, 1H), 4.13 (t, 2H, *J* = 6.7 Hz), 4.01 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 2.86 (t, 2H, *J* = 7.8 Hz), 2.10−2.02 (m, 5H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 187.3, 171.4, 161.8, 153.7, 150.9, 149.3, 141.9, 139.1, 124.6, 124.5, 123.8, 117.0, 113.4, 111.4, 106.8, 64.0, 56.3, 55.9, 33.0, 30.8, 21.2; HRMS-FAB (*m*/*z*): [M + H]+ calcd for $C_{23}H_{25}O_6S$, 429.1372; found, 429.1382.

*3-[3-Formyl-7-methoxy-2-(3,4,5-dimethoxyphenyl)benzo[b]-thiophen-5-yl]propyl Acetate (**20**).* Compound **17** (50 mg, 0.17 mmol) and **26c** (149 mg, 0.60 mmol) were treated in the same manner as described above to produce **20** (67 mg, 85%) as a yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 10.10 (s, 1H), 8.19 (s, 1H), 6.79 (s, 2H), 6.73 (s, 1H), 4.13 (t, 3H, *J* = 6.6 Hz), 4.02 (s, 3H), 3.94 (s, 3H), 3.92 (s, 6H), 2.86 (t, 2H, *J* = 6.6 Hz), 2.10 (s, 3H), 2.08−2.03 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 187.1, 171.4, 161.5, 153.7, 153.6, 142.0, 139.8, 139.0, 130.4, 127.3, 124.7, 117.1, 108.0, 106.9, 63.9, 61.2, 56.5, 55.9, 33.0, 30.8, 21.2; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{24}H_{26}O_7S$, 458.1399; found, 458.1398.

*3-[3-Formyl-7-methoxy-2-(3-nitrophenyl)benzo[b]thiophen-5-yl]propyl Acetate (**21**).* To dried KOAc were added **26d** (70 mg, 0.43 mmol) in DMA (4.0 mL), **17** (82 mg, 0.41 mmol), and $Pd(OAc)_2$ (0.45 mL, 0.002 mmol, 4.4 mM solution on DMA), and the mixture was stirred at 150 °C in an oil bath for 8 h under $N_2$. The reaction mixture was quenched with water and adjusted to pH 5 by the addition of saturated $NH_4Cl$ solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (6:1) to give **21** (70 mg, 40%) as a yellow oil. $^1$H NMR (600 MHz, $CDCl_3$): δ 10.03 (s, 1H), 8.48 (dd, 1H, *J* = 1.7 and 1.9 Hz), 8.39 (ddd, 1H, *J* = 8.1, 1.9, 1.0 Hz), 8.21 (s, 1H), 7.92 (ddd, 1H, *J* = 7.7, 1.7, 1.0 Hz), 7.73 (dd, 1H, *J* = 8.1, 7.7 Hz), 6.77 (s, 1H), 4.14 (t, 2H, *J* = 6.3 Hz), 4.03 (s, 3H), 2.87 (t, 2H, *J* = 7.7 Hz), 2.10 (s, 3H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 185.7, 171.2, 157.0, 153.6, 148.3, 142.4, 138.5, 136.2, 133.6, 131.2, 130.0, 125.10, 125.07, 124.5, 117.0, 107.2, 63.7, 55.9, 32.9, 30.6, 21.0; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{21}H_{20}O_6SN$, 414.1011; found, 414.1009.

**General Synthetic Procedure for Deprotection of Acetyl from Alcohol.** To a solution of **18** (9.0 mg, 0.02 mmol) in anhydrous MeOH (1.0 mL) and THF (0.5 mL) was added $K_2CO_3$ (22 mg, 0.16 mmol), and the mixture was stirred at rt under $N_2$. After being stirred for 1 h, the mixture was adjusted to pH 7 by the addition of saturated $NH_4Cl$ solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to afford **4** (7.2 mg, 95%).

*2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzo[b]thiophene-3-carbaldehyde (**4**).* Yellow solid. $^1$H NMR (400 MHz, $CDCl_3$): δ 10.05 (s, 1H), 8.19 (s, 1H), 7.12 (dd, 1H, *J* = 8.3, 2.1 Hz), 7.06 (d, 1H, *J* = 2.1 Hz), 7.04 (d, 1H, *J* = 8.3 Hz), 6.75 (s, 1H), 5.91 (bs, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.73 (t, 2H, *J* = 6.4 Hz), 2.88 (t, 2H, *J* = 7.8 Hz), 2.02−1.97 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): δ 187.1, 161.8, 153.5, 147.6, 146.7, 142.5, 138.9, 129.9, 124.3, 124.2, 123.9, 116.8, 114.8, 112.7, 106.8, 62.3, 56.2, 56.7, 34.7, 32.8; HRMS-FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{20}H_{21}O_5S$, 373.1110; found, 373.1103.

*2-(3,4-Dimethoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzo-[b]thiophene-3-carbaldehyde (**22**).* Compound **19** (11.2 mg, 0.026 mmol) was treated in the same manner as described above to produce **22** (10 mg, 99%) as a yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): δ 10.06 (s, 1H), 8.20 (s, 1H), 7.16 (dd, 1H, *J* = 8.4, 1.8 Hz), 7.09 (d, 1H, *J* = 1.8 Hz), 6.99 (d, 1H, *J* = 8.4 Hz), 6.75 (s, 1H), 4.01 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.73 (dt, 2H, *J* = 6.6, 4.8 Hz), 2.88 (t, 2H, *J* = 7.2 Hz), 2.02−1.97 (m, 2H), 1.36 (t, 1H, *J* = 4.8 Hz); $^{13}$C{$^1$H}


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NMR (100 MHz, $CDCl_3$): δ 187.1, 161.6, 153.5, 150.7, 149.1, 142.5, 138.9, 129.9, 124.4, 124.3, 123.6, 116.8, 113.2, 111.2, 106.8, 62.3, 56.1, 56.1, 55.7, 34.7, 32.8; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{21}H_{23}O_5S$, 387.1266; found, 387.1259.

*5-(3-Hydroxypropyl)-7-methoxy-2-(3,4,5-trimethoxyphenyl)-benzo[b]thiophene-3-carbaldehyde (**23**).* Compound **20** (67 mg, 0.026 mmol) was treated in the same manner as described above to produce **23** (49 mg, 78%) as a yellow solid. $^1H$ NMR (400 MHz, $CDCl_3$): δ 10.10 (s, 1H), 8.21 (s, 1H), 6.79 (s, 2H), 6.77 (s, 1H), 4.02 (s, 3H), 3.94 (s, 3H), 3.92 (s, 6H), 3.73 (m, 2H), 2.89 (t, 2H, $J$ = 7.8 Hz), 2.03−1.96 (m, 2H), 1.31 (brs, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 187.1, 161.5, 153.7, 153.5, 142.7, 139.8, 138.9, 130.4, 127.3, 124.5, 117.0, 108.0, 107.0, 62.4, 61.2, 56.5, 55.9, 34.8, 33.0; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{22}H_{25}O_6S$, 417.1372; found, 417.1376.

*5-(3-Hydroxypropyl)-7-methoxy-2-(3-nitrophenyl)benzo[b]-thiophen-3-carbaldehyde (**24**).* Compound **21** (10.4 mg, 0.025 mmol) was treated in the same manner as described above to produce **24** (9.9 mg, 100%) as a yellow oil. $^1H$ NMR (600 MHz, $CDCl_3$): δ 10.03 (s, 1H), 8.47 (dd, 1H, $J$ = 2.1, 1.5 Hz), 8.39 (ddd, 1H, $J$ = 8.3, 2.1, 1.0 Hz), 8.21 (s, 1H), 7.91 (ddd, 1H, $J$ = 7.8, 1.5, 1.0 Hz), 7.73 (dd, 1H, $J$ = 8.3, 7.8 Hz), 6.80 (d, 1H, $J$ = 1.0 Hz), 4.02 (s, 3H), 3.74 (t, 2H, $J$ = 6.4 Hz), 2.90 (t, 2H, $J$ = 7.8 Hz), 2.03−1.98 (m, 2H), 1.41 (s, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 185.7, 157.0, 153.6, 148.3, 143.1, 138.4, 136.2, 133.6, 131.1, 130.0, 125.06, 124.96, 124.5, 116.9, 107.4, 62.3, 55.8, 34.6, 32.8; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{19}H_{18}NO_5S$, 372.0906; found, 372.0922.

*3-Iodo-4-mercapto-5-methoxybenzaldehyde (**28**).* To a solution of 5-iodovanillin **27** (1.0 g, 3.7 mmol) in water (7.0 mL) was added KOH (4.4 mL, 4.4 mmol, 1.0 M in water) at 0 °C. After stirring for 30 min, dimethylthiocarbamoyl chloride (456 mg, 4.1 mmol) in THF (3.0 mL) was added slowly to the mixture. After stirring for 2 h, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (4:1) to give 4-(*N*,*N*-dimethylthiocarbamoyloxy)-3-iodo-5-methoxybenzaldehyde (1.24 g, 92%) as a colorless solid. $^1H$ NMR (600 MHz, $CDCl_3$): δ 9.89 (s, 1H), 7.89 (d, 1H, $J$ = 1.8 Hz), 8.39 (d, 1H, $J$ = 1.8 Hz), 3.90 (s, 3H), 3.49 (s, 3H), 3.43 (s, 3H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 189.9, 184.9, 153.3, 148.4, 135.9, 134.0, 111.2, 93.9, 56.6, 43.7, 39.3; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{11}H_{13}INO_3S$, 365.9661; found, 365.9655. 4-(*N*,*N*-dimethylthiocarbamoyloxy)-3-iodo-5-methoxybenzaldehyde (935 mg, 2.6 mmol) was irradiated in a microwave oven for 15.0 min at 220 °C. The solution was cooled and directly chromatographed on silica gel, eluting with hexane−EtOAc (3:1) to give 4-(*N*,*N*-dimethylcarbamoylthio)-3-iodo-5-methoxybenzaldehyde (654 mg, 70%) as a colorless solid. $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.91 (s, 1H), 8.02 (d, 1H, $J$ = 1.2 Hz), 7.42 (d, 1H, $J$ = 1.2 Hz), 3.92 (s, 3H), 3.18 (s, 3H), 3.03 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 190.3, 163.8, 161.2, 139.2, 134.4, 130.8, 111.5, 109.6, 57.0, 37.4, 37.2; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{11}H_{13}INO_3S$, 365.9661; found, 365.9675. 4-(*N*,*N*-dimethyl-carbamoylthio)-3-iodo-5-methoxybenzaldehyde (131 mg, 0.36 mmol) was dissolved in KOH (0.16 mL, 0.79 mmol, 5.0 M in water) and MeOH (5.0 mL). The mixture was heated to reflux for 3.0 h, and the reaction mixture was cooled and the MeOH was evaporated. The mixture and the organic layers were combined, washed with brine, dried with $Na_2SO_4$, filtered, and concentrated. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to afford **28** (38 mg, 36%, 60% brsm) as a yellow solid. $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.82 (s, 1H), 7.84 (d, 1H, $J$ = 1.6 Hz), 7.33 (d, 1H, $J$ = 1.6 Hz), 5.27 (s, 1H), 3.97 (s, 3H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 189.9, 154.4, 137.8, 135.1, 135.0, 107.4, 96.5, 56.9; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_8H_8IO_2S$, 294.9290; found, 294.9284.

*7-Methoxy-3-methylbenzo[b]thiophene-5-carbaldehyde (**29**).* Compound **28** (36 mg, 0.12 mmol) was suspended in aq NaOH solution (0.11 mL, 0.21 mmol, 1.9 M) and water (1.0 mL). To the mixture were added allyl bromide (16 μL, 0.19 mmol) in benzene (1.0 mL) and tetrabutylammonium bromide (4.1 mg, 0.013 mmol). After stirring at rt for 1.0 h, the mixture was poured into water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give 4-(allylthio)-3-iodo-5-methoxybenzaldehyde (33 mg, 79%) as a yellow solid. $^1H$ NMR (400 MHz, $CDCl_3$): δ 9.87 (s, 1H), 7.97 (d, 1H, $J$ = 1.2 Hz), 7.35 (d, 1H, $J$ = 1.2 Hz), 5.85−5.75 (m, 1H), 4.94−4.92 (m, 1H), 4.90−4.89 (m, 1H), 3.96 (s, 3H), 3.63 (dd, 2H, $J$ = 7.2, 0.8 Hz); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$): δ 190.2, 160.2, 137.7, 135.4, 134.8, 133.4, 117.7, 109.0, 108.7, 56.5, 38.3; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{11}H_{12}O_2SI$, 334.9603; found, 334.9614. To a solution of 4-(allylthio)-3-iodo-5-methoxybenzaldehyde (250 mg, 0.75 mmol) and $Pd(PPh_3)_4$ (44 mg, 0.038 mmol) in MeCN (20 mL) was added triethylamine (0.16 mL, 1.1 mmol) at rt. After stirring at 140 °C in an oil bath for 22 h, the mixture was cooled to rt and filtered with Celite. The residue was washed with EtOAc and the filtrate was evaporated. The resultant crude material was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **29** (65 mg, 42%) as a colorless solid. NMR (600 MHz, $CDCl_3$): δ 10.07 (s, 1H), 7.81 (d, 1H, $J$ = 1.2 Hz), 7.30 (d, 1H, $J$ = 1.2 Hz), 7.17 (q, 1H, $J$ = 1.8 Hz), 4.06 (s, 3H), 2.50 (d, 1H, $J$ = 1.8 Hz); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 192.3, 155.3, 141.3, 135.8, 135.2, 133.6, 123.7, 120.5, 100.9, 56.1, 14.1; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{11}H_{11}O_2S$, 207.0480; found, 207.0520.

*7-Methoxy-2-[3-methoxy-4,5-bis(methoxymethoxy)phenyl]-3-methylbenzo[b]thiophene-5-carbaldehyde (**30**).* Compound **29** (67 mg, 0.32 mmol), 5-iodo-1-methoxy-2,3-bis(methoxymethoxy)-benzene (58 mg, 0.16 mmol), silver oxide (38 mg, 0.16 mmol), sodium acetate (6.7 mg, 0.080 mmol), and $Pd(OAc)_2$ (0.15 mg, 0.4 mol %) were suspended in 1,1,1,3,3,3-hexafluoro-2-propanol (0.2 mL). The mixture was stirred at 35 °C in an oil bath for 18 h and filtered with Celite. The residue was washed with EtOAc and the filtrate was evaporated. The resultant crude material was purified by column chromatography on silica gel, eluting with hexane−EtOAc (4:1) to give **30** (36 mg, 51%, 100% brsm) as a colorless hard oil. $^1H$ NMR (600 MHz, $CDCl_3$): δ 10.08 (s, 1H), 7.82 (d, 1H, $J$ = 1.2 Hz), 7.31 (d, 1H, $J$ = 1.2 Hz), 7.01 (d, 1H, $J$ = 1.8 Hz), 6.80 (d, 1H, $J$ = 1.8 Hz), 5.25 (s, 2H), 5.21 (s, 2H), 4.07 (s, 3H), 3.90 (s, 3H), 3.66 (s, 3H), 3.53 (s, 3H), 2.54 (s, 3H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 192.3, 154.9, 153.6, 152.2, 142.8, 140.3, 135.8, 135.4, 134.2, 130.1, 128.6, 120.8, 111.2, 108.0, 101.4, 98.5, 95.7, 57.4, 56.5, 56.3, 56.2, 13.1; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{22}H_{24}O_7S$, 433.1321; found, 433.1336.

*5-(2-Hydroxyethyl)-7-methoxy-3-methyl-benzo[b]thiophene (**31**).* To a solution of trimethylphosphonium bromide (225 mg, 0.63 mmol) in THF (2.0 mL) was added *n*-BuLi (0.5 mL, 0.80 mmol, 1.6 M in hexane) at 0 °C. After stirring for 30 min, aldehyde **29** (65 mg, 0.32 mmol) in THF (1.0 mL) was added to the mixture. After stirring at rt for 1 h, the reaction mixture was concentrated and purified by column chromatography on silica gel, eluting with hexane−EtOAc (20:1) to give 7-methoxy-3-methyl-5-vinylbenzo[*b*]thiophene (29 mg, 45%) as a colorless solid. This intermediate (29 mg, 0.14 mmol) was dissolved in 9-BBN (4 mL, 2.0 mmol, 0.5 M in THF) and the mixture was refluxed at 80 °C in an oil bath for 1 h. After cooling to rt, EtOH (4 mL), aq NaOH solution (4 mL, 7 M), and $H_2O_2$ (4 mL, 30%) were added to the reaction mixture at 0 °C. After stirring at 50 °C in an oil bath for 1 h, saturated aq $NaHCO_3$ was added to the mixture, which was extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **31** (19 mg, 60%) as a colorless solid. $^1H$ NMR (400 MHz, $CDCl_3$): δ 7.19 (s, 1H), 7.05 (d, 1H, $J$ = 0.8 Hz), 6.67 (s, 1H), 3.99 (s, 3H), 3.93 (t, 2H, $J$ = 6.4 Hz), 3.00 (t, 2H, $J$ = 6.4 Hz), 2.41 (d, 3H, $J$ = 0.8 Hz), 1.52 (brs, 1H); $^{13}C\{^1H\}$ NMR (150 MHz, $CDCl_3$): δ 154.8, 141.8, 136.1, 132.5, 127.4, 122.4, 114.8, 105.6, 64.0, 55.8, 39.8, 14.2; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{12}H_{15}O_2S$, 223.0793; found, 223.0813.

*5-(2-Hydroxyethyl)-7-methoxy-2-(4-methoxyphenyl)-3-methyl-benzo[b]thiophene (**6**).* Compound **31** (19 mg, 0.085 mmol), 4-


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iodoanisole (10 mg, 0.043 mmol), silver oxide (10 mg, 0.043 mmol), sodium acetate (1.7 mg, 0.021 mmol), and $Pd(OAc)_2$ (0.38 mg, 4 mol %) were suspended in 1,1,1,3,3,3-hexafluoro-2-propanol (0.043 mL). The mixture was stirred at 35 °C in an oil bath for 18 h and filtered through Celite. The residue was washed with EtOAc and the filtrate was evaporated. The resultant crude material was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **6** (10 mg, 57%, 61% brsm) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.47 (d, 2H, $J$ = 8.8 Hz), 7.19 (s, 1H), 6.99 (d, 2H, $J$ = 8.8 Hz), 6.67 (s, 1H), 4.00 (s, 3H), 3.94 (dt, 2H, $J$ = 6.4, 4.8 Hz), 3.86 (s, 3H), 3.01 (t, 2H, $J$ = 6.4 Hz), 2.42 (s, 3H), 1.46 (t, 1H, $J$ = 4.8 Hz); $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ 159.5, 154.4, 143.4, 139.0, 136.3, 131.0, 127.4, 127.1, 115.1, 114.2, 105.7, 64.1, 55.9, 55.5, 39.9, 29.8, 13.0; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{19}H_{21}O_3S$, 329.1211; found, 329.1210.

*7-Methoxy-2-[3-methoxy-4,5-bis(methoxymethoxy)phenyl]-3-methyl-5-(1-propenyl)-benzo[b]thiophene (**32**).* To a solution of ethylphosphonium bromide (113 mg, 0.31 mmol) in THF (2.0 mL) was added PhLi (0.15 mL, 0.31 mmol, 2.0 M in dibutyl ether) at −78 °C. After stirring at rt for 15 min, aldehyde **30** (30 mg, 0.069 mmol) in THF (0.5 mL) was added to the mixture at −78 °C. After stirring for 15 min, the mixture was warmed to rt and then stirred for 30 min. To the reaction mixture were added conc. HCl (6.4 $\mu$L, 0.076 mmol) and KO*t*-Bu (9.3 mg, 0.083 mmol) at −78 °C, the mixture was warmed to rt and stirred for 15 min. To the mixture were added $H_2O_2$ (35 $\mu$L), water, and EtOAc, the organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was concentrated and purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **32** (25 mg, 80%, $E/Z$ = 2:1) as a colorless solid. **32**(***E***): $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.23 (s, 1H), 7.00 (d, 1H, $J$ = 1.6 Hz), 6.84 (s, 1H), 6.80 (d, 1H, $J$ = 1.6 Hz), 6.55 (dd, 1H, $J$ = 15.6, 1.2 Hz), 6.31 (dq, 1H, $J$ = 15.6, 6.4 Hz), 5.24 (s, 2H), 5.19 (s, 2H), 4.012 (s, 3H), 3.88 (s, 3H), 3.65 (s, 3H), 3.52 (s, 3H), 2.45 (s, 3H), 1.93 (dd, 3H, $J$ = 6.4, 1.6 Hz); $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ 154.3, 153.5, 151.0, 143.2, 138.7, 136.2, 135.4, 131.8, 130.9, 128.0, 126.2, 125.3, 113.0, 111.2, 108.0, 102.2, 98.5, 95.7, 57.3, 56.4, 56.2, 55.8, 18.6, 13.1; **32**(***Z***): $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.26 (s, 1H), 7.01 (d, 1H, $J$ = 2.0 Hz), 6.81 (d, 1H, $J$ = 2.0 Hz), 6.74 (s, 1H), 6.59 (dd, 1H, $J$ = 11.6, 2.0 Hz), 5.84 (dq, 1H, $J$ = 11.6, 6.8 Hz), 5.24 (s, 2H), 5.19 (s, 2H), 4.005 (s, 3H), 3.88 (s, 3H), 3.65 (s, 3H), 3.52 (s, 3H), 2.46 (s, 3H), 1.97 (dd, 3H, $J$ = 6.8, 2.0 Hz); $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$). As many peaks overlapped with **32**(***E***), different peaks are shown below. $\delta$ 153.9, 142.9, 135.6, 130.6, 126.6, 115.3, 105.8, 55.8, 14.9; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{24}H_{29}O_6S$, 445.1685; found, 445.1724.

*7-Methoxy-2-[3-methoxy-4,5-bis(hydroxy)phenyl]-3-methyl-5-(1-propenyl)-benzo[b]thiophene (**5**).* To a solution of compound **32** (25 mg, 0.056 mmol, $E/Z$ = 2:1) was added 1 M HCl (0.11 mL, 0.11 mmol), and the mixture was stirred at rt for 48 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was concentrated and purified by preparative thin-layer chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give **5** (20 mg. 100%, $E/Z$ = 2:1) as a colorless solid. Cis−trans isomers were separated using recycle high-performance liquid chromatography (HPLC) (CHRAL ART Cellulose-SC, YMC: 250 mm long × 10 mm inner diameter, OD: 254 nm) and eluted with hexane-isopropanol (80:20) (flow rate: 4.0 mL/min) to give **5**(***E***) (13 mg) and **5**(***Z***) (6.0 mg). **5**(***E***): $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.23 (s, 1H), 6.83 (s, 1H), 6.81 (d, 1H, $J$ = 1.8 Hz), 6.66 (d, 1H, $J$ = 1.8 Hz), 6.53 (dd, 1H, $J$ = 15.6, 1.8 Hz), 6.31 (dq, 1H, $J$ = 15.6, 6.6 Hz), 5.52 (s, 1H), 5.44 (s, 1H), 4.01 (s, 3H), 3.91 (s, 3H), 3.65 (s, 3H), 2.43 (s, 3H), 1.93 (dd, 3H, $J$ = 6.6, 1.8 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 154.3, 146.9, 143.9, 143.3, 138.9, 136.2, 132.5, 131.8, 127.6, 126.8, 126.1, 125.2, 113.0, 110.5, 105.1, 102.1, 56.4, 55.8, 18.6, 13.1; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{20}H_{21}O_4S$, 357.1161; found, 357.1177. **5**(***Z***): $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.25 (s, 1H), 6.82 (d, 1H, $J$ = 1.8 Hz), 6.73 (s, 1H), 6.67 (d, 1H, $J$ = 1.8 Hz), 6.59 (dd, 1H, $J$ = 11.4, 1.8 Hz), 5.84 (dq, 1H, $J$ = 11.4, 7.2 Hz), 5.51 (s, 1H), 5.42 (s, 1H), 4.00 (s, 3H), 3.92 (s, 3H), 2.44 (s, 3H), 1.98 (dd, 3H, $J$ = 7.2, 1.8 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 153.9, 147.0, 143.9, 143.0, 138.9, 135.6, 132.5, 130.6, 127.6, 126.8, 126.6, 125.6, 115.3, 110.5, 105.8, 105.1, 56.4, 55.8, 14.9, 13.1; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{20}H_{21}O_4S$, 357.1161; found, 357.1177.

*3-(2-(4-Acetoxy-3-methoxyphenyl)-3-(1,3-dioxolan-2-yl)-7-methoxybenzo[b]thiophen-5-yl)propyl Acetate (**33**).* To a solution of **18** (23 mg, 0.053 mmol) in anhydrous benzene (1.5 mL) were added *p*-TsOH·$H_2O$ (1.0 mg, 5.3 × 10$^{-3}$ mmol), MS 4 Å (31 mg), and ethylene glycol (44 $\mu$L, 0.79 mmol), and the mixture was refluxed under $N_2$. After being stirred for 1.0 h, the reaction mixture was diluted with EtOAc. The mixture was washed with water and brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (2:1) to afford **33** (27 mg, 100%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.46 (s, 1H), 7.17 (d, 1H, $J$ = 1.8 Hz), 7.14 (dd, 1H, $J$ = 8.1 and 1.8 Hz), 7.01 (d, 1H, $J$ = 8.1 Hz), 6.63 (s, 1H), 5.91 (s, 1H), 4.32−4.30 (m, 2H), 4.16 (t, 2H, $J$ = 6.5 Hz), 4.07−4.05 (m, 2H), 3.99 (s, 3H), 3.86 (s, 3H), 2.80 (t, 2H, $J$ = 7.9 Hz), 2.35 (s, 3H), 2.08 (s, 3H), 2.06−2.01 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.2, 169.0, 153.9, 150.9, 145.9, 140.1, 139.7, 139.4, 132.2, 126.8, 126.1, 122.8, 122.3, 116.0, 114.2, 105.4, 100.4, 65.4, 64.0, 56.0, 55.6, 32.9, 30.6, 21.0, 20.7; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{26}H_{29}O_8S$, 501.1583; found, 501.1602.

*3-[2-(4-Acetoxy-3-methoxyphenyl)-3-(acetoxymethyl)-7-methoxybenzo[b]thiophen-5-yl]propyl Acetate (**34**).* To a solution of **16** (134 mg, 0.53 mmol) in $CH_2Cl_2$ (10 mL) were added pyridine (90 $\mu$L, 0.98 mmol), DMAP (12 mg, 0.10 mmol), and $Ac_2O$ (0.11 mL, 1.2 mmol), and the mixture was stirred at rt. After being stirred for 2.5 h, DMAP (15 mg, 0.12 mmol) and $Ac_2O$ (45 $\mu$L, 0.48 mmol) were added and stirred for 30 min. The reaction mixture was poured into water and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (3:1) to afford 3-[3-(acetoxymethyl)-7-methoxybenzo[*b*]thiophen-5-yl]propyl acetate as a colorless oil (186 mg, 100%). $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.46 (s, 1H), 7.22 (s, 1H), 6.66 (s, 1H), 5.31 (s, 2H), 4.13 (t, 2H, $J$ = 6.5 Hz), 3.99 (s, 3H), 2.83 (t, 2H, $J$ = 7.7 Hz), 2.11 (s, 3H), 2.08 (s, 3H), 2.06−2.01 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.2, 171.0, 154.6, 139.8, 139.6, 131.0, 127.2, 127.1, 113.7, 105.5, 63.8, 60.2, 55.7, 32.8, 30.6, 21.0; HRMS-FAB ($m/z$): [M]$^+$ calcd for $C_{17}H_{20}O_5S$, 336.1031; found, 336.1030. 3-[3-(Acetoxymethyl)-7-methoxybenzo[*b*]thiophen-5-yl]propyl acetate (48 mg, 0.14 mmol) and **26a** (179 mg, 0.84 mmol) were treated in the same manner as described for the above direct arylation to produce **34** (35 mg, 49%) as colorless needles. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.26 (s, 1H), 7.21 (d, 1H, $J$ = 1.7 Hz), 7.16−7.12 (m, 2H), 6.67 (s, 1H), 5.32 (s, 2H), 4.13 (t, 2H, $J$ = 6.5 Hz), 4.01 (s, 3H), 3.87 (s, 3H), 2.84 (t, 2H, $J$ = 7.7 Hz), 2.35 (s, 3H), 2.11 (s, 3H), 2.09 (s, 3H), 2.06−2.01 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 170.9, 169.0, 154.2, 151.1, 145.0, 141.6, 140.1, 140.0, 132.3, 126.0, 123.1, 122.0, 114.3, 113.8, 105.8, 63.8, 58.5, 55.9, 55.7, 32.8, 30.6, 21.0, 20.7; HRMS-FAB ($m/z$): [M]$^+$ calcd for $C_{26}H_{28}O_8S$, 500.1505; found, 500.1528.

*3-[2-(4-Acetoxy-3-methoxyphenyl)-3-(hydroxymethyl)-7-methoxybenzo[b]thiophen-5-yl]propyl Acetate (**35**).* To a solution of **18** (16 mg, 0.039 mmol) in anhydrous THF (1.0 mL) was added sodium borohydride (2.1 mg, 0.055 mmol), and the mixture was stirred at 0 °C under $N_2$. After being stirred for 2.0 h, the reaction mixture was adjusted to pH 7 by the addition of saturated $NH_4Cl$ solution and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to afford **35** (15 mg, 85%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.36 (s, 1H), 7.29 (d, 1H, $J$ = 1.8 Hz), 7.21 (dd, 1H, $J$ = 8.0, 1.8 Hz), 7.12 (d, 1H, $J$ = 8.0 Hz), 6.65 (s, 1H), 4.86 (d, 2H, $J$ = 4.8 Hz), 4.14 (t, 2H, $J$ = 6.4 Hz), 4.01 (s, 3H), 3.88 (s, 3H), 2.84 (t, 2H, $J$ = 7.7 Hz), 2.35 (s, 3H), 2.07−2.02 (m, 5H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.3, 169.0, 154.2, 151.1, 143.3, 141.7, 140.0, 139.7, 132.7, 130.7, 125.7,


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123.0, 121.9, 114.2, 113.9, 105.6, 63.7, 57.0, 56.0, 55.7, 32.8, 30.5, 21.0, 20.7; HRMS-FAB ($m/z$): $[M]^+$ calcd for $C_{24}H_{26}O_7S$, 458.1399; found, 458.1398.

*4-[3-(Hydroxymethyl)-5-(3-hydroxypropyl)-7-methoxybenzo[b]-thiophen-2-yl]-2-methoxyphenol (**36**).* To a solution of **34** (16 mg, 0.033 mmol) in MeOH/THF (1:1, v/v, 1.0 mL) was added 2.0 M aq NaOH (0.14 mL, 0.28 mmol), and the mixture was stirred at rt. After being stirred for 3.5 h, the reaction mixture was adjusted to pH 4 by the addition of 1.0 M aq HCl and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was purified using column chromatography on silica gel, eluting with hexane−EtOAc (1:10) and HPLC (MeOH/water 3:1, 8 mL/min) to afford **36** (9.1 mg, 73%) as a colorless oil. $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 9.39 (s, 1H), 7.36 (s, 1H), 7.25 (d, 1H, $J$ = 1.8 Hz), 7.34 (dd, 1H, $J$ = 8.0, 1.8 Hz), 6.89 (d, 1H, $J$ = 8.0 Hz), 6.80 (s, 1H), 5.24 (t, 1H, $J$ = 5.2 Hz), 4.60 (d, 2H, $J$ = 5.2 Hz), 4.52 (t, 1H, $J$ = 5.2 Hz), 3.94 (s, 3H), 3.82 (s, 3H), 3.47 (dd, 2H, $J$ = 11.7, 6.2 Hz), 2.75 (t, 2H, $J$ = 7.7 Hz), 1.84−1.79 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, DMSO-$d_6$): $\delta$ 153.4, 147.7, 147.2, 142.2, 141.9, 140.5, 130.9, 124.7, 123.3, 122.0, 115.8, 114.7, 113.3, 105.8, 60.2, 55.7, 55.6, 55.3, 34.8, 32.4; HRMS-FAB ($m/z$): $[M]^+$ calcd for $C_{20}H_{22}O_5S$, 374.1188; found, 374.1183.

*2-(4-Acetoxy-3-methoxyphenyl)-5-(3-acetoxypropyl)-7-methoxybenzo[b]thiophene-3-carboxylic Acid (**37**).* To a solution of **18** (22 mg, 0.048 mmol) in acetone (1.0 mL) was added $KMnO_4$ (0.6 mL, 0.24 mmol, 0.40 M in water), and the mixture was stirred at 40 °C in an oil bath. After stirring for 8.0 h, the acetone was evaporated. The mixture was adjusted to pH 8 by the addition of 1.0 M aq $Na_2SO_3$ and 2.0 M aq NaOH and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined water layers were adjusted to pH 1−2 by the addition of 3.0 M aq HCl and extracted with $CH_2Cl_2$ (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:2) and recrystallized to afford a mixture of carboxylic acids (11.3 mg) as colorless needles. $^1$H NMR (400 MHz, $CDCl_3$, a mixture of two compounds, signals of the major are reported): $\delta$ 7.87 (s, 1H), 7.12−7.09 (m, 2H), 6.98 (d, 1H, $J$ = 8.3 Hz), 6.68 (s, 1H), 5.82 (s, 1H), 4.14 (t, 2H, $J$ = 6.4 Hz), 4.00 (s, 3H), 3.91 (s, 3H), 2.85 (t, 2H, $J$ = 7.8 Hz), 2.08−2.01 (m, 5H); HRMS-FAB ($m/z$): $[M + Na]^+$ calcd for $C_{22}H_{22}O_7SNa$, 453.0984; found, 453.0977. DMAP (0.31 mg, 2.6 × $10^{-3}$ mmol) and pyridine/$Ac_2O$ (1.9/2.2 $\mu$L) were added to the mixture (11.3 mg) and the reaction mixture was stirred at rt under $N_2$. After stirring for 1.0 h, the reaction was quenched with water and extracted with $CH_2Cl_2$ (2 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) and recrystallized to afford a carboxylic acid **37** (6.8 mg, 30% for two steps) as colorless needles. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.87 (s, 1H), 7.17 (d, 1H, $J$ = 1.8 Hz), 7.15 (dd, 1H, $J$ = 8.1, 1.8 Hz), 7.10 (d, 1H, $J$ = 8.1 Hz), 6.69 (s, 1H), 4.13 (t, 2H, $J$ = 6.5 Hz), 4.01 (s, 3H), 3.85 (s, 3H), 2.85 (t, 2H, $J$ = 7.7 Hz), 2.35 (s, 3H), 2.08 (s, 3H), 2.07−2.02 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.4, 169.0, 167.6, 154.3, 153.7, 150.8, 141.1, 140.6, 140.3, 132.7, 125.6, 122.7, 122.4, 121.9, 117.0, 114.3, 106.1, 64.0, 56.2, 55.9, 33.1, 30.8, 21.2, 20.9; HRMS-FAB ($m/z$): $[M]^+$ calcd for $C_{24}H_{24}O_8S$, 472.1192; found, 427.1175.

*Methyl 2-(4-Acetoxy-3-methoxyphenyl)-5-(3-acetoxypropyl)-7-methoxybenzo[b]thiophene-3-carboxylate (**38**).* $K_2CO_3$ (6.9 mg, 0.050 mmol) and MeI (1.0 mL, 0.040 M solution in anhydrous DMF) were added to compound **37** (6.8 mg, 0.015 mmol) and the reaction mixture was stirred at rt using a microvial. After stirring for 1.5 h, the reaction was quenched with water and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (2:1) and HPLC (MeOH/water 6:1, 8 mL/min) to afford **38** (3.3 mg, 45%) as colorless needles. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.74 (s, 1H), 7.12 (s, 1H), 7.088 (s, 1H), 7.087 (s, 1H), 6.67 (s, 1H), 4.14 (t, $J$ = 6.5 Hz), 4.00 (s, 3H), 3.86 (s, 3H), 3.76 (s, 3H), 2.84 (t, 2H, $J$ = 7.7 Hz), 2.35 (s, 3H), 2.09 (s, 3H), 2.07−2.02 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.2, 168.9, 164.6, 155.4, 153.6, 151.3, 150.6, 140.7, 140.23, 140.18, 132.9, 125.5, 123.2, 122.5, 122.0, 116.3, 113.7, 105.9, 63.9, 56.0, 55.7, 51.6, 32.9, 30.6, 21.0, 20.7; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{25}H_{27}O_8S$, 487.1427; found, 487.1422.

*3-[2-(4-Acetoxy-3-methoxyphenyl)-7-methoxy-3-(methylcarbamoyl)benzo[b]thiophen-5-yl]propyl Acetate (**39**).* To a solution of **37** (13 mg, 0.026 mmol) in MeCN (3.0 mL) were added *N*,*N*-diisopropylethylamine (11 $\mu$L, 0.066 mol) and HBTU (12 mg, 0.029 mmol), and the mixture was stirred at rt for 5 min. To the mixture was added 40% methylamine (6.1 mL, 0.078 mmol). After stirring for 3.0 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (2:1) to give **39** (10 mg, 78%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.48 (s, 1H), 7.20 (s, 1H), 7.18 (dd, 1H, $J$ = 8.0 and 2.4 Hz), 7.11 (d, 1H, $J$ = 8.0 Hz), 6.65 (d, 1H, $J$ = 0.8 Hz), 5.57 (q, 1H, $J$ = 4.8 Hz), 4.12 (t, 2H, $J$ = 6.4 Hz), 4.00 (s, 3H), 3.86 (s, 3H), 2.89 (d, 3H, $J$ = 4.8 Hz), 2.81 (t, 2H, $J$ = 7.2 Hz), 2.35 (s, 3H), 2.08 (s, 3H), 2.02 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.4, 169.2, 166.2, 154.0, 151.5, 143.4, 140.7, 140.61, 140.56, 132.1, 129.1, 125.7, 123.5, 121.5, 115.8, 113.2, 106.2, 64.0, 56.2, 55.9, 32.9, 30.8, 26.8, 21.2, 20.8; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{25}H_{28}NO_7S$, 486.1586; found, 486.1568.

*4-[3-(1-Hydroxyethyl)-5-(3-hydroxypropyl)-7-methoxybenzo[b]-thiophen-2-yl]-2-methoxyphenol (**40**).* MeMgBr (0.35 mL, 0.49 mmol, 1.4 M in THF/toluene (3:1)) was added dropwise to a solution of **4** (42 mg, 0.11 mmol) in THF (1.5 mL) at 0 °C. After stirring for 3 h, saturated $NH_4Cl$ solution was added to the reaction mixture and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to EtOAc only to give **40** (16 mg, 37%) as a colorless solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.69 (s, 1H), 6.99−6.94 (m, 3H), 6.66 (s, 1H), 5.72 (s, 1H), 5.29 (dq, 1H, $J$ = 6.6, 2.4 Hz), 3.99 (s, 3H), 3.92 (s, 3H), 3.74 (dt, 2H, $J$ = 5.4, 5.4 Hz), 2.86 (t, 2H, $J$ = 7.8 Hz), 1.99 (m, 2H), 1.86 (d, 1H, $J$ = 2.4 Hz), 1.73 (d, 3H, $J$ = 6.6 Hz), 1.31 (t, 1H, $J$ = 5.4 Hz); $^{13}$C{$^1$H} NMR (100 MHz, $CD_3OD$): $\delta$ 155.4, 148.9, 148.2, 141.6, 141.4, 141.0, 135.9, 127.2, 126.9, 123.9, 117.7, 116.3, 114.5, 106.1, 66.2, 62.3, 56.5, 56.0, 35.9, 33.8, 23.3; HRMS-FAB ($m/z$): $[M]^+$ calcd for $C_{21}H_{24}O_5S$, 388.1344; found, 388.1358.

*Methyl (E)-3-[2-(4-Hydroxy-3-methoxyphenyl)-5-(3-hydroxypropyl)-7-methoxybenzo[b]thiophen-3-yl]acrylate (**41**).* To a mixture of (methoxycarbonylmethyl)triphenylphosphonium bromide (76 mg, 0.18 mmol) and potassium *tert*-butoxide (25 mg, 0.21 mmol) was added **4** (40 mg, 0.11 mmol) in $CH_2Cl_2$ (6.0 mL). After refluxing at 60 °C in an oil bath for 2 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (2:1) to give **41** (38 mg, 78%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.98 (d, 1H, $J$ = 16.0 Hz), 7.49 (s, 1H), 7.06−7.00 (m, 3H), 6.71 (s, 1H), 6.53 (d, 1H, $J$ = 16.0 Hz), 5.80 (brs, 1H), 4.01 (s, 3H), 3.93 (s, 3H), 3.81 (s, 3H), 3.74 (t, 2H, $J$ = 6.0 Hz), 2.87 (t, 2H, $J$ = 7.6 Hz), 1.98 (m, 2H), 1.36 (brs, 1H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 168.1, 154.2, 148.5, 146.73, 146.67, 141.3, 140.1, 138.7, 126.5, 125.9, 125.5, 123.7, 119.0, 115.1, 114.9, 112.7, 106.0, 62.4, 56.2, 55.9, 51.8, 35.0, 33.1; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{23}H_{24}O_6S$, 428.1294; found, 428.1272.

*Methyl (E)-3-[5-(3-Hydroxypropyl)-7-methoxy-2-(3,4,5-trimethoxyphenyl)benzo[b]thiophen-3-yl]acrylate (**42**).* To a solution of **23** (49 mg, 0.12 mmol) in $CH_2Cl_2$ (10 mL) were added (methoxycarbonylmethyl)triphenylphosphonium bromide (76 mg, 0.18 mmol) and potassium carbonate (25 mg, 0.21 mmol). After refluxing at 60 °C in an oil bath for 19 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with


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hexane−EtOAc (1:1) to give **42** (51 mg, 93%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.95 (d, 1H, $J$ = 16.4 Hz), 7.50 (s, 1H), 6.74 (s, 2H), 6.73 (s, 1H), 6.55 (d, 1H, $J$ = 16.4 Hz), 4.02 (s, 3H), 3.93 (s, 3H), 3.89 (s, 3H), 3.82 (s, 3H), 3.74 (dt, 2H, $J$ = 6.0, 6.0 Hz), 2.88 (t, 2H, $J$ = 7.6 Hz), 1.99 (m, 2H), 1.34 (t, 1H, $J$ = 6.0 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 167.9, 154.2, 153.4, 148.0, 141.4, 140.0, 138.9, 138.6, 129.2, 126.9, 125.6, 119.3, 115.2, 107.5, 106.2 62.4, 61.1, 56.4, 55.9, 51.9, 34.9, 33.1; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{25}H_{28}O_7S$, 472.1556; found, 472.1534.

*Ethyl 3-(3-Formyl-7-methoxybenzo[b]thiophen-5-yl)propanoate (**43**).* To a stirred solution of **13** (55 mg, 0.16 mmol) in dry isopropyl alcohol (2.0 mL) was added 1.0 M hydrochloric acid/diethyl ether (0.26 mL, 0.26 mmol), and the mixture was stirred at 55 °C in an oil bath for 24 h under $N_2$. The reaction mixture was cooled to rt, adjusted to pH 7−8 by the addition of saturated $NaHCO_3$ solution, and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with brine, dried over $Na_2SO_4$, and concentrated in vacuo. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (2:1) to give ethyl 3-[3-(hydroxymethyl)-7-methoxybenzo[$b$]thiophen-5-yl]propanoate (41 mg, 88%) as a colorless oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.38 (s, 1H), 7.31 (s, 1H), 6.68 (s, 1H), 4.90 (d, 2H, $J$ = 5.7 Hz), 4.15 (dd, 2H, $J$ = 14.2, 7.3 Hz), 3.99 (s, 3H), 3.09 (t, 2H, $J$ = 7.8 Hz), 2.70 (t, 2H, $J$ = 7.8 Hz), 1.63 (t, 1H, $J$ = 5.7 Hz), 1.25 (t, 3H, $J$ = 7.3 Hz); HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{15}H_{19}O_4S$, 295.1004; found, 295.1015. To a solution of ethyl 3-[3-(hydroxymethyl)-7-methoxybenzo[$b$]thiophen-5-yl]propanoate (41 mg, 0.14 mmol) in anhydrous DMSO (1.0 mL) was added 2-iodoxybenzoic acid (72 mg, 0.26 mmol), and the mixture was stirred at rt under $N_2$. After being stirred for 1.5 h, the mixture was treated with water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water, aq $NaHCO_3$, and brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (8:1) to give **43** (40 mg, 100%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.1 (s, 1H), 8.28 (s, 1H), 8.10 (s, 1H), 6.77 (s, 1H), 4.15 (q, 2H, $J$ = 7.2 Hz), 4.00 (s, 3H), 3.11 (t, 2H, $J$ = 7.8 Hz), 2.71 (t, 2H, $J$ = 7.8 Hz), 1.26 (t, 3H, $J$ = 7.2 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 186.6, 172.8, 153.9, 143.7, 141.1, 137.0, 136.7, 127.6, 116.4, 106.9, 60.5, 55.7, 36.4, 31.5, 14.2; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{15}H_{17}O_4S$, 293.0848; found, 293.0860.

*Ethyl 3-[2-(4-Acetoxy-3-methoxyphenyl)-3-formyl-7-methoxybenzo[b]thiophen-5-yl]propyl Acetate (**44**).* To a solution of **43** (79 mg, 0.275 mmol) in anhydrous toluene (2.0 mL) were added **26a** (134 mg, 0.55 mmol), $Pd(OAc)_2$ (14.3 mg, 0.055 mmol), $PCy_3BF_4$ (30.5 mg, 0.083 mmol), PivOH (28.3 mg, 0.248 mmol), and $K_2CO_3$ (104 mg, 0.74 mmol), and the mixture was stirred at 110 °C in an oil bath under $N_2$. After being stirred for 24 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (5:1−3:1) to give **44** (99 mg, 80%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 10.07 (s, 1H), 8.21 (d, 1H, $J$ = 1.0 Hz), 7.172−7.166 (m, 2H), 7.15 (s, 1H), 6.77 (d, 1H, $J$ = 1.0 Hz), 4.16 (dd, 2H, $J$ = 14.3, 7.0 Hz), 4.00 (s, 3H), 3.89 (s, 3H), 3.12 (t, 2H, $J$ = 7.9 Hz), 2.72 (t, 2H, $J$ = 7.7 Hz), 2.37 (s, 3H), 1.27 (t, 3H, $J$ = 7.0 Hz); $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ 186.8, 172.9, 168.8, 160.3, 153.5, 151.3, 141.33, 141.25, 138.7, 130.5, 124.9, 123.3, 123.1, 116.8, 114.4, 106.9, 60.5, 56.1, 55.7, 36.4, 31.7, 20.7, 14.3; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{24}H_{25}O_7S$, 457.1321; found, 457.1337.

*Diethyl 3,3′-[3,3′-Diformyl-7,7′-dimethoxy-(2,2′-bibenzo[b]thiophene)-5,5′-diyl]dipropionate (**45**).* To a solution of **43** (11 mg, 0.038 mmol) in anhydrous toluene (1.0 mL) were added ethyl 3-(2-bromo-3-formyl-7-methoxybenzo[$b$]thiophen-5-yl)propanoate (22 mg, 0.058 mmol), $Pd(OAc)_2$ (1.6 mg, 0.0070 mmol), $PCy_3$ (2.5 mg, 0.0090 mmol), PivOH (3.8 mg, 0.037 mmol), and $K_2CO_3$ (19 mg, 0.14 mmol), and the mixture was stirred at 110 °C in an oil bath under $N_2$. After being stirred for 24 h, the mixture was treated with water and extracted with $CH_2Cl_2$ (3 × 10 mL). The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (4:1) and HPLC (acetone/water 4:1, 8 mL/min) to give **45** (6.1 mg, 26%) as a brown oil. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.07 (s, 2H), 8.24 (s, 2H), 6.84 (s, 2H), 4.17 (q, 4H, $J$ = 7.2 Hz), 4.02 (s, 6H), 3.14 (t, 4H, $J$ = 7.6 Hz), 2.73 (t, 4H, $J$ = 7.6 Hz), 1.28 (t, 6H, $J$ = 7.2 Hz); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 185.3, 172.7, 153.4, 146.9, 142.1, 138.0, 134.4, 126.8, 117.0, 107.8, 60.3, 55.8, 36.3, 31.6, 14.3; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{30}H_{31}O_8S_2$, 583.1460; found, 583.1452.

*3-[2-(4-Acetoxy-3-methoxyphenyl)-3-(1,3-dioxolan-2-yl)-7-methoxybenzofuran-5-yl]propyl Acetate (**52**).* To a solution of **51** (10 mg, 0.023 mmol) in toluene (1.5 mL) were added $p$-TsOH·$H_2O$ (1.2 mg, 6.9 $\mu$M) and ethylene glycol (19 $\mu$L, 0.34 mmol), and the mixture was refluxed in an oil bath using a Dean−Stark apparatus. After being refluxed for 9 h, the mixture was treated with water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with saturated $NaHCO_3$ solution and brine, dried over $MgSO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (2:1) to give **52** (8.3 mg, 75%) as a colorless oil. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.43 (d, 1H, $J$ = 1.8 Hz), 7.37 (dd, 1H, $J$ = 7.8, 1.8 Hz), 7.18 (d, 1H, $J$ = 1.2 Hz), 7.13 (d, 1H, $J$ = 7.8 Hz), 6.66 (d, 1H, $J$ = 1.2 Hz), 6.06 (s, 1H), 4.32−4.29 (m, 2H), 4.14 (t, 2H, $J$ = 6.6 Hz), 4.12−4.09 (m, 2H), 4.01 (s, 3H), 3.91 (s, 3H), 2.76 (t, 2H, $J$ = 7.8 Hz), 2.34 (s, 3H), 2.08 (s, 3H), 2.04−1.99 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 171.2, 168.9, 155.0, 151.2, 144.9, 142.4, 140.6, 137.2, 128.8, 128.6, 123.0, 121.0, 113.0, 112.9, 112.3, 107.8, 99.8, 65.5, 64.0, 56.13, 56.10, 32.7, 30.8, 21.0, 20.7; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_{26}H_{29}O_9$, 485.1812; found, 485.1803.

**Experimental Procedure for Known Compounds.** *4-(N,N-Dimethyl-carbamoylthio)-3-methoxybenzaldehyde (**8**).*[9a] A solution of dimethylthiocarbamoyl chloride (0.17 mL, 0.66 mmol, 3.9 M in THF) was slowly added to a stirred, ice-cooled solution of vanillin **7** (101 mg, 0.66 mmol) and KOH (0.66 mL, 0.66 mmol, 1.0 M in water) at 0 °C. The formed suspension was stirred in the cooling bath for 15 min and next at room temperature. After being stirred for 2.5 h, a solution of dimethylthiocarbamoyl chloride (0.08 mL, 0.31 mmol, 3.9 M in THF) was slowly added to the stirred solution. After being stirred for 30 min, the mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $Na_2SO_4$, and evaporated. The residue was purified by column chromatography on silica gel, eluting with hexane−EtOAc (3:1) to give 4-(*N*,*N*-dimethylthiocarbamoyloxy)-3-methoxybenzaldehyde (136 mg, 86%) along with recovered starting material **7** (5 mg, 5%). Colorless solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 9.96 (s, 1H), 7.51−7.49 (m, 2H), 7.23 (dd, 1H, $J$ = 6.5 and 0.7 Hz), 3.90 (s, 3H), 3.47 (s, 3H), 3.38 (s, 3H); 4-(*N*,*N*-dimethylthiocarbamoyloxy)-3-methoxybenzaldehyde (2.30 g, 9.6 mmol) in diphenyl ether (15.0 mL) was irradiated in a microwave oven for 15.0 min at 220 °C. The solution was cooled and directly chromatographed on silica gel to give **8** (2.22 g, 97%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.00 (s, 1H), 7.70 (d, 1H, $J$ = 7.8 Hz), 7.48−7.45 (m, 2H), 3.95 (s, 3H), 3.15 (s, 3H), 3.03 (s, 3H).

*4-Mercapto-3-methoxybenzaldehyde (**9**).*[9a] Compound **8** (2.22 g, 9.3 mmol) was dissolved in KOH (4.1 mL, 20.5 mmol, 5.0 M in water) and MeOH (20.0 mL). The mixture was heated to reflux for 1.0 h, and the reaction mixture was cooled and the MeOH was evaporated. The mixture and the organic layers were combined, washed with brine, dried with $Na_2SO_4$, filtered, and concentrated. The residue was chromatographed on silica gel, eluting with hexane−EtOAc (1:1) to afford **9** (1.55 g, 99%) as a yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 9.89 (s, 1H), 7.40 (dd, 1H, $J$ = 6.9, 1.4 Hz), 7.36−7.34 (m, 2H), 4.17 (s, 1H), 3.98 (s, 3H). $^{13}$C{$^1$H} NMR (100 MHz, $CDCl_3$): $\delta$ 189.9, 154.4, 137.8, 135.1, 135.0, 107.4, 96.5, 56.9; HRMS-FAB ($m/z$): [M + H]$^+$ calcd for $C_8H_8IO_2S$, 294.9290; found, 294.9284.

*Methyl (2R,3R)-2-(4-Hydroxy-3-methoxyphenyl)-7-methoxy-5-[(E)-3-methoxy-3-oxoprop-1-en-1-yl]-2,3-dihydrobenzofuran-3-carboxylate (**47**).*[14] To a solution of methyl ferulate **46** (2.1 g, 10.0 mmol) in benzene/methanol = 2:1 (30 mL) was added $Ag_2O$ (1.4 g,


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6.0 mmol), and the mixture was stirred at room temperature. After the reaction was complete, the mixture was filtered, concentrated, and purified by chromatography on silica gel, eluting with hexane−EtOAc (3:1) to afford **47** (1.00 g, 48%) as a colorless solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 7.65 (d, 1H, $J$ = 16.2 Hz), 7.19 (s, 1H), 7.02, (s, 1H), 6.93−6.89 (m, 3H), 6.32 (d, 1H, $J$ = 16.2 Hz), 6.11 (d, 1H, $J$ = 7.8 Hz), 5.64 (s, 1H), 4.35 (d, 1H, $J$ = 7.8 Hz), 3.92 (s, 3H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H).

*Methyl (E)-2-(4-Acetoxy-3-methoxyphenyl)-7-methoxy-5-(3-methoxy-3-oxoprop-1-en-1-yl)benzofuran-3-carboxylate (**48**).*[14] To a solution of **47** (1.0 g, 2.4 mmol) in pyridine (2.8 mL) were added $Ac_2O$ (2.8 mL) and DMAP (29 mg, 0.24 mmol), and the mixture was stirred at room temperature. After the reaction was complete, the mixture was poured into ice cold water and extracted with EtOAc. The organic layer was washed with 1 N HCl, sat. $NaHCO_3$, water, and brine, dried over $MgSO_4$, and evaporated. The obtained compound was dissolved in benzene (43 mL), and to the mixture was added DDQ (1.36 g, 6.0 mmol). After refluxing for 84 h, the mixture was evaporated. The residue was dissolved in EtOAc, washed with sat. $NaHCO_3$ and brine, dried over $MgSO_4$, concentrated, and purified by chromatography on silica gel, eluting with hexane−EtOAc (3:1) to afford **48** (533 mg, 50% from **47**) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.84 (s, 1H), 7.82 (d, 1H, $J$ = 16.0 Hz), 7.81 (s, 1H), 7.68 (d, 1H, $J$ = 8.0 Hz), 7.15 (d,1H, $J$ = 8.0 Hz), 7.04 (s, 1H), 6.47 (d, 1H, $J$ = 16.0 Hz), 4.06 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.84 (s, 3H), 2.35 (s, 3H).

*Methyl 2-[4-(Benzyloxy)-3-methoxyphenyl]-7-methoxy-5-(3-methoxy-3-oxopropyl)-benzofuran-3-carboxylate (**49**).*[14] To a solution of **48** (550 mg, 1.2 mmol) in MeOH (30 mL) were added Pd−C and $p$-TsOH (50 mg, 0.26 mmol) under a $N_2$ atmosphere. After charging with $H_2$, the mixture was stirred at 40 °C in an oil bath overnight and filtered with Celite. To the filtrate was added sat. $NaHCO_3$, and the mixture was stirred at room temperature for 1 h. After evaporation, the residue was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The obtained compound was dissolved in 2-butanone (50 mL) and to the solution were added BnBr (154 $\mu$L, 1.3 mmol) and $K_2CO_3$ (224 mg, 1.6 mmol). The mixture was refluxed overnight, to which water (100 mL) was added, extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified by chromatography on silica gel, eluting with hexane−EtOAc (3:1) to afford **49** (534 mg, 87% from **48**) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 7.70 (d, 1H, $J$ = 1.6 Hz) 7.60 (dd, 1H, $J$ = 8.8, 1.6 Hz), 7.46−7.43 (m, 3H), 7.38 (t, 2H, $J$ = 8.0 Hz), 7.31 (t, 1H, $J$ = 8.0 Hz), 6.96 (d, 1H, $J$ = 8.8 Hz), 6.70 (s, 1H), 5.24 (s, 2H), 4.01 (s, 3H), 3.98 (s, 3H), 3.94 (s, 3H), 3.70 (s, 3H), 3.06 (t, 2H, $J$ = 8.0 Hz), 2.71 (t, 2H, $J$ = 8.0 Hz).

*2-[4-(Benzyloxy)-3-methoxyphenyl]-5-(3-hydroxypropyl)-7-methoxybenzofuran-3-carbaldehyde (**50**).*[14] To a solution of **49** (100 mg, 0.20 mmol) in THF (10 mL) was added $LiAlH_4$ (1.0 M solution in THF) (1.0 mL, 1.0 mmol) at −5 °C and the mixture was stirred for 10 min and then warmed to room temperature. After the reaction was complete, aqueous THF and sat. $NH_4Cl$ were added. The mixture was extracted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The obtained compound was dissolved in EtOAc (15 mL) at 40 °C in an oil bath, and to the solution was added $MnO_2$ (3.0 g, 35 mmol). The mixture was stirred at room temperature for 20 min, filtered, and concentrated. The residue was purified by chromatography on silica gel, eluting with hexane−EtOAc (1:1) to afford **50** (78 mg, 88% from **49**) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.28 (s, 1H), 7.76 (s, 1H), 7.48−7.32 (m, 7H), 7.02 (d, 1H, $J$ = 8.0 Hz), 6.76 (s, 1H), 5.26 (s, 2H), 4.03 (s, 3H), 3.99 (s, 3H), 3.72 (dt, 2H, $J$ = 4.8, 5.6 Hz), 2.83 (t, 2H, $J$ = 8.0 Hz), 1.97 (m, 2H).

*Salvinal (**1**).*[14] To a solution of **50** (14 mg, 0.030 mmol) in $CH_2Cl_2$ was added $TiCl_4$ (0.3 mL, 2.7 mmol), and the mixture was stirred for 20 min at room temperature. The reaction was quenched with ice and 3 N HCl, and stirred for 30 min. The mixture was extracted with EtOAc, washed with sat. $NaHCO_3$, water, and brine, dried over $MgSO_4$, and concentrated. The residue was purified by chromatography on silica gel, eluting with hexane−EtOAc (1:1) to afford **1** (10 mg, 90%) as a pale yellow solid. $^1$H NMR (600 MHz, $CDCl_3$): $\delta$ 10.29 (s, 1H), 7.67 (d, 1H, $J$ = 1.2 Hz), 7.40 (dd, 1H, $J$ = 8.4, 2.4 Hz), 7.38 (d, 1H, $J$ = 2.4 Hz), 7.08 (d, 1H, $J$ = 8.4 Hz), 6.76 (d, 1H, $J$ = 1.2 Hz), 5.98 (s, 1H), 4.03 (s, 3H), 4.01 (s, 3H), 3.72 (t, 2H, $J$ = 6.0 Hz), 2.84 (t, 2H, $J$ = 7.8 Hz), 1.94 (m, 2H).

*3-[2-(4-Acetoxy-3-methoxyphenyl)-3-formyl-7-methoxybenzofuran-5-yl]propyl Acetate (**51**).*[16] To a solution of **1** (10 mg, 0.028 mmol) in $CH_2Cl_2$ (0.5 mL) were added pyridine (7.9 $\mu$L, 0.098 mmol), DMAP (1.7 mg, 0.014 mmol), and $Ac_2O$ (8.0 $\mu$L, 0.084 mmol), and the mixture was stirred at rt. After being stirred for 1 h, the reaction mixture was poured into water and extracted with EtOAc (3 × 10 mL). The combined organic layers were washed with 1 N HCl, saturated $NaHCO_3$ solution, water, and brine, dried over $MgSO_4$, and concentrated in vacuo to give **51** (12 mg, 98%) as a colorless solid. $^1$H NMR (400 MHz, $CDCl_3$): $\delta$ 10.33 (s, 1H), 7.68 (d, 1H, $J$ = 1.2 Hz), 7.46−7.43 (m, 2H), 7.22 (d, 1H, $J$ = 8.4 Hz), 6.75 (d, 1H, $J$ = 1.2 Hz), 4.12 (t, 2H, $J$ = 6.8 Hz), 4.04 (s, 3H), 3.95 (s, 3H), 2.81 (t, 2H, $J$ = 7.6 Hz), 2.37 (s, 3H), 2.09 (s, 3H), 2.07−2.00 (m, 2H); $^{13}$C{$^1$H} NMR (150 MHz, $CDCl_3$): $\delta$ 186.6, 171.2, 168.6, 164.7, 151.7, 144.7, 142.2, 142.0, 139.5, 127.2, 127.1, 123.6, 122.2, 117.7, 113.7, 112.7, 109.1, 63.8, 56.3, 56.1, 32.6, 30.7, 21.0, 20.7; HRMS-FAB ($m/z$): $[M + H]^+$ calcd for $C_{24}H_{25}O_8$, 441.1549; found, 441.1551.

**Antiproliferative Activity Assay.** The cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 25 mM $N$-(2-hydroxyethyl)piperazine-$N'$-ethanesulfonic acid (HEPES), 0.25% sodium bicarbonate, 10% fetal bovine serum, and 1× antibiotic−antimycotic (Gibco). Freshly trypsinized cell suspensions were seeded in 96-well microtiter plates at densities of 4000−11 000 cells per well, with compounds added from DMSO stock solutions and then successively diluted into the medium. The highest concentration of DMSO in the cultures (0.4% v/v) was used without the effect on cell growth under the culture conditions. After 72 h in culture, the attached cells were fixed in cold 10% trichloroacetic acid and then stained with 0.04% sulforhodamine B. The absorbance at 515 nm was measured using a microplate reader (ELx800, BioTek) after solubilizing the bound dye. The mean $IC_{50}$ is the concentration of the agent that reduced the cell growth by 50% under the experimental conditions and is the average from at least three independent and similar determinations.

**Flow Cytometric Analysis.** KB-VIN and MDA-MB-231 ($7 \times 10^4$ cells/well) cells were seeded in a 12-well plate 24 h prior to treatment with the compound for 24 or 48 h. **1** was used at 7.5 $\mu$M (1 × $IC_{50}$) or 22.5 $\mu$M (3 × $IC_{50}$) for MDA-MB-231 and 5 $\mu$M (1 × $IC_{50}$) or 15 $\mu$M (3 × $IC_{50}$) for KB-VIN. **4** was used at 0.95 $\mu$M (1 × $IC_{50}$) or 2.85 $\mu$M (3 × $IC_{50}$) for MDA-MB-231 and 0.57 $\mu$M (1 × $IC_{50}$) or 1.71 $\mu$M (3 × $IC_{50}$) for KB-VIN. **33** was used at 8.5 $\mu$M (1 × $IC_{50}$) or 25.5 $\mu$M (3 × $IC_{50}$) for MDA-MB-231 and 5 $\mu$M (1 × $IC_{50}$) or 15 $\mu$M (3 × $IC_{50}$) for KB-VIN. 25 $\mu$M or 35 $\mu$M 5-fluorouracil (5-FU) was used at 1 × $IC_{50}$ for MDA-MB-231 or KB-VIN, respectively. Combretastatin A-4 (CA-4) was used at 0.1 $\mu$M (3 × $IC_{50}$). The harvested and 70% EtOH-fixed cells were stained with propidium iodide (PI) containing RNase (BD Bioscience) subjected to flow cytometry (LSRFortessa operated by FACS Diva software, BD Bioscience).

**Immunocytochemistry.** MDA-MB-231 cells ($2.4 \times 10^4$ cells/well) were seeded in an eight-well chamber slide (Lab-Tech) for 24 h prior to treatment with the compound for 24 h. **1** or **33** was used at 22.5 or 25.5 $\mu$M (3 × $IC_{50}$), respectively. CA-4 was used at 0.1 $\mu$M (3 × $IC_{50}$) as a control for the tubulin polymerization inhibitor. Treated cells were fixed in 4% paraformaldehyde in PBS and stained with monoclonal antibody to $\alpha$-tubulin (B5-1-2, Sigma) and rabbit immunoglobulin G (IgG) to Ser10-phosphorylated histone H3 (p-H3) (#06-570, EMD Millipore), followed by labeling with fluorescein isothiocyanate (FITC)-conjugated antibody to mouse IgG (Sigma) and Alexa Fluor 549-conjugated antibody to rabbit IgG (Life Technologies), and DAPI for DNA as described previously.[17] The stained cells were observed using a confocal laser-scanning microscope (LSM700, Zeiss). The represented images were a projection of 16−18 optical sections processed by ZEN software (Zeiss). Final images were prepared using Adobe Photoshop.


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## ■ ASSOCIATED CONTENT

### Supporting Information

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Experimental procedure for known compounds and NMR spectra for new compounds (PDF)

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### Notes

The authors declare no competing financial interest.

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