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CONCISE SYNTHESIS AND EVALUATION OF *ORTHO*-NAPHTHOQUINONES CONTAINING A PHENOLIC HYDROXY MOIETY

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Abstract – A concise and efficient synthesis method for the preparation of anti-proliferative *ortho*-naphthoquinones is described. Notably, the synthesis of *ortho*-furanonaphthoquinone was achieved by utilizing a regioselective oxidative conjugate addition of dimethylamine and the Sonogashira coupling/cyclization reaction as the key steps. Additionally, an improved synthesis of hydroxy-β-lapachone was established and included a regioselective prenylation by directed *ortho*-lithiation. In vitro antiproliferative effects of the synthesized against a panel of 39 human cancer cell lines were evaluated and the results were directly compared to those previously obtained for **1**.

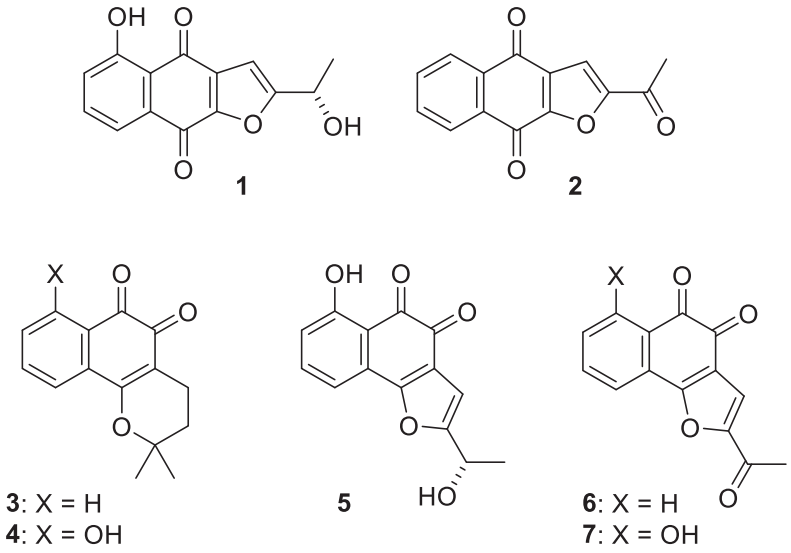
INTRODUCTION

Tabebuia avellanedae LORENTZ *ex* GRISEB[1] (Bignoniaceae) (syn. *Tabebuia impetiginosa*) is a well-known traditional medicine, which has been utilized in South America, particularly throughout Brazil and North Argentina since the Inca Era.[2] Remarkably, the stem bark of this plant shows a wide array of biological activities, such as antitumor, antibacterial, antifungal, and anti-inflammatory effects.[3] The discovery of its antitumor activities has made *T. avellanedae* an important medicinal resource.[4] A series of naphthoquinones and anthraquinones as well as a number of simple benzoic acid derivatives, lignans, and iridoid glycosides have been isolated as secondary metabolites of this plant species.[5] Consequently, the recent focus of our research involves evaluation of the bioactive constituents isolated from *T. avellanedae*.[6] Among these natural products, we previously reported the synthesis of (–)-5-hydroxy-2-(1-hydoxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**), which exhibited potent antiproliferative activity against various tumor cells.[7]

Furthermore, β-lapachone (**3**) is a natural naphthoquinone isolated from *T. avellanedae* and has also been reported as an effective antitumor agent.[8] Importantly, its prodrug is currently in clinical trials for the treatment of advanced solid tumors and pancreatic cancer.[9,10] In 2012, Padrón et al. reported the synthesis of a series of α- and β-lapachones containing hydroxy or methoxy functionalities on the benzene ring.[11] The conducted SAR study indicates that the antiproliferative activity is favored by naphthoquinones possessing a hydroxy moiety on the aromatic ring in close proximity to the quinone oxygen. The assessment of the antiproliferative activities of this series of analogs in human solid tumor cell lines provided 7-hydroxy-β-lapachone (**4**) as the lead compound, which displayed enhanced activity in comparison to the parent drug β-lapachone (**3**). Moreover, the same group also determined that the formation of reactive oxygen species (ROS) and DNA damage are the critical factors in the cellular toxicity caused by exposure to the β-lapachone analog. It is suggested that this may be the leading mechanism of action of this compound. Nevertheless, the drawback of β-lapachone analogs bearing phenolic hydroxy groups, including **4**, was a low synthetic yield in the steps involving the introduction of the prenyl moiety to the substrate quinones.

Additionally, *para*-furanonaphthoquinones, such as napabucasin (**2**), are also known to be constituents of *T. avellanedae*. Recent research has demonstrated that **2** is a potent inhibitor of stem cell activity in cancer cells.[12] Its mode of action is associated with inhibiting the signal transducer and activator of transcription 3 (STAT3) pathway.[13] More recently, Müller et al. described the synthesis of *ortho*-furanonaphthoquinones, including isonapabucasin (**6**), as well as *in vitro* evaluation of these compounds against human cancer cells.[14] The group demonstrated that **6** was twice as potent against STAT3 as napabucasin (**2**) in a homogeneous time-resolved fluorescence assay, with an IC_{50} value in the submicromolar range. These results are in good agreement with the potency of **2** and **6** to inhibit the growth of MDA-MB-231 cells. Although introduction of the phenolic hydroxy group on the aromatic ring in close proximity to the quinone oxygen is crucial for antiproliferative activities, *ortho*-furanonaphthoquinones, such as **7**, have not been synthesized.

Based on the promising results of the above studies and motivated by the significance of naphthoquinone derivatives, we decided to explore the synthesis and antiproliferative properties of *ortho*-furanonaphthoquinones bearing a phenolic hydroxy moiety. Thus, in the present study, we describe a concise synthetic method for the preparation of *ortho*-furanonaphthoquinone and β-lapachone analogs bearing a phenolic hydroxy group. *In vitro* antiproliferative activities of the synthesized analogs utilizing a panel of 39 human cancer cell lines were also evaluated and the results were directly compared to those previously obtained for **1**.

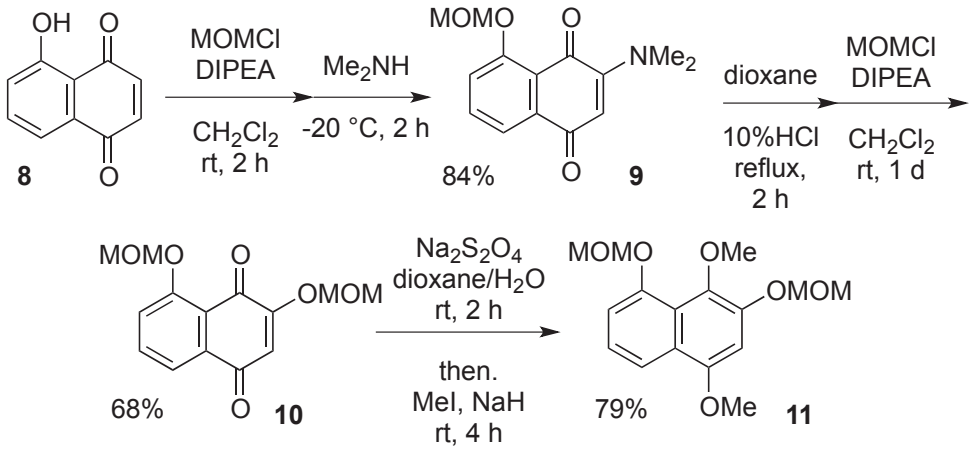


Scheme 1. Structures of *para*- and *ortho*-naphthoquinones

RESULTS AND DISCUSSION

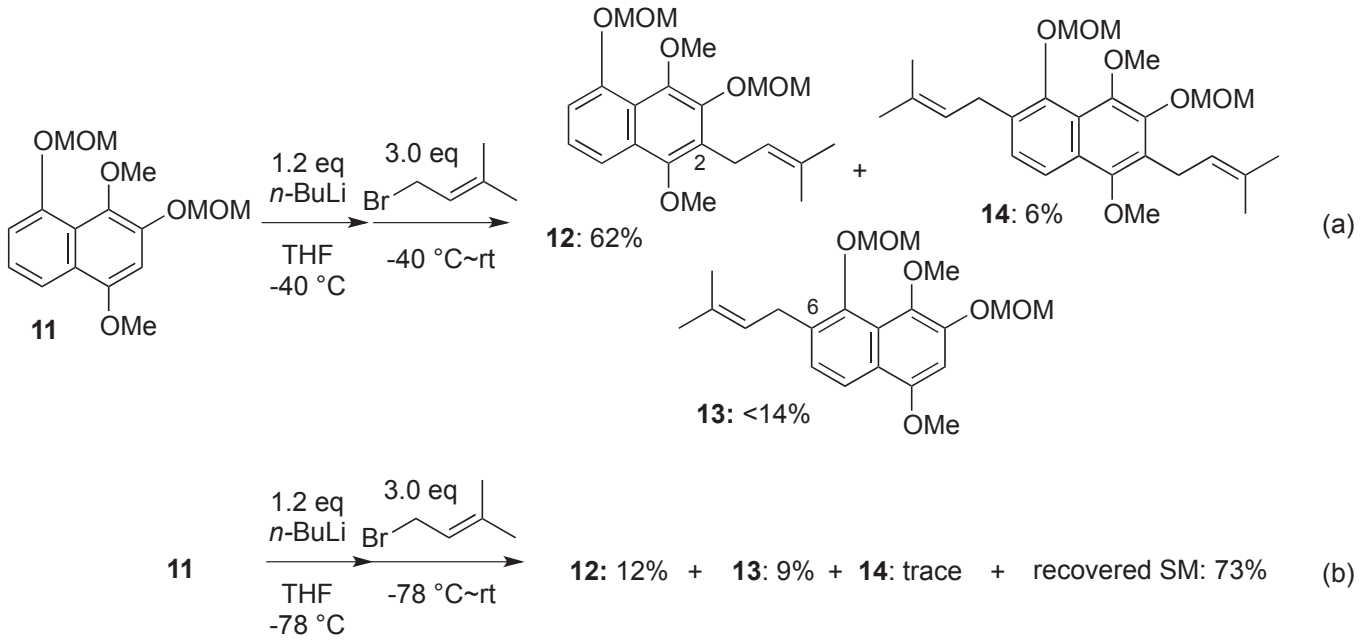
During the initial assessment of the previously reported synthetic methods for the preparation of **4**,[15] we unexpectedly encountered low chemical yields associated with the introduction of the prenyl group. Moreover, the purification of the resulting prenylated compounds by silica gel column chromatography was also challenging due to the physical properties of the analogs. Consequently, in the first instance, we focused on exploring improved synthesis methods for **4**. Based on the analysis of the approaches described in the literature, we decided to apply regioselective prenylation by directed *ortho*-lithiation as the key step.[16]

The regioselective oxidative conjugate addition of dimethylamine to MOM-protected juglone, which was synthesized from commercially available juglone (**8**) and MOMCl, gave the conjugate adduct **9** in 84% yield over 2 steps.[17] The hydrolysis of dimethylamine **9** using 10% HCl in refluxing dioxane, followed by a MOM protection of the two hydroxy groups afforded **10** in 68% yield. Reduction of the quinone with $Na_2S_2O_4$, followed by protection of the phenolic hydroxy moieties afforded **11** in a good overall yield (Scheme 2).



Scheme 2. Synthetic route to the key intermediate **11**

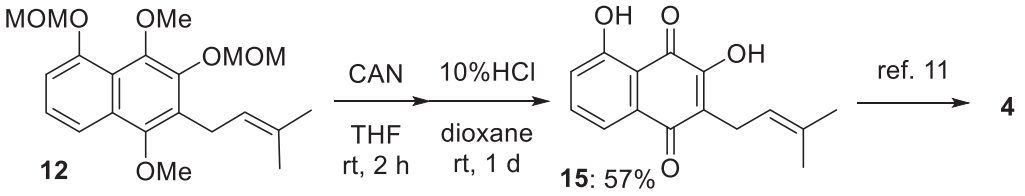
The following regioselective prenylation involving a directed *ortho*-lithiation gave the desired compound **12** in 62% yield as the major product. The formation of the 6-prenylated product **13** and 2,6-bis-prenylated product **14** as minor products was also observed (Scheme 3a). Under the described conditions, selection of the optimal reaction temperature was crucial for the success of the *ortho*-lithiation. At −78 °C, the *ortho*-lithiation reaction did not proceeded well and the starting material was recovered in 73% yield (Scheme 3b).



Scheme 3. Regioselective prenylation involving a directed *ortho*-lithiation

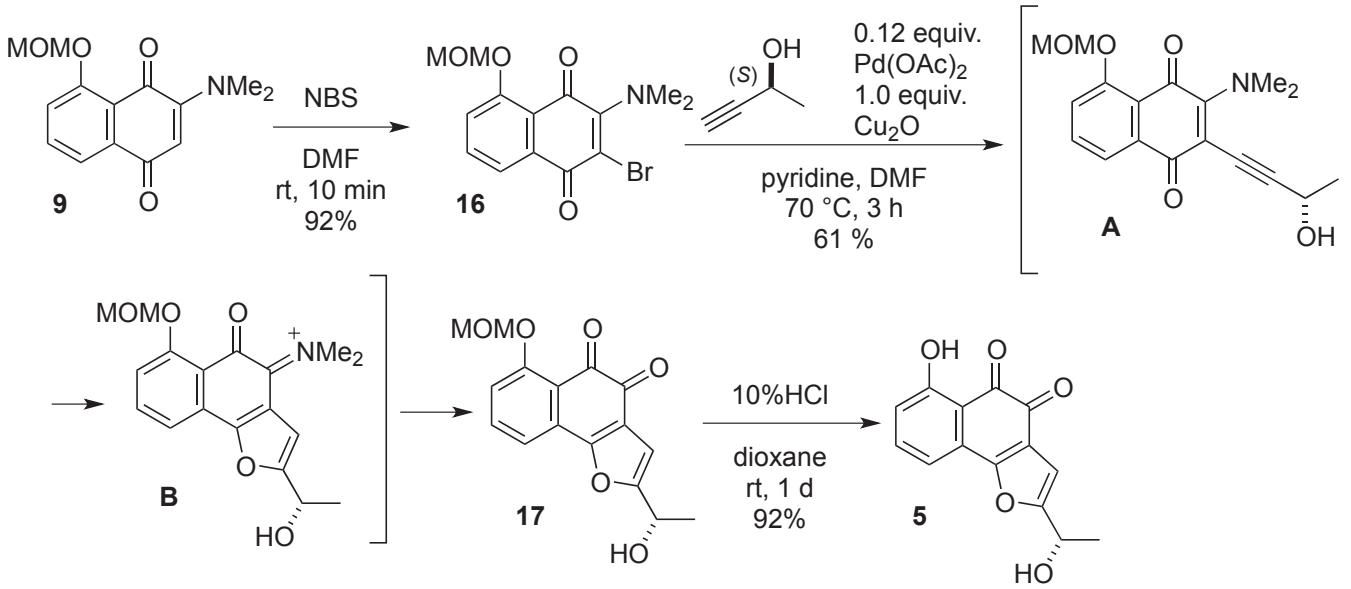
Oxidation of **12** with CAN, followed by exposure of the crude reaction mixture to 10% HCl successfully afforded the deprotected *para*-naphthoquinone **15**. According to the previously reported procedure,[11] treatment of **15** with methanesulfonic acid led to the formation of cyclized *ortho*-naphthoquinone **4**, along

with a small amount of *para*-naphthoquinone. The mixture of products was easily separated by silica gel column chromatography (Scheme 4).



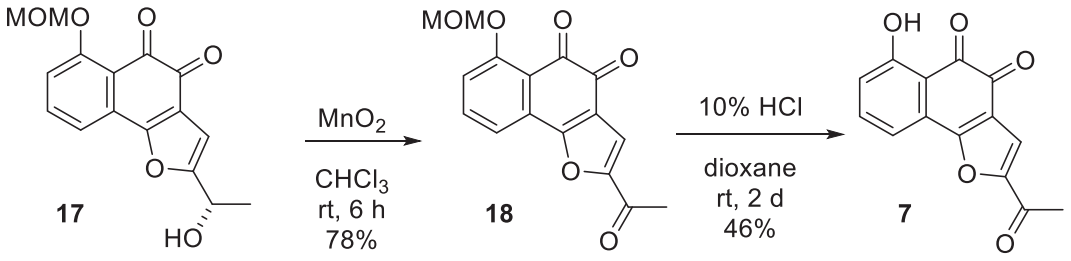
Scheme 4. Synthetic route to **4**

Subsequently, our focus shifted to the development of a method for the synthesis of *ortho*-furanonaphthoquinones **5**. In the first step, bromination of **9** afforded compound **16** in 92% yield. We have previously reported that halogenated quinones reacted smoothly with terminal acetylenes to give the desired coupling products under the optimized Sonogashira coupling reaction conditions.[18] During the investigation of the Sonogashira coupling reaction, *ortho*-quinone **17** was unexpectedly obtained as a sole product under heating (70 °C). We speculate that the reaction proceeds through intermediates **A** and **B**. Ultimately, removal of the MOM protecting group from the phenolic MOM ether under acidic conditions afforded the desired product **5** in 92% yield (Scheme 5).



Scheme 5. Synthetic route to **5**

Furthermore, oxidation of compound **17** with 10 equivalents of MnO_2, followed by the removal of the MOM group under the aforementioned reaction conditions, afforded ketone **7** (Scheme 6).



Scheme 6. Synthetic route to **7**

Further evaluation involved utilizing the COMPARE analysis to confirm the anticancer effects of **1**, **4**, **5**, and **7** and to estimate the correlation coefficient between the fingerprints of the test analogs and those of various reference compounds. It is well established in a panel of 39 human cancer cell lines (JFCR39) that a pair of compounds can potentially share the same mode of action when they have fingerprints and growth inhibitory profiles across JFCR39, which closely resemble each other. The COMPARE analysis is carried out by calculation of the Pearson correlation coefficient (*r* value) between the fingerprints of compounds X and Y.[19-21] Figure **1** demonstrates 50% growth inhibitory concentrations (GI_{50}) relative to the control. In these investigations, furanonaphtoquinones **1**, **5**, and **7** exhibited relatively strong inhibitory growth activity against a large number of cancer cell lines. Moreover, the compounds were more potent than β-lapachone-type naphtoquinone **4** against nearly all of the investigated cancer cell lines (mean of $LogGI_{50}$ values for all 39 cell lines (MG-MID) of **1**: −6.28, **4**: −5.98, **5**: −6.23, **7**: −6.43). The COMPARE analysis revealed that the fingerprints of compounds **1** and **5** correlated with those of Dolastatin and Navelbine, respectively, which are known as tubulin polymerization inhibitors (**1**: r = 0.596, **5**: r = 0.521). In addition, compound **4** correlated with Interferon-α (r = 0.58), whereas analog **7** did not correlate with any molecular target ($r < 0.5$). Generally, an r value of $0.5 < r < 0.75$ between two agents suggests that they might have a similar mechanism of action. Thus, these data suggest that *para*-naphthoquinone **1** and the corresponding *ortho*-naphthoquinone **5** may exhibit analogous modes of action. We recently reported that compound **1** displays potent STAT3 phosphorylation inhibitory activities.[22] Conversely, *ortho*-naphthoquinone **3** shows no inhibitory activity against phosphor-STAT3, even at 10 μM. As mentioned above, both napabucasin (**2**) and isonapabucasin (**6**) were identified to target the STAT3 phosphorylation. Hence, compounds containing the furanonaphthoquinone scaffold, both *para*- and *ortho*-quinones, including analog **5**, are suspected to exhibit STAT3 phosphorylation inhibitory activities. It has been reported that redox activation of β-lapachone (**3**) by NAD(P)H:quinone oxidoreductase 1 (NQO1) to generate ROS appears to account for its anticancer properties.[23] Compound **4** also displays a similar mechanism of action.[11]

MDA-MB-231 breast cancer cells have been reported to exhibit elevated levels of phosphorylated STAT3[24,25] and are widely utilized as a convenient model for the identification of STAT3 inhibitors.

Conversely, MCF-7 cells exhibit low levels of phosphorylated STAT3. Contrary to our expectation based on these reports, compounds **1**, **5**, and **7** were determined to show higher growth inhibitory effects against MCF-7 (Log GI_{50} (M) of **1**: −6.33, **5**: −6.67, **7**: −6.7) than against the MDA-MB-231 cells (Log GI_{50} (M) of **1**: −6.07, **5**: −6.33, **7**: −6.3). This implied the existence of other molecular targets and/or mechanisms of action for the furanonaphthoquinone analogs containing a phenolic hydroxy moiety. Thus, the detailed mechanism of these compounds remains unclear at this stage and further studies are necessary to elucidate the precise mode of action.

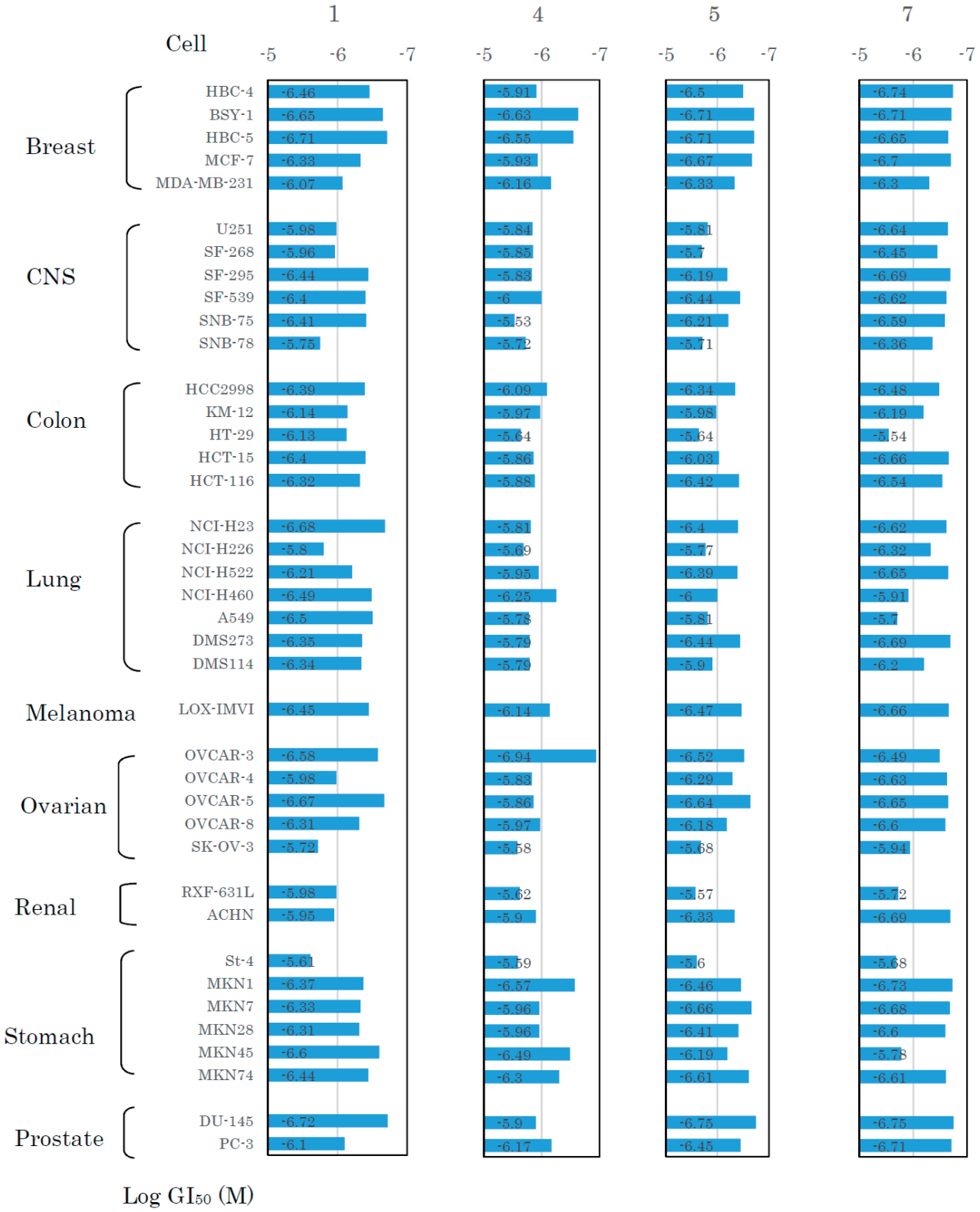


Figure 1. Growth inhibitory profiles of **1**, **4**, **5**, and **7** across the JFCR39 cell lines. Cell growth inhibition was assessed by measuring the changes in the total cellular protein levels following treatment with the given test compounds for 48 h using the sulforhodamine B colorimetric assay. Fingerprints were produced by processing 50% growth inhibition (GI_{50}) values using a computer. The x-axis represents the logarithm of GI_{50} values for the 39 cell lines. CNS = central nervous system.

EXPERIMENTAL

^{1}H- and ^{13}C-NMR spectra were acquired with Bruker-Biospin Avance III 400 MHz NMR spectrometer and taken in $CDCl_3$, unless otherwise noted. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Coupling constants J values are presented in Hz. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were recorded with a Shimadzu IRAffinty-1S spectrometer. IR spectroscopy of oil sample was measured as neat liquid film. The wave-numbers of maximum absorption peaks of IR spectroscopy are presented in cm–1. MS (ESI) is presented in m/z. Extracts were washed with brine and then dried over sodium sulfate. Silica gel column chromatography was used for purification. Compound **1**[6] was prepared according to a known literature procedure. Compounds **8** and (*S*)-3-butyn-2-ol were purchased from Tokyo Chemical Industry Co., Ltd.

2-(Dimethylamino)-8-(methoxymethoxy)naphthalene-1,4-dione (9). To a solution of **8** (4.35 g, 25 mmol) and DIPEA (10.9 mL, 62.5 mmol) in CH_2Cl_2 (40 mL) was added MOMCl (4.75 mL, 62.5 mmol) at 0 °C. After completion of reaction, dimethylamine (2.0 M in THF, 37.5 mL, 75 mmol) was added dropwise at –20 °C. After stirred for additional 2 h at –20 °C, the mixture was concentrated. The crude product was chromatographed on silica gel. Yield 84% (5.47 g). Yellow solid. Rf (hexane/EtOAc = 1/2) = 0.5. ^{1}H-NMR: δ 7.75 (d, 1H, *J* = 7.7 Hz), 7.57 (dd, 1H, *J* = 8.4, 7.7 Hz), 7.37 (d, 1H, *J* = 8.4 Hz), 5.74 (s, 1H), 5.32 (s, 2H), 3.55 (s, 3H), 3.16 (s, 6H). ^{13}C-NMR: δ 182.8 (C), 182.3 (C), 156.4 (C), 155.5 (C), 135.3 (C), 134.5 (CH), 122.1 (C), 120.0 (CH), 119.4 (CH), 104.5 (CH), 95.1 (CH_2), 56.5 (CH_3), 42.1 (CH_3). IR: 3066, 3034, 2924, 2855, 1748, 1263, 1192, 914, 843, 756, 698. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{14}H_{15}NNaO_4]^+$, 284.0899; Found, 284.0891.

2,8-Bis(methoxymethoxy)naphthalene-1,4-dione (10). To a solution of **9** (6.87 g, 23.9 mmol) in dioxane (36 mL) were added 10% HCl (4.0 mL). The mixture was stirred under reflux. After 2 h, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was used in next reaction without further purification. To a solution of crude and DIPEA (7.21 mL, 41.4 mmol) in CH_2Cl_2 (40 mL) was added MOMCl (2.62 mL, 34.5 mmol) at 0 °C. After 1 d, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 68% (1.3 g). Yellow solid. Rf (hexane/EtOAc = 2/1) = 0.25. ^{1}H-NMR: δ 7.80 (d, *J* = 7.5 Hz, 1H), 7.64 (dd, *J* = 7.5, 8.5 Hz, 1H), 7.47 (d, *J* = 8.5 Hz, 1H), 6.38 (s, 1H), 5.35 (s, 2H), 5.28 (s, 2H), 3.56 (s, 3H), 3.52 (s, 3H). ^{13}C-NMR: δ 184.9 (C), 178.7 (C), 158.3 (C), 157.5 (C), 135.1 (CH), 134.1 (C), 121.7 (CH), 120.2 (CH), 119.9 (C), 111.4 (CH), 95.1 (CH_2), 94.8

(CH_2), 56.7 (CH_3), 57.1 (CH_3). IR: 3853, 1680, 1653, 1616, 1585, 1149, 1149, 1024, 979. HRMS (ESI) m/z: $[M+Na]^+$ calcd for $[C_{14}H_{14}NaO_6]^+$, 301.0688; Found, 301.0689.

1,4-Dimethoxy-2,8-bis(methoxymethoxy)naphthalene (11). To a solution of **10** (1.39 g, 5.0 mmol) in dioxane (10 mL) and H_2O (10 mL) were added $Na_2S_2O_4$ (1.74 g, 10 mmol). After stirred for 2 h, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was used in the next step without further purification. To a solution of the crude product in DMF (20 mL) were added MeI (1.25 mL, 20 mmol) and NaH (360 mg, 20 mmol). After stirred for 4 h, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 79% (1.2 g). Colorless oil. Rf (hexane/EtOAc = 2/1) = 0.60. ^{1}H-NMR: δ 7.89 (d, *J* = 8.3 Hz, 1H), 7.25 (dd, *J* = 7.7, 8.3 Hz, 1H), 7.14 (d, *J* = 7.7 Hz, 1H), 6.82 (s, 1H), 5.30 (s, 2H), 5.29 (s, 2H), 3.96 (s, 3H), 3.86 (s, 3H), 3.60 (s, 3H), 3.59 (s, 3H). ^{13}C-NMR: δ 152.7 (C), 152.2 (C), 147.5 (C), 138.3 (C), 124.7 (C), 123.9 (CH), 122.1 (C), 116.7 (CH), 114.1 (CH), 98.7 (CH), 96.7 (CH_2), 96.7 (CH_2), 61.8 (CH_3), 56.5 (CH_3), 56.4 (CH_3), 55.8 (CH_3). IR: 2931, 1598, 1423, 1357, 1257, 1151, 1055, 1020, 808, 761. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{16}H_{20}NaO_6]^+$, 331.1158; Found, 331.1155.

1,4-Dimethoxy-3,5-bis(methoxymethoxy)-2-(3-methylbut-2-en-1-yl)naphthalene (12). A solution of **11** (420 mg, 1.36 mmol) in THF (5.0 mL) was added to a solution of BuLi (1.6 M in hexane, 1.28 mL, 2.0 mmol) at -40 °C. After stirred for 1 h, 1-bromo-3-methyl-2-butene (0.47 mL, 4.1 mmol) was added dropwise. After stirred for 1 d at rt, the mixture was treated with aqueous NH_4Cl solution, and extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 62% (317 mg). Colorless oil. Rf (hexane/EtOAc = 4/1) = 0.25. ^{1}H-NMR: δ 7.71 (dd, *J* = 8.6, 1.1 Hz, 1H), 7.30 (dd, *J* = 8.6, 7.6 Hz, 1H), 7.10 (dd, *J* = 7.6, 1.1 Hz, 1H), 5.32 (s, 2H), 5.30 (m, 1H), 5.24 (s, 2H), 3.86 (s, 3H), 3.86 (s, 3H), 3.61 (s, 3H), 3.59 (s, 3H), 3.59 (m, 2H), 1.83 (s, 3H), 1.70 (s, 3H). ^{13}C-NMR: δ 153.2 (C), 150.2 (C), 147.4 (C), 144.1 (C), 131.6 (C), 128.2 (C), 127.8 (C), 125.0 (CH), 123.0 (CH), 120.5 (C), 116.5 (CH), 111.7 (CH), 100.0 (CH_2), 96.1 (CH_2), 62.0 (CH_3), 61.5 (CH_3), 57.6 (CH_3), 56.4 (CH_3), 25.7 (CH_3), 24.3 (CH_2), 18.0 (CH_3). IR: 1713, 1261, 1231, 1136, 1171, 1130, 750, 698. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{21}H_{28}NaO_6]$+, 399.1784; Found, 399.1778.

1,4-Dimethoxy-2,8-bis(methoxymethoxy)-7-(3-methylbut-2-en-1-yl)naphthalene (13). Yield 14% (70 mg). Colorless oil. Rf (hexane/EtOAc = 4/1) = 0.20. ^{1}H-NMR: δ 7.91 (d, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 8.8

Hz, 1H), 6.78 (s, 1H), 5.38 (m, 1H), 5.31 (s, 2H), 5.07 (s, 2H), 3.95 (s, 3H), 3.80 (s, 3H), 3.63 (d, J = 7.0 Hz, 2H), 3.61 (s, 3H), 3.58 (s, 3H), 1.77 (s, 3H), 1.76 (s, 3H). ^{13}C-NMR: δ 152.4 (C), 148.8 (C), 147.5 (C), 136.8 (C), 133.3 (C), 132.4 (C), 125.6 (CH), 123.2 (C), 123.1 (C), 123.0 (CH), 118.2 (CH), 101.4 (CH_2), 97.1 (CH), 96.2 (CH_2), 61.6 (CH_3), 57.6 (CH_3), 56.2 (CH_3), 55.6 (CH_3), 28.5 (CH_2), 25.6 (CH_3), 17.8 (CH_3). IR: 2932, 1601, 1450, 1354, 1153, 1042, 976, 822, 733. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{21}H_{28}NaO_6]^+$, 399.1784; Found, 399.1786.

1,4-Dimethoxy-3,5-bis(methoxymethoxy)-2,6-bis(3-methylbut-2-en-1-yl)naphthalene (14). Yield 6% (36 mg). Colorless oil. Rf (hexane/EtOAc = 4/1) = 0.30. ^{1}H-NMR: δ 7.75 (d, J = 9.3 Hz, 1H), 7.24 (d, J = 9.3 Hz, 1H), 5.38 (m, 1H), 5.29 (m, 1H), 5.25 (s, 2H), 5.03 (s, 2H), 3.85 (s, 3H), 3.79 (s, 3H), 3.62 (d, J = 6.6 Hz, 2H), 3.62 (s, 3H), 3.61 (s, 3H), 3.57 (d, J = 6.6 Hz, 2H), 1.82 (s, 3H), 1.78 (s, 3H), 1.76 (s, 3H), 1.69 (s, 3H). ^{13}C-NMR: δ 150.5 (C), 149.2 (C), 147.5 (C), 140.0 (C), 132.7 (C), 132.1 (C), 131.5 (C), 127.1 (C), 126.9 (CH), 126.6 (C), 123.2 (CH), 123.2 (CH), 122.5 (C), 118.5 (CH), 101.3 (CH_2), 100.0 (CH_2), 62.1 (CH_3), 61.5 (CH_3), 57.7 (CH_3), 57.6 (CH_3), 28.6 (CH_2), 25.9 (CH_3), 25.7 (CH_3), 24.2 (CH_2), 18.0 (CH_3), 18.0 (CH_3). IR: 2932, 1593, 1447, 1385, 1339, 1157, 1041. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{26}H_{36}NaO_6]^+$, 467.2400; Found, 467.2410.

5-Hydroxy-2-(3-methylbut-2-en-1-yl)naphthalene-1,4-dione (15).[15] To a solution of **9** (373 mg, 1.0 mmol) in THF (20 mL) was added cerium(IV) ammonium nitrate (1.37 mg, 2.5 mmol) in one portion. The resulting solution was stirred at rt for 2 h. The reaction mixture was diluted by addition of H_2O, and extracted with EtOAc. The organic extracts were washed with brine, and then dried over Na_2SO_4. The crude product was used in the next reaction without further purification. To a solution of crude in dioxane (36 mL) was added 10% HCl (4.0 mL). After stirred for 1 d at rt, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 57% (148 mg). pale yellow solid. Rf (hexane/EtOAc = 4/1) = 0.40. ^{1}H-NMR: δ 11.10 (s, 1H), 7.62 (m, 2H), 7.19 (m, 2H), 5.18 (t, J = 7.3 Hz, 1H), 3.29 (d, J = 7.4 Hz, 1H), 1.78 (s, 3H), 1.68 (s, 3H). ^{13}C-NMR: δ 185.0, 183.7, 161.1, 152.4, 137.5, 134.0, 132.6, 124.7, 123.1, 119.6, 119.4, 112.9, 25.8, 22.6, 17.8.

7-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene-5,6-dione (4).[11] To a solution of **15** (285 mg, 1.1 mmol) in CH_2Cl_2 (5.0 mL) at –20 °C was added methanesulfonic acid (720 mg, 11 mmol) in one portion. After it was stirred for 0.5 h at –20 °C, the mixture was quenched by addition of H_2O, and extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 51% (146 mg). red solid. Rf

(hexane/EtOAc = 4/1) = 0.30. ^{1}H-NMR: δ 11.99 (s, 1H), 7.52 (dd, *J* = 7.6, 8.5 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 1H), 2.55 (t, *J* = 6.9 Hz, 2H), 1.83 (t, *J* = 6.9 Hz, 2H), 1.45(s, 6H). ^{13}C-NMR: δ 183.2, 178.2, 164.4, 161.6, 137.9, 132.4, 121.5, 116.8, 113.6, 112.8, 79.3, 31.5, 26.7, 16.2.

2-Bromo-3-(dimethylamino)-5-(methoxymethoxy)naphthalene-1,4-dione (16). To a stirred solution of **9** (1.5 g, 5.7 mmol) in dry DMF (27 mL) at rt was added NBS (1.12 g, 6.3 mmol). After stirred for 10 min at rt, the reaction was quenched by addition of H_2O, and extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 92% (1.80 g). Orange solid. Rf (hexane/EtOAc = 1/2) = 0.60. ^{1}H-NMR: δ 7.82 (d, 1H, *J* = 7.7 Hz), 7.56 (dd, 1H, *J* = 7.7, 7.5 Hz), 7.41 (d, 1H, *J* = 7.5 Hz), 5.34 (s, 2H), 3.54 (s, 3H), 3.25 (s, 6H). ^{13}C-NMR: δ 180.7 (C), 178.0 (C), 156.6 (C), 155.5 (C), 134.6 (CH), 133.6 (C), 120.9 (C), 120.6 (CH), 120.6 (CH), 109.2 (C), 95.0 (CH_2), 56.6 (CH_3), 44.7 (CH_3). IR (KBr): 1678, 1639, 1569, 1469, 1367, 1296, 1261, 1240, 1203, 1085, 1033, 985, 923, 902, 835, 786, 752, 734, 549. HRMS (ESI) m/z: $[M+Na]^+$ calcd for $[C_{14}H_{14}NNaO_4Br]^+$, 362.0004; Found, 361.9998.

(*S*)-2-(1-Hydroxyethyl)-6-(methoxymethoxy)naphtho[1,2-*b*]furan-4,5-dione (17). Under Ar atmosphere, a mixture of Cu_2O (72 mg, 0.50 mmol), (*S*)-3-butyn-2-ol (117 μL, 1.50 mmol), and pyridine (8.0 mL, 100 mmol) was stirred for 1 h at rt. A solution of **16** (170 mg, 0.50 mmol) and $Pd(OAc)_2$ (13.4 mg, 0.060 mmol) in DMF (5.0 mL) was added to this suspension, and the reaction mixture was stirred for 3 h at 70 °C. The mixture was filtered through a pad of Celite. The Celite was washed with EtOAc, and the combined organics were concentrated. The crude product was diluted with EtOAc. The organic phase were washed with H_2O and brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 61% (93 mg). Orange solid. Rf (hexane/EtOAc = 1/2) = 0.35. ^{1}H-NMR: δ 7.54 (dd, 1H, *J* = 7.6, 7.5 Hz), 7.41 (d, 1H, *J* = 7.5 Hz), 7.24 (d, 1H, *J* = 7.6 Hz), 6.68 (s, 1H), 5.35 (s, 2H), 4.96 (q, 1H, *J* = 6.6 Hz), 3.54 (s, 3H), 2.03 (s, 1H), 1.62 (d, 3H, *J* = 6.6 Hz). ^{13}C-NMR (DMSO): δ 179.3 (C), 174.3 (C), 160.9 (C), 160.4 (C), 160.0 (C), 136.5 (CH), 130.0 (C), 121.5 (C), 118.6 (CH), 117.4 (C), 116.1 (CH), 103.9 (CH), 94.7 (CH_2), 61.5 (CH), 56.8 (CH_3), 21.3 (CH_3). IR (KBr): 3487, 1676, 1562, 1458, 1404, 1373, 1261, 1234, 1195, 1153, 1087, 1062, 1010, 964, 923, 900, 881, 840, 790, 704, 528. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{16}H_{14}NaO_6]^+$, 325.0688; Found, 325.0689.

(*S*)-6-Hydroxy-2-(1-hydroxyethyl)naphtho[1,2-*b*]furan-4,5-dione (5). To a solution of **17** (128 mg, 0.4 mmol) in dioxane (12 mL) was added 10% HCl (1.2 mL). After stirred for 1 d at rt, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 92% (95 mg).

Reddish solid. Rf (hexane/EtOAc = 1/2) = 0.50. ^{1}H-NMR: δ 11.98 (s, 1H), 7.52 (dd, 1H, *J* = 7.3, 8.8 Hz), 7.25 (d, 1H, *J* = 7.3 Hz), 7.00 (d, 1H, *J* = 8.8 Hz), 6.69 (s, 1H), 4.94 (q, 1H, *J* = 6.6 Hz), 2.08 (s, 1H), 1.62 (d, 3H, *J* = 6.6 Hz). ^{13}C-NMR: δ 184.2 (C), 174.1 (C), 165.7 (C), 161.1 (C), 159.5 (C), 138.8 (CH), 127.9 (C), 121.7 (C), 121.5 (CH), 115.0 (CH), 112.2 (C), 103.8 (CH), 63.4 (CH), 21.3 (CH_3). IR (KBr): 3469, 1685, 1631, 1560, 1485, 1442, 1367, 1286, 1222, 1105, 1012, 916, 835, 796, 752, 715, 422. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{14}H_{10}NaO_5]^+$, 281.0426; Found, 281.0432.

2-Acetyl-6-(methoxymethoxy)naphtho[1,2-*b*]furan-4,5-dione (18). To a solution of **17** (40 mg, 0.13 mmol) in $CHCl_3$ (10 mL) was added MnO_2 (115 mg, 1.3 mmol). After stirred for 6 h at rt, the mixture was filtered through a pad of Celite. The Celite was washed with $CHCl_3$, and the combined organics were concentrated. The crude product was chromatographed on silica gel. Yield 78% (30 mg). Orange solid. Rf (hexane/EtOAc = 1/2) = 0.40. ^{1}H-NMR: δ 7.63 (m, 2H), 7.52 (s, 1H), 7.37 (d, 1H, *J* = 7.3 Hz), 5.38 (s, 2H), 3.55 (s, 3H), 2.57 (s, 3H). ^{13}C-NMR (DMSO): δ 186.0 (C), 178.3 (C), 174.1 (C), 162.0 (C), 160.7 (C), 153.2 (C), 136.7 (CH), 128.8 (C), 121.7 (C), 120.0 (CH), 118.2 (C), 117.2 (CH), 114.7 (CH), 94.7 (CH_2), 56.9 (CH_3), 26.4 (CH_3). IR (KBr): 1674, 1569, 1529, 1458, 1261, 1186, 1149, 1087, 1068, 1004, 952, 833, 800, 715, 638. HRMS (ESI) *m/z*: $[M+Na]^+$ calcd for $[C_{16}H_{12}NaO_6]^+$, 323.0532; Found, 323.0529.

2-Acetyl-6-hydroxynaphtho[1,2-*b*]furan-4,5-dione (7). To a solution of **18** (30 mg, 0.10 mmol) in dioxane (7.5 mL) was added 10% HCl (7.5 mL). After stirred for 2 d at rt, the mixture was diluted with H_2O, extracted with EtOAc. The organic extracts were washed with brine, dried over Na_2SO_4, and then concentrated. The crude product was chromatographed on silica gel. Yield 46% (12 mg). Orange solid. Rf (hexane/EtOAc = 1/2) = 0.55. ^{1}H-NMR: δ 12.02 (s, 1H), 7.64 (dd, *J* = 7.5, 8.6 Hz, 1H), 7.52 (s, 1H), 7.50 (d, *J* = 7.4 Hz, 1H), 7.15 (d, *J* = 8.6 Hz, 1H), 2.58 (s, 3H). ^{13}C-NMR (DMSO): δ 186.0 (C), 183.4 (C), 174.0 (C), 166.1 (C), 161.3 (C), 153.4 (C), 139.0 (CH), 126.7 (C), 123.1 (CH), 121.8 (C), 116.3 (CH), 114.5 (CH), 113.1 (C), 26.4 (CH_3). IR (KBr): 3120, 1687, 1554, 1568, 1529, 1485, 1452, 1367, 1271, 1215, 1164, 879, 800, 736, 711. HRMS (ESI) *m/z*: $[M-H]^-$ calcd for $[C_{14}H_7O_5]^-$, 255.0293; Found, 255.0297.

Determination of cell growth inhibition profiles. This experiment was carried out at the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research. The screening panel consisted of the following 39 human cancer cell lines (JFCR39): breast cancer HBC-4, BSY-1, HBC-5, MCF-7, and MDAMB-231; brain cancer U251, SF-268, SF-295, SF-539, SNB-75, and SNB-78; colon cancer HCC2998, KM-12, HT-29, HCT-15, and HCT-116; lung cancer NCI-H23, NCI-H226, NCI-H522,

NCI-H460, A549, DMS273, and DMS114; melanoma LOX-IMVI; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, and SK-OV-3; renal cancer RXF-631L and ACHN; stomach cancer St-4, MKN1, MKN7, MKN28, MKN45, and MKN74; and prostate cancer DU-145 and PC-3. Inhibition of cell growth was assessed by measuring changes in total cellular protein levels following 48 h treatment with a given test compound, using the sulforhodamine B colorimetric assay. The molar concentration of a test compound required for 50% growth inhibition (GI_{50}) of cells was calculated as reported previously. A detailed method is described elsewhere.[19-21] COMPARE analysis was performed by calculating the Pearson correlation coefficient (r) between the GI50 mean graphs of compounds X and Y using the following formula: $r = ((x_i - x_m)\ (y_i - y_m))/((x_i - x_m)^2\ (y_i - y_m)^2)^{1/2}$, where x_i and y_i are Log GI_{50} of the two compounds, respectively, for each cell line, and x_m and y_m are the mean values of x_i and y_i, respectively (n = 39). The Pearson correlation coefficients were used to determine the degree of similarity. The larger the coefficient is, the higher the similarity between X and Y is.[26]

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