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Synthesis, Characterization, and Antileukemic Properties of Naphthoquinone Derivatives of Lawsone

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Naphthoquinones are considered privileged structures for anticancer drug molecules. The Heck reaction of 2-hydroxy-1,4-naphthoquinone (lawsone) with 1-bromo-3-methyl-2-butene offered easy access to lapachol. Several naturally occurring linear and angular heterocyclic quinoids (α-lapachone, β-lapachone, dunnione, and related analogues) were prepared from lapachol. Furthermore, we demonstrated that the synthetic naphthoquinones inhibit cell proliferation in human leukemia HL-60 cells. In particular, angular-type derivatives were found to possess moderate cytotoxicity and to elevate the levels of intracellular glutathione disulfide (GSSG). Our work highlights the significant potential of naturally occurring angular-series naphthoquinones as antileukemic agents.

Introduction

Quinone molecules are widespread in nature, and some of them have been used to treat cancer (e.g., doxorubicin, daunorubicin, and mitomycin C).[1] The exploration of natural active constituents for the treatment of cancer has attracted substantial worldwide attention.[2] Compounds that preferentially exhibit cytotoxicity against neoplastic cells may be used for developing drugs that are effective against cancer in humans.

α-Lapachone (2,2-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*b*]-pyran-5,10-dione) was initially isolated from the heartwood of *Tabebuia avellanedae* (*Bignoniaceae* family).[3] This quinone, classified as a pyrano-1,4-naphthoquinone, has been reported to exhibit activity against strains of both methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*,[4] and to inhibit initial noncovalent binding of topoisomerase II to DNA in multidrug-resistant tumors.[5] β-Lapachone (2,2-dimethyl-3,4-dihydro-2*H*-naphtho[2,3-*b*]pyran-5,6-dione), which is an *ortho*-quinoid isolated from the bark of *T. avellanedae*, has been recognized as a very promising drug candidate for the treatment of cancer.[6–8] It was evaluated in phase II clinical trials for the treatment of pancreatic adenocarcinomas.[9] β-Lapachone was also reported to show a number of important biological activities, including trypanocidal,[10,11] anti-inflammatory,[12] antibacterial,[13] and antifungal properties,[14] which are linked to the formation of reactive oxygen species (ROS).

In 2013 we described the synthesis of furonaphthoquinones via Sonogashira coupling and intramolecular cyclization, and reported their inhibitory effects on human leukemia U937 and HL-60 cell proliferation.[15] Methoxylated furonaphthoquinones were found to possess moderate cytotoxicity. The aim of this study was to determine the structure–activity relationships of naturally occurring naphthoquinones and related analogues in human leukemia cells.

Results and Discussion

Preparation of lapachols and synthesis of β-lapachones and dunniones

In the preparation of α-lapachone, β-lapachone, and related compounds, lapachol was thought to be the best starting material. Because it has been considered that isoprenyl pyrophosphate couples with 2-hydroxy-1,4-naphthoquinone (lawsone, **1a**) to form lapachol in *Bignoniaceous* plants,[16] we initially embarked on the synthesis of lapachol from lawsone. Kazantzi et al. reported the $Pd(PPh_3)_4$-catalyzed alkylation of lawsone with 1-bromo-3-methyl-2-butene in acidic conditions at reflux;[17] however, the yield was less than 45%. By reference to this method, we used basic Heck conditions: reaction of compound **1a** with 1-bromo-3-methyl-2-butene (5.0 equiv) in the presence of 10 mol% $Pd(PPh_3)_4$ with triethylamine (3.0 equiv) in dioxane at room temperature for 4 h afforded lapachol (**2a**) in 61% yield, along with dunniol (**3a**)[18] as a byproduct in 17% yield (Scheme 1). 6- and 7-Methoxylated lapachols **2b** and **2c** were prepared from 6- and 7-methoxylawsones **1b** and **1c**[15] in 41 and 40% yields, respectively. After the successful formation of lapachols **2a**–**2c**, the stage was set for the synthesis of various naturally occurring naphthoquinone analogues. H_2SO_4-mediated cyclization of lapachol (**2a**)[19] smoothly furnished β-

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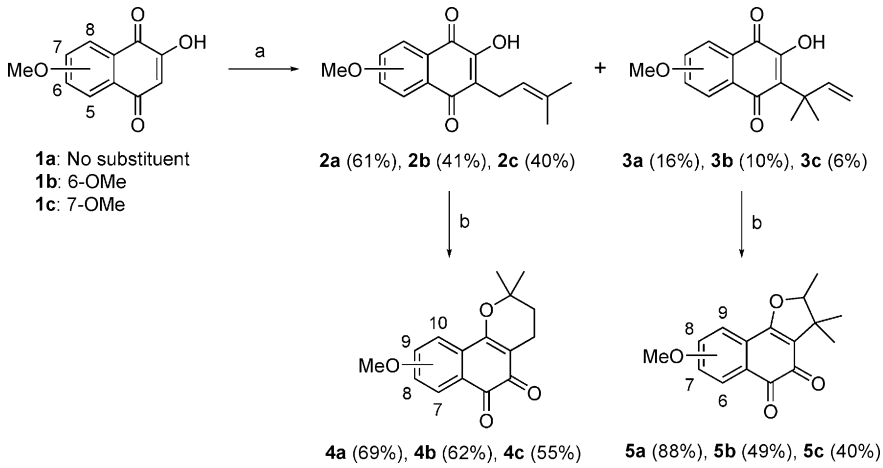


Scheme 1. Preparation of lapachols via the Heck reaction and synthesis of β-lapachones and dunniones. *Reagents and conditions*: a) 1-bromo-3-methyl-2-butene, $Pd(PPh_3)_4$, Et_3N, dioxane, RT, 4 h; b) H_2SO_4, CH_2Cl_2, 0 °C, 1 h.

lapachone (**4a**) in 69% yield. Under the same conditions, dunniol (**3a**) was cyclized to dunnione (**5a**)[20] in 88% yield through the Markovnikov rule.

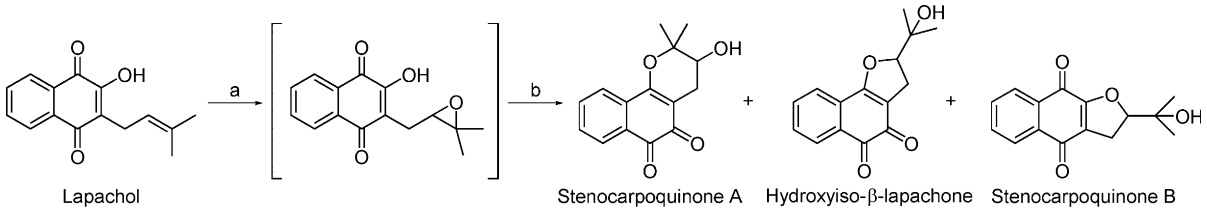
Synthesis of furonaphthoquinones and pyranonaphthoquinones from lapachols

The synthesis commenced with cyclization of a hydroxy group at the C2 position and an isoprenyl group at the C3 position. Oxidation of lapachol (**2a**) with *m*CPBA (1.2 equiv) proceeded at room temperature to afford an epoxide intermediate, which was immediately converted into stenocarpoquinone A (**6a**, angular type, 12%),[21] hydroxyiso-β-lapachone (**7a**, angular type, 39%), and stenocarpoquinone B (**8a**, linear type, 29%)[21] by subsequent cyclization with acetic acid (Table 1, entry 3). To confirm the structure of **6a**, we conducted an X-ray diffraction analysis of this compound (CCDC 1051828). An ORTEP image, depicted in Scheme 2, shows the molecular structure of stenocarpoquinone A (**6a**). It is interesting to examine the differences in the rates of the products formed (Table 1). Without acid, the amount of *m*CPBA largely affected selectivity. Upon treatment with *m*CPBA at 1.2 equiv, **7a** was obtained as the major product in 42% yield (entry 1), whereas an additional equivalent of *m*CPBA gave **8a** in 49% yield (entry 2). In the presence of trifluoroacetic acid (TFA), lapachol (**2a**) was transformed into **6a** in 51% yield (entry 4); as a strong Brønsted acid, TFA promotes the protonation of epoxide oxygen. Even with the use of Lewis acids (BF_3·Et_2O[19] and $AlCl_3$), **6a** was the major product (entries 5 and 6). Although the reaction with 0.1 wt. equiv of solid acids (Kaolin and Montmorillonite K10) resulted in poor selectivity of the products (entries 7 and 8), the additional amount of Mont. K10 (1.0 wt. equiv) led to high selectivity and good yield (**6a**, 89%, entry 9).

On the other hand, the reactions with 6- and 7-methoxylapachols **2b** and **2c** respectively afforded the 8- and 9-methoxylated stenocarpoquinone A analogues **6b** and **6c** as the major products under nearly every condition (Table 2). Although we used no acid conditions, methoxylated stenocarpoquinone B analogues **8** were not generated.

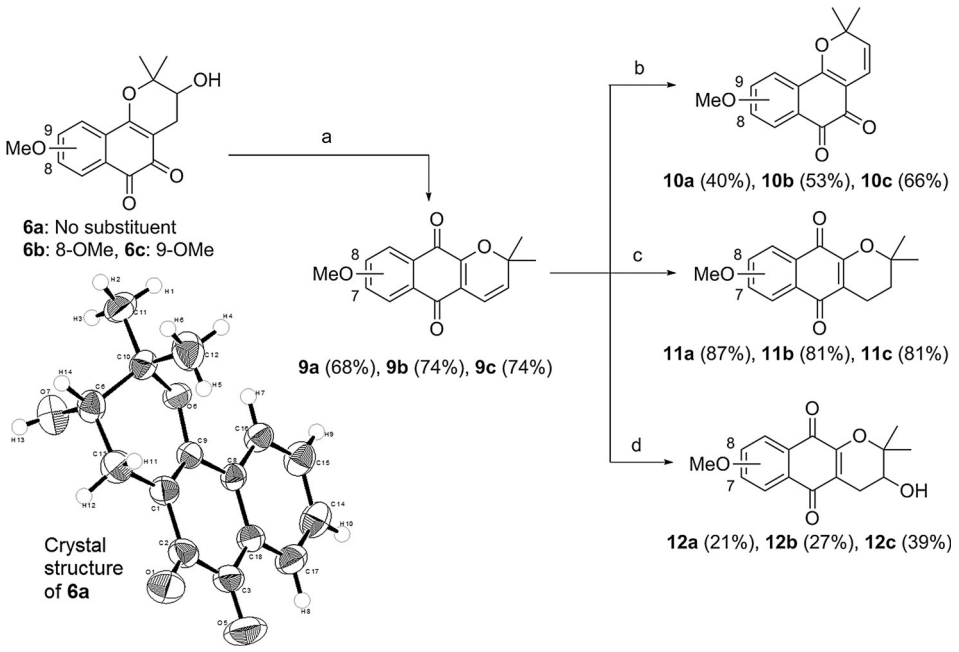
The next step in the synthesis involved dehydration of stenocarpoquinone A (**6a**), which was allowed to react with PPh_3 in CCl_4/MeCN at reflux (Scheme 2). However, these reaction conditions surprisingly provided dehydro-α-lapachone (**9a**,

Table 1. Reactivity of lapachol through oxidative cyclization.[a]



Entry	*m*CPBA [equiv]	Acid	Yield [%]			Selectivity		
			6a	**7a**	**8a**	**6a**	**7a**	**8a**
1	1.2	None	7	42	28	0.17	1	0.67
2	2.0	None	5	2	49	0.1	0.04	1
3	1.2	AcOH (3 equiv)	12	39	29	0.31	1	0.75
4	1.2	TFA (3 equiv)	51	12	2	1	0.24	0.04
5	1.2	BF_3·Et_2O (3 equiv)	38	5	0	1	0.13	0
6	1.2	$AlCl_3$ (3 equiv)	53	0	0	1	0	0
7	1.2	Kaolin (0.1 wt. equiv)	21	34	45	0.47	0.76	1
8	1.2	Mont. K10 (0.1 wt. equiv)	31	10	35	0.89	0.29	1
9	1.2	Mont. K10 (1.0 wt. equiv)	89	11	0	1	0.12	0

[a] *Reagents and conditions*: a) *m*CPBA, CH_2Cl_2, RT, 0.5 h; b) acid, 1 h.



Scheme 2. Synthesis of various pyranonaphthoquinones. *Reagents and conditions*: a) PPh_3, CCl_4/MeCN, reflux, 3 h; b) $TiCl_4$, CH_2Cl_2, 0 °C, 2 h; c) H_2, Pd/C, EtOAc, RT, 20 min; d) 1. *m*CPBA, CH_2Cl_2, 0 °C, 6 h, 2. $NaBH_3CN$, $BF_3{\cdot}OEt_2$, 2 h.

68%);[22] its carbon signal at the C10a position appeared at $\delta_C = 152.5$ ppm, which is a characteristic signal of linear-type pyranonaphthoquinones. 7- and 8-Methoxydehydro-α-lapachones **9b** and **9c** were prepared in 74 and 74% yield, respectively. To synthesize dehydro-β-lapachone **10a**,[23] dehydro-α-lapachone **9a** was treated with $TiCl_4$ in CH_2Cl_2 at 0 °C to give the target product **10a** in 40% yield. Presumably, the ring-opening reaction was driven by the in situ generation of a titanium(IV)–dione complex, which was subsequently cyclized on the other side. Catalytic hydrogenation of **9a** furnished α-lapachone **11a** in 87% yield. Reaction of **9a** with *m*CPBA effected the epoxide, which was reduced with $NaBH_3CN$ in the presence of $BF_3{\cdot}Et_2O$[24] to yield rhinacanthin A (**12a**)[25] in 21% yield. These procedures allowed the syntheses of the methoxylated analogues (Scheme 2).

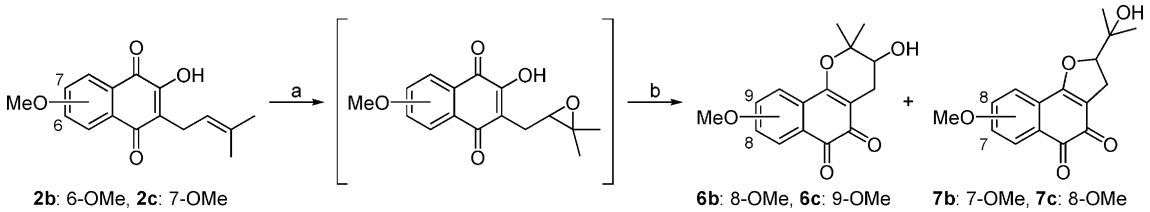
^{13}C NMR spectroscopic characteristics of naphthoquinone derivatives

It is difficult to determine the structures of either angular- or linear-type pyranonaphthoquinones with 1D NMR spectroscopy. The ^{13}C NMR chemical shift of C10b in the angular series, corresponding to C10a in the linear series, proved to be the most helpful for solving this problem. The δ values in $CDCl_3$ are summarized in Table 3. Deshielding effects in the angular pyranonaphthoquinones (**4a**–**4c**, **6a**–**6c**, and **10a**–**10c**) relative to the linear pyranonaphthoquinones (**9a**–**9c**, **11a**–**11c**, and **12a**–**12c**) were observed. In general, the ^{13}C NMR peaks of the angular series appeared downfield (C10b, $\delta_C >$ 160.0 ppm) relative to those of the linear series (C10a, δ_C between 150.0 and 155.0 ppm). The size of the heterocyclic ring (furan or pyran) was also found to influence these key carbon atoms, and the same effects were observed. The chemical shifts of C9b in the angular furonaphthoquinones were close to $\delta_C = 170.0$ ppm. Although stenocarpoquinone B (**8a**) belongs to the linear type, its C9a signal appeared at $\delta_C =$ 160.0 ppm. The information listed in Table 3 facilitates the structural determination of these compounds.

Synthesis of dimeric derivatives

To expand these investigations, we turned our attention to the synthesis of dimeric derivatives of the prepared naphthoquinones (Scheme 3). Oxidative coupling of lawsone (**1a**) by treatment with $(NH_4)_2S_2O_8$ gave bislawsone **13a**[26] in 25% yield

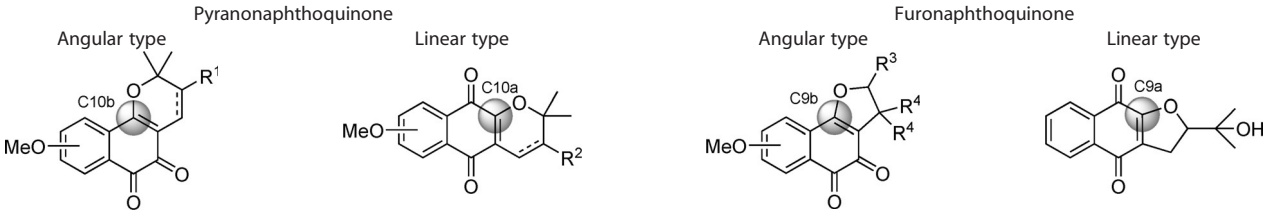
Table 2. Reactivity of methoxylated lapachols through oxidative cyclization.[a]



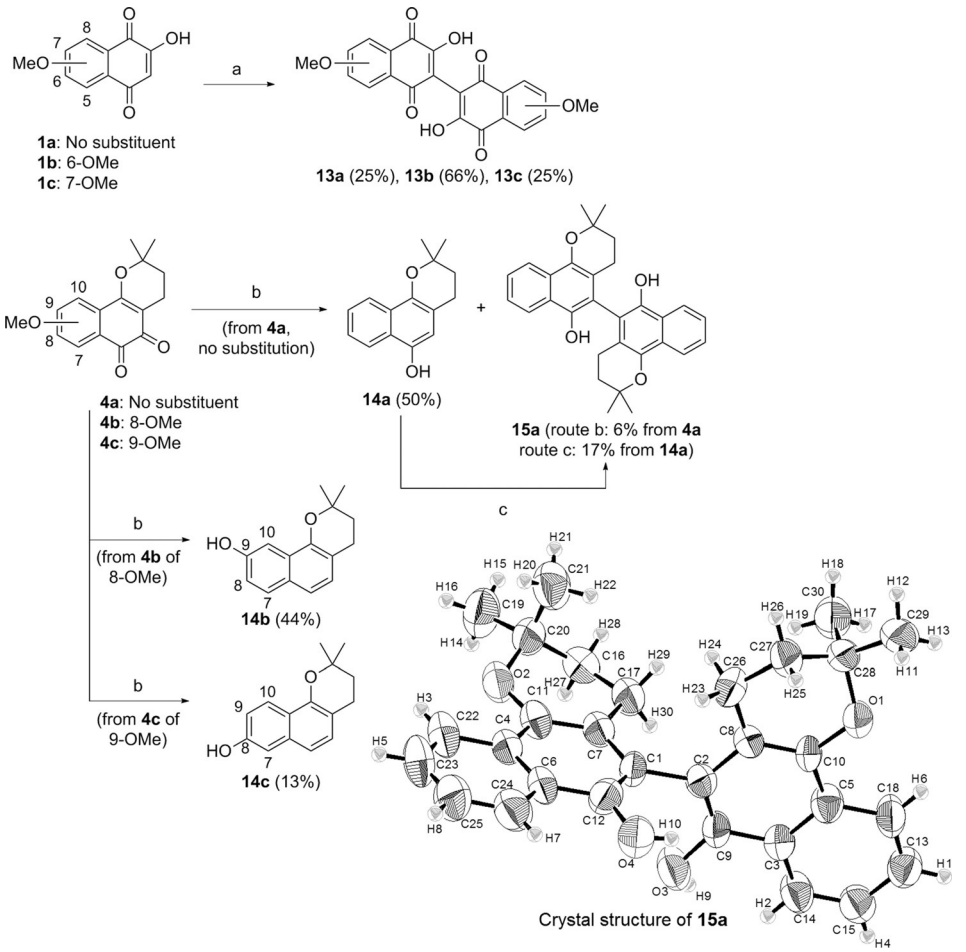
Entry	*m*CPBA [equiv]	Acid	Yield [%]				Selectivity			
			6b	**7b**	**6c**	**7c**	**6b**	**7b**	**6c**	**7c**
1	2.0	None	16	19	22	20	0.84	1	1	0.91
2	1.2	$AlCl_3$ (3 equiv)	37	0	55	0	1	0	1	0
3	1.2	Mont. K10 (0.1 wt. equiv)	33	19	49	39	1	0.58	1	0.65
4	1.2	Mont. K10 (1.0 wt. equiv)	36	17	32	19	1	0.47	1	0.59

[a] *Reagents and conditions*: a) *m*CPBA, CH_2Cl_2, RT, 0.5 h; b) acid, 1 h.

Table 3. ^{13}C NMR chemical shifts (from TMS) of key carbon atoms for structural determination.



Compd	δ_C [ppm] (C10b)	Compd	δ_C [ppm] (C10a)	Compd	δ_C [ppm] (C9b)	Compd	δ_C [ppm] (C9a)
4a	162.1	**9a**	152.5	**5a**	168.3	**8a**	160.0
4b	161.2	**9b**	152.9	**5b**	167.5		
4c	162.9	**9c**	152.2	**5c**	169.1		
6a	161.7	**11a**	154.7	**7a**	169.9		
6b	160.7	**11b**	155.0	**7b**	169.1		
6c	162.2	**11c**	154.4	**7c**	170.7		
10a	162.1	**12a**	153.8				
10b	161.2	**12b**	154.1				
10c	163.2	**12c**	153.6				



Scheme 3. Synthesis of bislawsone analogues and tetrahydrotectol and reaction of β-lapachones with HI. *Reagents and conditions*: a) $(NH_4)S_2O_8$, $MeCN/H_2O$, 80 °C, 3 h; b) HI, AcOH, reflux, 2 h; c) 1. TBSCl, DIPEA, THF, RT, 2 h, 2. $Et_3N{\cdot}3HF$, 24 h.

without chromatographic purification. 6,6′- and 7,7′-Dimethoxybislawsones **13b** and **13c** were prepared in 66 and 25% yield, respectively. The formation of tetrahydrotectol **15a**, which is a constituent of *Tabebuia* plants,[27] has been reported to occur by HI-promoted dimerization of β-lapachone (**4a**).[28] In the report, **15a** was obtained in 96%. However, by following this method, we obtained nordihydrolapachenol (**14a**, monomer, 50%)[27] as a major product instead of tetrahydrotectol (**15a**, 6%). TBS protection of the monomer and reaction with triethylamine trihydrogen fluoride ($NEt_3{\cdot}3HF$) led to the production of **15a** in 17%.[29] Scheme 3 includes an ORTEP image of the structure of tetrahydrotectol (**15a**; CCDC 1051827). One β-lapachone moiety was observed to be roughly perpendicular to the other. Unexpectedly, the dimerization of 8- and 9-methoxy-β-lapachones **4b** and **4c** did not result in formation of the desired tetrahydrotectol analogues. Notably, the reaction of 8-methoxy-β-lapachone **4b** with HI yielded 9-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (**14b**) in 44% yield. A doublet proton ($\delta_H = 7.53$ ppm, $J = 2.8$ Hz) of H-10 correlated with a carbon signal ($\delta_C =$ 147.6 ppm) of C10b in HMBC. This reaction includes ring opening, rearrangement, cyclization, decarbonylation, and demethylation. Likewise, 9-methoxy-β-lapachone **4c** was converted into

8-hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (**14c**) in 13% yield.

Cytotoxic evaluation against human leukemia HL-60 cells

As a crucial step for structure–activity relationships, we evaluated the synthetic naphthoquinone derivatives for in vitro cytotoxicity against HL-60 cells using the CCK-8 assay method.[30] Cells in the rapid phase of growth were exposed to the compounds for 48 h. At a concentration of 25.0 μM, most compounds exhibited cytotoxicity. In particular, β-lapachones (**4a**, 7.0%; **4b**, 5.9%; **4c**, 6.6%), dunniones (**5a**, 5.7%; **5b**, 4.9%; **5c**, 5.4%), stenocarpoquinone A analogues (**6a**, 6.4%; **6b**, 4.7%; **6c**, 6.3%), hydroxyiso-β-lapachones (**7a**, 7.2%; **7b**, 5.0%; **7c**, 4.5%), and stenocarpoquinone B (**8a**, 5.0%) potently inhibited cell proliferation with quite low cell survival rates (Figure 1). Their inhibitory effects were higher than that of paclitaxel (PTX, 14.0%).

The IC_{50} values of the potent compounds are as follows: **4a** (7.1 μM), **4b** (9.4 μM), **4c** (2.1 μM); **5a** (0.9 μM), **5b** (4.3 μM), **5c** (2.0 μM); **6a** (14.6 μM), **6b** (8.5 μM), **6c** (5.5 μM); **7a** (2.3 μM), **7b** (4.3 μM), **7c** (4.7 μM); **8a** (12.9 μM). In contrast, dehydro-β-lapachones **10a**–**10c** possessed weaker cytotoxicity than other angular-type compounds; in other words, the presence of a double bond at C3=C4 decreases potency. Although stenocarpoquinone B (**8a**) belongs to the linear type, its potency was excellent. With the exception of compounds **8a** and **10a**–**10c**, angular-type derivatives generally tend to show higher cytotoxicity than linear-type derivatives. The redox cycle of naphthoquinones leads to the generation of unstable semiquinone radicals and ROS, which in turn inflict great damage to cells.[31–33] We gathered from the data that the potent compounds were more easily reduced than others. Indeed, by using a quantum chemical approach, Paulino et al. indicated that the electronic asymmetry and polarity of the C=O bond are greater in the angular-type than in the linear-type compounds.[34,35] It has been reported that 2-chloro-2′-hydroxy dimeric naphthoquinones have anticancer potential;[36] however, bislawsones **13a**–**13c** were found to be nearly inactive at this concentration (25 μM), and tetrahydrotectol (**15a**) showed a cell survival rate of 46.6%.

Analysis of the intracellular glutathione content

In cells, naphthoquinones can undergo metabolism via single-electron reduction to form unstable semiquinone radicals,[37] which transfer electrons to molecular oxygen under backformation of the original quinones.[33] The semiquinone redox cycle generates a superoxide anion, which can be transformed into hydrogen peroxide, followed by the production of a hydroxyl radical. Moreover, glutathione peroxidase degrades hydrogen peroxide to water, often with the concomitant oxidation of reduced glutathione (GSH) into oxidized glutathione (GSSG).[31,38] Therefore, we assessed total glutathione (tGSH) and GSSG levels in HL-60 cells by using the enzymatic recycling method.[39] The ratio of GSSG to tGSH is expected to be roughly proportional to the extent of semiquinone radical generation. Cells were treated with β-lapachone (**4a**), dunnione (**5a**), and α-lapachone (**11a**) for 12 h, and the results are given in Table 4. The GSSG/tGSH ratios for **4a** and **11a** were 6.7- and 3.0-fold higher than blank (DMSO alone). Dunnione (**5a**) significantly elevated relative GSSG/tGSH by 19.9-fold. This tendency means that the higher the relative GSSG/tGSH value, the greater the cytotoxicity. It is believed that a drastic increase in intracellular GSSG levels and redox imbalance caused by **5a** profoundly inhibits cell proliferation, which implies that **5a** has the potential to be the most immediately reduced among the other compounds.

Table 4. IC_{50} and relative GSSG/tGSH values of β-lapachone (**4a**), dunnione (**5a**), and α-lapachone (**11a**).

Compound	IC_{50} [μM]	Rel. GSSG/tGSH
4a	7.1	6.7
5a	0.9	19.9
11a	19.4	3.0
blank (DMSO alone)	–	1

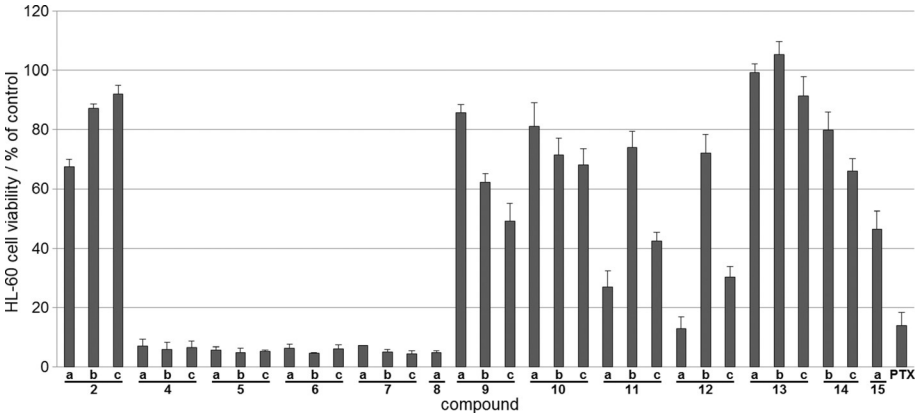


Figure 1. Cytotoxic effects of furonaphthoquinones, pyranonaphthoquinones, their methoxylated analogues, and the dimers against HL-60 cells (final compound concentration: 25 μM; data are the mean ± SEM, $n = 9$).

Conclusions

Herein we have presented the efficient and practical syntheses of naturally occurring furonaphthoquinones, pyranonaphthoquinones, their methoxylated analogues, and the dimers from lawsones. According to cytotoxicity assays, our findings suggest that the angular-type naphthoquinone derivatives act as in vitro antiproliferative agents in HL-60 cells. Notably,

dunnione (**5a**) was found to exert potent efficacy through substantial elevation in intracellular GSSG levels. Dunnione analogues have the potential for further development as chemotherapeutic drugs for the treatment of hyperproliferative disorders.

Experimental Section

General chemical procedures: All solvents and reagents were purchased from commercial suppliers and used without further purification. IR spectra were recorded on a JASCO FT/IR-460 Plus spectrophotometer. MS spectra were obtained with a JEOL JMS-700/GI spectrometer and a Waters UPLC–MS system (Aquity UPLC XevoQ-Tof). The purities of compounds were assessed as >95% using analytical UPLC–MS. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded with a JEOL ECX 400 spectrometer with tetramethylsilane as an internal standard. X-ray diffraction measurements were carried out on a Rigaku AFC-7R Mercury CCD diffractometer with graphite-monochromated $Mo_{K\alpha}$ radiation (λ = 0.71069 Å). Structures were solved by direct methods SIR97 and refined with Shelxl97 using the interface program Yadokari 2009. The final least-squares cycle included non-hydrogen atoms with anisotropic thermal parameters. Silica gel column chromatography (CC) was performed on silica gel N-60 (40–50 μm). Thin-layer chromatography (TLC) spots on plates pre-coated with silica gel 60 F_{254} were detected with a UV lamp (λ 254 nm). Fractionations for all CCs were based on TLC analyses.

CCDC 1051827 and 1051828 contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

Preparation of lapachols 2a–2c and dunniols 3a–3c: $Pd(PPh_3)_4$ (10 mol%) and Et_3N (1.71 mmol) were added to a solution of lawsones (**1a–1c**, 0.57 mmol) and 1-bromo-3-methyl-2-butene (2.87 mmol) in 1,4-dioxane (15 mL). After stirring for 4 h at RT, the resulting solution was poured into 10% aqueous HCl, partitioned with $CHCl_3$, and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluted with $CHCl_3$/*n*-hexane (1:1) to yield lapachols **2a–2c** and dunniols **3a–3c**.

Lapachol (2a): Yellow amorphous powder, 61% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (3H, s, Me), 1.79 (3H, s, Me), 3.31 (2H, d, J = 7.3 Hz, -CH_2-), 5.19–5.23 (1H, m, -CH=C(Me)$_2$), 7.67 (1H, td, J = 1.4 and 7.3 Hz, H-6), 7.74 (1H, td, J = 1.4 and 7.3 Hz, H-7), 8.06 (1H, dd, J = 0.9 and 7.3 Hz, H-5), 8.12 ppm (1H, dd, J = 0.9 and 7.3 Hz, H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.0, 22.7, 25.8, 119.7, 123.5, 126.1, 126.8, 129.2, 129.9, 132.9, 133.9, 134.9, 152.8, 181.8, 184.6 ppm; IR (film): $\tilde{\nu}$ = 3352, 1659, 1639, 1591, 1369, 1239 cm^{-1}; EIMS: *m/z* 242 $[M]^+$, 227 $[M-CH_3]^+$, 199 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{14}O_3$: 242.0943, found: 242.0935.

6-Methoxylapachol (2b): Yellow amorphous powder, 41% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (3H, s, Me), 1.79 (3H, s, Me), 3.28 (2H, d, J = 7.3 Hz, -CH_2-), 3.95 (3H, s, OMe) 5.16–5.20 (1H, m, -CH=C(Me)$_2$), 7.11 (1H, dd, J = 2.8 and 8.7 Hz, H-7), 7.57 (1H, d, J = 2.8 Hz, H-5), 8.00 ppm (1H, d, J = 8.7 Hz, H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.0, 22.7, 25.8, 56.1, 110.8, 119.1, 119.9, 122.7, 128.6, 128.8, 133.7, 135.5, 153.1, 165.2, 180.4, 184.6 ppm; IR (film): $\tilde{\nu}$ = 3371, 1653, 1593, 1253 cm^{-1}; EIMS: *m/z* 272 $[M]^+$, 257 $[M-CH_3]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1021.

7-Methoxylapachol (2c): Yellow amorphous powder, 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.68 (3H, s, Me), 1.79 (3H, s, Me), 3.28 (2H, d, J = 7.8 Hz, -CH_2-), 3.93 (3H, s, OMe), 5.16–5.21 (1H, m, -CH=C(Me)$_2$), 7.19 (1H, dd, J = 2.7 and 9.2 Hz, H-6), 7.49 (1H, d, J = 2.7 Hz, H-8), 8.04 ppm (1H, d, J = 9.2 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 18.0, 22.7, 25.8, 56.0, 110.0, 120.0, 121.0, 123.3, 126.3, 129.1, 131.2, 133.8, 152.6, 163.3, 181.9, 184.1 ppm; IR (film): $\tilde{\nu}$ = 3357, 1644, 1596, 1361, 1328 cm^{-1}; EIMS: *m/z* 272 $[M]^+$, 257 $[M-CH_3]^+$, 229 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1033.

Dunniol (3a): Yellow amorphous powder, 16% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.57 (6H, s, 2Me), 4.96 (1H, dd, J = 0.9 and 10.6 Hz, -CH=CH_2), 4.99 (1H, dd, J = 0.9 and 17.9 Hz, -CH=CH_2), 6.29 (1H, dd, J = 11.0 and 17.9 Hz, -CH=CH$_2$), 7.65 (1H, dd, J = 1.4 and 7.8 Hz, H-6), 7.74 (1H, dd, J = 1.4 and 7.8 Hz, H-7), 7.85 (1H, s, OH), 8.02–8.08 ppm (2H, m, H-5 and H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 (2C), 41.1, 109.7, 125.9, 127.1, 128.4, 128.5, 132.6, 134.2, 135.3, 148.1, 152.9, 182.3, 184.8 ppm; IR (film): $\tilde{\nu}$ = 3346, 1663, 1650, 1593, 1363, 1291 cm^{-1}; HR ESI ToF MS: *m/z* $[M+H]^+$ calcd for $C_{15}H_{15}O_3$: 243.1021, found: 243.1016, $[M+Na]^+$ calcd for $C_{15}H_{14}O_3Na$: 265.0841, found: 265.0826.

6-Methoxydunniol (3b): Yellow amorphous powder, 10% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 (6H, s, 2Me), 3.94 (3H, s, OMe), 4.95 (1H, dd, J = 0.9 and 10.6 Hz, -CH=CH_2), 5.00 (1H, dd, J = 0.9 and 17.9 Hz, -CH=CH_2), 6.27 (1H, dd, J = 11.0 and 17.9 Hz, -CH=CH$_2$), 7.09 (1H, dd, J = 2.8 and 8.7 Hz, H-7), 7.53 (1H, d, J = 2.8 Hz, H-5), 7.98 (1H, d, J = 8.8 Hz, H-8), 7.99 ppm (1H, s, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.2 (2C), 41.0, 56.0, 109.5, 110.8, 119.4, 121.8, 128.5, 128.6, 136.8, 148.3, 153.3, 165.6, 180.8, 184.8 ppm; IR (film): $\tilde{\nu}$ = 3346, 1664, 1648, 1595, 1361, 1282 cm^{-1}; HR ESI ToF MS: *m/z* $[M+H]^+$ calcd for $C_{16}H_{17}O_4$: 273.1127, found: 273.1126, $[M+Na]^+$ calcd for $C_{16}H_{16}O_4Na$: 295.0946, found: 295.0969.

7-Methoxydunniol (3c): Yellow amorphous powder, 6% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 (6H, s, 2Me), 3.92 (3H, s, OMe), 4.95 (1H, dd, J = 0.9 and 11.0 Hz, -CH=CH_2), 4.99 (1H, dd, J = 0.9 and 17.9 Hz, -CH=CH_2), 6.29 (1H, dd, J = 11.0 and 17.9 Hz, -CH=CH$_2$), 7.20 (1H, dd, J = 2.8 and 8.8 Hz, H-6), 7.46 (1H, d, J = 2.8 Hz, H-8), 7.75 (1H, s, OH), 7.99 ppm (1H, d, J = 9.2 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3 (2C), 41.1, 56.0, 109.1, 109.5, 121.6, 127.5, 128.0, 129.4, 130.1, 148.3, 152.8, 163.1, 182.5, 184.3 ppm; IR (film): $\tilde{\nu}$ = 3338, 1665, 1644, 1596, 1357, 1302 cm^{-1}; HR ESI ToF MS: *m/z* $[M+H]^+$ calcd for $C_{16}H_{17}O_4$: 273.1127, found: 273.1124, $[M+Na]^+$ calcd for $C_{16}H_{16}O_4Na$: 295.0946, found: 295.0951.

Synthesis of β-lapachones 4a–4c: Lapachols (**2a–2c**, 1.39 mmol) were dissolved in CH_2Cl_2/H_2SO_4 (1:4, 40 mL) at 0 °C. After stirring for 1 h, the resulting solution was carefully poured into iced water, partitioned with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:5) to yield β-lapachones **4a–4c**.

β-Lapachone (4a): Orange amorphous powder, 69% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.47 (6H, s, 2Me), 1.86 (2H, t, J = 6.9 Hz, H-3), 2.57 (2H, t, J = 6.9 Hz, H-4), 7.50 (1H, td, J = 1.4 and 7.8 Hz, H-8), 7.65 (1H, td, J = 1.4 and 7.8 Hz, H-9), 7.81 (1H, dd, J = 0.9 and 7.8 Hz, H-7), 8.05 ppm (1H, dd, J = 0.9 and 7.8 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.3, 26.8 (2C), 31.7, 77.5, 112.8, 124.2, 128.6, 130.2, 130.7, 132.7, 134.9, 162.1, 178.6, 180.0 ppm; IR (film): $\tilde{\nu}$ = 1644, 1601, 1570, 1119 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{14}O_3$: 242.0943, found: 242.0915.

8-Methoxy-β-lapachone (4b): Orange amorphous powder, 62% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (6H, s, 2Me), 1.84 (2H, t, J = 6.9 Hz, H-3), 2.55 (2H, t, J = 6.9 Hz, H-4), 3.93 (3H, s, OMe), 6.93 (1H, dd, J = 2.3 and 8.7 Hz, H-9), 7.27 (1H, d, J = 2.3 Hz, H-7), 8.02 ppm (1H, d, J = 8.7 Hz H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.3, 26.8 (2C), 31.7, 55.9, 79.2, 110.5, 113.1, 114.6, 123.6, 131.5, 134.9, 161.2, 165.1, 178.4, 179.2 ppm; IR (film): $\tilde{\nu}$ = 1640, 1589, 1386 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1043.

9-Methoxy-β-lapachone (4c): Orange amorphous powder, 55% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (6H, s, 2Me), 1.84 (2H, t, J = 6.9 Hz, H-3), 2.54 (2H, t, J = 6.9 Hz, H-4), 3.90 (3H, s, OMe), 7.12 (1H, dd, J = 2.8 and 8.7 Hz, H-8), 7.55 (1H, d, J = 3.2 Hz, H-10), 7.71 ppm (1H, d, J = 8.7 Hz H-7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.1, 26.8 (2C), 31.8, 55.9, 79.3, 110.7, 112.6, 121.0, 125.6, 126.0, 131.8, 161.7, 162.9, 178.7, 180.0 ppm; IR (film): $\tilde{\nu}$ = 1643, 1601, 1391, 1284, 1114 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1047.

Synthesis of dunniones 5a–5c: Dunniols (**3a–3c**, 0.21 mmol) were dissolved in CH_2Cl_2/H_2SO_4 (1:4, 6 mL) at 0 °C. After stirring for 1 h, the resulting solution was carefully poured into iced water, partitioned with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:1) to yield dunniones **5a–5c**.

Dunnione (5a): Red amorphous powder, 88% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (3H, s, Me), 1.46 (3H, s, Me), 1.48 (3H, d, J = 6.9 Hz Me), 4.68 (1H, q, J = 6.9 Hz, H-2), 7.54–7.58 (1H, m, H-8), 7.62–7.66 (2H, m, H-6 and H-7), 8.03 ppm (1H, d, J = 7.8 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.7, 20.4, 25.8, 44.2, 93.0, 123.4, 124.6, 128.0, 129.2, 130.8, 131.7, 134.6, 168.3, 175.5, 181.6 ppm; IR (film): $\tilde{\nu}$ = 1641, 1601, 1384, 1122 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{15}H_{14}O_3$: 242.0943, found: 242.0921.

7-Methoxydunnione (5b): Red amorphous powder, 49% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.24 (3H, s, Me), 1.42 (3H, s, Me), 1.45 (3H, d, J = 6.9 Hz Me), 3.91 (3H, s, OMe), 4.63 (1H, q, J = 6.9 Hz, H-2), 6.96 (1H, dd, J = 2.3 and 8.7 Hz,H-8), 7.09 (1H, d, J = 2.8 Hz H-6), 8.00 ppm (1H, d, J = 8.7 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.7, 20.4, 25.9, 44.3, 56.0, 92.8, 110.1, 116.2, 123.6, 124.1, 130.2, 132.1, 164.7, 167.5, 176.2, 180.2 ppm; IR (film): $\tilde{\nu}$ = 1639, 1583, 1384, 1152 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1051.

8-Methoxydunnione (5c): Red amorphous powder, 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (3H, s, Me), 1.44 (3H, s, Me), 1.46 (3H, d, J = 6.4 Hz Me), 3.91 (3H, s, OMe), 4.64 (1H, q, J = 6.9 Hz, H-2), 7.10 (1H, dd, J = 2.8 and 8.7 Hz, H-7), 7.54–7.57 ppm (2H, m, H-6 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.7, 20.5, 25.9, 44.0, 56.0, 92.9, 114.2, 120.2, 120.6, 121.3, 126.3, 132.8, 162.7, 169.1, 175.4, 181.8 ppm; IR (film): $\tilde{\nu}$ = 1645, 1604, 1402, 1281 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1019.

Synthesis of stenocarpoquinone A analogues 6a–6c, hydroxyiso-β-lapachone analogues 7a–7c, and stenocarpoquinone B (8a): *No-acid conditions*: *m*CPBA (containing 25% H_2O) was added to a solution of lapachols (**2a–2c**, 0.41 mmol) in CH_2Cl_2 (10 mL). After stirring for 1.5 h at RT, the resulting solution was poured into distilled H_2O, partitioned with EtOAc, washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:1) to yield stenocarpoquinone A analogues **6a–6c**, hydroxyiso-β-lapachones **7a–7c**, and stenocarpoquinone B (**8a**).

Acid conditions: *m*CPBA (containing 25% H_2O, 0.49 mmol) was added to a solution of lapachols (**2a–2c**, 0.41 mmol) in CH_2Cl_2 (10 mL). After stirring for 30 min at RT, various acids were added to the reaction mixture, and stirring was continued for 1 h. The resulting solution was poured into distilled H_2O, partitioned with EtOAc, and washed with saturated aqueous $NaHCO_3$ and brine (with the use of solid acids, the resulting solution was filtered through Celite instead of partitioning and washing). Other processes were the same as with the no-acid conditions.

Stenocarpoquinone A (6a): Orange amorphous powder; the yield is given in Table 1; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (3H, s, Me), 1.52 (3H, s, Me), 2.62 (1H, dd, J = 5.5 and 18.3 Hz, H-4α), 2.81 (1H, dd, J = 5.5 and 18.3 Hz, H-4β), 3.91 (1H, t, J = 5.5 Hz, H-3), 7.51 (1H, td, J = 1.4 and 7.8 Hz, H-8), 7.65 (1H, td, J = 1.4 and 7.8 Hz, H-9), 7.84 (1H, dd, J = 1.4 and 7.8 Hz, H-7), 8.04 ppm (1H, dd, J = 1.4 and 7.8 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2, 25.2, 25.4, 68.3, 81.6, 110.5, 124.5, 128.8, 130.5, 131.0, 132.2, 135.0, 161.7, 178.8, 179.6 ppm; IR (film): $\tilde{\nu}$ = 3373, 1636, 1597, 1570, 1388 cm^{-1}; EIMS: m/z 258 $[M]^+$, 240 $[M-H_2O]^+$, 230 $[M-CO]^+$; HR EI MS: m/z $[M]^+$ calcd for $C_{15}H_{14}O_4$: 258.0892, found: 258.0881.

8-Methoxystenocarpoquinone A (6b): Orange amorphous powder; the yield is given in Table 2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.45 (3H, s, Me), 1.51 (3H, s, Me), 2.61 (1H, dd, J = 5.0 and 18.3 Hz, H-4α), 2.80 (1H, dd, J = 5.0 and 18.3 Hz, H-4β), 3.92 (1H, t, J = 5.5 Hz, H-3), 3.94 (3H, s, OMe), 6.95 (1H, dd, J = 2.8 and 8.7 Hz, H-9), 7.30 (1H, d, J = 2.8 Hz, H-7), 8.05 ppm (1H, d, J = 8.7 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2, 25.1, 25.6, 56.0, 68.4, 81.4, 110.8 (2C), 115.0, 123.5, 131.8, 134.4, 160.7, 165.1, 178.1, 179.5 ppm; IR (film): $\tilde{\nu}$ = 3392, 1636, 1582, 1387, 1258 cm^{-1}; EIMS: m/z 288 $[M]^+$, 260 $[M-CO]^+$; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0985.

9-Methoxystenocarpoquinone A (6c): Orange amorphous powder; the yield is given in Table 2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.46 (3H, s, Me), 1.48 (3H, s, Me), 2.43 (1H, dd, J = 5.0 and 17.4 Hz, H-4α), 2.71 (1H, dd, J = 5.0 and 17.4 Hz, H-4β), 3.93 (3H, s, OMe), 4.30 (1H, t, J = 5.5 Hz, H-3), 7.27 (1H, dd, J = 2.7 and 8.7 Hz, H-8), 7.45 (1H, d, J = 2.8 Hz, H-10), 7.79 ppm (1H, d, J = 8.7 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.3, 25.2, 25.3, 55.9, 68.2, 81.9, 109.7, 113.1, 120.6, 125.7, 126.5, 132.4, 161.5, 162.2, 178.8, 179.8 ppm; IR (film): $\tilde{\nu}$ = 3394, 1643, 1603, 1562, 1391, 1287 cm^{-1}; EIMS: m/z 288 $[M]^+$, 260 $[M-CO]^+$; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0995.

Hydroxyiso-β-lapachone (7a): Red amorphous powder; the yield is given in Table 1; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (3H, s, Me), 1.39 (3H, s, Me), 3.05 (2H, dd, J = 1.8 and 11.0 Hz, H-3), 4.90 (1H, t, J = 9.6 Hz, H-2), 7.51–7.63 (3H, m, H-6, H-7, and H-8), 8.00 ppm (1H, d, J = 8.2 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.5, 25.9, 27.5, 71.8, 93.6, 116.1, 124.6, 127.4, 129.6, 130.7, 132.1, 134.7, 169.9, 175.4, 181.1 ppm; IR (film): $\tilde{\nu}$ = 3409, 1649, 1610, 1492, 1367 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{15}H_{14}O_4$: 258.0892, found: 258.0865.

7-Methoxyhydroxyiso-β-lapachone (7b): Red amorphous powder; the yield is given in Table 2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.28 (3H, s, Me), 1.41 (3H, s, Me), 3.08 (2H, dd, J = 1.4 and 7.3 Hz, H-3), 3.94 (3H, s, OMe), 4.91 (1H, t, J = 8.7 Hz, H-2), 6.99 (1H, dd, J = 2.8 and 8.7 Hz, H-8), 7.12 (1H, d, J = 2.8 Hz, H-6), 8.05 ppm (1H, d, J = 8.7 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.3, 25.9, 27.7, 56.1, 71.9, 93.4, 110.7, 115.9, 116.3, 123.9, 129.5, 132.5, 164.7, 169.1,

176.1, 179.7 ppm; IR (film): $\tilde{\nu}$ = 3409, 1643, 1584, 1454, 1245 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0968.

8-Methoxyhydroxyiso-β-lapachone (7c): Red amorphous powder; the yield is given in Table 2; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.25 (3H, s, Me), 1.39 (3H, s, Me), 3.03 (2H, dd, *J* = 5.5 and 10.1 Hz, H-3), 3.88 (3H, s, OMe), 4.88 (1H, t, *J* = 8.7 Hz, H-2), 7.08 (1H, dd, *J* = 2.8 and 8.3 Hz, H-7), 7.52 (1H, d, *J* = 2.3 Hz, H-9), 7.55 ppm (1H, d, *J* = 8.7 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.4, 25.9, 27.4, 56.0, 71.9, 93.7, 113.9, 114.6, 119.9, 120.2, 126.4, 132.6, 163.0, 170.7, 175.3, 181.3 ppm; IR (film): $\tilde{\nu}$ = 3401, 1644, 1482, 1415, 1283 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0991.

Stenocarpoquinone B (8a): Yellow amorphous powder; the yield is given in Table 1; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.26 (3H, s, Me), 1.41 (3H, s, Me), 3.16 (2H, dd, *J* = 1.4 and 9.2 Hz, H-3), 4.85 (1H, t, *J* = 10.6 Hz, H-2), 7.63–7.72 (2H, m, H-6 and H-7), 8.01–8.04 ppm (2H, m, H-5 and H-8); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 24.2, 25.9, 28.4, 71.7, 92.2, 125.1, 126.1, 126.4, 131.5, 132.96, 133.10, 134.3, 160.0, 177.8, 182.3 ppm; IR (film): $\tilde{\nu}$ = 3419, 1629, 1384 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{14}O_4$: 258.0892, found: 258.0869.

Synthesis of dehydro-α-lapachones 9a–9c: PPh_3 (0.38 mmol) was added to a solution of stenocarpoquinone A analogues (**6a–6c**, 0.19 mmol) in CCl_4/MeCN (1:1, 6 mL) at RT. After holding at reflux for 2 h, further PPh_3 (0.11 mmol) was added to the reaction mixture, and reflux was continued for an additional 1 h. The resulting solution was poured into distilled H_2O, partitioned with EtOAc, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:7) to yield dehydro-α-lapachones **9a–9c**.

Dehydro-α-lapachones (9a): Orange amorphous powder, 68% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 (6H, s, 2Me), 5.73 (1H, d, *J* = 10.1 Hz, H-3), 6.66 (1H, d, *J* = 10.1 Hz, H-4), 7.66–7.74 (2H, m, H-7 and H-8), 8.07–8.10 ppm (2H, m, H-6 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.5 (2C), 80.5, 115.5, 117.9, 126.3 (2C), 131.0, 131.56, 131.63, 133.3, 134.0, 152.5, 179.9, 181.9 ppm; IR (film): $\tilde{\nu}$ = 1674, 1646, 1592, 1570, 1332, 1274 1132 cm^{-1}; EIMS: *m/z* 240 $[M]^+$, 225 $[M-CH_3]^+$, 197 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{12}O_3$: 240.0786, found: 240.0782.

7-Methoxydehydro-α-lapachones (9b): Orange amorphous powder, 74% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.56 (6H, s, 2Me), 3.94 (3H, s, OMe), 5.69 (1H, d, *J* = 10.1 Hz, H-3), 6.63 (1H, d, *J* = 10.1 Hz, H-4), 7.12 (1H, dd, *J* = 2.8, 8.7 Hz, H-8), 7.52 (1H, d, *J* = 2.8 Hz, H-6), 8.02 ppm (1H, d, *J* = 8.7 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (2C), 56.0, 80.6, 110.2, 115.5, 117.5, 119.3, 125.0, 128.9, 130.3, 133.9, 152.9, 164.4, 178.9, 181.8 ppm; IR (film): $\tilde{\nu}$ = 1667, 1652, 1594, 1318, 1254, 1131 cm^{-1}; EIMS: *m/z* 270 $[M]^+$, 255 $[M-CH_3]^+$, 242 $[M-CO]^+$, 227 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{14}O_4$: 270.0892, found: 270.0873.

8-Methoxydehydro-α-lapachones (9c): Orange amorphous powder, 74% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.55 (6H, s, 2Me), 3.94 (3H, s, OMe), 5.72 (1H, d, *J* = 10.1 Hz, H-3), 6.65 (1H, d, *J* = 10.1 Hz, H-4), 7.16 (1H, dd, *J* = 2.3, 8.3 Hz, H-7), 7.53 (1H, d, *J* = 2.8 Hz, H-9), 8.02 ppm (1H, d, *J* = 8.7 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.4 (2C), 56.0, 80.3, 110.1, 115.7, 117.9, 120.1, 125.0, 128.6, 131.0, 133.5, 152.2, 163.8, 180.0, 181.3 ppm; IR (film): $\tilde{\nu}$ = 1675, 1646, 1599, 1333, 1281 cm^{-1}; EIMS: *m/z* 270 $[M]^+$, 255 $[M-CH_3]^+$, 242 $[M-CO]^+$, 227 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{14}O_4$: 270.0892, found: 270.0881.

Synthesis of dehydro-β-lapachones 10a–10c: $TiCl_4$ (1 M in CH_2Cl_2, 0.42 mmol) was added to a solution of dehydro-α-lapachones (**9a–9c**, 0.21 mmol) in CH_2Cl_2 (2 mL) at 0 °C. After stirring for 2 h, distilled H_2O was added to the reaction mixture, and stirring was continued for a few minutes. The resulting solution was poured into distilled H_2O, partitioned with CH_2Cl_2, washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with CH_2Cl_2 to yield dehydro-β-lapachones **10a–10c**.

Dehydro-β-lapachone (10a): Black amorphous powder, 40% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.58 (6H, s, 2Me), 5.56 (1H, d, *J* = 10.1 Hz, H-3), 6.63 (1H, d, *J* = 10.1 Hz, H-4), 7.56 (1H, td, *J* = 1.4 and 7.8 Hz, H-8), 7.68 (1H, td, *J* = 1.4 and 7.8 Hz, H-9), 7.86 (1H, dd, *J* = 0.9 and 7.8 Hz, H-7), 8.07 ppm (1H, dd, *J* = 0.9 and 7.8 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.7 (2C), 81.8, 111.9, 115.8, 124.4, 125.8, 128.9, 130.6, 131.5, 131.6, 135.0, 162.1, 175.5, 179.8 ppm; IR (film): $\tilde{\nu}$ = 1651, 1462, 1273 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{12}O_3$: 240.0786, found: 240.0766.

8-Methoxydehydro-β-lapachone (10b): Black amorphous powder, 53% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.57 (6H, s, 2Me), 3.95 (3H, s, OMe), 5.56 (1H, d, *J* = 10.6 Hz, H-3), 6.59 (1H, d, *J* = 10.1 Hz, H-4), 6.96 (1H, dd, *J* = 2.3 and 8.7 Hz, H-9), 7.28 (1H, d, *J* = 2.7 Hz, H-7), 8.03 ppm (1H, d, *J* = 8.7 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.7 (2C), 56.0, 81.7, 110.7, 112.1, 115.3, 115.8, 123.9, 125.9, 131.8, 133.8, 161.2, 165.1, 176.2, 178.2 ppm; IR (film): $\tilde{\nu}$ = 1644, 1596, 1563, 1317, 1254 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{14}O_4$: 270.0892, found: 270.0878.

9-Methoxydehydro-β-lapachone (10c): Black amorphous powder, 66% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.52 (6H, s, 2Me), 3.89 (3H, s, OMe), 5.48 (1H, d, *J* = 10.1 Hz, H-3), 6.57 (1H, d, *J* = 10.5 Hz, H-4), 7.10 (1H, dd, *J* = 2.8 and 8.7 Hz, H-8), 7.52 (1H, d, *J* = 2.7 Hz, H-10), 7.73 ppm (1H, d, *J* = 8.7 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.7 (2C), 56.0, 81.8, 110.1, 113.3, 115.9, 121.0, 124.2, 124.8, 126.4, 132.4, 162.5, 163.2, 175.4, 179.9 ppm; IR (film): $\tilde{\nu}$ = 1631, 1599, 1338, 1283 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{14}O_4$: 270.0892, found: 270.0894.

Synthesis of α-lapachones 11a–11c: Dehydro-α-lapachones (**9a–9c**, 0.42 mmol) and Pd/C (10 wt.%) were dissolved in EtOAc (3 mL) under H_2 atmosphere. After stirring for 20 min, the resulting solution was filtered through Celite and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:6) to yield α-lapachones **11a–11c**.

α-Lapachone (11a): Yellow amorphous powder, 87% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.44 (6H, s, 2Me), 1.83 (2H, t, *J* = 6.9 Hz, H-3), 2.62 (2H, t, *J* = 6.4 Hz, H-4), 7.60–7.69 (2H, m, H-7 and H-8), 8.06–8.09 ppm (2H, m, H-6 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.8, 26.6 (2C), 31.5, 78.2, 120.2, 126.0, 126.4, 131.2, 132.2, 133.0, 133.9, 154.7, 180.1, 184.4 ppm; IR (film): $\tilde{\nu}$ = 1678, 1645, 1612, 1269, 1204, 1116 cm^{-1}; EIMS: *m/z* 242 $[M]^+$, 227 $[M-CH_3]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{14}O_3$: 242.0943, found: 242.0936.

7-Methoxy-α-lapachone (11b): Yellow amorphous powder, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43 (6H, s, 2Me), 1.81 (2H, t, *J* = 6.9 Hz, H-3), 2.60 (2H, t, *J* = 6.9 Hz, H-4), 3.94 (3H, s, OMe), 7.10 (1H, dd, *J* = 2.8 and 8.7 Hz, H-8), 7.52 (1H, d, *J* = 2.8 Hz, H-6), 8.02 ppm (1H, d, *J* = 8.7 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.8, 26.6 (2C), 31.5, 56.0, 78.2, 109.9, 119.1, 119.6, 124.7, 129.0, 134.4, 155.0, 164.3, 179.1, 184.4 ppm; IR (film): $\tilde{\nu}$ = 1670, 1643, 1593, 1331, 1252 cm^{-1}; EIMS: *m/z* 272 $[M]^+$, 257 $[M-CH_3]^+$, 244 $[M-CO]^+$, 229 $[M-CH_3-CO]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1037.

8-Methoxy-α-lapachone (11c): Yellow amorphous powder, 81% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.43 (6H, s, 2Me), 1.81 (2H, t, J = 6.4 Hz, H-3), 2.60 (2H, t, J = 6.8 Hz, H-4), 3.93 (3H, s, OMe), 7.15 (1H, dd, J = 2.8 and 8.7 Hz, H-7), 7.51 (1H, d, J = 2.7 Hz, H-9), 7.99 ppm (1H, d, J = 8.7 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 16.8, 26.6 (2C), 31.5, 55.9, 78.0, 110.0, 120.1 (2C), 125.6, 128.3, 133.1, 154.4, 163.5, 180.1, 183.8 ppm; IR (film): $\tilde{\nu}$ = 1677, 1616, 1595, 1335, 1272 cm^{-1}; EIMS: m/z 272 $[M]^+$, 257 $[M-CH_3]^+$, 244 $[M-CO]^+$, 229 $[M-CH_3-CO]^+$; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_4$: 272.1049, found: 272.1025.

Synthesis of rhinacanthin A analogues 12a–12c: *m*CPBA (containing 25% H_2O, 0.23 mmol) was added to a solution of dehydro-α-lapachones (**9a–9c**, 0.21 mmol) in CH_2Cl_2 (3 mL) at 0 °C. After stirring for 6 h, $NaBH_3CN$ (0.83 mmol) and $BF_3{\cdot}Et_2O$ (0.62 mmol) were added to the reaction mixture, and stirring was continued for 2 h. The resulting solution was poured into distilled H_2O, partitioned with $CHCl_3$, washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:3) to yield rhinacanthin A analogues **12a–12c**.

Rhinacanthin A (12a): Yellow amorphous powder, 21% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.39 (3H, s, Me), 1.46 (3H, s, Me), 2.66 (1H, dd, J = 5.5 and 19.2 Hz, H-4α), 2.82 (1H, dd, J = 5.0 and 19.2 Hz, H-4β), 3.87 (1H, t, J = 5.5 Hz, H-3), 7.61–7.67 (2H, m, H-7 and H-8), 7.98–8.03 ppm (2H, m, H-6 and H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9, 24.8, 25.8, 68.3, 80.6, 118.3, 126.1, 126.5, 131.1, 132.0, 133.2, 134.1, 153.8, 179.6, 184.5 ppm; IR (film): $\tilde{\nu}$ = 3407, 1617, 1384, 1268, 1207, 1128 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{15}H_{14}O_4$: 258.0892, found: 258.0871.

7-Methoxyrhinacanthin A (12b): Yellow amorphous powder, 27% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (3H, s, Me), 1.49 (3H, s, Me), 2.67 (1H, dd, J = 5.0 and 18.8 Hz, H-4α), 2.81 (1H, dd, J = 5.0 and 19.2 Hz, H-4β), 3.92 (3H, s, OMe), 7.06 (1H, dd, J = 2.8 and 9.2 Hz, H-8), 7.41 (1H, d, J = 2.8 Hz, H-6), 7.93 ppm (1H, d, J = 8.7 Hz, H-9); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.1, 24.7, 25.8, 56.0, 68.3, 80.7, 109.8, 117.5, 119.4, 124.4, 129.0, 134.2, 154.1, 164.4, 178.5, 184.4 ppm; IR (film): $\tilde{\nu}$ = 3408, 1592, 1463, 1086 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0991.

8-Methoxyrhinacanthin A (12c): Yellow amorphous powder, 39% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (3H, s, Me), 1.49 (3H, s, Me), 2.67 (1H, dd, J = 5.0 and 19.2 Hz, H-4α), 2.82 (1H, dd, J = 5.0 and 19.4 Hz, H-4β), 3.92 (3H, s, OMe), 7.11 (1H, dd, J = 2.7 and 8.7 Hz, H-7), 7.45 (1H, d, J = 2.8 Hz, H-9), 7.93 ppm (1H, d, J = 8.7 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.0, 24.7, 25.8, 56.0, 68.4, 80.4, 110.1, 118.1, 120.2, 125.4, 128.4, 132.9, 153.6, 163.6, 179.6, 183.9 ppm; IR (film): $\tilde{\nu}$ = 3408, 1621, 1595, 1273, 1129 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{16}H_{16}O_5$: 288.0998, found: 288.0989.

Synthesis of bislawsones 13a–13c: Lawsones (**1a–1c**, 4.90 mmol) and $(NH_4)_2S_2O_8$ (9.80 mmol) were dissolved in MeCN/H_2O (1:1, 30 mL) at RT. After stirring at 80 °C for 3 h, the reaction mixture was completely cooled. The resulting solution was filtered, and the residual substance was washed with distilled H_2O and Et_2O, to yield bislawsones **13a–13c**.

Bislawsone (13a): Yellow amorphous powder, 25% yield; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.84–7.92 (4H, m, H-6, H-6′, H-7, and H-7′), 8.00 (2H, dd, J = 1.4 and 7.8 Hz, H-5 and H-5′), 8.10 ppm (2H, dd, J = 1.4 and 7.3 Hz, H-8 and H-8′); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 116.0 (2C), 126.4 (2C), 126.6 (2C), 130.7 (2C), 132.5 (2C), 134.0 (2C), 135.4 (2C), 156.9 (2C), 181.4 (2C), 182.7 ppm (2C); IR (film): $\tilde{\nu}$ = 3408, 1642, 1384 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{20}H_{10}O_6$: 346.0477, found: 346.0463.

6,6′-Dimethoxybislawsone (13b): Yellow amorphous powder, 66% yield; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 3.99 (6H, s, 2OMe), 7.32 (2H, dd, J = 2.8 and 8.7 Hz, H-7 and H-7′), 7.38 (2H, d, J = 2.8 Hz, H-5 and H-5′), 8.01 ppm (2H, d, J = 8.7 Hz, H-8 and H-8′); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 56.6 (2C), 110.9 (2C), 115.6 (2C), 119.5 (2C), 123.8 (2C), 129.4 (2C), 135.0 (2C), 157.1 (2C), 165.0 (2C), 180.1 ppm (4C); IR (film): $\tilde{\nu}$ = 3400, 1593, 1301 cm^{-1}; HR EI MS: m/z $[M]^+$ calcd for $C_{22}H_{14}O_8$: 406.0689, found: 406.0670.

7,7′-Dimethoxybislawsone (13c): Yellow amorphous powder, 25% yield; ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 3.96 (6H, s, 2OMe), 7.40 (2H, dd, J = 3.2 and 9.1 Hz, H-6 and H-6′), 7.51 (2H, d, J = 2.7 Hz, H-8 and H-8′), 7.94 ppm (2H, d, J = 8.7 Hz, H-5 and H-5′); ^{13}C NMR (100 MHz, [D_6]DMSO): δ = 56.5 (2C), 110.5 (2C), 115.9 (2C), 121.0 (2C), 125.8 (2C), 128.9 (2C), 132.5 (2C), 156.5 (2C), 163.6 (2C), 181.4 (2C), 182.2 ppm (2C); IR (film): $\tilde{\nu}$ = 3409, 1678, 1596, 1285 cm^{-1}; HR ESI ToF MS: m/z $[M+H]^+$ calcd for $C_{22}H_{15}O_8$: 407.0767, found: 407.0792.

Synthesis of nordihydrolapachenol (14a) and tetrahydrotectol (15a): HI (1.5 mL) was added to a solution of β-lapachones (**4a**, 0.54 mmol) in AcOH (5 mL) at RT. After holding at reflux for 2 h, the resulting solution was poured into 2% aqueous $Na_2S_2O_3$, partitioned with $CHCl_3$ (3×20 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with CH_2Cl_2/*n*-hexane (1:1) to yield nordihydrolapachenol (**14a**, 50%) and tetrahydrotectol (**15a**, 6%).

DIPEA (1.62 mmol) was added to a solution of nordihydrolapachenol (**14a**, 0.54 mmol) and TBSCl (1.08 mmol) in THF (5 mL) at RT. After stirring for 2 h, the resulting solution was poured into 10% aqueous HCl, partitioned with EtOAc, and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo overnight. $NEt_3{\cdot}3HF$ (2.70 mmol) was added to a solution of the reaction residue in THF (5 mL) at RT. After stirring for 24 h, the resulting solution was poured into distilled H_2O, partitioned with CH_2Cl_2, and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography, eluting with CH_2Cl_2/*n*-hexane (2:1) to yield nordihydrolapachenol (**14a**, 83%) and tetrahydrotectol (**15a**, 17%).

Nordihydrolapachenol (14a): Pale-brown amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.39 (6H, s, 2Me), 1.86 (2H, t, J = 6.9 Hz, H-3), 2.78 (2H, t, J = 6.9 Hz H-4), 5.05 (1H, s, H-5), 6.52 (1H, s, OH), 7.44 (2H, m, H-8 and H-9), 8.04 (1H, d, J = 9.6 Hz, H-7), 8.17 ppm (1H, d, J = 6.4 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.0, 26.9 (2C), 33.0, 74.1, 110.1, 113.9, 121.3, 121.7, 124.3, 125.1, 125.6, 126.5, 142.9, 144.0 ppm; IR (film): $\tilde{\nu}$ = 3434, 1597, 1376 cm^{-1}; HR ESI ToF MS: m/z $[M+H]^+$ calcd for $C_{15}H_{17}O_2$: 229.1229, found: 229.1207.

Tetrahydrotectol (15a): White amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (6H, s, 2Me), 1.41 (6H, s, 2Me), 1.79 (4H, t, J = 6.8 Hz, H-3 and H-3′), 2.23–2.50 (4H, m, H-4 and H-4′), 4.92 (2H, s, 2OH), 7.50–7.57 (4H, m, H-8, H-8′, H-9, and H-9′), 8.20 (2H, d, J = 7.8 Hz, H-7 and H-7′), 8.27 ppm (2H, d, J = 7.8 Hz, H-10 and H-10′); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.2 (2C), 26.6 (2C), 26.9 (2C), 32.9 (2C), 73.9 (2C), 112.3 (2C), 113.4 (2C), 121.7 (2C), 122.3 (2C), 123.7 (2C), 125.6 (2C), 126.4 (2C), 126.9 (2C), 142.8 (2C), 143.6 ppm (2C); IR (film): $\tilde{\nu}$ = 3408, 1592, 1392, 1122 cm^{-1}; EIMS: m/z 454 $[M]^+$,

441 $[M-CH_3]^+$; HR EI MS: *m/z* $[M]^+$ calcd for $C_{30}H_{30}O_4$: 454.2144, found: 454.2150.

Synthesis of 2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromenes 14b and 14c: HI (1.5 mL) was added to a solution of methoxy-β-lapachones (**4b** and **4c**, 0.54 mmol) in AcOH (5 mL) at RT. After holding at reflux for 2 h, the resulting solution was poured into 2% aqueous $Na_2S_2O_3$, partitioned with $CHCl_3$ (3×20 mL), and washed with brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by silica gel column chromatography, eluting with EtOAc/*n*-hexane (1:5) to yield 2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromenes **14b** and **14c**.

9-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (14b): Pale-red amorphous powder, 44% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.41 (6H, s, 2Me), 1.88 (2H, t, *J* = 6.8 Hz, H-4), 2.86 (2H, t, *J* = 6.9 Hz, H-3), 5.43 (1H, s, OH), 7.02 (1H, d, *J* = 8.7 Hz, H-5), 7.06 (1H, dd, *J* = 2.7 and 9.2 Hz, H-8), 7.25 (1H, d, *J* = 8.2 Hz, H-6), 7.53 (1H, d, *J* = 2.8 Hz, H-10), 7.65 ppm (1H, d, *J* = 8.7 Hz, H-7); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9, 27.0 (2C), 32.9, 74.6, 104.1, 115.0, 117.1, 118.9, 125.4, 126.8, 128.7, 129.5, 147.6, 153.2 ppm; IR (film): $\tilde{\nu}$ = 3399, 1607, 1384, 1218 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{16}O_2$: 228.1150, found: 228.1141.

8-Hydroxy-2,2-dimethyl-3,4-dihydro-2*H*-benzo[*h*]chromene (14c): Pale-brown amorphous powder, 13% yield; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.40 (6H, s, 2Me), 1.87 (2H, t, *J* = 6.9 Hz, H-4), 2.83 (2H, t, *J* = 6.9 Hz, H-3), 7.00 (1H, dd, *J* = 2.8 and 9.2 Hz, H-9), 7.04 (1H, d, *J* = 2.3 Hz, H-7), 7.10 (1H, d, *J* = 8.2 Hz, H-5), 7.14 (1H, d, *J* = 8.7 Hz, H-6), 8.11 ppm (1H, d, *J* = 9.2 Hz, H-10); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.7, 27.0 (2C), 33.0, 74.6, 109.4, 112.3, 116.4, 117.5, 121.1, 123.9, 128.8, 134.7, 149.0, 153.3 ppm; IR (film): $\tilde{\nu}$ = 3399, 1598, 1385 cm^{-1}; HR EI MS: *m/z* $[M]^+$ calcd for $C_{15}H_{16}O_2$: 228.1150, found: 228.1139.

Cell culture: HL-60 cells (DS Pharma Biomedical Co. Ltd., Osaka, Japan) were cultured in RPMI 1640 media (Wako Pure Chemical Industries Ltd., Osaka, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% antibiotics, penicillin/streptomycin (Gibco, Life Technologies, Thermo Fisher Scientific Inc., MA, USA). Cells were maintained at 37 °C under a humidified atmosphere of 5% CO_2.

CCK-8 assay: CCK-8 assays were performed essentially as described previously.[30] Cell counting kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). HL-60 cells (2×10^4 cells per mL, 100 μL) were seeded in 96-well plates. After 24 h incubation, solutions of test compounds were added to the culture. Following 48 h incubation, CCK-8 solution (10 μL) was added, and the plates were incubated for an additional 2 h. Visible absorption (λ 436 nm) was measured using a microplate reader (E_{max} precision microplate reader, Molecular Devices Japan, Tokyo, Japan).

Measurement of total glutathione (tGSH) and oxidized GSSG: To detect the presence of ROS derived from test compounds, oxidative stress measurements were performed. HL-60 cells (1×10^7 cells per mL) were seeded in 50 cm^2 culture flasks. After 24 h incubation, test compounds were added to the culture (final concentration: 25 μM). Following 12 h incubation, the cells were transferred into microfuge tubes and centrifuged at 200×*g* for 10 min. Collected cell pellets were washed with PBS and lysed by two cycles of freeze–thaw treatment in the presence of 8 mM HCl. 5-Sulfosalicylic acid (SSA) solution (5%, 40 μL) was added, and the supernatant of the lysate was obtained by centrifugation (8000×*g* for 10 min). Measurement of oxidative stress markers, tGSH and GSSG, were carried out using a GSH/GSSG quantification kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) according to the manufacturer's instructions. The concentrations of tGSH and GSSG were measured by using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) as a substrate in the absence and presence of GSH masking reagent, respectively. Visible absorption (λ 436 nm) was measured using a microplate reader. Standard curves were defined by using several fixed concentrations of substrate in each experiment, and the concentrations of tGSH and GSSH in test samples were calculated from the standard curves. Three replicates were performed for each compound and untreated control.

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