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Synthesis and evaluation of bioactive naphthoquinones from the Brazilian medicinal plant, *Tabebuia avellanedae*

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ABSTRACT

A series of naphthoquinones based on the naphtho[2,3-*b*]furan-4,9-dione skeleton such as (−)-5-hydroxy-2-(1′-hydoxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**) and its positional isomer, (−)-8-hydroxy-2-(1′-hydoxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**2**), which are secondary metabolites found in the inner bark of *Tabebuia avellanedae*, were stereoselectively synthesized and their biological activities were evaluated in conjunction with those of their corresponding enantiomers. Compound **1** exhibited potent antiproliferative effect against several human tumor cell lines, but its effect against some human normal cell lines was much lower than that of mitomycin. On the other hand, its enantiomer (*R*)-1 was less active toward the above tumor cell lines than **1**. The antiproliferative effect of **2** against all tumor cell lines was significantly reduced. These results indicated the presence of the phenolic hydroxy group at C-5 is of great important for increasing antiproliferative effect. In addition, **1** also showed higher cancer chemopreventive activity than **2**, while there were no significant differences between **1** and **2** in antimicrobial activity. Both compounds displayed modest antifungal and antibacterial activity (Gram-positive bacteria), whereas they were inactive against Gram-negative bacteria.

1. Introduction

Tabebuia avellanedae LORENTZ *ex* GRISEB[1] (Bignoniaceae) (syn. *Tabebuia impetiginosa*), which is widespread in South America throughout Brazil to north Argentina, has been well known as a traditional medicine since the Incan Era.[2] The stem bark of *T. avellanedae* has been used as a diuretic and as an astringent. In addition, the stem bark of this plant shows a wide array of biological activities such as antitumor, antibacterial, antifungal and anti-inflammatory activity.[3] In particular, the discovery of its antitumor activity has made *T. avellanedae* an important medicinal resource.[4] A series of naphthoquinones and anthraquinones, a number of simple benzoic acid derivatives and iridoid glycosides has been isolated as secondary metabolites of this plant.[5] Among these constituents, naphtho[2,3-*b*]furan-4,9-dione analogues such as (−)-5-hydroxy-2-(1′-hydoxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**) and its positional isomer, (−)–8-hydroxy-2-(1′-hydoxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**2**) are, in particular, worthy of attention because of their potent antiproliferative activity against various tumor cells as well as lapachol (**3**) and β-lapachone (**4**) that are congeners of **1** in this plant (Fig. 1).[6] In addition, compound **1** strongly inhibits Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cell[7] and TPA-induced tumor promotion on mouse skin initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) in two-stage carcinogenesis tests. Therefore, compound **1** is expected to be a potential cancer chemopreventer.[8]

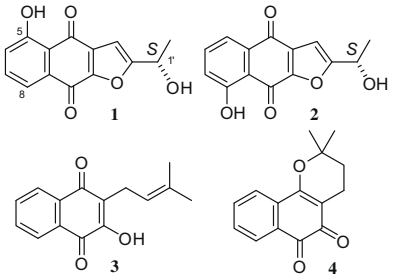

Figure 1.

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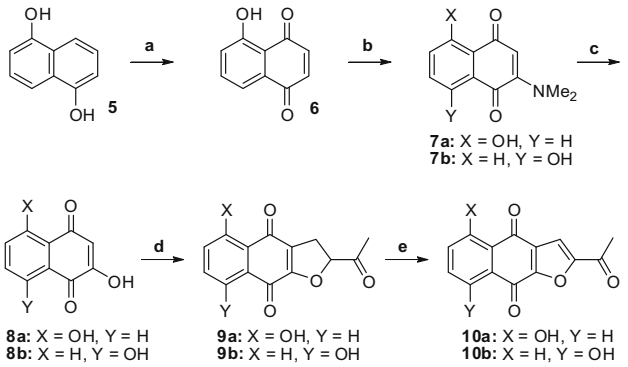
In our precedent paper,[9] we reported a concise and stereoselective synthesis of **1** and its antiproliferative effect toward several tumor cell lines and in vitro cancer preventive activity. Through our preliminary results, we clearly recognized that there were significant differences between **1** and (*R*)-**1** in their biological activities mentioned above. This finding promoted us to synthesize both **2** and its enantiomer (*R*)-**2** and to evaluate their biological activities since it has been reported by Wagner et al. that compound **2** was isolated in the state of a racemic mixture.[5c] In fact, we also could obtain **2** only as a racemate through repeated HPLC of a $CHCl_3$ extract of *T. avellanedae*. On the other hand, Fujimoto's group, who first obtained **1** and (*R*)-**1** together with **2** and (*R*)-**2** by optical resolution of the synthetic racemates **1** and **2**, isolated **2** from the same plant as a mixture in which the ratio of the (*R*)- and (*S*)-enantiomer was 3: 1. Furthermore, they reported that there were no differences between the two pairs of enantiomers in their antiproliferative activity toward L1210 cells.[6c] These results also encouraged us to advance our research program. Herein we report the stereoselective synthesis of two pairs of enantiomers described above via Noyori reduction as a key step and their antiproliferative effect against some human tumor cell lines and their corresponding human normal cell lines, in vitro cancer chemopreventive activity and antimicrobial activity.[10] In addition, in vivo cancer chemopreventive activity of **1** and (*R*)-**1** is also described.

2. Results and discussion

2.1. Chemistry

The gram-scale synthesis of **1** and **2** were accomplished similarly according to our preliminary synthesis of **1**.[9] The synthetic way to **1** and **2** is shown in Scheme 1. The synthesis of juglone (**6**) was started from commercially available 1,5-hydroxynaphthalene, which was oxidized with air in the presence of CuCl in the dark to give **6** in 47% yield.[11] Chemical transformations of **6** to **8** was carried out according to the reported methods[12] with some modifications. Oxidative amination of **6** with dimethylamine (2.0 M solution in THF) in toluene at −40 °C gave 2-dimethylaminojuglone (**7a**) and 3-dimethylaminojuglone (**7b**) in 42% and 11% yields, respectively. As reported in the precedent paper,[9] a THF solution of dimethylamine is suitable reagent for practical use, and moderate chemical yield and regioselectivity were observed. Deamination of 2-dimethylaminojuglone (**7a**) with 10% aqueous HCl gave 2,5-dihydroxy-1,4-naphthoquinone (**8a**) in quantitative chemical yield.[12b] The naphtho[2,3-*b*]furan-4,9-dione skeleton was constructed by the reaction of **8a** with 3,4-dibromobutan-2-one, which was synthesized from commercially available but-3-en-2-one and bromine, in the presence of DBU in THF according to the method reported by Hagiwara and others[13] to give naphthodihydrofuran **9a**. It should be noted that the gram-scale synthesis afforded only **9a** without the simultaneous formation of **10a** differently from the case of the milligram-scale synthesis.[9a] After the crude product was washed with MeOH, naphthodihydrofuran **9a** was treated with MnO_2 in chloroform to provide the desired naphthofuran **10a** in 50% yield in two steps. Compound **10b** was synthesized from **8b** via **9b** in the same manner as that described above. Subsequent Noyori reduction completed the stereoselective synthesis of **1** and **2** (Scheme 2).[14] Asymmetric transfer hydrogenation of naphthofurans **10a** and **10b,** which was catalyzed by a commercially available chiral Ru(II) complex, Ru [(*S*,*S*)-Tsdpen] (*p*-cymene)[15] in a formic acid–triethylamine mixture and CH_2Cl_2, successfully afforded the corresponding secondary alcohols **1** and **2** in high chemical yield and enantiomeric excess (89–97% yield, 95–97% ee). The physico-chemical properties of all the compounds synthesized in this study were identical in all respects to those reported previously.[13] The corresponding enantiomers (*R*)-**1** and (*R*)-**2** were similarly prepared using Ru [(*R*,*R*)-Tsdpen] (*p*-cymene) as a chiral catalyst.[16]



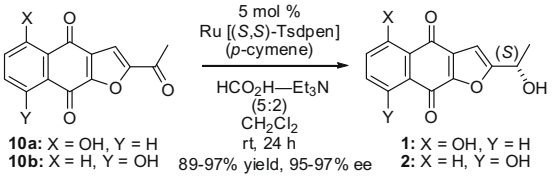
Scheme 1. Synthesis of **10**. Reagents and conditions: (a) CuCl, CH_3CN, air, rt, 47%; (b) Me_2NH, toluene, THF, −40 °C, 42% and 11% for **7a** and **7b**, respectively; (c) 10% HCl, dioxane, reflux, 97%; (d) 3,4-dibromobutan-2-one, DBU, THF, room temperature; (e) MnO_2, $CHCl_3$, reflux, 50–60% in two steps.

2.2. Biology

As reported so far, naphthoquinones isolated from the *Tabebuia* plants are recognized as antiproliferative compounds.[3b,6,7] Thus, the two pairs of enantiomers synthesized were also evaluated for their ability to suppress the growth of human tumor cell lines including PC-3 (prostate), A549 (lung) and MCF-7 (breast) (Table 1). Compound **1** exhibited rather potent antiproliferative activity against all the three cell lines (0.14–0.78 μM), which was almost equal to that of mitomycin (0.14–0.96 μM) and much higher than that of β-lapachone (1.13–9.96 μM). However, its antiproliferative effect against human normal cell lines including Hs888Lu (lung) and SVCT-M12 (breast) was noticeably lower than that of mitomycin (**1**: 3.36–54.5 μM, mitomycin: 0.56–1.46 μM) (Table 2).

On the other hand, the antiproliferative activity of its enantiomer (*R*)-**1** was discernibly reduced toward the tumor cell lines, in particular against MCF-7 cells, compared with that of **1**. These results suggested that the orientation of the hydroxy group at C-1′ is crucial. Interestingly, ketone **10a** was between **1** and (*R*)-**1** in antiproliferative activity. This finding suggests the superiority of the α-oriented hydroxy group of **1**. Regarding the four human normal cell lines tested (Table 2), the antiproliferative activity of (*R*)-**1** was somewhat higher than **1** only against IE cells.

The comparison between **1** and **2** showed that the position of a hydroxy group attached to the aromatic ring also affects their ability to suppress the growth of the tumor cell lines. As shown in Table 1, the antiproliferative effect of **2** that possesses a hydroxy function at C-8 was considerably lower than that of **1** (1.57–4.31 μM) although it was comparable with that of β-lapachone. Thus, the presence of a phenolic hydroxy group at C-5 seems to play an important role in increasing antiproliferative effect. A similar effect was also observed between **10a** and **10b**, supporting its role described above.



Scheme 2. Chiral Ru catalyst mediated asymmetric hydrogenation of naphthofuran **10**.

Table 1
Antiproliferative effects of **10a**, **10b**, **1**, (*R*)-**1**, racemate **1**, **2**, β-lapachone, lapachol and mitomycin against human tumor cell lines[a]

Compound	EC_{50} (μM) Human tumor cell lines		
	PC-3	A549	MCF-7
10a	0.84	2.0	0.78
10b	3.00	4.31	2.61
Racemate **1**	0.56	3.24	8.5
(*R*)-**1**	0.93	3.0	9.3
1	0.14	0.78	0.51
2	1.57	4.31	2.61
β-Lapachone	1.13	4.21	9.96
Lapachol	1.97	4.78	10.15
Mitomycin	0.14	0.43	0.96

[a] Human tumor cell lines: PC-3 = prostate, A549 = lung, MCF-7 = breast.

Table 2
Antiproliferative effects of **10a**, **10b**, **1**, (*R*)-**1**, racemate **1**, **2** and mitomycin against human normal cell lines[a]

Compound	EC_{50} (μM) Human normal cell lines					
	Fb	Hc	MPC-5	IE	Hs888Lu	SVCT-M12
10a	NT[b]	NT	NT	NT	7.7	7.7
10b	NT	NT	NT	NT	14.6	11.7
Racemate **1**	45.4	89.3	89.3	158	NT	NT
(*R*)-**1**	39.7	29.8	65.9	39.7	NT	NT
1	11.1	11.1	29.7	54.5	5.51	3.36
2	NT	NT	NT	NT	14.6	14.6
Mitomycin	0.93	1.46	2.1	1.46	0.56	0.96

[a] Human normal cell lines: Fb = skin, Hc = liver, MPC-5 = lung, IE = colon, Hs888Lu = lung, SVCT-M12 = breast.
[b] NT, not tested.

Unexpectedly, ketone **10a** exhibited potent antiproliferative effect toward the tumor cell lines (Table 1). In particular, it strongly suppressed the growth of MCF-7 cells, which was comparable to that of **1**. On the other hand, its antiproliferative effect against Hs888Lu and SVCT-M12 was not as high as that of **1** (Table 2). Therefore, **10a** is also eminently suitable for the development of anticancer drugs in addition to **1**.

The mechanism of action of compound **1** for antiproliferative effect is still unknown and now being under investigation. However, our preliminary results indicated that compound **1** affects E2F1, a multifunctional transcription factor. On the other hand, β-lapachone, which is a congener of **1**, has been shown to induce apoptosis in a variety of cancer cell lines and to be involved in transcription processes. Thus, we currently deduce that compound **1** also has a similar mechanism to that of β-lapachone.[6d]

Compound **1** has already been known to act as a cancer chemopreventive agent and showed potent inhibition on EBV-EA activation without cytotoxicity against Raji cells dose-dependently.[8] In order to compare in vitro cancer chemopreventive activity of (*R*)-**1**, **2** and (*R*)-**2** with that of **1**, they were evaluated for their ability to inhibit EBV-EA activation induced by TPA in Raji cells as a primary screening test for antitumor promoters along with β-lapachone and lapachol (Table 3). Among them, both **1** and (*R*)-**1** were found to be potent inhibitors with IC_{50} values (mol ratio/32 pmol TPA) of 33.2 and 38.9, respectively. In particular, compound **1** showed 10-fold higher inhibition than the positive control substance curcumin. Although (*R*)-**2**, which was unambiguously more potent than **2**, exhibited modest inhibitory effect that was comparable with that of β-lapachone, it was much less potent than **1**. These results strongly suggest that the hydroxy group at C-5 plays an important role in increasing the inhibitory effect as well as the antiproliferative effect against the tumor cell lines described above. Moreover, the values of cell viability at 1000 mol ratio in Table 3 indicate that the naphtho[2,3-*b*]furan-4,9-dione analogues are less cytotoxic than β-lapachone and lapachol.

Table 3
Inhibitory effect of **1**, (*R*)-**1**, racemate **1**, **2**, (*R*)-**2**, racemate **2**, β-lapachone and lapachol on TPA-induced EBV-EA activation

Compound	EBV-EA-positive cells (% viability)[a] Compound concentration (mol ratio/32 pmol TPA)				
	1000	500	100	10	IC_{50} (μM)
10a	0 (60)	7.1	23.7	56.0	37.2
10b	0 (70)	8.3	27.5	60.3	40.1
Racemate **1**	0 (60)[b]	6.2 (70)	20.7	52.9	34.9
(*R*)-**1**	0 (70)	9.7	24.7	59.4	38.9
1	0 (60)	4.4 (60)	16.9	50.0	33.2
Racemate **2**	8.9 (60)	28.4	59.0	84.6	306.5
(*R*)-**2**	4.2 (60)	20.4	52.5	76.3	201.1
2	7.4 (60)	26.5	58.3	80.5	271.0
β-Lapachone	4.7 (50)	21.7	50.4	73.1	210.3
Lapachol	8.9 (50)	32.8 (60)	65.2	86.6	311.4
Curcumin[c]	8.9 (60)	40.3	74.5	95.8	345

[a] Values represent relative percentage to the positive control value (100%).
[b] Values in parentheses represent viability percentages of Raji cells measured through trypan blue staining; unless otherwise stated, the viability percentage of Raji cells were more than 80%.
[c] Positive control substance.

In addition to in vitro cancer chemopreventive activity mentioned above, it has already been known that compound **1** exhibits in vivo cancer chemopreventive activity.[8a] These findings inevitably led to the idea that naphtho[2,3-*b*]furan-4,9-dione analogues are likely to prove extremely useful for the development of cancer chemopreventive agents. Therefore, we reexamined inhibitory effect of **1** that was the most active in vitro and compared with that of (*R*)-**1** on mouse skin tumor promotion in two-stage carcinogenesis experiments in which DMBA is used as an initiator and TPA as a promoter. Figure 2a shows the incidence (%) of papilloma-bearing mice during 20 weeks of promotion. In group 1, in which only TPA was applied to mouse skin initiated by DMBA (positive control), the first tumors were observed at 6 weeks with about 7% incidence. The rate of papilloma-bearing mice reached 100% in additional 4 weeks. On the other hand, those in groups II–IV which were treated with **1**, (*R*)-**1**, racemate **1** before application of TPA was approximately 7–20% at 10 weeks, 47–53% at 15 weeks and 67–80% even at 20 weeks, respectively. Twenty weeks of promotion allowed group I to form 8.6 papillomas per mouse, while groups II–IV possessed only 3.9–4.7 papillomas per mouse. The inhibitory activities of **1**, (*R*)-**1** and racemate **1** were also observed as the average numbers of papillomas per mouse during 20 weeks of promotion (Fig. 2b). These results clearly demonstrate the potential of naphtho[2,3-*b*]furan-4,9-dione analogues as cancer chemopreventive agents. It is noteworthy that (*R*)-**1** was somewhat more potent than **1** in this in vivo experiment model.

Antimicrobial activity of naphtho[2,3-*b*]furan-4,9-dione analogues synthesized was also examined because some constituents isolated from the *Tabebuia* plants including **3** and **4** are known to be active toward bacteria and fungi (Table 4). All compounds including a synthetic precursor **10a,** which is also one of constituents of *T. avellanedae,* were found to have modest or potent antibacterial activity against several Gram-positive bacteria (0.78–6.25 μg/mL) and antifungal activity (1.56–100 μg/mL) in comparison with penicillin G and amphotericin B. On the other hand, all compounds were not active against common Gram-negative bacteria at concentrations up to 100 μg/mL. From these results, it is likely that the presence of the hydroxy group at C-5 makes a slight contribution toward increasing antimicrobial activity, while the other hydroxy group is not so important although detailed structural requirements cannot be discussed.

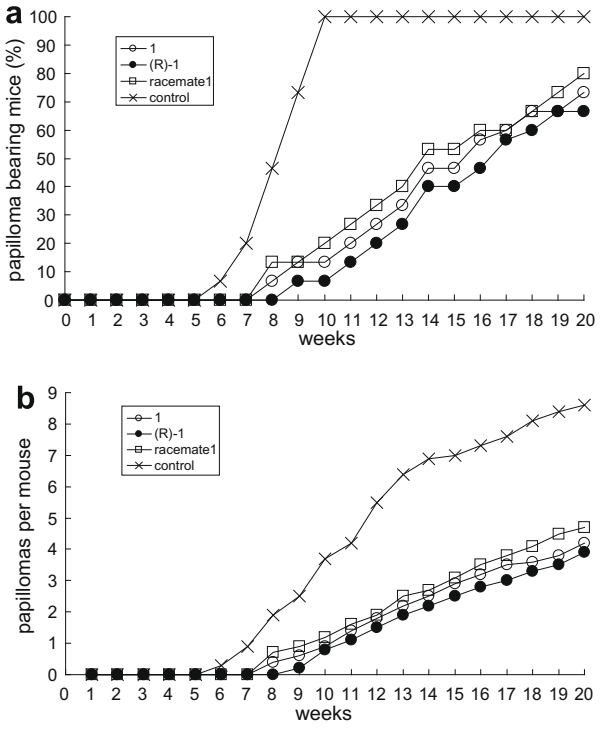


Figure 2. Inhibitory effect of **1** on mouse skin carcinogenesis induced by DMBA and TPA: X: control DMBA (390 nmol) and TPA (1.7 nmol) (group I); ○: DMBA (390 nmol), TPA (1.7 nmol) and **1** (85 nmol) (group II), ●: DMBA (390 nmol), TPA (1.7 nmol) and (*R*)-**1** (85 nmol) (group III), □: DMBA (390 nmol), TPA (1.7 nmol) and racemate **1** (85 nmol) (group IV). (a) Percentage of mice with papillomas. (b) Average number of papillomas per mouse. At 20 weeks of promotion, group II–IV were significantly different from group I (p <0.05) on papillomas per mouse.

3. Conclusions

In conclusion, the concise stereoselective synthesis of **1** and **2** starting from **5** was accomplished by using Noyori reduction as a key step, and the total yields of products **1** and **2** were 8.5% and 2.9%, respectively. Naphtho[2,3-*b*]furan-4,9-dione **1** synthesized in this study showed potent antiproliferative effect and in vitro and in vivo cancer chemopreventive activity. In addition, it displayed relatively strong antimicrobial activity against several Gram-positive bacteria and fungi. On the other hand, its positional isomer **2** was less active in antiproliferative effect and in vitro cancer chemopreventive activity, but exhibited similar antimicrobial activity to that of **1**. Furthermore, significant differences between **2** and (*R*)-**2** were recognized at least in the in vitro cancer chemopreventive activity. Interestingly, (*R*)-**1** showed somewhat higher cancer chemopreventive activity in vivo. Further modification toward structure–activity relationship studies is now in progress to better understand structural requirements for increasing their biological activities.

4. Experimental

^{1}H and ^{13}C NMR spectra were taken in $CDC1_3$ unless otherwise noted. Chemical shift values are expressed in ppm relative to internal tetramethylsilane. Abbreviations are as follows: s, singlet; d, doublet; t, triplet; m, multiplet. IR was expressed in cm^{-1}. The extract was dried over Na_2SO_4 unless otherwise noted. Purification was carried out using silica gel column chromatography. All reactions were carried out under an argon atmosphere unless otherwise stated.

5. Chemistry

5.1. 5-Hydroxy-1,4-naphthalenedione (6)[11]

This compound was prepared according to the reported procedure in 47% yield as yellow needles of mp 163–165 °C. ^{1}H NMR: 6.96 (1H, s), 7.29 (1H, dd, J = 2.0, 8.0 Hz), 7.62–7.67 (2H, m), 11.9 (1H, s). ^{13}C NMR: 114.9, 119.2, 124.5, 131.7, 136.6, 138.6, 139.6, 161.4, 184.3, 190.3.

5.2. 2-(Dimethylamino)-5-hydroxy-1,4-naphthalenedione (7a) and 2-(dimethylamino)-8-hydroxy-1,4-naphthalenedione (7b)[12a]

These compounds were prepared according to the reported procedure[12] with some modifications. To the solution of **6** (4.28 g, 24.5 mmol) in toluene (125 mL) was added dimethyl amine (18.4 mL, 2.0 M solution in THF, 36.8 mmol) at −40 °C. After the mixture was stirred for 12 h, the solvent was removed under reduced pressure. The residue was purified by column chromatography (hexane/EtOAc = 4/1) gave **7a** (2.23 g, 42% yield) as yellow needles of mp 145–146 °C and **7b** (0.58 g, 11% yield) as yellow needles of mp 155–157 °C.

5.3. 2,5-Dihydroxy-1,4-naphthalenedione (8a)[12b]

This compound was prepared from **7a** according to the reported procedure in 97% yield as yellow needles of mp >210 °C (dec.). ^{1}H NMR: 6.31 (1H, s),7.35 (1H, dd, J = 1.2, 8.5 Hz), 7.44 (1H, s), 7.59 (1H, t, J = 8.5 Hz), 7.69 (1H, dd, J = 1.2, 8.5 Hz), 12.33 (1H, s). ^{13}C

Table 4
Antimicrobial activity[a] of **1**, (*R*)-**1**, **2**, (*R*)-**2** and **10a**

	1	(*R*)-**1**	**2**	(*R*)-**2**	**10a**	PenicillinG[c]	AmphotericinB[c]
Bacillus subtilis NBRC3134	0.78	0.78	1.56	1.56	1.56	0.39	NT
Staphylococcus aureus NBRC13276	3.13	3.13	6.25	6.25	1.56	0.39	NT
Bacillus mycoides ATCC 11778	0.78	0.78	1.56	1.56	0.78	1.56	NT
Escherichia coli ATCC11775	>100	>100	>100	>100	>100	NT[b]	NT
Pseudomonas aeruginosa NBRC13275	>100	>100	>100	>100	>100	NT	NT
Salmonella enteritidis OPS10	>100	>100	>100	>100	>100	NT	NT
Candida albicans NBRC1060	25	25	25	25	>100	NT	1.56
Cryptococcus albidus NBRC0378	1.56	1.56	1.56	1.56	1.56	NT	1.56
Saccharomyces cerevisiae NBRC10114	3.13	3.13	3.13	3.13	1.56	NT	0.78
Aspergillus fumigatus NBRC4400	12.5	12.5	25	25	100	NT	1.56
Penicillium expansum NBRC8800	25	25	50	50	50	NT	1.56
Paecilomyces variotii NBRC4855	25	25	50	50	100	NT	0.78

[a] Values represent minimum growth inhibitory concentration, MIC (μg/mL).
[b] NT, not tested.
[c] Positive control substance.

NMR: 110.3, 114.5, 119.8, 126.7, 129.3, 135.2, 156.9, 161.4, 181.2, 191.2.

5.4. 2,8-Dihydroxy-1,4-naphthalenedione (8b)[12b]

Compound **8b** was prepared from **7b** according to the reported procedure in 97% yield as yellow needles of mp >215 °C (dec.). ^{1}H NMR: 6.31 (1H, s), 7.35 (1H, dd, J = 1.2, 8.5 Hz), 7.44 (1H, s), 7.59 (1H, t, J = 8.5 Hz), 7.69 (1H, dd, J = 1.2, 8.5 Hz), 12.33 (1H, s). ^{13}C NMR: 111.6, 113.1, 119.6, 123.4, 132.7, 138.2, 156.1, 161.6, 184.1, 185.1.

5.5. 2-Acetyl-2,3-dihydro-5-hydroxy-naphtho[2,3-*b*]furan-4,9-dione (9a)

This compound was prepared by the method of Hagiwara. To a solution of methyl vinyl ketone (215 g, 3.07 mol) in pentane (700 mL) was slowly added a solution of bromine (500 g, 3.13 mol) in pentane (600 mL) at −15 °C. After stirred for 10 min, concentration under reduced pressure gave 3,4-dibromobutan-2-one, which was immediately added to the solution of **8a** (97.3 g, 512 mmol) in THF (2.5 mL). To the solution was slowly added DBU (551 mL, 3.69 mol) at 0 °C, and the mixture was stirred for overnight at room temperature. The mixture was quenched with 10% HCl at 0 °C. The mixture was extracted with $CHCl_3$. The organic extracts were washed with brine, and then dried. Concentration and crystallization ($CHCl_3$/benzene = 2/1), then washing with MeOH gave **9a** (92 g, 70% yield) as yellow needles of mp 186–188 °C. ^{1}H NMR: 2.67 (3H, s), 7.32 (1H, dd, J = 1.0, 8.5 Hz), 7.59 (1H, s), 7.67 (1H, dd, J = 7.0, 8.5 Hz), 7.77 (1H, dd, J = 1.0, 7.0 Hz), 7.77 (1H, dd, J = 1.0, 7.0 Hz), 11.90 (1H, s). ^{13}C NMR: 26.6, 29.5, 87.4, 114.6, 119.7, 123.5, 126.0, 131.6, 135.4, 159.8, 161.3, 176.4, 187.5, 204.3. IR (KBr): 3400, 1717, 1678, 1647, 1616, 1450, 1234, 760. HRMS (ESI) m/z: $[M-H]^-$ calcd for $[C_{14}H_9O_5]^-$, 257.0450; found, 257.0452.

5.6. 2-Acetyl-2,3-dihydro-8-hydroxy-naphtho[2,3-*b*]furan-4,9-dione (9b)

Compound **9b** was prepared from **8b** using the same procedure as described for compound **9a**. Concentration and column chromatography (chloroform only) gave **9b** (72% yield) as yellow needles of mp 131–133 °C. ^{1}H NMR: 2.40 (3H, s), 3.40 (1H, d, J = 9.0 Hz), 3.40 (1H, d, J = 11.0 Hz), 5.29 (1H, dd, J = 9.0, 11.0 Hz), 7.23 (1H, dd, J = 3.5, 6.0 Hz), 7.63–7.61 (2H, m), 11.60 (1H, s). ^{13}C NMR: 26.4, 30.0, 87.2, 114.5, 119.4, 124.4, 124.6, 132.9, 137.1, 158.9, 162.1, 180.7, 181.8, 204.2. IR (KBr): 1736, 1650, 1620, 1439, 1246, 1177, 957, 756, 732. HRMS (ESI) m/z: $[M-H]^-$ calcd for $[C_{14}H_9O_5]^-$, 257.0450; found, 257.0465.

5.7. 2-Acetyl-5-hydroxy-naphtho[2,3-*b*]furan-4,9-dione (10a)[5c]

Under Ar atmosphere, a solution of compound **64** (20 g, 78 mmol) in chloroform (1.0 L) was heated with MnO_2 (90 g, <5 μm, activated, 1.04 mol) to reflux for 1 d. The mixture was passed through a silica gel column chromatography. Concentration gave **10a** (13.6 g, 71% yield) as yellow needles of mp >247 °C (dec.). ^{1}H NMR: 2.67 (3H, s), 7.32 (1H, dd, J = 1.0, 8.5 Hz), 7.60 (1H, s), 7.67 (1H, dd, J = 7.0, 8.5 Hz), 7.82 (1H, dd, J = 1.0, 7.0 Hz), 12.13 (1H, s). ^{13}C NMR: 26.8, 111.9, 115.3, 120.5, 125.9, 130.5, 132.7, 136.7, 152.9, 155.7, 162.7, 173.2, 185.6, 187.5.

5.8. 2-Acetyl-8-hydroxy-naphtho[2,3-*b*]furan-4,9-dione (10b)[5c]

Compound **10b** was prepared from **9b** using the same procedure as described for compound **10a**. Concentration gave **10b** (84% yield) as yellow needles of mp 220–221 °C. ^{1}H NMR: 2.67 (3H, s), 7.32 (1H, dd, J = 1.0, 8.5 Hz), 7.59 (1H, s), 7.67 (1H, dd, J = 7.0, 8.5 Hz), 7.77 (1H, dd, J = 1.0, 7.0 Hz), 11.90 (1H, s). ^{13}C NMR: 26.7, 112.4, 115.3, 120.5, 125.5, 131.3, 133.2, 137.0, 152.3, 155.8, 163.0, 178.8, 178.8, 187.2.

5.9. 5-Hydroxy-2-[(1*S*)-1-hydroxyethyl]-naphtho[2,3-*b*]furan-4,9-dione (1)[5c]

To a flask was added ketone **10a** (128 mg, 0.5 mmol), Noyori asymmetric transfer hydrogenation catalyst Ru [(*S*,*S*)-Tsdpen](*p*-cymene) (15 mg, 0.025 mmol, 5 mol %), CH_2Cl_2 (5.0 mL), formic acid/Et_3N (5:2, 1.3 ml). The resulting solution was stirred at room temperature for 24 h. The reaction mixture was diluted by addition of H_2O and 10% HCl aq, and extracted with $CHCl_3$. The organic extracts were washed with brine, and then dried over Na_2SO_4. Concentration and column chromatography (hexane/EtOAc = 2/1) gave **1** (115 mg, 89% yield, 96% ee) as yellow needles of mp 171–172 °C. $[\alpha]_D^{24}$ −22.7 (c 0.58, CH_3OH). 96% ee (HPLC, Daicel Chiralpak AD-H; hexane/*i*-PrOH = 9/1, 1.0 mL/min; 254 nm, minor 37.9 min and major 40.9 min). ^{1}H NMR: 1.66 (3H, d, J = 6.8 Hz), 2.31 (1H, br s), 5.05 (1H, m), 6.84 (1H, s), 7.27 (1H, dd, J = 1.0, 8.3 Hz), 7.62 (1H, dd, J = 8.0, 8.3 Hz), 7.75 (1H, dd, J = 0.9, 8.0 Hz), 12.18 (1H, s). ^{13}C NMR: 21.5, 63.8, 103.4, 115.2, 120.0, 125.3, 131.0, 132.6, 136.3, 152.0, 162.3, 165.4, 172.7, 186.5.

5.10. 5-Hydroxy-2-[(1*R*)-1-hydroxyethyl]-naphtho[2,3-*b*]furan-4,9-dione ((*R*)-1)

$[\alpha]_D^{24}$ +22.5 (c 0.42, CH_3OH).

5.11. 8-Hydroxy-2-[(1*S*)-1-hydroxyethyl]-naphtho[2,3-*b*]furan-4,9-dione (2)[5c]

Compound **2** was prepared from **10b** in a manner similar to that described for compound **10a**. Concentration and column chromatography (hexane/EtOAc = 2/1) gave 2 (97% yield, 97% ee) as yellow needles of mp 181–182 °C. $[\alpha]_D^{24}$ −22.3 (c 0.16, CH_3OH). 97% ee (HPLC, Daicel Chiralpak AD-H; hexane/*i*-PrOH = 9/1, 1.0 mL/min; 254 nm, minor 21.1 min and major 24.1 min). ^{1}H NMR: 1.66 (3H, d, J = 6.5 Hz), 2.61 (1H, br s), 5.05 (1H, q, J = 6.5 Hz), 6.85 (1H, s), 7.26 (1H, dd, J = 1.5, 8.0 Hz), 7.59 (1H, dd, J = 7.5, 8.0 Hz), 7.70 (1H, dd, J = 1.5, 7.5 Hz), 12.00 (1H, s). ^{13}C NMR: 21.5, 63.8, 104.7, 114.7, 120.1, 125.3, 131.9, 133.2, 136.3, 151.1, 162.6, 165.8, 178.5, 179.7.

5.12. 8-Hydroxy-2-[(1*R*)-1-hydroxyethyl]-naphtho[2,3-*b*]furan-4,9-dione ((*R*)-2)

$[\alpha]_D^{24}$ +21.7 (c 0.16, CH_3OH).

5.13. Antiproliferative effect assay

The antiproliferative effects of naphthoquinones were examined in cancer cell lines and normal cell lines derived from human origin. These cells were maintained in usual 10% fetal serum Dulbecco's minimum essential medium (DMEM) through experiments and exposed to four dose concentrations of naphthoquinones in a humidified atmosphere (37 °C, 5% CO_2) for 72 h. After reaction, cells were further incubated with 0.25% trypan blue dye for 20 min and counted for viable cells under light microscopic apparatus. IC_{50} values were calculated from separate experiments performed in triplicate.

5.14. In vitro EBV-EA activation assay

The inhibition of EBV-EA activation was assayed according to the reported method.[17] The cells were incubated for 48 h at 37 °C in a

medium containing *n*-butyric acid (4 mM), TPA (32 pmol) and various amounts of the test compounds. Smears were made from the cell suspension and the EBV-EA inducing cells were stained by means of an indirect immunofluorescence technique.[18] In each assay, at least 500 cells were counted and the number of stained cells (positive cells) among them was recorded. Triplicate assays were performed for each data point. The EBV-EA inhibitory activity of the test compound was compared with that of the control experiment (100%) with *n*-butyric acid plus TPA. In the experiments, the EBV-EA activities were ordinarily abound 40% and these values were taken as the positive control (100%). The viability of cells was assayed against treated cells by the trypan blue staining method. For the determination of cytotoxicity, the cell viability was required to be more than 60%.[19] Student's *t*-test was used for all statistical analyses.

5.15. In vitro antibacterial activity

MICs of (**1**, (*R*)-**1**, **2**, (*R*)-**2** and **10a**) were determined by slightly modified NCCLS M27-P broth dilution method.[20] A 50 μL aliquot of 0.85% sodium chloride solution containing 10^6/mL bacterial cells was added to 1 mL of Muller-Hinton broth (OXOID) containing (**1**, (*R*)-**1**, **2**, (*R*)-**2** and **10a**) at a concentration range of 0.1–100 μg/mL, followed by incubation for 24 h at 37 °C. Similarly, fungal conidia suspended in 50 μL of 0.05% Tween 80 solution (10^5/mL conidia concentration) were added to 1 mL of potato dextrose broth (DIFCO) containing (**1**, (*R*)-**1**, **2**, (*R*)-**2** and **10a**) at a concentration range of 0.1–100 μg/mL and incubated for 3–5 days at 28 °C.

5.16. In vivo two-stage carcinogenesis test on mouse skin papillomas promoted by TPA

Mice were divided into groups, with 15 females in each group and housed in a controlled environment. All mice were shaved with electric clippers 1 day before application of the agents to backs of the mice. 7,12-Dimethylbenz[*a*]anthracene (DMBA, 390 nmol) in 0.1 mL acetone were applied topically with pipet. After one week, the same skin portion was painted with **1**, (*R*)-**1**, racemate **1** (85 nmol) in acetone or acetone alone, 60 min before TPA (1.7 nmol). Results of tumor response are present as the average number of mice with papillomas and the percentage of mice with papillomas.

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References and notes

1. Gentry, A. H. *Ann. Missouri Bot. Garden* **1973**, *60*, 945.
2. Hashimoto, G. *Brazil Shokubutu-ki*; Teikoku Shoin: Tokyo, 1962.
3. (a) Hartwell, J. L. *Lloydia* **1968**, *31*, 71; (b) Rao, M. M.; Kingston, D. G. I. *J. Nat. Prod.* **1982**, *45*, 600; (c) Koyama, J.; Morita, I.; Tagahara, K.; Hirai, K. *Phytochemistry* **2000**, *53*, 869; (d) Park, B. S.; Lee, K. G.; Shibamoto, T.; Lee, S. E.; Takeoka, G. R. *J. Agric. Food Chem.* **2003**, *51*, 295; (e) Park, B. S.; Lee, H. K.; Lee, S. E.; Piao, X. L.; Takeoka, G. R.; Wong, R. Y.; Ahn, Y. J.; Kim, J. H. *J. Ethnopharmacol.* **2006**, *105*, 255; (f) Queiroz, M. L. S.; Valadares, M. C.; Torello, C. O.; Ramos, A. L.; Oliveira, A. B.; Rocha, F. D.; Arruda, V. A.; Accorci, W. R. *J. Ethnopharmacol.* **2008**, *117*, 228.
4. (a) Ueda, S.; Tokuda, H. *Planta Med.* **1990**, *56*, 669; (b) Zani, C. L.; Oliveira, A. B.; O1iveira, G. G. *Phytochemistry* **1991**, *30*, 2379.
5. For quinones and benzene derivatives: (a) Burnett, A. R.; Thomson, R. H. *J. Chem. Soc. C* **1967**, 1261; (b) Burnett, A. R.; Thomson, R. H. *J. Chem. Soc. C* **1967**, 2100; (c) Wagner, H.; Kreher, B.; Lotter, H.; Hamburger, M. O.; Cordell, G. A. *Helv. Chim. Acta* **1989**, *72*, 659; For iridoids: (d) Nakano, K.; Maruyama, K.; Murakami, K.; Takaishi, Y.; Tomimatsu, T. *Phytochemistry* **1993**, *32*, 37l; (e) Warasina, T.; Nagatani, Y.; Noro, T. *Phytochemistry* **2004**, *65*, 2003; (f) Warashina, T.; Nagatani, Y.; Noro, T. *Phytochemistry* **2005**, *66*, 589.
6. (a) Hussain, H.; Krohn, K.; Ahmad, V. U.; Miana, G. A.; Green, I. R. *Arkivoc* **2007**, 145; (b) Pardee, A. B.; Li, Y. Z.; Li, C. J. *Curr. Cancer Drug Targets* **2002**, *2*, 227; (c) Fujimoto, Y.; Eguchi, T.; Murasaki, C.; Ohashi, Y.; Kakinuma, K.; Takagi, H.; Abe, M.; Inazawa, K.; Yamazaki, K.; Ikekawa, N.; Yoshikawa, O.; Ikekawa, T. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2323; (d) Cragg, G. M.; Grothaus, P. G.; Newman, D. J. *Chem. Rev* **2009**, *109*, 3012.
7. Ueda, S.; Umemura, T.; Dohguchi, K.; Matsuzaki, T.; Tokuda, H.; Nishino, H.; Iwashima, A. *Phytochemistry* **1994**, *36*, 323.
8. Ueda, S. U.S. Patent 5663197, 2005; *Chem. Abstr.* **1994**, *121*, 50088.
9. (a) Yamashita, M.; Kaneko, M.; Iida, A.; Tokuda, H.; Nishimura, K. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6417; (b) Iida, A.; Tokuda, H.; Yamashita, M. JP Patent 4077863, 2008.
10. (a) Guiraud, P.; Steiman, R.; Campos-Takaki, G. M.; Seigle-Murandi, F.; Simeon de Buochberg, M. *Planta Med.* **1994**, *60*, 373; (b) Nagata, K.; Hirai, K.; Koyama, J.; Wada, Y.; Tamura, T. *Antimicrob. Agents Chemother.* **1998**, *42*, 700–702; (c) Eyong, K. O.; Folefoc, G. N.; Kuete, V.; Beng, V. P.; Krohn, K.; Hussain, H.; Nkengfack, A. E.; Saeftel, M.; Salem Ramadan Sarite, S. R.; Hoerauf, A. *Phytochemistry* **2006**, *67*, 605.
11. Tietze, L. F.; Güntner, C.; Gericke, K. M.; Schuberth, I.; Bunkoczi, G. *Eur. J. Org. Chem.* **2005**, 2459.
12. (a) Chaker, L.; Pautet, F.; Fillion, H. *Chem. Pharm. Bull.* **1994**, *42*, 2238; (b) Oliveira, A. B.; Ferreira, D. T.; Raslan, D. S. *Tetrahedron Lett.* **1988**, *29*, 155.
13. Hagiwara, H.; Sato, K.; Nishino, D.; Hoshi, T.; Suzuki, T.; Ando, M. *J. Chem. Soc., Perkin Trans. I* **2001**, 2946.
14. (a) Fujii, A.; Hashiguchi, S.; Uematsu, N.; Ikariya, T.; Noyori, R. *J. Am. Chem. Soc.* **1996**, *118*, 2521; (b) Noyori, R.; Hashiguchi, S. *Acc. Chem. Res.* **1997**, *30*, 97.
15. Tsdpen = *N*-(*p*-toluenesulfonyl)-1,2-diphenylethanediamine.
16. The purity of synthesized **1** and (*R*)-**1**, **2**, (*R*)-**2** used for biological evaluation is 95–97% ee.
17. Ito, Y.; Yanase, S.; Fujita, J.; Harayama, T.; Takashima, M.; Imanaka, H. *Cancer Lett.* **1981**, *13*, 29.
18. Henle, G.; Henle, W. *J. Bacteriol.* **1966**, *91*, 1248.
19. Ohigashi, H.; Takamura, H.; Koshimizu, K.; Tokuda, H.; Ito, Y. *Cancer Lett.* **1986**, *30*, 143.
20. National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast* (Proposal Standard M27-P); NCCLS: Villanova, PA, 1992.