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Stereoselective synthesis and cytotoxicity of a cancer chemopreventive naphthoquinone from *Tabebuia avellanedae*

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Abstract—Stereoselective synthesis of **1**, one of biologically active naphthoquinones from a Brazilian traditional medicine *Tabebuia avellanedae*, was achieved by utilizing Noyori reduction as a key step. Compound **1** displayed potent cytotoxicity against several human tumor cell lines, whereas it showed lower cytotoxicity against some human normal cell lines compared with that of mitomycin. On the other hand, its enantiomer was less active toward the tumor cell lines than **1**.

The Bignoniaceae plant, *Tabebuia avellanedae* Lorentz ex Griseb,[1] is a gigantic tropical tree native to South America from Brazil to north Argentina and has been known as a useful medicinal plant since the Incan Era.[2] The stem bark of *T. avellanedae* has been utilized as a diuretic and as astringent, and as a folk remedy for the treatment of cancer and various diseases.[3] Therefore, *T. avellanedae* is worthy of attention because of its highly promising therapeutic effects and has been extensively investigated as an important medicinal resource.[4]

Findings of the antitumor activity of an alcoholic extract of the stem bark of this plant[3b] and efforts to find clinically acceptable antitumor compounds led to the discovery of a series of naphthoquinones based on the naphtho[2,3-*b*]furan-4,9-dione skeltone such as (−)-5-hydroxy-2-(1′-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**1**) and its positional isomers, (±)-8-hydroxy-2-(1′-hydroxyethyl)-naphtho[2,3-*b*]furan-4,9-dione (**2**) (Fig. 1).[5] Extensive studies demonstrated that the constituent naphtho[2,3-*b*]-furan-4,9-dione congeners including compounds **1** and **2** showed potent cytotoxicity against numerous tumor cell lines.[6] Among these naphthoquinones, compound **1** exhibited remarkably potent inhibition against Epstein-Barr virus early antigen (EBV-EA) activation induced by the tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA).[7] Further compound **1** strongly inhibited TPA-induced tumor promotion on mouse skin initiated with 7,12-dimethylbenz[*a*]anthracene (DMBA) in two-stage carcinogenesis tests. Therefore, compound **1** was found also to act as a cancer chemopreventive agent.[6,8] According to previous studies on the chemical constituents of *T. avellanedae*, however, the yield of **1** from the inner bark of the tree was less than 0.001%.[5,7] On the other hand, the naphthoquinones described above can be obtained from the inner bark of only wild *T. avellanedae* plants of over 20 years old.[7] Moreover, artificial propagation of this plant is very difficult. These barriers have so far

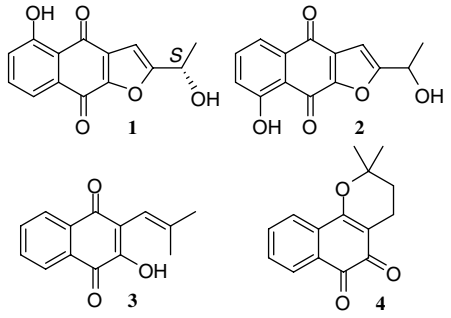

Figure 1.

Keywords: Stereoselective synthesis; Naphtho[2,3-*b*]furan-4,9-dione; *Tabebuia avellanedae*; Cancer chemoprevention; Antitumor activity.
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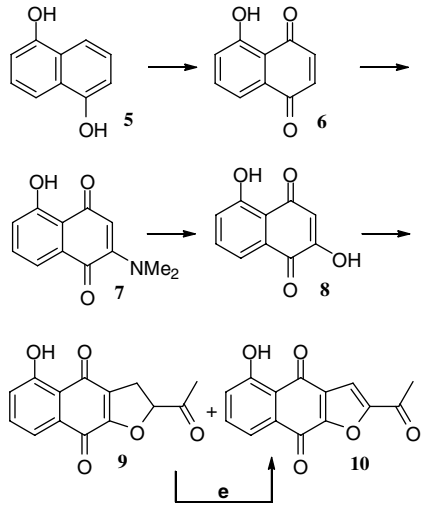
prevented further studies on biological properties of the naphtho[2,3-*b*]furan-4,9-dione congeners and consequently promoted us to synthesize **1**.

Although synthetic studies of other naphthoquinones isolated from the heartwood of this plant such as lapachol (**3**) and β-lapachone (**4**) have been extensively carried out,[9] the number of reports on the synthesis of naphtho[2,3-*b*]furan-4,9-diones is limited.[10] Among them, Fujimoto et al. obtained a mixture of racemates **1** and **2**, which were inseparable on silica gel chromatography.[10a] Separation of racemates **1** and **2** was accomplished through several steps including acylation, column chromatography, and alkaline hydrolysis. Finally, **1** and (*R*)-**1** were obtained by HPLC on a chiral column.[10a] In this paper, we report the stereoselective synthesis of **1** starting from 1,5-dihydroxynaphthalene (**5**). In addition, we also describe its cytotoxicity against several human tumor cell lines, human normal cells and in vitro cancer chemopreventive activity, comparing with those of racemate, **1** and (*R*)-**1**.

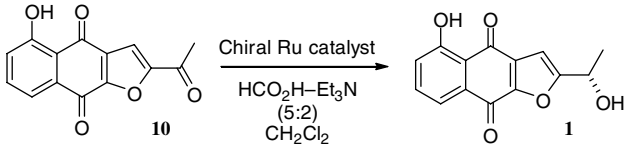
The first stereoselective synthesis of **1** was accomplished starting from commercially available 1,5-dihydroxynaphthalene (**5**) (Scheme 1). For the synthesis of juglone (**6**), compound **5** was oxidized with air in the presence of CuCl in the dark to give **6** in 47% yield.[11] Chemical transformations of **6** to **8** were 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 (**7**) and 3-dimethylaminojuglone in 48% and 10% yields, respectively. According to the previous paper,[12a] liquid dimethylamine (boiling point: −6 °C) was used for this step, whereas compound **7** was obtained also with a THF solution of dimethylamine, which is suitable for practical use, in comparable chemical yield and regioselectivity. Deamination of 2-dimethylaminojuglone (**7**) with 10% aqueous HCl gave



Scheme 1. Synthesis of **10**. Reagents and conditions: (a) CuCl, CH_3CN, air, rt, 47%; (b) Me_2NH, toluene, THF, −40 °C, 48% and 10% for **7** and 3-dimethylaminojuglone, respectively; (c) 10% HCl, dioxane, reflux, 97%; (d) 3,4-dibromobutan-2-one, DBU, THF, rt, 79% and 16% for **9** and **10**, respectively; (e) MnO_2, $CHCl_3$, reflux, 51%.

2-hydroxy-1,4-naphthoquinone (**8**) in improved yield compared with the previous method.[12b] The naphtho[2,3-*b*]furan-4,9-dione skeleton was constructed based on the method reported by Hagiwara et al.[10b] Thus, the reaction of **8** 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 afforded naphthodihydrofuran **9** in 79% yield and the desired natural naphthofuran **10** in 16% yield after separation by silica gel column chromatography. Naphthodihydrofuran **9** in chloroform was further treated with MnO_2 to provide the natural naphthofuran **10** in 51% yield along with 44% recovery of the dihydrofuran **9**. Subsequent Noyori reduction accomplished the stereoselective synthesis of **1** (Scheme 2). [13–16] Asymmetric transfer hydrogenation of naphthofuran **10** in a formic acid-triethylamine mixture and CH_2Cl_2 catalyzed by a commercially available chiral Ru(II) complex, Ru [(*S*,*S*)-Tsdpen] (*p*-cymene), RuCl [(*S*,*S*)-Tsdpen] (*p*-cymene), RuCl [(*S*,*S*)-Tsdpen] (mesitylene), RuCl [(*S*,*S*)-Msdpen] (*p*-cymene),[17] revealed that naphthofurane **10** can be reduced to the corresponding secondary alcohol in high chemical yield and enantiomeric excess (89–91% yield, 95–96% ee).

Compounds **1** and (*R*)-**1** were screened against a panel of human tumor cell lines including PC-3 (prostate), A549 (lung), and MCF-7 (breast), in order to explore their anticancer spectra.[18] The results are shown in Table 1. Compound **1** exhibited potent cytotoxicity against all three cell lines, especially PC-3 and A549, while (*R*)-**1** was less cytotoxic against all three cell lines. It is noteworthy that the cytotoxicity of **1** against PC-3 was comparable to that of mitomycin, which is known as a strong cytotoxic agent against a panel of human tumor cell lines. On the other hand, **1** and (*R*)-**1** revealed lower cytotoxicity toward a panel of human normal cell lines including Fb (skin), Hc (liver), MPC-5 (lung), and IE (colon) than mitomycin (Table 2). It is also noteworthy that **1** was less cytotoxic against all four cell lines (11.1–54.5 μM) than mitomycin that displayed potent



Scheme 2. Chiral Ru catalyst mediated asymmetric hydrogenation of naphthofuran **1**.

Table 1. Cytotoxic effect of racemate **1**, (*R*)-**1**, **1**, and mitomycin against human tumor cell lines[a,b]

Compound	EC_{50} (μM)		
	PC-3	A549	MCF-7
Racemate 1	0.56	3.24	8.5
(*R*)-**1**	0.93	3.0	9.3
1	0.14	0.96	3.5
Mitomycin	0.14	0.43	0.96

[a] Cell line: PC-3, prostate, A549, lung, MCF-7, breast.
[b] Cell viability was evaluated by the Trypan blue staining method.

Table 2. Cytotoxic effect of racemate **1**, (*R*)-**1**,**1**, and mitomycin against human normal cell lines[a,b]

Compound	EC_{50} (μM)			
	Fb	Hc	MPC-5	IE
Racemate 1	45.4	89.3	89.3	158
(*R*)-**1**	39.7	29.8	65.9	39.7
1	11.1	11.1	29.7	54.5
Mitomycin	0.93	1.46	2.1	1.46

[a] Cell line: Fb, skin; Hc, liver; MPC-5, lung; IE, colon.
[b] Cell viability was evaluated by the Trypan blue staining method.

Table 3. Inhibitory effects 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}[c] (μM)
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
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

[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 was more than 80%.
[c] IC_{50} value of curcumin, a positive control substance, was 345 μM.

cytotoxicity against the normal cell lines (0.93–1.46 μM). These results suggest that **1** could be a promising candidate for the development of anticancer drugs.

Compound **1** has already been known to act as a cancer chemopreventive agent.[8] In order to compare in vitro cancer chemopreventing activity of (*R*)-**1** with that of **1**, **1** and (*R*)-**1** were evaluated for their inhibitory effects on EBV-EA activation induced by TPA in Raji cells as a primary screening test for antitumor promoters (Table 3). In this assay, both **1** and (*R*)-**1** showed potent inhibition on EBV-EA activation without cytotoxicity against Raji cells dose-dependently (100%, 90–95%, 75–83%, 41–50% inhibition at 1000, 500, 100, and 10 mol ratio/TPA, respectively). In particular, **1** exhibited significantly potent inhibitory effects on EBV-EA activation. The inhibitory activities of these compounds were greater than those of β-lapachone and lapachol, which are known as congeners of **1** in *T. avellanedae*.

In conclusion, the concise stereoselective synthesis of **1** was completed by using Noyori reduction as a key step. Compound **1** showed potent cytotoxicity and cancer chemopreventive activity. Future progress on related series will be reported in due course.

Acknowledgments

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13. Synthesis of racemate **1**. Reagents and conditions: Reduction of **10** with $NaBH_4$ in CH_2Cl_2 at 0 °C provided racemate **1** in 79% yield.
14. The absolute stereochemistry of synthetic **1** was confirmed as *S* by comparing its specific rotation with the previously reported one.[10a]
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16. General experiment procedures: to a flask were added ketone **10** (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), and 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, SUMICHIRAL, OA-4500 (4.6 mmϕ × 250 mm), hexane/*i*-PrOH/MeOH = 95/4/1, 1 mL/min, 254 nm, minor 37.9 min and major 40.9 min). ^1H NMR ($CDCl_3$): 1.66 (3H, d, *J* = 6.8 Hz), 2.31 (1H, brs), 5.05 (1H, m), 6.84 (1H, s), 7.27 (1H, dd, *J* = 1.0, 8.3 Hz), 7.75 (1H, dd, *J* = 0.9, 8.0 Hz), 12.18 (1H, s). ^{13}C NMR ($CDCl_3$): 21.5, 63.83, 103.4, 115.2, 120.0, 125.3, 131.0, 132.6, 136.3, 152.0, 162.3, 165.4, 172.7, 186.5.
17. Tsdpen = *N*-(*p*-toluenesulfonyl)-1,2-diphenylethanediamine, Msdpen = *N*-(methanesulfonyl)-1,2-diphenylethanediamine.
18. Enantiomerically pure **1** and (*R*)-**1** (>99% ee) used for biological evaluation were prepared from racemate **1**

by HPLC separation using SUMICHIRAL OA-4500 (20 mmϕ × 250 mm).