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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

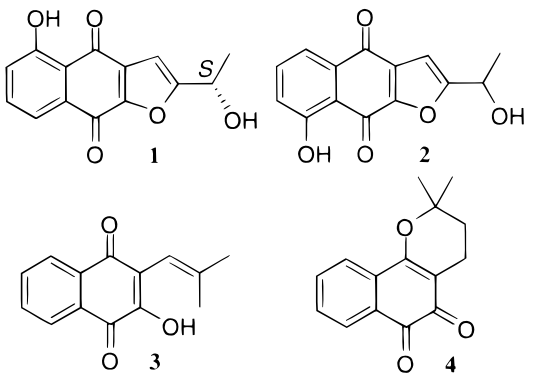
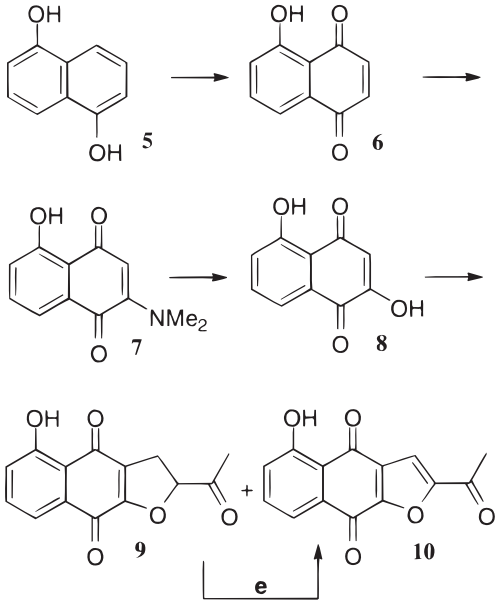



**Figure 1.**

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 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**.

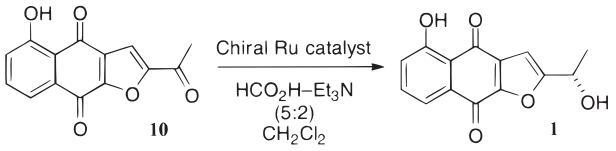
The first stereoselective synthesis of **1** was accomplished starting from commercially available 1,5-dihydroxynaphthalene (**5**) (Scheme 1). For the synthesis of juglone (**6**), compound **5** was oxidized with air in the presence of CuCl in the dark to give **6** in 47% yield.[11] Chemical transformations 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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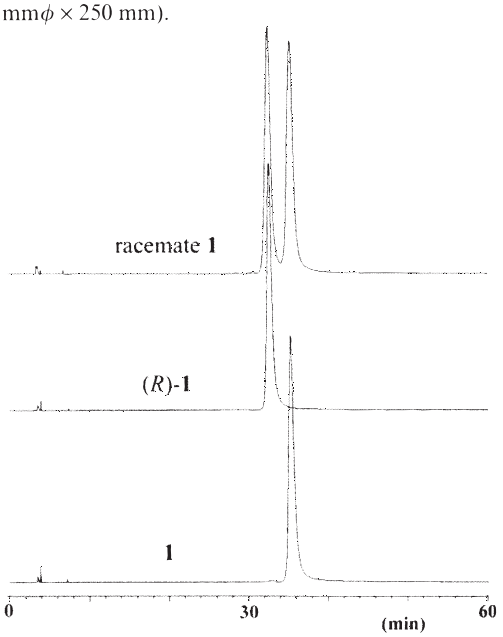
16. General experiment procedures: to a flask were added ketone **10** (128 mg, 0.5 mmol), Noyori asymmetric transfer hydrogenation catalyst Ru [(*S*,*S*)-Tsdpen](*p*-cymene) (15 mg, 0.025 mmol, 5 mol %), $CH_2Cl_2$ (5.0 mL), and formic acid/$Et_3N$ (5:2, 1.3 ml). The resulting solution was stirred at 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 mm$\phi$ × 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 mm$\phi$ × 250 mm).



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# タベブイアアベラネダエから単離されたがん予防活性ナフトキノンの立体選択合成と細胞毒性

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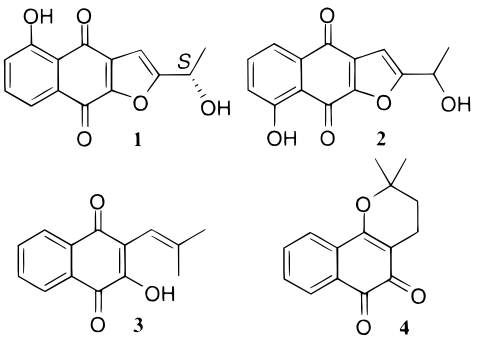
（要旨）
ブラジルの伝統薬物タベブイアアベラネダエから単離された生物活性ナフトキノンの一つである1の立体選択合成が野依還元をキーステップとして達成された。化合物1はいくつかのヒトがん細胞に対して強力な細胞毒性を示したが、ヒト正常細胞に対しては、マイトマイシンのそれと比較して、より低い細胞毒性を示した。一方、1のエナンチオマーは、がん細胞に対して、1ほど活性を示さなかった。

ノウゼンカズラ科植物タベブイアアベラネダエはブラジルから北アルゼンチンまでの南アメリカ原産の熱帯の巨木であり、インカの時代より有用薬用植物として知られている。タベブイアアベラネダエの樹皮は利尿剤や収斂剤として、またがんやさまざまな疾病の処置に対する民間薬として用いられてきた。それゆえ、タベブイアアベラネダエは、期待されるその高い療法効果から興味を引く価値あるものであり、重要な薬用資源として広く研究されてきた。

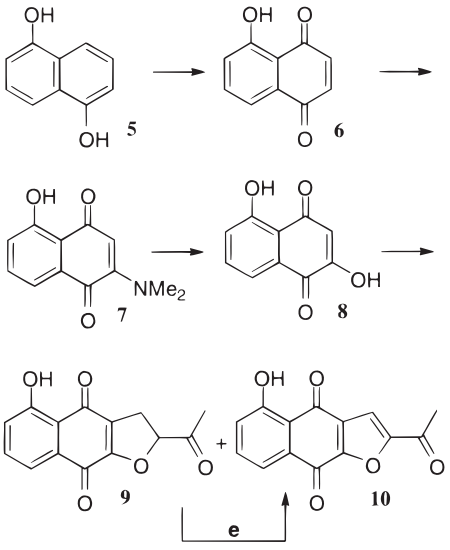
本植物の樹皮のアルコールエキスの抗腫瘍活性の発見と臨床に使用可能な抗腫瘍活性化合物を探索する努力により、1やその位置異性体2のような骨格構造を基本にした一連のナフトキノンが発見されるに至った（図1）。広範な研究により化合物1や2を含む類似成分が多くの腫瘍細胞に対して強力な細胞毒性を示すことが明らかとなった。これらナフトキノンの中で、化合物1は発がん促進物質TPAによって誘導されるEpstein-Barrウイルス初期抗原活性化を顕著に阻害した。さらに、化合物1は発がん2段階実験において、DMBAでイニシエーションを受けたマウス皮膚でのTPAによる発がん促進を強力に阻害した。それゆえ、化合物1は、がんの化学予防剤としても作用することがわかった。しかしながら、過去のタベブイアアベラネダエの成分研究によれば、内皮からの1の収率は0.001%以下であった。一方、上記ナフトキノンは樹齢20年以上の野生のタベブイアアベラネダエ



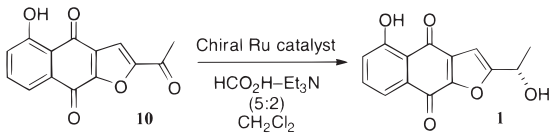

の内皮のみから得られる。さらに、この木の人工栽培は非常に難しい。これらの障害は、タベブイアアベラネダエのナフトキノン成分の生物活性の更なる研究を今まで妨げてきた。また、このことが我々に1を化学合成させるきっかけになった。

この木の心材から単離されたラパコール3やβ-ラパチョン4のようなナフトキノン類の合成研究は広く行われてきたが、化合物1の基本骨格をなすナフトキノン類の合成に関する報告の数は限られている。その中で、藤本らは、混合物（シリカゲルクロマトグラフィーでは分離不可能）を得ている。ラセミ体の1と2の分離は、アシル化、カラムクロマトグラフィー、アルカリ加水分解などの数行程を経て行われた。最終的に、1と鏡像体1はキラルカラムを用いたHPLC分離によって得られた。本論文では、化合物5から出発する1の立体選択的合成について報告する。

1の最初の立体選択的合成は市販の化合物5を用いて達成された（スキーム1）。ユグロン6は、化合物5を遮光下、塩化第一銅を用いて空気酸化することにより47％収率で得た。6から8への化学変換は、既存の方法に若干の修正を加え行われた。-40℃でトルエン中ジメチルアミン（2.0M THF 溶液）を用いた6の酸化的アミノ化により7とその位置異性体が、それぞれ48％と10％の収率で得られた。報告によれば、この工程に液体のジメチルアミン（沸点-6℃）が用いられたが、化合物7は使い勝手のよい実用的なジメチルアミンのTHF溶液を用いても、相当の化学収率と位置選択性で得ることができた。10％塩酸水溶液を用いた7の脱アミノ化は、報告の方法と比較しより改善した収率で8を与えた。ナフトキノン骨格は、長谷川らによって報告された方法に従って構築された。すなわち、THF中DBU存在下市販のブタ-3-エン-2-オンと臭素から合成される3,4-ジブロモブタン-2-オンを8と反応させ、シリカゲルカラムクロマトグラフィーで分離すると、ジヒドロ体9と望ましい天然型ナフトキノン10がそれぞれ79％と16％の収率で得られた。化合物9をさらにクロロフォルム中、二酸化マンガンで処理することにより、9を44％の収率で回収するとともに、望ましい10を51％で得た。続く野依還元により1の立体選択的合成が完成した（スキーム2）。ギ酸-トリエチルアミン混合物と塩化メチレン中、市販のキラルなルテニウム（Ⅱ）錯体（Ru[(S,S)-Tsdpen](p-cymene), RuCl[(S,S)-Tsdpen](p-cymene), RuCl[(S,S)-Tsdpen] (mesitylene), RuCl[(S,S)-Msdpen](p-cymene)など）によって触媒される10の不斉移動還元により、10は対応する2級アルコールの高収率（89-91％）、高エナンチオマー過剰率（95-96％）で還元された。




**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%.




**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.

化合物1とその鏡像体の抗腫瘍活性をヒトがん細胞（PC-3（前立腺がん）、A549（肺がん）、MCF-7（乳がん））を用いて調べた。結果を表1に示す。化合物1は3つすべての腫瘍細胞、特にPC-3とA549に対して有意な活性を示したが、その鏡像体の活性は低下した。1のPC-3に対する細胞毒性が強力な細胞毒性をもつ薬剤として知られているマイトマイシンの活性と同程度であったことは特筆される。一方、1とその鏡像体のヒト正常細胞（Fb（皮膚）、Hc（肝臓）、MPC-5（肺）、IE（小腸））に対する細胞毒性は、マイトマイシンより低かった（表2）。1が4つすべての細胞に対してマイトマイシンより細胞毒性が低かったことは特筆される。これらの結果は、1が抗がん剤開発のための有望な候補であることを示唆している。

化合物1ががんの化学予防剤として作用することはすでに知られている。鏡像体のインビトロのがん化学予防活性を1のそれと比較するために、抗発がん促進活性物質の初期のスクリーニングテストとしてラージ細胞の中でTPAによって誘導されるEBV-EA活性化に関する1とその鏡像体の阻害効果を評価した（表3）。この活性試験で、1とその鏡像体はともに、ラージ細胞に対して細胞毒性なくEBV-EA活性化を有意な容量依存的な阻害効果を示した。特に、1はEBV-EAの誘導を有意な阻害活性を示した。これらの化合物の阻害活性は、タベブイアアベラネダエに含まれる1の同族体として知られている$\beta$-ラパチョンやラパコールの活性よりも強力であった。

結論として、1の簡潔な立体選択的合成がキーステップとして野依還元を用いることにより完成された。化合物1は強力な細胞毒性とがんの化学予防効果を持つ薬剤として評価された。関連化合物に関するさらなる進展は、追って報告する。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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 mm$\phi$ × 250 mm), hexane/*i*-PrOH/MeOH = 95/4/1, 1 mL/min, 254 nm, minor 37.9 min and major 40.9 min). $^1$H 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 mm$\phi$ × 250 mm).