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Total Synthesis and Anticancer Activities of (−)- and (+)-Thespesone

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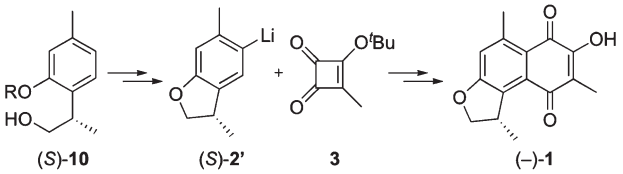

The natural *p*-naphthoquinone (−)-thespesone **1** and its (+)-enantiomer were synthesized for the first time by bisacylation of a 5-lithiodihydrobenzofuran **2′** with 4-methyl-3-*tert*-butoxycyclobut-3-ene-1,2-dione **3**. The racemate of the required 2-arylpropan-1-ol precursor **10** was kinetically resolved by an enzyme-catalyzed acetylation exclusively of the (*S*)-enantiomer. Saponification of this acetate mediated by the same enzyme, porcine pancreas lipase (PPL), afforded the (*S*)-2-arylpropan-1-ol in 96% ee. Its unreacted (*R*)-enantiomer could be obtained with 77% ee. (−)-(*S*)-Thespesone was far more efficacious against a panel of six cancer cell lines including multiresistant ones than its (+)-enantiomer and also when compared to thymoquinone, an established natural antitumoral *p*-quinone from *Nigella sativa*. Unlike the latter, (−)-thespesone was well tolerated by nonmalignant fibroblasts.

Introduction

The heartwood of portia or milo trees (*Thespesia populnea; malvaceae*) that grow in Asia, Africa, Hawaii, Florida, California, and the Caribbean Islands[1] has been traditionally used as a cardiac stimulant. It was also reported to have antifungal, antibiotic, antifertility, antibacterial, anti-inflammatory, antioxidant, purgative, hepatoprotective, and antitumor properties.[2−5] The constituents believed responsible for the majority of these effects are so-called mansonones,[6] highly oxo-functionalized sesquiterpenes of the cadinane type. They were isolated from extracts of the reddish brown heartwood.[7] Some of them have cytotoxic, antifungal, or antioxidant properties.[5−8] (−)-Thespesone **1** was first isolated in 1983[9] but was not fully characterized until 2004.[8] Herein we describe its total synthesis and the cytotoxicities of the two enantiomers and the racemate of thespesone against a panel of six cancer cell lines and nonmalignant foreskin fibroblasts. Scheme 1 outlines the retrosynthesis, which was based on a disconnection of the aromatic and the quinoid rings. *p*-Naphthoquinones had been previously obtained from halo benzenes by regioselective bisacylation of their lithiated derivatives with cyclobut-3-ene-1,2-diones such as **3**.[10−13] The required 5-bromo-dihydrobenzofuran **2** was to be prepared from 2-hydroxy-4-methyl-acetophenone **5** via functional group transformations[14] comprising the establisment of the *S*-configured stereocenter in the benzylic position of bromide **4** by an enzymatic resolution with porcine pancreas lipase.

Results and Discussion

Syntheses of Racemic and Enantiopure Thespesones 1. Commercially available 2-hydroxy-4-methyl-acetophenone

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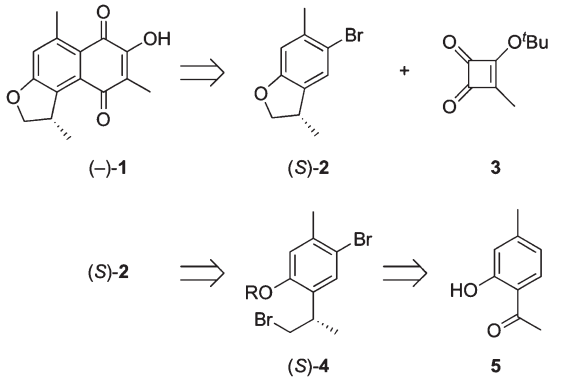
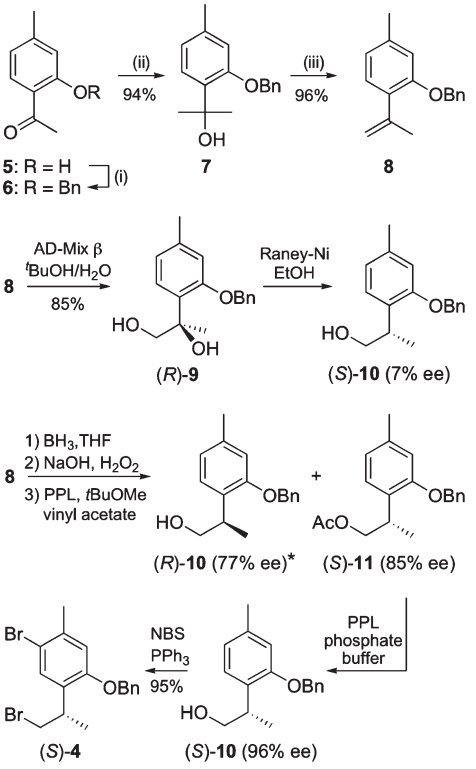
SCHEME 1. Retrosynthetic Analysis of (−)-Thespesone 1



5 was benzylated to give ether **6**, which was treated with methyl magnesium iodide to afford the tertiary alcohol **7**. Elimination of water using $KHSO_4$[15] left the styrene derivative **8** in 90% overall yield (Scheme 2). Initially, we intended to convert the latter to the enantiopure alcohol (*S*)-**10**, an immediate precursor to the required dibromide (*S*)-**4**, by means of an asymmetric dihydroxylation reaction followed by a selective reduction of the tertiary hydroxy group of diol (*R*)-**9** with Raney-Ni.[16−18] However, Sharpless dihydroxylation of **8** with AD-Mix β proceeded with low enantioselectivity. Product alcohol **10** was eventually isolated with 7% ee in favor of the *S*-enantiomer. This finding is in line with earlier reports on the low selectivity of AD-reactions of sterically encumbered styrenes.[19,20] Replacement of the benzoxy group of **8** by hydroxy or acetate residues gave insufficient conversion due to formation of a water-soluble phenolate, while an *ortho*-methoxy analogue did not react at all. An alternative attempt to prepare alcohol (*S*)-**10** by enantioselective hydroboration of **8** using Ipc-borane also failed.[21−25] More successful was an enzymatic resolution of racemic alcohol (±)-**10** as obtained by hydroboration of **8**

SCHEME 2. Synthesis of Chiral Precursors 4[a]



[a]Reagents and conditions: (i) BnBr, K_2CO_3, THF/DMF, reflux, 20 h, 99%; (ii) CH_3MgI, Et_2O, 0 °C to reflux (4 h), then sat. NH_4Cl; (iii) $KHSO_4$, DMF, 80 °C, 1 h. *ee after three runs.

with BH_3 in THF. Its acetylation mediated by porcine pancreas lipase (PPL)[26−30] in the presence of vinyl acetate and methyl *tert*-butyl ether as the solvent led to acetate (*S*)-**11** with 85% ee in 45% chemical yield. Residual **10** could be recovered and rereacted. After three runs, aside the acetate (*S*)-**11**, the unreacted (*R*)-**10** was isolated in an optical purity of 77% ee. The hydrolysis of the enriched (*S*)-**11** in aqueous phosphate buffer (pH 7) was also amenable to catalysis by PPL and afforded the target alcohol (*S*)-**10** with 96% ee, again with the option to repeat this procedure with recovered starting acetate until maximum conversion. Bromination of the enantiomers of alcohol **10** with NBS/PPh_3 afforded the respective dibromide **4**.[31] The enantiomeric purity of compounds **10** and **11** was in each case determined by gas chromatography on a Lipodex E column after deprotection of the hydroxy groups to give the respective stereoisomers of 2-(2-hydroxy-4-methylphenyl)propan-1-ol **10′**.

The benzyl ether of the enantiopure or racemic dibromide **4** had to be cleaved using $AlCl_3/PhNEt_2$[32] since the customary hydrogenation at Pd/C had failed. As some 5-bromo-3,6-dimethyldihydrobenzofuran **2** was inevitably formed as a side product of this deprotection, the crude dibromophenol was treated with K_2CO_3 in acetone right away to obtain pure **2** in over 80% yield. Scheme 3 shows this sequence for the

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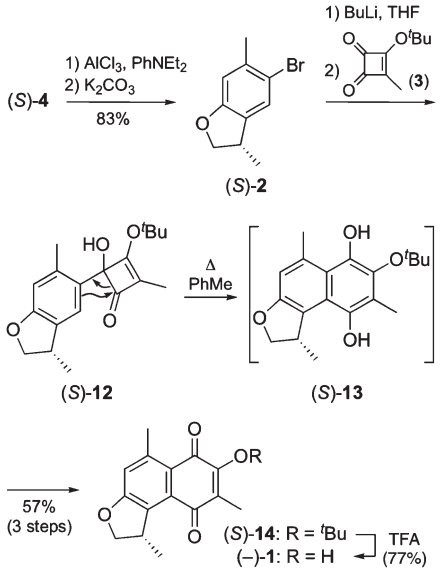
TABLE 1. Inhibitory Concentrations (μM) IC_{50}(72 h)[a] of (±)-1, (−)-1, (+)-1, and 15−17 When Applied to Cancer and Nonmalignant Cells

cell line/compound	518A2	HL-60	HT-29	HT-29/Colc	KB-V1/Vbl	MCF-7/Topo	HF
(±)-**1**	26 ± 4	71 ± 12		82 ± 25	17 ± 6	70 ± 12	> 100
(−)-**1**	20 ± 5	21 ± 13	> 100	67 ± 29	16 ± 6	11 ± 8	> 100
(+)-**1**	> 100	93 ± 10	> 100	84 ± 23	21 ± 25	> 100	> 100
15	28 ± 9[b]	28 ± 6[b]	47 ± 19	77 ± 1	32 ± 6[b]	27 ± 6[b]	33 ± 20[b]
16			> 20[c]		12[c]	3.3[c]	
17			0.7[c]		2.3[c]	0.2[c]	

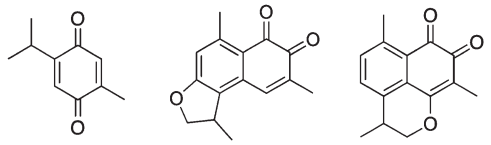
[a]Values are derived from concentration−response curves obtained by measuring the percentual absorbance of viable cells relative to untreated controls (100%) after 72 h of exposure of 518A2 melanoma, HL-60 leukemia, HT-29, and HT-29/Colc colon carcinoma, MCF-7/Topo breast carcinoma, and KB-V1/Vbl cervix carcinoma cells as well as human foreskin fibroblasts (HF) to the test compounds in the MTT assay. Values represent means of four independent experiments ± standard deviation. [b]Values taken from literature.[38,39] [c]SRB-assays (48 h) with sensitive wild-type cells; values taken from literature.[5]

SCHEME 3. Synthesis of (−)-(*S*)-Thespesone 1 from (*S*)-4



dibromide (*S*)-**4** that leads up to the natural (−)-(*S*)-thespesone. Li−halogen exchange of **2** with BuLi and subsequent trapping of the intermediate aryllithium compound with 3-*tert*-butoxy-4-methylcyclobut-3-ene-1,2-dione **3** left the acyloine **12**. This was not isolated but heated in toluene to initiate an electrocyclic opening−-closure sequence affording the hydroquinone **13**, which underwent an auto-oxidation to the quinone **14**.[10−13] Its *tert*-butyl group was finally removed with TFA in CH_2Cl_2.[12] Racemic and (+)-thespesone were synthesized analogously starting from either (±)-**4** or (*R*)-**4**.

In Vitro Cytotoxicities of the Thespesone Stereoisomers. Karalai et al.[5] reported the cytotoxicities of nine compounds isolated from *T. populnea* against four cancer cell lines (MCF-7, HeLa, HT-29, and KB carcinomas). For unknown reasons they left out thespesone **1**. Later on, Murugesan et al. reported an LD_{50}(7 d) > 10 μM for it when applied to MCF-7 breast carcinoma cells.[33] We now tested (−)-**1**, (+)-**1**, and (±)-**1** for growth inhibition of cells of human HL-60 leukemia, 518A2 melanoma, HT-29 colon carcinoma, and the multidrug-resistant variants of HT-29/Colc, MCF-7/Topo (breast), and KB-V1/Vbl (cervix; a HeLa-derivative), as well as of nonmalignant human foreskin fibroblasts (HF). We compared the results with those of thymoquinone **15**, a *p*-benzoquinone occurring in the seed oil of *Nigella sativa* that showed antitumor effects in various in vitro and animal studies,[34−37] and of Karalai's best perfomers, the *o*-quinoid mansonones D **16** and E **17**. IC_{50} values after 24 and 48 h of exposure to the test compounds are listed in the Supporting Information. Table 1 summarizes only the IC_{50}(72 h) values.



The natural (−)-**1** was generally far more active against the cancer cells than its (+)-enantiomer and even surpassed the efficacy of thymoquinone **15**. Unlike the latter, it was tolerated very well by the nonmalignant fibroblasts, which is interesting from a pharmacological viewpoint. While less active than mansonone E **17** in the colon, cervix, and breast carcinoma cell lines, (−)-**1** was comparable in activity to its close furanoid analogue **16**.

Conclusions

The first synthesis of natural (−)-thespesone **1** and its (+)-enantiomer was based upon an enzyme-mediated kinetic resolution of a chiral precursor alcohol and a known bisacylation of a 5-lithiodihydrobenzofuran with cyclobut-3-en-1,2-dione to establish the *p*-naphthoquinone. The resolution of racemic alcohol (±)-**10** via acetylation and rehydrolysis of the so-formed acetate, both mediated by porcine pancreas lipase (PPL), afforded the alcohol (*S*)-**10** with 96% ee in high chemical yield. The unreacted enantiomeric alcohol (*R*)-**10** could also be enriched to 77% ee by several more cycles of PPL-mediated acetylations. Unlike many other constituents of *T. populnea*, thespesone had not yet been screened for anticancer activity. We closed this gap and found that the natural (−)-enantiomer of thespesone was far more active against various cancer cell lines including multiresistant ones than its (+)-enantiomer and also when compared to the

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established antitumoral thymoquinone **15**. The large spread between efficacy against cancer cells and tolerance by non-malignant fibroblasts makes (−)-thespesone **1** a potential new candidate for anticancer therapy.

Experimental Section

General Remarks. Melting points are uncorrected. IR spectra were recorded on an FT-IR spectrophotometer equipped with an ATR sampling unit. Nuclear magnetic resonance (NMR) spectra were recorded under conditions as indicated. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard for ^{1}H and ^{13}C. Gas chromatograpy was conducted on a Lipodex E column (80−200 °C, rate 5 °C/min). For chromatography silica gel 60 (230−400 mesh) was used. All starting compounds were purchased from commercial sources and used without purification.

(±)-2-(2-(Benzyloxy)-4-methylphenyl)propan-1-ol (±)-10. A solution of compound **8** (6.7 g, 28.1 mmol) in dry THF (100 mL) was cooled to 0 °C and treated dropwise with BH_3 (1 M in THF, 28.7 mL). The resulting mixture was stirred at this temperature for 2 h, then treated with NaOH (10%, 36.8 mL) and H_2O_2 (30%, 19.2 mL), and finally stirred at room temperature for a further 1.5 h. Brine (80 mL) was added, and the mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The resulting crude product was purified by column chromatography (ethyl acetate/cyclohexane 1:4, silica gel 60) to yield 6.27 g (24.46 mmol, 87%) of (±)-**10** as a colorless oil; R_f 0.5 (cyclohexane/ethyl acetate 1:1); IR (ATR) ν_{max} 3389, 3031, 2922, 2870, 1610, 1578, 1505, 1453, 1412, 1381, 1288, 1255, 1162, 1134, 1016, 809, 732, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (d, J = 7.0 Hz, 3 H), 2.51 (s, 3 H), 2.76 (br, 1H, O*H*), 3.47 (ddq, J = 6.4 Hz, J = 6.6 Hz, J = 7.0 Hz, 1 H), 3.79 (dd, J = 17.0 Hz, J = 6.6 Hz, 1 H), 3.91 (dd, J = 17.0 Hz, J = 6.4 Hz, 1 H), 5.18 (s, 2 H), 6.96 (s, 1 H), 6.98 (d, J = 7.5 Hz, 1 H), 7.29 (d, J = 7.5 Hz, 1 H), 7.4−7.6 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.5, 21.0, 34.7, 67.0, 69.7, 112.5, 121.3, 126.9, 127.4, 128.2, 129.0, 136.6, 136.9, 155.9; m/z (EI) 256 (13) [M$^+$], 225 (11), 148 (14), 135 (26), 91 (100), 65 (9). Anal. Calcd for $C_{17}H_{20}O_2$: C, 79.65; H, 7.86. Found: C, 79.59, H, 8.06.

(*S*)-2-(2-(Benzyloxy)-4-methylphenyl)propyl Acetate (*S*)-11. A solution of alcohol (±)-**10** (5.75 g, 22.43 mmol) in methyl *tert*-butyl ether (100 mL) and vinyl acetate (3.05 mL, 2.84 g, 33.0 mmol) was treated with PPL (1.23 g), and the resulting mixture was stirred at room temperature for 6 days. It was filtered, and the filtrate was concentrated in a vacuum before being purified and separated from unreacted alcohol **10** by column chromatography (cyclohexane/ethyl acetate 8:1, silica gel 60). Yield: 1.5 g (5.0 mmol, 45%) of (*S*)-**11** as a colorless oil of R_f 0.8 (cyclohexane/ethyl acetate 1:1); $[\alpha]^{24}_D$ 13.7 (*c* 1, $CHCl_3$); 85% ee. IR (ATR) ν_{max} 3032, 2968, 1735, 1612, 1579, 1506, 1454, 1370, 1226, 1164, 1023, 810, 735 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40 (d, J = 7.1 Hz, 3 H), 2.06 (s, 3 H), 2.43 (s, 3 H), 3.72 (ddq, J = 6.3 Hz, J = 7.1 Hz, J = 7.5 Hz, 1 H), 4.30 (dd, J = 17.8 Hz, J = 7.5 Hz, 1 H), 4.38 (dd, J = 17.8 Hz, J = 6.3 Hz, 1 H), 5.14 (s, 2 H), 6.87 (s, 1 H), 6.88 (d, J = 7.9 Hz, 1 H), 7.21 (d, J = 7.9 Hz, 1 H), 7.4−7.6 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 16.9, 20.6, 21.1, 31.7, 68.3, 69.7, 112.6, 121.3, 126.9, 127.0, 127.5, 128.3, 137.1, 155.9, 170.7; m/z (EI) 298 (3) [M$^+$], 238 (11), 133 (21), 92 (12), 91 (100), 43 (12). Anal. Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.28; H, 7.51.

(*S*)-2-(2-(Benzyloxy)-4-methylphenyl)propan-1-ol (*R*)-10. The unreacted alcohol **10** was enantiomerically enriched to 77% ee by repeating the above reaction two more times to afford 1.99 g (7.76 mmol, 35%) of alcohol (*R*)-**10** as a colorless oil. $[\alpha]^{24}_D$ + 1.6 (*c* 1, $CHCl_3$).

(*S*)-2-(2-(Benzyloxy)-4-methylphenyl)propan-1-ol (*S*)-10. Acetate (*S*)-**11** (1.5 g, 5.0 mmol, 85% ee) was dissolved in methanol (42 mL) and phosphate buffer (pH 7, 126 mL) and treated with PPL (1.5 g) at 35 °C for 24 h to allow for ca. 50% conversion. The mixture was extracted with ethyl acetate (6 × 100 mL), and the extracts were washed with brine (100 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude product was purified by column chromatography (cyclohexane/ethyl acetate 6:1, silica gel 60) to yield 356 mg (1.39 mmol, 30%) of (*S*)-**10** as a colorless oil. $[\alpha]^{24}_D$ − 3.3 (*c* 1, $CHCl_3$); 96% ee; 2.68 mmol of starting acetate (*S*)-**11** was recovered and resubmitted to this protocol.

(*S*)-1-(Benzyloxy)-4-bromo-2-(1-bromopropane-2-yl)-5-methylbenzene (*S*)-4. A solution of alcohol (*S*)-**10** (220 mg, 0.86 mmol, 96% ee) in CH_2Cl_2 (20 mL) was cooled to 0 °C, treated with *N*-bromosuccinimide (489 mg, 2.75 mmol) and triphenylphosphane (270 mg, 1.03 mmol), and allowed to warm to room temperature overnight. The solvent was evaporated and the residue was taken up in ethyl acetate (2 mL) and purified by column chromatography (cyclohexane/ethyl acetate 4:1, silica gel 60). Yield: 325 mg (0.82 mmol, 95%); colorless oil; R_f 0.8 (cyclohexane/ethyl acetate 1:1); $[\alpha]^{24}_D$ −3.8 (*c* 1, $CHCl_3$). IR (ATR) ν_{max} 2924, 2954, 1726, 1602, 1492, 1454, 1385, 1249, 1174, 1091, 1024, 799, 733, 696 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (d, J = 6.7 Hz, 3 H), 2.38 (s, 3 H), 3.49 (dd, J = 15.8 Hz, J = 8.8 Hz, 1 H), 3.57 (ddq, J = 6.7 Hz, J = 8.8 Hz, J = 8.9 Hz, 1 H), 3.66 (dd, J = 15.8 Hz, J = 8.9 Hz, 1 H), 5.07 (s, 2 H), 6.84 (s, 1 H), 7.36 (s, 1 H), 7.4−7.5 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 18.5, 23.1, 35.3, 39.1, 70.4, 114.6, 115.8, 127.2, 128.1, 128.7, 131.1, 131.5, 136.7, 137.2, 155.3; m/z (EI) 397 (3) [M$^+$−1], 395 (4), 227 (8), 225 (8), 147 (4), 132 (3), 91 (100), 65 (5). Anal. Calcd for $C_{17}H_{18}Br_2O$: C, 51.28; H, 4.56. Found: C, 51.31; H, 4.63.

(*S*)-5-Bromo-3,6-dimethyl-2,3-dihydrobenzofurane (*S*)-2. Benzyl ether (*S*)-**4** (190 mg, 0.48 mmol) was dissolved in CH_2Cl_2 (15 mL), and $PhNEt_2$ (0.77 mL, 712 mg, 4.77 mmol) and $AlCl_3$ (191 mg, 1.43 mmol) were added. The mixture was stirred at room temperature for 1 h and then quenched with 1 M aqueous HCl (20 mL). It was extracted with diethyl ether (4 × 50 mL), and the extracts were washed with 1 M aqueous HCl and brine (100 mL each). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed in vacuum. The residue thus obtained was treated with K_2CO_3 (144 mg, 1.04 mmol) and acetone (50 mL), and the resulting mixture was heated under reflux overnight, then cooled to room temperature, filtered, and evaporated. The crude product was purified by column chromatography (cyclohexane/ethyl acetate 10:1, silica gel 60) to leave (*S*)-**2** (90 mg, 0.39 mmol, 83%) as a colorless oil; R_f 0.7 (cyclohexane/ethyl acetate 1:1); $[\alpha]^{24}_D$ −12.2 (*c* 1, $CHCl_3$). IR (ATR) ν_{max} 2921, 2852, 1737, 1659, 1633, 1463, 1377, 1260, 1092, 804, 721 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.28 (d, J = 6.8 Hz, 3 H), 2.31 (s, 3 H), 3.49 (ddq, J = 6.8 Hz, J = 8.6 Hz, J = 8.8 Hz, 1 H), 4.05 (dd, J = 16.0 Hz, J = 8.6 Hz, 1 H), 4.65 (dd, J = 16.0 Hz, J = 8.8 Hz, 1 H), 6.66 (s, 1 H), 7.24 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 19.3, 23.1, 36.3, 79.0, 111.7, 114.6, 127.3, 132.0, 137.2, 159.3; m/z (EI) 228 (44) [M$^+$+1], 226 (42), 147 (15), 132 (100), 115 (8), 91 (16), 63 (8), 51 (16); HRMS (EI) calcd for $C_{10}H_{11}BrO$ [M]$^+$ 225.9993, found 225.9980.

(*S*)-7-*tert*-Butoxy-1,5,8-trimethyl-1,2-dihydronaphtho[2,1-*b*]furan-6,9-dione (*S*)-14. A solution of (*S*)-**2** (63 mg, 0.28 mmol) in dry THF (15 mL) was cooled to −78 °C, treated dropwise with *n*-BuLi (2.1 M in hexane, 0.14 mL, 0.29 mmol) and stirred at this temperature for 1 h. A solution of dione **3** (69 mg, 0.42 mmol) in THF (10 mL) was added, stirring was continued for 90 min at −78 °C, and the mixture was finally quenched with saturated aqueous NH_4Cl (10 mL) and diluted with diethyl ether (25 mL). The phases were separated, and the aqueous one was extracted with diethyl ether (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The remainder was dissolved in toluene

(50 mL) and heated under reflux for 2 h. The reaction mixture was concentrated in vacuum, and the residue thus obtained was left overnight under ambient atmosphere at room temperature to give quinone **14**, which was purified by column chromatography (cyclohexane/ethyl acetate 4:1, silica gel 60). Yield: 50 mg (0.16 mmol, 57%); yellow oil of R_f 0.7 (cyclohexane/ethyl acetate 1:1); $[\alpha]^{24}_D$ −128.4 (*c* 1, $CHCl_3$). IR (ATR) ν_{max} 2962, 2929, 1659, 1600, 1566, 1457, 1365, 1259, 1105, 1016, 799 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.28 (d, J = 6.8 Hz, 3 H), 1.42 (s, 9 H), 2.04 (s, 3 H), 2.66 (s, 3 H), 4.06 (ddq, J = 6.8 Hz, J = 8.3 Hz, J = 8.7 Hz, 1 H), 4.37 (dd, J = 16.5 Hz, J = 8.7 Hz, 1 H), 4.57 (dd, J = 16.5 Hz, J = 8.3 Hz, 1 H), 6.82 (s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 10.6, 19.8, 23.6, 29.6, 37.1, 62.3, 80.2, 116.9, 123.5, 130.2, 132.9, 134.4, 157.9, 163.9, 183.6, 187.6; *m*/*z* (EI) 258 (56) [$M^+ - C_4H_8$], 216 (37), 159 (8), 111 (20), 91 (59), 57 (100). Anal. Calcd for $C_{19}H_{22}O_4$: C, 72.59; H, 7.05. Found: C, 72.61; H, 7.06.

(−)-7-Hydroxy-1,5,8-trimethyl-1,2-dihydronaphtho[2,1-*b*]furan-6,9-dione (−)-1. A solution of ether (*S*)-**14** (40 mg, 0.13 mmol) in CH_2Cl_2 (10 mL) was cooled to 0 °C, treated with TFA (0.5 mL), and stirred at this temperature for 1 h. The volatiles were removed in vacuum, and the crude product was purified by column chromatography (cyclohexane/ethyl acetate 4:1, silica gel 60) to yield (−)-**1** (26 mg, 0.1 mmol, 77%) as a yellow solid of mp 155−156 °C (lit.[9] 156−157 °C); R_f 0.6 (cyclohexane/ethyl acetate 1:1); $[\alpha]^{24}_D$ −151.5 (*c* 0.1, $CHCl_3$) [lit.[9] $[\alpha]^{29}_D$ −336 (*c* 0.01, $CHCl_3$)]; IR (ATR) ν_{max} 3318, 2956, 2923, 2840, 1640, 1598, 1560, 1464, 1385, 1351, 1259, 1094, 1020, 796 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.27 (d, J = 6.9 Hz, 3 H), 2.01 (s, 3 H), 2.69 (s, 3 H), 4.12 (ddq, J = 6.9 Hz, J = 8.1 Hz, J = 8.7 Hz, 1 H), 4.39 (dd, J = 16.5 Hz, J = 8.7 Hz, 1 H), 4.59 (dd, J = 16.5 Hz, J = 8.1 Hz, 1 H), 6.79 (s, 1 H), 7.71 (br, 1 H, O*H*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 8.3, 19.7, 23.8, 37.1, 80.4, 116.2, 117.7, 120.7, 128.8, 131.2, 134.2, 153.8, 165.6, 180.5, 186.3; *m*/*z* (EI) 258 (67) [M^+], 216 (42), 159 (12), 111 (16), 91 (43), 57 (100); HRMS (EI) calcd for $C_{15}H_{15}O_4$ [M + 1]$^+$ 259.0970, found 259.0965.

Cell Lines and Culture Conditions. The HL-60 cells were obtained from the German Collection of Biological Material (DSMZ), Braunschweig, the 518A2 cells from the Department of Oncology and Hematology of the Martin-Luther University Halle, Germany, the KB-V1/Vbl and the MCF-7/Topo cells from the Institute of Pharmacy of the University Regensburg, Germany, and the human foreskin (HF) fibroblasts as well as the HT-29 cells from the University Hospital Erlangen, Germany. The multidrug resistant cells were bred by repeated treatment with vinblastine sulfate (KB-V1/Vbl: 340 nM), topotecan hydrochloride (MCF-7/Topo: 550 nM), or colchicine (HT-29/Colc: 62.5 nM). The HL-60 and HT-29 cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B and 250 μg/mL gentamycine. The 518A2, the KB-V1/Vbl and the HF cells were cultured in Dulbecco's Modified Eagle Medium containing 10% FCS, 100 IU/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B and 250 μg/mL gentamycine. The MCF-7/Topo cells were grown in E-MEM medium supplemented with 2.2 g/L $NaHCO_3$, 110 mg/L sodium pyruvate and 5% FCS. The cells were maintained in a moisture-saturated atmosphere (5% CO_2) at 37 °C in 75-mL culture flasks. They were serially passaged following trypsinisation by 0.05% trypsin/0.02% EDTA. Mycoplasma contamination was routinely monitored, and only mycoplasma-free cultures were used.

Inhibition of Cell Growth and Metabolic Activity (MTT Assay). MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was used to identify viable cells which reduce it to a violet formazan. HL-60 cells (5×10^5/mL), and cells (5×10^4/mL) of 518A2, HT-29, KB-V1/Vbl, MCF-7/Topo carcinomas and foreskin fibroblasts (HF) were seeded in 96-well tissue culture plates and cultured for 24 h.[40] Incubation (5% CO_2, 95% humidity, 37 °C) of the cells following treatment with the test compounds was continued for 24, 48, or 72 h. Blank and solvent controls were treated identically. MTT in phosphate buffered saline (5 mg/mL) was added to a final concentration of 0.05% (HL-60, 518A2, HF) or 0.1% (KB-V1/Vbl, MCF-7/Topo, HT-29, HT-29/Colc). After 2 h the formazan precipitate was dissolved in 10% sodium dodecylsulfate in DMSO containing 0.6% acetic acid in the case of HL-60 cells. For the adherent 518A2, HT-29, HT-29/Colc, KB-V1/Vbl, MCF-7/Topo and HF cells the microplates were swiftly turned to discard the medium before adding the solvent mixture. The microplates were gently shaken in the dark for 30 min and absorbance at 570 and 630 nm (background) was measured with an ELISA plate reader. All experiments were carried out in quadruplicate; the percentage of viable cells was calculated as mean ± SD with controls set to 100%.

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Supporting Information Available: Syntheses and properties of compounds **6** − **10**, optical rotations of compounds (*R*)-**1**, (*R*)-**2**, (*R*)-**4**, (*R*)-**14**, ^1H and ^{13}C NMR spectra of compounds **1** − **14**; chiral GC of **10**, inhibitory concentrations IC_{50}(24 h/48 h) of (±)-/(−)-/(+)-**1** and **15**. This material is free of charge via the Internet at http://pubs.acs.org.

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