Brief Articles

Synthesis and Biological Evaluation of Dihydrobenzofuran Lignans and Related Compounds as Potential Antitumor Agents that Inhibit Tubulin Polymerization

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A series of 19 related dihydrobenzofuran lignans and benzofurans was obtained by a biomimetic reaction sequence involving oxidative dimerization of *p*-coumaric, caffeic, or ferulic acid methyl esters, followed by derivatization reactions. All compounds were evaluated for potential anticancer activity in an in vitro human disease-oriented tumor cell line screening panel that consisted of 60 human tumor cell lines arranged in nine subpanels, representing diverse histologies. Leukemia and breast cancer cell lines were relatively more sensitive to these agents than were the other cell lines. Compounds **2c** and **2d**, but especially **2b** (methyl (*E*)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate), the dimerization product of caffeic acid methyl ester, containing a 3′,4′-dihydroxyphenyl moiety and a hydroxyl group in position 7 of the dihydrobenzofuran ring, showed promising activity. The average GI_{50} value (the molar drug concentration required for 50% growth inhibition) of **2b** was 0.3 μM. Against three breast cancer cell lines, **2b** had a GI_{50} value of <10 nM. Methylation, reduction of the double bond of the C_3-side chain, reduction of the methoxycarbonyl functionalities to primary alcohols, or oxidation of the dihydrobenzofuran ring to a benzofuran system resulted in a decrease or loss of cytotoxic activity. Compound **2b** inhibited mitosis at micromolar concentrations in cell culture through a relatively weak interaction at the colchicine binding site of tubulin. In vitro it inhibited tubulin polymerization by 50% at a concentration of 13 ± 1 μM. The 2*R*,3*R*-enantiomer of **2b** was twice as active as the racemic mixture, while the 2*S*,3*S*-enantiomer had minimal activity as an inhibitor of tubulin polymerization. These dihydrobenzofuran lignans (2-phenyl-dihydrobenzofuran derivatives) constitute a new group of antimitotic and potential antitumor agents that inhibit tubulin polymerization.

Introduction

3′,4-Di-*O*-methylcedrusin (**4d**), 3-[2-(3,4-dimethoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol, was identified as one of the minor constituents of the red latex called 'dragon's blood' ('sangre de drago') in traditional medicine. This latex is obtained by slashing the bark of various South American *Croton* species (Euphorbiaceae). Lignan **4d**, belonging to the neolignans, was found to act as an inhibitor of cell proliferation[1] and has been assigned a 2*R*,3*S*-configuration.[2] Lignans and neolignans are formed in nature by the oxidative dimerization of various C_6C_3 phenols. While lignans are defined as those compounds in which the two C_6C_3 units are linked by a bond connecting the central (β) carbon atom of each side chain, in neolignans the two C_6C_3 units are not linked by a $\beta-\beta$ bond.[3] Lignans and neolignans display a wide variety of chemical structures, are widespread in nature, and exhibit a broad range of biological activities, including for some classes antitumor activities, e.g., the podophyllotoxin group of lignans.[4]

To explore the potential antiproliferative and antitumoral activity of 3′,4-di-*o*-methylcedrusin (**4d**), obtained in low yield from its natural source, a synthesis from various C_6C_3 precursors was devised. In this way both **4d** and a series of synthetic dihydrobenzofuran lignans were obtained. Their cytotoxicity was determined in an in vitro human disease-oriented tumor cell line screening panel. A structure−activity relationship could be established, and the mechanism of action was determined.

Chemistry

A series of 19 related dihydrobenzofuran lignans and benzofurans was synthesized as shown in Scheme 1. A biomimetic oxidative coupling of the *p*-coumaric (**1a**),

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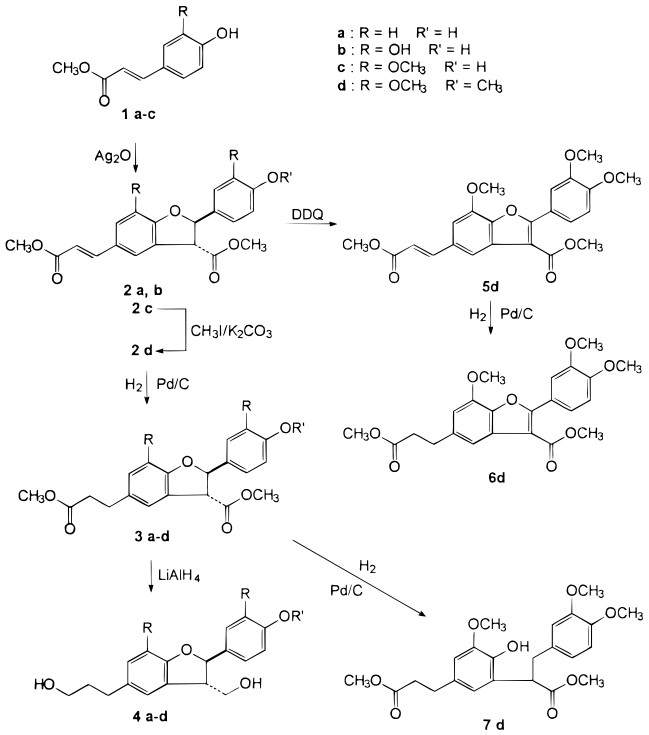
Scheme 1. Synthetic Routes



caffeic (**1b**), and ferulic acid (**1c**) methyl esters in the presence of silver oxide is the crucial step in the reaction sequence, generating the dihydrobenzofuran skeleton with a 2,3-*trans*-configuration.[5] The 4′-OH group of **2c** has been methylated with methyl iodide in the presence of potassium carbonate. Hydrogenation of the double bond of the propenoates **2a**−**d** in the presence of Pd−C yields the corresponding propanoates **3a**−**d**. Prolonged reaction times or large amounts of catalyst have to be avoided since this results in opening of the dihydrofuran ring as shown in the sequence **2d** → **3d** → **7d**. $LiAlH_4$ reduction of both ester functions of **3a**−**d** gave the primary alcohols **4a**−**d**. Two benzofuran lignans were prepared from **2d** by dehydrogenation to **5d** with DDQ, followed by hydrogenation of the side chain in the presence of Pd−C to **6d**.

The racemic products **2b** and **2d** have been resolved into their enantiomers by preparative-scale chiral HPLC.[2] The enantiomers of **3d** and **4d** have been synthesized from the corresponding enantiomers of **2d** by the same methodology as the racemic compounds (Scheme 1). The absolute configuration of the enantiomers of **2d**−**4d** was determined from a comparison of their CD spectra with the CD spectra of ephedradin A, a compound with a similar chromophore and with a known absolute configuration determined by anomalous dispersion X-ray crystallography.[6] In this way the naturally occurring 3′,4-di-*O*-methylcedrusin has been assigned a 2*R*,3*S*-configuration. This absolute configuration has been confirmed independently by chemical correlation with a (1*S*,4*R*)-camphanoyl derivative of a dihydrobenzofuran lignan whose absolute configuration was determined by an X-ray crystallographic study.[7] CD spectra of both enantiomers of **2b** have been recorded. Comparison with the CD spectra of the enantiomers of **2d**[2] allowed assignment of the absolute configuration (see Supporting Information, Figure 2). The first eluting enantiomer

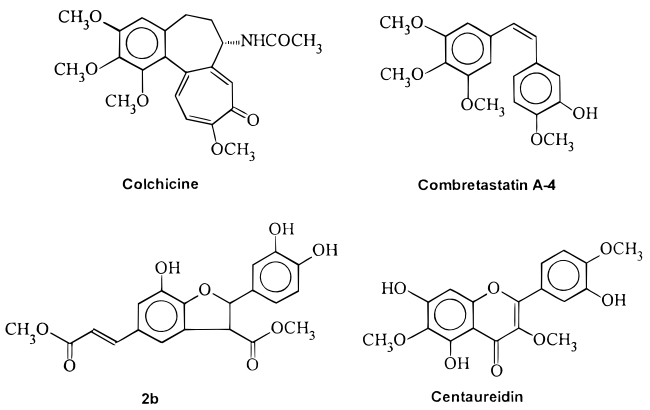


Figure 1.

of **2b** was assigned a 2*R*,3*R*-configuration and the second eluting enantiomer a 2*S*,3*S*-configuration.

Results and Discussion

(a) Evaluation of the Cytotoxicity of Dihydrobenzofuran Lignans. All dihydrobenzofuran lignans synthesized were evaluated in the in vitro human disease-oriented tumor cell line screening panel developed at the NCI. The average log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) calculated from all cell lines tested, as well as the log GI_{50} values for a series of representative cell lines, are listed in Table 1 (see Supporting Information). The complete set of data obtained in this screening panel showed that the leukemia cell lines and the breast cancer cell lines were relatively more sensitive to the cytotoxic dihydrobenzofuran lignans than were other cell lines. Three dihydrobenzofuran lignans (**2b**, **2c**, and **2d**(2*R*,3*R*)) showed an average log $GI_{50} < -5$ (corresponding to average GI_{50} values of 0.3, 3.3, and 5.1 μM, respectively) and were selected for further evaluation as potential antitumor agents. Compound **2b** was the dimerization product of caffeic acid methyl ester, containing a 3′,4′-dihydroxyphenyl moiety and a hydroxyl group in position 7 of the dihydrobenzofuran ring (Figure 1). Compound **2c** was the dimerization product of ferulic acid methyl ester, with only one free hydroxyl group left in position 4′; compound **2d** was the methylated derivative of **2c**. All three compounds still contained the methyl ester functionality and the double bond in the side chain. Apparently methylation of the hydroxyl groups reduced activity, since the caffeic acid derivative **2b** was 10 times more active than the ferulic acid derivative **2c**. Methylation of **2c** led to a further loss of activity. Of all the dihydrobenzofuran lignans tested, compound **2b** showed the greatest cytotoxicity against every cancer cell line examined. Against three breast cancer cell lines (MDA-MB-435, MDA-N, and BT-549) the GI_{50} for **2b** was <10 nM. Submicromolar GI_{50} values were observed against additional leukemia and breast cancer cell lines evaluated in the NCI screen as follows: 0.033 (CCRF−CEM), 0.081 (MOLT-4), 0.055 (RPMI-8226), 0.069 μM (SR) (leukemia cell lines); 0.029 (MCF7), 0.054 (MCF7/ADR−RES), 0.050 (MDA-MB-231/ATCC), 0.045 μM (HS 578 T) (breast cancer cell lines).

The series **2b** → **3b** → **4b** suggests the following structure−activity conclusions: reduction of the double bond in the C_3 side chain (**2b** → **3b**) caused over a 10-

fold decrease in activity (average log GI_{50} from −6.54 to −4.97), while additional reduction of the methyl ester functionality to a primary alcohol (**3b** → **4b**) led to an additional 10-fold loss in activity (average log GI_{50} −4.01 for **4b**). Most compounds tested were racemic mixtures, except for **2d**, **3d**, and **4d**. Compound **2d** allows us to conclude that activity was related to the stereochemistry of the dihydrofuran ring, the 2*R*,3*R*-isomer being the more active one. To our surprise the initial natural lead compound 3′,4-di-*O*-methylcedrusin (**4d**) showed no activity in the 60 cell line panel up to the highest concentration tested (100 μM).

For all compounds tested, including the most active ones, no TGI (total growth inhibition) or LC_{50} (50% cell kill) level was reached for most cell lines (log TGI and log $LC_{50} > -4.00$, 100 μM being the highest concentration tested). This indicated that their mechanism of action was cytostatic rather than cytotoxic within the 48 h time frame of the assay. The COMPARE algorithm,[8] a program that compares a complete set of cell sensitivities to those of standard agents or other agents present in the NCI database, showed a correlation (correlation coefficient = 0.735) between **2b** and a number of combretastatins,[9] which are known to act as antimitotic compounds by inhibiting tubulin polymerization (Figure 1). Correlation coefficients >0.6 can be considered significant. In addition, the flat configuration of the dose−response curves, obtained when TGI or LC_{50} values are not reached in the screening assays, is observed with many antitubulin agents.

(b) Interactions with Tubulin. Microtubules are an important target for anticancer chemotherapy. Chemicals that interact with tubulin, the major structural component of microtubules, can cause mitotic arrest by inhibiting (e.g., *vinca* alkaloids, colchicine) or stimulating (e.g., taxoids) microtubule assembly. Interestingly, all antimitotic agents currently in clinical use as anticancer drugs are natural products or semisynthetic derivatives.[10,11] Six compounds were initially examined for effects on the polymerization of purified bovine brain tubulin: **2b**, **2c**, **2d**(2*S*,3S), **2d**(2*R*,3*R*), **3b**, and **4b**. All of them, except for **2b**, showed an $IC_{50} > 40$ μM (50% inhibitory concentration). Compound **2b** was compared with the potent antimitotic drug combretatstatin A-4 and proved to be only about 1/10 as active: It inhibited the extent of polymerization by 50% at a concentration of 13 ± 1 (SD) μM ($n = 3$), while the IC_{50} value for combretastatin A-4, used as a positive control, was 1.2 ± 0.03 (SD) μM. Subsequent chromatographic resolution of **2b** into its enantiomers allowed their evaluation for antitubulin activity. The 2*R*,3*R*-enantiomer yielded an IC_{50} value of 6.0 ± 0.4 μM, while the 2*S*,3*S*-enantiomer was essentially inactive ($IC_{50} > 40$ μM).

Combretastatin A-4 as well as compound **2b** inhibited the binding of [^{3}H]colchicine to tubulin, and their relative activities were quantitatively consistent with their relative effects on the polymerization reaction. In reaction mixtures containing 1.0 μM tubulin and 5.0 μM [^{3}H]colchicine, binding of radiolabeled drug to the protein was inhibited (88 ± 4)% by 1.0 μM combretastatin A-4, and total inhibition occurred with 5.0 μM combretastatin A-4. Combretastatin A-4 is one of the most potent competitive inhibitors of colchicine binding to tubulin known to date. Compound **2b** was less potent: (14.0 ± 0.2)% inhibition occurred with the compound at 5.0 μM and (53 ± 3)% at 50 μM **2b**.

Compound **2b** was also evaluated for its effects on the growth of HL 60 human leukemia cells. An IC_{50} value of 0.2 μM for **2b** at 24 h on the growth of this cell line (increase in cell number from time zero) was obtained. Next the effect on the mitotic index at 10 μM was examined. After 24 h of growth, 35% of the cells displayed a mitotic configuration (condensed chromosomes), as compared with 2% in the control culture. The enantiomers were also examined for inhibitory effects on the growth of the HL 60 cells. The same relative activities were observed as with inhibition of tubulin polymerization. The IC_{50} value obtained with the 2*R*,3*R*-enantiomer was 0.08 μM, versus 0.6 μM with the 2*S*,3*S*-enantiomer. These values were obtained with freshly dissolved compounds. There is probably racemization of the enantiomers in solution. Preliminary experiments have yielded smaller differences when older solutions of the enantiomers were compared than when freshly prepared solutions were evaluated. Finally, the 2*R*,3*R*-enantiomer was similar to racemic **2b** in causing a marked increase in the mitotic index of HL 60 cells.

Structurally, compounds binding to the colchicine site of tubulin are much simpler than those binding to the *vinca* or taxoid domains. Many of them can be described as biaryl systems connected by a hydrocarbon bridge of variable length. These planar rings have to be tilted with respect to each other.[12] This may explain why the benzofuran derivative **5d** was not active in the 60 cell line panel, in contrast to its 2,3-saturated analogue **2d**. In addition to colchicine and combretastatins, other examples of naturally occurring or synthetic inhibitors of tubulin polymerization acting through the colchicine site of tubulin and possessing such a biaryl system include 2-aryl-1,8-naphthyridin-4(1*H*)-ones,[13] 2-phenyl-4-quinolones,[10] both with a N-containing heterocyclic unit, chalcones,[14] and flavonoids such as centaureidin, with a six-membered heterocyclic unit instead of a five-membered one as in dihydrobenzofurans. A recent screening of 79 flavones related to centaureidin (3,4′,6-trimethoxy-3′,5,7-trihydroxyflavone) (Figure 1) showed that maximum potencies for tubulin interaction were observed only with compounds possessing hydroxyl substituents at C-3′ and C-5 and methoxy groups at C-3 and C-4′.[15] The 3-methoxy group may be related to the loss of planarity, essential for binding to the colchicine site. It has already been observed by Cheng in 1986 that a tricyclic chemical structural pattern, consisting of a phenyl ring attached in position 2 of a naphthalene nucleus or composed of various heterocyclic units, was present in a large number of antineoplasic compounds.[16] In addition to the examples listed above, the dihydrobenzofuran lignans (2-phenyl-dihydrobenzofurans) discussed here constitute a new group of antimitotic agents that inhibit tubulin polymerization, fitting into this general pattern.

(c) Hollow Fiber Assay for Preliminary in Vivo Testing. Compounds **2b**, **2c**, and **2d**(2*R*,3*R*) were evaluated in the hollow fiber assay developed at the NCI. This is a preliminary in vivo screening tool for assessing the potential anticancer activity of compounds selected in the in vitro cell screen. However, none of the

compounds tested was sufficiently active for further in vivo testing in standard subcutaneous xenograft models.

Conclusion

In a series of synthetic dihydrobenzofuran lignans (2-phenyldihydrobenzofuran lignans), the dimerization product of caffeic acid methyl ester (**2b**) showed the highest activity in a screening panel consisting of 60 human tumor cell lines, with an average GI_{50} value of 0.3 μM and GI_{50} values <10 nM against some breast cancer cell lines. It appeared to inhibit mitosis at micromolar concentrations in cell culture through a relatively weak interaction at the colchicine binding site of tubulin, and these activities in the racemate probably derive from activity limited to the 2*R*,3*R*-enantiomer. These dihydrobenzofuran lignans (2-phenyldihydrobenzofuran derivatives) constitute a new group of antimitotic agents that inhibit tubulin polymerization. We think it likely that modification of substituents and/or the core structure of the molecule will yield more active agents, including compounds that will have antitumor activity.

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-545 melting point apparatus. ^{1}H and ^{13}C NMR spectra were measured on a Varian Unity 400 spectrometer. Additional HETCOR and long-range HETCOR measurements to verify the proposed assignments were performed on the same spectrometer. Chemical shifts are reported in δ (ppm). Numbering of the atoms is done in the same way as Lemière et al.[2] for ease of comparison.

DCI mass spectra were obtained on a Ribermag R-10-10B mass spectrometer. Column chromatography was performed on Merck silica gel 60, 0.040−0.063 mm, 230−400 mesh ASTM. Precoated silica gel plates (kieselgel 60, F_{254}, 0.2 mm) were used for TLC analysis. All products and reagents were puchased from Acros, Belgium.

Chiral separations were performed using a column (5 × 21 cm) packed with 0.25 kg of Chiralpack AD. Peak shaving and closed loop recycling techniques were used during the process. Ethanol was the solvent used for the chiral chromatography. Analytical HPLC using two independent systems was performed to check the purity of the products. Column A: Alltech Econosil C8 (4.6 × 250 mm). Column B: BIO-RAD Bio-Sil C18 (4.6 × 150 mm). Experimental details of the HPLC analysis are reported for all test compounds in the form column; mobile phase; retention time. The flow rate was 1 mL/min.

Preparation of Dihydrobenzofuran Lignans. Methyl cinnamates (**1a**−**c**) were prepared from a mixture of the corresponding cinnamic acid (4 g) and Dowex 50 W × 8200−400 (0.4 g) in 25 mL of absolute methanol. After the mixture was heated under reflux for 1 night, the mixture was filtered and evaporated under reduced pressure to afford the product as a solid (100%), which was used without further purification.

1a: amorphous, mp 136 °C; ^{1}H NMR (acetone-d_6) δ 8.80 (s, 1 H, 4-OH), 7.60 (d, J = 16.02 Hz, 1 H, H-7), 7.52 (d, J = 8.65 Hz, 2 H, H-2, H-6), 6.89 (d, J = 8.55 Hz, 2 H, H-3, H-5), 6.33 (d, J = 16.02 Hz, 1 H, H-8), 3.70 (s, 3 H, 9-OCH_3); ^{13}C NMR (acetone-d_6) δ 167.85 (C-9), 160.47 (C-4), 145.33 (C-7), 130.85 (C-2, C-6), 126.99 (C-1), 116.65 (C-3, C-5), 115.32 (C-8), 51.47 (9-OCH_3); DCI-MS (NH_3) m/z 179 (MH^+).

1b: amorphous, mp 158 °C; ^{1}H NMR (acetone-d_6) δ 8.3 (s, 2 H, 3-OH, 4-OH), 7.53 (d, J = 15.87 Hz, 1 H, H-7), 7.15 (d, J = 2.14 Hz, 1 H, H-2), 7.03 (dd, J = 8.09, 2.14 Hz, H-6), 6.86 (d, J = 8.09 Hz, 1 H, H-5), 6.27 (d, J = 16.02 Hz, 1 H, H-8), 3.70 (s, 3 H, 9-OCH_3); ^{13}C NMR (acetone-d_6) δ 167.82 (C-9), 148.75 (C-4), 146.33 (C-3), 145.68 (C-7), 127.75 (C-1), 122.51 (C-6), 116.43 (C-5), 115.28 (C-2), 115.21 (C-8), 51.45 (9-OCH_3); DCI-MS (NH_3) m/z 195 (MH^+).

1c: amorphous, mp 148 °C; ^{1}H NMR (acetone-d_6) δ 8.10 (s, 1 H, 4-OH), 7.59 (d, J = 16.02 Hz, 1 H, H-7), 7.29 (d, J = 1.89 Hz, 1 H, H-2), 7.12 (dd, J = 8.24, 1.98 Hz, 1 H, H-6), 6.87 (d, J = 8.24 Hz, 1 H, H-5), 6.38 (d, J = 15.94 Hz, 1 H, H-8), 3.91 (s, 3 H, 3-OCH_3), 3.68 (s, 3 H, 9-OCH_3); ^{13}C NMR (acetone-d_6) δ 167.87 (C-9), 149.98 (C-4), 148.66 (C-3), 145.66 (C-7), 127.42 (C-1), 123.75 (C-6), 116.06 (C-5), 115.55 (C-8), 111.42 (C-2), 56.35 (C-11), 51.49 (C-10); DCI-MS (NH_3) m/z 209 (MH^+).

Methyl (*E*)-3-[2-(4-hydroxyphenyl)-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate (2a) was prepared according to the method of Lemière et al.[2] using 4.86 g (27.3 mmol) of methyl *p*-coumarate, 4.53 g (19.5 mmol) of silver(I) oxide, 70 mL of anhydrous benzene, and 50 mL of anhydrous acetone and a reaction time of 65 h. The product was purified by column chromatography (3.8 × 30 cm, silica gel 60, 0.040−0.063 mm) with ethyl acetate−*n*-heptane (1:3) as the eluent. The product was obtained as an oil (23%). ^{1}H NMR (acetone-d_6) δ 8.55 (s, 1 H, 4-OH), 7.68 (d, J = 1.83 Hz, 1 H, H-6′), 7.55 (dd, J = 8.39, 1.83 Hz, 1 H, H-2′), 7.27 (d, J = 8.24 Hz, 2 H, H-2, H-6), 6.89 (d, J = 8.39 Hz, 1 H, H-3′), 6.87 (d, J = 8.24 Hz, 2 H, H-3, H-5), 6.64 (d, J = 7.33 Hz, 1 H, H-7′), 6.38 (d, J = 16.02 Hz, 1 H, H-8′), 6.02 (d, J = 7.33 Hz, 1 H, H-7), 4.37 (d, J = 7.33 Hz, 1 H, H-8), 3.80 (s, 3 H, 9-OCH_3), 3.72 (s, 3 H, 9′-OCH_3); ^{13}C NMR (acetone-d_6) δ 167.73 (C-9′), 162.10 (C-4), 158.61 (C-4′), 145.07 (C-7′), 131.84 (C-1), 131.54 (C-1′), 128.66 (C-2′), 128.38 (C-2, C-6), 116.34 (C-3, C-5), 126.69 (C-5′), 126.06 (C-6′), 116.08 (C-8′), 110.75 (C-3′), 87.72 (C-7), 55.60 (C-8), 52.97 (9-OCH_3), 51.55 (9′-OCH_3); DCI-MS (NH_3) m/z 355 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 5.14 min; Column B methanol−water, 50:20, 4.17 min.

Methyl (*E*)-3-[2-(3,4-dihydroxyhenyl)-7-hydroxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate (2b) was prepared according to the method of Lemière et al.[2] using 1.905 g (9.8 mmol) of methyl caffeate, 826 g (3.5 mmol) of silver(I) oxide, 40 mL of anhydrous benzene, and 20 mL of anhydrous acetone. The product was purified by column chromatography (3.8 × 30 cm, silica gel 60, 0.040−0.063 mm) with ethyl acetate−*n*-heptane (1:1) as the eluent. After evaporation and lyophilization, a white foam was obtained (33%); amorphous mp 159 °C; ^{1}H NMR (acetone-d_6) δ 8.05 (s, 2 H, 3-OH, 4-OH), 7.57 (d, J = 16.02 Hz, 1 H, H-7′), 7.14 (s, 1 H, H-6′), 6.90 (d, J = 1.95 Hz, 1 H, H-2), 6.84 (d, J = 8.24 Hz, 1 H, H-5), 6.79 (dd, J = 8.24, 1.98 Hz, 1 H, H-6), 6.33 (d, J = 16.02 Hz, 1 H, H-8′), 5.97 (d, J = 8.33 Hz, 1 H, H-7), 4.35 (d, J = 8.33 Hz, 1 H, H-8), 3.79 (s, 3 H, 9′-OCH_3), 3.71 (s, 3 H, 9-OCH_3), 3.38 (s, 1 H, 3′-OH); ^{13}C NMR (acetone-d_6) δ171.69 (C-9), 167.79 (C-9′), 150.07 (C-3′), 146.33 (C-4), 146.09 (C-3), 145.42 (C-7′), 142.53 (C-4′), 132.70 (C-1), 129.40 (C-1′), 127.33 (C-5′), 118.70 (C-6), 117.76 (C-2′), 117.33 (C-6′), 116.16 (C-5), 116.05 (C-8′), 113.93 (C-2), 87.88 (C-7), 56.29 (C-8), 52.95 (9-OCH_3), 51.55 (9′-OCH_3); DCI-MS (NH_3) m/z 387 (MH^+). Chiral HPLC analysis: Chiralpak AD, length 5 cm; *n*-hexane−ethanol, 60:40. Anal. ($C_{20}H_{18}O_6$) C, H.

Methyl (*E*)-3-[2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]-prop-2-enoate (2c) was prepared according to the method of Lemière et al.[2] using an improved workup. After evaporation, the residual brown oil was dissolved in methanol. The solution was left to stand overnight. Crystals (white, mp 151 °C) were formed, filtered, and washed with cold methanol (50%): ^{1}H NMR (acetone-d_6) δ 7.68 (s, 1 H, 4-OH), 7.63 (d, J = 16.02 Hz, 1 H, H-7′), 7.33 (s, 1 H, H-2′), 7.30 (s, 1 H, H-6′), 7.09 (d, J = 1.98 Hz, 1 H, H-2), 6.91 (dd, J = 8.09, 1.98 Hz, 1 H, H-6), 6.85 (d, J = 8.09 Hz, 1 H, H-5), 6.45 (d, J = 16.02 Hz, 1 H, H-8′), 6.03 (d, J = 8.02 Hz, 1 H, H-7), 4.48 (d, J = 8.02 Hz, 1 H, H-8), 3.93 (s, 3 H, 3′-OCH_3), 3.83 (s, 3 H, 3-OCH_3), 3.82 (s, 3 H, 9-OCH_3), 3.74 (s, 3 H, 9′-OCH_3); ^{13}C NMR (acetone-d_6) δ 171.73 (C-9), 167.86 (C-9′), 151.01 (C-4′), 148.75 (C-3), 148.21 (C-4), 145.80 (C-3′), 145.50 (C-7′), 131.81 (C-1), 129.42 (C-1′), 127.35 (C-5′), 120.18 (C-6), 119.02 (C-6′), 116.29 (C-8′), 116.02 (C-5), 113.46 (C-2′), 110.96 (C-2), 56.54 (3′-OCH_3), 56.35 (3-OCH_3), 55.92 (C-8), 53.07 (9-OCH_3), 51.67 (9′-OCH_3); DCI-MS (NH_3) m/z 415 (MH^+). HPLC analysis: Column A methanol−

water, 65:35, 5.01 min; Column B methanol−water, 50:20, 4.93 min. Anal. ($C_{22}H_{22}O_8$) C, H.

Methyl (*E*)-3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]prop-2-enoate (2d) was prepared using the method of Lemière et al.[2] using an improved workup. After evaporation of the reaction mixture, the resulting yellow oil was dissolved in methanol. The solution was left to stand overnight. Crystals (75%) were formed, filtered, and washed with cold methanol: White crystals, mp 135 °C; ^{1}H NMR (acetone-d_6) δ 7.65 (d, J = 15.91 Hz, 1 H, H-7′), 7.19 (s, 1 H, H-6′), 7.03 (s, 1 H, H-2′), 6.97 (dd, J = 15.91, 1.99 Hz, 1 H, H-6), 6.92 (d, J = 1.99 Hz, 1 H, H-2), 6.84 (d, J = 8.35 Hz, 1 H, H-5), 6.32 (d, J = 15.91 Hz, 1 H, H-8′), 6.13 (d, J = 8.15 Hz, 1 H, H-7), 4.35 (d, J = 8.15 Hz, 1 H, H-8), 3.87 (s, 3 H, 4-OCH$_3$), 3.86 (s, 3 H, 3-OCH$_3$), 3.84 (s, 3 H, 9-OCH$_3$), 3.80 (s, 3 H, 9′-OCH$_3$), 3.92 (s, 3 H, 3′-OCH$_3$); ^{13}C NMR (acetone-d_6) δ 170.74 (C-9), 167.55 (C-9′), 150.14 (C-4′), 149.62 (C-4), 149.49 (C-3), 144.85 (C-3′), 144.72 (C-6′), 132.18 (C-1), 128.76 (C-1′), 125.88 (C-5′), 118.82 (C-6),-117.99 (C-6′), 115.76 (C-8′), 112.53 (C-2′), 111.46 (C-5), 109.56 (C-2), 87.41 (C-7), 56.09 (3′-OCH$_3$), 56.09 (4-OCH$_3$), 56.05 (3-OCH$_3$), 52.83 (9-OCH$_3$), 51.56 (9′-OCH$_3$); DCI-MS (NH_3) m/z 429 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 5.06 min; Column B methanol−water, 50:20, 5.61 min. Anal. ($C_{23}H_{24}O_8$) C, H.

Methyl (*E*)-3-[2-(4-hydroxyphenyl)- 3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propanoate (3a) was prepared by dissolving 418 mg (1.18 mmol) of **2a** in 30 mL of acetone to which 220 mg of 5% Pd/C was added. The mixture was put in a Parr apparatus under 60 psi hydrogen pressure and was shaken for 20 min. After filtration and evaporation, the crude oil was purified by column chromatography (3 × 20 cm, silica gel 60, 0.040−0.063 mm) with ethyl acetate−heptane (3:1) as the eluent, resulting in a colorless oil (85%): ^{1}H NMR ($CDCl_3$) δ 7.26 (d, J = 8.70 Hz, 2 H, H-2, H-6), 7.17 (d, J = 1.83 Hz, 1 H, H-6′), 7.05 (dd, J = 8.24, 1.83 Hz, 1 H, H-2′), 6.81 (d, J = 8.70 Hz, 2 H, H-3, H-5), 6.74 (d, J = 8.24 Hz, 1 H, H-3′), 5.45 (s, 1 H, 4-OH), 4.24 (d, J = 7.78 Hz, 1 H, H-7), 3.81 (s, 3 H, 9-OCH$_3$), 3.68 (s, 3 H, 9′-OCH$_3$), 2.91 (t, J = 7.78 Hz, 2 H, H-8′), 2.61 (t, J = 7.78, 2 H, H-7′); ^{13}C NMR ($CDCl_3$) δ 173.55 (C-9′), 171.46 (C-9), 157.89 (C-4), 155.97 (C-4′), 132.70 (C-1′), 129.55 (C-2′), 127.51 (C-2, C-6), 124.93 (C-6′), 124.24 (C-5′), 115.63 (C-3, C-5), 109.74 (C-3′), 85.78 (C-7), 55.74 (C-8), 52.66 (C-9), 51.64 (C-9′), 36.22 (C-8′), 30.50 (C-7′); DCI-MS (NH_3) m/z 357 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 4.02 min; Column B methanol−water, 60:40, 9.22 min.

Methyl(*E*)-3-[2-(3,4-dihydroxyphenyl)-7-hydroxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propanoate (3b) was prepared from **2b** using the same method and concentrations as for the synthesis of **3a**. The crude oil was purified by column chromatography (3 × 20 cm, silica gel 60, 0.040−0.063 mm) with ethyl acetate−*n*-heptane (1:2) as the eluent, resulting in a colorless oil (95%): ^{1}H NMR (acetone-d_6) δ 7.95 (s, 2 H, 3-OH, 4-OH), 6.88 (d, J = 1.98 Hz, 1 H, H-2), 6.82 (d, J = 8.08 Hz, 1 H, H-5), 6.76 (dd, J = 8.08, 1.98 Hz, 1 H, H-6), 6.71 (s, 1 H, H-2′), 6.68 (s, 1 H, H-6′), 5.88 (d, J = 7.48 Hz, 1 H, H-7), 4.24 (d, J = 7.53 Hz, 1 H, H-8), 3.76 (s, 3 H, 9-OCH$_3$), 3.60 (s, 3 H, 9′-OCH$_3$), 2.80 (t, J = 7.63 Hz, 2 H, H-7′), 2.55 (t, J = 7.63 Hz, 2 H, H-8′); ^{13}C NMR (acetone-d_6) δ 173.45 (C-9′), 172.06 (C-9), 146.18 (C-4′), 146.08 (C-3), 145.41 (C-4), 142.00 (C-3′), 135.26 (C-1′), 133.36 (C-1), 126.48 (C-5′), 118.60 (C-6), 117.78 (C-6′), 116.20 (C-2′), 116.13 (C-5′), 113.9 (C-2), 87.95 (C-7), 56.40 (C-8), 52.94 (9-OCH$_3$), 51.53 (9′-OCH$_3$), 36.54 (C-8′), 31.20 (C-7′); DCI-MS (NH_3) m/z 389 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 6.25 min; Column B methanol−water, 60:40, 3.45 min.

Methyl 3-[2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propanoate (3c) was prepared from **2c** using the same method and concentrations as for the synthesis of **3a**. Workup was performed identically, resulting in a white powder (95%); mp 85−87 °C; ^{1}H NMR (acetone-d_6) δ 7.79 (s, 1 H, H-2′), 7.69 (s, 1 H, H-6′), 6.93 (d, J = 1.84 Hz, 1 H, H-2), 6.92 (d, J = 1.84 Hz, 1 H, H-2), 6.92 (d, J = 7.93 Hz, 1 H, H-6), 6.87 (dd, J = 7.93, 1.84 Hz, 1 H, H-5), 6.03 (d, J = 8.54 Hz, 1 H, H-7), 5.72 (s, 1 H, 4-OH), 4.30 (d, J = 8.54 Hz, 1 H, H-8), 3.98 (s, 3 H, 3′-OCH$_3$), 3.87 (s, 3 H, 3-OCH$_3$), 3.81 (s, 3 H, 9-OCH$_3$), 3.68 (s, 3 H, 9′-OCH$_3$), 2.91 (t, J = 7.78 Hz, 2 H, H-7′), 2.62 (t, J = 7.78 Hz, 2 H, H-8′); ^{13}C NMR (acetone-d_6) δ 173.21 (C-9′), 171.73 (C-9), 146.68 (C-4′), 146.43 (C-3), 145.92 (C-4), 144.57 (C-3′), 136.12 (C-8′), 134.19 (C-1′), 131.97 (C-1), 130.85 (C-7′), 125.25 (C-5′), 119.40 (C-6), 116.50 (C-6′), 114.44 (C-5), 113.16 (C-2′), 108.86 (C-2), 86.75 (C-7), 77.32 (C-8), 77.00 (3-OCH$_3$), 76.68 (3′-OCH$_3$), 52.53 (9-OCH$_3$), 51.50 (9′-OCH$_3$); DCI-MS (NH_3) m/z 417 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 3.88 min; Column B methanol−water, 50:20, 3.51 min.

Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-2,3-dihydro-1-benzofuran-5-yl]propanoate (3d) was prepared according to the method of Lemière et al.[2] The reaction was improved by using a Parr apparatus at 60 psi hydrogen pressure for 20 min. Workup was performed identically, resulting in a white powder (81%); mp 97−99 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (s, 1 H, H-6′), 7.70 (s, 1 H, H-2′), 6.97 (dd, J = 8.09, 1.99 Hz, 1 H, H-6), 6.95 (d, J = 1.84 Hz, 1 H, H-2), 6.83 (d, J = 8.09 Hz, 1 H, H-6), 6.05 (d, J = 8.39 Hz, 1 H, H-7), 5.70 (s, 1 H, 4-OH), 4.31 (d, J = 8.39 Hz, 1 H, H-8), 3.98 (s, 3 H, 3′-OCH$_3$), 3.86 (s, 3 H, 3-OCH$_3$), 3.85 (s, 3 H, 9-OCH$_3$), 3.68 (s, 3 H, 9′-OCH$_3$), 2.91 (t, J = 7.75 Hz, 2 H, H-7′), 2.62 (t, J = 7.78 Hz, 2 H, H-8′); ^{13}C NMR ($CDCl_3$) δ 173.16 (C-9′), 171.24 (C-9), 149.66 (C-4), 149.65 (C-3), 146.74 (C-4′), 144.50 (C-3′), 134.37 (C-1′), 133.04 (C-1), 125.51 (C-5′), 118.83 (C-6), 116.76 (C-6′), 113.74 (C-2′), 111.90 (C-5), 110.25 (C-2), 86.72 (C-7), 56.45 (3′-OCH$_3$), 56.23 (C-8), 56.20 (4-OCH$_3$), 56.15 (3-OCH$_3$), 52.52 (9-OCH$_3$), 51.46 (9′-OCH$_3$), 36.17 (C-7′), 30.96 (C-8′); DCI-MS (NH_3) m/z 431 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 4.50 min; Column B methanol−water, 50:20, 4.26 min.

3-[2-(4-Hydroxyphenyl)-3-hydroxymethyl-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol (4a) was prepared from **3a** according to the method of Lemière et al.[2] In the workup the crude green oil was dissolved in a few drops of ethyl acetate and left to stand overnight at 0 °C, resulting in the formation of white crystals (40%); mp 122−125 °C; ^{1}H NMR (acetone-d_6) δ 8.32 (s, 1 H, 4-OH), 7.68 (m, J = 1.40 Hz, 1 H, H-6′), 7.24 (d, J = 8.24 Hz, 2 H, H-2, H-6), 6.95 (dd, J = 8.08, 1.40 Hz, 1 H, C-2′), 6.89 (d, J = 8.08 Hz, 1 H, H-2′), 6.81 (d, J = 8.24 Hz, 2 H, H-3, H-5), 6.69 (d, J = 8.08 Hz, 1 H, H-3′), 5.50 (d, J = 6.10 Hz, 1 H, H-7), 4.37 (m, J = 6.10 Hz, 1 H, H-8), 3.85 (m, J = 10.83, 5.34 Hz, 1 H, H-9a), 3.78 (m, J = 10.83, 7.02 Hz, 1 H, H-9b), 2.89 (s, 2 H, 9-OH, 9′-OH), 2.61 (t, J = 7.98 Hz, 2 H, H-7′), 1.78 (m, J = 7.94, 6.24 Hz, 2 H, H-8′); ^{13}C NMR (acetone-d_6) δ 159.09 (C-4), 157.95 (C-4′), 135.49 (C-5′), 134.45 (C-1), 129.19 (C-2′), 128.87 (C-1′), 127.97 (C-2, C-6), 125.69 (C-6′), 116.06 (C-3, C-5), 109.35 (C-3′), 87.70 (C-7), 81.87 (C-9′), 65.09 (C-9), 54.79 (C-8), 35.99 (C-8′), 32.33 (C-7′); DCI-MS (NH_3) m/z 301(MH^+); DCI-MS (NH_3) m/z 283 (MH^+)−H_2O. HPLC analysis: Column A methanol−water, 65:35, 4.17 min; Column B methanol−water, 60:40, 2.53 min.

3-[2-(3,4-Dihydroxyphenyl)-3-hydroxymethyl-7-hydroxy-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol (4b) was prepared from **3b** according to the method of Lemière et al.[2] In the workup the crude oil was dissolved in 3 mL of ethyl acetate and left to stand overnight at 0 °C, resulting in the formation of white crystals (20%); mp 123 °C; ^{1}H NMR ($CDCl_3$) δ 6.84 (m, 1 H, H-2), 6.72 (m, 2 H, H-5, H-6), 6.60 (s, 1 H, H-6′), 6.56 (s, 1 H, H-2′), 5.53 (d, J = 5.95 Hz, 1 H, H-7), 3.81 (m, J = 10.99, 5.49 Hz, 1 H, H-9a), 3.73 (m, J = 10.99, 7.32 Hz, 1 H, H-9b), 3.56 (t, J = 6.57 Hz, 2 H, H-9′), 2.56 (t, J = 7.47 Hz, 2 H, H-7′), 1.80 (m, J = 7.48, 6.57 Hz, 2 H, H-8′); ^{13}C NMR ($CDCl_3$) δ 146.56 (C-4′), 146.37 (C-3), 146.14 (C-4), 141.76 (C-3′), 136.61 (C-1′), 135.34 (C-1), 129.88 (C-5′), 118.59 (C-6), 116.98 (C-2′), 116.71 (C-6′), 116.22 (C-5), 114.01 (C-2), 88.64 (C-7), 65.26 (C-9), 61.52 (C-9′), 55.68 (C-8), 35.73 (C-8′), 32.70 (C-7′); DCI-MS (NH_3) m/z 333 (MH^+); DCI-MS (NH_3) m/z 315 (MH^+)−H_2O. HPLC analysis: Column A methanol−water, 65:35, 3.01 min; Column B; methanol−water, 60:40, 2.41 min.

3-[2-(4-Hydroxy-3-methoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol (4c) was prepared from **3c** according to the method of Lemière et al.[2] The crude yellow oil was purified by column chromatography (3.8 × 30 cm, silica gel 60, 0.040−0.063 mm) with ethyl acetate−*n*-heptane (2:1) as the eluent, resulting in a light yellow oil (78%): ^{1}H NMR ($CDCl_3$) δ 6.93 (d, J = 1.83 Hz, 1 H, H-2), 6.90 (dd, J = 8.09, 1.83 Hz, 1 H, H-6), 6.86 (d, J = 8.09 Hz, 1 H, H-5), 6.67 (s, 1 H, H-2′), 6.65 (s, 1 H, H-6′), 5.54 (d, J = 7.48 Hz, 1 H, H-7), 3.94 (dd, J = 10.91, 4.42 Hz, 1 H, H-9a), 3.88 (dd, J = 10.91, 6.02 Hz, 1 H, H-9b), 3.87 (s, 1 H, 9-OH), 3.85 (s, 1 H, 9′-OH), 3.68 (t, J = 6.41 Hz, 2 H, H-9′), 3.59 (m, 1 H, H-8), 2.66 (t, 2 H, H-7′), 1.87 (m, 2 H, H-8′); ^{13}C NMR ($CDCl_3$) δ 146.70 (C-3), 149.07 (C-4), 146.62 (C-4′), 145.66 (C-4), 144.20 (C-3′), 135.37 (C-1′), 133.22 (C-1), 127.89 (C-5′), 119.36 (C-6), 116.07 (C-2′), 114.36 (C-5), 112.74 (C-6′), 108.92 (C-2), 87.84 (C-7), 64.04 (C-9), 62.26 (C-9′), 56.11 (3′-OCH_3), 56.01 (3-OCH_3), 53.83 (C-8), 34.57 (C-8′), 31.98 (C-7′); DCI-MS (NH_3) m/z 361(MH^+); DCI-MS (NH_3) m/z 343 (MH^+)−H_2O. HPLC analysis: Column A methanol−water, 65:35, 2.78 min; Column B methanol−water, 60:40, 1.35 min.

3-[2-(3,4-Dimethoxyphenyl)-3-hydroxymethyl-7-methoxy-2,3-dihydro-1-benzofuran-5-yl]propan-1-ol (4d) was prepared from **3d** according to the method of Lemière et al.[2] without further modification (56%). ^{1}H NMR ($CDCl_3$) δ 6.95 (m, 2 H, H-2, H-6), 6.82 (d, J = 8.70 Hz, 1 H, H-5), 6.67 (s, 1 H, H-2′), 6.65 (s, 1 H, H-6′), 5.54 (d, J = 7.17 Hz, 1 H, H-5), 3.93 (dd, J = 10.82, 6.14 Hz, 1 H, H-9b), 3.89 (s, 3 H, 4-OCH_3), 3.87 (dd, J = 10.80, 4.91 Hz, 1 H, H-9a), 3.84 (s, 3 H, 4-OCH_3), 3.67 (t, J = 6.34 Hz, 2 H, H-9′), 3.59 (m, 1 H, H-8), 2.66 (t, 2 H, H-7′), 1.87 (m, 2 H, H-8′); ^{13}C NMR ($CDCl_3$) δ 149.26 (C-3), 149.07 (C-4), 146.62 (C-4′), 144.19 (C-3′), 135.37 (C-1′), 133.92 (C-1), 127.90 (C-5′), 118.64 (C-6), 116.11 (C-6′), 112.78 (C-2′), 111.30 (C-5), 109.63 (C-2), 87.70 (C-7), 64.04 (C-9), 62.22 (C-9′), 56.12 (3′-OCH_3), 55.97 (C-10), 55.99 (C-11), 53.81 (C-8), 34.55 (C-8′), 31.96 (C-7′); DCI-MS (NH_3) m/z 375 (MH^+); DCI-MS (NH_3) m/z 357 (MH^+)−H_2O. HPLC analysis: Column A methanol−water, 65:35, 3.43 min; Column B methanol−water, 50:20, 2.01 min.

Methyl (*E*)-3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-1-benzofuran-5-yl]prop-2-enoate (5d) was prepared from 210 mg (0.49 mmol) of **2d**, 425 mg (1.87 mmol) of DDQ, and 25 mL of anhydrous dioxane. The resulting yellow-green solution was refluxed for 48 h under nitrogen. The mixture was cooled to room temperature, and the resulting light brown precipitate was removed by filtration. The filtrate was concentrated by evaporation and the residue purified by column chromatography (3 × 20 cm, silica gel 60, 0.040−0.063 mm) with dichloromethane. Recrystallization in acetone yielded white crystals (91%): mp 183−185 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, J = 15.86 Hz, 1 H, H-7′), 7.78 (d, J = 1.42 Hz, 1 H, H-6′), 7.74 (d, J = 2.13 Hz, 1 H, H-2), 7.71 (dd, J = 8.54, 2.13 Hz, 1 H, H-6), 7.00 (d, J = 1.37 Hz, 1 H, H-2′), 6.93 (d, J = 8.54 Hz, 1 H, H-5), 6.45 (d, J = 15.86 Hz, 1 H, H-8′), 4.04 (s, 3 H, 3′-OCH_3), 3.98 (s, 3 H, 3-OCH_3), 3.96 (s, 3 H, 4-OCH_3), 3.95 (s, 3 H, 9-OCH_3), 3.83 (s, 3 H, 9′-OCH_3); ^{13}C NMR ($CDCl_3$) δ 167.37 (C-9′), 164.16 (C-9), 161.68 (C-7), 151.19 (C-4), 148.52 (C-3), 145.45 (C-7′), 145.25 (C-3′), 144.08 (C-4′),131.45 (C-1′), 129.37 (C-5′), 123.18 (C-6), 121.65 (C-1), 117.13 (H-8′), 116.06 (C-6′), 112.75 (C-12′), 110.68 (C-5), 108.08 (C-8), 106.06 (C-2′), 56.12 (3-OCH_3), 56.14 (3′-OCH_3), 55.94 (4-OCH_3), 51.64 (9-OCH_3), 51.61 (9′-OCH_3); DCI-MS (NH_3) m/z 427(MH^+). HPLC analysis: Column A methanol−water, 65:35, 8.15 min; Column B methanol−water, 50:10, 6.89 min.

Methyl 3-[2-(3,4-dimethoxyphenyl)-7-methoxy-3-methoxycarbonyl-1-benzofuran-5-yl]propanoate (6d) was prepared from 190 mg (0.44 mmol) of **5d** and 80 mg of 5% Pd/C in 30 mL of THF. The mixture was shaken for 20 min in a Parr apparatus under 60 psi. After filtration and evaporation, an amorphous white powder was obtained which was purified by column chromatography with ethyl acetate−hexane (3:1) (2 × 10 cm, silica gel 60, 0.040−0.063 mm) (90%); ^{1}H NMR ($CDCl_3$) δ 7.74 (d, J = 1.98 Hz, 1 H, H-2), 7.71 (dd, J = 8.54, 1.98 Hz, 1 H, H-6), 7.43 (d, J = 1.42 Hz, 1 H, H-6′), 6.93 (d, J = 9.16 Hz, 1 H, H-5), 6.70 (d, J = 1.38 Hz, 1 H, H-2′), 4.02 (s, 3 H, 3′-OCH_3), 3.96 (s, 3 H, 3-OCH_3), 3.94 (s, 3 H, 4-OCH_3), 3.93 (s, 3 H, 9-OCH_3), 3.70 (s, 3 H, 9′-OCH_3), 3.06 (t, J = 8.09 Hz, 2 H, H-7′), 2.70 (t, J = 8.09 Hz, 2 H, H-8′); ^{13}C NMR ($CDCl_3$) δ 173.33 (C-9′), 164.61 (C-9), 161.15 (C-7), 150.97 (C-4), 148.52 (C-3), 144.82 (C-3′), 141.84 (C-4′), 137.46 (C-1′), 129.13 (C-5′), 123.13 (C-6), 122.21 (C-1), 113.81 (C-6′), 112.81 (C-2), 110.71 (C-5), 108.08 (C-2′), 77.24 (C-8), 56.20 (9′-OCH_3), 56.15 (3-OCH_3), 56.00 (9-OCH_3), 51.62 (3′-OCH_3), 51.55 (4-OCH_3), 36.40 (C-8′), 31.62 (C-7′); DCI-MS (NH_3) m/z 425 (MH^+). HPLC analysis: Column A methanol−water, 65:35, 8.61 min; Column B methanol−water, 50:10, 4.34 min.

Methyl 3-(2-[2-(3,4-dimethoxyphenyl)-1-methoxycarbonyethyl]-3-hydroxy-4-methoxyphenyl)propanoate (7d) is a side product of the hydrogenation of **2d** at prolonged reaction times without the use of a Parr apparatus. It is obtained when a solution of **2d** and Pd/C in ethyl acetate is bubbled through with hydrogen gas for 5 min. The reaction flask is then closed and stirred at room temperature. Reaction times more than 4 h show the appearance of compound **7d**. This product can be isolated by column chromatography using the same conditions as those for the separation of **2d**. The product is obtained as a colorless oil: ^{1}H NMR δ 6.74 (s, 1 H, H-2), 6.72 (s, 1 H, H-6′), 6.63 (m, 3 H, H-2, H-5, H-6), 5.92 (s, 1 H, 4′-OH), 4.24 (br t, J = 8.40, 7.02 Hz, 1 H, H-8), 3.84 (s, 3 H, 3-OCH_3), 3.82 (s, 3 H, 3′-OCH_3), 3.78 (s, 3 H, 4-OCH_3), 3.67 (s, 3 H, 9′-OCH_3), 3.61 (s, 3 H, 9-OCH_3), 3.28 (dd, J = 13.60, 8.40 Hz, 1 H, H-7a), 2.96 (dd, J = 13.60, 6.80 Hz, 1 H, H-7b), 2.84 (t, J = 7.60 Hz, 2 H, H-7′), 2.55 (t, J = 7.60 Hz, 2 H, H-8′); DCI-MS (NH_3) m/z 432 (MH^+).

Cytotoxicity Assays. The cytotoxic activity of test compounds was evaluated in the NCI's in vitro human disease-oriented antitumor screen. This screening panel consists of 60 human tumor cell lines, largely derived from solid tumors, plus some leukemia cell lines. Nine subpanels represent diverse histologies, i.e., nonsmall cell lung, colon, central nervous system, renal, ovarian, prostate, and breast cancers, melanoma, and leukemia. Compounds were tested at a minimum of five concentrations at 10-fold dilutions. Results are evaluated in terms of specificity and potency. The cytotoxic effects of each compound are expressed as the molar drug concentrations required for 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% cell kill (LC_{50}).[17]

Inhibition of Tubulin Polymerization. Details of the purification of tubulin and of the assays of tubulin polymerization and colchicine binding have been previously published.[18] Briefly, effects of test compounds on tubulin polymerization were evaluated in a system consisting of pure bovine brain tubulin (i.e., no microtubule-associated proteins) at 10 μM, in which assembly is induced by 0.8 M glutamate and requires GTP. Drug and tubulin are preincubated in the glutamate for 15 min at 30 °C and chilled on ice, GTP is added, and the assembly reaction started in a recording spectrophotometer with a 0−30 °C jump. After 20 min, the reaction mixtures are chilled to 0 °C to verify the extent of 'normal' assembly (as opposed to paclitaxel-induced assembly or aggregation reactions in which reversibility is generally affected). An IC_{50} value is determined based on the inhibition of the extent of assembly at a series of drug concentrations.

HL-60 human leukemia cells (a generous gift of Dr. T. R. Breitman, National Cancer Institute) were grown and examined for mitotic arrest as described before.[19] Combretastatin A-4 was generously provided by Dr. G. R. Pettit, Arizona State University.

Hollow Fiber Assay. The hollow fiber assay for assessing the potential anticancer activity of test compounds has been described by Hollingshead *et al.*[20] Briefly, human tumor cells were cultivated in polyvinylidene fluoride hollow fibers, and a sample of each cell line was implanted into each of two physiologic compartments (intraperitoneal and subcutaneous) in mice. After treatment with test compounds [**2b**, **2c**, and **2d**(2*R*,3*R*)] at each of two test doses (100 and 150 mg/kg/dose, intraperitoneal administration) using a QD × 4 schedule, fiber cultures were collected and the viable cell mass was deter-

mined using a formazan dye conversion assay. A scoring system was developed to simplify evaluation of the results. The cell lines used were: NCI-H23 and NCI-H522 (lung cancer), SF-295 and U251 (CNS cancer), LOX IMVI and UACC-62 (melanoma), OVCAR-3 and OVCAR-5 (ovarian cancer), COLO 205 and SW-620 (colon cancer), MDA-MB-231 and MDA-MB-435 (breast cancer).

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Supporting Information Available: CD spectra of both enantiomers of **2b** (Figure 2) and/or table of inhibition of in vitro tumor cell growth by synthetic dihydrobenzofuran lignans (Table 1). This material is available free of charge via the Internet at http://pubs.acs.org.

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