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Synthesis and Cytotoxic Activity Against a Non-small-cell Bronchopulmonary Carcinoma Line (NSCLC-N6) of Benzofuran Enantiomeric Derivatives

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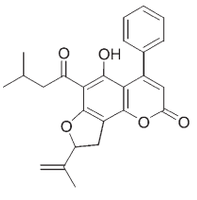
The synthesis of 2-isopropenyl-2,3-dihydrobenzofuranic enantioisomers is described. *Ortho*-(2-hydroxy-3-methylbut-3-enyl)phenol synthons are used as precursors to these structures. *In vitro* antitumor activity against a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6) of these enantioisomers has been investigated.

Keywords: NSCLC-N6; 2-Isopropenyl-2,3-dihydrobenzofuranic; Enantioisomers; Antitumor activity



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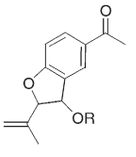
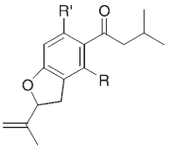


2 R = H, Angelate



3 R = OH, R' = H
4 R = H, R' = OH

INTRODUCTION

As part of our work on natural products isolation, diverse coumarinic compounds were isolated from *Calophyllum dispar*. Among these derivatives, **1**, a new furo[2′,3′:5,6]benzo[1,2-b]pyran-2-one was isolated.[1] This compound demonstrated a significant effect on a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6) (Table I).[2] The 2-isopropenyl-2,3-dihydrobenzofuran structure, characterized in **1**, has been already encountered in other natural products which have exhibited various biological activities.[3–6] Particularly, the tremetone derivatives **2** have showed antitumor activity against P388 cell lines.[7] In that work, we developed a new access to this benzofuran skeleton, in order to evaluate the cytotoxic activity against NSCLC-N6 cell lines of these original simplified molecules (**3**,**4**), related to **1**.

Methods for synthesizing such 2-isopropenyl-2,3-dihydrobenzofuranic structure involved two principal strategies. Whereas the first one yielded the desired structure in a one-step reaction,[8,9] the second involved synthesis of the benzofuran skeleton prior to formation of the isopropenyl substituent.[10,11]

We recently described a photo-oxygenation-reduction sequence as a straightforward access to *ortho*-(2-hydroxy-3-methylbut-3-enyl)phenols without the need for any phenolic protecting group.[12] In that work, we turned to our advantage the thermal instability of tertiary allylic hydroperoxyde intermediates in order to obtain secondary allylic alcohol derivatives as the sole oxidation products. As these derivatives contained the required functionalities, we considered that they could be the key intermediates in the synthesis of 2-isopropenyl-2,3-dihydrobenzofuranic structures *via* the Mitsunobu reaction.[13,14]

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TABLE I Antitumor activity against a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6) of **1**, **3a**, **3b** and **4a**, **4b**

Compd	IC_{50} (μg/ml)
(±)-1	3.3
(−)-3a	na*
(+)-3b	na
(−)-4a	15.0
(+)-4b	30.0

*na (no active).

MATERIALS AND METHODS

Melting points were determined on an Electrothermal 8100 melting point apparatus and are uncorrected. ^1H, ^{13}C NMR spectra experiments were recorded on a Jeol GSX WB 270 MHz and on a Bruker Avance DRX 500 MHz instruments using tetramethylsilane as the internal standard. IR spectra were recorded on a Bruker FT IR Vector 22 using potassium bromide disc for solids or neat liquid films for liquids. HREIMS (70 eV) were recorded on a Varian MAT 311 spectrometer.

Chemistry

We describe below each typical procedures used to obtain the described compounds.

Acylation

Aluminium trichloride (19.4 g, 4 eq.) was added to a stirred suspension of 1,3-dihydroxybenzene (resorcinol, 4 g, 36.4 mmol.) in carbon disulfide (34 ml). Nitromethane (18 ml) was then added over 30 min forming a homogeneous solution with evolution of hydrogen chloride. The solution was heated under reflux for 30 min, 5.26 g of isovaleryl chloride (1.2 eq.) in 5 ml nitromethane was then added over 30 min, and the solution was heated under reflux for a further 30 min before being allowed to cool with stirring. The mixture was poured onto ice-water (100 ml) and hydrochloric acid (3 M, 50 ml) was added. The aqueous layer was extracted with dichloromethane (2 × 70 ml) and AcOEt (2 × 70 ml). The combined organic layers were dried over sodium sulfate, filtered and evaporated under reduced pressure. The residue was chromatographed on silica gel column eluting with cyclohexane/AcOEt (75/25).

1-(2,4-Dihydroxyphenyl)-3-methylbutan-1-one **5**

Yield = 18%. (orange powder). Mp = 72–73°C. IR (cm^{-1}): 3359, 2959, 1628, 1264, 794. ^1H NMR ($CDCl_3$), δ (ppm): 1.01 (d, 6.5 Hz, 6H, CH_3), 2.27 (m, 1H, CH), 2.76 (d, 6.5 Hz, 2H, CH_2), 6.03 (s, OH), 6.38–6.42 (m, 1H, H arom.), 6.38–6.42 (m, 1H, H arom.), 7.66 (d, 9 Hz, 1H, H arom.), 12,98 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 22.7 (CH_3), 25.9 (CH), 46.9 (CH_2), 103.6 (CH arom.), 107.6 (CH arom.), 114.2, 132.6 (CH arom.), 162.4, 165.3, 205.0 (CO). HRMS (EI +): $M^+ = 194.0952$ (theoret. 194.0943).

Prenylation

To a dioxane solution (25 ml) of 1-(2,4-dihydroxyphenyl)-3-methylbutan-1-one **5** (5 g, 25.7 mmol) was added 1.07 ml (0.3 eq) of boron trifluoride etherate. Then, a dioxane solution (15 ml) of 2-methylbut-3-en-2-ol (2.7 ml, 1.2 eq) was added dropwise over a period of 50 min. The mixture was stirred for 3 days. An aqueous solution (15%, 30 ml) of sodium acetate was then added and the reaction mixture was concentrated under reduced pressure. Ethyl acetate (50 ml) was added and the organic phase was washed four times with a sodium acetate solution in water (15%, 55 ml), dried over sodium sulfate and filtered. A viscous crude oil was obtained after solvent removal. The mixture was then separated using liquid chromatography (silica, cyclohexane/AcOEt: 80/20).

1-[2,4-Dihydroxy-3-(3-methylbut-2-enyl)phenyl]-3-methylbutan-1-one **6**

Yield = 7%. (white powder). Mp = 86–87°C. IR (cm^{-1}): 3414, 1624, 1292, 1088, 792. ^1H NMR ($CDCl_3$), δ (ppm): 1.00 (d, 6.5 Hz, 6H, CH_3), 1.77 (s, 3H, CH_3), 1.84 (s, 3H, CH_3), 2.27 (m, 1H, CH), 2.76 (d, 7 Hz, 2H, CH_2), 3.45 (d, 7 Hz, 2H, CH_2), 5.38 (t, 7 Hz, 1H, CH), 6.02 (s, 1H, OH), 6.36 (d, 9 Hz, 1H, H_5), 7.57 (d, 9 Hz, 1H, H_6), 13.37 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 17.9 (CH_3), 21.7 (CH_2), 22.8 (CH_3), 25.8 (CH_3), 26.0 (CH), 46.8 (CH_2), 107.6 (C_5H), 113.7, 113.9, 121.0 (CH), 129.8 (C_6H), 135.9, 161.3, 162.8, 205.1 (CO). HRMS (EI +): $M^+ = 262.1541$ (theoret. 262.1568).

1-[2,4-Dihydroxy-5-(3-methylbut-2-enyl)phenyl]-3-methylbutan-1-one **7**

Yield = 22%. (white powder). Mp = 92–93°C. IR (cm^{-1}): 3263, 1628, 1352, 1243, 862. ^1H NMR ($CDCl_3$), δ (ppm): 1.01 (d, 6.5 Hz, 6H, CH_3), 1.79 (s, 3H, CH_3), 1.80 (s, 3H, CH_3), 2.26 (m, 1H, CH), 2.75 (d, 6.5 Hz, 2H, CH_2), 3.31 (d, 7 Hz, 2H, CH_2), 5.30 (t, 7 Hz, 1H, CH), 5.75 (s, 1H, OH), 6.37 (s, 1H, H_3), 7.46 (s, 1H, H_6), 12,76 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 17.9 (CH_3), 22.8 (CH_3), 25.8 (CH_3), 25.9 (CH), 29.0 (CH_2), 46.9 (CH_2), 103.9 (C_3H), 113.9, 118.5, 121.4 (CH), 131.7 (C_6H), 135.5, 161.3, 163.8, 204.8 (CO). HRMS (EI +): $M^+ = 262.1564$ (theoret. 262.1568).

Photo-oxidation-reduction Sequence

Dried air was bubbled through a solution of diphenolic prenylated compound (200 mg, 0.75 mmol) in dichloromethane (50 ml) containing tetraphenylporphine (6 mg, 0.009 mmol). The reaction mixture was water-cooled at 15°C and

irradiated with a halogen lamp (500 W) for 1.5 h. Triphenylphosphine (220 mg, 1.1 eq) was then added and the solution was stirred overnight at room temperature. After evaporation, the residue was chromatographed over silica gel, eluted with cyclohexane/AcOEt (75/25).

1-[2,4-Dihydroxy-3-(2-hydroxy-3-methylbut-3-enyl)phenyl]-3-methylbutan-1-one **8**

Yield = 75%. (yellow powder). Mp = 83–84°C. IR (cm^{-1}): 3442, 1620, 1370, 1093. ^1H NMR ($CDCl_3$), δ (ppm): 1.00 (d, 6.5 Hz, 6H, CH_3), 1.84 (s, 3H, CH_3), 2.24 (m, 1H, CH), 2.74 (dd, 1.5 Hz, 7 Hz, 2H, CH_2), 2.82 (dd, 8 Hz, 15 Hz, 1H, CH_2), 3.15 (dd, 2 Hz, 15 Hz, 1H, CH_2), 4.35 (dd, 2 Hz, 8 Hz, 1H, C*H*OH), 4.85 (br s, 1H, CH_2), 4.99 (br s, 1H, CH_2), 6.45 (d, 8.5 Hz, 1H, H_5), 7.56 (d, 8.5 Hz, 1H, H_6), 13.35 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 18.5 (CH_3), 22.8 (CH_3), 26.0 (CH), 28.4 (CH_2), 46.8 (CH_2), 77.6 (*C*HOH), 108.9 (C_5H), 110.4 (CH_2), 113.0, 113.4, 130.5 (C_6H), 146.8, 163.1, 163.3, 205.1 (CO). HRMS (EI +): M^+ = 278.1501 (theoret. 278.1518).

1-(5-Hydroxy-2,2-dimethyl-2*H*-chromen-6-yl)-3-methylbutan-1-one **9**

Yield = 16%. (amorphous mass). IR (cm^{-1}): 3419, 2959, 1613, 1322, 1113. ^1H NMR (CD_3OD), δ (ppm): 0.98 (d, 6.5 Hz, 6H, CH_3), 1.42 (s, 6H, CH_3), 2.21 (m, 1H, CH), 2.77 (d, 7 Hz, 2H, CH_2), 5.64 (d, 10 Hz, 1H, CH), 6.32 (d, 8.5 Hz, 1H, H_5), 6.67 (d, 10 Hz, 1H, CH), 7.66 (d, 8.5 Hz, 1H, H_6). ^{13}C NMR (CD_3OD), δ (ppm): 23.0 (CH_3), 27.1 (CH), 28.5 (CH_3), 47.6 (CH_2), 78.7, 109.3 (C_5H), 110.2, 114.9, 116.5 (CH), 129.4 (CH arom.), 132.9 (CH), 160.7, 160.8, 206.8 (CO).

1-[2,4-Dihydroxy-5-(2-hydroxy-3-methylbut-3-enyl)phenyl]-3-methylbutan-1-one **10**

Yield = 61%. (white powder). Mp = 82–83°C. IR (cm^{-1}): 3441, 1635, 1373, 1183. ^1H NMR ($CDCl_3$), δ (ppm): 1.00 (d, 6.5 Hz, 6H, CH_3), 1.82 (s, 3H, CH_3), 2.26 (m, 1H, CH), 2.72–2.76 (m, 1H, CH_2), 2.72–2.76 (m, 2H, CH_2), 2.92 (dd, 8 Hz, 15 Hz, 1H, CH_2), 4.40 (dd, 2 Hz, 8 Hz, 1H, C*H*OH), 4.91 (br s, 1H, CH_2), 5.01 (br s, 1H, CH_2), 6.46 (s, 1H, H_3), 7.38 (s, 1H, H_6), 9.09 (br s, 1H, OH), 12,74 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 18.2 (CH_3), 22.8 (CH_3), 25.7 (CH), 37.7 (CH_2), 46.6 (CH_2), 77.9 (*C*HOH), 105.2 (C_3H), 111,7 (CH_2), 113.7, 117.2, 133.4 (C_6H), 145.9, 163.2, 164.4, 204.5 (CO). HRMS (EI +): M^+ = 278.1500 (theoret. 278.1518).

1-[2,4-Dihydroxy-5-(3-hydroxy-3-methylbut-1-enyl)phenyl]-3-methylbutan-1-one **11**

Yield = 21%. (yellow powder). Mp = 138–140°C. IR (cm^{-1}): 3415, 2962, 1634, 1376. ^1H NMR (CD_3OD), δ (ppm): 1.00 (d, 7 Hz, 6H, CH_3), 1.39 (s, 6H, CH_3), 2.23 (m, 1H, CH), 2.82 (d, 7 Hz, 2H, CH_2), 6.28 (s, 1H, H_3), 6.35 (d, 16 Hz, 1H, CH), 6.74 (d, 16 Hz, 1H, CH), 7.83 (s, 1H, H_6). ^{13}C NMR (CD_3OD), δ (ppm): 23.0 (CH_3), 27.0 (CH), 30.0 (CH_3), 47.5 (CH_2), 71.7, 103.5 (C_3H), 114.4, 118.9, 121.7 (CH), 130.7 (C_6H), 137.8 (CH), 163.7, 165.3, 206.4 (CO).

Intramolecular Cyclisations Using the Mitsunobu Reaction

At 0°C and under N_2, triphenylphosphine (283 mg, 2.5 eq) was added to a THF solution (45 ml) containing the *ortho*-(2-hydroxy-3-methylbut-3-enyl)phenol derivative (120 mg, 0.43 mmol). A solution of diethyl azodicarboxylate (0.17 ml, 2.5 eq) in THF (3 ml) was added and the resulting mixture was stirred overnight at room temperature. After evaporation of the solvent, the oily residue was chromatographed (silica, cyclohexane/AcOEt: 96/4).

1-(4-Hydroxy-2-isopropenyl-2,3-dihydro-1-benzofuran-5-yl)-3-methylbutan-1-one **3a,b**

Yield = 52%. (amorphous mass). IR (cm^{-1}): 1644, 1618, 1307, 1246, 1087. ^1H NMR ($CDCl_3$), δ (ppm): 1.01 (d, 6.5 Hz, 6H, CH_3), 1.77 (s, 3H, CH_3), 2.27 (m, 1H, CH), 2.75 (d, 7 Hz, 2H, CH_2), 3.00 (dd, 7.5 Hz, 15.5 Hz, 1H, CH_2), 3.35 (dd, 10 Hz, 15.5 Hz, 1H, CH_2), 5.32 (dd, 7.5 Hz, 10 Hz, 1H, CH), 4.94 (br s, 1H, CH_2), 5.09 (br s, 1H, CH_2), 6.40 (d, 9 Hz, 1H, H_5), 7.63 (d, 9 Hz, 1H, H_6), 13.01 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 17.0 (CH_3), 22.8 (CH_3), 26.0 (CH), 31.0 (CH_2), 47.0 (CH_2), 88.0 (CH), 101.7 (C_5H), 112,5 (CH_2), 113.0, 114.7, 132.5 (C_6H), 143.3, 160.6, 166.7, 205.0 (CO).

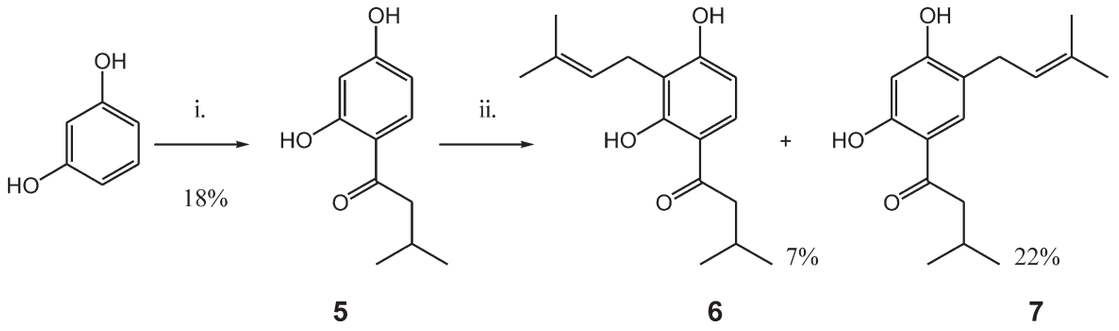
Enantioisomeric separation was achieved with a Chiralpack AD column, eluting with a hexane/isopropanol mixture (99.2/0.8):—enantioisomer ***3a***: retention time = 7.01 min, purity: 99.2% (0.8% of enantioisomer ***3b***), $[\alpha]_D = -78.4°$ ($c = 1.02 \times 10^{-3}$, $CHCl_3$);—enantioisomer ***3b***: retention time = 7.85 min, purity: 97.4% (2.6% of enantioisomer ***3a***), $[\alpha]_D = 73.2$ ($c = 0.82 \times 10^{-3}$, $CHCl_3$).

1-(6-Hydroxy-3-methyl-2,5-dihydro-1-benzoxepin-7-yl)-3-methylbutan-1-one **12**

Yield = 9%. ^1H NMR ($CDCl_3$), δ (ppm): 1.01 (d, 6.5 Hz, 6H, CH_3), 1.63 (s, 3H, CH_3), 2.27 (m, 1H, CH), 2.79 (d, 7.5 Hz, 2H, CH_2), 3.52 (d, 5.5 Hz, 2H, CH_2), 4.52 (br s, 2H, CH_2), 5.71 (t, 5.5 Hz, 1H, CH), 6.54 (d, 8.5 Hz, 1H, H arom.), 7.59 (d, 8.5 Hz, 1H, H arom.), 13.20 (s, 1H, OH).

1-(6-Hydroxy-3-methyl-2,5-dihydro-1-benzoxepin-9-yl)-3-methylbutan-1-one **13**

Yield = 21%. (amorphous mass). IR (cm^{-1}): 3327, 1647, 1588, 1292, 1066. ^1H NMR ($CDCl_3$), δ (ppm): 0.97 (d, 6.5 Hz, 6H, CH_3), 1.55 (s, 3H, CH_3), 2.27 (m, 1H, CH), 2.85 (d, 7 Hz, 2H, CH_2), 3.46 (d, 6 Hz, 1H, CH_2), 4.47 (br s, 2H, CH_2), 5.62 (m, 1H, CH), 5.76 (s, 1H, OH), 6.61 (d, 8.5 Hz, 1H, H_5), 7.50 (d, 8.5 Hz, 1H, H_6).^{13}C NMR ($CDCl_3$), δ (ppm): 19.7 (CH_3), 21.6 (CH_2), 22.7 (CH_3), 25.0 (CH), 51.8 (CH_2), 74.1 (CH_2), 111.7 (C_5H), 120.1 (CH), 124.5, 125.5, 128.6 (C_6H), 133.8, 155.8, 159.5, 205.1 (CO).



5 **6** **7**

SCHEME 1 i. $AlCl_3$, isovaleryl chloride, CS_2/CH_3NO_2 ii. 2-methylbut-3-en-2-ol, $BF_3{\cdot}Et_2O$, dioxane.

1-(6-Hydroxy-2-isopropenyl-2,3-dihydro-1-benzofuran-5-yl)-3-methylbutan-1-one **4a,b**

Yield = 61%. (amorphous mass). IR (cm^{-1}): 1635, 1483, 1228. ^1H NMR ($CDCl_3$), δ (ppm): 1.01 (d, 6.5 Hz, 6H, CH_3), 1.76 (s, 3H, CH_3), 2.27 (m, 1H, CH), 2.74 (d, 7 Hz, 2H, CH_2), 2.97 (dd, 7.5 Hz, 15 Hz, 1H, CH_2), 3.30 (dd, 9.5 Hz, 15 Hz, 1H, CH_2), 5.26 (dd, 7.5 Hz, 9.5 Hz, 1H, CH), 4.94 (br s, 1H, CH_2), 5.08 (br s, 1H, CH_2), 6.37 (s, 1H, H_3), 7.51 (s, 1H, H_6), 13.25 (s, 1H, OH). ^{13}C NMR ($CDCl_3$), δ (ppm): 17.0 (CH_3), 22.8 (CH_3), 25.9 (CH), 33.2 (CH_2), 46.9 (CH_2), 87.6 (CH), 98.2 (C_3H), 112,7 (CH_2), 113.7, 118.4, 126.2 (C_6H), 143.1, 166.1, 166.4, 204.2 (CO). HRMS (EI +): $M^+ = 260.1419$ (theoret. 260.1412).

Enantioisomeric separation was achieved with a Chiralpack AD column, eluting with a hexane/isopropanol mixture (99/1):—enantioisomer ***4a***: retention time = 7.44 min, purity: 97.2% (2.8% of enantioisomer ***4b***), $[\alpha]_D = -30.3°$ ($c = 1.98 \times 10^{-3}$, $CHCl_3$);—enantioisomer ***4b***: retention time = 7.97 min, purity: 95.1% (4.9% of enantioisomer ***4a***), $[\alpha]_D = 26.9°$ ($c = 1.16 \times 10^{-3}$, $CHCl_3$).

Pharmacology

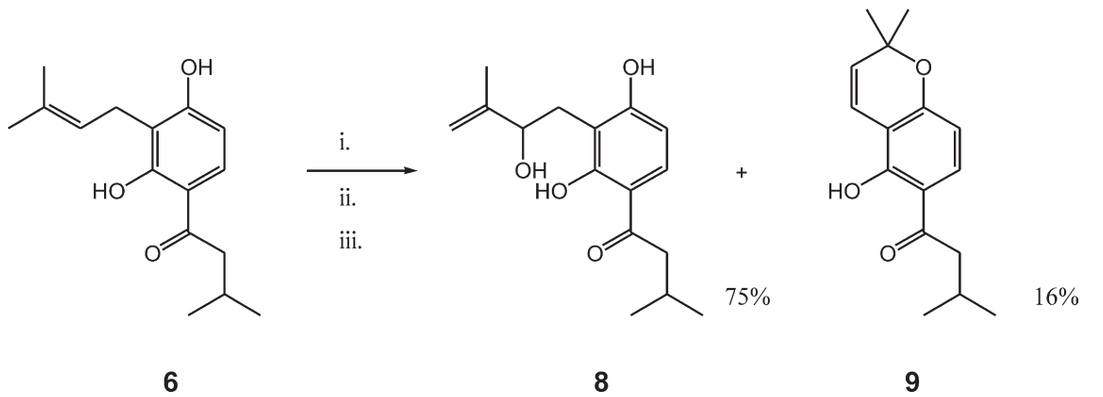
Cell Line and Cell Culture

The NSCLC-N6 line, derived from a human non-small-cell bronchopulmonary carcinoma (moderately differentiated, rarely keratinizing, and classified as $T_2N_0M_0$) was used for all experiments.[2] The cells were cultured in RPMI 1640 medium (Intermed) with 5% fetal calf serum to which were added 100 IU penicillin/ml, 100 μg streptomycin/ml and 2 mM glutamine. Cells (4×10^4/ml) were cultured at 37°C in a 95% air, 5% CO_2 atmosphere.

A 50-μL aliquot of the cell culture [NSCLC-N6 cells (2×10^4)] was mixed with a 50-μL aliquot of serial dilution of compounds and the mixture was incubated in a microtiter well plate (96-well Flacon 3072) for 72 h at 37°C in a humified incubator containing 5% CO_2 in air.

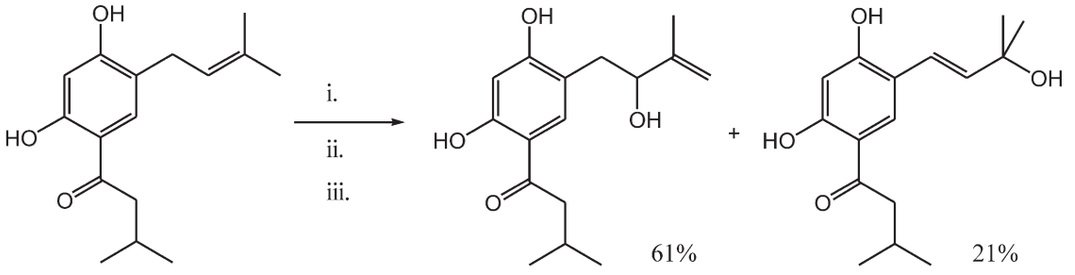
Cytotocity Determination

Continuous drug exposure: the experiment was performed in microwells using 2×10^5 cells/ml. 2-Isopropenyl-2,3-dihydrobenzofuran derivatives were tested at concentrations of 10, 3, 1, and 0.1 μg/ml. Cell growth was evaluated by a colorimetric test: 10 μL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma) was added. After 4 h the dark blue crystals of formazan, formed in mitochondria of living cells during the reduction of MTT[15], were solubilized with 100 μL of isopropanoic acid. Microplates were read by ELISA



6 **8** **9**

SCHEME 2 i. hν, CH_2Cl_2, TPP ii. PPh_3, CH_2Cl_2 iii. SiO_2: cyclohexane/ethyl acetate mixture (75/25).



SCHEME 3 i. hν, CH_2Cl_2, TPP ii. PPh_3, CH_2Cl_2 iii. SiO_2: cyclohexane/ethyl acetate mixture (75/25).

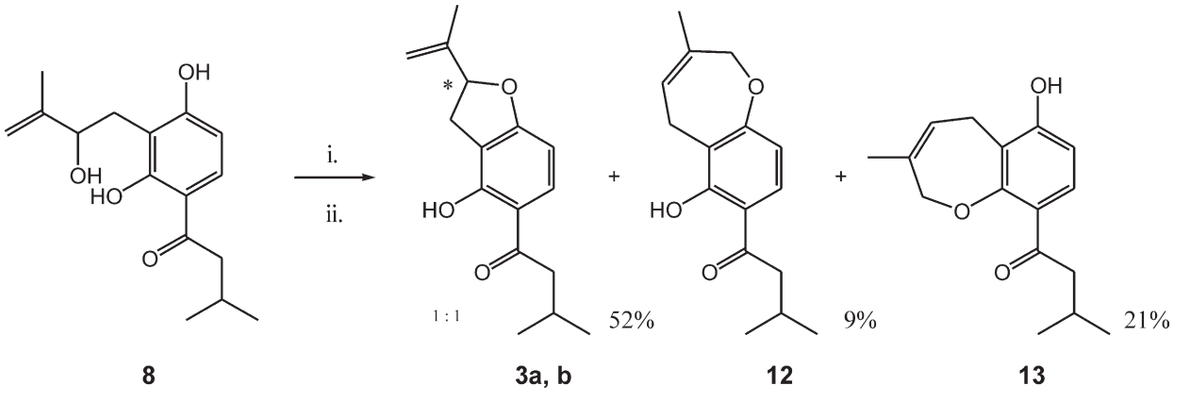
using a multiskar Titertek multiscan MK2 with a 570-nm filter.

RESULTS AND DISCUSSION

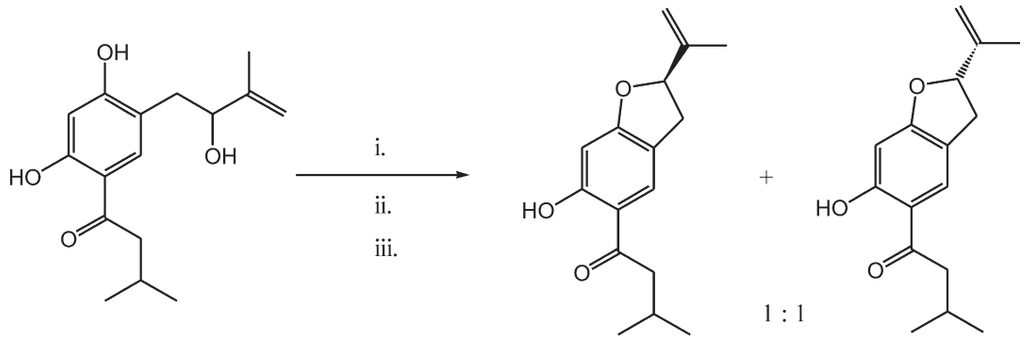
The Friedel-Crafts acylation of resorcinol (1,3-dihydroxybenzene) led to **5** in 82% yield. Isoprenylation of the diphenolic intermediate **5** yielded the two monoprenylated derivatives **6** and **7** in 7% and 22% respectively[16] (Scheme 1)

The photo-oxidation-reduction sequence, achieved at 15°C, furnished the secondary allylic alcohols **8** and **10** as the major products, from **6** and **7** respectively (Schemes 2 and 3). Unexpectedly, the ^{1}H NMR analysis of each reaction mixture showed the presence of the corresponding tertiary allylic alcohols. These results could be explained by a better stability of the hydroperoxide intermediates at 15°C. Liquid chromatography on silica gel allowed us to isolate the benzopyran derivative **9** in 16% yield. This compound resulted from an intramolecular cyclisation involving the 3-methyl-3-hydroxybut-1-enyl appendage and an *ortho*-phenolic group. The tertiary allylic alcohol **11** was found to be more stable on silica gel and was obtained in 21% yield.

The Mitsunobu reaction applied to **8** led to a mixture of three compounds **3**, **12** and **13** (Scheme 4). Under chromatographic conditions (SiO_2, cyclohexane/AcOEt: 96/4), **3** and **12**, as a 6/1 ratio mixture determined by ^{1}H NMR analysis, and **13** were obtained in 61% and 21% yield respectively. The 2-isopropenyl-2,3-dihydrobenzofuranic derivative **3** was isolated by further chromatographic purification from the mixture (silica Kromasil 10 μm, cyclohexane/CH_2Cl_2: 70/30). By doing so, **12** was never purified and its structure was determined by ^{1}H NMR spectrum analogy with **13**. The benzoxepine structure of **13** was elucidated by means of combined analytical methods including HREIMS, 1D and 2D NMR spectroscopy. The benzoxepine compounds **12** and **13** were formed *via* a minor S_N2' mechanism and were differentiated by the nature of the phenolic oxygen involved in the nucleophilic attack. Such benzoxepine structures were previously synthesized from primary allylic alcohol using the Mitsunobu reaction.[17] In that work, the authors considered this synthesis as a biomimetic approach to this kind of skeleton.



SCHEME 4 i. PPh_3, DEAD, THF, rt, 15 h ii. SiO_2: cyclohexane/ethyl acetate mixture (96/4).



SCHEME 5 i. PPh_3, DEAD, THF, rt, 15 h (61%) ii. SiO_2: cyclohexane/ethyl acetate mixture (96/4) (61%) iii. Chiral HPLC.

The 2-isopropenyl-2,3-dihydrobenzofuranic derivative **4** was obtained in 61% yield, as the sole product, from the alcohol **10**, under Mitsunobu conditions (Scheme 5).

Chiral HPLC purification (see experimental) was achieved and enantioisomers of **3** and **4** were separated. The antitumor activities of **1**, **3a** and **3b**, **4a** and **4b** derivatives were evaluated against a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6) (Table I). This biological study showed that the lack of the coumarin skeleton decreased the antitumor activity. These results also demonstrated a marked difference between the value of the IC_{50} of each enantioisomer **4a** and **4b**.

In conclusion, we have shown that 2-isopropenyl-2,3-dihydrobenzofuran structures could be synthesized from secondary allylic alcohols under Mitsunobu conditions. Our three-step strategy, involving *ortho*-prenylphenol derivatives as starting material, could be transposed to the synthesis of more complex natural products. The formation of 3-methyl-2,5-dihydrobenzoxepine derivatives was also observed. These results suggested that *ortho*-(2-hydroxy-3-methylbut-3-enyl)phenol compounds could be considered as biogenetic precursors of both benzofuran and benzoxepine structures. In this work, we also noted the formation of 2,2-dimethylbenzopyran derivatives from the *ortho*-prenylphenol appendage *via ortho*-(3-hydroxy-3-methylbut-1-enyl)phenol compounds. This result outlined a hypothetical biogenetic pathway leading to this kind of benzopyran structure.

The biological study of enantioisomers **3a** and **3b**, **4a** and **4b** showed that the presence of the lactone ring is responsible for the better antitumor activity of **1**. This study also demonstrated a difference between the IC_{50} values for both enantiomers of **4**. With these results in mind, we now wish to achieve the chiral HPLC separation of **1**, aiming at improving the antitumor potential of this derivative, isolated as a natural racemic mixture.

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