2-Arylbenzofuran Derivatives from *Morus cathayana*

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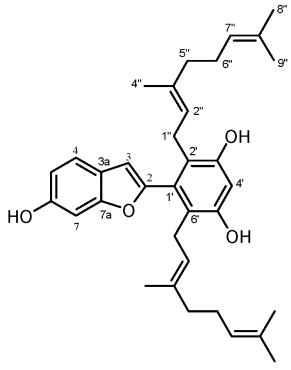
Four new 2-arylbenzofuran derivatives, cathafurans A (**1**), B (**2**), C (**3**), and D (**4**), were isolated from the stem bark of *Morus cathayana*. Their structures were determined by spectroscopic methods. Compounds **2** and **3** exhibited moderate activities against five human cancer cell lines, with IC_{50} values ranging from 6.17 to 9.60 μg/mL.

Morus cathayana Hemsley (Moraceae) root bark has been used in Chinese traditional medicine as a treatment for diabetes, arthritis, and rheumatism.[1] The constituents of its bark have been studied by many investigators, and a series of isoprenylated phenols have been isolated. Some of these phenolic compounds showed significant antimicrobial,[2] antioxidative,[3] anti-inflammation,[3] and cytotoxic activities.[4] Additional phytochemical investigation of an EtOH extract of *M. cathayana* bark has resulted in the isolation and structure elucidation of four new 2-arylbenzofuran derivatives (**1**−**4**), and evaluation of their cytotoxicity is described herein.



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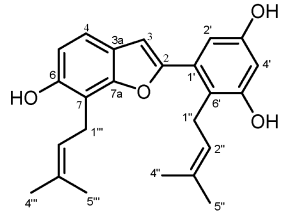

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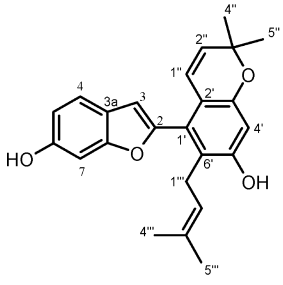

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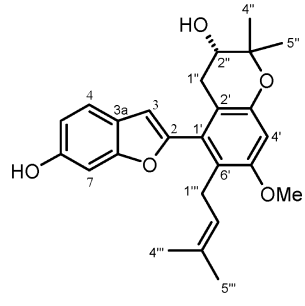

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Cathafuran A (**1**) was obtained as yellowish, amorphous powder and showed a positive reaction to the ferric chloride test. The molecular formula was determined to be $C_{34}H_{42}O_4$ by HRFABMS ($[M]^+$ at *m*/*z* 514.3096). The UV spectrum exhibited absorption maxima at 207, 253 (sh) and 295 nm and was similar to those of lakoochin B,[5] suggesting that **1** was a derivative of 2-arylbenzofuran. Its IR spectrum disclosed absorption bands assignable to OH (3407 cm^{-1}) and benzene ring (1624, 1598, and 1488 cm^{-1}) moieties. The ^1H NMR spectrum of **1** displayed the following signals: one set of ABX aromatic protons at δ 7.41 (1H, d, J = 8.0 Hz, H-4), 6.81 (1H, dd, J = 8.0, 2.0 Hz, H-5), and 6.95 (1H, d, J = 2.0 Hz, H-7), two singlets at δ 6.54 (1H, s, H-3) and 6.60 (1H, s, H-4′), which were assignable to a 2-arylbenzofuran moiety, and proton resonances corresponding to two geranyl groups at δ 1.35 (6H, s, H-4″), 1.53, 1.56, 1.57, 1.62 (each 3H, s, H-9″ and 10″), 1.86, 1.93 (each 2H, t, J = 7.5 Hz, H-5″), 1.97−2.04 (4H, m, H-6″), 3.14, 3.16 (each 2H, d, J = 8.5 Hz, H-1″), and 5.05−5.15 (4H, m, H-2″ and H-7″). The ^{13}C NMR spectrum (Table 1) revealed the presence of 34 carbons, which were similar to those of lakoochin B[5] except for the signals of geranyl groups. In the HMBC spectrum, the long-range correlations of H-1″/C-1′ and H-2″/C-2′,6′ suggested that two geranyl groups were connected to C-2′ and C-6′. Two symmetrical geranyl groups inhibited rotation of the phenyl moiety, which resulted in differences of their ^1H and ^{13}C NMR signals. The correlation of H-1″/H-4″ in the NOESY spectrum (Figures S1 and S2, Supporting Information) indicated geometry of the C-2″/3″ double bond in **1**. Assignments of all ^1H and ^{13}C NMR signals were accomplished by a combination of NOESY, HMQC, and HMBC spectra. Based on the above analysis and literature values,[5] the structure of cathafuran A (**1**) was determined to be as shown.

The UV spectrum of cathafuran B (**2**) exhibited absorption maxima at 204 and 309 nm, which were similar to those of mulberrofuran D[6] and suggested that **2** was probably a 2-arylbenzofuran derivative. Its IR spectrum had absorption bands indicating hydroxyl (3377 cm^{-1}) and benzene ring (1618, 1491 cm^{-1}) moieties. The HRFABMS spectrum of **2**, with a molecular ion at *m*/*z* 378.1824, indicated a molecular formula of $C_{24}H_{26}O_4$. The ^1H NMR spectrum of **2** exhibited the following proton signals: two pairs of doublets of aromatic protons at δ 6.79 (1H, d, J = 8.0 Hz, H-5), 7.29 (1H, d, J = 8.0 Hz, H-4), 6.43 (1H, d, J = 2.5 Hz, H-4′), and 6.77 (1H, d, J = 2.5 Hz, H-6′) and a singlet at δ 6.71 (1H, s, H-3), which were assignable to a 2-arylbenzofuran moiety, and 18 aliphatic proton resonances corresponding to two prenyl groups at δ 1.86 and 1.79 (each 3H, s, H-4″ or H-4‴), 1.77 (6H, s, H-5″ and 5‴), 3.53 (2H, d, J = 7.0 Hz, H-1″), 3.68 (2H, d, J = 7.0 Hz, H-1‴), 5.30 (1H, br t, J = 7.0 Hz, H-2″), and 5.40 (1H, br t, J = 7.0 Hz, H-2‴). The ^{13}C NMR spectrum (Table 1) revealed the presence of 24 carbons, which were similar to those of mulberrofuran D[6] except for the signals of prenyl groups. Long-range correlations of H-1″/C-6,7a and H-1″/C-1′,3′ indicated that the prenyl groups were connected to C-7 and C-2′, respectively. Analysis of the HMBC and HSQC spectra led to complete assignments of the proton and carbon signals in compound **2**. On the basis of the above analysis and literature values,[6] the structure of **2** was determined to be as shown in Figure 3, Supporting Information.

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Table 1. ^{13}C NMR Data (δ) of Compounds **1**−**4**

position	**1**[a]	**2**[a]	**3**[a]	**4**[b]
2	154.3	155.3	152.5	152.7
3	106.8	105.8	107.9	106.3
3a	122.4	122.5	122.0	122.3
4	121.5	118.8	121.8	121.0
5	112.7	112.9	112.9	111.8
6	156.0	153.3	156.2	153.3
7	98.5	112.1	98.3	98.3
7a	156.8	155.2	156.9	155.7
1′	131.5	132.9	129.5	131.7
2′	121.0	118.6	114.7	110.8
3′	154.4	157.5	153.0	151.4
4′	104.7	103.8	105.1	101.3
5′	154.4	156.8	156.6	157.2
6′	121.0	107.8	121.8	124.0
1″	27.0, 27.1	26.3	121.2	29.8
2″	125.2	125.4	128.5	69.8
3″	133.2, 135.2	130.9	76.2	76.6
4″	16.1	18.0	28.0	22.0
5″	40.4, 40.5	26.3	28.0	24.8
6″	27.3, 27.4			
7″	125.2			
8″	133.2, 129.9			
9″	17.7			
10″	25.8			
1‴		23.4	26.8	26.6
2‴		123.3	124.7	123.6
3‴		131.9	130.4	130.6
4‴		18.0	17.8	17.7
5‴		26.3	25.8	25.8

[a] In acetone-d_6. [b] In $CDCl_3$.

Cathafuran C (**3**) was obtained as a yellow powder. The UV spectrum exhibited absorption maxima at 208, 250 (sh), 274 and 299 nm, which were similar to those of **1**, suggesting that **3** was probably a derivative of 2-arylbenzofuran. Its IR spectrum disclosed absorption bands for OH (3403 cm^{-1}) and benzene ring (1624, 1599, and 1505 cm^{-1}) moieties. The HRFABMS of **3** indicated a molecular formula of $C_{24}H_{24}O_4$ with a pseudomolecular ion at *m/z* 377.1743 $[M + H]^+$. The ^{1}H NMR spectrum of **3** exhibited the following proton signals: one set of ABX aromatic protons at δ 6.97 (1H, d, J = 2.4 Hz, H-7), 6.84 (1H, dd, J = 8.4, 2.4 Hz, H-5), and 7.45 (1H, d, J = 8.4 Hz, H-4), as well as a singlet at δ 6.64 (1H, s, H-3), which were assignable to a benzofuran moiety; a pair of doublets at δ 5.48 (1H, d, J = 10.0 Hz, H-2″) and 6.08 (1H, d, J = 10.0 Hz, H-1″) and three signals at δ 1.37 (6H, s, H-4″ and 5″) and 6.46 (1H, s, H-4′), which were assignable to a 2,2-dimethylchromene moiety; and proton resonances attributable to a prenyl group at δ 1.40 (3H, s, H-4‴), 1.54 (3H, s, H-5‴), 3.22 (2H, d, J = 6.8 Hz, H-1‴), and 5.40 (1H, br t, J = 6.8 Hz, H-2‴). The ^{13}C NMR spectrum (Table 1) showed signals for 24 carbons. All ^{1}H and ^{13}C NMR signals were assigned on the basis of HMQC and HMBC spectroscopic analyses. In the HMBC spectrum, the long-range correlations between H-1‴ and C-1′ (or 5′), as well as H-2‴ and C-6′, indicated connection of the prenyl group to C-6′. The location of the 2,2-dimethylpyran unit was confirmed by the long-range correlations of H-1″/C-1′ and H-2″/C-2′ (Figure S4, Supporting Information). Thus, the structure of **3** was determined as shown.

Cathafuran D (**4**) was obtained as a yellow powder, $[\alpha]^{20}_D$ −13.5 (*c* 0.025, MeOH). The UV spectrum in methanol exhibited absorption maxima at 212, 271, and 296 nm, suggesting that **4** was a derivative of 2-arylbenzofuran. Its IR spectrum had absorption bands for OH and benzene ring moieties at 3376, 1624, 1596, and 1488 cm^{-1}. The HRFABMS indicated a molecular formula of $C_{25}H_{28}O_5$ with a pseudomolecular ion at *m/z* 409.2030 $[M + H]^+$. The ^{1}H NMR spectrum of **4** displayed the following proton signals: a singlet at δ 3.82 (3H, s, OMe-5′) for a methoxy group; one set of ABX aromatic protons at δ 7.42 (1H, d, J = 8.5 Hz, H-4), 6.98 (1H, br s, H-7), and 6.80 (1H, d, J = 8.5 Hz, H-5) and a singlet at δ 6.57(1H, s, H-3), which were assignable to a benzofuran moiety; one set of ABX protons at δ 3.71 (1H, m, H-2″), 2.81 (1H, dd, J = 5.0, 16.5 Hz, H-1″) and 2.50 (1H, dd, J = 5.0, 16.5 Hz, H-1″), as well as three singlets at δ 1.34 (3H, s, H-4″), 1.37 (3H, s, H-5″), and 6.51 (1H, s, H-4′), which were assignable to a 2,2-dimethylchroman moiety; and proton resonances attributable to a prenyl group at δ 1.44 (3H, s, H-4‴), 1.61 (3H, s, H-5‴), 3.20 (2H, d, J = 6.5 Hz, H-1‴), and 5.10 (1H, m, H-2‴). The ^{13}C NMR spectrum (Table 1) showed signals for 25 carbons. In the HMBC spectrum, correlations of H-1‴/C-1′,5′ and H-2‴/C-6′ identified the connection of the prenyl group to C-6′. The correlation between the methoxy protons and C-5′ confirmed the position of the methoxy group. The location of the hydroxydimethyldihydropyran unit was confirmed by the long-range correlations of H-1″/C-1′ and H-2″/C-2′ (Figure S5, Supporting Information). Complete assignments of the protons and carbons in **4** were accomplished by analysis of the HMBC and HSQC spectra. The configuration of the OH group at C-2″ was tentatively assigned as 2″*S* by comparison of the optical rotation of **4** with that of a similar compound, (*S*)-3,5-dihydroxy-2,2-dimethyl-7-(2-phenylethenyl)benzopyran.[7] On the basis of the above analysis, the structure of **4** was determined to be as shown.

Compounds **1**−**4** were assayed for their cytotoxic activities against five tumor cell lines (A549, Be-l7402, BGC-823, HCT-8, A2780) using the MTT assay. Cathafuran B (**2**) possessed nonselective antitumor activity against all five tested cell lines with IC_{50} values ranging from 6.27 to 9.60 μg/mL, while cathafuran C (**3**) selectively inhibited Bel7402, HCT-8, and A2780 cell lines with IC_{50} values 6.17, 7.33, and 7.61 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO P-2000 polarimeter. UV spectra were measured with a JASCO V-650 spectrophotometer. IR spectra were recorded on a Nicolet Impact-400 spectrometer by a microscope transmission method. NMR spectra were obtained on a Varian Mercury-Plus 500 or 400 MHz spectrometer with TMS as the internal standard. HRFABMS data were recorded on a VG Autospec-300 mass spectrometer. Silica gel (60−100 or 200−300 mesh, Qingdao Marine Chemical Factory), Sephadex LH-20 (Pharmacia), and ODS (40−60 μm, Merck) were used for column chromatography (CC). TLC was performed on GF254 plates (Qingdao Marine Chemical Factory) or TLC plates precoated with PR-18 F254s (Merck). HPLC was carried out using a Shimadzu System LC-10AD pump equipped with a Shimadzu UV-260 spectrophotometer and YMC-Pack ODS-A column (20 × 250 mm, 5 μm).

Plant Material. The stem bark of *Morus cathayana* was collected at Mountain Lu, Jiangxi Province, China. The sample was identified by Prof. Ce-Ming Tan, Jiujiang Institute of Forest Botany. A voucher specimen (No. 21037) has been deposited at the Herbarium of the Institute of Material Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, China.

Extraction and Isolation. The dried stem bark of *M. cathayana* (9 kg) was powdered and extracted with 95% aqueous EtOH three times at reflux. The solvent was removed under reduced pressure to give 700 g of residue. The residue was submitted to silica gel CC (60−100 mesh, 1.0 kg), eluting with petroleum ether (PE, 60−90 °C), $CHCl_3$, EtOAc, acetone, and MeOH, successively, and the fractions were concentrated to dryness. The $CHCl_3$-soluble fraction (100 g) was subjected to silica gel CC (200−300 mesh, 10 × 130 cm, 3 kg) and eluted with a gradient of petroleum ether−acetone [(10:1−8:1−6:1−4:1−2:1−1:1, v/v)]. The fractions were combined according to TLC profiles into five main fractions. Fraction B (6 g) was repeatedly subjected to silica gel CC (200−300 mesh, 4 × 60 cm, 180 g) eluted with PE−EtOAc (9:1−7:1−4:1−2:1−1:1, v/v) to give six subfractions. Fraction B-3 (600 mg) was separated on Sephadex LH-20 eluted with MeOH−$CHCl_3$ (1:1), to afford **1** (20 mg). Fraction B-4 (1.2 g) was separated by MPLC (ODS, 40−60 μm, MeOH−H_2O) and HPLC (YMC C-18, 20 × 250 mm, MeOH−H_2O, 80:20) to yield **2** (20 mg). Fraction C (31 g) was separated by silica gel CC (200−300 mesh, 6 × 100 cm, 800 g) eluted with PE−EtOAc (8:1−6:1−4:1−2:1−1:1, v/v) to give eight subfractions. Fraction C-2 (1.6 g) was purified by MPLC (ODS, 40−60 μm, MeOH−H_2O) and HPLC (YMC C-18, 20 × 250 mm, MeOH−H_2O,

75:25) to yield **4** (4 mg). Fraction D (19 g) was separated by silica gel CC eluted with PE−EtOAc (7:1−5:1−3:1−1:1, v/v) and purified by MPLC (ODS, 40−60 μm, MeOH−H_2O) and preparative TLC (PE−EtOAc, 3:1) to yield **3** (20 mg).

Cathafuran A (1): yellowish, amorphous powder; $FeCl_3$ test (brown); UV (MeOH) λ_{max} (log ε) 207 (4.71), 253 (3.98), 295 (4.12) nm; IR ν_{max} 3407, 2969, 2916, 1624, 1598, 1488, 1440, 1112 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.35 (6H, s, H-4″), 1.53, 1.56, 1.57, and 1.62 (each 3H, s, H-9″, 10″), 1.86 and 1.93 (each 2H, t, J = 7.5 Hz, H-5″), 1.97−2.04 (4H, m, H-6″, 6‴), 3.14 and 3.16 (each 2H, d, J = 8.5 Hz, H-1″), 5.05−5.15 (4H, m, H-2″, 7″), 7.41(1H, d, J = 8.0 Hz, H-4), 6.81 (1H, dd, J = 8.0, 2.0 Hz, H-5), 6.95 (1H, d, J = 2.0 Hz, H-7), 6.54 (1H, s, H-3), 6.60 (1H, s, H-4′); ^{13}C NMR (125 MHz, acetone-d_6) see Table 1; HRFABMS *m/z* 514.3096 (calcd for $C_{34}H_{42}O_4$, 514.3083).

Cathafuran B (2): white, amorphous powder; $FeCl_3$ test (brown); UV (MeOH) λ_{max} (log ε) 204 (4.43), 309 (4.15) nm; IR ν_{max} 3377, 2969, 2917, 1618, 1491, 1154 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.86 or 1.79 (6H, s, H-4″, 4‴), 1.77 (3H, s, H-5″), 1.77 (3H, s, H-5‴), 3.53 (2H, d, J = 7.0 Hz, H-1″), 3.68 (2H, d, J = 7.0 Hz, H-1‴), 5.30 (1H, br t, J = 7.0 Hz, H-2″), 5.40 (1H, br t, J = 7.0 Hz, H-2‴), 6.71 (1H, s, H-3), 6.79 (1H, d, J = 8.0 Hz, H-5), 7.29 (1H, d, J = 8.0 Hz, H-4), 6.43 (1H, d, J = 2.5 Hz, H-4′), 6.77 (1H, d, J = 2.5 Hz, H-6′); ^{13}C NMR (125 MHz, acetone-d_6), see Table 1; HRFABMS *m/z* 378.1824 (calcd for $C_{24}H_{26}O_4$, 378.1831).

Cathafuran C (3): yellow powder; $FeCl_3$ test (brown); UV (MeOH) λ_{max} (log ε) 208 (4.50), 250 (sh) (4.11), 274 (sh) (4.02), 299 (4.07) nm; IR ν_{max} 3403, 2976, 2927, 1624, 1599, 1505, 1488, 1439, 1111 cm^{-1}; ^1H NMR (400 MHz, acetone-d_6) δ 1.37 (6H, s, H-4″, 5″), 1.40 (3H, s, H-4‴), 1.54 (3H, s, H-5‴), 3.22 (2H, d, J = 6.8 Hz, H-1‴), 5.40 (1H, br t, J = 6.8 Hz, H-2‴), 5.48 (1H, d, J = 10.0 Hz, H-2″), 6.08 (1H, d, J = 10.0 Hz, H-1″), 6.46 (1H, s, H-4′), 6.64 (1H, s, H-3), 6.84 (1H, dd, J = 8.4, 2.4 Hz, H-5), 6.97 (1H, d, J = 2.4 Hz, H-7), 7.45 (1H, d, J = 8.4 Hz, H-4); ^{13}C NMR (100 MHz, acetone-d_6), see Table 1; EIMS *m/z* 376 $[M]^+$ (30), 361 (100), 317 (8), 305 (17), 277 (19), 58 (18); HRFABMS *m/z* 377.1743 (calcd for $C_{24}H_{25}O_4$, 377.1753).

Cathafuran D (4): yellow powder; $[\alpha]^{20}_D$ −13.5 (*c* 0.025, MeOH); $FeCl_3$ test (brown); UV (MeOH) λ_{max} (log ε) 212 (4.40), 271 (sh) (3.87), 296 (3.95) nm; IR ν_{max} 3376, 2976, 2930, 1624, 1596, 1488, 1445, 1116 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.34 (3H, s, H-4″), 1.37 (3H, s, H-5″), 1.44 (3H, s, H-4‴), 1.61 (3H, s, H-5‴), 2.50 (1H, dd, J = 5.0, 16.5 Hz, H-1″), 2.81 (1H, dd, J = 5.0, 16.5 Hz, H-1″), 3.20 (2H, d, J = 6.5 Hz, H-1‴), 3.71 (1H, m, H-2″), 3.82 (3H, s, OMe-5′), 5.10 (1H, m, H-2‴), 6.51 (1H, s, H-4′), 7.42 (1H, d, J = 8.5 Hz, H-4), 6.98 (1H, br s, H-7), 6.80 (1H, d, J = 8.5 Hz, H-5), 6.57 (1H, s, H-3); ^{13}C NMR (125 MHz, acetone-d_6), see Table 1. HRFABMS *m/z* 409.2030 (calcd for $C_{25}H_{29}O_5$, 409.2015).

Cytotoxicty Assay. Compounds **1**−**4** were tested for cytotoxicity against A549 (human lung carcinoma), Bel-7402 (human liver carcinoma), BGC-823 (human stomach carcinoma), HCT-8 (human colon carcinoma), and A2780 (human ovarian carcinoma) cells by means of the MTT assay. Briefly, these cells were plated in 96-well plates and cultured for 24 h. The appropriate test compounds and positive control were added into triplicate wells at concentrations of 0.1, 1.0, and 10.0 μg/mL and incubated for 4 days at 37 °C. MTT solution (10 μL, 5 mg/mL) was added into each well, and the plate was incubated for another 4 h. The resulting formazan crystals were dissolved in DMSO (100 μL), and the UV/vis absorbance (optical density: OD) was determined with a microplate spectrophotometer at 570 nm. The linear dependences between OD and the precent cell survival were calculated with Excel (Microsoft), and IC_{50} values were determined graphically as described previously.[8] The reference compound, 5-fluorouracil, exhibited activity toward the A549, Bel-7402, BGC-823, HCT-8, and A2780 cell lines with IC_{50} ranges of 0.2−0.7 μg/mL.

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Supporting Information Available: UV, IR, MS, and 1D and 2D NMR spectra of compounds **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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