Ebenfurans IV−VIII from *Onobrychis ebenoides*: Evidence that *C*-Prenylation is the Key Determinant of the Cytotoxicity of 3-Formyl-2-arylbenzofurans

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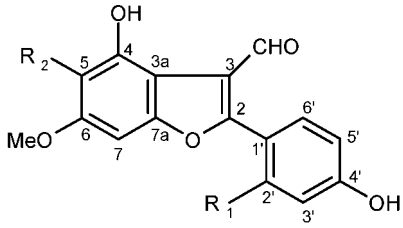
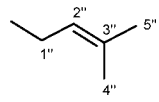
Phytochemical investigation of a methanol extract of *Onobrychis ebenoides* yielded five new 3-formyl-2-arylbenzofurans, namely, ebenfurans IV−VIII (**1**−**5**), together with the known compounds ebenfurans I, II (**6**), and III (**7**). Only **1** and **7** exhibited growth inhibitory activity against MCF-7 and Ishikawa cells, suggesting that the prenyl moiety at position C-5 is the key determinant of the cytotoxic activity of this group of compounds.

The genus *Onobrychis* (Leguminosae) contains over 160 species that are widespread in Europe, the Americas, West Asia, and North Africa.[1] Some species of the genus are cultivated for use as forage, with *Onobrychis viciifolia* being the most widely exploited for this purpose.[2] Previous phytochemical reports concerning plants of the genus *Onobrychis* are limited and have mainly referred to *O. viciifolia* due to its considerable economic importance.[3,4] *Onobrychis ebenoides* Boiss & Spruner, which is endemic to Greece,[5] has been examined in the present investigation.

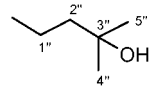
We previously reported the isolation of three 2-arylbenzofurans, ebenfurans I, II (**6**), and III (**7**),[6] where the latter two compounds carry an aldehyde moiety on C-3 of the furan ring. The 2-arylbenzofurans represent a small group of natural products, the classification of which has proven to be difficult due to their limited number, their structural similarity to nor- and neolignans, and the differences proposed for their biosynthetic routes that could be family dependent.[7−9]



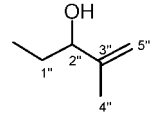
1 Ebenfuran IV R_1 = OMe R_2 =
7 Ebenfuran III R_1 =OH R_2 =



2 Ebenfuran V R_1 =OH R_2 =
3 Ebenfuran VI R_1 = OMe R_2 =



4 Ebenfuran VII R_1 =OH R_2 =
5 Ebenfuran VIII R_1 = OMe R_2 =



6 Ebenfuran II R_1 = OH R_2 = H

The biological and pharmacological properties of 2-arylbenzofurans are not well documented. Most of these are referred to as phytoalexins on the basis of their antifungal activity.[10,11] Also, antioxidant, antiplasmodial, anti-HIV, and estrogenic activities have been reported.[7,12−14] Recent attempts to explore the cytotoxic activities of 2-arylbenzofurans have yielded significant results. Some 2-arylbenzofurans, in particular the prenylated derivatives, were reported to be selectively cytotoxic against certain cancer cell lines.[15] In addition, Chang et al. reported on the cytotoxic activity of 3-formyl-2-arylbenzofurans against cancer cell lines.[16] Furthermore, we found that *C*-prenylation of 3-formyl-2-arylbenzofurans at position C-5 increases their cytotoxicity against a variety of cancer cells in a proliferation-dependent manner.[17] Herein, we show that oxidation and/or hydration of the prenyl moiety at the position C-5 of 3-formyl-2-arylbenzofurans pronouncedly decreases their cytotoxicity, suggesting that *C*-prenylation is the key determinant of the cytotoxic activity of these compounds.

In the present study, five new 3-formyl-2-arylbenzofurans were isolated from *O. ebenoides* and structurally characterized. These are 2-(2-methoxy-4-hydroxyphenyl)-5-(3-methylbuten-2-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran IV) (**1**), 2-(2,4-dihydroxyphenyl)-5-(3-hydroxy-3-methylbutyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran V) (**2**), 2-(2-methoxy-4-hydroxyphenyl)-5-(3-hydroxy-3-methylbutyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran VI) (**3**), 2-(2,4-dihydroxyphenyl)-5-(3-methyl-2-hydroxybuten-3-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran VII) (**4**), and 2-(4-methoxy-2-hydroxyphenyl)-5-(3-methyl-2-hydroxybuten-3-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran VIII) (**5**), and they were isolated together with the known compounds ebenfurans I, II (**6**), and III (**7**).[6] The structures of the isolated compounds were determined by spectroscopic data interpretation.

Ebenfuran IV (**1**) was isolated as an amorphous, yellow solid and exhibited a UV spectrum characteristic of a 3-formyl-2-arylbenzofuran, with maxima at 266 and 365 nm.[18] The CIMS of the compound exhibited a molecular ion peak at *m*/*z* 382 [M^+], and its molecular formula was determined as $C_{22}H_{22}O_6$ by HREIMS. The ^{1}H NMR spectrum indicated the presence of an ABX system consisting of a doublet (H-6′, J = 8.5 Hz) at 7.43 ppm, a double doublet (H-5′, J = 8.5, 1.9 Hz) at 6.57 ppm, and a doublet at 6.62 ppm (H-3′, J = 8.5 Hz). The carbon atoms of the aforementioned protons were found to resonate at 134.8 (C-6′), 110.1 (C-5′), and 101.6 ppm (C-3′), respectively. An additional downfield signal was observed in the ^{1}H NMR spectrum at 6.65 ppm and was allocated to C-7, while C-7 resonated at 87.8 ppm (HMQC spectrum). The deshielded aldehyde proton was observed as a singlet at 9.92 ppm and the corresponding carbon atom at 192.2 ppm. The HMBC spectrum revealed a correlation of the aldehyde moiety proton with C-3a (^3J) and C-3 (^2J), confirming the positions thereof.

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Table 1. ^{1}H NMR Spectroscopic Data (400 MHz, MeOD) for Ebenfurans IV−VIII (**1**−**5**)

	δ_H (J in Hz)				
position	ebenfuran IV (**1**)	ebenfuran V (**2**)	ebenfuran VI (**3**)	ebenfuran VII (**4**)	ebenfuran VIII (**5**)
2					
3					
3α					
4					
5					
6					
7	6.65, s	6.72, s	6.72, s	6.72, s	6.72, s
7α					
1′					
2′					
3′	6.62, d (1.9)	6.48, d (1.9)	6.64, d (1.9)	6.49, m	6.58, d (2.0)
4′					
5′	6.57, dd (8.5, 1.9)	6.49, dd (9.6, 1.9)	6.59, dd (8.5, 1.9)	6.49, m	6.44, dd (8.5, 2.0)
6′	7.43, d (8.5)	7.44, d (9.6)	7.48, d (8.5)	7.45, d (8.7)	7.47, d (8.5)
1″	3.37, d (7.0)	2.77, m	2.78, m		
1″a				3.07, dd (12.9, 6.6)	3.07, dd (13.0, 6.8)
1″b				2.94, dd (12.9, 7.1)	2.94, dd (13.0, 7.0)
2″	5.22, t (7.0)	1.67, m	1.67, m	4.40, dd (7.1, 6.6)	4.40, dd (7.0, 6.8)
3″					
4″	1.66, s	1.29, s	1.29, s	1.84, s	1.82, s
5″	1.79, s	1.29, s	1.31, s	4.66, br s	4.65, br s
CHO	9.92, s	9.93, s	9.77, s	9.93, s	9.78, s
OCH_3	3.84, s	3.87, s	3.86, s	3.87, s	3.86, s
OCH_3	3.84, s		3.88, s		3.86, s

Table 2. ^{13}C NMR Spectroscopic Data (50 MHz, MeOD) for Ebenfurans IV−VIII (**1**−**5**)

	δ_C, mult.				
position	ebenfuran IV (**1**)	ebenfuran V (**2**)	ebenfuran VI (**3**)[a]	ebenfuran VII (**4**)	ebenfuran VIII (**5**)
2	164.5, qC	167.1, qC	162.7, qC	165.8, qC	164.4, qC
3	119.9, qC	118.9, qC	118.6, qC	119.2, qC	119.1, qC
3α	108.7, qC	108.1, qC	106.9, qC	107.8, qC	107.2, qC
4	150.3, qC	150.2, qC	148.2, qC	149.8, qC	149.7, qC
5	113.8, qC	114.3, qC	113.2, qC	110.7, qC	110.5, qC
6	160.3, qC	159.9, qC	158.4, qC	160.3, qC	160.1, qC
7	87.8, CH	86.0, CH	86.4, CH	87.7, CH	87.5,CH
7α	156.4, qC	155.9, qC	154.2, qC	156.2, qC	156.0, qC
1′	109.5, qC	109.7, qC	109.7, qC	109.0, qC	109.0, qC
2′	161.6, qC	159.5, qC	159.5, qC	163.5, qC	160.0, qC
3′	101.6, CH	104.1, CH	99.7, CH	104.2, CH	101.4, CH
4′	163.8, qC	164.1, qC	158.5, qC	158.5, qC	158.9, qC
5′	110.1, CH	109.8, CH	107.9, CH	109.5, CH	110.0, CH
6′	134.8, CH	133.2, CH	132.9, CH	133.6, CH	133.8, CH
1″	24.1, CH_2	20.5, CH_2	18.2, CH_2	31.3, CH_2	31.2, CH_2
2″	125.4, CH_2	44.3, CH_2	42.1, CH	76.7, CH	76.5, CH
3″	132.1, qC	73.4, qC	71.5, qC	149.1, qC	149.0, qC
4″	19.0, CH_3	30.1, CH_3	29.6, CH_3	17.6, CH_3	17.8, CH_3
5″	27.2, CH_3	30.1, CH_3	29.3, CH_2	111.4, CH_2	111.2, CH_2
CHO	192.2	192.4	190.1	192.3	192.2
OCH_3	57.3	56.8	56.0	57.2	57.2
OCH_3	57.3		56.1		57.2

[a] $CDCl_3$ was used for the NMR experiments.

The isoprenyl side chain was established by the presence of a methylene group at 5.22 ppm (H-2″, $J = 7.0$ Hz), which appeared as a triplet and displayed a ^{1}H−^{1}H COSY correlation to the downfield methine at 3.37 ppm (H-1″, $J = 7.0$ Hz) and long-range correlations with two methyl groups at 1.66 (H-4″) and 1.79 ppm (H-5″). The ^{13}C, HMQC, and HMBC NMR spectra confirmed the presence of this specific side chain (Tables 2 and S1, Supporting Information). In addition, the HMBC spectrum revealed the correlation of H-1″ with C-5, thus indicating the position of the isoprenyl moiety in the basic skeleton. Finally, a 6H singlet at 3.84 ppm corresponded to the two methoxy groups of the molecule. In the HMQC spectrum, the methoxy group carbon atoms were both observed at 57.3 ppm, and the positions thereof were established at C-6 and C-2′ by COSY LR and HMBC NMR experiments. Thus, the structure of ebenfuran IV (**1**) was assigned as 2-(2-methoxy-4-hydroxyphenyl)-5-(3-methylbuten-2-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde.

Ebenfuran V (**2**) was also isolated as an amorphous, yellow solid with UV maxima at 263 and 361 nm. The CIMS showed a molecular ion peak at m/z 386 $[M]^+$, and its molecular formula was calculated as $C_{21}H_{22}O_7$. The NMR spectra of **2** were very similar to those of **1**, as expected from their structural resemblance. All the ^{1}H NMR signals corresponding to the basic skeleton were observed with δ values closely comparable to those of **1**. Spectroscopic variations were consistent with the lack of a ring B oxygenated methyl group and with signals that corresponded to a hydroxymethylbutyl group substituting for the isoprenyl group. The H-1″ and H-2″ methylene protons appeared as multiplets at 2.77 and 1.67 ppm, respectively. The corresponding carbon atoms resonated at 20.5 (C-1″) and 44.3 ppm (C-2″) (^{13}C NMR, HMQC) (Tables 2 and S1, Supporting Information). The position of the side chain on C-5 of **2** was confirmed with the HMBC experiment, where correlations of C-1″ with C-5 (^2J), C-4 (^3J), and C-6 (^3J) were observed. Accordingly, the structure of ebenfuran V (**2**) was established as 2-(2,4-dihydroxyphenyl)-5-(3-hydroxy-3-methylbutyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde (ebenfuran V).

Biosynthetic hypotheses suggest that isoflavonoids with a hydroxymethylbutyl side chain could be the result of hydration of the isoprenyl moiety following its addition to the basic skeleton of the isoflavonoid through the action of a prenyltransferase enzyme.[19]

In this case, ebenfuran V is possibly the result of the hydration of ebenfuran III (**7**), which was the major 2-arylbenzofuran of the *O. ebenoides* MeOH extract.[6] Furthermore, ebenfuran II (**6**), a 3-formyl-2-arylbenzofuran without a side chain, has also been isolated[6] and could be the hypothetical precursor compound of **7** (Figure S1, Supporting Information).

The third 3-formyl-2-arylbenzofuran to be isolated was ebenfuran VI (**3**) and was found to be the 2′-methyl ether of **2**. The UV spectrum of **3** was analogous to those of compounds **1** and **2**. The CIMS showed a molecular ion peak at *m/z* 400 $[M]^+$, and the molecular formula was calculated as $C_{22}H_{24}O_7$ by HREIMS. Due to the structural similarities of **2** and **3**, their NMR spectra were almost identical; a difference was in an additional peak in the ^{1}H NMR spectrum at 3.88 ppm, which corresponded to the protons of a second methoxy group of ring B. From the HMBC and COSY LR NMR spectra the position of the methoxy group was assigned to C-2′. Thus, ebenfuran VI (**3**) was determined as 2-(2-methoxy-4-hydroxyphenyl)-5-(3-hydroxy-3-methylbutyl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde.

Ebenfuran VII (**4**) is a derivative of **2** and **7**, in which the respective 3-hydroxy-3-methylbutyl and isoprenyl chains have been replaced with a 3-methyl-2-hydroxybuten-3-yl chain. The UV spectrum was in accordance with those of the preceding compounds, and a molecular ion peak at *m/z* 384 was clear in the CIMS. The HREIMS confirmed the molecular formula of $C_{21}H_{20}O_7$ for **4**. The NMR spectra of compound **4** exhibited all the typical peaks of a 3-formyl-2-arylbenzofuran. The only differences concerned the side chain, where the geminal protons of the C-5″ terminal double bond resonated together at 4.66 ppm as a broad singlet, while the protons of the C-1″ methylene group resonated separately as two double doublets at 3.07 (H-1″a, J = 7.1 and 12.9 Hz) and 2.94 ppm (H-1″b, J = 6.6 and 12.9 Hz). The oxymethine proton of the moiety was observed as a double doublet (J = 6.6 and 7.1 Hz) at 4.40 ppm, and the methyl group protons (H-4″) resonated at 1.84 ppm. The cross-peaks evident in the COSY and COSY LR spectra were used in the identification of the side chain. More specifically, a ^4J correlation between the C-4″ methyl group protons and the C-5″ protons was observed (COSY LR) along with a ^3J (COSY) and a ^5J (COSY LR) correlation of H-1″ with H-2″ and H-7, respectively. In addition, the HMBC spectrum exhibited correlations of H-1″ (^2J) and H-7 (^3J) with C-5 as well as those of H-4″ (^2J) and H-5″ (^2J) with C-3″. Therefore, the structure of ebenfuran VII (**4**) was established as 2-(2,4-dihydroxyphenyl)-5-(3-methyl-2-hydroxybuten-3-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde.

According to Tahara and Ibrahim,[19] in isoflavonoids the 3-methyl-2-hydroxybuten-3-yl side chain is also formed from the isoprenyl chain following generation of an unstable intermediate by oxidation, which then undergoes hydration and dehydration before yielding a 3-methyl-2-hydroxybuten-3-yl chain. This specific side chain found in compound **4** is very rare among natural products. Only a few isoflavonoids carrying this moiety have been reported including the isoflavones lupinisols A and β.[20] This is the first report of a 2-arylbenzofuran with such a side chain. On the basis of the compounds isolated here as well as the reported data on the biosynthesis of isoflavonoids[19] it is possible that 2-arylbenzofurans from the Leguminosae family follow a similar biosynthetic route (Figure S1, Supporting Information).

Ebenfuran VIII (**5**) was assigned as the 2′-methyl ether of **4**, and therefore these two compounds exhibited similar NMR spectra. The ESIMS revealed a pseudomolecular ion at *m/z* 397 (100) $[M - 1]^-$. HRESIMS allowed for the molecular formula to be calculated as $C_{22}H_{22}O_7$. The ^{1}H NMR spectrum of **5** indicated a 6H singlet at 3.86 ppm, which corresponded to two methoxy groups in the molecule. The COSY LR spectrum allowed the second methoxy group to be allocated to C-2′ of ring B. Thus, ebenfuran VIII (**5**) was assigned as 2-(4-methoxy-2-hydroxyphenyl)-5-(3-methyl-2-hydroxybuten-3-yl)-4-hydroxy-6-methoxybenzofuran-3-carbaldehyde. The proton signals of the ABX substitution system of ring B were clearly separated only with the appearance of this second methoxy group on the ring. This trend is observed in all such compounds isolated in this work (see Table 1), where lack of this group results in overlapping resonances.

Table 3. Effects of Compounds **1**−**7**, 17β-Estradiol, and ICI 182,780 on the Growth of MCF-7 Cells in the Absence or Presence of 0.1 nM 17β-Estradiol

	in the absence of 17β-estradiol		in the presence of 17β-estradiol	
compound	EC_{50}[a] (μM)	efficacy[b]	EC_{50}[a] (μM)	efficacy[b]
1	6.7	54 ± 16 (#)	3.9	31 ± 13 (#)
2	na	115 ± 5	na	98 ± 1
3	4.8	154 ± 14 (#)	na	118 ± 1
4	na	123 ± 4	na	79 ± 6
5	5.2	155 ± 1 (#)	na	98 ± 2
6	na	111 ± 9	5.5	64 ± 15
7	2.6	47 ± 12 (#)	2.9	31 ± 11 (#)
control		100		100
17β-estradiol	9.5*	163 ± 10 (#)	na	104 ± 5
ICI 182,780	na	103 ± 13	4.2**	63 ± 8 (#)

[a] EC_{50} values (calculated for statistically significant effects only) are compound concentrations required to achieve 50% of the efficacy. [b] The efficacy (mean ± SEM of at least three independent assays) of the test compounds, 17β-estradiol, or ICI 182,780 at 10 μM, 0.1 nM, or 1 μM, respectively, was calculated by $OD_{test\ compound} \times 100/OD_{control}$. $OD_{control}$, a measure of MTT conversion to formazan in the absence (vehicle only) or presence of 0.1 nM 17β-estradiol alone, was set as equal to 100. na = not applicable; *, pM; *, nM; (#), $p < 0.05$ vs vehicle.

We have previously reported that **7** is cytotoxic to cancer cells in a manner that is dependent on cell proliferation but independent of the estrogen receptor (ER) or multidrug resistance status of the cells, and that **6** is less effective in this respect.[17] In the present study, the cytotoxic activity of **1**−**5** against MCF-7 and Ishikawa cells (breast and endometrial adenocarcinoma cells known to express ERα and hardly any ERβ) was compared to that of **6** and **7** using the MTT and the sulforhodamine B assays.[17,21] Both assays gave very similar results. Table 3 shows that, while **1** and **7** reduced the growth of MCF-7 cells to 53% and 47% of that of vehicle-treated cells, the effect of the other 3-formyl-2-arylbenzofurans was either nonsignificant (p > 0,05; **2**, **4**,**6**) or to the opposite direction (**3**, **5**). As expected from previous findings,[17,21] 17β-estradiol (0.1 nM) stimulated cell growth, whereas ICI 182,780 (1 μM) had no effect under these conditions. Table 3 shows, in addition, that the rate of growth of MCF-7 cells in the presence of 0.1 nM 17β-estradiol was reduced by **1** and **7** to 33% and 29% of that of cells treated with the hormone alone. By contrast, the other 3-formyl-2-arylbenzofurans were either ineffective (p > 0,05; **2**−**5**) or much less effective in this respect (**6**). As expected,[19,21] ICI 182,780 inhibited cell growth whereas 17β-estradiol had no effect under these conditions. Similar results were obtained with ER-positive Ishikawa cells (data not shown). Using recombinant ERα and fluorescence polarization as previously described,[19] we determined that the binding activities of **1**−**7** for this receptor relative to that of 17β-estradiol (set equal to 100) were 0.46 ± 0.11, 0.23 ± 0.02, 0.06 ± 0.01, 0.94 ± 0.11, 0.04 ± 0.01, 0.18 ± 0.03, and 0.09 ± 0.01, respectively. These relative binding affinity values are obviously not associated with effects on cell growth, in accordance with the notion that the growth effects of **1**−**7** are largely independent of the ER status of the cells. These findings provide evidence that the prenyl moiety at position C-5, rather than the formyl group at C-3, is the key determinant of the cytotoxic activity of these 2-arylbenzofurans. In addition, these data show that removing (in the case of **6**) or replacing the prenyl moiety at C-5 with a 2-methoxy-4-hydroxyphenyl group (**2**) or a 3-methyl-2-hydroxybuten-3-yl group (**4**) abolished the cytotoxic activity of 2-arylbenzofurans and that substituting a 2-methoxy for the 2-hydroxy group at position C-2′ on top of replacing the C-5 prenyl

moiety gives rise to 2-arylbenzofurans (**3**, **5**) that promoted the growth of MCF-7 cells rather than inhibiting it.

Experimental Section

General Experimental Procedures. UV−vis spectra were obtained using spectroscopic grade EtOH/MeOH on a Shimadzu-160A spectrophotometer. IR spectra were recorded on a Perkin-Elmer, Paragon 500, FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 200 and 400 MHz spectrometers using solvents $CDCl_3$ and methanol-d_4 (Aldrich). The 2D-NMR experiments (COSY, COSY LR, HMQC, and HMBC) were performed using standard Bruker microprograms. CIMS were run on a Finnigan Trace DSQ using methane for the ionization procedure. EIMS were obtained with a Nermag R 10-10C instrument, and HREIMS were run on an AEI MS-902 spectrometer. Column chromatography was carried out using silica gel [Merck, 0.04−0.06 mm (flash) and 0.015−0.04 mm] with an applied pressure of 300 mbar. MPLC was performed with a Büchi model 688 apparatus on columns containing silica gel (Merck, 0.015−0.040). Precoated TLC silica 60 F_{254} plates (purchased from Aldrich) were used for thin-layer chromatography (0.25 and 2 mm layer thickness for analytical and preparative TLC, respectively). Spots were visualized using UV light and vanillin-sulfuric acid reagent.

Plant Material. Whole plants of *O. ebenoides* were collected in May 1998 from Mount Ymitos, Attica (Greece). A voucher specimen (no. NEK 006) was deposited in the herbarium of the Laboratory of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, University of Athens, and was identified by Assoc. Prof. Th. Constandinides.

Extraction and Isolation. The whole plant was dried, pulverized (1.8 kg), and then extracted with CH_2Cl_2 (2 L × 3) and MeOH (2 L × 5), for 48 h each. The MeOH extract was concentrated to a residue (50 g), which was subjected to vacuum-liquid chromatography over silica gel (0.015−0.04 mm). Elution with CH_2Cl_2/MeOH mixtures of increasing polarity yielded 11 fractions. Fraction 1 was subjected to column chromatography using a CH_2Cl_2/MeOH gradient system. Compounds **2** (6.7 mg) and **3** (2.6 mg) were isolated after purification by preparative TLC (CH_2Cl_2/cyclohexane, 80:20). Fractions 4 (1.15 g) and 5 (1.78 g) were combined and subjected to MPLC separation techniques. Elution with a CH_2Cl_2/MeOH gradient system yielded seven fractions (A−G). Fraction A gave ebenfuran I (20 mg), and fraction C gave ebenfuran II (26 mg) and ebenfuran III (28 mg).[6] Fraction B was further examined and underwent separation with CH_2Cl_2/MeOH, providing 15 fractions. Compound **5** (2.1 mg) was purified from fraction B2, while **4** (3.4 mg) was obtained from fraction B6. Fraction B15 yielded compound **1** (1.2 mg), which underwent further purification by preparative TLC (CH_2Cl_2/MeOH, 99:1).

Ebenfuran IV (1): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 266 (4.24), 365 (3.86) nm; IR (Nujol) ν_{max} 3450−3200 (OH), 1654 (CO), 1604 cm^{-1}; ^1H and ^{13}C NMR (MeOD, 400 and 50 MHz, respectively) in Tables 1 and 2; CIMS(+) *m/z* 382 $[M]^+$ (100), 383 (42); HRESIMS *m/z* 382.7890 (calcd for $C_{22}H_{22}O_6$, 382.7897).

Ebenfuran V (2): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 263 (4.01), 361 (3.66) nm; IR (Nujol) ν_{max} 3458−3189 (OH), 1648 (CO), 1601 cm^{-1}; ^1H and ^{13}C NMR (MeOD, 400 and 50 MHz, respectively) in Tables 1 and 2; CIMS(+) *m/z* 386 $[M]^+$ (100), 387 (32); HRESIMS *m/z* 386.7890 (calcd for $C_{21}H_{22}O_7$, 386.7897).

Ebenfuran VI (3): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 263 (4.22), 362 (3.81) nm; IR (Nujol) ν_{max} 3512−3250 (OH), 1656 (CO), 1611 cm^{-1}; ^1H and ^{13}C NMR (MeOD, 400 and 50 MHz, respectively) in Tables 1 and 2; CIMS(+) *m/z* 400 $[M]^+$ (100), 401 (32); HRESIMS *m/z* 400.7890 (calcd for $C_{22}H_{24}O_7$, 400.7897).

Ebenfuran VII (4): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 264 (4.39), 362 (3.65), 364 (3.65) nm; IR (Nujol) ν_{max} 3500−3222 (OH), 1649 (CO), 1612 cm^{-1}; ^1H and ^{13}C NMR (MeOD, 400 and 50 MHz, respectively) in Tables 1 and 2; ESIMS(−) *m/z* 383 $[M - 1]^-$ (100), 384 (44); HRESIMS *m/z* 384.7890 (calcd for $C_{21}H_{20}O_7$, 384.7897).

Ebenfuran VIII (5): amorphous, yellow solid; UV (MeOH) λ_{max} (log ε) 216 (4.43), 265 (4.37), 350 (3.81) nm; IR (Nujol) ν_{max} 3432−3200 (OH), 1654 (CO), 1615 cm^{-1}; ^1H and ^{13}C NMR (MeOD, 400 and 50 MHz, respectively) in Tables 1 and 2; ESIMS(−) *m/z* 397 $[M - 1]^-$ (100), 398 (31); HRESIMS *m/z* 398.7890 (calcd for $C_{22}H_{22}O_7$ 398.7897).

Effects on Cell Growth. 2-Arylbenzofuran effects on the proliferation of MCF-7 cells and ER-positive Ishikawa cells (purchased from ATCC and ECACC, respectively) were assessed following a 3-day incubation in 96-well flat-bottomed microculture plates using either the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, as previously reported,[22] or the sulforhodamine B assay, as described by Fang et al.[22] Briefly, MCF-7 cells growing in phenol red-free MEM (minimal essential medium) and 5% DCC-FBS (fetal bovine serum treated with dextran-coated charcoal to remove endogenous steroids), supplemented with 0.1 nM 17β-estradiol or with vehicle (DMSO) alone, were treated with increasing concentrations of the test compounds, 17β-estradiol, or the pure antiestrogen ICI 182,780 (all dissolved in DMSO) and were assessed spectrophotometrically (absorbance at 550 nm) using MTT conversion to a colored formazan as a means to assess relative numbers of viable cells. The efficacies (mean ± SEM of at least three independent MTT assays) of the test compounds, 17β-estradiol, and ICI 182,780 were tested at 10 μM, 0.1 nM, and 1 μM, respectively.

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Supporting Information Available: Table of 2D-HMBC NMR data for **1**−**5**, a figure showing biogenetic relationships, and 1D- and 2D-NMR spectra for **1**−**5**. This material is provided free of charge via the Internet at http://pubs.acs.org.

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