Synthesis of 2-Phenylbenzofuran Derivatives and Selective Binding Activities on Estrogen Receptor

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Received October 18, 2011; accepted November 25, 2011; published online November 30, 2011

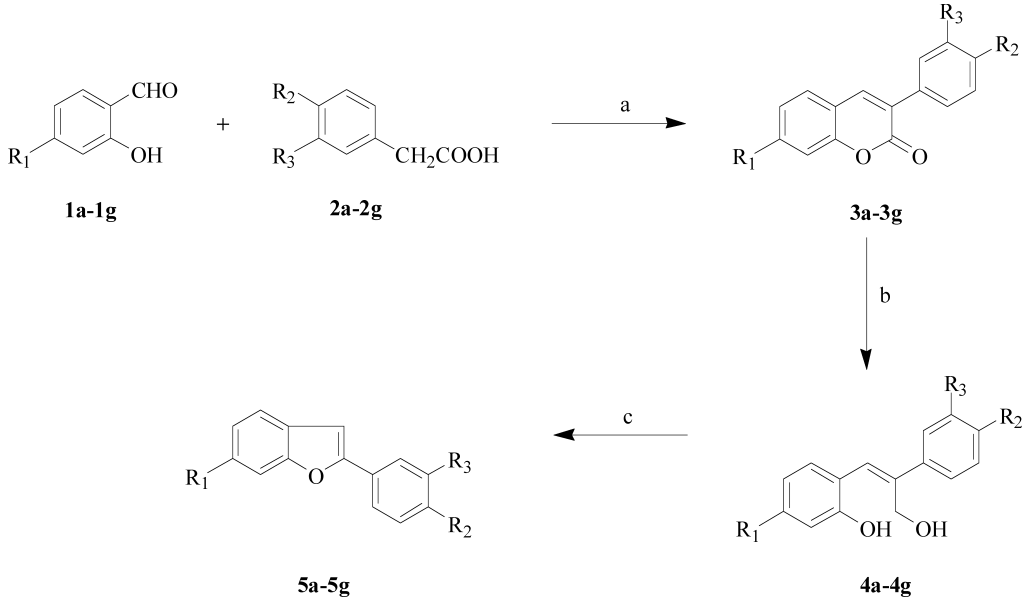
An improved chemical reaction protocol with short time and easy work-up was described here for 2-phenylbenzofuran derivatives. The final purified products, 2-phenylbenzofuran derivatives 5a—g and the intermediate diols 4a—g, were evaluated for their estrogen receptor (ER) binding affinity and selective activity *in vitro*. Among these fourteen tested compounds, 4g and 5g showed higher binding affinity on ER subtypes, ER*α* and ER*β*. Compound 4g exhibited preferable ER*α* binding, while 5g was more estrogen selective for ER*β*. The molecular docking was also performed to explore the detailed interactive interface between ER and the compounds.

Key words 2-phenylbenzofuran; diol; estrogen receptor; binding affinity; selectivity

2-Phenylbenzofuran compounds, a very important family of heterocycles, are widely found in natural products. Due to their effective pharmacological activities, these compounds have been extensively studied.[1—5] A literature survey revealed that most 2-phenylbenzofurans with hydroxy and methoxy groups demonstrated multi-bioactivities, including antitumor,[6,7] anti-microbial,[8—10] antivirus,[11] adenosine A_1 receptor antagonists[12,13] and immunosuppressant properties.[14] In recent years, it is found that 2-phenylbenzofurans have significant binding affinity and selectivity on estrogen receptor (ER)*β*.[15] Here, we reported an improved synthetic method for 2-phenylbenzofurans **5a**—**g** from 3-phenylcoumarins **3a**—**g**. After purification, these compounds were evaluated for ER binding affinity and selectivity *in vitro*. Moreover, we described the preliminary structure–activity relationship (SAR) and carried out molecular docking study for the interactions of these compounds with ER.

Results and Discussion

Chemistry As shown in Chart 1, 3-phenylcoumarin **3** was first synthesized by the Perkin reaction of hydroxylated benzaldehyde **1** with phenylacatic acid derivative **2**, then reduced into 2-(3-hydroxy-propenyl)-phenol **4** with diisobutylaluminium hydride (DIBAL-H). Compound **4** was finally cyclized into 2-phenylbenzofurans **5** in the presence of 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) at reflux temperature. Comparing to the method reported,[16] the procedure was modified by using DIBAL-H, a more easily-handled material,[17] as the reducing agent in stead of using aluminum chloride ($AlCl_3$) and lithium aluminium hydride ($LiAlH_4$). In addition, toluene was used as the solvent of cyclization in stead of using toxic reagent such as benzene. In this study, the



1a-1g **2a-2g** **3a-3g** **4a-4g** **5a-5g**

Reagents and conditions: (a) anhydrous CH_3COOK, Ac_2O, reflux, 4 h, 50—60% yield; (b) DIBAL-H (1.1 M solution in cyclohexane), dry CH_2Cl_2, 20°C, 3 h, 67—96% yield; (c) DDQ, dry toluene, reflux, 3 h, 40—60% yield.

Chart 1. Synthesis of Compounds **4a**—**g** and **5a**—**g**

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Table 1. Reactions of 3-Phenylcoumarins with DDQ for the Synthesis of 2-Phenylbenzofuran Derivatives **5a—g**

Entry	R_1	R_2	R_3	Yield of **4** (%)[a]	Yield of **5** (%)[a]
1	H	OCH_3	H	**4a**, 96	**5a**, 60
2	OCH_3	OCH_3	H	**4b**, 89	**5b**, 65
3	H	OCH_3	OCH_3	**4c**, 71	**5c**, 40
4	OCH_3	OCH_3	OCH_3	**4d**, 67	**5d**, 58
5	H	Br	H	**4e**, 93	**5e**, 57
6	OCH_3	Br	H	**4f**, 87	**5f**, 48
7	H	OH	H	**4g**, 79	**5g**, 54

a) Isolated yields.

Table 2. Relative Binding Affinity and Selectivity of Compounds **4g** and **5g** for ERα and ERβ

Compound	IC_{50} for ERα (μM)[a]	RBA[b] for ERα (%)	IC_{50} for ERβ (μM)	RBA for ERβ (%)	RBA ERα/ERβ[c]	RBA ERβ/ERα[d]
17β-Estradiol	0.019±0.007	100.00	0.004±0.001	100.00	1.00	1.00
Tamoxifen	0.553±0.129	3.73±0.51	0.224±0.072	1.90±0.63	2.0	0.5
4a	>10.00	—[e]	>10.00	—	—	—
4b	>10.00	—	>10.00	—	—	—
4c	>10.00	—	>10.00	—	—	—
4d	>10.00	—	>10.00	—	—	—
4e	>10.00	—	>10.00	—	—	—
4f	>10.00	—	>10.00	—	—	—
4g	1.351±1.007	1.86±0.66	1.004±0.103	0.40±0.08	4.7	0.2
5a	>10.00	—	>10.00	—	—	—
5b	>10.00	—	>10.00	—	—	—
5c	>10.00	—	>10.00	—	—	—
5d	>10.00	—	>10.00	—	—	—
5e	>10.00	—	>10.00	—	—	—
5f	>10.00	—	>10.00	—	—	—
5g	2.805±0.280	0.50±0.06	0.360±0.040	1.11±0.23	0.5	2.2

a) The values given are the average±S.D. of three experiments. *b*) RBA (relative binding affinity)=(IC_{50} 17β-estradiol/IC_{50} test compound)×100. *c*) RBA ERα/ERβ=RBAα/RBAβ. *d*) RBA ERβ/ERα=RBAβ/RBAα. *e*) Not determined.

lactone ring opening took place in a stereoselective manner, the diol **4a** with a (*Z*)-configuration was obtained as the only reaction product confirmed by H–H nuclear Overhauser effect spectroscopy (NOESY) spectrum.[18] The products **5a—g** and the intermediate diols **4a—g** were characterized by spectroscopic analyses which were collected in the experimental part.

Estrogen Receptor Binding Affinity The binding affinity of this series of 2-phenylbenzofurans **5a—g** on ER was determined by fluorescence polarization technology. The binding affinity is normalized as relative binding affinity (RBA) using estradiol as reference with 100% affinity. We focus on the diols **4** that have a stilbene skeleton. Stilbenes, an important phytoestrogen, exhibit estrogen activity. The stilbene skeleton is one of the important structures to construct the selective estrogen receptor modulators (SERMs).[19—22] For example, Tamoxifen and Raloxifen, which are usually applied in clinic as SERMs, have a conjugated stilbene skeleton in their structures. In addition, our previous studies of resveratrol analogues also indicated that all these compounds with a conjugated stilbene skeleton possessed a certain estrogen-like activity.[23] Therefore, in this study we selected seven new diols **4a—g** as the test compounds for ER binding affinity study. Tamoxifen was used as the positive control. As indicated in Table 2, 17β-estradiol (E_2), the known ER ligand, binds to both ERα and ERβ equally well. Tamoxifen is more selective binding to ERα. Compounds **4a—f** and **5a—f** with methoxy or halogen substituents appeared no binding affinity for ERα or ERβ with $IC_{50}\geq 10.0\,\mu$M. Interestingly, we found that both **4g** and **5g** demonstrated higher binding affinity for ERα and ERβ. Compared to Tamoxifen, **4g** showed 5-fold more selectivity for ERα while **5g** was about 2-fold higher ERβ selectivity, although the overall binding affinity was decreased.

In an attempt to understand the molecular interaction between synthesized compounds and ER, the molecular docking study was performed using the Discovery Studio 2.1/Flexible Docking protocol with published crystal structure of E_2/ERα complex (PDB ID:1A52) or E_2/ERβ complex (PDB ID:3OLS), respectively. Based on methods within CHARMm to sample side-chain and ligand conformations, the side-chains of amino acids in the ligand-binding domain (LBD) were allowed to move during docking in an induced-fit model. During the docking and subsequent scoring, all the parameters were remained the default setting except the Best Conformation Method for the Generate Ligand Conformations.

As seen in Fig. 1, the phenolic hydroxy of **4g** formed three hydrogen bonds with ERα Glu_{353} and Arg_{394} (Fig. 1a). The phenolic hydroxy of **5g** formed a hydrogen bonding network with ERα Glu_{353}, Arg_{394} and a water molecule (Fig. 1b). In contrast, **4a** or **5a**, lacking the phenolic hydroxy, formed only one hydrogen bond with ERα residues (Figs. 1c, d). This observation could explain the significant ER binding affinity for **4g** and **5g**, hence elucidate the importance of the phenol functionality in terms of hydrogen bonding. Since the ligand binding pockets of ERα and ERβ are highly conserved,[24,25] the binding mode

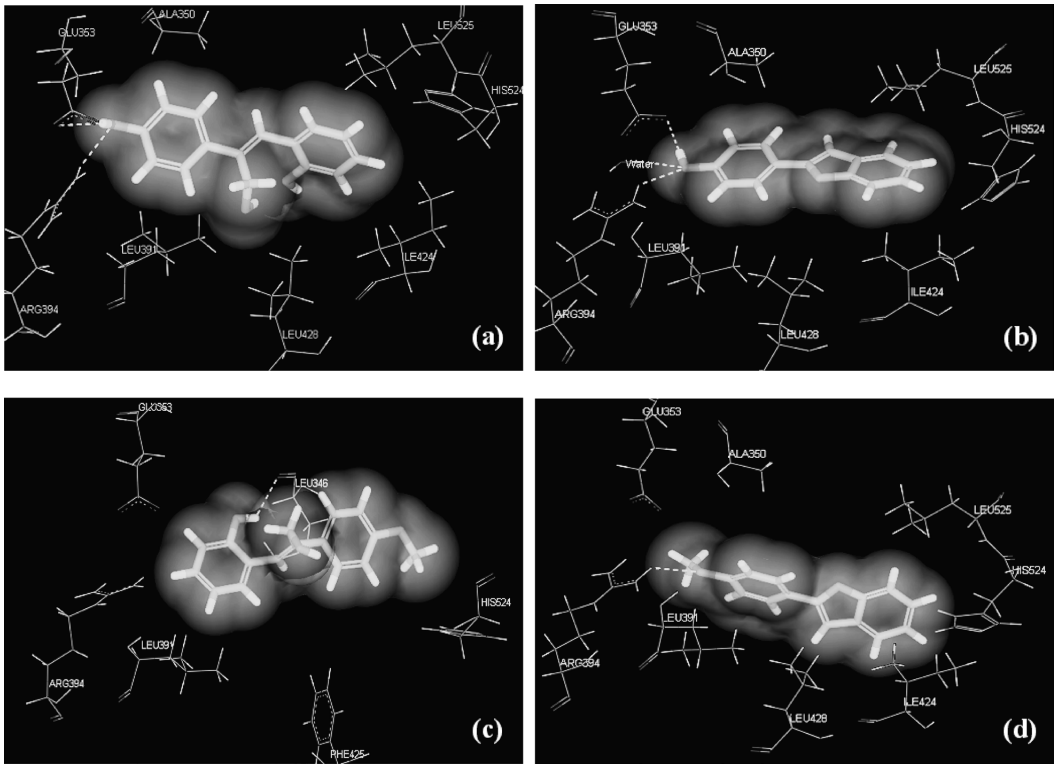

Fig. 1. The Binding Patterns of **4g** (a), **5g** (b), **4a** (c) and **5a** (d) into ERα

Only key residues are shown for simplicity. Hydrogen binds to key residues are shown as dotted lines.

of these compounds with ERβ was similar to that of ERα (the docking models of ERβ-ligand were not shown). In addition, compounds **4g** and **5g** exhibited similar binding free energy ($\Delta G_{\mathbf{4g}}$=−68.59 kcal/mol, $\Delta G_{\mathbf{5g}}$=−72.86 kcal/mol). Replacement of the phenolic hydroxy by a methoxy group, however, resulted in a significant decrease for **4a** and **5a** in binding free energy ($\Delta G_{\mathbf{4a}}$=−27.91 kcal/mol, $\Delta G_{\mathbf{5a}}$=−38.96 kcal/mol) compared to E_2 (ΔG_{E2}=−91.38 kcal/mol). This also indicated that the phenolic hydroxy groups had a high contribution to the overall binding.

The study of molecular docking could also provide explanation of ER subtypes preference for **4g** and **5g**. The binding cavity in ERα-E_2 complex had a volume of approximately 255.0 Å^3 with our docking method. This binding cavity size was slightly larger than that of ERβ-E_2, which had a volume of 207.1 Å^3. The result was in accordance with ERβ with a smaller ligand binding pocket than ERα suggested by Hubbard and Katzenellenbogen.[24,26,27] The molecule volume of **4g** was about 178.0 Å^3, while **5g** was 150.3 Å^3 by calculation. Moreover, compound **5g** exhibited more planar profile with ERβ relative to **4g**. These observations could partially explain that **5g** was selective for ERβ while **4g** was selective for ERα.

Conclusion

In summary, we have developed an efficient and practical protocol for synthesis of 2-phenylbenzofurans from 3-phenylcoumarins, with short reaction time and easy work-up. The ER binding affinity and selectivity of these synthesized 2-phenylbenzofurans and intermediate diols had been evaluated and a clear SAR was also described. The binding affinities of **4g** and **5g** on ER subtypes were significant. While **4g** showed a preference for ERα, **5g** showed a better ERβ selectivity. The two compounds could be served as promising structural leads for future study of novel ER subtype-selective ligands.

Experimental

General All reactions were carried out using oven-dried glassware. The starting materials and reagents were obtained from commercial suppliers without further purification. Dichloromethane (CH_2Cl_2) and toluene were distilled from calcium hydride (CaH_2) immediately prior to use. All reactions were monitored by TLC with Merck silica gel coated plates ($60F_{254}$, Qingdao, China) using UV-light (254 nm). Column chromatography was carried out using Silica gel (HG/T2354-92) purchased from Qingdao. Melting points were determined with an X-4 apparatus and are uncorrected. Infrared spectra (IR) were measured on a Bruker Vector22 spectrometer. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker Avance spectrometer using tetramethylsilane (TMS) as internal standard. Chemical shifts (δ) are reported in ppm, and coupling constants (J) are in Hz. Mass spectra (electron ionization (EI), 70 eV) were recorded on an Agilent 5975 inert mass selective detector. High resolution (HR) mass spectra (EI, 70 eV) were obtained by the corresponding service at Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences using a Waters Micromass GCT apparatus.

Reduction of 3-Phenylcoumarins to the Corresponding (*Z*)-2-(3-Hydroxy-2-phenylpropenyl)phenol Derivatives (4a—g) A solution of 3-phenylcoumarins (2.93 mmol) in anhydrous CH_2Cl_2 (8 mL) was cooled to −10°C under a nitrogen atmosphere. DIBAL-H (1.1 M solution in cyclohexane, 8 mL, 8.8 mmol) was added dropwise over a 40 min period, while the reaction temperature was maintained at −10°C. The reaction

was stirred at 20°C for 3 h and checked by TLC. After completed, the reaction mixture was allowed to cool to −10°C and quenched by addition of methanol, followed by full quenched with dilute hydrochloric acid. The mixture was agitated at room temperature for 1 h and filtered. The filtration was washed with water and target products were then dried. The filtrate was extracted with ethyl acetate, and the combined organic phase was washed with water and brine. The solvent was evaporated *in vacuo* and separated by column chromatography (petroleum ether/ethyl acetate) on silica gel.

(*Z*)-2-(3-Hydroxy-2-(4-methoxyphenyl)propenyl)phenol (**4a**): White solid, mp 124—125°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.57—7.55 (2H, d, J=8.8 Hz), 7.46—7.44 (1H, d, J=8.0 Hz), 7.11 (1H, t, J=7.6 Hz), 6.94—6.91 (3H, m), 6.87—6.79 (2H, m), 4.42 (2H, s), 3.76 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 158.9 (s), 155.8 (s), 139.0 (s), 134.1 (s), 130.4 (s), 128.8 (s), 127.9 (q), 124.6 (s), 119.1 (s), 115.6 (s), 114.0 (d), 58.9 (s), 55.5 (s). IR (KBr) cm^{-1}: 3450, 3298, 1602, 1451, 1234, 1002, 827, 757. MS (EI) *m/z* (%): 256 (M^+), 237, 225, 165, 121. HR-MS (EI) Calcd for $C_{16}H_{16}O_3$: 256.1099. Found: 256.1103.

(*Z*)-2-(3-Hydroxy-2-(4-methoxyphenyl)propenyl)-5-methoxyphenol (**4b**): White solid, mp 128—129°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.52 (2H, d, J=8.8 Hz), 7.40 (1H, d, J=8.0 Hz), 6.92 (2H, d, J=9.2 Hz), 6.84 (1H, s), 6.43—6.41 (2H, m), 4.39 (2H, s), 3.75 (3H, s), 3.70 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.1 (s), 158.7 (s), 156.9 (s), 137.4 (s), 134.5 (s), 1301.0 (s), 127.7 (d), 124.4 (s), 117.5 (s), 114.0 (d), 104.7 (s), 101.4 (s), 59.1 (s), 55.3 (d). IR (KBr) cm^{-1}: 3494, 3268, 1610, 1432, 1232, 1019, 839, 811. MS (EI) *m/z* (%): 286 (M^+), 267, 253, 161, 137. HR-MS (EI) Calcd for $C_{17}H_{18}O_4$: 286.1205. Found: 286.1203.

(*Z*)-2-(2-(3,4-Dimethoxyphenyl)-3-hydroxypropenyl)phenol (**4c**): White solid, mp 154—156°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.44 (1H, d, J=7.6 Hz), 7.16—7.08 (3H, m), 6.93 (1H, d, J=8.2 Hz), 6.88 (1H, s), 6.84—6.78 (2H, m), 4.39 (2H, s), 3.78 (3H, s), 3.75 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 155.8 (s), 148.9 (s), 148.6 (s), 139.2 (s), 134.8 (s), 130.5 (s), 128.9 (s), 125.0 (s), 124.6 (s), 119.2 (s), 119.1 (s), 115.7 (s), 112.0 (s), 110.7 (s), 58.8 (s), 55.9 (d). IR (KBr) cm^{-1}: 3467, 3214, 1598, 1456, 1243, 1004, 897, 860, 803, 762. MS (EI) *m/z* (%): 286 (M^+), 267, 237, 165, 151, 73, 57. HR-MS (EI) Calcd for $C_{17}H_{18}O_4$: 286.1205. Found: 286.1207.

(*Z*)-2-(2-(3,4-Dimethoxyphenyl)-3-hydroxypropenyl)-5-methoxyphenol (**4d**): Violescent solid, mp 143—145°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.42 (1H, d, J=8.4 Hz), 7.17—7.12 (2H, m), 6.95 (1H, d, J=8.4 Hz), 6.86 (1H, s), 6.45—6.43 (2H, m), 4.40 (2H, s), 3.80 (3H, s), 3.77 (3H, s), 3.72 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.1 (s), 156.9 (s), 148.8 (s), 148.5 (s), 137.8 (s), 135.0 (s), 131.0 (s), 124.8 (s), 119.0 (s), 117.5 (s), 112.0 (s), 110.5 (s), 104.8 (s), 101.4 (s), 59.1 (s), 55.9 (d), 55.3. IR (KBr) cm^{-1}: 3370, 1608, 1458, 1248, 1031, 849, 814. MS (EI) *m/z* (%): 316 (M^+), 297, 283, 267, 161. HR-MS (EI) Calcd for $C_{18}H_{20}O_5$: 316.1311. Found: 316.1312.

(*Z*)-2-(2-(4-Bromophenyl)-3-hydroxypropenyl)phenol (**4e**): White solid, mp 135—136°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.57 (4H, s), 7.44 (1H, d, J=7.6 Hz), 7.14 (1H, s, J=7.6 Hz), 7.00 (1H, s), 6.88—6.81 (2H, m), 4.43 (2H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 155.9 (s), 141.1 (s), 138.6 (s), 131.5 (d), 130.4 (s), 129.3 (s), 128.9 (d), 126.8 (s), 124.1 (s), 120.5 (s), 119.1 (s), 115.7 (s), 58.7 (s). IR (KBr) cm^{-1}: 3355, 3053, 1599, 1449, 1246, 1074, 1008, 829, 754. MS (EI) *m/z* (%): 306 (M^++2), 304 (M^+), 287, 207, 178, 165, 131, 107. HR-MS (EI) Calcd for $C_{15}H_{13}O_2Br$: 304.0099. Found: 304.0100.

(*Z*)-2-(2-(4-Bromophenyl)-3-hydroxypropenyl)-5-methoxyphenol (**4f**): Grayish solid, mp 118—119°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 7.53 (4H, s), 7.39 (1H, d, J=8.8 Hz), 6.94 (1H, s), 6.43—6.41 (2H, m), 4.39 (2H, s), 3.70 (3H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 160.5 (s), 157.2 (s), 141.4 (s), 136.9 (s), 131.5 (d), 131.1 (s), 128.7 (d), 126.6 (s), 120.1 (s), 117.0 (s), 104.9 (s), 101.4 (s), 58.8 (s), 55.4 (s). IR (KBr) cm^{-1}: 3367, 1610, 1431, 1241, 1074, 1012, 859, 814, 762, 718. MS (EI) *m/z* (%): 336 (M^++2), 334 (M^+), 317, 161, 137. HR-MS (EI) Calcd for $C_{16}H_{15}O_3Br$: 334.0205. Found: 334.0211.

(*Z*)-2-(2-(4-Hydroxyl)-3-hydroxypropenyl)phenol (**4g**): Violescent solid, mp 161—162°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 9.48 (1H, s), 9.42 (1H, s), 7.43 (3H, d, J=8.0 Hz), 7.09 (1H, t, J=7.6 Hz), 6.84—6.75 (5H, m), 4.38 (2H, s). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 157.1 (s), 155.8 (s), 139.2 (s), 132.5 (s), 130.3 (s), 128.7 (s), 127.9 (d), 124.8 (s), 123.8 (s), 119.1 (s), 115.4 (q), 58.9 (s). IR (KBr) cm^{-1}: 3379, 3231, 1608, 1223, 1001, 808, 753. MS (EI) *m/z* (%): 242 (M^+), 223, 165, 131, 107, 73, 57. HR-MS (EI) Calcd for $C_{15}H_{14}O_3$: 204.0943. Found: 204.0947.

General Procedure for the Preparation of 2-Phenylbenzofurans (5a—g) Under a nitrogen atmosphere, a mixture of (*Z*)-2-(3-hydroxy-2-phenylprop-1-enyl)phenol derivatives (2 mmol), DDQ (3.6 mmol) and dry toluene (45 mL) was refluxed for 3 h. The reaction was monitored by TLC. After removing the precipitates by filtration, the filtrate was concentrated and crystallized from methanol and dried.

2-(4-Methoxyphenyl)benzofuran (**5a**): White solid, mp 148—149°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.77 (2H, d, J=8.4 Hz), 7.54—7.47 (2H, m), 7.23—7.17 (2H, m), 6.95 (2H, d, J=8.4 Hz), 6.85 (1H, s), 3.82 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 159.9 (s), 156.0 (s), 154.6 (s), 129.4 (s), 126.3 (d), 123.7 (s), 123.3 (s), 122.7 (s), 120.5 (s), 114.2 (d), 110.9 (s), 99.6 (s), 55.3 (s). IR (KBr) cm^{-1}: 2838, 1608, 1501, 1450, 1245, 1175, 1022, 836, 801, 744. MS (EI) *m/z* (%): 224 (M^+), 209, 181, 152. HR-MS (EI) Calcd for $C_{15}H_{12}O_2$: 224.0837. Found: 224.0839.

6-Methoxy-2-(4-methoxyphenyl)benzofuran (**5b**): White solid, mp 160—161°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.74 (2H, d, J=8.8 Hz), 7.41 (1H, d, J=8.4 Hz), 7.06 (1H, s), 6.96 (2H, d, J=8.8 Hz), 6.88—6.86 (1H, m), 6.80 (1H, s), 3.86 (3H, s), 3.84 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 159.6 (s), 157.7 (s), 155.7 (s), 155.3 (s), 125.9 (d), 123.6 (s), 122.8 (s), 120.6 (s), 114.2 (d), 111.6 (s), 99.5 (s), 95.9 (s), 55.7 (s), 55.3 (s). IR (KBr) cm^{-1}: 2838, 1605, 1505, 1445, 1340, 1250, 1227, 1139, 1019, 938, 858, 799, 752. MS (EI) *m/z* (%): 254 (M^+, 100.0), 239 (35.0), 168 (26.7), 139 (28.9). HR-MS (EI) Calcd for $C_{16}H_{14}O_3$: 254.0943. Found: 254.0946.

2-(3,4-Dimethoxyphenyl)benzofuran (**5c**): White solid, mp 124—125°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.59—7.53 (2H, m), 7.47—7.44 (1H, m), 7.40 (1H, d, J=1.6 Hz), 7.31—7.23 (2H, m), 6.96—6.92 (2H, m), 4.01 (3H, s), 3.95 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 155.9 (s), 154.7 (s), 149.6 (s), 149.2 (s), 129.4 (s), 123.8 (s), 123.5 (s), 122.8 (s), 120.6 (s), 117.9 (s), 111.4 (s), 110.9 (s), 108.1 (s), 100.0 (s), 56.0 (s), 55.9 (s). IR (KBr) cm^{-1}: 2838, 1605, 1505, 1445, 1340, 1250, 1227, 1139, 1019, 938, 858, 799, 752. MS (EI) *m/z* (%): 254 (M^+), 239, 168, 139. HR-MS (EI) Calcd for $C_{16}H_{14}O_3$: 254.0943. Found: 254.0947.

2-(3,4-Dimethoxyphenyl)-6-methoxy Benzofuran (**5d**): White solid, mp 112—113°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ:

7.42—7.36 (2H, m), 7.33 (1H, s), 7.06 (1H, s), 6.93—6.91 (1H, m), 6.87—6.85 (1H, m), 6.82 (1H, s), 3.98 (3H, s), 3.92 (3H, s), 3.86 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 157.7 (s), 155.6 (s), 155.1 (s), 149.1 (s), 123.7 (s), 122.7 (s), 120.6 (s), 117.3 (s), 111.6 (s), 111.3 (s), 107.6 (d), 99.7 (s), 95.8 (s), 55.9 (d), 55.6 (s). IR (KBr) cm^{-1}: 2838, 1619, 1571, 1504, 1462, 1340, 1268, 1144, 1023, 953, 818, 761. MS (EI) *m/z* (%): 284 (M^+), 269, 254, 142. HR-MS (EI) Calcd for $C_{17}H_{16}O_4$: 284.1049. Found: 284.1055.

2-(4-Bromophenyl)benzofuran (**5e**): White solid, mp 147—149°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.73 (2H, d, J=8.0 Hz), 7.61—7.52 (4H, m), 7.34—7.26 (2H, m), 7.02 (1H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 154.9 (s), 154.7 (s), 131.9 (d), 129.4 (s), 129.0 (s), 126.3 (d), 124.6 (s), 123.1 (s), 122.5 (s), 121.0 (s), 111.2 (s), 101.8 (s). IR (KBr) cm^{-1}: 3046, 1605, 1578, 1212, 1072, 945, 906, 818, 753. MS (EI) *m/z* (%): 274 (M^++2), 272 (M^+), 165, 84. HR-MS (EI) Calcd for $C_{14}H_9BrO$: 271.9837. Found: 271.9838.

2-(4-Bromophenyl)-6-methoxy Benzofuran (**5f**): White solid, mp 122—124°C. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.65 (2H, d, J=8.4 Hz), 7.53 (2H, d, J=8.0 Hz), 7.43 (1H, d, J=8.8 Hz), 7.04 (1H, s), 6.93 (1H, s), 6.87 (1H, dd, J=2.4, 2.0 Hz), 3.86 (3H, s). ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 158.3 (s), 155.9 (s), 154.0 (s), 131.9 (d), 129.6 (s), 125.8 (d), 122.3 (s), 121.8 (s), 121.1 (s), 112.2 (s), 101.7 (s), 95.8 (s), 55.7 (s). IR (KBr) cm^{-1}: 2973, 2903, 1621, 1489, 1225, 1071, 911, 818. MS (EI) *m/z* (%): 304 (M^++2), 302 (M^+), 291, 289, 152, 57. HR-MS (EI) Calcd for $C_{15}H_{11}BrO_2$: 301.9942. Found: 301.9939.

4-(Benzofuranyl)phenol (**5g**): Yellowish solid, mp 189—191°C. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 10.04 (1H, s), 7.72 (2H, d, J=8.8 Hz), 7.56 (2H, t, J=7.2 Hz), 7.23—7.18 (2H, m), 7.13 (1H, s), 6.92 (2H, d, J=8.8 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 158.9 (s), 156.4 (s), 154.3 (s), 129.7 (s), 126.7 (d), 124.1 (s), 123.4 (s), 121.2 (s), 121.0 (s), 116.3 (d), 111.2 (s), 99.7 (s). IR (KBr) cm^{-1}: 3375, 2925, 1609, 1507, 1445, 1251, 1027, 1001, 749. MS (EI) *m/z* (%): 210 (M^+), 181, 152, 84, 66. HR-MS (EI) Calcd for $C_{14}H_{10}O_2$: 210.0681. Found: 210.0679.

Estrogen Receptor Binding Affinity Assay Tamoxifen was purchased from International Laboratory. The binding affinities were assessed by fluorescence polarization technology using a Synergy 2 SLFPA MODEL Multi-Detection Microplate Reader (Bio Tek Instruments).[28] The IC_{50} of 17β-estradiol and test compounds were determined by inhibiting the binding of the fluorescent estrogen ES2 (Invitrogen) to the isolated recombinant human ERα or ERβ (Invitrogen) by 50%. These values were used to normalize as RBA. IC_{50} values were 17β-estradiol or test compounds concentrations capable of inhibiting the binding of the fluorescent estrogen ES2 (9 nM) to ERα and ERβ by 50%. The RBAα and RBAβ of 17β-estradiol were set equal to 100. Curve fitting was performed using GraphPad Prism® software from GraphPad™ Software Inc.

Acknowledgments This work was supported by Ministry of Health of Zhejiang Province (WKJ2010-2-001), the Health Bureau of Zhejiang Province (XKQ-01001), Zhejiang Provincial Natural Science Foundation of China (Y2111012), Science Technology Department of Zhejiang Province (2011F10015) and National Natural Science Foundation of China (No. 81102380).

References

1) Ziegert R. E., Toräng J., Knepper K., Bräse S., *J. Comb. Chem.*, **7**, 147—169 (2005).
2) De Luca L., Nieddu G., Porcheddu A., Giacomelli G., *Curr. Med. Chem.*, **16**, 1—20 (2009).
3) Pang J. Y., Xu Z. L., *Chin. J. Org. Chem.*, **25**, 25—33 (2005).
4) Pu W. C., Wang F., Wang C., *Chin. J. Org. Chem.*, **31**, 155—165 (2011).
5) Dean F. M., Sargent M. V., *Comprehensive Heterocyclic Chemistry*, **4**, 531—597 (1984).
6) Ni G., Zhang Q. J., Zheng Z. F., Chen R. Y., Yu D. Q., *J. Nat. Prod.*, **72**, 966—968 (2009).
7) Dat N. T., Jin X. J., Lee K., Hong Y. S., Kim Y. H., Lee J. J., *J. Nat. Prod.*, **72**, 39—43 (2009).
8) Pacher T., Seger C., Engelmeier D., Vajrodaya S., Hofer O., Greger H., *J. Nat. Prod.*, **65**, 820—827 (2002).
9) Akgul Y. Y., Anil H., *Phytochemistry*, **63**, 939—943 (2003).
10) Jiang X. Z., Liu W. L., Zhang W., Jiang F. Q., Gao Z., Zhuang H., Fu L., *Eur. J. Med. Chem.*, **46**, 3526—3530 (2011).
11) Thompson L. U., Rickard S. E., Orcheson L. J., Seidl M. M., *Carcinogenesis*, **17**, 1373—1376 (1996).
12) Hutchinson S. A., Luetjens H., Scammells P. J., *Bioorg. Med. Chem. Lett.*, **7**, 3081—3084 (1997).
13) Scammells P. J., Baker S. P., Beauglehole A. R., *Bioorg. Med. Chem.*, **6**, 1517—1524 (1998).
14) Iwasaki T., Kondo K., Kuroda T., Moritani Y., Yamagata S., Sugiura M., Kikkawa H., Kaminuma O., Ikezawa K., *J. Med. Chem.*, **39**, 2696—2704 (1996).
15) Collini M. D., Kaufman D. H., Manas E. S., Harris H. A., Henderson R. A., Xu Z. B., Unwalla R. J., Miller C. P., *Bioorg. Med. Chem. Lett.*, **14**, 4925—4929 (2004).
16) Kinoshita T., Ichinose K., *Heterocycles*, **65**, 1641—1654 (2005).
17) "Technical Bulletin," Akzo Nobel (Asia) Co., Ltd., Shanghai, 2006, pp. 1 – 14.
18) See the supplemental information.
19) Cassidy A., Hanley B., Lamuela-Raventos R. M., *J. Sci. Food Agric.*, **80**, 1044—1062 (2000).
20) Waibel M., De Angelis M., Stossi F., Kieser K. J., Carlson K. E., Katzenellenbogen B. S., Katzenellenbogen J. A., *Eur. J. Med. Chem.*, **44**, 3412—3424 (2009).
21) Xiao C. F., Tao L. Y., Sun H. Y., Wei W., Chen Y., Fu L. W., Zou Y., *Chin. Chem. Lett.*, **21**, 1295—1298 (2010).
22) Bertini S., Calderone V., Carboni I., Maffei R., Martelli A., Martinelli A., Minutolo F., Rajabi M., Testai L., Tuccinardi T., Ghidoni R., Macchia M., *Bioorg. Med. Chem.*, **18**, 6715—6724 (2010).
23) Ma Z., Wang Z. Y., Zheng X. L., Shen Z. R., Hu Y. Z., *Chin. JMAP*, **24**, 362—365 (2007).
24) Pike A. C. W., Brzozowski A. M., Hubbard R. E., Bonn T., Thorsell A.-G., Engström O., Ljunggren J., Gustafsson J.-Å., Carlquist M., *EMBO J.*, **18**, 4608—4618 (1999).
25) Mosselman S., Polman J., Dijkema R., *FEBS Lett.*, **392**, 49—53 (1996).
26) Zhou H. B., Sheng S., Compton D. R., Kim Y., Joachimiak A., Sharma S., Carlson K. E., Katzenellenbogen B. S., Nettles K. W., Greene G. L., Katzenellenbogen J. A., *J. Med. Chem.*, **50**, 399—403 (2007).
27) Minutolo F., Macchia M., Katzenellenbogen B. S., Katzenellenbogen J. A., *Med. Res. Rev.*, **31**, 364—442 (2011).
28) Alexi X., Kasiotis K. M., Fokialakis N., Lambrinidis G., Meligova A. K., Mikros E., Haroutounian S. A., Alexis M. N., *J. Steroid Biochem. Mol. Biol.*, **117**, 159—167 (2009).