# Synthesis and Structure–Activity Relationship Studies of Furan-ring Fused Chalcones as Antiproliferative Agents

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**Abstract.** *As part of our continuing investigation of flavonoid derivatives as potential anticancer substances, the synthesis of 25 cinnamoyl derivatives of benzofuran as furan-fused chalcones was carried-out and these compounds were further evaluated for their antiproliferative activity towards HL60 promyelocytic leukemia cells. In comparison with 2',4'-dihydroxychalcone, attachment of a furan moiety on the A-ring enhanced activity by more than twofold. Benzofurans may be useful in the design of biologically active flavonoids.*

Chalcones, consisting of open-chain flavonoids in which the two aromatic rings are joined by a three-carbon α,β-unsaturated carbonyl system, are considered to be a crucial precursor of flavonoid and isoflavonoid biosyntheses. Due to the importance of these compounds, diverse studies on syntheses and biological activities of molecules containing the chalcone ring system have been reported. Studies revealed that compounds with a chalcone-based structure possess a wide variety of biological activities (1), such as anti-inflammatory (2), anti-bacterial (3, 4), anti-fungal (5-7), anti-oxidant (2, 8) and anticancer activities (9, 10).

In particular, the prenyloxychalcones, which contain a prenyl-type side-chain with different length, including 2,2-dimethylallyl, geranyl or farnesyl chains, have shown the interesting antitumoral activity (11). Although the prenyloxychalcones were thought to be precursors to furan-ring fused chalcones (FFCs), the occurrence of FFCs, which are rarely found in nature, has only been reported in the genera of *Lonchocarpus* (12) and *Derris* (13). Little is known about the effect of A-ring moiety of FFCs on their biological activity, whereas several efforts have been made to obtain new FFCs possessing modified B-ring structure and the relationship between B-ring and biological activity was discussed (14).

In the course of our investigation on the structure–activity relationship of flavonoids (15-17), the effects of A-ring structure on biological activity of FFCs drew our attention. In order to obtain a better understanding over the biological activity of FFCs, we synthesized 25 FFCs and determined their activity.

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## Materials and Methods

*General procedures.* Chemicals and solvents from commercial sources were used without further purification unless specified. Reactions were carried-out under argon and monitored by thin-layer chromatography on silica gel (mesh size 60, $F_{254}$) with visualization under UV light. Standard and flash column chromatography procedures were not optimized. Nuclear magnetic resonance (NMR) spectra were recorded on a 400-MHz JEOL ECP-400 spectrometer (JOEL, Tokyo, Japan), and chemical shifts values are expressed in parts per million shift (δ value) based on the residual $^1$H signal of the solvents. Signal patterns are indicated as s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. Coupling constant (*J*) are given in hertz. Unless otherwise specified, compounds were dissolved in $^2HCCl_3$. Electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) mass spectrometry were performed on Thermo Exactive (Thermo Fisher Scientific K.K, Yokohama, Japan) and Hitachi M8000 instruments (Hitachi, Tokyo, Japan), respectively.

*Synthesis of 5-cinnamoyl-6-hydroxy-3-phenylbenzofuran and 5-cinnamoyl-4-hydroxy-3-phenylbenzofuran.* General procedure for synthesis of FFCs is shown in Figure 1. To a solution of 2',4'-dihydroxyacetophenone (2.0 g, 13.1 mmol) in acetone (80 ml) in a round-bottomed flask, $K_2CO_3$ (5.43 g, 39.3 mmol) and KI (1.09 g, 6.5 mmol) were added and stirred at room temperature. To the above mixture, 2-chloro-1-phenylethan-2-one (2.42 g, 15.7 mmol) was added dropwise. The reaction mixture was refluxed for 4 h. After being cooled to room temperature, the organic solvent was removed under reduced pressure and the crude intermediate was recrystallized from ethanol to obtain the desired ether intermediate as a white solid (2.24 g, yield 63.2%). The ether intermediate (1.00 g, 3.67 mmol) was dissolved in ethanol (100 ml). To the solution, KOH (0.561 g) was

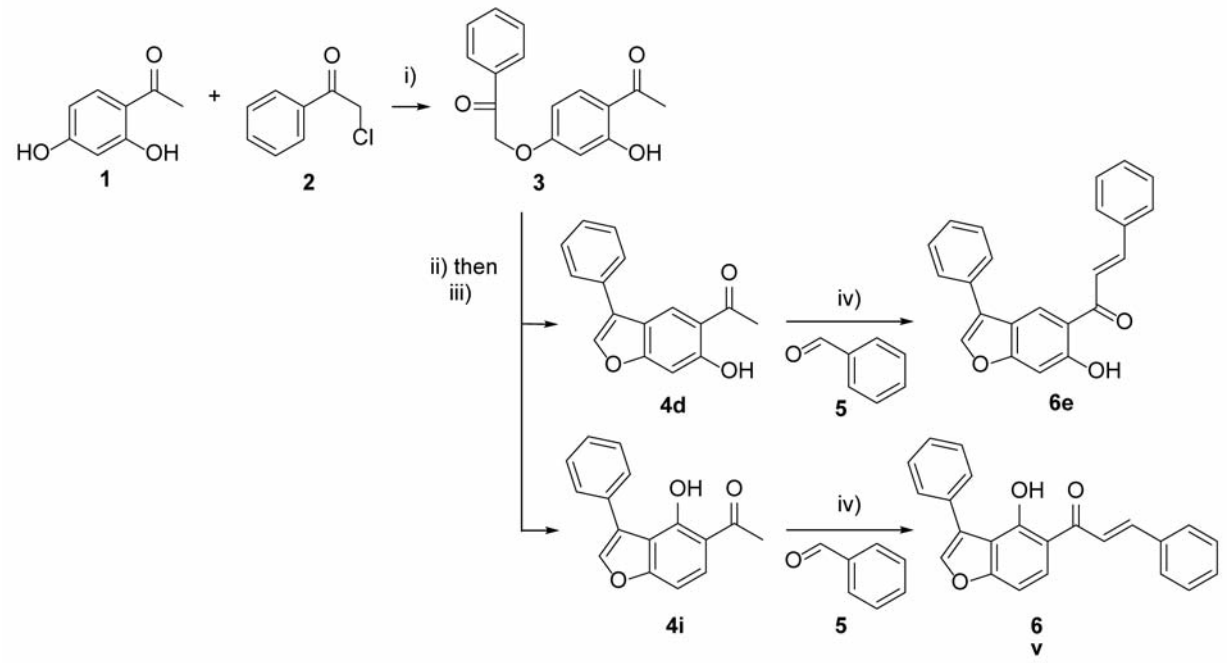


Figure 1. *Reagents and conditions for synthesis of* ***6e*** *and* ***6v****: i) $K_2CO_3$, KI, acetone, reflux, 8 h; ii) KOH in ethanol, reflux, 72 h; iii) separation on silica gel; iv) benzaldehyde (5), KOH in ethanol, rt, 72 h.*

added and refluxed for 19 h. After being cooled to room temperature, the reaction mixture was neutralized by 3 M hydrochloric acid, and was partitioned between ethyl acetate and water. The organic phase was dried over anhydrous $MgSO_4$ and the solvent was removed under reduced pressure. The residue was chromatographed over silica gel (hexane/dichloromethane; 95:5) to obtain 1-(6-hydroxy-3-phenylbenzofuran-5-yl)ethanone (**3d**; 0.204 g, yield 22.0%) as a yellowish crystal and 1-(4-hydroxy-3-phenylbenzofuran-5-yl)ethanone (**3i**; 0.088, yield 9.5%) as a light-yellowish crystal.

For **3d**: $^1$H-NMR ($CDCl_3$) δ 12.4 (1H, s), 8.06 (1H, s), 7.65 (2H, m), 7.48 (3H, m), 7.25 (1H, s), 6.98 (1H, s), 2.71 (3H, s). HRMS $(M + H)^+$: calcd for $C_{16}H_{13}O_3$, 253.0865; found 253.0863. For **3i**: $^1$H-NMR ($CDCl_3$) δ 13.8 (1H, s), 7.82 (1H, d, *J*=8 Hz), 7.68 (2H, m), 7.52 (3H, m), 7.44 (1H, s), 7.01 (1H, d, *J*=8 Hz), 2.56 (3H, s). HRMS $(M + H)^+$: calcd for $C_{16}H_{13}O_3$, 253.0865; found 253.0862.

To a solution of **3d** (0.390 g, 1.55 mmol) dissolved in 5% KOH/ethanol (w/w) (15 ml) in a 50-ml round-bottomed flask, benzaldehyde (**5**; 0.377 g, 3.09 mmol) was added dropwise and stirred at room temperature for 72 h. The reaction was quenched by acidification with 3 M hydrochloric acid. The solvent of the mixture was remove under reduced pressure and the orange residue was preliminarily chromatographed by a silica gel column eluting with hexane-ethyl acetate 9:1. The crude product was recrystallized from ethanol to afford 3-phenyl-5-cinnamoyl-6-hydroxybenzofuran (**6e**) as an orange solid (0.062 g, yield 12%). Compound **3i** was similarly treated as mentioned above to give 3-phenyl-5-cinnamoyl-4-hydroxybenzofuran (**6v**) as an orange solid (0.0455 g, yield 48.5%).

*Cell proliferation assay.* HL60 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum. The level of cellular proliferation for HL60 cells grown in 96-well microplate was measured by using alamar blue (Life Technologies Ltd., Tokyo, Japan). To each well, 100 μl of HL60 cell suspension ($1.0\times10^4$ cells/100 μl) was inoculated and the 100 μl of medium containing serial dilution of the samples to be assayed. After three days of incubation, 20 μl of alamar blue was aseptically added to each well, and cells were further incubated for approximately 20 h. Cellular proliferation as a percentage that of the untreated control was calculated with the following equation:

$$\text{Proliferation (\%)} = \frac{[(A_{570}-A_{595}) \text{ of test agent dilution}]-[(A_{570}-A_{595}) \text{ of blank}]}{[(A_{570}-A_{595}) \text{ of positive growth control}]-[(A_{570}-A_{595}) \text{ of blank}]}$$

where $A_{570}$ and $A_{595}$ are the absorbance at 570 nm and 595 nm, respectively.

## Results and Discussion

*Chemistry.* In the present article, 24 FFCs (**6a-6x** and **7**) were synthesized. The substitution position of FFCs was numbered as a benzofuran derivative since FFCs can be considered cinnamoyl derivatives of benzofuran. Synthesis of 3-phenyl-5-cinnamoyl-6-hydroxybenzofuran (**6e**) and 3-phenyl-5-cinnamoyl-4-hydroxybenzofuran (**6v**) is illustrated in Figure 1 as a general procedure for FFC synthesis. Synthesis of FFCs was largely divided into two steps; the first step was the construction of furan-ring fused acetophenone, and the second was the Claisen-Schmidt condensation between the furan-ring fused acetophenone and benzaldehyde derivatives. In the

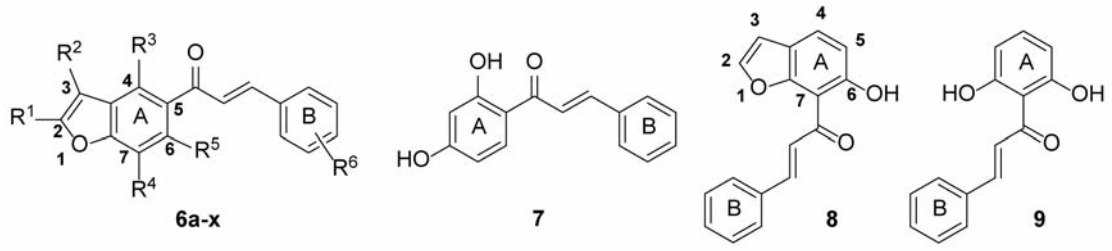


Figure 2. *Structures of furan-fused chalcones.*

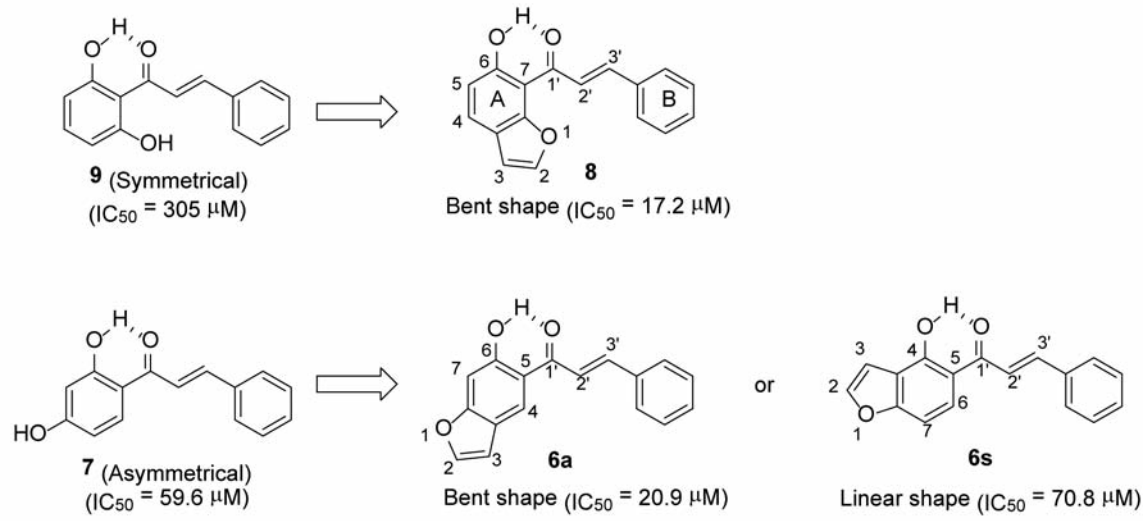


Figure 3. *Preferred conformations of the synthesized furan-ring fused chalcones. Dashed lines between phenolic hydrogen and carbonyl oxygen indicate hydrogen bonds.*

present study, furan-ring fused acetophenones were synthesized through Williamson's etherification of dihydroxyacetophenones by 2-bromo-1,1-diethoxyethane or several α-haloketones and the nucleophilic addition to the carbonyl carbon by C3' or C5' aromatic carbon which was followed by the spontaneous dehydration. Etherification selectively occurred at 4'-position of 2',4'-dihydroxyacetophenone and 2'-hydroxyl groups did not react with the methylation reagent, presumably because of the weakened nucleophilicity of 2'-hydroxyl oxygen atom due to its hydrogen bonding to the oxygen atom of the acetyl carbonyl group. The obtained mixture of 4- and 6-alkoxyacetophenones was treated under basic conditions without purification to give a mixture of the corresponding benzofuran derivatives which was then chromatographed to pure forms of 5-acetyl-4-hydroxybenzofuran (**2a**) and 5-acetyl-6-hydroxybenzofuran (**2b**).

To avoid such isomer production, the methylated analog of dihydroxyacetophenone, which was synthesized from the Friedel-Crafts acetylation of 2-methylresorcinol, was also examined as a starting compound. However, the methyl group on the aromatic ring did not affect yield although 6-hydroxybenzofuran was obtained as the sole product.

The combination of 20 acetylbenzofurans and several benzaldehyde derivatives led to a total of 25 FFCs being synthesized. Spectral data for **6d** are shown in Table I along with other synthetic FFCs.

*Antiproliferative activity*. Antiproliferative activity of 25 synthetic FFCs along with 2,4-dihydroxychalcone was determined using promyelocytic leukemia HL60 cells based on the alamar blue assay. The results are summarized in Figure 2.

Among the compounds tested, 5-cinnamoyl-6-hydroxy-3-phenlybenzofenone (**6e**) demonstrated the most potent activity [half maximal inhibitory concentration ($IC_{50}$)=12.3 μM], followed by 5-cinnamoyl-6-hydroxy-7-methylbenzofuran (**6f**, $IC_{50}$=16.1 μM). Furthermore 7-cinnamoyl-6-hydroxy-benzofuran (**8**, $IC_{50}$=17.2 μM), 5-cinnamoyl-6-hydroxy-3,7-dimethylbenzofuran (**6g**, $IC_{50}$=18.3 μM) and 3-tert-butyl-5-cinnamoyl-6-hydroxybenzofuran (**6d**, $IC_{50}$=18.5 μM) demonstrated significant antiproliferative activity.

Table I. *Analytical data of synthesized furan-fused chalcones.*

| Compound | 5-Cinnamoyl-6-hydroxybenzofuran (**6a**) | 3-*Tert*-butyl-5-cinnamoyl-6-hydroxy-7-methylbenzofuran (**6i**) | 5-(2,4-Dimethoxycinnamoyl)-6-hydroxy-2,3-dimethylbenzofuran (**6q**) |
|---|---|---|---|
| Weight (yield) | 0.285 g (63.3%) | 0.0377 g (29.8%) | 0.394 g (76.1%) |
| HRMS $(M + H)^+$ | Calcd for $C_{17}H_{13}O_3$, 265.0865; found 265.0864 | Calcd for $C_{22}H_{23}O_3$, 335.1647; found 335.1645 | Calcd for $C_{21}H_{21}O_5$, 353.1389; found 353.1385 |
| 1H-NMR | δ 12.8 (1H, s), 8.19 (1H, s), 7.94 (1H, d, *J*=15.2 Hz), 7.72 (1H, d, *J*=15.6 Hz), 7.68 (2H, m), 7.57 (1H, d, *J*=2.0 Hz), 7.44 (3H, m), 7.09 (1H, s), 6.75 (1H, d, *J*=1.6 Hz). | δ 13.2 (1H, s), 8.08 (1H, s), 7.94 (1H, d, *J*=15.6 Hz), 7.69 (1H, d, J=15.6 Hz), 7.68 (2H, m), 7.47 (3H, m), 7.30 (1H, s), 2.39 (3H, s), 1.45 (9H, s). | δ 13.2 (1H, s), 7.90 (1H, d, *J*=16 Hz), 7.88 (1H, s), 7.59 (1H, d, *J*=16 Hz), 7.33 (1H, dd, *J*=8, 2 Hz), 7.20 (1H, d, *J*=2 Hz), 6.96 (1H, s), 6.94 (1H, d, J=8 Hz), 3.99 (3H, s), 3.96 (3H, s), 2.36 (3H, s),2.18 (3H, s). |
| Compound | 5-Cinnamoyl-6-hydroxy-3-methylbenzofuran (**6b**) | 5-Cinnamoyl-6-hydroxy-3-phenyl-7-methylbenzofuran (**6j**) | 5-(3,4-Dimethoxycinnamoyl)-6-hydroxy-2,3-dimethylbenzofuran (**6r**) |
| Weight (yield) | 0.072 g (27.4%) | 0.0772 g (70.7%) | 0.368 g (71.1%) |
| HRMS $(M + H)^+$ | calcd for $C_{18}H_{15}O_3$, 279.1021; found 279.1019 | calcd for $C_{24}H_{19}O_3$, 355.1334; found 355.1330 | nd |
| $^1$H-NMR | δ 13.0 (1H, s), 8.03 (1H, s), 7.96 (1H, d, *J*=15.2 Hz), 7.74 (1H, d, *J*=15.6 Hz), 7.71 (2H, br. t), 7.46 (3H, m), 7.35 (1H, d, *J*=1.2 Hz), 7.03 (1H, s), 2.27 (3H, d, *J*=1.6 Hz). | δ 12.8 (1H, 2), 8.18 (1H, s), 7.96 (1H, d, *J*=15.6 Hz), 7.73 (1H, s), 7.69 (1H, d, *J*=15.6 Hz), 7.64 (4H, m), 7.54 (2H, m), 7.43 (4H, m), 2.45 (3H,s). | δ 13.3 (1H, s), 8.20 (1H, d, J=15.2 Hz), 7.89 (1H, s), 7.74 (1H, d, J=15.6 Hz), 7.65 (1H, d, J=8.4 Hz), 6.94 (1H, s), 6.57 (1H, dd, J=8.4, 2.4 Hz), 6.50 (1H, d, J=2.4 Hz), 3.94 (3H, s), 3.88 (3H, s), 2.55 (3H, s),2.17 (3H, s). |
| Compound | 5-Cinnamoyl-6-hydroxy-2,3-dimethylbenzofuran (**6c**) | 5-Cinnamoyl-6-hydroxy-3-(4-hydroxyphenyl)-7-methylbenzofuran (**6k**) | 5-Cinnamoyl-4-hydroxybenzofuran (**6s**) |
| Weight (yield) | 0.111 g (81.1%) | 0.0542 g (44.3%) | 0.385 g (87.1%) |
| HRMS $(M + H)^+$ | nd | nd | nd |
| 1H-NMR | δ 13.1 (1H, s), 7.96 (1H, s), 7.90 (1H, d, *J*=16.0 Hz), 7.74 (1H, d, *J*=16.0 Hz), 7.70 (2H, m), 7.45 (3H, m), 6.96 (1H, s), 2.35 (3H, s), 2.17 (3H, s). | δ 12.7 (1H, s), 8.13 (1H, s), 7.95 (1H, d, *J*=16.2 Hz), 7.68 (1H, d, *J*=16.2 Hz), 7.66 (3H, m), 7.55 (2H, m), 7.44 (3H, m), 7.06 (2H, m), 3.89 (3H, s), 2.44 (3H, s). | δ 13.4 (1H,s), 7.93 (1H, d, *J*=15.5 Hz), 7.86 (1H, d, *J*=8.7 Hz), 7.69 (1H, d, *J*=15.5 Hz), 7.68 (2H, m), 7.58 (1H, d, *J*=2 Hz), 7.44 (3H, m), 7.09 (1H, d, J=8.7 Hz), 7.02 (1H, d, *J*=2 Hz). |
| Compound | 3-*Tert*-butyl-5-cinnamoyl-6-hydroxybenzofuran (**6d**) | 5-Cinnamoyl-6-hydroxy-4,7-dimethoxybenzofuran (**6l**) | 5-Cinnamoyl-4-hydroxy-3-methylbenzofuran (**6t**) |
| Weight (yield) | 0.0076 g (19.7%) | 0.0731 g (35.5%) | 0.117 g (97.4 %) |
| HRMS $(M + H)^+$ | nd | nd | calcd for $C_{18}H_{15}O_3$, 279.1021; found 279.1020 |
| $^1$H-NMR | δ 13.1 (1H, s), 8.22 (1H, s), 7.95 (1H, d, *J*=15.2 Hz), 7.69 (2H, m), 7.67 (1H, d, *J*=15.2 Hz), 7.47 (3H, m), 7.28 (1H, s), 7.04 (1H, s), 1.45 (9H, s). | δ 13.2 (1H, s), 7.89 (1H, d, *J*=15.4 Hz), 7.84 (1H, d, *J*=15.4 Hz), 7.64 (2H, m), 7.51 (1H, d, *J*=2.4 Hz), 7.42 (3H, m), 6.87 (1H, d, J=2.4 Hz), 4.08 (3H, s), 4.03 (3H, s). | δ 13.2 (1H, s), 7.86 (1H, d, *J*=15.4 Hz), 7.80 (1H, d, J=8 Hz) 7.69 (2H, m), 7.67 (1H, d, *J*=15.4 Hz), 7.47 (3H, m), 7.04 (1H, s), 6.99 (1H, d, J=8 Hz), 2.44 (3H, s). |
| Compound | 5-Cinnamoyl-6-hydroxy-3-phenylbenzofuran (**6e**) | 5-Cinnamoyl-6-methoxybenzofuran (**6m**) | 5-Cinnamoyl-4-hydroxy-2,3-dimethylbenzofuran (**6u**) |
| Weight (yield) | 0.0624 g (11.9%) | 0.018 g (33.8%) | 0.115 g (80.4%) |
| HRMS $(M + H)^+$ | calcd for $C_{23}H_{17}O_3$, 341.1178; found 341.1177 | calcd for $C_{18}H_{15}O_3$, 279.1021; found 279.1019 | nd |
| $^1$H-NMR | δ 13.1 (1H, s), 8.32 (1H, s), 7.97 (1H, d, *J*=15.6 Hz), 7.71 (1H, s), 7.68 (1H, d, *J*=15.6 Hz), 7.63 (4H, m), 7.54 (2H, m), 7.44 (4H, m), 7,14 (1H, s). | δ 8.19 (1H, s), 7.63 (1H, d, *J*=15.6 Hz), 7.60 (2H, m), 7.57 (1H, d, *J*=1.6 Hz), 7.40 (1H, d, J=15.6 Hz), 7.37 (3H, m), 7.10 (1H, s), 6.75 (1H, d, J=1.6 Hz), 3.92 (3H, s). | δ13.4 (1H, s), 7.91 (1H, d, *J*=15.4 Hz), 7.73 (1H, d, J=9 Hz), 7.68 (1H, d, *J*=15.4 Hz), 7.67 (2H, m), 7.43 (3H, m), 6.93 (1H, d, J=9 Hz), 2.36 (3H, s), 2.35 (3H, s). |

Table I. *continued*

Table I. *continued*

| Compound | 5-Cinnamoyl-6-hydroxy-7-methylbenzofuran (**6f**) | 6-Hydroxy-5-(2-methoxy-cinnamoyl)-2,3-dimethylbenzofuran (**6n**) | 5-Cinnamoyl-4-hydroxy-3-phenylbenzofuran (**6v**) |
|---|---|---|---|
| Weight (yield) | 0.170 g (72.0%) | 0.331 g (69.9%) | 0.0455 g (38.3%) |
| HRMS $(M + H)^+$ | nd | nd | nd |
| $^1$H-NMR | δ 13.1 (1H, s), 8.07 (1H, s), 7.94 (1H, d, *J*=15.6 Hz), 7.75 (1H,d, *J*=15.6 Hz ), 7.69 (2H, m), 7.59 (1H, d, *J*=2.0 Hz), 7.45 (3H, m), 6.75 (1H, d, *J*=2.0 Hz), 2.43 (3H, s). | δ 13.2 (1H, s), 8.26 (1H, d, *J*=15.8 Hz), 7.89 (1H, s), 7.82 (1H, d, *J*=15.8 Hz), 7.71 (1H, dd, J=8, 2 Hz), 7.42 (1H, dt, J=8, 2 Hz), 7.03 (1H, dt, J=8, 2 Hz), 6.97 (1H, dd, J=8, 2 Hz), 6.95 (1H, s), 3.95 (3H, s), 2.35 (3H, s), 2.16 (3H, s). | δ 13.3 (1H, s), 7.94 (1H, d, *J*=15.4 Hz), 7.91 (1H, d, *J*=9.2 Hz), 7.71 (1H, d, *J*=15.4 Hz), 7.69 (4H, m), 7.62 (1H, s), 7.43 (6H, m), 7.11 (1H, d, J=9.2 Hz). |
| Compound | 5-Cinnamoyl-6-hydroxy-3,7-dimethylbenzofuran (**6g**) | 6-Hydroxy-5-(3-methoxycinnamoyl)-2,3-dimethylbenzofuran (**6o**) | 5-Cinnamoyl-4-hydroxy-3-(4-hydroxyphenyl)benzofuran (**6w**) |
| Weight (yield) | 0.0854 g (59.6%) | 0.291 g (61.5%) | 0.0488 g (48.5%) |
| HRMS $(M + H)^+$ | nd | nd | nd |
| $^1$H-NMR | δ 12.7 (1H, s), 7.95 (1H, d, *J*=15.6 Hz), 7.90 (1H, s), 7.76 (1H, d, *J*=15.6 Hz), 7.70 (2H, m), 7.46 (3H, m), 7.37 (1H, d), 2.39 (3H, s), 2.26 (3H, s). | δ 13.0 (1H, s), 8.26 (1H, d, *J*=15.4 Hz), 7.88 (1H, s), 7.71 (1H, d, *J*=15.4 Hz), 7.38 (1H, t, J=8 Hz), 7.31 (1H, dd, *J*=8, 2 Hz), 7.22 (1H, t, *J*=2 Hz), 7.00 (1H, dd, J=8, 2 Hz), 6.96 (1H, s), 3.89 (3H, s), 2.36 (3H, s), 2.18 (3H, s). | δ 12.8 (1H, s), 7.93 (1H, d, *J*=15.2 Hz), 7.90 (1H, d, *J*=8.8 Hz), 7.70 (1H, d, *J*=15.2 Hz), 7.67 (2H, m), 7.64 (2H, br d, *J*=9.2 Hz), 7.57 (1H, s), 7.44 (3H, m), 7.10 (1H, d, *J*=8.8 Hz), 7.49 (2H, br d, *J*=9.2 Hz), 3.87 (3H, s). |
| Compound | 5-Cinnamoyl-6-hydroxy-2,3,7-trimethylbenzofuran (**6h**) | 6-Hydroxy-5-(4-methoxycinnamoyl)-2,3-dimethylbenzofuran (**6p**) | 5-Cinnamoyl-4-methoxy-benzofuran (**6x**) |
| Weight (yield) | 0.136 g (81.5%) | 0.323 g (68.1%) | 0.030 mg (71.2%) |
| HRMS $(M + H)^+$ | nd | nd | calcd for $C_{18}H_{15}O_3$, 279.1021; found 279.1018 |
| $^1$H-NMR | δ 13.2 (1H, s), 7.92 (1H, d, *J*=15.6 Hz), 8.07 (1H, s), 7.74 (1H, d, *J*=15.6 Hz), 7.73 (1H, s),7.70 (2H, m), 7.44 (3H, m), 2.37 (3H, s), 2.36 (3H, s), 2.61 (3H, s). | δ 13.3 (1H, s), 7.92 (1H, d, *J*=16 Hz), 7.88 (1H, s), 7.67 (2H, br d, *J*=8.6 Hz), 7.62 (1H, d, *J*=16 Hz), 6.97 (2H, br d, J=8.6 Hz), 6.95 (1H, s), 3.88 (3H, s), 2.35 (3H, s), 2.18 (3H, s). | δ 7.86 (1H, s), 7.66 (1H, d, *J*=16 Hz), 7.62 (1H, d, *J*=8 Hz), 7.60 (2H, m), 7.47 (1H, d, *J*=16 Hz), 7.39 (3H, m), 7.28 (1H, d, *J*=8 Hz), 7.26 (1H, d, *J*=0.8 Hz), 6.99 (1H, d, *J*=0.8 Hz), 4.08 (3H, s). |

HRMS; High-resolution mass spectrum, $(M + H)^+$; molecular mass of protonated molecule, $^1$H-NMR; $^1$H nuclear magnetic resonance, s; singlet, d; doublet, dd; double doublet, t; triplet, m; multiplet, J; coupling constant given in hertz, nd; not determined.

*Structure and activity relationship*. Importance of the fused furan ring on the A ring moiety of chalcone was clearly indicated by the comparison between **8** and **9**; the biological activity of **8**, which was the furan-fused derivative of **9**, was significantly enhanced ($IC_{50}$=17.2 μM for **8** and 305 μM for **9**) (Table II). Similar potentiation of antiproliferative activity by attachment of a furan-ring was observed for several coumarins (16). However, the effect of furan-ring attachment on **7** was not simple, because **7**, which has an asymmetrical structure, gave two structural isomers through the furan-ring attachment, namely **6a** and **6s**. The furan-ring attachment increased activity in **6a**, while reduced the activity in **6s**; $IC_{50}$ values for **7**, **6a** and **6s** were 59.6, 20.9 and 70.8 μM, respectively. These results suggested the importance of the relative position of benzofuran and phenyl moieties in FFCs. The hydrogen bond between the phenolic hydrogen and the carbonyl oxygen fixed the free rotation of the C5-C1' bond and determined the orientation of the cinnamoyl group, causing the linear shape of **6s** and the bent shapes of **6a** and **8** (Figure 3). Similarly, comparison of the activity of **6b** *versus* **6t**, **6c** *versus* **6u** and **6e** *versus* **6v** showed that the bent shape enhanced the activity of FFCs. The existence of the hydrogen bond was indicated by the characteristic values of chemical shifts of the phenolic hydrogen; for example δ12.8 for **6a** and δ 13.4 for **6s**.

Table II. *Half-maximal inhibitory concentration and log P of fused-furan chalcones.*

| Compd# | Substituents | | | | | | $IC_{50}$ (μM) | log *P* |
|---|---|---|---|---|---|---|---|---|
| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | $R^6$ | | |
| **6a** | H | H | H | H | OH | H | 20.9 | 2.81 |
| **6b** | H | Me | H | H | OH | H | 22.2 | 3.30 |
| **6c** | Me | Me | H | H | OH | H | 29.7 | 3.63 |
| **6d** | H | t-Bu | H | H | OH | H | 18.5 | 4.51 |
| **6e** | H | Ph | H | H | OH | H | 12.3 | 4.48 |
| **6f** | H | H | H | Me | OH | H | 16.1 | 3.30 |
| **6g** | H | Me | H | Me | OH | H | 18.3 | 3.78 |
| **6h** | Me | Me | H | Me | OH | H | >200 | 4.12 |
| **6i** | H | t-Bu | H | Me | OH | H | 41.5 | 5.00 |
| **6j** | H | Ph | H | Me | OH | H | 47.5 | 4.97 |
| **6k** | H | 4-OH-Ph | H | Me | OH | H | 77.9 | 4.36 |
| **6l** | H | H | OMe | OMe | OH | H | 58.3 | 2.56 |
| **6m** | H | H | H | H | OMe | H | 22.2 | 3.07 |
| **6n** | Me | Me | H | H | OH | 2-OMe | 46.3 | 3.51 |
| **6o** | Me | Me | H | H | OH | 3-OMe | >200 | 3.51 |
| **6p** | Me | Me | H | H | OH | 4-OMe | >200 | 3.51 |
| **6q** | Me | Me | H | H | OH | 2,4-diOMe | >200 | 3.38 |
| **6r** | Me | Me | H | H | OH | 3,4-diOMe | 42.6 | 3.38 |
| **6s** | H | H | OH | H | H | H | 70.8 | 2.81 |
| **6t** | H | Me | OH | H | H | H | 75.2 | 3.30 |
| **6u** | Me | Me | OH | H | H | H | >400 | 3.63 |
| **6v** | H | Ph | OH | H | H | H | 66.8 | 4.48 |
| **6w** | H | 4-OH-Ph | OH | H | H | H | 98.3 | 4.36 |
| **6x** | H | H | OMe | H | H | H | 19.9 | 3.07 |
| **7** | - | - | - | - | - | - | 59.6 | 2.81 |
| **8** | - | - | - | - | - | - | 17.2 | 2.81 |
| **9** | - | - | - | - | - | - | 305 | 2.81 |

$IC_{50}$; Half-maximal inhibitory concentration (μM), *P*; octanol-water partition coefficient. Calculation of log *P* was carried out by ChemBioDraw Ultra 12.0 (Cambridge Soft).

Substitution of phenyl (**6e**) or *tert*-butyl group (**6d**) at C3 position of 6a enhanced activity; $IC_{50}$ values for **6a**, **6d** and **6e** were 20.9, 18.5 and 12.3 μM, respectively. However, this did not happen in the case of C7 methylated derivatives; the phenyl (**6j**) or *tert*-butyl (**6i**) substituted derivatives showed decreased activity when compared with **6f**; $IC_{50}$ values for **6f**, **6i** and **6j** were 16.1, 41.5 and 47.5 μM, respectively. The difference could partly be attributed to the elevated lipophilicity of C7-methylated compounds; log *P* values of **6i** and **6j** were higher than those of the corresponding C7-hydrogen compounds; log *P* values for **6i**, **6j**, **6d** and **6e** were 5.00, 4.97, 4.51 and 4.48, respectively. C7 methylation enhanced the activity of **6f**, **6g** and **6h**, whereas weakened **6i** and **6j**.

The B-ring methoxylated derivatives (**6n**, **6o**, **6p**, **6q** and **6r**) showed weakened activity when compared with **6c**. Similar activity-weakening phenomena were reported in 1-azaflavanones (17) and polymethoxyflavones (18).

In summary, the data presented herein demonstrate the importance of the spatial relationship between benzofuran and B rings of chalcone for their antiproliferative activity. We hope that these results present an insight into the importance of aryl ring orientation about the rotatable bonds between two aromatic rings in influencing antiproliferative activity.

## References


1 Dimmock JR, Elias DW, Beazely MA and Kandepu NM: Bioactivities of Chalcones. Curr Med Chem *6*: 1125-1149, 1999.

2 Cheng JH, Hung CF, Yang SC, Wang JP, Won SJ and Lin CN: Synthesis and cytotoxic, anti-inflammatory, and anti-oxidant activities of 2',5'-dialkoxychalcones as cancer chemopreventitative agents. Bioorg Med Chem *16*: 7270-7276, 2008.

3 Avila HP, Smânia EF, Monache FD and Smânia A Jr: Structure–activity relationship of antibacterial chalcones. Bioorg Med Chem *16*: 9790-9794, 2008.

4 Tomar V, Bhattacharjee G, Kamaluddin and Kumar A: Synthesis and antimicrobial evaluation of new chalcones containing piperazine or 2,5-dichlorothiophene moiety. Bioorg Med Chem Lett *17*: 5321-5324, 2007.

5 López SN, Castelli MV, Zacchino SA, Domínguez JN, Lobo G, Charris-Charris J, Cortés JC, Ribas JC, Devia C, Rodríguez AM and Enriz RD: *In vitro* antifungal evaluation and structure-activity relationships of a new series of chalcone derivatives and synthetic analogues, with inhibitory properties against polymers of the fungal cell wall. Bioorg Med Chem *8*: 1999-2013, 2001.
6 Vargas MLY, Castelli MV, Kouznetsov VV, Urbina GJM, Lopez SN, Sortino M, Enriz RD, Ribas JC and Zacchino S: *In vitro* antifungal activity of new series of homoallylamines and related compounds with inhibitory properties of the synthesis of fungal cell wall polymers. Bioorg Med Chem *11*: 1531-1550, 2003.
7 Sortino M, Delgado P, Juarez S, Quiroga, J, Abonia R, Insuasty B, Nogueras M, Rodero L, Garibotto FM, Enriz RD and Zacchino SA: Synthesis and antifungal activity of (Z)-5-arylidenerhodanines. Bioorg Med Chem *15*: 484-494, 2007.
8 Katsori AM and Hadjipavlou-Litina D: Chalcones in cancer: Understanding their role in terms of QSAR. Curr Med Chem *16*: 1062-1081, 2009.
9 Achanta G, Modzelewska A, Feng L, Khan SR and Huang P: A boronic-chalcone derivative exhibits potent anticancer activity through inhibition of the proteasome. Mol Pharmacol *70*: 426-433, 2006.
10 Modzelewska A, Pettit C, Achanta G, Davidson NE, Huang P and Khan SR: Anticancer activities of novel chalcone and bis-chalcone derivatives. Bioorg Med Chem *14*: 3491-3495, 2006.
11 Nimmanapalli PR, Aparoya P, Mohan Reddy C, Achari C, Ramu Sridhar P and Reddanna P: Design, synthesis, and biological evaluation of prenylated chalcones as 5-LOX inhibitors. Bioorg Med Chem *18*: 5807-5815, 2010.
12 Magalhães AF, Tozzi AM, Magalhães EG, Blanco IS and Soriano MD: Flavonoids from Lonchocarpus muehlbergianus. An Acad Bras Cienc *76*: 651-661, 2004.
13 Decharchoochart P, Suthiwong J, Samatiwat P, Kukongviriyapan V and Yenjai C: Cytotoxicity of compounds from the fruits of Derris indica against cholangiocarcinoma and HepG2 cell lines. J Nat Med *68*: 730-736, 2014.
14 Rao RR, Tiwari AK, Reddy PP, Babu KS, Suresh G, Ali AZ, Madhusudana K, Agawane SB, Badrinarayan P, Sastry GN and Rao JM: Synthesis of antihyperglycemic, α-glucosidase inhibitory, and DPPH free radical scavenging furanochalcones. Med Chem Res *21*: 760-774, 2012.
15 Kawaii S, Tomono Y, Katase E, Ogawa K and Yano M: Antiproliferative activity of flavonoids on several cancer cell lines. Biosci Biotechnol Biochem *63*: 896-899, 1999.
16 Kawaii S, Tomono Y, Ogawa K, Sugiura M, Yano M, Yoshizawa Y, Ito C and Furukawa H: Antiproliferative effect of isopentenylated coumarins on several cancer cell lines. Anticancer Res *21*: 1905-1911, 2001.
17 Kawaii S, Endo K, Tokiwano T and Yoshizawa Y: Relationship between structure and antiproliferative activity of 1-azaflavanones. Anticancer Res *32*: 2819-2825, 2012.
18 Kawaii S, Ikuina T, Hikima T, Tokiwano T and Yoshizawa Y: Relationship between structure and antiproliferative activity of polymethoxyflavones towards HL60 Cells. Anticancer Res *32*: 5239-5244, 2012.

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