Synthesis and biological evaluation of the novel antitumor agent 3'-monosubstituted chalcone derivatives

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Abstract. Seven novel 3'-mono-substituted chalcone derivatives were synthesized from 2,4-dihydroxyacetophenone in 4 steps in 18-31% overall yield. Their structures were confirmed by ^{1}H NMR. The anticancer activity of these new target compounds **(4~10)** against human leukemia K562 cells were evaluated by MTT assay *in vitro*. This study leads to the identification of one highly active molecule, compounds **10** (IC_{50} = 5.60 μM) against K562.

Introduction

Chalcones are natural or synthetic compounds belonging to the flavonoid family [1]. Chalcones possess many pharmacological activities [2], antitumor [3], anti-microbial [4], antimalarial [5], anti-bacterial [6], anti-oxidant [7], anti-tubercular [8], anti-angiogenic [9] and antileishmanial [10] properties. In the past decades, many research work on this kind of compounds have been reported, however most of them were focused on hydroxyl group-related derivatives, synthesis and biological evaluation of 3 ' mono-substituted carbon-carbon coupling chalcone derivatives have rarely been reported. In this paper, we would like to report the synthesis and biological evaluation of the novel antitumor agent 3'-monosubstituted chalcone derivatives.

Materials and methods

Synthetic route of chalcone derivatives.

The synthetic approaches of target compounds **1~10** were demonstrated in Figure1.

Synthesis of 1-(2,4-dihydroxy-3-iodophenyl)ethanone (compound 1)

2,4-dihydroxyacetophenone (5.00 g) in 20 mL ethanol was added I_2 (4.20 g) and KIO_3 (0.70 g) in 5 mL H_2O. The reaction was stirred at room temperature for 4~6 h. The reaction was diluted with water and extracted with ethyl acetate, the excess I_2 of the reaction was removed with a saturated solution of sodium thiosulfate. The organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 5:1) to afford compound **1** (7.50 g , 84%).

Synthesis of 1-(3-iodo-2,4-dimethoxyphenyl)ethanone (compound 2)

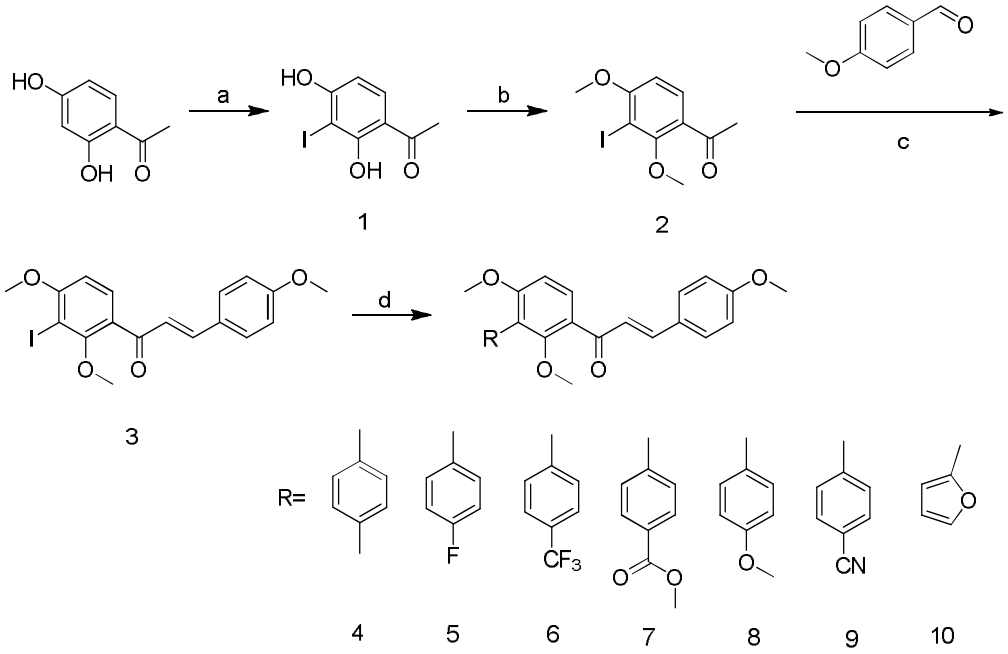

Fig.1 Synthetic route of chalcone derivatives

Reagents and conditions: (a) I_2 , KIO_3 , EtOH , H_2O , r.t. 4~6 h (b) CH_3I , DMF, r.t.6 h (c) 60% KOH , EtOH , r.t. 12 h (d) KOAc , (dppf) $PdCl_2$, 100 ℃ , 5h

1-(2,4-dihydroxy-3-iodophenyl)ethanone (5 g) in 20 mL DMF was added K_2CO_3 (3.23 g) and CH_3I (3 g). The reaction was stirred at room temperature for 6 h. Then the reaction mixture was poured into ice-water and extracted with ethyl acetate for 3 times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 5:1) to afford compound **2** (4.50 g , 81%).

Synthesis of (E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 3)

1-(3-iodo-2,4-dimethoxyphenyl)ethanone (2.00 g) in 10 mL ethanol was added anisic aldehyde (0.94 g) and KOH (5 mL, 60% aq) at 0 ℃. Then the reaction mixture was warmed to room temperature and stirred for 12 h. The reaction was diluted with water and extracted with ethyl acetate, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 15:1) to afford compound **3** (2.50 g , 90%).

Synthesis of (E)-1-(2,6-dimethoxy-4'-methyl-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 4)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-methylphenylboronic acid (96.1 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h in sealed tube. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **4** (60 mg , 35%).

Synthesis of (E)-1-(4'-fluoro-2,6-dimethoxy-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 5)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-fluorophenylboronic acid (98.9 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h. The reaction was diluted with water and extracted with ethyl acetate for 3 times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **5** (100 mg , 45%).

Synthesis of (E)-1-(2,6-dimethoxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 6)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-(trifluoromethyl) phenylboronic acid (134 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h in sealed tube. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **6** (80 mg , 40%).

Synthesis of (E)-methyl 2',6'-dimethoxy-3'-(3-(4-methoxyphenyl)acryloyl)-[1,1'-biphenyl]-4-carboxylate (compound 7)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-(methoxycarbonyl) benzeneboronic acid (135.7 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **7** (55 mg , 28%).

Synthesis of (E)-3-(4-methoxyphenyl)-1-(2,4',6-trimethoxy-[1,1'-biphenyl]-3-yl)prop-2-en-1-one (compound 8)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-methoxyphenylboronic acid (107.5 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h in sealed tube. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **8** (95 mg , 50%).

Synthesis of (E)-2',6'-dimethoxy-3'-(3-(4-methoxyphenyl)acryloyl)-[1,1'-biphenyl]-4-carbonitrile (compound 9)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 4-cyanophenylboronic acid (103.9 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h in sealed tube. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **9** (70 mg , 35%).

Synthesis of (E)-1-(3-(furan-2-yl)-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 10)

(E)-1-(3-iodo-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (200 mg) in 2 mL DMSO was added 2-furanylboronic acid (79.1 mg), KOAc (138 mg) and (dppf) $PdCl_2$ (17 mg). The reaction was stirred at 100℃ for 5 h. The reaction was diluted with water and extracted with ethyl acetate for three times, the organic layer was dried over Na_2SO_4. The solvent was removed under vacuum to provide the crude which was purified by flash column chromatography (silica gel, petroleum ether/ethyl acetate 8:1) to afford compound **10** (65 mg , 41%).

Biological assay.

K562 cells (100 μL) were cultured in 96-well plates at a density of 5×10^4 cells/mL for 2 hours. 0.5 μL of compounds solved in DMSO were added to each well to culture for additional 48 hours. MTT assay was then performed using Thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The IC_{50} values for each compound were calculated from three independent experiments by nonlinear regression using Graph Pad Prism 5.0.

Results and discussion

Characterize target chalcone derivatives (compound 4~10) by ^{1}HNMR.

(E)-1-(2,6-dimethoxy-4'-methyl-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 4) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 2.41(s, 3H), 3.39(s, 3H),3.80(s, 3H), 3.84(s, 3H),6.83(d, *J* = 8.8Hz, 1H), 6.90(d, *J* = 8.8Hz, 2H), 7.24~7.31(m, 3H), 7.45(d, *J* = 15.6Hz, 1H), 7.56(d, *J* = 8.8Hz, 2H), 7.6~7.75(m, 2H)

2(E)-1-(4'-fluoro-2,6-dimethoxy-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 5) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.38 (s, 3H), 3.81 (s, 3H), 3.84 (s, 3H), 6.84 (d, *J* = 8.8Hz, 1H), 6.91 (d, *J* = 8.8Hz, 2H), 7.13 (t, *J* = 8.8Hz, 2H), 7.36~7.44 (m, 3H), 7.57 (d, *J* = 8.8Hz, 2H), 7.70~7.80(m, 2H)

(E)-1-(2,6-dimethoxy-4'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 6) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.40(s, 3H), 3.81(s, 3H), 3.84(s, 3H), 6.86(d, *J* = 8.8Hz, 1H), 7.42(d, *J* = 15.6Hz, 1H), 7.52~7.58(m, 4H), 7.68~7.74(m, 3H), 7.79(d, *J* = 8.8Hz, 1H)

(E)-methyl 2',6'-dimethoxy-3'-(3-(4-methoxyphenyl)acryloyl)-[1,1'-biphenyl]-4-carboxylate (compound 7) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.38(s, 3H), 3.80(s, 3H), 3.83(s, 3H), 3.94(s, 3H), 6.85(d, *J* = 8.8Hz, 1H), 6.91(d, *J* = 8.8Hz, 1H), 7.42(d, *J* = 16Hz, 1H), 7.50(d, *J* = 8.4Hz, 2H), 7.57(d, *J* = 8.8Hz, 2H), 7.72(d, *J* = 8.8Hz, 1H), 7.77(d, *J* = 8.8Hz, 1H), 8.11(d, *J* = 8.4Hz, 2H)

(E)-3-(4-methoxyphenyl)-1-(2,4',6-trimethoxy-[1,1'-biphenyl]-3-yl)prop-2-en-1-one (compound 8) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.38(s, 3H), 3.81(s, 3H), 3.84(s, 3H), 3.86(s, 3H), 6.83(d, *J* = 8.8Hz, 1H), 6.90(d, *J* = 8.8Hz, 2H), 6.90(d, *J* = 8.8Hz, 2H), 6.99(d, *J* = 8.8Hz, 1H), 7.34(d, *J* = 8.8Hz, 1H), 7.45(d, *J* = 16Hz, 1H), 7.57(d, *J* = 8.8Hz, 2H), 7.68~7,74(m, 2H),

(E)-2',6'-dimethoxy-3'-(3-(4-methoxyphenyl)acryloyl)-[1,1'-biphenyl]-4-carbonitrile (compound 9) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.39 (s, 3H), 3.82 (s, 3H), 3.85 (s, 3H), 6.86 (d, *J* = 8.8Hz, 1H),

6.92 (d, *J* =8.8Hz, 2H), 7.39 (d, *J* = 15.6Hz, 1H), 7.53~7.58 (m, 4H), 7.69~7.74 (m, 4H), 7.78 (d, *J* = 8.8Hz, 1H)

(E)-1-(3-(furan-2-yl)-2,4-dimethoxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (compound 10) by ^{1}HNMR.

^{1}HNMR(400MHz,$CDCl_3$):δ/ppm 3.58 (s, 3H), 3.84 (s, 3H), 3.89 (s, 3H), 6.55 (dd, *J* = 3.2Hz,2Hz, 1H), 6.64 (dd, *J* = 3.2Hz,0.4Hz, 1H), 6.82 (d, *J* = 8.8Hz, 1H), 6.90 (d, *J* = 8.8Hz, 2H), 7.44 (d, *J* = 15.6Hz, 1H), 7.56~7.60 (m, 3H), 7.68~7.74 (m, 2H)

Anticancer activity assay.

All the target compounds were tested for their in vitro anticancer activity against K562 cells by MTT assay. The results were presented in Table 1.

Table 1 Inhibition Activity of the compounds **4**~**10** against K562 cells

Compounds	Chalcone	**4**	**5**	**6**	**7**	**8**	**9**	**10**
IC_{50}, μM	>10	>10	>10	>10	>10	>10	>10	5.60

Conclusion

In summary, we synthesized a series of 3'-mono-substituted chalcone derivatives.The *in vitro* antitumor activities of the newly chalcone derivatives on human tumor cell K562 were evaluated by MTT assay. Among them, compound **10** has good antitumor activity against K562 cells (IC_{50} = 5.60 μM) .Further modification based on compound **10** was undergoing in our lab.

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