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Inhibitory activity of prostaglandin E_2 production by the synthetic 2′-hydroxychalcone analogues: Synthesis and SAR study

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ABSTRACT

A series of 2′-hydroxychalcones has been synthesized and screened for their in vitro inhibitory activities of cyclooxygenase-2 catalyzed prostaglandin production from lipopolysaccharide-treated RAW 264.7 cells. Structure–activity relationship study suggested that inhibitory activity against prostaglandin E_2 production was governed to a greater extent by the substituent on B ring of the chalcone, and most of the active compounds have at least two methoxy or benzyloxy groups on B ring. The relationship between chalcone structures and their PGE_2 inhibitory activities was also interpreted by docking study on cyclooxygenase-2.

Chalcones, originally isolated from natural sources and considered as precursors of flavonoids and isoflavonoids, are abundant in edible plants. Chalcones are open-chain flavonoids in which two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-ones). Being as a minority subgroup of the flavonoid family, like other members, chalcones have been reported responsible for a variety of biological activities, including antiviral,[1,2] anticancer,[3–7] antimicrobial,[8,9] anti-inflammatory,[3,10–17] antioxidative,[6,7] antimalarial,[18] anti-leishmania,[19] antinociceptive,[20] and antiproliferative[21] activities. Hence, chalcones are considered as a class of important therapeutic potentials.

With respect to anti-inflammatory activity, several studies indicated that chalcone derivatives potently inhibit NO production by the inducible nitric oxide synthase (iNOS) catalyzed NO production by the different cellular mechanisms, iNOS down-regulation and/or iNOS inhibition, depending on their chemical structures.[12,15,16] However, chalcone derivatives also inhibited cyclooxygenase-2 (COX-2) catalyzed prostaglandin production[14] as well as 5-lipoxygenase.[11]

In this study, various 2′-hydroxychalcones with different substituents in the B ring were synthesized using a classical base catalyzed condensation reaction and tested for anti-inflammatory activities as well as for cytotoxicity.

The preparation of the 2′-hydroxychalcone derivatives (Table 1) was carried out via Claisen–Schmidt condensation (Scheme 1). Thus, an appropriate aryldehyde derivative was reacted with 2′-hydroxyl-acetophenone in MeOH/KOH to give the corresponding 2′-hydroxychalcone precipitated as the potassium salt. Subsequent treatment with HCl yielded the desired product (**1–18**) with an average yield of 46–88% (Table 1).[14,22] Their structures, established with ^{1}H NMR spectra, showed that the *E*-isomers were specifically generated following the reaction. The physical and analytical data for the new compounds **7** and **12** are presented in references and notes.[23,24]

All synthesized 2′-hydroxychalcone analogues were screened for their activities on PGE_2 production in the mouse macrophage-like cell line RAW 264.7, stimulated by lipopolysaccharide (LPS) at concentration of 10 μM.[14,25,26] The inhibitory activity of prostaglandin E_2 (PGE_2) production are contributed by two enzymes including COX-2 and PG synthase (down-regulating COX-2 induction). However in this study, RAW cells were pre-incubated with LPS for COX-2 induction, after that test compounds and arachidonic acid were added to examine the direct inhibitory activity against COX-2.[27] The inhibitory activities of synthetic chalcones on COX-2 catalyzed PGE_2 production from LPS-induced RAW 264.7 cells were estimated and shown in Table 1. Among 18 synthesized chalcones, six compounds including 4-benzyloxy-2′-hydroxychalcone (**9**), 3,4-dibenzyloxy-2′-hydroxychalcone (**13**), 3-benzyloxy-4-methoxy-2′-hydroxychalcone (**14**), 2,3-dimethoxy-2′-hydroxychalcone (**15**), 2,4-dimethoxy-2′-hydroxychalcone (**16**), and 3,4,5-trimethoxy-2′-hydroxychalcone (**17**) showed potential

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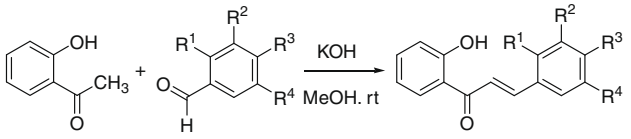
Table 1
Chemical structures, reaction conditions and biological activities of 2′-hydroxychalcone analogues

Chalcone	B ring substituents				Yield (%)	Reaction conditions		PGE_2 inhibition @ 10 μM[a] (%)	IC_{50}[a] (μM)	Cell viability @ 10 μM[a] (%)
	R^1	R^2	R^3	R^4		Reaction time (h)	Sol. for crystallization			
1	H	H	H	H	46	16	MeOH	60	—	119
2	H	H	Cl	H	85	12	MeOH	60	—	141
3	H	H	Br	H	58	24	MeOH/CH_2Cl_2	24	—	162
4	H	H	CH_3	H	74	16	MeOH	62	—	152
5	H	H	OCH_3	H	65	24	MeOH	32	—	115
6	H	H	SCH_3	H	58	12	MeOH/CH_2Cl_2	62	—	85
7	H	H	OCF_3	H	68	12	MeOH/CH_2Cl_2	18	—	87
8	H	H	Ph	H	63	12	MeOH	11	—	135
9	H	H	OBn	H	58	16	MeOH/CH_2Cl_2	*102*	4.1	99
10	H	Br	H	H	66	12	MeOH	51	—	90
11	H	Cl	Cl	H	88	12	MeOH	48	—	155
12	H	Br	OCH_3	H	65	12	MeOH/CH_2Cl_2	55	—	159
13	H	OBn	OBn	H	62	10	MeOH/CH_2Cl_2	*100*	4.6	103
14	H	OBn	OCH_3	H	59	16	MeOH/CH_2Cl_2	*93*	—	102
15	OCH_3	OCH_3	H	H	58	28	MeOH/CH_2Cl_2	*101*	3.8	93
16	OCH_3	H	OCH_3	H	67	12	MeOH/CH_2Cl_2	*98*	—	107
17	H	OCH_3	OCH_3	OCH_3	55	16	MeOH/CH_2Cl_2	*102*	6.2	65
18	H	–O–CH_2–O–		H	59	12	MeOH	< 0	—	118
NS-398[b]	—	—	—	—	—	—	—	109	0.05	104
Wogonin[b]	—	—	—	—	—	—	—	103	1.07	111

[a] All values represented here were mean of three experiments.
[b] Wogonin (5,7-dihydroxy-8-methoxyflavone) and NS-398 (*N*-(2-cyclohexyloxy)-4-nitromethanesulfonamide) were used as reference compounds.



Scheme 1. Synthesis of 2′-hydroxychalcone derivatives.

inhibitory activity against PGE_2 production with inhibition values larger than 90% (italicized in Table 1) and IC_{50} in range 3.8–6.2 μM. In term of structure–activity relationship (SAR), most of the active compounds possess at least two alkoxy groups (methoxy and/or benzyloxy) in the B ring, except compound **9** which has an unique benzyloxy group at 4-position.

The chalcone **9** (IC_{50} = 4.1 μM) also showed 1.6- to 9.2-fold stronger of inhibitory activity against COX-2 catalyzed prostaglandin production than compounds **1–8** which possess a 4-substituent of either hydro/chloro/bromo/methyl/methoxy/methylthio/trifluoromethy/phenyl group, respectively. Introducing either a strong electron withdrawal (Cl, Br, CF_3) group or a weak electron donating group (SCH_3, CH_3, OCH_3) to B ring of 2′-hydroxychalcone (**1**) does not influent PGE_2 production inhibition. However, the presence of a strong electron donating group at 4-position of 2′-hydroxychalcone (e.g., chalcone **9** containing benzyloxy moiety) exhibited a stronger activity than that of other groups. Bioactivity results indicated that the benzyloxy group at 4-position of 2′-hydroxychalcone contributed an important effect on the PGE_2 inhibitory activity. Docked chalcone-**9**:COX-2 (pdb 1cx2) complexes indicated the importance of oxygen of the benzyloxy moiety at 4-position of 2′-hydroxychalcone (Fig. 1).[28,29] This substituent leads to formation of both hydrogen bond and/or π-cation interaction with Arg120 residue, thus plays a critical role on the interaction between COX-2 and **9**. Conversely, no hydrogen bond was observed for chalcone **8** with the phenyl moiety at the same position versus compound **9** that explained why chalcone **8** achieved a weak inhibitory activity of prostaglandin E_2 production (Fig. 1).

Chalcone, containing more than two alkoxy groups on B ring in which one group substituted at 4-position and the other substituted at 3- or 5-position of B ring may enhance inhibitory activity toward PGE_2 production. However, the presence of 3,4-dioxymethylene in B ring (**18**) destroyed anti-inflammatory activity of chalcones. Chalcone **17** (2′-hydroxy-3,4,5-trimethoxychalcone) with

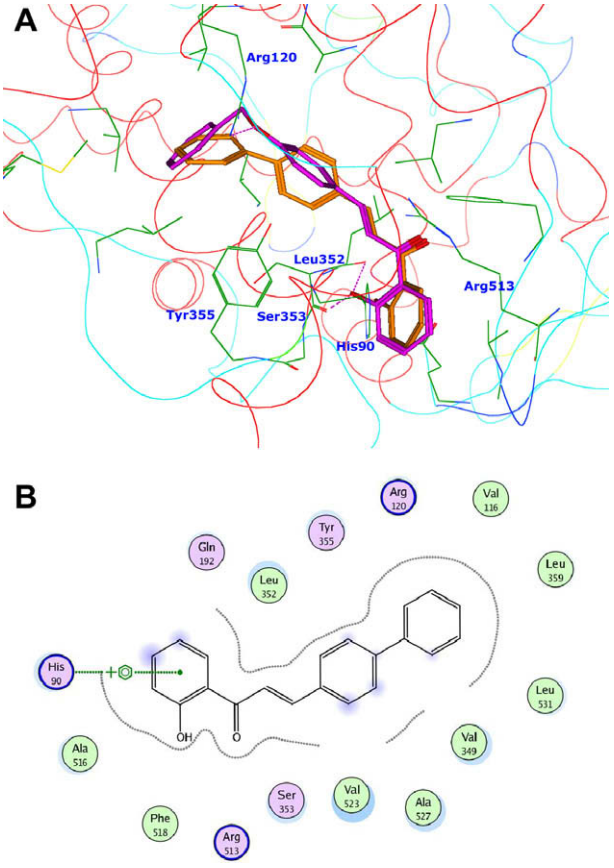


Figure 1. Relative position of chalcone **9** (magenta carbon) and chalcone 8 (orange carbon) in the active site of COX-2 generated by MOE docking (A). The hydrogen bonds (magenta dotted lines) within the binding site are indicated for compound **9**. The benzyloxy moiety at 4-position of 2′-hydroxychalcone established a strong interaction with Arg120 of COX-2 via both H-bond and π-cation interaction. 2D interactions between **8** and COX-2 showed in (B) with no hydrogen bond observed.

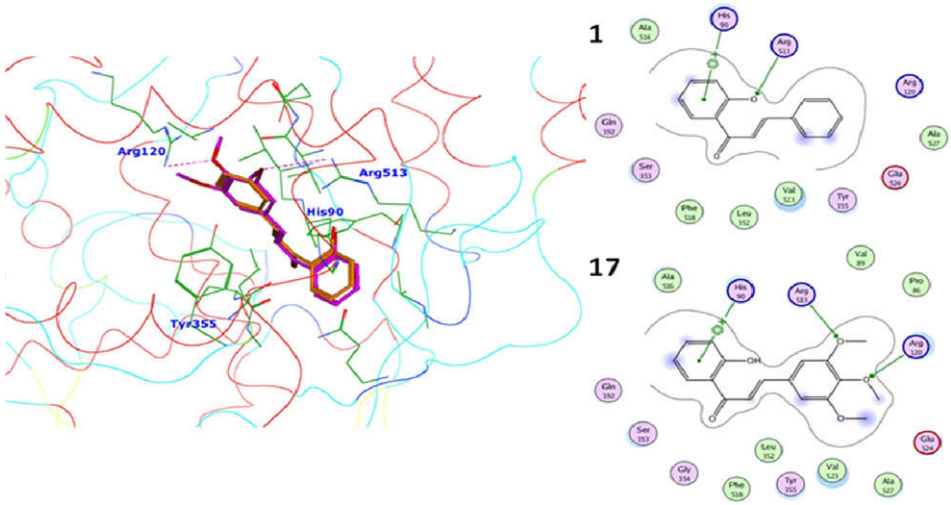


Figure 2. Docked conformation alignment of chalcone **17** (magenta carbon) and its original scaffold **1** (orange carbon) in COX-2 binding site generated by MOE docking (left side). In right side, 2D ligand-interactions between these chalcones and COX-2 are also showed. Three methoxy moieties presented in B ring of **17** contributed the additional hydrogen bonds (magenta dotted lines) with Arg120 and Arg513 of COX-2.

three methoxy groups in B ring formed additional hydrogen bonds (magenta dotted lines) with Arg120 and Arg513 of COX-2 in comparison with its original scaffold **1** (Fig. 2). At concentration of 10 μM, chalcone **17** markedly inhibit the PGE_2 production (102.3%). In addition by MTT cell viability assay,[29] compound **17** also exhibited a cytotoxic effect that may provide a template to design new novels with a dual activity of anti-inflammation and anticancer. Except chalcone **17**, most synthetic 2′-hydroxychalcones did not show cytotoxicity or less than 10% cell reduction as assessed by MTT assay indicating that they were not significant cytotoxicity against RAW 264.7 cells even in the presence or absence of LPS (Table 1).[30] Moreover, the significant increase of cell viability by synthesized chalcones (**1–5, 7, 11, 12, 16, 18)** in the MTT test were observed. Direct cell counting test is currently investigating for these compounds to figure out which reason, due to an increased cell number or an increased metabolism of the dehydrogenase, leads to the increase of cell viability.

In the literature, 5,7-diacetylflavone, which is obtained by means of the subsequent ring closure of the corresponding 2′-hydroxychalcone, strongly inhibited COX-2 (IC_{50} = 2.7 μM).[31] Molecular modeling showed that 5,7-diacetylflavone fits well into the binding pocket of COX-2 and their model suggested that a hydrogen bond exists between the oxygen of the ketone group at the 7-position and the hydroxy group of Tyr355.[31] Docking into the COX-2 active site, 2′-hydroxychalcone analogues showed a positioning similar to 5,7-diacetylflavone with A ring tend to place near Tyr355 more than B ring. However, no hydrogen bond with Tyr355 was formed for 2′-hydroxychalcone derivatives. It seems not surprising that the synthetic compounds do not contain any substituent in A ring and also no hydrogen bond with Tyr355 was observed for other derivatives of chrysin.[31] Conversely, hydrogen bonds were formed between those 2′-hydroxychalcone compounds and Arg120, Arg513.

In summary, 18 2′-hydroxychalcone derivatives were synthesized and evaluated for their PGE_2 inhibitory activities and cytotoxicity. Among them, six chalcones showed better desired biological activities than that of parent compound 2′-hydroxychalcone. The structural requirements for the inhibitory activity of 2′-hydroxychalcone analogues on PGE_2 production from RAW 264.7 cells may be drawn as follows: (i) the concomitance of at least two alkoxy groups on B ring of chalcones in which one group substituted at 4-position and the other substituted at 3- or 5-position of B ring may enhance inhibitory activity toward PGE_2 production; (ii) the benzyloxy moiety plays an important role on establishing strong interactions between chalcone and COX-2; (iii) the inhibition of PGE_2 production from RAW 264.7 cells of 2-hydroxychalcone derivatives is not associated with their cytotoxicity. The SAR information is meaningful to design and develop new compounds with higher anti-inflammatory activity.

Acknowledgments

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22. *General procedure*: 2′-hydroxyacetophenone (5 mmol) and benzaldehyde derivatives (5 mmol) were dissolved in methanol (10 mL) with stirring. Potassium hydroxide (15 mmol) was added in portions to give a blood-red solution. Resulting solution was stirred for 8–28 h, during which 2′-hydroxychalcone precipitated as the potassium salt. The solution/suspension was poured into cold 1 N HCl (10 mL), and further concentrated HCl was added until the solution was acidic. The resulting yellow solid was filtered, washed with water (2×20 mL), and recrystallized from corresponding solvent (MeOH or MeOH/CH_2Cl_2) to give the product.
23. *2′-Hydroxy-4-trifluoromethoxychalcone* (**7**): The product was obtained as yellow solid, mp 83–85 °C. UV (MeOH, λ_{max}): 203; 221.5 and 309.5 nm. IR (ν cm^{-1}) 1643.2; 1577.7; 1205.4; 1157.2; 754.1 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$), δ: 12.94 (s, 1H, 2′-OH), 7.92–7.97 (d, 1H, J = 8.0 Hz, H6′); 7.86–7.94 (d, 1H, J = 15.4 Hz, H_β); 7.68–7.72 (d, 1H, J = 8.6 Hz, H4′); 7.67–7.73 (m, 2H, J = 8.8 Hz, 2.8 Hz, H2, H6); 7.52–7.59 (d, 1H, J = 15.2 Hz, H_α), 7.48–7.56 (d, 1H, J = 8.4 Hz, 1.6 Hz, H5′); 7.26–7.30 (d, 1H, J = 8.2 Hz, H3′); 6.92–7.06 (m, 2H, J = 8.8 Hz, 8.2 Hz, 8.2 Hz, 2.8 Hz, H2 and H6). ^{13}C NMR (50 Hz, $CDCl_3$, ppm), δ: 194.2 (C4); 164.4 (C4 and C2′); 144.3 (C_β); 137.3 (C4′); 133.9 (C6′); 130.8 (C1, C2, and C6); 130.3 (C1′) and 121.9 (C_α); 121.7 (CF_3); 120.6 (C5′); 119.6 (C3′); 119.4 (C3, C5). Anal. ($C_{16}H_{11}F_3O_2$) C, H, O. m/z 309 (M^+, 70), 308 (100), 307 (95), 188 (68), 147 (94), 121 (94), 120 (96), 101 (70).
24. *3-Bromo-2′-hydroxy-4-methoxychalcone* (**12**): The product was obtained as yellow solid. Mp 137-139 °C. UV (MeOH, λ_{max}): 204; 249.5; 339.5 and 354 nm. IR (ν cm^{-1}) 1637.5, 1596.9; 1207.5, 1012.6; 767.6. ^1H NMR (200 MHz, $CDCl_3$), δ: 12.85 (s, 1H, 2′-OH); 7.95–8.0 (dd, 1H, J = 8.8 Hz, 1.8 Hz, H6′); 7.91–7.95 (d, 1H, J = 8.0H, 1.7 Hz, H2); 7.78–7.86 (d, 1H, J = 15.6 Hz, H_β); 7.49–7.57 (d, 1H, J = 15.6 Hz, H_α); 7.46–7.59 (m, 2H, H4′, H6); 6.96–7.05 (m, 2H, H3′, H5′); 6.92–6.96 (d, 1H, J = 8.2 Hz, H5); 3.98 (s, 3H, OCH_3). Anal. ($C_{16}H_{13}BrO_3$) C, H, O.
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26. RAW 264.7 cells obtained from American Type Culture Collection were cultured with DMEM supplemented with 10% FBS and 1% CO_2 at 37 °C and activated with LPS (*Escherichia coli* O127:B8). All 2′-hydroxychalcone analogues were screened at concentration of 10 μM for their activity on PGE_2 production in RAW 264.7 cells stimulated by LPS. Briefly, cells were plated in 96-well plates (2×10^5 cells/well). Each synthetic chalcone was dissolved in dimethyl sulfoxide (DMSO) and LPS (1 mg/mL) were added and incubated for 24 h to allow the expression of COX-2 and then, were washed with culture medium. Test compounds were added at 10 μM and incubated for 2 h in fresh culture medium supplemented with arachidonic acid. PGE_2 concentration in the medium was measured using EIA kit for PGE_2 according to the manufacturer's recommendation. All experiments were carried out at least three times.
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29. Molecular modeling and docking study. *Preparation of molecular structures.* The 3D structure of chalcone derivatives were prepared using the build molecule module in MOE. The structures of molecules are optimized by energy minimization until converged to a maximum derivative of 0.001 kcal mol^{-1} $Å^{-1}$. The lowest-energy conformer of each molecule was selected and stored in mdb database. *Preparation of target enzyme structure and docking.* The X-ray crystal structure of COX-2:SC-558 complex (pdb 1cx2) was retrieved from the RCSB Protein Data Bank (www.rcsb.org). The active site was defined as all the amino acid residues enclosed within 6.5 Å radius sphere centered by the bound ligand, SC-558 (1-phenylsulfonamied-3-trifluoromethyl-5-parabromophenyl-pyrazole, a selective COX-2 inhibitor) and 'site finder' in MOE was used to determine the binding site. The docking and subsequent scoring were performed using the MOE docking programs. The final of 30 docked conformations per ligand were analyzed and used to create the illustrative figures.
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