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2'-Hydroxychalcone Analogues: Synthesis and Structure-PGE_2 Inhibitory Activity Relationship

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Abstract— A series of 2'-hydroxychalcones was synthesized and screened for their *in vitro* inhibitory effects on PGE_2 production from RAW 264.7 cells induced by LPS. Structure–activity relationship study suggested that inhibitory activity of PGE_2 formation was governed to a greater extent by the substituent on B ring of the chalcone template, and most of the active compounds have at least two methoxyl or benzyloxyl groups on B ring. The relationship between chalcone structures and their PGE_2 inhibitory activity was also interpreted by docking study on cyclooxygenase 2.

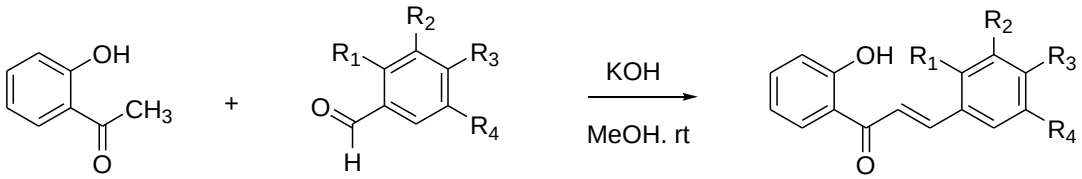
Chalcones, originally isolated from natural plant sources, considered as precursors of flavonoids and isoflavonoids are abundant in edible plants. Chemically, chalcones are open-chain flavonoids in which two aromatic rings are joined by a three carbon α,β-unsaturated carbonyl system (1,3-diphenyl-2-propen-1-ones). Being a majority subgroup of the flavonoid family, chalcones have been reported to have a variety of biological activities, including antiviral and anticancer,[1-3] anti-microbial,[4, 5] anti-inflammatory,[3, 6, 7] anti-ulcer and spasmolytic[8, 9] and antiproliferative[10] activities. Hence, chalcones is considered as a class with important therapeutic potentials.

In this study, various 2'-hydroxychalcones having different substituents in B-ring were synthesized using a classical base catalyzed condensation reaction and tested for anti-inflammatory activity via the inhibition of PGE_2 production from RAW 264.7 cells as well as for cytotoxicity at concentration of 10 μM.

RESULTS AND DISCUSSION

Chemistry

The preparation of the 2'-hydroxychalcone derivatives (Table 1) was carried out via Claisen-Schmidt condensation (Scheme 1). Thus, an appropriate aryldehyde derivaties was reacted with 2'-hydroxyacetophenone in MeOH/KOH to give the corresponding 4',6'-diprotected-2'-hydroxychalcones precipitated as the potassium salt. Subsequent treatment with an HCl yielded the desired product (CH.01-CH.20) with an average yield of 45-80% (Table 1). Their structures established with ^{1}H-NMR spectra showed that the E-isomers were specifically generated via this reaction. The spectral data of the synthesized compounds as well as detailed procedure for the synthesis are presented in experimental section.



Scheme 1. Synthesis of 2'-hydroxychalcone derivatives

Table 1. Chemical structures, yields and reaction conditions of 2'-hydroxychalcone analogues							
Product	B-ring substituents				Yield	Reaction Conditions	
	R_1	R_2	R_3	R_4	(%)	Reaction time (h)	Sol. for crystalization
CH.01	H	H	Cl	H	85	12	MeOH
CH.02	H	Cl	Cl	H	88	12	MeOH
CH.03	H	H	H	H	46	16	MeOH
CH.04	H	H	OCH_3	H	65	24	MeOH
CH.05	H	H	CH_3	H	74	16	MeOH
CH.06	H	H	Br	H	58	24	MeOH and CH_2Cl_2
CH.07	H	OCH_3	OBn	H	59	24	MeOH and CH_2Cl_2
CH.08	H	OBn	OBn	H	62	10	MeOH and CH_2Cl_2
CH.09	H	H	OBn	H	58	16	MeOH and CH_2Cl_2
CH.10	H	OBn	OCH_3	H	59	16	MeOH and CH_2Cl_2
CH.11	H	OCH_3	OCH_3	OCH_3	55	16	MeOH and CH_2Cl_2
CH.12	OCH_3	OCH_3	H	H	58	28	MeOH and CH_2Cl_2
CH.13	H	OCH_3	OCH_3	H	56	28	MeOH and CH_2Cl_2
CH.14	OCH_3	H	OCH_3	H	67	12	MeOH and CH_2Cl_2
CH.15	H	H	SCH_3	H	58	12	MeOH and CH_2Cl_2
CH.16	H	H	OCF_3	H	68	12	MeOH and CH_2Cl_2
CH.17	H	Br	OCH_3	H	65	12	MeOH and CH_2Cl_2
CH.18	H	-O-CH_2-O-		H	59	12	MeOH
CH.19	H	H	Ph	H	63	12	MeOH
CH.20	H	Br	H	H	66	12	MeOH

Biological activities

All synthesized 2'-hydroxychalcone analogues were screened for their activity on PGE_2 production in RAW 264.7 cells stimulated by lipopolysaccharide (LPS) at concentration of 10 μM. The inhibitory activities of synthetic chalcones on cyclooxygenase-2 (COX2) catalyzed PGE_2 production from LPS-induced RAW 264.7 cells were estimated and shown in Table 2. In addition, MTT cell viability assay also performed and all tested compounds showed no or less than 10% reduction indicating that those compounds were not significantly cytotoxicity to RAW 264.7 cells in the presence or absence of LPS (Table 2).

Table 2. Biological activities of chalcone analogues					
Chalcone	Biological activities (10 μM)		Chalcone	Biological activities (10 μM)	
	PGE_2 inhibition (%)[a]	Cell viability (%)[a]		PGE_2 inhibition (%)[a]	Cell viability (%)[a]
CH.01	59.68	140.5	CH.11	<u>102.3</u>	64.90
CH.02	48.05	154.6	CH.12	<u>100.9</u>	92.80
CH.03	60.30	119.1	CH.13	26.59	88.20
CH.04	32.22	115.3	CH.14	<u>98.30</u>	107.3
CH.05	62.07	152.2	CH.15	62.12	85.00
CH.06	23.56	161.5	CH.16	18.10	86.70
CH.07	< 0	102.0	CH.17	54.62	158.8
CH.08	<u>99.56</u>	102.7	CH.18	< 0	117.6
CH.09	<u>101.7</u>	98.90	CH.19	10.86	135.3
CH.10	<u>92.9</u>	102.4	CH.20	51.23	90.20
NS-398[b]	109.1	103.6	Wogonin[b]	102.6	111.1

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

These compounds inhibited PGE_2 production at 10 μM concentration with values in percentage (%) range. In those 20 synthesized chalcones, 6 compounds including CH.08-12 and CH.14 were indicated as the most potential inhibited PGE_2 production (inhibition values larger than 90% were underlined in Table 2). In term of structure-activity relationship (SAR), most of active compounds possess more than two alkoxy groups (methoxy and/or benzyloxy) in the B ring, excepting the CH.09 having an unique benzyloxy group at 4-position. The chalcone CH.09 also showed three-fold stronger than that observed for CH.04 (possessing a methoxy group at the same position).

The 2'-hydroxychalcone analogues possessing the unique substituent at 4-position of B-ring such as CH.01, CH.04-06, CH.15, CH.17, CH.19 (having a substituent either chloro/ methoxy/methyl/bromo/methylthio/trifluoromethyl/phenyl group, respectively) showed the same or less inhibitory activity than non-substituted 2'-hydroxychalcone (CH.03). Introducing either a strong electron withdrawal (Cl, Br, CF_3) group or a weak electron donating group (SCH_3, CH_3, OCH_3) to ring B of 2'-hydroxychalcone (CH.03) does not indicate the effect on PGE2 inhibition. However, a strong electron donating group at 4-position of 2'-hydroxychalcone (for example chalcone CH.09 containing benzyloxy moiety) exhibited a stronger activity than that of other 4-substituted-2'-hydroxychalcone analogues. Bioactivity results indicated that the benzyloxy group at 4-position of 2'-hydroxychalcone was contributed an important effect on the PGE_2 inhibitory activity. Docked CH.09:COX2 complexes indicated the important of oxygen of the benzyloxy moiety at 4-position of 2'-hydroxychalcone (Figure 1). This substituent formed both hydrogen bond and π-cation interaction with Arg120 thus plays the major role on the interaction between COX2 and CH.09. Conversely, no hydrogen bond was observed for compound CH.19 with phenyl moiety at the same position *versus* CH.09 that explained a weak anti-inflammatory activity (Figure 1).

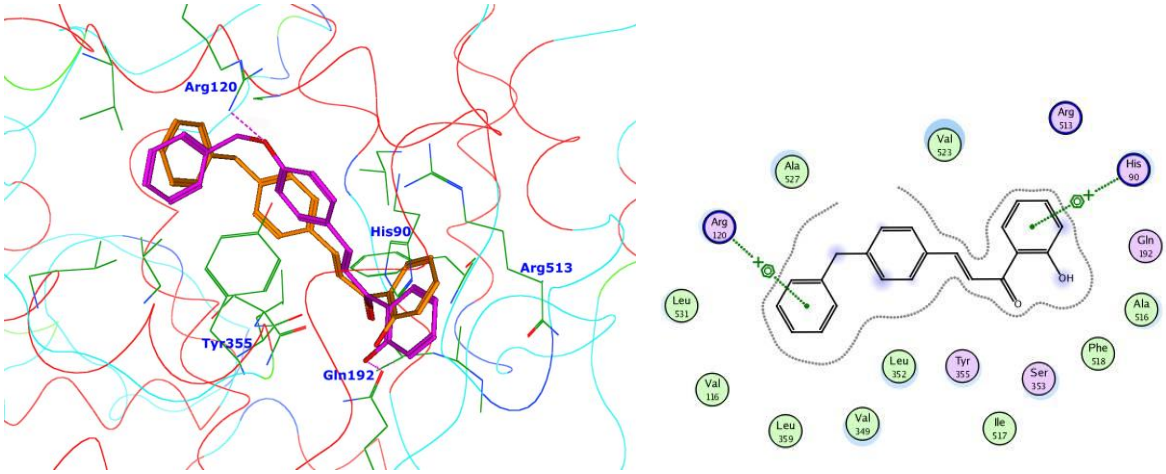


Figure 1. Relative position of CH.09 (magenta carbon) and CH.19 (orange carbon) in the active site of COX2 generated by MOE docking (left side). 2D interactions between CH.19 and COX2 is showed in right side with no H-bond observed. The hydrogen bonds (magenta dotted lines) within the binding site are indicated for compound CH.09. The benzyloxy moiety at 4-position of 2'-hydroxychalcone established a strong interaction with Arg120 of COX2 via both H-bond and π-cation interaction.

Compound CH.11 (2'-hydroxy-3,4,5-trimethoxychalcone) with 3 methoxyl groups in ring B formed additional hydrogen bonds (magenta dotted lines) with Arg120 and Arg513 of COX2 in comparison with its original scaffold CH.03 (Figure 2). At concentration of 10μM, CH.11 proved strong effect to inhibit the PGE_2 production (102.3%). However, CH.11 also indicated the effect on cell viability that provided a template to design new novels with a dual activity of anti-inflammation and anticancer. Except CH.11, most synthetic 2'-hydroxychalcones did not showed cytotoxicity which no or less than 10% cell reduction during MTT assay. Hence, the inhibition of PGE_2 production by chalcone derivatives might be not associated with their cytotoxicity at 10 μM.

In summary, twenty 2'-hydroxychalcone derivatives were synthesized and evaluated for their PGE_2 inhibitory activity and cytotoxicity. Among them, six chalcones showed better biological activities than that of 2'-hydroxychalcone. The structural requirements for the inhibitory activity of 2'-hydroxychalcone analogues on PGE_2 production from RAW 264.7 cells are drawn as follow: (*i*) the concomitance of more than two alkoxy groups on B ring in which one group sustituted at 3-position and the other substituted at 2- or 4-position of B-ring may enhance inhibitory activity of PGE_2 production; (*ii*) the benzyloxy plays a role as good legand, that established strongly interactions between compound and drug target; (*iii*) the inhibition of PGE_2 from RAW 264.7 cells is not associated with their cytotoxicity.

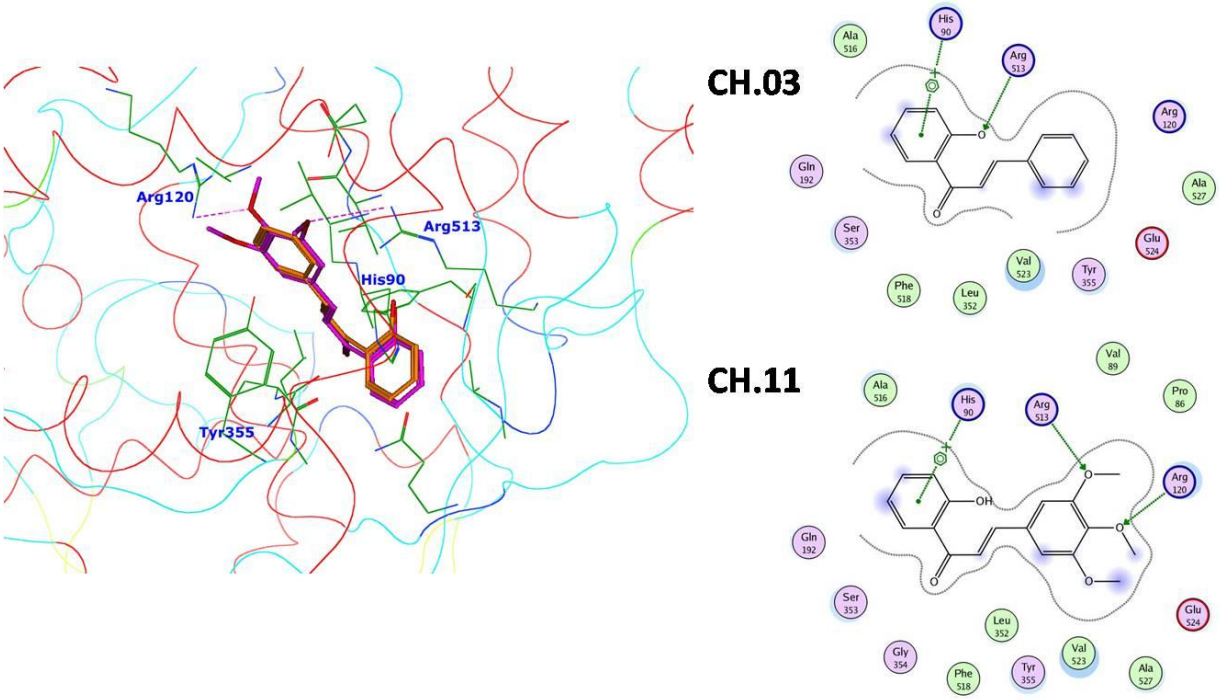


Figure 2. Docked conformation alignment of CH.11 (magenta carbon) and its original scaffold CH.03 (orange carbon) in COX2 binding site generated by MOE docking (left side). In right side, 2D ligand-interactions between these chalcones and COX2 are also showed. Three methoxyl moieties presented in ring B of CH.11 contributed the additional hydrogen bonds (magenta dotted lines) with Arg120 and Arg513 of COX2.

EXPERIMENTAL

Chemistry

All chemicals were obtained from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agent under nitrogen gas. All solvents used for chromatography were purchased and directly applied without further purification. ^{1}H-NMR spectra were recorded on a Varian Gemini 2000 instrument (200 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane as an internal standard. Peak splitting patterns are abbreviated as m (multiplet), s (singlet), bs (broad singlet), d (doublet), bd (broad doublet), t (triplet) and dd (doublet of doublets). Analytical thin-layer chromatography was performed using commercial glass plate with silica gel 60F254 purchased from Merck.

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 8-12 hours, 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 x 20 ml), and recrystallized from corresponding solvent (MeOH or MeOH/CH_2Cl_2) to give the product (Table 1).

CH.01: 4-chloro-2'-hydroxychalcone. Yellow crystals, m.p. 139 ^{o}C. UV (methanol, λmax): 203.5; 225 and 319 nm. IR (KBr, cm^{-1}): 1639.4; 1564, 752.8 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ: 12.76 (s, 1H, 2'-OH), 7.84-7.92 (d, 1H, *J* = 15.6 Hz, H_β,); 7.63-7.67 (d, 2H, *J* = 8 Hz, H3 and H5); 7.44-7.51 (d, 1H, *J* = 15.6 Hz, Hα); 7.48-7.67 (m, 3H, H2, H6 and H6'); 7.40-7.44 (m, 3H, H3', H4' and H5'); 6.92-7.06 (m, 2H, H3 and H5). Anal. C,H,O.

CH.02: 3,4-dichloro-2'-hydroxychalcone. Yellow crystals, m.p. 142 ^{o}C. UV (methanol, λmax): 204, 249 and 351 nm. IR (KBr, cm^{-1}): 1645.2, 1589, 750.3 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$,), δ: 12.68 (s, 1H, 2'-OH), 7.85-7.94 (t, 2H, *J* = 8.4 Hz, 16.2 Hz, H_β and H6'), 7.78 (s, 1H, H2), 7.60-7.67 (d, 1H, *J* = 15.6 Hz, H_α), 7.46-7.59 (m, 3H, H4', H5 and H6), 6.94-7.04 (m, 2H, H3'and H5'). Anal. C,H,O.

CH.03: 2'-hydroxychalcone. Yellow crystals, m.p. 78 °C. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.78 (s, 1H, 2'-OH), 7.91-7.96 (d, 1H, *J* = 7.6 Hz, and 1.8 Hz, H6'); 7.91-7.96 (dd, 1H, *J* = 14.4 Hz, H_β), 7.35-7.70 (m, 7H, H_α, H4', H2, H3, H4, H5, H6); 7.02-7.09 (m, 2H, H3', and H5'). Anal. C,H,O.

CH.04: 4-methoxy-2'-hydroxychalcone. Yellow crystals, m.p. 94 °C; UV (methanol, λmax): 205; 240; 339,5 and 365 nm. IR (KBr, cm^{-1}): 1641.5, 1608.9; 1211.5, 827,4; 763 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.95 (s, 1H, 2'-OH), 7.88-7.96 (d, 1H, *J* = 15.2 Hz, H_β), 7.87-7.91 (d, 1H, *J* = 7.4 Hz, H6'); 7.59-7.66 (d, 1H, *J* = 15.2 Hz, H_α); 7.62-7.66 (d, 2H, *J* = 8.8 Hz, H2 and H6); 7.45-7.49 (t, 1H, *J* = 8.8 Hz, and 7.6 Hz, H4'); 6.90-7.05 (m, 4H, H3, H5, H3' and h5'); 3.87 (s, 3H, OCH_3). Anal. C,H,O.

CH.05: 4-methyl-2'-hydroxychalcone. Yellow solid, m.p.120 °C. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.88 (s, 1H, 2'-OH), 7.87 – 9.95 (d, 1H, *J* = 15.2 Hz, H_β); 7.91-7.95 (d, 1H, *J* = 9.2 Hz, H6'); 7.58-7.66 (d, 1H, *J* = 15.2 Hz, H_α); 7.46-7.59 (m, 3H, *J* = 7.8 Hz, 8.0 Hz, 1.2 Hz, H2, H4'and H6); 7.22-7.26 (d, 2H, *J* = 8.0 Hz, H3 and H5); 6.90-7.01 (m, 2H, *J* = 8.0 Hz, 1.0 Hz, H3'and H5'); 2.40 (s, 3H, CH_3). Anal. C,H,O.

CH.06: 4-bromo-2'-hydroxychalcone. Yellow solid, m.p. 136 °C. UV (methanol, λmax): 203; 223.5; and 320 nm. IR (KBr, cm^{-1}): 1639; 1575; 1205.5; 758 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.87 (s, 1H, 2'-OH), 7.92 – 8.0 (d, 1H, *J* = 15.6 Hz, H_β); 7.92-7.96 (d, 1H, *J* = 7.6 Hz, H6'); 7.68-7.76 (d, 1H, *J* = 15.6 Hz, H_α); 7.55-7.65 (m, 3H, *J* = 7.8 Hz, 8.0 Hz, H2, H4' and H6); 7.92-7.96 (m, 2H, *J* = 8.2 Hz, H3 and H5); 6.90-7.05 (m, 2H, *J* = 8.0 Hz, H3' and H5'). Anal. C,H,O.

CH.07: 4-benzyloxy-3-methoxy-2'-hydroxychalcone. Yellow solid. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.87 (s, 1H, 2'-OH), 8.17-8.25 (d, 1H, *J* = 15.6 Hz, H_β); 7.90-7.94 (d, 1H, *J* = 8.0 Hz, H6'); 7.50-7.72 (d, 1H, *J* = 15.6 Hz, H_α); 7.27-7.31 (t, 1H, *J* = 6.2 Hz, 7.8 Hz, 1.8 Hz, H4'), 7.20-7.43 (m, 7H, H4', H5', Aryl); 6.90-7.05 (m, 3H, H2, H5 and H6), 5.25 (s, 2H, Ar-CH_2-); 3.95 (s, 3H, OCH_3). Anal. C,H,O.

CH.08: 3,4-dibenzyloxy-2'-hydroxychalcone. Yellow solid. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.91 (s, 1H, 2'-OH), 7.85-7.89 (d, 1H, *J* = 7.2 Hz, H6'); 7.78-7.89 (d, 1H, *J* = 15.2 Hz, H_β); 7.43-7.47 (d, 1H, *J* = 7.2 Hz, H4'); 7.39-7.47 (d, 1H, *J* = 15.2 Hz, H_α), 7.20-7.36 (m, 12H, H3', H5', 2xAryl); 6.95-7.05 (m, 3H, H2, H5 and H6), 5.24 (s, 4H, 2xAr-CH_2-). Anal. C,H,O.

CH.09: 4-benzyloxy-2'-hydroxychalcone. Yellow solid, m.p. 103-105 °C; UV (methanol, λmax): 204; 241; 379 nm. IR (KBr, cm^{-1}): 1637.5, 1562.2; 1174.6; 821.6; 765.7 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.94 (s, 1H, 2'-OH), 7.91-7.95 (d, 1H, *J* = 8.6 Hz, H6'); 7.87-7.95 (d, 1H, *J* = 14.8 Hz, H_β); 7.62-7.66 (d, 1H, *J* = 8.2 Hz, H4'); 7.59-7.66 (d, 1H, *J* = 14.8 Hz, H_α), 7.74-7.51 (m, 7H, H3', H5', Aryl); 6.93-7.05 (m, 4H, *J* = 8.8 Hz, 8.2 Hz, 8.2 Hz, 2.8 Hz, H2, H3, H5 and H6), 5.14 (s, 2H, Ar-CH_2-). Anal. C,H,O.

CH.10: 3-benzyloxy-4-methoxy-2'-hydroxychalcone. Yellow solid, m.p. 105 °C. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.93 (s, 1H, 2'-OH), 7.91-7.95 (d, 1H, *J* = 7.4 Hz, H6'); 7.82-7.92 (d, 1H, *J* = 15.2 Hz, H_β); 7.47-7.55 (d, 1H, *J* = 15.2 Hz, Hα), 7.20-7.43 (m, 7H, H4', H5', Aryl); 6.90-7.05 (m, 3H, H2, H5 and H6), 5.25 (s, 2H, Ar-CH_2-); 3.95 (s, 3H, OCH_3). Anal. C,H,O.

CH.11: 3,4,5-trimethoxy-2'-hydroxychalcone. Yellow solid, m.p. 157 °C; UV (methanol, λmax): 215.5; 252; 339.5 and 363.5 nm. IR (KBr, cm^{-1}): 1637.5, 1566.7; 1296.1; 1026.1; 835.1; 769.5 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.84 (s, 1H, 2'-OH), 7.92-7.96 (d, 1H, *J* = 7.8 Hz, H6'); 7.83-7.90 (d, 1H, *J* = 14.4 Hz, H_β); 7.50-7.58 (d, 1H, *J* = 14.4 Hz, H_α), 7.48-7.53 (d, 1H, H4'); 6.93-7.06 (m, 2H, H3' and H5'); 6.90 (s, 2H, H2' and H6'); 3.95 (s, 9H, 3xOCH_3); 3.92 (s, 3H, OCH_3). Anal. C,H,O.

CH.12: 2,3-dimethoxy-2'-hydroxychalcone. Yellow solid, m.p. 107 °C. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.89 (s, 1H, 2'-OH), 8.18-8.26 (d, 1H, *J* = 15.6 Hz, H_β); 7.91-7.95 (d, 1H, *J* = 8.2 Hz, H6); 7.72-7.80 (d, 1H, *J* = 15.6 Hz, H_α), 7.47-7.55 (t, 1H, H4'); 7.28-7.32 (d, 1H, H3'); 6.92-7.16 (m, 4H, H4, H5. H6 and H6'); 3.92 (s, 6H, 2xOCH_3). Anal. C,H,O.

CH.13: 3,4-dimethoxy-2'-hydroxychalcone. Yellow solid, m.p. 115 °C. IR (KBr, cm^{-1}): 1629.7; 1598.9; 1028; 744.5. ^{1}H-NMR (200 MHz, $CDCl_3$,, δ ppm: 12.88 (s, 1H, 2'-OH), 8.18-8.26 (d, 1H, *J* = 15.6 Hz, H_β); 7.91-7.95 (d, 1H, *J* = 8.2 Hz, H6'); 7.49-7.57 (d, 1H, *J* = 15.4 Hz, H_α); 7.46-7.54 (m, 1H, *J* = 8.6 Hz, *J* = 1.6 Hz, H4'); 7.18-7.30 (m, 2H, *J* = 8.2 Hz, 1.8 Hz, H3' and H5'); 6.90-7.05 (m, 3H, H2, H4, H5); 3.97, 3.95 (s, 6H, 2x OCH_3). Anal. C,H,O.

CH.14: 2,4-dimethoxy-2'-hydroxychalcone. Yellow solid, m.p. 111 °C; UV (methanol, λmax): 208; 258.5 and 383.5 nm. IR (KBr, cm^{-1}) 1629.7; 1560.3; 1203.5; 1024.1; 769.5 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 13.10 (s, 1H, 2'-OH), 8.14-8.22 (d, 1H, *J* = 15.4 Hz, H_β); 7.90-7.94 (d, 1H, *J* = 8.2 Hz, H6'); 7.64-7.75 (d, 1H, *J* = 15.4 Hz, H_α); 7.58-7.62 (d, 1H, *J* = 8.6 Hz, H6); 7.44-7.52 (m, 1H, *J* = 8.0 Hz, H4'); 6.99-7.03 (d, 1H, *J* = 8.6 Hz, H5'); 6.93-6.97 (d, 1H, *J* = 8.4 Hz, H3'); 6.55-6.59 (d, 1H, *J* = 7.6 Hz, H5), 6.50 (ds, 1H, *J* = 1.4 Hz, H3); 3.93, 3.87 (s, 6H, 2x OCH_3). Anal. C,H,O.

CH.15: 4-methylthio-2'-hydroxychalcone. Yellow solid, m.p. 85 °C; UV (methanol, λmax): 203; 253 and 375.5 nm. IR (KBr, cm^{-1}) 1637.5, 1566.7; 1201.5; 812.0; 758 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.87 (s, 1H, 2'-OH); 7.90-7.95 (dd, 1H, *J* = 7.8 Hz, 1.8 Hz, H6'); 7.86-7.94 (d, 1H, *J* = 15.6 Hz, H_β); 7.58-7.65 (d, 1H, *J* = 15.6 Hz, H_α); 7.46-7.65 (m, 3H, H2, H4', H6); 7.25-7.29 (d, 2H, *J* = 8.6 Hz, 2.0 Hz, H3, H5); 6.91-7.05 (m, 2H, H3' and H5'); 2.53 (s, 3H, SCH_3).

Anal. C,H,O.

CH.16: 4-trifluoromethoxy-2'-hydroxychalcone. Yellow solid, m.p. 81 ^{o}C; UV (methanol, λmax): 203; 221.5 and 309.5 nm. IR (KBr, cm^{-1}): 1643.2; 1577.7; 1205.4; 1157.2; 754.1 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 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). Anal. C,H,O.

CH.17: 3-bromo-4-methoxy-2'-hydroxychalcone. Yellow solid, m.p. 137-139 ^{o}C; UV (methanol, λmax): 204; 249.5; 339.5 and 354 nm. IR (KBr, cm^{-1}) 1637.5, 1596.9; 1207.5, 1012.6; 767.6. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 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,H,O.

CH.18: 3,4-dioxymethylene-2'-hydroxychalcone. Yellow solid, m.p. 108 ^{o}C; UV (methanol, λmax): 208; 266 and 373 nm. IR (KBr, cm^{-1}) 1641.3, 1566; 1242.1; 1037.6; 759.9 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$), δ ppm: 12.90 (s, 1H, 2'-OH); 7.92-7.96 (dd, 1H, *J* = 8.2 Hz, 1.4 Hz, H6'); 7.82-7.89 (d, 1H, *J* = 15.2 Hz, H_β); 7.68-7.72 (m, 1H, H4'); 7.46-7.54 (d, 1H, *J* = 15.2 Hz, H_α), 6.84-7.15 (m, 5H, H2, H3', H5, H5', H6); 6.0 (s, 2H,-O-CH_2-O-). Anal. C,H,O.

CH.19: 4-phenyl-2'-hydroxychalcone. Yellow solid, m.p. 147 ^{o}C; UV (methanol, λmax): 204; 249 and 351 nm. IR (KBr, cm^{-1}) 1637.5, 1571.9; 1203.5, 989,4; 756 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$, ppm), δ ppm: 12.71 (s, 1H, 2'-OH); 7.88-7.95 (d, 1H, *J* = 14.0 Hz, H_β); 7.87-7.91 (d, 1H, *J* = 7.6 Hz, H6'); 7.68 (s, 1H, H2); 6.93-7.60 (m, 7H, H_α, H4, H5, H3', H4', H5', H6). Anal. C,H,O.

CH.20: 3-bromo-2'-hydroxychalcone. Yellow solid, m.p. 139 ^{o}C; UV (methanol, λmax): 206 and 308 nm. IR (KBr, cm^{-1}): 1641.3; 1577.7; 1205.4; 1022.2; 761 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$, ppm), δ ppm: 12.88 (s, 1H, 2'-OH); 7.93-8.0 (dd, 1H, *J* = 15.6 Hz, H_β); 7.92-7.96 (d, 1H, *J* = 8.0 Hz, H6'); 7.69-7.76 (d, 1H, *J* = 15.6 Hz, H_α), 7.38-7.62 (m, 9H, H2, H3, H5, H6, Aryl); 6.99 -7.05 (m, 1H, *J* = 8.2 Hz, H5'); 6.92-6.96 (d, 1H, *J* = 8.0 Hz, H3'). Anal. C,H,O.

Biological Assays

PGE_2 inhibitory assay. The PGE_2 inhibitory assay was performed according to the previous published procedure.[11] RAW 264.7 cells obtained from American Type Culture Collection were cultured with DMEM supplemented with 10% FBS and 1% CO_2 at 37 ^{o}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 ($2x10^5$ 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 COX2 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 twice and they gave similar results.

Cytotoxicity. Cell viability was assessed with MTT assay based on the experimental procedures described previously.[12]

Molecular modeling and docking study

Preparation of molecular structures. The 3D structure of 20 chalcones were prepared using the build molecule module in MOE.[13] 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 COX2:L-758048 complex (pdb 2cx2) was retrieved from the RCSB Protein Data Bank.[14] The active site was defined as all the amino acid residues enclosed within 6.5Å radius sphere centered by the bound ligand, L-758048 (a benzyl-indole COX2 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.[13] The final of 30 docked conformations per ligand were analyzed and used to create the illustrative figures.

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