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Design, synthesis, biological evaluation, and molecular docking of chalcone derivatives as anti-inflammatory agents

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Abstract: In this study, two series of 35 new chalcone derivatives containing aryl-piperazine or aryl-sulfonyl-piperazine fragment were synthesized and their structures were characterized by ^{1}H, ^{13}C and ESI-MS. The *in vivo* and *in vitro* anti-inflammatory activities of target compounds were evaluated by using classical para-xylene-induced mice ear-swelling model and ELISA assays. Furthermore, docking studies were performed in COX-2 (4PH9). The *in vivo* anti-inflammatory assays indicated that most of the target compounds showed significant anti-inflammatory activities. Docking results revealed that the anti-inflammatory activities of compounds correlated with their docking results. Especially, compound **6o** exhibited the most potent anti-inflammatory activity *in vivo* with the lowest docking score of -17.4 Kcal/mol and could significantly inhibit the release of LPS-induced IL-6 and TNF-α in a dose-dependent manner *in vitro*.

Keywords: inflammation, chalcone, derivatives, anti-inflammatory activity, COX-2

Inflammation is a complex biological process which seriously threatens human health. Exaggerated and prolonged inflammation may cause various diseases, such as arthritis, sepsis, and even cancer.[1] At present, the most widely used drugs in treating

inflammation are non-steroidal anti-inflammatory drugs (NSAIDs), which account for 35% of the global market for prescription of pain medications.[2-3] Common NSAIDs, such as aspirin and indomethacin can inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2)[4-5], displaying significant toxicity to gastrointestinal tract and kidney.[6] For that reason, it is of great importance and urgent need to develop new anti-inflammation drugs.[7]

Chalcone which belongs to a flavonoid family is widely distributed in plants such as vegetables, fruits, tea and spices. It has been demonstrated that chalcone posses many important bioactivities including anti-oxidant[8], anti-cancer[9], anti-bacterial[10], anti-fungal[11], and anti-inflammation[12]. Recently, chalcone and its derivatives have been reported to show anti-inflammatory activities in acute lung injury[13-14] and could protect liver from hepatic injury by inhibition of hepatic inflammation and fibrosis.[15] Based on its favorable anti-inflammatory activity, chalcone framework has been used for chemical modification to find novel derivatives with better pharmacological or pharmacokinetic profiles.[16]

N-aryl piperazine moieties belong to an important class of organic compounds which is widely used in medicinal chemistry.[17] Recently, *N-aryl* piperazine derivatives have been attracting considerable attentions for their versatile properties in pharmacology[18-19] and anti-inflammatory activities in *vitro*[20] and in *vivo*[21]. Based on these data, we introduced *N-aryl* piperazine moiety into the chalcone skeleton to synthesize series I of chalcone derivatives. It is well recognized that selective COX-2 inhibitors (the coxibs) exhibit moderate gastrointestinal toxicity comparing with traditional NSAIDs.[22] In view of the structures of coxibs, we discover that the coxibs bear an arylsulfonyl moiety (Fig. 1), implying that compounds bearing arylsulfonyl moiety might show selective COX-2 inhibition activity. Thus, to make COX-2 selective compounds, series II was synthesized with an arylsulfonyl moiety insertion based on the structures of compounds from series I. The anti-inflammatory activities of these two series were screened *in vivo* and *in vitro*. Molecular modeling with COX-2 was also studied.

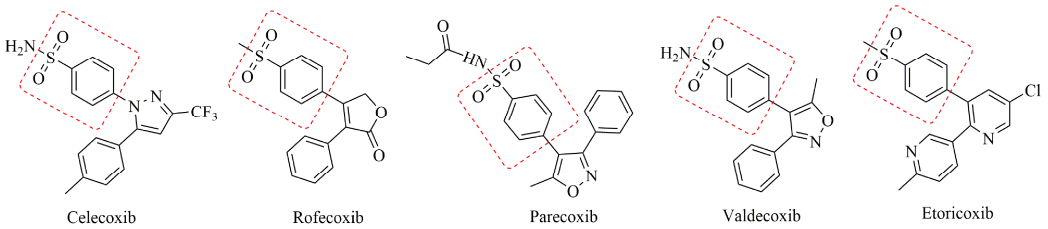
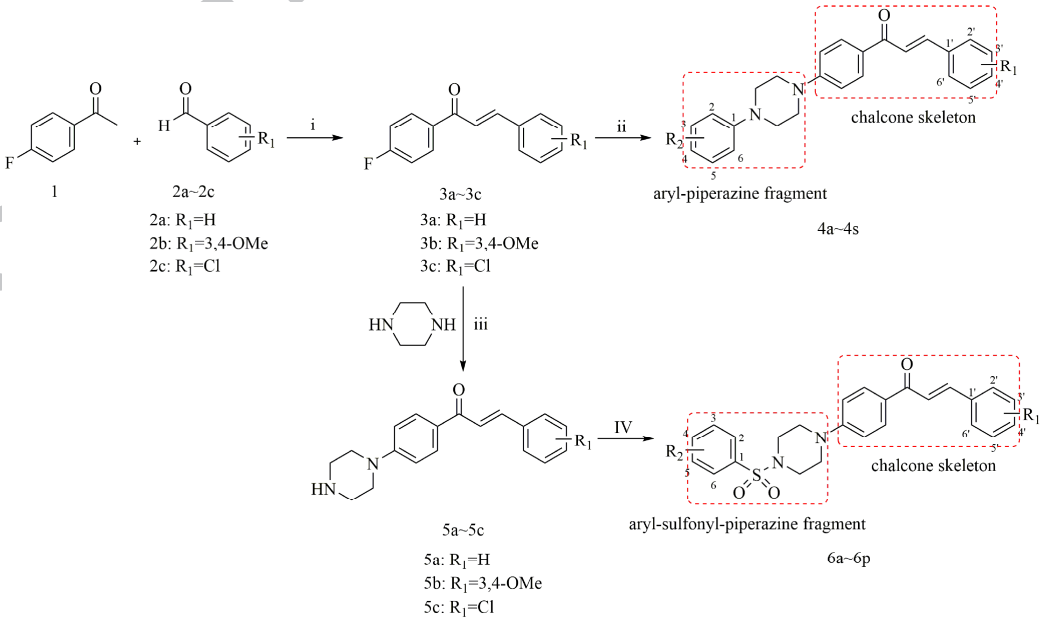


Figure 1. Structures of selective COX-2 inhibitors (coxibs).

The synthesis of target compounds is outlined in Scheme 1. Condensation of 4-fluoro acetophenone (**1**) with substituted benzaldehyde **2a~2c** in the presence of sodium hydroxide solution in enthanol at room temperature afforded the substituted chalcone **3a~3c.**[23] For series I containing aryl-piperazine fragment, intermediates **3a~3c** reacted with substituted aryl-piperazine in the presence of DMF solution at 120 ℃ for 12-16 h to give compounds **4a~4s** under nitrogen protection. For series II containing aryl-sulfonyl-piperazine fragment, treatment of **3a~3c** with piperazine by heating in the presence of potassium carbonate under nitrogen protection provided the intermediate products **5a~5c**. Especially, the reaction time of this step must be controlled under 5 to 6 h otherwise unprecedented complicated products obtained. Then intermediates **5a~5c** reacted with arenlsulfonyl to give compounds **6a~6p** as reported.[24] The structures and characterization of target compounds were depicted in Table 1 and supplementary information, respectively.



Scheme 1. Synthetic routes of chalcone derivatives. Reagents and conditions: (i) 10% NaOH, EtOH, rt; (ii) aryl piperazine derivatives, K_2CO_3, DMF, N_2, 12~16 h; (iii) piperazine, K_2CO_3, 120 °C, N_2, 4 h; (IV) (**6a~6n**): arenelsulfonyl chloride, K_2CO_3, acetone, rt, 24h; (**6o~6p**): arenelsulfonyl chloride, K_2CO_3, DMF, 50 °C, 12 h.

Table 1. Structures of synthesized chalcone derivatives.

Comp	R_1	R_2	Yields	Comp	R_1	R_2	Yields
4a	-	3,4-Cl	58%	**4s**	4'-Cl	3-OCH_3	57%
4b	-	4-NO_2	56%	**6a**	4'-Cl	4-Cl	78%
4c	-	2-F	53%	**6b**	4'-Cl	4-CF_3	77%
4d	-	1-Benzyl	56%	**6c**	-	4-CH_3	81%
4e	-	2,4-CH_3	63%	**6d**	-	4-Cl	68%
4f	-	3-Cl	51%	**6e**	-	3-(CH=CH)-4	71%
4g	-	2,3-Cl	58%	**6f**	-	4-Br	79%
4h	-	3-OCH_3	52%	**6g**	-	4-OCH_3	80%
4i	-	4-CH_3	55%	**6h**	-	2,4,6-CH_3	81%
4j	-	2-Cl	51%	**6i**	-	-	75%
4k	-	3-CF_3	57%	**6j**	-	4-OCF_3	76%
4l	-	-	60%	**6k**	-	2,4,6-isopropyl	78%
4m	3',4'-OCH_3	3,4-Cl	57%	**6l**	-	4-Butyl	69%
4n	3',4'-OCH_3	2,4-CH_3	61%	**6m**	-	4-CF_3	66%
4o	4'-Cl	2,4-CH_3	56%	**6n**	-	3,4-OCH_3	73%
4p	4'-Cl	3-Cl	54%	**6o**	3',4'-OCH_3	4-Br	76%
4q	4'-Cl	4-OCH_3	58%	**6p**	3',4'-OCH_3	4-OCH_3	72%
4r	4'-Cl	2-OCH_3	57%				

The *in vivo* anti-inflammatory activities of target compounds were evaluated using *in vivo* para-xylene-induced mice ear-swelling model. All the target compounds were administered at a dose of 40 mg/kg and aspirin (100 mg/kg) and celecoxib (5 mg/kg) were used as reference drugs. The results showed that for series I, only compounds **4b**,

4m, **4n**, and **4q** exhibited significant anti-inflammatory activities ($p < 0.05$). While for series II, almost all the derivatives displayed significant anti-inflammatory activities except compound **6l** ($p < 0.05$) (Table 2). Compound **6o** displayed the most potent anti-inflammatory activity which was better than that of aspirin and even equal to that of celecoxib. Preliminary structure activity relationship (SAR) analysis indicated that: 1) In view of the structures of compounds **4i** with **6c** and **4l** with **6i**, we found compounds **6c** and **6i** contained an extra sulfonyl moiety over compounds **4i** and **4l**, respectively. The *in vivo* anti-inflammatory activities of these compounds were **6c** >> **4i**, **6i** >> **4l**. These results implied that sulfonyl group could improve compounds anti-inflammatory activity. 2) Comparing compounds **4b** and **4i**, the *in vivo* anti-inflammatory activity was **4b** >> **4i**. Comparing compounds **6d**, **6f**, **6g**, **6j**, **6m** and **6c**, **6l**, the *in vivo* anti-inflammatory activities were **6d**, **6f**, **6g**, **6j**, **6m**, **6m** > **6c**, **6l**. These results implied that substitution at 4 position of aryl-piperazine or aryl-sulfonyl-piperazine with electron-withdrawing group showed advantage over electron-donating group in improving compound's anti-inflammatory activity. 3) Comparing compounds **4f** and **4p**, **4h** and **4s**, the *in vivo* anti-inflammatory activities were **4f** > **4p**, **4h** > **4s**. These results indicated that substitution at 4' position of benzene ring could not benefit from improving compound's anti-inflammatory activity.

Table 2. Anti-inflammatory acitivites of chalcone derivatives

Compound	Dose (mg/kg)	Swelling degree (mg)	Inhibition (%)	Docking Score (Kcal/mol)	p value
control		7.0 ± 1.6			
celecoxib	5	1.6 ± 0.7	77.03	-14.53	$p < 0.001$
aspirin	100	2.5 ± 0.8	64.91	-9.47	$p < 0.001$
chalcone	40	5.5 ± 0.7	21.68	-9.25	$p = 0.07$
4a	40	6.1 ± 1.5	13.41	-11.12	$p = 0.36$
4b	40	3.1 ± 1.3	55.35	-13.22	$p < 0.001$
4c	40	7.0 ± 3.4	0.57	-10.57	$p = 0.98$

4d	40	5.8 ± 1.0	17.69	-10.37	p = 0.15
4e	40	6.0 ± 0.7	14.98	-11.27	p = 0.19
4f	40	6.0 ± 1.4	14.12	-11.18	p = 0.29
4g	40	4.9 ± 2.4	30.67	-11.12	p = 0.10
4h	40	5.9 ± 0.8	15.98	-10.75	p = 0.17
4i	40	6.8 ± 1.3	2.85	-10.81	p = 0.82
4j	40	6.7 ± 2.8	4.71	-10.20	p = 0.80
4k	40	5.3 ± 3.1	24.96	-12.03	p = 0.21
4l	40	6.8 ± 2.0	3.71	-10.31	p = 0.81
4m	40	2.6 ± 0.8	63.48	-14.99	p < 0.001
4n	40	3.6 ± 1.9	48.50	-13.33	p = 0.008
4o	40	5.2 ± 2.6	25.68	-12.35	p = 0.17
4p	40	6.6 ± 2.8	5.85	-10.90	p = 0.77
4q	40	3.2 ± 1.5	55.06	-13.44	p < 0.001
4r	40	6.6 ± 1.2	6.13	-11.31	p = 0.61
4s	40	6.9 ± 2.2	2.28	-12.08	p = 0.88
6a	40	3.0 ± 1.4	56.63	-13.04	p < 0.001
6b	40	2.8 ± 1.0	59.63	-13.56	p < 0.001
6c	40	4.9 ± 0.9	30.81	-11.50	p = 0.01
6d	40	3.7 ± 2.1	46.79	-11.33	p = 0.005
6e	40	4.6 ± 0.9	34.95	-12.52	p = 0.007
6f	40	3.7 ± 0.9	47.36	-12.64	p = 0.02
6g	40	3.8 ± 0.9	45.22	-12.57	p = 0.02
6h	40	3.1 ± 1.1	55.49	-13.01	p < 0.001
6i	40	4.9 ± 0.7	29.96	-11.20	p = 0.01
6j	40	3.7 ± 2.6	47.36	-12.86	p = 0.02
6k	40	2.8 ± 0.7	60.06	-13.92	p < 0.001
6l	40	5.3 ± 3.0	23.97	-12.68	p = 0.25
6m	40	3.7 ± 0.8	47.93	-12.98	p < 0.001

6n	40	3.1 ± 1.1	55.49	-12.99	$p < 0.001$
6o	40	1.7 ± 0.8	75.46	-17.40	$p < 0.001$
6p	40	2.5 ± 1.0	64.05	-15.98	$p < 0.001$

The statistical significance was calculated between each compound group and control group by using student's t-test of unpaired data. P values < 0.05 were considered statistically significant.

The structure and favorable *in vivo* anti-inflammatory activities of compounds led us to study the molecular docking with COX-2 enzyme. The results indicated that the anti-inflammatory activities of compounds might correlate with their docking results with COX-2 (Table 2). Compound **6o**, **6p**, **4m**, and **6k** which had a lower docking score than others displayed better anti-inflammatory activities than other compounds. Especially, compound **6o** had the lowest docking score of -17.4 Kcal/mol and the best anti-inflammatory activity (inhibition rate, 75.46%) which showed two hydrogen bonds (H-bonds) and two chemical bonds of arene-cation with COX-2 (Fig.2 and Fig. 3). H-bonds were formed between oxygen atom of methoxyl group with amino acid Tyr386 and Ser531 at distances of 2.46 and 2.78 Å, respectively. The intensities of H-bonds between compound **6o** with COX-2 were 98% and 67%, respectively. Chemical bonds of arene-cation were formed between benzene ring of compound **6o** and amino acid Arg121 of COX-2. To validate the results of molecular docking, further enzyme studies on COX-2 are needed.

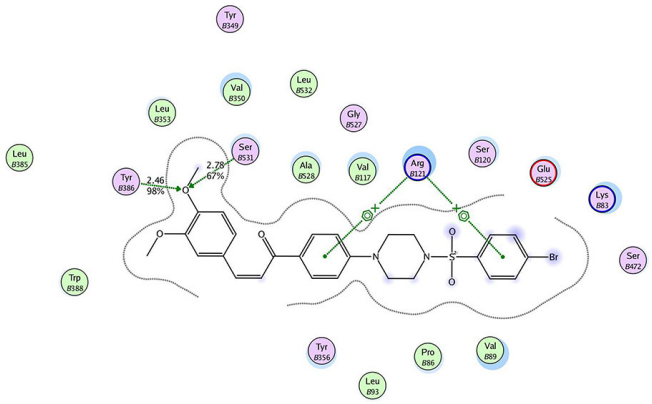


Figure 2. 2D docking model of compound **6o** with COX-2.

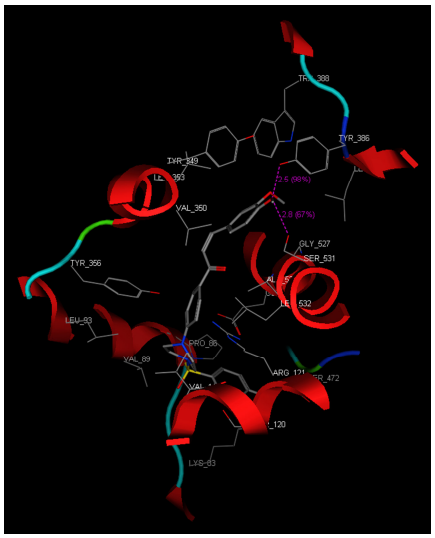


Figure 3. 3D docking model of compound **6o** with COX-2.

Considering the favorable *in vivo* anti-inflammatory activity and docking results in silico, we chose compound **6o** for further studies. To reveal the cytotoxity of compound **6o**, MTT assay was conducted in mouse RAW264.7 macrophages. The results indicated that compound **6o** showed no significant cytotoxity with a concentration less than 50 μM (Fig. 4a). To further detect the effects of compound **6o** on pro-inflammatory factors (TNF-α and IL-6) secretion, RAW264.7 macrophages were pretreated with different doses (10, 20, 40 μM) of compound **6o** for 2 h and then exposed to LPS (1 μg/ml) for additional 22 h. The levels of TNF-α and IL-6 in the media were determined. The results displayed that compound **6o** significantly inhibited TNF-α and IL-6 release by RAW264.7 macrophages in a dose-dependent manner (Fig. 4b).

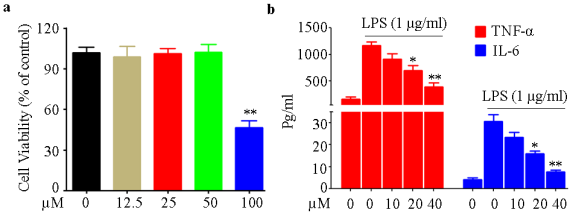


Figure 4. Cytotoxicity and *in vitro* anti-inflammatory activity of compound **6o**. (a) MTT assays of compound **6o** in mouse RAW264.7 macrophages. (b) Compound **6o** inhibited LPS-induced TNF-α and IL-6 release by RAW264.7 macrophages in a dose-dependent manner in mouse RAW264.7 macrophages.

In summary, two series of 35 new chalcone derivatives containing aryl-piperazine or aryl-sulfonyl-piperazine fragment were synthesized. The *in vivo* anti-inflammatory results revealed that most of the target compounds exhibited potent anti-inflammatory activity. Especially, compound **6o** exhibited the most potent anti-inflammatory activity *in vivo* and could significantly inhibit the release of LPS-induced IL-6 and TNF-α by RAW264.7 macrophages in a dose-dependent manner *in vitro*. Furthermore, docking results indicated that the anti-inflammatory activities of compounds correlated with their docking results. Our finding might provide information on developing potentially new and safe anti-inflammatory agents.

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