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Original article

Rational design, synthesis, and pharmacological properties of pyranochalcone derivatives as potent anti-inflammatory agents

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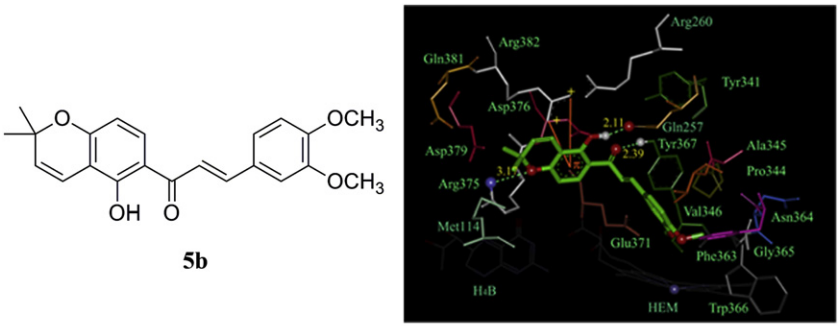
HIGHLIGHTS

- Two natural pyranochalcones and their derivatives were synthesized and evaluated for anti-inflammatory activities.
- **5b** showed remarkable *in vitro* and *in vivo* anti-inflammatory activity comparable to indomethacin.
- Molecular docking was employed to investigate the binding mode of the significant inhibitor **5b**.
- Structure–activity relationship is discussed.

GRAPHICAL ABSTRACT



Compound **5b** is a significant inhibitor to inflammation and can perfectly bind to the active site of murine iNOS.

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ABSTRACT

24 derivatives (**5a**–**x**) derived from natural pyranochalcones (**I** and **II**) were designed and evaluated for their inhibitory potency on the production of nitric oxide (NO) in LPS-stimulated RAW264.7 cells. Among them, four compounds (**5b**, **5d**, **5f**, and **5h**) exhibited more potent inhibitory effects on iNOS activity and iNOS-mediated NO production than a positive control indomethacin. Furthermore, **5b** could significantly suppress the progression of carrageenan-induced hind paw edema compared to indomethacin at a dosage of 10 mg/kg/day, and dose-dependently ameliorated the development of adjuvant-induced arthritis (AIA) validated by arthritic scores and H&E staining of joints. In addition, docking study confirmed that **5b** was an iNOS inhibitor with binding to the active site of murine iNOS.

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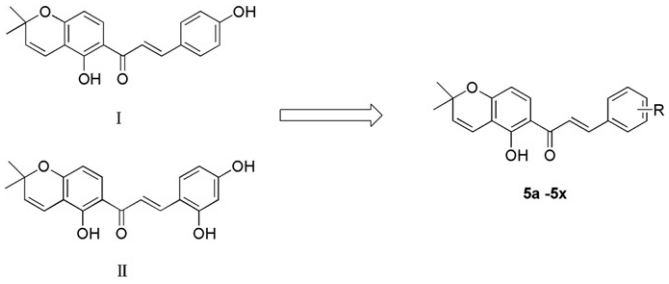


Fig. 1. Structure of **I**, **II**, and pyranochalcone derivatives.

1. Introduction

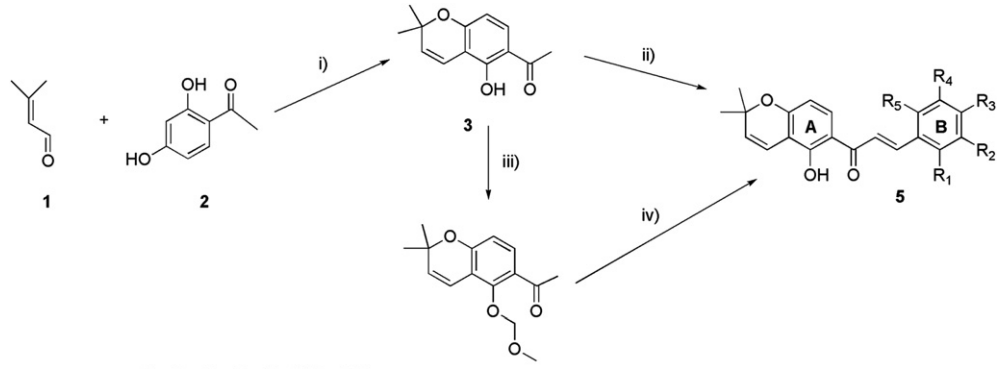
Inflammation is a central feature of many pathological conditions and always companies with physiological signs such as pain, redness, swelling, and even loss of organ functions [1]. During the progression of multiple inflammatory diseases, the activation of macrophages is crucial through the excessive release of pro-inflammatory cytokines and mediators such as nitric oxide (NO), tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and prostaglandin E2 (PGE_2) [2]. NO, being an endogenous free radical, is produced by the oxidation of L-arginine to L-citrulline which primarily catalyzed by inducible nitric oxide synthase (iNOS). Normally, NO is at a low level. While stimulated by pro-inflammatory cytokines or lipopolysaccharide (LPS), the iNOS could produce a high expression of NO and further induce joint injury at the inflammatory site. Since the suppression of the over-production of iNOS-mediated NO improves the incidence and severity of chronic inflammatory diseases like rheumatoid arthritis, inflammatory bowel disease, and psoriatic arthritis, iNOS could be recognized as a drug target of anti-inflammatory agents [3–5].

Pyranochalcones are an abundant class of flavonoids and widely distributed in nature. Comprehensive literatures have been reported that pyranochalcones possessed various pharmacological activity including radical scavenging activity [6], antimicrobial activity [7], and the inhibition of matrix metalloproteinase-2 (MMP-2) secretion and nicotinamide adenine dinucleotide (NADH), etc [8,9]. However, only few study focused on their structural modifications associated with the potential anti-inflammatory potency [10,11]. In our laboratory, two typical representatives of pyranochalcones, 4-hydroxyloncarpin (**I**) and 3″,3″-dimethylpyrano[3′,4′]2,4,2′-trihydroxychalcone (**II**), isolated from *Millettia ferruginea* and *Artocarpus communis*, were found to exhibit potent inhibitory activity against the production of NO in LPS-stimulated RAW264.7 cells and potential anti-inflammatory activity in *vivo* (Fig. 1).

As our continuing effort to explore for new anti-inflammatory chemical entities [5,12–14], a series of pyranochalcone derivatives were designed, synthesized and investigated for their inhibitory effects on NO production in LPS-induced RAW 264.7 cells and iNOS activity. Among these derivatives, four compounds (**5b**, **5d**, **5f**, and **5h**) showed more potent inhibitory activity against NO production than a positive control indomethacin at a concentration of 10 μM (78.4%, 81.3%, 80.4%, 79.9% *vs.* 59.2%, respectively). Furthermore, **5b** significantly suppressed the progression of inflammation in both two experimental models of carrageenan-induced hind paw edema and adjuvant-induced arthritis (AIA). Additionally, **5b** was confirmed as a potent iNOS inhibitor (IC_{50}: 7.9 μM) with binding to the active site of murine iNOS by docking study.

2. Chemistry

Two pyranochalcone occurrences **I**, **II** and 24 derivatives were synthesized according to previous reported procedures with the yield of 30–70% (Scheme 1) [15,16]. Briefly, the key intermediate **3** was obtained by the condensation of 2,4-dihydroxylacetophenone (**2**) with 3-methylcrotonaldehyde (**1**) in anhydrous pyridine at 120 °C in a good yield [17,18]. Then, two methods were developed to synthesize these desired compounds. *Method A*: The mixture of **3**, hydroxyl benzaldehydes protected by chloro(methoxy)methane (MOM–Cl), tetrabutyl ammonium bromide (TBAB), and sodium hydroxide was carried out using the microwave apparatus for 5 min



Ⅰ. R_1=R_2=R_4=R_5=H,R_3=OH
Ⅱ. R_2=R_4=R_5=H, R_2=R_3=OH
5a. R_1=R_2=R_4=R_5=H,R_3=OCH_3
5b. R_1=R_4=R_5=H,R_2=R_3=OCH_3
5c. R_2=R_5=H,R_1=R_3=R_4=OCH_3
5d. R_1=R_5=H,R_2=R_3=R_4=OCH_3
5e. R_1=R_2=R_4=R_5=H,R_3=benzyl
5f. R_1=R_2=R_3=R_5=H,R_4=F
5g. R_1=R_2=R_4=R_5=H,R_3=Cl
5h. R_1=R_2=R_4=R_5=H,R_3=Br
5i. R_1=R_2=R_4=H,R_3=OCH_3,R_5=F
5j. R_1=R_2=R_4=R_5=H,R_3=pyrrolidinyl
5k. R_1=R_2=R_4=R_5=H,R_3=diphenylamino
5l. R_1=R_2=R_3=R_4=R_5=H
5m. R_1=R_2=R_5=H, R_3=OH,R_4=OCH_3
5n. R_2=R_3=R_4=H, R_1=R_5=OH
5o. R_1=R_4=R_5=H, R_2=R_3=OH
5p. R_1=R_2=R_5=H, R_3=OH,R_4=OCH_2CH_3
5q. R_1=R_2=R_5=H,R_3=OCH_3,R_4=OH
5r. R_2=R_3=R_5=H,R_1=OH,R_4=Cl
5s. R_1=R_3=R_4=R_5=H,R_2=OH
5t. R_1=R_5=H,R_2=R_4=OCH_3,R_3=OH
5u. R_1=R_2=R_4=R_5=H,R_3=F
5v. R_1=R_3=R_4=R_5=H,R_2=OCH_3
5w. R_1=R_2=R_4=R_5=H,R_3=diethylamino
5x. R_4=R_5=H,R_1=R_2=R_3=OCH_3

Scheme 1. General synthesis for the twenty-six compounds. Reagents and conditions: (i) pyridine, reflux, 120 °C, 12 h; (ii) a. various MOM-protected benzaldehydes, NaOH (50%), TBAB, microwave, 5 min; b. MeOH, HCl (20%), reflux, 65 °C, 1 h. (iii) DMF, NaH, MOM–Cl, rt, 2 h; (iv) a. substituted aromatic aldehyde, MeOH, KOH (50%), rt, 24 h; b. MeOH, HCl (20%), reflux, 65 °C, 1 h.