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Synthesis and biological evaluation of novel pyrazoline derivatives as potent anti-inflammatory agents

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ABSTRACT

Twenty-eight pyrazoline derivatives, which originated from pyranochalcones, have been synthesized and evaluated for their inhibitory potency on the production of inflammatory mediator nitric oxide (NO) in LPS-stimulated RAW 264.7 cells. Among them, three compounds (**1c**, **11c**, and **15c**) exhibited potent inhibitory effects on NO production and iNOS activity superior to positive control *Indomethacin*, with **1c** being most efficacious. Furthermore, **1c** could suppress the progress of carrageenan-induced hind paw edema at a dosage of 50 mg/kg/day and dose-dependently ameliorate the development of adjuvant-induced arthritis (AIA). Docking study confirmed that **1c** was an iNOS inhibitor with good binding into the active site of murine iNOS.

Inflammation is a key process in the host defense system, involving multiple regulator of cellular and plasma to restrict its action to the time and place where necessary.[1,2] Inflammatory process can lead to various diseases, including rheumatoid arthritis, chronic inflammatory bowel disease, neurodegenerative disorder, and septic shock syndrome when beyond control.[3] Nitric oxide (NO), since its discovery as a biologically active molecule in 1987,[4] has been found to be an important and unique signal molecule involved in the regulation of many physiological and pathological processes, including vasodilation, nonspecific immune response, and acute or chronic inflammation.[5–7] Of note, as a physiological intercellular messenger, the paradoxical role of NO in the pathogenesis of inflammation is generally concentration-dependent.[8,9] NO is synthesized via the oxidation of L-arginine to L-citrulline catalyzed by a family of nitric oxide synthase (NOS) enzymes in the existence of nicotinamide adenine dinucleotide phosphate (NADPH) and O_2 as co-substrates and other co-factors.[10] The NOS family has been structurally classified as inducible NOS (iNOS) and constitutive NOS (cNOS), which is subdivided into neuronal NOS (nNOS) and endothelial NOS (eNOS) for its localization in the vascular endothelium and in the brain, respectively.[11] Because the induction of iNOS is responsible for the elevation of NO production, suppressing iNOS activity has become a way for drug development in anti-inflammation. NO possesses protective effects such as modulation of vascular smooth muscle tone, inhibition of leukocyte adhesion and platelet aggregation, and reduction of radicals at a normally low level in some inflammatory responses.[12] While under conditions of stress or upon the release of proinflammatory cytokines (TNF-α, IL-1β) and lipopolysaccharides (LPS),[13] iNOS can produce high level of NO, which further induces joint injury at the inflammatory site.[14,15] Therefore, pharmacological interference with the overproduction of NO and iNOS activity is appreciated as a promising strategy to obtain potent anti-inflammatory agents.

Pyrazolines are an abundant class of five-membered heterocycle and constitute numerous agents presenting a wide range of pharmacological activities. Comprehensive literatures have been reported that pyrazolines possess various pharmacological activities including antibacterial, antifungal, and anticancer,[16–18] etc. In our laboratory, two typical pyranochalcones, 4-hydroxyloncarpin and 3″,3″-dimethylpyrano [3′,4′]2,4,2′-trihydroxychalcone, isolated from natural products, were found to exhibited potent inhibitory activity against LPS-induced NO production and potential anti-inflammatory activity in vivo.[19] Based on these benzopyran structures, the anti-inflammatory effects of these 5-methoxy-2*H*-chromen analogs were reported in our previous achievements and some other convincing documents.[20–22] What's more, among

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the entities synthesized by our group, both compounds (**5d**[19] and **5**[22]) exhibited great biological activities when Part B turned out to be 3,4,5-trimethoxyphenyl (Fig. 1). However, limited research has been done with respect to whether the part of chalcones was necessary for biological activity or not. Therefore, we chose pyranochalcone **5** as leading compound and continued our further study. To explore new anti-inflammatory chemical entities, we used a common cyclization step to achieve a series of pyrazoline derivatives.

The general synthetic route of the pivotal intermediate pyranochalcone **5** is outlined in Scheme 1 using the same strategy as previously reported.[19,22,23] As revealed, the *N*-phenyl-substituted pyrazolines (**1a**–**6a**) were prepared directly by a cyclization of pyranochalcone **5** with commercially available substituted hydrazines in the presence of TBAB as catalyst.[24] A two-step sequence was determined and performed to prepare another 22 pyrazoline analogs (**1b**–**7b** and **1c**–**15c**).[25] Firstly, treatment of the pyranochalcone (**5**) with hydrazine hydrate (wt. 30%) and catalytic TBAB while refluxing in ethanol for 3 h afforded the appropriate 4,5-dihydro-1*H*-pyrazole (**I**) in excellent yields (>90%).[26] Secondly, compound **I** was treated with corresponding acyl chlorides or sulfonyl chlorides at 80 °C in the solvent of pyridine for 3 h to give the final *N*-substituted targeted compounds.

NO generation induced by LPS or cytokines plays an important role in the inflammatory conditions. We firstly accessed the inhibitory effects on NO production of twenty-eight pyrazoline compounds, with anti-inflammatory drug *Indomethacin* as a positive control. As depicted in Table 1, three compounds (**1c**, **11c**, **15c**)

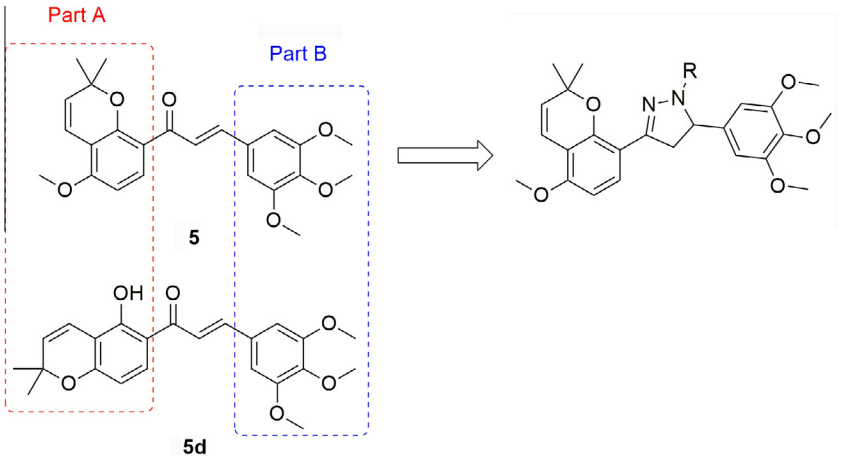
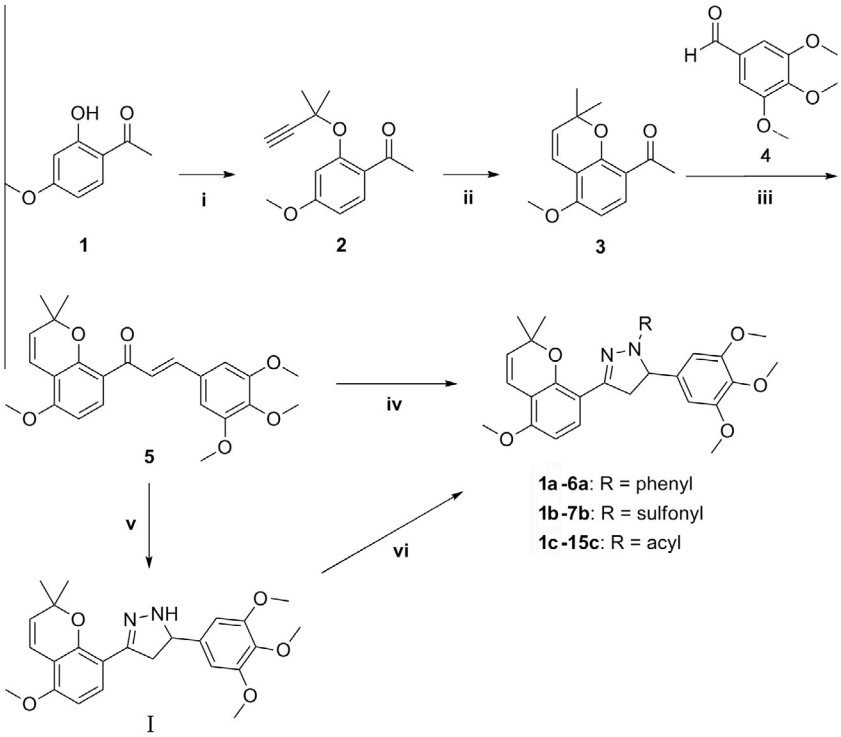


Figure 1. Chemical structure of compounds **5** and **5d**.



Scheme 1. Reagents and conditions: (i) 3-chloro-3-methyl-1-butyne, $CuCl_2{\cdot}H_2O$, DBU, CH_3CN, 0 °C, 5 h; (ii) pyridine 120 °C, overnight; (iii) KOH, methanol, two days, rt; (iv) substituted hydrazine, EtOH, TBAB, reflux, overnight; (v) $N_2H_4{\cdot}H_2O$ (30%), TBAB, 3 h; (vi) acyl chloride or sulfonyl chloride, pyridine, 80 °C, 3 h.