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Synthesis and anti-inflammatory activity of paeonol analogues in the murine model of complete Freund's adjuvant induced arthritis

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ABSTRACT

A new series of paeonol alkyl ether analogues were synthesized and confirmed with IR, ^{1}H NMR, ^{13}C NMR and HRMS spectra. They have shown anti-inflammatory activities by scavenging mediator of free radicals and inhibiting lipid mediator of inflammation on complete Freund's adjuvant (CFA) induced arthritis in mice. The in vitro and in vivo scavenging ability of free radicals was determined by using chemical analysis and commercial assay kits, respectively. The in vivo inhibiting lipid mediator of inflammation was examined by ELISA. Our results indicated that the substitution of the hydrogen in hydroxyl group at C_2 position of paeonol **1** by short carbon chain, in the presence or absence of bromo atom at C_5 position, decreased its scavenging ability on radicals (**3a** or **4a** vs **1**), while the long alkyl substitution (C_n >14) increased the activity. Compared with **3a** or **4a**, scavenging abilities of **3a**–**h** or **4a**–**h** gradually increased following the length elongation of alkyl carbon chain. Compounds **3h** and **4h** showed great scavenging ability on ·OH, $O_2^{\cdot-}$, DPPH, $ATBS^{+}$ and MDA, and good promotion on T-AOC and SOD. The results of the in vivo inhibiting lipid mediator of inflammation also demonstrated that **3h**, **4h** exhibited substantial inhibition on enzyme activity of COX-2, PGE2. Therefore, **3h** and **4h** have great potential to be the novel anti-inflammatory drug candidates for the therapy of arthritis.

Rheumatoid arthritis (RA), which affects nearly 1% of the world's population and places a significant burden upon society and patients,[1] is a serious, chronic and debilitating autoimmune disease characterized by inflammation and progressive joint destruction. Disease progression leads to bone, cartilage, and ligament destruction resulting in pain, stiffness, and deformity.

The drug treatment focuses on the use of nonsteroidal antiinflammatory drugs (NSAIDs) and disease-modifying anti-rheumatic drugs (DMARDs).[2] The main target for NSAID is to inhibit cyclooxygenase (COX). COX is one of the key enzymes involved in the degradation of arachidonic acid to prostaglandins (PG_S).[3] COX-2 is inducible and expressed mainly in inflammatory cells and COX-1 is cytoprotective and constitutively expressed in many tissues such as stomach, kidney, and platelets. Although NSAIDs are widely used to treat pain, fever, and inflammatory conditions, they can offer only palliative relief and do not halt disease progression.[4] Disease-modifying antirheumatic drugs (DMARDs) remain the first line of treatment for the majority of patients due to low cost and ability to retard disease at early onset.[5] However, there are still more drawbacks related to production efficiency and administration by injection. Furthermore, side effects tend to be serious and opportunistic infections may arise with fatal consequences.

Now treatment for RA has made significant advances over the last several decades, particularly since the introduction of biological therapies. Despite the discovery of many biological agents, there is still significant unmet medical need for safe and efficacious treatments for inflammatory, autoimmune diseases, and with different mechanism of action for patients unresponsive to current therapies.[6]

Moutan cortex radicis (MC), the roots of *Paeonia suffruticosa* Andrews (Ranunculaceae), are traditionally used in Chinese herbal medicine as an anti-inflammatory, antibacterial, antioxidant, antipyretic and analgesic agents for more than a thousand years.[7] The chemical constituents of MC include paeonolide, paeoniflorin, polysaccharides, steroids, and gallic acid. Paeonol (2-hydroxy-4-methoxyacetophenone), the major phenolic component of MC,

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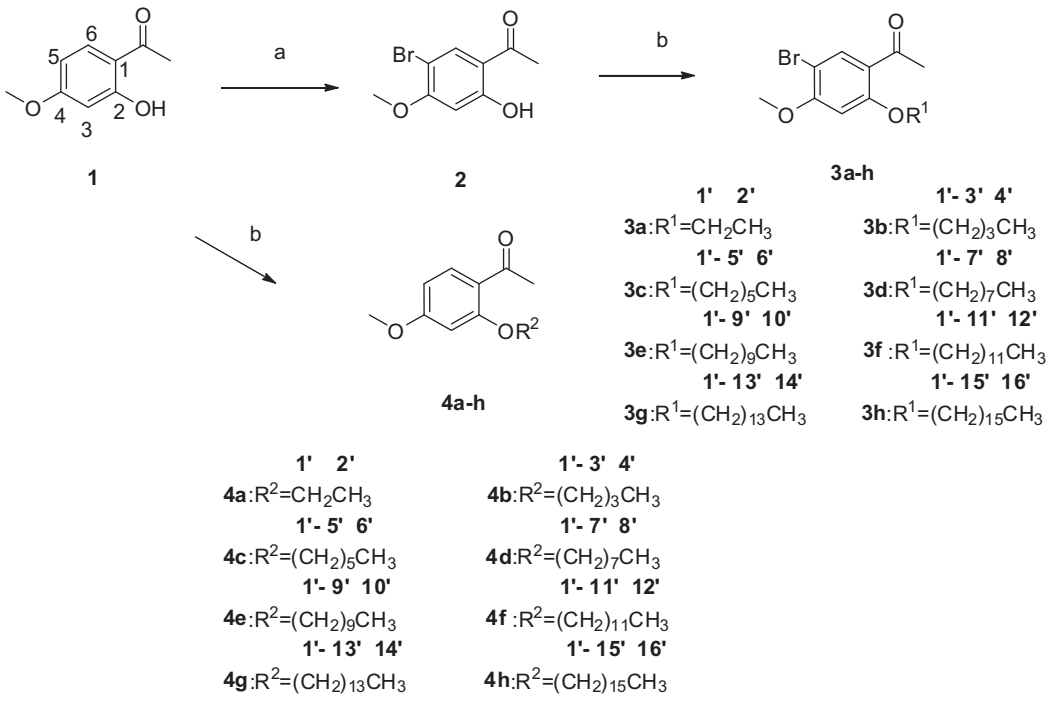

Figure 1. Protocol for synthesis of paeonol analogues. (a) Br_2, anhydrous $AlCl_3$, $CHCl_3$, ice-water bath; (b) Bromoalkanes, K_2CO_3, DMF, acetone, 60 °C.

Table 1
The half maximal inhibitory concentrations (IC_{50}) of scavenging activity of title compounds and positive control (mM) ($\bar{x} \pm s$, $n = 6$)

Compd	$O_2^{\cdot-}$ (mM)	DPPH (mM)	·OH (mM)	$ABTS^+$ (mM)
Trolox	0.6 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.0
1	5.8 ± 0.0	42.8 ± 2.6	0.2 ± 0.1	16.0 ± 1.0
2	15.6 ± 0.5	41.8 ± 8.4	3.0 ± 0.1	31.4 ± 2.2
3a	37.9 ± 0.2	46.8 ± 3.0	57.7 ± 1.6	36.0 ± 0.4
3b	38.2 ± 0.5	42.3 ± 8.1	56.9 ± 1.9	34.1 ± 0.8
3c	34.0 ± 1.0	42.4 ± 9.9	53.1 ± 2.5	35.7 ± 4.0
3d	30.9 ± 1.0	39.0 ± 2.0	38.1 ± 15.0	32.0 ± 2.7
3e	15.0 ± 0.3	35.1 ± 5.6	29.7 ± 13.2	34.7 ± 5.8
3f	10.6 ± 0.4	29.5 ± 1.7**	23.1 ± 10.8	32.7 ± 4.2
3g	6.8 ± 0.4	16.1 ± 0.6**	13.2 ± 5.3	8.7 ± 1.2**
3h	1.8 ± 0.1**	6.5 ± 0.4**	0.1 ± 0.0**	4.6 ± 0.9**
4a	6.6 ± 0.5	39.6 ± 5.1	35.3 ± 8.4	37.7 ± 2.5
4b	15.6 ± 0.5	43.3 ± 2.3	57.2 ± 2.0	36.3 ± 1.5
4c	18.0 ± 0.3	42.6 ± 3.6	84.4 ± 5.6	42.1 ± 0.9
4d	21.2 ± 0.9	40.0 ± 9.5	69.4 ± 3.1	38.3 ± 1.0
4e	26.6 ± 0.5	40.1 ± 7.7	57.4 ± 4.0	41.7 ± 3.2
4f	28.5 ± 0.5	37.4 ± 5.6	65.6 ± 10.8	40.7 ± 2.5
4g	35.9 ± 1.1	43.6 ± 4.1	57.0 ± 7.2	38.0 ± 1.7
4h	39.4 ± 0.5	39.4 ± 0.9	61.5 ± 6.7	36.3 ± 4.9

** $P < 0.01$ versus compd **1**. All experiments were run in triplicate.

has been reported to possess anti-inflammatory,[8] anti-hepatitis B virus,[9] reducing myocardial damage,[10] antibacterial,[11] antidiabetic[12] and antioxidant[13] properties. In the regard of anti-inflammation, paeonol can inhibit the expression of cell surface adhesion molecules, reactive oxygen species production, proinflammatory cytokines such as TNF-α and IL-1β.[14] Nowadays in Chinese medical market, paeonol has been prepared as different drug formations (tablets, ointments, injections) to treat skin pruritus, pain and rheumatoid arthritis. In addition, a large number of studies have also demonstrated that paeonol derivatives are associated with several biological activities. Qin[15] reported that paeonol Schiff-base derivatives could form complexes with copper ions and showed high antioxidant activity, moderate DNA-binding activity, and excellent tumor cell cytotoxicity. Zhu[16] presented that paeonol thiosemicarbazone derivatives are potential mushroom tyrosinase inhibitors. However, there is little literature to study the relationship between paeonol derivatives and their anti-inflammatory activities.

Therefore, we aim at synthesizing these compounds and evaluating their abilities of scavenging free radicals and inhibiting inflammatory factors in the murine model of complete Freund's adjuvant induced arthritis.

As illustrated in Figure 1, the bromination of paeonol **1** to important intermediate **2** followed by the etherification with different substituted alkyl halides in the presence of K_2CO_3 and acetone, afforded paenol ether analogues **3a**–**h**. Direct etherification of paeonol **1** obtained paenol ether analogues **4a**–**h**. Characterizations of these synthesized compounds were performed by