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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·$^-$, DPPH, $ATBS^+$ and MDA, and good

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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.

Keywords: Paeonol analogues; Free radicals; Anti-inflammatory; Cyclooxygenase-2

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, paeonifolorin, polysaccharides, steroids, and gallic acid. Paeonol (2-hydroxy-4-methoxyacetophenone) , the major phenolic component of MC, 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 infrared spectroscopies (IR), high resonance mass spectrometry (HRMS) and nuclear magnetic resonance (NMR). Their physical properties were summarized in Figure **S1**.

The *in vitro* scavenging abilities of synthetic compounds on superoxide radical ($O_2\cdot^-$), hydroxyl radical (·OH), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2′-azino-bis-3-ethylbenzthi-azoline-6-sulphonic acid ($ABTS^+$) radical were measured by

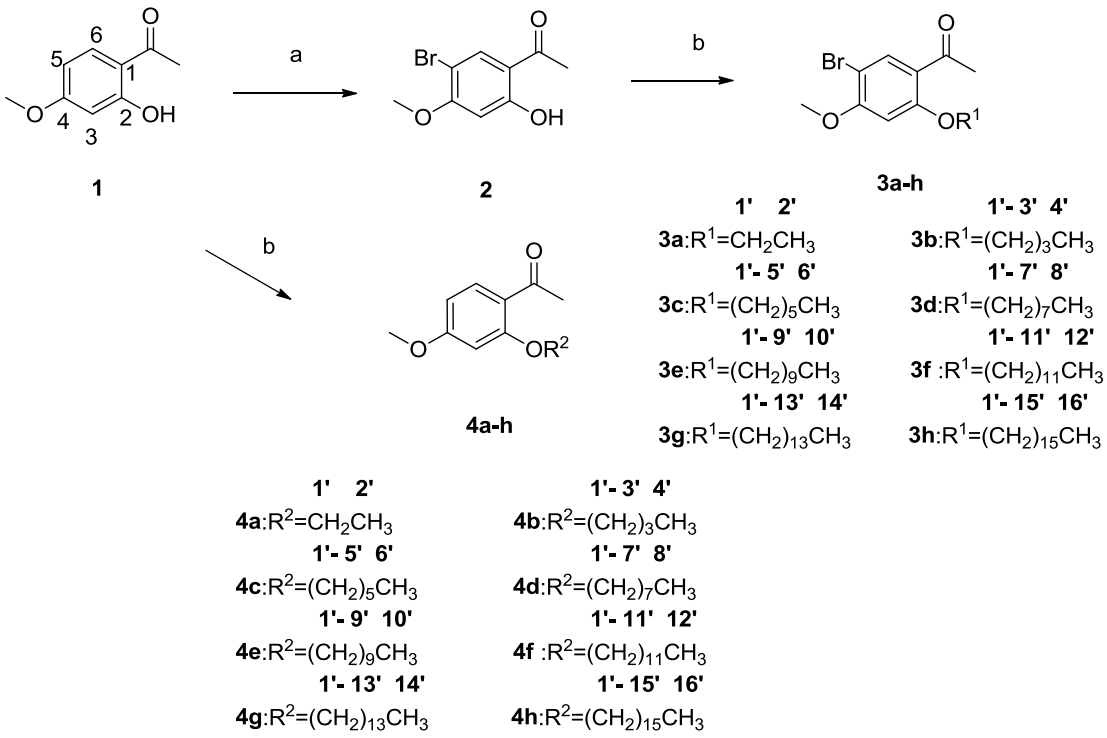

Figure 1 Protocol for synthesis of paeonol analogues

(a) Br_2, anhydrous $AlCl_3$, $CHCl_3$, ice-water bath; (b) Bromoalkanes, K_2CO_3, DMF, acetone, 60℃

the modified method of Pyrogallol autotrophic[17], Yu[18], Sachindra[19] and Feng[20], respectively. Trolox was used as a positive control. The data were depicted in Table **1**.

Compared to the lead compound **1**, the scavenging ability of **3a-3h** and **4a-h** on free radicals firstly decreased and then increased with the carbon chain extension. **3h** exhibited extremely significant difference (**3h** vs **1**, $P < 0.01$); **4h** had no difference (**4h** vs **1)**. When **3a-h** and **4a-h** compared with **3a and 4a** respectively**,** the scavenging ability on free radicals increased with the length elongation of carbon chain. The scavenging effects **of 3a-h** showed better than its corresponding compounds **4a-h**. The IC_{50} values of **3h** on $O_2\cdot^-$, DPPH, ·OH and $ABTS^+$ were 1.8, 6.5, 0.1, 4.6 mM, respectively; the IC_{50} values of **4h** on $O_2\cdot^-$, DPPH, ·OH and $ABTS^+$ were 39.4, 39.4, 61.5 and 36.3 mM, respectively. Based on the above findings, the introduction of long alkyl group (C_n >14) and bromine moiety into the lead compound could increase the *in vitro* scavenging abilities on free radicals. The influences of the title compounds on paw swelling degrees were observed in the murine model of complete Freund's adjuvant (CFA)-induced arthritis (Figure **S_2**).

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 < 0.05, **P < 0.01 vs compd. **1**. All experiments were run in triplicat.

After the administration of the CFA, paw swelling degrees in arthritis mice gradually increased till the maximum degree at the thirteenth day. Therefore, the administrations of these compounds were performed seven days after the model was established successfully, to evaluate the effects of synthetic compounds on paw edema in mice.

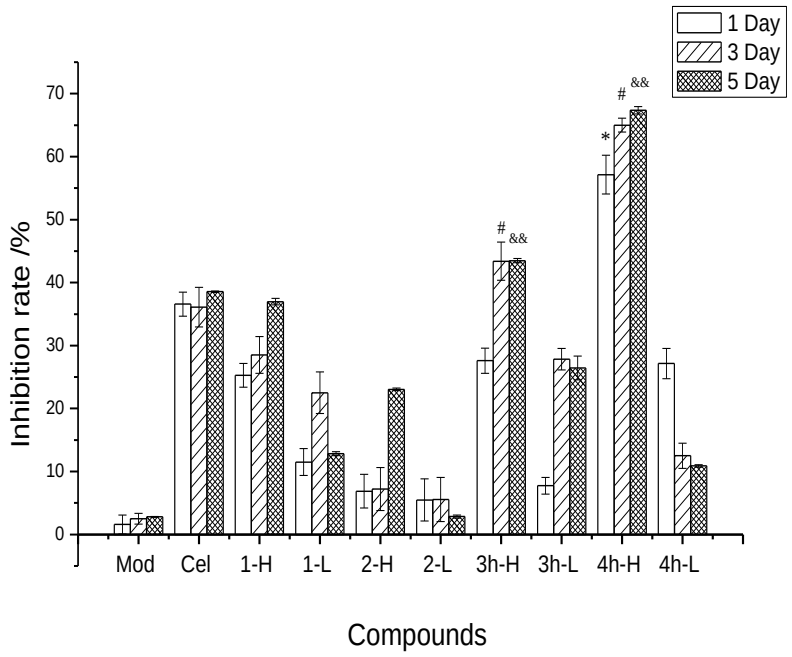

Figure 2 Effects of different compounds on paw swelling inhibition in the murine model of complete Freund's adjuvant (CFA) induced paw edema. Results are expressed as mean±SD and analyzed by two-way ANOVA followed by multiple comparison tests. *, $^{\#}$, $^{\&}P < 0.05$ considered to be significant difference compared to Celecoxib group. **, $^{\#\#}$, $^{\&\&}P < 0.01$ considered to be extremely significant difference compared to Celecoxib group.

In order to study the effects of different compounds on paw swelling inhibition, we employed the mice model of CFA-induced paw edema with Celecoxib as positive control. As shown in Figure **2**, compounds **1**, **2**, **3h**, **4h** had good inhibitory activity on mice paw edema. The high dose (100 $mg \cdot kg^{-1} \cdot d^{-1}$) of these compounds were better than the low dose (25 $mg \cdot kg^{-1} \cdot d^{-1}$). The paw swelling inhibitions were up to 45% and 65% at the high dose of **3h**, **4h**, respectively. Compared to Celecoxib (Cel) group, the inhibition of paw swelling with the high dose of **3h** exhibited significant difference ($P < 0.05$) and the high dose of **4h** had shown extremely significant difference ($P < 0.01$)

after the third day. Compounds **3h** and **4h** had excellent inhibition on mice paw edema.

The effects of these synthetic compounds on the *in vivo* activity of COX-2, prostaglandin E2 (PGE2), malondialdehyde (MDA) and superoxide dismutase (SOD), and total anti-oxidation capacity (T-AOC) in the mice serum were evaluated with chemical assay kit using Cel as a positive control, shown in Table **2**.

Table 2 Influence of different compounds on the in vivo concentrations of COX-2, PGE2, MDA, T-AOC and SOD in the serum of mice ($\bar{x} \pm s$, n =6)

Compd.	COX-2 (ng/L)	PGE2 (ng/L)	MDA (nmol/ml)	T-AOC (U/ml)	SOD (U/ml)
Control	60.4 ± 1.4	112.4 ± 1.6	6.1 ± 0.3	6.9 ± 1.1	90.8 ± 1.3
Model	85.3 ± 0.1	207.0 ± 2.1	15.5 ± 1.1	2.9 ± 0.5	66.7 ± 0.9
Cel	61.6 ± 0.4**	135.3 ± 1.2**	7.3 ± 0.7**	6.3 ± 0.3**	78.0 ± 2.1*
1-H	61.1 ± 2.4**	137.1 ± 5.0**	7.9 ± 0.3**	4.1 ± 0.7	68.1 ± 3.7
1-L	66.2 ± 0.5*	155.5 ± 4.1**	8.9 ± 0.1**	4.6 ± 0.2*	62.5 ± 2.4
2-H	57.6 ± 4.8**	142.3 ± 7.3**	6.4 ± 0.2**	6.0 ± 0.1**	78.1 ± 1.3*
2-L	65.4 ± 2.2**	179.5 ± 3.5*	9.9 ± 0.2**	3.8 ± 0.7	76.3 ± 2.0*
3h-H	64.7 ± 2.3**	150.1 ± 3.1**	5.8 ± 0.3**	11.6 ± 0.5**	76.2 ± 3.1*
3h-L	63.9 ± 0.2**	189.3 ± 6.4*	7.5 ± 0.1**	6.5 ± 0.3**	73.9 ± 4.3
4h- H	64.4 ± 2.4**	159.4 ± 2.3**	8.8 ± 0.4**	16.9 ± 0.4**	80.7 ± 1.1*
4h-L	65.2 ± 0.8**	182.2 ± 4.3*	9.7 ± 0.5*	11.1 ± 0.3**	72.1 ± 3.2

The dose of Celecoxib group was 30 $mg \cdot kg^{-1} \cdot d^{-1}$; The high dose of compounds group were 100 $mg \cdot kg^{-1} \cdot d^{-1}$; The low dose of compounds group were 25 $mg \cdot kg^{-1} \cdot d^{-1}$; *$P$ < 0.05, **P < 0. 01 vs Model group.

Compounds **1**，**2**，**3h**, **4h** could inhibit the *in vivo* levels of COX-2, PGE2 and MDA activities, and increase their T-AOC and SOD activities, and especially the high dose (100 $mg \cdot kg^{-1} \cdot d^{-1}$) of these compounds were better than the lowers (25 $mg \cdot kg^{-1} \cdot d^{-1}$). The biological effects of those high dose of compounds **2**，**3h**, **4h** showed either significant differences (P < 0.05) or extremely significant differences (P < 0. 01) compared to the lead compound **1**.

Compared to **1,** the scavenging ability of **3h** exhibited extremely significant difference (P < 0.01); **4h** had no difference. The introduction of bromine on paeonol might be responsible for the promotion of pharmacological activity. Furthermore, when compared with **1**, the scavenging ability of **3a-h** and **4a-h** on free radicals firstly decreased and then increased with the carbon chain extension. 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 the bromine 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, which might result from the change of lipophilic/hydrophobic property and stereoscopic size. As known that, homologous effect in those analogues resulted in physical and chemical properties changing in sequence.[21] If the biological activity of those analogues was related to water solubility, in other words, those compounds only in a water soluble state displayed their biological activities, the higher water solubility attributed to the stronger biological activity. Because the hydroxyl group possessed a hydrophilic property and the short alkoxyl group had a lipophilic property, paeonol **1** had higher water solubility than its derivatives **3a** and **4a**, resulting in the stronger scavenging ability of paeonol **1** than them.

The scavenging abilities of **3a-h** or **4a-h** gradually increased following the length elongation of alkyl carbon chain, compared with **3a** or **4a**. When the number of carbon exceeded fourteen (C_n >14), the biological activity of **3h** and **4h** became good. The *in vivo* experimental data had also demonstrated **3h** and **4h** exhibited the best biological activities. Taking the data of COX-2 as an example, there was supplementary effect between the ligand homologue and the receptor COX-2 enzyme according to induced-fit theory *in vivo*[22]. If the active site of COX-2 enzyme receptor had enough space, when the volume of alkyl partition in homologue increased and was fitted to the supplement with the stereoscopy of COX-2 enzyme receptor, the biological activity promoted with the increase of carbon number. That was the most likely explanation for **3a-h** or **4a-h**, which scavenging abilities gradually increased with the extension of alkyl carbon chain.

As we known, inflammatory disease was resulted from the stimulation of inflammatory mediators such as free radicals and lipid mediators.[23] From the preliminary results of scavenging abilities of those analogues on free radicals *in vitro*, **3h** and **4h** had good ability, however, **3h** exhibited better ability among them. According to the screening result, the in vivo inhibition of **3h**, **4h** on CFA-induced

paw swelling in mice had been observed. They also showed good inhibition, and especially **4h** had stronger inhibition. Thus, **3h** and **4h** should be considered as the lead compounds for further investigation on anti-inflammatory drug candidates.

Although the anti-inflammatory activities of those new synthetic compounds with the introduction of alkyl side chains into the paeonol scaffold were not as good as Cel with the same dosage, their biological activities have increased a lot compared to the lead compound **1**. In other words, the etherification of paeonol with the long alkyl chain could promote the anti-inflammatory activity of paeonol. In order to further study the relationship between the structures and the anti-inflammatory activity, more paeonol derivatives needed to be synthesized.

In conclusion, inspired by the functional scaffold of paeonol, we have demonstrated that some alkyl ether analogues of bromo-paeonol exhibited anti-inflammatory activities. The introduction of bromine and the long alkyl chain group on paeonol might be the major factor contributing to their pharmacological properties. Therefore, **3h** and **4h** have great potential to be the novel anti-inflammatory drug candidates for the therapy of arthritis.

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Supplementary data

Supplementary data (General procedures and spectral data) associated with this article can be found on the attachment.

References and notes

1. Isaacs, J.D. *Nature Rev. Immunol.* 2010, 10, 605.
2. Miwatashi, S.; Arikawa, Y.; Kotani, E.; Miyamoto, M.; Naruo, K.; Kimura, H.; Tanaka, T.; Asahi, S.; Ohkawa, S. *J. Med. Chem*. 2005, 48, 5966.
3. (a) Chowdhury, M.A.; Abdellatif, K.R.A.; Dong, Y.; Das, D.; Suresh, M.R.; Knaus, E.E. *J. Med. Chem*. 2009, 52,1525. (b)Almansa, C.; Bartrolí, J.; Belloc, J.; Cavalcanti, F.L.; Ferrando, R.; Gómez, L.A.; Ramis, I.; Carceller, E.; Merlos, M.; García-Rafanell, J. *J. Med. Chem*. 2004, 47, 5579.
4. (a) Portevin, B.; Tordjman, C.; Pastoureau, P.; Bonnet, J.; Nanteril, G.D. *J. Med. Chem*. 2000, 43, 4582. (b)Koch, K.; Melvin, L.S.; Reiter, L.A.; Biggers, M.S.; Showell H.J.; Griffiths, R.J.; Pettipher, E.R.; Cheng J.B.; Milici, A.J.; Breslow, R.; Conklyn, M.J.; Smith, M.A.; Hackman, B.C.; Doherty, N.S.; Salter, E.; Farrell, C.A.; Schulte, G. *J. Med. Chem.* 1994, 37, 3197.
5. Safina, B.S.; Baker, S.; Baumgardner, M.; Blaney, P.M.; Chan, B.K.; Chen, Y.H.; Cartwright, M.W.; Castanedo, G.; Chabot, C.; Cheguillaume, A.J.; Goldsmith, P.; Goyal, B.; Hancox, T.; Handa, R.K.; Iyer, P.S.; Kaur, J. Kondru, R.; Kenny, J.R.; Krintel, S,L.; Li, J.; Sohal, S. Sweeney, Z.K.; Tay, S.; Tivitmahaisoon, P.; Waszkowycz, B.; Wei, B.Q.; Yue, Q.; Zhang,C.H.; Sutherlin, D.P. *J. Med. Chem.* 2012, 55, 5887.
6. Lou, Y.; Han, X.; Kuglstatter, A.; Kondru, R.K.; Sweeney, Z.K.; Soth, M.; McIntosh, J.; Litman, R.; Suh, J.; Kocer, B.; Davis, D.; Park, J.; Frauchiger, S.; Dewdney, N.; Zecic, H.; Taygerly, J.P.; Sarma, K.; Hill, R.J. Gabri, T.; Goldstein, D.M.; Owens, T.D. *J. Med. Chem*. 2015, 58, 512.
7. (a) Baumgartner, R.R.; Steinmann, D.; Heiss, E.H.; Atanasov, A.G.; Ganzera, M.; Stuppner, H.; Dirsch, V.M. *J. Nat. Prod.* 2010, 73, 1578. (b)Ha, D.T.; Ngoc, T.M.; Lee, I.S.; Lee, Y.M.; Kim, J.S.; Jung, H.J.; Lee, S.M.; Na, M.K.; Bae, K.H. *J. Nat. Prod.* 2009, 72, 1465.
8. Chou, T.C. *Brit. J. Pharmacol.* 2003, 139, 1146.
9. Huang, T.J.; Chuang, H.; Liang, Y.C.; Lin, H.H.; Horng, J.C.; Kuo, Y.C.; Chen, C.W.; Tsai, F.Y.; Yen, S.C.; Chou, S.C.; Hsu, M.H. Eur. *J. Med. Chem.* 2015, 90, 428.
10. Nizamutdinova, I.T.; Jin, Y.C.; Kim, J.S.; Yean, M.H.; Kang, S.S.; Kim, Y.S.; Lee, J.H.; Seo, H.G.; Kim, H.J.; Chang, K.C. *Planta Med.* 2008, 74, 8.
11. Kim, H.K.; Tak, J.H.; Ahn, Y.J. *J. Agric. Food Chem.* 2004, 52, 7857.

12. Lau, C.H.; Chan, C.M.; Chan, Y.W.; Lau, K.M.; Lau, T.W.; Lam, F.C.; Law, W.T.; Che, C.T.; Leung, P.C.; Fung, K.P.; Ho, Y.Y.; Lau, C.B.S. *Phytomed.* 2007, 14, 778.
13. Chen, B.; Ning, M.; Yang, G. *Molecules*. 2012, 17, 4672.
14. (a) Pan, L.L.; Dai, M. *Phytomed*. 2009, 16, 1027. (b) Hsieh, C.L.; Cheng, C.Y.; Tsai, T.H.;Lin, I.H.; Liu, C.H.; Chiang, S.Y.; Lin, J.G.; Lao, C.J.; Tang, N.Y. *J. Ethnopharmacol*, 2006, 106, 208.
15. Qin, D.D.; Yang, Z.Y.; Zhang, F.H.; Du, B.; Wang, P.; Li, T.R. *Inorg. Chem. Commun.* 2010, 13, 727.
16. Zhu, T.H.; Cao, S.W.; Yu, Y.Y. *Int. J. Biol. Macromolec.* 2013, 62, 589.
17. Li, X.; Lin, J.; Gao, Y.; Han, W.; Chen, D. *Chem. Cent. J.* 2012, 6,140.
18. Yu, F.; Xu, D.; Lei, R.; Li, N.; Li, K. *J. Agric. Food. Chem.* 2008, 56, 730.
19. Sachindra, N.M.; Sato, E.; Maeda, H.; Hosokawa, M. Niwano, Y.; Kohno, M.; Miyashita, K. *J. Agric. Food. Chem.* 2007, 55, 8516.
20. Feng, J.Y.; Liu, Z.Q. *J. Agric. Food. Chem.* 2009, 57, 11041.
21. Zhang B.S.; Ye X.L.; Chen Z.; Yao B.; Tan P.; Li X.G. *Acta Pharm. Sin.* 2011, 46, 811.
22. Koshland Jr D.E.; Neet K.E. *Annu Rev Biochem.* 1968, 37(1), 359-411.
23. Wang D.X.; Jin H.M. *Human Pathophysiology*. 3rd ed. Beijing: People's Medical Publishing House, 2008: 386.

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