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Synthesis and evaluation of hydroxychalcones as multifunctional non-purine xanthine oxidase inhibitors for the treatment of hyperuricemia

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ABSTRACT

A series of hydroxychalcone derivatives have been designed, synthesized and evaluated for human xanthine oxidase (XO) inhibitory activity. Most of the tested compounds acted moderate XO inhibition with IC_{50} values in the micromolar rang. Molecular docking and kinetic studies have been performed to explain the binding modes of XO with the selected compounds. In addition, *in vitro* antioxidant screening results indicated that some of the hydroxychalcones possessed good anti-free radical activities. Furthermore, the preferred compounds **16** and **18** were able to significantly inhibit hepatic xanthine oxidase activity and reduced serum uric acid level of hyperuricemic mice *in vivo*. In summary, compounds **16** and **18** with balanced activities of antioxidant, XO inhibition and serum uric acid reduction, which are promising candidates for the treatment of hyperuricemia and gout.

Hyperuricemia is a common metabolic disease characterized by elevated serum uric acid (UA) which is the final oxidation product of purine catabolism in human.[1] Epidemiologic data indicate that the prevalence of hyperuricemia has been increasing in recent decades, but its treatment is still limited.[2,3]

In the process of the evolution, human and great apes lost the uricase enzyme which can convert uric acid to 5-hydroxyisourate, and eventually to the more soluble allantoin.[4] As a consequence, serum uric acid level in human is higher and can be altered more easily by various factors than that in other mammals.[5] Hepatic over-production or renal under-excretion of uric acid can both contribute to hyperuricemia and further cardiovascular and metabolic disorders associated with hyperuricemia.[6,7] Xanthine oxidase (XO) is a key enzyme that catalyzes the oxidation of xanthine and hypoxanthine into uric acid. As overproduction of uric acid is the primary cause of hyperuricemia, XO has always been considered as the most promising target for treatment of this condition.[8] Nowadays, allopurinol and febuxostat are clinically available to reduce serum uric acid levels by inhibiting XO, but more and more toxicity cases and side effects are reported during the use of them.[9,10] So, new XO inhibitors with more specific effects and fewer side effects than allopurinol and febuxostat are needed for treating hyperuricemia and gout.

Hyperuricemia has been increasingly reported to be associated with the pathogenesis of a wide range of chronic diseases such as hypertension, diabetes mellitus, metabolic syndrome, and renal and cardiovascular disease.[11,12] Recent research indicates that oxidative stress may play a major pathophysiological role in the development of hyperuricemia involving diseases mentioned above, especially renal and cardiovascular disease.[13,14] Uric acid, while being a potent antioxidant in the extracellular environment, is a pro-oxidant inside the cell where it oxidizes lipids, reduces nitric oxide availability in endothelial cells, and increases reactive oxygen species (ROS).[15] As a result, high levels of serum uric acid cause an increase of cardiovascular and renal risk.[16,17] ROS overproduction may be also caused by the enhancement of XO expression and activity. While hepatic XO catalyzes the oxidation of hypoxanthine and xanthine to uric acid, superoxide ion and hydrogen peroxide are also formed in this process.[18] The production of radical species by XO activity has been intensively studied in conjunction with kidney injuries and various forms of ischemic and vascular injuries.[19,20] These mechanisms suggest that there is a need for novel antioxidant approaches preventing ROS formation to attenuate renal and cardiovascular disorders associated with hyperuricemia.

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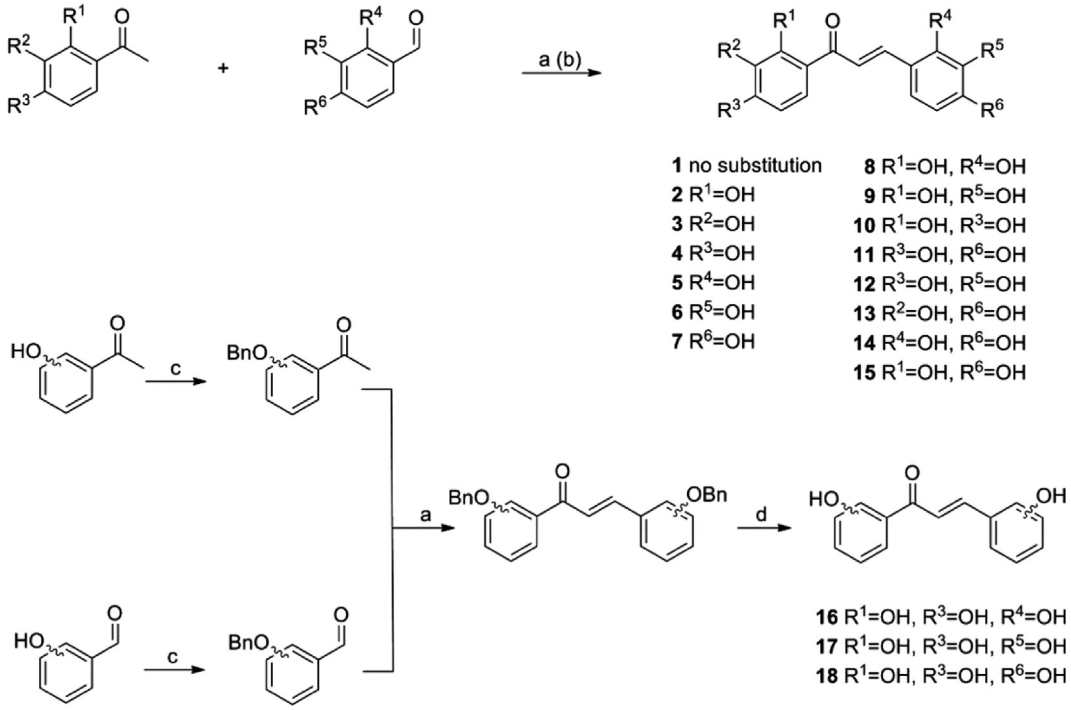

Scheme 1-2. Synthesis of the hydroxychalcone derivatives **1**–**18**.

Chalcones, a group of naturally occurring compounds, have attracted increasingly widespread attention in present-day society, since they possess a wide range of pharmacological properties possess a variety of bioactivities, including antitumor, free radical scavenging, antibiosis, antivirus, antiulcer and spasmolysis.[21–25] In the past years, some natural and synthetic hydroxychalcones have been reported as XO inhibitors.[26–30] Additionally, many flavone compounds such as rutin, quercetin and mulberroside have been found with hypouricemic effects in experimental hyperuricemia animals.[31–35] Based on these findings, we deduce that the XO inhibition and hypouricemic effect are impacted by the hydroxyl substitution in chalcone skeleton. Accordingly, we have designed and synthesized a series of hydroxychalcone derivatives and evaluated their XO inhibitory, antioxidant potency and the structure-activity relationship (SAR) studies, as well as hypouricemic action in a mouse model of hyperuricemia.

The hydroxychalcone derivatives (**1**–**15**) were synthesized along with the pathway shown in Scheme 1 with two methods.[36] The commercially available starting materials hydroxyacetophenone reacted with the appropriate hydroxylbenzaldehydes in the presence of 20% KOH (method A) or piperidine heated to 85 °C (method B) to give the target compounds in moderate to good yields. Compounds **16**–**18** were synthesized by a hydroxyl-protecting strategy in Scheme 2. Benzyl-protected hydroxyacetophenone reacted with benzyl-protected hydroxylbenzaldehydes in the presence of 20% KOH to afford the benzyloxychalcones, then benzyloxychalcones were treated with BBr_3 under ice-bath to give compounds **16**–**18**. All compounds were purified by column chromatography. The structures of the compounds were verified by ^1H NMR, ^{13}C NMR and mass spectrometry as cited in the experimental section.

The XO inhibitory activities of compounds **1**–**18** were measured spectrophotometrically under aerobic conditions using xanthine as the substrate by following the increase in absorbance at 295 nm, with Allopurinol as a Refs. 28,37. The corresponding IC_{50} values were demonstrated in Table 1. Based on the screening data, it could be seen that most of the tested compounds were able to inhibit XO with IC_{50} values in the micromolar range. Among the compounds, **2**, **8**, **9**, **12**, **15**, **16** and **18** showed good inhibitory activities, indicating that hydroxyl-substitution at 2-position was in favor of XO inhibition. Compared with different hydroxyl-substituted

Table 1
In vitro XO inhibition and antioxidant activities of the synthesized compounds.

Compounds	XO inhibition IC_{50} (μM)[a]	ABTS assay (trolox equivalent)[b]	DPPH assay inhibitory rate[c]
1	N[d]	N[f]	18.4%
2	71.3 ± 8.6	N[f]	13.5%
3	165 ± 10	N[f]	11.7%
4	N[d]	N[f]	12.5%
5	145 ± 6.6	N[f]	10.8%
6	228 ± 11	N[f]	11.3%
7	159 ± 5	N[f]	25.7%
8	87.4 ± 3.1	0.17	28%
9	91.8 ± 2.1	0.93	108 ± 7
10	107 ± 11	0.38	197 ± 16
11	117 ± 9	0.43	35.9%
12	88.3 ± 6.3	0.18	24.3%
13	133 ± 11	0.16	19.7%
14	N[d]	N[f]	10.8%
15	79.4 ± 2.7	0.24	23.1%
16	56.8 ± 2.3	0.87	128 ± 10 μM
17	108 ± 8	0.45	38.6%
18	47.3 ± 4.1	0.51	287 ± 17 μM
Allopurinol	19.6 ± 1.8	N[f]	20.5%
Resveratrol	n.t.[e]	0.65	95.1 ± 5.3 μM

[a] IC_{50}: 50% inhibitory concentration (means ± SEM of three experiments).
[b] Data are expressed as (mmol trolox)/(mmol tested compound).
[c] Inhibitory rates of the compounds at 2000 μM.
[d] Inactive at 1000 μM (highest concentration tested), at higher concentrations the compounds precipitate.
[e] n.t. = not tested.
[f] Trolox equivalent lower than 0.1.