Bioorganic & Medicinal Chemistry xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Discovery of novel curcumin derivatives targeting xanthine oxidase and urate transporter 1 as anti-hyperuricemic agents

Gui-Zhen Ao [a], Meng-Ze Zhou [b], Yu-Yao Li [a], Si-Ning Li [a], Hua-Nian Wang [a], Qian-Wen Wan [a], Huan-Qiu Li [a,*], Qing-Hua Hu [b,*]

[a] Department of Medicinal Chemistry, College of Pharmaceutical Sciences, Soochow University, Suzhou 215123, PR China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 5 September 2016
Revised 17 October 2016
Accepted 18 October 2016
Available online xxxx

Keywords:
Xanthine oxidase
Urate transporter 1
Anti-hyperuricemic
Uricosuric
Curcumin derivatives

ABSTRACT

A series of curcumin derivatives as potent dual inhibitors of xanthine oxidase (XOD) and urate transporter 1 (URAT1) was discovered as anti-hyperuricemic agents. These compounds proved efficient effects on anti-hyperuricemic activity and uricosuric activity in vivo. More importantly, some of them exhibited proved efficient effects on inhibiting XOD activity and suppressing uptake of uric acid via URAT1 in vitro. Especially, the treatment of **4d** was demonstrated to improve uric acid over-production and under-excretion in oxonate-induced hyperuricemic mice through regulating XOD activity and URAT1 expression. Docking study was performed to elucidate the potent XOD inhibition of **4d**. Compound **4d** may serve as a tool compound for further design of anti-hyperuricemic drugs targeting both XOD and URAT1.

1. Introduction

Uric acid is the end product of purine metabolism in human beings because a gene encoding uricase has undergone mutational silencing during hominoid evolution.[1–3] Hyperuricemia, characterized by the high level of serum uric acid, is a pathological condition which may originate from excessive production and/or impaired excretion of uric acid.[4] Persistent hyperuricemia is widely accepted as the primary risk factor for urate deposition diseases, such as gout and renal damage.[5,6] Reduction of the uric acid would play a causal role in the treatment of urate-related disease. Therefore, development of anti-hyperuricemic drugs has become increasingly important.

Currently, there are several drug strategies to control urate levels (Fig. 1). For example, the xanthine oxidase inhibitor allopurinol and febuxostat have been the most commonly used urate-lowering drug. Xanthine oxidase (XOD) in liver is the key enzyme to catalyze uric acid production, which catalyzes the oxidation of hypoxanthine to xanthine and further catalyze the oxidation of xanthine to uric acid.[7] Inhibitors of xanthine oxidase block conversion of xanthine to uric acid, exhibiting potentially effects on hyperuricemia.[8,9] Nevertheless, only about 40% of patients are able to meet treatment goals via allopurinol, and it occasionally causes Stevens Johnson syndrome, which may be fatal.[10] In addition, febuxostat has been associated with cardiovascular complications causing the Food and Drug Administration (FDA) to require a cautionary statement on the drug insert.[11] Thus, it requires more diversity of treatment option for hyperuricemia.

On the other hand, benzbromarone, a drug with potent uricosuric activity, effectively reduces serum urate levels through targeting urate transporter 1 (URAT1), with most people achieving normal uric acid values.[12–14] URAT1 (SLC22A12) at the luminal membrane, is the main renal-specific transporter involved in urate reabsorption in kidneys, which has been considered as a efficient target for treating hyperuricemia.[15] Hypouricemia was induced by an increase in urate urinary excretion and modest inhibition of XO. Urate urinary excretion was observed to be due to inhibition of URAT1 but not GLUT9. KUX-1151 is a potential XO and URAT1 dual inhibitors currently undergoing phase II trials (Kissei Pharmaceutical CO).[16] Given these considerations, the development of novel compounds that acted by dual inhibition of XOD and URAT1 could be a promising approach for treating hyperuricemia.

Curcumin, a polyphenolic compound derived from dietary spice turmeric, possesses diverse pharmacologic effects including anti-inflammatory, antioxidant[17–19], antiproliferative[20] and uricosuric activities.[21,22] In our previous publication[23], a series of a,b-unsaturated curcumin cyclohexanone analogous their antiproliferative activities have been studied. Recently, the uricosuric activity of curcumin has been investigated; it is potentially useful for treat-

* Corresponding authors.
E-mail addresses: huanqiuli@suda.edu.cn (H.-Q. Li), huqh@cpu.edu.cn (Q.-H. Hu).

http://dx.doi.org/10.1016/j.bmc.2016.10.022
0968-0896/

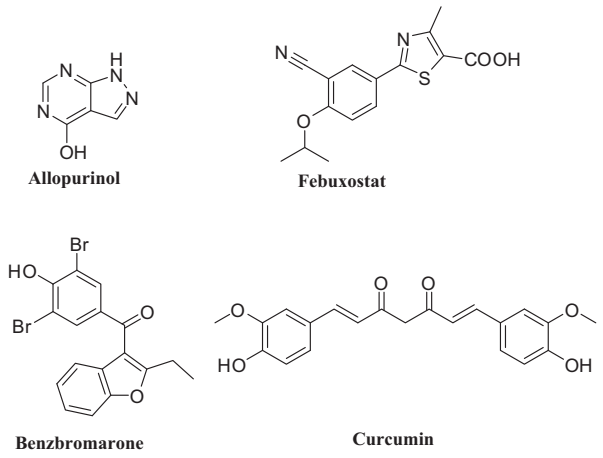


Figure 1. Chemical structures for allopurinol, febuxostat, benzbromarone and curcumin.

ment of hyperuricemia or gout as a new URAT1 inhibitor.[21] Therefore, in continuation of our earlier studies focus on the diverse biological activity of curcumin analogs, the a,b-unsaturated curcumin cyclohexanone analogous had been screened for their anti- hyperuricemia activity and some compounds showed potent anti-hyperuricemia activity. Thus, in this manuscript, we discovered a series of α,β-unsaturated cyclohexanone and cyclopentanone analogous as anti-hyperuricemia agents. Notably, compound **4d** is the XOD/URAT1 dual inhibitors with excellent in vitro and in vivo anti-hyperuricemia potency.

2. Chemistry

Twenty-two α,β-unsaturated cyclohexanone and cyclopentanone analogous of curcumin were synthesized to be screened for the anti-hyperuricemia. The preparation of the α,β-unsaturated cyclohexanone analogous have been reported in our previous publication.[23] As show in Scheme 1, the intermediates **3** or **6** was prepared by the Stork reaction. Enamines **2** or **5** were synthesized by cyclohexanone/cyclopentanone and morpholine in benzene. **3** or **6** were subsequently obtained by hydrolysis of 3,5-dimethoxybenzaldehyde and **2** or **5**. Then the α,β-unsaturated cyclohexanone and cyclopentanone analogous were synthesized by Claisen-Sch midt reaction, various substitute benzaldehydes and **3** or **6** were dissolved in 10% NaOH ethanol solution at room temperature to give the target compounds **4a**–**4j** and **7a**–**7l**. All compounds were purified by silicagel column and identified by elemental H and C NMR and HRMS.

3. Results and discussion

3.1. Anti-hyperuricemia activity in vivo

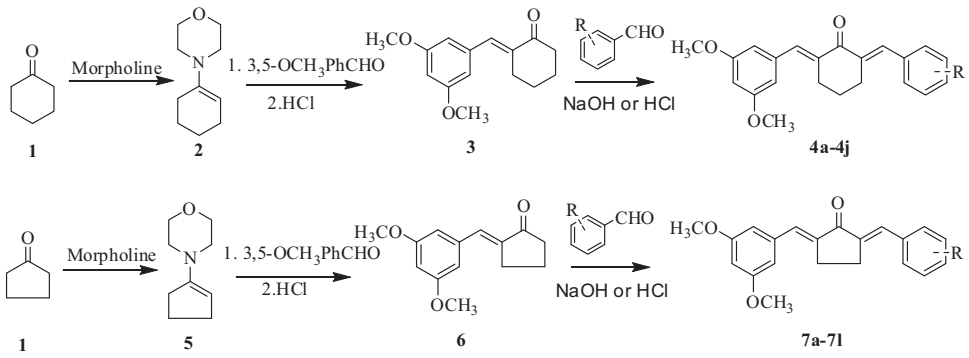
In order to monitor the efficacy of different synthesized α,β-unsaturated cyclohexanone and cyclopentanone analogous of curcumin, serum uric acid levels were determined using hyperuricemic mice models induced by oxonate treatment, allopurinol and benzbromarone, reported to inhibit xanthine oxidase and URAT1, respectively, were also screened under identical conditions for comparison. The inhibition ratios exhibited the anti-hyperuricemia activities of the synthesized curcumin derivatives and the results were summarized in Table 1. As expected, these curcumin α,β-unsaturated cyclohexanone and cyclopentanone derivatives exhibited remarkable anti-hyperuricemia activity comparable to the positive control.

Preliminary SAR (Structure Activity Relationship) studies were performed to deduce how the structure variation and modification could affect the anti-hyperuricemia activity. Generally, the results in Table 1 showed that those cyclohexanone derivatives would perform better than cyclopentanone analogous. As to cyclohexanone derivatives **4a**–**4j**, the electron withdrawing substituents on the phenyl ring at R1 position input substantial effects on the antiproliferative capability of the compounds. Compounds **4a**–**4e** and **4j** exhibited strong uric acid lowering activity (32.1–92.4%), which is slightly more potent compared to other compounds with electron-donating group at R1 position (**4f**–**4i**). **4f** and **4g** with strong electron-donating hydroxyl substitution on R-phenyl ring, showed low in vivo efficacy (7.7% and 11.8%, respectively).

Secondly, as for compounds with halogen substitution on R-phenyl ring, we could perceive the tendency that Br>Cl in the series. Compounds with R1 substitution at the *para* position (**4d**, **4i**, **7d** and **7l**) showed better activities than those with substitution at the *meta* position (**4c**, **4e**, **7c** and **7a**). Especially, compounds **4d** showed the most potent inhibitory activity (uric acid lowering activity 92.4%), and comparable to the positive control allopurinol (148.32%) and benzbromarone (99.7%).

3.2. Uricosuric activity in vivo

To confirm whether the anti-hyperuricemia activities of synthesized curcumin derivatives were attributed to their uricosuric effects, we detected uric acid excretion in 24 h of hyperuricemic mice with or without drug treatment. The results were summarized in Table 1. Taken together, the designed and synthesized curcumin α,β-unsaturated cyclohexanone analogous did demonstrate fairly potent uricosuric activity in vivo. Compounds **4c**, **4d** and **4j** displayed the most potent uricosuric in the in vivo assay, with uric acid excretion in 24 h elevation of 44.7%, 75.7%, and 36.4%, respec-



Scheme 1. Synthesis of target compounds.