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Development of benzoxazole deoxybenzoin oxime and acyloxylamine derivatives targeting innate immune sensors and xanthine oxidase for treatment of gout

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ABSTRACT

Both the inhibition of inflammatory flares and the treatment of hyperuricemia itself are included in the management of gout. Extending our efforts to development of gout therapy, two series of benzoxazole deoxybenzoin oxime derivatives as inhibitors of innate immune sensors and xanthine oxidase (XOD) were discovered in improving hyperuricemia and acute gouty arthritis. *In vitro* studies revealed that most compounds not only suppressed XOD activity, but blocked activations of NOD-like receptor (NLRP3) inflammasome and Toll-like receptor 4 (TLR4) signaling pathway. More importantly, (*E*)-1-(6-methoxybenzo[*d*]oxazol-2-yl)-2-(4-methoxyphenyl)ethanone oxime (**5d**) exhibited anti-hyperuricemic and anti-acute gouty arthritis activities through regulating XOD, NLRP3 and TLR4. Compound **5d** may serve as a tool compound for further design of anti-gout drugs targeting both innate immune sensors and XOD.

1. Introduction

Gout is an inflammatory arthritis characterized by abrupt self-limiting attacks of inflammation triggered by deposition of monosodium urate crystals (MSU) in the joint secondary to longstanding hyperuricemia, which has been associated with poor quality of life.[1] Therefore, prevention and treatment of gout should be studied and solved urgently.

Both the inhibition of inflammatory flares and the treatment of hyperuricemia itself are included in the management of gout.[2] For anti-inflammation, colchicine, corticosteroids and NSAIDs could effectively treated acute exacerbations of gout, nevertheless, broad drug toxicities, particularly in subjects with significant co-morbidities limited their application in clinic.[3] In addition, although blocking therapies, such as anakinra, canakinumab and rilonacept, have an increasing role in the management of difficult-to-treat gout, high cost and inconvenient route of administration made this type of drug difficult to be promoted.[4] Recent studies have demonstrated that innate immune sensors including Nod-like receptor 3 (NLRP3) and Toll-like receptor 4 (TLR4) are involved in sensing MSU deposition and subsequent activation of the downstream inflammatory response.[5] Assembly of NLPR3 inflammasome composed by NLRP3, apoptosis-associated speck-like protein (ASC) and caspase-1 led to cleavage of interleukin 1 beta (IL-1β) precursor to produce active IL-1β, playing a critical role in the pathogenesis of acute gouty arthritis.[6] On the other hand, TLR4 – Myeloid differentiation primary response gene 88 (MyD88) – NF-κB signaling also contributed to development of acute inflammation in primary gout patients.[7] Consistently, several natural compounds or traditional Chinese medicines exhibited beneficial effects on urate-related diseases through inhibition of NLRP3 and TLR4.[8,9] Moreover, some NLRP3 inhibitors for gout treatment have entered the phase of clinical research, such as bucillamine. Nevertheless, there are no dual inhibitors targeting NLRP3 and TLR4 were discovered for gout therapy.

More importantly, in spite of anti-inflammatory therapies successfully suppress MSU-induced inflammatory cascade, the serum uric acid still maintains a high level, which might lead to recurrence of gout attack.[10] Xanthine oxidase (XOD) involved in uric acid production, catalyzes the oxidation of xanthine to uric acid, which has been regarded as an effective target for treatment of hyperuricemia and gout. Febuxostat and allopurinol have been developed as XOD-targeted anti-hyperuricemia drugs commonly used in clinic.[11] Nevertheless, both febuxostat and allopurinol exhibited serious side effects, while they seem to be not effective for acute gouty arthritis.[12,13] Whereas there are no dual functional drugs which have anti-inflammatory and anti-hyperuricemic activities for gout therapy till now, the development of novel

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compounds that act by dual inhibition of innate immune sensors and XOD could be a promising approach for treatment of gout.

Interestingly, evidences from previous studies suggested that several kinds of flavonoids, such as quercetin and morin, not only exhibited potential anti-hyperuricemia effects in oxonate-treated mice by inhibiting XOD activities, but attenuated MSU-induced acute gouty arthritis through regulating innate immune sensors in rats.[14,15] Isoliquiritigenin, another type of flavones derivatives, has been regarded as a dual inhibitor of NLRP3 and TLR4, but high toxicity limited its clinical application.[16,17] Additionally, deoxybenzoins, intermediates in the synthesis of flavones, their oximes derivatives also exhibited similar biological activities including anti-hyperuricemic and immunosuppressive activities in our previous reports.[18,19] In view of the above, based on the deoxybenzoins oximes skeleton, we designed two series of benzoxazole deoxybenzoin oxime derivatives as dual inhibitors of innate immune sensors and XOD (Scheme 1). Notably, compound **5d** is multi-targeting inhibitor of NLRP3, TLR4 and XOD with excellent potency in treating hyperuricemia and acute gouty arthritis.

2. Chemistry

The preparation of the benzoxazole deoxybenzoin oxime derivatives is shown in Scheme 1. As summarized in Scheme 2, our chemical synthesis utilized a series of commercially available 2-aminophenols **1a**–**1f**. Compounds **1a**–**1f** reacted with an equal equivalent of lactic acid to yield **2a**–**2f**. The intermediates **2a**–**2f** were then oxidized by an equal equivalent of chromium trioxide in acetic acid under reflux to afford benzoxazole ketones **3a**–**3f**. Compounds **3a**–**3f** reacted with bromobenzene under $Pd(dba)_2$/DTPF-catalyzed R-arylation reaction to afford benzoxazole deoxybenzoin **4a**–**4n**. The treatment of selected deoxybenzoins derivatives **4a–4n** with hydroxylamine hydrochloride in ethanol afforded the benzoxazole deoxybenzoin oxime derivatives **5a–5n** with sodium acetate as catalyst and alkaline environment provider.

As shown in Scheme 3, benzoxazole acyloxylamines **7a**–**7h** with an oxime linking moiety were synthesized by reaction of appropriate acid chloride with oxime **6**, which was itself prepared from the corresponding benzoxazole ketone **3a** by condensation with hydroxylamine hydrochloride (Scheme 3).

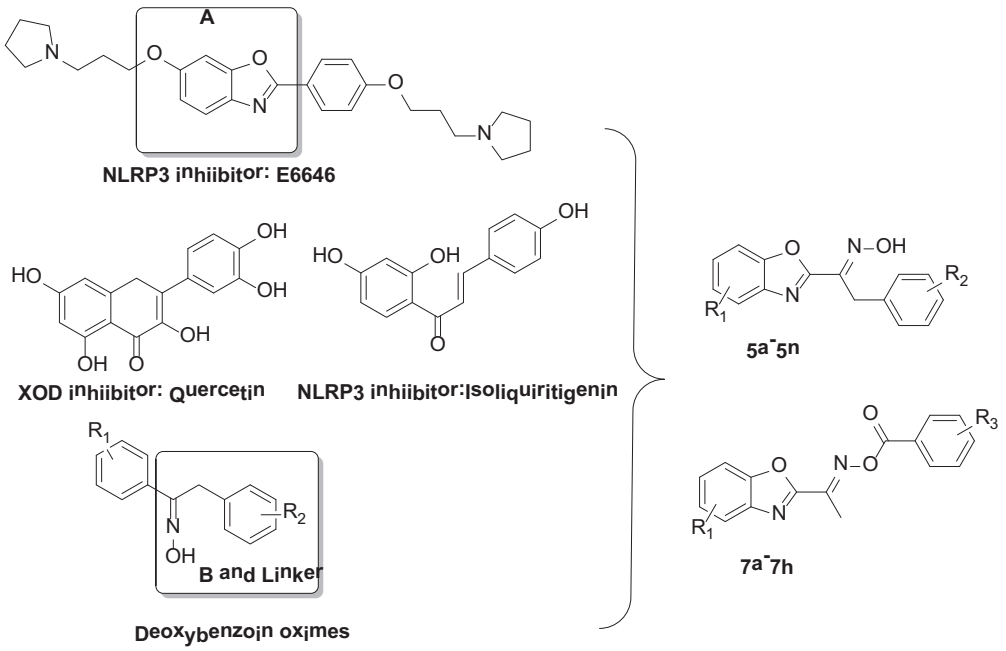
3. Results and discussion

3.1. In vitro XOD inhibitory activities screen and the binding modes

Since the xanthine oxidase (XOD) was the rate-limiting enzyme involved in uric acid production, we examined XOD inhibitory activities of the synthesized compounds. The results were summarized in Table 3. Preliminary SAR (Structure Activity Relationship) studies were performed to deduce how the structure variation and modification could affect the XOD inhibitory activities. As shown in Table 1, several benzoxazole deoxybenzoin oxime derivatives (e.g., **5d**, **5e**, **5h**, **5i**, **5k** and **5l**) showed good to excellent XOD inhibitory activity. As to compounds **5a**–**5n**, the electron-donating substituents on the phenyl ring at R_1 and R_2 position input substantial effects on the XOD inhibitory capability of the compounds. **5a** with no substituent for R_1 and R_2 exhibited IC_{50} value of 39.1 μM, and **5d** with methoxy group for R_1 and R_2 showed much increased inhibitory activity (IC_{50} = 3.7 μM), comparable to the positive control allopurinol (IC_{50} = 2.9 μM). Introduction of a chloro group on the R_1 led to the loss of XOD inhibitory activity. Compound **5e** (R_1 = 6-Cl) showed lower activity (IC_{50} = 17.8 μM) than **5d** (R_1 = 6-OMe), so the methoxy group is beneficial for increasing XOD inhibitory activity.

Secondly, benzoxazole acyloxylamines **7a**–**7 h** showed moderate XOD inhibitory activity (IC_{50} range: 15.2 μM-64.3 μM). Compounds with R_2 substitution at the *para* position (**7c**, **7f** and **7g**) showed better activities than others. Especially, compound **7f** with *para*-methoxy group on phenyl ring showed potent inhibitory activity and similar to the tendency for benzoxazole deoxybenzoin oxime derivatives.

Enzyme kinetics studies were performed for the representative compound **5d**. The Lineweaver-Burk plot (Fig. 1) revealed that compound **5d** acted as a competitive-type inhibitor of XOD with a Ki value of 2.89 μM. To investigate the binding modes between benzoxazole deoxybenzoin oxime derivatives and XOD,



Scheme 1. Design of the target compounds.