Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and synthesis of novel 2-(indol-5-yl)thiazole derivatives as xanthine oxidase inhibitors

Jeong Uk Song [a,b], Sung Pil Choi [b], Tae Hun Kim [b], Cheol-Kyu Jung [b], Joo-Youn Lee [b], Sang-Hun Jung [a,*], Geun Tae Kim [b,*]

[a] College of Pharmacy, Chungnam National University, Daejeon 305-764, Republic of Korea
[b] Research & Development, LG Life Sciences Ltd, 188 Munji-ro, Yuseong-gu, Daejeon 305-380, Republic of Korea

ARTICLE INFO

Article history:
Received 6 July 2014
Revised 11 January 2015
Accepted 22 January 2015
Available online xxxx

Keywords:
Gout
Hyperuricemia
Xanthine oxidase inhibitors
2-(Indol-5-yl)thiazole

ABSTRACT

Xanthine oxidase (XO) inhibitors have been widely used for the treatment of gout. Indole rings are frequently used as active scaffold in designing inhibitors for enzymes. Herein, we describe the structure–activity relationship for novel xanthine oxidase inhibitors based on indole scaffold. A series of novel tri-substituted 2-(indol-5-yl)thiazole derivatives were synthesized, and their in vitro inhibitory activities against xanthine oxidase and in vivo efficacy lowering uric acid level in blood were measured. Among them, 2-(3-cyano-2-isopropylindol-5-yl)-4-methylthiazole-5-carboxylic acid exhibits the most potent XO inhibitory activity (IC_{50} value: 3.5 nM) and the excellent plasma uric acid lowering activity. Study of structure activity relationship indicated that hydrophobic moiety (e.g., isopropyl) at 1-position and electron withdrawing group (e.g., CN) at 3-position of indole ring and small hydrophobic group (CH_3) at 4-position of the thiazole ring enhanced the XO inhibitory activity. Hydrophobic substitution such as isopropyl at 1-position of the indole moiety without any substitution at 2-position has an essential role for enhancing bioavailability and therefore for high in vivo efficacy.

Gout is commonly recognized as recurrent pains of acute inflammatory arthritis. High level of uric acid in blood, or hyperuricemia causes such symptoms by inducing the precipitation of monosodium urate crystals.[1] The disease occurs by overproduction of uric acid and/or impaired renal excretion of uric acid.[1–3] Uric acid is a product of purine metabolism, which involves xanthine oxidase (XO) in the two final steps (from hypoxanthine to xanthine, then to uric acid). Therefore inhibition of this enzyme has been an obvious target to control uric acid level in blood and eventually to cure gout. In this regard, allopurinol (Fig. 1) has been clinically used to treat hyperuricemia for more than forty years. Recently, febuxostat (Fig. 1) has been introduced. Both of these drugs target xanthine oxidase to inhibit its function. The XO inhibitors block the biosynthesis of uric acid through competitive inhibition, leading to lower uric acid level in blood.[4,5] Although both of them are inhibitors of XO, their mechanism of action is diverged. The purine analog, allopurinol is a mechanism-based inhibitor of xanthine oxidase.[5,6] Allopurinol itself is a weak XO inhibitor in vitro. However, it is rapidly oxidized by XO in vivo to form an active metabolite called oxypurinol, which is a potent XO inhibitor. On the other hand, febuxostat is a 2-phenylthiazole analog and displays higher inhibitory activity by strong non-competitive binding to active site of XO. However, it has not been proved to be clinically more beneficial compared with allopurinol.[7–9] Another approach to treat gout is to use recombinant urate oxidase, which directly metabolizes uric acid to allantoin. Pegloticase, approved in 2010, is a pegylated urate oxidase that treats refractory chronic gout.[3] Uric acid levels may also be lowered by uricosurics, which increase the urinary excretion of uric acid by interfering the urate absorption from kidney to blood.

Although the link between hyperuricemia and gout has been known for more than hundred years, the intrinsic metabolic relationship between hyperuricemia and diabetes was recently established.[10,11] Since hyperuricemia becomes serious health problem, it calls for more diverse treatment option. In the recent years, many synthetic scaffolds comprising triazole,[12] isocytosines,[13,14] pyrimidone[15] and imidazole[16] have been reported to display XO inhibition in the literatures. In this context, we have made an effort to discover novel XO inhibitors based on indolethiazole **6** as shown in Figure 1. The indole rings are frequently utilized as active scaffold in designing target molecules or inhibitors for enzymes and receptors.[17–19]

Although little was known about the effect of indole scaffold on XO inhibition, it can be found easily in promising anticancer,

* Corresponding authors. Tel.: +82 42 821 5939; fax: +82 42 823 6566 (S.-H.J.); tel.: +82 42 866 2247; fax: +82 42 861 2566 (G.T.K.).
E-mail addresses: jungshh@cnu.ac.kr (S.-H. Jung), gtakim@lgls.com (G.T. Kim).

http://dx.doi.org/10.1016/j.bmcl.2015.01.055
0960-894X/

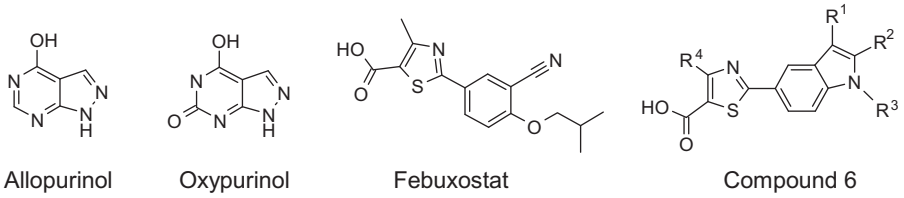


Figure 1. Structures of allopurinol, oxypurinol, febuxostat and compound **6** as xanthine oxidase inhibitors.

antimicrobial, anti-inflammatory, analgesic, anticonvulsants, antioxidant and antidiabetic agents,[17–19] indicating that indole may serve as a prospective scaffold in inhibiting xanthine oxidase.

In order to obtain an agent with better pharmacological profile and in vivo efficacy for the treatment of gout, we incorporated indole moiety into **6** as an isosteric replacement of phenyl moiety of febuxostat as shown in Figure 1.

The general synthetic route to **6** is shown in Scheme 1. Amide **2** was synthesized from acid **1** using HBTU [2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate] and ammonium chloride. Amide **2** was converted to thioamides **3** by refluxing with Lawesson's agent in THF. Compound **3** and ethyl 2-chloroacetoacetate were reacted with a catalytic amount of pyridine by refluxing in ethanol to afford indole-5-thiazole **4** in a reasonable yield of 70–85%. Compound **4** was reacted with alkyl bromide and sodium hydride in DMF at room temperature to furnish compound **5**, and this type of reaction allowed the modification of R^3 group. Ester **5** was hydrolyzed with sodium hydroxide in aqueous THF and methanol, and the final compound **6** was obtained in a good yield.

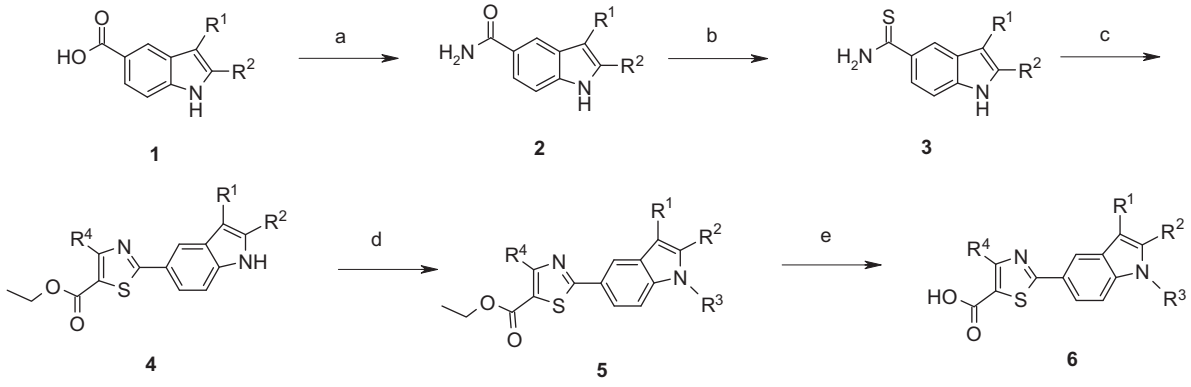
The established synthesis led us to evaluate in vitro xanthine oxidase inhibition of **6**. Assay of in vitro XO inhibition activity was performed by measuring the inhibitor concentration needed for 50% inhibition.[20] IC_{50} values of **6e**, **6k**, **6m** and **6n** showed the similar levels to febuxostat. Tables 1–3 represent the inhibitory activities against XO of the test compound.

The activities of indole **6a–g** were initially investigated as shown in Table 1. Compound **6a** (IC_{50} = 110 nM) with hydrogen for R^1 and isobutyl group for R^3 was found as a hit compound. The previous investigation indicated that an electron-withdrawing group such as nitro, chloro and cyano groups at 2-position in febuxostat plays an important role on xanthine oxidase inhibition.[23] Thus, chloro and nitro groups were introduced at 3-position of **6a**. Chloro compounds **6b**, **6c** and **6d** showed remarkably low IC_{50} values of 5.7 nM, 7.3 nM and 16.0 nM, respectively. Unfortunately, **6b** and **6d** were not metabolically stable (Table 1). Although **6c** exhibited highly potent inhibitory activity (IC_{50} = 7.3 nM), it displayed in vivo uric acid-lowering activity of only 18.9%. Compound **6e** with nitro group showed IC_{50} values of 4.2 nM. However, **6e** was less metabolically stable than **6g** (Table 1). Compound **6f** exhibited moderate inhibitory activity. Compound **6g** with cyano group showed an IC_{50} value of 5.5 nM which was ten times more potent in activity than the original hit compound **6a**. Thus, It was confirmed that substitution with electron-withdrawing group at 3-position of the indole ring increased in vitro XO inhibitory activity.

In order to examine the effect of the substitution at 3-position of the thiazole ring, analogs **6h–k** were prepared and their XO inhibitory activities were tested as shown in Table 2. Based on 50% cytotoxic concentration (CC_{50}) using primary rat hepatocytes, **6k** (CC_{50} = 200 μM) was less cytotoxic than **6g** (CC_{50} = 82 μM). Therefore, isopropyl group was fixed at 1-position for comparison. Compound **6i** with trifluoromethyl group exhibited high IC_{50} value of 895.0 nM, and **6j** with methoxy group much increased activity (IC_{50} = 90.0 nM). Compound **6h** (R^4 = H) and **6k** (R^4 = CH_3) displayed better inhibitory activities compared with **6i** and **6j**. Oral exposure in rats with **6h** and **6k** revealed that **6k** exhibited high C_{max} and AUC values as shown in Table 2. Therefore, we focused on the modification of **6k** comprising cyano group with various substituents for R^2 and R^3.

The substitution effect on XO inhibition is summarized in Table 3. Most of the compounds in Table 3 showed good XO inhibitory activity. Further selection was carried out on the basis of in vivo uric acid reduction at 10 mg/kg.

In order to check whether the R^2 substituent is worthwhile to be varied, **6l** (R^2 = CH_3) was prepared. It exhibited moderate inhibitory activity (IC_{50} = 10.7 nM) and low in vivo efficacy. Therefore, our interest was focused on the R^3 substituent. Introduction of fluoroisopropyl, hydroxyl isopropyl and methoxy isopropyl group afforded **6m**, **6n** and **6o**, showing potent IC_{50} values of 4.9 nM, 3.0 nM and 9.0 nM, respectively. Unfortunately, those compounds displayed weak uric acid lowering activity compared to **6k**. Compounds **6p** (R^3 = methanesulfonylethyl) and **6q** (R^3 = acetylaminoethyl) exhibited moderate inhibitory activities of 9.0 nM and



Scheme 1. Reagents and conditions: (a) HBTU, NH_4Cl, Et_3N, DMF, 74–90%; (b) Lawesson's agent, THF, reflux, 90–95%; (c) ethyl 2-chloroacetoacetate, pyridine, EtOH, reflux, 70–85%; (d) alkyl bromide, sodium hydride, DMF, 70–80%; (e) THF/MeOH = 1:1, 1 N NaOH, 90%.

Please cite this article in press as: Song, J. U.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.01.055