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Discovery of 2-phenylthiazole-4-carboxylic acid, a novel and potent scaffold as xanthine oxidase inhibitors

Xue Xu[a], Liming Deng[b], Lu Nie[c], Yueming Chen[b,d,e], Yanzhi Liu[b], Rongrong Xie[b,*], Zheng Li[b,d,*]

[a] Guangzhou General Pharmaceutical Research Institute Co., Ltd., Guangzhou 510240, PR China
[b] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[c] Jiujiang Vocational University, 88 Lian Xi Road, Jiujiang, Jiangxi 332000, PR China
[d] Key Laboratory of New Drug Discovery and Evaluation of Ordinary Universities of Guangdong Province, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[e] Guangzhou Key Laboratory of Construction and Application of New Drug Screening Model Systems, Guangdong Pharmaceutical University, Guangzhou 510006, PR China

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ABSTRACT

The xanthine oxidase (XO) plays an important role in producing uric acid, and therefore XO inhibitors are considered as one of the promising therapies for hyperuricemia and gout. We have previously reported a series of XO inhibitors with pyrazole scaffold to extend the chemical space of current XO inhibitors. Herein, we describe further structural optimization to explore the optimal heterocycle by replacing the thiazole ring of Febuxostat with 5 heterocycle scaffolds unexplored in this field. All of these efforts resulted in the identification of compound **8**, a potent XO inhibitor (IC_{50} = 48.6 nM) with novel 2-phenylthiazole-4-carboxylic acid scaffold. Moreover, lead compound **8** exhibited hypouricemic effect in potassium oxonate-hypoxanthine-induced hyperuricemic mice. These results promote the understanding of ligand-receptor interaction and might help to design more promising XO inhibitors.

In the purine metabolic pathway, xanthine oxidase (XO) is a key enzyme to produce the uric acid, while the hyperuricemia is an underlying cause of gout. Gout is an inflammatory disease resulting from the urate crystal deposition in tissues and joints, which is often related to hypertension, diabetes, and cardiovascular disease.[1] Since gout can be treated by decreasing the levels of uric acid, the uricosuric or XO inhibitors are considered as promising therapies for the treatment of hyperuricemia and gout.[2,3]

Recently, a lot of XO inhibitors have been reported in the literatures (Fig. 1). Febuxostat, a classical XO inhibitor, has approved by FDA for the treatment of chronic gout.[4] Besides the thiazole ring in Febuxostat, other heterocycle scaffolds were also explored in this field, such as pyrazoles[5], isoxazoles[6] and imidazoles.[7] Moreover, Topiroxostat, a XO inhibitor with triazole scaffold, has also been approved in Japan for the management of hyperuricemia.[8] In our previous study, the chemical space of XO inhibitor with pyrazole scaffold has also been systematically explored.[9] Herein, another 5 heterocycle scaffolds unexplored in this field were designed and synthesized to explore the optimal heterocycle for further structure-activity relationship (SAR) studies.

The synthetic routes of designed compounds **1–8** are summarized in Scheme 1. Treated ethyl 2-chloroacetoacetate with **1a** or **3a–b** formed oxazole **2a** or thiazole **4a–b**.[10] Thiazole **5a–b** was generated from ring-closure reaction of ethyl bromopyruvate and **3a–b**, which were obtained by treating benzamide with Lawesson's reagent.[11] The basic hydrolysis of intermediates **2a**, **4a–b**, and **5a–b** in the presence of lithium hydroxide, afforded target compounds **1–3**, **7**, and **8**. Cyclization of **6a** with **7a** provided thiazole ester **8a**. The synthesis of **11a** was obtained from ring-closure reaction of **9a** and **10a**.[12] While isoxazole **14a** was prepared by cyclization using hydroxylamine hydrochloride and **13a**, which was obtained by Claisen condensation.[13,14] These obtained esters **8a**, **11a**, and **14a** were subsequently converted to target compounds **4–6** in the presence of lithium hydroxide.

To obtain an optimal heterocycle for extending the chemical space of XO inhibitors, five heterocycles were introduced to replace the thiazole ring of Febuxostat. The XO inhibitory activities were investigated at the concentration of 10 μM. As shown in Table 1, the unsubstituted derivative of Febuxostat (compound **1**) revealed potent inhibitory activity against XO despite it is inferior to that of Febuxostat.

Abbreviations: XO, xanthine oxidase; FDA, the Food and Drug Administration; SAR, structure-activity relationship; THF, tetrahydrofuran; NMR, nuclear magnetic resonance; PBS, phosphate buffer saline; SD, standard deviation

* Corresponding authors at: School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China.
E-mail addresses: happyyunyun_1985@126.com (R. Xie), li.zheng.sky@163.com (Z. Li).

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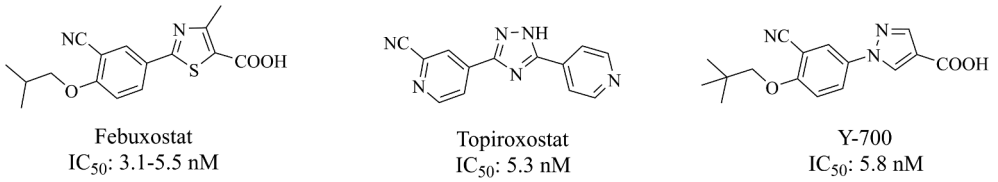
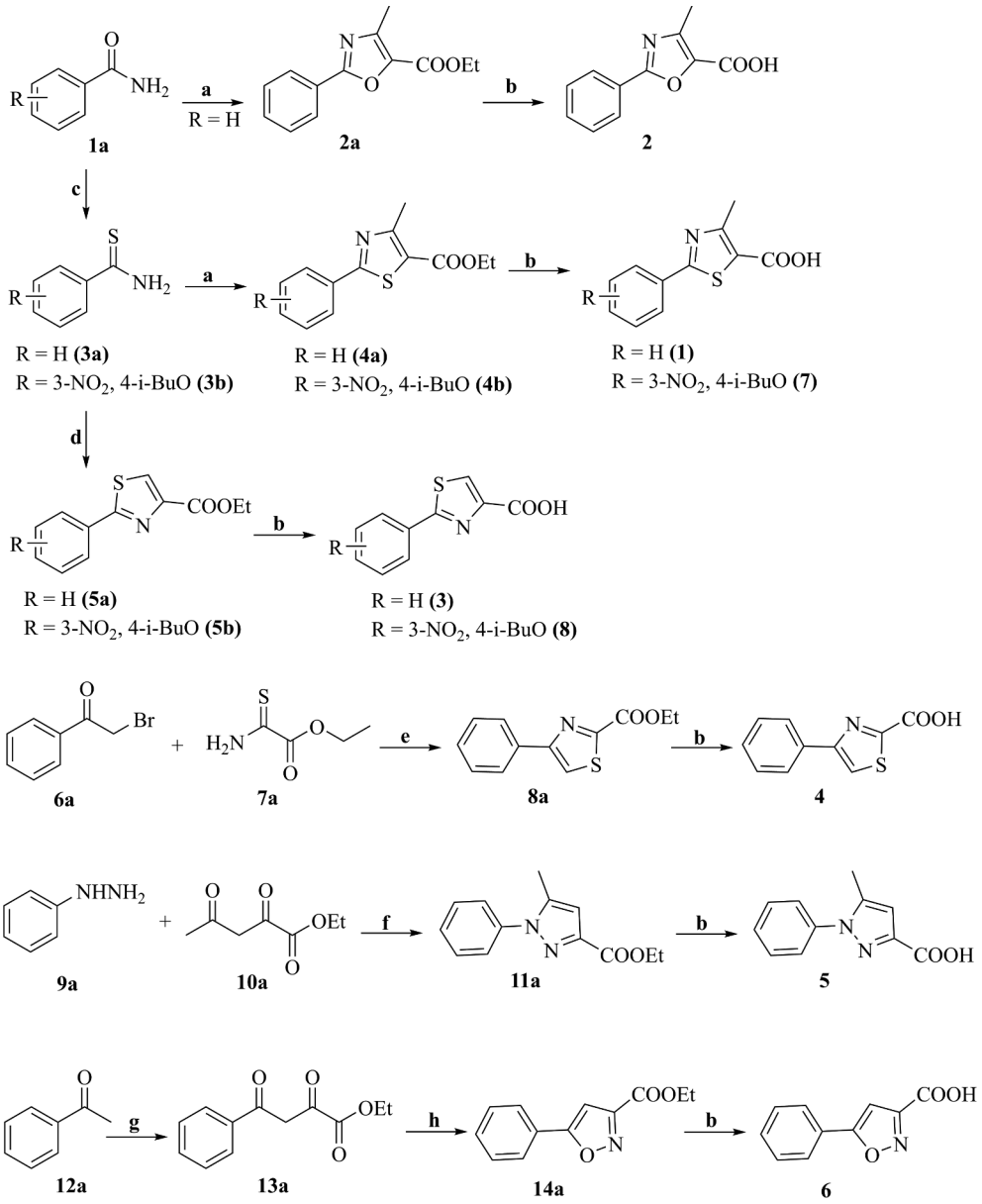


Fig. 1. The structures of representative non-purine XO inhibitors.

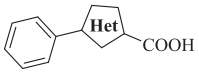


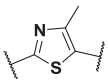
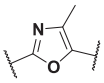
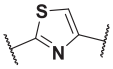
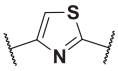
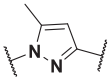
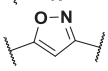
Scheme 1. Reagents and conditions: (a) ethyl 2-chloroacetoacetate, EtOH, reflux, 16 h; (b) LiOH·H_2O, THF/MeOH/H_2O, r.t., 4 h; (c) Lawesson's reagent, THF, reflux, 4 h; (d) ethyl bromopyruvate, EtOH, reflux, 4 h; (e) EtOH, reflux, 6 h; (f) EtOH, reflux, 2 h; (g) diethyl oxalate, NaOEt, 0-rt, 18 h; (h) hydroxylamine hydrochloride, EtOH, reflux, 2 h.

Although there was slightly difference between heterocycles, the oxazole analog **2** indicated a decreased inhibitory activity compared to compound **1**. A possible explanation is that the oxazole ring is a more hydrophilic heterocycle compared to that of thiazole ring, which decreased the hydrophobic interaction with the receptor. Interestingly, the inhibitory activity of compound **3** was recovered by switching the location of sulphur and nitrogen in compound **1**, which further verified our hypothesis above. However, position exchange of sulphur atom in compound **3** to afford compound **4** appeared to diminish the inhibitory activity, might attributing to the strict interaction in the binding pocket of XO. Compound **5**, the methyl analog of Y-700, resulted in a significant loss in potency compared with compounds **1** and **3**. The potency losses might be related to the change in conformation between phenyl group and pyrazole ring. Meanwhile, the isoxazole scaffold (compound **6**) was also not appropriate for the building of XO inhibitor, which might be attributed to the decreased hydrophobic interaction in presence of hydrophilic isoxazole ring.

Based on these positive results, two thiazole scaffolds of compound **1** and **3** were selected as our starting point for further modification (Table 2). In this field of XO inhibitors, the nitro group was usually

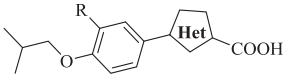
Table 1
In vitro xanthine oxidase inhibitory activities of six heteroaromatics.

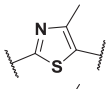
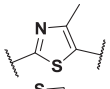
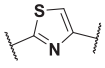


Compd	Het	XO inhibitory activity (%)[a]
Febuxostat		98.5
1		37.6
2		12.5
3		32.8
4		18.6
5		13.9
6		4.2

[a] Xanthine oxidase inhibitory activity at the concentration of 10 μM in three independent experiments.

Table 2
The inhibitory activities of the derivatives **7** and **8**.



Compd	R	Het	IC_{50}[a] (nM)
Febuxostat	CN		4.8
7	NO_2		33.4
8	NO_2		48.6

[a] Values are mean of three independent experiments.

introduced as a bioisosteres of cyan group.[5] As expected, the nitro analog of Febuxostat (compound **7**) revealed a significant improvement on inhibitory activity compared with compound **1** but, nevertheless, result in nearly 7-fold decrease in potency compared to Febuxostat. Consistent with the finding above, incorporation of nitro and isobutoxy groups (compound **8**) led to an increased inhibitory activity rise to the level of compound **7**. These results indicated that the 2-phenylthiazole-4-carboxylic acid scaffold might be introduced as a bioisosteres of thiazole ring in Febuxostat.

To better clarify the binding mode of compounds **7** and **8**, the induced-fit docking studies were performed based on the X-ray structure of XO (PDB code: 1VDV). As shown in Fig. 2, compounds **7** and **8** docked very well to the same binding site for Febuxostat. The carboxylic acid of Febuxostat formed hydrogen-bond network with residues Arg880 and Thr1010, and the thiazole ring formed interactions with Phe914 and Thr1010 (Fig. 2). Similarly, the thiazole ring of compounds **7** and **8** also revealed interactions with residues Glu802 and Thr1010, respectively. However, the carboxylic acid moiety of them

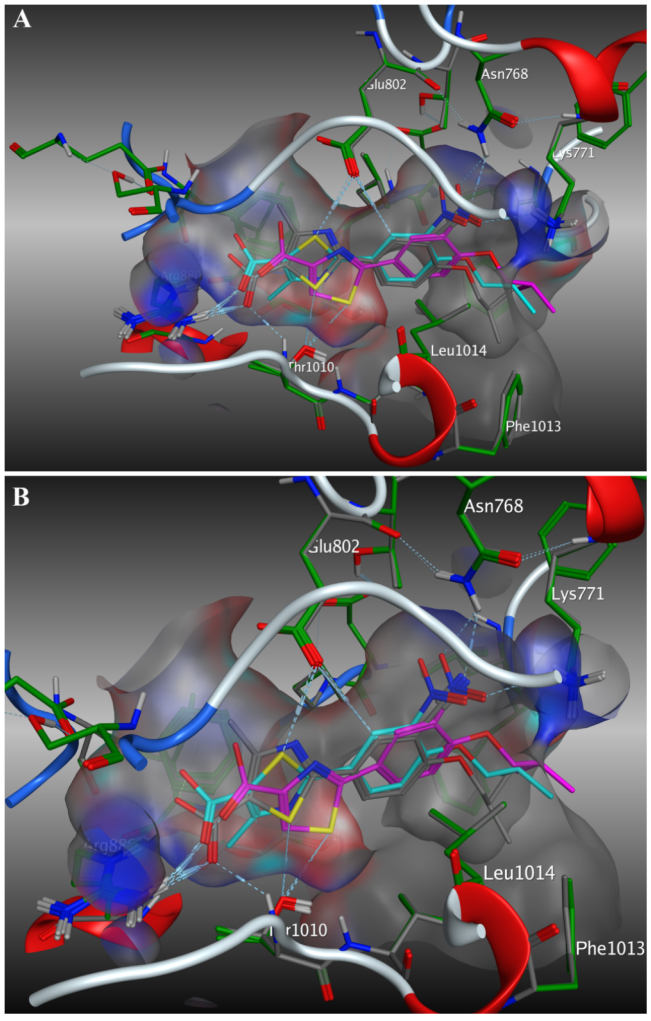


Fig. 2. The aligned docking poses of compound **7** (silvery), **8** (purple brown) and Febuxostat (light black) at the binding site of XO. Key residues are labeled in white, and interactions are represented by dashed lines.

only formed hydrogen-bond network with Arg880 (Fig. 2). These absences of hydrogen bond interactions were reasonably explained that the inhibitory activities of compounds **7** and **8** were inferior to that of Febuxostat. Moreover, the nitrile group of Febuxostat forming hydrogen bond with Asn768, but there was no direct interaction for the nitro group of compound **7**. Interestingly, the nitro group of compound **8** formed two hydrogen bonds with Asn768 and Lys771.

Based on their favorable inhibitory activities, compounds **7** and **8** were selected to evaluate hypouricemic effect in potassium oxonate-hypoxanthine-induced hyperuricemic mice. As shown in Fig. 3, subcutaneous injection of potassium oxonate (300 mg/kg) combines with intraperitoneal injection of hypoxanthine (500 mg/kg) induced an acute hyperuricemia in mice. As expected, the increases of serum uric acid levels were significantly suppressed in the treated groups, despite the hypouricemic effects of compounds **7** and **8** were inferior to that of Febuxostat, the most advanced drug in this field.

With the aim of exploring potent XO inhibitor with optimal heterocycle, we have identified a new 2-phenylthiazole-4-carboxylic acid scaffold by comprehensive evaluating 5 heterocycle scaffolds unexplored in this field. The lead compounds **7** and **8** exhibited potent XO inhibitory activity *in vitro* and hypouricemic effect in potassium oxonate-hypoxanthine-induced hyperuricemic mice. Moreover, compounds **7** and **8** fitted very well to the same binding site of Febuxostat in the induced-fit docking studies. All of these results indicated that the new 2-phenylthiazole-4-carboxylic acid scaffold was meaningful for

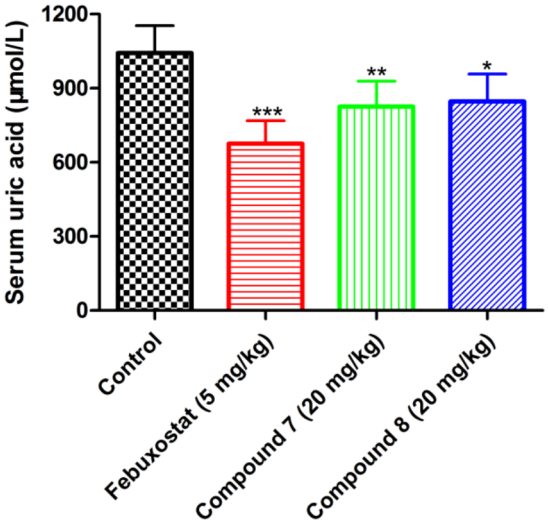


Fig. 3. Mice were treated with the potassium oxonate and hypoxanthine 1 h before drug administration, and the serum uric acid levels was measured at 1 h after drug administration. Results are mean ± SD (n = 6 per group). $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.001$ compared to control group by Student's *t* test.

further evaluation, and the information obtained from this work might help to design more promising XO inhibitors that are structurally related.

Notes

The authors confirm that this article content has no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2019.01.005.

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