Bioorganic & Medicinal Chemistry Letters 27 (2017) 3812–3816

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and evaluation of 1-phenyl-1*H*-1,2,3-triazole-4-carboxylic acid derivatives as xanthine oxidase inhibitors

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ARTICLE INFO

Article history:
Received 5 April 2017
Revised 17 May 2017
Accepted 22 June 2017
Available online 23 June 2017

Keywords:
1,2,3-Triazole
Xanthine oxidase inhibitor
Hyperuricemia

ABSTRACT

This study mainly focused on the modification of the X^2 position in febuxostat analogs. A series of 1-phenyl-1*H*-1,2,3-triazole-4-carboxylic acid derivatives (**1a-s**) with an N atom occupying the X^2 position was designed and synthesized. Evaluation of their inhibitory potency *in vitro* on xanthine oxidase indicated that these compounds exhibited micromolar level potencies, with IC_{50} values ranging from 0.21 μM to 26.13 μM. Among them, compound **1s** (IC_{50} = 0.21 μM) showed the most promising inhibitory effects and was 36-fold more potent than allopurinol, but was still 13-fold less potent than the lead compound Y-700, which meant that a polar atom fused at the X^2 position could be unfavorable for potency. The Lineweaver-Burk plot revealed that compound **1s** acted as a mixed-type xanthine oxidase inhibitor. Analysis of the structure-activity relationships demonstrated that a more lipophilic ether tail (e.g., *meta*-methoxybenzoxy) at the 4′-position could benefit the inhibitory potency. Molecular modeling provided a reasonable explanation for the structure–activity relationships observed in this study.

Xanthine oxidase (XO) is an important enzyme that catalyzes the hydroxylation of both hypoxanthine and xanthine in the last two steps of urate biosynthesis in humans.[1] The over-production of uric acid can lead to hyperuricemia, which is the key cause of gout. Thus, XO is considered the most promising target for treating this pathological condition.[2]

During purine oxidation, XO delivers an electron to the molecular oxygen and generates reactive oxygen species (ROS). An excess of ROS could evoke various pathological states including inflammation, metabolic disorders, atherosclerosis, cancer and chronic obstructive pulmonary disease.[3] Thus, inhibiting XO could reduce the production of ROS and benefit the treatment of these diseases.[4]

Allopurinol is a prototypical XO inhibitor and has been widely used in the treatment of hyperuricemia and gout for several decades. However, in some cases, it has been reported that allopurinol and its analogs, which possess a purine backbone, can lead to severe life-threatening side effects.[5] Therefore, searching for novel non-purine XO inhibitors with more potent XO inhibitory potency but fewer side effects has always been a hotspot. Y-700 is a classic non-purine XO inhibitor that was first reported by Ishibuchi S. et al.[6] Although it has not introduced into the market, the excellent activity of Y-700 has widely attracted researchers. Febuxostat[7] is another non-purine XO inhibitor that bears a thiazole moiety and has an outstanding inhibitory potency as well as acceptable side effects and was approved by the US Food and Drug Administration (FDA) in 2009. Thereafter, febuxostat analogs, which are characterized by a five-member aromatic ring linking a benzonitrile moiety and a carboxyl group, have been reported, such as selenazoles,[8] imidazoles[9] and isoxazoles[10] (Fig. 1). Furthermore, other XO inhibitors with different structural classes have also been recently reported, including topiroxostat (approved in Japan in 2013),[11] isocytosines,[3,12] 2-(indol-5-yl)thiazoles,[13] *N*-(1,3-diaryl-3-oxo-propyl)amides,[14] *N*-acetyl pyrazolines,[15] hydroxylated chalcones,[16] 9-deazaguanines,[17] flavonoids,[18] fraxamoside,[19] pyrano[3,2-*d*]pyrimidine,[20] 2-arylbenzo[*b*]furan[21] and benzaldehydes.[22]

In our previous report, we drew attention to febuxostat analogs of imidazoles[9] and changes in their inhibitory effects due to isosteric replacement at the X^1 position. In this study, we mainly focused on modifications and structure activity relationships (SARs) at the X^2 position. According to the co-crystal structure of XO in complex with Y-700,[23] X^2 is the closest point on the ligand from the molybdenum-pterin, which is the catalytic center of XO.

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http://dx.doi.org/10.1016/j.bmcl.2017.06.059
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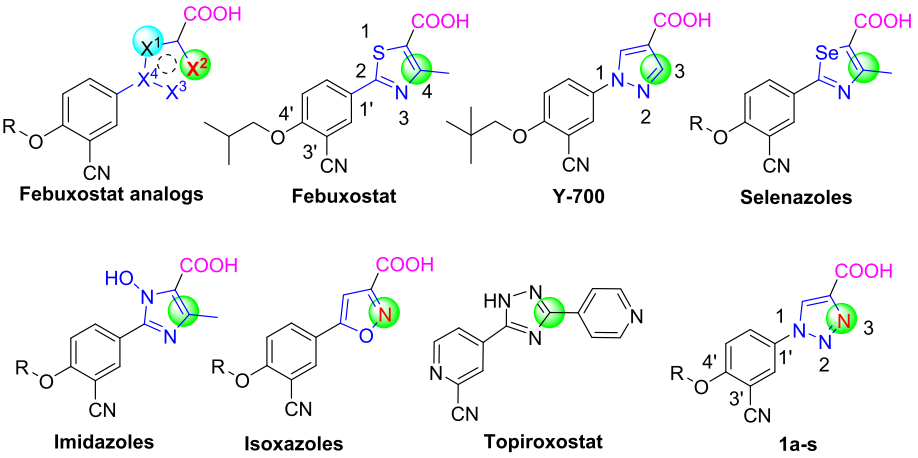


Fig. 1. The chemical structures of some XO inhibitors and designed compounds **1a-s**.

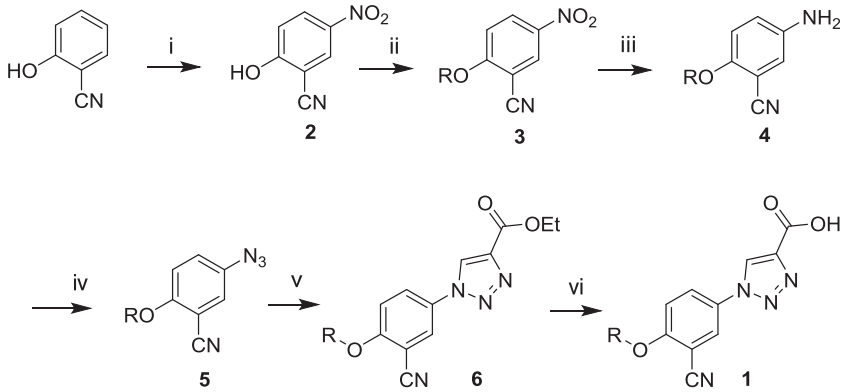
Between them, W498 was observed as a water bridge linked by the H-bonds to the molybdenum-pterin hydroxyl and the Glu1261 carboxyl group.[23] These interactions inspired us and suggest that a polar N atom fixed at the X^2 position may promote some interactions with molybdenum-pterin (e.g., link the molybdenum-pterin by a water bridge) and contribute to the inhibitory potency. Therefore, we designed and synthesized a series of XO inhibitors (**1a-s**) based on 1,2,3-triazole and investigated the effects of a fixed nitrogen atom at the X^2 position in the lead compound Y-700.

The synthesis strategy of target compounds **1** was performed as outlined in Scheme 1. Commercially available 2-hydroxybenzonitrile was nitrated in a conc. HNO_3/AcOH system to provide 2-hydroxy-5-nitrobenzonitrile (**2**). The reaction was triggered at 50 °C by the addition of a small amount of HNO_3 and maintained by the continuous addition of the remaining HNO_3. During the nitration process, it was essential to carefully control the HNO_3 dropping rate such that the reactive temperature was no higher than 70 °C to prevent the generation of oxidative by-products. In this reaction, a nitro group could also be introduced at the 3-position and generate the by-product 2-hydroxy-3-nitrobenzonitrile. This by-product was difficult to separate out due to its similar polarity, so we employed the mixture directly. A continuous three-step procedure of alkylation with the corresponding alkyl bromide or alkyl chloride followed by reduction using iron powder and isolation with column chromatography effortlessly provided pure 2-alkoxy-5-aminobenzonitriles (**4**). The diazotization of **4** was performed by treatment with sodium azide, which yielded the key intermediate 2-alkoxy-5-azidobenzonitriles (**5**). Cyclization of **5** with ethyl propiolate in the presence of copper sulfate and vitamin C via the Husigen reaction under microwave conditions produced ethyl 1-(4-alkoxy-3-cyanophenyl)-1*H*-1,2,3-triazole-4-carboxylates (**6**), which were hydrolyzed with sodium hydroxide and then acidified to give **1**. The structures of the synthesized compounds were elucidated by ^{1}H NMR, ^{13}C NMR and MS. All the spectral data were in accordance with the assumed structures.

K_2CO_3, KI, and DMF at 60 °C overnight; (iii) 1) Fe, NH_4Cl, EtOH and H_2O reflux for 3 h followed by 2) isolation by column chromatography (ethyl acetate:petroleum ether = 1:5 to 1:1); (iv) 1) $NaNO_2$, AcOH, and H_2O at -10 °C for 30 min followed by 2) NaN_3 at 0 °C for 3 h; (v) Ethyl propiolate, $CuSO_4$, and vitamin C at 50 °C under microwave conditions for 8 min; and (vi) NaOH, MeOH, and H_2O at 50 °C for 1.5 h, followed by the addition of 1 M HCl.

Bovine XO *in vitro* inhibitory potencies by target compounds **1a**–**s** were determined by spectrophotometrically measuring uric acid levels at 294 nm.[11,24] Allopurinol and Y-700 were included as reference compounds. The results are shown in Table 1.

According to the literature, an ether side chain containing 3–5 carbons at the 4′-position of febuxostat analogs has been proven to be welcome. Therefore, we initially synthesized a set of compounds bearing an ether chain with 3–5 carbons (**1a-g**) to examine the influence of the various R groups on XO inhibitory potency. Despite having potencies below Y-700 (IC_{50} = 0.016 μM), most of these compounds (except **1a**, IC_{50} > 30 μM) showed higher activities compared to allopurinol (IC_{50} = 7.56 μM), which encouraged us to carefully investigate the SAR to identify more effective compounds with potencies comparable to Y-700. Among these compounds, **1g** (IC_{50} = 1.62 μM), which bears an iso-pentyl R group,



Scheme 1. Reagents and conditions: (i) HNO_3 and AcOH at 50 °C for 4 h; (ii) RCl or RBr.