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Design, synthesis, and pharmacological and pharmacokinetic evaluation of 3-phenyl-5-pyridyl-1,2,4-triazole derivatives as xanthine oxidoreductase inhibitors

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ABSTRACT

In an effort to find a potent xanthine oxidoreductase (XO) inhibitor, we discovered the best compound 2-[2-(2-methoxy-ethoxy)-ethoxy]-5-[5-(2-methyl-pyridin-4-yl)-1*H*-[1,2,4]triazol-3-yl]-benzonitrile **28**. Here, we describe the following: (1) the design, synthesis, and structure–activity relationship of a series of 3-phenyl-5-pyridyl-1,2,4-triazole derivatives by in vitro studies of XO inhibitory activity in bovine milk and in vivo studies of serum uric acid (UA) reductive activity in rats, (2) a drug interaction study by a cytochrome P450 3A4 (CYP3A4) assay, and (3) a pharmacokinetic (PK) study. Compound **28** exhibits potent XO inhibitory activity, serum UA-lowering activity in rats, weak CYP3A4 inhibitory activity, and moderate PK profile.

Gout is a painful disease that is mainly caused by the deposition of monosodium urate crystals in joints. Various factors are suggested to contribute to the sustained elevation of the uric acid (UA) level in blood, termed hyperuricemia. Normally, UA is dissolved in blood. As a result of hyperuricemia, insoluble UA forms microscopic crystals in the capillary vessels of joints. These crystals cause inflammation and sharp pain, which is termed acute gouty arthritis or acute gout, and result in a significant degradation in the quality of life of patients. Moreover, some epidemiologic studies suggest that hyperuricemia is an independent risk factor for cardiovascular diseases such as myocardial infarction and cerebral infarction.[1] There are two types of drugs for controlling serum UA—xanthine oxidoreductase (XO) inhibitors and uricosuric agents. XO inhibitors block the terminal step in UA biosynthesis.[2] The only clinically available XO inhibitor is Allopurinol (**1**) that has been widely used in clinical practice because it is commonly tolerated as compared to uricosuric agents (Fig. 1). However, in patients with renal insufficiency, the plasma half life of oxipurinol, which is the major active metabolite of allopurinol, is prolonged. This is because oxipurinol is mainly removed by renal excretion. Moreover, as a consequence of its structural similarities to purine compounds, rare but severe hypersensitivity to Allopurinol (**1**) has been reported.[3] Thus, we have initiated the development of a novel safe XO inhibitor with non-purine isosteres that is removed by nonrenal excretion.

Thus far, we have investigated many compounds reported to exhibit XO inhibitory activities, such as Febuxostat (**2**) and Y-700 (**3**) (Fig. 1). We focused on the structural similarities of these XO inhibitors and prepared various types of acidic compounds; we found oxanilide-type compound **4** that exhibited weak XO inhibitory activity[4] (Fig. 2). We then considered introducing a heterocyclic ring moiety for activity reinforcement. Compound **4** was modified to 4-pyridyl compound **5** that exhibited strong in vitro

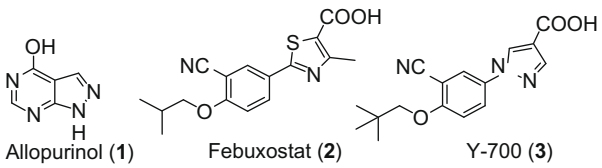

Figure 1. Structures of Allopurinol **1**, Febuxostat **2**, and Y-700 **3**.

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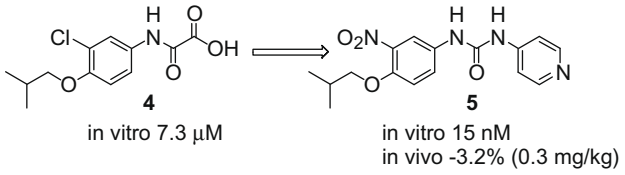


Figure 2. Oxanilide compound and urea-type compound.

XO inhibitory activity; however, Compound **5** did not decrease serum UA level in vivo[5] (Fig. 2). It was assumed that a urea moiety was metabolized into inactive form in vivo; therefore, we tried to convert this urea moiety into a stable cyclic structure. Compound **6** with triazole moiety exhibited the strongest in vitro activity and considerable in vivo activity (Fig. 3). Diaryl triazole derivatives have already been reported to exhibit XO inhibitory activity.[6] These reported compounds exhibit serum UA-lowering activity using spider monkeys, but the doses were as high as 5 mg/kg/day.[7] We failed to detect their potent serum UA-lowering activities.[8] The acute oral toxicity of **6** was very low in mice; however, **6** potently inhibited human cytochrome P450 (CYP) 1A2, 2C9, 2C19, 2D6, and 3A4. Especially, inhibition of CYP3A4 was most potent (80.9%; 10 μM),[9] which was suggestive of causing a serious drug–drug interaction. Some nitro compounds are afraid of having toxicities such as carcinogenicity and mutagenicity. Moreover, the plasma concentration of **6** was very low. The maximum drug concentration in plasma (C_{max}) was 13.8 ng/mL (1 mg/kg po).[10] Thus, we modified **6**.

The general synthesis of the diaryltriazole derivative **12** for determining the structure–activity relationship (SAR) is shown in Scheme 1.[6] The condensation of isonicotinic hydrazide **8** and imino ether **10** produced acylamidrazone **11**, which was thermally cyclized to yield **12**.

First, we attempted to resolve the most serious problem pertaining to the inhibition of CYP enzymes (Table 1). CYP3A4 is the most abundant CYP in adult human liver and small intestine and

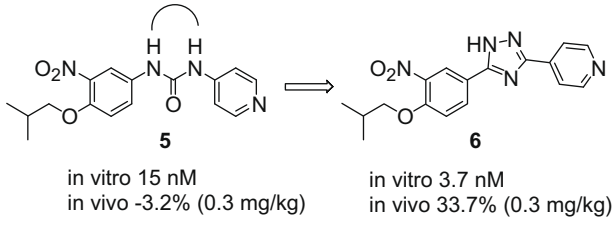
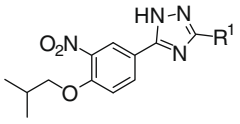
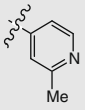
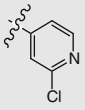
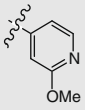
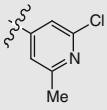


Figure 3. Modification to triazole compound.

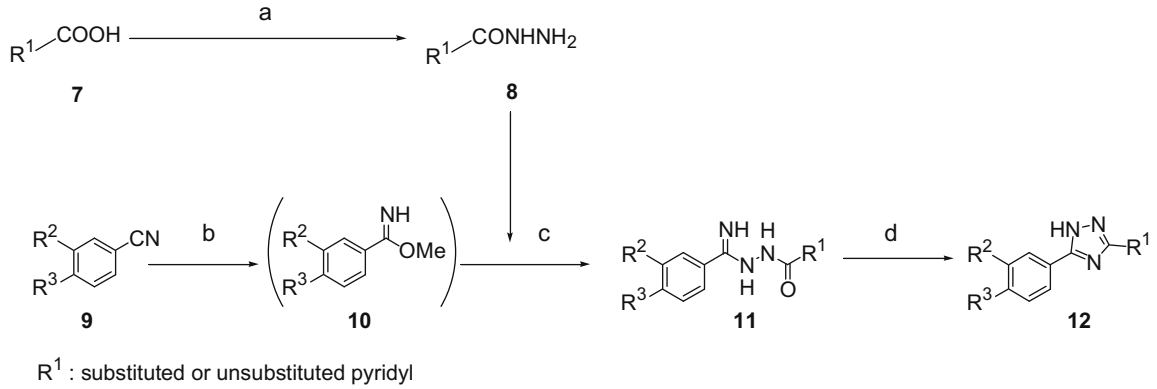
Table 1
SAR on the pyridyl moiety substituent.



Compound	R^1	In vitro IC_{50} (nM)[4]	In vivo (%)[5] at 0.3 mg/kg	Inhibition of CYP3A4 (%)[8] at 10 μM
6		3.7	33.7	80.9
13		5900	NT	18.2
14		>10,000	NT	NT
15		10	39.1	7.7
16		10	40.6[a]	7.1
17		>10,000	NT	6.4
18		>10,000	NT	10.9

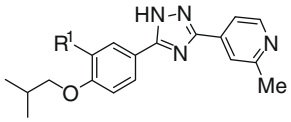
[a] Test compound was administered orally at 1 mg/kg; NT, not tested.

oxidizes numerous clinically important drugs among CYP enzymes,[11] and the SAR trends for inhibition of other CYP enzymes are typically similar to that for CYP3A4.[12] We therefore selected CYP3A4 as an index of inhibitory effect on CYP enzymes. We expected that this activity was mainly caused by the 4-pyridyl moiety.[12] Therefore, we synthesized and examined 3-pyridyl and 2-pyridyl compounds (**13 and 14**); however, these compounds did not inhibit the XO activity. We found that the 4-pyridyl moiety was essential for the XO inhibitory activity; therefore, we attempted the introduction of some substituent in position 2 (and 6) of the 4-pyridyl moiety (Table 1). As a result, we could reduce the CYP3A4 inhibitory activity by the substitution of groups such



Scheme 1. Reagents and conditions: (a) NH_2NH_2, 1,1′-carbonyldiimidazole, THF, rt; (b) NaOMe, MeOH, rt; (c) MeOH, reflux; (d) 200 °C.

Table 2
SAR at the 3-position on the benzene ring.



Compound	R^1	In vitro IC_{50}[4] (nM)	In vivo (%)[5] at 0.3 mg/kg	Inhibition of CYP3A4 (%)[9] at 10 μM
15	NO_2	10	39.1	7.7
19	CN	17	46.0	16.8
20	COOEt	20	42.4	NT
21	COOH	>10,000	NT	NT

NT, not tested.

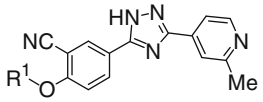
as methyl (**15**) or chloro groups (**16**) at position 2 without greatly reducing the XO inhibitory activity. On the other hand, the XO inhibitory activity of the methoxy-substituted compound (**17**) and the 2,6-disubstituted compound (**18**) disappeared.

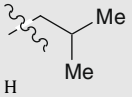
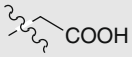
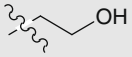
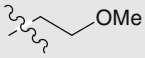
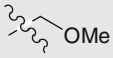
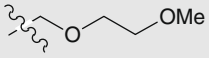
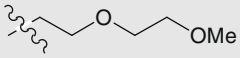
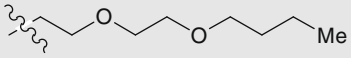
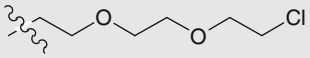
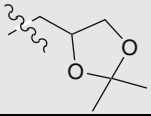
Next, the nitro group of compound **15** was replaced with other groups (Table 2). The same electron-withdrawing groups such as cyano (**19**) and carboethoxy (**20**) groups retained in vitro activity, however, carboxyric acid compound (**21**) which was expected to be the metabolite of **20** did not exhibit potent XO inhibitory activity. Cyano compound **19** exhibited more potent in vivo activity than the nitro group. However, the C_{max} value of **19** was very low (4.5 ng/mL, 1 mg/kg, po) likewise **15** (7.8 ng/mL, 1 mg/kg, po).[10]

Some studies suggested that low aqueous solubility leads to poor absorption from gut. We assumed that a low drug concentration in blood for these series of compounds was a result of their low aqueous solubility. Therefore, we modified the alkyl chain of the alkoxy group of **19** to hydrophilic groups (Table 3). Regrettably, phenol-type (**22**), carboxylic-type (**23**), and hydroxyl-type (**24**) compounds did not reduce UA level in vivo. However, the methyl-capped ether-type compound (**25**) retained the in vivo activity. Therefore, we fabricated polyethers of various lengths, which had alkyl-capped hydroxyl groups at the end of the alkyl chain (**26–31**). As a result, we obtained three polyether compounds (**27, 28**, and **30**) exhibiting potent in vivo activity. The pharmacokinetic (PK) profiles of these polyethers were greatly improved over that of **19** (Table 4). The best compound (**28**) had the most potent serum UA-lowering activity (52% decrease) and moderate PK profile (C_{max} 712 ng/mL, $t_{1/2}$ 0.97 h, 3 mg/kg, po) without CYP3A4 inhibition (−6.7%; 10 μM).

In conclusion, a series of pyridyl-triazole-phenyl-type compounds have been synthesized for the development of a novel safe XO inhibitor with a non-purine structure. In the course of SAR studies, we have found the pharmacophore of these compounds. The extent of CYP3A4 inhibition is decreased by introducing substituents in position 2 of the 4-pyridyl moiety. The nitro group was replaced with the cyano group. Further, the PK profile is improved by the replacement of the alkoxy substituents by alkyl-capped polyether groups. These optimizations led to the best compound **28** possessing potent in vivo serum UA-lowering activity and moderate PK profile without CYP3A4 inhibition.

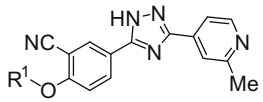
Table 3
SAR at the 4-position on the benzene ring.

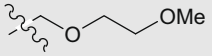
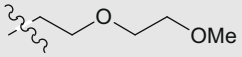
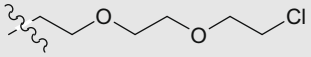


Compounds	R^1	In vitro IC_{50} (nM)[4]	In vivo (%)[5] at 0.3 mg/kg
19		17	46.0
22	H	43	−8.2
23		110	NT
24		40	10.9
25		64	36.5
26		24	27.0
27		24	40.0
28		31	52.0
29		26	10.1
30		21	40.7
31		60	27.9

NT, not tested.

Table 4
Comparison of **19** and polyether compounds.



Compounds	R^1	In vitro IC_{50}[4] (nM)	In vivo (%)[5] at 0.3 mg/kg	Inhibition of CYP3A4 (%)[9] at 10 μM	C_{max} (ng/mL)[10] at 3 mg/kg	$t_{1/2}$[10] (h)
19		17	46.0	16.8	16.7	0.37
27		24	40.0	16.5	360.1	0.45
28		31	52.0	−6.7	712.2	0.97
30		21	40.7	NT	684.4	0.52

NT, not tested.

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4. The IC_{50} values for the XO inhibitory activity of the test compounds were determined as follows: bovine milk XO (2 mU/mL) was incubated with 15 μM xanthine in a 50 mM phosphate buffer (pH 7.4) with or without the test compound at 25 °C. Uric acid formation was determined by the increase in absorbance at 292 nm using a spectrophotometer. The initial rate was calculated from 0 to 2.5 min.
5. The serum UA-lowering activity was measured as follows: the test compounds were administered orally to rats, and their blood was drawn from the orbital sinus 6 h after the administration. The blood was allowed to clot for 1 h at room temperature and centrifuged. The serum UA level was measured by the phosphotungstic acid method.
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8. UA-lowering activities were less than 26% at 0.3 mg/kg, po[5] (unpublished data).
9. The effects of the test compounds on the CYP3A4-mediated hydroxylation of testosterone were examined using human liver microsome. The amount of 6β-hydroxytestosterone produced in the reaction was determined by HPLC analysis.
10. The test compounds were orally administered to rats, and their blood samples were collected from jugular veins at predetermined times. All blood samples were centrifuged to obtain plasma. Aliquots of plasma samples were mixed with acetonitrile containing an appropriate internal standard and centrifuged. Aliquots of the supernatant were measured by the LC–MS system.
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