Bioorganic & Medicinal Chemistry Letters 19 (2009) 6225–6229

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of 3-(3-cyano-4-pyridyl)-5-(4-pyridyl)-1,2,4-triazole, FYX-051-a xanthine oxidoreductase inhibitor for the treatment of hyperuricemia

Takahiro Sato [a,*], Naoki Ashizawa [b], Koji Matsumoto [b], Takashi Iwanaga [b], Hiroshi Nakamura [c], Tsutomu Inoue [a], Osamu Nagata [b]

[a] Research Laboratories 1, Research Department, Medical R&D Division, Fuji Yakuhin Co., Ltd, 3936-2 Sashiougi, Nishi-ku, Saitama-shi, Saitama 331-0047, Japan
[b] Research Laboratories 2, Research Department, Medical R&D Division, Fuji Yakuhin Co., Ltd, 636-1 Iidashinden, Nishi-ku, Saitama-shi, Saitama 331-0068, Japan
[c] Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane-shi, Chiba 283-8555, Japan

ARTICLE INFO

Article history:
Received 9 July 2009
Revised 26 August 2009
Accepted 28 August 2009
Available online 2 September 2009

Keywords:
Gout
Xanthine oxidoreductase inhibitor
XO
Hyperuricemia
Triazole derivatives
CYP3A4
Structure–activity relationships

ABSTRACT

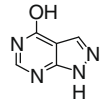
Our previous study identified 2-[2-(2-methoxy-ethoxy)-ethoxy]-5-[5-(2-methyl-4-pyridyl)-1*H*-[1,2,4]-triazol-3-yl]-benzonitrile (**2**) as a safe and potent xanthine oxidoreductase (XOR) inhibitor for the treatment of hyperuricemia. Here, we synthesized a series of 3,5-dipyridyl-1,2,4-triazole derivatives and, in particular, examined their in vivo activity in lowering the serum uric acid levels in rats. As a result, we identified 3-(3-cyano-4-pyridyl)-5-(4-pyridyl)-1,2,4-triazole (FYX-051, compound **39**) to be one of the most potent XOR inhibitors; it exhibited an extremely potent in vivo activity, weak CYP3A4-inhibitory activity and a better pharmacokinetic profile than compound **2**. Compound **39** is currently being evaluated in a phase 2 clinical trial.

© 2009 Elsevier Ltd. All rights reserved.

Hyperuricemia is a metabolic disorder in which blood uric acid (UA) levels are elevated for a sustained period of time; it is caused by various genetic and environmental factors.[1] In a normal person, UA is dissolved in the blood; however, in a person with hyperuricemia, insoluble UA forms microscopic crystals in the capillary vessels of joints. These crystals cause joint inflammation and sharp pain which are symptoms of acute gout; this condition significantly degrades the quality of life of patients.

Some epidemiological studies have suggested that hyperuricemia is an independent risk factor for cerebral infarction and cardiovascular diseases such as myocardial infarction.[2] Thus, the amelioration of hyperuricemia is of great importance for preventing the occurrence of gout and cardiovascular diseases. Two types of drugs are used for controlling the blood UA levels—xanthine oxidoreductase (XOR) inhibitors and uricosuric agents. XOR inhibitors are ideal for treating hyperuricemia because XOR catalyzes the terminal step in UA biosynthesis,[3] and patients lacking only XOR (xanthinuria type 1) do not exhibit any severe disorders except for xanthine urolithiasis.[4] Allopurinol (**1**), the only clinically available XOR inhibitor until recently, has been widely used in clinical practice because it is generally well tolerated by patients (Fig. 1).

However, in patients with renal impairment, the use of allopurinol (**1**) is restricted because the plasma half-life of oxipurinol, a major active metabolite of allopurinol (**1**) that is mainly eliminated via renal excretion, becomes prolonged. Moreover, rare but severe cases of hypersensitivity to allopurinol (**1**), which is a purine analogue, have been reported.[5] Therefore, there has been a considerable demand for a novel and safe XOR inhibitor. Recently, one such novel inhibitor—febuxostat—that was approved by the European Medicines Evaluation Agency (EMEA) and the Food and Drug Administration (FDA), has attracted worldwide attention. We also have been attempting to develop a novel and safe XOR inhibitor with nonpurine isosteres that can be eliminated by nonrenal excretion. In our previous research, we identified certain 4-pyridyltriazole derivatives that can potently inhibit the XOR in vitro[6] and reduce the serum UA levels in vivo;[7] for example, at a dose of 0.3 mg/kg, compound **2** decreased the serum UA levels of rats by 52% (Fig. 2).[8]

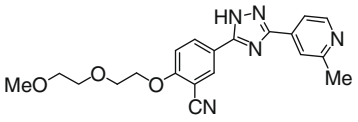


Allopurinol (**1**)

Figure 1. Structure of allopurinol (**1**).

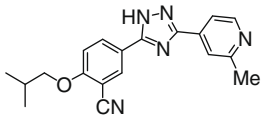
* Corresponding author. Tel.: +81 48 625 0071; fax: +81 48 625 0072.
E-mail address: takahiro@fujiyakuhin.co.jp (T. Sato).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.08.091



2

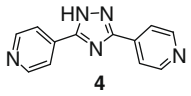
IC_{50} = 31 nM in vitro
52 ± 2.9 % (0.3 mg/kg) in vivo
*C*max = 712 ng/mL (3 mg/kg)



3

IC_{50} = 17 nM in vitro
46 ± 1.8% (0.3 mg/kg) in vivo
*C*max = 4.5 ng/mL (1 mg/kg)

Figure 2. Potent XOR inhibitors identified in our previous study.

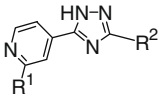


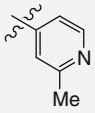
4

IC_{50} = 170 nM in vitro
*C*max = 4.50 μg/mL (3 mg/kg) ±

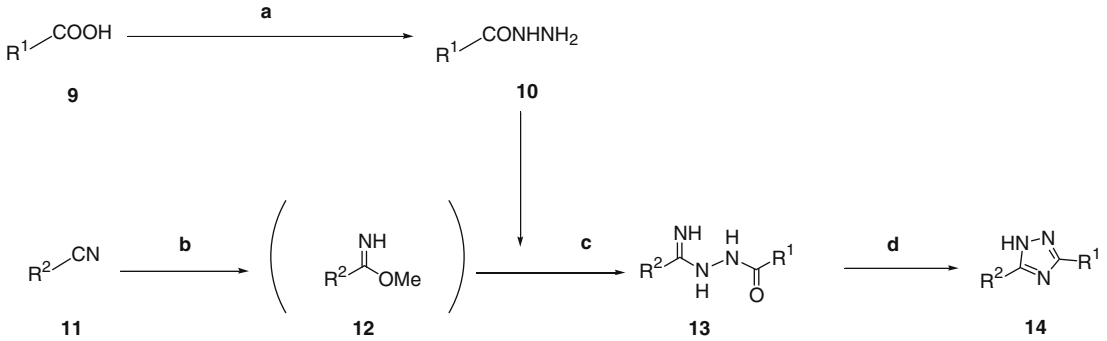
Figure 3. XOR inhibitor with high C_{max} value.

Table 1
3,5-Dipyridyl-1,2,4-triazole compounds with XOR-inhibitory activity



Compound	R^1	R^2	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h	Inhibition of CYP3A4 (%)[12] at 10 μM
4	H		170	−6.8 ± 30.9[a]	18.5
5	H		130	−7.7 ± 22.6[a]	10.4
6	H		31% inhibition (10 μM)	NT	20.3
7	Me		28	−0.4 ± 5.9	24.7
8	Me		310	26.1 ± 12.0	15.3

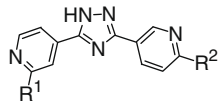
[a] Test compound was administered orally at a dose of 1 mg/kg; NT: Not tested.

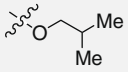
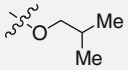
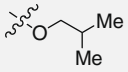


R^1, R^2: substituted or unsubstituted 3- or 4-pyridyl

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

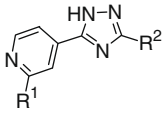
Table 2
SAR analysis of 3-(3-pyridyl)-5-(4-pyridyl)-1,2,4-triazole compounds



Compound	R^1	R^2	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h
5	H	H	130	−7.7 ± 22.6[a]
15	H	CN	38	−4.0 ± 12.5
16	H		24	13.4 ± 7.4[b]
17	Me		170	13.0 ± 6.7
18	CN		590	−6.1 ± 5.2[b]

[a] Test compound was administered orally at a dose of 1 mg/kg.
[b] Test compound was administered orally at a dose of 3 mg/kg.

Table 3
Comparison of the in vitro and in vivo activities of mono-substituted 4-pyridyl compounds and their methyl-substituted derivatives



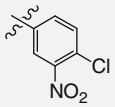
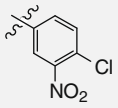
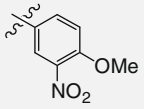
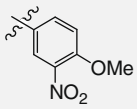
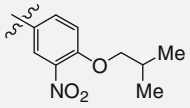
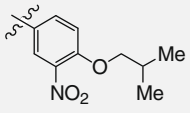
Compound	R^1	R^2	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h
19	H		4.7	0.9 ± 22.8
20	Me		30	19.8 ± 8.6
21	H		3.3	19.6 ± 15.1
22	Me		17	19.3 ± 5.5
23	H		3.7	33.7 ± 5.7
24	Me		10	39.1 ± 2.4

Table 3 (*continued*)

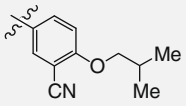
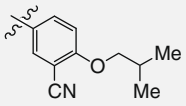
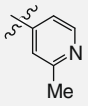
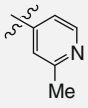
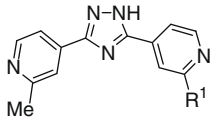
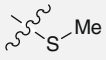
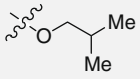
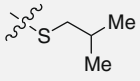
Compound	R^1	R^2	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h
25	H		4.9	36.3 ± 8.2
3	Me		17	46.0 ± 1.8
7	H		28	−0.4 ± 5.9
8	Me		310	26.1 ± 12.0

Table 4
SAR analysis of 3-(3-methyl-4-pyridyl)-5-(4-pyridyl)-1,2,4-triazole compounds



Compound	R^1	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h	C_{max} (μg/mL)[9] at 3 mg/kg
7	H	28	−0.4 ± 5.9	1.24[a]
8	Me	310	26.1 ± 12.0	NT
26	Cl	89	32.9 ± 6.0	4.07
27	CN	59	41.1 ± 1.3	3.97
28		170	19.2 ± 5.8	NT
29	Ph	59	8.0 ± 8.4	NT
30		63	2.3 ± 8.7	1.44
31		46	4.5 ± 7.1	NT

[a] Test compound was administered orally at a dose of 1 mg/kg; NT: Not tested.

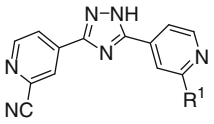
The pharmacokinetic profile of compound **2**, such as the maximum blood concentration (C_{max} 712 ng/mL (3 mg/kg))[9] and the half-life in the blood ($t_{1/2}$, 0.97 h),[9] can be improved further.

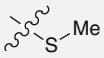
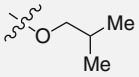
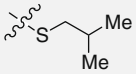
In light of such issues, we performed pharmacokinetic assays on other compounds that exhibited XOR-inhibitory activity. This led us to the identification of a 3,5-dipyridyl-1,2,4-triazole compound **4**, possessing a high C_{max} value (Fig. 3).

Certain 3,5-dipyridyl-1,2,4-triazole compounds with XOR-inhibition properties have been described previously,[10] and they exhibited serum UA-lowering activity at a dose of 5 mg/kg/day.[11] In our assay, they lowered the UA in rats by 26% or less at a dose of 0.3 mg/kg (Table 1).[7]

On the other hand, we found that these compounds possess good pharmacokinetic potential with an adequate C_{max} value. The

Table 5
SAR analysis of 3-(3-cyano-4-pyridyl)-5-(4-pyridyl)-1,2,4-triazole compounds



Compound	R^1	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h	Inhibition of CYP3A4 (%)[12] at 10 μM	C_{max} (μg/mL)[9] at 3 mg/kg
27	Me	59	41.1 ± 1.3	4.7	3.97
32	OMe	640	2.3 ± 3.2[a]	NT	NT
33		160	−3.7 ± 11.5	NT	NT
34		130	1.9 ± 6.1	NT	NT
35		53	−1.4 ± 10.6	NT	0.20
36	Ph	37	4.2 ± 8.1	NT	NT
37	Cl	39	33.6 ± 5.1	3.6	5.34
38	CN	18	32.0 ± 14.1	NT	NT
39	H	5.3	51.1 ± 1.2	18.6	4.62

[a] Test compound was administered orally at a dose of 3 mg/kg; NT: Not tested.

CYP3A4-inhibitory activities of these compounds were relatively low although an unsubstituted 4-pyridyl compound tends to inhibit CYP3A4.[8,13]

Considering the abovementioned characteristics, we modified these compounds in order to enhance their in vivo activities. Scheme 1 shows the general procedure used to synthesize 3,5-dipyridyl-1,2,4-triazole derivative **14** for determining the structure–activity relationship (SAR).[10] Acylamidrazone (**13**) was synthesized by the condensation of isonicotinic hydrazide (**10**) and iminoether (**12**), and it was then thermally cyclized to yield compound **14**.

First, the in vitro and in vivo activities of 3- and 4-dipyridyltriazole compounds with relatively low CYP3A4-inhibitory activity were examined (Table 2).

Although the in vitro activity increased noticeably by the introduction of cyano (**15**) and isobutoxy (**16**) groups at position 6 of the 3-pyridyl moiety, the in vivo activity did not. The introduction of methyl (**17**) and cyano (**18**) groups at position 2 of the 4-pyridyl moiety did not yield beneficial effects. We found that the presence of a methyl group at position 2 of the 4-pyridyl moiety tended to enhance the in vivo activity despite their less-potent in vitro activities (Table 3).[8] Compounds **16** and **17** provided similar results (Table 2).

Therefore, we subsequently examined the activities of different 4,4-dipyridyltriazole compounds with the 3-methyl-4-pyridyltriazole moiety in common (Table 4).

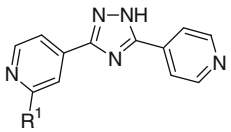
Compounds containing less bulky steric groups exhibited potent UA-lowering activity (**8**, **26**, and **27**). On the other hand, compounds containing bulky substituents were weak despite their potent in vitro activity (**29**, **30**, and **31**).

The compound containing a cyano group at position 2 of the 4-pyridyl moiety (**27**) exhibited marked in vivo activity in lowering the serum UA levels and it had a high C_{max} value. Therefore, the methyl group in the pyridyl moiety of compound **27** was replaced with various substituents (Table 5).

The substitution of the methyl group by electron-donating groups (**32**, **33**, **34**, and **35**) and bulky groups (**36**) decreased the in vivo activity. Compounds containing less bulky steric and electron-withdrawing groups (**37** and **38**) exhibited more than 30% UA-lowering activity. Unexpectedly, the mono-substituted R_1 compound (**39**) exhibited the most potent activity both in vitro and in vivo. Therefore, we examined various mono-substituted 4,4-dipyridyltriazole compounds (Table 6).

These mono-substituted compounds exhibited highly potent in vitro activity; however, except **39**, none of these compounds exhibited potent in vivo activity.

Table 6
SAR analysis of mono-substituted 4,4-dipyridyltriazole compounds



Compound	R^1	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg, 6 h	Inhibition of CYP3A4 (%)[12] at 10 μM	C_{max} (μg/mL)[9] at 3 mg/kg
7	Me	28	−0.4 ± 5.9	24.7	1.24[a]
39	CN	5.3	51.1 ± 1.2	18.6	4.62
40	Cl	7.9	26.5 ± 25.4[a]	54.1	4.36
41	Br	9.9	−6.2 ± 6.3	NT	NT
42	F	48	−0.2 ± 2.1	40.2	NT
43	Ph	6.8	−0.4 ± 12.6	NT	NT

[a] Test compound was administered orally at a dose of 1 mg/kg; NT: Not tested.

Table 7
Comparison of in vitro and in vivo activities of compound **39** and allopurinol (**1**)

Compound	In vitro IC_{50} (nM)[6]	In vivo (%)[7] at 0.3 mg/kg	
		2 h	6 h
39	5.3	59.9 ± 1.4	51.1 ± 1.2
1	2300	35.9 ± 4.0	−3.3 ± 2.7

As compared to allopurinol (**1**), 39 exhibited more potent in vitro and in vivo activities and had a more sustained in vivo effect than allopurinol (**1**) (Table 7).

These potent and more sustained effects of **39** have been confirmed by a crystallographic analysis of XOR-39 complex. The cyano group of compound **39** has been reported to play an important role in the binding activity between **39** and XOR. This is attributable to the formation of a hydrogen bond between Asn 768 of XOR and the cyano group of compound **39**, as revealed by X-ray crystal structure analysis (PDB code 1V97).[14]

Compound **39** exhibited a weak CYP3A4-inhibitory activity (18.6%); its C_{max} and bioavailability were as high as 4.62 μg/mL (3 mg/kg) and 69.6%, respectively. Moreover, the $t_{1/2}$ value of **39** was greater (19.7 h) than that of compound **2** (0.97 h). Since 39 is mainly excreted in the urine as triazole N_1- and N_2-glucuronides in monkeys and humans,[15] it is expected to be a safe drug in patients with renal impairment.

In conclusion, we modified a series of 3,5-pyridyl-1,2,4-triazole compounds in order to improve their in vivo activities. The SAR analysis of the methyl-substituted compounds confirmed the important role of the cyano substituent in the expression of the in vivo activity. The optimization of the series of compounds led to the identification of the mono-substituted compound **39** (FYX-051). This compound exhibits extremely potent effect in lowering the serum UA levels in vivo and it has good pharmacokinetic properties; moreover, it exhibits a weak CYP3A4-inhibitory activity. It is expected to be a beneficial drug for patients with hyperuricemia.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.091.

References and notes

1. Yamamoto, T. *Nippon Rinsho* **2008**, *66*, 636.
2. Krishnan, E.; Kwoh, C. K.; Schumacher, H. R.; Kuller, L. *Hypertension* **2007**, *49*, 298.
3. Emmerson, B. T. *N. Eng. J. Med.* **1996**, *334*, 445.
4. Ichida, K.; Amaya, Y.; Kamatani, N.; Nishino, T.; Hosoya, T.; Sakai, O. *J. Clin. Invest.* **1997**, *99*, 2391.
5. (a) Hande, K. R.; Noone, R. M.; Stone, W. J. *Am. J. Med.* **1984**, *76*, 47; (b) Bradlow, A. *Prescribers' J.* **1997**, *37*, 29.
6. The IC_{50} values for the XOR-inhibitory activity of the test compounds were determined as follows: bovine milk XOR (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. UA 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.
7. 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 2 or 6 h after the administration. The blood was allowed to clot for 1 h at room temperature and centrifuged. The serum UA levels were measured by the phosphotungstic acid method. Data are represented as the means ± SD of at least three rats.
8. Sato, T.; Ashizawa, N.; Iwanaga, T.; Nakamura, T.; Matsumoto, T.; Inoue, T.; Nagata, O. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 184.
9. 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.
10. Baldwin, J. J.; Kasinger, P. A.; Novello, F. C.; Sprague, J. M. *J. Med. Chem.* **1975**, *18*, 895.
11. Duggan, D. E.; Noll, R. M.; Baer, J. E.; Novello, F. C.; Baldwin, J. J. *J. Med. Chem.* **1975**, *18*, 900.
12. The effects of the test compounds on the CYP3A4-mediated hydroxylation of testosterone were examined at a concentration of 10 μM using human liver microsome. The amount of 6β-hydroxytestosterone produced in the reaction was determined by HPLC analysis.
13. (a) Miwatashi, S.; Arikawa, Y.; Kotani, E.; Miyamoto, M.; Naruo, K.; Kimura, H.; Tanaka, T.; Asahi, T.; Ohkawa, S. *J. Med. Chem.* **2005**, *48*, 5966; (b) Venkatakrishnan, K.; von Moltke, L. L.; Greenblatt, D. *J. Clin. Pharmacokinet.* **2000**, *38*, 111.
14. Okamoto, K.; Matsumoto, K.; Hille, R.; Eger, B. T.; Pai, E. F.; Nishino, T. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7931.
15. Nakazawa, T.; Miyata, K.; Omura, K.; Iwanaga, T.; Nagata, O. *Drug Metab. Dispos.* **2006**, *34*, 1880.