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Synthesis and Structure–Activity Relationships of 1-Phenylpyrazoles as Xanthine Oxidase Inhibitors

Seigo Ishibuchi,[a,*] Hiroshi Morimoto,[b] Takanori Oe,[b] Tsuguo Ikebe,[b] Hiroyoshi Inoue,[c] Atsushi Fukunari,[b] Miho Kamezawa,[b] Ichimaro Yamada[b] and Yoichi Naka[a]

[a]*Drug Discovery Research Laboratories, Welfide Corporation, Shodai-Ohtani 2-chome, Hirakata-shi, Osaka 573-1153, Japan*
[b]*Drug Development Laboratories, Welfide Corporation, 955 Koiwai, Yoshitomi-cho, Chikujo-gun, Fukuoka 871-8550, Japan*
[c]*Radioisotope Institute for Basic and Clinical Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*

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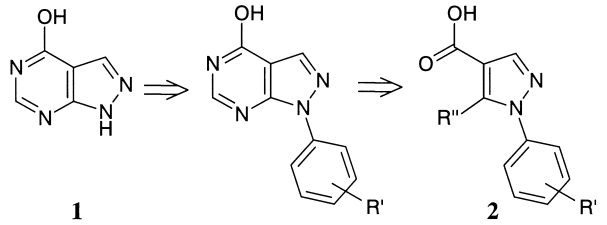
Abstract—A series of 1-phenylpyrazoles was evaluated for inhibitory activity against xanthine oxidase in vitro. Of the compounds prepared, 1-(3-cyano-4-neopentyloxyphenyl)pyrazole-4-carboxylic acid (Y-700) had the most potent enzyme inhibition and displayed longer-lasting hypouricemic action than did allopurinol in a rat model of hyperuricemia induced by the uricase inhibitor potassium oxonate.

Introduction

Since gout can be prevented by reducing plasma levels of uric acid (UA), therapy is possible with uricosuric drugs that increase the urinary excretion of UA, or with xanthine oxidase (XO) inhibitors that block the terminal step in UA biosynthesis.[1] The purine analogue, allopurinol is the only XO inhibitor administered in clinical situations, and has been used for the therapy of both the primary hyperuricemia of gout and that secondary to haematological disorders or antineoplastic therapy.[2] Unfortunately, it has been reported to be associated with an infrequent but severe hypersensitivity,[3] in which rises in the blood concentration of allopurinol metabolites point to involvement of drug toxicity, although the exact mechanism or causative agent has not been identified.[4] Allopurinol and its actual metabolite, oxipurinol, are substrates for N-1 ribosyl derivative-forming phosphoribosyl transferases.[5] Oxipurinol is also converted by uridine phosphorylase to the N-7 ribosyl derivative.[5] Because of the possible deleterious effects of these metabolites in long-term allopurinol administration, there have been efforts to find novel potent XO inhibitors with non-purine isosteres,[6] but so far none has been made clinically available.

Via chemical modification of allopurinol (**1**) as shown in Figure 1, we attempted to design novel XO inhibitors (**2**) possessing no purine nuclei. The rationale for the synthesis of these compounds included the following considerations: (1) The introduction of a phenyl group at the N-1 position in pyrazolo[3,4-*d*]pyrimidines would exclude the possibility of these compounds being converted, like allopurinol, to unnatural nucleotides; (2) As a model for the mechanism of XO inhibition by purine analogues, it was hypothesized that the molybdenum site of the enzyme becomes attached to the oxygen of their pyrimidine rings;[7] we therefore replaced the 4-hydroxypyrimidine moiety with a carboxyl group, the presence of which might enhance coordination with molybdenum.

Here, we describe the synthesis and structure–activity relationships of 1-phenylpyrazole derivatives as novel XO inhibitors.



1
allopurinol

2

Figure 1.

*Corresponding author. Tel.: +81-72-856-9283; fax: +81-72-857-5020; e-mail: ishibuchi@welfide.co.jp

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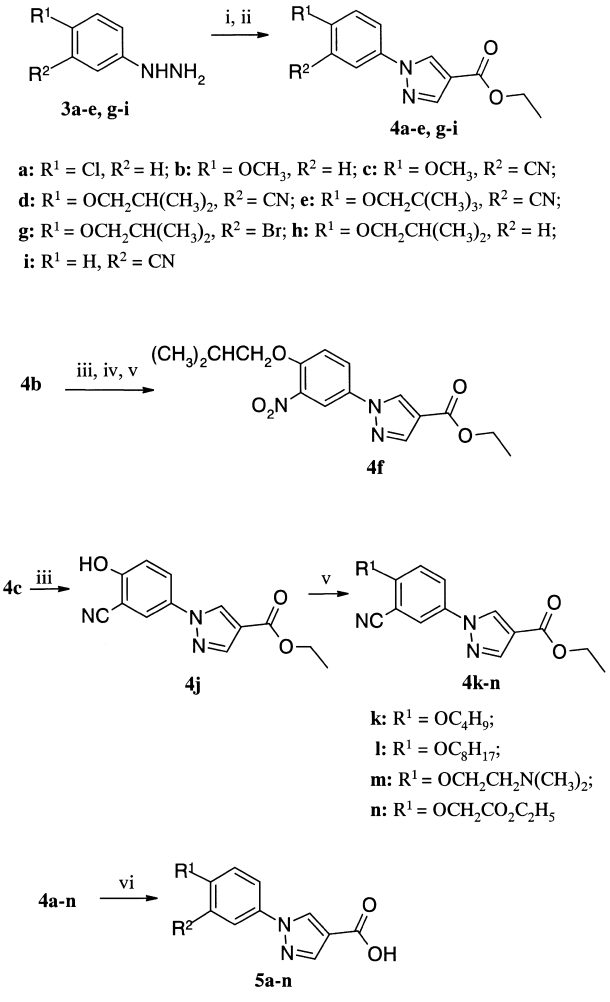
Synthesis

A general scheme for the synthesis of 1-phenylpyrazole derivatives is given in Scheme 1.

Condensation of a series of phenylhydrazines (**3a**–**e**, **g**–**i**) with ethyl ethoxymethylenecyanoacetate in refluxing ethanol followed by deamination with isoamyl nitrite gave **4a**–**e**, **g**–**i**. Demethylation of **4b** with $AlCl_3$ followed by nitration with HNO_3 and alkylation with isobutyl bromide afforded **4f**. Similarly, demethylation of **4c** with $AlCl_3$ gave the phenol derivative **4j**, which was treated with the corresponding alkyl bromide to afford **4k**–**n**. The pyrazole-4-carboxylic acid derivatives **5a**–**n** were synthesized by hydrolysis of **4a**–**n**.



Scheme 1. (i) EtOCH=C(CN)CO_2Et EtOH; (ii) isoamyl nitrite, THF; (iii) $AlCl_3$, $ClCH_2CH_2Cl$; (iv) NHO_3, AcOH; (v) alkyl bromide, K_2CO_3, DMF; (vi) 1 N NaOH, EtOH.

Biological Assays

Inhibitory activity against XO

Xanthine-oxygen reductase activity of XO was measured spectrophotometrically by following the absorbance of UA at 292 nm. The enzyme assay was conducted with a 1.5 mL reaction mixture containing Tris–HCl buffer (pH 8.1), 0.1 mol/L; XO from bovine milk (Sigma Chemical Co.), 0.0014 units; and xanthine, 0.12 mol/L, at various concentrations, or absent for the purpose of the control reaction. The assays were carried out aerobically at 37 °C. The reactions were started by adding xanthine to the mixture of the enzyme and the test drug, and terminated by addition of trichloroacetic acid at a final concentration of 10% (w/v). Duplicate assays were repeated three to four times. The inhibitory activity of each test compound against XO was indicated in terms of IC_{50} value (concentration of compound producing 50% inhibition compared to control reaction). IC_{50} and 95% confidence limit were calculated based on a non-linear regression analysis.

Hypouricemic effect in potassium oxonate-treated rats

Male Sprague–Dawley rats (6 weeks old, $n=8$) were repeatedly treated with the uricase inhibitor potassium oxonate (250 mg/kg, sc) 1 h before oral dosing (0.3–10 mg/kg) with the test compounds, and 3, 7 and 23 h afterward. One hour and immediately before administration of the test compounds, and 2, 4, 6, 10 and 24 h afterward, blood samples were taken individually under light anaesthetization with halothane. UA plasma level was determined with phosphotungstic acid colorimetry using a commercial kit (Uric Acid Test Kit, Wako Pure Chemical Industries Ltd). The area under the effect–time curves from time 0 to time 6 h ($AUEC_{0-6\,h}$) or 24 h ($AUEC_{0-24\,h}$) after test compound administration was calculated from mean UA plasma level at each time point. The efficacy of the hypouricemic action of each test compound was presented in terms of ED_{20} value (effective dose reducing $AUEC_{0-6\,h}$ or $AUEC_{0-24\,h}$ of control group by 20%), which was calculated based on a linear regression analysis.

Table 1. Inhibitory activity of 1-phenylpyrazole derivatives against XO in vitro

Compd	R^1	R^2	XO inhibitory activity[a] IC_{50} (nmol/L)
4d	$OCH_2CH(CH_3)_2$	CN	NA[b]
5a	Cl	H	NA
5b	OCH_3	H	NA
5c	OCH_3	CN	460 (350–590)
5d	$OCH_2CH(CH_3)_2$	CN	7.1 (6.2–8.1)
5e (Y-700)	$OCH_2C(CH_3)_3$	CN	5.8 (5.4–6.3)
5f	$OCH_2CH(CH_3)_2$	NO_2	33 (22–49)
5g	$OCH_2CH(CH_3)_2$	Br	43 (37–50)
5h	$OCH_2CH(CH_3)_2$	H	4700 (2400–11,000)
5i	H	CN	3300 (2500–4400)
5j	OH	CN	3600 (3400–3800)
5k	OC_4H_9	CN	15 (12–19)
5l	OC_8H_{17}	CN	21 (11–40)
5m	$OCH_2CH_2N(CH_3)_2$	CN	700 (350–1400)
5n	OCH_2CO_2H	CN	1500 (1400–1600)
Allopurinol (**1**)			260 (220–310)

[a]95% Confidence limits for IC_{50} values are shown in parentheses.
[b]NA = Not active < 50% inhibition @10,000 nmol/L.

Pharmacokinetics in potassium oxonate-treated rats

The oxonate-treated rats described above were used. The test compound was orally administered at doses of 0.3 and 3 mg/kg to animals. Blood samples were collected at designated time intervals and were centrifuged for the separation of plasma. Plasma concentration of test compound was analyzed by the HPLC method with reversed-phased C-18 column (Shim-pack CLC-ODS(M), 150×4.6 mm i.d., Shimadzu). The area under the plasma concentration–time curves from time 0 to time 6 h ($AUC_{0-6\,h}$) or 24 h ($AUC_{0-24\,h}$) was calculated by the linear trapezoidal method. The apparent oral clearance (CL/F) was calculated as dose/$AUC_{0-\infty\,h}$.

Table 2. Hypouricemic activity of **5d**, Y-700 (**5e**) and allopurinol (**1**)

Compd	ED_{20} (mg/kg, po)[a]	
	0–6 h	0–24 h
5d	2.5 (1.9–3.3)	>10
Y-700 (**5e**)	0.55 (0.33–0.79)	1.4 (1.1–1.9)
Allopurinol (**1**)	3.5 (2.0–8.8)	>10

[a]95% Confidence limits for ED_{20} values are shown in parentheses.

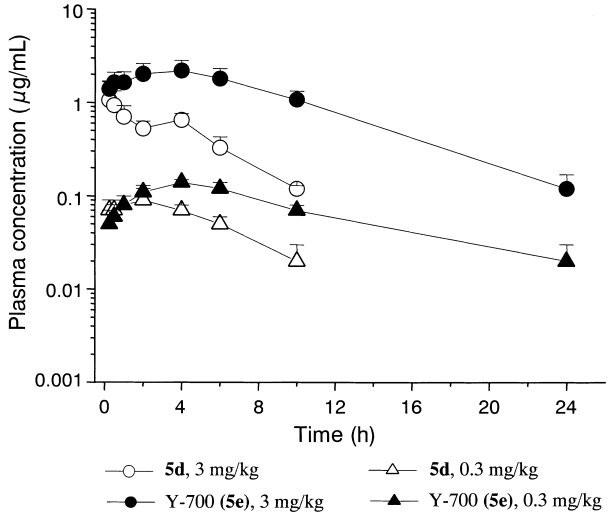


Figure 2. Plasma concentration of **5d** and Y-700 (**5e**) after oral administration to potassium oxonate-treated rats (mean±SD, $n=4$).

Results and Discussion

The SARs of the 1-phenylpyrazole derivatives newly synthesized as XO inhibitors are summarized in Table 1. The 4-monosubstituted analogues **5a**,**b** had no inhibitory activity against XO, but addition of a cyano group as an electrophilic substituent at the 3-position of the 4-methoxyphenyl unit produced apparent inhibitory activity (**5b** vs **5c**).

We therefore proceeded to examine the influence of substitution at the 4-position of the 3-cyanophenyl moiety with compounds **5c**–**e** and **5i**–**n**. It was found that increasing the size of the 4-substituent from hydrogen to butoxy and octyloxy groups improved XO inhibitory activity (**5i** vs **5k**, **l**), but that compounds with polar groups (**5m**, **n**) had much less potent inhibitory activity. Specifically, the introduction of bulky groups such as isobutoxy and neopentyloxy at the 4-position produced optimal results in vitro (**5d**, **e**). These compounds showed much more potent inhibitory activity than allopurinol ($IC_{50}=7.1$ and 5.8 nmol/L, respectively). Other electrophilic substituents in the form of bromo and nitro groups at the 3-position of the phenyl ring were tolerated, but a cyano group containing a π bond was best (**5f**,**g** vs **5d**). A free carboxyl group at the 4-position of the pyrazole ring was found to be essential for XO inhibition (**4d** vs **5d**). Since the 4-isobutoxy analogue **5h** and 3-cyano analogue **5i** were much less potent than the 3-cyano-4-isobutoxy analogue **5d**, the combination of a cyano group and a bulky alkoxy group was concluded to be of great importance for the XO inhibitory activity of the 1-phenylpyazole-4-carboxylic acids. However, the exact mechanism of enzyme inhibition by 1-phenylpyazole derivatives remains to be clarified.

Since the isobutoxy analogue **5d** and the neopentyloxy analogue **5e** (Y-700) showed more potent inhibitory activity than all other compounds tested in vitro, their hypouricemic effects were investigated and compared in vivo with that of allopurinol. Repeated subcutaneous injection of potassium oxonate to rats caused a marked UA plasma level increase which was maintained throughout the experiment (data not shown). Compound **5d** showed hypouricemic effects in hyperuricemic rats. However, the potency of the effects proved unsatisfactory with both ED_{20} (0–6 h) and ED_{20} (0–24 h) values of **5d** nearly equal to those of allopurinol (Table 2).

Table 3. Pharmacokinetic parameter of **5d** and Y-700 (**5e**) after oral administration to potassium oxonate-treated rats[a]

Compd	Dose (mg/kg)	Pharmacokinetic parameter					
		C_{max} (μg/mL)	t_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-6\,h}$ (μg·h/mL)	$AUC_{0-24\,h}$ (μg·h/mL)	$AUC_{0-\infty\,h}$ (μg·h/mL)
5d	0.3	0.09±0.03	1.1±0.6	3.3±1.0	0.41±0.09	0.65±0.11	0.62±0.10
	3	1.16±0.57	1.3±1.8	3.5±2.0	3.56±0.26	5.38±0.23	5.16±0.55
Y-700 (**5e**)	0.3	0.14±0.01	3.5±1.9	6.0±0.8	0.66±0.04	1.64±0.16	1.77±0.22
	3	2.29±0.68	3.5±1.0	4.5±0.7	11.44±2.87	25.63±5.97	26.40±6.17

[a]Mean±SD, $n=4$.

Y-700, on the other hand, showed potent and long-lasting hypouricemic effects, although the XO inhibitory activity of Y-700 was almost equipotent to that of **5d** in vitro (Table 1 and 2). Plasma concentration–time curves for Y-700 and **5d** after oral administrations are shown in Figure 2. The $AUC_{0-6\,h}$ and $AUC_{0-24\,h}$ for Y-700 at 3 mg/kg were 11.44 ± 2.87 and 25.63 ± 5.97 μg·h/mL, respectively; values for **5d** were 3.56 ± 0.26 and 5.38 ± 0.23 μg·h/mL, respectively (Table 3). The CL/F for Y-700 (0.12 ± 0.04 L/h/kg) was much lower than that for **5d** (0.59 ± 0.06 L/h/kg), indicating the sterically bulky neopentyloxy group of Y-700 contributed to the favourable pharmacokinetic properties in vivo. The differences between in vivo pharmacological activity for Y-700 and **5d** should be at least due to the differential pharmacokinetics of the two compounds. Further, toxicological studies including antigenicity and mutagenicity tests in experimental animals produced no results prohibitive to the study of Y-700 in humans.

To conclude, we prepared a novel series of 1-phenylpyrazole derivatives and discovered a number of compounds among them with more potent inhibitory activity than allopurinol. Specifically, the 1-(3-cyano-4-neopentyloxyphenyl)pyrazole-4-carboxylic acid Y-700 was found to be an orally effective XO inhibitor with potent and long-lasting hypouricemic effect in a rat model of hyperuricemia. Y-700 thus appears to be a promising candidate for the treatment of hyperuricemia, gout and other diseases in which XO may be involved.

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