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Synthesis and Bioactivities of Novel 5,6-Bis(4-methoxyphenyl)-2*H*-pyridazin-3-one Derivatives: Inhibitors of Interleukin-1 Beta (IL-1β) Production

Takayuki Matsuda,* Taro Aoki, Tomoyuki Koshi, Masao Ohkuchi and Hiromichi Shigyo

Tokyo Research Laboratories, Kowa Company Ltd., 2-17-43, Noguchi-Cho, Higashimurayama, Tokyo 189-0022, Japan

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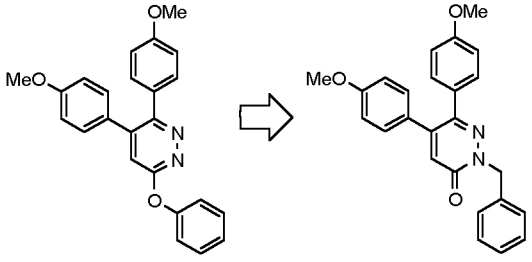
Abstract—New 5,6-bis(4-methoxyphenyl)-2*H*-pyridazin-3-one derivatives were prepared, and their abilities to inhibit IL-1β production were evaluated. Some compounds showed potent inhibitory activity against IL-1β production in HL-60 cells stimulated with lipopolysaccharide (LPS). The synthesis and structure–activity relationships of these compounds are described.

Introduction

Interleukin-1β (IL-1β) plays a pivotal role in the pathogenesis of inflammatory joint destruction, including rheumatoid arthritis (RA).[1] Joosten and co-workers reported that anti-TNF-α monoclonal antibody (mAb) is effective in the early stages of fully established type-II collagen-induced arthritis (CIA) in DBA/1J mice, whereas anti-IL-1β mAbs are most effective in the later stages of the disease.[2,3]

Previously, we reported the synthesis and ability to inhibit IL-1β production of 3,4-bis(4-methoxyphenyl)-6-phenoxypyridazine **1** ($IC_{50} = 0.10\,\mu M$).[4] To find other pyridazine-related derivatives, we planned to exchange the pyridazine ring of the compound **1** for a pyridazinone ring, and evaluated the activities of these compounds. We found a compound effective in CIA. Here, we describe synthesis and the structure–activity relationship of the 5,6-bis(4-methoxyphenyl)-2*H*-pyridazin-3-one analogues.



1 **5a**

Chemistry

The majority of the compounds listed in Table 1 were prepared from the common intermediate **2**. This compound was synthesized previously by Nannini et al.,[5] but it could be prepared in higher yield under the milder conditions shown in Scheme 1. Tartaric acid was oxidized with sodium periodate to generate glyoxylic acid in situ, and the reaction mixture was added to a suspension of desoxyanisoin **3** under basic conditions to provide aldol adduct **4**. Compound **4** was cyclized with hydrazine, followed by dehydration with *p*-toluenesulfonic acid to give the key intermediate **2**.

2-Substituted derivatives **5** were synthesized from the corresponding halide and intermediate **2** in the presence of potassium bicarbonate (Scheme 2).[6]

*Corresponding author. Tel.: +81-42-391-6211; fax: +81-42-395-0312; e-mail: tamatuda@kowa.co.jp

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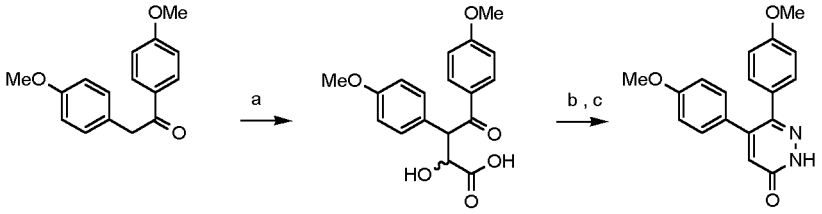


Scheme 1. Reagents and conditions: (a) (i) tartaric acid, $NaIO_4$; (ii) NaOH, EtOH, 40 °C, 17 h to 70 °C, 1 h, 93%; (b) H_2NNH_2 monohydrate, EtOH, 100 °C, 6 h; (c) *p*-TsOH, toluene, 120 °C, 3 h, 73%.

Results and Discussion

Compounds were evaluated for their abilities to inhibit IL-1β production in HL-60 cells stimulated with lipopolysaccharide (LPS). IC_{50} values were determined by comparison of yield with a control to which no test compound was added.[7] Results are summarized in Table 1.

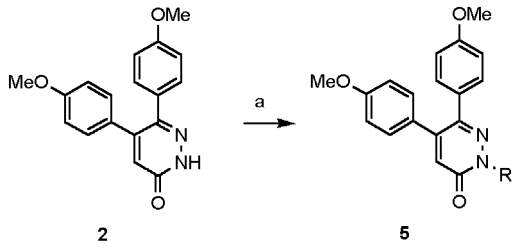
Previously, we reported that the pyridazine derivative **1** has inhibitory activity against IL-1β production.[4] However, it was less effective in vivo. We planned to convert the pyridazine ring of compound **1** to a pyridazinone ring, with a hydrophobic substituent such as a benzyl group at the 2-position.

The activity of the initially designed compound **5a** was more potent than that of unsubstituted compound **2** and similar to that of compound **1**. Some halogenated compounds, **5b**–**c**, showed increased activities, while others, **5e**–**i**, showed decreased activities. These results suggested that the bulkiness of chlorine atoms on the *N*-substituent might obstruct the interaction between the *N*-substituent and a narrow pocket in the drug receptor.

On the other hand, among the alkylated compounds, examined ethyl **5k**, cyclopentyl **5p** and cyclopentylmethyl **5q** were potent inhibitors, while the other analogues, **5j** and **5l**–**o**, showed decreased activity. These observations suggested that a planar substituent at the 2-position on the pyridazinone ring seems to be favorable for activity.

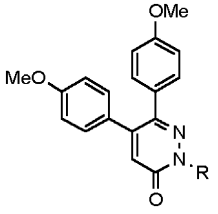
Furthermore, analogues possessing a planar substituent such as an allyl, pyridinylmethyl or cinnamyl group were synthesized and their activities were evaluated. Pyridinylmethyl analogues **5s** and **5t**, and cinnamyl derivative **5v** showed potent inhibitory activity against IL-1β production. However, the allyl derivative **5r** was not potent. An aromatic group in the *N*-substituent seems to play an important role in the inhibitory effect against IL-1β production. Moreover, the introduction of one or two halogen atoms on the phenyl group in the cinnamyl moiety provided the most potent compounds in the series of pyridazinones. 4-Chlorocinnamyl and 2,4-difluorocinnamyl analogues showed the same potency as compound **1**.

In the oral administration study, a series of 5,6-bis(4-methoxyphenyl)-2*H*-pyridazin-3-ones were practically insoluble in water and showed poor oral absorption in rats. However, an olive oil solution of 4-chlorocinnamyl



Scheme 2. Reagents and conditions: (a) halide, K_2CO_3, DMF, 70–80 °C.

Table 1. IL-1β inhibition assay results for compounds



Compd	R	IL-1β Inhibition IC_{50} (μM)[a]
2	H	31.6
5a	Bn	0.26
5b	4-FBn	0.15
5c	2,4-F_2Bn	0.18
5d	3,4-Cl_2Bn	0.21
5e	4-ClBn	0.53
5f	3,4-F_2Bn	0.54
5g	2,4-Cl_2Bn	1.10
5h	2,6-Cl_2Bn	3.16
5i	2,4,6-Cl_3Bn	9.40
5j	Me	1.13
5k	Et	0.61
5l	iPr	1.32
5m	iBu	>100
5n	cPr	7.98
5o	cPrCH_2	1.80
5p	cPn	0.51
5q	cPnCH_2	0.11
5r	CH_2=$CHCH_2$	>100
5s	3-PyCH_2	0.43
5t	4-PyCH_2	0.52
5u	2-PyCH_2	1.48
5v	Cinnamyl	0.62
5w	2,4-F_2Cinnamyl	0.10
5x	4-ClCinnamyl	0.10
5y	4-FCinnamyl	0.17
5z	2,4-ClCinnamyl	0.27
Prednisolone		0.76

[a]Concentration (μM) required for 50% inhibition of production of IL-1β.

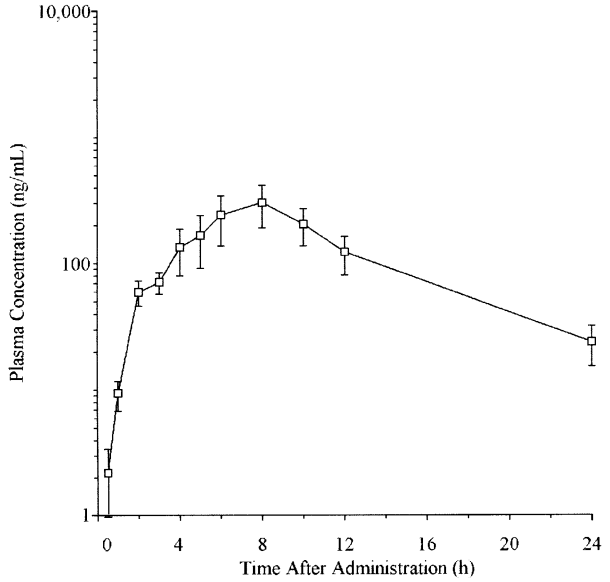


Figure 1. Pharmacokinetics of **5x**. Plasma concentration of **5x** after oral administration at a dose of 10 mg/kg in olive oil to rats. Data are the mean and SE of three rats.

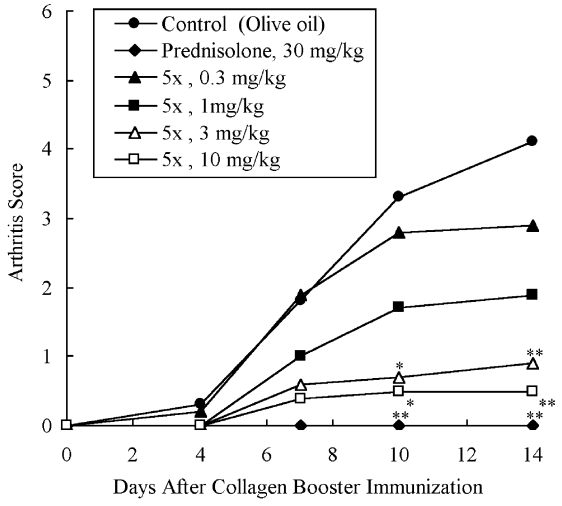


Figure 2. Evolution of CIA. Values represent the means of groups of 10 mice. Significantly different from the control: $*p<0.05$; $**p<0.01$.

Table 2. Pharmacokinetic parameters of **5x** at the Fig. 1 experiments

Compd	T_{max} (h)	C_{max} (ng/mL)	AUC (μg h/mL)	$t_{1/2}$
5x	7.0±1.0	316.3±108.0	3.90±0.98	4.90±0.78

Data are the means±SE of three rats.

derivative **5x** was absorbed in rats (Fig. 1). The pharmacokinetic results of **5x** are summarized in Table 2.

Among the 5,6-bis(4-methoxyphenyl)-2*H*-pyridazin-3-ones evaluated by CIA, strong oral activities were observed for **5x** (Fig. 2).[8] Furthermore, **5x** was checked for adverse effects by monitoring the behavior of mice treated with this compound at a dose of 30 mg/kg ip, and did not show any undesirable symptoms.

In conclusion, a novel class of 5,6-bis(4-methoxyphenyl)-2*H*-pyridazin-3-ones were synthesized and shown to be strong inhibitors of IL-1β production. Among these compounds, **5x** showed substantial plasma concentration profiles after oral administration in rats and the highest potency in the in vivo test without adverse effects. On the basis of these observations, we selected compound **5x** for further evaluation.

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8. 8-week-old DBA/1J mice were used for the arthritis experiments. Mice were immunized by intradermal injection at the base of the tail with 100 μg of bovine type-II collagen emulsified in an equal volume of Freund's complete adjuvant. Three weeks later, mice received a booster intradermal injection at the base of the tail with 100 μg of the same emulsified collagen. Test compounds were dissolved in olive oil, and administrated po twice a day from 1 day after first immunization to 14 days after collagen booster immunization. The mice were examined for clinical arthritis and scored by grading each paw on a scale for 0–3 based on erythema and swelling of the joint (0 = no erythema and swelling; 1 = erythema and swelling of one toe; 2 = erythema and swelling of two or more toes; 3 = complete erythema, swelling of the entire paw and incapacity to bend the ankle). As four legs were scored, the highest score possible was 12.