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Synthesis and Bioactivities of Novel Pyridazine Derivatives: Inhibitors of Interleukin-1 Beta (IL-1β) Production

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Abstract—New pyridazine 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 diseases.[1,2] Recently, an endogenous IL-1 receptor antagonist (IL-1Ra) was isolated,[3] and has been shown to be effective in animal models of septic shock,[4] endotoxin shock,[5] and rheumatoid arthritis (RA).[6] Moreover, Gabay[7] reported that IL-1β played a major role in synovitics and in the mechanisms that lead to the progressive joint destruction in RA. Inhibition of IL-1β thus offers an attractive target for the design of antirheumatic agents.

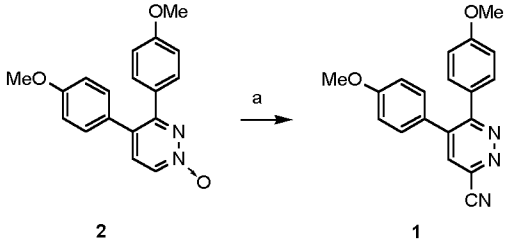
Screening of our in-house compounds on inhibition of IL-1β production allowed us to identify 3,4-bis(4-methoxyphenyl)-6-cyanopyridazine **1** as a potent inhibitor. However, compound **1** showed acute toxicity in mice. To reduce its toxicity, pyrimidine, pyrazine and other analogues of compound **1** were synthesized. In this paper, we describe synthesis and the structure–activity relationships on 3,4-bis(4-methoxyphenyl)-pyridazine analogues.

Chemistry and Biology

Cyanopyridazine derivative **1** was synthesized from pyridazine-*N*-oxide **2**[8] according to the method described in the literature (Scheme 1).[9]

Compound **6** was synthesized as shown in Scheme 2. Dimethoxybenzil **3** was condensed with glycinamide to provide pyrazinone **4** followed by chlorination with phosphorus oxychloride for conversion to chloropyrazine **5**.[10] Compound **5** was treated with tetrakis(triphenylphosphine)palladium in the presence of potassium cyanide to produce cyanopyrazine **6**.[11]

Preparation of cyanopyrimidine **12** is shown in Scheme 3. Although the synthesis of 2-cyano-4,5-diphenylpyrimidine was reported previously by Yamanaka et al.,[12] the main product was the 6-cyano isomer, and not the 2-cyano isomer. 2-Cyanopyrimidine **12** was obtained in good yield by a procedure similar to that described above for the synthesis of pyrazine **6**. Desoxyanisoin **7** was treated with ethyl formate under basic conditions to produce formylate **8**.[13–15] Condensation of **8** with *N*-methylurea afforded pyrimidinone **9** and intermediate **10**.[13–16] The intermediate **10** was also converted to **9** by dehydration using *p*-TsOH.[15] The pyrimidinone **9** was treated with phosphorus oxychloride and phosphorus



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Scheme 1. Reagents and conditions: (a) KCN, BnCl, H_2O, under N_2, 0 °C, 69%.

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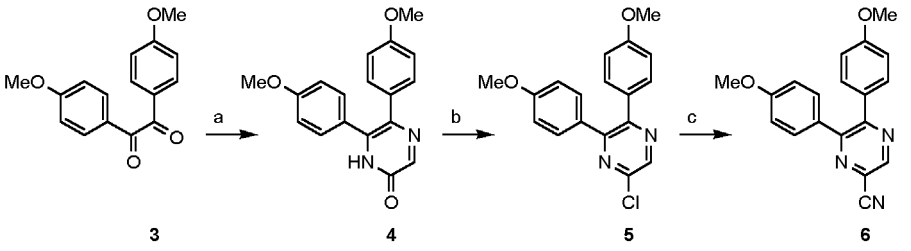
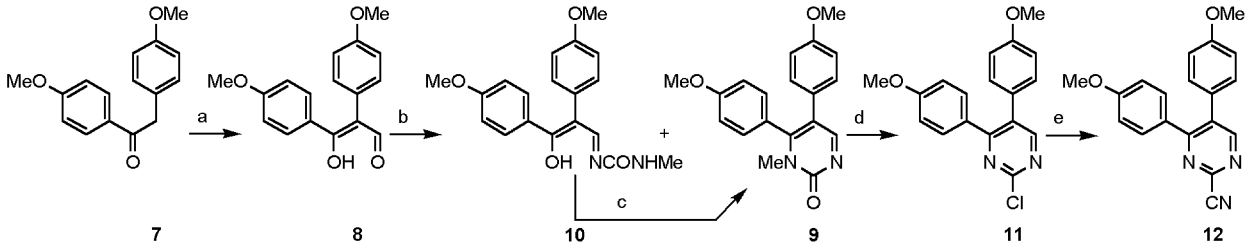
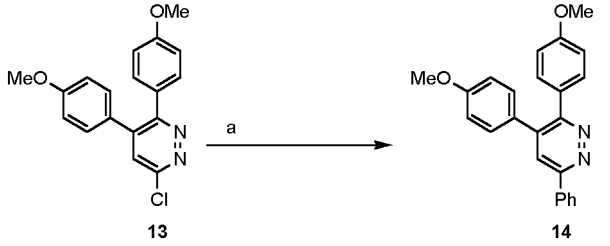


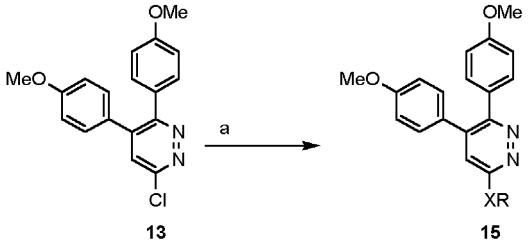
Scheme 2. Reagents and conditions: (a) $H_2NCOCH_2NH_2HCl$, NaOH, EtOH, 80 °C, 85%; (b) $POCl_3$, benzene, reflux, 27%; (c) KCN, $Pd(PPh_3)_4$, DMF, 150 °C, 81%.



Scheme 3. Reagents and conditions: (a) NaOEt, HCO_2Et, EtOH, 0 °C, 47%; (b) $H_2NCONHMe$, *p*-TsOH, toluene, reflux (**9**: 38%, **10**: 50%); (c) *p*-TsOH, xylene, reflux, 76%; (d) PCl_5, $POCl_3$, reflux, 57%; (e) KCN, $Pd(PPh_3)_4$, DMF, under Ar, 150 °C, 60%.



Scheme 4. Reagents and conditions: (a) PhMgBr, $Pd(PPh_3)_4$, benzene, 60 °C, 36%.



Scheme 5. Reagents and conditions: (a) RXH, K_2CO_3, DMF. Yields are shown in Table 2.

pentachloride to give 2-chloropyrimidine **11**.[17] Reaction of **11** with potassium cyanide and tetrakis(triphenylphosphine)palladium mainly gave 2-cyanopyrimidine **12**.

6-Phenylpyridazine was prepared by reaction of 3,4-bis-(4-methoxyphenyl)-6-chloropyridazine **13**[8] and phenylmagnesium bromide in the presence of tetrakis(triphenylphosphine)palladium (Scheme 4).

Pyridazine derivatives modified at the 6-position on the pyridazine ring were prepared as shown in Scheme 5. 6-Substituted derivatives **15a–x** were synthesized from compound **13** and the corresponding amines, thiophenols or phenols in the presence of potassium bicarbonate.[18,19]

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.[20]

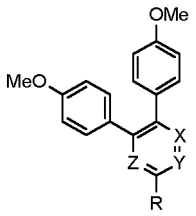
Acute toxicity of compounds was measured by Irwin's method; that is the general observation of behavior in mice treated at a dose of 30 mg/kg ip.[21]

Results and Discussion

The initial lead compound **1** is a potent IL-1β inhibitor, but shows many serious symptoms in mice. To avoid these adverse effects, analogues of compounds **1** and **14** were synthesized and their abilities to inhibit IL-1β production were evaluated as a preliminary evaluation. From the results of these tests, two analogues **12** and **14** were found to be non-toxic, but their potencies were reduced by over one order of magnitude compared to compound **1** (Table 1). These results supported the hypothesis that the toxicity of compound **1** is due to the substituent at the 6-position on the pyridazine nucleus. This hypothesis prompted us to investigate 3,4-bis(4-methoxyphenyl)-6-substituted-pyridazines, and consequently we found some potent inhibitors without adverse effects.

The compounds tested are shown in Table 2. In the in vitro assay, the benzylamino analogue **15d** and the 3,4,5-trimethoxyphenylamino analogue **15c** were not potent. *N*-Alkylation of 2,4-difluorophenylamino analogue **15b** decreased the inhibitory potency compared with non-alkylated analogue **15a**. As substituents on the

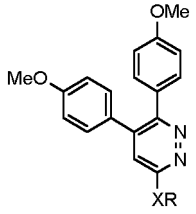
Table 1. Biological data for the compounds investigated



Compd	R	X	Y	Z	IL-1β Inhibition IC_{50} (μM)[a]	Symptoms[b] 30 mg/kg ip
1	CN	N	N	C	0.09	+ + + (Death, 6/8[c])
6	CN	N	C	N	6.60	+
12	CN	C	N	N	2.88	—
14	Ph	N	N	C	2.81	—
Prednisolone					0.76	—

[a]Concentration (μM) required for 50% inhibition of production of IL-1β.
[b]General observation of behavior in mice (+ + + = tremor, dyspnea and difficulty in walking, + + = depression of spontaneous locomotor activity, + = weak sedation, — = no significant change, NT = not tested).
[c]Number of dead mice/total number of mice used.

Table 2. Biological data for the compounds investigated



Compd **15**	RX	IL-1β Inhibition IC_{50} (μM)[a]	Symptoms[b] 30 mg/kg ip	Yield (%)	Temperature (°C)	Time (h)
15a	2,4-F_2PhNH	1.92	—	97.0	100	12
15b	2,4-F_2PhNPr	3.16	—	54.1	170	10
15c	3,4,5-$(MeO)_3$PhNH	> 100	—	71.3	140	5
15d	$PhCH_2NH$	> 100	NT	100.0	120	19
15e	3,4,5-Cl_3PhS	0.20	—	40.6	100	72
15f	2,4-F_2PhS	1.87	+	37.4	80	7
15g	PhS	20.5	—	12.9	100	7
15h	2,3,4,5,6-F_5PhO	0.04	+ +	60.5	150	24
15i	PhO	0.10	—	70.7	120	22
15j	2,3-F_2PhO	0.12	—	90.0	150	19
15k	2-CNPhO	0.15	—	88.1	150	24
15l	3-CNPhO	0.16	NT	75.2	150	19
15m	2,5-F_2PhO	0.18	NT	91.5	150	24
15n	2,3,5,6-F_4PhO	0.19	—	37.8	150	12
15o	2,6-F_2PhO	0.35	—	39.3	150	72
15p	4-MeOPhO	0.45	NT	94.7	150	24
15q	3,4-F_2PhO	0.46	—	99.1	150	14
15r	4-NO_2PhO	0.63	—	74.1	150	15
15s	4-PyO	1.24	—	85.2	150	22
15t	3,5-F_2PhO	1.38	—	98.0	150	6
15u	2,4-F_2PhO	2.32	+ +	52.5	120	13
15v	3-NO_2PhO	2.36	—	99.9	150	17
15w	2,4-Cl_2PhO	2.54	—	93.9	150	15
15x	4-CNPhO	> 100	—	57.5	150	13

[a]Concentration (μM) required for 50% inhibition of production of IL-1β.
[b]General observation of behavior in mice (+ + + = tremor, dyspnea and difficulty in walking, + + = depression of spontaneous locomotor activity, + = weak sedation, — = no significant change, NT = not tested).

phenylamino moiety, electron-withdrawing groups seemed to be favorable (**15a** vs **15c**). As substituents on the phenylthio moiety, electron-withdrawing groups were also favorable (**15e**, **15f** vs **15g**). The phenoxy analogue **15i** (IC_{50} = 0.1 μM) was more effective than the phenylthio analogue **15g** (IC_{50} = 20.5 μM). Introduction of electron-withdrawing groups onto the phenoxy moiety is expected to be useful for development of more

potent inhibitors. Compounds with electron-withdrawing groups **15j–n** were approximately equipotent with non-substituted phenoxy analogue **15i**. However, other analogues **15p–r** and **15t–x** showed reduceed inhibitory potency. Analogue **15s** containing a pyridinyl moiety with electron-withdrawing properties and analogue **15p** also showed decreased inhibitory potency. Interestingly, increases in the number of halogen atoms led to stronger activity, that is the pentafluorophenoxy analogue **15h** was the most effective compound.

Most of the compounds tested were not toxic as determined by observation of the behavior of mice. It is particularly noteworthy that compounds **15f**, **15h** and **15u** possessing fluorine atoms at 2- and 4-positions on the phenyl rings of phenylthio and phenoxy groups showed some undesirable effects, but 2,4-difluorophenylamino analogues **15a** and **15b**, and 2,4-dichlorophenoxy analogue **15w** did not.

In conclusion, a number of analogues with pyridazine, primidine and pyrazine rings instead of the pyridazine ring of compound **1** were evaluated for their abilities to inhibit IL-1β production. Starting from the lead compound **1**, we found potent inhibitors without toxicity among 6-substituted pyridazine compounds, especially 3,4-bis(4-methoxyphenyl)-6-phenoxypyridazine. Therefore, efforts to expand the structure–activity relationship are currently in progress in our laboratory using pyridazines as substrates.

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