PERKIN 1

Novel xanthine oxidase inhibitor studies. Part 3.[1] Convenient and general syntheses of 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones as a new class of potential xanthine oxidase inhibitors

Tomohisa Nagamatsu,*[a] Takayuki Fujita[a] and Kazuki Endo[b]

[a] *Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan*
[b] *Biology Laboratory, Research and Development Division, Yamasa Syoyu Co., Choshi, Chiba 288-0056, Japan*

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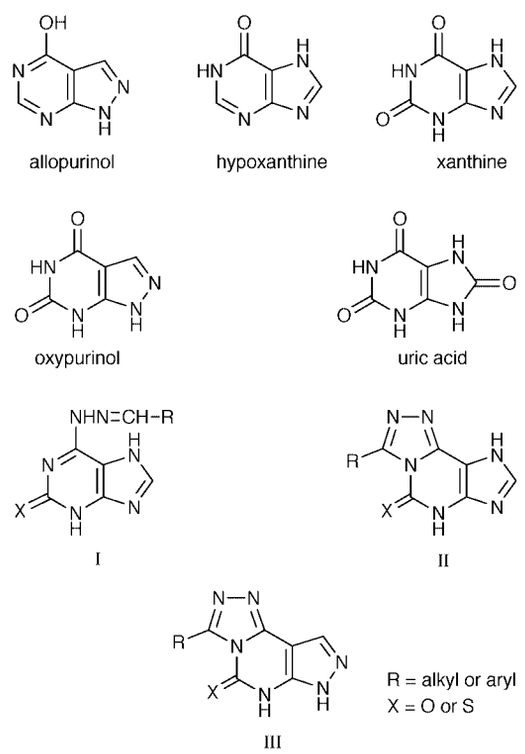
Convenient and general syntheses of 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**12**), a new class of potent xanthine oxidase inhibitors, involving the oxidative cyclisation of 6-substituted 4-alkylidenehydrazino- or 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidines (**3** and **11**) with 70% nitric acid as the key step, are reported. The hydrazones **3** and **11** were obtained by a versatile synthetic route *via* the key intermediates, 6-chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine **2** or oxypurinol **4**, starting from 2,4,6-trichloropyrimidine-5-carbaldehyde **1**. Their inhibitory activities against bovine milk xanthine oxidase *in vitro* are also described; *i.e.* the pyrazolotriazolopyrimidines **12** were several hundred times more potent than allopurinol.

Introduction

Allopurinol, a well known drug clinically used for treatment of gout and hyperuricemia resulting from uric acid,[2–4] has been reported as a potential inhibitor of xanthine oxidase (XO), which catalyzes the conversion of hypoxanthine and xanthine to uric acid.[5] Allopurinol is relatively non-toxic and does not appear to interfere with anabolic processes within the cell, as judged by its lack of inhibition of the growth of bacteria or tumors.[6] However, some allopurinol toxicities[7] and a life-threatening toxicity syndrome have been reported after its use.[8] Although XO inhibitory activities have recently been discovered in some newly synthesized compounds and previously known compounds,[9–15] no clinically effective XO inhibitors for the treatment of hyperuricemia have been developed since allopurinol was introduced for clinical use in 1963.[2,6] We have recently discovered that 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines (I) and the angular type purine analogues, 9*H*-1,2,4-triazolo[3,4-*i*]purines (II), have exhibited more potent bovine milk XO inhibitory activities than that of allopurinol.[1,16,17]

In our recent communication,[18] we reported the facile and general syntheses of 3- and/or 5-substituted 7*H*-pyrazolo-[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines (III) as a new class of potential xanthine oxidase inhibitors. Herein we report full details of the versatile and general syntheses of the 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (III), involving the oxidative cyclisation of 6-substituted 4-alkylidenehydrazino- or 4-arylmethylidene-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidines as the key step. Furthermore, we also report here their inhibitory activities against bovine milk xanthine oxidase in comparison with allopurinol *in vitro*.

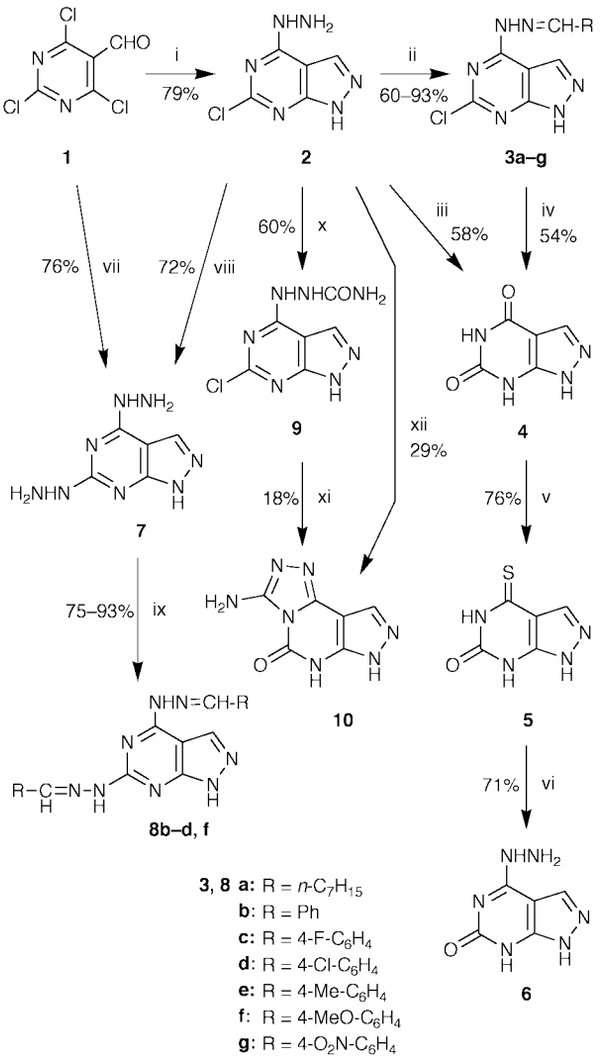


Results and discussion

In the preceding paper,[1] we have clarified that 9*H*-1,2,4-triazolo[3,4-*i*]purines (II), especially the 5-oxo or 5-thioxo derivatives, showed more potent bovine milk XO inhibitory activities than allopurinol. Therefore, in this paper we tried to prepare 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]-pyrimidin-5(6*H*)-ones (III), which are analogous to the triazolopurines (II), as another new class of potential XO inhibitors. Few methods for synthesis of the pyrazolotriazolopyrimidines (III) have been reported in the journal[19,20] or patent[21] literature and several derivatives have been synthesised. However, none of the 5-substituted derivatives has been prepared up to now.

In the first place we tried to synthesise the key intermediate, 4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one **6** derived

from barbituric acid. The requisite starting material, 2,4,6-trichloropyrimidine-5-carbaldehyde **1**, was prepared according to a literature method.[22] Treatment of **1** with anhydrous hydrazine (4 equiv.) in 2-methoxyethanol at 0 °C afforded 6-chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine **2** in 79% yield (Scheme 1). Subsequent reaction of compound **2** with



Scheme 1 *Reagents and conditions*: i, anh. NH_2NH_2, 2-methoxyethanol, 0 °C, 0.5 h; ii, RCHO, DMF, r.t., 10 h; iii, conc. HCl reflux, 1 h; iv, 10% HCl, reflux, 3 h; v, P_2S_5, pyridine, reflux, 2 h; vi, 50% ethanolic NH_2NH_2, reflux, 10 min; vii, anh. NH_2NH_2, 2-methoxyethanol, 100 °C, 5 h; viii, 80% aq. NH_2NH_2, 80–90 °C, 4 h; ix, RCHO, DMF, r.t., 10 h; x, urea, 2-ethoxyethanol, reflux, 5 h; xi, urea, 2-ethoxyethanol, reflux, 10 h; xii, urea, 2-ethoxyethanol, 36 h.

appropriate aldehydes (1.2 equiv.) in dimethylfumamide (DMF) at room temperature gave the corresponding hydrazones **3a**–**g** in 60–93% yields as shown in Tables 1 and 2. Further, heating compound **2** in concentrated hydrochloric acid (50 parts) under reflux for 1 hour gave oxypurinol **4** (58% yield), which was confirmed by direct comparison with an authentic sample.[23] The oxypurinol **4** was also obtained in a similar yield by heating the hydrazone **3b** (R = Ph) in 10% hydrochloric acid (100 parts) for 3 hours. Thiation of oxypurinol **4** by phosphorous pentasulfide gave the 6-oxo-4-thioxo derivative **5** (76% yield) following a literature procedure[23] and the reaction of **5** with excess 50% ethanolic hydrazine under reflux yielded the desired intermediate, 4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one **6**, in 71% yield.

On the other hand, heating compound **1** with excess anhydrous hydrazine at 100 °C or heating compound **2** with excess 80% hydrazine hydrate at 80–90 °C afforded the 4,6-dihydrazino derivative **7** in good yields. Subsequent reaction of compound **7** with appropriate aldehydes (3.0 equiv.) in DMF at room temperature gave the corresponding hydrazones **8b**–**d**,**f** in excellent yields as shown in Tables 1 and 2. Next, in an attempt to convert the 6-chloro-4-hydrazino compound **2** to the 6-oxo-4-hydrazino derivative **6**, compound **2** was reacted with urea (4.0 equiv.) in 2-ethoxyethanol under reflux for 5 hours. However, owing to the stability of the chloro group towards hydroxy substitution by urea or alkali, the intended compound **6** was not obtained, but 4-carbamoylhydrazino-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine **9**, which resulted from carbamoylation of the hydrazino group at the 4-position, was formed in 60% yield. Heating under reflux the product **9** with urea (3.0 equiv.) in 2-ethoxyethanol afforded the desired tricyclic compound, 3-amino-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-one **10**, in 18% yield. This compound **10** (29% yield) was also obtained by prolonged heating of **2** with urea under the same reaction conditions as mentioned above.

All new compounds **2**, **3** and **6**–**10** exhibited satisfactory elemental combustion analyses except for **2** and **6** and FAB-MS, IR and ^{1}H NMR spectral data consistent with the structures. In particular, the structure of the product **10** was confirmed by the presence of a two-proton broad singlet signal at δ 7.94 and one-proton signals at δ 12.27 and 13.10 in the ^{1}H NMR spectrum attributable to the amino and imino groups and by the presence of peaks at 3370 (ν_{as} NH), 3260 (ν_s NH), 1720 (ν C=O) and 1685 (δ NH) cm^{-1} in the IR spectrum attributable to amino and carbonyl groups. It was clarified that the substitution reaction of the chloro group by hydroxy was difficult in the pyrazolopyrimidine ring **2**, while in the pyrazolotriazolopyrimidine ring **10** it was easy.

The 4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one **6** as noted above was a versatile intermediate for the preparation of the 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidine ring system. Thus the hydrazinopyrazolopyrimidine **6** could be converted to the hydrazones **11b**, **e**–**r** (63–95% yields) by reaction with an appropriate aldehyde (1.5 equiv.) in DMF at room temperature (Scheme 2 and Tables 3 and 4). The hydrazones **11b**, **e**–**p**, **r** were subsequently cyclised to the corresponding 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones **12b**, **e**–**p**, **r** by heating with 70% nitric acid (1.2 equiv.) at 100 °C in 60–91% yields (*Method A*) (Tables 5 and 6). In the case of compound **11q** possessing a 4-hydroxybenzylidenehydrazino group as the substituent at the 4-position, the 3-(4-hydroxy-3-nitrophenyl) derivative **12s** was obtained by oxidative cyclisation–nitration in 52% yield. Oxidative cyclisation was also accomplished by heating compounds **11e**, **g**, **h**, **k**, **m**–**o**, **q** with diethyl azodicarboxylate (DEAD) (3–7 equiv.) under reflux in 50–67% yields (*Method B*). Moreover, the 3-alkyl derivatives **12a**–**d** were synthesised by treatment of compound **6** with an appropriate trialkyl orthoester (5.0 equiv.) in trifluoroacetic acid at room temperature (*Method C*) or heating compound **6** with trialkyl orthoesters (3.0 equiv.) in DMF at 100 °C (*Method D*) in 54–83% yields. In the light of this multiple step synthesis, a one-pot oxidative cyclisation starting from the 6-chloro-4-hydrazones **3a**–**g** would be attractive. Indeed, heating the hydrazones **3a**–**g** with 70% nitric acid (5.0 equiv.) in DMF at 100 °C afforded the desired 3-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones **12e**, **g**, **h**, **k**, **m**, **n**, **r** accompanied by hydrolytic dechlorination in 60–85% yields (*Method E*).

All new compounds **11** and **12** exhibited satisfactory elemental combustion analyses and FAB MS, IR and ^{1}H NMR spectral data consistent with the structures as indicated in Tables 3–6.

Xanthine oxidase inhibitory results

The novel pyrazolopyrimidines **2**, **3**, **6**, **8** and **11** and pyrazolo-

Table 1 Preparative, physical and analytical data for the compounds **3a–g**, **8b–d**,**f**

Compound (Formula)	Yield (%)	Mp/°C	Recrystn. solvent[a] (R_f, solvent system[b])	Found (%) (Required) C	H	N	*m/z* MH^+
3a $C_{13}H_{19}ClN_6$	60	250	AcOEt (0.78, A)	52.9 (53.0)	6.5 (6.5)	28.5 (28.5)	295/297
3b $C_{12}H_9ClN_6$	93	>300	DMF (0.56, A)	52.4 (52.85)	3.7 (3.3)	30.6 (30.8)	273/275
3c $C_{12}H_8ClFN_6$	89	>300	EtOH–DMF (0.60, A)	49.9 (49.6)	2.8 (2.8)	29.2 (28.9)	291/293
3d $C_{12}H_8Cl_2N_6$	76	>300	EtOH–DMF (0.64, A)	46.7 (46.9)	2.4 (2.6)	27.0 (27.4)	307/309/311
3e $C_{13}H_{11}ClN_6$	78	>300	EtOH–DMF (0.61, A)	54.0 (54.5)	4.1 (3.9)	29.1 (29.3)	287/289
3f $C_{13}H_{11}ClN_6O$	79	>300	EtOH–DMF (0.57, A)	51.7 (51.6)	3.8 (3.7)	27.7 (27.8)	303/305
3g $C_{12}H_8ClN_7O_2$	70	>300	EtOH–DMF (0.49, A)	45.6 (45.4)	2.7 (2.5)	30.6 (30.9)	318/320
8b $C_{19}H_{16}N_8{\cdot}H_2O$	87	298–300	EtOH–DMF (0.60, B)	60.7 (60.95)	4.8 (4.85)	29.9 (29.9)	357
8c $C_{19}H_{14}F_2N_8{\cdot}3/2\ H_2O$	93	>300	EtOH–DMF (0.61, B)	54.1 (54.4)	4.1 (4.1)	26.4 (26.7)	393
8d $C_{19}H_{14}Cl_2N_8{\cdot}3/2\ H_2O$	75	>300	EtOH–DMF (0.68, B)	50.2 (50.45)	3.7 (3.8)	24.6 (24.8)	425/427/429
8f $C_{21}H_{20}N_8O_2{\cdot}H_2O$	77	276	EtOH–DMF (0.62, B)	57.7 (58.1)	5.1 (5.1)	25.7 (25.8)	417

[a] All compounds **3** and **8** were obtained as colourless or pale yellow powdery crystals except for **3g** (yellow). [b] Solvent systems: (A) AcOEt–*n*-hexane (4 : 3 v/v), (B) AcOEt–EtOH (9 : 1 v/v).

Table 2 1R and ^{1}H NMR spectroscopic data for the compounds **3a–g**, **8b–d**, **f**

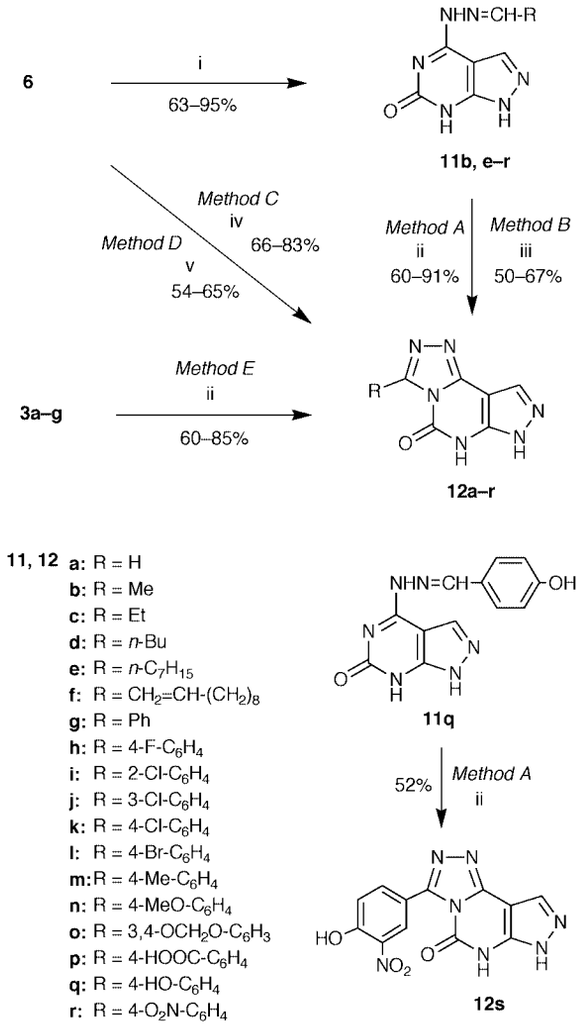
Compound	ν_{max}(Nujol)/cm^{-1}	δ_H[60 MHz; $(CD_3)_2SO$; Me_4Si]
3a	3180, 3100 (NH)	0.86 (3 H, *J* 6.8, $CHCH_2[CH_2]_5CH_3$), 1.31 (10 H, br s, $CHCH_2[CH_2]_5CH_3$), 2.15–2.60 (2 H, m, $CHCH_2[CH_2]_5CH_3$), 7.61 (1 H, t, *J* 6.6, $CHCH_2[CH_2]_5CH_3$), 8.18 (1 H, s, 3-H), 12.00 (1 H, br, 4-NH), 12.90 (1 H, br, 1-NH)
3b	3190, 3080 (NH)	7.40–7.60 (3 H, m, Ph-*m*,*p*H), 7.70–7.95 (2 H, m, Ph-*o*H), 8.28 (1 H, s, 3-H), 8.40 (1 H, s, C*H*-Ar), 12.44 (1 H, s, 4-NH), 13.75 (1 H, br s, 1-NH)
3c	3200, 3100 (NH)	7.32 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.1, Ar-*m*H), 7.90 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.9, Ar-*o*H), 8.27 (1 H, s, 3-H), 8.40 (1 H, s, C*H*-Ar), 12.45 (1 H, s, 4-NH), 13.70 (1 H, br, 1-NH)
3d[a]	3180, 3080 (NH)	7.55 (2 H, d, *J* 8.6, Ar-*m*H), 7.85 (2 H, d, *J* 8.6, Ar-*o*H), 8.26 (1 H, s, 3-H), 8.40 (1 H, s, C*H*-Ar), 12.54 (1 H, br, 4-NH), 13.70 (1 H, br, 1-NH)
3e	3190, 3080 (NH)	2.37 (3 H, s, CH_3), 7.30 (2 H, d, *J* 7.9, Ar-*m*H), 7.70 (2 H, d, *J* 7.9, Ar-*o*H), 8.29 (1 H, s, 3-H), 8.38 (1 H, s, C*H*-Ar), 12.40 (1 H, br, 4-NH), 13.50 (1 H, br, 1-NH)
3f	3210, 3100 (NH)	3.84 (3 H, s, OCH_3), 7.06 (2 H, d, *J* 8.8, Ar-*m*H), 7.78 (2 H, d, *J* 8.8, Ar-*o*H), 8.24 (1 H, s, 3-H), 8.39 (1 H, s, C*H*-Ar), 12.35 (1 H, br s, 4-NH), 13.60 (1 H, br, 1-NH)
3g[a]	3190, 3090 (NH)	8.09 (2 H, d, *J* 8.8, Ar-*o*H), 8.32 (2 H, d, *J* 8.8, Ar-*m*H), 8.35 (1 H, s, 3-H), 8.46 (1 H, s, C*H*-Ar), 12.78 (1 H, br s, 4-NH), 13.90 (1 H, br, 1-NH)
8b	3240, 3180, 3140 (NH)	7.26–7.83 (10 H, m, Ph-H × 2), 8.19 (1 H, s, 6-C*H*-Ar), 8.24 (1 H, s, 3-H), 8.32 (1 H, s, 4-C*H*-Ar), 10.81 (1 H, br s, 6-NH), 11.78 (1 H, br, 4-NH), 13.07 (1 H, br, 1-NH)
8c	3250, 3170, 3130 (NH)	7.27 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.1, 6-Ar-*m*H), 7.32 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.1, 4-Ar-*m*H), 7.74 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.9, 6-Ar-*o*H), 7.87 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.8, 4-Ar-*o*H), 8.20 (1 H, s, 6-C*H*-Ar), 8.26 (1 H, s, 3-H), 8.33 (1 H, s, 4-C*H*-Ar), 10.93 (1 H, br s, 6-NH), 11.80 (1 H, br, 4-NH), 12.90 (1 H, br, 1-NH)
8d	3250, 3180, 3120 (NH)	7.45 (2 H, d, *J* 8.4, 6-Ar-*m*H), 7.51 (2 H, d, *J* 8.5, 4-Ar-*m*H), 7.74 (2 H, d, *J* 8.4, 6-Ar-*o*H), 7.80 (2 H, d, *J* 8.5, 4-Ar-*o*H), 8.17 (1 H, s, 6-C*H*-Ar), 8.28 (1 H, s, 3-H), 8.32 (1 H, s, 4-C*H*-Ar), 10.95 (1 H, br, 6-NH), 11.80 (1 H, br, 4-NH), 13.00 (1 H, br, 1-NH)
8f	3250, 3170, 3140 (NH)	3.75 (3 H, s, 6-OCH_3), 3.81 (3 H, s, 4-OCH_3), 6.95 (2 H, d, *J* 8.5, 6-Ar-*m*H), 7.06 (2 H, d, *J* 8.8, 4-Ar-*m*H), 7.60 (2 H, d, *J* 8.5, 6-Ar-*o*H), 7.77 (2 H, d, *J* 8.8, 4-Ar-*o*H), 8.12 (1 H, s, 6-C*H*-Ar), 8.20 (1 H, s, 3-H), 8.24 (1 H, s, 4-C*H*-Ar), 10.59 (1 H, br s, 6-NH), 11.66 (1 H, br, 4-NH), 12.83 (1 H, br, 1-NH)

[a] This compound was measured at 200 MHz.

triazolopyrimidines **12** prepared in this study were tested as inhibitors of bovine milk xanthine oxidase in a similar assay method[14] as previously reported. The inhibition (%) and IC_{50} (μM) values of the compounds tested against bovine milk xanthine oxidase are shown in Table 7. Thus the introduction of both an aryl aldehyde hydrazone at the 4-position and an oxo group at the 6-position of the 1*H*-pyrazolo[3,4-*d*]pyrimidine ring led to markedly better activities in xanthine oxidase inhibition, these compounds being two orders of magnitude more active than allopurinol: IC_{50} values for **11g–k** and **11m–q** were *ca.* 0.08–0.4 μM, whereas that for allopurinol was 24.3 μM. Most of the pyrazolotriazolopyrimidines **12a–s** showed potent inhibitory activities, being two or three orders of magnitude more active than allopurinol. Of these compounds, **12k** (R = 4-ClC_6H_4) was the most active; it showed a 760-fold (IC_{50} = 0.032 μM) more potent bovine milk XO inhibitory activity than that of allopurinol.

Conclusion

Thus, this simple and general methodology provided a facile and convenient route to the preparation of 3-substituted 7*H*-



Scheme 2 *Reagents and conditions*: i, RCHO, DMF, r.t. or 40 °C, 10 h; ii, 70% HNO_3, DMF, 100 °C, 1–9 h; iii, DEAD, DMF, reflux, 5–9 h; iv, $RC(OEt)_3$, TFA, r.t., 1 h; v, $RC(OEt)_3$ or $RC(OMe)_3$, DMF, 100 °C, 1 h.

pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**12**), which were obtained by oxidative cyclisation of the corresponding 4-aldehyde hydrazones of 1*H*-pyrazolo[3,4-*d*]pyrimidines (**3** and **11**) with 70% nitric acid as the key step, as a new class of potential xanthine oxidase inhibitors. Their inhibitory activities against bovine milk xanthine oxidase *in vitro* were investigated, and some 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-ones (**11**) exhibited from several times to several hundred times more potent activities than allopurinol. In addition, the tricyclic compounds, 3-aryl-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**12**) showed potent inhibitory activities, being *ca.* three orders of magnitude more active than allopurinol. They did not show any appreciable inhibition against the proliferation of T-cell acute lymphoblastic leukemia (CCRF-HSB-2) however.† Biological testing of the compounds *in vivo* is now ongoing and the results will be reported later.

Experimental

General

Mps were obtained on a Yanagimoto micro melting point apparatus and were uncorrected. Microanalyses were measured by a Yanaco CHN Corder MT-5 apparatus. Mass spectra were recorded at 70 eV ionizing voltage with FAB ionization using a VG-70SE spectrometer and 3-nitrobenzyl alcohol or glycerol as a matrix. IR spectra were recorded using a JASCO FT/IR-200 spectrophotometer as Nujol mulls. ^{1}H NMR spectra were obtained using Hitachi FT-NMR R-1500 (60 MHz) and Varian VXR 200 MHz spectrometers. In all cases, chemical shifts are in ppm relative to $SiMe_4$ as internal standard and *J* values are given in Hz. All reagents were of commercial quality from freshly opened containers and were used without further purification. Organic solvents were dried by standard methods and distilled before use. Reaction progress was monitored by analytical thin layer chromatography (TLC) on pre-coated glass plates (silica gel 70 FM Plate-Wako) using the following solvent systems: (A) AcOEt–EtOH (4:1 v/v), (B) EtOH, (C) MeOH and others cited in the Tables. The products were visualized by UV light. Column chromatography was run on Daisogel IR-60 (63/210 μM, Daiso Co.). The reaction temperatures are indicated as the temperature of oil bath.

6-Chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine 2

To a stirring solution of 2,4,6-trichloropyrimidine-5-carbaldehyde **1**[22] (3.0 g, 14.2 mmol) in 2-methoxyethanol (20 cm^3) at 0 °C was added a solution of anhydrous hydrazine (1.82 g, 56.8 mmol) diluted with 2-methoxyethanol (18 cm^3) in limited amounts for 30 min. After the reaction was complete, the precipitated crystals were collected by filtration and washed with water and EtOH to afford the *pyrazolopyrimidine* **2** (2.06 g, 79%) as pale yellow powdery crystals, mp > 300 °C; R_f (A) 0.64; ν_{max}/cm^{-1} 3350 and 3260 (NH_2), 3160 and 3100 (NH) and δ_{max}/cm^{-1} 1660 (NH_2); δ_H [60 MHz; $(CD_3)_2SO$] 4.20 (2 H, br, NH_2), 8.50 (1 H, s, 3-H), 9.45 (1 H, br, 4-NH) and 13.40 (1 H, br, 1-NH); *m*/*z* (FAB, 3-nitrobenzyl alcohol matrix) 185 (MH^+) and 187 ($MH^+ + 2$). The product **2** was obtained as a single compound and was used for the following reactions without further purification because it was difficult to purify since it was insoluble in usual solvents.

4-Alkylidenehydrazino- and 4-arylmethylidenehydrazino-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidines 3a–g; General procedure

A mixture of the hydrazinopyrazolopyrimidine **2** (1.0 g, 5.42 mmol) and an appropriate alkyl aldehyde or aryl aldehyde (6.50 mmol) in DMF (50 cm^3) was stirred at room temperature for 10 hours. After the reaction was complete, the solution was evaporated under reduced pressure and the residue was triturated with EtOH or AcOEt to give crystals, which were collected by filtration and recrystallized from an appropriate solvent to afford the corresponding *hydrazones* **3a–g** as shown in Tables 1 and 2.

1*H*-Pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione 4 (oxypurinol)

(1) A mixture of the hydrazino derivative **2** (0.20 g, 1.08 mmol) with concentrated hydrochloric acid (10 cm^3) was heated under reflux for 1 hour. After the reaction was complete, the solution was treated with activated charcoal and evaporated under reduced pressure; the residue was recrystallized from water to afford *oxypurinol* [95 mg, 58%; mp > 300 °C; R_f (A) 0.48; ν_{max}/cm^{-1} 3180, 3150 and 3120 (NH) and 1720 (C=O)], which was identical with an authentic sample.[23]

(2) The hydrazone **3b** (0.50 g, 1.83 mmol) in 10% aqueous HCl (50 cm^3) was heated under reflux for 3 hours. After the reaction was complete, the solution was treated with activated charcoal and cooled to afford a deposit, which was collected by filtration. The filtrate was evaporated under reduced pressure and the residue was recrystallized from water to get the second

† We have found that some derivatives exhibited poor inhibitory activities against the proliferation of T-cell acute lymphoblastic leukemia (CCRF-HSB-2): the IC_{50} for **11j**, 11 μM; for **11o**, 45 μM; for **12i**, 25 μM; for **12j**, 14 μM; for **12k**, 35 μM; for **12l**, 23 μM; for **12n**, 36 μM; for **12q**, 34 μM; for **12r**, 36 μM; for **12s**, 35 μM and for arabinosylcytosine, 0.061 μM.

Table 3 Preparative, physical and analytical data for the compounds **11b**, **e**–**r**

Compound (Formula)	Reaction temp/°C	Yield (%)	Mp/°C	Recrystn. solvent[a] (R_f, solvent system[b])	Found (%) (Required) C	H	N	*m/z* MH^+
11b $C_7H_8N_6O$	r.t.	89	>300	EtOH–DMF (0.47, A)	43.4 (43.75)	4.2 (4.2)	43.65 (43.7)	193
11e $C_{13}H_{20}N_6O\cdot1/5\ H_2O$	r.t.	80	>300	EtOH–DMF (0.65, A)	55.8 (55.8)	7.2 (7.35)	30.3 (30.0)	277
11f $C_{16}H_{24}N_6O$	r.t.	63	>300	EtOH–DMF (0.47, B)	60.3 (60.7)	7.4 (7.65)	26.3 (26.6)	317
11g $C_{12}H_{10}N_6O\cdot1/5\ H_2O$	r.t.	85	>300	water–DMF (0.60, A)	55.8 (55.9)	4.1 (4.1)	32.8 (32.6)	255
11h $C_{12}H_9FN_6O\cdot1/4\ H_2O$	r.t.	74	>300	water–DMF (0.64, A)	52.2 (52.1)	3.6 (3.5)	30.5 (30.4)	273
11i $C_{12}H_9ClN_6O$	40	76	>300	EtOH–DMF (0.64, A)	49.5 (49.9)	3.5 (3.1)	28.7 (29.1)	289/291
11j $C_{12}H_9ClN_6O\cdot1/5\ H_2O$	40	95	>300	EtOH–DMF (0.65, A)	49.3 (49.3)	3.4 (3.2)	28.9 (28.75)	289/291
11k $C_{12}H_9ClN_6O\cdot1/5\ H_2O$	r.t.	95	>300	water–DMF (0.63, A)	49.2 (49.3)	3.4 (3.2)	28.7 (28.75)	289/291
11l $C_{12}H_9BrN_6O$	r.t.	83	>300	EtOH–DMF (0.65, A)	43.3 (43.3)	3.1 (2.7)	24.9 (25.2)	333/335
11m $C_{13}H_{12}N_6O\cdot1/5\ H_2O$	r.t.	93	>300	DMF (0.67, A)	57.4 (57.4)	4.5 (4.6)	31.1 (30.9)	269
11n $C_{13}H_{12}N_6O_2\cdot1/5\ H_2O$	r.t.	87	>300	water–DMF (0.62, A)	54.4 (54.2)	4.3 (4.3)	29.2 (29.2)	285
11o $C_{13}H_{10}N_6O_3$	40	85	>300	water–DMF (0.67, A)	52.1 (52.35)	3.6 (3.4)	27.7 (28.2)	299
11p $C_{13}H_{10}N_6O_3$	40	80	>300	water–DMF (0.64, C)	52.6 (52.35)	3.4 (3.4)	28.2 (28.2)	299
11q $C_{12}H_{10}N_6O_2$	40	85	>300	EtOH–DMF (0.64, A)	53.2 (53.3)	4.0 (3.7)	30.7 (31.1)	271
11r $C_{12}H_9N_7O_3\cdot H_2O$	r.t.	88	>300	EtOH–DMF (0.60, A)	45.45 (45.4)	3.5 (3.5)	30.8 (30.9)	300

[a] All compounds **11** were obtained as colourless powdery crystals except for **11m**, **r** (pale yellow). [b] Solvent systems: (A) AcOEt–EtOH (4:1 v/v), (B) AcOEt–EtOH (9:1 v/v), (C) AcOEt–*n*-hexane–AcOH (8:4:1 v/v).

crop. The product was identical with oxypurinol (150 mg, 54%).

4-Hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-one 6

To a mixture of hydrazine monohydrate (5.0 g, 99.9 mmol) and ethanol (5 cm^3) was added 4,5-dihydro-4-thioxo-1*H*-pyrazolo-[3,4-*d*]pyrimidin-6(7*H*)-one **5**[23] (1.0 g, 5.95 mmol) and the mixture was heated under reflux for 10 min. After the reaction was complete, the precipitated crystals were collected by filtration and washed with water and EtOH to afford the *hydrazino derivative* **6** (0.70 g, 71%) as colourless powdery crystals, mp > 300 °C; R_f (B) 0.28; ν_{max}/cm^{-1} 3360 and 3310 (NH_2), 3200, 3150 and 3100 (NH), 1710 (C=O) and δ_{max}/cm^{-1} 1670 (NH_2); δ_H [60 MHz; CF_3CO_2D] 8.72 (1 H, s, 3-H); *m/z* (FAB, 3-nitrobenzyl alcohol matrix) 167 (MH^+). The product **6** was obtained as a single compound and was used for the following reactions without further purification because it was difficult to purify since it was insoluble in usual solvents.

4,6-Dihydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine 7

(1) To a stirring solution of 2,4,6-trichloropyrimidine-5-carbaldehyde **1**[22] (3.0 g, 14.2 mmol) in 2-methoxyethanol (20 cm^3) at 0 °C was added anhydrous hydrazine (9.1 g, 283.9 mmol) dropwise. Then, the stirred mixture was heated at 100 °C for 5 hours. After the reaction was complete, the precipitated crystals were collected by filtration, washed with water and EtOH and recrystallized from water to afford the *dihydrazino derivative* **7** (1.94 g, 76%) as colourless powdery crystals, mp > 300 °C (Found: C, 33.1; H, 4.55; N, 61.5. $C_5H_8N_8\cdot1/5\ H_2O$ requires C, 32.7; H, 4.6; N, 61.0%); R_f (C) 0.20; ν_{max}/cm^{-1} 3335, 3270 and 3230 (NH_2), 3180, 3120 and 3110 (NH) and δ_{max}/cm^{-1} 1650 and 1640 (NH_2); δ_H [60 MHz; CF_3CO_2D] 8.81 (1H, s, 3-H); *m/z* (FAB, 3-nitrobenzyl alcohol matrix) 181 (MH^+).

(2) To a stirring solution of 80% aqueous hydrazine hydrate (20 cm^3, 320 mmol) was added 6-chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine **2** (2.0 g, 10.8 mmol) and the mixture was heated at 80–90 °C for 4 hours. After the same work-up as noted above, recrystallization of the crude crystals from water gave the *dihydrazino derivative* **7** (1.40 g, 72%).

4,6-Bis(arylmethylidenehydrazino)-1*H*-pyrazolo[3,4-*d*]pyrimidines 8b–d, f. General procedure

A mixture of the dihydrazino derivative **7** (0.60 g, 3.33 mmol) and an appropriate aldehyde (9.99 mmol) in DMF (20 cm^3) was stirred at room temperature for 10 hours. After the reaction was complete, the solution was evaporated under reduced pressure and the residue was triturated with EtOH to give crystals, which were collected by filtration and recrystallized from a mixture of EtOH and DMF to afford the corresponding *bishydrazones* **8b**–**d**, **f** as shown in Tables 1 and 2.

4-Carbamoylhydrazino-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine 9

A mixture of the hydrazinopyrazolopyrimidine **2** (0.5 g, 2.71 mmol) and urea (0.65 g, 10.8 mmol) in 2-ethoxyethanol (25 cm^3) was heated under reflux for 5 hours. After the reaction was complete, the solution was evaporated under reduced pressure to afford a solid. The solid was collected by filtration, washed with water and recrystallized from water to afford the *carbamoylhydrazino derivative* **9** (0.37 g, 60%) as colourless powdery crystals, mp > 300 °C (Found: C, 31.1; H, 2.9; N, 43.0. $C_6H_6ClN_7O\cdot1/7\ H_2O$ requires C, 31.3; H, 2.75; N, 42.6%); R_f (B)

Table 4 IR and ^{1}H NMR spectroscopic data for the compounds **11b**, **e**–**r**

Compound	ν_{max}(Nujol)/cm^{-1}	δ_H [200 MHz; $(CD_3)_2SO$; Me_4Si]
11b	3175, 3130, 3070 (NH); 1700 (C=O)	2.01 (3 H, d, *J* 5.4, CHC*H*$_3$), 7.69 (1 H, q, *J* 5.4, C*H*CH$_3$), 8.35 (1 H, s, 3-H), 10.40 (1 H, br, 4-NH), 10.80 (1 H, br s, 7-NH), 12.85 (1 H, br, 1-NH)
11e	3175, 3135, 3070 (NH); 1710 (C=O)	0.86 (3 H, t, *J* 6.4, CHCH$_2$CH$_2$[CH$_2$]$_4$C*H*$_3$), 1.29 (8 H, br s, CHCH$_2$CH$_2$[C*H*$_2$]$_4$CH$_3$), 1.44–1.66 (2 H, m, CHCH$_2$C*H*$_2$[CH$_2$]$_4$CH$_3$), 2.24–2.42 (2 H, m, CHC*H*$_2$CH$_2$[CH$_2$]$_4$CH$_3$), 8.05 (1 H, t, *J* 5.4, C*H*CH$_2$CH$_2$[CH$_2$]$_4$CH$_3$), 8.28 (1 H, s, 3-H), 10.22 (1 H, br, 4-NH), 10.82 (1 H, br s, 7-NH), 12.95 (1 H, br, 1-NH)
11f	3175, 3135, 3070 (NH); 1700 (C=O)	1.28 (10 H, br s, CHCH$_2$CH$_2$[C*H*$_2$]$_5$CH$_2$CH=CH$_2$), 1.44–1.64 (2 H, m, CHCH$_2$C*H*$_2$[CH$_2$]$_5$-CH$_2$CH=CH$_2$), 1.90–2.08 (2 H, m, CHCH$_2$CH$_2$[CH$_2$]$_5$C*H*$_2$CH=CH$_2$), 2.22–2.42 (2 H, m, CHC*H*$_2$-CH$_2$[CH$_2$]$_5$CH$_2$CH=CH$_2$), 4.86–5.06 (2 H, m, CHCH$_2$CH$_2$[CH$_2$]$_5$CH$_2$CH=C*H*$_2$), 5.64–5.90 (1 H, m, CHCH$_2$CH$_2$[CH$_2$]$_5$CH$_2$C*H*=CH$_2$), 7.60–7.72 (1 H, m, C*H*CH$_2$CH$_2$[CH$_2$]$_5$CH$_2$CH=CH$_2$), 8.28 (1H, s, 3-H), 10.28 (1 H, br, 4-NH), 10.82 (1 H, br s, 7-NH), 12.92 (1 H, br, 1-NH)
11g	3200, 3120, 3080 (NH); 1680 (C=O)	7.40–7.53 (3 H, m, Ph-*m*,*p*H), 7.80–7.90 (2 H, m, Ph-*o*H), 8.40 (1 H, s, 3-H), 8.46 (1 H, s, C*H*-Ar), 10.40 (1 H, br, 4-NH), 11.01 (1 H, br, 7-NH), 13.10 (1 H, br, 1-NH)
11h	3180, 3140, 3070 (NH); 1680 (C=O)	7.30 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.0, Ar-*m*H), 7.30 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.8, Ar-*o*H), 8.40 (1 H, s, 3-H), 8.45 (1H, s, C*H*-Ar), 10.40 (1 H, br, 4-NH), 11.00 (1 H, br, 7-NH), 13.09 (1 H, br, 1-NH)
11i[a]	3200, 3120, 3080 (NH); 1700 (C=O)	7.33–7.60 (3 H, m, 3′-H, 4′-H and 5′-H), 8.12–8.28 (1 H, m, 6′-H), 8.48 (1H, s, 3-H), 8.72 (1 H, s, C*H*-Ar), 10.35 (1 H, br s, 4-NH), 11.10 (1 H, br, 7-NH), 13.40 (1 H, br, 1-NH)
11j	3170, 3140, 3060 (NH); 1720 (C=O)	7.40–7.54 (2 H, m, 4′-H and 5′-H), 7.80–7.92 (2 H, m, 2′-H and 6′-H), 8.40 (1 H, s, 3-H), 8.43 (1 H, s, C*H*-Ar), 10.50 (1 H, br s, 4-NH), 11.01 (1 H, br s, 7-H), 13.10 (1 H, br s, 1-NH)
11k	3170, 3140, 3070 (NH); 1680 (C=O)	7.48 (2 H, d, *J* 8.6, Ar-*m*H), 7.87 (2 H, d, *J* 8.6, Ar-*o*H), 8.37 (1 H, s, 3-H), 8.45 (1 H, s, C*H*-Ar), 10.50 (1 H, br, 4-NH), 11.00 (1 H, br, 7-NH), 13.14 (1 H, br, 1-NH)
11l	3170, 3140, 3060 (NH); 1685 (C=O)	7.66 (2 H, d, *J* 8.6, Ar-*m*H), 7.80 (2 H, d, *J* 8.6, Ar-*o*H), 8.38 (1H, s, 3-H), 9.44 (1 H, s, C*H*-Ar), 10.50 (1 H, br, 4-NH), 11.00 (1 H, br s, 7-NH), 13.11 (1 H, br, 1-NH)
11m	3170, 3140, 3060 (NH); 1685 (C=O)	2.36 (3 H, s, CH$_3$), 7.28 (2 H, d, *J* 7.8, Ar-*m*H), 7.72 (2 H, d, *J* 7.8, Ar-*o*H), 8.35 (1 H, s, 3-H), 8.44 (1 H, s, C*H*-Ar), 10.45 (1 H, br, 4-NH), 11.00 (1 H, br, 7-NH), 13.10 (1 H, br, 1-NH)
11n[a]	3180, 3140, 3080 (NH); 1680 (C=O)	3.80 (3 H, s, OCH$_3$), 6.74 (2 H, d, *J* 8.5, Ar-*m*H), 7.58 (2 H, d, *J* 8.5, Ar-*o*H), 7.94 (1 H, s, 3-H), 7.99 (1 H, s, C*H*-Ar), 10.06 (1 H, br s, 4-NH), 10.92 (1 H, br s, 7-NH), 12.90 (1 H, br s, 1-NH)
11o	3180, 3140, 3070 (NH); 1655 (C=O)	6.09 (2 H, s, OCH$_2$O), 7.00 (1 H, d, $J_{5',6'}$ 8.2, 5′-H), 7.27 (1 H, d, $J_{5',6'}$ 8.2, $J_{2',6'}$ 1.6, 6′-H), 7.45 (1 H, d, $J_{2',6'}$ 1.6, 2′-H), 8.30 (1 H, s, 3-H), 8.45 (1 H, s, C*H*-Ar), 10.35 (1 H, br, 4-NH), 11.03 (1 H, br s, 7-NH), 13.04 (1 H, br, 1-NH)
11p[a]	3165, 3120, 3050 (NH); 1650, 1630 (C=O)	8.00 (4 H, br, Ar-H), 8.50 (2 H, s, 3-H and C*H*-Ar), 11.25 (1 H, br, 4-NH), 11.95 (1 H, br, 7-NH), 12.35 (1 H, br, COO*H*), 13.55 (1 H, br, 1-NH)
11q	3160, 3140, 3050 (NH); 1640 (C=O)	6.84 (2 H, d, *J* 8.4, Ar-*m*H), 7.65 (2 H, d, *J* 8.4, Ar-*o*H), 8.26 (1 H, s, 3-H), 8.41 (1 H, s, C*H*-Ar), 9.67 (1 H, br s, OH), 10.86 (1 H, br s, 4-NH), 11.02 (1 H, br s, 7-NH), 12.90 (1 H, br, 1-NH)
11r	3165, 3120, 3080 (NH); 1690 (C=O)	8.10 (1 H, d, *J* 8.8, Ar-*o*H), 8.30 (1 H, d, *J* 8.8, Ar-*m*H), 8.50 (1 H, s, 3-H), 8.52 (1 H, s, C*H*-Ar), 10.65 (1 H, br, 4-NH), 11.11 (1 H, br, 7-NH), 13.21 (1 H, br, 1-NH)

[a] This compound was measured at 60 MHz.

0.70; ν_{max}/cm^{-1} 3410 and 3360 (NH_2), 3200, 3100 and 3040 (NH), 1675 (C=O) and δ_{max}/cm^{-1} 1675 (NH_2); δ_H [200 MHz; $(CD_3)_2SO$] 6.25 (2 H, br, NH_2), 7.94 (1 H, s, 3-H), 8.66 and 10.00 (each 1 H, each br s, 2 × NH) and 13.67 (1 H, br s, 1-NH); *m*/*z* (FAB, glycerol matrix) 228 (MH^+) and 230 ($MH^+ + 2$).

3-Amino-7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 10

(1) The reaction mixture in the same reaction and under the same conditions as in the above preparation for **9** was heated under reflux for 36 hours. After the same work-up as noted above, recrystallization of the crude crystals from water gave the *pyrazolotriazolopyrimidine* **10** (0.15 g, 29%) as colourless powdery crystals, mp > 300 °C (Found: C, 37.1; H, 3.1; N, 49.9. $C_6H_5N_7O$·1/4 H_2O requires C, 36.8; H, 2.8; N, 50.1%); R_f (A) 0.57; ν_{max}/cm^{-1} 3370 and 3260 (NH_2), 3180 and 3100 (NH), 1720 (C=O) and δ_{max}/cm^{-1} 1685 (NH_2); δ_H [200 MHz; $(CD_3)_2SO$] 7.83 (1 H, s, 9-H), 7.94 (2 H, br s, NH_2), 12.27 (1 H, br s, 6-NH) and 13.10 (1 H, br s, 7-NH); *m*/*z* (FAB, glycerol matrix) 192 (MH^+).

(2) A mixture of the pyrazolopyrimidine **9** (0.2 g, 0.88 mmol) and urea (0.16 g, 2.66 mmol) in 2-ethoxyethanol (10 cm^3) was heated under reflux for 10 hours. After the same work-up as noted above, recrystallization of the crude crystals from water gave the *pyrazolotriazolopyrimidine* **10** (30 mg, 18%).

4-Alkylidenehydrazino- and 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(7*H*)-ones 11b, e–r. General procedure

A mixture of the hydrazinopyrazolopyrimidine **6** (1.0 g, 6.02 mmol) and an appropriate alkyl aldehyde or aryl aldehyde (9.03 mmol) in DMF (50 cm^3) was stirred at room temperature or 40 °C for 10 hours. After the reaction was complete, the solution was evaporated under reduced pressure and the residue was triturated with EtOH or AcOEt to give crystals, which were collected by filtration and recrystallized from an appropriate solvent to afford the corresponding *hydrazones* **11b**, **e**–**r** as shown in Tables 3 and 4.

7*H*-Pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-one 12a and its 3-substituted derivatives 12b–s. General procedure

(1) *Method A*: A mixture of an appropriate 4-alkylidenehydrazino- or 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]-pyrimidin-6(7*H*)-one **11b**, **e**–**r** (2.0 mmol) with 70% nitric acid (0.22 cm^3, 2.4 mmol) in DMF (30–50 cm^3) was heated at 100 °C for 1–9 hours. After the reaction was complete, the precipitated crystals were collected by filtration and combined with further material obtained by concentration of the filtrate under reduced pressure. The crystals were recrystallized from an appropriate solvent to afford the corresponding *pyrazolotriazolopyrimidines* **12b**, **e**–**p**, **r**, **s** as shown in Tables 5 and 6.

(2) *Method B*: A mixture of an appropriate 4-alkylidenehydrazino- or 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]-pyrimidin-6(7*H*)-one **11e**, **g**, **h**, **k**, **m**–**o**, **q** (2.0 mmol) with DEAD (0.35 g, 2.0 mmol) in DMF (50 cm^3) was heated under reflux. After heating for several hours, further DEAD (2.0 mmol amounts; total 3–7 equiv.) was added to the heated solution at hourly intervals until the hydrazone **11** disappeared. After the reaction was complete, the solution was evaporated under reduced pressure to leave a solid, which was purified by

Table 5 Preparative, physical and analytical data for the compounds **12a**–**s**

Compound (Formula)	Reaction conditions[a]			Yield[a] (%)	Mp/°C	Recrystn. solvent[b] (R_f, solvent system[c])	Found (%) (Required)			*m/z* MH^+
	Method	Temp/°C	Time/h				C	H	N	
12a	(*C*)	r.t.	1	66	>300	DMF	39.9	2.7	46.5	177
$C_6H_4N_6O·1/4\ H_2O$	(*D*)	100	1	65		(0.32, A)	(39.9)	(2.5)	(46.5)	
12b	(*A*)	100	2.5	64	>300	EtOH–DMF	43.5	3.5	43.7	191
$C_7H_6N_6O·1/5\ H_2O$	(*C*)	r.t	1	83		(0.38, A)	(43.4)	(3.3)	(43.4)	
	(*D*)	100	1	55						
12c	(*D*)	100	1	54	>300	EtOH–DMF	46.8	4.2	41.4	205
$C_8H_8N_6O$						(0.45, A)	(47.1)	(3.95)	(41.2)	
12d	(*D*)	100	1	55	>300	EtOH–DMF	51.1	5.5	36.0	233
$C_{10}H_{12}N_6O·1/5\ H_2O$						(0.55, A)	(50.9)	(5.3)	(35.6)	
12e	(*A*)	100	3	77	>300	EtOH–DMF	55.7	6.7	30.2	275
$C_{13}H_{18}N_6O·1/4\ H_2O$	(*B*)	reflux	7	60		(0.64, A)	(56.0)	(6.7)	(30.1)	
	(*E*)	100	1	75						
12f	(*A*)	100	2.5	67	>300	EtOH–DMF	60.3	7.35	26.3	315
$C_{16}H_{22}N_6O·1/4\ H_2O$						(0.34, B)	(60.3)	(7.1)	(26.35)	
12g	(*A*)	100	1	91	>300	water–DMF	55.9	3.45	32.75	253
$C_{12}H_8N_6O/1/4\ H_2O$	(*B*)	reflux	5	60		(0.60, A)	(56.1)	(3.3)	(32.7)	
	(*E*)	100	1	85						
12h	(*A*)	100	1	91	>300	EtOH–DMF	52.6	2.9	30.65	271
$C_{12}H_7FN_6O·1/4\ H_2O$	(*B*)	reflux	8	65		(0.62, A)	(52.5)	(2.75)	(30.6)	
	(*E*)	100	3	72						
12i	(*A*)	100	2	70	>300	water–DMF	50.0	2.8	28.9	287/289
$C_{12}H_7ClN_6O$						(0.63, A)	(50.3)	(2.5)	(29.3)	
12j	(*A*)	100	2	76	>300	water–DMF	49.9	2.8	29.2	287/289
$C_{12}H_7ClN_6O·1/5\ H_2O$						(0.64, A)	(49.65)	(2.6)	(28.95)	
12k	(*A*)	100	2	90	>300	water–DMF	49.7	2.8	29.2	287/289
$C_{12}H_7ClN_6O·1/5\ H_2O$	(*B*)	reflux	8	67		(0.63, A)	(49.65)	(2.6)	(28.95)	
	(*E*)	100	1	71						
12l	(*A*)	100	5	74	>300	EtOH–DMF	43.2	2.5	24.9	331/333
$C_{12}H_7BrN_6O·1/5\ H_2O$						(0.64, A)	(43.1)	(2.2)	(25.1)	
12m	(*A*)	100	9	60	>300	water–DMF	58.0	4.0	31.3	267
$C_{13}H_{10}N_6O·1/5\ H_2O$	(*B*)	reflux	9	54		(0.67, A)	(57.9)	(3.9)	(31.1)	
	(*E*)	100	3	67						
12n	(*A*)	100	3	88	>300	water–DMF	54.5	3.9	29.3	283
$C_{13}H_{10}N_6O_2·1/4\ H_2O$	(*B*)	reflux	9	57		(0.60, A)	(54.45)	(3.7)	(29.3)	
	(*E*)	100	3	61						
12o	(*A*)	100	1	81	>300	EtOH–DMF	52.2	3.1	28.0	297
$C_{13}H_8N_6O_3·1/4\ H_2O$	(*B*)	reflux	9	55		(0.62, A)	(51.9)	(2.85)	(27.9)	
12p	(*A*)	100	1.5	69	>300	EtOH–DMF	51.8	3.2	27.6	297
$C_{13}H_8N_6O_3·1/3\ H_2O$						(0.64, C)	(51.7)	(2.9)	(27.8)	
12q	(*B*)	reflux	9	50	>300	EtOH–DMF	52.8	2.9	31.0	269
$C_{12}H_8N_6O_2·1/4\ H_2O$						(0.51, A)	(52.85)	(3.1)	(30.8)	
12r	(*A*)	100	5	60	>300	EtOH–DMF	48.1	2.8	32.2	298
$C_{12}H_7N_7O_3·1/4\ H_2O$	(*E*)	100	5	60		(0.60, A)	(47.8)	(2.5)	(32.5)	
12s	(*A*)	100	1	52	>300	DMF	45.4	2.6	30.6	314
$C_{12}H_7N_7O_4·1/4\ H_2O$						(0.46, A)	(45.4)	(2.4)	(30.9)	

[a] The reaction conditions and yields depend on the particular method. [b] All compounds **12** were obtained as colourless powdery crystals except for **12b**, **h**, **k**, **l**, (colourless needles) and **12r**, **s** (yellow powder). [c] Solvent systems: (A) AcOEt–EtOH (4 : 1 v/v), (B) AcOEt–EtOH (9 : 1 v/v), (C) AcOEt–*n*-hexane–AcOH (8 : 4 : 1 v/v).

column chromatography on silica gel using AcOEt as eluent and recrystallized from an appropriate solvent to give the corresponding *pyrazolotriazolopyrimidines* **12e**, **g**, **h**, **k**, **m**–**o**, **q** as shown in Tables 5 and 6.

(3) *Method C*: A mixture of the hydrazinopyrazolopyrimidine **6** (0.60 g, 3.6 mmol) with an appropriate triethyl orthoester (18.0 mmol) in trifluoroacetic acid (9 cm^3) was stirred at room temperature for 1 hour. After the reaction was complete, the precipitated crystals were collected by filtration and recrystallized from an appropriate solvent to afford the corre-

Table 6 IR and ^{1}H NMR spectroscopic data for the compounds **12a**–**s**

Compound	ν_{max}(Nujol)/cm^{-1}	δ_H [200 MHz; $(CD_3)_2SO$; Me_4Si]
12a	3120, 3030 (NH); 1740 (C=O)	8.34 (1 H, s, 9-H), 8.62 (1 H, s, 3-H), 12.58 (1 H, br s, 6-NH), 13.60 (1 H, br s, 7-NH)
12b	3110, 3050 (NH); 1700 (C=O)	2.40 (3 H, s, CH_3), 8.54 (1 H, s, 9-H), 12.46 (1 H, br s, 6-NH), 13.57 (1 H, br s, 7-NH)
12c	3100, 3060 (NH); 1700 (C=O)	1.29 (3 H, t, *J* 7.6, CH_2CH_3), 2.77 (2 H, q, *J* 7.6, CH_2CH_3), 8.57 (1 H, s, 9-H), 12.43 (1 H, br s, 6-NH), 13.55 (1 H, br s, 7-NH)
12d	3090, 3060 (NH) 1700 (C=O)	0.92 (3 H, t, *J* 7.6, $CH_2CH_2CH_2CH_3$), 1.36 (2 H, sextet, *J* 7.6, $CH_2CH_2CH_2CH_3$), 1.72 (2 H, quintet, *J* 7.6, $CH_2CH_2CH_2CH_3$), 2.74 (2 H, t, *J* 7.6, $CH_2CH_2CH_2CH_3$), 8.57 (1 H, s, 9-H), 12.44 (1 H, br s, 6-NH), 13.55 (1 H, br s, 7-NH)
12e[a]	3110, 3070 (NH); 1700 (C=O)	0.86 (3 H, t, *J* 6.5, $CH_2CH_2[CH_2]_4CH_3$), 1.30 (8 H, br s, $CH_2CH_2[CH_2]_4CH_3$), 1.50–1.80 (2 H, m, $CH_2CH_2[CH_2]_4CH_3$), 2.50–2.95 (2 H, m, $CH_2CH_2[CH_2]_4CH_3$), 8.53 (1 H, s, 9-H), 12.41 (1 H, br s, 6-NH), 13.50 (1 H, br, 7-NH)
12f	3150, 3070 (NH); 1710 (C=O)	1.28 (10 H, br s, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 1.62–1.80 (2 H, m, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 1.92–2.06 (2 H, m, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 2.72 (2 H, t, *J* 7.3, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 4.87–5.04 (2 H, m, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 5.66–5.89 (1 H, m, $CH_2CH_2[CH_2]_5CH_2CH=CH_2$), 8.57 (1 H, s, 9-H), 12.43 (1 H, br s, 6-NH), 13.55 (1 H, br s, 7-NH)
12g[a]	3150, 3050 (NH); 1720 (C=O)	7.40–7.70 (3 H, m, Ph-*m*,*p*H), 7.90–8.35 (2 H, m, Ph-*o*H), 8.68 (1 H, s, 9-H), 12.60 (1 H, br s, 6-NH), 13.60 (1 H, br, 7-NH)
12h[a]	3110, 3090 (NH); 1710 (C=O)	7.37 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.1, Ar-*m*H), 8.22 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.9, Ar-*o*H), 8.66 (1 H, s, 9-H), 12.59 (1 H, br s, 6-NH), 13.65 (1 H, br, 7-NH)
12i	3150, 3070 (NH); 1710 (C=O)	7.44–7.78 (3 H, m, 3′-H, 4′-H and 5′-H), 7.95–8.09 (1 H, m, 6′-H), 8.69 (1 H, s, 9-H), 12.67 (1 H, br s, 6-NH), 13.58 (1 H, br, 7-NH)
12j[a]	3150, 3100 (NH); 1720 (C=O)	7.54–7.63 (2 H, m, 4′-H and 5′-H), 7.95–8.25 (2 H, m, 2′-H and 6′-H), 8.67 (1 H, s, 9-H), 12.62 (1 H, br s, 6-NH), 13.60 (1 H, br s, 7-NH)
12k	3160, 3100 (NH); 1700 (C=O)	7.62 (2 H, d, *J* 8.6, Ar-*m*H), 8.17 (2 H, d, *J* 8.6, Ar-*o*H), 8.70 (1 H, s, 9-H), 12.62 (1 H, br s, 6-NH), 13.66 (1 H br s, 7-NH)
12l	3150, 3060 (NH); 1720 (C=O)	7.76 (2 H, d, *J* 8.6, Ar-*m*H), 8.10 (2 H, d, *J* 8.6, Ar-*o*H), 8.69 (1 H, s, 9-H), 12.62 (1 H, br s, 6-NH), 13.65 (1 H, br s, 7-NH)
12m	3180, 3100 (NH); 1720 (C=O)	2.39 (3 H, s, CH_3), 7.35 (2 H, d, *J* 8.0, Ar-*m*H), 8.05 (2 H, d, *J* 8.0, Ar-*o*H), 8.66 (1 H, s, 9-H), 12.54 (1 H, br s, 6-NH), 13.61 (1 H, br s, 7-NH)
12n[a]	3160, 3100 (NH); 1730 (C=O)	3.85 (3 H, s, OCH_3), 7.10 (2 H, d, *J* 8.8, Ar-*m*H), 8.12 (2 H, d, *J* 8.8, Ar-*o*H), 8.64 (1 H, s, 9-H), 12.60 (1 H, br s, 6-NH), 13.50 (1 H, br, 7-NH)
12o	3160, 3050 (NH); 1720 (C=O)	6.13 (2 H, s, OCH_2O), 7.08 (1 H, d, $J_{5',6'}$ 8.1, 5′-H), 7.58 (1 H, d, $J_{2',6'}$ 1.6, 2′-H), 7.72 (1 H, d, $J_{5',6'}$ 8.1, $J_{2',6'}$ 1.6, 6′-H), 8.68 (1 H, s, 9-H), 12.55 (1 H, br s, 6-NH), 13.62 (1 H, br s, 7-NH)
12p[a]	3160, 3090 (NH); 1700, 1660 (C=O)	8.10 (2 H, d, *J* 8.8, Ar-*m*H), 8.32 (2 H, d, *J* 8.8, Ar-*o*H), 8.69 (1 H, s, 9-H), 12.70 (1 H, br s, 6-NH), 13.50 (2 H, br, 7-NH and COOH)
12q	3160, 3050 (NH); 1718 (C=O)	6.90 (2 H, d, *J* 8.8, Ar-*m*H), 7.99 (2 H, d, *J* 8.8, Ar-*o*H), 8.66 (1 H, s, 9-H), 9.94 (1 H, s, OH), 12.51 (1 H, br s, 6-NH), 13.60 (1 H, br, 7-NH)
12r[a]	3170, 3100 (NH); 1720 (C=O)	8.39 (4 H, br s, Ar-H), 8.69 (1 H, s, 9-H), 12.70 (1 H, br s, 6-NH), 13.60 (1 H, br 7-NH)
12s	3160, 3080 (NH); 1720 (C=O)	7.31 (1 H, d, $J_{5',6'}$ 8.8, 5′-H), 8.28 (1 H, dd, $J_{5',6'}$ 8.8. $J_{2',6'}$ 2.2, 6′-H), 8.60 (1 H, d, $J_{2',6'}$ 2.2, 2′-H), 8.70 (1 H, s, 9-H), 11.59 (1 H, s, OH), 12.62 (1 H, br s, 6-NH), 13.65 (1 H, br s, 7-NH)

[a] This compound was measured at 60 MHz.

sponding *pyrazolotriazolopyrimidines* **12a**, **b** as shown in Tables 5 and 6.

(4) *Method D*: A mixture of the hydrazinopyrazolopyrimidine **6** (0.60 g, 3.6 mmol) with an appropriate triethyl or trimethyl orthoester (10.8 mmol) in DMF (30–40 cm^3) was heated at 100 °C for 1 hour. After the reaction was complete, the solution was evaporated under reduced pressure and the residue was triturated with EtOH or AcOEt to give crystals, which were collected by filtration and recrystallized from an appropriate solvent to afford the corresponding *pyrazolotriazolopyrimidines* **12a**–**d** as shown in Tables 5 and 6.

(5) *Method E*: A mixture of an appropriate 4-alkylidenehydrazino- or 4-arylmethylidenehydrazino-6-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine **3a**–**g** (2.0 mmol) with 70% nitric acid (0.9 cm^3, 10.0 mmol) in DMF (30–50 cm^3) was heated at 100 °C for 1–5 hours. After the reaction was complete, the precipitated crystals were collected by filtration and further crystals were obtained by concentration of the filtrate under reduced pressure. The combined crystals were recrystallized from an appropriate solvent to afford the corresponding *pyrazolotriazolopyrimidines* **12e**, **g**, **h**, **k**, **m**, **n**, **r** as shown in Tables 5 and 6.

Xanthine oxidase assay

All test compounds and allopurinol were dissolved in dimethyl sulfoxide (DMSO) and diluted with 50 mM sodium phosphate buffer (pH 7.4) for *in vitro* experiments. The final concentration of DMSO in the reaction solution was 0.1%.

Bovine milk xanthine oxidase (XO) (10 mU ml^{-1}) was incubated with 100 μM xanthine in the presence and absence of the test compound (0.003–10 μM) at 25 °C for 15 min. Uric acid formation was determined by absorbance at 292 nm using a Hitachi 228-A spectrophotometer, and the inhibition rate (%) for the formation of uric acid and IC_{50} values of the test compounds were determined. The inhibition rate (*I*) of the test compound at each concentration was calculated by eqn. (1),

$$I\,(\%) = 100 - [(D - D_B)/T] \times 100 \qquad (1)$$

where *T* is the optical density of a solution of xanthine and XO, *D* is the optical density of a solution of test compound, xanthine and XO and D_B is the optical density of a solution of test compound and XO.

The inhibitory activity of allopurinol against bovine milk xanthine oxidase was also examined as a positive control. Each experiment was repeated at least twice at different concentrations (0.003–10 μM). The values of IC_{50}, *i.e.* the μM concentration of inhibitor necessary for 50% inhibition, were determined from the dose–response curve from the relation of the logarithmic concentration (μM) and the inhibition (%).

Table 7 Inhibitory activities of the compounds **2**, **3**, **4**, **6**, **8**, **11** and **12** against bovine milk xanthine oxidase in comparison with allopurinol

Compound No.	Inhibition (%)						IC_{50}/μM
	10	3	1	0.3	0.1	0.03	
2	21.4	11.8	8.3				>10
3b	7.6						>10
3c	21.0	16.0	11.5				>10
3d	12.0	9.5	9.4				>10
3e	14.2	13.8	10.8				>10
3f	41.0						>10
4[a]	39.8						22.1
6	24.1						>10
8b	7.2						>10
8c	16.3						>10
8d	12.4						>10
8f	17.2						>10
11b	58.6	45.0	34.8	15.8	6.9	6.2	4.670
11e	71.7	69.2	60.6	44.6	25.8	13.3	0.450
11f	53.3	36.5	22.4	13.0	9.7	4.6	7.894
11g	75.6	73.0	66.3	49.8	29.9		0.305
11h	68.5	69.9	63.6	47.0	29.7		0.373
11i	68.9	69.6	68.5	64.4	53.8	36.3	0.077
11j	67.6	66.4	63.4	54.2	38.7	20.7	0.223
11k	66.1	65.6	62.9	53.8	39.5	22.7	0.224
11l[b]	45.1	44.2	42.1	49.6	40.1	23.9	>10
11m	68.9	66.5	63.8	52.8	37.0	18.6	0.247
11n	74.5	73.3	70.0	59.2	41.0		0.172
11o	68.4	65.9	61.6	47.0	29.0	14.1	0.385
11p	68.1	65.2	60.3	46.8	29.3	14.8	0.399
11q	62.8	66.2	60.3	48.2	40.3	16.7	0.359
11r	57.3	52.1	46.9	39.5	28.7	14.0	1.925
12a	69.2	67.5	65.2	56.6	41.8	23.9	0.184
12b	71.5	68.8	65.1	52.5	37.3	20.7	0.250
12c[c]	57.0	55.2	52.0	42.2	28.8	17.8	0.782
12d[d]	57.6	55.1	53.0	46.1	34.9	18.9	0.529
12e	69.8	69.1	67.9	62.8	54.4	40.2	0.069
12f	66.8	65.0	63.4	59.6	48.4	32.0	0.117
12g	67.8	65.3	64.2	59.8	49.7	32.4	0.103
12h	69.5	68.8	67.7	64.6	56.7	39.6	0.062
12i	68.5	68.6	67.1	63.5	54.8	38.9	0.070
12j	68.9	69.6	68.8	66.7	61.3	47.4	0.038
12k[e]	72.3	70.6	70.3	68.3	62.9	49.3	0.032
12l[f]	70.1	66.4	67.4	63.9	60.4	48.9	0.034
12m[g]	72.2	70.7	70.0	67.0	60.9	46.0	0.041
12n	71.6	71.0	70.4	66.9	58.6	42.3	0.053
12o	70.2	69.6	68.6	66.6	61.0	46.3	0.041
12p	69.0	67.1	65.7	60.6	50.2	34.3	0.098
12q[h]	67.6	66.0	65.4	62.9	57.0	42.9	0.055
12r	69.8	68.2	68.7	64.4	57.5	39.6	0.060
12s	69.1	69.7	69.0	67.7	64.0	46.9	0.037
Allo[i]	38.2	19.9	9.9	4.6	3.2		24.3

[a] 30 μM: 53.9%, 100 μM: 63.9%. [b] This value is inaccurate because of insolubility in DMSO. [c] 0.01 μM: 12.6%. [d] 0.01 μM: 9.6%. [e] 0.01 μM: 30.4%, 0.003 μM, 16.4%. [f] 0.01 μM: 37.3%. [g] 0.01 μM: 28.2%, 0.003 μM: 13.6%. [h] 0.01 μM: 29.0%. [i] Allo: allopurinol.

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References

1 Part 2, T. Nagamatsu, H. Yamasaki, T. Fujita, K. Endo and H. Machida, *J. Chem. Soc.*, *Perkin Trans. 1*, 1999, 3117.
2 R. W. Rundles, J. B. Wyngaarden, G. H. Hitchings, G. B. Elion and H. R. Silberman, *Trans. Assoc. Am. Physicians*, 1963, **76**, 126.
3 T. F. Yü and A. B. Gutman, *Am. J. Med.*, 1964, **37**, 885.
4 J. R. Klinenberg, S. E. Goldfinger and J. E. Seegmiller, *Ann. Intern. Med.*, 1965, **62**, 639.
5 G. B. Elion, *Ann. Rheumat. Dis.*, 1966, **25**, 608.
6 G. B. Elion, S. Callahan, H. Nathan, S. Bieber, R. W. Rundles and G. H. Hitchings, *Biochem. Pharmacol.*, 1963, **12**, 85.
7 J. L. Young, R. B. Boswell and A. S. Nies, *Arch. Intern Med.*, 1974, **134**, 553.
8 K. R. Hande, R. M. Noone and W. J. Stone, *Am. J. Med.*, 1984, **76**, 47.
9 D. E. Duggan, R. M. Noll, J. E. Baer, F. C. Novello and J. J. Baldwin, *J. Med. Chem.*, 1975, **18**, 900.
10 R. L. Wortmann, A. S. Ridolfo, R. W. Lightfoot Jr. and I. H. Fox, *J. Rheumatol.*, 1985, **12**, 540.
11 A. Bindoli, M. Valente and L. Cavallini, *Pharmacol. Res. Commun.*, 1985, **17**, 831.
12 T. Spector, W. W. Hall, D. J. Porter, C. U. Lambe, D. J. Nelson and T. A. Krenitsky, *Biochem. Pharmacol.*, 1989, **38**, 4315.
13 S. Sato, K. Tatsumi and T. Takahashi, *Purine and Pyrimidine Metabolism in Man VII*, *Part A: Chemotherapy*, *ATP Depletion and Gout*, eds. R. A. Harkness, G. B. Elion and N. Zöllner, Plenum Press, New York, 1991, p. 135.
14 Y. Osada, M. Tsuchimoto, H. Fukushima, K. Takahashi, S. Kondo, M. Hasegawa and K. Komoriya, *Eur. J. Pharmacol.*, 1993, **241**, 183.
15 G. Biagi, I. Giorgi, O. Livi, V. Scartoni, I. Tonetti and L. Costantino, *Farmaco*, 1995, **50**, 257.
16 T. Nagamatsu, Y. Watanabe, K. Endo and M. Imaizumi, *PCT Int. Appl. WO* 96 26,208/1996 (*Chem. Abstr.*, 1996, **125**, 247848j).
17 T. Nagamatsu, T. Abiru, Y. Watanabe and K. Endo, *Jpn. Kokai Tokkyo Koho JP* 07,242,694/1995 (*Chem. Abstr.*, 1996, **124**, 117896s).

18 T. Nagamatsu and T. Fujita, *Chem. Commun.*, 1999, 1461.
19 G. A. Bhat and L. B. Townsend, *J. Chem. Soc.*, *Perkin Trans. 1*, 1981, 2387.
20 F. Gatta, M. Luciani and G. Palazzo, *J. Heterocycl. Chem.*, 1989, **26**, 613.
21 U. D. Treuner and H. Breuer, *USP* 4 053 474, 1977 (*Chem. Abstr.*, 1977, **88**, 37826s), *USP* 4 124 764, 1978 (*Chem. Abstr.*, 1978, **90**, 87508b) *Ger. Offen.* 2 838 029, 1979 (*Chem. Abstr.*, 1979, **91**, 39492r).
22 F. Yoneda, Y. Sakuma, S. Mizumoto and R. Ito, *J. Chem. Soc.*, *Perkin Trans. 1*, 1976, 1805.
23 R. K. Robins, *J. Am. Chem. Soc.*, 1956, **78**,784.

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