Novel xanthine oxidase inhibitor studies. Part 2.[1] Synthesis and xanthine oxidase inhibitory activities of 2-substituted 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines and 3- and/or 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines

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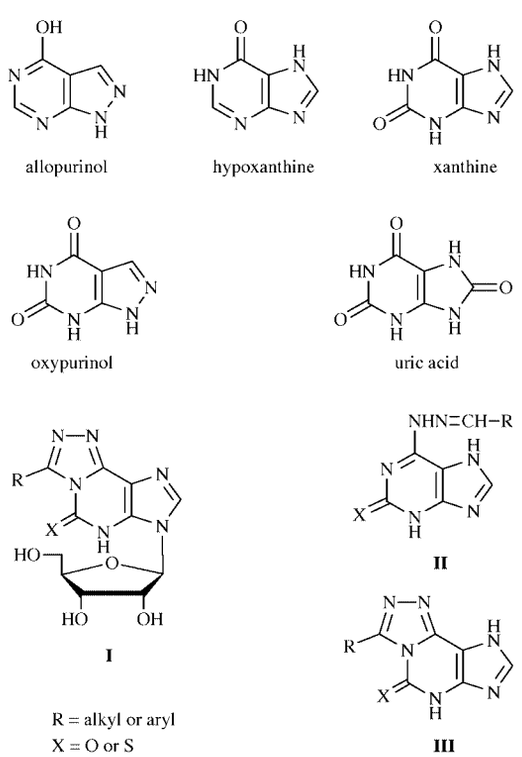
The facile and general synthesis of 2-substituted 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines and 3- and/or 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines, which were obtained by oxidative cyclisation of the corresponding 6-aldehyde hydrazones of 7*H*-purine, as a new class of potential xanthine oxidase inhibitors are reported. Their inhibitory activities against bovine milk xanthine oxidase *in vitro* were also investigated, and some purines **2** and **6** and triazolopurines **7** exhibited from several times to several hundred times more potent activities than allopurinol.

Introduction

Allopurinol (4-hydroxy-1*H*-pyrazolo[3,4-*d*]pyrimidine), a structural analogue of hypoxanthine, is both a substrate for and a potent inhibitor of xanthine oxidase (XO), which catalyzes the conversion of hypoxanthine and xanthine to uric acid.[2] The product of the enzymatic oxidation of allopurinol is oxypurinol which is the xanthine analogue. By thus inhibiting the formation of uric acid, allopurinol has been used widely for the clinical control of uric acid production in gout and hyperuricemia.[3–5] However, severe allopurinol toxicity[6] and a life-threatening toxicity syndrome have been reported after its use, which includes vasculitis, rash, eosinophilia, hepatitis and progressive renal failure.[7] Although xanthine oxidase/xanthine dehydrogenase inhibitory activity has recently been discovered in some newly synthesized compounds and previously known compounds,[8–14] no clinically effective XO inhibitors for the treatment of hyperuricemia have been developed since allopurinol was introduced for clinical use in 1963.[15] During the course of our work on the synthesis[1,16,17] and biological evaluation[18–20] of novel fused pyrimidines and purines, we initiated investigations aiming at designing new XO inhibitors. Among the fused purines prepared, the angular type purine analogues, 7β-D-ribofuranosyl-7*H*-1,2,4-triazolo[3,4-*i*]purines (I), have recently been investigated in our laboratory for their potential XO inhibitory activities.[21] Later we found that the 6-aldehyde hydrazones of 7*H*-purine (II) generally showed more potent bovine milk XO inhibitory activities than those of 9*H*-1,2,4-triazolo[3,4-*i*]purines (III)[22] (Scheme 1). We report here a facile and general synthesis of 2-substituted 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines and 3- and/or 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines as a new class of potential XO inhibitors and their bovine milk XO inhibitory activities.

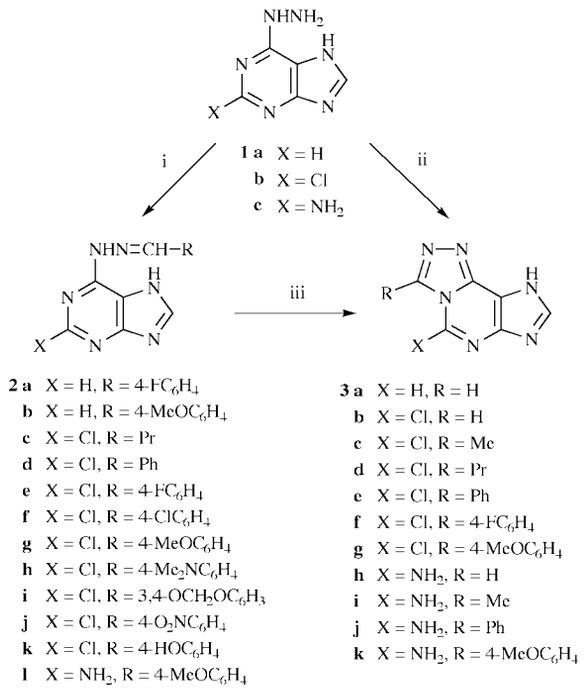


Scheme 1

Results and discussion

As we reported in preliminary work described in a patent,[22] most 2-substituted 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines (II) showed more potent bovine milk XO inhibitory activities than those of 9*H*-1,2,4-triazolo[3,4-*i*]purines (III), which were obtained by oxidative cyclisation of II, and allopurinol. Therefore, in the first place we tried to synthesise various 7*H*-purine derivatives possessing a 6-alkylidenehydrazino or 6-arylmethylidenehydrazino group at the 6-position as the substituent in order to explore the XO inhibitory activity. The requisite starting materials, 6-hydrazino-7*H*-purines **1a**–**c**, were prepared by the reaction of

their 6-chloro- or 6-thio-derivatives with excess hydrazine hydrate according to the literature procedure.[23] Treatment of the 6-hydrazino derivatives **1a**–**c** thus obtained with an appropriate alkylaldehyde or arylaldehyde in ethanol, 1,4-dioxane or glacial acetic acid at room temperature afforded the corresponding 6-aldehyde hydrazones **2a**–**l** in 70–90% yields as indicated in Scheme 2 and Tables 1 and 2. All new compounds

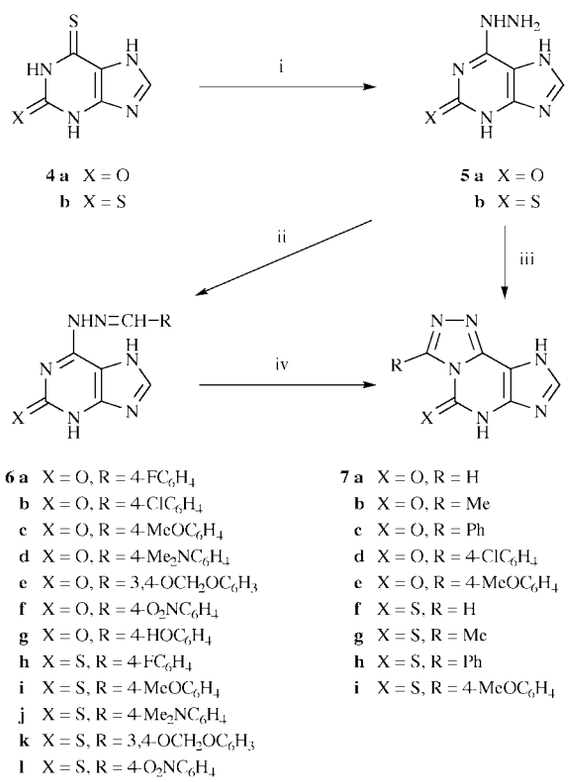


Scheme 2 *Reagents and conditions*: i, R-CHO, EtOH, 1,4-dioxane or AcOH, room temp., 3–10 h; ii, $RC(OEt)_3$, TFA, room temp. or DMF, 150–160 °C, 3–5 h; iii, $Pb(OAc)_4$, 1,4-dioxane, room temp. or AcOH, 120 °C, 2–5 h.

2a–**l** exhibited satisfactory elemental combustion analyses and FAB-MS, IR and ^{1}H NMR spectral data consistent with the structures.

Moreover, we have also elucidated that some 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines (III), especially the 5-oxo or 5-thioxo derivatives, showed more potent bovine milk XO inhibitory activities than that of allopurinol.[22] Although three reports[24–26] for the synthetic approach to the 9*H*-1,2,4-triazolo[3,4-*i*]purine ring system have hitherto appeared in the literature in addition to our previous work,[1] only several derivatives *i.e.* 5-oxo derivatives[26] were prepared. We wish to present now the details of the facile and general synthesis of 3- and/or 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines. Thus, heating 6-hydrazino-7*H*-purine **1a** with excess triethyl orthoformate under reflux gave the parent ring system, 9*H*-1,2,4-triazolo[3,4-*i*]purine **3a**,[24] in 70% yield. Similarly the 5-chlorotriazolopurines **3b**–**d** were prepared by the reaction of 2-chloro-6-hydrazino-7*H*-purine **1b** with appropriate triethyl orthoesters (40 parts) in trifluoroacetic acid (5 parts) at room temperature in moderate yields. Treatment of 6-arylmethylidenehydrazino-2-chloro-7*H*-purines **2d**,**e**,**g** with lead tetraacetate (1.5 equiv.) in 1,4-dioxane at room temperature yielded the corresponding 3-aryl derivatives **3e**–**g** owing to oxidative cyclization of **2d**,**e**,**g** in moderate yields. In addition, the 5-aminotriazolopurines **3h**–**k** were synthesised by heating 2-amino-6-hydrazino-7*H*-purine **1c** with an appropriate triethyl orthoester (40–60 parts) in DMF at 150–160 °C or treatment of its aldehydehydrazone **2l** with lead tetraacetate (1.5 equiv.) in glacial acetic acid (50 parts) at 120 °C in moderate yields. All new compounds **3b**–**k** exhibited satisfactory elemental combustion analyses and spectral data consistent with structures indicated.

Xanthine oxidase has been known to oxidise hypoxanthine and xanthine to uric acid. The inhibition of xanthine oxidase may serve to decrease the production of uric acid and prevent the formation of superoxide radical, which is generated as a by-product as the reduced enzyme is reoxidized by oxygen.[27] It was clarified that oxypurinol might be superior to allopurinol as an inhibitor of the xanthine oxidase-catalyzed production of superoxide radical[27] and is complexed very tightly with partially reduced xanthine oxidase in which the molybdenum was in the Mo(IV) state.[28,29] Oxypurinol {1*H*-pyrazolo[3,4-*d*]-pyrimidine-4,6(5*H*,7*H*)-dione} is a structural isomer of xanthine {7*H*-purine-2,6(1*H*,3*H*)-dione} and both possess two oxo groups in a similar position on the rings, while allopurinol possesses only one oxo group at the 4-position (see Scheme 1). Hence, it was assumed that the presence of an oxo group at the 6-position on the pyrazolopyrimidine ring was important for the formation of the complex between the reduced xanthine oxidase and allopurinol. That is to say, in order to investigate the XO inhibitory activities of the 2-oxo derivatives of 7*H*-purine (equivalent to the 6-oxo derivative of pyrazolopyrimidine), several 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purin-2(3*H*)-ones **6a**–**g** were prepared as indicated in Scheme 3. Indeed, the 2-oxopurines **6a**–**g** exhibited



Scheme 3 *Reagents and conditions*: i, aq. NH_2NH_2 (80%), reflux, 10 min; ii, R-CHO, AcOH, room temp., 4 h; iii, $RC(OEt)_3$, DMF, 150–160 °C, 5 h; iv, $Pb(OAc)_4$, 1,4-dioxane, reflux, 4 h.

more potent inhibitory activities than those of the purines **2a**–**l** without the 2-oxo group, as described below. In analogy with the 2-oxo derivatives, the 2-thioxopurines **6h**–**l** were also prepared. Thus the starting key compounds **4a**,**b** were obtained by thiation of xanthine according to the previously reported procedures.[30,31] Then, treatment of the 2-oxo-6-thioxo- **4a** and 2,6-dithioxo-purine **4b** with 80% hydrazine hydrate under reflux afforded the corresponding 6-hydrazino derivative **5a** and **5b** in *ca.* 60% yields. The subsequent treatment of **5a** and **5b** thus obtained with appropriate aldehydes (1.5 equiv.) in glacial acetic acid at room temperature afforded the corresponding 2-oxo- **6a**–**g** and 2-thioxo-6-aldehydehydrazones **6h**–**l** in good yields as given in Tables 3 and 4. On the other hand, heating the

Table 1 Preparative, physical and analytical data for compounds **2a**–**l** and **3a**–**k**

Compound (Formula)	Reaction conditions			Yield (%)	Mp/°C	Recrystn. solvent[a]	Found (%) (Required)			*m/z* MH^+
	Solvent	*T*/°C	Time/h				C	H	N	
2a $C_{12}H_9FN_6$	EtOH	r.t.	7	76	>280 (Decomp.)	EtOH–DMF	56.4 (56.25)	3.4 (3.5)	32.6 (32.8)	257
2b $C_{13}H_{12}N_6O$	EtOH	r.t.	7	78	>261 (Decomp.)	EtOH–DMF	58.4 (58.2)	4.5 (4.5)	31.1 (31.3)	269
2c $C_9H_{11}ClN_6$	Dioxane	r.t.	10	70	>300	EtOH	45.0 (45.3)	4.8 (4.65)	35.1 (35.2)	239/241
2d $C_{12}H_9ClN_6$	Dioxane	r.t.	5	86	>300	DMF	52.75 (52.85)	3.5 (3.3)	30.85 (30.8)	273/275
2e $C_{12}H_8ClFN_6$	Dioxane	r.t.	5	90	>300	DMF	49.4 (49.6)	3.1 (2.8)	28.8 (28.9)	291/293
2f $C_{12}H_8Cl_2N_6$	Dioxane	r.t.	5	69	>300	DMF	46.85 (46.9)	2.75 (2.6)	27.4 (27.4)	307/309
2g $C_{13}H_{11}ClN_6O$	Dioxane	r.t.	4	89	>300	DMF	51.7 (51.6)	3.7 (3.7)	27.9 (27.8)	303/305
2h $C_{14}H_{14}ClN_7$	Dioxane	r.t.	3	79	>300	DMF	53.0 (53.25)	4.5 (4.5)	30.8 (31.05)	316/318
2i $C_{13}H_9ClN_6O_2$	Dioxane	r.t.	5	81	>300	DMF	49.3 (49.3)	2.9 (2.9)	26.3 (26.5)	317/319
2j $C_{12}H_8ClN_7O_2$	Dioxane	r.t.	5	88	>300	DMF	45.5 (45.4)	2.2 (2.5)	30.8 (30.9)	318/320
2k $C_{12}H_9ClN_6O$	Dioxane	r.t.	5	87	>300	DMF	49.7 (49.9)	3.2 (3.1)	28.9 (29.1)	289/291
2l $C_{13}H_{13}N_7O{\cdot}H_2O$	AcOH	r.t.	4	66	>300	DMF	52.0 (51.8)	4.9 (5.0)	32.7 (32.5)	284
3a[b] $C_6H_4N_6$	None	Reflux	9	70	>300	DMF	45.2 (45.0)	2.7 (2.5)	52.3 (52.5)	161
3b $C_6H_3ClN_6$	TFA	r.t.	3	48	>300	Water	37.2 (37.0)	1.8 (1.55)	43.0 (43.2)	195/197
3c $C_7H_5ClN_6$	TFA	r.t.	3	44	>300	Water	40.2 (40.3)	2.1 (2.4)	40.4 (40.3)	209/211
3d $C_9H_9ClN_6{\cdot}H_2O$	TFA	r.t.	5	67	>300	Water	42.7 (42.4)	4.3 (4.35)	33.1 (33.0)	237/239
3e $C_{12}H_7ClN_6$	Dioxane	r.t.	2	54	>300	Water	53.3 (53.25)	2.85 (2.6)	30.8 (31.05)	271/273
3f $C_{12}H_6ClFN_6$	Dioxane	r.t.	2	58	>300	Water	49.9 (49.9)	2.0 (2.1)	29.2 (29.1)	289/291
3g $C_{13}H_9ClN_6O$	Dioxane	r.t.	2	62	>300	Water	52.0 (51.9)	3.25 (3.0)	27.8 (27.95)	301/303
3h $C_6H_5N_7$	DMF	150	5	61	280 (Decomp.)	DMF	41.35 (41.1)	3.0 (2.9)	55.9 (56.0)	176
3i $C_7H_7N_7$	DMF	160	5	66	274 (Decomp.)	DMF	44.65 (44.4)	3.9 (3.7)	51.7 (51.8)	190
3j $C_{12}H_9N_7$	DMF	160	5	60	>300	DMF	57.1 (57.4)	3.4 (3.6)	39.2 (39.0)	252
3k $C_{13}H_{11}N_7O$	AcOH	120	5	46	225 (Decomp.)	Water	55.5 (55.5)	3.7 (3.9)	34.9 (34.9)	282

[a] All compounds were obtained as colourless powdery crystals except for **2h** (orange) and **2j** (yellow). [b] Ref. 24 (lit., mp > 264 °C).

Table 2 IR and ^{1}H NMR spectroscopic data for the compounds **2a**–**l** and **3a**–**k**

Compound	ν_{max} (KBr)/cm^{-1}	δ_H [60 MHz; $(CD_3)_2SO$; Me_4Si]
2a	3370, 3160 (NH)	7.32 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 9.1, Ar-*m*H), 8.06 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.5, Ar-*o*H), 8.46 (1 H, s, N=C*H*Ar), 8.55 (2 H, s, 2- and 8-H), 12.50 (2 H, br s, 2 × NH)
2b	3380, 3210 (NH)	3.85 (3 H, s, OMe), 7.05 (2 H, d, *J* 8.8, Ar-*m*H), 8.00 (2 H, d, *J* 8.8, Ar-*o*H), 8.52 (1 H, s, N=C*H*Ar), 8.63 (1 H, s, 2-H), 8.65 (1 H, s, 8-H), 12.90 (2 H, br s, 2 × NH)
2c	3410, 3180 (NH)	0.95 (3 H, t, *J* 6.8, $CHCH_2CH_2CH_3$), 1.26–1.84 (2 H, m, $CHCH_2CH_2CH_3$), 2.25–2.57 (2 H, m, $CHCH_2$-CH_2CH_3), 7.51 (1 H, br t, *J* 6.2, $CHCH_2CH_2CH_3$), 8.34 (1 H, s, 8-H), 11.74 (1 H, s, NH), 12.24 (1 H, br s, NH)
2d	3400, 3200 (NH)	7.33–7.66 (3 H, m, Ph-*m*,*p*H), 7.73–8.13 (2 H, m, Ph-*o*H), 8.27 (1 H, s, N=C*H*Ph), 8.43 (1 H, s, 8-H), 12.15 (2 H, br s, 2 × NH)
2e	3450, 3200 (NH)	7.31 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 8.8, Ar-*m*H), 7.99 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.3, Ar-*o*H), 8.25 (1 H, s, N=C*H*Ar), 8.42 (1 H, s, 8-H), 12.13 (2 H, br s, 2 × NH)
2f	3350, 3200 (NH)	7.51 (2 H, d, *J* 8.8, Ar-*m*H), 7.94 (2 H, d, *J* 8.8, Ar-*o*H), 8.25 (1 H, s, N=C*H*Ar), 8.42 (1 H, s, 8-H), 12.16 (2 H, br s, 2 × NH)
2g	3420, 3200 (NH)	3.83 (3 H, s, OMe), 7.03 (2 H, d, *J* 8.8, Ar-*m*H), 7.87 (2 H, d, *J* 8.8, Ar-*o*H), 8.20 (1 H, s, N=C*H*Ar), 8.38 (1 H, s, 8-H), 12.00 (2 H, s, 2 × NH)
2h	3350, 3200 (NH)	3.00 (6 H, s, NMe_2), 6.75 (2 H, d, *J* 8.8, Ar-*m*H), 7.73 (2 H, d, *J* 8.8, Ar-*o*H), 8.13 (1 H, s, N=C*H*Ar), 8.35 (1 H, s, 8-H), 11.83 (2 H, s, 2 × NH)
2i	3350, 3200 (NH)	6.12 (2 H, s, OCH_2O), 6.97 (1 H, d, $J_{5',6'}$ 8.2, 5′-H), 7.21 (1 H, dd, $J_{5',6'}$ 8.2, $J_{2',6'}$ 1.8, 6′-H), 7.80 (1 H, d, $J_{2',6'}$ 1.8, 2′-H), 8.15 (1 H, s, N=C*H*Ar), 8.39 (1 H, s, 8-H), 12.01 (2 H, s, 2 × NH)
2j[a]	3410, 3200 (NH)	8.07 (2 H, d, *J* 8.8, Ar-*o*H), 8.46 (2 H, d, *J* 8.8, Ar-*m*H), 8.54 (1 H, s, N=C*H*Ar), 9.45 (1 H, s, 8-H)
2k	3410, 3190 (NH)	6.87 (2 H, d, *J* 8.2, Ar-*m*H), 7.76 (1 H, d, *J* 8.2, Ar-*o*H), 8.16 (1 H, s, N=C*H*Ar), 8.37 (1 H, s, 8-H), 9.89 (1 H, s, OH), 11.90 (2 H, s, 2 × NH)
2l	3370, 3320, 3190 (NH)	3.82 (3 H, s, OMe), 5.86 (2 H, br s, NH_2), 7.00 (2 H, d, *J* 8.8, Ar-*m*H), 7.75 (2 H, d, *J* 8.8, Ar-*o*H), 7.90 (1 H, s, N=C*H*Ar), 8.22 (1 H, s, 8-H), 11.30 (2 H, br s, 2 × NH)
3a	3060 (NH)	8.36 (1 H, s, 8-H), 9.27 (1 H, s, 5-H), 9.41 (1 H, s, 3-H), 13.94 (1 H, br s, NH)
3b	3040 (NH)	8.39 (1 H, s, 8-H), 9.51 (1 H, s, 3-H), 12.21 (1 H, br s, NH)
3c	3070 (NH)	2.97 (3 H, s, Me), 8.33 (1 H, s, 8-H), 12.00 (1 H, br s, NH)
3d	3070 (NH)	1.05 (3 H, t, *J* 7.3, $CH_2CH_2CH_3$), 1.72–2.10 (2 H, m, $CH_2CH_2CH_3$), 3.37 (2 H, t, *J* 7.1, $CH_2CH_2CH_3$), 8.32 (1 H, s, 8-H), 12.30 (1 H, br s, NH)
3e	3020 (NH)	7.50–7.76 (5 H, m, Ph-H), 8.41 (1 H, s, 8-H), 12.13 (1 H, br s, NH)
3f	3030 (NH)	7.39 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 8.8, Ar-*m*H), 7.79 (2 H, dd, $J_{H,H}$ 8.8, $J_{H,F}$ 5.3, Ar-*o*H), 8.42 (1 H, s, 8-H), 12.07 (1 H, br s, NH)
3g	3050 (NH)	3.86 (3 H, s, OMe), 7.09 (2 H, d, *J* 8.8, Ar-*m*H), 7.63 (2 H, d, *J* 8.8, Ar-*o*H), 8.40 (1 H, s, 8-H), 12.02 (1 H, br s, NH)
3h[a]	3320, 3280, 3100 (NH)	8.94 (1 H, s, 8-H), 9.67 (1 H, s, 3-H)
3i[a]	3310, 3100, 3060 (NH)	2.64 (3 H, s, Me), 8.93 (1 H, s, 8-H)
3j[a]	3300, 3120, 3070 (NH)	7.63–7.87 (3 H, m, Ph-*m*,*p*H), 8.15–8.38 (2 H, m, Ph-*o*H), 8.94 (1 H, s, 8-H)
3k	3300, 3170, 3100 (NH)	3.86 (3 H, s, OMe), 7.12 (2 H, d, *J* 8.8, Ar-*m*H), 7.58 (2 H, br s, NH_2), 8.22 (2 H, d, *J* 8.8, Ar-*o*H), 8.47 (1 H, s, 8-H), 12.09 (1 H, br s, NH)

[a] In CF_3CO_2D.

6-hydrazino derivative **5a** and **5b** with appropriate triethyl orthoesters (40–60 parts) under reflux afforded the corresponding 5-oxo- **7a**–**c** and 5-thioxo-triazolopurines **7f**–**h** in *ca.* 60% yields. The intramolecular cyclisation of **6b**,**c**,**i** to the corresponding **7d**,**e**,**i** (*ca.* 60% yields) were also accomplished by oxidation using lead tetraacetate (1.5 equiv.) in 1,4-dioxane at 120 °C in a similar manner as above. All new compounds **6a**–**l** and **7a**–**i** were fully characterised by various spectral analyses and satisfactory elemental combustion analyses as given in Tables 3 and 4. It is noteworthy that the compounds **3b**–**k** and **7a**–**i** were reasonably stable in acid or alkali solution due to the substituents at the 5-position.

Xanthine oxidase inhibitory results

The novel purine derivatives (**2**, **6**) and triazolopurine derivatives (**3**, **7**) prepared in this study were tested as inhibitors of bovine milk xanthine oxidase by a similar assay method to that previously reported.[13] The inhibition (%) and IC_{50} (μM) values of tested compounds against bovine milk xanthine oxidase are listed in Table 5. The introduction of arylaldehyde hydrazones at the 6-position of 7*H*-purine markedly increased their activities as inhibitors of xanthine oxidase, being one or two orders of magnitude more active than allopurinol. That is, the values of IC_{50} for **2a** and **2b** are 0.528 and 0.236 μM, respectively, while allopurinol is 24.3 μM. Moreover, the derivatives substituted by a chloro or amino group at the 2-position, *i.e.* compounds **2c**–**l**, showed a tendency to decrease the activity, but most of the 6-arylmethylidenehydrazino derivatives **2d**–**i**,**k** with a chloro group showed more inhibitory properties than allopurinol. On the other hand, the triazolopurines **3a**,**b**,**e**,**g**–**k** gave poor activities.

Oxypurinol (4,6-dioxopyrazolopyrimidine), which is a structural isomer of xanthine (2,6-dioxopurine), forms very tightly a reversible complex with electronically reduced xanthine oxidase due to the 6-oxo group as compared to allopurinol (4-oxo derivative).[32] Therefore, in order to investigate whether the inhibitory activity is reinforced by incorporating an oxo group at the 2-position of the purine ring, we synthesized the 2-oxo derivatives **6a**–**g** with 6-arylmethylidenehydrazino groups. As we would expect, all of them showed remarkable potent inhibitory activities, being three orders of magnitude more active than allopurinol. Among them, compound **6b** (X = O, R = 4-ClC_6H_4) (IC_{50}: 0.025 μM) was the most active; it exhibited 970-fold more potent bovine milk XO inhibitory activity than that of allopurinol (IC_{50}: 24.30 μM). In the case of the 6-thioxopurines **6h**–**l**, the inhibitory activities were less effective than the 6-oxopurines **6a**–**g**, but still showed several ten to hundred times greater activities than that of allopurinol. In contrast to the above purine derivatives, the triazolopurines **7a**–**i** generally showed more potent inhibitory activities than that of allopurinol, but less inhibitory activities compared with the purines **6a**–**l**, and yet some of them showed greater activity, *e.g.* compound **7d** (X = O, R = 4-ClC_6H_4) showed 370-fold (IC_{50}: 0.066 μM) more potent inhibitory activity than allopurinol. It was demonstrated that the oxo or thioxo group at the 2-position and the alkyl or arylmethylidenehydrazino group at the 6-position of the purines and the oxo or thioxo group at

Table 3 Preparative, physical and analytical data for compounds **6a–l** and **7a–i**

Compound (Formula)	Reaction conditions			Yield (%)	Mp/°C	Recrystn. solvent[a]	Found (%) (Required)			*m/z* MH^+
	Solvent	*T*/°C	Time/h				C	H	N	
6a $C_{12}H_9FN_6O$	AcOH	r.t.	4	77	275 (Decomp.)	DMF	52.7 (52.9)	3.4 (3.3)	30.6 (30.9)	273
6b $C_{12}H_9ClN_6O$	AcOH	r.t.	4	72	299 (Decomp.)	DMF	50.0 (49.9)	3.3 (3.1)	29.15 (29.1)	289/291
6c $C_{13}H_{12}N_6O_2$	AcOH	r.t.	4	71	291 (Decomp.)	DMF	54.8 (54.9)	4.4 (4.25)	29.6 (29.6)	285
6d $C_{14}H_{15}N_7O$	AcOH	r.t.	4	69	284 (Decomp.)	DMF	56.7 (56.6)	5.3 (5.1)	32.8 (33.0)	298
6e $C_{13}H_{10}N_6O_3$	AcOH	r.t.	4	73	299 (Decomp.)	DMF	52.2 (52.35)	3.6 (3.4)	27.9 (28.2)	299
6f $C_{12}H_9N_7O_3$	AcOH	r.t.	4	70	>300	DMF	47.9 (48.2)	3.2 (3.0)	32.7 (32.8)	300
6g $C_{12}H_{10}N_6O_2$	AcOH	r.t.	4	72	299 (Decomp.)	DMF	53.6 (53.3)	4.0 (3.7)	31.0 (31.1)	271
6h $C_{12}H_9FN_6S$	AcOH	r.t.	4	61	286 (Decomp.)	DMF	49.8 (50.0)	3.3 (3.15)	29.3 (29.15)	289
6i $C_{13}H_{12}N_6OS$	AcOH	r.t.	4	60	268 (Decomp.)	DMF	51.9 (52.0)	3.75 (4.0)	28.0 (28.0)	301
6j $C_{14}H_{15}N_7S$	AcOH	r.t.	4	66	283 (Decomp.)	DMF	53.6 (53.7)	4.7 (4.8)	31.5 (31.3)	314
6k $C_{13}H_{10}N_6O_2S$	AcOH	r.t.	4	59	289 (Decomp.)	DMF	49.8 (49.7)	3.1 (3.2)	26.7 (26.7)	315
6l $C_{12}H_9N_7O_2S$	AcOH	r.t.	4	51	252 (Decomp.)	DMF	45.4 (45.7)	3.0 (2.9)	30.85 (31.1)	316
7a $C_6H_4N_6O$	DMF	150	5	61	262 (Decomp.)	DMF	40.9 (40.9)	2.3 (2.3)	47.8 (47.7)	177
7b $C_7H_6N_6O$	DMF	160	5	58	277 (Decomp.)	DMF	44.2 (44.2)	3.3 (3.2)	44.45 (44.2)	191
7c $C_{12}H_8N_6O$	DMF	160	5	57	>300	DMF	57.0 (57.1)	3.5 (3.2)	33.6 (33.3)	253
7d $C_{12}H_7ClN_6O$	Dioxane	120	4	56	285 (Decomp.)	Water	50.3 (50.3)	2.5 (2.5)	29.4 (29.3)	287/289
7e $C_{13}H_{10}N_6O_2$	Dioxane	120	4	56	288 (Decomp.)	Water	55.3 (55.3)	3.4 (3.6)	29.7 (29.8)	283
7f $C_6H_4N_6S$	DMF	150	5	63	271 (Decomp.)	DMF	37.4 (37.5)	2.0 (2.1)	43.8 (43.7)	193
7g $C_7H_6N_6S$	DMF	160	5	60	266 (Decomp.)	DMF	40.7 (40.8)	2.8 (2.9)	40.7 (40.75)	207
7h $C_{12}H_8N_6S$	DMF	160	5	59	251 (Decomp.)	DMF	53.5 (53.7)	3.0 (3.0)	31.1 (31.3)	269
7i $C_{13}H_{10}N_6OS$	Dioxane	120	4	60	281 (Decomp.)	DMF	52.5 (52.3)	3.4 (3.4)	28.4 (28.2)	299

[a] All compounds were obtained as colourless powdery crystals except for **6d**, **6f**, **6j** (yellow) and **6l** (pale orange).

Table 4 IR and ^{1}H NMR spectroscopic data for the compounds **6a**–**l** and **7a**–**i**

Compound	ν_{max} (KBr)/cm^{-1}	δ_H [60 MHz; $(CD_3)_2SO$; Me_4Si]
6a	3350, 3140, 3025 (NH); 1690 (C=O)	7.26 (2 H, dd, $J_{H,H}$ 8.5, $J_{H,F}$ 9.1, Ar-*m*H), 7.83 (1 H, s, N=C*H*Ar), 8.08 (2 H, dd, $J_{H,H}$ 8.5, $J_{H,F}$ 5.6, Ar-*o*H), 8.40 (1 H, s, 8-H), 10.15, 11.20 and 11.70 (each 1 H, each br s, 3 × NH)
6b[a]	3360, 3140, 3050 (NH); 1690 (C=O)	7.52 (2 H, d, *J* 8.5, Ar-*m*H), 8.07 (2 H, d, *J* 8.5, Ar-*o*H), 8.62 (1 H, s, N=C*H*Ar), 8.67 (1 H, s, 8-H)
6c	3380, 3140, 3040 (NH); 1685 (C=O)	3.82 (3 H, s, OMe), 6.99 (2 H, d, *J* 8.2, Ar-*m*H), 7.86 (1 H, s, N=C*H*Ar), 7.92 (2 H, d, *J* 8.2, Ar-*o*H), 8.35 (1 H, s, 8-H), 10.40–11.80 (2 H, br s, 3 × NH)
6d	3405, 3140, 3050 (NH); 1685 (C=O)	2.99 (6 H, s, NMe_2), 6.73 (2 H, d, *J* 8.2, Ar-*m*H), 7.77 (2 H, d, *J* 8.2, Ar-*o*H), 7.78 (1 H, s, N=C*H*Ar), 8.28 (1 H, s, 8-H), 9.80, 10.50 and 11.24 (each 1 H, each br s, 3 × NH)
6e	3360, 3180, 3100 (NH); 1680 (C=O)	6.08 (2 H, s, OCH_2O), 6.94 (1 H, d, $J_{5',6'}$ 7.6, 5′-H), 7.27 (1 H, dd, $J_{5',6'}$ 7.6, $J_{2',6'}$ 1.2, 6′-H), 7.83 (1 H, d, $J_{2',6'}$ 1.2, 2′-H), 8.00 (1 H, s, N=C*H*Ar), 8.30 (1 H, s, 8-H), 10.25, 11.10 and 11.88 (each 1 H, each br s, 3 × NH)
6f[a]	3450, 3150, 3075 (NH); 1690 (C=O)	8.42 (4 H, s, Ar-H), 8.65 (1 H, s, N=C*H*Ar), 8.79 (1 H, s, 8-H)
6g	3450 (OH and NH); 3100 (NH); 1640 (C=O)	6.82 (2 H, d, *J* 7.9, Ar-*m*H), 7.81 (1 H, s, N=C*H*Ar), 7.83 (2 H, d, *J* 7.9, Ar-*o*H), 8.32 (1 H, s, 8-H), 9.80–11.70 (3 H, br s, 3 × NH)
6h[a]	3350, 3130, 3020 (NH)	7.23 (2 H, dd, $J_{H,H}$ 8.5, $J_{H,F}$ 9.4, Ar-*m*H), 8.07 (2 H, dd, $J_{H,H}$ 8.5, $J_{H,F}$ 5.6, Ar-*o*H), 8.66 (2 H, s, N=C*H*Ar and 8-H)
6i[a]	3420, 3200, 3120 (NH)	4.05 (3 H, s, OMe), 7.16 (2 H, d, *J* 8.8, Ar-*m*H), 7.98 (2 H, d, *J* 8.8, Ar-*o*H), 8.60 (1 H, s, N=C*H*Ar), 8.69 (1 H, s, 8-H)
6j[a]	3340, 3100, 3040 (NH)	3.54 (6 H, s, NMe_2), 7.81 (2 H, d, *J* 8.8, Ar-*m*H), 8.32 (2 H, d, *J* 8.8, Ar-*o*H), 8.63 (1 H, s, N=C*H*Ar), 8.76 (1 H, s, 8-H)
6k[a]	3300, 3130, 3000 (NH)	6.12 (2 H, s, OCH_2O), 6.99 (1 H, d, $J_{5',6'}$ 7.3, 5′-H), 7.39 (1 H, dd, $J_{5',6'}$ 7.3, $J_{2',6'}$ 1.2, 6′-H), 7.60 (1 H, d, $J_{2',6'}$ 1.2, 2′-H), 8.55 (1 H, s, N=C*H*Ar), 8.65 (1 H, s, 8-H)
6l[a]	3430, 3150, 3075 (NH)	8.24 (2 H, d, *J* 8.8, Ar-*m*H), 8.47 (2 H, d, *J* 8.8, Ar-*o*H), 8.64 (1 H, s, N=C*H*Ar), 8.79 (1 H, s, 8-H)
7a	3120, 3060 (NH); 1710 (C=O)	8.10 (1 H, s, 8-H), 8.36 (1 H, s, 3-H), 12.20 (2 H, br s, 2 × NH)
7b[a]	3120, 3000 (NH); 1715 (C=O)	2.83 (3 H, s, Me), 8.98 (1 H, s, 8-H)
7c	3250, 3140 (NH); 1705 (C=O)	7.40–7.75 (3 H, m, Ph-*m*,*p*H), 8.00–8.35 (2 H, m, Ph-*o*H), 8.13 (1 H, s, 8-H), 12.60 (2 H, br s, 2 × NH)
7d[a]	3240, 3100 (NH); 1720 (C=O)	7.60 (2 H, d, *J* 8.8, Ar-*m*H), 8.08 (2 H, d, *J* 8.8, Ar-*o*H), 8.63 (1 H, s, 8-H)
7e	3280, 3070 (NH); 1700 (C=O)	3.83 (3 H, s, OMe), 7.05 (2 H, d, *J* 8.8, Ar-*m*H), 7.82 (2 H, d, *J* 8.8, Ar-*o*H), 8.62 (1 H, s, 8-H), 12.15 (2 H, br s, 2 × NH)
7f	3350, 3100 (NH)	8.37 (1 H, s, 8-H), 8.58 (1 H, s, 3-H), 11.70 (2 H, br s, 2 × NH)
7g[a]	3320, 3100 (NH)	2.79 (3 H, s, Me), 8.96 (1 H, s, 8-H)
7h	3400, 3120 (NH)	7.40–7.80 (3 H, m, Ph-*m*,*p*H), 8.05–8.35 (2 H, m, Ph-*o*H), 8.30 (1 H, s, 8-H), 12.00 (2 H, br s, 2 × NH)
7i	3330, 3170 (NH)	3.89 (3 H, s, OMe), 7.13 (2 H, d, *J* 8.8, Ar-*m*H), 8.11 (2 H, d, *J* 8.8, Ar-*o*H), 8.36 (1 H, s, 8-H), 11.88 (2 H, br s, 2 × NH)

[a] In CF_3CO_2D.

the 5-position of the triazolopurines might be important for the inhibitory activity. Namely, it seems like that the inhibitory activity was influenced by the affinity of the substrates, the purines and triazolopurines, for the XO enzyme.

Conclusion

Thus, we have accomplished a general synthetic method for 2-substituted 6-alkylidenehydrazino- or 6-arylmethylidenehydrazino-7*H*-purines, and also for 3- and/or 5-substituted 9*H*-1,2,4-triazolo[3,4-*i*]purines as a new class of potential xanthine oxidase inhibitors. The triazolopurines were obtained by oxidative cyclisation of the corresponding 6-aldehyde hydrazones of 7*H*-purine using lead tetraacetate as an oxidizing agent. Their inhibitory activities against bovine milk xanthine oxidase *in vitro* were investigated, and purines **2** and **6** and triazolopurines **7** exhibited from several times to several hundred times more potent activities than allopurinol. In particular, 6-arylmethylidenehydrazino-7*H*-purin-2(3*H*)-ones **6a**–**g** showed remarkable potent inhibitory activities, being three orders of magnitude more active than allopurinol. Biological testing of the compounds prepared here *in vivo* is ongoing and the results will be reported later.

It is noteworthy that the purines **2** and **6** did not show any appreciable inhibition but some triazolopurines **3** exhibited inhibitory activity against the proliferation of T-cell acute lymphoblastic leukemia (CCRF-HSB-2) (IC_{50}: **3b**, 6.9 μM; **3e**, 1.6 μM; **3g**, 1.9 μM; **3j**, 19.5 μM; **3k**, 19.3 μM), KB cell (IC_{50}: **3e**, 4.5 μM; **3g**, 4.6 μM) and HT 1080 cell (IC_{50}: **3e**, 1.4 μM; **3g**, 1.5 μM).

Experimental

General

Mps were obtained on a Yanagimoto micro melting point apparatus and were uncorrected. Microanalyses were measured by Yanaco CHN Corder MT-5 apparatus. Mass spectra were recorded at 70 eV ionizing voltage with FAB ionization using a VG-70SE spectrometer. IR spectra were recorded on a JASCO IRA-102 spectrometer. ^{1}H NMR spectra were obtained using a Hitachi FT-NMR R-1500 (60 MHz) spectrometer with TMS as an internal standard. In all cases, chemical shifts are in ppm downfield to TMS. *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 aluminum-backed plates (Merck Kieselgel 60 F_{254}) and products were visualized by UV light. Column chromatography was run on Kieselgel 60 (70–230 mesh ASTM, Merck). The reaction temperatures are indicated as the temperature of the oil bath. The chemicals for the XO assay were purchased from Sigma Chemicals Co. (allopurinol and bovine milk xanthine oxidase) and Yamasa Syoyu Co. (xanthine).

6-(4-Fluorobenzylidenehydrazino)-7*H*-purine 2a and 6-(4-methoxybenzylidenehydrazino)-7*H*-purine 2b

A solution of 6-hydrazino-7*H*-purine **1a**[23] (1 g, 6.66 mmol) and 4-fluorobenzaldehyde (1.24 g, 9.99 mmol) or *p*-anisaldehyde (1.36 g, 9.99 mmol) in ethanol (80 cm^3) was stirred at

Table 5 Inhibitory activities of the compounds **2**, **3**, **6** and **7** against bovine milk xanthine oxidase in comparison with allopurinol

Compound No.	Inhibition (%)						
	10	3	1	0.3	0.1	0.03	IC_{50}/μM
2a	71.7	67.7	58.7	42.3	25.2	13.9	0.528
2b	77.0	72.7	68.8	54.4	34.1	19.7	0.236
2c	18.4						>10
2d	63.1	51.6	34.4	16.1	6.1		2.710
2e	64.5	56.7	39.4	22.0	13.1		1.960
2f	63.0	59.2	51.1	33.6	17.2		0.927
2g	61.6	57.6	53.6	34.1	16.2		0.801
2h	61.5	53.3	39.1	20.5	10.6		2.324
2i	64.7	59.7	48.6	27.9	15.0		1.149
2j	36.8						>10
2k	65.7	62.6	54.1	35.8	22.2		0.764
2l	22.2						>10
3a	9.4						>10
3b	37.0	10.4	4.4	1.9	2.0		>10
3e	3.1						>10
3g	9.8						>10
3h	5.2						>10
3i	2.7						>10
3j	5.7						>10
3k	16.7						>10
6a		81.1	80.3	77.5	69.7	45.2	0.038
6b	76.4	76.4	76.2	75.5	71.1	55.7	0.025
6c	64.6	62.2	61.6	60.5	57.9	41.4	0.057
6d		80.0	79.1	75.3	58.5	23.0	0.075
6e		80.0	79.6	76.1	63.8	28.4	0.063
6f		77.6	76.6	73.7	66.7	41.4	0.045
6g	79.8	80.7	79.4	77.2	70.8	39.2	0.045
6h	81.4	78.8	72.2	47.7	14.0	3.3	0.336
6i	64.1	61.0	50.6	28.0	13.6	7.7	0.969
6j	80.0	73.2	53.6	23.6	9.8	4.5	0.865
6k	79.6	78.5	75.4	59.7	15.8	10.9	0.235
6l	79.4	69.3	46.5	16.5	5.2		1.184
7a	28.2						>10
7b	48.2					4.3	>10
7c	76.6	54.3	23.8	9.8	4.7	28.9	2.570
7d	78.3	77.2	76.1	73.2	61.2		0.066
7f	11.0					1.8	>10
7g	56.3	24.0	8.9	4.5	2.6		7.907
7h	76.5	50.5	18.7	8.2	4.1		2.949
7i	56.4	44.0	25.2	8.6	3.3		5.372
Allo[a]	38.2	19.9	9.9	4.6	3.2		24.30

[a] Allo: allopurinol

room temperature for 7 hours. After the reaction was complete, the precipitates formed were collected by filtration and recrystallized from a mixture of ethanol and DMF to give the corresponding pure *hydrazones* **2a** and **2b** as shown in Tables 1 and 2.

6-Alkylidenehydrazino- and 6-arylmethylidenehydrazino-2-chloro-7*H*-purines 2c–k; general procedure

A mixture of 2-chloro-6-hydrazino-7*H*-purine **1b**[23] (1 g, 5.42 mmol) and an appropriate alkylaldehyde (8.1 mmol) or arylaldehyde (6.5 mmol) in 1,4-dioxane (40 cm^3) was stirred at room temperature for 3–10 hours. After the reaction was complete, the solvent was evaporated under reduced pressure and the residue was treated with ethanol to afford the crystals. The crystals were collected by filtration and recrystallized from DMF to give the corresponding pure *hydrazones* **2c**–**k** as shown in Tables 1 and 2.

2-Amino-6-(4-methoxybenzylidenehydrazino)-7*H*-purine 2l

A solution of 2-amino-6-hydrazino-7*H*-purine **1c**[23] (1 g, 6.05 mmol) and *p*-anisaldehyde (1.24 g, 9.11 mmol) in glacial acetic acid (40 cm^3) was stirred at room temperature for 4 hours. After the reaction was complete, the solvent was evaporated under reduced pressure to afford the solid, which was recrystallized from DMF to give the pure *hydrazone* **2l** as shown in Tables 1 and 2.

9*H*-1,2,4-Triazolo[3,4-*i*]purine 3a

A mixture of 6-hydrazino-7*H*-purine **1a** (1 g, 6.66 mmol) and triethyl orthoformate (50 cm^3, 337 mmol) was heated under reflux for 9 hours. After cooling, the precipitated crystals were collected by filtration, washed with ethanol and recrystallized from DMF to afford the pure *triazolopurines* **3a**. The melting point of product **3a** was over 300 °C, compared to the literature[24] mp of over 264 °C. The described structure was confirmed by satisfactory analytical and spectral data as shown in Tables 1 and 2.

5-Chloro-9*H*-1,2,4-triazolo[3,4-*i*]purine 3b and its 3-alkyl derivatives 3c,d; general procedure

To a mixture of 2-chloro-6-hydrazino-7*H*-purine **1b** (1 g, 5.42 mmol) with an appropriate triethyl orthoester (40 cm^3) at room temperature was added TFA (5 cm^3). After the reaction mixture was stirred at room temperature for 3–5 hours, the precipitates formed were collected by filtration and recrystallized from water to give the corresponding pure *triazolopurines* **3b**–**d** as shown in Tables 1 and 2.

3-Aryl-5-chloro-9*H*-1,2,4-triazolo[3,4-*i*]purines 3e–g; general procedure

A mixture of an appropriate 6-benzylidenehydrazino-2-chloro-7*H*-purine **2d,e,g** (3.5 mmol) with lead tetraacetate (2.35 g, 5.3 mmol) in 1,4-dioxane (30 cm^3) was stirred at room temperature for 2 hours. After the reaction was complete, water (100 cm^3) was added to the mixture and extracted with ethyl acetate (3 × 50 cm^3). The combined extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure to leave a solid, which was recrystallized from water to give the corresponding pure *triazolopurines* **3e**–**g** as shown in Tables 1 and 2.

5-Amino-9*H*-1,2,4-triazolo[3,4-*i*]purine 3h and its 3-substituted derivatives 3i,j; general procedure

To a solution of 2-amino-6-hydrazino-7*H*-purine **1c** (1 g, 6.05 mmol) in DMF (5 cm^3) was added an appropriate triethyl orthoester (40–60 mmol) and the mixture was heated at 150–160 °C for 5 hours. After cooling, the precipitated crystals were collected by filtration, washed with ethanol and recrystallized from DMF to give the corresponding pure *triazolopurines* **3h**–**j** as shown in Tables 1 and 2.

5-Amino-3-(4-methoxyphenyl)-9*H*-1,2,4-triazolo[3,4-*i*]purine 3k

A mixture of 2-amino-6-(4-methoxybenzylidenehydrazino)-7*H*-purine **2l** (1 g, 3.53 mmol) with lead tetraacetate (2.35 g, 5.30 mmol) in glacial acetic acid (50 cm^3) was heated at 120 °C under stirring for 5 hours. After the reaction was complete, the solid was removed by filtration and the filtrate was concentrated under reduced pressure to leave a solid, which was purified by column chromatography on silica using a mixture of ethyl acetate and ethanol as eluent to give the *triazolopurine* **3k** as shown in Tables 1 and 2.

6-Hydrazino-7*H*-purin-2(3*H*)-one 5a

A mixture of 1,2,3,6-tetrahydro-2-oxo-6-thioxo-7*H*-purine **4a**[30] (1 g, 5.95 mmol) and 80% hydrazine hydrate (10 ml) was heated under reflux for 10 min. After cooling, the precipitated crystals were collected by filtration, washed with water and recrystallized from water to yield the pure *hydrazino derivative* **5a** (0.62 g, 63%) as colourless powdery crystals, mp 278 °C (decomp.) (Found: C, 32.4; H, 4.5; N, 45.7. $C_5H_6N_6O·H_2O$

requires C, 32.6; H, 4.4; N, 45.6%); ν_{max} (Nujol)/cm^{-1} 3330 and 3260sh (NH_2), 3200, 3180 and 3135 (NH), 1685 (C=O) and δ_{max} (Nujol)/cm^{-1} 1660 (NH_2); δ_H [60 MHz; $(CD_3)_2SO$; Me_4Si] 4.40 (2 H, br s, NH_2), 7.74 (1 H, s, 8-H), 9.75 (1 H, br s, NH), 10.75 (1 H, br s, NH) and 11.30 (1 H, br s, NH); *m*/*z* (FAB, glycerol matrix) 167 (MH^+).

6-Hydrazino-7*H*-purine-2(3*H*)-thione 5b

A mixture of 1,2,3,6-tetrahydro-2,6-dithioxo-7*H*-purine **4b**[31] (1 g, 5.43 mmol) and 80% hydrazine hydrate (10 ml) was heated under reflux for 10 min. After cooling, the precipitated crystals were collected by filtration, washed with water and recrystallized from water to yield the pure *hydrazino derivative* **5b** as colourless powdery crystals (0.60 g, 61%), mp 292 °C (decomp.) (Found: C, 31.6; H, 3.8; N, 43.7. $C_5H_6N_6S\cdot1/2H_2O$ requires C, 31.4; H, 3.7; N, 43.95%); ν_{max} (KBr)/cm^{-1} 3440sh, 3260 and 3180 (NH_2 and NH) and δ_{max} (KBr)/cm^{-1} 1655sh (NH_2); δ_H [60 MHz; $(CD_3)_2SO + D_2O$; Me_4Si] 8.03 (1 H, s, 8-H); *m*/*z* (FAB, glycerol matrix) 183 (MH^+).

6-Arylmethylidenehydrazino-7*H*-purin-2(3*H*)-ones 6a–g; general procedure

A mixture of 6-hydrazino-7*H*-purin-2(3*H*)-one **5a** (0.5 g, 3.0 mmol) and an appropriate arylaldehyde (4.5 mmol) in glacial acetic acid (30 cm^3) was stirred at room temperature for 4 hours. After the reaction was complete, the solvent was evaporated under reduced pressure and the residue was treated with ethanol to afford the solid. The solid was collected by filtration and recrystallized from DMF to give the corresponding pure *hydrazones* **6a**–**g** as shown in Tables 3 and 4.

6-Arylmethylidenehydrazino-7*H*-purine-2(3*H*)-thiones 6h–l; general procedure

A mixture of 6-hydrazino-7*H*-purin-2(3*H*)-thione **5b** (0.5 g, 2.74 mmol) and an appropriate arylaldehyde (4.1 mmol) in glacial acetic acid (30 cm^3) was stirred at room temperature for 4 hours. After the reaction was complete, the precipitated solid was collected by filtration, washed with ethanol and recrystallized from DMF to give the corresponding pure *hydrazones* **6h**–**l** as shown in Tables 3 and 4.

9*H*-1,2,4-Triazolo[3,4-*i*]purin-5(6*H*)-one 7a and its 3-substituted derivatives 7b,c; general procedure

A mixture of 6-hydrazino-7*H*-purin-2(3*H*)-one **5a** (1 g, 6.02 mmol) with an appropriate triethyl orthoester (40–60 mmol) was heated at 150–160 °C for 5 hours. After cooling, the precipitated solid was collected by filtration, washed with ethanol and recrystallized from DMF to give the corresponding pure *triazolopurines* **7a**–**c** as shown in Tables 3 and 4.

3-Aryl-9*H*-1,2,4-triazolo[3,4-*i*]purin-5(6*H*)-ones 7d,e; general procedure

A mixture of an appropriate 6-arylmethylidenehydrazino-7*H*-purin-2(3*H*)-one **6b,c** (1.8 mmol) with lead tetraacetate (1.17 g, 2.64 mmol) in 1,4-dioxane (30 cm^3) was heated under reflux and stirring for 4 hours. After the reaction was complete, the solid was removed by filtration and the filtrate was concentrated under reduced pressure to leave a solid, which was purified by column chromatography on silica using a mixture of ethyl acetate and ethanol as eluent to give the corresponding *triazolopurines* **7d**,**e** as shown in Tables 3 and 4.

9*H*-1,2,4-Triazolo[3,4-*i*]purine-5(6*H*)-thione 7f and its 3-substituted derivatives 7g,h; general procedure

A mixture of 6-hydrazino-7*H*-purine-2(3*H*)-thione **5b** (1 g, 5.49 mmol) with an appropriate triethyl orthoester (40–55 mmol) in DMF (5 cm^3) was heated at 150–160 °C for 5 hours. After cooling, the precipitated solid was collected by filtration, washed with ethanol and recrystallized from DMF to give the corresponding pure *triazolopurines* **7f**–**h** as shown in Tables 3 and 4.

3-(4-Methoxyphenyl)-9*H*-1,2,4-triazolo[3,4-*i*]purine-5(6*H*)-thione 7i

A mixture of 6-(methoxybenzylidenehydrazino)-7*H*-purine-2(3*H*)-thione **6i** (0.5 g, 1.66 mmol) with lead tetraacetate (1.10 g, 2.49 mmol) in 1,4-dioxane (30 cm^3) was heated under reflux and stirring for 4 hours. After the reaction was complete, the solid was removed by filtration and the filtrate was concentrated under reduced pressure to leave a solid, which was purified by column chromatography on silica using a mixture of ethyl acetate and ethanol as eluent to give the *triazolopurine* **7i** as shown in Tables 3 and 4.

Xanthine oxidase assay

All test compounds and allopurinol were dissolved in DMSO and diluted with 50 mM sodium phosphate buffer (pH 7.4) for the *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 (0.001–10 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. The values of IC_{50} *i.e.* the μM concentration of inhibitor necessary for 50% inhibition, were determined by plotting V_0/V_I against the concentration of the inhibitor reading the absorbance change at 292 nm.

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