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Facile and general syntheses of 3- and/or 5-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines as a new class of potential xanthine oxidase inhibitors†

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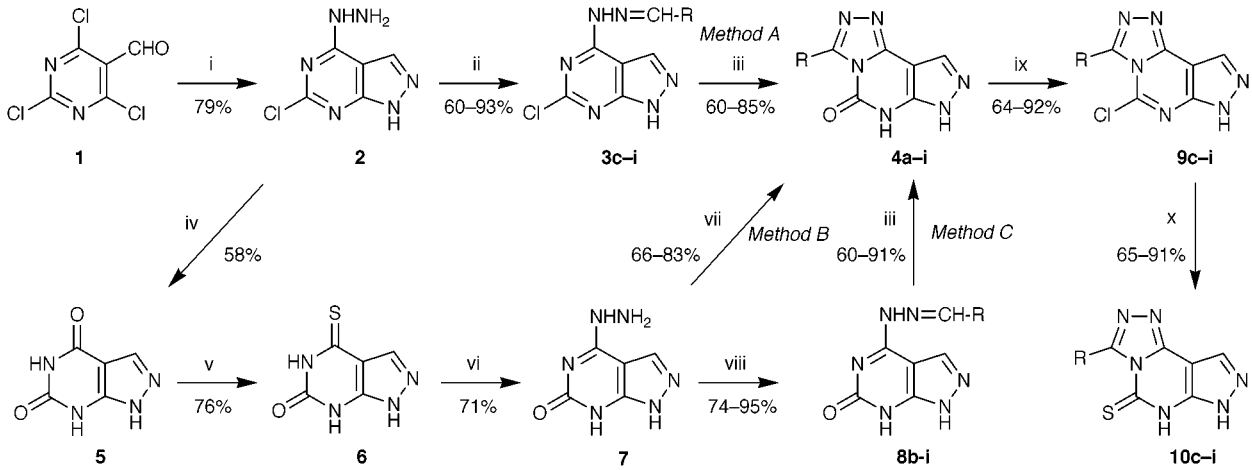
Convenient syntheses of 3- and/or 5-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines as a new class of potent xanthine oxidase inhibitors, involving the oxidative cyclisation of 4-alkylidenehydrazino- or 4-arylmethylidenehydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidines with 70% nitric acid as the key step, are described.

As part of our studies on the synthesis[1] and biological evaluation[2] of novel fused pyrimidines, we initiated investigations aiming at designing new xanthine oxidase (XO) inhibitors. Among the fused purines prepared, the angular type purine analogues, 7*H*-1,2,4-triazolo[3,4-*i*]purines have been recently investigated for their potential XO inhibitory activities.[3] Allopurinol is known to inhibit XO[4] and is now widely employed in treatment of gout and hyperuricemia resulting from uric acid.[5–7] Although XO inhibitory activities have recently been discovered in some synthetic compounds,[8–10] no clinically effective XO inhibitors for the treatment of hyperuricemia have been developed since allopurinol was introduced for clinical use in 1963.[4] Herein, we report a facile strategy for general syntheses of the title compounds as a new class of potent XO inhibitors.

We have elucidated that 5-substituted 7*H*-1,2,4-triazolo[3,4-*i*]purines, especially the 5-oxo or 5-thioxo derivatives,[3] showed more potent bovine milk XO inhibitory activities than allopurinol. In contrast, few synthetic ways to prepare 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines, which are analogous to them, have been reported in the literature[11,12] or in patents[13] and several derivatives have been synthesized. However, none of the 5-substituted derivatives have been prepared up to now.

Our synthetic approach to the title compounds **4a**–**i** involved the preliminary synthesis of 6-chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**) (79% yield) through the reaction of 2,4,6-trichloropyrimidine-5-carbaldehyde (**1**)[14] with anhydrous hydrazine (4 equiv.) in 2-methoxyethanol at 0 °C, followed by the reaction of **2** with an appropriate aldehyde (1.2–1.5 equiv.) in DMF at room temperature to yield the corresponding hydrazones **3c**–**i** in 60–93% yields as shown in Scheme 1.‡ In the light of this multiple step synthesis, a one-pot oxidative cyclisation starting from **3c**–**i** would be really attractive. Indeed, heating the hydrazones **3c**–**i** thus obtained with 70% nitric acid (*ca.* 5 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(6H)-ones (**4c**–**i**) accompanied with hydrolytic dechlorination in 60–85% yields (*Method A*).§ On the other hand, heating compound **2** with concentrated hydrochloric acid (50 parts) under reflux gave oxypurinol **5** (58% yield), which was confirmed by direct comparison with an authentic sample.[15] Then, treatment of the 4-thioxo derivative **6**, obtained by thiation of **5** according to the previously reported procedure,[15] with hydrazine monohydrate (17 equiv.) in ethanol under reflux afforded the 4-hydrazino derivative **7** in 71% yield. Compound **7** was subsequently cyclised to the corresponding **4a**,**b** (66–83% yields) by stirring with appropriate triethyl orthoesters (5 equiv.) in trifluoroacetic acid at room temperature (*Method B*).¶ Further, treatment of **7** with an appropriate aldehyde (1.5 equiv.) in DMF at room temperature gave the corresponding hydrazones **8b**–**i** in 74–95% yields. The intramolecular cyclisation of **8b**–**i** to the corresponding **4b**–**i** was also accomplished by

† Details of bovine milk xanthine oxidase inhibition by **4** and **10** are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/1461/



a R = H; **b** R = Me; **c** R = *n*-C_7H_{15}; **d** R = Ph; **e** R = 4-F-C_6H_4; **f** R = 4-Cl-C_6H_4; **g** R = 4-Me-C_6H_4; **h** R = 4-MeO-C_6H_4; **i** R = 4-O_2N-C_6H_4

Scheme 1 *Reagents and conditions*: i, anh. NH_2NH_2, $MeOCH_2CH_2OH$, 0 °C, 30 min; ii, RCHO, DMF, rt, 2–10 h; iii, 70% HNO_3, DMF, 100 °C, 1–5 h; iv, conc. HCl, reflux, 1 h; v, P_2S_5, pyridine, reflux, 2 h; vi, $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 10 min; vii, $RC(OEt)_3$, TFA, rt, 1 h; viii, RCHO, DMF, rt, 10 h; ix, $POCl_3$, reflux, 1–4 h; x, $(H_2N)_2C{=}S$, Bu^{s}OH, reflux, 0.5–2 h.

oxidation using 70% nitric acid (*ca.* 1.2 equiv.) in 60–91% yields in a similar manner as above (*Method C*).

In addition, we tried to prepare the 5-thioxo derivatives **10c**–**i**. Thus the key starting materials, 5-chloro derivatives **9c**–**i**, were readily prepared by refluxing the appropriate 5-oxo derivatives **4c**–**i** with phosphoryl chloride (100 parts) in 64–92% yields.‖ Then, thiation by reaction of **9c**–**i** with thiourea (1.2 equiv.) in butan-2-ol under reflux afforded the corresponding 5-thioxo derivatives **10c**–**i** in 65–91% yields.**

In conclusion, we accomplished the facile and general syntheses of not only oxypurinol **5** and 3- and/or 5-substituted 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines (**4** and **10**) as a new class of potential XO inhibitors but also 6-chloro-4-hydrazino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**2**), which was an useful intermediate. The compounds (**4** and **10**) exhibited 100–760 fold more potent bovine milk XO inhibitory activities than that of allopurinol† and did not show any appreciable inhibition against the proliferation of T-cell acute lymphoblastic leukemia (CCRF-HSB-2). Further investigation of the present synthetic and XO inhibitory study is in progress and will be reported in detail shortly.

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Notes and references

‡ All new compounds **2**, **3c**–**i**, **7** and **8b**–**i** exhibited satisfactory elemental combustion analyses and mass and ^{1}H NMR spectral data consistent with structures indicated, and showed mps over 300 °C except for **3c** (mp 250 °C).

§ *Typical procedure*: A solution of **3c** (0.3 g, 1.02 mmol) with 70% nitric acid (0.5 ml, 5.55 mmol) in DMF (30 ml) was heated at 100 °C for 1 h. After the reaction was complete, the solution was concentrated to dryness *in vacuo* and treated with MeOH to afford the crystals **4c** (75%), which were collected by filtration and recrystallized from a mixture of DMF and EtOH. Other derivatives **4d** (85%), **4e** (72%), **4f** (71%), **4g** (67%), **4h** (61%) and **4i** (60%) were prepared in a similar manner to **4c** and recrystallized from a mixture of DMF and EtOH or water. The compounds **4c**–**i** showed mps above 300 °C, respectively. δ_H[60 or 200 MHz, $(CD_3)_2SO$] for **4c**: 0.86 (3H, t, *J* 6.54), 1.30 (8H, br s), 1.50–1.80 (2H, m), 2.50–2.95 (2H, m), 8.53 (1H, s), 12.41 (1H, br s), 13.50 (1H, br); for **4d**: 7.40–7.70 (3H, m), 7.90–8.35 (2H, m), 8.68 (1H, s), 12.60 (1H, br s), 13.60 (1H, br); for **4e**: 7.37 (2H, dd, *J* 8.82, 9.06), 8.22 (2H, dd, *J* 8.82, 5.88), 8.66 (1H, s), 12.59 (1H, br s), 13.65 (1H, br); for **4f**: 7.62 (2H, d, *J* 8.60), 8.17 (2H, d, *J* 8.60), 8.70 (1H, s), 12.62 (1H, br s), 13.66 (1H, br s); for **4g** (CF_3CO_2D): 2.54 (3H, s), 7.51 (2H, d, *J* 8.76), 8.03 (2H, d, *J* 8.76 Hz), 8.94 (1H, s); for **4h**: 3.85 (3H, s), 7.10 (2H, d, *J* 8.76), 8.12 (2H, d, *J* 8.76), 8.64 (1H, s), 12.60 (1H, br s), 13.50 (1H, br); for **4i**: 8.39 (4H, br s), 8.69 (1H, s), 12.70 (1H, br s), 13.60 (1H, br).

¶ *General procedure*: A solution of **7** (0.2 g, 1.2 mmol) with an appropriate triethyl orthoester (6 mmol) in trifluoroacetic acid (3 ml) was stirred at room temperature for 1 h. After the reaction was complete, the deposit was collected by filtration and recrystallized from DMF to yield the corresponding **4a** (mp > 300 °C, 66%) and **4b** (mp > 300 °C, 83%). δ_H[200 MHz, $(CD_3)_2SO$] for **4a**: 8.34 (1H, s), 8.62 (1H, s), 12.58 (1H, br s), 13.60 (1H, br s); for **4b**: 2.40 (3H, s), 8.54 (1H, s), 12.46 (1H, br s), 13.57 (1H, br s).

‖ All new compounds **9c**–**i** exhibited satisfactory elemental combustion analyses and mass and ^{1}H NMR spectral data consistent with structures indicated, and showed mps over 300 °C except for **9c** (mp 150 °C).

** *Typical procedure*: A solution of **9c** (0.3 g, 1.02 mmol) with thiourea (93 mg, 1.22 mmol) in Bu^{s}OH (15 ml) was heated under reflux for 1 h. After the reaction was complete, the solution was concentrated to dryness *in vacuo* and treated with EtOH to afford crystals of **10c** (mp 260–261 °C, 65%), which were collected by filtration and recrystallized from a mixture of DMF and water. Other derivatives **10d** (81%), **10e** (71%), **10f** (71%), **10g** (74%), **10h** (91%) and **10i** (77%) were prepared in a similar manner to **10c** and recrystallized from a mixture of DMF and EtOH or water. The compounds **10d**–**i** showed mps above 300 °C. It is noteworthy that the compounds **4**, **9** and **10** were reasonably stable in acid or alkali solution due to the substituents at the 5-position on the rings. δ_H[60 or 200 MHz, $(CD_3)_2SO$] for **10c**: 0.90 (3H, t, *J* 6.30), 1.31 (8H, br s), 1.55–2.10 (2H, m), 2.64–3.08 (2H, m), 8.63 (1H, s), 14.0 (1H, br), 14.38 (1H, br); for **10d**: 7.50–7.64 (3H, m), 8.10–8.30 (2H, m), 8.84 (1H, s), 14.0 (1H, br s), 14.35 (1H, br s); for **10e**: 7.40 (2H, dd, *J* 8.84, 8.90), 8.25 (2H, dd, *J* 8.84, 5.54), 8.75 (1H, s), 14.01 (1H, br), 14.37 (1H, br); for **10f**: 7.63 (2H, d, *J* 8.57), 8.20 (2H, d, *J* 8.57), 8.81 (1H, s), 14.01 (1H, br), 14.38 (1H, br); for **10g**: 2.40 (3H, s), 7.35 (2H, d, *J* 7.92), 8.10 (2H, d, *J* 7.92), 8.75 (1H, s), 13.90 (1H, br), 14.40 (1H, br); for **10h**: 3.85 (3H, s), 7.09 (2H, d, *J* 8.82), 8.14 (2H, d, *J* 8.82), 8.75 (1H, s), 14.0 (1H, br), 14.55 (1H, br); for **10i**: 8.41 (4H, br s), 8.77 (1H, s), 13.75 (1H, br), 14.25 (1H, br).

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