HETEROCYCLES, Vol. 73, 2007, pp. 777 - 793. © The Japan Institute of Heterocyclic Chemistry
Received, 2nd August, 2007, Accepted, 5th September, 2007, Published online, 7th September, 2007. COM-07-S(U)58

SYNTHESIS OF THIENO[3,2-*e*][1,2,4]TRIAZOLO[1,5-*c*]PYRIMIDIN-5(6*H*)-ONES *VIA* THEIR [1,2,4]TRIAZOLO[4,3-*c*]PYRIMIDINE COMPOUNDS AS NEW RING SYSTEMS BY DIMROTH-TYPE REARRANGEMENT

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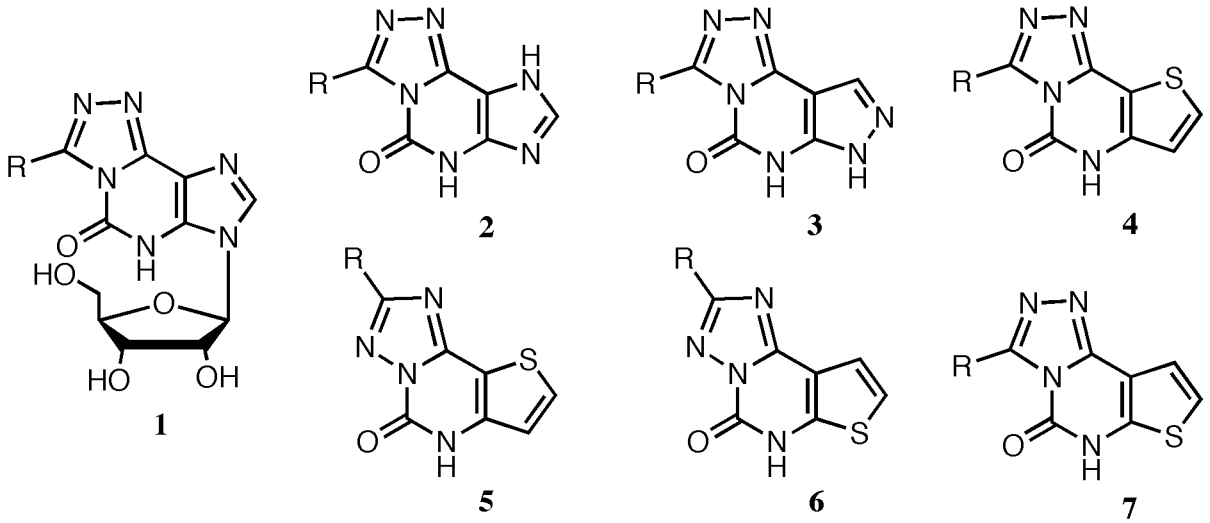
Abstract – General and facile syntheses of thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]-pyrimidin-5(6*H*)-one (**6a**) and its 2-substituted derivatives (**6b–j**) produced by instantaneous isomerization of their [4,3-*c*] compounds (**7a–j**), which were prepared by condensation of 4-hydrazinothieno[2,3-*d*]pyrimidin-2(1*H*)-one (**10**) with appropriate triethyl orthoesters or by oxidative cyclization of 4-(benzylidenehydrazino)thieno[2,3-*d*]pyrimidin-2(1*H*)-ones (**11c–j**), are described as novel ring systems and as a new class of potential xanthine oxidase inhibitors. The [1,5-*c*] isomers (**6a–c**) were further prepared by condensation of 3-amino-4-imino-2-oxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine (**14**) with appropriate triethyl orthoesters as a synthetic method for a reliable structure of the tricyclic ring systems.

INTRODUCTION

In connection with ongoing work aimed at synthesis and biological evaluation of novel tricyclic fused pyrimidine or purine systems,[1–6] we have recently reported the potential xanthine oxidase (XO) inhibitory activities of the angular type analogues such as 7β-D-ribofuranosyl-7*H*-[1,2,4]triazolo[3,4-*i*]purines (**1**),[7] 9*H*-[1,2,4]triazolo[3,4-*i*]purines (**2**),[8] and 7*H*-pyrazolo[4,3-*e*]-1,2,4-triazolo[4,3-*c*]pyrimidines (**3**).[9] Moreover, we have reported the reliable synthesis of thieno[2,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**4**) and thieno[2,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**5**) as a new class of potential XO

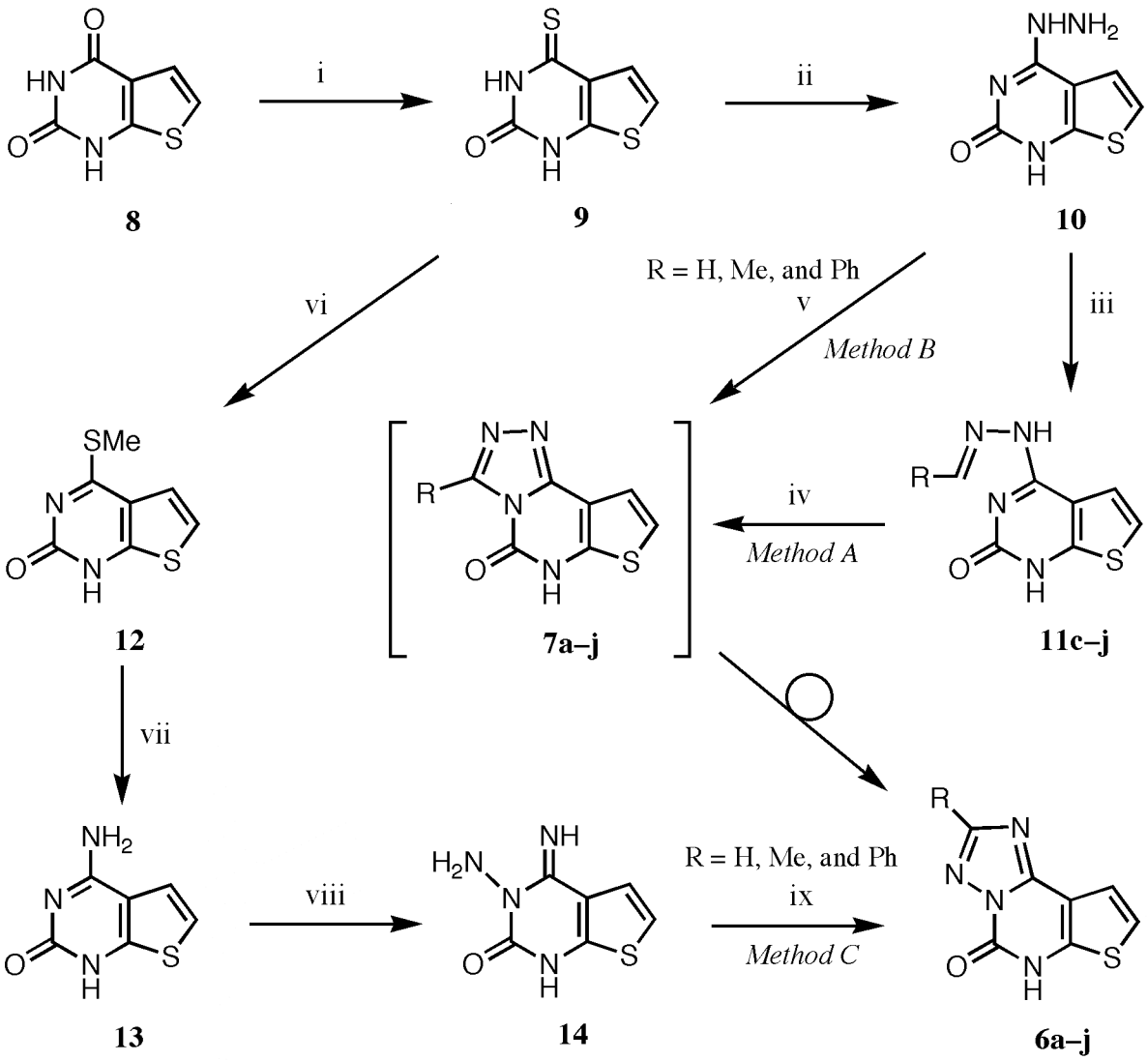
inhibitors.[10] Allopurinol has been reported as a potential inhibitor of XO,[11] which catalyzes the conversion of hypoxanthine and xanthine to uric acid, and been used extensively for the clinical control of uric acid production in gout and hyperuricemia.[12] Although allopurinol is a medicine which toxicity is comparatively rare, a life-threatening toxicity syndrome such as vasculitis, rash, eosinophilia, hepatitis, progressive renal failure has been reported after its use.[13] The XO inhibitory activities have been discovered in some newly synthesized compounds and previously known compounds.[14] However, the clinically effective XO inhibitors for the treatment of hyperuricemia and gout have not been developed yet since allopurinol was introduced for clinical use in 1963.[12,15] Therefore, such tricyclic hetero analogs (**1–5**) of allopurinol have aroused considerable recent interest for us. Recently, we have found the [1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one ring system was easily isomerized into the corresponding [1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one ring system.[16] In this paper, we report a reliable and general synthesis of 2-substituted thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**6**) *via* 3-substituted thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**7**) by Dimroth-type rearrangement as novel ring systems and a new class of the XO inhibitors.



Scheme 1

RESULTS AND DISCUSSION

In the first place we tried to synthesize the key intermediate, 4-hydrazinothieno[2,3-*d*]pyrimidin-2(1*H*)-one (**10**), which could be led through several processes from barbituric acid. The requisite starting material, thieno[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-dione (**8**), was prepared according to a literature method.[17] Treatment of **8** with Lawesson's reagent in 1,4-dioxane under reflux afforded 2-oxo-4-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine (**9**) in 82% yield, which was identified by its physical data with a



a: R = H; **b:** R = Me; **c:** R = Ph; **d:** R = 4-F-C_6H_4; **e:** R = 4-Cl-C_6H_4; **f:** R = 4-Br-C_6H_4; **g:** R = 4-Me-C_6H_4 ; **h:** R = 4-MeO-C_6H_4; **i:** R = 4-HO-C_6H_4; **j:** R = 4-O_2N-C_6H_4

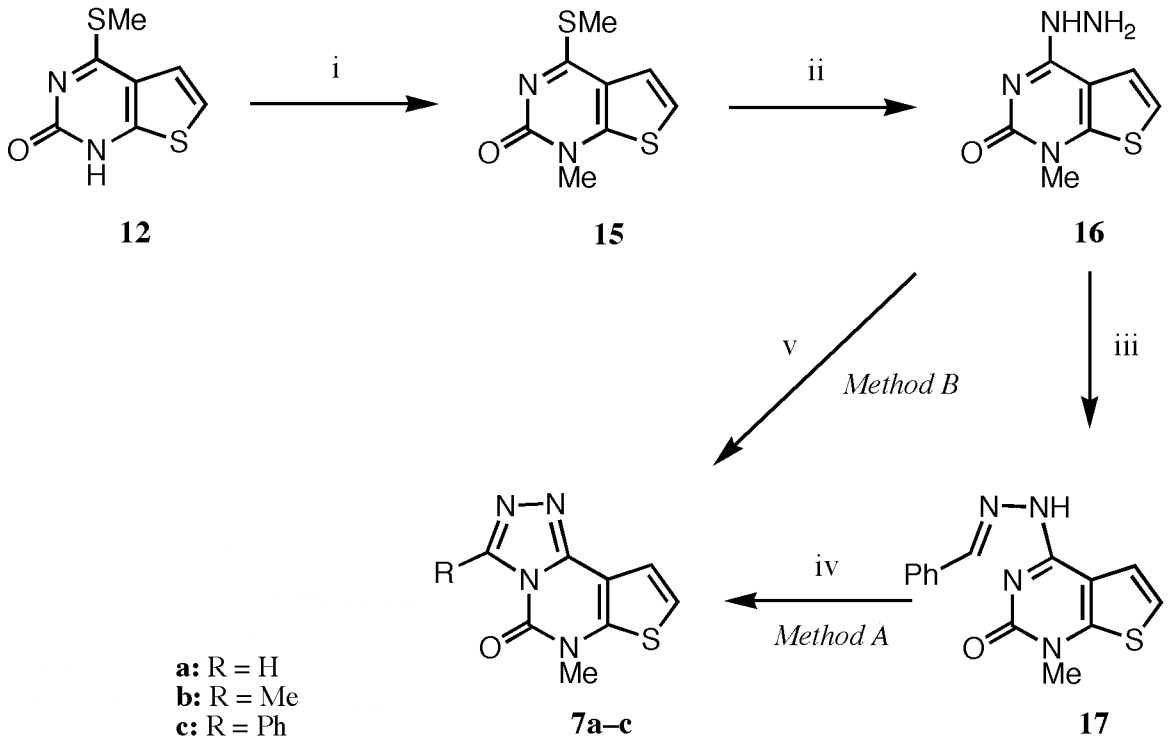
Scheme 2 *Reagents and conditions*: i, Lawesson's Reagent, 1,4- dioxane, reflux, 20 min; ii, $NH_2NH_2 \cdot H_2O$, water, reflux, 1.5 h; iii, ArCHO, MeOH, rt, 8–10 h; iv, chloranil, AcOH, reflux, 15–60 min; v, $RC(OEt)_3$, DMF, reflux, 30 min; vi, MeI, 2N NaOH, 5 °C, 10 min; vii, 28% aq. NH_3, sealed tube, 150 °C, 7 h; viii, H_2N-O-SO_3H, 1N NaOH, rt, 1.5 h; ix, $RC(OEt)_3$, AcOH, 80 °C, 10–60 min.

compound prepared by reaction of 2-aminothiophene-3-carbonitrile with carbonyl sulfide,[18] as shown in the sequential synthetic pathway of Scheme 2. Then, refluxing of **9** with excess hydrazine hydrate in water afforded the desired 4-hydrazinothieno[2,3-*d*]pyrimidin-2(1*H*)-one **(10)** in 70% yield. The compound **(10)** was anticipated as a versatile intermediate for the preparation of thieno[3,2-*e*][1,2,4]-triazolo[4,3-*c*]pyrimidine ring system (**7**). However, the expected ring system (**7**) was isomerized instantaneously to the thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine ring system (**6**) by Dimroth-type rearrangement. Consequently, the hydrazinothienopyrimidine **(10)** was converted to the hydrazones **(11c–j)** by reaction with an appropriate aldehyde in MeOH at room temperature in 72–90% yields (Tables

1 and 2). The hydrazones (**11c–j)** were cyclized oxidatively to the corresponding 2-arylthieno[3,2-*e*]-[1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**6c–j)** in 52–65% yields by heating with chloranil in acetic acid (*Method A*). In case of oxidation for **11** using 70% HNO_3, the drastic reactions took place and it was difficult to isolate pure compounds from many by-products. Furthermore, the 2-unsubstituted, 2-methyl, and 2-phenyl derivatives **(6a–c)** were synthesized by treatment of the compound (**10)** with an appropriate triethyl orthoester in DMF under refluxing in 68–95% yields (*Method B*).

We have recently reported that the fused heterocycles having the triazolo[4,3-*c*]pyrimidine ring system with an oxo or a thioxo group at the 5-position easily undergo Dimorth-type rearrangement to yield their triazolo[1,5-*c*] isomers.[10,16] In order to determine to be either the [1,5-*c*] system (**6**) or the [4,3-*c*] system (**7**) as the structure of products which were prepared by *Methods A* and *B*, we tried another reliable synthetic route (*Method C*) starting from 2-oxo-4-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine **(9)**. At first, treatment of **9** with CH_3I in 2N NaOH solution gave the 4-(methylthio)thieno[2,3-*d*]pyrimidin-2(1*H*)-one **(12)** in 56% yield. Then, reaction of **12** with 28% aqueous NH_3 in steel sealed tube at 150 °C afforded the 4-aminothieno[2,3-*d*]pyrimidin-2(1*H*)-one **(13)** in 67% yield. Thus, the second key intermediate, 3-amino-4-imino-2-oxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine **(14)**, was prepared in 39% yield by *N*-amination of reaction of **13** with excess hydroxylamine-*O*-sulfonic acid in 1N NaOH solution at room temperature. Finally, heating **14** thus obtained with appropriate triethyl orthoesters (*Method C*) gave the corresponding thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**6a–c)**, which were identical to the compounds **(6a–c)** prepared by *Methods A* or *B*, respectively. Therefore, the structure of the triazolo moiety for the tricyclic compounds (**7**) was assigned as not the triazolo[4,3-*c*] system but the triazolo[1,5-*c*] system. All new compounds (**6** and **10–14**) exhibited satisfactory elemental combustion analyses except for **14** and IR and ^{1}H NMR spectral data consistent with the structures indicated as shown in Tables 1-4 and experimental section.

Since the 1,2,4-triazolo[4,3-*c*]pyrimidine ring systems with an oxo or thiox group at the 5-position were rapidly isomerized into their [1,5-*c*] isomers, it was difficult to isolate the compounds having the [4,3-*c*] systems without isomerization.[16] We have found recently that the 6-methyl derivatives of 1,2,4-triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones, *i.e.* the methylated imide compounds, were quite stable.[16] Therefore, in order to isolate the thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidine ring system (**7**) without isomerization, we tried to prepare the 6-alkylated derivatives of compound (**7**). As shown in Scheme 3 as the sequential pathway, methylation of **12** was carried out with MeI-NaH solution in the usual way to get the 1-methyl-4-(methylthio)thieno[2,3-*d*]pyrimidin-2(1*H*)-one **(15)** in 75% yield. Then, treatment of **15** with 80% hydrazine hydrate in ethanol under reflux afforded the 4-hydrazino derivative (**16**) in 52% yield. The subsequent treatment of **16** with benzaldehyde in DMF at room temperature yielded the 4-benzaldehydehydrazone (**17**) in 66% yield. The intramolecular cyclization of **17** thus obtained to the



Scheme 3 *Reagents and conditions*: i, MeI, NaH, DMF, 40 °C, 12 h; ii, $NH_2NH \cdot H_2O$, EtOH, reflux, 2.5–3 h; iii, C_6H_5CHO, DMF, rt, 12 h; iv, chloranil, AcOH, reflux, 1 h; v, $RC(OEt)_3$, DMF, reflux, 1–1.5 h.

3-phenylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-ones (**7c**) was accomplished by oxidation using chloranil in acetic acid at reflux in 57% yield as shown in Tables 5 and 6. On the other hand, heating the 4-hydrazino derivative (**16**) with appropriate triethyl orthoesters in DMF under reflux afforded the corresponding thieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)- ones (**7a**) and its 3-methyl (**7b**) and 3-phenyl (**7c**) derivatives in 75–83% yields. All new compounds (**7a–c** and **15–17**) exhibited satisfactory elemental combustion analyses and IR, ^{1}H-NMR, and UV spectral data consistent with the structures indicated as shown in Tables 5-7 and experimental section. Especially, each isomer of the [1,5-*c*]pyrimidines (**6**) and the [4,3-*c*]pyrimidines (**7**) was distinguishable by ^{1}H-NMR and UV spectra. In ^{1}H-NMR [$(CD_3)_2SO$] spectra, the most prominent peak of the [1,5-*c*]pyrimidines (**6a**) was observed at δ 8.43 as a singlet signal attributed to the proton at the 2-position, while the similar singlet signal attributed to the proton at the 3-position of the [4,3-*c*]pyrimidines (**7a**) was observed at δ 9.26 in more downfield. In a similar manner as above, the methyl protons observed at δ 2.44 as singlet signal for the 2-methyl derivative (**6b**) appeared in higher field than those observed at δ 2.77 for the 3-methyl derivative (**7b**). It is noteworthy that the chemical shift of the proton or substituent at the 2-position for the [1,5-*c*]pyrimidines (**6**) was observed in higher field in comparison with it at the 3-position for the [4,3-*c*]pyrimidines (**7**). We have found the similar phenomenon in similar ring systems before.[10,16] The

Table 1 Physical and analytical data for the compounds (**11c–j**)

Compd No.	R	Yield (%)	Mp[a] (°C)	Formula (R_f)[b]	Analysis (%) Calcd (Found) C	H	N
11c	Ph	84	268	$C_{13}H_{10}N_4OS \cdot 1/4H_2O$ (0.60, A)	56.82 (56.97	3.85 3.93	20.39 20.44)
11d	4-F-C_6H_4	72	> 277 (decomp.)	$C_{13}H_9FN_4OS$ (0.69, A)	54.16 (53.79	3.15 3.31	19.43 19.03)
11e	4-Cl-C_6H_4	84	> 300	$C_{13}H_9ClN_4OS$ (0.70, C)	51.23 (51.14	2.98 3.19	18.38 18.33)
11f	4-Br-C_6H_4	84	> 300	$C_{13}H_9BrN_4OS$ (0.63, A)	44.71 (44.42	2.60 2.77	16.04 15.96)
11g	4-Me-C_6H_4	90	278–280	$C_{14}H_{12}N_4OS \cdot 1/10\ H_2O$ (0.70, A)	58.77 (58.42	4.30 4.30	19.58 19.31)
11h	4-MeO-C_6H_4	76	258–260	$C_{14}H_{12}N_4O_2S$ (0.60, A)	55.99 (55.83	4.03 4.16	18.65 18.74)
11i	4-HO-C_6H_4	75	> 270 (decomp.)	$C_{13}H_{10}N_4O_2S \cdot 3/5\ H_2O$ (0.70, B)	52.55 (52.25	3.80 3.75	18.86 18.69)
11j	4-O_2N-C_6H_4	72	> 295 (decomp.)	$C_{13}H_9N_5O_3S \cdot 3/5\ H_2O$ (0.62, A)	47.88 (47.94	3.15 2.90	21.47 21.12)

[a]All compounds were recrystallized from EtOH or DMF and were obtained as colorless powdery crystals except for **11h** (yellow). [b]Solvent system for TLC: (A) AcOEt, (B) AcOEt : EtOH (6:1 v/v), (C) AcOEt : EtOH (4:1 v/v).

UV spectrum of **6a** in ethanol had maxima at 225, 271, and 280 nm, while those of **7a** were at 244, 251, and 273 nm. As a general rule, a bathochromic shift of the respective maximum absorption of 2–20 nm was observed in each UV spectrum for the [1,5-*c*] compounds (**6**) compared to the corresponding [4,3-*c*] isomers (**7**). Thus, compounds **6b** (3-Me) and **6c** (3-Ph) showed the maxima at 233, 272, and 280 nm and the maxima at 241, 279, and 291 nm, respectively, whereas compounds **7b** (3-Me) and **7c** (3-Ph) showed the maxima at 245, 253, and 274 nm and the maxima at 250, 277, and 285 nm, respectively.

BIOLOGICAL EVALUATION

Antitumor activity: The new compounds (**6** and **11**) prepared here were evaluated *in vitro* for the growth inhibitory effect against CCRF-HSB2 (human T-cell acute lymphoblastoid leukemia) and KB

Table 2 IR and ^{1}H-NMR spectroscopic data for the compounds (**11c–j**)

Compd No.	ν_{max} (Nujol) /cm^{-1}	δH [300 MHz; [$(CD_3)_2SO$; Me_4Si]
11c	3150, 3110 (NH) 1690 (C=O)	7.15 (2H, br s, 5-H and 6-H), 7.45–7.50 (3H, m, Ph-*m*,*p*H), 7.84–8.21 (2H, m, Ph-*o*H), 8.47 (1H, s, N=C*H*Ar), 10.32 (1H, br s, 4-NH), 11.15 (1H, br s, 1-NH).
11d	3160, 3050 (NH) 1690 (C=O)	7.08 (1H, d, *J* 5.4, 5-H), 7.14 (1H, d, *J* 5.4, 6-H), 7.23 (2H, dd, $J_{H,H}$ 8.7, $J_{H,F}$ 9.0, Ar- *m*H), 8.10 (2H, dd, $J_{H,H}$ 8.7, $J_{H,F}$ 6.0, Ar-*o*H), 8.43 (1H, s, N=C*H*Ar), 10.40 (1H, br s, 4-NH), 11.42 (1H, br s, 1-NH).
11e	3160, 3040 (NH) 1690 (C=O)	7.05 (1H, d, *J* 5.4, 5- H), 7.14 (1H, d, *J* 5.4, 6-H), 7.43(2H, d, *J* 8.4, Ar-*m*H), 8.04 (2H, d, *J* 8.4, Ar-*o*H), 8.40 (1H, s, N=C*H*Ar), 10.40 (1H, br s, 4-NH), 11.45 (1H, br s, 1-NH).
11f	3160, 3040 (NH) 1690 (C=O)	7.05 (1H, d, *J* 5.4, 5-H), 7.13 (1H, d, *J* 5.4, 6-H), 7.57 (2H, d, *J* 8.4, Ar-*m*H), 7.97 (2H, d, *J* 8.4, Ar-*o*H), 8.39 (1H, s, N=C*H*Ar), 10.38 (1H, br s, 4-NH), 11.44 (1H, br s, 1-NH).
11g	3150, 3050 (NH) 1690 (C=O)	2.35 (3H, s, Me), 7.08 (1H, d, *J* 5.7, 5-H), 7.14 (1H, d, *J* 5.7, 6-H), 7.23 (2H, d, *J* 8.1, Ar- *m*H), 7.90 (2H, d, *J* 8.1, Ar-*o*H), 8.40 (1H, s, N=C*H*Ar), 10.25 (1H, br s, 4-NH), 11.41 (1H, br s, 1-NH)
11h	3160, 3060 (NH) 1695 (C=O)	3.80 (3H, s, OMe), 6.97 (2H, d, *J* 8.1, Ar-*m*H), 7.08 (1H, d, *J* 5.4, 5-H), 7.13 (1H, d, *J* 5.4, 6-H), 7.95 (2H, d, *J* 8.1, Ar-*o*H), 8.38 (1H, s, N=C*H*Ar), 10.20 (1H, br s, 4-NH), 11.41 (1H, br s, 1-NH).
11i	3440 (OH) 3210, 3050 (NH) 1680 (C=O)	6.79 (2H, d, *J* 8.4, Ar-*m*H), 7.07 (1H, d, *J* 5.7, 5-H), 7.13 (1H, d, *J* 5.7, 6-H), 7.82 (2H, d, *J* 8.4, Ar-*o*H), 8.33 (1H, s, N=C*H*Ar), 9.86 (1H, br s, OH), 10.08 (1H, br s, 4-NH), 11.35 (1H, br s, 1-NH).
11j	3160, 3060 (NH) 1695 (C=O)	7.12 (1H, d, *J* 5.7, 5-H), 7.17 (1H, d, *J* 5.7, 6-H), 8.23 (2H, d, *J* 8.7, Ar-*o*H), 8.33 (2H, d, *J* 8.7, Ar-*m*H), 8.55 (1H, s, N=C*H*Ar), 10.76 (1H, br s, 4-NH), 11.58 (1H, br s, 1-NH).

(human oral epidermoid carcinoma) cells by the modified MTT [3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay[19] for cellular growth and survival application method developed by Mosmann.[20] The results, *i.e.* IC_{50} (μg/mL) of each compound against the both cells, are summarized in Table 8. Most compounds of 2-substituted thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**6a**) showed 50% inhibitory activity against CCRF-HSB-2 and KB cancer cells at the concentration *ca.* 15–40 μg/mL and *ca.* 12–57 μg/mL, respectively.

Xanthine oxidase inhibitory activity: The xanthine oxidase (XO) inhibitory activities of the compounds (**6a–j** and **7a–c**) against bovine milk xanthine oxidase *in vitro* were investigated significantly.

Table 3 Physical and analytical data for the compounds (**6a–j**)

Compd No.	R	Yield (%)	Mp[a] (°C)	Formula (R_f)[b]	Analysis (%) Calcd (Found) C	H	N
6a	H	95 (*B*) 45 (*C*)	>300	$C_7H_4N_4OS \cdot 1/10H_2O$ (0.55, B)	43.34 (43.28	2.18 2.37	28.88 28.84)
6b	Me	66 (*B*) 33 (*C*)	>290 (decomp.)	$C_8H_6N_4OS$ (0.50, B)	46.59 (46.57	2.93 3.20	27.17 27.07)
6c	Ph	74 (*A*) 68 (*B*) 62 (*C*)	>282 (decomp.)	$C_{13}H_8N_4OS$ (0.69, B)	58.20 (57.97	3.01 3.32	20.88 20.56)
6d	4-F-C_6H_4	55 (*A*)	>300	$C_{13}H_7FN_4OS$ (0.77, B)	54.54 (54.48	2.46 2.71	19.57 19.44)
6e	4-Cl-C_6H_4	52 (*A*)	>300	$C_{13}H_7ClN_4OS \cdot 1/10H_2O$ (0.62, A)	51.27 (51.48	2.38 2.71	18.40 18.18)
6f	4-Br-C_6H_4	56 (*A*)	>300	$C_{13}H_7BrN_4OS \cdot 2/5\ H_2O$ (0.70, A)	44.06 (44.46	2.22 2.33	15.81 15.42)
6g	4-Me-C_6H_4	58 (*A*)	>300	$C_{14}H_{10}N_4OS \cdot 1/4\ H_2O$ (0.60, B)	58.62 (58.66	3.69 3.61	19.53 19.28)
6h	4-MeO-C_6H_4	62 (*A*)	>300	$C_{14}H_{10}N_4O_2S \cdot 1/4\ H_2O$ (0.54, B)	55.53 (55.74	3.49 3.61	18.50 18.38)
6i	4-HO-C_6H_4	60 (*A*)	>300	$C_{13}H_8N_4O_2S \cdot H_2O$ (0.75, A)	51.65 (51.25	3.33 3.46	18.53 18.31)
6j	4-O_2N-C_6H_4	65 (*A*)	>300	$C_{13}H_7N_5O_3S \cdot H_2O$ (0.73, A)	47.13 (46.93	2.74 2.43	21.14 21.18)

[a]Each yield of (*A*), (*B*) and (*C*) was obtained by *Method A*, *Method B*, *Method C*, respectively, as shown in **Scheme 2**. [b]All compounds were recrystallized from EtOH or DMF and were obtained as colorless powdery crystals or needles except for **6j** (yellow). [c]Solvent system for TLC: (A) AcOEt, (B) AcOEt : EtOH (4:1 v/v).

Some of them (**6a–j**) showed more potential XO inhibitory activity than that of allopurinol. Further investigations along this line are now in progress and the results of the inhibitory activities of the compounds described in this paper will be reported elsewhere.

CONCLUSION

Table 4 IR and ^{1}H-NMR spectroscopic data for the compounds (**6a–j**)

Compd No.	ν_{max} (Nujol) /cm^{-1}	δH [300 MHz; [$(CD_3)_2SO$; Me_4Si]
6a	3120 (NH) 1760 (C=O)	7.41 (1H, d, *J* 5.7, 9-H), 7.47 (1H, d, *J* 5.7, 8-H), 8.43 (1H, s, 2-H), 13.14 (1H, br s, NH)
6b	3070 (NH) 1740 (C=O)	2.44 (3H, s, Me), 7.36 (1H, d, *J* 5.7, 9-H), 7.40 (1H, d, *J* 5.7, 8-H), 13.00 (1H, br s, NH)
6c	3060 (NH) 1720 (C=O)	7.44 (1H, d, *J* 5.8, 9-H), 7.50–7.70 (4H, m, Ph-*m,p*H and 8-H), 8.15–8.35 (2H, m, Ph-*o*H), 13.15 (1H, br s, NH).
6d	3080 (NH) 1700 (C=O)	7.37 (2H, dd, $J_{H,F}$ 9.0, $J_{H,H}$ 8.1, Ar-*m*H), 7.41 (1H, d, *J* 5.7, 9-H) 7.51 (1H, d, *J* 5.7, 8-H), 8.22 (2H, dd, $J_{H,H}$ 8.1, $J_{H,F}$ 5.7, Ar-*o*H), 13.12 (1H, br s, NH).
6e	3080 (NH) 1715 (C=O)	7.15 (1H, d, *J* 5.4, 9-H), 7.20 (1H, d, *J* 5.4, 8-H), 7.42 (4H, br s, Ar-*o,m*H), 11.90 (1H, br s, NH).
6f	3090 (NH) 1720 (C=O)	7.38 (1H, d, *J* 5.7, 9-H), 7.48 (1H, d, *J* 5.7, 8-H), 7.71 (2H, d, *J* 8.7, Ar-*m*H), 8.12 (2H, d, *J* 8.7, Ar-*o*H), 13.12 (1H, br s, NH).
6g	3070 (NH) 1715 (C=O)	2.40 (3H, s, Me), 7.36 (2H, d, *J* 8.1, Ar-*m*H), 7.41 (1H, d, *J* 5.7, 9-H), 7.51 (1H, d, *J* 5.7, 8-H), 8.09 (2H, d, *J* 8.1, Ar-*o*H),13.09 (1H, br s, NH).
6h	3090 (NH) 1710 (C=O)	3.84 (3H, s, OMe), 7.09 (2H, d, *J* 8.7, Ar-*m*H), 7.40 (1H, d, *J* 5.7, 9-H), 7.51 (1H, d, *J* 5.7, 8-H), 8.12 (2H, d, *J* 8.7, Ar-*o*H), 13.05 (1H, br s, NH).
6i	3480 (OH) 3090 (NH) 1720 (C=O)	6.90 (2H, d, *J* 8.7, Ar-*m*H), 7.39 (1H, d, *J* 5.7, 9-H), 7.50 (1H, d, *J* 5.7, 8-H), 8.01 (2H, d, *J* 8.7, Ar-*o*H), 9.91 (1H, s, OH), 13.02 (1H, br s, NH).
6j	3095 (NH) 1730 (C=O)	7.43 (1H, d, *J* 5.7, 9-H), 7.53 (1H, d, *J* 5.7, 8-H), 8.38 (2H, d, *J* 9.0, Ar-*o*H), 8.43 (2H, d, *J* 9.0, Ar-*m*H), 13.18 (1H, br s, NH).

We have established an efficient and reliable synthesis of thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-ones (**6a**) and its 2-substituted derivatives (**6b–j**) by instantaneous isomerization of their [4,3-*c*] compounds (**7a–j**). The structures of some compounds (**6a–c**) were confirmed by synthesis from the reaction of 3-amino-4-imino-2-oxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine (**14**) with appropriate triethyl orthoesters. The isomer, 6-methylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(6*H*)-one (**7a**), and its 2-substituted derivatives (**7b,c**) were prepared in a similar manner as above. Antitumor activities for compounds (**6**) against CCRF-HSB-2 and KB cancer cells were evaluated *in vitro* and the most

Table 5 Physical and analytical data for the compounds (**7a–c**)

Compd No.	R	Yield (%)	Mp[a] (°C)	Formula (R_f)[b]	Analysis (%) Calcd (Found) C	H	N
7a	H	82% (*B*)	240	$C_8H_6N_4OS$ 0.48	46.59 (46.57	2.93 3.13	27.17 26.80)
7b	Me	83% (*B*)	265	$C_9H_8N_4OS \cdot 2/5\ H_2O$ 0.50	47.52 (47.88	3.90 3.75	24.63 24.52)
7c	Ph	57% (*A*) 75% (*B*)	195	$C_{14}H_{10}N_4OS$ 0.51	59.56 (59.57	3.57 3.75	19.85 19.62)

[a]Each yield of (A) and (B) was obtained by *Method A and Method B,* respectively, as shown in **Scheme 3**. [b]All compounds were recrystallized from EtOH and were obtained as colorless needles. [c]Solvent system for TLC: AcOEt.

Table 6 IR and ^{1}H-NMR spectroscopic data for the compounds (**7a–c**)

Compd No.	ν_{max} (Nujol)/cm^{-1}	δH [300 MHz; [$(CD_3)_2SO$; Me_4Si]
7a	1705 (C=O)	3.64 (3H, s, Me), 7.52 (1H, d, *J* 5.4,9-H), 7.61 (1H, d, *J* 5.4, 8-H), 9.26 (1H, s, 3-H)
7b	1710 (C=O)	2.77 (3H, s, C-Me), 3.59 (3H, s, N-Me), 7.45 (1H, d, *J* 4.8, 9-H), 7.52 (1H, d, *J* 4.8, 8-H)
7c	1720 (C=O)	3.59 (3H, s, Me), 7.48–7.53 (3H, m, Ph-*m,p*H), 7.60 (1H, d, *J* 5.4, 9-H), 7.63 (1H, d, *J* 5.4, 8-H), 7.71–7.73 (2H, m, Ph-*o*H)

compounds showed moderate antitumor activities. The compounds (**6a–j** and **7a–c**) were also examined for the XO inhibitory activities against bovine milk xanthine oxidase *in vitro* and **6a–j** were mostly found better activities compared to allopurinol. However, the compounds (**7a–c**) were slightly less active than that of allopurinol.

EXPERIMENTAL

Mps were determined using a Yanagimoto digital-micro-melting point apparatus MP 500D and were uncorrected. IR spectra were recorded using a JASCO FT/ IR-200 spectrophotometer as Nujol mulls.

Table 7 Ultraviolet spectra of compounds (**6a–c** and **7a–c**)

Compound No.	ν_{max}/nm (log ε/dm^{3}mol^{-1}cm^{-1})[a]	Compound No.	ν_{max}/nm (log ε/dm^{3}mol^{-1}cm^{-1})[a]
6a	*225* (4.44), 271 (4.03), *280* (4.04)	**7a**	*244* (4.27), *251* (4.20), 273 (4.32)
6b	233 (4.50), 272 (4.09), 280 (4.08)	**7b**	*245* (4.40), *253* (4.28), 274 (4.40)
6c	241 (4.62), *279* (4.01), *291* (3.96)	**7c**	*250* (4.34), *277* (4.34), 285 (4.36)

[a]All UV spectra were measured in EtOH. The italic values refer to wave lenths at which shoulders or inflections occur in the absorption.

Table 8 Evaluation of antitumor activities in vitro for compounds (**11c** and **6c–j**)

Compd No.	Inhibitory concentration against tumor cell lines [IC_{50} (μg/mL)]		Compd No.	Inhibitory concentration against tumor cell lines [IC_{50} (μg/mL)]	
	CCRF-HSB-2	KB		CCRF-HSB-2	KB
11c	>100	>100	**6g**	40.7	57.7
6c	20.6	17.9	**6h**	21.1	25.7
6d	22.3	19.3	**6i**	17.5	12.1
6e	>100	92.3	**6j**	16.6	22.8
6f	20.5	19.6	**Ara-C**[a]	0.047	0.23

[a]Ara-C: arabinosylcytidine.

Mass spectra were recorded at 70eV ionizing voltage with FAB ionization using a VG-70SE spectrometer and glycerol as a matrix. ^{1}H-NMR spectra were obtained using a Varian VXR 300 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. UV spectra were recorded in EtOH with a Hitachi U-2001 spectrophotometer. Microanalyses were measured by a Yanako CHN Corder MT-5 apparatus. All reagents were of commercial quality from freshly opened containers and were used without further purification. Reaction progress was monitored by analytical thin layer chromatography (TLC) on pre-coated glass plates (silica gel 70 FM Plate-Wako) using the solvent systems of A [AcOEt], B [AcOEt : EtOH (6:1)], C [AcOEt : EtOH (4:1)], D [AcOEt : EtOH (1:1)], and E [EtOH] unless being cited in the table and products were visualized by UV light. Column chromatography was accomplished on Daisogel IR-60 (63/ 210 μm, Daiso Co.). The reaction temperatures are indicated as the temperature of the oil bath.

2-Oxo-4-thioxo-1,2,3,4-tetrahydrothieno[2,3-*d*] pyrimidine (9)

A mixture of thieno[2,3-*d*]pyrimidin-2,4(1*H*,3*H*)-dione **(8)**[17] (0.5 g, 2.97 mmol) and 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent 1.25 g, 2.97 mmol) in 1,4-dioxane (10 mL) was heated under reflux for 20 min. After the reaction was complete the solution was evaporated *in vacuo* and the residue was triturated with AcOEt to give crystals, which were collected by filtration and recrystallized from EtOH to afford the 4-thioxo derivative **(9)** (0.45 g, 82%) as pale yellow powdery crystals, mp 303–305 °C (lit.[18] mp 300 °C); R_f (B) 0.70; IR (Nujol) ν_{max}/cm^{-1}: 3160, 3080 (NH), 1700 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 7.12 (1H, d, *J* 5.6, 5-H), 7.26 (1H, d, *J* 5.6, 6-H), 12.49 (2H, br s, 2 x NH); *Anal*. Calcd for $C_6H_4N_2OS_2$: C, 39.11; H, 2.19; N, 15.20, Found: C, 39.26; H, 2.38; N, 15.19; MS (FAB, glycerol matrix): m/z = 185 (MH^+).

4-Hydrazinothieno[2,3-*d*]pyrimidin-2(1*H*)-one (10)

A mixture of the 4-thioxo derivative **(9)** (1.0 g, 5.43 mmol) and hydrazine monohydrate (0.8 g, 16 mmol) in water (10 mL) was heated under reflux for 1.5 h. Upon cooling to rt after the reaction was complete, the resulting precipitates were collected by filtration, washed with water and EtOH, and crystallized from water to afford the hydrazine derivative **(10)** (0.69 g, 70%) as colorless powdery crystals, mp 238–241 °C; R_f (C) 0.30; IR (Nujol) ν_{max} or δ_{max}/cm^{-1}: 3320 (ν_{as}, NH_2), 3200 (ν_s, NH_2), 3100, 3065 (ν, NH), 1650 (ν, C=O), 1620 (δ, NH_2); ^{1}H-NMR [$(CD_3)_2SO$] δ: 5.83 (2H, br s, NH_2), 6.96 (1H, d, *J* 5.4, 5-H), 7.13 (1H, d, *J* 5.4, 6-H), 9.65 (2H, br s, 2 x NH); *Anal*. Calcd for $C_6H_6N_4OS$: C, 39.55; H, 3.32; N, 30.75, Found: C, 39.41; H, 3.56; N, 30.48; MS (FAB, glycerol matrix): m/z = 183 (MH^+).

General procedure for the preparation of 4-(benzylidenehydrazino)thieno[2,3-*d*]pyrimidin-2(1*H*)-one (11c) and its 4-(4-substituted benzylidenehydrazino) derivatives (11d–j)

A mixture of the hydrazine derivative (**10**) (0.2 g, 1.1 mmol) and an appropriate arylaldehyde (1.31 mmol) in MeOH (20 mL) was stirred at rt for 8-10 h. After the reaction was complete, the precipitated crystals were collected by filtration and washed with AcOEt, and recrystallized from a mixture of EtOH and DMF to afford the corresponding hydrazones **(11c–j)** as shown in Tables 1 and 2.

General procedure for the preparation of thieno[3,2-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5(6*H*)-one (6a) and its 2-substitued derivatives (6b–j)

(1) *Method A*: a solution of an appropriate arylaldehyde hydrazone **(11c–j)** (0.6 mmol) with chloranil (0.22 g, 0.9 mmol) in AcOH (20–30 mL) was refluxed for 15 min except for **11g**, which was refluxed for 60 min. After the reaction was complete, the solvent was evaporated *in vacuo* to afford the solid. The resulting solid was washed with EtOH–Et_2O mixture and crystallized from an appropriate solvent to give the corresponding thienotriazolopyrimidines **(6c–j)** as shown in Tables 3 and 4.

(2) *Method B*: a mixture of the hydrazino derivative **(10)** (0.2 g, 1.1 mmol) with an appropriate triethyl orthoester (1.32 mmol) in DMF (4 mL) was refluxed for 30 min. After cooling, the precipitated crystals were collected by filtration, washed with EtOH and recrystallized from EtOH to give the corresponding thienotriazolopyrimidines **(6a–c)** as shown in Tables 3, 4 and 7.

(3) *Method C*: a mixture of 3-amino-4-imino-2-oxo-1,2,3,4-tetrahdrothieno[2,3-*d*]pyrimidine **(14)** (0.2 g, 1.1 mmol) with an appropriate triethyl orthoester (11 mmol) in AcOH (5 mL) was heated at 80 °C for 10–60 min. After cooling, the precipitated crystals were collected by filtration, washed with EtOH-H_2O mixture and recrystallized from EtOH to give the corresponding products **(6a–c)** as shown in Tables 3 and 4.

4-(Methylthio)thieno[2,3-*d*]pyrimidin-2(1*H*)-one (12)

To a cooling solution of the 4-thioxo derivative **(9)** (0.5 g, 2.71 mmol) in 2N NaOH solution (5 mL) at 5 °C was added MeI (1.15 g, 8.10 mmol) dropwise. The solution was vigorously shaken for 10 min to afford precipitates. The precipitates were collected by filtration, dissolved in hot water and neutralized with 10% HCl solution. The precipitated crystals thus obtained were collected by filtration and dried under reduced pressure to yield the 4-methylthio derivative **(12)** (0.30 g, 56%) as colorless powdery crystals (from EtOH), mp > 277 °C (decomp.); R_f (C) 0.65; IR (Nujol) ν_{max}/cm^{-1}: 3080 (NH), 1640 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 2.58 (3H, s, Me), 7.19 (1H, d, *J* 5.7, 5-H), 7.29 (1H, d, *J* 5.7, 6-H), 12.22 (1H, br s, NH); *Anal*. Calcd for $C_7H_6N_2OS_2 \cdot 1/3H_2O$: C, 41.16; H, 3.29; N, 13.71, Found: C, 41.13; H, 3.00; N, 13.61; MS (FAB, glycerol matrix): m/z = 199 (MH^+).

4-Aminothieno[2,3-*d*]pyrimidin-2(1*H*)-one (13)

A mixture of the 4-methylthio derivative **(12)** (1.0 g, 5.04 mmol) and 28% aqueous NH_3 (50 mL) was heated in a steel sealed tube at 150 °C (10 kg/cm^2) pressure for 7 h. After the reaction was complete, the precipitated crystals were collected by filtration, washed with EtOH and recrystallized from 70% aqueous DMF to afford the 4-amino derivative **(13)** (0.56 g, 67%) as colorless powdery crystals, mp > 300 °C; R_f

(C) 0.60; IR (Nujol) ν_{max}/cm^{-1}: 3320 (ν_{as}, NH_2), 3120 (ν_s, NH_2), 3080 (ν NH), 1650 (ν, C=O), 1630 (δ, NH_2); ^{1}H-NMR [$(CD_3)_2SO$] δ: 6.97 (1H, d, *J* 5.4, 5-H), 7.32 (1H, d, *J* 5.4, 6-H), 7.70 (2H, br s, NH_2), 11.50 (2H, br s, 2 x NH); *Anal.* Calcd for $C_6H_5N_3OS \cdot 1/4H_2O$: C, 41.97; H, 3.23; N, 24.47, Found: C, 42.24; H, 3.20; N, 24.35; MS (FAB, glycerol matrix): m/z = 168 (MH^+).

3-Amino-4-imino-2-oxo-1,2,3,4-tetrahydrothieno[2,3-*d*]pyrimidine (14)

To a solution of 4-amino derivative **(13)** (0.3 g, 1.97 mmol) in 1N NaOH solution (9 mL) at rt was added a solution of hydroxylamine-O-sulfonic acid (0.6 g, 5.31 mmol) in water (6 mL) dropwise. Then, the solution was stirred at rt for 1.5 h to give the precipitates. The precipitates were collected by filtration and washed with water to afford the 3-amino derivative **(14)** (0.13 g, 39%) as colorless powdery crystals, mp 260 °C; R_f (D) 0.50; IR (Nujol) ν_{max}/cm^{-1}: 3400 (ν_{as}, NH_2), 3320 (ν_s, NH_2), 3170, 3080 (ν NH), 1665 (ν, C=O), 1620 (δ, NH_2); ^{1}H-NMR [$(CD_3)_2SO$] δ: 6.86 (1H, d, *J* 6.0, 5-H), 7.28 (1H, d, *J* 6.0, 6-H), 7.46 (2H, br s, NH_2), 8.05 and 8.45 (each 1H, each br s, 2 x NH); MS (FAB, glycerol matrix): m/z = 183 (MH^+). The product **(14)** 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.

1-Methyl-4-(methylthio)thieno[2,3-*d*]pyrimidin-2(1*H*)-one (15)

To a solution of 4-(methylthio)thieno[2,3-*d*]pyrimidin-2(1*H*)-one **(12)** (0.25 g, 1.26 mmol) in dry DMF (10 mL) were added 60% NaH (oil dispersion, 0.07 g, 1.75 mmol) and MeI (1.15 g, 8.10 mmol) and the solution was heated 40 °C for 12 h. After the reaction was complete, the solution was evaporated in *vacuo*. The residue was treated with water and the resulting crystals were collected by filtration, washed with water and recrystallized from AcOEt to afford 1-methyl-4-(methylthio)thieno[2,3-*d*]pyrimidin-2-(1*H*)one **(15)** (0.2 g, 75%) as colorless fine crystals, mp 257–258 °C; R_f (A) 0.38; IR (Nujol) ν_{max}/cm^{-1}: 1640 (C=O); ^{1}H-NMR ($CDCl_3$) δ: 2.67 (3H, s, S-Me), 3.66 (3H, s, N-Me), 6.92 (1H, d, *J* 5.7, 5-H), 7.19 (1H, d, *J* 5.7, 6-H); *Anal.* Calcd for $C_8H_8N_2OS_2$: C, 45.26; H, 3.80; N, 13.20, Found: C, 45.29; H, 3.83; N, 13.11.

4-Hydrazino-1-methylthieno[2,3-*d*]pyrimidin-2(1*H*)-one (16)

A mixture of the 4-methylthio derivative **(15)** (0.25 g, 1.18 mmol) and 80% hydrazine monohydrate (0.07 g, 1.18 mmol) in EtOH was refluxed for 2.5–3 h. During the reaction was carrying out, the crystals

deposited gradually. After ensuring complete consumption of the starting material **(15)**, the mixture was cooled at rt. The resulting crystals were collected by filtration, washed with EtOH-H_2O mixture and recrystallized from EtOH-DMF mixture to yield the hydrazino derivative **(16)** (0.12 g, 52%) as colorless powdery crystals, mp > 300 °C; R_f (E) 0.12; IR (Nujol) ν_{max} or ν_{max}/cm^{-1}: 3260 (ν_{as}, NH_2), 3200 (ν_s, NH_2), 3100, 3080 (ν NH), 1685 (ν, C=O), 1620 (δ, NH_2); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.39 (3H, s, N-Me), 6.77 (2H, br s, NH_2), 7.11 (1H, d, *J* 5.4, 7-H), 7.32 (1H, d, *J* 5.4, 6-H), 10.03 (1H, br s, NH); *Anal*. Calcd for $C_7H_8N_4OS \cdot 5/9H_2O$: C, 40.77; H, 4.45; N, 27.17, Found: C, 41.03; H, 4.08; N, 26.82.

4-(Benzylidenehydrazino)-1-methylthieno[2,3-*d*]pyrimidin-2(1*H*)-one (17)

A mixture of the hydrazino derivative **(16)** (0.2 g, 1.02 mmol) and benzaldehyde (0.14 g, 1.32 mmol) in DMF was stirred at rt for 12 h. After the reaction was complete, the precipitate was collected by filtration and crystallized from EtOH to afford the blue powdery crystals of the 4-benzylidenehydrazino derivative **(17)** (0.19 g, 66%), mp 210–212 °C; R_f (A) 0.27; IR (Nujol) ν_{max}/cm^{-1}: 3280, 3120 (NH), 1690 (C=O); ^{1}H-NMR [$(CD_3)_2SO$] δ: 3.40 (3H, s, N-Me), 7.22 (1H, d, *J* 5.4, 6-H), 7.26 (1H, d, *J* 5.4, 5-H), 7.41–7.43 (3H, m, Ar-*m*,*p*H), 8.01–8.04 (2H, m, Ar-*o*H), 8.44 (1H, s, N=C*H*Ar), 10.57 (1H, br s, NH); *Anal*. Calcd for $C_{14}H_{22}N_4OS$: C, 59.14; H, 4.25; N, 19.70, Found: C, 58.94; H, 4.36; N, 19.64.

General procedure for the preparation of 6-methylthieno[3,2-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-5(1*H*)-one (7a) and its 3-substituted derivative (7b,c)

(1) *Method A*: a solution of the benzaldehyde hydrazone (0.2 g, 0.71 mmol) **(17)** with choloranil (0.22 g, 0.9 mmol) in CH_3CO_2H (10 mL) was refluxed for 30 min. After the reaction was complete, water was added to the solution and the crystals deposited immediately were collected by filtration. The product was purified by column chromatography (eluent: AcOEt) to give the pure thienotriazolopyrimidine **(7c)** as shown in Tables 5 and 6.

(2) *Method B*: a mixture of the hydrazino derivatives **(16)** (0.2 g, 1.02 mmol) with an appropriate triethyl orthoester (2 mmol) in DMF (4 mL) was refluxed for 30 min. After the reaction was complete, small amount of water was added to the solution and the solution was kept overnight. The needless fine crystals deposited were collected by filtration, washed with H_2O and recrystallized from EtOH to afford the thienotriazolopyrimidine **(7a–c)** as shown in Tables 5, 6 and 7.

ACKNOWLEDGEMENTS

The authors are indebted to the SC-NMR Laboratory of Okayama University for the NMR spectral measurements.

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