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Thiazolo[5,4-*d*]pyrimidine Derivatives as Amplifiers of Phleomycin against *Escherichia coli*

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Abstract

The acetylation of 5-amino-6-methylpyrimidine-2,4-dithione (1; R = H) followed by cyclodehydration gave 2,7-dimethylthiazolo[5,4-*d*]pyrimidine-5-thione (2; R = Me) which underwent *S*-alkylation by methyl iodide, 2-chloroacetamide, etc. to afford the corresponding 5-alkylthio derivatives (4a–c). Treatment of the same substrate with carbon disulphide provided 7-methylthiazolo[5,4-*d*]pyrimidine-2,5-dithione (6) and thence the corresponding 2,5-bisalkylthio derivatives (4d–g). Similar reactions with 5-amino-6-mercapto-2-methylpyrimidine-4-thione (5; R^1 = Me, R^2 = SH, R^3 = H) gave 7-alkylthio-2,5-dimethylthiazolo[5,4-*d*]pyrimidines (4h–p) and 2,7-bisalkylthio-5-methylthiazolo[5,4-*d*]pyrimidines [(4q) and (4r)]. Two 2-alkylthio-7-methoxythiazolo-[5,4-*d*]pyrimidines, (4s) and (4t), were made by similar methods.

The foregoing compounds were tested *in vitro* as amplifiers of phleomycin against *E. coli*. Some of the more soluble compounds showed activities comparable with those of the best purine amplifiers previously tested.

Introduction

In previous publications, we have described how the antibacterial activity of phleomycin toward *Escherichia coli* was enhanced *in vitro* by the addition of certain purines.[1–4] To develop the possibility[1] of using phleomycin and a purine amplifier against infections of the kidney or urinary tract, the metabolism of several alkylthiopurines was studied both in mice[2,5–7] and in rats.[7] Although these purines showed encouraging activity *in vitro*, for use *in vivo* it was clearly essential that amplifiers reach the urinary tract, either unaltered or as active metabolites. The introduction of *C*-methyl substituents into the purine ring was found to discourage metabolic hydroxylation but the alkylthiopurines still underwent oxidative metabolism in mice to yield the corresponding (inactive) sulphoxides;[2] this was partly corrected by the incorporation of a carbamoyl group on to the alkylthio substituent.[5–7]

[1] Grigg, G. W., *Mol. Gen. Genet.*, 1970, **107**, 162; Grigg, G. W., Edwards, M. J., and Brown, D. J., *J. Bacteriol.*, 1971, **107**, 599; Grigg, G. W., *J. Gen. Microbiol.*, 1972, **70**, 221.
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[6] Bhushan, K., Brown, D. J., Lister, J. H., Stephanson, L. G., and Yoneda, F., *Aust. J. Chem.*, 1975, **28**, 2553.
[7] Brown, D. J., and Stephanson, L. G., *Aust. J. Chem.*, 1976, **29**, 1031.

In our search for effective amplifiers of phleomycin we are extending our studies to include hetero analogues of the active purines. In the present paper we report syntheses and potentiating activities for some methylthiothiazolo[5,4-*d*]pyrimidines (4), some of which have one or two carbamoyl groups attached to the *S*-methyl substituent. In addition, initial observations on the toxicity and metabolism of one highly active thiazolo[5,4-*d*]pyrimidine (4p) are recorded.

Syntheses

Preparations of some simple 2-, 5-, and 7-alkylthiothiazolo[5,4-*d*]pyrimidines, and of 2,5- and 2,7-bisalkylthiothiazolo[5,4-*d*]pyrimidines have been reported[8–11] but none of their 5- or 7-methylated derivatives has been made. In view of the fact that such *C*-methylation proved essential to prevent metabolic hydroxylation in purines,[2] we have made a selection of 5-alkylthio- and 2,5-bisalkylthio-7-methylthiazolo[5,4-*d*]-pyrimidines and also 7-alkylthio- and 2,7-bisalkylthio-5-methylthiazolo[5,4-*d*]-pyrimidines for evaluation as amplifiers of phleomycin.

Refluxing 5-amino-6-methylpyrimidine-2,4(1*H*,3*H*)-dithione[12] (1; R = H) with acetic anhydride gave the acetyl derivative (1; R = Ac), which remained uncyclized on prolonged refluxing in acetic anhydride, on fusion, or even on sublimation at 280°/1 mm. However, cyclodehydration occurred, on stirring in concentrated sulphuric acid at 25° or on refluxing with phosphorus pentasulphide in pyridine, to give the thiazolo[5,4-*d*]pyrimidine-5-thione (2; R = Me). Alkylation thence gave the thioethers (4a–c). An alternative and unexpected synthesis of the thioether (4a) occurred on refluxing the bismethylthio derivative (3; R = Me) with phosphorus pentasulphide in pyridine; this presumably occurred through a thioacetamido intermediate which underwent displacement of its 4-methylthio substituent by the nucleophilic sulphur atom of the 5-thioacetamido group.

In contrast, refluxing the dithione (1; R = H) with formic acid gave the formylated pyrimidine (1; R = CHO) which did not cyclize on treatment with concentrated sulphuric acid or with phosphorus pentasulphide in refluxing pyridine. In the latter case, a thioformamido derivative could not be detected in the reaction products. However, treatment of the *S*-methylated formyl derivative (3; R = H) under the same conditions with phosphorus pentasulphide gave *N*-(4′-mercapto-6′-methyl-2′-methylthiopyrimidin-5′-yl)formamide (5; R^1 = SMe, R^2 = Me, R^3 = CHO), probably by cyclization to the fused thiazole followed by hydrolysis during the aqueous workup.

An attempt was made to increase the water solubility of compounds (4a–c) by introducing hydrophilic hydroxymethyl substituents: fusion of the pyrimidine-dithione (1; R = H) with glycolic acid did not give any identifiable products but the α-hydroxyacetylated pyrimidine (1; R = $COCH_2OH$) was prepared by treating the amine (1; R = H) with acetoxyacetyl chloride[13] in pyridine at room temperature followed by hydrolysis of the acetoxy group under acidic conditions. Cyclization to the fused thiazole (2; R = CH_2OH) was effected in sulphuric acid but subsequent

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[9] Naito, T., and Inoue, S., *Chem. Pharm. Bull.*, 1958, **6**, 338.
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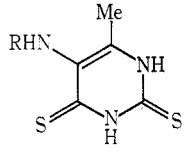
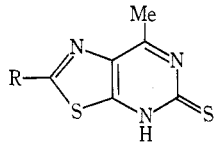
[13] Gatenbeck, S., *Acta Chem. Scand.*, 1955, **9**, 709.

methylation under mildly basic conditions did not give the methylthio derivative; instead it caused hydrolytic fission at the 2-position to afford the bismethylthiopyrimidine (3; R = CH_2OH).



(1)



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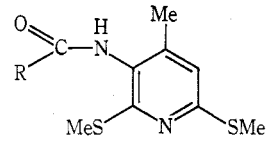

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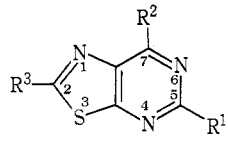
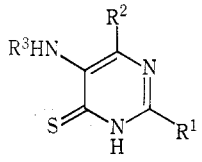


	R^1	R^2	R^3		R^1	R^2	R^3
(4a)	SMe	Me	Me	(4k)	Me	SMe	Me
(4b)	SCH_2CONH_2	Me	Me	(4l)	Me	SCH_2CONH_2	Me
(4c)	$SCHMeCONH_2$	Me	Me	(4m)	Me	$SCHMeCONH_2$	Me
(4d)	SMe	Me	SMe	(4n)	Me	SMe	CH_2OH
(4e)	SCH_2CONH_2	Me	SCH_2CONH_2	(4o)	Me	SCH_2CONH_2	CH_2OH
(4f)	$SCH(CONH_2)_2$	Me	$SCH(CONH_2)_2$	(4p)	Me	$SCHMeCONH_2$	CH_2OH
(4g)	SMe	Me	SCH_2CONH_2	(4q)	Me	SMe	SMe
(4h)	Me	SMe	H	(4r)	Me	SCH_2CONH_2	SCH_2CONH_2
(4i)	Me	SCH_2CONH_2	H	(4s)	Me	OMe	SMe
(4j)	Me	$SCHMeCONH_2$	H	(4t)	Me	OMe	SCH_2CONH_2



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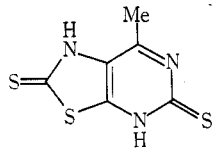

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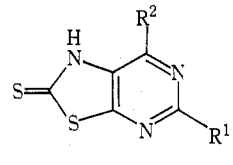

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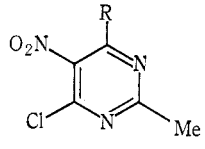

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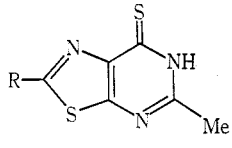

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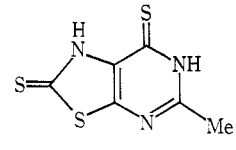

(10)

7-Methylthiazolo[5,4-*d*]pyrimidine-2,5(1*H*,4*H*)-dithione (6) was prepared from the pyrimidine (1; R = H) and potassium methylxanthate; subsequent treatment with two moles of appropriate alkylating agent gave the bisalkylthiothiazolopyrimidines (4d–f). Direct treatment of the formylated bismethylthiopyrimidine (3; R = H) with

potassium methylxanthate gave 7-methyl-5-methylthiothiazolo[5,4-*d*]pyrimidine-2(1*H*)-thione (7; R^1 = SMe, R^2 = Me) by addition of CS_2 with loss of the formyl and a methylthio group. Methylation of the thione gave the bismethylthio derivative (4d), whereas alkylation with chloroacetamide afforded the amide (4g) with two different alkylthio substituents.

5-Amino-6-mercapto-2-methylpyrimidine-4(3*H*)-thione (5; R^1 = Me, R^2 = SH, R^3 = H) prepared by the action of sodium hydrogen sulphide on the pyrimidine (8; R = Cl) readily gave the thiazolopyrimidine-7-thiones (9; R = H or Me) on refluxing in formic acid or acetic anhydride respectively. The reaction of the same pyrimidinethione with acetoxyacetyl chloride followed by boiling in dilute hydrochloric acid also gave the thiazolopyrimidine (9; R = Me). In these reactions none of the acyl intermediates was detected. The apparent ease with which 5-amino-6-mercapto-4-thiones gave cyclized products, compared with the isomeric 5-aminopyrimidine-2,4-dithiones, has been noted previously.[10,11]

Treatment of the thiones (9; R = H, Me or CH_2OH) with appropriate alkylating agents gave the 7-alkylthiothiazolopyrimidines (4i–p). Hydrolytic fission of the thiazole ring was not observed in any of these reactions.

The action of potassium methylxanthate on the pyrimidinethione (5; R^1 = Me, R^2 = SH, R^3 = H) gave 5-methylthiazolo[5,4-*d*]pyrimidine-2,7(1*H*,6*H*)-dithione (10) which afforded the bisalkylthio derivatives (4q) and (4r). The 7-methoxy analogues (4s) and (4t) were prepared by the sequence (8; R = Cl) → (8; R = OMe) → (5; R^1 = Me, R^2 = OMe, R^3 = H) → (7; R^1 = Me, R^2 = OMe) → (4s) and (4t) in which only the first step was known.

Table 1. Activities of thiazolo[5,4-*d*]pyrimidines as amplifiers of phleomycin

Measured at 2 mM; for definitions of activity and adjusted activity (A_{ad}) see ref.[4]

Compound[A]	Activity	A_{ad}	Compound[A]	Activity	A_{ad}
(4a)	++++	*300*	(4l)	++++	*178*
(4e)	++++	*650*	(4o)	++	*23*
(4i)	++++	*260*	(4p)	++++	*14*, 710[B]
(4j)	++	*20*	(4t)	+++	*54*
(4k)	++	*12*	caffeine	++	*11*, 30[B]

[A] Compounds omitted from series (4a–t) were soluble < 2 mM. [B] Measured at 8 mM; others too insoluble.

Biological Results and Discussion

All the thiazolo[5,4-*d*]pyrimidines (4a–t) were submitted to *in vitro* testing as amplifiers of phleomycin, by techniques previously outlined.[4] The results for those which proved sufficiently soluble (Table 1) indicated five very highly active amplifiers of which four (4e,i,l,p) bore carbamoylalkylthio groups, known to discourage metabolic *S*-oxidation. The activities for two of these, (4e) and (4p), were at least comparable with values[4] for the best group of purines tested to date: thus, inclusion of 2 mM (4e) or 8 mM (4p) in a culture of stationary-phase *E. coli* cells containing phleomycin (2μg/ml) induced a 600- to 700-fold decrease in viable cell count within two hours.

The compound (4p) also showed a remarkable phenomenon: its adjusted activity at 2 mM rose no less than 50-fold on increasing the concentration a mere 4-fold, to

8 mM; for comparison, previous amplifiers have shown[2] only a 1·2- to 11-fold increase over the same concentration range. This underlines the fact[4] that our pragmatic method of testing has taken little account of the optimum concentration for each amplifier or of any optimum value for [amplifier]/[phleomycin], mainly due to the very limited aqueous solubilities inherent in the more active purines and thiazolo[5,4-*d*]-pyrimidines.

Before undertaking any quantitative *in vivo* studies on toxicity or metabolism of the active thiazolo[5,4-*d*]pyrimidines, a preliminary trial was made as follows. An oral dose of the relatively soluble amplifier (4p) was administered to four mice, corresponding to 500 mg/mouse kg: none showed any ill effect. Their urine was collected for 48 h and concentrated prior to t.l.c. (alumina; chloroform/ethanol) and paper chromatography (butanol/acetic acid): only a minute fraction of the ingested amplifier appeared as an appropriate spot on each system, suggesting wholesale metabolic modification or destruction. In view of the above, *in vivo* investigations on thiazolo-[5,4-*d*]pyrimidines have been suspended in favour of other hetero analogues which appear to combine equally high *in vitro* activity and low toxicity with good aqueous solubility, and high metabolic stability.

Experimental

Analyses were performed by the Australian National University Analytical Services Unit. Most of the intermediate pyrimidine- and thiazolo[5,4-*d*]pyrimidine-thiones proved unusually difficult both to purify and to analyse satisfactorily: accordingly, they have been characterized properly only as their *S*-alkylated derivatives. The infrared spectra (cm^{-1}) were recorded from Nujol mulls on a Unicam SP200 instrument. All melting points were uncorrected. Mass spectra were kindly measured by Dr M. D. Fenn or by Dr J. K. MacLeod (A.N.U. Research School of Chemistry). N.m.r. spectra (chemical shifts in δ; J values in Hz; peaks singlets except when otherwise indicated) were measured in $(CD_3)_2SO$ at 60 MHz and 35° on a Varian T60A instrument with Me_4Si as internal standard.

N-(*2′,4′-Dimercapto-6′-methylpyrimidin-5′-yl*)*acetamide* (*1*; *R* = *Ac*)

5-Amino-6-methylpyrimidine-2,4-dithione (1; R = H)[12] and acetic anhydride (50 ml) were boiled under reflux for 30 min. On cooling, the product was collected and washed with a little acetic acid followed by water. Purification by dissolution in 0·5 N sodium hydroxide (250 ml), treatment with charcoal, and acidification with acetic acid, gave the *pyrimidinylacetamide* (61%) as yellow flakes, m.p. 344–347° (dec.) (Found: C, 39·3; H, 4·3; N, 19·3. $C_7H_9N_3OS_2$ requires C, 39·1; H, 4·2; N, 19·5%). ν_{max} 1670 (CO). N.m.r. 1·97, Ac; 2·20, 6′-Me. The substance was unchanged by sublimation at 280°/1 mm.

N-(*2′,4′-Dimercapto-6′-methylpyrimidin-5′-yl*)*formamide* (*1*; *R* = *CHO*)

The pyrimidinedithione (1; R = H) (6·0 g) and formic acid (98%; 50 ml) were boiled under reflux for 2 h and then chilled. Purification as for the homologue above gave the *pyrimidinylformamide* (62%), m.p. 285–287° (dec.) (Found: C, 35·4; H, 3·7. $C_6H_7N_3OS_2$ requires C, 35·8; H, 3·5%). ν_{max} 1660 (CO). N.m.r. 2·11, 6′-Me; 8·18, CHO. It was unaffected by sublimation at 260°/1 mm.

N-(*4′-Methyl-2′,6′-bismethylthiopyrimidin-5′-yl*)*acetamide* (*3*; *R* = *Me*)

N-(2′,4′-Dimercapto-6′-methylpyrimidin-5′-yl)acetamide (1; R = Ac) (2·15 g), methyl iodide (3·12 g), and sodium hydrogen carbonate (2·0 g) were shaken in water (30 ml) at 25° for 30 min. The colourless *bismethylthiopyrimidinylacetamide* (79%) had m.p. 171–173° (from water) (Found: C, 44·5; H, 5·4; N, 17·3. $C_9H_{13}N_3OS_2$ requires C, 44·4; H, 5·4; N, 17·3%). M^+ 243; ν_{max} 1670 (CO). N.m.r. 2·05, Ac; 2·18, 4′-Me; 2·45, 2·52, $(SMe)_2$.

N-(*4'-Methyl-2',6'-bismethylthiopyrimidin-5'-yl)formamide* (*3*; *R* = *H*)

Methylation of *N*-(2',4'-dimercapto-6'-methylpyrimidin-5'-yl)formamide (1; R = CHO) as above gave the *bismethylthiopyrimidinylformamide* (73%), m.p. 180–181° (Found: C, 42·2; H, 4·7; N, 18·6. $C_8H_{11}N_3OS_2$ requires C, 41·9; H, 4·8; N, 18·3%). M^+ 229; ν_{max} 1660 (CO). N.m.r. 2·13, 4'-Me; 2·40, 2·45, $(SMe)_2$.

N-(*4'-Methyl-2',6'-bismethylthiopyrimidin-5'-yl)glycolamide* (*3*; *R* = CH_2OH)

(i) The pyrimidinedithione (1; R = H) (1·73 g) was dissolved in dry pyridine (15 ml) and acetoxyacetyl chloride[13] (1·64 g) was added dropwise with stirring at 0°. Stirring was continued for an additional 30 min. The residue from evaporation was diluted with 2 N hydrochloric acid (30 ml) and the mixture was heated under reflux for 30 min. Cooling gave a solid which was dissolved in dilute sodium hydroxide (carbon) and reprecipitated with acid to give *N*-(2',4'-dimercapto-6'-methylpyrimidin-5'-yl)glycolamide (1; R = $COCH_2OH$) (44%) as a yellow powder, m.p. 293–295°. N.m.r. 2·02, Me; 3·97, CH_2. This compound (693 mg) was shaken with MeI (0·5 g) and 1 N sodium hydroxide (20 ml) for 45 min at 25°. The resulting *bismethylthiopyrimidinylglycolamide* (88%) had m.p. 183–184° (from ethanol) (Found: C, 41·8; H, 5·4; N, 16·1. $C_9H_{13}N_3O_2S_2$ requires C, 41·7; H, 5·1; N, 16·2%). ν_{max} 1685 (CO). N.m.r. 2·17, 4'-Me; 2·43, 2·50, $(SMe)_2$; 4·02, CH_2.

(ii) The above dimercaptopyrimidinylglycolamide (2·0 g) was stirred at 0° in concentrated sulphuric acid (10 ml) for 1 h. Dilution with ice (50 g) gave crude 2-hydroxymethyl-7-methylthiazolo-[5,4-*d*]pyrimidine-5-thione (2; R = CH_2OH) (66%), m.p. >300° (dec.). N.m.r. 2·09, Me; 3·99, CH_2. This compound (852 mg), methyl iodide (1·42 g) and sodium hydrogen carbonate (420 mg) were boiled for 30 min in a mixture of methanol (10 ml) and water (20 ml). Evaporation to 5 ml and subsequent addition of water (20 ml) gave the glycolamide (3; R = CH_2OH) (82%), identified by m.m.p.

2,7-Dimethyl-5-methylthiothiazolo[*5,4*-d]*pyrimidine* (*4a*)

(i) *N*-(2',4'-Dimercapto-6'-methylpyrimidin-5'-yl)acetamide (640 mg) and phosphorus pentasulphide (340 mg) were boiled under reflux for 5 h in anhydrous pyridine (25 ml). Water (10 ml) was added and heating was continued for 5 min. The residue from evaporation was diluted with water (20 ml) and crystallization was induced by the addition of several drops of dilute hydrochloric acid. The crude yellow 2,7-dimethylthiazolo[5,4-*d*]pyrimidine-5-thione (2; R = Me) (78%) had m.p. 320–322° (dec.). N.m.r. 2·49, 7-Me; 2·61, 2-Me. The same compound was prepared also by stirring the same substrate (3·0 g) in concentrated sulphuric acid (30 ml) for 1 h at 20°. Dilution with ice (300 g) gave the product (79%).

This thione (300 mg), methyl iodide (0·5 ml), sodium hydrogen carbonate (2 g), water (20 ml), and methanol (20 ml) were heated under reflux for 30 min. Evaporation to 10 ml gave the *dimethylmethylthiothiazolopyrimidine* (81%) as colourless needles, m.p. 88–90° (from light petroleum) (Found: C, 45·8; H, 4·1; N, 20·1. $C_8H_9N_3S_2$ requires C, 45·5; H, 4·3; N, 19·9%). M^+ 211. N.m.r. 2·53, SMe; 2·72, 7-Me; 2·75, 2-Me.

(ii) *N*-(4'-Methyl-2',6'-bismethylthiopyrimidin-5'-yl)acetamide (2·43 g) and phosphorus pentasulphide (1·22 g) were boiled under reflux in pyridine (50 ml) for 2 h. Water (50 ml) was added to the residue from evaporation. The solution was heated for 5 min on the steam bath and the product separated on cooling. Recrystallization gave the same product (44%) as in (i).

2-(2',7'-Dimethylthiazolo[*5,4*-d]*pyrimidin-5'-ylthio)acetamide* (*4b*)

Crude 2,7-dimethylthiazolo[5,4-*d*]pyrimidine-5-thione (740 mg), sodium hydrogen carbonate (420 mg), and 2-chloroacetamide (374 mg) were heated under reflux in water (50 ml) for 30 min. Chilling gave the *dimethylthiazolopyrimidinylthioacetamide* (61%), m.p. 187–188° (from water) (Found: C, 40·3; H, 4·3; N, 20·8. $C_9H_{10}N_4OS_2,0{\cdot}75H_2O$ requires C, 40·4; H, 4·3; N, 20·9%). N.m.r. 2·70, 7'-Me; 2·78, 2'-Me; 3·88, CH_2.

2-(2',7'-Dimethylthiazolo[*5,4*-d]*pyrimidin-5'-ylthio)propionamide* (*4c*)

A similar procedure using 2-bromopropionamide gave the *dimethylthiazolopyrimidinylthiopropionamide* (67·0%), m.p. 201–202° (Found: C, 44·5; H, 4·6; N, 20·7. $C_{10}H_{12}N_4OS_2$ requires C, 44·8; H, 4·5; N, 20·9%). N.m.r. 1·53, d, *J* 6·4, Me of Et; 2·77, 7'-Me; 2·82, 2'-Me; 4·50, q, *J* 6·4, CH.

N-(4'-Mercapto-6'-methyl-2'-methylthiopyrimidin-5'-yl)formamide (*5*; R^1 = *SMe*, R^2 = *Me*, R^3 = *CHO*)

N-(4'-Methyl-2',6'-bismethylthiopyrimidin-5'-yl)formamide (1·15 g), phosphorus pentasulphide (0·56 g) and pyridine (25 ml) were heated under reflux for 6 h. Water (10 ml) was added and heating was continued for 5 min. Evaporation and addition of 1 N hydrochloric acid (20 ml) gave crude mercaptomethylmethylthiopyrimidinylformamide as a yellow powder, m.p. 248–250° (dec.). ν_{max} 1665 (CO). N.m.r. 2·49, 6'-Me; 2·65, SMe. The identity of this product was confirmed by *S*-methylation to give back the original substrate in 86% yield.

7-Methyl-2,5-bismethylthiothiazolo[*5,4*-d]*pyrimidine* (*4d*)

The pyrimidinedithione (1; R = H) (10·5 g) was boiled under reflux for 12 h in a solution of potassium hydroxide (12·6 g), methanol (200 ml), water (8 ml) and carbon bisulphide (13·5 g). The residue from evaporation was diluted with water (150 ml) and treated with charcoal. Addition of acetic acid gave crude 7-methylthiazolo[5,4-*d*]pyrimidine-2,5-dithione as a yellow powder, m.p. >260° (dec.). N.m.r. 2·43, Me. *S*-Methylation (methyl iodide/1 N sodium hydroxide) gave the *methylbismethylthiothiazolopyrimidine* (72%), m.p. 126–128° (Found: C, 39·5; H, 4·1; N, 17·2. $C_8H_9N_3S_3$ requires C, 39·5; H, 3·7; N, 17·3%). M^+ 243. N.m.r. 2·51, 7-Me; 2·70, 5-SMe; 2·81, 2-SMe.

2-(5'-Carbamoylmethylthio-7'-methylthiazolo[*5,4*-d]*pyrimidin-2'-ylthio)acetamide* (*4e*)

The dithione (6) (1·08 g), sodium hydrogen carbonate (1·0 g), 2-chloroacetamide (0·94 g), and water (30 ml) were boiled for 1 h. Cooling gave the *product* (4e) (78%) as colourless prisms, m.p. 212–213° (from ethanol) (Found: C, 36·7; H, 3·5; N, 21·4. $C_{10}H_{11}N_5O_2S_3$ requires C, 36·5; H, 3·4; N, 21·3%).

2-{5'-(Dicarbamoylmethyl)thio-7'-methylthiazolo[*5,4*-d]*pyrimidin-2'-ylthio}malondiamide* (*4f*)

Similar treatment of the dithione (6) with 2-bromomalondiamide gave the *product* (4f), m.p. 243–245° (from ethanol) (Found: C, 34·6; H, 3·4; N, 23·3. $C_{12}H_{13}N_7O_4S_3$ requires C, 34·7; H, 3·2; N, 23·6%).

2-(7'-Methyl-5'-methylthiothiazolo[*5,4*-d]*pyrimidin-2'-ylthio]acetamide* (*2g*)

N-(4'-Methyl-2',6'-bismethylthiopyrimidin-5'-yl)formamide (1·15 g), potassium hydroxide (560 mg), carbon bisulphide (760 mg), and methanol (50 ml) were heated under reflux for 18 h. Evaporation, addition of water (50 ml) and acidification gave crude 7-methyl-5-methylthiothiazolo[5,4-*d*]-pyrimidine-2-thione (7; R^1 = SMe, R^2 = Me) (90%), m.p. 234–236°. N.m.r. 2·47, 2·52, Me_2. Treatment with 2-chloroacetamide in the usual way gave the *methylmethylthiothiazolopyrimidinylthioacetamide* (79%), m.p. 220–221° (from methanol) (Found: C, 37·8; H, 3·7; N, 19·4. $C_9H_{10}N_4OS_3$ requires C, 37·8; H, 3·5; N, 19·6%). M^+ 286. N.m.r. 2·52, 7-Me; 2·67, SMe; 4·12, CH_2.

The composition of the above thione (7; R^1 = SMe, R^2 = Me) was confirmed by *S*-methylation to the bismethylthio derivative (4d) (83%), described above.

5-Methyl-7-methylthiothiazolo[*5,4*-d]*pyrimidine* (*4h*)

4,6-Dichloro-2-methyl-5-nitropyrimidine[14] (30 g) in dioxan (100 ml) was added to stirred 2 M sodium hydrogen sulphide (750 ml) saturated with hydrogen sulphide. After 30 min the mixture was warmed on the steam bath with stirring for 2 h. Acidification of the cooled solution gave a solid. This was partly purified by dissolution in dilute sodium hydroxide, treatment with charcoal and reprecipitation with acetic acid to give crude 5-amino-6-mercapto-2-methylpyrimidine-4-thione (5; R^1 = Me, R^2 = SH, R^3 = H) (95%), m.p. ≮360° (dec.). This compound (5·0 g) and formic acid (98%; 50 ml) were boiled under reflux for 3 h. Cooling gave crude 5-methylthiazolo[5,4-*d*]pyrimidine-7-thione (9; R = H) (81%), m.p. 267–269°. N.m.r. 2·50. Me; 9·27, H2. Subsequent *S*-methylation gave the *methylmethylthiothiazolopyrimidine* (89%), m.p. 173–174° (from aqueous ethanol) (Found: C, 42·7; H, 4·0; N, 21·5. $C_7H_7N_3S_2$ requires C, 42·6; H, 3·6; N, 21·3%). M^+ 197. N.m.r. 2·65, 5-Me; 2·70, SMe; 9·45, H2.

[14] Albert, A., Brown, D. J., and Wood, H. C. S., *J. Chem. Soc.*, 1954, 3832.

*2-(5′-Methylthiazolo[5,4-*d*]pyrimidin-7′-ylthio)-acetamide and -propionamide (4i) and (4j)*

5-Methylthiazolo[5,4-*d*]pyrimidine-7-thione (550 mg), sodium hydrogen carbonate (420 mg), water (50 ml) and either 2-chloroacetamide (327 mg) or 2-bromopropionamide (460 mg) were warmed for 30 min. Cooling gave the *substituted acetamide* (4i) (70%), m.p. 224–226° (from water) (Found: C, 39·0; H, 3·5; N, 23·5. $C_8H_8N_4OS_2$ requires C, 40·0; H, 3·4; N, 23·3%); or the *homologous propionamide* (4j) (71%), m.p. 210–211° (Found: C, 42·3; H, 4·0; N, 21·6. $C_9H_{10}N_4OS_2$ requires C, 42·5; H, 4·0; N, 22·0%). N.m.r. 1·62, d, *J* 6·3, Me of Et; 2·77, 5′-Me; 4·80, q, *J* 6·3, SCH; 9·13, H2.

*2,5-Dimethyl-7-methylthiothiazolo[5,4-*d*]pyrimidine (4k)*

The above 5-amino-6-mercapto-2-methylpyrimidine-4-thione (5·0 g) was boiled for 3 h in acetic anhydride (50 ml). Refrigeration gave a solid which was dissolved in dilute sodium hydroxide, decolorized, and reprecipitated by acidification: the crude 2,5-dimethylthiazolo[5,4-*d*]pyrimidine-7-thione (9; R = Me) had m.p. 258–261°. N.m.r. 2·43, 2-Me; 2·70, 5-Me. Subsequent *S*-methylation gave the *dimethylmethylthiothiazolopyrimidine* (70%), m.p. 138–139° (from aqueous acetone) (Found: C, 45·7; H, 4·5; N, 20·1. $C_8H_9N_3S_2$ requires C, 45·5; H, 4·3; N, 19·9%). M^+ 211. N.m.r. 2·62, SMe; 2·65, 5-Me; 2·80, 2-Me.

*2-(2′,5′-Dimethylthiazolo[5,4-*d*]pyrimidin-7′-ylthio)-acetamide and -propionamide (4l) and (4m)*

Treatment of the above thione (9; R = Me) with 2-chloroacetamide or 2-bromopropionamide as for the analogues (4i) and (4j) gave the *substituted acetamide* (4l) (72%), m.p. 214–216° (from water) (Found: C, 42·4; H, 4·1; N, 21·9. $C_9H_{10}N_4OS_2$ requires C, 42·5; H, 4·0; N, 22·0%) (n.m.r. 2·60, 5′-Me; 2·77, 2′-Me; 3·97, CH_2); or the *homologous propionamide* (4m) (75%), m.p. 209–210° (Found: C, 44·4; H, 4·6; N, 20·8. $C_{10}H_{12}N_4OS_2$ requires C, 44·8; H, 4·5; N, 20·9%) (n.m.r. 1·63, d, *J* 6·3, Me of Et; 2·68, 5-Me; 2·80, 2-Me; 4·75, q, *J* 6·3, SCH) respectively.

*2-Hydroxymethyl-5-methyl-7-methylthiothiazolo[5,4-*d*]pyrimidine (4n)*

The pyrimidine (5; R^1 = Me, R^2 = SH, R^3 = H) (3·46 g) and pyridine (30 ml) were treated dropwise with stirring at 0° with acetoxyacetyl chloride[13] (3·28 g). Stirring was continued for 30 min at 20–25°. The residue from evaporation and 2 N hydrochloric acid (30 ml) were heated under reflux for 45 min. The solid which resulted on cooling was partly purified through alkali/acid treatment to give 2-hydroxymethyl-5-methylthiazolo[5,4-*d*]pyrimidine-7-thione (9; R = CH_2OH) (68%), m.p. 267–268°. N.m.r. 2·65, 5-Me; 4·91, CH_2. *S*-Methylation thence gave the *hydroxymethylmethylmethylthiothiazolopyrimidine* (90%), m.p. 223–226° (Found: C, 42·5; H, 4·2; N, 18·8. $C_8H_9N_3OS_2$ requires C, 42·3; H, 4·0; N, 18·5%). M^+ 227. N.m.r. 2·63, 2·67, Me_2; 4·87, CH_2.

*2-(2′-Hydroxymethyl-5′-methylthiazolo[5,4-*d*]pyrimidin-7′-ylthio)-acetamide and -propionamide (4o) and (4p)*

The above thione (9; R = CH_2OH) was treated with 2-chloroacetamide or 2-bromopropionamide as for the analogues (4i) and (4j) to give the appropriate *acetamide* (4o) (72·0%), m.p. 223–224° (from water) (Found: C, 39·9; H, 3·9; N, 20·6. $C_9H_{10}N_4O_2S_2$ requires C, 40·0; H, 3·7; N, 20·7%) (n.m.r. 2·63, Me; 4·02, SCH_2; 4·87, CH_2OH); or the corresponding *propionamide* (4p) (68%), m.p. 170–171° (Found: C, 42·0; H, 4·3; N, 19·4. $C_{10}H_{12}N_4O_2S_2$ requires C, 42·3; H, 4·3; N, 19·7%) (n.m.r. 1·55, d, *J* 6·2, Me of Et; 2·65, 5-Me; 4·72, q, *J* 6·2, SCH; 4·88, CH_2) respectively.

*5-Methyl-2,7-bismethylthiothiazolo[5,4-*d*]pyrimidine (4q)*

The pyrimidinethione (5; R^1 = Me, R^2 = SH, R^3 = H) was treated with alkaline carbon bisulphide, as for the preparation of the intermediate (6), to give crude 5-methylthiazolo[5,4-*d*]-pyrimidine-2,7-dithione (10) (69%), m.p. > 300° (dec.). N.m.r. 2·41, Me. Subsequent *S*-methylation gave the *methylbismethylthiothiazolopyrimidine* (72%), m.p. 126–128° (from aqueous methanol) (Found: C, 39·5; H, 4·1; N, 17·2. $C_8H_9N_3S_3$ requires C, 39·5; H, 3·7; N, 17·3%). M^+ 243. N.m.r. 2·60, 2·63, 2·78, Me_3.

*2-(7'-Carbamoylmethylthio-5'-methylthiazolo[5,4-*d*]pyrimidin-2'-ylthio)acetamide (4r)*

The above dithione (10) (654 mg) was allowed to react with 2-chloroacetamide as for the isomer (4e). The resulting *acetamide* (4r) (67%) had m.p. 275–276° (from dimethyl sulphoxide) (Found: C, 36·8; H, 3·4; N, 21·3. $C_{10}H_{11}N_5O_2S_3$ requires C, 36·5; H, 3·4; N, 21·3%).

*7-Methoxy-5-methyl-2-methylthiothiazolo[5,4-*d*]pyrimidine (4s)*

The pyrimidine (8; R = OMe)[15] (20 g) in dioxan (60 ml) was added to 2 M sodium hydrogen sulphide (400 ml). The mixture was stirred at 25° for 1 h and then at 100° for 2 h. Acidification gave a solid, partly purified by acid/alkali treatment to give crude 5-amino-6-methoxy-2-methylpyrimidine-4-thione (5; R^1 = Me, R^2 = OMe, R^3 = H), m.p. 192–193°. N.m.r. 2·33, 2-Me; 3·88, OMe. This compound (5·14 g) was heated with potassium hydroxide (2·4 g), carbon bisulphide (2·56 g), water (6 ml) and methanol (40 ml) for 12 h. The residue from evaporation was dissolved in water (100 ml) and acidified to give crude 7-methoxy-5-methylthiazolo[5,4-*d*]pyrimidine-2-thione (7; R^1 = Me, R^2 = OMe) (79%), m.p. >230° (dec.). N.m.r. 2·50, 5-Me; 3·98, OMe. *S*-Methylation gave the *methoxymethylmethylthiothiazolopyrimidine* (64%), m.p. 112–113° (from water) (Found: C, 41·6; H, 4·3; N, 18·4. $C_8H_9N_3OS_2,0·25H_2O$ requires C, 41·5; H, 4·1; N, 18·1%). M^+ 227. N.m.r. 2·58, 5-Me; 2·78, SMe; 4·08, OMe.

*2-(7'-Methoxy-5'-methylthiazolo[5,4-*d*]pyrimidin-2'-ylthio)acetamide (4t)*

The above intermediate (7; R^1 = Me, R^2 = OMe) was treated with 2-chloroacetamide as for the analogue (4i). The *methoxymethylthiazolopyrimidinylthioacetamide* (80%) had m.p. 203–204° (Found: C, 38·8; H, 4·0; N, 20·4. $C_9H_{10}N_4O_2S_2,0·5H_2O$ requires C, 38·7; H, 4·0; N, 20·1%). N.m.r. 2·55, 5-Me; 4·02, OMe; 4·12, CH_2.

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