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Studies on Pyrimidine Derivatives. VIII.[1] Synthesis of Some Alkylamino- and Alkylthio-, Thiazolo(5,4-*d*)pyrimidines. (I)

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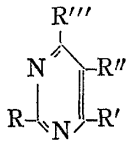
The antibacterial and anticancer activities of thiazolo(5,4-*d*)pyrimidines and related compounds reported in the previous papers[3] have been screened[4] for *Strep. pyogenes* C 203, *Staph. aureus* UC 76, *M. tuberculosis* H 37 RV, human epidermoid carcinoma (HEP 3 and FL 74), hypernephroma (HN) and mouse sarcoma (S 180).

Although unsubstituted thiazolo(5,4-*d*)pyrimidine and its 2-methyl-, 2-phenyl-, 2-hydroxy-, and 2-mercapto derivatives have shown no activity against them, some other derivatives have shown their activities. For example, when a mercapto group was attached to the 5-position in 2-methylthiazolo(5,4-*d*)pyrimidine, *i.e.*, 5-mercapto-2-methylthiazolo-(5,4-*d*)pyrimidine, it has shown the activities at 2.5 μg/ml *vs. Strep.* and 20 μg/ml *vs. Staph.* and *M.tuber.* Other results obtained up to the present time were summarized in Table I.

Upon these results no specific relation between chemical structure and chemotherapeutic activity could be drawn. However, since their related compounds had shown some activities as mentioned above, the preparations of 2-(or 5-)alkylamino-5-(or 2-)alkylthiothiazolo-(5,4-*d*)pyrimidines from 2,5-dichlorothiazolo(5,4-*d*)pyrimidine (III) by two-step reaction were undertaken.

It was concluded from the following experiment that replacement of one of the two chlorine atoms in III by a nucleophilic reagent gave 2-monosubstituted derivative. Thus, by treatment with a calculated amount of potassium hydrogen sulfide, 2,5-dichlorothiazolo(5,4-*d*)pyrimidine (III) was converted to the monomercapto derivative. The product was found to be identical

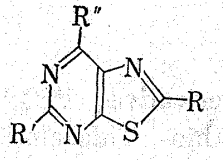
Table Ia. Antibacterial Activities of Thiazolo(5,4-*d*)pyrimidines and Related Compounds



Substituents				Organism	Days incub.	μg/ml Causing		Standard control	
R	R′	R″	R‴			Complete inhibition	Partial inhibition	Compound	μg/ml Compl.
Cl	SH	NH_2	H	*Strep.*	1	20	5	CM[a]	0.78
NH_2	SH	NH_2	H	*Strep.*	1	5	—	CM	0.78
NH_2	SH	NH_2	H	*Staph.*	1	20	—	CM	3.13
H	SH	NH_2	$SCH_2C_6H_5$	*Strep.*	1	2.5	1.25	CM	0.78
SC_2H_5	SH	$NHCOCH_3$	H	*Strep.*	1	10	2.5	CM	0.78

1) Part VII: *Chem. Pharm. Bull.* (Tokyo), **6**, 675 (1958).
2) Location: *Yagotourayama, Tenpaku-cho, Showa-ku, Nagoya.*
3) T. Takahashi, T. Naito, and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 334 (1958); T. Naito and S. Inoue, *ibid.*, **6**, 338 (1958); S. Inoue, *ibid.*, **6**, 343, 346, 349, 352, 675 (1958).
4) Screening tests were done by Dr. M. Fisher (antibacterial activities) and Dr. O.D. Bird (anticancer activities) at Parke, Davis Research Division.

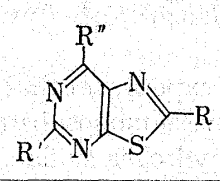
Table Ib.



Substituents R	R′	R″	Organism	Days incub.	μg/ml Causing Complete inhibition	Partial inhibition	Standard control Compound	μg/ml Compl.
Cl	Cl	H	*M. tuber.*	7	20	—	INH[b]	0.024
NH_2	SC_2H_5	H	*Strep.*	1	10	10	CM	0.78
NH_2	SC_2H_5	H	*Staph.*	1	10	—	CM	3.13
NH_2	SC_2H_5	H	*M. tuber.*	7	20	—	INH	0.024
$NHCOCH_3$	SC_2H_5	H	*Strep.*	1	0.16	—	CM	0.78
$NHCOCH_3$	SC_2H_5	H	*M. tuber.*	7	20	10	INH	0.024
SH	SH	H	*Strep.*	1	5	2.5	CM	0.78
SH	SC_2H_5	H	*Strep.*	1	1.25	—	CM	0.78
SH	H	SH	*Strep.*	1	2.5	—	CM	0.78
SH	NH_2	H	*Strep.*	1	5	—	CM	0.78
CH_3	SH	H	*Strep.*	1	2.5	—	CM	0.78
CH_3	SH	H	*Staph.*	1	20	—	CM	3.13
CH_3	SH	H	*M. tuber.*	7	20	—	INH	0.024

a) Chloramphenicol *b*) Isonicotinic acid hydrazide

Table Ic. Minimum Concentration(μg/ml) for Anticancer Activities[c]



Substituents R	R′	R″	Malignant lines HEP #3	FL-74	HN	S-180
CH_3	SH	H	100	100	100	100
SC_2H_5	NH_2	H[d]	100	100	100	—
NHC_6H_5	NH_2	H[e]	100	200	100	—
SCN	H	H[f]	100	—	—	—
SH	H	SH	100	—	—	—
OH	H	$SCH_2C_6H_5$	100	100	100	—

c) Tests were done by an *in vitro* tissue culture, established by Parke, Davis Research Division, antitumor screening program. They are looking for selective toxicity toward various malignant lines as compared with normal controls. Details concerning them will be reported in the near future.
d) This compound has shown activity against Yoshida sarcoma (unpublished).
e) Shown activities against Ehrlich ascites carcinoma and Yoshida sarcoma (unpublished).
f) Low grade, broad toxity.

with 5-chloro-2-mercaptothiazolo(5,4-*d*)pyrimidine(IV) prepared from 5-amino-2-chloro-4-mercaptopyrimidine (I) and potassium methylxanthate. Furthermore, III reacted with one mole of sodium ethylmercaptide to give monoethylthio derivative, 5-chloro-2-ethylthiothiazolo(5,4-*d*)pyrimidine(V), which was also found to be identical to the reaction product of the potassium salt of IV and ethyl bromide. Treatment of V with ethylamine afforded 5-ethylamino-2-ethylthiothiazolo(5,4-*d*)pyrimidine (VIb). Similar treatment of V with several amines gave the corresponding 5-alkylamino-2-ethylthio derivatives as shown in Table II.

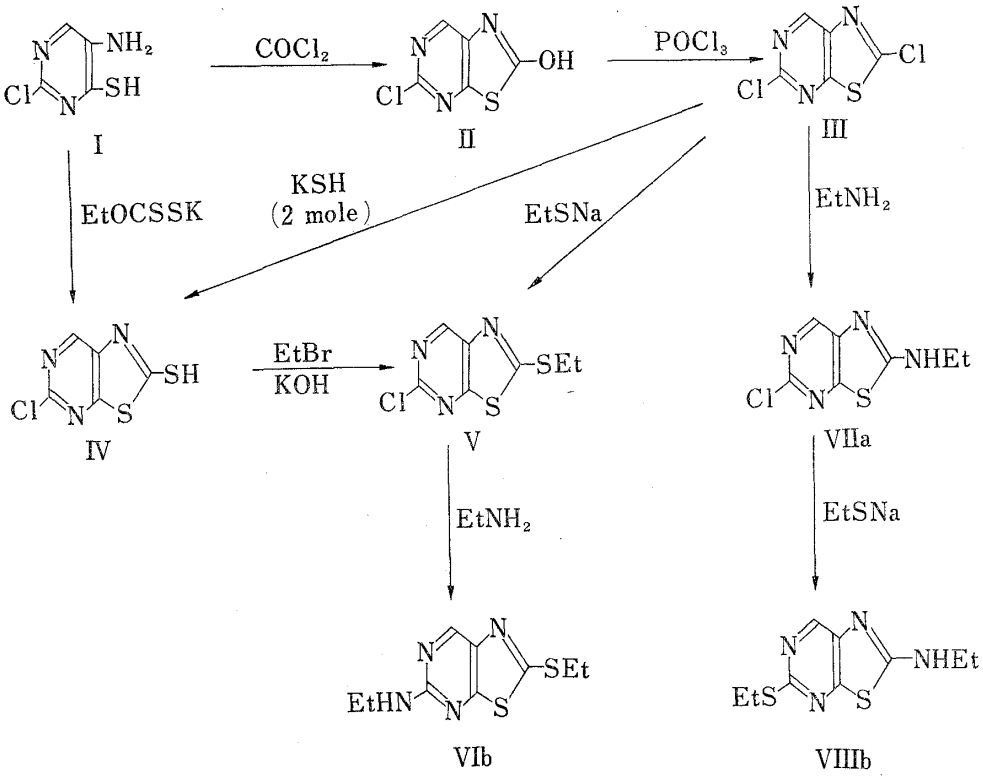
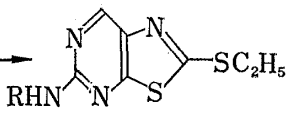
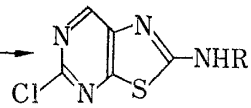


Chart 1

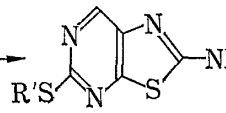
Table II. V →  VIa–h

No.	Substituents R	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VIa	H	96	colorless needle	EtOH	168	$C_7H_8N_4S_2$ a)	39.62	3.80	—	39.69	3.51	—
VIb	C_2H_5	98	colorless prism	MeOH	119—121	$C_9H_{12}N_4S_2$	45.00	5.00	—	44.90	5.10	—
VIc	C_3H_5	98	colorless needle	benzene–petr. benzin	110—111	$C_{10}H_{12}N_4S_2$	47.62	4.76	22.22	47.57	4.70	22.03
VId	C_3H_7	98	colorless pillar	MeOH	106	$C_{10}H_{14}N_4S_2$	47.24	5.51	22.05	47.18	5.41	22.05
VIe	*iso*-C_3H_7	96	colorless needle	petr. benzin	94— 96	$C_{10}H_{14}N_4S_2$	47.24	5.51	22.05	47.24	5.60	21.90
VIf	C_4H_9	98	colorless needle	benzene–petr. benzin	106	$C_{11}H_{16}N_4S_2$	49.25	5.97	20.90	49.47	5.75	20.80
VIg	*iso*-C_4H_9	98	colorless pillar	benzene–petr. benzin	115	$C_{11}H_{16}N_4S_2$	49.25	5.97	20.90	49.32	5.86	21.13
VIh	$C_6H_5CH_2$	98	colorless needle	benzene–petr. benzin	127—128	$C_{14}H_{14}N_4S_2$	55.63	4.63	18.54	55.70	4.47	18.71

a) This compound was prepared by heating of V with 15% ethanolic NH_3 at 150° for 3 hr. The product was identical with an authentic sample obtained from 5-amino-2-mercaptothiazolo(5,4-*d*)pyrimidine and EtBr (T. Naito and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 338 (1958)).

Table III. III ⟶  VIIa—c

No.	Substituents R	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Formula	Analysis (%) Calcd. N	Found N
VIIa	C_2H_5	100	colorless scale	benzene	159	$C_7H_7N_4ClS$	26.05	26.16
VIIb	C_3H_7	98	colorless needle	benzene–petr. benzin	118—119	$C_8H_9N_4ClS$	24.45	24.44
VIIc	C_4H_9	98	colorless needle	benzene–petr. benzin	102—104	$C_9H_{11}N_4ClS$	23.05	22.89

Table IV. VIIa—c ⟶  VIIIa—j

No.	Substituents R	Substituents R′	Crude yield (%)	Appearance	Recrystn. solv.	mp (°C)	Formula	Analysis (%) Calcd. N	Found N
VIIIa	C_2H_5	CH_3	82	colorless scale	dil. MeOH	125—129	$C_8H_{10}N_4S_2$	24.78	25.13
VIIIb	C_2H_5	C_2H_5	100	colorless scale	benzene–petr. benzin	128	$C_9H_{12}N_4S_2$	23.33	23.42
VIIIc	C_2H_5	C_3H_7	95	colorless scale	benzene–petr. benzin	114—116	$C_{10}H_{14}N_4S_2$	22.05	22.11
VIIId	C_2H_5	C_4H_9	89	colorless scale	petr. benzin–MeOH	110.5—113	$C_{11}H_{16}N_4S_2$	20.90	21.05
VIIIe	C_3H_7	CH_3	93	colorless scale	petr. benzin	118—118.5	$C_9H_{12}N_4S_2$	23.33	24.35
VIIIf	C_3H_7	C_2H_5	90	colorless scale	petr. benzin	99—100.5	$C_{10}H_{14}N_4S_2$	22.05	22.26
VIIIg	C_3H_7	C_3H_7	90	colorless scale	petr. benzin	98—100	$C_{11}H_{16}N_4S_2$	20.90	20.68
VIIIh	C_3H_7	C_4H_9	85	colorless scale	petr. benzin	112—113	$C_{12}H_{18}N_4S_2$	19.86	20.01
VIIIi	C_4H_9	CH_3	80	colorless scale	petr. benzin–MeOH	129.5—130.5	$C_{10}H_{14}N_4S_2$	22.05	21.98
VIIIj	C_4H_9	C_2H_5	83	colorless scale	petr. benzin–MeOH	89.5—91	$C_{11}H_{16}N_4S_2$	20.90	21.07

On the other hand, when being used two moles of ethylamine instead of sodium ethylmercaptide, monoethylamination took place smoothly with III to give 5–chloro–2–ethylamino derivative (VIIa) and subsequent treatment with sodium ethylmercaptide then gave a positional isomer of VIb, 2–ethylamino–5–ethylthiothiazolo(5,4–*d*)pyrimidine (VIIIb). Similarly, several 2–alkylamino–5–alkylthio derivatives were synthesized from 2,5–dichlorothiazolo(5,4–*d*)pyrimidine (III) through 2–alkylamino derivatives (VIIa—c). Results thus obtained were shown in Table III and IV.

Experimental[5]

2,5-Dichlorothiazolo(5,4-*d*)pyrimidine (III)——A mixture of 2 g of 5–chloro–2–hydroxythiazolo(5,4–*d*)pyrimidine (II),[6] 10 ml of $POCl_3$ and 2 ml of dimethylaniline was refluxed for 7 hr. The excess $POCl_3$ was removed in vacuum. The residue was poured on crushed ice, and extracted with ether. The extract was washed with dil. NH_4OH and H_2O, dried, and evaporated to yield 1.8 g of a crystalline residue. It was recrystallized from dil. MeOH to give III as colorless scales, mp 127—128°. *Anal.* Calcd. for $C_5HN_3Cl_2S$: C, 29.13; H, 0.49. Found: C, 29.19; H, 0.70.

5-Chloro-2-mercaptothiazolo(5,4-*d*)pyrimidine (IV)——Method A: A solution of 2 g of I[7] and 3.6 g of EtOCSSK in 100 ml of BuOH was refluxed for 15 hr. After cool, the reaction mixture was shaken with H_2O, and the H_2O extract was then shaken with ether to remove BuOH dissolved in the H_2O extract. The

5) All melting points are uncorrected.
6) S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 675 (1958).
7) S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **6**, 343 (1958).

H_2O layer was acidified with AcOH to precipitate yellow crystals of IV, mp above 300°. Yield, almost theoretical. Recrystallization of this compound was difficult and accordingly it was converted to the 2-ethylthio derivative (V) without purification as mentioned below.

Method B: A solution of 0.36 g of III in 15 ml of EtOH containing 0.26 g of KSH was heated at 60° for 2 hr. After removal of the solvent, a small amount of H_2O was added, treated with charcoal, and acidified with AcOH to yield 0.3 g of IV.

5-Chloro-2-ethylthiothiazolo(5,4-*d*)pyrimidine (V)——From IV: To a solution of 0.2 g of IV (crude) in 6 ml of MeOH containing 0.05 g of KOH was added 0.12 g of EtBr and the mixture was refluxed for 20 min. Removal of the solvent left an oily residue which was solidified soon. It was recrystallized from petr. ether–MeOH to give 0.2 g of V as colorless needles, mp 85—88°. *Anal.* Calcd. for $C_7H_6N_3ClS_2$: C, 36.21; H, 2.59; N, 18.10. Found: C, 36.28; H, 2.31; N, 18.20.

From III: To a solution of EtSNa (prepared from 0.14 g of Na, 0.5 g of EtSH and 10 ml of EtOH) was added a solution of 1.2 g of III in 30 ml of EtOH and the reaction mixture was refluxed for 20 min. After evaporation of the solvent, a small amount of H_2O was added, the separated crystals were recrystallized from petr. ether–MeOH to colorless needles. Yield, 1.1 g. This product was identical with V prepared from IV.

5-Ethylamino-2-ethylthiothiazolo(5,4-*d*)pyrimidine (VIb)——To 5 ml of MeOH containing 0.07 g of NaOH was added 0.14 g of $EtNH_2HCl$ and after shaking for a few minutes, 0.2 g of V was added to this solution. The reaction mixture was heated at 100° for 5 hr in a sealed tube. MeOH was distilled off in vacuum and addition of a small amount of H_2O to the residue gave 0.23 g of the crystalline product of VIb. Recrystallization from MeOH gave colorless prisms, mp 119—121°. *Anal.* Calcd. for $C_9H_{12}N_4S_2$: C, 45.00; H, 5.00. Found: C, 44.90; H, 5.10. All the other 5-alkylamino-2-ethylthio derivatives in Table II were prepared by the same method.

5-Chloro-2-ethylaminothiazolo(5,4-*d*)pyrimidine (VIIa)——A mixture of two moles of $EtNH_2$ (0.45 g) and 2 g of III in 60 ml of EtOH was refluxed for 4 hr. The reaction mixture was treated similarly as described under the reaction of VIb with $EtNH_2$. The product was recrystallized from benzene to give colorless scales of VIIa, mp 159°. Yield, 1.9 g. *Anal.* Calcd. for $C_7H_7N_4ClS$: N, 26.05. Found: N, 26.16. Other 2-alkylamino derivatives, shown in Table III, were prepared by essentially the same method.

2-Ethylamino-5-ethylthiothiazolo(5,4-*d*)pyrimidine (VIIIb)——A solution of 1 g of VIIa in 10 ml of EtOH was added to a solution of EtSNa (prepared from 0.11 g of Na, 0.5 g of EtSH and 10 ml of EtOH) and the mixture was heated under reflux for 2 hr. After removal of the solvent, a small amount of H_2O was added to the residue and the separated crystalline solid was collected. Recrystallization from benzene–petr. benzin gave colorless scales, mp 128°. *Anal.* Calcd. for $C_9H_{12}N_4S_2$: N, 23.33. Found: N, 23.42. All the other 2-alkylamino-5-alkylthiothiazolo(5,4-*d*)pyrimidines prepared from 2-alkylamino-5-chlorothiazolopyrimidines were listed in Table IV.

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Studies on Pyrimidine Derivatives. IX.[1] Synthesis of Some Alkylamino- and Alkylthio-, Thiazolo(5,4-*d*)pyrimidines. (2)

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(Received September 28, 1967)

Since 2-amino-5-anilinothiazolo(5,4-*d*)pyrimidine has shown some anticancer activities as reported in the foregoing paper,[3] we have prepared several 5-alkylamino-2-amino derivatives and related compounds in the hope of finding more beneficial change in their activities. Synthesis of these derivatives was carried out by the same procedure as in the case of 2-amino-

1) Part VIII: *Chem. Pharm. Bull.* (Tokyo), **16**, 741 (1968).
2) Location: *Yagotourayama, Tenpaku-cho, Showa-ku, Nagoya.*
3) S. Sugiura, E. Suzuki, T. Naito, and S. Inoue, *Chem. Pharm. Bull.* (Tokyo), **16**, 741 (1968).