Anthelmintic Quaternary Salts. III. Benzothiazolium Salts

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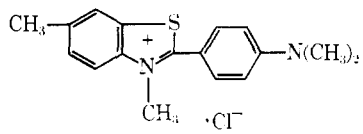
The synthesis and anthelmintic activity of a number of benzothiazolium salts analogous to the dye thioflavin T are described. The structural requirements for activity include a 2-phenyl substitutent with a basic group in the *para* position, and a 3-alkyl group no larger than ethyl (preferably methyl). The isomeric salts in which the site of quaternization is the exocyclic nitrogen, as well as the unquaternized benzothiazoles, are devoid of activity. The benzothiazole nucleus may be substituted with alkyl, alkoxy, or methylthio groups, but not with halogen. The most active compounds are of interest because they provide both lung and liver protection to swine by inhibiting the migration of *Ascaris suum* larvae in swine. Thioflavin and two analogs also showed activity against gastrointestinal nematodes in sheep.

The benzothiazolium dye, thioflavin T[1] (**1**), has



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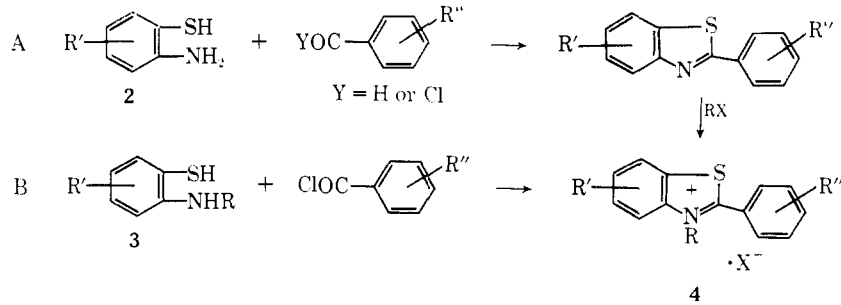
recently been shown to have anthelmintic properties.[2] The dye has also been claimed to have antibacterial activity[3] and growth-inhibitory activity on fungi of the candida group.[4] Its properties as a fluorescent staining agent,[5] acid–base indicator,[6] and photooxidation sensitizer[7] have been investigated.

Thioflavin is of considerable interest as an anthelmintic because it is highly effective in protecting swine from liver and lung damage caused by the larvae of *Ascaris suum*, whereas earlier antiascaris agents were effective only against the adult stages.

This paper is concerned with structure–activity relationships among benzothiazolium salts related to thioflavin.

Chemistry.—Thioflavin T was originally prepared[1a] by condensing sulfur with *p*-toluidine to form dehydrothiotoluidine followed by treatment with methyl chloride at elevated temperatures to give the product as the chloride salt. As this method was not adaptable to the preparation of analogs, recourse was had to the synthetic methods for benzothiazolium salts illustrated below. In method A, an *o*-aminobenzenethiol is condensed with an aldehyde or an acid chloride to yield a 2-substituted benzothiazole which is then quaternized. In method B, the product is obtained directly from the condensation of an *o*-alkylaminobenzenethiol with an acid chloride.



2-Aminobenzenethiols (**2**) were prepared by a variety of routes, the choice being determined by the availability of starting materials (Scheme I). Examples of each route are described in the Experimental Section. The aminobenzenethiols were converted in three steps to 3-alkyl- (or aralkyl-) benzothiazoline-2-thiones (**5**). New compounds of this type which were prepared in this program are listed in Table I.

Alkaline hydrolysis of **5** yielded the 2-alkylaminobenzenethiols **3**, which were required for the direct synthesis of benzothiazolium salts by method B.

The previously unreported 2-(*p*-dimethylaminophenyl)-5-methylbenzothiazole was prepared by condensing zinc 2-amino-4-tolylmercaptide (obtained from 4-chloro-3-nitrotoluene) with *p*-dimethylaminobenzaldehyde. Other new benzothiazoles which were prepared as intermediates in method A are listed in Table II. The 2-(*p*-diethylaminophenyl) analog **7** was prepared in the usual way by reaction of *p*-diethylaminobenzaldehyde with *o*-aminobenzenethiol. The 2-(ω-carboxyalkyl) derivatives **8** and **9** were prepared by the reaction of dicarboxylic acid anhydrides with *o*-aminobenzenethiol, and the N,N-dimethylamides **10**–**13** were obtained by treating the carboxylic acids with dimethylamine at 200°.

(1) Basic yellow 1 (Colour Index No. 49005): (a) A. G. Green, *Ber.*, **22**, 968 (1889); (b) M. T. Bogert and W. S. Taylor, *Collect. Czech. Chem. Commun.*, **3**, 480 (1931).

(2) (a) American Cyanamid Co., Netherlands Patent 6,501, 092 (Sept 8, 1965); (b) Abbott Laboratories, U. S. Patent Application AL56,869/65; (c) Dow Chemical Co., U. S. Patent 3,336,329 (Aug 15, 1967).

(3) G. J. Martin, *Am. J. Pharm.*, **119**, 432 (1947).

(4) F. Miyazawa, *Eisei Shikensho Hokoku*, **74**, 341 (1956); *Chem. Abstr.*, **51**, 8277 (1957).

(5) P. S. Vassar, C. F. A. Culling, and H. E. Taylor, *Am. J. Pathol.*, **35**, 718 (1959).

(6) O. W. Kolling and T. L. Stevens, *Anal. Chem.*, **34**, 1635 (1962).

(7) G. Oster, J. S. Bellin, R. W. Kimball, and M. E. Schrader, *J. Amer. Chem. Soc.*, **81**, 5095 (1959).

SCHEME I

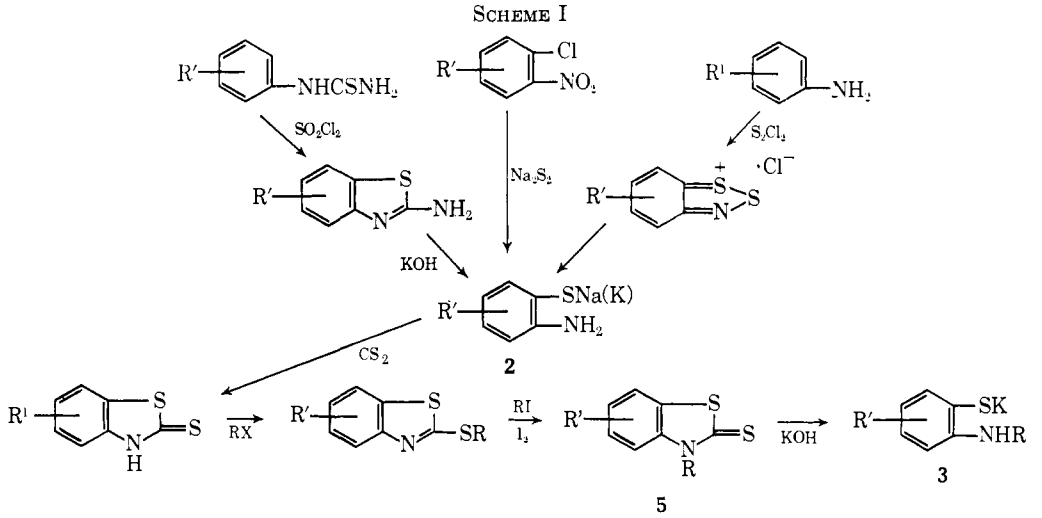
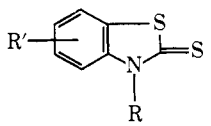


TABLE I

3-ALKYL- (AND -ARALKYL-) BENZOTHIAZOLINE-2-THIONES

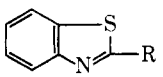


R	R′	Mp, °C	Yield, %	Formula[a]
CH_3	6-Cl	130–131	57	$C_8H_6ClNS_2$
CH_3	5-CH_3	188.5–189.5	79	$C_9H_9NS_2$
CH_3	6-C_2H_5	74–75	51	$C_{10}H_{11}NS_2$
CH_3	6-OC_2H_5	133–134	96	$C_{10}H_{11}NOS_2$
C_2H_5	6-CH_3	108–109	55	$C_{10}H_{11}NS_2$
n-C_3H_7	6-CH_3	103–104	22	$C_{11}H_{13}NS_2$
n-C_4H_9	H	53	30	$C_{11}H_{13}NS_2$
n-C_4H_9	6-CH_3	96–97	72	$C_{12}H_{15}NS_2$
$C_6H_5CH_2$	6-CH_3	154	93	$C_{15}H_{13}NS_2$
o-$CH_3C_6H_4CH_2$	6-CH_3	85–87	43	$C_{16}H_{15}NS_2$
p-$CH_3C_6H_4CH_2$	6-CH_3	88–89	62	$C_{16}H_{15}NS_2$
1-Naphthylmethyl	6-CH_3	222–223	71	$C_{19}H_{15}NS_2$
2-Methyl-1-naphthylmethyl	6-CH_3	204–206	30	$C_{20}H_{17}NS_2$

[a] All compounds analyzed correctly for C, H, N, S.

TABLE II

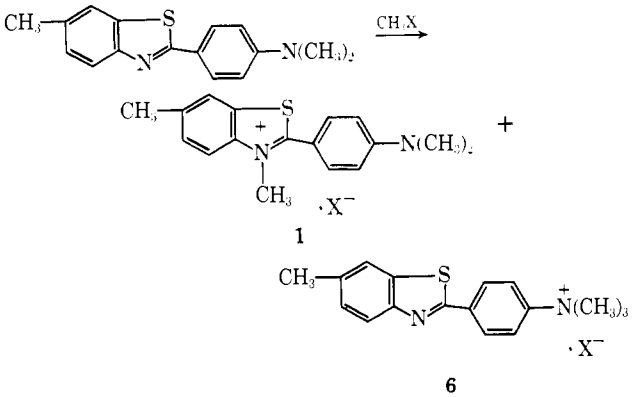
2-SUBSTITUTED BENZOTHIAZOLES



No.	R	Mp, °C	Yield, %	Formula[b]
7	p-$C_6H_4NEt_2$	125–126	30	$C_{17}H_{18}N_2S$
8	$(CH_2)_4COOH$	133–134	35	$C_{12}H_{13}NO_2S$
9	$(CH_2)_5COOH$	72–73	48	$C_{13}H_{15}NO_2S$
10	$(CH_2)_2CON(CH_3)_2$	192 (1.2 mm)[a]	61	$C_{18}H_{17}N_5O_8S$
	picrate	151–152		
11	$(CH_2)_3CON(CH_3)_2$	67–68	86	$C_{13}H_{16}N_2OS$
12	$(CH_2)_4CON(CH_3)_2$	44–45	84	$C_{14}H_{18}N_2OS$
13	$(CH_2)_5CON(CH_3)_2$	58–60	60	$C_{15}H_{20}N_2OS$

[a] Boiling point. [b] All compounds analyzed correctly for C, H, N, S.

All of the benzothiazolium salts listed in Table III were prepared by treating acid chlorides with N-alkylaminobenzenethiols (method B). Both methods A and B were used to prepare the p-dimethylaminophenylbenzothiazolium salts listed in Table IV. However, difficulties were encountered with method A because, as in the case of thioflavin itself, both possible isomers **1** and **6** were formed on alkylation. The relative yields



of the two isomers depended on the reaction conditions.[8] Under mild conditions the anilinium isomer **6** predominated, but when temperature and reaction time were increased, the benzothiazolium isomer **1** was formed preferentially. In some cases it was possible to convert the anilinium isomer to the benzothiazolium isomer in good yield by refluxing in hexanol. The isomers could be readily distinguished by their uv spectra. The benzothiazolium isomers had maxima at 413–420 mμ, as contrasted with maxima at 300–313 mμ for the anilinium isomers. The benzothiazolium isomers usually crystallized with water of hydration and formed stable insoluble complexes with phenols, particularly resorcinol. In the case of **31** (Table V), the isomers were separated most conveniently by the addition of resorcinol in aqueous solution to precipitate the benzothiazolium chloride–resorcinol complex.

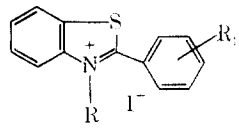
In general, method B was the preferred method for the compounds in Table IV because the products were obtained without contamination by the anilinium isomers.

A number of miscellaneous benzothiazolium salts are listed in Table V. 2-(3-Pyridylvinyl)benzothiazole dimethiodide (**35**) and the 2-(4-methoxystyryl) derivative **36** were prepared by reaction of 2,3-dimethylbenzothiazolium iodide with 3-pyridinecarboxaldehyde methiodide and p-anisaldehyde, respectively. The other

(8) D. L. Garmaise and G. Y. Paris, *Chem. Ind.* (London), 1645 (1967).

TABLE III

2-ARYL-3-ALKYLBENZOTHIAZOLIUM IODIDES

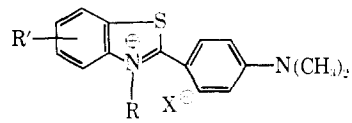


R	R_1	Mp, °C	Yield, %	Formula[a]
CH_3	2-Cl	203	53	$C_{14}H_{11}ClINS$
CH_3	4-Cl	224–225	64	$C_{14}H_{11}ClINS$
CH_3	3,4-Cl_2	204–205	55	$C_{14}H_{10}Cl_2INS$
CH_3	4-F	194	47	$C_{14}H_{11}FINS$
CH_3	3-Br	214–215	70	$C_{14}H_{11}BrINS$
CH_3	4-NO_2	230–231	66	$C_{14}H_{11}IN_2O_2S$
CH_3	3,5-$(NO_2)_2$	233	44	$C_{14}H_{10}IN_3O_4S$
CH_3	2-CH_3	213–214	27	$C_{15}H_{14}INS$
CH_3	3-CH_3	197–198	61	$C_{15}H_{14}INS$
CH_3	4-CH_3	202–203	50	$C_{15}H_{14}INS$
CH_3	4-$CH(CH_3)_2$	197	48	$C_{17}H_{18}INS$
CH_3	2-OCH_3	195	56	$C_{15}H_{14}INOS$
CH_3	4-OCH_3	199	53	$C_{15}H_{14}INOS$
CH_3	4-OC_2H_5	197	58	$C_{16}H_{16}INOS$
CH_3	4-$O(CH_2)_4CH_3$	132	31	$C_{19}H_{22}INOS$
n-C_3H_7	4-OCH_3	192–193 dec	48	$C_{17}H_{18}INOS$
$CH_2CH{=}CH_2$	4-OCH_3	153–155	30	$C_{17}H_{16}INOS$

[a] All compounds analyzed correctly for C, H, I, N, S.

TABLE IV

2-(*p*-DIMETHYLAMINOPHENYL)-3-ALKYLBENZOTHIAZOLIUM SALTS

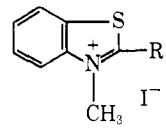


No.	R	R′	X	Mp, °C	Yield, %	Formula	Analyses
14	CH_3	H	Br	198–199	90	$C_{16}H_{17}BrN_2S$	C, H, Br, N, S
			Br–resorcinol complex	224–225	44	$C_{22}H_{23}BrN_2O_2S$	C, H
			3-Hydroxy-2-naphthoate	190–191		$C_{27}H_{24}N_2O_3S$	C, H
			0.5Pamoate	210–212 dec		$C_{55}H_{48}N_4O_6S_2 \cdot H_2O$	C, H, N, S
15	CH_3	6-Cl	I	210–211	85	$C_{16}H_{16}ClIN_2S$	C, H, I, N, S
16	CH_3	5-CH_3	I	198–199		$C_{17}H_{19}IN_2S$	C, H, I, N, S
			3-Hydroxy-2-naphthoate	193–194	81	$C_{28}H_{28}N_2O_3S \cdot H_2O$	C, H, N, S
			0.5Pamoate	273–274 dec		$C_{57}H_{52}N_4O_6S_2$	C, H
1	CH_3	6-CH_3	Cl	212 dec		$C_{17}H_{19}ClN_2S$	C, H, Cl, N, S
			HSO_4	250 dec		$C_{17}H_{20}N_2O_4S_2$	C, H, N, S
			3-Hydroxy-2-naphthoate	197–198	99	$C_{28}H_{26}N_2O_3S$	C, H, N, S
			0.5Pamoate	280–281		$C_{57}H_{52}N_4O_6S_2$	C, H, N, S
17	CH_3	6-C_2H_5	I	178–179 dec	57	$C_{18}H_{21}IN_2S$	C, H, N, S
18	CH_3	6-n-C_4H_9	I	163–164	10	$C_{20}H_{25}IN_2S$	C, H, I, N, S
19	CH_3	6-OCH_3	Cl	190–191 dec	84	$C_{17}H_{19}ClN_2OS$	C, H, Cl, N, S
			Cl–resorcinol complex	242–243	53	$C_{23}H_{25}ClN_2O_3S$	C, H, Cl, N, S
20	CH_3	6-OC_2H_5	I	198–200	95	$C_{18}H_{21}IN_2OS$	C, H, I, N, S
			Cl	173–174	78	$C_{18}H_{21}ClN_2OS$	C, H, N, S
21	CH_3	6-SCH_3	I	201–202 dec	29	$C_{17}H_{19}IN_2S_2$	C, H, I, N, S
22	C_2H_5	H	I	210–211	50	$C_{17}H_{19}IN_2S$	C, H, I, N, S
23	C_2H_5	6-CH_3	I	237	65	$C_{18}H_{21}IN_2S$	C, H, I, N, S
24	$CH_2CH{=}CH_2$	H	I	194	50	$C_{19}H_{19}IN_2S$	C, H, I, N, S
25	$(CH_2)_2CH_3$	H	I	233–234 dec	87	$C_{19}H_{21}IN_2S$	C, H, I, N, S
26	$(CH_2)_2CH_3$	6-CH_3	I	231–232	50	$C_{20}H_{23}IN_2S$	C, H, I, N, S
27	$(CH_2)_3CH_3$	H	I	218	25	$C_{20}H_{23}IN_2S$	C, H, I, N, S
28	$(CH_2)_3CH_3$	6-CH_3	I	220–221	60	$C_{21}H_{25}IN_2S$	C, H, I, N, S
29	$CH_2C_6H_5$	H	Cl	175–177	33	$C_{24}H_{21}ClN_2S$	C, H, Cl, N, S
30	$CH_2C_6H_5$	6-CH_3	I	188–189	55	$C_{24}H_{23}IN_2S$	C, H, I, N, S

compounds in the table were prepared by methods already described.

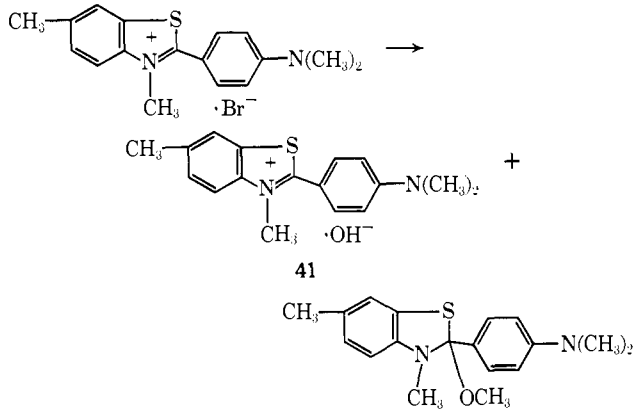
Behavior of Thioflavin in Alkaline Media.—When a solution of thioflavin bromide in MeOH was treated with Amberlite IRA-400 (OH form), a mixture of the quaternary hydroxide **41,** mp 159–160° dec, and the pseudobase **42,** mp 96–97°, was obtained. The hydroxide was a yellow solid with the characteristic

TABLE V: MISCELLANEOUS 2-SUBSTITUTED 3-METHYLBENZOTHIAZOLIUM SALTS



No.	R	Mp, °C	Yield, %	Method	Formula	Analyses
31	4-Diethylaminophenyl[a]	202–204 dec	53	A	$C_{24}H_{27}ClN_2O_2S$	C, H, Cl, N, S
32	3-Dimethylaminophenyl	180–181	62	B	$C_{16}H_{17}IN_2S$	C, H, I, N, S
33	2-Furyl	203–204	52	B	$C_{12}H_{10}INOS$	C, H, I, N, S
34	2-Thienyl	206	40	B	$C_{12}H_{10}INS_2$	C, H, I, N, S
35	3-Pyridylvinyl (methiodide)	226–228	85		$C_{16}H_{16}I_2N_2S$	C, H, I, N, S
36	4-Methoxystyryl	234–235	60		$C_{17}H_{16}INOS$	C, H, I, N, S
37	2-(N,N-Dimethylcarbamoyl)ethyl	157–158	95	A	$C_{13}H_{17}IN_2OS$	C, H, I, N, S
38	3-(N,N-Dimethylcarbamoyl)-*n*-propyl	186–187	89	A	$C_{14}H_{19}IN_2OS$	C, H, I, N, S
39	4-(N,N-Dimethylcarbamoyl)-*n*-butyl	167	93	A	$C_{15}H_{21}IN_2OS$	C, H, I, N, S
40	5-(N,N-Dimethylcarbamoyl)-*n*-pentyl	87–89	93	A	$C_{16}H_{23}IN_2OS \cdot 0.5H_2O$	C, H, I, N, S

[a] Chloride salt, resorcinol complex.

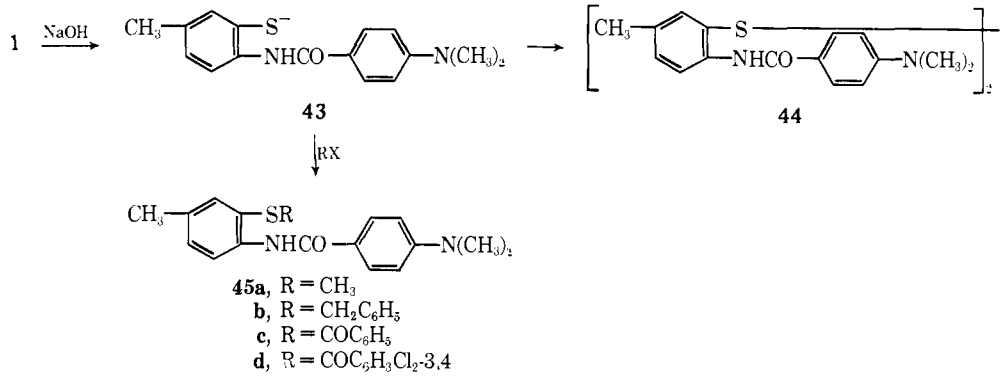


41

42

uv absorption (λ_{max} 416 mμ) of thioflavin. The 2-methoxybenzothiazoline was colorless and had λ_{max} 310 mμ in ligroin, but its solution in MeOH gradually turned yellow and had λ_{max} 416 mμ. (2-Ethoxy-2-phenyl-3-methylbenzothiazoline has been reported[9] to have λ_{max} 308 mμ.) Compound **42** was only very slightly soluble in water and formed a stable aqueous suspension under neutral conditions. It could be obtained more conveniently by treating thioflavin bromide with NaOMe in MeOH.

Treatment of thioflavin bromide with hot aqueous alkali gave a solution of the sodium salt of the thiol **43**, which was converted to the disulfide **44** and was also alkylated and acylated to give the derivatives **45a–d**.



43 44

45a, R = CH_3
b, R = $CH_2C_6H_5$
c, R = COC_6H_5
d, R = $COC_6H_3Cl_2$-3,4

Biological Results.—The compounds were tested for therapeutic activity against *Nematospiroides dubius* in mice and six gastrointestinal nematodes in lambs, and for prophylactic activity against *Ascaris suum* in mice and swine. The results are summarized in Table VI.

Compounds which showed activity were all closely related to thioflavin (**1**) in that they had a 2-(*p*-dialkylaminophenyl) substituent and were quaternized on the heterocyclic nitrogen atom. None of the benzothiazoles was active. The anilinium isomer (**6**) of thioflavin, the 3-dimethylamino analog **32**, the benzothiazoline **42**, the ring-opened derivatives **44** and **45**, and all the compounds in Tables III and V (except for the *p*-diethylamino analog **31**) were inactive.

The 6-methyl group was not critical for good activity; replacement by hydrogen, ethyl, butyl, methoxyl, ethoxyl, and methylthio gave compounds which were very active against *A. suum*. In their prophylactic action against *A. suum* in swine, these compounds eliminated lung pathology as effectively as thioflavin itself, but only the 6-ethyl analog was as active as thioflavin in preventing the development of liver lesions. Replacement of the 6-methyl by 6-chloro gave an inactive product.

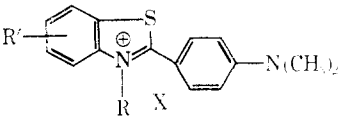
Less latitude was possible in replacing the 3-methyl substituent. The 3-ethyl analogs **22** and **23** showed some activity against *N. dubius* and *A. suum* in mice, but the introduction of groups larger than ethyl (**25–30**) resulted in complete loss of activity.

The basic substituent on the 4 position of the phenyl group was essential, as evidenced by the total inactivity of the isosteric 4-methoxy and 4-isopropyl analogs. However, the methyl substituents on the 4-amino group could be replaced by ethyl, as in **31**, which was very active on *A. suum* in both mice and swine.

(9) J. Metzger, H. Larivé, R. Dennilauler, R. Baralle, and C. Gaurat, *Bull. Soc. Chim. France*, 2868 (1964).

TABLE VI

ANTHELMINTIC ACTIVITY OF BENZOTHIAZOLIUM SALTS



No.	R	R'	X	GI nematodes in lambs[b]		*A. suum* in mice[c]	*A. suum* in swine[d]	
				N. dubius in mice,[a] activity	Dosage, mg/kg	Activity	Lung lesions, % redn	Liver lesions, % redn	Larvae in lungs, % redn
14	CH_3	H	Cl	5	150	5	80	87	95
15	CH_3	6-Cl	I	0					
16	CH_3	5-CH_3	I		50	0		97	100
		0.5 Pamoate					80	97	100
1	CH_3	6-CH_3	Cl	5	25	5	100	99	100
17	CH_3	6-C_2H_5	I		150	5	80	100	100
18	CH_3	6-*n*-C_4H_9	I				90		
19	CH_3	6-OCH_3	Cl	5	200	0	80	92	99
20	CH_3	6-OC_2H_5	Cl	5	200	1	80	97	100
21	CH_3	6-SCH_3	I	1	150	1	90	80	98
22	C_2H_5	H	I	0	200	1	40		
23	C_2H_5	6-CH_3	I	0			70		
24	$CH_2CH{=}CH_2$	H	I	0			0		
25	$(CH_2)_2CH_3$	H	I	0	150	0			
26	$(CH_2)_2CH_3$	6-CH_3	I	0	150	0			
27	$(CH_2)_3CH_3$	H	I						
28	$(CH_2)_3CH_3$	6-CH_3	I	0	100	0			
29	$CH_2C_6H_5$	H	Cl	0	100	0			
30	$CH_2C_6H_5$	6-CH_3	I	0	150	0	50		
31[e]				0			100	98	100

[a] Each of three mice which had been infected with 50 *N. dubius* larvae several weeks earlier was administered a dose of 15–50 mg/kg orally. A similar dose was administered on the following day. On the seventh day the mice were sacrificed and the test compounds were graded on the basis of the number of worms remaining in the intestine: 5 = total or near total elimination, 1 = moderate reduction, 0 = no activity. [b] The compounds were tested on sheep which had been experimentally infected with six species of gastrointestinal nematodes: *Haemonchus contortus, Cooperia curticei, Trichostrongylus colubriformis, Trichostrongylus axei, Ostertagia circumcincta,* and *Nematodirus spathiger*. The compounds were administered in two equal doses on consecutive days, and the reduction of the worm burden was estimated after determination of the egg count per gram of feces. The activity recorded is the average of the activity against the six helminths tested: 5 = total or near total elimination of the worm burden, 1 = moderate reduction, 0 = no activity. [c] A dose of 10 mg/kg was administered orally to each of three mice, followed by the administration of an infection of 100,000 embryonated *Ascaris suum* eggs. A second dose of 10 mg/kg was administered 4 hr later. After 8 days the mice were sacrificed and the extent of lung lesions was determined by gross examination of the lungs for the number and size of hemorrhagic areas due to the migration of the *Ascaris* larvae. The table lists the percentage reduction in lung lesions of the treated animals as compared with the unmedicated controls. [d] The test compounds were administered at a level of 0.01% in feed for a period of 10 days to two pigs in concrete-floored pens. An infection of 100,000 embryonated *Ascaris suum* eggs was administered 3 days after the start of the inclusion of the test compound in the feed. The animals were sacrificed after 10 days. The percentage reduction in liver lesions due to migrating *Ascaris* larvae in treated animals as compared with controls was determined by counting the small white scars ("milk spots") found on the surface of the liver. The procedure used to determine the number of larvae in the lungs of the pigs was based on the method described for mice by D. K. Hass (Ph.D. Thesis, University of Wisconsin, 1962). [e] 2-(4-Diethylaminophenyl)-3-methylbenzothiazolium chloride–resorcinol complex.

Thioflavin was also very active against the gastrointestinal nematodes of sheep at 25 mg/kg. Two close analogs (**14** and **17**) were also classified as being very active, but at the relatively high dosage of 150 mg/kg. In general, the compounds were significantly less active against the sheep nematodes than against *A. suum*.

A number of the most active compounds were tested as the pamoate and 3-hydroxy-2-naphthoate salts as well as the halide salts. The less soluble salts were less toxic to mice on oral administration, but showed no significant difference in anthelmintic potency.

Experimental Section[10]

3-Substituted Benzothiazoline-2-thiones (Table I). From Arylthioureas. 6-Ethyl-3-methylbenzothiazoline-2-thione.—*p*-Ethylphenylthiourea (75 g, 0.4 mole) (prepared in 45% yield from *p*-ethylaniline hydrochloride and KCNS) was dissolved in chlorobenzene (250 ml) and SO_2Cl_2 (67.5 g, 0.5 mole) was added dropwise at 70°. The mixture was stirred at 70° for 2 hr and then cooled and filtered to yield 2-amino-6-ethylbenzothiazole hydrochloride, mp 213–214°. An aqueous solution of the hydrochloride was basified with NH_4OH to give the free base, mp 115–116° (from EtOH), in 64% yield. *Anal.* ($C_9H_{10}N_2S$) C, H, N, S.

A solution of the 2-amino derivative (40 g, 0.22 mole) in 50% aqueous KOH solution was refluxed for 6 hr. The solution was cooled and treated with CS_2 (22.8 g, 0.3 mole). After refluxing for 6 hr, the solution was cooled and neutralized with AcOH to give 6-ethylbenzothiazoline-2-thione, mp 138–139° (from $CHCl_3$–petroleum ether (bp 60–90°)), yield 23 g (53%). *Anal.* ($C_9H_9NS_2$) C, H, N, S. MeI (10 ml) was added to a solution of 6-ethylbenzothiazoline-2-thione (22.4 g, 0.11 mole) in 12% NaOH (65 ml) and the mixture was stirred for 2 hr. The intermediate S-methyl derivative was separated by extraction with CCl_4 and the evaporated extract was rearranged to the 3-methyl derivative by heating at 200° for 1 hr with MeI (10 ml) and a crystal of I_2. The cooled reaction mixture was extracted with 250 ml of concentrated HCl, and the insoluble residue was crystallized from EtOH to give the product, mp 74–75°, yield 11.8 g (51%). This preparation was based on the general procedure described by Moore and Waight.[11]

(10) Melting points were taken on a Thomas–Hoover melting point apparatus and are corrected.

(11) C. G. Moore and E. S. Waight, *J. Chem. Soc.*, 4237 (1952).

From Chloronitrobenzenes. 3,5-Dimethylbenzothiazoline-2-thione.—4-Chloro-3-nitrotoluene (171.6 g, 1.0 mole) was refluxed with $Na_2S \cdot H_2O$ (648 g, 2.7 moles) in 1600 ml of H_2O for 24 hr. The solution was cooled and CS_2 (70 ml) was added dropwise. The mixture was heated on the steam bath for 1 hr and was then cooled and acidified with AcOH to give crude 5-methylbenzothiazoline-2-thione, mp 170–175°. Recrystallization from EtOH raised the melting point to 179–180°, yield 57.6 g (32%). *Anal.* ($C_8H_7NS_2$) C, H, N.

Treatment of 5-methylbenzothiazoline-2-thione (52 g) dissolved in 250 ml of 1.6 *N* aqueous NaOH with MeI (31 ml) gave 2-methylthio-5-methylbenzothiazole, mp 54–55°, yield 52 g (91%). *Anal.* ($C_9H_9NS_2$) C, H, N.

The 2-methylthio derivative (46 g) was rearranged as described above to the 3-methyl-2-thio isomer, mp 188.5–189.5°, in 79% yield.

3-Methyl-6-ethoxybenzothiazoline-2-thione.—Me_2SO_4 (80 ml) was added at 40° to a solution of the commercially available 6-ethoxybenzothiazoline-2-thione (140 g, 0.66 mole) and NaOH (105 ml of 50% NaOH) in 650 ml of H_2O. The reaction mixture was heated at 70° for 1 hr and then cooled giving the crude 2-methylthio-6-ethoxybenzothiazole, mp 39–42°. Recrystallization from dilute EtOH raised the melting point to 46–47°, yield 60%. *Anal.* ($C_{10}H_{11}NOS_2$) C, H, N.

The 2-methylthio derivative (58.8 g) was added to MeI (57 g) and a crystal of I_2. The MeI was slowly removed by distillation and the temperature of the residue was raised to 210°. After 30 min at that temperature the reaction mixture was cooled and extracted with 200 ml of concentrated HCl. The insoluble residue was crystallized from $CHCl_3$–petroleum ether to give the pure product, mp 133–134°, in 96% yield.

Substituted benzothiazoles (Table II). 5-(2-Benzothiazolyl)-*n*-pentanoic Acid (8).—The procedure used was a modification of the method described by Babichev and Derkach[12] for lower homologs. *o*-Aminobenzenethiol (42 g, 0.33 mole) in C_6H_6 (150 ml) was added dropwise to a solution of the anhydride[13] of adipic acid (42 g, 0.33 mole) in C_6H_6 (150 ml) at room temperature, and the solution was refluxed for 1.5 hr. The cooled solution was extracted with 10% NaOH and the alkaline extract was acidified to give the product, mp 133–134° (from dilute MeOH), yield 27 g (35%).

6-(2-Benzothiazolyl)-*n*-hexanoic Acid (9).—Pimelic acid was refluxed in an excess of Ac_2O for 3 hr, and the excess Ac_2O was removed under reduced pressure. The residue of pimelic anhydride[14] was treated with *o*-aminobenzenethiol as described above to give the product, mp 72–73°, in 48% yield.

2-(3-N,N-Dimethylcarbamylpropyl)benzothiazole (11).—4-(2-Benzothiazolyl)butyric acid[12] (25 g, 0.1 mole) was heated at 200° while Me_2NH was bubbled through the melt for 2 hr. The mixture was extracted with C_6H_6, and the C_6H_6 solution was washed with 5% K_2CO_3. Concentration of the solution gave the product, mp 67–68°, in 86% yield (from petroleum ether).

The other N,N-dimethylamides listed in Table II were prepared by the same method, which is based on the procedure described by McMillan and King.[15]

2-(*p*-Dimethylaminophenyl)-5-methylbenzothiazole.—A mixture of 4-chloro-3-nitrotoluene (171.6 g, 1.0 mole) and $Na_2S \cdot H_2O$ (648 g, 2.7 moles) in H_2O (1600 ml) was refluxed, with stirring, for 20 hr. The mixture was filtered, and to the clear filtrate was added H_2O_2 (160 ml of 30% solution) at 50–55° during 2 hr. The precipitated 2,2′-diamino-4,4′-dimethyldiphenyl disulfide was filtered and recrystallized from MeOH, giving 75 g (54%), mp 68–70.

The disulfide was added to EtOH (300 ml) and 7% HCl (750 ml) and the solution was heated to boiling. Zn powder (19.8 g, 0.3 g-atom) was added in portions until a clear solution resulted. The excess Zn was filtered, and NaOAc was added to the filtrate to precipitate zinc 2-amino-4-tolylmercaptide, yield 57 g. *p*-Dimethylaminobenzaldehyde (48.5 g, 0.34 mole) was added to a suspension of the zinc mercaptide (57 g, 0.17 mole) in AcOH (1.5 l.) and the mixture was heated on the steam bath for 30 min. The solution was filtered and diluted with H_2O to give the product, mp 182–184° (from Me_2CO), yield 42 g (46%). *Anal.* ($C_{16}H_{16}N_2S$) C, H, N, S.

2-Aryl-3-alkylbenzothiazolium Iodides (Table III). 2-(*p*-Chlorophenyl)-3-methylbenzothiazolium Iodide (Method B).—A solution of *p*-chlorobenzoyl chloride (17.5 g, 0.1 mole) in C_6H_6 (40 ml) was added dropwise to a stirred solution of 2-methylaminobenzenethiol (13.9 g, 0.1 mole)[16] in C_6H_6 (70 ml) at 20°. The mixture was stirred for 1 hr and 150 ml of H_2O was added. The aqueous layer was separated and treated with KI (30 g) giving the product, mp 224–225° (from MeOH), yield 24.7 g (64%).

2-*p*-Anisyl-3-*n*-propylbenzothiazolium Iodide.—3-*n*-Propylbenzothiazoline-2-thione[17] (19.5 g, 0.09 mole) was refluxed for 20 hr in EtOH (2 l.) containing 95 g (1.7 moles) of KOH. The mixture was filtered and the filtrate was concentrated. The residue was dissolved in H_2O (800 ml), neutralized to pH 7, and extracted with $CHCl_3$ (200 ml). The $CHCl_3$ extract was dried and evaporated, giving 2-*n*-propylaminobenzenethiol as a yellow oil which was used without further purification. The thiol was condensed with *p*-anisoyl chloride as previously described for method B to give the product, mp 192–193°, in 48% yield.

2-*p*-Anisyl-3-allylbenzothiazolium Iodide.—3-Allylbenzothiazoline-2-thione[11] (33 g, 0.16 mole) was added to a refluxing solution of KOH (45 g, 0.8 mole) in EtOH (350 ml). The mixture was allowed to reflux overnight (17 hr) with vigorous stirring. Most of the EtOH was then distilled and the residue was treated with 300 ml of H_2O. The aqueous solution was carefully neutralized with concentrated HCl (cooling) and extracted with three 100-ml portions of $CHCl_3$. The $CHCl_3$ extract was dried and concentrated under reduced pressure at 40°, giving 2-allylaminobenzenethiol as a dark red oil. The crude thiol was treated with *p*-anisoyl chloride to give the product, mp 153–155°, in 30% yield.

2-(*p*-Dimethylaminophenyl)-3-alkylbenzothiazolium Salts (Table IV). 2-(*p*-Dimethylaminophenyl)-3,6-dimethylbenzothiazolium Chloride (1) and *p*-(6-Methyl-2-benzothiazolyl)phenyltrimethylammonium Chloride (6) (Method A).—2-(*p*-Dimethylaminophenyl)-6-methylbenzothiazole[1a] (20 g, 0.075 mole) was heated with MeI (30 ml) in a pressure bottle at 90–95° for 4 hr. Since the chloride salt was desired for evaluation purposes, the crude methiodide (30.6 g) was added to Amberlite IRA-400 (chloride form, 250 ml) suspended in 1500 ml of MeOH and the suspension was boiled for 30 min. Concentration of the methanolic solution gave the mixture of chloride salts (23.4 g, yield quantitative), mp 203–204.5°. The crude product, which gave two main spots on tlc (HOAc–H_2O–EtOAc, 65:12:23 on silica gel G), was fractionated by extraction with $CHCl_3$. The $CHCl_3$-insoluble fraction consisted of **6**, mp 205°, λ_{max} 313 mμ. *Anal.* ($C_{17}H_{19}ClN_2S$) C, H, Cl, N, S.

The $CHCl_3$-soluble fraction was concentrated and crystallized from H_2O, giving the benzothiazolium chloride as the dihydrate, mp 212° dec, λ_{max} 413 mμ. The product was identical with that prepared by method B. Both isomers dequaternized on melting and reverted to the starting benzothiazole, mp 204.5–205°.

2-(*p*-Dimethylaminophenyl)-3-methylbenzothiazolium Chloride (Method B).—*p*-Dimethylaminobenzoyl chloride (29 g, 0.16 mole) was dissolved in 100 ml of C_6H_6, and the solution was added to 2-methylaminobenzenethiol[16] (22 g, 0.16 mole) in C_6H_6 (50 ml). The reaction mixture was allowed to stand overnight and the product was filtered (chloride salt), mp 179–180° dec, yield 45.5 g (95%).

The chloride (1 g) dissolved in H_2O was treated with excess aqueous NaI, giving the iodide salt, mp 223–224°, lit.[1b] mp 223–224°.

2-(*p*-Diethylaminophenyl)-3-methylbenzothiazolium Chloride–Resorcinol Complex (31).—2-(*p*-Diethylaminophenyl)benzothiazole (**7**) (20.0 g) was heated with MeI (24 ml) at 100° for 2.5 hr in a pressure bottle. The product was crystallized from MeOH–Et_2O giving 30.0 g (quantitative) of crude product, mp 183–185°. The uv spectrum indicated the presence of both the benzothiazolium isomer (420 mμ) and the anilinium isomer (300 mμ). The mixture of iodides (12.8 g, 0.03 mole) was stirred in 250 ml of Amberlite IRA-400 (Cl^- form) at room temperature for 1 hr. The resin was removed by filtration and the filtrate was concentrated to give 8.9 g (quantitative) of the product as a mixture of chloride salts.

Resorcinol (3.3 g) was added to a solution of the mixture of chlorides (8.9 g) in 500 ml of H_2O causing the precipitation of

(12) F. S. Babichev and N. Ya. Derkach, *Ukr. Khim. Zh.*, **22**, 208 (1956); *Chem. Abstr.*, **51**, 373 (1957).

(13) J. W. Hill, *J. Amer. Chem. Soc.*, **52**, 4110 (1930).

(14) J. W. Hill and W. H. Carothers, *ibid.*, **55**, 5023 (1933).

(15) F. H. McMillan and J. A. King, *ibid.*, **73**, 3165 (1951).

(16) A. I. Kiprianov and Z. N. Pazenko, *Zh. Obshch. Khim.*, **19**, 1523 (1949); *Chem. Abstr.*, **44**, 3488 (1950).

(17) F. P. Reed, A. Robertson, and W. A. Sexton, *J. Chem. Soc.*, 473 (1939).

the resorcinol complex of the benzothiazolium isomer (6.2 g, 53%), mp 202–204°. The uv spectrum of the complex showed only the peak (420 mμ) characteristic of the benzothiazolium isomer.

2-(3-Pyridylvinyl)benzothiazole Dimethiodide (35).—A solution of pyridine-3-carboxaldehyde methiodide[18] (5.0 g, 0.02 mole) and 2,3-dimethylbenzothiazolium iodide (5.8 g, 0.02 mole) in EtOH (50 ml) containing 0.3 ml of piperidine was refluxed for 2 hr. The product separated on standing, mp 226–228°, yield 85%.

Compound **36** was prepared similarly from *p*-anisaldehyde.

Thioflavin Hydroxide (41) and 2-Methoxy-2-(*p*-dimethylaminophenyl)-3,6-dimethylbenzothiazoline (42).—Thioflavin bromide dihydrate (300 g, 0.75 mole), dissolved in MeOH (6 l.), was passed through a column of IRA-400 (OH^- form) (1 l.) previously washed with MeOH. The effluent was evaporated to dryness, and the residual solid, consisting of a mixture of **41** and **42**, was extracted with Et_2O (1 l.). The insoluble hydroxide was removed by filtration, mp 159–160°, yield 100 g (45%). *Anal.* ($C_{17}H_{20}N_2OS$) C, H, N, O, S.

The extract was evaporated, and the residue was recrystallized from EtOH–Et_2O at room temperature to give pure **42**, mp 97–98°, yield 125 g (52%). *Anal.* ($C_{18}H_{22}N_2OS$) C, H, N, O, S.

Compound **42** was also prepared by adding MeONa [from 0.23 g (0.01 g-atom) of Na] to thioflavin bromide (4.0 g, 0.01 mole) in MeOH (50 ml) at room temperature. The product gradually separated, mp 95–97°, yield 2.7 g (86%).

Derivatives of 2-(*p*-Dimethylaminobenzoylamino)-5-methylbenzenethiol (43).—Thioflavin bromide dihydrate (40 g, 0.1 mole) was added to 400 ml of 5% aqueous NaOH and the suspension was boiled until all the solid had dissolved. One-tenth of this solution (0.01 mole) was treated with I_2 (2.5 g) in 15 ml of 5% NaOH and the solution was boiled for 2 hr. The precipitate obtained on cooling was crystallized from MeOH, giving the disulfide **44**, mp 200–202°, yield 2.0 g (67%). *Anal.* ($C_{36}H_{38}N_4O_2S_2$) C, H, N, O, S.

A further portion of the alkaline solution of the thiol was treated with MeI to give the S-methyl derivative **45a**, mp 126–128°, in 90% yield. *Anal.* ($C_{18}H_{22}N_2OS$) C, H, N.

The S-benzyl derivative **45b** was obtained by treatment of **43** with benzyl bromide, mp 122–123° (80%). *Anal.* ($C_{24}H_{26}N_2OS$) C, H, N.

Addition of benzoyl chloride in Me_2CO to the thiol gave the S-benzoyl derivative **45c**, mp 129.5–130.5° (95%). *Anal.* ($C_{24}H_{24}N_2O_2S$) C, H, N.

Similarly, the 3,4-dichlorobenzoyl derivative **45d**, mp 150–152°, was obtained in 32% yield. *Anal.* ($C_{24}H_{22}Cl_2N_2O_2S$) C, H, N.

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Catalysis by Poly-L-lysine of Aminolysis of Penicillin by Tris(hydroxymethyl)aminomethane

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The rate of penicillin loss in solutions containing both poly-L-lysine (PLL) and Tris in the pH range 7–9 is much more rapid than with either Tris or PLL alone. The rate is directly proportional to PLL concentration at low concentrations of PLL but becomes independent of Tris at high concentrations of the latter. Evidence is presented to show that the reaction taking place is aminolysis of penicillin by Tris catalyzed by PLL. Based on these results a mechanism is proposed which involves complex formation between PLL and penicillin prior to attack by Tris. This reaction may be a model for aminolysis of penicillin *in vivo*, leading to formation of antigen involved in penicillin allergy.

The principal antigenic determinant in penicillin allergy is the penicilloyl group bound by amide linkage to ε-amino groups of lysine residues on proteins.[1] One pathway by which formation of this hapten–protein conjugate may occur is the direct aminolysis of penicillin by the amino group.[2,3] Investigation of the mechanism of this reaction in a model system, using glycine as the amine, revealed that general base catalysis by a second molecule of glycine anion is required.[4] Further study of the reaction of benzylpenicillin with diamines suggested that intramolecular general base catalysis by one of the amino groups was involved and could increase reaction rate.[5] The present work was initiated to study the reaction of benzylpenicillin with poly-L-lysine (PLL), where it was thought that the large number of amino groups on the same molecule would accelerate the rate and perhaps be a better model for *in vivo* conditions.

As will be seen, however, in the presence of Tris buffer the reaction which takes place is aminolysis of the penicillin by Tris and this reaction is markedly catalyzed by PLL.

(1) A. L. DeWeck and G. Blum, *Int. Arch. Allergy*, **21**, 221 (1965).
(2) F. R. Batchelor, J. M. Dewdney, and D. Gazzard, *Nature*, **206**, 362 (1965).
(3) C. H. Schneider and A. L. DeWeck, *ibid.*, **206**, 57 (1965).
(4) M. A. Schwartz and G. M. Wu, *J. Pharm. Sci.*, **55**, 550 (1966).
(5) M. A. Schwartz, *ibid.*, **57**, 1209 (1968).

Results and Discussion

In the presence of 0.167 *M* Tris the rate of penicillin loss from solution at pH 8.8 is directly proportional to the concentration of PLL as shown in Figure 1. The small intercept represents reaction rate due to 0.167 *M* Tris alone at this pH. In Figure 2 is shown the dependence of reaction rate upon Tris concentration at several pH values when PLL concentration was kept constant at 9.0×10^{-5} *M* (0.04 *M* monomer). There is a saturation effect of the Tris and these curves can be fit by an equation of the form

$$k_{obsd} = \frac{a + b(T)}{c + (T)} \qquad (1)$$

where (T) represents Tris concentration and *a*, *b*, and *c* are constants. Plots of the reciprocal of k_{obsd} *vs.* 1/(T) were nonlinear as would be expected from eq 1.

The pH dependence of reaction rate at both high Tris concentration and in absence of Tris is given in Figure 3.