activity in the inhibition of carrageenin-induced edema in the rat paw. II, III, IV, azathioprine, and indomethacin were effective in suppression of both local and systemic lesions in adjuvant-induced arthritis. II, III, IV, and azathioprine were more effective in suppressing systemic lesions than in suppressing local lesions; whereas indomethacin was equally effective in suppressing local and systemic lesions.

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Antiparasitic Thiocyanatobenzothiazoles

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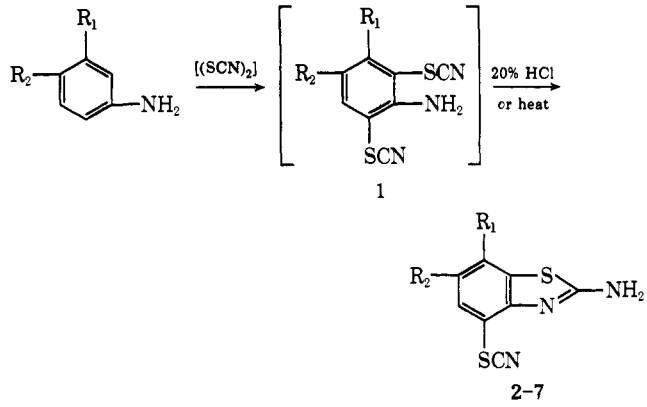
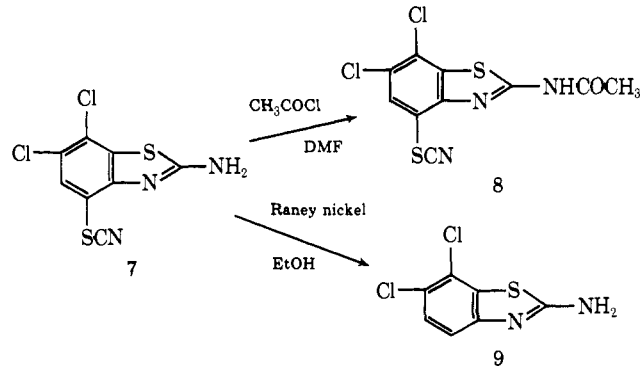
As part of a continuing program directed toward the development of novel antiparasitic agents,[1,2] a series of thiocyanatobenzothiazoles was synthesized.

The general method for the preparation of the benzothiazoles **2–7** involves the reaction of a suitably substituted aniline with excess thiocyanogen in anhydrous methanol solution.[3,4] The thiocyanogen was generated *in situ* by the action of bromine on sodium thiocyanate in methanol. The intermediate dithiocyanatoanilines **1** were occasionally isolable in this procedure but were generally smoothly cyclized in high yields in 20% HCl or by heating.[4] Slight modifications of the work-up procedure were necessary depending on the aniline substitution and are described in the Experimental Section. The synthesis of **2–7** is shown in Scheme I, and the structures are shown in Table I.

Since the cyclization of **1** can proceed to give either the 5,6- or 6,7-disubstituted benzothiazole derivatives, it became necessary to assign the proper structure. The chlorine atoms in **7** were found to be in the 6 and 7 positions as shown after removal of the thiocyanato group by boiling with Raney nickel in alcohol. The resulting product was 2-amino-6,7-dichlorobenzothiazole (**9**), which had been previously characterized.[3] The structures of the other disubstituted analogs (**5**, **6**) were assigned on the basis of the chemical shift in the nmr spectrum of the 5-proton which was in agreement with that of **7**. Details of thiocyanate removal and the evidence for structural assignment are included in the Experimental Section.

Scheme I





Acylation of **7** with acetyl chloride in DMF provided the 2-acetamido compound **8** in moderate yield. The position of acylation in 2-aminobenzothiazoles has been previously demonstrated to be on the 2-amino group.[5]

The thiocyanatobenzothiazoles **2–8** were tested for anthelmintic and antifungal activity against several test organisms, and the results are shown in Table I. The activities of the reference drugs, *dl*-tetramisole (**10**), bunamidine (**11**), and nystatin (**12**), are included for comparison.

Anthelmintic Testing. The anthelmintic activity of the compounds **2–8** was determined against *Ascaris suum* and *Hymenolepis nana* in the mouse, and the results of the testing are shown in Table I. The biologic method and the reference standards have been previously reported.[1,2]

The activity data of **2–8** against these two helminths failed to establish any structure–activity relationships. Only compound **3** had comparable activity to the reference drugs **10** and **11**.

Antifungal Testing. The antifungal activity of **2–8** was determined in Sabouraud's liquid medium BBL and the MIC values against a number of yeast species are shown in Table I.[6] The most active compounds tested were **5** and **7**, and, in addition, both significantly inhibited the growth of *Candida albicans* and *Microsporum canis* in the agar diffusion–cylinder cup test.[6]

Experimental Section

Melting points were determined in open capillary tubes using a Mel-Temp melting point apparatus and are uncorrected. The

Table I. Anthelmintic and Antifungal Activity of 2-Amino-4-thiocyanatobenzothiazoles

No.	R	R_1	R_2	Mp, °C	Yield, %	Recrystn solvent	Formula[a]	Anthelmintic act.[b] *in vivo*, % reductions				Antifungal act., MIC[c]					
								A. suum		*H. nana*		*T. glabrata* (VM-22)	*C. tropicalis* (VM-25)	*C. krusei* (VM-29B)	*C. guilliermondi* (VM-42)	*C. albicans* (VM-71)	*C. albicans* (VM-81)	*C. albicans* (M-3)
								300 mg/kg	100 mg/kg	300 mg/kg	100 mg/kg							
2	H	H	Cl	205–207	74	DMF–H_2O	$C_8H_4ClN_3S_2$	31	I'	75	I	10	>50	30	10	40	30	40
3	H	H	C_2H_5	191–192	53	MeOH	$C_{10}H_9N_3S_2$	92	69	93	30	>50	>50	>50	>50	>50	>50	>50
4	H	H	SCH_3	205–207	46	MeOH–DMF	$C_9H_7N_3S_2$	25	I	I	I	>50	>50	>50	>50	>50	>50	>50
5	H	Cl	F	196–199	4	CH_3NO_2	$C_8H_3ClFN_3S_2$	I	I	94	50	10	30	10	30	10	10	10
6	H	CH_3	F	224–226	21	MeOH	$C_9H_6FN_3S_2$	I	I	100	78	>50	>50	>50	>50	>50	>50	>50
7	H	Cl	Cl	243–245	26	EtOH	$C_8H_3Cl_2N_3S_2$	I	I	58	I	<1	40	2	<1	<1	<1	2
8	$COCH_3$	Cl	Cl	285–287	72	DMF–H_2O	$C_{10}H_5Cl_2N_3OS_2$	I	I	100	90[d]	>50	>50	>50	>50	>50	>50	>50
10	*dl*-Tetramisole							Toxic	100	Toxic	I							
11	Bunamidine							Toxic	11	Toxic	100							
12	Nystatin[e]											≤10	≤10	≤10	20	≤10	≤10	≤10

[a] Analyses for C, H, and N were obtained for all compounds and were within ±0.4% of the theoretical values. [b] *A. suum* infected mice were dosed twice a day for 5 days. *H. nana* infected mice were dosed twice a day for 3 days. [c] Minimal inhibitory concentration against indicated species of yeast (Norwich Pharmacal culture number) in Sabouraud's liquid medium BBL, μg/ml. [d] Dosed at 25 mg/kg three times b.i.d. [e] Potency = 4162 units/mg. [f] I = Inactive.

nmr spectra were determined with a Varian Associates Model A-60A spectrometer (Me_4Si).

2-Amino-6-chloro-4-thiocyanatobenzothiazole (2). A mixture of NaSCN (200 g, 2.5 mol) in anhydrous MeOH (1200 ml) was chilled to −7° in a Dry Ice–acetone bath. To the stirred mixture was added dropwise a chilled solution of Br_2 (200 g, 1.25 mol) in NaBr-saturated MeOH (300 ml). The temperature was maintained between −7 and −10° throughout the addition. After the addition was complete *p*-chloroaniline (65 g, 0.50 mol) was poured into the mixture and stirring continued at room temperature overnight. The reaction mixture was filtered and the filtrate was poured into H_2O (3 l.) and made basic with NH_4OH to precipitate the product. The product was removed by filtration and washed with H_2O and EtOH to give a tan solid (90 g, 74%).

Compounds 3 and 4 in Table I were prepared in a similar manner using the appropriately substituted anilines.

2-Amino-6,7-dichloro-4-thiocyanatobenzothiazole (7). A solution of NaSCN (162 g, 2.0 mol) in anhydrous MeOH was stirred below −15° in a Dry Ice–acetone bath. To it was added a cooled solution of Br_2 (60 ml, 1.0 mol) in NaBr-saturated MeOH (400 ml) over 5–10 min, maintaining the temperature below −10° and shielding from light. After the addition was complete, 3,4-dichloroaniline (65 g, 0.40 mol) was rapidly added to the stirred mixture at −10 to −15°. The stirred reaction mixture was allowed to come to room temperature slowly, and stirring was continued for an additional 6 hr. The solid was removed by filtration, suspended in H_2O, and made basic with NH_4OH. The suspension was filtered and the solid boiled in 1 l. of EtOH and filtered into a warm flask. The product crystallized on cooling and was collected by filtration (28 g, 26%); nmr (DMSO) δ 7.57 ppm (s, 1, 5-proton).

Compounds 5 and 6 in Table I were prepared in a similar manner using the appropriately substituted anilines: nmr (DMSO) of 5 δ 7.44 ppm (d, 1, J = 9.5 Hz, 5-proton) and of 6 δ 7.22 ppm (d, 1, J = 9.5 Hz, 5-proton).

2-Acetamido-6,7-dichloro-4-thiocyanatobenzothiazole (8). Acetyl chloride (8.5 ml, 0.12 mol) was added dropwise to a stirred mixture of 7 (27 g, 0.10 mol) and anhydrous NaOAc (20 g, 0.25 mol) in DMF (150 ml) at room temperature. The temperature rose to 45° and the mixture was then heated on a steam bath for 2 hr, cooled, and poured into an ice–water mixture. The solid was collected by filtration and recrystallized from DMF–H_2O to give analytical material (23 g, 72%).

2-Amino-6,7-dichlorobenzothiazole (9). To a solution of 7 (2.76 g, 0.01 mol) in absolute EtOH (150 ml) was added Raney nickel No. 28 (4 g). The stirred mixture was heated under reflux for 4 hr and then filtered while hot. The alcohol filtrate was subjected to gas chromatographic analysis and the results indicated the presence of only 9 and no evidence of 2-amino-5,6-dichlorobenzothiazole.[3] Evaporation of the solvent *in vacuo* gave material (1.3 g, 60%) which was identical with an authentic sample of 9.[3] The identification was made on the basis of nmr and ir spectral comparisons, melting point and mixture melting point data, and the previously mentioned gas chromatographic analysis. Experimental conditions for the gas chromatographic analyses have been reported previously.[3]

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