modates only about three cupric ions, which are relatively tightly bound, while the other accommodates about 16 cupric ions, which are rather weakly bound. It is possible that the first three cupric ions bind intimately by penetrating the protein while the rest of the cupric ions bind weakly to the surface of the protein.

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Synthesis of 2-(4-Arylthiosemicarbazidocarbonylthio)benzthiazoles and Their Monoamine Oxidase Inhibitory and Anticonvulsant Properties

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Abstract □ Ten 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles were synthesized, characterized, and evaluated for their monoamine oxidase inhibitory and anticonvulsant activities. All substituted benzthiazoles inhibited activity of monoamine oxidase in rat brain homogenate where the degree of enzyme inhibition was higher with kynuramine as compared to tyramine and 5-hydroxytryptamine as the substrates. All substituted benzthiazoles possessed measurable anticonvulsant activity against pentylenetetrazol-induced convulsions.

Keyphrases □ 2-(4-Arylthiosemicarbazidocarbonylthio)benzthiazoles—synthesis, characterization, monoamine oxidase inhibitory and anticonvulsant activities □ Monoamine oxidase inhibitory activity—substituted benzthiazoles, relationship between enzyme inhibitory and anticonvulsant activities, rat brain homogenate □ Anticonvulsant activity—substituted benzthiazoles, relationship to monoamine oxidase inhibitory activity, mice

Benzthiazoles have been reported to possess diverse pharmacological properties (1, 2). Recent studies indicated anticonvulsant and monoamine oxidase [EC 1.4.3.4 monoamine: O_2 oxidoreductase (deaminating)] inhibitory properties of substituted benzthiazoles (3). In addition, the ability of hydrazines (4), semicarbazides (5, 6), and thiosemicarbazides (7) to inhibit monoamine oxidase and the anticonvulsant activities possessed by the inhibitors of monoamine oxidase (8) led to the synthesis of 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles. In the present study, all substituted benzthiazoles possessed anticonvulsant activity and inhibited monoamine oxidase activity of rat brain homogenate.

The various substituted benzthiazoles were synthesized by following the methods outlined in Scheme I.

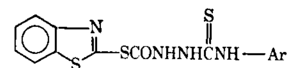
EXPERIMENTAL[1]

Chemistry—Ten 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles were synthesized from 2-mercaptobenzthiazole. 2-Mercaptobenzthiazole was first treated with ethyl chloroformate in the presence of dry acetone and anhydrous potassium carbonate to yield 2-(ethoxycarbonylthio)benzthiazole, and then the resulting product was converted into 2-(hydrazinocarbonylthio)benzthiazole by refluxing with hydrazine hydrate on a steam bath in absolute ethanol. The hydrazide thus formed was refluxed with suitable arylisothiocyanates in dry benzene to form the desired 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles (Compounds I–X).

2-(Ethoxycarbonylthio)benzthiazole—A mixture of 2-mercaptobenzthiazole (0.01 mole) in dry acetone, containing anhydrous potassium carbonate (0.011 mole) and ethyl chloroformate (0.01 mole), was refluxed on a steam bath for 16–18 hr. The mixture was filtered and the excess acetone from the filtrate was removed by distillation. The solid mass, which separated on cooling, was collected by filtration, washed with water, and recrystallized from ethanol, mp 63° [lit. (9) mp 64°].

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes with partial immersion thermometer and are corrected.

Table I—Physical Constants of 2-(4-Arylthiosemicarbazidocarbonylthio)benzthiazoles



Compound	Ar	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Found
I	C_6H_5	241°	68	$C_{15}H_{12}N_4OS_3$	C 50.00	50.24
					H 3.33	3.28
					N 15.55	15.59
II	o-$CH_3C_6H_4$	250°	55	$C_{16}H_{14}N_4OS_3$	C 51.33	51.25
					H 3.74	3.68
					N 14.97	14.91
III	m-$CH_3C_6H_4$	258°	58	$C_{16}H_{14}N_4OS_3$	C 51.33	51.42
					H 3.74	3.82
					N 14.97	14.98
IV	p-$CH_3C_6H_4$	244°	85	$C_{16}H_{14}N_4OS_3$	C 51.33	51.28
					H 3.74	3.75
					N 14.97	14.85
V	o-$OCH_3C_6H_4$	242°	52	$C_{16}H_{14}N_4O_2S_3$	C 49.23	48.28
					H 3.59	3.56
					N 14.36	14.28
VI	p-$OCH_3C_6H_4$	230°	86	$C_{16}H_{14}N_4O_2S_3$	C 49.23	49.32
					H 3.59	3.62
					N 14.36	14.31
VII	p-ClC_6H_4	255°	65	$C_{15}H_{11}ClN_4OS_3$	C 45.63	45.48
					H 2.79	2.76
					N 14.19	14.15
VIII	p-BrC_6H_4	240°	90	$C_{15}H_{11}BrN_4OS_3$	C 41.00	41.42
					H 2.56	2.51
					N 12.76	12.66
IX	p-IC_6H_4	256°	75	$C_{15}H_{11}IN_4OS_3$	C 37.04	37.34
					H 2.26	2.23
					N 11.52	11.47
X	α-$C_{10}H_7$	260°	70	$C_{19}H_{14}N_4OS_3$	C 55.61	55.58
					H 3.41	3.37
					N 13.66	13.55

[a] Melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

2-(Hydrazinocarbonylthio)benzthiazole—To a solution of 2-(ethoxycarbonylthio)benzthiazole (0.01 mole) in absolute ethanol was added 99–100% hydrazine hydrate (0.02 mole), and the mixture was refluxed on a steam bath for 10–12 hr. Excess ethanol was distilled and the precipitated crude product was collected, dried, and recrystallized from ethanol, mp 199–200° [lit. (9) mp 201°].

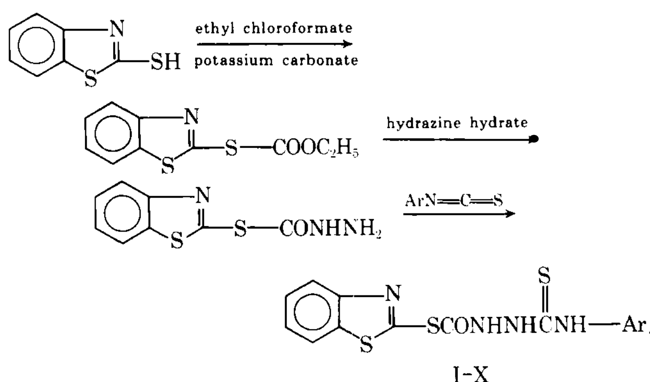
2-(4-Arylthiosemicarbazidocarbonylthio)benzthiazoles (I–X)—Equimolar portions of 2-(hydrazinocarbonylthio)benzthiazole and the suitable arylisothiocyanate were mixed in dry benzene, and the mixture was refluxed on a steam bath for 6 hr. The mixture was concentrated by removing benzene by distillation. The crude product, which separated on cooling, was collected by filtration; it was washed first with ether, then with dilute hydrochloric acid, and finally with water and was recrystallized from dimethylformamide (Table I).

Determination of Monoamine Oxidase Activity—*Spectrophotofluorometric Method*—Male albino rats, 100–150 g, were allowed food and water *ad libitum* until they were sacrificed by decapitation. The brains were removed immediately and homogenized[2] in the ratio of 1:9 (w/v) in ice-cold 0.25 *M* sucrose. The monoamine oxidase activity of rat brain homogenate was determined by incubation at 37° in air for 30 min, using kynuramine as the substrate (10). The reaction mixture in a total volume of 3 ml consisted of 0.5 ml of phosphate buffer (0.2 *M*, pH 7.5), 0.1 m*M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg wet weight of the tissue).

The various 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles were dissolved in propylene glycol (100%) and added to the brain homogenate to produce a final concentration of 0.5 m*M*. An equivalent amount of propylene glycol was added to the control tubes, and the mixture was incubated for 10 min before the addition of kynuramine. After the addition of kynuramine, the mixture was further incubated for 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid (w/v), and the precipitated proteins were removed by centrifugation. Suitable 1-ml aliquots of the supernatant solution were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline, formed during oxidative deamination of kynuramine by rat brain homogenate.

The 4-hydroxyquinoline formed was measured fluorometrically[3], using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm. An increase in absorbance provided a direct measurement of 4-hydroxyquinoline formation, which was taken as an index of the monoamine oxidase activity. The percentage inhibition was calculated from the decrease observed in absorbance, and this value provided an index of the inhibitory property of these substituted benzthiazoles.

Warburg Manometric Method—The monoamine oxidase activity of rat brain homogenate was determined by the conventional Warburg manometric technique, using tyramine and 5-hydroxytryptamine as the substrates (10). The decrease in oxygen uptake for 1 hr during oxidative deamination of tyramine or 5-hydroxytryptamine in the presence of the substituted benzthiazoles was used as an index of enzyme inhibition. The reaction mixture in a final concentration consisted of 50 m*M* phosphate buffer (pH 7.5),



Scheme I

[2] Potter–Elvehjem homogenizer.

[3] Aminco Bowman spectrophotofluorometer.

Table II—Monoamine Oxidase Inhibitory and Anticonvulsant Activity of 2-(4-Arylthiosemicarbazidocarbonylthio)benzthiazoles

Compound	Monoamine Oxidase Inhibition[a], %			Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], % after 24 hr
	Kynuramine	Tyramine	5-Hydroxytryptamine		
I	30.0 ± 1.0	12.1 ± 0.7	14.8 ± 0.9	20	40
II	71.4 ± 0.8	29.2 ± 1.0	18.8 ± 0.9	20	60
III	61.4 ± 0.8	39.0 ± 0.9	28.3 ± 0.7	30	50
IV	74.3 ± 0.7	28.2 ± 0.9	19.2 ± 0.8	30	50
V	87.1 ± 0.6	24.6 ± 0.6	29.3 ± 1.0	50	50
VI	70.0 ± 1.0	29.6 ± 0.7	49.9 ± 0.8	10	90
VII	68.6 ± 1.0	23.8 ± 0.7	30.1 ± 0.7	50	70
VIII	65.0 ± 0.8	17.8 ± 0.9	26.0 ± 0.5	50	40
IX	77.9 ± 0.5	36.5 ± 0.6	46.9 ± 0.9	30	50
X	34.3 ± 0.8	7.9 ± 0.8	8.7 ± 1.1	60	40

[a] The reaction mixture and assay procedures are described in the text. Each experiment was done in triplicate, and the values are the means of three separate experiments ± *SE*. [b] Screening procedures for the determination of anticonvulsant activity are described in the text. [c] Mortality in pentylenetetrazol-treated mice was observed during 24 hr.

10 m*M* tyramine or 5-hydroxytryptamine, and rat brain homogenate (equivalent to 250 mg of fresh tissue weight) in a total volume of 3 ml.

The various substituted benzthiazoles were dissolved in propylene glycol (100%), used at a final concentration of 1 m*M*, and were incubated with the enzyme preparation for 20 min before tyramine or 5-hydroxytryptamine was added. The enzyme system was then incubated for an additional hour at 37°, using oxygen as the gaseous phase. Readings of oxygen uptake were made every 10 min.

Determination of Anticonvulsant Activity—Anticonvulsant activity of substituted benzthiazoles was determined (11) in mice, 25–30 g, of either sex. The mice were divided in groups of 10, keeping the group weights as near the same as possible. Each test compound was suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v) and was injected in a group of 10 mice at a dose of 100 mg/kg ip. Four hours after the administration of the substituted benzthiazoles, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol not only produced convulsions in almost all untreated mice but also exhibited 100% mortality during 24 hr. No mortality was observed during 24 hr in animals treated with 100 mg/kg alone of the test compounds.

The mice were observed 60 min for seizures. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during 60 min were considered protected. The number of mice protected in each group was recorded, and the anticonvulsant activity of 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles was represented as percent protection. The mice were then observed for 24 hr, and their mortality was recorded.

RESULTS AND DISCUSSION

The inhibitory effects of 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles on the monoamine oxidase activity of rat brain homogenate during oxidative deamination of different substrates and their ability to afford protection against pentylenetetrazol-induced convulsions are recorded in Table II. The results indicated greater sensitivity of these substituted benzthiazoles toward monoamine oxidase during oxidative deamination of kynuramine, as reflected by higher inhibition of monoamine oxidase compared to inhibition observed when tyramine or 5-hydroxytryptamine was the substrate. Similar changes in the degree of monoamine oxidase inhibition also have been observed with other inhibitors during oxidative deamination of different substrates (7, 12).

The presence of substituents on the phenyl group of the thiosemicarbazide moiety influenced their ability to inhibit monoamine oxidase. Introduction of a substituent in the phenyl ring of these substituted benzthiazoles increased the degree of monoamine oxidase inhibition, with the exception of α-naphthyl substitution (X), which caused lowering of inhibition when tyramine or 5-hydroxytryptamine was the substrate. Among halogen-substituted benzthiazoles (VII–IX), the iodo-substituted benzthiazole (IX) possessed maximum monoamine oxidase inhibitory activity.

All 2-(4-arylthiosemicarbazidocarbonylthio)benzthiazoles possessed measurable anticonvulsant activity and exhibited some protection against pentylenetetrazol-induced 24 hr mortality in experimental mice (Table II).

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