Antihemolytic and Anticonvulsant Activities of 1-(2,4-Dichloro/2,4,5-Trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and Their Inhibition of NAD-Dependent Oxidations and Monoamine Oxidase

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Abstract □ Several 1-(2,4-dichloro and 2,4,5-trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides were synthesized and characterized by their sharp melting points and elemental analyses. All substituted thiosemicarbazides protected *in vitro* hypoosmotic hemolysis of dog red blood cells. These thiosemicarbazides selectively inhibited nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate and α-ketoglutarate, while NAD-independent oxidation of succinate was not affected. These compounds inhibited the activity of monamine oxidase in rat brain homogenate, and the degree of inhibition ranged from 26.5 to 89.2% at a final concentration of 0.03 m*M*, with kynuramine as the substrate. Almost all thiosemicarbazides possessed anticonvulsant activity; protection against pentylenetetrazol-induced convulsions in mice ranged from 10 to 70% at a dose of 100 mg/kg ip. These results provided evidence of some similarity between the membrane-stabilizing property of these substituted thiosemicarbazides with their ability to exhibit selective inhibition of NAD-dependent oxidations and inhibition of monoamine oxidase. On the other hand, the anticonvulsant activity possessed by these substituted thiosemicarbazides was unrelated to their *in vitro* antihemolytic and enzyme inhibitory properties.

Keyphrases □ Thiosemicarbazides, 1-(2,4-dichloro and 2,4,5-trichlorophenoxyacetyl)-4-alkyl/aryl—synthesis, antihemolytic and anticonvulsant activities, and relationship to inhibition of cellular respiratory activity and inhibition of monoamine oxidase □ Structure–activity relationships—thiosemicarbazides, antihemolytic activity, anticonvulsant activity, enzyme inhibitory properties □ Antihemolytic activity—thiosemicarbazides synthesized and tested □ Cellular respiratory activity—thiosemicarbazides, selective inhibition of NAD-dependent oxidation of pyruvate and α-ketoglutarate, monoamine oxidase, rat brain homogenate □ Monoamine oxidase inhibition—thiosemicarbazides, relationship to antihemolytic (membrane stabilizing) and anticonvulsant properties, inhibition of cellular respiratory activity, selective inhibition of NAD-dependent oxidation

Various hydrazines (1) and semicarbazides (2, 3) have been shown to inhibit monoamine oxidase [EC 1.4.3.4 monoamine O_2 oxido-reductase (deaminating)]. Monoamine oxidase inhibitors also have been shown to possess anticonvulsant properties (4). Furthermore, studies have indicated a relationship between the *in vitro* monoamine oxidase inhibitory property of α-benzoylamino-*N*-[(4-aryl semicarbazide/thiosemicarbazide)benzoyl]-4-substituted cinnamides and their ability to afford protection against pentylenetetrazol-induced convulsions (5). Numerous psychotropic agents have been proposed to affect the physicochemical properties of cell membranes which account for the basis of their mechanism of action (6), and various drug responses have been indicated as the probable result of drug–receptor interactions (7).

Stabilization of the red cell membranes has also been observed in various central nervous system depressants and local anesthetics (8, 9), tricyclic tranquilizers (9, 10), nonphenothiazine tranquilizers (8), and biogenic amines (11). Recently, the synthesis of substituted anilino-[3-methoxy-4-(4-arylthiosemicarbazidocarbonylmethyleneoxy)]benzylidines was reported, and attempts were made to correlate their monoamine oxidase inhibitory effectiveness and their ability to afford protection against hypoosmotic hemolysis in dog erythrocytes with their anticonvulsant property (12). A decrease in the cellular respiratory activity of brain tissue by the inhibition of the mitochondrial membrane-bound oxidase systems was observed with hypnotics (13–15) and anticonvulsants (16, 17), where these changes are presumably responsible for reduced energy production, leading ultimately to depressed neuronal activity.

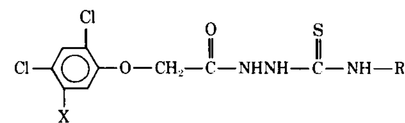
In the present study, various 1-(2,4-dichlorophenoxyacetyl)-4-substituted thiosemicarbazides and 1-(2,4,5-trichlorophenoxyacetyl)-4-substituted thiosemicarbazides were synthesized and evaluated for: (*a*) their *in vitro* ability to stabilize the red cell membrane; (*b*) their effects on the cellular respiratory activity during oxidation of pyruvate, α-ketoglutarate, NADH, and succinate by rat brain homogenates, and (*c*) their effects on the activity of rat brain monoamine oxidase. The ability of these substituted thiosemicarbazides to provide protection against pentylenetetrazol-induced seizures was also investigated in an attempt to correlate the anticonvulsant activity exhibited by these compounds with their ability to inhibit various enzyme systems as a basis of their biochemical mechanism of action.

EXPERIMENTAL[1]

Preparation of 1-(2,4-Dichlorophenoxyacetyl- or 2,4,5-Trichlorophenoxyacetyl)-4-substituted Thiosemicarbazides—The various 1-(2,4-dichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and 1-(2,4,5-trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides were synthesized by refluxing a mixture of an appropriate substituted chlorophenoxyacethydrazide (0.01 mole) and alkyl/aryl isothiocyanate (0.01 mole) in 25 ml of absolute ethanol for 2 hr. The solid mass, which separated on cooling, was collected by filtration, washed with petroleum ether (bp 40–60°), and recrystallized from ethanol (Table I).

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

Table I—Physical Constants of 1-(2,4-Dichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and 1-(2,4,5-Trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides



Compound	X	R	Melting Point[a]	Yield, %	Formula	Analysis, % Calc.	Analysis, % Found
I	H	C_2H_5	148°	50	$C_{11}H_{13}Cl_2N_3O_2S$	C 41.01	40.98
						H 4.07	4.00
						N 13.05	12.83
II	H	n-C_4H_9	126°	65	$C_{13}H_{17}Cl_2N_3O_2S$	C 44.58	44.44
						H 4.89	4.78
						N 12.00	11.94
III	H	n-C_7H_{15}	152°	55	$C_{16}H_{23}Cl_2N_3O_2S$	C 48.98	48.87
						H 5.86	5.87
						N 10.71	10.48
IV	H	o-$CH_3C_6H_4$	210°	45	$C_{16}H_{15}Cl_2N_3O_2S$	C 50.01	49.90
						H 3.94	3.89
						N 10.94	10.80
V	H	m-$CH_3C_6H_4$	162°	45	$C_{16}H_{15}Cl_2N_3O_2S$	C 50.01	50.00
						H 3.94	3.87
						N 10.94	10.80
VI	H	p-BrC_6H_4	183–185°	55	$C_{15}H_{12}BrCl_2N_3O_2S$	C 40.11	40.07
						H 2.69	2.65
						N 9.36	9.29
VII	H	p-FC_6H_4	162°	50	$C_{15}H_{12}Cl_2FN_3O_2S$	C 46.41	46.32
						H 3.12	3.00
						N 10.83	10.54
VIII	H	CH_2—CH=CH_2	158°	40	$C_{12}H_{13}Cl_2N_3O_2S$	C 43.13	43.00
						H 3.92	3.91
						N 12.58	12.20
IX	H	C_6H_{11}	198°	60	$C_{15}H_{19}Cl_2N_3O_2S$	C 47.88	47.85
						H 5.09	5.07
						N 11.17	11.20
X	Cl	C_2H_5	206°	65	$C_{11}H_{12}Cl_3N_3O_2S$	C 37.05	36.96
						H 3.39	3.37
						N 11.79	11.40
XI	Cl	n-C_4H_9	168°	55	$C_{13}H_{16}Cl_3N_3O_2S$	C 40.59	40.54
						H 4.19	4.00
						N 10.93	10.58
XII	Cl	n-C_7H_{15}	205–207°	40	$C_{16}H_{22}Cl_3N_3O_2S$	C 45.03	45.00
						H 5.20	5.09
						N 9.85	9.63
XIII	Cl	o-$CH_3C_6H_4$	165°	50	$C_{16}H_{14}Cl_3N_3O_2S$	C 45.90	45.85
						H 3.37	3.29
						N 10.04	9.90
XIV	Cl	m-$CH_3C_6H_4$	185°	60	$C_{16}H_{14}Cl_3N_3O_2S$	C 45.90	45.87
						H 3.37	3.36
						N 10.04	9.98
XV	Cl	p-BrC_6H_4	195°	45	$C_{15}H_{11}BrCl_3N_3O_2S$	C 37.25	37.20
						H 2.29	2.19
						N 8.69	8.53
XVI	Cl	p-FC_6H_4	160°	55	$C_{15}H_{11}Cl_2FN_3O_2S$	C 42.62	42.59
						H 2.62	2.60
						N 9.94	10.00
XVII	Cl	CH_2—CH=CH_2	166°	50	$C_{12}H_{12}Cl_3N_3O_2S$	C 39.10	39.02
						H 3.28	3.19
						N 11.40	11.28
XVIII	Cl	C_6H_{11}	175°	55	$C_{15}H_{18}Cl_3N_3O_2S$	C 43.86	43.56
						H 4.42	4.41
						N 10.23	10.04

[a] Melting points were taken in open capillary tubes and are corrected.

Determination of Hypoosmotic Hemolysis—Assay of hypoosmotic hemolysis was carried out by following a slightly modified published procedure (10) in which blood was used without oxygenation. Fresh heparinized blood of healthy mongrel dogs was used. To a 0.1-ml aliquot of dog blood was added 3 ml of a buffer solution (0.425% Na_2HPO_4–NaH_2PO_4 buffer, 5 mM, pH 7.4; total osmolality 135 mosmoles/liter) containing the test compounds. The tubes were shaken gently two or three times and were allowed to stand at room temperature (26–28°) for exactly 10 min. These tubes were then centrifuged for 5 min at 1000×g to separate the cells.

Under these conditions, hemolysis of the blood cells occurred but in no case exceeded 50%. The absorbance of the supernate was read at 540 nm in a colorimeter. Percent protection was calculated by comparing the absorbance values observed in the presence of the test compounds with those observed in the control tubes without the test compounds. The control value was represented as 100% hypoosmotic hemolysis. Absorbance values for the blank containing 3 ml of 0.85% NaCl (normal saline) were subtracted from the values for both the control and experimental tubes.

All test compounds were dissolved in the buffered saline solution of pH 7.4 (osmolality 135 mosmoles/liter) and were used at a final concentration of 0.05 or 0.1 mM. The effective dose$_{50}$ (ED_{50}), the concentration of the the test compound exhibiting 50% antihemolytic activity, was evaluated graphically using different concentrations of these substituted thiosemicarbazides, and this procedure provided comparative effectiveness of the test compounds. From the relationship between the concentration of the test compound and its relative antihemolytic activity, it was possible to determine, either by extrapolation or interpolation, the ED_{50} values

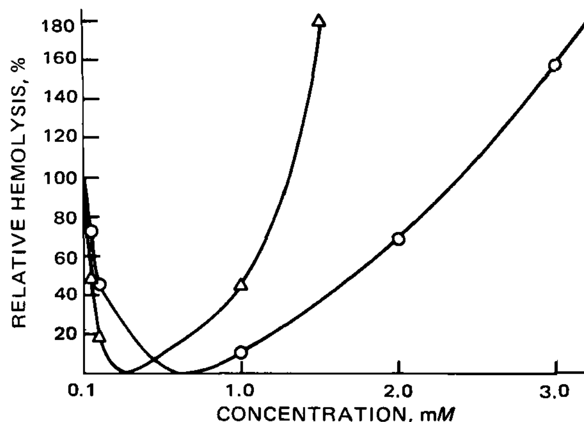


Figure 1—*Biphasic response of varying concentrations of 1-(2,4-dichlorophenoxyacetyl)-4-*m*-tolylthiosemicarbazide (V, △) and 1-(2,4,5-trichlorophenoxyacetyl)-4-ethylthiosemicarbazide (X, ○) on relative hypoosmotic hemolysis of red blood cells of dog. Assay procedures and contents of the reaction mixture are as indicated in the text.*

of these substituted thiosemicarbazides.

Assay of Respiratory Activity of Rat Brain Homogenate[2]—Male albino rats[3], kept on *ad libitum* diet, were used. Rats weighing 150–200 g were sacrificed by decapitation. The brains were taken out immediately and homogenized[4] in the ratio of 1:9 (w/v) in 0.25 *M* cold sucrose. Respiratory activity was determined by measuring the oxygen uptake by the conventional Warburg manometric method at 37° with air as the gas phase (16). Fresh brain homogenate of healthy albino rats, equivalent to 125 mg of wet tissue weight, was used in each flask.

The reaction mixture, in a final volume of 3 ml, consisted of 20 m*M* Na_2HPO_4 buffer (pH 7.4), 6.7 m*M* $MgSO_4$, 1 m*M* AMP (sodium salt), 33 m*M* KCl, and 500 µg of cytochrome c. The central well contained 0.2 ml of 20% KOH solution. Pyruvate, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*, and the final concentration of NADH was 0.5 m*M*. All substituted thiosemicarbazides were dissolved in propylene glycol (100%) and were used at a final concentration of 1 m*M*. An equivalent amount of propylene glycol was added to the control vessels.

Determination of Monoamine Oxidase Activity—A spectrophotofluorometric method was used for the determination of monoamine oxidase activity of rat brain homogenate, using kynuramine as the substrate (18). The 4-hydroxyquinoline formed during oxidative deamination of kynuramine was measured fluorometrically in a spectrophotofluorometer[5], using activating light of 315 nm and measuring fluorescence at the maximum of 380 nm.

Male albino rats[3], 150–200 g, were allowed food and water *ad libitum* and were sacrificed by decapitation. The brains were removed immediately and homogenized in the ratio of 1:9 (w/v) in ice-cold 0.25 *M* sucrose. The reaction mixture, in a total volume of 3 ml, consisted of phosphate buffer (0.5 ml, pH 7.5; 0.2 *M*), 0.1 m*M* kynuramine, and 0.5 ml of brain homogenate (equivalent to 10 mg of wet brain weight). The monoamine oxidase activity of rat brain homogenate was determined by incubation for 30 min at 37° in air.

The various substituted thiosemicarbazides were dissolved in propylene glycol (100%), added to the brain homogenate at a final concentration of 0.33 m*M*, and then incubated for 10 min before the addition of kynuramine. After the addition of kynuramine, the mixture was incubated for an additional 30 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid solution (w/v), and the precipitated proteins were removed by centrifugation.

Suitable 1-ml aliquots of the supernate were taken in 2 ml of 1 *N* NaOH solution and were assayed for 4-hydroxyquinoline. 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 thiosemicarbazides.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined against pentylenetetrazol-induced seizures in albino mice[3] of either sex weighing 25–30 g. The mice were divided into groups of 10, and the group weights were kept as near the same as possible. All substituted thiosemicarbazides were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compounds were injected intraperitoneally in a dose of 100 mg/kg to one group of 10 mice. Four hours after the administration of these compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol has been shown to produce convulsions in almost all untreated mice and to exhibit 100% mortality in these animals during 24 hr. No mortality was observed during 24 hr in mice treated with 100 mg/kg of the test compounds alone.

The mice were observed for 60 min for the occurrence of convulsions. An episode of clonic convulsion persisting 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 these substituted thiosemicarbazides was represented as the percentage protection. The animals were then observed for 24 hr and the mortality was recorded.

Table II—Protection of Hypoosmotic Hemolysis of Dog Erythrocytes by 1-(2,4-Dichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and 1-(2,4,5-Trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides

Compound	Protection[a], %		ED_{50}[b], m*M*
	0.05 m*M*	0.1 m*M*	
I	22.6 ± 0.38	48.1 ± 1.11	0.103
II	17.4 ± 0.58	39.5 ± 0.95	0.125
III	13.9 ± 0.77	28.3 ± 0.48	0.180
IV	22.3 ± 0.48	43.5 ± 0.73	0.118
V	51.5 ± 0.21	82.3 ± 1.25	0.048
VI	31.5 ± 0.35	44.4 ± 1.40	0.130
VII	35.6 ± 0.76	58.6 ± 0.88	0.078
VIII	40.0 ± 0.66	71.5 ± 0.64	0.064
IX	52.1 ± 0.99	82.5 ± 0.22	0.047
X	26.8 ± 0.41	53.4 ± 1.41	0.094
XI	41.3 ± 0.62	69.0 ± 0.85	0.063
XII	24.3 ± 0.59	44.2 ± 0.63	0.117
XIII	59.6 ± 0.30	85.3 ± 0.58	0.040
XIV	29.4 ± 0.99	63.4 ± 0.66	0.080
XV	41.3 ± 1.21	66.3 ± 0.73	0.066
XVI	48.9 ± 0.87	80.3 ± 0.11	0.052
XVII	42.5 ± 0.78	74.6 ± 1.7	0.061
XVIII	46.5 ± 0.55	80.1 ± 1.33	0.054

[a] Each experiment was done in duplicate. Assay conditions and the contents of the reaction mixture were as reported in the text. All values represent mean values of percent protection with ±*SE* calculated from three separate experiments. [b] Represents the concentration calculated graphically that would reduce the relative hypoosmotic hemolysis to one-half of the hemolysis of dog erythrocytes.

RESULTS

The membrane-stabilizing property of various 1-(2,4-dichlorophenoxyacetyl)-4-substituted thiosemicarbazides and 1-(2,4,5-trichlorophenoxyacetyl)-4-substituted thiosemicarbazides (Table I) was evaluated by determining the decrease in the degree of hypoosmotic hemolysis of dog red blood cells. All substituted thiosemicarbazides protected hypoosmotic hemolysis when used at the final concentrations of 0.05 and 0.1 m*M*.

As is evident from Table II, an increase in the ability of 2,4-dichloro-substituted thiosemicarbazides to protect against hypoosmotic hemolysis was mostly observed by the introduction of an ad-

[2] Commercial chemicals were used. Sodium pyruvate, sodium α-ketoglutarate, sodium succinate, NADH, adenosine monophosphate (AMP), cytochrome c, and kynuramine were obtained from Sigma Chemical Co., St. Louis, Mo.
[3] Animal Supply House, Lucknow, India.
[4] Potter-Elvehjem homogenizer.
[5] Aminco-Bowman.

Table III—Effect of 1-(2,4-Dichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and 1-(2,4,5-Trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides on the Cellular Respiratory Activity of Rat Brain Homogenate

Compound	Inhibition[a], %			
	Pyruvate	α-Ketoglutarate	NADH	Succinate
I	53.6 ± 1.2	58.5 ± 1.2	50.7 ± 0.7	Nil
II	81.5 ± 0.3	80.0 ± 0.9	60.3 ± 0.8	Nil
III	47.8 ± 1.3	52.3 ± 1.5	41.2 ± 0.6	Nil
IV	20.9 ± 1.3	31.3 ± 0.9	19.4 ± 0.2	Nil
V	29.1 ± 0.5	35.8 ± 0.7	25.3 ± 0.4	Nil
VI	37.2 ± 0.6	45.1 ± 0.4	29.2 ± 0.7	Nil
VII	49.9 ± 1.9	55.5 ± 1.1	32.8 ± 0.7	Nil
VIII	79.4 ± 0.9	84.7 ± 0.8	64.8 ± 1.0	Nil
IX	89.3 ± 1.5	87.8 ± 1.6	69.2 ± 1.5	Nil
X	51.8 ± 0.2	55.7 ± 0.5	45.8 ± 0.8	Nil
XI	79.9 ± 1.1	82.4 ± 1.8	69.3 ± 0.8	Nil
XII	56.1 ± 1.6	60.3 ± 1.0	55.8 ± 0.8	Nil
XIII	91.0 ± 1.2	94.3 ± 0.9	78.4 ± 0.4	Nil
XIV	35.0 ± 1.3	42.1 ± 1.2	32.0 ± 1.1	Nil
XV	79.7 ± 1.6	85.1 ± 1.1	66.4 ± 1.7	Nil
XVI	81.4 ± 0.3	86.2 ± 1.0	71.8 ± 1.5	Nil
XVII	76.0 ± 1.2	78.1 ± 1.5	65.4 ± 1.3	Nil
XVIII	84.2 ± 1.5	90.2 ± 1.3	72.1 ± 1.7	Nil

[a] Each experiment was done in triplicate. Assay conditions and vessel contents were as reported in the text. All values represent mean values of percent inhibition with ±*SE* of the mean calculated from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake with 125 mg wet weight of brain/hr. Pyruvate, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*, while the concentration of NADH was 0.5 m*M*. All substituted thiosemicarbazides were used at a final concentration of 1 m*M*.

ditional chloro substituent (2,4,5-trichloro-substituted thiosemicarbazides) at position 5 of the phenyl nucleus. In the present study, greatest antihemolytic activity was observed with Compounds V, IX, and XIII while 1-(2,4-dichlorophenoxyacetyl)-4-*n*-heptylthiosemicarbazide (III) was the least effective compound. With the use of higher concentrations of two of these thiosemicarbazides (V and X), an increase in hypoosmotic hemolysis, rather than in protection of hemolysis, of the red blood cells was observed (Fig. 1). In the present study, both antihemolytic (Table II) and hemolytic (Fig. 1) properties of these substituted thiosemicarbazides were concentration dependent.

Effects of these substituted thiosemicarbazides on the cellular respiratory activity of the rat brain homogenate are shown in Table III. All thiosemicarbazides at a final concentration of 1 m*M* elicited various degrees of inhibition during oxidation of pyruvate, α-ketoglutarate, and NADH whereas NAD-independent oxidation of succinate remained unaffected. Introduction of a chloro substituent at position 5 of the phenyl nucleus of the 2,4-dichloro-substituted thiosemicarbazides (IV–VII) resulted in an increased inhibitory effectiveness of the corresponding 2,4,5-trichloro-substituted thiosemicarbazides (XIII–XVI). No change in the inhibitory effectiveness was observed by the introduction of the chloro substituent on 4-alkyl- and cyclohexyl-substituted thiosemicarbazides (I–III, VIII–XII, XVII, and XVIII).

The monoamine oxidase inhibitory activity of these substituted thiosemicarbazides and the anticonvulsant activity possessed by these compounds are recorded in Table IV. All thiosemicarbazides inhibited *in vitro* monoamine oxidase activity of rat brain homogenate during oxidative deamination of kynuramine. In the present study, the 2,4,5-trichlorophenoxy-substituted thiosemicarbazides (X, XI, and XIII–XVII) produced greater inhibition of monoamine oxidase than their corresponding 2,4-dichlorophenoxy-substituted derivatives (I, II, and IV–VIII) when used at a final concentration of 0.33 m*M*, with the exception of heptyl- (III and XII) and cyclohexyl- (IX AND XVIII) substituted thiosemicarbazides where the 2,4-dichlorophenoxy-substituted thiosemicarbazides (III and IX) produced higher degree of monoamine oxidase inhibition.

The anticonvulsant activity possessed by these substituted thiosemicarbazides, as evidenced by the protection afforded by these compounds against pentylenetetrazol-induced seizures at a dose of 100 mg/kg, ranged from 10 to 70% (Table IV). Maximum anticonvulsant activity was observed with 1-(2,4-dichlorophenoxyacetyl)-4-*p*-bromophenylthiosemicarbazide (VI) while 1-(2,4-dichlorophenoxyacetyl)-4-*o*-tolylthiosemicarbazide (IV) showed no protection against pentylenetetrazol-induced convulsions. In general, 2,4,5-trichlorophenoxy-substituted thiosemicarbazides afforded greater protection than their corresponding 2,4-dichlorophenoxy-substituted thiosemicarbazides (Table IV). The data on anticonvulsant activity of these compounds and their 24-hr pentylenetetrazol-induced mortality did not indicate clearly an association between increased protection from convulsions and decreased mortality in experimental animals (Table IV).

DISCUSSION

The antihemolytic property of phenothiazines (9), reserpine (9), haloperidol (9), imipramine (10), amitriptyline (10), barbiturates (11), and amphetamine (11) has been proposed to reflect their effects on cell membrane functions. Thus, these thiosemicarbazides presumably prevent or diminish various processes in the cell membranes during protection of hypoosmotic hemolysis. As is evident from Fig. 1, these compounds exhibit a biphasic effect on erythrocyte membrane stabilization and lysis, as has been observed with psychotropic agents (19–21). Such biphasic changes are not abrupt in nature, since membrane stabilization and lysis were found to be concentration dependent and lysis was observed only after a peak of 100% stabilization.

Other studies have indicated that the cells with higher area to volume ratios are associated with a lower osmotic fragility and, consequently, less hypoosmotic hemolysis (22). Thus, the rigidity of the cell membranes may presumably account for the biphasic action of thiosemicarbazides; during the protection of hypoosmotic hemolysis, the surface area to volume ratio is not high enough to warrant the dispersing of the membrane molecules to cause lysis. An increase in the concentration of thiosemicarbazides possibly dominates the dispersing effect which leads to the lysis of the red blood cells.

These thiosemicarbazides seem to possess all necessary structural requirements of other membrane-stabilizing compounds for an

Table IV—Monoamine Oxidase Inhibitory and Anticonvulsant Properties of 1-(2,4-Dichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides and 1-(2,4,5-Trichlorophenoxyacetyl)-4-alkyl/arylthiosemicarbazides

Compound	Monoamine Oxidase Inhibition[a], %	Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[b], %
I	44.1 ± 0.78	10	70
II	51.6 ± 0.33	20	80
III	71.2 ± 0.25	10	50
IV	26.5 ± 0.88	0	60
V	55.4 ± 1.10	20	50
VI	43.3 ± 1.00	70	10
VII	44.1 ± 0.98	10	70
VIII	46.5 ± 0.35	10	80
IX	61.7 ± 0.25	30	60
X	55.4 ± 0.66	30	80
XI	64.9 ± 0.78	40	50
XII	47.3 ± 0.22	10	90
XIII	89.2 ± 1.22	30	50
XIV	70.2 ± 0.77	20	60
XV	82.1 ± 0.81	30	90
XVI	80.6 ± 0.72	40	50
XVII	67.6 ± 0.45	30	70
XVIII	35.2 ± 0.35	40	60

[a] Assay procedure and the contents of the reaction mixture were as indicated in the text. All substituted thiosemicarbazides were used at a final concentration of 0.33 m*M*. Each experiment was done in duplicate, and figures indicate mean values of three separate experiments with ±*SE* of the mean. [b] Screening procedure was as indicated in the text. Each thiosemicarbazide was used at the dose of 100 mg/kg ip, and pentylenetetrazol-induced mortality was observed during 24 hr. In the present study, administration of an equivalent amount of 5% gum acacia solution was found to possess no anticonvulsant activity.

effective antihemolytic agent. These include a basic hydrazino group at one end, a chain of carbon atoms, and a phenyl ring structure at the opposite end. Introduction of an electronegative chloro substituent at position 5 of the phenyl nucleus of 2,4-dichlorophenoxy-substituted thiosemicarbazides in general resulted in a greater antihemolytic property, as reflected by their lower ED_{50} values (Table II). An increase in the number of carbon atoms in the alkyl chain of 2,4-dichlorophenoxy-substituted thiosemicarbazides caused a simultaneous decrease in their antihemolytic effectiveness (I–III); in 2,4,5-trichlorophenoxy-substituted thiosemicarbazides, an increase in activity was observed with *n*-butyl substitution (XI) and further extension of the alkyl chain decreased their antihemolytic activity (XII). Halogen substitution was shown to play some role in enhancing the activity, while thiosemicarbazides possessing a hydrophobic cyclohexyl group (IX and XVIII) possessed significant antihemolytic activity.

The ability of thiosemicarbazides to cause selective inhibition of NAD-dependent oxidations has indicated possible inactivation of the electron-transport chain at the site of the transfer of the electrons from NADH to FAD (17, 23). Introduction of a chloro substituent at position 5 of the 2,4-dichlorophenyl nucleus caused greater inhibition of the cellular respiratory activity by these 2,4,5-trichlorophenoxy-substituted thiosemicarbazides. This increased inhibition of the oxidation of pyruvate, α-ketoglutarate, and NADH was only observed with aryl-substituted compounds since no significant change was observed with alkyl-, allyl-, and cyclohexyl-substituted thiosemicarbazides.

The monoamine oxidase inhibitory effectiveness of 2,4-dichlorophenoxy-substituted thiosemicarbazides was increased by the introduction of a chloro substituent at position 5 of their phenyl nucleus to form 2,4,5-trichlorophenoxy-substituted thiosemicarbazides, with the exception of XII and XVIII where a decrease in enzyme inhibition was observed (Table IV). An increase in the length of the alkyl chain in 2,4-dichlorophenoxy-substituted thiosemicarbazides alone resulted in a gradual increase in their enzyme inhibitory activity. On the other hand, elongation of the alkyl chain from *n*-butyl to *n*-heptyl showed lowering of monoamine oxidase inhibition in 2,4,5-trichlorophenoxy-substituted thiosemicarbazides. The low anticonvulsant activity of these thiosemicarbazides, with the exception of VI, failed to reflect the structure–activity relationship of these compounds.

These results have provided indications of some similarity between the membrane-stabilizing property of the thiosemicarbazides and their ability to inhibit cellular respiratory activity selectively and monoamine oxidase. The membrane-stabilizing property may be unspecific, and some functional groups in thiosemicarbazide molecules could presumably share common properties for cell membrane attachment to exhibit enzyme inhibitory and antihemolytic properties.

REFERENCES

(1) J. H. Biel, A. Horita, and A. E. Drukker, in "Psychopharmacological Agents," vol. 1, M. Gordon, Ed., Academic, New York, N.Y., 1964, p. 359.

(2) *Ibid.*, p. 414.

(3) L. A. Cates, *J. Med. Chem.*, **10,** 925(1967).

(4) D. J. Prockop, P. A. Shore, and B. B. Brodie, *Ann. N.Y. Acad. Sci.*, **80,** 643(1959).

(5) S. S. Parmar, A. K. Chaturvedi, A. Chaudhari, and R. S. Misra, *J. Pharm. Sci.*, **61,** 78(1972).

(6) M. Weatherall, in "Recent Advances in Pharmacology," 4th ed, J. M. Robson and R. S. Stacey, Eds., Churchill, London, England, 1968, p. 1.

(7) R. B. Barlow, in "Introduction to Chemical Pharmacology," 2nd ed., R. B. Barlow, Ed., Wiley, New York, N.Y., 1964.

(8) P. Seeman, *Biochem. Pharmacol.*, **15,** 1753(1966).

(9) P. Seeman and J. Weinstein, *ibid.*, **15,** 1737(1966).

(10) A. Despopoulos, *ibid.*, **19,** 2907(1970).

(11) W. Mikikits, A. Mortara, and R. G. Spector, *Nature (London)*, **225,** 1150(1970).

(12) C. Dwivedi, R. D. Harbison, B. Ali, and S. S. Parmar, *J. Pharm. Sci.*, **63,** 1124(1974).

(13) S. S. Parmar and P. K. Seth, *Can. J. Biochem.*, **43,** 1179(1965).

(14) J. H. Quastel, *Physiol. Rev.*, **19,** 135(1939).

(15) J. H. Quastel, *Trans. Faraday Soc.*, **39,** 348(1943).

(16) S. S. Parmar, C. Dwivedi, and B. Ali, *J. Pharm. Sci.*, **61,** 1366(1972).

(17) S. S. Parmar, C. Dwivedi, B. Ali, and R. S. Misra, *J. Med. Chem.*, **15,** 846(1972).

(18) M. Krajl, *Biochem. Pharmacol.*, **14,** 1684(1965).

(19) L. L. M. VanDeenen and R. A. Demel, *Biochim. Biophys. Acta*, **94,** 314(1965).

(20) H. Hauser and R. M. C. Dawson, *Biochem. J.*, **109,** 909(1968).

(21) L. G. Abood, I. Koyama, and H. Kimizuka, *Nature (London)*, **107,** 367(1963).

(22) R. P. Rand and A. C. Burton, *J. Cell. Comp. Physiol.*, **61,** 245(1963).

(23) S. S. Parmar, C. Dwivedi, A. Chaudhari, and T. K. Gupta, *J. Med. Chem.*, **15,** 99(1972).

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