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Anticonvulsant Activity and Selective Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations by 10-(2-Arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines

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Abstract □ Several 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and their corresponding cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were synthesized and characterized by their sharp melting points and elemental analyses. All compounds inhibited nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate and α-ketoglutarate selectively, whereas NAD-independent oxidation of succinate remained unaltered. All phenothiazine derivatives exhibited anticonvulsant activity, which was reflected by the 20–60% protection observed against pentylenetetrazol-induced convulsions in mice. The ability of substituted thiosemicarbazidophenothiazines to inhibit cellular respiratory activity was reduced considerably by cyclization to the corresponding substituted thiazolidinophenothiazines. On the other hand, cyclization generally resulted in increased anticonvulsant activity. Thus, the anticonvulsant activity possessed by these substituted phenothiazines bore no relationship with their ability to inhibit selectively the NAD-dependent oxidations. Selective inhibition of NAD-dependent oxidation of pyruvate and α-ketoglutarate in isolated rat brain mitochondria by some 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines was concentration dependent and competitive in nature.

Keyphrases □ Phenothiazines—synthesis, effects on NAD-dependent oxidations, anticonvulsant activity screened, mice □ Oxidation, NAD dependent—pyruvate and α-ketoglutarate, effect of phenothiazines □ Anticonvulsant activity—phenothiazines, synthesized, screened, mice □ Structure–activity relationships—substituted phenothiazines, anticonvulsant activity

The striking clinical success of chlorpromazine as an antipsychotic agent led to the synthesis of numerous phenothiazine derivatives. Chlorpromazine has been shown to diminish convulsant effects of nicotine and cocaine, whereas no protection was provided by chlorpromazine against convulsions induced by the administration of strychnine, caffeine, and pentylenetetrazol (1, 2). Earlier studies indicated that small differences in the chemical structure of the phenothiazine compounds produce significant changes in their pharmacological properties (3).

Furthermore, the ability of thiazolidone derivatives to afford protection against pentylenetetrazol-induced convulsions (4, 5) and of phenothiazines and thiazolidones to inhibit cellular respiratory activity of brain homogenates (4, 6) prompted synthesis of phenothiazine derivatives possessing the thiazolidone moiety at position 10 of the phenothiazine portion of their molecular structure. Anticonvulsant activity of these substituted thiazolidonylphenothiazines and their precursor thiosemicarbazidophenothiazines was determined against pentylenetetrazol-induced convulsions in an attempt to show the effect of cyclization on the anticonvulsant activity of these phenothiazines.

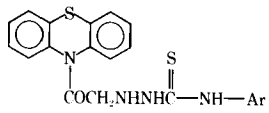
The ability of these substituted phenothiazines to inhibit cellular respiratory activity of brain homogenates was also investigated with a view to study the biochemical mechanism of action of these compounds. The various 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were synthesized as outlined in Scheme I.

EXPERIMENTAL[1]

10-Chloroacetylphenothiazine—Following the method of Ekstrand (7), a mixture of phenothiazine (0.3 mole) and chloroacetyl chloride (0.45 mole) in 100 ml of dry benzene was refluxed on a steam bath for 4 hr. Excess benzene was removed by distillation.

[1] All compounds were analyzed for their carbon, hydrogen, and nitrogen content. Melting points were taken in open capillary tubes and are corrected. IR spectra were obtained with Perkin-Elmer Infracord spectrophotometer model 137 equipped with sodium chloride optics in potassium bromide films in the 700–3500-cm^{-1} range.

Table I—Physical Constants of 10-(1-Acetyl-4-arylthiosemicarbazido)phenothiazines



Compound	Ar	Melting Point[a]	Yield, %	Molecular Formula	Analysis, % Calc.	Found
I	C_6H_5	130°	79	$C_{21}H_{18}N_4OS_2$	C 62.07	61.68
					H 4.43	4.35
					N 13.79	13.72
II	2-$CH_3C_6H_4$	164°	68	$C_{22}H_{20}N_4OS_2$	C 62.86	62.38
					H 4.76	4.57
					N 13.33	13.19
III	3-$CH_3C_6H_4$	155°	76	$C_{22}H_{20}N_4OS_2$	C 62.86	62.54
					H 4.76	4.48
					N 13.33	13.46
IV	4-$CH_3C_6H_4$	170°	88	$C_{22}H_{20}N_4OS_2$	C 62.86	62.72
					H 4.76	4.69
					N 13.33	13.40
V	2-$OCH_3C_6H_4$	175°	63	$C_{22}H_{20}N_4O_2S_2$	C 60.55	60.58
					H 4.59	4.55
					N 12.84	12.72
VI	4-$OCH_3C_6H_4$	182°	85	$C_{22}H_{20}N_4O_2S_2$	C 60.55	60.23
					H 4.59	4.29
					N 12.84	12.93
VII	4-ClC_6H_4	174°	82	$C_{21}H_{17}ClN_4OS_2$	C 57.20	57.55
					H 3.86	3.66
					N 12.71	12.53
VIII	4-BrC_6H_4	210°	95	$C_{21}H_{17}BrN_4OS_2$	C 51.96	51.87
					H 3.50	3.48
					N 11.55	11.59
IX	4-IC_8H_4	144°	90	$C_{21}H_{17}IN_4OS_2$	C 47.37	47.17
					H 3.19	3.22
					N 10.53	10.67
X	1-$C_{10}H_7$ (α-Naphthyl)	162°	83	$C_{25}H_{20}N_4OS_2$	C 65.79	65.51
					H 4.39	4.33
					N 12.28	12.47

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

The solid mass which separated on cooling was collected by filtration, washed with water to remove chloroacetyl chloride, dried, and recrystallized from benzene (yield, 75%), mp 114° [lit. (7) mp 115–116°].

10-Hydrazinoacetylphenothiazine—A mixture of 10-chloroacetylphenothiazine (0.3 mole) and hydrazine hydrate (99%, 0.45 mole) in 100 ml of absolute ethanol was refluxed on a steam bath for 18 hr. Excess ethanol was removed by distillation under reduced pressure. The crude product which separated on cooling was collected by filtration, dried, and recrystallized from ethanol (yield, 54%), mp 180°.

Anal.—Calc. for $C_{14}H_{13}N_3OS$: C, 61.99; H, 4.79; N, 15.49. Found: C, 62,24; H, 4.68; N, 15.38.

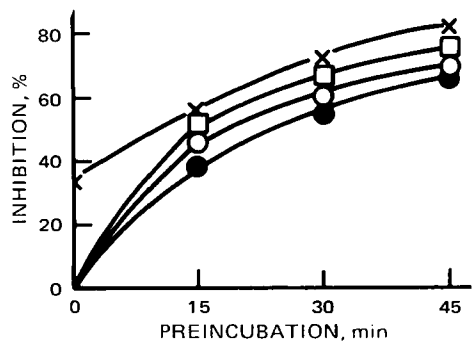


Figure 1—*Effect of preincubation of substituted phenothiazines (III, VIII, and X) and chlorpromazine with isolated rat brain mitochondrial preparations for various times prior to the addition of pyruvate. Zero-time experiments represent those where both the test compound and pyruvate were added simultaneously to the mitochondrial preparations. Assay procedures and the contents of the reaction vessels are described in the text. Key:* ○, *III (0.4 mM);* □, *VIII (0.4 mM);* ●, *X (0.4 mM); and* ×, *chlorpromazine (1 mM).*

10-(1-Acetyl-4-arylthiosemicarbazido)phenothiazines—A mixture of 10-hydrazinoacetylphenothiazine (0.01 mole) and the appropriate aryl isothiocyanates (0.01 mole) in 25 ml of absolute ethanol was refluxed on a steam bath for 6 hr. Excess ethanol was removed by distillation, and the solid mass which separated on cooling was collected by filtration, washed with ether and dilute hydrochloric acid, dried, and recrystallized from ethanol (Table I).

10-(2-Arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines—Following the method reported earlier (5, 8), a mixture of 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazine (0.01 mole), chloroacetic acid (0.01 mole), and fused anhydrous sodium acetate (0.015 mole) was dissolved in 25 ml of acetic acid. Then the mixture was refluxed on a free flame for 4–5 hr. The reaction mixture was poured over ice-cold water and kept in a refrigerator for 24 hr. The crude products which separated were collected by filtration, washed several times with water, dried, and recrystallized from ethanol (Table II).

Determination of Respiratory Activity of Rat Brain Homogenates and Isolated Mitochondria[2]—Male albino rats, kept on an *ad libitum* diet, were used in all experiments. Rats, 100–150 g, were sacrificed by decapitation. The brains were removed immediately and homogenized[3] in ice-cold 0.25 *M* sucrose solution in a ratio of 1:9 (w/v). Brain mitochondria were isolated by using differential centrifugation techniques (9). Rat brain homogenate was centrifuged[4] at 600×*g* for 10 min to sediment nuclei and cell debris.

The mitochondria were separated by centrifugation of the supernatant fraction at 10,000×*g* for 15 min. The isolated mitochondria were washed and recentrifuged twice and then suspended in a known volume of 0.25 *M* cold sucrose. All incubations were carried out at 37° in the conventional Warburg manometric apparatus,

[2] Commercial chemicals were used for the determination of cellular respiratory activity of rat brain homogenates and isolated mitochondria. Sodium pyruvate, sodium α-ketoglutarate, adenosine monophosphate (sodium salt), nicotinamide adenine dinucleotide, and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo.

[3] Potter–Elvehjem.

[4] International refrigerated centrifuge model B-20.

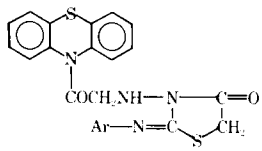


Table II—Physical Constants of 10-(2-Arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines

Compound	Ar	Melting Point[a]	Yield, %	Molecular Formula	Analysis, % Calc.	Found
XI	C_6H_5	122°	56	$C_{23}H_{18}N_4O_2S_2$	C 61.88	61.46
					H 4.04	3.82
					N 12.56	12.22
XII	2-$CH_3C_6H_4$	177°	54	$C_{24}H_{20}N_4O_2S_2$	C 62.60	62.44
					H 4.35	4.17
					N 12.17	12.08
XIII	3-$CH_3C_6H_4$	148°	55	$C_{24}H_{20}N_4O_2S_2$	C 62.60	62.35
					H 4.35	4.21
					N 12.17	11.88
XIV	4-$CH_3C_6H_4$	180°	62	$C_{24}H_{20}N_4O_2S_2$	C 62.60	62.68
					H 4.35	4.28
					N 12.17	11.78
XV	2-$OCH_3C_6H_4$	169°	58	$C_{24}H_{20}N_4O_3S_2$	C 60.50	60.48
					H 4.20	4.22
					N 11.76	11.57
XVI	4-$OCH_3C_6H_4$	185°	60	$C_{24}H_{20}N_4O_3S_2$	C 60.50	60.63
					H 4.20	3.87
					N 11.76	11.35
XVII	4-ClC_6H_4	179°	61	$C_{23}H_{17}ClN_4O_2S_2$	C 57.46	57.33
					H 3.54	3.29
					N 11.65	11.68
XVIII	4-BrC_6H_4	145°	64	$C_{23}H_{17}BrN_4O_2S_2$	C 52.57	52.59
					H 3.24	3.28
					N 10.67	10.53
XIX	4-IC_6H_4	132°	58	$C_{23}H_{17}IN_4O_2S_2$	C 48.25	48.00
					H 2.97	2.75
					N 9.79	9.66
XX	1-$C_{10}H_7$ (α-Naphthyl)	128°	56	$C_{27}H_{20}N_4O_2S_2$	C 65.32	65.38
					H 4.03	4.20
					N 11.29	11.31

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

using air as the gas phase (10). The oxygen uptake was measured at 10-min intervals.

Fresh rat brain homogenate (1 ml) or mitochondrial preparation (0.5 ml), equivalent to 100 mg wet brain weight, was added to the chilled Warburg vessels containing 6.7 m*M* magnesium sulfate, 20 m*M* sodium hydrogen phosphate buffer solution (pH 7.4), 1 m*M* adenosine monophosphate (sodium salt), 33 m*M* potassium chloride, and 500 μg of cytochrome c in a final volume of 3 ml unless otherwise stated. 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*. It was presumed that the endogenous nicotinamide adenine dinucleotide (NAD) present in brain homogenates was sufficient for the cellular respiratory activity. In mitochondrial preparations, NAD was added to the reaction mixture in a final concentration of 0.5 m*M*.

All 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and the corresponding cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were dissolved in propylene glycol (100%) and used at a final concentration of 2 m*M*. An equal volume of propylene glycol was added to the control vessels.

In preincubation studies, isolated rat brain mitochondrial preparations in the incubation mixture were incubated with or without some 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines at 37° for 15, 30, and 45 min prior to the addition of pyruvate or α-ketoglutarate. The zero-time experiments represent those in which the substituted phenothiazines and pyruvate or α-ketoglutarate were added simultaneously to the reaction mixture containing appropriate rat brain mitochondrial preparations. These preincubation studies were also carried out with chlorpromazine, which was used as the reference drug for comparative evaluation.

The I_{50} values (concentration producing 50% inhibition) were determined graphically for some 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines from the values obtained for the inhibition of the cellular respiratory activity of rat brain mitochondria during oxidation of pyruvate and α-ketoglutarate by the use of different concentrations of these substituted phenothiazines.

In the present study, the nature of inhibition of cellular respiratory activity of isolated rat brain mitochondria during oxidation of pyruvate was evaluated by following the graphic method of Lineweaver and Burk (11) as modified by Dixon (12).

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in albino mice of either sex, 25–30 g, against pentylenetetrazol-induced convulsions. The mice were divided in groups of 10, keeping the group weights as near the same as possible. All 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were suspended in 5% aqueous gum acacia to give a concentration of 1% (w/v). These test compounds were administered in a dose of 100 mg/kg ip to a group of 10 mice.

Four hours after the administration of the test compounds, the

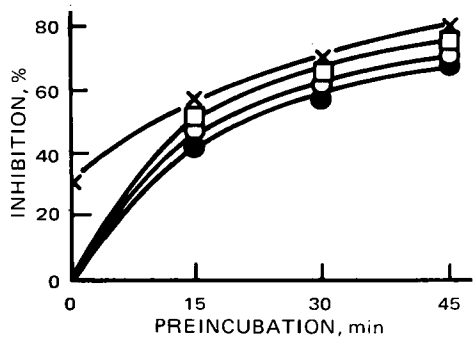


Figure 2—*Effect of preincubation of substituted phenothiazines (III, VIII, and X) and chlorpromazine with isolated rat brain mitochondrial preparations for various times prior to the addition of α-ketoglutarate. Zero-time experiments represent those where both the test compound and α-ketoglutarate were added simultaneously to the mitochondrial preparations. Assay procedures and the contents of the reaction vessels are described in the text. Key:* ○, *III (0.4 mM);* □, *VIII (0.4 mM);* ●, *X (0.4 mM); and* ×, *chlorpromazine (1 mM).*

Table III—Inhibition of Cellular Respiratory Activity of Rat Brain Homogenates by 10-(1-Acetyl-4-arylthiosemicarbazido)phenothiazines and Their Anticonvulsant Activity

Compound	Inhibition of Respiratory Activity[a], %		Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], %
	Pyruvate	α-Ketoglutarate		
I	54.8 ± 1.2	61.2 ± 1.3	20	40
II	45.7 ± 1.0	54.4 ± 0.9	40	10
III	76.4 ± 1.4	79.4 ± 1.3	60	10
IV	15.3 ± 0.5	30.0 ± 0.6	30	30
V	15.0 ± 0.4	14.3 ± 0.5	30	40
VI	50.0 ± 0.9	48.2 ± 0.9	60	Nil
VII	54.0 ± 0.9	51.2 ± 1.0	20	50
VIII	81.8 ± 1.2	88.2 ± 1.3	40	50
IX	87.0 ± 1.3	74.1 ± 1.1	50	30
X	94.3 ± 1.3	83.4 ± 1.3	50	50

[a] Assay procedures are as indicated in the text. Each experiment was done in duplicate, and the 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/100 mg wet tissue weight/hr. All substituted phenothiazines were used at a final concentration of 2 m*M*. Pyruvate and α-ketoglutarate were used at a concentration of 10 m*M*. The percent inhibition with succinate was nil. [b] Screening procedures are as indicated in the text. Each compound was used at a dose of 100 mg/kg ip. In the present study, administration of an equivalent amount of 5% gum acacia solution was found to possess no anticonvulsant activity. [c] Represents mortality during 24 hr in each group of animals administered 90 mg/kg sc of pentylenetetrazol.

mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol was shown to produce convulsions in almost all untreated mice, and the mice exhibited 100% mortality during 24 hr (5). No mortality was observed during 24 hr in mice treated with 100 mg/kg ip of the substituted phenothiazines.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm persisting for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during the 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of substituted phenothiazines was represented as the percent protection. The mice were then observed for 24 hr, and their mortality was recorded.

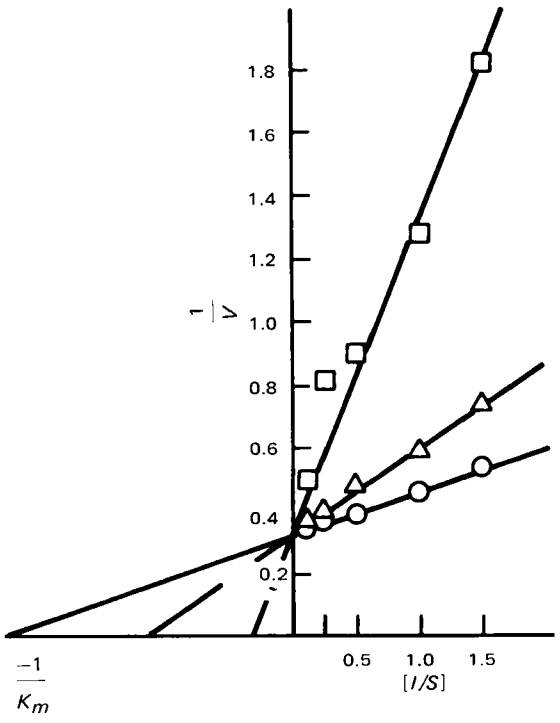
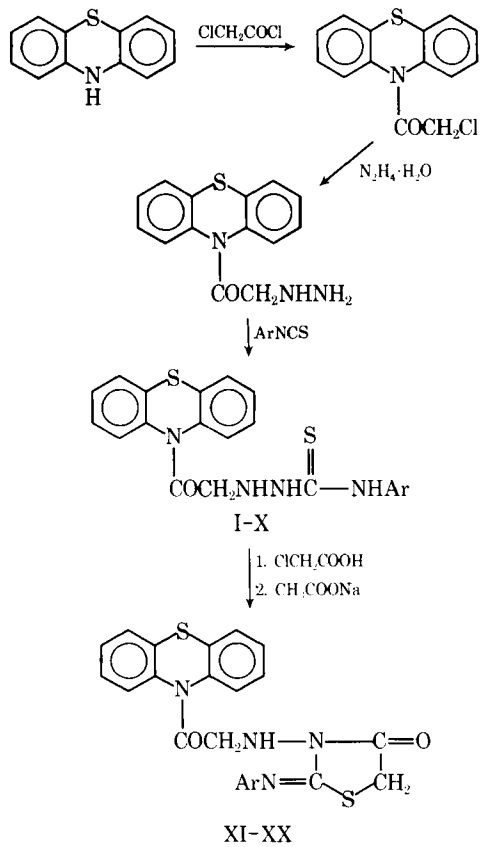


Figure 3—*Competitive inhibition of the cellular respiratory activity of isolated rat brain mitochondria by X during pyruvate oxidation. Assay procedures and the contents of the reaction vessels are described in the text.* [S] *denotes the molar concentration of pyruvate, and 1/V represents the reciprocal of the units of oxygen uptake equivalent to 100 mg of wet brain weight for mitochondrial preparations/30 min. Each experiment was done in duplicate, and the values are the means of three separate experiments. Key:* ○, *pyruvate control;* △, *0.4 m*M *X; and* □, *1 m*M *X. The* K_m *value for pyruvate was 0.4 m*M.

RESULTS AND DISCUSSION

In the present study, various 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines (Table I) were synthesized and converted into their corresponding cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines (Table II). The presence of the characteristic bands of C=O (1640 cm^{-1}), NH (3400 cm^{-1}) and C=S (1050 cm^{-1}) groups in the IR spectra of 10-(1-acetyl-4-phenylthiosemicarbazido)phenothiazine (I) and C=O (1630 cm^{-1}), NH (3400 cm^{-1}), and C=N (1690 cm^{-1}) groups in the IR spectra of 10-(2-phenylimino-3-acetylamino-4-thiazolidonyl)phenothiazine (XI) provided further support for the molecular structure of substituted phenothiazines. All of these compounds were evalu-



Scheme I

Table IV—Inhibition of Cellular Respiratory Activity of Rat Brain Homogenates by 10-(2-Arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines and Their Anticonvulsant Activity

Compound	Inhibition of Respiratory Activity[a], %		Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality[a], %
	Pyruvate	α-Ketoglutarate		
XI	29.0 ± 0.7	22.1 ± 0.4	40	50
XII	30.2 ± 0.6	32.1 ± 0.5	60	20
XIII	47.2 ± 0.7	36.9 ± 0.6	30	50
XIV	12.9 ± 0.4	23.1 ± 0.5	40	40
XV	5.2 ± 0.4	8.1 ± 0.4	60	20
XVI	15.7 ± 0.5	27.5 ± 0.5	60	30
XVII	22.5 ± 0.5	16.3 ± 0.4	60	20
XVIII	44.8 ± 0.7	46.8 ± 0.6	40	50
XIX	34.1 ± 0.7	45.2 ± 0.6	60	30
XX	49.9 ± 0.6	57.7 ± 0.8	50	40

[a] Details of the experimental methods for the determination of the cellular respiratory activity of rat brain homogenates, screening of the anticonvulsant activity, and pentylenetetrazol mortality are as described in Table III. The percent inhibition with succinate was nil.

ated for anticonvulsant activity and their ability to inhibit the cellular respiratory activity of rat brain homogenates.

As is evident from Table III, all 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines (I–X) inhibited selectively the NAD-dependent oxidation of pyruvate and α-ketoglutarate; NAD-independent oxidation of succinate remained unaltered. Cyclization of these compounds into corresponding 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines (XI–XX) retained their selective inhibitory effectiveness (Table IV), but the degree of inhibition was significantly lowered by cyclization during oxidation of both pyruvate and α-ketoglutarate.

In both series of substituted phenothiazines, α-naphthyl-substituted compounds (X and XX) exhibited maximum inhibitory activity. The presence of the substituents in the phenyl nucleus of these substituted phenothiazines significantly altered the ability of these compounds to inhibit selectively the oxidation of pyruvate and α-ketoglutarate. Moreover, in both series of substituted phenothiazines, the presence of bromo (VIII and XVIII) or iodo (IX and XIX) at the *para*-position of the phenyl nucleus resulted in a significantly greater degree of inhibition of the cellular respiratory activity as compared to phenothiazines possessing an unsubstituted phenyl nucleus in their structure (I and XI). The degree of inhibition, in general, was found to bear no relationship with the electronegativity of these halogen substituents on the phenyl nucleus.

Introduction of methyl (II–IV and XII–XIV) and methoxy (V, VI and XV, XVI) substituents on the phenyl nucleus caused decreased inhibition, with the exception of *m*-tolyl-substituted phenothiazines (III and XIII) which produced greater inhibition of the oxidation of pyruvate and α-ketoglutarate by rat brain homogenates as compared to compounds devoid of substituents in their phenyl nucleus (I and XI). The selective inhibitory effects of 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines were not found to be related with their chemical structure, so a definite structure–activity relationship was not exhibited.

The inhibitory effects of some of the more potent 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines were investigated on the respiratory activity of isolated rat brain mitochondria. As is evident from Table V, increase in the concentration of 3-methylphenyl- (III), 4-bromophenyl- (VIII), and α-naphthyl- (X) substituted phenothiazines caused greater inhibition of the oxidation of pyruvate and α-ketoglutarate. The comparative inhibitory effectiveness of these compounds was also reflected by their I_{50} values. The low I_{50} values of 0.6 and 0.56 m*M* observed for 10-[1-acetyl-4-(4-bromophenyl)thiosemicarbazido]phenothiazine (VIII) corresponded well with its maximum inhibitory effectiveness during oxidation of pyruvate and α-ketoglutarate, respectively.

In the present study, preincubation effects of three 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines (III, VII, and X) were investigated on the respiratory activity of isolated rat brain mitochondria at a concentration of 0.4 m*M*. Chlorpromazine, used as a standard drug for comparative evaluation, was used at a higher concentration of 1 m*M*. All substituted phenothiazines exhibited greater inhibitory activity as compared to the inhibitory activity of chlorpromazine during oxidation of pyruvate (Fig. 1) and α-ketoglutarate (Fig. 2).

Preincubation of these substituted phenothiazines as well as chlorpromazine with the mitochondrial preparations for a varying length of time prior to the addition of pyruvate or α-ketoglutarate resulted in progressive increases in their degree of inhibition. Maximum inhibition was observed when preincubation with appropriate mitochondrial preparations was carried out for 45 min before the addition of the substrate. On the other hand, simultaneous addition of the substituted phenothiazines and the substrate (zero-time experiments) to the reaction vessel containing brain mitochondrial preparation produced no inhibition of the oxidation of pyruvate and α-ketoglutarate.

These results, indicating the irreversible nature of inhibition by substituted phenothiazines, prompted kinetic studies using 10-[1-acetyl-4-(α-naphthyl)thiosemicarbazido]phenothiazine (X) to determine the nature of its inhibition during oxidation of pyruvate by isolated rat brain mitochondrial preparations. These kinetic studies (Fig. 3) revealed the competitive nature of inhibition by this compound. The intercept at the $1/S$ axis was taken as $-1/K_m$ (Fig. 3), and the value of 0.4 m*M* was obtained as the Michaelis

Table V—Inhibition of Cellular Respiratory Activity of Isolated Rat Brain Mitochondria by 10-(1-Acetyl-4-arylthiosemicarbazido)phenothiazines

Compound	Substrate	Inhibition by Substituted Phenothiazines[a], %				
		0.6 m*M*	0.8 m*M*	1.2 m*M*	1.5 m*M*	I_{50}, m*M*[b]
II	Pyruvate	30.0 ± 0.8	42.4 ± 0.6	91.8 ± 1.2	100	0.86
VIII	Pyruvate	50.5 ± 0.9	85.4 ± 1.0	94.6 ± 1.2	100	0.60
X	Pyruvate	20.5 ± 0.9	29.1 ± 0.8	94.0 ± 1.0	96.2 ± 1.1	0.90
II	α-Ketoglutarate	32.4 ± 0.8	62.4 ± 0.9	71.5 ± 0.6	98.2 ± 0.7	0.72
VIII	α-Ketoglutarate	55.4 ± 1.2	80.0 ± 0.9	88.4 ± 0.6	100	0.56
X	α-Ketoglutarate	32.4 ± 0.4	52.0 ± 0.7	66.6 ± 0.8	90.4 ± 0.8	0.79

[a] Assay procedures and the contents of the reaction mixture are as indicated in the text. Each experiment was done in duplicate, and the 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/100 mg wet weight of the tissue equivalent to mitochondrial preparation/hr. Pyruvate and α-ketoglutarate were used at a concentration of 10 m*M*. [b] Calculated graphically.

constant (K_m) in these experiments with pyruvate as the substrate. The irreversible nature of inhibition by these substituted phenothiazines, as indicated by preincubation studies, and the competitive nature of inhibition observed with kinetic studies indicated that these substituted phenothiazines react relatively irreversibly with rat brain mitochondrial preparation to produce "nonequilibrium antagonism," as reported earlier for monoamine oxidase inhibitors (13, 14).

The anticonvulsant activity possessed by these substituted phenothiazines was reflected by their ability to afford protection against pentylenetetrazol-induced convulsions in mice. The degree of protection ranged from 20 to 60% with 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines (Table III) and from 30 to 60% with their corresponding cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines. Contrary to the decrease in the degree of selective inhibition of NAD-dependent oxidations, the anticonvulsant activity, in general, increased on cyclization. Such an increase in the anticonvulsant activity, however, remained constant on cyclization with three compounds (XVI, XVIII, and XX), and less protection was observed with one cyclized phenothiazine (XIII) as compared to its precursor (III). These results do not provide evidence for the structural requirements in the molecular makeup of these substituted phenothiazines responsible for their anticonvulsant activity.

The results presented in this study have failed to provide a correlation between the anticonvulsant activity of these substituted phenothiazines and their ability to inhibit selectively the NAD-dependent oxidations of pyruvate and α-ketoglutarate by rat brain homogenates and isolated mitochondria. Detailed pharmacological and toxicological studies and further study of the effects of these substituted phenothiazines on other enzyme systems possibly may reflect a biochemical basis for the anticonvulsant activity of 10-(1-acetyl-4-arylthiosemicarbazido)phenothiazines and their cyclized 10-(2-arylimino-3-acetylamino-4-thiazolidonyl)phenothiazines.

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