internal standard in the drug-containing samples of the same urine (recovery) or subject.

Recovery—The validity of the method was verified and the recovery was determined by adding various known amounts of 6-demethylgriseofulvin to human urine. Each urine standard, as well as the blank urine, was analyzed eight times according to the method described (Table I).

The mean recoveries varied from 87 to 112% of the added amount. Recovery was independent of the concentration of 6-demethylgriseofulvin and averaged 98%.

Because of its simplicity, this method is well suited for the routine analysis of a large number of samples. It measures total, free, and conjugated 6-demethylgriseofulvin. Free metabolite can be determined by omitting the initial hydrolysis step. The method also allows griseofulvin, if present, to be determined simultaneously.

The method was used successfully in several bioavailability studies, and the results will be reported elsewhere.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 15, 1975, from the *Drug Metabolism Section, Sandoz Pharmaceuticals, East Hanover, NJ 07936*

Accepted for publication May 27, 1975.

The authors thank Ms. Monica Tuttle for technical assistance.

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CNS Depressant Activity of Pyrimidylthiazolidones and Their Selective Inhibition of NAD-Dependent Pyruvate Oxidation

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Abstract □ Several 1-aryl-3-(2-pyrimidyl)thiocarbamides and their corresponding cyclized 2-arylimino-3-(2-pyrimidyl)thiazolid-4-ones were synthesized and characterized by their sharp melting points and elemental analyses. These thiocarbamides and thiazolidones possessed anticonvulsant activity against pentylenetetrazol-induced convulsions and potentiated pentobarbital-induced hypnosis in mice. Most of these thiocarbamides and thiazolidones selectively inhibited nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate, where the use of added NAD decreased the degree of inhibition. The NAD-independent oxidation of succinate, on the other hand, remained unaltered. The anticonvulsant activity of thiocarbamides and thiazolidones was unrelated to their ability to inhibit the respiratory activity of rat brain homogenates during oxidation of sodium pyruvate. Cyclization of thiocarbamides to the corresponding thiazolidones in general enhanced their CNS depressant and enzyme inhibitory effectiveness.

Keyphrases □ Thiazolidones—synthesis, evaluation of CNS activity, effect on NAD-dependent oxidation □ Thiocarbamides—synthesis, evaluation of CNS activity, effect on NAD-dependent oxidation □ CNS activity—synthesis and evaluation of several thiocarbamides and thiazolidones □ Enzyme activity—effect of thiocarbamides and thiazolidones on NAD-dependent oxidation □ Anticonvulsants—synthesis and evaluation of several thiocarbamides and thiazolidones

Considerable interest recently has been focused in this laboratory on substituted thiazolidones that have been shown to possess anticonvulsant (1–3), hypnotic (4), and local anesthetic (5) properties. Earlier studies indicated diverse pharmacological profiles of pyrimidine derivatives, including diuretic (5), local anesthetic (6), and anticonvulsant (7, 8) properties. Piperazinothiocarbamides also have been shown to possess effects on the central nervous system (CNS) activity (9, 10). These observations prompted synthesis of substituted thiocarbamides, which were cyclized into the corresponding 2-arylimino-3-(2-pyrimidyl)thiazolid-4-ones.

In the present study, these thiocarbamides and thiazolidones were evaluated for their anticonvulsant activity and their ability to potentiate pentobarbital-induced hypnosis and to inhibit cellular respiratory activity of rat brain homogenates, with a view to studying their biochemical mechanism of action. The various thiazolidones were synthesized by following the methods outlined in Scheme I (3).

EXPERIMENTAL [1]

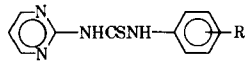
The various 1,3-disubstituted thiocarbamides (Table I) were prepared by refluxing equimolar quantities of 2-aminopyrimidine and the appropriate arylisothiocyanate in dry benzene. These substituted thiocarbamides were cyclized into the corresponding 2-arylimino-3-(2-pyrimidyl)thiazolid-4-ones by refluxing with chloroacetic acid and anhydrous sodium acetate in acetic acid by the following method.

1-Aryl-3-(2-pyrimidyl)thiocarbamides (I–VIII)—2-Aminopyrimidine (0.01 *M*) was mixed with a suitable arylisothiocyanate (0.01 *M*) in 15 ml of dry benzene, and the mixture was refluxed on a steam bath for 4 hr. The reaction mixture was concentrated by distilling the excess benzene under reduced pressure. The solid mass which separated on cooling was filtered, washed with ether and dilute hydrochloric acid, dried, and recrystallized from ethanol. All thiocarbamides, characterized by their sharp melting points and elemental analyses, are recorded in Table I.

2-Arylimino-3-(2-pyrimidyl)thiazolid-4-ones (IX–XVI)—A

[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-Aryl-3-(2-pyrimidyl)thiocarbamides



Compound	R	Melting Point[a]	Yield, %	Molecular Formula	Analysis, %	Calc.	Found
I	H	199°	85	$C_{11}H_{10}N_4S$	C	57.39	57.54
					H	4.34	4.39
					N	24.34	24.76
II	2-CH_3	214°	90	$C_{12}H_{12}N_4S$	C	59.01	59.34
					H	4.91	4.82
					N	22.95	22.95
III	4-CH_3	212°	84	$C_{12}H_{12}N_4S$	C	59.01	58.79
					H	4.91	5.02
					N	22.95	23.26
IV	2,4-$(CH_3)_2$	218°	65	$C_{13}H_{14}N_4S$	C	60.46	60.72
					H	5.42	5.34
					N	21.70	21.56
V	2,6-$(CH_3)_2$	228°	73	$C_{13}H_{14}N_4S$	C	60.49	60.29
					H	5.42	5.38
					N	21.70	21.84
VI	4-OCH_3	215°	75	$C_{12}H_{12}N_4OS$	C	55.38	55.72
					H	4.61	4.69
					N	21.53	21.75
VII	4-Br	220°	80	$C_{11}H_9BrN_4S$	C	42.71	42.92
					H	2.91	2.86
					N	18.12	18.18
VIII	4-Cl	225°	72	$C_{11}H_9ClN_4S$	C	49.90	50.21
					H	3.40	3.57
					N	21.17	21.42

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

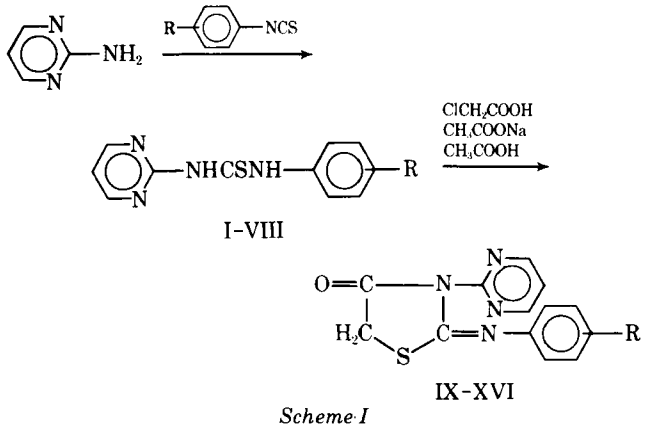
mixture of 1-aryl-3-(2-pyrimidyl)thiocarbamide (0.01 *M*), chloroacetic acid (0.01 *M*), and anhydrous sodium acetate (0.015 *M*) in 15 ml of acetic acid was refluxed on a free flame for 5–6 hr. The reaction mixture was poured into water, and the contents were allowed to stand overnight in a refrigerator. The crude thiazolidones which separated were filtered, washed several times with water, and recrystallized from ethanol (Table II).

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in albino mice of either sex weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as nearly the same as possible. All thiocarbamides and thiazolidones were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v).

The test compounds were injected at a dose of 100 mg/kg ip. Four hours later, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol produced convulsions in all untreated mice and caused 100% mortality within 24 hr.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion (11). Transient intermittent jerks and tremulousness were not counted. Animals devoid of threshold convulsions during the 60-min period were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of the compounds was represented as percent protection. The mice were then observed for 24 hr and mortality was recorded.

Potentiation of Pentobarbital (Sodium) Sleeping Time—The method of Winter (12) was followed to investigate the ability



Scheme I

of thiocarbamides and thiazolidones to potentiate pentobarbital-induced hypnoses. Albino mice, 20–25 g, were divided into groups of six animals. One group was used for each compound, while another group served as the control. Pentobarbital, when administered in a dose of 40 mg/kg ip to the control group, produced ataxia and no sleep. Increasing the dose of pentobarbital produced sleep in the mice. All thiocarbamides and thiazolidones were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v) and were injected at a dose of 100 mg/kg ip 1 hr prior to the administration of pentobarbital (40 mg/kg).

The animals were observed for sleep as evidenced by the loss of the righting reflex. The degree of potentiation produced by these compounds was calculated by the mean average time of sleep observed in experimental animals.

Determination of Respiratory Activity of Rat Brain Homogenate[2]—Male albino rats[3], kept on an *ad libitum* diet, were used. Rat brains, isolated from decapitated animals, were immediately homogenized in ice-cold 0.25 *M* sucrose in a homogenizer[4] in a ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured using air as the gas phase (13). Fresh rat brain homogenate (1 ml), equivalent to 100 mg wet weight, was added to 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 and succinate were used at a final concentration of 10 m*M*. NAD was used at a final concentration of 0.5 m*M*. It was presumed that the endogenous NAD, present in this homogenate, was sufficient for these oxidative processes. All compounds (final concentration of 2 m*M*) under assay were dissolved in propylene glycol (100%), and an equal volume of the solvent was added to the control vessels.

RESULTS AND DISCUSSION

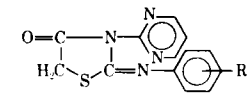
In the present study, 1-aryl-3-(2-pyrimidyl)thiocarbamides and their corresponding cyclized 2-arylimino-3-(2-pyrimidyl)thiazolid-

[2] Commercial chemicals were used in this study. Adenosine monophosphate (sodium salt), nicotinamide adenine dinucleotide, pyruvate, succinate, and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from British Drug House, Bombay, India.

[3] Animal Supply House, Lucknow, India.

[4] Potter-Elvehjem homogenizer.

Table II—Physical Constants of 2-Arylimino-3-(2-pyrimidyl)thiazolid-4-ones



Compound	R	Melting Point[a]	Yield, %	Molecular Formula	Analysis, %	
					Calc.	Found
IX	H	260° dec.	64	$C_{13}H_{10}N_4OS$	C 57.77	57.60
					H 3.70	3.59
					N 20.74	20.82
X	2-CH_3	209°	58	$C_{14}H_{12}N_4OS$	C 59.15	59.26
					H 4.22	4.42
					N 19.71	19.60
XI	4-CH_3	140°	52	$C_{14}H_{12}N_4OS$	C 59.15	59.25
					H 4.22	4.18
					N 19.71	19.35
XII	2,4-$(CH_3)_2$	175°	54	$C_{15}H_{14}N_4OS$	C 60.40	60.72
					H 4.69	4.50
					N 18.79	18.92
XIII	2,6-$(CH_3)_2$	199°	62	$C_{15}H_{14}N_4OS$	C 60.40	60.50
					H 4.69	4.43
					N 18.79	18.49
XIV	4-OCH_3	264°	67	$C_{14}H_{12}N_4O_2S$	C 56.00	56.21
					H 4.00	3.96
					N 18.66	18.72
XV	4-Br	234°	70	$C_{13}H_9BrN_4OS$	C 44.69	44.52
					H 2.57	2.60
					N 16.04	16.13
XVI	4-Cl	250°	55	$C_{13}H_9ClN_4OS$	C 51.23	51.27
					H 2.95	3.04
					N 18.39	18.45

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

4-ones were synthesized and evaluated for their CNS depressant activity. Anticonvulsant activity was determined against pentylenetetrazol-induced convulsions. The degree of protection afforded by thiocarbamides (100 mg/kg) ranged from 20 to 50% (Table III). The data in general indicate a trend of more protection from convulsions with less mortality. These thiocarbamides, in a dose of 100 mg/kg, potentiated pentobarbital sleeping time in mice, which was of a low order and ranged from 10 to 24 min. With the exception of II, IV, and VIII, these thiocarbamides showed low inhibition of the oxidation of pyruvate by rat brain homogenates. On the other hand, oxidation of succinate remained unaltered.

As is evident from Table IV, cyclization of thiocarbamides to their corresponding thiazolidones resulted in increased anticonvulsant activity with the exception of X, XI, and XVI. The anticonvulsant activity of substituted thiazolidones in doses of 100 mg/kg ranged from 20 to 80%, while XI failed to provide any protection against pentylenetetrazol-induced convulsions. The data seem to indicate a definite trend of more protection from convulsions with less mortality with the exception of XV, where 30% anticonvulsant activity was associated with low pentylenetetrazol-induced mortality of only 20%.

The cyclization of thiocarbamides to thiazolidones exhibited a significant increase in the ability of thiazolidones to potentiate pentobarbital-induced sleeping time in mice. Such an increase was two- to fivefold with thiazolidones as compared to substituted thiocarbamides. All thiazolidones inhibited the oxidation of pyruvic acid by rat brain homogenate, while the oxidation of succinate remained unaltered.

The results recorded in Table IV clearly show the increased inhibition of cellular respiratory activity by thiazolidones as compared to their precursor thiocarbamides. The presence of added NAD not only increased the cellular respiratory activity of rat brain homogenates during oxidation of pyruvate but also decreased the inhibitory effectiveness of substituted thiazolidones. These results, which are in good agreement with earlier reports (14, 15), thus provide evidence for a possible competition between the thiazolidones and NAD for the active site(s) on the enzyme molecule. Therefore, these results have indicated the selective inhibition of NAD-dependent oxidation of pyruvate by thiocarbamides (Table III) and thiazolidones (Table IV).

There was no definite relationship between the chemical structure of thiocarbamides and thiazolidones and their anticonvulsant effects or the inhibition of oxidation of pyruvate. Inhibition of certain metabolic processes in the brain has been proposed as a biochemical basis for the mechanism of action of various CNS depressants (16, 17), where a parallelism was observed between greater *in*

Table III—Pharmacological and Biochemical Properties of 1-Aryl-3-(2-pyrimidyl)thiocarbamides

Compound	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality in 24 hr[a], %	Potentiation of Pentobarbital Sleeping Time[b], min	Inhibition[c], %	
				Pyruvate	Succinate
I	50	30	18 ± 2	20.1 ± 1.6	Nil
II	40	70	10 ± 3	Nil	Nil
III	20	80	24 ± 6	24.9 ± 1.4	Nil
IV	30	70	17 ± 4	Nil	Nil
V	40	70	17 ± 2	15.6 ± 1.3	Nil
VI	30	50	22 ± 7	13.9 ± 1.2	Nil
VII	20	100	12 ± 2	22.1 ± 1.2	Nil
VIII	50	20	Nil	Nil	Nil

[a] Substituted thiocarbamides were administered (100 mg/kg ip) 4 hr before the administration of pentylenetetrazol (90 mg/kg sc). [b] Pentobarbital (40 mg/kg ip) produced ataxia and no sleep. Substituted thiocarbamides were administered (100 mg/kg ip) 1 hr prior to the administration of pentobarbital. The values represent the mean ± standard error from six mice. [c] Each experiment was done in duplicate. All values represent mean ± standard error from three experiments. The oxygen uptake was measured at 5-min intervals during 1 hr of incubation. The percent inhibition was calculated from the decrease in oxygen uptake per 100 mg wet brain weight.

Table IV—Pharmacological and Biochemical Properties of 2-Arylimino-3-(2-pyrimidyl)thiazolid-4-ones

Compound	Anticonvulsant Activity[a], % Protection	Pentylenetetrazol Mortality in 24 hr[a], %	Potentiation of Pentobarbital Sleeping Time[a], min	Inhibition[a], %		
				Pyruvate		Succinate
				Without Added NAD	With Added NAD[b]	
IX	80	30	51 ± 6	25.6 ± 0.8	15.3 ± 1.4	Nil
X	30	60	50 ± 9	48.8 ± 1.2	37.3 ± 1.5	Nil
XI	Nil	100	70 ± 8	35.5 ± 1.7	29.6 ± 1.7	Nil
XII	70	50	51 ± 5	40.3 ± 1.4	35.8 ± 1.3	Nil
XIII	50	50	54 ± 7	39.3 ± 1.7	32.4 ± 1.1	Nil
XIV	60	40	60 ± 4	38.9 ± 0.7	30.7 ± 1.7	Nil
XV	30	20	46 ± 10	27.9 ± 1.6	21.3 ± 1.8	Nil
XVI	20	70	27 ± 6	16.9 ± 1.2	11.8 ± 1.5	Nil

[a]Details of the experiments are as described in Table III. The various substituted thiazolidones were administered in a dose of 100 mg/kg ip for studying pharmacological properties and were used at a final concentration of 2 m*M* for biochemical studies. [b]The final concentration of the added NAD in the incubation mixture was 0.5 m*M*.

vivo hypnotic activity of some compounds and their ability to cause greater *in vitro* inhibition of cellular respiratory activity (18, 19).

The present study has failed to provide any definite correlation between anticonvulsant activities of thiocarbamides and thiazolidones and their abilities to potentiate pentobarbital-induced hypnosis and inhibit selectively NAD-dependent oxidation of pyruvate. Similar results were reported earlier with 3,4,5-trimethoxybenzamides (20). However, the results have indicated that cyclization of thiocarbamides to the corresponding thiazolidones in general resulted in increased CNS depressant activity, which correlated well with the increased ability of thiazolidones to inhibit selectively cellular respiratory activity of rat brain homogenates as compared to their precursor thiocarbamides.

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ACKNOWLEDGMENTS AND ADDRESSES

Received April 3, 1975, from the *Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow, 226003, India, and the ‡Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37232

Accepted for publication May 22, 1975.

Supported in part by the Department of Atomic Energy, Government of India, Bombay, India, Council of Scientific and Industrial Research, New Delhi, India. A. K. Chaturvedi and B. V. R. Sastry were supported by U.S. Public Health Service Research Grant NS-04699 and General Research Support Grant RR-05424.

The authors thank Professor K. P. Bhargava and Dr. J. P. Barthwal for their advice and encouragement. Grateful acknowledgment is made to the Department of Atomic Energy, Government of India, Bombay, India, for providing a Research Fellowship to S. K. Chaudhary.

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