h = effective diffusion layer thickness
L = length of tablet; parallel to flow
r = radius of tablet surface
R = rate of dissolution
t = time
v = volume of solution
$\mathbf{V}$ = vector describing liquid flow
V_x = x component of liquid velocity
x,y,z = cartesian coordinates
α = rate of shear in boundary layer
Γ = gamma function
β = dimensionless variable

REFERENCES

(1) D. E. Wurster and P. W. Taylor, *J. Pharm. Sci.*, **54**, 169(1965).

(2) W. I. Higuchi, *ibid.*, **56**, 315(1967).

(3) J. G. Wagner, *ibid.*, **50**, 359(1961).

(4) P. Singh, S. J. Desai, D. R. Flanagan, A. P. Simonelli, and W. I. Higuchi, *ibid.*, **57**, 959(1968).

(5) G. Levy, *ibid.*, **52**, 1039(1963).

(6) V. Levich, "Physicochemical Hydrodynamics," Prentice-Hall, Englewood Cliffs, N.J., 1962.

(7) H. Schlichting, "Boundary Layer Theory," 6th ed., McGraw-Hill, New York, N.Y., 1968.

(8) R. B. Bird, W. E. Stewart, and E. N. Lightfoot, "Transport Phenomena," Wiley, New York, N.Y., 1960.

(9) A. C. Shah, C. B. Peot, and J. F. Ochs, *J. Pharm. Sci.*, **62**, 671(1973).

(10) A. C. Shah and J. F. Ochs, *ibid.*, **63**, 110(1974).

(11) S. H. Yalkowsky, G. L. Flynn, and T. G. Slunick, *ibid.*, **61**, 852(1972).

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* Present address: College of Pharmacy, University of Minnesota, Minneapolis, MN 55455

‡ To whom inquiries should be directed.

Substituted Thiazolidones: Selective Inhibition of Nicotinamide Adenine Dinucleotide-Dependent Oxidations and Evaluation of Their CNS Activity

SUNIL K. CHAUDHARI *, MAHIMA VERMA *, ARVIND K. CHATURVEDI *, and SURENDRA S. PARMAR *‡x

Abstract □ Eight 2-arylimino-3-(3-*N*-morpholinopropyl)thiazolid-4-ones were synthesized from the corresponding 1-aryl-3-(3-*N*-morpholinopropyl)thiocarbamides, characterized, and tested for their effects on the cellular respiratory activity of rat brain homogenates. All substituted 4-thiazolidones selectively inhibited nicotinamide adenine dinucleotide (NAD)-dependent oxidations of pyruvate, citrate, DL-isocitrate, α-ketoglutarate, malate, β-hydroxybutyrate, L-glutamate, and NADH, while the NAD-independent oxidation of succinate remained unaltered. All thiazolidones possessed some degree of anticonvulsant activity against pentylenetetrazol-induced convulsions, and the protection afforded by these compounds at a dose of 100 mg/kg ranged from 30 to 80%. The low toxicity possessed by most of these thiazolidones was reflected by their approximate LD_{50} values from 300 mg/kg to greater than 1000 mg/kg. In the present study, the anticonvulsant activity possessed by these substituted 4-thiazolidones was unrelated to their ability to inhibit selectively the NAD-dependent oxidations by rat brain homogenates. These thiazolidones exhibited depression of the CNS activity which, in some cases, was associated with the increase in respiration. All thiazolidones potentiated pentobarbital (sodium) sleeping time in mice when administered in a dose of 100 mg/kg.

Keyphrases □ Thiazolidones, 2-arylimino-3-(3-*N*-morpholinopropyl)—synthesis, inhibition of NAD-dependent oxidations and relationship to anticonvulsant activity □ Structure–activity relationships—thiazolidones, anticonvulsant activity, inhibition of NAD-dependent oxidations, rats □ Anticonvulsant activity, thiazolidones—relationship to inhibition of NAD-dependent oxidations

Thiazolidones have been shown to possess diverse biological properties including hypnotic (1), local anesthetic (2), and anticonvulsant (3, 4) activities. Recent studies indicated the anticonvulsant activity of piperazinothiocarbamides (5, 6) and their ability to inhibit nicotinamide adenine dinucleotide (NAD)-dependent oxidations. Furthermore, compounds possessing a morpholino group attached to the heterocyclic nuclei have been shown to confer greater activity and less toxicity (7). These observations led to the synthesis of some 2-arylimino-3-(3-*N*-morpholinopropyl)thiazolid-4-ones and correlation of certain pharmacological properties with their ability to inhibit NAD-dependent oxidations.

EXPERIMENTAL[1]

The various 1,3-disubstituted thiocarbamides were prepared by refluxing an equimolar quantity of 3-*N*-morpholinopropylamine and different arylisothiocyanates in dry benzene. These substituted thiocarbamides, when refluxed with chloroacetic acid and anhydrous sodium acetate in absolute ethanol, formed the desired substituted thiazolidones (8).

1 - Aryl - 3 - (3 - *N* - morpholinopropyl)thiocarbamide—3 - *N* - Morpholinopropylamine (0.01 mole) was mixed with suitable aryl isothiocyanate (0.01 mole) in 15 ml of dry benzene, and the mixture was refluxed on a steam bath for 2 hr. The reaction mixture was concentrated by removing benzene by distillation under reduced pressure. The solid that separated on cooling was filtered, washed with ether and dilute hydrochloric acid, dried, and recrystallized from ethanol. All thiocarbamides were characterized by their sharp melting points and elemental analyses (Table I).

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

Table I—Physical Constants of Substituted Thiocarbamides

Ar—NHCSNHCH$_2$CH$_2$CH$_2$—N⟨O⟩

Compound	Ar	Melting Point[a]	Yield, %	Molecular Formula	Analysis, % Calc.	Found
I	C_6H_5	128°	80	$C_{14}H_{21}N_3OS$	C 60.21	60.40
					H 7.52	7.35
					N 15.05	15.22
II	2-CH_3—C_6H_4	188°	72	$C_{15}H_{23}N_3OS$	C 61.43	61.80
					H 7.84	7.49
					N 14.33	14.50
III	4-CH_3—C_6H_4	112°	87	$C_{15}H_{23}N_3OS$	C 61.43	61.75
					H 7.84	7.98
					N 14.33	14.20
IV	2,4-$(CH_3)_2$—C_6H_3	105°	83	$C_{16}H_{25}N_3OS$	C 62.54	62.75
					H 8.14	8.34
					N 13.67	13.59
V	2,6-$(CH_3)_2$—C_6H_3	105–107°	89	$C_{16}H_{25}N_3OS$	C 62.54	62.41
					H 8.14	8.37
					N 13.67	13.40
VI	4-OCH_3—C_6H_4	111°	82	$C_{15}H_{23}N_3O_2S$	C 58.25	58.25
					H 7.44	7.52
					N 13.59	13.69
VII	4-Cl—C_6H_4	135°	75	$C_{14}H_{20}ClN_3OS$	C 53.58	53.46
					H 6.37	6.50
					N 13.39	13.68
VIII	α-Naphthyl	115°	69	$C_{18}H_{23}N_3OS$	C 65.65	65.79
					H 6.99	7.12
					N 12.76	12.86

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

2-Arylimino-3-(3-*N*-morpholinopropyl)thiazolid-4-ones—A mixture of 1-aryl-3-(3-*N*-morpholinopropyl)thiocarbamide (0.01 mole), chloroacetic acid (0.01 mole), and anhydrous sodium acetate (0.015 mole) in 25 ml of absolute ethanol was refluxed on a steam bath for 4 hr. The mixture was cooled and poured into crushed ice, and the resulting solution was treated with 15% (w/v) solution of sodium carbonate until the solution was alkaline. The crude product that separated was filtered, washed several times with water, dried, and recrystallized from ethanol (Table II).

Assay of Respiratory Activity of Rat Brain Homogenate[2]—Male albino rats, 100–150 g, kept on an *ad libitum* diet, were used in all experiments. Rat brains isolated from decapitated animals were immediately homogenized in ice-cold 0.25 *M* sucrose in a Potter-Elvehjem homogenizer in a ratio of 1:9 (w/v). All incubations were carried out at 37°, and the oxygen uptake was measured by the conventional Warburg manometric technique using air as the gas phase (5). Fresh brain homogenate, 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 total volume of 3 ml unless otherwise stated.

The central well contained 0.2 ml of 20% KOH solution. Pyruvate, citrate, DL-isocitrate, α-ketoglutarate, malate, β-hydroxybutyrate, L-glutamate, NADH, and succinate were used at a final concentration of 10 m*M*, while the concentration of NADH was 0.5 m*M*. All thiazolidones 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.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined against pentylenetetrazol-induced convulsions in mice of either sex weighing 25–30 g. The mice were divided into groups of 10, keeping the group weights as near the same as possible. All thiazolidones were suspended in 5% aqueous gum acacia to give a concentration of 0.25% (w/v). The test compounds were administered to a group of 10 mice in a dose of 25–100 mg/kg ip. Four hours after the administration of the test compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol was shown to induce convulsions in almost all untreated mice and produced 100% mortality during a 24-hr period. On the other hand, no mortality was observed during 24 hr in animals treated with these doses of substituted thiazolidones alone.

The mice were observed for 60 min for the occurrence of seizures. An episode of clonic convulsion persisting for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were disregarded. 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 substituted thiazolidones was represented as percent protection.

Potentiation of Pentobarbital Sleeping Time—The method of Winter (9) was followed to investigate the ability of substituted thiazolidones to potentiate pentobarbital-induced hypnosis. Mice, 20–25 g, were used in groups of six animals. Each compound was tested in a group of six mice while one group served as a control for pentobarbital alone. Pentobarbital (sodium), when administered in a dose of 30 mg/kg ip to the control group, produced ataxia and no sleep; an increase in the dose of pentobarbital produced sleep in normal mice.

All thiazolidones were administered in a dose of 100 mg/kg ip 1 hr prior to the administration of pentobarbital. The animals were observed regularly for sleep as evidenced by loss of the righting reflex until the animals awakened. The time of administration of pentobarbital in both the control and experimental mice treated with thiazolidones was recorded. The mean average sleeping time in each group was calculated.

Determination of Approximate LD_{50} Values—The approximate LD_{50} values of these substituted thiazolidones were determined by intraperitoneal administration in albino mice, 25–30 g, following the method reported by Smith (10).

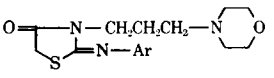
Determination of Behavioral Effects—The effects of these thiazolidones were investigated in albino mice, 25–30 g, using a dose of 100 mg/kg ip. The various effects observed were general depression, paralysis of the hindlimb associated with slight depression and an increase in the rate of respiration, and circular movements of the thiazolidone-treated mice.

RESULTS AND DISCUSSION

Inhibition of certain metabolic processes in the brain has been reported to be the mechanism of various central nervous system (CNS) depressants (11, 12). Earlier studies indicated a parallelism between *in vitro* and *in vivo* effects, since greater hypnotic activity

[2] Commercial chemicals were used in the present study. Sodium salts of pyruvic acid, citric acid, DL-isocitric acid, α-ketoglutaric acid, succinic acid, β-hydroxybutyric acid, DL-malic acid, and NADH were obtained from Sigma Chemical Co., St. Louis, Mo. Other common chemicals were obtained from the British Drug House, Bombay, India.

Table II—Physical Constants of Substituted 4-Thiazolidones



Compound	Ar	Melting Point[a]	Yield, %	Molecular Formula	Analysis, %	
					Calc.	Found
IX	C_6H_5	107°	65	$C_{16}H_{21}N_3O_2S$	C 60.19	60.47
					H 6.58	6.72
					N 13.16	13.44
X	2-CH_3—C_6H_4	73°	54	$C_{17}H_{23}N_3O_2S$	C 61.26	61.05
					H 6.90	6.72
					N 12.61	12.43
XI	4-CH_3—C_6H_4	82°	60	$C_{17}H_{23}N_3O_2S$	C 61.26	61.47
					H 6.90	7.12
					N 12.61	12.84
XII	2,4-$(CH_3)_2$—C_6H_3	53°	52	$C_{18}H_{25}N_3O_2S$	C 62.24	62.04
					H 7.20	7.10
					N 12.10	12.19
XIII	2,6-$(CH_3)_2$—C_6H_3	88°	64	$C_{18}H_{25}N_3O_2S$	C 62.24	62.16
					H 7.20	7.29
					N 12.10	11.98
XIV	4-OCH_3—C_6H_4	76°	59	$C_{17}H_{23}N_3O_3S$	C 58.45	58.60
					H 6.59	6.78
					N 12.03	12.23
XV	4-Cl—C_6H_4	55°	62	$C_{16}H_{20}ClN_3O_2S$	C 54.31	54.80
					H 5.65	6.72
					N 11.88	11.95
XVI	α-Naphthyl	112°	58	$C_{20}H_{23}N_3O_2S$	C 65.05	65.34
					H 6.23	6.19
					N 11.38	11.50

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

of some agents was reflected by their greater *in vitro* inhibition of respiration (13, 14). The inhibitory effects of substituted thiazolidones on the cellular respiratory activity of rat brain homogenates are presented in Table III. All thiazolidones selectively inhibited *in vitro* NAD-dependent oxidation of pyruvate, citrate, DL-isocitrate, α-ketoglutarate, malate, β-hydroxybutyrate, and L-glutamate, whereas NAD-independent oxidation of succinate remained unaltered. These results, exhibiting selective inhibition of NAD-dependent oxidations, are in agreement with earlier studies with 2-methyl-3-*o*-tolyl-4-quinazolone (15).

In the present study, *in vitro* inhibition of the oxidation of NADH was observed as was reported earlier for nitrobenzamides (16). These results have indicated possible inactivation of the transfer process of electrons in the respiratory chain by these thiazolidones by acting presumably at a site of transfer of electrons from NADH to flavine adenine dinucleotide. The enzyme inhibitory effectiveness of these thiazolidones was not uniform with regard to their ability to produce maximum inhibition during oxidation of various substrates. Compound IX, possessing an unsubstituted phenyl group attached to the thiazolidone nucleus, was the least effective compound during oxidation of all of the substrates used in the present study. The maximum inhibition of the oxidation of pyruvate and α-ketoglutarate by Compound XV, of citrate, DL-isocitrate, and malate by Compound XI, of L-glutamate and NADH by Compound X, and of β-hydroxybutyrate by Compound XII observed in these experiments failed to provide correlation between inhibitory effects and the chemical structure of these thiazolidones.

The results in Table IV compare the anticonvulsant activity possessed by these thiazolidones and their ability to potentiate pentobarbital sleeping time. All thiazolidones possessed anticonvulsant activity, which was reflected by the 30–80% protection afforded by these compounds against pentylenetetrazol-induced convulsions. Compounds IX and XV showed maximum protection, while Compound XIII afforded the least protection from pentylenetetrazol-induced convulsions. Data on anticonvulsant activity and 24-hr pentylenetetrazol-induced mortality (Table IV) indicated some association between increased protection from convulsions and decreased pentylenetetrazol mortality in experimental animals.

In the present study, intraperitoneal administration of 30 mg/kg of pentobarbital (sodium) produced ataxia and no sleep in mice. Administration of thiazolidones in a dose of 100 mg/kg ip 1 hr prior to the administration of pentobarbital produced sleep in mice. Thus, all thiazolidones potentiated pentobarbital sleeping time and the duration of sleep ranged from 21.6 to 98.6 min (Table IV). The greater potentiation of sleep observed with Compounds IX and XV, possessing maximum anticonvulsant activity, has pro-

Table III—Inhibition of the Respiratory Activity of Rat Brain Homogenate by Substituted 4-Thiazolidones

Compound	Inhibition[a], %								
	Pyruvate	Citrate	DL-Isocitrate	α-Ketoglutarate	Malate	β-Hydroxybutyrate	L-Glutamate	NADH	Succinate
IX	59.2 ± 0.7	17.4 ± 0.5	22.5 ± 0.9	35.3 ± 1.0	42.4 ± 1.4	44.0 ± 1.0	36.6 ± 1.2	30.7 ± 1.3	Nil
X	78.6 ± 0.8	43.9 ± 1.4	50.4 ± 0.7	56.9 ± 1.2	58.8 ± 0.8	60.5 ± 1.3	69.2 ± 1.5	50.1 ± 0.7	Nil
XI	72.1 ± 1.2	71.0 ± 1.5	78.1 ± 0.8	60.6 ± 1.4	59.0 ± 1.5	74.4 ± 1.5	55.0 ± 0.7	45.4 ± 0.9	Nil
XII	69.1 ± 0.9	68.0 ± 1.0	68.8 ± 1.4	40.8 ± 0.9	50.6 ± 0.9	83.7 ± 1.0	55.9 ± 0.9	45.3 ± 0.7	Nil
XIII	68.2 ± 0.8	56.7 ± 0.9	62.8 ± 0.9	44.3 ± 1.1	49.9 ± 1.0	76.3 ± 0.9	59.3 ± 0.9	40.9 ± 0.8	Nil
XIV	71.4 ± 0.9	55.4 ± 0.9	57.4 ± 1.0	57.9 ± 1.3	53.3 ± 0.9	78.3 ± 1.3	52.6 ± 1.4	40.3 ± 1.4	Nil
XV	83.3 ± 1.5	55.8 ± 1.3	60.7 ± 0.7	70.3 ± 1.5	55.4 ± 0.8	79.2 ± 1.0	65.0 ± 1.3	45.7 ± 1.3	Nil
XVI	60.4 ± 0.8	57.9 ± 1.4	60.3 ± 0.5	36.3 ± 0.9	52.6 ± 1.3	56.4 ± 1.0	53.7 ± 0.9	48.6 ± 0.9	Nil

[a] Each experiment was done in duplicate. All values represent mean values of percent inhibition with ± standard error of the mean from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake/100 mg wet tissue weight/hr. Different substrates and NADH were used at a final concentration of 10 and 0.5 m*M*, respectively. All substituted thiazolidones were used at a final concentration of 2 m*M*. Vessel contents and assay procedure are as described in the text.

Table IV—Pharmacological Properties of Substituted 4-Thiazolidones

Compound	Approximate LD_{50}, mg/kg ip	Anticonvulsant Activity[a], % Protection	Pentylene-tetrazol Mortality[b], %	Potentiation of Pento-barbital Sleeping Time[c], min	Behavioral Effects[d]
IX	500	80	20	98.6 ± 10	A
X	1000	60	30	45.3 ± 9	B
XI	500	50	20	21.6 ± 5	B
XII	>1000	50	20	44.2 ± 8	C
XIII	300	30	60	77.0 ± 11	A
XIV	>1000	50	20	67.0 ± 9	A
XV	>1000	80	20	88.2 ± 7	A
XVI	>1000	50	30	51.0 ± 6	A

[a] Anticonvulsant activity was determined at a dose of 100 mg/kg ip as described in the text. [b] Represents mortality during 24 hr in each group of animals administered pentylenetetrazol. [c] Administration of pentobarbital in a dose of 30 mg/kg produced ataxia and no sleep. Sleeping time was observed in animals treated with substituted thiazolidones (100 mg/kg ip) 1 hr prior to the administration of pentobarbital. [d] Behavioral effects were observed in animals treated with substituted thiazolidones in a dose of 100 mg/kg ip, and the various effects observed are indicated as: A, slight depression; B, ataxia in hindlimbs associated with slight depression and an increase in the rate of respiration; and C, circular movements. Various screening procedures for determination of the pharmacological properties of these substituted thiazolidones are as described in the text.

vided a relationship between the anticonvulsant activity of these thiazolidones and their ability to potentiate pentobarbital-induced sleep. This relationship, however, was not sufficiently uniform to make a definite conclusion since significant potentiation of sleep was observed with Compound XIII (77.0 min) which possessed the minimum anticonvulsant activity of only 30%.

The behavioral effects observed with the administration of these thiazolidones and their approximate LD_{50} values are recorded in Table IV. The various effects observed were depression of CNS activity, which was sometimes associated with the increase in respiration, paralysis of the hindlimb, and production of circular movements. The approximate LD_{50} values of these thiazolidones were found to be from 300 to >1000 mg/kg. Most compounds possessing approximate LD_{50} values >1000 mg/kg have indicated low toxicity associated with these thiazolidones.

Results presented in the present study have failed to provide any definite correlation between selective inhibition of NAD-dependent oxidations by thiazolidones and their abilities to provide protection against pentylenetetrazol-induced convulsions and to potentiate pentobarbital-induced hypnosis. It is hoped that further detailed pharmacological and toxicological studies and investigations of the effects of substituted thiazolidones on other enzyme systems may reflect a biochemical basis for their CNS activity.

REFERENCES

(1) W. J. Doran and H. A. Shouli, *J. Org. Chem.*, **33**, 193(1938).

(2) A. R. Surrey, *J. Amer. Chem. Soc.*, **71**, 3105(1949).

(3) H. D. Troutman and L. M. Long, *ibid.*, **70**, 3436(1948).

(4) C. Dwivedi, T. K. Gupta, and S. S. Parmar, *J. Med. Chem.*, **15**, 533(1972).

(5) A. K. Chaturvedi and S. S. Parmar, *Biochem. Pharmacol.*, **22**, 2355(1973).

(6) A. K. Chaturvedi and S. S. Parmar, *Curr. Sci.*, **41**, 253(1972).

(7) P. N. Bhargava and M. G. R. Nair, *J. Indian Chem. Soc.*, **34**, 42(1957).

(8) P. L. Rylander and E. Campaigne, *J. Org. Chem.*, **15**, 249(1950).

(9) C. A. Winter, *J. Pharmacol. Exp. Ther.*, **94**, 1(1948).

(10) C. C. Smith, *ibid.*, **100**, 408(1950).

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

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

(13) J. H. Quastel and A. H. M. Wheatley, *Proc. Roy. Soc. (London)*, **112**, 60(1932).

(14) F. A. Furham and J. Field, *J. Pharmacol. Exp. Ther.*, **77**, 392(1943).

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

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

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[x] To whom inquiries should be directed (at the University of North Dakota).