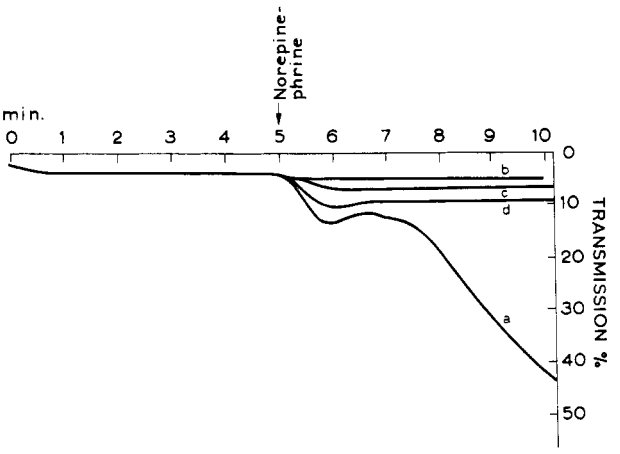


Figure 2. Effect of dansylcadaverine (1) on norepinephrine-induced platelet aggregation: a, no inhibitor; b, $3.1 \times 10^{-4} M$; c, $1.6 \times 10^{-4} M$; d, $6.2 \times 10^{-5} M$. Norepinephrine, $6.2 \times 10^{-6} M$.

soln (1–9 ml of blood). The blood was centrifuged at 200*g* for 15 min to prep platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obtd by centrifugation for 20 min at 3200*g*. All these operations were performed at room temp and completed within 45 min after the blood had been drawn. Platelets were counted using a Büchner chamber and a phase contrast microscope. Counts for PRP varied between 300,000 and 500,000 cells/mm^3.

Adenosine diphosphate (ADP) Na salt (Sigma Chemical Co.) was dissolved in Tris buffer (0.05 *M*) to a concn of $5 \times 10^{-3} M$. The soln was divided into aliquots and stored at −70°. Norepinephrine (Sigma Chemical Co.) was made up to $6.2 \times 10^{-6} M$ immediately before the expt and was kept at 0° until used.

The aggregation expts were carried out as follows. PRP (3 ml) and PPP (3 ml) were transferred into 2 sep cuvettes. The recorder was adjusted to 95% transmission against PPP and to 5% transmission against PRP; 0.1 ml of saline (0.08 *M* NaCl) was added to the cuvette contg PRP. It was allowed to stand in the titrator with stirring for 5 min, when aggregation was initiated by adding 0.1 ml of the ADP soln.

In the expts with inhibitors, saline was replaced by 0.1 ml of a soln of one of the compds **1–3**, dissolved in the Tris buffer. Adenosine (Sigma Chemical Co.) was employed as a "standard" inhibitor.

A "blank recording" using saline instead of inhibitor soln was run in the beginning and the end of each exptl series so that the effects of storing PRP could be observed. All expts were completed within 3–4 hr after drawing the blood.

For each plasma sample the suitable ADP concn had to be detd. A sufficiently low concn was chosen to give a typical two-phase curve showing a second wave of platelet aggregation. This concn was usually of the order of 10^{-6} *M*.

Chemical Methods and Materials. Melting points were detd with calibrated Anschütz thermometers in an electrically heated metal block. Ir spectra were run for identification purposes on a Perkin-Elmer 237 spectrophotometer. New compds, which were analyzed for C, H, and N, gave values within ±0.4% of the theoretical ones. Dansyl chloride was commercially available.

5-Benzylmethylaminopentylamine. 5-Benzylmethylaminovaleronitrile[10] (5 g; 24.7 mmoles) was reduced in 100 ml of dry Et_2O using LAH (1.14 g; 30 mmoles). The mixt was refluxed for 6 hr and worked up as usual yielding 4 g of product, bp 114–115° (1.5 mm) [lit.[10] 107–109° (0.5 mm)].

***N*-(5-Benzylmethylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide.** This compd was prepd in 85% yield from dansyl chloride and 5-benzylmethylaminopentylamine as previously described for **1**.[1] The dihydrochloride had mp 175° dec (from EtOH–Et_2O). *Anal.* ($C_{25}H_{33}N_3O_2S \cdot 2HCl$): C, H, and N.

***N*-(5-Methylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (2).** The preceding benzylamino deriv (1 g; 2.3 mmoles) in EtOH (50 ml) was hydrogenated over 0.1 g of 10% Pd/C at room temp overnight in a Parr app with an initial H_2 pressure of 3 kg/cm^2. After filtration and evapn, the amine (0.5 g; 63% yield) was converted to its dihydrochloride, mp 245–248° (from EtOH-ether). *Anal.* ($C_{18}H_{27}N_3O_2S \cdot 2HCl$): C, H, and N.

***N*-(5-Diethylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide (3).** This compound was prepared in 59% yield from dansyl chloride and 5-diethylaminopentylamine as previously described for **1**.[1] The dihydrochloride had mp 168–170° dec (from EtOH–ether). *Anal.* ($C_{21}H_{33}N_3O_2S \cdot 3HCl$): C, H, and N.

Acknowledgments. At the Faculty of Pharmacy, University of Uppsala, this work has been kindly supported by a grant from AB Kabi, Stockholm. The work at Northwestern University was aided by a U.S. Public Health Service Research Career Award and by grants from the National Institutes of Health and the American Heart Association.

References

(1) J. L. G. Nilsson, P. Stenberg, Ch. Ljunggren, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica,* in press (part III).
(2) (a) G. V. R. Born, and M. J. Cross, *J. Physiol.*, **170**, 397 (1964); (b) M. J. Cross, *Thromb. Diath. Haemorrh.*, **12**, 524 (1964).
(3) (a) L. Lorand, and K. Konishi, *Arch. Biochem. Biophys.*, **105**, 58 (1964); (b) L. Lorand, J. Downey, T. Gotoh, A. Jacobsen, and S. Tokura, *Biochem. Biophys. Res. Commun.*, **31**, 222 (1968).
(4) (a) K. Buluk, *Pol. Tyg. Lek.*, **10**, 191 (1955); (b) E. F. Lüscher, *Schweiz. Med. Wochenschr.*, **87**, 1220 (1957).
(5) (a) H. Bohn, *Thromb. Diath. Haemorrhag.*, **23**, 455 (1970); (b) J. McDonagh and R. H. Wagner, *Amer. J. Physiol.*, **219**, 1955 (1970).
(6) K. Buluk, T. Januszko, and J. Olbromski, *Nature (London)*, **191**, 1093 (1961).
(7) (a) L. Lorand, N. G. Rule, H. H. Ong, R. Furlanetto, A. Jacobsen, J. Downey, N. Öner, and J. Bruner-Lorand, *Biochemistry*, **7**, 1214 (1968); (b) J. L. G. Nilsson, P. Stenberg, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica*, **7**, 441 (1970); (c) L. Lorand, *Thromb. Diath. Haemorrh., Suppl.*, **39**, 75 (1970).
(8) W. B. Lacefield, R. G. Herrmann, J. Mills, W. M. Mills, and J. D. Frank, *J. Med. Chem.*, **14**, 133 (1971).
(9) G. V. R. Born, *Nature (London)*, **194**, 927 (1962).
(10) A. R. Surrey, A. S. Olivet, and J. O. Hoppe, *J. Amer. Chem. Soc.*, **76**, 4920 (1954).

Substituted Thiazolidones and Their Selective Inhibition of Nicotinamide-Adenine Dinucleotide Dependent Oxidations†

Surendra S. Parmar,* C. Dwivedi,‡ A. Chaudhari,§ and T. K. Gupta

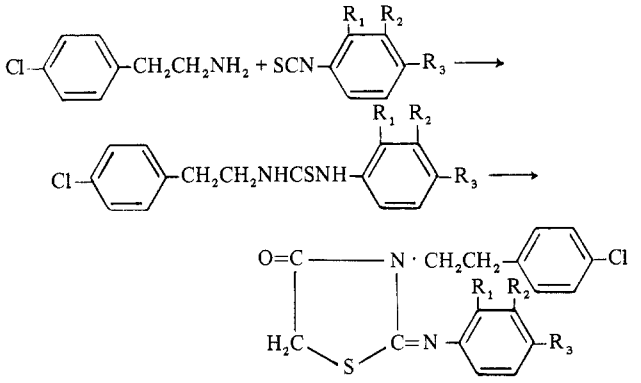
Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow-3, India.
Received June 28, 1971

CNS depressants that have profound effects on the activity of the brain are believed to inhibit certain metabolic processes.[1,2] Interactions with receptor surfaces[3] may account for structure-activity relationships of various psychopharmacological agents. In the present study the ability of thiazolidone derivatives to exhibit a wide variety of pharmacological properties, including anticonvulsant activity,[4,5] led us to investigate the ability of such thiazolidones to inhibit oxidation of the substrates of the tricarboxylic acid cycle like pyruvate, α-ketoglutarate and citrate, and β-hydroxybutyrate with a view of studying their biochemical mechanism of action. The anticonvulsant activity of these compounds was determined to correlate pharmacological properties with their enzyme inhibitory properties. The various substituted thiazolidones have been synthesized according to the methods outlined in Scheme I.

†This investigation was supported in part with the financial assistance obtained from the Indian Council of Medical Research, New Delhi, and the State Council of Scientific and Industrial Research.

‡Junior Research Fellow of I.C.M.R., New Delhi.
§Junior Research Fellow of State C.S.I.R., Lucknow.

Scheme I



Experimental Section

1-Aryl-3-(*p*-chlorophenethyl)thiocarbamide. A mixt of *p*-chlorophenethylamine (0.01 mole) and suitable aryl isothiocyanates (0.01 mole) in 15 ml of dry PhH was refluxed on a steam bath for 2 hr. The reaction mixt was concd under reduced pressure. On cooling the solid mass which sepd out was filtered, washed (Et_2O, dil HCl), dried, and recrystd from EtOH. All compounds were characterized by their sharp melting points and elemental analyses (Table I).

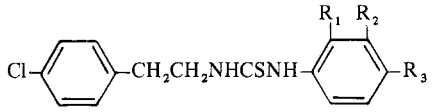
2-Arylimino-3-(*p*-chlorophenethyl)thiazolid-4-one. 1-Aryl-3-(*p*-chlorophenethyl)thiocarbamides (0.01 mole), $ClCH_2CO_2H$ (0.01 mole), and fused NaOAc (0.015 mole) were mixed in 15 ml of glacial AcOH and refluxed for 4–5 hr. The reaction mixt was poured into H_2O and kept overnight at 0°. The crude product which sepd out was filtered, washed several times (H_2O), and recrystd from EtOH (see Table II).

Biochemical Studies. Materials and Methods. Commercial chemicals were used in the present study. AMP, cytochrome *c*, NAD, and α-ketoglutarate were obtained from Sigma Chemical Co., St. Louis, Mo., sodium β-hydroxybutyrate from Mann Research Laboratories Inc., New York, N. Y., sodium pyruvate from E. Merck, Darmstadt. Sodium citrate, sodium succinate, and other common chemicals were purchased from British Drug House, Bombay.

Effect of Thiazolidones on the Respiratory Activity of Rat Brain Homogenate. Male albino rats kept on an ad libitum diet were sacrificed by decapitation. The brains were taken out immediately and homogenized in a Potter-Elvehjem homogenizer in 0.25 *M* cold sucrose in the ratio of 1:9. All incubations were carried out at 37° and O_2 uptake was measured by the conventional Warburg manometric technique with air as the gas phase. The central well contd 0.2 ml of 20% KOH. Various substrates and thiazolidones were used at a final concn of 10 m*M* and 1 m*M*, respectively. Thiazolidones were dissolved in propylene glycol (100%) and an equiv amt of the solvent was added to the control vessels.

Anticonvulsant activity was detd in mice of either sex weighing 25–30 g. The mice were divided in groups of 10, keeping the group wts equal as far as possible. Thiazolidones (100 mg/kg) were injected ip in 5% aq suspension of gum acacia to one group of 10 animals. Four hours after the administration of thiazolidones the mice were injected with pentylenetetrazole (80 mg/kg) sc under the loose skin of the back. This dose of pentylenetetrazole has been shown to produce convulsions in almost all untreated mice. The mice were then observed for the following 60 min for the occurrence of seizures. One episode of clonic spasm which persisted for a minimum period of 5 sec was considered a threshold convulsion. Transient intermittent jerks or tremulousness were not taken into account. Animals devoid of even a threshold convulsion during the period of 60 min were considered protected. The number of animals protected in each group was recorded and the anticonvulsant activity of these thiazolidones was represented as per cent protection.

Table I. Substituted Thiocarbamides



R_1	R_2	R_2	Mp,[a] °C	Yield, %	Formula[b]
H	H	H	110	75	$C_{15}H_{15}ClN_2S$
H	CH_3	H	140	85	$C_{16}H_{17}ClN_2S$
H	H	CH_3	120	92	$C_{16}H_{17}ClN_2S$
H	CH_3	CH_3	122	89	$C_{17}H_{19}ClN_2S$
OCH_3	H	H	125	64	$C_{16}H_{17}ClN_2OS$

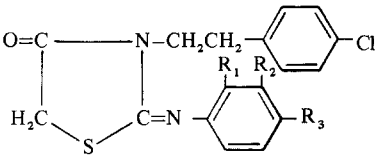
[a]Melting points were taken in open capillary tubes and are corrected graphically. [b]All compds were analyzed for C, H, and N, and analyses were found within limits.

Results and Discussion

The effects of various substituted thiazolidones on the oxidation of the substrates of the tricarboxylic acid cycle and β-hydroxybutyrate by rat brain homogenate are reported in Table III. All the thiazolidones inhibited the oxidation of α-ketoglutarate, citrate, β-hydroxybutyrate, and pyruvate. Maximum inhibition was observed with the compd where an unsubstituted Ph group was attached at position 2 of the thiazolidone nucleus. Introduction of a Me substituent at R_2 and/or R_3 position(s) of this Ph moiety was found to have no significant effect on the inhibitory property of these thiazolidones. On the other hand a MeO substituent at position R_1 of the Ph moiety attached at position 2 of the thiazolidone ring produced a low inhibitory effect under similar experimental conditions.

All these thiazolidones were, however, devoid of any inhibitory effects on the respiratory activity of rat brain homogenate when sodium succinate was used as a substrate. These studies have thus provided selective inhibition of NAD-dependent oxidation as was observed earlier with anticonvulsant substituted quinazolones.[6,7] Studies with added NAD during oxidation of pyruvic acid were found to indicate protection against the inhibitory effect of thiazolidones like the one observed with quinazolones[6,7] and β-amino ketones,[8] since definite reduction was observed in the degree of inhibition. These observations have thus provided

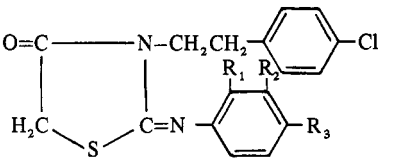
Table II. Substituted-4-thiazolidones and Their Anticonvulsant Activity



R_1	R_2	R_3	Mp,[a] °C	Yield, %	Formula[b]	Protection, %	LD_{50}, mg/kg
H	H	H	56	62	$C_{17}H_{15}ClN_2OS$	20	1600
H	CH_3	H	86	60	$C_{18}H_{17}ClN_2OS$	30	550
H	H	CH_3	52	64	$C_{18}H_{17}ClN_2OS$	40	> 1600
H	CH_3	CH_3	82	60	$C_{19}H_{19}ClN_2OS$	20	550
OCH_3	H	H	104	58	$C_{18}H_{17}ClN_2O_2S$	20	> 1600

[a,b]See footnotes to Table I.

Table III. Effect of 2-Arylimino-3-(*p*-chlorophenethyl)thiazolid-4-ones on the Oxidation of Different Substrates of Tricarboxylic Acid Cycle and β-Hydroxybutyrate by Rat Brain Homogenate[a]



			Pyruvate					
R_1	R_2	R_3	−NAD⁺	+NAD⁺	β-Hydroxy-butyrate	Citrate	α-Keto glutarate	Succinate
H	H	H	54.73 ± 0.54	42.15 ± 0.68	53.98 ± 0.84	53.05 ± 0.85	65.12 ± 0.93	Nil
H	CH_3	H	50.04 ± 0.67	36.15 ± 0.90	49.42 ± 0.78	47.93 ± 0.84	62.18 ± 0.61	Nil
H	H	CH_3	47.48 ± 0.81	41.32 ± 0.82	46.33 ± 0.89	50.14 ± 0.76	58.43 ± 0.98	Nil
H	CH_3	CH_3	49.78 ± 0.92	32.21 ± 0.84	44.10 ± 0.67	43.87 ± 0.78	56.82 ± 0.90	Nil
OCH_3	H	H	32.75 ± 1.00	26.44 ± 0.98	33.20 ± 0.54	28.12 ± 0.92	34.10 ± 0.85	Nil

[a]The O_2 uptake was measured at 10-min intervals. The reaction mixt (in a total vol of 3 ml) contains 6.7 m*M* $MgSO_4$, 20 m*M* Na_2HPO_4 in a buffer sol of pH 7.4, 2 m*M* adenylic acid (Na salt) 33 m*M* KCl and 500 μg of cytochrome *c*. The percentage inhibition was calcd from the decrease in O_2 uptake per hr per 100 mg wet wt. The final concn of substrates and 4-thiazolidones were 10 m*M* and 1 m*M*, resp. NAD^+ was used at a final concn of 0.5 m*M*.

evidence that thiazolidones inhibit the oxidative processes where participation of NAD is a limiting factor. At present it is not yet possible to define their exact site of action.

The anticonvulsant activity exhibited by substituted thiazolidones at 100 mg/kg is shown in Table II. All thiazolidones were able to afford protection which, however, was not of a high order. Anticonvulsant activity of these thiazolidones ranged from 20 to 40%, compounds having a Me substituent at the R_3 position of the Ph moiety exhibiting maximum anticonvulsant activity of 40%. The low toxicities of these compounds were reflected by high values of their approximate LD_{50} which in 3 of these thiazolidones was either 1600 mg/kg or higher. In the present investigation no definite correlation could be observed between the anticonvulsant property exhibited by these thiazolidones and their ability to inhibit NAD-dependent oxidations of the substrates of the tricarboxylic acid cycle as well as of β-hydroxybutyrate. These results have also failed to show structure-activity relationships of these thiazolidones with respect to their anticonvulsant or enzyme inhibitory properties.

Acknowledgments. The authors wish to express their thanks to Professor K. P. Bhargava and Professor D. Dayal for their advice and encouragement and to Dr. M. L. Dhar and Dr. Nitya Anand of the Central Drug Research Institute, Lucknow, for providing microanalysis facilities. Grateful acknowledgment is made to Dr. M. A. Davis of Ayerst Research Laboratories, Montreal, for the generous gift of research chemicals.

References

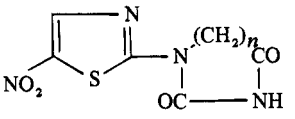
(1) J. H. Quastel, *Physiol. Rev.*, **19**, 135 (1939).
(2) J. H. Quastel, *Trans. Faraday Soc.*, **39**, 348 (1943).
(3) R. P. Barlow, "An Introduction to Chemical Pharmacology," 2nd ed, Wiley, New York, N. Y., 1964.
(4) H. D. Troutman and L. M. Long, *J. Amer. Chem. Soc.*, **70**, 3436 (1948).
(5) R. Kumar, T. K. Gupta, and S. S. Parmar, *J. Prakt. Chem.*, **312**, 201 (1970).
(6) S. S. Parmar and P. K. Seth, *Can. J. Biochem.*, **43**, 1179 (1965).
(7) P. K. Seth and S. S. Parmar, *Can. J. Physiol. Pharm.*, **43**, 1019 (1965).
(8) R. S. Varma, B. Ali, S. S. Parmar, and W. L. Nobles, *J. Med. Chem.*, **13**, 147 (1970).

Substituted Thiazolylureas

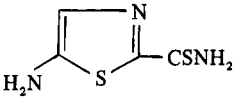
Peter J. Islip,* Michael D. Closier, M. R. Johnson, and Martin C. Neville

Chemistry Department, Division of Medical and Scientific Affairs, Parke, Davis and Company, Hounslow, Middlesex, England.
Received March 22, 1971

2-Amino-5-nitrothiazole derivatives are known to exhibit antiamebic,[1] antihistomonal,[2] antitrichomonal,[3] and antischistosomal[4] activities. Recently the preparation of some 1-(5-nitro-2-thiazolyl)hydantoins and -hydrouracils (I) possessing antibacterial and antiparasitic activity has been described.[5]



I; n = 1-2



II

This note describes the synthesis of some (5-substituted-2-thiazolyl)acylureas (III) (Table I) and -hydrouracils (V) (Table III) from the corresponding 5-substituted-2-aminothiazoles, and also some acylureas (IV) (Table II) and hydantoins from 5-aminothio-2-thiazolecarboxamide (chrysean) (II). The preparation of acylureas from aminothiazoles and acyl isocyanates and cyclization of the appropriate (2- or 3-haloacyl)ureas to the hydantoin or hydrouracil with NaH in DMF are described below.

Experimental Section†

The physical properties of the compds prepd are collected in Tables I–III.

*To whom inquiries should be addressed at the Chemical Research Laboratory, The Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS, England.

†Melting points are corrected and were determined in a capillary tube. Analytical results were obtained for C, H, and N for all compounds, and, unless otherwise stated, were within ±0.4% of the theoretical values.