

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

soln (1–9 ml of blood). The blood was centrifuged at 200g for 15 min to prep platelet-rich plasma (PRP). Platelet-poor plasma (PPP) was obt'd by centrifugation for 20 min at 3200g. 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/mm³.

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 det'd. 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 det'd 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 $\pm 0.4\%$ of the theoretical ones. Dansyl chloride was commercially available.

5-Benzylmethylaminopentylamine. 5-Benzylmethylaminovaleronitrile¹⁰ (5 g; 24.7 mmoles) was reduced in 100 ml of dry Et₂O 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 $114-115^\circ$ (1.5 mm) [lit.¹⁰ $107-109^\circ$ (0.5 mm)].

N-(5-Benzylmethylaminopentyl)-5-dimethylamino-1-naphthalenesulfonamide. This compd was prep'd in 85% yield from dansyl chloride and 5-benzylmethylaminopentylamine as previously described for 1¹. The dihydrochloride had mp 175° dec (from EtOH-Et₂O). Anal. (C₂₅H₃₃N₃O₂S · 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 H₂ pressure of 3 kg/cm². After filtration and evapn, the amine (0.5 g; 63% yield) was converted to its dihydrochloride, mp $245-248^\circ$ (from EtOH-ether). Anal. (C₁₈H₂₇N₃O₂S · 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 $168-170^\circ$ dec (from EtOH-ether). Anal. (C₂₁H₃₃N₃O₂S · 3HCl): C, H, and N.

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

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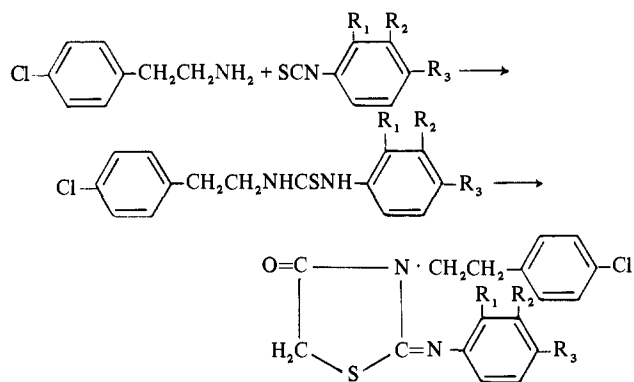
CNS depressants that have profound effects on the activity of the brain are believed to inhibit certain metabolic processes.^{1,2} Interactions with receptor surfaces³ may account for structure-activity relationships of various psychopharmacological agents. In the present study the ability of thiazolidone 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.

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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 (Et₂O, 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), ClCH₂CO₂H (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 H₂O and kept overnight at 0°. The crude product which sepd out was filtered, washed several times (H₂O), 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.

Table I. Substituted Thiocarbamides

			R ₁	R ₂	R ₃	Mp, ^a °C	Yield, %	Formula ^b
H	H	H	H	H	H	110	75	C ₁₅ H ₁₅ ClN ₂ S
H	CH ₃	H	H	H	H	140	85	C ₁₆ H ₁₇ ClN ₂ S
H	H	CH ₃	H	H	H	120	92	C ₁₆ H ₁₇ ClN ₂ S
H	CH ₃	CH ₃	H	H	H	122	89	C ₁₇ H ₁₉ ClN ₂ S
OCH ₃	H	H	H	H	H	125	64	C ₁₆ H ₁₇ ClN ₂ OS

^aMelting points were taken in open capillary tubes and are corrected graphically. ^bAll compds were analyzed for C, H, and N, and analyses were found within limits.

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	H	H	H	56	62	C ₁₇ H ₁₅ ClN ₂ OS	20	1600
H	CH ₃	H	H	H	H	86	60	C ₁₈ H ₁₇ ClN ₂ OS	30	550
H	H	CH ₃	H	H	H	52	64	C ₁₈ H ₁₇ ClN ₂ OS	40	> 1600
H	CH ₃	CH ₃	H	H	H	82	60	C ₁₉ H ₁₉ ClN ₂ OS	20	550
OCH ₃	H	H	H	H	H	104	58	C ₁₈ H ₁₇ ClN ₂ O ₂ S	20	> 1600

^{a, b}See footnotes to Table I.

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 O₂ 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 mM and 1 mM, 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.

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 R₂ and/or R₃ 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 R₁ 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 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		β -Hydroxybutyrate	Citrate	α -Keto glutarate	Succinate
R ₁	R ₂	R ₃	-NAD ⁺	+NAD ⁺				
H	H	H	54.73 \pm 0.54	42.15 \pm 0.68	53.98 \pm 0.84	53.05 \pm 0.85	65.12 \pm 0.93	Nil
H	CH ₃	H	50.04 \pm 0.67	36.15 \pm 0.90	49.42 \pm 0.78	47.93 \pm 0.84	62.18 \pm 0.61	Nil
H	H	CH ₃	47.48 \pm 0.81	41.32 \pm 0.82	46.33 \pm 0.89	50.14 \pm 0.76	58.43 \pm 0.98	Nil
H	CH ₃	CH ₃	49.78 \pm 0.92	32.21 \pm 0.84	44.10 \pm 0.67	43.87 \pm 0.78	56.82 \pm 0.90	Nil
OCH ₃	H	H	32.75 \pm 1.00	26.44 \pm 0.98	33.20 \pm 0.54	28.12 \pm 0.92	34.10 \pm 0.85	Nil

^aThe O₂ uptake was measured at 10-min intervals. The reaction mixt (in a total vol of 3 ml) contains 6.7 mM MgSO₄, 20 mM Na₂HPO₄ in a buffer sol of pH 7.4, 2 mM adenylic acid (Na salt) 33 mM KCl and 500 μ g of cytochrome *c*. The percentage inhibition was calcd from the decrease in O₂ uptake per hr per 100 mg wet wt. The final concn of substrates and 4-thiazolidones were 10 mM and 1 mM, resp. NAD⁺ was used at a final concn of 0.5 mM.

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 R₃ 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 LD₅₀ 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.

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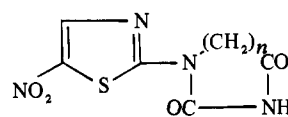
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Substituted Thiazolylureas

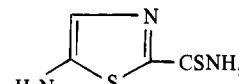
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2-Amino-5-nitrothiazole derivatives are known to exhibit antiamebic,¹ antihistomonal,² antitrichomonal,³ and antischistosomal⁴ activities. Recently the preparation of some 1-(5-nitro-2-thiazolyl)hydantoin and -hydrouracils (I) possessing antibacterial 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 hydantoin 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.

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†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 $\pm 0.4\%$ of the theoretical values.