Anticonvulsant and Antiproteolytic Properties of 3,5-Disubstituted Oxadiazole-2-thiones and Their Inhibition of Respiration in Rat Brain Homogenates

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Abstract □ Eight 5-(3,4-methylenedioxyphenyl)-3-arylaminomethyl-1,3,4-oxadiazole-2-thiones were synthesized, characterized by their sharp melting points, elemental analyses, and IR spectra, and evaluated for anticonvulsant activity. All substituted oxadiazole-2-thiones possessed anticonvulsant activity, which was reflected by their ability to provide 10–70% protection against pentylenetetrazol-induced convulsions in mice at 100 mg/kg ip. These compounds inhibited *in vitro* nicotinamide adenine dinucleotide (NAD)-dependent oxidation of pyruvate, α-ketoglutarate, and NADH by rat brain homogenates as well as NAD-independent oxidation of succinate by rat brain homogenates. Antiproteolytic activity of these substituted oxadiazole-2-thiones was reflected by their ability to inhibit trypsin hydrolysis of bovine serum albumin. These results indicated that the inhibition of cellular respiration and antiproteolytic activity of these substituted oxadiazole-2-thiones is not the biochemical basis for their anticonvulsant activity.

Keyphrases □ Oxadiazole-2-thiones, substituted—synthesized, evaluated for anticonvulsant activity in mice, effect on NAD-dependent and independent oxidations and antiproteolytic activity *in vitro* □ Anticonvulsant activity—various substituted oxadiazole-2-thiones evaluated in mice □ Oxidations, NAD dependent and independent—various substituted oxadiazole-2-thiones evaluated in rat brain homogenate □ Antiproteolytic activity—various substituted oxadiazole-2-thiones evaluated for effect on trypsin hydrolysis of bovine serum albumin □ Structure–activity relationships—various substituted oxadiazole-2-thiones synthesized, evaluated for anticonvulsant activity in mice, effect on NAD-dependent and independent oxidations and antiproteolytic activity *in vitro*

Earlier studies (1–4) suggested the anticonvulsant activity of substituted 1,3,4-oxadiazoles. Oxadiazoles also possess analgesic (5), muscle relaxant (6), tranquilizing (7), and anti-inflammatory (8) activities. The ability of oxadiazoles to inhibit trypsin hydrolysis of bovine serum albumin also was reported (2, 8).

These observations prompted the synthesis of 5-(3,4-methylenedioxyphenyl) -3- arylaminomethyl -1,3,4-oxadiazole-2-thiones by the methods outlined in Scheme I. These compounds also were evaluated for their anticonvulsant activity and their ability to inhibit cellular respiratory activity of rat brain homogenates and trypsin hydrolysis of bovine serum albumin with a view to studying their biochemical mechanism of action.

EXPERIMENTAL

Chemistry—3,4-Methylenedioxybenzoic acid (Ib) was prepared (9) by the oxidation of 3,4-methylenedioxybenzaldehyde (Ia) and was converted to its methyl ester (Ic) by refluxing with absolute methanol (10). The methyl ester, on refluxing with hydrazine hydrate (11), yielded 3,4-methylenedioxybenzohydrazide (Id), which was cyclized to the corresponding 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole-2-thione (Ie) in the presence of carbon disulfide and potassium hydroxide in ethanol (12). The various 5-(3,4-methylenedioxyphenyl)-3-arylaminomethyl-1,3,4-oxadiazole-2-thiones (II–IX, Table I) were synthesized through a Mannich reaction (13) from the thione (Ie).

Analyses for carbon, hydrogen, and nitrogen were performed; melting points were taken in open capillary tubes with a partial immersion thermometer and are corrected.

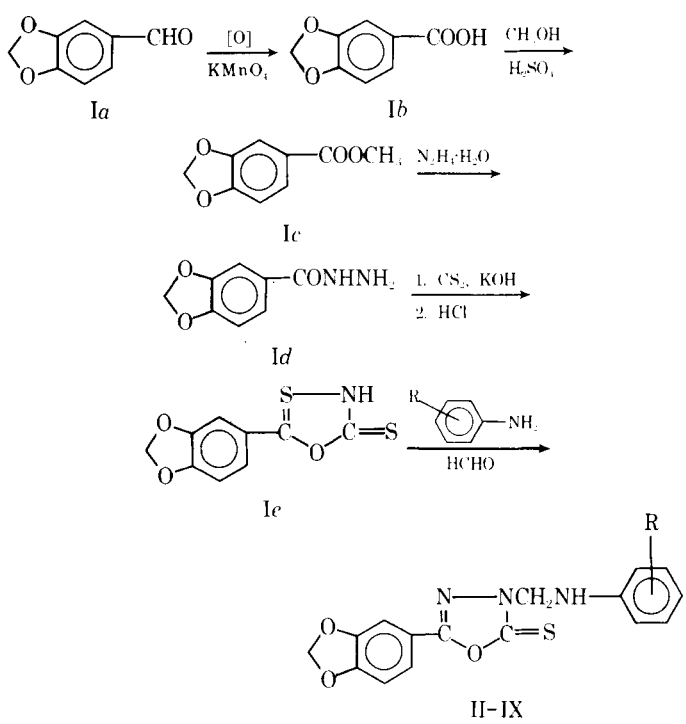
5-(3,4-Methylenedioxyphenyl)-1,3,4-oxadiazole-2-thione (Ie)—A mixture of 3,4-methylenedioxybenzohydrazide (0.2 mole), potassium hydroxide (0.2 mole), carbon disulfide (40 ml), and ethanol (200 ml) was refluxed on a steam bath until hydrogen sulfide evolution ceased. Excess ethanol was removed by distillation under reduced pressure. The residue was stirred with water, and the contents were filtered. The filtrate was acidified with dilute hydrochloric acid. The resulting compound was collected by filtration, washed with water, dried, and recrystallized with ethanol, yielding 80%, mp 238°.

Anal.—Calc. for $C_9H_6N_2O_3S$: C, 48.64; H, 2.70; N, 12.6. Found: C, 48.69; H, 2.62; N, 12.54.

5-(3,4-Methylenedioxyphenyl) -3- arylaminomethyl-1,3,4-oxadiazole-2-thiones (II–IX)—To an ethanolic solution of 5-(3,4-methylenedioxyphenyl)-1,3,4-oxadiazole-2-thione (0.01 mole) and formaldehyde (0.015 mole, 40%) was added slowly, with stirring, an ethanolic solution of the appropriate aromatic primary amine (0.01 mole). The reaction mixture was stirred for 1 hr at room temperature and left overnight in a refrigerator. The solid mass thus obtained was filtered, washed with cold ethanol, dried, and recrystallized with the appropriate solvent.

Compounds II–IX (Table I) were characterized by their sharp melting points and elemental analyses. The presence of the characteristic bands of C=S (~1090 cm^{-1}), C=N (~1600 cm^{-1}), and NH (~3300 cm^{-1}) in their IR spectra provided further support of their molecular structure.

Determination of Anticonvulsant Activity—Anticonvulsant activity was determined in albino mice, 25–30 g, of either sex. The mice were



Scheme I

0022-3549/78/1100-1507$01.00/0

Table I—Physical Constants of 5-(3,4-Methylenedioxyphenyl)-3-arylaminomethyl-1,3,4-oxadiazole-2-thiones

Compound[a]	R	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found	Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], % after 24 hr
II	H	142°	70	$C_{16}H_{13}N_3O_3S$	C 58.71	58.60	10	70
					H 3.97	4.00		
					N 12.84	12.92		
III	2-CH_3	164°	72	$C_{17}H_{15}N_3O_3S$	C 59.82	59.62	70	10
					H 4.39	4.40		
					N 12.31	12.20		
IV	3-CH_3	138°	68	$C_{17}H_{15}N_3O_3S$	C 59.82	59.94	50	30
					H 4.39	4.44		
					N 12.31	12.40		
V	4-CH_3	152°	65	$C_{17}H_{15}N_3O_3S$	C 59.82	59.65	30	30
					H 4.39	4.28		
					N 12.31	12.30		
VI	2-OCH_3	124°	53	$C_{17}H_{15}N_3O_4S$	C 57.14	57.00	70	Nil
					H 4.20	4.24		
					N 11.76	11.70		
VII	3-OCH_3	114°	62	$C_{17}H_{15}N_3O_4S$	C 57.14	57.26	30	50
					H 4.20	4.10		
					N 11.76	11.92		
VIII	4-OCH_3	106°	60	$C_{17}H_{15}N_3O_4S$	C 57.14	57.28	10	50
					H 4.20	4.20		
					N 11.76	11.70		
IX	4-Cl	198°	65	$C_{16}H_{12}ClN_3O_3S$	C 53.11	53.26	50	30
					H 3.31	3.34		
					N 11.61	11.58		

[a] These compounds were recrystallized with ethanol (II, IV, V, and IX), benzene–petroleum ether (VI and VIII), or ethanol–benzene (III). [b] The screening procedures are as indicated in the text. Administration of pentylenetetrazol (90 mg/kg sc) produced convulsions in all untreated mice and exhibited 100% mortality during 24 hr. [c] Mortality in pentylenetetrazol-treated mice was observed during the 24-hr period.

divided into groups of 10; group weights were kept as near the same as possible. Each oxadiazole-2-thione was suspended in 5% aqueous gum acacia at 1% (w/v). All test compounds were administered to a group of 10 mice at 100 mg/kg ip.

Four hours after the drug administration, the mice were injected with pentylenetetrazol (90 mg/kg sc). In the present experiments, this dose produced convulsions in all untreated mice and also exhibited 100% mortality during 24 hr (14). No mortality was observed during 24 hr in animals treated with the test compounds alone.

The mice were then observed for 60 min for seizures. An episode of clonic spasm that persisted for at least 5 sec was considered a threshold convulsion. Transient intermittent jerks and tremulousness were not counted. Animals devoid of the threshold convulsions during 60 min were considered protected. The number of animals protected in each group was recorded, and the anticonvulsant activity of these oxadiazole-2-thiones was represented by percent protection. No anticonvulsant activity was observed in animals treated with 5% aqueous gum acacia solution alone. The animals were then observed for 24 hr, and their mortality was recorded.

Assay of Cellular Respiratory Activity of Rat Brain Homogenates[1]—Albino rats, 100–150 g, were kept on an *ad libitum* diet. Rat brains isolated from decapitated animals were homogenized[2] immediately in ice-cold 0.25 *M* sucrose in a ratio of 1:9 (w/v). All incubations were conducted at 37°, and the oxygen uptake was measured by the conventional Warburg manometric technique with air as the gas phase (14).

Fresh brain homogenates equivalent to 100 mg wet weight were added to chilled Warburg vessels containing 6.7 mmoles of magnesium sulfate, 20 mmoles of dibasic sodium phosphate buffer (pH 7.4), 1 mmole of adenosine monophosphate (sodium salt), 33 mmoles of 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. Pyruvate, α-ketoglutarate, and succinate were used at a final concentration of 10 m*M*; the concentration of NADH was 0.5 m*M*. It was presumed that the endogenous NAD, present in the brain homogenates, was sufficient for these oxidative processes.

All oxadiazole-2-thiones were dissolved in propylene glycol (100%) and were incubated with rat brain homogenates at 37° for 10 min prior to the addition of different substrates. An equal volume of the solvent (0.2 ml) was added to the control vessels. The oxygen uptake was measured every 10 min for 60 min.

Assay of Proteolytic Activity of Trypsin—The antiproteolytic activity of substituted oxadiazole-2-thiones was determined by measuring their ability to inhibit trypsin hydrolysis of bovine serum albumin. The reaction mixture consisted of 0.05 *M* tromethamine buffer (pH 8.2), 0.075 mg of crystalline trypsin[3] (1 g of this crystalline trypsin preparation is capable, under certain conditions, of hydrolyzing 250 g of casein), and 0.03 m*M* bovine serum albumin[3] (substrate) in a total volume of 1 ml. The test compounds were dissolved in dimethylformamide and were used at final concentrations of 0.1, 0.5, and 1 m*M*. An equivalent amount of dimethylformamide, added to the control tubes, had no effect on the *in vitro* activity of trypsin with bovine serum albumin as the substrate.

The test compounds were incubated with trypsin for 10 min prior to the addition of bovine serum albumin (15). The reaction was stopped after 5 min by the addition of 0.5 ml of 15% (w/v) trichloroacetic acid. The acid-soluble products of protein breakdown, obtained after centrifugation in the supernatant fraction, were determined by the method of Lowry *et al.* (16). The decrease in the formation of protein breakdown products in the presence of the oxadiazole-2-thiones was used to determine their antiproteolytic activity.

RESULTS AND DISCUSSION

Table I illustrates the anticonvulsant activity of oxadiazole-2-thiones (100 mg/kg ip) against pentylenetetrazol-induced convulsions in mice. The anticonvulsant activity ranged from 10 to 70%; 5-(3,4-methylenedioxyphenyl) -3- (2-methylphenylaminomethyl)-1,3,4-oxadiazole-2-thione (III) and 5-(3,4-methylenedioxyphenyl)-3-(2-methoxyphenylaminomethyl)-1,3,4-oxadiazole-2-thione (VI) exhibited the greatest protection. 5-(3,4-Methylenedioxyphenyl)-3-phenylaminomethyl-1,3,4-oxadiazole-2-thione (II) and 5-(3,4-methylenedioxyphenyl)-3-(4-methoxyphenylaminomethyl)-1,3,4-oxadiazole-2-thione (VIII) possessed the least anticonvulsant activity. All compounds except II exhibited 50–100% protection against 24-hr pentylenetetrazol-induced mortality.

The effects of oxadiazole-2-thiones on the *in vitro* respiratory activity of rat brain homogenates are recorded in Table II. All compounds inhibited NAD-dependent oxidation of pyruvate, α-ketoglutarate, and NADH. These compounds also inhibited FAD-dependent oxidation of succinate by rat brain homogenates. The greatest inhibition of the oxidation of pyruvate and NADH was observed with III. The greatest inhibition of the oxidation of α-ketoglutarate and succinate was observed with II and VI, respectively.

The inhibitory effects of these compounds on the hydrolysis of bovine serum albumin by trypsin are recorded in Table III. All compounds

[1] Sodium pyruvate, sodium α-ketoglutarate, sodium succinate, NADH, adenosine monophosphate (sodium salt), and cytochrome c were obtained from Sigma Chemical Co., St. Louis, Mo.

[2] Potter-Elvehjem homogenizer.

[3] Sigma Chemical Co., St. Louis, Mo.

Table II—Inhibition of Respiratory Activity of Rat Brain Homogenates by 5-(3,4-Methylenedioxyphenyl)-3-arylaminomethyl-1,3,4-oxadiazole-2-thiones

Compound	Inhibition of Respiratory Activity[a], %			
	Pyruvate Oxidation	α-Ketoglutarate Oxidation	NADH Oxidation	Succinate Oxidation
II	67.3 ± 1.3	64.8 ± 1.3	51.9 ± 1.4	32.1 ± 1.0
III	78.6 ± 1.2	38.0 ± 1.2	84.0 ± 1.0	63.2 ± 1.1
IV	69.9 ± 1.4	26.4 ± 1.3	74.6 ± 1.3	17.6 ± 1.3
V	69.9 ± 1.4	33.5 ± 1.3	60.6 ± 1.4	49.1 ± 1.3
VI	76.6 ± 1.0	47.1 ± 1.3	68.1 ± 1.1	73.0 ± 1.5
VII	40.6 ± 1.1	21.4 ± 1.4	79.0 ± 1.0	55.4 ± 1.3
VIII	30.9 ± 1.3	13.1 ± 1.2	49.7 ± 1.2	16.2 ± 1.2
IX	55.9 ± 1.3	38.4 ± 1.4	66.7 ± 1.5	64.5 ± 1.4

[a] Assay procedures and the contents of the reaction mixture are as indicated in the text. Each experiment was done in duplicate. All values represent mean values of percent inhibition with ±*SEM* from three separate experiments. Inhibition was determined by the decrease in the oxygen uptake/100 mg of wet tissue weight/hr. All substituted oxadiazole-2-thiones were used at a final concentration of 2 m*M*. Different substrates and NADH were used at a final concentration of 10 and 0.5 m*M*, respectively.

possessed antiproteolytic activity, and the inhibition of trypsin activity was concentration dependent. The greatest inhibition in the activity of trypsin was observed with VI.

These results provided evidence for the changes in anticonvulsant activity of oxadiazole-2-thiones (Table I) on the introduction of a substituent in the phenyl nucleus of the arylamino moiety. In general, an increase in anticonvulsant activity was observed with the introduction of CH_3, OCH_3, or 4-Cl substituents; with VIII, however, no change in the anticonvulsant activity was observed as compared to the unsubstituted compound (II). The compounds possessing either a CH_3 (III) or OCH_3 (VI) group at position 2 of the phenyl nucleus provided the greatest protection against convulsions as compared to the unsubstituted compound.

The degree of protection against pentylenetetrazol-induced convulsions was decreased by the introduction of a CH_3 or OCH_3 substituent at position 2, 3, or 4 of the phenyl nucleus. Compounds with 4-CH_3 and 4-OCH_3 substituents showed low anticonvulsant activity. The order of the anticonvulsant activity of these compounds observed with respect to the substitution was 2-CH_3 or 2-OCH_3 > 3-CH_3 or 3-OCH_3 > 4-CH_3 or 4-OCH_3 > unsubstituted compound. The 4-Cl-substituted compound (IX) showed increased ability to provide protection as compared to the unsubstituted compound. The anticonvulsant activity of these substituted oxadiazole-2-thiones was related to their ability to provide protection against pentylenetetrazol-induced mortality during 24 hr.

Table III—Inhibition of Trypsin-Induced Hydrolysis of Bovine Serum Albumin by 5-(3,4-Methylenedioxyphenyl)-3-arylaminomethyl-1,3,4-oxadiazole-2-thiones

Compound	Inhibition[a], %		
	0.1 m*M*	0.5 m*M*	1 m*M*
II	23.2 ± 1.4	53.6 ± 1.2	62.6 ± 1.4
III	8.0 ± 0.2	15.9 ± 1.0	35.1 ± 0.6
IV	25.4 ± 1.3	50.7 ± 1.2	83.3 ± 1.7
V	32.0 ± 1.0	53.6 ± 1.2	65.2 ± 1.3
VI	40.4 ± 1.0	59.4 ± 1.7	90.5 ± 1.4
VII	8.0 ± 0.6	30.4 ± 1.3	40.5 ± 1.3
VIII	25.4 ± 1.3	33.3 ± 0.9	53.6 ± 1.4
IX	10.0 ± 1.4	13.0 ± 0.4	33.7 ± 0.7

[a] Assay procedures and the contents of the reaction mixture are as indicated in the text. Each experiment was done in triplicate. The figures indicate mean values of percent inhibition of trypsin activity during hydrolysis of bovine serum albumin with ±*SEM* calculated from three separate experiments. All substituted oxadiazole-2-thiones were used at final concentrations of 0.1, 0.5, and 1 m*M*.

Introduction of various substituents in the phenyl nucleus of the arylaminomethyl moiety of these substituted oxadiazole-2-thiones influenced their ability to inhibit respiratory activity of rat brain homogenates (Table II). The ability of these compounds to inhibit oxidation of pyruvate and NADH by rat brain homogenates corresponded well with the changes observed in their anticonvulsant activity due to substitution effects.

All substituted oxadiazole-2-thiones inhibited the activity of trypsin during hydrolysis of bovine serum albumin. These results (Table III) failed to show any definite role of substituents on antiproteolytic activity.

These results failed to provide a definite structure–activity relationship with respect to anticonvulsant activity, inhibition of cellular respiratory activity of rat brain homogenates, and antiproteolytic activity. However, determination of the activity of these substituted oxadiazole-2-thiones with other animal model systems could presumably prove useful in elucidating the relationship of molecular structure with anticonvulsant activity.

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