Anticonvulsant Activity of Substituted 4-Thiazolidones

S. P. SINGH*, THEODORE K. AUYONG‡, and SURENDRA S. PARMAR*‡ˣ

Abstract □ Several 3-(3,4-dimethoxyphenylethyl)-4-oxothiazolin-2-yl substituted hydrazones were synthesized and evaluated for their anticonvulsant activity. All thiazolidones were found to afford protection against pentylenetetrazol-induced seizures.

Keyphrases □ Hydrazones, 3-(3,4-dimethoxyphenylethyl)-4-oxothiazolin-2-yl substituted—synthesized and screened for anticonvulsant activity □ Thiazolidones—synthesized and screened for anticonvulsant activity □ Anticonvulsants, potential—synthesis and screening of 3-(3,4-dimethoxyphenylethyl)-4-oxothiazolin-2-yl substituted hydrazones

Diverse biological properties have been shown to be associated with substituted thiazolidones. These include anticonvulsant (1, 2), hypnotic (3), and local anesthetic (4) activities. Recent observations of the depression of the central nervous system (CNS) by thiazolidones, as evidenced by reduced locomotor activity, ataxia, hindlimb weakness, and loss of the righting reflex (5), led to the present synthesis of 3-(3,4-dimethoxyphenylethyl)-4-oxothiazolin-2-yl substituted hydrazones and evaluation of their anticonvulsant activity.

EXPERIMENTAL

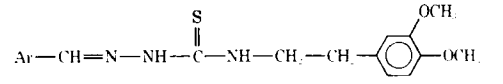
4-(3,4-Dimethoxyphenylethyl)-3-thiosemicarbazide—To an ethanolic solution of 3,4-dimethoxyphenylethylamine (0.1 mole) was slowly added 16.5 ml of concentrated ammonia solution (sp. gr. 0.91). After the mixture was cooled below 30°, carbon disulfide (7.5 ml) was added dropwise over 20 min and the mixture was allowed to stand at room temperature for 1 hr. To this mixture was added an aqueous solution of the sodium salt of monochloroacetic acid (0.1 mole). This resulted in an exothermic reaction, and hydrazine hydrate (0.1 mole, 80%) was then added to the warm solution. The mixture was cooled overnight and the crude thiosemicarbazide which separated out was filtered and recrystallized from ethanol, mp 140°, yielding 80%.

Anal.—Calc. for $C_{11}H_{17}N_3O_2S$: C, 51.76; H, 6.67; N, 16.86. Found: C, 51.24; H, 6.38; N, 16.66.

4-(3,4-Dimethoxyphenylethyl) 1-Substituted 3-Thiosemicarbazones—An ethanolic solution of 4-(3,4-dimethoxyphenylethyl)-3-thiosemicarbazide (0.01 mole) was added to a boiling solution of the suitable aldehyde (0.01 mole) in ethanol. The mixture was refluxed for 2 hr on a steam bath, and the reaction mixture was concentrated under reduced pressure. The solid mass which separated out on cooling was recrystallized from ethanol. All compounds were characterized by their sharp melting points and elemental analyses (Table I).

3-(3,4-Dimethoxyphenylethyl)-4-oxothiazolin-2-yl Substituted Hydrazones—4-(3,4-Dimethoxyphenylethyl) 1-substituted 3-thiosemicarbazone (0.005 mole), monochloroacetic acid (0.005

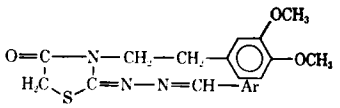
Table I—Physical Constants of 4-(3,4-Dimethoxyphenylethyl) 1-Substituted 3-Thiosemicarbazones



Compound Number	Ar	Melting Point[a]	Yield, %	Formula	Analysis, %	
					Calc.	Found
I	C_6H_5	125°	80	$C_{18}H_{21}N_3O_2S$	C 62.97 H 6.12 N 12.24	62.88 6.08 12.14
II	2-Cl—C_6H_4	167°	55	$C_{18}H_{20}ClN_3O_2S$	C 57.22 H 5.29 N 11.13	57.00 5.24 11.24
III	4-Cl—C_6H_4	135°	90	$C_{18}H_{20}ClN_3O_2S$	C 57.22 H 5.29 N 11.13	57.22 5.26 11.00
IV	3-Cl, 4-Cl—C_6H_3	198°	84	$C_{18}H_{19}Cl_2N_3O_2S$	C 52.43 H 4.61 N 10.14	52.28 4.58 10.17
V	2-CH_3—C_6H_4	115°	60	$C_{19}H_{23}N_3O_2S$	C 63.86 H 6.44 N 11.76	63.72 6.32 11.56
VI	4-OCH_3—C_6H_4	110°	88	$C_{19}H_{23}N_3O_3S$	C 61.13 H 6.17 N 11.26	61.12 6.15 11.24
VII	3-OCH_3, 4-OCH_3—C_6H_3	115°	78	$C_{20}H_{25}N_3O_4S$	C 59.55 H 6.23 N 10.42	59.34 6.00 10.34
VIII	3-OCH_3, 4-OC_2H_5—C_6H_3	122°	75	$C_{21}H_{27}N_3O_4S$	C 60.43 H 6.47 N 10.07	60.13 6.31 10.00
IX	4-$(CH_3)_2N$—C_6H_4	124°	82	$C_{20}H_{26}N_4O_2S$	C 62.18 H 6.74 N 14.50	61.88 6.55 14.37
X	C_6H_5—CH=CH	140°	68	$C_{20}H_{23}N_3O_2S$	C 65.04 H 6.23 N 11.38	64.98 6.18 11.29

[a] Melting points were taken in open capillary tubes and are uncorrected.

Table II—Physical Constants of 3-(3,4-Dimethoxyphenylethyl)-4-oxothiazoline-2-yl Substituted Hydrazones and Their Anticonvulsant Activity



Compound Number	Ar	Melting Point[a]	Yield, %	Formula	Analysis, %		Anticonvulsant Activity[b], % Protection	Pentylenetetrazol Mortality[c], %
					Calc.	Found		
XI	C_6H_5	130°	60	$C_{20}H_{21}N_3O_3S$	C 62.66 H 5.48 N 10.97	62.32 5.50 10.86	60	30
XII	2-Cl—C_6H_4	143°	45	$C_{20}H_{20}ClN_3O_3S$	C 57.48 H 4.79 N 10.60	57.21 4.63 10.78	40	50
XIII	4-Cl—C_6H_4	165°	75	$C_{20}H_{20}ClN_3O_3S$	C 57.48 H 4.79 N 10.60	57.42 4.70 10.58	60	30
XIV	3-Cl, 4-Cl—C_6H_3	185°	66	$C_{20}H_{19}Cl_2N_3O_3S$	C 53.09 H 4.20 N 9.29	52.92 4.00 9.24	30	40
XV	2-CH_3—C_6H_4	132°	48	$C_{21}H_{23}N_3O_3S$	C 63.48 H 5.79 N 10.58	63.42 5.58 10.36	60	40
XVI	4-OCH_3—C_6H_4	145°	70	$C_{21}H_{23}N_3O_4S$	C 61.02 H 5.57 N 10.17	61.00 5.44 10.22	50	30
XVII	3-OCH_3, 4-OCH_3—C_6H_3	150°	68	$C_{22}H_{25}N_3O_5S$	C 59.59 H 5.64 N 9.48	59.49 5.66 9.38	60	40
XVIII	3-OCH_3, 4-OC_2H_5—C_6H_3	138°	66	$C_{23}H_{27}N_3O_5S$	C 60.39 H 5.90 N 9.19	60.11 5.82 9.12	20	50
XIX	4-$(CH_3)_2$—N—C_6H_4	166°	72	$C_{22}H_{26}N_4O_3S$	C 61.97 H 6.10 N 13.15	61.87 6.00 13.12	10	80
XX	CH=CH—C_6H_5	144°	62	$C_{22}H_{23}N_3O_3S$	C 64.55 H 5.62 N 10.27	64.33 5.48 10.25	70	20

[a] Melting points were taken in open capillary tubes and are uncorrected. [b] Anticonvulsant activity was determined at a dose of 100 mg/kg as described under *Experimental*. [c] Represents mortality during 24 hr in each group of animals administered pentylenetetrazol.

mole), and fused sodium acetate (0.0075 mole) were mixed in 15 ml of acetic acid, and the mixture was refluxed for 6 hr. The reaction mixture was poured over ice-cold water and refrigerated overnight. The solid mass which separated out was filtered, washed with water, and recrystallized from acetic acid. All compounds (Table II) were characterized by their sharp melting points and elemental analyses.

Determination of Anticonvulsant Activity—Anticonvulsant activity against pentylenetetrazol-induced seizures was determined in mice of either sex weighing 25–30 g. The mice were divided into groups of 10, with weights being kept nearly the same as possible. Various 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 animals at a dose of 100 mg/kg ip. Four hours after the administration of these compounds, the mice were injected with pentylenetetrazol (90 mg/kg sc). This dose of pentylenetetrazol had been shown not only to produce convulsions in almost all untreated mice but also to exhibit 100% mortality during the 24-hr period. On the other hand, no mortality was observed during 24 hr in animals treated with 100 mg/kg of the test compounds alone. The mice were then observed for seizures for 60 min. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion. Transient intermittent jerks or tremulousness was not counted. Animals not exhibiting threshold convulsions during 60 min were considered protected. The number of animals protected in each group was noted, and the anticonvulsant activity of these thiazolidones was represented as percent protection. The animals were then observed for 24 hr and their mortality was recorded.

RESULTS AND DISCUSSION

The anticonvulsant activity exhibited by these thiazolidones at a dose of 100 mg/kg against pentylenetetrazol-induced seizures and the mortality due to the toxic effects of pentylenetrazol during 24 hr are shown in Table II. All 3-(3,4-dimethoxyphenylethyl)-4-oxothiazolin-2-yl substituted hydrazones afforded protection ranging from 10 to 70%. Maximum protection was observed with Compound XX; Compound XIX exhibited the lowest anticonvulsant activity. There appears to be some connection in these data but no uniform trend toward a correlation between the percent protection against pentylenetetrazol-induced seizures provided by these thiazolidones and their percent protection against pentylenetetrazol mortality (*e.g.*, Table II: 60% protection = 30 or 40% mortality, 50% protection = 30% mortality, and 30% protection = 40% mortality). These results have failed to supply evidence for a specific structural requirement for anticonvulsant activity in the molecular makeup of these thiazolidones.

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Forks, ND 58201

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[x] To whom inquiries should be directed.

Cannabis sativa L. (Marijuana) V: Pharmacological Evaluation of Marijuana Aqueous Extract and Volatile Oil

ALVIN B. SEGELMAN*[x], R. DUANE SOFIA‡, FLORENCE P. SEGELMAN*, JOHN J. HARAKAL‡, and LINDA C. KNOBLOCH‡

Abstract □ The aqueous extract (marijuana tea) and volatile oil prepared from marijuana were compared with (−)-*trans*-Δ^9-tetrahydrocannabinol for their effect on hexobarbital sleeping time and analgesic action in mice. All three substances prolonged hexobarbital sleeping time with an order of potency of (−)-*trans*-Δ^9-tetrahydrocannabinol > aqueous extract > volatile oil. Each agent produced significant analgesic activity. However, the potencies of the aqueous extract and the volatile oil were similar to each other but only 1/200 that of (−)-*trans*-Δ^9-tetrahydrocannabinol.

Keyphrases □ *Cannabis sativa* L.—pharmacological evaluation of marijuana aqueous extract and volatile oil, hexobarbital sleeping time and analgesia, mice □ Marijuana—pharmacological evaluation of aqueous extract and volatile oil, hexobarbital sleeping time and analgesia, mice

A recent report (1) described the preparation of marijuana[1] tea, a simple aqueous extract that was obtained by continuously heating *Cannabis* plant material in water under reflux for 6 hr. In addition, a volatile oil was removed and retained. Since the use of marijuana tea has been stated to prolong and intensify significantly the effects resulting from smoking marijuana (1), the effects of marijuana tea were compared to (−)-*trans*-Δ^9-tetrahydrocannabinol (I), the major psychoactive constituent of marijuana (2, 3). Two pharmacological parameters known to be affected by I (4, 5) were used, *i.e.*, hexobarbital sleeping time and alteration of painful stimuli. Moreover, since no pharmacological data have been reported for the volatile oil, it was also included.

EXPERIMENTAL

Test Animals—Male, albino mice[2] (CD-1 strain) weighing 20–28 g at the time of testing were used. The animals, housed in groups of eight each, were allowed at least 5 days of acclimation to laboratory housing conditions of 12 hr of light and 12 hr of dark at a temperature of 21–23°, with food and water continuously available up until the time of testing.

[1] The term marijuana as used here refers generally to the crushed and broken tops, including leaves and small stems, derived from flowering or nonflowering *Cannabis sativa* L. plants of either sex.

[2] Charles River Breeding Laboratories, Wilmington, Mass.

Table I—Effects of I, III, and IV on Hexobarbital Sleeping Time in Mice

Test Drug	Dose, mg/kg ip	Mice	Sleeping Time, Minutes (Mean ± *SE*)	Increase, %
Propylene glycol vehicle	—	16	49 ± 4	—
I	5.0	8	49 ± 2	0
	10.0	8	100 ± 7[a]	104
	20.0	8	105 ± 12[a]	114
IV	40.0	8	53 ± 5	8
	80.0	8	75 ± 6[a]	53
	160.0	8	87 ± 6[a]	78
	320.0	8	140 ± 11[a]	186
Distilled water vehicle	—	16	41 ± 2	—
III	12.5	8	44 ± 4	7
	25.0	8	58 ± 5[a]	41
	50.0	8	59 ± 3[a]	44
	100.0	8	81 ± 8[a]	98

[a] When compared with its respective vehicle-treated control group, $p \leq 0.05$.

Preparation of Aqueous Extract from Marijuana Tea—Marijuana tea was prepared from 100 g of marijuana[3] and lyophilized as described previously (1) to give 30 g of aqueous extract (II). Then II was purified by stirring with petroleum ether (bp 30–60°) to remove trace amounts of I and other petroleum ether-soluble fast blue B reactive substances, which were detected by TLC (6). The petroleum ether-extracted II was dried *in vacuo* at 40° to give a cannabinoid-free aqueous extract (III)[4].

Preparation of Volatile Oil—Marijuana plant material[3] was subjected to direct steam distillation. The water-insoluble volatile constituents were recovered from the aqueous distillate using an oil separator and dried (anhydrous sodium sulfate) to give a pale yellow aromatic oil, yielding 0.18% (w/w) (n_D^{20} 1.494 and d_{20}^{20} 0.8788) (IV). This IV was found to contain no detectable amounts of cannabinoids[5] as indicated by TLC (6).

Dosage Forms—All doses of I[6], III, and IV were prepared in a concentration to permit a constant volume of 0.1 ml/10 g body

[3] Female plant material representing a Mexican strain of *C. sativa* (1).

[4] Extract III was used for all pharmacological studies, although preliminary pharmacological experiments showed no significant differences in results when II and III were compared.

[5] The volatile oil obtained by water distillation during the preparation of marijuana tea (1) was shown by TLC (6) to contain significant amounts of I and thus was not used in the present pharmacological study.

[6] Obtained from the National Institute of Mental Health, Rockville, Md.