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Evaluation of semicarbazones for anticonvulsant and sedative-hypnotic properties

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A series of semicarbazones and thiosemicarbazones were synthesized and evaluated for anti-convulsant activity. Some compounds provided significant protection against Maximal Electroshock (MES) and subcutaneous strychnine induced seizures. Compound **1** was the most active in the series with activity in a dose of 30 mg/kg in the strychnine seizure pattern test and an ED50 of 10 mg/kg in the MES test. Hence it could serve as a prototype molecule for future development. Also compounds with a *p*-nitrophenyl substitution in place of the amino hydrogen of semicarbazone moiety showed activity in a dose of 30 mg/kg and an ED50 of 83 mg/kg in the MES test.

1. Introduction

Studies have shown that 1% of the population suffers from some kind of epilepsy and that 25% of them have seizures that are resistant to available medical therapies [1]. Furthermore, the anticonvulsant drugs presently used in clinical practice show a broad range of adverse effects. Consequently there is a need for antiepileptic substances having greater specificity and fewer side effects.

In the past, semicarbazones have been shown to possess excellent anticonvulsant activity [2–4]. It has been proposed that for activity in the MES test, a compound should have a large hydrophobic group in close proximity to at least two electron donor atoms [5].

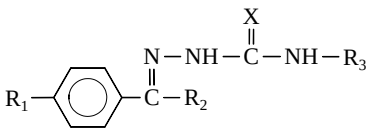
In view of these requirements for activity a number of aryl semicarbazones and thiosemicarbazones were prepared (Table 1). These semicarbazones containing a hydrophobic moiety (aryl ring) as well as two electron donor atoms in the semicarbazone group, have been shown to possess activity in the MES as well as in the subcutaneous pentylenetetrazole (scPTZ) screen. The aryl semicarbazones displaying activity interact with a specific binding site. The semicarbazono group and the aryl ring align, on a macromolecular complex, *in vivo*. These areas have been referred to as the hydrogen bonding area and the aryl binding site respectively. A third area has been proposed which gives an auxiliary binding area [6].

The principal aim of the present study was to investigate and optimize the structural requirements for different binding areas. With this aim, different substituents, in particular electron withdrawing groups (NO_2) were placed on the aryl ring, the amino hydrogen of the semicarbazono group was replaced by a 4-nitrophenyl group.

2. Investigations, results and discussion

The compounds were screened at 10, 30, 100 and 300 mg/kg in the MES test. The data reported in Table 2 revealed that 87% of the compounds synthesised afforded protection in the MES test, while 42% of these derivatives were active in the strychnine seizure pattern test. Hence the compounds displayed some MES selectivity. Compounds **1**, **6**,

Table 1: Physical properties of semicarbazones and thiosemicarbazones



Compd.	R_1	R_2	X	R_3	Mol. formula	M.p. (°C)	R_f	Part. coeff.
1	H	CH_3	O	H	$C_9H_{11}N_3O$	215	0.426	2.66
2	-3-NH_2	CH_3	O	H	$C_9H_{12}N_4O$	203	0.554	1.85
3	-4-NH_2	CH_3	O	H	$C_9H_{12}N_4O$	276	0.624	2.44
4	-4-Cl	CH_3	O	H	$C_9H_{10}N_3OCl$	205	0.400	1.85
5	-2-OH	H	O	H	$C_8H_9N_3O_2$	225	0.620	0.72
6	-2-NO_2	H	O	H	$C_8H_8N_4O_3$	256	0.562	1.65
7	-3-OCH_3 -4-OCH_3	H	O	H	$C_{10}H_{13}N_3O_3$	176	0.428	1.92
8	-4-OH -3-OCH_3	H	O	H	$C_9H_{11}N_3O_3$	239	0.525	1.12
9	-4-$N(CH_3)_2$	H	O	H	$C_{10}H_{14}N_4O$	220	0.794	1.94
10	H	CH_3	S	H	$C_9H_{11}N_3S$	91	0.750	1.85
11	-3-NH_2	CH_3	S	H	$C_9H_{12}N_4S$	110	0.746	2.03
12	-4-NH_2	CH_3	S	H	$C_9H_{12}N_4S$	180	0.724	2.27
13	-4-Cl	CH_3	S	H	$C_9H_{10}N_3SCl$	86	0.833	2.10
14	-2-OH	H	S	H	$C_8H_9N_3O$	231	0.800	0.78
15	H	H	O	p-NO_2-C_6H_4	$C_{14}H_{12}N_4O_3$	115	0.792	2.78
16	H	CH_3	O	p-NO_2-C_6H_4	$C_{15}H_{14}N_4O_3$	140	0.742	2.54

Partition-coefficient in chloroform/phosphate buffer (pH – 7.4)

Table 2: Anticonvulsant activity of semicarbazones and thiosemicarbazones

Compd.	Minimum active dose (mg/kg)					
	MES test			Strychnine test	Picrotoxin test	Neurotoxicity test
	0.5 h	4.0 h	ED_{50} (0.5 h)			
1	10	30	10.0	30	100	100
2	100	100	152.0	>300	–	100
3	10	>300	15.0	–	–	300
4	100	100	83.0	>300	100	30
5	>300	100	73.0	>300	–	–
6	300	300	200.0	300	–	>300
7	>300	>300	–	–	–	–
8	100	100	83.0	>300	–	30
9	100	300	15.0	100	–	30
10	10	30	–	60	–	100
11	300	300	–	300	–	100
12	100	>300	15.0	–	–	30
13	100	100	200.0	100	–	–
14	>300	>300	65.0	>300	–	100
15	300	30	83.0	100	–	300
16	100	100	–	>300	–	>300
Phenytoin	5	–	6.32	–	–	>300

(–) indicates: no data

9, **10**, **13**, and **15** were active in both the tests. Compound **1** was the most active, at the dose of 100 mg/kg in the MES test. Compound **5** showed activity at a dose of 100 mg/kg whereas compound **15** showed activity a 300 mg/kg.

In general, anticonvulsant activity was noted at the end of 30 min rather than 4 h i.e. the onset of action was rapid. Only compounds **5** and **14** showed activity at the end of 4 h while showing no activity at the end of 30 min. Thus in the case of compound **5** onset of action appears to be delayed which might be due to the low lipophilicity of the compound (partition coefficient 0.72) and in case of **15**, it is due to delayed metabolism.

Comparison between the semicarbazones **1**, **2**, **3**, **4** and **5** and the corresponding thiosemicarbazones **10**, **11**, **12**, **13** and **14** revealed that the semicarbazones were active at lower doses as compared to the corresponding thiosemicarbazones. In addition, the semicarbazones showed neurotoxicity at higher doses compared to the corresponding thiosemicarbazones (high protective index). Hence future development should utilize the semicarbazone pharmacophore.

Replacement of the amino hydrogen with *p*-nitrophenyl (compounds **15** and **16**) resulted in compounds with excellent activity. This is in sharp contrast to compounds synthesized earlier.

The activity of some compounds in the strychnine test showed that the semicarbazones can act through inhibitory glycine receptors. The protection offered in the picrotoxin test suggests that they may act on GABA receptors associated with chloride channels.

Of the 12 compounds tested for sedative hypnotic activity, **4**, **9**, **10**, **12**, **13**, **15** and **16** were found to potentiate the activity of pentobarbitone sodium (Table 3).

It appears that thiosemicarbazones have a higher sedative-hypnotic potential than the corresponding semicarbazones. Compounds with a CH_3-substituent near the semicarbazone moiety have a higher sedative-hypnotic potential than the compounds with a H-substitution.

3. Experimental

3.1 Chemistry

The m.p.'s are uncorrected. The purity of the compounds was confirmed by TLC using Silica Gel as stationary phase and chloroform-methanol (5 : 5) as solvent system. NMR spectra were recorded on a Jeol FX90Q, Fourier Transform spectrometer and IR spectra were recorded on a Jasco IR report 100 in KBr discs. The partition coefficients were determined using a chloroform-phosphate buffer system (pH 7.4).

3.1.1. Synthesis of semicarbazones

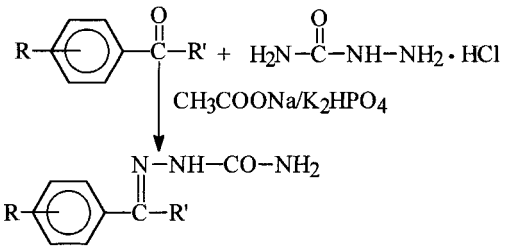
A solution of semicarbazide hydrochloride (0.01 mol), sodium acetate (0.01 mol) or dibasic potassium phosphate (0.01 mol) and water (20 ml)

Table 3: Test: Sedative-hypnotic activity

Compd.	Meantime taken (min)		Mean sleeping time (min)	Statistical significance*
	Loss of righting reflex	Gain of righting reflex		
Control	7.5 ± 3.24	154 ± 4.10	146.5 ± 5.11	insignificant
1	8.0 ± 3.13	150 ± 3.54	142 ± 6.23	significant
3	11 ± 2.35	132 ± 3.92	121 ± 4.21	significant
4	3.5 ± 1.90	182.5 ± 4.75	179 ± 4.69	insignificant
6	9 ± 2.73	160 ± 3.39	151 ± 4.31	significant
8	8 ± 2.54	127 ± 5.21	119 ± 5.29	significant
9	3 ± 3.25	178.5 ± 4.82	175.5 ± 5.35	significant
12	5 ± 3.31	217.5 ± 5.37	212.5 ± 4.61	insignificant
13	9 ± 4.20	154 ± 4.37	145 ± 5.41	significant
14	7 ± 3.39	198.5 ± 4.12	191.5 ± 3.97	significant
15	6.5 ± 4.29	194 ± 3.73	197.5 ± 5.10	significant

* Significant indicates significant difference in mean sleeping time with respect to control. Student's t-test was performed and statistical significance was observed at 95% confidence limits. n = 6, p = 0.5

Scheme 1

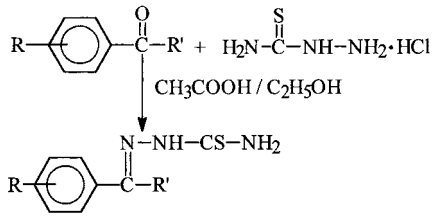


R = 3 NH_2, 4 NH_2, 4 Cl, 2-OH, 2-NO_2, 4-N $(CH_3)_2$ etc.
R′ = H, CH_3

was added slowly to a stirring solution of the appropriate carbonyl compound (0.01 mol) in ethanol (95%, 5 ml). The reaction mixture was stirred at room temperature for a time ranging from a few minutes to 2 h. The precipitate formed was collected, washed with water and dried. Recrystallization from ethanol (95%) gave the semicarbazone (Scheme 1).

3.1.2. Synthesis of thiosemicarbazones

Scheme 2



R = 3 NH_2, 4 NH_2, 4 Cl, 2-OH, 2-NO_2, etc.
R′ = H, CH_3

A solution of thiosemicarbazide (0.01 mol) in ethanol (10 ml) was added slowly to a stirred solution of the appropriate carbonyl compound (0.01 mol) in ethanol (15 ml) containing acetic acid (2 ml). The reaction mixture was stirred at room temperature for a time ranging from a few minutes to several hours. On cooling, the precipitate was collected, washed with water and dried. Recrystallization from ethanol gave thiosemicarbazone.

3.1.3. Synthesis of substituted semicarbazones

A solution of sodium cyanate (0.1 mol) in warm water (50 ml) was added to a stirred solution of *p*-nitroaniline (0.01 mol) in glacial acetic acid (10 ml). The precipitate obtained after cooling was washed with water, dried and recrystallized from boiling water to give *p*-nitrophenyl urea. This, on refluxing for 1.5 h with an equimolar quantity of hydrazine hydrate, NaOH (2 g) and EtOH (5 ml) gives 4-(4′-nitrophenyl) semicarbazide. To a solution of the substituted semicarbazide (0.01 mol) in water, was added an ethanolic solution of the equimolar quantity of a carbonyl compound. The pH of the reaction mixture was adjusted between 5–6 by adding glacial acetic acid. The reaction mixture was refluxed for a few minutes. The product obtained after cooling was filtered and recrystallized from ethanol.

3.2. Anticonvulsant screening

3.2.1. Electro shock method

Maximal seizures were introduced by application of a electrical current across the brain via corneal electrodes. The stimuli of parameters for rats were 150 mA, AC in a pulse of 60 Hz for 200 ms (0.2 s). The rats were previously administered with the test drug solution in polyethylene glycol. The abolition of hind limb tonic extensor spasm was recorded as a measure of anticonvulsant activity [7].

3.2.2. Chemo shock method

3.2.2.1. Strychnine seizure pattern test

Animals of the control group received just vehicle (polyethylene glycol). The other groups were administered experimental drug solution (i.p.). After 1 h all the animals of both groups were injected subcutaneously with strychnine (40 mg/kg) and observed for 45 min. The dose at which hindleg tonic extensor component abolished was noted.

3.2.2.2. Picrotoxin seizure

The test was performed as described above. The seizures were produced by injecting picrotoxin (s.c.) (3.15 mg/kg). Abolition of hind limb tonic seizures was taken as end point. Test was performed only for compounds **1** and **4** in a dose of 100 mg/kg.

3.3. Neurotoxicity test: rotorod test

The mice were trained to stay on an accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were given test compounds intraperitoneally in doses of 30, 100 and 300 mg/kg. After 30 min the mice were placed on the rotorod to measure the drug effects on motor performance [8]. The dose at which animals fell off the rotorod was determined.

3.4. Sedative-hypnotic activity

This test [9] was performed using the test substance in a dose of 100 mg/kg only. The drug was administered to a group of 6 animals. 30 min after drug administration, animals were injected with a solution of pentobarbitone sodium (in saline), in a dose of 30 mg/kg. The animals were then placed on their back and loss of righting reflex was taken as onset of sleep. The time taken by the animals to awake was noted. A control was also performed after pretreatment with test substance vehicle (polyethylene glycol).

3.5. NMR spectral study

Compound **1**: δ 1.6 (t, 6 H, of cyclohexane ring), 2.3 (t, 4 H, at ortho position of cyclohexane ring), 5.85 (s, 2 H, $CONH_2$), 8.8 (s, 1 H, NH).
Compound **4**: δ 1.8 (t, 3 H, CH_3), 5.90 (s, 2 H, $CONH_2$), 7.40 (d, 2 H, meta to aromatic chloro group), 7.90 (d, 2 H, ortho to aromatic chloro group), 8.60 (s, 1 H, NH).
Compound **12**: δ 2.2 (t, 3 H, CH_3), 3.90 (s, 2 H, $ArNH_2$), 6.30 (s, 2 H, $CSNH_2$), 6.70 (d, 2 H, meta to aromatic amino group), 7.60 (d, 2 H, ortho to aromatic amino group), 8.80 (s, 1 H, NH).

3.6 IR spectral study

Compound **1**: A chraracteristic IR band at 3470 cm^{-1} indicates a N–H stretch. A C=O stretch appears at 1740 cm^{-1}. C=N stretch and Ar–H band appear at 1610 cm^{-1} and 750 cm^{-1}, respectively.
Compound **4**: A characteristic IR band at 3450 cm^{-1} indicates a N–H stretch and a C=O stretch appears at 1690 cm^{-1}. C=N stretch and Ar-H band appears at 1610 cm^{-1} and 750 cm^{-1}, respectively.
Compound **12**: A characteristic IR band at 1610 cm^{-1} and 1190 cm^{-1} indicates a C=N stretch and a C=S stretch, respectively.
Compound **15**: A characteristic IR band at 1562 cm^{-1} indicates a asymmetric $ArNO_2$ stretch and the peak at 840 cm^{-1} indicates a C–N stretch for $ArNO_2$.

References

1 Saxena, A. K.; Saxena, M.: Developments in anticonvulsants. Progr. Drug Res. **44**, 185 (1995)
2 Tripathi, S.; Pandey, B. R.; Ramank; Barthwal, J. P.; Kishor, K.; Bhargava, K. P.: Res. Commun. Chem. Pathol Pharmacol. **22** (2), 291 (1998)
3 Dimmock, J. R.; McColl, J. M.; Wonko, S. L.; Thayer, R. S.; Hancock, D. S.: Pharmazie **46**, 529 (1991)
4 Dimmock, J. R.; Pandeya, S. N.; Quail, J. W.; Pugazhethi, U.; Allen, T. M.; Kao, G. Y.; Balzarini, J.; De Clercq, E.: Eur. J. Med. Chem. **30**, 303 (1995)
5 Jones, G. L.; Woodbury, D. M.: Drug Dev. Res. **2**, 333 (1987)
6 Dimmock, J. R.; Puthucode, N. R.; Smith, M. J.; Hetherington, M.; Quail, J. W.; Pugazhenthi, U.; Lechler, T.; Stables, J. P.: J. Med. Chem. **39**, 3984 (1996)
7 Krall, R. L., J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A.: Epilepsia **19**, 409 (1978)
8 Meza-Toleddo, S. E.; Zenteno-Gracia, M. T.; Juarez-Carvajal, E.; Martinez-Munoz, D.; Carvajal-Sandoval, G.: Arzneim-Forsch/Drug Res. **40**, 1289 (1990)
9 Srivastava, K.; Pandeya, S. N.: Bioorg. Med. Chem. Letters **3**, 547 (1993)

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