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Evaluation of p-nitrophenyl substituted semicarbazones for anticonvulsant properties

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A series of *p*-nitrophenyl substituted semicarbazones has been synthesised and their anticonvulsant activity was screened against MES, scPTZ and scSTY. 4(4′-Nitrophenyl)-*o*-nitrobenzaldehyde semicarbazone has been found to be the most active in all these tests. The studies revealed that a primary amino function is not essential for anticonvulsant activity in the semicarbazone series of compounds. Presumably these compounds could further act on glycine receptors.

1. Introduction

Epilepsy is a common neurological disorder. Different types of seizures may have different neurological bases and are to be controlled with different medications. In this regard, hydantoins, benzodiazepines and more recently remacemide have been used. For the last few years semicarbazones have been investigated as novel anticonvulsants [1, 2]. These compounds have been evaluated for their anticonvulsant properties using two screens namely the maximal electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) test. These compounds have been shown greater protection in the MES test than in the scPTZ screen. Further these compounds were inactive in the scPTZ screen after oral administration to rats.

Recently a theory has been promulgated, thereby, for MES activity the aryl ring and semicarbazono group interact at two locations on a receptor. These two locations are called the aryl binding site and the hydrogen bonding area. The terminal $-CONH_2$ group of the semicarbazono group is implicated in the hydrogen bonding process. Recently in our laboratory certain semicarbazones were evaluated for anticonvulsant and sedative hypnotic properties. In this study the terminal amino group of the semicarbazone was replaced by a *p*-NO_2-phenyl group. This was done in order to confirm whether this primary amino group is essential or not for anticonvulsant properties. The results obtained by a *p*-NO_2-phenyl substitution in place of the amino hydrogen of the semicarbazone moiety showed activity in a dose of 30 mg/kg and ED_{50} of 83 mg/kg in the MES test [3]. In a similar study Dimmock et al. revealed that the primary amino group of anticonvulsant arylsemicarbazones was not essential for their anticonvulsant activity. The present study was undertaken to further confirm the role of this primary amino group of aryl semicarbazones in hydrogen bonding at the receptor site. Thus, a series of *p*-NO_2-phenyl substituted derivatives at the terminal amino group of arylsemicarbazones have been prepared according to the Scheme. The anticonvulsant activity of these compounds has been screened against MES, scPTZ and scSTY (subcutaneous strychnine test).

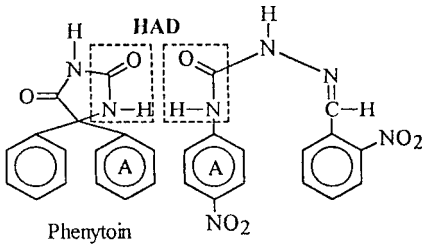
2. Investigations, results and discussion

The compounds were screened at 30, 100 and 300 mg/kg in the MES, scPTZ and scSTY test. The data reported in Table 1 revealed that 70% of the compounds afforded protection in the MES test, 46% of these derivatives were active in the subcutaneous pentylenetetrazole test and 80% of these derivatives were active in the strychnine seizure pattern test. The compounds **7**, **10** and **13** were active only

Table 1: Compounds displaying activity in the maximal electroshock (MES), subcutaneous pentylenetetrazole and neurotoxicity (NT) screens after intraperitoneal injection in mice

Compd.	Intraperitoneal injection in mice[a]						
	MES Screen		scPTZ	scSTY Screen		NT Screen	
	0.5 h	4.25 h	0.5 h	0.5 h	4 h	0.5 h	4 h
1	100	–	300	30	30	300	–
2	–	–	–	–	–	300	300
3	–	–	–	300	300	300	–
4	300	–	300	100	100	300	–
5	–	–	–	100	100	300	–
6	–	–	–	–	–	30	30
7	300	100	–	30	30	300	–
8	300	100	300	300	300	300	–
9	300	–	300	100	100	300	300
10	300	–	300	NOT	–	300	–
11	100 (33%) 300	–	–	NOT	–	300	300
12	300	–	–	NOT	–	300	100
13	300	–	300	NOT	–	–	–
Phenytoin	30	–	–	NOT	–	100	100
Carbamazipine	30	–	100	NOT	–	100	300

[a] Doses of 30, 100 and 300 mg/kg of each compound were administered. The figures in the Table reveal the lowest dose at which bioactivity was demonstrated in 50% or more of the animals. The lines denote an absence of anticonvulsant activity (or) neurotoxicity. NOT denotes not tested. Only compound **12** showed protection after 4 h in the MES test



2-Nitro benzylidine p-NO_2 Phenyl semicarbazone
HAD: Hydrogen bond acceptor/donar unit

in the scPTZ and the MES test. Compounds **3** and **5** were active only in strychnine seizure pattern test. The compounds **11** and **12** were active only in MES test. The compounds **10** and **11** were active at 100 mg/kg in the MES test. The compounds **1** and **7** were active at 30 mg/kg in the strychnine test.

In general, anticonvulsant activity was noted after 30 min rather than 4 h i.e. the onset of action was rapid. The compounds **7** and **8** showed activity after 4 h and 15 min. Thus in the case of compounds **7** and **8** the onset of action appears to be delayed.

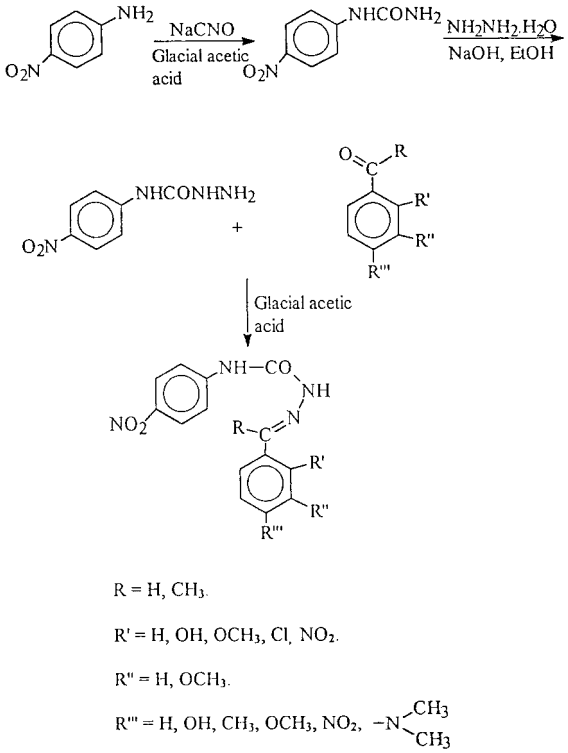
The activity by most of the compounds in the strychnine test shows that semicarbazones can act through inhibitory glycine receptors. The compounds **1** and **7** showed protection at a lower dose level (30 mg/kg) up to 24 h in the strychnine test.

All the compounds showed neurotoxicity at higher doses. The compounds **1**, **3**, **4**, **5**, **8** and **10** showed neurotoxicity only up to 30 min not up to 4 h. Compound **11** showed only 50% neurotoxicity for 30 min and 12.5% neurotoxicity after 4 h.

From these studies three results emerged

- The primary amino function of the semicarbazone derivatives expected to be responsible for anticonvulsant activity by hydrogen bonding at the receptor site was found to be definitely not essential for anticonvulsant activity. Alternatively the hydrogen bonding domain may be constituted by three atoms, that is, −CONH−N= pharmacophore. Incidently this is present in many anticonvulsant drugs like diphenylhydantion.

Scheme 1



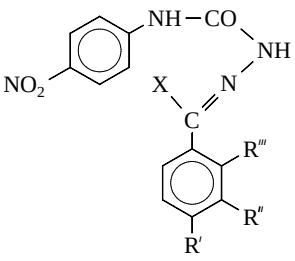
R = H, CH_3.

R' = H, OH, OCH_3, Cl, NO_2.

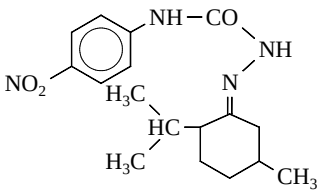
R'' = H, OCH_3.

R''' = H, OH, CH_3, OCH_3, NO_2, $-N(CH_3)_2$

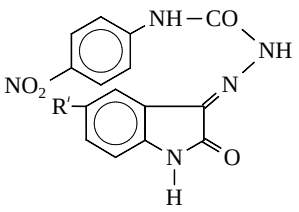
Table 2: Physical properties of p-nitrophenyl semicarbazones



(1–9)



(10)



(11–13)

Compd.	R′	R″	R‴	X	Mol Formula	m.p. (°C)	R_f	log P
1	NO_2	H	H	H	$C_{14}H_{11}N_5O_5$	135	0.59	+1.86
2	Cl	H	H	H	$C_{14}H_{11}N_4O_3Cl$	144	0.51	+1.26
3	H	H	CH_3	CH_3	$C_{16}H_{16}N_4O_3$	155	0.68	+1.33
4	OH	H	H	H	$C_{14}H_{12}N_4O_4$	153	0.60	+2.00
5	H	OCH_3	OCH_3	H	$C_{16}H_{16}N_4O_5$	148	0.65	+1.34
6	H	H	NO_2	H	$C_{14}H_{11}N_5O_5$	138	0.78	−0.94
7	OCH_3	H	H	H	$C_{15}H_{14}N_4O_4$	170	0.57	+1.92
8	H	H	$-N(CH_3)_2$	H	$C_{16}H_{17}N_5O_3$	150	0.54	+1.82
9	H	OCH_3	OH	H	$C_{15}H_{14}N_4O_5$	145	0.44	+1.72
10	–	–	–	–	$C_{17}H_{11}N_5O_4$	132	0.68	–
11	H	–	–	–	$C_{15}H_{11}N_5O_4$	135°	0.76	–
12	Cl	–	–	–	$C_{15}H_{10}N_5O_4Cl$	182°	0.74	–
13	NO_2	–	–	–	$C_{15}H_{10}N_5O_6$	160°	0.78	–

Partition coefficient in chloroform-phosphate buffer system (pH 7.4)

- The hydrophobic region as indicated by ring A should have an electron withdrawing group at the para position.
- These compounds might also act through glycine receptors because of their profound activity in the scSTY test.

Thus these studies have thrown a new light on the anticonvulsant activity of semicarbazone compounds. The terminal primary amino group of the semicarbazone moiety is not essential for anticonvulsant activity. In ring A the presence of an electron withdrawing group will be beneficial for anticonvulsant activity.

3. Experimental

3.1. Chemistry

3.1.1. General procedures for the synthesis and spectroscopy of the semicarbazones

The m.p.'s are uncorrected. The purity of the compounds was confirmed by TLC uing silica gel as stationary phase and benzene-ethyl acetate (90 : 10) solvent system. NMR spectra were recorded on a JEOL FX90Q, Fourier Transform Spectrometer and IR Spectra were recorded on a JASCO IR report 100 KBr discs. The partition coefficients were determined using a chloroform-phosphate buffer system (pH 7.4). *p*-Nitrophenylsemicarbazide was synthesised according to the literature [3] (m.p. 180–185 °C, yield 74%).

3.1.2. Synthesis of p-nitrophenyl substituted semicarbazones

To a solution of *p*-nitrophenyl semicarbazide (0.1 mol) in water was added an ethanolic solution of an equimolar quantity of the carbonyl compound. The pH of the reaction mixture was adjusted between 5–6 by adding glacial acetic acid. The reaction mixture was refluxed for 30 min. The products obtained after cooling were filtered and recrystallised from ethanol. Physical properties are described in Table 2.

3.1.3. Spectral data

1: Characteristic IR band at 3450 cm^{-1} indicates NH stretch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1600 cm^{-1}, Ar–H bend at 820 cm^{-1}.
2: ^1H NMR ($CDCl_3$) δ 6.0 (S, 1 H), 7.1–7.5 (m, 8 H), 8.8– (S, 1 H), 9.1 (m, 1 H). Characteristic IR band at 3450 cm^{-1} indicates NH stretch, C=O stretch appears at 1620 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 840 cm^{-1}.
3: Characteristic IR band at 3460 cm^{-1} indicates NH stetch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1600 cm^{-1}, Ar–H bend at 820 cm^{-1}.
4: Characteristic IR band at 3400 cm^{-1} indicates NH stretch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 820 cm^{-1}.
5: Characteristic IR band at 3400 cm^{-1} indicates NH stretch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 820 cm^{-1}.
6: Characteristic IR band at 3450 cm^{-1} indicates NH stretch, C=O stretch appears at 1500 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 860 cm^{-1}.
7: Characteristic IR band at 3460 cm^{-1} indicates NH stretch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 820 cm^{-1}.
8: ^1H NMR ($CDCl_3$) δ 3.0 (S, 6 H), 5.9 (S, 1 H), 7.2–7.8 (m, 8 H), 8.75 (S, 1 H), 9.8 (S, 1 H). Characteristic IR band at 3460 cm^{-1} indicates NH stretch, C=O stretch appears at 1640 cm^{-1}, C=N stretch appears at 1600 cm^{-1}, Ar–H bend at 840 cm^{-1}.
9: Characteristic IR band at 3460 cm^{-1} indicates NH stretch C=O stretch appears at 1630 cm^{-1}, C=N stretch appears at 1590 cm^{-1}, Ar–H bend at 840 cm^{-1}.

3.2. Pharmacological screening

The data in Table 1 were generated by the National Institute of Neurological Disorder and Stroke, NIH, USA.

3.2.1. Electroshock method

Maximal seizures were introduced by the application of electrical current to the brain via corneal electrodes. The stimulus parameters for mice was 50 mA. AC in a pulse of 60 Hz for 200 ms. The mice were 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.

3.2.2. Subcutaneous strychnine seizure pattern test

Animals of the control group received drug 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 (2 mg/kg) and observed for the hind leg tonic extensor component abolition. The dose at which hind leg tonic extensor component was ablished was noted for 4 h.

3.2.3. Subcutaneous pentylenetetrazole seizure pattern test

A pentylenetrazole dose of 85 mg/kg subcutaneously in mice causes seizures in more than 97% of the animals. This is called convulsive dose 97 or CD_{97}. The test was carried out by making the pentylenetetrazole injection approximately 10 min before the anticipated time of peak anticonvulsant drug action. The animals were observed during the following 4 h for the occurrence of seizures. A threshold convulsion is defined as one episode of clonic spasms which persists for at least 5 s. Absence of even a threshold convulsion during the period of observation is taken as the endpoint in this test.

3.2.4. 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 intraperitoneally test compounds in doses of 30, 100 and 300 mg/kg after 30 min and 4 h. The mice were placed on the rotorod to measure the effect of drug on motor performance. The dose at which animals fell off the rotorod was determined.

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