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Design of semicarbazones and their bio-isosteric analogues as potential anticonvulsants

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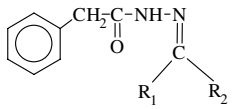
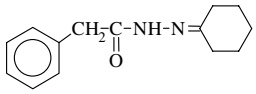
A series of semicarbazones and hydrazones were prepared and evaluated for anticonvulsant activity. Some compounds provided significant protection against maximal electroshock (MES) and subcutaneous strychnine induced seizures (ScSty). Compound **2a** emerged as the most active compound at a dose of 30 mg/kg in ScSty test. The compounds **1a**, **1g** and **2a**–**e** showed significant potentiation of sedative and hypnotic activity of pentobarbitone sodium. Thus compound **2a** could serve as a prototype for future developments.

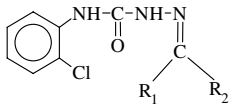
Table 1: Structure, physical properties of phenylacetic acid hydrazones and 2-chlorophenyl semicarbazones



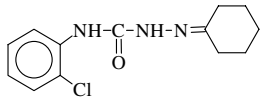
1a-c, 1e-h



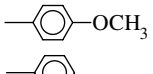
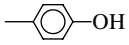
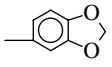
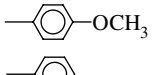
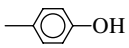
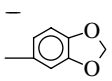
1d



2a-c, 2e



2d

Compound	R_1	R_2	M.p. (°C)	Formula	Yield (%)	Rf	Partition coefficient
1a	–H		153	$C_{16}H_{15}O_2N_2$	19.77	0.33[1]	0.9542
1b	–H		168	$C_{15}H_{14}O_2N_2$	29.52	0.31[1]	0.6020
1c	$-CH_3$		162	$C_{16}H_{15}O_2N_2$	61.00	0.87[2]	−1.3802
1d	–	–	119	$C_{14}H_{18}ON_2$	34.23	0.96[2]	0.2596
1e	–H		198	$C_{14}H_{14}O_3N_2$	95.74	0.80[2]	0.5598
1f	$-CH_3$		118	$C_{16}H_{16}ON_2$	83.33	0.83[2]	0.4189
1g	–H		133	$C_{15}H_{14}ON_2$	70.58	0.56[1]	0.6543
1h	–H		137	$C_{15}H_{14}ON_2Cl$	81.61	0.48[1]	0.2185
2a	–H		160	$C_{15}H_{14}O_2N_3Cl$	10.06	0.84[2]	0.9815
2b	–H		169	$C_{14}H_{12}O_2N_3Cl$	14.53	0.75[2]	0.5113
2c	$-CH_3$		199	$C_{15}H_{14}O_2N_3Cl$	23.76	0.64[2]	−1.4121
2d	–	–	138	$C_{13}H_{16}ON_3Cl$	18.11	0.88[2]	0.3296
2e	–H		122	$C_{15}H_{12}O_3N_3Cl$	18.92	0.74[2]	0.7712

R_f: Mobile phase: 1. Benzene : Ethyl acetate (9 : 1), 2. Chloroform : Methanol (8 : 2),
Partition Coefficient: Chloroform-Phosphate buffer (pH 7.4)

1. Introduction

Epilepsy is characterised by seizures, which are due to abrupt discharge of a large population of neurons firing synchronously [1]. It is estimated that in developed countries 0.5-1% of the population suffer from epilepsy [1]. However, with the available antiepileptic drugs (AEDs) on the market, about 70% of the people with epilepsy achieve satisfactory seizure control. During the past five years, several new drugs have been approved or in the process of being approved [2] e.g. lamotrigine, gabapentin, tiagabine and milacemide. Although these drugs have been shown to be effective in reducing seizures in a number of patients, their efficacy does not appear to be superior to that of the drugs developed earlier [3]. Therefore, the need for more effective and less toxic antiepileptic drugs still exists.

The past decade has witnessed a resurgence of interest in the development of anticonvulsant drugs. Recently Dimmock et al. have synthesised a number of aryl, arylidene and aryloxy aryl semicarbazones and proposed a model for anticonvulsant activity [4]. The proposed structural features include a hydrophobic pocket, a hydrogen bonding area along with a two electron donor system represented by a carbimino-carbon atom. In these studies the terminal $-CONH_2$ group of the semicarbazone was suggested for hydrogen bonding property. In a study from this laboratory, some semicarbazones have been synthesised in which the hydrophobic pocket with a 4-nitrophenyl group was considered for excellent anticonvulsant activity [5]. The aim of the present study was to see whether a 2-chlorophenyl group can be a substitute for the 4-nitrophenyl group, because of the increased π value of the 2-chloro group (0.71) as compared to the 4-nitro group (-0.28) [6], to increase the lipophilicity of the hydrophobic pocket. This 2-chlorophenyl substituent was placed at the terminal amino group of the semicarbazide molecule. In this $-NH-CO$-system has been implicated for hydrogen bonding. Further, in this study the $-NH-$ was replaced by a $-CH_2-$ group which has reduced hydrogen bonding characteristics. This led to the synthesis of phenylacetic acid hydrazones (Table 1).

2. Investigations, results and discussion

The compounds were screened for activity in the MES, ScMet, and NT tests, 0.5 h and 4 h after intraperitoneal (i.p.) injection to mice using doses of 30, 100, 300 mg/kg (Table 2). Compounds **1d**, **1g**, **2a**, **2b**, **2d** and **2e** afforded 100% protection at 300 mg/kg in the mice i.p. MES test 0.5 h after administration. Compound **2a** afforded 20% protection in the ScMet test at a dose level of 100 mg/kg after 0.5 h. Compound **1e** showed 25% toxicity at 100 mg/kg and 300 mg/kg, while 25% and 50% toxicity was observed for **1f**, at dose levels of 100 mg and 300 mg/kg, respectively, in the NT test. Compounds **2a**–**e** afforded 100% protection at 30 mg/kg in the NT test. Compounds **2a** and **2b** afforded 100% protection in the ScSty test at a dose of 30 mg/kg.

Compounds **2a** and **2b**, were tested orally in rats (MES). Toxicity tests were also performed (not represented in Table 2). Both the compounds afforded more than 50% protection even after 1 h in the MES test, at 30 mg/kg. At the same dose, both the compounds afforded 100% protection 2 h after administration in the NT test.

Of the thirteen compounds tested for sedative – hypnotic activity, **1a**, **1g** and **2a**–**e**, were found to potentiate the activity of pentobarbitone sodium (Table 3).

The 2-chlorophenyl substituted derivatives have shown activity in the MES test, therefore the proposition of Dimmock et al. [4], that a terminal $-CONH_2$ group is required for anticonvulsant activity is not consistent with the present results. Only the $-NH-$ group can act as a hydrogen bonding entity. Replacement of this $-NH-$ with $-CH_2-$ has completely abolished activity in the MES test, in the majority of phenylacetic acid hydrazones except **1d** and **1g**. All the compounds are inactive in the ScMet test. The compounds have exhibited activity in the ScSty seizure test and could act on the inhibitory glycine receptors. From this study compound **2a**, has emerged as the lead compound, with activity in MES, ScMet and ScSty test, at the lowest doses as compared to other compounds in the series. Further molecular modification of this molecule may lead to the discovery of new potential anticonvulsant agents.

Table 2: Anticonvulsant activity of phenylacetic acid hydrazones and 2-chlorophenyl semicarbazones

Compd.	Intraperitoneal injection in mice/rats					
	MES		ScMet	NT		ScSty
	0.5 h	4 h	0.5 h	0.5 h	4 h	0.5 h
1a	–	–	–	30	30	100
1b	–	–	–	–	–	100
1c	–	–	–	–	–	100
1d	300	300	–	30	–	300
1e	–	–	–	30	30	–
1f	–	–	–	30	30	30
1g	300	300	–	30	30	100
1h	–	–	–	30	30	–
2a	100	–	100	30	30	30
2b	300	–	–	30	30	30
2c	–	–	–	30	30	100
2d	300	–	300	30	30	100
2e	300	–	–	30	30	300

Table 3: Sedative – hypnotic activity

Compd.	Mean time taken (min)		Mean sleeping time (min)
	Loss of righting reflux	Gain of righting reflux	
1a	22.66 ± 3.59	192.00 ± 6.00	169.33 ± 3.90
1g	36.5 ± 4.19	221.66 ± 10.30	185.16 ± 8.11
2a	19.66 ± 1.97	136.66 ± 6.34	117.16 ± 5.75
2b	36.83 ± 5.52	215.16 ± 10.28	178.66 ± 6.74
2c	14.16 ± 2.40	180.16 ± 6.30	166.00 ± 4.43
2d	40.33 ± 3.44	155.0 ± 4.43	116.33 ± 3.29
2e	16.16 ± 4.01	274.66 ± 8.53	258.50 ± 5.73
Control	7.50 ± 1.70	63.66 ± 3.73	56.16 ± 3.18

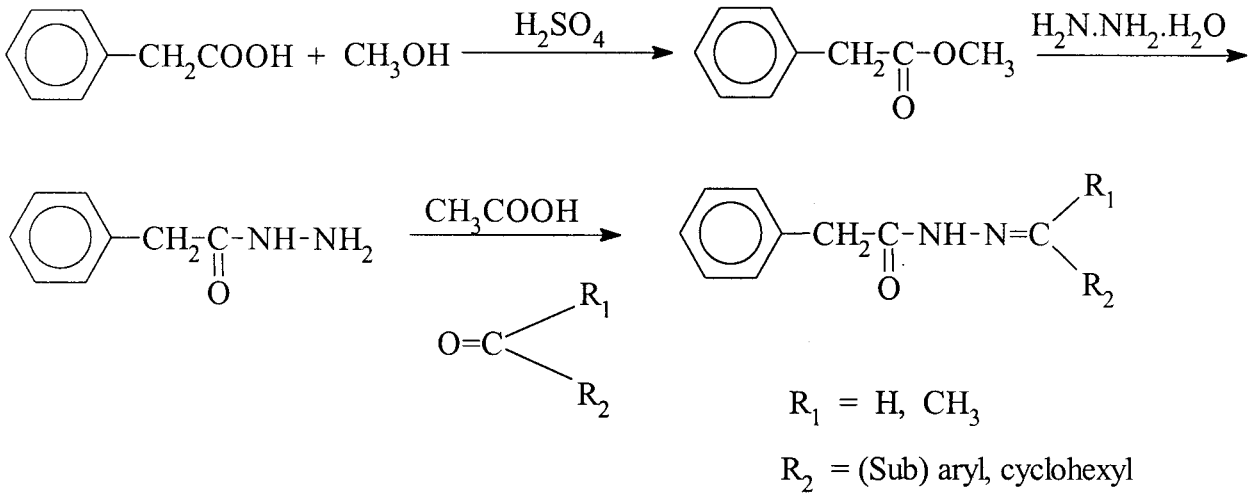
All compounds represented above indicated significant difference in mean sleeping time with respect to control. Student's t-test was performed and statistical significance was observed at 95% confidence limits n = 6, p = 0.05.

3. Experimental

3.1. Chemistry

The melting points are uncorrected. The purity of the compounds was confirmed by TLC using silica gel as stationary phase and benzene : ethylacetate (9 : 1) and chloroform : methanol (8 : 2) as solvent system. ^{1}H NMR spectra were recorded on a Jeol FX90Q Fourier Tranform spectrometer using $DMSOd_6$ as solvent and TMS as internal standard and IR spectra were recorded on a Jasco IR report 200 in KBr discs. The partition co-efficients were determined using a chloroform-phosphate buffer system (pH 7.4).

Scheme 1



3.1.1. Synthesis of phenylacetic acid hydrazones

The intermediate ester and hydrazides required in the synthesis of **1a**–**h**, were synthesised as follows: A solution of phenylacetic acid (0.01 mol), H_2SO_4 (0.002 mol) and CH_3OH (100 ml) was heated under reflux with stirring for 24 h. On cooling, $NaHCO_3$ solution (0.01 mol) was added to neutralise any acid present. Extraction with diethyl ether and evaporation of organic solvents produced the methyl ester, which was used directly. The methyl ester (0.01 mol) and hydrazine hydrate (0.05 mol) in 95% ethanol (50 ml) were refluxed with stirring for 24 h. On cooling, the precipitate was collected. The identity of the intermediate hydrazide was confirmed by IR spectroscopy and m.p. (115 °C) in comparison with the literature value (116 °C) [7]. An ethanolic solution of aryl hydrazide (0.01 mol) and the appropriate carbonyl compound (0.01 mol) and a few drops of glacial acetic acid were refluxed with stirring for 2 h. On cooling, the precipitate was collected, dried and recrystallised from ethanol (95%) to give the phenylacetic acid hydrazones **1a**–**h** (Scheme 1).

3.1.2. Synthesis of 2-chlorophenyl semicarbazones

A solution of sodium cyanate (0.1 mol) in warm water (50 ml) was added with stirring to a solution of 2-chloroaniline (0.1 mol) in glacial acetic acid (10 ml). The precipitate obtained after cooling was washed with water, dried and recrystallized from boiling water to give 2-chlorophenyl urea. The identity of the intermediate urea was confirmed by IR spectroscopy and m.p. (149 °C) in comparison to the literature value (152 °C) [8]. Equimolar quantities of the urea and hydrazine hydrate, NaOH (2g) and 95% ethanol (5 ml) were refluxed for 24 h to give the 4-(2′-chlorophenyl) semicarbazide (m.p. 179 °C, Anal: $C_7H_8N_3OCl$) A solution of the substituted semicarbazide (0.1 mol) and an equimolar quantity of the appropriate carbonyl compound were refluxed for 3 h in the presence of glacial acetic acid (1–1.5 ml). The product obtained after cooling was filtered and recrystallised from 95% ethanol to give 2-chlorophenyl semicarbazones (Scheme 2).

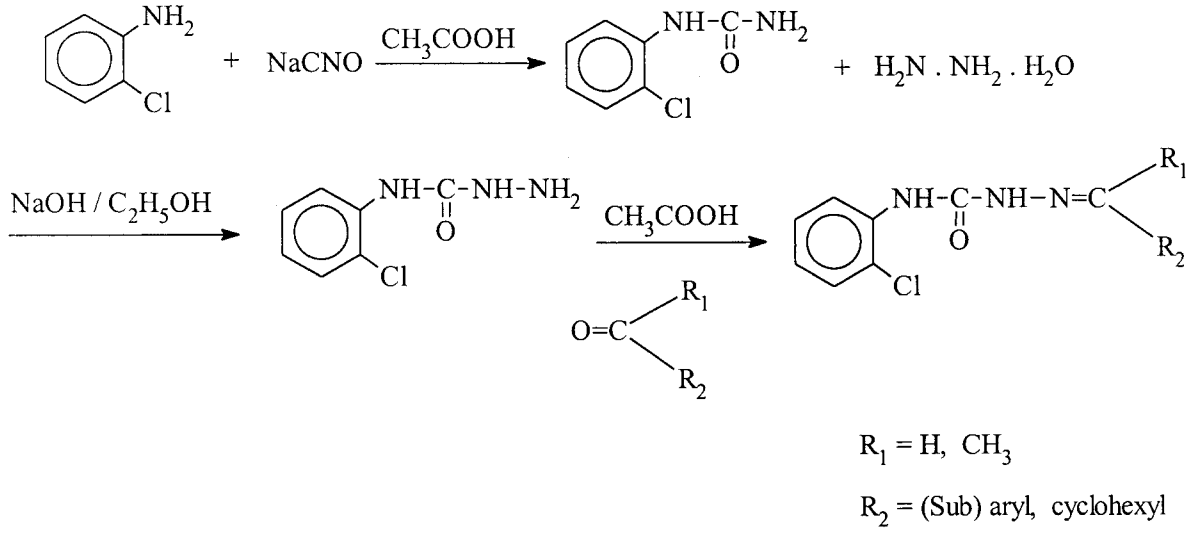
3.2. Anticonvulsant screening

The data in Table 2 (except ScSty test) were generated by the National Institute of Neurological Disorders and Stroke, NIH, USA using their protocols [9]. Compounds **2a** and **2b**, were also administered orally to rats and the results were noted at the end of 0.25 h, 0.5 h, 2 h, and 4 h. The figures in Table 2 reveal the lowest dose at which bioactivity was demonstrated and the lines indicate the absence of anticonvulsant activity.
Strychnine seizure pattern test (ScSty): Animals of the control group received vehicle (polyethylene glycol). Experimental drug solution (i.p.) was administered i.p. to the other groups. After 1 h all the animals of both groups were injected subcutaneously with strychnine (4 mg/kg) and observed for 45 min. The dose at which hindleg tonic extensor component was abolished was noted (Table 2).

3.3. Sedative – hypnotic activity

The drug was administered at a dose of 30 mg/kg to a group of six animals. The animals were injected with a solution of 30 mg/kg pentobarbitone sodium in PEG 200 after 30 min. The animals were then placed on their back and loss of righting reflex was taken as the onset of sleep. The time taken by the animals to awake was noted. A control was also performed after pretreatment with test substance vehicle (PEG 200) (Table 3).

Scheme 2



3.4. ^1H NMR spectral study

2a: ($DMSOd_6$) δ 3.3 (s, 1 H, ArCH=N−), 3.81 (s, 3 H, OCH_3), 6.3–6.36 (1s, 1H−CONH, D_2O exchangeable) 7.12–7.25 (m, 4 H, 2-chlorophenyl), 7.3–7.5 (m, 4 H, 4-methoxy phenyl), 8–8.18 (s, 1 H, ArNH, D_2O exchangeable).
2b: ($DMSOd_6$) δ 3.3 (s, 1 H, ArCH=N), 6.3–6.36 (s, 1 H, CONH, D_2O exchangeable), 7.12–7.25 (m, 4 H, 2-chlorophenyl), 7.84–8 (m, 4 H, 2-hydroxy phenyl), 8–8.18 (s, 1 H, ArNH, D_2O exchangeable), 8.9–9 (s, 1 H, −OH).

3.5. IR spectral study

2a: A characteristic IR band at 3400 cm^{-1} indicates N−H stretch, C=O stretch at 1650 cm^{-1}, C=N stretch and Ar−H band appears at 1600 and 810 cm^{-1} respectively.
2b: A characteristic IR band at 3400 cm^{-1} indicates N−H stretch, C=O stretch at 1640 cm^{-1}, C=N stretch and Ar−H band appears at 1600 and 810 cm^{-1} respectively.
2d: A characteristic IR band at 3350 cm^{-1} indicates N−H stretch, C=O stretch at 1640 cm^{-1}, C=N stretch and Ar−H band appears at 1600 and 810 cm^{-1} respectively.

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