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Original research paper

Synthesis of N^4-(2,4-dimethylphenyl) semicarbazones as 4-aminobutyrate aminotransferase inhibitors

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Several 2,4-dimethylphenyl substituted semicarbazones were synthesized in three steps involving aryl urea and aryl semicarbazide formation. The structures were confirmed by spectral and elemental analyses. All the compounds were evaluated for anticonvulsant activity by using a series of test models, including maximal electroshock seizure, subcutaneous pentylenetetrazole and subcutaneous strychnine seizure threshold tests. The compounds were also evaluated for behavioural impairement and depression activity. In the neurochemical investigation, potent compounds were evaluated for their effects on rat brain γ-aminobutyric acid (GABA) levels and *in vitro* γ-aminobutyrate transaminase (*Pseudomonas fluorescens*) activity. Preliminary studies suggest that these compounds exhibit anticonvulsant activity via a GABA-mediated mechanism.

Keywords: 2,4-dimethylphenyl semicarbazones, anticonvulsants, GABA, GABA-transaminase, maximal electroshock, pentylenetetrazole, strychinine

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Approximately fifty million people worldwide have epilepsy, making the condition the second leading neurological disorder. With optimal drug therapy, epilepsy is controlled in about 75% of the patients, but about 10% continue to have seizures at intervals of one month or less, since they are not responsive to conventionally available medical therapies (1). Moreover, the current drug therapy is associated with adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, gingival hyperplasia, hirsutism and megaloblastic anaemia (2). In the last twenty-five years, particularly in the last decade, there have been many advances relating to all aspects of epilepsy. Noteworthy has been the progress in terms of understanding the established antiepileptic drugs (AEDs) and introduction of several newer agents developed rationally, on the basis of available information on the biochemical changes in the epileptic brain. Aryl semicarbazones have documented consistent advances in the design of novel anticonvulsant agents (3–6). If the aryl semicarbazones displaying activity in the maximal electroshock seizure (MES) screen interact at a specific binding site, it is likely that the semicarbazone group

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(NHCONHN=) and the aryl ring, referred to as the hydrogen bonding area and the aryl binding site, respectively, align at complementary areas on a macromolecule complex *in vivo*. In addition, a number of substituted aryl ureas have exhibited potent anticonvulsant activity (7, 8). Substitution in the 2-position of the phenyl ring with electron-donating groups was generally beneficial to activity; exceptions appear to be those groups which are capable of hydrogen bonding, such as the OH and NH_2, where activity was found to be reduced (9). The importance of the *ortho*-methyl group for anticonvulsant activity has been emphasized in many studies (10–13), including the recently marketed drug tiagabine. MES and subcutaneous pentylenetetrazole (scPTZ) tests have become the two most widely employed seizure models for the early identification and high throughput screening of investigational antiepileptic drugs. These tests, albeit extremely effective in identifying new antiepileptic drugs that may be useful for the treatment of human generalized tonic-clonic and generalized myoclonic seizures, may miss novel antiepileptic drugs that may be useful for the treatment of therapy resistant partial seizures. It is also important to note that several clinically effective drugs, including primidone and vigabatrin, are not capable of blocking seizures in MES and scPTZ tests (14, 15). In addition to our studies on various aryl semicarbazones (13, 16–18), the present work is an attempt to study the effect of 2,4-dimethyl substitution on anticonvulsant activity. Based on our recent research outcomes (19), potent compounds were studied for their effect on γ-aminobutyric acid (GABA) levels in rat brain and *in vitro* GABA-transaminase activity.

EXPERIMENTAL

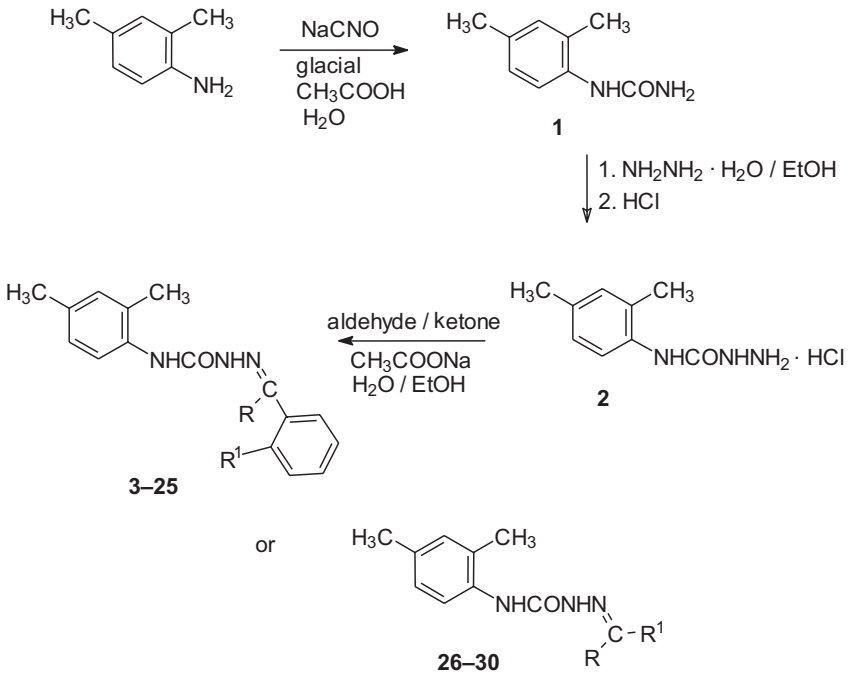
Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus (Büchi Laboratories, Switzerland) and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds by using Jasco IR Report 100 (KBr) (Jasco, UK) and Brucker Avance 300 MHz instruments (Brucker Biospin, Germany), respectively. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. All exchangeable protons were confirmed by addition of D_2O. Elemental analysis (C, H, N) was performed with a Perkin Elmer Model 240C analyser (Perkin Elmer, USA).

*Synthesis of 2,4-dimethylphenyl semicarbazide hydrochloride (**2**)*

The 2,4-dimethylphenyl urea (**1**) was synthesized according to the earlier reported procedure (13). Compound **1** (11.48 g, 0.07 mol) was refluxed in ethanol with double quantity of hydrazine hydrate (7 mL) for 24 h. The resultant precipitate was filtered off and dried. 0.06 mol of **2** was dissolved in sufficient quantity of methanol by warming and 50 mL of concentrated hydrochloric acid was added slowly, and cooled in a freezer for 2 h. The precipitate obtained was filtered off and dried (Scheme 1).

*Synthesis of 2,4-dimethyl phenyl semicarbazones (**3–30**). General procedure.*

To a solution of **2** hydrochloride (0.55 g, 0.003 mol) in methanol, an equimolar quantity of sodium acetate in water and the appropriate aldehyde or ketone in methanol were



Scheme 1

added. The mixture was stirred under heating on a magnetic stirrer for 30 min and the resultant precipitate was filtered off and dried. The product was recrystallized from 95% ethanol. The chemical and spectral data of the compounds synthesized are presented in Table I.

The synthesized compounds (Scheme 1) show characteristic absorption bands in IR spectra: 3450, 3300–3260 (NH), 3040, 2940, 1650–1670 (CONH), 1550–1570 (C=N, imine), 1300–1400 (C-N, Ar-NH) and 835–845 cm^{-1}. The spectral data of all the synthesized compounds are presented in Table II.

Pharmacology

Male albino mice (CF-1 strain, 18–25 g, 3 months) and male albino rats (Sprague Dawley/Wistar, 100–150 g, 6 months) were used as experimental animals. The Institutional Animal Ethical Committee reviewed and approved all animal procedures. The animals were housed in metabolic cages, and allowed free access to food and water. The synthesized compounds **1–30** were suspended in a 0.5% methyl cellulose/water mixture or in polyethylene glycol (PEG 200).

Anticonvulsant screening. – Anticonvulsant evaluations were undertaken using the reported procedures (20–22). Initially, all compounds were administered *i.p.* at doses of 30, 100 and 300 mg kg^{-1} to one to four mice [(4 animals for doses 30 and 100 mg kg^{-1} and 1 animal for 300 mg kg^{-1}; if protected, tested in the remaining 3 animals (NIH standard

Table I. Physical data of the synthesized compounds

Compound	R	R^1	Yield (%)	M.p. (°C)	Molecular formula	M_r
1	–	–	70	207–209	$C_9H_{12}N_2O$	164.20
2	–	–	67	157–159	$C_9H_{13}N_3O$	179.22
3	H	H	69	182–184	$C_{16}H_{17}N_3O$	267.33
4	H	2-Cl	67	193–195	$C_{16}H_{16}N_3OCl$	301.77
5	H	2-OH	88	211–213	$C_{16}H_{17}N_3O_2$	283.32
6	H	2-NO_2	73	216–218	$C_{16}H_{16}N_4O_3$	312.32
7	H	3-Cl	67	194–196	$C_{16}H_{16}N_3OCl$	301.77
8	H	3-NO_2	57	229–231	$C_{16}H_{16}N_4O_3$	312.32
9	H	4-Br	97	224–226	$C_{16}H_{16}N_3OBr$	346.22
10	H	4-OH	86	209–211	$C_{16}H_{17}N_3O_2$	283.32
11	H	4-NO_2	80	251–253	$C_{16}H_{16}N_4O_3$	312.32
12	H	4-CH_3	89	195–197	$C_{17}H_{19}N_3O$	281.35
13	H	4-OCH_3	77	139–141	$C_{17}H_{19}N_3O_2$	297.35
14	H	4-$N(CH_3)_2$	68	208–210	$C_{18}H_{22}N_4O$	310.39
15	H	3-OCH_3, 4-OH	75	191–193	$C_{17}H_{19}N_3O_3$	313.35
16	CH_3	H	62	204–206	$C_{17}H_{20}N_3O$	281.35
17	CH_3	2-OH	68	217–219	$C_{17}H_{19}N_3O_2$	297.35
18	CH_3	3-NH_2	73	219–221	$C_{17}H_{20}N_4O$	296.37
19	CH_3	4-Cl	62	224–226	$C_{17}H_{18}N_3OCl$	315.80
20	CH_3	4-OH	53	177–179	$C_{17}H_{19}N_3O_2$	297.35
21	CH_3	4-NO_2	86	247–249	$C_{17}H_{18}N_4O_3$	326.35
22	CH_3	4-NH_2	55	184–186	$C_{17}H_{20}N_4O$	296.37
23	CH_3	4-CH_3	68	175–177	$C_{18}H_{21}N_3O$	295.38
24	C_6H_5	H	59	189–191	$C_{22}H_{21}N_3O$	343.42
25	C_6H_5	4-Br	51	67–69	$C_{22}H_{20}N_3OBr$	422.32
26	CH_3	CH_3	55	158–160	$C_{12}H_{17}N_3O$	219.28
27	CH_3	C_2H_5	57	147–149	$C_{13}H_{19}N_3O$	233.31
28	CH_3	CH_2COCH_3	56	84–86	$C_{14}H_{19}N_3O_2$	261.32
29	CH_3	$CH_2CH(CH_3)_2$	60	102–104	$C_{15}H_{23}N_3O$	261.36
30	R-R^1 = cyclohexyl		71	173–175	$C_{15}H_{21}N_3O$	259.35

protocol)]. Activity was established using the MES, scPTZ and subcutaneous strychnine (scSTY) and neurotoxicity tests. Compounds **5**, **6**, **10–12** and **22** were challenged in the 6 Hz, 32 mA current for 3 s to examine antipsychomotor seizure activity in mice (23). The reference compounds employed were phenytoin, carbamazepine and sodium valproate in doses of 30, 100 and 300 mg kg^{-1} (*i.p.* in a 0.5% methylcellulose/water mixture). Some selected derivatives described in this study were examined for oral activity in the rat MES screen (24).

Table II. Elemental and spectral analyses of the synthesized compounds

Compound	Calculated/found (%)			^{1}H NMR (300 MHz, δ ppm)								
	C	H	N	Ar-CH_3	Ar-CH_3	ArH	ArNH	imine H	CONH	$NHNH_2$	NH_2	Others
1	65.83/65.71	7.37/7.35	17.06/17.01	2.16	2.22	6.90–7.22	8.90	–	–	–	6.20	–
2	60.32/60.21	7.31/7.30	23.45/23.41	2.16	2.20	6.80–8.0	9.0	–	9.72	10.80	5.80	–
3	71.89/71.64	6.41/6.39	15.72/15.69	2.15	2.22	6.82–8.12	8.91	7.89	9.89	–	–	–
4	63.68/63.44	5.34/5.32	13.92/13.89	2.17	2.25	6.83–8.22	8.98	7.65	9.76	–	–	–
5	67.83/67.62	6.05/6.04	14.83/14.80	2.19	2.29	6.83–8.40	8.87	7.54	9.89	–	–	–
6	61.53/61.34	5.16/5.15	17.94/17.89	2.23	2.34	6.79–8.16	9.01	7.54	9.91	–	–	–
7	63.68/63.51	5.34/5.32	13.92/13.89	2.30	2.21	6.78–8.19	9.12	7.79	9.86	–	–	–
8	61.53/61.32	5.16/5.14	17.94/17.90	2.22	2.16	6.76–8.12	9.04	7.69	9.76	–	–	–
9	55.51/55.32	4.66/4.64	12.14/12.10	2.17	2.21	6.76–8.22	9.10	7.66	9.89	–	–	–
10	67.83/67.62	6.05/6.04	14.83/14.80	2.21	2.23	6.89–8.18	8.59	7.89	10.89	–	–	–
11	61.53/61.31	5.16/5.15	17.94/17.89	2.20	2.24	6.96–8.23	8.63	8.01	11.00	–	–	–
12	72.57/72.30	6.81/6.79	14.94/14.89	2.16	2.20	6.76–7.84	8.48	7.90	9.72	–	–	2.24 (Ar-CH_3)
13	68.67/68.45	6.44/6.42	14.13/14.10	2.22	2.29	6.59–7.21	8.47	7.77	9.75	–	–	3.77 (Ar-OCH_3)
14	69.65/69.40	7.14/7.12	18.05/17.99	2.21	2.27	6.79–7.32	8.91	7.54	9.91	–	–	3.10 (Ar-$N(CH_3)_2$)
15	65.16/64.94	6.11/6.09	13.41/13.37	2.16	2.20	6.76–7.84	8.47	7.78	9.89	–	–	3.82 (Ar-OCH_3), 9.76 (Ar-OH)
16	72.57/72.39	6.81/6.79	14.94/14.91	2.20	2.31	6.54–7.55	8.67	7.78	9.76	–	–	2.14 (CH_3)
17	68.67/68.43	6.44/6.42	14.13/14.09	2.31	2.32	6.45–7.59	8.76	7.89	9.45	–	–	2.19 (CH_3), 9.88 (Ar-OH)
18	68.89/68.65	6.80/6.78	18.90/18.84	2.20	2.21	6.54–7.89	8.39	7.76	9.89	–	–	2.17 (CH_3), 5.29 (Ar-NH_2)
19	64.66/64.47	5.75/5.73	13.31/13.26	2.23	2.33	6.67–7.56	8.21	7.88	9.45	–	–	2.17 (CH_3)
20	68.67/68.51	6.44/6.42	14.13/14.10	2.29	2.34	6.54–7.89	8.37	7.78	9.84	–	–	2.17 (CH_3), 9.78 (Ar-OH)

Table II. cntd.

Compound	Calculated/found (%)			^{1}H NMR (300 MHz, δ ppm)								
	C	H	N	Ar-CH_3	Ar-CH_3	ArH	ArNH	imine H	CONH	$NHNH_2$	NH_2	Others
21	62.57/62.38	5.56/5.55	17.17/17.13	2.34	2.19	6.34–7.56	8.34	7.67	9.69	–	–	2.17 (CH_3)
22	68.89/68.72	6.80/6.78	18.90/18.85	2.20	2.22	6.54–7.67	8.44	7.90	9.55	–	–	2.14 (CH_3), 5.32 (Ar-NH_2)
23	73.19/72.98	7.17/7.16	14.23/14.19	2.29	2.31	6.51–7.66	8.34	7.67	9.66	–	–	2.19 (CH_3), 2.29 (Ar-CH_3)
24	76.94/76.72	6.16/6.14	12.24/12.20	2.27	2.28	6.45–7.69	8.45	7.56	9.45	–	–	6.49–7.81 (ArH)
25	62.57/62.36	4.77/4.75	9.95/9.84	2.31	2.35	6.41–7.55	8.56	7.67	9.76	–	–	–
26	65.73/65.51	7.81/7.79	19.16/19.11	2.18	2.24	6.80–7.12	8.24	7.90	9.54	–	–	1.85 (CH_3), 1.95 (CH_3)
27	66.92/66.71	8.21/8.19	18.01/17.95	2.22	2.26	6.82–7.18	8.20	7.92	9.44	–	–	1.50 (CH_3), 1.84–1.90 (CH_2), 1.98 (CH_3)
28	64.35/64.14	7.33/7.31	16.08/16.04	2.34	2.21	6.69–7.34	8.19	7.91	9.43	–	–	1.53 (CH_3), 1.93 (CH_2), 2.08 (CH_3)
29	68.93/68.69	8.87/8.85	16.08/16.03	2.33	2.19	6.69–7.22	8.21	7.92	9.46	–	–	1.53 (CH_3), 1.30 (CH_2), 1.12 ($2CH_3$), 1.78 (CH)
30	69.47/69.27	8.16/8.14	16.20/16.15	2.29	2.22	6.56–7.25	8.27	7.94	9.44	–	–	1.32 (4H of *o*-position in cyclohexyl), 1.66 (6H of cyclohexyl)

Table III. Anticonvulsant activity and neurotoxicity (mg kg^{-1}) of the synthesized compounds

Compound	MES screen[a]		scPTZ screen[a]		scSTY screen[a]		Toxicity screen[a]	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1	100	–	300[d]	–	–	–	300	–[f]
2	100	–	300[d]	–[e]	–	–	100	100
5	–	–[c]	–	–	–	–	300	–
9	–	–	300	–	100	300	300	100
10	–[c]	–	–	–	–	–	–	–
12	300	300	–	–	75	75	300	–
23	–	–	–	–	100	100	–	300
25	300[b]	–	300[d]	–	150	150	300	–
26[g]	100	300	300[d]	300[b]	300	300	100[f]	300
27[g]	300	–	300[d]	–	100	–	300	–
28	–	–	–[d]	–	–	–	–	–
29	100	300	100	300	300	300	100	300
30	300	300	300	–[d]	300	300	300	300
Phenytoin	30	30	–	–	–	100	100	100
Carbamazepine	30	100	100	300	–	–	100	300
Na-valproate	300	–	300	–	300	–	–	–

[a] Doses of 30, 100 and 300 mg kg^{-1} were administered in 0.5% methylcelluose/water mixture. Numbers in the table indicate the minimum dose by which bioactivity was demonstrated in half or more of the mice. The dash (–) indicates the absence of activity at the maximum dose administered (300 mg kg^{-1}).

[b] In the MES screen, the compound showed protection at 100 mg kg^{-1} in 1/3 mice.

[c] In the MES screen, compounds **5** and **10** at 30 mg kg^{-1} showed protection of 1/3 mice after 1 and 0.25 h, respectively.

[d] In the scPTZ screen at a dose of 100 mg kg^{-1}, compounds that showed protection were **1**, **2**, **25** and **27** (0.25 h), **26** (0.25 and 1 h), **28** (0.5 h) and **30** (1 h).

[e] Compound **2** at 100 mg kg^{-1} caused death following continuous seizure 1 h period after administration.

[f] Compounds **1** and **26** showed neurotoxicity at 100 mg kg^{-1} 1 h after administration.

Neurotoxicity screening. – Minimal motor impairment was measured in mice (groups of 4) by the rotorod test. The mice were trained to stand on an accelerating rotarod rotating at 10 rpm. The rod diameter was 3.2 cm. Trained animals were given *i.p.* injections of the test compounds in doses of 30, 100 and 300 mg kg^{-1}. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials.

Behavioral testing. – The titled compounds (30 mg kg^{-1}) were screened for their behavioral effects using an actophotometer (25) at 30 min and 1 h after injection in each group of six animals. The behavior of animals inside the photocell was recorded as a digital score. Increased scores suggested good behavioral activity. The control animals

Table IV. Behavioral study on some selected compounds using an actophotometer

Compound[a]	Activity score[b]		
	Control[d]	Post treatment (h)	
	(24 h prior)	0.5	1
25	197.67 ± 13.24	120.33 ± 11.35	41.33 ± 3.25
26	282.33 ± 29.08	100.33 ± 13.11	51.17 ± 6.80
27	301.5 ± 27.67	137.33 ± 11.33	93.17 ± 5.93
29	154.83 ± 16.11	70.33 ± 6.43	55 ± 5.99
30	155.17 ± 26.98	60.17 ± 5.04	71 ± 7.45
Phenytoin[c]	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44

[a] Compounds were tested at a dose of 100 mg kg^{-1} (*i.p.*).

[b] Each score represents the mean ± SEM of six mice, significantly different from the control score at $p < 0.05$ (Student's t test).

[c] Tested at 30 mg kg^{-1} (*p.o.*, in 0.5% methylcelluose/water mixture).

[d] Control animals were administered PEG (*i.p.*).

were administered PEG. Phenytoin was used as reference for comparison at a dose of 30 mg kg^{-1} (*p.o.*, in a 0.5% methylcellulose/water mixture*)*. The observations are tabulated in Table IV.

CNS depressant study. – The forced swim pool method described earlier was followed (26). Wistar rats (six animals in each group) were placed in a chamber (diameter 45 cm, height 20 cm) containing water up to the height of 15 cm at 25 ± 2 °C. Two swim sessions were conducted, an initial 15 min pre-test, followed by a 5 min test session 24 h later. The animals were administered an *i.p.* injection (30 mg kg^{-1}) of the test compound 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which are necessary to keep its head above the surface) during the 5 min test period were measured. Carbamazepine was used as a reference for comparison at a dose of 30 mg kg^{-1} (*i.p.*, in a 0.5% methylcellulose/water mixture). The control animals were administered PEG. The results are presented in Table V.

Isolation of rat brain regions and GABA assay. – The GABA assay for compounds **2**, **26**, **27** and **29** was performed in brain tissue extracts enzymatically as previously described (27). Adult Wistar rats (groups of six animals) were used for this purpose. After 2 h of drug administration (30 mg kg^{-1}, *i.p.*), the animal was sacrificed by decapitation, and the brain regions, mid brain, olfactory lobe, cerebellum and medulla oblongata were dropped into separate vials containing 4–6 mL of ice-cold 80% ethanol and processed further as described previously (28).

In vitro *GABA transaminase activity.* – The GABA-T enzyme was prepared from *Pseudomonas fluorescens* according to the method reported earlier (29). The *in vitro* enzyme inhibition assay was performed up to a 4 h period for compounds **2**, **26**, **27**, and **29** according to the previously reported procedure (30).

Table V. CNS study on selected compounds in a forced swim pool test

Compound[a]	Immobility time (s)	
	Control (24 h prior)[b]	Post treatment (60 min after)[c]
PEG	143.5 ± 11.42	157.83 ± 10.04[NS]
25	163.33 ± 8.53	208.33 ± 9.92
26	151 ± 6.39	212.33 ± 6.89
27	142.17 ± 6.27	197.83 ± 8.44
28	147.33 ± 5.82	225.5 ± 6.99
30	139.33 ± 6.67	192.17 ± 6.47
Carbamazepine[d]	131.5 ± 9.32	207.33 ± 8.49

[a] Compounds were tested at a dose of 100 mg kg^{-1} (*i.p.*).

[b] Control animals were administered PEG (*i.p.*).

[c] Each value represents the mean ± SEM of six mice significantly different from the control at $p < 0.008$ (NS – not significant).

[d] Tested at 30 mg kg^{-1} (*i.p.*, in 0.5% methylcelluose/water).

RESULTS AND DISCUSSION

All the thirty compounds synthesized, including the intermediates 2,4-dimethylphenyl urea (**1**) and semicarbazide (**2**) derivatives, were evaluated for anticonvulsant activity. The results are presented in Table III, along with the data on phenytoin, carbamazepine and sodium valproate. The 2,4-dimethylphenyl urea (**1**) and the semicarbazide (**2**) and some semicarbazone derivatives showed anti-MES activity, indicative of their ability to prevent seizure spread. At a dose of 100 mg kg^{-1}, compounds that showed protection in half or more of the tested mice were **1**, **2**, **26** and **29** after 0.5 h of drug administration. Compounds **12**, **26**, **29** and **30** were capable of preventing seizure spread over a 4 h period at a dose of 300 mg kg^{-1}. All other compounds were inactive. In the rat *i.p.* MES screen, compounds **5** and **10** at 30 mg kg^{-1} showed protection after 1 h and 0.25 h of administration, respectively. In the rat oral MES screen (30 mg kg^{-1}), compounds that were active were **26** (0.5 h) and **27** (0.5, 1 and 2 h).

Compounds **1**, **2**, **9**, **25–27**, **29** and **30** were found to be active in the scPTZ test, a test used to identify compounds that elevate the seizure threshold. Compound **29** showed activity at a dose of 100 mg kg^{-1} comparable with carbamazepine and higher potency than sodium valproate. Death following continuous seizure was observed in one animal after 1 h of administration of compound **2** (100 mg kg^{-1}), though the compound exhibited protection after 0.5 h (Table III). All compounds were also evaluated in the scSTY tests. The activity of compounds **9**, **12**, **23**, **25–27 29** and **30** in the scSTY test showed that semicarbazones could also act through inhibitory glycine receptors, as reported earlier (13). Compound **12** was able to prevent scSTY-induced seizure at a dose of 75 mg kg^{-1} over a 4 h period. Compounds that showed anti-scSTY activity at 100 mg kg^{-1} include **9**

(0.5 h), **27** (0.5 h) and **25** (1 h) at 150 mg kg^{-1}. Compounds **26**, **29** and **30** showed protection at 300 mg kg^{-1}, and compounds **29** and **30** showed loss of the righting reflex after 0.25 h of drug administration.

In the neurotoxicity test, compounds **12** and **28** did not show toxicity at the highest administered dose of 300 mg kg^{-1} while compounds **1**, **5**, **23**, **25**, **27** and **30** showed lower toxicity than the standard drugs phenytoin and carbamazepine. Mice were unable to grasp the rotorod after administration of compounds **2** (300 mg kg^{-1}, 0.5 h), **5** (300 mg kg^{-1}, 0.5 h), **12** (300 mg kg^{-1}, 0.5 h), **26** (100 mg kg^{-1}, 0.5 h and 1 h and at 300 mg kg^{-1} 0.5 and 4 h) and **30** (300 mg kg^{-1}, 0.5 and 4 h). With compound **29**, loss of the righting reflex was observed after 0.5 and 4 h after a 300 mg kg^{-1} dose was administered, and with compound **30** (300 mg kg^{-1}) slight tremors were noted. Compounds **4**, **6–8**, **10**, **11**, **13–22** and **24** did not exhibit anticonvulsant activity or neurotoxicity.

The lower levels of neurotoxicity associated with compounds **5**, **23**, **25–27** and **28** and the absence of toxicity with **4**, **6–8**, **12–22** and **28** clearly indicate the difference in activity due to the carbimino terminal substituents, which were also responsible for the difference in bioactivity of the semicarbazones. When one of the substituents at the carbimino terminal was hydrogen or methyl, the other substituents seemed to be responsible for the activity. As regards substituents, an electron donating group that increases the lipophilicity of the ring, as in **9** and **12**, showed good protection in MES, scSTY, scPTZ and scSTY screens, respectively. In the case of compound **5**, toxicity was observed which showed that the compound had sufficient hydrophobicity to cross the blood brain barrier. As this compound was inactive, the importance of *para* substitution comes to the front. But in the case of compound **10**, the absence of activity could be attributed to the fact that the *p*-OH is highly prone to metabolic attack (O-glucuronide). The *para* methyl group in **12** with relatively higher resistance to metabolic attack may have made the compound active. In general, it appeared that small, lipophilic, non-hydrogen bonding groups at the 4-position showed activity.

Replacement of the proton in the carbimino carbon atom by methyl (**17–23,** and **26–29**) or phenyl (**24** and **25**), leading to an increase in the size of the group at this position of the molecule, has shown variation in activity. This modification might increase the anticonvulsant activity due to additional van der Waals bonding or alternatively steric impedance to alignment at a binding site, leading to reduction or abolition of activity (5). As regards **24** and **25,** the former did not show any anticonvulsant activity whereas **25** with a *para* bromo group showed activity. This is consistent with the report that 4-bromo phenyl derivatives showed better anticonvulsant activity (3, 13).

Results obtained with levetiracetam in the 6 Hz test suggest that it may identify a compound that is not active by either the MES or scPTZ test and thus detect active substances that would have been missed by a more traditional identification procedure (31, 14). In the present study, some selected compounds (**5**, **6**, **10–12** and **22**) were screened in the 6 Hz psychomotor seizure model. Compounds **6**, **11** and **12** showed protection in 25% mice at 100 mg kg^{-1} after 0.5, 2, and 0.25 h, respectively. Recent studies completed by the anticonvulsant screening program, NIH (23), have revealed that 6 Hz seizures appear to be somewhat resistant to phenytoin and other sodium channel blockers.

It is conceivable from this study that the structural features essential for interaction at the binding site were a lipophilic moiety (2,4-dimethylphenyl ring) and a hydrogen bonding domain (amide function, NHCO), as proposed earlier (32). The distal aryl ring

Table VI. Effect of compounds on the GABA system

Compound	Concentrations of GABA (μg per 100 mg protein)				*In vitro* GABA-T activity		
	Mid brain	Olfactory lobe	Cerebellum	Medulla oblongata	Concentration (μmol L^{-1})	Time point (h)	Inhibition (%)
Control[d]	46.49 ± 3.34	14.85 ± 1.03	29.54 ± 1.85	47.89 ± 9.15	–	–	–
2	48.91 ± 3.77	29.79 ± 0.93[a]	45.54 ± 2.20[a]	38.77 ± 11.25	62.5	3	90
26	63.39 ± 2.77[a]	19.79 ± 3.22	19.36 ± 2.22[a]	37.64 ± 6.77	250	4	44
27	36.09 ± 5.05	27.85 ± 2.89[b]	38.92 ± 3.34[c]	46.87 ± 3.34	62.5	3	89
29	102.27 ± 11.37[a]	18.71 ± 0.63	25.82 ± 3.46	41.08 ± 2.96	125	3	51

Each value represents the mean ± SEM of six rats significantly different from the control at [a] $p < 0.01$, [b] $p < 0.02$, [c] $p < 0.03$.

[d] Control animals were administered PEG (*i.p.*).

at the carbimino terminal (benzylidene ring) may be essential for the pharmacokinetic properties of the compounds, since variation in the substituents at the distal aryl ring was found to affect the biological activity.

In the behavioral despair test, some selected compounds (**26**–**28**, **30**) were tested and were found to decrease the motor activity, as indicated by the actophotometer scores (Table IV). Similar results were obtained in Porsolt's swim pool test with compounds **26** and **27** and **29** and **30**, in which an increase in the immobility time by the compounds indicated the CNS depression effect (Table V).

In order to explore the mechanism of anticonvulsant activity, some selected compounds that were highly active were subjected to neurochemical investigation to study their effects on the levels of GABA in different regions of rat brain, *viz.*, mid brain, olfactory lobe, cerebellum, and medulla oblongata. Compounds **2** and **27** showed an increase of GABA levels in the olfactory lobe and cerebellum, while compounds **26** and **29** showed a significant increase in GABA levels in the mid brain region of rat brain (Table VI). These compounds did not have a significant effect on the GABA levels in medulla oblongata. Further, compounds **2**, **26**, **27** and **29** were carried on to preliminary *in vitro* GABA-T (*Pseudomonas fluorescens*) inhibition studies. Compound **2** and **27** emerged as potent inhibitors of the enzyme (90 and 89%, respectively) and **26** and **29** showed 44 and 57% inhibition, respectively. These studies confirm our earlier report (19) on aryl semicarbazones acting through a GABA-mediated mechanism. These data corroborate that the compounds align at a specific binding site and are not structurally nonspecific.

CONCLUSIONS

The presented results indicated that the 2,4-dimethylphenyl semicarbazones were effective in MES, scPTZ and scSTY models and were found to act through GABA-mediation.

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S A Ž E T A K

Sinteza N^4-(2,4-dimetilfenil) semikarbazona kao inhibitora 4-aminobutirat aminotransferaze

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Sintetizirano je nekoliko 2,4-dimetilfenil supstituiranih semikarbazona u tri sintetska koraka koji uključuju aril uree i aril semikarbazide. Strukture spojeva su potvrđene spektroskopskim metoda i elementarnom analizom. Ispitano je antikonvulzivno djelovanje novih spojeva nakon izazivanja konvulzija elektrošokom te supkutanom primjenom pentilentetrazola ili strihnina. Osim toga, testirano je antidepresivno djelovanje te učinak tih spojeva na ponašanje štakora. Praćen je njihov utjecaj na koncentraciju γ-aminomaslačne kiseline (GABA) u mozgu štakora te *in vitro* na aktivnost γ-aminobutirat transaminaze (*Pseudomonas fluorescens*). Preliminarni pokusi ukazuju da antikonvulzivno djelovanje ovih spojeva uključuje GABA-ergički sustav.

Ključne riječi: 2,4-dimetilfenil semikarbazoni, antikonvulzivi, GABA, GABA-transaminaza, maksimalni elektrošok, pentilentetrazol, strihnin

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