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Microwave-assisted synthesis, anticonvulsant activity and quantum mechanical modelling of N-(4-bromo-3-methylphenyl) semicarbazones*

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Abstract: Objective: To study the effect of halo substitution on disubstituted aryl semicarbazones on the anticonvulsant potential and model the activity based on quantum mechanics. Methods: A series of twenty-six compounds of N^4-(4-bromo-3-methylphenyl) semicarbazones were synthesized and evaluated for the anticonvulsant activity in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ) seizure threshold tests. Some potential compounds were also tested in the subcutaneous strychnine (scSTY) and subcutaneous picrotoxin (scPIC) seizure threshold tests. The synthesized compounds were tested for behavioral impairment and CNS (central nervous system) depression in mice. Quantum mechanical modelling was carried out on these compounds to gain understanding on the structural features essential for activity. Results: Some compounds possessed broad spectrum anticonvulsant activity as indicated by their effect in pentylenetetrazole, strychnine, picrotoxin and maximal electroshock seizures models in resemblance to other aryl semicarbazone derivatives reported earlier. The higher the difference in HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy levels was, the greater was the activity profile. Conclusion: The pharmacophoric requirements for compounds to exhibit anticonvulsant activity that includes one aryl unit in proximity to a hydrogen donor-acceptor domain and an electron donor have been justified with the molecular orbital surface analysis of the synthesized compounds.

Key words: Anticonvulsants, Aryl semicarbazones, Quantum mechanics (QM), Molecular orbital surfaces
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INTRODUCTION

Epilepsy is an episodic neurological disorder that affects up to 1% of the population, and is perhaps best characterized as a family of disorders. Although the specific mechanism underlying this group of disorders is diverse, they all appear to have in common, the feature of aberrant synchronized discharge of neurons leading to hyperexcitability in the central nervous system (Dichter, 1998). In the recent years, much effort has been devoted to explore the novel approaches by elucidating the cellular and molecular mechanisms of the hyperexcitability to provide specific target for novel therapies and as a result several new drugs such as vigabatrin, lamotrigine, gabapentin, tiagabine have appeared in the market (Willmore, 2005). These new agents have been proved to be mainly for adjunctive therapy of partial and secondarily generalized seizures, some of them however undoubtedly have a wide spectrum of efficacy, but uncontrolled seizures and medication toxicity are still major problems of antiepileptic drug treatment. Thus there is clearly a need for additional drugs, as well as for new strategies for preventing epilepsy.

We have recently been investigating various aryl-substituted semicarbazones as potential anticonvulsant agents (Pandeya *et al*., 2000; Yogeeswari *et al*., 2003; 2004a; 2004b; 2005a; Thirumurugan *et*

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al., 2006) and compound N^1-(2,6-dimethylphenyl)-N^4-(4-N,N-dimethyl aminobenzaldehyde) semicarbazones showed an ED_{50} (effective dose at 50% of the total population) of 18.33 mg/kg and TD_{50} (toxic dose at 50% of the total population) greater than 250 mg/kg, resulting in a high protection index of >13.64 when compared to phenytoin (Yogeeswari *et al*., 2005b). The rationale behind the development of semicarbazones is their structural dissimilarity to exciting antiepileptic drugs, so it was hoped that such novel compounds would not have the side effects seen with many of the currently available medications (Kadaba, 1984). Aryl semicarbazones have also been shown to possess excellent anticonvulsant activity in the maximal electroshock seizure (MES) in both mice and rats and also against clonic seizures induced by pentylenetetrazole (PTZ) in mice, being more active than some conventional antiepileptic drugs, beside their low neurotoxicity.

While fitting semicarbazones to the binding site hypothesis, it is likely that two-electron donor atoms in semicarbazones group and aryl ring align at the complimentary area on a macromolecular complex with the receptor in vivo (Yogeeswari *et al*., 2005b). Our analysis of the distance relationship showed that aryl semicarbazones fulfilled the essential demands of the pharmacophore when compared with the phenytoin, carbamazepine, denzinamide, and remacemide (Yogeeswari *et al*., 2005a; 2005b). The presence of electron-rich atom/group attached at the *para* position of the aryl ring showed increased potency in the MES screen. Substitution in the aryl ring by halogens led to a number of semicarbazones with low ED_{50} values in the rat oral MES screen accompanied by high protective index values (Dimmock *et al*., 1995). Hence, in our earlier study, 4-bromophenyl group was considered important and led to compounds with better activity profile with very low neurotoxicity and no sedative-hypnotic activity (Pandeya *et al*., 2000). Recently we reported on variously substituted aryl semicarbazones with the order of activity being found to be 4-F>2-Br=3-Br=4-Cl>4-CH_3>4-Br>3-Cl>3-CH_3 with respect to the primary aryl group (Yogeeswari *et al*., 2006). We also reported some 3-chloro-2-methylphenyl semicarbazones as potential anticonvulsant agents (Yogeeswari *et al*., 2004a). In continuation of our work on disubstituted aryl semicarbazones, the present work focuses on the synthesis and anticonvulsant evaluation of newer 4-bromo-3-methylphenyl semicarbazones for exploring the structure-activity relationships of aryl semicarbazones.

MATERIALS AND METHODS

Chemistry

Melting points were determined in one end open capillary tubes using Buchi 530 melting point apparatus and were uncorrected. The homogeneity of the compounds was monitored by thin-layer chromatography (TLC), on silica gel G (Merck) coated aluminium plates, using chloroform: methanol (9:1) as solvent system. Elemental analyses (C, H, and N) were undertaken with Perkin-Elmer model 240C analyzer. InfraRed (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR report 100 (KBr) and Brucker Avance (300 MHz) instruments respectively. Chemical shifts are reported in δ using tetramethylsilane as internal standard. All exchangeable protons were confirmed by the addition of D_2O. Log*P* values were determined using Alchemy and Scilog P software (Tripos Co., UK).

Preparation of 4-bromo-3-methylphenyl urea: Substituted aniline (0.1 mol) was dissolved in 25 ml of glacial acetic acid and 12.5 ml of water. To this 0.1 mol of sodium cynate (6.5 g) in 25 ml of warm water was added with stirring. The mixture was allowed to stand for 30 min, then cooled in ice and filtered with suction and dried, recrystallised from boiling water to yield substituted aryl urea. KBr pellets 3400 cm^{-1}, 1700 cm^{-1}, 780 cm^{-1}, 750 cm^{-1}, ^{1}H-NMR (DMSO-d_6, δ, 300 MHz) 2.4 (s, 3H, $ArCH_3$), 7.3~7.5 (m, 3H, ArH), 7.98 (s, 1H, ArNH, D_2O exchangeable), 9.88 (s, 2H, CONH, D_2O exchangeable).

Synthesis of 4-bromo-3-methylphenyl semicarbazide: The 4-bromo-3-methylphenyl urea (0.05 mol) and excess of hydrazine hydrate (0.1 mol) in ethanol were refluxed for 24 h. Two thirds volume of alcohol was distilled by vacuum distillation unit and poured in to ice. The resultant precipitate was filtered, washed with water and dried. The solid was recrystallised with 90% alcohol. KBr pellets 3400 cm^{-1}, 3280 cm^{-1}, 1640 cm^{-1}, 760 cm^{-1}, ^{1}H-NMR (DMSO-d_6, δ, 300 MHz) 2.18 (s, 3H, $ArCH_3$), 7.4~7.5 (m, 3H, ArH),

5.60 (s, 2H, NH_2, D_2O exchangeable), 7.92 (s, 1H, ArNH, D_2O exchangeable), 9.94 (s, 1H, CONH, D_2O exchangeable).

Synthesis of 4-bromo-3-methylphenyl semicarbazones (**1**~**26**, Fig.1): The conversion of semicarbazide to semicarbazones was carried out using microwave irradiation (domestic microwave Matrix LG input 220 V, 50 Hz, 980 W, 4.7 A, frequency 2450 MHz). To a solution of 4-bromo-3-methylphenyl semicarbazide (0.003 mol), in ethanol was added an equimolar quantity of appropriate aldehydes or ketone. The pH of the reaction mixture was adjusted to 5~6 by adding glacial acetic acid, to facilitate the nucleophilic substitution. The reaction mixture was exposed to microwave irradiation for time period of 2~3 min (*I*=80%). The product obtained after cooling was filtered and recrystallised from 95% ethanol. The physical data for the synthesized compounds are presented in Table 1. The elemental analyses data are presented in Table 2. The IR spectra of the semicarbazone derivatives were identical in the following aspects: IR (KBr) cm^{-1} 3450, 3250~3300, 1650, 1595, 840; ^{1}H-NMR (300 MHz) spectra of some representative compounds are as follows:

1. 2-hydroxybenzaldehyde

N-(4-bromo-3-methylphenyl) semicarbazone (**3**): Yield: 61%, m.p.: 166 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.28 (s, 3H, $ArCH_3$), 6.81~7.80 (m, 7H, ArH), 8.25 (s, 1H, ArNH, D_2O exchangeable), 8.67 (s, 1H, ArOH, D_2O exchangeable), 10.06 (s, 1H, CONH, D_2O exchangeable).

2. 4-nitrobenzaldehyde

N-(4-bromo-3-methylphenyl) semicarbazone (**8**): Yield: 75.5%, m.p.: 180 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.26 (s, 3H, $ArCH_3$), 7.20~7.81 (m, 7H, ArH), 8.70 (s, 1H, imine H), 9.4 (s, 1H, ArNH, D_2O exchangeable), 10.6 (s, 1H, CONH, D_2O exchangeable).

3. 4-methoxybenzaldehyde

N-(4-bromo-3-methylphenyl) semicarbazone (**9**): Yield: 67%, m.p.: 155 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.20 (s, 3H, $ArCH_3$), 3.80 (s, 3H, OCH_3) 7.02~8.12 (m, 7H, ArH), 8.24 (s, 1H, imine H), 8.86 (s, 1H, ArNH, D_2O exchangeable), 9.62 (s, 1H, CONH, D_2O exchangeable).

4. 3-hydroxy-4-methoxybenzaldehyde

N-(4-bromo-3-methylphenyl) semicarbazone (**12**): Yield: 45%, m.p.: 148 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.10 (s, 3H, $ArCH_3$), 3.64 (s, 3H, OCH_3), 7.24~7.32 (m, 6H, ArH), 7.76 (s, 1H, imine H), 8.82 (s, 1H, ArNH, D_2O exchangeable), 9.26 (s, 1H, OH, D_2O exchangeable), 10.05 (s, 1H, CONH, D_2O exchangeable).

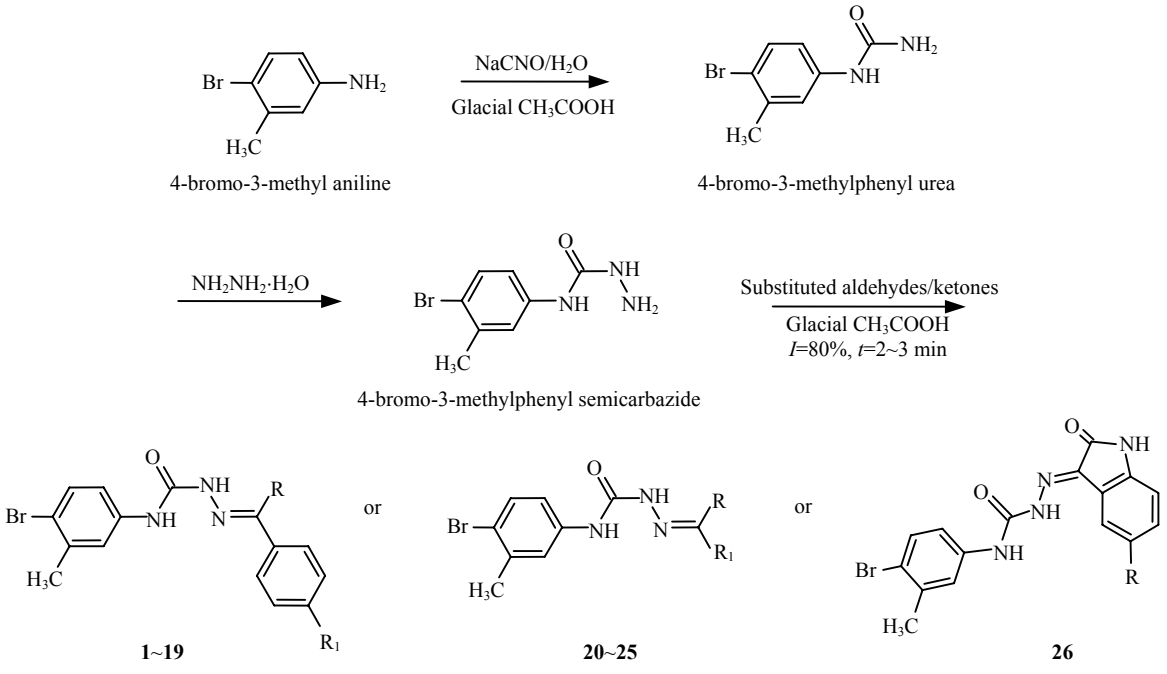

Fig.1 Scheme for synthesis of 4-bromo-3-methylphenyl semicarbazones

Table 1 Physical and biological data of 4-bromo-3-methylphenyl-semicarbazones

Compound	R	R_1	Yield (%)	m.p. (°C)[a]	*Rf*[b]	Intraperitoneal injection in mice[c]				
						MES screen		scPTZ screen		Toxicity screen
						0.5 h	4 h	0.5 h	4 h	0.5 h
1	H	H	51	158	0.82	100	300	300[e]	300	300[f]
2	H	2-NO_2	75	142	0.88	−	−	−	−	−
3	H	2-OH	61	166	0.76	300	−	300	−	300
4	H	2-OCH_3	58	138	0.89	−	−	−	−	−
5	H	2-Cl	56	154	0.64	300	−	300	−	300
6	H	2-CH_3	50	145	0.89	300[d]	300	300	−	300[f]
7	H	3-NO_2	62	151	0.75	300	−	−	−	300[f]
8	H	4-NO_2	75.5	180	0.82	300	−	−	−	−
9	H	4-OCH_3	67	155	0.84	100	−	300	−	300[f]
10	H	4-Br	62	172	0.73	300	−	−	−	300[f]
11	H	4-$N(CH_3)_2$	52	150	0.85	300	−	300	−	300
12	H	3-OCH_3, 4-OH	55	148	0.88	300[d]	−	300	−	300[f]
13	CH_3	H	57	170	0.90	300[d]	300	300	−	100
14	CH_3	3-NH_2	62	185	0.82	300	−	300	−	300
15	CH_3	4-NH_2	67	135	0.87	300	−	300[e]	−	300[f]
16	CH_3	4-NO_2	82	189	0.71	100	−	300[e]	−	300
17	CH_3	4-OH	57	145	0.80	100	−	300[e]	−	100
18	CH_3	4-CH_3	40	178	0.88	100	−	300[e]	−	300
19	C_6H_5	H	60	138	0.74	100	300[d]	300	−	300[f]
20	CH_3	CH_3	57	181	0.70	100	300	300	300	300[f]
21	CH_3	C_2H_5	53	161	0.66	300	−	300	−	100[f]
22	CH_3	CH_2COCH_3	50	180	0.71	300[d]	−	−	−	300
23	CH_3	$CH_2CH(CH_3)_2$	56	160	0.69	300	−	300	−	100[f]
24	CRR_1=cylopentylene		54	138	0.89	300	−	300	−	300[f]
25	CRR_1=cylohexylene		58	185	0.92	100	300	300[e]	−	100
26	H		68	142	0.64	100	300	300	−	100
Phenytoin			−	−	−	30	30	−	−	−
Ethsuximide			−	−	−	−	−	300	−	−

[a]Melting points of the compounds at their decomposition; [b]Rention factor (Rf) with TLC using mobile phase $CHCl_3$:CH_3 (9:1); [c]Doses of 30, 100 and 300 mg/kg were administered to one to four mice. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (−) indicates an absence of activity at maximum dose administered (300 mg/kg); [d]In the MES screen, at a dose of 100 mg/kg, compounds **6**, **12**, **13**, **19**, **22** showed protection in 1/3 mice at 0.5 h; [e]In the scPTZ screen, at a dose of 100 mg/kg, compounds that showed protection were **1**, **15**, **18**, **25** (2/5, 0.25 h) **16**, **17** (1/4, 0.5 h); [f]In the neurotoxicity screen, at a dose of 100 mg/kg, compounds that showed toxicity were **1**, **7** (1/8, 0.5 h), **6**, **9**, **10**, **19**, **24** (1/4, 0.5 h) **15**, **20**, **23** (3/8, 0.5 h) and **12**, **21** (3/5, 0.25 h)

5. 1-(4-aminophenyl)ethan-1-one

N-(4-bromo-3-methylphenyl) semicarbazone (**15**): Yield: 67%, m.p.: 135 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.88 (s, 3H, CH_3), 2.24 (s, 3H, $ArCH_3$), 5.32 (s, 2H, NH_2, D_2O exchangeable), 7.14~7.26 (m, 7H, ArH), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.38 (s, 1H, CONH, D_2O exchangeable).

6. 1-(4-hydroxyphenyl)ethan-1-one

N-(4-bromomethylphenyl) semicarbazone (**17**): Yield: 57%, m.p.: 145 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.04 (s, 3H, CH_3), 2.27 (s, 3H, $ArCH_3$), 7.24~7.91 (m, 7H, ArH), 8.98 (s, 1H, ArNH, D_2O exchangeable), 9.51(s, 1H, ArOH, D_2O exchangeable), 9.84 (s, 1H, CONH, D_2O exchangeable).

7. 1-(4-methylphenyl)ethan-1-one

N-(4-bromo-3-methylphenyl) semicarbazone (**18**): Yield: 40%, m.p.: 178 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.94 (s, 3H, CH_3), 2.28 (s, 6H, 2-$ArCH_3$), 7.12~7.30 (m, 7H, ArH), 8.78 (s, 1H, ArNH, D_2O exchangeable), 10.01 (s, 1H, CONH, D_2O exchangeable).

8. Acetone

N-(4-bromo-3-methylphenyl) semicarbazone

Table 2 Elemental analysis of 4-bromo-3-methylphenyl semicarbazones

Compound	Molecular formula	Molecular weight	Calculated (%)			Found (%)		
			C	H	N	C	H	N
1	$C_{15}H_{14}N_3OBr$	332	54.23	4.25	12.65	53.35	3.98	12.24
2	$C_{15}H_{13}N_4O_3Br$	377	47.76	3.47	14.85	48.04	3.69	15.11
3	$C_{16}H_{19}N_5O$	348	51.74	4.05	12.07	52.60	4.86	11.16
4	$C_{16}H_{16}N_3O_2Br$	362	53.05	4.45	1.60	54.50	4.48	12.04
5	$C_{15}H_{13}N_3OClBr$	366.5	49.14	3.57	1.46	50.08	3.12	10.98
6	$C_{16}H_{16}N_3OBr$	346	55.51	4.66	12.14	56.62	3.82	13.21
7	$C_{15}H_{13}N_4O_3Br$	377	47.76	3.47	14.85	48.19	4.12	14.40
8	$C_{15}H_{13}N_4O_3Br$	377	47.76	3.47	14.85	48.11	3.86	14.20
9	$C_{16}H_{16}N_3O_2Br$	362	53.05	4.45	1.60	54.88	4.12	11.98
10	$C_{15}H_{13}N_3OBr$	411	43.82	3.19	10.22	45.88	4.56	12.68
11	$C_{16}H_{16}N_3OBr$	346	54.41	5.10	14.93	55.12	6.18	13.63
12	$C_{17}H_{19}N_4OBr$	375	50.81	4.26	11.11	51.43	5.82	10.56
13	$C_{16}H_{16}N_3O_3Br$	378	55.51	4.66	12.14	56.62	3.88	11.36
14	$C_{16}H_{16}N_3OBr$	346	53.20	4.74	15.51	54.12	5.13	14.92
15	$C_{16}H_{16}N_4OBr$	361	53.20	4.74	15.51	55.00	6.12	15.11
16	$C_{16}H_{17}N_4OBr$	361	49.12	3.86	14.32	50.23	4.12	13.66
17	$C_{16}H_{15}N_4O_3Br$	391	53.05	4.45	11.60	54.12	3.88	12.43
18	$C_{16}H_{16}N_3O_2Br$	362	56.68	5.04	11.66	55.12	6.68	11.20
19	$C_{17}H_{18}N_3OBr$	360	61.78	4.44	10.29	62.28	5.12	11.45
20	$C_{21}H_{18}N_3OBr$	408	46.50	4.97	14.79	46.00	5.69	13.64
21	$C_{11}H_{14}N_3OBr$	284	48.34	5.41	14.09	49.16	6.68	13.22
22	$C_{12}H_{16}N_3OBr$	298	47.87	4.94	12.88	48.14	5.88	13.62
23	$C_{13}H_{16}N_3O_2Br$	326	51.54	6.18	12.88	52.07	8.43	11.43
24	$C_{14}H_{21}N_3OBr$	327	50.34	5.20	13.55	51.23	6.12	14.13
25	$C_{13}H_{16}N_3OBr$	310	51.86	5.60	12.46	52.16	6.18	13.78
26	$C_{14}H_{18}N_3OBr$	324	51.44	3.51	15.01	51.13	5.01	16.24

(**20**): Yield: 57%, m.p.: 181 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.90 (s, 6H, 2-CH_3), 2.14 (s, 3H, ArCH_3), 6.80~7.13 (m, 3 H, ArH), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.80 (s, 1H, CONH, D_2O exchangeable).

9. Butan-2-one

N-(4-bromo-3-methylphenyl) semicarbazone (**21**): Yield: 53%, m.p.: 161 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.50~1.53 (t, 3H, CH_3), 1.92~1.98 (q, 2H, CH_2), 2.02 (s, 3H, CH_3), 2.20 (s, 3H, ArCH_3) 7.19~7.26 (m, 3H, ArH), 8.46 (s, 1H, ArNH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable).

10. Heptan-2-one

N-(4-bromo-3-methylphenyl) semicarbazone (**22**): Yield: 50%, m.p.: 180 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.50~1.52 (t, 3H, CH_3), 1.74~1.88 (m, 8H, CH_2), 2.04 (s, 3H, CH_3), 2.22 (s, 3H, ArCH_3), 7.19~7.24 (m, 3H, ArH), 8.30 (s, 1H, ArNH, D_2O exchangeable), 9.68 (s, 1H, CONH, D_2O exchangeable).

11. N-(4-bromo-3-methylphenyl)

2-(2-oxoindolin-3-ylidine) hydrazine carboxamide (**26**): Yield: 68%, m.p.: 142 °C, ^{1}H-NMR (DMSO-d_6) δ: 2.32 (s, 3H, ArCH_3), 7.10~7.75 (m, 7H, ArH), 8.88 (s, 1H, ArNH, D_2O exchangeable), 10.22 (s, 1H, CONH, D_2O exchangeable), 11.02 (s, 1H, CONH of isatin, D_2O exchangeable).

Pharmacology

Preliminary anticonvulsant evaluation was done using reported procedures (Krall *et al*., 1978; Porter *et al*., 1984; Kupferberg, 1989). Male albino mice (CF-1 Strain, 18~25 g) and male albino rats (Sprague-Dawely, 100~150 g) were used as experimental animals. All the test compounds were suspended in 30% PEG. The animals were kept at 24 °C, in groups of 5 per cage receiving chow pellets and water. The light dark cycle was 12 h:12 h. Efforts were made to avoid any unnecessary distress to the animals. All the

animal tests were performed in accordance with the Animal Ethics Society of the Institute.

Anticonvulsant screening: All the test compounds were administered intraperitoneally in a volume of 0.01 ml/g body weights for mice and 0.004 ml/g body weights for rats at doses of 30, 100, 300 mg/kg to one to four mice. Anticonvulsant activity assessed after 30 min and 4 h intervals of administration. Activity was established using MES, scPTZ (subcutaneous pentylenetetrazole), scSTY (subcutaneous strychnine) and subcutaneous picrotoxin tests and data are presented in the Tables 1 and 3.

Table 3 Biological data of some selected 4-bromo-3-methylphenyl semicarbazones

Compound	Intraperitoneal injection in mice			
	scSTY screen		Picrotoxin screen	
	0.5 h	4 h	0.5 h	4 h
1	300	300	−	−
4	−	−	−	−
5	300	−	300	300
8	−	−	−	−
10	300	−	−	−
11	−	−	−	−
12	300	300	300	−
13	−	−	−	−
15	100	100	100	100
16	100	100	100	100
17	300	−	100	100
19	100	100	300	300
20	100	100	300	300
21	−	−	−	−
22	−	−	−	−
23	100	100	300	−
25	−	−	100	100
26	100	100	300	300
Phenytoin	−	−	×	×
Ethsuximide	300	−	−	−

The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (−) indicates an absence of activity at maximum dose administered (300 mg/kg). The cross (×) indicates the compounds not tested in animals. Numbers of animals used in each doses were one to four

Neurotoxicity screen: Rotarod test was done to detect the motor deficit in mice. Animals were divided in groups (**4**~**8**) and trained to stay on accelerating Rotarod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals (able to stay on the Rotarod for at least 2 consecutive periods of 90 s) were given an i.p. injection of the test compounds in the doses of 30, 100 and 300 mg/kg. Neurological deficit 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. The dose at which the animal fell off the rod was determined, and data are presented in the Table 1.

Behavioral test: CNS (central nervous system) depression was measured by evaluating the locomotor activity of the animal using actophotometer (Boisser and Simon, 1965). Animals were acclimatized to the dark environment 24 h before the test. Activity was noted after 30 min and 1 h of drug injection (100 mg/kg, i.p.). The control was administered with the 30% PEG only. The behaviour of the animal inside the photocell was recorded as digital score. Increased score represented good behavioral activity (Table 4).

CNS depressant study: Behavioral depression was studied by swimming despair test (Porsolt *et al*., 1978). Mice were dropped, one at a time in Plexiglas's cylinder (diameter: 45 cm, height: 20 cm), containing water up to a height of 15 cm at (25±2) °C. After a brief spell of vigorous activity, they showed a posture of immobility, characterized by floating motionless in water making only those movements necessary to keep head over water. This immobility reflects state of depression. After allowing one minute for acclimatization each mouse was observed for 5 min for immobility. Thus immobility time (i.e. total duration of immobility in a period of 5 min) was recorded. Each mouse was subjected to this test 24 h prior to (control) and 60 min after the drug treatment (100 mg/kg, i.p.). Mean immobility time for each group of 6 mice was recorded and presented in Table 4.

GABA (γ-aminobutyric acid) level estimation in the brain tissues: The GABA assay was performed in brain tissue extracts enzymatically as previously described. Adult Wistar rats were divided into three groups of three animals each. After 2 h of drug administration (100 mg/kg, i.p.), the animals were decapitated and the brain regions, olfactory lobe, mid brain, cerebellum and medulla oblongata were dropped into separate vials containing 4~6 ml of ice cold 80% ethanol and processed further as described previously (Roberts, 1962).

Table 4 CNS study of 4-bromo-3-methylphenyl semicarbazones

Compound[a]	Actophotometer (locomotor activity score)[b]		Immobility time (s)[c]	
	0.5 h	1 h	Control	Test (after 1 h)
Control	318.00±13.68	288.50±11.31	–	–
1	174.67±2.93	133.50±4.26	161.00±9.04	190.50±6.40***
2	169.00±4.46	135.00±4.24	142.00±5.14	183.02±4.70
3	181.50±3.82	150.00±4.94	103.00±16.68	174.50±4.71
4	235.50±4.93	217.00±6.21	149.83±4.14	188.00±4.74
5	209.50±7.34	193.33±3.91	142.50±17.55	143.00±18.93 NS
6	181.00±4.72	161.00±4.46	151.00±17.58	144.00±11.95 NS
7	170.01±4.08	130.00±5.03	206.00±4.32	217.83±1.17
8	235.50±4.93	217.00±6.21	149.83±4.14	188.00±4.74
9	200.00±11.00	170.50±5.06	186.00±10.60	206.50±5.60 NS
10	194.50±5.59	140.57±3.94	174.50±5.59	204.17±3.94
11	212.50±9.32	179.00±6.08	203.33±3.21	208.00±3.44 NS
12	214.00±5.37	151.00±3.33	94.00±15.91	131.00±16.99 NS
13	347.33±14.35 NS	280.50±17.49 NS	164.00±17.69	152.50±14.91 NS
14	190.17±4.73	201.50±5.67	121.00±9.36	114.50±2.09 NS
15	196.00±19.73	175.00±8.12	100.50±5.41	186.00±2.92***
16	203.50±5.89	191.50±5.73	158.00±10.99	166.50±6.08 NS
17	281.33±11.42 NS	208.20±9.77	126.00±11.54	155.00±15.46 NS
18	280.83±18.94 NS	274.50±11.77 NS	193.50±10.00	165.83±8.27 NS
19	192.50±4.54	172.00±4.77	106.67±13.11	150.00±11.64***
20	207.00±9.13	182.00±5.45	162.00±4.36	189.00±4.83
21	228.50±16.57*	173.00±5.87	150.00±10.20	169.00±11.26 NS
22	231.50±15.70**	263.00±15.70 NS	149.00±3.53	150.50±2.88 NS
23	192.00±4.91	160.00±7.75	137.00±7.74	182.00±4.38
24	178.00±6.65	158.00±9.27	94.00±11.32	121.50±17.35 NS
25	255.67±16.68*	223.00±17.82**	187.00±3.12	183.00±5.34 NS
26	206.00±4.47	175.50±4.88	139.00±14.36	186.83±5.16***
Phenytoin[d]	104.11±14.56	106.23±12.44	–	–
Carbamazepine[d]	–	–	131.50±9.32	207.33±8.49

[a]The compounds were tested at the dose level of 100 mg/kg; [b]Each value represents the mean±*SEM* of six mice significantly different from the control at $P<0.0005$, $^{*}P<0.005$ and $^{**}P<0.05$ and NS denotes the values which are not significant (Student *t*-test); [c]Each value represents the mean±*SEM* of six mice significantly different from the control at $P<0.005$ and $^{***}P<0.05$ and NS denotes the values which are not significant (Student *t*-test); [d]The compounds were tested at the dose level of 30 mg/kg

Quantum mechanical calculations

Quantum mechanical (QM) calculations were carried out using Argus Lab version 4.0.1. The 3D structures of the compounds were geometry optimized using Hamiltonian PM3 (Parameterized Method 3) semi-empirical QM method (Dewar and Walter, 1977). For the estimation of HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energy and surfaces, the single-point energy calculation using Hamiltonian ZINDO (Zerner's intermediate neglect of differential overlap) and RHF-SCF (restricted Hartree-fock-single consistent field) method (Basis set STO-6G) (Zerner, 1991) was employed. The HOMO surfaces were visualized using a contour value of 0.05 in opaque mode using black and gray for positive and negative phase of the orbital in space.

RESULTS AND DISCUSSION

The initial anticonvulsant evaluation of the

4-bromo-3-methylphenyl semicarbazones was established by electrical and chemical tests, using the standard protocol. The electrical test employed was maximal electroshock seizure (MES) pattern test and chemical test was scPTZ seizure threshold test. The acute neurological toxicity was determined by the Rotarod test. The anticonvulsant and neurotoxicity test results for the titled compounds are reported in Table 1, along with the literature data for the standard drugs. The titled semicarbazones (**1**~**26**) were administered at 30, 100, 300 mg/kg doses intraperitoneally. All the compounds except **2** and **4** exhibited activity in the preliminary MES screen, indicative of their ability to prevent seizure spread. At the dose of 100 mg/kg, compounds that showed protection in half or more of the tested mice were **1**, **9**, **16**~**20**, **25**, and **26** whereas other active compounds showed protection at 300 mg/kg. Compounds **1**, **6**, **13**, **19**, **20**, **25**, **26** were active at both 0.5 h and 4 h intervals i.e. exhibited prolonged duration of action whereas other compounds showed rapid onset (0.5 h) with a shorter duration of action.

In the scPTZ test, a test used to identify compounds that elevate seizures threshold, all of the compounds except **2**, **4**, **7**, **8**, **10**, and **22** showed protection. Only compounds **1** and **20** showed activity for a prolonged period and all other active compounds were active for a shorter duration only. In the acute neurological toxicity screen, compounds **2**, **4** and **8** were found to be devoid of any neurotoxicity at the highest dose administered (300 mg/kg). There was no separation between the anticonvulsive dose and the neurotoxic dose, but the neurotoxicity exhibited was only for a short duration (0.5 h). Compounds **13**, **17**, **21**, **23**, and **25**~**26** were found to be more neurotoxic (100 mg/kg).

Some selected compounds were carried onto the second phase of anticonvulsant screening in the scSTY and subcutaneous picrotoxin (scPIC) induced threshold models. Compounds except **4**, **8**, **11**, **13**, **21**, **22** and **25** exhibited protection against scSTY model. The compounds that showed anti-scSTY activity at 100 mg/kg include **15**, **16**, **19**, **20**, **23** and **26**. Except compounds **5**, **10** and **17** other compounds exhibited a long duration of action i.e. at both 0.5 h and 4 h time period. In the subcutaneous picrotoxin screen, compounds except **1**, **4**, **8**, **10**, **11**, **13**, **21**, **22** exhibited protections, pinpointing the GABA mediated mechanism of the synthesized compounds. Compounds **15**~**17** and **25** showed protections at 100 mg/kg and other active compounds showed activity at 300 mg/kg. Except compounds **12** and **23**, all other compounds showed protection in both 0.5 h and 4 h intervals.

In the behavioral despair test, except compounds **13**, **17**, **18**, and **22**, all others showed significant decrease in motor activity as indicated by the actophotometer scores. The standard drug phenytoin also showed significant decrease in the locomotor activity. The semicarbazones derivatives were also studied for CNS depressant effect in the Porsolt's forced swim pool test and compared with carbamazepine. Most of the active compounds except **1**~**4**, **7**, **8**, **10**, **15**, **19**, **20**, **23**, and **26** did not show any significant increase in the immobility time as compared to the control, indicating lesser CNS depressant effect than the conventional drugs.

In order to explore the mechanism of anticonvulsant activity of these derivatives, two compounds (**20** and **25**) were subjected to neurochemical investigation to determine GABA level in the different regions of the brain, as there is regional difference in GABA concentration within the CNS. Both the compounds were found to show significant increase in the GABA level in the olfactory lobe and cerebellum regions of rat brain with respect to the control (Table 5). These results suggest that aryl semicarbazones may exert their anticonvulsant effect through GABA-mediated mechanism.

In this paper, it is clear that N^4-(4-bromo-3-methylphenyl) semicarbazones have the potential to

Table 5 GABA concentration in μg/100 g weight of rat brain

Compound	GABA concentrations in different region of rat brain[a]			
	Olfactory lobe	Mid brain	Cerebellum	Medulla oblongata
Control	40.5±0.28	101.6±0.30	39.3±0.05	93.4±0.02
20	112.4±0.45	52.1±0.40	108.3±0.54	71.8±0.35
25	29.9±0.09	74.0±0.47	102.1±0.24	72.1±0.08
Clobazam	143.0±0.15	713.0±0.47	235.0±0.25	188.0±0.30

[a]Each value represents the mean±*SEM* of three rats significantly different from the control at $P<0.0001$

treat a wide range of seizure types by their multiple mechanism of action as indicated by their activity in four animal models of seizures. Nine compounds (**5**, **12**, **15**~**17**, **19**, **20**, **23**, and **26**) showed activity in all the screens (MES, scPTZ, scSTY, and scPIC), exhibiting a broad spectrum of anticonvulsant activity.

QM modelling methods predict the behavior of electrons. They are thus the most fundamental and accurate theoretical tools available to predict molecular properties. In theory, QM methods enable completely accurate prediction of any property; there are some important classes of behavior (notably reactivity, electronic, magnetic, and optical behavior) that can only be modelled using QM methods, because they are determined by electronic behavior that cannot be approximated well using other methods.

In the present study the titled compounds were geometry optimized by semi-empirical PM3 QM method and subjected to single-point energy calculation to determine their HOMO and LUMO energies (E_{HOMO} and E_{LUMO}). The greater E_{HOMO} is, the greater the electron-donating capability; conversely, the smaller E_{LUMO} is, the smaller the resistance to accept electrons. Compounds that present larger values of E_{HOMO} are more electron donor and the compounds that present smaller values of E_{LUMO} are more electron acceptor. These variables are interpreted as measures of molecular reactivity and stability. As E_{HOMO} increases (relative to other molecules), the molecule is less stable and more reactive. For E_{LUMO}, the situation is the opposite (Chen *et al*., 2005). The higher energy values (E_{HOMO} and E_{LUMO}) for alkyl ketone compounds when compared to aryl aldehyde/ketone compounds could be due to the absence of resonance stability which is seen with the latter. The difference (ΔE) in E_{HOMO} and E_{LUMO} was also determined and presented in Table 6. The higher the ΔE, the higher is the anticonvulsant activity as seen with compounds **16** and **18**.

Analysis of the molecular orbital surfaces also reveals the extent of conjugation, and elucidates the nature of extended pi-systems in particular. Electron-rich and electron-poor species tend to reveal the localization or delocalization of the partial or full charge by the shape of the HOMO or LUMO. Molecular orbitals, when viewed in a qualitative graphical representation, can provide insight into the nature of reactivity, and some of the structural and physical properties of molecules. Well known concepts such as conjugation, aromaticity and lone pairs are well illustrated by molecular orbitals.

Table 6 E_{HOMO}, E_{LUMO} and ΔE values for 4-bromo-3-methylphenyl semicarbazones

Compound	E_{HOMO} (eigenvalues)	E_{LUMO} (eigenvalues)	ΔE
1	−0.342	−0.033	−0.309
2	−0.338	−0.144	−0.194
3	−0.339	−0.056	−0.283
4	−0.344	−0.061	−0.283
5	−0.339	−0.052	−0.287
6	−0.337	−0.048	−0.289
7	−0.359	−0.073	−0.286
8	−0.361	−0.077	−0.284
9	−0.340	−0.051	−0.238
10	−0.345	−0.069	−0.275
11	−0.332	−0.052	−0.280
12	−0.342	−0.057	−0.285
13	−0.330	−0.044	−0.286
14	−0.326	−0.052	−0.274
15	−0.335	−0.049	−0.286
16	−0.357	−0.062	−0.295
17	−0.316	−0.047	−0.269
18	−0.335	−0.044	−0.291
19	−0.334	−0.047	−0.287
20	−0.346	−0.011	−0.335
21	−0.346	−0.021	−0.325
22	−0.352	−0.025	−0.327
23	−0.348	−0.020	−0.328
24	−0.348	−0.021	−0.327
25	−0.349	−0.019	−0.330
26	−0.350	−0.056	−0.294

E_{HOMO}, E_{LUMO} and ΔE calculation using Hamiltonian ZINDO and RHF-SCF method

The HOMO surface was visualized for the titled compounds and the following observations were made. As proposed earlier, the anticonvulsant pharmacophore requirements for aryl semicarbazones include an aryl ring, a hydrogen-bonding domain and an electron donor system. HOMO surface analysis also confirmed this hypothesis wherein compounds **1** and **9** showed a delocalized orbital surface over an aryl ring, imine group (electron donor), and the amide moiety (hydrogen acceptor-donor unit) (Fig.2). Similar surfaces were observed with all active compounds. Compounds which did not comply with this hypothesis were found to be inactive. Undelocalized or splitting of electron cloud on the aryl ring was

found to be not favorable for activity as represented in the figure for compound **2** and **4**. It was also seen that unsymmetrical molecular orbital surfaces resulted in decrease in activity. This observation was made with other less active and inactive compounds. This is a preliminary report on quantum mechanical modelling on aryl semicarbazones. In future further studies on other well-known aryl semicarbazones have to be dealt in detail.

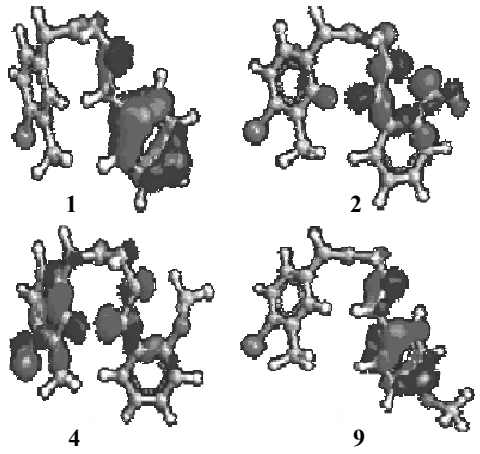

Fig.2 HOMO surface visualization (contour value= 0.05) of some representative compounds in opaque mode. The colors indicate the phase of the orbital in space (black for positive and gray for negative)

CONCLUSION

Thus the present study demonstrated that 3-bromo-4-methylphenyl semicarbazones possess broad spectrum anticonvulsant activity as indicated by their effect in pentylenetetrazole, strychnine, picrotoxin and maximal electroshock seizures models in resemblance to other aryl semicarbazone derivatives reported earlier (Yogeeswari *et al*., 2004a; 2005b; Thirumurugan *et al*., 2006), hence these compounds in addition to their action at the sodium channels, might also act through the GABA or glycine receptors. The substitution with bromo group in combination with the methyl group was found to be beneficial for the anticonvulsant activity. The QM modelling results confirmed the importance of the 4-point pharmocophore requirement as proposed earlier for aryl semicarbazones (Yogeeswari *et al*., 2005b).

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