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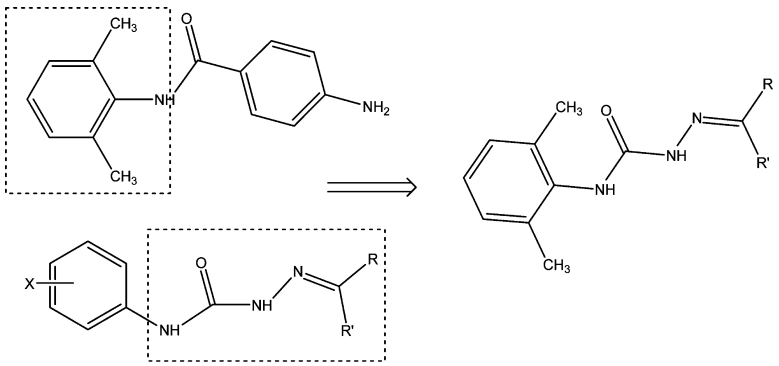

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Discovery of *N*-(2,6-Dimethylphenyl)-Substituted Semicarbazones as Anticonvulsants: Hybrid Pharmacophore-Based Design

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Epilepsy is the most common primary neurological disorder known. In the past decade, various aryl semicarbazones have been designed that were structurally dissimilar from many common anticonvulsants containing the dicarboximide function (CONRCO), which may contribute to toxic side effects. In the present work various N^4-(2,6-dimethylphenyl) semicarbazones were designed as pharmacophore hybrids between the aryl semicarbazones and ameltolide. A three-dimensional four-point pharmacophore model was developed for anticonvulsants, and the title compounds were found to match with ralitoline. All of the compounds exhibited anticonvulsant activity in the maximal electroshock test when administered by both intraperitoneal and oral routes. Compound N^1-(2,6-dimethylphenyl)-N^4-(2-hydroxybenzaldehyde) semicarbazone (**9**) emerged as a prototype with wide spectrum anticonvulsant agent active in five models of seizure with no neurotoxicity and hepatotoxicity. Compound **9** increased the 4-aminobutyric acid (GABA) level by 118% and inhibited the GABA transaminase enzyme both in vitro and ex vivo.

Introduction

Epilepsy is a collective term that includes over 40 different types of human seizure disorders.[1] Approximately 1% of the world population at any one time (∼50 million people worldwide) is afflicted with this serious neurological disorder.[2] Although the current drugs provide adequate seizure control in many patients, it is roughly estimated that up to 28−30% of patients are poorly treated with the available antiepileptic drugs (AEDs). Moreover, many AEDs have serious side effects,[3,4] and lifelong medication may be required. Hence, with all of these factors in mind, it has been suggested that the focus of epilepsy research should be directed to identifying the underlying mechanism of epileptogenesis and the subsequent "expression" of seizure activity, rather than resorting primarily to symptom control, that is, mere suppression of seizures.[5]

In the past decade, aryl semicarbazones have been designed that were structurally dissimilar from many common anticonvulsants containing the dicarboximide function (CONRCO), which may contribute to toxic side effects.[6] Consistent advances in the design of novel anticonvulsant agents have been obtained through the works of Dimmock and his collegues,[7−10] which included various aryl semicarbazones and (aryloxy) aryl semicarbazones. The terminal primary amino group was implicated in hydrogen bonding.[11] Using the semicarbazone template, Pandeya and co-workers[12−16] demonstrated through a series of successive works the significant anticonvulsant potential in animal epilepsy models for the N^4-(substituted phenyl) semicarbazones. These aryl semicarbazones were found to be less neurotoxic than conventional AEDs and were found to show no sedative−hypnotic activity.[12] Recently, 4-sulfamoylphenyl-substituted aryl semicarbazones were reported to exhibit greater potency than sodium valproate in maximal electroshock seizure (MES) and subcutaneous strychnine (scSTY)-induced seizure threshold tests.[17]

Although the mechanism of action of aryl semicarbazones was never completely studied, it was reported that similarly substituted semicarbazides had comparable efficacy and seem to be acting by inhibition of sodium ion channels.[18] In another study on 4-bromobenzaldehyde semicarbazone was found to have no effect on the rat brain γ-aminobutyric acid (GABA) concentration and the GABA transaminase (GABA-T) enzyme.[19] A representative semicarbazone was reported to possess anticonvulsant activity by means of its sodium current inhibiting capacity in the nerve cells.[20] Recent studies in our laboratory on various *N*-(3-bromophenyl)- and *N*-(4-ethoxyphenyl)-substituted semicarbazones have revealed for the first time that N^4-(substituted phenyl) semicarbazones could act through GABA mediation[21] and inhibit the enzyme GABA-T.[22]

Substitution in the 2-position of the phenyl ring with electron-donating groups was generally beneficial to activity, and the exception appears to be those groups that are capable of hydrogen bonding such as OH and NH_2, where activity was found to be decreased.[23] The importance of the *o*-methyl group in the aryl ring for anticonvulsant activity had been depicted in many studies.[24−28] Clark et al.[29,30] synthesized a number of benzamides of aminobenzoic acids having potent activity against MES seizures in mice. The 2,6-dimethylanilide ameltolide proved to be the most potent compound arising from these studies with an ED_{50} of 2.6 mg/kg

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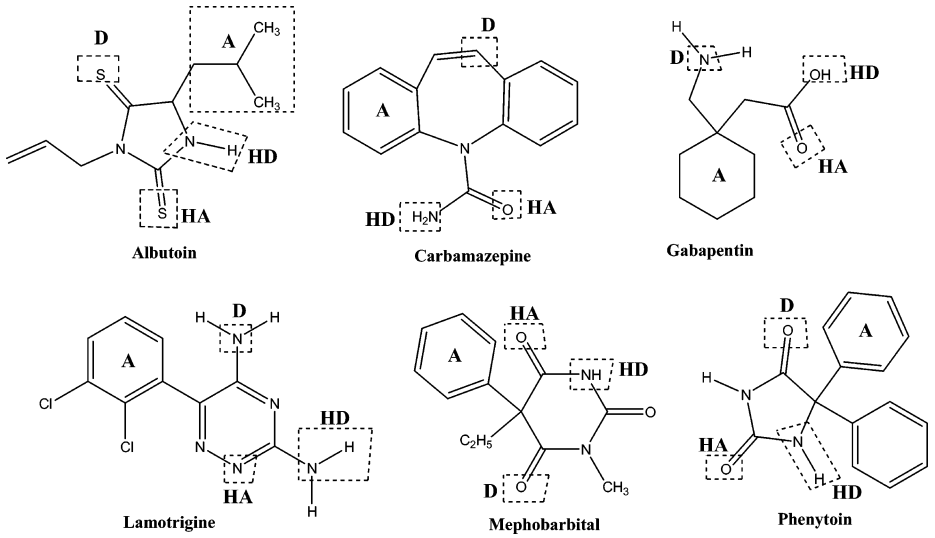


Figure 1. Structures of well-known anticonvulsants.

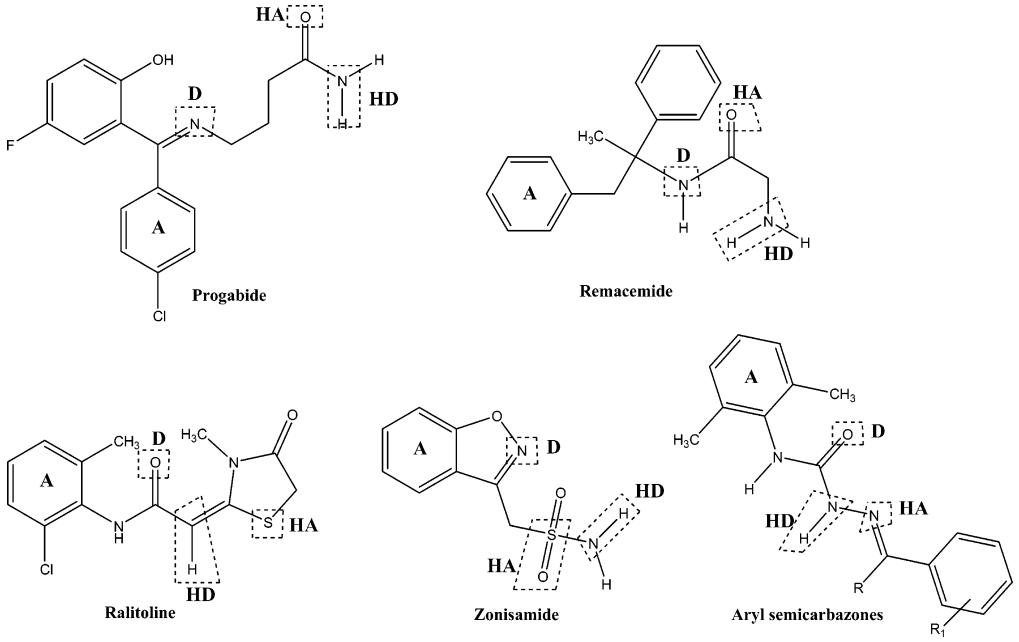


Figure 2. Structures of well-known anticonvulsants.

when administered by an intraperitoneal (ip) route in mice. 4-Amino-*N*-(2,6-dimethylphenyl)phthalimide was previously designed from the models of ameltolide and thalidomide.[31] Because the N^4-(substituted phenyl) semicarbazones represent a novel class of compounds that exhibit anticonvulsant activity, further structural modification of the substituted phenyl moiety was of interest. Various N^4-(2,6-dimethylphenyl)-substituted semicarbazones were prepared to find a superior compound that would exhibit a broad spectrum of anticonvulsant activity with less or no toxicity. These compounds were evaluated for central nervous system (CNS) depression, motor dysfunction, and hepatotoxicity and compared with phenytoin. These compounds were found to exhibit anticonvulsant activity through GABA mediation as evident from the elevation of GABA levels in rat brain and inhibition of GABA-T in vitro (*Pseudomonas fluorescens*) and ex vivo (rat brain tissue). This paper is the first of its kind to report on semicarbazones with no neurotoxic and hepatotoxic side effects.

Chemistry and Molecular Design

Earlier two-dimensional (2D) modeling on anticonvulsants has identified that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a special spatial arrangement are to be recommended for anticonvulsant activity.[32−34] However, the conclusions of these studies were not related to other substance classes acting at the same receptor site. In the past a pharmacophore model had been suggested on anticonvulsant acting through blocking of voltage-gated sodium channel.[35] In the present study, the 10 well-known and structurally different compounds with anticonvulsant activity, albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, and zonisamide (Figures 1 and 2) with different mechanisms of action, were selected so as to propose a generalized pharmacophore model. To build a pharmacophore based on the structures of anticonvulsant compounds, two methods were applied. In the first method a set of minimum energy conforma-

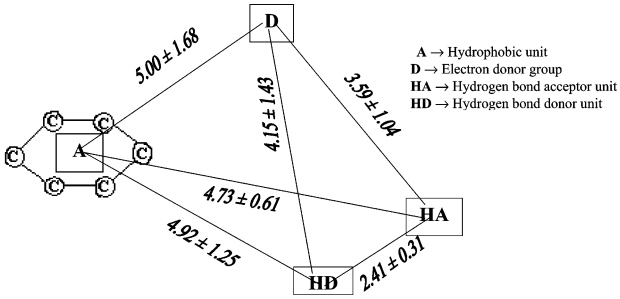


Figure 3. Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 (Alchemy 2000) and CHARMm (ACD) parametrization.

tions for each structure was generated and the common structural features were noted. In another method all possible conformations for each structure were considered to evaluate shared orientations of the common functional groups. The pharmacophore group's distance estimation was done by molecular mechanics calculations with the force fields based on both CHARMm force fields and MM3 parametrization. In the present work, energy minimization was performed on albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, zonisamide, and the aryl semicarbazones using both CHARMm (ACD 3D viewer) and MM3 (Alchemy 2000) parametrization. A systemic conformational search was performed using the Alchemy 2000 program. The crucial structural components that were included in the four-point pharmacophore model (Figure 3) were the aryl ring center or the lipophilic group (A), an electron donor atom (D), a hydrogen bond acceptor (HA), and a hydrogen bond donor (HD). In agreement with all of the compounds, we determined the center of the aromatic ring as the reference point for A. The distance between the four pharmacophoric points was calculated for a minimum of four different conformations and represented as mean ± standard deviation (Table 1). An average distance range for every point was obtained and compared for aryl semicarbazones. The drugs albutoin (A−HD), remacemide (HA−HD), and progabide (A−D, HD−D, HA−D) showed distances out of range when compared to the average distance. Now it may be interesting to examine whether the aryl semicarbazones reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that the aryl semicarbazones did fulfill the essential demands of the pharmacophore when compared to the average

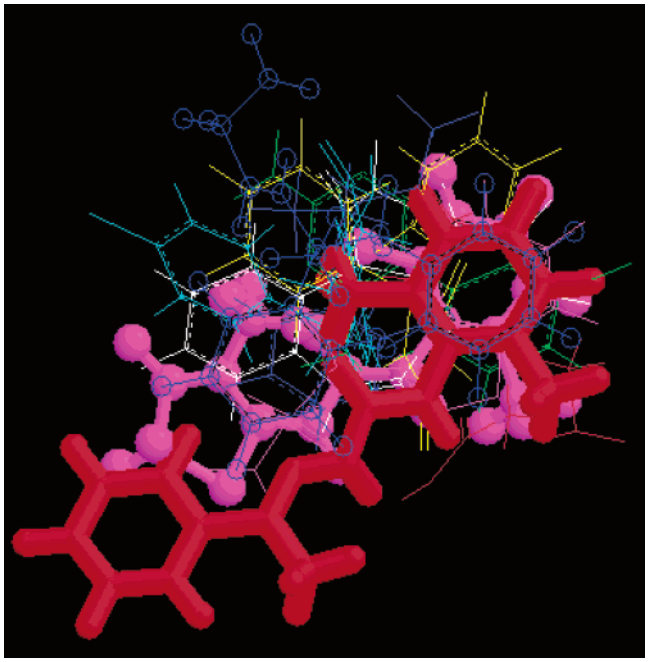


Figure 4. Pharmacophore mapping of aryl semicarbazone (**13**, red tube) with nine standard drugs that include carbamazepine (white), gabapentin (red stick), Lamotrigine (green), mephobarbital (blue sticks), phenytoin (yellow), progabide (cyan), ralitoline (pink ball-and-stick), remacemide (blue thin ball-and-stick), zonisamide (pink stick).

distance requirement. A representative of the proposed aryl semicarbazone (**13**) was superimposed with the nine standard drugs by merging the energy-minimized structures by MM3 force fields and is represented in Figure 4. In this superimposition study, albutoin was not included, as the number of atoms in the hydrophobic area did not match with other compounds under study. The proposed aryl semicarbazone (**13**) seems to fit better with ralitoline. With this as background, the present work highlights the importance of the synthesis of prototypes of aryl semicarbazones.

The synthesis of N^4-(2,6-dimethylphenyl) semicarbazones was accomplished as presented in Figure 5. The method was based on an earlier reported procedure.[36] The starting material, 2,6-dimethylaniline, was treated with phenyl chloroformate in chloroform at room temperature to yield phenyl *N*-(2,6-dimethylphenyl) carbamate. The carbamate on condensation with hydrazine hydrate in methylene chloride gave the N^4-(2,6-dimethylphenyl) semicarbazide. The semicarbazone derivatives

Table 1. Distance Ranges between the Essential Structural Elements A, D, and HA−HD[a]

compd	A−HA	A−HD	A−D	HA−HD	HD−D	HA−D
albutoin	5.06 ± 0.05	2.53 ± 0.04	4.81 ± 1.66	2.72 ± 0.22	4.16 ± 1.45	4.35 ± 1.65
carbamazepine	3.73 ± 1.02	4.41 ± 0.09	3.86 ± 0.68	2.28 ± 0.06	5.24 ± 0.03	3.88 ± 1.03
gabapentin	4.78 ± 0.50	4.64 ± 0.12	4.00 ± 0.45	2.26 ± 0.21	2.64 ± 0.05	3.36 ± 0.03
lamotrigine	5.17 ± 0.70	5.70 ± 1.94	4.50 ± 0.88	2.42 ± 0.01	3.48 ± 0.74	2.53 ± 0.26
mephobarbital	5.50 ± 1.69	5.04 ± 0.20	4.64 ± 0.53	2.26 ± 0.01	3.19 ± 1.18	3.88 ± 1.12
phenytoin	4.82 ± 0.06	4.97 ± 1.22	4.51 ± 0.03	2.26 ± 0.12	4.15 ± 0.82	2.43 ± 0560
progabide	4.02 ± 0.28	4.32 ± 2.14	8.96 ± 0.68	2.67 ± 0.06	7.40 ± 1.12	5.73 ± 0.72
ralitoline	4.24 ± 0.05	4.43 ± 1.54	6.89 ± 1.42	2.29 ± 0.05	2.84 ± 0.15	3.47 ± 1.13
remacemide	5.55 ± 1.51	7.39 ± 1.64	4.54 ± 0.95	3.01 ± 0.25	3.40 ± 0.06	2.29 ± 0.00
zonisamide	4.51 ± 0.13	5.82 ± 0.90	3.29 ± 0.49	1.89 ± 0.53	5.02 ± 1.09	4.07 ± 0.89
av distance	**4.73 ± 0.61**	**4.92 ± 1.25**	**5.00 ± 1.68**	**2.41 ± 0.31**	**4.15 ± 1.43**	**3.59 ± 1.04**
aryl semicarbazone (**13**)	4.19 ± 0.18	3.96 ± 1.58	6.59 ± 0.91	2.26 ± 0.03	2.95 ± 1.43	3.20 ± 0.85

[a] Distances calculated for 3D optimized structures at least for each four conformations using MM3 parametrization (Alchemy 2000, Tripos Co.) and represented as mean ± SD in angstroms.

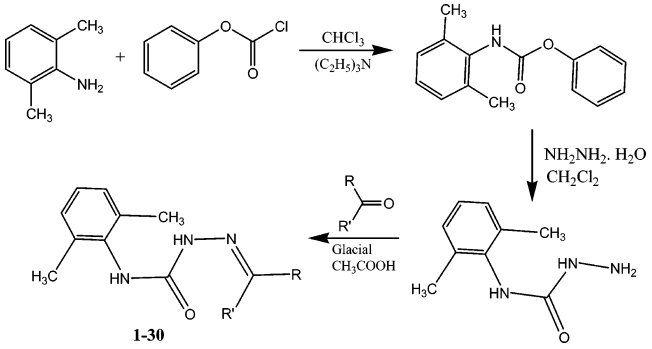
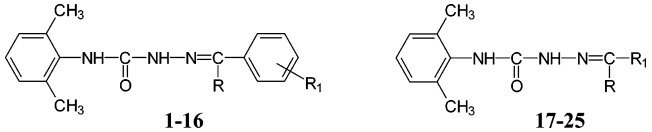


Figure 5. Synthetic protocol of N^4-(2,6-dimethylphenyl) semicarbazones.

Table 2. Physical Data of the 2,6-Dimethylphenyl Semicarbazones



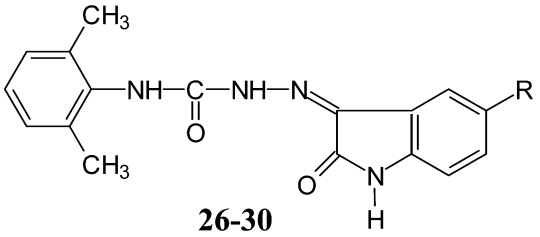
compd	substituent R	substituent R_1	yield (%)	mp (°C)	R_f (chloroform/methanol 9:1)
1	H	H	57	213	0.82
2	H	4-CH_3	52	199	0.80
3	H	4-OCH_3	62	201	0.78
4	H	4-*N*-$(CH_3)_2$	64	209	0.81
5	H	4-NO_2	85	256	0.90
6	H	4-OH, 3-OCH_3	77	236	0.77
7	H	3-NO_2	83	224	0.91
8	H	2-Cl	53	208	0.85
9	H	2-OH	81	214	0.81
10	H	2-NO_2	83	233	0.93
11	CH_3	H	60	205	0.79
12	CH_3	4-OH	50	200	0.82
13	CH_3	4-NH_2	53	194	0.76
14	CH_3	4-NO_2	64	256	0.90
15	CH_3	3-NH_2	51	198	0.77
16	CH_3	2-OH	52	230	0.75
17	H	2-furfuryl	59	>270	0.70
18	CH_3	CH_3	60	>270	0.71
19	CH_3	CH_2CH_3	57	>270	0.72
20	CH_3	$CH_2CH(CH_3)_2$	54	>270	0.69
21	CH_3	CH_2COCH_3	56	250	0.68
22	C_6H_5	C_6H_5	82	202	0.88
23	$C_6H_5CH_2$	$C_6H_5CH_2$	90	205	0.76
24	CRR_1 = cyclohexylene		58	>270	0.82
25	CRR_1 = cyclopentylene		57	>270	0.81

(**1**−**30**; Tables 2 and 3) were prepared by reaction of the appropriate aryl/alkyl aldehyde or ketone or isatin derivatives with the N^4-(2,6-dimethylphenyl) semicarbazide. In general, the IR spectra showed the C=N peak at 1638−1590 cm^{-1} and the NH stretching vibrations at 3450 cm^{-1}. The synthesized compounds exhibited characteristic amide bonds at 3300−3190 and 1700−1680 cm^{-1}.

Results and Discussion

As with any other class of drugs, the preclinical discovery and development of a new chemical entity for the treatment of epilepsy rely heavily on the use of predictable animal models. At the present time, there are three in vivo models that are routinely used by most AED discovery programs. They include the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazol (scPTZ), and the kindling model. Of these, the MES and scPTZ seizure models represent the two

Table 3. Physical Data of the 2,6-Dimethylphenyl Semicarbazones with Isatin Derivatives



compd	R	yield (%)	mp (°C)	R_f (chloroform/methanol 9:1)
26	H	77	190	0.87
27	F	63	182	0.89
28	Cl	64	191	0.90
29	Br	60	222	0.89
30	CH_3	66	183	0.84

animal seizure models most widely used in the search for new AEDs.[37−39] All of the titled compounds were evaluated initially in the MES, scPTZ, and subcutaneous strychnine (scSTY) seizure models. The acute neurological toxicity (NT) was determined in the rotorod test. The obtained results are listed in Table 4. After ip injection in mice using doses of 30, 100, and 300 mg/kg, the following observations may be made. All of the compounds were active in the MES test, indicative of their ability to prevent seizure spread. At a dose of 100 mg/kg all compounds showed protection in half or more of the tested mice except **10**, **14**, and **23**. In the scPTZ screen all of the compounds showed activity at 0.5 h except **15**, **20**, **23**, **24**, **26**, and **29**, and none of the compounds showed protection in the 4 h period. There was no separation between the anticonvulsant dose against scPTZ and the neurotoxic dose (300 mg/kg). Promising data were obtained in the scSTY screen wherein the percentages of compounds that were active at the minimum doses of 30, 100, and 300 mg/kg were 60, 30, and 7, respectively, indicative of their ability to prevent seizure spread and possible interaction with glycine receptors. Therefore, these compounds may be useful in treating not only generalized tonic−clonic and complex partial seizures but also absence seizures. Compound **25** was found to be inactive in the scSTY screen. The neurological deficit caused by these compounds when compared with the standard drugs such as phenytoin and carbamazepine was less as these compounds exhibited neurotoxicity at the maximum dose utilized and after only 0.5 h after administration. All of the compounds except **15**, **20**, **23**−**26**, and **29** showed protection in the three animal models of seizures. Compounds **2**, **6**, **9**, **11**, **12**, **16**, and **19**, **20** emerged as anticonvulsants with no neurotoxicity.

A valuable property of a candidate anticonvulsant is its ability to inhibit convulsions when given by the oral route. All of the compounds were also examined for activity in the rat oral MES screen, and the data are presented in Table 4. Initially, a dose of 30 mg/kg was employed. The compounds have been compared with respect to the onset of action and duration of effect for which the number of animals protected at each time point is crucial, which could give some information on the biotransformation of the compounds under study. When compared with the total number of rats protected, compounds **6**, **9**, **18**, **19**, and **21** have shown greater

Table 4. Anticonvulsant Activity and Minimal Motor Impairment of 2,6-Dimethylphenyl Semicarbazones

	intraperitoneal administration to mice[a]						oral administration to rats[b]				
	MES		scPTZ	scSTY		NT[c]					
compd	0.5 h	4 h	0.5 h	0.5 h	2 h	0.5 h	0.25 h	0.5 h	1 h	2 h	4 h
1	100	100	300	30	100	300	1	3	3	3	4
2	100	300	300	100	100	–	0	2	3	4	3
3	100	300	300	30	100	300	1	4	3	3	3
4	100	300	300	100	100	300	1	2	2	3	4
5	100	300	300	30	100	300	1	1	2	1	1
6	100	300	300	30	100	–	3	3	4	3	4
7	100	300	300	30	100	300	1	1	3	1	3
8	100	300	300	30	100	300	2	1	1	1	4
9	100	300	300	30	100	–	1	3	3	4	4
10	300	300	300	100	–	300	2	2	2	2	2
11	100	300	300	30	100	–	1	2	4	3	3
12	100	300	300	30	100	–	1	2	4	3	4
13	100	300	300	30	100	300	1	3	4	2	4
14	300	300	300	100	300	300	1	2	2	3	1
15	100	300	–	100	300	300	1	3	1	2	3
16	100	300	300	100	300	–	3	1	2	3	4
17	100	300	300	100	300	300	0	2	1	4	3
18	100	300	300	100	100	300	3	4	4	3	2
19	100	300	300	30	100	–	1	4	4	3	3
20	100	300	–	30	100	–	1	2	2	4	2
21	100	300	300	100	300	300	2	4	2	4	4
22	100	300	300	300	–	300	1	1	1	1	0
23	300	300	–	300	–	300	0	1	1	0	0
24	100	300	–	100	300	300	1	1	2	4	3
25	100	300	300	–	–	300	2	2	1	3	1
26	100	300	–	30	100	300	2	2	1	1	3
27	100	300	300	30	100	300	1	1	1	1	3
28	100	300	300	30	100	300	1	1	0	1	1
29	100	300	–	30	100	300	1	1	0	2	0
30	100	300	300	30	100	300	1	1	3	3	3
phenytoin	30	30	–	–	–	100	1	4	3	3	3
carbamazepine	30	100	100	–	–	100	–	–	–	–	–

[a] Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice (three in each group). The animals were examined at 0.5 and 4.0 h. (For scSTY test, 0.5 and 2.0 h examinations were made.) A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg). [b] The data in the oral MES screen indicate the number of rats of four that were protected at a dose of 30 mg/kg. [c] NT indicates neurotoxicity screening using rotorod test.

Table 5. Anticonvulsant Activity of Some Selected Compounds against ScPIC-Induced Seizure Threshold Test after Intraperitoneal Injection into Mice

	intraperitoneal injection in mice[a]	
compd	scPIC screen	neurotoxicity screen
9	30	–
12	30	–
13	30	300
21	30	300
phenytoin	–	100
phenobarbital	30	100
valproic acid	30	300

[a] Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby bioactivity was deter-0 ined. A dash indicates no protection at the maximum dose of 300 mg/kg.

potency than phenytoin (14 animals protected) and compounds **1**, **3**, **12**, and **13** were equipotent with phenytoin.

Some selected compounds (**9**, **12**, **13**, and **21**) were evaluated in the subcutaneous picrotoxin (scPIC)-induced seizure threshold test, in which all of the compounds exhibited promising activity at the minimum dose of 30 mg/kg, and compounds **9** and **12** exhibited no neurotoxicity, indicative of the possible involvement of GABA in the anticonvulsant action (Table 5).

More complete data were obtained from the quantitative MES evaluation in mice and rats dosed intraperitoneally and orally, respectively. Results of the quantitative test for selected compounds, along with the data on the standard drug phenytoin, are reported in Table 6. The maximum dose administered for the generation of TD_{50} data was different for each compound due to problems in solubilization of the higher doses. In the mice ip MES screen, among the tested compounds (**4**, **6**, **7**, **10**, and **12**), compound **4** gave an ED_{50} of 18.33 mg/kg and a TD_{50} of >250 mg/kg, resulting in a high protection index (PI), that is, TD_{50}/ED_{50}, of >13.64 when

Table 6. Quantification Studies of 2,6-Dimethylphenyl Semicarbazones in the MES and Neurotoxicity Test in Rats

compd	route	t^a (h)	MES[b] ED_{50} (mg/kg)	neurotoxicity[b] TD_{50} (mg/kg)	PI[c]
2	oral	2.0	33.1 ± 1.34	>120	>3.62
4	ip	2.0	18.3 ± 0.69	>250	>13.6
6	ip	1.0	26.2 ± 1.08	>117	>4.4
7	ip	1.0	29.7 ± 1.80	>120	>4.0
9	oral	4.0	19.8 ± 1.11	>500	>25.1
10	ip	0.5	23.2 ± 1.27	>100	>4.3
12	ip	0.25	41.6 ± 0.94	>68	>1.6
13	oral	4.0	29.1 ± 1.00	>464	>15.9
19	oral	4.0	29.0 ± 0.81	>500	>17.2
21	oral	2.0	19.8 ± 0.60	>216	>10.8
phenytoin	oral	2.0	23.2 ± 4.28	>500	>21.6
	ip	2.0	6.48 ± 3.60	42.8	6.6

[a] Time to peak effect. [b] The compound was examined 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h after administration in 6−12 rats, and results are represented as mean ± SEM at 95% confidence limit. [c] Protection index, i.e., TD_{50}/ED_{50}.

Table 7. Hippocampal Kindling Screen

compd	animal	dose (mg/kg)	seizure score		ADD[a] (s)	
			predrug	drug	predrug	drug
7	rat 1	100	5	1	26−42	52
	rat 2		4−5	5	11−39	59
9	rat 1	130	5	1	26−39	59
	rat 2		5	1	30−39	25

[a] After discharge duration (time of maximum effect, 45 min). Result suggests ability to prevent/modify fully kindled seizures.

compared to phenytoin. The time to peak effect for compound **4** was found to be 2 h, similar to that of phenytoin. With an ED_{50} of 41.64 mg/kg and a TD_{50} > 68, compound **12** was less effective and more toxic than the other compounds. Quantitative data in the rat oral MES screen showed that compounds **9** and **21** were equipotent to phenytoin. The times to peak effect for compounds **9** and **21** were 4 and 2 h, respectively, when compared to 2 h for phenytoin. In the present work, compound **9** emerged as the most active compound with respect to PI of >25.19.

The convulsions produced in the kindled rat test are believed to constitute a suitable model for complex partial seizures evolving into generalized motor seizures in humans.[40,41] In the preliminary hippocampal-kindling screen in rats (ip), compounds **7** and **9** were administered at a single dose of 100 and 130 mg/kg, respectively, and the after discharge thresholds (ADT) at either 30, 60, or 90 min or more after administration were recorded. Seizure score and after discharge (AD) duration in seconds were also recorded, and the results are given in Table 7. The seizure severity was classified behaviorally according to Racine[42] as (1) immobility, eye closure, twitching of vibrissae, sniffing, and facial clonus; (2) head nodding associated with more severe facial clonus; (3) clonus of one of the fore limbs; (4) rearing often accomplished by bilateral forelimb clonus; and (5) all of the above plus loss of balance and falling, accomplished by generalized clonic seizures. The AD duration was the duration of limbic (stages 1−2) and/or motor seizures (stages 3−5). Behavioral alterations after administration of compounds **7** and **9** were determined at different time points after injection up to 2 min before amygdala stimulation. Time of maximum effect was found to be 45 min. The results suggest that compound **9** possessed an ability to prevent or modify fully kindled seizures. Compound **7** needs to be studied further with a third animal before its effectiveness in this model can be conclusively determined.

Some of the compounds were evaluated for the behavioral study using an actophotometer-scoring technique at a dose of 30 mg/kg. Compounds **7** and **26**−**30** did not show any behavioral despair effect in which all other compounds including the standard drug phenytoin showed a significant decrease in the locomotor activity as represented in Table 8. In a similar study using the swimming pool test, the immobility times after administration of some selected compounds were compared with carbamazepine (Table 8). Compounds **4**, **9**, **12**, **13**, and **19** were found to show no significant CNS depression compared with the control at $p < 0.05$. All other compounds tested were found to emerge as CNS depressants as they increased the immobility time. Some selected compounds (**2**, **4**, **6**, **7**, **9**, **10**, **12**, **13**, **19**, and **21**) were evaluated for the sedative−hypnotic effect using the pentobarbital-induced narcosis model. Except for compounds **2**, **7**, **10**, and **12** all other tested compounds did not potentiate or induce narcosis as presented in Table 8.

Hepatotoxic adverse drug reactions have contributed to the decline of many promising therapies. Importantly, the mortality rate of hepatic idiosyncratic drug reactions is quite high. Drug-induced liver disease is typically unpredictable, idiosyncratic, and rare. Studies have shown that phenytoin is a possible cause of acetaminophen hepatotoxicity,[43] and anticonvulsants such as carbamazepine[44] and valproic acid[45] were also found to enhance or show hepatotoxic side effects. In the present study some representative compounds (**2**,

Table 8. CNS Study of the Selected Compounds

compd[c]	activity score using actophotometer[a]			immobility time in Porsolt's swimpool test[b]		mean sleeping time[d] (min)
	control (24h before)	post-treatment 0.5 h after	post-treatment 1.0 h after	control (24 h before)	post-treatment (60 min after)	
2	268 ± 25.5	175 ± 16.6	133 ± 14.6	146 ± 21.3	225 ± 07.8	150 ± 19.5
4	245 ± 14.9	147 ± 8.1	124 ± 08.0	182 ± 14.2	207 ± 08.5 NS	−[e]
6	238 ± 16.8	157 ± 12.6	130 ± 16.0	143 ± 16.7	207 ± 10.1	68 ± 12.6 NS
7	202 ± 25.7	129 ± 14.3 NS	131 ± 10.6 NS	173 ± 15.9	231 ± 11.1	147 ± 13.0
9	217 ± 19.4	155 ± 14.3	135 ± 12.5	190 ± 16.5	191 ± 10.1 NS	−
10	281 ± 28.3	177 ± 21.2	152 ± 17.4	132 ± 15.7	190 ± 13.0	141 ± 17.5
12	260 ± 25.9	205 ± 11.0	198 ± 12.5	164 ± 21.5	198 ± 17.0 NS	108 ± 15.7
13	258 ± 26.5	202 ± 8.4	187 ± 5.3	184 ± 17.2	204 ± 15.1 NS	−
19	224 ± 20.8	188 ± 12.1	195 ± 18.4	176 ± 22.6	199 ± 11.6 NS	61 ± 14.9 NS
21	214 ± 26.9	127 ± 12.2	144 ± 15.2	159 ± 11.5	228 ± 12.0	−
26	270 ± 20.9	224 ± 16.7 NS	217 ± 17.6 NS			
27	224 ± 27.1	204 ± 16.5 NS	194 ± 19.6 NS			
28	280 ± 31.4	263 ± 17.0 NS	267 ± 21.4 NS			
29	201 ± 17.7	172 ± 16.1 NS	176 ± 12.0 NS			
30	372 ± 28.2	355 ± 20.6 NS	368 ± 24.3 NS			
phenytoin	247 ± 21.1	104 ± 14.5	106 ± 12.4			
carbamazepine				131 ± 09.3	207 ± 08.4	
phenobarbital						54 ± 15.7

[a] Each score represents the mean ± SEM of six mice, significantly different from the control score at $p < 0.05$, and NS denotes not significant at $p < 0.05$ (Student's t test). [b] Each value represents the mean ± SEM of six mice, significantly different from the control at $p < 0.05$, and NS denotes not significant at $p < 0.05$ (Student's t test). [c] The compounds were tested at a dose of 100 mg/kg (ip), except phenytoin, carbamazepine (30 mg/kg), and phenobarbital (40 mg/kg), in six aminals. [d] Each value represents the mean ± SEM of six mice, significantly different from the control at $p < 0.05$, and NS denotes not significant at $p < 0.05$ (Student's t test). [e] Reversal of pentobarbitone-induced narcosis.

Table 9. Effects of 2,6-Dimethylphenyl Semicarbazones on Serum Levels of Transaminases in Six Rats

compd[a]	SGPT[b] (units/mL)	SGOT[b] (units/mL)
control[c]	45.0 ± 6.24	73.1 ± 5.98
2	47.3 ± 3.19	70.2 ± 4.78
9	43.3 ± 4.81	64.8 ± 5.30
13	44.6 ± 6.57	71.3 ± 4.06
19	49.3 ± 4.32	79.6 ± 5.71
phenytoin[d]	52.6 ± 3.48	84.1 ± 6.50

[a] The compounds were tested at a dose of 30 mg/kg (po) for 14 days. [b] Each value represents the mean ± SEM of six rats, not significant from the control value at $p < 0.05$ (Student's t test). [c] Control animals (six rats) were treated with 0.5% methylcellulose for 14 days. [d] Tested at 25 mg/kg (po) for 14 days.

9, **13**, and **19**) were administered chronically to rats for 2 weeks and the serum levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were measured (Table 9). The compounds did not show any significant increase in the levels of these enzymes when compared to the control.

To explore the mechanism of anticonvulsant activity of these derivatives, seven compounds (**2**, **4**, **6**, **7**, **9**, **10**, and **12**) were subjected to neurochemical investigation to determine the GABA levels in the whole rat brain. The study was conducted in groups of six animals, in two independent experiments. In the first set of study the brains were removed after a 2 h administration of the drug, which was given by the ip route. In another set of animals, the drug was given by oral route for 7 days, and their brains were removed after 4 h of the seventh day of administration of drug. The GABA levels were measured in the whole brain and are given in Table 10. All of the tested compounds were found to increase the GABA levels significantly when compared to control animals at $p < 0.05$ in both experiments. These compounds were also studied for possible inhibition of the enzyme GABA-T in vitro and ex vivo. Decreasing concentrations from 5, 2.5, 1.25, and 0.625 μM/100 μL were employed in the enzyme inhibition assay, and percentage inhibition with respect to the concentration of reduced nicotinamide adenine dinucleotide phosphate was recorded at different time points from 1 to 6 h. The results are presented in Table 10 along with the data on phenelzine. All of the compounds showed inhibition of the enzyme in both in vitro and ex vivo systems except compound **7**, which showed 88% inhibition in the in vitro model only. These results suggest that aryl semicarbazones exert anticonvulsant activity through a GABA-mediated mechanism.

Structure−Activity Relationship

In the preliminary anticonvulsant screening, because all of the compounds showed protection when administered by the ip route, a structure−activity relationship on this series of compounds could be derived from the oral MES screen in rats. The bioevaluation led to an understanding of the importance of the size of the group at the carbimino carbon atom. Replacement of the proton on the carbimino carbon atom by methyl (**11**−**16** and **18**−**21**) had shown anticonvulsant activity similar to that of compounds with carbimino hydrogen. However, when replaced with bulkier groups such as phenyl (**22**) or benzyl (**23**), the potency decreased, which might be due to steric hindrance to binding by the additional bulkier groups. Variation in the substituents on the aryl group at the carbimino end was found to affect the biological activity. At the 2-position, introduction of the hydroxyl function (**9**) was found to show better activity than substitution with chloro (**8**) or nitro (**10**), when the activity was reduced. Additionally, at the 3- or 4-position also, nitro (**5**, **7**, or **14**) substitution decreased the activity when compared to the unsubstituted compound **1**. Introduction of electron-donating groups such as methyl (**2**), methoxy (**3**), and *N*,*N*-dimethylamino (**4**) at the 4-position was found to improve the activity profile compared to electron-withdrawing group introduction. Replacement of the distal phenyl ring with alkyl groups such as methyl (**18**), ethyl (**19**), and acetyl methyl (**21**) displayed a better activity profile compared to the phenyl conjugates.

In conclusion, the present study revealed that some of the 2,6-dimethylphenyl semicarbazones possessed a broad spectrum of anticonvulsant activity with less neurotoxicity and no hepatotoxicity compared to clinically used drugs. Compound **9** emerged as a prototype, being effective in both ip and oral MES screens and also exhibiting activity against scPTZ, scSTY, and scPIC and in the kindling model of seizure. The compound was also found to increase the GABA levels in rat brain by 118% compared to the control and was also found to inhibit GABA-T enzyme both in vitro and ex vivo.

Table 10. Effect of Selected Compounds in the GABA System

	GABA level in rat whole brain concn[b] (μg/100 mg of tissue)		% inhibition of GABA-T enzyme activity[a]							
			in vitro				ex vivo			
compd[c]	2 h post-treatment[d]	7 days post-treatment[e]	concn (μM)	3 h	4.5 h	6 h	concn (μM)	3 h	4.5 h	6 h
control	48.4 ± 5.05	50.5 ± 2.68								
2	90.2 ± 4.30	100 ± 3.73	5.0	55	70	0	1.25	32	75	48
4	75.5 ± 6.18**	68.1 ± 4.77***	0.625	0	64	0	1.25	0	0	9
6	91.2 ± 7.67*	106 ± 3.68	5.0	0	56	23	2.50	0	56	12
7	79.8 ± 5.37*	87.9 ± 6.87	0.625	88	0	0	5.0	0	0	0
9	92.1 ± 8.75**	110 ± 2.73	1.25	58	0	9	2.50	90	43	0
10	84.7 ± 6.39**	81.6 ± 1.88	0.625	53	0	40	1.25	0	98	23
12	81.1 ± 7.61**	93.6 ± 4.52	2.50	0	0	55	1.25	0	25	60
clobazam	101 ± 6.77	110 ± 4.83								
phenelzine			1.25	0	75	62	1.25	25	85	65

[a] Concentrations of 5, 2.5, 1.25, and 0.625 μM/100 μL were used for all of the tested compounds. The data indicate the minimum concentration whereby at least 50% inhibition was demonstrated in one or more time points. [c] The compounds were tested at a dose of 100 mg/kg (ip) except **10** (300 mg/kg) and clobazam (30 mg/kg). [d] Each value represents the mean ± SEM of six rats, significantly different from the control at $p < 0.001$; *, $p < 0.004$; **, $p < 0.01$; and ***, $p < 0.008$ (Student's t test). [e] The compounds were tested at a dose of 30 mg/kg (po) for 7 days.

Experimental Section

Chemistry. Melting points were measured in open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Advance (300 MHz) instruments, 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. Mass spectra were measured with a Shimadzu GC-MS-QP5000 spectrophotometer. Only molecular ions (M^+) and base peaks are given. Elemental analyses (C, H, and N) were undertaken with a Perkin-Elmer model 240C analyzer, and all analyses were consistent with theoretical values (within ±0.4%) unless indicated. The homogeneity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel G (Merck) coated aluminum plates, visualized by iodine vapor and UV light. Developing solvent was chloroform/methanol (9:1).

Synthesis of Phenyl-*N*-(2,6-dimethylphenyl) Carbamate. Phenylchloroformate (0.1 mol, 12.1 mL) was dissolved in 40 mL of chloroform. To this solution were slowly added 2,6-dimethylaniline (0.1 mol, 12.3 mL) and triethylamine (0.1 mol, 13.9 mL), and the mixture was stirred at room temperature for 5 h. Then the reaction mixture was concentrated to one-third volume and, after cooling, 100 mL of petroleum ether (bp 55 °C) was added to the above solution. The precipitate appeared immediately, which was filtered, washed with a large quantity of water, and again filtered and dried: mp 106 °C; IR (KBr) ν_{max} 3260, 3040, 2850, 1710, 1600−1540, 1240 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.28 (s, 6H, Ar-2CH_3), 7.02−7.87 (m, 8H, ArH), 8.44 (s, 1H, ArNH, D_2O exchangeable); MS, *m/z* 225 (M^+, 100). Anal. Calcd ($C_{15}H_{15}NO$) C, H, N.

Synthesis of 2,6-Dimethylphenyl Semicarbazide. Phenyl-*N*-(2,6-dimethylphenyl) carbamate (0.05 mol, 12.05 g) was dissolved in 100 mL of methylene chloride. To this solution was added 4.85 mL of hydrazine hydrate (0.1 mol), and the mixture was stirred at room temperature for 24 h. The precipitate of 2,6-dimethylphenyl semicarbazide was separated out, filtered, washed with dichloromethane, and dried: mp 184 °C; IR (KBr) ν_{max} 3450, 3300, 2980, 1660, 1620, 1590−1540, 1360, 1310, 1210 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.24 (s, 6H, Ar-2CH_3), 5.46 (s, 2H, NH_2, D_2O exchangeable), 7.18−7.36 (m, 3H, ArH), 8.38 (s, 1H, ArNH, D_2O exchangeable), 9.52 (bs, 1H, $NHNH_2$, D_2O exchangeable); MS, *m/z* 179 (M^+, 100). Anal. Calcd ($C_9H_{13}N_3O$) C, H, N.

General Procedure for the Synthesis of 2,6-Dimethylphenyl Semicarbazones (1−30). To a solution of 2,6-dimethylphenyl semicarbazide (0.003 mol, 0.54 g) in ethanol (20 mL) was added an equimolar quantity of appropriate alkyl/aryl aldehydes or ketones (including isatin derivatives) in ethanol (5−6 mL), and the mixture was stirred for 1−3 h (24−48 h reflux for compounds **22**−**30**) until the completion of the reaction. The resultant precipitate was filtered, dried, and recrystallized from 95% ethanol. The physical data of the compounds are presented Table 2. The IR spectra of the compounds were identical in the following aspects: 3420−3200, 2950−2900, 1680−1660, 1620−1520, 1350, 1210, and 840 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz, δ) spectra and *m/z* of the some of the representative compounds are as follows.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(4-methylbenzaldehyde) semicarbazone (2):** δ 2.14 (s, 6H, Ar-2CH_3), 2.32 (s, 3H, ArCH_3), 6.56−6.58 (d, 2H, J = 7.6 Hz), 7.04 (s, 3H, ArH), 7.23−7.27 (d, 2H, J = 7.6 Hz), 8.02 (s, 1H, imine-H), 8.34 (s, 1H, ArNH, D_2O exchangeable), 9.42 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 281.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(4-*N*,*N*-dimethylaminobenzaldehyde) semicarbazone (4):** δ 1.82 [s, 6H, Ar−N(CH_3)$_2$], 2.16 (s, 6H, Ar-2CH_3), 6.54−6.56 (d, 2H, J = 7.5 Hz), 7.07 (s, 3H, ArH), 7.18−7.21 (d, 2H, J = 7.5 Hz), 8.20 (s, 1H, imine-H), 8.32 (s, 1H, ArNH, D_2O exchangeable), 9.62 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 310.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(4-nitrobenzaldehyde) semicarbazone (5):** δ 2.18 (s, 6H, Ar-2CH_3), 7.14 (s, 3H, ArH), 7.42−7.44 (d, 2H, J = 7.5 Hz), 7.87 (s, 1H, imine-H), 8.13−8.16 (d, 2H, J = 7.5 Hz), 8.57 (s, 1H, ArNH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 312.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(4-hydroxy,3-methoxybenzaldehyde) semicarbazone (6):** δ 2.20 (s, 6H, 2-CH_3), 3.81 (s, 3H, OCH_3), 6.75−6.78 (d, 1H, J = 7.5 Hz), 7.03−7.08 (m, 4H, aryl-H), 7.48 (s, 1H, aryl-H), 7.80 (s, 1H, imine-H), 8.41 (s, 1H, aryl NH D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable), 10.34 (s, 1H, OH, D_2O exchangeable); MS, *m/z* 313.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(2-hydroxybenzaldehyde) semicarbazone(9):** δ 2.21 (s, 6H, 2-CH_3), 6.79−6.88 (m, 2H, aryl-H), 7.08 (s, 3H, aryl-H), 7.16−7.21 (m, 1H, aryl-H), 7.91−7.94 (d, 1H, aryl-H, J = 7.5 Hz), 8.24 (s, 1H, imine-H), 8.42 (s, 1H, aryl-NH, D_2O exchangeable), 9.98 (s, 1H, CONH, D_2O exchangeable), 10.49 (s, 1H, OH, D_2O exchangeable); MS, *m/z* 283.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-[1-(4-hydroxyphenyl)ethan-1-one] semicarbazone (12):** δ 2.14 (s, 3H, CH_3), 2.20 (s, 6H, 2-CH_3), 6.74−6.76 (d, 2H, J = 7.6 Hz), 7.05 (s, 3H, aryl-H), 7.63−7.65 (d, 2H, J = 7.6 Hz), 8.30 (s, 1H, aryl-NH, D_2O exchangeable), 9.72 (s, 1H, CONH, D_2O exchangeable), 10.34 (s, 1H, OH, D_2O exchangeable); MS, *m/z* 297.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-[1-(4-aminophenyl)ethan-1-one] semicarbazone (13):** δ 2.14 (s, 3H, CH_3), 2.19 (s, 6H, 2-CH_3), 5.32 (s, 2H, NH_2, D_2O exchangeable), 6.52−6.54 (d, 2H, J = 7.5 Hz), 7.07 (s, 3H, aryl-H), 7.61−7.64 (d, 2H, J = 7.5 Hz), 8.30 (s, 1H, aryl-NH, D_2O exchangeable), 9.34 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 296.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(propan-2-one) semicarbazone (18):** δ 1.76 (s, 3H, CH_3), 1.85 (s, 3H, CH_3), 2.18 (s, 6H, 2-CH_3), 7.07−7.13 (t, 1H, J = 7.5 Hz), 7.20−7.22 (d, 2H, J = 7.5 Hz), 8.42 (s, 1H, aryl-NH, D_2O exchangeable), 9.52 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 219.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(butan-2-one) semicarbazone (19):** δ 1.01−1.03 (t, 3H, CH_3, J = 7.6 Hz), 2.14 (s, 3H, CH_3), 2.73−2.77 (q, 2H, J = 7.6 Hz), 2.20 (s, 6H, 2-CH_3), 7.11−7.14 (m, 3H, aryl-H), 8.40 (s, 1H, aryl-NH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 233.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(1-acetylpropan-2-one) semicarbazone (21):** δ 1.64 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.12 (s, 2H, CH_2), 2.21 (s, 6H, 2-CH_3), 7.07−7.11 (m, 3H, aryl-H), 8.38 (s, 1H, aryl-NH, D_2O) exchangeable), 9.64 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 261.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(diphenyl ketone) semicarbazone (22):** δ 2.12 (s, 6H, 2-CH_3), 7.05−7.66 (m, 13H, aryl-H), 8.90 (s, 1H, aryl-NH, D_2O exchangeable), 10.55 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 343.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(cyclohexanone) semicarbazone (24):** δ 1.3−1.6 (m, 10H, cyclohexyl), 2.13 (s, 6H, 2-CH_3), 7.0−7.5 (m, 3H, aryl-H), 8.88 (s, 1H, aryl-NH, D_2O exchangeable), 10.50 (s, 1H, CONH, D_2O exchangeable); MS, *m/z* 259.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(indole-2,3-dione) semicarbazone (26):** δ 2.13 (s, 6H, 2-CH_3), 7.07−7.70 (m, 7H, aryl-H), 8.69 (s, 1H, aryl-NH, D_2O exchangeable), 9.55 (s, 1H, CONH, D_2O exchangeable), 10.11 (s, 1H, isatinyl CONH, D_2O exchangeable); MS, *m/z* 308.

***N*^{1}-(2,6-Dimethylphenyl)-*N*^{4}-(5-fluoroindole-2,3-dione) semicarbazone (27):** δ 2.33 (s, 6H, 2-CH_3), 6.77−7.60 (m, 6H, aryl-H), 8.90 (s, 1H, aryl-NH, D_2O) exchangeable), 9.55 (s, 1H, CONH, D_2O exchangeable), 10.18 (s, 1H, isatinyl CONH, D_2O exchangeable); MS, *m/z* 326.

Pharmacological Methods. Male albino mice (CF-1 strain, 18−25 g) and male albino rats (Sprague−Dawley, 100−150 g) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The aryl semicarbazones were suspended in 30% poly(ethylene glycol) 400 or a 0.5% methylcellulose/water mixture.

1. Anticonvulsant Screening. The anticonvulsant evaluations were undertaken partly by the National Institutes of Health, using their reported procedures.[46,47] Initially all compounds were administered ip at doses of 30, 100, and 300 mg/kg to one to four mice. Activity was established using the

MES, scPTZ, scSTY,[48] and scPIC[49] tests. Some selected compounds described in this study were examined for oral activity in the MES screen in rats.

2. Neurotoxicity Screening. Minimal motor impairment was measured in mice by the 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 an ip injection of the test compounds in doses of 30, 100, and 300 mg/kg. 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.

3. Quantification Studies. Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50}, groups of 6−12 rats were given a range of ip (**4**, **6**, **7**, **10**, and **12**) and oral (**2**, **9**, **13**, **19**, and **21**) doses of the test drug until at least three points were established in the range of 10−90% seizure protection or minimal observed neurotoxicity. From the plot of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line, and standard error of the slope were calculated by means of a computer program written at NINDS, NIH.

4. Preliminary Hippocampal-Kindling Screen. The kindling procedure for drug experiments in fully kindled rats was according to that of Löscher and Honack.[50] Briefly, the rats were anesthetized with chloral hydrate (360 mg/kg ip) for implantation of a bipolar electrode into the right hemisphere aimed at the basolateral amygdala. Electrical stimulation of the amygdala was initiated after a recovery period of 2 weeks after surgery. Threshold for induction of amygdala after discharge (prekindling ADT) was determined, and constant-current stimulations (500 μA, 1 ms, monophasic square-wave pulse, 50/s for 1 s) were delivered to the amygdala at intervals of 1 day until at least 10 consecutive fully kindled seizures were elicited. Compounds **7** and **9** were administered at a single dose of 100 or 130 mg/kg, respectively, ip, and the ADT was determined either 30, 60, 90 min or more after administration. Seizure score and AD duration (ADD) in seconds were recorded. Behavioral alterations after administration of test compounds were determined at different times after injection up to 2 min before amygdala stimulation.

5. Behavioral Test Using Actophotometer. The test compounds (100 mg/kg) were screened for their behavioral effects using a photoactometer (INCO, Ambala, India) at 30 min and 1.0 h after ip injection to mice. The behavior of the animals inside the photocell was recorded as a digital score.[51] The photoactometer was placed in a soundproof box, and mice were placed inside. The duration of the experimental observation was up to 10 min (2 + 8). After an initial period of 2 min during which the animals became accustomed to the new environment, the counter was reset and the remaining 8 min reading was noted. After each trial, the base was cleaned with 20% v/v ethyl alcohol.

6. Porsolt's Swim Test. The method adopted was a modification of that described by Porsolt et al.[52] The animals were subjected to a 15 min swimming session 24 h prior to the conduct of a 6 min test. The animals were given an ip injection (100 mg/kg) of the test compounds 30 min before the test session. The duration of immobility (passive floating without struggling and making only those movements necessary to keep the head above the surface of water) was recorded during the last 4 min of the 6 min testing period (for rats, the last 5 min of the total 7 min period).

7. Pentobarbitone-Induced Narcosis. The method of barbiturate hypnosis potentiation activity as described earlier[53] was employed. Swiss albino mice of either sex (20−25 g) were divided into groups of six, and food was withdrawn 12 h before the start of the experiment. Each animal of the control and test (100 mg/kg) group was injected with pentobarbital sodium (40 mg/kg, ip), and the sleeping time of each mouse was noted as the interval between loss and gain of righting reflex. The criteria for the loss and gain of righting reflex were taken as the inability or ability, respectively, of the mice to right themselves within 30 s, in three successive trials when placed on their backs.

8. Hepatotoxicity Studies. The animals were divided into groups of six, and the control group received a basal diet and vehicle. Other groups were administered the test drug in a dose of 30 mg/kg/day po (in PEG 400 or 2% methylcellulose) for 14 days. After the stipulated period, each animal was anesthetized by anesthetic ether, and blood was collected by cardiac puncture to assess the transaminase activity. The in vitro determination of transaminase activity was carried out according to the 2,4-dinitrophenyl hydrazine method[54,55] using SPAN diagnostic reagent kits.

9. Isolation of Rat Brain Regions and GABA Assay. The GABA assay was performed in brain tissue extracts enzymatically as previously described. Adult Wistar rats were divided into three groups of six animals each. After 2 h of drug administration (100 mg/kg, ip), the animals were decapitated and the brains were dropped into separate vials containing 4−6 mL of ice-cold 80% ethanol and processed further as described previously.[56] A chronic study was also carried out after po administration of the test compounds (30 mg/kg) for 7 days.

10. In Vitro and ex Vivo GABA-T Enzyme Inhibition Assay. The GABA-T enzyme was prepared from *Pseudomonas fluorescens* according to the method reported earlier.[57] The in vitro (bacterial) and ex vivo (rat brain) enzyme inhibition assay was performed for a 6 h time period at four different concentrations of the test compounds (5, 2.5, 1.25, and 0.625 μM) according to the previously reported procedure.[19,58]

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Supporting Information Available: Elemental analyses of compounds **1**−**30**. This material is available free of charge via the Internet at http://pubs.acs.org.

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