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2,4-Dimethoxyphenylsemicarbazones with anticonvulsant activity against three animal models of seizures: Synthesis and pharmacological evaluation

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Abstract—Various 2,4-dimethoxyphenylsemicarbazones were synthesized starting from 2,4-dimethoxyaniline via a phenylcarbamate intermediate. The structures were confirmed by spectral and elemental analyses. The anticonvulsant activity of the synthesized compounds was established after intraperitoneal administration in three seizure models in mice which include maximal electroshock seizure, subcutaneous pentylenetetrazole, and subcutaneous strychnine-induced seizure screens. Nine compounds exhibited protection in all the three seizure models, and N^1-(2,4-dimethoxyphenyl)-N^4-(propan-2-one)semicarbazone (**17**) emerged as the most active compound with no neurotoxicity. These compounds were found to elevate γ-aminobutyric acid (GABA) levels in the midbrain and medulla oblongata regions equipotent to clobazam.

1. Introduction

Epilepsy is not a disease, but a syndrome of different cerebral disorders of the central nervous system (CNS), which is characterized by paroxysmal, excessive, and hypersynchronous discharges of large numbers of neurons.[1] In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million population.[2] Despite the optimal use of available antiepileptic drugs (AEDs), many patients with epilepsy fail to experience seizure control and others do so only at the expense of significant toxic side effects.[3] The limitations with the conventional AEDs highlighted the need for developing newer agents for epilepsies and the AED search has come a long way, particularly over the last two decades.

Aryl semicarbazones have documented increasing advances in AED drug design and were found to act by blocking the voltage-gated sodium ion channels.[4–8] We have recently been investigating various aryl-substituted semicarbazones as potential anticonvulsant agents.[9–15] While attempting to establish some correlation between structure and the activity of these aryl semicarbazones, we observed that the structural requirement for activity in the maximal electroshock (MES) test was the presence of at least one phenyl ring in close proximity to two-electron donor atoms (semicarbazono group). And compounds displaying anti-pentylenetetrazole (PTZ) activity, often possess an alkyl substituent close to two-electron donor atoms.[16]

In view of these general requirements for activity, we have designed aryl-substituted semicarbazones with various aryl and alkyl aldehydes and ketones to be active in a variety of convulsant stimuli. Surprisingly, these aryl semicarbazones were devoid of sedative-hypnotic activity and exhibited anticonvulsant activity with lesser neurotoxicity.[9] These aryl semicarbazones do not possess the dicarboximide group as found in conventional drugs like barbiturates, hydantoins, oxazolidindiones, etc., which may be associated with toxicity and side effects.[17] In this paper, we report the synthesis and pharmacological evaluation of 2,4-dimethoxyphenylsemicarbazones and compared its potential with those of established drugs. These aryl-substituted semicarbazones have

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elevated the γ-aminobutyric acid levels in different regions of brain.

2. Results and discussion

2.1. Synthesis

The synthesis of 2,4-dimethoxyphenylsemicarbazones was accomplished as presented in Scheme 1. The 2,4-dimethoxyaniline was treated with phenyl chloroformate in the presence of chloroform to yield phenyl-*N*-(2,4-dimethoxyphenyl)carbamate. The carbamate on condensation with hydrazine hydrate in methylene dichloride gave the 2,4-dimethoxyphenylsemicarbazide (**1**). Finally the required 2,4-dimethoxyphenylsemicarbazones were prepared by the reaction between appropriate aryl/alkyl aldehydes or ketones and **1** in the presence of glacial acetic acid in ethanol. The physical properties of the synthesized compounds are presented in Table 1. The structures were characterized by both spectral and elemental analyses and the data were within ±0.4% of the theoretical values.

2.2. Pharmacological activity

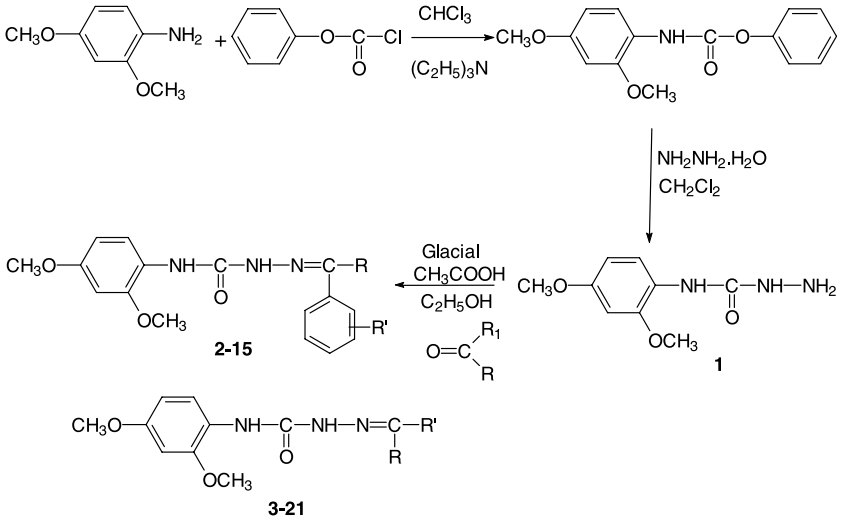
The new derivatives (**1**–**21**) obtained from the reactional sequence were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ), subcutaneous strychnine threshold test (scSTY), and neurotoxicity screens, using doses of 30, 100, and 300 mg/kg, and observation carried out at two different time intervals (0.5 and 4 h). These data are presented in Table 2. All the compounds except **6**, **9**, and **16**, showed anti-MES activity indicative of their ability to prevent seizure spread. Compounds that showed protection against MES model at 100 mg/kg include **4**, **7**, **8**, and **10** (0.25 h), **17**–**19**, and **20** (0.25 h). The compounds **4**, **5**, **8**, **15**, and **17** showed activity both at 0.5 h and 4.0h periods. Most of the compounds showed activity only at 0.5 h, indicating that they have rapid onset and shorter duration of action.

Compounds **1**, **4**, **8**, **9**, **14**–**18**, and **20**–**21** were found to be active in the scPTZ test, a test used to identify compounds that elevate seizure threshold. Compounds **1**, **8**, and **21** showed activity at a dose of 100 mg/kg comparable with Ethosuximide. Compounds that showed moderate protection at a dose of 100 mg/kg include **9** (0.25 h, 1/5), **15** (0.25 h, 2/5), **16** (0.25 h, 1 h, 1/5), and **18** (0.25 h, 1/5). All these compounds showed 100% protection at a dose of 300 mg/kg at 0.5 h. So these compounds have quick onset of action but for shorter duration.

The compounds were also screened in the scSTY pattern test. All the compounds except **8**, **11**–**12**, and **21** showed protection against scSTY-induced seizure threshold test, indicative of their ability to prevent seizure. Compounds **1**, **4**, **9**, **14**, **17**, and **18** showed activity at 100 mg/kg at 0.5 h in which compound **17** showed activity up to 4.0 h. Compounds that showed activity at 300 mg/kg include **1**, **4**, **9**, **10**, **14**, **16**, and **18** (4 h), **2**, **3**, **5**, **13**, and **19** (0.5 h), and **6**, **7**, **15**, and **20** at both the time points.

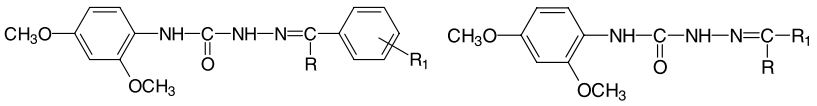
In the neurotoxicity screen, compounds **1**, **5**, **7**, **13**, **17**, and **21** did not show neurotoxicity in the maximum administered dose (300 mg/kg) and the compounds **4** and **18** were less neurotoxic compared to Phenytoin. On the other hand, compounds **2**, **8**, **10**, **15**, **16**, and **19**–**20** were neurotoxic at the anticonvulsant dose and compounds **3**, **6**, **9**, and **14** were more neurotoxic. Compounds **11** and **12** exhibited neither anticonvulsant activity nor neurotoxicity.

Among the compounds, the unsubstituted aryl derivative (**2** and **13**) exhibited activity against MES and scSTY models. When the C-4 position of the aryl ring was substituted with donating groups (**3**–**5** and **14**–**15**), the compounds exhibited activity in more than one model with the compounds **4**, **14**, and **15** being active in all the three models. The compound with an electron-withdrawing group at C-4 position (**6**) exhibited activity only in the scSTY model, while the similar compound with carbimino methyl group (**16**) exhibited activity against scPTZ and scSTY. The 3-chloro (**7**)



Scheme 1. Synthesis of the title compounds.

Table 1. Physical data of the 2,4-dimethoxyphenylsemicarbazones



2-16 **17-21**

Compound	Substituents		Yield (%)	Mp (°C)	Molecular formula[a]	Molecular weight	R_f	log P[b]
	R	R_1						
2	H	H	62	188	$C_{16}H_{17}N_3O_3$	299.33	0.71	1.88
3	H	4-CH_3	55	159	$C_{17}H_{19}N_3O_3$	313.36	0.62	2.07
4	H	4-OCH_3	52	155	$C_{17}H_{19}N_3O_4$	329.35	0.64	1.48
5	H	4-N–$(CH_3)_2$	50	137	$C_{18}H_{22}N_4O_3$	342.40	0.64	1.27
6	H	4-NO_2	66	173	$C_{16}H_{16}N_4O_5$	344.33	0.72	2.21
7	H	3-Cl	64	139	$C_{16}H_{16}N_3O_3Cl$	333.77	0.70	2.21
8	H	3-NO_2	69	200	$C_{16}H_{16}N_4O_5$	344.33	0.74	2.19
9	H	2-Cl	60	179	$C_{16}H_{16}N_3O_3Cl$	333.77	0.68	2.19
10	H	2-CH_3	61	127	$C_{17}H_{19}N_4O_3$	313.35	0.69	1.97
11	H	2-OH	57	156	$C_{16}H_{17}N_3O_4$	315.33	0.65	1.52
12	H	2-NO_2	65	145	$C_{16}H_{16}N_4O_5$	344.32	0.74	2.13
13	CH_3	H	61	161	$C_{17}H_{19}N_3O_3$	313.35	0.71	1.85
14	CH_3	4-Cl	58	166	$C_{17}H_{18}N_3O_3Cl$	347.80	0.74	1.95
15	CH_3	4-CH_3	53	151	$C_{18}H_{21}N_3O_3$	327.38	0.63	2.06
16	CH_3	4-NO_2	67	118	$C_{17}H_{18}N_4O_5$	358.35	0.77	2.05
17	CH_3	CH_3	54	159	$C_{12}H_{17}N_3O_3$	251.28	0.69	1.51
18	CH_3	C_2H_5	52	138	$C_{13}H_{19}N_3O_3$	265.31	0.62	1.79
19	CH_3	$CH_2CH(CH_3)_2$	52	141	$C_{15}H_{23}N_3O_3$	293.36	0.61	2.13
20	CH_3	CH_2COCH_3	50	156	$C_{14}H_{19}N_3O_4$	293.32	0.66	1.62
21	CRR_1 = cyclohexyl		51	147	$C_{15}H_{21}N_3O_3$	291.35	0.67	1.76

[a] Elemental analyses for C, H, and N were within ±0.4% of the theoretical values.
[b] log P was generated using Alchemy 2000 and SciLogP software.

and 3-nitro (**8**) substituted derivatives exhibited activity in more than one models, viz., MES and scSTY, and MES and scPTZ, respectively. The 2-hydroxy (**11**) and 2-nitro (**12**) substituted derivatives were inactive, while a 2-chloro (**9**) and 2-methyl (**10**) were active in scPTZ and scSTY, and Mes and scSTY models, respectively.

When the carbimino terminal aryl ring was replaced with alkyl groups like methyl (**17**), ethyl (**18**), and acetomethyl (**20**), the compounds were found to exhibit activity against all the three animal models, whereas isobutyl derivative (**19**) was active in MES and scSTY models. The cyclohexyl derivative (**21**) showed activity in both MES and scPTZ models. Hence, the compounds **1**, **4**, **14**, **15**, **17**, **18**, and **20** exhibited a broad spectrum of anticonvulsant activity, viz., MES, scPTZ and scSTY. With regard to neurotoxicity, the compounds **1**, **15**, **17**, and **18** showed no or lesser neurotoxicity.

In comparison to the 2,6-dimethylphenylsemicarbazones reported earlier,[15] the 2,4-dimethoxyphenylsemicarbazones were less effective except compound **5** and **17** which were not neurotoxic compared to the 2,6-dimethylphenyl analogues.

Four compounds (**1**, **15**, **17**, and **18**) were evaluated orally in rats for activity in scPTZ test at several time points (Table 3). The compounds were tested at 50 mg/kg and compared with standard drug Ethosuximide. Compounds **15**, **17**, and **18** showed better protection than the standard drug Ethosuximide. These compounds did not exhibit neurotoxicity at the tested dose of 50 mg/kg.

Some selected compounds (**4**, **5**, **13**, **15**, and **17**–**20**) were studied for the CNS behavioral activity in mice using actophotometer and Porsolt's swim pool test in mice and the results are presented in Table 4. In the behavioral study, using actophotometer, the compounds **4** and **19** showed no behavioral despair effect after 1.0 h when compared to Phenytoin. All other compounds were found to decrease the activity of the animals. In a similar study using Porsolt's swim test, the immobility time after the administration of the test compounds was compared with that of Carbamazepine. The compound **20** was found to show no significant CNS depression and all other compounds tested were found to emerge as CNS depressants as they increased the immobility time.

In order to explore the mechanism of anticonvulsant activity, some selected compounds (**4**, **15**, and **17**), which were more active, were subjected to neurochemical investigation to study their effects on the levels of GABA in different regions of rat brain viz., olfactory lobe, mid brain, medulla oblongata, and cerebellum, as there are regional differences in GABA concentration within the CNS[18] (Table 5). The compounds were found to increase the GABA level in the midbrain region significantly. In other brain regions, there was no significant increase in the level of GABA, except for

Table 2. Anticonvulsant activity and minimal motor impairment of 2,4-dimethoxyphenylsemicarbazones

Compound	Intraperitoneal injection in mice[a]							
	MES screen		scPTZ screen		scSTY screen		Neurotoxicity screen	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	2.0 h	0.5 h	4.0 h
1	300	—[b]	100	—	100	300	—	—
2	300	—	—	—	300	—	300	—
3	300	—	—	—	300	—	100	100
4	100	300	300	—	100	300	300	—
5	300	300[b]	—	—	300	—	—	—
6	—	—	—	—	300	300	30	100
7	100	—	—	—	300	300	—	—
8	100	300	100	—	—	—	100	300
9	—	—	300[c]	—	100	300	100	100
10	300[b]	—	—	—	—	300	300	—
11	—	—	—	—	—	—	—	—
12	—	—	—	—	—	—	—	—
13	300	—	—	—	300	—	—	—
14	300	—	300	—	100	300	100	—
15	300	300	300[c]	—	300	300	300	—
16	—	—	300[c]	—[c]	—	300	300	—
17	100	100	300	—	100	100	—	—
18	100	—	300[c]	—	100	300	300	—
19	100	—	—	—	300	—	300	—
20	300[b]	—	300	—	300	300	300	—
21	300	—	100	—	—	—	—	—
Phenytoin	30	30	—	—	—	—	100	100
Ethosuximide	—	—	100	300	300	—	—	—

[a] Doses of 30, 100, and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4.0 h (scSTY test, 0.5 and 2.0 h period study) after injections were administered. The dash (—) indicates the absence of activity at maximum dose administered (300 mg/kg) and (x) indicates not tested.

[b] In the MES screen at a dose of 100 mg/kg, compounds that showed protection were **1** (1 h, 1/3), **10**, and **20** (0.25 h, 1/3).

[c] In the scPTZ screen, at a dose of 100 mg/kg, compounds that showed protection were **9** (0.25 h, 1/5), **15** (0.25 h, 2/5), **16** (0.25, 1 h, 1/5), and **18** (0.25 h, 1/5).

Table 3. Evaluation of some compounds in the scPTZ test after oral administration (50 mg/kg) to rats

Compound	Oral administration to rats[a]			
	0.25 h	0.5 h	1.0 h	2.0 h
1	1	2	0	0
15	2	1	1	1
17	3	3	2	1
18	2	3	1	1
Ethosuximide	0	2	1	1

[a] The figures indicate the number of rats out of four which were protected.

compounds **15** and **17**, which increased the GABA level in the medulla oblongata region similar to Clobazam.

3. Conclusion

The present study revealed that some of the 2,4-dimethoxyphenylsemicarbazones possessed a broad spectrum of anticonvulsant activity with less or no neurotoxicity. Nine compounds exhibited protection in all the three seizure models, viz., MES, scPTZ, and scSTY models and N^1-(2,4-dimethoxyphenyl)-N^4-(propan-2-one)semicarbazone (**17**) emerged as the most active compound in these three models with no neurotoxicity. These compounds were found to elevate γ-aminobutyric acid (GABA) levels in the midbrain and medulla oblongata regions equipotent to clobazam.

4. Experimental

4.1. Chemistry

Melting points were determined with a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr). ^{1}H NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer. Chemical shifts were expressed in parts per million (δ) with tetramethylsilane as an internal standard. The elemental analyses (C, H, N) were performed using Perkin-Elmer model 240 C analyzer, and all values were within ±0.4% of the theoretical compositions. The purity of the compounds was monitored by ascending thin-layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapour. Developing solvents used in TLC were chloroform/methanol (9:1). The log *P* for all the synthesized compounds were calculated using SciLogP software in Alchemy 2000 (Tripos Co.).

4.1.1. Synthesis of phenyl-*N*-(2,4-dimethoxyphenyl)carbamate. Phenylchloroformate (0.1 mol, 12.6 ml) was dissolved in 40 ml of chloroform and equimolar quantities of 2,4-dimethoxyaniline (0.1 mol, 12.3 ml) and triethylamine (0.1 mol, 13.9 ml) were added dropwise and stirred in the room temperature for 5 h. The reaction mixture was concentrated to one-third volume and

Table 4. CNS studies on selected compounds

Compound[a]	Actophotometer activity score[b]			Immobility time[c] (s)	
	Control (24 h before)	Post treatment		Control (24 h prior)	Post treatment (60 min after)
		0.5 h after	1.0 h after		
4	350.67 ± 17.43	207.00 ± 09.56	305.67 ± 11.64 NS	154.33 ± 6.06	209.50 ± 6.23
5	406.67 ± 19.71	282.00 ± 11.08	350.33 ± 15.75	157.33 ± 7.97	197.17 ± 8.03
13	395.33 ± 17.48	231.17 ± 12.68	264.50 ± 10.90	170.67 ± 7.57	204.33 ± 7.87
15	417.50 ± 15.68	268.67 ± 07.53	326.00 ± 11.94	162.33 ± 3.88	218.67 ± 6.08
17	488.00 ± 12.57	276.50 ± 08.09	308.33 ± 13.12	158.50 ± 4.90	219.00 ± 5.01
18	414.17 ± 17.59	323.33 ± 07.91	340.33 ± 09.20	165.50 ± 6.53	187.33 ± 7.32
19	322.00 ± 10.58	240.67 ± 07.85	303.33 ± 09.28 NS	176.00 ± 6.94	202.33 ± 7.80
20	338.00 ± 11.43	154.17 ± 06.44	225.50 ± 09.63	171.67 ± 8.44	193.33 ± 6.09 NS
Phenytoin[d]	247.32 ± 21.12	104.11 ± 14.56	106.23 ± 12.44	—	—
Carbamazepine[d]	—	—	—	131.50 ± 9.32	207.33 ± 8.49

[a] The compounds were tested at a dose of 100 mg/kg (ip).
[b] Each score represents the means ± 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).
[c] Each value represents the means ± 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).
[d] Tested at 30 mg/kg (ip).

Table 5. Effect of selected compounds on GABA system

Compound[a]	Concentration of GABA (mg/100 mg tissue)[b]			
	Olfactory lobe	Midbrain	Medulla oblongata	Cerebellum
Control	12.01 ± 1.74	44.31 ± 1.78	33.28 ± 2.04	21.93 ± 2.52
4	13.00 ± 2.56	58.87 ± 2.61*	39.51 ± 2.74	29.25 ± 3.40
15	12.71 ± 1.80	63.77 ± 2.82*	42.18 ± 2.54*	29.79 ± 3.08
17	14.58 ± 1.78	64.72 ± 3.48*	44.32 ± 2.92*	27.99 ± 2.83
Clobazam[c]	17.67 ± 2.62	63.57 ± 2.83*	45.91 ± 2.16*	28.73 ± 3.12

[a] The compounds were tested at a dose of 100 mg/kg (ip).
[b] Each value represents the means ± SEM of six rats significantly different from the control at $*P < 0.05$ and the remaining values are not significant at $P < 0.05$ (Student's *t*-test).
[c] Tested at 30 mg/kg (ip).

100 ml of petroleum ether was added. The precipitate, which appeared, was washed with water, filtered, and dried, mp 126 °C, IR (KBr) ν_{max} 3400, 3100–3060, 2940, 1700, 1320, 845 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 3.74 (s, 3H, $ArOCH_3$), 3.78 (s, 3H, $ArOCH_3$), 6.86–7.67 (m, 8H, ArH), 8.32 (s, 1H, ArNH, D_2O exchangeable).

4.1.2. Synthesis of 2,4-dimethoxyphenylsemicarbazide (1). Phenyl-*N*-(2,4-dimethoxyphenyl)carbamate (0.05 mol, 12.05 gm) was dissolved in 100 ml of dichloromethane. To this solution, 4.85 ml of hydrazine hydrate (0.1 mol) was added and refluxed with stirring for 24 h. The precipitate of 2,4-dimethoxyphenylsemicarbazide (**1**) was separated by vacuum filtration and washed with dichloromethane, and dried, mp 190 °C, IR (KBr) ν_{max} 3390, 3090, 2860, 1680, 1320, 840 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz, δ ppm) 3.68 (s, 3H, $ArOCH_3$), 3.72 (s, 3H, $ArOCH_3$), 5.42 (s, 2H, NH_2, D_2O exchangeable), 7.12–7.37 (m, 3H, ArH), 8.36 (s, 1H, ArNH, D_2O exchangeable), 9.48 (br s, 1H, $NHNH_2$, D_2O exchangeable).

4.1.3. General procedure for the synthesis of 2,4-dimethoxyphenylsemicarbazones (2–21). To a solution of 2,4-dimethoxyphenylsemicarbazide (0.003 mol, 0.84 g) in 25 ml of ethanol, an equimolar quantity of appropriate aldehyde or ketone in 5 ml ethanol and glacial acetic acid (1–2 drops) was added. The mixture was stirred with heating for 1–4 h until the completion of the reaction and the resultant precipitate was filtered and dried. The product was recrystallized from 95% ethanol. The physical data of the compounds are presented in Table 1. The IR spectra of the compounds were identical in the following aspects: 3400–3320, 2900–2850, 1690–1670, 1620–1540, 1320, 840 cm^{-1}. The spectral and elemental analyses of some representative compounds are as follows:

4.1.4. N^1-(2,4-Dimethoxyphenyl)-N^4-(4-methylbenzaldehyde)semicarbazone (3). Yield: 55%; mp: 159 °C; IR (KBr): 3340, 2890, 1670, 1610, 1590–1530, 1320 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 2.24 (s, 3H, $ArCH_3$), 3.64 (s, 3H, $ArOCH_3$), 3.68 (s, 3H, $ArOCH_3$), 6.78–7.86 (m, 7H, ArH), 7.92 (s, 1H, imine H), 8.46 (s, 1H, ArNH, D_2O exchangeable), 9.70 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{17}H_{19}N_3O_3$: C, 65.16; H, 6.11; N, 13.41; found: C, 64.91; H, 6.09; N, 13.36.

4.1.5. N^1-(2,4-Dimethoxyphenyl)-N^4-(4-nitrobenzaldehyde)semicarbazone (6). Yield: 66%; mp: 173 °C; IR (KBr): 3350, 2900, 1690, 1605, 1590–1540, 1435, 1330, 1310, 1210 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.70

(s, 3H, $ArOCH_3$), 3.74 (s, 3H, $ArOCH_3$), 6.84–7.96 (m, 7H, ArH), 8.02 (s, 1H, imine H), 8.62 (s, 1H, ArNH, D_2O exchangeable), 10.94 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{16}H_{16}N_4O_5$: C, 55.81; H, 4.68; N, 16.27; found: C, 55.60; H, 4.66; N, 16.21.

4.1.6. N^1-(2,4-Dimethoxyphenyl)-N^4-(2-chlorobenzaldehyde)semicarbazone (9). Yield: 60%; mp: 179 °C; IR (KBr): 3350, 2890, 1690, 1590–1540, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.70 (s, 3H, $ArOCH_3$), 3.73 (s, 3H, $ArOCH_3$), 6.78–7.58 (m, 7H, ArH), 8.02 (s, 1H, imine H), 8.89 (s, 1H, ArNH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{16}H_{16}N_3O_3Cl$: C, 57.58; H, 4.83; N, 12.59; found: C, 57.54; H, 4.86; N, 12.66.

4.1.7. N^1-(2,4-Dimethoxyphenyl)-N^4-(2-hydroxybenzaldehyde)semicarbazone (11). Yield: 57%; mp: 156 °C; IR (KBr): 3350, 2900, 1690, 1590–1540, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 3.70 (s, 3H, $ArOCH_3$), 3.73 (s, 3H, $ArOCH_3$), 6.55–7.28 (m, 7H, ArH), 8.10 (s, 1H, imine H), 8.90 (s, 1H, ArNH, D_2O exchangeable), 9.40 (s, 1H, CONH, D_2O exchangeable), 9.88 (s, 1H, OH, D_2O exchangeable); Calculated for $C_{16}H_{17}N_3O_4$: C, 60.94; H, 5.43; N, 13.33; found: C, 61.14; H, 5.60; N, 13.66.

4.1.8. N^1-(2,4-Dimethoxyphenyl)-N^4-(4-methylacetophenone)semicarbazone (15). Yield: 53%; mp: 151 °C; IR (KBr): 3352, 2910, 1690, 1590–1540, 1312, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 1.3 (s, 3H, Carbimino-CH_3), 2.34 (s, 3H, CH_3), 3.72 (s, 3H, Ar-OCH_3), 3.74 (s, 3H, $ArOCH_3$), 6.35–7.66 (m, 7H, ArH), 8.90 (s, 1H, ArNH, D_2O exchangeable), 10.05 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{18}H_{21}N_3O_3$: C, 66.04; H, 6.47; N, 12.84; found: C, 66.10; H, 6.64; N, 12.96.

4.1.9. N^1-(2,4-Dimethoxyphenyl)-N^4-(propan-2-one)-semicarbazone (17). Yield: 54%; mp: 159 °C; IR (KBr): 3370, 2890, 1670, 1590–1545, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 1.82 (s, 3H, CH_3), 1.93 (s, 3H, CH_3), 3.66 (s, 3H, $ArOCH_3$), 3.70 (s, 3H, $ArOCH_3$), 6.72–6.84 (m, 3H, ArH), 8.22 (s, 1H, ArNH, D_2O exchangeable), 9.54 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{12}H_{17}N_3O_3$: C, 57.36; H, 6.82; N, 16.72; found: C, 57.14; H, 6.80; N, 16.66.

4.1.10. N^1-(2,4-Dimethoxyphenyl)-N^4-(acetylacetone-semicarbazone (20). Yield: 50%; mp: 156 °C; IR (KBr): 3350, 2900, 1670, 1590–1545, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 1.52 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.54 (s, 2H, CH_2), 3.70 (s, 3H, $ArOCH_3$), 3.76 (s, 3H, $ArOCH_3$), 6.66–6.84 (m, 3H, ArH), 8.88 (s, 1H, ArNH, D_2O exchangeable), 10.24 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{14}H_{19}N_3O_4$: C, 57.33; H, 6.53; N, 14.33; found: C, 57.56; H, 6.60; N, 14.48.

4.1.11. N^1-(2,4-Dimethoxyphenyl)-N^4-(cyclohexanone)-semicarbazone (21). Yield: 51%; mp: 147 °C; IR (KBr): 3350, 3000, 2900, 1670, 1590–1545, 1500, 1320, 1200 cm^{-1}; ^1H NMR (DMSO-d_6) δ (ppm): 1.32 (m, 4H, *o*-methyl protons of cyclohexyl ring), 1.66 (m, 6H, *m*- and *p*-methyl protons of cyclohexyl ring), 3.72 (s, 3H, $ArOCH_3$), 3.77 (s, 3H, $ArOCH_3$), 6.36–6.74 (m, 3H, ArH), 8.93 (s, 1H, ArNH, D_2O exchangeable), 10.55 (s, 1H, CONH, D_2O exchangeable); Calculated for $C_{15}H_{21}N_3O_3$: C, 61.84; H, 7.27; N, 14.42; found: C, 61.86; H, 7.46; N, 14.50.

4.2. Pharmacology

Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague–Dawley/Wistar, 100–150 g) were used as experimental animals. The animals were housed in metabolic cages, and allowed free access to food and water. The synthesized compounds (**1**–**21**) were suspended in 0.5% methylcellulose/water mixture or in polyethylene glycol (PEG 200).

4.2.1. Anticonvulsant screening. The anticonvulsant evaluations were undertaken by the National Institute of Health, using their reported procedures.[19–21] 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, and scSTY tests. Some selected derivatives described in this study were examined for oral activity in the rat scPTZ screen.

4.2.2. Neurotoxicity screening. Minimal motor impairment was measured in mice by the rotarod test.[22] The mice were trained to stay on an accelerating rotarod that rotates at 10 rpm. The rod diameter was 3.2 cm. Trained animals were given 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.

4.2.3. Behavioral testing. Some selected compounds (100 mg/kg) were screened for their behavioral effects using actophotometer[23] at 30 min and 1 h after injection. The behavior of animals inside the photocell was recorded as a digital score. Increased scores suggest good behavioral activity. The control animal was administered PEG. The observations are tabulated as Table 3.

4.2.4. CNS depressant study. The forced swim pool method described earlier was followed,[24] mice were placed in a chamber (diameter: 45 cm, height: 20 cm) containing water up to a 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 ip injection (100 mg/kg) of the test compounds 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 of water) during the 5 min test period was measured. The results are presented in Table 4.

4.2.5. Isolation of rat brain regions and GABA assay. The GABA assay for compounds **4**, **15**, and **17** was performed in brain tissue extracts enzymatically as previously described.[25] Adult Wistar rats were used for this purpose. After 2 h of drug administration (30 mg/kg,

ip), the animal was sacrificed by decapitation and the brain regions, midbrain, 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.[26]

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