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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of some novel methylene-bridged aryl semicarbazones as potential anticonvulsant agents

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Abstract A series of aryl semicarbazones containing a methylene bridge in their skeleton were synthesized as anticonvulsant agents. The strategy of introducing a methylene bridge was to increase the flexibility of the structures because of sp^3 hybridization. Pharmacological evaluations of the compounds were performed by determination of their effects on pentylenetetrazole-induced seizure parameters and neurotoxicity in mice. The statistical analysis indicated that most of the synthesized compounds showed significant anticonvulsant activity in comparison with the control group. No remarkable neurotoxicity was observed in rotorod test. A QSAR study was performed using multiple linear regressions. The results of the QSAR study confirmed that the compounds with Br at *para* position of these new derivatives are more potent than *para*-ethoxy series.

Keywords Semicarbazones · Methylene bridge · Anticonvulsant · Neurotoxicity · Pentylenetetrazole

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Introduction

Epilepsy is the third most common neurologic disorder after stroke and dementia, and as a general term includes more than 40 different types of seizure disorders characterized by recurrent episodes of abnormal events. About 0.5–1% of the world population are involved in this serious neurological disorder at any one time (Sander, 2003; Yogeeswari *et al.*, 2005a, b). Despite an optimal drug therapy, approximately one quarter of epileptic patients find an insufficient control for their convulsions. Epilepsy is controlled in about 75% of the patients, but 10% continue to have seizures at intervals of 1 month or less. This happens because of the lack of response toward conventionally available medical therapies. In addition, the present drug therapies are associated with some adverse effects, such as fatigue, drowsiness, headache, mental confusion, cognitive impairment, ataxia, hirsutism, gingival hyperplasia, megaloblastic anemia, and gastrointestinal disturbances. More than 25% of the patients discontinue the treatment because of the adverse drug reactions caused by initial antiepileptic drugs (AED) (Perucca, 2005; Yogeeswari *et al.*, 2006; Kennedy and Lhatoo, 2008).

In the last 20 years, a novel designed group of compounds, aryl semicarbazones and N_4-substituted phenyl aryl semicarbazones, have shown considerable anticonvulsant activities. Furthermore, these new compounds showed no remarkable sedative-hypnotic effects and were found to be less neurotoxic than usual AEDs (Pandeya *et al.*, 2000; Yogeeswari *et al.*, 2003; Yogeeswari *et al.*, 2004; Yogeeswari *et al.*, 2005a, b; Yogeeswari *et al.*, 2006; Shafiee *et al.*, 2009). Recognized modeling on anticonvulsants has identified that one aryl unit, one or two electron donor atoms, and one NH group in correct spatial arrangement are necessary for anticonvulsant activity. A four-point pharmacophore model for anticonvulsant activity of AEDs that has been developed previously (Yogeeswari *et al.*, 2005a, b) includes the following:

(i) The aryl ring center or the lipophilic group with a halogen substituent at *para* position (A)
(ii) An electron donor atom (D)
(iii) A hydrogen bond acceptor (HA)
(iv) A hydrogen bond donor (HD).

The limited lipophilic group (L) at N_4-substitution (Fig. 1) regulates the pharmacokinetic properties of aryl semicarbazones. It could be considered as a subordinate or the fifth pharmacophore together with the four essential ones mentioned above. Compounds with the alkyl groups such as methyl, ethyl, and acetomethyl replaced in L position have displayed better anticonvulsant effects against standard animal protocols (Yogeeswari *et al.*, 2004; Thirumurugan *et al.*, 2006). In their suggested pharmacophore model, the center of the aromatic ring is determined as the reference point for A (Yogeeswari *et al.*, 2005a, b; Rajak *et al.*, 2010). Several research groups have synthesized different derivatives of these compounds by changing the L and A binding sites to obtain more active and less toxic anticonvulsant agents. On the contrary, very few studies have been done about the distances and spatial conformations in the pharmacophore structure of aryl semicarbazones (Dimmock *et al.*, 2000; Pandeya *et al.*, 2000; Yogeeswari *et al.*, 2004; Yogeeswari *et al.*, 2005a, b; Yogeeswari *et al.*, 2006; Azam *et al.*, 2009; Azam *et al.*, 2010; Kaushik *et al.*, 2010). The average distance range between these four pharmacophore points has been previously calculated (Yogeeswari *et al.*, 2005a, b). The common structure of aryl semicarbazones has been superimposed with nine standard AEDs. It has been verified that aryl semicarbazones fit to the essential parts of the pharmacophore (Yogeeswari *et al.*, 2005a, b). However, the distances relating to pharmacophore A in aryl semicarbazones are considerably different from those in routine AEDs.

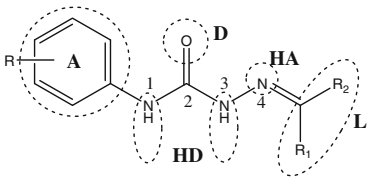


Fig. 1 Proposed pharmacophore in aryl semicarbazones

One notable fact about aryl semicarbazones is attributed to potential resonances in –NHCO– groups. It causes duality in H-donor/-acceptor identity of the moieties at 1, 3, and 4 positions because of the unstable amide protons (Fig. 1). Therefore, the spatial requirements of all pharmacophores can be anticipated by aryl semicarbazones. In this regard, a new series of aryl semicarbazones with an additional methylene bridge between N_1 and the carbonyl group (C_2) of the semicarbazone skeleton were synthesized. The reason for this modification was to introduce a different distance between pharmacophores and a more flexibility and lipophilicity in aryl semicarbazone structure. The anticonvulsant and neurotoxicity properties of the synthesized compounds were evaluated in comparison with the control group.

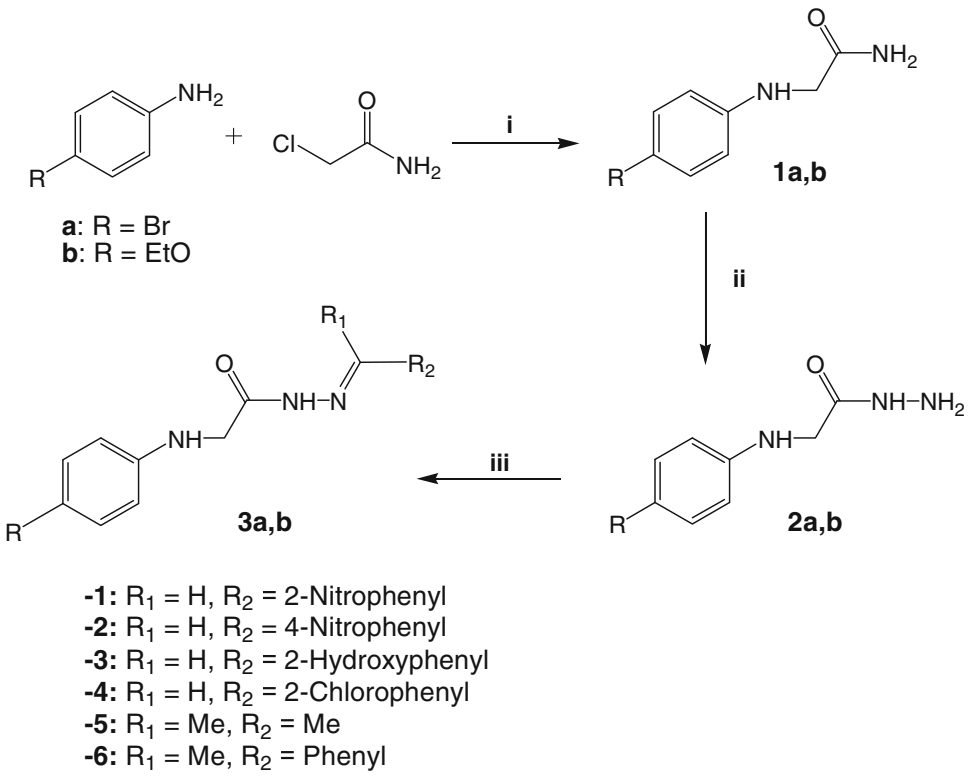
Results and discussion

Synthesis

Scheme 1 shows the synthesis of arylsemicarbazone derivatives **3a(1–6)** and **3b(1–6)**. The 4-substituted aniline was reacted with 2-chloroacetamide in the presence of Na_2CO_3 to give **1a**, **b**. Acetohydrazide derivatives **2a**, **b** were prepared by an uncommon and slow reaction of **1a**, **b** with hydrazine hydrate as previously reported (Pandeya *et al.*, 1999; Dumciute *et al.*, 2006; Singh *et al.*, 2011). Finally, aryl semicarbazones **3a(1–6)** and **3b(1–6)** were synthesized by condensation of **2a** and **2b** with appropriate aldehydes and ketones. The physicochemical characteristics of the target compounds are given in Table 1.

Pharmacology

The anticonvulsant mechanism of the semicarbazones is not clearly defined. However, it is proposed that they act through inhibition of the GABA transaminase enzyme (GABA-T) and consequently increase the GABA levels in the brain. These compounds also affect seizure activity by blocking the voltage-gated sodium ion channels (Dimmock *et al.*, 2000; Yogeeswari *et al.*, 2005a, b; Thirumurugan *et al.*, 2006). The above functions are similar to well-established mechanisms of anticonvulsant agents (Treiman, 2001; Bölcskei *et al.*, 2008). Pentylenetetrazole (PTZ) by affecting the $GABA_A$-gated chloride channels induces seizure activities through stimulation of epileptogenic



Scheme 1 Synthesis of aryl semicarbazone derivatives (**3a**, **b**). Reagents and conditions: (*i*) Na_2CO_3, EtOH, refluxed for 24 h; (*ii*) NH_2NH_2, EtOH, refluxed for 24 h; (*iii*) Appropriate aldehyde or ketone, EtOH, refluxed for 4 h

Table 1 Physicochemical characteristics of finally synthesized compounds

Compounds	*R*	R_1	R_2	mp (°C)	Yield (%)	Mol. Formula	Mol. Wt.
3a-1	Br	H	2-Nitrophenyl	201–203	51	$C_{15}H_{13}N_4O_3Br$	377.2
3a-2	Br	H	4-Nitrophenyl	238–240	75	$C_{15}H_{13}N_4O_3Br$	377.2
3a-3	Br	H	2-Hydroxyphenyl	238–241	87	$C_{15}H_{14}N_3O_2Br$	348.2
3a-4	Br	H	2-Chlorophenyl	190–192	53	$C_{15}H_{13}N_3OBrCl$	366.6
3a-5	Br	Me	Me	155–157	84	$C_{11}H_{14}N_3OBr$	284.2
3a-6	Br	Me	Phenyl	187–189	75	$C_{16}H_{16}N_3OBr$	346.2
3b-1	EtO	H	2-Nitrophenyl	126–128	56	$C_{17}H_{18}N_4O_4$	342.3
3b-2	EtO	H	4-Nitrophenyl	215–216	64	$C_{17}H_{18}N_4O_4$	342.3
3b-3	EtO	H	2-Hydroxyphenyl	187–189	42	$C_{17}H_{19}N_3O_3$	313.4
3b-4	EtO	H	2-Chlorophenyl	185–188	43	$C_{17}H_{18}N_3O_2Cl$	331.8
3b-5	EtO	Me	Me	143–145	52	$C_{13}H_{19}N_3O_2$	249.3
3b-6	EtO	Me	Phenyl	146–148	47	$C_{18}H_{21}N_3O_2$	311.4

centers in the brain. PTZ-mediated seizure was used as a clinically relevant experimental animal model for evaluation of seizure latency, seizure duration, and death time (DT) through acute administration of PTZ at CD_{97} (a dose of PTZ to produce clonic seizures lasting for a period of at least 5 s in 97% of animals tested) (Gholizadeh *et al.*, 2007).

The target compounds were evaluated for anticonvulsant activity against PTZ-induced tonic-clonic generalized seizure. Their neurotoxicity was also measured by rotorod test. The results for all derivatives of **3a**, **b** on PTZ-induced seizure parameters are reported in Tables 2 and 3. The onset of seizure was 184 ± 32 s for the control group. Our results showed that administration of compounds **3a-1**, **3a-5**, **3a-6**, **3b-1**, **3b-3**, and **3b-5** ($P < 0.01$) and **3b-4** and **3b-6** ($P < 0.05$) with a dose of 300 mg/kg significantly increased seizure latency in comparison with control animals. In addition, intraperitoneal injection of 100 mg/kg of compounds **3b-2** ($P < 0.01$) and **3a-2**, **3a-3**, **3b-3**, and **3b-5** ($P < 0.05$) significantly increased seizure latency. These results are probably caused by an elevation of GABA levels in the brain for –NO_2–, –OH–, and alkyl-substituted derivatives (Yogeeswari *et al.*, 2005a, b, 2006; Rajak *et al.*, 2010). No significant alteration on seizure latency was found with

the dose 50 mg/kg for some synthesized compounds (Table 2).

The mean of total seizure duration for the control group was 9.9 ± 1.3 s. Evaluation of seizure duration indicated that injection of compounds **3a-1**, **3a-5**, and **3b-5** ($P < 0.01$) and **3a-2** and **3a-6** ($P < 0.05$) with dose 300 mg/kg significantly decreased total seizure duration compared with control animals. Compounds **3a-2** and **3b-5** caused significant ($P < 0.05$) decrease on the total seizure duration at the dose of 100 mg/kg in comparison with control group (Table 2). Based on our results, it can be deduced that substitution of 4-Br at *R* position in combination with each group of nitrophenyl, methyl, or phenyl at R_2 position of these new synthesized compounds leads to a better anticonvulsant effect compared with the other functional groups.

Table 2 Effects of methylene bridge containing aryl semicarbazones on PTZ-induced seizure parameters

Compound	Dose (mg/kg)	Onset (s)	Total seizure time (s)
Control (vehicle)	2 ml/kg	184 ± 32	9.9 ± 1.3
3a-1	100	144 ± 29	11 ± 2.0
	300	1,073 ± 224**	2.8 ± 1.1**
3a-2	50	640 ± 229	6.1 ± 1.5
	100	809 ± 293*	3.8 ± 1.4*
	300	596 ± 240	3.7 ± 0.81*
3a-3	50	724 ± 290	7.7 ± 2.2
	100	884 ± 276*	6.6 ± 2.0
	300	562 ± 271	8.6 ± 2.2
3a-4	100	403 ± 101	6.4 ± 1.5
	300	514 ± 330	4.5 ± 1.5
3a-5	100	224 ± 50	10 ± 2.0
	300	1,098 ± 212**	3.3 ± 1.1**
3a-6	100	498 ± 229	11 ± 2.7
	300	915 ± 268**	3.1 ± 1.1*
3b-1	100	275 ± 84	8.7 ± 1.7
	300	626 ± 217**	6.8 ± 1.7
3b-2	50	603 ± 205	5.3 ± 1.2
	100	885 ± 219**	5.2 ± 1.0
	300	643 ± 243	7.3 ± 2.5
3b-3	50	275 ± 46	11 ± 1.5
	100	616 ± 106*	6.4 ± 0.92
	300	903 ± 198**	5.2 ± 1.8
3b-4	100	436 ± 230	8.5 ± 1.9
	300	590 ± 192*	9.3 ± 1.6
3b-5	100	373 ± 70*	4.9 ± 0.67*
	300	425 ± 66**	4.2 ± 0.74**
3b-6	100	243 ± 67	5.9 ± 0.92
	300	414 ± 101*	5.8 ± 0.70
Phenobarbital[a]	15	1,088 ± 319**	2.2 ± 1.0**
Sodium valproate[b]	220	1,207 ± 267**	2.3 ± 1.1**

All compounds were injected intraperitoneally to groups of mice at presented doses 30 min before PTZ injection (80 mg/kg). Control group received DMSO solution as vehicle. [a] Phenobarbithal and [b] sodium valproate were injected as standard drugs. Data were shown as mean ± SEM of eight animals in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared with control animals

The mean time for incidence of death following PTZ injection (death time) was 416 ± 58.2 s in the control group. It was indicated that DT significantly increased after administration of 300 mg/kg of **3a-1**, **3a-5**, and **3b-2** ($P < 0.001$), **3a-6** and **3b-3** ($P < 0.01$), and **3a-2** and **3a-3** ($P < 0.05$). Pretreatment of animals with 100 mg/kg of **3a-2**, **3a-3**, **3a-6**, and **3b-2** ($P < 0.001$), **3b-1** and **3b-4** ($P < 0.01$), and **3a-4** and **3a-5** ($P < 0.05$) also caused significant increase in DT (Table 3). These results were also confirmed with findings of onset of seizure and seizure duration.

It is important to consider that the substitution of –OH group at R_2 position of the 4-bromo series (**3a-3**) increased DT at lower doses (50 and 100 mg/kg, $P < 0.001$) more than higher concentration (300 mg/kg, $P < 0.05$). One tentative explanation for this event could be related to increased hydrogen bonding interactions with the receptors at lower doses (Table 3).

Our findings also indicated that the compounds with 4-bromo group at *R* moiety are more effective than their counterparts with 4-ethoxy at the same position (Tables 2, 3). It seems that the lipophilicity of these molecules plays an important role in their anticonvulsant activity. The lipophilic property of the 4-bromo substituent is better than 4-ethoxy and this effect might lead to more affinity toward hydrophobic region of the receptor at A position of the proposed pharmacophore. One more possible reason is the improved diffusion of 4-Br-substituted compounds across the blood−brain barrier.

High dose (300 mg/kg) of compounds **3a-1**, **3a-2**, **3a-3**, **3a-5**, **3a-6**, **3b-2**, and **3b-3** that emerged as promising anticonvulsant agents was further tested for acute neurological toxicity by the rotorod method. Evaluation of all tested compounds on minimal motor impairment indicated no significant neurotoxicity except for **3b-2** (Fig. 2). No delayed mortality was observed during 1 week after neurotoxicity assessment in treated mice.

Quantitative structure activity relation

To investigate the role of structural features on the anti-seizure activity of the synthesized compounds, quantitative structure activity relation (QSAR) studies were conducted. The QSAR method was first developed by Hansch and Fujita as a way of identifying the relationship between compound structures and their activities (Kumar *et al.*, 2007). QSAR is based on this assumption that biological activity is a mathematical function of molecular properties

Table 3 Effects of methylene bridge containing aryl semicarbazones on protection (%), survived (%), and death time parameters in PTZ-induced seizure

Compound	Dose (mg/kg)	Protection %[a]	Survived %[b]	Death time (s)
Control (vehicle)	2 ml/kg	0	0	416 ± 58.2
3a-1	100	0	38	897 ± 276
	300	33	33	1,265 ± 171***
3a-2	50	0	62	1,400 ± 205***
	100	38	62	1,456 ± 188***
	300	16	50	1,122 ± 305*
3a-3	50	25	100	1,800 ± 0.00***
	100	38	100	1,800 ± 0.00***
	300	25	25	832 ± 237*
3a-4	100	0	25	885 ± 225*
	300	14	29	781 ± 242
3a-5	100	0	25	945 ± 203*
	300	45	64	1,428 ± 160***
3a-6	100	14	86	1,577 ± 223***
	300	38	50	1,161 ± 254**
3b-1	100	0	12	1,011 ± 206**
	300	14	14	734 ± 194
3b-2	50	16	50	1,137 ± 313*
	100	22	44	1,286 ± 201***
	300	16	67	1,528 ± 182***
3b-3	50	0	12	659 ± 183
	100	0	0	896 ± 200
	300	22	33	1,055 ± 225**
3b-4	100	14	43	1,189 ± 291**
	300	0	12	651 ± 180
3b-5	100	0	0	623 ± 130
	300	0	0	606 ± 106
3b-6	100	0	0	310 ± 71.3
	300	0	0	483 ± 93.6
Phenobarbital	15	50	100	1,800 ± 0.00***
Sodium valproate	220	50	100	1,800 ± 0.00***

All compounds were injected intraperitoneally to groups of mice at presented doses 30 min before PTZ injection (80 mg/kg). Control group received DMSO solution as vehicle. Phenobarbithal and sodium valproate were injected as standard drugs. Data were shown as mean ± SEM of eight animals in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ show significant differences compared with control animals

[a] Protection % = (number of mice was not afflicted with seizure/number of tested mice in each group) × 100

[b] Survived % = (number of mice was alive/number of tested mice in each group) × 100. The time of death after PTZ injection was shown as death time

called descriptors (Kumar *et al.*, 2007). The quality of a QSAR model depends on many factors including biological data, the choice of the descriptors, and statistical methods. The common approaches used in QSAR studies are normally based on linear methods such as multiple linear regression (MLR) and principal component regression (PCR) or non-linear methods such as support vector machines, neural networks, and fuzzy mappings (Jalali-Heravi and Asadollahi-Baboli, 2009; Afiuni-Zadeh and Azimi, 2010). For this purpose, the ligands were drawn in chemdraw and saved as cdx file type. A primary 3D generation of the structures was done through iterative runs of OpenBabel in Linux (Mandriva) operating system. The structures were then entered into Hyperchem for geometry optimization using AM1 semiempirical method. The conjugate gradient algorithm Polak Ribiere with the RMS value 0.1 kcal/Å·mol was used during minimization. Automation of minimization for different compounds was done by means of an application implemented in VB.net through dynamic data exchange with Hyperchem. The resulted ligands were then subjected to Dragon Software for calculation of descriptors. During this stage, up to 436 descriptors were calculated for each ligand. The descriptors included constitutional, functional, properties, topological, and 2D autocorrelations indices. The matrix of descriptors (each row vector representing one ligand) together with the p-function of the total seizure time at 300 mg/kg (activity vector) was entered into Matlab for further statistical evaluations. To decrease autocorrelations in the data, a pretreatment step was performed. For this purpose, Pearson's correlation for all descriptors was calculated. Among the two descriptors with high co-linearity (correlation > 0.85), the one with more correlation with the activity vector was retained and the other was excluded. The number of the resulted descriptors after this step was reduced to 28 descriptors. Correlations of some remained descriptors with the activity vector are shown in Table 4. The resulted matrix of the descriptors and the activity vector were subsequently entered into SPSS for MLR analysis using stepwise method. The two descriptors, namely, T (N..Br) and MATS7e were selected to be in good relationship with the activity vector. To obtain a more reasonable model compound, **3b-3** was considered as an outlier and excluded from the final model. The final MLR equation is as follows:

$$\begin{aligned} -\log(\text{activity}) &= \{0.06(\text{TN..Br}) \pm 0.002\} \\ &+ \{-1.493(\text{MATS7e}) \pm 0.260\} \\ &+ \{-1.082 \pm 0.56\} \\ r &= 0.941 \end{aligned}$$

To evaluate the predictive activity of the model, leave one out cross-validation (LOO-CV) internal test was used. During this procedure, iterative cycles of ligand elimination and model building were preceded by predicting the activity of the eliminated ligand. The two metrics R_{cv} and predicted residual error sum of squares (PRESS) were 0.88

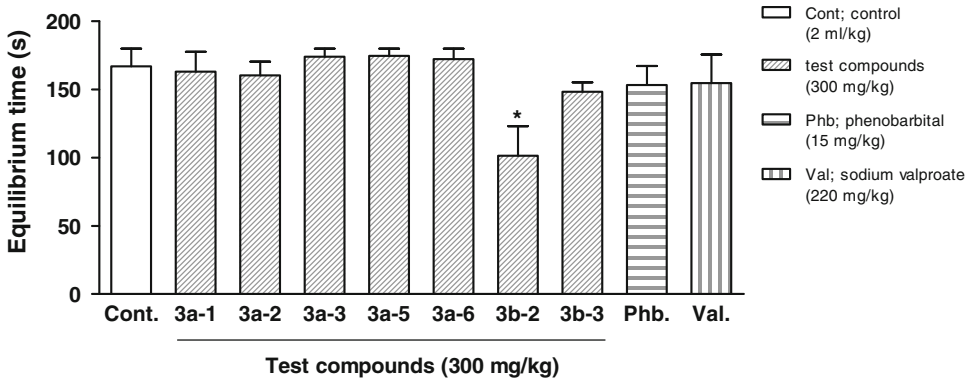


Fig. 2 Evaluation of effective synthesized compounds on neurotoxicity. Tested compounds were intraperitoneally injected to groups of mice at presented doses. Neurologic toxicity was defined as the failure of animals to remain on the rod for three trials. Each trial continues for 1 min. The equilibrium time or time that each animal remains on the rod was measured for total duration of three trials (180 s). Each value represents the mean ± SEM of seven animals. Control group received vehicle with the same volume (DMSO solution). Phenobarbithal (15 mg/kg) and sodium valproate (220 mg/kg) were intraperitoneally injected as standard drugs. $*P < 0.05$ shows significant difference compared with control group

and 0.073, respectively (Fig. 3). The result of CV internal validation revealed the stability of the model to be in a reasonable level.

The descriptor T (N..Br) corresponds to sum of topological distances between N and Br and is among topological descriptors. MATS7e corresponds to Moran autocorrelation −lag7/weighted by atomic Sanderson's electronegativites and is among 2D autocorrelation descriptors. The result of this study is in accord with the fact that the compounds with bromine at *para* position are more potent than their counterparts with ethoxy group. It seems that the presence of different substituents on the second phenyl ring can alter the conformation of the resulted structures (Fig. 4). In this regard, the topological distances between Br and N and consequently the activity of the final compounds could be influenced.

Conclusion

In this study, a number of methylene-bridged aryl semicarbazones were designed, synthesized, and characterized by spectral analysis. They were evaluated for preliminary anticonvulsant activity using PTZ animal model and rotorod neurotoxicity screening after intraperitoneal administration.

In general, **3a-1**, **3a-5**, **3a-6**, **3b-1**, **3b-3**, **3b-5**, and **3b-6** derivatives exhibited better anticonvulsant activities against PTZ-induced seizure at high dose (300 mg/kg) in comparison with the lower concentrations (100 or 50 mg/kg). The activity was related to the existence of methyl groups as R_1 substituent and 2-nitrophenyl, methyl, phenyl, and 2-hydroxyphenyl groups as R_2 substituent in new derivatives structure. Administration of moderate dose (100 mg/kg) of compounds **3a-2** and **3a-3** showed better anticonvulsant capacity in PTZ-induced seizure compared with the higher concentration (300 mg/kg). This fact is more obvious at death time. This kind of activity was attributed to the presence of 4-nitrophenyl and 2-hydroxyphenyl groups as R_2 substituent of these new derivatives in combination with 4-Br as R substituent. The result of the QSAR study indicates that the compounds with bromine at *para* position are more potent than their counterparts with ethoxy group. As a consequence of QSAR study, two structural characteristics of these compounds are important in their activities. The first is the topological distances between N and Br and the second one is atomic Sanderson's electronegativity of the substituent. The obtained mathematical equation can be used for designing of other structures in the future studies. In all experiments, none of these new derivatives showed more protection than the standard drugs. However, **3a-1**, **3a-5**, **3a-6**, and **3b-3** at the dose of 300 mg/kg and **3a-2**, **3a-3**, and **3b-2** at the dose of 100 mg/kg were comparable with the standard drugs. Neurotoxicity evaluation by rotorod test showed that all compounds except **3b-2** did not cause any neurological toxic effect in comparison with control group and standard drugs.

Experimental

Chemistry

All reagents and solvents were used from commercially available sources (Sigma and Merck). Capillary tubes on an electrothermal melting point were used to determine melting point. Proton nuclear magnetic resonance (^{1}H NMR), ^{13}C

Table 4 Autocorrelation in the matrix of resulted descriptors and activity after pretreatment procedure

MSD'	'X4A'	'S3K'	'PW5'	'C1C2'	'C1C3'	'VEA1'	'T(N..Br)'	'BEHv4'	'MATS2v'	'MATS8v'	'MATS1e'	'MATS2e'	'MATS7e'	'GATS2m'	'GATS7e'	'Hy'	Activity'
1	0.7	0.3	−0.7	0.2	0.6	−0.2	−0.2	−0.4	0.6	0.5	−0.3	−0.1	0.1	0.1	−0.3	0.2	0
	1	0.3	−0.5	0.2	0.4	0.3	−0.6	0.1	0.3	0.2	−0.7	−0.7	0.4	−0.2	−0.1	0.3	−0.4
		1	−0.1	0.2	0.4	−0.1	−0.6	0.4	0.1	0	0.4	0	0.2	−0.8	−0.6	−0.4	−0.3
			1	0.4	0.1	0.1	−0.2	0.4	0	0.1	0.2	0.1	−0.5	−0.2	0.5	−0.4	0.2
				1	0.8	0	−0.3	0.3	0.5	0.7	0	0.1	−0.6	−0.1	0.5	−0.3	0.3
					1	−0.3	−0.4	0	0.8	0.8	−0.1	0.1	−0.5	−0.1	0.1	−0.2	0.3
						1	−0.4	0.6	−0.3	−0.3	−0.4	−0.6	0.5	−0.3	0.3	0.1	−0.5
							1	−0.5	−0.2	0	0.3	0.6	−0.3	0.8	0.2	0.1	0.6
								1	−0.4	−0.3	0.1	−0.4	0.3	−0.7	0	−0.3	−0.4
									1	0.8	−0.2	0.2	−0.6	0.2	0.3	−0.2	0.3
										1	−0.1	0.4	−0.7	0.4	0.3	0	0.5
											1	0.8	−0.3	−0.2	−0.3	−0.6	0.3
												1	−0.7	0.4	0	−0.4	0.7
													1	−0.4	−0.5	0.5	−0.7
														1	0.5	0.3	0.6
															1	−0.2	0.4
																1	−0.2
																	1

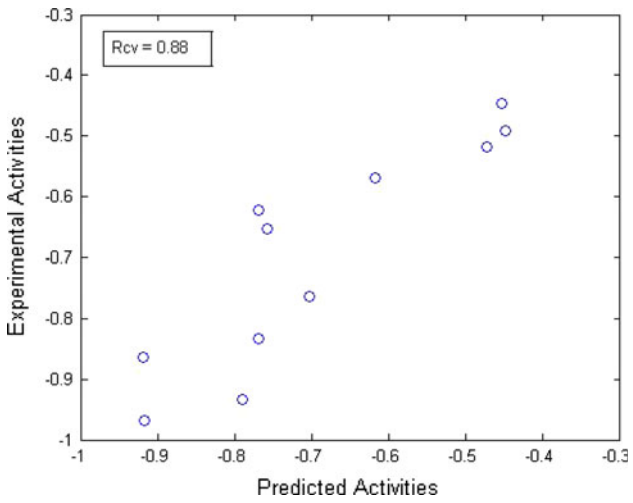


Fig. 3 Plot of predicted values versus experimental values for the activity vector

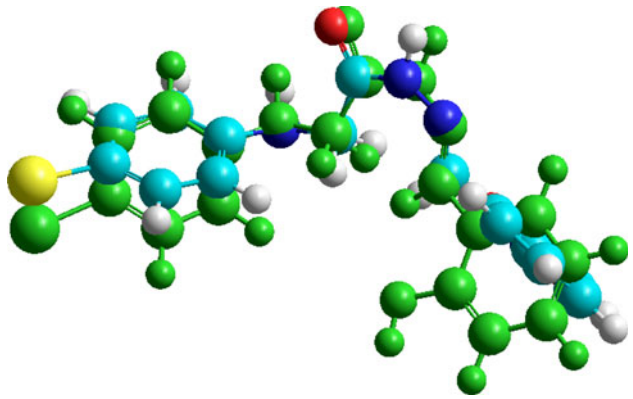


Fig. 4 Superimposition of compounds **3a-1** (*front molecule*) and **3a-3** (*back molecule*). The presence of different substituents on the second ring can affect the activity of the resulted structures because of changes in molecular features such as T(N..Br)

nuclear magnetic resonance (^{13}C NMR), and infrared were used for structure elucidation. ^{1}H NMR and FT-IR spectra were obtained by Brucker advance (500 MHz) and Mgna-IR-550 instruments, respectively. ^{13}C NMR spectra were obtained by Brucker advance (125 MHz). Chemical shifts (δ) are reported in parts per million (ppm) by using tetramethyl silane as internal standard. Elemental analyses (C, H, and N) were undertaken with Perkin–Elmer model 240C analyzer. Ascending thin layer chromatography (TLC) on silica gel G (Merck) was used to follow-up the reactions for purity and completion. Iodine vapor and UV light were used for visualization of TLC spots. Purification of derivatives was performed on silica gel 60 (particle size 0.06–0.2).

*General procedure for the synthesis of 2-(arylamino) acetamide (**1a**,**b**)*

In a one-neck flask containing 100 ml absolute ethanol, 4-substituted aniline (0.05 mol), sodium carbonate (2.65 g, 0.025 mol), and 2-chloroacetamide (4.67 g, 0.05 mol) were added and refluxed for 24 h with stirring (Pandeya *et al.*, 2000). The solvent was evaporated and 40 ml distilled water was added to residue. The precipitate was filtered off, washed with extra water, and recrystallized from 90% ethanol.

*2-(4-Bromophenylamino) acetamide (**1a**)* Yield 60%, mp: 136–138°C. IR (KBr, *v*max, cm^{-1}): 3400 (N–H, NH_2), 3217 (C–H, Ar), 1670 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) ?δ: 7.66 (s, 1H, $CONH_2$), 7.13 (d, 2H, $J = 8.1$ Hz, Ar–H), 6.72 (s, 1H, $CONH_2$), 6.45(d, 2H, $J = 8.1$ Hz, Ar–H), 4.40(s, 2H, CH_2), 3.61(s, 1H, Ar–NH); Elem. Anal. Calcd. for $C_8H_9N_2OBr$; C: 41.95, H: 3.96, N: 12.23, found C: 42.17, H: 3.59, N: 12.45

*2-(4-Ethoxyphenylamino) acetamide (**1b**)* Yield 56%, mp: 146–148°C. IR (KBr, *v*max, cm^{-1}): 3399 (N–H, NH_2), 2982 (C–H, CH_2), 2902 (C–H, CH_3), 1647 (C=O), 1515 (C=C, Ar); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 7.28 (s, 1H, $CONH_2$), 7.07 (s, 1H, $CONH_2$), 6.69 (d, 2H, $J = 8.4$ Hz, Ar–H), 6.46 (d, 2H, $J = 8.4$ Hz, Ar–H), 5.45 (s, 1H, Ar–NH), 3.86 (q, 2H, $J = 6.4$ Hz, CH_2O), 3.49 (s, 2H, CH_2), 1.23 (tr, 3H, $J = 6.4$ Hz, CH_3); Elem. Anal. Calcd. for $C_{10}H_{14}N_2O_2$; C: 61.84, H: 7.27, N: 14.42. found C: 61.97, H: 7.51, N: 14.12.

*General procedure for the synthesis of 2-(arylamino) acetohydrazide (**2a**,**b**)*

A solution of compound **1a-b** (0.03 mol) in 50 ml of absolute ethanol was added dropwise to hydrazine hydrate (14.5 ml, 10 mol) with vigorous stirring and the mixture was refluxed for 24–36 h until the reaction was completed (Pandeya *et al.*, 2000; Dumciute *et al.*, 2006). The solvent was evaporated and distilled water (50 ml) was added to the mixture. The white resultant precipitate was filtered, washed with extra water, and crystallized in 90% ethanol twice.

*2-(4-Bromophenylamino) acetohydrazide (**2a**)* Yield 50%, mp: 143–145°C. IR (KBr, *v*max, cm^{-1}): 3322 and 3296 (N–H), 1684 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 9.14 (s, 1H, CONH), 7.18 (d, 2H, $J = 8.0$ Hz, Ar–H), 6.51 (d, 2H, $J = 8.0$ Hz, Ar–H), 6.08 (s, 1H, Ar–NH), 4.26 (s, 2H, CH_2), 3.63(s, 2H, NH_2); Elem. Anal. Calcd. for $C_8H_{10}N_3OBr$; C: 39.37, H: 4.13, N: 17.22. found C: 39.05, H: 4.20, N: 17.33.

*2-(4-Ethoxyphenylamino) acetohydrazide (**2b**)* Yield 44%, mp: 132–134°C. IR (KBr, *v*max, cm^{-1}): 3346 and 3301 (N–H), 2980 (C–H, CH_2), 2869 (C–H, CH_3), 1655 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 9.03 (s, 1H, CONH), 6.70 (d, 2H, $J = 8.0$ Hz, Ar–H), 6.47 (d, 2H, $J = 8.0$ Hz, Ar–H), 5.45 (s, 1H, Ar–NH), 4.21 (bs, 2H, NH_2), 3.83 (q, 2H, $J = 6.8$ CH_2O), 3.54 (s, 2H, CH_2), 1.23 (t, 3H, $J = 6.8$, CH_3); Elem. Anal. Calcd. for $C_{10}H_{15}N_3O_2$; C: 57.40, H: 7.23, N: 20.08. found C: 57.35, H: 7.55, N: 20.40.

*General procedure for the synthesis of **1–6** derivatives of **3a** and **3b***

Appropriate aldehyde or ketone (5 mmol) was added dropwise to solution of **2a**,**b** (5 mmol) in 40 ml of ethanol and the result mixture was refluxed for 3–4 h. The reaction mixture was concentrated to half of the volume and allowed to cool to room temperature (Pandeya *et al.*, 2000; Yogeeswari *et al.*, 2004). The precipitate was filtered, washed with water, and recrystallized from 90% ethanol twice. ^{1}HNMR of **1–6** derivatives **3a** and **3b** showed the presence of both Z and E isomers. The efforts for separation of these isomers by column chromatography or TLC were unsuccessful. Therefore, a mixture of two isomers was used for biological studies.

*2-(4-Bromophenylamino)-N′-(2-nitrobenzylidene)acetohydrazide (**3a-1**)* Yield 51%, mp: 201–203°C. IR (KBr, *v*max, cm^{-1}): 3365 and 3254 (N–H), 3088 (C–H, Ar), 1695 (C=O), 1513 and 1380 (N–O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.78 (bs, 1H, CONH), 8.65 and 8.35 (s, 1H, imine-H), 8.09–8.01 (m, 2H, Ar–H), 7.75–7.65 (m, 2H, Ar–H), 7.18 (m, 2H, Ar–H), 7.57 (m, 2H, Ar–H), 6.30 and 6.01 (s, 1H, Ar–NH), 4.21 and 3.81 (s, 2H, CH_2); Elem. Anal. Calcd. for $C_{15}H_{13}N_4O_3Br$; C: 47.76, H: 3.47, N: 14.85. found C: 48.09, H: 3.81, N: 14.43.

*2-(4-Bromophenylamino)-N′-(4-nitrobenzylidene)acetohydrazide (**3a-2**)* Yield 75%, mp: 238–240°C. IR (KBr, *v*max, cm^{-1}): 3407 and 3185 (N–H), 3080 (C–H, Ar), 1679 (C=O), 1525 and 1340 (N–O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.77 (bs, 1H, CONH), 8.33 and 8.09 (s, 1H, imine-H), 8.25–8.18 (m, 2H, Ar–H), 7.95–7.85 (m, 2H, Ar–H), 7.25–7.15 (m, 2H, Ar–H), 6.70–6.65 (m, 2H, Ar–H), 6.21 and 6.02 (s, 1H, Ar–NH), 4.25 and 3.82 (s, 2H, CH_2); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 172.06 and 167.62 (C=O), 148.22 and 148.08 (C_4, nitrophenyl), 144.91 (CH-imine), 141.65, 141.04, 140.87, 131.85, 131.75, 128.43, 128.32, 124.48 (aromatic), 114.74 (C_2, bromophenyl), 107.63 and 107.14 (C_4, bromophenyl), 46.23 and 44.26 (CH_2); Elem. Anal. Calcd. for $C_{15}H_{13}N_4O_3Br$; C: 47.76, H: 3.47, N: 14.85. found C: 47.37, H: 3.70, N: 14.51.

*2-(4-Bromophenylamino)-N′-(2-hydroxybenzylidene) acetohydrazide (**3a-3**)* Yield 87%, mp: 238–241°C. IR (KBr, *v*max, cm^{-1}): 3438 and 3175 (N–H), 3081 (C–H, Ar), 1676 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.40 (bs, 2H, Ar–OH and CONH), 8.44 and 8.29 (s,1H, imine-H), 7.70–7.47 (m, 1H, Ar–H), 7.30–7.10 (m, 3H, Ar–H), 6.90–6.80 (m, 2H, Ar–H), 6.55–6.50 (m, 2H, Ar–H), 6.25 and 5.98 (s, 1H, Ar–NH), 4.17 and 3.81 (s, 2H, CH_2); Elem. Anal. Calcd. for $C_{15}H_{14}N_3O_2Br$; C: 51.74, H: 4.05, N: 12.07. found C: 51.65, H: 3.82, N: 12.45.

*2-(4-Bromophenylamino)-N′-(2-chlorobenzylidene)acetohydrazide (**3a-4**)* Yield 53%, mp: 190–192°C. IR (KBr, *v*max, cm^{-1}): 3404 and 3188 (N–H), 3071 (C–H,Ar), 1682 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.74 (bs, 1H, CONH), 8.63 and 8.39 (s, 1H, imine-H), 8.05–7.93 (m, 1H, Ar–H), 7.50–7.35 (m, 2H, Ar–H),7.25–7.18 (m, 1H, Ar–H), 6.55–6.50 (m, 2H, Ar–H), 6.26 and 6.01 (s, 1H, Ar–NH), 4.22 and 3.80 (s, 2H, CH_2); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 171.27 (C=O), 145.42 (CH-imine), 144.80 (C_1, bromophenyl), 141.65, 134.53, 132.79, 132.46, 132.07, 131.65, 131.51, 130.59, 130.12, 129.75, 128.01, 127.21, 127.16, 127.10 (aromatic), 115.19 (C_4, bromophenyl), 45.43 (CH_2); Elem. Anal. Calcd. for $C_{15}H_{13}N_3OBrCl$; C: 49.14, H: 3.57, N: 11.46. found C: 49.41, H: 3.60, N: 11.55.

*2-(4-Bromophenylamino)-N′-(propan-2-ylidene)acetohydrazide (**3a-5**)* Yield 84%, mp: 155–157°C. IR (KBr, *v*max, cm^{-1}): 3424 and 3216 (N–H), 3120 (C–H, Ar), 1673 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 10.30 and 10.03 (s, 1H, CONH), 7.17 (d, 2H, $J = 8$ Hz, Ar–H), 6.52 (d, 2H, $J = 8$ Hz, Ar–H), 6.13 and 5.90 (s, 1H, Ar–NH), 4.04 and 3.76 (s, 2H, CH_2), 1.85 (s, 3H, CH_3), 1.81 (s, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 171.35 and 165.82 (C=O), 150.90 (C-imine), 146.57 (C_1, phenyl), 132.31 and 131.92 (C_3, phenyl), 115.11 and 114.67 (C_2, phenyl), 109.30 (C_4, phenyl), 48.53 and 45.16 (CH_2), 25.46 and 16.06 (CH_3); Elem. Anal. Calcd. for $C_{11}H_{14}N_3OBr$; C: 46.50, H: 4.97, N: 14.79. found C: 46.23, H: 4.65, N: 14.90.

*2-(4-Bromophenylamino)-N′-(1-phenylethylidene)acetohydrazide (**3a-6**)* Yield 75%, mp: 187–189°C. IR (KBr, *v*max, cm^{-1}): 3401 and 3172 (N–H), 3093 (C–H, Ar), 1662 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 10.75 and 10.40 (s, 1H, CONH), 7.80–7.70 (m, 2H, Ar–H), 7.45–7.35 (m, 3H, Ar–H), 7.25–7.10 (m, 2H,Ar–H), 6.60–6.50 (m, 2H, Ar–H), 6.20 and 6.00 (s, 1H, Ar–NH), 4.24 and 3.85 (s, 2H, CH_2), 2.25 (s, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 171.70 and 171.22 (C=O), 148.52 and 147.89 (C-imine), 146.71 (C_1, bromophenyl), 142.88, 139.46, 137.86, 133.19, 133.00, 131.35, 130.11, 129.87, 129.18, 128.91, 128.64, 128.28, 128.20, 128.12, 127.93, 126.26, 125.81,

124.20 (aromatic), 114.39, 114.28 (C_2, bromophenyl) 107.96, 107.86 (C_4, bromophenyl), 46.65, 46.42 (CH_2), 13.62, 13.35 (CH_3); Elem. Anal. Calcd. for $C_{16}H_{16}N_3OBr$; C: 55.51, H: 4.66, N: 12.14. found C: 55.38, H: 4.44, N: 12.37.

*2-(4-Ethoxyphenylamino)-N′-(2-nitrobenzylidene)acetohydrazide (**3b-1**)* Yield 56%, mp: 126–128°C. IR (KBr, *v*max, cm^{-1}): 3331 (N–H), 2984 (C–H, CH_2), 2909 (C–H, CH_3), 1703 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.76 (bs, 1H, CONH), 8.65 and 8.35 (s, 1H, imine-H), 8.00–7.90 (m, 2H, Ar–H), 7.75–7.60 (m, 2H, Ar–H), 6.70–6.50 (m, 4H, Ar–H), 5.55 and 5.30 (s, 1H, Ar–NH), 4.14 and 3.76 (s, 2H, CH_2), 3.86 (m, 2H, CH_2O), 1.33–1.23 (m, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 172.53 and 168.30 (C=O), 150.91 and 150.63 (C_4, ethoxyphenyl), 148.38 (C_2, nitrophenyl), 142.91 (C-imine), 139.18, 134.10, 133.81, 130.97, 130.82, 129.17, 128.97, 128.46, 125.07, 124.85, 115.74, 113.84 (phenyl), 63.72 (OCH_2), 47.41 and 45.00 (CH_2), 15.29 (CH_3–C=N); Elem. Anal. Calcd. for $C_{17}H_{18}N_4O_4$; C: 59.64, H: 5.30, N: 16.37. found C: 59.42, H: 5.56, N: 16.04.

*2-(4-Ethoxyphenylamino)-N′-(4-nitrobenzylidene)acetohydrazide (**3b-2**)* Yield 64%, mp: 215–216°C. IR (KBr, *v*max, cm^{-1}): 3366 (N–H), 2936 (CH, CH_3), 1671 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.75 (bs, 1H, CONH), 8.35–8.25 (m, 3H, imine-H and Ar–H), 8.10–7.91 (m, 2H, Ar–H), 6.80–6.69 (m, 2H, Ar–H), 6.65–6.50 (m, 2H, Ar–H), 5.55 and 5.35 (s, 1H, Ar–NH), 4.19 and 3.81(s, 2H, CH_2), 3.88 (m, 2H, CH_2O), 1.35–1.24 (m, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 172.68 and 168.34 (C=O), 150.91 and 150.58 (C_4, ethoxyphenyl), 148.16 (C_4, nitrophenyl), 144.85 (CH-imine), 143.03, 142.80, 141.50, 141.08, 140.91, 128.42, 128.30, 124.52, 115.76, 113.87, 113.82 (aromatic), 63.78 (OCH_2), 47.41 and 45.02 (CH_2), 15.35 (CH_3); Elem. Anal. Calcd. for $C_{17}H_{18}N_4O_4$; C: 59.64, H: 5.30, N: 16.37. found C: 59.50,H: 5.21, N: 16.10.

*2-(4-Ethoxyphenylamino)-N′-(2-hydroxybenzylidene) acetohydrazide (**3b-3**)* Yield 42%, mp: 187–189°C. IR (KBr, *v*max, cm^{-1}): 3322 and 3276 (N–H), 2975 (C–H, CH_2), 2903 (C–H, CH_3), 1679 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 11.75–11.10 (bs, 2H, Ar–OH and CONH), 8.46 and 8.31 (s, 1H, imine-H), 7.41–7.30 (m, 2H,Ar–H), 7.05–6.94 (m, 2H, Ar–H), 6.78–6.70 (m, 2H, Ar–H), 6.68–6.55 (m, 2H, Ar–H), 5.65 and 5.33 (s, 1H, Ar–NH), 4.13 and 3.76 (s, 2H, CH_2), 3.83 (m, 2H, CH_2O), 1.24 (m, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 171.78 and 167.73 (C=O), 157.80 and 156.86 (C_2, hydroxyphenyl), 150.94 and 150.61 (C_4, ethoxyphenyl), 147.93 (CH-imine), 143.00, 142.85, 141.71, 131.76, 131.56, 129.87, 127.07, 120.25, 119.05, 119.92, 119.77, 116.81, 116.58, 115.79, 113.88, 113.81 (aromatic), 63.75 (OCH_2), 47.73 and 45.08 (CH_2), 15.32 (CH_3); Elem. Anal. Calcd. for $C_{17}H_{19}N_3O_3$; C: 65.16, H: 6.11, N: 13.41. found C: 65.45, H: 5.83, N: 13.45.

*2-(4-Ethoxyphenylamino)-N′-(2-chlorobenzylidene)acetohydrazide (**3b-4**)* Yield 43%, mp: 187–189°C. IR (KBr, *v*max, cm^{-1}): 3407 (N–H), 2979 (C–H, CH_2), 2903 (C–H, CH_3), 1707 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 9.40 (bs, 1H, CONH), 8.30 (bs, 1H, imine-H), 7.78–7.72 (m, 2H, Ar–H), 7.57–7.47 (m, 2H, Ar–H), 7.40–7.35 (m, 2H, Ar–H), 6.85–6.70 (m, 2H, Ar–H), 6.55 (d, 2H, phenyl), 4.55 (bs, 1H, Ar–NH), 4.15 (m, 2H, CH_2O), 4.00 and 3.90 (s, 2H, CH_2), 1.23 (m, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 165.64 (C=O), 152.27 (C_4, ethoxyphenyl), 148.93 (CH-imine), 138.21, 135.42, 135.15, 134.45, 132.00, 131.46, 130.60, 129.80, 129.20, 127.80, 127.12, 126.49, 115.82, 113.68 (aromatic), 64.01 (OCH_2), 52.20 (CH_2), 15.08 (CH_3); Elem. Anal. Calcd. for $C_{17}H_{18}N_3O_2Cl$; C: 61.54, H: 5.47, N: 12.66. found C: 61.85, H: 5.15, N: 12.54.

*2-(4-Ethoxyphenylamino)-N′-(propan-2-ylidene)acetohydrazide (**3b-5**)* Yield 52%, mp: 143–145°C. IR (KBr, *v*max, cm^{-1}): 3414 and 3201 (N–H), 2973 (C–H, CH_2), 2901 (C–H, CH_3), 1671 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 10.27 and 9.96 (s, 1H, CONH), 6.75–6.65 (m, 2H, Ar–H), 6.53–6.43 (m, 2H,Ar–H), 5.50 and 5.19 (s, 1H, Ar–NH), 3.99 and 3.71 (s, 2H, CH_2), 3.85 (m, 2H, CH_2O), 1.90 (m, 3H, CH_3), 1.78 (m, 3H, CH_3), 1.25 (m, 3H, CH_3); Elem. Anal. Calcd. for $C_{13}H_{19}N_3O_2$; C: 62.63, H: 7.68, N: 16.85. found C: 62.45,H: 7.83, N: 16.62.

*2-(4-Ethoxyphenylamino)-N′-(1-phenylethylidene)acetohydrazide (**3b-6**)* Yield 47%, mp: 146–148°C. IR (KBr, *v*max, cm^{-1}): 3414 and 3187 (N–H), 2978 (C–H, CH_2), 2902 (C–H, CH_3), 1669 (C=O); ^{1}HNMR (500 MHz, DMSO-d_6) (ppm) δ: 10.75 and 10.38 (s, 1H, CONH), 7.85–7.70 (m, 2H, Ar–H), 7.47–7.35 (m, 3H, Ar–H), 6.75–6.69 (m, 2H, Ar–H), 6.65–6.50 (m, 2H, Ar–H), 5.50 and 5.30 (s, 1H, Ar–NH), 4.21 and 3.63 (s, 2H, CH_2), 3.85 (m, 2H, CH_2O), 2.22 (bs, 3H, CH_3), 1.24 (bs, 3H, CH_3); ^{13}CNMR (500 MHz, $CDCl_3$) (ppm) δ: 173.00 and 167.80 (C=O), 150.55 (C_4, ethoxyphenyl), 148.36 (C-imine), 143.07 (C_1, ethoxyphenyl), 138.60, 129.74, 129.54, 128.89, 128.78, 126.77, 126.55, 116.00, 113.93, 113.76 (aromatic), 63.75 (OCH_2), 47.00 and 45.46 (CH_2), 15.34 (CH_3), 14.01 (CH_3–C=N); Elem. Anal. Calcd. for $C_{18}H_{21}N_3O_2$; C: 69.43, H: 6.80, N: 13.49. found C: 69.75, H: 6.59, N: 13.70.

Determination of anticonvulsant activity

Male albino mice (from Pasteur Institute of Iran) weighing 20–28 g were used for these experiments. Animals were

kept at controlled room temperature (25°C) with 12 h light/12 h dark cycle. They were placed in standard cages and allowed for free access to food and water except during the experiment. All animal manipulations were carried out according to the ethical committee for the use and care of laboratory animals of Tehran University of Medical Sciences. On the day of experiment, the animals were transferred into separate cages randomly and allowed to habituate for 30 min before any administration. Anticonvulsant activity was evaluated according to the standard method (Campagna *et al.*, 1993; Shafaroodi *et al.*, 2004; Narayana *et al.*, 2006; Amin *et al.*, 2008; Abdel-Aziz *et al.*, 2009; Wagle *et al.*, 2009; Hosseini-Zare *et al.*, 2011). In brief, each test compound was intraperitoneally injected (ip) to groups of eight mice 30 min before PTZ (Sigma, USA) injection. An acute intraperitoneal injection of PTZ (80 mg/kg, CD97 for generalized tonic-clonic seizure in the current experiment) was administered in a freshly prepared solution for induction of convulsions in mice. Each animal was placed into the cylinder ($30 \times 25 \times 25$ cm^3) instantly after PTZ administration and its behavior was observed carefully for 30 min to determine the latency of onset of generalized tonic-clonic seizure (myoclonic jerks and other pre-convulsive chewing behavior were not noted), total seizure duration, and DT. A latency of 1,800 s was recorded for instances in which no generalized tonic-clonic seizure was occurred. Protection percent (number of mice was not afflicted with seizure/number of tested mice in each group) $\times$ 100 and Survived percent (number of mice was alive/number of tested mice in each group) $\times$ 100 were calculated in each group.

PTZ was dissolved in distilled water (DW) (0.1 ml/10 g of body weight of the mouse) and the test compounds were dissolved or suspended in DMSO solution (0.02 ml/10 g of body weight of the mouse). The control group received the same concentration of vehicle. Phenobarbital (15 mg/kg dissolved in DMSO: 0.02 ml/10 g of body weight of the mouse) and sodium valproate (220 mg/kg dissolved in DMSO: DW, 1:1) were administered as reference drugs.

For the compounds that showed significant changes with dose of 100 mg/kg, the effects of lower dose (50 mg/kg) were also evaluated.

Evaluation of neurotoxicity-minimal motor impairment (MMI)

The rotorod test was used to evaluate minimal motor impairment (neurotoxicity) in mice. The male albino mice (20–28 g) were trained to stay on an accelerating knurled plastic rod rotating at seven revolutions per minute (Acceler Rota-Rod 7650, UGO Basile,Varese, Italy). Non-toxic animals can remain on a rod rotating at this speed indefinitely. Previously trained mice were given selected test compounds intraperitoneally at the dose of 300 mg/kg. Neurologic toxicity was defined as the failure of animals to remain on the rod for one block. Each block includes three trials and each trial continues for 1 min. The equilibrium time or time that each animal remains on the rod was measured for total duration of three trials (180 s).

Statistical analysis

Data were presented as mean ± SEM of eight animals in each group. One-way analysis of variance followed by Dunnett's multiple comparison post hoc test were used for comparison between treated groups and control animals. The differences less than 0.05 were considered statistically significant.

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