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Laboratory note

Design, synthesis and in vivo anticonvulsant screening in mice of Novel phenylacetamides

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Abstract

A set of seven novel N-substituted 2-anilinophenylacetamides were designed by pharmacophore generation and using flexible alignment module of MOE software. The novel molecules were synthesized and screened for anticonvulsant activity in Swiss albino mice by MES and ScPTZ induced seizure tests. Test compounds were found to be potent in MES test. Compounds **12** and **14** were found to be more potent with ED_{50} values 24.0 and 8.0 mg kg^{-1}, respectively, and their activity was comparable to standard drugs (Phenytoin, Carbamazepine). Test compounds did not show significant activity in ScPTZ test. Compounds **12** and **14** also exhibited higher protective indices (20.3 and 87.5, respectively) when assessed for neurotoxicity by rotarod test as compared to the standards.

Keywords: Anticonvulsants; phenylacetamides; 2-anilinophenylacetamides; MES test; ScPTZ test

1. Introduction

Epilepsy is the most common serious disorder of the brain and is characterized by recurrent unprovoked seizures. It is estimated that there are 50 million people with epilepsy worldwide and the majority of cases are in the developing countries [1,2]. The disorder, if untreated, can lead to impaired intellectual function or death and is typically accompanied by psychopathological consequences such as loss of self-esteem [3]. The established antiepileptic drugs like phenytoin, carbamazepine, ethosuximide, valproic acid and barbiturates though widely prescribed, produce adverse effects such as ataxia, hepatotoxicity, gingival hyperplasia and megaloblastic anaemia [4,5]. Studies indicate that a significant group of patients (20–30%) are resistant to the currently used therapeutic agents. Thus, there is a constant need for improved antiepileptic drugs i.e. agents with more effective anticonvulsant activity and exhibiting lower toxicity.

Intensive study into the physiological and biochemical events that take place during epileptic seizures has provided insight into the molecular mechanisms by which these might be controlled [6]. Inhibition of neuronal cation conductance via block of voltage gated sodium channels is a proven mechanism by which anticonvulsants phenytoin, carbamazepine and valproic acid act to control seizures [7].

The present study aims to generate pharmacophore for potent anticonvulsant activity and from it design the structure of a lead molecule. It is then proposed to synthesize a series of analogs of the lead molecule and screen them for anticonvulsant activity using maximal electroshock seizure (MES) and sub-cutaneous pentylenetetrazole (ScPTZ) induced seizure models.

2. Results and discussion

2.1. Design and synthesis of test series

A group of potent anticonvulsants which act by binding to the Na^+ ion channel were selected for pharmacophoric studies. A two-point pharmacophore was then generated wherein an aromatic center separated from an H-bonding acceptor/donor group was found to be essential for activity (Fig. 1). 3D database searching lead to identification of phenylacetamide as the

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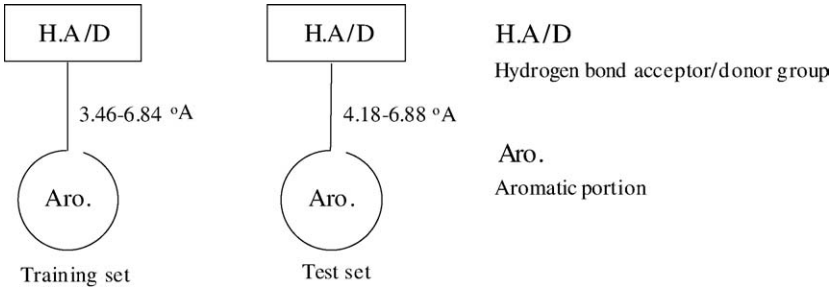


Fig. 1. Pharmacophore for anticonvulsant activity.

lead molecule. To further refine the search, a flexible alignment study was carried out using structures of potent anticonvulsants flunarizine and PD85639. Seven substituted phenylacetamides which showed good alignment (low scores) were chosen for further development (Table 1 and Fig. 2).

The substituted phenylacetamides were synthesized using 2-anilinophenylacetic acid (2-[2-(phenylamino) phenyl] acetic acid) as an important intermediate. Synthesis of 2-[2-(phenylamino) phenyl] acetic acid was achieved by two different routes (Methods A and B, Scheme 1), where the percentage yield in Method A was 43% and in Method B was 85.5%. Method B had advantages with respect to not only yield but also in the efficiency of the process (cost and number of steps). The condensation of diphenylamine with chloroacetylchloride (Method B) was achieved by using DMF in catalytic quantity and without the use of solvent. It led to improved yields of 1-(diphenylamino)-2-chloro-ethan-1-one. The condensation of diphenylamine with oxalyl chloride (Method A), was similarly achieved in the absence of solvent and without any reduction in yield when compared with the literature process [9]. Thus the use of solvent was avoided and cost effectiveness achieved.

The Wolff–Kishner reduction of *N*-phenyl-indolin-2,3-dione was achieved by refluxing with hydrazine hydrate in alcohol for 6 hours, effectively reducing the reaction time and avoiding the use of any high boiling solvents. In the final step 2-[2-(phenylamino) phenyl] acetyl chloride was condensed with various amines readily at room temperature except in the case of pyrrolidin-2-one which required high temperature due to its decreased reactivity.

2.2. Anticonvulsant screening

2.2.1. MES test

The results of preliminary anticonvulsant screening are given in Table 2. Compound **12** and **14** were active at 30 mg kg^{-1} body weight dose. Compound **10** and **13** were active at 100 mg kg^{-1} body weight dose while compound **9** was active only at a dose of 300 mg kg^{-1} body weight. Compounds **11**, **12** and **13** induced drowsiness to varying degrees. Compound **11** was active at a dose of 100 mg kg^{-1} body weight but activity could be recorded at 4 h as the compound induced severe drowsiness in the initial stages.

2.2.2. ScPTZ test

No seizure abolition could be achieved with any of the test compounds. However, compound **14** could reduce the severity of the seizures and prevent death in 75% mice at 100 mg kg^{-1} body weight dose, whereas Compounds **12**, **13** could prevent death in 50% mice at a dose of 300 mg kg^{-1} body weight.

2.2.3. Neurotoxicity test

The Median minimal neurotoxic dose (TD_{50}) in mice was determined by the rotarod procedure. No ataxia, sedation, hyperexcitability was observed for groups treated with compound **12** (up to 400 mg kg^{-1}) and compound **14** (up to 600 mg kg^{-1}). As the dose was further increased mice were found to be lethargic.

On the basis of their preliminary anticonvulsant potential, compounds **12** and **14** were selected for quantitative evaluation of their pharmacological parameters. Median effective doses

Table 1
Pharmacophore generation and flexible alignment studies

Pharmacophore studies				Flexible alignement		
Training set		Test set		Test set	Score	
Compound	Dist.[a] (°A)	Compound	Dist.[a] (°A)	Compound	PD85639	Flunarizine
Phenytoin	3.46	**8**	4.26	**8**	208.47	218.63
Carbamazepine	3.43	**9**	4.18	**9**	210.32	225.26
Flunarizine	4.42	**10**	4.49	**10**	117.47	129.04
Zonisamide	4.46	**11**	4.14	**11**	223.37	236.29
Lamotrigine	4.46	**12**	6.71	**12**	117.37	133.28
Nafimidone	4.70	**13**	6.88	**13**	119.34	136.70
Rufinamide	4.75	**14**	5.23	**14**	131.15	137.49
Remacemide	5.20					
Dezinamide	6.37					
Denzimol	6.84					

[a] Distance between centroid of aromatic portion and centroid of H.A/D group.

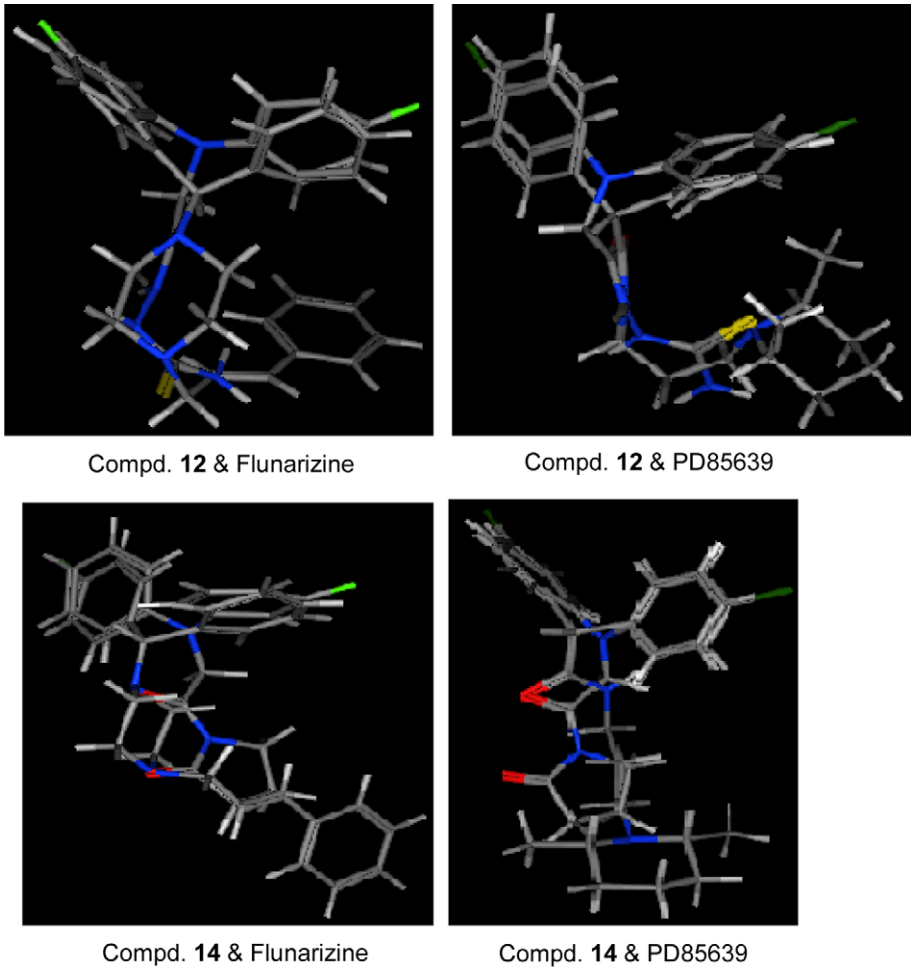


Fig. 2. Flexible alignment of Compounds **12** and **14** with Flunarizine and PD85639.

(ED_{50}s) and median neurotoxic doses (TD_{50}s) were determined at the time of peak anticonvulsant and neurotoxic effect and this data is given in Table 3. The data includes the corresponding values for phenytoin and carbamazepine taken from literature [13,16].

3. Conclusions

Potent anticonvulsant profile was displayed by compounds **12** (ED_{50} = 24.0 mg kg^{-1}) and **14** (ED_{50} = 8.0 mg kg^{-1}). They also had higher protective index, Compound**12** (P.I. = 20.3) and **14** (P.I = 87.5) when compared to standard drugs. Good separation between anticonvulsant activity and neurotoxicity was thus observed.

4. Experimental protocols

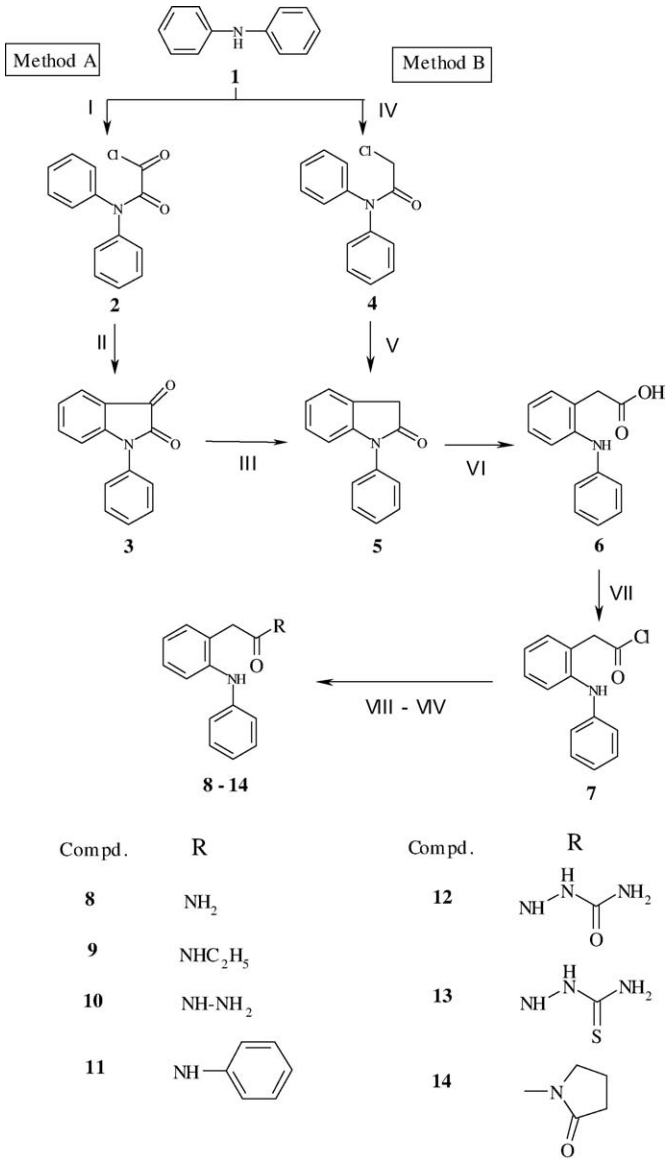
4.1. Design

4.1.1. Generation of pharmacophore for potent anticonvulsant activity

A training set of 10 molecules known to possess potent anticonvulsant activity was utilized for pharmacophoric study and it included phenytoin, carbamazepine, remacemide, flunarizine, rufinamide, zonisamide, nafimidone, dezinamide, denzimol and lamotrigine. The software 'Molecular Operating Environment' was used. (MOE of Chemical Computing Group. Inc. on a Pentium IV 1.6 GHz workstation). Upon superposition of minimum energy conformers of the training set molecules, the presence of at least one phenyl ring and a hydrogen bond acceptor/donor (H.A/D) group could be identified in each molecule. Therefore, the distance between the centroid of phenyl ring/aromatic moiety and H.A/D unit was measured and based on this; a two point pharmacophore model was built up (Table 1 and Fig. 1). Further, 3-D database searching to find molecules obeying the above distance constraints resulted in identification of substituted phenylacetic acid derivatives.

4.1.2. Flexible alignment

Phenylacetamides in general were found to have sodium channel blocking activity, one such derivative PD 85639 was equipotent with flunarizine in neuroprotection assays [8]. Therefore, the designed molecules were subjected to flexible alignment with PD 85639 and flunarizine using descriptors like aromaticity, H-bond acceptor/donor groups, Log*P*, molar refractivity and volume. Out of twenty molecules used in the



Scheme 1. (I) Oxalyl chloride, DCM, RT, 2 h; (II) $AlCl_3$, CS_2, RT, 2 h; (III) hydrazine hydrate, EtOH, reflux, 6 h; (IV) chloroacetyl chloride, DMF, 80 °C, 2 h; (V) $AlCl_3$, 180 °C, 1 h; (VI) alkaline hydrolysis, EtOH; (VII) $SOCl_2$, RT, 1 h; (VIII) aq. ammonia, RT, 2 h; (IX) 70% ethylamine, RT, 2 h; (X) 90% hydrazine hydrate, RT, 2 h; (XI) aniline, RT, 2 h; (XII) Semicarbazide HCl, $(C_2H_5)_3N$, RT, 2 h; (XIII) thiosemicarbazide, RT, 2 h; (XIV) pyrrolidin-2-one, pyridine, 90 °C, 2.5 h.

study, seven molecules showed good alignment with PD 85639 and flunarizine (Table 1, Fig. 2).

4.2. Synthesis

Progress of all the reactions was monitored by thin-layer chromatography using Merck precoated silica gel GF254. Compounds were purified by Column Chromatography using silica gel 60 (0.062–0.200 mm) from Merck. Melting points were recorded on an electrically heated melting point apparatus. All intermediates were characterized by infrared spectroscopy (KBr disc method) on a JASCO FT/IR 5300. The ^1H NMR spectrum was recorded on Varian–VXR-300S at 300 MHz. Elemental analysis results were within ± 0.4% of theoretical values.

Table 2
Anticonvulsant screening of test compounds **8**–**14** in MES and ScPTZ models

Compound administered	Protection against Maximal Electroshock induced seizures		Protection against subcutaneous Pentylenetetrazole induced seizures	
	0.5 h	4.0 h	0.5 h	4.0 h
8	+ +	+ +	–	–
9	+	+	–	–
10	+ +	+ +	–	–
11	N.D.	+ +	N.D.	–
12	+ + +	+ +	–	–
13	+ +	+ +	–	–
14	+ + +	+ + +	–	–
Phenytoin	+ + +	+ + +	–	–
Carbamazepine	+ + +	+ + +	–	–
Diazepam	–	–	+ + +	+ + +
Phenobarbitone	–	–	+ + +	+ + +
Vehicle treated	–	–	–	–

Statistical analysis was conducted by the method of Litchfield and Wilcoxon [15], $P < 0.025$ compared to vehicle treated control groups; Key: + + +: activity at 30 mg kg^{-1}; + +: activity at 100 mg kg^{-1}; +: activity at 300 mg kg^{-1}; –: no activity even at 300 mg kg^{-1}; N.D.: not determined.

Table 3
Quantitative anticonvulsant data of test compounds **12** and **14** in mice

Compound	MES ED_{50}[a]	TD_{50}[a]	Protective index (PI)[b]
12	24.0 [0.5]	500 [0.5]	20.33
14	8.0 [0.5]	700 [0.5]	87.5
Phenytoin	9.5 [2.0]	66 [2.0]	6.9
Carbamazepine	11.7 [0.5]	71.6 [0.5]	6.11

[a] ED_{50} and TD_{50} values are in mg kg^{-1}. Numbers in parentheses are 95% confidence intervals. The dose data was obtained at the 'time of peak effect' (indicated in hours in square brackets).
[b] PI calculated as (TD_{50}/ED_{50}).

*4.2.1. Synthesis of 2-[2-(phenylamino) phenyl] acetic acid (**6**) (Scheme 1)*

4.2.1.1. Method A

*4.2.1.1.1. 1-(Diphenylamino)-2-chloro-ethanedione (**2**).* Diphenylamine **1** (20 g, 0.11 mol) was added in small portions to cold oxalyl chloride 17 g (11 ml, 0.4 mol) with stirring and the mixture was stirred at room temperature for 2 hours and excess of oxalyl chloride was removed on a rotary evaporator. The residue on recrystallization from absolute ethanol gave pale white colored crystals. Yield: 30.06 g (98%), m.p.: 140 °C; IR (KBr) cm^{-1} 3069, 2934, 1776, 1684, 1587, 1491, 670.

*4.2.1.1.2. N-phenyl-indolin-2,3-dione (**3**).* To a solution of 30 g (0.11 mol) of **2** in 160 ml of carbon disulfide at 10 °C was added 47 g (0.35 mol) of anhydrous aluminum chloride in

small portions. The mixture was stirred at room temperature for 2 hours and was poured into a mixture of ice and 50 ml of 2 N HCl. The solid obtained was filtered, washed well with water and air-dried. Recrystallization using ethanol yielded pale buff colored crystals. Yield: 24.90 g (83%), m.p.: 121 °C; IR (KBr) cm^{-1} 3061, 2930, 1739, 1610, 1500, 1464.

*4.2.1.1.3. N-phenyl-indolin-2-one (**5**)*. To a solution of 20 g (0.07 mol) of **3** in 60 ml of ethanol in a round bottom flask fitted with a reflux condenser was added 30 ml of 90% hydrazine hydrate. The mixture was heated under reflux for 6 hours, the excess hydrazine hydrate was removed by distillation and the residual solution concentrated. The residual mixture was then diluted with water. The precipitated crystals were filtered, washed with water and air-dried. Recrystallization from ethanol and water mixture (80:20) gave white colored leaflets. Yield: 11.6 g (58%), m.p.: 119 °C (115–118 °C) [9]; IR (KBr) cm^{-1} 3005, 2945, 1682, 1589; H^1 NMR ($CDCl_3$) δ ppm 7.53–7.36 (m, 5H, Ar), 7.21, 7.10–7.08, 6.90–6.80 (d, 4H, Ar), 3.72 (s, 2H, CH_2). Anal. Calcd. for $C_{14}H_{11}NO$: C, 80.36; H, 5.30; N, 6.69. Found: C, 80.40; H, 5.27; N, 6.67.

*4.2.1.1.4. 2-[2-(Phenylamino) phenyl] acetic acid (**6**)*. A solution of 10 g of **5** in 33 ml of 2 N NaOH and 33 ml of ethanol was refluxed for 4 hours. Ethanol was removed by distillation. The residual mixture was then acidified using hydrochloric acid. The precipitate obtained was then filtered, washed with water and air-dried. Recrystallization from ethanol-water mixture using decolorizing charcoal offered colorless crystals. Yield: 9.0g (90%), m.p.: 117 °C (114–116 °C) [10]; IR (KBr) cm^{-1} 3398, 2926, 1711, 1367; H^1 NMR ($CDCl_3$) δ ppm 7.55–7.42 (m, 5H, Ar), 7.32, 7.10–7.08, 6.95–6.78 (d, 4H, Ar), 3.7 (s, 2H, CH_2), 2.17 (s, 1H, Ar–N*H*). Anal. Calcd. for $C_{14}H_{13}NO_2$: C, 73.99; H, 5.77; N, 6.16. Found: C, 74.05; H, 5.81; N, 6.13.

4.2.1.2. Method B

*4.2.1.2.1. 1-(Diphenylamino)-2-chloro-ethan-1-one (**4**)*. To a solution of 20 g (0.11 mol) of **1** and chloroacetyl chloride (16.0 ml, 0.14 mol) was added 3 ml of *N,N*-dimethylformamide (DMF) and heated at a temperature of 80 °C on water bath for a period of 2 hours. The reaction mixture was then diluted with water wherein the product precipitated out. It was filtered, washed with water, dried and recrystallized using ethanol to get pale white crystals. Yield: 28.05 g (97%). m.p.: 115 °C (113–116 °C) [9]; IR (KBr) cm^{-1} 3005, 2945, 1680, 1589, 1491, 700.

*4.2.1.2.2. N-phenyl-indolin-2-one (**5**)*. A mixture of 25 g (0.10 mol) of **4** and 31 g (0.23 mol) of anhydrous aluminum chloride was heated in flask fitted with $CaCl_2$ guard tube until an internal temperature of 180–190 °C was attained. There was copious evolution of hydrogen chloride; mixture was heated for an additional 10 min. The molten mass was allowed to cool and then treated with crushed ice and 70 ml of 1 N hydrochloric acid with stirring. The crude product, which solidified, was filtered, washed well with water and air-dried. Recrystallization from absolute ethanol gave pale golden colored crystals. Yield: 18.75 g (75%), m.p.: 119 °C (117–119 °C) [9].

*4.2.2. General procedure for the synthesis of N-substituted 2-[2-(phenylamino) phenyl] acetamides (**8**–**11**) (Scheme 1)*

2-[2-(Phenylamino) phenyl] acetic acid **6** (1 g, 0.004 mol) was reacted with redistilled thionyl chloride (0.38 ml, 0.005 mol) for an hour. The 2-[2-(phenylamino) phenyl] acetyl chloride **7** thus obtained was cooled and reacted in turn with various amines (0.008 mol) stirred for 2 hours. On dilution with water, the product precipitated out. It was filtered, washed with sodium bicarbonate solution, followed by washing with water and then air-dried. Products were recrystallized with ethanol.

*4.2.2.1. 2-[2-(Phenylamino) phenyl] acetamide (**8**)*. Reaction of **7** (1.15 g, 0.04 mol) and 30% aq. ammonia solution gave **8**. Yield: 0.7 g (71.08%), m.p.: 272–274 °C; IR (KBr) cm^{-1} 3405, 3060, 2950, 1695, 1500; H^1 NMR ($CDCl_3$) δ ppm 9.23 (d, 1H, CONH), 7.42–7.50 (m, 5H, Ar), 7.32, 7.27, 7.10 (m, 3H, Ar), 6.76 (d, 1H, Ar), 3.44 (s, 2H, CH_2), 3.88 (s, 2H, NH_2,), 2.2 (s, 1H, Ar-N*H*). Anal. Calcd. for $C_{14}H_{14}N_2O$: C, 74.31; H, 6.24; N, 12.38. Found: C, 74.27; H, 6.19; N, 12.42.

*4.2.2.2. N-ethyl-2-[2-(phenylamino) phenyl] acetamide (**9**)*. Reaction of **7** (1.15 g, 0.04 mol) and 70% aq. ethylamine solution gave **9**. Yield: 0.81 g (73.28%), m.p.: 129–131 °C; IR (KBr) cm^{-1} 3398, 3058, 2944, 1697, 1500; H^1 NMR ($CDCl_3$) δ ppm 9.23 (d, 1H, CONH), 7.46–7.55 (m, 5H, Ar), 7.36, 7.31, 7.10 (m, 3H, Ar), 6.76 (d, 1H, Ar), 3.44 (s, 2H, CH_2), 3.22 (s, 2H, CH_2,), 2.2 (s, 1H, Ar–N*H*), 1.23 (2H, CH_3). Anal. Calcd. for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.01. Found: C, 75.49; H, 7.02; N, 10.94.

*4.2.2.3. 2-{[2-(Phenylamino) phenyl] acetyl} hydrazine (**10**)*. Reaction of **7** (1.15 g, 0.04 mol) and 90% aq. hydrazine hydrate solution gave **10**. Yield: 0.69 g (66%), m.p.: 95 °C; IR (KBr) cm^{-1} 3393, 3057, 2947, 1699, 1500; H^1 NMR ($CDCl_3$) δ ppm 9.23 (d, 1H, CONH), 7.46–7.55 (m, 5H, Ar), 7.36, 7.31, 7.10 (m, 3H, Ar), 6.76 (d, 1H, Ar), 3.47 (s, 2H, CH_2), 3.08 (s, 2H, NH_2, hydrazide), 2.2 (s, 1H, Ar-N*H*). Anal. Calcd. for $C_{14}H_{15}N_3O$: C, 69.69; H, 6.27; N, 17.41. Found: C, 69.74; H, 6.30; N, 17.44.

*4.2.2.4. N-phenyl-2-[2-(phenylamino) phenyl] acetamide (**11**)*. Reaction of **7** (1.15 g, 0.04 mol) with aniline (0.401 ml, 0.004 mol) gave **11**. Yield: 0.74 g (55.71%), m.p.: 125–127 °C; IR (KBr) cm^{-1} 3327, 3038, 2930, 1626, 1500; H^1 NMR ($CDCl_3$) δ ppm 7.54–7.42 (m, 6H, Ar), 7.32(d, 1H, Ar), 7.24, 7.05 (m, 2H, Ar), 7.18–7.08 (m, 4H, Ar), 6.81 (d, 1H, Ar), 3.72 (s, 1H, CON*H*), 3.5 (s, 2H, CH_2), 2.2 (s, 1H, Ar-

N*H*). Anal. Calcd. for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.26. Found: C, 79.50; H, 6.08; N, 9.23.

4.2.3. 2-{[2-(Phenylamino) phenyl] acetyl} hydrazine carboxamide (**12**)

Semicarbazide HCl (0.9 g, 0.008 mol) was taken in 2 ml of DMF and to it was added 1.16 ml (0.008 mol) of triethylamine and the mixture warmed on a water-bath for 20 min. This mixture was added slowly to a cold solution of **7** (1.15 g, 0.04 mol) and then stirred at room temperature for 2 hours. It was then diluted with water when the product precipitated out. The precipitate was filtered off, washed with $NaHCO_3$ solution followed by washing with water and air-dried. Recrystallization with ethanol offered dark brown crystals. Yield: 0.67 g (53.62%), m.p.: 100–101 °C; IR (KBr) cm^{-1} 3398, 3204, 3059, 2930, 1697, 1500; H^1 NMR ($CDCl_3$) δ ppm 9.23 (d, 1H, CONH), 7.58–7.45 (m, 5H, Ar), 7.30, 7.26 (d, 2H, Ar), 7.08 (m, 1H, Ar), 6.76 (d, 1H, Ar), 3.7 (s, 2H, CH_2), 2.2(s, 1H, Ar–N*H*), 2.17 (s, 2H, –$CONH_2$), 1.4 (s, 1H, N–NH). Anal. Calcd. for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.31; H, 5.62; N, 19.68.

4.2.4. 2-{[2-(Phenylamino) phenyl] acetyl} hydrazine thiocarboxamide (**13**)

To the cold acid chloride **7** (1.15 g, 0.04 mol) was added 0.8 g (0.008 mol) of thiosemicarbazide in 2 ml of DMF and stirred at room temperature for 2 hours. Reaction mixture was then diluted with water when the product precipitated out, which was filtered off, washed with $NaHCO_3$ solution, followed by washing with water and air-dried. The product was purified by column chromatography (petroleum ether 70: chloroform30), the second fraction being the pure product. Yield: 0.83 g (62.84%), m.p.: 240–242 °C; IR (KBr) cm^{-1} 3408, 3238, 3144, 2928, 1699, 1500, 1180,1105; H^1 NMR ($CDCl_3$) δ ppm 9.23 (d, 1H, CONH), 7.54–7.44 (m, 5H, Ar), 7.30, 7.26 (d, 2H, Ar), 7.05 (m, 1H, Ar), 6.73 (d, 1H, Ar), 3.0 (s, 2H, CH_2), 2.9 (s, 1H, Ar–N*H*), 2.7 (s, 2H, –$CSNH_2$), 1.25 (s, 1H, N–NH). Anal. Calcd. for $C_{15}H_{16}N_4OS$: C, 59.98; H, 5.37; N, 18.65; S, 10.68. Found: C, 60.02; H, 5.40; N, 18.63; S, 10.61.

4.2.5. 1-{[2-(Phenylamino) pheny] acetyl}-2-pyrrolidinone (**14**)

To the cold acid chloride **7** (1.15 g, 0.04 mol) was added (0.36 ml, 0.0048 mol) of pyrrolidin-2-one in 5 ml of dry pyridine and heated at 90 °C for 2.5 hours. Pyridine was removed on rotary evaporator. The solid obtained was washed with 5% HCl, washed with $NaHCO_3$ solution, followed by washing with water and air-dried. The product was purified by column chromatography (petroleum ether 70: chloroform 30), the second fraction being the pure product. Yield: 0.82 g (64.51%), m. p.: 160–163 °C; IR (KBr) cm^{-1} 3398, 3059, 2924, 1720, 1500; H^1 NMR ($CDCl_3$) δ ppm 7.55–7.44 (m, 5H, Ar), 7.36, 7.24 (d, 2H, Ar), 7.15 (m, 1H, Ar), 6.76 (d, 1H, Ar), 4.4 (s, 2H, CH_2), 2.17 (s, 1H, Ar–N*H*), 1.6 (m, 2H, –CH_2–), 1.27 (m, 2H, –CH_2–), 0.88 (m, 2H, –CH_2–). Anal. Calcd. for $C_{18}H_{18}N_2O_2$: C, 73.45; H, 6.16; N, 9.52. Found: C, 73.41; H, 6.13; N, 9.49.

4.3. Anticonvulsant screening

The Institutional Ethics Committee, Regd. No: 242/CPCSEA approved the use of animals and animal models. Swiss albino (male) mice in the weight range 25–30 g were used. Mice were housed in cages. Temperature was maintained at 25 ± 3 °C and humidity at 50–60%. Lighting was artificial with alternate 12 hour light and 12 hour dark cycle. Mice were fed on standard laboratory diet and supplied with drinking water [11].

4.3.1. MES test [12,13]

Mice were divided into 10 groups with four mice in each group. Suspension of test compounds **8**–**14**/standard (phenytoin, carbamazepine) in 0.5% tween80 in saline were administered intraperitoneally (i.p.) at three dose levels (30, 100, 300 mg kg^{-1}). The untreated group was administered the same volume of the vehicle. A drop of 0.9% saline was instilled in each eye prior to application of electrodes (Centroniks Electroconvulsiometer). The mice were subjected to electrical shock delivered through the corneal electrodes for 0.2 s (40 mA, 50 Hz, ac). Failure to extend the hindlimbs to an angle with the trunk greater than 90° is defined as protection. Seizure pattern was recorded at 0.5 and 4 hours after administration of the dose (Table 2).

4.3.2. ScPTZ test [13,14]

Mice were divided into 10 groups of four mice each. Suspension of test compounds **8**–**14**/standard (diazepam, phenobarbitone) in 0.5% tween80 in saline were administered intraperitoneally at three dose levels (30, 100, 300 mg kg^{-1}). Thirty minutes later, pentylenetetrazole at a dose of 85 mg kg^{-1} was administered subcutaneously to all groups. One group with vehicle served as control. Protection was defined as a failure to observe a single episode of clonic spasms of at least 5-s duration during a 30-min period following administration of test/standard compound. The observations made are given in Table 2.

4.3.3. Neurotoxicity test [14]

The median minimal neurotoxic dose (TD_{50}) in mice was determined by the rotarod procedure. Mice were divided into eight groups of four mice each. Suspensions of test compounds **12** and **14** in 0.5% tween80 in saline were administered intraperitoneally with dose levels in increasing order (200–800 mg kg^{-1}). The mice were placed on a 1-inch diameter knurled plastic rod rotating at 6 rpm. Unimpaired mice can easily remain on a rod rotating at this speed. The inability of the mice to maintain their balance for at least 1 min in three successive trials was interpreted as demonstration of motor impairment or toxicity.

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References

[1] S. Jain, Epilepsia 46 (2005) 46–47.
[2] M.T. Medina, R.M. Duron, L. Martinez, J.R. Osorio, A.L. Estrade, C. Zuniga, K.R. Holden, Epilepsia 46 (2005) 124–131.
[3] D. Rosenbloom, A.R.M. Upton, Can. Med. Assoc. J. 128 (1983) 261–270.
[4] M.J. Brodie, M.A. Dichter, N. Engl, J. Med. 334 (1996) 168–175.
[5] W.J. Currey, D.L. Kulling, Epilepsia 57 (1998) 513–520.
[6] B.S. Meldrum, Epilepsia 37 (1996) S4.
[7] C.P. Taylor, Curr. Pharm. Des. 2 (1996) 375–381.
[8] I. Roufos, S.J. Hays, D.J. Doolay, R.D. Schwarz, G.W. Campbell, A.W. Probert, J. Med. Chem. 37 (1994) 268–274.
[9] R. Sarges, R.H. Howard, K.B. Koe, J. Med. Chem. 32 (1989) 437–444.
[10] P. Moser, A. Sallmann, I. Wiesenberg, J. Med. Chem. 33 (1990) 2358–2368.
[11] OECD (Organization for Economic Cooperation and Development), Guidelines, USA, 1998.
[12] B.B. Gallagher, in: J.A. Vida (Ed.), Anticonvulsants: A series of monographs Vol 15, Academic Press, London, 1977.
[13] R.L. Krall, J.K. Penry, B.G. White, H.J. Kupferberg, E.A. Swinyard, Epilepsia 19 (1978) 409–428.
[14] C.R. Clark, M.J.M. Wells, R.T. Sansom, G.N. Norris, R.C. Dockens, W.R. Ravis, J. Med. Chem. 27 (1984) 779–782.
[15] J.T. Litchfield, F.A. Wilcoxon, J. Pharmacol. Exp. Ther. 96 (1949) 99–113.
[16] I.O. Edafiogho, K.R. Scott, in: M.E. Wolff (Ed.), Burger's Medicinal Chemistry, John Wiley & Sons, New York, 1996, pp. 175–260.