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Original article

Design & synthesis of 2-(substituted aryloxy)-5-(substituted benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one as potential anticonvulsant agents

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ABSTRACT

A series of 2-(substituted aryloxy)-5-(substituted benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one were designed & synthesized using appropriate synthetic route keeping in view the structural requirement of pharmacophore and evaluated for anticonvulsant activity and CNS activities. After intraperitoneal injection to mice, some synthesized derivatives were examined in the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazol (scPTZ) induced seizure and neurotoxicity screens. Those found potent were also evaluated for behavioural impairment and depression activity. Among the compound tested, **5 eIX** showed protection from seizures in both the animal models at dose level of 30 mg/kg while **5 bII** & **5 cII** showed protection against scPTZ model at same dose level. Some titled compounds exhibited lesser CNS depression and neurotoxicity compared to clinically effective drug.

1. Introduction

The improvement in the treatment of epilepsy over the past decade is mainly associated with the development of new antiepileptic drugs (AEDs), taking advantage of the pharmacophoric requirement specifically on a single target [1]. There is currently a need for improved agents for the treatment of seizure disorders, as there is a significant group of patients who develop refractory epilepsy or are resistant to the available antiepileptic drugs. The long established AEDs control seizures in 50% of patients developing partial seizures and in 60–70% of those developing generalized seizures [2–4], rest are not responsive to conventionally available medical therapies. Moreover, the current drug therapy is associated with adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, gingival hyperplasia, hirsutism and megaloblastic anaemia [5]. This fact necessitates the search for new anticonvulsant.

Searching for new AED on the basis of the knowledge of the pharmacophoric pattern in terms of interaction at the binding site, as suggested by Dimmock et al. [6,7] proved to be promising. Based on this consideration, we have reported [8–11] several heterocyclic compounds, which have shown considerable anticonvulsant activities. As a part of our continuous investigation in this area, we designed and synthesized several new 2-(substituted-aryloxy)-5-(substituted-benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one as a part of refinement of lamotrigine, a phenyltriazine derivative, is a novel antiepileptic drug that shares similar mode of action on neuronal sodium channels as phenytoin [12].

Unverferth et al. identified a common pharmacophore model based on some well known voltage-gated sodium channel blockers including phenytoin and lamotrigine [13].

All the synthesized titled compounds comprised of the essential pharmacophoric elements (Fig. 1) that are necessary for good anticonvulsant activity as suggested by Unverferth et al. [13]. The essential structural features which could be responsible for an interaction with the active site of voltage-gated sodium channels were a hydrophobic HP unit (R), an electron donor (D) group, and a hydrogen donor/acceptor (HBD) unit [14].

Our work also highlights the distance mapping and matching of the synthesized compounds with the help of the given model.

2. Chemistry

The titled compounds were synthesized as presented in Scheme 1 by refluxing 4-(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-one and substituted acid hydrazide, which in turn were synthesized from the acid via its esterification followed by its nucleophilic substitution reaction i.e. hydrazinolysis with hydrazine hydrate. 4-

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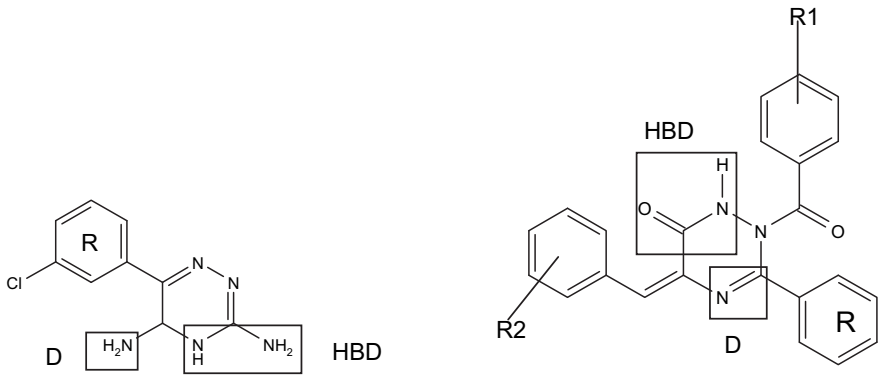
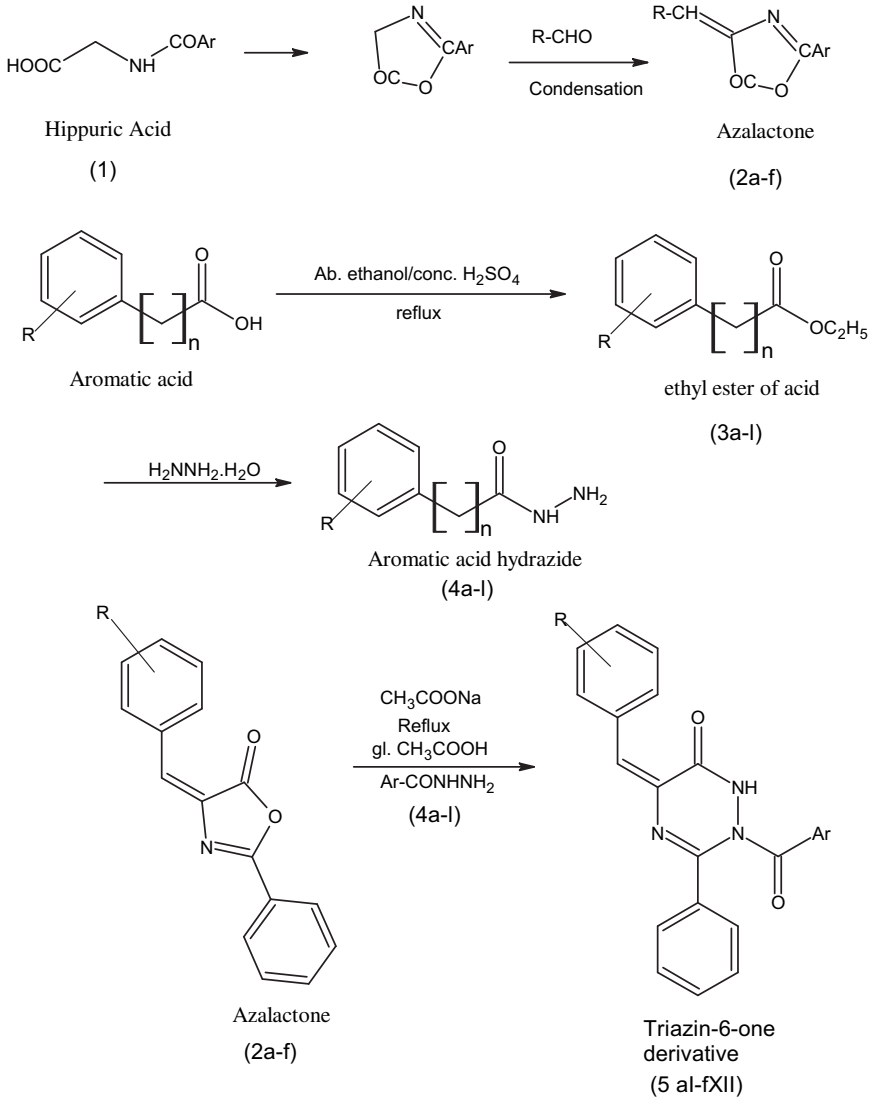


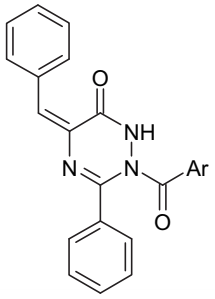
Fig. 1. Structures of sodium channel modulators. The essential structure elements for the pharmacophore of Unverferth et al. are indicated by rectangles.

(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-one derivatives were synthesized from the hippuric acid & substituted aromatic aldehyde by Erlenmeyer–Plochl azalactone synthesis while hippuric acid was synthesized from glycine by Schotten–Baumann benzoylation reaction. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. The physical data for the newly synthesized compounds are presented in Table 1.



Scheme 1. Scheme for the synthesis 2-(substituted aryloxy)-5-(substituted benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one.

Table 1
Physical data of 2-(substituted aryloxy)-5-(substituted benzylidene)-3-phenyl-2,5-dihydro-1*H*-[1,2,4] triazin-6-one derivatives



Compds.	R	Ar	Yield (%)	M.P[a] (°C)	Molecular formula[b] (M.W)
5 aI	H	Phenyl	57	228–232	$C_{23}H_{17}N_3O_2$ (367.41)
5 aII	H	p-CH_3-Phenyl	51	223–225	$C_{24}H_{19}N_3O_2$ (381.44)
5 aIII	H	p-NO_2-Phenyl	65	245–248	$C_{23}H_{16}N_4O_2$ (412.41)
5 aIV	H	4-Pyridyl	48	158–161	$C_{22}H_{16}N_4O_2$ (368.40)
5 aV	H	p-Cl-Benzyl	53	205–207	$C_{24}H_{18}ClN_3O_2$ (415.88)
5 aVI	H	Naphthalen-1-yl-methylene	59	275–276	$C_{28}H_{21}N_3O_2$ (431.50)
5 aVII	H	p-OCH_3-Phenyl	62	214–217	$C_{24}H_{19}N_3O_3$ (397.44)
5 aVIII	H	P-Cl-Phenyl	54	220–222	$C_{23}H_{16}ClN_3O_2$ (401.86)
5 aIX	H	o-Cl-Phenyl	57	194–197	$C_{23}H_{16}ClN_3O_2$ (401.86)
5 aX	H	p-F-Phenyl	49	162–164	$C_{23}H_{16}FN_3O_2$ (385.40)
5 aXI	H	Phenoxymethylene	53	173–175	$C_{24}H_{19}N_3O_3$ (397.44)
5 aXII	H	[p-(isobutyl)α-methyl] benzyl	55	177–179	$C_{29}H_{29}N_3O_2$ (451.57)
5 bI	CH_3	Phenyl	57	218–222	$C_{24}H_{19}N_3O_2$ (381.44)
5 bII	CH_3	p-CH_3-Phenyl	62	210–214	$C_{25}H_{21}N_3O_2$ (395.47)
5 bIII	CH_3	p-NO_2-Phenyl	65	215–219	$C_{24}H_{18}N_4O_4$ (426.44)
5 bIV	CH_3	4-Pyridyl	51	189–193	$C_{23}H_{18}N_4O_2$ (382.43)
5 bV	CH_3	p-Cl-Benzyl	59	194–196	$C_{25}H_{20}ClN_3O_2$ (429.91)
5 bVI	CH_3	Naphthalen-1-yl-methylene	53	251–254	$C_{29}H_{23}N_3O_2$ (445.53)
5 bVII	CH_3	p-OCH_3-Phenyl	59	234–236	$C_{25}H_{21}N_3O_3$ (411.46)
5 bVIII	CH_3	P-Cl-Phenyl	63	224–230	$C_{24}H_{18}ClN_3O_2$ (415.88)
5 bIX	CH_3	o-Cl-Phenyl	65	212–215	$C_{24}H_{18}ClN_3O_2$ (415.88)
5 bX	CH_3	p-F-Phenyl	51	240–246	$C_{24}H_{18}FN_3O_2$ (399.43)
5 bXI	CH_3	Phenoxymethylene	58	123–128	$C_{25}H_{21}N_3O_3$ (411.46)
5 bXII	CH_3	[p-(isobutyl)α-methyl] benzyl	58	138–142	$C_{30}H_{31}N_3O_2$ (465.60)
5 cI	OH	Phenyl	63	105–108	$C_{23}H_{17}N_3O_3$ (383.41)
5 cII	OH	p-CH_3-Phenyl	57	151–155	$C_{24}H_{19}N_3O_3$ (397.44)
5 cIII	OH	p-NO_2-Phenyl	65	184–191	$C_{23}H_{16}N_4O_5$ (428.41)
5 cIV	OH	4-Pyridyl	47	155–161	$C_{22}H_{16}N_4O_3$ (384.40)
5 cV	OH	p-Cl-Benzyl	55	159–162	$C_{24}H_{18}ClN_3O_3$ (431.88)
5 cVI	OH	Naphthalen-1-yl-methylene	51	169–173	$C_{28}H_{21}N_3O_3$ (447.50)
5 cVII	OH	p-OCH_3-Phenyl	54	148–153	$C_{24}H_{19}N_3O_4$ (413.44)
5 cVIII	OH	P-Cl-Phenyl	60	135–138	$C_{23}H_{16}ClN_3O_3$ (417.86)
5 cIX	OH	o-Cl-Phenyl	62	125–130	$C_{23}H_{16}ClN_3O_3$ (417.86)
5 cX	OH	p-F-Phenyl	53	134–138	$C_{23}H_{16}FN_3O_3$ (401.40)
5 cXI	OH	Phenoxymethylene	44	145–148	$C_{24}H_{19}N_3O_4$ (413.44)
5 cXII	OH	[p-(isobutyl)α-methyl] benzyl	58	133–141	$C_{29}H_{29}N_3O_3$ (467.57)
5 dI	OCH_3	Phenyl	56	152–156	$C_{24}H_{19}N_3O_3$ (397.44)
5 dII	OCH_3	p-CH_3-Phenyl	59	216–220	$C_{25}H_{21}N_3O_3$ (411.46)
5 dIII	OCH_3	p-NO_2-Phenyl	62	235–242	$C_{24}H_{18}N_4O_5$ (442.43)
5 dIV	OCH_3	4-Pyridyl	55	153–154	$C_{23}H_{18}N_4O_3$ (398.42)
5 dV	OCH_3	p-Cl-Benzyl	54	185–191	$C_{25}H_{20}ClN_3O_3$ (445.91)
5 dVI	OCH_3	Naphthalen-1-yl-methylene	59	227–231	$C_{29}H_{23}N_3O_3$ (461.52)
5 dVII	OCH_3	p-OCH_3-Phenyl	51	224–227	$C_{25}H_{21}N_3O_4$ (427.46)
5 dVIII	OCH_3	P-Cl-Phenyl	49	216–219	$C_{24}H_{18}ClN_3O_3$ (431.88)
5 dIX	OCH_3	o-Cl-Phenyl	58	266–268	$C_{24}H_{18}ClN_3O_3$ (431.88)
5 dX	OCH_3	p-F-Phenyl	53	214–216	$C_{24}H_{18}FN_3O_3$ (415.43)
5 dXI	OCH_3	Phenoxymethylene	56	154–158	$C_{25}H_{21}N_3O_4$ (427.46)
5 dXII	OCH_3	[p-(isobutyl)α-methyl] benzyl	57	161–168	$C_{30}H_{31}N_3O_3$ (481.60)
5 eI	o-Cl	Phenyl	55	173–178	$C_{23}H_{18}ClN_3O_2$ (401.86)
5 eII	o-Cl	p-CH_3-Phenyl	53	196–198	$C_{24}H_{18}ClN_3O_2$ (415.88)
5 eIII	o-Cl	p-NO_2-Phenyl	67	184–187	$C_{23}H_{15}ClN_4O_4$ (446.85)
5 eIV	o-Cl	4-Pyridyl	63	204–206	$C_{22}H_{15}ClN_4O_2$ (402.84)
5 eV	o-Cl	p-Cl-Benzyl	58	204–208	$C_{24}H_{17}Cl_2N_3O_2$ (450.33)
5 eVI	o-Cl	Naphthalen-1-yl-methylene	56	254–255	$C_{28}H_{20}ClN_3O_2$ (465.94)
5 eVII	o-Cl	p-OCH_3-Phenyl	51	238–242	$C_{24}H_{18}ClN_3O_3$ (431.88)
5 eVIII	o-Cl	P-Cl-Phenyl	53	251–256	$C_{23}H_{15}Cl_2N_3O_2$ (436.30)
5 eIX	o-Cl	o-Cl-Phenyl	55	201–204	$C_{23}H_{15}Cl_2N_3O_2$ (436.30)
5 eX	o-Cl	p-F-Phenyl	45	189–193	$C_{23}H_{15}ClFN_3O_2$ (419.85)

Table 1 (*continued*)

Compds.	R	Ar	Yield (%)	M.P[a] (°C)	Molecular formula[b] (M.W)
5 eXI	o-Cl	Phenoxymethylene	58	153–157	$C_{24}H_{18}ClN_3O_3$ (431.88)
5 eXII	o-Cl	[p-(isobutyl)α-methyl] benzyl	59	189–191	$C_{29}H_{28}ClN_3O_2$ (486.02)
5 fI	p-$N(CH_3)_2$	Phenyl	57	281	$C_{25}H_{22}N_4O_2$ (410.8)
5 fII	p-$N(CH_3)_2$	p-CH_3-Phenyl	53	242–245	$C_{26}H_{24}N_4O_2$ (424.51)
5 fIII	p-$N(CH_3)_2$	p-NO_2-Phenyl	63	270–274	$C_{25}H_{21}N_5O_4$ (455.48)
5 fIV	p-$N(CH_3)_2$	4-Pyridyl	55	282–284	$C_{24}H_{21}N_5O_2$ (411.47)
5 fV	p-$N(CH_3)_2$	p-Cl-Benzyl	56	236–237	$C_{26}H_{23}ClN_4O_2$ (458.95)
5 fVI	p-$N(CH_3)_2$	Naphthalen-1-yl-methylene	51	207–208	$C_{30}H_{26}N_4O_2$ (474.57)
5 fVII	p-$N(CH_3)_2$	p-OCH_3-Phenyl	51	264–266	$C_{26}H_{24}N_4O_3$ (440.51)
5 fVIII	p-$N(CH_3)_2$	P-Cl-Phenyl	54	244–248	$C_{25}H_{21}ClN_4O_2$ (444.92)
5 fIX	p-$N(CH_3)_2$	o-Cl-Phenyl	55	238	$C_{25}H_{21}ClN_4O_2$ (444.92)
5 fX	p-$N(CH_3)_2$	p-F-Phenyl	58	216	$C_{25}H_{21}F_9N_4O_2$ (428.47)
5 fXI	p-$N(CH_3)_2$	Phenoxymethylene	61	220–224	$C_{26}H_{24}N_4O_3$ (440.51)
5 fXII	p-$N(CH_3)_2$	[p-(isobutyl)α-methyl] benzyl	52	210–213	$C_{31}H_{34}N_4O_2$ (494.64)

[a] Melting point of the compounds at their decomposition.
[b] Solvent of recrystallization – ethanol.

3. Pharmacology

Some of the new derivatives obtained by the above mentioned procedure were undertaken for the initial anticonvulsant studies by the anticonvulsant drug development (ADD) program protocol [15,16]. The profile of anticonvulsant activity was established after i. p. injections into mice and evaluated in the maximal electroshock (MES), subcutaneous pentylenetetrazole (scPTZ) and neurotoxicity screens, using doses of 30, 100 and 300 mg/kg at two different time intervals. These data are presented in Table 2. Some selected compounds were evaluated for their CNS behavioural activity in mice using actophotometer and CNS depressant study using Porsolt's forced swim pool test. The results are presented in Tables 3 and 4 respectively.

4. Computational parameter

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD freeware 3D viewer 8.04 version. Along with this CLOGP for selected synthesized compounds were calculated by using Pallas demo version 3112 which was then compared with the experimental Log *P* data of these selected compounds.

Table 2
Anticonvulsant and neurotoxicity screening of compounds. Evaluation of compounds in the mouse intraperitoneal MES, scPTZ and NT screens.[a]

Compd. No	MES screen		PTZ screen		Neurotoxicity screen	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
5 aVIII	100	300	100	300	300	300
5 bII	100	100	30	30	300	–
5 bV	100	300	100	300	300	300
5 bVIII	100	300	100	100	–	–
5 bXI	300	300	300	–	×	×
5 cII	30	100	100	300	–	–
5 cX	300	–	300	–	×	×
5 dII	100	100	30	30	300	300
5 dVIII	100	–	100	300	300	300
5 eIX	30	30	30	100	300	300
Phenytoin	30	30	×	×	100	100
Sodium Valporate	×	×	300	–	–	–

[a] Doses of 30, 100 and 300 mg/kg of the compound were administered and the protection and neurotoxicity measured after 0.5 and 4 h. The figures indicate the minimal dose required to cause protection or neurotoxicity in 50% or more of the animals. The dash (–) indicates the absence of anticonvulsant activity or neurotoxicity. × denotes not tested.

5. Results and discussions

5.1. Anticonvulsant activity

All the tested compounds showed protection against MES test indicative of their ability to inhibit the seizure spread. Compounds **5 cII** and **5 eIX** showed protection against the MES model at 30 mg/kg while some compounds **5 aVIII, 5 bII, 5 bV, 5 bVIII, 5 d II, 5 dVIII** showed protection at dose level of 100 mg/kg. The compound **5 eIX** showed activity both at 0.5 h and 4 h period at dose level of 30 mg/kg indicating the compound to be highly potent and long acting. Similarly compound **5 cII** was also found to be highly potent but short acting as 4 h protection requires the dose of 100 mg/kg. The compounds **5 bII** and **5 dII** showed activity both at 0.5 h and 4 h period at dose level of 100 mg/kg indicating that compounds are potent and long acting while, remaining compounds showed activity only at 0.5 h, indicating that these are having rapid onset and shorter duration of action.

All the tested compounds of this series were found to be active in the scPTZ test, a test used to identify compounds that elevate seizure threshold. Compounds **5 bII, 5 dII, 5 eIX** showed activity at

Table 3
Behavioural study on some selected compounds using Actophometer.

Compounds[a]	Activity score	Post treatment (locomotor activity score)[b]	
	Control (24 h prior)	0.5 h after	1 h after
5 bII	327.17 ± 12.37	259.00 ± 13.37	228.50 ± 8.80
5 bVIII	289.00 ± 11.16	256.17 ± 21.48[NS]	265.50 ± 13.65
5 cII	143.33 ± 9.09	129.50 ± 14.33	150.17 ± 18.60[NS]
5 dII	267.50 ± 14.85	213.33 ± 17.57	224.33 ± 9.37
5 eIX	262.00 ± 19.41[NS]	195.33 ± 11.52	210.33 ± 14.84
Phenytoin[c]	119.33 ± 17.43	78.87 ± 16.66	97.17 ± 13.49

[a] The compounds were tested at a dose level of 100 mg/kg (i.p.).
[b] Each score represents the mean ± SEM of six mice, significantly different from control at $P < 0.05$ & NS denote the value, which were not significant (student's *t*-test).
[c] The compounds were tested at a dose level of 30 mg/kg (i.p.).

Table 4
CNS study on selected compounds in a forced swim pool test.

Compounds[a]	Immobility time (s)	
	Control[b] (24 h prior)	Post treatment[c] (1 h after)
PEG	160.00 ± 13.37	173.50 ± 11.54^{NS}
5 bII	148.17 ± 7.89	145.83 ± 9.63
5 cII	139.53 ± 12.61	147.33 ± 4.57
5 dII	137.33 ± 10.83	151.50 ± 6.72
5 eIX	142.50 ± 9.37	148.17 ± 13.70^{NS}
Carbamazepine[d]	143.33 ± 8.42	169.00 ± 11.63

[a] Compounds were tested at a dose of 100 mg/kg (*i.p.*).
[b] Control animals were administered PEG (*i.p.*).
[c] Each value represents the mean ± SEM of six mice significantly different from the control at $P < 0.05$ (NS − not significant).
[d] Tested at 30 mg/kg (i.p.).

a dose of 30 mg/kg while **5 aVIII, 5 bV, 5 bVIII, 5 cII** and **5 dVIII** showed activity at dose of 100 mg/kg, Among the tested compounds **5 bII** and **5 dII** were found to be highly potent having rapid onset and long duration of action. Compound **5 eIX** was found to be highly potent with rapid onset & intermediate duration of action. While, compound **5 bVIII** found to be potent with rapid onset and intermediate action. Compounds **5 aVIII, 5 bV, 5 cII** and **5 dVIII** displayed rapid onset and shorter duration of action. Compounds **5 bIX** and **5 cX** were found to have low potency and short duration of action.

Two general trends may be discerned. First, data for the MES and scPTZ tests revealed that 60% and 70% respectively, of the compounds had greater activity at the end of 0.5 h than after 4 h. thus; in general, these compounds are short acting anticonvulsant. Secondly, protection was afforded by all the tested compounds in the MES and scPTZ screen, respectively. Two compounds **5 bII** and **5 dII** showed greater activity in the scPTZ screen rather than MES test, while **5 cII** showed good activity in the MES model. For remaining cases equal activity was demonstrated.

In neurotoxicity screen, compounds **5 bVIII** and **5 cII** did not show neurotoxicity in the maximum administered dose (300 mg/kg) and the remaining compounds were found to be less neurotoxic as compared to phenytoin.

In the behavioural study using actophotometer, the compounds **5 bVIII** & **5 cII** showed no behavioural despair effect when compared to phenytoin as represented in Table 3. Compounds **5 dII** showed decreased locomotor activity in the 30 min interval but no significant effect on behavioural despair was observed during 1 h time period. All other compounds were found decreasing behavioural activity of the animals. Similarly results obtained in Porsolt's swim pool test with compounds **5 dII**, in which an increase in the slight immobility time by the compounds indicated the CNS depressant effect. Rest of the tested compounds showed no significant variation from control.

As observed through data analysis, all the compound with varied substitution i.e. electron withdrawing or releasing group are potent but compound with p-CH_3 substitution at arylcarboxy attached to nitrogen was found to be more potent in both the models and also possess long duration of action. Thus overall conclusion suggests that the whole moiety can be interpreted as the lead molecule from this data.

5.2. Computational parameter

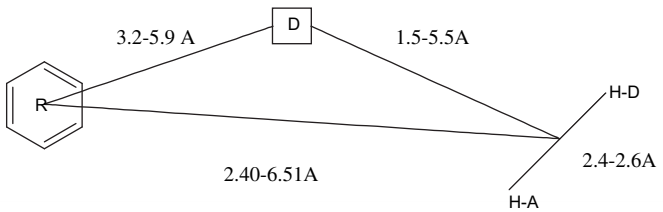
5.2.1. Distance mapping

The present work involves the correlation of the structural requirement of well known and structurally different anticonvulsant compounds with the titled compounds. The presence of at least one aryl (R) unit, one or two electron donor (D) atoms, and a hydrogen bond acceptor/donor unit (HBD). In the present study, four well known and structurally different compounds with anticonvulsant activity i.e phenytoin, carbamazepine, lamotrigine & diazepam were selected. In an initial study, calculations on the basis of molecular mechanics, with the force field based on CHARMM parameterization [17] were performed to obtain an overview on their minimum energy conformation. Table 5 shows the distances between the various groups postulated as essential for anticonvulsant action. Now it was interesting to evaluate whether the synthesized substituted 1,2,4-Triazin-6-one reflected the conditions of the derived pharmacophore model. Our analysis of the distance relationship showed that the titled compounds fulfil the essential demands of pharmacophores when compared with other well known anticonvulsant.

5.2.2. Log P determination

Some of the active compounds which were selected for the Log *P* and partition coefficient showed dependence of biological activity on lipophillic character in a congeneric series. In particular, for drugs acting on central nervous system to be potent, they have to cross blood brain barrier (BBB), thus potency has been correlated with optimum lipophilicity (Log *P*) near 2. In this study, we attempted to correlate the anticonvulsant activity of potent congeners with their combined calculated Log *P* value, CLOGP. The experimental Log *P* values were determined using the octanol-Phosphate buffer method. The data is presented in Table 6. As observed some of the experimental values were in good agreement with the theoretical values. Some compounds were very near to the theoretical value. All the selected compounds showed lipophillic character.

Table 5
Distance range between the essential structure elements R, D & HBD.



Compounds	R–HBD	R–D	D–HBD
Phenytoin	3.58	3.77	2.21
Carbamazepine	5.09	4.87	4.06
Lamotrigine	6.44	3.41	4.45
Diazepam	4.79	4.82	1.49
Lead moiety of **5 aI–fXII**	4.83	3.03	2.80

Distances calculated for 3D optimized structures using ACD freeware 3D viewer 8.04 version.

Table 6
CLOGP for selected synthesized compounds.

Compounds	Experimental Log *P*	Theoretical Log $P_{Combined}$ value
5 bII	2.13	3.63
5 bV	2.44	3.91
5 bVIII	2.37	3.97
5 cII	2.11	2.79
5 dII	2.31	3.24
5 dV	2.13	3.47
5 dVIII	2.27	3.46
5 eIII	2.13	2.94
5 eIX	2.41	3.10

Theoretical Log $P_{combined}$ was calculated by using Pallas demo version 3112.

6. Experimental protocols

6.1. Chemistry

Melting points were determined in an open end capillary tubes on Hicon digital melting point apparatus and are uncorrected. Infrared (IR) & proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded for the compounds on FT-IR Rez Bio Rad Win-IR (KBr) and Brucker DRX-300 instruments, respectively. Chemical shifts were expressed in parts per million (ppm) relative to tetramethyl silane as an internal standard. The elemental analysis (C/N) were performed on Vario EL III CHNS analyzer using sulphanilic acid as a standard, and all the values were with in ±0.4% of the theoretical compositions. The Fast atom bombardment (FAB) spectra were recorded on Jeol SX 102/DA-600 mass spectra system using Argon/Xenon (6 kV 10 mA) as the FAB gas. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC), visualized by iodine vapour.

6.1.1. Synthesis of Hippuric acid (I)

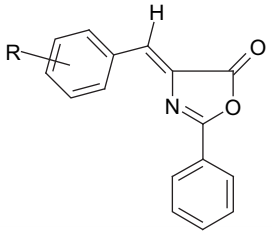
Dissolved 25 g (0.33 mol) of glycine in 250 ml of 10% sodium hydroxide solution contained in a conical flask. Added 54 g (45 ml, 0.385 mol) of benzoyl chloride in divided portions to the solution. Stoppered the flask and it was shaken vigorously after each addition until all the chloride reacted. Placed a few grams of crushed ice in the solution and then conc. hydrochloric acid was added slowly with continuous stirring until the mixture became acidic. Collected the resulting precipitated crude product, which might be contaminated with a little benzoic acid, Placed this solid in 100 ml of carbon tetrachloride and boiled gently for 10 min; this extracted any benzoic acid which might be present. Allowed the mixture to cool slightly, filtered under gentle suction and washed the product on filter with 10–20 ml of carbon tetrachloride. Recrystallised the dried product from boiling water. Collected the benzoylglycine and dried it. The practical yield of product was 80% and its m.p. 186 °C.

6.1.2. General procedure for the synthesis of 4-benzylidene-2-phenyl-4H-oxazol-5-one derivatives (**2a–f**)

A mixture of aromatic aldehyde (0.25 mol), hippuric acid (0.25 mol), acetic anhydride (0.75 mol) and anhydrous sodium acetate was taken in a 500 ml conical flask and heated on an electric hotplate with constant shaking. As soon as the mixture liquefied completely, transferred the flask to a water bath and heated for 2 h. Then added 100 ml of ethanol slowly to the contents of the flask and allowed the mixture to stand overnight. Filtered the crystalline product with suction and first washed with two 25 ml portions of ice-cold alcohol and then washed with two 25 ml portions of boiling water & dried at 100 °C. The physicochemical data are mentioned in Table 7.

These synthesized intermediates showed characteristic absorption bands in **IR spectra**: 1795–1785 (–C=O stretching of

Table 7
Physicochemical data of 4-(substituted benzylidene)-2-phenyl-4*H*-oxazol-5-one.



S. No.	R	M.P.[a] (°C)	% yield	Colour	Mol. formula[b] (M.W.)	^{1}H NMR (δ ppm, DMSO-d6)
a	H	158	60	Yellow needle	$C_{16}H_{11}NO_2$ (249.27)	8.31–8.29 (d, 2H, 2′, 6′ Ar-H), 8.14–8.12 (d, 2H, 2, 6 Ar-H), 7.74–7.52 (m, 6H, Ar-H), 7.35 (s, 1H, CH=C)
b	p-CH_3	134	58	Parrot green	$C_{17}H_{13}NO_2$ (263.20)	8.38–8.35 (d, 2H, 2′, 6′ Ar-H), 8.14–8.12 (d, 2H, 2, 6 Ar-H), 7.74–7.49 (m, 3H, Ar-H), 7.45 (s, 1H, CH=C), 7.28–7.26 (d, 2H, 3, 5 Ar-H), 2.37 (s, 3H, CH_3)
c	p-OH	172–173	62	Yellow	$C_{16}H_{11}NO_3$ (265.27)	10.62 (s, 1H, OH), 8.38–8.35 (d, 2H, 2′, 6′ Ar-H), 8.19–8.16 (d, 2H, 2, 6 Ar-H), 7.70–7.45 (m, 3H, Ar-H), 7.39 (s, 1H, CH=C), 6.94–6.91 (d, 2H, 3,5 Ar-H)
d	p-OCH_3	156	52	Yellow fluffy	$C_{17}H_{13}NO_3$ (279.30)	8.20–8.17 (d, 2H, 2′, 6′ Ar-H), 8.14–8.12 (d, 2H, 2,6 Ar-H), 7.63–7.43 (m, 3H, Ar-H), 7.39 (s, 1H, CH=C), 6.85–6.83 (d, 2H, 3, 5 Ar-H), 3.82 (s, 3H, OCH_3)
e	o-Cl	156–158	61	Yellow	$C_{16}H_{10}ClNO_2$ (283.72)	8.17–8.15 (d, 2H, 2′, 6′ Ar-H), 7.92–7.90 (d, 1H, 6 Ar-H), 7.66–7.56 (m, 5H, Ar-H), 7.48 (s, 1H, CH=C), 7.18–7.13 (d, 1H, Ar-H)
f	p-$N(CH_3)_2$	217–219	55	Dark brown	$C_{18}H_{16}N_2O_2$ (292.34)	8.20–8.17 (d, 2H, 2′, 6′ Ar-H), 8.08–8.06 (d, 2H, 2, 6 Ar-H), 7.67–7.61 (t, 3H, 3′, 4′, 5′ Ar-H), 7.24 (CH=C), 6.85–6.83 (d, 2H, 3, 5 Ar-H), 3.08 (s, 6H, $-N(CH_3)_2$)

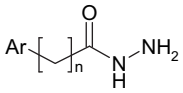
[a] Melting point of the compounds at their decomposition.
[b] Elemental analyses for C, N were within ± 0.4% of the theoretical values.

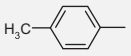
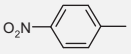
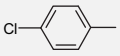
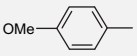
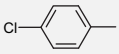
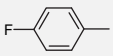
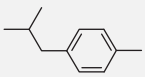
lactone), 1657–1648 (–C=N stretching) cyclic, 1606–1588, 1553–1498 (–C–C-(skeletal) stretching of benzene ring), 1039–1030 (–C–O stretching of ether), 881–851, 832–813 (Ar-H bending). The spectral data of all the synthesized compounds are presented in Table 7.

*6.1.3. General procedure for the synthesis of acid hydrazide (**4a–l**)*

Aromatic acids were first converted to its ester (***3a–l***) by esterification procedure using conc. sulphuric acid as a catalyst in absolute ethanol. The mixture of acid, absolute ethanol and conc. sulphuric acid were refluxed for 10–12 h, Distilled off about half of the alcohol on a water bath after this, diluted the residue with sufficient quantity of water and removed the upper layer of the crude ester and extracted the aqueous layer with ether. Later on, combined ethereal extract and crude ester were washed with water, then with saturated sodium hydrogen carbonate solution until effervescence ceased, and finally with water. Dried with anhydrous sodium sulphate & removed the ether on a water bath. To an alcoholic solution of aromatic ester (***3a–l***), hydrazine hydrate was added. The resulting reaction mixture was refluxed for

Table 8
Physicochemical characteristics of synthesized substituted hydrazide.



	Ar	*n*	R_f[c]	M.P. (°C)[a]	% yield	Colour	Mol. formula[b] (M.W.)
a		0	0.40	112–114	78	White	$C_7H_8N_2O$ (136.15)
b		0	0.16	117	82	White fluffy	$C_8H_{10}N_2O$ (150.17)
c		0	0.46	218	70	Yellow crystalline	$C_7H_7N_3O_3$ (181.15)
d		0	0.10	171–173	–	White crystalline	$C_6H_7N_3O$ (137.13)
e		1	0.17	149–153	80	White fluffy	$C_8H_9ClN_2O$ (184.62)
f		1	0.24	185–186	82	White fluffy	$C_{12}H_{12}N_2O$ (200.23)
g		0	0.28	136–140	80	White	$C_8H_{10}N_2O_2$ (166.17)
h		0	0.15	163–164	75	White fluffy	$C_7H_7ClN_2O$ (150.17)
i		0	0.38	118–120	66	White fluffy	$C_7H_7ClN_2O$ (170.59)
j		0	0.26	162–166	74	White	$C_7H_7FN_2O$ (154.14)
k		1	0.31	129–134	78	White	$C_7H_8N_2O_2$ (152.15)
l		2	0.61	92–94	77	Buff fluffy	$C_{13}H_{20}N_2O$ (220.31)

[a] Melting point of the compounds at their decomposition.
[b] Solvent of recrystallization – ethanol.
[c] Solvent system – toluene: ethyl acetate: formic acid (5:4:1).

10–12 h. The excess of alcohol was distilled off and cooled. Filtered off crystals of the acid hydrazide (***4a–l***), and recrytallised from ethanol. The physical data of the hydrazides are given in Table 8.

*6.1.4. General procedure for the synthesis of Triazinone (**5 aI–fXII**)*

An equimolar quantity of 4-benzylidene-2-phenyl-4*H*-oxazol-5-one (***2a–f***) derivative (0.01 mol), substituted acid hydrazide (***4a–l***) (0.01 mol) and sodium acetate (0.2 g) in gl. acetic acid (10 ml) was refluxed for 8–10 h. The reaction mixture was poured into crushed ice and stirred. The solid obtained was filtered, washed with water, dried and recrystallised from ethanol. The physical data of all the synthesized compounds are given in Table 1.

The synthesized compounds (Scheme 1) showed characteristic absorption bands in **IR spectra**: 3450, 3328–3303 (NH), 1737–1690 (CONH), 1696–1662 (C=O), 1628–1604 (C=N, imine), 1300–1400 (C–N, Ar-NH).

*6.1.4.1. 2-Benzoyl-5-benzylidene-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aI)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.57 (s, 1H, CONH), 8.38–8.35 (d, 2H, Ar-H), 8.22–8.19 (d, 2H, Ar-H), 7.97–7.95 (t, 2H, Ar-H), 7.88–7.86 (d, 2H, Ar-H), 7.62–7.60 (t, 1H, Ar-H), 7.48–7.35 (m, 6H, Ar-H), 7.27 (s, 1H, CH=C). **Mass spectra:** M^+ (367), M-1 (366), M-105, M-77.

*6.1.4.2. 5-Benzylidene-2-(4-methyl-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.61 (s, 1H, CONH), 8.27–8.25 (d, 2H, Ar-H), 8.03–8.00 (d, 2H, Ar-H), 7.81–7.7 (d, 2H, Ar-H), 7.65–7.56 (m, 4H, Ar-H), 7.42–7.34 (m, 4H, Ar-H), 7.29 (s, 1H, CH=C), 2.38 (s, 3H, CH_3).

*6.1.4.3. 5-Benzylidene-2-(4-nitro-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aIII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.54 (s, 1H, CONH), 8.40–8.38 (d, 2H, Ar-H), 8.26–8.24 (d, 2H, Ar-H), 7.95–7.93 (d, 2H, Ar-H), 7.76–7.54 (m, 5H, Ar-H), 7.47–7.39 (m, 3H, Ar-H), 7.26 (s, 1H, CH=C).

*6.1.4.4. 5-Benzylidene-3-phenyl-2-(pyridine-4-carbonyl)-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aIV)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.61 (s, 1H, CONH), 8.98–9.01 (d, 2H, Pyridine), 8.31–8.29 (d, 2H, Ar-H), 8.14–8.12 (d, 2H, Ar-H), 8.01–7.99 (d, 2H, pyridine), 7.73–7.64 (m, 3H, Ar-H), 7.55–7.51 (m, 3H, Ar-H), 7.29 (s, 1H, CH=C).

*6.1.4.5. 5-Benzylidene-2-[2-(4-chloro-phenyl)-acetyl]-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aV)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.56 (s, 1H, CONH), 8.32–8.29 (d, 2H, Ar-H), 8.14–8.12 (d, 2H, Ar-H), 7.86–7.47 (m, 6H, Ar-H), 7.33–7.35 (d, 2H, Ar-H), 7.28 (s, 1H, CH=C), 7.25–7.23 (d, 2H, Ar-H), 3.61 (s, 2H, CH_2).

*6.1.4.6. 5-Benzylidene-2-(2-naphthalen-1-yl-acetyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aVI)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.48 (s, 1H, CONH), 8.22–8.20 (m, 3H, Ar-H), 7.91–7.84 (m, 5H, Ar-H), 7.60–7.58 (d, 2H, Ar-H), 7.51–7.38 (m, 5H, Ar-H), 7.33–7.31 (d, 2H, Ar-H), 7.24 (s, 1H, CH=C), 4.11 (s, 2H, $-CH_2-$).

*6.1.4.7. 5-Benzylidene-2-(4-methoxy-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aVII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.56 (s, 1H, CONH), 8.28–8.26 (d, 2H, Ar-H), 8.03–8.01 (d, 2H, Ar-H), 7.73–7.51 (m,6H, Ar-H), 7.27–7.25 (d, 2H, Ar-H), 7.23 (s, 1H, CH=C), 7.12–7.09 (d, 2H, Ar-H), 3.85 (s, 3H, OCH_3).

*6.1.4.8. 5-Benzylidene-2-(4-chloro-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aVIII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.70 (s, 1H, CONH), 8.38–8.35 (d, 2H, Ar-H), 8.03–8.01 (d, 2H, Ar-H), 7.72–7.49 (m, 10H, Ar-H), 7.31 (s, 1H, CH=C).

*6.1.4.9. 5-Benzylidene-2-(2-chloro-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aIX)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.70 (s, 1H, CONH), 8.37–8.35 (d, 2H, Ar-H), 8.05–8.03 (d, 2H, Ar-H), 7.66–7.51 (m, 10H, Ar-H), 7.31 (s, 1H, CH=C).

*6.1.4.10. 5-Benzylidene-2-(4-fluoro-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aX)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.61 (s, 1H, CONH), 8.38–8.36 (d, 2H, Ar-H), 8.05–8.03 (d, 2H, Ar-H), 7.87–7.47 (m, 8H, Ar-H), 7.28 (s, 1H, CH=C), 7.24–7.22 (d, 2H, Ar-H).

*6.1.4.11. 5-Benzylidene-2-(2-phenoxy-acetyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aXI)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.56 (s, 1H, CONH), 8.25–8.23 (d, 2H, Ar-H), 8.07–8.05 (d, 2H, Ar-H), 7.76–7.48 (m, 6H, Ar-H), 7.27 (s, 1H, CH=C), 7.24–7.22 (d, 2H, Ar-H), 6.91–6.82 (m, 3H, Ar-H), 5.11 (s, 2H, CH_2).

*6.1.4.12. 5-Benzylidene-2-[2-(4-isobutyl-phenyl)-propionyl]-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 aXII)**.* **^{1}H NMR (DMSO-d$_6$) (δ, ppm)** 11.59 (s, 1H, CONH), 8.27–8.25 (d, 2H, Ar-H), 8.01–7.99 (d, 2H, Ar-H), 7.83–7.81 (d, 2H, Ar-H), 7.65–7.34 (m, 8H, Ar-H), 7.29 (s, 1H, CH=C), 3.95 (q, 1H, –CH(methyl)-), 2.59 (d, 2H, $-CH_2-$), 2.18 (m, 1H, CH), 1.57 (d, 3H, CH_3), 1.21 (d, 6H, 2 X CH_3).

*6.1.4.13. 2-Benzoyl-5-(4-methyl-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 bI)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.60 (s, 1H, CONH), 8.36–8.34 (d, 2H, Ar-H), 8.12–8.10 (d, 2H, Ar-H), 7.88–7.86 (d, 2H, Ar-H), 7.74–7.49 (m, 6H, Ar-H), 7.31 (s, 1H, CH=C), 7.28–7.26 (d, 2H, Ar-H), 2.37 (s, 3H, CH_3).

*6.1.4.14. 2-(4-Methyl-benzoyl)-5-(4-methyl-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 bII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.60 (s, 1H, CONH), 8.27–8.25 (d, 2H, Ar-H), 8.03–8.00 (d, 2H, Ar-H), 7.81–7.78 (d, 2H, Ar-H), 7.58–7.56 (m, 3H, Ar-H), 7.29 (s, 1H, CH=C), 7.18–7.14 (m, 4H, Ar-H), 2.38 (s, 6H, CH_3).

*6.1.4.15. 2-[2-(4-Chloro-phenyl)-acetyl]-5-(4-methyl-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 bV)**.* **^{1}H NMR (DMSO-d$_6$) (δ, ppm)** 11.64 (s, 1H, CONH), 8.35–8.33 (d, 2H, Ar-H), 8.14–8.12 (d, 2H, Ar-H), 7.88–7.49 (m, 5H, Ar-H), 7.35–7.33 (d, 2H, Ar-H), 7.28 (s, 1H, CH=C), 7.25–7.23 (d, 2H, Ar-H), 3.61 (s, 2H, CH_2), 2.38 (s, 3H, CH_3).

*6.1.4.16. 2-(4-Chloro-benzoyl)-5-(4-methyl-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 bVIII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.61 (s, 1H, CONH), 8.38–8.35 (d, 2H, Ar-H), 8.13–8.11 (d, 2H, Ar-H), 7.84–7.51 (m, 7H, Ar-H), 7.31 (s, 1H, CH=C), 7.18–7.16 (d, 2H, Ar-H), 2.37 (s, 3H, CH_3).

*6.1.4.17. 5-(4-Methyl-benzylidene)-2-(2-phenoxy-acetyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 bXI)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.66 (s, 1H, CONH), 8.25–8.23 (d, 2H, Ar-H), 8.03–8.01 (d, 2H, Ar-H), 7.73–7.41 (m, 6H, Ar-H), 7.24 (s, 1H, CH=C), 7.11–6.92 (m, 4H, Ar-H), 5.16 (s, 2H, CH_2), 2.37 (s, 3H, CH_3).

*6.1.4.18. 5-(4-Hydroxy-benzylidene)-2-(4-methyl-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 cII)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.65 (s, 1H, CONH), 10.48 (s, 1H, OH), 8.31–8.29 (d, 2H, Ar-H), 8.12–8.10 (d, 2H, Ar-H), 7.93–7.91 (d, 2H, Ar-H), 7.71–7.52 (m, 5H, Ar-H), 7.23 (s, 1H, CH=C), 7.03–7.01 (d, 2H, Ar-H), 2.34 (s, 3H, CH_3).

*6.1.4.19. 2-(4-Fluoro-benzoyl)-5-(4-hydroxy-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one **(5 cX)**.* **^{1}H NMR(DMSO-d$_6$) (δ, ppm)** 11.56 (s, 1H, CONH), 10.40 (s, 1H, OH), 7.28–7.26 (d, 2H, Ar-H),

7.10–7.08 (d, 2H, Ar-H), 7.83–7.56 (m, 5H, Ar-H), 7.29 (s, 1H, CH=C), 7.28–7.26 (d, 2H, Ar-H), 6.98–6.96 (d, 2H, Ar-H).

6.1.4.20. 5-(4-Methoxy-benzylidene)-2-(4-methyl-benzoyl)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one ***(5 dII).*** **^{1}H NMR(DMSO-d_6) (δ, ppm)** 11.52 (s, 1H, CONH), 8.21–8.19 (d, 2H, Ar-H), 8.05–8.02 (d, 2H, Ar-H), 7.94–7.92 (d, 2H, Ar-H), 7.79–7.56 (m, 5H, Ar-H), 7.31 (s, 1H, CH=C), 6.95–6.93 (d, 2H, Ar-H), 3.83 (s, 3H, OCH_3), 2.38 (s, 3H, CH_3).

6.1.4.21. 2-(4-Chloro-benzoyl)-5-(4-methoxy-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one ***(5 dVIII).*** **^{1}H NMR (DMSO-d_6) (δ, ppm)** 11.62 (s, 1H, CONH), 8.26–8.24 (d, 2H, Ar-H), 8.03–8.01 (d, 2H, Ar-H), 7.84–7.81 (d, 2H, Ar-H), 7.76–7.43 (m, 5H, Ar-H), 7.33 (s,1H, CH=C), 7.08–7.06 (d, 2H, Ar-H), 3.85 (s, 3H, OCH_3).

6.1.4.22. 2-(2-Chloro-benzoyl)-5-(2-chloro-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one ***(5 eIX).*** **^{1}H NMR(DMSO-d_6) (δ, ppm)** 11.46 (s, 1H, CONH), 8.22–8.20 (d, 2H, Ar-H), 7.99–7.97 (d, 2H, Ar-H), 7.81–7.53 (m, 7H, Ar-H), 7.43 (s, 1H, CH=C), 7.35–7.33 (d, 2H, Ar-H).

6.1.4.23. 2-Benzoyl-5-(4-dimethylamino-benzylidene)-3-phenyl-2,5-dihydro-1H-[1,2,4] triazin-6-one ***(5 fI).*** **^{1}H NMR(DMSO-d_6) (δ, ppm)** 11.57 (s, 1H, CONH), 8.38–8.35 (d, 2H, Ar-H), 8.22–8.19 (d, 2H, Ar-H), 7.97–7.95 (t, 2H, Ar-H), 7.88–7.86 (d, 2H, Ar-H), 7.48–7.35 (m, 4H, Ar-H), 7.17 (s, 1H, CH=C), 6.82–6.79 (d, 2H, Ar-H), 3.04 (s, 6H, Dimethyl) **Mass spectra:** M^+ (410), M-1 (409).

6.2. Pharmacology

Male albino mice (20–25 g) were used as experimental animals. The Institutional Animal Ethics committee (IAEC) reviewed and approved all the animal procedures adopted. The animals were housed at an ambient temperature of 25 ± 2 °C, in groups as required per metabolic cages and allowed free access to chow pellets and water. The light/dark cycle of 12 h: 12 h was maintained. All the synthesized test compounds were suspended in 30% polyethylene glycol (PEG 200).

6.2.1. Anticonvulsant screening

Anticonvulsant evaluations were undertaken using the reported procedures [15,18]. Initially all the test compounds were administered i.p. in a volume of 0.01 ml/g body weight of mice at doses of 30, 100, 300 mg/kg to 1–6 animals. Anticonvulsant activity was assessed after 30 min and 4 h intervals of administration. Activity was established using the MES and scPTZ tests.

6.2.2. Neurotoxicity screen

Minimal motor impairment was measured in mice by the rotorod test [19]. Animals were divided in groups of 4 animals and trained to stay on accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals (able to stay on the rotorod for at least two consecutive periods of 90 s) were given an i.p. injection of the test compounds in doses of 30, 100 and 300 mg/kg. Neurological deficit was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trials. The dose at which animal fell off the rod, was determined.

6.2.3. Behavioural test

Some of the titled compounds (30 mg/kg) were screened for their behavioural effects using an actophotometer [20] at 30 min and 1 h after injection in each group of 6 animals. Animals were acclimatized to the dark environment 24 h before the test. The control administered was 30% PEG only. The behaviour of the animal inside the photocell was recorded as digital score. Increased score represented good behavioural activity.

6.2.4. CNS depressant study

The forced swim pool method reported earlier by Porsolt et. al. was followed [21]. Mice (six animals in each group) were placed in chamber (diameter 45 cm, height 20 cm) containing water up to the 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 *i.p.* injection (30 mg/kg) of the test compound 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which were necessary to keep its head above the surface) during the 5 min test period were measured. This immobility reflected state of depression. Carbamazepine was used as a reference for comparison at a dose of 30 mg/kg (*i.p.*, in PEG). The control animals were administered 30% PEG.

6.3. Computational parameter

6.3.1. Distance mapping

In conformational analysis of the clinically effective anticonvulsant drugs such as phenytoin, carbamazepine, lamotrigine and diazepam, a molecular model was suggested on the basis of molecular dynamics distance estimations [22]. For the estimation of the molecular mechanics calculation of titled compounds, the ACD/3D viewer 8.04 version program was used for employing the CHARMM force field.

6.3.2. Log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature [23]. 10 mL of octanol and 10 mL phosphate buffer were taken in a glass stoppered graduated tube and 5 mg of accurately weighed drug was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to dynamic equilibrate for 6 h. The aqueous and octanol phase were separated and filtered through membrane filter and drug content in aqueous phase was analysed by UV spectroscopy.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the on-line version, at doi: 10.1016/j.ejmech.2010.05.051.

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