Arch. Pharm. Chem. Life Sci. 2015, *348*, 1–13

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Full Paper

Synthesis, Biological Activity, and Docking Study of Novel Isatin Coupled Thiazolidin-4-one Derivatives as Anticonvulsants

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A series of 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one derivatives were designed and synthesized under microwave irradiation, using an eco-friendly, efficient, microwave-assisted synthetic protocol that involves cyclocondensation of 3-substituted benzylidine-hydrazono-indolin-2-one **3a–j** with thioglycolic acid in dimethyl formamide (DMF) as solvent and anhydrous zinc chloride as a catalyst, keeping in view the structural requirement of the pharmacophore. The intermediate compounds **3a–j** were obtained by condensation of the hydrazone of indoline-2,3-dione with aromatic aldehydes. The synthesized derivatives were evaluated for CNS depressant activity and anticonvulsant activity in mice using the maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (sc-PTZ) induced seizure tests. All the derivatives showed good CNS depressant activity and showed protection in the MES test, indicative of their ability to inhibit the seizure spread. A histopathological study was performed to evaluate liver toxicity caused by the synthesized compounds. The compounds were nontoxic. A computational study was performed, in which log *P* values were calculated experimentally. Virtual screening was performed by molecular docking of the designed compounds into the ATP binding sites of the NMDA and AMPA receptors, to predict if these compounds have analogous binding modes.

Keywords: Anticonvulsants / Indoline-2,3-dione / MES test / Molecular docking / PTZ-induced seizure

Received: January 16, 2015; Revised: February 28, 2015; Accepted: March 6, 2015

DOI 10.1002/ardp.201500020

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Introduction

Epilepsy is a very common neurological affliction in men characterized by temporary high neuronal discharges resulting in uncontrolled seizure and affected millions of people worldwide [1]. Moreover, the recent drug therapy is full with adverse side effects such as gastrointestinal disturbances, drowsiness, ataxia, hirsutism, gingival hyperplasia, and megaloblastic anemia and lifelong medication may be required [2–4]. Therefore, there is continuous need for the search and development of more effective and reliable anticonvulsant drugs. As the literature survey reveals that indoline-2,3-dione (isatin) derivatives have exhibited potent CNS depressant and anticonvulsant activities in many research papers [5, 6] as well as the Schiff base with 4-thiazolidinone derivatives possess remarkable anticonvulsant activity [7–9] and in continuation of our earlier efforts [10–14] for search of novel anticonvulsant agents. In the present work our objective was to develop novel hybrid heterocyclic molecule having indoline-2,3-dione moiety coupled with thiazolidinone nucleus having appropriate pharmacophors with the hope to get novel compounds with significant and potential anticonvulsant activity and minimal toxic side effects. Thus, novel 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-

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thiazolidin-4-one derivatives, having optimum log *P* values, were designed and synthesized, which show similar mode of action on neuronal sodium channels as phenytoin. All the synthesized derivatives have essential pharmacophoric elements that are necessary for anticonvulsant activity as suggested by Nadeem et al. [15], which are shown in Fig. 1. The anticonvulsant drugs require basic groups like –CH, –C=O, –NH which could be responsible for interaction with the active site of receptors of brain were a hydrophobic unit (R), hydrogen donor (D) group [16]. Most of the anticonvulsant drugs are acting by two basic receptors mechanisms, AMPA and NMDA. The α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) is a non-NMDA-type ionotropic transmembrane receptor for glutamate that mediates fast synaptic transmission in the central nervous system. *N*-Methyl-D-aspartic acid (NMDA) is an amino acid derivative that acts as a specific agonist at the NMDA receptor mimicking the action of glutamate, the neurotransmitter which normally acts at that receptor [17, 18]. Hence, we have also tried to dock the designed compounds with the crystal structure of NMDA and AMPA receptors to explore their binding mode. Computational study was performed in which log *P* values were calculated experimentally. Histopathological study of liver was performed with the standard drug phenytoin and the synthesized compound **4e**, same compound **4e** shows excellent anticonvulsant activity, so as to compare the necrosis and inflammation caused and to study hepatotoxicity.

Results and discussion

Chemistry

The synthesis of the title compounds **4a–j** was carried out as presented in Scheme 1. Thiazolidin-4-one ring was built up on the indoline-2,3-dione by a series of reactions as follows: in

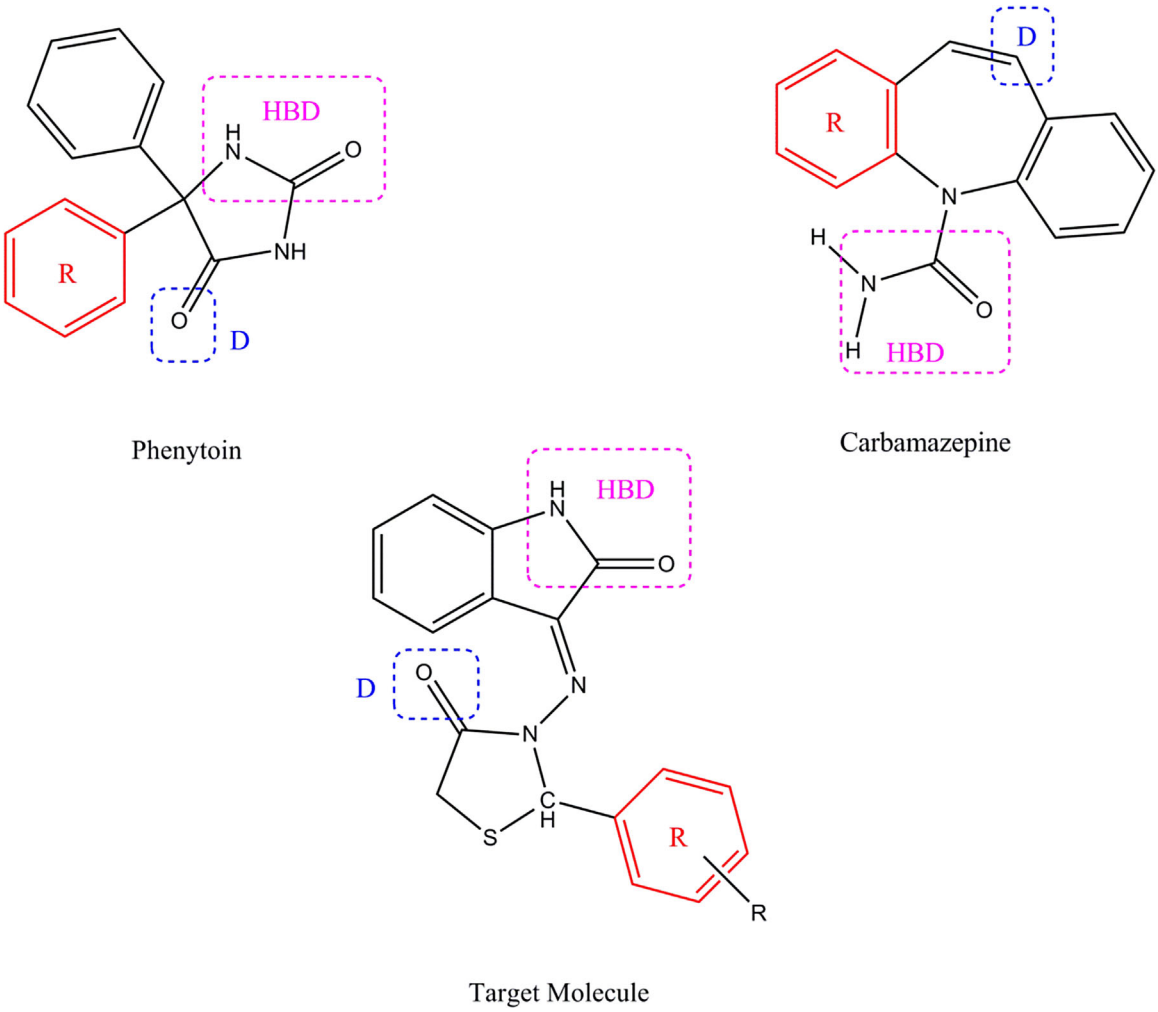
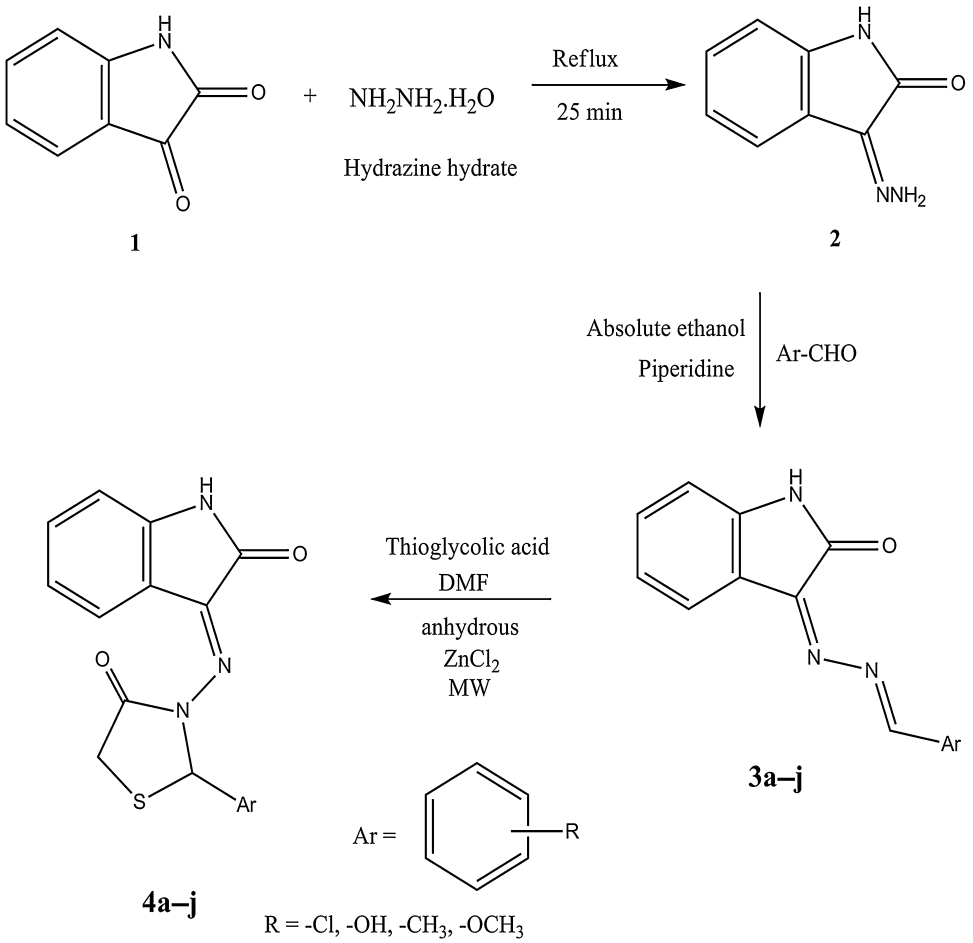


Figure 1. Structures of sodium channel modulators.



Scheme 1. Synthetic protocol for the title compounds.

the first step, indoline-2,3-dione was refluxed with hydrazine hydrate for 25 min in heating mental to give 3-hydrazonoindolin-2-one **2**; which was further refluxed with aromatic aldehydes in absolute ethanol in the presence of catalytic amount of piperidine to give 3-substituted benzylidine-hydrazono-indolin-2-ones **3a–j**. Final derivatives 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-thiazolidin-4-ones **4a–j** were obtained by cyclocondensation of **3a–j** with mercaptoacetic acid (thioglycolic acid) in dimethyl formamide (DMF) as solvent and anhydrous zinc chloride, as a catalyst, under microwave irradiation. The physicochemical parameters of the synthesized compounds are presented in Table 1. The data obtained from IR, mass, ^{1}H NMR elemental analysis confirmed the proposed structures.

A series of novel 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one derivatives **4a–j** were obtained under microwave irradiation in good yield and required shorter reaction time. Microwave-assisted synthesis of title compounds was carried out as it requires reduced reaction time, giving increased reaction rate, yield enhancement, cleaner, and greener, eco-friendly synthetic protocol [19, 20]; DMF is used as a solvent, instead of benzene, which is a proven carcinogenic solvent used in conventional synthesis of thiazolidinones. The conventional synthesis is carried out in Dean–Stark apparatus and also requires removal of benzene–water azotrope [21]. Thus, the microwave-assisted synthetic protocol is efficient and eco-friendly. All the synthesized compounds were evaluated for CNS depressant and anticonvulsant activity and have shown promising CNS activity.

Anticonvulsant activity

All the synthesized compounds have shown protection against MES test indicative of their ability to inhibit the seizure spread. Test compounds **4d**, **4e**, **4f**, **4i**, and **4j** have shown better protection against MES at 100 mg/kg, after 30 min and compounds **4e**, **4f**, **4h**, and **4i** have shown protection at dose level of 100 mg/kg at 30 min against PTZ-induced seizure. The compounds **4e**, **4f**, and **4i** have shown excellent activity in both MES and PTZ models at same dose of 100 mg/kg. The activity data are presented in Table 2.

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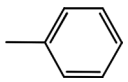
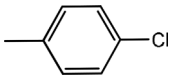
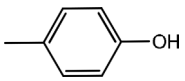
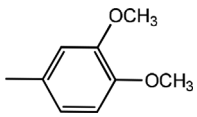
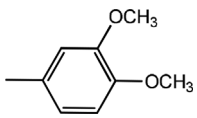
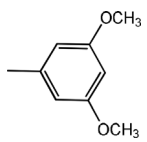
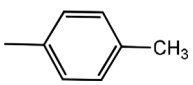
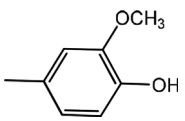
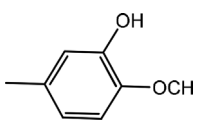
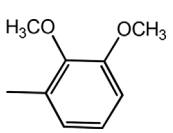
Table 1. **The physicochemical parameters of the synthesized compounds 4a–j.**

Code	Ar	Mol. weight	Yield (%)	M.P. (°C)	Log *P* value	R_f value	MW (min)
4a		323	86	132–134	1.92	0.45	18
4b		357	89	139–140	2.91	0.62	20
4c		339	87	149–151	1.93	0.42	19
4d		353	82	124–126	1.94	0.68	20
4e		383	80	179–181	1.98	0.72	22
4f		383	90	114–116	2.54	0.73	23
4g		337	75	129–131	2.06	0.80	20
4h		369	72	139–141	2.04	0.50	20
4i		369	74	159–161	2.28	0.69	22
4j		383	91	119–121	2.52	0.88	24

Solvent of recrystallization was ethanol; eluants used in TLC were petroleum benzene/methanol (0.5:4.5) for all compounds.

Table 2. Anticonvulsant activity and mortality percentage of compounds 4a–j.

Compounds	MES screen mean ± SEM		% Mortality R/D	Sc PTZ screen mean ± SEM onset of convulsion	
	30 mg/kg	100 mg/kg		30 mg/kg	100 mg/kg
Control	16.02 ± 0.344		1/4	40.44 ± 0.748**	
Standard (phenytoin)	12.21 ± 0.152**	9.12 ± 0.142**	5/0	55.48 ± 0.462*	86.84 ± 0.482
4a	X	15.36 ± 0.160**	2/3	X	61.22 ± 0.432**
4c	15.67 ± .0.130ns	11.24 ± 0.240**	4/1	43.25 ± 0.439**	52.36 ± 0.297**
4d	X	13.26 ± 0.169**	5/0	X	56.52 ± 0.439**
4e	16.21 ± .0.230**	11.26 ± 0.229**	4/1	43.84 ± 0.139**	71.46 ± 0.460**
4f	17.35 ± 0.168**	12.38 ± 0.193**	4/1	52.92 ± 0.339**	73.3 ± 0.249**
4g	X	14.98 ± 0.339**	3/2	X	63.45 ± 0.564**
4h	15.12 ± 0.339**	10.1 ± 0.114ns	3/2	56.22 ± 0.432**	71.4 ± 0.933**
4i	14.09 ± 0.159**	12.04 ± 0.242**	5/0	64.22 ± 0.729**	77.56 ± 0.729**
4j	X	15.72 ± 0.432**	4/1	X	61.82 ± 0.673**

A dose of 30 and 100 mg/kg of compound was administered and the protection measured after 30 min. The mean significant results were calculated by GraphPad and one-way ANOVA method. R, recovery; D, death; X, not done.
*$P < 0.05$, **$P < 0.01$, ns = non significant $P > 0.05$.

Behavioral study

In the behavioral activity using actophotometer, the compounds **4a**, **4b**, **4d**, **4e**, **4f**, and **4g** showed no behavioral despair effect when compared to diazepam at 1 h, and compounds **4c**, **4h**, **4i**, and **4j** have shown good CNS depressant activity at 30 mg/kg at 1 h when compared with standard (diazepam, 4 mg/kg). At 100 mg/kg, compounds **4c**, **4e**, **4f**, **4h**, and **4i** have shown good CNS depressant activity. The compounds **4c**, **4e**, **4h**, **4i**, and **4j** at dose level 30 mg/kg have shown excellent activity and compounds **4e**, **4f**, **4h**, **and 4i** at dose level 100 mg/kg have shown decreased locomotor activity in the 4 h interval but no despair was observed during 4 h time period when compared to diazepam. The results of these activities are shown in Tables 3 and 4 [22].

Muscle relaxant activity

All compounds in muscle relaxant activity have shown very good result except **4b** which has chloro group at 4-position. The compounds **4a**, **4c**, **4e**, **4f**, **4h**, **4i**, and **4j** have shown activity at dose level 100 mg/kg.

Histopathological evaluation

Histopathological study of liver T.S. shows that the standard drug phenytoin has caused necrosis and inflammation and is

Table 3. Behavioral study of the synthesized compounds 4a–j.

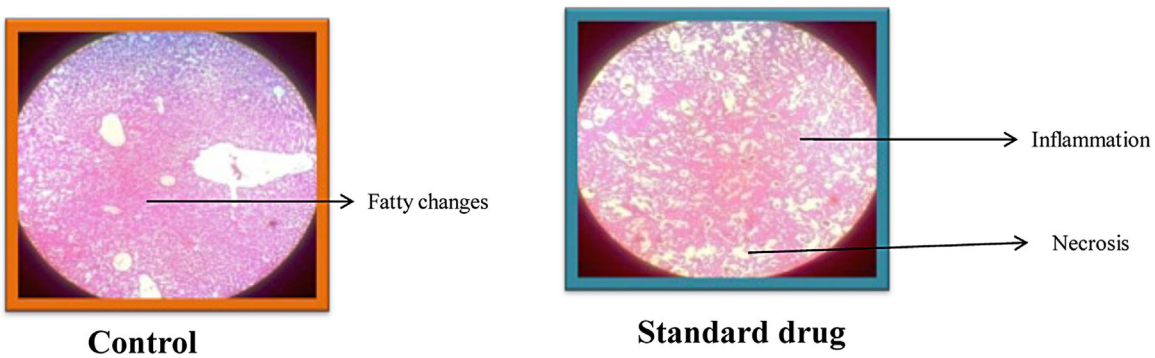
Compounds	Activity score using actophotometer post-treatment[a)]			
	1 h		4 h	
	30 mg/kg	100 mg/kg	30 mg/kg	100 mg/kg
4a	126.00 ± 10.242**	133.60 ± 1.400**	118.20 ± 10.336**	139.0 ± 1.000**
4b	182.00 ± 1.095ns	199.0 ± 1.517ns	202.40 ± 3.709ns	207.80 ± 3.917ns
4c	107.20 ± 12.114**	103.8 ± 7.179**	85.80 ± 0.860**	92.60 ± 1.208**
4d	122.00 ± 9.793**	118.2 ± 10.336**	129.20 ± 0.862**	159.0 ± 2.049*
4e	120.20 ± 1.772**	97.00 ± 11.480**	107.20 ± 12.11**	89.00 ± 1.225**
4f	118.20 ± 10.336**	87.00 ± 1.703**	125.00 ± 1.258**	80.60 ± 0.509**
4g	133.20 ± 3.043**	133.0 ± 2.049**	149.20 ± 2.354**	153.00 ± 1.00**
4h	104.00 ± 7.169**	62.01 ± 1.612**	96.00 ± 2.350**	57.00 ± 0.707**
4i	110.60 ± 9.304**	68.00 ± 1.225**	114.80 ± 12.944**	61.40 ± 1.631**
4j	116.40 ± 9.605**	120.0 ± 2.121**	113.40 ± 12.143**	127.40 ± 0.9274**
Standard[b)]	Diazepam 44.60 ± 0.509**		Diazepam 67 ± 0.3162**	
Control	224.2 ± 20.038		224.2 ± 20.038**	

[a)] Each value represents the mean ± SEM significantly different from the control at $P < 0.05$, NS denotes not significant at $P < 0.05$ (Student's *t*-test).
[b)] The compound was tested at dose level of 4 mg/kg (oral).
*$P < 0.05$, **$P < 0.01$, ns = non significant $P > 0.05$.

Table 4. **Locomotor activity of the synthesized compounds 4a–j.**

Compounds	% Decrease in locomotor activity			
	1 h		4 h	
	30 mg/kg	100 mg/kg	30 mg/kg	100 mg/kg
4a	51.1	86.34	47.75	80.59
4b	30.76	54.32	20.10	25.93
4c	49.96	91.8	41.10	86.55
4d	39.24	86.6	32.46	86.38
4e	51.66	90.44	49.96	88.42
4f	40.26	88.10	43.15	86.55
4g	32.50	85.32	26.14	79.40
4h	69.53	92.83	59.01	90.81
4i	63.07	92.15	68.80	88.12
4j	65.11	90.12	61.16	81.27
Standard	Diazepam 12.2 ± 0.663**		Diazepam 10.4 ± 0.509**	
Control	293.8 + 1.828			

The locomotor activity score was measured for 10 min.



Histopathology of mice liver (T.S. of liver) showing effect of control and standard drug

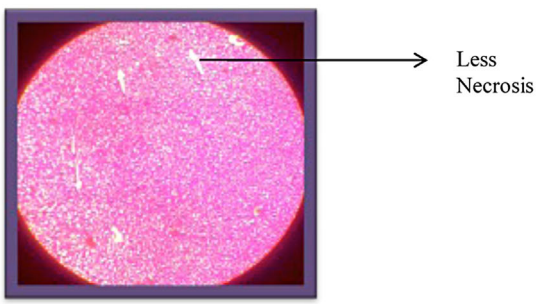
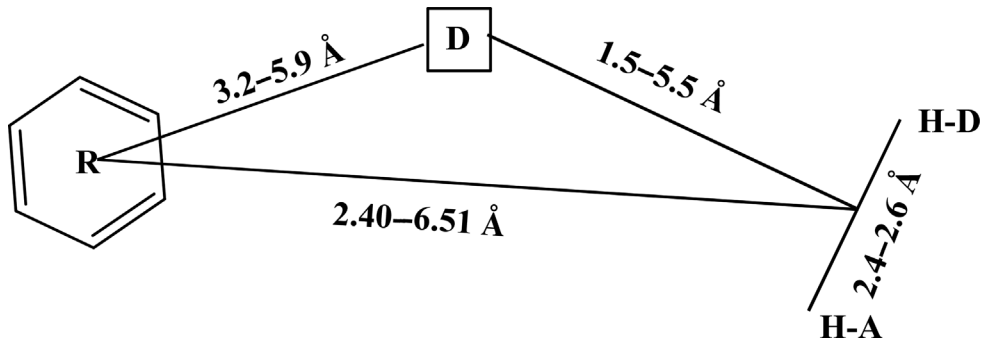


Figure 2. Microscopical examination.

Table 5. Distance mapping between the structure elements R, D, and HBD



Compounds	R-HD	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 **4a–j**	6.04	4.75	5.01

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

hepatotoxic. Control, 0.5% Tween 80 has shown fatty changes and necrosis, whereas liver of animals administered with synthesized compound, **4e**, has shown very less necrosis and no inflammation, as shown in Fig. 2. Thus, the synthesized compounds are less toxic than the standard drug.

Computational parameters

Distance mapping

The present work involves the correlation of the structural requirement of well-known and structurally different anticonvulsant compounds with the title 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) is structural requirement for anticonvulsant activity. The essential structural features which could be responsible for an interaction with the active site of voltage-gated sodium channels were a hydrophobic unit (R), an electron donor (D) group, and a hydrogen donor/acceptor (HBD) unit. Distance mapping between the structure elements R, D, and HBD is shown in Table 5.

Log P determination

Several classes of CNS active compounds showed dependence of biological activity on lipophilic character in a congeneric series. Hansch and Leo found that blood–brain barrier penetration is optimal when the log *P* values are in the range of 1.5–2.7 with the mean value of 2.1 [23, 24]. From the experimental log *P* determination, it was found that most of the compounds are having log *P* values between 1.5 and 2.7, which is requirement to cross the blood–brain barrier. All the values are shown in Table 6. Most of the compounds like **4b**, **4c**, **4e**, **4f**, **4h**, and **4i** have shown dependence of biological activity on lipophilic character in congeneric series. Hence, these compounds are showing excellent anticonvulsant activity.

Molecular docking methodology

In this study, we have used molecular docking tool, GLIDE (Schrödinger, Inc., USA) (2006) for automated flexible ligand docking of synthesized compounds with two established epilepsy molecular targets namely NMDA receptor and AMPA receptor. The low energy conformation of the ligands was selected and docked into the grid generated from protein structures using extra precision (XP) docking mode. The final

Table 6. Experimental and theoretical log *P* values of compounds 4a–j.

Compounds	Experimental log *P*	Theoretical log *P* combined value
4a	1.92	2.5
4b	2.91	3.1
4c	1.93	2.1
4d	1.94	2.4
4e	1.98	2.32
4f	2.54	2.32
4g	2.06	2.64
4h	2.04	2.06
4i	2.28	2.06
4j	2.52	2.3

Theoretical log *P* combined was calculated by using Pallas demo version 3112.

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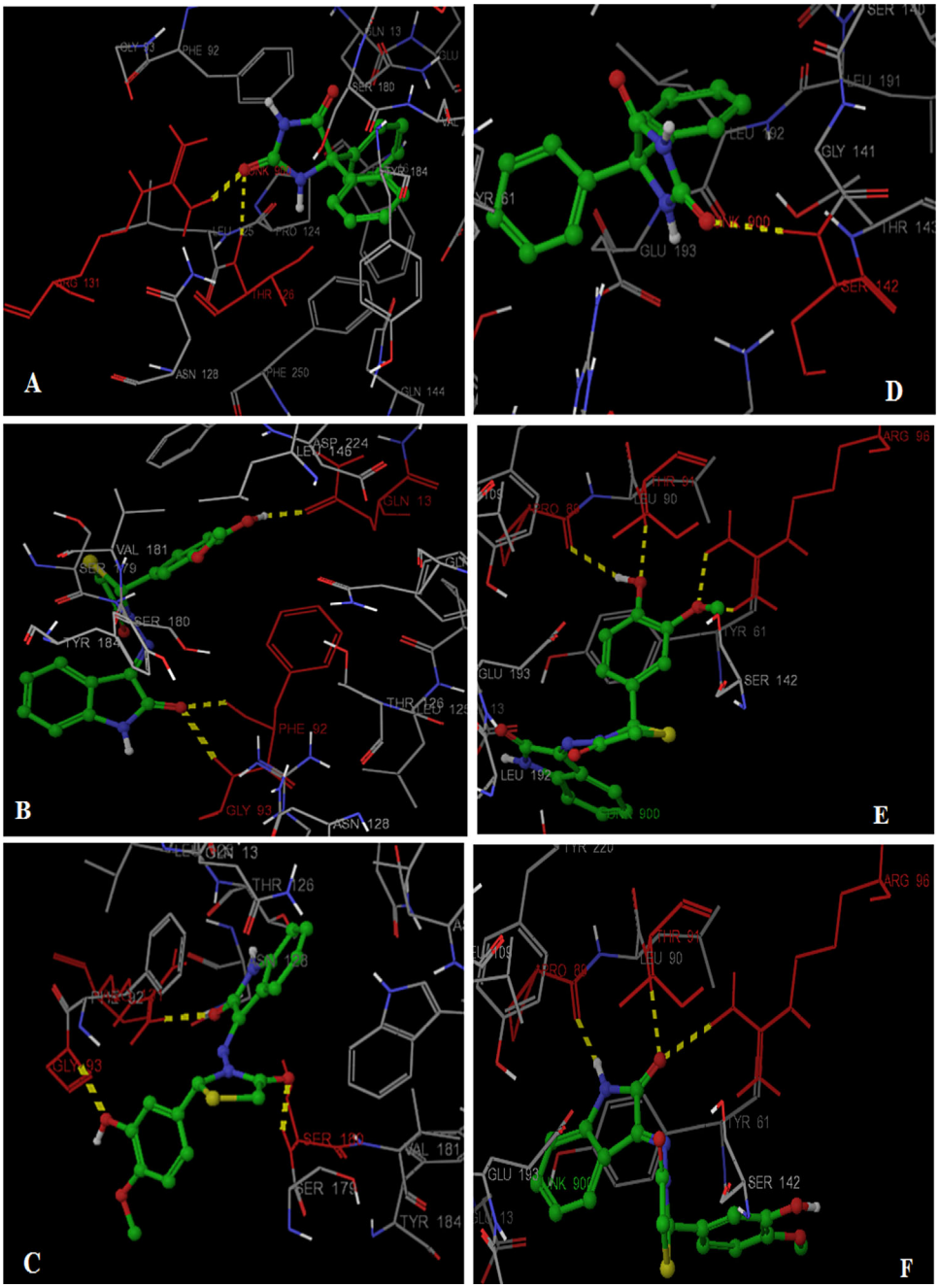

Figure 3. Binding mode of phenytoin (A), compound **4h** (B) and **4i** (C) showing H-bond interaction with amino acid residues of NMDA receptor (PDB: 1PBQ); binding mode of phenytoin (D), compound **4h** (E) and **4i** (F) showing H-bond interaction with amino acid residues of AMPA receptor (PDB: 1FTL); dotted yellow bond showing H-bond interaction with site residue.

evaluation is done on the basis of docking score, glide energy (kcal/mol), and probable hydrogen bonding interaction with amino acid residues. We docked phenytoin into the active site of both NMDA receptor (docking score −9.010283 and 3 H-bonds) and AMPA receptor (docking score −8.457558 and 1 H-bond) and then we replaced with our compounds to compare the binding mode of both the ligand and the test compounds. Compound **4h** has exhibited good binding properties with NMDA receptor (docking score −9.190447 and 3 H-bonds) and AMPA receptor (docking score −8.482894

Table 7. Results of glide docking based on glide dock score, glide energy, and hydrogen bonding interaction of synthesized compounds 4a–j with NMDA receptor.

Sr. no.	Docking score	Glide energy (kcal/mol)	H-bonding residues
4a	−8.267906	−66.717638	GLY 93, PHE 92
4b	−8.222225	−66.343933	GLY 93, PHE 92
4c	−8.696813	−68.998742	GLY 93, PHE 92, GLN 13
4d	−8.632272	−62.91166	GLY 93, PHE 92
4e	−8.63082	−69.3503	GLY 93, PHE 92
4f	−8.329504	−62.149948	GLY 93, PHE 92
4g	−8.188746	−60.289785	GLY 93, PHE 92
4h	−9.190447	−72.684925	GLY 93, PHE 92, GLN 13
4i	−9.006918	−70.632847	GLY 93, PHE 92, SER 180
4j	−8.649542	−66.1017	GLY 93, PHE 92
Phenytoin	−9.010283	−70.945541	ARG 131, THR 126

and 3 H-bonds). Compound **4i** has shown prominent interaction with amino acid residues of NMDA receptor (docking score −9.006918 and 3 H-bonds) and AMPA receptor (docking score −8.170842 and 3 H-bonds). The docking images are given in Fig. 3. The purpose of docking study was to co-relate the obtained activity data and explain the possible interactions which might take place between the synthesized derivatives **4a–j** and epilepsy molecular targets. Results of glide docking based on glide dock score, glide energy, and hydrogen bonding interaction of synthesized compounds **4a–j** with NMDA receptor and AMPA receptor are shown in Tables 7 and 8.

Conclusion

By using eco-friendly, efficient, microwave-assisted synthetic protocol, 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)-amino)-thiazolidin-4-one derivatives were synthesized in good yield. These derivatives were evaluated for anticonvulsant and CNS depressant activity with the hope of getting novel hybrid compounds serving as potential anticonvulsant agents. SAR study predicts that compounds like **4h** and **4i** have shown promising activity. Compounds with smaller electron donating, polar groups like –OH (**4c**), $-OCH_3$ (**4d**), attached to phenyl ring at *para*-position show significant activity in MES screening model. Experimental calculation of log *P* values suggests that compounds having log *P* values in the range 1.5–2.5, have exhibited better activities than the compounds having higher log *P* values. Compounds **4c**, **4h**, **4i**, and **4j** have shown good CNS depressant activity at 30 mg/kg. The synthesized derivative **4e** having experimental log *P* value of 1.98 has shown good protection against MES and PTZ seizures and has shown less hepatotoxicity than the standard drug phenytoin. Some of the synthesized derivatives like **4b**, having higher log *P* value, i.e., **4b**, has exhibited lesser CNS depressant and anticonvulsant activity. The results of docking studies also support the postulation that our active compounds may act on the epilepsy molecular targets through hydrogen bonding. Thus, all the experimental observations of biological activity, docking studies, log *P* calculations suggest that the synthesized compounds 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one are potential anticonvulsants, cause CNS depression and muscle relaxation.

Table 8. Results of glide docking based on glide dock score, glide energy, and hydrogen bonding interaction of synthesized compounds 4a–j with AMPA receptor.

Sr. no.	Docking score	Glide energy (kcal/mol)	H-bonding residues
4a	−7.408415	−57.9229	PRO 89, THR 91
4b	−7.395962	−55.0516	PRO 89, THR 91
4c	−7.948836	−62.7822	PRO 89, THR 91, ARG 96
4d	−7.073684	−53.0707	PRO 89, THR 91
4e	−7.189234	−54.3548	PRO 89, THR 91
4f	−7.110536	−55.6056	PRO 89, THR 91
4g	−7.063207	−53.5356	PRO 89, THR 91
4h	**−8.482894**	**−62.8598**	PRO 89, THR 91, ARG 96
4i	**−8.170842**	**−64.9968**	PRO 89, THR 91, ARG 96
4j	−7.045262	−54.1236	PRO 89, THR 91
Phenytoin	**−8.457558**	**−65.2977**	SER 142

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In conclusion, the compounds **4e**, **4f**, **4h**, and **4i** can be further optimized and developed as lead molecules for future investigations.

Experimental

Chemistry

The structures of the final compounds of the series **4a–j** were confirmed by the spectral data and elemental analysis. Infrared (IR) was recorded for the compounds on JASCO FTIR (PS 4000) using KBr pellet. All ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Advance II (400 MHz) instruments. Chemical shifts are reported in parts per million (ppm) using TMS as an internal standard. Elemental analyses (C, H, and N) were undertaken with a Shimadzu's FLASHEA112 analyzer and all analyses were consistent with theoretical values (within ±0.5%) unless indicated otherwise.

*General procedure for synthesis of 3-substituted benzylidine-hydrazono-indolin-2-ones **3a–j***

Mixture of equimolar quantity of aromatic aldehyde (0.015 mol) and hydrazone **2** (0.01 mol) was refluxed for 3–6 h in the presence of 3–4 drops of piperidine as a catalyst in absolute ethanol on heating mental. The completion of the reaction was monitored by TLC. The reaction mixture was filtered and washed with water and cooled. The crude product was recrystallized from ethanol. The melting point and yield were recorded.

*General procedure for synthesis of 2-(substituted-phenyl)-3-(2-oxoindolin-3-ylidene)amino)-thiazolidin-4-ones **4a–j***

Microwave method: Schiff's base **3** (0.01 mol) in DMF (15 mL) and thioglycolic acid (0.01 mol) was taken in a dry Erlenmeyer flask. A pinch of anhydrous $ZnCl_2$ as a catalyst was added to the flask slowly. The reaction mixture was irradiated in synthetic microwave oven for about 12–14 min at 700 W and 82°C. The completion of reaction was monitored by TLC. The reaction mixture was poured in ice cold water with continuous stirring. The obtained solid was filtered, washed with water, and dried. The product was recrystallized from ethanol. The melting point and yield of the product was recorded. The structures of the synthesized final compounds of the series **4a–j** were confirmed by the spectral data and elemental analysis as given below.

*3-((2-Oxoindolin-3-ylidene)amino)-2-phenylthiazolidin-4-one (**4a**)*

IR (KBr ν_{max} in cm^{-1}): 3369 (N–H stretch), 3130 (C–H stretch Ar), 1630 (C=O stretch), 1243 (C–S stretching), 596 (C–S bending); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.23 (s, 2H, $-CH_2$ of thiazolidinone ring); 5.97 (s, 1H, –N–CH–Ar); 6.8–7.5 (m, 9H, $J = 1.25$ Hz, Ar–H); 8.2 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 34.9, 63.4, 117.6, 119.5, 124.5, 126.8, 126.9, 127.2, 128.6, 128.7, 129.5, 131.3, 133.2, 139.4, 141.4, 168.4, 171.2. MS *m/z* 323.12 (M+1); Anal. calcd. for $C_{17}H_{13}N_3O_2S$: C, 65.79; H, 4.55; N, 9.03; Found C, 65.77; H, 4.54; N, 9.01.

*2-(4-Chlorophenyl)-3-((2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one (**4b**)*

IR (KBr ν_{max} in cm^{-1}): 3337 (N–H stretch), 3081 (C–H stretch Ar), 2890 (C–H stretching of CH_2), 1665 (C=O stretch), 1242 (C–S stretching), 720 (C–S bending); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.96 (s, 2H, $-CH_2$ of thiazolidinone ring); 5.82 (s, 1H, –C–H of thiazolidinone ring); 7.1–7.8 (m, 8H, $J = 1.25$ Hz, Ar–H); 8.21 (s, 1H, $J = 1.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.2, 62.2, 117.6, 119.5, 124.6, 128.2, 128.3, 129.5, 130.2, 130.4, 131.0, 132.8, 133.2, 137.5, 141.2, 163.8, 172.1; MS *m/z* 357.12 (M+1); Anal. calcd. for $C_{17}H_{12}N_3O_2SCl$: C, 65.79; H, 4.55; N, 9.03; Found C, 65.76; H, 4.55; N, 9.05.

*2-(4-Hydroxyphenyl)-3-((2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one (**4c**)*

IR (KBr ν_{max} in cm^{-1}): 3495 (N–H stretch), 3261 (–OH stretch), 3060 (C–H stretch Ar), 1712 (C=O stretch), 724 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 4.23 (s, 2H, $-CH_2$ of thiazolidinone ring); 5.38 (s 1H –OH); 5.87 (s, 1H, –N–CH–Ar); 7.8–7.7 (m, 8H, $J = 1.25$ Hz, Ar–H); 8.2 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.9, 63.4, 115.8, 115.2, 117.7, 119.6, 124.6, 129.5, 130.2, 130.4, 131.0, 132.8, 133.2, 141.2, 156.9, 163.7, 172.1; MS *m/z* 339.01 (M+1); Anal. calcd. for $C_{17}H_{13}N_3O_3S$: C, 60.17; H, 3.86; N, 12.38; Found C, 60.25; H, 3.83; N, 12.40.

*2-(4-Methoxyphenyl)-3-((2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one (**4d**)*

IR (KBr ν_{max} in cm^{-1}): 3320 (N–H stretch), 3110 (C–H stretch Ar), 1672 (C=O stretch), 1242 ($-OCH_3$ stretch), 722 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.23 (s, 3H of $-OCH_3$); 4.23 (s, 2H, $-CH_2$ of thiazolidinone ring); 5.97 (s, 1H, –N–CH–Ar); 6.8–7.5 (m, 9H, $J = 1.25$ Hz, Ar–H); 8.2 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.8, 55.3, 63.4, 114.2, 114.6, 117.3, 119.6, 124.6, 128.5, 130.2, 130.4, 131.0, 132.8, 133.2, 141.3, 159.0, 163.8, 172.4; MS *m/z* 353.2 (M+1); Anal. calcd. for $C_{18}H_{15}N_3O_3S$: C, 61.18; H, 4.28; N, 11.89; Found C, 61.17; H, 4.25; N, 11.86.

*2-(3,4-Dimethoxyphenyl)-3-((2-oxoindolin-3-ylidene)-amino)-thiazolidin-4-one (**4e**)*

IR (KBr ν_{max} in cm^{-1}): 3360 (N–H stretch), 3045 (C–H stretch Ar), 2875 ($-CH_3$ stretch), 1690 (C=O stretch), 1242 ($-OCH_3$ stretch), 743 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.98 (s, 6H of $-OCH_3$); 4.01 (s, 2H, $-CH_2$ of thiazolidinone ring); 5.95 (s, 1H, –N–CH–Ar); 6.9–7.4 (m, 7H, $J = 1.25$ Hz, Ar–H); 8.0 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.9, 63.9, 56.1, 56.3, 112.0, 113.5, 117.5, 119.5, 122.2, 124.2, 129.8, 131.2, 132.9, 133.6, 148.3, 149.2, 141.7, 168.2, 172.8; MS *m/z* 383.2 (M+1); Anal. calcd. for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96; Found C, 59.55; H, 4.46; N, 10.93.

*2-(2,4-Dimethoxyphenyl)-3-((2-oxoindolin-3-ylidene)-amino)-thiazolidin-4-one (**4f**)*
IR (KBr ν_{max} in cm^{-1}): 3415 (N–H stretch), 3057 (C–H stretch Ar), 2890 (–CH_3 stretch), 1252 (–OCH_3 stretch), 1720 (C=O stretch), 718 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.38 (s,6H OF –OCH_3); 4.23 (s, 2H, –CH_2 of thiazolidinone ring); 5.97 (s, 1H, –N–CH–Ar); 7.2–7.9 (m, 7H, $J = 1.25$ Hz, Ar–H); 8.02 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 34.9, 52.9, 55.1, 56.8, 100.2, 106.8, 108.2, 116.5, 119.6, 124.2, 129.7, 159.2, 160.3, 130.5, 131.8, 133.7, 141.2, 168.2, 171.3; MS *m/z* 383.1 (M+1); Anal. calcd. for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96; Found C, 59.54; H, 4.48; N, 10.94.

*3-((2-Oxoindolin-3-ylidene)amino)-2-(p-tolyl)-thiazolidin-4-one (**4g**)*
IR (KBr ν_{max} in cm^{-1}): 3231 (N–H stretch), 3010 (C–H stretch Ar), 2895 (–CH_3 stretch), 1692 (C=O stretch), 752 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 2.40 (s 3H of –CH_3) 4.02 (s, 2H, –CH_2 of thiazolidinone ring); 5.98 (s, 1H, –N–CH–Ar); 6.8–7.8 (m, 8H, $J = 1.25$ Hz, Ar–H); 8.15 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 21.3, 35.1, 63.7, 117.7, 119.4, 124.2, 127.9, 128.6, 128.8, 128.9, 129.4, 131.1, 133.0, 136.7, 136.6, 141.4, 168.8, 172.2. MS *m/z* 337.2 (M+1); Anal. calcd. for $C_{18}H_{15}N_3O_2S$: C, 64.08; H, 4.48; N, 9.03; Found C, 64.12; H, 4.45; N, 9.00.

*2-(4-Hydroxy-3-methoxyphenyl)-3-((2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one (**4h**)*
IR (KBr ν_{max} in cm^{-1}): 3419 (N–H stretch), 3209 (OH stretch), 3052 (C–H stretch Ar), 2880 (–CH_3 stretch), 1250 (OCH_3 stretch), 1690 (C=O stretch), 716 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.23 (s 3H OF –OCH_3); 4.30 (s, 2H, –CH_2 of thiazolidinone ring); 5.39 (s,1H –OH); 5.97 (s, 1H, –N–CH–Ar); 6.9–7.5 (m, 7H, $J = 1.25$ Hz, Ar–H); 8.2 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.9, 63.8, 56.1, 114.2, 115.6, 117.5, 119.4, 122.2, 124.3, 129.3, 131.5, 132.8, 133.2, 141.3, 147.1, 147.7, 168.9, 170.1. MS *m/z* 369.3 (M+1); Anal. calcd. for $C_{18}H_{15}N_3O_4S$: C, 58.53; H, 4.09; N, 11.38; Found C, 58.55; H, 4.40; N,11.36.

*2-(3-Hydroxy-4-methoxyphenyl)-3-((2-oxoindolin-3-ylidene)amino)-thiazolidin-4-one (**4i**)*
IR (KBr ν_{max} in cm^{-1}): 3300 (N–H stretch), 3202 (OH stretch), 3085 (C–H stretch Ar), 2890 (–CH_3 stretch), 1687 (C=O stretch), 1225 (OCH_3 stretch), 722 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.34 (s 3H of –OCH_3); 4.23 (s, 2H, –CH_2 of thiazolidinone ring); 4.79 (s,1H of –OH); 5.87 (s, 1H, –N–CH–Ar); 7.3–7.7 (m, 7H, $J = 1.25$ Hz, Ar–H); 8.2 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.7, 56.2, 63.8, 112.7, 115.4, 116.2, 119.5, 122.3, 124.4, 129.4, 133.1, 131.2, 132.9, 147.1, 149.5, 141.2, 168.5, 172.1. MS *m/z* 369.2 (M+1); Anal. calcd. for $C_{18}H_{15}N_3O_4S$: C, 58.53; H, 4.09; N, 11.38; Found C, 58.57; H, 4.06; N, 11.32.

*2-(2,3-Dimethoxyphenyl)-3-((2-oxoindolin-3-ylidene)-amino)-thiazolidin-4-one (**4j**)*
IR (KBr ν_{max} in cm^{-1}): 3350 (N–H stretch), 3040 (C–H stretch Ar), 2885 (–CH_3 stretch), 1730 (C=O stretch), 1233 (OCH_3 stretch), 770 (C–S stretching); ^{1}H NMR (DMSO-d_6, 400 MHz) δ ppm: 3.34 (s,6H of –OCH_3); 4.02 (s, 2H, –CH_2 of thiazolidinone ring); 5.12 (s, 1H, –N–CH–Ar); 6.8–7.8 (m, 7H, $J = 1.25$ Hz, Ar–H); 8.00 (s, 1H, $J = 7.5$ Hz, –NH D_2O exchangeable); ^{13}C NMR (DMSO-d_2) δ ppm: 35.6, 57.9, 55.8, 60.3, 113.7, 117.5, 117.9, 119.8, 121.4, 124.5, 129.4, 131.5, 133.2, 145.6, 150.2, 141.2, 168.8, 172.9. MS *m/z* 383.12 (M+1); Anal. calcd. for $C_{19}H_{17}N_3O_4S$: C, 59.52; H, 4.47; N, 10.96; Found C, 59.56; H, 4.45; N, 10.91.

Pharmacology

Male Swiss Albino mice (CF-1 strain, 20–30 g) were used as experimental animals. Animals divided into each group of five animals were housed and allowed free access to water and food. A 12:12 h light/dark cycle was maintained throughout the experimental studies. All the tested compounds were administered in suspension made up to 0.5% Tween 80 in 0.9% sodium chloride solutions and are used for MES and sc-PTZ study and 30% PEG for behavioral activity. Pentylenetetrazole (80 mg/kg) dissolved in 0.9% sodium chloride solution was administered in the posterior midline of the mice and the onset and severity of convulsions were noted for control group. The test group was administered with the selected compounds 0.5 h prior to the administration of PTZ. The statistical analyses were performed by one-way ANOVA followed by Student's *t*-test to evaluate the results using GraphPad Prism software. All the values were expressed as mean ± SEM. All the tests were performed in accordance with guidelines laid out by the Institutional Animal Ethics Committee [25]. The approval of the Institutional Animal Ethics Committee (CPCSEA number CPCSEA/IAEC/Pharm. Chem/12/2011-12/54) of Y.B. Chavan college of Pharmacy, Aurangabad, Maharashtra, India, was taken prior to the start of the experiments.

Anticonvulsant effects in the maximal electroshock seizure (MES) test [26, 27]
All the test compounds and the standard drug phenytoin was injected intraperitonially in a volume of 0.01 mL/g at a dose of 30 and 100 mg/kg. Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. At 30 min after the administration of the compounds, the activities were evaluated in MES test. The animals were observed closely for 5 min. The disappearance of the hind limb extensor tonic convulsion was used as positive criterion. The results of test compounds were compared with the standard drug.

Sc-PTZ-induced seizures [28, 29]
All the test compounds and the standard drug phenytoin was injected intraperitonially in a volume of 0.01 mL/g at a dose 30 and 100 mg/kg and at 30 min after the administration of the test compounds, 85 mg/kg PTZ dissolved in saline was administered subcutaneously. The animals were placed in individual cages and observed for 30 min. The number of

clonic and tonic seizures as well as the number of deaths was noted. The delay onset of convulsion was referred to be anticonvulsant response. The results of test compounds were compared with the standard drug.

Behavioral screening

CNS depressant activity

The activity was measured on digital actophotometer with the oral administration of drug in a volume 0.2 mL at dose of 30 and 100 mg/kg to mice. The standard drug diazepam was injected at dose 4 mg/kg. The activity was assessed after 1 and 4 h of drug administration. The mice were placed in the box and behaviour was noted for 10 min and the basal activity scores for all the animals were recorded. The results of test compounds were compared with the standard drug.

Muscle relaxant activity

This activity was measured on rotarod. The treated mice were placed on rotarod at intervals and the time of fall from the rod was noted. A group of five mice was used for one compound and average reading was taken. The activity score was noted and based on these result percentage decrease in fall time was calculated [30].

Evaluation of histopathology of mice liver (T.S. of liver)

The synthesized compounds were evaluated for liver toxicity upon Swiss Albino mice with reference to standard phenytoin, 300 mg/kg. The synthesized compounds of 4-thiazolidinone derivatives **4e** and **4i** at 300 mg/kg were administered intraperitoneally to animal. The mice liver was removed after scarification of mice followed by the drug administration. The liver was washed with Kreb's solution and stored in 10% formalin; the specimen was subjected to microscopic visualization; it was found that the liver of the animal treated with standard resulted in hepatotoxicity. The liver treated with standard has shown hepatotoxicity while the liver of animals treated with synthesized compounds resulted in lesser inflammation and has shown lesser hepatotoxicity [31].

Computational parameters

Distance mapping

In the present study, four well-known and structurally different compounds with anticonvulsant activity, i.e., phenytoin, carbamazepine, lamotrigine, and diazepam were selected. Our analysis of the distance relationship showed that the title compounds fulfill the essential demands of pharmacophore when compared with other standard anticonvulsants. A molecular model was suggested on the basis of molecular dynamics distance estimation with the help of ACD/chem. sketch/3-D viewer 8.04 version program [32].

Log P determination

The partition coefficient between octanol and water buffer was determined at room temperature using a reported procedure [33]. A total of 10 mL of octanol and 10 mL of phosphate buffer were taken in glass stoppered graduated tube and 5 mg of accurately weight test drug was added. The mixture was then shaken with mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to equilibrate for 6 h. The aqueous and octanol phase were separated and the phases were filtered though membrane filter and drug content in aqueous phase was analysed by UV spectroscopy. Partition coefficient [34] was calculated by using the formula PC = (Ct − Ca)/Ca. PC = partition coefficient Ct = concentration of total drug. Ca = concentration of drug in aqueous phase.

Molecular modeling

The molecular docking tool, GLIDE (Schrödinger Inc., USA; 2006) was used for ligand docking studies into the NMDA and AMPA receptor binding pocket. The crystal structure of these receptors was obtained from a protein data bank (PDB ID: 1PBQ and 1FTL) [35, 36]. The protein preparation was carried out using "protein preparation wizard" in Maestro 9.0 in two steps: preparation and refinement. After ensuring chemical correctness, water molecules in the crystal structures were deleted, and hydrogens were added, where they were missing. Using the OPLS 2005, force field energy of crystal structure was minimized [37]. Grids were defined centering them on the ligand in the crystal structure using the default box size. The ligands were built using Maestro build panel and prepared by Ligprep 2.2 module, which produce the low-energy conformer of ligand using OPLS 2005 force field.

The authors are grateful to Mrs. Fatma Rafiq Zakaria, Chairman, Maulana Azad Education Trust and Principal for encouragement and support. The authors are thankful to Dr. S.J. Surana, Principal, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, for providing necessary facility for in silico study.

The authors have declared no conflict of interest.

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