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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Docking, synthesis, and pharmacological evaluation of isoindoline derivatives as anticonvulsant agents

Asghar Davood · Mohsen Amini · Leila Azimidoost · Somaieh Rahmatpour · Ali Nikbakht · Maryam Iman · Hamed Shafaroodi · Abdollah Ansari

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Abstract Eleven analogs of *N*-arylisoindoline pharmacophore were synthesized and evaluated for their anticonvulsant activities. The in vivo screening data acquired indicate that all the analogs have the ability to protect against pentylenetetrazole-induced seizure. Compounds **2**, **6**, and **11** elevated clonic seizure thresholds at 30 min which were more active than reference drug phenytoin, and compounds **2**, **7,** and **11** showed marked anticonvulsant activity on tonic seizure. The most potent compounds were **2** and **11** which had comparative activity to the phenytoin. Using a model of the open pore of the Na channel, we have docked all compounds. Docking studies have revealed that these compounds interacted mainly with residues II-S6 of NaV1.2 by making hydrogen bonds and have additional hydrophobic interactions with other domains in the channel's inner pore.

Keywords Anticonvulsant · Docking · Isoindoline · Na channel · Seizure

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Introduction

Epilepsy is a brain disorder involving repeated, spontaneous seizures of any type. Seizures are the physical findings or changes in behavior that occur after an episode of abnormal electrical activity in the brain (Chang and Lowenstein, 2003). Epilepsy affecting 0.5–1 % of the population worldwide, and every year new cases are added to this figure (Loscher, 1998; Leppik, 2008; Chen *et al.*, 2007). Antiepileptic drugs (AEDs) are used to reduce or prevent the occurrence of epileptic seizures, but up to 30 % of patients are resistant to the available medical therapies (Kubota and Sakakihora, 2000; French, 1999) and treatment of epilepsy still remains inadequate and patients suffer from a number of unfavorable side effects. Some types of seizures are still not adequately treated with AEDs (Ilangovan *et al.*, 2010; Malawska, 2005); phenytoin, carbamazepine, oxcarbazepine, and lamotrigine are effective in the control of partial and generalized tonic–clonic seizures and are ineffective in the treatment of generalized absence seizures (Rogawski, 2002). Therefore, in response to these limitations, the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.

Studies with mixtures of phenytoin, carbamazepine, and lamotrigine have revealed that these drugs bind to a common recognition site on sodium channels (Kuo, 1998). Although these three compounds are structurally dissimilar, they do contain a common motif of two phenyl groups separated by one to two C–C or C–N single bonds (1.5–3 A) (Fig. 1). These two phenyl groups probably are critical elements in binding (Rogawski, 2002). The proposed binding site is domain IV-S6 in the channel's inner pore (Lipkind and Fozzard, 2010, 2005). Docking studies of these drugs revealed that they show a common pharmacophore, including an aromatic ring that has an aromatic–aromatic

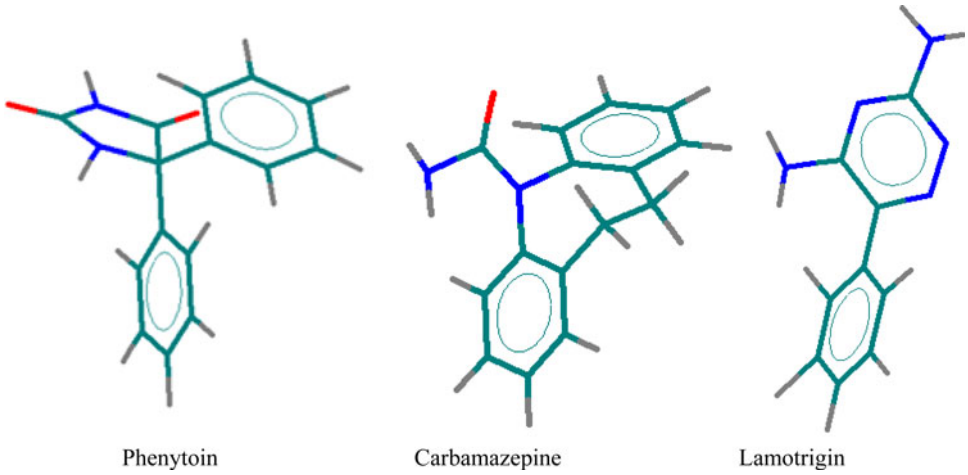


Fig. 1 Structure of antiepileptic drugs phenytoin, carbamazepine, and lamotrigine

interaction with Tyr-1771 of NaV1.2 and a polar amide or imides that interacts with the aromatic ring of Phe-1764 by a low energy amino-aromatic hydrogen bond. The second aromatic ring is almost at the right angle to the pharmacophore and fills the pore lumen, probably interacting with the other S6 segments and physically occluding the inner pore to block the Na permeation. The hydrophobic interactions with this second aromatic ring maybe an important contribution to the binding abilities of these anticonvulsants (Lipkind and Fozzard, 2010, 2005).

Recently, *N*-phenylisoindoline derivatives were designed by molecular hybridization approach based on ameltolide and thalidomide (Fig. 2) as they possess a similar degree of anticonvulsant potency due to their phenytoin-like profile (Vamecq *et al*., 2000, 1998; Davood *et al*., 2012). The ability of isoindolines to interact with the neuronal voltage-dependent sodium channels was studied in the batrachotoxin affinity assay (Vamecq *et al*., 2000). The presence of hydrophobic interactions of second aromatic ring (Chen *et al*., 2007) and a polar amide in the middle mainly contributes to the binding abilities of anticonvulsants and known properties of isoindoline derivatives (Vamecq *et al*., 2000, 1998; Davood *et al*., 2012). Therefore, we explored this idea further based on SAR studies of *N*-arylisoindoline and their drug–receptor interaction profiles (Davood *et al*., 2012). Ultimately, the new series of *N*-arylisoindoline derivatives were docked and synthesized to investigate the pharmacological activities.

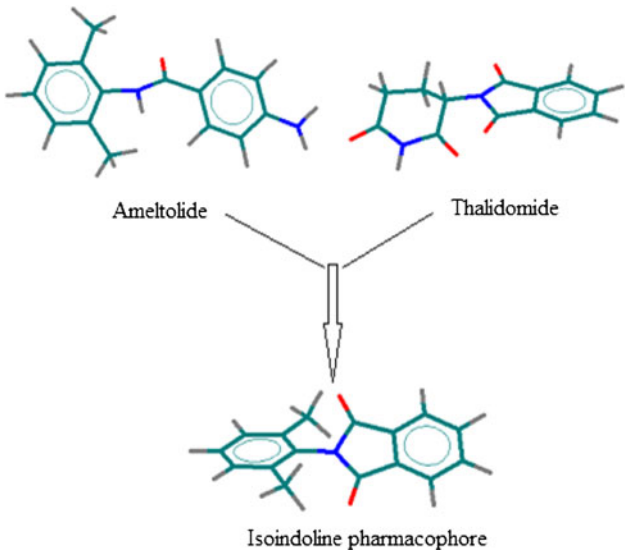


Fig. 2 Rational design of isoindoline pharmacophore by molecular hybridization approach

Materials and methods

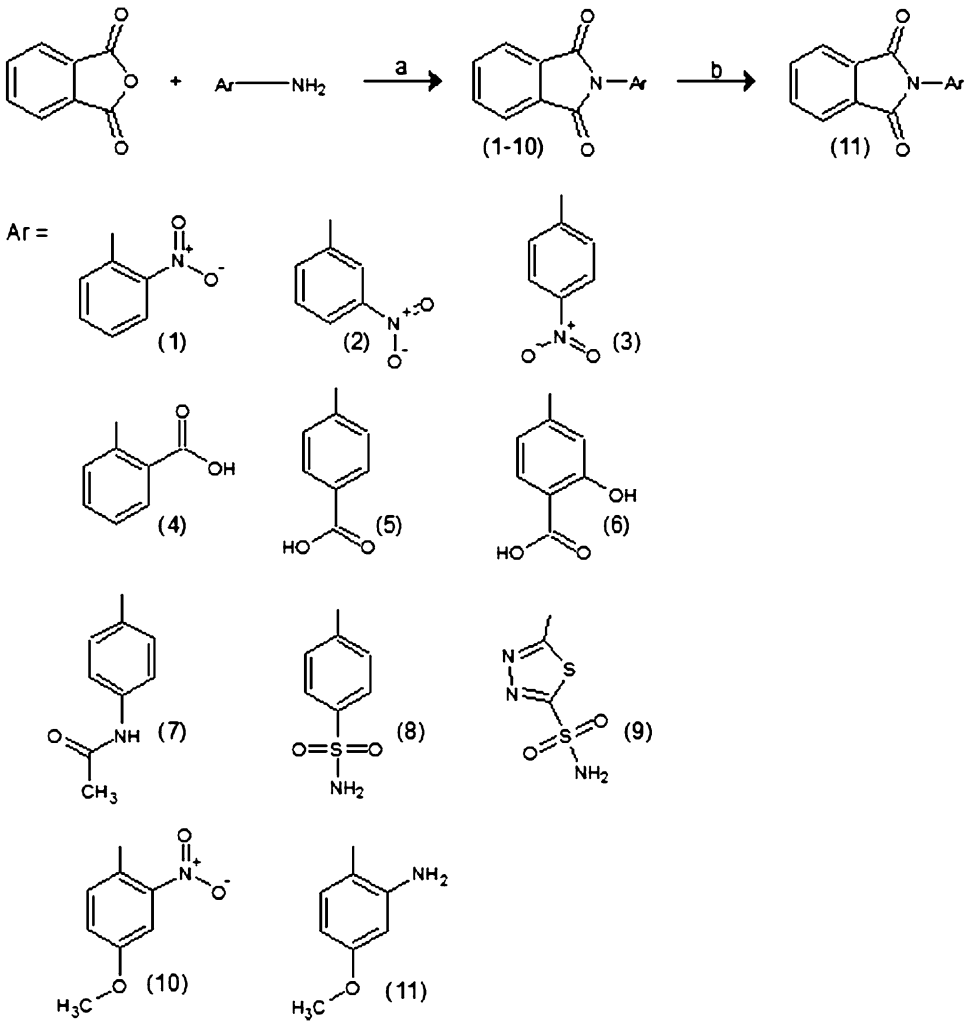
Chemistry

A group of *N*-aryl derivatives of the isoindoline (**1**–**11**), possessing a variety of substituent at the 2′-, 3′-, 4′-, and 5′-positions of the isoindole ring, were synthesized by condensation of the respective aromatic amine (homocycle or heterocycle) with phthalic anhydride in acetic acid at reflux temperature (Vamecq *et al*., 2000; Davood *et al*., 2012) (Scheme 1). Compound **10** was reduced to compound **11** using Pd/C and cyclohexene in 2-propanol at reflux temperature.

Reagents and solvents were obtained from Merck (Darmstadt, Germany). Pentylenetetrazole (PTZ) was purchased from Sigma (UK).

Spectroscopy and analytical procedures

Melting points were determined using a Thomas-Hoover capillary apparatus and were uncorrected. ^{1}HNMR spectra were recorded on a Bruker FT-500 spectrometer and TMS was used as an internal standard. Infrared spectra were acquired on a Nicolet 550-FT spectrometer. Elemental analysis was



Scheme 1 Reagents and conditions: (*a*) acetic acid glacial, reflux (*b*) 2-propanol, cyclohexene, Pd/C, reflux

carried out using a Perkin-Elmer model 240-C apparatus. The results of elemental analysis (C, H, and N) were within 0.4 % of the calculated amounts. Molecular modeling studies were carried out by HYPERCHEM and Autodock 4.2.3.

General procedure for the preparation isoindoline derivatives (**1**–**11**)

A solution of phthalic anhydride (0.5 g, 3.73 mmol) and arylamine (3.73 mmol) in glacial acetic acid (5 mL) was stirred and heated under reflux. The product of this reaction was precipitated by addition of water, filtered, dried, and recrystallized to give desired compounds.

*2-(2-Nitrophenyl) isoindoline-1,3-dione (**1**)*

By means of the general procedure, 2-nitroaniline provided the title compound after 19 h of reflux: yellow crystals, yield 56 %; mp 204–207.5 °C (ethanol). IR (KBr): ν cm^{-1}, 3098 (CH-aromatic), 1778, 1726 (CO), 1521, 1388 (NO_2); ^{1}H NMR ($CDCl_3$): δ 8.13 (d, J = 8.43 Hz, 1H, H_3-phenyl), 7.57–7.91 ppm (m, 7H, aromatic); Mass *m/z* (rel.int): 268 (M^+, 8), 220 (100), 164 (21), 103 (21), 75 (43); Anal. Calcd. for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44. Found: C, 62.60; H, 3.02; N, 10.41.

*2-(3-Nitrophenyl) isoindoline-1,3-dione (**2**)*

By means of the general procedure, 3-nitroaniline provided the title compound after 20 h of reflux: White crystals, yield 88.5 %; mp 174–176 °C (ethanol). IR (KBr): ν cm^{-1}, 3093 (CH-aromatic), 1721, 1735 (CO), 1526, 1378 (NO_2); ^{1}H NMR ($CDCl_3$): δ 8.79 (d, J = 2.46 Hz, 1H, H_2-phenyl), 8.62 (dd, J = 8.15, 2.47 Hz, 1H, H_4-phenyl), 7.75–8.04 (m, 5H, aromatic), 7.43 ppm (dd, J = 8.16, 4.98 Hz, 1H, H_5-phenyl); Mass *m/z* (rel.int): 268 (M^+, 6), 222 (50), 179 (44), 153 (7), 103 (35), 75 (100), Anal. Calcd. for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44. Found: C, 62.74; H, 3.02; N, 10.46.

*2-(4-Nitrophenyl) isoindoline-1,3-dione (**3**)*

Preparation of this compound has been reported already (Vamecq *et al*., 2000). By means of the general procedure, 3-nitroaniline provided the title compound after 10 h of reflux: yellow crystals, yield 81.5 %; mp 262–264 °C (ethanol). IR (KBr): ν cm^{-1}, 3119 (CH-aromatic), 1780, 1731 (CO), 1521, 1347 (NO_2); ^{1}H NMR ($CDCl_3$): δ 8.35(d, $J = 9.18$ Hz, 2H, $H_{3,5}$-phenyl), 7.71–7.94(m, 6H, aromatic); Mass *m/z* (rel.int): 268 (M^+, 43), 237 (21), 165 (14), 103 (36), 74 (100), Anal. Calcd. for $C_{14}H_8N_2O_4$: C, 62.69; H, 3.01; N, 10.44. Found: C, 62.57; H, 3.00; N, 10.42.

*2-(1, 3-Dioxoisoindolin-2-yl) benzoic acid (**4**)*

By means of the general procedure, 2-aminobenzoic acid provided the title compound after 8 h of reflux: White crystals, yield 61 %; mp 217–221 °C (chloroform). IR (KBr): ν cm^{-1}, 3200 (OH), 3083 (CH-aromatic), 1721, 1698 (CO); ^{1}H NMR (DMSO-*d*6): δ 8.16 (dd, $J = 6.60$, 2.30 Hz, 1H, H_3-phenyl)), 7.41–7.95 ppm (m, 7H, aromatic); Mass *m/z* (rel.int): 268 (M^+ +1, 18), 266(M^+- 1, 100), 221 (100), 195 (50), 177 (100), 102 (48), 139 (28), 104 (100), 74 (100), 49 (100); Anal. Calcd. for $C_{15}H_9NO_4$: C, 67.42; H, 3.39; N, 5.24. Found: C, 67.51; H, 3.40; N, 5.23.

*4-(1,3-Dioxoisoindolin-2-yl) benzoic acid (**5**)*

By means of the general procedure, 4-aminobenzoic acid provided the title compound after 17 h of reflux: White crystals, yield 80 %; mp 282–289.5 °C (ethanol). IR (KBr): ν cm^{-1}, 3225–3310 (OH), 3067 (CH-aromatic), 1726, 1695 (CO); ^{1}H NMR (DMSO-*d*6): δ 8.42–9.51 (brs, 1H, COOH), 8.17 (d, $J = 8.58$ Hz, 2H, $H_{3,5}$-phenyl), 7.75–8.03 (m, 4H, aromatic), 7.56 ppm (d, $J = 8.58$ Hz, 2H, $H_{2,6}$-phenyl);. Anal. Calcd. for $C_{15}H_9NO_4$: C, 67.42; H, 3.39; N, 5.24. Found: C, 67.55; H, 3.40; N, 5.25.

*2-Hydroxy-4-(1,3-dioxoisoindolin-2-yl) benzoic acid (**6**)*

By means of the general procedure, 4-amino-2-hydroxybenzoic acid provided the title compound after 2 h of reflux: White crystals, yield 40 %; mp 293–295 °C (methanol–chloroform). IR (KBr): ν cm^{-1} 2750–3700 (OH), 1716, 1676 (CO); ^{1}HNMR (DMSO-*d*6): δ 11.25–1155 (brs, 1H, COOH), 7.75–8.12 (m, 5H, aromatic), 7.06–7.39 ppm (m, 2H, $H_{2,6}$-phenyl); Mass *m/z* (rel.int): 283 (M^+, 25), 265 (60), 237 (19), 192(10), 132 (31), 103 (65), 76 (72), 75 (100).; Anal. Calcd. for $C_{15}H_9NO_5$: C, 63.61; H, 3.20; N, 4.95. Found: C, 63.70; H, 3.21; N, 4.96.

*N-(4-(1,3-Dioxoisoindolin-2-yl) phenyl) acetamide (**7**)*

By means of the general procedure, *N*-(4-aminophenyl) acetamide provided the title compound after 20 h of reflux: violet crystals, yield 85 %; mp 228–231 °C (methanol). IR (KBr): ν cm^{-1}, 3318 (NH), 3020 (CH-aromatic), 2919 (CH-aliphatic), 1787, 1710, 1675 (CO); ^{1}HNMR (DMSO-*d*6): δ 9.87(br, 1H, NH), 7.79–7.89(m, 4H, aromatic), 7.70(d, $J = 8.78$ Hz, 2H, $H_{3,5}$-phenyl), 7.28 (d, $J = 8.9$ Hz, 2H, $H_{2,6}$-phenyl), 2.12 ppm (s, 3H, CH_3); Mass *m/z* (rel.int): 280 (M^+, 22), 238 (29), 236 (43), 177 (14), 130 (6), 103 (50), 75 (100); Anal. Calcd. for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.49; H, 4.33; N, 9.97.

*4-(1,3-Dioxoisoindolin-2-yl) benzene sulfonamide (**8**)*

By means of the general procedure, 4-aminobenzenesulfonamide provided the title compound after 55 h of reflux: yellow crystals, yield 89 %; mp 185–187 °C (methanol). IR (KBr): ν cm^{-1}, 3365, 3257 (NH_2), 1721, 1680 (CO), 1301, 1157(SO_2); ^{1}H NMR (DMSO-*d*6): δ 7.96–8.09 (m, 6H, aromatic), 7.68 (d, $J = 8.27$ Hz, 2H, $H_{2,6}$-phenyl), 7.28 ppm (s, 2H, NH_2); Mass *m/z* (rel.int): 302(M^+, 29), 301 (M^+-1, 36), 237 (14), 222 (78), 166 (21), 103 (50), 75 (100); Anal. Calcd. for $C_{14}H_{10}N_2O_4S$: C, 55.62; H, 3.33; N, 9.27. Found: C, 55.72; H, 3.34; N, 9.25.

*5-(1,3-Dioxoisoindolin-2-yl)-1,3,4-thiadiazole-2-sulfonamide (**9**)*

By means of the general procedure, 5-amino-1,3,4-thiadiazole-2-sulfonamide provided the title compound after 5 h of reflux: White crystals, yield 47 %; mp 264–274 °C (ethanol). IR (KBr): ν cm^{-1}, 3329, 3180 (NH_2), 3078 (CH-aromatic), 1792, 1736 (CO); ^{1}H NMR (DMSO-*d*6): δ 7.90–8.02(m, 4H, aromatic); Mass *m/z* (rel.int): 310 (M^+, 14), 308 (30), 244 (25), 188 (9), 170 (33), 156 (10), 102 (58), 74 (100); Anal. Calcd. for $C_{10}H_6N_4O_4S_2$: C, 38.71; H, 1.95; N, 18.06. Found: C, 38.82; H, 1.94; N, 18.04.

*2-(4-Methoxy-2-nitrophenyl) isoindoline-1,3-dione (**10**)*

By means of the general procedure, 4-methoxy-2-nitroaniline provided the title compound after 7 h of reflux: yellow crystals, yield 62.5 %; mp 148–153 °C (ethanol). IR (KBr): ν cm^{-1}, 3080 (CH-aromatic), 2938 (CH-aliphatic), 1781, 1723 (CO), 1540, 1387(NO_2); ^{1}H NMR ($CDCl_3$): δ 7.69–7.93 (m, 4H, aromatic), 7.60 (d, $J = 2.56$ Hz, 1H, H_3-phenyl), 7.24(d, $J = 8.5$ Hz, 1H, H_6-phenyl), 7.16 (dd, $J = 8.70$, 2.6 Hz, 1H, H_5-phenyl), 3.83 ppm (s, 3H, OCH_3); Mass *m/z* (rel.int): 298 (M^+, 38), 252 (100), 209

(13), 153 (10); Anal. Calcd. for $C_{15}H_{10}N_2O_5$: C, 60.41; H, 3.38; N, 9.39. Found: C, 60.52; H, 3.39; N, 9.38.

*2-(2-Amino-4-methoxyphenyl) isoindoline-1,3-dione (**11**)*

2-(4-Methoxy-2-nitrophenyl) isoindoline-1,3-dione (**10**) (2.7 g, 5.7 mmol) was dissolved in 2-propanol (30 ml) and then 10 % palladium on charcoal (500 mg) and cyclohexene (150 ml) were added. The mixture was heated under reflux for 20 h and then filtered. The solvent was evaporated in vacuo, and the residue was recrystallized from an ethanol 96° to give the desired compound as a yellow crystal. (0.65 g, 43.3 % yield). mp 188–190 °C. IR (KBr): ν cm^{-1}, 3441, 3365(NH_2), 1716, 1695 (CO); ^1H NMR ($CDCl_3$) δ 7.72–7.98 (m, 4H, aromatic), 7.00(d, $J = 8.88$ Hz, 1H, H_6-phenyl), 6.43 (d, $J = 8.80$ Hz, 1H, H_5-phenyl), 6.38 (s, 1H, H_3-phenyl), 3.78 ppm (s, 3H, OCH_3); Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.16; H, 4.51; N, 10.44. Found: C, 67.30; H, 4.52; N, 10.46.

Molecular modeling and docking

Conformational analysis of the phenytoin and compound **8** was performed by Semi-empirical molecular orbital calculations (PM3) method by means of the HYPERCHEM software. Among all energy minima conformers, the global minimum was used in docking calculations.

Using a model of the open pore of the Na channel that has been developed by homology with the crystal structures of K channels (Lipkind and Fozzard, 2005), we have docked compounds **1**–**11** and phenytoin as a reference drug. Docking calculations were performed by means of Autodock software (version 4.2.3) and the implemented Lamarckin GL. The docking log (dlg) files were analyzed by the AutoDock Tools, graphical user interface of Autodock. The docked conformations of each ligand were ranked into clusters based on the binding energy. The top ranked conformations were visually analyzed.

Pharmacology and determination of anticonvulsant activity

Pharmacology

Male Swiss mice weighing 22–28 g (Pasteur Institute) were used throughout the study. Animals were housed in groups of 4–5 and were allowed free access to food and water except for the short time when animals were removed from their cages for testing. All behavioral experiments were conducted during the period between 10:00 and 13:00 with normal room light (12-h regular light/dark cycle) and temperature (22 ± 1 °C). All procedures were carried out in accordance with the institutional guidelines for animal care and use. Each mouse was used only once, and each treatment group consisted of at least eight animals.

Determination of clonic and tonic seizure thresholds

The infusion pump was adjusted to pump PTZ (0.5 %) with constant rate (1 ml/min) in all the experiments (NE 1000, New Era Pump System, Inc.). A 30-gauge butterfly needle allowing access to the tail vein of mice was connected to a pump by a flexible tube which made it possible to infuse PTZ (0.5 %) at a constant rate of 1 ml/min to unrestrained freely moving animals. Time was measured when forelimb clonus (clonic seizure) followed by forelimb extension (tonic seizure) were observed and the doses of PTZ administered (mg/kg of mice weight) were measured as an index of clonic and tonic seizure threshold (Loscher and Rundfeldt, 1991).

Results and discussion

Chemistry

Eleven derivatives of *N*-arylisoindoline analogs were synthesized in 40–89 % yield based on the method that is shown in Scheme 1. All the compounds were characterized by TLC followed by IR, Mass, elemental analysis, and proton NMR.

Molecular modeling and docking

Flexible docking was carried out on the active site of the open pore of the Na channel. The binding energies, hydrogen bonding, and Ki of all the compounds studied have been tabulated (Table 1). Lowest energy and maximum number of conformations per cluster was set as the criteria to predict the binding modes of the compounds. Our docking studies reveal while phenytoin interacted with the domain IV-S6 of NaV1.2 (Davood *et al*., 2012) but compounds **1**–**11**, interacted mainly with the domain II-S6 (Fig. 3). There is a hydrogen binding and additional hydrophobic interaction with domain I and II in the channel's inner pore. Oxygen of oxygen-containing functional group like as carbonyl, nitro, or carboxyl groups play main role in making hydrogen binding interaction with the OH of THR87 and SER84 or NH3 of LYS7. In the compounds **5** and **6** which contain *p*-COOH, there is a hydrogen bonding interaction between oxygen of OH and NH_3 of LYS7. In the compounds **1**, **4**, **7**, **8**, **10**, and **11,** there is a hydrogen bond between oxygen of carbonyl of imide and OH of THR87 or SER84 (Fig. 4); in the compounds **2** and **9,** there is a hydrogen bond between oxygen of nitro and nitrogen of NH_2 with the OH of THR87 (Fig. 3).

Table 1 The ability of isoindoline derivatives (**1–11**) to the protection against PTZ-induced seizure

Comp.	CST (mg/kg)[a]	TST (mg/kg)[b]	Binding energy	Ki (μM)	Atoms in hydrogen bonds	logP[c]
1	46.34 ± 3.51	65.66 ± 5.87	−5.29	133.56	Sod:G:THR87 (OH) with O (keton)	2.63
2	64.89 ± 9.25	96.84 ± 9.91	−6.36	21.8	Sod:G:THR87 (OH) with O (NO_2)	2.63
3	41.12 ± 3.85	61.6 ± 5.76	−5.71	65.56	No H.B formed	2.63
4	42.95 ± 3.1	58.08 ± 4.39	−4.74	337.8	Sod:G:THR87 (OH) with O (keton)	2,37
5	46.65 ± 5.5	79.94 ± 1.31	−5.86	50.4	Sod:LYS7 (NH_3) with O (COOH)	2,37
6	52.1 ± 4.32	64.97 ± 8.46	−5.6	78.42	Sod:LYS7 (NH3) with O (COOH)	2.09
7	40.37 ± 1.39	86.37 ± 8.37	−6.46	18.27	Sod:G:THR87 (OH) with O (keton)	1.52
8	33.40 ± 2.85	60.56 ± 2.44	−6.54	16.01	Sod:G:THR87 (OH) with O (keton)	1.56
9	45.14 ± 1.55	62.35 ± 1.44	−5.75	61.07	Sod:G:THR87 (OH) with N (NH_2)	2.22
10	49.4 ± 7.31	61.92 ± 5.96	−5.41	108.07	Sod:G:THR87 (OH) with O (keton)	2.37
11	56.34 ± 4.3	98.12 ± 12.44	−6.4	20.3	Sod:G:SER84 (OH) with O (keton)	1.64
Phenytoin	49.20 ± 1.09	115.00 ± 1.85	−5.83	53.37	Sod:E:SER83:O:H	2.26
Vehicle	38.9 ± 1.01	59.58 ± 1.01				

[a] Colonic seizure threshold—it was calculated as mg of PTZ which induced clonic seizure/weight of mouse (kg)

[b] Tonic seizure threshold—it was calculated as mg of PTZ which induced clonic seizure/weight of mouse (kg)

[c] logP values have been calculated by HYPERCHEM software

Aryl part of isoindoline pharmacophore forms a hydrophobic interaction with the hydrophobic pocket of receptor that mainly created by domains I, II (Fig. 3).

Binding energy analysis of compounds **1–11** (Table 1) has led us to similar structures for their complexes with the two domain IIS6 residues in the open inner pore of the Na channel that are known from mutagenesis studies to be critical for their blocking action (Davood *et al.*, 2012). Based on the predicated binding energies (Table 1), some designed compounds, like compounds **7**, **8**, and **11**, should be more potent than phenytoin; however, the experimental data did not confirm this possibly due to low logP of these compounds.

Protection against PTZ-induced seizure

The ability of the compounds **1–11** to protect against PTZ-induced seizure, tonic and colonic, was determined by an in vivo assay, and the results are summarized in Table 1. Each compound was dissolved in DMSO, injected intraperitoneally, and screened for anticonvulsant activities at doses of 10, 20, and 40 mg/kg compared with phenytoin as a positive control. The single doses of all compounds (40 mg/kg) were administered 15, 30 or 60 min prior to distinct groups of mice.

Further analysis showed that most of the compounds and phenytoin exerted their maximal effects 30 min after administration. The in vivo screening data indicated that except compound **8,** all the analogs have the ability to protection against PTZ-induced seizure (Table 1).

Phenytoin as a standard did not inhibit clonic seizure evoked by PTZ in lower doses (10, 20 mg/kg). Compounds **2**, **6**, and **11** elevated the clonic seizure thresholds at 30 min which were more active than phenytoin as a reference drug, and compounds **2**, **7,** and **11** showed marked anticonvulsant activity on tonic seizure. The most potent compounds were **2** and **11** that had comparative activity to the reference drug phenytoin. In the nitro series, compounds **1**, **2,** and **3**, meta derivative (comp. **2**) is more potent than ortho (comp. **1**) and para (comp. **3**) derivatives, reactivity in nitro analogs is according to meta > ortho > para. In the carboxylic acid series (compounds **4–6**), para analog is more potent then ortho analog. Reduction of nitro (comp. **10**) to amine (comp. **11**) increases the clonic and tonic activity.

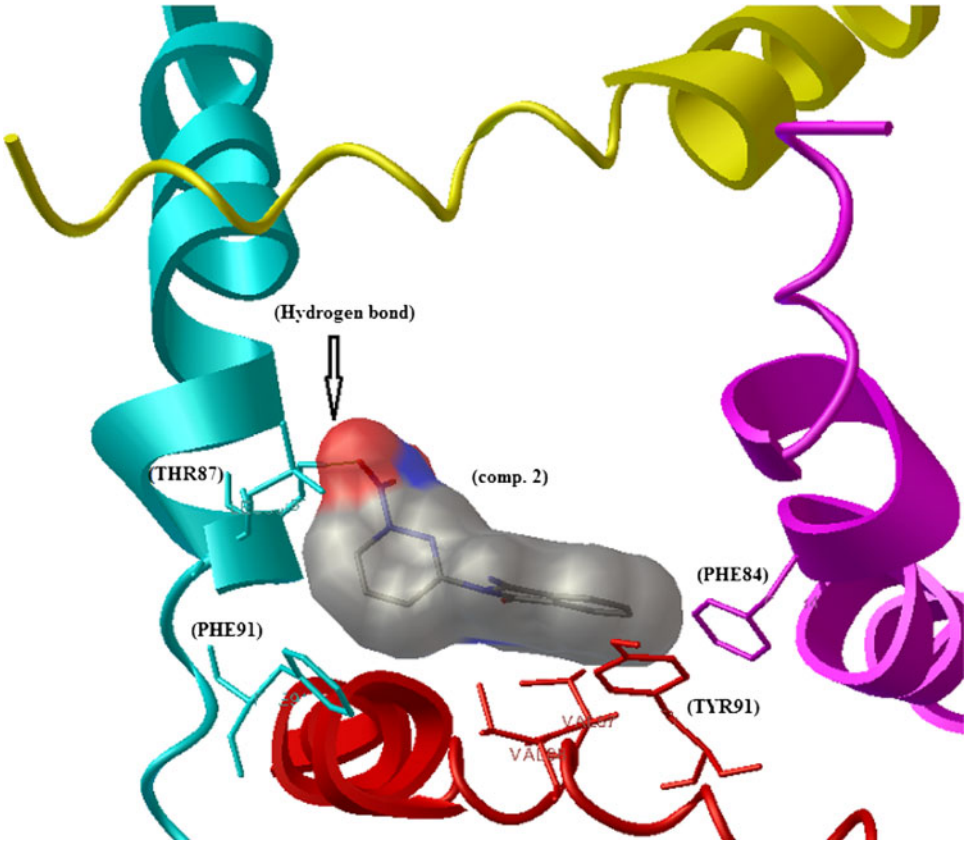


Fig. 3 Docked structure of **2** in the model of sodium channel (Nav1.2) (*top view*). The backbones of S6 α-helices of domains I–IV are shown by *red*, *green*, *yellow*, and *violet*, correspondingly. Hydrogen bond (distance 1.754 Å) is represented with *dashed green lines* (Color figure online)

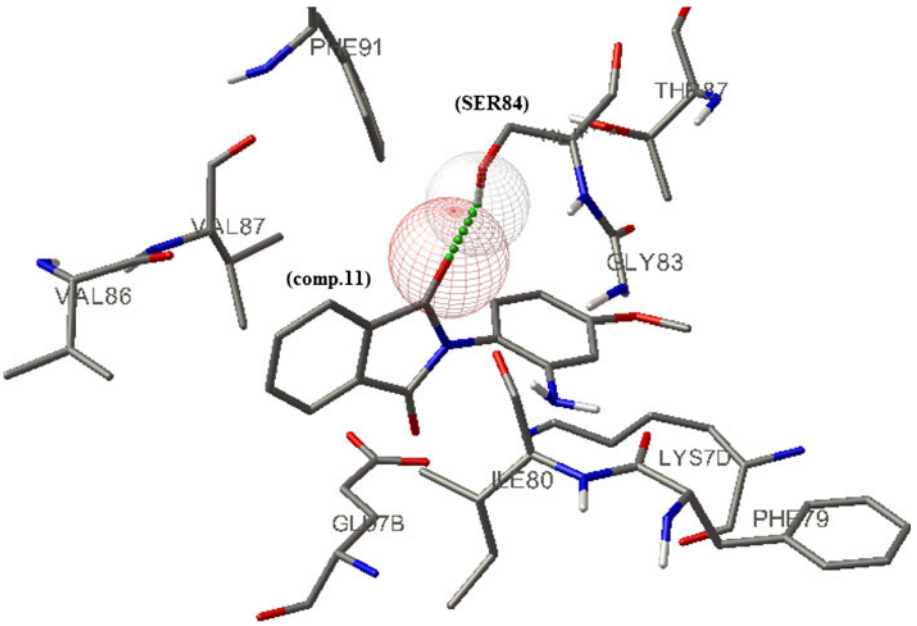


Fig. 4 Docked structure of 11 in the model of sodium channel (Nav1.2). Hydrogen bond (distance = 1.6 Å, energy = 1.724) is represented with *dashed green lines* (Color figure online)

Statistical analysis

The results are presented as mean ± SEM and the statistical significance between the groups was analyzed by means of variance followed by one-way ANOVA test. *P* values <0.05 were considered as indicative of significance.

Conclusion

Eleven analogs of *N*-arylisoindoline derivatives were synthesized, characterized by TLC followed by IR, Mass, elemental analysis, and proton NMR and tested for their ability to protection against PTZ-induced seizure in vivo in mice. The in vivo screening data acquired indicate that all

the analogs have the ability to protect against PTZ-induced seizure. These compounds exerted their maximal effects 30 min after administration. The most potent compounds were **2** and **11**.

Docking studies reveal that these compounds interacted mainly with the domain II-S6 of NaV1.2 by making a hydrogen bond and have additional hydrophobic interaction with domain I and II in the channel's inner pore where oxygen of oxygen-containing moieties plays a main role in creating hydrogen binding interaction with the residues THR87, SER84, and LYS7. Aryl parts of this pharmacophore form a hydrophobic interaction with the hydrophobic pocket of receptor which is mainly created by domains I and II.

For future studies, it is recommended that the isoindoline part should remain as it is and, to achieve a better potency, the *N*-aryl part should be replaced with other lipophilic aromatic moieties to improve the partition coefficients. Currently, our research group is exploring this idea for designing newer compounds with better anticonvulsant activities.

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