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Novel derivatives of phthalimide with potent anticonvulsant activity in PTZ and MES seizure models

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ABSTRACT

Objective(s): Phthalimide-based derivatives have anticonvulsant activity like as phenytoin by inhibition of sodium channel. In our previously research we mentioned about some phthalimide derivatives as potent anticonvulsant agents.

Materials and Methods: Fourteen analogs of 2-substituted phthalimide pharmacophore were synthesized and then were evaluated for the anticonvulsant activities in pentylenetetrazole-induced seizures (PTZ) and maximal electroshock seizure (MES) models.

Results: The *in vivo* screening results showed that all the analogs have the ability to protect against the maximal electroshock and PTZ. The compounds 3 and 9 elevated clonic seizure thresholds at 30 min which were more active than the standard medicine phenytoin. Compounds 3, 6, 7, 11, 13 and 14 with 100% protection were the most potent ones in tonic seizure. The most potent compound in the both PTZ and MES models was compound 3. Using a model of the open pore of sodium channel, all of the compounds were docked. Results of docking showed that the ligands 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.

Conclusion: Some of these compounds are more potent than phenytoin simultaneously in the clonic and tonic seizures.

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Introduction

Epilepsy affects near 50000000 people in the world, a serious neurological disorder that typically reveals as spontaneous convulsions and/or a loss of consciousness. Efforts devoted in recent years to develop novel therapeutic strategies resulted in the availability of several drugs as anticonvulsants (1, 2). However, the available antiepileptic drugs are not always effective and only in less than 80% of patients showed to reduce the severity and number of seizures (3). Moreover, treatment associated with undesirable side effects (4). So, new antiseizure drug development, with appropriate therapeutic properties, is an important experiment for medicinal chemists.

Sodium channel is one of the most appropriate targets in the treatment of epilepsy. Neuronal voltage-gated sodium channels (NVSC) have a key role in the action potentials in neurons and other nervous cells. Thus, NVSC blocking compounds have a characterization of a class of drugs treat pain, seizures and arrhythmia. Voltage-gated sodium channels are containing of an alpha subunit and the beta subunits (5). Expression of the alpha subunit alone is sufficient to produce a functional channel. The α-subunit contains four repeated domains, labeled I through IV, each containing six membrane-spanning regions, labeled S1 through S6. The family of sodium channels includes nine known members. The proteins of these channels are named Nav1.1 through Nav1.9 (6, 7).

Phthalimide pharmacophore is one of the new ligand that acts as sodium channel antagonist which designed and evaluated as anticonvulsant agents. Based on the structure-activity relationships for 4-amino-benzamide derivatives (especially ameltolide) and thalidomide, Vamecq *et al* studied N-phenyl phthalimide derivatives as rigidified analogues of ameltolide (Figure 1) and 4-amino-N-(2, 6-dimethylphenyl) phthalimide model was designed and subsequently phthalimide pharmacophore without the 4-amino group in the phthaloyl moiety was prepared (8). Similarly, to ameltolide, *N*-phenylphthalimide derivatives exhibit a phenytoin-like profile i.e. the interaction of phthalimide

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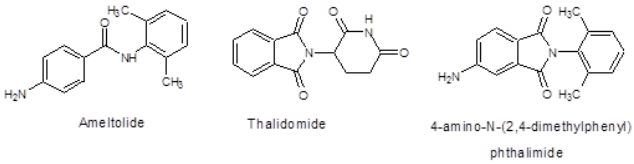


Figure 1. Structure of some of anticonvulsant agents

pharmacophore with NVSC channels was considered in the batrachotoxin affinity assay. These compounds are reasonably potent in the MES test and are impotent in the subcutaneous pentylenetetrazole (ScMet) test (9). Our docking studies revealed that while phenytoin interacts with the domain IV-S6 of NaV1.2, the phthalimide derivatives, mainly interact with the domain II-S6 of NaV1.2 in which the oxygen of carbonyl group plays a major role in the drug-receptor interactions (10-12). In the previous study we have reported new phthalimide derivatives with high anticonvulsant activity in the PTZ test (10-12). Based on the results of our reported study the activity of these compounds against pentylenetetrazole-induced seizure can significantly be influenced by the size and hydrophobicity of these compounds (10-12). Therefore, for more studies, it was recommended that the phthalimide pharmacophore should remain intact, and the N-aryl part should be replaced with more liphophilic and bulky aromatic moieties in order to achieve a higher potency (10-12). In the present study, our research group is exploring the idea of designing new compounds with more anticonvulsant activity in the both MES (tonic seizure) and IV-PTZ (clonic seizure) tests.

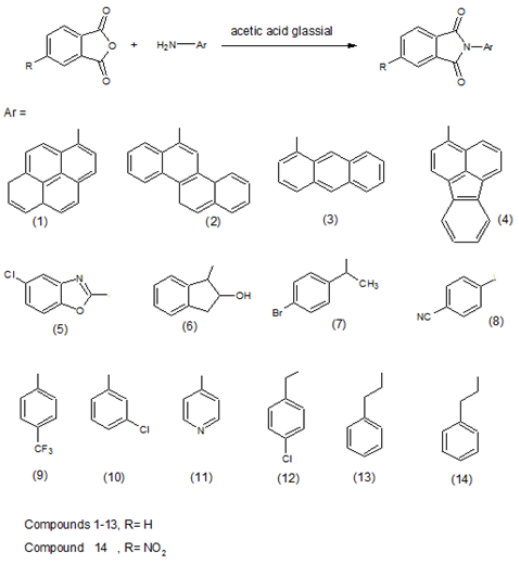
Materials and Methods

Chemistry

A group of 2-substituted phthalimide (**1-14**), was synthesized by condensation of the respective aromatic amine (homocycle or heterocycle) with phthalic anhydride in acetic acid at reflux temperature (scheme 1) (10, 11).

In vivo experiments

Anticonvulsant evaluation in the IV-PTZ tests (clonic convulsions), was performed as described previously (10, 11). Anticonvulsant evaluation in the MES test (tonic convulsions), was performed using following procedure (13). Tonic convulsions the hind extremities of mice were induced by passing alternating current (50 Hz, 35 mA and 0.2 sec) from an electroconvulsive therapy apparatus (Model 7800, Ugo Basile, Camerio, Italy) via ear electrodes. Electrodes were moistened by normal saline before attaching to the ear of mouse to improve electrode contact. The current which used was predetermined before the experiment and the current showed to cause hind limb extension in all the mice. Data are expressed in terms of percent protection which is the



Scheme 1. Synthesis of new derivatives of phthalimide

percentage of animals in each group that did not induce hind-limb extension or death after electroshock.

Computational study

Using HyperChem and AutoDock software (version 4.2.3) and a model of the open pore of the Na channel which was developed by homology with the crystal structures of K channels (13), conformational analysis and docking studies were performed as described previously (10-12).

Experimental protocols

Chemistry

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

Spectroscopy and analytical procedures

Melting points were determined using a Thomas-Hoover capillary apparatus which were uncorrected. ^{1}HNMR and ^{13}CNMR spectra were recorded on a Bruker FT-500 spectrometer TMS was used as an internal standard. Infrared spectra were acquired on a Nicolet 550-FT spectrometer. Elemental analysis was carried out with 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 using HyperChem and AutoDock 4.2.3.

General procedure for preparation of isoindoline derivatives (1-11)

A solution of phthalic anhydride (148 mg, 1 mmol) and arylamine (1 mmol) in glassial acetic acid (1.5 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-(Pyren -1-yl)-1H-isoindole-1,3(2H)-dione (1)

Using the general procedure and 1-aminopyren provided the title compound after 12 hr of reflux: Green crystals, yield 79.9%; mp 287-289 °C (ethanol).

^{1}HNMR ($CDCl_3$) : δ 8.324(d, *J*= 8.4Hz, 1H, aromatic), 8.276(d, *J*=7.6Hz, 1H, aromatic), 8.242(d, *J*=7.6Hz, 1H, aromatic), 8.123-8.201(m, 3H, aromatic), 8.053-8.098(m, 3H, H-4,7-phthalimide and aromatic), 7.962(d, *J*=8Hz, 1H, aromatic), 7.856-7.909 ppm (m, 3H, H-5,6-phthalimide and aromatic).; ^{13}CNMR ($CDCl_3$): δ 168.15(CO), 134.50, 132.19, 131.50, 131.01, 129.20, 128.80, 128.70, 127.50, 126.25, 126.15, 126.10, 125.80, 125.60, 124.80, 124.21, 122.04.; IR (KBr) : ῡ cm-1 ,3045 (CH-aromatic), 1776,1757, 1719(CO). Anal. ($C_{24}H_{13}NO_2$) C, H, N.

2-(Chrysen-1-yl)-1H-isoindole-1,3(2H)-dione (2)

Using the general procedure and 1-aminochrysen provided the title compound after 10 hr of reflux: Yellow crystals, yield 67.5%; mp 314-316 °C (ethanol). ^{1}HNMR (CD_2Cl_2) : δ 8.972(d, *J*=8.4Hz, 1H, aromatic), 8.852(d, *J*=9.2Hz, 1H, aromatic), 8.774(s, 1H, aromatic), 8.737(d, *J*=8.4Hz, 1H, aromatic), 8.194(d, *J*=9.6Hz, 1H, aromatic), 8.098-8.206(m, 3H, H-4,7-phthalimide and aromatic), 7.952-7.973(m, 2H, H-5,6-phthalimide), 7.830-7.873 (m, 2H, aromatic), 7.684-7.798 ppm (m, 3H, aromatic).; ^{13}CNMR (CD_2Cl_2): δ 168.193(CO), 162.358(CO), 134.903, 132.528, 132.388, 131.703, 130.508, 129.297, 129.197, 129.019, 128.896, 127.927, 127.782, 127.712, 127.436, 127.403, 127.168, 124.133, 124.088, 123.329, 123.272, 121.189. ; IR (KBr): ῡ cm-1, 3092, 3027(CH-aromatic), 1779, 1750, 1718(CO). Anal. ($C_{26}H_{15}NO_2$) C, H, N.

2-(Anthracen-1-yl)-1H-isoindole-1,3(2H)-dione (3)

Using the general procedure and 1-aminoanthracen provided the title compound after 14 hr of reflux: brownish crystals, yield 70.6%; mp 263-264 °C (ethanol). ^{1}HNMR ($CDCl_3$): δ 8.545(s, 1H, H-10'-aromatic), 8.163 (s, 1H, H-5'-aromatic), 8.155(d, *J*=6.4Hz, 1H, H-9'-aromatic), 8.059-8.081(m, 2H, H-4,7-phthalimide), 8.017(d, *J*=8.4Hz, 1H, H-6'-aromatic), 7.877-7.906 (m, 3H, H-5,6-phthalimide and aromatic), 7.550-7.589(m. 1H, aromatic), 7.436-7.483 ppm(m, 3H, aromatic). ;

^{13}CNMR ($CDCl_3$): δ 168(CO), 134.778, 132.12, 132.239, 130.680, 128.701, 128.650, 128.640, 128.280, 127.645, 127.072, 126.264, 126.211, 124.668, 124.302, 121.461.; IR (KBr): ῡ cm-1, 3052, 3020 (CH-aromatic), 1778, 1729, 1721 (CO). Anal. ($C_{22}H_{13}NO_2$) C, H, N.

2-(Fluoranthen-3-yl)-1H-isoindole-1,3(2H)-dione (4)

Using the general procedure and 3-aminofluoranthen provided the title compound after 10 hr of reflux: orange crystals, yield 70.5%; mp 256-257 °C (ethanol). ^{1}HNMR (CD_2Cl_2): δ 8.068(d, *J*=7.2Hz, 1H, aromatic), 8.026-8.048(m, 2H, H-4,7-phthalimide), 7.995-8.014 (m, 1H, aromatic), 7.952-7.987(m, 2H, aromatic), 7.888-7.909(m, 2H, H-5,6-phthalimide), 7.592-7.681 (m, 3H, aromatic), 7.438-7.460 ppm (m, 2H, aromatic).; ^{13}CNMR (CD_2Cl_2): δ 168.028(CO), 139.950, 138.882, 138.548, 137.637, 134.821, 133.410, 132.206, 129.126, 128.879, 128.417, 128.203, 127.531, 123.968, 122.975, 122.134, 121.985, 120.971, 120.002.; IR (KBr) : ῡ cm-1 , 3055, 3037 (CH-aromatic) , 1781, 1715 (CO). Anal. ($C_{24}H_{13}NO_2$) C, H, N.

2-(5-Chloro-1,3-benzoxazol-2-yl)-1H-isoindole-1,3(2H)-dione (5)

Using the general procedure and 2-amino-5-chlorobenzoxasole provided the title compound after 14 hr of reflux: light red crystals, yield 49.6%; mp 265-266 °C (ethanol). ^{1}HNMR (CD_3COCD_3): δ 7.536-7.540(m, 4H, aromatic), 7.517 (s, 1H, aromatic), 7.296 (dd, *J*=8.4Hz, 2.4Hz, 2H, aromatic).; IR (KBr) : ῡ cm-1 , 3090, 3035 (CH-aromatic) , 1777, 1655 (CO). Anal. ($C_{15}H_7ClN_2O_3$) C, H, N.

2-(2-Hydroxy-2,3-dihydro-1H-inden-1-yl)-1H-isoindole-1,3(2H)-dione (6)

Using the general procedure and 1-amino-2-hydroxy-2,3-dihydro-1H-inden provided the title compound after 14 hr of reflux: White crystals, yield 50.9%; mp 153-154 °C (ethanol). ^{1}HNMR ($CDCl_3$): δ 7.837-7.858 (m, 2H, H-4,7-phthalimide), 7.730-7.752(m, 2H, H-4,5-phthalimide), 7.325-7.335(m, 2H, H-4',7'-indol), 7.217-7.225 (m, 2H, H-5',6'-indol), 5.974(d, *J*= 7.6Hz, 1H, H-1'-indol), 5.693 (q, *J*= 7.2Hz, 1H, H-2'-indol), 3.485 ppm (d, *J*= 7.2Hz, 2H, H-3'-indol).; ^{13}CNMR ($CDCl_3$): δ 170.526, 167.974(CO), 141.223, 136.881, 134.358, 132.008, 129.336, 127.509, 125.126, 124.903, 123.621, 73.121 (C-1'-indol), 55.000 (C-2'-indol), 38.326 (C-3'-indol).; IR (KBr): ῡ cm-1 , 3450 (OH), 3067 (CH-aromatic) , 2977, 2932 (CH-aliphatic), 1780, 1739, 1712 (CO). Anal. ($C_{17}H_{13}NO_3$) C, H, N.

2-[1-(4-Bromophenyl)ethyl]-1H-isoindole-1,3(2H)-dione (7)

Using the general procedure and 1-(4-bromophenyl)ethanamine provided the title compound after 12 hr of reflux: white crystals, yield 58.5%; mp 125-126 °C (ethanol). ^{1}HNMR ($CDCl_3$) : δ 7.798-7.829 (m, 2H, H-4,7-phthalimide), 7.697-7.728 (m, 2H, H-5,6-phthalimide), 7.458 (tt, *J*= 8.4Hz, 2.4Hz, 2H, H-3',5'-phenyl),7.391 (tt, *J*= 8.4Hz, 2.4Hz, 2H, H-2',6'- phenyl), 5.526 (q, *J*= 7.2Hz, 1H, CHCH3), 1.91 ppm (d, *J*=7.2Hz, 3H, CHCH3).; ^{13}CNMR ($CDCl_3$): δ 168.275 (CO), 139.479, 134.267, 132.119, 131.826, 129.505, 123.502, 121.935, 49.236 ($CHCH_3$), 17.636($CHCH_3$).; IR (KBr) : ῡ cm-1, 3065, 3032 (CH-aromatic), 2982, 2900 (CH-aliphatic), 1774, 1755, 1710(CO). Anal. ($C_{16}H_{12}BrNO_2$) C, H, N.

4-(1,3-Dioxo-1,3-dihydro-2H-isoindol-2-yl) benzonitrile (8)

Using the general procedure and 4-aminobenzonitrile provided the title compound after 16 hr of reflux: white crystals, yield 56%; mp 182-184 °C (ethanol). ^{1}HNMR (CD_3COCD_3): δ 7.953-8.025 (m, 6H, aromatic), 7.826 ppm (dd, *J*= 8.4Hz, 1.2Hz, 2H, H-3', 5'-phenyl).; ^{13}CNMR (CD_3COCD_3): δ 166.638(CO), 136.861, 135.063, 132.956, 132.119, 127.592, 123.782, 118.290, 111.256.; IR (KBr): ῡ cm-1 , 3099, 3064 (CH-aromatic) , 2225(CN), 1783, 1743, 1724 (CO). Anal. ($C_{15}H_8N_2O_2$) C, H, N.

2-[4-(Trifluoromethyl)phenyl]-1H-isoindole-1,3(2H)-dione (9)

Using the general procedure and 4-(trifluoromethyl) benzamine provided the title compound after 16 hr of reflux: White crystals, yield 66%; mp 250-253oC (ethanol). ^{1}HNMR (CD_3COCD_3): δ 7.968-7.999 (m, 2H, H-4, 7-phthalimide), 7.901-7.935 ppm (m, 4H, H-5, 6-phthalimide and H-2', 6'-phenyl), 7.714 ppm (d, *J*=8.4Hz, H-3', 5'-phenyl).; ^{13}CNMR (CD_3COCD_3): δ 167.318(CO), 135.595, 132.231, 128.528, 126.648, 124.319.; IR (KBr) : ῡ cm-1 3066 (CH-aromatic) , 1793, 1752, 1726(CO). Anal. ($C_{15}H_8F_3NO_2$) C, H, N.

2-(3-Chlorophenyl)-1H-isoindole-1,3(2H)-dione (10)

Using the general procedure and 3-chloroaniline provided the title compound after 3 hr of reflux: white crystals, yield 54%; mp 157-161oC (ethanol). ^{1}HNMR ($CDCl_3$): δ 7.73-8.02(m, 4H, aromatic), 7.40-7.49(m, 4H, aromatic).; IR (KBr): ῡ cm-1 , 3075 (CH-aromatic) , 1767, 1721(CO). Anal. ($C_{14}H_8ClNO_2$) C, H, N.

2-(Pyridin-4-yl)-1H-isoindole-1,3(2H)-dione (11)

Using the general procedure and 4-aminopyridine provided the title compound after 28 h of reflux: white crystals, yield 74%; mp 176-179oC (ethanol). ^{1}HNMR ($CDCl_3$): δ 8.74(d, *J*=8 Hz, 2H, H3', 5'-pyridine), 7.76-8.06(m, 4H, phthalimide) 7.61(d, *J*=8 Hz, 2H, H2',6'-pyridine).; IR (KBr): ῡ cm-1 , 3057 (CH-aromatic), 1774, 1705 (CO). Anal. (C13H8N2O2) C, H, N.

2-(4-Chlorobenzyl)-1H-isoindole-1,3(2H)-dione (12)

Using the general procedure and 4-chlorobenzyl-amine provided the title compound after 10 hr of reflux: white crystals, yield 65%; mp 125-128 °C (ethanol). ^{1}HNMR ($CDCl_3$): δ 7.84 (dd, *J*=5.8 Hz, *J*=3.2 Hz, 2H, H-4, 7-phthalimide), 7.70 (dd, *J*=5.8 Hz, *J*=3.2 Hz, 2H, H-5, 6-phthalimide), 7.36(d, *J*=8 Hz, 2H, 3', 5'-phenyl), 7.27 (d, *J*=8 Hz, 2H, 2', 6'-phenyl), 4.8 ppm (s, 2H, CH2); IR (KBr): ῡ cm-1 , 3080 (CH-aromatic), 1772, 1704, 1669(CO). Anal. ($C_{15}H_{10}ClNO_2$) C, H, N.

2-(2-Phenylethyl)-1H-isoindole-1, 3(2H)-dione (13)

It has been reported already (10).

5-Nitro-2-(2-phenylethyl)-1H-isoindole-1,3(2H)-dione (14)

It has been reported already (10).

Molecular Modeling and Docking

Conformational analysis of the phenytoin and compounds **1-14** was performed through Semi-empirical molecular orbital calculations (PM3) method using the HYPERCHEM software. Among all energy minima conformers, the global minimum was used in docking calculations.

Docking calculations were performed using AutoDock software (version 4.2.3). Using a model of the open pore of the Na channel that has been developed by homology with the crystal structures of K channels (12); we have docked all compounds and phenytoin as a reference drug. Docking was performed using the implemented Lamarckin GL and the default parameters and ten independent docking runs were performed for each ligand.

Pharmacology, determination of anticonvulsant activity

Anticonvulsant evaluation in the PTZ test (Clonic convulsions) and the MES test (Tonic convulsions), was performed as described previously (10, 11).

Statistical analysis

The results are presented as mean±SEM, and the statistical significance between the groups was analyzed by mean of variance followed by one-way ANOVA (Tukey's test) and Chi-square. *P* values less than 0.05 were considered as indicative of significance.

Results

Chemistry

Fourteen derivatives of 2-substituted analogs of phthalimide were synthesized in 49.6 –79.9% yield based on method that is shown in scheme 1. All of the compounds characterized by TLC followed by FT-IR, elemental analysis and proton NMR and some of them additionally characterized by carbon NMR.

Molecular modeling and docking

Flexible docking was done on the active site of the Na channel open pore. The binding energies, Ki and other results of docking of all the compounds under study were 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. As it was previously reported, while phenytoin interacted with the domain IV-S6 of NaV1.2 (10, 11), compounds **1-14** interacted mainly with the domain II-S6. Oxygen of imide plays the main role in drug-receptor interaction by making hydrogen bond with the OH of Thr87 or Ser84. 2-Aryl part of phthalimide created a hydrophobic-hydrophobic interaction with receptor that mostly

Table 1. Docking results of phthalimides **1-14** using AutoDock 4.2 software

No	Binding energy (Kcal/mole)	Ligand efficiency	Inhibi-constant (nM)	Intermol energy	Vdw-hb-desolv energy	Electrostatic energy	Total internal	Torsional energy	Unbound energy
1	-8.13	-0.3	1.1	-8.43	-8.4	-0.03	-0.41	0.3	-0.41
2	-8.15	-0.28	1.06	-8.45	-8.42	-.03	-0.59	0.3	-0.59
3	-7.63	-0.31	2.57	-7.92	-7.93	0.01	-0.55	0.3	-0.55
4	-7.17	-0.27	5.5	-7.47	-7.45	-0.02	-0.54	0.3	-0.54
5	-7.0	-0.33	7.39	-7.3	-7.17	-0.13	0.04	0.3	0.04
6	-5.94	-0.28	44.55	-6.53	-6.51	-0.03	-0.58	0.6	-0.58
7	-6.36	-0.32	21.85	-6.95	-6.9	-0.05	-0.42	-0.6	-0.42
8	-5.65	-0.31	71.89	-5.95	-5.93	-0.02	-0.28	0.3	-0.28
9	-5.5	-0.26	92.8	-6.1	-6.11	0.02	-0.4	0.6	-0.4
10	-5.84	-0.32	52.12	-6.14	-6.12	-0.03	-0.35	0.3	-0.35
11	-5.14	-0.3	171.8	-5.43	-5.42	-0.02	-0.28	0.3	-0.28
12	-5.99	-0.32	40.35	-6.59	-6.54	-0.05	-0.38	0.6	-0.38
13	-6.14	-0.33	29.49	-7.08	-7.03	-0.05	-0.39	0.89	-0.39
14	-6.38	-0.29	21.17	-7.57	-7.49	-0.08	-0.49	1.19	-0.49
phen	-5.83	-0.31	53.37	-6.43	-6.38	-0.04	-0.71	0.6	-0.71

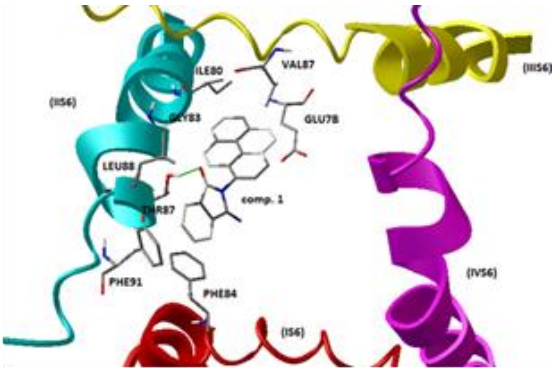


Figure 2. Docked structure of phthalimide in Model of Sodium Channel. Hydrogen bonds are represented with dashed green lines

made by domains I, II, in which in the compounds **1-4** that have more bulky and lipophilic moiety, the binding energy are more negative due to stronger hydrophobic interactions (Figure 2).

Pharmacology

The ability of the compounds 1-14 to protect against pentylenetetrazole-induced seizure, colonic, was determined using an *in vivo* assay; the results are summarized in Table 2 and Figures 3-5. Each compound was dissolved in DMSO, injected intraperitoneally and then were screened for anticonvulsant activities at doses of 20, 40 and 80 mg/kg compared with phenytoin as a positive control. To finding the time course of the effects the single dose of all compounds (40 mg/kg) was administered 15, 30 or 60 min prior to distinct groups of mice.

The ability of the compounds 1-14 to protect against MES induced seizure, tonic, was determined using an *in vivo* assay, and the results are summarized in Table 3 and Figure 6.

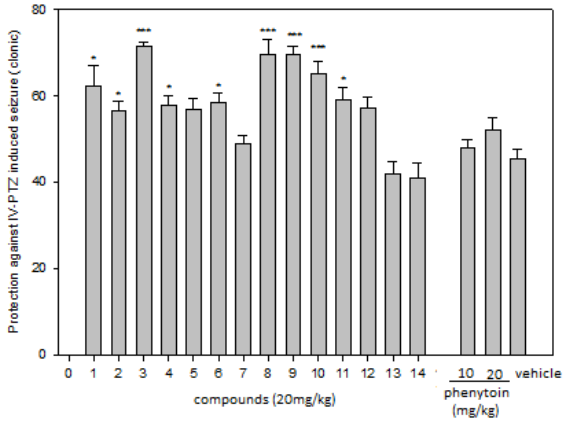


Figure 3. Effect of phenytoin (10 and 20 mg/kg) and compounds **1-14** (20 mg/kg) on clonic seizure threshold induced by PTZ in mice. Animal received vehicle or drugs 30 min before PTZ administration. Data are expressed as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle

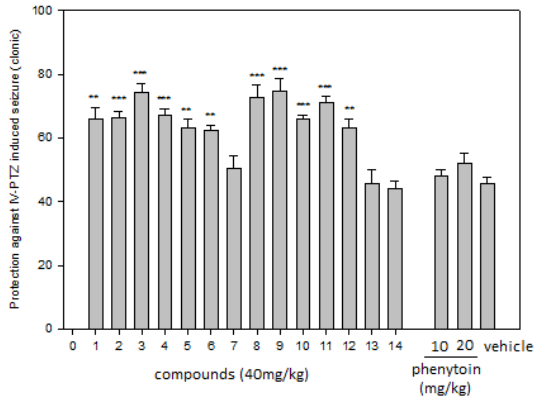


Figure 4. Effect of phenytoin (10 and 20 mg/kg) and compounds **1-14** (40 mg/kg) on clonic seizure threshold induced by PTZ in mice. Animal received vehicle or drugs 30 min before PTZ administration. Data are expressed as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle

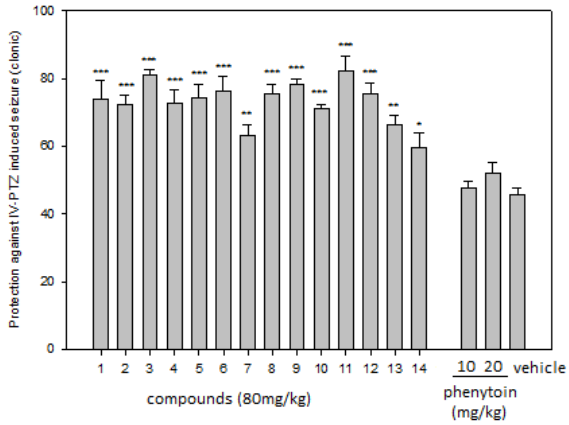


Figure 5. Effect of phenytoin (10 and 20 mg/kg) and compounds **1-14** (80 mg/kg) on clonic seizure threshold induced by PTZ in mice. Animal received vehicle or drugs 30 min before PTZ administration. Data are expressed as mean ±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle

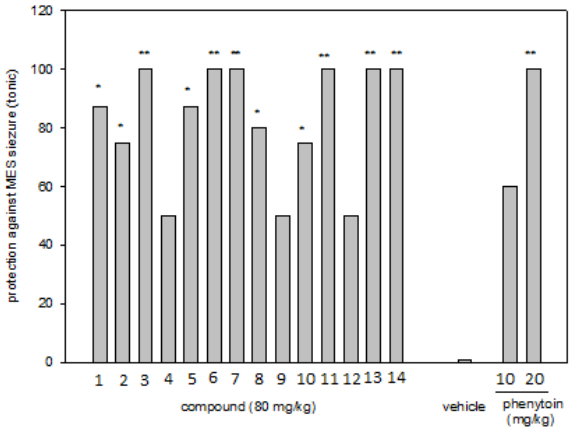


Figure 6. Effect of phenytoin (10 and 20 mg/kg) and compounds **1-14** (80 mg/kg) on tonic seizure threshold induced by MES in mice. Animal received vehicle or drugs 30 min before test. Data are expressed as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle

Table 2. The Ability of phthalimides (1-14) to Protect against pentylenetetrazole-induced seizure (clonic seizure) in IV-PTZ test

Compound	CST in IVPTZ test		
	20 mg/kg	40 mg/kg	80 mg/kg
1	62.36±4.60 [a,d,e]	66.03±3.67 [b]	73.88±5.62 [c]
2	56.63±2.26 [a]	66.43±1.69 [c]	72.37±2.63 [c]
3	71.42±1.06 [c,d,e,f,g]	74.14±2.93 [c]	81.28±1.49 [c]
4	57.75±2.21 [a]	67.07±1.91 [c]	72.72±3.19 [c]
5	56.98±2.58	63.27±2.51 [b]	74.29±4.12 [c]
6	58.34±2.39 [a]	62.30±1.56 [b]	76.21±4.37 [c]
7	49.00±2.01	50.49±4.05	63.24±2.98 [b]
8	69.76±3.35 [c]	72.75±3.90 [c]	75.34±3.16 [c]
9	69.69±1.88 [c,d,e,f,g]	74.81±3.73 [c]	78.20±1.81 [c]
10	65.06±2.91 [c,d,e,f]	65.81±1.5 [c]	71.25±1.26 [c]
11	59.06±2.99 [a]	70.95±2.12 [c]	82.19±4.62 [c]
12	57.29±2.53	63.29±2.77 [b]	75.36±3.39 [c]
13	42.04±2.85	45.59±4.53	66.23±3.03 [b]
14	40.99±3.44	43.91±2.57	59.61±4.37 [a]
Vehicle	45.56±2.02		
Phenytoin 10 mg/kg	47.93±1.98		
Phenytoin 20 mg/kg	52.02±3.08		

Data are expressed as mean±SEM. [a]*P*<0.05, [b]*P*<0.01, [c]*P*<0.001 compared to vehicle. [d]*P*<0.05, [e]*P*<0.01 dose 20 mg/kg compounds **1-14** compared to phenytoin 10 mg/kg. [f]*P*<0.05, [g]*P*<0.01 dose 20 mg/kg compounds 1-14 compared to phenytoin 20 mg/kg

Table 3. The effect of phthalimides (**1-14**) on the electroshock-induced seizure model (MES) in mice

Groups (N=15)	Tonic seizure protection (%)	Significancy	Mortality protection (%)
Compound 1 (20 mg/kg)	62.5	*P*<0.05	100
Compound 1 (40 mg/kg)	75		100
Compound 1 (80 mg/kg)	87.5		100
Compound 2 (20 mg/kg)	25		100
Compound 2 (40 mg/kg)	50	*P*<0.05	100
Compound 2 (80 mg/kg)	75		100
Compound 3 (20 mg/kg)	25		100
Compound 3 (40 mg/kg)	62.5	*P*<0.01	100
Compound 3 (80 mg/kg)	100		100
Compound 4 (20 mg/kg)	0		75
Compound 4 (40 mg/kg)	20	*P* >0.05	80
Compound 4 (80 mg/kg)	50		100
Compound 5 (20 mg/kg)	25		87.5
Compound 5 (40 mg/kg)	50	*P*<0.05	100
Compound 5 (80 mg/kg)	87.5		100
Compound 6 (20 mg/kg)	40		60
Compound 6 (40 mg/kg)	83.3	*P*<0.01	100
Compound 6 (80 mg/kg)	100		100
Compound 7 (20 mg/kg)	60		100
Compound 7 (40 mg/kg)	80	*P*<0.01	100
Compound 7 (80 mg/kg)	100		100
Compound 8 (20 mg/kg)	38		80
Compound 8 (40 mg/kg)	62	*P*>0.05	80
Compound 8 (80 mg/kg)	81		100
Compound 9 (20 mg/kg)	12.1		100
Compound 9 (40 mg/kg)	25.5	*P*>0.05	100
Compound 9 (80 mg/kg)	50		100
Compound 10 (20 mg/kg)	25		100
Compound 10 (40 mg/kg)	50	*P*<0.05	100
Compound 10 (80 mg/kg)	75		100
Compound 11 (20 mg/kg)	62.5		100
Compound 11 (40 mg/kg)	87.5	*P*<0.01	100
Compound 11 (80 mg/kg)	100		100
Compound 12 (20 mg/kg)	25		100
Compound 12 (40 mg/kg)	37.5	*P*>0.05	100
Compound 12 (80 mg/kg)	50		100
Compound 13 (20 mg/kg)	66.7		100
Compound 13 (40 mg/kg)	71.4	*P*<0.01	100
Compound 13 (80 mg/kg)	100		100
Compound 14 (20 mg/kg)	50		100
Compound 14 (40 mg/kg)	73	*P*<0.01	100
Compound 14 (80 mg/kg)	100		100
Vehicle	0		0
Phenytoin 10 mg/kg	60	*P*<0.01	100
Phenytoin 20 mg/kg	100		100

Note: Percentage of protection against incidence of tonic seizure and death subsequent electroshock was compared among groups using chi-square test

Discussion

Based on the predicated docking results (Table 1), some synthesized compounds predicted to be more potent than phenytoin, however the experimental data did not confirm this phenomena possibly due to partition coefficient of the compounds.

Time-course analysis showed that all of the compounds and phenytoin exerted their maximal effects 30 min after administration. *In vivo* screening data generated showed that at doses of 20 and 40 mg/kg except compound **7, 13** and **14**, all the analogs showed the ability to protect against PTZ-induced seizure and were more potent than phenytoin at doses 10 and 20 mg/kg (Figure 3 and Figure 4). All the phthalimides **1-14** at dose 80 mg/kg were more potent than phenytoin in protection against PTZ-induced seizure (Figure 5). *In vivo* screening data indicated that at doses of 20, 40 and 80 mg/kg, compounds **3** and **9** elevated clonic seizure thresholds at 30 min, which were, more active than phenytoin as a reference drug and were the most potent ones.

Our results reveals in the lipophilic series, compounds **1-4**, compound **3** is the most potent one. Compounds **8, 9**, and **11** contain a moiety with hydrogen binding ability in the *para* position of aryl ring which showed very potent activity. Structural analysis of compounds **7, 13** and **14** which showed the highest activity at different doses, indicate

insertion of ethyl moiety between phthalimide and aryl part, results in decreasing the activity.

In vivo screening data acquired indicated that except compound **4** which was inactive at doses 20 mg/kg, all the analogs have the ability to protect against MES induced seizure which compounds **3, 6, 7, 11, 13** and **14** at dose 80 mg/kg with 100% protection are the most potent ones (Figure 6).

Based on the results of IV-PTZ and MES tests, compound **3** is the most potent compound in the both of clonic and tonic seizures, which is more active than phenytoin as a reference drug and it candidate for more evaluation.

Conclusion

Fourteen analogs of 2-substituted analogs of phthalimid were synthesized, characterized by TLC followed by IR, elemental analysis and NMR and tested for their ability to protect against pentylenetetrazole-induced seizure and MES *in vivo* in mice. *In vivo* screening data acquired indicated that all the analogs have the ability to protect against IV-PTZ and MES induced seizure. These compounds exerted their maximal effects 30 min after administration. The most potent compounds in PTZ test were **3** and **9** and in the MES were compounds **3, 6, 7, 11, 13** and **14** with 100% protection. Compound **3** with high lipophilic property is the most potent compound in the clonic and tonic seizures, which is more active than phenytoin as a reference drug. Compound **3** has been chosen for further evaluation.

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