Design, Synthesis and Protection Against Pentylenetetrazole-induced Seizure of N-aryl Derivatives of the Phthalimide Pharmacophore

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Design, Synthesis and Protection Against Pentylenetetrazole-induced Seizure of *N*-aryl Derivatives of the Phthalimide Pharmacophore

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Abstract: A series of compounds including *N*-aryl substituents of phthalimide and 4-nitrophthalimide were synthesized and evaluated for their anticonvulsant properties. The *in vivo* screening data suggest that all the analogs have the ability to protect against pentylenetetrazole-induced seizures. These compounds exerted their maximal effects 30 min after administration. The most potent compound in both, tonic and clonic seizure was 1-naphthyl derivative (comp. 6), which was more active than the reference drug known as Phenytoin. Using an open pore model of the Na channel**,** these anticonvulsants were docked in the active site and examined in relation to the residues identified by mutagenesis as important for their binding energies. Docking studies revealed that all compounds (1-13) interacted mainly with residues II-S6 of NaV1.2 by making hydrogen bonds and additional hydrophobic interactions with domain I and II in the channel's inner pore.

Keywords: Phthalimide, Nitrophthalimide, Seizure, Phenytoin.

1. INTRODUCTION

Epilepsy is a common neurological condition affecting 0.5 to 1 % of the population worldwide (more than 60 million people) according to epidemiological studies. Every year approximately 250000 new cases are added to this Figure [1-3]. It is roughly estimated that significant number of patients (up to 30%) are resistant to the available medical therapies [4, 5]. Despite the development of several new antiepileptic drugs (AEDs) the treatment of epilepsy still remains inadequate and patients suffer from a number of unfavorable side effects. Although several new anticonvulsants are already in clinical use, some types of seizures are still not adequately treated with current therapies, have limitations and intolerable side effects [6, 7]. In response to these limitations; the development of new drugs to optimally manage seizures has been strongly advocated. Therefore, the search for new anticonvulsant drugs continues to be an active area of investigation in medicinal chemistry.

The tricyclic anticonvulsant drugs such as phenytoin and carbamazepine typically have a tricyclic structure, a polar amide in the middle (Fig. **1**) which blocks the neuronal voltage-gated Na channels. The proposed binding site is domain IV-S6 in the channel's inner pore [8, 9]. Docking studies of these drugs revealed that they show a common pharmacophore, including an aromatic ring that has an aromatic-aromatic interaction with Tyr-1771 of NaV1.2 and a polar amide or imide 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 contribute to the binding abilities of these anticonvulsants [8, 9].

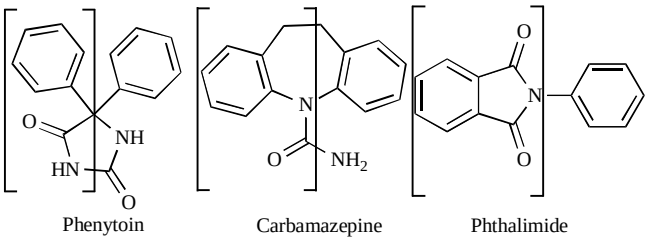

Fig. (1). Phenytoin, Carbamazepine and Phthalimide Pharmacophre that Typically have a Tricyclic structure, with a polar amide in the Middle.

Recently, N-phenylphthalimide derivatives were designed based on ameltolide and thalidomide as they possess a similar degree of anticonvulsant potency due to their phenytoin-like profile [10,11]. The ability of phthalimide to interact with the neuronal voltage-dependent sodium chan-

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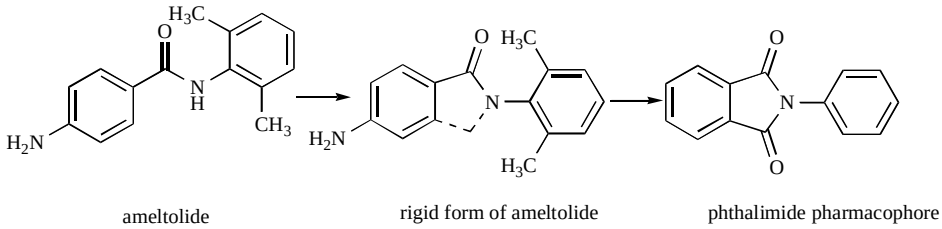


Fig. (2). Rational design of Phthalimide Pharmacophore from the model of Ameltolide.

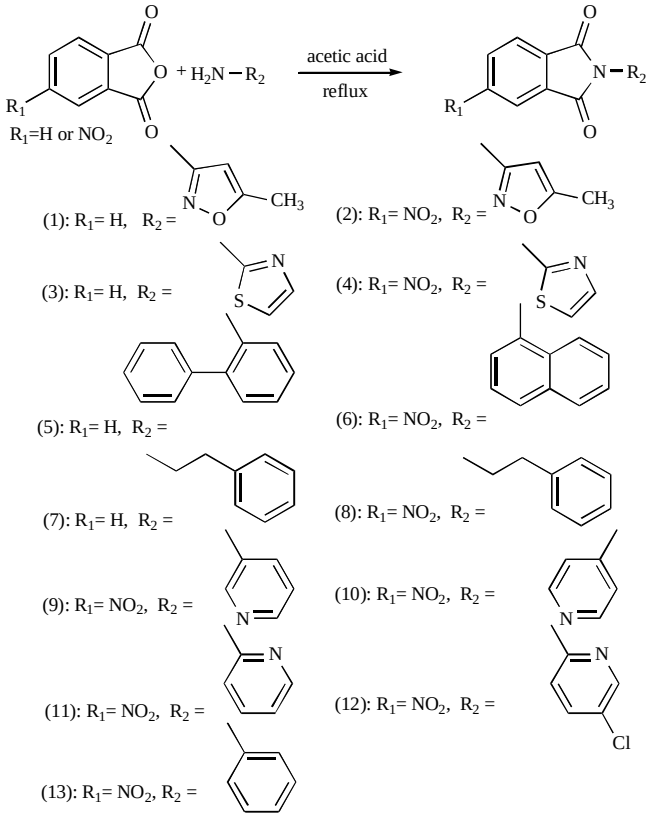


Fig. (3). Synthesis of New Derivatives of Phthalimide.

nels was studied in the batrachotoxin affinity assay [10]. Indeed, the phthalimide pharmacophore is a rigid form of ameltolide (Fig. **2**) and same as phenytoin having a tricyclic structure and aromatic ring, a polar amide and a second aromatic ring (Fig. **1**).

The presence of hydrophobic interactions of second aromatic ring [3] and a polar amide in the middle mainly contributes to the binding abilities of anticonvulsants and known properties of phthalimide derivatives [10-15]. Therefore, we explored this idea further based on SAR studies of N-phenylphthalimide and their drug-receptor interaction profiles. Ultimately, the new series of N-phenylphthalimide derivatives were docked and synthesized in order to investigate the pharmacological activities.

2. CHEMISTRY

Analogs of N-aryl derivatives of the phthalimide (1-13), 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 amines (homocycle and heterocyclic) with phthalic anhydride and nitro phthalic anhydride in acetic acid at reflux temperature (Fig. **3**).

3. PHARMACOLOGY

The 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 that animals were removed from their cages for testing. All behavioral experiments were conducted between 10:00 and 13:00 in a room with normal light (12h regular light/dark cycle) and temperature of 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.

4. COMPUTATIONAL STUDY

The chemical structure of desired phthalimides (1-13) was built by using HYPERCHEM software (version 7, Hypercube Inc.). Conformational analysisof the designed compounds were performed through semi-empirical molecular orbital calculations (PM3) method using the HYPERCHEM software. Among all energy minima conformers, the global minimum of compounds was used in docking calculations.

Docking calculations were performed using Autodock software (version 4.2.3). Using an open pore model of the Na channel that was developed based on homology model of the crystal structures of K channels [16]. The synthesized compounds were docked in to the active site as well as Phenytoin which was acting as our reference drug and validation of our technique. In order to assign the perfect grid of each ligand, grid box values that were obtained by trial and error and previous study by Gregory M. *et al.* [8, 16]. Finally docking was performed using the implemented Lamarckin GL and the default parameters and ten independent docking runs were performed for each phthalimide.

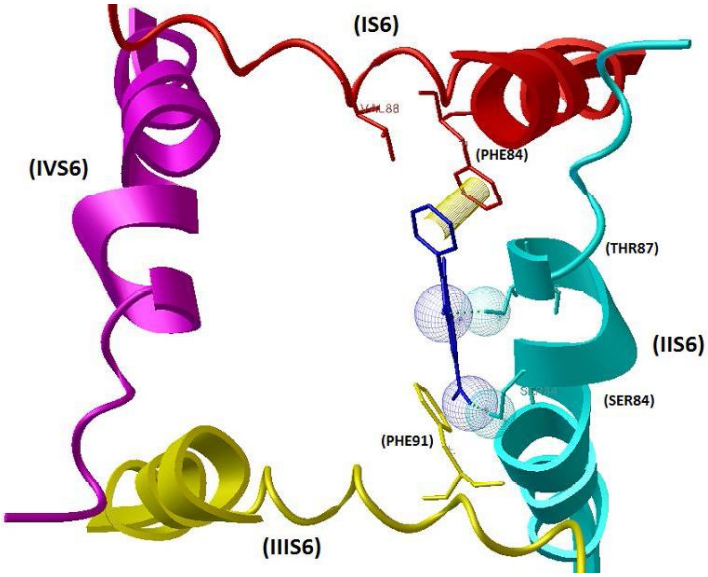
5. RESULTS AND DISCUSSION

5.1. Chemistry

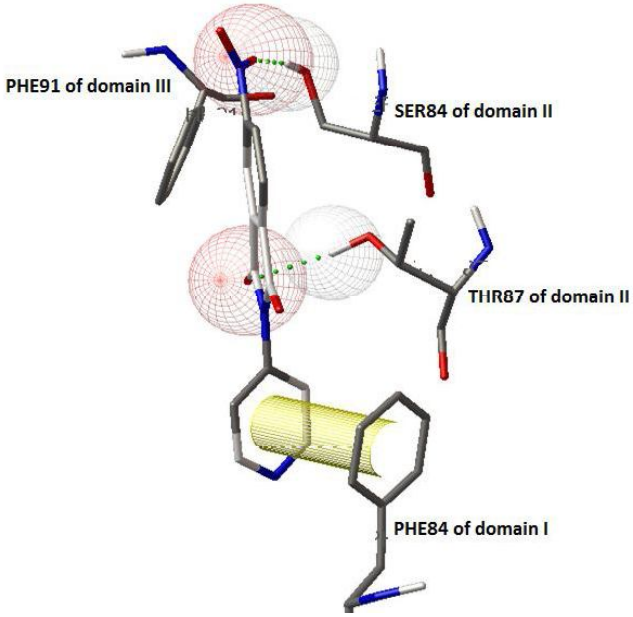
13 derivatives of N-aryl phthalimide and 4-nitrophthalimide analogs were synthesized in good yields (45-79%), as indicated in (Fig. **3**). All of compound characterized by TLC followed by IR, elemental analysis and proton NMR.

5.2. Molecular Modeling and Docking

Flexible docking of all synthesised compounds were carried out in the active site of the open pore of the Na channel. The predicted binding energies of these inhibitors in the ac-



(A)



(B)

Fig. (4). Docked structure of 10 in Model of Sodium Channel. Hydrogen bonds are Represented with Dashed green lines and Pi-pi Interaction Represented as a Yellow Cylinder.

tive site are listed in Table **1**. Our docking studies revealed that Phenytoin interacts with the domain IV-S6 of NaV1.2, however, the phthalimide, nitrophthalimide derivatives and synthesized compounds (1-13), mainly interacted with the domain II-S6 by making a hydrogen bond and additional hydrophobic interactions with the domain I, II and III in the channel's inner pore (Figs. **4a** and **5**). In compounds 1-13, the oxygen of carbonyl group plays a major role in making a hydrogen binding interaction with the OH group of THR87 (Fig. **4a**-**b**).

In the nitrophthalimide, compounds 2, 4, 6, 8, 9, 10, 11, 12, and 13, there is additional hydrogen binding interactions between oxygen of the nitro group in the ligand and the OH of SER84 or NH_2 of LYS7 (Figs. **4** and **5**). The N-aryl part of phthalimide pharmacophore forms a hydrophobic interaction with the hydrophobic pocket of the receptor made from PHE84 and PHE91 of domains I,II respectively (Figs. **4** and **5**). For compounds 6, 8, 9, 10, 11, 12, and 13, there is an efficient pi-pi interaction between N-aryl part and the aromatic ring of PHE84 of domain I (Fig. **5**). The Aryl part of phthalimide pharmacophore has a weak interaction with the PHE91 of domain III (Figs. **4b** and **5**).

The binding energy analysis of phthalimide pharmacophore has led us to aim for compounds with similar structures in order to make similar complexes with the two domain IIS6 residues in the open inner pore of the Na channel.

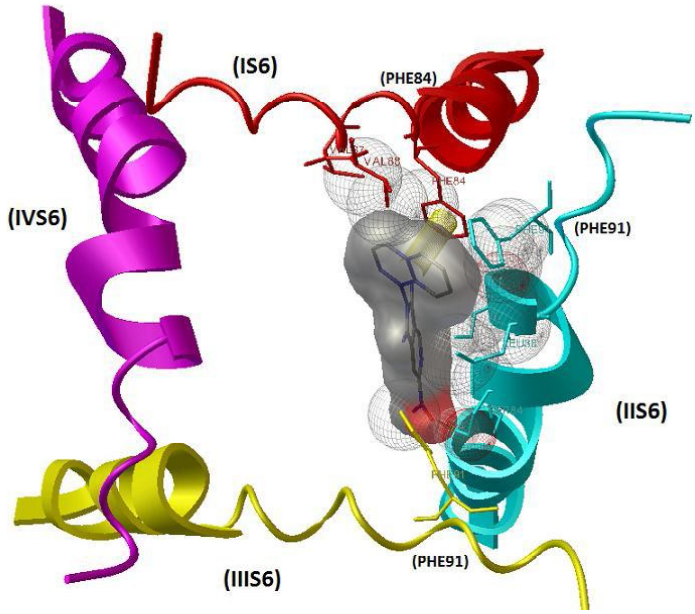


Fig. (5). Docked structure of Compound 6 in Model of sodium channel. Hydrogen Bonds are Represented with Dashed Green Lines and Pi-pi Interaction Represented as a Yellow Cylinder.

Table 1. The Ability of Phthalimide Drivatives (1-13) to Protection Against Pentylenetetrazole-induced Seizure

Comp.	Binding Energy [a]	CST [b]	TST [c]
1	-5.6	60.25 ± 3.70	87.75 ± 6.65
2	-6.37	66.40 ± 5.29	84.40 ± 6.67
3	-5.37	56.20 ± 2.35	83.60 ± 1.63
4	-5.89	63.50 ± 4.09	84.25 ± 5.45
5	-6.12	67.75 ± 5.02	90.75 ± 6.34
6	-7.43	66.60 ± 1.86	112.25 ± 4.99
7	-6.14	53.00 ± 4.06	75.75 ± 7.09
8	-6.38	48.40 ± 2.94	70.20 ± 4.67
9	-5.98	64.60 ± 4.08	77.60 ± 3.61
10	-5.95	63.40 ± 3.47	87.40 ± 5.11
11	-6.14	76.00 ± 5.42	94.50 ± 5.30
12	-6.12	68.67 ± 2.82	92.67 ± 4.34
13	-5.96	64.66 ± 2.80	96.16 ± 2.88
Vehicle		51.80 ±2.695	76.05 ± 4.97
Phenytoin	-5.83	64.55 ± 3.07	111.78 ± 5.05

[a] Predicted binding energy (kcal/mol)
[b] Colonic seizure threshold
[c] Tonic seizure threshold

According to the mutagenesis studies, these properties are critical for their blocking activities. The binding energies of these compounds with the side chains of Thr87 and Ser84 of domain IIS6 and Phe84 of domain IS6 are high. The alanine-scanning mutagenesis clearly shown that these three amino acid residues are important, allowing us to propose that they are essential to the channel's recognition of the anticonvulsant structures. Additionally, the orientation of the rigid drug structures at the interface cleft between domains I and III is also suggested to be critical and important.

The orientation of all compounds (1-13), in the active site of sodium channel were examined by docking experiment; the orientation of compounds 6 and 10 shown in Figs. (**4** and **5**) respectively. Our docking studies revealed that compound 6 with the binding energy of -7.43 kcal/mole should be more active than Phenytoin (binding energy = -5.83) and that was confirmed by experimental data. Based on the predicated binding energies (Table **1**), some designed compounds should be more potent than Phenytoin, however, the experimental data did not confirm this possibly due to low logP of these compounds.

5.3. Protection against Pentylenetetrazole-induced Seizure

The ability of phthalimides (1-13) to protect against pentylenetetrazole-induced, tonic and colonic seizure, was determined using an *in vivo* assay. The results are summarized in table 1. Each compound was dissolved in DMSO, injected intraperitoneally and screened for their anticonvulsant activities at doses of 10, 20 and 40 mg/kg compared with Phenytoin as a positive control. The single dose of 40mg/kg (best dose) for all compounds were administered at 15, 30 and 60 minutes time intervals. Data for compound 5 is shown in Fig. (**6**).

Further analysis showed that all compounds and Phenytoin exerted their maximal effects 30 minutes after administration. The *in vivo* screening data indicated that all the analogs have the ability to protect against pentylenetetrazole-induced seizure (Table **1**).

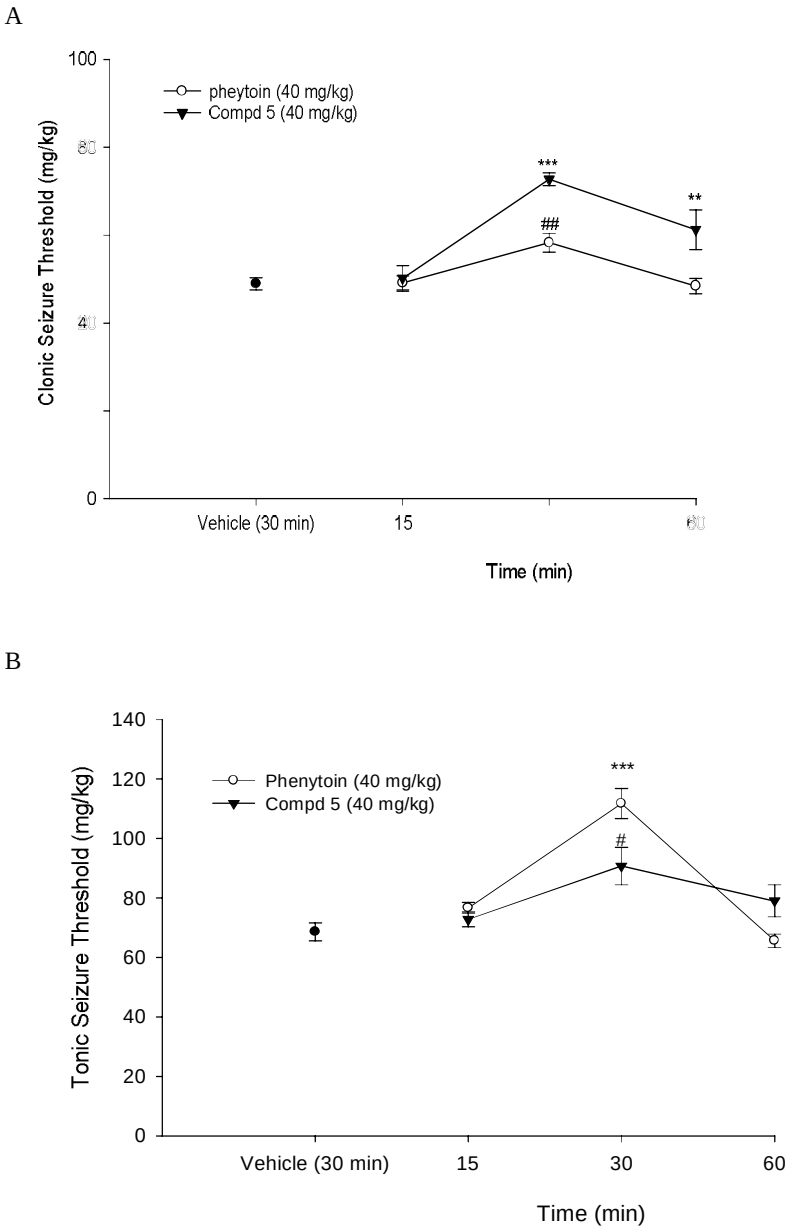


Fig. (6). The Time course of the Effects of Phenytoin (40 mg/kg) and Compound 5 (40 mg/kg) on tonic (A) and Clonic (B) Seizure Threshold by PTZ. Phenytoin or Compound 5 was Administered 15, 30 and 60 min before PTZ and their effect Compared to Vehicle (30 min before test). Data are Expressed as mean ± S.E.M. ***P*<0.01, ****P*<0.001, # *P*<0.01, ## *P*<0.001 Compared to Vehicle group (30 min).

Phenytoin did not prevent clonic seizure induced by PTZ in low doses (10, 20 mg/kg). Compounds 2, 5, 6, 9, 11, 12, and 13 elevated clonic seizure thresholds at 30 min but only compounds 6, 11, 12, and 13 showed significant anticonvulsant activity on tonic seizure (Figs. **7**-**10**). The most potent compound in both, tonic and clonic seizure was 1-naphthyl derivative (comp. 6), which was more active than the reference drug phenytoin.

The inhibition of benzodiazepine receptor by flumazenil, partially inhibited the anticonvulsant effects of compound 6 on both tonic and clonic PTZ-induced seizures (Fig. **11**), suggesting that probably GABA-A receptor can be an influential factor as well as Na channels properties [10, 11].

6. CONCLUSION

A Series of N-aryl phthalimide and 4-nitrophthalimide analogs were synthesized and characterized by TLC followed by IR and proton NMR. The elemental analysis has confirmed the purity of products. Ultimately, their ability to protect against pentylenetetrazole-induced seizure *in vivo* was investigated in mice. Based on our *in vivo* screening data all the synthesized analogs have the ability to protect against pentylenetetrazole-induced seizure. These compounds exerted their maximal effects after 30 min of administration. The most potent compound in both, tonic and clonic seizure was 1-naphthyl derivative (comp. 6), which was more active than the reference drug phenytoin.

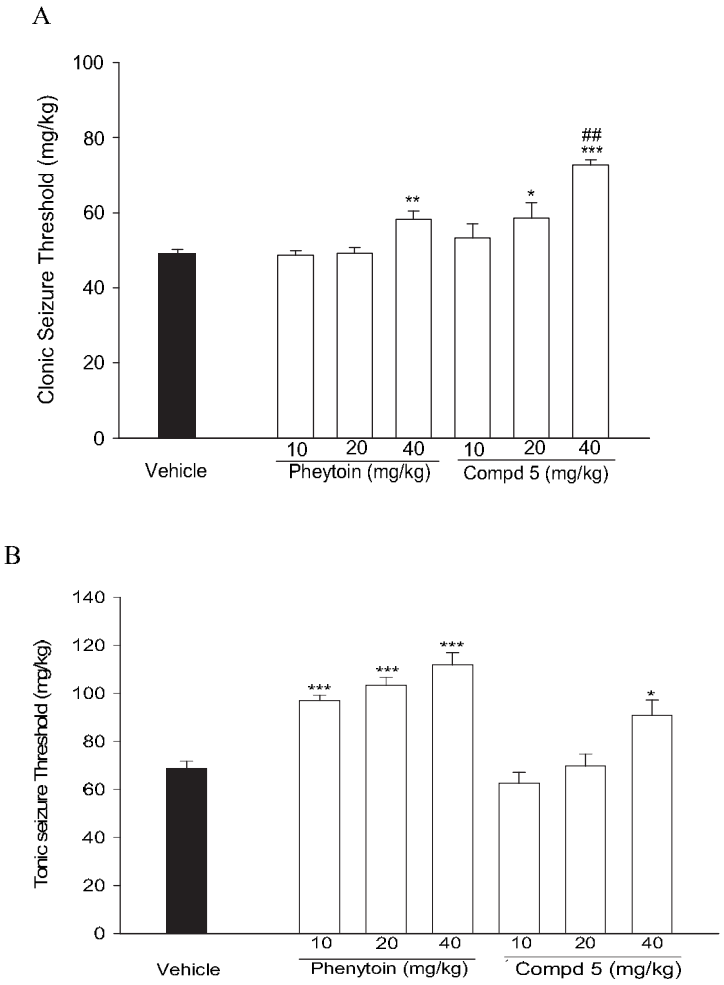


Fig. (7). Effect of Different doses of Phenytoin and Compound 5 on Clonic (**A**) and tonic (**B**) Seizure Threshold Induced by PTZ in Mice. Animal Received Vehicle or Drugs (10, 20, 40 mg/kg), 30 min before PTZ Administration. Data are Expressed as mean ±S.E.M. **P*<0.05, ***P*<0.01, ****P*<0.001 Compared to Vehicle, $^{\#\#}$ *P*<0. 01 Compared to Corresponding Phenytoin Group.

Docking studies revealed that all compounds interacted mainly with the domain II-S6 of NaV1.2 by making a hydrogen bond and have an additional hydrophobic interaction with domain I, II and III in the inner pore of the channel. All compounds (1-13), the oxygen of carbonyl group plays a major role in making hydrogen binding interactions with the OH of THR87. In the 4-nitrophthalimide derivatives, the oxygen of nitro group has an additional hydrogen binding interaction with the OH of SER84 or NH_2 of LYS7. The N-aryl part of phthalimide pharmacophore makes a hydrophobic interaction with the hydrophobic pocket of receptor, involving residues PHE84 and PHE91 of domains I and II respectively. The Aryl part of phthalimide has a weak interaction with the PHE91 of domain III. In compounds 6, 8, 9, 10, 11, 12 and 13, there is an efficient pi-pi interaction between N-aryl part and the aromatic ring of PHE84 of domain I.

The results so far revealed that the activity of these compounds against pentylenetetrazole-induced seizure can significantly be influenced by the size and hydrophobicity of these compounds. Therefore, for future studies, it is 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 better potency. Currently, our research group is exploring this idea for designing newer compounds with better anticonvulsant activities.

7. EXPERIMENTAL PROTOCOLS

7.1. Chemistry

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

7.2. 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 TMS which 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 with Hyperchem and Autodock4.2.

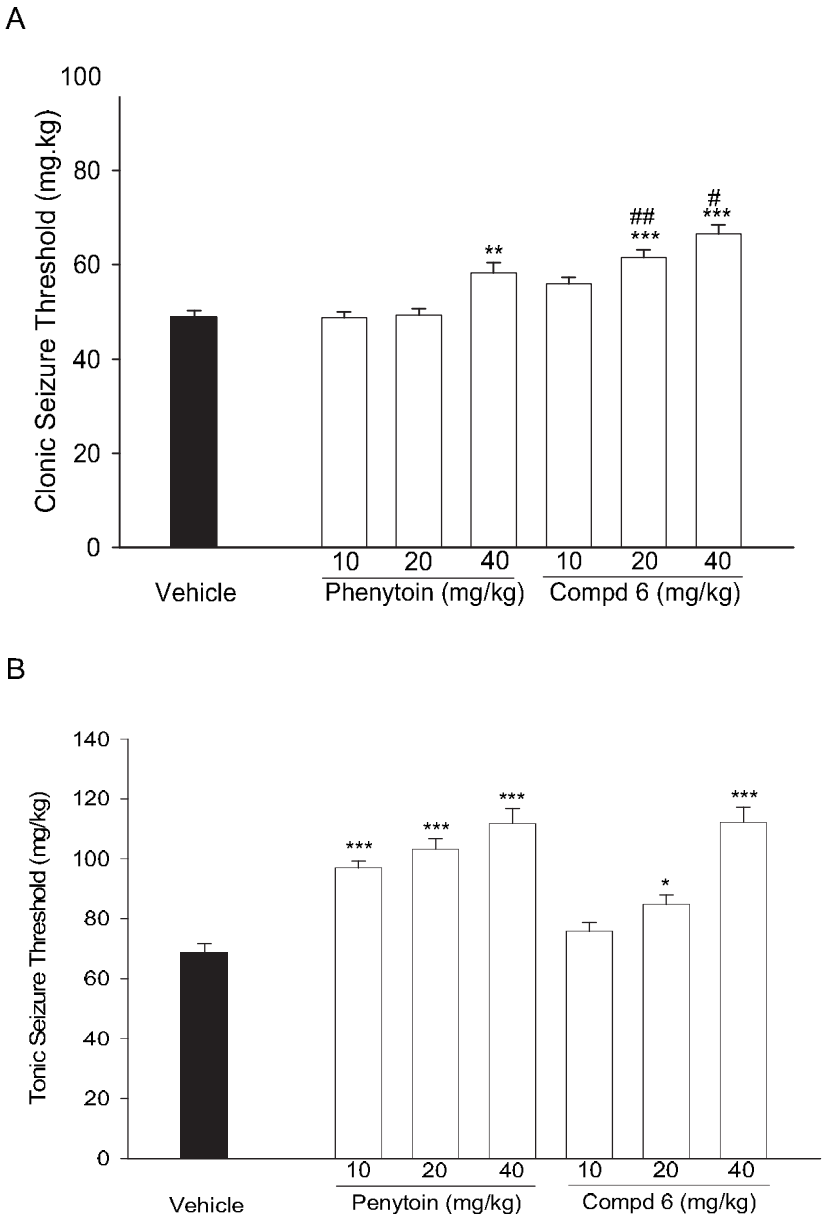


Fig. (8). Effect of Different doses of Phenytoin and Compound 6 on Clonic (**A)** and tonic (**B**) seizure threshold induced by PTZ in mice. Animal received vehicle or drugs (10, 20, 40 mg/kg), 30 min before PTZ administration. Data are expressed as mean ±S.E.M. **P*<0.05, ***P*<0.01, ****P*<0.001 compared to vehicle, $^{\#}$ *P*<0.05, $^{\#\#}$ *P*<0. 01 compared to corresponding phenytoin group.

7.3. Preparation Phthalimide Derivatives

7.3.1. 2-(5-methyl-1, 2-oxazol-3-yl)-1H-isoindole-1, 3(2H)-dione (1)

A mixture of 3-amino-5-methylisoxazol (380 mg, 3.88 mmol) and phthalic anhydride (575 mg, 3.88 mmol) in glassial acetic acid (8 mL) was stirred and heated under reflux for 10 h; the product of this reaction was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield of 1, 79 %; mp 193.5-195°C; ^{1}H NMR ($CDCl_3$+DMSO-d6) δppm 7.78-8.08(m,4H, aromatic), 6.52(s, 1H, H-4'), 2.50(s, 3H, CH3) ; IR(KBr, υ, cm-1) 3053(H-aromatic), 2914(H-aliphatic), 1790, 1726 (C= O phthalimide), Anal. ($C_{12}H_8N_2O_3$) C, H, N.

7.3.2. 2-(5-methyl-1, 2-oxazol-3-yl)-5-nitro-1H-isoindole-1, 3(2H)-dione (2)

A mixture of 3-amino-5-methylisoxazol (380 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) in glassial acetic acid (8 mL) was stirred and heated under reflux for 10 h; the product of this reaction was precipitated by addition of water, filtered, dried, and recrystallized from 95% ethanol: yield of 2, 54%; mp 195-198°C; ^{1}H NMR ($CDCl_3$+DMSO-*d*6) *δ*ppm 8.68-8.72(m, 2H, H-4 + H-6), 8.24(d, J=8.10 Hz, 1H, H-7), 6.55(s, 1H, H-4'), 2.52(s, 3H, CH_3); IR(KBr, *υ*, cm^{-1}) 3073(H-aromatic), 2924(H-aliphatic), 1798, 1736 (C= O phthalimide), 1547, 1347 (NO_2), Anal. ($C_{12}H_7N_3O_5$) C, H, N.

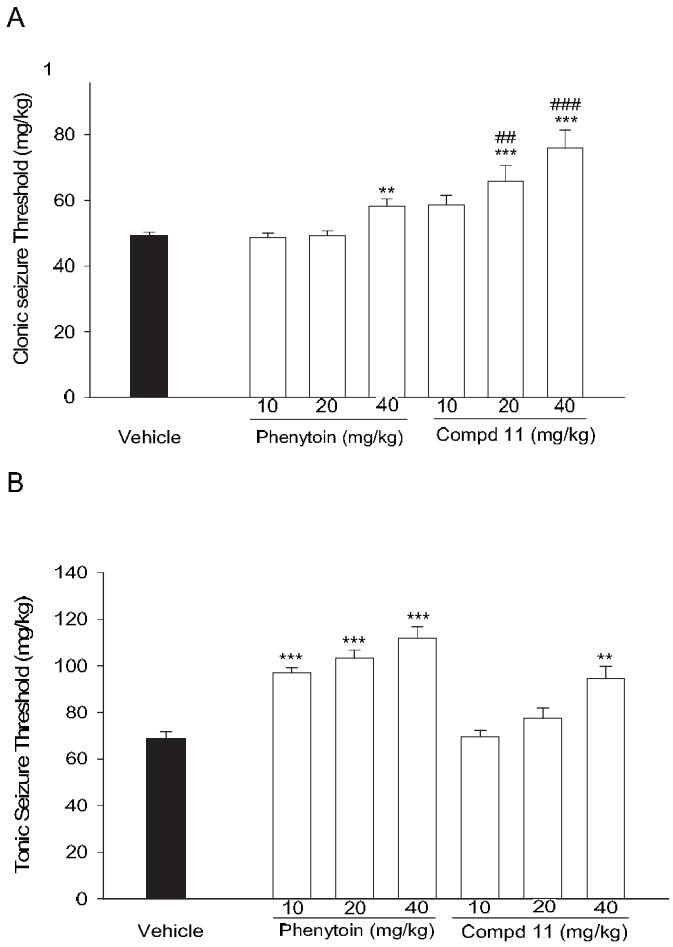


Fig. (9). Effect of Different doses of Phenytoin and Compound 11 on Clonic (A) and tonic (B) Seizure Threshold Induced by PTZ in Mice. Animal received vehicle or Drugs (10, 20, 40 mg/kg), 30 min before PTZ Administration. Data are Expressed as mean ±S.E.M. ***P*<0.01, ****P*<0.001 Compared to Vehicle, ## *P*<0.01, ### *P*<0.001 Compared to Corresponding Phenytoin Group.

7.3.3. 2-(1, 3-thiazol-2-yl)-1H-isoindole-1, 3(2H)-dione (3)

Using a procedure similar to that of **1**, 2-aminothiazole (389 g, 3.88 mmol) and phthalic anhydride (575 mg, 3.88 mmol) provided the title compound, after 21h and recrystallization from ethanol: yield 53%; mp 203-204°C; ^{1}H NMR ($CDCl_3$) δ ppm 7.75-8.02(m,4H, aromatic), 7.50-7.55(m, 2H, H-4' + H-5'); IR (KBr, υ, cm-1) 3109(H-aromatic), 1769, 1718, (C= O phthalimide), Anal ($C_{11}H_5N_3O_4S$) C, H, N.

7.3.4. 5-nitro-2-(1, 3-thiazol-2-yl)-1H-isoindole-1, 3(2H)-dione (4)

Using a procedure similar to that of **2**, 2-aminothiazole (389 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 18h and recrystallization from ethanol: yield 45%; mp 213-217 °C; ^{1}H NMR ($CDCl_3$) δ ppm 8.65-8.77(m, 2H, H-4 + H-6), 8.40(d, J=8.0 Hz, 1H, H-7), 7.50-7.55(m, 2H, H-4' + H-5'); IR (KBr, υ, cm^{-1}) 3114(H-aromatic), 1772, 1721, (C= O phthalimide), 1536, 1378, (NO_2). Anal($C_{11}H_5N_3O_4S$) C, H, N.

7.3.5. 2-(biphenyl-2-yl)-1H-isoindole-1, 3(2H)-dione (5)

Using a procedure similar to that of **1**, 2-aminobiphenyl (657 mg, 3.88 mmol) and phthalic anhydride (575 mg, 3.88 mmol) provided the title compound, after 15h and recrystallization from ethanol: yield 63%; mp 217-219 °C; ^{1}H NMR ($CDCl_3$) δ ppm 7.24-7.81 (m, 13H, aromatic) ; IR(KBr, υ, cm^{-1}) 3067 (H-aromatic)1782, 1710 (C= O phthalimide), Anal. ($C_{20}H_{13}NO_2$) C, H, N.

7.3.6. 2-(naphthalen-1-yl)-5-nitro-1H-isoindole-1, 3(2H)-dione (6)

Using a procedure similar to that of **2**, 1-aminonaphthalen (556 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 8h and recrystallization from ethanol: yield 78%; mp 224-227 °C; ^{1}H NMR ($CDCl_3$) δ ppm 8.67-8.81(m, 2H, H-4 + H-6), 8.19(d, J=8.0 Hz, 1H, H-7), 7.39-7.97(m, 7H, H-naphthyl); IR(KBr, υ, cm^{-1}) 3098, 3052(H-aromatic), 1770, 1731 (C= O phthalimide), 1536, 1367 (NO_2), Anal. ($C_{18}H_{12}N_2O_4$) C, H, N.

7.3.7. 2-(2-phenylethyl)-1H-isoindole-1, 3(2H)-dione (7)

Using a procedure similar to that of **1**, 2-phenylethanamine (471 mg, 3.88 mmol) and phthalic anhydride (575 mg, 3.88 mmol) provided the title compound, after 8 h and recrystallization from ethanol: yield 61%; mp 135-138 °C; ^{1}H NMR ($CDCl_3$) δ ppm 7.82(dd, J= 5.9 Hz, 3.2 Hz, 2H,

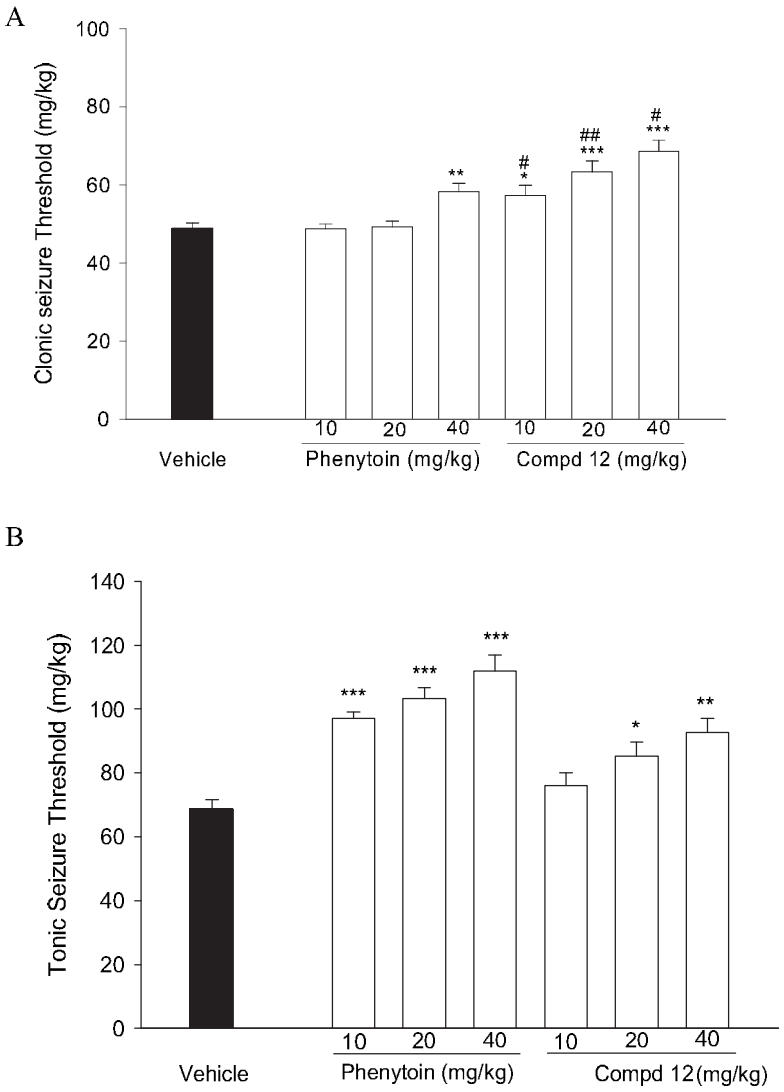


Fig. (10). Effect of Different doses of Phenytoin and Compound 12 on Clonic (**A**) and tonic (**B**) Seizure Threshold Induced by PTZ in Mice. Animal received vehicle or Drugs (10, 20, 40 mg/kg), 30 min before PTZ Administration. Data are Expressed as mean ± S.E.M. **P*<0.05, ***P*<0.01, ****P*<0.001 Compared to vehicle, $^{\#}$ *P*<0.05, $^{\#\#}$ *P*<0. 01 Compared to Corresponding Phenytoin Group.

H-4 + H-7), 7.70(dd, J= 5.9 Hz, 3.5 Hz, 2H, H-5 + H-6), 7.20(m, 5H, phenyl), 3.92(t, J= 7.6 Hz, 2H, NCH_2), 2.99(t, J= 7.6 Hz, 2H, NCH_2CH_2); IR(KBr, υ, cm^{-1}) 3050(H-aromatic), 2934,2950(H-aliphatic), 1771, 1696(C= O phthalimide), Anal. ($C_{16}H_{13}NO_2$) C, H, N.

7.3.8. 5-nitro-2-(2-phenylethyl)-1H-isoindole-1, 3(2H)-dione (8)

Using a procedure similar to that of **2**, 2-phenylethanamine (471 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 8 h and recrystallization from ethanol: yield 57%; mp 137-140 °C; ^{1}H NMR (DMSO-d6) δ ppm 8.61(dd, J= 8.0 Hz, 1.6 Hz, 1H, H-6), 8.47(d, J= 1.6 Hz, 1H, H-4), 8.10(d, J= 8.0 Hz, 1H, H-7), 7.20-7.40(m, 5H, phenyl), 3.92(t, J= 7.6 Hz, 2H, NCH_2), 2.99(t, J= 7.6 Hz, 2H, NCH_2CH_2) ; IR(KBr, υ, cm^{-1}) 3115, 3050(H-aromatic), 2944(H-aliphatic), 1772, 1709 (C=O phthalimide),1544, 1349 (NO_2), Anal. ($C_{16}H_{12}N_2O_4$) C, H, N.

7.3.9. 5-nitro-2-(pyridin-3-yl)-1H-isoindole-1, 3(2H)-dione (9)

Using a procedure similar to that of **2**, 3-aminopyridine (370 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 15h and re-crystallization from ethanol: yield 75%; mp 209-212 °C; ^{1}H NMR ($CDCl_3$ + DMSO-*d*6) δ ppm 8.41-8.79(m, 3H, H-4 + H-6 + H-2'), 8.15-8.28(m, 2H, H-7 + H-6'), 7.75-7.90(m, 2H, H-4' + H-5'); IR(KBr, υ, cm^{-1}) 3130(H-aromatic), 1772, 1721 (C= O phthalimide), 1536, 1347(NO_2), Anal. ($C_{13}H_7N_3O_4$) C, H, N.

7.3.10. 5-nitro-2-(pyridin-4-yl)-1H-isoindole-1, 3(2H)-dione (10)

Using a procedure similar to that of **2**, 4-aminopyridine (370 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 11h and recrystallization from ethanol: yield 51%; mp 213-215 °C; ^{1}H NMR ($CDCl_3$ + DMSO-*d*6) δ ppm 8.50-8.77(m, 5H, H-4 + H-6 + H-7 + H-2' + H-6'), 8.11-8.21(m, 2H, H3' + H-5');

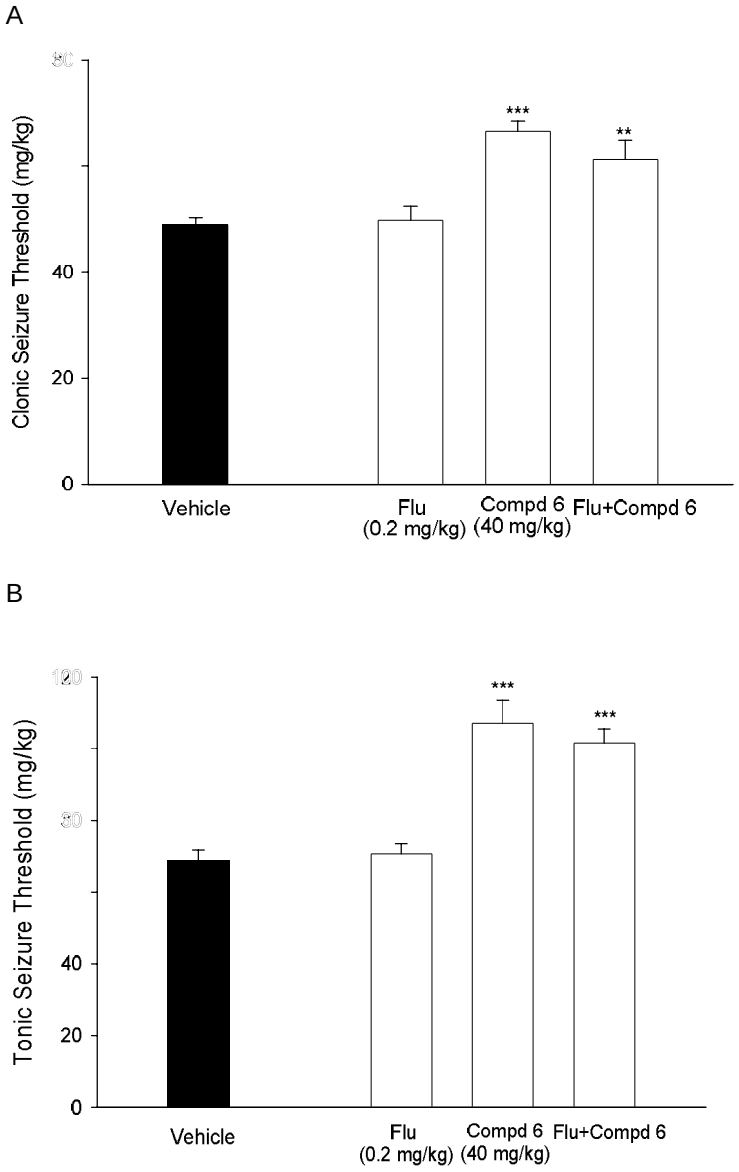


Fig. (11). Effect of Benzodiazepine Receptor Antagonist Flumazenil (0.2 mg/kg) on Anticonvulsant effect of Compound 6, (A): Clonic seizure threshold and (B) tonic seizure threshold. Flumazenil was Administered 15 min before Compound 6(40 mg/kg) or its vehicle and 45 min before PTZ. Data are Expressed as mean ± S.E.M. ***P*<0.01, ****P*<0.001 Compared to Vehicle.

H-6 + H-7 + H-2' + H-6'), 8.11-8.21(m, 2H, H3' + H-5'); IR(KBr,υ, cm^{-1}) 3108(H-aromatic), 1782, 1726(C= O phthalimide), 1536, 1347 (NO_2), Anal. ($C_{13}H_7N_3O_4$)C, H, N.

7.3.11. 5-nitro-2-(pyridin-2-yl)-1H-isoindole-1, 3(2H)-dione (11)

Using a procedure similar to that of **2**, 2-aminopyridine (370 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 12h and recrystallization from ethanol: yield 59%; mp 202-205 °C; ^{1}H NMR ($CDCl_3$) δ ppm 8.65-8.78(m, 3H, H-4 + H-6 + H-7), 8.17(dd, J=1.6Hz 7.6Hz, 1H, H-6'), 7.93(dd, J=2.0Hz, 8.10Hz, 1H, H-4'), 7.41-7.50(m, 2H, H-3' + H-5'); IR(KBr, υ, cm-1) 3097, 3047(H-aromatic), 1772, 1710(C= O phthalimide), 1541, 1378(NO_2), Anal. ($C_{13}H_7N_3O_4$) C, H, N.

7.3.12. 2-(5-chloropyridin-2-yl)-5-nitro-1H-isoindole-1, 3(2H)-dione (12)

Using a procedure similar to that of **2**, 5-chloro-2-aminopyridine (500 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88 mmol) provided the title compound, after 12h and recrystallization from ethanol: yield 61%; mp 189-192 °C; ; ^{1}H NMR ($CDCl_3$) δ ppm 8.62-8.77(m, 3H, H-4 + H-6 + H-6'), 8.17(d, J=8.01Hz, 1H, H-7), 7.90(dd, J=2.50Hz, 8.43Hz, 1H, H-4'), 7.43(d, J=8.49Hz, 1H, H-3') ; IR(KBr, υ, cm^{-1}) 3093, 3037(H-aromatic), 1765, 1726 (C= O phthalimide), 1536, 1388(NO_2), Anal. ($C_{13}H_6ClN_3O_4$)C, H, N.

7.3.13. 5-nitro-2-phenyl-1H-isoindole-1, 3(2H)-dione (13)

Using a procedure similar to that of 2, aniline (361 mg, 3.88 mmol) and 4-nitrophthalic anhydride (750 mg, 3.88

mmol) provided the title compound, after 10h and recrystallization from ethanol: yield 70%; ^{1}H NMR (DMSO-d6) δ ppm 8.65(dd, J= 8.0 Hz, 1.6 Hz, 1H, H-6), 8.50(d, J= 1.6 Hz, 1H, H-4), 8.15(d, J= 8.0 Hz, 1H, H-7), 7.50-7.60(m, 5H, phenyl), ; IR (KBr, υ, cm-1) 3090(H-aromatic), 1778, 1711 (C= O phthalimide),1542, 1347 (NO_2), Anal. ($C_{14}H_8N_2O_4$) C, H, N.

7.4. Molecular Modeling and Docking

The chemical structure of desired phthalimides (1-13) was built by using HYPERCHEM software (version 7, Hypercube Inc.). Conformational analysis of the designed compounds was performed through semi-empirical molecular orbital calculations (PM3) method using the HYPERCHEM software. Among all energy minima conformers, the global minimum of compounds 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 [16]; we have docked these 13 compounds and phenytoin as a reference drug. In order to assign the perfect grid of each ligand, grid box values that were obtained by trial and error and previous study by Gregory M. *et al.* [8, 16]. Finally docking was performed using the implemented Lamarckin GL and the default parameters and ten independent docking runs were performed for each phthalimide.

7.5. Pharmacology, Determination of Anticonvulsant Activity

7.5.1. 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 [17].

7.5.2. 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 less than 0.05 were considered as indicative of significance.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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