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Article *in* Pharmaceutical Chemistry Journal · June 2014

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Pharmaceutical Chemistry Journal, Vol. 48, No. 3, June, 2014 (Russian Original Vol. 48, No. 3, March, 2014)

DOCKING AND SYNTHESIS OF 2-ARYLISOINDOLINE-1,3-DIONE DERIVATIVES AS ANTICONVULSANT AGENTS

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Original article submitted April 29, 2013.

Antiepileptic drugs are used to prevent or reduce the occurrence of epileptic seizures but up to 30% of patients are resistant to the available medical therapies. Ten analogs of *2*-aryl substituent of isoindoline-1,3-dione were synthesized and evaluated for their anticonvulsant activities. The in vivo screening data acquired indicate that all the analogs have the ability to protection against pentylenetetrazole-induced Seizure. These compounds exerted their maximal effects 30 min after administration. The most potent compound was 4-triazoly derivative (compound VIII). Using a model of the open pore of the Na channel**,** we have docked compound VIII. Docking studies has revealed that this ligand interact mainly with residues II-S6 of NaV1.2 by making hydrogen bonds and have additional hydrophobic interactions with domain I and II in the channel's inner pore.

Keywords: Anticonvulsant, Arylisoindoline, Design, Phenytoin, Seizure**.**

Approximately 1% of the world's population (~50 million people) is affected by epilepsy, a serious neurological disorder that typically manifests as spontaneous convulsions and/or a loss of consciousness [1]. Most antiepileptic drugs are associated with adverse effects, such as sedation, ataxia and weight loss (e.g. topiramate) or weight gain (e.g. valproate, tiagabine, and vigabatrin). Rare adverse effects can be life threatening such as rashes leading to Stevens-Johnson syndrome (e.g. lamotrigine) or aplastic anaemia (e.g. felbamate) [2]. As about 30% of people affected by epilepsy have uncontrolled seizures, the development of safer and more effective new antiepileptic drugs (AEDs) is necessary. Despite the excitement that has accompanied the launch of new alternative drugs in the last 20 years; they have made little improvement on the number of patients who suffer from chronic and refractory epilepsy [1, 3, 4].

The anticonvulsant drugs such as phenytoin and carbamazepine that typically have a tricyclic structure, with a polar amide in the middle (Figure 1), block neuronal voltage-gated Na channels, that their binding sites is domain IV-S6 in the channel's inner pore [5, 6]. Docking studies of these drugs reveal that they show a common pharmacophore, including an aromatic ring and a polar amide or imide. The second aromatic ring is at almost right angles to the pharmacophore and hydrophobic interactions with this second aromatic ring may contribute an important component to binding for anticonvulsants [5, 6].

Phthalimide pharmacophore is one of the new ligand that acts as sodium channel antagonist with a phenytoin-like profile, which designed and evaluated as anticonvulsant agents [7 – 9]. Indeed this pharmacophore, same as phenytoin, have a tricyclic structure and they show a common pharmacophore, including an aromatic ring, a polar imide and a second aromatic ring (Figure 1). The ability of arylisoindoline pharmacophore to interact with neuronal voltage-dependent sodium channels was studied in the batrachotoxin affinity assay [7]. In the previous study we have reported the new phthalimide derivatives with good anticonvulsant activity in the pentylenetetrazole (PTZ) test [9], Based on the efficient interactions of second aromatic ring [7] that contribute an important component to binding, and also some of arylisoindoline derivatives were relatively active [7 – 14], to extend the SAR of this pharmacophore, and finding their profile in drug-receptor interaction we undertook to design and synthesis of a new series of 2-arylisoindoline-1,3-dione derivatives.

A group of 2-aryl derivatives of the isoindoline-1,3-dione (I-X), possessing a variety of substituent at the 2′-, 3′-, 4′ and 5′ -positions of the isoindoline ring, were synthesized

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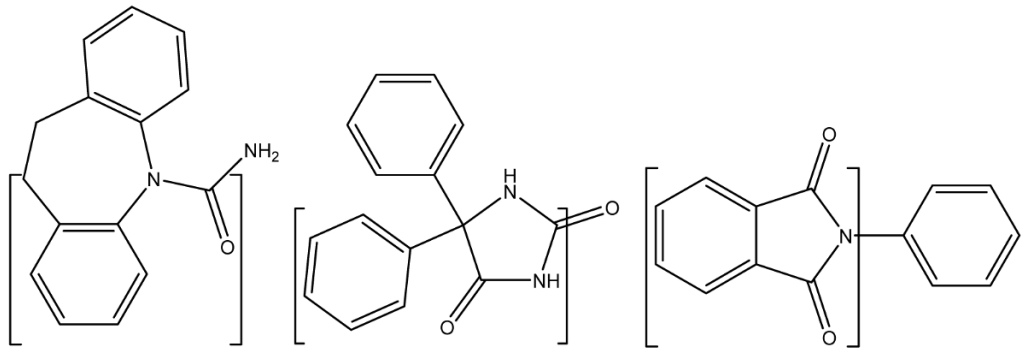

Carbamazepine Phenytoin isoindoline

Fig. 1. Carbamazepine, phenytoin, and isoindoline pharmacophre that typically have a tricyclic structure, with a polar amide or imide in the middle.

by condensation of the respective aromatic amine (homocycle or hetrocycle) with phthalic anhydride in acetic acid at reflux temperature (Figure 2).

EXPERIMENTAL PART

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

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 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 Autodock 4.2.3.

General procedure for preparation of 2-arylisoindoline-1,3-dione derivatives (1 – 10)

A solution of phthalic anhydride (0.5 g, 3.73 mmol) and arylamine (3.73 mmol) in glassial 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 from 95% ethanol to give desired compound.

2-(6-methylpyridin-2-yl)isoindoline-1,3-dione (I)

Using the general procedure and 2-amino-6-methylpyridine provided the title compound after 12 h of reflux: White crystals, yield 38%; mp 193 – 193.5°C (ethanol). IR (KBr): í cm^{-1} , 3086 (CH-aromatic), 2920 (CH-aliphatic), 1774, 1720 (CO); ^{1}H NMR (DMSO-d_6): δ 7.99 – 7.97 (m, 2H, aromatic), 7.95 – 7.91 (m, 3H, aromatic), 7.39 (d, J = 7.64 Hz, 1H, H_3-pyridine), 7.36 (d, J = 7.73 Hz, 1H, H_5-pyridine), 2.51 ppm (s, 3H, CH_3). $C_{14}H_{10}N_2O_2$.

2-(4-methylpyridin-2-yl)isoindoline-1,3-dione (II)

Using the general procedure and 2-amino-4-methylpyridine provided the title compound after 18 h of reflux: White crystals, yield 71%; mp 218 – 222°C (ethanol). IR (KBr): ν cm^{-1} 3062 (CH-aromatic), 2909 (CH-aliphatic), 1721, 1777(CO); ^{1}H NMR ($CDCl_3$): δ 8.54 (d, J = 7.20 Hz, 1H, aromatic), 8.03 – 7.73 (m, 4H, aromatic), 7.22 – 7.16 (m, 2H, aromatic), 2.45 ppm (s, 3H, CH_3). $C_{14}H_{10}N_2O_2$.

2-(2-chloropyridin-3-yl) isoindoline-1,3-dione (III)

Using the general procedure and 3-amino-2-chloropyridine provided the title compound after 7 h of reflux: White crystals, yield 61%; mp 152 – 155°C (ethanol). IR (KBr): ν cm^{-1} 3059 (CH-aromatic), 1722, 1782 (CO).; ^{1}H NMR (CDCl3): δ 8.57 – 8.49 (dd, J = 1.9, 2.8 Hz, 1H, H_6-pyridine), 8.07 – 7.78 (m, 4H, aromatic), 7.69 (d, J = 1.9 Hz, 1H, H_4-pyridine), 7.51 – 7.35 ppm (dd, J = 3, 4.7 Hz, 1H, H_5-pyridine).; Mass *m/z* (rel. int): 257 (M^+, 78), 223 (100), 194 (13), 178 (65), 166 (84), 140 (44), 103 (88), 76 (90), 50 (74). $C_{13}H_7ClN_2O_2$.

2-(5-chloropyridin-2-yl)isoindoline-1,3-dione (IV)

Using the general procedure and 2-amino-5-chloropyridine provided the title compound after 9 h of reflux: White crystals, yield 57%; mp 149 – 152°C (ethanol). IR (KBr): ν cm^{-1} 3103, 3027 (CH-aromatic), 1726, 1757 (CO). ^{1}H NMR ($CDCl_3$): δ 8.64 – 8.61 (d, J = 2.5 Hz, 1H, H_6-pyridine), 8.04 – 7.74 (m, 5H, aromatic), 7.47 – 7.37 (d, J = 8.5Hz, 1H, H_3-pyridine). $C_{13}H_7ClN_2O_2$.

2-(pyridin-3-yl)isoindoline-1,3-dione (V)

Using the general procedure and 3-aminopyridine provided the title compound after 10 h of reflux: White crystals, yield 89%; mp 239 – 241°C (ethanol). IR (KBr): ν cmcm^{-1}, 3098 (CH-aromatic), 1726, 1755 (CO).; ^{1}H NMR (DMSO-d_6): δ 8.41 (s, 1H, H_2-pyridine), 8.3 – 8.2 (m, 3H, aromatic) 7.94 – 7.72 (m, 4H, aromatic).; Mass *m/z* (rel. int): 224 (M+, 42), 165 (20), 139 (14), 102 (50), 75 (100). $C_{13}H_8N_2O_2$.

2-(naphthalen-1-yl)isoindoline-1,3-dione (VI)

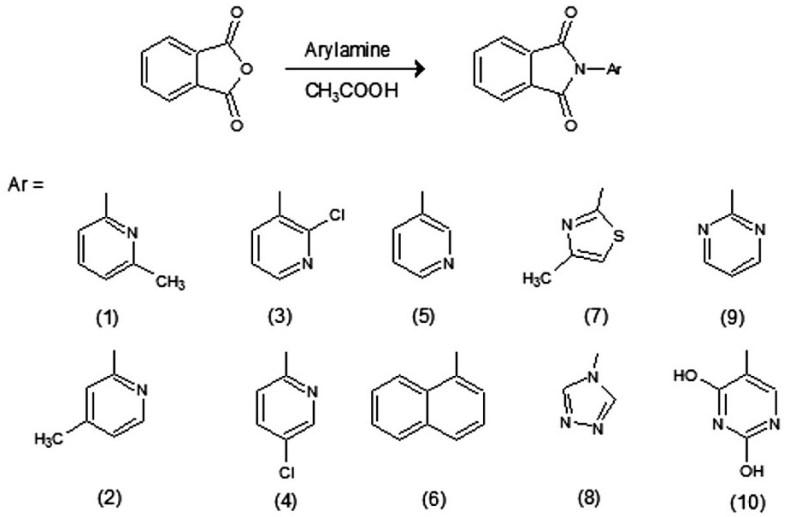

Fig. 2. Preparation of new derivatives of isoindoline pharmacophore.

Using the general procedure and 1-aminonaphthalen provided the title compound after 3 h of reflux: White crystals, yield 73%; mp 182 – 185°C (ethanol). IR (KBr): ν cmcm-1, 3062 (CH-aromatic), 1722, 1780 (CO); ^{1}H NMR (DMSO-d_6): δ 8.08 – 7.77 (m, 6H, aromatic), 7.70 – 7.42 (m, 5H, aromatic). $C_{18}H_{11}NO_2$.

2-(4-methylthiazol-2-yl)isoindoline-1,3-dione (VII)

Using the general procedure and 2-amino-4-methyl-1,3-thiazole provided the title compound after 14 h of reflux: brown crystals, yield 55%; mp 143°C (methanol-ethyl acetate). IR (KBr): ν cmcm^{-1}, 3058 (CH-aromatic), 2980 (CH-aliphatic), 1785, 1749 (CO).; ^{1}H NMR (DMSO-d_6): δ 8.012 – 7.987 (m, 2H, aromatic), 7.954 – 7.926 (m, 2H, aromatic), 7.341 (s, 1H, thiazole), 2.397 (s, 3H, CH_3). $C_{12}H_8N_2O_2S$.

2-(4H-1,2,4-triazol-4-yl)isoindoline-1,3-dione (VIII)

Using the general procedure and 4-amino-1,2,4-triazole provided the title compound after 10 h of reflux: White crystals, yield 55%; mp 269 – 270°C (ethanol). IR (KBr): ν cmcm^{-1}, 3127 (CH-aromatic), 1801, 1755 (CO); ^{1}H NMR (DMSO-d_6): δ 8.877(s, 2H, triazole), 8.104 – 8.087 (m, 2H, aromatic), 8.022 – 8.005 (m, 2H, aromatic); $C_{10}H_6N_4O_2$.

2-(pyrimidin-2-yl)isoindoline-1,3-dione (IX)

Using the general procedure and 2-aminopyrimidine provided the title compound after 12 h of reflux: White crystals, yield 38%; mp 221 – 221. 5°C (ethanol). IR (KBr) : ν cm^{-1}, 3099, 3047 (CH-aromatic), 1789, 1758.(CO).; ^{1}H NMR (DMSO-d_6): δ 9.046 (d, J = 4.89 Hz, 2H, H_4, H_6-pyrimidine), 8.043 – 8.018 (m, 2H, aromatic), 7.982 – 7.957(m, 2H, aromatic), 7.698 ppm (t, J = 4.86 Hz, 1H, H_5-pyrimidine).; ^{13C} NMR (DMSO-d_6): δ 166.529(CO), 160.578, 153.642, 136.300, 131.852, 124.937, 122.380. $C_{12}H_7N_3O_2$.

2-(2,4-dihydroxypyrimidin-5-yl)isoindoline-1,3-dione (X)

Using the general procedure and 5-amino-2,4-dihydroxypyrimidine provided the title compound after 14 h of reflux: White crystals, yield 86%; mp > 300°C (ethanol). IR (KBr): ν cm^{-1}, 3538 (OH), 3081, 3036 (CH-aromatic), 1785, 1725.(CO).; ^{1}H NMR (DMSO-d_6): δ 11.608 (s, 1H, OH), 11.363 (d, J = 5.2 Hz, 1H, OH), 7.99 – 7.96 (m, 2H, aromatic), 7.94 – 7.92 (m, 2H, aromatic), 7.87 (d, J = 6.13 Hz, 1H, pyrimidine). $C_{12}H_7N_3O_4$.

Pharmacology, determination of anticonvulsant activity

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 wa-

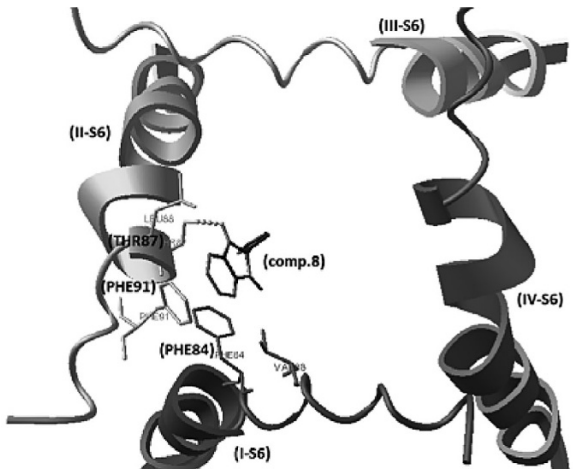

Fig. 3. Docked structure of VIII in Model of sodium channel (Nav1.2) (top view). The backbones of S6 á-helices of domains I – IV are shown by red, green, yellow, and violet, correspondingly. Hydrogen bond is represented with dashed green lines.

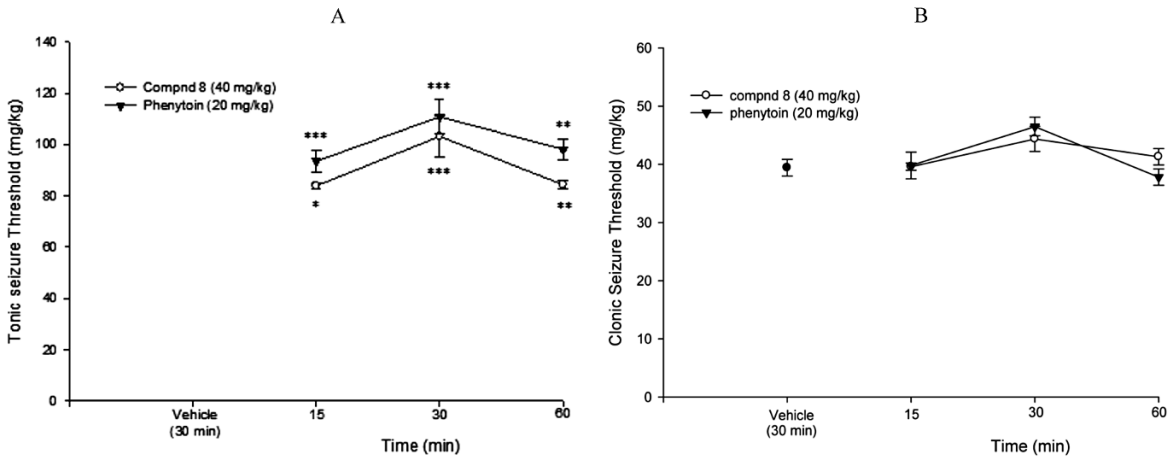

Fig. 4. The time course of the effects of phenytoin (20 mg/kg) and compound VIII (40 mg/kg) on tonic (A) and clonic (B) seizure threshold by PTZ. Phenytoin or Compound VIII was administered 15, 30 and 60 min before PTZ and their effect compared to vehicle (30 min before test). Data are expressed as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle.

ter except for the short time that 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.

Anticonvulsant evaluation in the PTZ test (Clonic and tonic convulsions) was performed as described previously [9].

TABLE 1. The ability of isoindoline derivatives (I – X, 40 mg/kg) to protection against pentylenetetrazole-induced Seizure.

Compound	CST [a]	TST [b]
I	44.64 ± 2.42	72.61 ± 4.67
II	43.52 ± 2.22	66.92 ± 3.56
III	42.34 ± 2.92	57.9 ± 5.56
IV	43.39 ± 6.63	65.52 ± 5.83
V	45.3 ± 2.91	58.8 ± 3.75
VI	48.37 ± 2/11	96.89 ± 5.65**
VII	39.52 ± 2.78	62.46 ± 5.44
VIII	44.35 ± 2.16	103.18 ± 3.94***
IX	43.72 ± 2.95	69.25 ± 8.8
X	47.72 ± 2.15	65.94 ± 3.43
Vehicle	38.9 ± 1.01	59.58 ± 1.01
Phenytoin (20mg/kg)	49.20 ± 1.09	115.00 ± 1.85***

[a] Colonic seizure threshold, [b] Tonic seizure threshold
Data are expressed as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle

Computational study

Using HyperChem and Autodock software (version 4.2.3) and a model of the open pore of the Na channel that has been developed by homology with the crystal structures of K channels [6], conformational analysis and docking studies were performed as described previously [9].

RESULTS AND DISCUSSION

Chemistry

10 derivative of 2-arylisoindoline-1,3-dione analogs were synthesized in 37 – 86% yield based on method that shown in Figure 2. All of compounds characterized by TLC followed by IR, 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. Already, we have reported while phenytoin interacted with the domain IV-S6 of NaV1.2 [9], compound 8, interacted mainly with the domain II-S6. Oxygen of imide play main role in drug-receptor interaction by making hydrogen bond with the OH of Thr87 (distance = 1.626, energy = -0.506) (Figure 3).

Aryl part of phthalimide pharmacophore forms a hydrophobic interaction with the hydrophobic pocket of receptor that created by PHE84 and PHE91 of domains I, II respectively (Figure 3). Triazole moiety orientated to the center of channel.

The energies of interaction of 8 with the side chains of Thr87 and Phe84 of domain IIS6 and Phe91 of domain IS6 are high and alanine-scanning mutagenesis has clearly shown that these three amino acid residues are important, allowing

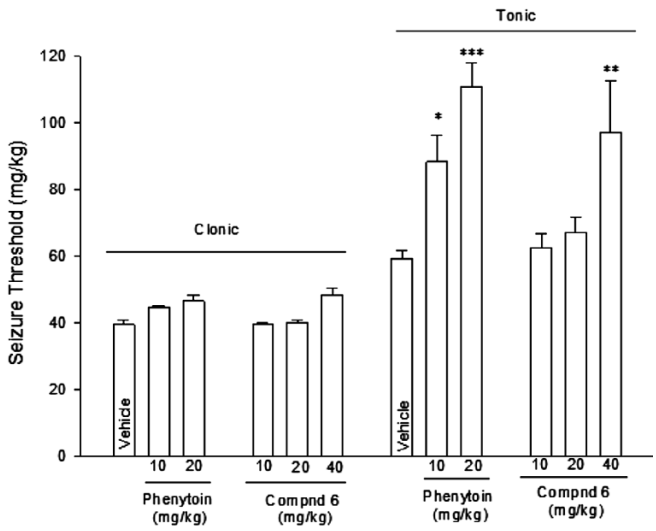

Fig. 5. Effect of different doses of phenytoin and compound VI on tonic and clonic seizure threshold induced by PTZ in mice. Animal received vehicle or drugs (10, 20, 40 mg/kg), 30 min before PTZ administration. Data are expressed as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle.

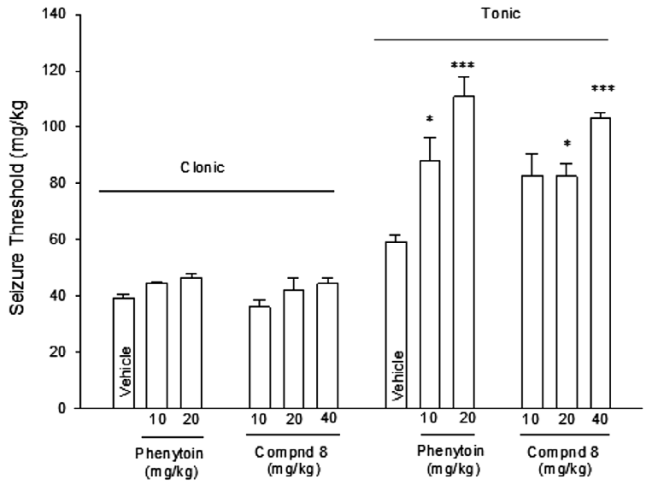

Fig. 6. Effect of different doses of phenytoin and compound VIII on tonic and clonic seizure threshold induced by PTZ in mice. Animal received vehicle or drugs (10, 20, 40 mg/kg), 30 min before PTZ administration. Data are expressed as mean ± S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to vehicle.

us to propose that they are essential to the channel′s recognition of the anticonvulsant structures and that they orient the rigid drug structures at the interface cleft between domains I and II.

Protection against pentylenetetrazole-induced Seizure

The ability of this group of isoindoline (I-X) to protection against pentylenetetrazole-induced Seizure, tonic and colonic, was determined using an in vivo assay, and the results are summarized in table.

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 that data for compound 8 has been shown in (Figure 4).

Further analysis showed that all compounds and phenytoin exerted their maximal effects 30 min after administration. The in vivo screening data acquired indicate that all the analogs have the ability to protection against pentylenetetrazole-induced Seizure (Table 1).

Phenytoin as a standard did not inhibit clonic seizure evoked by PTZ in lower doses (10, 20 mg/kg). Compounds VI and X elevated clonic seizure thresholds at 30 min but only compounds VI and VIII showed marked anticonvulsant activity on tonic seizure (Figures 5 – 6). The most potent compound was 4-triazoly derivative (comp. VIII), that had comparative activity to the reference drug phenytoin.

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.

CONCLUSION

A number of 2-arylisoindoline-1,3-dione analogs were synthesized, characterized by TLC followed by IR, elemental analysis and proton NMR and tested for their ability to protection against pentylenetetrazole-induced Seizure in vivo in mice. The in vivo screening data acquired indicate that all the analogs have the ability to protection against pentylenetetrazole-induced Seizure. These compounds exerted their maximal effects 30 min after administration. The most potent compound was 1-triazole derivative (comp. VIII).

Docking studies reveals this pharmacophore 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 that oxygen of carbonyl group plays a main role in creating hydrogen binding interaction with the OH of THR87. Aryl parts of isoindoline pharmacophore form a hydrophobic interaction with the hydrophobic pocket of receptor that created by PHE84 and PHE91 of domains I and II respectively and triazole has orientated to the center of channel.

For future studies, it is recommended that the isoindoline part should remain as it is and, in order to achieve a better potency, the 2-aryl part should be replaced with other aro-

matic moieties. Currently, our research group is exploring this idea for designing newer compounds with better anticonvulsant activities.

ACKNOWLEDGMENTS

We would like to thank Professor Arthur J. Olson and Professor A. Fozzard for their kindness in providing us with the AutoDock 4.2 program and model of sodium channel. Moreover, we appreciate the technical assistance of the Medicinal Chemistry Department of Azad University, Pharmaceutical Sciences Branch, in performing the computational studies.

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