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Synthesis, in vivo and in silico Studies of N-aryl-4-(1,3-dioxoisoindolin-2-yl)benzamides as an Anticonvulsant Agent

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ABSTRACT

Background: These days epilepsy is common neurological disorder, which can affect on quality of life by unpredictable seizure. Thalidomide is one of the drugs to control the epilepsy but side effects such as teratogenicity, made it difficult to use.

Methods: Six new analogues of N-aryl-4-(1,3-dioxoisoindolin-2-yl)benzamides were synthesized and tested for anti-seizure activity. To evaluate the anti-seizure activity of these new derivatives, 40 mice in 8 groups were received 10 mg/Kg of each new derivatives 30 min before the injection of pentylenetetrazole (PTZ, 70 mg/Kg) to induced seizures. Latency time to first symptom of seizure was measured and compared to vehicle and standard groups. Docking methodology was applied to study on mode of interaction between $GABA_A$ receptor and synthetized compounds.

Results: Structures of the all synthesized compounds were confirmed by NMR and mass spectroscopy. The latency time and mortality rate were individually measured for an hour after injection of pentylenetetrazole. Docking study revealed that synthesized compounds and thalidomide interact in similar conformation with $GABA_A$ receptor.

Conclusion: The experimental and docking results were found in good correlation and demonstrate that the most active compound (5a), with 3,4-dimethylphenyl residue increased the duration of seizure inhibition threshold in comparison with thalidomide.

Keywords:

Epilepsy

Molecular docking simulation

Phthalimide

Seizures

Thalidomide

Introduction

Recent development in epilepsy treatment have been raised due to need to an appropriate drug which has the least side effects. Epilepsy is a seizure that apparently occurs without a specific cause, but it is an abnormality in the body's nervous system that causes epileptic and non-epileptic seizures such as hypoglycemia, fever, hypotension, migraine and many others.[1-2] Side effects of drugs are the main reasons of patient dissatisfaction. For this reason, more studies are necessary to reduce drug side effects and enhance treatment quality. One of the most impressive examples of global medical disaster, is teratogenicity effect of thalidomide as a result of (S)- and (R)-enantiomer possess of carbon (Figure 1). However, studies show that only the (S)-enantiomer leads to teratogenic side, whereas no teratogenicity was observed for (R)-enantiomer.[3]

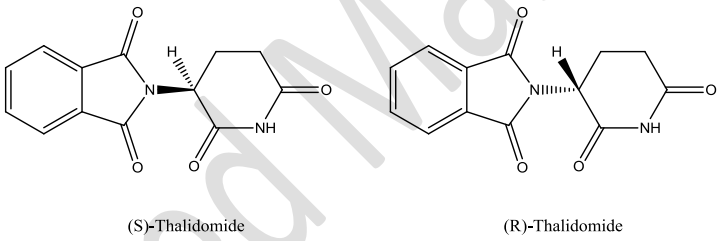


Figure 1. Thalidomide enantiomers

In recent years, thalidomide have been one of most notable topics in studies due to its numerous effects on neurological pathways.[3-5] Anti-epileptic drugs such as phenytoin, valproic acid, carbamazepine and thalidomide has multiple side effects, including diplopia, anemia and teratogenicity.[6,7] The word of teratogen is given to any peripheral or congenital factor that causes fetal harm and disorder in its development during prenatal period. Today, thalidomide usage is carefully monitored by successful schemes like S.T.E.P.S. (System for Thalidomide Education and Prescribing Safety Program).[4,6] Based on recent researches, thalidomide has anti-inflammatory effects and inhibits TNF-α.[7-10] Occasionally this factor leads immune system to attack normal tissues

that cause inflammation and damages.[8] According to this, it seems that TNF-α is one of the important factors in the development of epilepsy.[8,10]

Study on anticonvulsant activity of phthalimide derivatives shows the ability of these compounds to interact with $GABA_A$ receptor.[11] Based on this concept and with the goal of preserving the beneficial effects of thalidomide and eliminating its side effects, specially teratogenicity which causes it to be discontinued, six new non-chiral analogues of thalidomide are synthesized and anti-seizure effects and their docking pattern with $GABA_A$ receptor were evaluated.

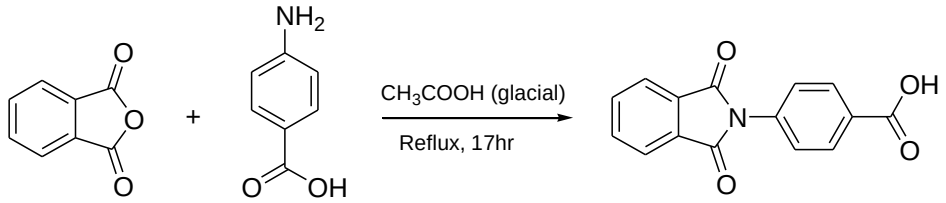
Materials and Methods

Chemicals

All chemicals which used in this study, including phthalic anhydride, 4-aminobenzoic acid, acetic acid, acetonitrile, hydroxybenzotriazole (HOBT), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), aniline, benzylamine, 4-chloroaniline, 2,6-dichloraniline, 2,4-difluoroaniline, 3,4-dimethylaniline, pentylenetetrazole (PTZ) purchased from Merck (Germany) and Sigma (U.S.A). All solvents were synthetic grade and distilled before use. Thalidomide and all synthesized compounds were suspended in a 10% aqueous DMSO (vehicle) solution for *in vivo* test.

Preparation of N-(4-carboxyphenyl)phthalimide

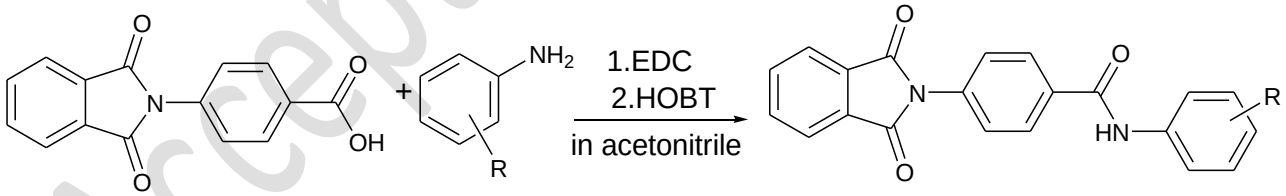
Phthalic anhydride (1, 0.01mol; 1.48g), 4-aminobenzoic acid (2, 0.01mol; 1.37g) and 20 ml glacial acetic acid were added in to a 100 ml round bottom flask [12,13]. The mixture was stirred at 100 °C and then refluxed for 17h. Progression of reaction was controlled by TLC. Finally, the solvent was removed by rotary evaporator at vacuum. Then residue washed with hot ethanol to remove excess para-amino-benzoic acid and filtered. The compound was recrystallized from ethanol and 2.1 g white crystals of N-(4-carboxyphenyl)phthalimide (3) were obtained (Scheme 1).[12,14]



Scheme 1. Reagents and conditions for the synthesis of N-(4-carboxyphenyl)phthalimide (3).

General method for preparation of N-(aryl)-4-(1,3-dioxoisoindolin-2-yl)benzamide

N-(4-carboxyphenyl)phthalimide (3, 1 mmol; 0.26g), EDC (1.22 mmol; 0.19g), HOBT (1.4 mmol; 0.19g) and 40 ml of acetonitrile were added in to a round bottom flask. After complete dissolving of all compounds were stirred for 7h at room temperature. Progression of the reaction was controlled by TLC ($CHCl_3$/MeOH; 4:1). When the reaction completed, 1 mmol of different aromatic amines (4) individually were added and stirred for 24h at room temperature.[15,16] The reaction was diluted with chloroform and washed respectively with brine, bicarbonate solution and citric acid. Finally, the organic layer was dried over $MgSO_4$ and removed by rotary evaporator in reduced pressure. The product was recrystallized from ethanol (Scheme 2).



R= 3,4-dimethyl (5a)
4-Cl (5b)
2,4-diF (5c)
H (5d)
benzyl (5e)
2,6-diCl (5f)

Scheme 2. Reagents and conditions for the synthesis of N-(aryl)-4-(1,3-dioxoisoindolin-2-yl)benzamide (5a-f)

N-(3-4-dimethylphenyl)-4-(1,3-dioxoisoindolin-2-yl)benzamide (5a)

Yield: 69%; Cream solid; m.p.> 250 ºC. IR (KBr, cm-1): 3346 (NH), 3056 (C-H aromatic), 2922 (C-H aliphatic), 1686 (C=O amid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 2.25 – 2.28 (S, 6H, $2CH_3$), 7.13 (d, j = 7.8 Hz, 1H, H-5''), 7.36 (d, j = 7.8, 1H , H-6''), 7.46 (S, 1H, H-2''), 7.63 (d, j = 8.2 Hz, 2H, H-2', 6'), 7.78 (S, 1H, NH amid), 7.83 (m, 2H, H-5,6), 7.98 (m, 2H,-4,7), 8.00 (d, j = 8.2 Hz, 2H , H-3',5′').

N-(4-chlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)benzamide (5b)

Yield: 68%; Cream solid; m.p.>250 ºC. IR (KBr, cm^{-1}): 3350 (NH), 3101(C-H aromatic), 3064 (C-H aromatic), 2985 (C-H aromatic), 1784 (C=O), 1724 (C=O amid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 7.47 (d, j = 7.6 Hz, 2H, H-2'',6''), 7.58 (d, j = 7.6 Hz, 2H, H-3'',5''), 7.84 (d, j = 8.5 Hz, 2H, H-2',6''), 7.87 (m, 2H, H-5,6), 8.02 (m, 2H, H-4,7), 8.13 (S, 1 H , NH), 8.44 (d, j = 8.0 Hz, 2H, H-3',5').

N-(2,4-difluo H-2'', H-2'',rophenyl)-4-(1,3-dioxoisoindolin-2-yl)benzamide (5c)

Yield: 64%; Bright pink solid; m.p.> 250 ºC. IR (KBr, cm^{-1}): 3392(NH), 3055 (C-H aromatic), 2929 (C-H aliphatic), 1742 (C=O), 1673 (C=O amid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 6.94 (d, j = 8.6 Hz, 1H, H-6''), 7.59 (d, j = 8.6 Hz, 1H, H-5''), 7.71 (d, j = 8.0 Hz, 2H, H2''), 7.84 (m , 2H , H5,6), 7.96 (s, 1H, NH), 7.99 (m, 2H, H-4,7), 8.02 (d, j = 8.0 Hz, 2H, H-3',5'), 8.41 (s, 1H, H-3'').

4-(1,3-dioxoisoindolin-2-yl)-N-phenylbenzamide (5d)

Yield: 60%; Cream solid; m.p.> 250 ºC. IR (KBr, cm^{-1}): 3355 (NH), 3053 (C-H aromatic), 2922 (C-H aliphatic), 1710 (C=O), 1671 (C=O Amid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 7.19 (t, J= 7.2 Hz, 1H, H-4''), 7.40 (t, j = 7.2, 2H, H-3'',5''),7.66 (m, 4H, H-2,2', 6, 6'), 7.81 (s, 1H, NH), 7.84 (m, 2H, H-5,6), 7.99 (m, 2H, H-4,7), 8.02 (d, j = 8.4 Hz, 2H, H-3',5').

N-benzyl-4-(1,3-dioxoisoindolin-2-yl)benzamide (5e)

Yield: 61%; Cream solid; m.p.> 250 ºC. IR (KBr, cm^{-1}): 3362 (NH), 3065 (C-H aromatic), 2934 (C-H aliphatic), 1775 (C=O), 1717 (C=O Amid). ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 4.53 (d, j = 6.6 Hz, 2H, CH_2), 7.24 – 7.36 (m, 5H, Ph), 7.53 (m, 2H, H-2',6'), 7.63 (S, 1H, NH), 7.78 (m, 2H, H-5,6), 7.90 (m, 2H, H-4,7), 8.40 (m, 2H, H-3',5').

N-(2,6-dichlorophenyl)-4-(1,3-dioxoisoindolin-2-yl)benzamide (5f)

Yield: 68%; Cream solid; mp> 250 ºC. IR (KBr, cm^{-1}): 3459 (NH), 2959 (CH-aromatic), 2918 (C-H aliphatic), 1783 (C=O), 1722 (C=O Amid), ^{1}H-NMR (500 MHz, $CDCl_3$): δ= 7.45-7.49 (m, 2H, H-3",5"), 8.58 (t, j = 7.9 Hz, 1H, H-4"), 7.84 (d, j = 8.6Hz, 2H, H-2',6'), 7.86 (m, 2H, H-5,8), 8.02 (m, 2H, H-4,7), 8.11 (s, 1H, NH), 8.43 (d, j = 8.6 Hz , 2H, H-3',5').

Docking methodology

Relying on present report based on $GABA_A$ receptor homology model and effect of phthalimide derivatives on $GABA_A$ receptor as anticonvulsant agent, docking simulation was used to predict the possible interaction between synthesized compounds and $GABA_A$ receptor.[17] MarvinSketch (Ver. 15.10.12) was applied to create 2D structures and Chem3D Ultra (Ver. 8.0) was used to generate 3D pdb format file. AutoDockTools (Ver. 1.5.6; ADT) was used for preparation of all input files for docking simulation and visualization of docking results in benzodiazepine (BZD) binding pocket. Polar hydrogens were added then all non-polar hydrogens were merged. Partial atomic charge was compute by Kollman method. Pdbqt files of receptor and ligands were saved. Grid-point spacing of 0.375 Å and grid box of 50×50 ×50 Å (x, y, and z) points were centered on BZD binding pocket with xyz-coordinates 43.321, 43.476, 8.701. Grid maps were generated by AutoGrid 4.2. Docking calculation parameters were set in default values and 100 Lamarckian genetic algorithm job runs were add to docking parameter file (.dpf). Docking calculation was done by AutoDock 4.2. Estimated docking binding energies were extracted from docking log files. Visualization was done by Discovery Studio Visualizer (Ver. 17.2) and Pymol (Ver. 1.1evel). LogP values were calculated by ChemBioDraw ultra (Ver. 12.0).

Determination of seizure threshold

To study the effect of synthesized compounds (5a-f) and thalidomide on the seizure induced by PTZ, 40 mice were separated in 8 groups of 5 mice (n=5). Group 1 was used as negative control group (vehicle; 10 mg/kg) and animals from group 2 received 10 mg/kg of thalidomide as positive control group. Group 3-8 received synthesized compounds 10 mg/kg.[18] The doses of synthesized compound (10 mg/Kg) and PTZ (70 mg/Kg) were chosen based on the study of Palencia et al.[18] 30 minutes later, each animal was injected intraperitoneally (IP) with a single dose of PTZ (70 mg/kg of body weight). The time elapsed after PTZ injection for the inhibition of the seizure (latency time) was individually measured for one hour.

Statistical analysis

Data are expressed as means ± SEM of 5 mice and analyzed using the SPSS statistical software package (Version 23.0). To compare the threshold seizures after peritoneal injection of all compounds, one-way analyses of variance (ANOVA) followed post hoc Tukey's tests were used to analyze the data.[19] $P<0.05$ was considered statistically significant.

Results

In first step, N-(4-carboxyphenyl)phthalimide (3) synthesis from phthalic anhydride (1) and 4-aminobenzoic acid (2) in good yield. In next step, different aromatic amines (4a-f) were conjugated with aid of EDC and HOBt to N-(4-carboxyphenyl)phthalimide (3) to obtain synthesized compounds 5a-f in good yield.

Docking simulation results

Docking results show that compounds 5d and 5a are the most active derivatives with binding energy -9.98 and -9.73 kcal/mol, respectively, which their interaction with receptor are shown in Figure 2. Graphical interaction with receptor shows that all the interactions of these compounds are same, π-π

interactions with Phe 77, His 101and H bond of carbonyl group and Thr 142 and another NH group has H bond with Thr 159. Just there is one extra π–π interaction of Phe 77 with pyrrolidine-2,5-dione in compound 5d. In addition, thalidomide was properly fit to the BZD-binding pocket with -7.17 kcal/mol binding energy.

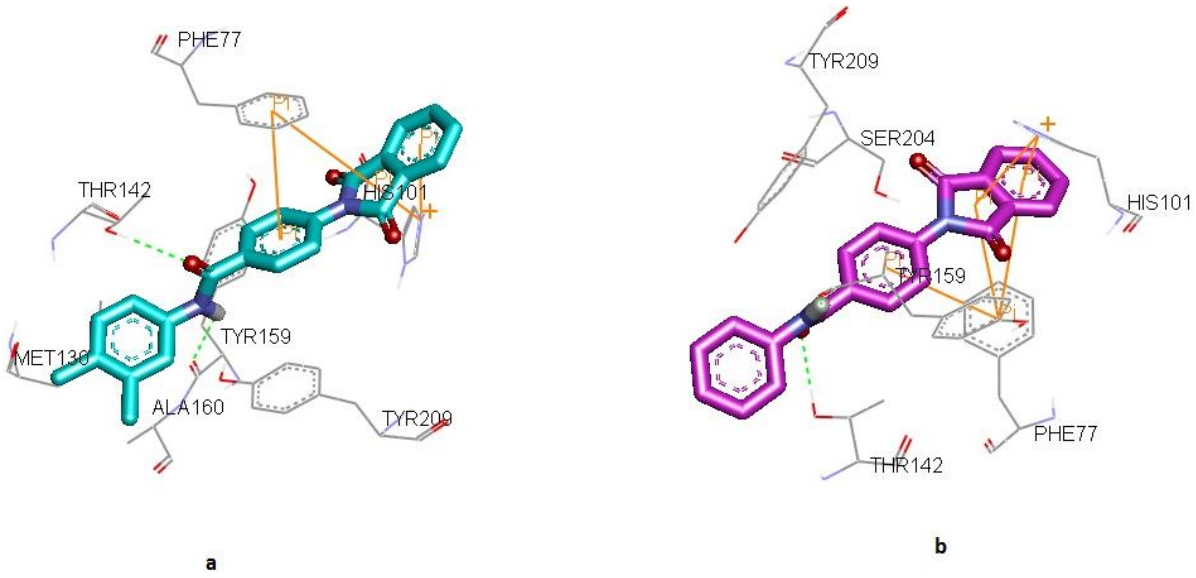


Figure 2. a) Interaction of compounds 5a (a; cyan) and 5d (b; magenta) with benzodiazepine (BZD) binding pocket.

Therefore, it seems that the difference between the binding energy of compounds 5d and 5a are related to conformational changes of methyl substitute of compound 5a, which increase the steric hindrance and decrease the correct placement of 5a in BZD-binding pocket (Figure 3).

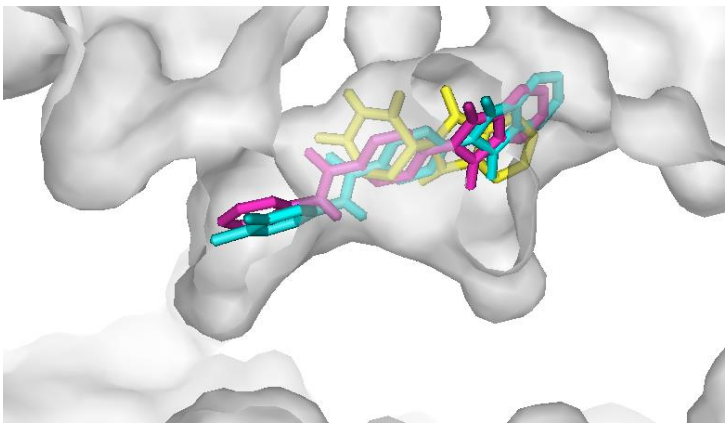


Figure 3. Docking pose of compound 5a (cyan), 5d (magenta) and thalidomide (yellow).

Effect of different N-aryl-4-(1,3-dioxoisoindolin-2-yl)benzamides on the seizure threshold

The results of anticonvulsant activities are presented in Figure 4 and Table 1. The seizure occurred in all groups after PTZ injection and the latency time and mortality rate were measured. In this test, each of these six synthesized compounds were compared with each other and with positive and negative control groups. The results are shown in Table 1.

In Figure 4, there is a clear trend of decreasing seizure and deaths due to PTZ administration in mice compared with negative control group. Peritoneal injection of 5a group with the dose of 10 mg/kg, increased the latency time of first seizure in mice. In addition, this group has a significant difference between all the groups and control groups ($P < 0.05$).

It seems that this group has the highest log P, following group 5f and because of highest lipophilicity, it caused a stronger binding and desirable effect (Table 1). Intraperitoneally injection of five other compounds with the dose of 10 mg/kg had a significant difference with vehicle however when compared with positive control group (thalidomide), only compound 5a has shown better anticonvulsant activity than thalidomide. The result of compound 5c is considered to be similar to the vehicle group by the ANOVA test. Results of 5b, 5d, 5e, and 5f are significantly higher than from what observed with the vehicle.

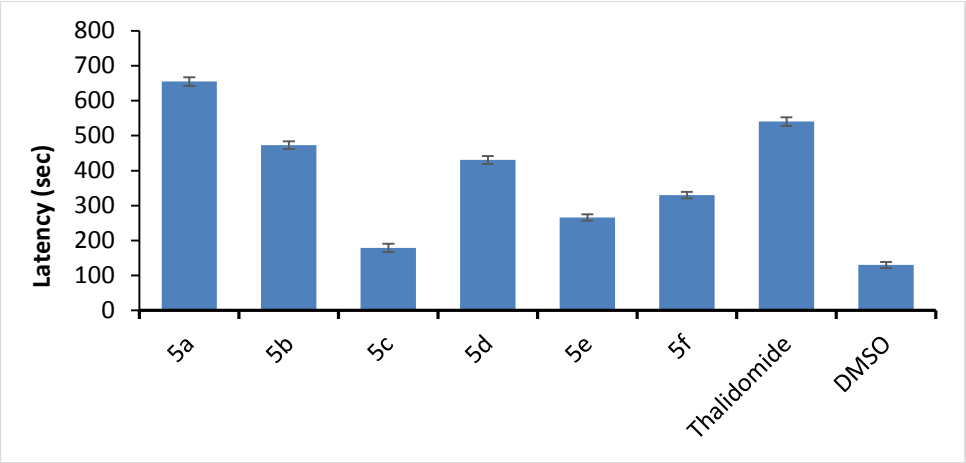


Figure 4. Effect of synthesized compounds (5a-f; 10 mg/kg) on pentylenetetrazole induced tonic-clonic seizures in mice in comparesion with positive control group (thalidomide) and negative control group (vehicle).

Table 1. Structure, binding energy of N-aryl-4-(1,3-dioxoisoindolin-2-yl)benzamide derivatives on pentylenetetrazole induced seizure threshold in mice

Compound	MW	Log p	Binding energy (kcal/mol)	Latency (Mean± S.E.M, Sec.)	Mortality
5a	370.4	4.43	-9.73	654.80±5.57	0
5b	376.79	4.01	-9.33	472.80±4.77	1
5c	378.33	3.77	-9.34	178.60±5.34	2
5d	342.35	3.46	-9.98	430.40±5.01	1
5e	356.37	3.53	-9.63	266.00±4.19	1
5f	411.24	4.57	-9.64	330.40±4.09	2
Thalidomide	258.23	-0.15	-7.17	540.40±5.42	1
Vehicle	_	_		129.80±3.78	4

Discussion

Anticonvulsants agent contain diverse pharmacological group to treatment the epileptic seizures and bipolar disorder. Recent studies approaches are based on structural modification of old medicines such as thalidomide. Therefore, many of researchers have focused on phthalimide pharmacophore to prepare new compounds such as N-phthaloyl GABA series.[20-22] The purpose of these modifications is preparing similar compounds to thalidomide structure and increasing the lipophilicity of synthetic molecules by capping the amine group in the GABA structure.[23-25] Although, studies showed there are no anticonvulsant activity for N-phthaloyl GABA.[24,25] Mendyk et al. synthesized various amide derivatives of N-phthaloyl GABA with aliphatic and benzyl amine moieties, to reducing the toxicity and improving the anticonvulsant activity of this series.[26]

Based on Mendyk et al. report,[26] in this work, six compound bearing phthalimide ring conjugated with aryl benzamide moieties were synthesized and their anti-epileptic effects were investigated. Docking study shows that the compounds 5d and 5a have highest binding energy respectively. But experimental results show that among six synthesized compounds, compound 5a with 3,4-dimethyl group enhanced the duration of seizure inhibition in male mice in compare with thalidomide. It seems that the difference between the binding energy of compounds 5a and 5d are related to conformational changes of methyl substitute of compound 5a which increases the steric hindrance and decreases the correct placement in BZD-binding pocket. Nevertheless, this methyl will increase the lipophilicity property and may increase the crossing the compound into BBB and duration of seizure inhibition in male mice.

Conclusion

According to the widespread use of thalidomide in the treatment of various diseases and its influence on different neural pathways, in order to reduce its complications including teratogenic and also to eliminate carbon chiral (racemization), similar compounds of thalidomide had been synthesized and its antiepileptic properties were investigated in vivo and in silico. In good agreement of docking and

experimental results, 5a with 3,4-dimethylphenylamine group can bind effectively to the BZD-binding pocket and enhanced the duration of seizure inhibition in male mice in compare with thalidomide. Inhibition of tonic-clonic seizure is the significant outcome of this study.

Ethical issue

We confirm that, we have read the journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

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