Original Article

Anticonvulsive evaluation and histopathological survey of thalidomide synthetic analogs on lithium-pilocarpine-induced status epilepticus in rats

Arash Amanlou[1,2], Faezeh Eslami[2,3], Maryam Shayan[2,3], Pejman Mortazavi[1,*], and Ahmad Reza Dehpour[2,3,*]

[1]*Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, I.R. Iran.*
[2]*Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, I.R. Iran.*
[3]*Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, Tehran, I.R. Iran.*

Abstract

Background and purpose: Status epilepticus is a severe neurological disorder that can be life-threatening. Thalidomide and its analogs have shown promising results to confront pentylenetetrazole-induced seizures. This study aimed to evaluate the potential effects of three synthesized thalidomide derivatives on lithium-pilocarpine-induced status epilepticus.

Experimental approach: To induce status epilepticus, rats received lithium chloride (127 mg/kg, i.p.) and pilocarpine HCl (60 mg/kg, i.p.) 20 h after lithium chloride injection. Thirty min before pilocarpine HCl administration, rats received hyoscine N-butyl bromide (1 mg/kg, i.p.) and concurrently one of the test compounds (5B, 5C, and 5D), diazepam, thalidomide, or vehicle (4% DMSO) to evaluate their anti-epileptic effects. Epileptic seizures scores were assessed through the Racine scale. Twenty-four h after injection of pilocarpine, brain samples were extracted for further histopathological evaluation.

Findings/Results: Results revealed that among tested compounds (5B, 5C, and 5D), only compound 5C (1 mg/kg) exhibited excellent anti-epileptic activity comparable to diazepam (10 mg/kg). Compound 5D (100 mg/kg) only demonstrated comparable anti-epileptic activity to thalidomide (1 mg/kg). Compound 5B did not have any anti-epileptic activity even at the dose of 100 mg/kg. The histopathological survey showed that compound 5C has more neuroprotective effects than diazepam and thalidomide in the cortex of the brain. In the cornu ammonis 1 region, thalidomide had higher protective properties and in the cornu ammonis 3 and dentate gyrus areas, diazepam had higher efficacy to prevent necrosis.

Conclusion and implications: Compound 5C is a good candidate for further studies regarding its potency, compared to thalidomide and diazepam.

Keywords: Histopathology; Hippocampus; N-Phthalimide; Status epilepticus; Thalidomide.

INTRODUCTION

More than 50 million people around the world are affected by epilepsy (1). About 35 million of this population are living in developing countries (2). In kids with epilepsy, it can significantly impact their quality of life and education (3). When seizures are repeated, it is defined as epilepsy, and the maximum state of epilepsy is status epilepticus (4). Different mechanisms are involved in a seizure attack. When they cannot stop the seizure attack, then status epilepticus occurs. The more a seizure attack is prolonged, the less it has the chance to cease (5). When a seizure attack is prolonged, it can cause more damage to the brain and be more life-threatening (6).

*Corresponding authors:
P. Mortazavi, Tel: +98-2144865154, Fax: +98-2144865119
Email: sp.mortazavi@gmail.com
A.R. Dehpour, Tel: +98-2188973652, Fax: +98-2166402569
Email: dehpour@yahoo.com

Despite the extensive research, the exact molecular basis for status epilepticus is unknown. A seizure typically happens when an imbalance between neuronal excitation and inhibition in the brain causing excitatory overload. Increasing Na^+ channel activity and glutamate concentration while decreasing gamma-aminobutyric acid (GABA) and K^+ channel activity is the main possible mechanism it is agreed upon. But other mechanism remains to discover (7,8).

Numerous drugs have been introduced to the clinic to prevent patients from starting or entering the status epilepticus phase. Still, a limited number has been able to control or return the patient to everyday life. Unfortunately, the potency of drugs decreases with continuous use, and drug resistance will develop, and the patient's life is endangered. As a result, it is essential to discover new drugs with the ability to inhibit this form of epilepsy, both in the prevention and treatment phase.

Thalidomide, a phthalimide glutamic acid derivative, was initially intended as a sedative or tranquilizer but was soon used for treating a wide range of other conditions, including colds, flu, nausea, and morning sickness in pregnant women. Although, pregnant patients had to discontinue it owing to its teratogenicity (9).

Utilization of thalidomide in diseases that had not been previously prescribed almost started in the early years of century 21 in patients with erythema nodosum leprosum in the USA. This drug has also presented immunomodulatory properties (10). It has been used in diseases and disorders such as graft versus host disease (GVHD), cancers, rheumatoid arthritis, sarcoidosis, and inflammatory bowel disease (11). Thalidomide has been used in patients suffering from refractory epilepsy (12). However, its most important side effect in pregnant women has limited its widespread use (13).

Considering the beneficial effects of thalidomide on refractory epilepsy as well as, the beneficial impact of this drug and related compounds in recent reports, the latest researches were designed with the intention to increase its anti-epileptic activity and reduce its main side effects (11,12,14-16).

In the present study, the anti-epileptic effects and histopathological evaluation of the three most potent phthalimide analogs of thalidomide (compounds 5B, 5C, and 5D) in which the glutarimide ring was replaced by non-aliphatic side chains were investigated employing the lithium-pilocarpine-induced status epilepticus model in rats and the results compared to previously reported anticonvulsive activity in the pentylenetetrazole (PTZ)-induced seizures model in mice (14-16).

MATERIALS AND METHODS

Drugs

Lithium chloride (Sigma, St Louis, MO, USA), pilocarpine HCl (Sina Daru Pharmaceutical Company, Tehran, Iran), diazepam, and scopolamine butylbromide (Hyoscine N-butylbromide, ChemiDarou Company, Tehran, Iran) were used as received. Thalidomide and three previously synthesized thalidomide derivatives (compound 5B, N-(4-chlorophenyl)-2-((1, 3-dioxoisoindolin-2-yl) methyl)-4, 5-dihydrothiazole-4-carboxamide; compound 5C, 2-(1,3-dioxoisoindolin-2-yl)-N,3-diphenylpropanamide; and compound 5D, N-(3,4-dimethylphenyl)-4-(1, 3-dioxoisoindolin-2-yl)benzamide) were received as gifts from the Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences (14-16).

The synthesized compounds and thalidomide were dissolved in an aqueous solution of dimethyl sulfoxide (DMSO, 4% v/v). Pilocarpine HCl, hyoscine N-butylbromide, and lithium chloride were dissolved in a normal saline solution at the appropriate concentration. All the test solutions were prepared on the day of testing. The route of all administration was intraperitoneal (i.p.).

Animals

Male Wistar rats with a weight ranging from 200-250 g were divided into six groups each comprising of eight animals. Rats were kept in each standard polycarbonate cage. The temperature was set at 24 ± 1 °C, and the 12/12-h light/dark cycle was set. Rats had free access to water and food. All the experiments done on the animals were favorable to the Guidelines for the Care and Use of Laboratory Animal Ethics Committee (Ethics code: IR.IAU.SRB.REC.1399.188) and the National Institutes of Health (NIH publication NO. 85-23; revised 1985).

Induction of status epilepticus by lithium-pilocarpine

Each rat received lithium chloride by intraperitoneal injection with a dosage of 127 mg/kg. After 20 h of lithium chloride administration, induction of status epilepticus was initiated by i.p. injection of pilocarpine HCl with 60 mg/kg dosage. Thirty min before injecting pilocarpine HCl, hyoscine N-butylbromide (1 mg/kg, i.p.) was injected in order to prevent the peripheral cholinergic side effects of pilocarpine HCl (17). Concurrently, thalidomide and the synthesized compounds (1 mg/kg for compound 5C and 100 mg/kg for 5B and 5D), diazepam as a positive control (10 mg/kg, i.p.), and vehicle (4% DMSO) as a negative control (1 mL/kg) were individually injected.

Assessment of status epilepticus was performed according to Racine's study done in 1972 (18). Five stages are: (1) moving the mouth and a condition called facial movement; (2) the second stage is when the rats start to nod their head; (3) the third stage is when the clonus of the forelimbs is observable; (4) rearing is defined as stage four; and (5) when a complete motor seizure is added to the loss of the body's normal position, this is the final and fifth stage (11). Examiner who gave the scores was blind to the study groups.

Study of the hippocampus histopathology using hematoxylin and eosin staining

Rats were perfused transcardially with phosphate-buffered saline (PBS) and 4% paraformaldehyde after an overdose with an anesthetic agent (3% pentobarbital) and were sacrificed by decapitation. Brains were isolated and stored in 10% formaldehyde for 2-3 days. Each hemisphere of the brain was cautiously separated. Paraffin blocks were made, and 8 μm coronal sections were cut, mounted, and used for hematoxylin and eosin histopathological scoring (19). Neurons are detected by features such as extensive eosinophilic cytoplasm, chromatin disintegration, and the absence of an integral nuclear membrane, which serve as a marker for irreversible neuronal damage.

Degenerative changes in the cornu ammonis 1 (CA1), CA3, and the dentate gyrus (DG) regions of the hippocampus and the cortex of the brain (20,21) was evaluated by the changes in the neurons, such as cytoplasmic vacuolation, nuclear chromatin clumping, hypereosinophilia, condensed cytoplasm, and fragmentation of the cells were used to determine the relative percentage of neuronal damage.

Counting of the neurons numbers and the image analysis of the cortex and the hippocampus regions was performed using the ImageJ software macro plugin (22). Five random fields for the cortex and three random fields for the hippocampus were chosen for the neuron's numeration. The microscopic zoom was 40 high power field (HPF).

Docking simulations and PTZ-induced seizures

Data for docking binding energies, lipophilicity index (ClogP), and PTZ-induced seizures latency times were extracted from recently published studies (12-14).

Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). GraphPad Prism (version 8.4.3) was used to analyze the data. One-way analysis of variance (ANOVA) with post hoc Tukey's test or Dunnett's post hoc test was used to compare three or more groups. It was considered statistically significant when a P value was less than 0.05.

RESULTS

Effects of synthesized compounds on status epilepticus

In vivo, anticonvulsant activity of the synthesized compounds was evaluated using the lithium-pilocarpine model of status epilepticus compared to the control group, diazepam, and thalidomide as reference anti-epileptic drugs. As shown in Fig. 1, compound 5C at a 1 mg/kg dose exhibited excellent anti-epileptic activity. While the other two test compounds (5B and 5D) did not produce any activity at similar doses, compound 5D demonstrated comparable anti-epileptic activity to thalidomide at 100 mg/kg. Compound 5B did not have any anti-epileptic activity even at the dose of 100 mg/kg (Fig. 1).
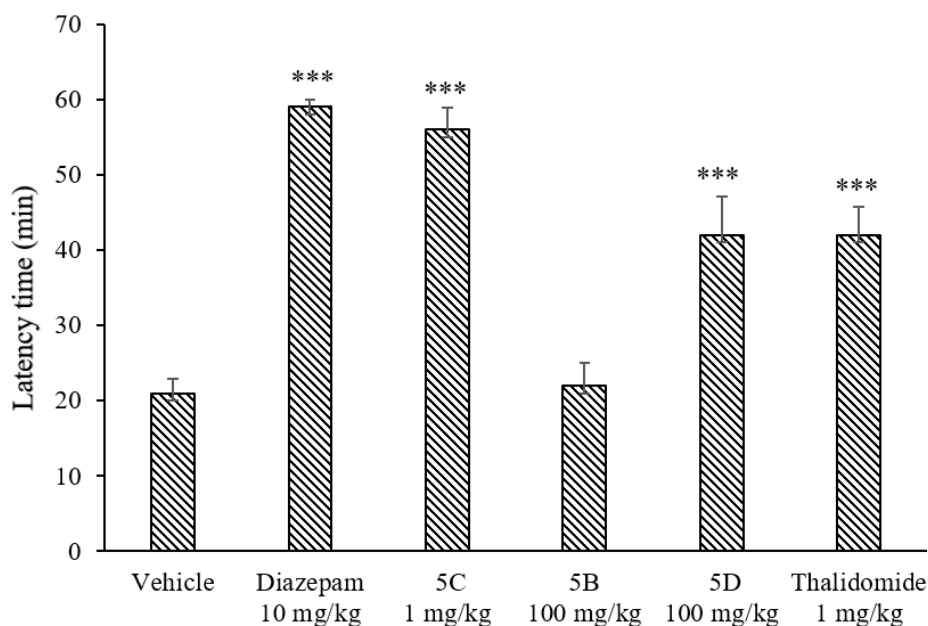
Fig. 1. The anti-epileptic activity of diazepam, compounds 5C, 5B, and 5D, and thalidomide in the lithium-pilocarpine model of status epilepticus in comparison with the control group (vehicle). n = 8-10. ***$P < 0.001$ Indicates significant differences compared to the vehicle group. Data represent mean ± SEM.

Table 1. Percentage of necrotic neurons to total neurons being counted 24 hours post status epilepticus induction in rat brain. Data are expressed as mean ± SEM, n = 5. ***$P < 0.001$ and **$P < 0.01$ indicate significant differences compared to the vehicle group.

	Dentate gyrus	Cornu ammonis 3	Cornu ammonis 1	Cortex
Compound 5C	17.38 ± 4.45**	22.02 ± 4.55**	16.43 ± 2.53***	33.58 ± 1.43***
Thalidomide	24.69 ± 8.98	21.40 ± 7.28**	9.98 ± 1.97***	37.69 ± 2.68***
Diazepam	16.34 ± 1.57**	18.74 ± 1.14***	22.67 ± 7.85***	39.82 ± 0.18***
Vehicle	30.84 ± 1.41	35.59 ± 6.18	36.40 ± 4.09	53.23 ± 7.42

As a reference drug, diazepam produced significant anti-epileptic activity at a dose of 10 mg/kg.

Effects of various treatments on degenerative changes and the histopathological score of the hippocampus

The vehicle solution did not protect neurons morphology in the control group; the neurons exhibited necrotic lesion morphology (Table 1, Figs. 2-5). As shown in Table 1, compound 5C (Fig. 3) protected temporal cells from necrosis compared to the vehicle group. Diazepam (Fig. 4) and thalidomide (Fig. 5) also protected cells from necrosis in a lower percentage than compound 5C. Diazepam and thalidomide groups showed similar activity in

the protection of neurons in this area. Similar patterns of action were observed for the CA1, CA3, and DG regions (Table 1).

Table 2 shows the structure of studied compounds, lipophilicity (Clogp), binding energy at GABAA active site, and anti-epileptic activity at two different seizure models. As shown in Table 2, compound 5C with the binding energy of -9.95 kcal/mol is the most active compound *in silico* and *in vivo* studies. This compound also had a higher latency time in the PTZ model of the seizure than other analogs. In anti-epileptic activity based on lithium-pilocarpine test compound 5C again had similar activity to the standard drug diazepam while those two other analogs did not pose any significant activity ($P > 1$).
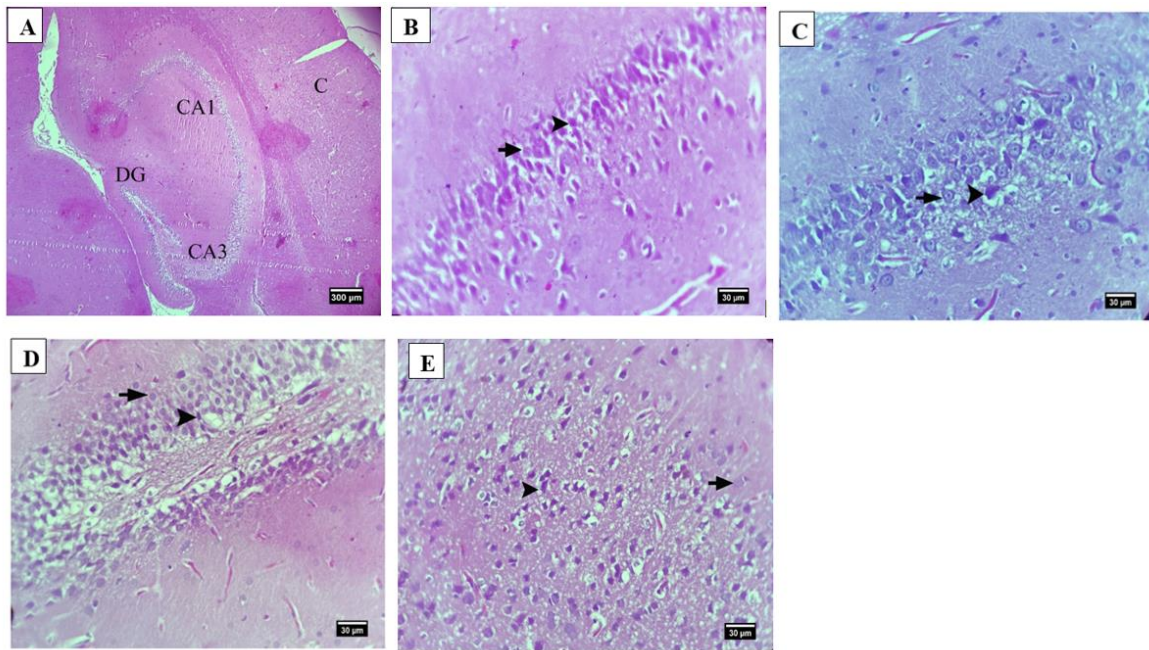
Fig. 2. Section of the cortex and the hippocampus region (CA1, CA3, and the DG) in (A) the control group, which shows a decrease in the population of neurons in different areas of the hippocampus. (B) CA1 cross-section of the hippocampus, which shows a reduction in the population of neurons and necrosis of neurons (arrow tip) compared to healthy neurons (arrow). (C) CA3 cross-section of the hippocampus, which shows a decrease in the population of neurons and necrosis of neurons (arrow tip) compared to healthy neurons (arrow). (D) DG cross-section of the hippocampus, which shows a decrease in the population of neurons and necrosis of neurons (arrow tip) compared to healthy neurons (arrow). (E) Cortex cross-section shows a decrease in the population of neurons and necrosis of neurons (arrow tip) compared to healthy neurons (arrow). C, Cortex; CA, cornu ammonis; DG, dentate gyrus.
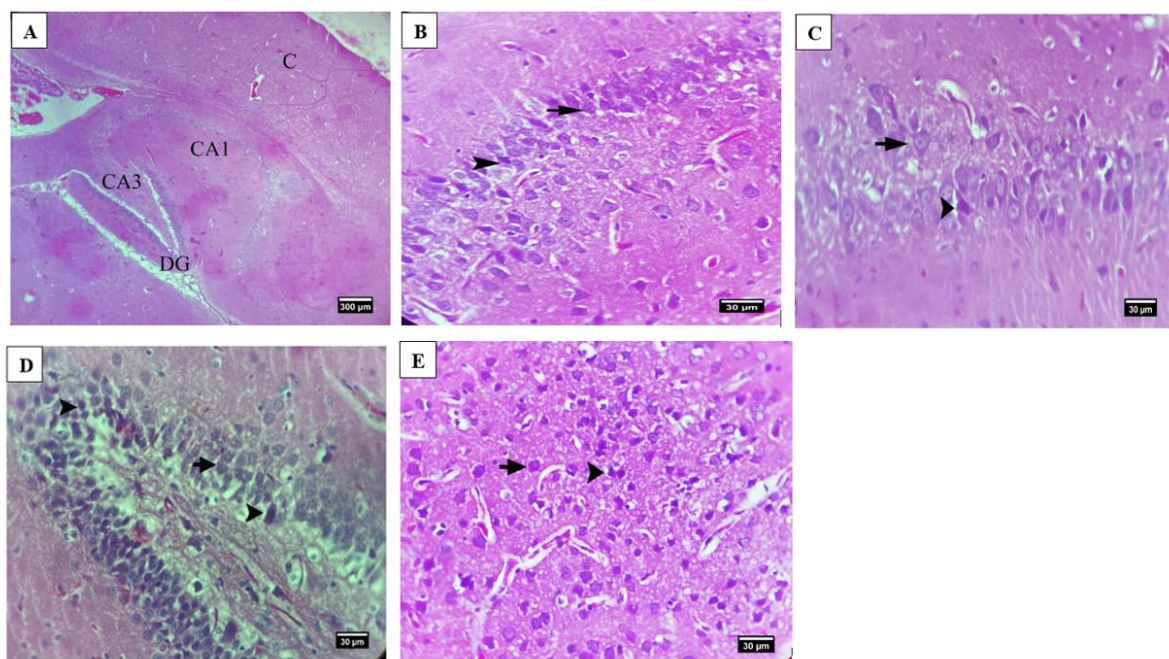

Fig. 3. Cortex and hippocampus sections (CA1), CA3), and DG) in compound 5C (1 mg/kg), where a decrease in the population of neurons is seen in some areas of (A) the hippocampus and (B) CA1 section of the hippocampus where necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). (C) CA3 cross-section of the hippocampus where necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow) and (D) DG cross-section of the hippocampus which shows a significant decrease in the population of neurons and necrosis of neurons (arrow tip) compared to healthy neurons (arrow). (E) Cortex section in which necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). C, Cortex; CA, cornu ammonis; DG, dentate gyrus.
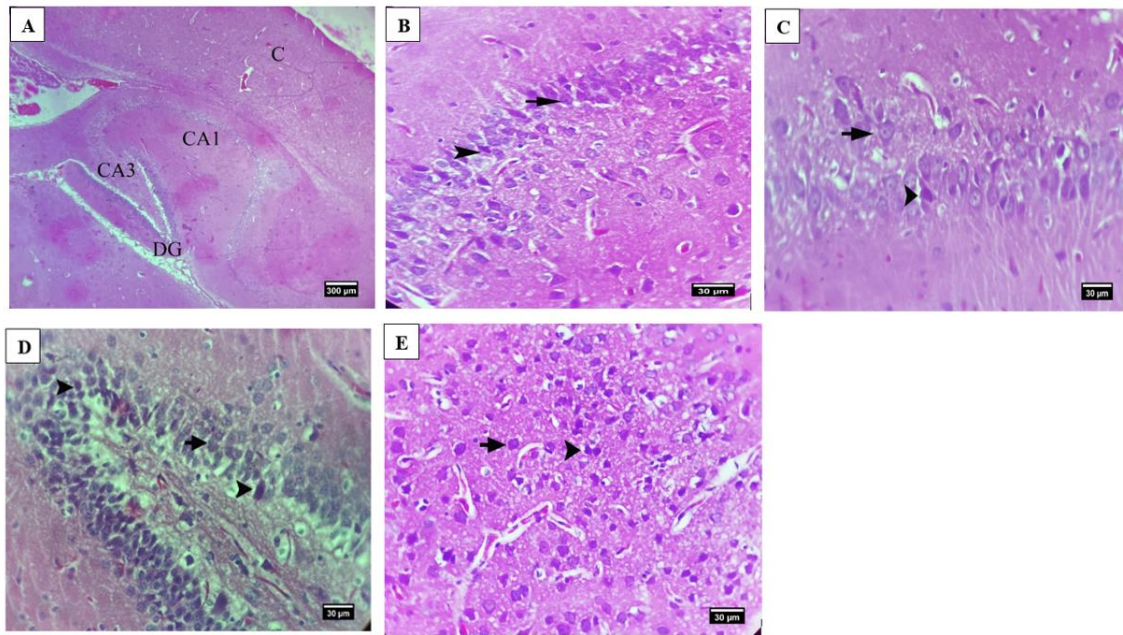
Fig. 4. Section of the cortex and hippocampus (CA1, CA3, and DG) in diazepam group (10 mg/kg), (A) where a significant population of neurons is seen in different areas of the hippocampus and (B) CA1 cross-section of the hippocampus where gliosis (yellow arrow), and necrosis of neurons (arrow tip) are seen in comparison with healthy neurons (arrow). (C) CA3 section of the hippocampus where mild necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow) and (D) DG cross-section of the hippocampus where significant necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). (E) Cortex section where necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). C, Cortex; CA, cornu ammonis; DG, dentate gyrus.
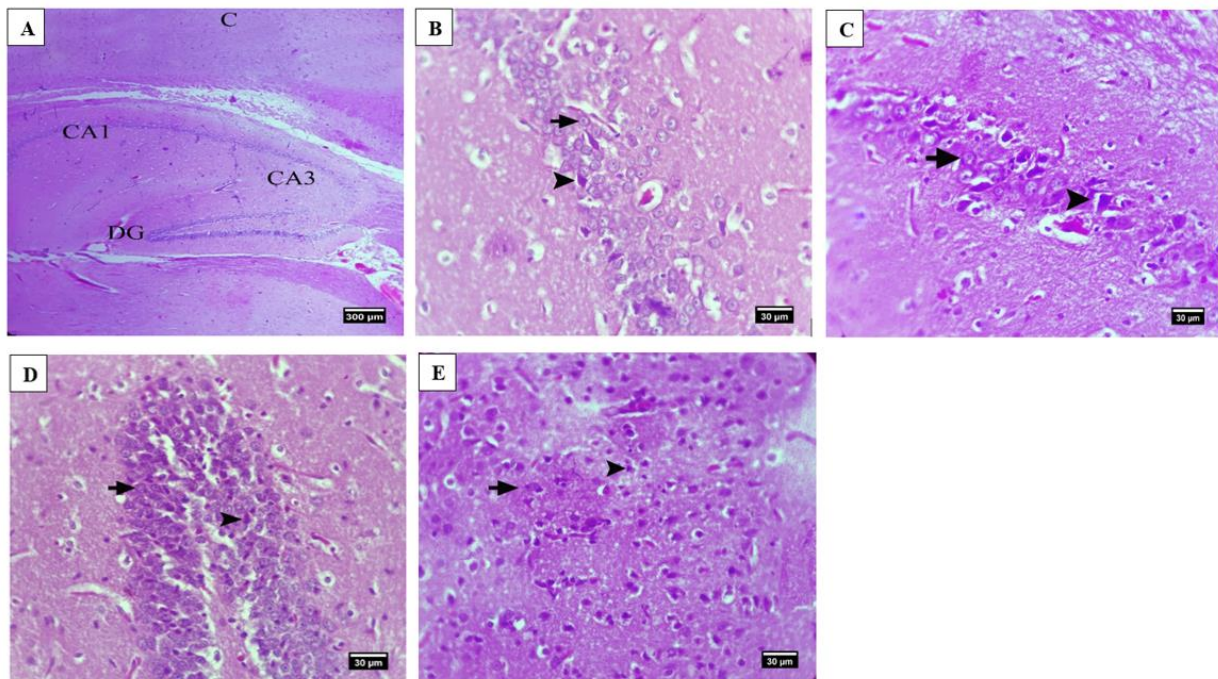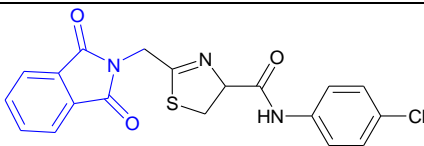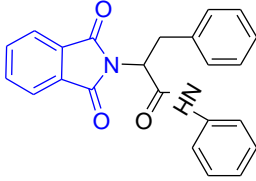

Fig. 5. Corte and hippocampus (CA1, CA3, and DG) in thalidomide group (1 mg/kg) where a significant population of neurons is seen in (A) different areas of the hippocampus and (B) CA1 cross-section of the hippocampus where mild necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). (C) CA3 section of the hippocampus which mild necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow) and (D) DG cross-section of the hippocampus where mild necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). (E) Cortex section in which necrosis of neurons (arrow tip) is seen compared to healthy neurons (arrow). C, Cortex; CA, cornu ammonis; DG, dentate gyrus.
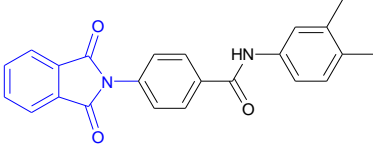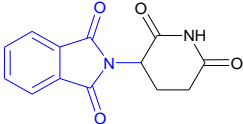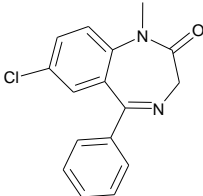
Table 2. Structure, lipophilicity, binding energy at γ-aminobutyric acid type A (GABA$_A$) active site, and anti-epileptic activity of the tested compound.

Compounds	Structures	Clogp[§]	Binding energy (kcal/mol)[§]	Latency time(sec)*,[§] (PTZ test)	Latency time (lithium-pilocarpine test) (min)* / mortality rate (%)[φ]
5B		2.52	-9.60	692.60 ± 5.71	22 ± 6.70 / (25)
5C		3.67	-9.95	780.8 ± 134.03	56 ± 6.52 (25)
5D		4.43	-9.73	654.80 ± 5.57	42 ± 11.50 (50)
Thalidomide		-0.15	-7.17	540.40 ± 5.42	42 ± 11.51 (37.5)
Diazepam		2.82	-7.68	1800 (cut-off time)	59 ± 2.24 (12.5)
Vehicle	DMSO/H$_2$O	-	-	129.80 ± 3.78	21±4.18 (25)

*Data are expressed as mean ± SEM; [§]data obtained from Ref. 12-14; [φ]mortality rate (%) 24 h post status epilepticus induction.

DISCUSSION

The treatment of status epilepticus involves the use of potent intravenous medications as quickly as possible. In the first 30 min of status epilepticus, catecholamine and glucose levels, heart rate, and cerebral blood flow increase. Besides, after 30 min of status epilepticus, acidosis, hypoglycemia, hyponatremia, and hypotension occur (23). Furthermore, brain damage will be initiated. Among the brain regions, the hippocampus placed on the brain's temporal lobe is one of the areas affected by seizures (2). After temporal lobe epilepsy, some of the brain's pathological findings are loss of neurons and gliosis in some of the hippocampus areas (6). In the DG, CA1, and CA3 regions of the hippocampus, loss of neurons after status epilepticus is detectable (24,25).

Different drugs are prescribed for patients suffering from seizures and epilepsy, depending on their general condition and medication history. Phenytoin, carbamazepine, sodium valproate, phenobarbital, ethosuximide are used as available anti-epileptic drugs. Commonly prescribed medications used in status epilepticus are paraldehyde, lidocaine, and diazepam (26). Intravenous administration of diazepam, lorazepam, and midazolam are the effective first-line therapy for treating status epilepticus. Phenobarbital is the second-line medication for status epilepticus (4).

Most of these drugs have serious side effects, and after repeated use, tolerance occurs. Some of the known side effects of anti-epileptic drugs are fever, rashes, and allergic reactions (27). There are also psychiatric side effects known in anti-epileptic therapy. In patients

taking barbiturates as medications, there is a chance of depression and attention deficit hyperactivity disorder (ADHD). Carbamazepine can cause mania or depression (27). These side effects encouraged researchers to find an effective, fast-acting, and safe medication.

There are various models for the induction of status epilepticus in rats, and these models are used to simulate temporal lobe epilepsy. Kainic acid, pilocarpine, and lithium-pilocarpine models of status epilepticus are three models used to simulate status epilepticus (6). Using lithium chloride coupled with pilocarpine can result in a lower dosage of pilocarpine being used. Also, it will cause rats to present better states of status epilepticus (28). Other chemicals that can cause status epilepticus are bicuculline and soman (24).

Numerous data are indicating that vascular and neuronal injury occurs as a result of status epilepticus. Moreover, activation of the glial cells, blood-brain barrier disruption, and metabolic disturbance are also observed in status epilepticus. A decreased GABAergic neurotransmission occurs both during and after status epilepticus. An increase in the N-methyl-D-aspartic acid or N-methyl-D-aspartate (NMDA) receptors activation is responsible for the elevated neuronal apoptosis and damage caused by status epilepticus. Interleukin-1β/toll-like receptor indirectly regulates NMDA receptors in epileptic seizures, mammalian target of rapamycin (mTOR) pathway, ATP level, unregulated expression of cyclooxygenase 2 were reported to be involved in neuronal damage of the brain (7,8). Glutamate receptor agonists (NMDA and kainate), low sodium and high levels of potassium and inflammation, and oxidative stress are involved in the pathophysiology of temporal lobe epilepsy and status epilepticus (24,29-31).

In search of new treatment of seizure and epilepsy, thalidomide has shown an anticonvulsive effect in both PTZ-induced seizure and pilocarpine-induced status epilepticus (32,33). Due to its potent activity of thalidomide in these tests, many attempts were made to design analogs of thalidomide to treat seizures and epilepsy. Different analogs are designed and tested in animal models of epilepsy to evaluate their ability to confront seizures (14-16,34). Among them, N-arylphthalimide analogs are wiedly studied. Some of them have shown an ability to hold against PTZ-induced seizures (34), N-aryl-4-(1,3-dioxoisoindolin-2-yl) benzamides (16), phthalimide (N-aryl-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanamide derivatives (14), phthalimide-4,5-dihydrothiazole-amide (15) revealed an anti-epileptic effect in PTZ-induced seizure.

Amongst different types of epilepsies, the treatment of temporal lobe epilepsy is one of the toughest ones. Various mechanisms are involved in the anticonvulsive activity of thalidomide including inhibition of nitric oxide, interleukin 6, and tumor necrosis factor-alpha action (14,33,35-38). In the amygdaloid kindling model, thalidomide has shown anti-epileptic properties, even in low dosages (10 mg/kg) compared to topiramate with a dosage of 80 mg/kg (36).

In recent studies, docking simulation has shown that thalidomide and its analogs act on the GABAA receptor (14-16). Based on the mentioned studies, in the present study, we investigated the anticonvulsive effects of three analogs of thalidomide in the lithium-pilocarpine model of status epilepticus in rats for the first time. These compounds recently showed a remarkable anticonvulsive impact on the PTZ model of seizure compared to thalidomide (14-16). While all these three compounds exhibited an anticonvulsive effect in the PTZ test, only compound 5C showed strong activity comparable to diazepam and thalidomide in the lithium-pilocarpine-induced status epilepticus model. Compound 5D exhibited its activity only at the very high dose of 100 mg/kg, and compound 5B had almost no activity at any test doses (even at 100 mg/kg).

Compound 5C, which is active in both tests, has higher binding energy in docking experiments than others. While higher lipophilicity is an essential factor for compounds to cross the blood-brain barrier, this compound has a log P of about 3.67, which is lower than log p for compound 5D (4.43). Still, compound 5D at the dose of 100 mg/kg only exhibited 70% of the activity of compound 5C. Then other factors such as molecular shape and active transport through amino acid transporter are involved in its higher activity.

Compound 5C (1 mg/kg) and diazepam (10 mg/kg) have similar activity in the used method. Histopathological survey (Table 1) in cortex neurons, which is an important area in status epilepticus, showed that compound 5C has more protective effects than thalidomide and even diazepam. In the CA1 region, thalidomide had higher protective properties than other regions. However, in CA3 and DG areas, diazepam has higher efficacy to prevent necrosis. Based on the *in silico* study, compound 5C has a good interaction with the GABAA receptor active site (Table 2). Therefore, the possible mechanism of action of compound 5C may be due to activation of the GABAergic pathway, preventing apoptosis, vascular and neuronal injury in the brain.

CONCLUSION

The results of the current study confirmed that compound 5C has potent anti-epileptic activity in lithium pilocarpine-induced status epilepticus in rats and exerts neuronal protective effects in brain regions, closely tied to the status epilepticus which is comparable to thalidomide and diazepam. This finding is aligned with the previous study that confirmed this compound has antiepileptic activity in PTZ-induced seizures in mice (14). These findings make compound 5C a potential candidate for further epilepsy research.

Conflict of interest statement
The authors declared no conflict of interest in this study.

Authors' contribution
The pharmacological study and manuscript writing had been conducted by A. Amanlou and supervised by A.R. Dehpour. F. Eslami and M. Shayan participated in the scoring study. Histopathological studies had been conducted by A. Amanlou and P. Mortazavi.

REFERENCES

1. Rojas A, Jiang J, Ganesh T, Yang MS, Lelutiu N, Gueorguieva P, *et al*. Cyclooxygenase-2 in epilepsy. Epilepsia. 2014;55(1):17-25.
   DOI: 10.1111/epi.12461.
2. Perucca E. Treatment of epilepsy in developing countries. BMJ. 2007;334(7605):1175-1176.
   DOI: 10.1136/bmj.39065.460208.80.
3. Smithson WH, Walker MC. What is Epilepsy? Incidence, Prevalence and Aetiology. In: ABC of Epilepsi. John Wiley & Sons; 2012. pp. 1-3.
4. Husain AM. Practical Epilepsy. Springer; 2015. Chapter 3, Epileptic Seizures; pp. 28-38. Chapter 4, Epilepsy Syndromes; pp. 39-53.
   DOI: 10.1891/9781617051876.
5. Walker MC. Pathophysiology of status epilepticus. Neurosci Lett. 2018;667:84-91.
   DOI: 10.1016/j.neulet.2016.12.044.
6. Pitkänen A, Buckmaster PS, Galanopoulou AS, Moshé SL. Models of Seizures and Epilepsy. 2nd ed. Elsevier; 2017. pp: 75,143,536.
7. Fountain NB. Status epilepticus: risk factors and complications. Epilepsia. 2000;41 Suppl 2:S23-S30.
   DOI: 10.1111/j.1528-1157.2000.tb01521.x.
8. Hong Z. Brain Damage Caused by Status Epilepticus. In: Wang X, Li S, editors. Refractory Status Epilepticus: Diagnosis and Treatment. Singapore, Springer; 2017. pp. 61-73.
   DOI: 10.1007/978-981-10-5125-8_3.
9. Jacobson JM. Thalidomide: a remarkable comeback. Expert Opin Pharmacother. 2000;1(4):849-863.
   DOI: 10.1517/14656566.1.4.849.
10. Melchert M, List A. The thalidomide saga. Int J Biochem Cell Biol. 2007;39(7-8):1489-1499.
    DOI: 10.1016/j.biocel.2007.01.022.
11. D'angio P, Maccarty J. Pharmaceutical compositions and dosage forms of thalidomide. United States Patents, 2007. No. US-7230012-B2. https://pubchem.ncbi.nlm.nih.gov/patent/US7230012.
12. Palencia G, Martinez-Juarez IE, Calderon A, Artigas C, Sotelo J. Thalidomide for treatment of refractory epilepsy. Epilepsy Res. 2010;92(2-3):253-257.
    DOI: 10.1016/j.eplepsyres.2010.10.003.
13. Science Museum. Thalidomide. 2019. Available from: https://www.sciencemuseum.org.uk/objects-and-stories/medicine/thalidomide.
14. Kiminejad Malaie P, Asadi M, Hosseini FS, Biglar M, Amanlou M. Synthesis, *in vivo* and *in silico* studies of N-aryl-4-(1,3-dioxoisoindolin-2-yl)benzamides as an anticonvulsant agent. Pharm Sci. 2020;26(1):38-44.
    DOI: 10.34172/PS.2019.54.
15. Tabatabaei Rafiei LS, Asadi M, Hosseini FS, Amanlou A, Biglar M, Amanlou M. Synthesis and evaluation of anti-epileptic properties of new phthalimide-4,5-dihydrothiazole-amide derivatives. Polycycl Aromat Compd. 2020:1-11.
    DOI: 10.1080/10406638.2020.1776345.
16. Asadollahi A, Asadi M, Hosseini FS, Ekhtiari Z, Biglar M, Amanlou M. Synthesis, molecular docking,

and antiepileptic activity of novel phthalimide derivatives bearing amino acid conjugated anilines. Res Pharm Sci. 2019;14(6):534-543.
DOI: 10.4103/1735-5362.272562.

17. Mante PK, Adongo DW, Woode E. Anticonvulsant effects of *antiaris toxicaria* aqueous extract: investigation using animal models of temporal lobe epilepsy. BMC Res Notes. 2017;10:167-175.
DOI: 10.1186/s13104-017-2488-x.

18. Racine RJ. Modification of seizure activity by electrical stimulation. I. After-discharge threshold. Electroencephalogr Clin Neurophysiol. 1972;32(3):269-279.
DOI: 10.1016/0013-4694(72)90176-9.

19. Nobakht M, Hoseini SM, Mortazavi P, Sohrabi I, Esmailzade B, Rahbar Rooshandel N, *et al*. Neuropathological changes in brain cortex and hippocampus in a rat model of Alzheimer's disease. Iran Biomed J. 2011;15(1-2):51-58.
PMID: 21725500.

20. Fujikawa DG, Pais ES, Aviles Jr ER, Hsieh KC, Bashir MT. Methamphetamine-induced neuronal necrosis: the role of electrographic seizure discharges. Neurotoxicology. 2016;52:84-88.
DOI: 10.1016/j.neuro.2015.11.001.

21. Kumari P, Singh N, Saha L. Potentiation of pentylenetetrazole-induced neuronal damage by dimethyl sulfoxide in chemical kindling model in rats. Indian J Pharmacol. 2018;50(2):84-87.
DOI: 10.4103/ijp.IJP_559_17.

22. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, *et al*. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676-682.
DOI: 10.1038/nmeth.2019.

23. Scott RC, Surtees R, Neville B. Status epilepticus: pathophysiology, epidemiology, and outcomes. Arch Dis Child. 1998;79(1):73-77.
DOI: 10.1136/adc.79.1.73.

24. Alldredge BK. Status epilepticus: a clinical perspective. Ann Pharmacother. 2006;40(3):577-577.
DOI: 10.1345/aph.1G518.

25. Griffiths T, Evans MC, Meldrum BS. Status epilepticus: the reversibility of calcium loading and acute neuronal pathological changes in the rat hippocampus. Neuroscience. 1984;12(2):557-567.
DOI: 10.1016/0306-4522(84)90073-3.

26. Faingold CL, Fromm GH. Drugs for Control of Epilepsy: Actions on Neuronal Networks Involved in Seizure Disorders. 1th ed. Boca Raton: CRC Press; 1992. pp: 195-211.
DOI: 10.1201/9780429262623.

27. McCandless DW. Epilepsy: Animal and Human Correlations. 1th ed. New York: Springer; 2012. pp: 309,437.
DOI: 10.1007/978-1-4614-0361-6.

28. Muller CJ, Bankstahl M, Groticke I, Loscher W. Pilocarpine vs. lithium-pilocarpine for induction of status epilepticus in mice: development of spontaneous seizures, behavioral alterations and neuronal damage. Eur J Pharmacol. 2009;619(1-3):15-24.

DOI: 10.1016/j.ejphar.2009.07.020.

29. Gonzalez-Reyes S, Santillan-Cigales JJ, Jimenez-Osorio AS, Pedraza-Chaverri J, Guevara-Guzman R. Glycyrrhizin ameliorates oxidative stress and inflammation in hippocampus and olfactory bulb in lithium/pilocarpine-induced status epilepticus in rats. Epilepsy Res. 2016;126:126-133.
DOI: 10.1016/j.eplepsyres.2016.07.007.

30. Terrone G, Frigerio F, Balosso S, Ravizza T, Vezzani A. Inflammation and reactive oxygen species in status epilepticus: biomarkers and implications for therapy. Epilepsy Behav. 2019;101(Pt B):106275,1-9.
DOI: 10.1016/j.yebeh.2019.04.028.

31. Ashhab MU, Omran A, Kong H, Gan N, He F, Peng J, *et al*. Expressions of tumor necrosis factor alpha and microRNA-155 in immature rat model of status epilepticus and children with mesial temporal lobe epilepsy. J Mol Neurosci. 2013;51(3):950-958.
DOI: 10.1007/s12031-013-0013-9.

32. Pourshadi N, Rahimi N, Ghasemi M, Faghir-Ghanesefat H, Sharifzadeh M, Dehpour AR. Anticonvulsant effects of thalidomide on pentylenetetrazole-induced seizure in mice: a role for opioidergic and nitrergic transmissions. Epilepsy Res. 2020;164:106362.
DOI: 10.1016/j.eplepsyres.2020.106362.

33. Islas-Espinoza AM, Campos-Rodriguez C, San Juan ER. Thalidomide protects against acute pentylenetetrazol and pilocarpine-induced seizures in mice. J Toxicol Sci. 2018;43(11):671-684.
DOI: 10.2131/jts.43.671.

34. Davood A, Shafaroodi H, Amini M, Nematollahi A, Shirazi M, Iman M. Design, synthesis and protection against pentylenetetrazole-induced seizure of N-aryl derivatives of the phthalimide pharmacophore. Med Chem. 2012;8(5):953-963.
DOI: 10.2174/157340612802084289.

35. Eslami SM, Ghasemi M, Bahremand T, Momeny M, Gholami M, Sharifzadeh M, *et al*. Involvement of nitrergic system in anticonvulsant effect of zolpidem in lithium-pilocarpine induced status epilepticus: evaluation of iNOS and COX-2 genes expression. Eur J Pharmacol. 2017;815:454-461.
DOI: 10.1016/j.ejphar.2017.10.002.

36. Palencia G, Rubio C, Custodio-Ramirez V, Paz C, Sotelo J. Strong anticonvulsant effect of thalidomide on amygdaloid kindling. Epilepsy Res. 2011;95(3):263-269.
DOI: 10.1016/j.eplepsyres.2011.04.008.

37. Payandemehr B, Rahimian R, Gooshe M, Bahremand A, Gholizadeh R, Berijani S, *et al*. Nitric oxide mediates the anticonvulsant effects of thalidomide on pentylenetetrazole-induced clonic seizures in mice. Epilepsy Behav. 2014;34:99-104.
DOI: 10.1016/j.yebeh.2014.03.020.

38. Peterson PK, Hu S, Sheng WS, Kravitz FH, Molitor TW, Chatterjee D, *et al*. Thalidomide inhibits tumor necrosis factor-alpha production by lipopolysaccharide- and lipoarabinomannan-stimulated human microglial cells. J Infect Dis. 1995;172(4):1137-1140.
DOI: 10.1093/infdis/172.4.1137.