Original Article

Synthesis, molecular docking, and antiepileptic activity of novel phthalimide derivatives bearing amino acid conjugated anilines

Azar Asadollahi[1], Mehdi Asadi[2,3], Faezeh Sadat Hosseini[2], Zeinab Ekhtiari[2], Mahmood Biglar[4], and Massoud Amanlou[2,4,5,*]

[1]*Department of Medicinal Chemistry, Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, I.R. Iran.*
[2]*Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, I.R. Iran.*
[3]*School of Pharmacy, Alborz University of Medical Sciences, Karaj, I.R. Iran.*
[4]*Drug Design and Development Research Center, The Institute of Pharmaceutical Sciences (TIPS), Tehran University of Medical Sciences, Tehran, I.R. Iran.*
[5]*Experimental Medicine Research Center, Tehran University of Medical Sciences, Tehran, I.R. Iran.*

Abstract

A series of N-aryl-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanamides derivatives were synthesized in two steps. Phthalic anhydride and phenylalanine are first reacted under microwave radiation to form 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid, which finally took part in an amidation reaction with different anilines. The final products were characterized by infrared, proton nuclear magnetic resonance (^{1}H NMR) and mass spectroscopy techniques. The antiepileptic activity of the synthesized compounds at a fixed dose of 10 mg/kg was evaluated by pentylenetetrazole at 70 mg/kg induced seizure threshold method in male mice (n = 5) and compared with aqueous DMSO (10 %, v/v; as negative control) and thalidomide (70 mg/kg; as positive control). The results indicated that compounds **5c, 5e,** and **5f** as well as thalidomide significantly have higher latency time than what observed with aqueous DMSO ($P < 0.05$). The seizure latency threshold for **5e** and **5f** were statistically similar to the results of thalidomide but compound **5c** showed significantly higher latency time than thalidomide. While, the electron-deficient benzene ring (**5a** and **5b**) has demonstrated the lowest activity but compound **5e**, which is the most electron rich product among tested compounds, showed good antiepileptic activity. Molecular docking was performed in order to understand how the synthetized compounds, interact with gamma-aminobutyric acid (GABA)$_A$ receptor. Docking results were in good harmony with experimental data and indicated that lowest binding energy belongs to compound **5c**, which has strongest interactions with the active site of GABA$_A$ receptor. Compound **5c** could be used for further investigation.

Keywords: Antiepileptic agent; Microwave synthesis; Molecular docking simulation; Phenylalanine; Phthalimide derivatives.

INTRODUCTION

Epilepsy is a group of neurological disorders, which is characterized by epileptic seizures (1). The exact cause of seizures is unknown, but can be intensified by brain injury, stroke, brain tumors, infections, and birth defects (2,3). In general, excessive and abnormal nerve cells activity in the cerebral cortex leads to epileptic seizures (4).

A number of clinically available antiepileptic drugs such as phenytoin, phenobarbital, and ethosuximide contain a heterocyclic ring possessing nitrogen atom bonded to two carbonyl groups. The structure of some antiepileptic class drugs can be observed in Fig. 1. Thalidomide is an immunomodulatory drug, which is used in the treatment of epilepsy. The structure of thalidomide contains a phthalimide ring conjugated to a glutarimide. Thalidomide is shown to be active in the erythema nodosum leprosum control (5), inflammatory, and autoimmune diseases, including Crohn's disease (6) and rheumatoid arthritis but different side effects prevents its general use (7).

Phthalimide conjugated to an amide bond, has been of the interest in several drug discovery efforts (8-10) due to its wide biological activities (11). Microwave energy caused a great development in modern organic synthesis because of its advantageous features (12,13). Microwave synthesis is fast, efficient, with low side products, and therefore, is keenly interested in drug discovery (14,15). Regarding these advantages, microwave assisted reactions has the potential to influence medicinal chemistry in three major phases of the drug discovery, generation of a discovery library, hit-to-lead efforts and lead optimization (16,17).

Based on biological activities of the skeleton containing phthalimide conjugated to an amide bond, hereby, we introduce a new class of compounds containing phthalimide amino acid conjugate via microwave synthesis.

In this study, the phthalimide was conjugated to phenylalanine and converted to final product by amidation reaction whose structure is depicted in Fig. 2. Molecular docking was performed in order to understand how the synthetized compounds, interact with gamma-aminobutyric acid (GABA)$_A$ receptor (18). Anticonvulsant activity was evaluated by tonic-clonic generalized seizure induced by pentylenetetrazole (PTZ) on male mice.
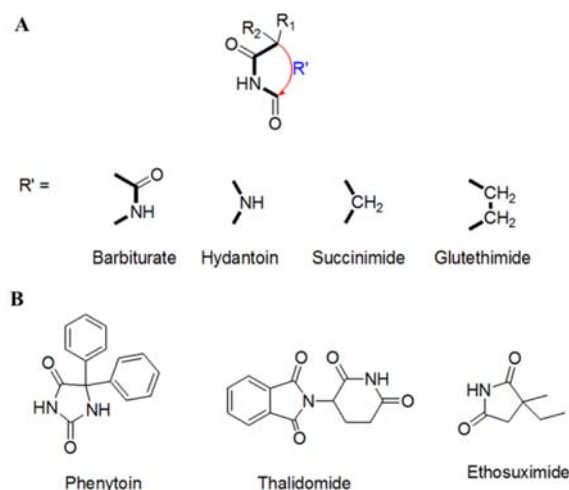
Fig. 1. General structure of different (A) antiepileptic drugs and (B) structure of drugs used in clinics.
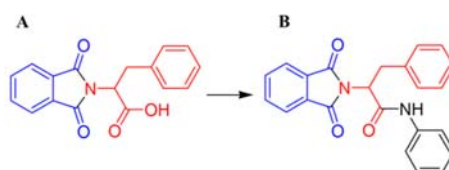
Fig. 2. Structure of (A) 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid as intermediate compound and (B) N-aryl-2-(1,3-dioxoisoindolin-2-yl)- 3-phenylpropanamide as final products.
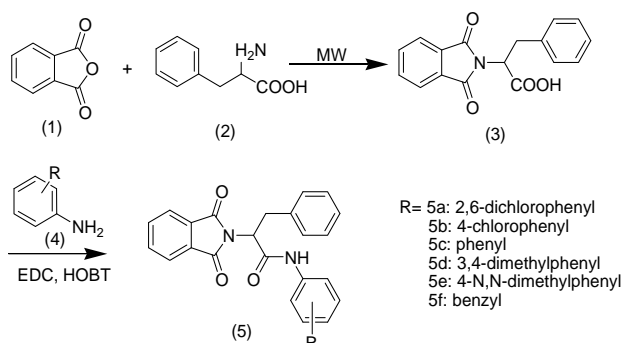
Scheme 1. Synthesis of N-aryl-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanamides.

MATERIALS AND METHODS

Instrumentation

All commercially solvents, reagents, and chemicals were purchased from Merck (Germany) and Sigma-Aldrich (USA) chemical companies and used as received without further purification. The infrared (IR) spectra were obtained on a Nicolet Fourier-transform (FT)-IR Magna 550 spectrophotometer (Nicolet, USA, potassium bromide disks). Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker FT-500 spectrometer (Germany) at 500 MHz using tetramethylsilane (TMS) as internal standard in pure deuterated solvents. Chemical shifts are given in the δ scale in parts per million (ppm). Mass spectra of the products were obtained with a HP (Agilent technologies) 5937 mass selective detector (USA). Thin layer chromatography (TLC) analyses were performed on Merck silica gel-60F$_{254}$ aluminum-backed plates.

Procedure for the synthesis of 2-(1,3-dioxo-isoindolin-2-yl)-3-phenylpropanoic acid (3)

For the synthesis of compound **3**, phthalic anhydride (10 mmol, 1.48 g) and phenylalanine (10 mmol, 1.65 g) were mixed well together and then, silica gel (1.48 g) as dehydrating agent added to the mixture. The reaction mixture was transferred to a 50 mL flask and irradiated by microwave radiation for 5 min and repeated if necessary (Scheme 1). The progress of reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and poured on 10 g of ice. The title compound (**3**) was separated by filtration and recrystallized from ethanol to obtain a white solid with the yield of 85%. Melting point: 176-178 °C. IR (KBr): 3424, 3284, 1665, 1624 cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz): 3.59 (d, J = 8.9 Hz, 2H, CH$_2$), 5.23 (m, 1H, CH-$_{Chiral}$), 7.14-7.19 (m, 5H, Ph), 7.69 (td, J_1 = 5.4 Hz, J_2 = 3.0 Hz, 2H, H$_{5,6}$), 7.78 (dd, J_1 = 5.4 Hz, J_2 = 3.0 Hz, 2H, H$_{4,7}$) (19-23).

Procedure for the synthesis of 2-(1,3-dioxo-isoindolin-2-yl)-N,3-diphenylpropanamide derivatives (5)

To the solution of 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**3**) (10.0 mmol) in dimethylformamide (25 mL) at 0 °C, hydroxybenzotriazole (12.0 mmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (11.0 mmol) were added and stirred for 1 h. Afterward, to the former product, a solution of different anilines (10.0 mmol) in dimethylformamide (10 mL) was added and stirred at room temperature for 24 h (Scheme 1). After completion of the reaction, the reaction quenched by adding water and dichloromethane. The mixture washed with HCl (1M, 3 × 25 mL), NaHCO$_3$ solution (10% w/v, 3 × 25 mL) and brine (25 mL). The organic layer was collected and dried over MgSO$_4$ and concentrated in reduced pressure. The product was recrystallized from ethanol (20-23).

Molecular docking

Docking simulations using AutoDock 4.2 (24) were performed on thalidomide and synthetized compounds in benzodiazepine (BZD)-binding pocket. Homology modeled GABA$_A$ molecular structure was obtained from Richter *et al.* (25).

To prepare two dimensional (2D) structures of compounds (**5a-f**) MarvineSketch version 15.10.12 (26) was used and 3D structures were obtained by Chem3D ultra version 8.0 (27). All structures were minimized with MM2 algorithm and then converted to pdb format file. AutoDockTools 1.5.6 (ADT), Autogrid 4.2, and Autodock 4.2 (24) were used to prepare input files, calculate grid maps and docking simulations. Grid-point spacing of 0.375 Å and grid box of 50 × 50 × 50 Å (x, y, and z) points with the xyz-coordinated 43.321, 43.476, and 8.701 was used. After merging all non-polar hydrogen, Kollman charged were added to the receptor. All other values were set as defaults and Lamarckian genetic algorithm (GLA) search for 100 run job were used (28). Discovery Studio visualizer version 17.2 (29) and Pymol version 1.1evel were used for visualization (30).

Determination of pentylenetetrazole induced seizure threshold

Antiepileptic activity of compounds **5a-f** was evaluated by PTZ seizure threshold test (31). For studying the tonic-colonic seizure, 8 groups of 5 male mice (weighing 20-30 g) were selected and maintained at a controlled temperature (25 ± 2 °C) and 12/12-h light/dark cycles. The animals were allowed free access to food and water except when removed from their cages for the experimental procedure. Injections were performed to the negative and positive control, and the test groups. The solutions of the synthesized compounds (10 mg/kg), thalidomide (70 mg/kg, positive control) in aqueous DMSO (10 %; v/v; vehicle; negative control) were prepared and injected subcutaneously. After 30 min PTZ (70 mg/kg of mice) was injected and the animals' first tonic-clonic seizure episode and death were recorded during 60 min post injection.

This study was carried out in accordance with the guidelines for the care and use of laboratory animals of the Tehran University of Medical Sciences. Study procedures were approved by the Animal Ethics Committee of Tehran University of Medical Sciences, Tehran, I.R. Iran under the ethical No. IR.TUMS.REC.1394.1516.

RESULTS

N- (2 ,6 - dichlorophenyl) – 2 - (1 ,3 - dioxo-isoindolin-2-yl)-3-phenylpropanamide (5a)

White powder, yield 67%, melting point > 250 °C. IR (KBr): 3424 (NH), 3084(CH aromatic), 1665 (C=O), 1624 (C=C aromatic) cm^{-1}. ^{1}H NMR (500 MHz: CDCl$_3$): 3.60 (d, J = 6.25 Hz, 2H, CH$_2$), 5.2 (t, J = 6.25 Hz, 1H, CH$_{Chiral}$), 7.16-7.24 (m, 5H, Ph), 7.61 (d, J = 8.5 Hz, 1H, H$_{4''}$), 7.69 (d, J = 5.0 Hz, 2H, H$_{5,6}$), 7.77 (t, J = 8.5 Hz, 2H, H$_{3'',5''}$), 7.88 7.69 (d, J = 5.0 Hz, 2H, H$_{4,7}$), 8.07 (s, 1H, NH-$_{Amid}$). MS: m/z: 438.16. Anal. calcd. for C$_{25}$H$_{23}$N$_3$O$_3$: C, 62.89; H, 3.67; N, 6.38. Found: C, 62.74; H, 3.38; N, 6.45.

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

White powder, yield 71%, melting point: 198-200 °C. IR (KBr): 3430 (NH), 3008 (CH aromatic), 1686 (C=O), 1620 (C=C aromatic) cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz): 3.66 (dd, J_1 = 13.8 Hz, J_2 = 6.5 Hz, 2H, CH$_2$-diastropic), 3.62 (dd, J_1 = 13.8 Hz, J_2 = 6.5 Hz, 2H, CH$_2$-diastrotopic), 5.24 (m, 1H, CH-chiral), 7.18 (t, J = 7.5 Hz, 1H, H$_{4'}$), 7.23 (d, J = 7.5 Hz, 2H, H$_{2',6'}$), 7.25-7.28 (m, 4H, H$_{3',3'',5'5''}$), 7.43 (d, J = 8.0 Hz, 2H, H$_{2'',6''}$), 7.72 (td, J_1 = 5.0 Hz, J_2 = 3.0 Hz, 2H, H$_{5,6}$), 7.81 (dd, J_1 = 5.0 Hz, J_2 = 3.0 Hz, 2H, H$_{4,7}$), 8.30 (s, 1H, NH-amid). MS: m/z (%) = 404.16. Anal. calcd. for C$_{23}$H$_{16}$Cl$_2$N$_2$O$_3$: C, 68.24; H, 4.23; N, 6.92. Found: C, 68.41; H, 4.45; N, 7.08.

2-(1, 3-dioxoisoindolin-2-yl)-N, 3-diphenyl-propanamide (5c)

White powder, yield 63%, melting point: 212-214 °C. IR (KBr): 3435 (NH), 3022 (CH aromatic), 1685 (C=O), 1618 (C=C aromatic) cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz): 3.62 (dd, J_1 = 14.2 Hz, J_2 = 7.5 Hz, 2H, CH$_2$-diastrotopic), 3.62 (dd, J_1 = 14.2 Hz, J_2 = 7.5 Hz, 2H, CH$_2$-diastrotopic), 5.25 (m, 1H, CH-chiral), 6.77 (d, J = 7.5 Hz, 2H, H$_{2',6'}$), 6.83 (t, J = 7.2 Hz, 1H, H$_{4''}$), 7.11 (t, J = 7.5 Hz, 1H, H$_{4'}$), 7.19 (d, J = 7.5 Hz, 2H, H$_{3',5'}$), 7.31 (d, J = 7.2 Hz, 2H, H$_{3'',5''}$), 7.48 (d, J = 7.2 Hz, 2H, H$_{2'',6''}$), 7.71 (dd, J_1 = 5.5 Hz, , J_2 = 3.0 Hz, 2H, H$_{5,6}$), 7.81 (dd, J_1 = 5.5 Hz, , J_2 = 3.0 Hz, 2H, H$_{4,7}$), 8.24 (s, 1H, NH-Amid). MS: m/z (%) = 370.18. Anal. calcd. for C$_{23}$H$_{17}$ClN$_2$O$_3$: C, 72.62; H, 5.61; N, 10.16. Found: C, 72.85; H, 5.80; N, 10.38.

N, N-(3, 4-dimethylphenyl)-2-(1, 3-dioxo-isoindolin-2-yl)-3-phenylpropanamide (5d)

White powder, yield 65%, melting point > 250 °C. IR (KBr): 3440 (NH), 3025(CH aromatic), 1688 (C=O), 1619 (C=C aromatic) cm^{-1}. ^{1}H NMR (CDCl$_3$, 500 MHz): 221-2.23 (s, 6H, 2 × CH$_3$), 3.62 (dd, J_1 = 14.1 Hz, J_2 = 6.6 Hz, 1H, CH$_2$-diastrotopic), 3.67 (dd, J_1 = 14.1 Hz, J_2 = 6.6 Hz, 1H, CH$_2$-diastrotopic), 5.23 (m, 1H, CH-chiral), 7.05 (d, J = 8.0 Hz, 1H, H$_{5'}$), 7.18 (d, J = 8.0 Hz, 2H, H$_{6'}$), 7.21-7.23 (m, 5, Ph), 7.28 (s, 1H, H$_{2'}$), 7.72 (td, J_1 = 5.2 Hz, J_2 = 3.0 Hz, 2H, H$_{5,6}$), 7.81 (dd, J_1 = 5.2 Hz, J_2 = 3.0 Hz, 2H, H$_{4,7}$), 8.03 (s, 1H, NH-amid). MS: m/z (%) = 398.26. Anal. calcd. for

$C_{25}H_{22}N_2O_3$: C, 75.36; H, 5.57; N, 7.03. Found: C, 75.59; H, 5.82; N, 7.34.

N- (3, 4-dimethylphenyl) -2 - (1, 3-dioxo-isoindolin-2-yl)-3-phenylpropanamide (5e)

White powder, yield 62%, meltin point > 250 °C. IR (KBr): 3434 (NH), 3014(CH aromatic), 1668 (C=O), 1618 (C=C aromatic) cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): 3.06 (s, 6H, 2×CH₃), 3.53 (d, *J* = 7.5 Hz, 2H, CH₂), 5.24 (m, 1H, CH-chiral), 7.15 (d, *J* = 8.5 Hz, 2H, H₃″,₅″), 7.19-7.22 (m, 5H, Ph), 7.43 (d, *J* = 8.5 Hz, 1H, H₂″,₆″), 7.68 (d, *J* = 5.0 Hz, 2H, H₅,₆), 7.76 (d, *J*= 5.0 Hz, 2H, H₄,₇), 8.60 (s, 1H, NH-amid). MS: m/z (%) = 398.26. Anal. calcd. for $C_{25}H_{23}N_3O_3$: C, 72.62; H, 5.61; N, 10.16. Found: C, 72.39; H, 5.42; N, 10.35.

N-benzyl-2-(1, 3-dioxoisoindolin-2-yl)-3-phenylpropanamide (5f)

White powder, yield 70%, melting point : 202-204 °C. IR (KBr): 3430 (NH), 3028 (CH aromatic), 1688 (C=O), 1622 (C=C aromatic) cm⁻¹. ¹H NMR (CDCl₃, 500 MHz): 3.26 (dd, *J₁* = 14.25 Hz, *J₂* = 7.5 Hz, 1H, CH₂-diastrotopic), 3.31 (dd, *J₁* = 14.25 Hz, *J₂* = 7.5 Hz, 1H, CH₂-diastrotopic), 4.41(dd, *J₁* = 11.25 Hz, *J₂* = 5.0 Hz, 2H, N-CH₂), 4.92 (m, 1H, CH-chiral), 7.67 (t, *J* = 8.5 Hz, 1H, H₄″), 7.69-7.74 (m, 5H, Ph), 7.91 (d, *J* = 5.0 Hz, 2H, H₅,₆), 7.96 (d, *J* = 5.0 Hz, 2H, H₄,₇), 8.27 (s, 1H, NH-amid). MS: m/z (%) = 398.26. Anal. calcd. for $C_{24}H_{20}N_2O_3$: C, 74.98; H, 5.24; N, 7.29. Found: C, 75.21; H, 5.44; N, 7.60.

Molecular docking simulation

Docking results showed that amongst all docked compounds to BZD binding pocket of GABA_A, compound **5c** presented appropriate interaction with active site pocket with lowest binding energy (Table 1). Interactions of thalidomide and compound **5c** with BZD binding pocket of GABA_A are shown in Figs. 3 and 4. Phthalimide part of thalidomide plays effective role in three H bonds with Glu189, Ser 204, and Tyr 159, and two π–π interactions with key residues His 101 and Phe 77. Carbonyl group has two H bonds with Thr 142 and Thr 206. NH group also has H bond with Thr 206 (Fig. 3).

In comparison with thalidomide, compound **5c** had two same π–π interactions with residues His 101 and Phe 77 and one H bonds with Tyr 159. His 101 showed extra π–π interactions with benzyl and pyrrolidine-2, 5-dione. In addition, there were two π–σ interactions between Tyr 159 and compound **5c** and also phenyl ring show new hydrophobic interaction with Met 130 (Fig. 4).
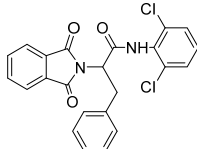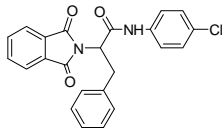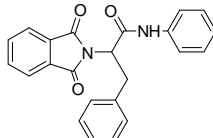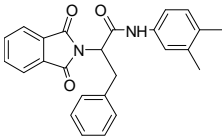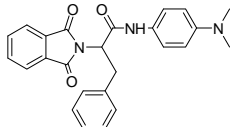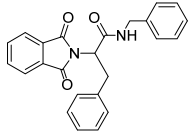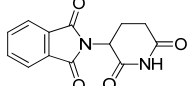
It seems that the difference between the binding energy of thalidomide and compound **5c** is related to conformational changes which were arising from phenyl ring lead to getting appropriate interaction of compound **5c** with BZD-binding pocket in comparison with thalidomide (Fig. 5).

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

The antiepileptic activity of the compounds **5a-f** was evaluated by PTZ induced seizure threshold method (31). The behaviors of the animals were evaluated for 60 min. The results of the antiepileptic activity of the test compounds are presented in Fig. 6. As can be observed, most of the compounds showed antiepileptic activity in the PTZ induced seizure threshold test. Among the tested compounds, compound **5c** showed the highest activity (Table 1). For better understanding the activity of the tested compounds, their antiepileptic activities were compared with thalidomide as a known antiepileptic agent.

It is notable that, the electron poor compounds showed the lowest activity. Among the tested compounds, 2,6-dichloro- and 4-chloro- derivatives had been the least active materials. In addition, N,N-dimethylamino derivative (**5e**), which is the most electron rich compound among the tested substances had shown good antiepileptic activity. On the other hand, the most active compound was non-substituted derivative (**5c**) with lowest lipophilic character. Therefore, it can be assumed that for proper interaction with the receptor, the compound must be linear without a substitution on ortho position and lower lipophilicity.

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Table 1. Chemical structure and calculated docking binding energy of compounds **5a-f.**

Compounds	Structures	Log p	Binding energy (kcal/mol)
5a		4.79	-9.61
5b		4.23	-9.16
5c		3.67	-9.95
5d		4.64	-9.51
5e		3.95	-8.97
5f		3.74	-9.34
Thalidomide		0.15	-7.17
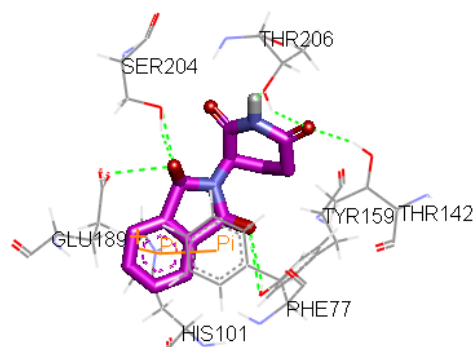
Fig. 3. Interaction of thalidomide with gamma-aminobutyric acid A (GABA$_A$) receptor benzodiazepine binding pocket.
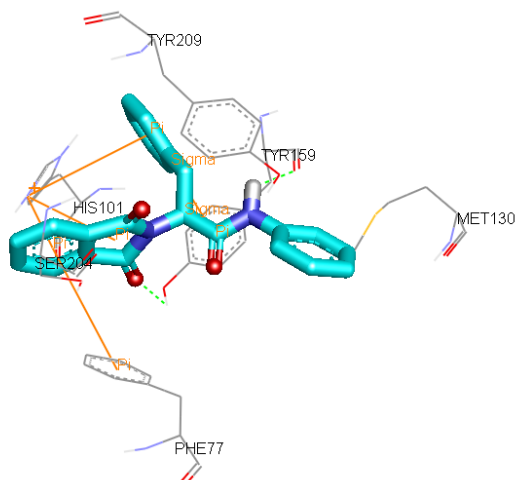
Fig. 4. Interaction of compound **5c** with gamma-aminobutyric acid (GABA$_A$) A receptor benzodiazepine binding pocket.
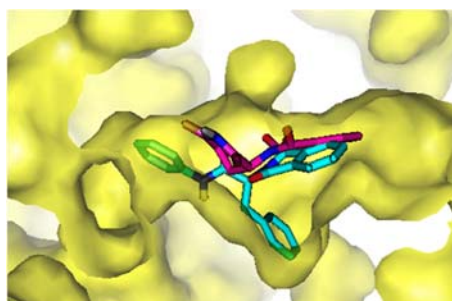
Fig. 5. Superimposition of thalidomide and compound **5c** in gamma-aminobutyric acid (GABA$_A$) A receptor benzodiazepine binding pocket.
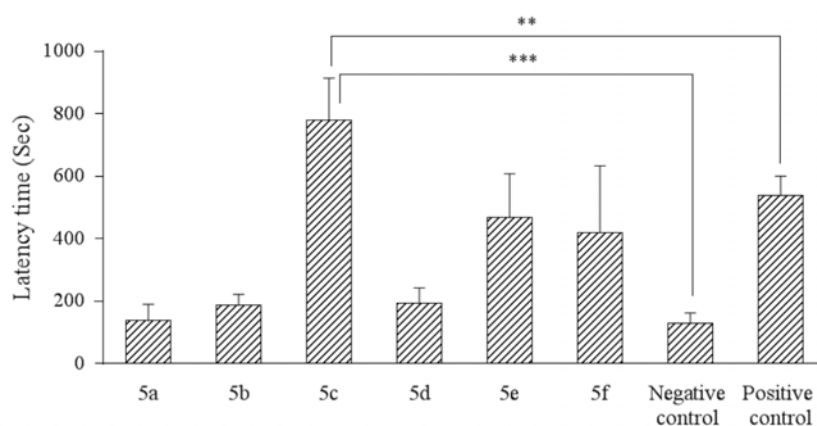
Fig. 6. Antiepileptic activity of 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid derivatives. The solutions of the synthesized compounds **5a-f** (10 mg/kg), thalidomide (70 mg/kg, positive control) in aqueous DMSO (10 %; v/v; vehicle; negative control) were used. $**P < 0.01$ and $***P < 0.001$ show significant differences between indicated groups.

The detailed results, based on the raw data obtained in this research, related to latency times following the exposure to PTZ on different treatment groups, average, and SEM of each group were calculated and the data was plotted as column chart with error bars showing SEM value (Fig. 6). A one-way analysis of variance (ANOVA) was conducted and followed by Homl-Sidak post-hoc test, using Sigma-Plot software (version 12.5.0.38). Normality test by Shapiro-Wilk method was Passed (P = 0.628). Difference in the mean values among treatment groups was significant ($P \leq 0.001$) and power of the performed analysis with alpha was 0.050:1.000. Comparison of different factors by the performed Holm-Sidak method can be more described as below. Results of treatment by aqueous DMSO (negative control) and thalidomide (positive control) groups showed a statistically significant difference (P <0.05) which can be considered as an overall validity proof for the performed animal study. Average results of latency time for compounds **5a, 5b,** and **5d** group was 138.0, 187.0, and 192.4 sec consequently, which is not significantly different from the results of aqueous DMSO (P > 0.05). Therefore, it can be deduced that the *in vivo* response of these groups are not statistically different from that of negative control group. In addition, the results of latency times of **5c**, **5e,** and **5f** groups are significantly higher than that of aqueous DMSO group (P < 0.05) which was indicative of an *in vivo* response different from negative control group. The result of **5e** group is less than the latency results of thalidomide though nonsignificant. Although average latency of **5c** treated group was highest amongst all tested compounds, the results for this group was statistically different from that of thalidomide group. Therefore, the *in vivo* results of **5c** and thalidomide were considered not similar (Fig. 6).

DISCUSSION

In recent years, the synthesis of compounds from the phthalimide family has been extensively developed to achieve antiepileptic and anticancer compounds (32-33). Regarding the importance of compounds containing phthalimide ring, we hereby reported a facile, two-step strategy for the synthesis of this class of organic compounds. The synthesis route is presented in Scheme **1**. The first step, contains microwave assisted reaction of phthalic anhydride (**1**) with phenylalanine (**2**) to form desire 2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanoic acid (**3**). This step benefits the advantages of microwave mediated organic synthesis which performed very fast and the products obtained in high isolated yields. The second step involved amidation reaction between carboxylic acid of compound (**3**) and the amine group of different anilines (**4**). To get more desirable reaction, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and hydroxybenzotriazole were used for the activating the carboxylic acid in the amidation reaction (34).

Using the advantages of two efficient reactions for the synthesis of compounds containing phthalimide, six derivatives containing different functional groups were synthesized. Different anilines bearing electron donating and electron withdrawing functionalities successfully participated in the amidation reaction and gave the N-aryl-2-(1,3-dioxoisoindolin-2-yl)-3-phenylpropanamide derivatives (**5a-f**). The anilines were selected based on the Hansch and Hammett principles, which specified the physicochemical properties and induction effects of compounds.

The title compounds contained different substituents with different electronic environments, as shown in Table 1. They contained different hydrophilic and hydrophobic groups, either electron-deficient or electron-rich groups, to study and compare their antiepileptic activity and physicochemical properties.

The antiepileptic screening for the newly synthesized compounds displayed that all of them were active in PTZ test, and **5c** showed maximum latency time 780.8 sec which was more active than recently introduced compound "N-(3-4-dimethylphenyl)-4-(1, 3-dioxoisoindolin-2-yl)benzamide" (35). It should be indicated that the latency time of

both these compounds was better than thalidomide. In addition, compounds **5e** and **5f** approximately showed similar protection, while compounds **5a**, **5b**, and **5d** showed fewer protections than thalidomide. These results indicated that the compound **5c**, which has no substitutions on phenyl ring, induced the best interaction with the GABA_A receptor in comparison to other compounds. Therefore, the molecular size of this series may play important role to interact with the receptor. This result was completely compatible with docking results in which, compound **5c** showed the lowest binding energy among all compounds. In addition, the role of dimethylamine and methylene in benzylic position as electron-donating groups on the phenyl ring, for **5e** and **5f** is evident. This effect has been reduced for **5d** due to both electronic effects and spatial density of two methyl groups in *meta* and *para* positions. Therefore, the compound **5c** could be considered a new hope in the management of epilepsy; of course further studies are required to investigate the safety and pharmacological effects of this compound.

CONCLUSION

In conclusion, six different N-aryl-2-(1, 3-dioxoisoindolin-2-yl)-3-phenylpropanamides derivatives were synthesized in good yields and their antiepileptic activity were evaluated by PTZ induced seizure threshold method. Obtained results indicated that antiepileptic activity of compound **5c** significantly higher from what observed for aqueous DMSO and thalidomide. Molecular docking simulation confirmed the experimental results and revealed that the proper interaction with active site of GABA_A receptor and appropriate lipophilicity of compound **5c** was responsible for this effect. So this compound would be a good candidate for further investigations and optimization to develop new antiepileptic drug.

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