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Research Article

DESIGN, SYNTHESIS AND ANTICONVULSANT ACTIVITY OF PHTHALIMIDE DERIVATIVES

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ABSTRACT

A novel series of 2-(4-((2-alkylhydrazinyl)methyl)phenyl) isoindoline-1,3-dione **(6a-f)** and *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **(7a-b)** have been synthesized. The structures of synthesized compounds were confirmed by spectral (FT-IR, ^{1}H NMR, ^{13}C NMR and Mass) data. The title compounds (**6a-f** and **7a-b**) were evaluated for *in vivo* anticonvulsant activity using behavioral method. Among synthesized derivatives, compound **6e** exhibited good activity when compared with reference standard phenytoin, while **6a** and **7b** did not exhibit activity**.** Compounds **6b, 6c, 6d, 6f** and **7b** exhibited moderate to weak activity.

Keywords: Phthalimide, phenytoin, behavioral method, anticonvulsant activity.

INTRODUCTION

Epilepsies are a group of disorders of CNS characterized by paroxysamal cerebral dysrhythmia, manifesting as brief episodes (seizures) of loss or disturbance of consciousness, with or without characteristic body movements (convulsions), sensory or psychiatric phenomenon.[1] The majority of epileptic seizures are controlled through drug therapy, particularly anticonvulsant drugs. The type of treatment prescribed will depend on several factors including the frequency and severity of the seizures as well as the person's age, overall health, and medical history. An accurate diagnosis of the type of epilepsy is also critical to choosing the best treatment. However, current estimates indicate that 20 - 30% of patients with epilepsy are refractory to all forms of medical therapy. These medically intractable patients are candidates for surgical treatment in an attempt to achieve better seizure control. Another group of patients who might benefit are those whose seizures may be relatively well controlled but who have certain characteristic presentations or lesions that strongly suggest surgical intervention might be curative.[2] From the study of structures of clinically established drugs, it can be concluded that the anticonvulsant properties have been displayed by various hydrazones (=N-NH), amides (-CONH2) and carbamides(-NHCO-NH-). Anti-seizure medications may have some side effects. Mild side effects include: Fatigue, dizziness, weight gain, loss of bone density, skin rashes, loss of coordination, speech problems, memory and thinking problems. More severe but rare side effects include: depression, suicidal thoughts and behaviors, severe rash, inflammation of certain organs such as liver.[3-4]

Since current drug therapy is associated with so many side effects, continued research and exploration of newer, safer and effective alternatives are needed for the treatment of epilepsies. According to the studies several of *N*-phenylphthalimides were shown to possess a phenytoin-like profile and strong activity in the MES test.[5] In the present study compounds with the isoindoline-1,3-dione (phthalimide) system as a core fragment have been synthesized and evaluated for their antiepileptic activity. Among heterocyclic scaffolds, phthalimides are of particular biological interest and have been reported for their antimycobacterial[6], anticonvulsant[7], antiproliferative[8], MAO inhibitory[9] and antiinflammatory[10] activity. In view of these observations we planned to synthesize pthalimide derivatives having amide linkage in order to get better anticonvulsant activity with less side effects.

MATERIALS AND METHODS

All chemicals and reagent used were of laboratory grade and were purchased from Sigma-Aldrich Ltd, Molychem chemicals Ltd, SD Fine Chemicals Ltd, India. Solvents used were dried and purified as and when required. The reactions were monitored by Thin Layer Chromatography. TLC was performed on Silica gel G plates activated for 30 min (120 ^{0}C) and developed using various solvent systems. The spots were visualized by exposure to iodine vapours. Melting points of synthesized compounds were determined by Thiele's tube melting point apparatus and are uncorrected. FT-IR spectra were recorded on Schimadzu IR AFFINITY-1 spectrophotometer by using KBr pellets. The ^{1}H-NMR and ^{13}C-NMR was recorded on Bruker Avance II 400 NMR spectrophotometer by using $DMSOd_6$ as solvent and Tetra Methyl Silane (TMS) as internal standard, chemical shifts are expressed as δ values (ppm). The mass spectra were recorded on Waters, Q-TOF Micromass (LC-MS).

Synthesis of 2-(4-methylphenyl)isoindoline-1,3-dione(3)

Equimolar quantities of phthalic anhydride **1** and 4-methylaniline **2** were dissolved in glacial acetic acid (10 mL) and the resulting solution was refluxed for 2 h. The product separated on cooling was separated, washed with water and recrystallized from ethanol.[11] White solid, Yield 72.29 %, m. p. 186-188 ^{0}C, IR data (KBr, cm^{-1}) 3041.74 (aromatic C-H), 2916.37 (aliphatic C-H), 1710.86 (C=O).

Synthesis of 2-(4-(bromomethyl)phenyl)isoindoline-1,3-dione(4)

The 2-(4-methylphenyl)isoindoline-1,3-dione **(3,** 0.1 mol) in glacial acetic acid was heated at 80-100 ^{o}C with constant stirring and to this bromine (0.1 mol) dissolved in glacial acetic acid (20 mL) was added drop wise over a period of 1 h maintaining the temperature at 80-100 ^{0}C. The heating was continued till the color of solution changes slightly. The solution was then cooled and poured in ice cold water and the product thus obtained was washed thoroughly with cold water and recrystallized from ethanol. [12]

Pale yellow solid, Yield 65.83 %, m. p. 158-160 ^{0}C, IR data (KBr, cm^{-1}) 3041.74 (aromatic C-H), 2916.37 (aliphatic C-H), 1716.65 (C=O), 721.38 (CH-Br).

Synthesis of 2-(4-(hydrazinylmethyl)phenyl)isoindoline-1,3-dione(5)

Compound 2-(4-(bromomethyl)phenyl)isoindoline-1,3-dione **(4,** 0.1 mol**)** was taken in 25ml of ethanol in a round bottom flask and hydrazine hydrate (0.1 mol) was added to it drop wise. The resulting mixture was refluxed for 1 h. The solid obtained on cooling was separated by filtration and recrystallized from ethanol. [13]

Pale white solid, Yield 67 %, m. p. 338-340^{0}C, IR data (KBr, cm^{-1}) 3338.78 (-NH), 3020.53 (aromatic C-H), 2896.08 (aliphatic C-H), 1658.78 (C=O).

General experimental procedure for synthesis of 2-(4-((2-alkylhydrazinyl)methyl) phenyl)isoindoline-1,3-dione derivatives (6a-f)

A mixture of 2-(4-(hydrazinylmethyl)phenyl)isoindoline-1,3-dione **(5,** 0.1 mol**),** triethylamine (0.2 mol) in 30 ml of ethanol was stirred vigorously for 5 min at room temperature. To the resulting solution, alkyl halide (0.1 mol) dissolved in 2 ml ethanol was added, and the mixture was stirred for 6 h at room temperature. The reaction mixture was quenched by addition of water and diluted with ethyl acetate. The organic layer was washed with brine two times and dried over magnesium sulphate. The solution was filtrated and concentrated to get solid product. The crude products were recrystallized from appropriate solvents to furnish compounds **6 a-f.**[14-15]

2-(4-((2-isopropylhydrazinyl)methyl)phenyl) isoindoline-1,3-dione(**6a**)

Pale brown solid; Yield 65.5 %; m.p. 324-326^{0}C; IR data (KBr, cm^{-1}): 3165.32 (-NH), 3012.94 (aromatic,C-H), 2894.31 (aliphatic C-H), 1661.75 (C=O).

2-(4-((2-(2-chloroethyl)hydrazinyl)methyl) phenyl)isoindoline-1,3-dione (**6b**)

Yellow solid; Yield 40.32 %; m.p. >355 ^{0}C; IR data (KBr, cm^{-1}): 3185.32 (-NH),3016.80 (aromatic, C-H), 2895.28 (aliphatic C-H),1659.82 (C=O), 655.92 (C-Cl).

2-(4-((2-isobutylhydrazinyl)methyl)phenyl) isoindoline-1,3-dione (**6c**)

Dark brown solid; Yield 83.3 %; m.p. 318-320 ^{0}C; IR data (KBr, cm^{-1}): 3162.32 (-NH), 3015.83 (aromatic, C-H), 2894.31 (aliphatic C-H), 1664.64 (C=O).

2-(4-((2-(tert-butyl) hydrazinyl) methyl) phenyl) isoindoline-1,3-dione (**6d**)
Dark brown solid; Yield 58.3 %; m.p. 330-332 ^{0}C; IR data (KBr, cm^{-1}): 3165.32 (-NH), 3015.83 (aromatic, C-H), 2894.31 (aliphatic C-H), 1664.64 (C=O).

2-(4-((2-butylhydrazinyl)methyl)phenyl) isoindoline-1,3-dione (**6e**)
Pale brown solid; Yield 81.3 %; m.p. 348-350 ^{0}C; IR data (KBr, cm^{-1}): 3165.32 (-NH),3015.83 (aromatic,C-H), 2921.32 (aliphatic C-H), 1664.64(C=O).

2-(4-((2-(4-methylbenzyl) hydrazinyl) methyl) phenyl)isoindoline-1,3-dione (**6f**)
Pale brown solid; Yield 73.58 %; m.p. 328-330^{0}C; IR data (KBr, cm^{-1}): 3165.32 (-NH), 3012.94 (C-H,Ar), 2921.32 (C-H, aliphatic), 1660.78 (C=O); ^{1}H NMR (DMSO d_6): δ 11.97 (s, 1H, NH), 11.58 (s, 1H, NH), 7.1-7.9 (d,10H,aromatic-H), δ 8.1-8.3 (t,2H,aromatic-H), 3.07-3.2 (s,4H,CH_2), 2.27-2.36 (s,3H,CH_3); ^{13}C NMR (DMSO-d_6): δ 137.14- 123.24 (18C, aromatic-C); 150.3, 154.66 (2C, C=O); 78-79 (2C,C-N), 20.74 (1C,CH_3); Mass spectra: m/z 371.34 (M^+).

General experimental procedure for synthesis of *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide (7a-b)
A mixture of 2-(4-(hydrazinylmethyl)phenyl)isoindoline-1,3-dione **(5,** 0.1 mol**)** and triethylamine (0.2 mol) in 30 ml of dioxane was stirred vigorously for 5 min at room temperature. To the resulting solution, acid chloride (0.1 mol) dissolved in 2 ml dioxane was then added, and the mixture was stirred for 6 h at room temperature. The reaction mixture was quenched by addition of water and diluted with ethyl acetate. The organic layer was washed with brine two times and dried over magnesium sulphate. The solution was filtered. The crude products were obtained by concentrating the filtrate. The crude products were recrystallized from suitable solvents to afford compounds **7a-b**.[14-15]

***N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl) benzohydrazide** (**7a**)
Pale brown solid; Yield 80 %; m.p. 160 ^{0}C; IR data (KBr, cm^{-1}): 3165.32 (-NH), 3014.74 (aromatic, C-H), 2897.08 (aliphatic, C-H), 1662.64 (C=O); ^{1}H NMR (DMSO d_6): δ 12.61 (s,1H,NH), 7.45-7.94 (d,8H,aromatic-H), 7.96-8.35 (t,5H,aromatic-H), 3.70, 3.72 (s,2H,CH_2) ; ^{13}C NMR (DMSO-d_6): 159.48, 164.05, 167.27 (C=O, 3C), 145.01-123.1 (18C, aromatic-C), 78.70 (C-N, 1C); Mass spectra: m/z 371.31 (M^+).

***N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl) acetohydrazide** (**7b**)
Pale brown solid; Yield 80 %; m.p. 194-196 ^{0}C; IR data (KBr, cm^{-1}): 3165.19 (-NH), 3015.83 (aromatic, C-H), 2935.66 (aliphatic C-H), 1662.64 (C=O).

Anticonvulsant activity (Behavioral method)
In vivo anticonvulsant activity of 2-(4-((2-alkylhydrazinyl)methyl) phenyl)isoindoline-1,3-dione derivatives **(6a-f)** and *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **(7a-b)** derivatives were determined by behavioral method using actophotometer and the results are presented in Table 1 and Figure 1. Phenytoin (20 mg/kg) was used as a reference standard. Randomize the animals into groups according to the body weights. Each group consists of six mice. Synthesized derivatives (100 mg/kg) and phenytoin (20 mg/kg) were dissolved in 10% aqueous solution of Tween 80. Control animals received 10% aqueous solution of Tween 80. The sample solutions were administered orally in standard volume of 0.8 ml/kg body weight 1h prior to the test. The mice were placed in the box and the behavior was noted for 10 min. The activity score was noted and based on these results, percentage decrease in locomotor activity was calculated.[16]

RESULTS AND DISCUSSION
The synthetic routes of compounds are outlined in **Scheme I**. The phthalimide **1** was reacted with 4-methylaniline **2** to gave 2-(4-methylphenyl)isoindoline-1,3-dione **3** which on bromination gave 2-(4-(bromomethyl)phenyl)isoindoline-1,3-dione **4**. Compound **4** on further reaction with hydrazine hydrate yielded 2-(4-(hydrazinylmethyl)phenyl) isoindoline-1,3-dione **5.** Compound **5** on reaction with various alkyl halides and acid chlorides form 2-(4-((2-alkylhydrazinyl)methyl) phenyl)isoindoline-1,3-diones **6a-f** and *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **7a-b** respectively.
All the synthesized derivatives were characterized by their physical properties and spectral data. The purity and homogeneity of synthesized compounds were confirmed by TLC. Spectral analysis (IR, ^{1}H, ^{13}C NMR and mass spectrometry) of the compounds adequately supported the structures of synthesized compounds. The formation of intermediates and successful completion of

reaction was confirmed by chemical tests and determination of melting point.

The formation of 2-(4-((2-alkylhydrazinyl)methyl) phenyl)isoindoline-1,3-dione derivatives **(6a-f)** was supported by presence of a band between 3250-3100 cm^{-1} (CH-NH); 3100-3050 (aromatic C-H); 3000-2800 (aliphatic C-H) in the IR spectra. The ^{1}H-NMR spectra of 2-(4-((2-(4-methylbenzyl)hydrazinyl)methyl)phenyl) isoindoline-1,3-dione (**6f)** showed characteristic peaks corresponding to the protons of different group and functionalities in the molecule. A singlet was observed at δ 11.5-11.9 due to C-NH, 10 doublets and 2 triplets between δ 7.1-8.3 due to aromatic-H, singlet at 2.2-2.3 due to CH_3 in ^{1}H-NMR spectra. The ^{13}C-NMR showed peaks between δ 137-123 for aromatic carbons, peaks between δ 78-79 for two C-N and δ 20.74 for CH_3. The mass spectra showed M^+ peak at 371.34.

The formation of *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **(7a-b)** was supported by presence of a band between 3250-3100 cm^{-1} (CH-NH); 3100-3050 (aromatic C-H); 3000-2800 (aliphatic C-H),1600-1700 cm^{-1} (C=O) in the IR spectra. The ^{1}H-NMR spectra of *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl) benzohydrazide (**7a)** showed characteristic peaks corresponding to the protons of different group and functionalities in the molecule. A singlet was observed at δ 12.6 due to C-NH, 8 doublets and 5 triplets between δ 7.4-8.3 due to aromatic-H, singlet at 3.7-3.72 due to CH_2 in ^{1}H-NMR spectra. The ^{13}C-NMR showed a peaks between δ 145-123 for aromatic carbons, peaks between δ 78-79 for two C-N and δ 167-159 for C=O. The mass spectra showed M^+ peak at 371.31.

Biological studies

Different *in vivo* methods have been reported for evaluation of anticonvulsant activity. From those methods, behavioral method was considered, for evaluation of *in vivo* anticonvulsant activity of synthesized phthalimide derivatives. This method has been widely used in research and is a low cost, fast and reliable model to predict anticonvulsant activity.

In the present study six albino mice were employed for each set of compound against a control set. The dose employed for derivatives **(6a-f and 7a-b)** was 100 mg/kg body weight orally. The reference standard phenytoin was administered orally at 20 mg/kg body weight. Compound **6e** along with phenytoin showed significant decrease in locomotor activity while compounds **6a** and **7b** did not show any behavioral despair effect when compared with the control.

CONCLUSION

In the present studies, 2-(4-((2-alkylhydrazinyl)methyl) phenyl)isoindoline-1,3-dione derivatives **(6a-f)** and *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **(7a-b)** derivatives were obtained successfully in convenient steps in good yield and evaluated for anticonvulsant activity using behavioral method.

Compound **6e** with *n*-butyl substitution at- NH showed good activity when compared to phenytoin as reference standard in 2-(4-((2-alkylhydrazinyl)methyl)phenyl)isoindoline-1,3-dione **(6a-f)** series. Other compounds such as **6b, 6c** with *tert*-butyl and sec-butyl substitution at -NH showed moderate activity. Compound **6f** with *p*-methyl benzyl substitution at -NH showed weak activity. Compound **6a** with *sec*-propyl substitution at -NH did not show any activity.

In *N'*-(4-(1,3-dioxoisoindolin-2-yl)benzyl)aryl/alkylcarbonyl hydrazide **(7a-b)** derivatives only compound **7a** with benzoyl group substitution at –NH showed moderate activity while **7b** with acetyl group substitution at –NH did not show any activity.

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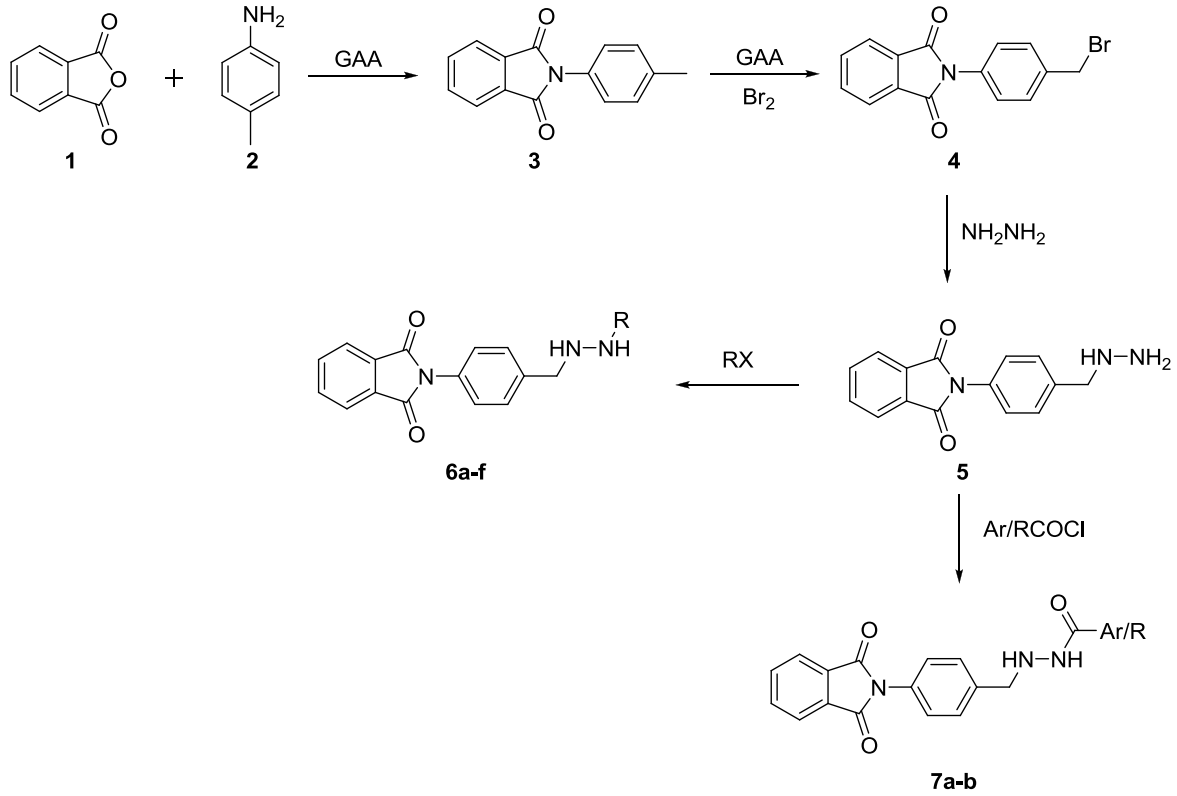

Scheme I: Synthesis of phthalimide derivatives

Table 1: Behavioral activity of compounds 6a-f and 7a-b using actophotometer

Compounds	Activity score using actophotometer	% Change in locomotor activity
6a	307±37.30	9.71(↓)
6b	211.25±21.86	40.21(↓)
6c	198.5±30.26	41.77(↓)
6d	211.5±15.56	37.87(↓)
6e	167.5±14.94	50.74(↓)
6f	263.3±29.46	22.65(↓)
7a	176.7±33.08	48.26(↓)
7b	324.3±55.68	4.62(↓)
Phenytoin (20 mg/kg)	147.5±3.23	56.62(↓)
Control	340±63.46	

Values are expressed as mean± SEM.
Compounds were tested orally at 100mg/kg dose level.
% Change is calculated as 100-[(Test×100)/Control]

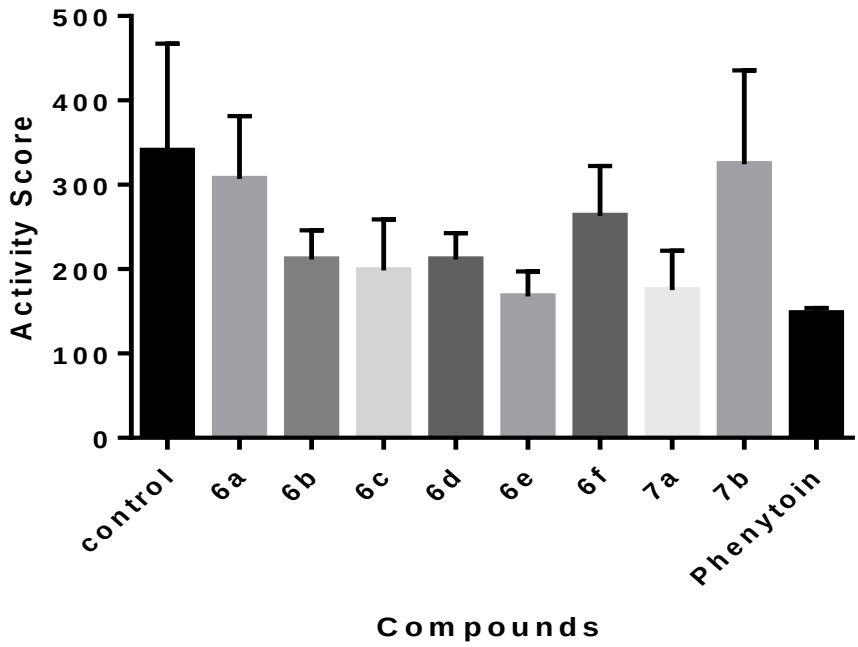

Fig. 1: Graphical representation of biological activity results

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