

Research Article



Pharmacological Screening of a Thiohydantoin Derivative for Epilepsy

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ABSTRACT

Though hydantoin nucleus serves as a common template in combinatorial chemistry libraries, only a few derivatives have been evolved so far. Thiohydantoin is a sulphur analogue which possesses hypolipidemic, anticarcinogenic, antimutagenic, antithyroidal and anti-inflammatory properties. The present study aimed to synthesize a thiohydantoin derivative, 5,5-diphenylthiohydantoin (DPTH) and to investigate the anticonvulsant potential of the compound using primary screening methods like Maximal electroshock seizure, MES and Pentylentetrazole, PTZ models as per antiepileptic drug development program. The rotarod ataxia method assessed the neurotoxicity of the compound. The synthesis of the compound was carried out by microwave irradiation method. The compound, DPTH was found to be more active in PTZ model rather than MES; Estimation of neurotransmitter levels of GABA and AchE in brain tissue homogenates was also done to further substantiate the results obtained.

Keywords: Convulsion, diphenylthiohydantoin, rotarod, PTZ, MES.

INTRODUCTION

Epilepsy is a complex neurodegenerative disease marked by abnormal neuronal discharges that are usually accompanied by some alterations of consciousness and atypical behavior for a limited duration¹. The conventional anti epileptic drugs² provide control only over one third of the patients and a majority of them are still complicated with the development of chronic toxicity, neuropsychological and psychiatric disorders, teratogenicity and shortened life expectancy.

The antiepileptic treatment aims to provide adequate control of seizures though drug resistance is commonly observed among them. Thus, there is a need for new drugs that are more safe and tolerable, when compared to the existing antiepileptic agents³. The hydantoin⁴ nucleus is present in wide range of biologically significant compounds including antiarrhythmics, anti tumour and anti convulsants. Thiohydantoin is the Sulphur analogue with one or both carbonyl group replaced. In spite of being structurally very similar to phenytoin, only very little amount of information is available regarding the anticonvulsant potential of thiohydantoin derivatives.

In perspective of the above, objective of the present work focused on exploring the anticonvulsant property of aryl derivative of thiohydantoin by testing their ability to protect mice against electroshock and chemically induced seizure and to evaluate the neuroprotective effects too.

MATERIALS AND METHODS

Animals

Swiss albino mice (Animal house, Medical college, Thiruvananthapuram: IAEC No.03/11/2014/MCT) of either sex weighing between 20-25g were used for the

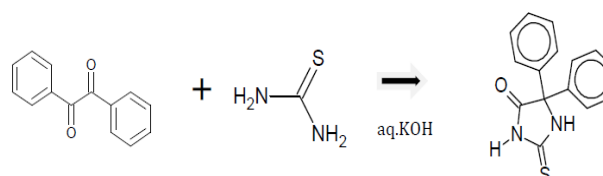
present study. The animals were housed in polypropylene cages under a 12h light/dark cycle and in controlled room temperature with free access to standard rodent diet. All procedures and techniques were in accordance with the Committee for the purpose of control and supervision on experimental animals, with every attempt made to minimize pain and distress to the animals.

Chemicals

The chemicals were procured from Himedia labs, Mumbai. Solvents were obtained from Central drug house, India. Melting points were determined in open capillary tubes on digital melting point apparatus and are uncorrected. The characterization of the compounds were carried out by both IR Spectrometer (Thermonicolete Avatar 375) and NMR spectrometer (Bruker Avance III, 400MHz).

Synthesis of diphenylthiohydantoin by microwave activation⁵

To a mixture of 20.2g benzil and 10.03g thiourea dissolved in 40ml ethanol, 25ml of 1.2M aq. KOH were added. The mixture was stirred for 5 min following an initial pulse of 90sec. 30sec pulses were applied for a period of 30 min stirring in between them. The precipitate was formed upon addition of 300ml cold water. Filtered and later acidified with glacial acetic acid. The test compound was collected and dried. Recrystallized was carried out from ethanol.



Toxicity studies⁶

Acute oral toxicity test was carried out according to OECD (Organisation for economic cooperation and development) guidelines for testing of chemical number 425. OECD 425 guideline minimizes the number of animals required to estimate the acute oral toxicity of a chemical. Healthy female albino rats were used for the study. Care was taken to ensure that rats used for the study were non pregnant. Animals were fasted prior to dosing. (food but not water should be withheld during night)

Animals were dosed at 175, 550, 1750 and 5000 mg/kg and were tested for mortality.

Screening for anticonvulsant activity**Maximal electroshock seizure method⁷***Grouping of animals*

Swiss albino mice weighing between 20-25g were randomly divided into 4 groups of 6 animals each. Group I-control (0.5% CMC suspension) Group II-Phenytoin treated group (39mg/kg) Group III-Test drug Diphenylthiohydantoin (1.55mg/kg) Group IV-Test drug (Diphenylthiohydantoin 0.78mg/kg).

Procedure

The test compound and standard drug were administered orally as 0.5% CMC suspension. After one hour of drug administration, an electrical stimulus of 50mA was applied through transauricular electrodes for a duration of 0.2 seconds with the help of an electroconvulsimeter. Maximal seizure was defined by the tonic extension of the hind limb to an angle close to 180° to the plane of the body axis. The duration of extensor phase, recovery phase and percentage of animals protected from seizures were recorded.

Pentylentetrazol seizure method⁸*Grouping of animals*

Swiss albino mice weighing between 20-25g were randomly divided into 4 groups of 6 animals each. Group I-control (0.5% CMC suspension) Group II-diazepam treated group (4mg/kg ip) Group III-Test drug Diphenylthiohydantoin (1.55mg/kg) Group IV-Test drug (Diphenylthiohydantoin 0.78mg/kg).

Procedure

The animals were placed in plexiglass arena (30cm x 30cm x 30cm) on the day of the experiment. They were observed for 30minutes after PTZ (60mg/kg sc) administration Mortality was evaluated by the percentage of the death in 1 hour. The time to onset of clonic convulsions, and seizure duration along with its severity were determined for each animal.

Neurotoxicity estimation by Rotarod method⁹

Neurological impairment is easily detected by rotarod ataxia test, proposed by Dunham and Miya (1957). Untreated control mice when placed on a 6 r.p.m. rotating rod maintains their equilibrium for a longer period.

Procedure

The apparatus consisted of a horizontal rod with 3.6 cm diameter that moves on its axis at 15 rpm and subdivided into 5 compartments by plexiglass disks. The mice were trained to stay on an accelerating rotarod that rotates at 6 revolutions/min. The drug was administered orally to each group and the observations were recorded at the peak time of activity of drug; 1 hr after the drug administration. Fall of time is taken as the end point.

Biochemical investigations

The dosing was carried out for a period of 21 days following which the mice were subjected to chemical induced convulsions, PTZ method and other biochemical investigations.

Estimation of GABA neurotransmitter¹⁰

The brain aminobutyric acid, GABA content was estimated according to the method of Lowe et al. The fluorescence was recorded at 377/455 nm.

Estimation of Acetylcholinesterase activity¹¹

The AchE activity was measured by photometric method as proposed by Ellman et al. The absorbance was measured at 412 nm.

Statistical Analysis

Values are shown as mean ± standard error mean for all the groups of six animals. The results were interpreted by ANOVA, one way analysis of variance followed by Dunnett's t test.

Docking studies

Docking studies of thiohydantoin derivative is described in another paper.

RESULTS

5,5-diphenylthiohydantoin was synthesized by microwave irradiation method and structures were determined by IR, ¹H-NMR analysis and the compound was evaluated for anticonvulsant property and the level of neurotransmitters in the brain tissue were also estimated and quantified.

Spectral data

DPTH; IR(KBr) 765.80 (C-H out of plane), 1377.93(C-N str.), 1739.34 (C=O str), 1157.81(C-S str), 3071.36(C-Hstr), 3268.58 cm⁻¹ (N-H str). ¹H-NMR (δ,ppm) 7.31-7.45 (m,10H,Ar-H), 7.87(s,1H,N-H)

Assessment of anticonvulsant activity

Maximum electroshock Seizure model: The duration of tonic hindlimb extension in mice treated with vehicle was 16.7833±0.93 sec. The DPTH in doses of 0.78mg/kg and 1.55mg/kg did not protect animals from seizures but the duration was reduced; though the standard drug Phenytoin completely abolished the extensor phase (Figure 1, 2).

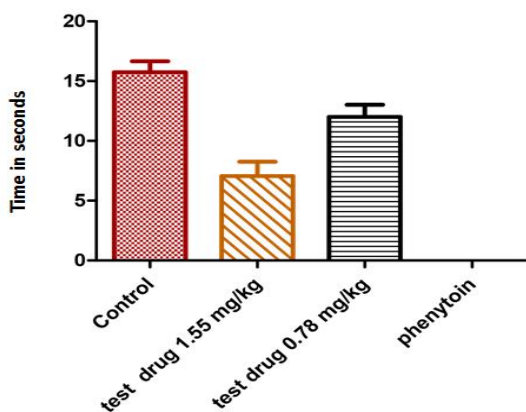


Figure 1: Duration of extensor phase in MES model

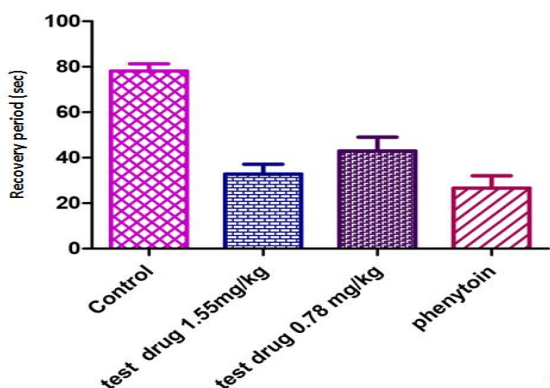


Figure 2: Duration of recovery phase in MES model

Pentylenetetrazol-induced seizures: In animals treated with vehicle, clonic convulsions appeared 255.12±0.69 sec after PTZ and half of the animals died after seizures. The DPTH significantly inhibited the duration and onset of convulsions in a dose dependent manner. Mortality was observed in the group treated with 1.55mg/kg after 24 h with a percentage protection of 83.33%.(Figure. 3,4)

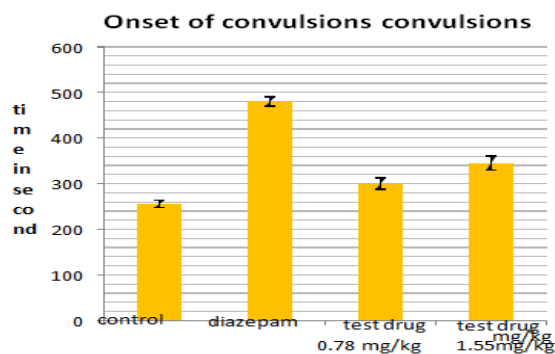


Figure 3: Onset of convulsions in PTZ model

Neurotoxicity estimation: The neurotoxicity of the compound was assayed by rotarod ataxia method where animals treated with DPTH at doses of 0.78mg/kg and 1.55 mg/kg did not exhibit any signs of neurotoxicity even at the 4th hour (Table 1).

duration of convulsions

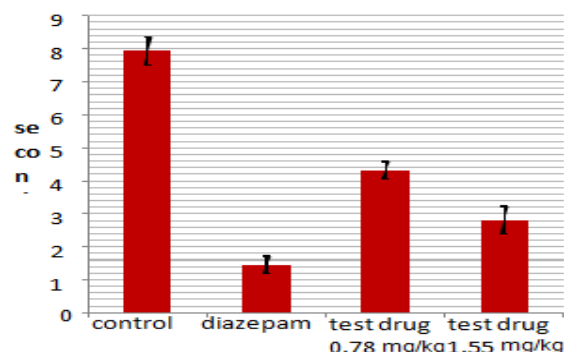


Figure 4: Duration of convulsions in PTZ model

Table 1: Rotarod Ataxia test.

	Fall of Time(Sec)		
	0H	1H	4H
Control	165.167±3.17	162±3.06	156±5.90
Test Drug DPTH (1.55mg/kg)	163.5±2.48	161.33±2.44**	157.67±2.26##
Test Drug DPTH (0.78 mg/kg)	160.16±2.67	159±4.25**	158±3.14##
Phenytoin	159.5±3.92	159.67±3.57**	152±5.17##

n=6, values are expressed in Mean ± SEM. ##P<0.01,**P<0.001.All groups were compared to control (vehicle treated) One way ANOVA followed by Dunnett’s t- test.

Biochemical estimations

The levels of GABA were seen to be elevated in the group of animals treated with test drug, DPTH at a dose of 1.55 mg/kg where the absorbance value was 213.97±1.30 compared to the control value 179.09±0.68. (Table 2)

Table 2: Estimation of GABA levels in brain homogenate.

	GABA Level
Control	179.09±0.68
Test Drug DPTH (1.55mg/kg)	213.97±1.30**
Test Drug DPTH (0.78 mg/kg)	176.46±3.73 ^{ns}
Phenytoin	307.67±0.74**

n=3, values are expressed in Mean ± SEM. **P<0.001.All groups were compared to control (vehicle treated) One way ANOVA followed by Dunnett’s t- test. P>0.05, ns: not significant compared to control

The test drug, DPTH, when compared to phenytoin showed a decrease in acetylcholinesterase level suggesting that it may not be associated with memory deficits similar to phenytoin. (Table 3)



Table 3: Estimation of Acetylcholinesterase levels

	Before addition of substrate	After three minutes	Change in absorbance	Amount of AChE
Control	0.9928±0.0012	0.8784±0.0029	0.1144±0.0052	3.28±0.1785
Phenytoin	0.9828±0.0096	0.8330±0.1736	0.1498±0.0083	4.30±0.1596*
Test 1.55mg/kg	1.1523±0.1932	1.0341 ±0.0021	0.1182±0.0099	3.55±0.2835*
Test 0.78mg/kg	1.2250±0.0078	1.1010±0.0063	0.1240±0.1467	3.39±0.2961*

n=3, values are expressed in Mean ± SEM. *P<0.001. All groups were compared to control (vehicle treated) and are expressed as μ mole of acetylcholine/g wet wt of tissue. One way ANOVA followed by Dunnett's t- test.

DISCUSSION

The observations emanated in the present study clearly indicated that the anticonvulsant potential of the compound against seizures induced by MES and PTZ models in a dose dependent way. However it could not completely abolish the hind limb extensions of MES effective against MES induced seizures.

The test drug, diphenylthiohydantoin is structurally very similar to diphenylhydantoin except in the fact that the oxygen is replaced by sulphur at the second position. The drug obeys all the features required to exhibit anti convulsant activity. The reason for its diminished activity in MES test may be due to the fact that when carbonyl group was substituted with sulphur moiety, the probability to form hydrogen bond decreased. These considerations indicate conclusively that the degree of motional freedom at phenyl groups and potential to form hydrogen bond are important SAR features in antiepileptic phenytoin-like compounds^{xii}.

The drug, diphenylthiohydantoin was found to be active in PTZ model suggesting its possible role in petit mal seizures rather than grand mal epilepsy. PTZ may be exerting its convulsant effect by inhibiting the activity of GABA at GABA A receptors. The promising anticonvulsant potential elicited by the test drug in PTZ model of seizures extends its suitability to possess GABAergic mechanism of action. GABA levels in the mice brain were estimated after a dosing of 21 days followed by PTZ induced seizure and decapitation. Enhancement in GABA levels were seen for the test drug, which substantiates the above data. However, if the GABAergic mechanisms could be proved by further docking studies, it may help in expanding our knowledge on the mechanism of action of the test drug.

Previous literature evidences suggest a link between cognitive dysfunction and low levels of acetylcholine (ACh) in brain^{xiii}. Though, seizure induction in both PTZ and MES caused a significant decrease in AChE and BChE enzyme activities, pretreatment with DPTH brought the enzyme activities towards the normal control. DPTH, when compared to phenytoin shows a decrease in

acetylcholinesterase level. Further studies on various memory models in animals may substantiate the above suggested data.

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