Synthesis, Characterization and Anti-epileptic Activity of Thiohydantoin Derivatives

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ABSTRACT

A vast number of biological properties have been recognised to Thiohydantoin derivatives, have attracted continuing interest because of their various biological activities, namely anticonvulsant, hypolipidemic activity, anti-thyroidal activity, and anti-microbial actions. In this look upon, multiple numbers of Thiohydantoin derivatives are synthesised besides anti-epileptic mechanisms have been investigated. A series of 3-substituted 2-thiohydantoin Derivatives were synthesised from Benzil via Benzil-Benzilic Acid Rearrangement, characterised and evaluated for their anti-epileptic activity by Maximal Electroshock Seizure (MES) model for the anti-epileptic activity of synthesised compounds was less when compared to standard. Among the activity, synthesised compounds 1 indicated more anti-epileptic activity. With this confirmation, we revised integrated a series of innovative 3-substituted 2-thiohydantoin derivatives with a different substituent on the construction and studied the structural activity relationship. The outcomes indicated with the purpose of all the tested compounds have displayed evident anti-epileptic activity. Among the series compounds, 1 shows more anti-epileptic activity than other synthesised compounds.

INTRODUCTION

The chemistry and biological activity of Thiohydantoins and derivatives have been investigated for more than 150 years. The Thiohydantoin moiety, which is present in various pharmacologically active compounds represents a pharmaceutical significance. Thiohydantoins are sulphur compound of hydantoins with carbonyl groups substitute by sulphur group (Wolters Kluwer, 2006). Among the Thiohydantoins, 2 Thiohydantoins be imperative acknowledged due to various pharmacological application like antihyperlipidemic, anticancer, anti-thyroidal, antiviral, anti-mutagenic, anti-fungal, anti-bacterial, antiulcer, and anti-inflammatory. (Wang et al., 2006). Thiohydantoin and its derived compound are furthermore essential intermediates in the production of several amino acids; besides they are the primary material of different generation of long-lasting high temperature established epoxy resins (Asif, 2014). Several Thiohydantoin derivative is made use of rare consumer commodities such as hair spray foundation, some time ago make the most of drug and photographic films (Faghihi et al., 2004). In current work, we description about 3-substituted 2-thiohydantoin was synthesised through benzil-benzilic acid reac-
tion (Gangadhar et al., 2013), via Benzil and variety of Alkyl/Aryl-Thiourea and in the presence of 10% sodium hydroxide along with ethanol. Synthesised derivatives were assessing for anti-epileptic activity through Maximal Electroshock Seizure induced epileptic in an animal model. (Kulkarni, 2007)

MATERIALS AND METHODS

All the chemicals and reagents, solvents utilise for this effort were obtained from Merck chemicals Pvt limited Bengaluru, India. All the chemicals and reagents were obtained from an industrial source and used further without purification.

Melting points were found out by the electrothermal melting point apparatus with an open capillary tube. The finishing point of the reaction and purity of the compounds was observed by Thin Layer Chromatography. Infrared (IR) spectra were confirmation for the derivative on JASCO 4100 FT-IR using Potassium Bromide pellet disc technique. NMR spectra were confirmation on a Bruker advance spectrometer.

Synthesis

Synthesis of Thiohydantoin derivative reaction was carried out resembling scheme-1

Step 1: Synthesis of substituted thiourea

To dissolve 2 grams appropriate aryl/alkyl amine and 2 ml of concentrated hydrochloric acid the resulting solution was warmed than add slowly saturated solution of Ammonium thiocyanate (30 grams of Ammonium thiocyanate dissolved 30 ml of water) to the above thermal solution. After addition of saturated solution completely reflux mixture for 45 min. in 250 ml round bottom flask then boil the solution got turbid. The resulting turbid solution was poured in cold water. The resulting thiourea derivatives precipitated was filtered. (Gangadhar et al., 2013)

Step 2: synthesis of 2-Thiohydantoins

The two grams of Benzil was placed in a 250 ml round-bottomed flask with 1gm N-substituted thiourea derivative than dissolve with support of 24 ml of absolute ethanol, and 2.5 ml of 30% aqueous sodium hydroxide were added gradually to this reaction mixture. In the help of Boiling chips were in addition to the reaction mixture and a condenser was attached following packaging the ground glass joint by way of Teflon tape furthermore two hours heated at 110-120°C under a reflux condenser. This reaction solution was allowed to cool at room temperature and poured the reaction solution in 200ml of ice-cold water. The reaction mixture was unclear, so the suspended materials were eliminated by filtration. Then the apparent solution was carefully acidified with concentrated hydrochloric acid, and the product brought together by vacuum filtration and thoroughly washed with water (Liton and Islam, 2006). Various substituted Thiohydantoin synthesised and their physiochemical properties recorded in Table 2.

Anti-epileptic activity

Animal selection

Either sex of SD albino rats were acquired from the Karpagam University Experimental Animal House in Coimbatore. The animals were provided standard diet water and chow maintained under the standard procedure of temperature (22±2°C) light (12-h light/12-h dark cycle) and humidity (55±5%). The rats were randomly allocated to different treatment and control groups. This experiment was agreed by institutional animal ethical committee (KFMSR/M.Pharm/03/2019-20-IAECNO), under the procedures of (CPSCEA) Committee for the Perseverance of Supervision and Control of Experimental procedure for Animals with every attempt made to minimise pain and distress to the animals.

Grouping of animals

SD albino rats weighing between 200-250g were divided into six groups of six animals each.

Assessment of antiepileptic activity

The Corneal electrodes technique is used for the bilateral delivery system of the electrical stimulus. 50 mA for 0.2 sec of Electroconvulsive shock was provided through a corneal electrode to prompt Hind Limb Tonic Extensor phase (HLTE) in rats. There are five divisions observed in mice after delivering maximal electroshock.

The five different phases are

A. Flexor
B. Extensor
C. Convulsion
D. Stupor and
E. Death or Recovery are noted and correspondingly the time consumed by mice in each division.

After mentioned to delivery, the current output was checked by an exhausting multimeter. The electrical inducement was functional using a stimulator apparatus for six groups of six animals, respectively. The positioning for the anticonvulsant effect was closing down of HLTE within 10 sec after delivery of the shock inducement. Kumar et al. (2013) Delivery of using electroconvulsiometer to rat with the assistance of corneal electrode phases occur after giv-
Figure 1: Synthesis of Thiohydantoin derivative

Figure 2: Graphical representation of anti-epileptic activity of on maximal electroshock (MES) induced seizures in albino rats.

Table 1: Grouping of Animals

<table>
<thead>
<tr>
<th>S.no</th>
<th>Grouping</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Group I</td>
<td>0.5% CMC suspension</td>
</tr>
<tr>
<td>02</td>
<td>Group II</td>
<td>Phenytoin -39mg/kg</td>
</tr>
<tr>
<td>03</td>
<td>Group III</td>
<td>N-phenyl 5,5diphenyl thiohydantoin-1.55mg/kg</td>
</tr>
<tr>
<td>04</td>
<td>Group IV</td>
<td>O-amino N-phenyl 5,5diphenyl thiohydantoin-1.55mg/kg</td>
</tr>
<tr>
<td>05</td>
<td>Group V</td>
<td>N-phenyl 5,5diphenyl thiohydantoin 4’carboxylic acid-1.55mg/kg</td>
</tr>
<tr>
<td>06</td>
<td>Group VI</td>
<td>N-amino phenyl 5,5diphenyl thiohydantoin-1.55mg/kg</td>
</tr>
</tbody>
</table>
Table 2: Physio chemical properties of Thiohydantoin derivatives

<table>
<thead>
<tr>
<th>Compound No</th>
<th>Molecular Formula</th>
<th>Colour and nature of compound</th>
<th>Percentage yield</th>
<th>Molecular weight</th>
<th>Rf value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C₂₁H₁₆N₂OS</td>
<td>White colour and amorphous</td>
<td>53.33%</td>
<td>334g</td>
<td>0.80</td>
</tr>
<tr>
<td>2</td>
<td>C₂₁H₁₈N₃OS</td>
<td>Violet colour and amorphous</td>
<td>58.82%</td>
<td>360g</td>
<td>0.76</td>
</tr>
<tr>
<td>3</td>
<td>C₂₂H₁₆N₂O₂S</td>
<td>Puff colour and amorphous</td>
<td>88.23%</td>
<td>356g</td>
<td>0.78</td>
</tr>
<tr>
<td>4</td>
<td>C₂₁H₁₇N₃O₂S</td>
<td>Light brown colour and amorphous</td>
<td>94.12%</td>
<td>359g</td>
<td>0.86</td>
</tr>
</tbody>
</table>


Table 3: Effect of synthesised Thiohydantoin compounds on Anti-Epileptic activity by Maximal Electroshock (MES) induced epileptic in SD albino rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Treatment</th>
<th>Duration of various phases of epileptic (Seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Phase I</td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>8±0.45</td>
</tr>
<tr>
<td>2</td>
<td>Standard</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>Compound 1</td>
<td>3±0.16</td>
</tr>
<tr>
<td>4</td>
<td>Compound 2</td>
<td>3±0.17</td>
</tr>
<tr>
<td>5</td>
<td>Compound 3</td>
<td>3±0.15</td>
</tr>
<tr>
<td>6</td>
<td>Compound 4</td>
<td>3±0.19</td>
</tr>
</tbody>
</table>

R = Recovery, D = Death, A = Absent

RESULTS AND DISCUSSION

Various substituted Thiohydantoin was synthesised, and their melting point and analytical data are given below

**Compound 1: N-phenyl 5,5 diphenylthiohydantoin**

Mp: 205-208°C, MS (EI): 334[M]+, 1HNMR (300MHz): 7.46 ppm (s,15H), 9.56 ppm (s,1H). IR (KBr): 1236.48 cm⁻¹ (C=S stretch) 3056.35 cm⁻¹ (C-H aromatic stretch), 1587.52 cm⁻¹ (C=O stretch), 1171.76 cm⁻¹ (C-N stretch). 3468.13 cm⁻¹ (N-H stretch)

**Compound 2: O-amino N-phenyl 5, 5 diphenylthiohydantoin**

Mp: 226-228°C, MS (EI): 360[M]+, 1HNMR (300MHz): 3.96 ppm (s, 3H), 7.2-7.70 ppm (m, 14H), 9.60 ppm (s, 1H). IR (KBr): 3060.75 cm⁻¹ (C-H aromatic stretch), 1235.48 cm⁻¹ (C=S stretch), 1677.18 cm⁻¹ (C=O stretch), 1171.76 cm⁻¹ (C-N stretch). 3461.38 cm⁻¹ (N-H stretch)

**Compound 3: N-phenyl 5, 5 diphenylthiohydantoin 4 carboxylic acid**

1H NMR (300 MHz): 7.2-7.7 δ ppm (m, 14H), 9.5 δ ppm (s, 1H). IR (KBr): 3076.56 cm⁻¹ (C-H stretch), 1234.48 cm⁻¹ (C=S stretch), 1523.59 cm⁻¹ (C=C stretch, aromatic), 1687.77 cm⁻¹ (C=O stretch), 1172.76 cm⁻¹ (C-N stretch). 3438.42 cm⁻¹ (N-H stretch).

**Compound 4: N-hydroxyl 5, 5 diphenylthiohydantoin**

Mp: 212-215°C. MS (EI): 359[M]+. 1H NMR (300 MHz): 2.4 δ ppm (s, 2H), 7.3 δ ppm (s, 15H). IR (KBr): 3390.49 cm⁻¹ (N-H stretch), 3132.23 cm⁻¹ (C-H stretch, aromatic), 2947.33 cm⁻¹ (C-H stretch, aliphatic), 1669.11 cm⁻¹ (C=O stretch), 1597.47 cm⁻¹ (C=C stretch, aromatic), 1240.0 cm⁻¹ (C=N stretch), 1132.76 cm⁻¹ (C-N stretch).

Several thioureas were designed by using corresponding aromatic amine with Ammonium Thiocyanate than simple base catalysed condensation of Benzil with Thiourea. The formation of 3-substituted 2-thiohydantoin derivatives these products follows the benzil-benzilic acid rearrangement that is shown in the Scheme I. Purity of all designed compounds was checked by Thin Layer Chromatography and Melting Point. All newly synthesised compounds were investigated by Fourier Transform Infrared, Mass Spectroscopy (EI), 1HNMR. The anti-epileptic activity was carried out by using maximal Electroshock seizure induced epileptic in the rat model. Phenytoin is used as an anti-epileptic activity standard drug. Results outcome are listed in Figure 1 and Table 3 displayed that all newly synthesised compounds of 3-substituted 2-thiohydantoin derivatives possess considerable Anti-Epileptic activity—compounds one more potent anti-epileptic activity than other newly synthesised compounds.

**CONCLUSIONS**

The simple appropriate technique for the synthesis of 2-thiohydantoins was improved on benzil-benzilic acid rearrangement. Synthesised derivatives were confirmed by Fourier Transform Infrared, Mass Spectroscopy (EI), 1HNMR, all synthesised compounds of 3-substituted 2-thiohydantoin displays noticeable anti-epileptic activity. Lipophilic groups such as the amino group in the para position of 3 phenyl ring displays increase the activity, while hydrophilic groups such as carboxyl on Para position of 3 phenyl ring outcome decrease in an anti-epileptic activity. Further wide-ranging study is required to authorise to the achievement of anti-epileptic agents in pharmaceutical field and studies to enhance the effectiveness of Thiohydantoin derivatives.

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**Conflict of interest**

The authors declare that they have no conflict of interest for this study.

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