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Original Research Article

Fabricating Hydantoin / Thiohydantoin Hybrids from A Natural Product, Cuminaldehyde: New Players for Anti-Convulsant Therapeutics

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Abstract: While designing some anti-convulsant hybrid molecules from a natural product, novel hydantoin **(5)** / thiohydantoin **(8)** based analogs were synthesized rationally from cuminaldehyde **(1)**, the principal product of *Cuminum cyminum* (cumin) and also secondary components present in eucalyptus, cassia, and myrrh. The research was planned as literature reports potent anti-seizure activity of cumin oil, probably due to the presence of cuminaldehyde. The starting material, cuminaldehyde **(1)**, the safe natural product was converted into intermediate Schiff's base analogs **(3)** and **(7)** employing semicarbazide **(2)** / thiosemicarbazide **(6)**. The molecule **(8)** demonstrated the most potent anti-MES activity at 30 mg/kg (0.5 hr) and 100 mg/kg (4 hr), respectively. Successively, the analog was cyclized by ethyl chloroacetate **(4)** in thr presence of fused alcoholic sodium acetate to form the compound of interest. The sophisticated analytical tools (FT-IR, ^{1}H-NMR, and Mass) and elemental analyses provided all essential characteristics and evidence of the fabricated analogs. The hydantoin hybrids were screened for their anticonvulsant activity employing the Maximal Electroshock Seizure (MES) Test. The derivative **(5)** exhibited lower anti-MES activity as compared to the derivative **(8)** and standard drug, phenytoin. The compound presented anti-MES activity at 100 mg/kg (0.5 hr) and 300 mg/kg (4 hr), respectively. The lipophilicity accounts to be the decisive factor in the expression of anti-convulsant activity. The study demonstrated new possibilities in designing pharmacologically active analogs from a natural product in the near future.
Keywords: Hydantoin; Thiohydantoin; Cuminaldehyde; Antiepileptic; Epilepsy; Seizure.

INTRODUCTION

Epilepsy is one of the world's oldest recognized conditions and the most common neurological disorders that have affected more than 50 million people across the globe of all ages. It is a heterogeneous group of chronic disorder of the brain primarily characterized by hypersynchronous neuronal firing and hyperexcitability [1]. In latest clinical practice, more than 25 anti-epileptic drugs (AEDs) are in therapeutics, but still 35% of the patient population experience uncontrolled and associated seizures [2]. To the figure of 50 million, 200 thousand new cases are arriving every year, representing resistant to the current pharmacotherapy. The modern treatment for the management of seizures involved constant medication for a longer duration, which often the precipitates numerous adverse effects like anemia, gastrointestinal disturbance, drowsiness, nausea, hyperplasia, ataxia, etc [3]. Therefore, in recent times a shift from complete synthetic drug based therapeutics to complete or partial natural therapy have been observed, which compelled to design a molecule based on a natural product.

From the beginning of drug discovery, organic chemists have remained fascinated by the largely diversified unexplored classes of substances distributed in the flora and fauna [4]. In the due course of time, Natural Products (NPs) have been identified to play a key role in drug discovery; especially in the therapeutic areas of cancer, immunosuppression, CNS diseases, metabolic diseases, etc. [5]. Since the last several decades, progressive findings have been reported in the literature regarding the biological potentials of either semi-synthetic drugs or various natural products. A number of NP derived molecules are in current therapeutic practice for treating central nervous system (CNS) disorders [6]. Following those avenues, cuminaldehyde ($C_{10}H_{12}O$), the principal product of *Cuminum cyminum* (cumin) and also secondary components present in eucalyptus, cassia, and myrrh was chosen as literature reports potent anti-seizure

activity of cumin oil, probably due to the presence of cuminaldehyde [7].

The present research aimed at synthesizing some novel (thio-)hydantoin derivatives from the natural product, cuminaldehyde. The compound of interest was achieved by transforming the staring material into hydantoin via Schiff's base intermediates and screened for anti-convulsant activity using Maximal Electroshock Seizure (MES) Test.

MATERIALS AND METHODS

Materials

Cuminaldehyde, thiosemicarbazide, semicarbazide, and ethyl chloroacetate were procured from Sigma-Aldrich Ltd., Germany. All other chemical derivatives, solvents, and analytical grade reagents employed in synthesis were procured from Merck and HiMedia.

Instruments

The melting points of the synthesized derivatives were measured on Perfit melting point apparatus. Thin layer chromatography was carried out using silica gel G-coated TLC plates (Merck). The FT-IR spectra were recorded in KBr discs on the IRAffinity-1 instrument. The ^{1}H-NMR (400 MHz) spectra were recorded using Bruker spectrospin NMR DPX-300. The TMS (Sigma-Aldrich) was used as an internal standard. The mass spectra were obtained on JEOL-JMS-DX 303 instrument. The elemental analyses were performed on Perkin-Elmer 240C analyzer.

Animals

Male Albino Swiss mice of weight 20-25 g were employed for the screening of fabricated derivatives after approval from Department Ethical Committee and CPCSEA. The animals were kept in polypropylene cages (6 mice in each cage) under a controlled room temperature of 25–26ºC and humidity 50–55% with proper hygienic conditions. A 12/12 hr light/dark cycles were followed during the conduct of the experiment. The animals were fed on standard mice pellet and water *ad libitum*.

Synthesis of target compounds

The hydantoin-based analogs were synthesized rationally from cuminaldehyde **(1)**. For synthesizing the preferred derivatives **(5)** and **(8)**, the -CHO (aldehydic group) of cuminaldehyde **(1)** was first converted into a Schiff's base (C=N) form by substituting the carbonyl group by an azomethine function. The route for synthesizing hydantoin **(5)** involved the reaction of cuminaldehyde **(1)** with semicarbazide **(2)** to form an intermediate product Schiff's base analog **(3)**. Successively, the analog was cyclized by ethyl chloroacetate **(4)** in the presence of fused alcoholic sodium acetate to form the hydantoin hybrid **(5)**. The thiohydantoin **(8)** was prepared quite similarly to the previous reaction. Instead, thiosemicarbazide **(6)** was employed to introduce the sulfur function into the hybrid. The terminal step involved cyclization of Schiff's base intermediate product **(7)** by ethyl chloroacetate **(4)** to fabricate the thiohydantoin **(8)** analog. The **Scheme 1** describes the outline of synthesis.

Synthetic protocol for 2-(4-isopropylbenzylidene) hydrazinecarboxamide **(3)**

An equal quantity (0.01 M) of cuminaldehyde **(1)** and semicarbazide **(2)** were refluxed in ethanolic media with continuous stirring for 12 hr in the presence of few drops of glacial acetic acid. The content was cooled successively and the precipitate was collected suitably. The obtained product was washed with cold water thoroughly, dried and recrystallized with an aqueous ethanolic solution.

76% yield; FTIR (KBr) υ (cm^{-1}): 3331 (NH_2), 3202 (NH), 3057 (C-H, aromatic), 1695 (C=O), 1647 (C=N, azomethine), 1605 (C=C, aromatic), 1527 (-NH, bending). ^{1}H NMR (δ, ppm, $CDCl_3$): 8.27 (azomethine, 1H), 7.2-7.8 (aromatic, 4H), 6.58 (amine, 2H), 6.12 (amide, 1H), 1.26 (methyl group, 6H). MS: M^+ 205. Anal. Calcd. for $C_{11}H_{15}N_3O$: C, 64.37; H, 7.37; N, 20.47. Found: C, 64.21; H, 7.19; N, 20.22.

Synthetic protocol for 3-((4-isopropylbenzylidene) amino) imidazolidine-2,4-dione **(5)**

An equimolar mixture (0.1 M) of 2-(4-isopropylbenzylidene)hydrazinecarboxamide **(3)**, ethyl chloroacetate **(4)** and fused sodium acetate in ethanolic media were refluxed for 6 hr. The reaction content was cooled further and consecutively poured into the crushed ice with vigorous stirring. The acquired solid product was filtered, dried duly, and recrystallized from absolute ethanol.

45% yield; FTIR (KBr) υ (cm^{-1}): 3113 (-NH, stretch), 3149 (C-H, aromatic), 1674 (C=O), 1653 (C=N, azomethine), 1629 (C=C, aromatic), 1536 (-NH, bending). ^{1}H NMR (δ, ppm, $CDCl_3$): 9.11 (azomethine, 1H), 7.3-7.7 (aromatic, 4H), 6.38 (amide, 1H), 1.22 (methyl group, 6H). MS: M^+ 245. Anal. Calcd. for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 4.38; N, 17.38. Found: C, 62.99; H, 4.31; N, 17.17.

Synthetic protocol for 2-(4-isopropylbenzylidene) hydrazinecarbothioamide **(7)**

Equal quantity (0.01 M) of cuminaldehyde **(1)** and thiosemicarbazide **(6)** were refluxed in ethanolic media with continuous stirring for 12 hr in the presence of few drops of glacial acetic acid. The content was cooled successively and the precipitate was collected suitably. The obtained product was washed with cold water thoroughly, dried and recrystallized with aqueous ethanolic solution.

66% yield; FTIR (KBr) υ (cm^{-1}): 3398 (-NH_2), 3177 (-NH, stretch), 3034 (C-H, aromatic), 1681 (C=N,

azomethine), 1622 (C=C, aromatic), 1592 (-NH, bending), 1154 (C=S). ^{1}H NMR (δ, ppm, $CDCl_3$): 8.67 (amine, 2H), 8.42 (azomethine, 1H), 7.1-7.9 (aromatic, 4H), 6.03 (amide, 1H), 1.25 (methyl group, 6H). MS: M^+ 221. Anal. Calcd. for $C_{11}H_{15}N_3S$: C, 59.69; H, 6.83; N, 18.99. Found: C, 59.42; H, 6.54; N, 18.63.

Synthetic protocol for 3-((4-isopropylbenzylidene) amino)-2-thioxoimidazolidin-4-one **(8)**

Equimolar mixture (0.1 M) of 2-(4-isopropylbenzylidene)hydrazinecarbothioamide **(7)**, ethyl chloroacetate **(4)** and fused sodium acetate in ethanolic media were refluxed for 6 hr. The reaction content was cooled further and consecutively poured into the crushed ice with vigorous stirring. The acquired solid product was filtered, dried duly, and recrystallized from absolute ethanol.

54% yield; FTIR (KBr) υ (cm^{-1}): 3168 (-NH, stretch), 3145 (C-H, aromatic), 1666 (C=N, azomethine), 1657 (C=C, aromatic), 1512 (-NH, bending), 1179 (C=S). ^{1}H NMR (δ, ppm, $CDCl_3$): 9.14 (azomethine, 1H), 7.49 (amide, 1H), 7.3-7.8 (aromatic, 4H), 1.28 (methyl group, 6H). MS: M^+ 261. Anal. Calcd. for $C_{13}H_{15}N_3OS$: C, 59.74; H, 5.79; N, 16.08. Found: C, 59.55; H, 5.66; N, 16.01.

Anti-convulsant activity

The hydantoin hybrids were screened for their anticonvulsant activity employing the Maximal Electroshock Seizure Test (MES) using the protocols given by National Institute of Neurological Disorders and Stroke, NIH (USA) [8]. The protocol involved supra-maximal electroshocks (50 mA, 60 Hz) for the duration of 0.2 sec after the administration of 30, 100, and 300 mg/kg doses of experimental compounds. The anticonvulsant activity was measured by the abolition of the hind limb tonic extensor spasm.

RESULT AND DISCUSSION

Chemistry

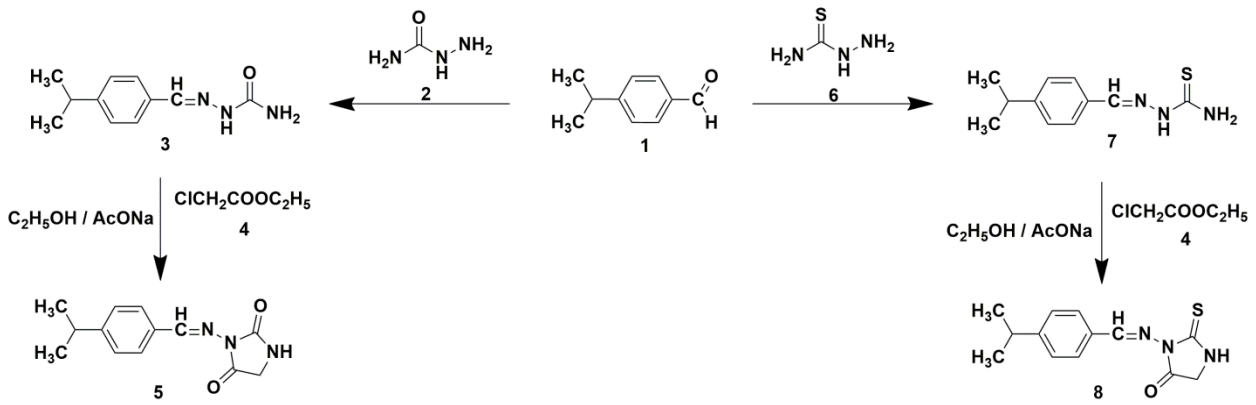
The sophisticated analytical tools provided all essential characteristics and evidences of the fabricated analogs. The stretching of the amines and amides appeared prominently in the range of 3145-3398 cm^{-1}. The amide bending was detected at 1512-1592 cm^{-1}. The C-H and C=C stretching of the aromatic ring were observed in the ranges 3034-3145 cm^{-1} and 1605-1657 cm^{-1}, respectively. The formation of the intermediate derivatives, i.e. Schiff's base based compounds (**3** and **7**) was confirmed by the appearance of sharp characteristic peaks in the range 1649-1681 cm^{-1}. The peak at 1150 cm^{-1} was noticed to be the C=S group, representing the thiosemicarbazide based origin. The formation of the final derivative was validated by the disappearance of the NH_2 peak in the spectra and appearance of amide peak after 3100 cm^{-1}.

The ^{1}H NMR-based study of the compounds revealed that appearance of peaks at specified regions of the spectra, illustrating the chemical reaction scenario. The two methyl groups of cuminaldehyde pharmacophore emerge in the range of 1.2-1.3 ppm. The continuous appearance of these peaks in all spectra inferred the presence of methyl groups in every derived analog. The Schiff's base components were characterized by the location of azomethine protons at 8.5 ppm, confirming the conversion of the aldehydic group of the starting material. A key differentiating feature involved the elevated value of Schiff's base proton from 8.55 ppm of intermediate to 9.0 ppm of the terminal compounds. The NMR spectra of the desired compound(s) illustrated the presence of amide proton in the range of 6-6.5 ppm. This marked variation strongly supported the formation of amide after cyclization. The aromatic protons were predominantly recognized from the appeared peaks in the range of 7.2-7.8 ppm.

The mass spectra demonstrated the emergence of the base peak of the derivatives which corresponds to the molecular weight. Numerous small fragment peaks also appeared in the spectra having m/z in the range of 100-125. The characterization data fully supported the practical existence of the proposed compounds. The % practical estimation of elemental analyses of the intermediates and analogs were found to be in a very close conformity compared to theoretical values.

Anti-convulsant activity

In the screening process, the experimental derivatives were administered by i.p. route at three dose levels of 30, 100, and 300 mg/kg, respectively, in male Albino Swiss mice. The anti-convulsant activity was measured after 0.5 and 4 hr intervals against maximal electroshock-induced seizure (MES) threshold test. The molecule **(8)** demonstrated the most potent anti-MES activity at 30 mg/kg (0.5 hr) and 100 mg/kg (4 hr), respectively. The derivative **(5)** exhibited lower anti-MES activity as compared to the derivative **(8)** and standard drug, phenytoin. The compound presented anti-MES activity at 100 mg/kg (0.5 hr) and 300 mg/kg (4 hr), respectively. In the biological activity, lipophilicity accounts to be the most decisive factor. It might be believed that the analog **(5)**, having log P of 2.13, was not able to cross the biological membrane fully to exhibit the activity and thus resulted in lower activity [9]. In a general rule, log P of more than 2.5 is essential to exhibit CNS activity [10]. In contrast, analog **(5)**, having log P of 3.12, expressed an optimum log P value essential for exhibiting anti-convulsant action. Literature has reported that for exhibiting optimized activity, the designed molecule must have log P value of less than 4 [11]. Therefore, the molecule represented potent and quite comparable anti-seizure activity than that of reference drug, phenytoin. The anticonvulsant activity of the derivatives is summarized in **Table 1**.



Scheme 1. The synthetic outline.

Table 1. Anticonvulsant and neurotoxicity evaluation of compounds.

Compounds	MES Screening	
	0.5 hr	4 hr
5	100	300
8	30	100
Phenytoin	30	30

CONCLUSION

In an attempt to develop some potent analogs with anti-seizure perspectives from a natural starting material, we identified molecule **(8)** demonstrated the most potent anti-MES activity at 30 mg/kg (0.5 hr) and 100 mg/kg (4 hr) whereas the derivative **(5)** exhibited lower anti-MES activity as compared to the derivative **(8)** and standard drug; phenytoin, with anti-MES activity at 100 mg/kg (0.5 hr) and 300 mg/kg (4 hr). The characterization of the compounds was found to be in full agreement with that of the structures. This study will certainly promote researchers in the rational synthesis of hybrid molecules from natural materials (or natural products) having some biological activity and molecular target inhibitory perspectives.

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