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Synthesis of Novel Spirohydantoins and Evaluation of their Anticonvulsant Activity

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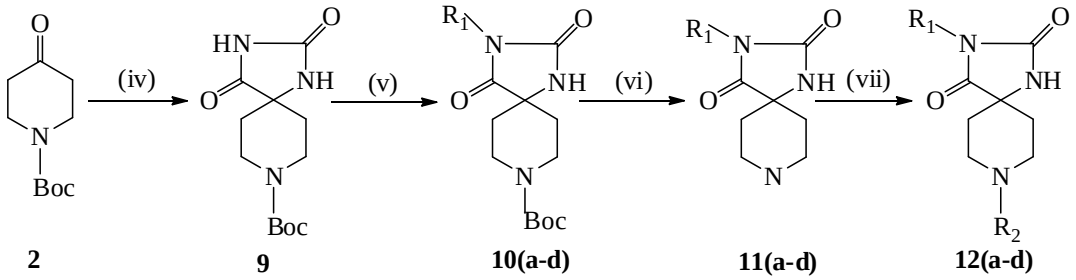
ABSTRACT

A series of novel triazaspiro anddiazaspiro compounds were synthesized and biologically evaluated for their anticonvulsant activity in the maximal electroshock seizure (MES). Most of the tested compounds exhibited moderate to weak activity in the MES screen test. A few compounds are able to protect seizure effect significantly higher than other derivatives. This effect was similar to standard drug treated rats.

Graphical Abstract



2 **9** **10(a-d)** **11(a-d)** **12(a-d)**

Scheme 2

Keywords: Hydantoins, Triazaspiro hydantoins, Anticonvulsant, Maximal electroshock, Phenytoin

INTRODUCTION

Epilepsy, one of the most frequent neurological afflictions in man characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion, requires special medical attention. Though several new anticonvulsants are introduced, some types of seizures are still not adequately treated with current therapy. Toxicity, intolerance, and lack of efficacy for certain types of seizures are some of the limitations of the current medications [1].Hydantoins, a class of cyclic imides, have been demonstrated to possess good anticonvulsant property [2,3]. Depending on the nature of substitution on the hydanto in ring, a wide range of other pharmacological properties, e.g., fungicidal [4], herbicidal [5], antitumor [6], anti-inflammatory [6], anti-HIV [7], hypolipidemic [8], and antihypertensive [9] activities, have also been identified. The anticonvulsant activity of 5,5-diphenylhydantoin, commonly known as Phenytoin, has been known since 1938 as one of the most useful anticonvulsant drugs and is still regarded as the drug of choice for treating generalized tonic-clonic seizure. However, a number of side effects have limited its use [3, 10-12]. In continuation of our research on hydantoins [13-16], the synthesis and evaluation of anticonvulsant activity of derivatives 6(a-t) is reported.

MATERIALS AND METHODS

The instrumental details are same as reported [13] in our earlier publication.

Synthesis of triazaspiro hydantoins 8(a-d) and 12(a-d): Triazaspiro hydantoins 8(a-d) and 12(a-d)were synthesised as outlined in the Scheme 1 and 2 respectively. The Boc protected 4-piperidinone (2) was converted in to azepinone, a seven membered ring by using BF_3 etherate and ethyl diazoacetate with 60% yield. Triazaspiro hydantoin 5 was synthesized under Bucherer-Bergs conditions using potassium cyanide and ammonium carbonate in 72% yield. The selective *N*-alkylation was carried at 3rd position of hydantoin ring alkyl/aryl halides using K_2CO_3 and DMF. Target key intermediates 7(a-d) were accomplished by deprotection of Boc group from the compounds 6(a-d). The amine functionalization of the azepinone ketone ring in the fifth step was carried out using chloroethyl piperidine to result to the desired compounds 8(a-d). This was furnished by normal condensation reaction. Similarly compounds 12(a-d) were also synthesised using similar procedure. Their structures were confirmed by ^{1}H NMR, LCMS and IR spectra. The chemical structures, purity and physical data of novel compounds are in table 1.

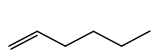
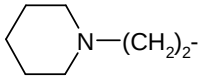
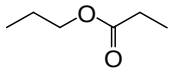
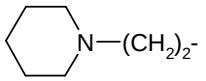
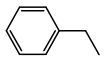
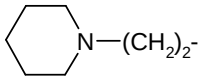
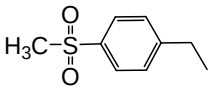
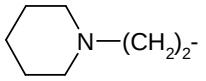
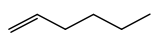
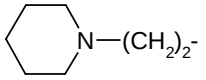
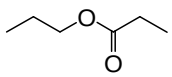
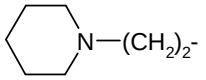
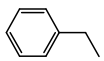
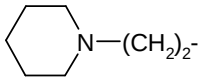
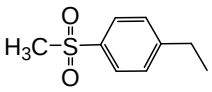
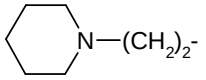
Synthesis of 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester (2): A solution of piperidin-4-one (1) (20 g, 201.7 mmol) was taken in dry THF, cooled to 0-5 ^oC. Triethylamine (50.8 g, 502.5 mmol) and Boc anhydride (43.8 g, 201.7 mmol) were added. The reaction mixture was allowed to stir at room temperature for 5 h. The progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. Finally organic layer was washed with water and dried over anhydrous Na_2SO_4. The organic layer was evaporated to get 4-oxo-piperidine-1-carboxylic acid *tert*-butyl ester in good yield (75%, 40.0 g).

Synthesis of 4-oxo-azepane-1,3-dicarboxylic acid-1-*tert*-butyl ester 3-ethyl ester (3): To a solution of 1-Boc-4-piperidone (2) (20g, 100.3 mmol) in dry ether (100 mL), simultaneous addition of drop wise solutions of $BF_3.OEt_2$ (18.4 g, 130 mmol) and ethyl diazoacetate (17.2 g, 150 mmol) in dry ether was carried out at -30ºC. Reaction mixture was stirred for 1 h at –20^{o}C.Then it was brought to 0 ºC and 30% K_2CO_3 solution was added till it was basic. Reaction mixture was extracted with ethyl acetate. Combined organic layer was dried over Na_2SO_4 and concentrated. Crude was purified by column chromatography over silica gel (3:7 ethyl acetate - pet ether) to yield 3 as pale yellow liquid (17.1 g, 60%).

Synthesis of 4-oxo-azepane-1-carboxylic acid *tert*-butyl ester (4): To a solution of the above keto-ester (16 g, 56.0 mmol) in THF (60 mL), LiOH (8.0 g, 336 mmol) in water (30 mL) was added and

the mixture was heated at 80 ºC for 18 h. Reaction mixture was neutralized with citric acid solution and extracted with EtOAc. Combined organic layer was dried over Na_2SO_4 and concentrated. Crude was purified by column chromatography over silica gel (Pet ether/EtOAc) to yield 4 as paleyellow liquid (7.7 g, 65%).

Table 1. Chemical structure, physical data and purity of the compounds 8(a-d) and 12(a-d)

Compound	R_1	R_2	M.P (°C)	Yield (%)
8a			168-170	67
8b			142-144	70
8c			181-183	74
8d			101-103	70
12a			139-141	64
12b			156-158	66
12c			117-119	72
12d			119-121	70

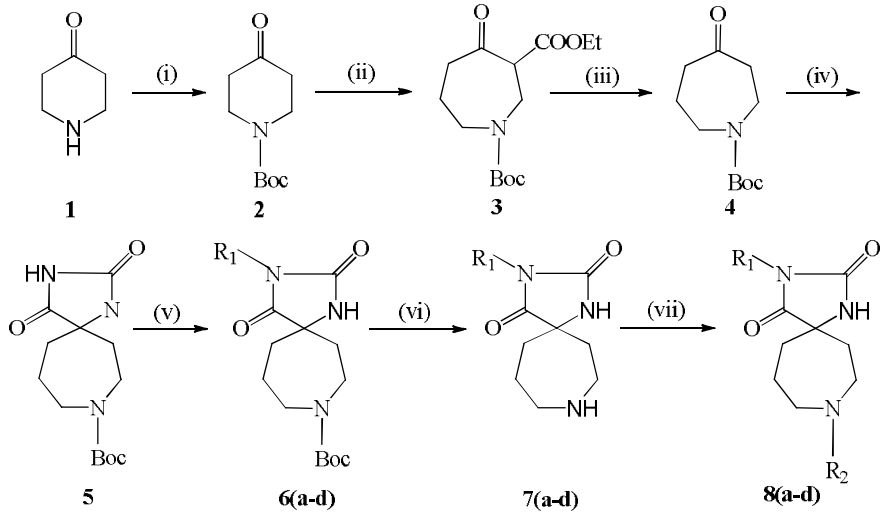
Synthesis of 2, 4-dioxo-1,3,8-triazaspiro[4,6]undecane-8-carboxylic acid *tert*-butyl ester (5): A solution 4-oxo-azepane-1-carboxylic acid *tert*-butyl ester(4)(7.0 g, 32.8 mmol), ammonium carbonate (9.22 g, 96 mmol) were taken in ethanol and water. Then potassium cyanide (6.25 g, 96 mmol) dissolved in water and it was added the above solution. The reaction mixture was stirred for 2 days. The progress of the reaction was monitored by TLC. Upon completion, the reaction mixture was filtered and washed with water to get hydantoin product 5 in good yield (6.4 g, 72%). Similarly, compound 9was synthesised with good yield (7.5 g, 71%).

General procedure for the synthesis 6(a-d) and 10(a-d): A solution of 2,4-dioxo-1,3,8-triazaspiro[4,6]undecane-8-carboxylic acid *tert*-butyl ester **(5)** (1.0 eq) in *N*,*N*-dimethyl formamide was taken, anhydrous K_2CO_3 (3.0 eq) was added to the solution and stirred for 10 min. Alkyl/aryl halide (1-1.2 eq) was added. The reaction mixture was stirred at room temperature for 6-8 h and progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. Finally water wash was given to the organic layer and dried over anhydrous sodium sulphate. The solvent was evaporated, crude product was purified by column chromatography using chloroform: methanol (9:1) as an eluent. Similarly, compounds 10(a-d)was synthesised from the intermediate 9.

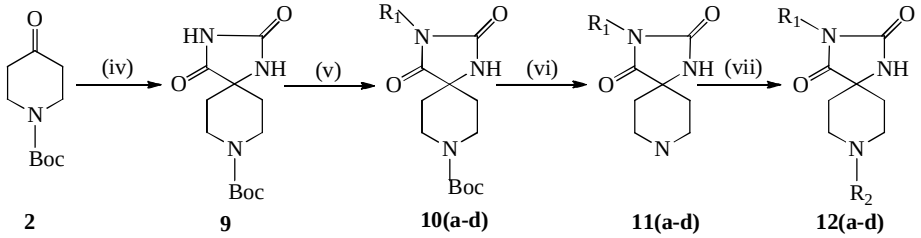
General procedure for the synthesis of compounds 7(a-d) and 11(a-d): Compound 6a/6b/6c/6d was taken in dry MDC, cooled to 0^0C. Ether in HCl was added and allowed to stir at room temperature for 3 h. Deprotected salt compound was basified with $NaHCO_3$ solution and extracted with ethyl acetate, organic layer was concentrated to get desired compound. Similar experimental procedure was followed for the compounds 11(a-d).

General procedure for the synthesis of triazaspiro hydantoin derivatives 8(a-d) and 12(a-d): A solution of 7a/7b/7c/7d(1.0 eq) in *N*,*N*-dimethyl formamide was taken, anhydrous K_2CO_3 (3.0 eq) was added to the solution and stirred for 10 min. 1-(2-chloro-ehtyl)-piperidine (1.0 eq) was added. The reaction mixture was stirred at room temperature for 10 h and progress of the reaction was monitored by TLC. Upon completion, the solvent was removed under reduced pressure and residue was taken in water and extracted with ethyl acetate. Finally water wash was given to the organic layer and dried over anhydrous sodium sulphate. The solvent was evaporated, crude product was purified by column chromatography using chloroform: methanol (9:1) as an eluent. Similarly, compounds 12(a-d) obtained from 11a/11b/11c/11d.

Characterization of 3-pent-4-enyl-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,6] undecane-2,4-dione (8a): (0.287 g, pale yellow crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.77 (s, 1H, -NH), 5.84-5.73 (m, 1H, =CH), 5.03-4.96 (m, 2H, CH_2=), 3.37-3.32 (t, 2H, -CH_2-), 3.17-3.11 (m, 4H, -CH_2-), 2.88-2.83 (m, 2H, -CH_2-), 2.74-2.68 (m, 2H, -CH_2-), 2.37-2.30 (m, 4H, -CH_2-), 1.97-1.94 (m, 2H, -CH_2-), 1.74-1.64 (m, 4H, -CH_2-), 1.61-1.55 (m, 4H, -CH_2-), 1.36-1.32 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 363.1. IR (KBr, cm^{-1}): 3322, 1651.Anal. calcd for $C_{20}H_{34}N_4O_2$: C, 66.26; H, 9.45; N, 15.46; found: C, 66.23; H, 9.42; N, 15.43.



Scheme 1



Scheme 2

Reagents and Conditions:(i). $(Boc)_2$, TEA, dry THF, r.t. (ii). $BF_3.OEt_2$ and ethyl diazoacetate in dry ether and 30 % K_2CO_3 (iii). LiOH, water, 80°C, 18 hr (iv). KCN, $(NH_4)_2CO_3$, ethanol: water, r.t. (v). 5(a-d), K_2CO_3, DMF (vi). Ether in HCl, MDC (vii). 1-(2-chloro-ehtyl)-piperidine, K_2CO_3, DMF.

Characterization of [2,4-dioxo-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,6]undec-3-yl]-acetic acid propyl ester (8b): (0.287 g, pale brown crystalline solid); ^{1}H NMR (DMSO-d_6, 400MHz) δ: 8.71 (s, 1H, -NH), 4.16 (s, 2H, -CH_2-), 4.03 (t, 2H, -CH_2-), 3.15-3.07 (m, 4H, -CH_2-), 2.89-2.83 (m, 4H, -CH_2-), 2.35-2.29 (m, 2H, -CH_2-), 1.99-1.94 (m, 2H, -CH_2-), 1.78-1.63 (m, 8H, -CH_2-), 1.62-1.54 (m, 4H, -CH_2-), 1.35-1.31 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 395. IR (KBr, cm^{-1}): 3320, 1730, 1649.Anal. calcd for $C_{20}H_{34}N_4O_4$: C, 60.89; H, 8.69; N, 14.20; found: C, 60.86; H, 8.67; N, 14.23.

Characterization of 3-benzyl-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,6] undecane-2,4-dione (8c): (0.31 g, white crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.47 (s, 1H, -NH), 7.22-7.12 (m, 5H, Ar-H), 4.23 (s, 2H, -CH_2-), 3.14-3.09 (m, 4H, -CH_2-), 2.37-2.30 (m, 2H, -CH_2-), 1.99-1.94 (m, 2H, -CH_2-), 1.73-1.60 (m, 8H, -CH_2-), 1.63-1.54 (m, 4H, -CH_2-), 1.38-1.30 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 385.2. IR (KBr, cm^{-1}): 3330, 1647.Anal. calcd for $C_{22}H_{32}N_4O_2$: C, 68.72; H, 8.39; N, 14.57; found: C, 68.75; H, 8.36; N, 14.54.

Characterization of 3-(4-methanesulfonyl-benzyl)-8-(2-piperidin-1-yl-ethyl)-1,3,8-triaza spiro [4,6]undecane-2,4-dione (8d): (0.27 g, pale brown crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.55 (s, 1H, -NH), 7.36-7.20 (m, 4H, Ar-H), 4.54 (s, 2H, -CH_2-), 3.26 (s, 3H, -CH_3), 3.19-3.09 (m, 4H, -CH_2-), 2.37-2.32 (m, 4H, -CH_2-), 1.99-1.95 (m, 2H, -CH_2-), 1.77-1.64 (m, 8H, -CH_2-), 1.61-1.55 (m, 4H, -CH_2-), 1.37-1.32 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 463.1. IR (KBr, cm^{-1}): 3340, 1652, 1330, 1277.Anal. calcd for $C_{23}H_{34}N_4O_4S$: C, 59.72; H, 7.41; N, 12.11; found: C, 59.75; H, 7.44; N, 12.15.

Characterization of 3-pent-4-enyl-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,5] decane-2,4-dione

(12a): (0.27 g, white crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.75 (s, 1H, -NH), 5.82-5.74 (m, 1H, =CH), 5.05-4.94 (m, 2H, CH_2=), 3.35-3.31 (t, 2H, -CH_2-), 3.15-3.10 (m, 4H, -CH_2-), 2.87-2.82 (m, 2H, -CH_2-), 2.72-2.66 (m, 2H, -CH_2-), 2.35-2.30 (m, 4H, -CH_2-), 1.99-1.94 (m, 2H, -CH_2-), 1.74-1.67 (m, 2H, -CH_2-), 1.61-1.54 (m, 4H, -CH_2-), 1.37-1.34 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 349.1. IR (KBr, cm^{-1}): 3345, 1657.Anal. calcd for $C_{19}H_{32}N_4O_2$: C, 65.48; H, 9.26; N, 16.08; found: C, 65.45; H, 9.29; N, 16.05.

Characterization of [2,4-dioxo-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,5]dec-3-yl]-acetic acid propyl ester (12b): (0.27 g, pale brown crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.70 (s, 1H, -NH), 4.17 (s, 2H, -CH_2-), 4.04 (t, 2H, -CH_2-), 3.13-3.08 (m, 4H, -CH_2-), 2.87-2.82 (m, 4H, -CH_2-), 2.34-2.30 (m, 2H, -CH_2-), 1.99-1.94 (m, 2H, -CH_2-), 1.77-1.63 (m, 6H, -CH_2-), 1.60-1.54 (m, 4H, -CH_2-), 1.36-1.32 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 381.2. IR (KBr, cm^{-1}): 3330, 1738, 1657.Anal. calcd for $C_{19}H_{32}N_4O_4$: C, 59.98; H, 8.48; N, 14.73; found: C, 59.95; H, 8.45; N, 14.70.

Characterization of 3-benzyl-8-(2-piperidin-1-yl-ethyl)-1,3,8-triazaspiro[4,5] decane-2,4-dione (12c): (0.30 g, white crystalline solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.50 (s, 1H, -NH), 7.2-7.11 (m, 5H, Ar-H), 4.24 (s, 2H, -CH_2-), 3.14-3.09 (m, 4H, -CH_2-), 2.35-2.30 (m, 2H, -CH_2-), 1.98-1.94 (m, 2H, -CH_2-), 1.74-1.63 (m, 6H, -CH_2-), 1.62-1.55 (m, 4H, -CH_2-), 1.37-1.31 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 371. IR (KBr, cm^{-1}): 3310, 1657.Anal. calcd for $C_{21}H_{30}N_4O_2$: C, 68.08; H, 8.16; N, 15.12; found: C, 68.06; H, 8.19; N, 15.15.

Characterization of 3-(4-methanesulfonyl-benzyl)-8-(2-piperidin-1-yl-ethyl)-1,3,8-triaza spiro[4,5]decane-2,4-dione (12d): (0.275 g, white solid); ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 8.53 (s, 1H, -NH), 7.35-7.21 (m, 4H, Ar-H), 4.52 (s, 2H, -CH_2-), 3.25 (s, 3H, -CH_3), 3.17-3.09 (m, 4H, -CH_2-), 2.36-2.31 (m, 4H, -CH_2-), 1.97-1.94 (m, 2H, -CH_2-), 1.75-1.64 (m, 6H, -CH_2-), 1.60-1.55 (m, 4H, -

CH_2-), 1.35-1.32 (m, 2H, -CH_2-). MS (ESI + ion): m/z = 449.2. IR (KBr, cm^{-1}): 3320, 1649, 1325, 1274.Anal. calcd for $C_{22}H_{32}N_4O_4S$: C, 58.91; H, 7.19; N, 12.49; found: C, 58.94; H, 7.16; N, 12.46.

PHARMACOLOGY

Maximal electroshock seizure Model (MES): Maximal electroshock (MES) seizure model was used in the present study to evaluate the anticonvulsant activity of the drugs. Seizures were induced in ten weeks old male wistar rats(200-220 g) (procured from National Institute of Nutrition, Hyderabad) by delivering electro shock of 150 mA for 0.2 sec by means of a convulsiometer through a pair of ear clip electrodes. The test compounds (1-10, 100 mg/kg) were administered by oral route 30 mins before the maximal electroshock seizure test by suspending in carboxymethylcellulose (1%). The animals were observed closely for 2 min. The percentage of inhibition of seizure relative to control was recorded and calculated [17]. Phenytoin (100 mg/kg, p.o) was used as a standard drug. The data was analysed by one way ANOVA followed by Dunnett's test (Table 2).

Table 2. Results and effect of the compounds in the MES test

Treatment	E/F[a]	% Protection
Control(Vehicle)	7.77±1.11	
Standard	0.06±0.01	99.14
8a	0.68±0.11	91.25*
8b	2.42±0.12	68.85*
8c	1.72±0.11	77.86
8d	1.98±0.13	74.51
12a	1.02±0.01	86.87
12b	3.08±0.14	60.36
12c	2.19±0.11	71.81
12d	2.35±0.13	69.75

Values are expressed as mean ± SE. n = 6 animals in each group.
[a]E/F = Extension/Flexion.
*p<0.05 when compared to standard drug treated rats.

RESULTS AND DISCUSSION

All the compounds have shown promising and significant protective effect on maximal electroshock induced seizures when compared to vehicle treated control rats. Compound 8a and 12a are able to protect seizure effect significantly higher and this effect was similar when compared to standard drug treated rats. Whereas, compounds 8b, 8c, 8d, 12b, 12c and 12d have shown moderate protective effect on seizure (Table 2).

Among the compounds showing anticonvulsant activity, compounds with more lipophilic *N*-substitution like 1-pentene group in the hydantoin molecule, are more active than other *N*-substitution in the hydantoin ring. Compounds with hydrophobic phenyl substitutions in the hydantoin ring shows decreases in the anticonvulsant activity compared to those of propyl substitution derivatives. The rapid onset of action is believed to be due to the substitution of more lipophilic group in the *N*-substitution in the hydantoin moiety. The substitution of more lipophilic tosyl group in the distal phenyl moiety in the *N*-substitution alters the anticonvulsant activity compared to phenyl substitution. Evidently, this distal hydrophobic centre alters the bioavailability of the molecules.

In the present study, the anticonvulsant activity of newly synthesized diazaspiro derivatives in MES models of seizures in rats was investigated. Antiepileptic drug research has for several decades focused on identifying new potential drugs based on their anticonvulsant activity against single acute seizures induced by various stimulators, usually in mice and rats. All established antiepileptic drugs have anticonvulsant activity in at least MES model [18]. Thus, this test may in some way distinguish the potential utility of compounds against different seizure types. In the present series of compounds many of them show good anticonvulsant activity at 100 mg/kg compared to Phenytoin. So there is an

increase in anticonvulsant activity by our molecular modifications. In the semicarbazone series and urelyene anticonvulsants Dimmock [19, 20] proposed a binding site hypothesis for these compounds eliciting anticonvulsant activity.

A scrutiny for certain selected structures for active anticonvulsants has been shown to possess a hydrophobic unit, an electron donor group and hydrogen donor acceptor unit. In our present series of compounds the active compounds possess all the requirements essential for anticonvulsant activity as proposed by Dimmock and others.

APPLICATION

From the results of this study, the structure-activity relationships could be derived. Compounds 8a-d exhibited promising inhibition effect compare to 12a-d derivatives, this might be due to the ring size effect. Because with the same substitution of ethyl piperidine at the cyclic amine ring and varying the substitution at the hydantoin ring in both derivatives, compounds 8a-dshows promising activity. All the compounds have shown promising and significant protective effect on maximal electroshock induced seizures when compared to vehicle treated control rats.

CONCLUSIONS

A series of novel triazaspiro hydantoins 8a-d and 12a-d were synthesized in good yield and their anticonvulsant activities have been evaluated. The results are encouraging and show that the hydantoins are more potential molecules for the treatment of seizure effect. Approximately 70% of patients can achieve complete freedom from seizures with appropriate treatment [21]. If there is lipophilic moiety, then MES activity is favoured. The compounds in the study have shown promising and significant protective effect on maximal electroshock induced seizures when compared to vehicle treated control rats.

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