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Research Article

# DESIGN, SYNTHESIS AND ANTIEPILEPTIC EVALUATION OF 5-(ARYL)-N-PHENYL-1,3,4-THIADIAZOL-2-AMINE

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**ABSTRACT**

Epilepsy is one of the leading neurological disorders, which is a major threat to public health. Though, several new antiepileptic drugs are developed, the treatment of epilepsy remains still inadequate, and the patient suffers from a lot of side effects. Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy. So here an indirect type of molecular modeling study was carried out to find out the 3D structural similarity between some reported antiepileptic drugs and the newly designed 1,3,4-thiadiazole derivatives. Thus, a new series of 1,3,4-thiadiazole derivatives were synthesized by cyclization of N-phenylthiosemicarbazide with various aromatic acids. Both conventional and microwave irradiated synthesis of 1,3,4-thiadiazole derivatives have been carried out to compare their yield and reaction time. Microwave technology enables the reaction to be simple, rapid, high yielding with at most pure and led environmentally benign synthesis. All the newly synthesized compounds were characterized by I.R, $^1$H NMR, and LC-MASS and also evaluated for their antiepileptic activity by MES model in rats.

**Keywords:** N-phenylthiosemicarbazide, Aromatic carboxylic acid, 1,3,4-thiadiazole, Microwave synthesis, Antiepileptic activity.

## INTRODUCTION

Epilepsy is a very common chronic and progressive neurological disorder characterized by *seizures* which result from episodic neuronal discharge[1]. Approximately 50 million people worldwide suffer from epilepsy, making this condition the second leading neurological disorder[2]. Despite the development of several new anticonvulsants, the treatment of epilepsy remains still inadequate[3]. The current drug therapy of epilepsy is associated with side effects such as drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism, megaloblastic anemia and teratogenicity etc[4,5]. Therefore, there is an enormous demand for development of new anticonvulsant agents with lower toxicity and fewer side effects[6,7]. Foroumadi A. *et. al.* reported that 5-[4-chloro-2-(2-chlorophenoxy)phenyl]-*N*-ethyl-1,3,4-thiadiazol-2-amine (**4c**) was the most active compound in the series against convulsion[8]. Further the 3D structural similarity between 5-[4-chloro-2-(2-chlorophenoxy)phenyl]-*N*-ethyl-1,3,4-thiadiazol-2-amine and the newly designed 1,3,4-thiadiazoles was checked by indirect type of molecular modeling studies[9,10,11]. Low r.m.s.d (root mean square distance) value suggested good 3D structural similarity between these compounds. This prompted us to synthesize 1,3,4-thiadiazoles derivatives by cyclisation of 4-phenylthiosemicarbazide with various aromatic carboxylic acids[12]. On the other hand, numerous studies have been performed with the aim of exploring anticonvulsant properties of 1,3,4-thiadiazole analogues[13]. The above studies found that 1,3,4-thiadiazole analogues possess comparable anticonvulsant activity in comparison to the respective standard drugs employed. The search for better anticonvulsant drug and the importance of thiosemicarbazide and 1,3,4-thiadiazoles as anticonvulsant pharmacophore encouraged us to carry out the synthesis of some novel thiadiazole derivatives[14]. So various innovative technologies has been devised to speed up the chemical reactions. In the last few years, microwave-induced organic reaction enhancement (MORE) chemistry has gained popularity as a non-conventional technique for rapid synthesis[15,16]. The usage of microwave energy to accelerate the organic reactions is of increasing interest and offers several advantages over conventional heating technique. Synthesis of drug molecules which normally require a long time can be achieved conveniently and rapidly in microwave oven [17]. Less reaction time, easy work up and cleaner products are the major advantages of microwave heating[18,19]. Further more the reactions can be carried out under solvent free conditions which holds strategic positions as the solvents are often very toxic, expensive, problematic to use. Solvent free condition is especially suitable for microwave activation. Thus the use of microwave energy for the synthesis of new drug molecules forms a part of green chemistry[20,21].

### Chemistry

N-phenylthiosemicarbazide **(2)** was prepared by the treatment of aniline with $CS_2$ in presence of ethanol-ammonia solution, followed by reaction with hydrazine hydrate. Then N-phenylthiosemicarbazide undergoes cyclisation with various aromatic acids to produce corresponding 1,3,4-thiadiazoles derivatives **(3)** as depicted in Fig. 1.

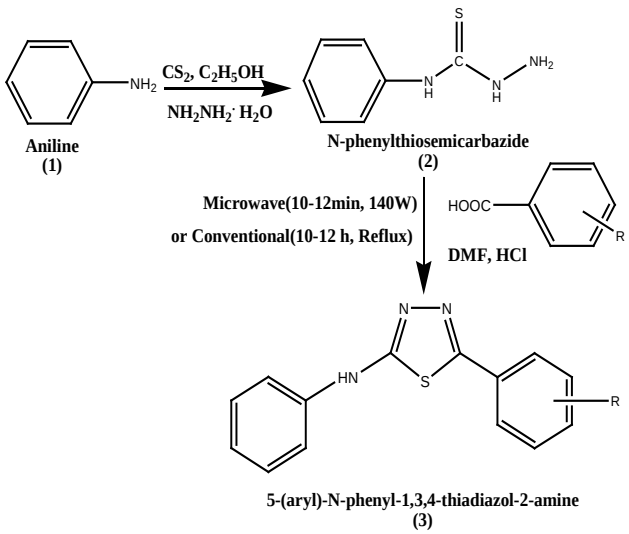
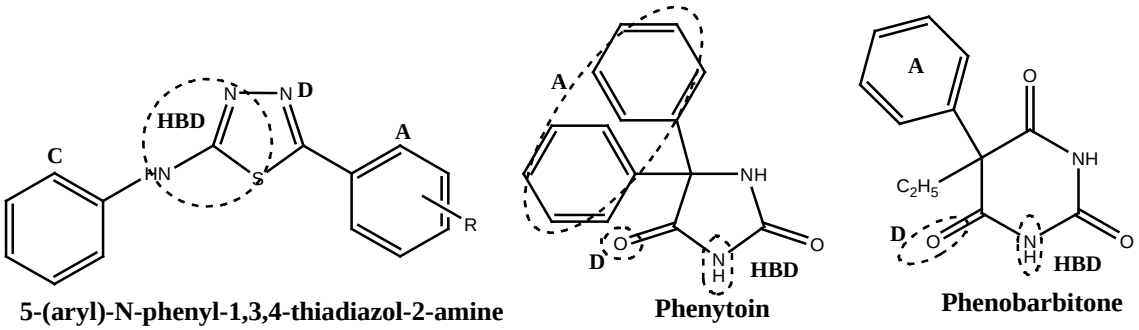



**Fig. 1: Scheme for the synthesis of title compounds.**

### Pharmacophoric Structural features of 1,3,4-Thiadiazoles

The search for better antiepileptic drug and the importance of semicarbazides and 1,3,4-thiadiazoles as anticonvulsant pharmacophore encouraged us to carry out the synthesis of above title compounds. The conformational investigation of the existing antiepileptic drugs such as Phenytoin, Carbamazepine, Lamotrigine, Rufinamide and Phenobarbitone has lead to the proposal of a general model for antiepileptic activity[22,23]. The semicarbazides based pharmacophore model comprises of following four vital binding sites such as (a) An aryl hydrophobic binding site (**A**) with halo substituent preferably at *para* position; (b) A hydrogen bonding domain (**HBD**); (c) An electron donor group (**D**) and (d) Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the antiepileptic drugs (**C**) as shown in Fig. 2.




**5-(aryl)-N-phenyl-1,3,4-thiadiazol-2-amine** **Phenytoin** **Phenobarbitone**

**Fig. 2: Pharmacophoric pattern in title compounds and well-known antiepileptic drugs**

**Molecular Modelling**

The 3D structural similarity between 5-[4-chloro-2-(2-chlorophenoxy)phenyl]-*N*-ethyl-1,3,4-thiadiazol-2-amine (**4c**) and the newly designed 5-(aryl)-N-phenyl-1,3,4-thiadiazol-2-amine were checked by indirect type of molecular modeling studies as shown in Fig. 3 & 4. All the structures of newly synthesized compounds were generated; energy minimized and superimposed using PC based molecular modeling software ChemDraw Ultra (Version 8.0, Cambridge Soft Corporation, and USA). All geometries were fully optimized by minimizing the energy with respect to geometrical variables without symmetry constraints, using a 0.01 Kcal/mol gradient.

The r.m.s.d. observed was around 0.112. The low r.m.s.d. value suggested good 3D similarity between these compounds which prompted us to synthesize some 1,3,4-thiadiazoles derivatives as antiepileptic agent.

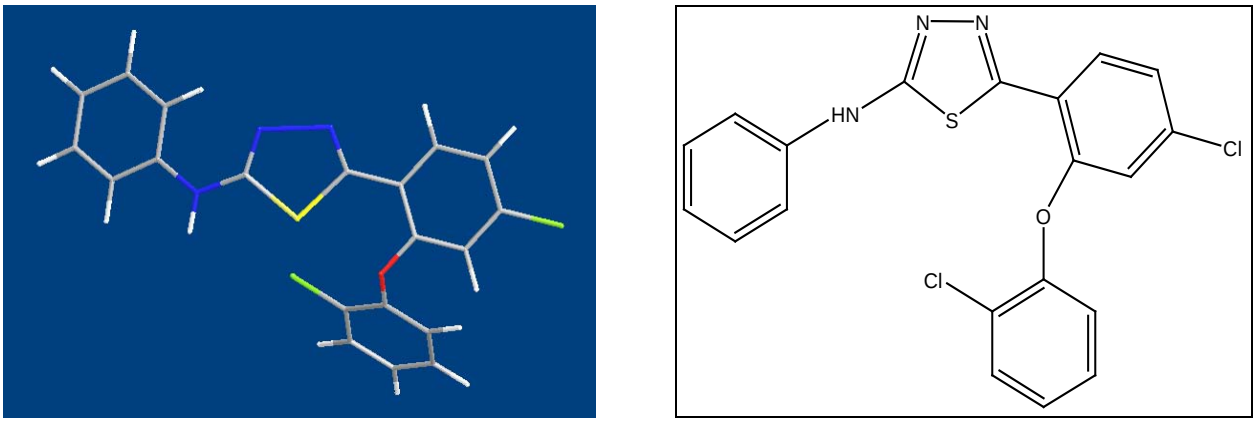



**Fig. 3: Structure of 5-[4-chloro-2-(2-chlorophenoxy)phenyl]-*N*-ethyl-1,3,4-thiadiazol-2-amine (4c)**

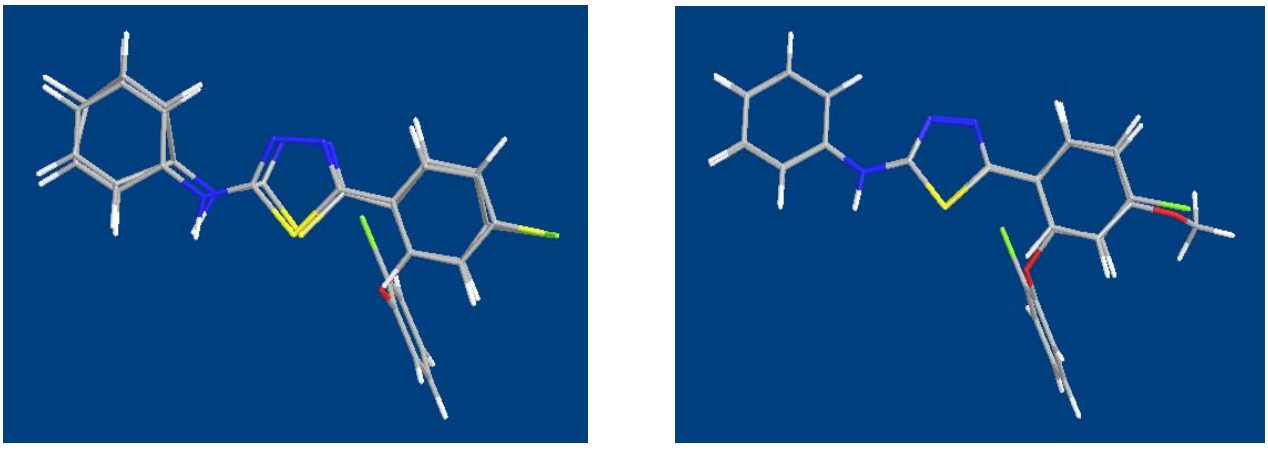


**Fig. 4: 3D structure superimposition of compound 4c & 3d and 4c & 3e**

**MATERIALS AND METHODS**

**Materials**

The chemicals and solvents used for the experimental work were commercially procured from Sigma Aldrich, Merck, Loba Chem without further purification. All the melting points were taken in open capillaries and are uncorrected. Follow up of the reactions and checking the purity of the compounds was made by TLC on pre-coated Silica gel-aluminum plates (Type 60 $F_{254}$, Merck, Darmstadt, Germany) and were visualized by exposure to UV-light (254 nm) or iodine vapor for few seconds. The IR spectra of the compounds were recorded on FT-IR Spectrophotometer, model IR Affinity-1 (SHIMADZU), using KBr powder and the values are expressed in $cm^1$. $^1H$ NMR spectra of selected

compounds were recorded on multinuclear FT NMR Spectrometer, model Advance-II (Bruker), (at 400 MHz) using tetramethylsilane (TMS) as an internal standard and DMSO-$d_6$ as a solvent. The multiplicities of the signals are denoted with the symbols *s, d, t and m* for singlet, doublet, triplet and multiplet, respectively. The LC-MS spectra were recorded on Waters Micromass Q-Tof Micro (70 eV), a hybrid quadrupole time of flight mass spectrometer. The microwave irradiated synthesis was performed in scientific microwave oven, Catalyst System (operating between 140-700W). All the reactions were carried out at power level-1, which corresponds to 140W.

**Synthesis of N-phenylthiosemicarbazide (2)**

Aniline (0.1mole, 9g) was dissolved in ethanol (95%, 20 ml) and ammonia (20 ml). Then Carbon disulfide (0.1mole, 7.6 g) was added drop wise and stirred for 30 min. To this, hydrazine hydrate (0.1mol, 4.86 ml) was added and the reaction mixture was refluxed on water bath. In between, the completion of reaction was checked by TLC. After completion of reaction, the reaction mixture was allowed to cool to room temperature and kept overnight in freezing condition to get the solid product **(2)**. The separated solid product was filtered, dried and recrystallised from methanol.

**General Synthesis of 5-(aryl)-N-phenyl-1,3,4-thiadiazol-2-amine (3a-e)**

**Conventional method**

N-phenylthiosemicarbazide **(2)** (0.01mol, 1.67g), various aromatic acids (0.01mol) in DMF (25ml) were taken and refluxed on water bath for 10-12 h. In between TLC was checked to the check completion of reaction. After that the reaction mixture was slowly poured into crushed ice and kept over night. The separated solid was filtered, washed with water, dried and purified by recrystallization from methanol.

**Microwave assisted method**

N-phenylthiosemicarbazide **(2)** (0.01mol, 1.67g), various aromatic acids (0.01mol) in DMF (25ml) were taken and subjected for microwave irradiation for 10-12 min. at power level 140 watts. In between TLC was checked to check completion of reaction. After that the reaction mixture was slowly poured into crushed ice and kept overnight. The separated solid was filtered, washed with water, dried and purified by recrystallization from methanol.

**Spectral data of 5-(aryl)-N-phenyl-1,3,4-thiadiazol-2-amine derivatives (3a-e)**

**N, 5-diphenyl-1,3,4-thiadiazol-2-amine (3a)**

**IR:** 3344 (NH sec.), 3086 (Ar CH), 2358 (C-S-C) 1670 (C=N), 750 ($C_6H_5$). **$^1$H NMR:** 8.07 (s, 1H, NH), 7.01-8.05 (m, 10H, Ar-H)

**5-(2-chlorophenyl)-N-phenyl-1,3,4-thiadiazol-2-amine (3b)**

**IR:** 3344 (NH sec.), 3068 (Ar CH), 2358 (C-S-C) 1693 (C=N), 761 ($C_6H_5$)**,** 729 (C-Cl)

**5-(4-aminophenyl)-N-phenyl-1,3,4-thiadiazol-2-amine (3c)**

**IR:** 3331 (NH sec.), 3105 (Ar-CH), 2358 (C-S-C), 1651 (C=N), 1600 (Primary. $NH_2$), 761 ($C_6H_5$). **$^1$H NMR:** 9.73 (s, 1H, NH), 6.59 (s, 2H, $NH_2$), 7.22-8.00 (m, 9H, Ar-H).

**5-(4-fluorophenyl)-N-phenyl-1,3,4-thiadiazol-2-amine (3d)**

**IR:** 3450(NH sec.), 3066 (Ar CH), 2358 (C-S-C), 1604 (C=N), 1161 (C-F), 769 ($C_6H_5$). **$^1$H NMR:** 8.12 (s, 1H, NH), 7.05-8.09 (m, 9H, Ar-H). **MS:** 272.5($M^+$)

**5-(4-methoxyphenyl)-N-phenyl-1,3,4-thiadiazol-2-amine (3e)**

**IR:** 3450 (NH sec.), 3072 (Ar CH), 2358 (C-S-C), 1606 (C=N), 1176 ($OCH_3$), 744 ($C_6H_5$). **$^1$H NMR:** 11.74 (s, 1H, NH), 3.85 (s, 3H, $OCH_3$), 6.84-7.98 (m, 9H, Ar-H). **MS:** 283.5(M), 284.5($M^+$).

**RESULTS AND DISCUSSION**

All the synthesized compounds were characterized by their physical, chemical and spectral data (**Tables 1, 2**). As compared to conventional heating, microwave heating provides high yield with purity product in less reaction time.

**Biological Evaluation**

**Animals**

The antiepileptic activity was carried out on albino rats (100-150 g) of either sex as experimental animal. The animals were housed under standard conditions and allowed free access to standard pellet diet and water (*ab libitum*), except during the experiment. The test compounds and standard drug were suspended in 0.5% methyl cellulose/water mixture.

**Antiepileptic activity**

The antiepileptic activity of the newly synthesized compounds was done according to the protocols of National Institute of Neurological Disorders and Stroke, NIH (USA). The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) constituted under Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (Regd. No. 926/ab/06/CPCSEA). The maximal electroshock (MES) method was performed to induce the seizures in order to evaluate the antiepileptic activity [24,25]. Rats were randomly distributed into various groups of six animals each (n=6). Group I served as control, Group II served as standard (Phenytoin sodium 25mg/kg body weight) and all the synthesized compounds were injected to consecutive group of animals with dose of 300mg/kg body weight respectively. The standard drug was administered i.p. 30 min. before and the test compounds were administered orally, 1h prior to induce the convulsion and Electro convulsive shock (150 mA for 0.2 sec) was delivered through corneal electrode to induce convulsions to each group of rats. The various phases of convulsion which were produced are Flexion, Extension, Clonus and Stupor. Prior to delivery, current output was checked by multimeter. After the electric stimulation occurrence, the duration of phases was noted and HLTE (Hind limb tonic extension) phase was compared with control group. Decrease in duration of hind limb extension was considered as protective action **(**Table 3**)**. The percentage potency and percentage protection in convulsion activity was calculated in each group of animals. [26]

$$\% \text{ Potency} = (MEPD_{nc} - MEPD) / (MEPD_{nc} - MEPD_{Std}) \times 100$$

$$\% \text{ Protection} = (MEPD_{nc} - MEPD) / MEPD_{nc} \times 100$$

Where MEPD*nc* is the mean extensor phase duration of normal control in sec., MEPD is the mean extensor phase duration of sample or standard in sec.and $MEPD_{Std}$ is the mean extensor phase duration of standard in sec. The percentage protection of test compounds in convulsion activity was shown in Fig. 5.

**Table 1: Comparison of conventional method with microwave assisted method.**

| Comp. C Code | Conventional Synthesis Energy | | | Microwave synthesis Energy | | |
|---|---|---|---|---|---|---|
| | Temp ($^0$C) | Time (hr) | Yield (%) | (Power. Watt) | Time (min.) | Yield (%) |
| 3a. | 90-100 | 11 | 57.54 | 210 | 12 | 78.29 |
| 3b. | 90-100 | 10 | 67.53 | 210 | 11 | 85.26 |
| 3c. | 90-100 | 10 | 55.87 | 210 | 12 | 76.11 |
| | 90-100 | 11 | 55.67 | 210 | 12 | 82.56 |

**Table 2: Characterization Data of Synthesized Compounds 3(a-e)**

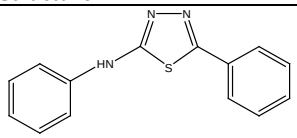
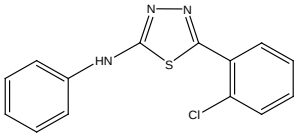
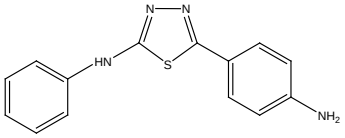
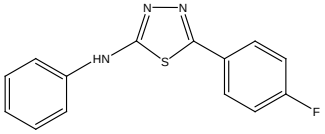
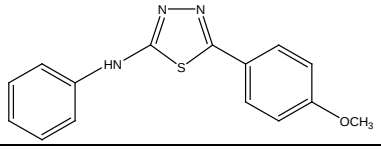
| Comp. Code | Structure | MF | MW | mp (°C) | $R_f$ Value |
|---|---|---|---|---|---|
| 3a. |  | $C_{14}H_{11}N_3S$ | 253.32 | 120-123 | 0.59 |
| 3b. |  | $C_{14}H_{10}ClN_3S$ | 287.77 | 130-134 | 0.54 |
| 3c. |  | $C_{14}H_{12}N_4S$ | 268.34 | 155-157 | 0.63 |
| 3d. |  | $C_{14}H_{10}FN_3S$ | 271.31 | 134-136 | 0.53 |
| 3e. |  | C15H13N3OS | 283.35 | 145-148 | 0.62 |

**Table 3: Antiepileptic activity of synthesized compounds 3(a-e)**

| Group ( n=6) | Treatment | Dose (mg/kg) | HLTE phase Duration (sec) | % Potency | % Protection | Recovery/Death |
|---|---|---|---|---|---|---|
| Group I | Control (Normal saline) | 25 | 12 | 100 | 78.57 | Recovered |
| Group II | Standard (Phenytoin) | | | | | |
| Group III | 3a. | 300 | 28 | 63.63 | 50 | Recovered |
| | 3b. | 300 | 21 | 79.54 | 62.5 | Recovered |
| | 3c. | 300 | 25 | 70.45 | 55.35 | Recovered |
| | 3d. | 300 | 21 | 79.54 | 62.5 | Recovered |
| | 3e. | 300 | 20 | 81.81 | 64.28 | Recovered |

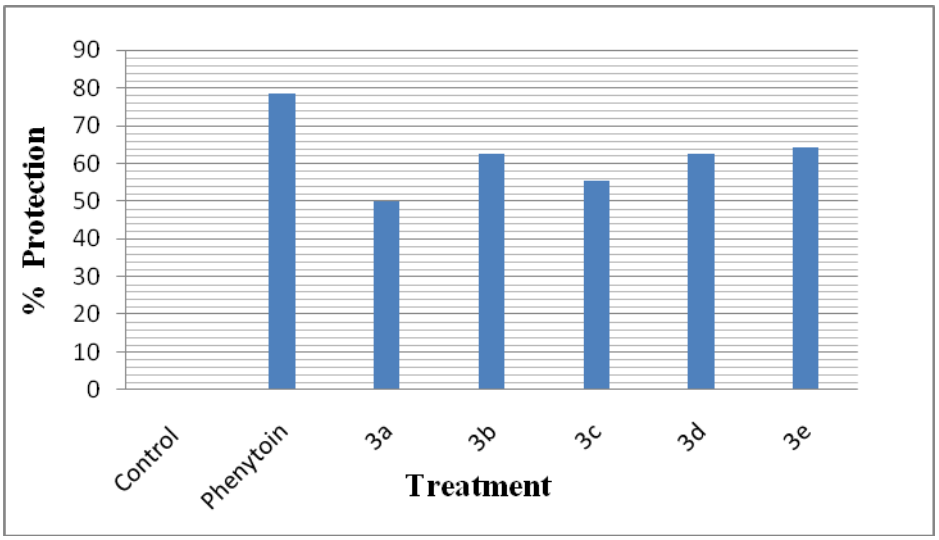



**Fig. 5: Antiepileptic activity of 1,3,4-Thiadiazole Derivatives**

## CONCLUSION

A new series of 1,3,4-thiadiazole derivatives were synthesized successfully by both conventional and microwave irradiated method to compare their yield. Microwave irradiated synthetic technology made the reaction simple, rapid, with high yield. Some of the synthesized compounds showed significant activity against convulsion. And also the low r.m.s.d. value of some newly synthesized compound suggested good 3D similarity to confirm their antiepileptic activity.

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