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Comparative Study of Anticonvulsant Property among Different Fluoro Substituted Synthesized Benzothiazole Derivatives

I. Shanmukha[1*], A. M. Madhusudhana[1], E. Jayachandran[1], M. Vijay Kumar[1] and B. Prakash[2]

[1]Department of Pharmacology, S.C.S. College of Pharmacy, Harapanahalli, Karnataka, India.
[2]Department of Physiology, Basaveshwara Medical College, Chitradurga, Karnataka, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author IS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AMM and EJ managed the analyses of the study. Authors MVK and BP managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Objective: The present study is to evaluate antiepileptic activity of some fluoro benzothiazolo oxadiazolo quinazoline and sulfonamido quinazoline derivatives.
Background of the Study: Epilepsy is a chronic disorder of the brain, characterized by the periodic and unpredictable occurrence of seizures. Epilepsies affect around 1–2% of the world population including the fact that the convulsions of approximately 25% of epileptics are inadequately controlled by medication.
Materials and Methods: Albino mice (weighing 20-25g) of either sex were used in this study. MES seizures were induced in mice by delivering electroshock of 60ma for 0.2 seconds by means of an electro- convulsiometer transauricularly through a pair of ear clip electrodes. Both test animals and standard group received diazepam (5mg/kg) p.o strychnine nitrate (1 mg/kg) was administered.
Results and Discussion: MES induced convulsion the mentioned dose was administered one hour prior to MES elicitation. It was observed that all SSBDs except ap-7, bz-10 and s-5 shown significant anticonvulsant effect. The results are compiled in the

**Corresponding author: Email: shannu76@rediffmail.com;*

Table 1 and graphically depicted in Figs 1 and 2 respectively.
Conclusion: The present investigation revealed that the SSBDS ap-3, ap-6 and bz-9 were shown significant anticonvulsant activity against both MES and strychnine induced models.

Keywords: Epilepsy; fluoro benzothiazoles; convulsions; strychnine; MES; SSBD's.

1. INTRODUCTION

Epilepsy is a chronic disorder of the brain, characterized by the periodic and unpredic Table occurrence of seizures. Epilepsies are common and frequently devastating and affect around 1–2% of the world population. Present drug treatment for epilepsy patients have a number of disadvantages/limitations, including the fact that the convulsions of approximately 25% of epileptics are inadequately controlled by medication. In recent years, the field of antiepileptic drug development has become quite dynamic, affording many promising research opportunities [1]. Therefore, the continued search for safer and more effective anti-epileptic drugs is necessary. As per the literature survey reveals that, various derivatives of Benzothiazole, quinazoline derivatives have shown promising anticonvulsant activity, along with other pharmacological activities [2]. Further the degree of success varies as a function of seizure type, cause and other factors. Disappointingly, despite all efforts, the complete control of seizures can be achieved in up to 50% of patients [3]. Most of the epileptic patients not only suffer from stigmatization, they usually suffer from depression, muscular spasm, strange sensations, abnormal behavioural changes, convulsions, loss of consciousness and are highly prone to suicide and sudden, unexpected death [4]. Benzothiazole derivatives have been studied and found to have various chemical reactivity and biological activity [5].

Now a day's vast number of compounds with Fluorobenzene moiety features in diverse areas like antibacterial, antifungal, anti-inflammatory, psychoactive agents, anti-convulsant and anti-allergic activities etc.

2. MATERIALS AND METHODS

2.1 Animals

Albino mice (weighing 20-25g) of either sex were used in this study. They were housed in polypropylene cages and maintained at 27°C±2°C under 12 hrs dark / light cycle. Ethical clearance for usage of the animals was obtained from the Institutional animal ethical committee (Certificate reference no: 681/A 2010-11 dated: 30-11-2010) prior to the beginning of the project work.

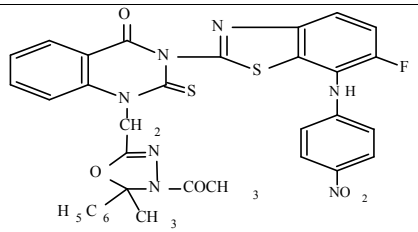
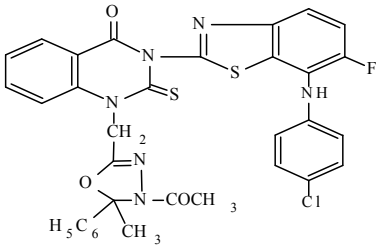
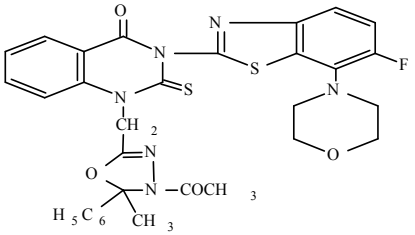
2.2 Source of Compounds

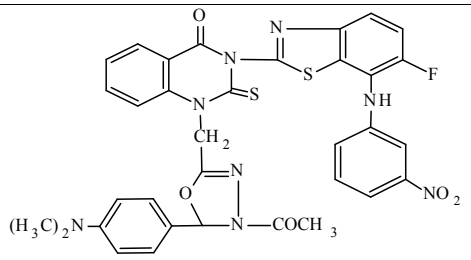
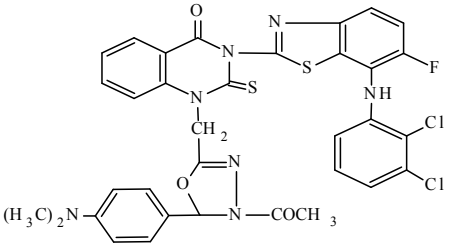
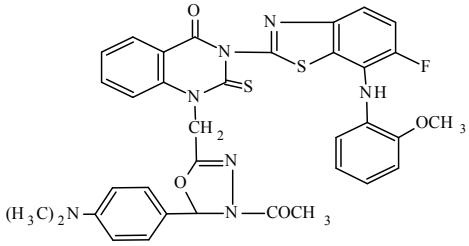
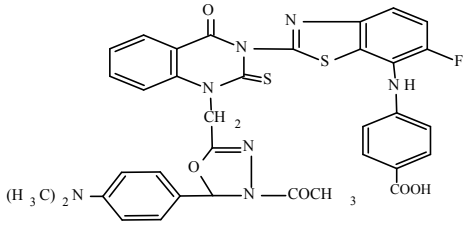
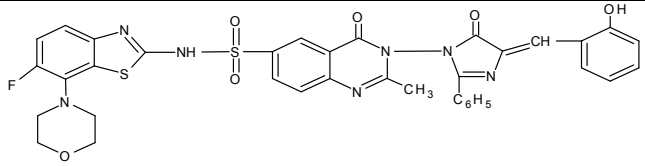
The novel synthesized substituted Benzothiazole derivatives (SSBDs) were synthesized by Mr. Gajaraja Sharma and Mr. Sri Ranga T. under the guidance of Dr. E. Jayachandran, Professor and head, PG Department of Pharmaceutical Chemistry, S.C.S. College of Pharmacy, Harapanahalli. The same SSBDs were procured for screening anticonvulsant and antiallergic activities in validated experimental animal model.

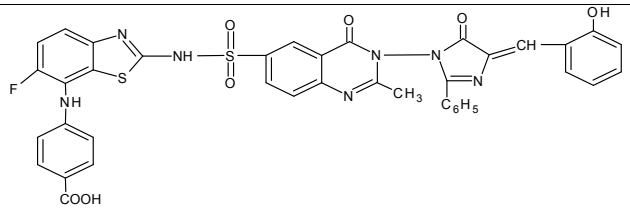
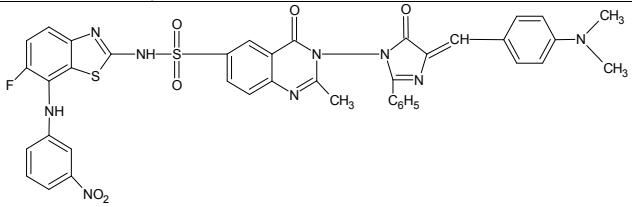
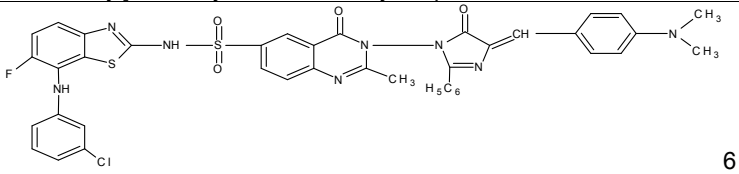
2.3 Acute Toxicity

The toxicity of SSBDs were determined by using female albino mice (20-25g), maintained under standard husbandry conditions. The animals were fasted for 3-4 hours prior to the experiment. Animals were administered with single dose of synthesized substituted Benzothiazole derivatives observed up to 48 hours study period for its mortality (short term toxicity). Based on the short-term toxicity profile, the next dose were determined as per OECD guidelines No 420 [6]. The minimum effective dose of SSBDs was determinated at dose of 30, 100, 150 and 250 mg/kg body weight of an animal to carry out screening of anticonvulsant and antiallergic activities.

Table 1. List of SSBDs procured

Compound no.	Compound code	Structure and chemical name
1	AP - 3	 3-{6-fluoro-7-[(4-nitrophenyl)amino]-1,3-benzothiazol-2-yl}-1-[(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-2-thioxo-2,3-dihydroquinazoline-4(1H)-one.
2	AP - 6	 3-{7-[(4-chlorophenyl)amino]-6-fluoro-1,3-benzothiazol-2-yl}-1-[(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-2-thioxo-2,3-dihydroquinazoline-4(1H)-one.
3	AP - 7	 3-(6-fluoro-7-morpholin-4-yl-1,3-benzothiazol-2-yl)-1-[(4-acetyl-5-methyl-5-phenyl-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-2-thioxo-2,3-dihydroquinazoline-4(1H)-one.

4	BZ - 2	 3-{(6-fluoro-7-[(3-nitrophenyl)amino]-1,3-benzothiazol-2-yl}-1-({4-acetyl-5-[4-(dimethylamino)phenyl]-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one.
5	BZ - 7	 3-{7-[(2,3-dichlorophenyl)amino]-6-fluoro-1,3-benzothiazol-2-yl}-1-({4-acetyl-5-[4-(dimethylamino)phenyl]-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one.
6	BZ - 9	 3-{(6-fluoro-7-[(methoxyphenyl)amino]-1,3-benzothiazol-2-yl}-1-({4-acetyl-5-[4-(dimethylamino)phenyl]-4,5-dihydro-1,3,4-oxadiazol-2-yl}methyl)-2-thioxo-2,3-dihydroquinazolin-4(1H)-one.
7	BZ - 10	 4-{[6-fluoro-1-({4-acetyl-5-[4-(dimethylamino)phenyl]-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-2-(4-oxo-2-thioxo-1,4-dihydroquinazoline-3(2H)-yl)-1,3-benzothiazol-7-yl]amino}benzoic acid.
8	V - 11	 6[6-fluoro-7-morpholino-(1,3)benzothiozolyl-2-aminosulphonyl] (5z)-5[2-hydroxybenzylidine-2-phenyl-3,5-dihydro-4H-4-imidazolonyl]-2-methyl-4-oxo-3-4-dihydroquinazoline

9	V - 13	 6[6-fluoro-7-p-carboxylanilino-(1,3)benzothiozolyl-2-aminosulphonyl] (5z)-5[2-hydroxybenzylidine-2-phenyl-3,5-dihydro-4H-4-imidazolonyl]-2-methyl-4-oxo-3-4-dihydroquinazoline
10	S – 2	 6 [6-fluoro-7-m-nitroanilino-(1,3)-benzothiozolyl-2-aminosulphonyl] (5Z)-5[4-(dimethylamino) benzylidine-2-phenyl-3,5-dihydro-4H-4-imidazolonyl]-2-methyl-4-oxo-3,4-dihydroquinazoline
11	S - 5	 6 [6-fluoro-7-m-chloroanilino-(1,3)-benzothiozolyl-2-aminosulphonyl] (5Z)-5[4-(dimethylamino) benzylidine-2-phenyl-3,5-dihydro-4H-4-imidazolonyl]-2-methyl-4-oxo-3,4-dihydroquinazoline

3. EXPERIMENTAL DESIGN

3.1 Maximal Electro Shock (MES) Induced Convulsions

Seizures were induced in mice by delivering electroshock of 60mA for 0.2 seconds by means of an electro- convulsiometer transauricularly through a pair of ear clip electrodes. Normal group administered with 2% w/v gum acacia and receive MES. The test animals (n=6) received orally with compound 1–11 at a dose of 100 mg/kg and standard group received phenytoin (25 mg/kg) injected i.p. and tested after one hour for MES induced seizure response. All the experimental groups were compared with the control. This current intensity should elicit complete tonic extension of the hind limbs in control mice. The onset time of seizures, duration of tonic hind limb extension and mortality for each animal was observed. Decrease in duration of hind limb extension was considered as a protective action [7,8].

3.2 Strychnine Induced Convulsions in Mice

Strychnine nitrate 1 mg/kg was administered subcutaneously induce clonic-tonic convulsions in mice. The test animals (n=6) received orally with compound 1–11 at a dose of 100 mg/kg and standard group received Diazepam (5mg/kg p.o strychnine nitrate 1 mg/kg) was administered, 60 min after the administration of drug. Each animal was then placed into individual plastic cages during test session. Mice that did not convulse 30 min after

strychnine administration were considered to be protected. The parameters such as onset of seizures, duration of tonic-clonic seizures and percentage of mortality for each animal were observed during test session [9,10].

4. RESULTS AND DICUSSION

MES and Strychnine are commonly used models for screening of potential anticonvulsant drugs. The MES test is considered to be one of the predictor of likely therapeutic efficacy against generalized tonic-clonic seizures whereas the Strychnine model represents a valid method for human generalized myoclonic and also absence seizures. Epilepsy is exhibited due to an array of underlying pathological phenomenon including kindling, deregulation of sequential firing of neurons, over expression of Na^+ channel, inhibition of glycine synthesis, down regulation of nitroso-oxidative stress leading to flexion and extension of limbs. These therapeutic strategies were traced in our investigation [11].

MES induced convulsions in mice conducted at a dose of 100mg/kg body weight of an animal. The mentioned dose was administered one hour prior to MES elicitation. It was observed that all SSBDs except AP-7, BZ-10 and S-5 shown significant anticonvulsant effect as compared to control by increasing onset time of seizures and reducing the duration of tonic extensor phase. The standard drug phenytoin (25mg/kg) exhibited a significant anticonvulsant activity. The results of the study are compiled in the Table 2 and graphically depicted in Figs. 1 and 2 respectively.

Table 2. Mean data of effect of synthesized substituted benzothiazole derivatives on MES induced convulsions in mice

Sl. No.	Treatment	Dose	Onset of tonic clonic seizures(min.)	Duration of tonic clonic seizures (sec.)
1	Control (2% Gum acacia p.o)	-----	1.070±0.1092	22.60±2.040
2	Standard (Phenytoin)	25mg/kg (p.o.)	4.090±0.3011***	10.80±4.128***
3	AP-3	100mg/kg (p.o.)	2.140±0.1240**	14.64±1.404*
4	AP-6	100mg/kg (p.o.)	3.133±0.1265***	12.92±1.548**
5	AP-7	100mg/kg (p.o.)	1.073±0.0486 NS	18.95±1.299 NS
6	BZ-2	100mg/kg (p.o.)	2.030±0.2285**	15.26±1.208*
7	BZ-7	100mg/kg (p.o.)	1.908±0.2244*	15.00±1.439*
8	BZ-9	100mg/kg (p.o.)	2.173±0.1140***	13.20±1.392**
9	BZ-10	100mg/kg (p.o.)	1.073±0.0523 NS	16.82±1.542 NS
10	V-11	100mg/kg (p.o.)	1.937±0.2064*	14.51±1.180*
11	V-13	100mg/kg (p.o.)	1.907±0.2927*	14.89±1.044*
12	S-2	100mg/kg (p.o.)	1.808±0.1840*	14.74±0.9139*
13	S-5	100mg/kg (p.o.)	1.072±0.1161 NS	16.47±0.7678 NS

*The data are presented as mean ± SEM, n=6. Statistical analysis were performed using One-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: *P=0.05, **P<0.01, P<***0.001 compared to control group*

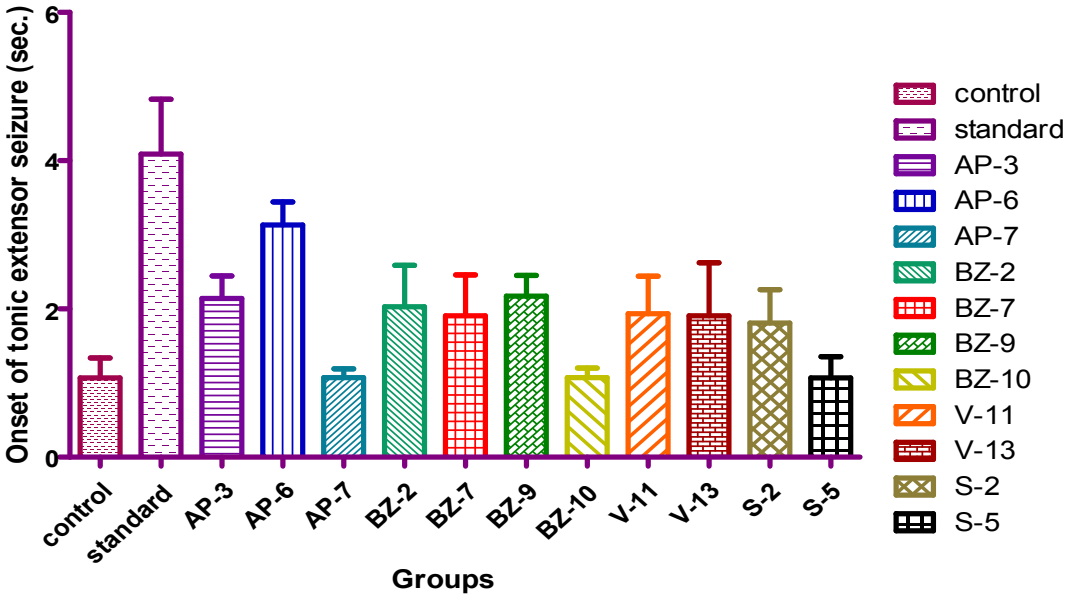


Fig. 1. Onset of tonic extensor seizure (sec.) in MES model

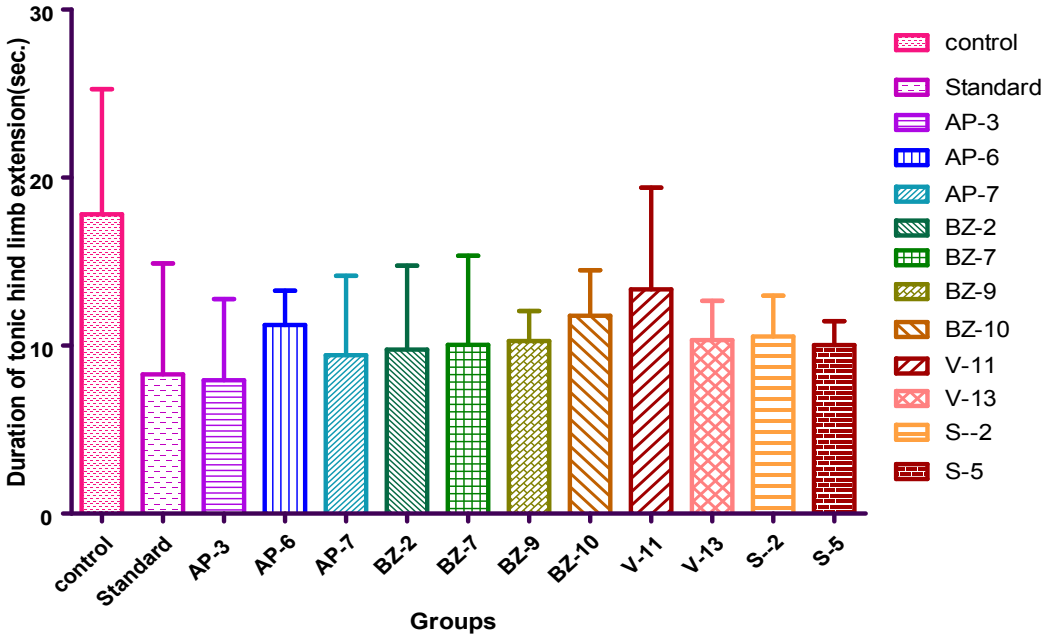


Fig. 2. Duration of tonic hind limb extension (sec.) in MES model

The mentioned dose was administered one hour prior to seizure elicitation. It was observed that all SSBDs except BZ-2, BZ-10, V-11 and S-2 shown significant anticonvulsant effect as compared to control by increasing onset of seizures and reducing the duration of tonic-clonic

seizures. The standard drug diazepam (5mg/kg) exhibited a significant anticonvulsant activity.

The results of the study are compiled in the Table 3 and graphically depicted in Figs. 3 and 4 respectively. Besides many antiepileptic drugs (AEDs) such as phenobarbital, phenytoin, carbamazepine, vigabatrin, valproate, felbamate and lamotrigine, which are effective toward only 60-80% of patients, have some undesirable side effects, such as headache, nausea, anorexia, ataxia, hepatotoxicity, drowsiness, gastrointestinal disturbance, gingival hyperplasia and hirsutism [12-15]. Despite the availability of many AEDs, there is still an urgent need for the development of more effective and safer AEDs, since about 30% of epileptic patients are not seizure-free with the existing AEDs [16].

Table 3. Mean data of effect of synthesized substituted benzothiazole derivatives on strychnine induced convulsions in mice

Sl. No.	Treatment	Dose	Onset of tonic clonic seizures(min.)	Duration of tonic clonic seizures (sec.)	% protection (1hr)
1	Control (2% Gum acacia)	---	3.592±0.2058	17.82±3.041	0 %
2	Standard (Diazepam)	5mg/kg (p.o.)	11.91±1.334***	8.288±2.695**	50 %
3	AP-3	100mg/kg (p.o.)	11.09±1.749***	7.943±1.972**	16.66 %
4	AP-6	100mg/kg (p.o.)	10.47±0.6275**	11.22±0.8376 NS	0 %
5	AP-7	100mg/kg (p.o.)	10.02±1.870**	9.430±1.927*	16.66 %
6	BZ-2	100mg/kg (p.o.)	6.545±0.7460 NS	9.763±2.041*	0 %
7	BZ-7	100mg/kg (p.o.)	9.287±1.780*	10.05±2.162*	16.66 %
8	BZ-9	100mg/kg (p.o.)	8.955±0.9031*	10.27±0.7310*	16.66 %
9	BZ-10	100mg/kg (p.o.)	6.567±0.9315 NS	11.78±1.111 NS	0 %
10	V-11	100mg/kg (p.o.)	5.670±1.114 NS	13.35±2.471 NS	0 %
11	V-13	100mg/kg (p.o.)	8.702±0.5248*	10.33±0.9537*	0 %
12	S-2	100mg/kg (p.o.)	6.180±1.421 NS	10.54±0.9957 NS	0 %
13	S-5	100mg/kg (p.o.)	8.957±1.399*	10.04±0.5794*	0 %

*The data are presented as mean±SEM, n=6. Statistical analysis were performed using One-way analysis of variance (ANOVA), followed by Dunnet's multiple comparison test. Levels of significance: *P<0.05, **P<0.01, P<***0.001 compared to control group*

4.1 MES Induced Convulsive Model

Antiepileptic drugs act by blocking seizure spread, moreover MES induced tonic extension can be prevented either by drugs that inhibit voltage dependant Na^+ channels or block glutaminergic excitation mediated by the N-methyl- D-aspartate (NMDA) receptor. It was observed that phenytoin as a standard drug (25mg/kg) exhibit extremely significant effect (***P<0.001). AP-6 and BZ-9 exhibit very significant effect (**P<0.01). AP-3, BZ-2, BZ-7, V-11, V-13 and S-2 exhibit significant (*p<0.05) anticonvulsant effect as compared to control, by increasing onset time of seizures and reducing the duration of tonic extensor phase and tonic-clonic seizures. Hence, the anticonvulsant activity of SSBDs against MES induced convulsions involve blockade of seizure spread, which perhaps occurred by inhibiting voltage dependant na^+ channels.

4.2 Strychnine Induced Convulsive Model

Glycine is an inhibitory neurotransmitter in the CNS and strychnine is a competitive antagonist of the glycine receptor. Strychnine produces convulsion by antagonizing the inhibitory spinal cord and brainstem reflexes of glycine and anticonvulsant drugs should delay the seizure produced by strychnine [17].

It was observed that AP-3 and diazepam as a standard drug (5mg/kg) exhibit extremely significant (***P<0.001). AP-6 and AP-7 exhibit very significant effect (**P<0.01). BZ-7, BZ-9, V-13 and S-5 exhibit significant (*P<0.05) anticonvulsant effect as compared to control.

4.3 Statistical Analysis

Results were expressed as mean ± SEM, (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test by using graph pad instat software. p value less than 0.05 was considered to be statistically significant. *p<0.05, **p<0.01 and ***p<0.001, when compared with control and toxicant group as applicable.

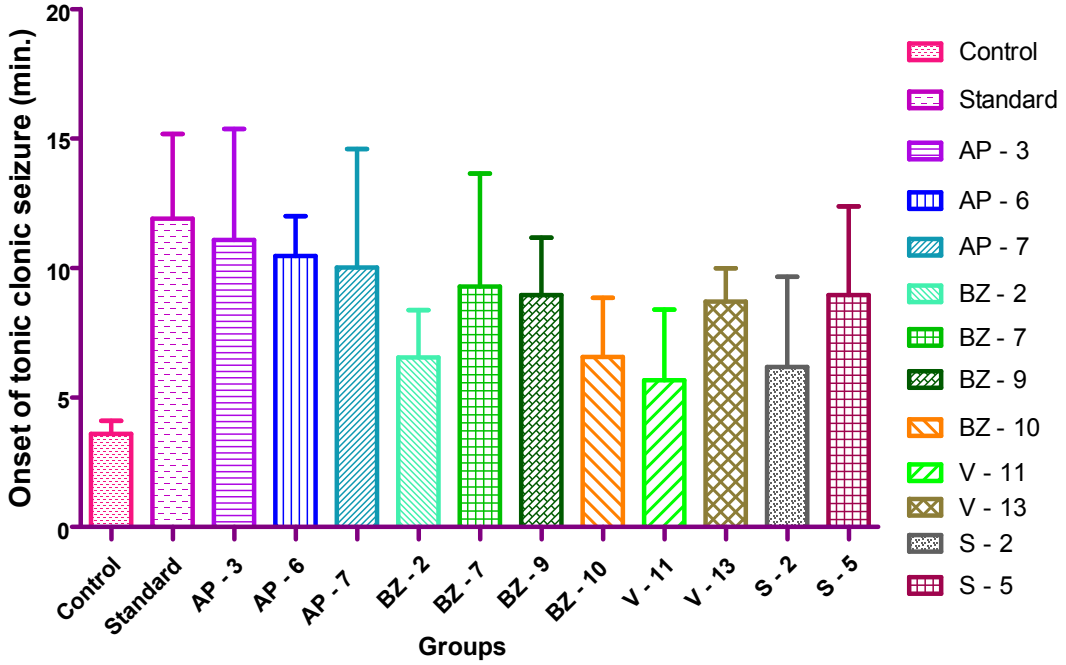


Fig. 3. Onset of tonic-clonic seizure (min.) in Strychnine model

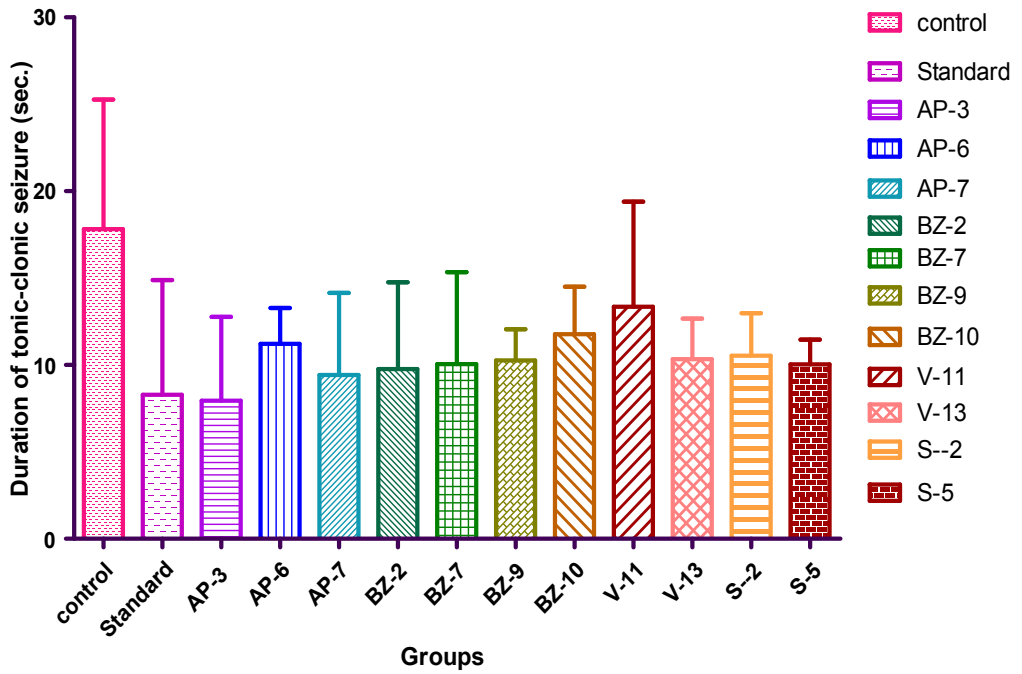


Fig. 4. Duration of tonic-clonic seizure (sec.) in strychnine model

5. CONCLUSION

The present investigation revealed that the SSBDs exhibit anticonvulsant activity. The anticonvulsant action of SSBDs is better against strychnine induced seizures than MES seizures. AP-3, AP-6 and BZ-9 were shown significant anticonvulsant activity against both MES and strychnine induced models.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Siddiqui N, et al. Synthesis and preliminary screening of benzothiazol-2-yl thiadiazole derivatives for anticonvulsant activity. Acta Pharm. 2009;59:441–451.

2. Sachin S Laddha, Satyendra P Bhatnagar. Novel fused quinazolinones: further studies on the anticonvulsant activity of 1,2,9,11-tetrasubstituted-7H-thieno [2´,3´:4,5] pyrimido [6,1-b]-quinazolin-7-one and 1,3,10,12-tetrasubstituted-8H-pyrido [2´,3´:4,5] pyrimido [6,1-b]quinazolin-8-one. Future medicinal chemistry, Future science. 2010;2(4):565-573. Available: http://en.wikipedia.org/wiki/Convulsion. Access date 25/12/2012
3. Azikiwe CCA, Siminialayi IM, Brambaifa N, Amazu LU, Enye JC, Ezeani MC Anticonvulsant activity of the fractionated extract of Crinum jagus bulbs in experimental animals. Asian Pacific Journal of Tropical Disease. 2012;446-452.
4. Priyanka Chaudhary, Pramod Kumar Sharma, Anjana Sharma, Jonish Varshney. Recent advances in pharmacological activity of benzothiazole derivatives. Int J Curr Pharm Res. 2010;2(4):511.
5. Acute oral toxicity-Fixed Dose Procedure. OECD guidelines 420 for testing of chemicals; 2001.
6. Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther. 1952;106:319-330.
7. Monocha A, Sharma KK, Mediratta PK. Possible mechanism of anticonvulsant effect of ketamine in mice. Indian Journal of Experimental Biology. 2001;39:1002-8.
8. Ngo Bum E, Ngoupaye GT, Talla E, Dimo T, Nkantchoua GCN, Pelankenand MM, Taiwe GS. The anticonvulsant and sedative properties of stems of Cissus quadrangularis in mice Afr. J Pharm Pharmacol. 2008;2(3):042-047.
9. Om Prakash, Jyoti2 Amit Kumar and Rajiv Gupta. Evaluation of anticonvulsant activity of Artocarpus heterophyllus Lam. leaves (Jackfruit) in mice, Der Pharmacia Lettre. 2013;5(1):217-220.
10. Tejas P. Gosavi, Amit D. Kandhare, Pinaki Ghosh2, Subhas L. Bodhankar. Anticonvulsantactivity of Argentum metallicum. A homeopathic preparation Der Pharmacia Lettre. 2012;4(2):626-637.
11. Meador KJ, Clin J. Psychiatry. 2003;64(8):30-34.
12. Lin Z, Kadaba PK. Med. Res. Rev. 1997;17:537-572.
13. Wagner ML. Am J. Hosp. Pharm. 1994;51:1657-1666.
14. Zaccara G, Franciotta D, Perucca E. Epilepsia. 2007;48:1223-1244.
15. Bialer M, Walker MC, Josemir MS. CNS Drugs. 2002;16:285-289.
16. Adeyemi OO, Akindele AJ, Yemintan OK, Aigbe FR, Fagbo FI. Anticonvulsant, anxiolytic and sedative activities of the aqueous root extracts of Securidaca longepedunculata Fresen. Journal of Ethnopharmacology. 2003;(130):191-95.

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