

Synthesis, Characterization and Anticonvulsant Activity of Some Novel 4, 5-Disubstituted-1, 2, 4-Triazole Derivatives

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ABSTRACT

The current therapy of epilepsy is associated with a number of side effects including sedation and hypnosis. Newer improved molecules are needed with lesser side effects and with improved physical properties. In view of these facts, a series of novel 4, 5-disubstituted-1,2,4-triazoles (6a-o) were synthesized on refluxing hydrazinecarbothiomides with an aqueous solution of sodium hydroxide starting from methyl-4-hydroxy benzoate via synthesis of an intermediate methyl 3-amino-4-hydroxybenzoate, methyl 2-substitutedphenyl-1,3-benzoxazole-5-carboxylates and 2-substitutedphenyl-1,3-benzoxazole-5-carbohydrazides. The structure of synthesized compounds was confirmed on the basis of their elemental analysis and spectral data results. All these compounds were screened for anticonvulsant activity using Maximal Electroshock and subcutaneous pentylenetetrazole method. Among the tested compounds 6g, 6h and 6m showed potent activity comparable to that of standard drugs phenytoin and carbamazepine. Compounds 6g, 6h, 6i, 6k and 6m successfully passed the rotarod test without any sign of neurological deficit.

INTRODUCTION

Epilepsy is not a disease, but a syndrome of different cerebral disorders of central nervous system, and it is characterized by paroxysmal, excessive and hypersynchronous discharges of large numbers of neurons (McNamara, 1999; Kaushik *et al.*, 2010). Being one of the world's oldest recognized disorders, it is surrounded by fear, discrimination, social and frightening manifestation (Daras *et al.*, 2007). In fact, epilepsy is the second most prevalent neurological disorder after stroke in the industrial world (Korczyń *et al.*, 2015). It is one of the most common neurological disorders, affecting 0.5-1% of the population worldwide (45-100 million people)

(Njamnshi *et al.*, 2010; Blum, 1998; Bell and Sander, 2002; Husain *et al.*, 2011; Wlaz and Loscher, 1998; Scheurer and Pedley, 1990). Every year approximately 250000 new cases are added to this figure (Siddiqui *et al.*, 2007). Many drugs have been marketed recently for the treatment of epilepsy (Sabers and Gram, 2000; Britton and So, 1995; Loscher, 1998).

Several newer antiepileptic drugs (such as pregabalin, stiripentol, zonisamide, tiagabine, lamotrigine, levetiracetam, topiramate) are greatly compromised by severe side effects such as vertigo, ataxia, headache, hirsutism, hepatotoxicity, gastrointestinal and cardiovascular. Moreover about 30% of patients have uncontrolled seizures (Kwan and Brodie, 2000; Spear, 2001). The insufficient information on the cellular mechanism of epilepsy in humans and the complex mechanism of action of most of the antiepileptic drugs makes it difficult to use rational methodologies in the field of drug discovery. There is a substantial need for the development of new, more effective and less toxic antiepileptic drugs (Smith *et al.*, 2007).

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The literature survey revealed that the anticonvulsant activity is mainly attributed due to the presence of aryl binding site (A) with aryl/alkyl hydrophobic group, hydrogen bonding domain (HBD) and electron donor group (D) (Dimmock *et al.*, 2000a,b). Pandeya *et al.*, (2002) while investigating the semicarbazone series, proposed a new pharmacophore model with four binding sites essential for anticonvulsant activity (Figure 1). These sites are:

- A hydrophobic aryl ring,
- A hydrogen-bonding domain,
- An electron donor acceptor system,
- Another hydrophobic aryl ring responsible for metabolism

These groups were found in the structures of well-established antiepileptics such as phenytoin, albutoin, rufinamide, phenobarbital, diazepam, carbamazepine and lamotrigine (Figure 2) (Unverferth *et al.*, 1998).

Therefore, continued search for novel antiepileptic drugs with less toxicity and more selectivity continues to be an

area of investigation in the field of medicinal chemistry. Many studies revealed that the triazole ring is an important lead moiety in the field of agriculture, microbiology and medicine which exhibits a broad spectrum of biological activities such as anticancer (Li *et al.*, 2009), anti-inflammatory (Salgin-Goksen *et al.*, 2007; Kumar *et al.*, 2008), analgesic (Salgin-Goksen *et al.*, 2007), antimicrobial (Gumrukcuoglu *et al.*, 2007), antitubercular (Klimesova *et al.*, 2004), bactericidal (Guzeldemirci *et al.*, 2010), fungicidal (Siddiqui *et al.*, 2005), insecticidal (Chai *et al.*, 2003), herbicidal (Ma *et al.*, 2006) and CNS stimulant (Nagai *et al.*, 1998) activities. Literature survey reveals that 4, 5-disubstituted-2,4 dihydro-3H-1,2,4-triazole derivatives have not been paid much attention for their anticonvulsant properties.

These exciting manifold activities of triazole derivatives stimulated us to synthesize a series of 4, 5-disubstituted-2,4 dihydro-3H-1,2,4-triazole derivatives for their potential antiepileptic properties by using pharmacophoric features with aromatic hydrophobic aryl (A), NH-C=S as hydrogen bonding domains (HBD), nitrogen atom as electron donor (D) and phenyl as distal aryl ring (C) (Figure 2).

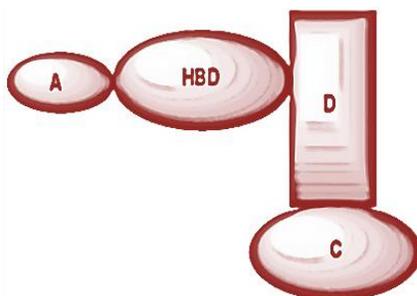


Fig. 1: Suggested pharmacophore model for anticonvulsant activity. A: hydrophobic domain, HBD: hydrogen bonding domain, C: distal hydrophobic domain, D: electron donor moiety.

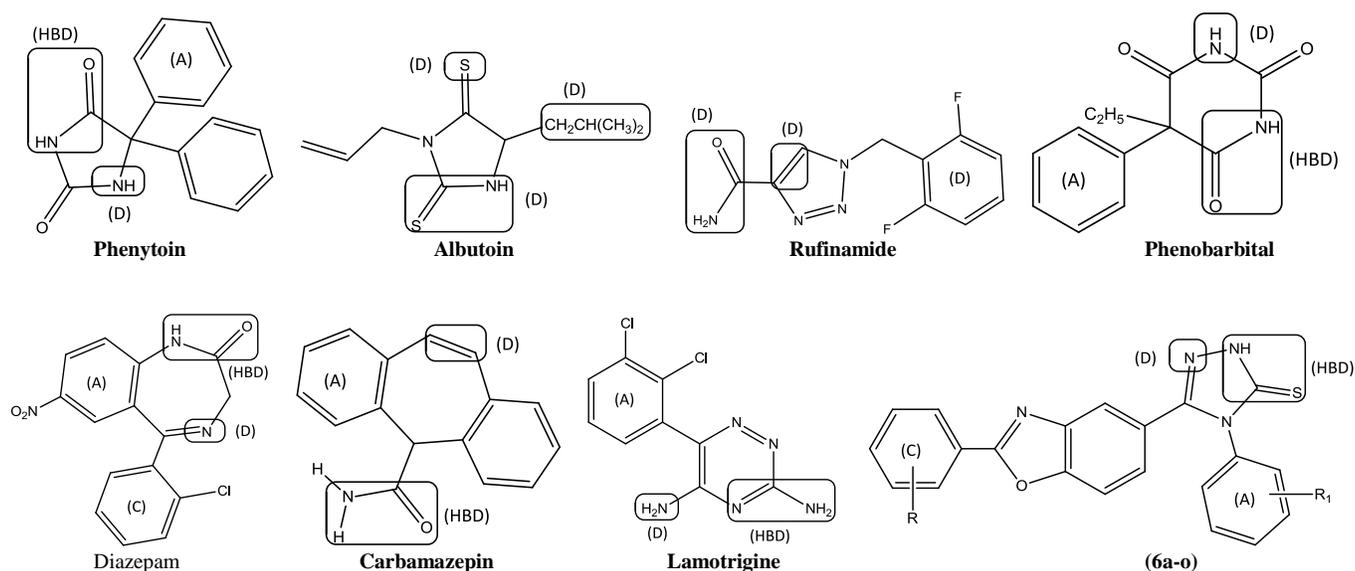


Fig. 2: Structure of proposed general pharmacophore model of the synthesized compound and reported chemical drugs.

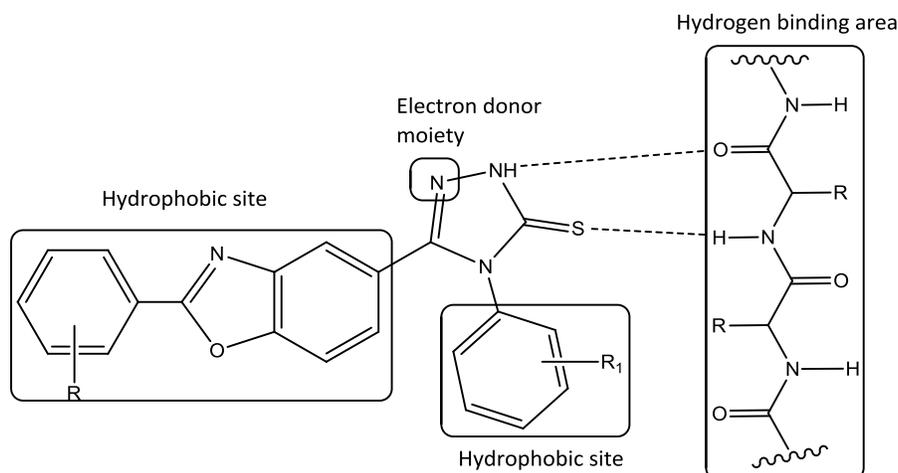


Fig. 3: Proposed binding interactions of the title compounds using putative binding site theory (Dimmock *et al.*, 2000a, b; Gibson *et al.*, 2009; Pandeya *et al.*, 2002).

Table 1: Physicochemical parameters of synthesized compounds (6a-o).

CodeNo.	R	R ¹	Mol. formula ^a	M.P. ^b (°C)	% yield	Log P ^c	R _f ^d Value
6a	C ₆ H ₅	2-CH ₃ C ₆ H ₄	C ₂₂ H ₁₆ N ₄ OS	100-102	40	0.71	0.50
6b	C ₆ H ₅	3-CH ₃ C ₆ H ₄	C ₂₂ H ₁₆ N ₄ OS	115-117	55	0.80	0.61
6c	C ₆ H ₅	4-CH ₃ C ₆ H ₄	C ₂₂ H ₁₆ N ₄ OS	95-97	45	0.81	0.67
6d	3-ClC ₆ H ₄	2-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	120-122	65	1.95	0.79
6e	3-ClC ₆ H ₄	3-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	110-112	59	1.04	0.70
6f	3-ClC ₆ H ₄	4-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	125-127	61	1.89	0.71
6g	4-ClC ₆ H ₄	2-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	125-127	67	1.71	0.69
6h	4-ClC ₆ H ₄	3-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	140-142	79	1.86	0.56
6i	4-ClC ₆ H ₄	4-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ ClN ₄ OS	135-137	39	1.79	0.88
6j	3-BrC ₆ H ₄	2-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	145-147	48	1.98	0.71
6k	3-BrC ₆ H ₄	3-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	130-132	67	1.83	0.67
6l	3-BrC ₆ H ₄	4-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	125-127	70	0.99	0.77
6m	4-BrC ₆ H ₄	2-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	140-142	71	1.96	0.46
6n	4-BrC ₆ H ₄	3-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	145-147	56	1.87	0.61
6o	4-BrC ₆ H ₄	4-CH ₃ C ₆ H ₄	C ₂₂ H ₁₅ BrN ₄ OS	150-152	50	1.80	0.71

^aSolvent of crystallization — ethanol.

^bMelting point of the compounds at their decomposition.

^cLog P was calculated using absorbance data, chloroform / phosphate buffer at 28 °C.

^dSolvent system — benzene : acetone (8 : 2, 7 : 3 and 6 : 4), toluene : ethylacetate : formic acid (5 : 4 : 1).

Table 2: Anticonvulsant and motor impairment screening of synthesized compounds (6a-o).

Code No.	Intraperitoneal injection in mice ^a				Neurotoxicity screen ^a	
	MES screen		scPTZ screen		0.5h	4h
	0.5h	4h	0.5h	4h		
6a	—	—	—	—	×	×
6b	300	—	300	—	300	—
6c	—	—	—	—	×	×
6d	100	300	300	—	—	300
6e	300	—	—	—	300	—
6f	100	300	300	—	300	—
6g	30	300	300	—	—	—
6h	30	300	300	300	—	—
6i	100	300	—	300	—	—
6j	100	300	—	300	300	300
6k	100	300	300	—	—	—
6l	300	—	300	—	—	300
6m	30	300	—	300	—	—
6n	100	300	—	300	—	300
6o	100	300	300	300	300	300
Phenytoin ^b	30	30	—	—	100	100
Carbamazepine ^b	30	100	100	300	300	300

^aDoses of 30, 100 and 300 mg/kg were administered to mice through intraperitoneal route. The figures in the table indicate the minimum dose where by bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4 hrs after the drug administration. The dash (—) indicates an absence of activity at maximum dose administered (300mg/kg) and cross (×) denotes not tested. Propylene glycol (0.1ml, i.p.) was used as control solvent.

^bData of Phenytoin and Carbamazepine, used as standard drugs, were obtained referring, Dimmock *et al* 1995 and White *et al* 1995.

MATERIALS AND METHODS

All the chemicals and solvents used were mostly of laboratory grade obtained from Merck, CDH and SD Fine Chemicals Limited. The reactions were monitored by thin layer chromatography (TLC) using benzene: acetone (8:2, 7:3 and 6:4) and toluene: ethyl acetate: formic acid (5:4:1) as solvent systems. Iodine chamber and UV lamp were used for visualization of TLC spots. The ¹H-NMR spectra were recorded on DRX-300 NMR and BRUKER 400 Ultra Shield™ spectrometer (chemical shifts in δ, ppm) in DMSO-*d*₆ using TMS as internal reference, mass spectra were recorded on a UPLC-MS/MS (WATERS, Mass Lynx version 4.1) spectrometer and IR spectra were recorded in KBr pellets on (BIO-RADS) FTS-135 spectrometer. Microanalyses of the compounds were done on Perkin-Elmer model 240 analyzer and the values were found within ± 0.4% of the theoretical values. The melting points were determined in open glass capillary using Kjeldahl flask containing liquid paraffin and are uncorrected. The physicochemical parameters and anticonvulsant screening of the synthesized compounds are presented in Table 1 and 2 respectively.

EXPERIMENTAL PROTOCOL

Method of synthesis

Synthesis of methyl 4-hydroxy-3-nitrobenzoate (1)

To a solution of aluminium nitrate (20gm) in acetic acid: acetic anhydride (1:1) mixture (80mL) was added an appropriate methyl-4-hydroxy benzoate (20gm) in small portions, while cooling and shaking occasionally. The reaction mixture was left at room temperature for 2 hrs while shaking the contents intermittently to complete the nitration. The resulting brown solution was diluted with ice cold water (250mL) and acidified with concentrated nitric acid (20mL) to get a bulky yellow precipitate. It was filtered and washed with small quantity of methanol and purified by recrystallization from alcohol to get a yellow crystalline compound (1) (Gopalakrishna *et al.*, 2005).

Synthesis of Methyl-3-amino-4-hydroxybenzoate (2)

Methyl 4-hydroxy-3-nitrobenzoate (15gm) was dissolved in boiling ethanol (200mL) and sodium dithionite was added to this boiling alcohol until it becomes almost colorless. Then the solvent was reduced to one third of its volume by distillation and the residual liquid was triturated with crushed ice. Usual work up of the reaction mixture gave pure compound (2) (Gopalakrishna *et al.*, 2005).

General procedure for synthesis of methyl 2-substitutedphenyl-1,3-benzoxazole-5-carboxylates (3a-e)

A mixture of Methyl-3-amino-4-hydroxybenzoate (0.01mol) and an appropriate aryl acid (in excess) was refluxed for 15 hrs. The reaction mixture was cooled and poured onto the crushed ice with stirring to obtain the compounds (3a-e) (Ampati *et al.*, 2010).

General procedure for synthesis of 2-substitutedphenyl-1,3-benzoxazole-5-carbohydrazides (4a-e)

A mixture of compound (3a) (0.01mol), hydrazine hydrate (99%, 0.01mol) was heated under reflux in absolute ethanol for 15-20 hrs. The reaction mixture was cooled and the solids obtained were filtered and crystallized from ethanol to get white crystalline product (4a).

Similarly other compounds (4b-e) were also prepared by above specified method (Husain *et al.*, 2009).

General procedure for synthesis of N-substitutedphenyl-2-[(2-substitutedphenyl-1,3-benzoxazol-5-yl)carbonyl]hydrazinecarbothioamides (5a-o)

An alcoholic solution of compound (4a) (0.002mol) and substituted phenyl isothiocyanate (0.002mol) was refluxed for 2-4 hrs. The contents were concentrated and poured onto crushed ice, filtered and dried to get hydrazinecarbothioamides (5a).

Similarly other compounds (5b-o) were also prepared by above specified method (Mavrova *et al.*, 2009; Siddiqui *et al.*, 2005).

General procedure for synthesis of 4-substitutedphenyl-5-(2-substitutedphenyl-1,3-benzoxazol-5-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thiones (6a-o)

A suspension of compound (5a) (0.002mol) in ethanol (25mL) was dissolved in aqueous sodium hydroxide (8%, 20mL) and gently refluxed for 5-6 hrs. The resulting solution was concentrated, cooled and filtered. The filtrate was adjusted to pH 5-6 with dilute acetic acid and was kept aside for 1 hr. The crystals produced were filtered, washed with water, dried and recrystallized from ethanol.

The compounds (6b-o) were also synthesized by similar method using reagents in proper mole ratio (Mavrova *et al.*, 2009).

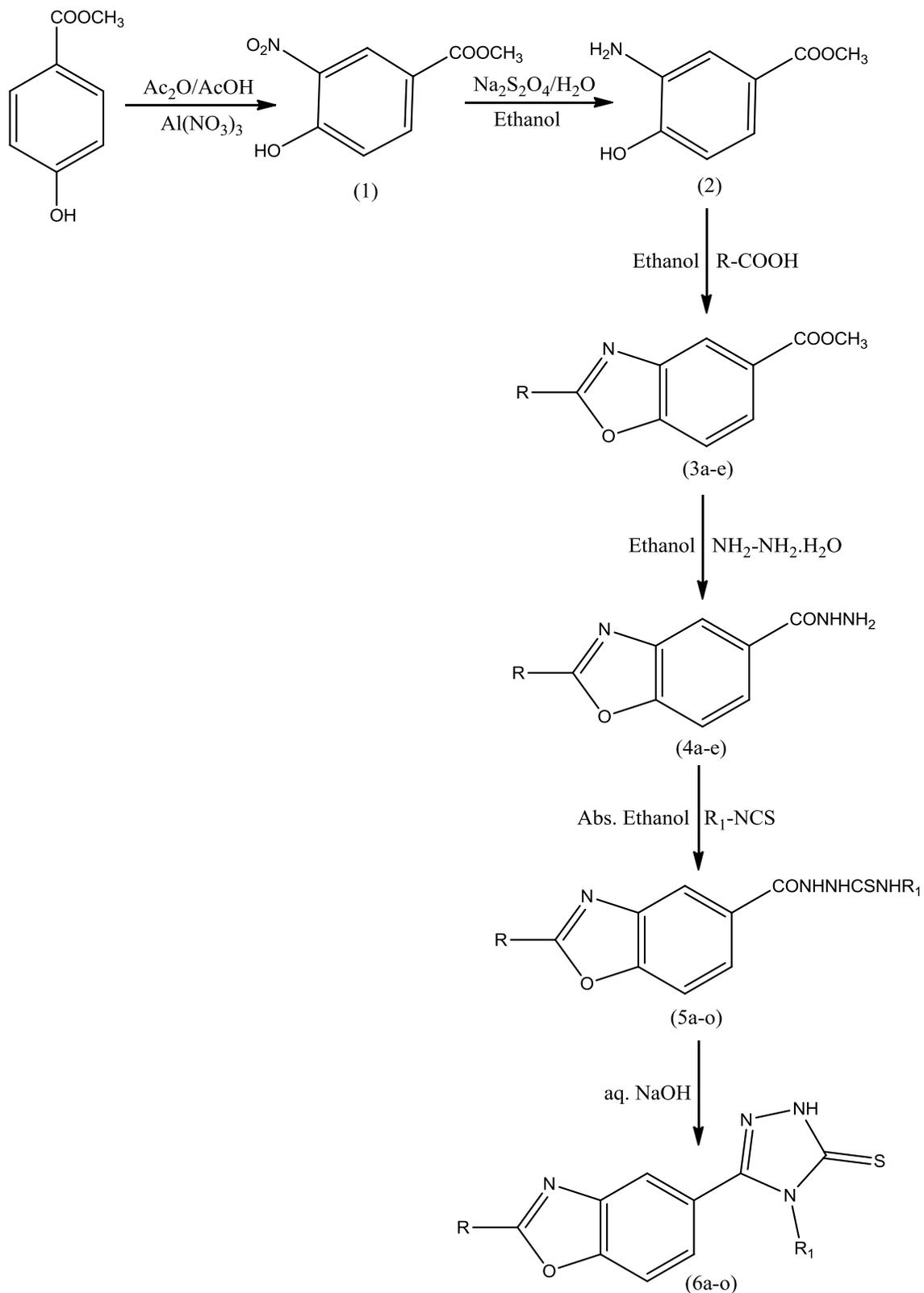
The synthetic route of the compounds is shown in Scheme 1.

4-(2-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6a)

IR (KBr, cm⁻¹): 3314 (NH), 3002 (CH), 1607 (C=N), 1177 (C=S), ¹H-NMR (DMSO-*d*₆, δ, ppm): 9.55 (s, 1H, triazole), 6.89-8.04 (m, 12H, Ar-H), 2.48 (s, 3H, CH₃), EI-MS: 385 (M+1). Anal. calcd for C₂₂H₁₆N₄OS : C, 68.76; H, 4.27; N, 14.45. Found C, 68.73; H, 4.19; N, 14.57.

4-(3-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6b)

IR (KBr, cm⁻¹): 3315 (NH), 3084 (CH), 1625 (C=N), 1182 (C=S). ¹H-NMR (DMSO-*d*₆, δ, ppm): 9.81 (s, 1H, triazole), 6.58-8.26 (m, 12H, Ar-H), 2.40 (3H, s, CH₃). Anal. calcd for C₂₂H₁₆N₄OS : C, 68.96; H, 4.01; N, 14.90; Found C, 68.73; H, 4.19; N, 14.57.



Scheme 1: Synthesis of 4, 5-disubstituted-2, 4 dihydro-3H-1, 2, 4-triazole derivatives (6a-o).

4-(4-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6c)

IR (KBr, cm^{-1}): 3314 (NH), 3045 (CH), 1631 (C=N), 1228 (C=S). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.79 (s, 1H, NH, triazole), 7.57-8.87 (m, 12H, Ar-H), 2.49 (3H, s, CH_3). EI-MS: 385 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{16}\text{N}_4\text{OS}$: C, 68.96; H, 4.01; N, 14.90. Found C, 68.73; H, 4.19; N, 14.57.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(6d)

IR (KBr, cm^{-1}): 3315 (NH), 3053 (CH), 1668 (C=N), 1237 (C=S), 740 (C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.80 (s, 1H, NH, triazole), 6.97-7.99 (m, 11H, Ar-H), 2.59 (s, 3H, CH_3). EI-MS: 419 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 62.74; H, 3.12; N, 3.66. Found C, 63.08; H, 3.61; N, 3.37.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(3-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6e)

IR (KBr, cm^{-1}): 3307 (NH), 3011 (CH), 1634 (C=N), 1243 (C=S), 734(C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.80 (s, 1H, triazole), 7.06-8.11 (m, 12H, Ar-H), 2.60 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 63.50; H, 3.76; N, 3.72. Found C, 63.08; H, 3.61; N, 3.37.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6f)

IR (KBr, cm^{-1}): 3307 (NH), 3007 (CH), 1649 (C=N), 1275 (C=S), 726 (C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.77 (s, 1H, triazole), 6.76-8.01 (m, 12H, Ar-H), 2.53 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 63.00; H, 3.21; N, 3.00. Found C, 63.08; H, 3.61; N, 3.37.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6g)

IR (KBr, cm^{-1}): 3289 (NH), 3085 (CH), 1600 (C=N), 1246 (C=S), 741 (C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.40 (s, 1H, triazole), 7.04-8.20 (m, 12H, Ar-H), 2.61 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 63.00; H, 3.21; N, 3.00. Found C, 63.08; H, 3.61; N, 3.37.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(3-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6h)

IR (KBr, cm^{-1}): 3390 (NH), 3011 (CH), 1596 (C=N), 1243 (C=S), 702 (C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.55 (s, 1H, triazole), 6.86-7.26 (m, 12H, Ar-H), 2.59 (3H, s, CH_3). EI-MS: 419 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 63.15; H, 3.98; N, 3.74. Found C, 63.08; H, 3.61; N, 3.37.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6i)

IR (KBr, cm^{-1}): 3330 (NH), 3058 (CH), 1633 (C=N), 1243 (C=S), 736 (C-Cl). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.41 (s, 1H, triazole), 7.00-7.98 (m, 12H, Ar-H), 2.88 (3H, s, CH_3). Anal. calcd

for $\text{C}_{22}\text{H}_{15}\text{ClN}_4\text{OS}$: C, 63.45; H, 3.77; N, 3.66. Found C, 63.08; H, 3.61; N 3.37.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-4-(2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione(6j)

IR (KBr, cm^{-1}): 3327 (NH), 3017 (CH), 1574 (C=N), 1234(C=S), 539 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.44 (s, 1H, triazole), 6.77-7.04 (m, 12H, Ar-H), 2.34 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 56.89; H, 3.09; N, 12.45. Found C, 57.03; H, 3.26; N, 12.09.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-4-(3-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6k)

IR (KBr, cm^{-1}): 3364 (NH), 3092 (CH), 1595 (C=N), 1248 (C=S), 533 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.00 (s, 1H, triazole), 6.99-8.00 (m, 12H, Ar-H), 2.77 (3H, s, CH_3). EI-MS: 464 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 56.43; H, 3.44; N, 11.90. Found C, 57.03; H, 3.26; N 12.09.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6l)

IR (KBr, cm^{-1}): 3316 (NH), 3088 (CH), 1527 (C=N), 1249 (C=S), 563 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.81 (s, 1H, triazole), 6.58-8.26 (m, 12H, Ar-H), 2.48 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 56.71; H, 3.35; N, 12.40. Found C, 57.03; H, 3.26; N, 12.09.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-4-(2-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6m)

IR (KBr, cm^{-1}): 3319 (NH), 2980 (CH), 1593 (C=N), 1212 (C=S), 533 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.51 (s, 1H, triazole), 6.10-8.01 (m, 12H, Ar-H), 2.55 (3H, s, CH_3). EI-MS: 464 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 57.39; H, 2.90; N, 12.18. Found C, 57.03; H, 3.26; N 12.09.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-4-(3-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6n)

IR (KBr, cm^{-1}): 3318 (NH), 3029 (CH), 1599 (C=N), 1212 (C=S), 547 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.43 (s, 1H, triazole), 6.07-7.97 (m, 12H, Ar-H), 2.57 (3H, s, CH_3). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 57.29; H, 3.56; N, 11.79. Found C, 57.03; H, 3.26; N, 12.09.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-4-(4-methylphenyl)-2,4-dihydro-3H-1,2,4-triazole-3-thione (6o)

IR (KBr, cm^{-1}): 3380 (NH), 3056 (CH), 1600 (C=N), 1244 (C=S), 533 (C-Br). $^1\text{H-NMR}$ (DMSO- d_6 , δ , ppm): 9.66 (s, 1H, triazole), 6.14-8.11 (m, 12H, Ar-H), 2.71 (3H, s, CH_3). EI-MS: 464 (M+1). Anal. calcd for $\text{C}_{22}\text{H}_{15}\text{BrN}_4\text{OS}$: C, 56.89; H, 3.01; N, 12.33. Found C, 57.03; H, 3.26; N 12.09.

ANTICONVULSANT SCREENING

The anticonvulsant screening of the synthesized compounds were performed according to the standard protocol

provided by epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by Antiepileptic Drug Development (ADD) program (Krall *et al.*, 1978; Stables *et al.*, 1997; Kupferberg *et al.*, 1998). Swiss albino mice (20-25gm) of either sex were used as experimental animals. All experimental protocols were carried out with permission from the Institutional Animal Ethics Committee (IAEC).

Animals were obtained from the Central Animal House Facility, Jamia Hamdard University, New Delhi, India. The mice were kept under standard conditions at an ambient temperature of $25 \pm 2^\circ\text{C}$ and allowed free access to food and water except at the time they were brought out of the cage. The synthesized compounds were suspended in polyethylene glycol (PEG-400).

Maximal electroshock test (MES)

The anticonvulsant activity in MES test was indicated by the lower dose which protected the hind limb tonic extension in more than half of the animals. Each animal received i.p. injection of the test compounds (30/100/300 mg/kg) followed by electroshock with 60 Hz of 50 mA for 0.2sec via ear clip electrode through electroconvulsometer as per the reported procedure and activity was assessed at 0.5 and 4 hrs after administration (Krall *et al.*, 1978; Porter *et al.*, 1984).

Subcutaneous pentylenetetrazole seizure test (scPTZ)

The subcutaneous pentylenetetrazole test was performed according to the known protocol (Swinyard *et al.*, 1989; Chen *et al.*, 2007; Kucukguzel *et al.*, 2004). Subcutaneous injection of the pentylenetetrazole produces clonic seizures in laboratory animals. It detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals were pretreated with various doses of the test compound given by i.p. injection. The dose of pentylenetetrazole (75mg/kg) which induces convulsions in >95% of animals is injected into a loose fold of the skin in the midline of the neck. The animals were placed in isolation cages to minimize stress and observed for the next 30 min for the presence or absence of a seizure. Failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 sec duration) was defined as protection.

Neurotoxicity screening

To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotarod procedure (Dunham and Miya, 1957; Kucukguzel *et al.*, 2004) is used to disclose minimal muscular or neurological impairment. The mice were trained to stay on an accelerating rotarod that rotated at 6 rpm and its diameter was 3.2 cm. Only those mice were taken for the test which could stay on the revolving rod for at least one minute. Trained animals were injected i.p. with the test compounds at doses of 300 mg/kg. The inability of the animal to maintain

equilibration on the rod for at least one minute indicated neurotoxicity.

Log P determination

Log P (partition coefficient) is an imperative physicochemical marker of drug permeability across the blood brain barrier for an inadequate drug concentration in crucial brain areas (Kwan and Brodie, 2005). Pharmacological activity is dependent on the lipophilic character of the drug. Anticonvulsant activities of different type of compounds were correlated with lipophilicity (Lien *et al.*, 1979). However, it has been observed that the maximum potency of the drugs which act on the central nervous system is obtained with congeners having an optimum lipophilicity (log P) near 2. In general the optimal hydrophobicity (log P \approx 2) of the molecules is essential for anticonvulsant activity without any neurotoxicity. Therefore, partition coefficient of all the compounds were determined by the procedure described in the literature (Farrar *et al.*, 1993) and to establish the correlation between log P and anticonvulsant activity.

RESULTS AND DISCUSSION

The synthetic route used to synthesize title compounds is outlined in Scheme 1. The methyl- 3-amino-4-hydroxybenzoate (2) was prepared according to the method reported in the literature (Gopalakrishna *et al.*, 2005). The methyl-3-amino-4-hydroxybenzoate underwent cyclization with an appropriate aryl acids to afford methyl-2-substitutedphenyl-1,3-benzoxazole-5-carboxylates (3a-e). Furthermore, reaction of methyl 2-substitutedphenyl-1,3-benzoxazole-5-carboxylates with hydrazine hydrate followed by treatment with substituted arylisothiocyanates resulted in the formation of hydrazinecarbothioamides (5a-o). Finally, hydrazinecarbothioamides were cyclized with an aqueous solution of sodium hydroxide to give the titled compounds (6a-o), was confirmed by appearance of a singlet at around 9.80 ppm due to -NH proton of triazole ring and absence of a singlet at around 10.27 and 8.67 ppm attributed to -CONH- and -NHCSNH- protons of hydrazinecarbothioamides respectively. In all the cases the TLC of the product showed the single spot confirming the chromatogram for only one product. The chemical structures of the synthesized compounds were established by elemental analysis and spectral data results are reported in experimental protocols. The elemental analysis results were within $\pm 0.4\%$ of the theoretical values.

The preliminary anticonvulsant activity of the target compounds (6a-o) were determined according to the phase I tests of the Antiepileptic Drug Development (ADD) program. ADD program was developed by National Institute of Neurological Disorders and Stroke (NINDS) and it includes the subcutaneous pentylenetetrazole (Swinyard *et al.*, 1989) and themaximal electroshock seizure screen (Porter *et al.*, 1984). scPTZ and MES screens are considered as the "gold standard" seizure model screens where they are used to identify compounds that elevate seizure threshold and to indicate the ability of the test prevent

seizure spread, respectively. Additionally, acute toxicity from antiepileptic drugs in rodents is almost invariably manifested by neurological deficits. These include sedation, altered motor activity, ataxia, and impaired righting reflexes. These effects of antiepileptic drugs are often summarized by the term "neurotoxicity." Minimal neurological deficit, such as impaired motor function, can be detected by standardized test, that is, by the rotarod test (Dunham and Miya, 1957). Data is presented in Table 2 after the 0.5 and 4 hrs time intervals at the dose level of 30, 100 and 300 mg/kg. Phenytoin and carbamazepine were used as the standard drugs for the comparison.

Maximal electroshock seizure test is a proven method to check the hind limb tonic extension seizure and identifies clinical candidates that prevent seizure spread. All the compounds except 6a and 6c were found to exhibit protection in both MES and scPTZ tests making them useful for broad spectrum of seizure types. Compounds that showed protection against MES model at 100 mg/kg include 6d, 6f, 6i, 6j, 6k, 6n and 6o. Compounds 6d, 6f, 6g, 6h, 6i, 6j, 6k, 6m, 6n and 6o showed activity both at 0.5 and 4.0 hrs. Thus, only three compounds 6g, 6h and 6m showing activity at a lower dose of 30 mg/kg seems to be very potent in anticonvulsant MES screening. Some of the compounds showed activity only at 0.5 h, indicating that they have rapid onset and shorter duration of action.

Insc PTZ screening, all the compounds except 6a, 6c and 6e showed activity indicative of their ability to prevent seizure spread. Compounds 6b, 6d, 6f, 6g, 6k and 6l showed 100% protection at a dose of 300 mg/kg at 0.5 h. So these compounds have quick onset but for shorter duration of action. Some compounds (6i, 6j, 6m and 6n) were also active after 4.0 hrs extended period of activity. Only two compounds 6h and 6o showed activity at the dose level of 300 mg/kg at both time intervals.

In the neurotoxicity screening, compounds 6g, 6h, 6i, 6k and 6m do not show any toxicity at the dose of 300 mg/kg. Compounds 6j and 6o were toxic at 0.5 and 4.0 hrs., whereas three compounds 6b, 6e and 6f showed toxicity after 0.5 h and do not show toxicity after 4.0 hrs. Three compounds (6d, 6l and 6n) showed delayed toxicity i.e., toxicity only after 4.0 hrs, which is comparable with that of carbamazepine (300mg/kg). However, all the compounds were less toxic than phenytoin (100mg/kg). There have been several attempts to provide insight into pharmacophore modeling of the putative MES receptor showing several common structural features essential for activity. Although, the chemical diversity and various mechanisms of action of anticonvulsants make it difficult to identify a common pharmacophore, the essential structural elements in the four-point pharmacophore model were assumed as lipophilic aryl ring center (A), =N- as an electron donor atom (D), -NHC=S as a hydrogen-bonding domain (HBD) and another hydrophobic aryl ring (C) responsible for metabolism (Figure 2) (Unverferth *et al.*, 1998). All the molecules are 3D optimized and were conformers which bring the above-mentioned groups closely together. Partition coefficient is an imperative physicochemical marker of drug permeability across

the blood brain barrier (BBB) for an inadequate drug concentration in crucial brain areas (Kwan and Brodie 2005). Therefore, partition coefficients of all the compounds were determined to establish the correlation between log P and anticonvulsant activity. Compounds 6d, 6f, 6g, 6h, 6i, 6j, 6k, 6m, 6n and 6o were found to be more lipophilic having potent anticonvulsant activity. The other compounds 6e and 6l were also lipophilic having some potency. Compounds 6a, 6b and 6c were less lipophilic and were less active in MES test.

On correlating the structures of the sample candidate with their biological activities, it has been observed that, out of various phenyl substituted derivatives, three compounds (6g, 6h and 6m) have significance toward both MES and scPTZ activities (30 and 300mg/kg). The nature of the substituted group on the phenyl ring appeared to greatly influence the antiepileptic activity. On analyzing the antiepileptic activity of all the compounds, the following SAR was gained. Presence of halogens at para position greatly increased antiepileptic activity. Among halogen analogs chloro derivatives exhibited better activity followed by bromo, whereas the presence of a methyl substituent showed moderate activity and unsubstituted derivatives exhibited least activity.

CONCLUSION

A series of 4, 5-disubstituted-1,2,4-triazoles were synthesized and characterized by spectral techniques. The compounds were subjected to antiepileptic screening by standard methods with drug phenytoin and carbamazepine as standards. All the compounds except 6a and 6c were found to exhibit protection in both MES and scPTZ tests making them useful for broad spectrum of seizure types. The triazole derivatives displayed moderate to good anticonvulsant activity. Here the activity is attributed to the presence of favorable structural environment such as aryl binding site with a hydrophobic group, hydrogen bonding domain group, electron donor, electron withdrawing group and another hydrophobic aryl ring. Rather increase in the hydrophobicity in the synthesized molecules brings about same degree of activity in the series. The compounds 6g, 6h and 6m found to be the most promising analogs displaying protection in MES model without neurotoxicity and emerged as lead in these series. Further, the 6d, 6f, 6i, 6j, 6k, 6n and 6o come out as potential candidates for further investigation. Some compounds (6d, 6f, 6g, 6h, 6i, 6j, 6k, 6m, 6n and 6o) showed more lipophilic character and were more active. The compounds 6e and 6l were also lipophilic but were less active in MES test. Finally, it can be readily conclude that the substitution pattern in the phenyl ring influences the activity as well astoxicity of the different substituted triazoles.

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