Research Article

Anticonvulsant and Neurotoxicity Evaluation of Some Fused 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole Derivatives

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ABSTRACT

Keeping in view the structural requirements suggested in the pharmacophore model for anticonvulsant activity, a new series of 3,6-disubstituted-1,2,4-triazolo-1,3,4-thiadiazole derivatives (6a-z) were synthesized with aromatic hydrophobic aryl ring (A), –O– as hydrogen bonding domain (HBD), nitrogen atom as electron donor (D), and phenyl as distal aryl ring (C). Synthesized compounds were characterized on the basis of their elemental analysis and spectral data results. Preliminary in vivo anticonvulsant screening was performed by two most adopted seizure models, maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (scPTZ). Based on anticonvulsant screening results, only two compounds, IVg and IVm were found to be most active; they exhibited activity comparable to standard drugs phenytoin and carbamazepine. Compounds IVb, IVc, IVk, IVl, IVm and IVn successfully passed the rotorod test without any sign of neurological deficit.

Keywords: Fused triazolothiadiazoles, triazole, anticonvulsants, neurotoxicity, lipophilicity.

INTRODUCTION

Epilepsy is the name of a brain disorder characterized predominantly by recurrent and unpredictable interruptions of normal brain function. It is a common neurological condition, affecting 0.5-1% of the population worldwide and is a main cause of disability and mortality.[1-6] Every year about 2.4 million new cases are added to these figures. [7-8] Studies have reported that more than 10 million people in India are afflicted with epilepsy[9]. The prevalence of epilepsy is higher in the rural (1.9%) as compared with the urban population (0.6%)[10-11]. It is roughly estimated that 75-80% of epileptic patients provided with adequate seizure control with available AEDs. The therapeutic failure in 20-25% of patients and serious side effects in the available AEDs have stimulated intensive search on novel AEDs[12-13].

The fused triazolothiadiazole nucleus is incorporated in wide variety of therapeutically agents, such as anti-inflammatory[14-16], antimicrobial[17-18] and anticancer[19-20]. Although the current drugs provide adequate seizure control in many patients, it is roughly estimated that up to 28-30% of patients are poorly treated with the available AEDs[21-22]. Many AEDs have serious side effects like neurotoxicity, depression, other CNS related diseases, cosmetic (gingival hyperplasia) to life threatening (hepatotoxicity, megaloblastic anemia)[23-29] and lifelong medication may be required. Toxicity, intolerance, and lack of efficacy are the limitations of the current AEDs. Therefore, the continued search for safer and more effective new AEDs is necessary[30]. In recent years, the field of antiepileptic drug development (ADD) has become quite dynamic, affording many promising research opportunities, and there is a continuing demand for new anticonvulsant agents as it has not been possible to control every kind of seizure with currently available antiepileptic drugs.

Literature survey showed that fused triazolothiadiazole, a heterocyclic compound of varied biological activities were found to be one of the new classes of anticonvulsant effects during its initial screening in the MES test[31]. From the study of structures of clinically established drugs, it can be concluded that the anticonvulsant properties have been displayed due to the presence of at least one hydrophobic unit, one or two electron donor atoms, and/or an NH group[32-35]. It was deduced recently that the requirement for anticonvulsant activity includes (Figure 1)[36]

1. An aryl hydrophobic binding site (A) with halogen substituent preferably at para position
2. A hydrogen bonding domain (HBD)
3. An electron donor group (D)
4. Another hydrophobic-hydrophilic site controlling the pharmacokinetic properties of the anticonvulsant (C).

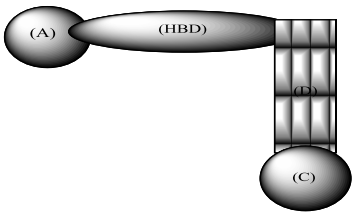
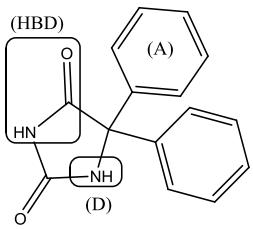


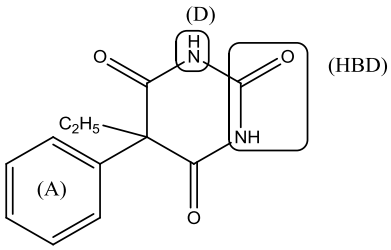
Figure 1: Suggested pharmacophore model for anticonvulsant activity. Hydrophobic domain (A), hydrogen bonding domain (HBD), distal hydrophobic domain (C) and electron donor moiety (D).

The prime need was to search for a new molecule that could complement all the above pharmacophores in one.

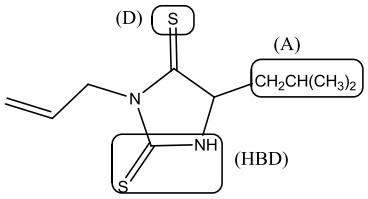
In view of these general requirements for activity; we have design and synthesize the first combination of triazolothiadiazole as a basic nucleus incorporated with substituted benzoxazole moiety within a single molecule. The titled compounds possessed all the required pharmacophoric elements (Figure 2) as the benzoxazole have aryl ring can be referred to the aryl ring (A), the –N- of the triazolothiadiazole can act as a electron donor system (D) and the –O– of the benzoxazole ring constitute the HBD unit. Benzoxazole[37] and triazolothiadiazole nucleus[31] both have anticonvulsant properties separately. Our research on benzoxazole nucleus as anticonvulsant agents encourage us to synthesizing newer derivatives that contain substituted benzoxazole nucleus and fused 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole in the same compound. Such combination is hoped to develop compounds with increased lipophilic character having potential anticonvulsant activity.



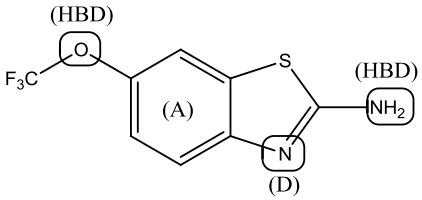
Phenytoin



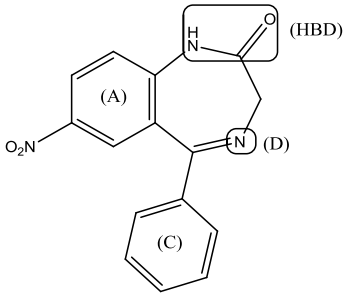
Phenobarbital



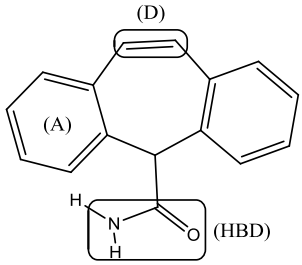
Rufinamide



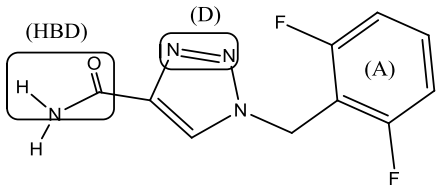
Riluzole



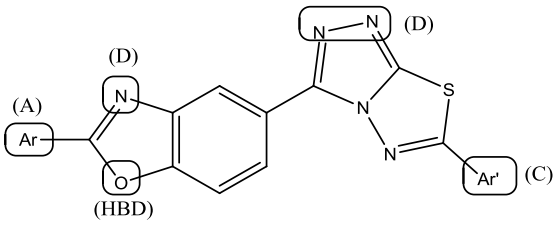
Diazepam



Carbamazepine



Rufinamide



(IVa-n)

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

MATERIALS AND METHODS

Chemistry

All the chemicals and solvents used were mostly of AR grade obtained from Spectrochem, Merck, CDH and S.D. Fine Chem. Ltd. The melting points were determined in open glass capillary tubes using Kjedahl flask containing liquid paraffin and are uncorrected. Thin layer chromatography (TLC) was carried out using Silica gel G (Merck) and the solvent system benzene-acetone (9:1 and 8:2, v/v) and toluene-ethylacetate-formic acid (TEF) (5:4:1, v/v/v); the spots were visualized under iodine vapors or UV light. Microanalysis of the compounds was done on Perkin-Elmer model 240 analyzer and the values were found within ±0.4% of the theoretical values. The FT-IR Spectra were recorded in KBr pellets on (BIO-RAD FTS), FT-IR spectrophotometer. ^{1}H-NMR spectra were recorded on DPX-300 NMR spectrometer and BRUKER-400 Ultra Shield™ spectrometer. Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS). Mass spectrometry was recorded on UPLC-MS/MS (WATERS, Mass Lynx version 4.1) spectrometer. The spectral data of the synthesized compounds are presented in experimental protocols. The

physicochemical parameters and anticonvulsant screening of the synthesized compounds are presented in Table 1 and 2 respectively.

Synthesis

Synthesis of methyl 2-(3-chlorophenyl)-1, 3-benzoxazole-5-carboxylate and their hydrazide (I)

These compounds were obtained from 4-carbomethoxy-2-aminophenol by the method reported in the literature[37,38].

Synthesis of potassium 2-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]carbonylhydrazine dithiocarbazate (II)

Potassium hydroxide (0.03 mol) was dissolved in absolute ethanol (50 mL). The solution was cooled in an ice bath and acid hydrazide **I** (0.02 mol) was added with stirring. To this, carbon disulfide (0.025 mol) was added in small portions with constant stirring. The reaction mixture was stirred continuously for 12 hrs at room temperature. The precipitated potassium dithiocarbazate was collected by filtration, washed with anhydrous ether and dried in vacuum. The potassium salt thus obtained was used in the next step without further purification[31].

Synthesis of 4-amino-5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-4*H*-1,2,4-triazole-3-thiol (III)

A suspension of compound **II** (0.02 mol) and hydrazine hydrate (0.04 mol) in water (50 mL) was refluxed for 10-15 hrs with occasional shaking. The color of the reaction mixture changed to light green with evolution of hydrogen sulfide gas. A homogenous mixture was obtained during the reaction process. The reaction mixture was cooled to room temperature and diluted with cold water (20 mL). On acidification with dil. HCl a white precipitate was obtained. It was filtered, washed with cold water, dried and recrystallized from ethanol. The compound was found pure in TLC analysis using toluene: ethylacetate: formic acid (5:4:1, v/v/v) as solvent system[14,31].

General procedure for synthesis of 3,6-disubstituted-[1,2,4]-triazolo-[3,4-*b*]-1,3,4-thiadiazoles (IVa-n)

An equimolar mixture of **III** (0.01 mol) and aromatic acids in phosphorous oxychloride (10 mL) was refluxed for 5 hrs. After completion of reaction, the reaction mixture was cooled to room temperature and then poured onto crushed ice with stirring. The mixture was allowed to stand overnight and a solid mass separated out was filtered, treated with aq. ammonia, washed with cold water, dried and crystallized from ethanol to get crystalline solid **IVa**.

The compounds **(IVb-n)** were also synthesized by similar method using reagents in proper mole ratio[31].

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

2-(3-chlorophenyl)-5-(6-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl)-1,3-benzoxazole (IVa)

IR (KBr, cm^{-1}): 3063 (C-H), 1622 (C=N), 1458 (C-N), 1223 (N-N=C), 745 (C-Cl), 714 (C-S-C). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.04-8.14 (12H, complex, m, Ar H). MS: m/z 429 (M^+), (M^+ +1), (M^+ +2). Elemental analysis: Calcd for $C_{22}H_{12}ClN_5OS$: C, 61.18; H, 3.01; N, 15.83% Found: C, 61.47; H, 2.81; N, 16.29%.

2-(3-chlorophenyl)-5-[6-(2-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVb)

IR (KBr, cm^{-1}): 3011 (C-H), 1631 (C=N), 1452 (C-N), 1212 (N-N=C), 766 (C-Cl), 710 (C-S-C). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.04-8.14 (11H, m, Ar H). Elemental analysis: Calcd for $C_{22}H_{11}Cl_2N_5OS$: C, 56.88; H, 2.11; N, 15.23% Found: C, 56.91; H, 2.39; N, 15.08%.

2-(3-chlorophenyl)-5-[6-(4-chlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVc)

IR (KBr, cm^{-1}): 3034 (C-H), 1645 (C=N), 1459 (C-N), 1220 (N-N=C), 733 (C-Cl), 699 (C-S-C). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.95-8.03 (11H, m, Ar H). MS: m/z 464 (M^+). Elemental analysis: Calcd for $C_{22}H_{11}Cl_2N_5OS$: C, 56.60; H, 2.11; N, 15.23% Found: C, 56.91; H, 2.39; N, 15.08%.

2-(3-chlorophenyl)-5-[6-(2,6-dichlorophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVd)

IR (KBr, cm^{-1}): 3010 (C-H), 1601 (C=N), 1489 (C-N), 1258 (N-N=C), 758 (C-Cl), 692 (C-S-C). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.20-8.11 (10H, m, Ar H). MS: m/z 498 (M^+), (M^+ +1). Elemental analysis: Calcd for $C_{22}H_{10}Cl_3N_5OS$: C, 53.11; H, 2.01; N, 14.21% Found: C, 52.98; H, 2.02; N, 14.04%.

5-[6-(2-chlorobenzyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-2-(3-chlorophenyl)-1,3-benzoxazole (IVe)

IR (KBr, cm^{-1}): 3017 (C-H), 1644 (C=N), 1431 (C-N), 1265 (N-N=C), 700 (C-Cl). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.95-8.03 (11H, m, Ar H), 2.15-2.29 (2H, m, CH_2). MS: m/z 478 (M^+), (M^+ +1), (M^+ +2). Elemental analysis: Calcd for $C_{23}H_{13}Cl_2N_5OS$: C, 57.90; H, 3.01; N, 14.83% Found: C, 57.75; H, 2.74; N, 14.64%.

5-[6-(2-bromophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-2-(3-chlorophenyl)-1,3-benzoxazole (IVf)

IR (KBr, cm^{-1}): 3011 (C-H), 1631 (C=N), 1452 (C-N), 1213 (N-N=C), 766 (C-Cl), 710 (C-S-C), 545 (C-Br). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.95-8.03 (11H, m, Ar H). MS: m/z 508 (M^+), (M^+ +1), (M^+ +2). Elemental analysis: Calcd for $C_{22}H_{11}BrClN_5OS$: C, 52.07; H, 1.96; N, 14.03% Found: C, 51.94; H, 2.18; N, 13.77%.

5-[6-(3-bromophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-2-(3-chlorophenyl)-1,3-benzoxazole (IVg)

IR (KBr, cm^{-1}): 3011 (C-H), 1631 (C=N), 1452 (C-N), 1226 (N-N=C), 766 (C-Cl), 710 (C-S-C), 545 (C-Br). ^1H NMR (DMSO-d_6, δ, ppm): 7.12-8.21 (11H, m, Ar H). Elemental analysis: Calcd for $C_{22}H_{11}BrClN_5OS$: C, 52.07; H, 1.96; N, 14.03% Found: C, 51.94; H, 2.18; N, 13.77%.

5-[6-(4-bromophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-2-(3-chlorophenyl)-1,3-benzoxazole (IVh)

IR (KBr, cm^{-1}): 3011 (C-H), 1631 (C=N), 1452 (C-N), 1244 (N-N=C), 766 (C-Cl), 710 (C-S-C), 545 (C-Br). ^1H NMR (DMSO-d_6, δ, ppm): 6.98-8.01 (11H, m, Ar H). MS: m/z 508 (M^+), (M^+ + 1). Elemental analysis: Calcd for $C_{22}H_{11}BrClN_5OS$: C, 52.07; H, 1.96; N, 14.03% Found: C, 51.94; H, 2.18; N, 13.77%.

2-{3-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl} aniline (IVi)

IR (KBr, cm^{-1}): 3277 (N-H), 3018 (C-H), 1617 (C=N), 1420 (C-N), 1237 (N-N=C), 718 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 6.89-8.09 (11H, m, Ar H), 5.98 (2H, s, NH_2). MS: m/z 444 (M^+), (M^+ +1). Elemental analysis: Calcd for $C_{22}H_{13}ClN_6OS$: C, 59.18; H, 3.12; N, 18.77% Found: C, 59.39; H, 2.95; N, 18.89%.

4-{3-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl} aniline (IVj)

IR (KBr, cm^{-1}): 3288 (N-H), 3046 (C-H), 1608 (C=N), 1488 (C-N), 1246 (N-N=C), 736 (C-Cl), 711 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 6.60-7.97 (11H, m, Ar-H), 6.04 (2H, s, NH_2). Elemental analysis: Calcd for $C_{22}H_{13}ClN_6OS$: C, 59.18; H, 3.12; N, 18.77% Found: C, 59.39; H, 2.95; N, 18.89%.

2-(3-chlorophenyl)-5-[6-(4-nitrophenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVk)

IR (KBr, cm^{-1}): 3034 (C-H), 1604 (C=N), 1445 (C-N), 1226 (N-N=C), 787 (C-Cl), 701 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 7.14-8.11 (11H, m, Ar-H). MS: m/z 474 (M^+), (M^+ +1). Elemental analysis: Calcd for $C_{22}H_{11}ClN_6O_3S$: C, 56.01; H, 2.14; N, 18.03% Found: C, 55.64; H, 2.33; N, 17.70%.

4-{3-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl} phenol (IVl)

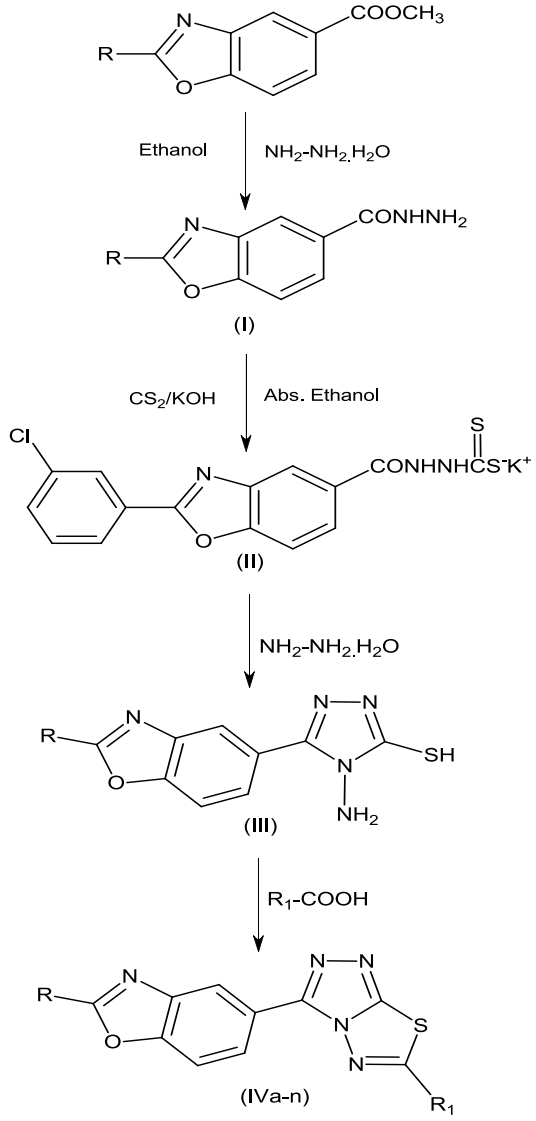
IR (KBr, cm^{-1}): 3555 (OH), 3011 (C-H), 1617 (C=N), 1460 (C-N), 1235 (N-N=C), 787 (C-Cl), 688 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 9.87 (1H, s, OH), 6.68-7.61 (11H, complex, m, Ar-H). Elemental analysis: Calcd for $C_{22}H_{12}ClN_5O_2S$: C, 58.91; H, 3.01; N, 15.83% Found: C, 59.26; H, 2.71; N, 15.71%.

2-(3-chlorophenyl)-5-[6-(2-methylphenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVm)

IR (KBr, cm^{-1}): 3011 (C-H), 1606 (C=N), 1472 (C-N), 1241 (N-N=C), 741 (C-Cl), 709 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 7.25-8.08 (11H, m, Ar-H), 2.58 (3H, s, CH_3). MS: m/z 443 (M^+), (M^+ +1), (M^+ +2). Elemental analysis: Calcd for $C_{23}H_{14}ClN_5OS$: C, 62.34; H, 3.21; N, 16.02% Found: C, 62.23; H, 3.18; N, 15.78%.

2-(3-chlorophenyl)-5-[6-(4-methylphenyl)[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-3-yl]-1,3-benzoxazole (IVn)

IR (KBr, cm^{-1}): 3031 (C-H), 1586 (C=N), 1485 (C-N), 1258 (N-N=C), 745 (C-Cl), 712 (C-S-C). ^1H NMR (DMSO-d_6, δ, ppm): 7.15-7.95 (11H, m, Ar-H), 2.55 (3H, s, CH_3). Elemental analysis: Calcd for $C_{23}H_{14}ClN_5OS$: C, 62.34; H, 3.21; N, 16.02% Found: C, 62.23; H, 3.18; N, 15.78%.



Scheme 1: Synthesis of 3,6-disubstituted -1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives

Table 1: Physicochemical parameters of synthesized compounds (**IVa-n**)

Code No.	R	R_1	Mol. formula[a]	M.P[b] (°C)	Log *P*[c]	R_f[d] Value
IVa	3-ClC_6H_4	C_6H_5-	$C_{22}H_{12}ClN_5OS$	110-112	0.59	0.81
IVb	3-ClC_6H_4	2-ClC_6H_4	$C_{22}H_{11}Cl_2N_5OS$	115-117	1.04	0.83
IVc	3-ClC_6H_4	4-ClC_6H_4	$C_{22}H_{11}Cl_2N_5OS$	130-132	0.98	0.73
IVd	3-ClC_6H_4	2,6-di$Cl_2C_6H_3$	$C_{22}H_{10}Cl_3N_5OS$	180-182	1.95	0.80
IVe	3-ClC_6H_4	2-$ClC_6H_4CH_2$	$C_{23}H_{13}Cl_2N_5OS$	190-192	0.80	0.57
IVf	3-ClC_6H_4	2-BrC_6H_4	$C_{22}H_{11}BrClN_5OS$	140-142	0.56	0.75
IVg	3-ClC_6H_4	3-BrC_6H_4	$C_{22}H_{11}BrClN_5OS$	110-112	2.07	0.80
IVh	3-ClC_6H_4	4-BrC_6H_4	$C_{22}H_{11}BrClN_5OS$	135-137	1.86	0.72
IVi	3-ClC_6H_4	2-$NH_2C_6H_4$	$C_{22}H_{13}ClN_6OS$	150-152	1.70	0.68
IVj	3-ClC_6H_4	4-$NH_2C_6H_4$	$C_{22}H_{13}ClN_6OS$	140-142	1.67	0.91
IVk	3-ClC_6H_4	4-$NO_2C_6H_4$	$C_{22}H_{11}ClN_6O_3S$	150-152	1.78	0.72
IVl	3-ClC_6H_4	4-OHC_6H_4	$C_{22}H_{12}ClN_5O_2S$	160-162	1.45	0.69
IVm	3-ClC_6H_4	2-$CH_3C_6H_4$	$C_{23}H_{14}ClN_5OS$	185-187	2.16	0.81
IVn	3-ClC_6H_4	4-$CH_3C_6H_4$	$C_{23}H_{14}ClN_5OS$	180-182	1.91	0.75

[a]Solvent of crystallization — ethanol.

[b]Melting point of the compounds at their decomposition.

[c]Log P was calculated using absorbance data, chloroform / phosphate buffer at 28 °C.

[d]Solvent system — benzene : acetone (8 : 2, v/v), benzene : ethanol (2 :0.5, v/v), toluene : ethylacetate : formic acid (5 : 4 : 1, v/v/v).

Pharmacological Activity

Anticonvulsant Screening

The experiments were carried out on Swiss albino mice (20-25 g) of either sex obtained from the Institutional Animal Ethics committee, R.V. Northland Institute, Dadri, Greater Noida, Uttar Pradesh, India, under the proposal number RVNI/IAEC/2017/05. The mice were housed in standardized conditions (12 h light/dark cycle) with free access to food and water. They were habituated in the animal facilities for at least one week. The synthesized compounds were suspended in polyethylene glycol (PEG-400).

We followed 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[39-42]. This program aims to encourage and facilitate the discovery of new therapeutic agents for epilepsy. According to this program, anticonvulsant screening is performed at the beginning with qualitative screens following doses of 30, 100 and 300 mg/kg of test compound, using maximal electroshock seizure test, subcutaneous pentylenetetrazole test and acute toxicity assessment of motor impairment. The anticonvulsant and neurotoxicity data of the compounds are reported in Table 2.

Maximal electroshock test (MES)

The compounds were screened for their anticonvulsant activity by electroshock seizure method[39-41]. Supramaximal electroshock of current intensity of 50 mA, 60Hz for 0.2 sec. duration was applied to mice, with the doses of test compounds 30, 100, 300 mg/Kg. The abolition of the hind limb tonic extensor spasm was recorded as anticonvulsant activity.

Subcutaneous pentylenetetrazole seizure test (*sc*PTZ)

The *sc*PTZ test utilized a dose of pentylenetetrazole 75 mg/kg. This produced clonic seizures lasting for a period of at least 5 sec. At the anticipated time the convulsant was administered subcutaneously. Animals were observed over a 30 min period. Absence of clonic spasm in half or more of the animals in the observed time period indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold[43].

Neurotoxicity screening

In mice, the rotorod procedure is used to identify minimal muscular or neurological impairment[44]. The motor coordination was tested in mice using rotorod procedure. Inability of a treated mouse to maintain equilibrium for at least 1 min on a 3.2cm diameter slowly rotating rod (6 rpm) was used as the endpoint indicating motor impairment.

Lipophilicity determination

The dependence of biological activity in the set of congeneric agents or lipophilic character has been shown in many types of drug action in particular, the reports by Lien and co-workers indicated that the anticonvulsant activity of different types of compounds was correlated with lipophilicity[45]. However, it has been observed that the maximum potency of the drugs which act on the central nervous system (CNS) is obtained with congeners having an optimum lipophilicity (log P) near 2. In this study, we attempted to correlate the anticonvulsant activity of the 3,6-disubstituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazoles with the calculated log P value, were determined using chloroform phosphate buffer method[46].

Table 2: Anticonvulsant and motor impairment screening of synthesized compounds (**IVa-n**)

Code No.	Intraperitoneal injection in mice[a]				Neurotoxicity screen[a]	
	MES screen		scPTZ screen			
	0.5h	4h	0.5h	4h	0.5h	4h
IVa	—	—	—	—	×	×
IVb	300	—	300	—	—	—
IVc	300	—	300	—	—	—
IVd	100	300	300	—	—	300
IVe	—	300	—	—	×	×
IVf	—	—	300	—	×	×
IVg	30	300	300	—	—	300
IVh	100	300	—	300	—	300
IVi	300	—	—	—	×	×
IVj	300	300	300	—	300	—
IVk	300	—	300	—	—	—
IVl	300	—	300	—	—	—
IVm	30	300	300	300	—	—
IVn	100	300	—	300	—	—
Phenytoin[b]	30	30	—	—	100	100
Carbamazepine[b]	30	100	100	300	300	300

[a]Doses of 30, 100 and 300 mg/kg were administered to mice through intraperitoneal route. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined 0.5 and 4.0 hrs after the drug administration. The dash (−) indicates an absence of activity at maximum dose administered (300 mg/kg) and cross (×) denotes not tested. Propylene glycol (0.1 ml, i.p.) was used as control solvent.

[b]Data from reference[47-48].

RESULTS AND DISCUSSION

The synthetic route used to synthesize title compounds is outlined in Scheme 1. The acid hydrazide **(I)** was prepared from the reaction of 4-carbomethoxy-2-aminophenol with substituted aromatic acid followed by treatment with hydrazine hydrate.[37] The acid hydrazide was react with carbon disulfide in the presence of potassium hydroxide to afford potassium dithiocarbazate salt (II). This salt underwent cyclization with an excess of hydrazine hydrate to give 4-amino-3-substituted-5-mercapto- (4H)-1,2,4-triazole **(III)**. The resultant triazole was further converted to titled compounds through one-pot reaction by condensation with different aromatic/aroyl acids in the presence of phosphorous oxychloride. In all the cases the TLC of the product showed the single spot confirming the chromatogram for only one product. The physical constants of the title compounds are presented in Table 1. The structure of compound **(III)** was confirmed by ^{1}H-NMR spectral data results. The ^{1}H-NMR spectra showed a downfield singlet at around 13.8 ppm attributed to -SH group, while the -NH_2 group appeared as a singlet at around 5.6 ppm. The absence of signals for -NH_2 and -SH protons confirmed that the triazole was converted into triazolothiadiazoles **(IVa-n)** by reacting with the -COOH group of aromatic acids. The structures of the synthesized compounds were established by elemental analysis and spectral data results are reported in experimental protocols.

The anticonvulsant activity of the newly synthesized compounds **(IVa-n)** were determined 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 and it includes the

maximal electroshock seizure[43] and the subcutaneous pentylenetetrazole.[44] Additionally, acute toxicity of antiepileptic drugs in rodents is almost invariably manifested by neurological deficits can be detected by the rotarod test.[45] Data is presented in Table 2 after the 0.5 and 4.0 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.

In the anticonvulsant screening, every compound indicated empowering action except **IVa** and **IVf**. Compounds **IVg** and **IVm** were observed to be very dynamic against MES test at a dose level of 30 mg/kg at 0.5 h time interval characteristic of their capacity to avoid seizure spread at generally bring down dosage. Compounds that showed protection at a moderate level against MES model at 100 mg/kg include **IVd, IVh** and **IVn**. The compounds **IVd, IVg, IVh, IVj, IVm** and **IVn** showed activity both at 0.5 and 4.0 hrs time intervals. In this way, the larger part of the compounds demonstrated empowering anticonvulsant activity at 0.5 h interval showed that they have a fast onset and shorter duration of activity.

In chemo shock examination, all the compounds except **IVa, IVe** and **IVi** showed activity indicative of their ability to prevent seizure spread. Compounds **IVb, IVc, IVd, IVf, IVg, IVj, IVk** and **IVl** 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 (**IVh** and **IVn**) were also active after 4.0 hrs extended period of activity. Only one compound **IVm** showed activity at the dose level of 300 mg/kg at both time intervals.

In neurotoxicity study, rotarod test was employed to estimate the undesired effects such as sedation and ataxia produced by the compounds. Compounds **IVb, IVc, IVk, IVl, IVm** and **IVn** did not show any toxicity at the dose of 300 mg/kg. No any compounds were toxic at 0.5 and 4.0 hrs., whereas only one compound **IVj** showed toxicity after 0.5 h and do not show toxicity after 4.0 hrs. Some compounds **IVd, IVg** and **IVh** 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).

Compounds **IVd, IVg, IVh, IVm** and **IVn** were found to be more lipophilic having potent anticonvulsant activity. The other compounds **IVb, IVc, IVi, IVj, IVK** and **IVl** were also lipophilic having some potency. Compounds **IVe** and **IVf** were very less lipophilic and were less or negligible active in MES and scPTZ test.

CONCLUSION

Various 3,6-disubstituted-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazole derivatives (**IVa-n**) were synthesized and exhibited anticonvulsant activity in MES and scPTZ screens. All the compounds exhibited anticonvulsant activity in MES and scPTZ screens**.** In the primary MES screening compounds **IVd, IVg, IVh, IVj, IVm** and **IVn** showed activity both at 0.5 and 4.0 hrs against seizures confirming their potential utility as prototypic molecules. The anticonvulsant activity data revealed that all the compounds showed remarkable reduction of hind limb tonic extensor phase and compounds **IVg** and **IVm** were found to be the most potent compounds in the series. Moreover, anticonvulsant activities of the other tested compounds were found to be much less effective than phenytoin and carbamazepine used as standard drug. According to the results obtained it seems that presence of halosubstituted aryl at benzoxazole moiety and halo (like chloro and bromo) and alkyl group attached on aryl ring at triazolothiadiazole ring displayed the best anticonvulsant activity and favorable high protection. There were some compounds like **IVd, IVg, IVh, IVm** and **IVn** that showed more lipophilic character and were more active. The compounds **IVb, IVc, IVi, IVj, IVK** and **IVl** were also lipophilic but were less active in MES test. Some of above mentioned compounds have shown higher degree of protection and obviously may have future commitment.

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