Research Article

Synthesis of Some New 2, 5-Disubstituted - 1, 3, 4 -Thiadiazole Derivatives and Investigation of their Anticonvulsant Activities

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ABSTRACT

Epilepsy is a common neurological disorder throughout the world which is characterized by recurrent unprovoked epileptic seizures. A need for the development of newer antiepileptic drugs with improved efficacy and tolerability with lesser side effects, as several of the currently available antiepileptic drugs have been associated with severe side effects including neurotoxicity, depression and other CNS related diseases. In view of these facts various substituted thiadiazoles were synthesized by the reaction of hydrazine carbothiomides with concentrated sulfuric acid starting from methyl-3-amino-4-hydroxy benzoate via synthesis of an intermediate methyl- 2 –substituted aryl- 1, 3-benzoxazole-5-carboxylates and 2-substitutedaryl-1,3-benzoxazole-5-carbohydrazides. The structure of the synthesized compounds was confirmed by spectral data and elemental analysis. All the compounds were tested for anticonvulsant activity utilizing pentylenetetrazole induced seizure and Maximal Electroshock Seizure methods. Anticonvulsant activity was shown by majority of the synthesized compounds, among these two compounds IVg and IVm were considered to have potent anticonvulsant activity at 30 mg/kg dose level with lesser neurotoxicity comparable to that of standard drugs.

Keywords: Anticonvulsant, Neurotoxicity, Lipophilicity, Thiadiazoles.

INTRODUCTION

Epilepsy is a chronic neurological disorder, affecting a large section of people of all ages across the world. It is characterized by unpredictable and periodic seizures.[1-2] In fact, epilepsy is the third most frequent neurological disorder encountered in the elderly after cerebrovascular disease and dementia.[3] Epilepsies are common and frequently devastating and affect around 60 million of the global population. On an average every year 0.25 million new cases are added to these figures.[4-7] Epilepsy also possesses a considerable economic burden on the society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to the treatment.[8-9] A number of antiepileptic drugs (AEDs) is available which provide adequate seizure control, however, 25-30% of patients are still estimated to be poorly treated and they are pharmacoresistant to the available therapy.[10-11] Recently many newer and effective AEDs have been developed such as pregabalin, stiripentol, zonisamide, lamotrigine, topiramate, valproate, tiagabine, levetiracetam etc. as promising anticonvulsants.[12-14] However, therapeutic benefits of these drugs are associated with undesirable side effects such as headache, nausea, hepatotoxicity, anorexia, ataxia, drowsiness, gastrointestinal disturbances and hirsutism.[15-18] Several in vivo and in vitro animal models have been proposed and many new antiepileptic drugs have been marketed recently, but large numbers of patients are still pharmacoresistant.[19] These observations reinforce the need for developing newer agents for epilepsies.

The search for antiepileptic drugs with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry. A rational drug design process of a new anticonvulsant could be achieved in several ways. The first strategy is the identification of new targets through better understanding of molecular mechanisms of epilepsy. Another way is to modify already existing drugs and formulations.[20-21] The long established AEDs control seizures in 50% of patients developing partial seizures and 60-70% of those developing generalized seizures.[22-24] Hence there is an urgent need to develop new, more effective and less toxic antiepileptic drugs.[25]

In recent years, the fields of antiepileptic drug development have become quite dynamic, affording many promising research opportunities. Mechanistic approaches are increasingly being facilitated by the new wave of research in epileptics.[26] Recent studies revealed that the substituted thiadiazole derivatives have attracted much attention due to their broad spectrum of pharmacological activities such asanticancer,[27-28]anti-inflammatory,[29-30] antimicrobial,[31-32] antifungal,[33-34] antiviral,[35-36] antitubercular,[37-38] antidepressant,[39-40] and anticonvulsant activities[41-43] probably resulting from its planar and compact structure.

Thiadiazoles, heterocyclic compounds of varied biological activities were found to be one of the new classes of anticonvulsant agents as revealed by literature survey.[43] Hence, the present studies were undertaken to study the anticonvulsant potential of substituted thiadiazoles. In this paper, we report the synthesis, anticonvulsant

activity and neurotoxicity of a series of 2,5-disubstituted-1,3,4-thiadiazoles (IVa-o).

MATERIALS AND METHODS

Experimental procedure

All the chemicals and solvents used were mostly of AR grade obtained from Merck, CDH and S.D. Fine Chemicals Limited. The melting points were determined in open glass capillary using kjeldahl flask containing liquid paraffin and are uncorrected. The FT-IR spectra were recorded in KBr pellets on (BIO-RAD FTS), FT-IR spectrophotometer (λ-max in cm^{-1}). ^{1}H-NMR were recorded on DRX-300 NMR spectrometer and BRUKER 400 Ultra Shield™ (chemical shifts (δ) in ppm) in DMSO-d_6/$CDCl_3$ using tetra methylsilane (TMS) as internal reference. Mass spectrometry was recorded on UPLC-MS/MS (WATERS, Mass Lynx version 4.1) spectrometer. Elemental analysis was undertaken with a Perkin-Elmer model 240 analyzer, and all analyses were consistent with theoretical values within ±0.4% of the theoretical value. The homogeneity of the compounds was checked by thin layer chromatography (TLC) on silica gel G coated plates by using benzene: acetone (8:2), benzene: ethanol (2:0.5), toluene: ethylacetate: formic acid (5:4:1) as solvent systems. Iodine chamber and UV lamp were used for the visualization of TLC spots.

General procedure for synthesis of 2-substituted-5-carbomethoxy benzoxazoles (Ia-e)

Methyl-3-amino-4-hydroxy benzoate (0.01 mol) was dissolved in a mixture of an appropriate aryl acid and ethanol (50 mL) and refluxed for 15 hrs. The reaction mixture was cooled and poured onto the crushed ice with stirring to obtain the compound **Ia**.

Similarly other compounds **(Ib-e)** were also prepared by above specified method.[44]

General procedure for synthesis of 2-substitutedbenzoxazole-5-carboxylic acid hydrazides (IIa-e)

An equimolar quantity of **Ia** (0.01 mol) and hydrazine hydrate (99%, 0.01 mol) was taken in absolute ethanol (30 mL) and refluxed for 20 hrs. After this reaction mixture was poured to crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol to give compound **IIa**.

Similarly other compounds (**IIb-e**) were also prepared by above specified method.[45]

General procedure for synthesis of N-phenyl-2-[(2-phenyl-1, 3-benzoxazol-5-yl) carbonyl]

hydrazinecarbothiomide (IIIa-o)

An equimolar quantity of compound **IIa** (0.002 mol) and substituted aryl isothiocyanate (0.002 mol) was refluxed for 2-4 hrs. The contents were concentrated and poured to crushed ice, filtered and dried to give compound IIIa.

Similarly other compounds (**IIIb-o**) were also prepared by above specified method.[46]

General method for synthesis of 5-(2-substitutedaryl-1,3-benzoxazol-5-yl) -2- (substituted aryl amino) -1, 3, 4-thiadiazoles (IVa-o)

Each thiosemicarbazide **IIIa** (0.002mol) was added gradually under stirring to cooled concentrated sulphuric acid (15mL) at 0°C for 30 min. The reaction mixture was further stirred for another 2-3 hrs in an ice bath. It was then poured over crushed ice under stirring. The obtained precipitate was filtered, washed with water, dried and recrystallized with ethanol to furnish compound **IVa**.

The compounds **(IVb-o)** were also synthesized by similar method using reagents in proper mole ratio.[46] The synthetic route and the physicochemical parameters of synthesized compounds are presented in Scheme 1 and Table 1 respectively.

N-(2-methylphenyl)-5-(2-phenyl-1, 3-benzoxazol-5-yl)-1,3,4-thiadiazol-2-amine (IVa)

IR (KBr, cm^{-1}): 3383 (NH), 3001 (CH), 1599 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.24(s, 3H, CH_3), 7.13-7.76 (m, 12H, Ar-H), 8.18 (s, 1H, NH). MS: m/z (M+1) 385. Elemental analysis: Calcd for $C_{22}H_{16}N_4OS$: C, 68.55; H, 4.01; N, 14.90% Found: C, 68.73; H, 4.19; N, 14.57%.

N-(3-methylphenyl)-5-(2-phenyl-1, 3-benzoxazol-5-yl)-1,3,4-thiadiazol-2-amine (IVb)

IR (KBr, cm^{-1}): 3360 (NH), 3013 (CH), 1602 (C=N).^{1}H NMR (DMSO-d_6, δ, ppm): 2.55 (s, 3H, CH_3), 7.17-7.88 (m, 12H, Ar-H), 8.31(s, 1H, NH). MS: m/z (M+1) 385. Elemental analysis: Calcd for $C_{22}H_{16}N_4OS$: C, 69.05; H, 4.32; N, 14.34% Found: C, 68.73; H, 4.19; N, 14.57%.

N-(4-methylphenyl)-5-(2-phenyl-1, 3-benzoxazol-5-yl)-1, 3, 4-thiadiazol-2-amine (IVc)

IR (KBr, cm^{-1}): 3404 (NH), 3041 (CH), 1604 (C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.30 (s, 3H, CH_3), 7.27-7.96 (m, 12H, Ar-H), 8.25 (s, 1H, NH). MS: m/z (M+1) 385. Elemental analysis: Calcd for $C_{22}H_{16}N_4OS$: C, 68.23; H, 3.91; N, 15.21% Found: C, 68.73; H, 4.19; N, 14.57%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-thiadiazol-2-amine (IVd)

IR (KBr, cm^{-1}): 3377 (NH), 3043 (CH), 1618 (C=N), 744 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.40 (s, 3H, CH_3), 7.40-8.14 (m, 11H, Ar-H), 8.32 (s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.12; H, 3.95; N, 13.59% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-thiadiazol-2-amine (IVe)

IR (KBr, cm^{-1}): 3345 (NH), 3010 (CH), 1604 (C=N), 729 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 7.33-7.88 (m, 11H, Ar-H), 8.16 (s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.44; H, 4.00; N, 13.09% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-thiadiazol-2-amine (IVf)

IR (KBr, cm^{-1}): 3407 (NH), 2997 (CH), 1596 (C=N),725 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.41 (s, 3H, CH_3), 7.26-8.01 (m, 11H, Ar-H), 8.23 (s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.00; H, 3.90; N, 13.33% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-thiadiazol-2-amine (IVg)

IR (KBr, cm^{-1}): 3389 (NH), 3035 (CH), 1600 (C=N), 744 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 7.31-7.89 (m, 11H, Ar-H), 8.21(s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.48; H, 4.01; N, 13.62% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-thiadiazol-2-amine (IVh)

IR (KBr, cm^{-1}): 3392 (NH), 3014 (CH), 1598 (C=N), 712 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.52 (s, 3H, CH_3), 7.50-8.11 (m, 11H, Ar-H), 8.27 (s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.34; H, 3.11; N, 13.09% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-thiadiazol-2-amine (IVi)

IR (KBr, cm^{-1}): 3340 (NH), 3051 (CH), 1610 (C=N), 730 (C-Cl). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.44 (s, 3H, CH_3), 7.26-8.02 (m, 11H, Ar-H), 8.20 (s, 1H, NH). MS: m/z (M+1) 419. Elemental analysis: Calcd for $C_{22}H_{15}ClN_4OS$: C, 63.23; H, 3.36; N, 13.11% Found: C, 63.08; H, 3.61; N, 13.37%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-thiadiazol-2-amine (IVj)

IR (KBr, cm^{-1}): 3388 (NH), 3017 (CH), 1594 (C=N), 544 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.44 (s, 3H, CH_3), 7.24-8.11 (m, 11H, Ar-H), 8.27 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 57.40; H, 3.04; N, 12.15% Found: C, 57.03; H, 3.26; N, 12.09%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-thiadiazol-2-amine (IVk)

IR (KBr, cm^{-1}): 3404 (NH), 3092 (CH), 1611 (C=N), 534 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.55 (s, 3H, CH_3), 7.14-7.98 (m, 11H, Ar-H), 8.19 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 57.20; H, 3.14; N, 12.11% Found: C, 57.03; H, 3.26; N, 12.09%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-thiadiazol-2-amine (IVl)

IR (KBr, cm^{-1}): 3356 (NH), 3081 (CH), 1580 (C=N), 561 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.34 (s, 3H, CH_3), 7.21-8.19 (m, 11H, Ar-H), 8.32 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 56.98; H, 3.24; N, 12.33% Found: C, 57.03; H, 3.26; N, 12.09%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-thiadiazol-2-amine (IVm)

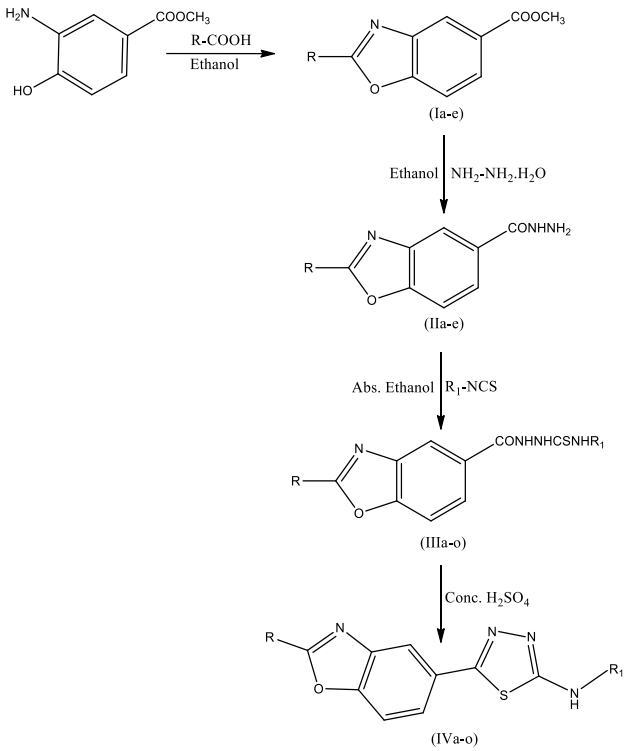
IR (KBr, cm^{-1}): 3389 (NH), 3066 (CH), 1594 (C=N), 531 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.44 (s, 3H, CH_3), 7.31-8.03 (m, 11H, Ar-H), 8.24 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 57.40; H, 3.22; N, 12.33% Found: C, 57.03; H, 3.26; N, 12.09%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-thiadiazol-2-amine (IVn)

IR (KBr, cm^{-1}): 3319 (NH), 3020 (CH), 1594 (C=N), 538 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.46 (s, 3H, CH_3), 7.36-8.00 (m, 11H, Ar-H), 8.21 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 57.34; H, 3.55; N, 12.35% Found: C, 57.03; H, 3.26; N, 12.09%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-thiadiazol-2-amine (IVo)

IR (KBr, cm^{-1}): 3380 (NH), 3056 (CH), 1600 (C=N), 525 (C-Br). ^{1}H-NMR (DMSO-d_6, δ, ppm): 2.47 (s, 3H, CH_3), 7.29-7.91 (m, 11H, Ar-H), 8.29 (s, 1H, NH). MS: m/z (M+1) 464. Elemental analysis: Calcd for $C_{22}H_{15}BrN_4O_2$: C, 56.88; H, 3.01; N, 11.83% Found: C, 57.03; H, 3.26; N, 12.09%.



Scheme 1: Synthesis of 2, 5-disubstituted - 1, 3, 4 - thiadiazole derivatives (IVa-o)

Pharmacological Activity

Anticonvulsant Screening

Swiss albino mice (20-25 g) 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, and New Delhi, India.

Table 1: Physicochemical parameters of title compounds (IVa-o)

Code No.	R	R_1	Mol. formula[a]	M.P[b] (°C)	Log *P*[c]	R_f[d] Value
IVa	C_6H_5	2-$CH_3C_6H_4$	$C_{22}H_{16}N_4OS$	150-152	0.76	0.55
IVb	C_6H_5	3-$CH_3C_6H_4$	$C_{22}H_{16}N_4OS$	145-147	0.74	0.78
IVc	C_6H_5	4-$CH_3C_6H_4$	$C_{22}H_{16}N_4OS$	145-147	0.69	0.65
IVd	3-ClC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	140-142	2.01	0.60
IVe	3-ClC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	155-157	1.98	0.70
IVf	3-ClC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	135-137	1.58	0.67
IVg	4-ClC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	150-152	2.11	0.74
IVh	4-ClC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	145-147	1.95	0.70
IVi	4-ClC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4OS$	160-162	0.88	0.65
IVj	3-BrC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	155-157	1.99	0.80
IVk	3-BrC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	145-147	1.76	0.80
IVl	3-BrC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	150-152	0.86	0.85
IVm	4-BrC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	165-167	2.11	0.69
IVn	4-BrC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	150-152	1.99	0.75
IVo	4-BrC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4OS$	160-162	2.08	0.77

[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).

The pharmacological testing of the final compounds were performed according to the standard protocol given by epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) following the protocol adopted by the Antiepileptic Drug Development (ADD) program. Compounds were administered intraperitoneally as a solution in polyethylene glycol (PEG-400). The anticonvulsant and neurotoxicity data of the compounds are reported in Table 2.

Maximal Electroshock (MES) Test

Maximal electroshock seizure was elicited with a current intensity of 50 mA, 60Hz for 0.2 sec *via* ear clip electrodes, with the doses of test compounds (30, 100, 300 mg/kg). The maximal seizure typically consists of a short period of tonic extension of the hind limbs and a final clonic episode. The abolition of the hind limb tonic extensor component of the seizure due to the drug treatment is defined as anticonvulsant activity.[47-48]

Subcutaneous Pentylenetetrazole (scPTZ) Seizure Threshold Test

The subcutaneous dose of pentylenetetrazole (75 mg/kg) at which 95% of the animals showed convulsive reaction was determined by a dose-percent effect curve. The synthesized compounds were administered at the three graded doses (30, 100, 300 mg/Kg) intraperitoneally. At the anticipated time, PTZ was then administered subcutaneously in the posterior midline of mice. 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.[49-50]

Neurotoxicity Screening

The neurotoxicity of all the test compounds was evaluated using rota rod test. Mice were trained to balance on the rotating rod (3.2 cm diameter) that rotates at 6 rpm. Trained animals were treated with test compounds at a dose of 300 mg/kg administered intraperitoneally. Neurotoxicity was determined by the inability of the animal to maintain equilibration on the rod for at least for one minute.[50]

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.[51] Pharmacological activity is dependent on the lipophilic character of the drug. Anticonvulsant activities of different types of compounds were correlated with lipophilicity.[52] 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≈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 and to establish the correlation between log P and anticonvulsant activity.[53]

Table 2: Anticonvulsant and Neurotoxicity screening of the title compounds (IVa-o)

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

Number of animals in each group (n) = 4; [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 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 of Phenytoin and Carbamazepine used as standard drugs and were obtained from the reference.[54-55]

RESULTS AND DISCUSSION

A pragmatic approach to synthesize new series of thiadiazole derivatives in satisfactory yields was illustrated in Scheme 1 and their structures were characterized by elemental and spectral analysis. The physicochemical properties of synthesized compounds are presented in Table 1. Methyl 3-amino-4-hydroxybenzoate are cyclized to 2-substituted-5-carbomethoxy benzoxazoles (Ia-e) on treatment with substituted aryl acids in ethanol, which on treatment with hydrazine hydrate followed by treatment with substituted arylisothiocyanates, afforded hydrazine carbothioamides (IIIa-o). These compounds were cyclized to titled compounds (IVa-o) with cold concentrated sulphuric acid. The IR spectrum of compound **IVa** showed absorption peak at 1599 cm^{-1} due to C=N stretching vibration. In the ^1H NMR spectrum of the compound the singlet of CSNH and CONH of thiosemicarbazide disappeared and a multiplet was obtained in the aromatic region at δ7.13-7.76 ppm for 12 ArH protons. The NH proton was observed as a singlet at δ8.18 confirming the structure of compound. The mass spectra of the compound **Iva** showed molecular ion peak (M+1) at *m/z* 385 corresponding to molecular formula $C_{22}H_{16}N_4OS$**.** In all the cases the TLC of the product showed the single spot confirming the chromatogram for only one product.

In preliminary screening, the test compounds were dissolved in propylene glycol 400 and administered by *i.p.* injection at three dose levels (30, 100 and 300 mg/kg) in albino mice and the activity was examined after 0.5 and 4.0 hrs intervals against maximal electroshock-induced seizure and subcutaneous pentylenetetrazole tests. The anticonvulsant and neurotoxicity data of the compounds are reported in Table 2.

All the compounds except **IVc** and **IVl** were found to exhibit protection in MES test making them useful for broad spectrum of seizure types. Compounds that showed protection against MES model at 100 mg/kg include **IVd, IVe, IVh, IVj, IVn** and **IVo**. Compounds **IVa, IVe, IVg, IVh, IVj, IVm, IVn** and **IVo** showed activity both at 0.5 and 4.0 hrs. Thus, only two compounds **IVg** and **IVm** showing activity at a lower dose of 30 mg/kg seems to be very potent in anticonvulsant MES screening. Some of the compounds that were active only at 0.5 h included **IVb, IVd, IVf, IVi** and **IVk** representing that they have rapid onset and short duration of action.

The synthesized compounds challenged the *sc*PTZ test to predict their potential against seizure threshold. All the

compounds except **IVa, IVc, IVe, IVi** and **IVo** showed activity indicative of their ability to prevent seizure spread. Compounds **IVb, IVf, IVg, IVk** and **IVl** were active against seizures after 0.5 h at a dose of 300 mg/kg. So these compounds have quick onset but for shorter duration of action. Some compounds **IVh** and **IVn** were active at 300 mg/kg after 4.0 hrs, indicative of the extended period of action. Only three compounds **IVd, IVj** and **IVm** were active at the dose of 300mg/kg at both time intervals indicative of the longer duration of action.

In the neurotoxicity screening, most of the compounds did not show any toxicity at the dose of 300mg/kg. Compounds **IVf, IVj** and **IVo** revealed toxicity at a dose of 300 mg/kg after 0.5 h only, and compounds **IVd, IVg** and **IVh** exhibited delayed toxicity i.e., only after 4.0 hrs, which is comparable with that of carbamazepine (300 mg/kg). However, all the compounds were less toxic than phenytoin (100 mg/kg).

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

CONCLUSION

The present work indicates that halosubstituted aryl at benzoxazole and alkylsubstituted aryl at thiadiazole moiety have given impetus to the present investigation and showed favored anticonvulsant activity as compared to unsubstituted rings. Thus, a number of 5-(2-substitutedaryl-1,3-benzoxazol-5-yl) -2- (substituted aryl amino) -1,3,4-thiadiazole derivatives were synthesized successfully, and tested for anticonvulsant activity using MES and scPTZ screens. The results of the investigations suggest that two compounds i.e., **IVg** and **IVm** of the newly synthesized series of thiadiazoles displayed significant anticonvulsant activity with lesser neurotoxicity than the standard drugs. These compounds represent valuable leads in the exploration of agents controlling both treatment of seizures and intoxication during epilepsy and can be considered as lead molecules for future investigations. Some compounds **IVd, IVe, IVg, IVh, IVj, IVm, IVn** and **Ivo** showed more lipophilic character and were more active. The compounds **IVa, IVb, IVc**, **IVi** and **IVl** were also lipophilic but were less active in MES test. Hence, we may conclude that reported series of substituted thiadiazole derivatives may be promising for the development of potential anticonvulsant agents after further optimization.

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