Synthesis, Anticonvulsant and Neurotoxicity Screening of Some Novel 2, 5-Disubstituted - 1, 3, 4 – Oxadiazole Derivatives

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ABSTRACT

A series of 2, 5-disubstituted - 1, 3, 4 – oxadiazoles (4a-o) were synthesized on refluxing hydrazine carbothioamides with iodine and potassium iodide in ethanolic sodium hydroxide solution starting from methyl-3-amino-4-hydroxy benzoate via synthesis of an intermediate methyl-2-substitutedaryl-1, 3-benzoxazole-5-carboxylates and 2-substitutedaryl-1, 3-benzoxazole-5-carbohydrazides. The newly synthesized compounds were characterized on the basis of spectral (FT-IR, ^{1}H-NMR, MS) and elemental analysis. All these compounds were screened for anticonvulsant activity using Maximal Electroshock (MES) and subcutaneous pentylenetetrazole (scPTZ) method. Anticonvulsant activity was shown by majority of the synthesized compounds when given *i.p.* to mice. Among the tested compounds 4e, 4j and 4o were considered to have potent anticonvulsant activity comparable to that of standard drugs Phenytoin and Carbamazepine. Compounds 4e, 4g, 4h, 4i, 4k and 4m passed the rota rod test successfully without any sign of neurological deficit.

Keywords: Anticonvulsant, Neurotoxicity, Lipophilicity, 1, 3, 4-Oxadiazoles.

INTRODUCTION

Convulsion is a heterogeneous group of disorders characterized by the neuronal hyper excitability and hyper synchronous neuronal firing presented with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. [1-2] The insufficient information on the cellular mechanism of human epilepsy 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; hence the present drug therapy is rather concerned only with control of epilepsy symptoms than cure. [3] Epilepsy is one of the most common disorders of the brain, affecting around 1-2% of the world population. [4-9] Every year approximately 250,000 new cases are added to this figure. [10] Although conventional antiepileptic drugs (AEDs): primidone, phenytoin, carbamazepine, phenobarbitol, valproic acid, ethosuximide and benzodiazepine, are already in clinical use, some types of seizures are still not adequately treated with current therapy and have limitations or intolerable side effects. [11-13] In response to these limitations several new drugs like lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin, levetiracetam, oxcarbazepine, zonisamide, fosphenytoin, vigabatrin and felbamate have been strongly advocated to optimally manage seizures. [14-15] However, there is a significant group of patients (up to

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40%) who are resistant to the available antiepileptic drugs. [16-17] These facts make the field of anticonvulsant drug discovery a high priority. [18]

The search for antiepileptic compounds 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. [19-20] 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. The long established AEDs control seizures in 50% of patients developing partial seizures and 60-70% of those developing generalized seizures. [21-23] Hence there is an urgent need to develop new anticonvulsants. [24]

During recent years, there has been intense investigation of different classes of oxadiazole compounds and many of them were found to be pharmacologically active. The substituted oxadiazoles have attracted much attention due to their prominent utilization as anti-inflammatory, [25-29] antimicrobial, [30-32] antifungal, [33] antimalarial, [34] anticancer, [35] antitubercular, [36-38] antidepressant [39] and anticonvulsant activity [40-42] probably resulting from its planar and compact structure.

Oxadiazoles, heterocyclic compounds of varied biological activities were found to be one of the new classes of anticonvulsant agents as revealed by literature survey. [41] 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. In the present work it was therefore thought to design and synthesize the first combination of oxadiazole as a basic nucleus incorporated with substituted benzoxazole moiety within a single molecule. Such combination is hoped to develop compounds with lipophilic character having potential anticonvulsant activity.

MATERIALS AND METHODS

All the chemicals and solvents used were mostly of AR grade obtained from Merck, CDH and S.D. Fine Chemicals Limited. The melting points of synthesized compounds were determined in open glass capillaries using Kjeldahl flask containing liquid paraffin and are uncorrected. Thin layer chromatography using Silica gel G (Merck) plates was used to access the reaction and purity of the synthesized compounds. Elemental analysis was obtained for all the synthesized compounds 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 (λ-max in cm^{-1}). The proton magnetic resonance spectra (^{1}H-

NMR) were recorded on DRX- 300 NMR spectrometer and BRUKER 400 Ultra Shield™. Chemical shifts (δ) are expressed in ppm in DMSO-d$_6$/CDCl$_3$ using tetramethylsilane (TMS) as internal reference. Mass spectrometry was recorded on UPLC-MS/MS (WATERS, Mass Lynx version 4.1) spectrometer.

General procedure for synthesis of 2-substituted-5-carbomethoxy benzoxazoles (1a-e)

Methyl-3-amino-4-hydroxy benzoate (0.001mol) was dissolved in a mixture of an appropriate aryl acid (in excess) and ethanol (50 ml) and refluxed for 15 hours. The reaction mixture was cooled and poured onto the crushed ice with stirring to obtain the compound 1a. Similarly other compounds (1b-e) were also prepared by above specified method. [43]

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

An equimolar quantity of 4-carbomethoxy-2-aminophenol (0.01mol) and hydrazine hydrate (99%, 0.01mol) was taken in absolute ethanol (30 ml) and refluxed for 20 hours. After this reaction mixture was poured to crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol to give compound 2a. Similarly other compounds (2b-e) were also prepared by above specified method. [44]

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

An equimolar quantity of compound (2a) (0.002mol) and substituted phenyl isothiocyanate (0.002mol) was refluxed for 2-4 hours. The contents were concentrated and poured into crushed ice, filtered and dried to give compound 3a. Similarly other compounds (3b-o) were also prepared by above specified method. [45-46]

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

A suspension of corresponding hydrazinecarbo thiomide (3a) (0.002mol) in ethanol (30 ml) was dissolved in 5N aq. NaOH solution (2 ml) under cooling and stirring, resulting in the formation of a clear solution. To this iodine in potassium iodide solution (5%) was added gradually under stirring till the color of iodine persisted at room temperature. The reaction mixture was then refluxed for 3-5 hours on water bath. It was then concentrated, cooled and poured over crushed ice. The solid mass that was precipitated was filtered, dried and recrystallized from ethanol to get crystalline solid 4a. The compounds (4b-o) were also synthesized by similar method using reagents in proper mole ratio.

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

N-(2-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-1,3,4-oxadiazol-2-amine (4a)

IR (KBr, cm^{-1}): 3367 (NH), 3013 (CH), 1560 (C=N); ^{1}H NMR (DMSO-d$_6$, δ, ppm): 2.48 (s, 3H, CH$_3$), 7.53-8.18 (m, 12H, Ar-H), 9.80 (s, 1H, NH); EIMS: m/z (M+1) 369;

Elemental analysis: Calcd for $C_{22}H_{16}N_4O_2$: C, 71.55; H, 4.01; N, 15.59% Found: C, 71.73; H, 4.38; N, 15.21%.
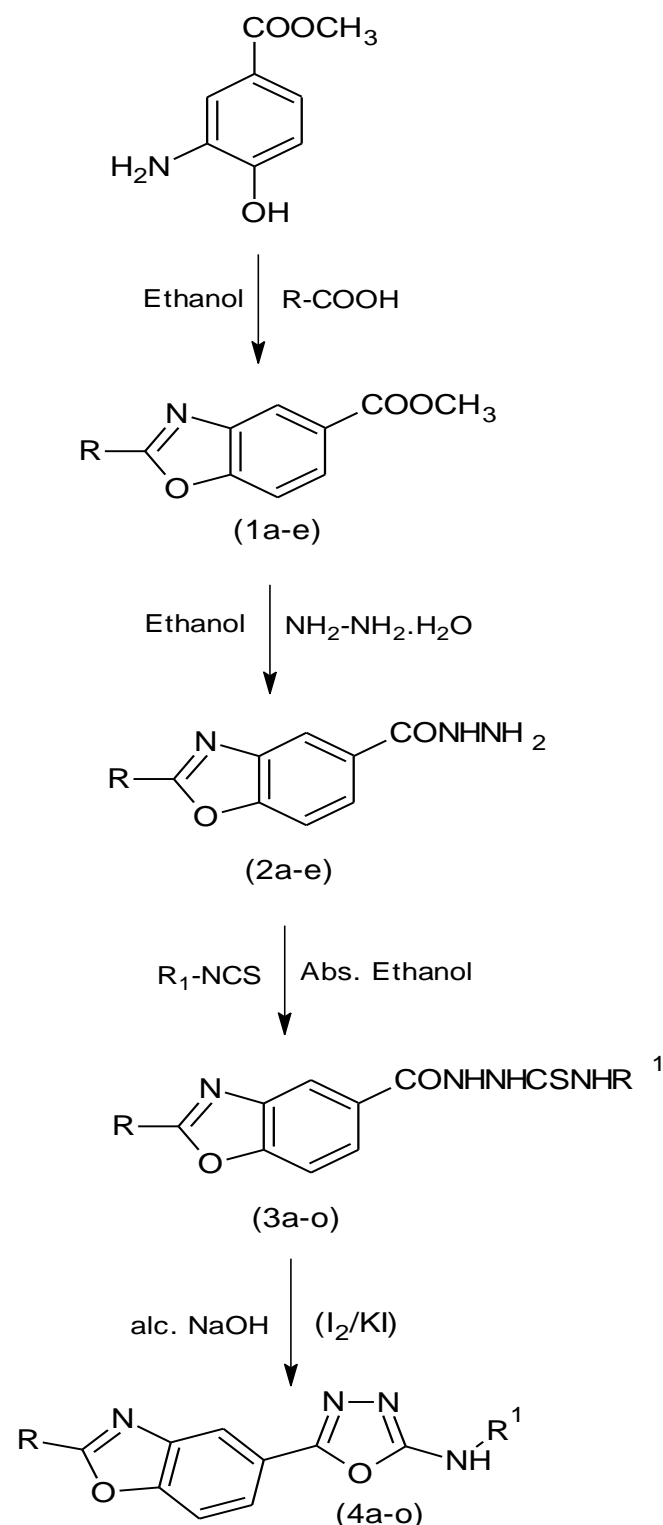
Scheme 1: Synthesis of 2, 5-disubstituted - 1, 3, 4 - oxadiazole derivatives (4a-o)

N-(3-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-1,3,4-oxadiazol-2-amine (4b)
IR (KBr, cm⁻¹): 3301 (NH), 2995 (CH), 1588 (C=N); ¹H NMR (DMSO-d₆, δ, ppm): 2.35 (s, 3H, CH₃), 7.27-8.11 (m, 12H, Ar-H), 9.78 (s, 1H, NH); EIMS: m/z (M+1) 369; Elemental analysis: Calcd for $C_{22}H_{16}N_4O_2$: C, 71.55; H, 4.01; N, 15.59% Found: C, 71.73; H, 4.38; N, 15.21%.

N-(4-methylphenyl)-5-(2-phenyl-1,3-benzoxazol-5-yl)-1,3,4-oxadiazol-2-amine (4c)
IR (KBr, cm⁻¹): 3314 (NH), 3044 (CH), 1601 (C=N); ¹H NMR (DMSO-d₆, δ, ppm): 2.30 (s, 3H, CH₃), 7.27-7.96 (m, 12H, Ar-H), 9.92 (s, 1H, NH); EIMS: m/z (M+1) 369; Elemental analysis: Calcd for $C_{22}H_{16}N_4O_2$: C, 71.55; H, 4.01; N, 15.59% Found: C, 71.73; H, 4.38; N, 15.21%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-oxadiazol-2-amine (4d)
IR (KBr, cm⁻¹): 3301 (NH), 3022 (CH), 1578 (C=N), 1230 (C=S), 744 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.40 (s, 3H, CH₃), 7.40-8.04 (m, 12H, Ar-H), 9.55 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-oxadiazol-2-amine (4e)
IR (KBr, cm⁻¹): 3311 (NH), 3022 (CH), 1604 (C=N), 1249 (C=S), 741 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.49 (s, 3H, CH₃), 7.33-8.13 (m, 12H, Ar-H), 9.66 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(3-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (4f)
IR (KBr, cm⁻¹): 3300 (NH), 3011 (CH), 1641 (C=N), 1266 (C=S), 733 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.31 (s, 3H, CH₃), 7.26-8.20 (m, 12H, Ar-H), 9.33 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-oxadiazol-2-amine (4g)
IR (KBr, cm⁻¹): 3299 (NH), 3045 (CH), 1608 (C=N), 1239 (C=S), 738 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.51 (s, 3H, CH₃), 7.35-8.23 (m, 12H, Ar-H), 9.55 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(3-methylphenyl)-1,3,4-oxadiazol-2-amine (4h)
IR (KBr, cm⁻¹): 3366 (NH), 3023 (CH), 1586 (C=N), 1241 (C=S), 723 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.50 (s, 3H, CH₃), 7.50-8.31 (m, 12H, Ar-H), 9.47 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(4-chlorophenyl)-1,3-benzoxazol-5-yl]-N-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (4i)
IR (KBr, cm⁻¹): 3311 (NH), 3047 (CH), 1590 (C=N), 1248 (C=S), 732 (C-Cl). ¹H-NMR (DMSO-d₆, δ, ppm): 2.38 (s, 3H, CH₃), 7.26-8.20 (m, 12H, Ar-H), 9.40 (s, 1H, NH); EIMS: m/z (M+1) 403; Elemental analysis: Calcd for $C_{22}H_{15}ClN_4O_2$: C, 65.88; H, 4.06; N, 14.09% Found: C, 65.59; H, 3.75; N, 13.91%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-N-(2-methylphenyl)-1,3,4-oxadiazol-2-amine (4j)
IR (KBr, cm⁻¹): 3307 (NH), 3011 (CH), 1570 (C=N), 1235 (C=S), 530 (C-Br). ¹H-NMR (DMSO-d₆, δ, ppm): 2.45 (s,

3H, CH$_3$), 7.25-8.16 (m, 12H, Ar-H), 9.53 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-*N*-(3-methylphenyl)-1,3,4-oxadiazol-2-amine (4k)

IR (KBr, cm^{-1}): 3360 (NH), 3088 (CH), 1582 (C=N), 1241 (C=S), 536 (C-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.34 (s, 3H, CH$_3$), 7.44-8.23 (m, 12H, Ar-H), 9.66 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

5-[2-(3-bromophenyl)-1,3-benzoxazol-5-yl]-*N*-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (4l)

IR (KBr, cm^{-1}): 3323 (NH), 3087 (CH), 1569 (C=N), 1246 (C=S), 561 (C-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.34 (s, 3H, CH$_3$), 7.21-8.19 (m, 12H, Ar-H), 9.41 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-*N*-(2-methylphenyl)-1,3,4-oxadiazol-2-amine (4m)

IR (KBr, cm^{-1}): 3311 (NH), 3023 (CH), 1590 (C=N), 1241 (C=S), 539 (C-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.34 (s, 3H, CH$_3$), 7.33-8.23 (m, 12H, Ar-H), 9.44 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-*N*-(3-methylphenyl)-1,3,4-oxadiazol-2-amine (4n)

IR (KBr, cm^{-1}): 3334 (NH), 3090 (CH), 1592 (C=N), 1237 (C=S), 544 (C-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.46 (s, 3H, CH$_3$), 7.36-8.11 (m, 12H, Ar-H), 9.48 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

5-[2-(4-bromophenyl)-1,3-benzoxazol-5-yl]-*N*-(4-methylphenyl)-1,3,4-oxadiazol-2-amine (4o)

IR (KBr, cm^{-1}): 3384 (NH), 3044 (CH), 1611 (C=N), 1242 (C=S), 536 (C-Br). ^{1}H-NMR (DMSO-d$_6$, δ, ppm): 2.47 (s, 3H, CH$_3$), 7.39-8.21 (m, 12H, Ar-H), 9.76 (s, 1H, NH); EIMS: m/z (M+1) 448; Elemental analysis: Calcd for C$_{22}$H$_{15}$BrN$_4$O$_2$: C, 59.40; H, 3.04; N, 12.15% Found: C, 59.08; H, 3.38; N, 12.53%.

Pharmacological Activity
Anticonvulsant Screening

The anticonvulsant screening of the final compounds was done according to the protocols of National Institute of Neurological Disorders and Stroke, NIH, USA. 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, New Delhi, India. The mice were kept under standard conditions at an ambient temperature of 25 ± 2°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)

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 seizure test (scPTZ)

The subcutaneous pentylenetetrazole test was performed according to the known protocol. [49-51] This method utilizes pentylenetetrazole (75 mg/kg) administered as a 0.5% solution subcutaneously in the posterior midline that produces seizures in >95% of animals. The animals were observed for 30 min. 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

The minimal motor impairment was measured in mice by the rota rod test procedure. [51-52] The mice were trained to stay on an accelerating rota rod that rotated at 10 rotations/min 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. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least for one minute.

LogP 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. [53] Pharmacological activity is dependent on the lipophilic character of the drug. Anticonvulsant activities of different types of compounds were correlated with lipophilicity. [54] 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 [55] 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 required starting material, methyl 3-amino-4-hydroxybenzoate was prepared according to the method reported in the literature [43] which on cyclization with substituted aryl acids gave the product, 2-substituted-5-carbomethoxy benzoxazoles (1a-e). Furthermore, reaction of

compound 2-substituted-5-carbomethoxy benzoxazoles with hydrazine hydrate followed by treatment with substituted arylisothiocyanates resulted in the formation of hydrazinecarbothioamides (3a-o). The hydrazinecarbothioamides were oxidatively cyclized to 2, 5-disubstituted -1,3,4-oxadiazoles (4a-o) by elimination of H_2S using iodine and potassium iodide in ethanolic sodium hydroxide. The IR spectrum of oxadiazole 4a showed absorption peak at 1560 cm^{-1} due to C=N stretch vibration. The structure was further supported by its ^{1}H-NMR spectrum, which showed a multiplet at δ 7.53-8.18 ppm for 12 ArH and singlet at δ 9.80 ppm for 1 NH protons. The mass spectra showed molecular ion peak M+1 at m/z 369 corresponding to molecular formula $C_{22}H_{16}N_4O_2$. 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 ±0.4% of the theoretical values. The physicochemical properties of the titled compounds are presented in Table 1.

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 (Swiss strain) and the activity was examined after 0.5 and 4.0 hours intervals against maximal electroshock-induced seizure and subcutaneous pentylenetetrazole tests. The anticonvulsant and neurotoxicity data of the compounds and standard drugs are presented in Table 2.

Table 1: Physicochemical parameters of synthesized compounds (4a-o)

Code No.	R	R^1	Mol. formula^a	M.P^b (°C)	Log P^c	R$_f$^{d} Value
4a	C_6H_5	2-$CH_3C_6H_4$	$C_{22}H_{16}N_4O_2$	110-112	0.71	0.72
4b	C_6H_5	3-$CH_3C_6H_4$	$C_{22}H_{16}N_4O_2$	110-112	0.77	0.70
4c	C_6H_5	4-$CH_3C_6H_4$	$C_{22}H_{16}N_4O_2$	120-122	0.81	0.55
4d	3-ClC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	130-132	1.95	0.77
4e	3-ClC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	125-127	2.11	0.61
4f	3-ClC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	140-142	2.09	0.76
4g	4-ClC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	130-132	1.66	0.70
4h	4-ClC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	120-122	1.96	0.55
4i	4-ClC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}ClN_4O_2$	130-132	1.70	0.51
4j	3-BrC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	150-152	2.05	0.71
4k	3-BrC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	140-142	2.01	0.59
4l	3-BrC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	145-147	0.74	0.71
4m	4-BrC_6H_4	2-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	135-137	1.68	0.72
4n	4-BrC_6H_4	3-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	150-152	1.96	0.67
4o	4-BrC_6H_4	4-$CH_3C_6H_4$	$C_{22}H_{15}BrN_4O_2$	130-132	2.08	0.78

^{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).

Table 2: Anticonvulsant and motor impairment screening of synthesized compounds (4a-o)

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

n = 4; Where n = number of mice
^{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 hours 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. [56-57]

All the compounds except 4a, 4c and 4l 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 4d, 4f, 4h, 4k and 4n. Compounds 4d, 4e, 4f, 4h, 4j, 4k, 4n and 4o showed activity both at 0.5 and 4.0 hours. Thus, only three compounds 4e, 4j and 4o showing activity at a lower

dose of 30 mg/kg seems to be very potent in anticonvulsant MES screening. Some of the compounds (4b, 4g, 4i and 4m) showed activity only at 0.5 hour, indicating that they have rapid onset and shorter duration of action.

In scPTZ screening, all the compounds except 4a, 4b and 4c showed activity indicative of their ability to prevent seizure spread. Compounds 4d, 4e, 4f, 4g, 4k and 4l showed 100% protection at a dose of 300 mg/kg at 0.5 hour. So these compounds have quick onset but for shorter duration of action. Some compounds (4i, 4m and 4n) were also active after 4.0 hours extended period of activity. Only two compounds 4h and 4o showed activity at the dose level of 300 mg/kg at both time intervals.

In the neurotoxicity screening, compounds 4e, 4g, 4h, 4i, 4k and 4m do not show any toxicity at the dose of 300 mg/kg. Only one compound 4o was toxic at 0.5 and 4.0 hours, whereas two compounds 4f and 4j showed toxicity after 0.5 hour and do not show toxicity after 4.0 hours. Two compounds (4d and 4n) showed delayed toxicity i.e., toxicity only after 4.0 hours, which is comparable with that of carbamazepine (300 mg/kg). However, all the compounds were less toxic than phenytoin (100 mg/kg).

Compounds 4d, 4e, 4f, 4h, 4j, 4k, 4n and 4o were found to be more lipophilic having potent anticonvulsant activity. The other compounds 4g, 4i and 4m were also lipophilic having some potency. Compounds 6a, 6b, 6c and 4l were very less lipophilic and were less or negligible active in MES and scPTZ test.

In conclusion, the present results have revealed that halo substituted aryl at benzoxazole ring and alkyl substituted aryl at oxadiazole ring were essential for activity. Thus a number of 2, 5-disubstituted -1,3,4-oxadiazoles exhibited anticonvulsant activity in MES and scPTZ screens. In the primary MES screening compound 4d, 4e, 4f, 4h, 4j, 4k, 4n and 4o showed activity both at 0.5 and 4.0 hourrs 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 4e, 4j and 4o 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 halo groups like chloro and bromo group attached on aryl ring increase the potency. There were some compounds like 4d, 4e, 4f, 4h, 4j, 4k, 4n and 4o that showed more lipophilic character and were more active. The compounds 4g, 4i and 4m 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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REFERENCES

1. Stafstrom CE. Epilepsy: A review of selected clinical syndromes and advances in basic science. J Cereb Blood Flow Metab. 2006; 26: 983-1004.
2. Sharma AK, Khosla R, Mehta VL, Kela AK. Antiepileptic agents: newer generation. Ind J Pharmacol. 1996; 28: 1-l0.
3. Kenda BM, Matagne AC, Talaga PE, Pasau PE, Differding E, Lallemand BI, Frycia AM, Moureau FG, Klitgaard HV, Gillard MR, Fuks B, Michel P. Discovery of 4-substituted pyrrolidone butanamides as new agents with significant antiepileptic activity. J Med Chem. 2004; 47: 530-549.
4. Njamnshi AK, Bissek ACZK, Yepnjio FN, Tabah EN, Angwafor SA, Kuate CT, Dema F, Fonsah JY, Acho A, Kepeden MN, Azinwi YH, Kuwoh PB, Angwafor FF, Muna WF. A community survey of knowledge, perceptions, and practice with respect to epilepsy among traditional healers in the Batibo Health District, Cameroon. Epilep Behav. 2010; 17: 95-102.
5. Blum DE. New drugs for persons with epilepsy. Adv Neurol. 1998; 76: 57-87.
6. Bell GS, Sander JW. The epidemiology of epilepsy: The size of the problem. Seizure. 2002; 11 (Suppl. A): 306-314.
7. Husain A, Siddiqui N, Sarafroz M, Khatoon Y, Rasid M, Ahmad N. Synthesis, anticonvulsant and neurotoxicity screening of some novel 1,2,4-trisubstituted-1*H*-imidazole derivatives. Acta Polo Pharm Drug Res. 2011; 68: 657-663.
8. Wlaz P, Loscher W. Weak anticonvulsant effects of two novel glycine B receptor antagonists in the amygdala-kindling model in rats. Eur J Pharmacol. 1998; 342: 39-46.
9. Scheurer ML, Pedley TA. The evaluation and treatment of seizures. N Engl J Med. 1990; 323: 1468-1474.
10. Husain A, Naseer MA, Sarafroz M. Synthesis and anticonvulsant activity of some novel fused heterocyclic 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives. Acta Polo Pharm Drug Res. 2009; 66: 135-140.
11. Brodie MJ, Dichter MA. Antiepileptic drugs. N Engl J Med. 1996; 334: 168-175.
12. Deckers CLP, Genton P, Sills GJ, Schmidt D. Current Limitations of Antiepileptic Drug Therapy: A Conference Review. Epilep Res. 2003; 53: 1-17.
13. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000; 342: 314-319.
14. Brazil CW, Pedly TA. Advances in the medical treatment of epilepsy. Ann Rev Med. 1998; 49: 135-162.
15. McCabe PH. New Anti-epileptic drugs for the 21st Century. Expert Opinion Pharmacother. 2000; 1: 633-674.
16. Lopes LJM. The new drugs and the strategies to manage epilepsy. Curr Pharmac Design. 2000; 6: 873-878.
17. Berk M, Segal J, Janet L, Vorster M. Emerging options in the treatment of bipolar disorders. Drugs. 2001; 61: 1407-1414.
18. Porter RJ, Rogawski MA. New antiepileptic drugs: from serendipity to rational discovery. Epilepsia. 1992; 33 Suppl 1: S1-6.
19. Bruno-Blanch L, Galvez J, Garcia-Domenech R. Topological virtual screening: a way to find new anticonvulsant drugs

from chemical diversity. Bioorg Med Chem Lett. 2003; 13: 2749-2754.

20. Malawska B, Scatturium A. Application of Pharmacophore Models for the Design and Synthesis of New Anticonvulsant Drugs. Mini Rev Med Chem. 2003; 3: 341-348.

21. Lopes LJM. The new drugs and the strategies to manage epilepsy. Curr Pharmac Design. 2000; 6: 873-878.

22. Berk M, Segal J, Janet L, Vorster M. Emerging options in the treatment of bipolar disorders. Drugs. 2001; 61: 1407-1414.

23. Duncan JS. The promise of new antiepileptic drugs. Br J Clin Pharmacol. 2002; 53: 123-131.

24. Szelenyi I, Horvath K, Howes JF, Mazarati AM. The treatment of epilepsy: future possibilities. Drugs Fut. 2003; 28: 925-936.

25. Akhter M, Husain A, Azad B, Ajmal M. Aroylpropionic acid based 2,5-disubstituted-1,3,4-oxadiazoles: synthesis and their anti-inflammatory and analgesic activities. Eur J Med Chem. 2009; 44: 2372-2378.

26. Amir M, Kumar S. Synthesis and evaluation of anti-inflammatory, analgesic, ulcerogenic and lipid peroxidation properties of ibuprofen derivatives. Acta Pharm. 2007; 57: 31-45.

27. Kumar H, Javed SA, Khan SA, Amir M. 1,3,4-Oxadiazole/thiadiazole and 1,2,4-triazole derivatives of biphenyl-4-yloxy acetic acid: synthesis and preliminary evaluation of biological properties. Eur J Med Chem. 2008; 43: 2688-2698.

28. Bhandari SV, Bothara KG, Raut MK, Patil AA, Sarkate AP, Mokale VJ. Design, synthesis and evaluation of anti-inflammatory, analgesic and ulcerogenicity studies of novel S-substituted phenacyl-1,3,4-oxadiazole-2-thiol and Schiff bases of diclofenac acid derivatives. Bioorg Med Chem. 2008; 16: 1822-1831.

29. Kucukguzel SG, Kucukguzel I, Tatar E, Rollas S, Sahin F, Gulluce M, De Clercq E, Kabasakal L. Synthesis of some novel heterocyclic compounds derived from diflunisal hydrazide as potential anti-infective and anti-inflammatory agents. Eur J Med Chem. 2007; 42: 893-901.

30. Padmavathi V, Reddy GS, Padmaja A, Kondaiah P, Shazia A. Synthesis, antimicrobial and cytotoxic activities of 1,3,4-oxadiazoles, 1,3,4-thiadiazoles and 1,2,4-triazoles. Eur J Med Chem. 2009; 44: 2106-2112.

31. Karthikeyan MS, Prasad DJ, Mahalinga M, Holla BS, Kumari NS. Antimicrobial studies of 2,4-dichloro-5-fluorophenyl containing oxadiazoles. Eur J Med Chem. 2008; 43: 25-31.

32. Sahin G, Palaska E, Ekizoglu M, Ozalp M. Synthesis and antimicrobial activity of some 1, 3, 4-oxadiazole derivatives. I L Farmaco. 2002; 57: 539-542.

33. Chen CJ, Song BA, Yang S, Xu GF, Bhadury PS, Jin LH, Hu DY, Li QZ, Liu F, Xue W, Lu P, Chen Z. Synthesis and antifungal activities of 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-thiadiazole and 5-(3,4,5-trimethoxyphenyl)-2-sulfonyl-1,3,4-oxadiazole derivatives. Bioorg Med Chem. 2007; 15: 3981-3989.

34. Zareef M, Iqbal R, De Dominguez NG, Rodrigues J, Zaidi JH, Arfan M, Supuran CT. Synthesis and antimalarial activity of novel chiral and achiral benzenesulfonamides bearing 1, 3, 4-oxadiazole moieties. J Enz Inhi Med Chem. 2007; 22: 301-308.

35. Aboraia AS, Abdel-Rahman HM, Mahfouz NM, EL-Gendy MA. Novel 5-(2-hydroxyphenyl)-3-substituted-2,3-dihydro-1,3,4-oxadiazole-2-thione derivatives: promising anticancer agents. Bioorg Med Chem. 2006; 14: 1236-1246.

36. Navarrete-Vazquez G, Molina-Salinas GM, Duarte-Fajardo ZV, Vargas-Villarreal J, Estrada-Soto S, Gonzalez-Salazar F, Hernandez-Nuneza E, Said-Fernandez S. Synthesis and antimycobacterial activity of 4-(5-substituted-1,3,4-oxadiazol-2-yl)pyridines. Bioorg Med Chem. 2007; 15: 5502-5508.

37. Kucukguzel SG, Oruc EE, Rollas S, Sahin F, Ozbek A. Synthesis, characterization and biological activity of novel 4-thiazolidinones, 1,3,4-oxadiazoles and some related compounds. Eur J Med Chem. 2002; 37: 197-206.

38. Mamolo MG, Zampieri D, Vio L, Fermeglia M, Ferrone M, Pricl M, Scialino G, Banfi E. Antimycobacterial activity of new 3-substituted 5-(pyridin-4-yl)-3H-1,3,4-oxadiazol-2-one and 2-thione derivatives. Preliminary molecular modeling investigations. Bioorg Med Chem. 2005; 13: 3797-3809.

39. Mazouz F, Lebreton L, Milcent R, Burstein C. 5-Aryl-1,3,4-Oxadiazol-2(3H)-one derivatives and sulfur analogues as new selective and competitive monoamine oxidase type B inhibitors. Eur J Med Chem. 1990; 25: 659-671.

40. Zarghi A, Faizi M, Shafaghi B, Ahadian A, Khojastehpoor HR, Zanganeh V, Tabatabai SA, Shafiee A. Design and synthesis of new 2-substituted-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles as benzodiazepine receptor agonists. Bioorg Med Chem Lett. 2005; 15: 3126-3129.

41. Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A, Shafiee A. Synthesis and anticonvulsant activity of new 2-substituted-5- [2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles. Bioorg Med Chem Lett. 2004; 14: 6057-6059.

42. Lankau HJ, Unverferth K, Grunwald C, Hartenhauer H, Heinecke K, Bernoster K, Dost R, Egerland U, Rundfeldt C. New GABA-modulating 1,2,4-oxadiazole derivatives and their anticonvulsant activity. Eur J Med of Chem. 2007; 42: 873-879.

43. Gopalakrishna B, Ranghunandan N, Rao VJ, Bari S, Venkatesham A and Sarangapani M. Synthesis and anti-inflammatory activity of some new benzoxazole schiff bases. Ind Drug. 2005; 6: 369-374.

44. Husain A, Naseer MA, and Sarafroz M. Synthesis and anticonvulsant activity of some novel fused heterocyclic 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives. Acta Polo Pharm Drug Res. 2009; 66: 135-140.

45. Mavrova AT, Wesselinova D, Tsenov YA, Denkova P. Synthesis, cytotoxicity and effects of some 1,2,4-triazole and 1,3,4-thiadiazole derivatives on immunocompetent cells. Eur J Med Chem. 2009; 44: 63-69.

46. Siddiqui AA, Arora A, Siddiqui N, Misra A. Synthesis of some 1,2,4-triazoles as potential antifungal agents. Ind J Chem. 2005; 44: 838-841.

47. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia. 1978; 19: 409-428.

48. Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B, White BG. Antiepileptic drug development program. Cleve Clin Q. 1984; 51: 293-305.

49. Swinyard EA, Woodhead JH, White HS, Franklin MR. General principles: experimental selection, quantification, and evaluation of anticonvulsants. In: Levy RH, Mattson RH, Melrum B, Penry JK, Dreifuss FE. Antiepileptic drugs. Edn 3, Raven-Press, New York, 1989, 85–102.

50. Chen J, Sun XY, Chai KY, Lee JS, Song MS, Quan ZS. Synthesis and anticonvulsant evaluation of 4-(4-alkoxylphenyl)-3-ethyl-4H-1,2,4-triazoles as open-chain analogues of 7-alkoxyl-4,5-dihydro[1,2,4]triazolo[4,3-a]quinolines. Bioorg Med Chem. 2007; 15: 6775-6781.

51. Kucukguzel I, Kucukguzel SG, Rollas S, Sanis GO, Ozdemir O, Bayrak I, Altug T, Stables JP. 3-(aryl alkyl thio)-4-alkyl/aryl-5-(4-aminophenyl)-4h-1,2,4-triazole derivatives and their anticonvulsant activity. I L Farma. 2004; 59: 893-901.

52. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rat and mice. J Am Pharm Asso Sci. 1957; 46: 208–209.

53. Kwan P, Brodie MJ. Potential role of drug transporters in the pathogenesis of medically intractable epilepsy. Epilepsia. 2005; 46: 224-35.

54. Lien EJ, Liuo RCH, Shinoucla HG. Quantitative structure-activity relationships and dipole moments of

anticonvulsants and CNS depressants. J Pharm Sci. 1979; 68: 463-468.

55. Farrar VA, Ciechanowicz-Rutkowska M, Grochowski J, Serda P, Pilati T, Filippini G, Hinko CN, El-Assadi A, Moore JA, Edafiogho IO, Andrews CW, Cory M, Nicholson JM, Scott KR. Synthesis and CLOGP correlation of imidooxy anticonvulsants. J Med Chem. 1993; 36: 3517-3525.

56. Dimmock JR, Pandeya SN, Quail JW, Pugazhenthi U, Allen TM, Kao GY, Balzarini J, De Clercq E. Evaluation of the semicarbazones, thiosemicarbazones and biscarbohydrazones of some aryl alicyclic ketones for anticonvulsant and other biological properties. Eur J Med Chem. 1995; 30: 303-314.

57. White HS, Woodhead JH, Franklin MR, Mattson RH, Meldrum BS. Antiepileptic drugs. Edn 4, Raven Press, New York, 1995: 99.

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