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DESIGN AND SYNTHESIS OF NOVEL SUBSTITUTED BENZIMIDAZOLE ANALOGS AS POTENT ANTICONVULSANT AGENTS

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ABSTRACT
Based on pharmacophore model requirement a series of novel heterocyclic substituted benzimidazole derivatives were synthesized as potent anticonvulsant agents. The synthesized compounds were characterized by FT-IR, ^{1}H-NMR, Mass spectroscopy and bases of elemental analysis. MES test and *sc*PTZ test were performed for screening anticonvulsant activity. Moreover all potent compounds were examined for its neurotoxicity by rota rod test. Results revealed that entire series of compounds exhibited mild to good anticonvulsant activity. In general it was found that the pyrazolone derivatives **Va-Vi** exhibited higher antiepileptic activity than the isoxazolone derivatives **III** and pyridimidinone derivatives **IVa-IVb.** Among all test compounds the most active compound was 4-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-1-(4-methoxyphenyl)-3-amino-1*H*-pyrazol-5(4*H*)-one **Ve** and the least active was 4-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-3-aminoisoxazol-5(4*H*)-one **III**.

KEYWORDS: Benzimidazole, Isoxazole, Pyrimidine, Pyrazole, MES test, *sc*PTZ test.

INTRODUCTION
Epilepsy is a chronic neurological disorder, characterized by paroxysmal cerebral dysrhythmia, manifested as brief episodes (seizures) of loss or disturbance of consciousness, frequently followed by convulsions.[1] About 1% of the world populations have epilepsy, with almost 90% of these people being in developing countries.[2] Epilepsy also affects about 5% of individuals over their life time. In fact, epilepsy is the 3rd most often spread neurological disorder identified in the elderly after dementia and cerebrovascular diseases.[3]

It is well documented that seizures have different types that mainly depends on which part and how much of the brain is affected by the electrical disturbance. As on date there is no permanent cure for epilepsy. Current treatment options for epilepsy includes direct or indirect electrical stimulation, seizure suppression through invasive surgery and/or use of antiepileptic drugs (AED_S). It has been estimated that 20% of the patients with epilepsy using the first generation AED_S like phenytoin, sodium valproate, phenobarbitone and carbamazepine.[4] Unfortunately, despite the development of handful of new antiepileptics, at least 30% of patients associated with epilepsy continue to show experience seizures and many others do so only at the expense of significant dose-related toxicity and severe side effects that range in harshness from minimal brain impairment, megaloblastic anemia and hepatic failure.[5] These limitations with current AED_S demand the necessary for the development of more selective and lower toxicity AED_S in the field of medicinal chemistry.

The importance of heterocyclic compounds has long been recognized in the field of synthetic organic chemistry. Currently, heterocyclic compounds have been extensively studied due to their important properties and applications. It is well known that a number of heterocyclic compounds containing nitrogen exhibited a wide variety of biological activity. Among these compounds, benzimidazole derivatives have become especially noteworthy in recent years due to their wide spectrum of biological activity such as anti-microbial,[6] analgesic,[7] anti-inflammatory,[8] antiviral,[9] anticancer,[10] anti-tubercular,[11] and other activities.[12-13] Benzimidazole represents a good template for preparations of some new anticonvulsant agents since such a heterocyclic system possess the required pharmacophoric moiety.[14-15] On the other hand isoxazole, pyrimidine, and pyrazoles have gained importance because of physiological and pharmacological activities associated with them.[16-21] The anticonvulsant action of isoxazole, pyrimidine, and pyrazole moieties are ascribed to its unique properties of being an electron donor system and ability to act as a constrained pharmacophore at the receptor site.

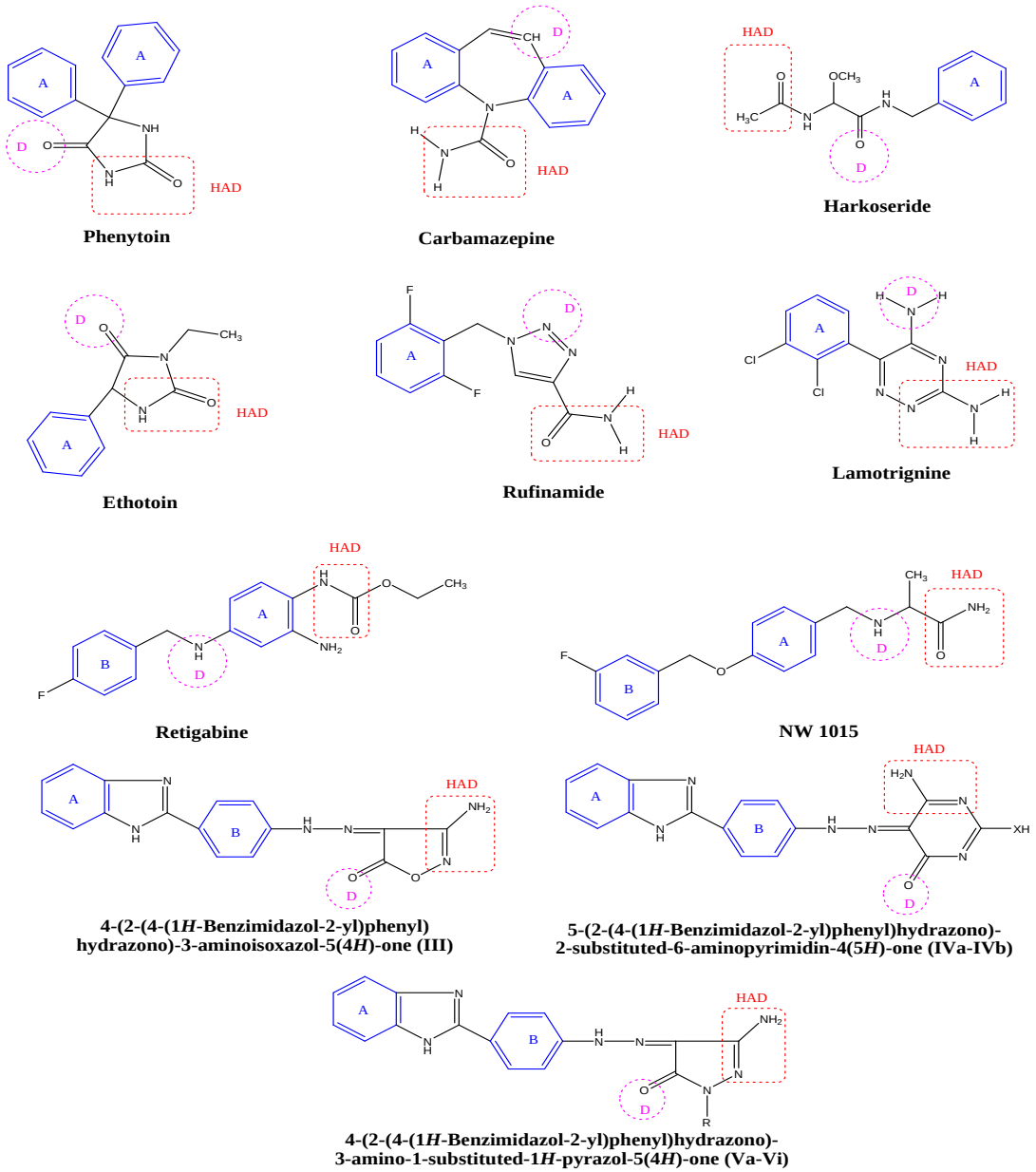


Fig. 1. Pharmacophoric pattern of well known antiepileptics and title compounds with its vital structural features: (A) hydrophobic aryl ring system, (HAD) hydrogen bond acceptor/donor domain, (D) electron donor moiety and (B) distal aryl ring.

In past, it is well documented in literature that, the essential structural features which could be responsible for an interaction with the active site of voltage gated Na^+ channels were a presence of one electron donor atom (D), Hydrogen donor/acceptor unit (HAD) and a hydrophobic domain (A).[22-23] This common template was found in the structures of first generation AED_S such as carbamazepine or phenytoin, among the newest drugs felbamate or in the second generation AED_S and the drugs in preclinical or in clinical development (Fig. 1). Much efforts devoted in the recent years based on the pharmacophore model for the development of novel therapeutics resulted in the availability of several newer drugs (such as zonisamide, topiramate, levetiracetam, tiagabine, lamotrigine, pregabalin, stiripentol) as promising antiepileptics.[24]

Based on the above findings and considering the wide applications of benzimidazole molecule in medicinal chemistry an attempt has been made to synthesize different substituted benzimidazole derivatives containing isoxazole/pyrimidine/pyrazole moiety as antiepileptic agents.

MATERIALS AND METHODS

General

The chemicals and reagents used were obtained from various chemical units Qualigens, E. Merck India Ltd., CDH and SD Fine Chem. The solvents used were of LR grade and purified before their use. The silica gel G used for analytical chromatography (TLC) was obtained from E. Merck India Ltd. All the melting points were taken in open glass capillary and are uncorrected. ^{1}H-NMR

spectra were recorded at 500 MHz on Bruker Avance-500 NMR spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an internal standard. The chemical shifts are reported in ppm scale. Mass spectra were obtained on a JEOL-SX-102 instrument using electron impact ionization. All the IR spectra were recorded in KBr pellets on a Jasco FT-IR 410 spectrometer. Elemental analyses were performed on a Perkine Elmer model 2400C analyzer and were within±0.4% of the theoretical values.

Synthesis of 4-(1H-benzimidazol-2-yl)benzenamine (I)

A mixture of *o*-phenylenediamine **1** (1.08g, 0.01mol) and *p*-aminobenzoic acid **2** (1.37g, 0.01mol) were stirred in a syrupy *o*-phosphoric acid (15ml) at 200°C for 2h. The reaction mixture was cooled and poured on crushed ice. The bulky white precipitate obtained was stirred in cold water (150ml) and sodium hydroxide solution (5M) was added until the pH 7. The resulting solid obtained **I** was filtered and recrystallized from methanol. Yield = 70%, m.p. 246-248°C. IR (KBr) cm^{-1}: 3357 (NH), 2985 (Ar-CH), 1675 (C=N), 1586 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.09-8.24 (m, 8H, Ar-CH), 5.78 (s, 1H, NH of benzimidazole), 4.30 (s, 2H, NH_2). EI-MS *m/z*: 209 (M^+). *Anal*. Calcd for $C_{13}H_{11}N_3$: C, 74.62; H, 5.30; N, 20.08. Found: C, 74.86; H, 5.28; N, 20.01.

Synthesis of ethyl 2-(2-(4-(1H-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate (II)

4-(1*H*-Benzimidazol-2-yl)benzenamine **I** (2.09 g; 0.01 mol) was dissolved in a mixture of concentrated hydrochloric acid (5ml) and water (5ml). The solution is cooled to 5°C by immersing the flask in a mixture of ice and water. The solution of powdered sodium nitrite (1.38g; 0.02mol) dissolved in water (5ml) was drop wise added to the solution of 4-(1*H*-benzimidazol-2-yl)benzenamine hydrochloride with stirring. The stirring was continued for 30min after complete addition of sodium nitrite. The obtained diazonium salt was added to a solution of ethyl 2-cyanoacetate (1.13g; 0.01mol) in ethanol (10ml) with stirring. Then the reaction mixture was stirred for 5h magnetically at room temperature and kept aside at room temperature for further 2 h. The solid product **II**, so formed, was collected by filtration and recrystallised from ethanol. Yield = 78%, m.p. 212-214 °C. IR (KBr) cm^{-1}: 3394 (NH), 3090 (Ar-CH), 2944 (CH_3-CH), 1755 (C=O), 1695 (C=N), 1551 (C=C); 1092 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.25-8.31 (m, 8H, Ar-CH), 7.03 (s, 1H, NH of hydrazone), 5.38 (s, 1H, NH of benzimidazole), 4.16-4.42 (t, 2H, CH_2), 1.04-1.40 (t, 3H, CH_3). EI-MS *m/z*: 333 (M^+). *Anal*. Calcd for $C_{18}H_{15}N_5O_2$: C, 64.86; H, 4.54; N, 21.01. Found: C, 65.07; H, 4.52; N, 20.94.

Synthesis of 4-(2-(4-(1H-benzimidazol-2-yl)phenyl)hydrazono)-3-aminoisoxazol-5(4H)-one (III)

A mixture of ethyl 2-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate **II** (3.33 g; 0.01 mol) and hydroxylamine hydrochloride (1.04 g; 0.015 mol) in ethanol (20ml) was refluxed for 12 h in water bath. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water and stirred well. The product obtained **III** was filtered, washed with water twice, dried and recrystallised. Yield = 72%, m.p. 236-237°C. IR (KBr) cm^{-1}: 3395 (NH), 3043 (Ar-CH), 1757 (C=O), 1695 (C=N), 1551 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 6.82-7.96 (m, 8H, Ar-CH), 6.49 (s, 1H, NH of hydrazone), 5.07 (s, 1H, NH of benzimidazole), 2.51 (s, 2H, NH_2). EI-MS *m/z*: 320 (M^+). *Anal*. Calcd for $C_{16}H_{12}N_6O_2$: C, 60.00; H, 3.78; N, 26.24. Found: C, 59.81; H, 3.79; N, 26.31.

Synthesis of 5-(2-(4-(1H-benzimidazol-2-yl)phenyl)hydrazinyl)-2-substituted-6-aminopyrimidin-4(5H)-one (IVa-IVb)

A mixture of ethyl 2-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate **II** (3.33g; 0.01mol), urea/thiourea (0.015 mol) and potassium carbonate (0.2g) in ethanol (20ml) was refluxed for 15 h on a water bath. On cooling the solid separated out was filtered. The residue was dissolved in hot water and filtered when hot. The filtrate was neutralized with acetic acid and the solid precipitated out **Iva-IVb** was filtered and recrystallised from ethanol.

5-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-2-hydroxy-6-aminopyrimidin-4(5H)-one (IVa)

Yield = 81%, m.p. 203-205 °C. IR (KBr) cm^{-1}: 3545 (OH), 3397 (NH), 3018 (Ar-CH), 1754 (C=O), 1693 (C=N), 1658 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.16-8.14 (m, 8H, Ar-CH), 6.72 (s, 1H, NH of hydrazone), 5.45 (s, 1H, NH of benzimidazole), 2.16 (s, 2H, NH_2), 2.08 (s, 1H, OH). EI-MS *m/z*: 347 (M^+). *Anal*. Calcd for $C_{17}H_{13}N_7O_2$: C, 58.79; H, 3.77; N, 28.23. Found: C, 58.95; H, 3.78; N, 28.14.

5-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-2-mercapto-6-aminopyrimidin-4(5H)-one (IVb)

Yield = 74%, m.p. 222-223 °C. IR (KBr) cm^{-1}: 3397 (NH), 3087 (Ar-CH), 2523 (SH), 1757 (C=O), 1695 (C=N), 1654 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.31-8.37 (m, 8H, Ar-CH), 6.90 (s, 1H, NH of hydrazone), 5.29 (s, 1H, NH of benzimidazole), 2.38 (s, 2H, NH_2), 1.72 (s, 1H, SH). EI-MS *m/z*: 363 (M^+). *Anal*. Calcd for $C_{17}H_{13}N_7OS$: C, 56.19; H, 3.61; N, 26.98. Found: C, 56.38; H, 3.60; N, 27.05.

Synthesis of 4-(2-(4-(1H-benzimidazol-2-yl)phenyl)hydrazinyl)-3-amino-1-substituted-1H-pyrazol-5(4H)-one (Va-Vi)

A mixture of ethyl 2-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate **II** (3.33 g; 0.01 mol) and various hydrazine hydrochloride (0.015 mol) in ethanol (20ml) was refluxed for18 h in water bath. After removal of ethanol in vacuum, the oil obtained was poured into ice cold water and stirred well. The product separated **Va-Vi** was filtered, dried and recrystallised.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-3-amino-1H-pyrazol-5(4H)-one (Va)
Yield = 79%, m.p. 247-249°C. IR (KBr) cm^{-1}: 3395 & 3234 (NH), 3042 (Ar-CH), 1763 (C=O), 1681 (C=N), 1656 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.04-7.83 (m, 8H, Ar-CH), 6.61 (s, 1H, NH of hydrazone), 6.39 (s, 1H, NH of pyrazole), 5.05 (s, 1H, NH of benzimidazole), 2.13 (s, 2H, NH_2). EI-MS *m/z*: 319 (M^+). *Anal*. Calcd for $C_{16}H_{13}N_7O$: C, 60.18; H, 4.10; N, 30.70. Found: C, 60.03; H, 4.09; N, 30.81.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-3-amino-1-phenyl-1H-pyrazol-5(4H)-one (Vb)
Yield = 77%, m.p. 234-235°C. IR (KBr) cm^{-1}: 3372 & 3257 (NH), 3065 (Ar-CH), 1722 (C=O), 1671 (C=N), 1628 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.28-8.10 (m, 13H, Ar-CH), 7.16 (s, 1H, NH of hydrazone), 5.41 (s, 1H, NH of benzimidazole), 2.59 (s, 2H, NH_2). EI-MS *m/z*: 395 (M^+). *Anal*. Calcd for $C_{22}H_{17}N_7O$: C, 66.82; H, 4.33; N, 24.80. Found: C, 67.01; H, 4.35; N, 24.74.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-(4-chlorophenyl)-3-amino-1H-pyrazol-5(4H)-one (Vc)
Yield = 72%, m.p. 219-220°C. IR (KBr) cm^{-1}: 3392 & 3310 (NH), 3015 (Ar-CH), 1749 (C=O), 1679 (C=N), 1601 (C=C), 835 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 6.70-7.76 (m, 12H, Ar-CH), 6.28 (s, 1H, NH of hydrazone), 5.13 (s, 1H, NH of benzimidazole), 2.25 (s, 2H, NH_2). EI-MS *m/z*: 431 (M^{+2}), 429 (M^+). *Anal*. Calcd for $C_{22}H_{16}ClN_7O$: C, 61.47; H, 3.75; N, 22.81. Found: C, 61.29; H, 3.76; N, 22.89.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-(4-fluorophenyl)-3-amino-1H-pyrazol-5(4H)-one (Vd)
Yield = 78%, m.p. 241-244°C. IR (KBr) cm^{-1}: 3392 & 3365 (NH), 3006 (Ar-CH), 1730 (C=O), 1694 (C=N), 1626 (C=C), 1061 (C-F). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.17-8.20 (m, 12H, Ar-CH), 6.81 (s, 1H, NH of hydrazone), 5.36 (s, 1H, NH of benzimidazole), 2.40 (s, 2H, NH_2). EI-MS *m/z*: 413 (M^+). *Anal*. Calcd for $C_{22}H_{16}FN_7O$: C, 63.92; H, 3.90; N, 23.72. Found: C, 64.10; H, 3.89; N, 23.75.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-(4-methoxyphenyl)-3-amino-1H-pyrazol-5(4H)-one (Ve)
Yield = 80%, m.p. 250-252°C. IR (KBr) cm^{-1}: 3341 & 3259 (NH), 3094 (Ar-CH), 2976 (CH_3-CH), 1715 (C=O), 1684 (C=N), 1632 (C=C), 1051 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.01-8.35 (m, 12H, Ar-CH), 6.67 (s, 1H, NH of hydrazone), 5.04 (s, 1H, NH of benzimidazole), 3.79 (s, 3H, OCH_3), 2.02 (s, 2H, NH_2). EI-MS *m/z*: 425 (M^+). *Anal*. Calcd for $C_{23}H_{19}N_7O_2$: C, 64.93; H, 4.50; N, 23.05. Found: C, 64.77; H, 4.52; N, 23.11.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-(3-chlorophenyl)-3-amino-1H-pyrazol-5(4H)-one (Vf)
Yield = 78%, m.p. 199-201 °C. IR (KBr) cm^{-1}: 3389 & 3302 (NH), 3049 (Ar-CH), 1724 (C=O), 1688 (C=N), 1640 (C=C), 856 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 6.78-8.09 (m, 12H, Ar-CH), 6.42 (s, 1H, NH of hydrazone), 5.27 (s, 1H, NH of benzimidazole), 2.57 (s, 2H, NH_2). EI-MS *m/z*: 431 (M^{+2}), 429 (M^+). *Anal*. Calcd for $C_{22}H_{16}ClN_7O$: C, 61.47; H, 3.75; N, 22.81. Found: C, 61.67; H, 3.74; N, 22.74.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-(3-fluorophenyl)-3-amino-1H-pyrazol-5(4H)-one (Vg)
Yield = 73%, m.p. 275-276°C. IR (KBr) cm^{-1}: 3395 & 3366 (NH), 3094 (Ar-CH), 1753 (C=O), 1700 (C=N), 1592 (C=C), 1073 (C-F). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.23-8.18 (m, 12H, Ar-CH), 6.79 (s, 1H, NH of hydrazone), 5.40 (s, 1H, NH of benzimidazole), 2.29 (s, 2H, NH_2). EI-MS *m/z*: 413 (M^+). *Anal*. Calcd for $C_{22}H_{16}FN_7O$: C, 63.92; H, 3.90; N, 23.72. Found: C, 64.07; H, 3.91; N, 23.65.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-3-amino-5-oxo-4,5-dihydropyrazole-1-carbothioamide (Vh)
Yield = 74%, m.p. 208-210 °C. IR (KBr) cm^{-1}: 3358 & 3324 (NH), 3085 (Ar-CH), 1719 (C=O), 1672 (C=N), 1617 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 6.69-7.95 (m, 8H, Ar-CH), 6.34 (s, 1H, NH of hydrazone), 5.12 (s, 1H, NH of benzimidazole), 2.80 (s, 2H, NH_2), 2.46 (s, 2H, NH_2). EI-MS *m/z*: 378 (M^+). *Anal*. Calcd for $C_{17}H_{14}N_8OS$: C, 53.96; H, 3.73; N, 29.61. Found: C, 53.79; H, 3.74; N, 29.70.

4-(2-(4-(1H-Benzimidazol-2-yl)phenyl)hydrazono)-1-isonicotinoyl-3-amino-1H-pyrazol-5(4H)-one (Vi)
Yield = 77%, m.p. 227-229°C. IR (KBr) cm^{-1}: 3391 & 3315 (NH), 3102 (Ar-CH), 1759 (C=O), 1701 (C=N), 1601 (C=C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.36-8.42 (m, 12H, Ar-CH), 6.85 (s, 1H, NH of hydrazone), 5.38 (s, 1H, NH of benzimidazole), 2.34 (s, 2H, NH_2). EI-MS *m/z*: 424 (M^+). *Anal*. Calcd for $C_{22}H_{16}N_8O_2$: C, 62.26; H, 3.80; N, 26.40. Found: C, 62.43; H, 3.79; N, 26.35.

Biological activities

Pharmacology

All the synthesized compounds were evaluated for their antiepileptic effects using male albino mice (Swiss, 18-25g) and rat (Wistar 100-150g). The primary qualitative evaluations were performed in mice involved two epilepsy tests (MES and *sc*PTZ test). Acute neurological toxicity induced by the compounds in mice was assessed through standardized rotorod test. In the initial screening, candidate compounds were screened for their antiepileptic potential through MES and *sc*PTZ models in mice at a dose level of 30, 100 and 300mg/kg by intraperitoneal (*i.p*) route and the groups of mice are tested at different time points (i.e., 0.5 and 4h) post administration of the test candidate. It is generally acknowledged that the MES model, which uses an electrical stimulus, induces generalized tonic-clonic seizures. Through electrical induction, it is used to help recognize those compounds which prevent seizure

spread. The *sc*PTZ is a model where the myoclonic seizures induced by chemical induction. It helps in identifying those compounds that might act by increasing seizure threshold. Each group consisted of six animals. The animals were maintained in colony cages at 25±2°C, relative humidity of 45–55%, under a 12h light and dark cycle; were fed standard animal feed.[25] All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

Antiepileptic activity

The maximal electroshock test (MES)

The MES is a model for generalized tonic-clonic seizures and provides a hint of a compound's ability to stop seizure spread when all neuronal circuits in the brain are maximally active. These seizures are extremely reproducible and are electro physiologically reliable with human seizures. For the MES, a drop of anesthetic and electrolyte solution (tetracaine hydrochloride (0.5%) in saline (0.9%)) was applied to the eyes of individual animal before to placement of the corneal electrodes. The electrical stimulus in the MES test was 50 milli Ampere, 60 Hz, for mice and 150 milli Ampere, 60 Hz, for rats delivered for 0.2 seconds by an apparatus similar to previously reported method.[26-27] Abolition of the hindleg tonic extensor component of the seizure was used as the endpoint. Mice are initially tested with different doses of 30, 100 and 300mg/kg of test compound given by *i.p.* injection at various intervals while rats are initially screened at a fixed dose of 30mg/kg given by oral route.

The subcutaneous pentylenetetrazole seizure test (scPTZ)

Subcutaneous injection of the convulsant Pentylenetetrazole produces clonic seizures in laboratory animals. The scPTZ test detects the ability of test compounds to raise the seizure threshold of an animal and thus protect it from exhibiting a clonic seizure. Animals are pretreated with various doses of the test compound given by *i.p.* injection. The dose of Pentylenetetrazole which induce convulsions in 97% of animals (CD_{97}: 85mg/kg mice) is injected into a loose fold of skin in the midline of the neck. The animals are placed in isolation cages to minimize stress and observed for the next 30 min for the presence or absence of a seizure. An episode of clonic spasms, approximately 3-5 seconds, of the fore and/or hindlimbs, jaws, or vibrissae is taken as the endpoint. Animals which do not meet this criterion are considered protected.[28]

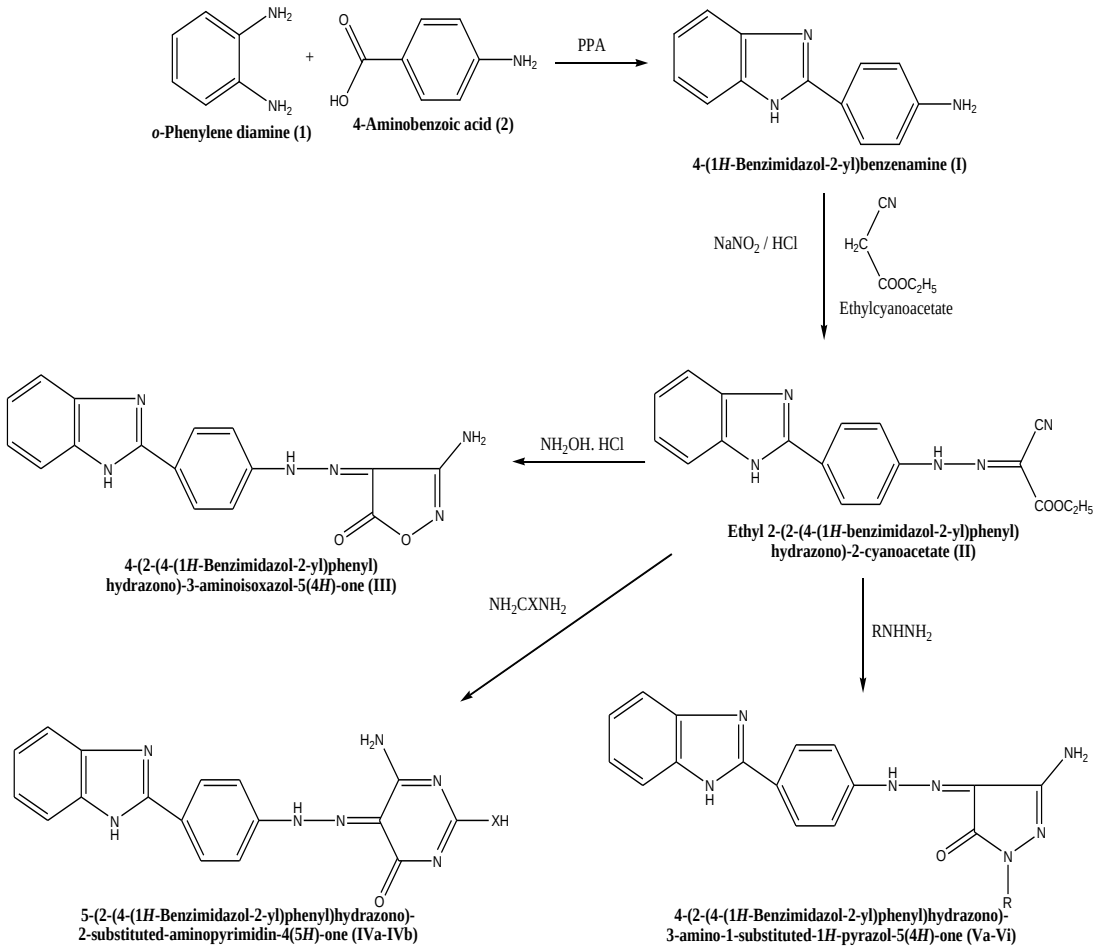
Acute toxicity-minimal motor impairment

To assess a compound's undesirable side effects (toxicity), animals are monitored for overt signs of impaired neurological or muscular function. In mice, the rotorod procedure is used to disclose minimal muscular (MMI) or neurological impairment (MNI).[29] When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for long periods of time. The animal is considered toxic if it falls off this rotating rod three times during a 1 min period. In addition to MMI, animals may exhibit a circular or zigzag gait, abnormal body posture and spread of the legs, tremors, hyperactivity, lack of exploratory behavior, somnolence, stupor, catalepsy, loss of placing response and changes in muscle tone.

RESULTS AND DISCUSSION

Chemistry

The title compounds **III, IVa-IVb & Va-Vi** were synthesized as per the protocol shown in Scheme 1. By substituting different groups at imine nitrogen, a sequence of novel 1-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazone analogs were synthesized in the present study. From *o*-phenylenediamine & *p*-amino benzoic acid, a sequence of novel heterocyclic substituted 1-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono analogs **III, IVa-IVb & Va-Vi** were synthesized by a multistep synthesis. Primarily *o*-phenylenediamine **1** was treated with *p*-amino benzoic acid **2** in presence of polyphosphoric acid (PPA) to obtain 4-(1*H*-benzimidazol-2-yl)benzenamine **I** by cyclisation reaction. In the second step, obtained amino derivative **3** was diazotized using sodium nitrite and hydrochloric acid. Subsequently the diazotized salt was treated with ethylcyanoacetate to produce ethyl 2-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate **II** through intramolecular rearrangement reaction. In the final step, the obtained keto ester **II** undergoes dehydrative cyclisation with various amine derivatives such as hydroxylamine hydrochloride, urea/thiourea, and different hydrazine hydrochloride analogs and produces corresponding isoxazole **III**, pyrimidine **IVa-IVb**, and pyrazole derivatives **Va-Vi**, respectively. To optimize the reactions for purity and completion, TLC was performed throughout the reactions.



IVa) X = O; IVb) X = S; Va) R = H; Vb) R = C_6H_5; Vc) R = *p*-ClC_6H_4; Vd) R = *p*-FC_6H_4; Ve) R = *p*-$OCH_3C_6H_4$; Vf) R = *m*-ClC_6H_4; Vg) R = *m*-FC_6H_4; Vh) R = $CSNH_2$; Vi) R = C_5H_4NCO

Scheme 1: Synthesis of novel substituted benzimidazole analogs

Assigned structures of the test compound were confirmed using various spectral studies (IR, NMR, mass spectra, and elemental analyses). Presence of particular groups was identified from IR spectra by means of some characteristic absorption peaks. Absence of a broad absorption peak between 3000-3500 cm^{-1} corresponds to COOH and presence of absorption peak at 3357 cm^{-1} in IR due to presence of NH stretching, confirms the formation of 4-(1*H*-benzimidazol-2-yl)benzenamine **I.** It is further supported by appearance of singlet in its ^{1}H-NMR spectra at δ 5.78 and 4.30 ppm for one and two proton which might be assigned to NH and NH_2 proton, respectively. The formations of keto ester **II** was confirmed from the appearance of sharp peak at 1755 cm^{-1} in IR corresponds to C=O stretching and appearance of two singlet peaks at δ 7.03, and 5.38 ppm for one proton each which might be assigned to NH of hydrazone, and NH of benzimidazole proton, respectively. This is further confirmed from NMR spectroscopy by the appearance of tetret peak at δ 4.16-4.42ppm for two protons which might be assigned to CH_2 of C_2H_5 and triplet peak at δ 1.04-1.40 ppm for three protons which might be assigned to CH_3 of C_2H_5. The formation of novel heterocyclic substituted 1-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazone analogs **III, IVa-IVb & Va-Vi** from ethyl 2-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-2-cyanoacetate **II** can be recognized from the disappearance of tetret and triplet peaks in its ^{1}H-NMR spectra corresponds to the CH_2 and CH_3 of C_2H_5. The structure of title compounds **III, IVa-IVb & Va-Vi** were further confirmed by appearance of various other peaks in NMR spectroscopy corresponds to assigned structure. Mass spectrum further confirmed their molecular weight and purity.

Biological activities

Antiepileptic activity

The maximal electroshock test (MES)

For the identification of antiepileptic activity in mice, test compounds were administered *i.p.* and challenged by maximal electroshock (MES) and subcutaneous pentylenetetrazole seizure **(***sc*PTZ) test. Compounds found to be active in these seizure challenges are generally regarded to be significantly useful candidates in treatment of partial, generalized and even absence seizures. The data regarding the antiepileptic screening of all the compounds are reported in Table 1.

Table 1: Antiepileptic activity and neurotoxicity of compounds III, IVa-IVb & Va-Vi administered intraperitoneally to mice

Compound	MES[a] screening		*sc*PTZ[b] screening		NT[c] screening	
	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]	0.5 h[d]	4.0 h[d]
III	-	-	-	-	ND	ND
IVa	300	-	-	-	ND	ND
IVb	-	300	-	-	ND	ND
Va	100	300	-	300	ND	ND
Vb	30	100	100	300	-	-
Vc	100	100	300	300	300	-
Vd	100	100	300	300	-	300
Ve	30	30	100	300	-	-
Vf	100	100	300	300	300	-
Vg	100	100	300	300	-	300
Vh	300	300	300	-	ND	ND
Vi	300	300	-	300	ND	ND
Phenytoin[e]	30	30	-	-	100	100
Ethosuximide[f]	-	-	100	300	-	-

a Maximal electroshock test (administered intraperitoneally to mice at doses ranging from 30 to 300mg/kg).
b Subcutaneous pentylenetetrazole test (administered intraperitoneally to mice at doses ranging from 30 to 300mg/kg).
c Neurotoxicity (administered intraperitoneally to mice at doses ranging from 30 to 300mg/kg).
d Time of test after drug administration.
e Reference drug, data for phenytoin ref.[30]
f Reference drug, data for ethosuximide ref.[31]
The sign - (mdash) represents an absence of activity at maximum dose administered (300mg/kg).
ND - Not determined.

Out of several tested compounds, two compounds **Vb** and **Ve** were found to be significantly active in the electroshock investigation as they showed protection at the lowest dose of 30mg/kg after 0.5h. But at same dose (30mg/kg) compound **Ve** continued to show the activity after 4.0h; whereas compound **Vb** needs 100mg/kg, indicating the rapid onset as well as long duration of action of these compounds. The promising activity of the compounds may be attributed to the electron donor group present in phenyl ring attached at C-1 of pyrazole. After 0.5h, at 100mg/kg compounds **Va, Vc, Vd, Vf** and **Vg** were showed protection indicating the ability of these compounds to protect from seizures at relatively lower dose. These compounds except **Va** were also active after 4.0h at 100mg/kg dose; whereas **Va** was found to be active at 300mg/kg after 4.0 h. Compounds **IVa, IVb, Vh** and **Vi** were found to be active at a dose of 300mg/kg either after 0.5 h and/ 4.0 h. Rest of test compound **III** doesn't showed protection at any of the tested dose.

The subcutaneous pentylenetetrazole seizure test (scPTZ)

Most of the compounds exhibited moderate to good antiepileptic activity in the *sc*PTZ screening. Compounds that revealed protection in the *sc*PTZ test indicating the ability of substance to increasing seizure threshold. At a dose of 100mg/kg compounds **Vb** and **Ve** showed protection after 0.5 h. But at higher doses (300mg/kg) these compounds continued to show the activity after 4.0 h indicating the rapid onset as well as long duration of action of these compounds. The above results were comparable to results obtained for ethosuximide which is recognized as reference AED for this screen. Compounds **Vc, Vd, Vf** and **Vg** showed protection at 300mg/kg after 0.5 h and 4.0 h. Except **III, IVa** and **IVb** rest of compounds **Va, Vh** and **Vi** were active at 300mg/kg either after 0.5 h or 4.0 h. It was observed that in this method, the most active compound have electron donor group substituted phenyl ring at C-1 of pyrazole ring resulted in increased antiepileptic activity.

Except compound **III** rest of other tested compounds that are, **IVa-IVb,** and **Va-Vi** were showed activity in either MES or *sc*PTZ model at any one of the tested dose after 0.5h. The study reveals that 75% of the compounds that is **Va-Vi** were shown activity in *sc*PTZ test, whereas in MES screening except compound **III,** rest of 92% of the compounds were active at any one of the tested dose. These reports revealed that maximum of compounds possessed some MES selectivity.

Acute toxicity-minimal motor impairment

Neurotoxicity study was evaluated by rotorod test in mice. The study revealed that most of the candidate compounds exhibited neurotoxicity at doses higher than widely prescribed drugs Phenytoin or Carbamazepine. But while evaluating an antiepileptic compounds, separation between antiepileptic and neurotoxic dose is desirable. All the compounds evaluated for its neurotoxicity study except **III, IVa-IVb, Va, Vh** and **Vi,** due to its poor response in antiepileptic activity. In neurotoxic study at 100mg/kg dose **Vc** was found to be neurotoxic. Compounds **Vd, Vf** and **Vg** were showed neurotoxicity at 300mg/kg, while all other compounds

Vb and **Ve** were not found to be neurotoxic at maximum administered dose.

The maximal electroshock test (MES) of selected compounds by oral route

Ability to inhibit epilepsy when given by the oral route is a valuable property of candidate antiepilepsy. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of antiepileptic activity or neurotoxicity. We identified two compounds **Vb** and **Ve** from the initial screen that were further evaluated for oral availability using the MES acute seizure model and neurotoxicity in rats at a dose of 30mg/kg. The results obtained are presented in Table 2.

Table 2 Antiepileptic activity and toxicity of compounds Vb and Ve administered orally (30mg/kg) to rats.

Compound	MES[a]					TOX[b]
	0.25 h[c]	0.5 h[c]	1 h[c]	2 h[c]	4 h[c]	
Vb	1/4	2/4	2/4	3/4	3/4	0/4 (-)[d]
Ve	1/4	2/4	3/4	4/4	4/4	0/4 (-)[d]
Phenytoin[e]	1/4	4/4	3/4	3/4	3/4	0/4 (-)[d]

[a] *maximal electroshock test (dose of 30mg/kg was administrated. The data indicate: number of rats protected /number of rats tested).*
[b] *Neurotoxicity (number of rats protected /number of rats tested).*
[c] *Time after drug administration.*
[d] *(-) No neurotoxicity at dose tested.*
[e] *Reference drug, data for phenytoin ref.*[30]

From these data it was observed that the most active compound is **Ve** which protected 100% (4/4) of rats at time points 2h and 4h, 75% (3/4) at 1 h, 50% (2/4) at 0.5 h and 25% (1/4) at 0.25 h. These molecules were more active and showed longer duration of satisfactory action than Phenytoin. While, compounds **Vb** were found moderately effective in rat MES oral screen and protected only 75% (3/4) of rats at time points 2 h and 4 h, 50% (2/4) at 0.5h and 1 h and 25% (1/4) at 0.25h. All derivatives tested were found devoid of neurotoxicity when given orally at a dose of 30mg/kg. The *in vivo* data in rats confirmed absorption of compounds from gastrointestinal tract and also their penetration to central nervous system. The inhibition of electrically induced seizures that is characteristic for Phenytoin and Phenytoin-like drugs may indicate the influence of compounds on voltage depended Na^+ channels as the most plausible mechanism of antiepileptic action.

Structure activity relationship

On correlating the structures of the sample candidate with their biological activity, it has been observed that, out of twelve tested compounds, two compounds **Vb** and **Ve** exhibited better activity in MES and/ *sc*PTZ test. These two above mentioned compounds possess pyrazolone nucleus coupled benzimidazole hydrazone. The nature of substituted ring on C_2 of benzimidazole ring appeared to greatly influence the antiepileptic activity; the pyrazolone derivatives **Va-Vi** exhibited higher antiepileptic activity than the isoxazolone derivatives **III** and pyridimidinone derivatives **IVa-IVb.** Among pyrazolone substituted compounds, Phenyl substituted compounds **Vb-Vg** exhibited better activity than unsubstituted derivatives **Va,** carbothioamide derivatives **Vh** and isonicotinoyl analogs **Vi**. Within phenyl substituted pyrazolone derivatives **Vb-Vg,** test compound **Ve** exhibited highest activity. The increases in antiepileptic activity of compound **Ve** may be attributed to the presence of electron releasing group (methoxy) on phenyl ring (which is absent in rest of derivatives) might be accountable for additional bonding with the binding site. In general electron releasing group substituted phenyl ring containing pyrazolone analogs **Ve** exhibited highest activity followed by unsubstituted phenyl ring containing pyrazolone analogs **Vb** and electron donating group substituted phenyl ring containing pyrazolone analogs **Vc-Vd** and **Vf-Vg**.

CONCLUSION

In conclusion, a series of novel heterocyclic substituted benzimidazole derivatives **III, IVa-IVb, Va-Vi** were synthesized and characterized by IR, NMR, mass spectroscopy, and elemental analyses. Entire test compounds were evaluated for their antiepileptic activity by MES and *sc*PTZ model along with its neurotoxicity. All title compounds exhibited various degree of antiepileptic and neurotoxicity activities. In this series, generally it was found that the pyrazolone derivatives exhibited higher antiepileptic activity than the isoxazolone derivatives and pyridimidinone derivatives. Among pyrazolone substituted compounds, phenyl substituted compounds exhibited better activity than unsubstituted derivatives, carbothioamide derivatives and isonicotinoyl analogs. Within phenyl substituted pyrazolone derivatives electron releasing group substituted phenyl ring containing pyrazolone analogs exhibited highest activity followed by unsubstituted phenyl ring containing pyrazolone analogs and electron donating group substituted phenyl ring containing pyrazolone analogs. From the study we identified two compounds **Vb** and **Ve** as potent compounds that were further evaluated for oral availability using the MES acute seizure model and neurotoxicity in rats at a dose of 30mg/kg. Compound **Ve** exhibited better antiepileptic activity in oral dose than standard drug phenytoin. Among all test compounds the most active compound was 4-(2-(4-(1*H*-benzimidazol-2-yl)phenyl)hydrazono)-1-(4-methoxyphenyl)-3-amino-1*H*-pyrazol-5(4*H*)-one **Ve**

that revealed protection in the electrically induced seizures at a dose of 30mg/kg (*i.p.*) after 0.5 h and 4 h. This molecule also provided protection in the *sc*PTZ at a dose of 100mg/kg and 300mg/kg after 0.5 h and 4 h, respectively. Thus the compound **Ve** emerged out as the lead molecule with a wide spectrum of antiepileptic activity without any neurotoxicity.

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REFERENCES

1. McNamara JO. Emerging insights into the genesis of epilepsy. Nature, 1999; 339: A15-22.
2. Fisher R, Boas WVE, Blume W, Elger C, Genton P, Lee P, Engel J. Epileptic seizures and epilepsy: Definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia, 2005; 46: 470-2.
3. Kramer G. Epilepsy in the elderly: Some clinical and pharmacotherapeutic aspects. Epilepsia, 2001; 42: 55-9.
4. Mattson RH, Levy R, Mattson RH, Meldrum R. Antiepileptic drugs. Raven Press, New York, 1995; 123-35.
5. Brown TR, Holmes GLN. Epilepsy. N Eng J Med., 2001; 344: 1145-51.
6. Sri Krishnanjaneyulu I, Saravanan G, Vamsi J, Supriya P, Udaya Bhavana J, Venkata Sunil Kumar M. Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives. J Adv Pharm Tech Res., 2014; 5: 21-7.
7. Monika G, Chander M. Design, synthesis and biological evaluation of novel 1,2,5-substituted benzimidazole derivatives as gastroprotective anti-inflammatory and analgesic agents. Med Chem., 2015; 5: 58-63.
8. Kaur G, Kaur M, Silakari O. Benzimidazoles: An ideal privileged drug scaffold for the design of multitargeted anti-inflammatory ligands. Mini Rev Med Chem., 2014; 14: 747-67.
9. Jun C, Jiangtao X, Xianjin L. Synthesis and antiviral activity against Coxsackie virus B3 of some novel benzimidazole derivatives. Bioorg Med Chem Lett., 2005; 15: 267-9.
10. Castro AR, Rivera IL, Rojas LC, Vazquez GN, Rodríguez AN. Synthesis and preliminary evaluation of selected 2-aryl-5(6)-nitro-1*H*-benzimidazole derivatives as potential anticancer agents. Arch Pharm Res., 2011; 34: 181-9.
11. Yoon YK, Ali MS, Wei AC, Choon TS, Ismail R. Synthesis and evaluation of antimycobacterial activity of new benzimidazole aminoesters. Eur J Med Chem., 2015; 93: 614-24.
12. Swiqtkiewicz KB, Olasik EM. Some characteristics of activity of potential chemotherapeutics - Benzimidazole derivatives. Adv Med Sci., 2015; 60: 125-32.
13. Akhtar W, Khan MF, Verma G, Shaquiquzzaman M, Rizvi MA, Mehdi SH, Akhter M, Alam MM. Therapeutic evolution of benzimidazole derivatives in the last quinquennial period. Eur J Med Chem., 2017; 126: 705-53.
14. Bhrigu B, Siddiqui N, Pathak D, Alam MS, Ali R, Azad B. Anticonvulsant evaluation of some newer benzimidazole derivatives: Design and synthesis. Acta Pol Pharm., 2012; 69: 53-62.
15. Siddiqui N, Alam MS, Ali R, Yar SM, Alam O. Synthesis of new benzimidazole and phenylhydrazinecarbothiomide hybrids and their anticonvulsant activity. Med Chem Res., 2016; 25: 1390-402.
16. Jagdish K, Mymoona A, Chanda R, Gita C. Design, synthesis and neuropharmacological evaluation of thiophene incorporated isoxazole derivatives as antidepressant and antianxiety agents. Int J Pharm Chem Anal, 2015; 2: 74-83.
17. Tochiro T, Kazuhisa NA, Shuji K, Shinji I, Hiroshi M, Hiroshi YA, Michinao MB, Takao SB, Hideomi F. Isoxazole derivatives as centrally acting muscle relaxants. III. Synthesis and activity of conformationally restricted analogs. Chem Pharm Bull, 1987; 35: 3676-90.
18. Mona MH. New pyrimido[5,4-*e*]pyrrolo[1,2-*c*]pyrimidines: Synthesis, 2D-QSAR, anti-inflammatory, analgesic and ulcerogenicity studies. Eur J Med Chem., 2012; 55: 12-22.
19. Bhimanna K, Hesham F. Thiazolo[4,5-*d*]pyrimidines as a privileged scaffold in drug discovery. Eur J Med Chem., 2016; 113: 198-213.
20. Alagarsamy V, Saravanan G. Synthesis and anticonvulsant activity of novel quinazolin-4(3*H*)-one derived pyrazole analogs. Med Chem Res., 2012; 22: 1711-22.
21. Panneer Selvam T, Vijayaraj Kumar P, Saravanan G, Prakash CR. Microwave assisted synthesis, characterization and biological activity of novel pyrazole derivatives. J Saudi Chem Soc., 2014; 18: 1015-21.
22. Bruno-Blanch L, Galvez J, Garcia-Domenach R. Topological virtual screening: A way to find new anticonvulsant drugs from chemical diversity. Bioorg Med Chem Lett., 2003; 13: 2749-54.
23. Estrada E, Pena A. In silico studies for the rational discovery of anticonvulsant compounds. Bioorg Med Chem., 2000; 8: 2755-70.
24. Stefan H, Feuerstein TJ. Novel anticonvulsant drugs. Pharmacol Ther., 2007; 113: 165-83.

25. Olfert ED, Cross BM, McWilliam AA. Canadian council of animal care guide to the care and use of experimental animals, 1993; 1: 2nd edn.
26. Woodbury LA, Davenport VD. Design and use of a new electroshock apparatus and analysis of factors altering seizure threshold and pattern. Arch Int Pharmacodyn Ther., 1952; 92: 97-107.
27. White HS, Johnson M, Wolf HH, Kupferberg HJ. The early identification of anticonvulsant activity: The role of maximal electroshock and subcutaneous pentylenetetrazole seizure models. Ital J Neurol Sci., 1995; 16: 73-7.
28. Swinyard EA, Clark LD, Miyahara JT, Wolf HH. Studies on the mechanism of amphetamine toxicity in aggregated mice. J Physiol, 1961; 132: 97-102.
29. Dunham MS, Miya TA. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Sci., 1957; 46: 208–9.
30. Yogeeswari P, Sriram D, Ragavendran JV, Thirumurugan R. The GABA shunt: An attractive and potential therapeutic target in the treatment of epileptic disorders. Curr Drug Metab, 2005; 6: 127-39.
31. Rajak H, Deshmukh R, Aggarwal N, Kashaw S, Kharya MD, Mishra P. Synthesis of novel 2,5-disubstituted-1,3,4-thiadiazoles for their potential anticonvulsant activity: Pharmacophoric model studies. Arch Pharm, 2009; 342: 453-61.