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Biheterocyclic Benzothiazoles with Anticonvulsant, Antidepressant and Antinociceptive Activity: Design, Synthesis & Pharmacological Evaluation

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Abstract
*A novel series of N-thiazol-2-yl-benzamide derivatives **3a-u** was synthesized and evaluated in vivo for anticonvulsant, antidepressant, and antinociceptive activity. Anticonvulsant activity was assessed using the maximal electroshock and pentylenetetrazole-induced seizure tests in mice, with sodium valproate and phenytoin sodium as reference drugs. The majority of compounds demonstrated robust activity against MES, and all exhibited promising activity against PTZ-induced seizures; compounds **3d** and **3g** provided complete protection. Acute neurotoxicity was evaluated using the rotarod test. Compounds **3d** and **3g** exhibited the greatest potency without neurotoxicity compared to traditional pharmacotherapies. Compound **3d** exhibited a median hypnotic dose of 867 mg/kg, approximately twice its TD50 value, while compound **3g** displayed an HD50 of 632 mg/kg and an LD50 of 834 mg/kg. Compound **3d** demonstrated significant antinociceptive activity ($p < 0.01$) with an increased reaction time. The physicochemical properties and pharmacokinetic profiles of the target compounds were also evaluated.*
Key-words: Benzothiazole, In-vivo Anticonvulsant, Antiepileptic, Antidepressants, Antinociceptive

INTRODUCTION

Epilepsy is a severe neurological disorder that carries social stigma, often occurs with psychiatric conditions, and imposes substantial economic costs. It affects approximately 1% of the global population, with 2.4 million new cases reported annually [1]. Despite the availability of various anti-seizure drugs, about one-third of patients with epilepsy remain drug-resistant [2] their seizures cannot be adequately controlled even with multiple medications. The development of new, more effective treatments is crucial to address this unmet medical need.

The underlying mechanisms of drug resistance in epilepsy are complex and multifaceted. Several potential factors have been implicated, including altered expression or function of drug transporters, changes in drug targets, and activation of signalling pathways that promote seizure generation [3].

The high global burden of this debilitating disorder and the significant proportion of patients who fail to achieve adequate seizure control with current therapies underscore the critical need for continued research and development of novel, more effective treatment approaches to improve outcomes for individuals living with epilepsy. Existing evidence suggests that genetic factors may play a crucial role in determining an individual's response to anti-seizure medications, with certain genetic variants potentially contributing to drug resistance. As such, the exploration of substitution therapy, where first-line anti-seizure medications are used as a replacement for second-line or third-line options, is a promising avenue of investigation that could lead to improved seizure control and better quality of life for patients with treatment-resistant epilepsy.

Barbiturates and benzodiazepines are widely used as anti-epileptic drugs; however, these are inefficient in controlling seizures in more than 30% of the patient and have a low therapeutic window, drug-drug interaction, and various types of adverse effects. Consequently, the search for new derivatives to design more selective and potent anti-epileptic agents with minimal side effects continues to be an important area of research [4].

The key mechanisms underlying drug resistance in epilepsy include altered drug transport, target modification, and changes in drug metabolism [5]. Currently available anti-seizure drugs, such as gabapentin, pregabalin, vigabatrin, lacosamide, lamotrigine, and levetiracetam, have been linked to a range of adverse effects, including gastrointestinal disturbances, nausea, hirsutism, hepatotoxicity, weight gain, drug resistance, and neurotoxicity. As a result, there is an ongoing quest for the development of newer, more effective antiepileptic drugs with improved safety profiles.

To address the limitations of current anti-seizure drugs, researchers have explored the use of molecular hybridization, a rational drug design approach that combines two or more pharmacophores or bioactive moieties

into a single molecular entity. This strategy aims to harness the synergistic effects of the combined pharmacophores, potentially leading to enhanced therapeutic efficacy, improved pharmacokinetic properties, and reduced adverse effects [6]. Benzothiazole is a common heterocyclic ring system with a wide range of biological activities, many 1,2,4-triazole derivatives have been patented and are widely used in agriculture, and they are known to have anti-inflammatory, antiviral, analgesic, antimicrobial, anticonvulsant, and antidepressant activities [7].

The prevalence of the benzothiazole moiety in a wide array of bioactive natural compounds and established pharmaceuticals underscores its utility as a privileged structure in medicinal chemistry, making it a valuable starting point for the design and synthesis of new chemical entities with enhanced pharmacological profiles. Because of their adaptability in molecular design and their capacity to engage with a variety of biological targets, benzothiazole derivatives are still a focus of intense research and development initiatives [8]. Substituted benzothiazole have been investigated for their anticonvulsant activity, and it has been discovered that certain derivatives exhibit considerable efficacy in animal models of epilepsy. It has been demonstrated that the endocyclic sulfur and nitrogen functionalities within this heterocyclic moiety are crucial for anticonvulsant efficacy [9].

Further, researchers have found that the presence of an oxadiazole moiety in the heteroyclic ring shows potent anticonvulsant properties. 1,3,4-Oxadiazole is a five-membered ring containing one oxygen and the synthesized compounds were evaluated for their anticonvulsant properties using the standard MES (maximal electroshock seizure) and scPTZ (subcutaneous pentylenetetrazole) models. Oxadiazole-containing heterocyclic compounds have demonstrated a diverse array of biological functions, encompassing antioxidant [10], antibacterial [11], antimicrobial [12], anti-inflammatory [13], antituberculosis [14], antiviral and anticancer [15].

Aiming to maximize the pharmacological potential of benzothiazole and benzoyl chloride structural units, we synthesized a series of novel hybrid compounds, considering the structural requirements for anticonvulsant activity. The synthesized compounds were evaluated for anticonvulsant efficacy using MES and scPTZ protocols, as well as for potential neurotoxicity (rotarod test) and antinociceptive effects (thermal stimulus).

2. MATERIALS AND METHODS

2.1. Chemistry

The chemical substances and laboratory materials that we required were obtained from reputable suppliers of the chemical such as S. D. Fine Chemicals Ltd., Sigma Aldich, CDH, and E. Merck. They use the stationary phases of silica gel G and the solvents systems,

benzene : acetone and toluene : ethyl acetate : formic acid for these experiments and can verify the purity of the synthesized compounds. In order to identify and confirm the synthesized chemical substances as pure, iodine vapour was used as the visualizing agent. Thiele tube apparatus was used to perform the melting points. The Fourier transform infrared spectra, were obtained on Bruker FTIR spectrometer, using KBr pellet samples. Samples were dissolved in $CDCl_3$ and ^1H NMR and ^{13}C NMR spectra recorded on NMR spectrometer (300 MHz, Bruker-400 Ultra shield TM) and TMS [$(CH_3)_4Si$] used as the internal standard. Types of signal splitting pattern observed were s (singlet), d (doublet), t (triplet) and m (million); synthesized compounds were analysed with Chem Draw 21 software for their physicochemical as well as pharmacokinetic attributes.

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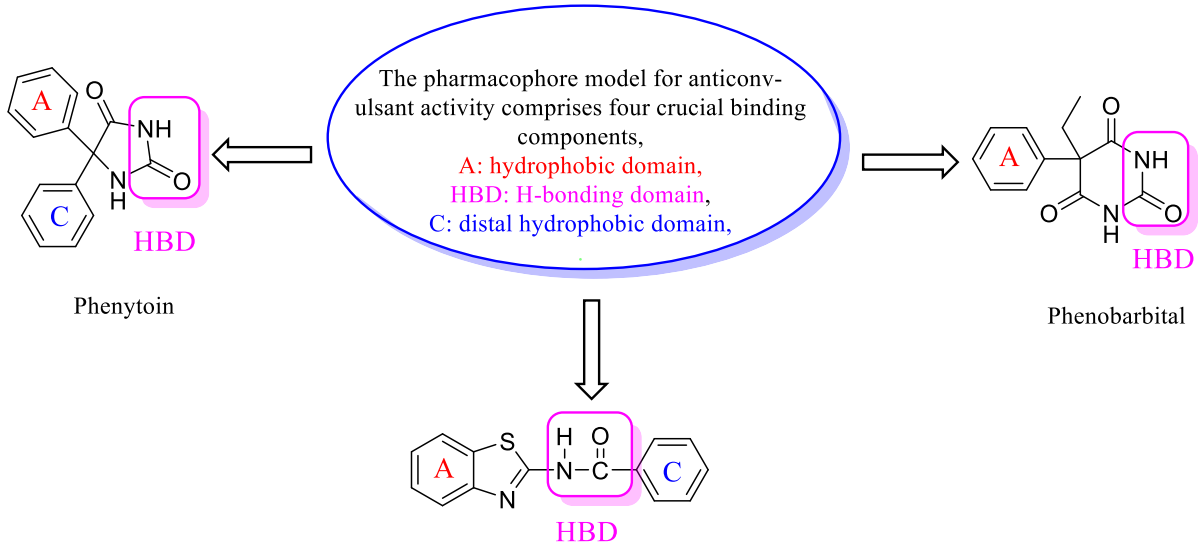


Fig. 1 Pharmacophore model for anticonvulsant activity

General procedure for the synthesis of titled compounds of Scheme 3 (3a-o)

Synthesis of 4-Substituted-1,3-benzothiazol-2-amines (1a-d).

Glacial acetic acid, substituted aniline (0.01 mol), potassium thiocyanate (0.01 mol) were mixed, stirred, and allowed to stand with the mixture cooled. Then 0.01 mol of bromine was added dropwise at a rate to keep the reaction temperature below 10 °C during addition. Thirty more hours of stirring was continued. Filtration, washing with acetic acid and drying separated the resulting hydroxyl Chloride salt. This product was dissolved in hot water, neutralized by adding 25% aqueous ammonia solution, filtered, washed with water and dried. It was then recrystallized with benzene finally.

Synthesis of 4-Substituted benzoyl chloride (2a-d)

The mixture of substituted benzoic acid (0.01 mol) and thionyl chloride (0.01 mol) in dimethyl formamide is refluxed and stirred for 3h when the reaction mixture becomes transparent and clear in solution, is cooled and poured over cold dil. HCl (50%). The resulting carbamate was then recrystallized from ethanol to obtain the target compounds.

Synthesis of N-[4-(4-Substituted-phenyl)- (benzo[d]thiazol-2-yl)benzamide (3a-u)

The final compounds 3a-o were obtained when 4- substituted benzothiazoles 1a-d (0.02 mol) and substituted benzoyl chloride 2a-d (0.02 mol) were refluxed in benzene for 5 h, solid material obtained was filtered and recrystallized from the benzene.

Synthesis of N-(benzo[d]thiazol-2-yl) benzamide (3a)

Yield: 65%; m.p.: 268-269 ° C. FT-IR (KBr) υmax cm-1: 3489 (NH str.), 3020 (CH-Ar str.), 1794 (C=N str), 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 2.11 (3H, s), 6.68 (1H, s), 7.18-7.57 (6H, 7.25 (m, *J* = 8.2, 0.5 Hz), 7.34 (m, *J* = 8.2, 0.5 Hz), 7.40 (d, *J* = 8.1, 1.6 Hz), 7.51 7.95 (1H, d, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.80, 148.30, 137.90, 124.70, 114.20, 49.30, 23.70; ESI-MS m/z = 254.10 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-2-hydroxybenzamide (3b)

Yield: 60%; m.p.: 264-265 °C. FT-IR (KBr) υmax cm-1: 3495 (NH str.), 3064 (CH-Ar str.), 1694 (C=N str), 1675 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.13 (3H, s), 6.67 (1H, s), 7.16-7.59 (6H, 7.22 (m, *J* = 8.1, 7.7, 0.5 Hz), 7.33 (m, *J* = 8.1, 0.5 Hz), 7.38 (t, *J* = 8.2, 1.6 Hz), 7.92(1H, d, *J* = 8.0, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.78, 148.29, 137.91, 124.69, 114.21, 49.28, 23.71; ESI-MS m/z = 270.05 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-4-hydroxybenzamide (3c)

Yield: 62%; m.p.: 238-239 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3467 (NH str.), 3064 (CH-Ar str.), 1824 (C=N str), 1646 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): ^{1}H NMR: δ 2.14 (3H, s), 6.66 (1H, s), 7.18-7.57 (6H, 7.21 (m, *J* =

8.0, 0.5 Hz), 7.32 (m, *J* = 8.0, 0.5 Hz), 7.40 (d, *J* = 8.1, 1.6 Hz), 7.92 (1H, d, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.79, 148.31, 137.89, 124.70, 114.19, 49.29, 23.70; ESI-MS m/z = 270.06 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-2-chlorobenzamide (3d)

Yield: 57%; m.p.: 266-267 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3228 (NH str.), 3033 (CH-Ar str.), 1804 (C=N str), 1690 (C=O), 767 (C-Cl); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.12 (3H, s), 6.62 (1H, s), 7.12-7.53 (6H, 7.24 (m, *J* = 8.2, 0.5 Hz), 7.30 (m, *J* = 8.2, 0.5 Hz), 7.39 (d, *J* = 8.2, 1.6 Hz), 7.91 (1H, d, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.81, 148.31, 137.91, 124.69, 114.20, 49.30, 23.69; ESI-MS m/z = 288.01 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-3-chlorobenzamide (3e)

Yield: 55%; m.p.: 264-265 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3228 (NH str.), 3033 (CH-Ar str.), 1804 (C=N str);1690 (C=O), 767 (C-Cl); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): ^{1}H NMR: δ 2.09 (3H, s), 6.64 (1H, s), 7.20-7.58 (6H, 7.23 (m, *J* = 8.2, 0.5 Hz), 7.34 (m, *J* = 8.2, 0.5 Hz), 7.40 (t, *J* = 8.1, 7.5, 1.6 Hz), 7.92 (1H, t, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.82, 148.29, 137.90, 124.67, 114.19, 49.31, 23.70; ESI-MS m/z = 288.03 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-4-chlorobenzamide (3f)

Yield: 62%; m.p.: 276-277 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3358 (NH str.), 3033 (CH-Ar str.), 1804 (C=N str), 1690 (C=O), 764 (C-Cl); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): ^{1}H NMR: δ 2.12 (3H, s), 6.62 (1H, s), 7.19-7.57 (6H, 7.25 (m, *J* = 8.1, 0.5 Hz), 7.42 (t, *J* = 7.6, 1.6, 0.5 Hz, 7.92 (1H, t, *J* = 8.1, 1.3, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.79, 148.31, 137.89, 124. 71, 114.21, 49.31, 23.69; ESI-MS m/z = 288.04 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-2-nitrobenzamide (3g)

Yield: 66%; m.p.: 267-268 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3408 (NH str.), 3063 (CH-Ar str.), 1823 (C=N str), 1674 (C=O), 1337 (NO_2); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 2.10 (3H, s), 6.64 (1H, s), 6.95 (1H,s), 7.18-7.57 (6H, 7.25 (m, *J* = 8.2, 0.5 Hz), 7.40 (t, *J* = 8.1, 1.6 Hz, 7.91 (1H, t, *J* = 8.1, 1.3, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.81, 148.30, 137.89, 124. 70, 114.22, 49.29, 23.70; ESI-MS m/z = 299.02 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-4-nitrobenzamide (3h)

Yield: 65%; m.p.:258-259 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3438 (NH str.), 3043 (CH-Ar str.), 1784 (C=N str), 1666 (C=O), 1367 (NO_2); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.12 (3H, s), 6.68 (1H, s), 6.93 (1H s), 7.20-7.58 (6H, 7.29 (m, *J* = 8.1, 0.5 Hz), 7.44 (t, *J* = 8.2, 1.6 Hz, 7.93 (1H, t, *J* = 8.1, 1.3, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.78, 148.29, 137.81, 124. 69, 114.19, 49.31, 23.69; ESI-MS m/z = 299.04 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-2-methoxybenzamide (3i)

Yield: 61%; m.p.: 245-246 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3398 (NH str.), 3083 (CH-Ar str.), 1644 (C=N str); 1635 (C=O), ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.14 (3H, s), 6.65 (1H, s), 6.95 (1H s), 7.18-7.57 (6H, 7.28 (m, *J* = 8.1, 0.5 Hz), 7.42 (m, *J* = 8.1, 1.6 Hz), 7.95 (1H, t, *J* = 8.2, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.79, 148.30, 137.85, 124.70, 114.21, 49.30, 23.71; ESI-MS m/z = 284.03 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-2,3-dimethoxybenzamide (3j)

Yield: 62%; m.p.: 276-277 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3315 (NH str.), 3096 (CH-Ar str.), 1635 (C=O), 1623 (C=N str); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.13 (3H, s), 6.68 (1H, s), 6.95 (1H s), 7.21-7.56 (6H, 7.25 (m, *J* = 8.2, 0.5 Hz), 7.44 (m, *J* = 8.2, 0.5 Hz), 7.94 (1H, t, *J* = 8.1, 0.5 Hz).). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.82, 148.27, 137.44, 124.69, 114.22, 49.29, 23.68; ESI-MS m/z = 284.05 (M + 1)

Synthesis of N-(benzo[d]thiazol-2-yl)-4-isopropylbenzamide (3k)

Yield: 62%; m.p.: 274-275 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3395 (NH str.), 3035 (CH-Ar str.), 1844 (C=N str); 1653 (C=O), ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 2.11 (3H, s), 6.68 (1H, s), 6.92 (1H s), 7.18-7.54 (6H, 7.23 (m, *J* = 8.2, 0.5 Hz), 7.41 (m, *J* = 8.2, 0.5 Hz), 7.93 (1H, t, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.79, 148.29, 137.42, 124.71, 114.20, 49.30, 23.68; ESI-MS m/z = 296.10 (M + 1)

Synthesis of 4-methyl-N-(5-methylbenzo[d]thiazol-2-yl)benzamide (3l)

Yield: 74%; m.p.: 278-279 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3489 (NH str.), 3020 (CH-Ar str.), 1794 (C=N str); 1685 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): δ 2.14 (3H, s), 6.66 (1H, s), 6.93 (1H s), 7.19-7.56 (6H, 7.22 (m, *J* = 8.1, 0.5 Hz), 7. 43 (m, *J* = 8.2, 0.5 Hz), 7.92 (1H, t, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.81, 148.31, 137.39, 124.72, 114.19, 49.31, 23.69; ESI-MS m/z = 282.08 (M + 1)

Synthesis of 4-methoxy-N-(5-methylbenzo[d]thiazol-2-yl)benzamide (3m)

Yield: 85%; m.p.: 240-241 ° C. FT-IR (KBr) υmax cm-1: 3495 (NH str.), 3064 (CH-Ar str.), 1694 (C=N str), 1675 (C=O); 1H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 2.12 (3H, s), 6.68 (1H, s), 6.94 (1H s), 7.18-7.57 (6H, 7.24 (m, *J* = 8.2, 0.5 Hz), 7.44 (m, *J* = 8.2, 0.5 Hz), 7.94 (1H, t, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.79, 148.29, 137.41, 124.70, 114.21, 49.29, 23.70; ESI-MS m/z = 284.32 (M + 1)

Synthesis of 4-hydroxy-N-(5-methylbenzo[d]thiazol-2-yl)benzamide (3n)

Yield: 82%; m.p.: 256-257 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3465 (NH str.), 3023 (CH-Ar str.), 1674 (C=N str), 1668 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 2.11 (3H, s), 6.67 (1H, s), 6.95 (1H s), 7.20-7.58 (6H, 7.25 (m, *J* = 8.4, 0.5 Hz), 7. 42 (m, *J* = 8.2, 0.5 Hz), 7.92 (1H, t, *J* = 8.1, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.81, 148.31, 137.38, 124.68, 114.18, 49.27, 23.69; ESI-MS m/z = 284.30 (M + 1)

Synthesis of 1-(1H-benzo[d]imidazol-2-yl)-N-(1-(2-chlorophenyl)ethyl)hydrazine-1-carboxamide (3o) Yield: 78%; m.p.: 241-242 ° C. FT-IR (KBr) υ_{max} cm^{-1}: 3436 (NH str.), 3066 (CH-Ar str.), 1812 (C=N str), 1684 (C=O); ^{1}H-NMR (DMSO-d_6, 300MHz) δ (*ppm*): 1.34 (3H, s), 6.68 (1H, s), 6.89 (1H s), 7.20-7.58 (6H, 7.12 (m, *J* = 8.2, 0.5 Hz), 7. 45 (m, *J* = 8.1, 0.5 Hz), 7.93 (1H, t, *J* = 8.2, 0.5 Hz). ^{13}C NMR (75 MHz, DMSO-d_6): δ ppm: 159.75, 148.28, 137.38, 124.65, 114.21, 49.29, 23.74; ESI-MS m/z = 329.82 (M + 1)

2.2. Anticonvulsant activity

Newly synthesized compounds **3a-o** were assessed for anticonvulsant activity using the MES and scPTZ models, following established protocols [16]. The preliminary Phase I pharmacological screening included the MES and scPTZ tests, as well as a minimal motor impairment assessment [17]. Compounds were administered intraperitoneally (i.p.) as solutions in polyethylene glycol at initial doses of 30, 100, and 300 mg/kg in mice [18].

2.3. Neurotoxicity screening

Minimal motor impairment in mice was determined using the standard rotarod test, as previously described [19]. Mice were trained to maintain balance on the rotarod, revolving at 10 rpm. Subsequently, the test substances were administered intraperitoneally at a dose of 100 mg/kg. Neurotoxicity was indicated by the inability of an animal to remain on the rod for at least one minute in each of the four trials

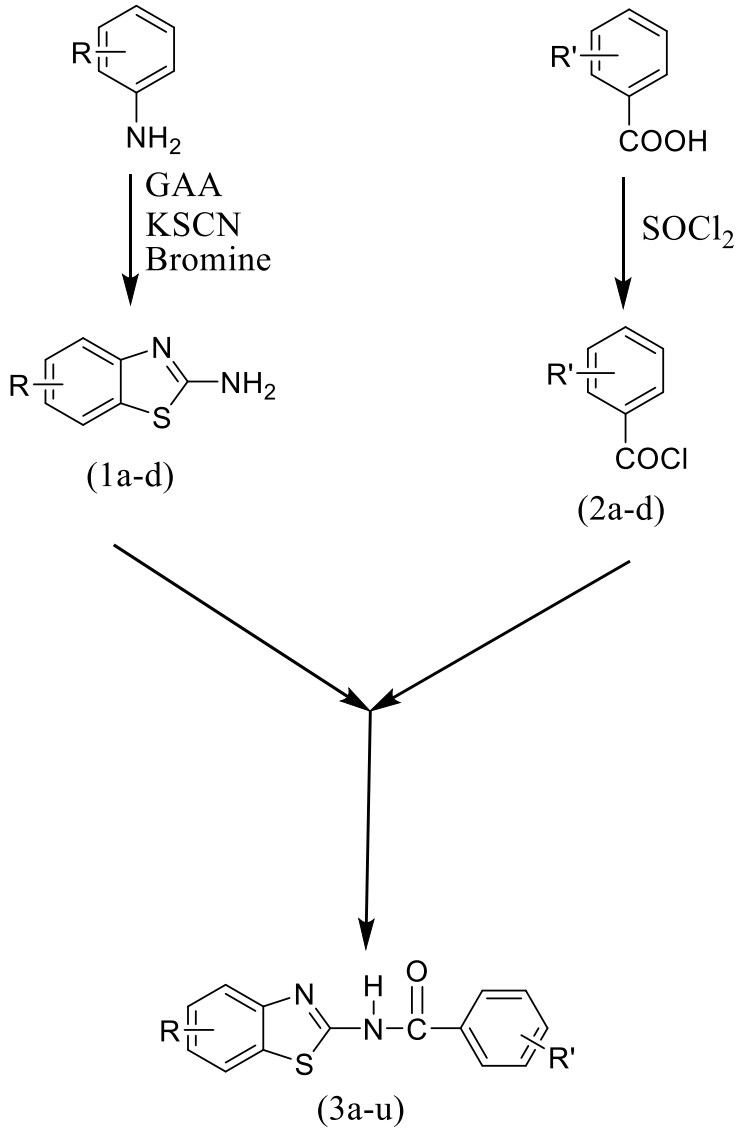
2.4. Antidepressant activity

Antidepressant activity was assessed using the forced swim test, also known as the despair swim test, in groups of six albino rats of either sex (110-140 g) [20]. The FST consisted of two swimming sessions: a 15-minute training session followed 24 hours later by a 5-minute test session, with drug administration 1 hour prior to the test. During each session, rats were individually placed in plexiglass cylinders (40 cm high, 20 cm in diameter) containing water (24-26 °C, 30 cm deep) [21].

2.5. Antinociceptive activity

The antinociceptive activity was assessed using a thermal stimulus technique. Investigations were performed on groups of six albino mice under standard laboratory conditions. Test compounds were administered orally at a dose of 20 mg/kg, suspended in a 0.5% methyl cellulose-water mixture. Antinociceptive activity was evaluated 4 hours post-administration by gently immersing the tail of each mouse in a thermostatically controlled water bath at 55 °C. The latency to withdraw the tail from the hot water was recorded for both control and treated groups [22].

Scheme 1. Synthetic route for the synthesis of compounds 3a-u.



3. RESULTS AND DISCUSSION

3.1. Chemistry

The target compounds 3a-o were prepared using the outlined methods in Scheme 1. In the initial step, 4-substituted aniline was reduced to yield 4-substituted-1,3-benzothiazol-2-amines. Subsequently, substituted benzoic acid was reacted with thionyl chloride in the presence of DMF to obtain 4-substituted benzoyl chloride. Finally, condensation of the 4-substituted benzothiazoles with the substituted benzoyl chloride produced the final compounds 3a—o. The synthesis of all the compounds was monitored by thin-layer chromatographic analysis in

n-hexane : ethyl acetate/chloroform systems under UV. The structures of all the compounds were confirmed using ESI-HRMS, ^{1}HNMR, and ^{13}CNMR spectroscopy.

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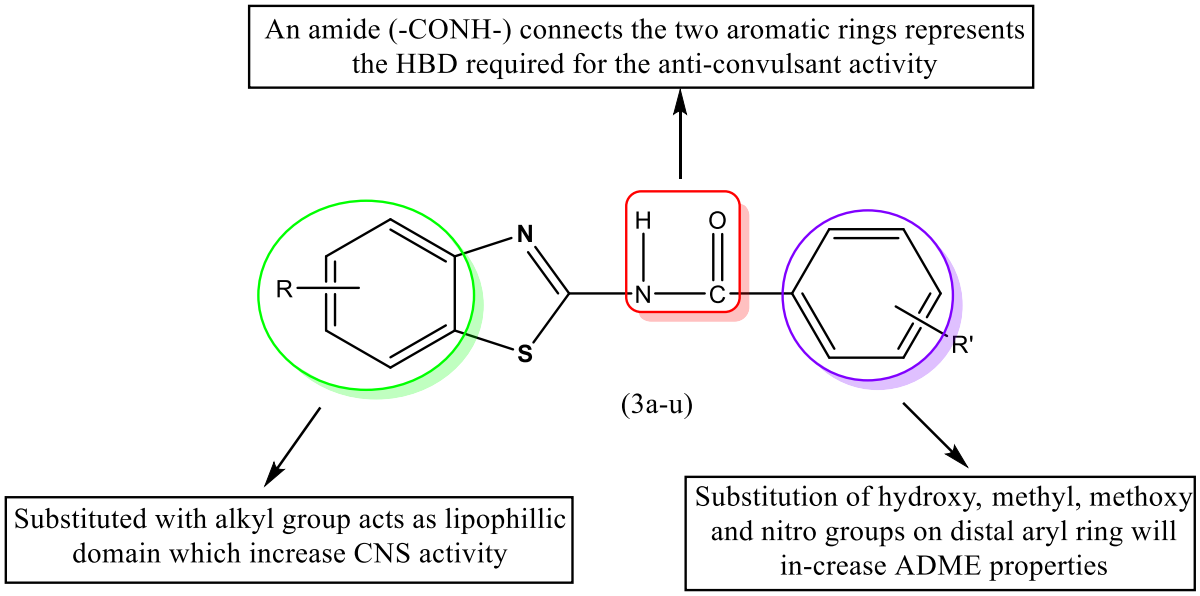

Fig 2. Strategies for developing target compounds

3.2. In Vivo Studies

3.2.1 Anticonvulsant activity

The anticonvulsant efficacy of the final compounds 3a-o was evaluated using two well-established animal models: Maximal Electroshock Seizure (MES) and Pentylenetetrazole-Induced Seizure (Sc-PTZ). Mice were administered all the synthesized compounds, intraperitoneally at doses of 30, 100 and 300 mg/kg and observations made at two different times, 1 and 24 h post injection. The rotorod test was used to assess motor impairment, and the data are found in Table 1.

All the compounds protected at least to some degree in the MES test as a component of preliminary phase I anticonvulsant screening, reflecting their ability to prevent seizure spread. Of these, compounds 3d and 3g showed the highest attained seizure protection at the lowest dose 30 mg/kg at 0.5 h. of particular interest, compound 3g retained anticonvulsant activity at 4.0 h, which might indicate rapid onset and a long duration of action. Also Compound 3d was active at 4.0 h but at higher doses of 100 mg/kg.

None of these compounds caused any significant motor impairment. Subsequent to 0.5 h, 100 mg/kg was an effective dose for the majority of the compounds: 3a, 3c, 3k, 3l, 3m and 3n. In addition to active, 3c, 3k and 3m were also active at the same dose 4.0 h after.

Active compounds for the scPTZ screen were 3c, 3d, 3f, 3g, 3k and 3l 3c, 3f, and 3k were active at 100 mg/kg at 0.5 h. Compounds 3f and 3k were active after 4.0 h, but only at the higher dose of 300 mg/kg. Active compounds 3d, 3g, 3l, 3q were active at 300 mg/kg after 0.5h.

Table 1- Anticonvulsant activity and minimal motor impairment of the synthesized compounds

Compound	Intraperitoneal injection in mice[a]					
	MES screen		scPTZ screen		Minimal motor impairment screen	
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h
3a	100	–[b]	–	300	300	300
3b	300	300	–	–	–	300
3c	100	100	100	–	300	100
3d	30	100	300	–	–	–
3e	–	300	–	–	–	–
3f	30	30	100	300	–	–
3g	30	30	100	300	–	–
3h	300	300	–	–	100	300
3i	300	300	–	–	–	–
3j	–	300	–	–	–	300

3k	100	100	100	300	300	100
3l	100	300	300	—	100	100
3m	100	100	—	—	100	300
3n	100	300	—	300	—	300
3o	—	300	—	—	300	300
Phenytoin	30	30	—	—	100	100
Ethosuximide	—	—	100	300	—	—
Phenobarbital	100	30	—	—	100	300

aNumber of animals used = 6; Solvent used- Polyethylene glycol; Dose of 30, 100 and 300 mg/kg were administered i.p.
The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice.
The animals were examined at 0.5h and 4h after injections were administered.
bThe dash (—) indicates an absence of activity at maximum dose administered (300 mg/kg)

A study of the structure activity relationship revealed that derivatives of the nitro group at the phenyl ring were most active of the series and produced the greatest motor impairment. Derivatives with attachment of methoxy group at para position of phenyl ring were comparatively more effective than those having hydroxyl group. Least motor impairment was shown by unsubstituted derivatives.

Following the phase II anticonvulsant screening, we quantitatively compared the two most potent compounds 3d and 3g for their anticonvulsant efficacy and severity of motor impairment. Table 2 presents the results. Analogues of both compounds showed similar anticonvulsant activity and higher protective index than the standard drugs. However, Compound 3i exhibited very robust anti-MES activity (ED50 = 13.4 mg/kg) comparable with the reference antiepileptic phenytoin and carbamazepine, and superior to phenobarbital and valproate. Animals treated with 3i had minimal motor impairment, and significantly lower than all of the comparator drugs. The protective index equalled 51.0, which was significantly higher than the range of 1.6 – 8.1 found for the existing anti-epileptic drugs.

Table 2- Phase II quantitative anticonvulsant evaluation of most active compounds

Compound	ED_{50}[a]		TD_{50}[b]	PI[c]	
	MES	scPTZ		MES	scPTZ
3d	23.9 (19.2-28.4)[d]	178.6 (126.3-244.6)	439.3 (311.4-564.3)	18.3	2.4
3g	13.4 (9.8-17.1)	81.6 (53.4-113.3)	684.1 (451.3-908.5)	51.0	8.3
Phenytoin	9.5 (8.1-10.4)	>300	65.5 (52.5-72.9)	6.9	<0.22
Carbamazepine	8.8 (5.5-14.1)	>100	71.6 (45.9-135)	8.1	<0.22
Phenobarbital	21.8 (21.8-25.5)	13.2 (5.8-15.9)	69 (62.8-72.9)	3.2	5.2
Valproate	272 (247-338)	149 (123-177)	426 (369-450)	1.6	2.9

Number of animals used = 10; Solvent used: polyethylene glycol (0.1 mL, i.p.).
aDose in milligrams per kilogram body mass.
bMinimal toxicity which was determined by rotorod test 30 min after the test drug was administered.
cPI = Protective index (TD50/ED50).
dData in parentheses are the 95% confidence limits.

3.2.2. Antidepressant activity

The antidepressant activity data of the compounds are presented in Table 3. Compounds 3b and 3i were found to be the most potent of the series as it showed the significant antidepressant activity ($p < 0.01$). Compounds 3e, 3j, 3k, and 3n also showed statistically significant decrease in the immobility time ($p < 0.05$). Other tested compounds failed to show any significant antidepressant activity. It was observed that the substitution at the aryl ring attached to the benzothiazole ring at fourth position by electron releasing groups increases the activity

irrespective of the size of the substituent. Substitution on the aryl ring attached to the Benzothiazole ring did not have much effect on the activity.

Table 3- Antidepressant activity data of the active compounds

Compound	Mean average immobility time (s)[a] Mean ± SEM	
	Untreated	Treated
3b	39.6 ± 1.2	25.6 ± 1.3**
3d	36.9 ± 1.2	33.9 ± 1.2
3e	52.3 ± 1.6	40.4 ± 1.1*
3g	37.4 ± 1.2	34.1 ± 1.2
3i	41.3 ± 1.4	28.2 ± 1.5**
3j	42.5 ± 1.4	35.4 ± 1.7*
3k	29.3 ± 1.2	21.1 ± 1.1*
3m	38.4 ± 1.6	34.3 ± 1.4
3n	31.2 ± 1.3	23.2 ± 1.2*
3o	53.1 ± 1.7	49.5 ± 1.8
Fluoxetine	43.1 ± 1.2	24.1 ± 1.2***

aDose = 30 mg/kg (p.o.); *p < 0.05, **p < 0.01, ***p < 0.001. Data was analyzed by unpaired students‘t’ test

3.2.3. Antinociceptive activity

The antinociceptive (pain-relieving) activity of the compounds that demonstrated significant anticonvulsant (seizure-reducing) activity was evaluated using the thermal stimulus technique. Specifically, the compounds found to be active at 30 and 100 mg/kg doses were selected for this assessment and compared to the standard drug diclofenac at a 20 mg/kg dose. Antinociceptive activity was measured as the mean average reaction time in seconds, with the data presented as Mean ± SEM. Statistical analysis was conducted using Student's t-test, comparing the treated compounds to their respective untreated controls.

The antinociceptive activity of the ten active compounds were evaluated and found that a compound 3d was significantly active ($p < 0.01$) and have shown interesting increase in the reaction time. Two more compounds, 3g and 3n displayed statistically significant ($p < 0.05$) results. All other compounds tested were devoid of the activity and have shown non-significant changes in the reaction time.

Table 4 p-substituted N-(benzo[d]thiazol-2-yl) benzamide

Compound	Mean average reaction time (s)[a] Mean ± SEM	
	Control	Treated
3d	1.2 ± 0.01	2.6 ± 0.12**
3g	1.6 ± 0.06	2.1 ± 0.07*
3i	0.8 ± 0.02	1.0 ± 0.03
3k	0.9 ± 0.05	1.2 ± 0.04
3l	1.8 ± 0.04	2.1 ± 0.05
3n	1.6 ± 0.10	2.7 ± 0.04*
3o	1.1 ± 0.03	1.5 ± 0.07
3q	1.7 ± 0.07	1.6 ± 0.04
3r	1.3 ± 0.08	1.0 ± 0.03
3s	1.5 ± 0.07	1.9 ± 0.06
Diclofenac	0.7 ± 0.03	5.2 ± 0.04***

aDose = 20 mg/kg (p.o); *p < 0.05, **p < 0.01, ***p < 0.001. Data was analyzed by unpaired students‘t’ test.

4. CONCLUSIONS

In a quest for enhanced antiepileptic agents, a novel series of N-thiazol-2-yl)benzamide derivatives was designed and synthesized. These compounds displayed favorable drug-like characteristics and met key structural requirements for antiepileptic activity. In vivo studies confirmed their anticonvulsant potential, with compounds 3d and 3g demonstrating the most significant antiepileptic activity at 30 mg/kg, observed at 0.5 and 4 hours post-administration, without inducing neurotoxicity. Antidepressant screening indicated minimal impact on

locomotor activity compared to standard drugs. Furthermore, compound 3d exhibited significant antinociceptive activity ($p < 0.01$), leading to a notable increase in reaction time, suggesting a promising multi-target profile for this biheterocyclic benzamide derivative.

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