

IDENTIFICATION OF NOVEL IMIDAZOLE DERIVATIVES WITH PROMISING ANTIEPILEPTIC AND ANTIDEPRESSANT ACTIVITIES: NEUROPHARMACOLOGICAL AND IN SILICO STUDIES

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ABSTRACT

Imidazole scaffolds are well-established pharmacophores in widely used anticonvulsant and antidepressant medications, including Dizimol, Nafimidone, and Napamezole hydrochloride. In the present study, two imidazole derivatives (C1 and C3) were synthesised via a green chemistry route using diketones and substituted aldehydes and were evaluated for neuropharmacological activity. Molecular docking was performed against the Gamma-aminobutyric acid receptor (PDB ID: 8G4N), sodium channel inactivator (PDB ID: 5VB8), and the monoamine transporter complex (PDB ID: 2QJU) to elucidate their potential mechanisms of action. Compound C1 exhibited a stronger affinity toward the sodium channel (−9.4 kcal/mol), while C3 showed comparable binding across all targets. Free radical scavenging activity, assessed using the 2,2-diphenyl-1-picrylhydrazyl assay, revealed superior antioxidant potential for C3, with a radical scavenging activity of 84.15%. Neuropharmacological activities were evaluated in Swiss albino mice using an Actophotometer, rotarod, and pentylenetetrazol-induced seizure model at doses of 50 and 100 mg/kg. Both C1 and C3 produced significant Central Nervous System depressant and muscle-relaxant effects, with C1 showing greater protection against Pentylenetetrazol induced seizures at a dose of 100 mg/kg. Overall, these results suggest that C1 and C3 possess dose-dependent anticonvulsant, antidepressant, and neuroprotective properties, which supports SDG-3. These derivatives can be potential lead candidates for multifunctional neuroactive drug development in the future.

Keywords: Synthetic Imidazole Derivatives, CNS Depressant, Anticonvulsant, Antioxidant, Docking

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INTRODUCTION

Epilepsy is a chronic neurological disorder characterised by unwanted seizures that are abnormal and caused by excessive neuronal discharge in the brain. According to the World Health Organisation (WHO), around 50 million people are affected worldwide, making it one of the most widespread neurological conditions globally.¹ The epilepsy disorder is clinically dissimilar, which occurs when focal seizures arise from a specific cerebral region, whereas generalised seizures involve both hemispheres simultaneously.² The etiological pathogenesis of epilepsy involves multiple genetic factors related to ion channel mutations, traumatic brain injury, infections, cortical malformations, metabolic abnormalities, and autoimmune factors.³ The pathophysiology of epilepsy involves an imbalance between excitatory and inhibitory GABAergic neurotransmission, resulting in hyperexcitability and hypersynchrony of neurons.⁴ Antiepileptic drugs (AEDs) are currently used for treatment, such as phenytoin, carbamazepine, valproate, benzodiazepines, and levetiracetam, which enhance GABAergic transmission, modulate channels, such as voltage-gated sodium or calcium channels, and inhibit glutamate release. These medicines demonstrate antiepileptic effects via all these mechanisms.⁵ The need for newer AEDs is because, despite having all the AEDs that have significant therapeutic effects, approximately 30% of patients are resistant to AEDs in epilepsy, which indicates the urgent need for a novel therapeutic molecule that should have improved efficacy, fewer adverse effects, and broader mechanistic targets that can be targeted for antiepileptic activity.⁶ Depression is another major neurological illness, such as epilepsy, which affects the mind

psychologically and is one of the leading causes of global disability, characterized by persistent sadness, lack of interest, and cognitive and physiological disturbances.⁷ Depression, also known as major depressive disorder (MDD), is a common but serious mood disorder characterized by persistent sadness, loss of interest or pleasure in activities, and a range of emotional and physical symptoms that impair daily functioning. This affects how a person feels, thinks, and behaves, often leading to significant distress and reduced quality of life. A decrease in the synaptic levels of serotonin (5-HT), norepinephrine (NE), and dopamine (DA) causes depressive symptoms.^{8,9}

Unlike epilepsy, depression has a more complex pathophysiology, including hypothalamic-pituitary-adrenal (HPA) axis dysregulation, neuroinflammatory activation, altered GABAergic and glutamatergic signalling, and impaired synaptic plasticity.^{10,11} Reduced brain GABA concentrations and abnormal GABA-A receptor activity are observed in patients with major depressive disorder, suggesting that inhibitory neurotransmission plays a crucial role in mood regulation.¹² Available standard antidepressant therapies, such as selective serotonin reuptake inhibitors (SSRIs), serotonin norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, and monoamine oxidase inhibitors (MAOIs), which target monoaminergic systems, have a slow therapeutic onset, a significant relapse rate, and treatment resistance remains common in most patients.¹³

All of these factors create an urge to search for new compounds or scaffolds capable of acting through multiple model neuropharmacological pathways. Depression and epilepsy can occasionally coexist, with overlapping neurochemical and molecular pathways that comprise disruptions in monoaminergic transmission and GABAergic signalling.¹⁴ The compound or scaffold serves as a base scaffold for anticonvulsant and antidepressant drugs and showcases both activities in these conditions. The imidazole scaffold has attracted significant attention due to its pharmacological versatility and structural adaptability, as imidazole is a 5-membered heterocyclic ring containing two nitrogen atoms capable of hydrogen bonding, electron delocalisation, and π - π stacking interactions, which confer favourable physicochemical and pharmacokinetic properties. Clinically proven standard drugs with imidazole nuclei, such as losartan, ketoconazole, metronidazole, and cimetidine, have demonstrated medicinal relevance. Some drugs, such as Denzimol, Nafimidone, and Napamezole hydrochloride, are imidazole scaffold-based, showing antiepileptic and antidepressant activities. Furthermore, imidazole derivatives possess lipophilicity suitable for blood-brain barrier (BBB) penetration and have shown promising neuropharmacological activities, including anticonvulsant, anxiolytic, and antidepressant effects.¹⁵ Their structural flexibility allows for substitution patterns that can be fine-tuned to optimise receptor binding and biological activity. *In silico* computational methods can facilitate the interaction of a compound or its derivatives with three key central nervous system targets, including the GABA receptor (PDB ID: 8G4N), sodium channel inactivator (PDB ID: 5VB8), and monoamine transporter complex (PDB ID: 2QJU). In the brain, rapid inhibitory neurotransmission is mediated by the GABA-A receptor, a pentameric ligand-gated chloride channel.¹⁶ Positive allosteric modulation of GABA-A receptors hyperpolarises neuronal membranes, resulting in reduced excitability and anticonvulsant, anxiolytic, and antidepressant effects.¹⁷ Sodium channels are essential for the generation of action potentials, and their inhibition or stabilisation in an inactivated state can effectively reduce neuronal hyperexcitability. (5VB8) provides information regarding potential channel-blocking interactions.^{18,19}

Monoamine transporters (2QJU) are responsible for the reuptake of neurotransmitters from the synaptic cleft, thereby regulating mood and affective tone. Their inhibition is mostly achieved by antidepressant drugs, which act as templates for modifying ligand binding at human monoamine transporter sites.^{20,21} In continuation of our earlier research on the synthesis of imidazole derivatives using a green chemistry approach, the present work was designed to address this gap by integrating the environmentally sustainable synthesis, molecular docking, and *in vivo* pharmacological evaluation into a single drug discovery framework. Three imidazole derivatives, C1, C2, and C3, were synthesised using a green chemistry approach, and their interactions with epilepsy- and depression-related molecular targets were investigated through molecular docking. *In silico* molecular docking was performed against the GABA receptor (PDB ID: 8G4N), sodium channel inactivator (PDB ID: 5VB8), and monoamine transporter complex (PDB ID: 2QJU) for lead optimization and to reduce the need for extensive trial-and-error

synthesis. Based on good binding scores and interactions with different amino acids, compounds C1 and C3 were studied for neuropharmacological activity using the various models. The hypothesis is to combine the green chemistry approach and in vivo pharmacological evaluation for the establishment of novel imidazole derivatives with potent antiepileptic and antidepressant activities. This research directly supports SDG-3: good health and well-being by advancing the discovery of novel therapeutic candidates for neurological and mental disorders. Furthermore, the use of a green chemistry approach aligns with the principle of sustainable pharmaceutical development by reducing the environmental impact associated with conventional synthesis.

EXPERIMENTAL

Chemicals and Reagents

All experiments used essential reagents, including Tween 80, DPPH (2,2-diphenyl-1-picrylhydrazyl), 2-aminothiazole, ammonium acetate, benzil, benzaldehyde, ascorbic acid, and pentylenetetrazol (PTZ). CAN (Ceric ammonium nitrate) and copper sulphate as catalysts. At the same time, ethanol, glacial acetic acid, and methanol were obtained as solvents from the institutional laboratory stock of St. John Institute of Pharmacy and Research, Vevoor, Palghar, India.

The standard Central nervous system (CNS) drugs diazepam (2 mg/kg) and phenytoin (1 mg/kg), manufactured by Abbott Healthcare Pvt. Ltd., India, and Pentoxifylline (2 mg/kg), manufactured by Zentiva Pvt. Ltd., India, were procured from a local pharmacy. All chemicals and reagents used were of analytical grade. FT-IR Spectrophotometer (Bruker Alpha II) and ¹H-NMR Spectrometer (500 MHz Bruker Ascend 500) were used for spectral characterisation.

In Silico Study

Prediction of the Probability of Activity of Novel Imidazole Derivatives

The probability of activity and inactivity was predicted using the PASS Online Software for the structure of novel synthetic imidazole derivatives.

Docking Study

Computational studies were carried out to elucidate the molecular basis of interactions between novel synthetic imidazole derivatives and the GABA receptor (PDB ID: 8G4N), sodium channel inactivator (PDB ID: 5VB8), and neurotransmitter transporter (SERT, NET, DAT) blockers (PDB ID: 2QJU).

Steps for Docking

Preparation of Ligand

First, the structures of all ligands were drawn in ChemDraw Ultra 8.0 and saved as a mol file. The file was then opened in ChemDraw 3D, and the ligand energies were minimised using the MMFF94 force field. This file was saved in PDB format.

Preparation of Protein

The downloaded protein file was opened in Discovery Studio Visualizer v 3.1, and the heteroatoms and water molecules were removed. This file was saved in the PDB format, as with FIX.

Run Molecular Docking in PyRx

The cleaned protein structure was first loaded into the docking software and prepared using AutoDock Tools by selecting the macromolecule option. The ligand structures were prepared using Open Babel, where the ligand molecules were imported and subjected to energy minimisation to obtain stable conformations. After minimisation, the ligand files were converted to the pdbqt format, which is required for docking calculations. Subsequently, the Vina Wizard was opened, and the prepared protein and ligand files were loaded. The grid box was calculated to cover the active site.

After setting the grid parameters, the docking process was initiated to calculate the ligand-target protein binding affinity. Upon completion of the docking simulation, results were generated and binding affinity values recorded. The output data were then exported and saved as a CSV file, which was subsequently opened and stored as an Excel file for further analysis and interpretation.

Visualisation of Ligand and Protein Interaction

Open the output file of the docked ligand in Discovery Studio and minimise it. Then opened PC and then opened Discovery Studio to user and then selected our folder macromolecules, selected the protein file (underdo FIX), then dragged that file and opened it in the Discovery Studio. Clicked the label in Discovery Studio, selected Add, and then applied the amino acid. Clicked Ligand Interaction, clicked SAS, then showed distance, and then clicked minima, showing a 3D diagram. Clicked on the 2D diagram to display it, and then clicked on the interaction to show the distance. The 2D and 3D diagrams were saved as ligand and image files.

Synthesis of Novel Synthetic Imidazole Derivatives

Ten imidazole derivatives were rationally designed using a validated strategy previously employed by our research group. Of these, C1, C2, and C3 were identified as promising candidates through *in silico* studies and were subsequently synthesised and characterised by TLC, melting point determination, and FT-IR and ¹H-NMR spectroscopy.

Procedure for the Synthesis of Novel Synthetic Imidazole Derivatives

Synthesis of 1,2,4,5-tetrasubstituted imidazole derivatives (C1-C3) using green chemistry approach

Benzil (0.012 mol), substituted aromatic aldehyde (0.014 mol), and ammonium acetate (0.06 mol) were introduced into a 250 mL round-bottom flask containing glacial acetic acid (40 mL). A 2% (w/v) aqueous copper sulphate solution was prepared separately, and 2.5 mL was added dropwise to the reaction mixture. Copper sulfate acts as a Lewis acid catalyst, enhancing carbonyl activation and facilitating imine formation, followed by cyclocondensation.²² The reaction mixture was refluxed for 3 h. After completion of the reaction, the mixture was cooled to 50-60 °C and carefully poured into distilled water (125 mL) with gentle stirring. The resulting mixture was allowed to stand for 15 min to ensure complete precipitation. The completion of the reaction was monitored by TLC (Mobile Phase: Methanol: Chloroform: Acetic acid 7:2:1). The solid product was isolated by vacuum filtration, washed with ice-cold water to remove residual impurities, dried, and recrystallised from methanol (20 mL)^{23,24} (Fig. 1).

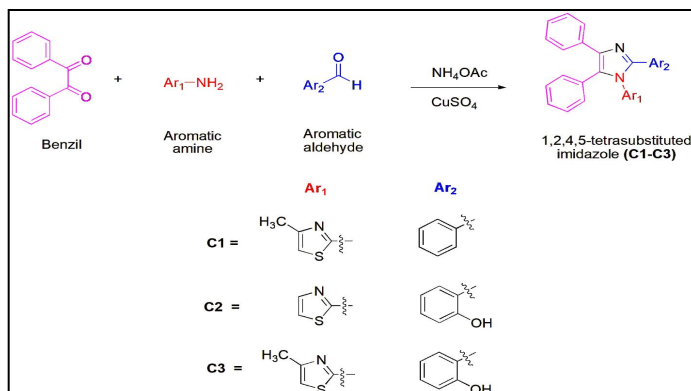


Fig.-1: Scheme of Synthesis of Substituted Imidazole Derivative (C1-C3)

1-(4-methylthiazol-2-yl)-2,4,5-triphenyl-1H-imidazole (C1)

Yellow crystals from methanol: Yield: 50.89 %, m.p.: 120-122 °C, FT-IR (cm⁻¹): 3037.12 (Ar-H), 2920.77 (CH₃), 1649.53 (C=N), 1586.11 (Ar C=C), 1322.58 (CH bend), 1027.14 (C-N); ¹H NMR (400 MHz, DMSO-d₆) δ = 7.33-6.58 (m, 15H, Ar-H), 6.17 (s, 1H, C=CH thiazole), 3.07 (s, 3H, CH₃).

2-(4,5-diphenyl-1-(thiazol-2-yl)-1H-imidazol-2-yl)phenol (C2)

Buff crystals from methanol: Yield: 91.63 %, m.p.: 160-162 °C, FT-IR (cm⁻¹): 3471.29 (OH), 3059.77 (Ar-H), 1657.64 (C=N), 1591.60 (Ar C=C), 1292.67 (CH bend), 1172.94 (C-O Phenolic), 1070.45 (C-N); ¹H-NMR (400 MHz, DMSO-d₆): δ = 12.79 (s, 1H, OH), 8.09-8.08 (d, 1H, N-C=CH thiazole), 7.77-7.52 (m, 4H, Ar-H Phenolic), 7.51-7.29 (m, 10H, Ar-H), 7.23-7.21 (d, 1H, S-C=CH thiazole).

2-(1-(4-methylthiazol-2-yl)-4,5-diphenyl-1H-imidazol-2-yl)phenol (C3)

Buff crystals from methanol: Yield: 66.58 %, m.p.: 102-104 °C, FT-IR (cm⁻¹): 3569.32 (OH), 3028.68 (Ar-H), 2935.38 (CH₃), 1656.0 (C=N), 1594.60 (Ar C=C), 1310.99 (CH bend), 1209.91 (C-O Phenolic), 1070.27 (C-N); ¹H-NMR (400 MHz, DMSO-d₆): δ = 12.95 (s, 1H, OH), 8.04-7.81 (m, 4H, Ar-H Phenolic), 7.79-6.82 (m, 10H, Ar-H), 7.46 (s, 1H, C=CH thiazole), 3.74 (s, 3H, CH₃).

Selection of Animals for Neuropharmacological Study

Healthy adult Swiss Albino mice, aged 8–9 weeks, weighing 20-30 g, were obtained from Lacsmi Biofarms Pvt. Ltd. [Registration No. 1277/PO/RcBt/S/09/CCSEA]. They were housed in a controlled environment at a temperature of 22 ± 2 °C on a 12-hour light/dark cycle, and humidity was maintained between 50% and 60% (but not below 30% or exceeding 70%). The mice were provided unrestricted access to a commercial standard diet and water. The animals were allowed to acclimatise for 24 h under laboratory conditions before beginning the study. All experimental procedures were conducted during the light period between 8:00 AM and 4 PM.²⁵ The experimental protocols and procedures used in the study were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the St. John Institute of Pharmacy and Research, Vevoor, Palghar, Maharashtra, India (SJIPR/IAEC/07/24-25). A total of 42 mice were evenly distributed into seven groups (n = 6/group). The treatment groups were as follows: Control, Disease Control (diazepam), standard (pentoxifylline), test group I (C1, 50 mg/kg), test group II (C1, 100 mg/kg), test group III (C3, 50 mg/kg), and test group IV (C3, 100 mg/kg).

Acute Toxicity

An acute toxicity study of synthetic imidazole derivatives (C1 and C3) was conducted according to the Organisation for Economic Co-operation and Development (OECD) Guideline No. 425, which refers to the “Acute Oral Toxicity-Up and Down Procedure” (UDP), assessing the safety of chemicals and substances.²⁶

Dose Preparation

All the doses of Synthetic Imidazole derivatives and standard drugs were prepared in a 2% v/v aqueous solution of Tween 80. Synthetic Imidazole derivatives were evaluated for CNS depressant and anticonvulsant effects at oral doses of 50 and 100 mg/kg.

Antioxidant Activity**DPPH Radical Scavenging Assay**

Antioxidant activity was evaluated using the DPPH assay in accordance with the method of Choi et al. (2002), with some modifications. Different concentrations of the test compounds (10–500 μM) were mixed with methanolic DPPH solution (85 μM) and incubated at 30 °C for 30 min. The absorbance was recorded at 517 nm using a Shimadzu UV-1900 spectrophotometer (Japan).^{27,28} Ascorbic acid was used as the standard. The % inhibition and IC₅₀ values were calculated. All experiments were performed in triplicate.²⁹

Percentage of DPPH radical scavenging activity was calculated using the formula:

$$\text{Scavenging effect (\%)} = \frac{\text{Control (absorbance)} - \text{Sample (absorbance)}}{\text{Control (absorbance)}} \times 100$$

Evaluation of CNS Depressant Activity**Antidepressant Activity by Actophotometer**

Locomotor activity was evaluated using a Digital Actophotometer (Japson/India) based on the photobeam interruption. The enclosed area of the Actophotometer where mice move is 30 cm × 30 cm × 30 cm. The baseline activity was recorded before the administration of any treatment, before acclimatisation. Mice were randomly assigned to seven groups (n = 6/group) and received treatment by the peroral (p.o.) route using oral gavage 30 min before the test. The control, disease control, and standard groups received saline solution, diazepam (2 mg/kg), and pentoxifylline (2 mg/kg), respectively. The test groups were treated with C1 and C3 at 50 and 100 mg/kg doses of diazepam (2 mg/kg). Each mouse was placed in an Actophotometer for 10 min, and activity scores were recorded.^{30,31}

The percentage inhibition of locomotor activity was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Antidepressant Activity by Rotarod

The motor coordination effect was evaluated using a rotarod apparatus (Dolphin/India) rotating at 25 revolutions per minute (rpm). Mice were randomly divided into seven groups (n = 6/group), and before any experimental treatment, the mice were trained in multiple sessions. The baseline readings were recorded before treatment. The control, disease control, and standard groups received saline solution (0.2 mL, orally), diazepam (2 mg/kg), and pentoxifylline (2 mg/kg), respectively. The test group was treated with C1 and C3 at 50 and 100 mg/kg doses plus diazepam (2 mg/kg) by oral gavage (p.o.) 60 min before the test.^{32,33} All groups were subjected to 5 minutes in the apparatus. Fall-off time was measured in seconds during the test.³¹ The percentage inhibition of time spent on the rotarod was calculated using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Evaluation of Anticonvulsant Activity

PTZ-induced Seizures

The anticonvulsant activity was evaluated using the PTZ-induced seizure paradigm. Mice were randomly divided into seven groups (n = 6 per group). Seizures were induced by intraperitoneal (i.p.) administration of PTZ (75 mg/kg) 30 min after administration of the test compound. The control group received saline (2 mg/kg, p.o.), the disease control group received PTZ only, and the standard group received phenytoin (1 mg/kg, p.o.). The test groups were treated with C1 and C3 at doses of 50 and 100 mg/kg (p.o.) before the PTZ challenge. Following PTZ injection, the animals were observed individually for 30 min in plexiglass cages (25 × 15 × 10 cm). Latency to onset of tonic-clonic convulsions, duration of seizures, and mortality were recorded. Seizure protection was defined as a delayed onset, reduced duration, or complete absence of seizures during the observation period.³⁴⁻³⁷

Statistical Analysis

All data obtained are expressed as the mean ± Standard Error of the Mean (SEM). Data from behavioural tests were statistically analysed using two-way repeated measures ANOVA. In both cases, Tukey's multiple range test was used for the post-hoc analysis. A value of p < 0.05 is considered statistically significant.

RESULTS AND DISCUSSION

Acute Toxicity

Acute oral toxicity was assessed according to the OECD Guideline 425 UDP procedure. Both Synthetic Imidazole derivatives (C1 and C3) were administered at a dose of 2000 mg/kg to Swiss albino mice. During the 14 days, there were no signs of toxicity or mortality. No behavioural or physiological abnormalities were observed in any animals; therefore, derivatives of Imidazole C1 and C3 were found to be safe at 2000 mg/kg.

In Silico Molecular Docking

The selected protein and ligand structures were docked using PyRx, and their binding affinities were predicted. The binding affinities of C1, C2, and C3 were higher than those of the other compounds, indicating their stronger interactions with the target (Table-1).

Compounds ranging from C1-C3 were docked with the help of the PyRx software tool and interacted with GABA (PDB ID 8G4N), a sodium channel inactivator (PDB ID 5VB8), and antidepressants (PDB ID 2QJU). Ligands C1 and C3 showed moderate binding affinities across all three targets compared with the other compounds. For the protein ID 8G4N, the binding affinity scores are -8.3 and -8.1 kcal/mol for C1 and C3, respectively.

However, for the sodium channel inactivator protein (5VB8), both C1 (-9.4) and C3 (-9.3) exhibited strong affinities, comparable to those of the best-performing ligands, suggesting good potential for

channel inhibition. Their binding (2QJU) affinities (-8.4 each) were moderate and consistent with those of most ligands. (Table-1). However, the derivatives C1 and C3 showed good binding affinities. Therefore, these were selected for further studies. (Fig.-2a to 2i)

Table-1: Binding Affinities of Ligands of C1 to C10 against Different Proteins

S. No.	Ligand	Binding affinities of receptors and ligands		
		GABA receptor PDB ID (8G4N)	Sodium channel inactivator PDB ID (5VB8)	Neurotransmitter transporters (SER, NET, DAT) blocker PDB ID (2QJU)
1	C1	-8.3	-9.4	-8.4
2	C2	-8.5	-9.3	-8.2
3	C3	-8.1	-9.3	-8.4
4	C4	-8.8	-8.6	-8.2
5	C5	-8.9	-9.1	-9.2
6	C6	-8.2	-9	-8.4
7	C7	-9	-8.8	-9.4
8	C8	-9	-8.9	-8.4
9	C9	-9.4	-9.1	-8.3
10	C10	-8.7	-8.4	-9.2

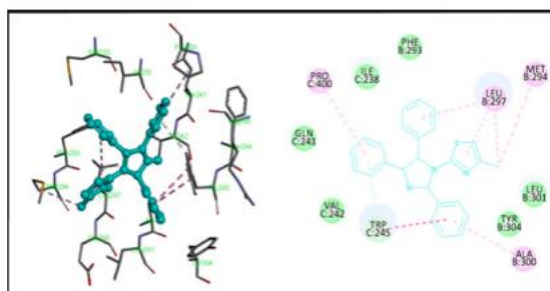


Fig.-2a: 2D and 3D Images of Ligand C1 and Target 8G4N Receptor Interaction

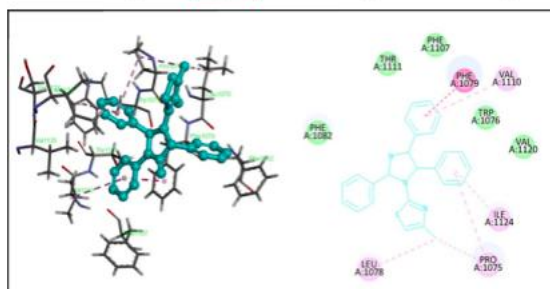


Fig.-2b: 2D and 3D images of Ligand C1 and Target 5VB8 Receptor Interaction

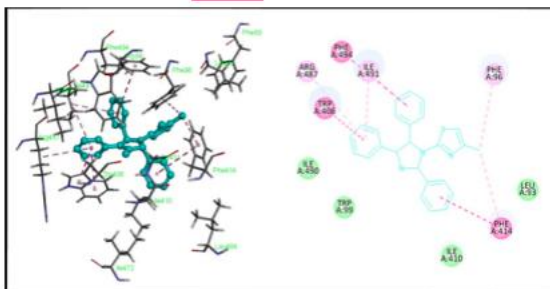
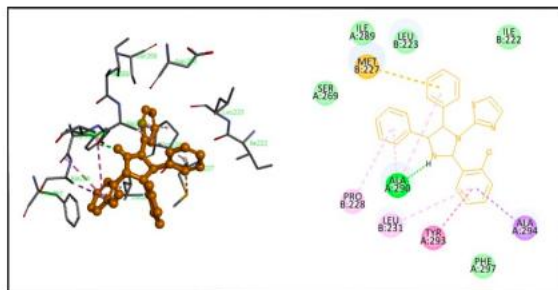
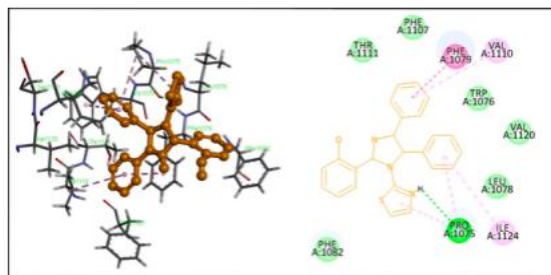
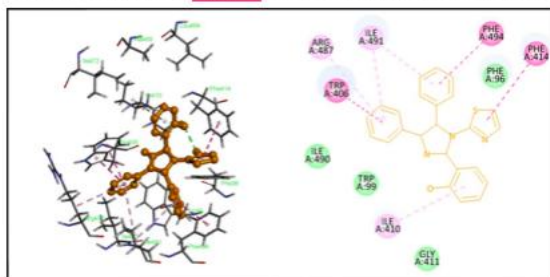
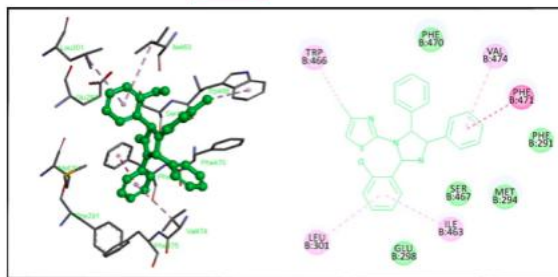
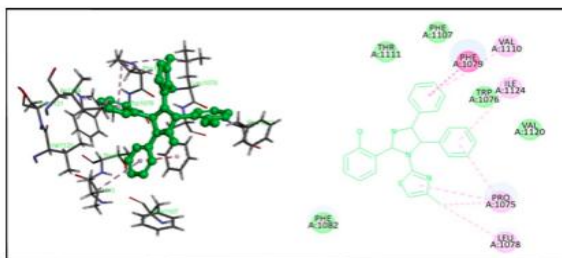
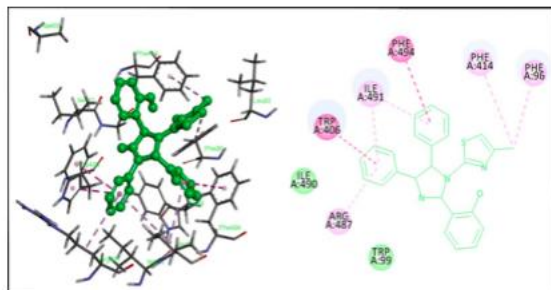


Fig.-2c: 2D and 3D images of Ligand C1 and Target 2QJU Receptor Interaction

Fig.-2d: 2D and 3D images of Ligand C2 and Target 8G4N Receptor InteractionFig.-2e: 2D and 3D images of Ligand C2 and Target 5VB8 Receptor InteractionFig.-2f: 2D and 3D images of Ligand C2 and Target 2QJU Receptor InteractionFig.-2g: 2D and 3D images of Ligand C3 and Target 8G4N Receptor InteractionFig.-2h: 2D and 3D images of Ligand C3 and Target 5VB8 Receptor Interaction



Antioxidant Activity (%RSA) of Test Compounds C1 and C3 using DPPH Assay

Table-2: Percentage RSA of C1 and Standard

S. No.	Concentration in ppm	%RSA of C1 $\pm$ SEM	%RSA of Standard $\pm$ SEM
1	20	12.66 $\pm$ 0.26	22.9 $\pm$ 0.77
2	40	13.60 $\pm$ 0.24	32.84 $\pm$ 0.33
3	60	14.62 $\pm$ 2.68	42.09 $\pm$ 0.72
4	80	14.70 $\pm$ 3.76	52.35 $\pm$ 0.25
5	100	17.16 $\pm$ 3.4	57.69 $\pm$ 0.13

S. No.	Concentration in ppm	%RSA of C3 $\pm$ SEM	%RSA of Standard $\pm$ SEM
1	20	26.95 $\pm$ 0.68	39.11 $\pm$ 1.63
2	40	33.40 $\pm$ 1.93	44.62 $\pm$ 0.55
3	60	42.01 $\pm$ 1.02	49.86 $\pm$ 2.04
4	80	47.45 $\pm$ 2.38	55.91 $\pm$ 1.63
5	100	56.28 $\pm$ 1.02	60.61 $\pm$ 0.34

Evaluation of CNS Depressant Activity using Actophotometer

2065

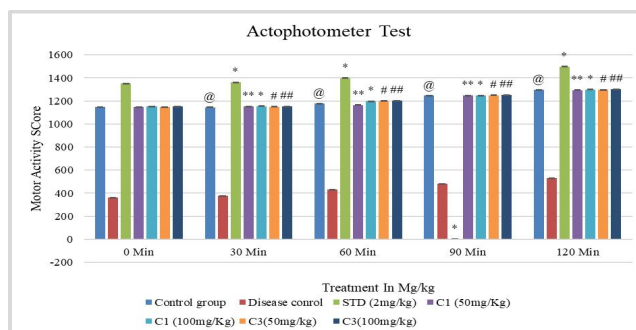


Fig. 3: Effect of C1 and C3 Derivatives on Locomotor Activity in the Actophotometer

Evaluation of CNS Depressant Activity using the Rotarod

We assessed motor coordination and balance using the rotarod apparatus. The disease control group exhibited a significant decrease in the fall latency (11 ± 0.31 s), confirming motor impairment. In contrast, the standard group maintained normal motor performance (44.6 ± 0.43 s), comparable to the control group (44.5 ± 0.29 s). Among the test compounds, C3 at 100 mg/kg showed the most significant improvement in motor coordination, with a fall latency of 46.6 ± 0.54 s, surpassing both the control and standard groups. C1 (100 mg/kg) and C3 (50 mg/kg) exhibited moderate activity with fall times of (34.4 ± 0.33 s) and (29.4 ± 0.33 s), respectively, while C1 at 50 mg/kg showed the least improvement (28 ± 0.92 s) (Table-4). These results suggest that C3 has significantly higher activity, particularly at 100 mg/kg, exerts a protective effect on motor coordination, and shows stronger muscle relaxant activity, indicating that CNS depressants potentially reverse motor deficits. This study supports SDG 3 by developing and evaluating novel imidazole derivatives as potential antiepileptic and antidepressant agents, with the goal of improving therapeutic options for neurological and mental health disorders.

Table-4: Effect of C1 and C3 Derivatives on Motor Coordination in Rotarod

Groups	Mice fall from the rotarod in seconds $\pm$ SEM
Control group	44.5 ± 0.288675
Disease control group	11 ± 0.307318
STD group	44.6 ± 0.428174
C1(50mg/kg)	28 ± 0.918937
C1(100mg/kg)	34.4 ± 0.333333
C3(50mg/kg)	29.4 ± 0.333333
C3(100mg/kg)	46.6 ± 0.542627

[Data are presented as mean $\pm$ standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by multiple comparisons]

Evaluation of Anticonvulsant Activity

Pentylenetetrazol (PTZ) Induced Seizures

In the PTZ-induced seizure model, the control group exhibited rapid seizure onset (30.5 ± 0.5 s) with no protection. The standard drug (STD) significantly delayed seizure onset (240.33 ± 0.5 s, $p < 0.01$) and prolonged seizure duration (5.32 ± 0.26 s), offering $2 \pm 0.01\%$ protection. Among test compounds, C1 (100 mg/kg) demonstrated significant anticonvulsant activity with delayed onset (30.66 ± 0.3 s, $p < 0.01$), increased seizure duration (2.33 ± 0.12 s), and $1.6 \pm 0.03\%$ protection. C3 (100 mg/kg) also significantly delayed seizure onset (38.8 ± 0.3 s, $p < 0.01$) and offered $1.3 \pm 0.01\%$ protection, although seizure duration remained relatively short (1.8 ± 0.29 s) (Table-5).

Table-5: Effect of C1 and C3 Derivatives on the Onset of Seizure, Duration of Seizure, and % Protection of Seizure in the Anticonvulsant Model

Groups	Onset of seizure	Duration of seizure	% Protection
Control group	30.5 ± 0.5	0 ± 0.39	0 ± 0
STD group	$240.33 \pm 0.5^{**}$	$5.32 \pm 0.26^{**}$	$2 \pm 0.01^{**}$
C1(50mg/kg)	$20.5 \pm 0.3^*$	$1.66 \pm 0.43^*$	$0 \pm 0.0^*$
C1(100mg/kg)	$30.66 \pm 0.3^{**}$	$2.33 \pm 0.12^{**}$	$1.6 \pm 0.03^{**}$

C3(50mg/kg)	35 ± 0.3*	1.2 ± 0.45*	0 ± 0*
C3(100mg/kg)	38.8 ± 0.3**	1.8 ± 0.29**	1.3 ± 0.01**

[Data are presented as mean ± standard error of the mean (SEM). Statistical significance was assessed using one-way analysis of variance (ANOVA) followed by multiple comparisons. Symbols indicate significance compared to the disease control group: *p<0.01 and **p<0.05]

Lower doses of C1 and C3 were less effective, with C1 (50 mg/kg) showing minimal anticonvulsant activity and C3 (50 mg/kg) showing no protection. These findings suggest that both C1 and C3 at 100 mg/kg exhibit moderate anticonvulsant effects, with C1 showing slightly greater efficacy in seizure suppression.

CONCLUSION

The present study on the neuropharmacological evaluation of novel synthetic imidazole derivatives demonstrated significant pharmacological activities, supporting the medical relevance of imidazole scaffold-based antiepileptic and antidepressant activities. The imidazole derivatives C1 and C3 were selected based on their binding energies using a computational *in silico* method, such as docking. The radical scavenging antioxidant assay (DPPH assay) revealed that C3 exhibited superior free radical scavenging activity compared with C1, which could contribute to its neuroprotective effects. Neuropharmacological evaluations showed both derivatives produced dose-dependent CNS depressant effects by reducing locomotor activity and impairing motor coordination, with C3 showing stronger activity. Furthermore, in the PTZ-induced seizure model, C1:1-(4-methylthiazol-2-yl)-2,4,5-triphenyl-1H-imidazole and C3:2-(1-(4-methylthiazol-2-yl)-4,5-diphenyl-1H-imidazol-2-yl) phenol (100mg/kg) significantly suppressed seizures and offered substantial protection. Acute toxicity studies confirmed the safety of both C1 and C3 at doses up to 2000 mg/kg. This study validated the neuropharmacological potential of imidazole derivatives C1 and C3 in Swiss Albino mice, emphasising C3 as a natural CNS-active antiepileptic and antidepressant agent. This study supports SDG-3 by developing and evaluating novel imidazole derivatives as potential antiepileptic and antidepressant agents to improve therapeutic options for neuropharmacological and mental health disorders. These findings provide a direction for further studies of both C1 and C3 synergistic compounds, mechanisms of action, and clinical relevance in neurological disorders.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3 of Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. World Health Organisation, *Epilepsy Fact Sheet*, Published online 2024. <https://www.who.int/news-room/fact-sheets/detail/epilepsy>
2. R.S. Fisher, J.H. Cross, J.A. French, N. Higurashi, E. Hirsch, F.E. Jansen, L. Lagae, S.L. Moshé, J. Peltola, E.R. Perez, I.E. Scheffer, S.M. Zuberi, *Epilepsia*, **58(4)**, 522(2017), <https://doi.org/10.1111/epi.13670>
3. O. Devinsky, A. Vezzani, S. Najjar, N.C. De Lanerolle, M.A. Rogawski, *Trends in Neurosciences*, **36(3)**, 174(2013), <https://doi.org/10.1016/j.tins.2012.11.008>
4. C.E. Stafstrom, L. Carmant, *Cold Spring Harbour Perspectives in Medicine*, **5(6)**, a022426(2015), <https://doi.org/10.1101/cshperspect.a022426>
5. M. Bialer, H.S. White, *Nature Reviews Drug Discovery*, **9(1)**, 68(2010), <https://doi.org/10.1038/nrd2997>
6. P. Kwan, A. Arzimanoglou, A.T. Berg, M.J. Brodie, W.A. Hauser, G. Mathern, S.L. Moshé, E. Perucca, S. Wiebe, J. French, *Epilepsia*, **51(6)**, 1069(2010), <https://doi.org/10.1111/j.1528-1167.2009.02397.x>
7. World Health Organisation, *Depression Fact Sheet*, Published online, 2025, <https://www.who.int/news-room/fact-sheets/detail/depression>
8. K.J. Ressler, C.B. Nemeroff, *Depress Anxiety*, **12(S1)**, 2(2000), [https://doi.org/10.1002/1520-6394\(2000\)12:1+<2::AID-DA2>3.0.CO;2-4](https://doi.org/10.1002/1520-6394(2000)12:1+<2::AID-DA2>3.0.CO;2-4)
9. S. Antonina, C. Paweł, J. Maciej, G. Marta, G. Jakub, *Medical Science*, **29(163)**, 1(2025), <https://doi.org/10.54905/disssi.v29i163.e178ms3706>
10. V. Krishnan, E.J. Nestler, *Nature*, **455(7215)**, 894(2008), <https://doi.org/10.1038/nature07455>
11. R.S. Duman, G.K. Aghajanian, *Science*, **338(6103)**, 68(2012), <https://doi.org/10.1126/science.1222939>
12. B. Luscher, Q. Shen, N. Sahir, *Molecular Psychiatry*, **16(4)**, 383(2011), <https://doi.org/10.1038/mp.2010.120>
13. G.I. Papakostas, W.C. Jackson, R. Rafeyan, M.H. Trivedi, *Journal of Clinical Psychiatry*, **81(3)** (2020), <https://doi.org/10.4088/JCP.OT19037COM5>
14. A.M. Kanner, *Epilepsia*, **52(S1)**, 21(2011), <https://doi.org/10.1111/j.1528-1167.2010.02907.x>
15. F. Al-blewi, S.A. Shaikh, A. Naqvi, F. Aljohani, M.R. Aouad, S. Ihmaid and N. Rezki, *International Journal of Molecular Sciences*, **22(3)**, 1162(2021), <https://doi.org/10.3390/ijms22031162>
16. E. Sigel, M.E. Steinmann, *Journal of Biological Chemistry*, **287(48)**, 40224(2012), <https://doi.org/10.1074/jbc.R112.386664>
17. R.W. Olsen, W. Sieghart, *Neuropharmacology*, **56(1)**, 141(2009), <https://doi.org/10.1016/j.neuropharm.2008.07.045>
18. W.A. Catterall, *The Journal of Physiology*, **590(11)**, 2577(2012), <https://doi.org/10.1113/jphysiol.2011.224204>
19. J. Payandeh, T. Scheuer, N. Zheng, W.A. Catterall, *Nature*, **475(7356)**, 353(2011), <https://doi.org/10.1038/nature10238>
20. G.E. Torres, R.R. Gainetdinov, M.G. Caron, *Nature Reviews Neuroscience*, **4(1)**, 13(2003), <https://doi.org/10.1038/nrn1008>
21. A. Yamashita, S.K. Singh, T. Kawate, Y. Jin, E. Gouaux, *Nature*, **437(7056)**, 215(2005), <https://doi.org/10.1038/nature03978>
22. V.K. Yadav, M. Bhandari, S. Singh, *Rasayan Journal of Chemistry*, **17(4)**, 1727(2024) <https://doi.org/10.31788/RJC.2024.1748967>
23. T.S. Vasantha, U. Maheshwari, F. Sultana, R. Begum, *World Journal of Biology Pharmacy Health Sciences*, **18(2)**, 386(2024), <https://doi.org/10.30574/wjbphs.2024.18.2.0294>
24. S.B. Patel, K. R.K. Sutarsandhiya, *Rasayan Journal of Chemistry*, **17(4)**, 2067(2024), <https://doi.org/10.31788/RJC.2024.1748975>

25. CPCSEA, *Guidelines for Laboratory Animal Facility*, Committee for Control and Supervision of Experiments on Animals, Ministry of Environment, Forest and Climate Change, Government of India, Published online, 2025, <http://cpcsea.gov.in>
26. OECD, *Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure*, OECD, 2022. <https://doi.org/10.1787/9789264071049-en>
27. M. Rahman, B. Islam, M. Biswas, K. Alam, *BMC Research Notes*, **8(1)**, 621(2015), <https://doi.org/10.1186/s13104-015-1618-6>
28. P. Molyneux, *Songklanakarin Journal of Science and Technology*, **26** (2004).
29. K. Alper, K.A. Schwartz, R.L. Kolts, A. Khan, *Biological Psychiatry*, **62(4)**, 345(2007), <https://doi.org/10.1016/j.biopsych.2006.09.023>
30. U. Bhosale, R. Yegnanarayan, P. Prachi, M. Zambare, R.S. Somani, *Annals of Neurosciences*, **18(2)**, 44-7 (2011), <https://doi.org/10.5214/ans.0972.7531.1118204>
31. G.L. Krauss, P. Klein, C. Brandt, S.K. Lee, I. Milanov, M. Milovanovic, B.J. Steinhoff, M. Kamin, *The Lancet Neurology*, **19(1)**, 38(2020), [https://doi.org/10.1016/S1474-4422\(19\)30399-0](https://doi.org/10.1016/S1474-4422(19)30399-0)
32. A.N. Shuvaev, O.S. Belozor, O.I. Mozhei, A.G. Mileiko, L.D. Mosina, I.V. Laletina, I.G. Mikhailov, Y.V. Fritsler, A.G. Teschemacher, and S. Kasparov, *Brain Sciences*, **12(4)**, 495(2022), <https://doi.org/10.3390/brainsci12040495>
33. H.M. Shan, M.A. Maurer, M.E. Schwab, *BMC Biology*, **21(1)**, 177(2023), <https://doi.org/10.1186/s12915-023-01679-y>
34. H. Hosseinzadeh, G.R. Karimi, M. Rakhshanizadeh, *Journal of Ethnopharmacology*, **98(1-2)**, 207(2005), <https://doi.org/10.1016/j.jep.2004.12.007>
35. J. Duan, J. Wang, Q. Zhao, D. Wu, Y. Liu, *Pharmacognosy Magazine*, **20(1)**, 347(2024), <https://doi.org/10.1177/09731296231203790>
36. I.E. Scheffer, S. Berkovic, G. Capovilla, M.B. Connolly, J. French, L. Guilhoto, E. Hirsch, S. Jain, G.W. Mathern, S.L. Moshé, D.R. Nordli, E. Perucca, T. Tomson, S. Wiebe, Y.H. Zhang, S.M. Zuberi, *Epilepsia*, **58(4)**, 512(2017), <https://doi.org/10.1111/epi.13709>
37. W. Löscher, H. Klitgaard, R.E. Twyman, D. Schmidt, *Nature Reviews Drug Discovery*, **12(10)**, 757(2013), <https://doi.org/10.1038/nrd4126>

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