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Novel 1,4-Dihydropyridine–1,3,4-Oxadiazole Hybrids as Potential Anticonvulsant Agents: Microwave-Assisted Synthesis, *In Silico* Studies, And Biological Evaluation

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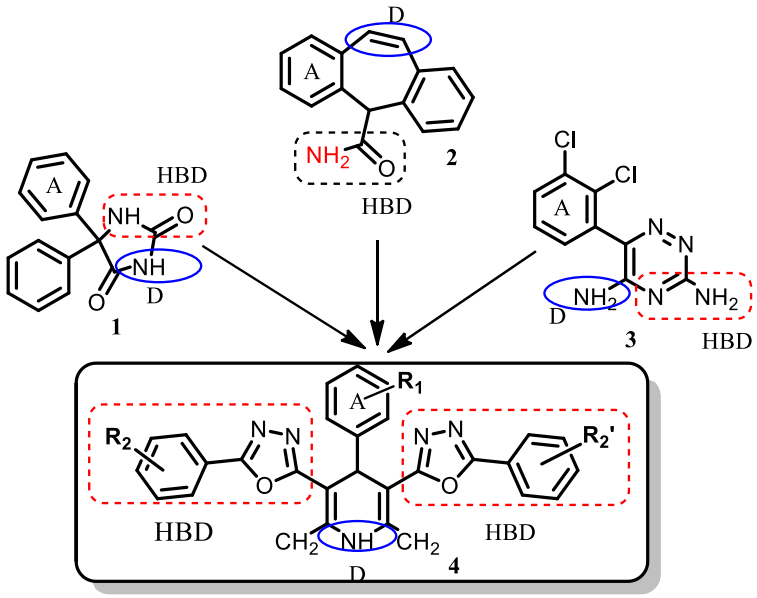
ABSTRACT

Here in the design, in silico analysis, green synthesis, and biological evaluation of novel hybrid compounds containing 1,4-dihydropyridine (DHP) and 1,3,4-oxadiazole (Ox) as potential anticonvulsant agents has been reported. A focused library of DHP–Ox derivatives was screened using SwissADME and molecular docking against the voltage-gated sodium channel protein (PDB ID: 5HVX). Based on the docking results, three lead compounds (DP01–DP03) were selected and synthesized using a microwave-assisted green approach. The synthesized compounds were characterized by TLC, FTIR, ^{1}H-NMR, and LC–MS. Docking studies showed strong binding affinities (−9.3 to −10.3 kcal/mol), which were better than the standard drug phenytoin (−7.5 kcal/mol). Acute toxicity studies revealed no mortality up to 2000 mg/kg. In the strychnine-induced seizure model, all compounds showed significant dose-dependent anticonvulsant activity. DP01 exhibited the highest activity, significantly increasing seizure latency and providing complete protection against convulsions. These findings identify DHP–Ox hybrids as promising lead molecules for the development of safer and more effective anticonvulsant drugs.

Key words: Microwave assisted synthesis, *In-silico* Study, Dihydropyridine, 1,3,4-Oxadiazole, Anticonvulsant.

1. Introduction:

Epilepsy is a chronic neurological condition characterized by persistent and unprovoked seizures arising from abnormal neuronal hyperexcitability in the brain. It affects nearly 50 million people worldwide and remains one of the most widespread neurological disorders linked with significant morbidity and condensed quality of life [1,2]. Although antiepileptic drugs (AEDs) are clinically accessible, approximately 30% of epileptic patients continue to suffer from pharmacoresistant seizures, while long-term therapy is often associated with adverse effects such as hepatotoxicity, neurotoxicity, sedation, cognitive impairment, and behavioral instabilities [3–5]. Therefore, the development of safer and more effective anticonvulsant agents continues to be an imperative objective in medicinal chemistry and neuropharmacological investigation.Voltage-gated sodium channels (VGSCs) play a perilous role in neuronal depolarization and propagation of action potentials and are among the most validated molecular targets for anticonvulsant therapy [6,7]. Undue sodium influx fallouts in repetitive neuronal firing and sustained membrane depolarization, contributing to seizure commencement and epileptogenesis [8]. Clinically established anticonvulsants such as Phenytoin (1), Carbamazepine (2), and Lamotrigine (3) exert their anticonvulsant effects primarily through modulation of voltage-gated sodium channels. Structural analysis of these agents revealed essential pharmacophoric features responsible for anticonvulsant activity, including a hydrophobic aryl binding domain, hydrogen-bonding functionality, and electron-donating groups. Based on these structural requirements, the present DHP–Ox scaffold (4) was rationally designed by integrating the pharmacophoric features (Figure 1) of clinically active anticonvulsants into a single molecular framework [9-10]. The incorporation of the 1,4-dihydropyridine and 1,3,4-oxadiazole nuclei was therefore expected to enhance sodium channel interaction and contribute toward improvedanticonvulsant activity.

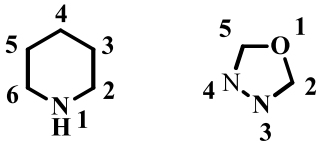


Where:

A- Hydrophobic domain; HBD-Hydrogen Bonding Domain; D- Electron donar moeity

Figure 1. Pharmacophoricprovisions for anticonvulsant Drug discovery

Among various nitrogen-containing heterocyclic systems, 1,4-dihydropyridine (DHP) derivatives have gained considerable attention because of their broad spectrum of pharmacological activities including antihypertensive [11], anti-inflammatory [12], anticancer [13], neuroprotective [14], and anticonvulsant properties [15-17]. DHP derivatives are widely recognized as calcium channel modulators; however, recent studies have demonstrated that substituted DHP analogs can significantly influence neuronal ion homeostasis and central nervous system (CNS) activity [15-16].



1,4-dihydropyridine **1,3,4-oxadiazole**

Structural modification of the DHP nucleus through electron-donating and electron-withdrawing substituents has been reported to alter receptor affinity, lipophilicity, and anticonvulsant efficacy [16]. Hydroxyl- and methoxy-substituted DHP derivatives have shown promising anticonvulsant potential in experimental seizure models owing to enhanced receptor interaction and improved hydrogen-bonding characteristics [17,18].Similarly, the 1,3,4-oxadiazole ring system is an important privileged scaffold extensively explored in medicinal chemistry due to its favorable

pharmacokinetic profile, metabolic stability, and broad spectrum of biological activities including antimicrobial, anti-inflammatory, anticancer, analgesic, and anticonvulsant effects [19–21]. Incorporation of the oxadiazole nucleus into hybrid pharmacophores has been shown to improve lipophilicity, receptor-binding interactions, and overall biological potency [22]. Recent investigations have demonstrated that oxadiazole-containing derivatives possess significant anticonvulsant activity through interaction with neuronal receptors and ion channels involved in seizure regulation [23].

Molecular hybridization is an effective medicinal chemistry strategy involving the integration of two or more bioactive pharmacophores into a single molecular framework to obtain compounds with enhanced pharmacological activity and improved physicochemical properties [24]. In addition, computer-aided drug design approaches such as molecular docking and ADME prediction have become valuable tools for rational lead identification and optimization by predicting receptor interactions, binding affinity, and pharmacokinetic behavior prior to synthesis [25]. Furthermore, microwave-assisted organic synthesis has emerged as an efficient and environmentally favorable technique due to its reduced reaction times, improved yields, operational simplicity, and cleaner reaction conditions compared with conventional synthetic methods [26-27].

Based on these considerations, the present study was undertaken to design novel DHP–1,3,4-oxadiazole hybrid derivatives as potential anticonvulsant agents targeting voltage-gated sodium channels. A focused library of derivatives was computationally screened using molecular docking and ADME prediction studies. Selected lead compounds exhibiting favorable in silico profiles were synthesized using a microwave-assisted approach, characterized by spectroscopic techniques, and evaluated for anticonvulsant activity using the strychnine-induced seizure model in mice. Additionally, structure–activity relationship (SAR) analysis was performed to investigate the influence of aromatic substitutions on anticonvulsant activity.

2. Materials and Methods

2.1. Chemicals and Reagents

All chemicals and reagents used in the present study were of analytical reagent (AR) grade and procured from reputed commercial suppliers including Thomas Baker, Loba Chemie, SDFCL Fine-Chem Ltd., and Avra Synthesis Pvt. Ltd. The reagents were used without further purification unless otherwise specified.

Melting points of the synthesized compounds were determined using an electrical melting point apparatus by the open capillary tube method, and the reported values are uncorrected. Reaction progress and purity of the compounds were monitored by thin-layer chromatography (TLC) using silica gel precoated aluminum plates as the stationary phase. The solvent system employed for TLC analysis consisted of n-hexane and ethyl acetate (7:3, v/v). TLC spots were visualized under ultraviolet (UV) light and iodine vapor exposure. The presence of a single spot under different solvent conditions 1. confirmed the purity of the synthesized compounds.

Infrared (IR) spectra were recorded using a Shimadzu IR Affinity-1 FTIR spectrophotometer by the potassium bromide (KBr) pellet method, and absorption frequencies were expressed in cm^{-1}. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL 600 MHz NMR spectrometer using DMSO-d6 as the solvent, and chemical shifts (δ) were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Multiplicity patterns were designated as singlet (s), doublet (d), triplet (t), and multiplet (m). Mass spectral analysis was carried out using LC–MS instrumentation for molecular weight confirmation.

2.2. *In Silico* Studies

2.2.1. Software and Computational Tools

Molecular modeling and docking studies were performed using Discovery Studio Visualizer(Version 21.1.0), PyMOL(Version 0.8), and AutoDockTools(Version 1.5.7). Ligand structures were prepared using ChemDraw Ultra 8.0 and Chem3D software. [28]

2.2.2. ADME Prediction Studies

The physicochemical and pharmacokinetic properties of the designed compounds were evaluated using SwissADME. Parameters including molecular weight, lipophilicity (LogP), topological polar surface area (TPSA), hydrogen bond donors and acceptors, molar refractivity, gastrointestinal (GI) absorption, blood–brain barrier (BBB) permeability, Lipinski's rule of five, bioavailability score, and synthetic accessibility were predicted. The BOILED-Egg model was employed to assess passive gastrointestinal absorption and brain penetration characteristics of the designed derivatives. [29]

2.2.3. Protein Preparation

The crystal structure of the voltage-gated sodium channel complexed ligand (PDB ID: 5HVX) was retrieved from the RCSB Protein Data Bank. The protein structure was imported into Discovery Studio Visualizer for preprocessing. Water molecules, heteroatoms, and co-crystallized ligands were removed from the protein structure to obtain the target receptor. Polar hydrogen atoms were added to simulate physiological conditions, and the optimized protein structure was subsequently saved in PDB format for docking studies. [30]

2.2.4. Ligand Preparation

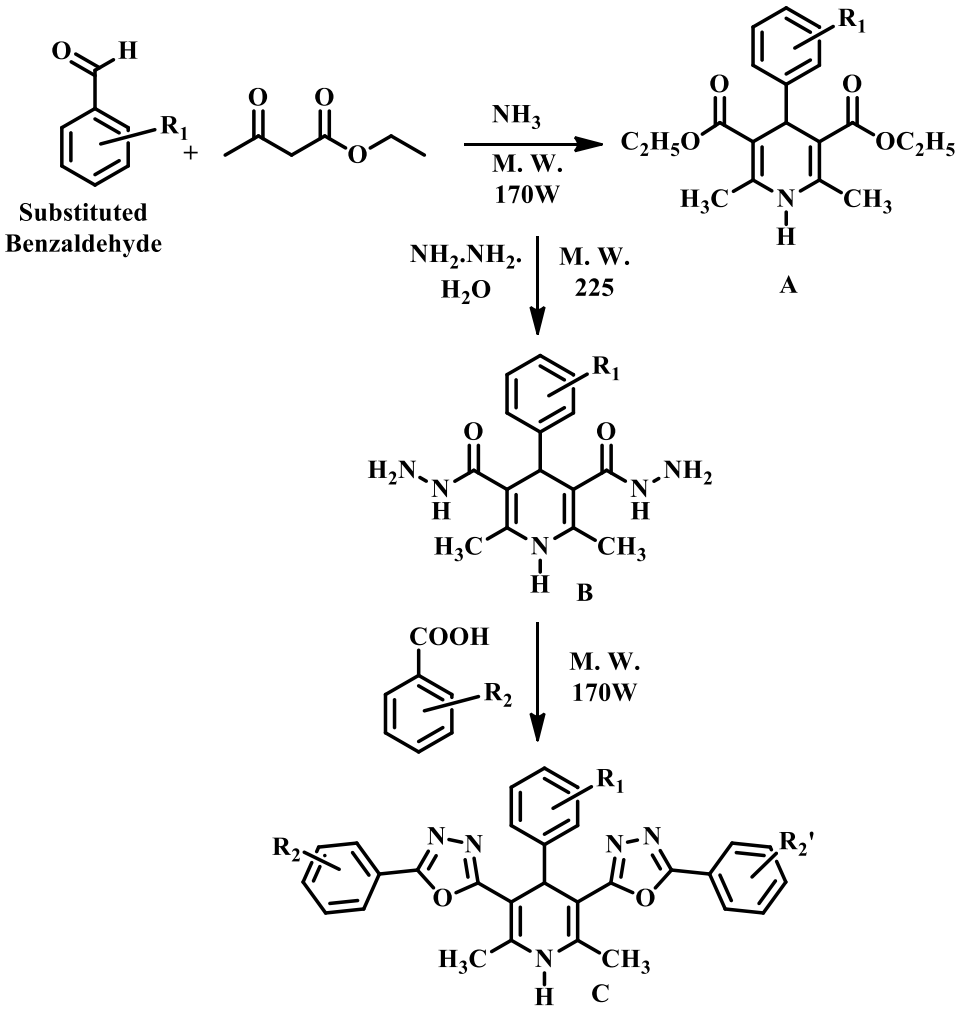
The chemical structures of the designed DHP–Ox derivatives were drawn using ChemDraw Ultra 8.0 software. The corresponding SMILES notation was generated and imported into Chem3D Ultra for energy minimization using the MM2 molecular mechanics force field. After geometry optimization, the minimized ligand structures were saved in PDB format for molecular docking analysis.

2.2.5. Molecular Docking Studies

Molecular docking studies were carried out using AutoDockTools-1.5.7 to evaluate the binding affinity of the designed compounds toward the voltage-gated sodium channel. The prepared protein and ligand structures were converted into PDBQT format prior to docking. Docking simulations were performed to identify the optimal ligand conformations and calculate binding affinity values expressed in kcal/mol. The docked complexes were visualized using PyMOL and Discovery Studio Visualizer to analyze intermolecular interactions such as hydrogen bonding, hydrophobic interactions, and bond distances between ligands and amino acid residues within the active site. [31]

2.3. Chemistry

2.3.1. Synthetic Scheme



Scheme 1. Microwave-assisted syntheticway for the preparation of novel substituted 1,4-dihydropyridine–1,3,4-oxadiazole hybrid derivatives.

2.3.2. General Procedure for the Microwave Assisted Synthesis of Diethyl 2,6-dimethyl-4-substitutedphenyl-1,4-dihydropyridine-3,5-dicarboxylate [A] (Step I)

A mixture of substituted benzaldehyde (10 mmol), ethyl acetoacetate (20 mmol), and ammonia (30 mmol) was transferred into a microwave-compatible round-bottom flask. The reaction mixture was subjected to microwave irradiation using a domestic microwave system (Catalyst System CATA 2R) at 170 W for 15–18 min. After completion of the reaction, the mixture was cooled to room temperature and transferred into chloroform. The contents were filtered through a sintered glass funnel containing sodium bicarbonate to neutralize residual acidity. The organic layer was separated, and the crude product was recrystallized using ethanol to obtain purified crystals. The

purity of the synthesized compounds was confirmed by TLC using n-hexane:ethyl acetate (7:3 v/v) as the mobile phase.[32]

2.3.2.1. General Procedure for the Microwave Assisted Synthesis of 2,6-Dimethyl-4-substitutedphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide [B] (Step II)

Diethyl 2,6-dimethyl-4-substitutedphenyl-1,4-dihydropyridine-3,5-dicarboxylate was dissolved in 95% ethanol in a round-bottom flask fitted with a reflux condenser. Equimolar quantities of hydrazine hydrate (85%) were added dropwise with continuous stirring for approximately 10 min. The reaction mixture was then subjected to microwave irradiation at 225 W for 25–30 min. Reaction progress was monitored by TLC using n-hexane:ethyl acetate (7:3 v/v). Upon completion, the reaction mixture was cooled, and crushed ice was added to precipitate the product. The resulting solid was filtered, washed, and dried. [33]

2.3.2.3. General Procedure for the Synthesis of 2,6-Dimethyl-4-Substitutedphenyl-3,5-bis(5-substitutedphenyl-1,3,4-oxadiazol-2-yl)-1,4-dihydropyridine [C] (Step III)

An equimolar mixture of 2,6-dimethyl-4-substitutedphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide and substituted aromatic acid was dissolved in ethanol, followed by the addition of a few drops of concentrated sulfuric acid as a catalyst. The reaction mixture was stirred and subjected to microwave irradiation at 170 W for 15–20 min. Completion of the reaction was monitored by TLC using n-hexane:ethyl acetate (7:3 v/v). After completion, crushed ice was added to the reaction mixture to induce precipitation of the product. The precipitated solid was filtered, dried, and purified for further characterization. [34]

2.4. Pharmacological Evaluation

2.4.1. Experimental Animals

Healthy Swiss albino male mice weighing 22–30 g were used for the pharmacological studies. The animals were housed in polypropylene cages under standard laboratory conditions with controlled temperature (25 ± 2 °C), relative humidity (55 ± 5%), and a 12 h light/dark cycle. Animals were provided with standard pellet diet and water ad libitum throughout the experimental period. Bedding material consisting of rice husk was replaced twice weekly to maintain hygienic conditions. Prior to experimentation, animals were acclimatized to laboratory conditions for at least 30 min. All experimental protocols were carried out in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of MET's Institute of Pharmacy, Nashik (Registration No. 1344/PO/RE/S/10/CPCSEA).

2.4.2. Acute Oral Toxicity Study

Acute oral toxicity studies of the synthesized compounds were performed according to OECD Guideline 425 (Up-and-Down Procedure) for determination of median lethal dose (LD_{50}). The synthesized compounds were administered orally in a stepwise manner at dose levels of 55, 175, 550, and 2000 mg/kg body weight. The animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h with special attention during the initial 4 h, and daily thereafter for a total period of 14 days for signs of toxicity, behavioral changes, and mortality. LD_{50} values were estimated using AOT425Stat software (Version 1.0). [35]

The test compounds were suspended in 5% carboxymethyl cellulose (CMC) immediately before administration. Statistical analysis of the experimental data was carried out using one-way ANOVA followed by Dunnett's post hoc test using GraphPad Prism software. The results were expressed as mean ± SEM, and values of $p < 0.05$ were considered statistically significant.

2.4.3. Evaluation of Anticonvulsant Activity [36]

The anticonvulsant activity of the synthesized compounds was evaluated using the strychnine-induced seizure model in mice. The animals were randomly divided into different experimental groups consisting of six animals in each group. The disease control group received strychnine (2 mg/kg), whereas the standard group received Diazepam (2 mg/kg, p.o.). The test groups received synthesized compounds DP01, DP02, and DP03 at a dose of 200 mg/kg orally mention [37] Table 1.The synthesized compounds were freshly suspended in 5% CMC prior to administration. Following treatment, convulsions were induced by administration of strychnine, and the animals were observed for seizure onset, number of convulsions, duration of convulsions, and percentage protection against seizures. The latency to convulsion was recorded in minutes. Animals were continuously monitored during the observation period, and anticonvulsant activity was assessed by comparing the treated groups with the disease control group.

Table 1. Experimental Design for Anticonvulsant Activity

Group	Treatment	Dose (mg/kg, p.o.)	Number of Animals
I	Disease Control (Strychnine)	2	6
II	Standard (Diazepam)	2	6
III	DP01	200	6
IV	DP02	200	6
V	DP03	200	6

Statistical analysis of anticonvulsant activity data was performed using one-way ANOVA followed by Dunnett's multiple comparison test. Results were expressed as mean ± SEM, and $p < 0.05$ was considered statistically significant.

3. Result and discussion:

3.1 Chemistry

A focused library of novel 1,4-dihydropyridine–1,3,4-oxadiazole (DHP–Ox) hybrid derivatives (DP01–DP10) **Scheme 1**.was designed as potential anticonvulsant agents targeting voltage-gated sodium channels. Based on molecular docking affinity and predicted pharmacokinetic characteristics, selected lead compounds (DP01–DP03) were synthesized successfully through a microwave-assisted synthetic protocol. The synthetic route involved sequential Hantzsch condensation, hydrazide formation, and cyclodehydration reactions leading to formation of the target DHP–Ox hybrid scaffold.

Microwave-assisted synthesis significantly reduced reaction time and improved reaction efficiency compared with conventional heating methods. The synthesized compounds were obtained in good to excellent yields ranging from 73–95%, indicating the suitability of microwave irradiation for rapid synthesis of heterocyclic derivatives. Thin-layer chromatography performed using n-hexane:ethyl acetate (7:3 v/v) revealed single spots for all synthesized compounds, confirming completion of the reaction and purity of the isolated products.

Hydroxyl-substituted derivatives such as DP01, DP03, and DP09 (Table 2.) demonstrated comparatively higher yields (93–95%), which may be attributed to favorable electronic effects facilitating cyclization and stabilization of the oxadiazole ring system. In contrast, the chloro-substituted derivative DP05 showed relatively lower yield (79%), possibly due to the electron-withdrawing nature of chlorine affecting reaction progression.

Table 2. Substitution pattern of designed DHP–Ox derivatives

Compound	R_1	R_2, R_2'
DP01	2-OH	2,3,4-trihydroxy
DP02	2-OH	2-OH
DP03	4-OH	2,3,4-trihydroxy
DP04	4-OH	2-OH
DP05	4-OH	2-Cl
DP06	H	H
DP07	H	4-OCH_3
DP08	H	2-OH
DP09	4-OCH_3	2,3,4-trihydroxy
DP10	4-OCH_3	2-OH

Compound DP01: 4-(5-(4-(2-hydroxyphenyl)-2,6-dimethyl-5-(5-(2,3,4-trihydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1,4-dihydropyridin-3-yl)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol; molecular formula: $C_{29}H_{23}N_5O_9$; molecular weight: 585.15 g/mol; yield: 93%; white solid; mp 168–170°C; Rf = 0.75 (n-hexane:ethyl acetate, 7:3). FTIR (KBr, cm^{-1}): 3556, 3043, 2910, 1944, 1487, 1273, 752. ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.1 (s, 1H, NH), 7.28–8.54 (m, Ar–H), 4.90–5.20 (m, OH), 4.39 (s, 1H, DHP-H), 1.70 (s, 6H, CH_3). ESI-MS (ESI^+): m/z 587 $[M+H]^+$.

Compound DP02: 2-(5-(4-(2-hydroxyphenyl)-5-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-2,6-dimethyl-1,4-dihydropyridin-3-yl)-1,3,4-oxadiazol-2-yl)phenol; molecular formula: $C_{29}H_{23}N_5O_5$; molecular weight: 521.17 g/mol; yield: 80%; white solid; mp 126–128°C; Rf = 0.65 (n-hexane:ethyl acetate, 7:3). FTIR (KBr, cm^{-1}): 3606 (O–H stretching), 3065 (Ar–C–H stretching), 2926 (aliphatic C–H stretching), 1683 (C=O stretching), 1618 (C=C/C=N aromatic stretching), 1233 (C–O stretching), 754 (Ar–C–H bending). ^{1}H-NMR (500 MHz, DMSO-d_6, δ ppm): 11.2 (s, 1H, NH of DHP), 6.10–7.50 (m, Ar–H), 4.90–5.20 (m, phenolic OH), 4.39 (s, 1H, C4 proton of DHP ring), 1.70 (s, 6H, CH_3). ESI-MS (ESI^+): m/z 521 $[M+H]^+$.

Compound DP03: 4-(5-(4-(4-hydroxyphenyl)-2,6-dimethyl-5-(5-(2,3,4-trihydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1,4-dihydropyridin-3-yl)-1,3,4-oxadiazol-2-yl)benzene-1,2,3-triol; molecular formula: $C_{29}H_{23}N_5O_9$; molecular weight: 585.15 g/mol; yield: 95%; white solid; mp

120–122 °C; Rf = 0.68 (n-hexane:ethyl acetate, 7:3). FTIR (KBr, cm^{-1}): 3554 (O–H stretching), 2974 (aliphatic C–H stretching), 1707 (C=O stretching), 1616 (C=N stretching), 1533 (C=C aromatic stretching), 1095 (C–O stretching), 1039 (Ar–C–H bending). ^1H NMR (400 MHz, DMSO-d_6, δ ppm): 10.8 (s, 1H, NH of DHP), 6.90–7.10 (m, Ar–H), 4.90–5.20 (m, phenolic OH), 4.20 (s, 1H, C4 proton of DHP ring), 1.60 (s, 6H, CH_3). ESI-MS (ESI^+): m/z 585 $[M+H]^+$.

3.2 Physicochemical and *In Silico* ADME Analysis

The physicochemical and pharmacokinetic properties of the designed DHP–Ox derivatives were predicted using SwissADME in order to obtain preliminary insight (Table 3.) into their drug-likeness and absorption characteristics. The calculated physicochemical parameters demonstrated that the molecular weights of the designed derivatives ranged from 473.19–599.17 g/mol, while consensus LogP values suggested moderate lipophilicity favorable for receptor interaction. Hydroxyl-rich derivatives such as DP01 and DP03 exhibited comparatively higher topological polar surface area (TPSA) values due to the presence of multiple hydrogen bond donor and acceptor functionalities.

Table 3. Predicted physicochemical and ADME properties of selected DHP–Ox derivatives

Compound	MW (g/mol)	TPSA (Å²)	HBA	HBD	Consensus LogP	GI Absorption	BBB Permeant	Lipinski Violations
DP01	585.15	231.48	13	8	1.42	Low	No	3
DP02	521.17	150.56	9	4	3.15	Low	No	1
DP03	585.15	231.48	13	8	1.42	Low	No	3
DP04	521.17	150.56	9	4	3.15	Low	No	1
DP05	557.10	110.10	7	2	5.04	Low	No	2

SwissADME analysis predicted comparatively low gastrointestinal absorption and limited passive blood–brain barrier permeability for most of the designed compounds. The elevated TPSA values

and multiple hydroxyl substitutions present in compounds DP01 and DP03 may reduce passive membrane diffusion characteristics. Furthermore, several derivatives exhibited Lipinski rule violations owing to increased molecular polarity and hydrogen bonding potential.

The BOILED-Egg (Figure 2.) pharmacokinetic model indicated that the majority of the designed derivatives were positioned outside the optimal BBB-permeable region, suggesting limited passive CNS penetration. In addition, some compounds were predicted to behave as P-glycoprotein substrates, which may further influence CNS distribution and pharmacokinetic behavior. However, these computational predictions should be interpreted cautiously, as SwissADME represents a preliminary predictive model and may not completely account for complex *in-vivo* biological transport mechanisms and pharmacodynamic responses.

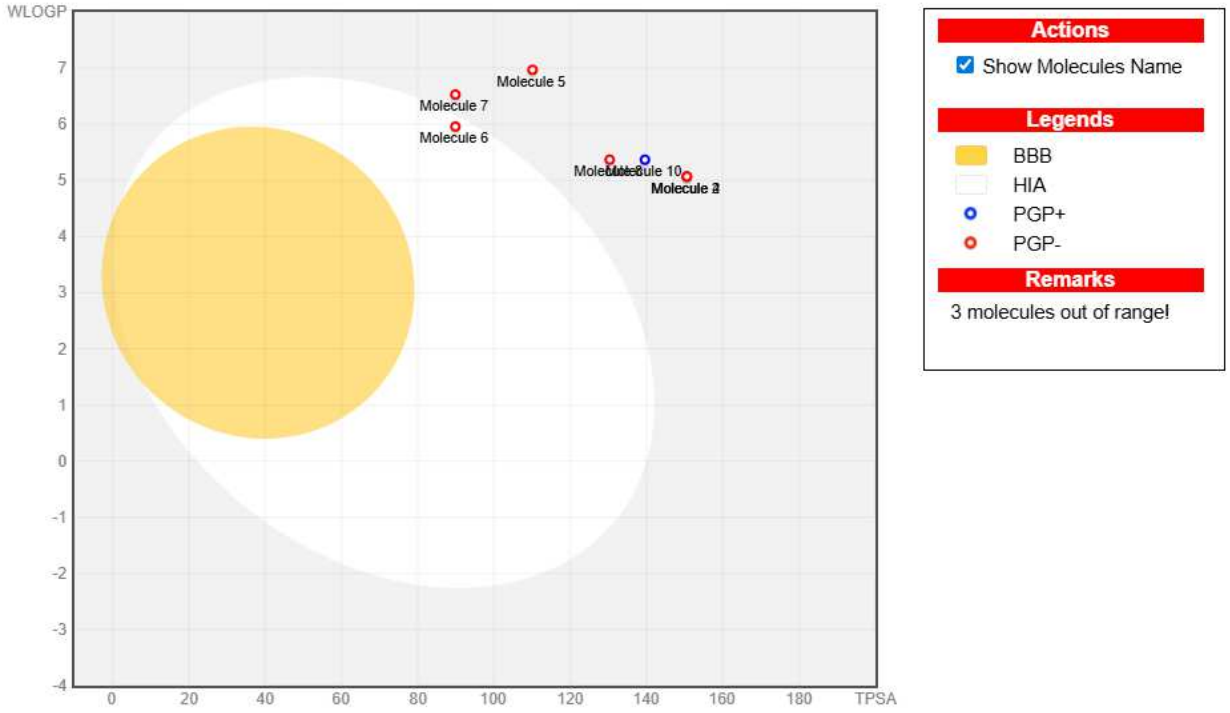


Figure 2. BOILED-Egg model prediction of gastrointestinal absorption and blood–brain barrier permeability of designed DHP–Ox derivatives generated using SwissADME.

Interestingly, despite the predicted limitations in BBB permeability, synthesized compounds DP01–DP03 demonstrated significant anticonvulsant activity in the strychnine-induced seizure model. The observed *in vivo* activity suggests that the synthesized derivatives may still attain pharmacologically relevant CNS exposure under physiological conditions. This apparent discrepancy between computational prediction and biological activity may be attributed to limitations of *in-silico* prediction models, possible carrier-mediated transport mechanisms, partial

BBB permeation, or dynamic in vivo pharmacokinetic behavior not fully represented by computational tools.

Among the designed derivatives, DP02 and DP04 demonstrated comparatively balanced lipophilicity and TPSA values with fewer Lipinski violations, indicating relatively favorable pharmacokinetic characteristics. In contrast, hydroxyl-rich derivatives exhibited superior docking affinity and anticonvulsant activity, suggesting that enhanced receptor-binding interactions and hydrogen-bonding capability may compensate for reduced permeability characteristics.

Overall, the *in silico*ADME analysis provided useful preliminary pharmacokinetic insight into the designed DHP–Ox derivatives and supported further optimization of the scaffold for improved CNS drug-likeness and anticonvulsant potential.

3.3 Spectral Characterization

The structures of the synthesized DHP–Ox hybrid derivatives were confirmed using FTIR, ^{1}H-NMR, and LC–MS spectral analyses. The spectral data *(see supplementary data)*obtained were in good agreement with the proposed molecular structures.

3.3.1 FTIR Spectral Analysis

The FTIR spectra of compounds DP01–DP03 exhibited characteristic absorption bands corresponding to important functional groups present within the synthesized derivatives. Broad absorption bands observed in the range of 3554–3606 cm^{-1} confirmed the presence of phenolic O–H stretching vibrations. Aromatic C–H stretching frequencies appeared between 3043–3065 cm^{-1}, whereas aliphatic C–H stretching vibrations corresponding to methyl groups of the DHP nucleus appeared between 2910–2974 cm^{-1}.

Characteristic C=N stretching vibrations of the oxadiazole ring were observed around 1616 cm^{-1}, confirming successful cyclization and formation of the oxadiazole nucleus. Aromatic C=C stretching frequencies appeared within the range of 1487–1618 cm^{-1}, while aromatic C–O stretching bands were observed between 1095–1273 cm^{-1}. The disappearance of hydrazide-associated peaks and appearance of oxadiazole-specific absorption bands confirmed successful formation of the target DHP–Ox derivatives.

3.3.2 ^{1}H-NMR Spectral Analysis

The ^{1}H-NMR spectra of compounds DP01–DP03 further supported the proposed structures of the synthesized derivatives. A characteristic singlet corresponding to the N–H proton of the 1,4-

dihydropyridine nucleus appeared at δ 10.8–11.2 ppm. Multiplet signals observed within the aromatic region at δ 6.1–7.5 ppm was assigned to aromatic protons of substituted phenyl rings attached to the DHP–Ox scaffold.

Phenolic hydroxyl protons appeared as multiplet signals around δ 4.9–5.2 ppm, confirming hydroxyl substitution on the aromatic rings. The proton associated with the C4 position of the 1,4-dihydropyridine ring appeared as a characteristic singlet around δ 4.2–4.5 ppm. Furthermore, methyl protons attached to the DHP nucleus appeared as singlet signals around δ 1.6–1.7 ppm.

3.3.3 LC–MS Spectral Analysis

LC–MS analysis further confirmed the molecular structures and purity of compounds DP01 and DP03. Compound DP01 exhibited a prominent molecular ion peak at m/z 587 corresponding to the protonated molecular ion $[M+H]^+$ consistent with the proposed molecular formula $C_{29}H_{23}N_5O_9$. Additional fragment ion peaks observed at m/z 448.126, 434.123, 355.092, and 307.107 supported sequential fragmentation of substituted oxadiazole-linked aromatic moieties and partial cleavage of the DHP nucleus.

Similarly, compound DP03 displayed a characteristic molecular ion peak at m/z 585 corresponding to its molecular formula $C_{29}H_{23}N_5O_9$. Fragment ions observed at m/z 543.115 and 200.108 further confirmed fragmentation of substituted oxadiazole and dihydropyridine moieties.

Collectively, FTIR, ^{1}H-NMR, and LC–MS analyses conclusively confirmed successful synthesis and structural integrity of the designed DHP–Ox hybrid derivatives.

3.4 Molecular Docking Studies

Molecular docking studies were carried out to investigate the binding affinity and intermolecular interactions of the designed DHP–Ox derivatives with the voltage-gated sodium channel protein (PDB ID: 5HVX). The docking scores of the designed compounds ranged from −9.3 to −10.3 kcal/mol, which were significantly better than the standard anticonvulsant Phenytoin (−7.5 kcal/mol), suggesting strong receptor-binding potential. Figure 3 shows molecular docking interactions for the standard, Phenytoin.

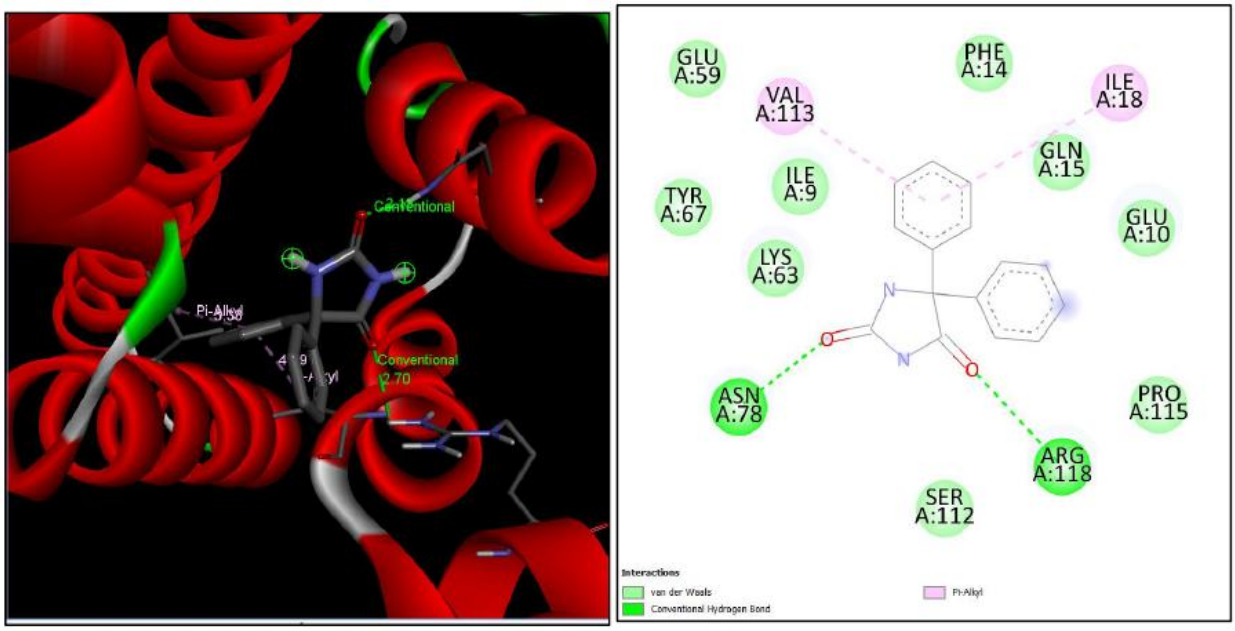


Figure 3. 3D & 2D interaction diagram of Phenytoin with voltage-gated sodium channel (5HVX)

Table 4. Docking score of designed DHP–Ox derivatives against voltage-gated sodium channel (5HVX)

Compound	**Docking Score (kcal/mol)**
Phenytoin	−7.5
DP01	−9.5
DP02	−10.3
DP03	−10.1
DP04	−9.7
DP05	−9.4
DP06	−9.3
DP07	−9.7
DP08	−9.6
DP09	−9.7
DP10	−9.6

Among the designed derivatives, DP02 demonstrated the highest binding affinity (−10.3 kcal/mol), followed by DP03 (−10.1 kcal/mol) Table 4. Interaction analysis revealed that the synthesized

compounds formed multiple conventional hydrogen bonds, π-cation interactions, π-donor hydrogen bonds, π-π stacked interactions, and π-alkyl interactions (Table 5) with amino acid residues present within the receptor active site.

Table 5. Molecular docking interactions of designed DHP–Ox derivatives with voltage-gated sodium channel (PDB ID: 5HVX)

Compound	Docking Score (kcal/mol)	Amino Acid Residue	Type of Interaction
DP01	−9.5	Arg118	Conventional hydrogen bond
		Asn78	Conventional hydrogen bond
		Lys63	Conventional hydrogen bond
		Arg118	π–Cation interaction
		Gln112	π–Donor hydrogen bond
		Val113, Ile18, Tyr67	π–Alkyl interaction
DP02	−10.3	Arg118, Asn78, Lys63, Ile9	Conventional hydrogen bond
		Arg118	π–Cation interaction
		Gln122	π–Donor hydrogen bond
		Ala232, Val113, Ile18, Tyr67	π–Alkyl interaction
DP03	−10.1	Arg118, Gln228, Gln122, Glu231, Glu78, Ser63	Conventional hydrogen bond
		Arg118	π–Cation interaction
		Val113, Gln15	π–Donor hydrogen bond
		Ala232	π–Sigma interaction
		Pro115	π–Alkyl interaction

Compound	Docking Score (kcal/mol)	Amino Acid Residue	Type of Interaction
DP04	−9.7	Arg118	Conventional hydrogen bond
		Val113	Carbon hydrogen bond
		Val63	π–Cation interaction
		Tyr122	π–π stacked interaction
		Ile18, Arg74	π–Alkyl interaction
DP05	−9.4	Arg118, Gln15	Conventional hydrogen bond
		Gln112	Halogen interaction
		Val113	π–Sigma interaction
		Tyr67	π–Donor hydrogen bond
		Pro115, Ile18	π–Alkyl interaction
DP06	−9.3	Arg118, Gln15	Conventional hydrogen bond
		Lys63	π–Cation interaction
		Val113	π–Sigma interaction
		Tyr67	π–π stacked interaction
		Ile18, Arg74	π–Alkyl interaction
DP07	−9.7	Asn78, Phe72	Conventional hydrogen bond
		Ser75	Carbon hydrogen bond
		Lys118, Arg63	π–Cation interaction

Compound	Docking Score (kcal/mol)	Amino Acid Residue	Type of Interaction
		Tyr67	π–π T-shaped interaction
		Ile113, Val18, Trp77	π–Alkyl interaction
DP08	−9.6	Arg118	Conventional hydrogen bond
		Val113	Carbon hydrogen bond
		Lys63	π–Cation interaction
		Val113	π–Donor hydrogen bond
		Tyr122	π–π stacked interaction
		Ile18, Arg74	π–Alkyl interaction
DP09	−9.7	Arg118, Arg119, Glu122, Gln59	Conventional hydrogen bond
		Gln228	Carbon hydrogen bond
		Gln15	π–Donor hydrogen bond
		Ala232, Pro115, Val113	π–Alkyl interaction
DP10	−9.6	Arg118, Asn78, Lys63	Conventional hydrogen bond
		Gln122	π–Donor hydrogen bond
		Ala232, Arg119, Val113, Tyr67, Ile18	π–Alkyl interaction

Common interacting residues observed among active compounds included Arg118, Val113, Ala232, Gln122, Tyr67, Ile18, and Lys63, suggesting their critical role in stabilizing ligand binding within the sodium channel binding pocket.

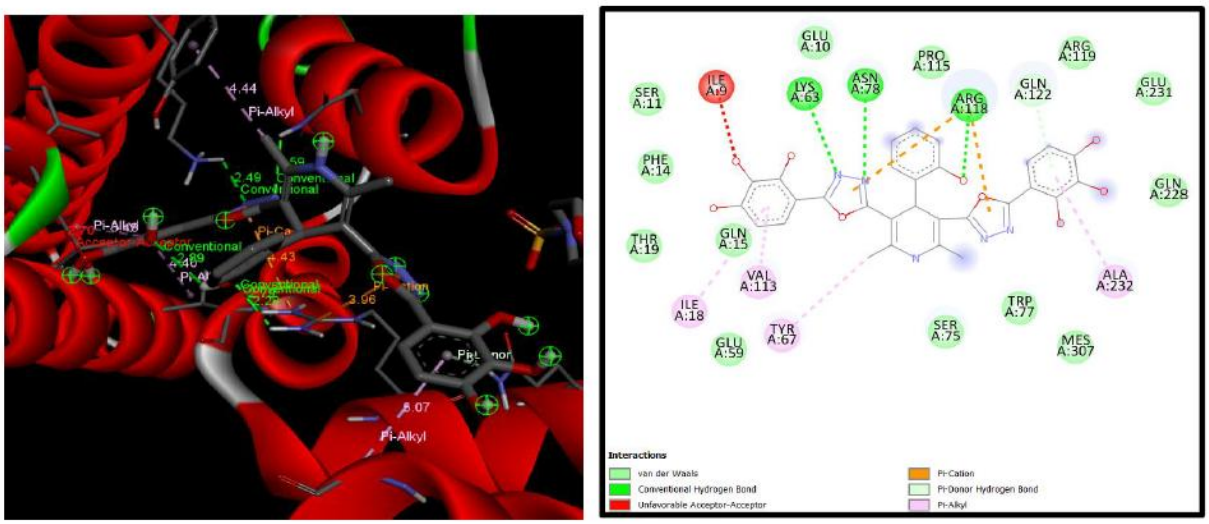


Figure 4. 3D& 2D interaction diagram of DP01 with voltage-gated sodium channel (5HVX).

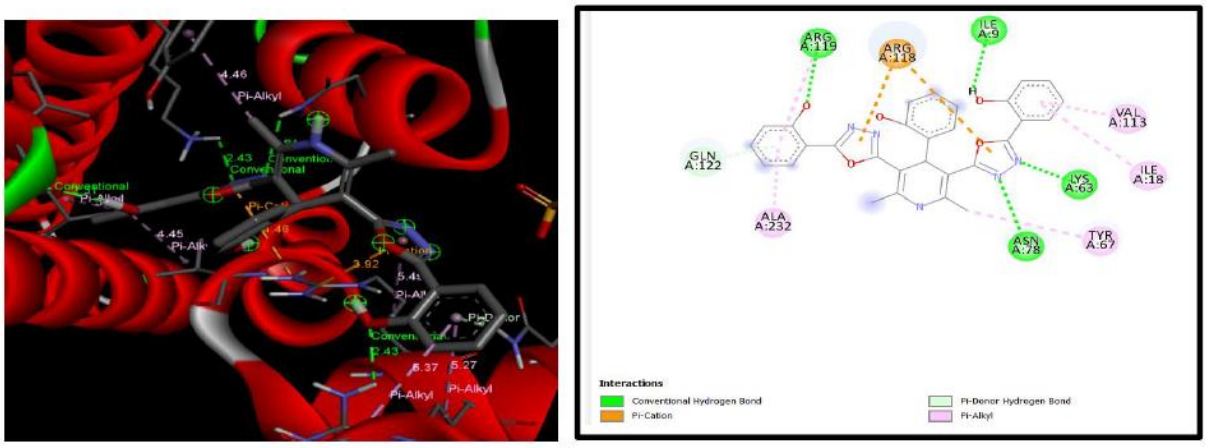


Figure 5. 3D & 2D interaction diagram of DP02 with voltage-gated sodium channel (5HVX).

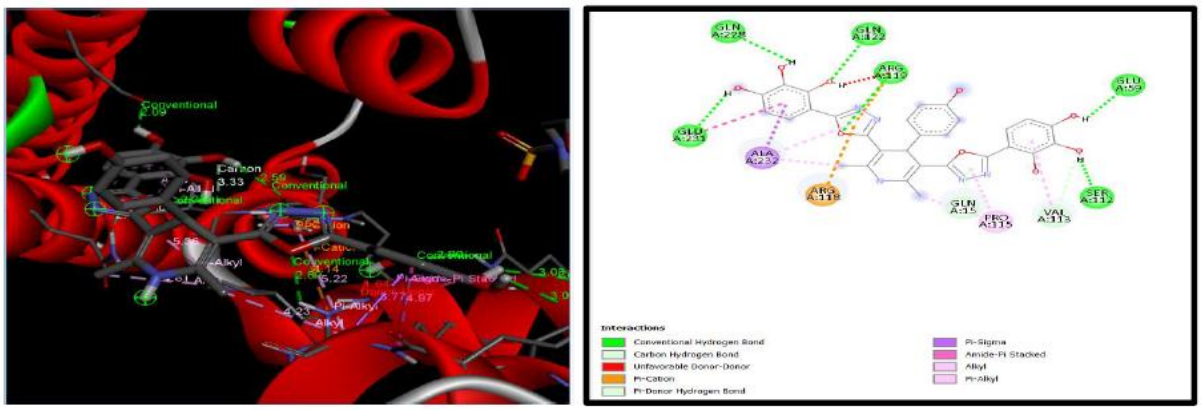
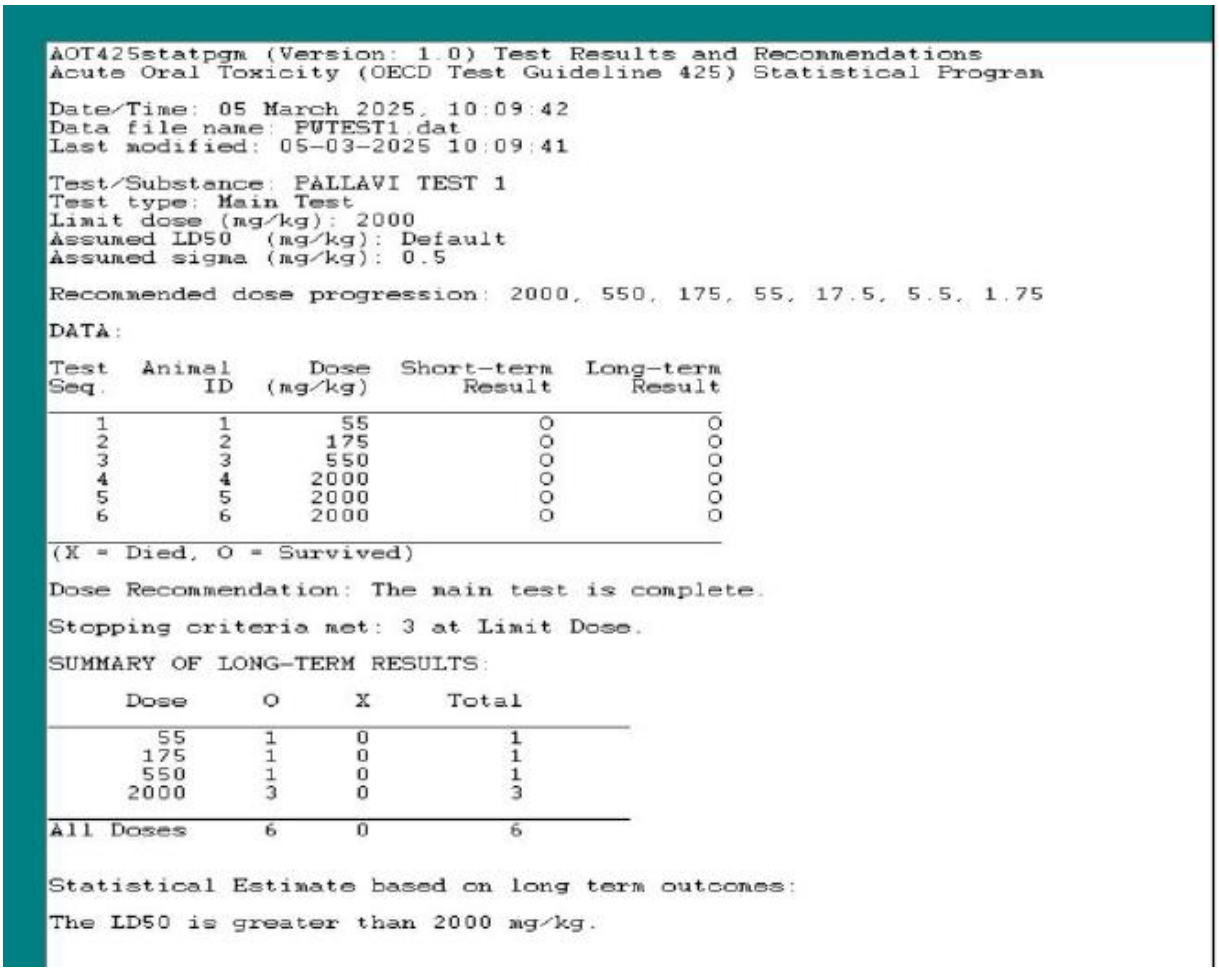


Figure 6. 3D& 2D interaction diagram of DP03 with voltage-gated sodium channel (5HVX).

The observed docking results correlated well with the pharmacological findings, where DP01, DP02 and DP03 (Figure 4,5,6) demonstrated superior anticonvulsant activity. Hydroxyl-rich derivatives exhibited enhanced binding affinity, possibly due to increased hydrogen-bonding capability and favorable electronic interactions within the receptor cavity.

3.5 Acute Oral Toxicity Study

Acute oral toxicity studies were performed according to OECD Guideline 425 using AOT425Stat software (Version 1.0) Figure 7. No mortality or significant behavioral abnormalities were observed in animals treated with the synthesized compounds up to a limit dose of 2000 mg/kg body weight. The LD_{50} value was estimated to be greater than 2000 mg/kg, indicating a favorable preliminary safety profile of the synthesized DHP–Ox derivatives.



AOT425statpgm (Version: 1.0) Test Results and Recommendations
Acute Oral Toxicity (OECD Test Guideline 425) Statistical Program

Date/Time: 05 March 2025, 10:09:42
Data file name: PVTEST1.dat
Last modified: 05-03-2025 10:09:41

Test/Substance: PALLAVI TEST 1
Test type: Main Test
Limit dose (mg/kg): 2000
Assumed LD50 (mg/kg): Default
Assumed sigma (mg/kg): 0.5

Recommended dose progression: 2000, 550, 175, 55, 17.5, 5.5, 1.75

DATA:

Test Seq.	Animal ID	Dose (mg/kg)	Short-term Result	Long-term Result
1	1	55	O	O
2	2	175	O	O
3	3	550	O	O
4	4	2000	O	O
5	5	2000	O	O
6	6	2000	O	O

(X = Died, O = Survived)

Dose Recommendation: The main test is complete.

Stopping criteria met: 3 at Limit Dose.

SUMMARY OF LONG-TERM RESULTS:

Dose	O	X	Total
55	1	0	1
175	1	0	1
550	1	0	1
2000	3	0	3
All Doses	6	0	6

Statistical Estimate based on long term outcomes:

The LD50 is greater than 2000 mg/kg.

Figure 7. AOT425-Stat report for acute oral toxicity study of synthesized DHP–Ox derivatives.

The absence of toxic manifestations such as tremors, sedation, respiratory distress, or convulsions further suggested that the synthesized compounds possess a wide therapeutic safety margin.

3.6 Anticonvulsant Activity

The anticonvulsant activity of synthesized compounds DP01, DP02, and DP03 were evaluated using the strychnine-induced seizure model in mice Table 6. The disease control group exhibited rapid onset of convulsions with seizure latency of 3.32 ± 0.04 min along with seven convulsive episodes within the first 30 min of observation.

Treatment with synthesized DHP–Ox derivatives significantly prolonged seizure latency and reduced the number of convulsions compared with the disease control group, indicating pronounced anticonvulsant activity.

Table 6. Anticonvulsant activity of synthesized DHP–Ox derivatives using strychnine-induced seizure model

Compound	Dose (mg/kg)	Latency to Convulsion (min ± SEM)	Number of Convulsions	% Protection
Control	2	3.32 ± 0.04	7	0
DP01	200	11.54 ± 0.06**	2	100
DP01	400	25.67 ± 0.09**	1	100
DP02	200	9.20 ± 0.01***	3	83
DP02	400	22.67 ± 0.03***	1	100
DP03	200	7.01 ± 0.007***	5	66
DP03	400	15.67 ± 0.01***	3	83

Values are expressed as Mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's post hoc test.**p < 0.01; ***p < 0.001 compared with disease control group.

Among the tested compounds Figure 8,9,10.,DP01 exhibited the most potent anticonvulsant activity by significantly prolonging seizure latency to 25.67 ± 0.09 min at 400 mg/kg with complete protection against convulsions. DP02 also demonstrated strong anticonvulsant activity with protection ranging from 83–100%, whereas DP03 showed comparatively moderate anticonvulsant activity.

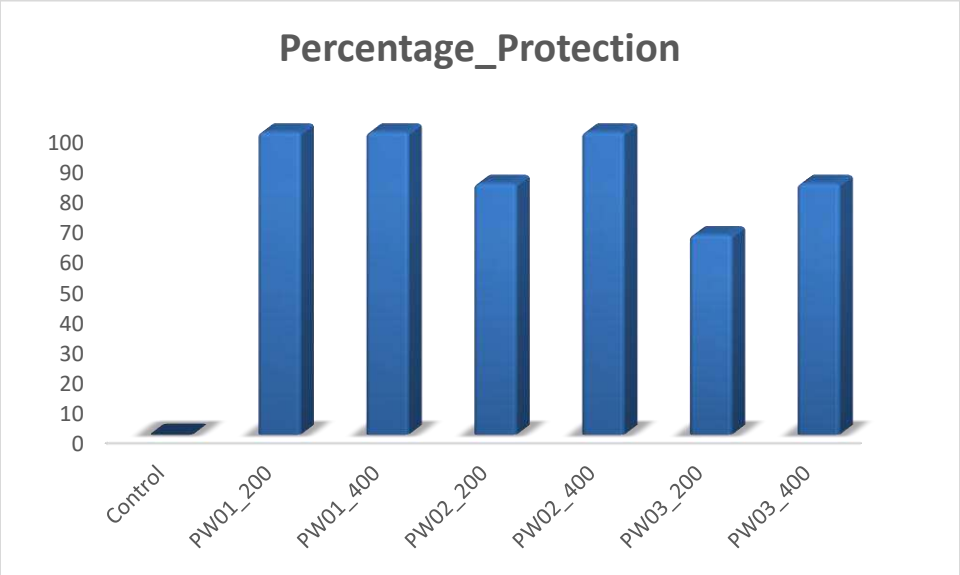


Figure 8. Percentage protection exhibited by synthesized DHP–Ox derivatives in the strychnine-induced seizure model.

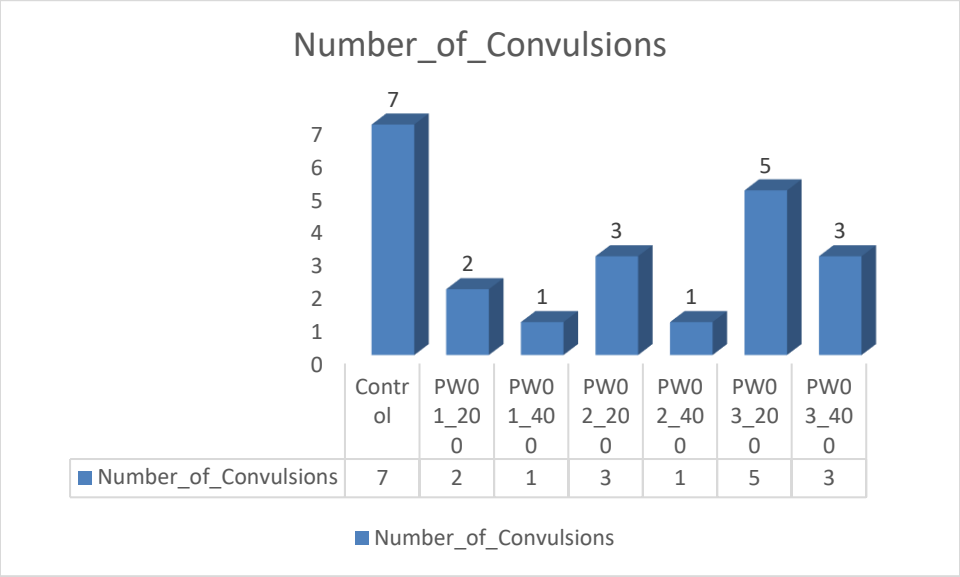


Figure 9. Effect of synthesized DHP–Ox derivatives on the number of convulsions in mice.

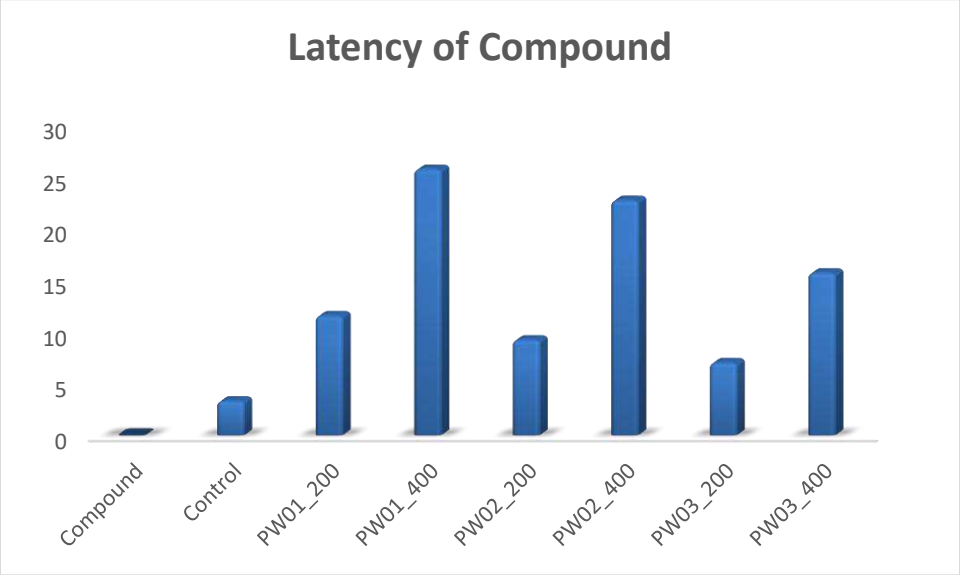


Figure 10. Effect of synthesized DHP–Ox derivatives on seizure latency in the strychnine-induced seizure model.

The superior anticonvulsant activity of DP01 and DP02 may be attributed to the presence of hydroxyl-rich aromatic substitutions capable of forming stronger hydrogen-bonding interactions with amino acid residues present within the sodium channel binding pocket. Furthermore, the observed dose-dependent activity suggested effective modulation of neuronal ion channel activity leading to suppression of neuronal hyperexcitability.

3.7 Structure–Activity Relationship (SAR)

Structure–activity relationship analysis revealed that the nature and position of substituents present on the aromatic rings significantly influenced docking affinity and anticonvulsant activity of the designed DHP–Ox derivatives. Hydroxyl-substituted derivatives exhibited superior receptor-binding affinity and biological activity compared with unsubstituted or halogen-substituted analogs.

Compound DP01 possessing ortho-hydroxyl substitution at R_1 and 2,3,4-trihydroxyl substitution at R_2, R'_2 demonstrated the highest anticonvulsant activity and favorable receptor interaction profile. Similarly, DP03 containing para-hydroxyl substitution at R_1 and polyhydroxyl substitution at R_2, R'_2 also exhibited significant anticonvulsant activity.

In contrast, the unsubstituted derivative DP06 displayed comparatively lower docking affinity, suggesting that the absence of polar functional groups reduced stabilization within the receptor cavity. These findings indicated that hydroxyl substitution positively contributes toward anticonvulsant activity through enhanced hydrogen bonding, electronic stabilization, and stronger ligand–receptor interactions with voltage-gated sodium channels.

4. Conclusion

4.1. Synthesis and Structural Characterization

A series of novel substituted 1,4-dihydropyridine–1,3,4-oxadiazole (DHP–Ox) hybrid derivatives were successfully designed and synthesized using a microwave-assisted synthetic approach involving sequential Hantzsch condensation, hydrazide formation, and cyclodehydration reactions. The adopted synthetic methodology offered reduced reaction time, operational simplicity, and good to excellent yields ranging from 73–95%. The synthesized compounds exhibited satisfactory physicochemical properties including distinct melting point ranges and acceptable Rf values. Structural characterization using FTIR, ^{1}H-NMR, and LC–MS spectral analyses confirmed successful formation of the desired DHP–Ox hybrid scaffold and validated the proposed molecular structures of the synthesized derivatives.

4.2. Molecular Docking and *In-Silico* Evaluation

Molecular docking studies performed against the voltage-gated sodium channel protein (PDB ID: 5HVX) demonstrated significant binding affinity of the designed derivatives, with docking scores ranging from −9.3 to −10.3 kcal/mol compared with the standard anticonvulsant Phenytoin (−7.5 kcal/mol). The docking analysis revealed important ligand–receptor interactions involving key

amino acid residues such as Arg118, Val113, Ala232, Gln122, Tyr67, and Lys63, indicating stable accommodation of the synthesized derivatives within the receptor binding cavity.

Preliminary *in silico* ADME evaluation using SwissADME suggested moderate drug-likeness characteristics of the designed derivatives. Although certain hydroxyl-rich compounds exhibited elevated TPSA values and limited predicted passive blood–brain barrier permeability, the significant anticonvulsant activity observed experimentally suggests that computational prediction models may not completely represent the complex in vivo pharmacokinetic and pharmacodynamic behavior of CNS-active molecules. Thus, the observed biological activity highlights the importance of experimental validation in anticonvulsant drug discovery.

4.3. Correlation of Molecular Docking with *In-Vivo* Anticonvulsant Activity

A meaningful correlation was observed between molecular docking affinity and *in vivo* anticonvulsant activity. Compounds DP01, DP02, and DP03, which demonstrated superior docking scores and favorable receptor-binding interactions, also exhibited significant anticonvulsant activity in the strychnine-induced seizure model. Among the evaluated derivatives, DP01 exhibited the most potent anticonvulsant activity by significantly prolonging seizure latency and providing complete protection against convulsions at 400 mg/kg. DP02 also demonstrated strong anticonvulsant activity with complete seizure protection, whereas DP03 exhibited comparatively moderate anticonvulsant efficacy.

The observed correlation between docking affinity, receptor-binding interactions, and pharmacological activity suggests that modulation of voltage-gated sodium channels may contribute to the anticonvulsant mechanism of the synthesized DHP–Ox derivatives. Furthermore, the dose-dependent anticonvulsant activity observed during biological evaluation supports the pharmacological relevance of the designed scaffold.

4.4. Structure–Activity Relationship (SAR)

Structure–activity relationship analysis indicated that the nature and position of aromatic substitutions significantly influenced receptor-binding affinity and anticonvulsant activity of the synthesized derivatives. Hydroxyl-rich aromatic substitutions positively contributed toward biological activity by enhancing hydrogen-bonding interactions within the receptor binding cavity.

Compounds containing hydroxyl substitutions demonstrated improved docking affinity and superior anticonvulsant activity compared with unsubstituted or halogen-substituted analogs.

DP01 and DP03, containing polyhydroxyl substitutions, exhibited enhanced receptor interactions and favorable anticonvulsant activity, indicating that hydroxyl functionalities play an important role in stabilizing ligand–receptor interactions. In contrast, derivatives lacking polar substitutions demonstrated comparatively lower docking affinity and reduced pharmacological activity.

4.5. Pharmacological Evaluation and Safety Profile

Acute oral toxicity studies performed according to OECD Guideline 425 demonstrated that the synthesized derivatives possess a favorable preliminary safety profile, with LD_{50} values estimated to be greater than 2000 mg/kg. Pharmacological evaluation using the strychnine-induced seizure model revealed significant dose-dependent anticonvulsant activity of the synthesized compounds, supporting their potential therapeutic applicability as anticonvulsant agents.

Future Perspective

Overall, the present investigation successfully established DHP–Ox hybrid derivatives as promising lead candidates for anticonvulsant drug development. The integrated correlation observed between molecular docking studies, in silico prediction, structure–activity relationship analysis, and *in vivo* anticonvulsant activity provides a strong scientific basis for the pharmacological potential of the synthesized scaffold. The combination of favorable receptor-binding affinity, significant anticonvulsant activity, and acceptable preliminary safety profile highlights the therapeutic relevance of these derivatives.

Further structural optimization, pharmacokinetic investigation, mechanistic studies, and advanced CNS permeability evaluation are warranted to improve drug-likeness characteristics and establish the clinical potential of these compounds as novel anticonvulsant agents.

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Ethics Statement:

All experimental protocols were carried out in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (IAEC) of MET's Institute of Pharmacy, Nashik (Approval No. 1344/PO/RE/S/10/CPCSEA).

Declaration on the Use of Generative AI

The authors used ChatGPT (OpenAI) solely to assist with language editing, grammar correction, and improvement of manuscript readability. The AI tool was not used for study design, data collection, data analysis, interpretation of results, or generation of scientific conclusions. All scientific content, results, and interpretations were reviewed, verified, and approved by the authors, who take full responsibility for the accuracy and integrity of the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing financial interests, personal relationships, or potential conflicts of interest that could have influenced the research, authorship, or publication of this work.

Data Availability:

The data supporting the findings of this study are available from the authors upon reasonable request.

Abbreviations

DHP-1,4-Dihydropyridine

Ox-1,3,4-Oxadiazole

ADME- Absorption, Distribution, Metabolism, and Excretion

BBB- Blood–Brain Barrier

CNS- Central Nervous System

TLC- Thin Layer Chromatography

FTIR- Fourier Transform Infrared Spectroscopy

NMR- Nuclear Magnetic Resonance

LC–MS- Liquid Chromatography–Mass Spectrometry

SAR- Structure–Activity Relationship

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