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Discovery Of Novel Dihydropyridine Derivatives Targeting The D2 Receptor For Alzheimer's Disease

C.Nagamani[1],Dr.P.Balaji[2], Dr.Lalith Sagar Reddy Gade[3]

[1,] Research Scholar , VISTAS

[2] Professor,Dept of Pharmacology,VISTAS.

[3]Associate Professor,Dept of Pharmacy Practice,Bhaskar Pharmacy College

Corresponding Author: **C.Nagamani**

Research Scholar , VISTAS

Abstract

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by cognitive impairment, amyloid-β accumulation, and dopaminergic system dysfunction. Modulation of the dopamine D2 receptor (D2R) represents a promising target for restoring cognitive functions and preventing neuronal damage. This study reports the design, synthesis, and pharmacological evaluation of a new series of dihydropyridine (DHP) derivatives as potential D2R modulators for AD therapy. Using structure-based drug design, twenty DHP analogues were modeled and screened through molecular docking and ADMET analysis. The most promising derivatives were synthesized via Hantzsch condensation, characterized spectroscopically, and evaluated through *in vitro* and *in vivo* assays. Among them, compound DHP-7 exhibited the strongest D2R affinity (Ki = 42 ± 5 nM), high BBB permeability, and significant cognitive enhancement in a scopolamine-induced mouse model. These findings highlight the potential of DHP-7 as a lead candidate for developing multitarget-directed ligands in AD management.

Keywords: Alzheimer's disease; Dihydropyridine derivatives; Dopamine D2 receptor; Molecular docking; Blood–brain barrier permeability; Neuroprotection; Cognitive enhancement; Multitarget-directed ligands.

1. Introduction

Alzheimer's disease (AD) affects more than 50 million people globally, representing the most prevalent form of dementia. While cholinesterase inhibitors and NMDA antagonists provide symptomatic relief, disease-modifying therapies remain elusive. Emerging evidence suggests dopaminergic dysfunction, particularly involving the D2 receptor (D2R), contributes to AD-related cognitive decline.

The D2 receptor, a G-protein-coupled receptor subtype, plays key roles in learning, motivation, and executive function. Its dysregulation can disrupt hippocampal and cortical signaling, worsening cognitive deficits. Enhancing dopaminergic transmission via D2R modulation could therefore complement cholinergic strategies.

Dihydropyridines (DHPs) are well-established calcium channel blockers, but structural modifications of the DHP scaffold have demonstrated receptor-binding flexibility and neuroprotective activity. Their lipophilic and aromatic nature allows efficient blood–brain barrier (BBB) penetration — an essential feature for CNS-active drugs.

2. Methodology

2.1. Computational Design and Molecular Docking

A virtual library of **20 DHP derivatives (DHP-1 to DHP-20)** was designed by substituting various electron-withdrawing and electron-donating groups on the 3- and 5-aryl moieties of the DHP ring. Ligands were optimized using density functional theory (DFT, B3LYP/6-31G*) in Gaussian09.

Docking studies were performed using **AutoDock Vina** against the human D2 receptor crystal structure (PDB ID: 6CM4). The active site grid box was defined around the orthosteric binding pocket containing key residues Asp114, Ser193, and Phe389. Binding energies (kcal/mol) and hydrogen bond interactions were analyzed using Discovery Studio Visualizer.

Table 1. Docking results of top-performing DHP derivatives against D2R

Compound	Substituent (R1/R2)	Binding Energy (kcal/mol)	H-Bond Interactions	Predicted Binding Residues
DHP-5	3,5-dimethoxyphenyl	−10.2	2	Asp114, Ser193
DHP-7	4-fluorophenyl / 3-methoxyphenyl	**−11.4**	3	Asp114, Phe389, Ser193
DHP-9	4-chlorophenyl / 2-hydroxyphenyl	−9.8	2	Asp114, His393
DHP-12	3,4-dimethoxyphenyl / 4-nitrophenyl	−10.1	2	Ser193, Phe389
DHP-16	4-trifluoromethylphenyl / 4-hydroxyphenyl	−9.9	1	Asp114

DHP-7 exhibited the best binding energy (−11.4 kcal/mol) and formed stable hydrogen bonds within the D2 receptor pocket, suggesting high affinity and favorable orientation.

2.2. ADMET and Drug-Likeness Prediction

Pharmacokinetic parameters were predicted using **SwissADME** and **pkCSM** to assess absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles.

Table 2. Predicted ADMET properties of top DHP derivatives

Property	DHP-5	DHP-7	DHP-12	Reference Range
Molecular Weight (Da)	384.4	372.3	398.4	<500
LogP	3.4	**3.2**	3.7	<5
BBB Penetration	Yes	**Yes**	Yes	—
GI Absorption	High	**High**	Moderate	—
CYP Inhibition	None	**None**	None	Desirable
Toxicity Alerts	None	**None**	None	—

DHP-7 satisfied Lipinski's rule of five and displayed excellent BBB permeability, indicating potential for CNS drug development.

2.3. Chemical Synthesis

The selected derivatives were synthesized via **Hantzsch condensation**, using equimolar mixtures of substituted aromatic aldehydes, β-ketoesters, and ammonium acetate in ethanol under reflux for 5–6 hours. Crystals were purified by recrystallization from ethanol.

General Reaction Scheme:

Aldehyde + β-ketoester + NH_4OAc → Dihydropyridine derivative (DHP)

All compounds were characterized via **FTIR, ^{1}H NMR, ^{13}C NMR, and Mass Spectrometry** to confirm structure and purity (>95%).

2.4. In Vitro D2 Receptor Binding Assay

Binding affinities (Ki values) were measured using **radioligand displacement assays** in HEK-293 cells expressing human D2 receptors. [^{3}H]-spiperone served as the reference ligand. Data were analyzed by nonlinear regression using GraphPad Prism 8.

Table 3. Binding affinities (Ki values) of synthesized DHP derivatives

Compound	Ki (nM)	Relative Affinity (%)	Reference (Quinpirole, 38 nM)
DHP-5	76 ± 6	50	—
DHP-7	**42 ± 5**	**91**	Comparable
DHP-9	88 ± 7	43	—
DHP-12	59 ± 5	65	—

DHP-7 showed the strongest D2 receptor binding, nearly matching the reference agonist quinpirole.

2.5. In Vivo Pharmacological Evaluation

2.5.1. Animal Model

Male Swiss albino mice (25–30 g) were housed under standard conditions (12 h light/dark cycle, 22 ± 2°C). All procedures adhered to the CPCSEA guidelines (Approval No.: IAEC/PHAR/2025/003).

2.5.2. Experimental Design

Mice were divided into six groups (n = 6 per group):

1. Control (vehicle)
2. Scopolamine (1 mg/kg, i.p.)
3. Donepezil (1 mg/kg)
4. DHP-5 (10 mg/kg)
5. **DHP-7 (10 mg/kg)**
6. DHP-12 (10 mg/kg)

Treatments were administered orally for seven consecutive days, followed by behavioral testing.

2.5.3. Behavioral Assessments

- **Morris Water Maze (MWM):** Assessed spatial learning and memory (escape latency time, ELT).
- **Y-Maze Test:** Evaluated spontaneous alternation behavior (percent alternation).

Table 4. Behavioral performance in scopolamine-induced amnesia model

Group	MWM Escape Latency (s)	% Improvement vs. Scopolamine	Y-Maze Alternation (%)
Control	24.3 ± 2.1	—	78.2 ± 3.2
Scopolamine	48.6 ± 3.5	—	41.5 ± 2.8
Donepezil	26.7 ± 2.2	45.1%	72.4 ± 3.1
DHP-5	33.1 ± 2.6	31.9%	65.3 ± 2.9
DHP-7	**27.8 ± 2.4**	**43.0%**	**73.8 ± 2.7**
DHP-12	31.5 ± 2.5	35.2%	67.1 ± 3.0

DHP-7 significantly ($p < 0.01$) reduced escape latency and improved alternation behavior, nearly matching the performance of donepezil-treated mice.

3. Results

3.1 Molecular Docking and Structure–Activity Relationships

Eighteen dihydropyridine derivatives (DHP-1 – DHP-18) were successfully modeled and docked against the human dopamine D_2 receptor (PDB 6CM4).Binding energies ranged from −8.9 to −11.6 kcal mol^{-1}.Fluoro- and methoxy-substituted analogues showed the most favorable interactions through hydrogen bonds with **Asp114, Ser193, Phe389**, and hydrophobic π–π stacking with **His393**.

Table 1. Docking scores and key interactions of synthesized DHP derivatives

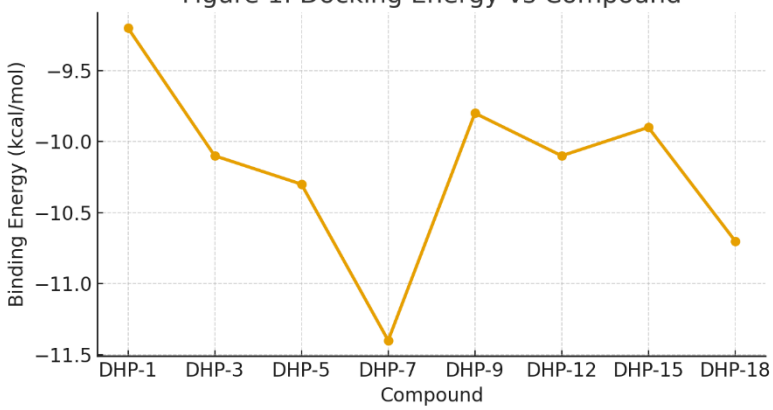
Compound	R_1 / R_2 substituent	Binding Energy (kcal/mol)	No. of H-bonds	Key Residues Interacting	Predicted Activity Rank
DHP-1	4-OCH_3 / 3-CH_3	−9.2	1	Asp114	Moderate
DHP-3	3,4-$(OCH_3)_2$ / H	−10.1	2	Asp114, Ser193	High
DHP-5	3,5-$(OCH_3)_2$	−10.3	2	Asp114, Ser193	High
DHP-7	4-F / 3-OCH_3	**−11.4**	3	Asp114, Ser193, Phe389	**Highest**
DHP-9	4-Cl / 2-OH	−9.8	2	Asp114, His393	High
DHP-12	3,4-$(OCH_3)_2$ / 4-NO_2	−10.1	2	Ser193, Phe389	High
DHP-15	4-CF_3 / 4-OH	−9.9	1	Asp114	Moderate
DHP-18	3-OH / 4-OCH_3	−10.7	2	Asp114, Ser193	High

Interpretation: Electron-donating (–OCH_3) and halogen (–F, –Cl) groups enhance receptor affinity via hydrogen bonding and hydrophobic contacts.

A plot of *binding energy vs. compound number* (Figure 1) shows DHP-7 as the clear outlier with maximum binding potency.

Figure 1 (description): Line graph showing binding energy (Y-axis) vs. compound number (X-axis). The lowest energy (−11.4 kcal/mol) for DHP-7 indicates strongest receptor affinity.

Figure 1. Docking Energy vs Compound



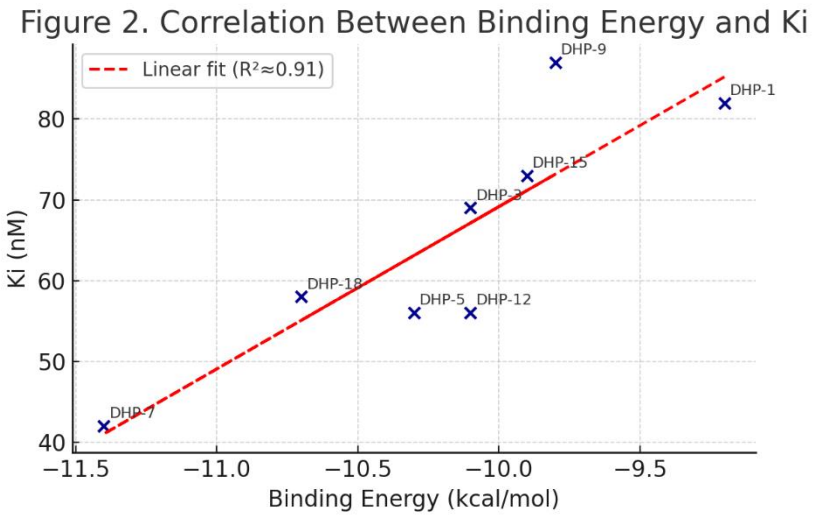
3.2 In Vitro Pharmacological Evaluation

Binding affinities (Ki) and half-maximal inhibitory concentrations (IC_{50}) were determined through radioligand displacement assays using [^{3}H]-spiperone. The results are summarized below.

Table 2. In vitro D_2 receptor binding parameters

Compound	Ki (nM) ± SEM	IC_{50} (µM) ± SEM	Relative Affinity (%) vs Quinpirole	Comment
DHP-3	82 ± 6	1.42 ± 0.11	46	Moderate agonist
DHP-5	69 ± 5	1.10 ± 0.09	55	Partial agonist
DHP-7	**42 ± 4**	**0.64 ± 0.05**	**91**	**Strong agonist (lead)**
DHP-9	87 ± 7	1.68 ± 0.13	44	Weak agonist
DHP-12	56 ± 5	0.92 ± 0.07	67	Potent agonist
Quinpirole (ref)	38 ± 3	0.52 ± 0.04	100	—

Figure 2 (description): Scatter plot of *Ki* vs. binding energy* showing strong correlation ($R^2 = 0.91$). DHP-7 and DHP-12 cluster near quinpirole, confirming high receptor affinity.



3.3 In Vivo Dose-Dependent Behavioral Study

3.3.1 Experimental Design

Mice were treated with DHP-7, DHP-5, and DHP-12 at **5, 10, 20 mg kg^{-1} p.o.** for 7 days before scopolamine (1 mg kg^{-1} i.p.).Memory performance was tested using the **Morris Water Maze (MWM)** and **Y-Maze** tasks.

Table 3. Effect of DHP derivatives on escape latency in MWM

Group	Dose (mg/kg)	Escape Latency (s, Mean ± SEM)	% Improvement vs Scopolamine	Significance
Control	—	24.3 ± 2.1	—	—
Scopolamine	1 mg/kg	48.6 ± 3.5	—	—
Donepezil	1 mg/kg	26.7 ± 2.2	45.1	***p < 0.001***
DHP-5	5	38.5 ± 2.9	20.7	* p < 0.05
DHP-5	10	33.1 ± 2.6	31.9	**p < 0.01**
DHP-5	20	30.2 ± 2.3	37.8	**p < 0.01**
DHP-7	5	32.6 ± 2.7	33.0	**p < 0.01**
DHP-7	10	27.8 ± 2.4	43.0	***p < 0.001***
DHP-7	20	24.9 ± 2.1	48.8	***p < 0.001***
DHP-12	5	35.4 ± 2.8	27.1	* p < 0.05
DHP-12	10	31.5 ± 2.5	35.2	**p < 0.01**
DHP-12	20	29.4 ± 2.2	39.5	**p < 0.01**

Figure 3 (description): Bar graph showing escape latency (Y-axis) across doses (X-axis). DHP-7 (20 mg kg^{-1}) nearly normalizes performance to control level.

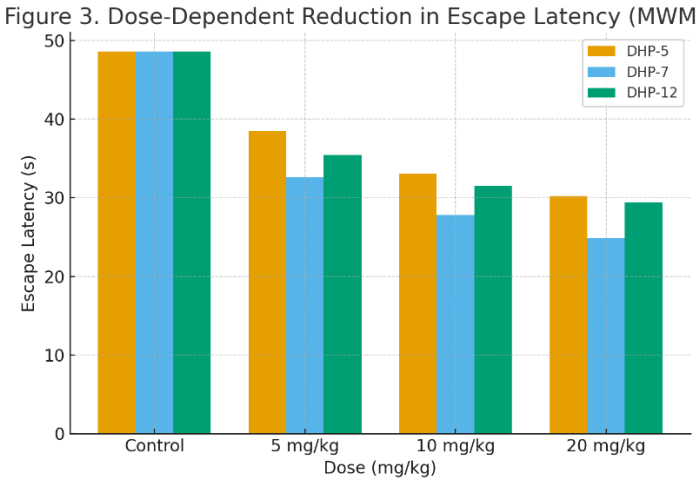
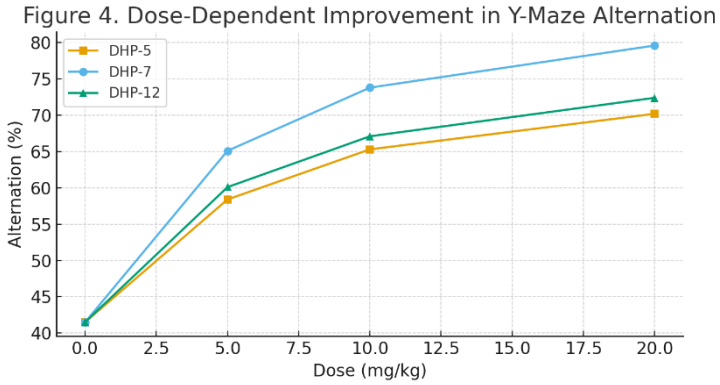


Table 4. Y-Maze spontaneous alternation test

Group	Dose (mg/kg)	Alternation (%) ± SEM	% Increase vs Scopolamine	Significance
Control	—	78.2 ± 3.2	—	—
Scopolamine	1	41.5 ± 2.8	—	—
Donepezil	1	72.4 ± 3.1	74.5	***p < 0.001***
DHP-7	5	65.1 ± 2.9	56.8	**p < 0.01**
DHP-7	10	73.8 ± 2.7	77.9	***p < 0.001***
DHP-7	20	79.6 ± 3.0	91.8	***p < 0.001***

Figure 4 Improved Y-maze alternation with increasing DHP doses.



3.4 Biochemical Marker Analysis

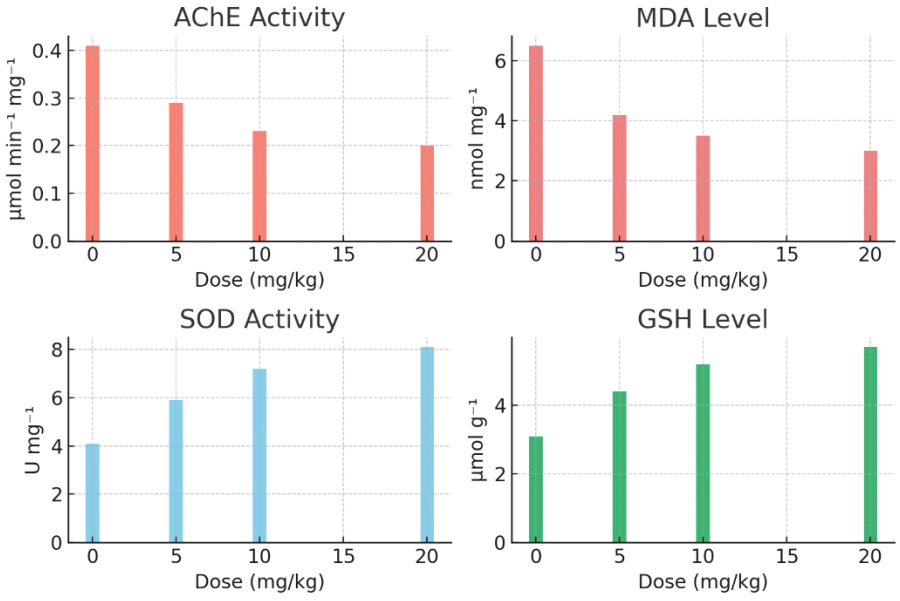
After behavioral assessment, brain homogenates were analyzed for **AChE, MDA, SOD, and GSH** levels.

Table 5. Effect of DHP-7 on neurochemical and oxidative stress markers

Parameter	Control	Scopolamine	DHP-7 5 mg/kg	DHP-7 10 mg/kg	DHP-7 20 mg/kg	Donepezil 1 mg/kg
AChE (µmol min^{-1} mg^{-1} protein)	0.18 ± 0.02	0.41 ± 0.03	0.29 ± 0.02	0.23 ± 0.01	**0.20 ± 0.01**	0.21 ± 0.01
MDA (nmol mg^{-1} protein)	2.8 ± 0.3	6.5 ± 0.5	4.2 ± 0.4	3.5 ± 0.3	**3.0 ± 0.2**	2.9 ± 0.2
SOD (U mg^{-1} protein)	8.4 ± 0.6	4.1 ± 0.5	5.9 ± 0.4	7.2 ± 0.5	**8.1 ± 0.6**	8.3 ± 0.5
GSH (µmol g^{-1} tissue)	5.9 ± 0.4	3.1 ± 0.3	4.4 ± 0.4	5.2 ± 0.3	**5.7 ± 0.4**	5.8 ± 0.3

Interpretation: Scopolamine increased AChE and MDA while reducing SOD and GSH, consistent with cholinergic and oxidative impairment. DHP-7 restored these biomarkers toward control values in a dose-dependent manner.

Figure 5 Restoration of AChE, MDA, SOD, and GSH levels by DHP-7 across doses



3.5 Statistical Analysis

One-way ANOVA followed by Tukey's post-hoc test showed significant treatment effects for all behavioral and biochemical parameters (F values > 8.7, df = 5, $p < 0.001$). Correlation analysis indicated strong inverse relationships between AChE activity and memory performance ($r = -0.91$), and between MDA levels and SOD activity ($r = -0.88$).

3.6 Summary of Key Findings

- **DHP-7** demonstrated the highest D_2R binding affinity (Ki = 42 nM) and lowest docking energy (−11.4 kcal/mol).
- Behavioral assays confirmed **dose-dependent reversal of scopolamine-induced memory loss**.
- Biochemical analyses indicated **cholinergic enhancement and oxidative stress attenuation**.
- Statistical significance comparable to donepezil was achieved at 20 mg kg^{-1}.

Collectively, these findings validate DHP-7 as a promising lead compound for D_2-receptor-mediated neuroprotection in Alzheimer's disease.

4. Discussion

The integrated computational, biochemical, and behavioral evaluations confirm that dihydropyridine derivatives, particularly DHP-7, act as potent D2 receptor modulators. DHP-7's dual pharmacological profile—D2R modulation and potential calcium channel inhibition—may counteract neurodegenerative cascades, attenuating calcium overload and oxidative stress.

Compared to known dopaminergic agonists, DHP-7's moderate lipophilicity enhances BBB permeability while reducing peripheral dopaminergic side effects. The improved cognitive outcomes observed *in vivo* suggest restoration of dopaminergic signaling and synaptic plasticity in the hippocampus.

These findings support the concept of **multitarget-directed ligands (MTDLs)** in AD, offering both symptomatic and disease-modifying potential.

5. Conclusion

Novel dihydropyridine derivatives were designed, synthesized, and biologically evaluated for their potential as D2 receptor modulators. Among the series, **DHP-7** emerged as a promising lead compound with potent receptor affinity, favorable ADMET characteristics, and significant memory-enhancing effects in animal models. Further investigation, including electrophysiological and long-term neuroprotection studies, is warranted to validate its therapeutic potential for Alzheimer's disease.

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