

**Synthesis, characterization, *in silico* ADMET study and anticholinesterase activity of novel naphthyridines**Anita Yadav^{1*}, Brijesh Prajapati¹, Rahul Chauhan¹¹R.K. College of Pharmacy, Sathiaon, Azamgarh, Uttar Pradesh, India**Abstract**

The present study aimed to synthesize, characterize, and evaluate the anticholinesterase potential of novel 1,8-naphthyridine derivatives along with their physicochemical and pharmacokinetic properties. A series of five compounds, namely 4-methyl-1,8-naphthyridine-2,6-diyl dibenzoate derivatives (ANB₁₋₅), were synthesized through a multistep synthetic pathway involving the formation of 2,6-dihydroxy-4-methyl-1,8-naphthyridine followed by esterification with substituted benzoyl chlorides. Anticholinesterase activity was determined using Ellman's colorimetric method, while drug-likeness and ADMET properties were predicted using SwissADME and pkCSM online tools. The synthesized derivatives exhibited moderate to good yields (67-71%) and demonstrated dose-dependent inhibition of acetylcholinesterase enzyme. Among the tested compounds, ANB₃ exhibited the highest anticholinesterase activity with 65.07% inhibition at 10 mM concentration and the lowest IC₅₀ value (6.89 mM), indicating superior potency compared to the other derivatives. In silico studies revealed favorable pharmacokinetic profiles with excellent intestinal absorption (>95%), compliance with Lipinski's rule of five, and acceptable drug-likeness characteristics. The ADMET analysis suggested that ANB₃ possessed the most balanced profile in terms of absorption, lipophilicity, and biological activity. Overall, the findings identify ANB₃ as a promising lead candidate for further optimization and development as a potential anticholinesterase agent for the management of neurodegenerative disorders.

Keywords: 1,8-naphthyridine, anti-cholinesterase, Ellman's method, *in-silico*, Alzheimer's disease

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Email id: anitayadav9231@gmail.com**JOURNAL OF PHARMACOLOGY AND BIOMEDICINE**

ISSN No. 2456-8244

Publication Hosted by

www.jpbiomed.com**Cite this article as**Yadav A, Prajapati B, Chauhan R. Synthesis, characterization, *in silico* ADMET study and anticholinesterase activity of novel naphthyridines. J Pharmacol Biomed. 2026; 10(4): 1001-1010

Introduction

Neurodegenerative disorders represent a growing global health challenge, with Alzheimer's disease (AD) being the most prevalent cause of dementia among the elderly population. AD is characterized by progressive cognitive decline, memory impairment, and behavioral disturbances that significantly affect the quality of life of patients and their caregivers. One of the major pathological features associated with AD is the deficiency of the neurotransmitter acetylcholine (ACh) in the brain due to increased activity of the enzyme acetylcholinesterase (AChE). Consequently, inhibition of AChE remains an established therapeutic strategy for improving cholinergic neurotransmission and alleviating symptoms associated with cognitive dysfunction.^{1,2}

Currently available cholinesterase inhibitors such as donepezil, rivastigmine, and galantamine provide symptomatic relief but are often associated with limitations including adverse effects, short duration of action, and limited efficacy in halting disease progression. Therefore, the search for novel heterocyclic compounds possessing potent anticholinesterase activity and favorable pharmacokinetic properties continues to attract considerable attention in medicinal chemistry research.

Among various nitrogen-containing heterocyclic systems, 1,8-naphthyridines constitute an important class of bioactive molecules exhibiting a broad spectrum of pharmacological activities, including antimicrobial, anti-inflammatory, anticancer, antiepileptic, antioxidant, and central nervous system activities. The structural resemblance of the naphthyridine nucleus to other biologically active heterocycles allows it to interact effectively with multiple biological targets. Furthermore, strategic substitution on the naphthyridine scaffold can modulate physicochemical properties and biological activity, making it an attractive framework for the development of novel therapeutic agents³⁻⁸.

Recent advances in computer-aided drug design and pharmacokinetic prediction tools have also facilitated the early evaluation of drug-likeness, absorption, distribution, metabolism, excretion, and toxicity (ADMET) characteristics of newly synthesized molecules. Such *in silico* approaches not only reduce experimental costs but also assist in identifying promising lead candidates with improved safety and efficacy profiles before extensive biological investigations.

In view of the therapeutic importance of naphthyridine derivatives and the continuing need for effective cholinesterase inhibitors, the present study was undertaken to synthesize a series of novel 4-methyl-1,8-naphthyridine-2,6-diyl dibenzoate derivatives (ANB1–ANB5). The synthesized compounds were characterized using spectroscopic techniques including FTIR and ¹H-NMR studies. Furthermore, their physicochemical properties and drug-likeness were assessed through

in silico ADMET analysis, while their potential as cholinesterase inhibitors was evaluated using Ellman's method. The study aims to explore the influence of different benzoate substitutions on anticholinesterase activity and identify promising lead molecules for further development as agents against neurodegenerative disorders.

Material and Methods

The experimental scheme was adapted from the previous reports by modification in conditions and optimized for obtaining highest yield.^{9,10}

The multistep reaction pathway involved the synthesis of 2-amino-5-hydroxypyridine followed by the synthesis of 2,6-dihydroxy-4-methyl-1, 8-naphthyridine and finally the formation of 4-methyl-1,8-naphthyridin-2,6-diyl dibenzoate derivatives.

Synthesis of 2-amino-5-hydroxypyridine

Combine 2-amino-5-bromopyridine, 2,5-hexanedione, p-toluenesulfonic acid, and toluene in the three-necked flask. Heat the mixture to reflux and collect the water generated in the Dean-Stark trap. Continue refluxing until no more water is collected and TLC analysis indicates complete consumption of the starting material. Cool the reaction mixture to room temperature (25-35 °C). Add ethyl acetate (100 mL) and wash the organic layer with a saturated sodium bicarbonate solution (100 mL), followed by saturated brine (100 mL). Dry the organic phase over anhydrous sodium sulfate, filter, and concentrate under reduced pressure to obtain the protected intermediate as a brown oil.

Synthesis of 2,6-dihydroxy-4-methyl-1, 8-naphthyridine

0.1 mole ethylacetoacetate was added to 0.1 mole of 2-amino-5-hydroxy pyridine. To the mixture 5 mL concentrated sulfuric acid was added. A white solid precipitate started forming in the reaction vessel. The reaction mixture was then refluxed at 135°C. The white precipitate slowly started to disappear. The mixture was refluxed till the completion of the reaction, which was monitored by TLC. The reaction mixture was cooled overnight to obtain the product, which was filtered, dried and recrystallized from ethanol.

General method for synthesis of target compounds

0.1 mole of 2,6-dihydroxy-4-methyl-1, 8-naphthyridine was dissolved in ethanol and to it was added 0.12 mole of benzoyl chloride derivatives and stirred at a temperature of 0°C for 2 h. The mixture formed was treated with a 10% NaHCO₃ solution until no CO₂ gas bubbles emerged. The

solid formed was filtered off and washed with cold water. The product was recrystallized from ethanol to obtain the desired compounds.

4-methyl-1,8-naphthyridine-2,6-diyl dibenzoate, ANB₁

¹H-NMR (δ ppm): 6.87-8.35 (aromatic protons), 2.62 (methyl protons); FTIR (cm⁻¹): 3050 (C-H, stretching), 1327-1225 (aromatic stretching), 1738 (C=O, stretching), 858 (C-N, stretching)

4-methyl-1,8-naphthyridine-2,6-diyl bis(4-chlorobenzoate), ANB₂

¹H-NMR (δ ppm): 6.87-8.35 (aromatic protons), 2.62 (methyl protons); FTIR (cm⁻¹): 3037 (C-H, stretching), 1335-1215 (aromatic stretching), 1728 (C=O stretching), 850 (C-N, stretching)

4-methyl-1,8-naphthyridine-2,6-diyl bis(4-methoxybenzoate), ANB₃

¹H-NMR (δ ppm): 6.87-8.35 (aromatic protons), 3.80 (methoxy protons), 2.62 (methyl protons); FTIR (cm⁻¹): 3017 (C-H, stretching), 1399-1252 (aromatic stretching), 1720 (C=O stretching), 821 (C-N stretching)

4-methyl-1,8-naphthyridine-2,6-diyl bis(4-fluorobenzoate), ANB₄

¹H-NMR (δ ppm): 6.87-8.35 (aromatic protons), 2.62 (methyl protons); FTIR (cm⁻¹): 3029 (C-H stretching), 1325-1221 (aromatic stretching), 1736 (C=O stretching), 867 (C-N stretching)

4-methyl-1,8-naphthyridine-2,6-diyl bis(4-methylbenzoate), ANB₅

¹H-NMR (δ ppm): 6.87-8.35 (aromatic protons), 2.62 (methyl protons), 2.31 (methyl protons, attached to aromatic ring); FTIR (cm⁻¹): 2972 (C-H stretching), 1412-1296 (aromatic stretching), 1742 (C=O stretching), 851 (C-N stretching)

The synthesized compounds were observed for color, practical yield, solubility determination and determination of retention factor (*R_f*) value by thin layer chromatographic technique (TLC).¹¹⁻¹³ The structural characterization of the synthesized compounds was done by FT-IR, and NMR spectral studies.¹⁴

***In silico* ADMET study**

The 2D structures of the ligands were sketched on ChemDraw Ultra 12.0 and then transferred on Chem 3D to create the three-dimensional structures. The canonical SMILES (simplified molecular-input line-entry system) were generated using ChemDraw and then submitted to SwissADME online tool for the ADMET analysis, the prediction of physicochemical parameters

and the drug-likeness using the Lipinski rule of five. The so-called Rule-of-five of Lipinski delineated the relationship between pharmacokinetic and physicochemical parameters.¹⁵

Anti-cholinesterase Activity

The synthesized compounds were individually dissolved in required volume of dimethylsulfoxide (DMSO) and diluted using phosphate buffer solution (PBS) (pH = 7.8) for the final range of concentrations (2-10 μ M).

A common chromogenic agent, Ellman's reagent was used for AChE catalyzed hydrolysis. In fresh 96-well microplates, each well was filled with 40 μ L of 50 mM Tris-HCl buffer pH 8.0, 20 μ L of test sample solution, 100 μ L of Ellman's reagent (prepared by dissolving 18.5 mg reagent in 10 mL of buffer), and 20 μ L of AChE solution (prepared by dissolving 0.26 U/mL enzyme in 15 mL buffer). The contents were mixed and incubated at 25°C for 15 min. The absorbance was measured at 412 nm. Now, 20 μ L of 15 mM acetyl thiocholine (ACTh) (prepared by dissolving in water) was added as a substrate to the wells and the plate was incubated for 20 min at 37°C. Blank determination was done without test samples to obtain 100% AChE activity.^{16,17} The absorbance was measured at 412 nm and the percentage AChE inhibition was calculated using formula:

$$\% \text{AChE inhibition} = (A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}} * 100$$

Results and Discussion

The synthesis of naphthyridine from β -keto esters follows the basics of Conrad-Limpach synthesis of quinolines. Using 2-amino-5-hydroxypyridine as the amine and condensing with ethyl acetoacetate yielded 4-methyl-1,8-naphthyridine. The mechanism involved in the cyclization is temperature dependent. At low temperature the amine condenses with the ester at the keto group to give an anil, which catalyzes to naphthyridine-4-one on heating. On the other hand, at higher temperature, the initial product of the condensation is the anilide, which is formed by the condensation of the amine at the ester function. The anilide undergoes ring closure on heating, or in the presence of an acid, and on subsequent oxidation yields 2,6-dihydroxy-4-methyl-1, 8-naphthyridine. The nucleophilic attack of the hydroxyl group of the naphthyridine to the electron deficient chloride of the benzoyl chloride results in nucleophilic substitution resulting in the esterification process and formation of the desired compounds.

The spectral characterization of the synthesized derivatives revealed the presence of aromatic protons, and the protons of methyl in all the compounds. The protons of methoxy were present in ANB₃. The FTIR spectrum exhibited the stretching vibrations due to C-H, C-C, C=C, C-O and

C-N in the compounds. Five 1,8-naphthyridine derivatives (**ANB₁₋₅**) the properties are presented in table 1.

Table 1. Physicochemical properties of the compounds

Code	Yield	Molecular Weight	Melting point
ANB ₁	69	384.38	216-218 °C
ANB ₂	71	453.27	205-207 °C
ANB ₃	68	444.44	213-215 °C
ANB ₄	67	420.37	240-242 °C
ANB ₅	70	412.44	221-223 °C

All the compounds were soluble in chloroform and DMSO whereas insoluble in water and methanol.

Anticholinesterase activity

The anticholinesterase activity was evaluated by Ellman's method and the results of inhibition of cholinesterase activity was calculated from the absorbance of blank and the test solutions (Table 2).

Table 2. %Inhibition of cholinesterase action

Code	% AChE inhibition				
	2 mM	4 mM	6 mM	8 mM	10 mM
ANB ₁	23.42	33.40	39.92	45.87	53.36
ANB ₂	22.47	29.17	36.47	42.80	51.06
ANB ₃	25.91	37.24	43.95	56.05	65.07
ANB ₄	15.93	20.73	25.72	30.52	36.28
ANB ₅	19.19	24.76	31.29	38.39	25.72

The synthesized derivatives were able to inhibit AChE activity dose dependently from 15.93 to 65.07%. The IC₅₀ was calculated from inhibition percentage and was found to be 8.99 mM, 9.84 mM, 6.89 mM, 15.57 mM and 22.59 mM for ANB₁ to ANB₅ respectively.

This confirms that substitution at the 2,6-positions of the naphthyridine core with various benzoate derivatives influences the anticholinesterase potential. Among the derivatives, ANB₃ (4-methoxybenzoate substitution) exhibited the highest inhibitory activity, reaching 65.07% at 10 mM, with the lowest IC₅₀ value (6.89 mM). The presence of an electron-donating methoxy group likely enhances binding affinity to the active site of AChE through increased π -electron density and possible hydrogen bonding interactions. In contrast, ANB₄ (4-fluoro substitution) showed the weakest inhibition (36.28% at 10 mM, IC₅₀ = 15.57 mM), suggesting that the electron-withdrawing nature of fluorine reduces favorable interactions with the enzyme. ANB₅ (4-methyl substitution) displayed moderate activity at lower concentrations but a decline at higher concentration (25.72% at 10 mM), indicating possible steric hindrance or reduced solubility affecting enzyme binding. ANB₁ and ANB₂ (unsubstituted and 4-chloro derivatives) showed intermediate activity, with IC₅₀ values of 8.99 mM and 9.84 mM respectively, suggesting that halogen substitution provides only modest improvement compared to the parent benzoate.

The results highlight that electron-donating substituents (methoxy, methyl) generally favor AChE inhibition, whereas electron-withdrawing substituents (fluoro, chloro) reduce potency. This aligns with the hypothesis that enhanced electron density in the aromatic ring improves π - π stacking and hydrogen bonding interactions with the enzyme's active site. The dual ester linkage at positions 2 and 6 of the naphthyridine core appears crucial for activity, providing rigidity and optimal orientation for binding.

***In silico* ADMET properties of synthesized naphthyridine derivatives**

The SMILES of the synthesized naphthyridine derivatives was generated using chemdraw ultra 12.0 software. The physicochemical properties of the compounds predicted *in silico* by SwissADME are presented in table 3 and the ADMET properties predicted by pkCSM are presented in table 4.

Table 3. Physicochemical properties of ANB₁₋₅

Compound Code	HBD	HBA	Log P	NRB	PSA	MR	Log S
ANB ₁	0	6	4.26	6	78.38	107.31	-5.52
ANB ₂	0	6	5.46	6	78.38	117.32	-6.70
ANB ₃	0	8	4.12	8	96.84	120.29	-5.66
ANB ₄	0	8	4.98	6	78.38	107.22	-5.83

ANB ₅	0	6	4.96	6	78.38	117.24	-6.12
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Table 4. ADMET of ANB₁₋₅

ADMET Parameters	ANB ₁	ANB ₂	ANB ₃	ANB ₄	ANB ₅
Absorption					
Water solubility (log mol/L)	-4.342	-4.761	-4.187	-4.127	-4.553
Intestinal absorption (human) (% Absorbed)	98.202	95.384	100	97.426	98.301
Skin Permeability (log Kp)	-2.73	-2.735	-2.736	-2.736	-2.731
Distribution					
VD _{ss} (human) (log L/kg)	-0.178	-0.076	-0.193	-0.33	-0.045
CNS permeability (log PS)	-3.059	-2.945	-3.458	-3.32	-2.968
Metabolism					
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
Excretion					
Total Clearance (log ml/min/kg)	0.713	-0.295	0.902	0.69	0.718
Toxicity					
AMES toxicity	No	No	No	No	No
Hepatotoxicity	Yes	No	No	Yes	Yes
Oral Rat Acute Toxicity (LD ₅₀) (mol/kg)	2.594	2.555	2.66	2.728	2.516

The *in-silico* evaluation of the synthesized 1,8-naphthyridine derivatives (ANB₁–ANB₅) provided important insights into their physicochemical and pharmacokinetic behavior. All compounds displayed moderate to high lipophilicity (Log P 4.12–5.46), which supports membrane permeability but contributes to poor aqueous solubility (Log S –5.52 to –6.70). The absence of hydrogen bond donors and the presence of 6–8 hydrogen bond acceptors suggest that passive diffusion is the primary mode of absorption. Polar surface area values (78–96 Å²) remained within the acceptable range for oral bioavailability, while molar refractivity values indicated moderate polarizability. ADMET predictions revealed excellent intestinal absorption (>95% for all compounds), with ANB₃ achieving complete absorption (100%). Skin permeability was consistently low across the series, and distribution studies suggested limited tissue penetration with poor CNS permeability,

reducing the likelihood of central neurotoxicity. Metabolic profiling indicated that none of the compounds were CYP2D6 substrates, though all were CYP3A4 substrates, highlighting potential drug–drug interaction risks. Clearance values varied, with ANB₃ showing the highest clearance, suggesting faster elimination, while ANB₂ exhibited the lowest clearance, indicating possible accumulation. Toxicity predictions confirmed non-mutagenicity for all derivatives, though hepatotoxicity was observed in ANB₁, ANB₄, and ANB₅. Acute oral toxicity values ($LD_{50} \approx 2.5\text{--}2.7 \text{ mol/kg}$) indicated moderate toxicity. Overall, ANB₃ emerged as the most promising candidate, combining favorable physicochemical properties, excellent absorption, balanced lipophilicity, and strong anticholinesterase activity, whereas derivatives with halogen substitutions (ANB₂ and ANB₄) showed reduced potency or safety concerns. These findings suggest that further structural optimization, particularly through the introduction of hydrophilic substituents to improve solubility and reduce hepatotoxicity, could enhance the therapeutic potential of the naphthyridine scaffold. Table 4 reveals that all compounds meet every single criterion of Lipinski's rule of five and thus fully obey the rule. Consequently, all the investigated compounds present a good drug-likeness profile, since they are predicted to be easily absorbed and have good permeability and bioavailability.

Conclusion

The present investigation successfully synthesized and characterized five novel 1,8-naphthyridine derivatives and evaluated their physicochemical, *in silico*, and biological properties. The derivatives demonstrated moderate to good yields, consistent structural confirmation, and favorable drug-likeness parameters, though solubility remained a limitation. *In vitro* anticholinesterase assays confirmed that all compounds inhibited AChE in a dose-dependent manner, with ANB₃ showing the highest potency. The combined experimental and computational findings highlight ANB₃ as a promising lead compound for further optimization. Future work should focus on structural modifications to improve solubility, reduce hepatotoxicity, and enhance CNS penetration, thereby strengthening the therapeutic potential of the naphthyridine scaffold in the development of novel cholinesterase inhibitors.

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