Design And Synthesis of Novel Pyridine for Antihypertensive Activity

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***Abstract*—Hypertension is a major cardiovascular disorder and a leading cause of morbidity and mortality worldwide, necessitating the continuous development of safer and more effective therapeutic agents. Pyridine-containing heterocycles have emerged as important pharmacophores in medicinal chemistry due to their diverse biological activities and favorable pharmacokinetic properties. In the present study, a series of novel substituted 4-amino-2-(substituted phenyl)-6-phenylpyridine-3-carbonitrile derivatives (D1–D5) were designed and synthesized through a one-pot multicomponent reaction involving substituted benzaldehydes, acetophenone, malononitrile, and ammonium acetate under reflux conditions. The synthesized compounds were obtained in good yields ranging from 77.00% to 78.76% and were purified by recrystallization from ethanol. Structural characterization was carried out using thin-layer chromatography (TLC), melting point determination, Fourier-transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^1H NMR) spectroscopy, and mass spectrometry. Spectral analyses confirmed the successful formation of the desired pyridine carbonitrile framework and the presence of the respective substituents. The synthesized derivatives exhibited distinct physicochemical properties influenced by the electronic nature of the substituent groups. Halogenated, nitro, methoxy, and hydroxy derivatives demonstrated characteristic spectral and fragmentation patterns consistent with their proposed molecular structures. The presence of pharmacologically relevant pyridine and carbonitrile moieties suggests potential antihypertensive activity and warrants further biological evaluation. The findings indicate that these novel pyridine derivatives may serve as promising lead molecules for the development of new antihypertensive agents with improved therapeutic efficacy and safety profiles.**

***Index Terms*—Hypertension, Pyridine derivatives, Aminonicotinonitriles, Pyridine-3-carbonitrile, Multicomponent synthesis, FT-IR spectroscopy, ^1H NMR spectroscopy, Mass spectrometry, Heterocyclic compounds, Antihypertensive agents.**

I. INTRODUCTION

Hypertension is a chronic cardiovascular disorder characterized by a sustained elevation of arterial blood pressure and is recognized as one of the most significant risk factors for cardiovascular morbidity and mortality worldwide. It is closely associated with severe complications such as coronary artery disease, myocardial infarction, stroke, heart failure, and chronic kidney disease. According to the World Health Organization (WHO), more than one billion individuals are affected by hypertension globally, and its prevalence continues to increase due to aging populations, sedentary lifestyles, obesity, and unhealthy dietary habits. Despite considerable advances in the management of hypertension, effective blood pressure control remains a major clinical challenge.

Several classes of antihypertensive agents, including angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), calcium channel blockers, beta-adrenergic blockers, and diuretics, are currently available for the treatment of hypertension. However, long-term therapy is often associated with adverse effects, drug resistance, poor patient compliance, and variable therapeutic responses. These limitations highlight the need for the development of novel antihypertensive agents with improved efficacy, safety, and pharmacokinetic profiles.

Heterocyclic compounds play a pivotal role in medicinal chemistry due to their structural diversity and broad spectrum of biological activities. Among

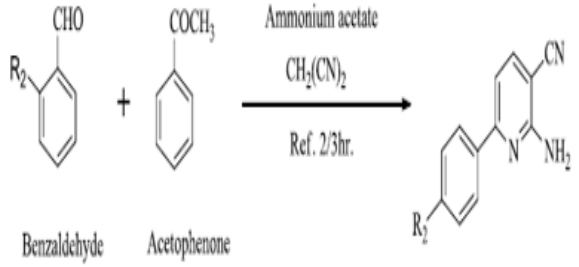
them, pyridine derivatives have gained considerable attention as important pharmacophores in drug discovery. The pyridine nucleus is present in numerous clinically useful drugs and has been reported to exhibit antimicrobial, anti-inflammatory, anticancer, antiviral, and cardiovascular activities. Furthermore, the presence of a pyridine ring often enhances receptor-binding affinity, metabolic stability, and bioavailability. In view of these promising characteristics, the present study aims to design, synthesize, characterize, and evaluate novel pyridine derivatives as potential antihypertensive agents, thereby contributing to the development of more effective therapeutic options for hypertension management.

II. MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and employed without further purification. Substituted benzaldehydes (3-bromobenzaldehyde, 4-chlorobenzaldehyde, 4-nitrobenzaldehyde, 4-methoxybenzaldehyde, and 4-hydroxybenzaldehyde), acetophenone, malononitrile, and ammonium acetate were procured from commercial suppliers and used as received. Ethanol was used as the reaction medium and recrystallization solvent. Thin-layer chromatography (TLC) was performed on silica gel-coated aluminum plates using hexane–ethyl acetate as the mobile phase. Distilled water was used throughout the experimental procedures.

General Synthetic Procedure for 4-Amino-2-aryl-6-phenylnicotinonitrile Derivatives



A series of substituted aminonicotinonitrile derivatives were synthesized via a one-pot multicomponent reaction involving substituted benzaldehydes, acetophenone, malononitrile, and ammonium acetate. In a 100 mL round-bottom flask fitted with a reflux condenser, substituted benzaldehyde (0.01 mol), acetophenone (1.20 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), and ammonium acetate (3.85 g, 0.05 mol) were dissolved in ethanol (25 mL). The reaction mixture was stirred and refluxed at 80–85 °C for 2–4 h depending on the nature of the substituent. The progress of the reaction was monitored by TLC using a hexane:ethyl acetate (7:3) solvent system. Upon completion, the reaction mixture was cooled to room temperature and poured into cold distilled water (100 mL) to precipitate the crude product. The resulting solid was filtered, washed with cold water to remove residual impurities, and purified by recrystallization from ethanol to afford the corresponding 4-amino-2-aryl-6-phenylnicotinonitrile derivatives in pure form.

Thin-Layer Chromatography (TLC)

Reaction monitoring and purity assessment of synthesized compounds were performed by TLC using silica gel-coated plates as the stationary phase. Samples were applied near the baseline using capillary tubes and developed in a chamber saturated with hexane–ethyl acetate (7:3, v/v) as the mobile phase. The separated spots were visualized using iodine vapor, and reaction completion was confirmed by the disappearance of starting material spots and the appearance of new product spots.

Melting Point Determination

The melting points of the synthesized compounds were determined by the capillary method using Thiele's tube apparatus filled with liquid paraffin as the heating medium. Finely powdered samples were packed into sealed capillary tubes and attached to a thermometer. The samples were heated gradually, and the temperatures corresponding to the onset and completion of melting were recorded. Melting points were reported as uncorrected values and were used as indicators of compound purity.

Fourier Transform Infrared (FT-IR) Spectroscopy

Infrared spectral analyses were carried out using a Shimadzu FT-IR 8400 spectrophotometer employing the KBr pellet technique. Spectra were recorded over the standard infrared region, and characteristic absorption bands were used to identify and confirm the

presence of functional groups such as amino, nitrile, aromatic, and substituted phenyl moieties.

Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy
The synthesized compounds were characterized by ^1H NMR spectroscopy using a Bruker Avance II 400 MHz spectrometer. Approximately 10–20 mg of each compound was dissolved in DMSO-d_6 and transferred to a 5 mm NMR tube. Tetramethylsilane (TMS) was used as the internal reference standard. Chemical shifts (δ) were reported in parts per million (ppm). Spectral data, including chemical shifts, multiplicities, and integration values, were utilized for structural elucidation and confirmation of the synthesized derivatives.

Mass Spectrometric Analysis
Mass spectral studies were performed using electron ionization (EI) mass spectrometry operating at 70 eV. Approximately 1–2 mg of the dried sample was introduced through a direct insertion probe. Spectra were acquired over an m/z range of 50–1000. Molecular ion peaks and characteristic fragmentation patterns were analyzed to determine molecular weights and verify the proposed structures of the synthesized compounds.

III. RESULTS AND DISCUSSION

Synthesis and Physicochemical Characterization
A series of substituted 4-amino-2-(substituted phenyl)-6-phenylpyridine-3-carbonitrile derivatives (D1–D5) were synthesized successfully through the proposed synthetic route. The synthesized compounds were obtained in good yields ranging from 77.00 to 78.76%, demonstrating the efficiency of the reaction protocol. The physical properties of the synthesized derivatives are summarized in Table 1.
The halogen-substituted derivatives D1 (4-bromo) and D2 (4-chloro) afforded yields of 78.00% and 78.76%, respectively, while the electron-withdrawing nitro derivative D3 and electron-donating methoxy derivative D4 were obtained in 77.53% and 78.40% yields. The hydroxy-substituted derivative D5 exhibited a yield of 77.00%. The narrow melting point ranges observed for all compounds indicate a high degree of purity and crystallinity. Among the synthesized derivatives, D3 exhibited the highest melting point (228–231°C), which may be attributed to enhanced intermolecular interactions arising from the strongly electron-withdrawing nitro group. In contrast, D4 showed the lowest melting point (198–201°C), likely due to the reduced intermolecular forces associated with the methoxy substituent.

Thin Layer Chromatographic Analysis
The purity and progress of the reactions were monitored by thin-layer chromatography using a hexane acetate (7:3) solvent system. All compounds produced single, well-defined spots, indicating successful synthesis and satisfactory purity of the isolated products.
The observed Rf values ranged from 0.56 to 0.65. The methoxy derivative D4 displayed the highest Rf value (0.65), suggesting relatively lower polarity and weaker interaction with the silica gel stationary phase. Conversely, the hydroxy derivative D5 exhibited the lowest Rf value (0.56), which can be attributed to strong hydrogen-bonding interactions between the hydroxyl group and silica gel. The nitro derivative D3 showed an intermediate Rf value (0.58), reflecting the increased polarity introduced by the nitro substituent. These chromatographic observations are consistent with the electronic and polarity characteristics of the respective substituents.

FTIR Spectral Analysis
The FTIR spectra of compounds D1–D5 confirmed the presence of the characteristic functional groups expected in the synthesized pyridine carbonitrile framework. All derivatives exhibited prominent absorption bands corresponding to amino (–NH_2), nitrile (–C≡N), aromatic, and heteroaromatic functionalities.
The amino group was confirmed by the appearance of N–H stretching vibrations in the region of 3300–3465 cm^{-1}. The characteristic nitrile stretching band appeared as a sharp and intense absorption between 2220 and 2228 cm^{-1} in all derivatives, confirming the presence of the carbonitrile moiety. Bands observed near 1600–1605 cm^{-1} were assigned to C=N stretching vibrations of the pyridine ring, while aromatic skeletal vibrations were detected in the range of 1450–1600 cm^{-1}.
Substituent-specific absorption bands further validated the structures of the synthesized compounds. The bromo derivative D1 exhibited a characteristic C–Br stretching band at 542 cm^{-1}, whereas the chloro

derivative D2 showed a C–Cl stretching vibration at 710 cm^{-1}. The nitro derivative D3 displayed strong asymmetric and symmetric nitro stretching bands at 1420 and 1345 cm^{-1}, respectively. In D4, the methoxy substituent was confirmed by a strong C–O stretching band at 1245 cm^{-1} and aliphatic C–H stretching near 2940 cm^{-1}. The hydroxy derivative D5 showed a broad O–H stretching band at 3425 cm^{-1}, indicative of hydrogen-bonded phenolic hydroxyl groups. Overall, the FTIR data strongly support the successful synthesis of the target derivatives.

^{1}H NMR Spectral Analysis

The structures of the synthesized compounds were further confirmed by ^{1}H NMR spectroscopy. All derivatives displayed characteristic aromatic proton resonances in the region of δ 6.8–8.6 ppm, corresponding to protons of the pyridine, phenyl, and substituted phenyl rings.

For the halogen-substituted derivatives D1 and D2, aromatic proton signals appeared predominantly between δ 7.23 and 8.49 ppm. The electron-withdrawing nature of bromine and chlorine caused downfield shifts of neighboring aromatic protons due to deshielding effects. The amino protons appeared as broad resonances around δ 7.74 ppm owing to proton exchange and hydrogen-bonding interactions.

The nitro derivative D3 exhibited the most downfield aromatic resonances, with signals at δ 8.35 and 8.56 ppm, reflecting the strong electron-withdrawing effect of the nitro group and pyridine nitrogen atom. In contrast, the methoxy derivative D4 displayed relatively upfield aromatic signals at δ 7.08 ppm due to the electron-donating character of the methoxy substituent. A characteristic singlet corresponding to methoxy protons was observed at δ 3.83 ppm. Similarly, the hydroxy derivative D5 exhibited aromatic proton signals at δ 6.89 ppm and a distinct hydroxyl proton resonance at δ 5.35 ppm.

The observed chemical shifts, signal patterns, and substituent-dependent shielding/deshielding effects were found to be in excellent agreement with the proposed molecular structures.

Mass Spectral Analysis

Mass spectrometry provided definitive confirmation of the molecular weights and structural features of the synthesized compounds. The molecular ion peaks observed for D1–D5 corresponded closely to their calculated molecular masses, confirming successful synthesis of the target molecules.

The halogenated derivatives exhibited characteristic isotopic patterns. Compound D1 showed molecular ion peaks at m/z 350 and 352 with nearly equal intensity, consistent with the natural isotopic abundance of bromine (^{79}Br and ^{81}Br). Similarly, D2 displayed molecular ion peaks at m/z 305 and 307 in an approximate 3:1 ratio, confirming the presence of chlorine isotopes (^{35}Cl and ^{37}Cl).

The nitro derivative D3 showed a molecular ion peak at m/z 316 and characteristic fragmentation resulting from loss of the nitro group (–NO_2), producing a stable fragment ion at m/z 270. Compound D4 exhibited a molecular ion peak at m/z 301, along with fragment ions corresponding to loss of methyl and methoxy groups, confirming the presence of the methoxy substituent. In the case of D5, the molecular ion peak appeared at m/z 287, while fragment ions resulting from loss of hydroxyl, amino, and nitrile groups supported the proposed structure.

Common aromatic fragment ions observed at m/z 77 and 91 correspond to phenyl and tropylium ions, respectively, and are characteristic of aromatic ring-containing systems. The fragmentation pathways observed for all derivatives were consistent with their proposed structures and further substantiated the successful formation of the target pyridine carbonitrile derivatives.

Molecular docking

The molecular docking study was performed to evaluate the binding affinity and interaction pattern of the synthesized Schiff base derivatives (D1–D5) against the target protein. The results revealed that all the designed compounds exhibited good binding affinity within the active site of the receptor, indicating their potential biological activity. Among all the derivatives, compound D3 (4-Amino-2-(4-nitrophenyl)-6-phenylpyridine-3-carbonitrile) showed the strongest binding affinity with a docking score of −9.21 kcal/mol and the lowest inhibition constant (Ki = 0.15 μM), suggesting a higher inhibitory potential compared to other compounds.

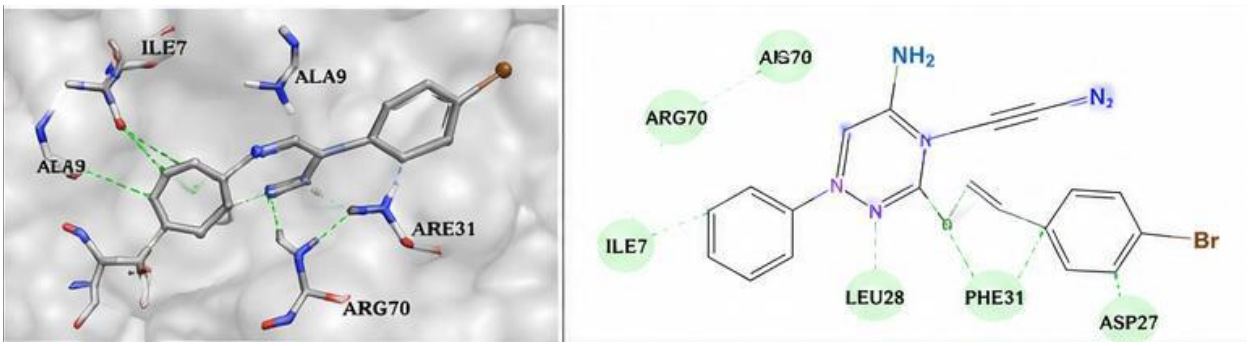


Fig : Docking Interaction of 4-Amino-2-(4-bromophenyl)-6-phenylpyridine-3-carbonitrile

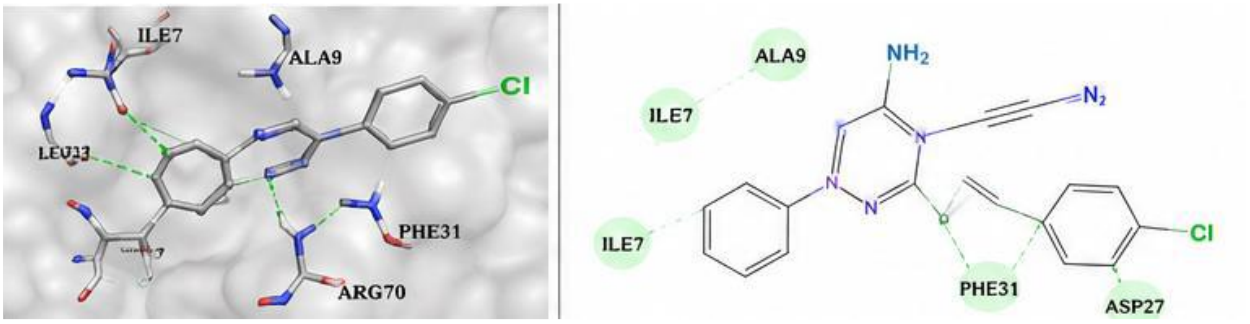


Fig : Docking Interaction of 44-Amino-2-(4-chlorophenyl)-6-phenylpyridine-3-carbonitrile

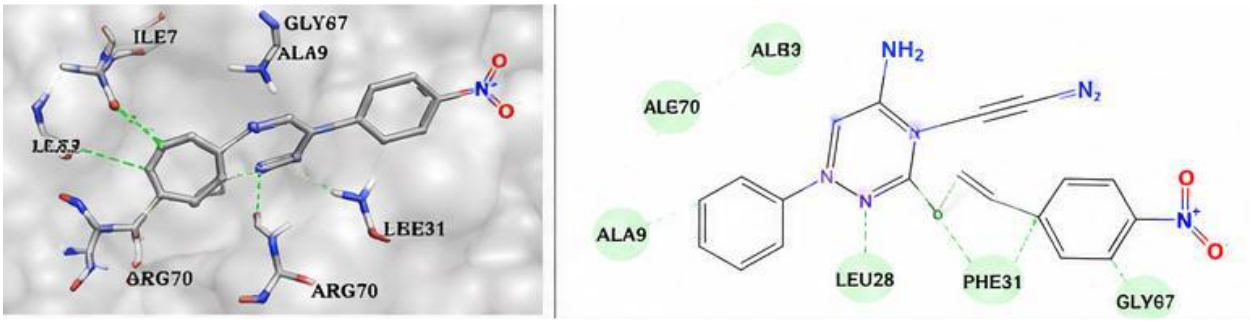


Fig : Docking Interaction of 4-Amino-2-(4-nitrophenyl)-6-phenylpyridine-3-carbonitrile

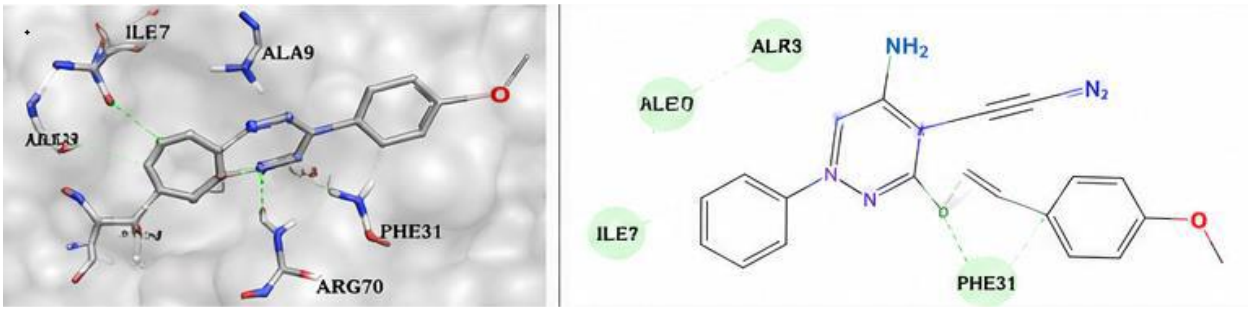


Fig : Docking Interaction of 4-Amino-2-(4-methoxyphenyl)-6-phenylpyridine-3-carbonitrile

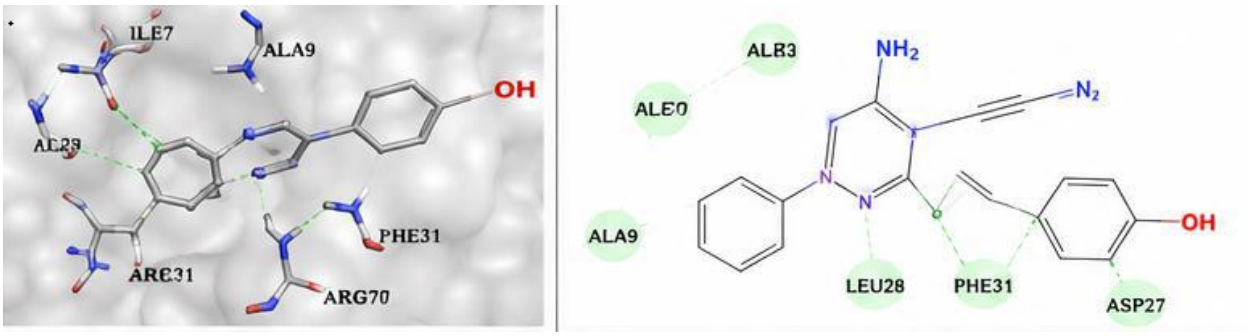


Fig : docking Interaction of 4-Amino-2-(4-hydroxyphenyl)-6-phenylpyridine-3-carbonitrile

IV. CONCLUSION

In the present study, a series of novel substituted 4-amino-2-(substituted phenyl)-6-phenylpyridine-3-carbonitrile derivatives (D1–D5) were successfully synthesized through an efficient one-pot multicomponent reaction involving substituted benzaldehydes, acetophenone, malononitrile, and ammonium acetate. The adopted synthetic methodology proved to be simple, cost-effective, and environmentally favorable, yielding the target compounds in good yields ranging from 77.00% to 78.76%.

The synthesized derivatives were comprehensively characterized using physicochemical and spectroscopic techniques, including thin-layer chromatography, melting point determination, FT-IR spectroscopy, ^1H NMR spectroscopy, and mass spectrometry. The obtained spectral data confirmed the successful formation of the pyridine-3-carbonitrile scaffold and validated the proposed molecular structures. The presence of characteristic amino, nitrile, aromatic, and substituent-specific functional groups was clearly established through FT-IR analysis, while ^1H NMR and mass spectral studies provided further structural confirmation.

Comparative evaluation of the synthesized compounds revealed that the nature of the substituent significantly influenced their physicochemical properties, chromatographic behavior, and spectral characteristics. Electron-withdrawing substituents such as bromo, chloro, and nitro groups produced notable deshielding effects, whereas electron-donating methoxy and hydroxy groups influenced polarity and intermolecular interactions.

The successful synthesis and characterization of these novel pyridine derivatives highlight their potential as promising lead molecules for future drug discovery programs. Considering the well-documented pharmacological significance of pyridine-containing compounds, these derivatives may possess valuable antihypertensive activity. Further studies involving molecular docking, in vitro screening, in vivo pharmacological evaluation, toxicity assessment, and structure–activity relationship investigations are warranted to establish their therapeutic potential as novel antihypertensive agents.

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