

Novel Picolinic Acid-Derived Pyridine Carboxamides: Synthesis, Characterization and Evaluation of Cytotoxic and Antibacterial Activities

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ABSTRACT

Picolinic acid represents a versatile nitrogen-containing heteroaromatic scaffold with considerable potential for structural diversification and biological investigation. In the present study, a series of ten picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj) was designed, synthesized, and evaluated for their *in vitro* cytotoxic and antibacterial activities. The target compounds were prepared through a multistep synthetic sequence involving conversion of picolinic acid to 4-chloropyridine-2-carbonyl chloride, formation of 4-chloro-*N,N*-disubstituted pyridine-2-carboxamide intermediates, synthesis of *N*-(4-nitrobenzylidene)benzene-1,2-diamine, and subsequent coupling to afford the final derivatives. The synthesized compounds were obtained in 62–75% yields, with melting points ranging from 197 to 239 °C. Structural characterization was supported by infrared spectroscopy, ¹H NMR spectroscopy, and molecular ion data. The cytotoxic activity was evaluated against MCF-7, HT-29, and A549 cancer cell lines using the MTT assay. Among the synthesized derivatives, VIh exhibited the most potent cytotoxic activity, with IC₅₀ values of 9.2, 8.4, and 16.6 μM against MCF-7, HT-29, and A549 cells, respectively, followed by VIg with IC₅₀ values of 11.4, 13.5, and 18.9 μM. Antibacterial activity was evaluated against *Bacillus thermophilus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella paratyphi* A using the cup-plate method. VIh demonstrated the broadest antibacterial activity, producing inhibition zones of 13, 15, 11, and 14 mm, respectively, while VIg produced zones of 12, 10, 9, and 12 mm. Although the activity of the lead derivatives remained lower than that of the respective reference standards, the results demonstrate that structural modification of the picolinic acid-derived pyridine carboxamide scaffold can produce compounds with promising dual biological activity. Overall, VIg and VIh emerged as the most promising derivatives and may serve as useful lead structures for further optimization and mechanistic investigation.

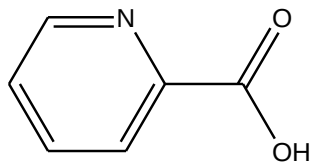
Keywords: Antibacterial activity; Anticancer activity; Cytotoxicity; Heterocyclic compounds; Picolinic acid; Pyridine carboxamides.

INTRODUCTION

Picolinic acid (pyridine-2-carboxylic acid, Fig. 1) is a nitrogen-containing heteroaromatic carboxylic acid and a positional isomer of nicotinic acid. Structurally, it consists of a pyridine ring bearing a carboxylic acid group at the 2-position, which provides a distinctive combination of aromaticity, nitrogen-based coordination ability, hydrogen-bonding capacity, and carboxyl functionality [1, 2]. These structural characteristics make

picolinic acid an attractive scaffold for medicinal and synthetic chemistry. The pyridine nitrogen and carboxyl group can participate in diverse intermolecular interactions and can be further functionalized to generate structurally diverse derivatives with modified physicochemical and biological properties [3]. The chemical versatility of this scaffold has consequently stimulated considerable interest in the design and synthesis of picolinic acid-based molecules for

pharmacological applications. The present study was undertaken to explore this scaffold through the synthesis of substituted picolinic acid derivatives and evaluation of their biological activities [4].



Picolinic acid

Fig. 1. Chemical structure of Picolinic acid.

Heteroaromatic scaffolds containing nitrogen are extensively investigated in drug discovery because incorporation of nitrogen atoms into aromatic systems can influence molecular polarity, hydrogen-bonding interactions, electronic distribution, aqueous solubility, and target-binding characteristics [5]. Among these systems, pyridine derivatives occupy an important position because appropriately substituted pyridine rings can establish favorable interactions with biological macromolecules. Modification of the pyridine nucleus at different positions provides an opportunity to tune molecular properties and biological responses. In particular, introduction of amide functionalities into pyridine-based frameworks can provide additional hydrogen-bond donor and acceptor sites and may enhance interactions with biological targets. Accordingly, the development of substituted pyridine carboxamides represents a rational strategy for generating novel biologically active molecules [6].

Cancer remains a major global health challenge, and the identification of new small molecules with selective and potent anticancer activity continues to be an important objective of medicinal chemistry. Conventional anticancer agents are frequently associated with limitations including systemic toxicity, inadequate selectivity, resistance, and adverse effects. Therefore, continuous efforts have been directed toward the discovery of structurally novel chemical entities capable of interfering with cancer-cell proliferation and survival [7]. Heterocyclic compounds are particularly valuable in this context because their structural diversity allows systematic modification of molecular characteristics and exploration of structure–activity relationships (SAR). Evaluation against different cancer cell lines provides an initial indication of the influence of structural modifications on cellular sensitivity and can help identify promising lead structures for further optimization [8].

The MCF-7, HT-29, and A549 cell lines represent widely used experimental models of breast, colorectal, and lung cancer, respectively [9]. Evaluation of

candidate molecules against these distinct cancer cell types is useful for determining whether biological activity is broad or preferential toward a particular cellular model. The MTT assay is a commonly employed colorimetric method for preliminary assessment of cell viability and cytotoxicity. In this assay, metabolically active cells reduce the yellow tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to insoluble purple formazan through cellular reductive activity. The quantity of formazan formed is related to cellular metabolic activity and can therefore be used to estimate the cytotoxic response following treatment with test compounds. In the present investigation, the synthesized derivatives were evaluated for their cytotoxic potential using MCF-7, HT-29, and A549 cancer cell lines, with IC₅₀ values used as a quantitative measure of their inhibitory activity. The study material reports the use of cisplatin as a reference compound for comparison of cytotoxic potency [10].

In addition to anticancer activity, heteroaromatic compounds containing appropriately positioned nitrogen, amide, and other functional groups may exhibit antimicrobial properties. The increasing emergence of antimicrobial resistance has intensified the search for alternative chemical entities with antibacterial activity. Structural diversification of established heterocyclic frameworks can generate compounds with different physicochemical and target-interaction profiles and may provide useful starting points for antimicrobial lead discovery [11]. Assessment against both Gram-positive and Gram-negative bacteria is particularly informative because differences in cell-envelope architecture can substantially influence the susceptibility of bacterial organisms to small molecules [12]. Therefore, the present study also investigated the antibacterial activity of the synthesized picolinic acid derivatives against *Bacillus thermophilus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella paratyphi* A using the cup-plate method, with ciprofloxacin employed as the reference antibacterial agent.

From a synthetic perspective, the present work was designed around functionalization of the picolinic acid nucleus followed by incorporation of additional pharmacophoric elements. Picolinic acid was converted to 4-chloropyridine-2-carbonyl chloride, which served as a key intermediate for subsequent amide formation. Reaction with dimethylamine or secondary amines afforded 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediates. In parallel, condensation of 4-nitrobenzaldehyde with 1,2-phenylenediamine generated an imine-containing intermediate, N-(4-nitrobenzylidene)benzene-1,2-diamine. Subsequent coupling of these intermediates afforded the target 4-(2-(4-nitrobenzylideneamino)phenylamino)-N,N-

disubstituted pyridine-2-carboxamide framework. Ten derivatives, designated VIa–VIj, were synthesized through variation of the substituent associated with the amide moiety. The synthesized compounds were subsequently characterized using their reported physicochemical and spectroscopic parameters before biological evaluation [13].

The structural design of these derivatives incorporates several potentially important molecular features, including the pyridine nitrogen, carboxamide functionality, anilino linkage, imine group, and para-nitrophenyl moiety. The combination of these functionalities provides an opportunity to investigate how structural variation influences both physicochemical characteristics and biological activity [14]. Comparative evaluation of the synthesized derivatives may therefore provide preliminary SAR information and identify structural features associated with enhanced cytotoxic or antibacterial effects. Such information is valuable for guiding subsequent structural optimization and mechanistic investigations. Accordingly, the objective of the present study was to synthesize and characterize a series of novel picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj) and systematically investigate their *in vitro* cytotoxic and antibacterial activities. The cytotoxic potential was assessed against MCF-7, HT-29, and A549 cancer cell lines using the MTT assay, while antibacterial activity was evaluated against selected Gram-positive and Gram-negative bacterial strains using the cup-plate method. Comparative analysis of the biological findings, together with the structural characteristics of the synthesized derivatives, was intended to establish preliminary structure–activity relationships and identify promising compounds for further pharmacological and medicinal-chemistry investigations.

MATERIALS AND METHODS

Chemicals and reagents

Picolinic acid, thionyl chloride, anhydrous N,N-dimethylformamide (DMF), toluene, tetrahydrofuran (THF), methanol, potassium tert-butoxide, potassium carbonate, ethyl acetate, sodium sulfate, 4-nitrobenzaldehyde, 1,2-phenylenediamine, 3 Å molecular sieves, dimethylamine and appropriate secondary amines were used for the synthesis of the target compounds. Analytical-grade solvents and reagents were used throughout the synthetic procedures. For biological evaluation, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, trypsin, ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], and other required cell-culture reagents were used. Nutrient broth and

nutrient agar were used for antibacterial evaluation. Ciprofloxacin and cisplatin were used as reference standards for antibacterial and cytotoxicity studies, respectively. The source document specifies Sigma-Aldrich (St. Louis, MO, USA) for DMEM, MTT, trypsin and EDTA, Arrow Labs for FBS, and Tarsons for 96-well tissue-culture plates.

General synthetic procedure

The synthetic route involved sequential preparation of the key pyridine carboxamide intermediate, preparation of the substituted imine intermediate, and subsequent coupling to furnish the final picolinic acid derivatives. The overall synthetic sequence consisted of four principal transformations: (i) conversion of picolinic acid to 4-chloropyridine-2-carbonyl chloride, (ii) conversion of the acid chloride to 4-chloro-N,N-disubstituted pyridine-2-carboxamide derivatives, (iii) condensation of 4-nitrobenzaldehyde with 1,2-phenylenediamine to obtain N-(4-nitrobenzylidene)benzene-1,2-diamine, and (iv) coupling of the two intermediates to produce the target compounds VIa–VIj [15, 16].

Synthesis of 4-chloropyridine-2-carbonyl chloride (II)

Anhydrous DMF (6 mL) was added slowly to thionyl chloride (1.68 mol) at 40–50 °C under a nitrogen atmosphere. The reaction mixture was stirred at the same temperature for 10 min. Picolinic acid (I) (0.048 mol) was then added portion-wise over a period of 30 min. During addition, the reaction mixture underwent a characteristic color change from green to orange and subsequently purple. The reaction mixture was heated to 72 °C, accompanied by vigorous evolution of sulfur dioxide. After 16 h, a yellow solid was formed. The mixture was allowed to cool to room temperature and diluted with toluene (500 mL), followed by concentration to approximately 200 mL. The dilution and concentration procedure was repeated twice, after which the mixture was concentrated to near dryness. The resulting solid was filtered, washed with toluene, and dried under high vacuum for 4 h to afford 4-chloropyridine-2-carbonyl chloride (II). The reported yield was 80%, with a melting point of 151–154 °C [17].

Synthesis of 4-chloro-N,N-disubstituted pyridine-2-carboxamide (III)

The synthesized 4-chloropyridine-2-carbonyl chloride (II) (0.03 mol) was subsequently treated with dimethylamine or an appropriate secondary amine solution (2.0 mol/L) prepared in a THF–methanol solvent system. The reaction was initiated at 0 °C under an argon atmosphere and the mixture was subsequently stirred at approximately 3 °C for 4 h. After completion of the reaction, the mixture was concentrated to near

dryness to obtain the corresponding 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediate (III). The reported yield was 78%, and the product exhibited a melting point of 166–168 °C [18].

Synthesis of N-(4-nitrobenzylidene)benzene-1,2-diamine (V)

The imine intermediate was prepared by condensation of 4-nitrobenzaldehyde with 1,2-phenylenediamine. 4-Nitrobenzaldehyde was dissolved in methanol and added to 1,2-phenylenediamine dissolved in methanol in the presence of 3 Å molecular sieves. The reaction mixture was stirred at room temperature for 72 h. Following completion of the reaction, the product was isolated and recrystallized from ethanol to afford N-(4-nitrobenzylidene)benzene-1,2-diamine (V). The reported yield was 71%, with a melting point of 181–184 °C.

Synthesis of 4-(2-(4-nitrobenzylideneamino)phenylamino)-N,N-disubstituted pyridine-2-carboxamides (VIa–VIj)

Compound V (0.05 mol) was dissolved in dry DMF and treated with potassium tert-butoxide. The reaction mixture was stirred at room temperature for 2 h. Subsequently, 4-chloro-N,N-disubstituted pyridine-2-carboxamide (III) (0.05 mol) was added, followed by potassium carbonate. The resulting reaction mixture was heated at 80 °C under an argon atmosphere for 6 h. After completion of the reaction, the mixture was cooled to room temperature and poured into ethyl acetate and brine. The organic and aqueous layers were separated, and the aqueous phase was extracted again with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, and concentrated to obtain the desired 4-(2-(4-nitrobenzylideneamino)phenylamino)-N,N-disubstituted pyridine-2-carboxamide derivative (VI). Using this general synthetic procedure, ten derivatives designated VIa–VIj were prepared by varying the substituent associated with the N,N-disubstituted carboxamide moiety. The reported isolated yields ranged from 62% to 75%, while the melting points ranged from 197 to 239 °C. The physical characteristics and molecular formulas of the individual derivatives were documented for structural comparison [19].

Physicochemical characterization of synthesized compounds

The synthesized compounds were characterized using their physicochemical and spectroscopic properties. Melting points were recorded for the synthesized derivatives and reported in degrees Celsius. The molecular formulas and molecular ion information were used to support the proposed molecular structures. The intermediate 4-chloropyridine-2-carbonyl chloride (II)

showed a reported molecular ion peak at m/z 176.17 (M^+), whereas the 4-chloro-N,N-disubstituted pyridine-2-carboxamide intermediate (III) showed a reported molecular ion peak at m/z 185.21 ($M^+ + 1$). Compound V exhibited a reported molecular ion peak at m/z 242.25 ($M^+ + 1$). For the representative final derivative VI, infrared spectroscopy (KBr) showed absorption bands at 3030.9 cm^{-1} corresponding to aromatic C–H stretching, 1615–1513.1 cm^{-1} associated with C=C and C=N stretching, 1245.0 cm^{-1} corresponding to C–N stretching, 1641.3 cm^{-1} attributed to the carbonyl group, and bands reported in the 1542–3185.8 cm^{-1} region associated with the nitro functionality. The ^1H NMR spectrum recorded in DMSO- d_6 exhibited multiplet signals at δ 7.5–6.8 ppm corresponding to aromatic protons, a singlet at δ 5.0 ppm attributable to the NH proton, and a singlet at δ 2.5 ppm corresponding to the N-methyl groups in the representative dimethylamide derivative. The reported molecular ion peak of this derivative was m/z 390.61 ($M^+ + 1$) [20, 21].

***In vitro* cytotoxicity evaluation**

Cell lines and culture conditions

The cytotoxic activity of compounds VIa–VIj was evaluated against three human cancer cell lines: MCF-7 (breast cancer), HT-29 (colon cancer), and A549 (lung cancer). The cell lines were procured from the National Centre for Cell Science (NCCS), Pune, India. The cells were maintained as adherent monolayers in DMEM supplemented with 10% FBS, 100 $\mu\text{g/mL}$ penicillin, 200 $\mu\text{g/mL}$ streptomycin, and 2 mM L-glutamine. Cell cultures were maintained in a humidified incubator at 37 °C under 5% CO_2 conditions.

Preparation of test solutions

A stock solution of each synthesized compound was prepared at a concentration of 10 mg/mL in DMSO. The stock solutions were subsequently diluted with sterile water to obtain working concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/mL}$. These working solutions were used for evaluation of concentration-dependent cytotoxic effects [22].

MTT assay

Cytotoxicity was determined using the MTT colorimetric assay. Briefly, viable cells were quantified using the trypan blue exclusion method, and approximately 1×10^4 viable cells were seeded into each well of 96-well tissue-culture plates. The cells were allowed to establish and were subsequently treated with different concentrations of the test compounds. The treated cells were incubated for the specified treatment period under cell-culture conditions at 37 °C in medium supplemented with 10% FBS. Following treatment, the culture medium was replaced with 90 μL of fresh serum-

free medium and 10 μ L of MTT reagent (5 mg/mL). The plates were incubated at 37 °C for 4 h to permit formation of purple formazan crystals by metabolically active cells. After incubation, the medium containing residual MTT was removed and 200 μ L of DMSO was added to each well to dissolve the formed formazan crystals. The plates were further incubated at 37 °C for 10 min. Absorbance was subsequently measured at 570 nm using a SpectraMax spectrophotometer (Molecular Devices). Cell viability was determined relative to the untreated control, and IC₅₀ values were calculated from concentration–response relationships. The cytotoxic activity was expressed as IC₅₀ values, defined as the concentration of test compound required to inhibit cellular viability by 50% under the experimental conditions. Cisplatin was included as the reference cytotoxic agent. The reported IC₅₀ values for compounds VIa–VIj were compared with those of cisplatin to assess their relative cytotoxic potency against the three cancer cell lines [23].

***In vitro* antibacterial activity**

Test microorganisms

The antibacterial activity of compounds VIa–VIj was evaluated against two Gram-positive bacterial strains, *Bacillus thermophilus* (ATCC 7953) and *Staphylococcus aureus* (ATCC 25923), and two Gram-negative bacterial strains, *Klebsiella pneumoniae* (MTCC 3384) and *Salmonella paratyphi* A (ATCC 11511). The antibacterial activity was assessed using the cup-plate method, with ciprofloxacin used as the reference antibacterial agent.

Culture media

Nutrient broth was used for preparation of bacterial inocula, whereas nutrient agar was employed for the antibacterial screening. The nutrient agar medium contained peptone (5.0 g/L), sodium chloride (5.0 g/L), beef extract (1.5 g/L), yeast extract (1.5 g/L), and agar (15.0 g/L), with distilled water added to a final volume of 1000 mL. The pH of the medium was adjusted to 7.4 $\pm$ 0.2.

Preparation and maintenance of bacterial cultures

The test organisms were subcultured on nutrient agar. Sterilized culture medium was inoculated with the respective bacterial strains and incubated at 37 $\pm$ 1 °C for 24 h. Stock cultures were subsequently maintained under refrigerated conditions. For preparation of bacterial inocula, a loopful of each stock culture was transferred into 100 mL of nutrient broth contained in 250-mL conical flasks and incubated at 37 $\pm$ 1 °C for 48 h before antibacterial evaluation [24].

Preparation of test solutions

Each synthesized compound was dissolved in analytical-grade dimethylformamide (DMF) by dissolving 10 mg of compound in 10 mL of solvent. Ciprofloxacin was used as the reference standard and was prepared separately in sterile distilled water. A DMF solvent control was also included to assess any antibacterial effect attributable to the solvent [25].

Cup-plate antibacterial assay

Nutrient agar medium was sterilized by autoclaving at 121 °C and 15 lb/in² pressure for 15 min. Petri plates, test tubes, and cotton-plugged flasks were sterilized separately using a hot-air oven at 160 °C for 1 h. Approximately 27 mL of molten nutrient agar was transferred aseptically into each sterile Petri plate. The medium was inoculated with the respective bacterial culture, using 6 mL of inoculum per 300 mL of nutrient agar medium, and the plates were allowed to stand at room temperature until the medium solidified. Sterile cork borers were used to prepare wells in the solidified agar medium. An aliquot of 0.1 mL of each test solution was aseptically introduced into the corresponding well. The plates were maintained undisturbed under refrigerated conditions for at least 2 h to facilitate diffusion of the test compounds into the agar matrix. The plates were subsequently incubated at 37 $\pm$ 1 °C for 24 h. Following incubation, the diameter of the zone of inhibition surrounding each well was measured using an antibiotic zone reader. All antibacterial experiments were performed in triplicate. A solvent control containing 0.1 mL DMF was maintained concurrently, while ciprofloxacin at 10 μ g/cup served as the reference standard. The antibacterial activity was expressed as the diameter of the zone of inhibition in millimetres (mm). The reported concentration of each test compound was 200 μ g/cup. The antibacterial response of compounds VIa–VIj was compared with the ciprofloxacin reference standard against each bacterial strain [26].

Data analysis

The cytotoxicity results were expressed as IC₅₀ values, while antibacterial activity was expressed as the diameter of the zone of inhibition in millimetres. Comparative evaluation was performed across the synthesized derivatives and the respective reference standards. Antibacterial experiments were performed in triplicate as reported in the experimental procedure. The source material does not provide sufficient information regarding the number of independent biological replicates for the MTT assay or a specific statistical test; therefore, no additional statistical methodology has been introduced beyond the reported experimental design.

Statistical Analysis

Cytotoxicity results were expressed as IC₅₀ values, while antibacterial activity was expressed as the diameter of the inhibition zone in millimetres. Antibacterial experiments were performed in triplicate. The available study material did not provide sufficient information regarding the number of independent biological replicates for the MTT assay or a specific statistical test; therefore, no additional statistical methodology was introduced beyond the reported experimental design.

RESULTS AND DISCUSSION

Chemistry and synthesis

A series of ten picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj) was synthesized through a convergent synthetic sequence involving preparation of key intermediates followed by their coupling to generate the final heteroaromatic derivatives. The synthetic strategy was designed to incorporate a pyridine carboxamide pharmacophore together with an anilino-imine and *p*-nitrophenyl structural unit. The overall synthetic pathway is presented in Scheme 1. Initially, picolinic acid (I) was converted into 4-chloropyridine-2-carbonyl chloride (II) using thionyl chloride in the presence of anhydrous DMF. The reaction afforded the acid chloride intermediate in 80% yield, with a reported melting point of 151–154 °C. The formation of this activated acyl intermediate provided a suitable electrophilic center for subsequent amide formation. The reported molecular ion peak at m/z 176.17 (M^+) further supported the formation of intermediate II (Fig. 2).

The acid chloride intermediate was subsequently reacted with dimethylamine or appropriate secondary amines to produce 4-chloro-*N,N*-disubstituted pyridine-2-carboxamide derivatives (III). This transformation afforded the corresponding intermediates in a reported yield of 78%, with a melting point of 166–168 °C. The conversion of the acid chloride into the corresponding carboxamide introduced the variable *N*-substituted region that was subsequently used for structural diversification of the final compounds. The reported molecular ion peak at m/z 185.21 ($M^+ + 1$) was consistent with the formation of the representative carboxamide intermediate. In a parallel reaction sequence, *N*-(4-nitrobenzylidene)benzene-1,2-diamine (V) was prepared through condensation of 4-nitrobenzaldehyde with 1,2-phenylenediamine in methanol using 3 Å molecular sieves. The reaction was conducted at room temperature for 72 h and afforded the desired imine intermediate in 71% yield, with a reported melting point of 181–184 °C. The molecular ion peak at m/z 242.25 ($M^+ + 1$) supported the assigned molecular structure. The final derivatives VIa–VIj were obtained by coupling intermediate V with the corresponding 4-chloro-*N,N*-disubstituted pyridine-2-carboxamide intermediates (III) in dry DMF in the presence of potassium tert-butoxide and potassium carbonate. The reaction was performed at 80 °C under an argon atmosphere. The resulting compounds were isolated following extraction, washing, drying and concentration. The reported yields of the final derivatives ranged from 62% to 75%, indicating that the coupling strategy was applicable to a range of *N*-substituted analogues.

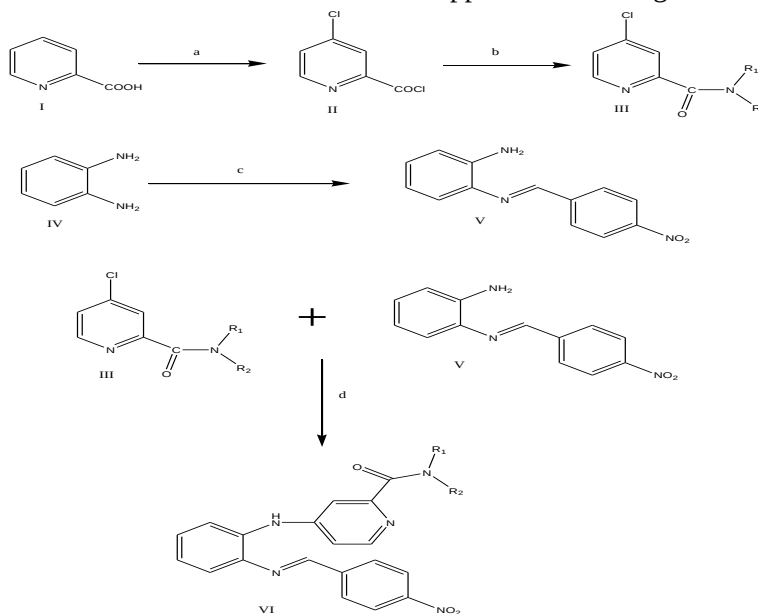


Fig. 2. Overall synthetic route for the preparation of picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj) through sequential formation of 4-chloropyridine-2-carbonyl chloride (II), 4-chloro-*N,N*-disubstituted pyridine-2-carboxamide intermediates (III), *N*-(4-nitrobenzylidene)benzene-1,2-diamine (V), and subsequent coupling to afford the target derivatives VIa–VIj.

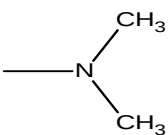
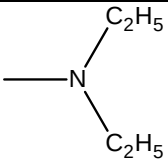
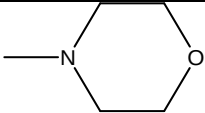
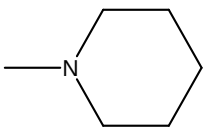
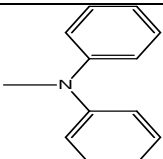
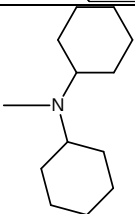
Physicochemical characteristics of compounds VIa–VIj

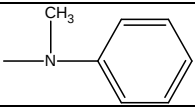
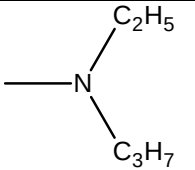
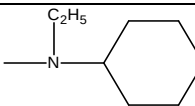
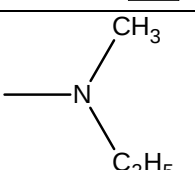
The synthesized compounds displayed melting points ranging from 197 to 239 °C, indicating the formation of relatively high-melting crystalline products. The individual yields ranged from 62% to 75%. Compound VIa was obtained in 65% yield with a melting point of 211–213 °C, whereas VIb was obtained in 72% yield with a melting point of 197–199 °C. VIc showed a yield of 62% and melting point of 221–224 °C, while VId was obtained in 64% yield with a melting point of 228–231 °C. Compounds VIe and VIf were obtained in 72% and 75% yields, respectively, with melting points of 235–237 and 218–220 °C. VIg, VIh, VIi and VIj exhibited

yields of 70%, 66%, 63% and 68%, respectively, with melting points of 237–239, 215–217, 226–227 and 209–211 °C. Overall, the relatively consistent synthetic yields demonstrate the reproducibility of the final coupling reaction despite structural variation among the derivatives. The variation in melting points may reflect differences in molecular packing, intermolecular interactions and the electronic or steric characteristics of the substituents incorporated into the carboxamide portion of the molecules. However, because the complete substituent assignments corresponding to VIa–VIj are not provided in the available experimental data, a definitive substituent-specific interpretation of these melting-point differences is not possible (**Table 1**).

Table 1. Physical and physicochemical characteristics of the synthesized picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj).

The table presents the compound code, substituent (R), molecular formula, melting point, and isolated percentage yield of the synthesized derivatives.

S.No.	Comp.	R	Mol. Formula	M.P. (°C)	Yield%
1	VIa		C ₂₁ H ₁₉ N ₅ O ₃	211-213	65
2	VIb		C ₂₃ H ₂₃ N ₅ O ₃	197-199	72
3	VIc		C ₂₂ H ₂₁ N ₅ O ₃	221-224	62
4	VId		C ₂₃ H ₂₃ N ₅ O ₃	228-231	64
5	VIe		C ₂₆ H ₂₁ N ₅ O ₃	235-237	72
6	VIf		C ₂₇ H ₂₉ N ₅ O ₃	218-220	75
7	VIg		C ₃₁ H ₃₅ N ₅ O ₃	237-239	70

					
8	VIh		C ₃₁ H ₂₃ N ₅ O ₃	215-217	66
9	VIIi		C ₂₃ H ₂₁ N ₅ O ₄	226-227	63
10	VIj		C ₂₄ H ₂₃ N ₅ O ₃	209-211	68

Spectroscopic characterization

The structural assignment of the synthesized compounds was further supported by spectroscopic characterization. For the representative final derivative VI, the FTIR spectrum displayed an aromatic C–H stretching band at 3030.9 cm⁻¹. Absorption bands in the 1615–1513.1 cm⁻¹ region were assigned to C=C and C=N stretching vibrations, supporting the presence of the aromatic and imine functionalities. The band at 1641.3 cm⁻¹ was attributed to the amide carbonyl group, while the reported C–N stretching absorption occurred at 1245.0 cm⁻¹. The reported bands associated with the nitro functionality further supported incorporation of the *p*-nitrophenyl moiety. The ¹H NMR spectrum of the representative compound recorded in DMSO-*d*₆ exhibited a multiplet in the δ 7.5–6.8 ppm region corresponding to aromatic protons. A singlet at δ 5.0 ppm was assigned to the NH proton, while the signal at δ 2.5 ppm corresponded to the N-methyl groups of the representative dimethylamide derivative. These signals were consistent with the proposed aromatic, anilino and N,N-disubstituted carboxamide structural framework. The reported molecular ion peak at *m/z* 390.61 (M⁺ + 1) provided additional support for the proposed structure.

In vitro cytotoxic activity

The cytotoxic potential of compounds VIa–VIj was investigated against three human cancer cell lines, namely MCF-7, HT-29 and A549, using the MTT assay. The IC₅₀ values obtained from the concentration–response studies are summarized in **Table 2**. Lower IC₅₀ values indicate greater inhibition of cellular metabolic activity and, consequently, greater cytotoxic potency under the experimental conditions. Cisplatin was included as the reference cytotoxic agent. The synthesized compounds demonstrated considerable variation in cytotoxic activity across the three cell lines, indicating that structural modification of the picolinic

acid-derived scaffold influenced the biological response. Among the tested compounds, VIg and VIh were particularly prominent, exhibiting substantially lower IC₅₀ values than most of the other synthesized derivatives. Compound VIg showed IC₅₀ values of 11.4, 13.5 and 18.9 μM against MCF-7, HT-29 and A549 cells, respectively. Compound VIh exhibited IC₅₀ values of 9.2, 8.4 and 16.6 μM against MCF-7, HT-29 and A549 cells, respectively. Thus, VIh represented the most potent derivative overall based on its lowest IC₅₀ values across the three evaluated cell lines.

Compound VIj also displayed comparatively strong cytotoxic activity, with IC₅₀ values of 27.2, 20.3 and 35.37 μM against MCF-7, HT-29 and A549 cells, respectively. VIa and VIb exhibited moderate activity, with IC₅₀ values of 38.4, 33.7 and 50.1 μM and 40.6, 35.6 and 55.7 μM against MCF-7, HT-29 and A549 cells, respectively. VIc demonstrated weaker activity than VIa and VIb, with IC₅₀ values of 80.5, 60.4 and 70.9 μM against MCF-7, HT-29 and A549 cells, respectively. VIe and VIf also showed moderate-to-weak cytotoxicity, with IC₅₀ values of 91.2, 78.4 and 59.3 μM for VIe and 78.9, 84.1 and 92.2 μM for VIf against MCF-7, HT-29 and A549 cells, respectively. VId showed limited cytotoxic activity, with an IC₅₀ value of 142.6 μM against MCF-7, while no measurable activity was reported against HT-29 and A549 under the tested conditions. Similarly, VIIi showed an IC₅₀ value of 73.2 μM against MCF-7 and 41.7 μM against A549, whereas no activity was reported against HT-29. The reference drug cisplatin exhibited IC₅₀ values of 8.61, 6.56 and 14.31 μM against MCF-7, HT-29 and A549, respectively. Although VIh did not surpass cisplatin against all three cell lines, its IC₅₀ values were relatively close to those of the reference compound, particularly against MCF-7 and HT-29. The results therefore identify VIh as a promising lead among the synthesized derivatives, warranting further investigation.

Table 2. *In vitro* cytotoxic activity of the synthesized picolinic acid-derived derivatives (VIa–VIj) against human cancer cell lines.

IC₅₀ values (μM) represent the concentration of each test compound required to inhibit 50% of cellular viability against MCF-7 (breast cancer), HT-29 (colon cancer), and A549 (lung cancer) cells, as determined by the MTT assay. Cisplatin was used as the reference cytotoxic agent. NA, no activity.

Compound	IC ₅₀ (μm)		
	MCF-7	HT-29	A 549
VIa	38.4	33.7	50.1
VIb	40.6	35.6	55.7
VIc	80.5	60.4	70.9
VI d	142.6	NA	NA
VIe	91.2	78.4	59.3
VI f	78.9	84.1	92.2
VI g	11.4	13.5	18.9
VI h	9.2	8.4	16.6
VI i	73.2	NA	41.7
VI j	27.2	20.3	35.37
Cisplatin	8.61	6.56	14.31

Comparative cytotoxic response and preliminary structure–activity relationship

A comparative analysis of the IC₅₀ data suggests that cytotoxic activity was strongly dependent on the structural characteristics of the synthesized derivatives. VIg and VIh showed the most favorable activity profile, whereas VI d showed the weakest overall response. This marked difference indicates that modification of the N,N-disubstituted carboxamide region can substantially influence the biological behavior of the common picolinic acid-derived scaffold. The common structural framework of the synthesized compounds contains a pyridine ring, carboxamide group, anilino linkage, imine functionality and *p*-nitrophenyl group. These functionalities provide multiple sites capable of participating in hydrogen bonding, dipolar interactions and other noncovalent interactions with biological targets. The improved activity observed for VIg and VIh may therefore be associated with a favorable balance of steric, electronic and physicochemical properties produced by their respective substituents. However, because the available source does not explicitly provide the complete identity of the R groups corresponding to each derivative, a precise substituent-specific SAR interpretation cannot be established without introducing unsupported structural assignments.

An additional observation is that the relative sensitivity of the cell lines varied among the compounds. Several derivatives exhibited lower IC₅₀ values against HT-29 than against A549, whereas other derivatives showed the opposite trend. VIh displayed particularly strong activity against both MCF-7 and HT-29 cells, suggesting that its activity was not restricted to a single cancer-cell model. Conversely, VI f showed comparatively weak activity

across all three cell lines. These findings demonstrate the importance of evaluating the derivatives against multiple cancer models when identifying promising lead structures.

In vitro antibacterial activity

The antibacterial activity of compounds VIa–VIj was evaluated against two Gram-positive bacteria, *Bacillus thermophilus* and *Staphylococcus aureus*, and two Gram-negative bacteria, *Klebsiella pneumoniae* and *Salmonella paratyphi* A. The results were expressed as the diameter of the zone of inhibition in millimetres at a test concentration of 200 μg/cup. Ciprofloxacin at 10 μg/cup was used as the reference standard. All ten derivatives exhibited measurable antibacterial activity against at least some of the tested microorganisms, although the magnitude of inhibition varied substantially among the compounds and bacterial strains. VIa produced inhibition zones of 4, 3, 5 and 3 mm against *B. thermophilus*, *S. aureus*, *K. pneumoniae* and *S. paratyphi* A, respectively. VIb showed a comparatively improved response, with inhibition zones of 6, 5, 8 and 7 mm against the four organisms, respectively.

VIc showed inhibition zones of 3, not detected, 5 and 2 mm against *B. thermophilus*, *S. aureus*, *K. pneumoniae* and *S. paratyphi* A, respectively. VI d exhibited inhibition zones of 6, 2, 7 and 9 mm, respectively, demonstrating a comparatively stronger response against *S. paratyphi* A. VIe showed inhibition zones of 5, 8, 4 and no activity against the respective organisms. VI f displayed relatively weak antibacterial activity, with inhibition zones of 2 and 3 mm against *B. thermophilus* and *S. aureus*, respectively, no detectable activity against *K. pneumoniae*, and 2 mm against *S. paratyphi* A. In

contrast, VIg exhibited a marked increase in antibacterial activity, producing zones of 12, 10, 9 and 12 mm against *B. thermophilus*, *S. aureus*, *K. pneumoniae* and *S. paratyphi* A, respectively. Compound VIh exhibited the highest antibacterial activity among the synthesized derivatives. It produced inhibition zones of 13, 15, 11 and 14 mm against *B. thermophilus*, *S. aureus*, *K. pneumoniae* and *S. paratyphi* A, respectively. The comparatively broad activity of VIh across both Gram-positive and Gram-negative organisms identifies it as the most promising antibacterial derivative within the synthesized series.

VIi produced inhibition zones of 6, 3, 5 and 7 mm, whereas VIj exhibited zones of 3, 1, 2 and 2 mm against the four bacterial strains, respectively. Thus, VIj displayed relatively weak antibacterial activity despite showing comparatively favorable cytotoxic activity. Ciprofloxacin produced inhibition zones of 18, 20, 16 and 18 mm against *B. thermophilus*, *S. aureus*, *K. pneumoniae* and *S. paratyphi* A, respectively. Therefore, although VIh exhibited the highest antibacterial activity among the synthesized compounds, its activity remained lower than that of ciprofloxacin under the reported assay conditions (Table 3).

Table 3. *In vitro* antibacterial activity of the synthesized picolinic acid-derived derivatives (VIa–VIj).

The antibacterial activity is expressed as the diameter of the zone of inhibition (mm) against *Bacillus thermophilus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Salmonella paratyphi* A at a test concentration of 200 µg/cup. Ciprofloxacin (10 µg/cup) was used as the reference antibacterial agent. NA, no activity.

Compound	Gram +ve		Gram –ve	
	<i>Bacillus thermophilus</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Salmonella paratyphi</i> A
VIa	4	3	5	3
VIb	6	5	8	7
VIc	3	NA	5	2
VI d	6	2	7	9
VIe	5	8	4	NA
VI f	2	3	NA	2
VIg	12	10	9	12
VIh	13	15	11	14
VIi	6	3	5	7
VIj	3	1	2	2
Ciprofloxacin 10µg/cup	18	20	16	18

Comparative antibacterial structure–activity relationship

The antibacterial results revealed a clear difference in activity among the derivatives despite the presence of a common heteroaromatic framework. VIg and VIh were substantially more active than most of the other synthesized compounds, indicating that the structural modification introduced at the variable portion of the molecule can markedly influence antibacterial activity. The particularly strong activity of VIh against all four organisms suggests that its molecular architecture may provide a favorable combination of lipophilicity, electronic characteristics and functional-group orientation for interaction with bacterial cellular components. The activity of VIh was observed against both Gram-positive and Gram-negative organisms, indicating that its antibacterial effect was not limited to a particular bacterial cell-envelope class. However, the present zone-of-inhibition assay provides only a preliminary measure of antibacterial activity and does not directly establish the molecular mechanism of action.

The enhanced activity observed for VIg and VIh should therefore be considered an important lead-finding observation rather than definitive evidence of a specific antibacterial target. The comparatively low activity of VIj further emphasizes that cytotoxic and antibacterial activities were not necessarily correlated. VIj displayed appreciable cytotoxic activity, particularly against HT-29 cells, but produced only small inhibition zones against the tested bacteria. This difference suggests that the biological properties of these compounds are strongly assay- and target-dependent.

Integrated biological evaluation

When the cytotoxic and antibacterial results are considered together, VIh emerged as the most promising compound in the investigated series. It exhibited IC₅₀ values of 9.2, 8.4 and 16.6 µM against MCF-7, HT-29 and A549 cells, respectively, together with inhibition zones of 13, 15, 11 and 14 mm against the four bacterial strains. VIg represented the second notable derivative, showing IC₅₀ values of 11.4, 13.5 and 18.9 µM against

the three cancer cell lines and antibacterial inhibition zones of 12, 10, 9 and 12 mm. The simultaneous cytotoxic and antibacterial activity observed for VIg and VIh suggests that these derivatives possess a favorable biological profile and may serve as useful lead structures for subsequent medicinal-chemistry optimization. Nevertheless, the present findings represent preliminary *in vitro* evidence. Further investigations involving mechanistic studies, selectivity evaluation against nonmalignant cells, determination of minimum inhibitory concentrations, apoptosis-related assays, and molecular target studies would be necessary to establish their therapeutic potential. Collectively, the synthetic investigation successfully generated ten picolinic acid-derived pyridine carboxamide derivatives with moderate-to-good isolated yields. The physicochemical and spectroscopic findings supported the proposed structures of the intermediates and representative final derivative. Biological screening demonstrated substantial differences in activity across the synthesized series. In the cytotoxicity assay, VIh was the most active derivative, followed by VIg and VIj, whereas VId exhibited the weakest response. In the antibacterial assay, VIh showed the greatest and broadest inhibition, followed by VIg. The concordant cytotoxic and antibacterial profiles of VIg and VIh highlight these compounds as the most promising members of the series. The results also indicate that structural diversification of the picolinic acid-derived carboxamide framework can produce marked changes in biological activity. The present findings provide a useful preliminary SAR foundation for further optimization of this scaffold and support continued investigation of selected derivatives, particularly VIg and VIh, as potential leads for anticancer and antibacterial drug discovery.

CONCLUSION

A series of ten novel picolinic acid-derived pyridine carboxamide derivatives (VIa–VIj) was successfully synthesized through a multistep synthetic approach and evaluated for their *in vitro* cytotoxic and antibacterial activities. The target compounds were obtained in moderate-to-good yields of 62–75%, and their structures were supported by physicochemical and spectroscopic characterization. The biological evaluation demonstrated considerable variation in activity among the synthesized derivatives, indicating that structural modification of the pyridine carboxamide scaffold substantially influenced their biological properties. Among the synthesized compounds, VIh emerged as the most promising derivative, exhibiting potent cytotoxic activity against MCF-7, HT-29, and A549 cancer cells, with IC_{50} values of 9.2, 8.4, and 16.6 μ M, respectively. VIh also demonstrated the broadest antibacterial activity against

the tested Gram-positive and Gram-negative organisms. VIg showed a similarly favorable biological profile, with IC_{50} values of 11.4–18.9 μ M and appreciable antibacterial activity. Although the activities of VIg and VIh were lower than those of the respective reference standards, their overall biological profiles identify them as promising lead compounds. These findings provide preliminary structure–activity relationship insights and support further optimization of the picolinic acid-derived pyridine carboxamide scaffold. Further studies involving mechanistic investigations, selectivity assessment, minimum inhibitory concentration determination, and *in vivo* evaluation are warranted to establish the therapeutic potential of these lead derivatives.

Ethics Statement

This study involved *in vitro* evaluation of synthesized chemical compounds using established cancer cell lines and bacterial strains. No human participants or experimental animals were involved in the study; therefore, ethical approval was not applicable.

Informed Consent Statement

Not applicable, as the study did not involve human participants or identifiable human data.

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Conflict of Interest

The authors declare that they have no conflict of interest related to this study.

Author Contributions

Shalini Thakkallapelly: Conceptualization, methodology, synthesis, investigation, data collection, analysis, and manuscript preparation.
Lalitha Repudi: Supervision, scientific guidance, interpretation of results, critical revision, and final approval of the manuscript. Both authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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Data Availability Statement

The data supporting the findings of this study are contained within the article. Additional information may

be obtained from the corresponding author upon reasonable request.

Additional Characterization Information

The complete identities of the variable substituents (R groups) corresponding to derivatives VIa–VIj should be provided to enable a reliable compound-by-compound structure–activity relationship analysis. The current information does not permit unsupported assignment of these substituents.

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